Medicinal Chemistry rescuing the empowerment of science to promote human health.

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20-24th september
Atlântico Búzios Convention
Búzios, Rio de Janeiro - Brazil

II BrazMedChem 2025



Annals


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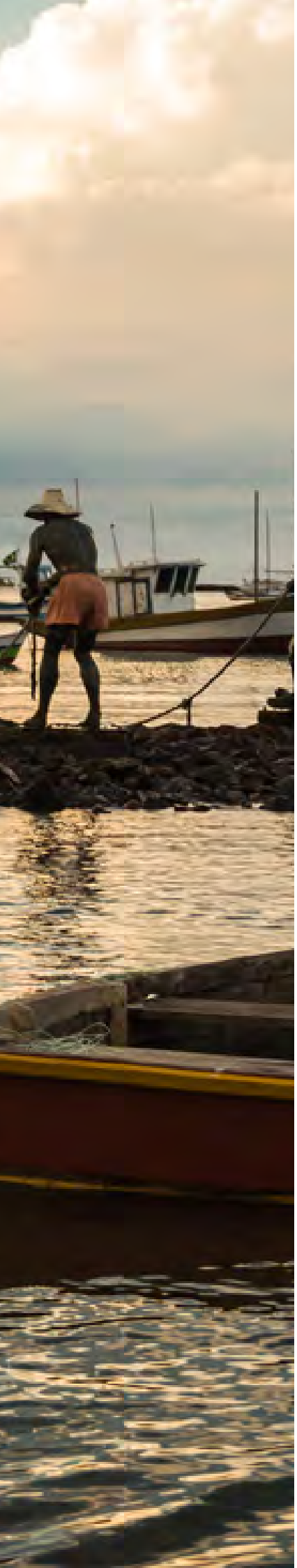
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Presentation

We and the organizing committee, have worked tirelessly for you to have the best experience in this edition of BrazMedChem. It is important to highlight that this event is the result of the hard work of many people, and I'd like to take a moment to say thanks to all colleagues that contributed to BrazMedChem 2025. We'd like to start by expressing my sincere gratitude to the organizing committee, namely:

- Prof. Arthur Kummerle
- Prof. Carlos Maurício Sant'Anna
- Prof. Luzineide Tinoco
- Prof. Sabrina Ferreira

We also want to acknowledge all members of the scientific committee, through its chairs Prof. Glaucius Oliva and Prof. Rafael Guido. A special thanks to Prof. Stefan Laufer, president of our international scientific committee.

We are deeply grateful to all invited speakers who agreed to come to BrazMedChem, all of them costing flight tickets at their own expense. Furthermore, we'd like to thank all the sponsors for believing in the potential of this event and the funding agencies that supported BrazMedChem 2025: The National Council for Scientific Research and Development – CNPq and The Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro-FAPERJ.

We must thank our secretariat team led by Alessandra Leitão from Creacteve Eventos and Web and Graphic Designer Rogerio Kaiser whose support was essential for making this event happen. We cannot forget to recognize the volunteer work of the social networks team, Gabriel Alves and Manuelle Cunha, along with all monitors that you be seeing for the next days handling mics and offering help to other participants.

Now, in its 12th edition, the Brazilian Symposium on Medicinal Chemistry (BrazMedChem) is an international event organized by the Brazilian scientific community and recognized by the Brazilian Chemistry Society and its Medicinal Chemistry division.

We believe the professional career of many participants can be confounded with the history of BrazMedChem, as mine can. Since its inception in 2001, this biannual event has brought together internationally renowned researchers and students for over 20 years. While BrazMedChem is a mature event, a benchmark in Brazil for practitioners of the interdisciplinary field of Medicinal Chemistry, the latter remains a science in constant evolution. As a fundamental science in the process of drug innovation, events that promote the updating and discussion of ideas in Medicinal Chemistry are essential to contributing to the advancement of this field. After a recent period of denial and attacks on the essential role of science in promoting human health, the 2025 edition of BrazMedChem will focus

on the theme "Medicinal Chemistry: Rescuing the Empowerment of Science to Promote Human Health."

We are proud to say that the current edition attracted nearly 350 participants to the beautiful City of Buzios, among students, academic researchers and industry professionals. It was our absolute priority to offer a scientific program of the highest academic standard. Featuring an intense agenda over 3 days, including 25 talks by internationally renowned speakers, distributed across four thematic sessions, 4 lectures and the opening conference, BrazMedChem offers its participants access to the state of the art in the field.

We also would like to highlight the roundtable bringing together highly prestigious members of the academy and industry from both private and public sectors in Brazil to discuss “New Challenges in the Brazilian Pharma Industry”. Moreover, with 228 accepted abstracts we will have two poster sessions and twelve oral presentations of selected abstracts.

With special attention to the students and all the newcomers to the field of medicinal chemistry we have offered 3 high-level short courses, free for all registered participants, as pre-event activities yesterday and earlier today. The organizing committee is deeply grateful to all instructors and trainers coming from different states in Brazil and as far away as Scotland! We also had technical talks by our sponsors Clarivate and ACS as well as the ACS on Campus for the first time in BrazMedChem. The current edition of BrazMedChem was also home to important recognitions and celebrations, such as a Special Session in tribute to prof. Carlos Alberto Manssour Fraga, who prematurely left us last year.

An initiative that has been continuously supported by BrazMedChem is the Workshop of Young Researchers in Drug Design and Development. In its sixth edition the workshop has been an important forum for the new generations of medicinal chemists in Brazil. Through the person of Prof. Pedro de Sena I’d like to thank the Workshop’s organizing committee.

All these activities offered plenty of opportunities for participants to embark on fruitful discussions with other colleagues and specialists.

Sincerely,

Dr. Floriano Paes Silva Junior – Chair (FIOCRUZ, Rio de Janeiro-RJ)
Dra. Lídia Moreira Lima – Chair (UFRJ, Rio de Janeiro-RJ)


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Participant



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The Chemical Library Space: From Compound Chemography to Management of Portfolio of DNA-Encoded Libraries.

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Chemography, Generative Topographic Mapping, In Silico Library Design, DNA-encoded Libraries.

Introduction

Chemography[1] – the cartography of chemical space – is a chemist-friendly approach to represent molecules on a human-readable 2D map, a projection of the initial high-dimensional molecular descriptor space, nevertheless expected to preserve neighborhood (neighbors in the initial space should be projected close to each other on the map). Among the many[2] -linear or non-linear – algorithms allowing such a projection, Generative Topographic Mapping[3] (GTM) stands out as the most polyvalent. It works like a fuzzy-logics-driven grid-based approach, where the map is described as regular grid of "nodes" and items are mapped by assignment to their specific nodes. Unlike in the very popular Kohonen maps, where each item is unambiguously placed in its one "residence" node, in GTM items are assigned fractional "residence weights" (technically termed Responsibilities R) for several nodes. Responsibility R_{mn} may be intuitively understood as the "level of attachment" of molecule m to node n, with the sum of responsibilities of m over all nodes being equal to one. Due to this fuzzy, continuous approach, GTMs can accommodate property landscapes, where nodes are colored by the R-weighted means of the properties of their residents – which make them valuable QSAR tools.
Molecules that are similarly "split" between a same pool of nodes (have a same "Responsibility Pattern" RP) naturally form a cluster, and usually share some clearly identifiable common structural "signature" – which may be a common specific substructure, or scaffold, or topological pharmacophore, This spontaneous, unsupervised ordering by underlying chemotypes makes GTM an outstandingly potent tool for library comparison[4]. The Cumulated Responsibility CR vectors obtained by summing up R values of library members (with or without molecular property-driven weighing) will define the library, in the sense of implicitly describing which "chemotypes" are likely present in there, and in which relative proportions. Comparing two libraries can be reduced to an instant comparison of two CR vectors, no longer requiring tedious pairwise comparison of individual members. Libraries can be hence treated as individual objects – which is particularly valuable with DNA-encoded Libraries (DELs) and any other mixture libraries, where individual compounds cannot be easily separated.

Results and Discussion

Herein we introduce the concept of Chemical Library Space (CLS) in which resident items are individual chemical libraries. We define and compare several CR-based vectorial library encodings (Figure 1).

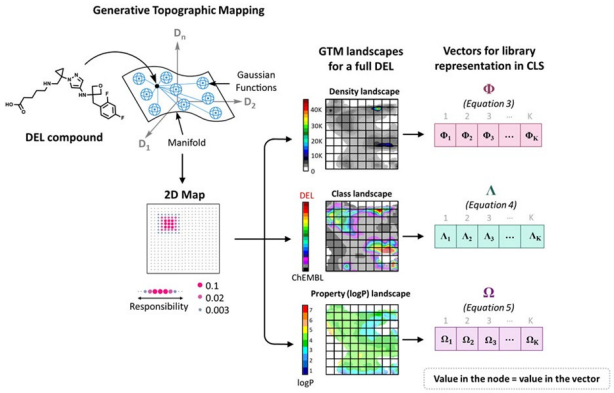


Figure 1: Scheme depicting how each of the vectorial library representations (Φ, Λ, Ω) are derived from the GTM

These allow effective comparison of libraries, with the ability to tune and chemically interpret the similarity relationships. In particular, property-tuned CLS encodings enable to simultaneously compare libraries with respect to both property and chemotype distribution. We apply the various CLS encodings for the selection problem of DELs that optimally "match" a reference collection (here ChEMBL release 28), showing how the choice of the CLS descriptors may help to fine-tune the "matching" (overlap) criteria (Figure 2). It is clear that library comparison in terms of mutual chemical space coverage is much richer than the state-of-art comparisons of property distribution histograms. In Figure 3, the two compared DELs may seem as equivalent choices in terms of logP distribution patterns – yet, they are radically different with respect to the chemical space they cover, with DEL1 being much more diverse, touching significant zones of the ChEMBL-covered chemical space.

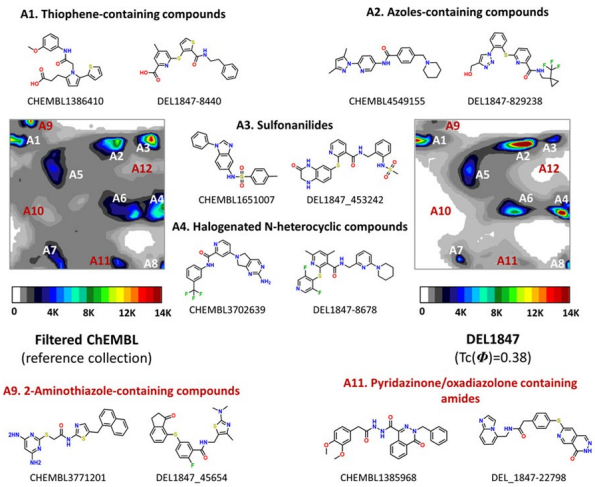


Figure 2: Interpretation of the similarity between ChEMBL and DEL1847 via structural analysis of the density landscapes of those libraries. Areas A1-A8 (labeled in white) correspond to the peaks of high density in ChEMBL space that were reproduced in DEL1847. Areas A9-A12 (labeled in red) represent mismatched zones.

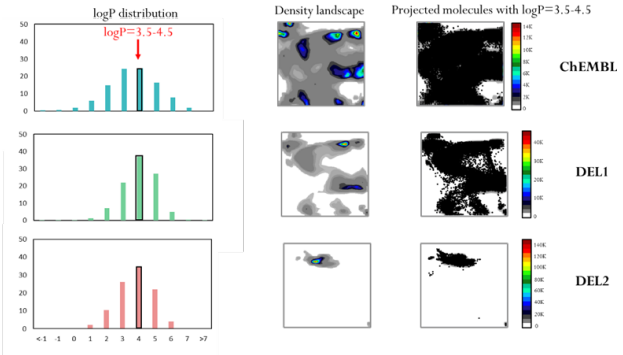


Figure 3: Looking only at logP distribution histograms conveys the impression that the two DELs are equivalent - whereas their coverage of chemical space is radically different

Even so, library enumeration and mapping required to obtain their vectorial representations may become a bottleneck as soon as sizes of billions of compounds come into play. However, Artificial Intelligence (AI) can be used to bypass this lengthy enumeration process. A graph-convolutional Combinatorial Library Neural Network[5] (CoLiNN) was developed to predict the vectorial representation of any combinatorial library from the reaction rules and building block sets directly, with excellent accuracy and in times shortened by several orders of magnitude.

The CLS is therefore useful for the management of a portfolio of libraries, just like Chemical Space (CS) helps managing a portfolio of molecules. Given the steadily growing number of DEL designs, the CLS becomes "crowded", and requires analysis tools beyond pairwise library comparison. An option is the cartography of CLS on meta-(μ)GTMs[6] – "meta" to remind that these are maps of the CLS, itself based on responsibility vectors issued by regular CS GTMs. 2,5K DELs and ChEMBL (reference) were projected on the μGTM, producing landscapes of library-specific properties. These describe both inter-library similarity and intrinsic library characteristics in the same view, herewith facilitating the selection of the best project-specific libraries.

Conclusions

Hence, the proposed CLS is a new efficient way for polyvalent analysis of thousands of chemical libraries. Selection of an easily accessible compound collection for drug discovery, as a substitute for difficult to produce reference library, can be tuned for either primary or target-focused screening, also considering property distributions of compounds. Alternatively, selection of libraries covering novel regions of the chemical space with respect to a reference compound subspace may serve for library portfolio enrichment.

Acknowledgements

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Dynamically chiral phosphonic acid-type metallo-β-lactamase inhibitors

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Key words: dynamic chirality, phosphonic acids, metallo-β-lactamase inhibitors, structural adaptability.

Introduction

Antimicrobial resistance is a critical global health threat. The resistance development against the worldwide most prescribed antibiotics, the β-lactams, is especially concerning as it not just affects our most frequently used antibiotics, such as amoxicillin and cephalexin, but also our “last-resort” drugs, the carbapenems.[1] This resistance is primarily caused by β-lactamase enzymes that dismantle β-lactam antibiotics.[2] Combination therapy has been proven successful in tackling the resistance caused by serine-β-lactamases[3], however, so far no effective inhibitor against metallo-β-lactamases (MBLs) has reached the market. MBLs are among the most distressing group of bacterial enzymes for the current clinical practice.[4]

Results and Discussion

We introduce a new strategy for the inhibition of MBLs by dynamically chiral phosphonic acids that interconvert between two bioactive enantiomers that bind the same binding site yet in different binding modes.[5] These compounds were designed to be stereochemically dynamic in order to adapt to the structural variations of different metallo-β-lactamase enzymes, which is a key challenge in the development of a broad-spectrum MBL inhibitor. A set of 13 new compounds were obtained through a straightforward 5 step synthetic route. They are soluble in water, penetrate the bacterial membrane and show promising inhibitory activity against the clinically most relevant MBLs VIM-2, NDM-1 and GIM-1 (IC_{50} ~10-200μM). Several of them inhibit multiple enzymes highlighting their broad-spectrum potential. Experimental and computational studies revealed a conserved yet adaptable binding mode, targeting MBL active-site zinc ions while mimicking the β-lactam hydrolysis transition-state. Computational analyses suggest that stereodynamic interconversion between two active isomers may be a fruitful strategy to counteract resistance via single point mutations.

Conclusions

The discovery of new, adaptive and broad spectrum MBL inhibitors may provide a long sought solution to tackle β-lactam resistance. Combination therapy with these agents is expected to restore the initial activity of β-lactams, an important class of antibiotics with a lowered risk of renewed rapid resistance development.

Acknowledgements

We acknowledge the Swedish Research Council (2013-8804, 2024-05496), NSFC-STINT (82211530060), Uppsala Antibiotic Center, Swedish Society for Medical Research (PD20-0191), Åke Wiberg Foundation (M22-0199), Max Planck Society, DFG (458246365), Federal Ministry of Education and Research Germany (01KI2114), Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund (project no. TKP2021-EGA-24 under TKP2021-EGA funding scheme).

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Development Of An Automated Computational Strategy For The Identification Of New Antiviral Compounds: The Nipah Virus Case

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Key words: Drug design, antiviral, pandemics, medicinal chemistry, automatization, virtual screening.

Introduction

Pandemics have a profound impact, as evidenced by the bubonic plague, Spanish influenza, HIV/AIDS, and SARS-CoV-2. Nipah virus (NiV), characterized by high lethality and zoonotic transmission, is a significant threat, responsible for outbreaks in Asia and Oceania, with no specific treatment or approved vaccine. The proposal aims to use computational tools to discover and optimize drugs against NiV, using molecular modeling, docking, and molecular dynamics to identify promising molecules and advance the research and development of effective therapies.

Results and Discussion

A general strategy was developed for this work, focusing on the use of ML/AI tools. Several molecular systems were established for methodological control, such as G protein, L-P protein complex, and M protein. These viral protein structures were obtained from databases or modeled using traditional methods (Modeller).

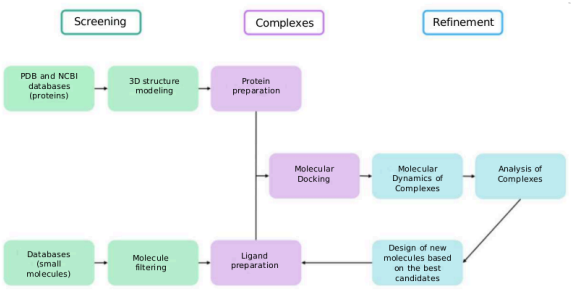


Figure 1: Computational workflow for therapeutic candidate identification.

Non-automated molecular docking was also performed to ensure that the data generated by the automated strategy was reliable. The virtual screening workflow produced protein-ligand poses, which were, then, submitted to the PLACER software to produce a complex ensemble. The ensembles generated were evaluated by the AEV-PLIG tool, allowing the calculation of binding affinities for each frame.

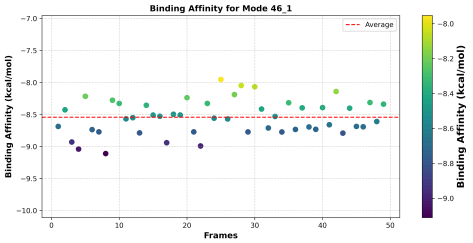


Figure 2: Example of the binding affinities calculated for one of the ensembles obtained from docking and PLACER results.

Conclusions

The workflow designed was able to obtain high-scoring binding-affinity predictions for several complexes. These data may be useful to identify relevant hits which can be further optimized into leads, working as a starting point for the discovery of novel inhibitors against NiV proteins.

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Probing the Protein Kinases′ Cysteinome by Covalent Fragments

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Keywords: Protein kinases, covalent inhibitors, cysteinome, fragment-based drug discovery, structural biology

Introduction

Selectivity remains a critical challenge in the development of new protein kinase inhibitors. A prominent strategy to achieve selectivity within the "kinome" involves targeting poorly conserved, non-catalytic cysteine residues adjacent to the ATP-binding pocket using electrophilic functional groups, often referred to as covalent "warheads" [1]. Despite significant progress in the development of such covalent kinase inhibitors, many accessible cysteine positions within the "cysteinome" of protein kinases remain unexplored and untargeted [2-4].

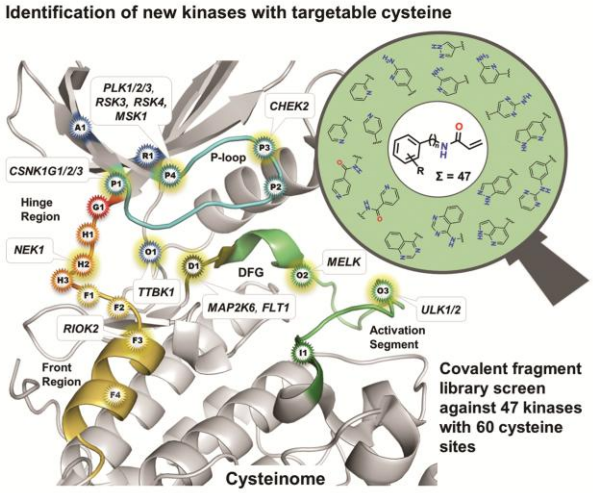


Figure 1: Overview of fragments and target cysteine positions addressed in this study.[5]

Results and Discussion

Here, we present a simple yet generalizable approach for sampling ATP-site cysteines using fragment-like covalent inhibitors [5]. To achieve this, we developed a kinase-focused covalent library comprising 47 compounds and systematically screened it against a panel of 47 protein kinases, encompassing 60 potentially accessible cysteines near the active site. Initial screening was conducted using intact protein mass spectrometry (MS) and differential scanning fluorimetry (DSF) assays. Promising hits were further validated by complementary techniques, including enzymatic assays, cellular NanoBRET assays, MS-based kinetic measurements to determine k_{inact}/K_I, and glutathione (GSH) reactivity assays. Binding sites were confirmed through tryptic digestion, mutagenesis studies, and X-ray crystallography.

Conclusions

Our findings significantly expand the repertoire of ligandable cysteines within protein kinases, identifying covalent binders for enzymes such as CSNK1G1/2/3, CHEK2, PLK1/2/3, MELK, ULK1/2, and RIOK2. They offer valuable insights into unique binding modes elucidated by X-ray crystal structures and deliver isoform-specific hits with promising profiles. These hits represent excellent starting points for the development of highly potent and selective covalent inhibitors, particularly for the less-explored regions of the kinome.

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In vitro pharmacological activity of inhibitors dipeptidyl nitrile derivatives in liver and pancreatic cancer cells.

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Key words: cysteine proteases, cellular assays, preclinical trials, liver and pancreatic cancer, antineoplastic.

Introduction

Pancreatic and liver cancers have high mortality and metastasis rates in Brazil, with limited chemotherapy effectiveness.[1] Cysteine cathepsin inhibitors, such as dipeptidyl nitrile derivates, show potential in targeting cathepsins B, L and S.[2]

This project aims to identify cytotoxic cysteine cathepsin inhibitors of sixteen dipeptidyl nitrile derivatives in pancreatic (MIA PaCa-2) and liver cancer (HepG2) cell lines, using Balb/3T3 clone A31 as a non-tumoral fibroblast.

Results and Discussion

Figure 1: Screening assays.

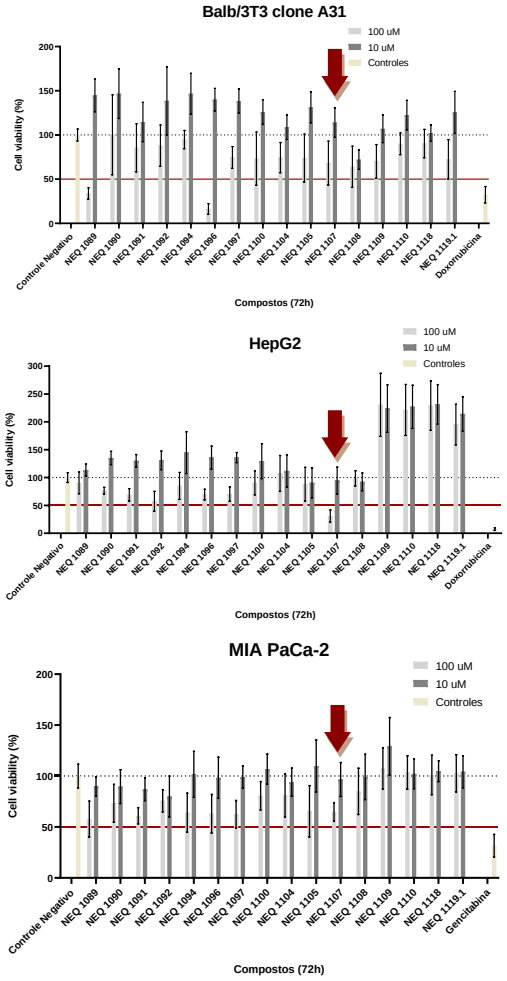


Figure 2: Concentration-response curve NEQ1107.

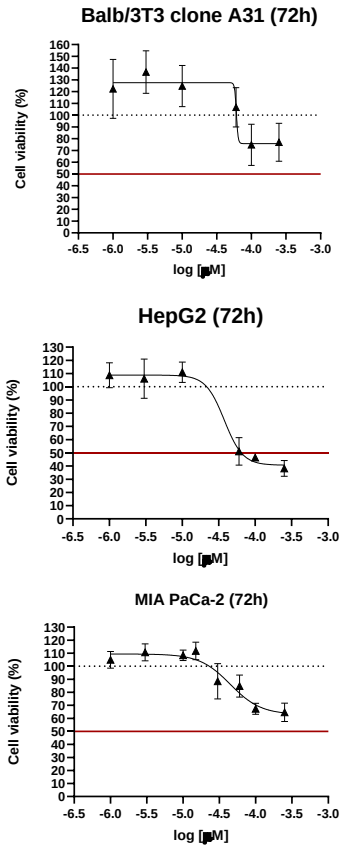


Table 1. Data from concentration-response curves NEQ1107.

Cell line	IC_{50} (μL)	SI
HepG2	38 ±3.7	>6.6
MIA Paca-2	>250	-
Balb/3T3 clone A31	>250	-

$$SI = IC_{50\ Balb} / IC_{50\ HepG2}$$

Conclusions

These results demonstrate that the NEQ1107 was the only cytotoxic compound. It was active and selective against HepG2 cells. Future studies will analyze the cell death mechanism and resistance of the remaining cells.

Acknowledgement

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Structure-Guided Discovery of Indolizine-Based Anticancer Agents: Targeting Tubulin Inhibition

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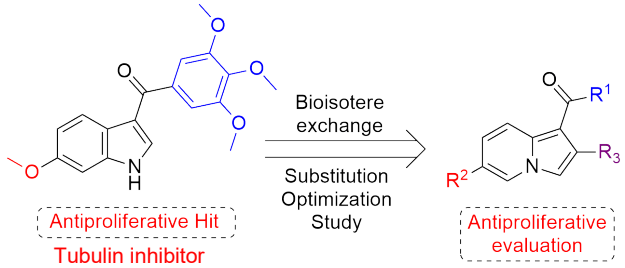
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Key words: Indolizine, Tubulin inhibitor, optimization, antiproliferative.

Introduction

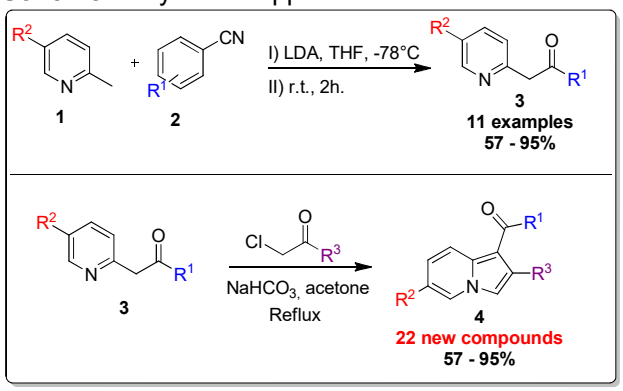
Indolizines are *N*-heterocycle compounds formed by the fusion of the pyridine ring and the pyrrole ring and have several scientific and technological applications.[1] A bioisotere of indolizine, indole with aryl ketone groups in the pyrrole ring, showed interesting antitumor activity [2,3], leading to a Hit compound that underwent clinical trials, but has been discontinued. Inspired by this, our work seeks to explore a bioisotere exchange strategy and synthesize a class of substituted indolizines with different structural patterns.

Figure 1. Project rationale inspired by indole structures.



Results and Discussion

Scheme 1. Synthetic approach for indolizines.



All synthesized compounds were screened at 50 µM against three cancer cell lines: BT-20 (breast carcinoma), HCG-27 (gastric carcinoma), and CAL-27 (tongue carcinoma). An inhibition threshold of 70% of inhibition in the initial screening was set forpIC$_{50}$ determination by dose-response curve. Based on the antiproliferative IC$_{50}$ values, four compounds were selected for evaluation in the tubulin inhibition assay.

Figure 2. Selected examples and IC$_{50}$ against cancer cell lines.

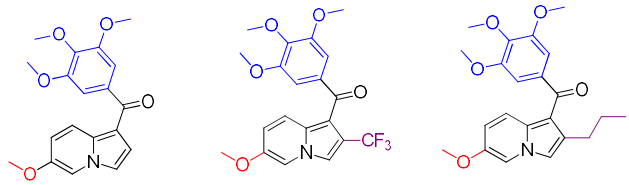
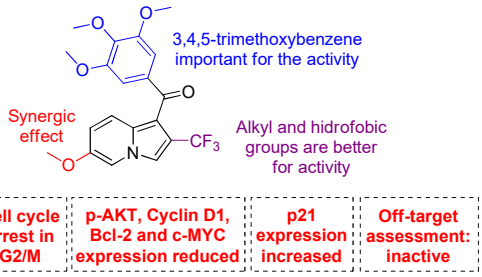


Figure 3. Key findings.



Conclusions

In summary, the synthesized polysubstituted indolizines exhibited promising antiproliferative effects. Compounds **4a** and **4b** exhibited strong antiproliferative effects through microtubule destabilization and G2/M arrest, comparable to the reference compounds Paclitaxel and Verubulin. They modulated pro-mitotic proteins (p-AKT, c-MYC, Cyclin D1) and increased p21, indicating the induction of apoptosis. Both were selective for tumor cells, sparing GNP15, with a selective index higher than 2

Acknowledgements

FAPESP (Proc. FAPESP 2022/06051-5; 2022/05327-7; 2023/16292-2), CAPES e CNPq.

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Synthesis and antitumoral activity of novel biaryl hydroxy-triazole and fluorene-triazole hybrids

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Key words: 1,2,3-triazoles, 9H-fluorenes, Molecular Hybridization, Leukemia, Cancer.

Introduction

Cancer remains a major global health concern, with some of the most common and fatal types including breast, prostate, lung, colorectal, and leukemia.[1] Drug resistance and non-selective toxicity are major barriers to effective chemotherapy, underscoring the need for new therapeutic scaffolds. In this context, 1,2,3-triazoles stand out for their stability, pharmacophoric versatility, and ability to serve as both bioactive cores and molecular linkers.[2]
Simultaneously, 9*H*-fluorene moieties exhibit rigid, aromatic frameworks that enhance drug-target interactions. By hybridizing triazoles with fluorene scaffolds, our goal was to develop novel compounds with antitumor potential, to be tested against HCT-116 (colorectal cancer), SNB-19 (astrocytoma), MDA-MB-231 (triple-negative breast cancer), and MOLM-13 (acute myeloid leukemia, FLT3-ITD mutant) cell lines.[3]

Results and Discussion

We synthesized 13 novel biaryl hydroxy-1,2,3-triazoles in yields ranging from 16% to 89% via a one-pot telescopic enaminone–azide cycloaddition followed by in situ reduction. Cyclization was achieved via a $BF_3{\cdot}OEt_2$-promoted intramolecular Friedel–Crafts reaction, affording 11 new 9H-fluorene-1,2,3-triazoles with isolated yields ranging from 20% to 97% (Figure 1). Cytotoxic evaluation was performed against HCT-116, SNB-19, MDA-MB-231, and MOLM-13 cancer lines, plus non-tumoral HEK-293 cells. Among the compounds evaluated, most exhibited higher potency against MOLM-1, with **LSO258**, **LSO272**, **LSO276**, and **LSO278** showing IC_{50} values of 25.5, 12.5, 24.8, and 18.7 µM, respectively. Notably, **LSO272** and **258** showed a selectivity index (SI) ≥ 2.
Notably, **LSO278** displayed versatility, exhibiting additional activity against HCT-116 (IC_{50} = 23.4 µM) and MDA-MB-231 (IC_{50} = 34.3 µM). The presence of bromine substituents and the triazole moiety were critical for activity, likely due to halogen bonding and improved membrane permeability. Interestingly, both rigid (fluorene) and flexible (biaryl) scaffolds were active, depending on substitution patterns. While a direct correlation between flexible ligands and rigid scaffolds could not be established, the presence of a triazole moiety and bromine substitution on the blue ring (derived from aryl azide) consistently emerged as key features, as all active compounds shared this structure.

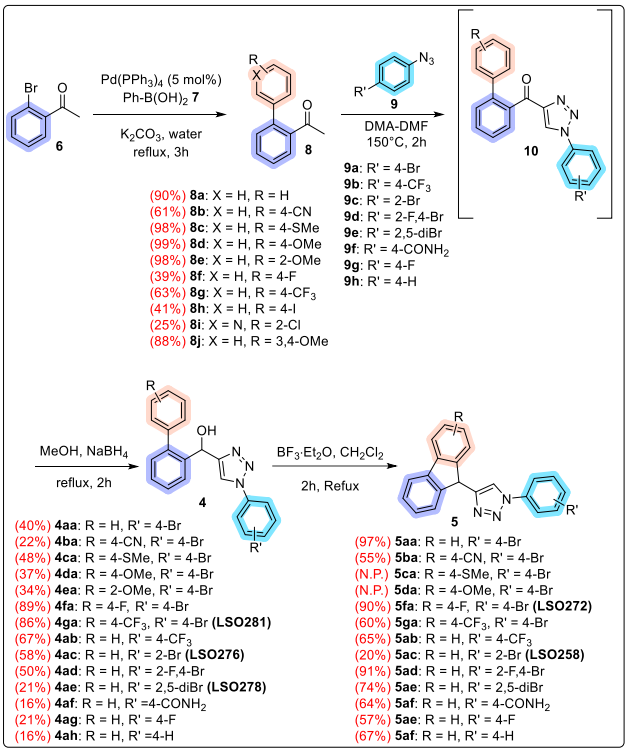


Figure 1: Synthesis of biaryl biaryl hydroxy-1,2,3-triazoles and 9*H*-fluorene-1,2,3-triazoles.

Conclusions

The synthesis of triazole-based hybrids incorporating fluorene scaffolds yielded promising antileukemic agents, with enhanced activity toward FLT3-ITD mutated leukemia (MOLM-13). Key pharmacophoric features—such as triazole linkages and brominated aryl rings—are likely responsible for the improved binding affinity and selectivity. This study highlights the relevance of molecular hybridization in drug design and underscores the potential of these new scaffolds in targeting resistant leukemia subtypes.

Acknowledgements

The authors would like to thank CAPES, CNPq and FAPERJ for their financial support. We would also like to thank PUC-Rio and CALPH for the structure that made the work possible

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Bioactive spirochromanes in the search for pre-clinical candidates for Chagas disease

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Key words: Chagas disease, Spirochromane.

Introduction

Chagas disease (CD) is a neglected tropical disease caused by the flagellated protozoan parasite *Trypanosoma cruzi.* It affects approximately 7 million people globally and claims more than 10,000 lives every year, especially in low-income countries. Only two drugs are currently approved for its treatment: benznidazole and nifurtimox, both of which have a long treatment period and severe adverse effects.[1]

The aim of this project was to develop novel, safer alternatives for the treatment of CD, and consisted of the synthesis and *in vitro* evaluation of molecules containing a spirocyclic chromane core. This class of compounds was identified by the Drugs for Neglected Diseases initiative (DNDi) via phenotypic screening; it has moderate potency that is not correlated with CYP51 inhibition.

Results and discussion

Starting with the two molecules represented below (Figure 1), the hit-to-lead campaign yielded more than 100 compounds – each evaluated for *T. cruzi* activity, cytotoxicity, lipophilicity, aqueous solubility, and metabolic clearance.

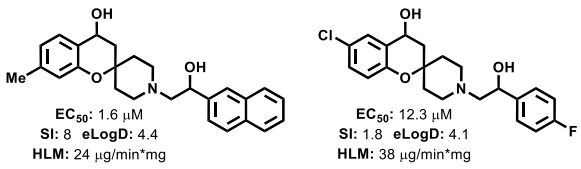


Figure 1: Initial hit compounds for the series.

Modifications were centered on four major areas of the scaffold: the spirochromane aromatic portion (pink), chromane benzylic position (light purple), linker (light green), and head group (orange) (Figure 2).

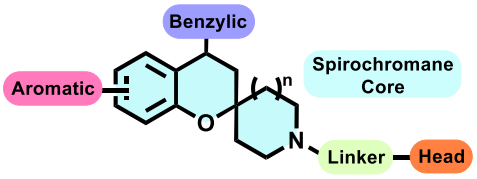


Figure 2: Major modification points.

The strategies employed included scanning of electron donating and withdrawing groups, substituent walk (position isomerism), classical and non-classical bioisosterism, homologation, truncation, and scaffold hopping. Some selected modifications are depicted in Figure 3, below:

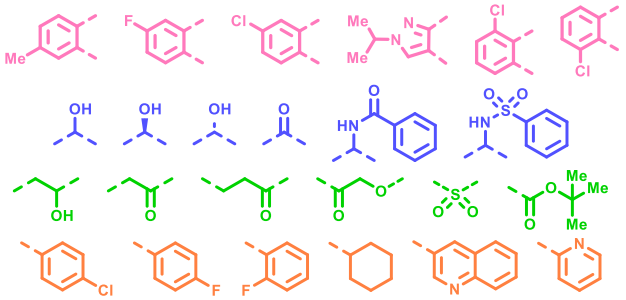


Figure 3: Examples of modifications in the series.

Conclusions

Although it was not possible to determine a clear correlation between the modifications made and the potency or drug-like properties of this series, some compounds showed improved activity along with good selectivity and could, therefore, be interesting starting points for new early drug discovery campaigns, as illustrated by the sulfonamide in Figure 4.

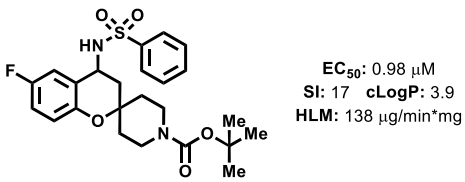


Figure 4: Promising analogue obtained during the campaign.

Acknowledgements

The São Paulo Research Foundation (FAPESP) for the financial support (2013/07600-3, 2015/50655-9, 2023/10715-9 and 2024/20141-2). DNDi is grateful to its donors, public and private, who have provided funding for all DNDi activities since its inception in 2003. A full list of DNDi's donors can be found at http://www.dndi.org/about/donors/.

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4-acyl-1.2.3-triazoles as key precursor of bioactive small molecules targeting new Cystic Fibrosis drug candidates

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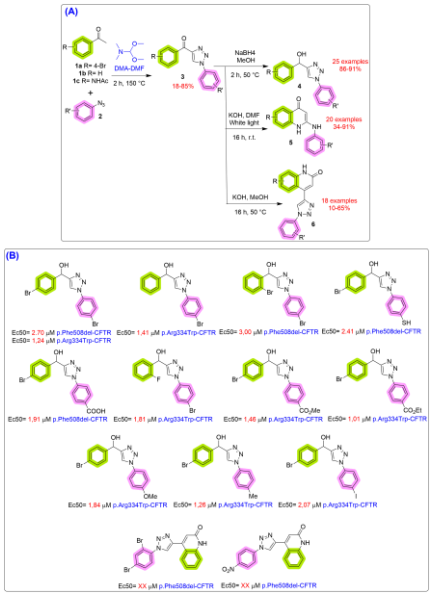
Key words: Cystic Fibrosis; Quinolone; Triazole; molecular hybridization.

Introduction

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Without a cure, the most effective treatment so far is a triple combination of drugs called Trikafta, whose treatment costs more than $300,000 per year.[1] This high cost underscores the need for more accessible therapeutic alternatives. Triazole-based compounds are notable for their versatility in medicinal chemistry.[2] In this context, the potential to use a single intermediate that can be readily converted into a variety of compound classes remains largely unexplored. Among these, 4-acyl-1,2,3-triazoles stand out due to their chemical properties, which make them suitable for diverse applications.[2] Since triazole-based compounds are an area of expertise for LabSint, supported by several reports demonstrating biological activity across various targets, this project aims to explore 4-acyl-1,2,3-triazoles as key intermediates for the synthesis of new hydroxy-1,2,3-triazoles, 2-(phenylamino)quinolin-4(1*H*)-ones, and triazole-based quinolin-2(1*H*)-one hybrid drug candidates.

Results and Discussion

4-acyl-1,2,3-triazoles (**3**) were synthesized from selected acetophenones (**1**) and various azidobenzene derivatives (**2**) in the presence of DMF-DMA for 2 hours at 150 °C, using a metal- and solvent-free methodology. The resulting compounds **3a–c** were obtained with 18%-85% yields. Hydroxy-1,2,3-triazoles (**4a–b**) were subsequently obtained through acyl reduction with NaBH4 in methanol for 2 hours, achieving yields between 85% and 91%. The formation of (phenylamino)quinolin-4(1*H*)-ones (**5**) required cyclization of the triazole intermediate. To promote C–N bond formation followed by a Dimroth-type rearrangement, compound **3c** was reacted with KOH in DMF under white LED irradiation. This procedure yielded 24 novel type **5** compounds, with isolated yields ranging from 34% to 91%. When light was replaced by heat as the activation source, quinolin-2(1*H*)-one–triazole hybrids (**6**) were obtained via an aldol condensation pathway. A selective formation of compound **6** was achieved when MeOH was used as the solvent under the same conditions. A total of 18 novel compounds were synthesized, with yields ranging from 10% to 65% (**Scheme 1a**). All synthesized compounds were tested against different CF mutations (p.Phe508del-CFTR and p.Arg334Trp-CFTR). Compounds of type **4** demonstrated greater versatility, with several molecules showing activity against both mutations. In contrast, compounds of type **5** showed no significant activity in preliminary assays. However, two representatives of type **6** exhibited promising activity in early evaluations. A summary of the structure–activity relationships is presented in **Scheme 1b**.



Scheme **1**: (a) 4-acyl-1.2.3-triazoles as key precursor of new drug candidates; (b) Activity and structure relationship of **4**,**5** and **6** compounds.

Conclusions

In conclusion, the versatility of 4-acyl-1,2,3-triazoles was demonstrated through their role as key intermediates for the synthesis of novel hydroxy-1,2,3-triazoles, 2-(phenylamino)quinolin-4(1H)-ones, and triazole-based quinolin-2(1H)-one hybrids, with yields reaching up to 98%. The ability of these new compounds to restore the function of mutated CFTR proteins was also demonstrated, particularly for the hydroxy-1,2,3-triazoles, with 12 compounds showing activity against both mutations and achieving EC50 values as low as 1.01 μM.

Acknowledgements

The authors would like to thank CAPES, CNPq and FAPERJ for their financial support. Also, we link to thank PUC-Rio and CALPH for the structure that made the work possible.

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Unraveling the Dual Inhibition of *Plasmodium falciparum* PI4K and PKG by Sapanisertib Through Alchemical Free Energy Calculations

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Key words: SAR, Molecular dynamics, alchemical calculations

Introduction

Malaria remains one of the deadliest infectious diseases worldwide, with 597,000 deaths reported in 2023 by the WHO. Among emerging strategies to combat *Plasmodium falciparum*, targeting protein kinases has gained attention due to their essential role in the parasite's life cycle.[1] Phosphatidylinositol 4-kinase (*Pf*PI4K) is already a clinically validated target, while cGMP-dependent protein kinase (*Pf*PKG) stands out for its low likelihood of resistance development. A dual inhibitor of *Pf*PI4K and *Pf*PKG could represent a significant advance in antimalarial therapy. Although previous efforts have identified potential dual inhibitors, their structural basis of action remains poorly understood, particularly for *Pf*PI4K, which lacks an experimentally determined structure. As a result, the molecular mechanisms behind dual inhibition of *Pf*PI4K and *Pf*PKG remain to be fully elucidated.

Results and Discussion

Sapanisertib (**SAP**, Figure 1) was recently identified as a potent compound against *P. falciparum* Dd2 strain (IC_{50}= 76 nM) and as a dual inhibitor of *Plasmodium vivax* PI4K (*Pv*PI4K, IC_{50} = 4 nM, ΔG_{bind} = −11.46 kcal/mol) and *Pf*PKG (IC_{50} = 20 nM, ΔG_{bind} = −10.50 kcal/mol).[2] To investigate the molecular basis of its dual inhibition, we employed a newly developed model of *Pf*PI4K. This truncated model, constructed and validated by us, demonstrated robust predictive performance in alchemical free energy calculations against a set of *Pv*PI4K inhibitors (*in press*), yielding r = 0.71, τ = 0.48, ρ = 0.69, with a mean absolute error of 0.78 ± 0.68 kcal/mol and a root mean square error of 0.92 ± 0.50 kcal/mol. For *Pf*PKG, we employed the crystal structure of the kinase domain (8EM8), with the C-terminal region predicted by AlphaFold2. To explore the binding modes of **SAP**, we first performed molecular docking, followed by triplicate 300 ns MD simulations (ff19SB+OPC+ GAFF2) for both targets. A representative conformation of each complex (Figure 2) was then extracted using an unsupervised machine learning-based clustering approach over all replicas. Next, we employed alchemical free energy calculations (ff14SB+TIP3P+GAFF2) to quantify the contribution of each substituent in **SAP** (Figure 3).

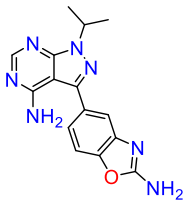


Figure 1: Sapanisertib.

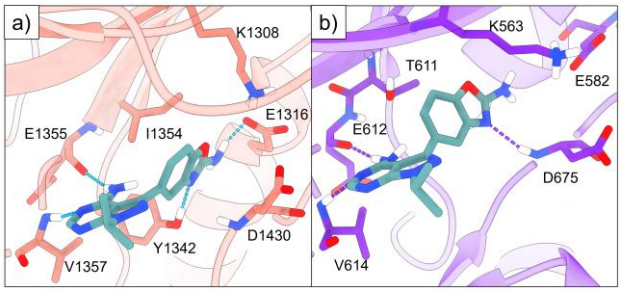


Figure 2: Putative binding mode of sapanisertib in the ATP-binding pocket of (a) PI4K and (b) PKG.

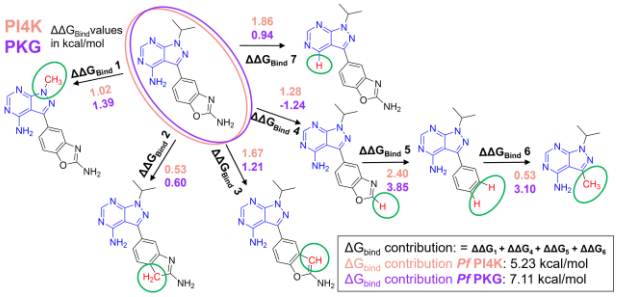


Figure 3: Alchemical mutations of sapanisertib against PI4K and PKG. Positive ΔΔG indicates that removing the group is energetically unfavorable.

Surprisingly, the decomposition revealed a more favorable profile for PKG. The amine on the benzoxazole forms a hydrogen bond with PI4K, but not in PKG, matching $\Delta\Delta G_4$. Still, the benzoxazole shows stronger PKG binding, likely due to van der Waals interactions in a hydrophobic back pocket made accessible by the small gatekeeper residue (T611). Yet, **SAP** is more potent against PI4K, likely due to its pyrazolopyrimidine core. This scaffold probably interacts more favorably with the PI4K hinge region, as anticipated by $\Delta\Delta G_7$ (~1 kcal/mol more potent). Absolute binding free energy calculations will explore the scaffold's importance.

Conclusions

Our computational SAR reveals that while **SAP's** substituents favor PKG, its higher potency for PI4K likely stems from scaffold–hinge interactions. Absolute binding free energy calculations will be conducted to confirm this preference. All transformations will be repeated in triplicate and extended to human mTOR, **SAP's** original target, to identify key modifications for enhancing selectivity in future dual-inhibitor design.

Acknowledgements

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Hierarchical intermolecular interaction profiling of nAChRs α3β4, α4β2 and α7: Enhanced comprehension of ligand recognition.

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Key words: nAChR; docking; machine-learning; virtual-screening; Alzheimer's disease; nicotine dependence.

Introduction

Recent advances in Alzheimer's disease and nicotine use disorder have targeted nicotinic acetylcholine receptors (nAChRs) aiming to improve neuroplasticity and mediate nicotine replacement through partial agonism and modulation, especially subtypes α3β4, α4β2, and α7 which are majorly expressed in the central nervous system[1]. Herein, a computer-aid drug discovery strategy, using post-processed docking data and machine learning, was employed to identify hierarchical interaction and structure patterns for binding sites of human nAChRs. These include: **(i)** α3β4 and α7 orthosteric sites, **(ii)** the α7 transmembranar allosteric site and **(iii)** the α4-β2 orthosteric sites of the α4β2[2].

Results and Discussion

In Figure 1A, for instance, model performance is shown, using data generated from the best docking condition with 122 training and 31 test ligands. The KNeighbords algorithm outperformed the linear model, improving the $R^2_{5\text{-fold}}$ from 0.586 to 0.717.

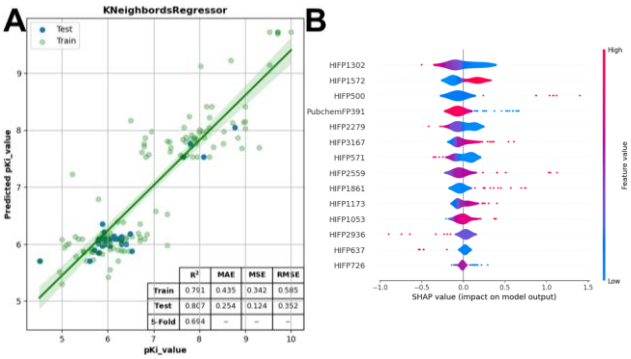


Figure 1. Regression model for nAChR $(α4)_2(β2)_3$ orthosteric site. **(A)** Model performance with evaluation metrics; **(B)** SHAP analysis of feature contributions to predictions.

Additionally, feature analysis in model construction (Figure 1B) showed HIFP500, HIFP1173, HIFP1572, HIFP1861, and PubchemFP391 as most influential, clearly separating positive and negative effects in ligand pKi predictions. Notably, PubchemFP391 emerges as a selective parameter between ortoshteric sites $(α4)_2(β2)_3$ and α7. Subtype $(α4)_2(β2)_3$ tends to avoid trisubstituted basic nitrogen, favoring H-bonds with hydroxyl of Y100 and carbonyl from W156 backbone, as also seen in HIFP1173 and HIFP1572 (Figure 2), as well as HIFP500 and HIFP1861 hydrophobic and π stacking interactions with F119 side chain.

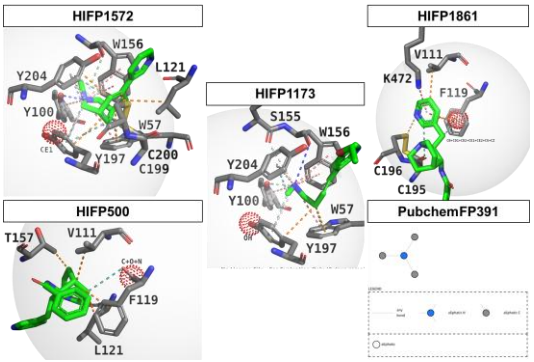


Figure 2. Key features of the selected regression model for nAChR $(α4)_2(β2)_3$ orthosteric site. Carbon atoms are in gray (protein) and green (ligands), oxygen in red, nitrogen in blue, and sulfur in yellow. Intermolecular interactions appear as dotted lines in color blue (H-bonds), light blue (weak H-bonds), yellow (chalcogen bonds), light green (cation–nucleophile), red (π stacking), pink (cation–π), and orange (hydrophobic).

The same was applied for all nAChRs in this work. We confirmed that intermolecular interactions with specific amino acids in nAChRs' sites are key for activity, as reported by structural data[2,3]. These involve π stacking with W149 in α3β4, H-bond with Y100 and π stacking with F119 in α4β2, H-bond with Y217 and hydrophobic contact with L131 in α7, plus H-bond or cation-nucleophile with W149/156/171 in α3β4, (α4)2(β2)3, and α7. Hydrophobic contacts also occur with N236 and F275 in α7 allosteric site.

Conclusions

Key fingerprints reflected specific interactions of nAChRs α3β4, α4β2, and α7, including H-bonds, π stacking, and hydrophobic contacts Y100, F119, W156, and N236.

Acknowledgements

We thank FAPESP, Regular Project, Process nº2018/00187-7, INCT INOFAR – Process INCT/CNPq nº 402176/2024-3, for the financial support and CNPq for the scholarship (Daniel A. G. Ruiz).

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A Comprehensive Hit Assessment Approach for Early-Stage Chagas disease Drug Discovery

Luiza R. Cruz (PQ)[1], Fanny Escudié (PQ)[1], Lorna MacLean (PQ)[2], John Thomas (PQ)[2], Christy Paterson (PQ)[2], Jennifer Riley (PQ)[2], Leah Torrie (PQ)[2], Lynsey Swan (PQ)[2], Mary Wheldon (PQ)[2], Michael Thomas (PQ)[2], Sandra Carvalho(PQ)[3], Eric Chatelain (PQ)[1], Jean-Robert Ioset (PQ)[1], Manu De Rycker (PQ)[2], Susan Wyllie (PQ)[3], Charles Mowbray (PQ)[1], Jadel M. Kratz (PQ)[1]*.

*[1]Drugs for Neglected Diseases initiative, Geneva, Switzerland. [2]Drug Discovery Unit, University of Dundee, Dundee, United Kingdom, [3] Wellcome Centre for Anti-Infectives Research, University of Dundee, Dundee, United Kingdom. *jkratz@dndi.org*

Key words: Chagas disease, drug discovery, hit assessment, hit-to-lead, lead optimization, mode of action

Introduction

Chagas disease (CD) is the neglected tropical disease (NTD) with the highest mortality rate in Brazil, accounting for 31,000 deaths between 2016 and 2020.[1] Despite its significant health impact, very few candidates for CD have been identified and progressed into clinical development. In fact, of all the diseases caused by trypanosomatids, new chemical entities (NCEs) have only reached registration or late clinical development stages for sleeping sickness, and there are just two of these: fexinidazole and acoziborole. This is a multifactorial problem, contributed to by high attrition rates during the discovery phase and the currently underdeveloped early-stage pipeline for CD. DNDi and its partners have made concerted efforts to explore different chemical spaces looking for chemically novel hits/leads and also to circumvent the high prevalence of hits with known or deprioritized modes of action (MoA) (such as CYP51 or cytochrome bc1 inhibitors) in high-throughput screen campaigns. These targets have shown limited translational success due to issues such as lack of sustained efficacy or parasite recrudescence.[2] Implementing early screening filters to flag hits acting via these mechanisms allows researchers to focus lead optimization (LO) efforts on novel or underexplored mechanisms with greater potential. We report herein a hit assessment strategy that increases the efficiency of the pipeline and reduces the risk of advancing compounds with known liabilities.

Results and Discussion

Using DNDi's compound database as the source, the team reevaluated hit compounds that were previously identified in high-throughput campaigns but not selected for further optimization. The following criteria were used during this initial prioritization effort: a. promising activity against *T. cruzi* (<10-15 µM); b. physicochemical and ADME properties (experimental or predicted) within an adequate range; c. low cytotoxicity and/or a minimum level of selectivity; d. diverse structure-activity relationship (SAR) possibilities; and e. chemical tractability. From this search, 13 novel chemical series were selected to enter the hit assessment phase. The initial step involved the resynthesis of hits already cataloged in the database. This process of structural and purity confirmation was crucial for reconfirming their anti-*T. cruzi* activity. In addition to the resynthesized hits, closely related analogues that were either already available or were specifically synthesized to expand the compound set were included in this stage. All synthesized compounds underwent testing against the standard intracellular *T. cruzi* amastigote assay and were also evaluated for CYP51 inhibition using an enzymatic assay. Compounds showing CYP51 inhibition correlated with trypanosomal activity were deprioritized.

Subsequently, representatives from each chemical series were assessed against a panel of other deprioritized mechanisms of action (e.g. cytochrome bc1, t-RNA synthetase, proteasome). This profiling facilitated the prioritization of series for the final cell-based time-kill assay. The study enabled differentiation between two activity profiles: fast-killing, associated with benznidazole, which is time- and concentration-dependent, or slow-killing, associated with posaconazole, with a mostly time-dependent activity profile.

Conclusions

After this comprehensive hit assessment stage, chemical series with novel chemical structural features, not associated with known/deprioritized MoAs, were progressed to the hit-to-lead stage. They are currently being optimized in collaboration with multiple partners within DNDi's global H2L/LO consortium. Efforts to deconvolute their molecular targets and/or MoA are ongoing in parallel.

Acknowledgements

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Re-styling an old scaffold: ebsulfur analogs with improved activity and selectivity against the infective stage of trypanosomes

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Key words: Trypanosomatid, redox biosensor

Introduction

Several species of trypanosomatids cause fatal and disabling diseases in humans and livestock animals. The current chemotherapy is limited and new drug candidates with improved efficacy and safety are needed. The benziso-thiazolone (e.g. Ebsulfur, EbS) and -selenazolone (e.g. Ebselen, EbSe) have been extensively investigated for their promising action towards transmissible and non-transmissible diseases.

Results and Discussion

In this work we synthetized 23 benzisothiazolones and tested their anti-trypanosomatid activity against the clinically relevant stages of three major trypanosomatid species (*Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania infantum*). Several compounds demonstrated improved activity and selectivity, presented nM or low µM activity and, at least a two-digit selectivity against Trypanosoma sp. (indices exceeding 6000 in some cases) but most proved inactive towards *L. infantum*. Structure-activity relationship analysis reveals that the chemotype of the top hits consisted of phenyl and benzyl rings occupying the N2 position of the benzisothiazolone scaffold and harboring polar substituents in the para position. Most compounds from these two clusters induced a rapid redox unbalance in the intracellular pool of low molecular weight thiols. None of the hits, but EbSe and EbS, affected Trypanothione synthetase activity. However, at large enzyme:compound ratios, some inhibited irreversibly (and covalently) Trypanothione reductase (TR). Some hits exerted a minor effect on the rate of glucose consumption. A therapeutic efficacy study in a murine model of acute African trypanosomiasis was performed for the most potent and selective benzisothiazolone against bloodstream T. brucei (EC_{50}=0.022 µM, SI=6772).

Conclusions

This study highlights benzisothiazolones as a promising chemical scaffold for antiparasitic drug development. The observed species-specific activity is likely consequence of differences in parasite biology. Two for which we find a correlation are the number of biological membranes the compounds must cross to reach their molecular targets and the differing proliferation rates of the parasites. Accordingly, the extracellular and rapidly dividing blood stream *T. brucei* (doubling time of ~6 h) is the most susceptible, followed by the intracytosolic *T. cruzi* amastigotes (~20 h doubling time), and finally *L. infantum* amastigotes (intraphagolysosomal, doubling time >60 h to 12 days). Some of the compounds presented a good correlation between biosensor oxidation, EC_{50} values, and inhibition of enzymes from the parasite thiol redox metabolism, suggesting a mode of action involving disruption redox homeostasis. Using EbS and one of TR inhibition compounds, mass spectrometry studies confirmed incorporation of the full compound into C221, identified as the target residue supporting a mechanism involving thiolate attack on the thiazolone ring and hetero-disulfide bond formation, similar to EbSe-GSH chemistry. Despite promising in vitro profiles, in vivo efficacy of the top hit remains limited, the hit was unable to reduce parasite burden monitored by in vivo imaging, but extended animal survival. Future efforts should focus on elucidating precise molecular targets, improving compound solubility and bioavailability, and exploring alternative delivery strategies or combinations therapies to improve therapeutic outcomes.

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We acknowledge financial support of CSIC, FOCEM (MERCOSUR), PEDECIBA, ANII and to Dr. Rosario Duran for technical advice and Drs. Alan Fairlamb and Diego Arias for expression constructs provided.

Structure–activity relationship (SAR) and ADMET study of β-pinene derivatives with antileishmanial activity using in silico methods.

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Key words: Leishmaniasis, Leishmania amazonensis, Beta-pinene derivatives, Structure-activity relationship, Molecular modeling, ADMET analysis.

Introduction

Leishmaniases are a group of diseases caused by protozoa of the genus Leishmania, classified by the World Health Organization (WHO) as Neglected Tropical Diseases (NTDs). The disease affects approximately 2 million people annually and threatens an additional 350 million in endemic regions[1]. Twenty Leishmania species are known to cause disease in humans. L. amazonensis is one of the primary etiological agents of CL. Due to the absence of an effective vaccine and treatment, along with the limited interest of the pharmaceutical industry in developing safer and more suitable drugs, there is an urgent need to explore new therapeutic strategies for leishmaniases. Monoterpene derivatives have shown promising antiparasitic properties due to their biochemical action against microorganisms and their ability to modulate host responses. In this context, a series of 11 beta-pinene derivatives were synthesized and tested against the promastigote form of L. amazonensis, compared to miltefosine, and studied in a structure-activity relationship and had their pharmacokinetic and toxicological profile evaluated.

Results and Discussion

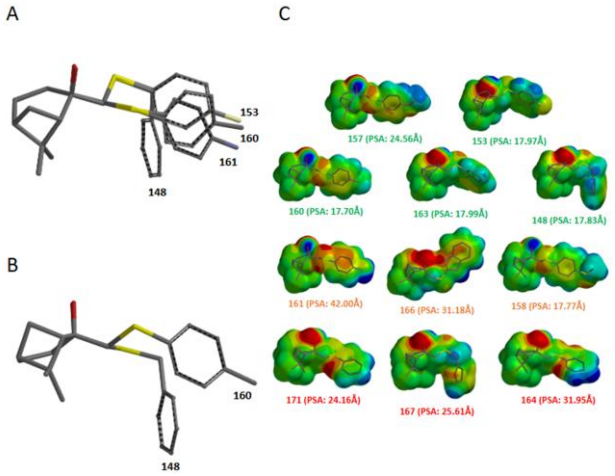


Figure1. (A) 3D structures of compounds 148, 153, 160 and 161; (B) Structures of compounds 148 and 160, C) Molecular Electrostatic potential (MEP) maps of the compounds, with the respective PSA values in Å²

SAR analysis showed that lipophilicity (clog P > 3.8) and low polar surface area (PSA < 25 Å²) enhance biological activity. Heterocyclic derivatives (GM166, GM171, GM167, GM164) had higher PSA and lower clog P, reducing membrane permeability and activity. An intramolecular hydrogen bond improved antiparasitic action. Promising derivatives (GM148, GM153, GM157, GM160, GM161) showed no hepatotoxicity, mutagenicity cardiotoxicity but were predicted as skin sensitizers and CYP inhibitors.

Compound	Ro5	HEP	MUT	hERG	CARC.	TD_{50} (ug/kg/day)	SKIN SENS.
GM157	0	No	No	No	No	65.4	Yes
GM153	1	No	No	No	No	96.6	Yes
GM160	0	No	No	No	No	57.5	Yes
GM163	0	No	No	No	No	58.4	Yes
GM148	0	No	No	No	No	121.9	Yes

Table1. pharmacokinetic and toxicological profile of the 5 best compounds.

After ADMET analysis, these promising compounds are safer than miltefosine, due to its carcinogenic potential[2].

Conclusions

Llipophilicity, molecular volume, and polar surface area are critical factors influencing the compounds' activity. ADMET assays demonstrated that the top five derivatives show good oral bioavailability and minimal toxicological risks, although cutaneous sensitization was observed. The findings suggest that compound 157 is the hit compound.

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Rational Design and Synthesis of Aminomethylenecycloalkanediones (AMCDs) as Potential Inhibitors of *T. brucei* Alternative Oxidase

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Key words: Neglected diseases, Trypanosoma brucei, Alternative oxidase (AOX), Tricarbonylated compounds, Sleeping sickness.

Introduction

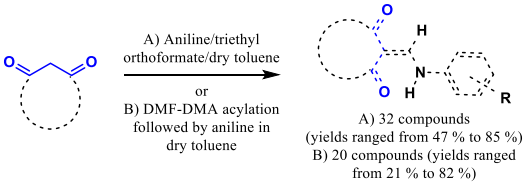
Human African Trypanosomiasis (HAT), or sleeping sickness, caused by *Trypanosoma brucei*, remains a serious health issue in tropical and subtropical regions.[1] Climate change has further increased the risk of vector dispersion and the emergence of new endemic areas.[2] Current treatments, including melarsoprol, an organoarsenic compound, are limited and highly toxic.[3] In 2021, fexinidazole became the first oral drug approved for both stages of HAT; however, its metabolism and central nervous system (CNS) penetration are associated with side effects.[4]

Alternative oxidase (AOX), a mitochondrial enzyme absent in humans, represents a selective and promising target.[5,6] This work focuses on the rational design and synthesis of 3-(alkyl/arylamino-methylene)-2,4-cycloalkanediones (AMCDs) as potential AOX inhibitors, using cost-effective, short-step routes based on cyclic 1,3-dicarbonyl precursors. Our approach includes the design of a compound library, SAR/QSAR studies, and pharmacodynamic evaluation.

Results and Discussion

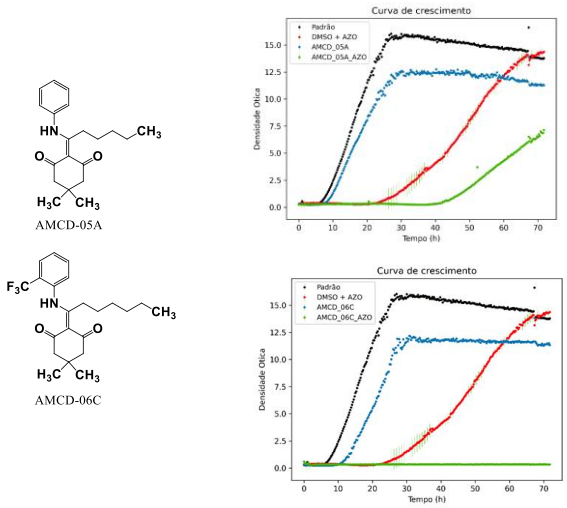
Virtual screening using the ZINC database identified AMCD scaffolds among the top 500 ranked hits. These fragments bear structural similarity to colletochlorin B, a natural AOX inhibitor, particularly in their hydrophobic tail (e.g., geranyl group) and a 2-hydroxy-5-halo benzaldehyde motif that mimics key hydroxyls in the natural substrate ubiquinol.

Three AMCD series were synthesized using dimedone, 1,3-cyclohexanedione, or 1,3-indanedione as starting materials, via two synthetic strategies: (A) a multicomponent reaction with aniline and triethyl orthoformate in dry toluene, or (B) initial acylation with DMF-DMA followed by aniline addition (Scheme 1). Compounds were easily isolated by rotary evaporation, filtration, and purification via hexane washing or recrystallization.



Scheme 1. General strategy for the synthesis of AMCDs.

Preliminary biological assays in the AOX-expressing model organism *Pichia pastoris* showed significant AOX inhibition by selected AMCDs, with low toxicity in the absence of azoxystrobin (Scheme 2). These findings support the viability of AMCDs as promising AOX inhibitors.



Scheme 2. Preliminary biological assays of AOX inhibition by AMCDs in *Pichia pastoris*.

Further biological evaluations using *T. brucei* are ongoing in collaboration with Prof. Dr. Ariel M. Silber (ICB-USP) and Dr. Arthur T. Cordeiro (LNBio–CNPEM).

Conclusions

To date, 52 AMCD derivatives have been synthesized. The preparation of analogs bearing longer alkyl chains (up to eight carbons) is in progress. Biological assays currently underway will determine their efficacy and safety as potential treatments for HAT.

Acknowledgements

CNPq Finep UNICAMP IQ Instituto de Química

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Application of cross-coupling reactions in the optimization of the synthesis of multitarget bioactive 1,2,4-triazoles for Alzheimer's disease.

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Key words: 3-amino-1,2,4-triazoles, Chan-Lam reaction, Suzuki coupling, Buchwald-Hartwig, Alzheimer's disease

Introduction

Alzheimer's disease (AD) is a progressive, multifactorial neurodegenerative condition of high clinical complexity, representing the leading cause of dementia in the elderly and one of the greatest global public health challenges. In this context, our research group has been focused on the development of compounds bearing 1,2,4-triazole cores aimed at inhibiting therapeutic targets related to Alzheimer's disease, with emphasis on the inhibition of the key enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The 3-amino-1,2,4-triazole series **(1)** exhibited IC_{50} values ranging from 0.386 µM to 18.09 µM for AChE, and from 0.416 µM to 13.3 µM for BuChE,[1,2] with the most active compounds acting via a mixed inhibition mechanism, enhancing their therapeutic potential by also interfering with β-amyloid plaque formation. Furthermore, these compounds were able to chelate metals involved in AD, such as Zn and Cu.

Despite their promising biological profile, the synthetic route previously employed by the group involves nine steps in a linear synthesis, posing limitations, as each variations at the R^1 or R^2 position, which requires restarting the synthesis from the initial steps, leading to long time synthesis and high reagent consumption. This work proposes an optimized synthetic strategy based on cross-coupling methodologies to efficiently access triazole derivatives in only three steps (Figure 1).

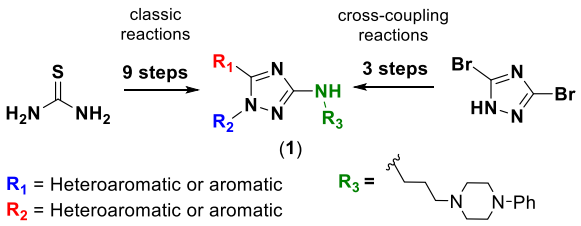
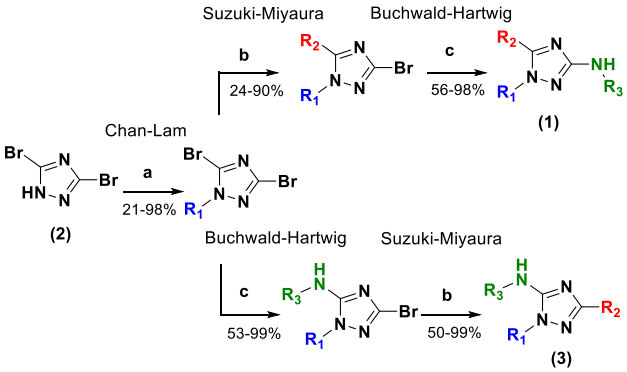


Figure 1: Synthesis of 3-amino-1,2,4-triazoles.

Results and Discussion

Cross-coupling reactions play a key role in the synthesis of bioactive molecules due to their broad applicability, high efficiency, selectivity, and compatibility with a wide range of functional groups. In this work, these methodologies were employed starting from the commercial reagent 3,5-dibromo-1H-1,2,4-triazole **(2)**, aiming to access and expand the scope of the previously developed series **(1)** through an optimized synthetic route. The first step involves a Chan–Lam amination (C–N arylation, copper-mediated) (**a**), followed by a Suzuki–Miyaura coupling (C–C bond formation, palladium-catalyzed) (**b**), and finally a convergent step using the Buchwald–Hartwig reaction (C–N bond formation, palladium-catalyzed) (**c**). Additionally, the inversion of steps (**b**) and (**c**) was also explored, enabling the synthesis of the corresponding regioisomers **(3)** without compromising yields, thus demonstrating the robustness and flexibility of the adopted methodology (Figure 2).



a) R_1-$B(OH)_2$ (1.3eq.), $Cu(OAc)_2$ (1eq.), Py (1.3eq.), DCM, 25°C, 16h; **b)** R_2-$B(OH)_2$ (0.5eq.), $Pd(PPh_3)_2$ (5mol%), K_2CO_3 (2eq.), MePh:EtOh:H_2O, 105°C, 1h; **c)** R_3-NH_2 (1,5eq.), $Pd[Cl(cinnamyl)]_2$ (5mol%), t-BuXPhos (0,1eq), NaOt-Bu (1,5eq.), MePh, 100°C,18h

Figure 2: Optimized synthetic route.

Conclusions

This new approach makes the synthesis of new derivatives with the 1,2,4-triazole core easier, eliminating protection steps, reducing the overall number of synthetic steps (9 to 3 steps), increasing the global yield, and enabling an efficient access to previously studied bioactive compounds in our group, as well as the design and synthesis of novel 1,2,4-triazoles with increased molecular diversity.

Acknowledgements

To CNPq, CAPES, FAPERJ, and UFRRJ for scholarships and financial support.

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Optimized HDAC inhibitors induce robust γ-globin gene expression and exhibit superior efficacy to hydroxyurea in Sickle Cell Disease models

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Key words: Sickle cell disease, histone deacetylase, fetal hemoglobin inducers.

Introduction

One of the most promising therapeutic approaches to mitigate the clinical manifestations of sickle cell disease (SCD) involves the pharmacological reactivation of fetal hemoglobin (HbF, $\alpha_2\gamma_2$), which effectively inhibits the polymerization of sickle hemoglobin (HbS) due to its non-sickling properties. Among the agents under investigation, epigenetic modulators such as histone deacetylase (HDAC) inhibitors have demonstrated the capacity to induce γ-globin gene expression, thereby increasing HbF levels in erythroid cells.[1]

Our research group has previously described that resveratrol derivatives designed as HDAC inhibitors induce γ-globin gene expression at levels comparable to those found for the approved drug hydroxyurea using CD34+ cells.[2,3] Building upon these prior findings and guided by molecular modeling techniques, we have undertaken the design, chemical synthesis, and biological assessment of a new series of HDAC inhibitors.[3]

Results and Discussion

A total of 24 intermediates and 7 final compounds were successfully synthesized, with overall yields varying between 29% and 85%. Among them, the two lead candidates demonstrated potent inhibitory activity against HDAC-1 and HDAC-2, with IC_{50} values ranging from 17 to 950 nM. In K562 erythroleukemic cells, these compounds induced a significant upregulation of γ-globin expression, achieving approximately a 7-fold increase at a concentration of 1 μM over 72 hours—markedly higher than the 2.5-fold induction observed with 30 μM hydroxyurea. Furthermore, in HUDEP cells, treatment with the final compounds at 800 nM for 72 hours resulted in γ-globin gene expression levels between 30- and 138-fold, without detectable cytotoxicity (Figure 1). ADME profiling of the most active analogs revealed logP values between 2.51 and 2.98, and metabolic stability assays in human liver microsomes showed low clearance rates (14–17%) and half-lives ranging from 216 to 266 minutes. Solubility (logS) ranged from –7.10 to –7.30, and passive permeability evaluated using the PAMPA model yielded Pe values (10^{-6} cm/s) from 14.6 to 15.5 across pH 6.5 to 7.4.

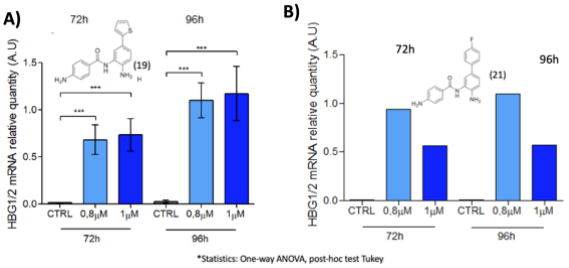


Figure 1: Effect of compounds (**19**) and (**21**) on HBG1/2 expression in HUDEP cells**.** Relative expression levels of *HBG1/2* were significantly induced after 72 h and 96 h of treatment with the compounds (**19**) (panel **A**, left) and (**21**) (panel **B,** right) compared to the control (CTL), as measured by RT-qPCR. $p < 0.001$ (one-way ANOVA followed by Tukey's post hoc test).

Conclusions

These findings indicate that the novel HDAC inhibitors exhibit favorable characteristics for the induction of fetal hemoglobin and may represent a promising therapeutic alternative to hydroxyurea in the management of sickle cell disease. Ongoing proof-of-concept experiments in transgenic sickle cell mouse models aim to establish an optimal dosing strategy capable of safely and effectively promoting HbF induction.

Acknowledgements

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Cereblon-recruiting Proteolysis Targeting Chimeras (PROTACs) for selective HDAC1 degradation as fetal hemoglobin inducers

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Key words: Sickle cell disease, histone deacetylase, fetal hemoglobin, PROTAC, epigenetics.

Introduction

Induction of fetal hemoglobin (HbF) represents a well-established and promising approach for the treatment of hemoglobinopathies such as Sickle Cell Disease (SCD). Among the strategies investigated, epigenetic modulation—particularly through inhibition of histone deacetylases (HDACs 1 and 2)—has demonstrated encouraging potential in elevating HbF levels.[1] Building upon our previous findings that HDAC-1 and -2 inhibition significantly increases HbF expression, the present study investigates whether targeted degradation of HDACs via the PROTAC strategy may offer a more effective alternative for HbF induction.

Results and Discussion

Forty intermediates and thirteen new cereblon-recruiting PROTACs were synthesized and characterized via analytical methods. For a preliminary screening, HCT116 cell culture was treated with all PROTACs at concentrations ranging from 0.01 to 10 μM and the measurement of degradation of HDACs 1, 2 and 3 was evaluated via western blot. Three of these compounds (ARP-26, ARP-37 and ARP-49) were able to degrade HDAC-1 more selectively, when compared to HDAC-2 and HDAC-3, achieving DC_{50} values of 2.5 μM. Interestingly, the PROTACs showed selective degradation of HDAC-1 over -3.[2] Then, these 3 PROTACs were selected for further studies in HUDEP-2 cell culture at concentrations of 500 and 750nM, and incubation time of 72 and 96h, to measure the HbF induction effect. Treatment with the tested compounds significantly increased HBG1/2 mRNA expression and the percentage of HbF-positive HUDEP-2 cells. At 72 hours, HBG1/2 mRNA levels rose by 26.9-fold and 35.5-fold at 500 nM and 750 nM, respectively; at 96 hours, the increases were 34-fold and 44.5-fold. HbF-positive cells increased 7.5-fold and 9-fold at 72 hours, and 8.37-fold and 10.6-fold at 96 hours, for 500 nM and 750 nM, respectively. Hydroxyurea (HU, 100 μM) served as a positive control, showing a 10-fold increase in HBG1/2 expression at 72 hours and HbF-positive cell increases of 8.5-fold (72h) and 8.9-fold (96h). All results were statistically significant ($p < 0.0001$).

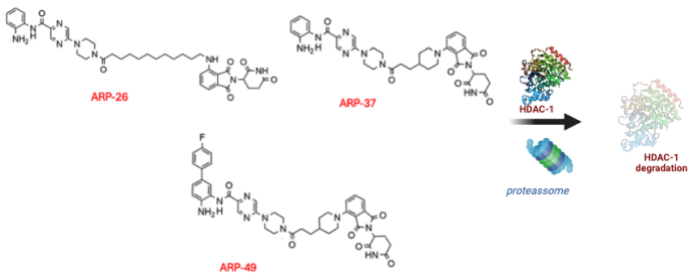


Figure 1: Chemical structures of the three most potent compounds inducing HbF expression (ARP-26, 37 and 49).

Conclusions

The compounds identified in this study demonstrated selective degradation of HDAC-1, a property not previously reported in the literature. Notably, ARP-49 significantly enhanced HBG1/2 expression in HUDEP-2 cells, surpassing the effect of hydroxyurea (HU), the current standard treatment for SCD, by 2.6-fold at 500 nM after 72 hours—despite being administered at a 200-fold lower concentration. These results highlight the promising potential of ARP-49 for further preclinical investigation as a novel strategy to promote HbF production.

Acknowledgements

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The compound LINS01022, a novel antagonist of histamine H3 receptor, is a potent analgesic in a murine neuropathic pain model

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Key words: Histamine H3 receptor, Neuropathic pain, Antihistamines, Analgesic.

Introduction

Neuropathic pain (NP), a disorder affecting the somatosensory nervous system, is much more challenging to treat than other types of chronic pain. Despite the availability of treatments, patients suffering from NP show only partial alleviation of pain, denoting the need still unmet for effectively treating NP. Literature evidence indicates that histaminergic system is a therapeutic target for the management of neuropathic pain, especially by using drugs that modulate the histamine H_3 receptor (H_3R). Recent studies indicate that antagonist or inverse agonists of H_3R may represent promising analgesics for treating NP. In this endeavor, our group described 1-(2,3-dihydrobenzofuran-2-yl)methylpiperazines (LINS01 compounds) as antagonists of H_3R. Considering the potential of H_3R as target to treat NP and the drug-like profile of the compound LINS01022,[1] a potent nanomolar affinity (pK_i 8.2) antagonist at H_3R, in this work we investigated the potential of this compound as analgesic in a murine NP model.

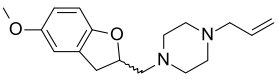


Figure 1: Structure of LINS01022 compound.

Results and Discussion

The compound was synthesized as previously described in literature, with adequate yields.[1] The final compound was converted into its di-hydrogenmaleate salt by reacting the free base with excess of maleic acid to increase the purity and water solubility. The stablished SAR for LINS01 compounds suggests that the methoxy group provides better solubility and selectivity towards H_3R, while the allyl group increased the antagonist potency, as indicated from modeling studies on H_3R. The analgesic potential of LINS01022 was assessed in a NP model using adult male Albino-Swiss CD-1 mice (chronic constriction injury to the sciatic nerve). After 7 days from the surgery, the animal developed hypersensitivity to mechanical and thermal stimuli, when the compound administration has started. The effects of single intraperitoneal (10, 20 and 30 mg/kg) injections of the compound were measured on mechanical (von Frey test) and thermal (cold plate and tail flick tests) stimuli.[2]

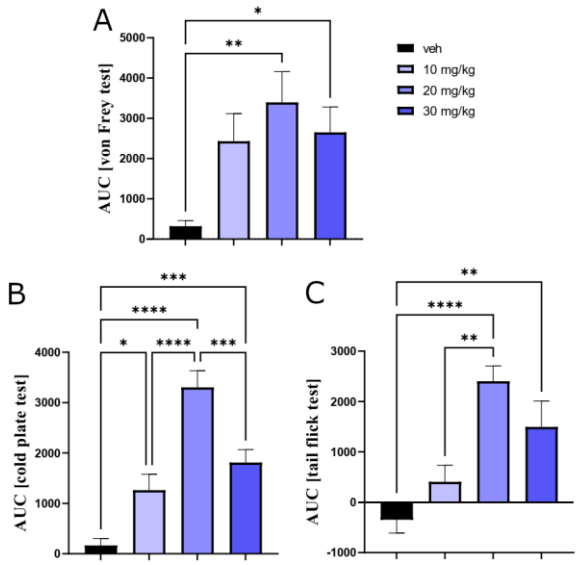


Figure 2: Results from von Frey, cold plate and tail flick tests after administration of 10, 20 and 30 mg/kg of LINS01022. p < *0.05; **0.01; ***0.001; ****0.0001.

The compound showed analgesic activity for both mechanical and thermal stimuli in all administered doses, being the strongest effect obtained with 20 mg/kg. The analgesic effect of LINS01022 is dose-dependent, that may be attributed to either central and peripheral effects. These effects were also observed to other H3R antagonists in previous works, such as E-98[2] and AR71,[3] denoting the involvement of H_3R in the mechanism of analgesic effect. Furthermore, no toxic effects to the animals were identified after administration of LINS01022.

Conclusions

This is the first report of the analgesic effect of LINS01022 in this murine NP model. The obtained results indicate that LINS01022 has the potential to alleviate sciatic nerve injury-induced NP.

Acknowledgements

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From *in silico* to *in vivo*: novel PKM2 inhibitor with antineoplastic effect against oral squamous cell carcinoma mitigates tumor growth.

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Key words: Drug repurpose, Glycolysis, Metabolism, Oral squamous cell carcinoma, Pyruvate kinase.

Introduction

Oral squamous cell carcinoma (OSCC) treatment has several adverse effects[1]. Pyruvate kinase (PK) converts phosphoenolpyruvate and ADP into pyruvate and ATP in glycolysis[2], and majority of cells express via alternative splicing either PKM1 or PKM2[3]. Non-proliferating epithelial cells rely on the M1 isoform. PKM2 is overexpressed in proliferating cells, adapting metabolism to provide building blocks or ATP as needed, and its nuclear translocation favors the expression of oncogenes [4]. Thereby, cancer cells benefit from PKM2, and our work aims to identify new hit molecules able to significantly bind to PKM2 and disrupt cancer cell metabolism, leading to a new treatment of OSCC and other neoplasms.

Results and Discussion

Screening through molecular docking in PKM2 was performed using DrugBank and SigmaAldrich molecule libraries. Docking literature described ligands resulted in two base pharmacophoric models, with 3494 fitting candidates. Duplicates and molecules containing toxic functional groups were removed, leaving 346 candidates. Predicted pharmacokinetics was considered, resulting in 13 possible hits. Literature was reviewed for biological effects, and 6 molecules were acquired. Using LDH coupled PKM2 enzymatic reaction, one potential inhibitor with IC50 of 2.7 ±0.09μM was identified. ATP production was measured and was impaired by the molecule with again an IC50 of 2.7μM, ensuring direct PKM2 inhibition. Cell viability accessed through MTT assay showed antineoplastic effect against OSCC lineages SCC4, SCC9, SCC25 with an average IC50 of 88.46μM, but revealed almost no selectivity, for its IC50 of 97.43μM in normal fibroblasts. Videomicroscopy time-lapse filming treated OSCC cells revealed morphological evidence of apoptotic cell death after 12h. Flow cytometry with Propidium iodide stanning revealed 69% of events on subG1, which may indicate DNA fragmentation of apoptotic bodies. Molecular docking and intermolecular interactions mapping using tetrameric PKM2 structure (PDB ID: 4FXF) in triplicate on the ADP site showed average binding score of -8.82 kcal.Mol-1, revealing predominantly electrostatic interactions, forming 6 hydrogen bonds and two salt bridges with nearby residues. Before in vivo experimentation, hemolysis in human blood was evaluated (CEP#43134721.4.0000.5626). No hemolytic potential was observed. Acute toxicity assay was performed using C57BL/6 mice (CEUA#2699110419), LD50 was characterized as 240 mg/kg. Hystopathological analysis revealed intestinal damage with crypt hypoplasia. No deaths or alterations occurred in 200 mg/kg, considered tolerated. A therapeutic test was conducted using Balb/c nude mice (CEUA#6497220421). Animals received a subcutaneous flank xenotransplant of SCC9 cells. Upon reaching 300 mm^3, animals were treated every 3 days, totaling 400 mg/kg (2x tolerated dose), fractioned in 12 doses. The group treated with the molecule showed significantly slower tumor growth, achieving an average final volume of 35% (65% inhibition of growth) when compared with the negative control, with no necropsy alteration.

Conclusions

In conclusion, a novel PKM2 inhibitor with antineoplastic effects *in vitro* induces apoptotic cell death and mitigates tumor growth *in vivo*. It may serve as a hit scaffold for lead optimization in the search of a new way of treating OSCC, and other tumors that benefit from PKM2 expression.

Acknowledgements

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A Greener and Cost-Effective Platform for Antiparasitic Drug Discovery: Microscale Parallel Synthesis Coupled with Biological Screening

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Keywords: Anti-infectives, heterocyclic, medicinal chemistry

Introduction

Guiding structural modification through structure-activity relationships (SARs) is essential, but laborious using conventional synthesis methods.[1] The **Microscale Parallel Synthesis (MPS)** approach enables rapid access to libraries of compounds. This platform, combined with **Medium-Throughput Screening** (MTS), was explored against a parasite panel including *Trypanosoma cruzi*, *T. brucei*, *T. rhodesiense*, as well as *Leishmania* species and cytotoxicity assays to unable fast access to libraries of structurally diverse heterocyclic compounds.

Results and Discussion

In this study, a miniaturized platform synthesis (MPS) approach, integrated with Medium-Throughput Screening (MTS), was employed to generate and evaluate heterocyclic compound libraries via urea and amide bond formations, as well as Groebke-Blackburn-Bienaymé reaction (GBB-3CR) in 96-well plates (**Figure 1** and **2**).

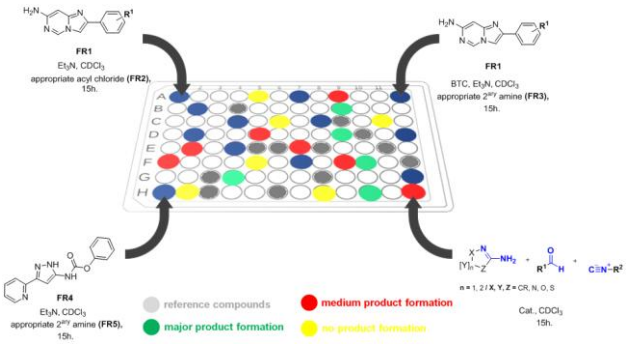


Figure 1. MPS in 96-well plates

Reaction products (S1–S96) were rapidly analyzed by LC-MS, classified by product formation efficiency [green: major (>70%), yellow: medium (~35%) and blue: no product], and quantified using semi-quantitative ^{1}H NMR. Only reactions with >70% conversion rate proceeded to biological assays, with samples standardized to a 1 mM concentration for screening. This strategy enabled the production of large libraries at the micromole scale without purification for initial biological assays, significantly reducing costs and promoting greener chemistry.

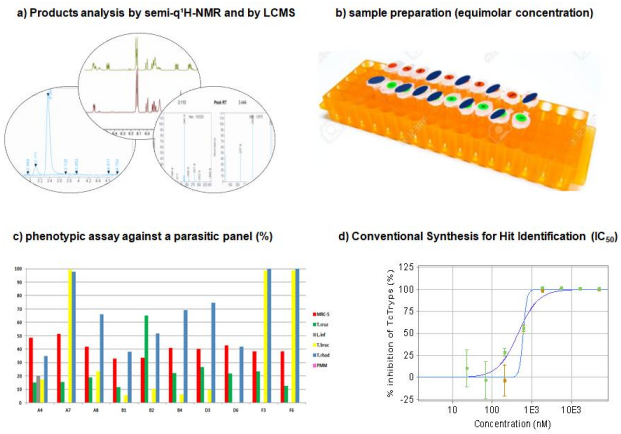


Figure 2. Overview of the overall workflow of the microscale parallel synthetic (MPS) approach combined with phenotypic assays against a parasitic panel.

Screening of unpurified reaction mixtures successfully identified active compounds, with subsequent resynthesis and purification confirming their antitrypanosomal activities. Notably, compounds reaction mixtures demonstrated inhibition of trypanosomal growth in crude screening, with a confirmed IC_{50} values after purification. These results validate the MPS-MTS platform as a powerful tool for efficient hit identification and optimization in antiparasitic drug discovery.

Conclusions

Our Microscale Parallel Synthesis (MPS) platform offers a greener, safer alternative to classical HTS by minimizing reagent use, waste, and exposure to hazardous materials. This innovative approach enables efficient selection of promising compounds for future translational research in infectious disease therapy.

Acknowledgements

We are grateful to the São Paulo Research Foundation (FAPESP) for funding (grant numbers: #2023/11804-5; #2024/08271-8).

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Synthesis and characterization of novel Nrf2 pathway activators

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Key words: Antioxidant, Keap1, Protein protein interaction, Drug discovery, Sulfonylhydrazone

Introduction

The transcription factor Nrf2 plays a central role in cellular defense against oxidative stress and in the maintenance of redox homeostasis. Under normal physiological conditions, Nrf2 is degraded via ubiquitination mediated by the Cul3 complex in association with the Keap1 protein. However, under oxidative stress, the Keap1-Nrf2 interaction is disrupted, allowing the stabilization and nuclear translocation of Nrf2, which activates antioxidant and cytoprotective genes, such as NQO1 and HO-1. The dysregulation of this molecular axis is associated with several diseases, including inflammatory, cardiovascular, neurodegenerative, and pulmonary diseases, such as Chronic Obstructive Pulmonary Disease (COPD). Given the relevance of this pathway for the development of new therapeutic approaches, the present study aimed to design, synthesize and characterize new inhibitors of the Keap1-Nrf2 protein-protein interaction and therefore to obtain new prototypes capable of activating this antioxidant pathway selectively and efficiently. In this context, sulfonylhydrazone derivatives were designed by Ligand Based Drug Design approach, followed by their synthesis and structural characterization by NMR, FTIR and MS. Then, compounds were evaluated using as model human peripheral blood mononuclear cells (PBMCs) derived from healthy donors (Ethical Committee approvement – CAAE 36880914.0.0000.5259). NQO1 protein expression was assessed by western blotting to test their ability to activate the Nrf2 pathway and then the cell viability was measured by MTT.

Results and Discussion

The results demonstrated that the compounds SHZ06 and SHZ17 were able to increase the protein expression of NQO1 in a dose-dependent manner, indicating the activation of the Nrf2 pathway. In addition, the cell viability assays showed that these sulfonylhydrazones did not compromise the viability of human monocytes at the concentrations evaluated (Figure 1). The expression and purification of the recombinant human Keap1 protein were successfully realized by cloning the Kealch domain of human Keap1 gene (UniProt Q14145, 321-609) in E. coli BL21(DE3) model, enabling future studies of protein-ligand interaction by STD-NMR.

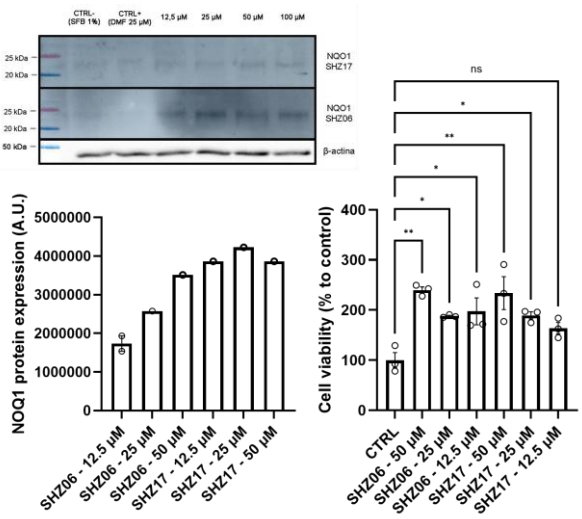


Figure 1: SHZ-induced NQO1 expression and cell viability in human PBMCs.

Conclusions

Until now, our data suggests that the synthesized compounds represent potential candidates for non-covalent activators of the Nrf2 pathway. Future studies will include protein-ligand interaction assays and structural optimization to improve the selectivity and pharmacokinetic properties of these molecules.

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DockTData: Automated Integration of Binding Affinity and Molecular Structure Data for Receptor–Ligand Interaction Modeling

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Key words: Binding affinity, Protein-ligand complexes, Structural bioinformatics, Data integration, Dataset

Introduction

Predicting receptor–ligand binding affinity is a central task in rational drug discovery and molecular modeling. However, integrating affinity data from heterogeneous sources remains challenging due to structural inconsistencies, limited standardization, and insufficient linkage between activity values and three-dimensional structures. The DockTData project addresses these issues through an automated pipeline for extracting, transforming, and integrating receptor–ligand data from public sources such as BindingDB, ChEMBL, and the Protein Data Bank (PDB). Proposing adherence to the FAIR principles (Findable, Accessible, Interoperable, Reusable), DockTData produces a dataset intended to support the development of machine learning models and scoring functions for affinity prediction.

High-quality datasets linking structural and energetic information are essential for training predictive models in computational drug discovery. In particular, machine learning algorithms designed to infer receptor–ligand affinity require consistent, large-scale, and well-annotated data sources that connect biochemical activity with three-dimensional molecular representations. Although databases like PDBbind have supported the development of scoring functions, their limited scope and lack of systematic adherence to open-access principles constrain their utility for machine learning applications. By linking structural and binding data from multiple repositories, DockTData facilitates data integration and reuse, enabling research workflows and supporting the development of open and publicly accessible machine learning pipelines for receptor–ligand affinity prediction.

Results and Discussion

The DockTData pipeline follows an ETL (Extract, Transform, Load) architecture, performing automated data retrieval via APIs (PDB), compressed tabular files (BindingDB), and relational databases (ChEMBL). The transformation stage includes unit normalization, record validation, and cross-referencing of molecular identifiers (UniProt ID, InChIKey, CCD). For PDB entries, structural and experimental metadata are harmonized, including resolution, determination methods, and detailed annotations of polymeric (proteins, peptides, nucleic acids) and non-polymeric entities (ligands, cofactors, oligosaccharides, small molecules) comprising the complexes. Data from ChEMBL and BindingDB are processed into a standardized format and integrated only when a strict correspondence with experimentally resolved structures in the PDB is established. For target proteins, UniProt accessions reported in ChEMBL and BindingDB are matched against those annotated in PDB entries, ensuring identity through exact sequence correspondence or high-confidence mappings. For ligands, chemical identity is verified by comparing standardized structural descriptors such as InChIKeys. In the case of BindingDB, ligand–protein complexes are linked to PDB structures when sequence identity is ≥85% and ligand structures are confirmed to match the Chemical Component Dictionary (CCD) records. The final output is an integrated dataset comprising receptor–ligand complexes with associated affinity data (such as Ki, Kd, and IC50), covering more than 30,000 PDB structures and around 14,000 distinct ligands. These ligands are primarily small molecules of pharmacological interest, typically with a molecular weight above 150 Da or explicitly identified as relevant by the authors who deposited the structures.

Conclusions

DockTData provides a scalable and reproducible framework for the integration of structural and functional molecular data, supporting computational modeling of receptor–ligand interactions. The resulting dataset is a valuable resource for predictive modeling and bioinformatics analysis, adhering to the FAIR and open-access principles. Future developments include the implementation of sequence-based automated mapping of molecular targets to PDB structures and the launch of a public web portal for data access, featuring a collaborative curation platform. We also plan to develop methodologies to integrate affinity data for peptide ligands, oligosaccharides, and multi-ligand complexes.

Acknowledgements

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Integrating Natural Products and Fragments in High-Throughput Crystallography for Drug Discovery in Brazil

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Key words: high-throughput crystallography, natural products, fragment screening

Introduction

Crystallographic fragment screening is a powerful structure-based drug discovery method that identifies low-molecular-weight ligands binding to protein targets. It offers high-resolution structural insights, reveals cryptic binding sites, and supports rational design, though it is limited by the weak affinities of fragments. In contrast, natural products (NPs), are shaped by evolution for biological activity, and offer rich and diverse scaffolds, providing a vast and underexplored chemical space, particularly within Brazil's exceptional biodiversity. High-throughput crystallography bridges these complementary strategies, enabling rapid identification of novel binding modes, allosteric sites, and interaction networks identification. At the MANACÁ beamline (Sirius/LNLS), we have implemented an automated pipeline for fragment screening and direct crystallographic NP capture from unpurified samples[1], by integrating high-throughput data collection, automated processing, and PanDDA[2] analysis to detect low-occupancy binders. By integrating Brazil's rich biodiversity, fragment libraries and cutting-edge synchrotron technology, this platform enables crystallographic NP- and fragment-based Drug Discovery projects in Brazil, supported by CNPEM's open-access infrastructure for academic and industrial drug discovery initiatives nationwide.

Results and Discussion

The CNPEM crystallographic pipeline integrates multiple open-access facilities, including automated crystallization plate preparation, ligand transfer, and high-throughput data collection at the MANACÁ beamline (Figure 1A). To validate the workflow, we screened 80 fragment-like molecules and 16 known TTR-binding natural products against transthyretin (TTR), a protein linked to amyloidosis. Analysis of over 250 crystals revealed clear electron density for six fragments, including one at the RBP site, and four natural products at the thyroxine (T4) binding site (Figure 1B).

When using a natural compound library as a compound source against a second protein target, we also detected clear ligand density for the bioactive NP, even in crude and early-stage fractions (Figure 1C). This streamline hit identification and guide downstream purification, thereby reducing overall isolation time of the bioactive compound.

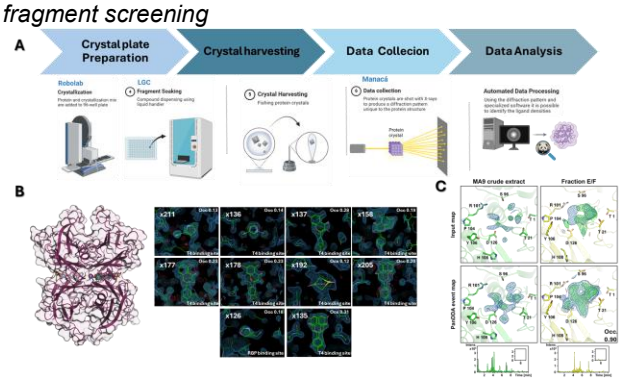


Figure 1: **A)** CNPEM's high throughput crystallography pipeline for both NP- and fragment-based campaigns. **B)** Fragment hits at the T4 thyroxine and RBP binding site of TTR. **C)** NP bioactive compound capture after soaking with crude extract and pre-fractionated sample.

Conclusions

By enabling direct crystallographic analysis of unpurified NP samples, our approach allows for the capture of bioactive compounds bound to protein targets without requiring prior compound isolation. This significantly reduces the time and effort traditionally needed in natural product-based drug discovery. The combination of natural product- and fragment-based crystallography accelerates rational lead development by leveraging the structural complexity of NPs and the modular simplicity of fragments. Therefore, this work establishes a new ground on NP- and fragment-based crystallographic pipeline at CNPEM, by integrating multiple open-access facilities, combining automation, high-throughput data collection, and rapid and robust data analysis to support both academic and industrial drug discovery research.

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Cytotox-profiling: a cell-based high-content multiparametric assay protocol for early drug cytotoxicity profiling in high throughput

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Key words: cytotoxicity, multiparametric, drug discovery

Introduction

Drug discovery is a long and expensive process[1], thus gaining knowledge of toxicity and off-target effects of a compound can mitigate the chances of failure or post-approval restrictions, saving time and resources[2]. Cell-based assays emerge as indispensable tools for dissecting the mechanisms of cytotoxicity and can be integrated into the early stages of drug discovery[2].

High Content Screening (HCS) combined with High Content Analysis (HCA) represents pivotal methodologies for rapidly identifying compounds with potential safety issues. In this study, we present the development and validation of Cytotox-profiling, a multiparametric cell-based assay designed to elucidate mechanism of cytotoxicity. The system encompasses the measurement of 7 parameters: cell viability, nuclear area, mitochondrial function, plasma membrane integrity, apoptosis, oxidative stress, and endoplasmic reticulum stress.

Results and Discussion

Cell staining protocols were optimized for each parameter to enhance detection and quantification. Hoechst was used to measure cell viability, CellROX to detect ROS, Mitotracker deep red (MTDR) to probe mitochondrial function, propidium iodine (PI) for membrane permeability, thioflavin T (ThT) for endoplasmic reticulum (RE) stress and CellEvent Caspase red to measure apoptosis.

Representative images of cells treated and untreated (control) with reference compounds are shown in Figure 1. Compounds with known mechanisms of action, such as digitonin (membrane lysis)[3], FCCP (mitochondrial uncoupler)[4], menadione (oxidative stress)[5] and Bortezomib (ER stress)[6] were employed to ensure robustness in parameter detection and measurement.

The Cytotox-profiling was formatted in two assays, Assay#1 to detect early unspecific mechanisms (18h – membrane integrity, ROS, mitochondrial function) and Assay#2 to detect late mechanisms (48h – apoptosis, and ER stress), both automated in a 384-well plate format.

The Cytotox-profiling accurately identified compounds with known mechanisms of cytotoxicity while also distinguishing between one or multiple cytotoxic mechanisms by evaluating compounds in concentration-response curves.

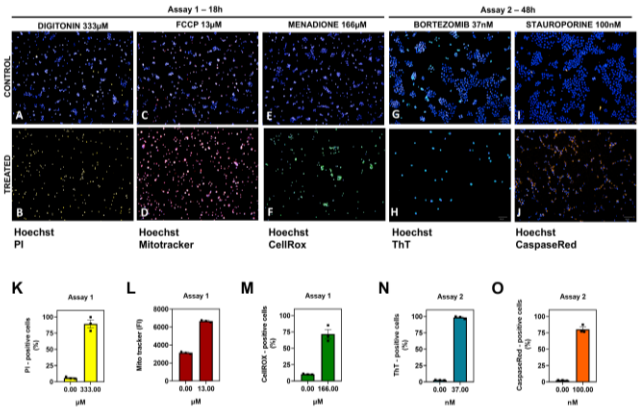


Figure 1: Representative images of the five different dyes used for mechanisms of cytotoxicity quantification in the two assays.

Conclusions

The information provided by this assay can contribute to detection of nonspecific and undesired toxicity, thereby aiding in the prioritization of lead compounds. Furthermore, the system exhibits flexibility to accommodate variations in cell type and fluorophores, rendering it adaptable to meet the specific requirements of diverse drug discovery programs.

Acknowledgements

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Latent Representations from Large Language Models for Ligand-Kinase Interaction Prediction in Drug Discovery

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Key words: Drug Discovery, Kinase, Deep Learning, Large Language Models, Embedding vectors

Introduction

Protein kinases are pivotal drug targets, yet screening the vast combinatorial landscape of >500 human enzymes against millions of ligands is experimentally prohibitive. We introduce a text-centric alternative that sidesteps the structural prerequisites of graph-based pipelines: ligands are expressed as SMILES strings embedded by IBM's SMI-TED, and kinases as amino-acid sequences embedded by Meta AI's ESM-2. Concatenating these n-dimensional vectors, a compact multilayer perceptron assigns interaction probabilities. By dispensing with 3-D structures and docking poses—requirements that limit current graph neural-network approaches [1], our method relies solely on readily available sequence and SMILES data, expanding target coverage and simplifying data preparation while preserving state-of-the-art accuracy.

Results and Discussion

The curated data set used in this work comprises 427,167 ligand–kinase pairs drawn from ChEMBL, each represented exclusively by a SMILES string and a FASTA sequence. Transformer-based embeddings were generated with SMI-TED (ligands) and ESM-2 (kinases), concatenated, and fed to a three-layer perceptron. Stratified five-fold training produced tightly clustered learning curves (Figure 1): median ROC-AUC values of 0.95 (train) and 0.93 (validation) and PR-AUC values of 0.94 and 0.92, respectively. Point metrics stabilised at F1 ≈ 0.86 and MCC ≈ 0.72, while the loss gap between splits remained below 0.05, indicating negligible over-fitting. Calibration was also sound, with Brier scores of 0.08–0.10. Evaluation on the held-out test set confirmed these trends. The confusion matrix of the test set (Figure S1) shows 19,458 true negatives (TN) and 17,516 true positives (TP) versus 2,785 false positives (FP) and 2,958 false negatives (FN). These counts translate to an overall accuracy of 86.6 %, precision of 86.3 %, recall of 85.6 %, specificity of 87.5 %, F1 of 85.9 %, and MCC of 0.73. The near-symmetry between FP and FN suggests that the decision boundary does not unduly favour either class, a desirable property when both wasted assays (FP) and missed actives (FN) carry significant costs.



Figure 1: Metrics

Embeddings distilled from language models evidently capture pharmacophoric and structural cues sufficient for high-confidence classification, even in the absence of explicit atomistic detail. The pipeline's engineering choices, dynamic GPU-to-RAM off-load, asynchronous batching, and checkpointing, allow billion-parameter backbones to operate on standard HPC nodes, ensuring that scalability keeps pace with continuously growing chemical libraries.

Conclusions

Combining SMI-TED and ESM-2 embeddings in a compact multilayer perceptron yields a fully text-based screen that classifies kinase–ligand pairs as active or inactive with 86 % accuracy, ROC-AUC above 0.90 and MCC ≈ 0.73 on 42,717 unseen test examples, all without relying on 3-D structures or docking; this scalable approach therefore accelerates early-stage kinase drug discovery by rapidly screening vast chemical libraries against multiple potential targets , with minimal data preparation, to identify both primary targets and off-target interactions.

Acknowledgements

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Mapping Chemical Space with Unsupervised Learning to Enhance Virtual Screening in Drug Discovery

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Keywords: Chemical Space, Unsupervised learning, Virtual Screening, ROCK, Kinase Inhibitors.

Introduction

The concept of chemical space is fundamental in cheminformatics, medicinal chemistry, natural product research, and drug discovery. Exploring chemical space enables the identification of structural and pharmacological trends, where data analysis and artificial intelligence play a crucial role in efficient navigation and interpretation [1]. In this work, we demonstrate how unsupervised learning can extract meaningful patterns to guide virtual screening. First, we apply it to cluster FDA-approved drugs. Second, we focus on the neurodegeneration-related target Rho-associated kinase (ROCK), exploring drug repurposing strategies to identify potential ROCK inhibitors. Finally, we highlight key chemotypes of ROCK inhibitors, which informed the virtual screening of an in-house compound library and the *in silico* combinatorial generation of new molecules for subsequent synthesis and evaluation.

Results and Discussion

First, we clustered FDA-approved drugs using molecular fingerprints as descriptors, revealing trends in prevalent chemotypes, particularly an increase in aromatic heterocycles, and shifts in target classes, such as kinases and emerging epigenetic modulators [2]. The chemical space of drugs and drug candidates was analyzed using cheminformatic tools. Molecular structures were converted into chemical fingerprints using RDKit and CDK within the KNIME platform. These fingerprints represent molecules as high-dimensional vectors, which were then projected into two-dimensional space for visualization using Uniform Manifold Approximation and Projection (UMAP) for dimensionality reduction.

Second, focusing on the neurodegeneration-related target ROCK, we integrated chemical space analysis with crystallographic data to develop pharmacophore and docking models for drug repurposing. Pharmacophore models were generated using ligand overlay in the CCDC Hermes package, which then guided docking constraints in CCDC GOLD for virtual screening. This approach identified six approved drugs with experimentally determined IC_{50} values, including ruxolitinib (ROCK1 IC_{50} = 25 nM; ROCK2 IC_{50} = 7 nM).

Third, applying similar virtual screening methods, we identified key chemotypes (7-azaindole, indazole) that informed the *in silico* combinatorial generation of novel molecules and the screening of an in-house library [3]. A KNIME workflow incorporating RDKit, Indigo, and CDK nodes was implemented for molecule generation. Docking prioritized 13 synthesized candidates, yielding four low-nanomolar ROCK inhibitors (*e.g.* LASSBio-2382: ROCK1 IC_{50} = 3.40 nM; ROCK2 IC_{50} = 1.09 nM), and one ROCK2-selective compound (LASSBio-2389: ROCK1 IC_{50} = 1143 nM; ROCK2 IC_{50} = 51 nM).

Conclusions

Collectively, these studies establish unsupervised learning as a powerful tool to (1) identify trends in drug discovery, (2) prioritize repurposing candidates, and (3) guide molecular design, connecting chemical space analysis with practical virtual screening outcomes.

Acknowledgements

We gratefully acknowledge funding support from CNPq and INCT-INOFAR.

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Novel Hybrid Xanthene and Pyrane Drugs Activity in Hormone-Dependent Tumor Models.

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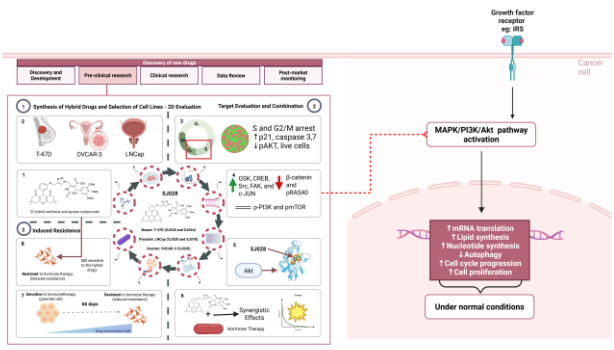
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Key words: hybrid drugs; antitumor

Introduction

The development of more effective oncologic therapies with fewer adverse effects relies on multidisciplinary collaboration but is often hindered by the intrinsic and acquired resistance of tumor cells. In this context, rationally designed hybrid drugs—formed by the combination of two bioactive compounds—represent a promising strategy, offering synergistic effects, dose reduction, and a lower risk of resistance.

Results and Discussion



Following in silico screening for binding affinity and pharmacological properties, the compounds were tested in in vitro cytotoxicity assays.

Cell Line	M3 (IC_{50})	M4 (IC_{50})	M10 (IC_{50})	SI M3	SI M4	SI M10
T-47D	3,44 ± 1,42	5,27 ± 1,08	-	>10,17	>6,64	-
BT-474	4,32 ± 1,05	5,62 ± 0,97	-	>8,10	>6,23	-
MDA-MB-231	3,94 ± 0,99	7,19 ± 0,88	-	>8,88	>4,87	-
SKBR-3	7,08 ± 0,87	36,11 ± 1,54	-	>4,94	<2	-
PC-3	8,92 ± 1,36	-	15,56 ± 1,41	>3,92	-	>2,34
LNCap	12,08 ± 1,27	-	17,38 ± 1,08	>2,90	-	<2
OVCAR-3	18,72 ± 0,95	-	-	2,19	-	-
SKOV-3	>35	-	-	<2		

M3 and M4 were the most effective against breast cancer cell lines, M3 and M10 against prostate, and M3 against ovarian cancer. For mechanistic studies, the most sensitive lines were selected: T-47D (breast), LNCaP (prostate), and OVCAR-3 (ovary), and analyzed by flow cytometry. In T-47D cells, M3 and M4 increased early apoptosis (24 h) and induced cell cycle arrest in S and G2/M phases (48 h); similar effects were observed with M3 and M10 in LNCaP cells, and with M3 in OVCAR-3.

	T-47D			T-47D			T-47D
	DMSO			M3			M4
	G1	S	G2/M	G1	S	G2/M	G1
	71,1	18,7	9,33	13,7	41,4	38	22,1
Compared to DMSO:	-	-	-	Decreased	Increased	Increased	Decreased
	LNCap			LNCap			LNCap
	DMSO			M3			M10
	G1	S	G2/M	G1	S	G2/M	G1
	67,5	14	15,8	18,7	79,6	1,7	35,7
Compared to DMSO:	-	-	-	Decreased	Increased	Decreased	Decreased
	OVCAR-3			OVCAR-3			
	DMSO			M3			
	G1	S	G2/M	G1	S	G2/M	
	34,1	23,4	19,9	3,77	75,4	11,4	
Compared to DMSO:	-	-	-	Decreased	Increased	Decreased	

The overexpression of p21 confirmed cell cycle arrest. Kinase array analysis revealed activation of GSK, CREB, Src, FAK, and c-JUN, along with a reduction in β-catenin and pRAS40, suggesting modulation of the PI3K/AKT pathway in T-47D cells treated with M3. The increase in γH2AX levels indicated DNA damage across all cell lines, and Western blot analysis confirmed reduced levels of phospho-AKT, with stable phospho-PI3K and mTOR levels. To evaluate AKT as a direct target, M3 and M4 were combined with the AKT inhibitor MK2206 in T-47D cells; M3 and M10 in LNCaP; and M3 in OVCAR-3, showing antagonistic interactions.

	T-47D		LNCap		OVCAR-3
	M3 + MK2206	M4 + MK2206	M3 + MK2206	M10 + MK2206	M3 + MK2206
ZIP	-37,56	-12,243	-46,842	-45,582	-17,406
Bliss	-36,038	-12,647	-45,96	-45,088	-16,572
Loewe	-27,258	-8,065	-30,834	-34,037	-12,817
Efeito	Antagonistic	Antagonistic	Antagonistic	Antagonistic	Antagonistic

In addition, AKT silencing via esiRNA abrogated the cytotoxic effects of the hybrids in previously sensitive cells. Docking analysis confirmed AKT as a potential molecular target of M3. In 3D models, the hybrids increased caspase-3/7 activity (24h) and expanded the necrotic core and the number of dead cells (72h), reinforcing their efficacy in more physiologically relevant models.

Co-administration of the hybrids with tamoxifen, fulvestrant, or letrozole yielded additive effects, reducing the IC_{50} by over 50%.

	Tamoxifen				Fulvestrant				Letrozole	
	T-47D		LNCap		OVCAR-3	T-47D		OVCAR-3	T-47D	
	M3	M4	M3	M10	M3	M3	M4	M3	M3	M4
ZIP	64,732	35,748	-2,105	-3,842	25,677	31,04	22,472	23,822	4,508	9,538
Bliss	64,58	35,702	-3,7	-6,278	24,299	30,565	21,99	23,467	5,088	9,147
Loewe	44,714	5,625	-23,809	-18,015	8,44	30,514	4,003	11,507	-14,452	3,702
Efeito	Synergistic	Synergistic	Additive	Additive	Synergistic	Synergistic	Synergistic	Synergistic	Additive	Additive

In the acquired resistance model, cells were continuously exposed to hormone therapy followed by recovery. Resistance was confirmed by reduced γH2AX expression and IC_{50} values greater than 35 µM for hormone therapy alone. Furthermore, resistant cells remained sensitive to the hybrids, with IC_{50} values comparable to parental cells, underscoring the potential of M3, M4, and M10 against hormone therapy-resistant phenotypes.

T-47D Resistant to Tamoxifen		T-47D Resistant to Fulvestrant		T-47D Resistant to Letrozole		LNCap Resistant to Tamoxifen		OVCAR-3 Resistant to Tamoxifen	OVCAR-3 Resistant to Fulvestrant
M3	M4	M3	M4	M3	M4	M3	M10	M3	M3
4,369	5,152	4,374	5,722	4,515	5,819	1,606	16,189	9,722	11,395

Conclusions

Thus, compounds M3, M4, and M10 demonstrated strong antitumor activity by inducing apoptosis, cell cycle arrest, and DNA damage, along with modulation of the PI3K/AKT pathway. Their efficacy was preserved in resistant models and enhanced in combination with standard therapies, highlighting their potential as therapeutic candidates for hormone-responsive and resistant tumors.

Acknowledgements

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Binding interactions of lupeol e derivatives with Leishmaniasis targets: In silico and in vitro studies.

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Key words: Lupeol, Leishmaniasis, target, disease, derivatives, pharmacology.

Introduction

Faced with leishmaniasis as a neglected disease featuring toxic and limited treatments, this research group pursued less toxic and more effective alternatives by focusing on lupeol, a natural compound. Using computational modeling, was developed two optimized lupeol derivatives. These derivatives were synthesized and tested in vitro, validating their initial potential as new drug candidates.

Results and Discussion

The analysis identified 675 targets associated with Leishmania and lupeol. Among these, 12 were common to both groups. The protein-protein interaction analysis highlighted 5 essential targets for the parasite.

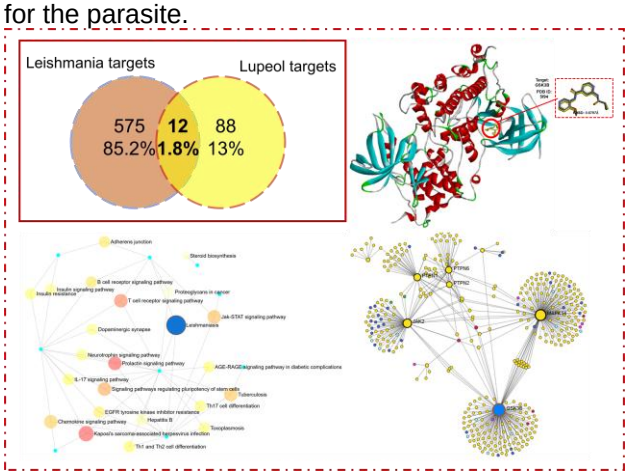


Figure 1: Venn diagram for target correlation; protein-protein interaction network; and redocking of target ID 5fp4 (Protein Data Bank).

Molecular docking was focused on the most promising target, GSK3 an enzyme regulating cellular processes.

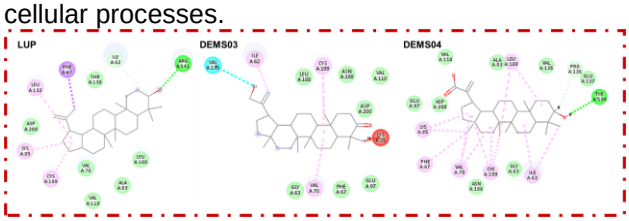


Figure 2: Molecular docking of lupeol and its derivatives.

The binding affinity of Lupeol was -4.6 kcal/mol, while for derivatives DEMS01 it was -5.5 kcal/mol and DEMS02 -1.4 kcal/mol.

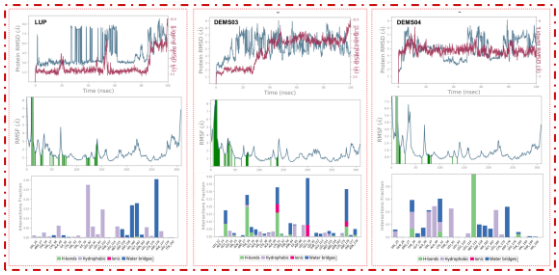


Figure 3: The RMSD, RMSF and Histogram of protein-ligand contacts.

The RMSD of the ligands remained aligned with that of the protein, with no significant diffusion from the binding site. The RMSF showed C-α and B-factor values below 8 Å. Hydrogen bonds predominated at residues MET115, LYS51, and TYR54. Water bridges were also significant.

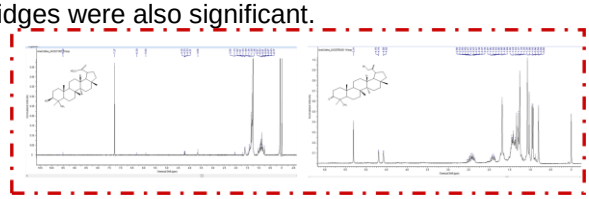


Figure 4: Synthesis of the derivatives.

To obtain the lupeol derivatives, the methodology described and established by research group[1] was applied.

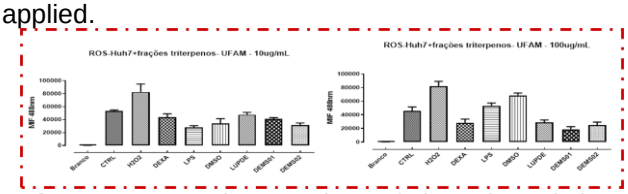


Figure 5: In vitro testing of lupeol and its derivatives.

The derivatives inhibited ROS better than lupeol and dexamethasone at both concentrations, but with distinct efficacy: DEMS02 in low stress, while DEMS01 stood out in high stress.

Conclusions

The derivatives showed promise, however, DEMS01 stood out in preliminary in vitro tests. The molecule is a candidate for future tests.

Acknowledgements

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Antitumor Activity and Toxicity Profile of Hybrid Chalcone–Quinazoline Compounds in Cervical Cancer Cell Lines

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Key words: HPV, hybrid molecules

Introduction

Cervical cancer is linked to infection by oncogenic Human Papillomavirus (HPV). Current treatments like cisplatin chemotherapy often face resistance and cause serious side effects, necessitating safer, more effective options[1]. Chalcones have antiproliferative effects, induce apoptosis, generate reactive oxygen species, and DNA damage[2]. Quinazolines act as tyrosine kinase inhibitors, blocking pathways that promote cell growth and survival[3]. Hybrid molecules that combine chalcones and quinazolines have demonstrated the ability to induce cell cycle arrest, oxidative stress, and DNA damage, leading to cancer cell death[4]. This study aims to explore chalcone-quinazoline hybrids as targeted therapies against cervical cancer driven by HPV oncoproteins E6 and E7 and tumors that are independent of these oncoproteins.

Results and Discussion

An initial screening of hybrid compounds 7A–7O was performed in cervical cancer cell lines (HCB514, CaSki, SiHa, HeLa, and C33-A) and non-tumorigenic cells (HFF-1) to identify selective cytotoxicity candidates. Compounds 7A, 7E, and 7N exhibited broad toxicity, whereas 7F significantly reduced cell viability in CaSki, SiHa, and C33-A cells, with minimal effects on non-tumorigenic cells. Structure-activity relationship (SAR) analysis revealed that the compound 7B, with a single methyl group, showed broad selectivity, while compounds with methoxy (–OCH_3) and/or chloro (–Cl) groups were selective for fewer cell lines.

In silico analyses indicated that 7B, 7F, and 7N possess high lipophilicity and low aqueous solubility. Molecular target prediction suggested potential affinity for EGFR, CDK4, and nuclear receptors, suggesting a possible transcriptional regulatory mechanism. Toxicity predictions showed a low risk for ocular, oral, and dermal toxicity. Notably, compound 7N was not associated with ototoxicity, whereas 7B and 7F exhibited moderate ototoxic potential.

Dose–response assays performed in SiHa and C33-A cells confirmed selective cytotoxicity (selectivity index [SI] ≥ 2). Due to the low selectivity observed in CaSki cells, this line was excluded from subsequent analyses.

To elucidate the mechanisms of cytotoxicity, cell cycle analysis was performed. In C33-A cells, compound 7B increased the sub-G1 population, indicative of apoptosis or DNA fragmentation, while compounds 7F and 7N induced minor G1 and sub-G2 arrest. In SiHa cells, all three compounds elevated the S-phase population, suggesting replication stress or arrest during DNA synthesis. To confirm the induction of DNA damage, Western blot analysis for γ-H2AX was performed. A significant increase in γ-H2AX phosphorylation level was observed in both cell lines, supporting the occurrence of DNA lesions. In parallel, the expression of phosphorylated AKT (p-AKT) was evaluated as a marker of PI3K/AKT pathway activation. The p-AKT levels remained unchanged across all treatments, indicating that the observed effects on genomic integrity are independent of this survival signaling pathway. Additionally, in C33-A cells, there was an increase in p27 expression, suggesting its involvement in cell cycle arrest induced by the hybrid compounds.

Conclusions

Compounds 7B, 7F, and 7N showed selective cytotoxicity in cervical cancer cells and induced DNA damage, as evidenced by γ-H2AX upregulation. Cell cycle alterations and replication stress suggest genotoxic mechanisms. These effects appear independent of AKT signaling. These findings support their potential as targeted anticancer agents and justify further investigation.

Acknowledgements

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Computational Studies and Preclinical Evaluation of Novel MGMT Inhibitors: An Integrated Strategy to Overcome Temozolomide Chemoresistance in High-Grade Malignant Gliomas

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Key words: *High-grade gliomas; MGMT; Temozolomide; Molecular dynamics; Virtual screening; Drug repositioning; Natural compounds.*

Introduction

High-grade malignant gliomas are central nervous system tumors characterized by high lethality and recurrence rates, largely due to their aggressive behavior and resistance to conventional therapies. These tumors are classified as grade IV by the World Health Organization (WHO). A key mechanism underlying this therapeutic resistance involves the methylation status of the *MGMT* gene. The MGMT protein plays a critical role in repairing DNA damage caused by alkylating agents, such as temozolomide, the frontline chemotherapeutic agent used in the treatment of gliomas, thereby contributing to reduced treatment efficacy in patients with unmethylated *MGMT* promoters.[1,2]

Justificative and Objective

MGMT expression is associated with poor therapeutic response and unfavorable patient prognosis, underscoring the urgent need for novel therapeutic strategies capable of selectively inhibiting this protein. This study aims to identify and evaluate natural compounds derived from Brazilian biodiversity, as well as repurposed drugs, with potential MGMT inhibitory activity through an integrated *in silico* and *in vitro* approach.

Methodology

Initially, three-dimensional models of the MGMT protein were generated using classical homology modeling methods. These models then underwent molecular dynamics simulations (triplicates of 1000 ns each) to assess their structural stability and conformational behavior. Structural conformations from each trajectory were analysed and selected by frame visual inspection and pocket analysis. Virtual screening will be conducted by consensus docking with AutoDock-GPU (empirical free-energy scoring function) and DiffDock (diffusion-based docking) using the Brazilian Biodiversity Natural Products Database (BrNPDB) and FDA-approved compounds from the DrugBank database with a total of 14.000 compounds to identify novel potential MGMT inhibitors. Promising compounds will subsequently be evaluated through *in vitro* assays, including 2D and 3D cell culture models.

Results

The three-dimensional MGMT models exhibited excellent structural quality metrics. Molecular dynamics simulations revealed significant conformational fluctuations in the active-site pocket, with surrounding residues modulating solvent accessibility and thus potentially affecting ligand binding. Active site pocket analysis identified distinct frames in which the active site was most accessible, these conformations were subsequently selected and prepared for ensemble docking studies. Based on protein–ligand interaction analysis by consensus molecular docking virtual screening with Brazilian natural products and reporpused drugs, the most promising compounds will be selected and acquired for *in vitro* validation in both 2D and 3D cell culture models to evaluate and validate the computational findings.

Conclusion

This study demonstrates the feasibility of integrating computational modeling and experimental validation to identify novel MGMT inhibitors. The findings highlight promising candidate compounds with potential therapeutic relevance for overcoming resistance in high-grade gliomas, paving the way for future clinical studies and drug development efforts targeting MGMT.

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Design, Synthesis and Evaluation of Coumarin Derivatives as Modulators of Histamine H_3 Receptors, and Cholinesterase and MAO-B Inhibitors for the Treatment of Alzheimer's Disease

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Key words: Alzheimer's disease, Coumarins, Histamine H_3 receptors, Acetylcholinesterase, Monoamine oxidase B.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, and complex not fully understood pathogenesis. As a multifactorial condition, AD involves multiple pathological mechanisms, which complicates the development of effective treatments. Consequently, recent research has increasingly focused on the design of multi-target-directed ligands (MTDLs)[1], typically combining cholinesterase (ChE) inhibition with modulation of other therapeutic relevant targets to AD, aiming to address the disease's multifaceted nature[2,3,4].

In this context, the present work describes the synthesis and pharmacological evaluation of coumarin derivatives functionalized with aromatic, heteroaromatic, and aliphatic substituents. These MTDLs were designed as histamine H_3 receptor (H_3R) modulators and ChE and monoamine oxidase B (MAO-B) inhibitors based on the recognizing that the literature's pharmacophore model for H_3R ligands and our LaDMol's ChE inhibitor model were complementary (Figure 1).

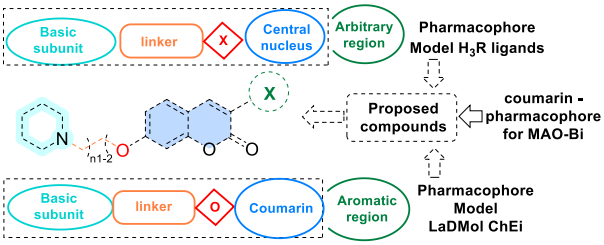
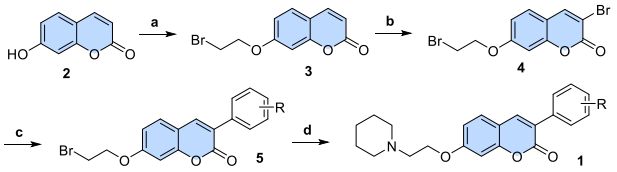


Figure 1. Molecular design of new MTDLs based on pharmacophore H_3R and ChE models.

Results and Discussion

The synthesis of compounds was divided into four distinct series, based on structural pattern of variations in the coumarin. Classical synthetic methodologies, including the Pechmann and Perkin reactions, were employed alongside cross-coupling techniques such as Suzuki-Miyaura and Buchwald–Hartwig reactions to introduce the desired substituents. One of the synthetic routes is exemplified in Figure 2. Thirty-two final compounds were synthesized, with pharmacological evaluations currently underway.



a) Br_2, AcOH/AcONa, 3h, r.t, 80%; b) 1,2-dibromoethane, K_2CO_3, acetone, 8h, 60°C, 78%; c) $ArB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, Toluene:EtOH:H_2O, 3-4h, 65°C, 72-90%; d) piperidine, MeCN, 3h, 60°C, 45-98%.

Figure 2. Suzuki Cross-coupling synthesis of designed 3-aryl-7-alcoxy-coumarins.

To date, twenty-two compounds have been fully evaluated on the three targets, presenting best IC_{50} values of 70 nM for AChE and 647 nM for BChE (generally with selectivity for AChE); MAO-B IC_{50} values of up to 75 nM; and high affinity for the histamine H_3R with K_i values ranging from 12 to 299 nM. These compounds were no neurotoxic in SH-SY5Y neuroblastoma cells at 10 µM. Compounds LF269, LF287, LF349, and LF353 stood out as promising MTDLs, exhibiting selective inhibition of AChE (IC_{50} = 240, 463, 74, and 330 nM) and MAO-B (IC_{50} = 561, 100, 75.6, and 1396 nM), along with excellent affinity for the H_3R (K_i = 28.7, 33.4, 33.4, and 18.1 nM), respectively. Molecular modeling studies for the three targets were conducted presenting interesting insights into the observed activity.

Conclusions

In summary, our MTDLs were successfully designed and in general our compounds were able to modulate at least two of the three proposed targets, with the main activity consisting of H_3R modulation. Notably, LF269, LF287, LF349, and LF353 emerged as strong candidates for further drug development as authentic MTDLs, with well-balanced potencies and affinity within the targets.

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Evaluation of the antitumor activity of hybrid drugs (Chalcones and Quinazolines) in pancreatic cancer cell lines

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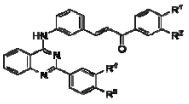
Key words: pancreatic cancer; hybrid molecules; cytotoxicity

Introduction

Hybrid molecules are promising in cancer therapy due to synergistic effects, lower required doses, and reduced resistance. Chalcones and quinazolines are promising candidates due to their ability to inhibit proteins linked to cell survival and therapy resistance. In previous work, 2-arylquinazolinechalcone hybrids showed strong antitumor activity against breast cancer cells in both 2D and 3D in vitro models, with high selectivity for tumor cells. These compounds induced G0/G1 cell cycle arrest and cell death, possibly through p27 upregulation, ATR inhibition, and increased H2AX phosphorylation, indicating DNA damage. In this study, we investigate whether these compounds also exhibit antitumor activity against pancreatic cancer cells. Given the high mortality and limited treatment options associated with this cancer type, evaluating the cytotoxicity and selectivity of these hybrids in pancreatic models is crucial for exploring their broader therapeutic potential.

Results and Discussion

From 15 compounds (7a–7o), four (7b, 7c, 7f, 7n) were selected for their selective cytotoxicity at 10 µM, inhibiting over 50% of Panc-1, MiaPaCa-2, and BxPC-3 cells, while sparing HFF-1 fibroblasts. Structure–Activity Relationship (SAR) analysis revealed that the presence of methoxy (–OCH_3) and chloro (–Cl) substituents on the arylquinazoline moiety conferred greater selectivity toward tumor cells compared to when these groups were present only on the chalcone portion of the molecules. However, the presence of chloride in combination with other substituents in the molecule resulted in reduced cytotoxicity across all tested cell lines (**Figure 1**). Previous studies have reported similar findings, including a prior investigation involving the same 2-arylquinazolinochalcone compounds in breast cancer models[1-3]. IC_{50} values confirmed that 7b, 7c, 7f, and 7n were more potent than 5-FU, especially against Panc-1, all with $IC_{50} < 10$ µM (7.19, 8.23, 7.74, and 5.88 µM, respectively).



Classification	Compound	Chalcone		Arylquinazoline	
		R¹	R²	R³	R⁴
Toxic to all cell lines	7a	H	H	H	H
	7c	CH_3	H	H	H
	7f	OCH_3	OCH_3	H	H
Toxic to Panc-1, Mia-paca-2 and HFF-1	7b	OCH_3	H	H	H
Toxic HFF 1	7i	OCH_3	H	OCH_3	H
Selective to Mia-paca-2	7m	OCH_3	OCH_3	OCH_3	H
Selective to Panc-1, Mia paca 2 and BXPC 3	7b	H	H	OCH_3	H
	7c	H	H	OCH_3	OCH_3
	7d	H	H	Cl	H
	7f	CH_3	H	OCH_3	H
	7n	OCH_3	OCH_3	OCH_3	OCH_3
Non Toxic	7g	CH_3	H	Cl	H
	7j	OCH_3	H	OCH_3	OCH_3
	7k	OCH_3	H	Cl	H
	7o	OCH_3	OCH_3	Cl	H

Figure 1. Structure–Activity Relationship (SAR) analysis of 2-Arylquinazolinechalcones in pancreatic cancer cell lines. The chemical structures and synthesis of these compounds have been previously described[3].

Selectivity indexes also showed higher values for PANC-1 considering both HFF-1 and HaCat healthy cell lines. In addition, 3D spheroid assays confirmed the cytotoxic potential of these compounds in more physiologically relevant models, with increased propidium iodide (PI) fluorescence and decreased calcein staining observed in Panc-1 and BxPC-3 spheroids. Considering the consistent and pronounced cytotoxicity observed for Panc-1 cells in both 2D and 3D models, this cell line was selected for the next stages of the study. Furthermore, due to their lower IC_{50} values, compounds 7b and 7n were also selected for subsequent investigations. Flow cytometry results showed that compounds 7b and 7n led to a reduction in the percentage of cells in the G0/G1 phase, with compound 7n also promoting an accumulation of cells in the S phase. Cell death analysis with 7AAD and annexinV-FITC, showed increasing in late apoptosis for 7n.

Conclusion

Together, these findings demonstrate that the 2-arylquinazoline hybrids, particularly compounds 7b and 7n, exhibit potent and selective cytotoxicity

against pancreatic cancer cells, especially the PANC-1 line, in both 2D and 3D models. SAR analysis highlighted the importance of methoxy and chloro substituents on the arylquinazoline moiety for enhanced selectivity. Flow cytometry revealed that compound 7n alters cell cycle dynamics by increasing the S phase population, and apoptosis assays confirmed its ability to induce late apoptotic cell death. These results support the continued investigation of 7b and 7n as promising therapies for pancreatic cancer.

Acknowledgements

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Three-Step Synthesis of *Tc*G6PDH Inhibitory Dihydroquinazolinones.

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Key Words: *Quinazolinones; Tricarbonylated compounds; Chagas' Disease.*

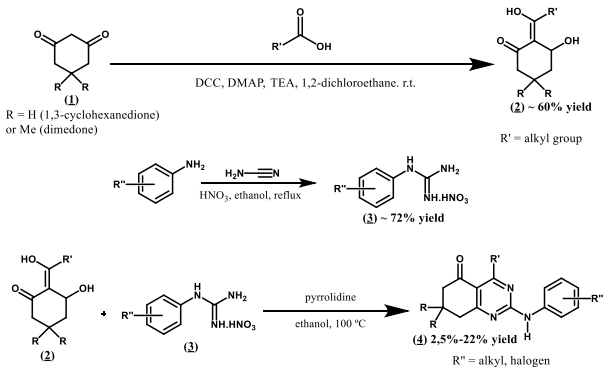
Introduction

This study presents a concise and modular approach to the synthesis of dihydroquinazolinones, aiming to explore their potential as inhibitors of *Trypanosoma cruzi* glucose-6-phosphate dehydrogenase (*Tc*G6PDH), a promising target for Chagas disease chemotherapy. Given the existence of human homologs of G6PDH, achieving selectivity toward the parasitic enzyme is a paramount requirement. The proposed synthetic route (Scheme 1) consists of three steps and utilizes easily accessible cyclic 1,3-diones, carboxylic acids, and aniline-derived guanidinium nitrates. The biological activity of the synthesized compounds was assessed through IC_{50} determination against *Tc*G6PDH.

Results and Discussion

Our approach builds on the previous work of Mercaldi and coworkers,[1] which reported dihydro-quinazolinones as effective *Tc*G6PDH inhibitors. The strategy enables systematic diversification at three scaffold positions, modulated by the choice of dione, acid, and aniline derivative.

As shown in Table 1, IC_{50} values for selected compounds were consistent with the literature, validating our methodology. Notably, compounds derived from acetic acid showed enhanced selectivity toward *Tc*G6PDH, while analogs from 5,5-dimethylcyclohexane-1,3-dione exhibited lower activity. Among aniline derivatives, *p*-fluoroaniline analogs demonstrated the highest potency.



Scheme 1. Applied strategy for the synthesis of dihydroquinazolinones.

In total, we prepared 19 quinazolinones and a summary of the biological data for selected compounds is presented below.

Table 1. IC_{50} values of selected prepared compounds.

Compounds	IC_{50} *Tc*G6PDH	IC_{50} *Hs*G6PDH	Yields
AC-P001	2.32 µM	67.49 µM	19%
AC-P002	>100 µM	>100 µM	17%
AC-P005	55.21 µM	>100 µM	17%
AC-P007	5.87 µM	>100 µM	16%

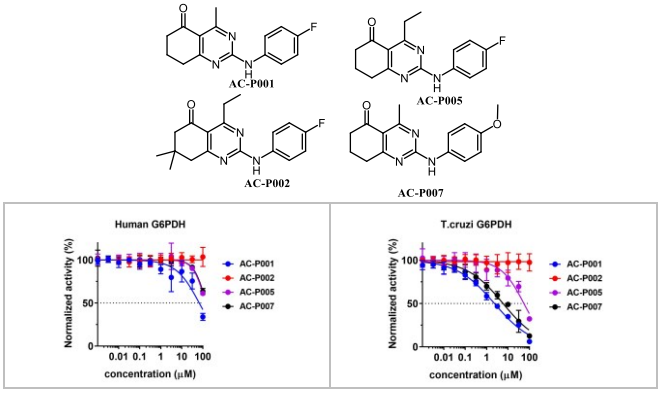


Figure 1. *Hs*G6PDH *vs. Tc*G6PDH inhibition.

Conclusions

Despite modest yields, the proposed synthesis efficiently provides diverse dihydroquinazolinones from inexpensive and readily available starting materials. Biological evaluation confirmed earlier findings and for a SAR evaluation, the methyl group present at the heterocycle (AC-P001) shows better results than using a bigger chain, like AC-P005. Another important feature on the structure is the fluorine atom on the phenyl substituent (AC-P001) in comparison with the methoxy group (AC-P007). The last modification on this series is the presence of two methyl substituents on the cyclohexane moiety, that if it's present on the structure (AC-P002), the activity decreases in comparison of it's absence (AC-P005). In particular, this work enables the study of this class of molecules by providing direct changes over three regions of the structure opening avenues for improving both selectivity and potency.

Acknowledgements

UNICAMP Instituto de Química · CNPq · CAPES · Finep

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Molecular docking and synthesis of quinoline-carboxamide derivatives as potential inhibitors of the SARS-CoV-2 main protease

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Key words: *Antiviral; CoViD-19; Cysteine Protease; Molecular Modeling; Medicinal Chemistry; Organic Synthesis*

Introduction

The main protease (M^{pro}) or 3-chymotrypsin-like protease (3CLpro) of SARS-CoV-2/CoViD-19 pandemic, is a crucial cysteine protease for viral replication and a pivotal target for antiviral drug development. Considering that amide bonds can be recognized and cleaved by proteases, and that the quinoline nucleus is found in certain antiviral compounds,[1] ten methyl (**1a-e**, R_1 = CH_3) and phenyl (**2a-e**, R_1 = Ph) quinoline-carboxamide compounds with two amide bonds were designed as potential M^{pro} inhibitors. We aimed to assess the binding modes of these ten compounds in the active site of the target protein through molecular docking simulations and to synthesize one of them.

Results & Discussion

Docking was carried out with the MCULE and GOLD programs.[2] The 3-D structure of the target protein, bound to a keto-benzothiazol reversible covalent inhibitor coded **V7G401**,[3] was obtained from the Protein Data Bank (PDB 7JKV, X-ray resolution = 1.25 Å).[3] In MCULE, which implements the AutoDock Vina standard protocol, the 2-D structures of the ten compounds and reference inhibitor (**V7G401**, considering both structures, before and after covalent binding) were drawn and converted to 3-D structures.

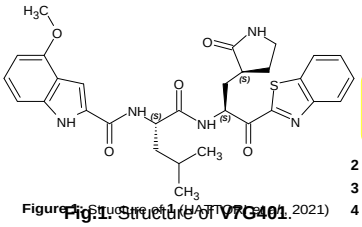


Fig.1. Structure of **V7G401**.

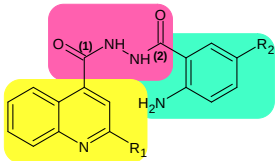


Fig.2. Structures of **1a-e** and **2a-e** (**1**, R_1 = CH_3; **2**, R_1 = Ph; **a**, R_2 = H; **b**, R_2 = CH_3; **c**, R_2 = F; **d** = R_2 = Cl; **e**, R_2 = Br).

Ligands were prepared for GOLD by constructing 3-D structures, optimizing geometries and performing conformational analysis (Monte Carlo) by molecular mechanics (MMFF) in SPARTAN.[2] In GOLD, considering the active site, the lowest-energy conformer of the reference inhibitor (**V7G401**) and the ten compounds were redocked and docked, respectively. The intermolecular interactions in the poses of the complexes resulting from docking in both programs (MCULE and GOLD) were analyzed in BIOVIA DSV.[2] In poses from MCULE, three derivatives stood out by interacting with the same residues as in the crystal structure. In GOLD, two binding mode hypotheses were considered: covalent and non-covalent.

Docked poses of methyl-substituted compounds (**1a-e**, R_1 = CH_3) have the shortest distance between the sulfur atom of catalytic Cys145 (nucleophilic residue) and the nearest carbonyl-amide group, potentially acting as covalent inhibitors. On the other hand, considering the non-covalent binding mode hypothesis, docked poses of phenyl-substituted compounds (**2a-e**, R_1 = Ph) showed higher GOLD-SCORE values, suggesting stronger binding affinity. A methyl-substituted quinolinic acid compound (**1a**) was synthesized by the Pfitzinger reaction, using isatin, KOH, and acetone (reflux, 6h30min), to obtain the quinoline core. Simultaneously, isatoic anhydride was synthesized from isatin and peracetic acid (room temperature, 19h). Subsequently, reaction of isatoic anhydride with hydrazine hydrate in acetonitrile (ice bath, 40min) yield the corresponding hydrazide. The target product (**1a**) (*N'*-(2-aminobenzoyl)-2-methyl-quinoline-4-carbohydrazide) was obtained by reacting the carboxylic acid group of the quinoline core with hydrazide, in presence of HOBt and EDC, in DMF (31h), achieving a yield of 32%. Preliminary antiviral assays were performed in human type II pneumocytes cell model (Calu-3) for the synthesized compound (**1a**), which exhibited promising values of 50% effective concentration (EC_{50} = 3.80 µM), 50% cytotoxic concentration (CC_{50} > 200 µM), and selectivity index (SI = CC_{50}/EC_{50} > 52.63).

Conclusions

Ten methyl/phenyl quinoline-carboxamides were docked into the active site of M^{pro} from SARS-CoV-2. Methyl derivatives (**1a-e**) showed a potential covalent binding mode, while a non-covalent one was pointed out for the phenyl derivatives (**2a-e**). The methyl derivative **1a** (R_1 = CH_3, R_2 = H) was synthesized and demonstrated promising antiviral activity (EC_{50} = 3.80 µM) in Calu-3 cells with SI > 52.63. Future investigations will involve evaluating the product against M^{pro} (*in vitro* enzymatic assay), and the molecular docking and synthesis of isatins hybrids with quinolines, coumarins, and naphthoquinones.

Acknowledgements

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Synthesis and Evaluation of Coumarin-Phenolic Acid Derivatives for Skin Aging Prevent Activities

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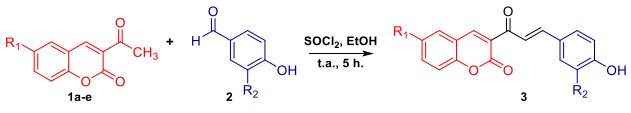
Key words: *Polyphenols; Coumarins; Skin-Aging Molecules.*

Introduction

Skin aging is one of the major dermatological concerns today, resulting from various factors that lead to physical, morphological, and physiological changes. Macroscopically, aged skin presents wrinkles, hyperpigmented spots, loss of elasticity, discoloration, dryness and slower regeneration, along with reduced protection against pathogens and increased susceptibility to skin cancer. Key processes involved in cellular senescence include glycation, solar radiation, and the action of free radicals, which act synergistically to exacerbate oxidative stress and structural damage. Addressing these mechanisms can help delay aging and promote skin health. In this context, coumarins and phenolic acids emerge as promising agents due to their broad range of pharmacological properties.[1-2] Based on this, the present study reports the synthesis of coumarin-phenolic acid hybrids, as well as the *in vitro* evaluation of their antioxidants, antiglycation, and photoprotective activities.

Results and Discussion

The compounds **(3a-j)** were obtained via an aldol condensation reaction between coumarin and the corresponding aldehyde derived from de phenolic acid, as described by Petrov (2008).[3] The yields of the resulting derivatives ranged from 52.0 to 95.0%, as illustrated in the scheme below:



3a 76,0%- R_1= H; R_2= OCH_3; **3f** 57,0%- R_1= H; R_2= OH;
3b 52,0%- R_1= Br; R_2= OCH_3; **3g** 52,0%- R_1= Br; R_2= OH;
3c 64,0%- R_1= Cl; R_2= OCH_3; **3h** 57,0%- R_1= Cl; R_2= OH;
3d 92,0%- R_1= OCH_3; R_2= OCH_3; **3i** 95,0%- R_1= OCH_3; R_2= OH;
3e 71,0%- R_1= NO_2; R_2= OCH_3; **3j** 73,0%- R_1= NO_2; R_2= OH;

Scheme 1. Synthesis of coumarin-phenolic acids derivatives **3a-j**.

After the synthesis, purification, and characterization, the antioxidant, photoprotective, and antiglycation activities of the compounds were evaluated to investigate their potential roles in combating premature aging. In the ^{1}H NMR spectra of all compounds, a singlet around 8.0 ppm and two doublets at approximately 7.0 and 7.5 ppm (J=16.0 Hz) were observed, corresponding to the most deshielded proton of the coumarin ring and the *trans*-olefinic hydrogens of the chalcone moiety. The ^{13}C NMR spectra confirmed the expected number of signals and their characteristic chemical shifts. Elemental analysis (CHN-S) revealed carbon and hydrogen contents in close agreement with theoretical values. FTIR analysis showed characteristic absorption bands at ~1730 cm^{-1} (C=O, lactone) and 1690 cm^{-1} (C=O, α,β-unsaturated ketone), consistent with the proposed structures. The sun protection factor (SPF) was determined using the method described by Mansur et al. (1986).[4] According to ANVISA Resolution RDC No. 30 (June 1, 2012), a substance is considered photoprotective when its SPF exceeds 6. Notably, hybrids **3g** and **3j** showed SPF values of 15.15 and 14.54, respectively. For comparison purposes, benzophenone-3, a commercial UV filter, exhibited lower SPF values under the same conditions. Antioxidant activity was assessed using the DPPH• and ABTS•$^+$ assays, which are based on the reduction of stable radicals by antioxidants.[5] Among the tested derivatives, compound **3f** showed the best performance in the DPPH• assay (EC_{50}= 19.76 μmol L^{-1}), while **3h** stood out in the ABTS•$^+$ assay (EC_{50} =16.14 μmol L^{-1}). These values were significantly lower than those of the standard antioxidant, which presented EC_{50} values of 89.31 μmol L^{-1} (DPPH) and 92.92 μmol L^{-1} (ABTS), highlighting the superior radical scavenging capacity of the hybrids. The antiglycation activity was evaluated using bovine serum albumin (BSA) assay, which measures the percentage inhibition of advanced glycation end-products (AGEs) formation. Compounds are considered efficient antiglycation agents when inhibition exceeds 80%, and inefficient when it falls below 20%.[5] At a concentration of 1 μmol L^{-1}, the best results were observed for **3a, 3b** and **3h**, with inhibition rates above 90%.

Conclusions

The assay results revealed notable photoprotective activity, as all ten synthesized compounds exhibited SPF values above 6 at 45 μmol L^{-1}, with **3g** and **3j** demonstrating the highest effectiveness. Regarding antiglycation activity, the molecular hybrids demonstrated inhibition rates above 70% at 1 μmol L^{-1}, with **3a, 3b** and **3h** standing out, each surpassing 90% inhibition. As for antioxidant potential, EC_{50} values in the DPPH assay ranged from 250 to 19 μmol L^{-1}, with **3f** being the most active. In the ABTS assay, EC_{50} values varied from 16.1 to 100 μmol L^{-1}, with **3h** demonstrating the highest efficiency. These findings underscore the potential of the synthesized derivatives as multifunctional agents for the development of new skincare products targeting the preventing skin aging.

Acknowledgements

LaSMMed Laboratório Síntese de Moléculas Medicinais; CAPES; UEL; CMLP; LabFitoBio

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Synthesis and preliminary biological evaluation of dual PI3Kδ/HDAC6 purine-benzohydroxamates for hematological malignancies

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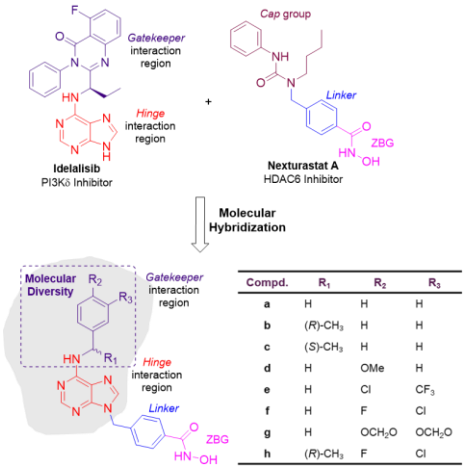
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Key words: HDAC6, PI3K, hybridization, multi-target approach, dual inhibitors, hybrids, hematological cancer.

Introduction

The enzymes PI3Kδ and HDAC6 are attractive therapeutic targets in hematologic malignancies due to their roles in cell proliferation, survival, and stress response. To explore a multitarget strategy, we designed and synthesized purine–benzohydroxamate hybrids that integrate pharmacophoric features of the selective inhibitors idelalisib (PI3Kδ) and nexturastat A (HDAC6). (**Figure 1**).

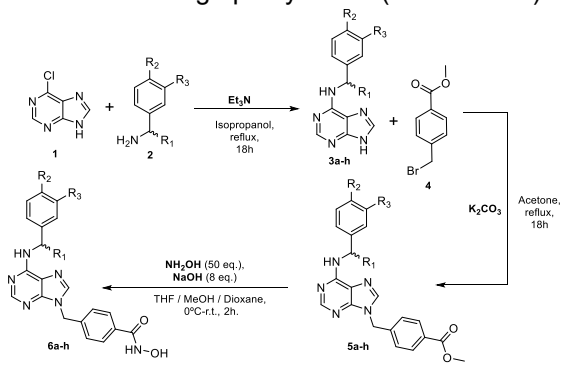


Compd.	R_1	R_2	R_3
a	H	H	H
b	(*R*)-CH_3	H	H
c	(*S*)-CH_3	H	H
d	H	OMe	H
e	H	Cl	CF_3
f	H	F	Cl
g	H	OCH_2O	OCH_2O
h	(*R*)-CH_3	F	Cl

Figure 1: Design of hybrid compounds.

Results and Discussion

Three-step synthetic route[1] (Scheme 1) successfully yielded sixteen intermediates and eight final compounds (**6a–h**), with overall yields ranging from 25% to 37% and high purity levels (95.3–99.4%).



Scheme 1: Synthetic route for hybrid compounds.

Biological evaluation (**Table 1**) revealed that compounds **6b** and **6f** exhibited the most potent cytotoxicity across hematologic tumor cell lines, including JAK2- and JAK3-mutant models (IC_{50} = 0.13–0.4 μM), surpassing ruxolitinib. Compound **6f** also showed low toxicity in normal colon and skin cells. A stereochemical preference was evident, with **6b** (*R*) outperforming its enantiomer **6c** (*S*). Additionally, **6e** showed selective activity in colorectal cancer cells, while **6d** displayed moderate potency with hematologic bias. Compounds **6g** and **6h** remain under investigation.

Table 1. Cytotoxicity against tumor and normal cells.

	Cell lines IC_{50} (μM)[ab]									
Comp.	Jurkat (LLA-T)	Namalwa (LLA-B)	SET2 (JAK2 mut.)	U937 (JAK3 mut.)	PC3 (Prostate)	HCT116 (Colon)	MDA (Breast)	A431 (Epidermoid Carcinoma)	CCD18CO (Normal Colon)	HaCat (Normal Skin)
6a	0.91	0.82	1.2	1.4	10.3	2.16	3.15	3.95	31	5.44
6b	0.14	0.13	0.15	0.4	1.69	1.2	1.01	0.93	2.35	0.55
6c	0.97	0.64	1.7	1.5	5.92	13.5	3.68	2.95	4.09	3.07
6d	1,01	0,68	2	2,4	12	2,15	10	9,06	41,4	6,86
6e	2,41	2,62	11,89	4,3	>50	1,92	33,5	30,7	>50	13,3
6f	0,58	0,37	1,8	1,3	6,8	0,24	2,35	3,63	27,1	1,89
Vor.[c]	0,72	0,79	1,54	1	>50	1,49	2,34	2,72	18,55	3,09
Rux.[c]	36,2	41,8	0,18	4	15,2	16,0	17,9	44,5	37,3	14,6

[a]Values obtained by MTT bioreduction assay. [b]The values are represented by a gradient from green to red to indicate compounds´ potency: very potent (IC_{50}≤1 μM, dark green), high potency (1 μM< IC_{50}≤4 μM, light green), moderate (4 μM< IC_{50}< 20 μM, yellow), low potency (20 μM≤IC_{50}< 50 μM, red), and inactive (IC_{50} ≥ 50 μM, dark red). [c]Vor=Vorinostat Rux=Ruxolitinib.

Conclusions

Compounds **6b** and **6f** are promising lead candidates for hematologic malignancies, combining potency, selectivity, and favorable preliminary safety. The influence of chirality and substitution on cellular activity provides a strong basis for further optimization. Future studies will include enzymatic assays to confirm the intended dual-target mechanism and therapeutic relevance.

Acknowledgements

FAPESP (grants #2021/08260-8, #2023/13499-5, and 2024/07723-2) and FCF–USP for their support.

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Chalcones and Pyrazolines as Potential Anti-Helminth Agents: Synthesis, Computational Modeling and Phenotypic Assays

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Keywords: Chalcones, Strongyloides venezuelensis, Pyrazolines, Organic Synthesis, Molecular Docking.

Introduction

The search for new compounds with anti-helminth activity is urgently needed due to emerging resistance to current drugs. Strongyloidiasis is classified as a neglected disease, mainly due to the absence of public health policies in the countries where they are endemic and limited availability of effective treatment[1]. In that regard, chalcones and derivatives show promise as compounds given their broad biological activities (antibacterial, antifungal, antiparasitic)[2]. Despite the urgent need for new drugs for human helminthiasis, there are numerous challenges in identifying targets of action. Molecular docking provides valuable predictive insights into compound-target interactions[3]. This work aimed to synthesize chalcones and pyrazoline derivatives, perform molecular docking and phenotypic assays to assess the potential anti-helminth against Nematoda (roundworms), *Strongyloides venezuelensis*, experimental model of Strongyloidiasis

Results and Discussion

Molecular docking was performed with 90 compounds (chalcones and pyrazolines) against three target proteins: Cathepsin B, Glutathione S-transferase, and Adenosine Kinase - all well-characterized in literature and associated with parasite development. The compounds showing the best binding scores were synthesized, purified, and spectroscopically characterized by NMR. Table 1 presents the binding score results for two of the most promising compounds with potential antiparasitic activity.

Table 1. Binding scores for the evaluated compounds alongside of albendazole, reference drug.

	Binding Score (kcal mol^{-1})		
	Cathepsin B	Glutathione s-transferase	Adenosine Kinase
CH18	-6,70	-6,46	-7,18
AP18	-6,70	-7,34	-8,32
Albendazol	-5,50	-7,37	-8,82

Based on molecular docking screening, phenotypic assays and *in vitro* mortality evaluations were conducted on *S. venezuelensis* female parasites at different time points (2, 24, 48, and 72 hours) following exposure to concentrations of 5, 10, and 20 µg mL^{-1} of Chalcone 18 (CH18) and Pyrazoline 18 (AP18). Phenotypic alterations, including a marked reduction in motility, darkening, and internal organ damage, were observed in parasites exposed to 10 µg mL^{-1} and 20 µg mL^{-1} of CH18 and AP18 after 48 and 72 hours of incubation, compared to control group (drug-free). The mortality rate increased proportionally with both concentration and exposure time. The concentration of 20 µg mL^{-1} resulted in 100% mortality into 24 hours for CH18 and into 48 hours for AP18, respectively. The concentration of 10 µg mL^{-1} of CH18 caused 66% mortality in 48 hours and 34% in 72 hours. While 10 µg mL^{-1} of AP18 caused 34% in 48 hours 66% in 72 hours. No mortality was observed in female parasites exposed to the concentration of 5 µg mL^{-1}. However, CH18 and AP18 induced phenotypic changes, notably a reduction in parasite motility. Females exposed to albendazole (pharmacological control), at the same concentrations, only presented changes in motility without mortality until the end of the experiment, 72 hours.

Conclusions

Considering the therapeutic limitations of albendazole against *Strongyloides stercoralis*, we present an innovative approach to the treatment of Strongyloidiasis, which could be extended to the treatment of other helminths.

Acknowledgements

This work was supported by CAPES. Furthermore, the authors are thankful to the Institutional Laboratory C-LABMU/UEPG for the use of the characterization facilities. To Profª Drª. Silmara Marques Allegretti of the Institute of Biology, (UNICAMP) for conducting antiparasitic activities.

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An integrated in silico approach for the identification of *Mycobacterium tuberculosis* pantothenate synthetase inhibitors

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Keywords: M. tuberculosis; Drug Discovery; Virtual Screening; Molecular Dynamics.

Introduction

Tuberculosis (TB) remains a primary cause of mortality from a single infectious agent worldwide, a threat significantly amplified by the emergence of multidrug-resistant *Mycobacterium tuberculosis* (*Mtb*) strains[1]. The enzyme pantothenate synthetase (PS), is critical for the biosynthesis of pantothenate[2]. This biosynthesis is critical in Mtb and notably absent in humans, represents a highly attractive and validated target for drug development[3]. In this study, we employed an integrated in silico workflow, combining pharmacophore modeling, molecular docking, and extensive molecular dynamics simulations, to identify and characterize novel small-molecule inhibitors of *Mtb*PS.

A shape-based pharmacophore model (SBPB) was developed in vROCS from known *Mtb*PS inhibitors. The SBPB was validated via ROC curve analysis, the model was employed for virtual screening of the MCULE database. Molecular docking studies were conducted using FRED, with the crystal structure of the target protein retrieved from the Protein Data Bank (PDB ID: 3IUB)[4].

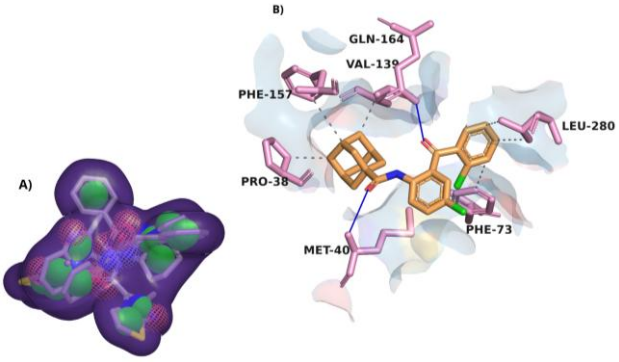
To investigate binding stability, the highest-scoring complex and the apo-enzyme were subjected to 100 ns molecular dynamics simulations in GROMACS (GROMOS force-field). Detailed protein-ligand interaction patterns were then elucidated using PLIP.

Results and Discussion

The SBPB (fig. 1A) demonstrated excellent predictive performance (AUC = 0.97; EF = 0.36). Application of this model to the MCULE database resulted in the identification of 183 compounds presenting molecular features compatible with known MtbPS inhibitors. Among these hits, compound KS1 was selected for further investigation based on its top-ranking molecular docking score (-10.2), obtained through a validated docking protocol (AUC = 0.80).

The complex exhibited high structural stability, reaching equilibrium within 30 ns and maintaining a low mean root-mean-square deviation (RMSD) of 0.28 ± 0.05 nm throughout the simulation. Residue-level fluctuation analysis (RMSF) revealed that KS1 binding did not induce significant conformational perturbations, with the complex (mean RMSF = 0.10 ± 0.04 nm) displaying a dynamic profile similar to that of the apoenzyme. Notably, the observed flexible regions, particularly the α3' helix (residues 74–88), are consistent with previously reported dynamic domains. Additionally, KS1 formed hydrophobic interactions with Phe73, Leu280, Pro38, Val139, and Phe157, along with two hydrogen bond acceptor interactions involving Gln164 and Met40.

Figure 1: (A) Pharmacophore model representing key molecular features: the spheres represent the pharmacophore characteristics: hydrogen acceptors in half-filled blue, hydrogen donors in half-filled red, cationic centers in filled blue and the presence of rings is represented in green. (B) Interaction map of the MtbPS-KS1 complex, where hydrogen bonds are depicted as solid blue lines and hydrophobic interactions as dashed gray lines.



Conclusions

This study identified KS1 as a novel potential inhibitor of *Mtb*PS through in silico screening and validation. Molecular dynamics simulations demonstrated stable binding via key interactions.

Acknowledgements

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Imidazolium-Based Topical Formulation Against Resistant Candida: From Design to Preclinical Proof-of-Concept

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Key words: Antimicrobial, yeast, resistant candidiasis, non-systemic

Introduction

Imidazolium-based ionic liquids (ILs) are organic salts with tunable physicochemical properties, and some have demonstrated biological activity. Among them, 1-hexadecyl-3-methylimidazolium chloride (C_{16}MImCl) has shown potent antifungal effects against multidrug-resistant dermatophytes (MIC ~0.05 µg/mL), along with low cytotoxicity and absence of genotoxicity in human cells[1,2]. ILs have also exhibited strong antifungal activity against emerging yeast pathogens, including multidrug-resistant clinical isolates of *Candida tropicalis*, *C. parapsilosis*, *C. glabrata*, and *Trichosporon asahii*. Notably, C_{16}MImCl demonstrated MIC values as low as 0.9 µg/mL and caused a significant reduction (33–89%) in ergosterol content, indicating inhibition of the lanosterol 14α-demethylase pathway[2,3]. This project evaluates a novel C_{16}MImCl-based topical formulation through synthesis, *in vitro* antifungal assays, cytotoxicity tests, and *in vivo* efficacy in a murine model of cutaneous candidiasis.

Results and Discussion

C_{16}MImCl (Fig. 1) was synthesized in 90% yield and 97.58% purity, confirmed by analytical techniques. Both the free salt and the formulation exhibited similar antifungal activity (MIC = 0.78 µg/mL; MFC = 1.24 µg/mL) and cytotoxicity (CC_{50} ≥ 4 µg/mL for L929 and ≥ 5 µg/mL for HaCaT), with selectivity indices > 10 for *C. glabrata* and *C. parapsilosis*, indicating a favorable safety margin. The clinical isolates used were previously characterized as resistant to fluconazole (MIC > 64 µg/mL) and, in some cases, to amphotericin B (MIC = 1–2 µg/mL)[4], including multidrug-resistant strains such as *C. krusei*, *C. auris*, and *C. albicans*. In vivo (Fig. 2C), the gel completely eradicated *C. albicans* in a murine superficial candidiasis model, reducing fungal load from ~8 log CFU/g to undetectable levels. Although moderate cytotoxicity was observed in vitro, this may overestimate toxicity compared to intact skin[5]. Ongoing studies are evaluating local tolerability, including skin irritation and corrosion.

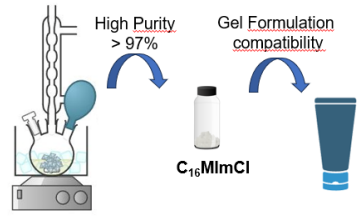


Fig. 1 - Synthesis and gel formulation of C_{16}MImCl.

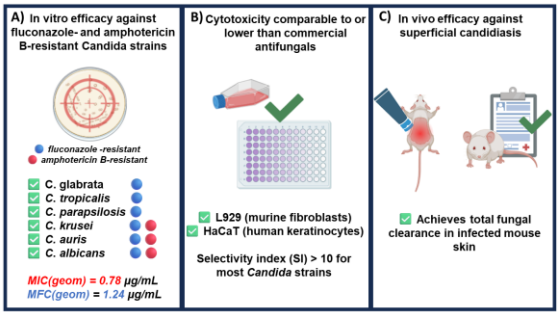


Fig. 2 - C_{16}MImCl formulation shows strong antifungal activity against resistant *Candida* (MIC 0.78 µg/mL) (A), low toxicity (SI > 10) (B), and full fungal clearance in a mouse model (C).

Conclusions

The C_{16}MImCl-based gel demonstrated potent and selective antifungal activity against multidrug-resistant *Candida* spp., maintaining efficacy and safety at 1% (w/w) concentration. The formulation preserved bioactivity without increasing cytotoxicity and showed complete in vivo eradication of infection. These results support its potential as a novel topical therapy currently under patent protection.

Acknowledgements

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Formation of β-Lapachone–β-Cyclodextrin Inclusion Complex Evaluated by Molecular Dynamics for Potential Antitumor Application.

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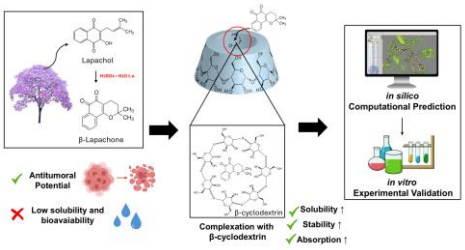
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Key words: β-lapachone, β-cyclodextrin, inclusion complex, molecular docking, molecular dynamics

Introduction

Naphthoquinone derivatives such as β-lapachone (βLP) show promising antitumor activity but are limited by poor solubility and bioavailability. β-cyclodextrin (βCD) offers a viable strategy by forming inclusion complexes that improve solubility, stability, and pharmacological efficacy.

Figure 1: βLAP and βCD: *in silico* and *in vitro* approach to improve solubility and bioavailability.

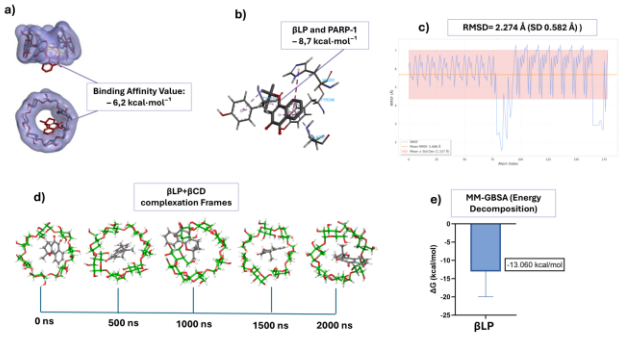


This study aimed to characterize the formation of an inclusion complex between βLP and βCD and evaluate its interaction and antitumor potential using *in silico* models.

Results and Discussion

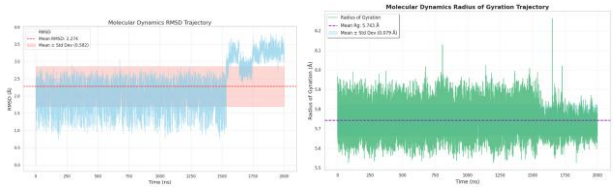
Analysis by molecular docking and dynamics revealed that βLP exhibited strong affinity, conformational stability, and favorable interactions within the βCD cavity.

Figure 2: Results from molecular docking (a,b) and dynamics (c,d,e).



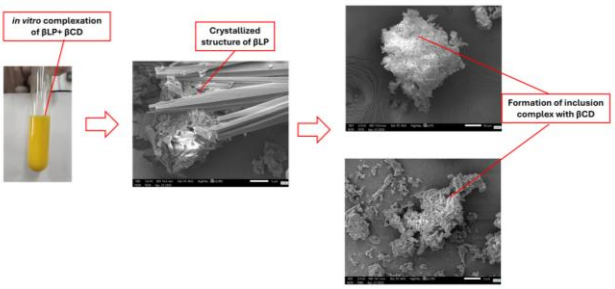
The RMSD analysis showed that the system reached stability in the final frames, and βLP remained mostly complexed with βCD, with energy decomposition revealed during the last 100 ns of simulation.

Figure 3: RMSD (left) and Gyr trajectory (right).



This result is further confirmed by the evaluation of complex formation through Scanning Electron Microscopy (SEM), as shown in Figure 3.

Figure 4: Formulation and evaluation of the inclusion complex through SEM.



Conclusions

in silico and preliminary *in vitro* analysis demonstrated that βLP fits efficiently into the cavity of βCD, forming a well-accommodated conformation, as supported by binding affinity and trajectory analysis. However, further experimental validation is still required to fully characterize the complexation.

Acknowledgements

This study was funded by resources from the Center for Innovation and Artificial Intelligence for Health (CI-IA Saúde), in part by resources from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Process No. 2020/09866-4, from the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) Process No. PPE-00030-21 and from UNIMED Belo Horizonte. Amazonas Research Support Foundation. Multiuser Center for Analysis of Biomedical Phenomena of the Amazonas State University. Tutorial Education Program.

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Uninterrupted Synthesis of Tetrahydroisoquinolines and Migrastatic Evaluation in Triple-Negative Breast Cancer Cells.

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Key words: Breast Cancer, tetrahydroisoquinolines

Introduction

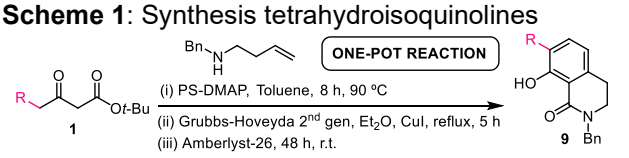
Breast cancer is the leading cause of cancer-related death among women worldwide. Among its subtypes, triple-negative breast cancer (TNBC) is distinguished by its high aggressiveness, rapid growth, and a pronounced tendency to metastasize to distant organs [1]. Unlike other breast cancer types, TNBC lacks both hormone receptors and HER2, severely limiting available therapeutic options. Current treatments focus primarily on reducing tumor size via chemotherapy but have proven largely ineffective at preventing metastatic spread [2].

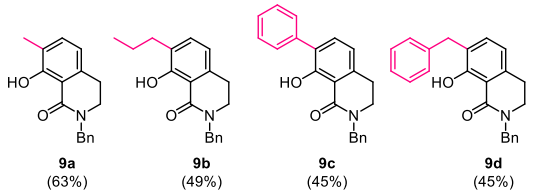
In light of this challenge, developing novel therapeutic strategies that inhibit cellular migration without harming normal cells is essential. Accordingly, this study is devoted to the synthesis of bioactive compounds with potential antimigratory, non-cytotoxic activity. We designed a simple, efficient synthetic methodology to access a series of tetrahydroisoquinoline derivatives via a one-pot tandem reaction sequence. This structural scaffold is well known for its presence in a variety of biologically active molecules, making it a promising platform for the development of innovative therapeutic agents.

Results and Discussion

The synthesis of the tetrahydroisoquinolines began with the formation of β-ketoamides, followed by a cross-metathesis reaction and a one-pot tandem sequence (Scheme 1). This sequence comprises an intramolecular Michael addition followed by a Robinson annulation, efficiently assembling the tetrahydroisoquinoline core.

Scheme 1: Synthesis tetrahydroisoquinolines





Preliminary biological analysis of compound 9c shows no cytotoxic effects on the MDA-MB-231 breast cancer cell line or the non-tumorigenic MCF10F cell line after 24 h of exposure (Figure 1). Moreover, a dose-dependent inhibition of cancer cell migration is observed after 24 h of treatment (Figure 2).

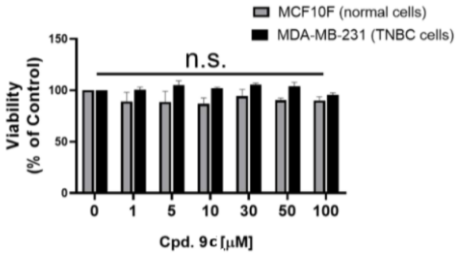


Figure 1. Effect of compound **9c** on the viability and proliferation of TNBC cells and normal mammary cells at 24h of exposition.

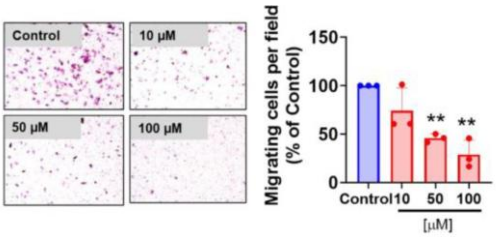


Figure 2. Effect of compound 8c on migration of TNBC cells at 24h of exposition.

Conclusions

The first tetrahydroisoquinoline analogue evaluated showed no cytotoxic effects and reduced the migration of triple-negative breast cancer cells in a dose-dependent manner. It is expected that the analysis of the remaining synthesized compounds will reveal similar activities, supporting the proposal of tetrahydroisoquinolines as a new class of chemical structures with selective migrastatic effects.

Acknowledgements

ANID Doctoral scholarship number: 21232281, Fondecyt N° 1251470, Fondecyt N° 1240262´.

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Identification of active small molecules targeting the endopeptidase RipA from *Mycobacterium tuberculosis*

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Key words: tuberculosis, cell-wall hydrolase, fragment-based drug discovery.

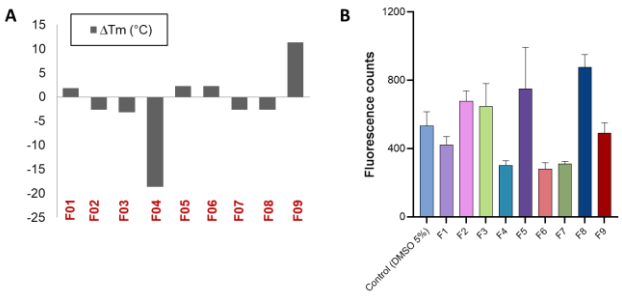
Introduction

Tuberculosis (TB) remains the global leading cause of mortality among infectious diseases. Although current treatments are effective, their prolonged duration and adverse effects compromise patient adherence, contributing to the emergence of drug-resistant strains. These challenges emphasize the urgent need for novel therapeutic strategies, including the identification of new drug targets. In this context, the cysteine peptidase RipA, which plays a central role in mycobacterial daughter cell separation and host-pathogen interactions[1-2], has emerged as a potential novel target for the development of new anti-TB drugs. This study aimed to identify small molecules targeting RipA using a Fragment-Based Drug Discovery (FBDD) approach.

Results and Discussion

From the initial fragment-library screening, 53 fragment hits were identified, with nine small molecules confirmed as RipA binders exhibiting dissociation constants (K_D) ranging from micromolar to low millimolar range. Structural clustering revealed three major chemotypes: aromatic compounds, oxygen-containing heterocycles, and pyrazoline ring derivatives.

Figure 1: Structural and functional modulation of RipA by the fragment ligands.



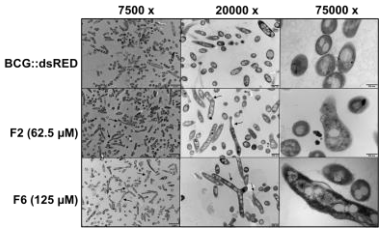
(A) Ligand-induced thermal shift of RipA. (B) RipA enzymatic activity in the presence of validated fragment ligands.

Notably, some ligands enhanced RipA activity, while two also inhibited the growth of BCG, H37Rv, and L5 strains. Microscopy analysis of treated samples revealed morphological changes, suggesting cell wall disruption and a possible impact on RipA activity.

Table 1: Minimum inhibitory concentration (MIC) determination of RipA inhibitors against mycobacterial strains.

Fragment	Strains		
	H37Rv	BCG	L5
F2	> 125 µM	> 250 µM	250 µM
F6	> 500 µM	1 mM	1 mM

Figure 2: Ligands F2 and F6 induce phenotypic changes in mycobacterial cells.



Conclusions

This study provides novel insights into RipA-ligand interactions and identifies promising molecular scaffolds for the development of new anti-TB agents. To our knowledge, this is the first study to identify small active molecules targeting RipA, with some compounds already demonstrating inhibitory effects against Mycobacteria reference strains.

Acknowledgements

FAPESP (2016/18721-4, 2024/02235-0, 2024/15878-6), CNPq (151725/2022-5).

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Synthesis and biological evaluation of dehydroxy isoquines and isotebuquines derivatives: new drug-like compounds against visceral Leishmaniasis

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[2] *Grupo de Química Orgánica Medicinal, Facultad de Ciencias, Universidad de la República, Uruguay.*

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Key words: isoquines, isotebuquines, Leishmaniasis, visceral.

Introduction

Leishmaniasis is first among the neglected tropical diseases and second, after Malaria, among the parasitic-associated diseases with major health impact measure as Disability-Adjusted Life Year[1]. The visceral form (VL) of the disease, caused by the *donovani* and *infantum* species, when it is symptomatic, is lethal without treatment. Human treatment is exclusively chemotherapeutic and depends on a handful of old-fashion drugs that show toxicity, resistance, differential susceptibility to species and strains/ isolates, low stability, high costs and/or require a complex administration.

With the goal to tackle VL, at the starting point of the scaffold 4-aminoquinoline[2], we have designed new dehydroxylated isoquines and isotebuquines derivatives, mostly drug-like molecules (SwissADME[3]), with structural modifications to modulate its basicity and lipophilicity. They were tested in the clinically relevant stage of the parasite (intracellular amastigote) infecting human macrophages (THP-1 derived monocytes). For the most promising compounds its activity was confirmed in infected Bone Marrow Derived Macrophages (BMDM). A bioluminescent line of *L. infantum*[4] was employed, following "standardized protocols"[5].

Results and Discussion

About 30 compounds derived from 4-aminoquinolines were synthesized: isoquines, isotebuquines and core control compounds (**Figure 1**).

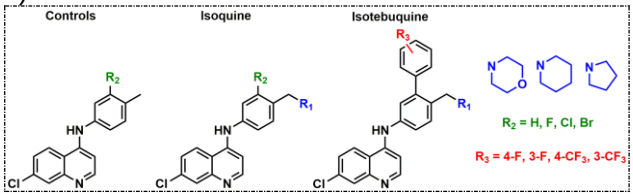


Figure 1. Controls, isoquine and isotebuquine derivatives.

Molecules containing a dialkylamino group showed a good bioactivity profile. The morpholinyl substituted derivatives reduces host cytotoxicity and improved solubility, while piperidinyl and pyrrolyl moieties increase anti-parasitic activity. Within the isoquine series, fluoride and chloride as R_2-substituent do not improve parasitic activity, whereas bromide substituent which increases selectivity in pyrrolyl and piperidinyl-derivatives, but not in morpholinyl ones. Regarding isotebuquine series, 3- and 4-CF_3 substituted-derivatives, are soluble and no toxic to the host cell, parasitic potency ranking: 4-F >3-F >3-CF_3 >4-CF_3 (last one is no active). Some promising hits showed equal of better profile than Miltefosine drug, EC_{50} ~ 5 µM, S.I. > 4) (**Figure 2**).

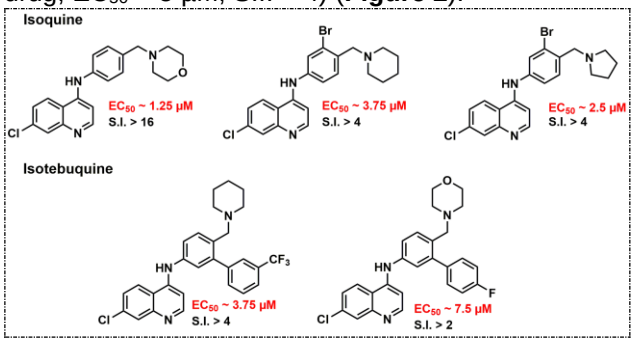


Figure 2. Promising hits.

Conclusions

We designed novel derivatives of the isoquine scaffold, and perform a SAR with the goal to explore a new chemical space against the clinically relevant stage of this parasitosis. Future plans point to study the possible accumulation of the compound in the phagolysosome[6] and therapeutic efficacy studies in an acute murine model of VL for the most prominent hits.

Acknowledgements

PEDECIBA, ANII, MEC and CSIC.

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Function-Oriented Synthesis of Hydroisoquinoline Scaffolds for Madangamine Alkaloid–Based Drug Discovery

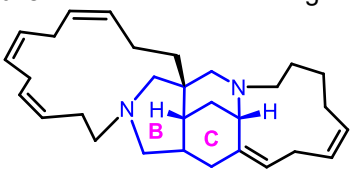
Valentina Ríos Sobarzo[1] (PG), Cristian Maulen[1] (PG), Héctor Hernández[1] (PG), Camila Linares Pipón[1] (PG), Maximiliano Martínez (PQ), Claudio Parra Montes[1] (PQ), Ben Bradshaw[2] (PQ),
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Organic Synthesis, Function-Oriented Synthesis, Tandem Reaction

Introduction

Madangamines are pentacyclic alkaloids characterized by a diazatricyclic nucleus and two macrocyclic rings, where the hydroisoquinoline nucleus is present in the BC rings of this alkaloid. Madangamine A has been reported to have potent in vitro cytotoxic activity with ED50 values of 0.93 μg mL^{-1} against murine leukemia P388, human lung A549, and brain U373, and 5.7 μg mL^{-1} against breast MCF-7.[1] To date, four total syntheses have been reported, which require long sequences with low yields and little flexibility to generate analogues. To overcome these limitations, Function-Oriented Synthesis (FOS) addresses the problem by focusing not on the structure of the natural product but on the biological function of the compound. This allows for the design of better pharmacophores. In the case of these alkaloids, no information has been reported, so this strategy allows us to evaluate which rings of the alkaloid are responsible for its biological activity.[2] In addition, obtaining the hydroisoquinoline nucleus would be the starting material for the construction of the diazatricyclic nucleus present in madangamine.

Figure 1: Structure of Madangamine

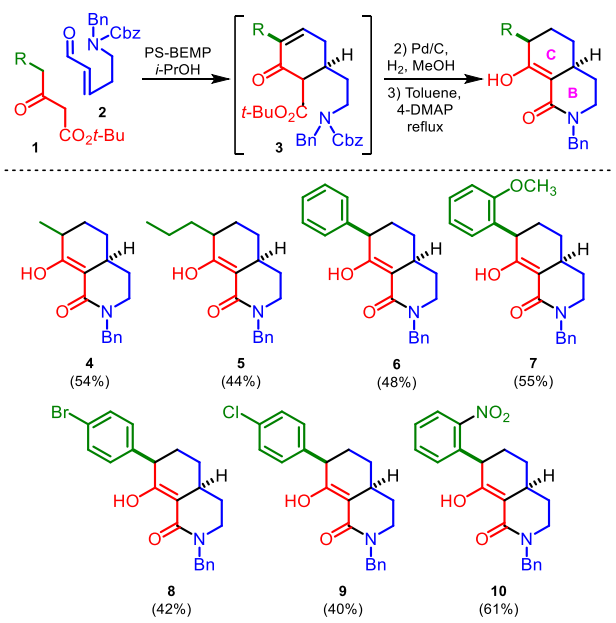


Madangamine A

Results and Discussion

The synthesis of the hydroisoquinoline rings was carried out by a tandem reaction, starting from a β-ketoester **1** and a bis-protected enal **2**. A Robinson-type reaction led to the formation of a cyclohexenone 3, followed by hydrogenation, which was essential to eliminate the double bond, prevent the aza-Michael reaction, and allow the removal of the Cbz group. This strategy yielded a free benzylamine, which subsequently cyclized over the ester to form the hydroisoquinoline compound. The synthesized bicycles were characterized by ^{1}H and ^{13}C NMR spectroscopy.

Figure 2: Synthesis of Hidroisoquinoline



In addition, a molecular docking study will be carried out to understand how the hydroisoquinoline nucleus binds, which will guide future structural modifications. These modifications aim to optimize the structure-activity relationship (SAR) with a view to enhancing its anticancer activity.

Conclusions

As part of this work, the proposed azabicyclic nuclei, which are one of the main objectives of my doctoral thesis, were successfully synthesized. Molecular modeling studies are currently being conducted to determine the key structural characteristics associated with biological activity, with the expectation of obtaining promising results, in line with those reported by Leese et al.[3]

Acknowledgements

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Synthesis and evaluation of hydrazides and quinazolinones: in search of new hits against *Leishmania infantum*

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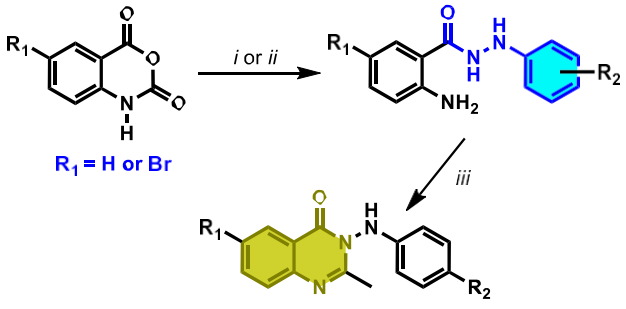
Key words: Hydrazides, Quinazolinones, Leishmanicidal, Leishmania infantum

Introduction

Currently, the treatment of leishmaniasis is based on a small arsenal of drugs, all presenting disadvantages in terms of toxicity, efficacy, price or treatment regime. To overcome these problems, efforts have been made in the search for new leishmanicidal drugs more effective and cost-effective. Moreover, the disease in question is considered neglected, since it affects mainly underdeveloped countries, therefore there is no incentive interest for the development of effective treatments by pharmaceutical industries, what has encouraged the researchers in this purpose. One of these researches, published by our group, demonstrated the activity of aromatic hydrazide against *Leishmania amazonensis*, opening space for new researches exploring these prototypes. [1,2] The objectives of this work are to synthesize a series of hydrazides and quinazolinones, including those previously evaluated in *L. amazonensis*, and investigate their leishmanicidal profile in the promastigota form of *L. infantum*.

Results and Discussion

Twelve compounds were prepared from the respective isatoic anhydrides and hydrazines commercially available, by means of a single step and without the use of catalyst (Scheme 1). Yields ranged from 45% to 95%.



i) EtOH, $PhNHNH_2$, Reflux, 2h ii) EtOH, $PhNHNH_2$.HCl, NaOH, Reflux, 2-9h; iii) AcCl, MeCN, r.t., 3h

Scheme 1

Two brominated hydrazides and the unsubstituted one were transformed into quinazolinones, using acetyl chloride as acylation agent, in MeCN as solvent, at room temperature. The yields of the products varied between 51 and 85%. These compounds were tested *in vitro* on *L. infantum* promastigotes by incubating different concentrations of the derivatives (0-128μM) for 72h with $2x10^6$ promastigotes/mL. Miltefosin was used as a reference drug. After incubation, the viability of the parasite was evaluated by fluorescent test with resazurin. The monosubstituted or dissubstituted bromine derivatives presented higher potency with IC_{50} below 10μM, similar to the results presented for *L. amazonensis* [2]. Interestingly the quinazolinones did not present activity until the maximum concentration tested, indicating that the amino group is probably essential for the leishmanicidal activity.

Conclusion

Among the prepared compounds the brominated hydrazides were the most active, with IC_{50} of 3.8μM and 3.8μM. On the other hand the quinazolinones were inactive. The most promising derivatives will be evaluated on the intracellular amastigote form. Other aromatic hydrazides are being prepared and will be evaluated, after characterization and evaluation of purity.

Acknowledgements

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Identification of chlorogenic acids from *Moringa oleifera* leaves as modulators of Prion Protein aggregation using AS-MS

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Key words: prion protein, affinity selection-mass spectrometry, natural products, aggregation assays, Moringa oleifera.

Introduction

Prion diseases are fatal neurodegenerative disorders caused by the misfolding and aggregation of the cellular prion protein (PrP^C) into its pathogenic form (PrP^{Sc}), leading to progressive neuronal damage. As no effective treatments currently exist, the search for therapeutic molecules is crucial. Potential strategies include preventing misfolding, inhibiting aggregation, disrupting existing fibrils, or enhancing the clearance of misfolded proteins[1]. In this context, small molecules capable of modulating PrP^C misfolding or promoting PrP^{Sc} disaggregation are promising therapeutic candidates for prion and other amyloidogenic diseases. Natural products have emerged as a rich source of anti-aggregation scaffolds, offering new opportunities for drug discovery[2]. In this study, we investigated *Moringa oleifera* extracts as a source of bioactive compounds with potential to modulate prion protein aggregation using affinity selection-mass spectrometry (AS-MS)[3].

Results and Discussion

We initially evaluated the inhibitory activity of six different extracts from the leaves and flowers of *M. oleifera* on prion protein aggregation via real time quaking-induced conversion (RT-QuIC). These extracts were added to the PrP^C substrate before adding PrP^{Sc} fibrils. The hydroethanolic extract from *Moringa oleifera* leaves prepared by infusion (MoLV-EHU) completely inhibited the conversion of PrP^C to PrP^{Sc}, and was selected for further investigation.
MoLV-EHU extract was analytical-scale microfractionated using an HPLC-UV system, and the PrP aggregation inhibition for each microfraction was assessed, resulting in a biochromatogram. The data revealed the highest PrP aggregation values at 18.5 and 22 min. Using AS-MS assay, several ligands were isolated due to their affinity to PrP^C. However, in the AS-MS assay, ligands are isolated based on their affinity for the protein, regardless of their functional effect. Therefore, the results from the biochromatogram of this same extract were used to guide the analysis of ligands with the desired functional effect. This approach identified chlorogenic and neochlorogenic acids as potent inhibitors of prion aggregation. These compounds exhibited significant antiprion activity, with IC_{50} values of 64.41 ± 12.12 µM and 35.34 ± 7.09 µM, respectively. In addition to inhibiting the conversion of PrP^C to PrP^{Sc}, both compounds could disaggregate pre-formed PrP^{Sc} fibrils *in vitro*.

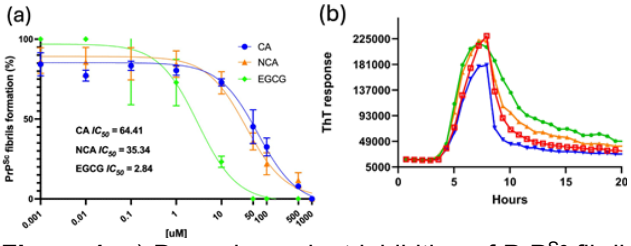


Figure 1: a) Dose-dependent inhibition of PrP^{Sc} fibril formation by chlorogenic acid, neochlorogenic acid, and epigallocatechin gallate. b) After PrP^{Sc} aggregation in the RT-QuIC assay (8 h), CA and NCA were added at 50 µM and 500 µM. ThT fluorescence was monitored for an additional 12 hours, showing an exponential decrease over time, indicating disaggregation of PrP^{Sc}.

AS-MS proved to be a valuable tool for isolating modulators of PrP aggregation directly from crude natural product extracts, avoiding the need for expensive and time-consuming fractionation and purification processes.

Conclusions

Identifying chlorogenic and neochlorogenic acids highlights the therapeutic potential of natural products in combating prion diseases and other amyloidogenic disorders. Our findings suggest that these bioactive compounds could serve as promising lead compounds for developing novel treatments for prion diseases. Further *in vivo* studies and pharmacokinetic optimization are warranted to explore their full therapeutic potential.

Acknowledgements

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Targeting the Ubiquitination Pathway: Development of a Fluorescence Anisotropy Assay for Identifying UBE2A Modulators

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Key words: High-throughput assay, fluorescence polarization, UBE2A, ubiquitination.

Introduction

Ubiquitination is a conserved post-translational modification critical for cellular homeostasis, regulating protein turnover, cell cycle, and signaling pathways[1] (Fig. 1). Selective modulators of ubiquitination enzymes, such as PROTACs and compounds identified by high-throughput screening (HTS), are of growing therapeutic interest[2,3]. UBE2A, an enzyme central to ubiquitination, is implicated in X-linked intellectual disability syndromes and cancer, turning it a valuable drug target[4,5]. To support the identification of chemical modulators, we developed a robust and reproducible fluorescence polarization (FP)-based biochemical assay to monitor UBE2A enzymatic activity *in vitro*, tailored for high-throughput screening applications.

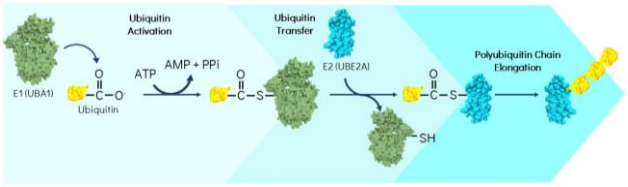


Figure 1. Graphic representation of the initial steps in the ubiquitin conjugation pathway.

Results and Discussion

To reconstitute the ubiquitination cascade, human recombinant proteins UBE2A (E2), UBA1 (E1), and ubiquitin (Ub) were expressed in *E. coli Rosetta 2* and purified via distinct chromatographic protocols. Ubiquitin was labeled with fluorescein isothiocyanate, generating the probe Flc-Ub for reaction monitoring by FP (Fig. 2A). Assays were miniaturized to 384-well plates (25 µL/well). Reactions were initiated with ATP and monitored using a ClarioStar® plate reader. FP changes were used to assess enzymatic complex formation and reaction kinetics. UBA1-Flc-Ub interaction was confirmed as concentration-dependent. Unlabeled ubiquitin stabilized the Flc-Ub/UBA1 complex. UBE2A titration induced a concentration-dependent increase in fluorescence anisotropy, with a specific range yielding stable curves and slower kinetics, favorable for modulator detection. Assay compatibility with DMSO (0–5%) was confirmed, with all concentrations maintaining consistent kinetic profiles and acceptable Z' values, typically above 0.5, indicating robust assay quality and reliability. (Fig. 2B). The assay was automated using the Multidrop® Combi dispenser system for the addition of the solutions. For system validation, a pilot screening was conducted using a library of 1,280 synthetic compounds possessing physicochemical properties consistent with blood–brain barrier permeability. Data collection was conducted at multiple time points after reaction initiation. At 100 minutes (Z' value =0.63 ± 0.07), a suitable timepoint for modulator identification, 4 potential activators and 40 potential inhibitors were identified based on the threshold of ±3 SD from the mean of positive controls. These hits will be further validated using orthogonal assays, including X-ray crystallography (Fig. 2C).

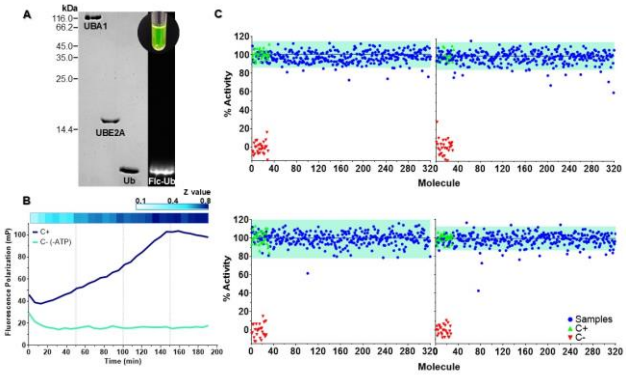


Figure 2. Recombinant proteins, reaction kinetics, and HTS-assay validation. (A) SDS-PAGE analysis of the recombinant proteins. (B) Kinetics of the polyubiquitination reaction catalyzed by UBE2A and analysis of Z'-value distribution. (C) Preliminary HTS assay performed over a 100-minute time course.

Conclusions

The developed HTS assay demonstrates promising sensitivity, specificity, and robustness for the identification of UBE2A modulators, which will be subsequently investigated in orthogonal assays.

Acknowledgements

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PDBs: 1UBQ, 7ZH9; 6CYO.

Fishing for Emerging Pharmacophores Against Anaplastic Thyroid Carcinoma: Synthesis, Characterization and Biological Evaluation of B-rich [1,2,4,5]Tetrazine Derivatives

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Key words: Anaplastic Thyroid Carcinoma, metallacarborane, carborane, tetrazine, ARNsec.

Introduction

Anaplastic thyroid carcinoma (ATC) is a highly undifferentiated malignancy that originates from follicular thyroid cells. Although it represents a small fraction, approximately 2%, of all thyroid cancer cases, ATC stands out as the most aggressive subtype. Most patients are diagnosed with significant locoregional invasion, and in 15% to 50% of cases, distant metastases are already present at the time of diagnosis. Consequently, all ATC cases are classified as stage IV according to the American Joint Committee on Cancer (AJCC) staging system. The prognosis remains dismal, with a median overall survival of only 5 months and a 1-year survival rate of just 20%.[1,2] n this context, we investigated the hybridization of two underexplored yet potent pharmacophores, boron-rich metallacarboranes and tetrazines, to generate novel entities with targeted antitumor activity.

Results and Discussion

Six new chemical entities combining a [1,2,4,5]tetrazine core and boron-rich carborane/metallacarborane moieties were synthesized (Figure 1) and fully characterized. Cytotoxicity assays were conducted on ATC (8505C) and normal human thyroid follicular cell lines (Nthy-ori 3-1). IC_{50} values and selectivity indexes (SI) revealed that two compounds showed unexpected selectivity towards the tumor line. One of these was found to be mutagenic under metabolic activation (Ames test, TA97 with S9), suggesting bioactivation by liver enzymes. Conversely, the second compound showed no mutagenicity under any condition and was selected for further evaluation. Intracellular distribution studies by confocal Raman microscopy revealed focal accumulation of the lead compound in tumor cells after 4h of incubation, suggesting specific subcellular interactions. RNA-seq analysis of 8505C cells treated with the lead compound revealed transcriptional signatures indicative of endoplasmic reticulum (ER) stress and unfolded protein response (UPR), likely culminating in intrinsic programmed cell death (Figure 2).

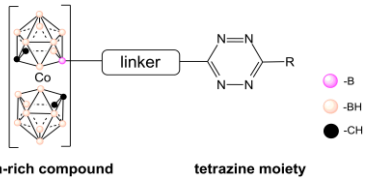


Figure 1: General structure of the synthesized [1,2,4,5]tetrazine–metallacarborane hybrids.

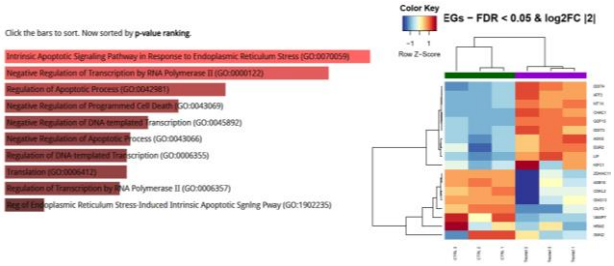


Figure 2: GO enrichment of upregulated DEGs highlights unfolded protein response and intrinsic apoptosis signaling (left). Heatmap of top differentially expressed genes (right) confirms treatment-specific expression patterns.

Conclusions

We identified a novel hybrid [1,2,4,5]tetrazine-metallacarborane derivative with selective cytotoxicity against ATC cells, non-mutagenic profile, and intracellular localization suggestive of target engagement. Transcriptomic data further support ER stress-mediated apoptosis as a possible mechanism of action, highlighting this compound as a lead candidate.

Acknowledgements

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Rational Design of the OGT Inhibitors as Anticancer Candidates

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Key words: cancer, leukemia, Virtual screening, docking studies, SBDD, LBDD, model validation, OGT, interactions.

Introduction

O-GlcNAc transferase (OGT) adds O-linked N-acetylglucosamine (O-GlcNAc) to proteins, regulating vital cellular functions. Altered O-GlcNAc levels are associated with aggressive tumor behavior and poor prognosis, especially in leukemia. It suggests OGT as a potential therapeutic target for anticancer agents. At the moment only one OGT inhibitor is in clinical trials, OSMI-4. The search for safe and non-toxic OGT inhibitors with favorable kinetics is essential to improve leukemia treatment outcomes.

Results and Discussion

Computational analysis of the OGT structure (4N3B) was performed in CavityPlus and identified cavity 1 as the primary binding site with a high druggability score (2966.00). Located between the TPR and catalytic domains, this cavity is supported by probe clustering and key interactions with HIS 498, LYS 842, and HIS 920, residues critical for UDP-GlcNAc binding.



Figure 1. The active site of 4N3B on Cavity Plus.

A five-feature pharmacophore model was developed based on the alignment of seventeen known OGT inhibitors interacting within cavity 1, using the HipHop algorithm in Discovery Studio, with OSMI-4 as the reference compound (Fig. 2). For validation, the inhibitors were divided into a training set (70%) and a testing set (30%), with 50 decoys generated per active compound. Validation was conducted using the Pharmit platform across five scenarios: once using the full dataset, and twice each with the 70% training and 30% testing splits. The model demonstrated strong performance (AUC: 0.9665, $EF_1\%$: 17.543), confirming its suitability for ligand-based virtual screening. A dataset of compounds from the purchasable ZINC database and cannabinoids was then screened and filtered based on pharmacophore features, binding scores, and mRMSD values.

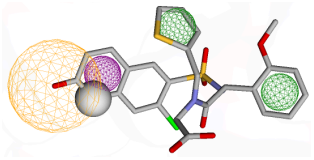


Figure 2. Figure showing the 5 pharmacophore features constructed on the reference query OSMI-4.

Twelve compounds (LBVS ten top-ranked from the ZINC database and two cannabinoids) were selected for the molecular docking performed using the DockThor platform. Based on binding interactions and key residues involved, 4 hits (3 from ZINC, 1 cannabinoid) were identified for further study.

Selected Compounds	Docking score (Kcal/mol)
2-methyl-2-(4-methylpent-3-enyl)-7-pentyl-3,4-dihydrochromen-5-ol	-10,359
ZINC12229686	-9,327
ZINC40146673	-9,505
ZINC30965045	-9,625

Table 1. Table of the 4 selected compounds with their docking scores.

Conclusions

In conclusion, this study identified cavity 1 as OGT's key druggable site and developed a validated five-feature pharmacophore model based on known inhibitors. Virtual screening and molecular docking revealed four ZINC compounds and one cannabinoid with strong interactions at the active site, offering promising hits for future OGT inhibitor development.

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Investigation of the *in vitro* Genotoxicity Structure Relationship of the Leishmanicidal Lead LASSBio-1483 and their structure analogues

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Key words: Genotoxicity; nitroaromatic compounds; leishmanicidal lead; metabolism-dependent genotoxicity; cyclic voltammetry

Introduction

LASSBio-1483 has been identified as a promising lead compound for anti-leishmanial therapy, demonstrating both cytotoxic activity and efficacy in vitro and in vivo[1]. This biological activity was initially attributed to the presence of a nitroaromatic substructure. However, data from the literature suggest that the bioreduction of the nitro group plays a critical role in the toxicological mechanisms of action of nitroaromatic compounds[2]. In this context, the present study aimed to investigate the relationship between the chemical structure and the potential in vitro genotoxicity of LASSBio-1483 and its structural analogues.

Results and Discussion

Nine LASSBio-1483 analogues were designed and synthesized and had their aqueous solubility determined by kinetic solubility assay. Then, comparative micronucleus assays were performed. The analysis of micronuclei in interphase cells, both with and without metabolic activation, was then used to assess genotoxicity using the OECD 487 procedure[3]. Lastly, cyclic voltammetry was used to clarify the chemical reactivity to oxidation-reduction systems, as this is a metric associated with metabolism-dependent genotoxicity. Therefore, the leishmanicidal activity was reassessed to dissociate the potential toxicophoric effect from the desired structure-based activity.

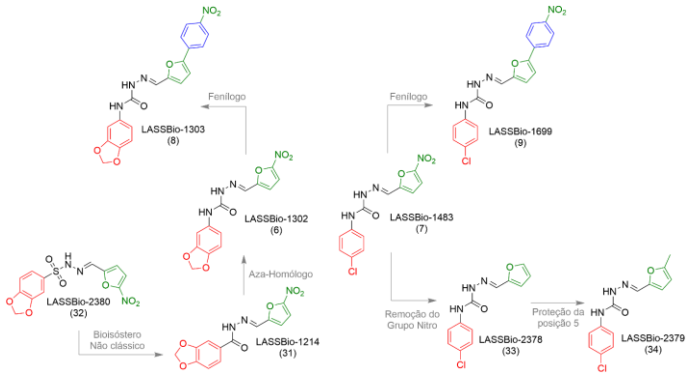


Figure 1: Substances avaliated in this study

All designed compounds were successfully synthesized in good yields. Despite minor differences, all compounds exhibited low aqueous solubility. In the in vitro genotoxicity assay, phenyl-homologated analogues showed reduced genotoxicity, presenting lower frequencies of micronucleated cells across all three conditions of the OECD 487 protocol. When comparing the furyl-methyl analogue with LASSBio-1483, the results indicated that genotoxicity is primarily dependent on the presence and positioning of the nitroaromatic moiety. No significant differences were observed among the compounds with different linkers in this assay. Cyclic voltammetry analysis revealed that the cathodic peak potentials of the phenyl-homologated analogues were shifted to more negative values compared to their lower phenyl counterparts, indicating lower redox susceptibility. However, in the ex vivo activity assay, the phenyl-homologated compounds, although safer, were less potent and failed to effectively kill the amastigote forms of *Leishmania amazonensis*.

Conclusions

The results suggest that phenyl homologation exerts a protective effect against in vitro genotoxicity. The reduction in genotoxic potential is directly related to the chemical reactivity of the nitro group. When attached directly to a furan ring, the nitro group exhibits higher reactivity compared to its attachment to an aryl moiety. These findings highlight the critical influence of structural context on nitroaromatic compound safety. The observed chemical reactivity is supported by a slight shift of the cathodic peak potential to more negative values in the cyclic voltammogram of the compound bearing the nitro group on the aryl ring, indicating decreased ease of reduction.

Acknowledgements

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Schistosomicidal and TRPM$_{PZQ}$-modulating activities of novel praziquantel analogues for schistosomiasis drug discovery

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Key words: Schistosomiasis, drug discovery, praziquantel analogues, phenotypic screening,TRPM$_{PZQ}$, TRPM$_{MCLZ}$.

Introduction

The discovery of a transient receptor potential ion channel of the melastatin subfamily activated by praziquantel (TRPM$_{PZQ}$) has opened new opportunities for target-based schistosomiasis drug discovery[1]. In this study, we evaluated the schistosomicidal potential of a novel series of 1*H*-1,2,3-triazole derivatives of praziquantel (PZQ) and their synthetic intermediates on larval (schistosomula), immature (juveniles) and adult forms of *Schistosoma mansoni* and tested their ability to modulate wild-type and mutant versions of schistosome TRPM$_{PZQ}$ (Sm_TRPM$_{PZQ}$), as well as other related ion channels.

Results and Discussion

In total, six out of eleven PZQ analogues showed a significant effect at 50 μM (72h) on *S. mansoni* schistosomula motility and/or phenotype: three triazoles (**5b**, **5c**, **5e**) and three synthetic intermediates (**2**,**3**,**4**). These compounds were selected for further analysis.

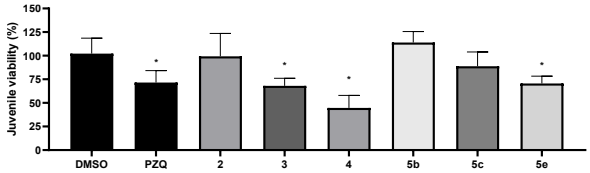


Figure 1 – Juvenile schistosome viability after 48h incubation with PZQ analogues at 100 μM. * p< 0.05

Table 1. Adult worm motility inibition activity and selectivity of PZQ analogues after 48h incubation.

Compound	Worms motility inhibition (EC_{50}; μM)		HepG2 cytotoxicity (CC_{50}; μM)	Selectivity Index (CC_{50}/EC_{50})	
	Male	Female		Male	Female
PZQ	0.25	ND	> 300	> 1200	ND
5c	ND	ND	88.1	ND	ND
5e	1.9	3.9	294	154.7	75.4
2	15.9	29.1	367.8	23.1	12.7
3	4.5	9.9	> 300	>66.7	>30.3
4	1.6	9.4	156.4	97.7	16.6
5b	49.2	95	28.5	0.6	0.3

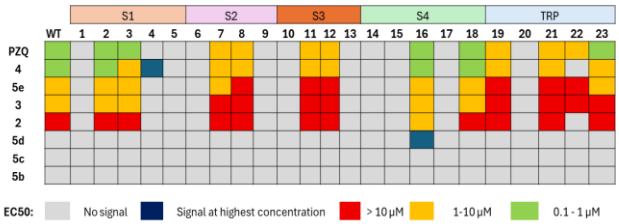


Figure 2: Activation profiles of PZQ analogues on wild-type (**WT**) and mutant versions (**1-23**) of Sm_TRPM$_{PZQ}$. Each single-point mutation targets a specific residue of the PZQ predicted binding site.

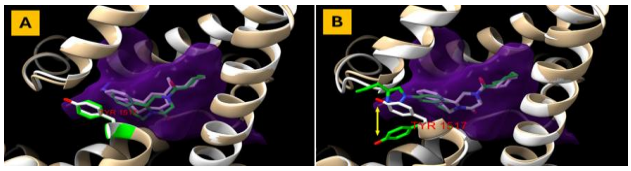


Figure 3: Binding poses of PZQ and **4** (A) or **5e** (B) in wild-type Sm_TRPM$_{PZQ}$. PZQ is represented in white and the analogues in green thick sticks. Yellow arrow indicates a positional shift of Tyr 1517.

Apart from **5b** and **5c**, all the other compounds activated TRPM$_{PZQ}$ from *Echinococcus granulosus* (EC_{50}: 0.21 – 30.1 μM), including **5d,** which was not effective on Sm_TRPM$_{PZQ}$. However, none of them activated TRPM$_{PZQ}$ from *Fasciola hepatica* or schistosome TRPM modulated bv meclonazepam (TRPM$_{MCLZ}$) under the concentrations tested.

Conclusions

Compounds **3**,**4** and **5e** were the most promising PZQ analogues, demonstrating efficacy against multiple life stages of schistosomes, along with good selectivity and TRPM$_{PZQ}$-modulating activity.

Acknowledgements

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Development of a novel high-content microscopy assay to evaluate compound toxicity in wild-type zebrafish embryos

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Key words: Zebrafish; zebrafish embryos; toxicity assay; high content analysis; artificial intelligence (AI)

Introduction

The teleost zebrafish (*Danio rerio*) is a well-established vertebrate model in toxicological studies[1]. Traditionally, the effects of chemical compounds on zebrafish are assessed using microscopy-based techniques[2], such as the OECD Test Guideline 236 (OCD236) for acute toxicity in fish embryos[3]. However, these methods are subjective, semi-quantitative and low-throughput. Here, we report the development of a novel automated high-content microscopy assay to evaluate compound-induced effects in wild-type zebrafish embryos.

Results and Discussion

The images of zebrafish embryos treated either with E3 medium or test compounds (DMSO, 3,4-dichloroaniline or $ZnSO_4$) were employed in the development of two key steps of the assay: image segmentation (**Figure 1**) and phenotype classification (**Figure 2**).

A

Dataset	Dice (Mean ± SD)		
	Whole body	Yolk sac	Pericardial area
Train	0.96 ± 0.009	0.97 ± 0.008	0.90 ± 0.09
Test	0.95 ± 0.003	0.80 ± 0.02	0.25 ± 0.08

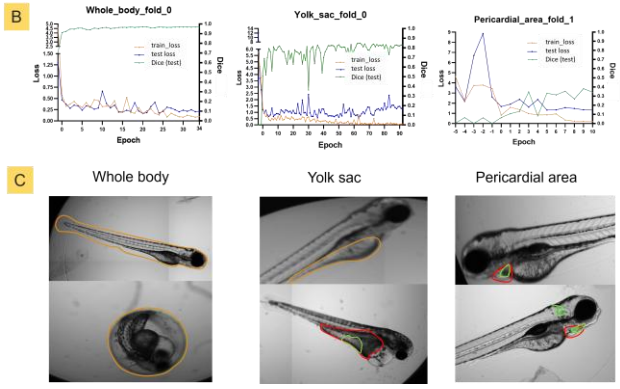


Figure 1: Training of convolutional neural network (CNN) models for segmentation of the whole body, yolk sac, and pericardial area of zebrafish embryos. (A) Model performance evaluated using nested cross-validation (nested CV). (B) Loss curves for the model version with the highest Dice score. (C) Comparison of semi-automated (red, ground truth) and predicted (green) segmentation masks, with orange outlines indicating regions of perfect overlap.

In order to automatically detect zebrafish embryos with morphological changes, initially each individual was visually classified as “normal”, “not hatched” or “altered”. These phenotypes were later used as target labels for an XGBoost model, while hundreds of morphological features, quantified by Cellprofiler software, served as input variables (**Figure 2**).

A

Dataset	MCC (Mean ± SD)	AUC (Mean ± SD)	F1 (Mean ± SD)	Precision (Mean ± SD)	Recall (Mean ± SD)	Balanced accuracy (Mean ± SD)
Train	0.91 ± 0.02	0.98 ± 0.006	0.93 ± 0.02	0.95 ± 0.01	0.92 ± 0.02	0.92 ± 0.02
Test	0.84 ± 0.05	0.96 ± 0.01	0.89 ± 0.03	0.91 ± 0.03	0.88± 0.03	0.88 ± 0.03

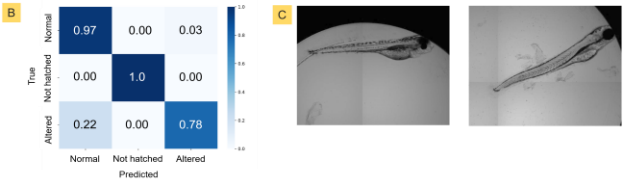


Figure 2: Training of an XGBoost model to classify zebrafish embryos phenotypes based on hundreds of morphological features quantified by Cellprofiler. (A) Model performance evaluated using nested CV. (B) Confusion matrix of the model version with the highest Matthews correlation coefficient (MCC). (C) Representative images of embryos with “altered” phenotype misclassified as “normal”.

Conclusions

All experiments conducted so far failed to meet one or more of the minimum assay validation criteria recommended for OECD236 due to issues with negative or positive control embryos. However, the whole-body segmentation AI model is ready to be incorporated into our assay pipeline, while the models for yolk sac and pericardial area still require improvement. The XGBoost classifier was effective in identifying “normal” and “not hatched” phenotypes but struggled to detect “altered” individuals exhibiting subtle morphological changes. Further experiments are needed to continue model training and to validate the assay.

Acknowledgements

Fiocruz, CNPq, FAPERJ.

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Investigation of the Synthesis of Hydrazide–Triazoloquinone Hybrids as Potential Antileishmanial Agents

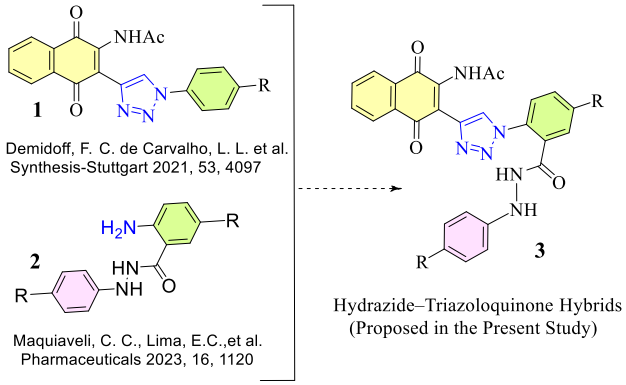
Natália M.A.M. Barros (IC),[1] Igor F. L. Ferraz (IC),[1] Edezio F. Cunha-Junior(PQ),[1] Leandro L. de Carvalho (PQ),*[1] Evanoel C. de Lima (PQ)[1]**

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***evanoel.crizanto@gmail.com / Key words: Naphthoquinone, Triazole, Click Chemistry, Antiparasitic*

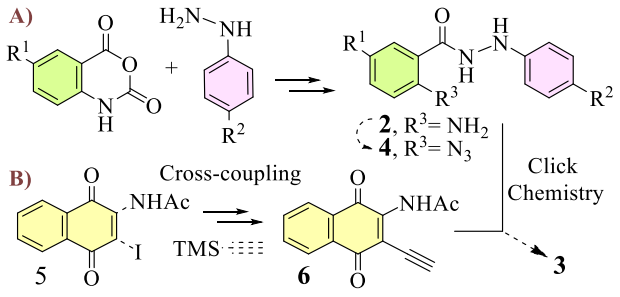
Introduction

Leishmaniasis is an infectious disease caused by protozoa of the *Leishmania* genus, transmitted by phlebotomine sandflies. Its clinical forms range from the more common cutaneous type to the more severe and potentially fatal visceral type, depending on the parasite species and the host's immune status. Current therapeutic options present significant limitations, such as high toxicity and elevated costs, which highlight the urgent need for new pharmacological approaches. In this context, our research group has been investigating hybrid compounds with leishmanicidal potential, including triazole–naphthoquinone hybrids[1] and aromatic hydrazides[2] derived from isatoic anhydride. Herein, the present study proposes the rational synthesis of hydrazide–triazoloquinone hybrids, aiming to evaluate their activity against the promastigote forms of *L. amazonensis* and *L. infantum*.



Results and Discussion

Route A: First, the isatoic anhydride was reacted with phenylhydrazine under reflux in ethanol, providing unsubstituted hydrazide **2** (80% yield). Next, three other hydrazides were prepared, namely with methyl (88%), bromine (61%), and fluoride (45%) substituent in the *para* position.[2]



To obtain azide **4** (unsubstituted), hydrazide **2** is being subjected to several azidation protocols via a diazotization reaction followed by SNAr with NaN_3. However, satisfactory yields have not yet been achieved (reactions monitored by TLC).

Route B: starting from halo-naphthoquinone **5**, the alkynyl-naphthoquinone **6** was obtained through a palladium-free cross-coupling reaction followed by desilylation of the alkyne moiety (74% yield over two steps).[1] With compounds **4** and **6** in hand, copper(I)-mediated 1,3-dipolar cycloaddition reactions will be investigated in order to obtain the desired triazole–naphthoquinone hybrids type **3**.

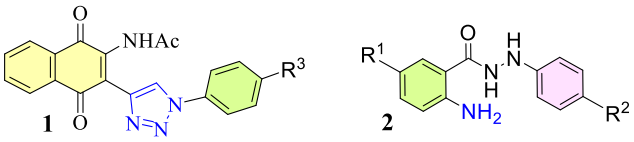


Table 1. Evaluation of leishmanicidal activity

Compound	*L. amazonensis* IC_{50} (μM)	*L. infantum* IC_{50} (μM)
1a, R^3= OMe	1,09	---
1b, R^3= F	10,0	---
2a, $R^{1,2}$ = H	10,0	---
2b, R^1= H, R^2= Br	5,3	4,7
2b, R^1= Br, R^2= H	4,2	3,8
2c, $R^{1,2}$ = Br	1,3	3,8

According to Table 1, both triazole-naphthoquinones (type **1**) and hydrazides (type **2**) exhibited noteworthy leishmanicidal activity. In the former case, the observed activity is likely due to oxidative stress induced by the naphthoquinone core. In the latter case, investigations are currently underway to propose a molecular target.

Conclusions

Based on the leishmanicidal activities exhibited by the template compounds,[1,2] the newly designed hybrids are expected to display an enhanced structure–activity relationship (SAR), due to the rational integration of pharmacophoric fragments.[3]

Acknowledgements

Agencies: FAPERJ & CNPq. Analytical centers: LAMAR/IPPN-UFRJ & Farmanguinhos-Fiocruz

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Computer-aided structure-based design of second generation inhibitors of SARS-CoV-2 Main Protease

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Keywords: *Covid, Inhibitor, Mpro, Drug Discovery.*

Introduction

The COVID-19 pandemic highlighted how quickly new strains of SARS-CoV-2 can appear underscoring the need for more antiviral treatments. Paxlovid, which contains Nirmatrelvir, targets the main viral protease (Mpro) essential for replication. However, emerging Mpro-resistant variants have reduced its effectiveness.

To address this, our team developed a computational approach combining generative modeling, machine learning, a classification model, and docking to identify new Mpro inhibitors[1]. Five compounds proved promising, with crystallographic data obtained for two (818 and 183) (Figure 1).

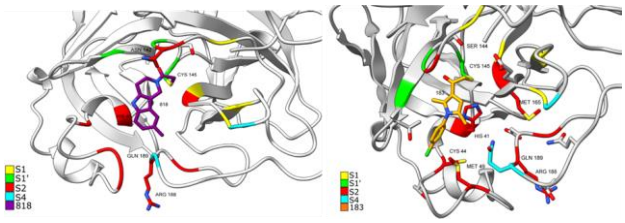


Figure 1: Crystallographic structures for the complexes of compounds 818 and 183 with Mpro (PDB:9OT4).

Given the rise of Nirmatrelvir-resistant variants, we are using structural insights to design new analogs. These will be tested against Mpro enzymes from resistant SARS-CoV-2 strains and other coronaviruses, aiming to develop effective broad-spectrum inhibitors and deepen our understanding of Mpro-targeting mechanisms.

Results and Discussion

To design new analogues, we used structural data alongside *in situ* design tools like AutoGrow4[2] and FastGrow[3] to optimize interactions with Mpro subsites. Promising candidates will undergo docking calculations informed by molecular dynamics simulations. The top-performing analogues will be evaluated for synthetic feasibility, and those viable for synthesis will proceed to in vitro testing for inhibitory activity.

With the help of the Fastgrow and Autogrow4 programs, more than 5,000 analogs were generated for compounds 818 and 183. Docking was performed using the GOLD program for these analogs, and the best results were obtained for each compound (Figure 2).

Docking against the inhibitor-resistant L50F mutant revealed 20 points drop in score and altered binding and orientation compared to 818, highlighting the need for improved inhibitors or further optimization.

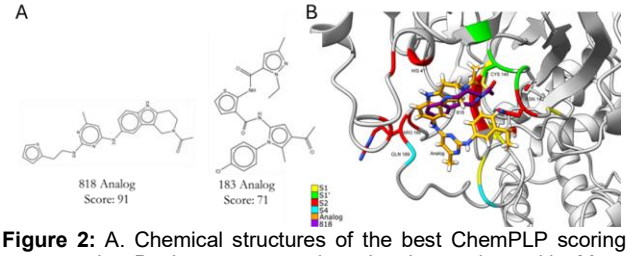


Figure 2: A. Chemical structures of the best ChemPLP scoring compounds. B. Image comparing the interaction with Mpro between the best analog of compound 818 and the original compound.

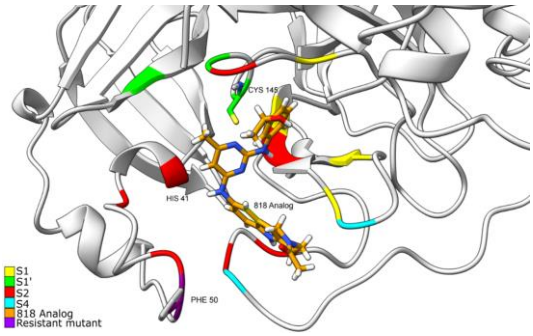


Figure 3: Model of the Mpro L50F structure (PDB:8u4y) interacting with the highest-scoring analog.

We performed rescoring with the results from the growth programs and found they did not match, so it is important to establish a new method to validate the generated analogs.

Conclusions

In conclusion, we designed analogues that preserve key binding interactions while extending into adjacent Mpro subsites, potentially enhancing affinity and specificity. These modifications offer a strong basis for further optimization. To improve prediction accuracy, we will incorporate molecular dynamics, ensemble docking, and a more robust validation framework integrating experimental data and machine learning.

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Design and Development of Carbamates as Potentially pan-HDAC Inhibitors Against Cancer

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Key words: HDAC inhibition, Carbamate Derivatives, Cancer.

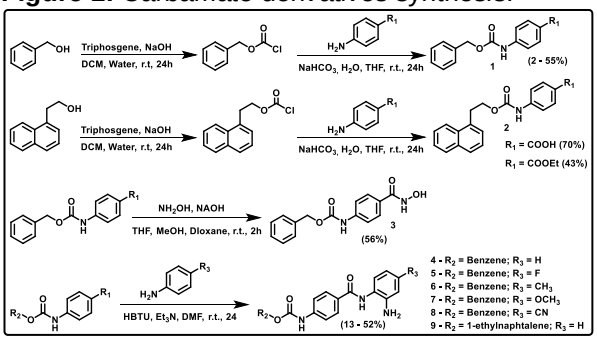
Introduction

Histone deacetylases (HDACs) and its many isoforms, play a crucial role in cancer pathology as they module gene expression through epigenetic mechanisms.[1] The HDACs from classes I, II and IV have a zinc ion as a cofactor, representing promising targets against many cancer types such as glioblastoma, leukemia, colorectal, triple-negative breast and prostate cancer.[2] The present work aimed the design, synthesis and biological evaluation as pan-HDAC inhibitors and anticancer agents of carbamate derivatives constituted of a hydrophobic linker that connects the CAP group, responsible for the enzyme recognition and selectivity, and the zinc-binding group (ZBG), which provides target complementarity.[2]

Results and Discussion

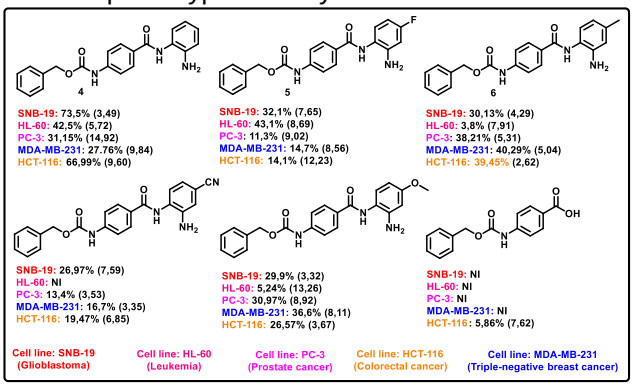
The synthesis of the carbamate derivatives was designed to modify both CAP scaffold and ZBG, aiming to generate CAPs with varying sizes and distinct zinc-binding groups. Initially, chloroformate intermediates were synthesized and subsequently reacted with anilines containing carboxylic acid or ester groups. After that, using the carbamate derivatives presenting both aforementioned chemical groups, two types of reactions were done: one to yield benzamides featuring electron-donating or electron-withdrawing substituents, and another to produce hydroxamic acids as alternative ZBGs. (**Figure 1**).

Figure 1: Carbamate derivatives synthesis.



Single-concentration (**20μM**) assays were conducted against various cancer cell lines expressing distinct HDAC isoforms to assess the biological activity of the carbamate derivatives (**Figure 2**).

Figure 2: Cell growth inhibition indexes obtained from the phenotypical assays.



The carbamates **4**, **5** and **6** emerged as the most promising against glioblastoma, prostate cancer and triple-negative breast cancer, respectively. However, the carbamate derivatives containing bulky CAPs and hydroxamic acid as ZBG remain to be tested.

Conclusions

Most of the synthesized compounds presented some anticancer activity against glioblastoma, leukemia, colorectal, triple-negative breast and prostate cancer cell lines, but the carbamates **4**, **5** and **6** exhibited the greatest tumor growth inhibition indexes against glioblastoma, leukemia and triple-negative breast cancer, respectively. Focusing on the optimization of those compounds is the main priority alongside the phenotypical screening of the remaining carbamates and enzymatic assays targeting HDACs from classes I, II and IV are ongoing to better characterize their therapeutic potential as pan-HDAC inhibitors against cancer.

Acknowledgements

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New potent LASSBio-1632 analogues and their physicochemical properties evaluation

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Key words Lead-optimization, Sulfonylhydrazone, N-methylation effect, Phosphodiesterase 4 inhibition, Molecular Modeling, DMPK.

Introduction

PDE4 is a hydrolase enzyme responsible for regulating intracellular concentration of cAMP. When inhibited, it increases cAMP levels, which is an interesting approach for the treatment of several inflammatory diseases[1,3]. Among PDE4 inhibitors, Nunes et al. have identified LASSBio-1632 as a sulfonylhydrazone derivative able to inhibit PDE4A with an IC_{50} = 500 nM, displaying great chemical stability under pH 2.0, poor aqueous solubility (9.5 × 10^{-3} g/mL) and short microsomal half-life ($t_{1/2}$ = 24 minutes).[2]

In this study, we describe the synthesis, anti-PDE4 activity and physicochemical properties evaluation of a series of new sulfonylhydrazones designed aiming to optimize LASSBio-1632 potency, solubility and permeability.

Results and Discussion

The design of LASSBio-1632 analogues was performed considering its binding mode within the active site of PDE4A (PDB ID: 3I8V, Figure 1). Compounds were synthetized by successive interconversions of functional groups, followed by *N*-alkylations. After structural characterization and purity checks, the compounds were tested to determine their anti-PDE4 potency (Table 1), kinetic solubility and permeability in PAMPA BBB and GIT (Table 2).

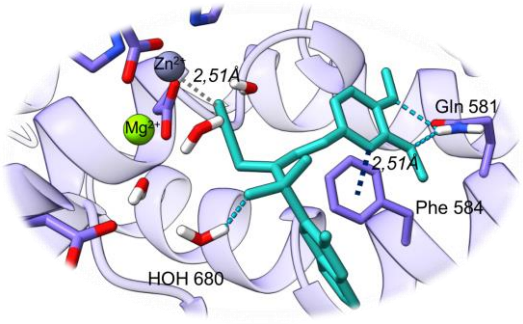


Figure 1: Binding mode of Compound 4 in the active site of PDE4A (PDB ID: 3I8V). Molecular docking was performed using GOLD software.

Table 1. Compounds potency Against PDE4 isoforms

	Compound IC_{50} (nM)			
Compounds	PDE4A	PDE4B	PDE4C	PDE4D
LASSBio-1632	124	38.61	1542	109.9
Compound 3	80.92	62.4	780.5	119.5
Compound 4	22.94	11.96	120.51	33.64
Compound 12	303.8	151.4	1775	256.4
Compound 13	95.49	76.26	1052	74.19

The analyses were made in Reaction Biology, with 10 µM compounds concentration, 1 µM cAMP and buffer 10 mM Tris, pH 7.5, 5 mM $MgCl_2$, 0.01% Brij 35, 1 mM DTT, and 1% DMSO.

Table 2. Experimental Physicochemical Properties

	Kinetic solubility [4]	PAMPA-GIT [5]	PAMPA-BBB [5]
Compounds	Aqueous sol. 4 hours (µM)	Class.	Class.
LASSBio-1632	< 1	N/D	CNS+
Compound 2	167,1	High	CNS+
Compound 4	< 1	N/D	CNS-
Compound 6	19,8	High	CNS-
Compound 8	< 1	High	CNS+
Compound 13	< 1	High	CNS+

Conclusions

In our studies, we were able to identify three new structural analogs equipotent to the LASSBio-1632 prototype and a more potent analog that lacks the ability to cross the blood-brain barrier. This promising data prompted further studies to better understand the DMPK and in vivo anti-inflammatory profile of compound 4.

Acknowledgements

FAPERJ, INCT-INOFAR, CAPES and CNPq

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Screening and inhibition characterization against the SARS-CoV-2 Main Protease (Mpro) of analogues of substituted 1-phenyl-2,5-dimethyl-1H-pyrrol-3-yl) fragments carrying an alpha-chloroketone warhead

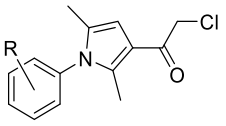
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Key words: COVID-19, SARS-CoV-2, Mpro inhibitors, Fragment-based drug discovery

Introduction

COVID-19, caused by the coronavirus SARS-CoV-2, is responsible for millions of deaths worldwide[1]. Paxlovid (nirmatrelvir plus ritonavir) is an antiviral drug used to treat mild to moderate COVID-19 by targeting the main protease (Mpro), a cysteine protease essential to virus replication cycle[2]. However, the emergence of Mpro mutant variants with reduced sensitivity to nirmatrelvir, along with the possibility of future mutations, highlights the need to discover novel Mpro inhibitors[3]. Previously, our group employed generative artificial intelligence (AI) models to predict molecular fragments with Mpro inhibitory activity. Some of these fragments showed *in vitro* Mpro inhibition (10 min incubation), including compound 183 (IC_{50} = 116.8 µM)[4], a 1-(4-chlorophenyl)-2,5-dimethyl-1H-pyrrol-3-yl) derivative carrying an alpha-chloroketone group (**Figure 1**). In addition to being active against the enzyme, this compound was also able to reduce the viral replication rate in a cellular model of SARS-CoV-2 infection (Calu-3), suggesting its pharmacological potential for the treatment of COVID-19.



R = H; 4-Cl; 4-NO_2; 4-OCH_3; 4-CH_3; 4-Br; 2-Cl-4-NO_2; 2-OCH_3,4-NO_2; 2-NO_2-4-CH_3; 2Cl-5-NO_2; 3NO_2-4-Cl;2,4-diCl; 3,4-diCl; 3,5-diCl; 2-CH_3-5-Cl

Figure 1: Chemical structure of compound 183 (R=4-Cl) and it's analogues.

In this study, we aim to screen analogues of compound 183 against SARS-CoV-2 Mpro and characterize their inhibition mechanism. As a first step, we have studied the reversibility of the wild-type (WT) Mpro inhibition by the parent compound 183.

Results and Discussion

In order to determine if compound 183 acts as a reversible or irreversible inhibitor of Mpro we performed a quick dilution assay (**Figure 2**). In this assay, the inhibitor (at a concentration 10x its IC_{50} value) is pre-incubated (20 min) with the enzyme at a concentration 100x higher than used in the usual assay condition. Then, the reaction is started by adding the substrate while resulting a 100-fold quick dilution.

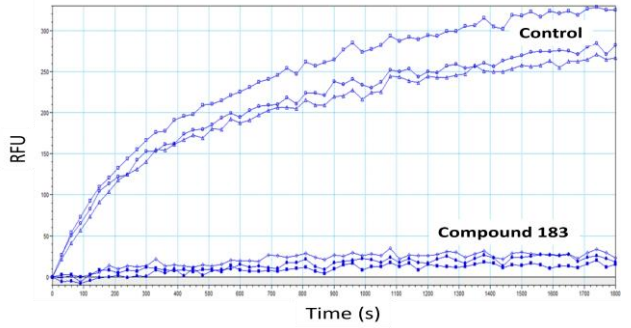


Figure 2: Progression reaction curves generated during the reversibility assay with Mpro incubated with either DMSO (control) or compound 183.

The progression reaction curves generated after incubation of Mpro with fragment 183 exhibited a low and steady slope, as shown in **Figure 2** —a behavior associated with irreversible inhibitors. This result is consistent with X-ray crystallography data generated by our group, which revealed that compound 183 binds covalently to Mpro through an interaction between the cysteine residue in the enzyme's catalytic site and its chloromethyl ketone moiety.

Conclusions

In this work, we determined that molecular fragment 183 is an irreversible inhibitor of Mpro. Experiments are currently underway to determine the kinetic constant (k_{inact}) that defines the interaction between the compound and the WT enzyme. The same analysis will also be conducted using Mpro mutant variants E166N, Q192V, and H172Y, which exhibit reduced sensitivity to nirmatrelvir, with IC_{50} values that are approximately 336, 25, and 31 times higher, respectively, compared to WT Mpro[3].

Acknowledgements

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Discovery of new antischistosomal piperine analog compounds acting as efflux pump inhibitors: *in silico*, *in vivo* and DMPK analysis.

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[2]*Laboratório de Síntese Orgânica e Prospecção Biológica - LaSOPB, IQ, UFRJ, Brazil;*
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Key words: Schistosomiasis, Triazole, Efflux pumps, Medicinal Chemistry.

Introduction

Schistosomiasis affects approximately 250 million people globally according to the World Health Organization. In Brazil, *Schistosoma mansoni* is the causing agent. The only available treatment is Praziquantel (PZQ), which is reasonably safe and effective. However, its extensive use in endemic regions raises concerns about the emergence of resistance. Efflux pumps, particularly ABC transporters, play a key role in resistance mechanisms. Studies have identified a connection between P-Glycoprotein (P-gp/ABCB1/SMDR2) and PZQ resistance in *S. mansoni*, highlighting the need for alternative therapies. In this work, we present a series of Piperine-derived compounds acting as schistosomicidal agents and evaluate their DMPK properties and activity against human P-gp.

Results and Discussion

Piperine and its analogues were docked using Gnina v 1.3 in a *S. mansoni* P-gp (SMDR2) model and their predicted binding energy is shown on Table 1. Effect on human P-gp activity at 50µM was evaluated using Promega Pgp-glo, Verapamil was used as stimulation control (competitive inhibition). Motility of adult worms of *S. mansoni* was also evaluated after 72h of compounds incubation at 50µM. Worms treated with Praziquantel were the positive control.

Table 1. *In silico*, *in vitro*, and *in vivo* profiles of tested compounds.

Compound	Docking score (kcal/mol)	P-gp (%)	Worm Motility reduction (%)
Verapamil	n.a.	173.3 ± 30.2*	n.a.
Piperine	-12.26	118.8 ± 12.8*	70.6 ± 14.1*
TPIPPh	-18.16	135.7 ± 4.5*	74.1 ± 10.2*
TMCloro	-23.06	108.8 ± 22.8	n.e.
TPCloro	-19.31	127.1 ± 30.7	n.e.
TMMetil	-22.89	101.9 ± 16.7	n.e.
TPMetil	-18.09	91.7 ± 17.4	n.e.
Praziquantel	n.a.	n.a.	97.03 ± 3.7*

n.a.: Not available; n.e.: Not effective; * Statistical difference from vehicle.

Of all molecules, only piperine and TPIPPh showed activity against adult worms. Therefore, we decided to evaluate the *in vitro* DMPK profile of all the molecules to understand if pharmacokinetics-related issues would explain why the docking results did not translate to schistosomicidal effect. We found that while TPIPPh had high solubility, TMMetil and Piperine had intermediate and the others showed low solubility. All molecules except Piperine are highly metabolized by microsomal enzymes which impact bioavailability. All molecules showed good permeability value, however, only soluble molecules like Piperine and TPIPPh would be available to penetrate the cells and inhibit the efflux pump.

Table 2. MDCK Permeability, Microsomal clearance and Solubility of test and control compounds.

Compounds	Solubility (µM)	Papp (nm/s)	Clearance (mL/min/g mouse liver)
Piperine	16.4 - 29.2	235.3 - 245.8	5.1 - 5.2
TPIPPh	87.6 - 210	244.2 - 241.2	24.2 - 28.7
TMMetil	33.9 - 36.9	210.6 - 224.2	18.8 - 20.5
TPCloro	2.4 - 2.9	164 - 227.7	20.7 - 22.3
TMCloro	4.6 - 4.8	199.9 - 236.2	29.4 - 34.6
Diethylstilbestrol	4.8 - 5.6	n.a.	n.a.
Trazadone	203 - 233	n.a.	n.a.
Atenolol	n.a.	13.8	n.a.
Bupropion	n.a.	250.4 - 257.9	n.a.
Warfarin	n.a.	n.a.	<0.5
Verapamil	n.a.	n.a.	16.8 – 18.1

n.a.: Not available

Conclusions

Our results demonstrate the antischistosomal activity of Piperine and its analogous compound, TPIPPh. Those compounds showed activity against human P-gp and *in silico* studies suggest that they may interact with SMDR2. We hope to identify new efflux pump inhibitors by using similar scaffolds and proposing synthesizable molecules guided by the molecular docking results. Associating DMPK results with the molecule structures, we may be able to select compounds with better pharmacokinetic profiles.

Acknowledgements

FAPERJ, CAPES, CNPq and FIOCRUZ

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Development of subunit-selective substrates for the *Schistosoma mansoni* proteasome using substrate specificity profiling

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Key words: proteasome, substrate specificity, parasite, drug discovery, protease inhibitor

Introduction

Schistosomiasis affects over 200 million people globally, yet treatment options remain limited[1]. Proteasome is a validated drug target in parasitic diseases, including Schistosomiasis[2]. We previously demonstrated that inhibitors of the *S. mansoni* proteasome (Sm20S) show strong antischistosomal activity[2]. We aimed to develop Sm20S-specific substrates over human proteasome substrates to enable more effective drug screening and functional studies. For this, Sm20S was purified and its substrate specificity characterized using Multiplex Substrate Profiling by Mass Spectrometry (MSP-MS), a rapid, high-throughput method that maps proteolytic activity via peptide library digestion[4]. This revealed three distinct cleavage profiles corresponding to Sm20S active subunits (β1, β2, and β5). So new fluorogenic substrates specific to each subunit were developed, providing valuable tools for selective Sm20S activity measurement and antischistosomal drug discovery. (**Figure 1**).

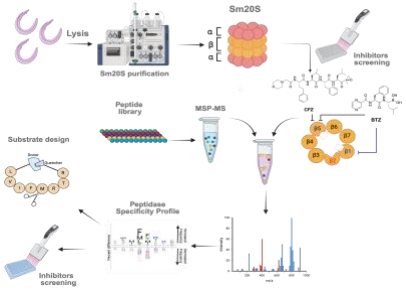


Figure 1: Workflow for the Development of Sm20S-Specific Fluorogenic Substrates using MSP-MS.

Results and Discussion

Dose-response curves revealed that carfilzomib (CFZ) preferentially inhibits the Sm20S β5 subunit, with lower potency against β1 and β2. Similarly, bortezomib (BTZ) has stronger inhibition of β5 and β1 over β2 (**Figure 2**).

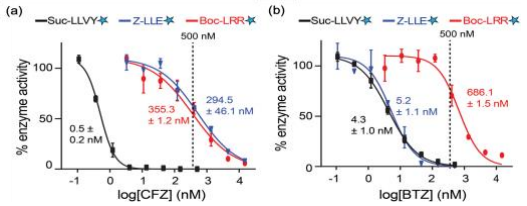


Figure 2: Determining the inhibitor concentration needed to inactivate Sm20S β5 or β5/β1 subunits.

We used MSP-MS in the presence of 500 nM CFZ or BTZ to map peptide cleavage and link substrate specificity to individual catalytic subunits (**Figure 3**).

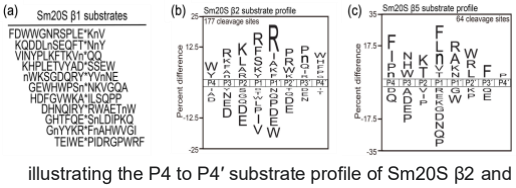


Figure 3: MSP-MS profiling of the Sm20S catalytic subunits. (a) List of Sm20S β1 substrates. (b) and (c) IceLogo plot illustrating the P4 to P4′ substrate profile of Sm20S β2 and β5, respectively.

These new substrates (β1: mca-VDQMDGW-K(dnp)-NH_2, β2: mca-FnKRR-K(dnp)-NH_2, and β5: 40 μM Ac-FNKL-amc) exhibit 9-fold or more improved activity compared to traditional human constitutive proteasome (c20S) substrates (β1: Z-LLE-Aamc, β2: Boc-LRR-amc, and β5: Suc-LLVY-amc) (**Figure 4**).

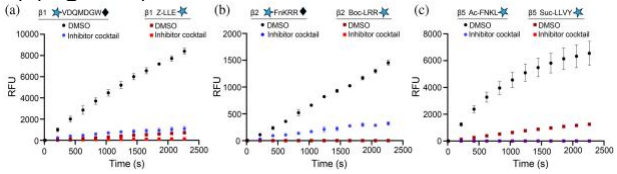


Figure 4: Evaluation of Sm20S substrates. (a–c) Proteolytic activity of Sm20S over time using the new and classic substrates in DMSO or the presence of a proteasome inhibitor cocktail (10 μM each of BTZ, CFZ, and MZB). The light blue star represents the amc fluorophore, and the black diamond represents the K(dnp) quencher.

The new substrates show similar cleavage rates across the three major human-infective *Schistosoma* species, supporting the potential for a single Sm20S-targeted inhibitor to act as a pan-schistosomicide (**Figure 5**).

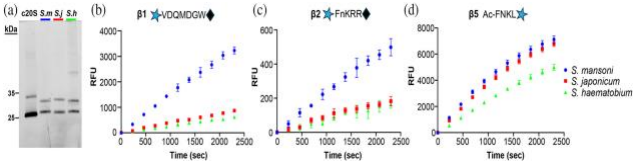


Figure 5: Comparison of the 20S activities from three *Schistosoma* species with the subunit-specific substrates.

Schistosome lysates were pre-incubated with proteasome inhibitors to test their cross-species inhibitory effects using the new substrates. Similar inhibition profiles were recorded for the three *Schistosoma* species (**Figure 6**).

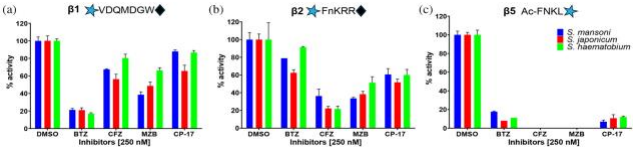


Figure 6: Comparison of proteasome inhibition across three Schistosoma species by cleavage of the fluorescent subunit-specific substrates.

Conclusions

The substrate and inhibition profiles for the proteasome from the three medically important *Schistosoma* species are similar. This suggests that Sm20S-focused inhibitor development can be efficiently extrapolated to the other *Schistosoma* species, leading to significant time and resource savings.

Acknowledgements

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Evaluation of the Activity of 4-Quinolones Against Multi-Life Stages of Plasmodium spp.

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Key words: malaria, vivax, antimalarials, resistance

Introduction

Malaria remains a critical global health problem. Parasite resistance to therapeutic interventions, particularly in the case of *Plasmodium falciparum*, and the scarcity of transmission-blocking drugs contribute to the aggravating burden of this disease. This study evaluates the efficacy of 4-quinolone[1] derivatives as multi-stage antiplasmodial compounds with activity against asexual intra-erythrocytic parasite forms and liver stages and their potential as transmission-blocking agents.

Results and Discussion

We demonstrate that the natural product 2-substituted-4-quinolone (compound 1), isolated from plants and microorganisms, along with its derivatives (compounds 2–6, Figure 1), exhibits significant activity against *Plasmodium vivax* sexual stages by inhibiting oocyst and sporozoite formation in *Anopheles darlingi* following treatment with 10 µM of compound **1**. This transmission-blocking activity was further confirmed in vivo using a *Plasmodium berghei* murine model treated at 50 mg/kg (Figure 2). Additionally, these compounds prevented hepatic cell infection, with compound **1** showing the highest inhibition across both sexual and asexual parasite stages (Table 1). Notably, this is the first report identifying this class of compounds as transmission-blocking agents. Previous studies have demonstrated their activity against asexual blood stages[1].

Table 1: Compounds liver stage inhibition

Compound	IC50 (µM)	Confluency [10µM]	Confluency [1µM]
1	0.25 ± 0.00	78.7 ± 2	96.9 ± 6
5	0.30 ± 0.00	146.8 ± 3	100.4 ± 1
6	0.38 ± 0.04	101.7 ± 11	105.2 ± 3

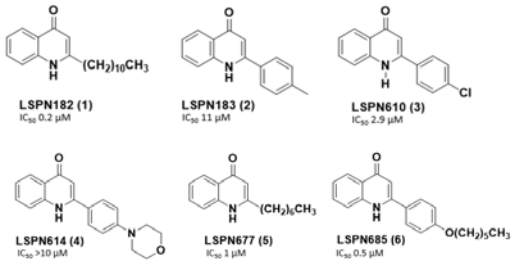


Figure 1. Chemical structure of 4-quinolone derivatives **1**-**6** . The IC_{50} value of each compound was calculated based on the antiplasmodial activity against the asexual blood stages of *Plasmodium falciparum* 3d7 sensitive strain.

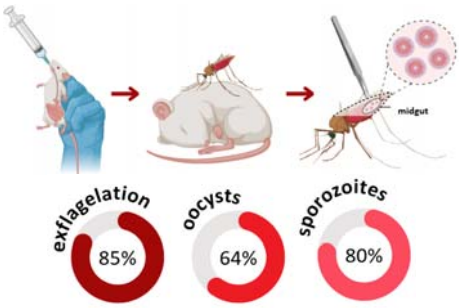


Figure 2: Inhibition of Compound **1** of *P. berghei* sexual phases [50 mg/kgx12h].
CEP: (n°#28176720.9.0000.0011)

Conclusions

The 2-substituted-4-quinolones can inhibit the entire parasite life cycle, highlighting their potential as promising candidates for future antimalarial drug development

Federal University of São Paulo; Institute of Hygiene and Tropical Medicine; FAPESP n°2023/02543-3 & 2022/05873-1

1de Souza, J. O. et al. Parasitological profiling shows 4(1H)-quinolone derivatives as new lead candidates for malaria. Eur. J. Med. Chem. Rep. 2021, https://doi.org/10.1016/j.ejmcr.2021.100012

A Novel Sulfonylhydrazone Derivative of LASSBio-1632 as Putative Treatment for Inflammatory Pulmonary Diseases.

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Key words: COPD, inflammation, PDE4 inhibitor.

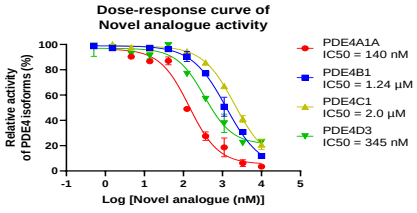
Introduction

Phosphodiesterase 4 (PDE4) has emerged as a promising therapeutic target for inflammatory lung diseases, particularly concerning chronic obstructive pulmonary disease (COPD)[1]. LASSBio-1632, a sulfonylhydrazone derivative with PDE4 inhibitory and anti-inflammatory activity[2], was used as a structural base to develop a more potent analogue. Docking simulations using GOLD software based on PDE4A crystal structure (PDB: 3I8V) showed that both LASSBio-1632 and the new analogue form hydrogen bonds with Gln581 and π–π interactions with Phe584. Notably, the novel compound demonstrates enhanced access to the metal-binding site compared to LASSBio-1632. This study investigates its pharmacological profile through in vitro and in vivo approaches. Due to confidentiality and intellectual property protection, the chemical name and structure of the analogue are undisclosed.

Results and Discussion

The novel analogue displayed potent inhibitory activity of PDE4 isoforms, with affinity toward PDE4A1A and PDE4D3, while maintaining activity against PDE4B1 and PDE4C1 (Figure 1).

Figure 1: Dose-response curve of the novel analogue activity on PDE4A1A, PDE4B1, PDE4C1, and PDE4D3 isoenzymes.



Moreover, the compound was shown to attenuate LPS-induced activation of alveolar macrophages by demonstrating a significant inhibition of TNF-α production *in vitro* (Figure 2). In the *in vivo* acute lung injury (ALI) model, the treatment with the analogue significantly reduced total leukocyte count and total protein levels in bronchoalveolar lavage (BAL) fluid (Figure 3A, B), demonstrating anti-inflammatory effects and protection against LPS-induced alveolar damage.

Figure 2: Effect of the novel analogue treatment on the activation of alveolar macrophages (AMJ2-C11 cell lineage) stimulated with LPS *in vitro*. Results are expressed as mean ± SEM. *P < 0.05 compared to LPS group.

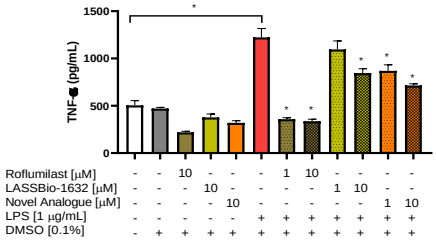
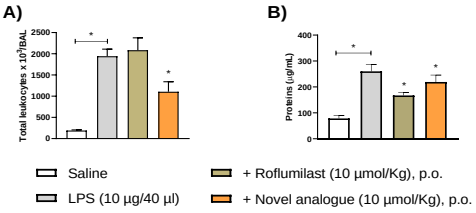


Figure 3: Effect of treatment with the new analog on LPA model on: A) Total leukocyte count in BAL. B) Quantification of total proteins in BAL supernatant. Results are expressed as mean ± SEM. *P < 0.05 compared to LPS group.



Conclusions

This novel sulfonylhydrazone analogue demonstrated a promising pharmacological profile as a selective PDE4 inhibitor, especially targeting the PDE4A1A isoform, exhibiting both anti-inflammatory and lung-protective effects, indicating a potential therapeutic application for inflammatory pulmonary diseases.

Acknowledgements

This work was supported by FIOCRUZ, FAPERJ, CAPES, and CNPq.

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Tail Elongation of OG-001 Enhances Selectivity Toward Tumor-Associated hCA IX and XII

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Key words: Indole, sulfonamide, tail-elongation, carbonic anhydrase

Introduction

Human carbonic anhydrase IX (hCA IX) and XII (hCA XII), also known as the tumor-associated CA, are (over)expressed in various cancer types, causing hypoxia-induced acidosis, metastasis, and chemoresistance, resulting in tumor progression. Consequently, they emerged as attractive biological targets for cancer therapy, leading to the discovery of many small potential molecules, including our previously synthesized 5-sulfamoylindole-based **OG-001**[1] (2-(hydrazinecarbonyl)-3-phenyl-1*H*-indole-5-sulfonamide) and **SLC-0111** developed by Supuran et al.[2], and has entered phase Ib/II clinical trials.[3] Therefore, hCA IX and hCA XII inhibitors have emerged as a promising research area for developing more effective cancer treatments that can combat acidosis and reduce the insensitivity of cancer cells to chemotherapy and radiotherapy. However, achieving selectivity over off-target isoforms, hCA I and II, remains a significant challenge.[4]

The most effective strategy for achieving isoform selectivity among hCAs is the "*tail approach*", which involves elongating a molecule with either a hydrophilic or hydrophobic group via various linkers. While the zinc-binding sulfonamide and the aromatic ring-attached anchors the inhibitor within the conserved catalytic site, the appended tail allows the molecule to interact with variable residues in the external and middle-external rims of the active site, which differ among isoforms.[5] One of the key differences in the external and middle-external rims is the bulky Phe131 residue in hCA II, which limits space and restricts access for larger substituents, whereas in hCA IX and XII, this residue is replaced by smaller amino acids (Val or Ala), creating a more open binding pocket (**Figure 1**).[6]

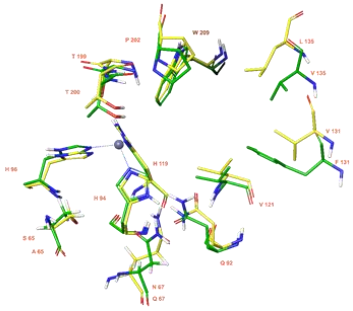


Figure 1: Comparison of hCA II (green) and hCA IX (yellow). Catalytic Zn^{2+} is shown as a sphere (grey)[7]

In this study, we report on the tail elongation and selectivity optimization of OG-001, a non-selective hCA IX and XII potent inhibitor, by adding benzenesulfonyl groups to the hydrazide part (**Figure 2**), obtaining SC derivatives, which are generally more selective for the tumor-associated hCA IX/XII over the off-targets cytosolic hCA I/II.

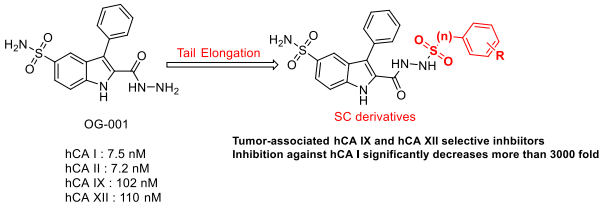


Figure 2: Tail-elongation of OG-001

Results and Discussion

All the synthesized and purified compounds were characterized with infrared, ^{1}H NMR, and ^{13}C NMR spectral analyses. The synthesized compounds and the reference acetazolamide (AAZ) were assessed for their hCA inhibitory properties. Among the compounds synthesized, compounds **SC4** and **SC9** emerged as the most selective against hCA II and hCA I, respectively, as shown in Table 1.

Table 1. Inhibition data of Compounds SC1, SC3, SC4, and SC9, and Standard (AAZ) against Isozymes hCA I, II, IV, and IX.

Entry	n	R_1	K_I (nM)[a]				Selectivity Index			
			hCA I	hCA II	hCA IX	hCA XII	hCA I / hCA IX	hCA I / hCA IX	hCA I / hCA IX	hCA I / hCA IX
SC1	0	-	25.4	5.8	6.2	8.0	4.1	3.2	0.9	0.7
SC3	0	4-F	23480	187.1	91.2	77	257.5	304.9	2.1	2.4
SC4	0	4-Cl	31550	215.3	88.5	100.3	356.5	314.6	2.4	2.1
SC9	1	-	49220	121.7	65.3	77.4	753.9	635.9	1.96	1.6
AAZ		-	250.0	12.0	25.0	5.7	10	43.9	0.5	2.13

Non-substituted benzenesulfonyl addition enhanced activity against hCA IX/XII but did not improve selectivity over hCA II. However, the addition of halogens to the benzene ring or increasing the

length by substituting the benzenesulfonyl with a benzylsulfonyl improved both the activity against the tumor-associated hCA IX/XII and the selectivity for hCA IX/XII over the cytosolic off-targets hCA I/II.

Conclusions

The tail elongation of OG-001 through the addition of arylsulfonyl derivatives enhanced selectivity towards hCA IX and XII while maintaining potent inhibitory activity. These findings support further investigation of OG-001 derivatives as selective anticancer agents targeting hCA IX/XII.

Acknowledgements

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Structure-activity relationships of inactive-conformation binding EGFR inhibitors: Linking the ATP and allosteric pockets

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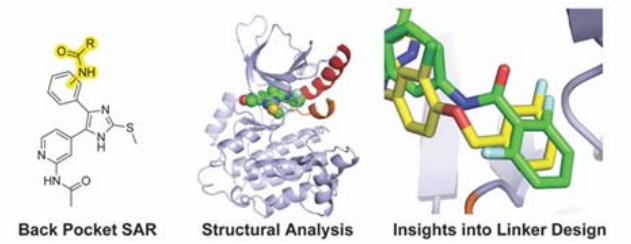
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Key words: Kinase inhibitors, Structure-guided.

Introduction

The epidermal growth factor receptor (EGFR) tyrosine kinase is a well-studied target in oncology and plays a central role in the development of targeted therapies for non-small cell lung cancer (NSCLC).[1] While EGFR tyrosine kinase inhibitors (TKIs) have demonstrated significant clinical success in treating EGFR-mutant NSCLC, their long-term efficacy is frequently compromised by acquired resistance mutations, including T790M and the subsequent C797S mutation.[2,3] These challenges underscore the urgent need for next-generation inhibitors and a recently emerged strategy to overcome EGFR drug resistance, proposed by our lab and others, involves the discovery of molecules that simultaneously bind to the ATP (orthosteric) and allosteric pockets, known as ATP-allosteric bispecific inhibitors (AABIs).[4,5]



Results and Discussion

To deepen our understanding of the linker site between the ATP and allosteric pockets, we investigated the incorporation of back pocket-binding functional groups into an ATP-site-targeting scaffold. We evaluated a series of trisubstituted imidazole-based EGFR inhibitors to determine how different back pocket moieties affect potency, particularly against EGFR mutants (L858R and L858R/T790M). The most potent compounds featured meta-substituted aniline linkers bearing 2,6-difluorophenyl and/or 3-hydroxyphenyl groups, exhibiting nanomolar IC_{50} values and effective suppression of EGFR phosphorylation in NSCLC cell lines. Additionally, X-ray co-crystallography of compound 6 revealed its binding to the inactive EGFR conformation, stabilized by π-stacking and hydrogen bonding interactions within the back pocket.

Conclusions

This study establishes that meta-substituted aniline linkers bearing specific aryl groups are optimal for engaging the EGFR back pocket in the inactive conformation. The linker architecture in these trisubstituted imidazoles is distinct from that of the more commonly applied quinazoline TKIs, highlighting the importance of alternative points of connection with different ATP-site groups and provides a structural and functional framework for designing next-generation Type I½ and ATP-allosteric bivalent inhibitors (AABIs) capable of overcoming key resistance mutations in EGFR-driven cancers like T790M and C797S.[6]

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Holistic Multitask Graph Learning for Screening Broad-Spectrum Antimycobacterial Compounds

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Key words: Artificial Intelligence, Antibacterial, Graph Neural Network, Drug Discovery, Virtual Screening.

Introduction

Infections caused by mycobacteria pose a persistent threat to global health, compounded by the emergence and dissemination of drug-resistant strains, including those unrelated to tuberculosis (TB). The limited efficacy and toxicity associated with current therapeutic regimens underscore the urgent need for innovative strategies to identify new antimycobacterial agents. In this study, we developed multitask learning models to screen novel broad-spectrum antimycobacterial compounds, harnessing the capacity of Graph Neural Networks (GNNs)[1] to capture complex, non-linear structure–activity relationships directly from molecular graph representations.

Results and Discussion

Initially, compounds tested against five different mycobacterial species and four different testing methods against *M. tuberculosis* were retrieved from ChEMBL. Subsequently, AttentiveFP and three GNNs powered by holistic mechanisms (GAT, MPNN, GIN) were trained for antimycobacterial activity prediction. The results demonstrated that the AttentiveFP method outperformed the other approaches in AUC and accuracy (Fig. 1).

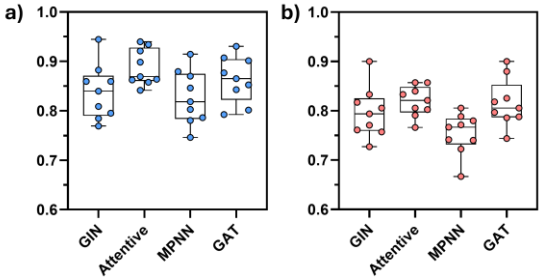


Figure 1: Benchmarking results of GNNs across nine classification tasks using random splits: (a) Area Under the Curve (AUC) and (b) Accuracy.

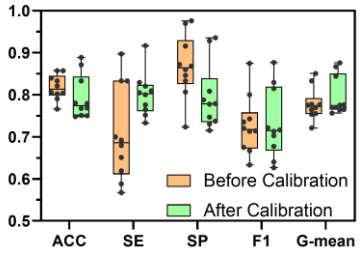


Figure 2: AttentiveFP model performance across multiple metrics before (orange) and after (green) calibration.

Subsequently, a threshold moving technique was applied to the AttentiveFP model to address the unbalanced data across multiple tasks, yielding a smooth improvement (Fig. 2).

Furthermore, the counterfactual explainability of the best model was calculated, using systematically perturbing atomic environments with isosteric and valence-based modifications and quantifying the resulting changes in predicted probabilities maps, highlighting regions with positive and negative influence of each molecule against mycobacterial species (Fig. 3).

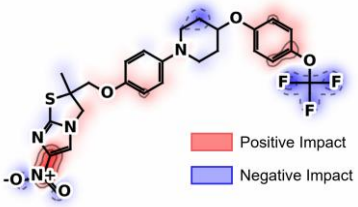


Figure 3: Counterfactual explainability analysis of a sample molecule from the test set. Regions in light red indicate regions contributing positively to inhibitory activity, while regions in light blue indicate those with a negative contribution.

The explainability of the AttentiveFP model shows a similar structure-activity relationship compared to the approved Anti-TB Delamanid[2], strengthening the predictive capacity of the model.

Conclusions

In summary, this study demonstrates the effective application of holistic multitask GNNs to predict broad-spectrum antimycobacterial activity with improved accuracy and generalizability. The integration of explainability modules further enabled the identification of substructural motifs associated with antimycobacterial activity, providing mechanistic insights that can guide rational lead optimization. Moving forward, these models will be applied to large-scale virtual screening campaigns to prioritize novel broad-spectrum antimycobacterial candidates for experimental validation, thereby accelerating the discovery pipeline against mycobacterial infections.

Acknowledgements

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Novel *N*-Acylhydrazone Quinolinylic Derivatives as Potential Multi-Target Candidates Against *Mycobacterium tuberculosis* and *Plasmodium falciparum*

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Key words: Key words: Mycobacterium tuberculosis, P. falciparum coinfection, quinolines, N-acylhydrazone.

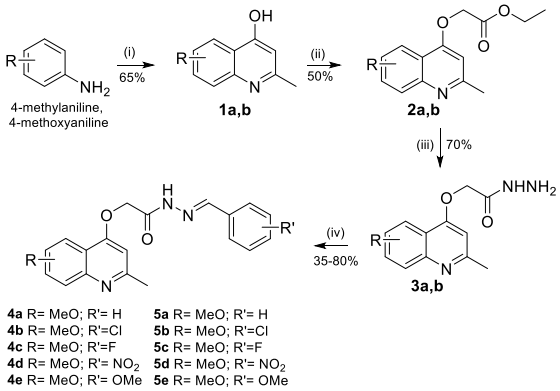
Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), and malaria, caused by Plasmodium spp., are among the leading causes of morbidity and mortality from infectious diseases.[1,2] Co-infection with TB and malaria represents an emerging global health concern. Although treatment options are available, the concurrent management of both infections poses challenges due to potential drug interactions and an increased risk of resistance.[3] In light of these limitations, we investigated a dual-target approach using novel compounds with potential activity against both pathogens.

Results and Discussion

Ten novel derivatives (**5a–e** and **6a–e**) were designed through a molecular hybridization strategy and synthesized in four steps, yielding satisfactory results (Figure 1)[4,5].

Figure 1: Synthetic route for the preparation of derivatives **5a–e** and **6a–e.**



(i) Ethyl acetoacetate, EtOH, AcOH, reflux, 3 h; then Dowtherm, 250 °C;
(ii) Acetone, K_2CO_3, KI, reflux, 10 h;(iii) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 2 h;
(iv) Benzaldehyde, EtOH, AcOH, room temperatur, 1 h.

PITTA et al; Journal of Medicinal Chemistry, 2016. BOECHAT et al; Journal of Medicinal Chemistry, v. 54, p. 5988–5999, 2011. ANGELOVA et al; Bioorganic & Medicinal Chemistry Letters, 2 set. 2016. OLIVEIRA, Fernanda A.; et al BMC Chemistry, v. 16, n. 50, 2022.

After structural characterization, the purity of the synthesized quinoline derivatives was confirmed by HPLC-UV, with values ranging from 96% to 99%.

All compounds underwent biological evaluation. Antimycobacterial activity against *Mtb* H37Rv was assessed using the MABA assay (Franzblau et al., 1998), in collaboration with INI/Fiocruz. The derivatives exhibited modest activity, possibly due to low solubility and precipitation issues. Antiplasmodial activity against *P.falciparum* was evaluated using the SYBR Green assay, in collaboration with IFSC/USP. Three derivatives demonstrated low micromolar IC_{50} values, highlighting their promising potential.

Cytotoxicity was evaluated in HepG2 cells, and the selectivity index (SI) was calculated.

Isoniazid, chloroquine, and artesunate were used as reference standards, and their inhibitory concentrations are included in the table for comparison.

Table 1. Purity and biological activities of derivatives **5a–e** and **6a–e**.

Compound	HPLC (%)	MIC (μg/mL) *Mtb* H37Rv	IC_{50} (μM) P. falciparum	CC_{50} (μM) HepG2	SI
5a	94.4	25	11.0 ± 1.0	>100	>9
5b	99.1	>100	4.9 ± 0.8	>100	>20
5c	98.9	>100	10.2 ± 0.3	45.0 ± 7.0	4
5d	98.9	>100	5.7 ± 0.8	>100	>18
5e	98.3	>100	>50	>100	>2
6a	97.0	25	24.3 ± 0.1	>100	>4
6b	98.5	>100	12.0 ± 2.0	>25	>2
6c	97.8	25	19.0 ± 0.3	>25	>1
6d	96.6	>100	3.9 ± 0.5	13.3 ± 0.7	3
6e	98.1	>100	>50	>100	>2
Isoniazid	—	0.3 μg/mL	—	—	—
Chloroquine	—	—	0.187 ± 0.018	—	—
Artesunate	—	—	0.020 ± 0.002	—	—

Legend: HPLC: purity (%). MIC: minimum inhibitory concentration against *M.tb* H37Rv. IC_{50}: 50% inhibition of *P. falciparum* growth. CC_{50}: 50% cytotoxic concentration in HepG2 cells. SI: selectivity index (CC_{50}/IC_{50}).

Conclusions

These results indicate the potential of a single molecule to exert dual biological activity against malaria–TB co-infection. However, further structural optimization is required to enhance potency and improve pharmacokinetic properties for potential therapeutic application.

Acknowledgements

Fiocruz, Farmanguinhos, UFRJ, CNPq; FAPERJ; CAPES.

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In Silico Evaluation of the Antifungal Potential of Metabolites from *Malpighia glabra* L.

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Key words: Malpighia glabra, Adenine phosphoribosyltransferase, Tubulina e Docking.

Introduction

Fungal infections are an increasing public health concern, causing high morbidity and mortality, particularly in immunocompromised patients such as transplant recipients, HIV carriers, and individuals undergoing cancer treatment. Resistance to conventional antifungals, drug toxicity, and the limited availability of new therapies hinder effective control. In this context, bioactive compounds of natural origin have gained relevance in the search for new antifungal candidates. Cruz et al. (2017) reported that extracts of Malpighia glabra L. (acerola), a fruit widely found in Brazil and rich in flavonoids and phenolic acids, exhibited antifungal activity against conidia and muriform cells of strains associated with chromoblastomycosis. Despite this evidence, studies investigating their interactions with specific molecular targets remain scarce. Therefore, this work evaluates, through molecular modeling, the interactions of M. glabra metabolites with the pharmacological targets of the antifungals flucytosine and griseofulvin.

Results and Discussion

Molecular docking simulations were performed using 16 major metabolites from Malpighia glabra L. against two enzymatic targets essential for the survival of fungal cells. One of these targets is adenine phosphoribosyltransferase, the enzyme targeted by flucytosine, which is involved in nucleotide synthesis. The binding affinity values highlight the three best-performing compounds, which were compared to the reference drug, as shown in Table 1.

Table 1. Molecular Docking Results for Bioactive Compounds and APRT (Flucytosine Target).

Molecules	Natural Source	Binding Affinity kcal/mol (PDB: 1G2P)
Rutin	Acerola	-7,5
Ellagic Acid	Acerola	-7,5
Cyanidin 3-glycoside	Acerola	-7,3
Flucytosine	Control	-5,8

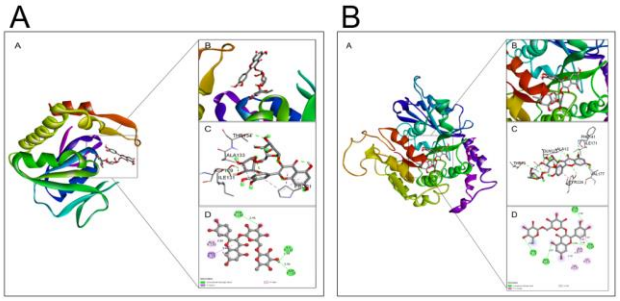
For tubulin, the target of griseofulvin which is essential for intracellular transport and mitotic cell division in fungal cells the binding energies obtained were compared to the reference drug, as shown in Table 2.

Table 2. Molecular Docking Results for Bioactive Compounds and Tubulin (Griseofulvin Target)

Molecules	Natural Source	Binding Affinity kcal/mol (PDB: 1TUB)
Rutin	Acerola	-8,8
Ellagic Acid	Acerola	-8,5
Cyanidin 3-glycoside	Acerola	-8,5
Griseofulvin	Control	-6,5

As no experimental binding energy values (in kcal/mol) for flucytosine and griseofulvin were found in the literature, we performed docking simulations with these reference drugs against their respective targets (adenine phosphoribosyltransferase – PDB: 1G2P and tubulin – PDB: 1TUB) to establish internal comparison parameters. The obtained values, –5.8 kcal/mol for flucytosine and –6.5 kcal/mol for griseofulvin, were adopted as references in this study. Rutin showed the best binding affinity among all compounds for both enzymatic targets, with –7.5 kcal/mol for the flucytosine target and –8.8 kcal/mol for the griseofulvin target. Compared to the reference drugs, it exhibited higher affinity in both cases, suggesting promising antifungal activity through in silico studies, as shown in Figure 1.

Figure 1. Molecular docking interactions of rutin with adenine phosphoribosyltransferase A (PDB: 1G2P) and B tubulin B (PDB: 1TUB).



Conclusions

The results indicate that metabolites from acerola (*Malpighia glabra* L.), especially rutin, exhibit stable interactions with enzymes essential for fungal survival. The high binding affinity suggests potential antifungal activity, although further studies, including molecular dynamics and ADME/Tox analyses, are needed to confirm their therapeutic applicability.

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Development of Novel Cysteine Protease B Inhibitors Based on the Bortezomib Scaffold

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Key words: Neglected Tropical Diseases, Leishmaniasis, Cysteine-protease, CPB inhibitors

Introduction

Leishmaniasis is a Neglected Tropical Disease (NTD) caused by protozoa of the genus Leishmania, present in over 98 countries and putting around 350 million at risk[1]. Currently, treatment options are limited and come with several drawbacks, including high toxicity and high costs, which highlights the need for new drug development. Proteases are widely studied as therapeutic targets[2], aiming, for example, at the treatment of infectious diseases. In *Leishmania* spp., the main cysteine protease is CPB, which is involved in various functions from nutritional processes to host cell infection. Recently, our research group identified that bortezomib, a drug approved for the treatment of multiple myeloma as a promising CPB inhibitor prototype with IC_{50} = 6.3 ± 1.1 µM; *L. mexicana* EC_{50} = 1.0 ± 0.2 µM (unpublished results), molecular modifications guided by computational methods were proposed to optimize its properties for better interaction with CPB. In this work, we focused on the design, synthesis, and evaluation of novel derivatives targeting CPB from *Leishmania* spp., based on bortezomib structure.

Results and Discussion

The design of the compounds was based on replacing the boronic acid group in bortezomib with a selective warhead capable of binding to the cysteine residue of CPB. The pyrimidine moiety was also replaced with two different nitro-heterocyclic groups and the resulting derivatives were evaluated with either an amide or a carbamate group (**Figure 1**).

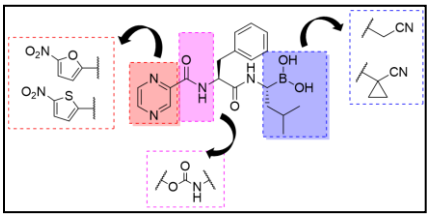


Figure 1: Design of the compounds based on bortezomib structure.

Molecular docking experiments were performed using Maestro Schrödinger in covalent binding mode for 8 designed compounds (PDB code: 6P4E). The nitro-heteroaromatic moieties showed a preferential occupation of the S2 pocket of the protease. The NO_2 group was able to perform a cation–π interaction with the Tyr-210 residue. Two compounds were initially synthesized, following the key steps: an amide bond formation between *L*-phenylalanine ester and 5-nitrofuran-2-carboxylic acid using EDC, HOBt, DIPEA, and DMF at 24 °C for 24 h, with 60% yield. After, the ester hydrolysis with LiOH in THF at 24 °C for 4 h affording 85% of yield and the last step with an amide bond formation under the same conditions described to introduce the warhead group (nitrile or cyclopropyl nitrile) with 15 to 20% yield, respectively. After NMR characterization, the compounds were evaluated against CPB with IC_{50} > 20 µM. These results suggest that the structural modifications in bortezomib may provide a promising starting point for the development of novel CPB inhibitors. The synthesis and biological evaluation of the remaining compounds will be essential to identify the most potent and promising candidate.

Conclusions

In this work, eight compounds were designed as CPB inhibitors based on the structure of bortezomib. Molecular docking experiments suggested that all compounds were able to interact with the protein's catalytic site. Two compounds were synthesized, characterized and evaluated against CPB, with IC_{50} > 20 µM. Further evaluation of the remaining compounds will assess their potential as CPB inhibitors.

Acknowledgements

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Study of the inhibition of the odor-binding protein for the control of *Anopheles* mosquitoes through in silico techniques

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Key words: Odor-binding protein, Anopheles, in silico techniques.

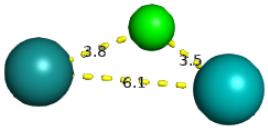
Introduction

Vector-borne diseases represent a serious public health problem. Mosquitoes and other hematophagous dipterans are the main vectors, transmitting pathogens such as protozoa, viruses, and bacteria[1]. The interaction between vector and host is mediated by the insect's olfactory system, in which odor-binding proteins (OBPs) play a fundamental role in odor detection. This study aimed to apply hierarchical virtual screening strategies, integrating both ligand-based and target-based methods, to identify compounds that interact with OBPs and potentially disrupt the transmission cycle[2].

Results and Discussion

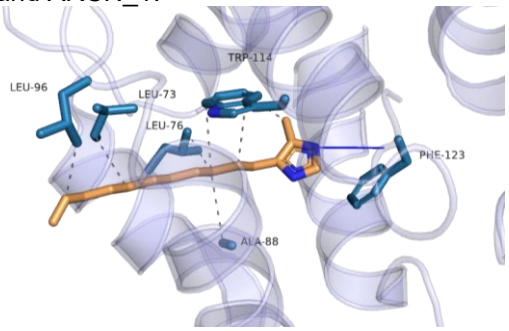
15 active OBP inhibitors were used to generate 10 pharmacophore models, analyzed through GALAHAD internal parameters and ROC curve (AUC). The selected model (Figure 1) contains one hydrogen bond acceptor and two hydrophobic regions, specificity = 3.4, Pareto = 0.0, energy = 38.7, sterics = 24.5, Hbond = 1.5 and AUC = 0.8.

Figure 1: Representation of the best pharmacophore model based on OBP inhibitors.



The model was used to filter 76,536 molecules from the Biogenic database (ZINC20). The results showed that only 850 molecules achieved QFIT values higher than those of known inhibitors. The filtered molecules were evaluated using physicochemical descriptors calculated on the ChemGPSNP online server, and only those with K-Nearest Neighbors = 3 were selected. The screening yielded 15 molecules, which were then used for analog generation through the insertion of fragments with similar shape and electrostatics. The structure design using vBROOD produced 59,087 molecules, which were submitted to molecular docking on the 3N7H target using the FRED program for ranking (Chemgauss score). The compound ARCN_1 showed the best docking score (Chemgauss score = -12.68). The complex shows a hydrogen bond donor interaction with Phe123, and hydrophobic interactions with Gly92, Leu80, Trp114, Ala88, His77, Leu96, Phe59, His111, Leu76, Met91, and Tyr122 (figure 2).

Figure 2: Intermolecular interaction map between OBP and ARCN_1.



The crystallographic ligand N,N-diethyl-meta-toluamide exhibits key interactions involved in target inhibition, some of which are also observed with the compound ARCN_1. Residues such as Phe123, Trp114, His111 and Gly92 are key residues considered essential for molecular target inhibition, and literature data indicate that they participate in hydrophobic interactions. Molecular docking results demonstrated that the compound ARCN_1 is positioned within the hydrophobic tunnel of the OBP protein[3].

Conclusions

Through the applied methods, compound ARCN_1 proved to be a promising candidate, establishing important intermolecular interactions for target inhibition.

Acknowledgements

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NatProDB: A digital repository of natural products Brazil's Semi-Arid Flora

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Keywords: NatProDB, Molecular database, Natural products.

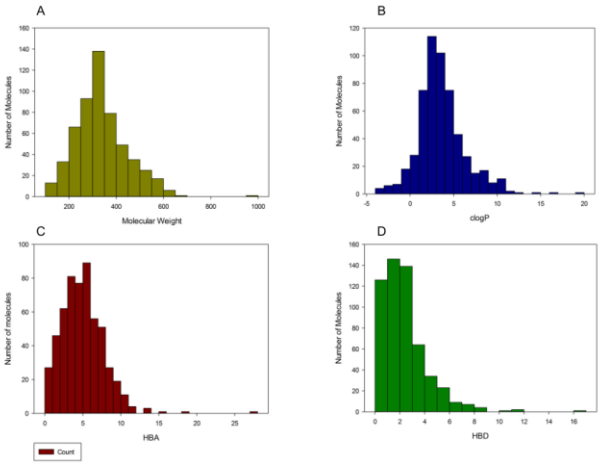
Introduction

Natural products (NP) have long been used in medicine and remain key in drug discovery due to their structural diversity and drug-like properties. Over one-third of FDA-approved new molecular entities (NME) are natural products or derivatives[1]. Given their complex structures and costly synthesis, virtual databases are valuable. This study introduces NatProDB – Natural Products Data Bank, a curated database of molecules from Brazilian semi-arid flora for Virtual Screening and Drug Discovery.

Results and Discussion

NatProDB contains 510 cataloged compounds, all sourced from scientific literature, will be annotated with the following information: molecular structure (in SMILES), compound identifier (ID), compound name, species name, and family and drug-likeness properties. NatProDB encompasses data on 98 vegetals species, spanning 47 distinct families. The Leguminosae family is the most represented in the database (23.5%), followed by Euphorbiaceae (13.7%) and Asteraceae (4.9%). The physicochemical properties of the NatProDB molecules are summarized in Figure 1. The molecular weights in the dataset range from 109.13 to 954.66 g/mol suggesting a predominance of mid-sized molecules. ClogP values in the dataset range from -3.24 to 19.88, indicating a wide spectrum of lipophilicity. The hydrogen bonding profile of the compound library was evaluated through the analysis of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD). The HBA values ranged from 0 to 27, while the HBD values varied between 0 and 16.

Figure 1. Distribution of molecular properties for compounds in NatProDB: Molecular weight (A), clogP (B), hydrogen bond acceptors (C), and hydrogen bond donors (D).



These characteristics, when considered together, suggest that a considerable portion of the library falls within drug-like chemical space. The database is stored in the Zinc database (ZINC UEFS Natural Products), but a specific homepage (natprodb.uefs.br) is being developed to access information from NatProDB.

Conclusions

Brazilian flora remains an invaluable source of chemical compounds with potential for new drug development. This work contributes to the study of semi-arid Brazilian plants by providing a curated set of molecules and tools to support virtual screening. The current version of the database includes 510 compounds and will continue to be expanded.

Acknowledgements

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Improved Route to a Key Intermediate for Dibenzosuberone-Based p38 Inhibitors via an Optimized Early-Stage Heck Coupling

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Key words: process development, scale up, p38 kinase inhibitors, skepinone-L, Heck coupling, Buchwald precatalysts

Introduction

The p38α MAP kinase has emerged as a key target in the treatment of inflammatory and oncologic diseases.[1,2] Dibenzosuberone-based p38 inhibitors, such as skepinone-L, have shown promising in vivo efficacy but require efficient synthetic access to their core scaffold. The original discovery route suffered from low overall yield and limited scalability due to problematic steps including a Wittig olefination and a Friedel–Crafts cyclization.[3] Therefore, a robust, scalable, and chromatography-free synthesis was needed to support further preclinical development.[4]

Results and Discussion

The bottleneck steps in the initial discovery route to the tricyclic core scaffold of dibenzosuberone-based p38 inhibitors were a Wittig reaction to install the central stilbene unit and a Friedel–Crafts cyclization to close the seven-membered ring. Both steps were associated with poor atom economy, relatively low yields, and challenging workups. Specifically, the Wittig step required stoichiometric triphenylphosphine, generating large amounts of triphenylphosphine oxide that complicated purification. The Friedel–Crafts acylation suffered from incomplete conversion, emulsion formation during quenching, and required column chromatography, making it unsuitable for scale-up.

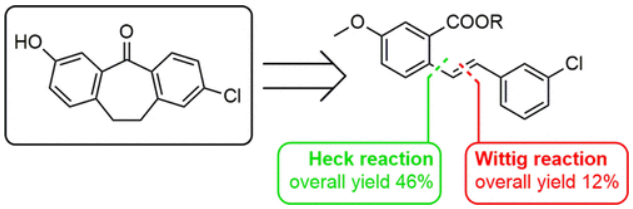


Figure 1: Key Modification of the initial discovery route.

To overcome these drawbacks, we redesigned the route to introduce a convergent and catalytically efficient Heck coupling early in the sequence. Using a Buchwald-type Pd(0) precatalyst (G3 μ-dimer) and tri-tert-butylphosphine under mild conditions, we achieved high coupling efficiency. Optimization allowed us to reduce Pd loadings to as low as 0.1 mol%, while maintaining isolated yields of 85–91% on up to 50 g scale. Environmentally friendlier solvents (EtOAc) and a simplified precipitative workup further increased the practicality of the process.

For ring closure, we replaced the problematic Friedel–Crafts acylation with a reaction using Eaton's reagent, which avoided phase separation issues and eliminated the need for chromatographic purification.

Overall, the new route significantly improved process robustness, sustainability, and yield. The full sequence proceeds in five linear steps with an overall yield of 46%, nearly quadrupling the output of the original method (12%).

Conclusions

The optimized synthesis offers a scalable and efficient route to a tricyclic key intermediate for dibenzosuberone-based p38 inhibitors. By eliminating chromatographic steps and reducing catalyst and solvent demands, the process is well-suited for preclinical supply and future manufacturing development. This work highlights the importance of early process optimization in translational medicinal chemistry.

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Mode-of-action investigation of a new generation of acridine/acridone-based antimalarials.

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Key words: Plasmodium falciparum, malaria, Pyronaridine, Quinacrine, SAR.

Introduction

Malaria remains a major global health concern due to its high morbidity and mortality. With increasing resistance to artemisinin-based therapies, there is an urgent need for new, safe, and effective antimalarial drugs. In this study, 18 novel acridine/acridone-based compounds were developed, inspired by the structures of pyronaridine and quinacrine, which are known for their activity against *Plasmodium* spp[1]. This study aimed to investigate their structure–activity relationships against *P. falciparum*, elucidate their modes of action, and evaluate potential synergistic interactions with existing antimalarial drugs.

Results and Discussion

On the basis of structure-activity relationship (SAR) studies, we identified derivatives with potent and selective properties, exhibiting comparable or enhanced selectivity indices compared to pyronaridine and quinacrine. The most promising candidates were found among the pyronaridine derivatives, which demonstrated efficacy against *P. falciparum* blood-stage parasites, equipotent *ex vivo* activity against *P. vivax* and *P. falciparum* field isolates from Brazil, and favorable therapeutic outcomes in a murine malaria model. Moreover, we conducted investigations into the mode of action of acridine/acridone-based compounds, revealing a distinct mechanism of action. Specifically, our findings demonstrated that pyronaridine analogs showed fast-acting activity, no cross-resistance against multidrug-resistant resistant parasites and resistance selection was not achieved even after prolonged exposure. In contrast, the acridone-based compound **5a** exhibited slow-acting behavior, a significant loss of potency against the atovaquone-resistant TM90C6B strain, and synergism with proguanil. In addition, compound **5a** disrupts membrane potential (Fig. 1) and targets the parasite's mitochondrial bc1 complex (IC_{50} = 2 µM), suggesting a mode of action comparable to atovaquone.

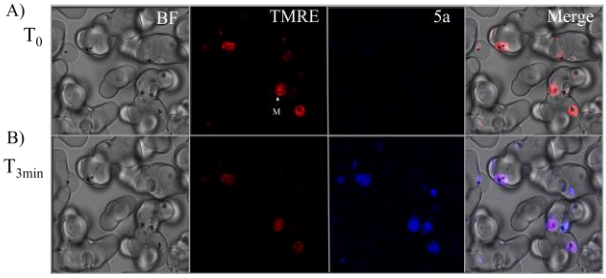


Figure 1. Effect of 5a on Δ Ψm from *P. falciparum*. Bright-field (BF) and 100 nM TMRE fluorescence (red) images of infected erythrocytes in A) the initial time (T_0) and B) 3 min after the addition of 10 µM of 5a (blue). 'M' indicates the parasite mitochondrion.

Compound **2d**, on the other hand, appears to target a different site than **5a**, close to parasite's digestive vacuole (Fig. 2) as observed for pyronaridine[1].

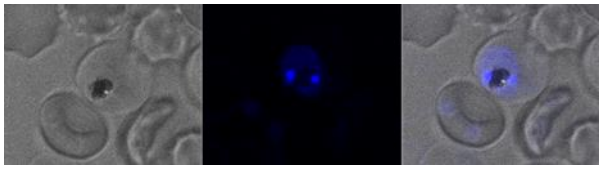


Figure 2. Confocal microscopy showing the distribution of the compound **2d** (10 µM) in *P. falciparum* infected erythrocytes.

Conclusions

Overall, our findings highlight scaffold-dependent mechanisms of action, with acridone-based **5a** acting on the parasite's mitochondria, and acridine derivatives acting rapidly, likely through heme detoxification pathways. These results underscore the potential of scaffold optimization to overcome resistance and guide future antimalarial development.

Acknowledgements

We thank the FAPESP (CEPID grant 2013/07600-3, 2020/12904-5, 2019/17721-9; 2024/04805-8) and fellowships (2015/20084-0, 2018/07287-7, 2021/03977-1 and 2020/14429-2).

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Rational Design of Proteasome Inhibitors as Potential Modulators of the NLRP3 Inflammasome Pathway

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Key words: Proteasome, N- acylhydrazone, NLRP3 inflamasome,

Introduction

Inflammatory diseases are often associated with excessive activation of the NLRP3 inflammasome pathway, making it a promising therapeutic target.[1,2] In this context, the proteasome plays a crucial role in regulating NLRP3 inflammasome activity, and its inhibition represents a viable strategy to modulate the inflammatory response.[3] Therefore, the development of new proteasome inhibitors may contribute significantly to the treatment of inflammatory disorders. This study aimed to perform design, synthesis, and molecular modelling studies of a series of *N*-acylhydrazone derivatives containing a phenylboronic acid group and some bioisosteres, planned as putative proteasome inhibitors, using a potent and indirect NLRP3 inflammasome inhibitor NIC-0102 as a prototype. [3,4]

Results and Discussion

Target compounds were designed considering NIC-0102 as prototype by the replacement of its peptide framework by an N-acylhydrazone subunit.[5] Then the alkyl boranic acid was substituted by a phenylboranic acid. Then the isosterism replacement of phenyl group by a thiophene ring was proposed.The synthesis of the target compounds was performed following the reaction conditions exemplified. All synthesized compounds were thoroughly characterized by NMR, mass spectrometry, and HPLC, showing an average purity between 98% and 99%. All the synthesized compounds were evaluated for their ability to inhibit the proteasome in assays conducted by the company Reaction Biology. However, none of them showed significant inhibitory activity against the target. To understand the molecular reason for the inactivity, molecular docking studies were performed using the GOLD software, using the crystallographic structure of human 20S proteasome complex with Bortezomib at 2.1. Docking studies revealed a favorable binding mode for phenylboronic acids, including key interactions with the catalytic threonine residue. However, thiophene-based analogs displayed a misalignment of the boron atom relative to threonine, preventing the covalent bond formation The calculations indicated that boronic acids bound to sp^2-hybridized carbons tend to drift away from the active site, thereby impairing the formation of the covalent bond with the catalytic threonine. In contrast, NIC-0102, which features a boronic acid attached to an sp^3-hybridized carbon, presents a more favorable geometry for effective proteasome inhibition. These findings highlight the crucial influence of the boron center's hybridization on the compound's inhibitory activity.

Conclusions

Although the synthesized compounds lacked proteasome inhibitory activity, docking and quantum mechanical calculations studies revealed key structural factors to explain the inactivity. The hybridization of the carbon bonded to the boronic acid group influences its positioning and interaction with the catalytic threonine residue. These insights support future structural optimization toward more effective inhibitors.

Acknowledgements

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Structure-based Drug Repurposing Against *Leishmania infantum*: Virtual Screening Targeting CRK12 Kinase

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Key words: leishmania infantum; drug repositioning; kinase; virtual screening; protein modelling

Introduction

Leishmaniasis is a neglected tropical disease, caused by parasites of the genus *Leishmania spp.* It is estimated that between 700,000 and 1 million new cases occur annually worldwide. Visceral leishmaniasis (VL) is the most severe form, in which parasites from the *L. donovani–L. infantum* complex migrate from the site of inoculation and proliferate in the liver, spleen, and bone marrow, resulting in host immunosuppression[1]. The sequencing of the *Leishmania* genome has enabled a better understanding of the parasite's viability and infectivity pathways, highlighting the relevance of eukaryotic protein kinases (ePKs). ePKs are essential for orchestrating the cellular signaling required for *Leishmania* survival throughout its life cycle, and therefore, they are considered potential therapeutic targets[2]. The kinase of interest in this study is CRK12, which was recently identified and validated as a therapeutic target for VL[3]. Drug repositioning is a cost-effective and time-saving strategy aimed at identifying new therapeutic uses for approved drugs with known safety profiles. Considering the urgent need for new treatments for leishmaniasis, structure-based drug repurposing targeting CRK12 may enable the identification of novel inhibitors as well as the redirection of already marketed drugs for the treatment of VL.

Results and Discussion

The primary structure of CRK12 was extracted from UNIPROT (ID: A0A6L0WJ69) and its 3D structure was obtained through different structural modeling approaches using the following servers: Modeller, Swiss-Model, RosettaCM, ColabFold and AlphaFold 3. For Modeller, Swiss-Model and RosettaCM, which uses comparative modelling approaches, the CDK9 protein (PDB ID: 4BCF) was used as a template due to its sequence homology with CRK12 (32.48% identity). The structural validation using the ProCheck Ramanchandran's plot indicated that the model generated by AlphaFold 3 exhibited the highest stereochemical quality, with 92.5% of its residues within most favorable regions. Structure-based Virtual Screening (SBVS) methods using GOLD and CarsiDock[4] were validated by assessing their ability to discriminate between active and decoy compounds in a pre-compiled test set of 12 CDK9 active compounds found in PDB, and 240 decoys generated in DEEPCOY[5]. The effectiveness of the SBVS protocol was demonstrated by the receiver operating characteristic curve (ROC) analysis, which effectively distinguished decoys from true ligands. As a result, Gold and CarsiDock showed a ROC curve with an area under the curve (AUC) of 0.52 and 0.72, respectively. This finding, thus, indicates that CarsiDock was able to accurately distinguish between known active and decoys, validating this protocol to further virtual screening campaigns.

Conclusions

In this work, we obtained and validated a three-dimensional model of CRK12 protein, in which the most accurate model was generated in AlphaFold 3 algorithm through deep learning strategies. We were also able to validate the virtual screening protocol, in which, CarsiDock was able to produce a ROC-AUC of 0.72, thus indicating its ability to distinguish between known CDK9 active compounds from decoys. The next step will be to start the SBVS with the approved drugs from DrugBank, utilizing the CarsiDock protocol. This project highlights the potential for developing new CRK12 inhibitors as promising anti-Leishmania agents. These compounds could contribute to more effective treatment strategies, leading to the improvement of the quality of life for individuals affected by leishmaniasis.

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Bank of Molecules: an initiative to leverage collaborative natural product-based drug discovery in Brazil

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Key words: Drug discovery; Brazilian biodiversity; Bioprospection, Compound management; Metabolomics; Bioassays.

Introduction

Molecules from Nature (NPs) continue to represent the main source of new drugs. Brazil is a biodiversity hotspot, with many molecules still to be discovered and developed as innovative drugs. The Brazilian scientific community is solid and needs to be connected to foster pharmaceutical innovation in Brazil. With this goal, we have stablished the Bank of Molecules, a collaborative initiative that aligns cutting-edge scientific infrastructure and expertise aimed to avoid bottlenecks in early-stage drug discovery and to promote collaborative pharmaceutical innovation throughout Brazil.

Results and Discussion

How it works: researchers interested in depositing their samples (extracts, fractions, pure compounds) to the Bank of Molecules (**depositor**) send their samples (2-10 mg) to LNBio/CNPEM, along with basic information that guarantees traceability of the sample (template worksheet available through contact). The samples are received by LNBio/CNPEM, where they are registered in a certified and trackable database, processed and stored at low temperature under inert atmosphere, following automated processes under GLP practices. In this first stage, all the samples are being sent to two main analysis at LNBio/CNPEM: i) untargeted metabolomic analysis - quality control at time zero and to sample the chemical space covered by the samples; and ii) biological evaluation in cellular assays (HCT-116, colon cancer, and HaCat, reference keratinocyte) – aiming to identify natural samples with anticancer potential and verify the cytotoxicity of the samples to normal cells. The first results are expected within 90 days, which will be delivered through standard reports on biological and chemical analyses, highlighting the main findings. From there, the parties involved can discuss the next steps of the collaboration, including the publication in scientific literature or further developments from the obtained data, which may include industrial and government partners, according to the interest of the **depositor**. The remaining samples are kept at LNBio/CNPEM for further analysis of the **depositor's** interest. The deposited samples remain property of the **depositor** and will not be used without their consent. At the same time, researchers interested in accessing the Bank of Molecules (**users**) can express their intents and will be connected to the **depositor** through our system. Once approved, the samples will be shipped to the **users** in "assay-ready plates", typically containing 2.5 nL of the approved chemical samples (384-well format preferred). The "assay-ready plates" can be promptly tested by the **users**, by dispensing their biological samples (bacteria, cells, target proteins, and so on) upon arrival of the plate at their laboratories. This enables the execution of medium to high-throughput screenings at different institutions in Brazil, avoiding bottlenecks in pipetting and trackability of chemical sample, thus speeding up bioprospection in the country. In this context, the **depositors** will have more information about the biological activity of their samples, whereas **users** will have access to more samples to test in their projects.

Conclusions

The Bank of Molecules has just been launched, attending researchers from all regions of Brazil. We invite researchers to contribute to the Bank of Molecules by depositing their chemical samples or by accessing them in their drug discovery projects in a collaborative fashion. For more information, please visit: https://lnbio.cnpem.br/ , or send an e-mail to: Banco.Moleculas@lnbio.cnpem.br.

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Machine learning-based regression models for the prediction of potential selective Cannabinoid Receptor 2 Agonists to treat osteoarthritis

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Key words: CB2 receptor, osteoarthritis, machine learning, regression models.

Introduction

The cannabinoid receptor 2 (CB2R) is a promising therapeutic target in the regulation of inflammatory, immunological and bone remodeling processes, with potential application in chronic joint diseases, such as osteoarthritis and rheumatoid arthritis.[1] This study aims to explore the prediction of bioactive and selective compounds for CB2R, integrating rigorous data curation, molecular descriptor generation and machine learning techniques[2].

Results and Discussion

A total of 11,751 molecules with reported experimental activity for CB2R were collected from ChEMBL and BindingDB, of which 2,750 comprised the final data set after curation with filters for duplication, inconsistencies, PAINS and Lipinski criteria[3]. Four data division approaches were tested: 1) random, 2) Kennard-Stone algorithm, 3) clustering with the K-Means algorithm and 4) clustering with the Kennard-Stone algorithm; this last provided the best chemical space representation (Fig. 1). Regression models (RF, SVR, XGBoost, KNN) were trained using PubChem, Morgan, and MACCS Keys fingerprints by *in house* Python scripts with pandas and numpy libraries, scikit-learn and RDKit for function implementation, pre-processing and hyperparameter optimization (Randomized SearchCV). From the KNN with PubChem fingerprints, we obtained models with the best R^2 value (0.7793) and the lowest MAE value (0.1897).

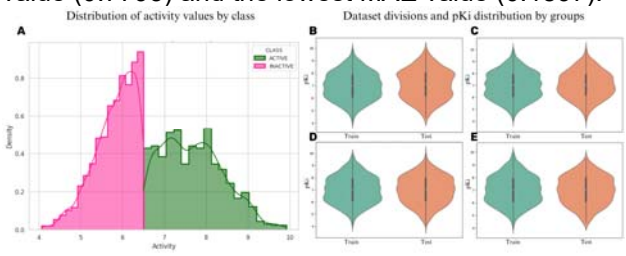


Figure 1: A) Distribution plot of activity values by class. Violin plot of the B) 1, C) 2, D) 3, E) 4 dataset divisions and pKi distribution by groups.

A virtual screening was performed in the NuBBEdb database, identifying 393 molecules with predicted pKi ≥ 6.5 nM (value considered active)[4]. These molecules were screened with the ALPACA platform, resulting in 34 selective compounds, superior to control (WIN55,212-2), with predicted pKi ≥ 7 nM[5]. These 34 molecules were compared with results from an internal classification model, confirming 11 molecules as active and pKi predicted by KNN ≥ 7 nM. The pharmacokinetic prediction (SwissADME) of these 11 molecules highlighted 4 compounds (NuBBE2366, NuBBE1962, NuBBE1595, NuBBE1344) with a high probability of permeating the blood-brain barrier (BBB) and without being P-gp substrates, representing high potential candidates[6] (Fig. 2).

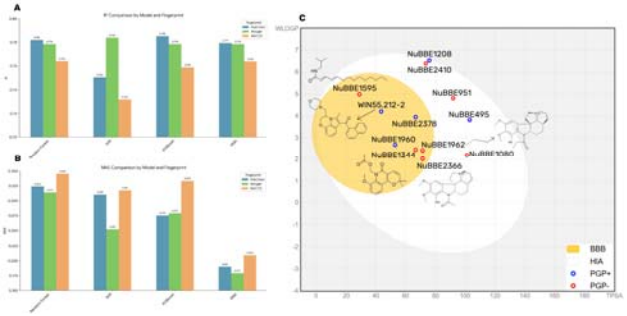


Figure 2: Statistical metrics plots of all generated models. A) R^2 and B) MAE for each model. C) BOILED-egg plot of the selected compounds and WIN55,212-2, with the 2D structure highlighted.

Conclusions

Results showed a robust computational pipeline combining curation, data splitting and supervised regression algorithms. This pipeline efficiently identified potential novel CB2-selective modulators with promising pharmacokinetic profiles for osteoarthritis therapy. Future large-scale membrane molecular dynamics simulations will be performed to investigate stability, and ligand affinity at the CB2 receptor.

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Breaking the Mold: 3D Carboranes as Non-Classical Bioisosteres in Erlotinib Analogs

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Key words: epidermal growth factor receptors, carborane, non-classical bioisosterism.

Introduction

Phenyl rings are ubiquitous in drug design but often impose limitations due to planarity, poor solubility, and metabolic instability.[1] As non-classical 3D bioisosteres, carboranes offer unique structural and physicochemical advantages, including σ-aromaticity, lipophilicity, and metabolic robustness.[2] Here, we applied a 3D bioisosteric switch to erlotinib, a known EGFR tyrosine kinase inhibitor, replacing its planar aryl moiety with dicarbaboranyl isomers. The resulting compounds displayed enhanced antiproliferative activity in glioblastoma cell lines, improved selectivity over non-tumoral cells, and moderate inhibition of both wild-type and mutant $EGFR_{T790M}$. Additional studies revealed favorable pharmacokinetic, safety, and mechanistic profiles, including induction of apoptosis and lack of mutagenicity. These results highlight the value of carboranes as 3D scaffolds with distinct structural and pharmacological advantages, offering a promising avenue for the development of novel anticancer agents aimed at challenging tumor types.

Results and Discussion

A small library of erlotinib analogs featuring 3D dicarbaboranyl moieties as non-classical bioisosteres of the phenyl group were synthesized and biologically evaluated. Among them, para-substituted derivatives (**13**) and (**17**) showed markedly improved cytotoxicity, up to 12-fold compared to erlotinib, and enhanced selectivity for glioblastoma cells over astrocytes. Meta isomers (**15**) and (**16**) were the most potent $EGFR_{WT}$ inhibitors in the series. Docking and molecular dynamics simulations confirmed stable binding within the EGFR ATP-binding pocket, showing a moderate correlation with *in vitro* enzymatic inhibition results (Figure 1). Compound (**17**) induced apoptosis and demonstrated favorable safety *in vivo* (LD_{50} > 2000 mg/kg) with no significant changes in blood or liver parameters. Absence of mutagenicity was confirmed via Ames test in four strains, both with and without metabolic activation. These findings support the potential of carborane-based scaffolds to improve both the efficacy and pharmacological profile of existing anticancer agents through 3D bioisosteric replacement.

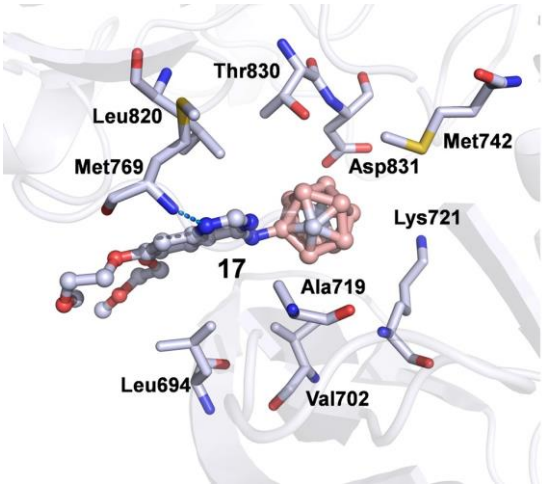


Figure 1: Representative structure of the 300 ns molecular dynamics simulations were used. Derivative (**17**) interact with ATP binding loop (residues 694 to 702), α-helix C (residues 730 to 745), hinge region (residues 762 to 773) and DFG motif (residues 830 to 833). Catalytic loop (residues 812 to 818) and activation loop (residues 833 to 852).

Conclusions

Derivative (**17**) demonstrated over 12-fold increased cytotoxicity and nearly 7-fold selectivity toward glioblastoma cells, combined with favorable EGFR inhibition and excellent *in vivo* safety, supporting its development as a lead anticancer agent.

Acknowledgements

This work was supported by the Agencia Nacional de Investigación e Innovación (ANII, Uruguay), CSIC-Udelar, Fondo Vaz Ferreira-MEC, and PEDECIBA.

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Guanidine Derivatives as New *Plasmodium falciparum* Inhibitors

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Key words: malaria, guanidine derivatives, P. falciparum.

Introduction

Malaria is a parasitic disease caused by the pathogenic protozoa *Plasmodium* spp., which has a significant global impact on human health.[1] The increasing resistance of *P. falciparum* strains to approved drugs highlights the urgent need for the discovery of new antimalarial candidates. Guanidine alkaloids emerged as promising natural products with antiparasitic activity, and a promising parasitological profile against malaria parasites.[2,3] Herein we report the results of investigating a series of synthetic guanidine derivatives with potent anti-plasmodial activity.

Results and Discussion

Based on our previous work with natural guanidine alkaloids batzelladines F and L,[3] we assessed the anti-plasmodial activity of 234 synthetic guanidine derivatives as new *P. falciparum* inhibitors (Figure1).

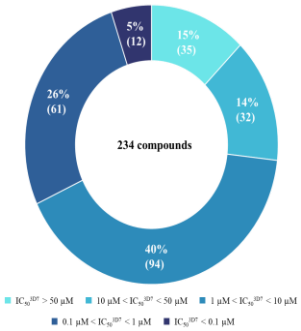


Figure 1: Percentage distribution of the activity of the 234 compounds evaluated against the 3D7 strain.

In this series, *c.a.* 5% of the derivatives (12 compounds) showed potent antiparasitic activity against *P. falciparum*, with IC_{50} values < 0.1 μM. These results represent a notable improvement in antiplasmodial activity when compared to the activity of batzelladines F and L.[3] Additionally, guanidine derivatives with IC_{50}^{3D7} values < 10 μM were tested against human HepG2 cell lines to evaluate liver cytotoxic effects. In general, the most potent compounds exhibited good to excellent selectivity index (SI) values (SI > 10). In order to investigate the mechanism of action of these derivatives, compounds of each subseries (**FFC41**, **FFC53**, **RMS496**, **RMS502**, and **TAC20B4**) were selected for parasitological profile studies. The investigation included speed of action (as fast or non-fast acting inhibitor), combination profile with artesunate, resistant panel (3D7, Dd2, K1, Dd2R_DSM265, 3D7R_MMV692848, and TM90C6B strains) and stage specificity assays. Figure 2 summarizes the main findings.

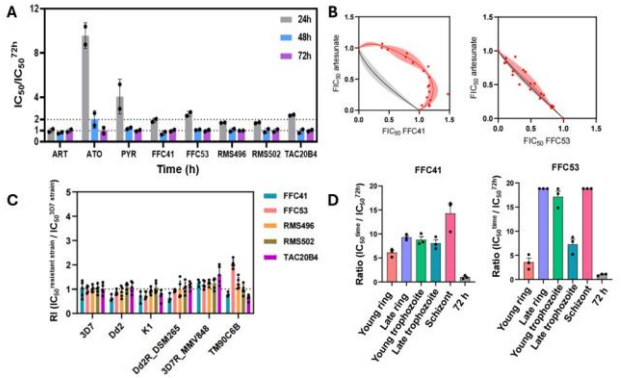


Figure 2. (**A**) Speed of action. (**B**) Representative combination profile with artesunate. (**C**) Resistant panel. (**D**) Representative stage specificity assay.

Guanidine-based compounds displayed unique parasitological profiles, indicating each subseries may act through a different mechanism (Figure 3).

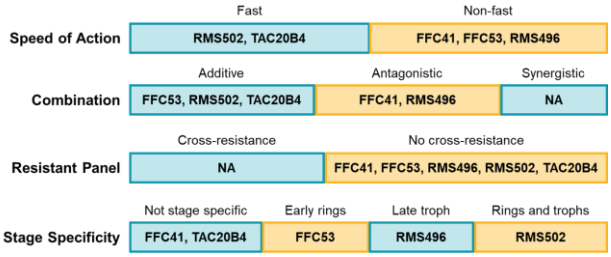


Figure 3: Schematic results for parasitological profile.

Conclusions

Among 234 synthetic guanidine derivatives assayed against *P. falciparum*, 5% showed inhibitory activity at low nanomolar concentrations. Five compounds of each subseries exhibited unique anti-plasmodial profiles, suggesting different mechanisms of action for these compounds. These results indicate that the guanidine derivatives are attractive candidates for drug development.

Disclaimer: The molecules described in this study are subject to a pending patent application.

Acknowledgements

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Exploring Ultra-Large Chemical Space: A Virtual Screening Approach to Identify New Cruzain Inhibitors

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Key words: Cruzain, ultra-large scale virtual screening, molecular docking, machine learning

Introduction

Chagas disease remains a significant public health challenge, marked by high mortality rates and the lack of effective treatment options. Since the late 1980s, cruzipains have been the most extensively studied molecular targets in the search for new prototypes to treat Chagas disease[1]. However, the development of new drugs remains a complex and time-consuming task. Commercially available compound libraries have expanded significantly and may now contain up to tens of billions of molecules. Virtual screening (VS) through molecular docking has proven to be an effective strategy for exploring large chemical libraries. However, applying molecular docking at ultra-large scale becomes computationally expensive. A potential solution to this limitation is the use of robust machine learning models as predictive filters to identify compounds with a high likelihood of favorable docking scores. In this study, we aimed to develop an ultra-large-scale virtual screening pipeline to identify novel cruzipain inhibitors with potential antichagasic activity.

and Discussion

Among the molecular docking software, DOCK6 stands out due to its intuitive interface, extensive documentation[2]. To validate the docking protocol, a set of 96 cruzain inhibitors was compiled from the literature based on their structural diversity and inhibitory potency (IC_{50}/Ki values ranging from 0.004 to 77.5 μM). Using DUDE-Z software, structurally diverse subset of 45 compounds (86.05% with Tc ≤ 0.3) were ultimately selected for the generation of 2,250 decoys. These active and decoy compounds were then used to validate the docking protocol. Docking results were evaluated using a ROC curve and enrichment analysis (AUC = 0.87; EF 1% = 16.1; EF 5% = 7.2; EF 10% = 5.8).

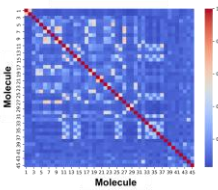


Figure 1 – Heatmap of active compounds

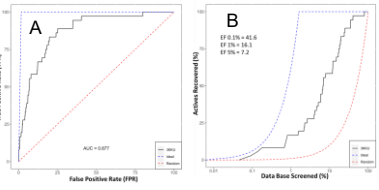


Figure 2 – A) ROC curves with AUC values. B) Enrichment curves with EF at

To develop a predictive model, we used a dataset of one million molecules with diverse physicochemical properties from the ZINC20 database. The data were split into training (70%) and test (30%) sets. The final model demonstrated strong predictive performance on external validation, achieving an R^2 of 0.95 on the 30% test set. Considering the success of the predictive model, we expanded the screening to a commercially available subset of the ZINC20 database, containing approximately 647 million compounds. The top 1% of compounds, ranked by predicted docking scores, were then subjected to docking simulations using DOCK6. The most promising candidates identified based on visual inspection will be prioritized for in vitro evaluation against the cruzain enzyme.

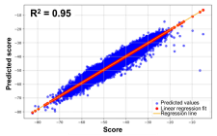


Figure 3 – Score values calculated by DOCK6.12 versus predicted score

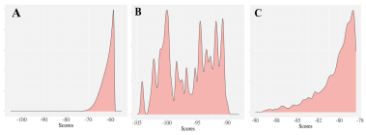


Figure 4 – Distribution of predicted docking score values based on the complete subset of commercially available ZINC20 molecules (A), scores between -105 and -90 (B), and between -90 and -75 (C).

Conclusions

We developed a fast multicore automation protocol for the DOCK6 molecular docking program. By integrating this method with a robust machine learning model (R^2 = 0.95), we efficiently screened a chemical library of 647 million compounds. This strategy enabled the identification of potential cruzain inhibitors, which are currently undergoing experimental validation.

Acknowledgements

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Machine Learning Models for the Identification of Natural Products with Anti-Schistosomiasis Activity

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Keywords: Schistosomiasis, Schistosoma mansoni, Machine Learning, Natural Products, ADMET.

Introduction

Schistosomiasis is one of the neglected diseases affecting millions of people in tropical and subtropical regions, remaining underexplored in research and the development of new therapies. Its treatment relies mainly on praziquantel, which presents significant limitations, such as ineffectiveness against immature forms of the parasite and emerging resistance. The scarcity of standardized and systematic experimental data in public databases hinders the discovery of new effective drugs. This study aims to apply machine learning models to predict the biological activity of natural products against *Schistosoma mansoni* and prioritize promising candidates from the LOTUS and NPAtlas libraries, totaling approximately 292,000 compounds.[1] Following rigorous data curation and balancing of the dataset using the SMOTE technique, different algorithms were trained, including Random Forest (RF), Support Vector Regression (SVR), XGBoost, and Deep Neural Networks (DNN).

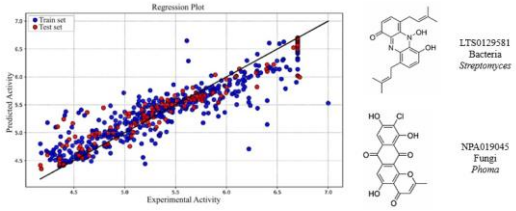
Results and Discussion

The machine learning models were developed using a curated dataset of 243 compounds from the ChEMBL database, labeled as active or inactive based on pIC_{50} values. To address class imbalance, the SMOTE technique was applied. Multiple molecular fingerprints were tested, and the best performance was obtained with the Random Forest (RF) model using AtomPair fingerprints. The model achieved R^2 of 0.9314 (cross-validation) and 0.8953 (external validation), with strong robustness confirmed by additional metrics such as MAE, Q^2, and CCC (Table 1). The predictive capacity of the RF model can be visualized in Figure 1, which shows the correlation between experimental and predicted pIC_{50} values for the training and test sets.

Table 1. Statistical validation metrics for the RF model.

r^2	MAEcv	$*q^2$	MAEfull
0.9314	0.1328	0.8953	0.1475
$testQ^2_{F1}$	$testQ^2_{F2}$	$testQ^2_{F3}$	testCCC
0.9314	0.9313	0.9314	0.9446

Figure 1. Regression plot illustrating the correlation between experimental and predicted activities (pIC_{50}) obtained by the RF model. Blue and red dots represent the training and test sets, respectively.



Feature importance analysis revealed that fused aromatic systems with heteroatoms (e.g., indoles, quinolines) and electron-donating groups (such as hydroxyl and methoxyl) were positively correlated with antiparasitic activity. In contrast, structural patterns related to molecular flexibility showed a negative correlation, suggesting that more rigid molecules tend to perform better. The validated RF model was used to screen natural products from the LOTUS and NPAtlas databases. Fourteen compounds were selected for further analysis, mainly from the flavonoid, alkaloid, diazanaphthalene, and polyphenolic families. Notably, LTS0129581 and NPA019045 presented structural features aligned with those identified as important by the model. Both contain aromatic rings with hydroxyl groups and low structural flexibility—features associated with enhanced predicted activity. These observations support the relevance of the model's fragment-based interpretation and illustrate how different natural scaffolds may converge in terms of physicochemical features relevant to anti-schistosomal potential .

Conclusions

This study presents the potential of integrating machine learning with medicinal chemistry to accelerate the identification of new therapies for neglected diseases, offering clear guidelines for the rational development of new drug candidates against schistosomiasis.

Acknowledgements

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Lost in the Noise: A Data Curation Framework for Building Robust Machine Learning Models against *Leishmania infantum*

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Keywords: Machine Learning, leishmaniasis, leishmania infantum, data curation.

Introduction

Visceral leishmaniasis, caused by *Leishmania infantum*, is a neglected tropical, potentially fatal and difficult to treat due to the toxicity and high cost of available drugs[1]. In this context, machine learning methods are promising in identifying new therapeutic candidates. However, the limited amount of data and its high variability hinder the development of robust and reliable models[2]. In this scenario, the ChEMBL and PubChem databases encompass over 1500 compounds with IC_{50} values determined against the amastigote form of *L. infantum*, based on cell-based assays using different methodologies[2], variations in strains, cell sources, incubation times, and detection methods. These factors lead to a significant variation in the biological activity of control compounds such as miltefosine (0.5 – 7.47 μM) and amphotericin B (0.01 – 0.32 μM). So, this study grouped the different cell-based assays according to the activity values of the control compounds and common methodological features, aiming to build datasets with lower inter-assay variability. The compounds were classified as active (< 10 μM) and inactive (> 12 μM). The compound dataset was divided in training/validation and test sets using the MASSA[3] software, which were used to train classification models with the MLJar package.

Results and Discussion

The complete dataset (D0) was initially separated according to the biological data of the control compounds, being divided into D1 (amphotericin B 0.01 – 0.32 μM), D2 (miltefosine 2.30 – 7.47 μM), D3 (miltefosine 2.30 – 2.84 μM), and D4 (miltefosine 5.71 – 7.47 μM). These sets were further divided according to similarities in the activity determination methods, notably D1.1 with compounds tested against murine cells, and D3.1 with compounds tested using the same biological protocol. These datasets and subsets were subjected to an exploratory analysis of the correlation between physicochemical properties and biological activity. We observed correlations between molecular weight and LogP with the biological data in the D1 set, and even more pronouncedly in the D1.1 subset, while no significant correlations were found in any other dataset. Six types of fingerprints were generated for each dataset and used to train models. The models that showed the lowest logarithmic loss function (LogLoss) during internal cross-validation were selected to evaluate the model performance on the external test set. The results (Table 1) indicate that the data curation led to a significant improvement in performance metrics on the test set. However, the reduced number of compounds in the subsets directly affected the robustness of the models.

Table 1. Metrics of the best models (using test set)

Dataset x fingerprint	LogLoss	F1-score	MCC	Acc
D0 ECFP4	0.41	0.81	0.63	0.82
D1 Torsions	0.30	0.89	0.79	0.90
D1.1 MACCS	0.16	0.93	0.86	0.93
D3 MACCS	0.28	0.86	0.72	0.87
D3.1 ECFP4	0.23	0.89	0.78	0.89

MCC (Matthews Correlation Coefficient); Acc (Accuracy).

To interpret the model, we employed the SHAP[4] analysis for the model trained with the D1.1 subset. With this analysis, we identified substructures that the most influence the biological activity. The results indicate that the activity is enhanced by characteristics associated with flexibility and specific groups. A positive correlation was observed with flexible aliphatic fragments, such as methylene chains (MACCS 155, 138, 116) and substituents in adjacent (vicinal) positions in rings (MACCS 150). In the same way, nitrogen-containing motifs such as N-*-N and N-O bond contribute to the activity (MACCS 77, 71). In contrast, the activity tends to decrease in the presence of characteristics that confer greater rigidity to the molecule. We also observed negative correlations between activity and molecular properties, such as carbon-nitrogen bonds in aromatic systems (MACCS 65) and fixed distances of three or four bonds between heteroatoms (MACCS 97, 79). So, these structural arrangements are unfavorable for biological interaction.

Conclusions

From the results, we can conclude that data curation is a critical step that not only enhances the predictive performance of the models but also increases the reliability of the interpretability analysis. This can yield effective tools for the selection of new candidates against *L. infantum*.

Acknowledgements

CAPES, FAPESP, CNPq, EACH-USP, and UFABC.

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Synthesis of Quinone-Fused Pyrrole Derivatives as Antitumor Candidates

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Key words: Cancer, quinone, pyrrole.

Introduction

Quinone derivatives, recognized as clinically approved anticancer agents, act primarily through redox cycling, a process in which one-electron reduction leads to the generation of reactive oxygen species (ROS), ultimately inducing oxidative stress and cancer cell death. In addition to redox activity, quinones act as electrophilic species capable of forming covalent adducts with cellular nucleophiles, such as proteins and DNA, thereby impairing vital cellular functions and inducing apoptosis in cancer cells.[1,2]

Our research group has focused on the annulation of quinone with a pyrrole nucleus to develop novel antitumor compounds. Annulated compounds reduce the formation of semiquinones and reactive oxygen species, which are major contributors to the cumulative cardiotoxicity commonly associated with various quinone derivatives.[1] Based on the structure of compound **1** (**Figure**), which exhibits significant antitumor activity against pancreatic tumor cell lines (Mia PaCa-2, Panc1, and BxPC-3) at nanomolar concentrations[3], we describe the synthesis of a series of quinone-fused pyrrole derivatives **2a–g**. The target compounds possess diverse functional groups at the N-1, C-2, and C-3 positions of the heterocyclic ring, and their cytotoxic activities will be assessed against a range of human tumor cell lines.

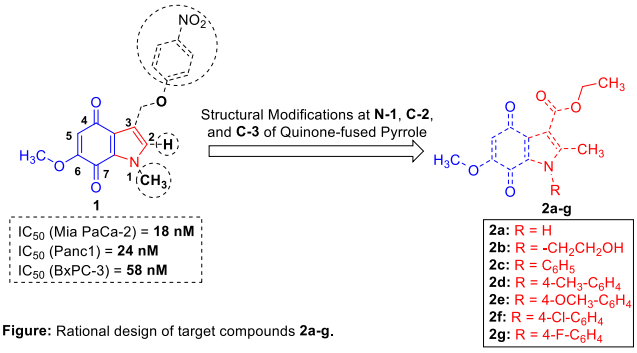


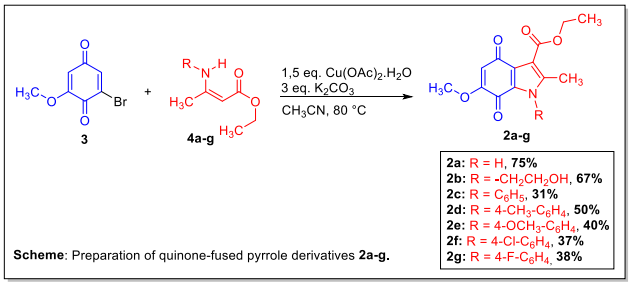
Figure: Rational design of target compounds **2a-g.**

Results and Discussion

The quinone-fused pyrrole derivatives **2a–g** were synthesized via a Michael addition reaction between bromobenzoquinone (**3**) and various β-enamino esters **4a–g** in refluxing acetonitrile. Copper acetate served as the oxidizing agent, while sodium carbonate was used as the base **(Scheme)**.[4] The products were obtained in yields ranging from 31% to 75%. The chemical structures of the target compounds **2a-g** were elucidated using spectroscopic methods, including Infrared Region (IR) and ^{1}H and ^{13}C NMR spectra.

Conclusion

The synthetic strategy employing the Michael reaction between bromobenzoquinone (**3**) and β-enamino esters **4a–g** afforded the target compounds in yields ranging from 31% to 75%. The product yields were influenced by the reactivity of the β-enamino esters. The synthesized compounds will be further evaluated for their cytotoxic activity against various human tumor cell lines.



Scheme: Preparation of quinone-fused pyrrole derivatives **2a-g.**

Acknowledgements

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Evaluation of Nitric Oxide-Releasing and Anti-proliferative Properties of alpha-Acyloxy-(1,2,5-oxadiazole-2-oxide)carboxamides Synthesized via Passerini Multicomponent Reaction

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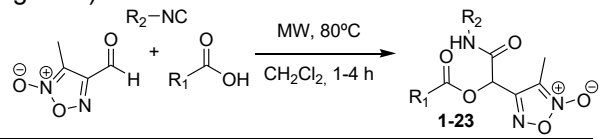
Key words: nitric oxide donors, furoxans, multicomponent reactions, Passerini reaction, anti-proliferative activity

Introduction

High intracellular levels of nitric oxide (NO) lead to cytotoxic effects against tumor cells.[1] In this context, organic compounds containing the 1,2,5-oxadiazole-2-oxide (furoxan) scaffold play an important role as exogenous NO donors under physiological conditions and, therefore, may act as potential antitumor agents.[2] Multicomponent reactions are considered useful synthetic tools for the rapid and efficient construction of structurally diverse organic compounds, such as furoxan hybrid molecules.[3] Herein, we report the Passerini multicomponent reaction applied to the synthesis of nitric oxide-releasing furoxan based alpha-acyloxy carboxamides as well as their NO release capacity and anti-proliferative activity.

Results and Discussion

Initially, a library of 23 structurally diverse a-acyloxy-(1,2,5-oxadiazole-2-oxide) carboxamides was synthesized in good to excellent yields (67-97%) via Passerini reaction between 3-methyl-4-furoxancarbaldehyde, aliphatic and aromatic carboxylic acids and isocyanides in dichloromethane under microwave heating at 80ºC for 1-4 hours (Figure 1).



1	R_1 = Me	R_2 = *t*Bu (84%)	**15**	R_1 = 3-Br-C_6H_4	R_2 = *t*Bu (92%)
2	R_1 = Et	R_2 = *t*Bu (87%)	**16**	R_1 = 2-Br-C_6H_4	R_2 = *t*Bu (92%)
3	R_1 = Pr	R_2 = *t*Bu (74%)	**17**	R_1 = 3-NO_2-C_6H_4	R_2 = *t*Bu (94%)
4	R_1 = 2-furanyl	R_2 = *t*Bu (89%)	**18**	R_1 = 2-NO_2-C_6H_4	R_2 = *t*Bu (95%)
5	R_1 = C_6H_5	R_2 = *t*Bu (91%)	**19**	R_1 = 2-Me-C_6H_4	
6	R_1 = 4-NO_2-C_6H_4	R_2 = *t*Bu (85%)		R_2 = CH_2CO_2Et	(95%)
7	R_1 = 4-OMe-C_6H_4	R_2 = *t*Bu (88%)	**20**	R_1 = 4-OMe-C_6H_4	
8	R_1 = 4-Me-C_6H_4	R_2 = *t*Bu (88%)		R_2 = CH_2CO_2Et	(87%)
9	R_1 = 4-Br-C_6H_4	R_2 = *t*Bu (94%)	**21**	R_1 = 4-OMe-C_6H_4	
10	R_1 = C_9H_{21}	R_2 = *t*Bu (91%)		R_2 = *c*C_6H_{11}	(93%)
11	R_1 = 3-Me-C_6H_4	R_2 = *t*Bu (87%)	**22**	R_1 = 2-Me-C_6H_4	
12	R_1 = 2-Me-C_6H_4	R_2 = *t*Bu (93%)		R_2 = *c*C_6H_{11}	(91%)
13	R_1 = 3-OMe-C_6H_4	R_2 = *t*Bu (97%)	**23**	R_1 = 4-OMe-C_6H_4	
14	R_1 = 2-OMe-C_6H_4	R_2 = *t*Bu (89%)		R_2 = 2-morpholinoethyl	(67%)

Figure 1: Scope of Passerini reaction.

The NO-releasing ability of compounds **1-23** was quantified by a chemiluminescent method in the presence of cysteine as a thiol-activating reagent. It was observed that all compounds were capable of releasing NO within the range of 0.8-7.4 μM (from a solution containing 100 μM of the compound). It is noteworthy that the isocyanide variation imposed interesting changes in the NO release ability of the studied a-acyloxy carboxamides. The use of ethyl isocyanoacetate for the synthesis of **19** and **20** led to the most active NO-releasing compounds (7,4 μM and 6,4 μM respectively). On the other hand, the products **21** and **22**, derived from cyclohexyl isocyanide, proved to be the less promising NO donors, releasing NO at very low concentrations (1.5 and 2.0 μM, respectively).

In vitro anti-proliferative activity of compounds **1-23** was evaluated against cells derived from breast adenocarcinoma (MCF-7), prostate adenocarcinoma (LNCaP), and malignant melanoma (SK-MEL-28), as well as normal cells (Primary Human Umbilical Vein Endothelial Cells - HUVEC), Among all tested compounds, **21** and **22** exhibited modest anti-proliferative activity against LNCaP cells with IC_{50} values of 95,5 μM and 66,6 μM respectively. It is noteworthy that none of the compounds tested so far showed significant anti-proliferative activity against the HUVEC cell line at their highest concentration (IC_{50} > 100 μM).

Conclusions

We reported the Passerini multicomponent reaction applied for the rapid and efficient synthesis of 23 α-acyloxy-(1,2,5-oxadiazole-2-oxide) carboxamides in good to excellent yields (67-97%) which produced levels of NO within the range of 0.8-7.4 μM. In vitro anti-proliferative experiments were carried out against three tumor cell lines and HUVEC normal cell line, which products **21** (IC_{50} = 95.5 μM) and **22** (IC_{50} = 66.6 μM) exhibited moderated anti-proliferative activity against LNCaP cells. This study demonstrated the potential of furoxan based α-acyloxy carboxamides as promising class of compounds to be explored for antitumor activity.

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Exploring 1,2-Naphthoquinone Derivatives for the development of Trypanocidal Agents

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Key words: Trypanosoma cruzi, naphthoquinones, phenols.

Introduction

Chagas disease, caused by *Trypanosoma cruzi*, is a neglected tropical disease,[1] affecting around 7 million people, particularly in underprivileged of Latin America regions.[2] The World Health Organization (WHO) recommends nifurtimox and benznidazole for acute-phase treatment, but their limited efficacy in chronic stages and significant side effects pose major therapeutic challenges.[3] Consequently, safer and more effective alternatives are urgently needed.

Naphthoquinone derivatives, such as aryloxy-1,4-naphthoquinones **1a–b** (**Figure**) show promising activity anti-*T. cruzi* activity, as evidenced by their low micromolar IC_{50} values. Based on these considerations, our research focuses on the synthesis of a series of aryloxy-1,2-naphthoquinone analogs **2a–h**, achieved by substituting the 1,4-naphthoquinone core of compounds **1a–b** with a 1,2-quinonoid scaffold[4].

Quinones often exert their biological activity through redox cycling, generating reactive oxygen species (ROS) that induce oxidative stress and cellular damage in parasites such as *T. cruzi*. Notably, the redox potential and efficiency of ROS production can vary between 1,2- and 1,4-quinone systems, potentially influencing the extent and specificity of oxidative damage inflicted on the parasite[5].

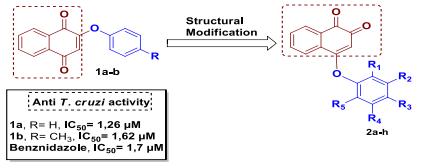
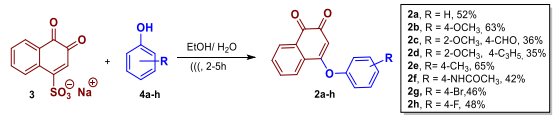


Figure. Strategy for the design of aryloxy-1,2-naphthoquinone derivatives **2a-h.**

Results and Discussion

The series of compounds **2a-h** was synthesized via nucleophilic substitution reaction between sodium 1,2-naphthoquinone-4-sulfonate (**3**) and phenolic derivatives (**4a-h**) in aqueous medium under ultrasonic irradiation (**Scheme**).



Scheme. Preparation of 4-aryloxy-1,2-naphthoquinone derivatives **2a-h.**

After silica gel column purification, yields ranged from 36–65%. The compounds **2a–h** were characterized by infrared spectroscopy (IR) and ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR).

Conclusion

In summary, compounds **2a–h** were synthesized in yields ranging from 36% to 65%, depending on the electronic characteristic of the phenolic derivatives **4a–h**. The antiparasitic potential of the target compounds will be evaluated, with a specific focus on their activity against the human pathogen *Trypanosoma cruzi*.

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Identification of potential selective BACE-1 inhibitors as Alzheimer's drug candidates through computational approaches

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Key words: Alzheimer, BACE-1, virtual-screening

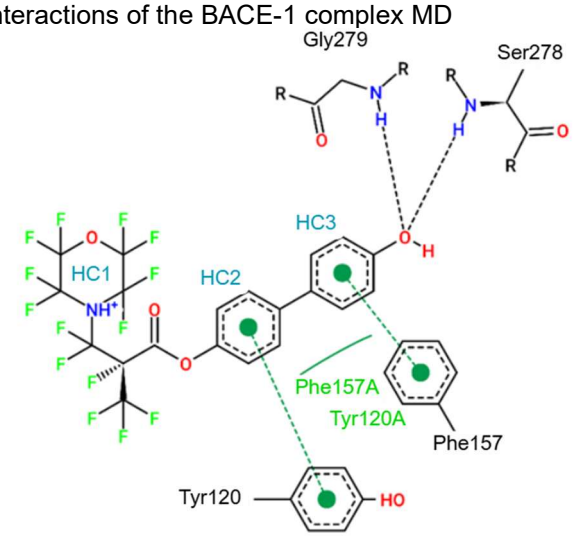
Introduction

Alzheimer's disease (AD) is a neurodegenerative disease with amyloid cascade as the main pathogenesis hypothesis, wherein β-amyloid peptides, generated by BACE-1 enzyme cleavage, are responsible for the disease. Therefore, BACE-1 has become a key target in AD treatment. BACE-1 has a homolog, BACE-2, which is unrelated to AD and involved in other pathways and so off-target inhibition can promote adverse effects. The present study aimed to identify stereo-electronic requirements for selective BACE-1 inhibitors using computational approaches. 214.446 molecules from the Sigma Aldrich® database obtained from the ZINC[15] database were applied in virtual screening with best pharmacophore models for BACE-1 and BACE-2 inhibitors, molecular docking in both enzymes with GOLD software and Molecular Dynamics (MD) with GROMACS.

Results and Discussion

Ten pharmacophore models for BACE-2 inhibitors were built with eight selective BACE-2 inhibitors (IC_{50} BACE-2 < 10.0 nM and Selective Index < 0.25) and validated with internal statistical parameters from GALAHAD and enrichment metric (AUC=0.83). The best pharmacophore model was used for the exclusion of molecules with QFIT > 0.0 at Sigma Aldrich database. The previously validated pharmacophore model for BACE-1 inhibitors was also applied (Do Bomfim *et al.,* 2023). Next, molecules with QFIT > 0.0 for BACE-1 and QFIT = 0.0 for BACE-2 were applied in molecular docking with both enzymes. In order to prioritize molecules with selective properties, a cutoff point was determined corresponding to the highest score achieved for known active inhibitors for BACE-1. Thus, ZINC98228398 (Z1), with elevated score for BACE-1 and the lowest score for BACE-2 were selected for MD. The RMSD was calculated for each system and revealed that the ligand can stabilize protein structures ($RMSD_{BACE\text{-}1}$ = 2,41 and $RMSD_{BACE\text{-}2}$ = 2,28). Finally, 2D plot of intermolecular interactions show that Z1 can stabilize at BACE-1 binding-site (Figure 1).

Figure 1. A representative snapshot of 2D binding interactions of the BACE-1 complex MD



The collective data showed the minimum requirements for BACE-1 inhibition: three hydrophobic centers (HC), two of them (HC2 and HC3) participate in π-stacking interactions with Phe157 and Tyr120; a positively charged group to form an electrostatic interaction with the catalytic aspartic acids; and groups capable of hydrogen bond interactions with Ser278 and Gly279. For selective inhibition, the distance between the hydrophobic centers (HC2 and HC3) could impact BACE-2 activity and capability of doing π-stacking interactions, These observations suggest that stereoelectronic effects play a key role in determining the selectivity profile.

Conclusions

With the use of virtual-screening methods combined with molecular dynamics, a potential BACE-1 selective inhibitor was identified and the steric requirements that can influence selectivity.

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Design, Synthesis, and *In Silico* Evaluation of Aurone Derivatives as Potential Antitumor Agents

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Key words: aurones, haspin kinase, docking molecular, antitumorais

Introduction

Cancer is a disease with high incidence and prevalence, remaining one of the leading causes of death worldwide. Despite significant advancements in anti-tumor therapy in recent years, there is still a critical need for new, more selective antineoplastic agents with fewer adverse effects. Haploid germline-specific nuclear protein kinase (Haspin) is a serine/threonine kinase that associates with chromosomes and specifically phosphorylates its sole substrate, histone H3, during mitosis. Haspin is overexpressed in cancer cells, making it a functional target for anti-cancer drugs. Furthermore, Haspin inhibition in tumor cell lines prevents chromosomal alignment and the completion of normal mitosis. Thus, more selective Haspin inhibitors could serve as novel antimitotic agents capable of preventing cancer cell proliferation while potentially causing fewer adverse effects compared to other anti-cancer agents.

Aurones, a subclass of flavonoids, are potent and highly selective Haspin inhibitors (IC_{50} = 0.15 nM), known to induce apoptosis and exhibit antiangiogenic and anti-tumor properties in human cancer cell lines. This study aims to synthesize novel aurones as candidates for new anti-tumor drugs, guided by molecular modeling studies and subsequent biological evaluation in anti-tumor cell lines

Results and Discussion

Initially, the 3D structure of *H. sapiens* Haspin kinase complexed with the inhibitor ZW282 (PDB: 7OPS) was retrieved from the Protein Data Bank. The binding mode and molecular interactions with important active site amino acid residues were evaluated. Redocking was performed using the GOLD SUITE program to validate the methodology. Subsequently, a chemical library of 160 aurones was subjected to molecular docking with Haspin, and their top-scoring poses were analyzed in comparison to known inhibitors. Aurones thatmaintained crucial interactions within the Haspin active site were selected, and their ADMET profiles were assessed through theoretical calculations. Molecules exhibiting superior physicochemical properties were then synthesized and will be submitted for antineoplastic activity evaluation.

Redocking calculations yielded an RMSD of 0.57 Å, validating the established methodology. In the molecular docking screening, out of 160 proposed aurones, 42 showed a binding mode similar to known protein inhibitors. ADMET profile analysis led to the exclusion of 12 compounds due to predicted *in silico* genotoxic (Ames) and cardiotoxic risks. Consequently, 30 aurones were selected for synthesis via aldol condensation between 5-hydroxybenzofuranone and corresponding heteroaromatic aldehydes, achieving yields ranging from 20% to 60%.

Conclusions

A virtual screening of a chemical library containing 160 aurones led to the selection of 20 compounds. These selected aurones adhered to the pharmacophore model and maintained crucial chemical interactions with key amino acid residues in the enzyme's active site. Furthermore, they did not present an *in silico* genotoxic or cardiotoxic risk. The synthesized aurones, featuring nitrogenated heterocycles in ring B and diverse substitution patterns in ring A, were obtained with yields ranging from 20% to 60%. These compounds will be evaluated for their antineoplastic activity against solid tumor cell lines. Through this rational drug design approach, we expect to identify aurones with potential as novel anti-tumor agents.

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Rational design and synthesis of bis-1,2,3-triazoles with potential biological activity against SARS-CoV-2 Mpro

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Agustina Grich *submitted the work.*

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Key words: COVID-19, SARS-CoV-2, Main protease, inhibitors, triazoles.

Introduction

The pandemic caused by SARS-CoV-2 continues to have a significant global health impact. Several therapeutic targets have been described for the treatment of SARS-CoV-2 through antiviral drug design. Among the most extensively studied is its main protease (Mpro), which is responsible for selectively hydrolyzing the viral polyprotein to generate the functional proteins required for the normal viral replication cycle.[1] This protein is highly conserved and shares strong homology across different coronaviruses, making its inhibitors potential broad-spectrum antiviral agents. Nirmatrelvir was the first covalent inhibitor of this enzyme approved by the FDA in late 2021 under emergency use authorization for the treatment of moderate to severe COVID-19 cases. One drawback of this drug is its suboptimal biopharmaceutical performance, largely due to the presence of peptide bonds, which negatively affect metabolic stability and bioavailability.[2]

The main objective of this work is design and develop a synthetic methodology towards selected bis-1,2,3-triazoles as potential targeted covalent inhibitors of SARS-CoV-2 main protease (Mpro) with improved pharmacokinetic properties compared to the drugs currently on the market.

Results and Discussion

The initial *in silico* studies resulted in the identification of a library of compounds that all maintain the bis-1,2,3-triazole structure (**Figura 1**), but differ in the warhead and the substituents R^2 and R^4. A focused virtual screening of the eMolecules library was carried out to select the substituents mentioned above. Tidyscreen[3] were used to filter the library based on drug-like properties, synthetic feasibility, and compatibility with the structural and electronic requirements of the receptor subsites involved in binding. The selection criteria were guided both by the constraints of the planned synthetic routes and by the specific features of the target binding pocket.

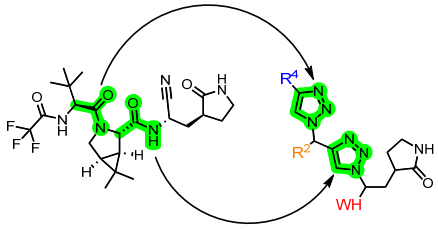


Figure 1: Bis-1,2,3-triazole scaffold of target compounds.

To identify the most potent binders, molecular docking was conducted in silico to predict the binding interactions of the proposed compounds with the Mpro binding site.

Molecular dynamics were employed to assess the stability and persistence of interactions between the designed inhibitors and the protein over time, complementing the data obtained from molecular docking. To quantitatively analyse these interactions, the Protein-Ligand Interaction Fingerprints (ProLIF) tool was used to generate interaction profiles from the docked protein-ligand complexes.

Several synthetic routes were designed and tested, and after iterative optimization, a final synthetic plan was established to obtain these kind of compounds.

Conclusions

A rational design and screening approach led to the selection of bis-1,2,3-triazole candidates with favorable properties. A viable synthetic route was established and will be used to obtain the selected compounds.

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Prospecting selective ligands of nucleoside hydrolase from green propolis through affinity selection mass spectrometry

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Key words: Neglected Tropical Diseases, Leishmania donovani, Natural Products, High throughput.

Introduction

The search for new therapeutic options against leishmaniasis remains a global health priority. Endemic in Brazil, visceral leishmaniasis (VL) is the most severe form of the disease and is fatal in up to 95% of untreated cases.[1] *Leishmania donovani* (*Ld*), main causative agent of VL, lacks the *de novo* purine synthesis and relies entirely on the purine salvage pathway. In this context, nucleoside hydrolase (NH) has emerged as a promising selective target for drug discovery due its key role in the purine salvage pathway.[2]

In this study, we employed Affinity Selection Mass Spectrometry (AS-MS) as a high throughput screening assay strategy to investigate ligands of *Ld*NH. Green propolis, a natural product rich in bioactive compounds, was selected for investigation due to its well-documented antimicrobial and antiparasitic properties[3], making it a promising candidate for ligand discovery.

Results and Discussion

Preliminary inhibition assays at 200 µg/mL showed that green propolis and geopropolis extracts inhibited LdNH activity by 59.0%-82.4%, and 28.3%-60.0%, respectively. Ethanolic and methanolic extracts of green propolis displayed IC_{50} of 0.66 ± 0.022 µg/mL and 40.3 ± 6.31 µg/mL, respectively. The ethanolic extract (EEP), which showed the highest inhibitory potential, was selected for further investigation by AS-MS. The enzyme *Ld*NH was immobilized in magnetic particles as reported in previous work[4] and incubated with EEP at 2 mg/mL for 15 minutes at room temperature. After magnetic separation, the supernatant containing unbound compounds was collected. High-affinity ligands were eluted using methanol under heat for 20 min to ensure complete dissociation of the protein–ligand complex. As a control assay, the same procedure was performed using heat-inactivated *Ld*NH to assess non-specific binding.

Bound compounds, named ligands, were analyzed by liquid chromatography-high resolution mass spectrometry (LC-HRMS). Data analysis revealed 18 selective ligands of *Ld*NH. Four of these have been annotated via the GNPS platform (Table 1). The remaining compounds are currently under analysis using spectral databases, including MassBank, and reference data from propolis LC-MS literature for tentative structural identification. The affinity ratio (AR) was determined by dividing the ligand peak area from S4 incubated with active *Ld*NH by the corresponding area from S4 incubated with inactive *Ld*NH. Only ligands with AR values ≥ 1.20 were considered potential binders.

Table 1. LC-HRMS/MS analysis in negative mode and structure annotation for the identified ligands.

RT (min)	*m/z*	AR	Annotation
4.47	353.0877	1.62	neochlorogenic acid
6.46	367.1034	1.76	methyl (1*S*,3*R*,4*R*,5*R*)-3-[(*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylate
11.07	515.1183	1.27	cynarine
37.83	329.0662	1.36	tricin

Once all structures are elucidated, the most promising ligands, those exhibiting the highest affinity for *Ld*NH, will be further evaluated for their inhibitory activity to validate their potential as enzyme inhibitors.

Conclusions

AS-MS proved to be a powerful strategy for selectively identifying bioactive ligands directly from complex natural matrices, such as propolis, underscoring its utility in accelerating early-stage drug discovery efforts.

Acknowledgements

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Novel potential drug scaffold against visceral Leishmaniasis discovered using a drug repurposing approach.

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Key words: Leishmania, amastigotes, CNS drugs, tricyclics compounds.

Introduction

From a repositioning approach of drugs selected based in their availability in the market of developing countries, we are looking for new drugs scaffolds for the treatment of visceral leishmaniasis (LV)[1].
After tested the drugs in models of VL employing a reference cell line of *L. infantum* (MHOM/MA/67/ITMAP263 strain) most of the bioactive chemical entities showed a tricyclic core and were originally developed to target central nervous system (CNS)[2]. The structural features of these compounds include polycyclic frameworks, basic amine moieties, and varying degrees of lipophilicity, which may contribute to their ability to first, send and concentrate the compounds to the phagolysosome[3] and second, interact with intracellular targets in the parasite.

Results and Discussion

A comparative analysis performed with 20 drugs against extracellular promastigote[4] and intracellular amastigote stages (infecting human macrophages derived from THP-1 monocytes and mouse Bone Marrow Derived Macrophages)[5], showed six of them with inhibitory concentrations (IC_{50}) ranging from 1 to 13 µM against amastigotes (with slow to null proliferation rate). These compounds share common structural motifs such as polycyclic aromatic systems (tricyclic nitrogen-containing rings), and halogen substitutions. The most potent compounds also contain-alkyl amine (secondary or tertiary) substituents that may favor phagolysosome accumulation.
In addition to the evaluation against intracellular amastigotes, leishmanicidal activity was also assessed against the promastigote stage (with rapid proliferation rate). The selected subset drugs demonstrated activity ranging from 17.0 to 75.0 µM. These findings suggest a potential stage-specific profile of action, which should be considered in further mechanistic studies. This study suggests that CNS-active scaffolds may be suitable templates for anti-leishmanial drug design.

Conclusions

The results highlight that specific structural features such as as the presence of tricyclic systems, basic nitrogen atoms, and hydrophobic substituents are associated with increased leishmanicidal activity. These findings support the potential repurposing of CNS-related scaffolds for the development of new antileishmanial agents.

Acknowledgements

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Molecular Modeling study of cruzain enzyme inhibition by 2-acetamidotiophene-3-carboxamide derivatives.

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Key words: Chagas Disease, Cruzain, Molecular Modeling, Molecular Dynamics, Non-covalent inhibitors.

Introduction

Chagas disease is a neglected tropical disease caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), responsible for approximately 12,000 deaths annually. Current therapies, such as nifurtimox and benznidazole, have limited effectiveness during the chronic phase and cause significant adverse effects, underscoring the urgent demand for safer and more effective therapeutic alternatives (Pérez-Molina, Molina, 2018).

In this context, cruzain (CRZ) emerges as a promising therapeutic target due to its expression in all life forms of *T. cruzi* metacyclogenesis and its essential role in parasite survival. Non-covalent inhibitors derived from the 2-acetamidothiophene-3-carboxamide class have already demonstrated potent trypanocidal activity with low mammalian toxicity (Wiggers et al, 2013). The present study aims to investigate the inhibition mechanism of CRZ by five selected derivatives of this class using Molecular Modeling techniques.

Results and Discussion

The docking protocol was validated by the redocking method using a cristallographic structure retrieved from the Protein Data Bank (PDB ID: 4KLB), consisting of the ligand AC1 bound to the cruzain enzyme. The softwares employed were the AutoDock Vina 1.2.0, AutoDock-GPU and DOCK6, and the top scored poses of 100 replicates for each program were evaluated. These validated protocols achieved pose accuracies (RMSD ≤ 2.0Å compared to the reference crystallographic pose) of 86%, 90% and 88%, respectively.

Subsequently, additional CRZ-ligand complexes were obtained through molecular docking of the remaining four compounds (AC2-5) selected from the work of Wiggers et al. (2013). The dockings were performed in decaplicates using the previously validated parameters. The ligands AC2-5 showed different hydrogen bonds when compared with the co-cristallyzed ligang AC1, interacting mostly with residues Ser61, Gly66 and Asn70. Ligand AC2 generally formed more hydrogen bonds than the other ligands. By using three different programs it was obtained a variety of possible ligand-enzyme complexes. After a group similarity analysis the best poses were selected.

The most promising complexes for each ligand were then subjected to Molecular Dynamics (MD) simulations using GROMACS 2024.1, performed in triplicate for a duration of 300 ns. System preparation for MD consisted of exclusion of water molecules from the tridimensional enzymatic structure, residue protonation adjustments (pH 5.5) and charge equilibration. Preliminary analysis suggest that the interaction with this class of ligands do not change significatively cruzain secondary structure or radius of gyration, and hydrogen bonds seem to be critical for ligand binding to the enzyme active site. Subsequent analyses will focus on enzyme structural dynamics and detailed enzyme-ligand interaction profiles.

Conclusions

The MD simulations are expected to provide detailed insights into the inhibition profile of the evaluated compounds, elucidating the molecular mechanism underlying CRZ inhibition. These findings may significantly enhance our understanding of CRZ inhibition mechanism and aid the rational design of novel inhibitors with improved anti-Chagasic activity.

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The authors thank the Brazilian governmental agencies CAPES, CNPq and Fiocruz for the funding provided.

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Virtual screening approaches for identification of novel cruzain inhibitors

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Key words: Cruzain, Virtual Screening, Molecular Docking, Molecular Dynamics, In Silico ADMET Evaluation.

Introduction

Chagas disease, an anthropozoonosis caused by the protozoan parasite *Trypanosoma cruzi*, remains a major global public health concern due to high infection rates and significant mortality. While existing treatments exhibit effectiveness during the acute phase, their efficacy substantially decreases in the chronic stage, highlighting the urgent need for novel therapeutic alternatives[1]. Among promising molecular targets, cruzain (CRZ) has emerged as particularly attractive because of its critical role throughout the parasite's life cycle. This study applied computational techniques, includiing virtual screening and Molecular Dynamics (MD) simulations, to explore structural and dynamic aspects of CRZ inhibition by five previously identified carbamoyl imidazole inhibitors[2]. Structural modifications aimed at enhancing inhibitor potency and safety profiles were explored.

Results and Discussion

Initially, structural modifications were conducted using the SwissBioisostere server, resulting in 9,078 novel derivatives candidates. These compounds underwent virtual screening performed in triplicate using complementary docking software packages (Molegro, Dock6, and Autodock Vina). The compounds were ranked using the Exponential Consensus Ranking methodology, and the top 91 candidates were further assessed for absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties using *pkCSM* and *ADMETlab2.0* platforms, resulting in the selection of 10 lead candidates. Subsequent MD simulations of the 10 selected compounds were carried out in triplicate using *Gromacs 2024.1* for 300 ns. RMSD analysis revealed minor positional changes of inhibitors within the active site without detachment. The binding of inhibitors did not significantly alter enzyme flexibility, protein compactness, or secondary structure throughout the simulation. Predominant hydrogen bond interactions were identified between the inhibitors and CRZ, resulting in subtle changes in the enzyme's movement patterns, indicative of enzyme inhibition. Binding free energy calculations were performed using the MM/GBSA method, selected for its favorable balance between computational efficiency and reasonable predictive accuracy in estimating ligand–receptor interactions. Although MM/GBSA does not provide absolute free energy values with high accuracy, it is widely accepted for ranking ligands based on relative affinities. The method was applied to representative snapshots from molecular dynamics simulations to account for conformational flexibility, and the results confirmed strong affinity for the enzyme's active site.

Table 1. Theoretical pIC_{50} values and their binding free energies calculated by MMGBSA (ΔG1= replica1, ΔG2= replica2, ΔG3= replica3, ΔG_{bind}= average and standard deviation).

Inhibitor	ΔG1 (kcal/mol	ΔG2 (kcal/mol	ΔG3 (kcal/mol	ΔG_{bind} (kcal/mol)	PIC_{50}
B95	-22.85	-20.51	-21.60	-21.65 ± 1.17	5.98
CRZ1	-16.23	-17.05	-16.72	-16.67 ± 0.41	5.18
CRZ2	-19.87	-21.77	-21.80	-21.15 ± 1.11	5.63
CRZ3	-22.65	-22.27	-22.77	-22.56 ± 0.26	5.61
CRZ4	-19.17	-15.30	-16.14	-16.87 ± 2.04	4.65
CRZ5	-23.51	-24.09	-23.57	-23.72 ± 0.32	5.20
CB-2501	-26.78	-26.95	-23.96	-25.90 ± 1.68	5.64
CB-2815	-24.48	-23.68	-21.34	-23.17 ± 1.63	5.29
CB-2977	-15.13	-15.43	-15.01	-15.19 ± 0.22	5.57
CB-6366	-14.78	-19.37	-15.79	-16.65 ± 2.41	6.03
CB-7328	-19.69	-19.80	-18.69	-19.39 ± 0.61	5.69
CB-7553	-13.27	-14.93	-19.69	-15.96 ± 3.33	5.45
CB-8278	-21.00	-17.02	-20.62	-19.55 ± 2.2	6.20
CB-8703	-20.07	-20.22	-21.11	-20.47 ± 0.56	6.01
CB-8743	-27.35	-26.57	-26.00	-26.64 ± 0.68	5.69
CB-8966	-20.20	-24.29	-20.90	-21.80 ± 2.19	5.36

Conclusions

In conclusion, this virtual screening approach successfully identified ten novel carbamoyl imidazole derivatives promising potency and favorable safety profiles, suitable for progression to experimental

validation and further development as anti-Chagasic agents.

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Natural products as platforms for molecular diversification: synthetic approaches to anti-infective compounds

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Key words: Natural compounds, biological activity, Complexity to Diversity, bioguided fractionation, anti-infectives.

Introduction

Natural products (NPs) and their derivatives are used in the treatment of numerous diseases.[1] Most of these compounds are structurally complex with a high percentage of sp^3-hybridized carbon atoms and multiple stereogenic centers.
Currently, there is renewed interest not only in the search for new bioactive cores, but also in the development of strategies for constructing large collections of compounds capable of mimicking the complexity of NPs. These molecules have historically been crucial in the identification and development of anti-infective agents. Interest in these systems has increased in recent years, but the rapid emergence and widespread spread of resistance has forced us to re-evaluate them as a means of identifying new chemical skeletons with anti-infective activity for drug development.

Results and Discussion

Our work is based on a massive screening of plant species using bioguided fractionation based on their evaluation as anti-infectives (antibacterial, antifungal, antiparasitic).
Once the pure bioactive compounds have been identified, a viable approach is used to create diverse compounds with high structural and stereochemical complexity. To this end, initially, ring distortion strategies are applied to these compounds;[3] subsequently, classical derivatization methodologies are applied to the obtained nuclei to generate compounds with a wide structural diversity. The anti-infective activity of the compound families obtained is evaluated in sensitive and resistant strains, proposing structure-activity relationships useful for the synthesis of new, more potent and less toxic compounds.[4,5]
This work presents the results obtained on sesquiterpene lactones and alkaloids isolated and derivatized following this multidisciplinary approach (see general workflow in Figure 1).
Following this logic for one of the most promising hits, we synthesized a series of non-previously reported rationally designed solanocapsine (a steroidal alkaloid)[6] derivatives using different synthetic methodologies.[7] These derivatives, along with the natural precursor, were evaluated against clinically relevant bacterial strains, including multidrug-resistant pathogens, to establish valuable structure-activity relationships. The results revealed potent antibacterial activity; certain derivatives exhibiting enhanced efficacy compared to the parent compound. Additionally, synergistic interactions with conventional antibiotics were explored, further highlighting their therapeutic potential.

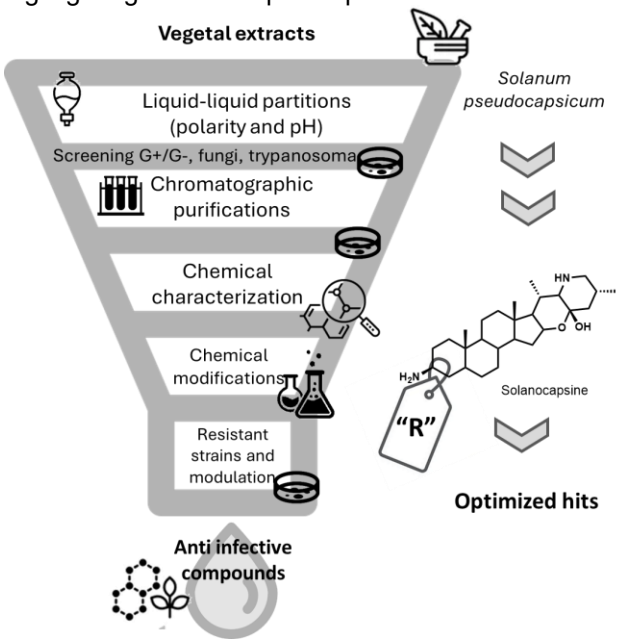


Figure 1: General workflow for anti-infective complementary bioguided fractionation.

Conclusions

Since the combinatorial chemical approaches employed in recent decades have not produced effective drugs, we propose that the development of new anti-infectives based on natural structures with proven antibacterial activity is the best strategy with the greatest possibility of success to address the growing crisis of resistance to these compounds.

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Development of New Potential Multi-Target Agents for the Inhibition of HIV-1 Replication

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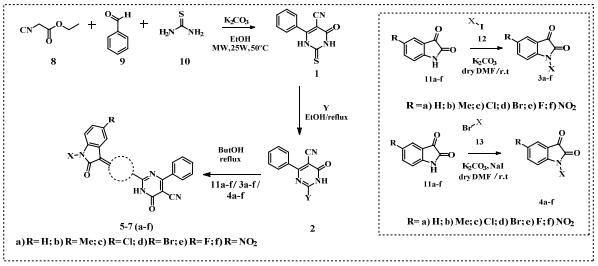
Keywords: AIDS, HIV, Multi-target, pyrimidinones, isatin

Introduction

The HIV replication cycle involves several events that are exclusively associated with viral components, which represent key targets for chemotherapeutic intervention [1]. Currently, first-line antiretroviral therapies target different viral enzymes, such as reverse transcriptase (RT) and integrase. However, the clinical efficacy of these drugs has been compromised by the emergence of resistant viral strains, often linked to poor treatment adherence. As a result, the development of innovative compounds, particularly multi-target agents, has become increasingly important. Multi-target drug design is a promising strategy that aims to simultaneously modulate multiple viral targets, potentially enhancing treatment efficacy, improving patient adherence, and reducing the likelihood of resistance development [2]. In this context, the present study aims to synthesize and evaluate the antiretroviral and cytotoxic activities of eighteen novel compounds with potential dual activity against HIV-1 RT and integrase. Notably, the catalytic site of RT shares topological similarities with the catalytic domain of integrase, making these two enzymes particularly attractive for the development of multi-target inhibitors [3].

Results and Discussion

The synthetic methodology for the preparation of the target compounds involves three sequential steps. First, the Biginelli reaction is employed to construct the pyrimidinone core. This is followed by an addition–elimination reaction. Finally, the resulting key intermediate undergoes a bimolecular nucleophilic substitution (S_N2) reaction with the respective isatin derivatives, which are synthesized in parallel. To date, fourteen intermediates (**1**, **2**, **3a–f**, and **4a–f**) have been successfully obtained, with reaction yields ranging from 58% to 92%, along with six final products (**5a–f**), with yields ranging from 59% to 75%. The structures of all compounds were confirmed by spectroscopic and spectrometric analyses.



Scheme 1. Synthetic methodology for the preparation of final compounds **5a–f**, **6a–f**, and **7a–f**.

Conclusions

The compounds proposed in this study were designed using the molecular hybridization strategy of Medicinal Chemistry, combining scaffolds with well-established antiretroviral activity as pharmacophoric fragments. To date, the synthetic route has proven feasible, and the project timeline remains on track. Upon completion of the synthesis and characterization of all targets final compounds **5a–f**, **6a–f**, and **7a–f**, the entire series will be evaluated for antiretroviral activity and cytotoxicity profiles.

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New biphenylpyrimidine derivatives as ponatinib analogs, designed as potential inhibitors of the Bcr-Abl1 tyrosine kinase with the T315I mutation

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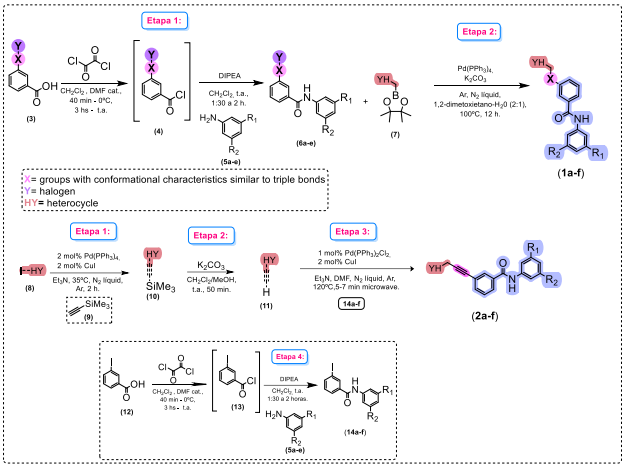
Keywords: Tyrosine kinase inhibitors, chronic myeloid leukemia, T315I mutation, ponatinib.

Introduction

The dysregulation of protein kinases plays a critical role in the pathogenesis of various cancers, with the Bcr-Abl1 oncoprotein being a key therapeutic target in chronic myeloid leukemia (CML).[1] Although imatinib was the first tyrosine kinase inhibitor (TKI) approved for CML, its effectiveness is limited by the emergence of resistance-conferring mutations, such as T315I.[2] Ponatinib, currently the only adenine-mimetic TKI effective against this mutation, is associated with severe adverse effects, including arterial occlusions and increased risk of mortality.[3] In this context, the present project proposes the development of 12 novel ponatinib derivatives incorporating isosteric modifications in the adenine-mimetic moiety, aiming to enhance selectivity and reduce toxicity. Two compound series based on heterocycles scaffolds were designed to improve binding affinity toward both the wild-type and T315I-mutant forms of Bcr-Abl1. The compounds were synthesized via a convergent approach involving two to four synthetic steps. This work aims to identify more effective and safer therapeutic candidates for the treatment of CML.

Results and Discussion

A convergent synthetic route was developed for the preparation of 12 novel derivatives, grouped into series **1a–f** and **2a–f**, involving two to four reaction steps. Series **1a–f** was synthesized from biphenyl-3-benzoic acid (**3**), which was converted into the corresponding acid chloride (**4**) and subsequently coupled with substituted anilines (**5a–e**), followed by Suzuki cross-coupling reactions with boronic acid derivatives. Series **2a–f** was obtained through a sequence comprising Sonogashira couplings of iodinated intermediates (**8**), desilylation of trimethylsilyl groups (**10**), amide bond formation with 3-iodobenzoic acid (**12**), and final Sonogashira couplings. These synthetic strategies enabled the efficient construction of the target compounds, as illustrated in Scheme 1.



Scheme 1. Proposed synthetic methodology for the new ponatinib derivatives **1a–f** and **2a-f**.

All 12 novel ponatinib derivatives were successfully synthesized, with isolated yields ranging from 39% to 80%. The compounds were fully characterized by electrospray ionization mass spectrometry (ESI-MS), infrared (IR) spectroscopy, and proton, carbon-13, and fluorine-19 nuclear magnetic resonance (^{1}H, ^{13}C, and ^{19}F NMR) spectroscopy. Chromatographic purity was determined by high-performance liquid chromatography (HPLC).

Conclusions

The novel ponatinib derivatives, designed through isosteric modifications in the adenine-mimetic region, were synthesized, fully characterized, and initially evaluated in K562 and HEK 293 cell lines, showing promising results. Currently, further cytotoxicity assessments are being conducted in HEK 293 cells, and enzymatic assays against both ABL1^{wt} and ABL1^{T315I} isoforms are underway.

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Alkaloids as Potential Acaricidal Agents Against *Rhipicephalus microplus* Acetylcholinesterase: A Hierarchical Virtual Screening Approach

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Key words:*Virtual screening; Natural acaricidal compounds; Acetylcholinesterase Inhibitors.*

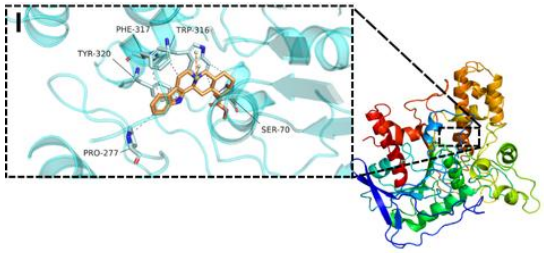
Introduction

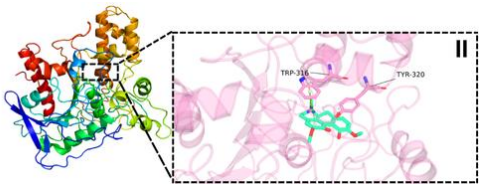
Infestations by *Rhipicephalus microplus* (Acari: Ixodidae) cause severe economic losses in livestock through reduced productivity, mortality, and pathogen transmission. With synthetic acaricide resistance now affecting over 90% of farms in endemic regions of Brazil there is an urgent need for novel alternatives.[1] The objective of this study was to evaluate the inhibitory activity of alkaloids against *R. microplus* acetylcholinesterase (RmAChE) through an in silico hierarchical virtual screening approach, employing computational OpenEye tools (vROCS and FRED). This approach aims to develop safer and more effective acaricides.

Results and Discussion

Twenty-six structures with biological activity against *R. microplus* or other organisms were selected, organized by their enzyme activity (Ki/IC50) or lethal dose (LD50) values and seven commercially available acaricidal drugs were also included. Shape-based superposition method was used for model design in vROCS program. The 33 inhibitors were randomly divided into 11 for the training set (GT) and 22 for the test set (GA). Eleven models were designed, and the model based on the Trichlorfon molecule was considered the best, with a Fitted ROC Area = 0.70. In the virtual screening, 20 alkaloids from the NatProDB database were submitted to the shape-based model. The top ranked alkaloids by molecular docking were yohimbine (-17.45) and magnoflorine (-16.25).

Figure 1: Representation of intermolecular interactions of the yohimbine-RmAChE (I) and magnoflorine-RmAChE (II) complexes.





The yohimbine-RmAChE complex (Figure 1-I) exhibits hydrophobic interactions between yohimbine's C11/C19 and Trp316, and between its quinolizidine structure and Phe317, similar to studies with a known acaricide.[2] A π-cation interaction occurred between the N-heterocyclic and Trp316. Additionally, a hydrophobic interaction was observed between Tyr320 and C6 of yohimbine. A donor hydrogen bond formed between the hydroxyl group of yohimbine and the oxygen of Ser70. Conversely, the magnoflorine-RmAChE complex (Figure 1-II) displays parallel π-stacking between Trp316 and magnoflorine's guaiacyl ring, π-cation interactions between the quaternary amine and the indole ring of Trp316, and a hydrophobic interaction between C4 of the isoquinoline nucleus and the aromatic ring of Tyr320.

Hydrophobic forces, particularly between aromatic rings and Phe317/Tyr320, are crucial for effective AchE inhibition. Figure 1-II further supports existing literature, emphasizing the significance of π-stacking, π-cation, and hydrophobic interactions for the stability and inhibitory potential of these compounds on RmAChE.[2]

Conclusions

Yohimbine and magnoflorine shows promise as new acaricides against R. microplus AChE, potentially reducing reliance on synthetic antiparasitics. Future research will support its activity via molecular dynamics simulations.

Acknowledgements

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Identifying candidate inhibitors of *Trypanosoma cruzi* proteasomefrom LASSBio chemical library byMolecular docking

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Key words: Molecular docking, Proteasome, Chagas disease, Virtual screening

Introduction

Chagas disease, caused by the protozoan *Trypanosoma cruzi,* affects approximately six to seven million people worldwide and leads to over 10,000 deaths annually[1]. The proteasome, a multi-subunit enzyme essential to the ubiquitin-proteasome system[2], represents a promising target fordrug development. While some proteasome inhibitors are already approved for cancer treatment **(9),** others are being explored for neglected tropical diseases such as leishmaniasis, Chagas disease **(1,2,3,5,6,7,8,10)** and sleeping sickness**(4)**[3]**(Fig.1)**. This study aimed to: I)Investigate by molecular docking the interaction of known proteasome inhibitors with the substrate-binding site of the *T. cruzi* proteasome; and II) Conduct a virtual screening of compounds from the LASSBio chemical library to identify potential new inhibitors.

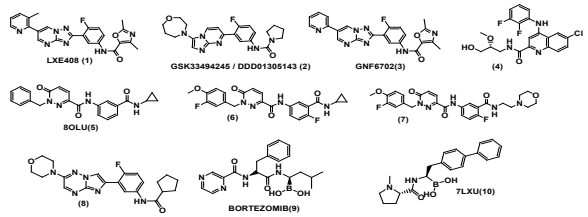


Figure 1: Chemical structures of known proteasome inhibitors selected for molecular docking into *T.cruzi* proteasome.

Results and Discussion

A redocking study was performed using the proteasome inhibitor LXE408 **(1)** in the active site of the *Leishmania tarentolae*proteasome**(PDB ID:6CTZ)**, employing GOLD 2023.2.0 (CCDC)[4]. The binding site was defined as an 18 Å radius around the Phe24 residue. The *L. tarentolae*proteasome shares 86.6% sequence identity with *T. cruzi* proteasome. Subsequently, the *T. cruzi* proteasome structure**(PDB ID:9F9T)** was used for molecular docking of ten selected compounds, using the GoldScore scoring function. Hydrogen atoms were added and water molecules removed from the crystallographic structure prior to docking.
Following this initial analysis with know inhibitors, a virtual screening was conducted with the *T. cruzi* proteasome using 1,931 compounds from the LASSBio chemical library, employing the same docking protocol with GOLD program[5]. The top 10 best results are presented in **table 1** and in **fig. 2**.

Table 1. Score values (GoldScore) of the 10 best compounds from the LASSBio chemical library docked into the substrate binding site of the *T. cruzi* proteasome.

COMPOUND	SCORE	COMPOUND	SCORE
LASSBio 458	83.94	LASSBio 460	80.40
LASSBio 1762	82.44	LASSBio 1944	80.26
LASSBio 864	81.89	LASSBio 863	80.05
LASSBio 845	81.70	LASSBio 1723	79.89
LASSBio 309	80.80	LASSBio 1616	78.87

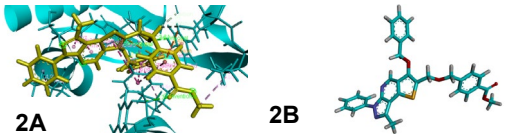


Figure 2: **2A**: Predicted interactions with the target. Compound LASSBio 458.**2B**:3D structure of coumpound **LASSBio 458.**

Conclusions

The redocking analysis revealed that GoldScore was the most accurate scoring function, presenting the lowest RMSD value (1.9 Å), compared to ChemScore (10.3 Å), ChemPLP (3.8 Å), and ASP (7.4 Å). All known proteasome inhibitors from the literature showed favorable predicted interactions with the proteasome binding site. Among them, the top-ranked compounds were compounds **6** (score: 79.66),**3** (score: 79.21) and **1** (score: 78.31). Remarkably, nine out of the ten best-ranked compounds from the LASSBio chemical library obtained higher docking scores than the literature inhibitors. **LASSBio 458** achieved the highest score (83.94). In its best docking pose, **LASSBio 458** formed hydrogen bonds with residues Tyr223 and Gly239 from chain L and Tyr 25 from chain K. In addition, π-stacking interactions were observed with Phe24, Tyr223 and Gly239 **(Fig. 2A,2B)**.

Acknowledgements

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IDENTIFICATION AND CHARACTERIZATION OF POTENTIAL ALLOSTERIC SITES FOR DESIGNING SELECTIVE INHIBITORS OF SRPK1 AND SRPK2

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Key words: Allosteric sites, Cancer, SRPK1, SRPK2.

Introduction

Tumor-related diseases are among the major groups of diseases requiring new therapies. SRPK1 and SRPK2 kinases play key roles for tumor development. SRPK1 acts in the VEGF regulatory pathway, associated with angiogenesis and tumor growth[1]. SRPK2 plays an important role in cell cycle regulation and apoptosis[2]. Overexpression of both kinases are associated with several types of cancer. Previous studies showed that, depending on the biochemical context, the inhibition of SRPK1 can promote or prevent tumor growth[3], highlighting the importance of selective inhibitors for each kinase. However, there are no selective inhibitors for SRPK1 or SRPK2. All inhibitors developed for SRPK1 share residual activity in SRPK2, due to the similarity of their orthosteric sites. Searching for allosteric sites in both kinases represents a possibility to develop selective inhibitors for SRPK1 and SRPK2. The main objective of this study is to identify and characterize potential allosteric sites in SRPK1 e SRPK2 that allows the screening and development of selective inhibitors for both kinases.

Results and Discussion

Atomic coordinates of both kinases were obtained from the Protein Data Bank under IDs 5MY8 (SRPK1) and 2X7G (SRPK2). Regions without electron density were modelled through SwissModel. A Normal Mode Analysis revealed that the 14 lowest-frequency modes (modes 7 to 20) accounted for approximately 80% of the motions in both kinases and revealed a strong correlation between distant domains in these enzymes. Additionally, the modes for the *apo* and *holo* states were highly similar, suggesting that the presence of the ligand in the ATP-binding site does not significantly restrict the global motions of the kinases. Based on that, we selected modes 7 to 20 in *apo* states for further analysis. For these 14 Modes, we created ensembles of energetically favorable conformations through energy minimizations and displacing the original conformation in steps of 0.2 Å, ranging from -2.0 to 2.0 Å. From these 14 favorable ensembles, we selected six with the highest conformational diversity observed through Principal Component Analysis to check for cavities. We found four cavities with allosteric potential in both kinases and were named Docking groove (already described in previous studies), DLG-site, C-site, and EVL-site. We combined one global mode (7 to 20) and two localized modes (21 to 100) to optimize opening movements of the cavities. Moreover, we observed that the EVL-site showed 58.82% of identity between SRPK1 and SRPK2, and the C-site showed an identity of 80%, representing a potential of selectivity in each kinase. On the other hand, the docking groove and the DLG-site were more similar between the kinases, showing an identity of 88% and 100%, respectively. A consurf analysis revealed that EVL-site, C-site, and docking groove are cavities with highly variable residues among 404 human protein kinases, also representing a potential of selectivity among kinases. Electrostatic potential evidenced that chemical space in EVL-site is greatly diverse between SRPK1 and SRPK2, and slightly different for C-site. SASA analysis showed that residues in all four sites are highly exposed to solvent and are accessible to inhibitors. Lastly, an ESSA analysis revealed that all four sites, in both kinases, have residues that, when interacting with a ligand, have the potential to modulate protein essential dynamics, indicating a potential allosteric activity. Based on these results, we hypothesize that EVL-site, and C-site exhibit the highest potential for mediating allosteric mechanisms involved in the selective inhibition of SRPK1 and SRPK2.

Conclusions

In conclusion, we characterized four cavities in SRPK1 and SRPK2 that possess sufficient volume to accommodate a ligand, exhibit lower identity compared to the ATP-binding site, contain variable residues across the protein kinase family, differ in chemical space, are solvent-exposed, and include residues with potential allosteric activity. Together, these results show that we found cavities that potentially can allow us to design selective inhibitors for SRPK1 and SRPK2.

Acknowledgements

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Discovery of novel calcineurin inhibitors via structure-based virtual screening for immunosuppressive therapy

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Keywords: calcineurin, virtual screening, docking, immunosuppressant.

Introduction

Calcineurin (CaN) is a cytosolic protein responsible for the activation of nuclear factor of activated T cells (NFAT), which promotes T cell activation. CaN is the target of immunosuppressants such as tacrolimus and cyclosporine. Its inhibition prevents graft rejection since T cell activation can lead to the rejection of transplanted organs.[1] However, chronic use of immunosuppressants can cause serious adverse effects, including neurotoxicity, nephrotoxicity, and metabolic disorders. Therefore, the development of new drugs with milder side effects could improve the quality of life for transplanted patients.[2] The virtual screening methodology provides a robust and efficient approach for identifying novel CaN inhibitors, thereby enhancing the drug discovery process.[3] This study aims to identify new CaN inhibitors from natural products in combination with computational screening techniques.[4]

Results and Discussion

Docking assays were carried out targeting the two pharmacologically relevant domains of CaN (LxVP and PxIxIT), using the 3D structure of human calcineurin expressed in T cells (PDB ID: 4OR9). Natural product compounds sourced from ZINC20, Pubchem, and FarManguinhos databases were screened using the DockThor and AutoDock Vina programs. Grid boxes were defined based on key amino acid residues reported in the literature.[5,6] Approximately 98,000 docking assays were conducted with DockThor for LxVP and PxIxIT domains of CaN, with top 100 compounds exhibiting scores from -10.243 to -9.322 and from -8.331 to -7.659 kcal/mol for the LxVP and PxIxIT domains, respectively. Using AutoDock Vina, approximately 116,000 docking assays were performed targeting the LxVP and PxIxIT domains of CaN, with top 100 compounds yielded scores ranging from -9.308 to -8.214 and -8.440 to -6.145 kcal/mol, respectively as shown in Table 1. These data suggest that several compounds exhibited greater affinity for CaN than the reference drug tacrolimus, which showed docking scores of −9.476 and -6.909 kcal/mol in DockThor and AutoDock Vina, respectively. The next step will include visual inspection, consensus analysis, and pharmacokinetic predictions, aiming to select 5 to 10 candidate compounds for subsequent *in vitro* validation.

Table 1. Summary of virtual screening assays for the identification of novel CaN inhibitors targeting the LxVP and PxIxIT binding domains.

	LxVP domain		PxIxIT domain	
	DockThor	**Vina**	**DockThor**	**Vina**
Number of tested compounds	49,000	49,000	58,000	58,000
Total score range (kcal/mol)	-10.243 to 26.559	-9.308 to 5.380	-8.331 to 52.215	-8.440 to 40.759
Top-100 score (kcal/mol)	-10.243 to -9.322	-9.308 to -8.214	-8.331 to -7.659	-8.440 to -6.145

Conclusions

In this study, over 214,000 docking assays were conducted to assess molecular interactions with compounds, that were classified and ranked according to their docking score values. The top-100 compounds are currently undergoing consensus evaluation to ensure consistency across docking methods as well as visual inspection and pharmacokinetic predictions. Based on these analysis, 5 to 10 candidate compounds will be selected for subsequent *in vitro* validation assays.

Acknowledgements

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New zidovudine-based NRTI derivatives as potential HIV-1 inhibitors

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Keywords: AIDS, HIV-1, reverse transcriptase, zidovudine, isatin.

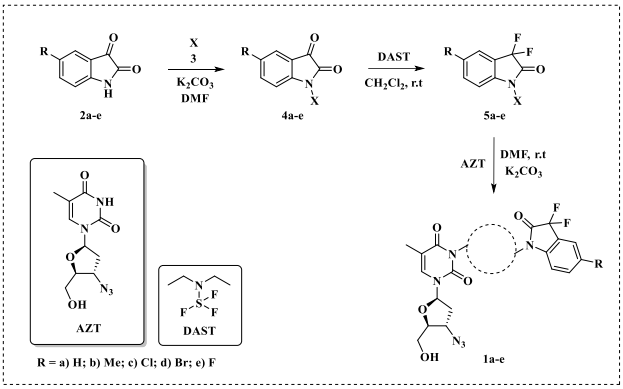
Introduction

The reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1) plays a pivotal role in the viral replication cycle by catalyzing the conversion of genomic RNA into proviral DNA. Owing to this essential function, RT is one of the primary targets for the development of antiretroviral agents. Inhibitors of this enzyme are classified as nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Among the NRTIs, zidovudine (AZT) was the first drug approved for the treatment of AIDS[1], noted for its initial efficacy. However, its clinical utility is limited by a short half-life, significant toxicity, and the emergence of resistant viral strains.[2,3] In light of these challenges, structural modification of AZT represents a promising strategy to obtain derivatives with improved pharmacological profiles. Previous studies have demonstrated that molecular hybridization of AZT with bioactive scaffolds, such as isatin, can yield compounds with antiretroviral potential.[4] In this context, the present work focuses on the synthesis, characterization, and biological evaluation of five novel molecular hybrids resulting from the conjugation of AZT with gem-difluorinated isatins. The synthesized compounds incorporate an alkyl spacer, a rational design element aimed at modulating lipophilicity, reducing toxicity, and enhancing antiretroviral activity.[5]

Results and Discussion

The synthetic methodology employed to obtain compounds **1a–e** consists of three sequential steps. Initially, a bimolecular nucleophilic substitution (S_N2) reaction is carried, yielding intermediates **4a–e**.[6] Subsequently, a gem-difluorination reaction using DAST is performed to afford intermediates **5a–e**.[4] Finally, the target compounds **1a–e** are synthesized via a second S_N2 reaction between the respective gem-difluorinated isatins **5a–e** and AZT[7] (Scheme 1). So far, eleven compounds (**4a–e**, **5a–e**, and **1a**) have been successfully synthesized, with reaction yields ranging from 25% to 55%, including the novel final product **1a**. The structures of these compounds were confirmed by spectrometric and/or spectroscopic techniques, supporting the successful formation of the intended chemical entities.

Scheme 1. Synthetic route for obtaining the final products **1a-e**.



Conclusions

The molecular hybridization strategy highlights the importance of rational modulation of physicochemical properties, such as lipophilicity and toxicity, with the goal of optimizing biological activity. Consequently, this work may contribute to innovation in the field of antiretroviral medicinal chemistry. The synthetic route has proven to be feasible, and the project timeline is progressing as planned. Following the synthesis and characterization of compounds **1a–e**, further studies will be carried out to evaluate their antiretroviral activity and cytotoxicity profiles.

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Discovery of novel natural $P2Y_2$ receptor antagonists by integrated virtual screening

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Keywords: $P2Y_2$ receptor, virtual screening, docking.

Introduction

$P2Y_2$ is a purinergic receptor widely expressed in humans, especially in epithelial, endothelial, muscle, bone, neuronal and immune cells, in which it is physiologically activated by extracellular ATP and UTP.[1,2] Its activation induces a series of physiological responses, such as intracellular calcium mobilization, the release of proinflammatory mediators, and cell proliferation and migration, which are associated with the development of inflammatory diseases, pain and cancer.[2] Despite being considered an important therapeutic target, there is still a shortage of selective antagonists for the $P2Y_2$ receptor, limiting its clinical use.[2,3] In this context, natural products have emerged as valuable sources for the discovery of new antagonists, and screening methodologies that allow rapid and efficient testing of a large number of compounds are needed.[4] Thus, the aim of this project is to use virtual screening through the docking technique to identify molecules originating from natural products that present affinity for the $P2Y_2$ receptor. With this, we will select the molecules that present the highest score values for the *in silico* prediction stage and, subsequently, for the *in vitro* testing stage to investigate whether they are capable of inhibiting the physiological responses from the $P2Y_2$ receptor.

Results and Discussion

Docking assays were initiated using the DockThor and AutoDock Vina programs in the orthosteric binding site of the $P2Y_2$ receptor, which includes the key residues Arg110, Phe113, Tyr114, Asp185, Phe261, and Arg292.[5] The three-dimensional structure of the $P2Y_2$ receptor used in the study was previously elucidated using the AlphaFold2 program with the protein sequence registered in UniProt (ID: P41231). We tested approximately 56,300 molecules derived from natural products available from the databases ZINC20, PubChem, and FarManguinhos. Of these, 15,332 were tested in DockThor, where the total score variation ranged from -9.923 to 47.392 kcal/mol. Another 41,030 molecules were tested in AutoDock Vina, with a score variation between -11.223 and 49.071 kcal/mol. After the docking tests are completed, a consensus analysis will be performed, and an affinity ranking will be created between the molecules tested and the $P2Y_2$ receptor, from which the molecules with the best score values will be selected for *in silico* predictions. After this, we will select 5 to 10 molecules for *in vitro* testing.

Conclusions

Considering the central role of the $P2Y_2$ receptor in inflammatory processes, pain, and cancer, this work contributes to the identification of new antagonists through an integrated approach that combines virtual screening with *in vitro* experimental validation. The application of this strategy allows the analysis of a large number of compounds to select those with the greatest affinity for the receptor.

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GeoProLib: A geopropolis-derived compound library and its application in COMT inhibitor screening

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Key words: GeoProLib; Geopropolis; Molecular dynamics; Virtual Screening.

Introduction

Geopropolis is a complex mixture produced by stingless bees from glandular secretions, plant resins, and soil. Due to its compositional variability and limited studies, its biological activity requires further investigation[1], particularly against relevant molecular targets like Catechol-O-methyltransferase (COMT), involved in neurotransmitter regulation and Parkinson's disease treatment[2].

This work focused on constructing GeoProLib, a database of 263 geopropolis-derived molecules. Compounds were obtained from literature, and collected data included ID, molecular structure (SMILES), species, and reference. Physicochemical properties were evaluated. An in silico approach combined molecular docking and dynamics of the top-ranked compound against COMT. Docking was performed using FRED. The target structure retrieved from the Protein Data Bank (PDB ID: 3BWY)[3].

To investigate binding stability, the highest-scoring complex and the apo-enzyme were subjected to 100 ns molecular dynamics simulations in GROMACS (GROMOS force-field). Ligand topologies were generated using the Automated Topology Builder (ATB). Detailed protein-ligand interaction patterns were then elucidated using PLIP.

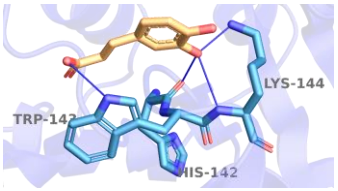
Results and Discussion

The construction of GeoProLib resulted in the cataloging of 263 isolated and identified compounds from geopropolis samples of various species and locations. The physicochemical analysis of the library revealed a wide spectrum of values for the evaluated properties. The molecular weights in the dataset range from 100.16 to 636.47 g/mol, suggesting a predominance of mid-sized molecules. ClogP values in the dataset range from -3.58 to 10.92, indicating a wide spectrum of lipophilicity. Hydrogen bond acceptor values ranged from 0 to 18, while donor values varied between 0 and 11.

All the molecules were subjected to molecular docking. Subsequent investigations were conducted with the G28 compound, which was the top-ranked hit in the molecular docking (-12.8), obtained through a validated docking protocol (AUC = 0.97).

The study showed excellent structural stability with a productive phase of 39 ns and a low mean root-mean-square deviation (RMSD) of 0.21 ± 0.01 nm throughout the simulation. G28 interacts with the active site of the enzyme through three hydrogen bonds, being a donor in the interaction with HIS142 and an acceptor with TRP143 and LYS144.

Figure 1: Interaction map of the COMT-G28 complex, where hydrogen bonds are depicted as solid blue lines.



Conclusions

This work established GeoProLib, a database of 263 geopropolis-derived compounds, highlighting its value for drug discovery. The results reinforce the need to further investigate geopropolis's complex chemical composition. Computational screening identified G28 as a promising COMT inhibitor, with molecular dynamics confirming its stable binding through key interactions.

Acknowledgements

We sincerely thank ChemAxon and OpenEye Scientific, as well as the National Council for Scientific and Technological Development (CNPq) and the Research and Graduate Program (PPPG) of the State University of Feira de Santana (UEFS) for the financial support.

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Identifying lead compounds for the P2X7 receptor: From algorithms to potential drugs

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Keywords: P2X7 receptor, virtual screening, Alphafold2, Gnina, AutoDock Vina, Dockthor.

Introduction

P2X7 is a purinergic receptor that is expressed mainly in immune and glial cells and plays significant roles in inflammation. P2X7 is physiologically activated by extracellular ATP at concentrations exceeding 100 µM, which is considered a danger signal, since these high concentrations are observed in situations of cell damage and cytolysis. P2X7 activation results in the opening of an ion channel that allows the flux of cations according to the electrochemical gradient. However, prolonged exposure to ATP also promotes the opening of a membrane pore with a cutoff of approximately 900 Da.[1] Several studies in the literature have associated the activation of this receptor with the development of inflammatory and neurodegenerative diseases as well as pain conditions, making it an important pharmacological target.[2,3] Although several antagonists for the P2X7 receptor have been developed, none have received approval for clinical use, thus perpetuating the therapeutic gap associated with this receptor.[3] In this context, the use of virtual screening techniques can aid in the identification of novel antagonists for the P2X7 receptor with desirable affinity and pharmacological characteristics. Recent studies have demonstrated the efficiency of using docking techniques combined with deep learning algorithms in the search for new drugs.[4] However, the application of these techniques is still significantly scarce in the field of purinergic receptors. Thus, the aim of this work is to perform virtual screening using docking techniques associated with deep learning algorithms.

Results and Discussion

Initially, we constructed five models of the human P2X7 receptor using AlphaFold2,[5] an advanced deep learning algorithm. After this step, we selected the Rank_1 model due to its superior results in inter-chain (ipTM) and global (pTM) confidence metrics. Subsequently, we compared the conformation of the selected model to the crystal structure of the rat P2X7 (6U9V)[1] in the APO state, resulting in an RMSD of 1.031 Å (Figure 1).[6] Additionally, we analyzed the average pLDDT score for residues reported as important in the literature as well as their surrounding areas for both the orthosteric and allosteric sites. The average pLDDT scores for the orthosteric and allosteric sites were 89.23 and 87.30, respectively (Table 1). Furthermore, we developed an algorithm to perform large-scale docking assays using the GNINA program.[7] Finally, we performed docking assays on approximately 320,000 natural product molecules using the GNINA, AutoDock Vina, and DockThor programs.

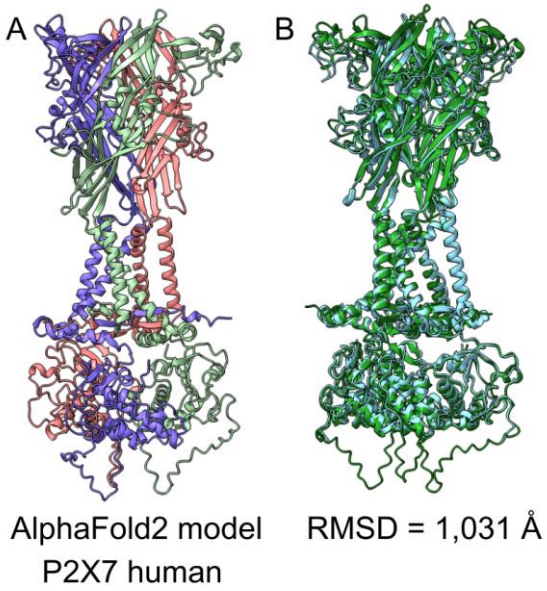


Figure 1. AlphaFold2 model of the human P2X7 receptor and structural superposition with crystallized rat P2X7 (PDB ID: 6U9V). (A) Representation of the AlphaFold2-predicted human P2X7 receptor structure with its three distinct subunits colored in purple, green, and pink for enhanced visualization. (B) Detailed structural superposition, where the predicted human P2X7 structure is shown in green, and the crystallized rat P2X7 structure (PDB ID: 6U9V) is presented in blue, highlighting conformational similarities and differences.

Table 1. Mean pLDDT values for key residues and their respective neighbors involved in P2X7 receptor interactions with orthosteric and allosteric binding sites.

Orthosteric site		Allosteric site	
Residue	**PLDDT (mean)**	**Residue**	**PLDDT (mean)**
Lys64	90.65	Phe88	90.65
Lys66	90.94	Phe95	77.73
Thr189	92.86	Phe103	85.82
Asn292	80.30	Met105	92.00
Arg294	86.87	Phe293	83.90
Lys311	93.78	Val312	93.72
Global	89.23	Global	87.30

Conclusions

Initially, we constructed a model of the human P2X7 receptor using AlphaFold2. The model was selected due to its superior confidence metrics and high structural similarity to the crystallized rat version (RMSD of 1.031 Å). The model's reliability for future docking assays was corroborated by the analysis of pLDDT scores at the orthosteric and allosteric sites. Subsequently, we performed approximately 320,000 docking assays with natural product molecules, using the GNINA, AutoDock Vina, and DockThor programs. The 100 molecules with the highest predicted affinity will be evaluated for their pharmacokinetic profiles and physicochemical properties in silico, with a final selection of 5 to 10 compounds for *in vitro* testing.

Acknowledgements

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Exploring Brazilian Chemical Space for Novel Modulators of α-Synuclein Aggregation in Parkinson's Disease

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Key words: Amyloid aggregation, anti-amyloid, molecular dynamics simulations

Introduction

The pathological hallmark of Parkinson's disease (PD) is the accumulation of misfolded α-synuclein (α-syn) in the brain. Currently, there is no cure or preventive therapy for PD, and available treatments offer only temporary symptom relief.[1]

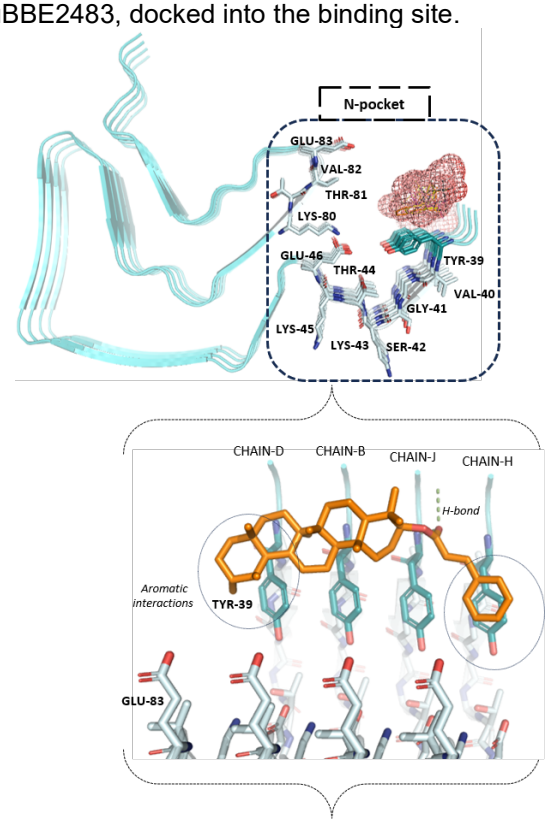
A growing body of evidence suggests that natural products (NPs) have potential as modulators of neurotoxicity associated with amyloid aggregates. Brazil is one of the most biodiverse countries in the world.[2] In this study, we aim to explore this rich chemical space using computational tools to identify novel modulators of the α-syn aggregation pathway. To this end, we performed a structure-based virtual screening of the NuBBE database (NuBBEDB), which contains a diverse array of secondary metabolites and their derivatives originating from Brazilian biodiversity.[2]

Results and Discussion

We then performed a structure-based virtual screening using AutoDock Vina, targeting the 3D α-syn protofibril structure (PDB ID: 7YNN). This structure represents a fibril formed *in vitro* from synthetic or recombinantly expressed peptides. Although amyloid fibrils generated *in vitro* can differ significantly from those found in *ex vivo* samples from patient brains, this *in vitro*–assembled α-syn fibril retains a conserved, druggable N-pocket that is also present in *ex vivo* α-syn fibrils.[3]

Therefore, our virtual screening specifically focused on the N-pocket as the docking site. In this pocket, a total of 2,363 compounds from NuBBEDB were docked, with the top 50 hits showed binding affinities ranging from –8.8 to –7.1 kcal/mol. Notably, these top hits form intermolecular interactions with the Tyr39 residue. Interestingly, Tyr39 acts like a valve in the N-pocket: when its side chain points outward (open state), the pocket forms a narrow, deep groove; when it points inward (closed state), the pocket becomes wider and shallower. The presence of ligands may interfere with this valve-like mechanism, potentially disrupting the structural dynamics of the N-pocket and affecting fibril stabilization.

Figure 1: The protofibril structure is shown in cartoon, with the top five docked ligands displayed in both line and mesh formats. A zoomed-in view highlights the binding pose of the top hit, NuBBE2483, docked into the binding site.



Conclusions

Currently, we are performing additional analyses, including pharmacophore modeling, drug-likeness profiling, and a literature review of previously reported evidence related to the selected hits. Our goal is to identify promising candidates for experimental validation using biophysical and imaging techniques.

Acknowledgements

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Medicinal Chemistry Analysis of Monoterpenoids: In Silico Discovery Pipelines Driving Rational Drug Development

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Key words: monoterpenoids, in silico screening, drug-likeness, ADMET profiling

Introduction

Monoterpenoids are a structurally diverse class of plant-derived compounds historically utilized for their broad pharmacological activities, including antimicrobial, anti-inflammatory, and antioxidant effects. Despite promising bioactivity, the therapeutic development of monoterpenoids has been hindered by poor solubility, limited oral bioavailability, and dose-dependent toxicities such as neurotoxicity and dermal sensitization. Advances in computational modeling have revitalized interest in these molecules by enabling systematic *in silico* screening of pharmacokinetic profiles and toxicity risks. This approach supports rational selection of candidates with favorable drug-like properties, overcoming key barriers to clinical translation.

Objectives

To computationally screen 1,175 monoterpenoids and identify structurally diverse compounds exhibiting optimal drug-like characteristics, predicted safety, and favorable pharmacokinetic behavior.

Methodology

Monoterpenoid SMILES were retrieved from PubChem using ChEBI classification. Thirteen computational tools, including pKCSM, SwissADME, ADMETlab, and ToxinPRED, were applied to predict physicochemical properties, ADMET parameters, and toxicological risks. Drug-likeness was evaluated with Lipinski, Veber, Pfizer, GSK, and Golden Triangle criteria. In total, 54 parameters were compiled, and compounds were filtered based on rigorous thresholds for solubility, permeability, metabolic stability, and safety. DataWarrior software was used for statistical analysis, Bravais–Pearson correlation calculation, and automatic SAR modeling. Finally, TargetNet predicted putative protein targets using ECFP4 fingerprints and a probability cutoff of ≥70% to ensure high-confidence and biologically meaningful interactions.

Results

From an initial dataset of 1,175 monoterpenoids retrieved via ChEBI ontology, systematic computational filtering progressively reduced the pool. Early physicochemical screening excluded 467 compounds, mainly due to excessive lipophilicity and suboptimal molecular weight or TPSA. Subsequent absorption and distribution evaluations eliminated additional candidates for poor Caco-2 permeability and high plasma protein binding. Metabolism profiling revealed widespread CYP inhibition and substrate liabilities, further narrowing the set to 365 molecules. Rigorous toxicological assessment, including mutagenicity, hepatotoxicity, and hERG inhibition prediction, identified critical safety concerns, reducing the dataset to 44 compounds. Application of medicinal chemistry filters such as Lipinski, Pfizer, GSK, and Golden Triangle criteria culminated in seven molecules with optimal profiles. These candidates demonstrated low predicted toxicity, favorable drug-likeness, and shared high-probability interactions with iNOS and APOBEC-3A, highlighting their translational potential.

Conclusions and Perspectives

This study identified seven monoterpenoids with optimal drug-like profiles, low predicted toxicity, and shared engagement with iNOS and APOBEC-3A. These findings highlight unexplored scaffolds and support future experimental validation and lead optimization efforts.

Acknowledgements

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Machine learning classification models for the prediction of selective CB2R agonists to treat osteoarthritis

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Keywords: CB2 receptor, osteoarthritis, machine learning, classification models.

Introduction

Osteoarthritis is an inflammatory disease that affects the joints, causing pain and obstructing movement[1]. Considering that the incidents of this affliction have severely risen due to the modern lifestyle, researches focused on developing new and more effective therapies become utterly indispensable. This study aims discovering compounds capable of interacting selectively with cannabinoid receptor 2, directed to osteoarthritis therapy, utilizing modern computational techniques.[2]

Results and Discussion

A curated dataset of 2,750 CB2R active molecules was derived from 11,751 entries from ChEMBL and BindingDB. Data curation includes filters for duplicates, PAINS and Lipinsky criteria. Four data division approaches were tested (random, Kennard-Stone, K-Means/ Kennard-Stone clustering); Kennard-Stone provided the best chemical space representation (Fig. 1). Random Forest, SVM, and KNN classification models were trained, with cross-validation, using the PubChem, Morgan and MACCS Keys fingerprints by internal Python scripts using pandas and numpy libraries, as well as sckit-learn and RDKit (function implementation and pre-processing). The model obtained with Random Forest and MACCS Keys fingerprints reached the best AUC values (0.97 - Fig. 2) and best accuracy values for active and inactive classes (0.956 and 0.968, respectively - Fig. 2).

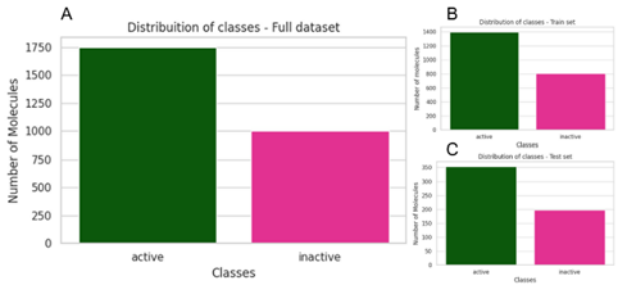


Figure 1: A) Distribution plot of number of molecules in each class for A) the full dataset; B) training set; C) test set.

A virtual screening was performed in the NuBBEdb database, identifying 453 molecules labeled as "active". These molecules were screened with the ALPACA platform, for selectivity analysis, using WIN55,212-2 as control[5]. Furthermore, the ALPACA output was crossed with results from an internal regression model, which confirmed 11 molecules being labeled as both active and selective and having a predicted pKi ≥ 7 nM. The pharmacokinetic prediction (SwissADME) of these 11 molecules highlighted four compounds (NuBBE2366, NuBBE1962, NuBBE1595, NuBBE1344) with a high probability of penetrating the blood-brain barrier (BBB) who were also not P-gp substrates, thus representing high potential candidates for key compounds[6].

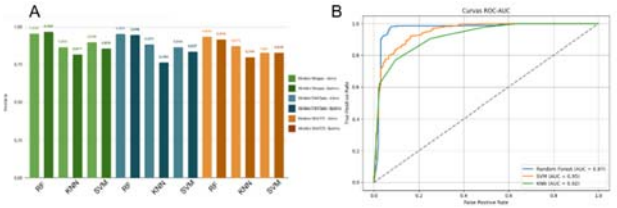


Figure 2: A) Accuracy plots of all generated models for active and inactive classes, respectively; B) AUC-ROC graphs for the Random Forest, KNN and SVM models generated with MACCS Keys fingerprints.

Conclusions

The results achieved in this study unravel the capability of supervised classification models to assist in the search for selective CB2R agonists, when paired with efficient data curation and rational training-test splitting. Additionally, the presented workflow evidences the overall potential of computational techniques to support rational drug design for osteoarthritis therapy.

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Identification and Characterization of Novel Inhibitory Molecules Targeting SARS-CoV-2 Proteases with Antiviral Activity

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Key words: SARS-CoV-2, Inhibitors, Proteases.

Introduction

The successful replication of SARS-CoV-2 depends on the activity of the viral cysteine-proteases MPro and PLPro. Therefore, they are considered attractive drug target candidates for the treatment of COVID-19. An *in silico* and empirical screening of an in-house compound library identified over 100 MPro inhibitors (IC_{50} ≤ 25 µM), 43% of which also inhibited PLPro. Thirty compounds exhibited antiviral activity comparable to Lopinavir and Remdesivir [1]. The present study aims to complete the screening of this chemolibrary and characterize the mode-of-inhibition, cytotoxicity.

Results and Discussion

MPro and PLPro were expressed and purified in *Escherichia coli* BL21 Gold (DE3), yielding 10 and 3 mg/L of protein, respectively, with 95% purity according to SDS-PAGE analysis. The activity of both proteases was measured by monitoring the hydrolysis of a fluorescent peptidic substrate.

In a screening of 491 compounds, 21 molecules (4%) were "hits" (≥50% inhibition at 25 µM), with IC_{50} values ranging from 0.14 to 25 µM. Cytotoxicity assay performed against human lung cells (cell line A549) identified four compounds that impaired cell viability at 50 and 100 µM. All "hits" were evaluated in serial dilutions on Vero cells infected with SARS-CoV-2; two showed antiviral activity without cytotoxicity, while the others were inactive or toxic to the infected cells.

Two families of compounds, rotenoids and naphthoquinones, outstand for their high hit rate against MPro (13% and 40%, respectively). Four rotenoids have $IC_{50,\ MPro}$ = 10-25 µM and lacked cytotoxic. Twelve out of 30 naphthoquinones have $IC_{50,\ MPro}$ = 3-15 µM, but most of them were cytotoxic. Only one of these "hits", a dimethylphenyl-pyperazin substituted naphthoquinone, showed true antiviral activity (Figure 1) ($IC_{50,\ MPro}$ = 16.4±1.3 µM; $EC_{50,\ SARS\text{-}CoV\text{-}2}$ = 36 µM; $CC_{50,\ Vero}$ ≥ 50 µM; SI > 1.4). The quinones inhibiting MPro were co-screened at 25 µM against PLPro to verify the selectivity for the molecular target. The following trend in potency was observed: a) inhibition MPro ~ PLPro for 6 compounds, b) inhibition MPro > PLPro for 6 compounds. This result suggests a higher selectivity of naphthoquinones for MPro.

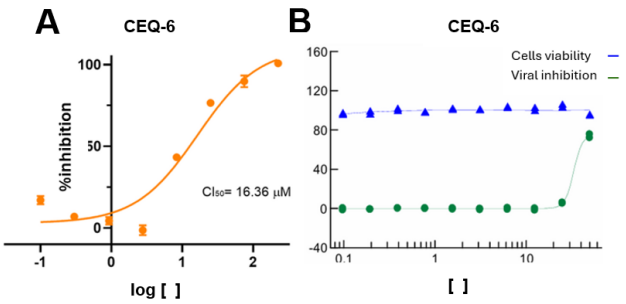


Figure 1: Enzymatic and biological assays of the best hit from the quinone family. **A)** IC_{50} plot for MPro. **B)** Anti-SARS-CoV-2 activity and host-cell cytotoxicity.

Conclusions

Compared to a computational-guided screening campaign performed for this chemolibrary (hit rate 21%) [1], the "blind" screening identified a lower number of MPro hits (hit rate 4%, 21 out 491 compounds). Two of the novel hits had antiviral activity. Rotenoids and naphthoquinones emerged as promising scaffolds to be optimized for improving their protease and antiviral potency, and selectivity, respectively.

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Molecular docking and pharmacophore mapping in the search for dengue NS2B-NS3 protease inhibitors

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Key words: molecular docking, pharmacophore mapping, NS2B-NS3 protease dengue, protein-ligand interactions.

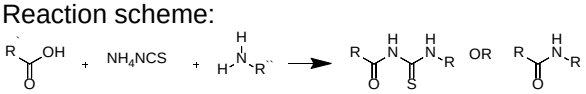
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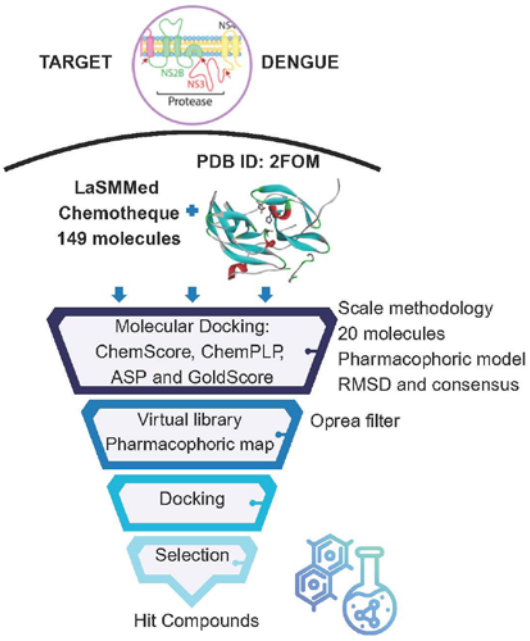
Currently, there are no specific antivirals approved for dengue in Brazil, and the available treatments are only palliative. Given the urgent need for effective antiviral therapeutics, our research focuses on identifying drugs that target the dengue virus. We specifically aimed at the NS2B-NS3 protease of dengue virus, conducting a virtual screening using molecular docking (Gold software) on the chemical library of the LaSMMed-UEL research group.

Results and Discussion

After docking (initial screening), the molecules were ranked according to the consensus-based scaling, and the top 20 molecules were then selected. This selection included different chemical classes: coumarin polyphenols, coumaryl chalcone, cinnamic acid thiourea, apocynin derivative, coumaryl amide, and chromone. This screening led to the identification of compounds with significant protease inhibition potential. The selected compounds belong to the thiourea class and were chosen because they constitute the largest number of compounds among the top 20. Based on this class, we generated virtual libraries containing approximately 8,000 molecules using the iLib Diverse software, which included the application of the OPREA filter and commercial reagent databases. The molecule chosen as a pharmacophoric model is also a thiourea derivative, and was chosen due to its proximity and interaction with the catalytic triad (His51, Asp75, and Ser135). We then created pharmacophoric maps using LigandScout software, screening virtual libraries based on these models to identify molecules with potential pharmacophoric characteristics. The selected molecules were subsequently docked in the protease (Gold software). The compounds selected in the second docking screening showed better interaction with the catalytic triad, with an optimization of their proximity compared to the results of the first screening. These new derivatives were synthesized from carboxylic acids, thiocyanate (linker), and amines, resulting in amides and thioureas, depending on the reaction conditions used.

Reaction scheme:





Conclusions

This comprehensive virtual screening process—which includes preliminary molecular docking, pharmacophore mapping, creation of virtual libraries with the desired pharmacophore characteristics, and subsequent molecular docking of the most promising virtual candidates—allowed us to identify prominent molecules (thiourea derivatives) that were then synthesized. Both the newly synthesized compounds and those identified during the initial docking study of our chemical library are currently undergoing testing to evaluate their antiviral activity against dengue virus.

Acknowledgements

The authors thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil) for financial support (Finance Code 001).

Evaluation of Natural Product Like-compounds obtained through Diversity-Oriented Synthesis as Anti-Trypanosomatid Agents

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Key words: natural product-like compounds (NPLCs), Diversity-Oriented Synthesis (DOS), T. cruzi, Leishmania spp.

Introduction

Chagas disease and leishmaniasis are major neglected tropical diseases that disproportionately affect low-income regions, where current treatments are limited, often ineffective, and toxic.[1] The rise of drug resistance and adverse side effects further underscores the urgent need for new therapeutic agents.[2,3] In this context, natural product-like compounds (NPLCs) have emerged over the last two decades as promising molecules for drug discovery for this pathologies. These compounds are designed to retain the privileged scaffolds and biological relevance of natural products while potentially exhibiting improved pharmacological properties such as enhanced activity, reduced toxicity, and better pharmacokinetics.[1,2] Furthermore, NPLCs can be efficiently accessed through Diversity-Oriented Synthesis (DOS) strategies, which enable rapid exploration of diverse regions of chemical space, resulting in libraries enriched with stereochemical and functional diversity capable of modulating various biological processes.[4,5]

Our research group has explored this concept using a straightforward DOS approach that combines tropolones as dienes with alkene or carbonyl-based dienophiles in key [4 + 2] cycloaddition, followed by photoisomerization and reductions steps. This methodology has yielded diverse polycyclic scaffolds such as homobarrelenones and norcaranes, dihydrofluorenones, which are structurally rich and suitable for biological screening. [6-8] Notably, some derivatives obtained using diazo or nitroso dienophiles have demonstrated antiparasitic and antitumor activity, supporting these frameworks' versatility and bioactivity potential.[8]

Building on this platform, we expanded our homobarrelenone library and evaluated their activity against *Trypanosoma cruzi* and *Leishmania infantum*. Furthermore, to gain insight into the possible mechanisms of action of the most promising compounds, target-based assays were performed, focusing on cruzipain and ergosterol biosynthesis.

Results and Discussion

We combined [4 + 2] Diels-Alder cycloadditions follow by photoisomerization and reductions reactions using tropolones as dienes, reacting with various dienophiles including *N*-methylmaleimide, acrylonitrile, and methyl acrylate. (**Figure 1**) A library of seventeen compounds was synthesized and evaluated against *Trypanosoma cruzi* (epimastigote form) and *Leishmania spp* (promastigote form).

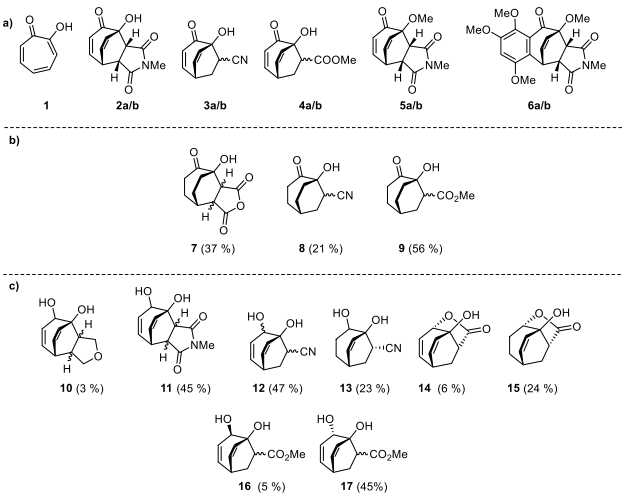


Figure 1. Compounds synthesized through DOS. a) Parent compounds: tropolone and corresponding Diels–Alder adducts. b) Scaffolds obtained after catalytic hydrogenation. c) Skeletons generated by $NaBH_4$ reduction.

Four compounds exhibited trypanocidal activity, with IC_{50} values ranging from 2 to 43 μM. To gain insight into their potential mechanisms of action, two key biological targets were evaluated: the ergosterol biosynthesis pathway and cruzipain, a major cysteine protease of *T. cruzi*. Among the active molecules, adduct **3** stood out for its high selectivity index against mammalian cells and its strong inhibition of ergosterol biosynthesis. Additionally, in the search for alternative molecular targets, two compounds **2** and **3** were identified as cruzipain inhibitors, suggesting a distinct mode of action within the series.

We also conducted a preliminary screening in *Leishmania infantum*-infected macrophages derived from the THP-1 monocyte, using a bioluminescent parasite strain.[9] Compound **5** appears as a promising hit.

Conclusions

Notably, four compounds exhibited activity in phenotypic assays against *T. cruzi* and *L. infantum*, with two of them demonstrating selective antiparasitic effects compared to mammalian cells. Furthermore, mechanistic studies revealed that one of the active compounds interferes with the ergosterol biosynthesis pathway, leading to the accumulation of squalene, a key intermediate in the sterol metabolism of the parasite. Additionally, two compounds were identified as cruzipain inhibitors, suggesting a distinct mode of action. These results reinforce the potential of our synthesized compounds as promising candidates for the development of new treatments against neglected tropical diseases.

Acknowledgements

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Computational prediction and design of inhibitors for neutralizing α-neurotoxins from Micrurus snake venoms

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Key words: structure-based drug design, nicotinic cholinergic receptors, α-Neurotoxins, Micrurus snake

Introduction

Human nicotinic acetylcholine receptors (nAChRs) are ion channels composed of multiple protein subunits, whose specific combination determines their biological function. The $(\alpha1)_2\beta1\delta\varepsilon$-type nAChRs present at the neuromuscular junction mediate muscle contraction via Na^+ influx and exhibit high affinity for α-neurotoxins from elapid snakes, such as *Micrurus coralinus* and *Micrurus frontalis*. These toxins block channel activity by competing with the endogenous ligand acetylcholine for the binding site, causing paralysis of motor nerves that can lead to death. Envenomation by elapid snakes causes severe and potentially fatal complications, and the difficulty in producing effective antivenoms underscores the need for alternative therapeutic strategies.

Objectives

To characterize the molecular basis of the interaction between α-neurotoxins from *M. coralinus* and *M. frontalis* and nAChRs, aiming to develop inhibitors with therapeutic potential for snakebite treatment.

Methodology

Initially, the sequences of the α1, β1, δ, and ε subunits of nAChRs junction nicotinic receptors (Q9UGM1, P17787, P07510, and Q07001), as well as those of α-neurotoxins from *M. coralinus* (C6JUP3 and C6JUP2) and *M. frontalis* (P86420 and P86422), were obtained from UniProt. AlphaFold2 and Modeller were used to model the structures and assemble the multimeric complexes. The generated models were evaluated using servers such as MolProbity, QMEAN, and VoroMQA. Molecular dynamics simulations were then performed to generate an ensemble of conformations suitable for interaction with the toxins. Conformation selection was based on Solvent Accessible Surface Area (SASA) and Principal Component Analysis (PCA). Molecular docking was carried out using the HADDOCK program, employing the α1–ε and δ–α1 subunit interfaces as active sites. Finally, the RING server was used to characterize the interactions between nAChRs and the α-neurotoxin.

Results

The predicted models exhibited Ramachandran plots without outliers, indicating adequate stereochemical quality. Global VoroMQA values were above average and close to 0.6, reinforcing the overall structural integrity of the models. Additionally, QMEAN scores near 1 suggest high reliability in the modeling process, reflecting good agreement with known experimental structures. Using PCA and SASA analyses, conformations capable of accommodating the toxins were identified, which was subsequently confirmed by successful docking at the α1–ε cavity, yielding HADDOCK scores ranging from −95.9 to −150.1. Interaction analysis using RING highlighted the 3FTx-2 toxin from *M. coralinus* as having the greatest interaction potential, forming a salt bridge—possibly crucial for complex stability—between residues E130 and K57.

Conclusions and Perspectives

Our findings highlight the robustness of the employed methodology, demonstrating the successful integration of structural modeling, molecular dynamics, and docking calculations to characterize the interactions between α-neurotoxins and nAChRs. This approach will now be extended to the δ–α1 interface, where new molecular dynamics simulations will be performed to assess toxin stability within the resulting complexes.

Acknowledgements

The authors thank CNPq and CAPES for financial support and Oswaldo Cruz Foundation – Ceará and State University of Ceará for structural support.

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Evaluation of antiplasmodial activity of atovaquone-based compounds against sensitive and multidrug-resistant *Plasmodium falciparum* strains.

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Key words: malaria, SAR, Atovaquone, resistant strain.

Introduction

Malaria, an ancient disease caused by *Plasmodium* protozoa, affected approximately 263 million people in 2023. The mortality rate remains nearly three times higher than the 75% reduction target set by the Global Technical Strategy for Malaria 2016–2030[1]. Increasing resistance to first-line drugs is one of the main challenges in controlling the disease, making the search for new compounds with antiplasmodial activity and new mode of actions urgent. In this study, we synthesized and evaluated a series of 13 atovaquone-based analogues (Figure 1), an effective antimalarial against *P. falciparum.*

Results and Discussion

The screening of the new derivatives identified 12 compounds with antiplasmodial activity in the low micromolar range and no observed cytotoxic effects in hepatic cell lines (HepG2), demonstrating considerable selectivity. Notably, **LaDMol376** showed an IC_{50}^{3D7} of 1.3 µM and selectivity index (SI) greater than 77. Structure–activity relationship (SAR) analysis guided the selection of four derivatives for testing against five multidrug-resistant strains of *P. falciparum*. No cross-resistance with standard antimalarials was observed, and the compounds maintained their potency compared to the drug-sensitive 3D7 strain. However, the IC_{50} values obtained for the TM90C6B strain, an atovaquone-resistant strain, showed 2- to 4-fold increase in potency compared to the sensitive 3D7 strain (Figure 2 and Table 1). These findings suggest that other sites within the parasite's mitochondrial bc1 complex may be explored as potential targets for these new compounds. Additionally, validated machine learning studies indicated promising ADME properties, such as high absorption and high bioavailability.

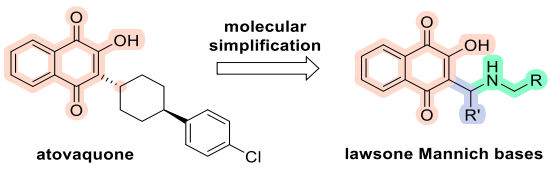


Figure 1. Scheme of the molecular design used for the development of 13 new atovaquone-based compounds.

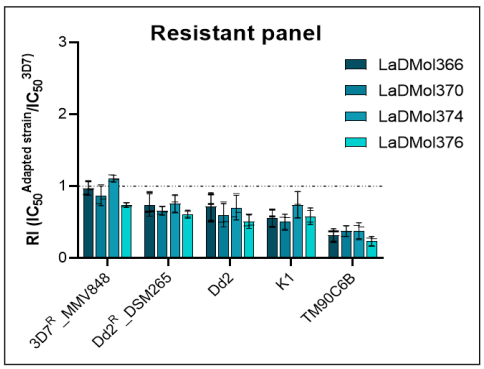


Figure 2. Resistance index of LaDMol compounds against a panel of multidrug-resistant *P. falciparum* strains ($3D7^R$ MMV848, $Dd2^R$_DSM265, Dd2, K1 and TM90C6B) in relation to the sensitive 3D7 strain.

Strains	LaDMol366		LaDMol370		LaDMol374		LaDMol376	
	IC_{50} (µM)	RI*	IC_{50} (µM)	RI*	IC_{50} (µM)	RI*	IC_{50} (µM)	RI*
3D7	2.3 ± 0.5		2.0 ± 0.3		1.5 ± 0.4		1.3 ± 0.2	
$3D7^R$_MMV848	1.9 ± 0.1	0.8	1.7 ± 0.3	0.8	1.65 ± 0.07	1.1	1.03 ± 0.04	0.8
$Dd2^R$_DSM265	1.4 ± 0.3	0.6	1.3 ± 0.1	0.6	1.1 ± 0.1	0.7	1.3 ± 0.7	1.0
Dd2	1.4 ± 0.3	0.6	1.2 ± 0.3	0.6	1.0 ± 0.2	0.6	0.7 ± 0.1	0.5
K1	1.1 ± 0.2	0.5	1.0 ± 0.2	0.5	1.1 ± 0.3	0.7	0.9 ± 0.1	0.7
TM90C6B	0.6 ± 0.2	0.2	0.7 ± 0.1	0.3	0.6 ± 0.1	0.4	0.33 ± 0.09	0.2

*RI= $IC_{50}^{Adapted\ strain}/IC_{50}^{3D7}$

Table 1. Evaluation of the *in vitro* antiplasmodial activity (IC_{50}) of LaDMol compounds against the sensitive 3D7 strain and five multidrug-resistant strains, along with their respective resistance indices (RI).

Conclusions

The synthesis and evaluation of new atovaquone-based analogues proved to be a successful approach. We identified potent and selective compounds against *P. falciparum*. The absence of cross-resistance with standard antimalarial, along with the improved potency observed against TM90C6B offer the opportunity for investigating an alternative mechanism of action underlying the antiplasmodial activity of the series.

Acknowledgements

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Imatinib–indolinone hybrids: synthesis of potential inhibitors for the treatment of Chronic Myeloid Leukemia

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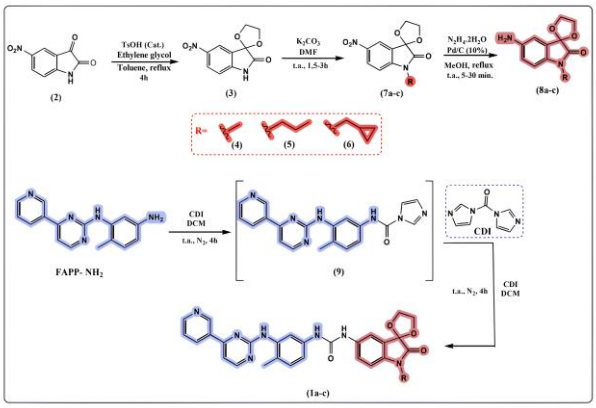
Key words: Tyrosine kinase inhibitors, chronic myeloid leukemia, imatinib.

Introduction

The dysregulation of protein kinases is involved in the pathogenesis of various cancers, with the Bcr-Abl1 oncoprotein being a key target in chronic myeloid leukemia (CML). Tyrosine kinase inhibitors (TKIs) targeting Bcr-Abl1 are the main therapy for CML, acting through competition at the ATP-binding site[1]. Imatinib (IMT) revolutionized CML treatment and became the first-line therapy for Ph+ CML[2]. However, point mutations led to the development of second-generation TKIs such as nilotinib (NLT), dasatinib (DAT), and bosutinib (BST), designed to overcome imatinib resistance. Although these agents offer faster and more effective molecular responses, challenges remain due to residual resistance and severe adverse events, particularly of arterio-occlusive nature[3]. These issues highlight the need for new Bcr-Abl1 inhibitors with improved efficacy and reduced toxicity.

Results and Discussion

A convergent four-step synthetic methodology was proposed for the preparation of target compounds (**1a–c)** (Scheme 1), starting from 5-nitroisatin (**2**) and the diamine FAPP-NH_2. Initially, 5-nitroisatin (**2**) undergoes ketone carbonyl protection via acetalization to yield intermediate **3**. This compound is then N-alkylated through an SN2 reaction with commercial bromides (**4–6**), producing key intermediates (**7a–c**). The nitro groups are subsequently reduced to afford the corresponding anilines (**8a–c**). In the final step, FAPP-NH_2 reacts with CDI to form the isocyanate intermediate in situ (**9**), which then undergoes a one-pot nucleophilic addition-elimination reaction with anilines (**8a–c**), leading to the formation of the final products (**1a–c**), as shown in Scheme 1.



Scheme 1. Proposed synthetic methodology for the new imatinib hybrids (**1a–f**) and (**2a-f**).

The 3 new imatinib hybrids have already been obtained, with yields ranging from 39% to 80%. The compounds were characterized by electrospray ionization mass spectrometry (ESI-MS), infrared (IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy of ^{1}H, ^{13}C, and ^{19}F. Chromatographic purities were assessed by high-performance liquid chromatography (HPLC).

Conclusions

New imatinib-derived hybrids developed using the molecular hybridization approach—a well-established strategy in medicinal chemistry—were synthesized, characterized, and evaluated for their activity in K562-sensitive, Lucena-1, and HEK 293 cells. The compounds showed promising results and will also be subjected to enzymatic assays against the wild-type ABL1 isoform (ABL1^wt).

Acknowledgements

To CNPq, PIBIC, and Farmanguinhos – FIOCRUZ, To my advisors Dr. Monica Macedo Bastos, Dr. Luiz Cláudio F. Pimentel, and Dr. Mayara Salles S. do Nascimento for all the knowledge and guidance.

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Synthesis and study of the anti-inflammatory activity of amides derived from NSAIDs and their derived *S*-Nitrosothiols.

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Keywords : Non steroidal anti-inflammatory drugs, Ketoprofen, Medicinal Chemistry, Prodrugs, coupling reactions.

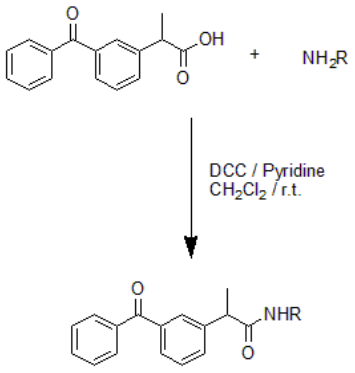
Introduction

Anti-inflammatory drugs are medicines used to control inflammation, which can be caused by various factors, such as infections and environmental stress. NSAIDs, which are often prescribed, act mainly by inhibiting cyclooxygenase enzymes (COX-1 and COX-2), which are essential in the synthesis of prostaglandins, mediators of inflammation. However, prolonged use of NSAIDs can lead to gastrointestinal side effects, since non-selective inhibition of COX-1, which protects the gastric mucosa, contributes to these problems. In addition, COX-2 has been associated with carcinogenesis, and studies suggest that selective COX-2 inhibitors may have chemo-preventive effects, especially in colon cancer. This project aims the synthesis and study of amides derived from nonsteroidal anti-inflammatory drug (NSAIDs) Ketoprofen. These compounds are potential prodrugs with less side effects, and appropriate selectivity to the different cyclooxygenase isoforms.

Results and Discussion

Amides were prepared by reacting the ketoprofen with heterocyclic aromatic amines by coupling reactions with carbodiimide DCC in dichlorometane (Scheme 1; Figure 1).

The products obtained were characterized using spectroscopic techniques, including ^{1}H NMR, ^{13}C NMR, FT-IR, as well as contouring techniques such as HSQC, to confirm the preparation of the compounds.



Scheme 1

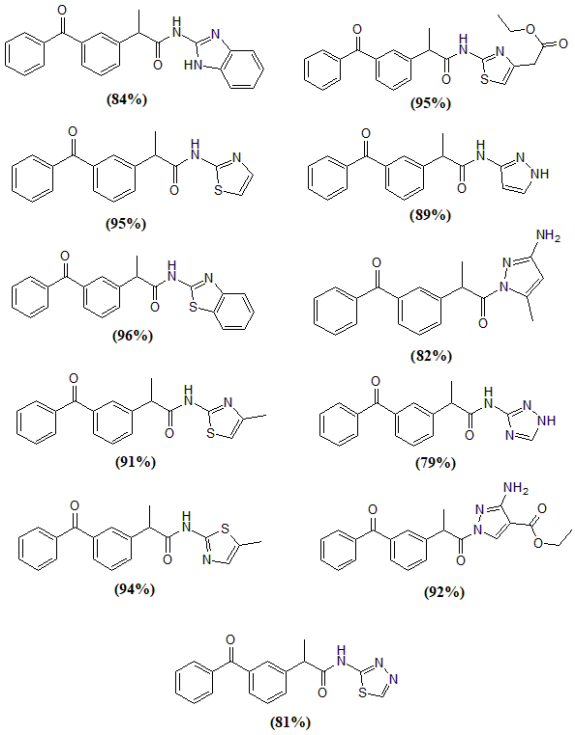


Figure 1: Heterocyclic amides derived from ketoprofen synthesized and yields (%).

In vitro biological activity obtained in our research group assays showed that the modified anti-inflammatory drugs did not exhibit toxicity different from that of the prototype molecules, and continued to exhibit anti-inflammatory characteristics. The anti-inflammatory activity assay was performed with macrophages seeded on plates at three different concentrations: 1, 10, and 100 µM, according to the procedure established by the Cayman Chemical kit entitled "COX Activity Assay Kit." A certain selectivity for COX-2 inhibition over COX-1 was also observed.The results obtained for the percentage inhibition of COX-1 showed that no amide derivative showed an inhibition rate of this isoform greater than 50%, with an average of between 30 and 40%.

We acknowledge the financial support given by the Brazilian funding Institutions Fapesp and CAPES.

Sehapal, S.; Prasad, D. N.; Singh, R. K.; *Mini-Reviews in Medicinal Chemistry*, **2018**, 18, 1199-1219.

NMR-based fragment screening to identify new ligands targeting nucleoside hydrolase from *Leishmania donovani*

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Key words: Visceral Leishmaniasis, NMR Spectroscopy, Drug Discovery, CPMG, WaterLOGSY, Fragments.

Introduction

Leishmaniasis remains a major public health issue, particularly in Latin America, where Brazil reports the highest number of Visceral Leishmaniasis (VL) cases[1]. Current therapies for VL are associated with toxicity, resistance, and limited efficacy, highlighting the urgent need for new therapeutic strategies. One promising molecular target is nucleoside hydrolase from *Leishmania donovani* (*Ld*NH), an essential enzyme in the parasite's purine salvage pathway[2]. As *Ld*NH is absent in mammalian cells, it presents a selective and attractive opportunity for Fragment-Based Drug Discovery (FBDD), a powerful strategy for developing new therapeutic agents through the identification of low-molecular-weight ligands that interact with biologically relevant targets. By starting from structurally simple fragments, FBDD enables the rational design and optimization of lead compounds with improved binding affinity and selectivity. Among the biophysical techniques employed in FBDD, nuclear magnetic resonance (NMR) spectroscopy plays a central role due to its high sensitivity in detecting weak protein-ligand interactions, even at low affinities. 1D NMR experiments such as CPMG, WaterLOGSY, and STD-NMR are particularly effective for fragment screening, hit validation, and epitope mapping, making them valuable tools for early-stage drug discovery[3]. To explore *Ld*NH as a therapeutic target, we employed an NMR-based FBDD approach using the commercial DSI-poised-iNEXT-G2 library containing 696 fragments.

Results and Discussion

For screening, fragments were organized into pools of 12 fragments per sample at a final concentration of 0.208 mM. Samples were prepared in 20 mM phosphate buffer (pH 7.4), 300 mM NaCl, 10 µM *Ld*NH dimer, and 10% D_2O. A total of 116 pooled samples were analyzed by ^1H, CPMG, and WaterLOGSY in the presence and absence (control) of *Ld*NH to evaluate the protein-ligand interaction. In the initial screening stage, comparative analysis of the spectra identified 33 possible ligands based on signal broadening and intensity reduction in the CPMG spectra. These 33 fragments were then evaluated individually under the same experimental conditions using CPMG, and WaterLOGSY to exclude false positives. Fragments that showed consistent interaction profiles, such as reduced signal intensity in CPMG and signal inversion in WaterLOGSY (figure 1), were considered ligands (hits).

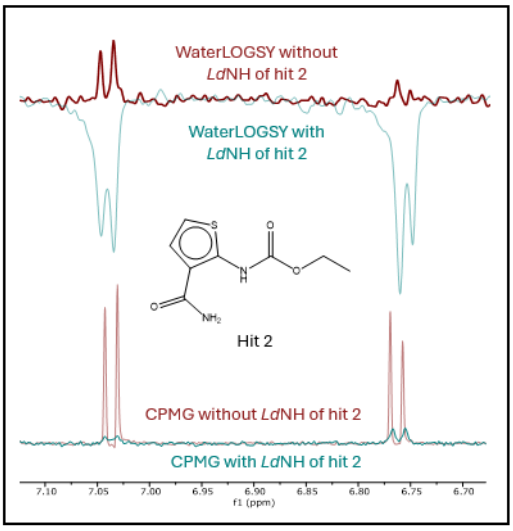


Figure 1: Protein–ligand interaction of hit 2 by CPMG and WaterLOGSY NMR.

This approach confirmed 10 hits, all containing at least one aromatic system, suggesting that aromaticity plays a critical role in *Ld*NH binding. Additionally, six hits feature amide groups, indicating synthesis via amine acylation and allowing retro-synthetic decomposition into their respective precursors, thus enabling the design and synthesis of analogs for future studies.

Conclusions

The successful screening identified promising fragments that, together with ongoing STD-NMR and enzymatic assays, will guide the rational design of optimized inhibitors and reinforce the potential of *Ld*NH as a valuable drug target for antileishmanial therapy.

Acknowledgements

CNPq, FAPERJ, CAPES.

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Mechanistic Insights into a Promising Drug Candidate Against Neglected Protozoan Diseases

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Key words: drug discovery, mode of action, trypanosomatids

Introduction

Neglected Tropical Diseases (NTDs) are a group of infections that predominantly affect populations living in poverty, where access to healthcare is limited. Among these, *Trypanosoma cruzi* and *Leishmania* spp.—the causative agents of Chagas disease and leishmaniasis, respectively—are endemic to Latin America. Currently, there are no available vaccines for these parasitic infections, and the existing treatments, developed decades ago, are associated with high toxicity and increasing drug resistance. Consequently, the search for new, effective, and safe therapeutic agents is a pressing need.
Our research group has synthesized and evaluated a series of compounds with promising activity against *T. cruzi* and *Leishmania* spp., demonstrating both in vitro efficacy in cultured parasites and in vivo reduction of parasitic load in animal models[1,2]. Among these, compound 1260 has emerged as the most promising, showing potent trypanocidal activity, high selectivity, low cytotoxicity, and no significant mutagenicity [1,2]. In addition to its comparable in vitro efficacy to current drugs such as Nifurtimox, Benznidazole, and Miltefosine, compound 1260 is also active against a local strain of *L. infantum* isolated from infected dogs in Uruguay[1].

Results and Discussion

To further understand the biological effects and mechanism of action of compound 1260, we employed a multidisciplinary approach, including cell viability assays, flow cytometry-based phenotypic analysis, transcriptomic profiling, and differential gene expression analysis.
Cell recovery assays revealed that compound 1260 exhibits trypanostatic activity at concentrations near the IC_{50}, while acting as a trypanocide at 5× and 10× IC_{50}. Morphological observations supported these findings: control parasites displayed senescent features by day 15, whereas treated parasites showed persistent structural alterations, particularly at 10× IC_{50}—consistent with trypanocidal action.
Flow cytometry analyses conducted 24 hours post-treatment demonstrated a dose-dependent reduction in calcein-positive (viable) cells and a corresponding increase in Annexin V-positive populations, indicative of apoptosis induction. In contrast, low levels of PI staining across all conditions suggest that necrosis is not the predominant mode of cell death.
Transcriptomic profiling in *T. cruzi* identified significant changes in gene expression upon treatment with compound 1260. Upregulated genes were enriched in pathways associated with sterol biosynthesis and mitochondrial function, while downregulated genes implicated pathways involved in parasite–host interaction and proliferative signaling. Gene Ontology enrichment analysis revealed increased expression of genes related to lipid metabolism, particularly enzymes of the mevalonate pathway and sterol production, which were mapped onto the sterol biosynthesis pathway.

Conclusions

Compound 1260 exerts a dose-dependent antiparasitic effect, being trypanostatic at low concentrations and trypanocidal at higher doses, primarily through the induction of programmed cell death. Transcriptomic analysis supports this phenotype and highlights the activation of sterol biosynthesis as a potential adaptive or compensatory mechanism in response to drug-induced stress. Further functional studies targeting the sterol biosynthetic genes identified in this work are necessary to elucidate their role in the drug's mechanism of action.

Acknowledgements

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Molecular Modeling and In Vitro Evaluation of Arylthioureas as Cryptococcus neoformans Urease Inhibitors

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Keywords: antifungal agents, urease, cryptococcosis, molecular docking.

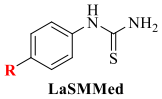
Introduction

Cryptococcus spp. are pathogenic ureolytic microorganisms that cause various disorders in the host organism and, in severe cases, lead to death. These pathogens use the urease enzyme as a key virulence factor, as it allows them to produce ammonia and mitigate the harsh pH conditions they encounter. In this context, this work aims to evaluate arylthioureas for their inhibitory potential against urease from *C. neoformans* through molecular docking and *in vitro* assays of the anti-ureolytic activities of these substances.[1]

Results & Discussion

Our previous research identified five arylthioureas (**LaSMMed 1-5**) as potent inhibitors of *Canavalia ensiformis* urease, with approximately 85% inhibition and IC_{50} values ranging from 0.464 to 0.575 mM.[2] In this work, we expanded our study to include fungal urease from *C. neoformans* (CNU). The compounds we tested demonstrated even better inhibition, achieving rates exceeding 92%, with IC_{50} values ranging from 0.342 to 0.378 mM. This range is slightly higher than that of the standard thiourea (IC_{50} = 0.265 mM). Among the compounds, **LaSMMed 1** and **LaSMMed 4** showed the strongest activity, with an IC_{50} of 0.34 mM. This suggests that non-bulky halogen or electron-donating groups enhance inhibition. In contrast, bulkier withdrawing groups, such as nitro or bromine, resulted in a slight reduction in activity (Table 1).

Table 1. Inhibitory activity (%I) and IC_{50} (mM) ± standard deviation (SD) of LaSMMed (10 mM) and standard thiourea against CNU.

 LaSMMed	R	%I ± SD	IC_{50} ± SD
LaSMMed 1	Cl	92.81 ± 0.54[a]	0.342 ± 0.005[a]
LaSMMed 2	Br	91.81 ± 0.18[a]	0.358 ± 0.008[b]
LaSMMed 3	NO_2	92.74 ± 0.18[a]	0.355 ± 0.003[b]
LaSMMed 4	OCH_3	93.24 ± 0.05[a]	0.340 ± 0.006[a]
LaSMMed 5	CH_3	92.89 ± 0.18[a]	0.378 ± 0.002[c]
Thiourea	-	92.19 ± 0.36[a]	0.265 ± 0.005[d]

Molecular docking simulations of **LaSMMed 1-5** with CNU revealed their key interactions within the enzyme's active site, emphasizing crucial functional groups and structure for targeting. All compounds exhibited a similar binding mode: the nitrogen atoms of the thiourea moiety formed hydrogen bonds with Gly543 and Asp626, while the sulfur atom interacted with nickel, which aligns with existing literature data.[1] Additionally, the phenyl ring, whether substituted or not, formed important hydrophobic interactions with residues like His400, His402, His485, His512, His538, Asn432, Ala433, Cys585, His586, Asp487, Asp629, and Met630 (Figure 1).

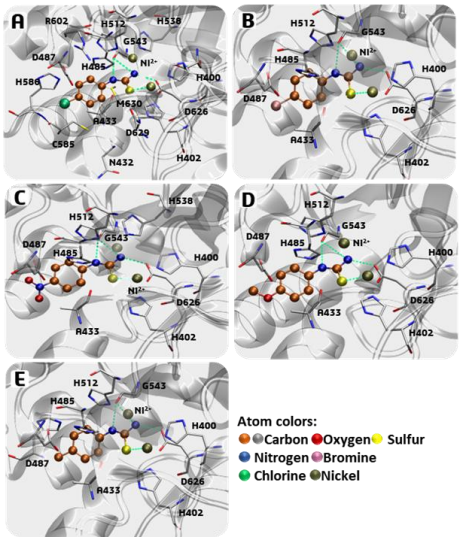


Figure 1. The best GOLD molecular docking poses (orange ball and sticks) of (**A**) LaSMMed 1, (**B**) LaSMMed 2, (**C**) LaSMMed 3, (**D**) LaSMMed 4, and (**E**) LaSMMed 5 within the substrate binding site of the CNU.

Conclusions

Results emphasize that arylthioureas could lead to the development of new anti-ureolytic agents.

Acknowledgements

INCT on Urease Inhibitors of Agricultural and Medicinal Interest - FAPERJ – CENAPAD-SP – CNPq – CAPES

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Synthesis and evaluation of alkyltriazoles as potential antiplasmodial agents

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Key words: Malaria, alkytriazoles, heme, antiplasmodial,

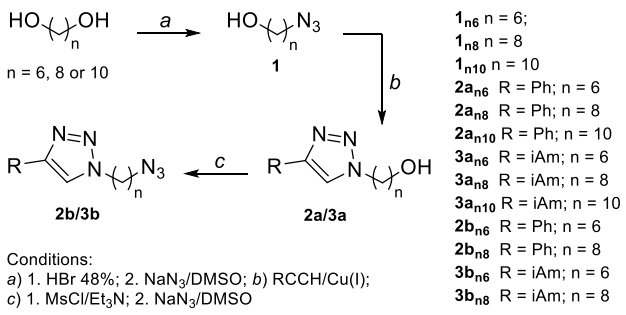
Introduction

Malaria remains a major global health issue and is caused by protozoa of the genus *Plasmodium*. In 2023, the WHO estimated 263 million cases and 597,000 deaths[1], highlighting that effective control measures are still lacking. The emergence of drug-resistant parasites[2] has made the search for new potential antimalarial agents increasingly urgent. Previous studies have shown that 3-alkylpyridines (3-APA) exhibit antiplasmodial activity against resistant strains of *Plasmodium falciparum* (IC_{50}: <5 μM) and demonstrate high affinity for the heme group (K_d: 22–38 μM)[3], although these compounds display a low cytotoxicity selectivity index (SI)[4]. It is hypothesized that molecules with high heme-binding affinity inhibit hemozoin formation, thereby maintaining free heme, which is toxic to the parasite and essential for its survival in the human host[2]. To identify active compounds with an improved SI, this study describes the synthesis and evaluation of a series of alkyltriazole analogues of 3-APA. Their interaction with heme was assessed by UV-vis titration[5] and Mass Spectrometry[3].

Results and Discussion

Alkyltriazoles were synthesized according to the synthetic route depicted in Figure 1. The products were obtained via a Cu(I)-catalyzed *click* reaction between an alkyne (phenylacetylene or 5-methyl-1-hexyne) and an alkylazide (**1**$_{n6}$, **1**$_{n8}$, **1**$_{n10}$).

Figure 1: Synthesis of alkyltriazole derivatives.



Conditions:
a) 1. HBr 48%; 2. NaN_3/DMSO; *b*) RCCH/Cu(I);
c) 1. MsCl/Et_3N; 2. NaN_3/DMSO

EM-ESI confirmed complex formation through the detection of a heme–ligand peak. For the alkyltriazole ligands, most of the synthesized compounds exhibited a corresponding peak, indicating interaction with heme in a 1:1 stoichiometry (one ligand molecule bound to one free heme). Dissociation constants (K_d) for these interactions were determined by UV-vis titration. The values obtained for the alkyltriazoles suggest high binding affinity toward the heme. Table 1 summarizes the global yields and K_d values for the tested compounds.

Table 1: Global yields and K_d of alkyltriazoles with heme.

Compd	Global Yield	K_d (μM)
2a$_{n6}$	22%	3,935±2,164
2a$_{n8}$	43%	2,547±0,918
2a$_{n10}$	18%	ND
3a$_{n6}$	33%	0,783±0,385
3a$_{n8}$	31%	0,944±0,764
3a$_{n10}$	22%	ND
2b$_{n6}$	20%	1,286±1,070
2b$_{n8}$	21%	2,278±0,819
3b$_{n6}$	26%	2,776±1,807
3b$_{n8}$	11%	3,870±2,584

None of these compounds showed antiplasmodial activity against *Plasmodium falciparum in vitro*. Despite high heme binding, limited efficacy may stem from poor membrane permeability, highlighting the need to optimize physicochemical properties and explore advanced delivery systems.

Conclusions

In this study, we demonstrated that the alkyltriazole compounds not only interact with the heme group (except for **3a**$_{n10}$ and **2a**$_{n10}$), but also exhibit high binding affinity, as indicated by their low K_d values. These compounds, despite not showing antiplasmodial activity, they act as valuable reference for future scaffold optimization.

Acknowledgements

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Computational studies involving GSK-3β inhibitors as therapeutic alternatives targeting Alzheimer's disease

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Key words: Alzheimer's disease, GSK3-β, Computational studies, Molecular docking, HeatMap/LUNA approach

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia, characterized by cognitive loss and neuronal death.[1] Different AD-related targets such as glycogen synthase kinase 3-beta (GSK-3β), a serine/threonine kinase, are expressed in the brain. Its role is involved in neurodevelopment, axon formation and synaptogenesis. However, hyperactivation of GSK-3β has been associated with various pathological features of AD, including hyperphosphorylation of the tau protein, neuroinflammation, oxidative stress damage, memory impairment and amyloid-β (Aβ) plaques deposition.[2,3] In this context, we describe herein computational studies involving molecular docking, followed by HeatMap and LUNA approaches to figure out the structural features relevant for the bioactivity in GSK-3β inhibitors.

Results and Discussion

In the molecular docking step, the 4 scoring functions available in the GOLD software (ASP, ChemPLP, ChemScore and GoldScore) were used for redocking validation, based on the orientation of the co-crystallized ligand and interactions with key amino acids residues. In this sense, the structure of the GSK-3β, was retrieved from PDB (ID: 8FF8). The reference ligand was constructed, protonated (pH 7.4) and optimized from the DSV, MarvinSketch and Mopac (PM7) softwares, respectively. In the validation process, an RMSD of 0.55 was obtained for the GSK-3β. The GoldScore score function presented the best result. Molecular dynamics studies are in progress to figure out the stability of the protein-ligand complexes. One of the goals is also to apply artificial intelligence to uncover new interaction patterns. Parallelly a total of 745 ligands with defined Ki values were extracted from the ChEMBL database to perform molecular docking under the same conditions described above for the co-crystallized structure. Figure 1 shows the docking result for the ligand presenting highest activity (pKi = 10.05). Intermolecular interactions were analyzed using the LUNA *toolkit*. Figure 2 displays heatmaps of the 30 ligands with the highest activity (pKi values ranging from 8.2 to 10.05), highlighting the most relevant interactions.

Figure 1: Molecular docking result for the ligand presenting the highest activity (pKi = 10.05).

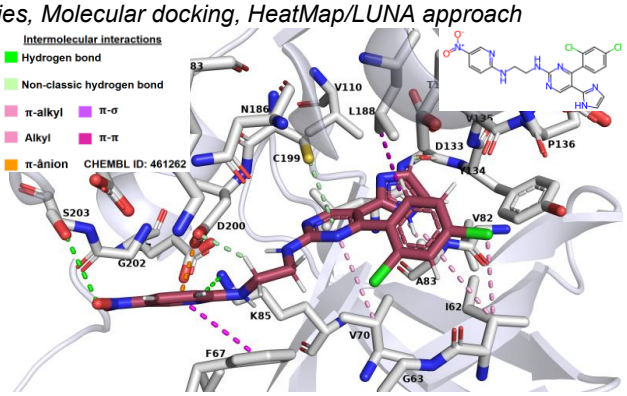
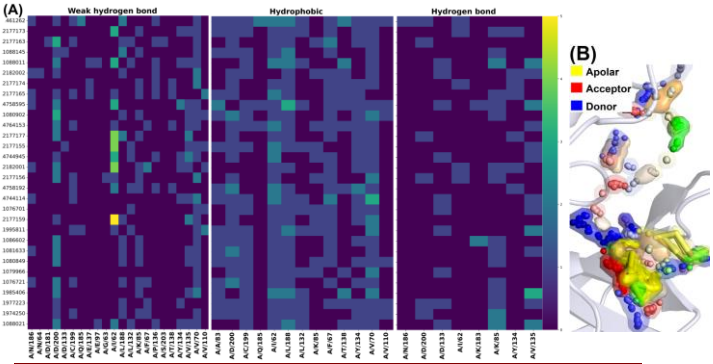


Figure 2: (A) Heatmap analysis of the key residues involved in the most relevant intermolecular interactions. X-axis: amino acids; Y-axis: ChEMBL IDs. Scale of intermolecular interactions ranges from purple (least relevant) to yellow (most relevant). (B) Analysis of binding pockets identified by E-FTMap.



Conclusions

The heatmap analysis revealed that the most active ligands predominantly are involved in hydrophobic interactions with key residues such as V110, I62, and L188, located in a central apolar cluster of the GSK-3β. Additionally, hydrogen bonds with residues D200, V135, and E137 contribute for the stabilization and proper orientation of the ligand. These findings were consistent with the mapping results obtained by E-FTMap, which reveal the distribution of polar and apolar regions within the GSK-3β binding site and show the agreement between biological activity and interaction profile of the analyzed compounds.

Acknowledgements

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In silico evaluation of *N*-aryl-1,10-phenanthroline-2-amines as potential inhibitors of *T. cruzi* GP63 zinc-metalloprotease by docking and molecular dynamics simulations

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Keywords: Neglected diseases, Chagas disease, Trypanosoma cruzi, Zinc-metalloprotease, Zinc-chelating agents, Molecular dynamics.

Introduction

Chagas disease, caused by *Trypanosoma cruzi*, affects millions of people in Latin America and still poses challenges for diagnosis and treatment. Current drugs are toxic and ineffective in the chronic stage, and the parasite shows growing resistance, making new therapies essential.[1]

This study evaluated *N*-aryl-1,10-phenanthroline-2-amine derivatives, which exhibit trypanocidal activity and zinc-chelating ability. Computational simulations were used to explore the interaction and stability of these compounds as potential inhibitors against *Tc*GP63, a key enzyme involved in cell invasion by the parasite during *T. cruzi* infection in Chagas disease.[1]

Results & Discussion

Based on the *in vitro* trypanocidal efficacy of synthesized *N*-aryl-1,10-phenanthroline-2-amines (**Phen1**-**20**), we explored their potential as ligands for our *T. cruzi* GP63 (*Tc*GP63) model.[1] Docking simulations with four GOLD scoring functions showed that *N*-(R-phenyl) derivatives (R = CH_3, OCH_3, CF_3, CN, NO_2, F, Cl, Br) had higher fitness scores than *N*-naphthyl ones. Among these, the six *para*-substituted derivatives (**Phen4**, *p*-CH_3; **Phen7**, *p*-OCH_3; **Phen10**, *p*-CN; **Phen14**, *p*-F; **Phen17**, *p*-Cl; **Phen18**, *p*-Br) (Figure 1) were most favorable compared to *ortho* or *meta-substituted compounds*.

Subsequent aqueous molecular dynamics simulation (GROMACS package, CHARMM36 force field, and TIP3P water model) of the protein-ligand complexes showed persistent interactions within the *Tc*GP63 active site, primarily through coordination with Zn(II)-cofactor, and H-bonding with catalytic **Glu221** and zinc binding **His224**. RMSD and RMSF analyses confirmed the stability of these interactions, particularly for compounds with electron-withdrawing groups by inductive effect, such as *p*-OCH_3 (**Phen7**) and *p*-CN (**Phen10**).

ΔG_{bind} calculations by the linear interaction energy (LIE) method corroborated the favorable interactions observed in simulations, highlighting **Phen7** and **Phen10** as the most promising candidates (Table 1).

Figure 1. The GOLD molecular docking poses of derivatives **Phen4** (A), **Phen7** (B), **Phen10** (C), **Phen14** (D), **Phen17** (E), and **Phen18** (F) within the active site of the *Trypanosoma cruzi* GP63 metalloprotease.

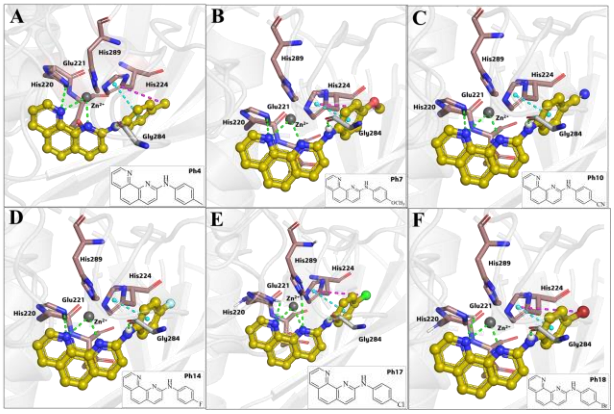


Table 1. The binding free energy (ΔG_{bind}, kcal/mol) estimated by the LIE method (standard coefficients α = 0.161 and β = 0.50).

Phen	R	ΔG_{bind}
4	*p*-CH_3	-8.30
7	*p*-OCH_3	-8.88
10	*p*-CN	-9.29
14	*p*-F	-6.82
17	*p*-Cl	-7.53
18	*p*-Br	-3.29

Conclusions

Results emphasize that *N*-phenyl-1,10-phenanthroline-2-amines could lead to the development of new trypanocidal agents against Chagas disease.

Acknowledgements

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Water-soluble porphyrins and metalloporphyrins: efficient photosensitizers for photodynamic therapy against breast cancer

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Keywords: Metallic complexes, light, cancer, cationic porphyrins.

Introduction

Triple negative breast cancer (TNBC) constitutes up to 20% of all cases of diagnosed breast cancer and presents a poor prognosis for patients, with high mortality and recurrence rates. Standard care for TNBC includes non-specific chemotherapy, radiotherapy, and surgeries. These approaches often lead to severe adverse effects, such as nausea, vomiting, hypersensitivity, myelosuppression, weight loss and secondary cancers[1,2]. The development of new treatment options for TNBC is of paramount interest. Photodynamic therapy (PDT) is one promising alternative for TNBC treatment, as it's a localized and minimally invasive therapy, reducing adverse effects. In the PDT protocol, a substance, known as a photosensitizer (PS), interacts with visible light, generating reactive oxygen species (ROS) that effectively kill the cancerous cells[3]. Porphyrins derivatives, and their metallic complexes, are commonly used as PS, due to their ability to absorb visible light and generate ROS[4], but solubility issues and ROS generation efficacy hamper the usage of porphyrins as PS in PDT. In this work, the use of the water-soluble 5,10,15,20-tetrakis(N-ethylpyridinium-3-yl)porphyrin chloride (H_2P), and its Zn^{2+} (ZnP), Cu^{2+} (CuP), Mn^{3+} (MnP), Sn^{4+} (SnP) and Au^{3+} (AuP) complexes as PS, with irradiation at 520±5 nm and 11,7 mW cm^{-2} total light dose, was evaluated. Notably, the CuP, SnP, and AuP complexes are new molecules. Their synthesis and biological assays against the TNBC cell line MDA-MB-231 were performed.

Results and Discussion

The porphyrin derivatives were synthesized and characterized according to Vieira et al.[5]

Firstly, a preliminary experiment, with a 100 µM concentration was performed to assess the most promising compounds. CuP and MnP did not show increased toxicity upon irradiation, due to their poor ability to generate ROS[5], and they were not further investigated. The IC_{50} and selectivity indexes (SI) of the H_2P, ZnP, SnP, and AuP were determined against the MDA-MB-231 TNBC cell line and HEK-293 (healthy cells), in presence or absence of light, as seen in Table 1.

Table 1. IC_{50} and SI of the tested compounds (µM±SD) in the presence (520±5 nm and 11,7 mW cm^{-2}) or absence of light.

Compound	HEK-293		MDa-MB-231		SI[a]
	Dark	Light	Dark	Light	
H_2P	>1000	0,68±0,11	>1000	0,21±0,13	3,2
ZnP	>1000	0,12±0,05	>1000	0,22±0,16	0,5
SnP	>1000	0,13±0,19	>1000	0,77±0,25	0,2
AuP	>1000	44,73±31,21	>1000	25,24±12,11	1,8

[c] *Selectivity index. SI= IC_{50} HEK-293/ IC_{50} MDA-MB-231.*

H_2P, ZnP, and SnP exhibited remarkable photodynamic activity, as seen by their IC_{50} values in nanomolar range. Although the SI of ZnP and SnP are <1,0, they are still promising candidates for PS, as the PDT approach is localized. The clonogenic assay, which predicts drug sensitivity/resistance, was performed by irradiating the compounds at their IC_{50} values and comparing clonogenic cells survival with a control. SnP completely prevented the survival of clonogenic cells, while H_2P and ZnP reduced colony survival by 50% and AuP by 30%, highlighting the potential of SnP as a PS. Furthermore, cell by cell flow cytometry indicated that the mechanism of cytotoxicity of the compounds may involve cell cycle arrest, followed by the cell death.

Conclusions

The present findings showcase the potential of water-soluble porphyrins, especially SnP, due to its remarkable IC_{50} and complete inhibition of clonogenic cell survival. It can be recognized as potential PS for the treatment of TNBC.

Acknowledgements

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Development and application of a novel screening method for dual-target inhibitors of cholinesterases

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Key words: enzyme inhibitors, screening assays, acetylcholinesterase, butyrylcholinesterase, enzyme immobilization.

Introduction

An estimated 55 million people worldwide are affected by some form of dementia, with Alzheimer's disease (AD) responsible for 60 to 80% of cases, a condition marked by the relentless and irreversible loss of neurons.[1] Among the hypotheses proposed to explain its pathophysiology, cholinergic system dysfunction stands out as a key factor. The cholinergic hypothesis attributes cognitive decline to reduced acetylcholine levels, caused by the combined action of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE predominates in early AD, while BuChE activity rises in later stages, making both enzymes complementary therapeutic targets.[2] Currently, AChE inhibitors are the main drugs used to treat AD, but their limited efficacy and adverse effects have prompted the search for safer and more effective alternatives. [3] In this context, dual-target cholinesterase inhibitors have emerged as a promising strategy to improve therapeutic outcomes. In this study, a modified Ellman-based approach was employed to screen a library of synthetic compounds.

Results and Discussion

To enable this screening, a high performance liquid chromatography based method was developed. AChE and BuChE were covalently immobilized onto commercial magnetic particles, and enzymatic hydrolysis was performed off-line. Thiocholine was indirectly detected by post-column derivatization with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent), yielding a yellow ion absorbing at 412 nm. The method used a YMC Triart C18 column (50 × 3.0 mm, 3.0 µm) with H_2O:MeOH (97:3, v/v) at 0.4 mL/min (pump A), and 0.25 mM DTNB in phosphate buffer (100 mM, pH 7.0) was infused post-column at 0.2 mL/min (pump B). It was validated according to ANVISA's RDC No. 167/2017, demonstrating linearity, precision, and accuracy. Enzyme kinetics assays, conducted in triplicate with increasing substrate concentrations, enabled the determination of Michaelis-Menten constants: 245.2 ± 50.5 µM for AChE and 877.4 ± 130.1 µM for BuChE. These values were used in inhibition assays to ensure optimal screening conditions. The method was validated for screening using donepezil as a model inhibitor, yielding IC_{50} values (64.91 ± 15.99 nM for AChE and 2202 ± 326 nM for BuChE) and a non-competitive inhibition mechanism for both enzymes in agreement with the literature. These results demonstrate the method's reliability for accurate inhibitor detection and discrimination of inhibition mechanisms. [4] Sixteen compounds were screened at 10 µM, and those showing inhibition greater than 60% were selected for IC_{50} determination: ten for AChE and four for BuChE. IC_{50} values were obtained from dose response curves, ranging from 0.64 to 980.81 nM for AChE and from 109.2 to 7738.7 nM for BuChE. Among these, AAPA34 and AAPA43 were identified as potent dual inhibitors (Table 1). Their inhibition mechanisms were elucidated through kinetic studies using varying concentrations of substrate and inhibitor, enabling the construction of Lineweaver Burk plots to support mechanism classification.

Table 1. IC_{50} and type of inhibition of the most potent inhibitors

AAPA	AChE		BuChE	
	IC_{50} (nM)	Type of inhibition	IC_{50} (nM)	Type of inhibition
34	0.6 ± 0.1	Competitive	109.2 ± 14.7	Competitive
43	1.0 ± 0.1	Competitive	1212.5 ± 144.0	Competitive

Conclusions

In conclusion, the analytical method was successfully developed and validated. Its ability to recognize and characterize cholinesterase inhibitors was confirmed using donepezil. It was then applied to the screening of synthetic compounds, identifying two promising candidates with dual-target potential that exhibited greater inhibitory potency than the model inhibitor.

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Development of Coumarin-1,2,3-Triazole Conjugates: Promising Leads for Anticancer Therapy Targeting PKM2.

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Keywords: OSCC, PKM2, Drug Development.

Introduction

Cancer constitutes one of the most significant challenges faced by health systems worldwide due to its high mortality rate and the difficulty in developing effective therapies.[1] Pkinase M2 (PKM2) is overexpressed in many tumors and has been associated with cancer resistance, tumor progression, and poor prognosis, making it a promising target for the development of anticancer therapeutics.[2] In this context, the structural combination of bioactive scaffolds, such as coumarin and 1*H*-1,2,3-triazole, has emerged as a valuable strategy for identifying new drugs candidates with potential antitumor activity.[3] In this study, twelve novel coumarin-based compounds will be synthesized and evaluated through *in vivo*, *in silico* and *in vitro* approaches to assess their biological activity against cancer cells and their potential as prototypes for PKM2 inhibition.

Results and Discussion

A novel coumarin-based compounds were synthesized through a multistep procedure as shown in **Figure 1**.

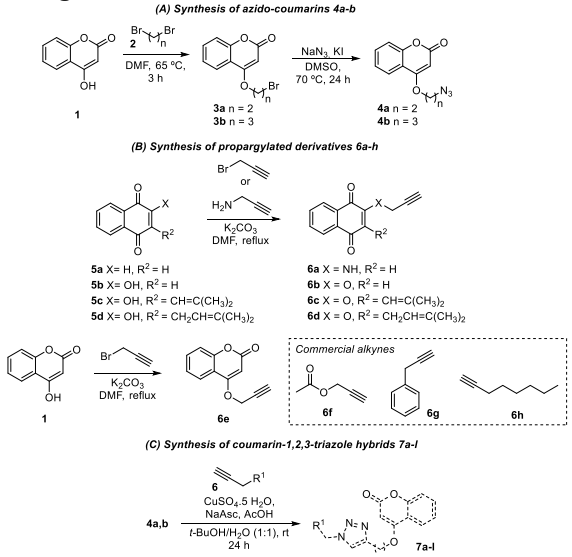


Figure 1: Synthesis of compounds **7a-l**.

All compounds were tested against SCC-9 oral squamous cell carcinoma cell line. Compound **7f** demonstrated the highest selectivity, with selectivity indices (SI) of 4.61 for B16F-10, 3.38 for HCT116, 2.98 for 4T1, and an average SI of 2.18 across SCC-4, SCC-9 and SCC-25 cell lines while it's antiproliferative effect is associated with oxidative stress-induced apoptosis. **7f** also exhibited low hemolytic activity and did not induce observable toxic effects in C57BL/6 mice at doses up to 400 mg/kg. Moreover, the analysis of the PKM2 inhibitory potential of **7f** was supported by molecular docking and *in vitro* biochemical assays, indicating that the compound may bind to PKM2 and inhibit its catalytic activity in a dose-dependent manner. Ultimately, *in silico* analyses of the physicochemical properties of **7f** revealed favorable pharmacological characteristics and predicted low toxicity.

Conclusions

The compounds will represent pioneering examples of a novel class of potent cytotoxic agents effective against oral squamous cell carcinoma (OSCC). Compound **7f** demonstrated potent and selective activity with low *in vivo* toxicity, a well-defined mechanism of action involving apoptosis and ROS production, and notable potential to inhibit PKM2.

Acknowledgements

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Combining drug repurposing and virtual screening to discover novel antagonists for P2X7 receptor

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Keywords: P2X7, antagonists, screening virtual, docking.

Introduction

Purinergic receptors play a key role in cell communication and the regulation of physiological processes.[1] Among them, the P2X7 receptor stands out for its involvement in inflammation and pain.[2-6] It is expressed in immune cells, such as macrophages and dendritic cells, and in central nervous system cells, including astrocytes and microglia, and contributes to inflammatory and neurodegenerative processes.[2-7] The activation of P2X7 by extracellular ATP at concentrations above 100 µM triggers ion flux and, with prolonged stimulation, induces membrane pore formation, leading to the release of proinflammatory cytokines.[2,8,9] Although preclinical studies with P2X7 antagonists have shown promising anti-inflammatory and analgesic effects,[4] no drugs targeting this receptor have been approved. In this context, this project applies virtual screening to identify new P2X7 antagonists from drug repositioning, aiming to discover safer and more effective therapeutic candidates.

Results and Discussion

Docking assays were performed using the human P2X7 receptor structure based on the model predicted by AlphaFold2. Initially, reference ligands such as agonists (ATP and BzATP) and antagonists (BBG, KN-62, AZ11645373, A740003, PPADS, and oATP) were tested to establish a comparative affinity range for the binding sites. Docking simulations were conducted using DockThor and AutoDock Vina, focusing on both the orthosteric and allosteric sites of the receptor. The score range for reference ligands was between -9.758 and -7.126 kcal/mol at the orthosteric site and between -11.629 and -7.187 kcal/mol at the allosteric site. For drug repurposing, 9,300 molecules from the DrugBank database were tested using DockThor program, with the top 100 molecules having scores ranging between -10.294 and -8.150 kcal/mol and between -13.302 and -10.920 kcal/mol in the orthosteric and allosteric sites, respectively. In AutoDock Vina, the scores ranged between -11.426 and -9.991 kcal/mol in the orthosteric site. Molecules ranked among the top 100 in both programs will undergo visual inspection and consensus analysis to select candidates for acquisition and subsequent *in vitro* experimental validation.

Conclusions

Structure-based virtual screening enabled the identification of novel candidate antagonists for the P2X7 receptor, a relevant target for the treatment of inflammation and chronic pain. Several compounds demonstrated higher binding affinities than known reference ligands did, indicating potential antagonistic activity. The next steps will focus on selecting the most promising candidates for *in vitro* experimental validation.

Acknowledgements

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Azoles for the control of *Meloidogyne incognita*: a telescoped synthesis of 4-aryl-2-aminothiazoles and 4-aryl-2-aminoselenazoles mediated by tribromoisocyanuric acid

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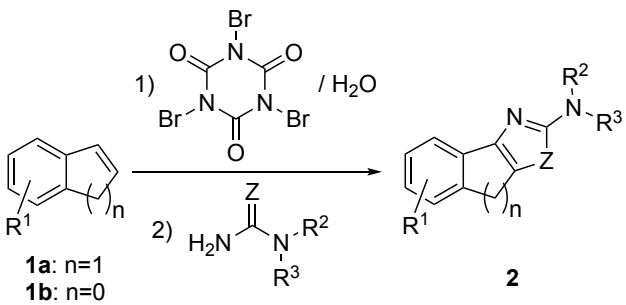
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Key words: thiazoles, selenazoles, organic synthesis, nematodes, Meloidogyne

Introduction

Currently, plant-parasitic nematodes (PPNs) pose a significant threat to global food production, accounting for more than 30% of crop yield losses worldwide.[1] Among the most destructive PPNs is *Meloidogyne incognita*, which causes severe losses in soybean production.[2] Therefore, the development of new products for PPN control is of great interest.
One of the possibilities for controlling PPNs is the use of thia- and selenazoles, which are traditionally prepared by the Hanztsch synthesis, which uses toxic reagents. To circumvent such problems, we developed a very convenient telescoped reaction[3] mediated by tribromoisocyanuric acid (TBCA) for the synthesis of such azoles,[4,5] thus minimizing the production of chemical waste, reducing operational costs, and avoiding contact with toxic and/or unstable intermediates. Once prepared, the azoles underwent bioassay with *M. incognita*.

Results and Discussion

The 4-aryl-2-aminoazoles **2** were prepared according to **Scheme 1**, using indene **(1a)** as substrate for thiazoles and styrenes **(1b)** for selenazoles. The protocol involves three reactions: a tandem co-bromination and oxidation of the formed bromohydrin by excess TBCA to give phenacyl bromides, followed by a telescoped condensation with thioureas and selenoureas to give the thiazoles **2a** (19 – 62 %) and selenazoles **2b** (25 – 65 %), respectively.



2a: n=1; Z=S; R^1=H; R^2=H, Ph; R^3=H, alkyl, allyl, aryl
2b: n=0; Z=Se; R^1=H, alkyl, halogen; R^2,R^3=H,Me

Scheme 1. Telescoped synthesis of -aminoazoles.

In vitro tests were conducted in 96-well polypropylene plates with 300 μL wells, where 20 μL of an aqueous suspension containing approximately 20 second-stage juveniles (J2s) of *M. incognita* and 100 μL of the thiazole or selenazole solution were added. After 48 h, J2 mortality and immobility were evaluated. The substances that showed nematicidal activity were selected to determine *in vitro* the lethal concentration capable of killing 50 % (LC_{50}) of *M. incognita* J2. An *in vivo* test was conducted with tomato plants (*Solanum lycopersicum* L. 'Santa Clara') to evaluate the number of galls after treatment with different concentrations of the substance selected in the *in vitro* assay. Among the azoles evaluated, one showed nematicidal activity against *M. incognita*, causing 100 % immobility of the nematodes (commercial nematicide: 100 %) and more than 60 % mortality of the nematodes (commercial nematicide: 45 %). In the *in vivo* test, there was a reduction of more than 50 % in the number of galls compared to the negative control.

Conclusions

We have developed a convenient telescoped synthesis of 4-aryl-2-aminothiazoles and 4-aryl-2-aminoselenazoles. Among the azoles studied, one showed nematicidal activity *in vitro* and *in vivo* against *M. incognita*. Therefore, it has potential for use in the development of new nematicides.

Acknowledgements

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Hit-to-lead for Chagas disease: Lessons learned with Benzoxazinone series

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Key words: Trypanosoma cruzi, ADME optimization

Introduction

The Lead Optimization Latin America (LOLA) consortium is part of a set of initiatives coordinated by the Drugs for Neglected Diseases initiative (DNDi) that aim to develop treatment alternatives for Chagas disease (CD)[1]. Current CD treatments have variable efficacy and adverse effects that affect the prevalence of the disease, affecting more than 70 million people in Latin America[2]. Therefore, the aim of this work is to present a hit-to-lead (H2L) campaign for the Benzoxazinone series, demonstrating how Drug Discovery programs can be conducted in academia.

Results and Discussion

The Benzoxazinone series was identified by High-Throughput Screening (HTS) with a commercial compound library, performed by DNDi collaborators. The hit stood out for its submicromolar trypanocidal potential and balanced ADME properties, in addition to not presenting CYP51 inhibition. An early *in vivo* pharmacokinetic study of the hit showed a high *in vivo* clearance, and this, coupled with *in vitro* metabolic stability data, directed the optimization efforts (Figure 1). In addition, the metabolite identification (MetID) study highlighted the sulfonamide and morpholine groups as the main soft spots, guiding efforts during the exploration of the SPR. The exploration of SAR and SPR were conducted aiming to increase the metabolic stability of the series and the potency against *Trypanosoma cruzi*. To this end, classical medicinal chemistry strategies were used to design the analogues during the various optimization cycles. The prediction of the Phase I metabolites by Stardrop software supported the selection on the commercial building blocks used to modulate metabolism. Multiparametric optimization of this series resulted in the synthesis of more than 90 derivatives that had their trypanocidal activity established. The physicochemical and ADME properties were also determined experimentally.

Conclusions

The Benzoxazinone series was discontinued from LOLA due to its low trypanocidal potential against most strains of the DTU panel. Nevertheless, it was demonstrated that the use of clear metabolism modulation strategies from the beginning, based on *in vitro* and *in vivo* data, was decisive for the progress of this H2L campaign.

Acknowledgements

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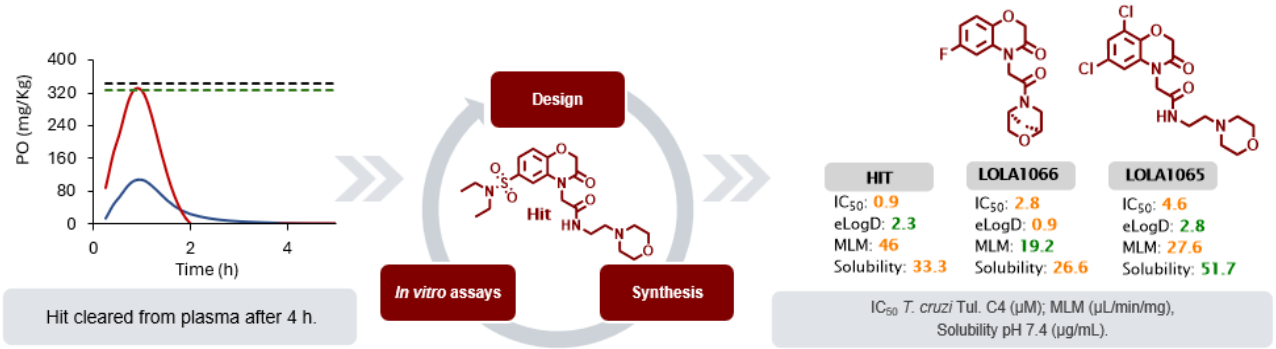


Figure 1: H2L Bezoxazinone series.

Structure-Based Design and *In Silico* ADMET Profiling of Lenalidomide Derivatives for Multiple Myeloma

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Key words: Modification, cancer, solubility, spacer

Introduction

The discovery that immunomodulatory imide drugs (IMiDs) exhibit potent antitumor properties has significantly advanced the treatment of specific hematological malignancies.[1] Among them, thalidomide, lenalidomide, and pomalidomide have proven effective in managing multiple myeloma, myelodysplastic syndrome (MDS) with deletion of chromosome 5q, and other related disorders. Recent findings have demonstrated that IMiDs exert their effects by binding to cereblon (CRBN), a substrate receptor of the CRL4 E3 ligase complex, thereby promoting the ubiquitination and subsequent degradation of the transcription factors IKZF1 and IKZF3 in multiple myeloma cells.[2] This mechanism contributes directly to their anti-myeloma efficacy. Considering the current therapeutic challenges, the development of novel agents remains crucial. In this regard, the rational design and synthesis of new lenalidomide derivatives represent a promising strategy for further investigation. Thus, this study aims to evaluate novel lenalidomide derivatives through in silico ADMET and docking analysis to guide future optimization.

Results and Discussion

Lenalidomide derivatives were designed by introducing aliphatic spacers [n = 1 (C1), 2 (C2), 4 (C4), 6 (C6), 8 (C8), 10 (C10)] at the solvent-exposed amine position of the isoindolinone ring (**Figure 1)**, outside the hydrophobic pocket. Molecular docking was conducted using AutoDock Vina, with redocking validation (RMSD = 0.4278 Å).

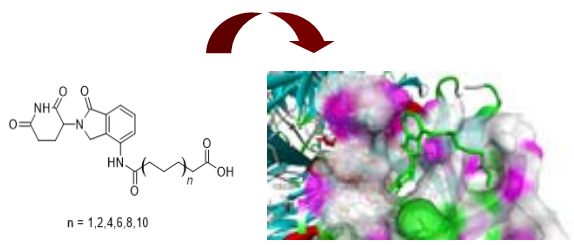


Figura 1: Representation of the chemical structures of lenalidomide derivatives and their interaction with the biological target.

The target protein (PDB: 4TZ4) was prepared at pH 7.4 using APBS-PDB2PQR. Ligand geometries were optimized with Avogadro (MMFF94). Binding affinities ranged from –8.0 to –6.4 kcal/mol, with the six-carbon spacer derivative showing the highest affinity. Crucial interactions with TRP380, HIS378, and ASN351 were maintained across all analogs.

In silico ADMET analysis (SwissADME and pkCSM) confirmed Lipinski's rule compliance and absence of mutagenicity (AMES test) for all compounds. Most analogs (C2–C10) displayed high gastrointestinal absorption; only C1 was predicted to be low. CYP3A4 inhibition emerged in C8 and C10. All compounds were predicted to be hepatotoxic, and oral LD_{50} values indicated low acute toxicity, ranging from 2.3 to 2.94 mol/kg. A clear trend of increasing lipophilicity (Log P: 0.13 to 2.76) and improved absorption was observed with longer spacers. Based on the obtained data, it was observed that the lipophilicity of the compounds increased as more carbon atoms were added to the chain, while still adhering to Lipinski's Rule. In the context of isoforms, it was found that as carbon atoms were incorporated into the chain, more CYP enzymes were inhibited. Regarding toxicity data, the compounds exhibited hepatotoxicity following the structural modification. Acute oral toxicity values ranged from 2.3 to 2.937 mol/kg, with the compounds being classified as low toxicity.

Conclusions

Spacer elongation in lenalidomide derivatives enhanced molecular interactions without compromising target binding, while also improving predicted ADMET properties. These findings support the feasibility of structural modification as a rational strategy to develop next-generation IMiDs with optimized pharmacokinetics and efficacy.

Acknowledgements

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Structure-based virtual screening and drug repurposing to identify immunosuppressive agents for liver transplantation

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Keywords: calcineurin, drug repurposing, virtual screening, immunosuppressants, docking, liver transplantation.

Introduction

Liver diseases represent a global public health problem and are responsible for 1 to 2 million deaths annually. Diseases such as viral infections, autoimmune hepatitis, and alcohol abuse can progress to cirrhosis, a condition that led to 1.5 million deaths in 2019.[1] Orthotopic liver transplantation is the only curative intervention for patients with end-stage liver disease. The success of transplantation is dependent on immunosuppressive therapy, which prevents graft rejection. The basis of this therapy is calcineurin inhibitors (CNIs), such as tacrolimus and cyclosporine. Although effective, their chronic use causes severe adverse effects, including nephrotoxicity, neurotoxicity, and metabolic disorders. Chronic kidney failure can affect up to 20% of patients within five years after transplantation, requiring reduction or suspension of treatment.[2] Given the urgent need for safer drugs, this work used virtual screening as a strategy for drug repurposing, aiming to identify new calcineurin inhibitors with better safety profiles.

Results and Discussion

The repurposing strategy focused on calcineurin as a pharmacological target, specifically at its binding sites with nuclear factor of activated T cells (NFAT) and the LxVP and PxIxIT domains. A curated library of approximately 9,000 compounds from DrugBank, comprising both approved and investigational drugs, was screened using docking. To enhance the robustness of the results, a consensus approach was applied by employing two docking programs, DockThor and AutoDock Vina. Initial screening with DockThor revealed that seven drugs had docking scores superior to that of tacrolimus (-9.476 kcal/mol), one of the reference CNIs. The consensus analysis, which compared the top 100 results from both programs, identified 21 common molecules for the LxVP domain and 10 for PxIxIT. Two molecules, RAF-265 (Figure 1) and LAN, both under investigation for cancer treatment, stood out for appearing in the top rankings for both domains in both programs, emerging as the most promising candidates. In the LxVP domain, the RAF-265 molecule presented binding scores of -9.285 and -7.835 kcal/mol, whereas the LAN molecule presented scores of -9.472 and -7.808 kcal/mol according to the DockThor and AutoDock Vina programs, respectively. In the PxIxIT domain, RAF-265 presented scores of -7.559 and -6.200 kcal/mol, whereas LAN displayed scores of -7.519 and -5.904 kcal/mol in DockThor and AutoDock Vina, respectively.

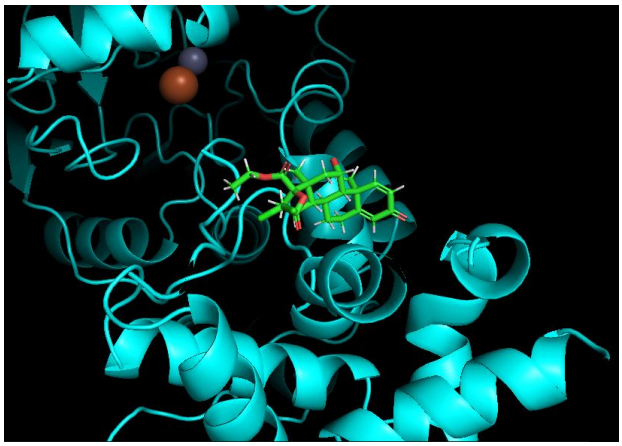


Figure 1: Best pose of the RAF-265 molecule interacting with the calcineurin LxVP site.

Conclusions

The virtual screening approach for drug repurposing was successful in identifying clinically relevant compounds as potential calcineurin inhibitors. The molecules RAF-265 and LAN, in particular, represent promising candidates for new therapeutic applications as immunosuppressants. The next steps of the project include the visual inspection of these drugs and experimental validation of their biological activity through *in vitro* lymphocyte proliferation inhibition assays.

Acknowledgements

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4-Quinolone-3-Carboxamide Derivatives via OPSS: A DNA-Targeted Study Combining In Silico and In Vitro Tools

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Key words: Quinolone derivatives, DNA binding, One-pot synthesis.

Introduction

4-Quinolones are heterocyclic compounds known for their broad biodynamic profile and are found in widely used antibiotics in clinical practice, such as ciprofloxacin and levofloxacin.[1] The carboxamide group, in turn, is an important pharmacophore present in several drugs and is associated with various biological activities. Thus, 3-carboxamide-4-quinolone derivatives have been extensively studied, with examples of compounds exhibiting antiproliferative profile.[2] Increasing concerns regarding environmental impact, toxicity, and the cost of organic solvents have encouraged the development of more sustainable synthetic routes.[3] In this context, the present work describes the development of a one-pot stepwise synthesis (OPSS) method (Scheme 1) for the synthesis of 3-carboxamide-4-quinolone derivatives, in addition of *in vitro* and *in silico* DNA-targeted studies.

Results and Discussion

All synthesized compounds were carefully characterized, and eight of them (10a, 10b, 10d, 10e, 10f, 10g, 10k, and 10n) were selected based on their electronic properties at the molecular termini for interaction studies with calf thymus DNA (CT-DNA). The interaction was initially evaluated in vitro through UV–Vis absorption, steady-state fluorescence, and viscometry, using the CT-DNA: EB complex—formed by the interaction of ethidium bromide (EB) with CT-DNA—as a reference. The results indicated that the compounds bind to DNA mainly through secondary interactions such as hydrogen bonding and van der Waals forces, with no evidence of intercalation between nitrogenous bases. Moreover, viscosity measurements indicated that the 3-carboxamide-4-quinolone derivatives do not induce changes in the length of the CT-DNA double helix, suggesting the absence of intercalation between the bases. These results indicate that these compounds bind to DNA through secondary interactions, without directly interfering with the helical structure. *In silico* calculations were performed using a molecular docking approach under physiological conditions. The 3-carboxamide-4-quinolone derivatives exhibited similar binding poses in the minor groove of DNA, consistent with the experimental binding constant values. Molecular docking analyses indicated that the interactions with DNA occur mainly through van der Waals forces and hydrogen bonds, especially between carboxyl groups of the nucleobases and amino groups of the compounds, with distances characteristic of strong interactions (ranging from 1.70 to 2.50 Å).

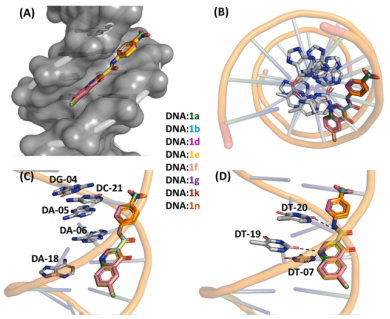


Figure 1. Superposition of the top-ranked docking pose in the DNA interaction site.

Conclusions

Fifteen 3-carboxamide-4-quinolone derivatives were synthesized in good yields using an efficient OPSS strategy. Eight compounds were selected for interaction studies with CT-DNA, demonstrating binding through secondary interactions (hydrogen bonds and van der Waals forces) without intercalation between the bases. Molecular modeling studies confirmed a preferential affinity for thymine and adenine bases, suggesting the potential of these compounds as DNA replication inhibitors, with possible applications in the development of antiproliferative drugs.

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Development of novel triazolylarylpyrimidine analogs as potential drug candidates for the treatment of HIV

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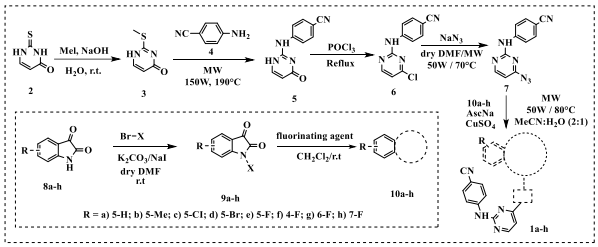
Keywords: AIDS, HIV, isatin, DAPY, LASFAR036

Introduction

HIV-1, the etiological agent of AIDS, remains a major global public health concern. One of the primary challenges in antiretroviral therapy is the emergence of viral variants resistant to currently available drugs, underscoring the ongoing need for novel therapeutic strategies[1]. In the pursuit of new non-nucleoside reverse transcriptase inhibitors (NNRTIs), our research group synthesized LASFAR036, a DAPY-class analog—a core scaffold shared by the NNRTIs etravirine and rilpivirine. This compound inhibited viral replication by over 80%, displayed an IC_{50} of 3.2 nM, and suppressed reverse transcriptase (RT) enzymatic activity by 94%. Molecular docking studies suggested that LASFAR036 interacts with a distinct site on RT, indicating a potentially novel mechanism of action. However, the compound exhibited a short plasma half-life of 1.13 hours, highlighting the need for structural optimization to improve its lipophilicity and metabolic stability[2]. To address these limitations, we proposed the synthesis, physicochemical characterization, and biological evaluation of a new series of fluorinated analogs **1a–h** derived from LASFAR036. The incorporation of fluorine atoms was guided by literature reports indicating their potential to enhance molecular lipophilicity, thereby favoring an extended plasma half-life[3].

Results and Discussion

The adopted synthetic route comprises seven reaction steps. Initially, thiouracil (**2**) undergoes S-alkylation to afford intermediate **3**. Subsequently, compound **3** reacts with 4-aminobenzonitrile (**4**) under microwave irradiation to yield intermediate **5**, which is then subjected to chlorination using $POCl_3$ to afford intermediate **6**. This intermediate undergoes a nucleophilic aromatic substitution (S_NAr) with sodium azide (NaN_3), resulting in the formation of compound **7**. In parallel, isatins (**8a–h**) undergo a bimolecular nucleophilic substitution reaction (S_N2) to generate intermediates **9a–h**, which are subsequently treated with a fluorinating agent to afford compounds **10a–h**. Finally, a copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC "click" reaction) is performed between intermediate **7** and compounds **10a–h** to obtain the final products **1a–h**. To date, six intermediates (**3**, **5**, **6**, **7**, **9a**, and **10a**) have been successfully synthesized with yields ranging from 61% to 95%, along with final compound **1a**, obtained in 61% yield. The intermediates were characterized by spectrometric methods, while final compound **1a** was characterized using both spectrometric and spectroscopic techniques, confirming the expected structures.



Scheme 1. Synthetic methodology for the preparation of final compounds **1a–h**.

Conclusions

Thus far, the proposed synthetic methodology has proven to be feasible, and the data obtained indicate that the route is reproducible. The project timeline is progressing as planned. The next steps include the completion of the synthesis and characterization of compounds **9b–h**, **10b–h**, and **1b–h**, followed by the execution of biological assays.

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Design the optimization of canabidiol bioavailability using chemoinformatics tools and bioisosteric strategies

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Key words: CBD, molecular modification, computacional chemistry, bioisosterism.

Introduction

Cannabidiol (CBD) is one of the cannabinoids present in *Cannabis sativa* and is among those with the highest concentration in its extract, accounting for around 40% of the composition.[1] CBD has also been shown to interact with several endocannabinoid systems present in the body. Regarding the mechanism of action of CBD, there is still no exact understanding of its functioning. However, it is known that it is directly related to the cannabinoids in the endocannabinoid system, which is present in several areas of the body, including the central nervous system, peripheral nervous system, and the brain. CBD is directly linked to CB1 and CB2 receptors, with CB1 being found in the pain pathways of the brain and spinal cord, thus showing good results in analgesia. CB2 receptors are mainly located in immune cells, where CBD directly influences the anti-inflammatory process. CBD can also act as a negative allosteric modulator of the CB1 cannabinoid receptor, a G-protein-coupled receptor that is highly abundant in the body. Cannabidiol has been used in the treatment of several pathological conditions, including epilepsy, Alzheimer's disease, and in reducing pain in patients with breast cancer, multiple sclerosis, glaucoma, among others. CBD is considered a drug with low oral bioavailability due to factors that interfere with both its absorption and systemic availability after ingestion.[2] The oral bioavailability of CBD is estimated to be between 6% and 19% in humans, depending on the formulation and administration conditions. The factors that interfere with its bioavailability is its extensive first passage hepatic metabolism; the hepatic enzymes CYP3A4 and CYP2C19 metabolize a large fraction of the compound. High lipophilicity, which makes it difficult to dissolve in the aqueous content of the gastrointestinal tract, and moderate permeability indicated in Caco-2 studies, interacts with P-glycoprotein, which pumps CBD back into the intestinal lumen.[3] The aim of this project is to do design the optimization of CBD bioavailability using chemoinformatics tools and bioisosteric strategies.

Results and Discussion

As a methodology for optimizing CBD in this work through the bioisosterism strategy, the swissbioisostere (www.swissbioisostere.ch) platform is being used. A substituent position was submitted to the platform, which generated 2,243 fragments that are being manually inspected from the data generated on the platform, which are: activity, qualitative classification of best, similar or worst, TPSA, logP and molecular mass. So far, 469 fragments have been selected, the main form of selection of which is by the activity endpoint represented by the colored bar, in which the green bar represents a significant increase in activity (>0.5log), the orange bar represents a neutral or very slight effect, and the red bar represents a significant decrease (<–0.5log). All 469 fragments selected so far have 100% of the green bar or at least 50% of the green bar and 50% of the orange bar. We are avoiding selecting fragments that have any percentage of the red bar. When submitting the CBD structure to the swissadme (http://www.swissadme.ch/) and admetsar (https://lmmd.ecust.edu.cn/) platforms to have a positive control, the theoretical result corroborates the literature, about logP (consensus = 5.20, high gastrointestinal absorption in swissadme, it is a substrate of CYP3A4 and CYP2C19 and of PgP, it has good permeability in Caco2 (-4.62). In a preliminary test of the compound with the first selected fragment, in the swissadme platform, the logP increased slightly to 5.8 and the solubility in water became even lower, which will probably maintain the same characteristics of CBD.

Conclusions

It has been concluded so far that it is still necessary to filter the fragments that can increase water solubility and prevent binding to P-glycoprotein, maintaining permeability in Caco2 cells, maintaining or improving the activity of the generated compound.

Acknowledgements

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Phthalimide as Protecting Group of Amines: Expanding the Toolbox of the DNA Encoded Chemical Library (DECL) Technology

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Key words: Drug Discovery, Biochemistry, DNA-encoded-Chemical library technology, method development

Introduction

To investigate the feasibility of phthalimide as an amine-protecting group on DNA. This will include the identification of reaction conditions for deprotection of the phthalimide group. Further aims are to demonstrate the utility of the developed method in a DECL synthetic sequence.
In our research group, we focus on discovering new antibacterial molecules. Antibiotic resistance is a severe global problem, where the medicines we use to fight infections become less effective against bacteria as they develop resistance against the drugs. Chemists have dedicated significant efforts to finding new, improved drugs and drug combinations to combat this emerging problem. DNA-encoded chemical library (DECLs) technology provides an efficient and cost-effective drug discovery tool for new starting points. In this approach, large libraries containing millions to billions of compounds attached to unique DNA barcodes are created and used to screen against various isolated drug targets for novel binders. As DNA is fragile, mild synthetic strategies are needed which can ensure selective reactions on building blocks attached to the DNA molecule. Here, DNA-compatible protecting groups of amines are for example limited. Protecting groups need to be stable in conditions suitably of choice, easily attached and removed.

Results and Discussion

We have investigated the DNA-compatibility method of deprotection of phthalimide. For this, we developed methods for attaching phthalimide to an amine on double-stranded DNA and for removal of the phthalimide, freeing the amine. This allows us to more easily work with DNA and gain better control over the use of amines on DNA molecules.

Conclusions

Successfully our objectives of this project, to develop reaction conditions for DNA-compatible phthalimide protection and deprotection were discovered. Future work within our project is to investigate the usage of phthalimide as a protecting group of amines on DNA for application in DNA-encoded library synthesis tested bacterial activity assays.

In vitro and in silico analysis of benzoylthiourea derivatives and their potential anti-*Mycobacterium tuberculosis* activity

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Key words: thioureas, HadAB, liposome encapsulation, molecular docking, minimal inhibitory concentration.

Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb), which infects approximately 10.8 million people worldwide, ranking first as the largest drug-resistant airborne epidemic in 2022[1]. This has led to an increase in antibiotic resistance and TB-related deaths, underscoring the need for new treatments that can provide a better quality of life for patients while being effective against resistant strains[1]. A key molecular target is the (3*R*)-hydroxyacyl-ACP dehydratase (HadAB) from Mtb, which is an enzyme involved in the biosynthesis of the cell wall mycolic acids, known for being the target of the former anti-TB drug isoxil (ISO), a thiourea derivate[2,3,4]. Therefore, the present work aimed to evaluate *in vitro* the minimal inhibitory concentration (MIC) of benzoylthioureas against Mtb (H37Rv), previously identified by our research group, as possible HadAB inhibitors through a virtual screening based on molecular docking[5]. These derivatives were also encapsulated in liposomes, aiming to improve their aqueous solubility. Afterwards, we performed another molecular docking analysis, applying the prior parameters, on the 13 best-ranked benzoylthioureas, now without the oxidation of the thiourea group, to establish new chosen molecules and thus carry out their *in vitro* assays.

Results and Discussion

In a previous study from our research group, 99 oxidized benzoylthioureas were screened using molecular docking, resulting in the selection of four molecules: LPS 05, 21, 152, and 153. Then, *in vitro* MIC assays were performed in Mtb H37Rv, a susceptible strain against first- and second-line TB drugs[6]. Resulting in the absence of inhibitory activity in concentrations ≤ 1 mM, rifampicin being the treatment reference (MIC = $3{,}645.10^{-6}$ mM). During those studies, it was noted that the high insolubility in water of these compounds prevented their solubilization in the aqueous culture medium; therefore, it was possible that this could interfere with the compound's activity. Encapsulation methodologies were carried out using the lipid film hydration technique, resulting in positively charged liposomes (polydispersity index: 17.3 to 27.2%; zeta potential: 34.03 to 53.26 mV; particle size: 182.72 to 578.36 nm, with LPS 152 being the exception at 867.56 nm)[7]. All achieved between 97.80% and 100% encapsulation efficiency. Once more, MIC assays were performed, this time using the encapsulated compounds (1 mM) and the empty liposome as a control; however, they did not yield different results from the previous assays. Other *in silico* studies were carried out with the same parameters, but without the oxidation of EthA to its respective sulfenic acids, as described in the literature for the ISO (pro-drug)[8]. Rendering a different ranking based on *fitness score* (FS), but all remained above the original ligand (fisetin) FS (60.21). When analyzing their interactions at the HadAB active site, LPS 154, 165, and 209 were the best choices, as they made at least one interaction with the catalytic amino acids (His41 and Asp36)[5]. Moreover, all interact with one or two of the four water molecules (HOH: 330, 339, 353, and 434) that are reported to be essential for the binder's stability[9].

Conclusions

LPS 05, 21, 152, and 153 did not show inhibitory activity on Mtb (≤ 1 mM). Having a possible molecule activation different from the reported (EthA), revealing the need for new studies using non-oxidized molecules to select new compounds and measure their activity. Given that, cytotoxicity assays will also be performed on LLCMK2 to demonstrate whether liposomes can reduce the cytotoxicity in mammalian cells.

Acknowledgements

UEL CAPES LabMult LaSMMed Laboratório Síntese de Moléculas Medicinais

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Identification of potential Aldose Reductase inhibitors from the Natural Products Database of the Bahia Semi-arid (NatProdB)

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Key words: Virtual Screening. Diabetes. Molecular Dynamic. Inhibition.

Introduction

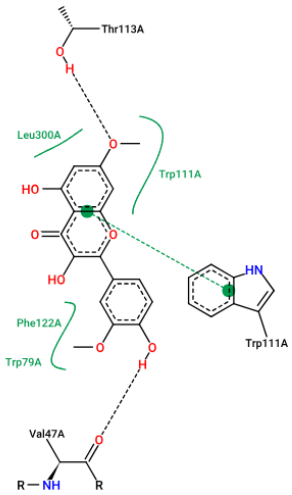
Diabetes Mellitus (DM) is a disease characterized by high levels of glucose, when not properly controlled, can result in various complications. Among the mechanisms of diabetes complications, there is Aldose Reductase (ALR2), a NADPH-dependent enzyme, present in the polyol pathway, where the glucose is converted to sorbitol, which accumulate in cell membranes, leading to disease complications such as retinopathy and vascular diseases.

Thus, ALR2 inhibition is important for preventing or reducing the progression of DM complications. This study had the aim of prioritize potential inhibitors against ALR2 at Natural Products Database of the Bahia Semi-Arid region using docking-based virtual screening.

Results and Discussion

ALR2 3D structure (PDB ID = 1US0; Resolution = 0.66 Å) was prepared using the Biopolymer module at SYBYL-X 2.1.1. for docking-based virtual screening. Next, parameters of Autodock Vina 1.1.2 was submitted to efficiency evaluation by redocking (RMSD = 1.36Å). In addition, enrichment factor by AUC/ROCurve was calculated using DUD-E (10 true positives and 500 false negatives) (AUC=0.73). These results demonstrate that the docking parameters is adequate to produce good poses and predictive for identification of potential active molecules. Natural Products Database (n=461) of the State University of Feira de Santana was collected from the COCONUT database and applied in docking routine with ALR2. Score values revealed that CNP0237570, a O-methylated flavonol, exhibited a highest binding affinity (-9.90 kcal/mol). In order to evaluate the intermolecular interactions, 2D plot using PoseView server was generated and it showed hydrogen bonding with Val47 and Thr113 and hydrophobic interactions with Trp79, Trp111, Phe122 and Leu300, all of them are key catalytic residues **(Figure 1).**

Figure 1. 2D interactions map of CNP0237570 with ALR2. Black dashed lines and green lines represent hydrogen bonding and hydrophobic interactions, respectively.



So, this natural product can be selected for further computational approach as Molecular Dynamics.

Conclusions

The virtual screening has identified CNP0237570, a metabolite found from green propolis of the *Mimosa tenuifora, a species* from the semi-arid region of Bahia as a promising potential inhibitor against ALR2.

Acknowledgements

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Identification of Novel Dual *Haemonchus contortus* PMT1/PMT2 Inhibitors by Molecular Docking

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Key words: anthelmintic, alkaloid, dual inhibitors, phosphoethanolamine N-methyltransferases

Introduction

Haemonchus contortus infections represent one of the most common constraints in small ruminant production[1]. The nematode resistance to various available drugs, along with concerns regarding drug residues in animal-derived food products and environmental contamination, has intensified the search for new antiparasitic agents. In this context, the identification of compounds that act on novel molecular targets, distinct from those of existing anthelmintics, represents a promising strategy.
The enzyme phosphoethanolamine N-methyltransferase from *H.contortus* , comprising the di-domain proteins *Hc*PMT1 and *Hc*PMT2, is essential for the phosphocholine biosynthesis, which is one of the main lipids in nematodes[2].
The aim of this study was to identify, alkaloids às potential dual inhibitors of *Hc*PMT1 and *Hc*PMT2 by molecular docking.

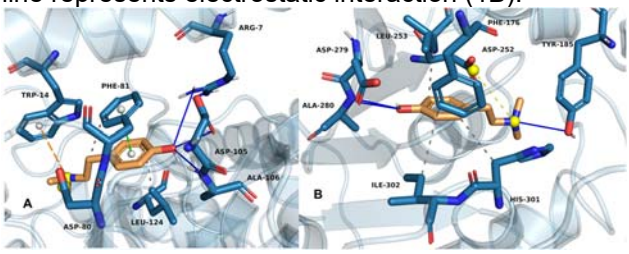
Results and Discussion

The experimental three-dimensional structure of PMT1 and PMT2 were collected from the Protein Data Bank (PDB IDs 4KRG and 4KRI). Receptor preparation was performed in Chimera (v.1.9) using the DockPrep module. Molecular docking was performed in DOCK (v.6.10) using the crystallographic ligand coordinates to construct the grid box. Pose ranking was performed using the GridScore (default) score. Considering the need to act on both enzyme isoforms, the scoring function values were summed, selecting the pose with the best combined score. Intermolecular interactions were analyzed using the PLIP server. Hordenine was the alkoloid with the best score for *Hc*PMT1 and *Hc*PMT2 (Table 1).

Table 1. Summed score of the poses of each alkaloid docked in the target proteins (PMT1 and PMT2) of *Haemonchus contortus.*

ALKALOIDS	PMT1	PMT2	Σ Grid Score
Hordenine	-34.18	-33.18	-67.36
Magnoflorine	-29.54	-34.37	-63.91
Palmatine	-29.86	-35.11	-64.97
Papaverine	-29.35	-31.44	-60.79

Figure 1: Intermolecular interactions for the hordenine complex with *HcPMT1* (1A) and *HcPMT2* (1B). Blue hydrogen bonds, gray dashed lines represent hydrophobic interactions, green dashed line demonstrates π-stacking (1A), yellow dashed line represents electrostatic interaction (1B).



In *Hc*PMT1 (1A), hydrogen bonds occurred between the hydroxyl group with Arg7, Asp105, Ala106; in the aromatic ring there is a hydrophobic interaction with Leu124 π-stacking with Phe81, which also interacts with a hydrophobic interaction at C7; finally, an electrostatic interaction occurs with AA Asp80 and T rp 14. For *Hc*PMT2 (1B), hydrogen bonds occur with Asp279, Ala280 and Tyr185; hydrophobic interactions between the aromatic ring and Leu253, Phe176 (2x), Ile302, His301 and an electrostatic interaction in the amine terminal portion with Asp252. Complex 1A showed important electrostatic interaction at residue Trp14, literature says that this residue is responsible for the space orientation at the site. For PMT2, hordenine interacted at residues His301 and Tyr185, close to those reported as part of the active site (His295 and Tyr191).

Conclusions

Among the alkaloids evaluated, hordenine exhibited the best ranked for both *Hc*PMT1 and *Hc*PMT2, with relevant intermolecular interactions, suggesting its potential as a dual inhibitor of these targets.

Acknowledgments

I would like to thank the Postgraduate Program in Pharmaceutical Sciences of State University of Feira de Santana and the funding agency CNPq

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Multiparametric optimization of pyrazole compounds with antimalarial activity

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Key words: Drug discovery, hit-to-lead, malaria, SAR.

Introduction

Despite significant progress in malaria control, the emergence of drug-resistant *Plasmodium falciparum* strains continues to undermine current therapies. The development of new chemical entities with novel mechanisms of action and improved pharmacological profiles remains a critical goal in global antimalarial efforts[1,2]. Herein, we describe the hit-to-lead optimization of a pyrazole-based series initially identified through phenotypic screening by Medicines for Malaria Venture (MMV). This collaborative study, part of the MINDI consortium, involved iterative chemical design, biological evaluation, and pharmacokinetic profiling, aiming to balance potency, metabolic stability, and drug-like properties.

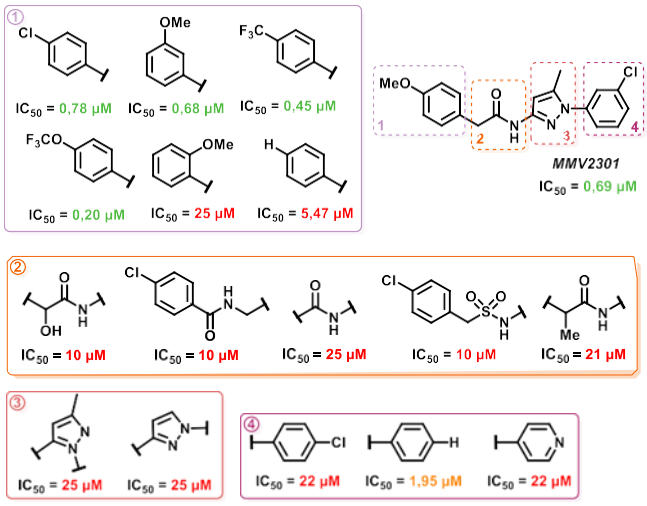
Results and Discussion

The campaign originated from MMV2301, a hit compound identified in a phenotypic screen against the asexual blood stage of *P. falciparum*. Approximately 40 analogs were synthesized with variations at the central core, right-hand side (RHS), and left-hand side (LHS) fragments. *In vitro* potency was determined against the *P. falciparum* 3D7 strain, generating a detailed SAR map (**Figure 1**). Several compounds achieved IC_{50} values in the low micromolar range. These potent analogs were further characterized for pharmacokinetic parameters, including microsomal stability and clearance in hepatocyte assays.

While potency improved significantly, most analogs displayed high lipophilicity (logD > 4), correlating with poor aqueous solubility, elevated clearance rates, and increased likelihood of CYP450 interactions[3]. Importantly, the compounds showed no cytotoxicity in HepG2 cells and lacked activity against hERG channels.

A metabolite identification (MetID) study revealed that amide hydrolysis was the main metabolic liability, explaining the rapid *in vivo* clearance. This observation is consistent with known vulnerabilities of amide bonds under physiological conditions[4]. To overcome this issue, structural modifications focused on either masking or replacing the amide moiety, with preliminary results indicating a reduction in metabolic susceptibility.

Figure 1: Representative analogs of the pyrazole series and their IC_{50} values against *P. falciparum* (3D7 strain).



Conclusions

This study successfully improved antimalarial potency, achieving compounds with IC_{50} values as low as 200 nM, and generated a comprehensive SAR profile. However, the metabolic instability associated with the amide functionality hindered further advancement into the lead optimization phase.

Acknowledgements

We thank Medicines for Malaria Venture (MMV), FAPESP (The State of São Paulo Research Foundation) – Project 2015/50655-9 and Process 2024/00815-9 for financial support. We are grateful to UNICAMP and USP for institutional support and infrastructure, and to TCGLS for DMPK analyses.

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Exploratory assessment of 4-allyl, 4-propargyl and 4-benzyl-piperazine amides as inhibitors of monoamine oxidase B

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Key words: MAO-B, Parkinson disease, Neurodegenerative diseases.

Introduction

Parkinson's Disease (PD) is the second most prevalent chronic neurodegenerative disease worldwide, manifesting a number of motor and non-motor symptoms that hinder the patient's life. The pathological mechanisms underlying PD are complex and multifactorial, but at the end the dopaminergic neurons of the substantia nigra die, leading to an imbalance of the dopaminergic-cholinergic neurotransmission. The current therapies are still based on recovering dopaminergic neurotransmission. Monoamine oxidase B (MAO-B) is a key enzyme in the pathogenesis of PD. It catalyzes the oxidation of dopamine, which produces reactive oxygen species and toxic metabolites that affect mitochondrial function and neuronal viability. Thus, the inhibition of MAO-B is a considerable strategy to treat PD. Several MAO-B inhibitors present the propargyl amine pharmacophore in the structure, related to the irreversible inhibition of MAO-B. However, the bioisosteric replacement for allyl and benzylamines would keep the interaction with the enzyme avoiding covalent inhibition. Considering this, our group designed a set 4-allyl and 4-benzyl-piperazine amides and its 4-propargyl counterparts (LINS06 compounds) to assess their potential as MAO-B inhibitors.

Results and Discussion

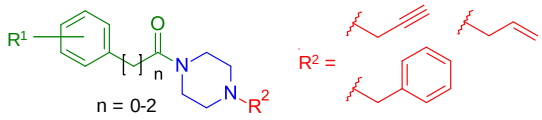


Figure 1: General structure of the designed LINS06 compounds.

The compounds were synthesized following well-stablished methods with moderate to good yields.[1,2] Aryl carboxylic acids were converted into the corresponding acyl chlorides using $SOCl_2$ or directly used in the reaction with Boc-piperazine using EDC/HOBt-catalyzed coupling reaction. The Boc intermediates were deprotected with TFA and then alkylated with corresponding alkyl halides to generate the final compounds LINS06 with allyl (**1a-e**), propargyl (**2a-e**) or benzyl (**3c-g**) piperazine moieties.

The compounds were tested for their inhibitory activity against human MAO-B by following the previously published method.[3]

Table 1. Results for the inhibitory activity of LINS06 compounds against human MAO-B.

Compound	% inhib (1 μM) ± SEM	IC_{50} (nM) ± SEM
1a	18 ± 1	n.d.
1b	0	n.d.
1c	0	n.d.
1d	2 ± 1	n.d.
1e	5 ± 1	n.d.
2a	0	n.d.
2b	0	n.d.
2c	2 ± 1	n.d.
2d	20 ± 3	n.d.
3c	0	n.d.
3f	0	n.d.
3g	57 ± 2	244.2 ± 19.9

The allyl-containing compounds (**1**) showed negligible inhibitory activity on the enzyme, while some of the propargyl counterparts presented a discrete increase in the activity. Just one benzyl piperazine compound (**3g**) showed relevant inhibitory activity, with nanomolar affinity, suggesting that the substitution pattern on R^1 led to increased affinity. This pattern will be further explored in a future set of compounds.

Conclusions

In summary, the present work reveals that 4-benzyl piperazine amide **3g** was an interesting prototype for further modification in the search for novel MAO-B inhibitors, suggesting that non-covalent MAO-B inhibitors with high potency can be achieved.

Acknowledgements

FAPESP (2023/03485-7), CNPq (307829/2021-9 and 302348/2025-5) and CAPES (code 001).

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Evaluation of Barbituric and Thiobarbituric Acid Derivatives as Covalent Inhibitors of *Leishmania braziliensis* DHODH

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Key words: leishmaniasis, covalent inhibitors, DHODH, mimetic core

Introduction

Leishmaniases are part of the group of Neglected Tropical Diseases (NTDs), prevalent in tropical and subtropical regions of developing countries and affecting around 2 billion people[1]. Targeting the enzyme dihydroorotate dehydrogenase (DHODH), essential for parasite survival, is a promising strategy for the design of antiparasitic compounds[2]. At CRAFT, researchers focused on covalent inhibition of *Lb*DHODH by designing inhibitors that mimic orotic acid and bind to the catalytic cysteine (*Cys*131)[2], a residue unique to class I DHODH[3]. Electrophilic warheads increase potency when combined with a substrate-mimicking core, as demonstrated by **QHM1036**, which showed time-dependent inhibition and revealed structural insights for drug development.

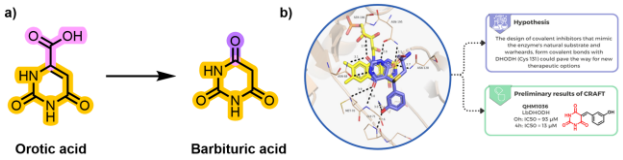


Figure 1: a) Barbituric acid as a mimetic nucleus of orotic acid; b) QHM1036 and crystal structure showing *Cys*131 attacking the exocyclic double bond.

Results and Discussion

With the aim of exploring the orotic acid-mimetic core and developing more potent covalent inhibitors of the *Lb*DHODH enzyme, a series of barbituric acids (BA) and thiobarbituric acids (TB) was synthesized (Figure 2). Both BA and TB analogs were evaluated in parallel for their inhibitory activity and through molecular docking studies.

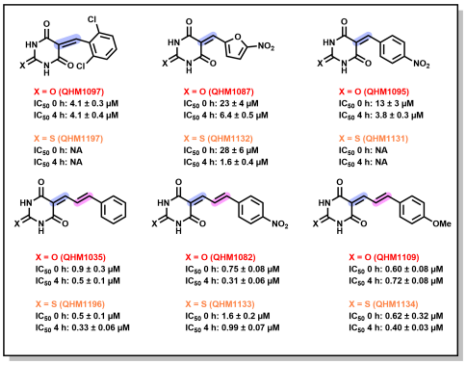


Figure 2: Synthesized compounds and their enzymatic results against *Lb*DHODH.

Molecular docking experiments were performed using Maestro (Schrödinger®), with LigPrep for ligand preparation, Protein Preparation Wizard for protein setup, and Glide for docking simulations. After preparing the three-dimensional structure of *Lb*DHODH, a docking protocol was applied to determine the binding mode between the ligand and the target in non-covalent docking mode.

Docking Scores (DS) ranged from -2.032 to -7.599. The compound with the best binding energy was QHM1035, with a DS of -7.599.

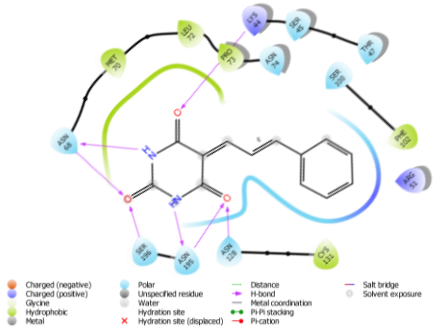


Figure 3: 2D representation of compound QHM1035 in the active site of *Lb*DHODH.

Overall, the molecules showed promising performance agains *Lb*DHODH, particularly compounds with exocyclic dienes as warheads (substituted or unsubstituted), in contrast to the initially identified hit. The introduction of a sulfur atom showed an impact on activity in some cases, with loss of activity observed in two compounds bearing only one exocyclic double bond.

Conclusions

Compounds demonstrated significant enzymatic activity, while molecular docking supported their favorable binding modes. These results provide valuable insights for the rational design of new antileishmanial agents targeting DHODH.

Acknowledgements

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Development of Fragments Aiming *M. tuberculosis* STPKs PknA, -B, -G and RipA Interaction in a Multitarget Approach

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Key words: Tuberculosis, FBDD, Scaffold hopping, Multitarget Approach, Organic Synthesis, Virulence Factor.

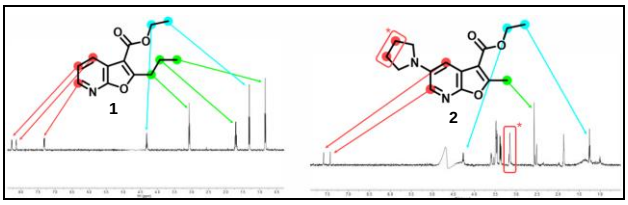
Introduction

Tuberculosis is a highly contagious airborne disease that affects millions of individuals annually. It is known that *M. tuberculosis* (*M.tb*) can remain dormant in the human body for years before reactivating and manifesting symptoms.[1] The RipA enzyme, crucial in bacterial reactivation, cell division, and cell wall maintenance, recently emerged as a promising target.[2] Also, some more studied mycobacterial Ser/Thr protein kinases (STPKs) play an important role in physiological maintenance as well as in the survival of *M.tb* within the host.[3,4] In this work, we propose the synthesis of heterocyclic compounds aiming interaction with these targets.

Results and Discussion

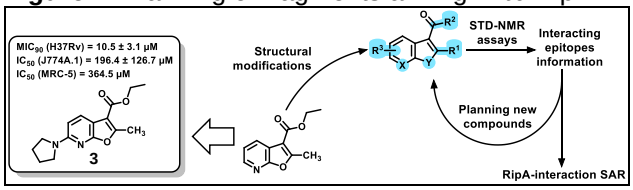
A fragment screening revealed a molecule from CRAFT's compounds library that exhibited notable interactions with the active site of RipA, as assessed by STD-NMR. Furthermore, the evaluation of compounds with different modifications in its furo[2,3-*b*]pyridine scaffold revealed new regions amenable to fragment growth that were identified by STD-NMR, taking fragments **1** and **2** as an example, presented in the **fig. 1** below.

Figure 1: Fragment-RipA interaction points detected via STD-NMR.



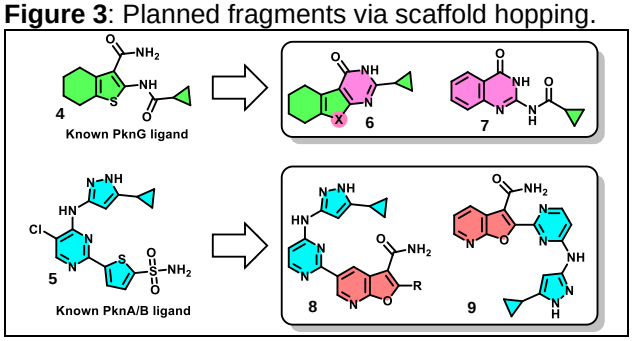
To enhance RipA interaction, our goal is to optimize these fragments and explore the reactivity of the furo[2,3-*b*]pyridine scaffold through various chemical reactions (**fig. 2**). Also, the core scaffold explored hre is similar to the fragment **3**, with known activity against *M.tb*, but unknown mechanism of action.[5]

Figure 2: Planning of fragments aiming *M.tb* RipA



One issue when working with the *M.tb* RipA is the lack of structural information about its active site, but having the known STPKs PknA, -B and -G ligands (**4, 5**) as a starting point, some of the modifications in the furo[2,3-*b*]pyridine scaffold are expected to result in compounds that mimic these fragment-STPK interaction through scaffold hopping, as well as keeping some interaction with the active site of the RipA (**6-9**). The computational studies of these fragments via molecular docking are ongoing, so molecules that exhibit good *in silico* interaction with PknA, -B and/or -G will be selected to be synthesized and tested against these enzymes as well as the RipA.

Figure 3: Planned fragments via scaffold hopping.



A double inhibition of any of these STPKs and RipA may cause the bacteria to be more vulnerable within the host, since these enzymes play an important role to keep the integrity of the cell wall, these bacteria's strongest defense. This might make some resistant *M.tb* strains more succeptive to antibiotics by drug association.

Conclusions

With the development of these compounds, a double inhibition of RipA/PknA, -B or -G is intended, resulted in the vulnerabilization of *M.tb* through a multitarget approach.

Acknowledgements

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Synthesis and antineoplastic activity of new analogues of LQB-461

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Key words: Nitrones, aldehydes, cancer

Introduction

In previous studies aiming at the discovery of new antiproliferative and antiparasitic compounds, we synthesized and evaluated several arylnitrones and their synthetic precursors, the corresponding aromatic aldehydes. Regarding the antiproliferative action, nitrone **LQB-278**, was the most active on Jurkat leukemia cells, inhibiting NO production, up-regulating p21CIP1/WAF1/Sdi1 protein expression, reducing cell growth, and inducing apoptosis (IC_{50} of 0.4 μM).[1] Interestingly, among the aromatic aldehydes, **LQB-461** was the most promising, inducing apoptosis in these cells through both extrinsic and intrinsic pathways (IC_{50} of 2.8 μM).[2,3] Since aldehyde oxidases (AOXs) are a group of enzymes involved in drug and xenobiotic metabolism and this enzyme is highly promiscuous and metabolizes a wide range of aldehydes, nitro-derivatives and *N*-oxides, we wondered if metabolites of **LQB-287** and **LQB-461** could be responsible for their antiproliferative action. Herein we report the antiproliferative effects of six derivatives of **LQB-461** (**1a-f**, Figure 1) on a series of human leukemia cell lines (Jurkat, K562, FEPS, and Lucena), as well as melanoma (MV3), prostate (PC3), and breast cancer (MCF7, MBA-MB231) cell lines.

Results and Discussion

New compounds (**1a-d**) were obtained through the *O*-alkylation of *ortho*-hydroxyaldehydes with halides (global yields 52-81%), while compounds **1e**,**f** are commercially available.

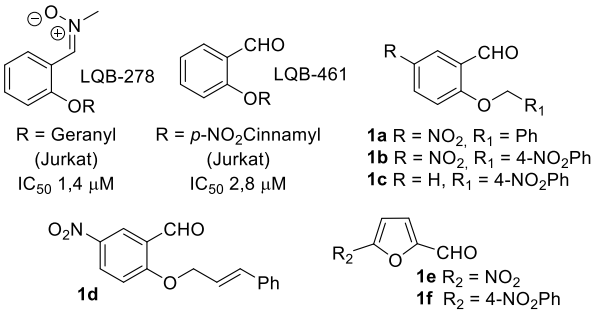


Figure 1: Lead compounds and derivatives evaluate in this work (**1a-f**).

Figure 2 provides the differential cytotoxic effects of compounds (**1a-f**) on various cancer cell lines (A-G) at a fixed concentration of 10 μM, as measured by their impact on cellular metabolic activity (viability). Except for **1e**, the other nitroaldehydes proved to be potent in Jurkat cells (A). However, in K562, FEPS and MV3, cell inhibition below 50% was not observed. Compound **1a** exhibited the most potent cytotoxicity across JURKAT, PC3, MCF7, and MBA-MB231 cell lines, with moderate effects on K562 and MV3.

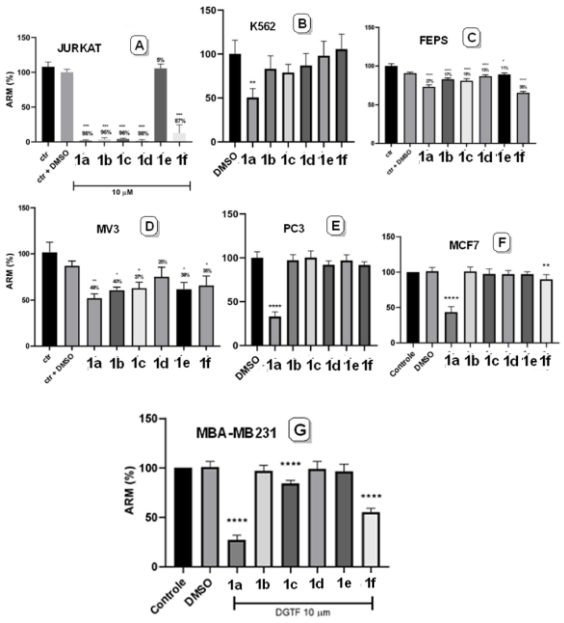


Figure 2: LQBs in cancer cells lines in 72h at 10 μM.

Conclusions

New derivatives of **1a**, as well as the corresponding nitrones, are being evaluated, with determination of IC_{50} value and their toxicity. The mechanism of action is under study using microarray data and cellular cycle inhibition. We also plan to use cyclic voltammetry to better understand the role of the nitrone and nitro groups in the redox process.

Acknowledgements

CNPQ, CAPES and FAPERJ for financial suport

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Discovery of bioactive $P2Y_2$ receptor antagonists from *Joannesia princeps* Vell.

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Keywords: $P2Y_2$, antagonist, calcium, Joannesia princeps, docking.

Introduction

Joannesia princeps Vell., a botanical species native to Brazil and belonging to the Euphorbiaceae family, is popularly known as “cotieira,” “boleira,” and “andá-assu”. It possesses medicinal properties, including laxative, antiparasitic, and antimicrobial activities.[1,2] Recent studies have shown that branch extracts of *J. princeps* inhibit purinergic P2Y receptors, particularly the $P2Y_2$ subtype.[3,4] This receptor is widely expressed in various human cell types, especially immune cells, where it plays roles in calcium mobilization and inflammation.[5,6] Moreover, $P2Y_2$ activation has been implicated in the development of cancer and neuropathic pain.[7-10] However, the scarcity of selective antagonists remains a significant challenge for therapeutic application. In this context, we initiated an ethnobotanical study of *J. princeps*, integrating *in vitro*, *in vivo*, and *in silico* approaches to explore its potential as a source of novel $P2Y_2$ receptor antagonists.

Results and Discussion

In a previous work, we reported that the crude branch extract of *J. princeps* inhibits calcium responses in J774.G8 cells triggered by UTP and MRS 4062, which are known agonists of the $P2Y_2$ receptor.[4] Importantly, this inhibitory effect was not attributed to fluorescence quenching or cytotoxicity, as confirmed by MTT viability assays. In murine models of formalin-induced nociception and paw edema, the extract significantly attenuated pain-related behaviors and paw swelling, indicating both analgesic and anti-inflammatory properties. To further explore its bioactive potential, we performed docking analyses on 23 compounds previously reported from this botanical species. The assays were conducted using the DockThor program with the human $P2Y_2$ receptor structure predicted by AlphaFold2. The docking scores ranged from -8.998 to -5.302 kcal/mol, and the compounds with greater affinity were isolariciresinol-9’-O-β-D-xylopyranoside (-8.998 kcal/mol), 2’’-O-galloylisovitexin (-8.891 kcal/mol), and quercetin 3-O-β-D-galactopyranoside (-8.455 kcal/mol). These compounds exhibit higher binding affinity than UTP (-7.03 kcal/mol), which served as the reference ligand. The next steps include visual inspection of these compounds and *in vitro* experimental validation through calcium assays.

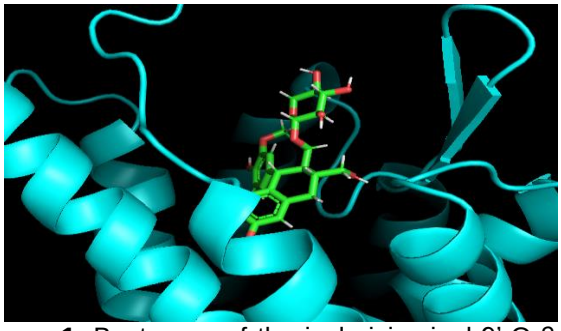


Figure 1. Best pose of the isolariciresinol-9’-O-β-D-xylopyranoside compound interacting with the orthosteric site of the $P2Y_2$ receptor.

Conclusions

These findings highlight the potential of *J. princeps* as a valuable source for the discovery of novel compounds with antagonistic activity toward the $P2Y_2$ receptor. These compounds may offer promising therapeutic applications for the treatment of inflammation and pain.

Acknowledgements

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In silico analysis of an aminopyrimidine-β-lapachone via RNA-seq, membrane diffusion by MD simulation, and docking into human EXOSC4

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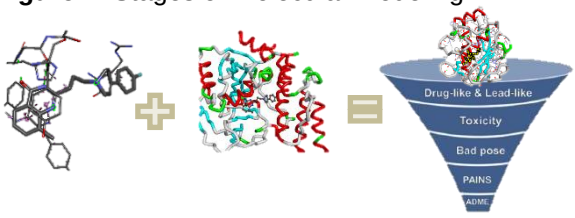
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Key words: Bioinformatics, Molecular modeling, Molecular dynamics, Exosome complex component RRP41, Cancer

Introduction

The use of transcriptomic data from RNA-Seq enables the identification of changes in gene expression profiles induced by compound exposure, allowing the creation of a database of differentially expressed genes and the inference of potentially modulated metabolic and signal transduction. Specific simulations of permeation through lipid bilayers or membrane channels allow the evaluation of predictive pharmacokinetic parameters[1].

Figure 1: Stages of molecular modeling.



This study aimed to analyze the mechanism of action and pharmacokinetic profile of a β-lapachone derivative through RNA-Seq and molecular dynamics focusing on membrane permeability.

Results and Discussion

As a result, higher binding affinity and lower energy levels were observed for the RNA-Seq-identified target (*EXOSC4*, PDB code 6D6R) [2].

Figure 2: RNA-seq analysis of Skmel-23 compared to MRC5.

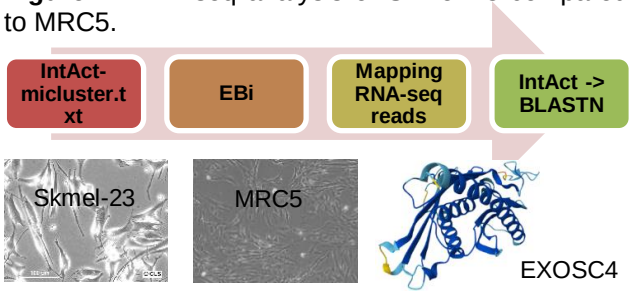
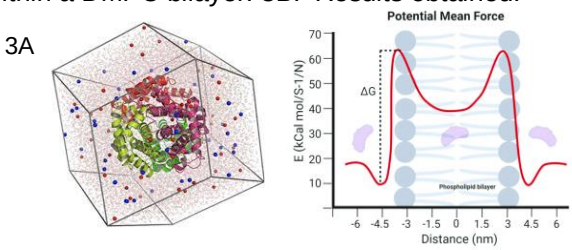
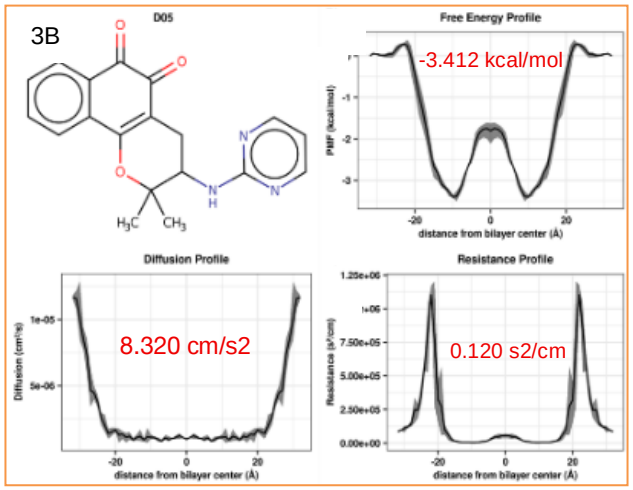


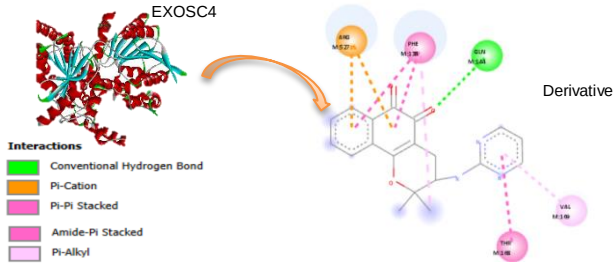
Figure 3: 3A: Membrane diffusion of the derivative within a DMPC bilayer. 3B: Results obtained.





The affinity of the derivative for the selected target was also demonstrated by molecular docking analysis, as shown in Figure 4.

Figure 4: Molecular docking of the derivative.



Conclusions

These findings highlight the therapeutic potential of β-lapachone derivatives in melanoma treatment, with specific molecular targets and a favorable permeability profile.

Acknowledgements

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Green Synthesis and Rational Design of Peptidic Triazoles Targeting the Orthosteric Site of PAR-2.

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Key words: PAR-2 antagonists, green chemistry, peptidic triazoles.

Introduction

Protease-activated receptors (PARs) are a subfamily of G protein-coupled receptors (GPCRs) activated by circulating proteases, inflammatory cells, and epithelial tissues. Among them, PAR-2 plays a crucial role in inflammation, pain, wound healing, angiogenesis, and the maintenance of epithelial barrier function. Its activation by pancreatic and extra-pancreatic trypsins, mast cell tryptase, and coagulation factors (VIIa, Xa)[1] links it to various chronic inflammatory diseases, such asthma, inflammatory bowel disease, and arthritis, as well as cancer progression by promoting tumor proliferation, invasion, and metastasis[2]. Despite its pathophysiological relevance, no approved drugs currently act directly on PAR-2, highlighting a significant unmet therapeutic need.

Results and Discussion

This study reports the design, synthesis, and characterization of a novel series of 32 peptidic triazole derivatives with potential activity as PAR-2 antagonists. The compounds were rationally designed through molecular docking studies targeting the orthosteric site of the receptor (Figure 1), prioritizing key interactions with conserved residues involved in ligand-induced activation[3,4]. Docking results guided the selection of candidates showing strong binding affinities and favorable conformational orientation in the active site.

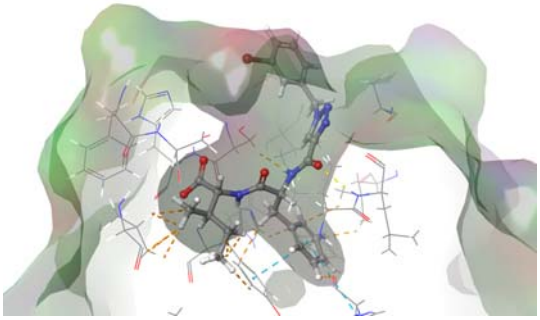


Figure 1: Principal interaction protein-ligand between orthosteric site of PAR-2 and the compound 2BrC3-Ph-L.

The synthesis followed green chemistry principles, utilizing a copper nanoparticle-catalyzed azide-alkyne cycloaddition under mild conditions and reduced energy consumption, achieved via microwave-assisted heating[5] (Figure 2). This approach minimized hazardous reagent use and reaction time, aligning with sustainable synthesis goals.

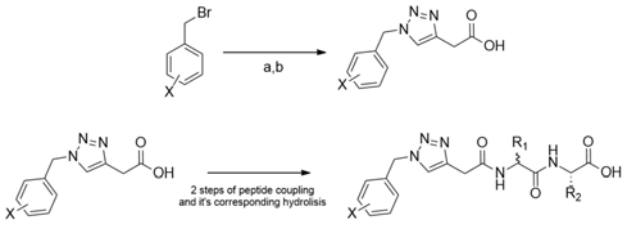


Figure 1: Scheme of synthesis of new peptidic triazole derivates. a) NaN_3, H_2O-THF, reflux, 3h. b) CuNPs, H_2O-MeOH,120°C MW, 30 min. Peptide coupling: a) HATU, EDC, amino acid, py, DCM,70°C MW, 30 min. b) LiOH 2M, THF, MeOH, 30 min.

Conclusions

These novel triazole derivatives represent a promising structural platform for the development of PAR-2-targeted therapeutic candidates. Their rational design and environmentally conscious synthesis highlight the potential of integrating modern computational tools with green methodologies in early-stage drug discovery.

In future studies, the biological activity of these compounds will be evaluated in vitro through cell viability and calcium mobilization assays in HEK293 cells overexpressing PAR-2 and stimulated with specific agonists. These assays will provide critical insights into their pharmacological profile and antagonist potential, helping to identify lead compounds for further development.

Acknowledgements

This research was funded by Fondecyt grant number 3240227 (MMC) and 1250920 (FZ)

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Synthesis, *in silico* and *in vitro* studies of *N*-benzyl-piperidine derivatives as inhibitors of cholinesterases and BACE1 for Alzheimer's disease

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Key words: dual, ligand, neurodegenerative, cholinesterase, beta-secretase 1.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia globally. The available pharmacological treatments primarily focus on symptomatic cognitive enhancement[1]. Consequently, there is a critical need for more effective therapies to delay the onset of AD or slow its progression[2]. In this context, the objective of this project was to design novel *N*-benzyl-piperidine derivatives, followed by computational analyses and enzymatic inhibition assays, with the aim of identifying multitarget drug candidates for the treatment of AD.

Results and Discussion

For the structural design, a molecular hybridization strategy was employed starting from the structures of the AChE inhibitor donepezil and the BACE1 inhibitor verubecestat. The designed derivatives (3a-d) had their physicochemical and pharmacokinetic parameters and their interaction with the cholinesterase and BACE1 enzymes evaluated by in silico methods. The designed derivatives showed adequate oral bioavailability and blood-brain barrier permeation predictions and formed stable complexes with the target enzymes. Then, these derivatives were evaluated for their inhibitory activity against AChE and BuChE in vitro and brain cell viability. Derivatives **3a** and **3d** were the most potent (3a, AChE IC50 2,08 ± 0,16 µM and BuChE IC50, 7,41 ± 0,44 µM; 3d, AChE IC50 17,68 ± 1,31 µM and 26,28 ± 2,06 µM) had their synthesis stablished together with novel structural analogs (**3e-m**).

The synthesized compounds had their chemical structures and purity confirmed by traditional analytical methods (1D and 2D NMR, HRMS and HPLC). The new analogs were also evaluated for their inhibitory activity against cholinesterases and **3m** were the most potent (AChE IC502,26 ± 0,04 µM and BuChE IC50, 7,31 ± 0,11 µM). The results demonstrated that, for this class of derivatives, substitutions on position 2 of the heterocycle are favorable for an inhibitory potency lower than 10 µM. Moreover, bulky substituents at position 4 also favor the inhibition and a nitrile substituent increases selectivity for AChE inhibition. The selected cholinesterase inhibitors are currently under evaluation as BACE1 inhibitors, aiming at the identification of multitarget bioactive agents for Central Nervous System action in AD management.

Among the designed and synthetized *N*-benzyl-piperidine derivatives, two dual cholinesterase inhibitors were initially identified, corroborating the *in silico* studies for their interaction with these enzymes. Additionally, these *N*-benzyl-piperidines have demonstrated drug likeness profiles and a putative interaction with BACE1 enzyme by *in silico* methods.

Acknowledgements

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Analysis of the drug-like properties of compounds with β-(1,3)-D-glucan synthase inhibitory activity

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Keywords: target, antifungal, database, molecular properties

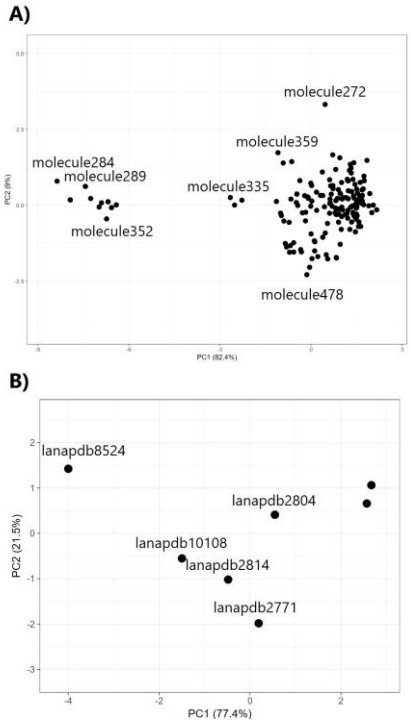
Introduction

The enzyme β-(1,3)-D-glucan synthase is an essential component of the cell wall of many pathogenic fungi, including species from the *Candida* and *Aspergillus*.[1] There are just four classes of systemically acting antifungals.[2] However, these antifungals have various drawbacks in terms of toxicity, spectrum of activity, safety and pharmacokinetic properties.[3] Therefore, this study aims to evaluate the drug-like properties of 180 compounds previously reported as active against the β-(1,3)-D-glucan synthase in the ChEMBL 35 database, using the SwissADME[4] platform. The results will be compared with the top 7 hits obtained through molecular docking simulations using the LANaPDB[5] database and the target.

Results and Discussion

The compounds were evaluated according to the drug-likeness rules proposed by Lipinski, Veber, Egan, and Ghose based on the analysis of number of hydrogen bond acceptors (HBA), molar refractivity (RF), topological polar surface area (TPSA), and lipophilicity (LogP).

Figure 1: **Multivariate statistical analysis (PCA) of compounds that inhibit β-(1,3)-D-glucan synthase.**
A) ChEMBL B) LANaPD



The PCA results indicated that most ChEMBL compounds exhibited a relatively homogeneous profile. However, some compounds, such as 284, 289, and 352, violated all the evaluated rules (Figure 1A). In contrast, the LANaPDB compounds exhibited a more diverse distribution; however, all of them violated at least two criteria (MW ≤ 500 and MR between 40 and 130 according to Lipinski and Ghose, respectively) (Figure 1B). Notably, eight compounds from ChEMBL fully complied with all evaluated parameters: 357, 363, 365, 371-374, and 450. The molecular weight (MW) of these compounds ranged from 418 to 482 g/mol and LogP 1.38 to 3.07. There are 501 compounds in LANaPDB complying with these parameters.
Analyzing the properties of the seven hits from LANaPDB obtained through molecular docking, LANaPDB10108 stood out for having the most favorable overall profile in terms of drug-likeness.

Conclusions

The drug-like properties evaluated within the parameters should provide better pharmacokinetic features. Among them, LANaPDB10108 exhibited the most promising properties and a molecular weight of 610.65 g/mol. It is important to note that the approved oral antifungal agent ibrexafungerp has a MW of 730.0 g/mol, indicating exceptions to the <500 g/mol guideline for favorable pharmacokinetics.

Acknowledgements

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Identification of key residues involving antagonist activity at μ, δ and ƙ opioid receptors targeting the Alcohol Use Disorder treatment

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Key words: Alcohol Use Disorder, opioid receptors, molecular docking, heatmap

Introduction

Ethanol directly influences the release of endogenous opioids, which are positively associated with craving in patients with alcohol use disorders (AUD)[1]. Recent studies have shown that individuals with moderate and severe AUD present dysregulation in the opioid system[2]. In this study, a computer-aid drug discovery approach, based on molecular docking and pharmacophore modeling[3], was performed to identify molecular interactions associated with antagonist activity involving key residues targeting μ, δ and ƙ opioid receptors.

Results and Discussion

Molecular docking was performed to obtain the best conformation for 99 ligands bonded to their opioid receptors, retrieved from ChEMBL database. The resulting structures were used to obtain complexes using pharmacophore models. Figure 1 shows the most active ligand for each receptor subtype.

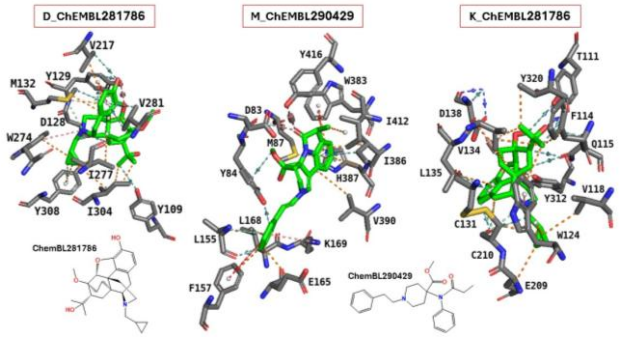


Figure 1: Intermolecular interactions for the most active ligands for delta, mu and kappa receptors. Carbon atoms are in gray (protein) and green (ligands), oxygen in red, nitrogen in blue, and sulfur in yellow. Intermolecular interactions appear as dotted lines in blue (H-bond), light blue (weak H-bond), yellow (chalcogen bond), light green (cation–nucleophile), red (π-stacking), pink (cation–π), and orange (hydrophobic).

The models present all interactions as heatmaps, correlating the amino acids residues with all ligands, sorted by activity. Furthermore, in addition to the key interactions already described for δ receptor, the model reveals two additional interactions among the active and highly active ligands. The first red box in Figure 2 highlights a cation-nucleophile interaction between some of the most active ligands and the residue M132. The same way, the second red box indicates a hydrophobic interaction with residue I304, which is prevalent among active ligands.

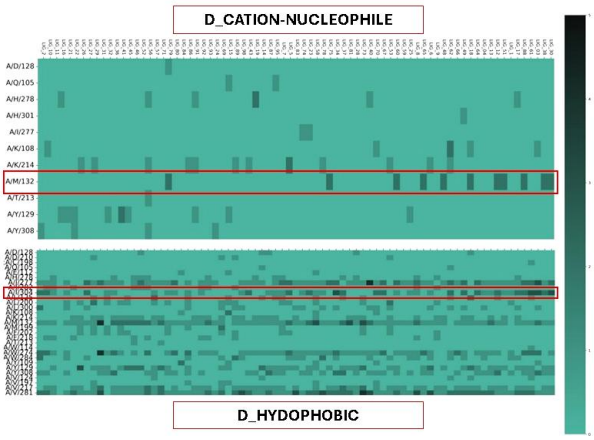


Figure 2: Heatmap of key intermolecular interactions of δ (D) receptor. The color scale is measured from the lightest shade at 1 to the darkest shade at 5.

The same analysis was applied to μ and ƙ receptors. For the ƙ receptors, a cation-nucleophile interaction with C210 and a cation-π interaction with W124 were identified. The μ receptor exhibited a consistent pattern of π-stacking interactions with H387, where face-to-edge interactions were observed among active ligands, while dislocated face-to-face predominated among the inactive or less active ligands.

Conclusions

The prevalence of interactions involving M132 and I304 in the δ, C210 and W124 for ƙ and H387 for μ may be associated with the antagonist activity at these opioid targets.

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Molecular Targeting of SARS-CoV-2 and Oropouche Virus: A Search for Antiviral Inhibitors

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Key words: SARS-Cov-2, Oropouche, RdRp, M^{pro}, PL^{pro}.

Introduction

Viral infections continue to pose a major global public health challenge due to their high incidence, rapid transmission, and the limited availability of effective treatments. The development of new antiviral agents depends on a detailed understanding of viral infection and replication mechanisms, enabling the identification of molecular targets capable of disrupting these processes. Current pharmacological research has focused on discovering compounds that block viral entry or interfere with key stages of the replication cycle, especially in the context of emerging viruses, which represent a growing threat to global health[1].

The COVID-19 pandemic, caused by SARS-CoV-2, has resulted in nearly 700 million cases and 7 million deaths worldwide, highlighting the impact of emerging viruses[2]. This positive-sense, single-stranded RNA virus encodes essential non-structural proteins such as the main protease (M^{pro}/nsp5) and the papain-like protease (PL^{pro}/nsp3), both considered important therapeutic targets[3].

In Brazil, the Oropouche virus (OROV), an arbovirus transmitted by mosquitoes, has also raised concern due to its potential for widespread transmission. OROV has a segmented, negative-sense single-stranded RNA genome comprising three segments: L (encoding the RNA-dependent RNA polymerase, RdRp), M (encoding envelope glycoproteins Gn and Gc, and the non-structural protein NSm), and S (encoding the nucleocapsid protein N and the non-structural protein NSs). The RdRp plays a central role in viral replication, making it a promising target for antiviral drug development[4]. This study focuses on the identification of inhibitory compounds targeting the M^{pro} and PL^{pro} proteases of SARS-CoV-2 and the RdRp of Oropouche virus, aiming to contribute to the discovery of novel antiviral agents.

Results and Discussion

In the search for SARS-CoV-2 protease inhibitors, 30 licochalcones derivatives were tested. At 100 µM, 10 compounds inhibited M^{pro} and 11 inhibited PLpro, though none exceeded 80% inhibition. Notably, 7 compounds (23%) were active against both proteases, suggesting potential for broad-spectrum antiviral activity.

A second set of 75 synthetic compounds based on natural compounds including flavonoids, quinones, alkaloids, lignans, terpenes, and benzothiazines—was screened. For the PL^{pro} protease eight compounds showed >80% inhibition at 100 µM and were selected for IC_{50} assays, with values ranging from 14 ± 1 to 64 ± 3 µM.

Regarding Oropouche virus (OROV), the development of inhibitors targeting the RNA-dependent RNA polymerase (RdRp) is still at an early stage, as the recombinant expression of this large, multi-domain protein (~262 kDa) has not yet been described. To address this, the full-length L segment (6852 bp) was successfully cloned using the Bac-to-Bac expression system, and recombinant baculoviruses were generated for expression in Sf9 cells. Additionally, a second construct encoding only the catalytic domain of RdRp (~91 kDa) was cloned for future fragment screening assays. Both constructs will be expressed and purified for biochemical and inhibitor screening assays.

Conclusions

These results highlight promising chemical scaffolds for SARS-CoV-2 protease inhibition and establish the necessary groundwork for future screening of RdRp inhibitors targeting Oropouche virus.

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Functional characterization of Uracil-DNA glycosylase from Monkeypox Virus as a target for antiviral development

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Key words: DNA polymerase; uracil-DNA glycosilase; kinetic assays

Introduction

In May 2022, the World Health Organization (WHO) was notified of a confirmed case of monkeypox in the United Kingdom, caused by the Monkeypox virus (MPXV) (1). Subsequently, the disease rapidly spread to other countries. A sharp and concerning surge in global cases has been observed over the past year, with reported infections rising from 117,663 in November 2024 to 138,029 by March 2025. Brazil alone accounted for 14,327 of the 142,151 confirmed cases worldwide as of April 2025 (2). MPXV belongs to the genus *Orthopoxvirus* and contains a double-stranded DNA genome, as well as encoding two essential polymerases: a DNA polymerase (DNApol) and a DNA-dependent RNA polymerase (DdRp) (3). Uracil-DNA glycosylase is a non structural protein (nspE4) that is a key enzyme of the DNApol replication complex that plays a key role in maintaining genomic stability by removing uracils incorrectly incorporated into DNA, thereby preventing mutations (4). Moreover, it is essential for viral DNA replication, making it a promising target in the search for antiviral compounds. Therefore, the objective of this study is to functionally characterize nspE4 through kinetic and structural assays.

Results and Discussion

The *nspE4* gene was cloned into the pET28a expression vector and transformed into *Escherichia coli* BL21 (DE3) cells. Protein expression was carried out in Terrific Broth (TB) medium and induced with 0.5 mM IPTG. The recombinant protein was purified by affinity chromatography using a Ni^{2+} column, yielding sufficient quantities for enzymatic assays.

The kinetic activity was assessed using the protein with a fluorescent hairpin-type substrate containing uracil as the target base (4). Optimization of the reaction conditions allowed the identification of ideal enzyme and substrate concentrations, enabling clear detection of the enzyme catalytic activity. Under these conditions, a progressive increase in fluorescence over time was observed, confirming the uracil removal activity of nspE4. These data were fitted (R^2 0.99) to the Michaelis-Menten equation using a single binding site model and the kinetic parameters were approximately K_M = 40 (± 3) nM, V_{max} = 10 µM/min and k_{cat} = 109 (± 3) min^{-1}.

A screening of 326 structurally diverse compounds was conducted to identify potential inhibitors of the nspE4 enzyme. Among those tested, eight compounds exhibited more than 70% inhibition compared to the control containing the same concentration of DMSO. All assays were performed in duplicate. For every 88 compounds tested, four activity controls and four background reactions were included. The quality of each plate was assessed by calculating the Z′-factor, and only plates with a Z′ value greater than 0.7 were considered valid. These eight hit compounds include coumarin-like derivatives originally developed for malaria treatment by the Global Health Compound Collection and hydantoin-like derivatives, known for their pharmacological potential.

Conclusions

These results contribute to the understanding of the MPXV replication mechanism and contribute to the search for new antiviral compounds that target essential viral enzymes.

Acknowledgements

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DockTDesign: Many-objective Optimization with Deep Generative Models for *de novo* Drug Design

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Key words: de novo drug design, GAs, VAE

Introduction

de novo drug design (dnDD) aims to generate novel molecules satisfying multiple conflicting objectives, making it inherently a many-objective optimization problem (MaOP), where more than three objectives must be optimized simultaneously. This study presents a generative chemistry framework combining a pre-trained variational autoencoder (VAE) with many-objective evolutionary algorithms (EAs) to effectively optimize multiple objectives in dnDD. Leveraging the generative potential of the VAE and the optimization strengths of EAs, our method navigates the latent space to concurrently optimize up to five drug design objectives, including docking-based binding affinity prediction. Among the evaluated EAs algorithms, NSGA-III consistently outperformed others in generating novel molecules aligned with the targeted properties. The source code is partially publicly available at https://github.com/gmmsb-lncc/generative-optim.

Results and Discussion

The proposed generative chemistry framework integrates a pre-trained deep generative model (HierVAE), with multi- and many-objective EAs (NSGA-II and NSGA-III) for generating molecules that meet multiple dnDD objectives. Molecules are encoded as real-valued vectors in the VAE's structured latent space, facilitating genetic operations such as crossover and mutation. Decoding these latent vectors into molecular structures (SMILES strings) allows evaluation of drug-design goals. By evolving solutions in latent space, this approach bypasses direct manipulation of discrete molecular representations, effectively generating diverse, non-dominated molecular candidates (Fig. 1). This empowers medicinal chemists with optimized solutions that meet multiple goals, enabling tailored drug design selections based on specific preferences.

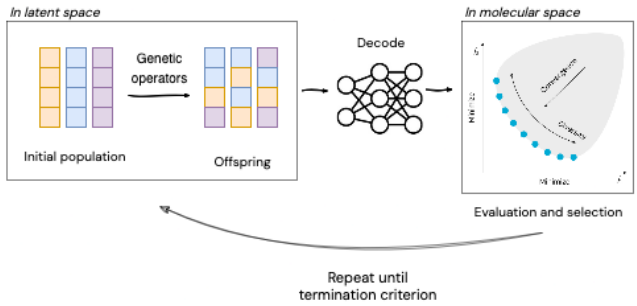


Figure 1. The proposed many-objective generative framework.

As a real-world example, five objectives were simultaneously optimized: maximizing drug-likeness (QED) and synthetic accessibility (SA), controlling molecular complexity, maximizing similarity to a known potent lpxC inhibitor, and minimizing similarity to a known human off-target inhibitor of matrix metalloproteinase-9 (MMP-9). Later, similarity measures were replaced by docking experiments using DockThor for pose prediction and DockTDeep for binding affinity estimation. Among the evaluated EAs, NSGA-III yielded superior results with the highest hypervolume (HV = 0.880) and lowest inverse generational distance (IGD = 0.031), indicating optimal convergence and diversity. Both NSGA-II and NSGA-III produced fully valid molecules. NSGA-II generated more unique and internally diverse structures, while NSGA-III showed higher novelty, with most of the generated compounds not found in the training set. Moreover, docking-guided optimization effectively produced selective molecules with strong predicted affinity for lpxC and weak affinity toward MMP-9. The obtained Pareto front contained 144 non-dominated solutions meeting all criteria simultaneously. From these, 24 promising compounds were selected, each having predicted affinity better than -10 kcal/mol against lpxC and worse than -8 kcal/mol against MMP-9, while maintaining overall adequate values for all the other objectives.

Conclusions

This study introduced a generative chemistry framework combining a pre-trained HierVAE model with many-objective EAs, successfully optimizing up to five simultaneous objectives, a scenario still underexplored in *de novo* drug design literature. These results emphasize the potential of many-objective optimization in accelerating innovative *de novo*-based drug discovery.

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Hit to Lead Optimization of heterocycles based on isoindolones as agents against Trypanosomiasis

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Key words: isoindolones, hit to lead, *Trypanosoma brucei rhodesiense.*

Introduction

The compound QHM845 were considered a hit in our previous studies, due to the potent activity against *Trypanosoma brucei rhodesiense*, very low cytotoxicity and excellent metabolic stability[1]. However, the compound showed very low bioavailability due to limited water solubility. Efforts focusing on expanding the library of new analogues with improved water solubility, by the addition of more polar groups have proven to be challenging. Based on previous knowledge of SAR (structure-activity relationship) and in the attempt to obtain better candidates in a hit to lead campaign, we designed and synthesized new isoindolone and phthalimide derivatives, whose trypanocidal activity is being evaluated. By maintaining potency and improving the desired parameters, it is expected that in the next period, studies of pharmacokinetics may lead to a better profile of the best compound.

Results and Discussion

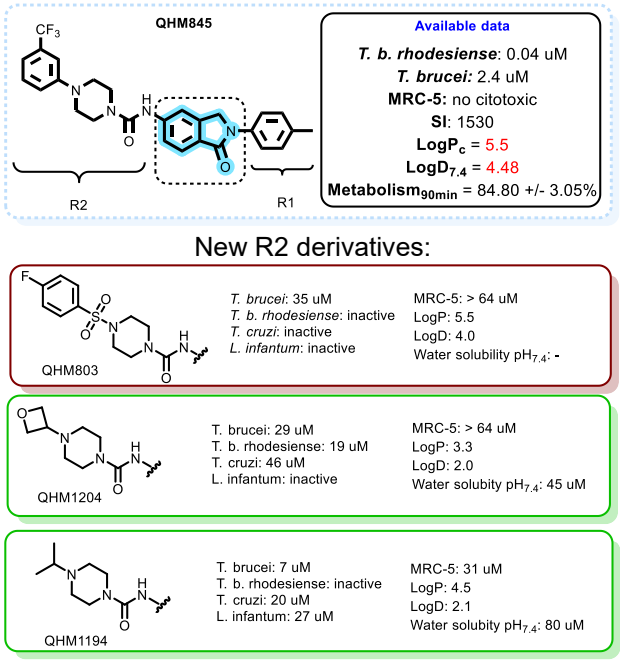


Figure 1: Hit QHM845 identified in previous studies and three new R2 derivatives evaluated

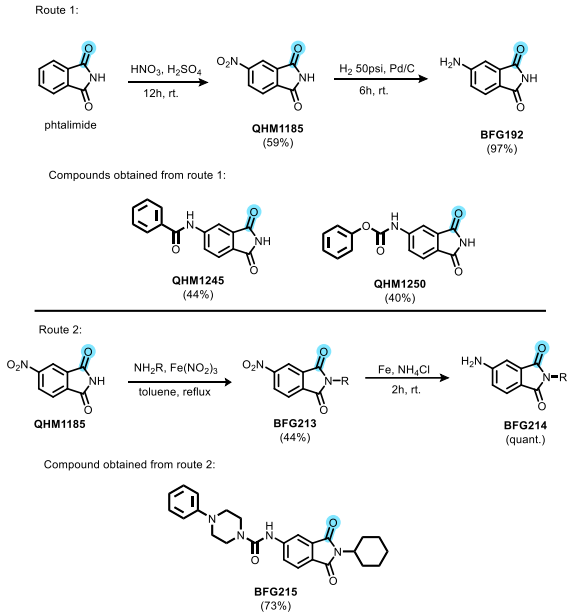


Figure 2. Synthetic route to obtain phtalimides derivatives

The addition of polar groups in the R2 of the isoindolone moiety brought a better profile of physicochemical properties, but the potency and selectivity were lost.
A small series of phtalimides was also synthesized varying R2 between amide, carbamate and urea. The activity against parasites is under evaluation.

Conclusions

We successfully synthesized new isoindolones and phthalimides with more polar groups and higher sp3 fraction, with improved molecular properties compared to QHM845. Ongoing antiparasitic activities will be important for increasing the library to find better antiparasitic activity.

Acknowledgements

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Optimization of diarylalkynes with antiviral potential: Design, synthesis and evaluation of activity of new *Hs*DHODH inhibitors

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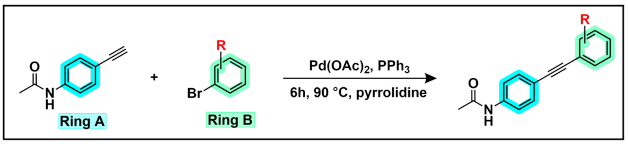
Key words: host-directed antivirals, molecular fragments, HsDHODH.

Introduction

Building upon previous 1,2-diarylethine scaffold, that inhibit human dihydroorotate dehydrogenase (*Hs*DHODH) and demonstrated antiviral activity[1], this work addresses the critical low water solubility of these compounds. To overcome this challenge, we are employing Sonogashira coupling reactions to synthesize a structurally diverse series of novel alkynes with different substituents on the ring B. This approach aims to develop compounds with improved physicochemical properties while maintaining or enhancing biological activity.

Results and Discussion

Using the main methodology[2] exemplified in **Scheme 1**, it was possible to obtain four fragments (**Figure 1**).



Scheme 1: Sonogashira coupling with the starting material N-p-ethynilphenylacetamide and an aryl bromide of interest to obtain diarylalkyne fragments.

Given that low yields have been a persistent limiting factor in these alkynes synthesis, we studied the optimization of the methodology to improve coupling efficiency. Despite multiple attempts, the copper-free methodology still displays the best yield obtained (37%). This result suggests that further exploration of alternative reaction conditions or catalytic systems may be necessary to achieve higher synthetic effectiveness for this particular scaffold **(Figure 1)**.

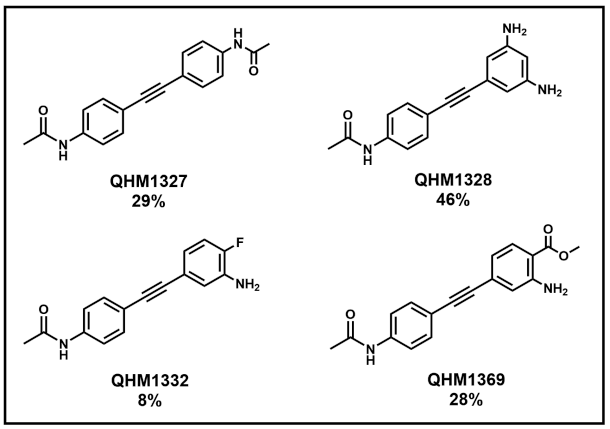


Figure 1: Diarylalkyne fragments successfully obtained with their respective yields.

The enzymatic inhibition of *Hs*DHODH is currently being evaluated to establish structure-activity relationship and pave the way to find optimized antiviral compounds.

Conclusions

Although several conditions of synthesizing 1,2-diarylethines were studied, the copper-free methodology remains the most effective synthetic approach for obtaining a diverse series of compounds. To date, four alkynes derivatives were synthesized and are under evaluation of enzymatic inhibition of *Hs*DHODH.

Acknowledgements

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Biochemical, biophysical and structural characterization of cGMP-dependent protein kinase (PKG) from *Plasmodium falciparum*

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malaria, kinase, PfPKG, crystallography, inhibitors, antiplasmodial

Introduction

Malaria, caused by protozoa of the genus *Plasmodium* spp., remains a major global health challenge, with an estimated 263 million cases and 597 thousand deaths in 2023. The increasing resistance to current antimalarials underscores the urgent need for new therapies with innovative mechanisms of action. Among the most promising molecular targets are parasite protein kinases, such as *Pf*PKG, which is essential for multiple stages of the *P. falciparum* life cycle. This work combines structural biology, medicinal chemistry, and inhibitor screening strategies to structurally and functionally characterize this kinase, including the resolution of its 3D structure and the evaluation of candidate inhibitors.

Results and Discussion

*Pf*PKG was produced in ExpiSf9 cells and purified using a three-step strategy: nickel affinity chromatography, His-tag cleavage with TEV protease, and size-exclusion chromatography(Fig. 1).

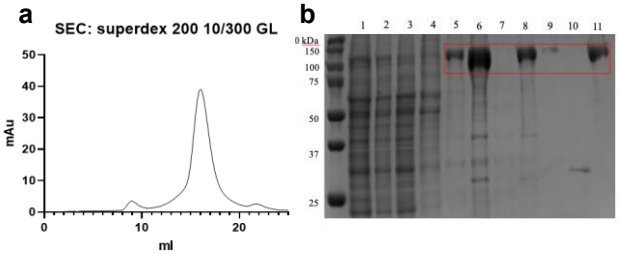


Figure 1. Large-scale expression and purification in insect cells. (a) Size-exclusion chromatography profile and (b) SDS-PAGE gel showing the different steps of the purification process.

The thermal stability of the enzyme was evaluated by Differential Scanning Fluorimetry (DSF), using different buffer conditions including variations in ionic strength, pH, and additives (Hampton Research). Additionally, DFS analyses were used to assess the stabilizing effect of inhibitors, revealing a significant increase in the enzyme stability in the presence of ibrutinib (ΔTm = 6.2 °C) and IBT6A (ΔTm = 7.9 °C).

Kinetic assays indicated K_M values of 130 ± 30 µM for ATP and 110 ± 20 µM for the peptide substrate. These K_M values agree with the reported values.[1] Inhibition screening assays indicated that sapanisertib (IC_{50} = 914 nM), ibrutinib (IC_{50} = 676 ± 30 nM), and IBT6A (IC_{50} = 139 ± 30 nM) were *Pf*PKG inhibitors. Ibrutinib and IBT6A also showed *in vitro* antiplasmodial activity against the chloroquine sensitive 3D7 strain, showing IC_{50}^{3D7} values of 3.1 ± 0.8 µM and 1.9 ± 0.3 µM for IBT6A, respectively.

The 3D structure of apo *Pf*PKG was elucidated by X-ray crystallography at a resolution of 2.31 Å. Comparative structural analysis identified notable differences in the catalytic site relative to human kinases, underscoring regions that may be explored for the discovery of selective inhibitors. These results constitute a significant advancement for structure-based drug discovery (SBDD) initiatives targeting novel *Pf*PKG inhibitors as antimalarial agents.

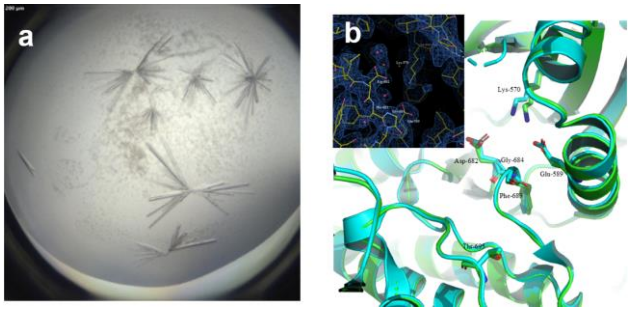


Figure 2. (a) *Pf*PKG crystals obtained in 0.1 M imidazole pH 7, 9% PEG 20K, and 0.2 M KCl (6.5 mg/mL), imaged using the Formulatrix Rock Imager. (b) Alignment of *Pf*PKG-apo (cyan) and 5DYK[1] (green) models with a $2F_o–F_c$ map (σ = 1.0) at the ATP-binding site.

Conclusions

We successfully expressed, purified, and structurally and functionally characterized *Pf*PKG as an attractive drug target. Our findings pave the way for the discovery of new *Pf*PKG inhibitors as lead candidates for antimalarial drug discovery.

Acknowledgements

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Synthesis and Molecular Modeling Study of New Multi-Target Inhibitors of ROCK (1/2) and PDE4

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Key words: RhoA-Kinase, Phosphodiesterase 4, Inhibitors, N-acylhydrazone, Sulfonylhydrazone, Neurodegenerative diseases

Introduction

Like other neurodegenerative diseases, Alzheimer's disease (AD) is a multifactorial condition, and the use of multitarget therapies in its early stages has emerged as a promising strategy for treatment. The modulation of new targets associated with AD pathogenesis has been explored as a potential approach to prevent or slow disease progression [1]. In this context, the present study describes the synthesis of new multitarget compounds designed as phosphodiesterase 4 (PDE4) and RhoA-kinase (ROCK) inhibitors, two relevant therapeutic targets for neuroinflammatory diseases, including AD [2,3].

Results and Discussion

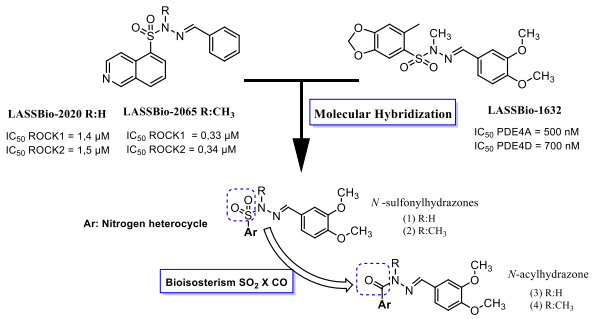


Figure 1: Design concept of target compounds.

The synthetic route for obtaining the target compounds involved three linear steps based on functional group interconversion reactions: (1) Yamada oxidation for the preparation of the desired methyl ester; (2) hydrazinolysis, using either the methyl ester or heteroaromatic chloride derivative as starting material; and (3) condensation of key intermediates (hydrazide or sulfonylhydrazide) with 3,4-dimethoxybenzaldehyde. Subsequently, N-methylated derivatives were obtained via alkylation with methyl iodide.

The compounds exhibited relative purity higher than 95% as determined by HPLC and had their solubility and permeability experimentally assessed. Kinetic solubility studies showed that the non-methylated NSH derivative (compound 1) displayed greater aqueous solubility compared to its analogs. Both NSH derivatives 1 and 2 were able to inhibit the two ROCK isoforms, with N-methylation resulting in a slight increase in biological activity (51% inhibition for ROCK1 and 64% inhibition for ROCK2 at 10 µM). However, only derivative 2 showed activity against PDE4 isoforms (88% inhibition for PDE4A, 93% inhibition for PDE4B, 71% inhibition for PDE4C, and 95% inhibition for PDE4D at 10 µM) indicating that N-methylation is essential for anti-PDE4 activity.

Conclusions

The compounds were synthesized in good yields using classical synthetic methodologies and were properly characterized by ^{1}H and ^{13}C NMR spectroscopy, infrared (IR) spectroscopy, and mass spectrometry (HRMS). Among the compounds evaluated, only the *N*-methyl *N*-sulfonylhydrazone derivative (compound 2) showed inhibitory activity against ROCK1, ROCK2, and PDE4B. As a perspective, further structural modifications of the *N*-methyl *N*-sulfonylhydrazone derivative 2 are planned in order to improve its enzymatic activity, particularly against ROCK.

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Screening of potential multitarget agents: bioisosteric modification on LINS05 compounds identified novel cholinesterase inhibitors

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Key words: acetylcholinesterase, butyrylcholinesterase, cognition, neurodegenerative disorders.

Introduction

Neurodegenerative disorders comprise a group of diseases characterized by functional impairments associated with progressive neuronal degeneration. Among the most prevalent are Alzheimer's disease and Parkinson's disease, highly debilitating and still lacking effective therapeutic options. These disorders are complex and multifactorial, often marked by cognitive decline linked to a significant reduction in acetylcholine (ACh) levels.[1,2] Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are key enzymes responsible for ACh hydrolysis, a key neurotransmitter related cognitive processes. Cholinesterases (ChEs) inhibition is still used as therapeutic strategy to improve cognition in these conditions.[3,4] In previous work, our group identified a set of arylamides (LINS05 series) as multitarget ChEs inhibitors, which motivated the design of a new set of bioisosteres. In this work a new set of 12 arylsulfonamides were synthesized and evaluated for their inhibitory activity against AChE and BChE using a methodology previously developed by our group.[4]

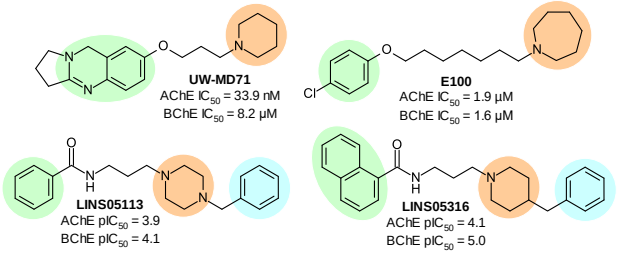
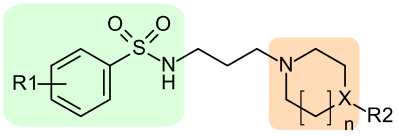


Figure 1. Compounds with AChE/BChE activity.

Results and Discussion



R1 = H, Cl, Me, MeO, 2,3-benzo
n = 1,2
X = CH, N
R2 = H, Bn

Figure 2. General structure of the new designed LINS05 compounds.

Five intermediate compounds (arylsulfonylalkyl halides) were synthesized with good yields, ranging from 78–89%. From these intermediates, 12 final compounds were prepared including unsubstituted (**1a**) and chlorine (**2a-d**), methyl (**3a-c**), naphthyl (**4a**) and methoxy (**5a-c**) substituted derivatives with yields ranging from 85–91%. All compounds were screened for their inhibitory activity against electric eel AChE (*ee*AChE) and equine BChE (*eq*BChE) at an initial concentration of 100 µM.

Table 1. Results for the inhibitory activity of novel LINS05 compounds eeAChE and eqBChE.

Compound	% Inhibition (100 µM) ± SEM	
	***ee*AChE**	***eq*BChE**
1a	47.2 ± 0.1	26.3 ± 0.7
2a	53.2 ± 2.9	86.9 ± 0.5
2b	49.4 ± 1.7	86.6 ± 0.4
2c	37.9 ± 0.6	30.1 ± 1.5
2d	42.2 ± 3.0	60.7 ± 1.0
3a	40.1 ± 1.0	31.8 ± 1.5
3b	50.7 ± 1.3	84.3 ± 0.5
3c	41.2 ± 1.8	87.2 ± 0.4
4a	30.0 ± 0.9	66.5 ± 0.8
5a	30.1 ± 0.6	22.0 ± 0.5
5b	45.5 ± 0.9	85.3 ± 0.1
5c	43.4 ± 0.4	78.2 ± 0.3

The compounds exhibited moderate inhibitory activity on AChE, while some compounds displayed strong inhibition on BChE, particularly those containing a second aromatic moiety (**2a-b**, **3b-c**, **5b-c**). Regarding the substituents on the aryl region, no important differences were noted in the inhibitory activity.

Conclusions

This study identified novel LINS05 derivatives that exhibited activity against BChE, and structural features such as benzyl substitution played the role in the activity. Selected compounds are under exploitation as prototypes for further optimization and screening in other selected targets.

Acknowledgements

The authors are thankful to CNPq (307829/2021-9 and 302348/2025-5) and CAPES (financial code 001).

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Comparative analysis of physicochemical and metabolic profile of LASSBio-294 and its isosteres analogues

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Key words: DMPK, metabolism, pharmacokinetics, physicochemical properties.

Introduction

Physicochemical properties determination and DMPK studies are important steps in the early drug discovery process. Understanding the structure-property relationship can help in hit to lead (H2L) and in the lead optimization stages, once it is possible to understand how structural variations affect solubility, permeability and stability of the molecules, and their impact on absorption, metabolism and clearance, predicting their impact on oral bioavailability[1]. In this context, the present study experimentally evaluated the physicochemical stability (pH-dependent stability, solubility, and permeability) and metabolic stability of the vasodilator prototype LASSBio-294 and its isosteres (LASSBio-897, LASSBio-787, LASSBio-2062, LASSBio-123, and LASSBio-129)[2,3,4].

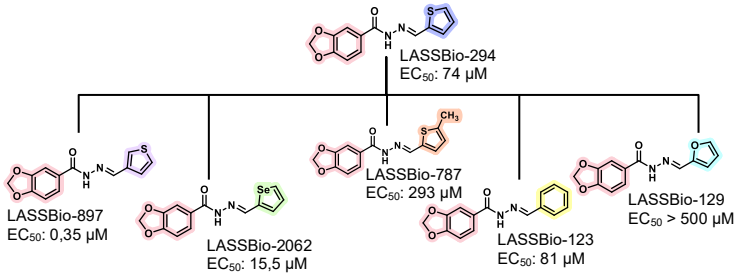


Figure 1: LASSBio-294 and its isosteres and their vasodilator potency

Results and Discussion

Table 1: Physical-chemical and metabolic profile of LASSBio-294 and its isosteres, obtained experimentally.

	LASSBio -294	LASSBio -897	LASSBio -787	LASSBio -2062	LASSBio -123	LASSBio -129
Stability pH 7.4 (%)	87.15	92.26	93.39	90.37	93.54	89.66
Stability pH 6.6 (%)	76.86	81.01	83.70	85.29	90.62	61.59
Solubility 4h (µM)	39.66	21.98	9.12	21.46	28.45	193.70
Solubility 24h (µM)	32.87	16.23	7.10	17.24	27.16	192.43
Pe. GIT	High	High	High	High	High	High
Pe. BBB	CNS+	CNS+	CNS+	CNS+	CNS+	CNS-
$t_{1/2}$ (min)*	10.33	32.38	18.98	20.87	20.56	42.26
Cl_{int} (mL/min/kg)*	2.36	0.85	1.16	1.18	1.45	0.57
$t_{1/2}$ (min)**	12.80	46.58	20.62	26.45	27.17	38.28
Cl_{int} (mL/min/kg)**	1.72	0.58	1.07	0.83	0.81	0.57

* With NADPH generating system; ** Without NADPH generating system.

All compounds were stable at plasma pH (7.4). At pH 6.6 (duodenal), LASSBio-294 and LASSBio-129 showed lower stability. LASSBio-129 had the highest aqueous solubility (>190 µM), while LASSBio-787 was the least soluble (<10 µM). All compounds showed high gastrointestinal permeability (by PAMPA-GIT assay). Only LASSBio-129 was not permeable to the blood-brain barrier (by PAMPA-BBB). Regarding the metabolic stability on rat liver microsomes, LASSBio-294 was the most unstable ($t_{1/2}$ = 10.33 min and 12.80 min, with and without NADPH, respectively). Metabolic analysis indicated *O*-dealkylation at the methylenedioxy group of benzodioxole ring as the major metabolite, with exception for LASSBio-787, which major metabolite was the alcohol formed by hydroxylation of the methyl group of the thiophene ring. The study of metabolic stability under non-oxidative conditions (in the absence of NADPH-generating system) enabled the hydrolysis of acylhydrazones, catalyzed by carboxylesterase, to be identified for all the compounds tested, generating benzo[d][1,3]dioxole-5-carboxylic acid as the main metabolite.

Conclusions

Our data suggest that LASSBio-129 has the best experimental physicochemical and metabolic properties. However, considering that DMPK data should always be aligned with the pharmacodynamic profile to indicate the most promising compound, in this study, LASSBio-897 stood out due to its better in vitro pharmacokinetic and pharmacodynamic profile.

Acknowledgements

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Development of a structure-activity relationship study for the Thiadiazole series

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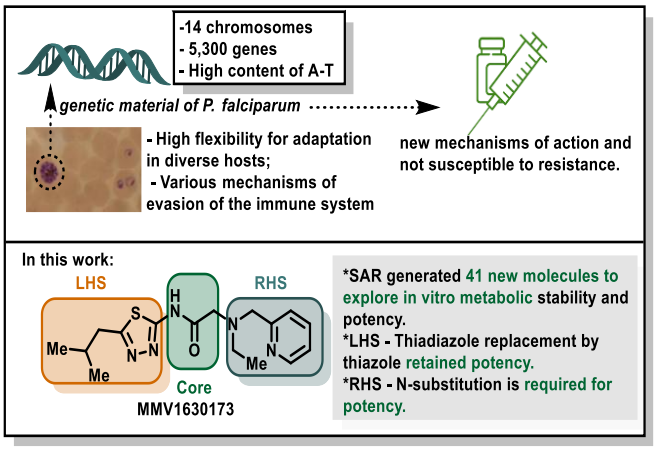
Key words: *Drug Discovery, SAR, Thiadiazole, Cross-Resistance, and Metabolism.*

Introduction

Malaria is an infectious disease caused by the protozoa of the genus *Plasmodium*, which are transferred to vertebrate hosts, including humans, by the bite of infected female mosquitoes of the genus Anopheles. Although the initial symptoms (fever, fatigue, myalgia, and malaise) are nonspecific and common to many infections, malaria can progress to severe and fatal conditions. In 2023, the disease resulted in approximately 263 million new cases and 597,000 deaths, with approximately 95% of cases consolidated in Sub-Saharan Africa.[1] Despite a mortality rate of 0.1-2%,[2] malaria imposes a significant burden on the host and society. Chronic infection and severe forms can lead to permanent neurological sequelae and severe chronic anemia, especially in children and pregnant women. These debilitating difficulties affect individual productivity, perpetuating a cycle of poverty. *Plasmodium falciparum*, the most virulent species responsible for the majority of malaria morbidity and mortality, has a compact genome. It consists of 14 chromosomes, with a limited number of introns and approximately 5,300 genes. A unique feature of its genome is the high content of (A-T), which reaches approximately 80% of its total sequence.[3] The high mutation rate in genes that encode surface proteins is an immune evasion strategy. These mutations enable the parasite to constantly alter its surface antigens, making it challenging for the host's immune system to recognize and eliminate the parasite.[4] Investigating new molecules that selectively target the dormant stages of the parasite within the hepatocyte, which have innovative mechanisms of action on essential biological targets across multiple phases of the parasite's complex life cycle, and demonstrate the absence of cross-resistance, is crucial for identifying a new drug candidate for positively impacting the treatment of malaria.

Results and Discussion

The collaboration between Medicines for Malaria Venture (MMV) and UNICAMP employed a phenotypic screening approach to identify compounds with inhibitory activity against Plasmodium falciparum in vitro. Through high-throughput screening (*HTS*), a promising molecule from the thiadiazole series, **MMV1630173**, was detected.



Scheme 1: *SAR* study of the thiadiazole series.

The *hit* demonstrated micromolar (μM) IC_{50} values of 3.0 μM for *Pf3D7* and 0.7 μM for *PfNF54*. Additionally, it exhibited a favorable toxicity profile, the absence of cross-resistance, and a novel mode of action —highly desirable characteristics for an antimalarial drug candidate. The molecule also exhibited good solubility and a moderate mortality profile, indicating its potential for further investigation. However, significant challenges were identified, including low intrinsic potency, high metabolism in both rat and human models (indicating rapid elimination from the body), and high activity in the hERG channel (potentially increasing the risk of cardiotoxicity). To improve the pharmacological properties of the hit, several synthetic methodologies were developed to explore the structure-activity relationship (*SAR*) around the thiadiazole core.

Conclusions

Due to the high risk associated with future structural alterations on the *SAR* and the deficiency of improvement in activity and metabolism, the thiadiazole series was discontinued, as it lacked the necessary potential to continue in the drug development pipeline.

Acknowledgements

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In silico strategies for identification of aryl hydrocarbon receptor antagonists

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Key words: Cancer, in silico, natural products, aryl hydrocarbon receptor.

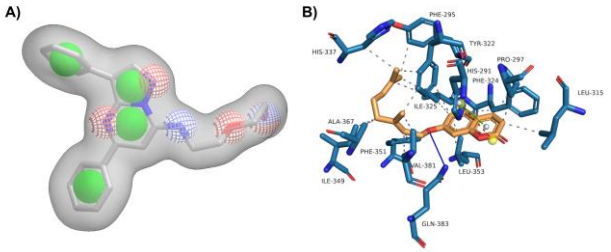
Introduction

The aryl hydrocarbon receptor (AHR), a transcription factor implicated in tumor progression, represents a promising therapeutic strategy to combat the high lethality of cancer[1]. This work aimed to identify novel AHR antagonists from a natural products database, employing a hierarchical virtual screening approach, to discover new scaffolds with antineoplastic potential.

Results and Discussion

A model based on shape and volume was design in the vROCS program *(OpenEye scientific),* using the antagonist MolPort-002-650-228 (IC_{50}: 0.650 μM) as a template. The validation stage using the ROC curve analysis method demonstrated excellent prediction capacity (AUC = 0.90). The model was used to screen the Database of Natural Products of the Semi-Arid Region of Bahia (NatProDB; http://natprodb.uefs.br/, access date: 10/17/2024), considering molecules with an average greater than or equal to TanimotoCombo ≥ 1.66. The molecular docking box was prepared in Make Receptor 4.1.1.0 *(OpenEye Scientific),* based on the spatial positioning of the crystallographic ligand JY6 (PDB ID: 7ZUB). The previously screened molecules were prepared in Omega 4.2.1.2 *(OpenEye Scientific)* and sent to molecular docking in the program FRED 4.1.1.0 *(OpenEye Scientific).* The standard classification function Chemgauss4 score (CG4) was applied to rank these structures, in which the compound auraptene (Nat351) (Figure 1B) from *Polygala paniculata L.* showed the best score (CG: -9.52). The analysis of intermolecular interactions elucidated a hydrogen acceptor bond with residue Gln383, hydrophobic interactions with Phe295, Pro297, Leu315, Tyr322, Phe324, Ile325, His337, Ile349, Phe351, Leu353, Ala367 and Val381; π-stacking interaction (perpendicular) and eletrostatic interactions with residue His291. Molecular recognitionis dictated by the planarity of the molecule, which optimizes π-stacking interactions. Planar moieties act as a determining feature for selectivity at the binding site, while the stabilization of the complex is performed by hydrophobic contacts (Leu349 and Val381)[2]. This pattern of interactions is corroborated by crystallographic data of AHR antagonists[2,3], attesting to the accuracy and predictive capacity of the implemented methodology.

Figure 1: (A) 3D similarity model generated in vROCS, based on the antagonist MolPort-002-650-228. (B) Analysis of the intermolecular interactions of the complex 7ZUB+Nat351. Hydrophobic bonds (gray dashed), acceptor hydrogen bonds (blue solid line), π_stacking interactions (red solid line) and salt bridges (green solid line).



Conclusions

The collected data allowed the identification of the molecule auraptene (Nat351), a promising natural product with important intermolecular interactions and potential antagonist activity against AHR.

Acknowledgements

The authors sincerely thank OpenEye Scientific for providing access to vROCs, vBrood, and FRED software. Thanks to the Graduate Program in Biotechnology. This work was financed by CAPES.

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Design and synthesis of novel triazolopyrimidine derivatives with potential activity against *Plasmodium Falciparum*

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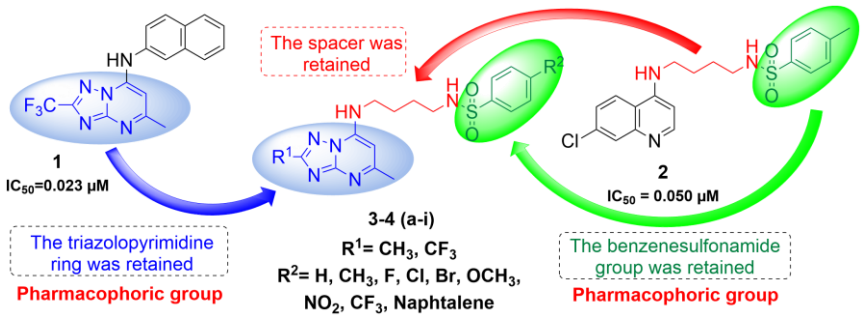
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Key words: malaria, Plasmodium falciparum, PfDHODH, triazolopyrimidine, molecular hybridization, chloroquine.

Introduction

Malaria, caused by *Plasmodium* spp., is one of the most prevalent parasitic diseases worldwide. Due to the disease's global impact, limitations in vector control, and the absence of an effective vaccine, antimalarial drugs remain the primary strategy for malaria control [1,2]. Although drugs such as primaquine, chloroquine, and mefloquine are currently used, their high toxicity and the emergence of resistant variants emphasize the need for new compounds with alternative mechanisms of action [3]. Recent studies involving molecules containing the triazolopyrimidine ring **(1)** and other sulfonamides **(2)** exhibited significant *in vitro* activity against *P. falciparum*, with IC_{50} values of 0.023 µM and 0.050 µM, respectively [4]. Considering this, and based on the molecular hybridization strategy used in medicinal chemistry, as illustrated in Figure 1, the combination of these two scaffolds was proposed **(3-4)** with the aim of enhancing activity against *P. falciparum*.

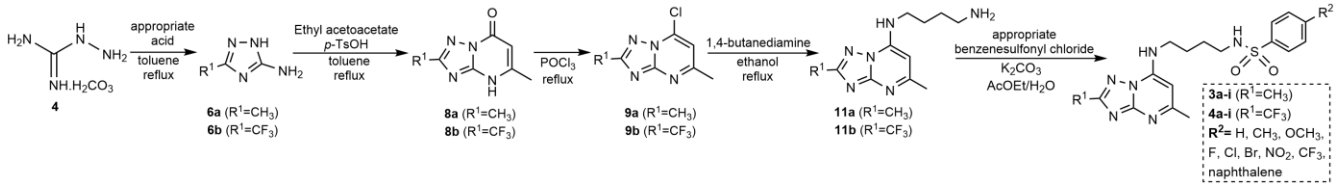
Figure 1. Design of new triazolopyrimidine derivatives **3-4 (a-i)**



Results and Discussion

The synthetic route to triazolopyrimidine derivatives **3-4 (a–i)** is showed in Scheme 1. Aminotriazoles (**6a-b**) were synthesized in 70–97% yields, followed by condensation with ethyl acetoacetate in the presence of catalytic *p*-toluenesulfonic acid, which afforded triazolopyrimidinones (**8a–b**) in 52–89% yields. At this stage, compounds **8a-b** were chlorinated with phosphorus oxychloride to afford chlorotriazolopyrimidines **9a-b**, which reacted with 1,4-butanediamine to give **11a-b** (27–92%). The final products were obtained through a Schotten–Baumann-like reaction with the respective benzenesulfonyl chlorides, in 13–83% yields. All synthesized compounds were characterized by mass spectrometry, and compounds **11a-b**, as well as the final products, were also analyzed by nuclear magnetic resonance (NMR). Purity was determined by high-performance liquid chromatography (HPLC), and, with the exception of two final products, all twelve compounds exhibited purities above 95% without the need for additional purification steps.

Scheme 1. Synthetic route for the production of new triazolopyrimidine derivatives



Conclusions

In this work, 8 intermediates and 18 final products have been obtained, with yields ranging from 12% to 97% and purities above 95%, except for two compounds that are yet to be purified. The next steps involve optimizing the synthetic route and submitting the final compounds for biological evaluation against *P. falciparum*.

Acknowledgements

The authors thank the the National Council of R&D of Brazil (CNPq) for the fellowships granted to the authors.

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A Novel Phthalazinone–Phenylthiazole Hybrid: Synthesis, *In Vitro* Anticancer Activity, and *In Silico* Investigation.

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Key words: Phthalazinone, thiazole, hepatocarcinoma, BAX, docking.

Introduction

Cancer is still the second leading cause of death globally. In 2022, approximately 20 million new cases and 9.7 million deaths were reported[1]. Liver cancer ranks third in cancer mortality, with hepatocarcinoma (HCC) being the most common histological type (75-85% of all diagnoses)[1]. Due to limited treatment options and unfavorable prognosis, new therapies are urgently needed. In this work, we report the synthesis of simple, low-molecular-weight phthalazin-1(*2H*)-one derivatives and evaluate their action against an HCC cell line (Huh-7).

Results and Discussion

All phthalazinones were synthesized from precursor **A1**. Notably, heating **A1** with thiosemicarbazide (**TSB**) yielded **A2**, which lacked the thioamide group, likely lost under the reaction conditions. A one-pot reaction rendered the thiazole derivative **A3**. **A4** and **A5** were easily formed by cyclization with aryl-hydrazines. All products were characterized by FTIR, ^{1}H and ^{13}C NMR, and the structure of **A3** was further confirmed by HRMS and X-ray diffraction.

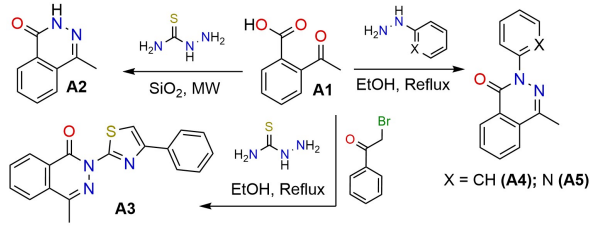


Figure 1: Synthesis of phthalazinones **A2-A5**.

A screening at 50 µM using the MTT assay[2] revealed that only **A3** reduced Huh-7 cell viability by more than 30%, highlighting the phenylthiazole moiety importance. Then, **A3**'s IC_{50} was determined as 14.79 ± 0.65 µM. To verify mitochondrial function, O_2 consumption was measured after 48 h of treatment with **A3** (7.5 µM)[2]. The most drastic effect was a ~99% reduction in ATP-linked respiration (1.698 ± 0.081 vs 0.018 ± 0.248 nmol O_2 min^{-1} 10^6 cells; p<0.001). Basal and maximum FCCP-induced respiration didn't change significantly, suggesting that **A3**, under the above conditions, impairs ATP synthesis without disrupting the electron transport chain – possibly a sign of increased membrane permeability and early apoptosis.

A literature search identified **BAM-7** and compound **16** – two known BAX activators described by Stornaiuolo and collegues[3] – as structurally related to **A3**. These molecules mimic BIM – a pro-apoptotic protein that binds to BAX protein, activating the apoptosis pathway. The calculated atom pair Tanimoto similarity[4] coefficients were 0.68 (**A3** vs **16**) and 0.46 (**A3** vs **BAM-7**), confirming their resemblance. An NMR structure of BAX was retrieved from PDB (2K7W) and the ligand structures (**A3**, **BAM7** and **16**) were built and energy-minimized by DFT (Spartan'24, Wavefunction, Inc.). Docking with the ChemScore function (GOLD 2023.2.0, CCDC), showed **A3** fitting well in the trigger site, making a hydrogen bond and a cation-π interaction with Lys-21, and hydrophobic contacts with Lys-21 and Leu-141. **A3** adopted a pose similar to the known activators **BAM-7** and **16**.

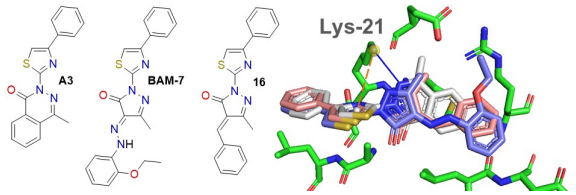


Figure 2: Overlay of **A3** (white), **BAM-7** (purple) and **16** (light pink). Interactions analyzed via PLIP[5]. Images rendered in PyMol (Delano Scientific LLC).

Conclusions

A new phthalazinone-phenylthiazole hybrid exhibited cytotoxicity against an HCC cell line, possibly disrupting ATP synthesis via mitochondrial impairment. Docking results suggest interaction with BAX protein as a possible mechanism of action for the most active compound **A3**. Cytotoxicity of **A3** in normal human cells is currently under investigation.

Acknowledgements

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Heterologous production of HsPD4B and enzymatic inhibition assays of novel *N*-aryl-*N*-acylhydrazones.

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Key words: PDE4B, iPDE4B, N-acylhydrazones, heterologous expression, enzymatic inhibition.

Introduction

Phosphodiesterases (PDEs) are key enzymes that regulate intracellular cyclic nucleotide levels by hydrolyzing cAMP and/or cGMP into their inactive 5'-monophosphate forms (5'AMP and/or 5'GMP). They play a crucial role in various inflammatory and neurodegenerative diseases. Among the 11 identified families, PDE4 selectively hydrolyzes cAMP and requires Zn^{2+} and Mg^{2+} ions for its catalytic activity. The PDE4B isoform is considered an interesting target for neuroinflammatory diseases, such as Alzheimer's. Several PDE4 inhibitors have been investigated; however, the occurrence of side effects, such as nausea and headaches, has prompted the search for more efficient inhibitors with improved efficacy and tolerability[1]. Based on previous results[2], in this study, we designed and synthesized a new series of *N*-arylated *N*-methyl-acylhydrazones (NAHs) as potential PDE4 inhibitors (Figure 1A-C).

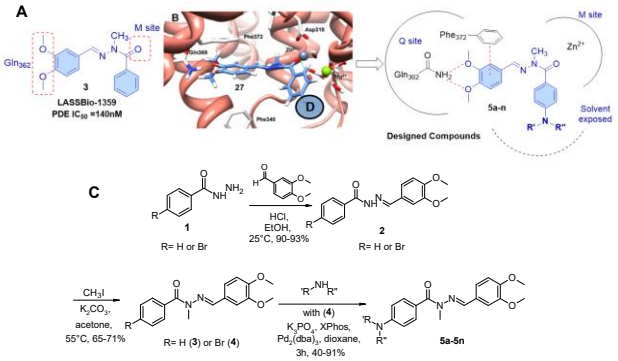


Figure 1: **A-** Lead compound; **B-** Point of *N*-arylation in D, region facing the solvent; **C-** Synthetic route of desired *N*-arylated-*N*-methyl-NAHs.

Results and Discussion

The synthetic route involved three key steps: condensation of the selected aldehyde with benzyl hydrazide to afford the corresponding *N*-acyl hydrazone; methylation of the hydrazone nitrogen[2]; and *N*-arylation via a Buchwald-Hartwig coupling reaction with diverse amines. Following this route, compounds 5a–n were synthesized with yields ranging from 40% to 90%. To evaluate the inhibitory activity of these compounds, the human PDE4B gene was subcloned into the pET28a expression vector, and heterologous expression was performed in *E. coli* BL21(DE3) strain. The resulting inclusion bodies were denatured, purified by affinity chromatography, and subsequently refolded. The recombinant protein was obtained at a concentration of 0.72 mg/mL, with its secondary and tertiary structures characterized and its catalytic activity confirmed. Finally, the inhibitory activity of the synthesized compounds was initially evaluated using a commercially available PDE4B enzyme, serving as a reference for future comparisons with the in-house produced enzyme. At 10 µM, the compounds showed inhibition values ranging from 80-100%.

Conclusions

The results obtained in this study contribute to the optimization of these compounds as PDE4 inhibitors and provide valuable insights for the identification of a lead compound.

Acknowledgements

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6,7-dimethoxy-4-aminoquinazolines designed as FLT3 inhibitors for the treatment of acute myeloid leukemia.

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Key words: fms-like tyrosine kinase 3; kinase inhibitors; quinazolines; acute myeloid leukemia.

Introduction

Protein kinases (PKs) are essential enzymes involved in the regulation of cellular processes through the transfer of phosphate groups from ATP to target proteins, participating in a wide range of signaling pathways. Dysregulation or aberrant expression of these enzymes are directly associated with the onset and progression of various diseases, including Acute Myeloid Leukemia (AML). Among the most clinically relevant targets in this context is FLT3, a receptor tyrosine kinase frequently mutated in AML patients. These mutations are strongly correlated with disease aggressiveness and poor prognosis, making FLT3 a compelling therapeutic target. In this context, the present project aims to perform the rational design of novel FLT3 inhibitors through the molecular hybridization of scaffolds previously reported in the literature, one of which belonging to the LASSBio Chemical Library.

Results and Discussion

The rational design of the new series of 6,7-dimethoxy-4-aminoquinazoline derivatives was based on the optimization of LASSBio-2166, a bioactive compound from the LASSBio Chemical Library that had previously shown activity against the FLT3-ITD mutation — the most prevalent in clinical settings and associated with poor prognosis. The design strategy involved molecular hybridization with a literature-reported quinoline inhibitor that exhibited nanomolar potency against FLT3-ITD and effectively inhibited the growth of cell lines expressing mutant forms of the enzyme, including the gatekeeper mutation (FLT3-F691L).

Six designed hybrids were synthesized through classical chemical reactions, including a final Nucleophilic Aromatic Substitution (SNAr) step with yields ranging from 60-90%, and were fully characterized using spectroscopic and spectrometric techniques, including infrared spectroscopy (IR), mass spectrometry (MS), and ^{1}H and ^{13}C nuclear magnetic resonance (NMR). Purity was confirmed by high-performance liquid chromatography (HPLC), with all compounds showing purity greater than 95%.

Two representatives of the series were already evaluated for inhibitory activity against FLT3 using enzymatic assays with FLT3-ITD and FLT3-F691L variants. In the case of the ITD mutation, one designed derivative exhibited an inhibitory potency (IC_{50} = 16 nM) approximately 30-fold higher than LASSBio-2166, indicating an increase in potency and highlighting the success of the hybridization strategy. On the other hand, no relevant inhibition was observed against FLT3-F691L for the compounds tested so far.

Ongoing docking studies aim to elucidate the molecular interactions between the designed hybrids and FLT3 through comparative analysis with approved inhibitors and the original prototypes. Kinetic solubility has been determined. In parallel, cytotoxicity assays are being initiated to evaluate the effects of the synthesized compounds on the viability of leukemic cell lines and human peripheral blood mononuclear cells.

Conclusions

The new 6,7-dimethoxy-4-aminoquinazoline derivatives demonstrated superior inhibitory potency against the FLT3-ITD mutation compared to LASSBio-2166, validating the rational design strategy based on molecular hybridization with a nanomolar inhibitor. Although no relevant activity was observed against the resistant FLT3-F691L variant, the promising results against FLT3-ITD support the continued optimization of this series. Ongoing studies on molecular interactions, cytotoxicity, and stability will be essential to guide the development of more effective FLT3 inhibitors.

Acknowledgements

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Antifungal Potential of the Imidazolium Salt C16BnImCl

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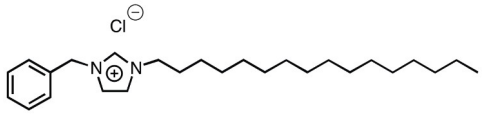
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Key words: Antimicrobial, imidazolium ionic liquid, resistant candidiasis

Introduction

Some imidazolium-based ionic liquids (Ils) are already recognized for their potential antimicrobial effect[1]. The IL 1-benzyl-3-hexadecylimidazolium chloride (C16BnImCl) was previously tested in vitro for its cytotoxicity in glioblastoma cell line[2]. Due to the limited availability of studies in the scientific literature assessing its antimicrobial activity, this study aimed to synthesize, characterize, and evaluate the in vitro efficacy of IL C16BnImCl against *Candida glabrata* M, *C. parapsilosis* LIP45, *C. tropicalis* MG6, *C. krusei* MG26, *C. Auris* 881, and *C. albicans* MG.



C_{16}BnImCl

Results and Discussion

C16BnImCl was synthesized from 1-hexadecylimidazole with benzyl chloride in toluene under reflux at 110 °C for 24 h. After solvent evaporation, the crude product was recrystallized in cold ethyl acetate, affording a white crystalline salt. ^{1}H and ^{13}C NMR spectroscopy confirmed the structural identity of C16BnImCl, with no significant impurities observed. The antifungal activity was evaluated through minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays using the broth microdilution in 96-well microtiter plates, following CLSI guidelines (document M27-A2). The results obtained with C16ImBnCl are presented in **Table 1**. These strains were evaluated in a previous study and showed resistance to fluconazole (MIC > 64 µg/mL), while *Candida auris*, *C. albicans*, and *C. krusei* also exhibited resistance to amphotericin B (MIC = 1–2 µg/mL)[3]. Although ILs like C_{16}BnImCl show potent antifungal activity, their amphiphilic structure can disrupt not only fungal but also mammalian cell membranes. In our previous work, cytotoxicity was noted in Vero cells at 2 µg/mL[2], confirming its slight selectivity between fungal and mammalian cells. Mechanistically, ILs may exhibit selectivity through differences in membrane composition fungal membranes contain ergosterol, while mammalian cells contain cholesterol. Future work will focus on tuning the alkyl chain length, counterion variation, and functional group modifications to enhance selective affinity toward fungal membranes while minimizing mammalian toxicity.

Table 1. Minimum inhibitory concentration (MIC in µg/mL) and minimum fungicidal conDcentration (MFC in µg/mL) in parentheses.

	C_{16}BnImCl	Miconazole	Ketoconazole
***C. glabrata* M**	0.39 (0.78)	1.56 (12.5)	0.09 (0.09)
***C. parapsilosis* LIP45**	0.39 (0.78)	0.09 (0.38)	0.09 (0.09)
***C. tropicalis* MG6**	0.78 (0.78)	1.56 (3.12)	0.09 (0.19)
***C. krusei* MG2**	0.78 (0.78)	1.56 (3.12)	0.09 (0.19)
***C. auris* 881**	1.56 (1.56)	6.25 (12.5)	0.39 (0.78)
***C. albicans* MG**	1.56 (1.56)	12.5 (12.5)	0.39 (3.12)
MIC/MFC	0.8 (0.98)	1.7 (4.4)	0.1 (0.3)

Conclusions

C_{16}BnImCl demonstrated potent antifungal activity against drug-resistant Candida strains and exhibited moderate selectivity for fungal cells over mammalian cells. Ongoing efforts are focused on structural optimization to improve fungal specificity and reduce cytotoxicity, with the aim of developing safer and more selective antifungal agents.

Acknowledgements

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Chalcones as Antibacterial Modulators in β-Lactamase Inhibition

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Keywords: β-Lactam antibiotic resistance, MIC Reduction, Antibacterial, Organic Synthesis, Molecular Docking.

Introduction

The emergence of β-lactamase-mediated resistance compromises the efficacy of β-lactam antibiotics such as ampicillin and cephalosporins. In this context, chalcones have emerged as promising antibacterial adjuvants. Studies show that some chalcone derivatives inhibit β-lactamases, restoring or enhancing antibiotic activity against resistant strains[1,2]. Additionally, certain chalcones exhibit synergistic effects with antibiotics, even without intrinsic antimicrobial activity. While the exact mechanisms remain unclear, available data support the potential of chalcones to combat resistance and enhance antimicrobial therapies[3].

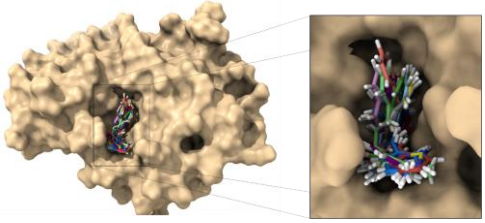
Results and Discussion

A molecular docking study was performed using the AutoDock software, included in the PyRx 0.8 interface[4], 50 chalcone derivatives bearing various substituents (–OH, $–NO_2$, $–CH_3$, $–OCH_3$, $–N(CH_3)_2$, and $–NH_3$) against the β-lactamase enzyme in complex with its endogenous ligand (PDB ID: 1GHM). The docking simulations also included the degraded products of cephaloridine and ampicillin as reference compounds. Based on the binding energy scores, the ten most promising chalcones were selected for synthesis, using the Claisen-Schmidt reaction of the aldolic cross-condensation type, followed by purification and structural characterization via nuclear magnetic resonance (NMR) spectroscopy[5]. Table 1 summarizes the binding energy results obtained from the docking analysis, while Figure 1 illustrates the binding orientation of the chalcone derivatives within the enzyme's active site.

Table 1. Binding scores for chalcones, cephaloridine **(a)** and ampicillin **(b)** obtained from AutoDock software. ***(kcal mol^{-1})***.

Chalcone	*Score*	Chalcone	*Score*	Chalcone	*Score*
02	-6.22	**16**	-6.21	**35**	-7.72
09	-6.32	**24**	-6.54	**36**	-7.66
10	-6.09	**30**	-6.03	**(a)**	-7.87
12	-6.09	**34**	-7.50	**(b)**	-5.73

Figure 1. Snapping of chalcones to the active site of β-Lactamase (PDB: 1GHM - Analysis ChimeraX and Discovery Studio programs).



The 10 synthesized chalcone derivatives were evaluated using an in vitro modulation assay to assess their ability to potentiate the activity of the antibiotic ampicillin against four bacterial strains[6]. A significant reduction in the minimum inhibitory concentration (MIC) of ampicillin was observed in all tested strains, with decreases exceeding 50% and reaching up to 87%. These results support the potential of chalcones as modulators of antibiotic activity. The data are presented in Table 2, which summarizes the MIC reductions. This enhanced activity may be attributed to the inhibition of β-lactamase enzymes, thereby preventing the enzymatic degradation of β-lactam antibiotics.

Table 2: Bacterial strains and MIC reduction.

	Decrease in MIC (%) of Ampicillin									
Chalcones	**02**	**09**	**10**	**12**	**16**	**24**	**30**	**34**	**35**	**36**
S. aureus	50	87	50	50	50	50	75	75	75	75
E. coli	50	50	50	50	50	50	50	50	50	50
B. cereus	87	75	-	50	50	-	50	87	87	75
P. aeruginosa	50	-	-	50	50	-	-	75	-	75

Conclusions

Given the growing threat of multidrug-resistant bacteria and the limitations associated with current antimicrobial therapies, modulation strategies have emerged as a viable approach to restore the efficacy of existing antibiotics, allowing for dose reduction and decreased treatment costs. In this context, chalcones have shown promise as modulators of β-lactam antibiotics, offering a potential means to enhance their antibacterial activity against resistant strains.

Acknowledgements

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Green Light for Medicinal Chemistry: Sustainable Synthesis of Versatile and Potentially Bioactive Pyrazoloquinolinone Derivatives

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Keywords: biological properties, green chemistry, multicomponent reactions, pyrazolo[3,4-b]quinolinone.

Introduction

Pyrazolo[3,4-*b*]quinolinone derivatives have emerged as promising frameworks in medicinal chemistry due to the broad spectrum of pharmacological activities they exhibit, including anticancer, antiviral, antimalarial, antibacterial, and parasiticidal effects[1-2]. A commonly employed strategy for their synthesis is via multicomponent reactions (MCRs). This approach offers several advantages, such as facilitating the assembly of complex molecules from simpler reactants and providing excellent atom-economy[3]. Moreover, MCRs can rapidly generate multiple analogues of a hit molecule, thus offering a cost- and time-effective strategy for drug discovery[3]. This becomes particularly important given that Green Chemistry has recently emerged as a highly relevant paradigm in medicinal chemistry. Reported methodologies of these compounds often present drawbacks, such as prolonged reaction times (2 to 24 hours)[2,4], complex workup, and the use of hazardous reagents like DMF[4]. Therefore, there is a pressing need to explore alternative synthetic routes aligned with the Green Chemistry Principles (GC 1-12) (**Figure 1**).

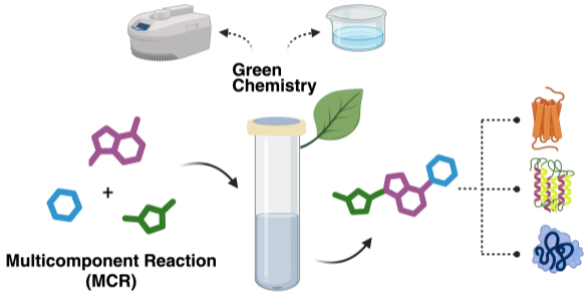
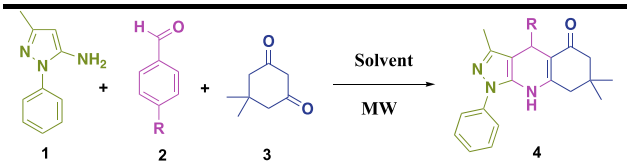


Figure 1: Green multicomponent synthesis of potentially bioactive pyrazoloquinolinone derivatives[5].

Results and Discussion

The developed methodology involves the reaction of various aldehydes, amino pyrazole derivatives, and different dimedones in a green solvent mixture under microwave irradiation at 90-100 °C for 10-15 minutes, followed by crude product filtration and subsequent recrystallization. We obtained 20 novel derivatives with good to excellent yields (>72%) (**Table 1**) which were structurally characterized using spectroscopic nuclear magnetic resonance (NMR) (^{1}H, ^{13}C, ^{19}F), high-resolution mass spectrometry (HRMS), and X-ray diffraction. Utilizing ADMETLab 3.0[6], we were able to determine high drug-likeness based on zero Lipinski Rule violations, as well as assess the probability of HEK293 cytotoxicity for compounds **4a-e** (**Table 1**). Their biological activity will be evaluated through *in vitro* assays to determine their potential against various targets.

Table 1. Isolated yield (%), drug-likeness and probability of HEK293 cytotoxicity of compounds **4a-e**.



Comp. **4**	**R**	Yield (%)	Lipinski Rule Violations	Probability of HEK293 Cytotoxicity
a	H	94	0	0.497
b	CH_3	72	0	0.460
c	4-NO_2	86	0	0.649
d	4-Cl	89	0	0.726
e	4-Br	98	0	0.494

Conclusions

We successfully developed a new methodology to access potentially bioactive pyrazoloquinolinone derivatives, featuring shorter reaction times, microwave-assisted irradiation under milder conditions (GC 6 and GC 2), and simplified purification (GC 1), thus providing a greener alternative to conventional protocols.

Acknowledgements

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Novel Primaquine Derivatives as Potential Candidates Against *Plasmodium vivax*

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Key words: malaria, primaquine derivatives, G6PD enzyme, Plasmodium vivax.

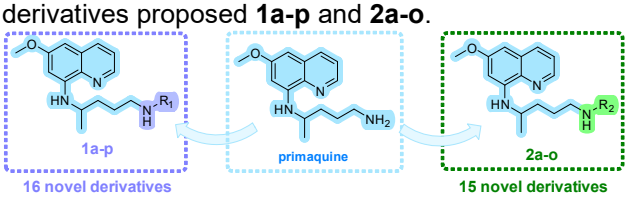
Introduction

Malaria is a parasitic disease with the highest morbidity and mortality in tropical and subtropical regions. Among the main drugs recommended by the WHO for malaria treatment are primaquine (PQ) and tafenoquine (TQ), along with chloroquine (CQ), that are the only drugs used in Brazil for *P. vivax* infection by inhibiting hypnozoites. However, PQ and TQ can induce severe hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. This adverse effect restricts the use of these drugs and can be fatal [2,3]. Therefore, to have a more effective and safer therapies this study explores novel primaquine derivatives based on molecular hybridization with some prototypes previously obtained in our research group.

Results and Discussion

The novel derivatives **1a-p** and **2a-o** (Figure 1) were designed exploring the molecular hybridization concept. The compounds were obtained from a two-step *one-pot* synthesis, with satisfactory yields.

Figure 1: Molecular design of novel 8-aminoquinoline derivatives proposed **1a-p** and **2a-o**.



After the structural characterization of the novel primaquine derivatives, purity was determined by HPLC-UV, reaching purity ranging from 95% to 99%. All 31 compounds were submitted to biological assays in collaboration with IHMT-NOVA-UNL (PT). The compounds were submitted to a primary *in vitro* screening assay (Table 1), in which three had satisfactory %inhibitory against *P. falciparum* 3D7-GFP. Antiplasmodium activity was evaluated using a dose-response assay to estimate IC_{50} using two strains: 3D7-GFP (CQ-susceptible strain), W2 (CQ resistant strain), allowing calculation of the resistance index (RI) based on IC_{50}s for both strains. A cytotoxicity assay in HepG2 cells using the MTT colorimetric method was executed too [4,5,6,7]. Data are presented in Table 2.

Table 1. Data from primary *in vitro* screening assay of derivatives **1a-p** and **2a-o**.

%inhibitory against *P. falciparum* 3D7-GFP					
Sub.	**10 µM**	**1 µM**	**Sub.**	**10 µM**	**1 µM**
1a	8,3±3,4	17,4±10,4	2a	20,8 ± 13,8	0,7 ± 6,6
1b	14,6±2,3	11,1±7,6	2b	6,2 ± 7,2	8,3 ± 9,4
1c	7,6±6,0	16,7±6,8	2c	-0,7 ± 16,1	-25,0 ± 7,6
1d	79,6±4,2	11,8±9,7	2d	89,0 ± 2,9	-34,0 ± 12,5
1e	2,1±10,3	20,8±6,1	2e	8,3 ± 6,2	2,1 ± 10,3
1f	11,1±8,3	19,4±7,1	2f	31,9 ± 3,1	6,2 ± 4,1
1g	37,5±10,1	13,9±2,8	2g	38,9 ± 3,4	9,7 ± 5,7
1h	22,2±5,2	18,1±5,0	2h	35,4 ± 6,6	15,3 ± 5,0
1i	31,9±3,1	18,1±9,3	2i	7,6 ± 7,2	9,7 ± 13,7
1j	26,4±5,0	14,6±3,6	2j	7,6 ± 7,4	3,5 ± 12,3
1k	11,1±4,4	17,4±9,3	2k	18,1 ± 4,2	18,1 ± 7,5
1l	23,6±5,7	10,4±6,3	2l	24,3 ± 5,3	10,4 ± 5,7
1m	34,7±2,4	4,9±7,2	2m	17,4 ± 10,5	25,0 ± 2,0
1n	10,4±8,6	17,4±7,4	2n	52,8 ± 2,8	17,4 ± 5,0
1o	11,1±7,1	11,8±6,9	2o	23,6 ± 9,3	0,0 ± 2,8
1p	15,3±2,4	19,4±6,2	-	-	-
CQ	86,3±3,6	87,7±1,8	CQ	86,3±3,6	87,7±1,8
PQ	63,9±5,2	21,5±3,6	PQ	63,9±5,2	21,5±3,6

Table 2. Purity and biological activities of derivatives **1d**, **2d** and **2n**.

Sub.	HPLC UV (%)	IC_{50} (µM) 3D7-GFP	IC_{50} (µM) W2	$(RI)_{CQ}$	CC_{50} (µM) HepG2	SI
1d	99	2.6 ± 0.5	4.3 ± 0.3	1.65	5.4 ± 0.9	2.08
2d	95	2.6± 0.5	4.1 ± 0.2	1.57	4.6 ± 3.7	1.76
2n	96	5.5± 0.6	6.3 ± 1.3	1.14	2.0 ± 1.0	0.37
CQ	-	0.007 ± 0.003	0.3 ± 0.0003	43.68	-	-
PQ	-	1.6 ± 0.7	0.5 ± 0.015	0.33	3.5 ± 1.1	2.18

Conclusions

These results indicate that others structural optimizations are required to improve potency and cytotoxicity. Also, PQ and **1d** have equivalent SI, thus, this compound can be a prototype for new modifications. However, the RI of compounds is around 1, so the RI does not indicate cross-resistance of substances with other current antimalarials.

Acknowledgements

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Molecular Docking and Quantum Mechanics simulations for understanding the interaction between Pt(II)-NHC complexes and DNA

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Keywords: Pt(II)-NHC complexes, molecular docking, DFT, QSAR, cancer.

Introduction

The search for new cancer drugs is driven by severe side effects and resistance to existing platinum drugs, such as cisplatin. In this context, a new class of compounds, platinum(II) complexes with N-heterocyclic carbene ligands (Pt(II)-NHC), emerges as a promising alternative due to their high efficacy. The study in question uses computational tools (molecular docking and quantum mechanics) to investigate how these new complexes interact with DNA, which is considered one of the main targets for their antitumor action.

Results and Discussion

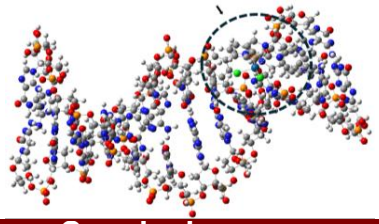
A set of 10 Pt(II)-NHC complexes were selected from the literature, based on a recent study that reported the synthesis, characterization, and biological activity of these complexes against cancer cell lines[2]. Molecular docking simulations, using a blind docking approach, where the search space was intentionally defined to encompass the entire DNA structure, were performed with the Autodock Vina 1.1.2 program against a ds-DNA (PDB ID: 1BNA). This strategy allows an unbiased exploration of all potential binding sites. The results generated a theoretical affinity ranking, with calculated binding energies between -6.1 and -6.9 kcal/mol, and identified the DNA minor groove as the preferred binding site. This binding mode has already been documented for other DNA-binding agents, including some Pt complexes, and the result found reinforces the biological relevance of the poses.

Table 1. Affinity energies (AE, kcal/mol) and IC_{50} (μM/L) of Pt(II)-NHC compounds evaluated by molecular docking

No	Group	IC_{50}	AE	No	Group	IC_{50}	AE
1	H	8.6	- 6.1	**6**	OMe	13.7	- 6.7
2	OMe	4	- 6.4	**7**	H	42.1	- 6.3
3	OMe	11.1	- 6.7	**8**	OMe	37.1	- 6.7
4	H, F	7.6	- 6.6	**9**	PPh3	0.43	- 6.1
5	OMe, F	3.2	- 6.9	**10**	PPh3, OMe	0.52	- 6.4

The Pt(II)-NHC compounds that exhibited the best AE were those substituted with methoxy groups (-OMe), suggesting an important role for this ligand in establishing the interaction with DNA. The standard molecular docking protocol used models the initial step of molecular recognition and non-covalent binding, a crucial prerequisite that guides the complex to its target and determines its orientation before any covalent reaction can occur. The interactions demonstrated patterns such as the predominance of van der Waals and pi interactions, signaling the types of interactions that may stabilize the complexes in the target. Furthermore, the covalent interaction of the compounds with DNA was studied using quantum mechanics methodologies, Figure 1 shows the optimized structure for Complex 1 using a DFT/semiempirical approach at the BLYP-D4/def2-SVP//GFN1-xTB protocol. The influence of replacing the chlorine ligands with water, and the adduct formation with nitrogen bases guanine and adenine were also considered and a comparison between the calculated thermodynamics parameters for cisplatin and oxaliplatin will be carried out. Finally, an analysis of the frontier molecular orbitals, HOMO and LUMO, was also performed.

Figure 1. Optimized structure of Pt(II)-NHC Complex 1 inserted into DNA



Conclusions

Molecular docking studies positioned the Pt-NHC complexes in the minor groove of DNA. Affinity energy analysis suggests a theoretical structure-activity relationship in which the substituents modulate the stability of the noncovalent bond responsible for the initial molecular recognition. These findings provide a rational starting point for understanding the initial recognition event between this class of compounds and their target. Finally, the covalent mechanism of interaction with guanine and adenine will also be presented.

Acknowledgements

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In silico studies of galactoporphyrins useful for photodynamic therapy

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Key words: porphyrins, galactose, in silico studies

Introduction

Porphyrins are important molecules for living organisms, and their basic structures consist of an aromatic macrocycle containing four pyrrole rings. Due to their ability to absorb radiant energy in the ultraviolet and visible regions, porphyrins have been used as photosensitizers in photodynamic therapy (PDT), including in cancer treatment[1]. In this context, C-glycoporphyrins derivatives, containing sugars bound at *meso* positions of the porphyrin system, have achieved prominence among third-generation porphyrins, and may have affinity for proteins overexpressed by tumor strains, such as galectins[1]. In this study we proposed to use *silico* approach to evaluate the electronic, conformational and *drug-like* properties of glycoporphyrin derivatives, such as digalacto-diphenylporphyrin, its O-substituted derivatives with methyl, benzyl, acetyl and benzoyl groups, and the olefin derivative of galactoporphyrin (Figure 1)[2]. Additionally, docking studies with Galectin-3 protein and digalactodiphenylporphyrin were performed to evaluate the affinity to this protein, which is present in bladder cancer cells.

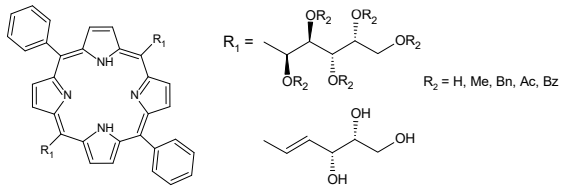


Figure 1: C-Galacoporphyrins

Results and Discussion

Using the BIOVIA Discovery Studio (2023) and Spartan24 software, the target compounds were designed and optimized, and the energies, including HOMO and LUMO, were calculated using the semi-empirical method PM6 and the quantum mechanical method DFT B3LYP[3], with base function 6-31G*. In addition, some calculations of physicochemical properties, known as *drug-like* properties, such as clogP and polar surface area (PSA), were performed. The results showed that the steric effects, especially for the derivatives O-benzoyl (and O-benzyl), can promote greater distortions in the aromatic system in the porphyrin nucleus, observed by the variations of the torsion angles of the aromatic system bonds and by the calculations of the energy variation between HOMO and LUMO orbital energies (slightly higher ΔE than the less hindered porphyrins). In turn, the olefin derivative of digalactodiphenilporphyrin showed lower variations in the torsion angles of the aromatic system and lower variations between HOMO and LUMO (2.54 eV), which may represent better photosensitizing properties. The PSA and clogP predicted showed good values, being 129.5 $Å^2$ and 3.17, respectively. Molecular docking studies of the digalacto-diphenilporphyrin on the Galectin-3 (PDB: 3zsj; Ligand: lactose; Res: 0.86 Å), using the Gold software (2024.3.0, CCDC) with ChemScore scoring function, were performed. The results revealed few similar interactions with the amino acid residues in comparison with the co-crystallized ligand. We also observed that water molecules contributed decisively to the possible interactions with the lactose binding site. Additional studies are being carried out to optimize these experiments and determine the possible interactions between galactoporphyrin derivatives and others galectin proteins.

Conclusions

Steric effects can affect negatively the photosensitizing potential of porphyrins, while conjugation effects can positively affect it. According to these results, the most promising derivatives were those containing double conjugated bond with the porphyrin nucleus. It was observed that sugar-derived groups may contribute to modulate the *drug-like* properties of porphyrins. Molecular docking with the galactoporphyrins of this work showed that interactions with galectin 3 are weak and water contribution is important. The size of these molecules may have influenced these results.

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4*H*-Chromene and Benzo[*f*]chromene Hybrids with 1,2,5-oxadiazol-2-oxide as Potencial Antitumor Nitric Oxide Donors.

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Key words: furoxan, nitric oxide donors, multicomponent reactions, cancer.

Introduction

High levels of nitric oxide (NO) promote cytotoxic effects in tumor cells, mainly by activating apoptosis mechanisms.[1] NO releasing compounds, such as those containing 1,2,5,oxadiazole-2-oxide (furoxan) moiety, are capable of generate NO under physiological conditions with potential antitumor activity.[2] 4*H*-Chromenes and benzo[*f*]chromenes are privileged structural units present in compounds with potential antitumor activity.[3] Multicomponent reactions are an efficient strategy for the synthesis of complex structures. Herein, we report the synthesis of new furoxan-based chromenes and benzochromenes and the measurement of their nitric oxide release ability.

Results and Discussion

We synthesized the furoxan-based compounds **2a-c** using dimedone, ethyl acetoacetate or 2-naphthol, malononitrile as component CH_2-acid, and 3-methyl-4-furoxancarbaldehyde (**1**). Compounds **2a-c** were obtained in good to excellent yields (85-95%) when the reaction was carried out in ethanol, using 10 mol% DABCO as a catalyst at room temperature (Figure 1).

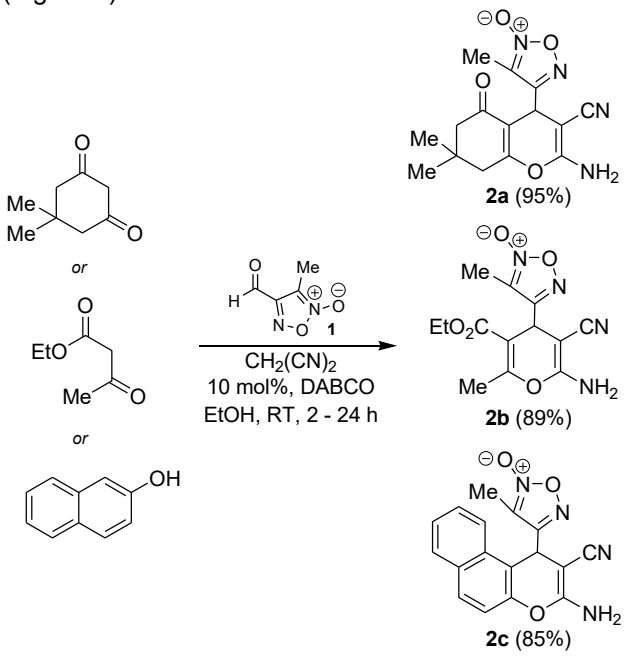


Figure 1: Synthesis method by multicomponent reaction to obtain compounds **2a-c**.

The NO-releasing ability of compounds **2a-c** was quantified by a chemiluminescent method. Firstly, a model study for NO release was carried out in order to understand the influence of the variables such as: type of thiol (*i.e.* cysteine, *N*-acetylcysteine and reduced glutathione), thiol and furoxan concentration, and incubation time. Therefore, the compounds **2a-c** (initial concentration 100 μM in PBS buffer) were incubated for 24 hours at 37ºC in the presence of cysteine (5 mM) as thiol-activating compound, being able to release NO in the range of 0.1–1.1 μM (Figure 2). As a positive control, for comparison purposes: the NO donor deta NONOate, at the same initial concentration, was able to release 18.4 μM of NO.

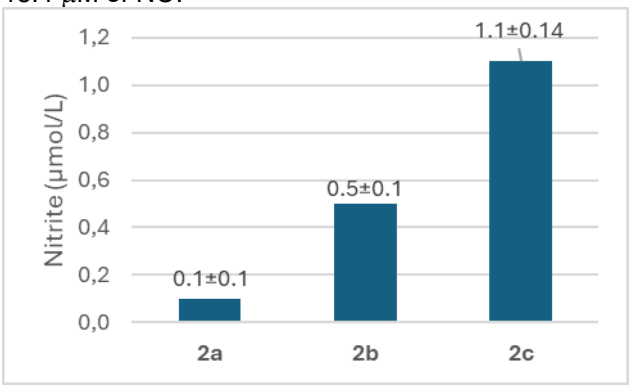


Figure 2: NO releasing profile of compounds **2a-c**.

Conclusions

In conclusion, we have described an efficient method for the preparation of 4*H*-chromenes and benzo[*f*]chromenes hybridized with the furoxan scaffold. The compounds obtained were able to release NO in the range of 0.1 – 1.1 μM. Antiproliferative activity studies are underway in our research group at this moment, so that we can evaluate the possible antitumor activity of these compounds against several cancer cell lines.

Acknowledgements

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In silico evaluation of the potential of spilanthol and derivatives against molecular targets of Leishmania spp.

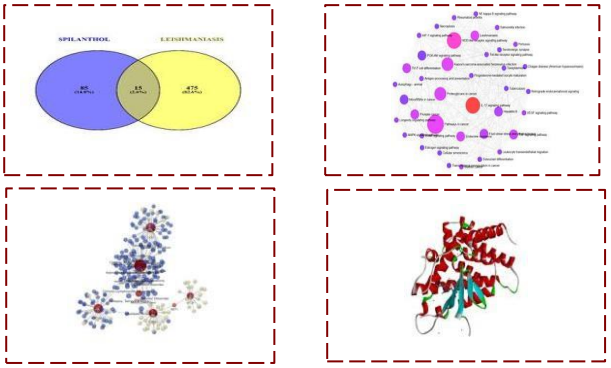
Yasmim D.N. Evangelista[1](IC), Douglas E.M. de Souza[1] (PG), Odoíza N.M.S. Lins[1] (IC), Gabrielly M. Ruiz[1] (IC), Iasmin A. Alves[1] (IC), Fernanda Guilhon-Simplicio[1,2*](PQ). [1]Universidade Federal do Amazonas. [2]Center for Innovation in Artificial Intelligence for Health (CIIA – Saúde/UFMG)

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Key words: Leishmaniasis, target, neglected tropical disease, target fishing, molecular docking, molecular dynamics simulations.

Introduction

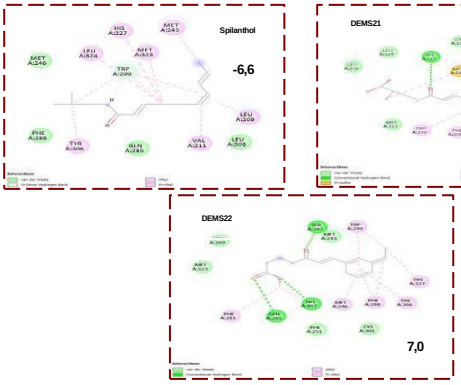
Leishmaniasis, an endemic disease of the Amazon, lacks effective treatments due to the toxicity and low selectivity of available therapies. Spilanthol, a bioactive substance from Acmella oleracea, has antiparasitic properties, but its physicochemical properties limit its clinical efficacy. Therefore, the development of new derivatives is necessary to optimize their pharmacological potential. In this context, computational simulations were used to evaluate the interaction of spilanthol and two derivatives with Leishmania spp. targets to improve binding affinity, active site stability, and physicochemical properties.

Results and Discussion



The platforms used included GeneCards©, SwissTargetPrediction©, Venny 2.1.0©, NetworkAnalyst©,DiscoveryStudio©.

Figure 1: Selection and analysis of molecular targets related to Spilanthol activity in Leshmaniasis.



The platforms used included AutodockVina© and DiscoveryStudio©. The binding energy is in kcal/mol.

Figure 2: Molecular docking poses and interactions of Spilanthol and its derivatives with the CCR1 protein (PDB ID: 4NY9). The platform used included Maestro©.

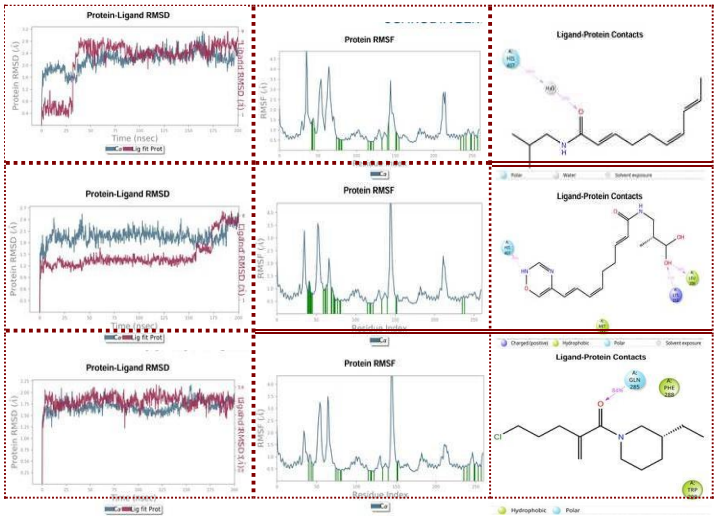


Figure 3: Molecular dynamics analysis and interactions of Spilanthol and its derivatives.

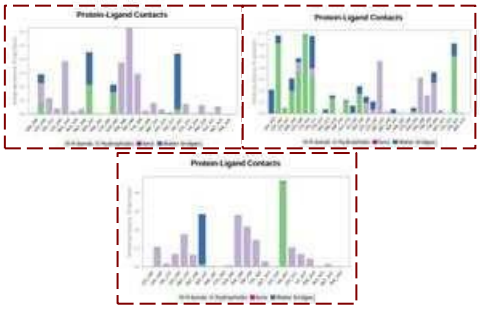


Figure 4: Analysis of hydrogen interactions of Spilanthol and its derivatives with the 4NY9 protein.

Conclusions

The results indicate that derivatives DEMS21 and DEMS22 presented the best results, demonstrating potential against leishmaniasis, but further experimental studies are needed to confirm their efficacy and safety before therapeutic applications.

Acknowledgements

This study was funded by resources from the Center for Innovation and Artificial Intelligence for Health (CI-IA Saúde), in part by resources from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Process No. 2020/09866-4, from the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) Process No. PPE-00030-21 and from UNIMED Belo Horizonte.. Amazonas Research Support Foundation; Multiuser Center for Analysis of Biomedical Phenomena of the Amazonas State University. Tutorial Education Program

VARADHARAJAN, V. et al. Binding interaction of spilanthol and UDA with malaria targets: Network pharmacology, ADME, molecular docking, and molecular dynamics simulation studies. Indian Journal of Biochemistry and Biophysics (IJBB), v. 61, n. 10, p. 604-619, 2024.

AI-Driven in silico screening of Lupeol derivatives as potential therapeutics against *Leishmania* spp.

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Key words: neglected diseases, molecular docking, virtual screening

Introduction

Leishmaniasis is a group of neglected tropical diseases caused by different trypanosomatid parasites of the genus Leishmania, being endemic in several countries and affecting 12 to 15 million people worldwide[1].

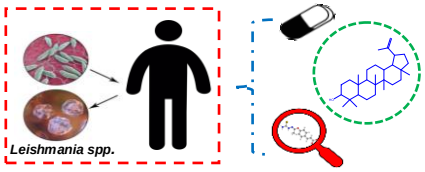


Figure 1: Research of new molecules with antiparasitic action.

Results and Discussion

45 lupeol derivatives were designed from modifications of the hydroxyl group at C-3.

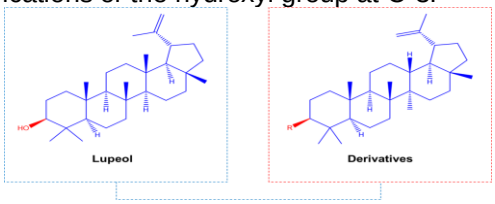


Figure 2: Structure of lupeol and derivatives.

Lupeol and derivatives were analyzed in silico according to the protocol and softwares outlined in Figure 3.

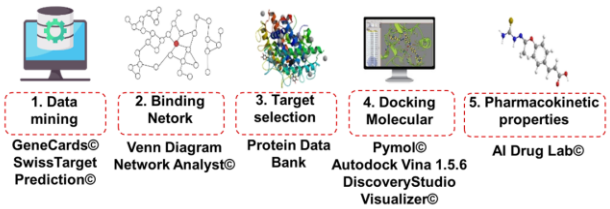


Figure 3: Structure of lupeol and derivatives.

From the protein-protein interaction analysis, 17 promising targets for lupeol were identified, pteridine reductase 1 and dihydrorate dehydrogenase were selected.

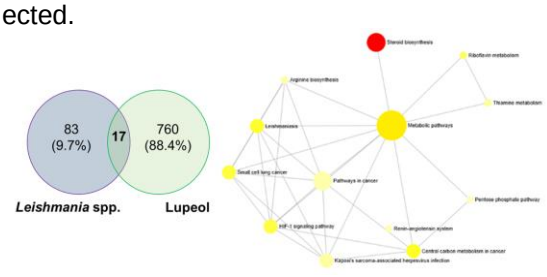
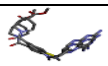
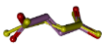


Figure 4: Biological target selection.

Table 1. Validation of the molecular docking protocol by redocking.

Target	PDB Code	Grid box dimension	RMSD (Å)	Redocking
Pteridine reductase 1	2QHX	12×12×18	1.2	
Dihydrorate dehydrogenase	3TQ0	12×34×26	0.3	

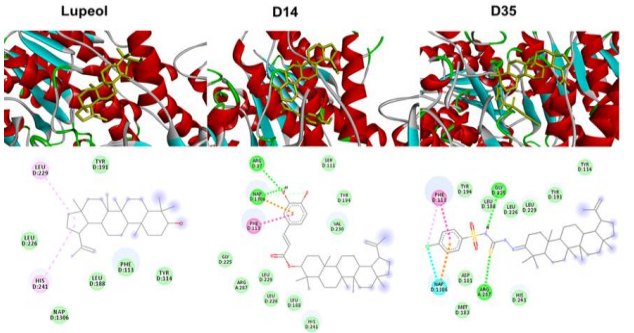


Figure 5: Analysis of the interactions of lupeol and derivatives D14 and D35 with the enzyme 2QHX.

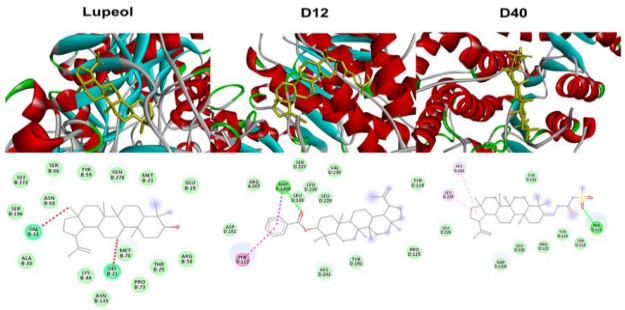


Figure 6: Analysis of the interactions lupeol and derivatives D12 and D40 with the enzyme 3TQ0.

All molecules showed moderate binding to plasma proteins, high skin permeability, and did not inhibit the Pgp protein.

Conclusions

Derivatives D12, D14, D35 and D40 have potential antileshmanial activity. However, in vitro and in vivo evaluations are required to confirm their biological activity.

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This study was funded by resources from the Center for Innovation and Artificial Intelligence for Health (CI-IA Saúde), in part by resources from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Process No. 2020/09866-4, from the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) Process No. PPE-00030-21 and from UNIMED Belo Horizonte.

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Cytotoxic and antiproliferative profile, DMPK study and PB-PK modeling of EGFR inhibitors

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Key words: EGFR inhibitors, DMPK, PB-PK modeling, cancer

Introduction

Lung cancer is a serious health problem, 85% of them are represented by the non-small cell lung cancer (NSCLC)[1]. Cancer therapies targeting epidermal growth factor receptor (EGFR), such as small-molecule kinase inhibitors and monoclonal antibodies, have been developed as standard therapies for NSCLC, since EGFR overexpression is observed in 40–80% of patients with NSCLC.[2] However, the emergence of resistance to EGFR inhibitors compromises their antitumoral efficiency[3]. To overcome this limitation covalent inhibitors of the doubly mutated EGFR enzyme have been developed. In this work we describe the study of the cytotoxic and antiproliferative activity of EGFR inhibitors on different human tumor cell lines and the in vitro study of metabolism and pharmacokinetics (DMPK) of the most potent compounds, in addition to PB-PK modeling to simulate the absorption, distribution, metabolism and excretion of compounds in the human body, considering anatomical and physiological characteristics.

Results and Discussion

The cytotoxic and antiproliferative of four covalent EGFR inhibitors and fine non-covalent EGFR inhibitors are investigated by MTT and SRB methodologies, using erlotinib, osimertinib and WZ4002, as standards control. The experiments were performed against three human NSCLC cell lines with different EGFR mutations (H292 *wt*, PC9-L858R and H1975-L858RT790M). First, all compounds and the standards were evaluated at a screening concentration of 10μM. Compounds that showed inhibition percentages ≥ 50% were selected to determine the concentration-response curve (CC_{50}). For the wild-type strain (H292 *wt*), none of the compounds reduced cell viability by more than 30%. Among the non-covalent EGFR inhibitors LN2725 emerged as the most potent one (CC_{50} = 1.28μM), being 10 times more potent than erlotinib, although less potent than osimertinib and WZ4002.

Regarding the covalent EGFR inhibitors, LN2600 and LN2601 were equipotent to osimertinib and WZ4002 against the PC9 strain. Remarkably, LN2601 (CC_{50} = 0.23μM) stood out in the H1975 strain, outperforming erlotinib by 53 times and achieving similar potency to osimertinib and WZ4002. Our results also showed a great cytotoxic selectivity index for the target compounds, since none of them affected cell viability of human PBMCs. Then, LN2725 and LN2601 had their DMPK profile studied. Both compounds were able to permeate the BBB in PAMPA assay. LN2725 proved to be more vulnerable to oxidative metabolism, while LN2601 was more susceptible to hydrolysis. This inhibitor also exhibited excellent stability over a range of pHs (2.0, 6.6 and 7.4), in contrast to LN2601 that was unstable at pH 2.0.

Conclusions

We were able to determine the comparative potency of new EGFR inhibitors with erlotinib, osimertinib and WZ4002 drugs against EGFR-mutated tumors cell lines. LN2725 exhibited the best DMPK profile, and its ability to permeate BBB indicates the possibility of repositioning it on glioblastomas. Now, PB-PK modeling studies are under investigation to simulate LN2725 and LN2601 absorption, distribution, metabolism and excretion in the human body.

Acknowledgements

LASSBio ®, INCT-INOFAR, CNPq, CAPES.

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Docking Benchmark Automation and 3D-QSAR Integration for Rational Designing of HDAC6-Selective Inhibitors.

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Key-words: Histone Deacetylase 6 Inhibitors, Molecular Docking, 3D-QSAR

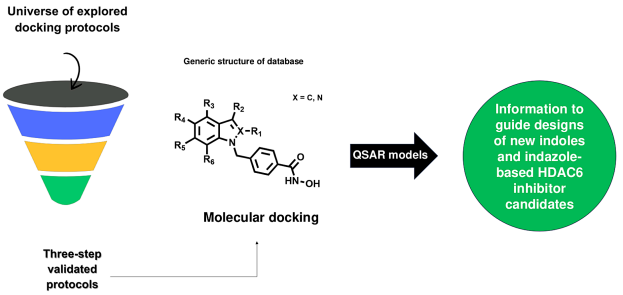
Introduction

Histone Deacetylase 6 (HDAC6) is an isoform within a family of enzymes that plays a crucial role in gene transcription and epigenetic regulation through chromatin remodeling and histone protein modifications. This isoform has emerged as a promising therapeutic target due to its ability to deacetylate non-histone substrates, including α-tubulin, HSP90, and cortactin, which are key cytoskeletal proteins involved in cellular structure and function. Although pan-HDAC inhibitors (HDACi) have gained approval for the treatment of various hematologic cancers, their lack of isoform selectivity leads to significant side and adverse effects, highlighting the need for designing selective inhibitors targeting specific isoforms such as HDAC6. To find selective HDAC6i, we developed a computational pipeline that integrates automatated molecular docking benchmark with predictive 3D-QSAR modeling to guide the rational design of compounds. To avoid limitations related to docking accuracy and parameter selection, we implemented Dockalibris, a Python-based platform that systematically automates the search for optimal docking parameters via AutoDock Vina.

Results and Discussion

Additional validation experiments are currently underway to further assess the Dockalibris platform's performance and to confirm the practical application of these computational results in developing selective HDAC6 inhibitors. A database of HDAC6 structures and inhibitors was built, and docking protocol validation was performed using the Dockalibris platform. This approach enabled systematic exploration of different docking configurations through redocking and cross-docking experiments to identify the most robust parameter sets. The validated protocols were subsequently applied to generate high-confidence docking poses for a series of HDAC6 inhibitors derived from indazole and indole scaffolds. These docking poses served as input for developing 3D-QSAR models (CoMFA and CoMSIA), which demonstrated robust predictive performance, with statistically significant internal and external validation metrics: $q^2 = 0.731$ and $r^2 = 0.900$ for the CoMFA model, and $q^2 = 0.540$ and $r^2 = 0.869$ for the CoMSIA model. The steric, electrostatic, and hydrophobic contour maps generated by these models provided valuable structural insights to guide scaffold functionalization. Based on these findings, new indole- and indazole-based candidates with promising predicted pIC_{50} values were proposed. Additional validation experiments are being conducted to further assess the Dockalibris platform's performance and to confirm the application of these computational results in developing of selective HDAC6 inhibitors.

Figure 1: Workflow of Docking Protocols and QSAR Modeling to Guide HDAC6 Inhibitor Designing.



Conclusions

In summary, this integrated workflow illustrates how automated docking benchmarking, combined with advanced 3D-QSAR modeling, can support the rational design of selective HDAC6i. Although still under development, the Dockalibris platform and the QSAR models have yielded promising preliminary results and valuable insights that will guide subsequent steps in the research program, including the synthesis of new candidates and experimental validation studies. Future work will focus on refining this computational approach and evaluating its broader applicability in structure-based drug design campaigns.

Acknowledgements

CNPq, CAPES, FAPESP and PUB-USP.

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Molecular Dynamics Studies on *Trypanosoma cruzi* Dihydroorotate Dehydrogenase Complexes: An Analysis of the Inhibitor Influence.

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Key words: TcDHODH, molecular dynamics, enzyme inhibition, Chagas disease.

Introduction

Chagas disease is a neglected tropical disease caused by *Trypanosoma cruzi*, still lacking effective treatments in the chronic stages. *T. cruzi* dihydroorotate dehydrogenase (*Tc*DHODH) is a promising drug target due to structural differences from the human homolog and its essential role in pyrimidine biosynthesis[1]. This study employed molecular dynamics simulations (MDS) to investigate the impact of ligands (**Fig. 1**) on the structure and dynamics of *Tc*DHODH[1].

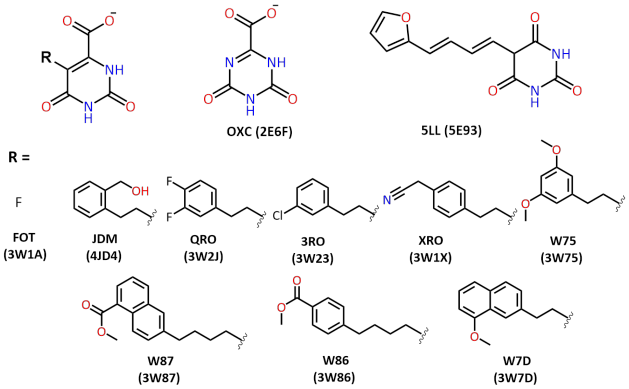


Fig.1: TcDHODH inhibitors' structures and PDB ID.

Results and Discussion

MDS (100 ns) of 11 TcDHODH-ligand complexes revealed that ligand volume and conformation influence active site flexibility and enzyme inhibition. RMSD-ligand analysis showed inhibitors < 200 Å^3 had a mean of 0.5 to 2 Å of SD. Ligands >200 Å^3 had higher mean RMSD (2.96 - 3.81 Å), except W7D (1.57 Å). Orotate-derived ligands with an ethylene spacer (JDM, QRO, 3RO, XRO, W75, and W7D) had SD < 0.5 Å, indicating stability. W86 and W87, with a butylene spacer, showed greater mobility (SD of 0.75 and 0.51 Å, respectively). Despite this mobility, none of the ligands showed a tendency to exit the active site during the simulation time. After evaluating the global movement of the holoenzyme and ligands, we conducted RMSF analysis to assess local fluctuations, as this allows for the observation of residue flexibility throughout the simulation. Two regions showed ΔRMSF values >1 Å: His87−Pro93 (green) and Leu128−Gln138 (red) (Fig.2A). The His87−Pro93 loop, distant from the active site and dimer interface (>7 Å), had decreased mobility upon ligand binding and appears unrelated to enzymatic action. In contrast, the Leu128−Gln138 loop exhibited variations exceeding 1 Å in W86 and 3RO, indicating high and medium residue mobility. This increased activity likely facilitates substrate/product entry or exit, potentially leading to a loss of enzymatic function[2].

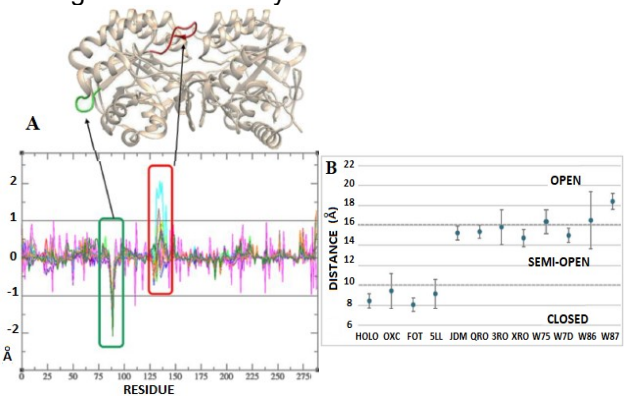


Fig.2: Structural mobility analysis. (A) ΔRMSF relative to MDS for all ligands. (B) Distance for the active loop (average and standard deviation) and the corresponding regions of the active site (closed, semi-open, and open).

In summary, smaller ligands (e.g., OXC, FOT) maintained the active site in a closed conformation, while larger ligands induced semi-open or open conformations, increasing the Pro134−Lys214 distance and active site volume. MM-PBSA calculations showed that ligands promoting open conformations (e.g., W86, W87) exhibited more negative ΔG_{bind} values (−59.98 to −52.29 kcal/mol), which correlated with higher inhibitory potency.

Conclusions

Ligand size and conformation modulate the active site flexibility of TcDHODH, influencing inhibitory potency. These insights support the rational design of new inhibitors targeting TcDHODH for the treatment of Chagas disease.

Acknowledgements

CAPES and FAPERJ for funding and CENAPAD-SP for computational resources.

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Computational Approach to Identify Modulators of Human Mu-Opioid Receptors in Pain Control

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Keywords: GPCR; Machine Learning-QSAR; Molecular simulation; Pharmacodynamics; Molecular docking

Introduction

Although distinct, acute and chronic pain are interconnected and represent a challenge in clinical and biomedical research. While acute pain is a transient and protective response, chronic pain persists beyond the healing time and may become an independent pathological condition. The transition between these phases involves complex pathophysiological changes, such as neuronal sensitization and dysfunctions in central inhibitory circuits, resulting in allodynia and hyperalgesia.[1]

This research aims to identify FDA-approved drugs (https://www.accessdata.fda.gov/) available in DrugBank (https://go.drugbank.com/) as potential modulators of the human mu-opioid receptor (hMOR), a G-protein-coupled receptor (GPCR), to propose therapeutic combinations that carry a lower risk of dependence.

To achieve this goal, we intend to build classification models using a machine learning-based quantitative structure-activity correlation (ML-QSAR)[2] technique, utilizing "active"/"inactive" compounds against hMOR (UniProt ID: P35372) available in ChEMBL (https://www.ebi.ac.uk/chembl/)[3], aiming at the virtual screening of FDA-approved drugs.

The 3D structure of hMOR by cryogenic electron microscopy, available in the Protein Data Bank (PDB) (8EFQ), in complex with the peptide agonist DAMGO (PubChem ID: 5462471), was analyzed in the Cavity module of the CavityPlus server (v.2022) (http://pkumdl.cn:8000/cavityplus/) to detect potential binding pockets in this receptor structure.

Results & Discussion

Chemical structure and bioactivity data from ChEMBL (chembl-webresource-client 0.10.9) were preprocessed using Google Colab and the Python programming language (v.3.10), with the RDKit, Pandas, NumPy, and Matplotlib libraries utilized. Only compounds showing defined EC_{50} values in functional (F) assays were selected to reflect the biological effect of a compound against the receptor. After data curation, including removal of duplicates and conversion of EC_{50} values to pEC_{50}, a total of 1.183 compounds were associated with the F assay against hMOR. The distribution of pEC_{50} exhibited a mean value of 7.215 M; compounds with a $pEC_{50} \geq 7.0$ M were classified as active (n = 648), while those below this value were classified as inactive (n = 535). The docking protocol was validated by redocking DAMGO (**Fig.1**), considering the protonated amine group, using the AutoDockTools program (v.1.5.6). All residues within the 50x50x50 Å grid box of cavity 1, as detected by CavityPlus, were considered as the binding site. This process yielded in a pose with an RMSD of 1.96 Å (**Fig.2**), achieved through 50 runs of the Lamarckian Genetic Algorithm (LGA).

Fig.1. DAMGO's 2D structure.

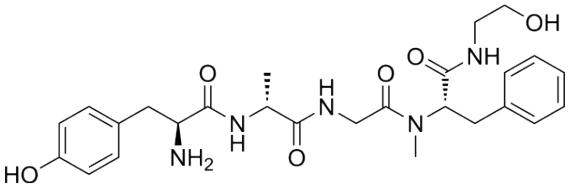
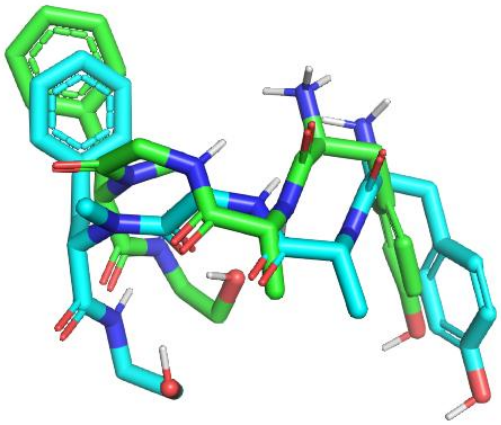


Fig. 2. Image of DAMGO overlay docking pose and crystal pose.



Conclusions

As a perspective, an ML-QSAR model will be developed for the virtual screening of FDA-approved drugs, followed by docking of the drugs best classified by the ML-QSAR model as active against hMOR, and finally, molecular dynamics simulations of the best-scoring poses (protein-ligand complexes).

Acknowledgements

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A new copper-hydrazide based complex: a potential metallodrug?

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Key words: copper complex, cytotoxicity, metallodrug

Introduction

The urgency in developing new anticancer drugs is highly recognized, as a matter of public health. In this context, new metallodrugs have been synthesized as promising tools to overcome some of the limitations associated with conventional chemotherapy.[1]

In special, copper complexes have exhibited significant biological effects, including antibacterial, antiviral and antitumor activities. A notable example is the Casiopeínas® family of compounds[2], which have demonstrated considerable biological activities, with at least one representative compound entering in clinical trials.[2] Inspired by this class of compounds, the present work describes the synthesis, characterization and preliminary biological studies of a new heteroleptic copper(II) complex with hydrazide and naphtoquinone based ligands.

Results and Discussion

The copper complex (Cu1) was synthesized as follows: first, copper(II) nitrate and 4-aminobenzoic hydrazide (4AMNH) were reacted in methanol under stirring for 20 minutes. Then, a methanolic solution of deprotonated lawsone (LAU) was added to the reaction. After 24 hours of stirring, the red solid obtained was filtered off, washed with methanol and distilled water, and dried in desiccator over P_2O_5.

The complex was characterized by elemental analysis, and the experimental values (%C: 42.41; %H: 3.08; %N: 12.68) are in accordance with the theoretical data for the composition $CuC_{17}H_{14}N_4O_7$ (%C: 45.39; %H: 3.14; %N: 12.45). The UV-Vis spectrum of the complex (see Fig. 1) exhibited the main absorption band in the same region as the free ligands. The band at 449 nm is assigned to an intramolecular charge transfer (CT), indicating lawsone deprotonation upon coordination.[3] The complex is stable in dimethylsulfoxide for 24 hours.

The infrared (IR) spectra support the proposed coordination of the ligands to the metal by the nitrogen and oxygen atoms. The NH stretching bands of 4AMNH appear shifted at higher energies, while the OH band from lawsone disappears. The C=O bands are shifted to lower energies, with a greater shift for the coordinated carbonyl.

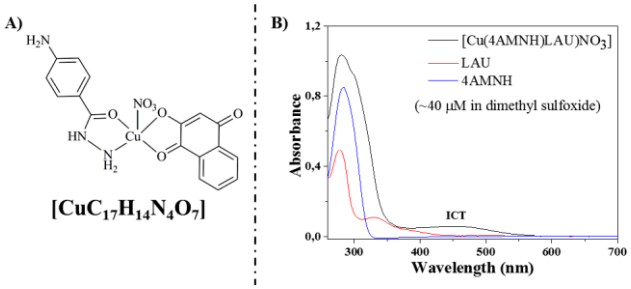


Figure 1: A) Proposed structure to complex. B) UV-Vis spectra to complex and free ligands.

The investigation of cytotoxic potential and possible biomolecular targets are ongoing, using 2D cell culture assays in tumoral and non-tumoral cell lines to assess cytotoxicity and selectivity. Direct interactions with biomolecules such as DNA and proteins are also under investigation.

Preliminary assays showed lower toxicity for the complex Cu1 than the reference drug doxorubicin, from *screening* in hepatocellular carcinoma (HepG2) cell line (commonly used to hepatotoxicity studies)[4].

Conclusions

A new heteroleptic Cu(II) complex was synthesized, and the characterization techniques led us to suggest the structure proposed in Figure 1. Ongoing biological studies aim to assess its potential antiproliferative activity over tumor cells. The compound has shown to be less toxic over HepG2 cell line than doxorubicin.

Acknowledgements

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Synthesis of new heterocyclic compounds as potential trypanosomicide agents

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Key words: Trypanosomiasis, Imidazoazines derivatives, Solubility optimization, DMPK

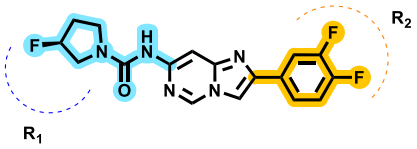
Introduction

Trypanosomiasis is considered a neglected tropical disease (NTD) by World Health Organization (WHO), and the species pathogenic to humans are *Trypanosoma cruzi*, responsible for Chagas disease, and *Trypanosoma brucei*, the etiologic agent of African sleeping sickness (also known as Human African Trypanosomiasis).[1] New imidazoazine derivatives were synthesized by our research group and showed potent biological activity against parasite cultures (*T. cruzi* and *T. brucei*), low cytotoxicity and high metabolic stability. It was observed that an imidazo[1,2-*c*]pyrimidine derivative exhibited excellent plasma exposure upon oral administration in rats, however, the low solubility remains to be improved.[2,3] Therefore, the aim of this work is to design and synthesize new compounds to improve solubility and select the best modifications through the construction of a structure-activity relationship study (SAR) with the phenotypic analysis against *T. cruzi* and *T. brucei.*

Results and Discussion

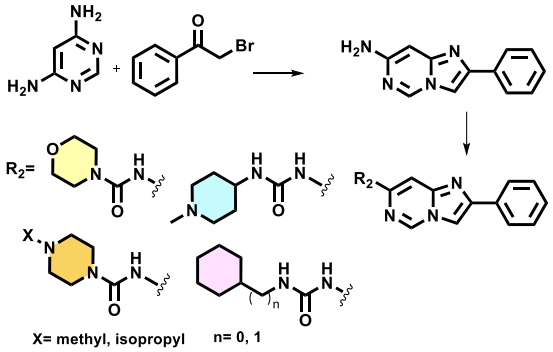
The hit compound (figure 1) discovered by Silva and co-workers (2017) showed a potent biological activity against *T. cruzi* (EC_{50}= 0.09 µM) and *T. brucei* (EC_{50}= 0.02 µM).

Figure 1: Imidazopyrimidine derivative with potential trypanosomicide activity.



Therefore, the scaffold was used as a reference for modifications, the phenyl ring in R_2 was maintained, and a variety of flexible six-membered heterocycles were added in R_1 to evaluate the influence of stereoelectronic effects in the activity, and to enhance solubility. The presence of additional heteroatoms (N, O) can increase polarity and basicity. Additionally, some of those flexible heterocycles possess aliphatic substitutions that enrich the molecule with sp^3 regions, which can improve solubility, reduce melting point, and off-target promiscuity, resulting in increased drug absorption and improved toxicological profiles.[4-6]
The synthesis was executed in a linear route of two steps (figure 2), the starting material was produced by the condensation of appropriate diaminopyrimidine and bromoacetophenone, followed by the formation of an isocyanate intermediate with triphosgene, which led to the final product with the addition of primary/secondary amines of interest.

Figure 2: Synthetic route of Imidazopyrimidine derivatives.



Finally, the compounds were confirmed by mass spectrometry, ^1H and ^{13}C NMR spectra, followed by characterization of physical-chemical properties, purity profile and evaluation of the biological activity towards *T. cruzi* and *T. brucei* by phenotypic assays.

Conclusions

The new derivatives showed promising antiparasitic activity, complementing SAR studies and directing new modifications in the imidazopyrimidine scaffold to achieve promising leads with favorable pharmacokinetic profiles against *T. cruzi* and *T. brucei*.

Acknowledgements

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CRAFT – Center for the Research and Advancement of Fragments and Molecular Targets

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Biological and Biophysical Investigation of *Plasmodium falciparum* Pyruvate Kinase II (PKII) as a New Molecular Target for Antimalarial drug Discovery

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Key words: Malaria, P. falciparum, Pyruvate Kinase

Introduction

Malaria remains one of the most prevalent infectious diseases globally, with high incidence and mortality rates. The emergence of *Plasmodium falciparum* strains resistant to primary antimalarial drugs is concerning and underscores the need for innovative therapies targeting new biological mechanisms. The parasite's apicoplast-resident enzyme, pyruvate kinase II (*Pf*PKII), is a promising yet underexplored molecular target due to significant challenges in its *in vitro* characterization. In this work, we engineered a stable and active recombinant *Pf*PKII construct, enabling robust biophysical analysis. Using this optimized enzyme, we identified suramin, an existing trypanocidal drug, as an inhibitor of *Pf*PKII, establishing a new foundation for structure-based drug design against this critical malaria target.

Results and Discussion

The plasmid encoding *Pf*PKII residues 42-745 was provided by Prof. Sean Prigge (Johns Hopkins University).[1] We developed an optimized construct (residues 70-745) without the N-terminal signal peptide. The recombinant protein was efficiently expressed and purified using four chromatography steps, yielding highly pure and stable tetrameric protein (Fig. 1a and 1b).

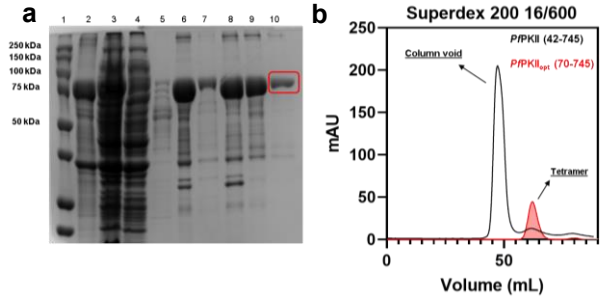


Figure 1. **(a)** SDS-PAGE gel electrophoresis of the *Pf*PKII purification process. Column 10 shows *Pf*PKII after purification steps. **(b)** Comparison of the SEC chromatogram profiles for the original *Pf*PKII (black) and optimized *Pf*PKII (*Pf*PKII$_{opt}$, red) constructs.

The tetrameric state of *Pf*PKII$_{opt}$ was determined using SEC column calibration (V_e = 62 mL, corresponding to 290 kDa) and SEC-MALS analysis (279 ± 6 kDa). The predicted mass of the monomer was approximately 79 kDa. Differential Light Scattering (DLS) indicated that the optimized protein was monodisperse (PDI = 0.07). The optimized *Pf*PKII was used for initial structural studies. Cryo Electron Microscopy (CryoEM) images of the sample revealed homogenous and monodisperse particles (Fig. 2a), enabling further data collection for initial density map reconstruction. *Pf*PKII$_{opt}$ exhibited catalytic activity, with a K_M^{App} value for PEP (substrate) comparable to that of the original construct (K_M^{App} = 26 μM). The optimized protein also demonstrated increased activity, producing a luminescence signal approximately ten times higher than the original construct (Fig. 2b). Preliminary inhibition screening identified suramin, a known trypanocidal agent, as an inhibitor of *Pf*PKII (Inhibition = 59 ± 4% at 80 μM). Further assays will be conducted to elucidate the mechanism of action of suramin on *Pf*PKII$_{opt}$.

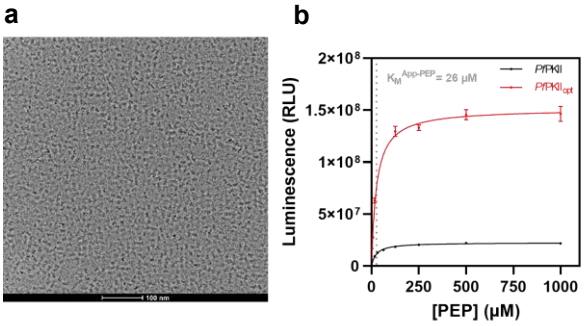


Figure 2. **(a)** Cryo-EM image of *Pf*PKII collected at CNPEM (Campinas/SP), using a Talos Arctica microscope (Thermo Fisher Scientific) operating at 200 kV. **(b)** Comparison of the concentration-response curve for the original *Pf*PKII (black) and optimized *Pf*PKII constructs (*Pf*PKII$_{opt}$) (red).

Conclusions

The optimized *Pf*PKII demonstrated greater stability and activity, making structural studies possible. Furthermore, suramin was identified as an inhibitor of *Pf*PKII. These findings indicate that *Pf*PKII is an attractive target for antimalarial drug discovery.

Acknowledgements

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Synthesis and Optimization of Indole-2-carboxamides for Enhanced Anti-Plasmodial Activity

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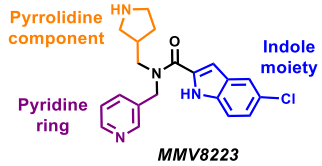
Key words: Malaria, Indoles, Carboxamides, Chloroquine, SAR studies, Cross-Resistance.

Introduction

Malaria, caused by *Plasmodium* parasites, disproportionately impacts the world's least economically developed countries. The disease made 597 thousand fatal victims in 2023, of those 95% registered in Africa[1]. The increasing resistance to the available chemotherapies is an obstacle to the treatment[1,2]. This study focusses on the synthesis and optimization of indole carboxamides compounds with the goal of providing a new pre-clinical antimalarial candidate.

Results and Discussion

The initial work on the series consisted of the hit identification by high-throughput screening of MMV libraries. The molecule returned an $IC_{50,Pf3D7}$ of 1.4 µM and no toxicity (hERG, HepG2) was detected. The hit was valided based on antimalarial criteria[3] and has its structure represented on **Figure 1**.



MMV8223

Figure 1: Molecular hit and its main exploration cores.

A synthetic route has been proposed to allow the series exploration in three different molecular cores – indole, pyridine and pyrrolidine. A 4-step, versatile route has been achieved, allowing the synthesis of around 40 analogs, building up a strong SAR. During the synthesis, secondary amines were obtained either by reductive amination of primary amines with aldehydes or through nucleophilic displacement using tosylated compounds and aminopyridines. These secondary amines reacted with carboxylic acids to afford the corresponding amides. N-methylated pyrrolidines and piperidines were also synthesized via reductive amination.

Regarding the structural modifications, replacing the indole moiety with a Cl-phenyl ring or benzimidazole led to reduced potency. In addition, substituting -Cl on indole by -H, -Cl, -F or -OMe also led to reduced potency, except to $-CF_3$ (IC_{50} = 0.4 µM). Any modification to the pyridine ring – including changes in the nitrogen atom position and ring substitution – as well as its replacement by an aliphatic chain resulted in loss of potency. N-Methylation of pyrrolidine nitrogen and removal of methylene linker increased potency. Furthermore, replacing pyrrolidine with piperidine also enhanced activity, especially when combined with a $-CF_3$ on the indole (IC_{50}= 0.22 µM). Also, chiral resolution and further test of the two pyrrolidine enantiomers returned similar potency (A(*R*) IC_{50} = 1.0 µM; A(*S*) IC_{50} = 2.0 µM).

The most promising analogs were submitted to ADME Tier 1 assays. In general, compounds have moderately low to good logD, and a solubility in the ideal range. Similarly to the hit, most of the compounds are also metabolically stable. On the other hand, indicatives of cardiotoxicity were noticed for a few analogs tested.

Some derivatives presented loss of potency when tested against a panel of resistant strains, indicating a cross-resistance profile. Two selected compounds were also tested in the presence of verapamil – a known efflux pump inhibitor – against the resistant Dd2 strain. Similar to chloroquine, their potency increased by 8-fold and 5-fold, respectively, compared to assays without verapamil.

Conclusions

The SAR exploration lead to the synthesis of active compounds against *Plamodium*, with an analog that demonstrated a sixfold improvement in potency over the initial hit, while also exhibiting enhanced metabolic stability and weak hERG channel activity. Nevertheless, the cross-resistance attributed a high risk to the series, leading to its discontinuity.

Acknowledgements

We gratefully acknowledge Unicamp and USP for the infrastructure as well as MMV and FAPESP (PITE 2015/50655-9) for their valuable support.

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Antibacterial Potential of Natural Compounds from Acerola (Malpighia glabra L.)

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Key words: Malpighia glabra, β-lactamase Inhibition, Dihydropteroate Synthase Docking

Introduction

Bacterial infections, caused by opportunistic pathogens or highly virulent bacteria, represent a significant global public health challenge. Although antibiotic therapy remains the primary treatment strategy, its effectiveness has been increasingly compromised by the alarming rise of bacterial resistance to existing antimicrobials. This growing resistance exacerbates the morbidity and mortality associated with bacterial infections and highlights the urgent need for new therapeutic alternatives. In this context, natural compounds derived from plant sources have emerged as promising candidates for the development of new bioactive molecules with antimicrobial potential. In a study by Cruz et al. (2017), the extract of acerola (Malpighia glabra L.) exhibited antifungal activity against conidia and muriform cells of strains associated with chromoblastomycosis. Based on these findings, the present study aims to evaluate the antibacterial potential of bioactive compounds extracted from acerola (Malpighia glabra L.) through molecular docking simulations, with the objective of identifying promising candidates for the development of alternative therapeutic strategies against bacterial infections. As molecular targets, the enzymes β-lactamase and dihydropteroate synthase (DHPS) were selected, as they are widely distributed among bacteria. Their crystallographic structures were obtained from the Protein Data Bank with PDB ID: 6DPZ for β-lactamase and PDB ID: 1AD4 for DHPS. Molecular docking was performed using AutoDock Vina software.

Results and Discussion

The ligands with the best performance were selected from the 16 docking simulations performed for each protein target, with particular emphasis on β-lactamase enzymes. The results were compared with standard therapeutic agents, including clavulanic acid, amoxicillin, and (1R,2S)-2-{[(pyrrolidin-1-yl)sulfonyl]amino}cyclooctane-1- carboxylic acid, as shown in Table 1.

Table of the best binding affinities for β-lactamase

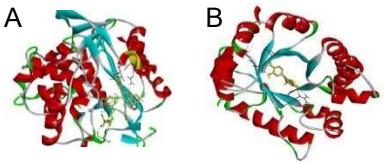
MOLECULE	NATURAL SOURCE	FORMULA	AFFINITY
Rutin	Acerola berry	C27H30O16	-9.3 kal/mol
Chlorogenic acid	Acerola berry	C16H18O9	-8.5 kal/mol
Quercetin	Acerola berry	C15H10O7	-8.0 kal/mol
Amoxicillin	Control	C16H19N3OS	-7.5 kal/mol
1R,2S)-2-{[(Pyrrolidin-1-yl)sulfonyl]amino}cyclooctane-1-carboxylic acid	Control	C13H24N2OS	-7.2 kal/mol
Clavulanic acid.	Control	C8H9NO5	-6.1 kal/mol

Additionally, the binding affinities of bioactive (DHPPP), promoting pyrophosphate release and the compounds were evaluated against the enzyme dihydropteroate synthase (DHPS), a key component of the folate biosynthesis pathway. This enzyme catalyzes the condensation of para-aminobenzoic acid (pABA) with 7,8-dihydropterin pyrophosphate formation of 7,8-dihydropteroate, an essential intermediate in folate synthesis. Among the compounds tested, quercetin exhibited the highest binding affinity with DHPS at -7.8 kcal/mol, followed by kaempferol and rutin, both with
-7.7 kcal/mol. These results suggest a potential competitive inhibitory effect of these flavonoids on the target enzyme, indicating promising in silico antibacterial activity. Detailed values are presented in Table 2

Table of the best binding affinities for dihydropteroate synthase.

MOLECULE	NATURAL SOURCE	FORMULA	AFFINITY
Quercetin	Acerola berry	C15H10O7	-7.8 kal/mol
Rutin	Acerola berry	C27H30O16	-7.7 kal/mol
Kaempferol	Acerola berry	C15H10O6	-7.7 kal/mol
Hydroxymethylpterin	Control	C7H9N5O8P2	-7.5 kal/mol
Sulfadoxine	Control	C12H14N4O4S	-6.3 kal/mol

Figure 01) 3D image of the β-lactamase protein with the ligand rutin, side A. On side B, the 3D image of the dihydropteroate synthase protein with the ligand quercetin.



Conclusions

In this study, 16 bioactive compounds from acerola (Malpighia glabra L.) were evaluated using molecular docking techniques. Three molecules (rutin, chlorogenic acid, and quercetin) demonstrated favorable interactions with the β-lactamase enzyme, while dihydropteroate synthase showed favorable interactions with quercetin, rutin, and kaempferol, indicating potential antibiotic activity. Further investigations into their metabolic pathways, cytotoxic effects, and pharmacokinetic properties should be conducted to ensure the safety and efficacy of these molecules as therapeutic candidates.

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Universidade Federal do Pará, PROPESP, and PPGQM3

Incorporating Receptor Flexibility into DockThor Using Ensemble Docking Approaches

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Key words: Receptor flexibility, Ensemble docking, Molecular docking program, DockThor

Introduction

Molecular recognition is a fundamental mechanism in biological systems, driven by the dynamic interaction between ligands and their molecular targets. Two principal theories — induced fit [1] and conformational selection [2] — explain how ligands achieve specificity and affinity by interacting with flexible receptor structures. Understanding these interactions is crucial in fields such as drug discovery and enables the elucidation of the molecular determinants underlying recognition and selectivity.

Molecular docking is a key computational strategy in drug design, facilitating insights into molecular recognition and enabling the prediction of the preferred orientation and binding affinity of ligands to their receptors [3]. However, accurately accounting for receptor flexibility remains one of the greatest challenges in docking methodologies, even among the most advanced approaches [4].

DockThor is a protein-ligand docking program developed by Brazilian students and researchers from the Grupo de Modelagem de Sistemas Biológicos (GMMSB, LNCC/MCTI), freely available via its web server (www.dockthor.lncc.br). DockThor demonstrates robust performance in re-docking, peptide docking, and cross-docking experiments, establishing itself as a competitive tool, particularly for highly flexible ligands [5]. Despite these strengths, DockThor has traditionally modeled the receptor as a rigid structure, which limits its ability to fully capture the complexities of molecular recognition.

In this work, we propose incorporating receptor flexibility into the DockThor docking algorithm and evaluate its performance using two ensemble docking strategies: (i) Combined Grid (CG), which generates a unified potential grid by combining grids derived from multiple protein conformations, and (ii) Multiple Grids (MG), which docks ligands independently against grids corresponding to different conformations.

Results and Discussion

Our previous cross-docking results using the Astex Non-Native benchmark demonstrate that the ensemble docking approach enhances docking accuracy with DockThor. To achieve this, we incorporated receptor flexibility in DockThor through an ensemble docking strategy.

The CG and MG approaches address different flexibility objectives: CG is suitable for experiments involving local flexibility movements or cases with a very large number of protein structures (*e.g.* sampling from molecular dynamics or nuclear magnetic resonance experiments), as it reduces the sampling space; in contrast, MG can capture both local and global flexibility without substantially increasing computational costs. Moreover, these strategies can be combined to optimize receptor flexibility modeling further while maintaining computational efficiency.

All implemented ensemble strategies either improved or maintained re-docking performance in our preliminary tests. When considering five protein conformations, the computational time increased by only ~2.4× compared to docking with a single structure.

Although further studies are required, our preliminary results suggest that the ensemble docking strategy is an effective method for accounting for receptor flexibility without incurring substantial computational costs.

Conclusions

- Grid-based ensemble docking allows receptor flexibility with a good balance between accuracy and computational cost.
- These enhancements improve the applicability of DockThor in more realistic molecular recognition experiments, reinforcing its value as a tool for drug discovery and computational biology.
- The updated version will be available at www.dockthor.lncc.br.

Acknowledgements

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Design, synthesis, and antiplatelet activity of new N-heterocyclic compounds.

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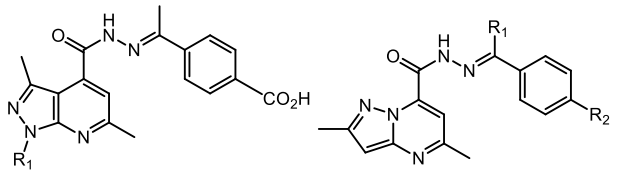
Key words: machine learning, molecular docking, virtual screening, antiplatelet, synthesis

Introduction

TXA2 is a potent platelet-aggregating prostanoid involved in thrombus formation and vasoconstriction, which can contribute to the development of cardiovascular diseases. The enzyme thromboxane A synthase (TXAS) is responsible for the synthesis of TXA2.[1] Virtual screening (VS), particularly through molecular docking, is a valuable tool for identifying active compounds by predicting ligand-protein binding affinity using scoring functions.[2] To further improve the classification of potential compounds, machine learning (ML) algorithms can be employed to develop classification models.[2] In this work, we present a study focused on the design of potential TXAS inhibitors by integrating molecular docking and ML approaches, with subsequent synthesis and in vitro evaluation to assess their antiplatelet activity.

Results and Discussion

We developed a machine learning classification model that integrated molecular docking scoring and molecular descriptors of ligands. Molecular docking studies at TXAS were conducted using the ChemPLP function of the GOLD software, and molecular descriptors were calculated via DataWarrior. The classification models were built using KNIME with the XGBoost algorithm. The ML model with the best performance was applied to the VS of a library of 142 proposed novel *N*-heterocycle compounds, structurally related to antiplatelet compounds from the literature.[3] These structures were fed into the binary classification model, which was designed to categorize them as either active or inactive. Of the screened structures, four were predicted by the classification model to have the highest probability of activity (P≥0.70). Then, these four designed compounds (1-4, Figure 1) were synthesized through a 3-step synthetic route, starting from 5-amino-3-methyl-1*H*-pyrazoles bearing different substituents at the N-1 position. The final synthetic step involved the nucleophilic addition of a carbohydrazide group to substituted benzaldehydes or acetophenone, yielding the benzylidene moiety, confirmed through spectroscopic techniques. These compounds were subsequently evaluated in vitro for their ability to inhibit arachidonic acid-induced platelet aggregation in human plasma. Compounds 2 and 3 (Figure 1) predicted by the ML model with a higher probability of TXAS inhibition (P>0.8) demonstrated inhibition of platelet aggregation at a concentration of 500 µM, suggesting a potential correlation between their predicted TXAS inhibitory activity and their observed antiplatelet effects.



(1) R_1 = Ph (P= 0.70)
(2) R_1 = CH_3 (P= 0.83)
(3) R_1 = H; R_2 = CO_2H (P= 0.82)
(4) R_1 = CH_3; R_2 = CN (P= 0.77)

Figure.1: New *N*-heterocycle compounds (1-4) and their predicted probability of TXAS inhibition (P).

Conclusions

Integrating molecular docking and ML, virtual screening identified promising compounds for inhibiting TXAS. The antiplatelet activity results were related to the enzyme inhibition potential (P) identified through VS, with this action proving concentration-dependent.

This approach streamlined the discovery process for bioactive compounds by reducing the number of compounds for synthesis and subsequent biological testing. These results underscore the increasing importance of artificial intelligence, particularly machine learning, in the design of bioactive compounds.

Acknowledgements

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Synthesis, computational study and biological evaluation of 1,4-dihydropyridines as DPP-4 inhibitors.

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Keywords: *Diabetes, Dipeptidyl peptidase-IV, Inhibitor, Multicomponent Reaction, Niobium.*

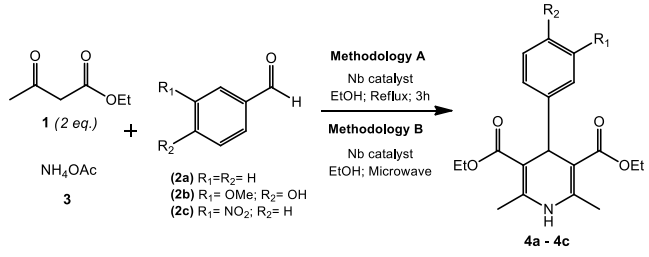
Introduction

Diabetes is a metabolic disorder that presents hyperglycemia and vascular complications due to the lack of insulin production or its inadequate use by the body. Dipeptidyl peptidase-4 (DPP-4) is an important biological target related to the treatment of type II diabetes, since DPP-4 inhibitors can lead to an increase in insulin levels and prolonged activity of glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby contributing to effective glycemic control.[1] Molecular modeling methods, which enable the evaluation of the main contributions of fragments to biological activity, allowed the development of a model with structural information, which in turn facilitated the design of new DPP-4 ligands. 1,4-Dihydropyridines (1,4-DHPs) are an important class of organic compounds in medicinal chemistry associated with a wide variety of therapeutic effects. 1,4-DHPs can be prepared via the Hantzsch multicomponent reaction (MCR), which involves the condensation of ketoester, aldehyde and ammonia. This study aims to synthesize a series of 1,4-DHPs to act as DPP-4 ligands, as well as to evaluate their enzymatic activity and toxicity through *in silico* and *in vivo* assays.

Results and Discussion

According to Scheme 1, the reactions were initially performed under refluxing ethanol (**Methodology A**) and three niobium catalysts were tested: niobium pentoxide (**NBO**), niobium phosphate (**NBP**) and ammonium oxalate (**ANO**). To compare a new Hantzsch MCR methodology employing niobium compound catalysis, the reactions were performed using a microwave reactor (**Methodology B**).

Scheme 1: Synthesis of 1,4-dihydropyridines.



In the microwave-assisted reactions, the molar ratios of the reagents were the same as those used under reflux conditions. The reagents and solvent were added to a sealed tube, which was then placed into a microwave reactor (Discover Proteomics CEM) operating at 300 W, 100 °C, in cycles of 3.5 min (2 min of ramping and 1.5 min of reaction time). The reactions were carried out using the two niobium catalysts that showed the best performance under reflux conditions. 1,4-DHPs were prepared in 3-h reflux reactions with yields ranging from 3-65% and in microwaves with yields from 45 to 87% (Table 1). The variation in yield is also related to the difficulty of purifying the compounds.

Table 1. Results of Hantzsch MCR.

Entry[a]	Aldehyde	Catalyst	Product	Yield (**A**)	Yield (**B**)
1[b]	**2a**	-	**4a**	25%	-
2	**2a**	NBO	**4a**	37%	64%
3	**2a**	ANO	**4a**	35%	58%
4	**2a**	NBP	**4a**	32%	-
5	**2b**	NBO	**4b**	65%	55%
6	**2b**	ANO	**4b**	63%	45%
7	**2b**	NBP	**4b**	24%	-
8	**2c**	NBO	**4c**	23%	87%
9	**2c**	ANO	**4c**	20%	81%

[a] Reactions: ethyl acetoacetate (2 eq.), aldehyde (1 eq.), 20% (w/w) of niobium catalyst (related to ethyl acetoacetate), ammonium acetate (1 eq.); [b] Reaction refluxed for 48 hours.

Synthetic 1,4-DHPs were subjected to enzymatic inhibition assays, evaluated using a luminescent enzymatic assay. The new compounds exhibited inhibition percentage lower than 50% at the concentration of 1 mM. The drug sitagliptin was used as a reference (IC_{50}: 31 nM). Cytotoxicity assays revealed that the compounds tested in the enzymatic assays are non-toxic. Among the *in silico* studies, the physicochemical properties related to the oral bioavailability of the compounds were calculated using SwissADME. The comparison between experimental and predicted values suggests that the model was not suitable for accurately predicting the inhibitory potency.

Conclusions

The results suggest that the ligands require structural modifications to achieve suitable activity profiles.

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Novel ROCK inhibitors and their corresponding chemical stability, solubility and permeability

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Keywords:*ROCK, docking molecular, PAMPA, chemical stability, aqueous solubility*

Introduction

Rho kinases (ROCK) are enzymes that play a crucial role in regulating the actin cytoskeleton and in cellular processes such as migration, adhesion and muscle contraction [1]. They are involved in various pathologies, including cardiovascular diseases, neurodegenerative diseases and cancer, making them promising therapeutic targets [2].

The present work deals with the identification of new ROCK1 and ROCK2 inhibitors based on the J0P prototype [3].

Results and Discussion

From these studies we identified compounds LASSBio-2346 (IC_{50} ROCK1 = 3.65 µM; IC_{50} ROCK2 = 7.47 µM), LASSBio-2350 (IC_{50} ROCK1 = 3.39 µM; IC_{50} ROCK2 = 7.24 µM) and LASSBio-2354 (IC_{50} ROCK1 = 4.13 µM; IC_{50} ROCK2 = 6.69 µM) presented kinetic solubility values above 50 µM at 4h and 24h. These compounds also showed high permeability in the gastrointestinal tract PAMPA assay and positive permeability in the blood-brain barrier PAMPA assay. It is also worth noting that these compounds show chemical stability at plasma pH, although they undergo 50 per cent hydrolysis at pH 2 in the first 30 minutes of the chemical stability test. Furthermore, these results suggest that LASSBio-2346, LASSBio-2350 and LASSBio-2354 could be investigated in the context of ageing diseases affecting the central nervous system.

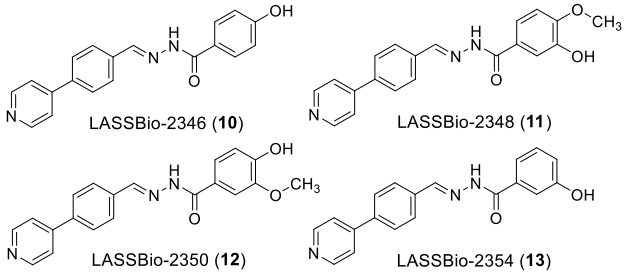


Figure 1. Chemical structures of compounds LASSBio-2346 (10), LASSBio-2348 (11), LASSBio-2350 (12), and LASSBio-2354 (13).

Table 1. Inhibitory activity against ROCK1 and ROCK2 kinase of compounds LASSBio-2346 (10), LASSBio-2348 (11), LASSBio-2350 (12), and LASSBio-2354 (13).

Compound	IC_{50} ROCK1 (µM)	IC_{50} ROCK2 (µM)
LASSBio-2346 (10)	3.65	7.47
LASSBio-2348 (11)	3.51	7.04
LASSBio-2350 (12)	3.39	7.24
LASSBio-2354 (13)	4.13	6.69

Table 2. Properties drug likeness of compounds LASSBio-2346 (10), LASSBio-2348 (11), LASSBio-2350 (12), and LASSBio-2354 (13).

Compound	Sol. 4h (µM)	Sol. 24h (µM)	Pe. GIT	Pe. BBB	Stability pH 7.4	Stability pH 2
LASSBio-2346 (10)	51.49	50.71	High	CNS +	90.87 ± 5.10%	35.04 ± 0.75%
LASSBio-2348 (11)	60.46	52.69	High	CNS +	80.35 ± 13.69%	25.71 ± 11.01%
LASSBio-2350 (12)	51.30	51.88	High	CNS +	96.62 ± 2.89%	16.27 ± 6.48%
LASSBio-2354 (13)	100.36	88.78	High	CNS +	87.35 ± 2.79%	22.02 ± 1.06%

Conclusions

The LASSBio compounds (**10** - **13**) represent promising N-acylhydrazone hits for further investigation *in vivo* models of aging-related diseases affecting the central nervous system, such as Alzheimer's and Parkinson's diseases.

Acknowledgements

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Indium(III)-sparfloxacin complex as radiosensitizing potential agent

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Key words: sparfloxacin, antitumoral cytotoxicity, radiosensitizing effect.

Introduction

Cervical cancer is the fourth most common and deadly type of cancer among women in Brazil[1]. Nowadays, cisplatin is the main drug used to treat high risk patients, but it has considerable kidney, hematologic and gastrointestinal toxicity[2].

Aiming to develop alternative treatments, fluorquinolones, which present cytotoxic activity thought DNA intercalation[3] emerge as interesting candidates. In this context, the present work describes the synthesis of an Indium(III) complex with sparfloxacin (SPAR), a DNA synthesis inhibitor, and its pharmacological and radiosensitization potential against human cervical adenocarcinoma cells (HeLa).

Results and Discussion

Sparfloxacin indium(III) complex (In-SPAR) was synthesized by reacting $InCl_3$ with sparfloxacin in methanol under reflux for 24h. After filtration and vacuum-dry, it was characterized by elemental analysis, IR, UV-VIS and NMR spectroscopy. HeLa cells were treated with SPAR and its complex in the absence or presence of gamma irradiation Co-60 (6Gy) and metabolic viability was evaluated using the MTT assay.

CHN analysis confirmed a 1:2 metal-to-ligand ratio. IR and ^1H NMR spectra in DMSO-d_6 suggested bidentate coordination through the carboxylate and ketone oxygen atoms. Based on these results, the complex is proposed as $[InCl_2(SPAR)_2]Cl$, where SPAR denotes sparfloxacin coordinated in its zwitterionic form (Fig. 1).

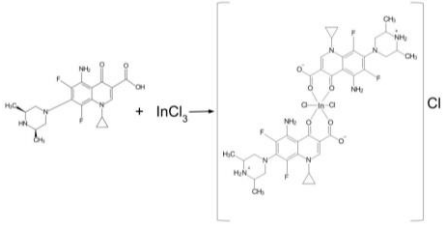


Figure 1. Chemical equation for the synthesis of $[InCl_2(SPAR)_2]Cl$

SPAR and In-SPAR showed dose-dependent cytotoxic activity against HeLa cells. In(III) coordination appears to potentiate SPAR's cytotoxicity (Fig. 2).

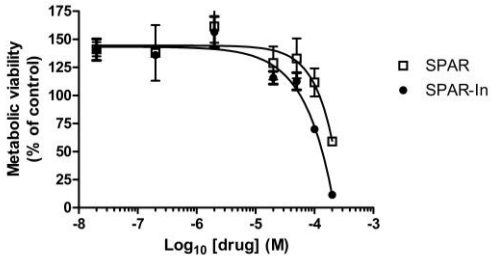


Figure 2: Cytotoxicity of SPAR and In-SPAR against HeLa cells.

Adenocarcinoma is known to be radioresistant. SPAR and In-SPAR were able to reduce the metabolic viability of radioresistant cells (Fig. 3) treated with gamma radiation (6 Gy of Co-60) with radiation enhancement factors of 1.5 and 4, respectively.

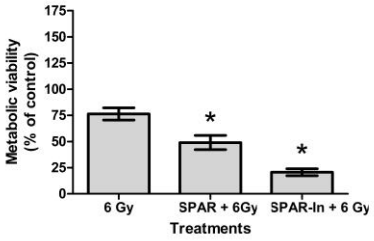


Figure 3. Radiosensitization of HeLa cells induced by combined treatment with Co-60 gamma radiation (6 Gy) and 1E-04 M drugs. [*p < 0,01]

Conclusions

The In-SPAR complex was synthesized with success. Coordination with In(III) increased SPAR's cytotoxicity against HeLa cells. Moreover, both compounds promoted radiosensitization up to 1.5 times for the SPAR and 4 times for the complex. These results indicate the potential of these compounds as radiosensitizing agents against cancer cells.

Acknowledgements

The authors acknowledge the financial support of CNPq, financial support and infrastructure provision of CDTN/CNEN and the cells' irradiation by Gamma Irradiation Lab. (LIG/CDTN).

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From Cancer to Alzheimer: *In silico* insights on novel potentially bioactive synthetic 1,4-acridinediones

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Key words: docking, synthesis, anticancer, 1,4-DHP, cholinesterase, inhibitor

Introduction

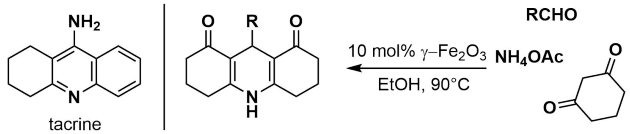
1,4-Dihydropyridines **(1,4-DHP**) and their fused structures, such as acridinediones (**ACD**), exhibit broad pharmacological activity, notably antitumor effects. Besides cancer, other biological targets have also been investigated, including cholinesterase enzymes, which are relevant in the context of Alzheimer's disease.[1]

In this work, **ACD** derivatives containing substituted 4-phenyl groups were studied to enhance interactions with the active sites of these enzymes. The approach involves *in silico* screening through molecular docking, followed by comparison of binding energy results with those obtained for tacrine, a reference drug. Based on the docking results, the most promising compounds will be synthesized for further investigation.

Results and Discussion

In silico analyses were conducted using AutoDock4 software[2] to evaluate the interaction profile of a series of **ACD** derivatives, along with the reference drug tacrine (**Figure 1**), against cholinesterase enzymes, acetylcholinesterase (**AChE**, PDB: 1CFJ) and butyrylcholinesterase (**BChE**, PDB: 6I0B). The **ACD** derivatives were then synthesized after the docking results analyses via a Hantzsch multicomponent reaction, based on a methodology previously established by our research group.[3] It is worth mentioning that all compounds were achieved in good yields and exhibit fluorescence emission.

Figure 1. Structure of tacrine and **ACD** derivatives obtained via Hantzsch multicomponent synthesis.



R: 1a - 2,3,5-trimethoxyphenyl
1b - 4-methoxybiphenyl
1c - 3-trifluoromethoxyphenyl

The molecular docking results revealed key interactions[4] between the ligands and active site residues of **AChE** and no significant binding was found for **BChE**, indicating selectivity. Tacrine interacted with Trp84 in the anionic site (**AS**) and Tyr121 in the peripheral anionic site (**PAS**), located at the entrance of the catalytic gorge. Similar patterns were observed for the acridinedione derivatives, which also interacted with Trp84 and other **AS** residues, crucial for blocking acetylcholine access to the catalytic triad (**CT**). Several compounds interacted with **PAS** residues such as Tyr334 and Asp72. Ligand **1b** showed a strong interaction with His440 of the **CT** (**Figure 2**), possibly explaining its enhanced stabilization energy of −11.61 kcal·mol^{-1}, the lowest among the series (**Table 1**). Additional interactions in the acyl pocket (**AP**) and oxyanion hole (**OH**) were identified, potentially contributing to ligand stabilization.

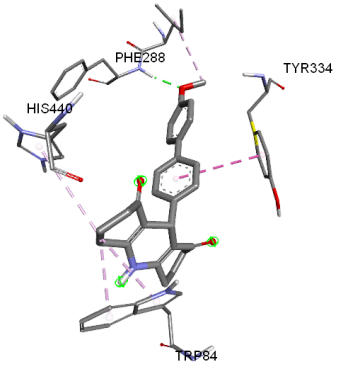


Figure 2: Binding pose of **1b** in **AChE** active site.

Table 1. Lowest binding energy values and interaction sites of derivatives on **AChE**.

Compound	Energy (kcal.mol^{-1})	Active Site Interactions
1a	-9,76	AS, PAS
1b	-11,61	CT, AS, AP, PAS
1c	-9,90	CT, AS, AP, OH, PAS
Tacrine	-7,64	AS, PAS

Conclusions

The acridinedione derivatives showed promising interactions with **AChE**, indicating potential as selective inhibitors. Future studies will involve other **1,4-DHP** derivatives to further optimize binding affinity and selectivity. Additionally, in vitro assays using Ellman's method are planned to experimentally validate the inhibitory activity of the synthesized compounds.

Acknowledgements

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In vitro evaluation of LASSBio-1586 and analogues hepatotoxicity in HepG2 cells using 2D and 3D models

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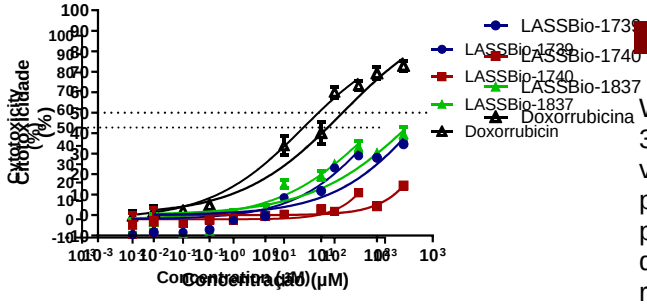
Key words: drug-induced liver injury, hepatotoxicity, 3D hepatic spheroids, HepG2 cells

Introduction

Drug-induced liver injury (DILI) is a challenge in drug development, often leading to the discontinuation of compounds in clinical trials or post-marketing.[1] It can occur through direct dose-dependent toxicity or unpredictable idiosyncratic reactions. Despite its relevance, data on DILI in Latin America is scarce. Studies such as LatinDILI point to high morbidity and mortality, highlighting the importance of early toxicity assessment.[2] This study evaluates the cytotoxicity of LASSBio-1586 and its structural analogues in 2D and 3D HepG2 models to identify hepatotoxic risks and safety profiles.

Results and Discussion

12 compounds were tested for their cytotoxic profile against HepG2 cells, at a screening concentration of 100 µM, using the 72-hour MTT method and doxorubicin as a positive standard. LASSBio-1586 and 8 more compounds wasn't cytotoxic, then the compounds that showed cytotoxicity ≥ 40% were selected for determination of the concentration-response curve. Thus, LASSBio-1738, LASSBio-1740 and LASSBio-1836 had their cytotoxic potency demonstrated in comparison to doxorubicin (Figure 1).



Compound	DOXORRUBICIN	LASSBio-1739	LASSBio-1740	LASSBio-1837
CC50 (µM) 72 h	24,35 (15,98-37,11) Emáx: 72,9%	>300 Emáx=34,6%	>300 Emáx=14,4%	>300 Emáx=39,9%

Figure 1: Dose-response curves and determination of the CC_{50} of the compounds in the 2D model.

Doxorubicin had a CC_{50} of 24.35 µM and a maximum effect of 72.9%, while the target compounds exhibited CC_{50} > 300 µM, suggesting low liver toxicity. Considering the inability of HepG2 cell in 2D model to predict undirect liver toxicity, we repeat the experiments using 3D model of HepG2 cells. In this model, we use additional positive standards as acetaminophen, known to have metabolism-dependent hepatotoxicity. As depicted in Figure 2, doxorubicin caused visible morphological changes in the spheroids, but its coloring interfered with the spectrophotometric reading. Acetaminophen remained non-cytotoxic in these tests at a screening concentration of 100 µM implying that the 3D model, at this stage, cannot explain indirect cytotoxicity.

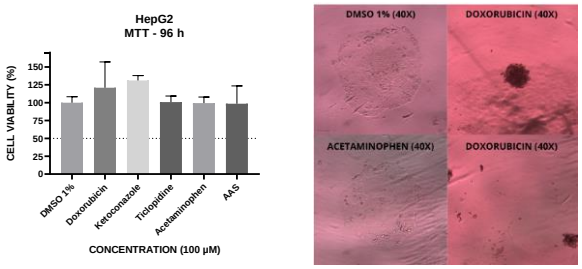


Figure 2: Morphological changes in HepG2 spheroids observed during screening at 100 µM with standards in the 3D model.

Conclusions

We demonstrate the use of HepG2 cells in 2D and 3D culture as a model to evaluate hepatotoxicity in vitro. In 2D models, we demonstrated that the prototypes LASSBio-1586 and analogues, previously identified in our laboratory showed no direct cytotoxic effect on HepG2 cells. Our 3D model results need to improve to reproduce indirect cytotoxicity.

Acknowledgements

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Ethinyl-benzamide derivatives as potential protein tyrosine kinase BCR-ABL1 inhibitors

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Key words: Ponatinib, Kinases, Leukemia, Cancer

Introduction

Protein tyrosine kinases (PTKs) play crucial roles in various cellular processes, including metabolism and cell cycle regulation. However, when they become constitutively active and ligand-independent, due to alterations such as mutations, overexpression, or chromosomal translocations, uncontrolled cell proliferation may occur, leading to the development of certain neoplasms, such as Chronic Myeloid Leukemia (CML) [Jiao *et al.*, 2018]. Imatinib mesylate was the first representative of the protein tyrosine kinase inhibitor (PTKI) class targeting BCR-ABL1, and it revolutionized the treatment of CML. However, the emergence of resistance has challenged its long-term efficacy. This led to the development of second- and third-generation inhibitors, including nilotinib, dasatinib, and bosutinib (second-generation), and ponatinib (third-generation) [Alves *et al.*, 2021]. Ponatinib is the only ATP-mimetic inhibitor capable of overcoming the T315I mutation in the BCR-ABL1 domain; however, its clinical use is limited by severe side effects [Chan et al., 2020]. Therefore, the aim of this project is the synthesis, characterization, and anti-myeloproliferative evaluation of fifteen novel ponatinib analogs, retaining the ethinyl-benzamide moiety, critical for overcoming the T315I mutation, while incorporating various substituents.

Results and Discussion

The synthetic approach for obtaining the target compounds follows a convergent pathway involving two to five reaction steps (Figure 1), yielding the final products in good to excellent overall yields. Biological assays for K562 cell line inhibition showed promising results, particularly for compounds **4e** and **6e** (Figure 2). These molecules were subsequently tested for cytotoxicity against HEK 293 cells, demonstrating greater selectivity compared to the reference drugs, imatinib and ponatinib (Figure 3).

Figure 1. Synthetical methodology applied to obtain the desired molecules.

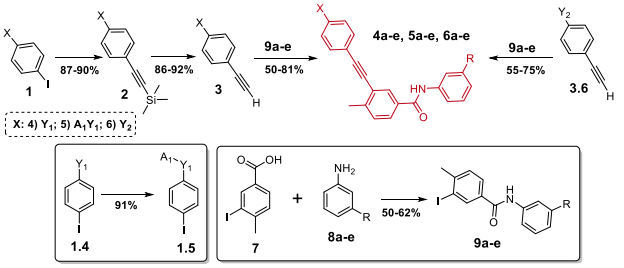


Figure 2. K562 cell inhibition results for the most potent compounds.

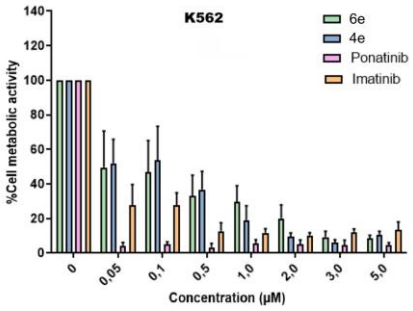
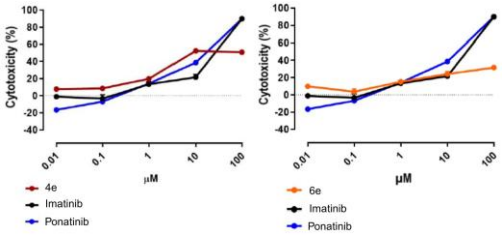


Figure 3. Evaluation of cytotoxic effects on HEK 293 cells.



Conclusions

In conclusion, the target compounds were synthesized in good yields and successfully characterized, confirming their chemical structures. Among them, compounds **4e** and **6e** exhibited the most potent inhibitory activity against K562 cells, along with favorable selectivity profiles.

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Development and chemoinformatic characterization of an expanded version of electronic chemical library "Brazilian Compound Library"

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Key words: chemical libraries, cheminformatics, BraCoLi, computer-aided drug design, compound screening.

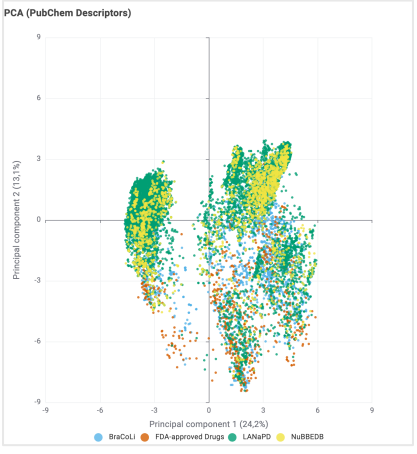
Introduction

The Brazilian Compound Library (BraCoLi) is a free-to-access electronic library aimed to manually curate and harmonize chemical and biological information of bioactive compounds obtained by research laboratories in Brazil. The first version of BraCoLi was launched in 2022, containing 1,176 chemical entities at the time only from the Federal University of Minas Gerais (UFMG)[1]. Some BraCoLi's entities have been evaluated on their antibacterial, anti-*Trypanosoma* and antiviral activities[2,3,4]. Therefore, we aimed to include new molecules to the catalog—focusing on gathering data from other institutions—including those from other geographical regions of Brazil—, as well as characterize the chemical diversity and drug-like profile of BraCoLi.

Results and Discussion

The current partial version of BraCoLi (titled version 1.5) contains 2,302 molecules, comprising synthetic, semisynthetic and natural products. We were able to expand the content from other universities, now having molecules obtained at UFBA, UFMG, UFOP, Unifal, and Unifesp. The addition of secondary metabolites to this version is an important highlight, especially flavonoids, shikimates, and triterpenes, not present at the first version of BraCoLi.

Figure 1: Chemical space visualization of structural properties of BraCoLi in comparison with FDA-approved drugs, NuBBEDB, and LANaPD, generated by Principal Component Analysis (PCA).



In terms of drug-like profiling, 58% of the entities fit four drug-like empirical rules (Lipinski, Veber, Ghose and Muegge), in which more than 80% are adequate according to at least Ro5. We also compared BraCoLi's chemical diversity with two databases (Latin American Natural Product Database or LANaPD, and NuBBE Database or NuBBEDB)[5,6,7], as well as drugs approved by the FDA from 1998 through 2017. Using Principal Component Analysis for dimensionality reduction and PubChem descriptors library, BraCoLi present a unique distribution, showing a vast structural diversity to be applied on computer-aided drug design strategies.

Conclusions

As BraCoLi expanded to include natural products, we can see an extension of its chemical diversity. We expect to establish new collaborations for data curation and compilation, make a tentative "version 2.0" available, and develop a web search database.

Acknowledgements

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Discovery and characterization of cell-permeable inhibitors of *Leishmania mexicana* CLK1 using an in-cell target engagement assay

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Key words: Protein kinase, infectious diseases, target engagement, leishmaniasis, kinase inhibitor, drug discovery.

Introduction

Leishmaniasis is a neglected tropical disease with limited treatment options and significant unmet medical need. Protein kinases have emerged as promising therapeutic targets in kinetoplastid parasites, including *Leishmania* species. In this context, *Leishmania mexicana* CLK1 (LmxCLK1) and its closely related paralogue LmxCLK2, which share an identical protein kinase domain, have been implicated as essential for parasite survival. Genetic and pharmacological evidence indicates that simultaneous deletion or inhibition of both LmxCLK1 and LmxCLK2 is lethal to the parasite. Despite their therapeutic potential, robust tools for validating target engagement of small-molecule inhibitors in *Leishmania* are lacking. In-cell target engagement assays have become essential for confirming compound binding to putative targets within living cells. These assays not only demonstrate cellular permeability but also provide direct evidence of target engagement. Here, we report the development of a bioluminescence resonance energy transfer (BRET)-based target engagement assay in live cells to identify and validate cell-permeable, ATP-competitive inhibitors of LmxCLK1.

Results and Discussion

Using our newly developed BRET assay, we screened a library of human kinase inhibitors and identified WZ8040, a third-generation human EGFR inhibitor, as a potent LmxCLK1 ligand. WZ8040 demonstrated robust target engagement in both promastigotes and macrophage-internalized amastigotes, displaying an EC_{50} of 2.1 µM for amastigote killing and minimal toxicity toward host macrophages (Figure 1). Biochemical assays confirmed that WZ8040 covalently binds and inhibits LmxCLK1, with mass spectrometry identifying Cys172 as the primary site of modification. Interestingly, genetic validation using overexpression and knockout parasite lines supports LmxCLK1 as the primary target of WZ8040. However, the retained activity of WZ8040 in mutant lines carrying the Cys172Ala substitution suggests that covalent binding at this residue is not essential for compound efficacy.

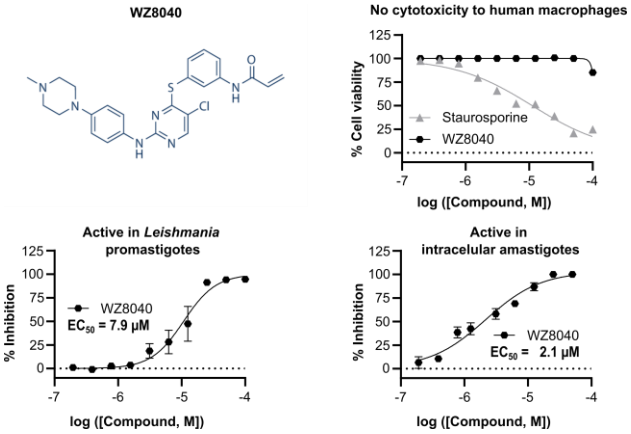


Figure 1: Phenotypic validation of WZ8040 activity in *Leishmania mexicana*.

Conclusions

Our findings highlight the utility of BRET-based assays for compound identification and target validation in kinetoplastid parasites, and underscore the potential of CLK1 as a druggable kinase in *Leishmania*. This integrated approach provides a framework for accelerating the discovery of novel anti-leishmanial agents through target engagement-guided strategies.

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Structural insights into ligand-binding at the ZIKV NS2B-NS3 protease active site

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Key words: Flavivirus, ZIKV, NS2B-NS3 protease, high-throughput screening, enzymatic assay, DENV

Introduction

Zika virus (ZIKV) is a flavivirus associated with neurological complications such as congenital Zika syndrome in newborns of infected pregnant women and Guillain-Barré syndrome in adults. Despite its significant public health impact, no specific antivirals or widely available vaccines have been approved to date. The viral NS2B-NS3 protease is essential for processing the viral polyprotein into individual mature proteins and represents an important target for antiviral development[1]. Structural studies of this protease in complex with small-molecule inhibitors can provide critical insights into drug design strategies against ZIKV and related flaviviruses.

Results and Discussion

The cZipro expression vector containing a bicistronic construct of ZIKV protease, in which the proteins NS2B and NS3 are produced separately and associate post-translationally, was used to transform *E. coli* BL21 for the expression of this enzyme. NS2B-NS3 was purified through affinity and size-exclusion chromatography. A high-throughput screening (HTS) of ~6,000 commercially available small-molecules was performed using a fluorogenic substrate (Bz-nKRR-AMC) at 10 µM (Figure 1). Nine compounds inhibited enzymatic activity by ≥80%, and six exhibited concentration-dependent inhibition with IC_{50} values ranging from 2.3 to 60 µM.

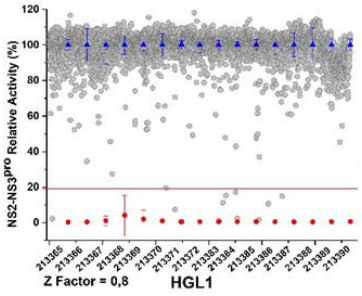


Figure 1: High-throughput screening of 5,632 compounds. Scatter plots show protease activity (%) at 10 µM. Light gray dots represent tested compounds; blue triangles and red dots indicate negative (DMSO) and positive (Aprotinin) controls, respectively. Error bars show standard deviation.

To investigate the structural basis of inhibitor-binding to ZIKV NS2B-NS3 protease, pre-formed protease crystals were soaked with the six active compounds. X-ray diffraction data were collected at the Brazilian Synchrotron Light Laboratory (LNLS), and structural refinement is in progress. Preliminary electron density maps revealed positive signals near the catalytic triad (His51, Asp75, Ser135), consistent with ligand binding at the active site (Figure 2)

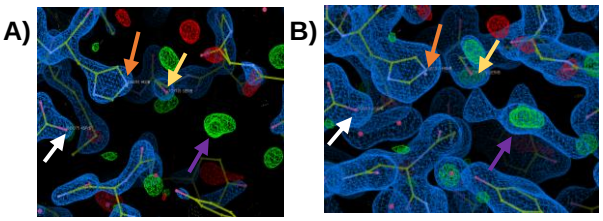


Figure 2: Electron density map of the complete catalytic triad in ZIKV NS2B-NS3 protease. Panels A and B show the same site with different zoom levels. The catalytic residues His51, Ser135, and Asp75 are indicated by orange, yellow, and white arrows, respectively. The purple arrow indicates the green density near the triad, enclosed by blue 2Fo–Fc contours suggesting the presence of a possible bound ligand.

Conclusions

The high-throughput screening of a large compound library identified promising inhibitors of the ZIKV NS2B-NS3 protease, which were further investigated through crystallographic experiments. The resulting electron density observed in the protease active site suggests specific interactions with these candidate molecules, providing important structural insights. This integrated approach provides valuable information to the understanding of ZIKV protease inhibition and supports ongoing efforts in antiviral research.

Acknowledgements

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2-Phenyl-2*H*-1,2,3-Triazole-Oxime: oral anti-hyperglycemic effect and *in vitro* DMPK properties

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Key words: Diabetes, glycosidases, triazole, anti-hyperglycemic agents.

Introduction

In our previous study, we reported the synthesis and preliminary *in vitro* evaluation of a series of 60 phenyl-1,2,3-triazole derivatives, as glycosidase inhibitors[1]. Building upon those initial findings, the present study aims to further investigate the antidiabetic potential of one of the most promising compounds from our previous work.

Results and Discussion

Here we report the *in vivo* inhibition of digestive glucosidases and *in vitro* DMPK properties of 2-phenyl-2*H*-1,2,3-triazole-oxime.

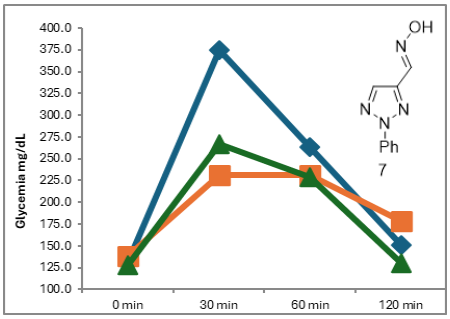


Figure 1: Effect of 2-Phenyl-2*H*-1,2,3-Triazole-Oxime (Compound 7, green) and acarbose (orange) on the hyperglycemic effect induced by maltose (blue).

A new batch of compound 7 was synthetized and the purity analysis was checked by UPLC-UV (254 nm)-MS, as 99% pure. A new IC_{50} curve was performed with porcine pancreatic amylase and similar results with the previous work were obtained (203 µM). Compound 7, as well as acarbose (positive control), were administered via oral gavage at a concentration of 10 mg/kg together with 4 g/kg of a maltose or starch solution. Mice treated with compound 7 and acarbose demonstrated a significant reduction in the postprandial blood glucose peak, when compared with control animals, which received only the maltose/starch solution (Fig.1).

In vitro DMPK properties of compound 7 were also evaluated (Table 1). The molecule showed around 200 µM of aqueous solubility, almost double off 100 µM cut-off. The compound presents significant cellular permeability in MDCK cell assay, higher than the permeable control bupropion. Considering that our drug has its effect on the gut lumen, it's preferable that it presents poor cell permeability. This effect could be mitigated by a fast microsomal hepatic metabolism, as we observed in our experiment. Considering the points raised by *in vitro* DMPK experiments, new derivatives can be proposed by adding polar groups to the molecule.

Table 1. MDCK Permeability, Microsomal clearance and Solubility of compound 7 and control compounds.

Solubility (µM)	
7	160 - 201
Diethylstilbestrol	4.84 - 5.57
Trazadone	203 - 233
Papp (nm/s)	
7	311.5 - 375.5
Atenolol	13.83 - 13.86
Bupropion	250.4 - 257.9
Clearence Values (mL/min/g mouse liver)	
7	16.0 - 16.7
Warfain	< 0.5
Verapamil	16.8 - 18.1

Conclusions

Our results demonstrate the antihyperglycemic activity and *In vitro* DMPK properties of 2-phenyl-2H-1,2,3-triazole-oxime. We hope to identify a glycosidase inhibitor molecular scaffold that can be used to control postprandial glycemia, and that can be easily synthesized. It is expected that these compounds may aid in the development of a new drug for the treatment of diabetes and metabolic syndrome.

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Identification of candidate *Trypanosoma cruzi* cruzain inhibitors by molecular docking-based virtual screening

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Key words: Docking studies; Anti-Trypanosoma cruzi compounds; Chagas disease; Virtual screening

Introduction and Objectives

Trypanosoma cruzi is a protozoan parasite responsible for Chagas disease (CD), also known as American trypanosomiasis, common in South America, Central America, and Mexico; there are also cases recorded in countries such as USA, Spain, and Japan, affecting about 8 million people globally.[1] CD has an acute febrile phase and a chronic phase, characterized by myocarditis, megaesophagus, among other symptoms.

Since there are only two drugs currently available for the treatment of CD, nifurtimox and benznidazole, and they are only effective in the disease acute phase, the search for new ones is urgently needed. There are many enzymes identified as essential to the parasite. Cruzipain, the most abundant cysteine protease of *T. cruzi*, is involved in metacyclogenesis, host cell invasion, and modulation of the host cell response.

We are developing an in-house virtual chemical library composed by compounds synthetized and natural products isolated in the theses of the Chemistry Graduate Program of UFRRJ since its foundation in 1966. The objective of the present study was the identification in this library of candidate inhibitors for cruzain by Structure-Based Virtual Screening (SBVS).

100 molecules of our chemical library had their protonation states defined with the pKa plugin of the Marvin program (Chemaxon) and were energy minimized with PM6 method of Spartan'24 (Wavefunction). The optimized structures were used in a molecular docking study with GOLD 2023.2.0 (CCDC) in a crystallographic structure of cruzain (PDB code 1ME4, 1.20 Å resolution)[2], bound to a hydroxymethyl ketone Inhibitor.

Results and Discussion

ChemPLP is the GOLD scoring function recommended for SBVS studies.[2] Before using it, we initially made a redocking analysis to define the pose prediction performance of this function with the 1ME4 structure. Since the cocrystallized ligand is covalently bound to the catalytic Cys25 residue, the GOLD covalent docking approach was used. Excellent results for the redocking were obtained, with a RMSD of 0.663 Å for the best pose (fitness score = 70.9). Based on this result, the docking study with the virtual library was performed using ChemPLP, from which 5 compounds were selected (Table 1). They exhibited fitness scores greater than 63 points, which corresponds to 90% of the reference score value. In fact, PPGQ202230220 (Fig. 1) presented a score value higher than the reference. Interestingly, this compound was previously identified as having excellent trypanocide activity (IC_{50} = 0,8 µM, 37°C).[3]

Table 1. Score values (ChemPLP) of selected compounds

Compounds	Fitness Value
PPGQ202230220	81.54
PPGQ202230208	69.20
PPGQ201810101	68.39
PPGQ201810110	67.51
PPGQ202230202	66.02

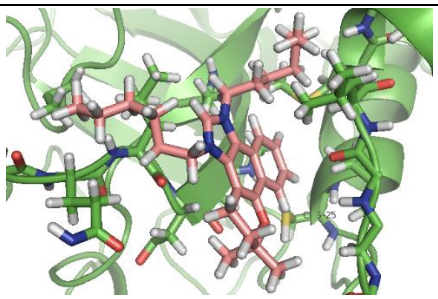
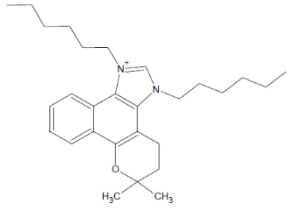


Figure 1: Predicted interactions of compound PPGQ20223020 (pink) with *T. cruzi* cruzain (green).



Conclusions

The molecular docking approach with *T. cruzi* cruzain applied to our in-house virtual chemical library allowed the identification of candidate ligands for this enzyme. The best ranked compound, PPGQ202230220, exhibited a higher fitness score than the reference co-crystallized ligand. PPGQ202230220 was previously identified as a trypanocide compound,[3] which indicates the effectiveness of the method.

Acknowledgements

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Photochemical Synthesis and Evaluation of the Interaction Between Quinoxaline Derivatives and DNA: In Vitro and In Silico Approaches

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Key words: quinoxalines, molecular docking, synthesis, DNA, antitumor

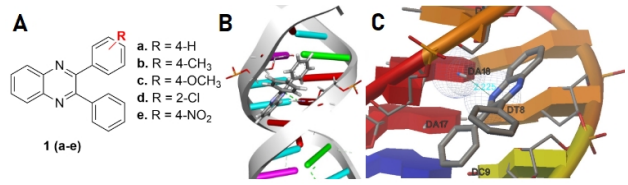
Introduction

Quinoxalines are benzopyrazines with fluorescent properties and potential pharmaceutical applications. Although their antitumor activity is known, the structure–activity relationship (SAR) and interaction with biological targets require detailed analysis to predict optimal binding modes.[1,2] In this study, molecular docking was used to evaluate the interaction of 2,3-diphenylquinoxaline derivatives with DNA. Based on promising results, a synthetic route was developed, and their interaction with ct-DNA was further examined through in vitro assays.

Results and Discussion

The *in silico* analyses were performed using AutoDock4 software,[3] with a series of 2,3-diphenylquinoxaline derivatives (Figure 1A) employed as ligands in complexes with DNA (PDB:5T4W). The results indicated a preferentially surface-binding mode (Figure 1B), and revealed that quinoxaline **1a** interacts with DNA through a hydrogen bond with adenine (DA18)(Figure 1C).

Figure 1: Compounds **1a-e** (A) and molecular docking between DNA and **1a** (B and C).



Analysis shows that the compounds **1a-e** exhibited affinity for both the minor and major grooves of the DNA with negative binding energy values, suggesting spontaneous and favorable interactions (Table 1).

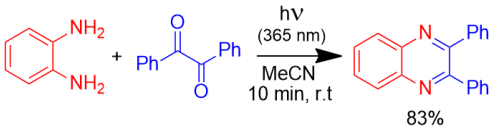
Table 1: Interaction data obtained by molecular docking.

Compound	Energy (kcal/mol)	Interaction with DNA
1a	-8.39	minor groove
1b	-8.76	major groove
1c	-8.50	minor groove
1d	-7.81	major groove
1e	-8.02	minor groove
DAPI	-7.94	minor groove

Also, permeability of **1a** through biological membranes were simulated by PerMM analysis yielding a minimum energy value of −6.5 kcal/mol.[4]

The compound **1a** was synthesized via photochemical route (Figure 2) as an alternative to traditional methods that use high temperatures and long reaction times, resulting in product formation within a short reaction period. Although the best yield was achieved in acetonitrile, high yields (60–80%) were also obtained in other solvents such as EtOH, DCM, and DMSO. Synthesis of **1b-e** compounds are still under investigation.

Figure 2: Photochemical synthesis.



83%

The interaction between ct-DNA and 2,3-diphenylquinoxaline **1a** was studied using UV-Vis and fluorescence spectroscopy. A constant ligand concentration was maintained, while the amount of ct-DNA was gradually increased to generate environments with low, moderate, and high levels of DNA saturation.[5] Spectroscopic analysis revealed a progressive hyperchromic effect as the ct-DNA concentration increased, suggesting changes in the interaction between the ligand and DNA. A decrease in fluorescence intensity was observed, except for the 1:1 molar ratio, suggesting that, at this proportion, the formed complex adopts a conformation more favorable to fluorescence emission. No intercalation occurs since quinoxaline emission at 418 nm is not affected by DNA which makes these compounds promising for imaging.

Conclusions

Molecular docking and spectroscopic findings indicate that compound **1a** exhibits effective binding to DNA grooves and shows potential for bioimaging applications. Future work should focus on optimizing photochemical synthesis and assessing the biological activity of the remaining derivatives.

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Targeting Schistosoma mansoni TGR: Structure-Guided Synthesis and Optimization of Novel Fragment-Based Inhibitors.

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Key words: Schistosomiasis, Schistosoma mansoni, SmTGR, FBDD, Linking, Growing.

Introduction

Schistosomiasis is a neglected tropical disease and affects over 250 million people worldwide, especially children.[1] It is caused by trematodes of the Schistosoma genus, with *S. mansoni* being the only species involved in Brazil. The current treatment relies exclusively on Praziquantel, a drug in use for over 40 years, which presents limitations such as lack of pediatric formulation, reduced efficacy in immature stages of the parasite's life cycle, and, most concerning, the potential emergence of drug-resistant parasites.[2] Given this scenario, the search for new molecular targets and bioactive compounds is extremely important. One molecular target is the *S. mansoni* Thioredoxin-Glutathione Reductase (*Sm*TGR), an essential enzyme that plays a key role in maintaining redox balance and protecting against oxidative stress. Inhibiting this enzyme impairs the parasite's ability to survive within the host.[3] In this work, fragment-based drug discovery (FBDD) strategies were employed, using both fragment linking and fragment growing approaches for the rational design of *Sm*TGR inhibitors.

Results and Discussion

In a previous study, 35 fragments interacting with *Sm*TGR were identified by X-ray crystallography, from which 5 fragments were selected for optimization. Three of them were chosen to be optimized via fragment linking approaches due to their proximity within enzyme's site. 50 molecules were designed through the combination of the 3 fragments with different linkers and 8 of them were chosen for the synthesis, as shown in **Figure 1**.

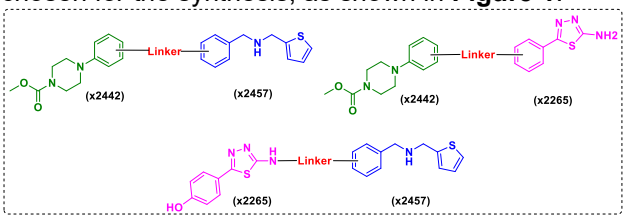


Figure 1: Fragments chosen to be linked.

Two of the fragments were chosen to be optimized using fragment growing approaches with the use of the program AutoGrow4. Five molecules were generated and chosen to be synthesized **(Figure 2)**.

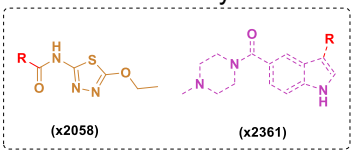


Figure 2: Fragments chosen for growing.

The synthesis of the molecules from the linking approach started with the preparation of the fragments x2442, x2457, x2265, and their respective derivatives via reductive amination, Buchwald-Hartig cross coupling reaction and thiadiazole synthesis, resulting in a total of 19 compounds with good yields. Once the fragments were assembled, they were linked either directly or via linkers such as 1,2,3-triazoles, aliphatic chains and non-aromatic heterocycles, generating 2 final products. Alternatively, the synthesis of molecules from the growing approach started with the preparation of the fragments x2058 and x2361, followed by a reaction with different isocyanates to obtain 1 final product containing a urea group and a brominated analogue.

Conclusions

Until now, a total of 41 compounds were synthesized, including synthetic intermediates among which are 38 novel molecules and 3 compounds previously described in the literature. So far 23 compounds, including only 3 final products have been tested for their ability to inhibit *Sm*TGR in enzymatic assays but none was effective. As other final products are obtained, a better understanding of this enzyme will allow the rational design of new molecules targeting *Sm*TGR, and providing a basis for the development of novel therapeutic strategies.

Acknowledgements

The authors would like to thank CAPES, CAPES PrInt, CNPq, FAPERJ and LABRMN.

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Molecular Dynamics of Phytocompounds from *Schinus terebinthifolius* as Potential Inhibitors of Acetylcholinesterase Related to Alzheimer's Disease

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Keywords: Brazilian peppertree, triterpenoids, molecular docking, Alzheimer disease, biflavonoids

Introduction

Schinus terebinthifolius Raddi is a species with medicinal properties. Phytochemical studies have characterized by HPLC-ESI-MSn of the active extract inhibitory activity against the AChE enzyme related to Alzheimer's disease. The biflavonoids I6, II8-apigenin (1), and naringenin-apigenin (2), as well as triterpenoids 3,7-dioxo-tirucalla-8,24Z-dien-27-oic acid (3), 3,21-dihydroxy-tirucalla-24Z-en-27-oic acid (4), 3β-hydroxy-tirucalla-7,24Z-dien-27-oic acid (Z-schinol) (5)[1,2]. This study aims to investigate the potential binding modes of the five phytocompounds (1-5) from the hydroethanolic extract of *S. terebinthifolius* to the human acetylcholinesterase (hAChE) enzyme by molecular docking (FRED program) and molecular dynamics simulations (GROMAC).[3]

Results and Discussion

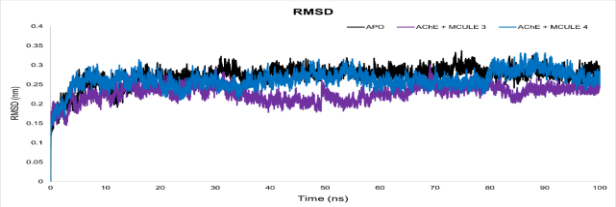
The molecular docking indicated that the terpenoids were the best ranked (Table 1). The main intermolecular interactions for each compound are shown in the Table 1.

Table 1: The Ebind, ChemGauss score between the (1 and 2) and (3, 4 and 5) in the active site and the ligand efficiency (LE) of the compounds were estimated.

Compound	Chemgauss score Ebind	Ligand efficiency LE	Active site residues interacting with ligands
1	-5.97	-0.14	Asp74, Gly122, Gly121
2	-5.69	-0.14	Asp74, Tyr75, Ser293
3	-7.01	-0.20	Tyr72, Ser203
4	-7.80	-0.23	Ser125, Phe295
5	-7.75	-0.23	His287

Based on the ligand efficiency values and the identification of important intermolecular interactions, (3) and (4) compounds were subjected to molecular dynamics simulations. The RMSD plot highlights (Figure 1) the conformational changes of the structures during molecular dynamics (t = 100ns), particularly in the stability phase of the system. However, the RMSD values are similar to those of previous studies, which obtained an average RMSD of 0.25 ± 0.03 nm for the APO form (only protein). However, the HOLO form in this current study presented similar behavior, with a decrease in the average RMSD value, suggesting a greater balance in the current simulations. In line with this, the results of the present study indicate that the binding of compound (3) promotes a more stable interaction, with less conformational fluctuation of AChE (RMSD = 0.23 ± 0.01 nm). On the other hand, compound (4) (RMSD = 0.25 ± 0.01 nm) seems to cause greater structural flexibility when compared to compound (3), being more stable than the native form of the protein. Binding mode analysis shows that both ligands retain key interactions with AChE binding site[4].

Figure 1. RMSD (backbone) of the APO structures (AChE) and the hAChE-(3) and hAChE-(4) complexes over 100 ns of molecular dynamics.



Conclusions

Triterpenoid compounds from a species from the Anacardiaceae family, have great biological potential. The results obtained indicate that these compounds may act as potential AChE inhibitors, and the combination of technologies is a viable alternative for the development of drugs for Alzheimer's disease.

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Development of New Triazole-Based Lonidamine Derivatives as Potential Agents Against Cancer

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Key words: Cancer, Indazole, Lonidamine, Synthesis, Triazole.

Introduction

Prostate cancer is the second most common cause of solid tumors among men, it´s characterized by anormal growth of cells that make up the prostate, which is a gland in the male reproductive system responsible for producing the fluid that nourishes sperm in semen. Lonidamine, a drug that is composed of an indazole ring, is an anti-cancer agent that has been used in combination chemotherapy and is currently undergoing clinical trials for the treatment of tumours that are refractory to conventional chemotherapy. The 1,2,3-Triazole group is found in several structures that have good biological properties because it´s a bioisostere of an amide. Previous results from the group showed that a molecule containing the triazole combined with an indazole nucleus had better biological activities against prostate cancer. So, the objective of this work is to synthesize new molecules derived from lonidamine, which have in their structures an indazole nucleus and a triazole group.

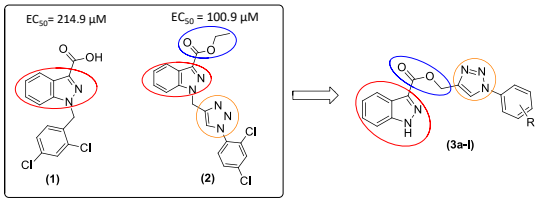


Figure 1. Planning of the final molecules.

Results and Discussion

The synthetic route is divided into three parts and begins with commercial anilines through a diazotization reaction followed by an addition-elimination reaction to form aromatic azides **(5a-l)**, obtaining yields ranging from 72% to 89%. Next, starting from indazole-3-carboxylic acid in a medium containing thionyl chloride, an acid chloride was formed in situ, which underwent a substitution reaction with propargyl alcohol to form an alkyne containing an indazole nucleus **(7)**, obtaining 82% yield.

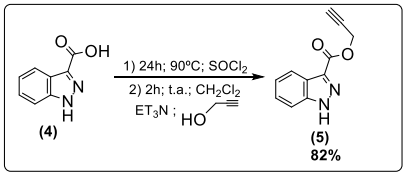


Figure 2. Second step of the synthetic route.

Then, a Huisgen 1,3-dipolar reaction catalyzed by copper was performed between this alkyne and the aromatic azides to form the final compounds **(3a-l)**, obtaining yields between 8% and 65%.

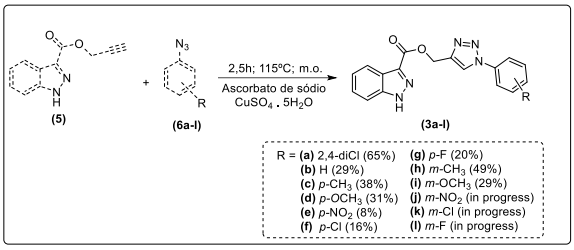


Figure 3. Third step of the synthetic route.

An example of their characterization by ^{1}H NMR is shown below.

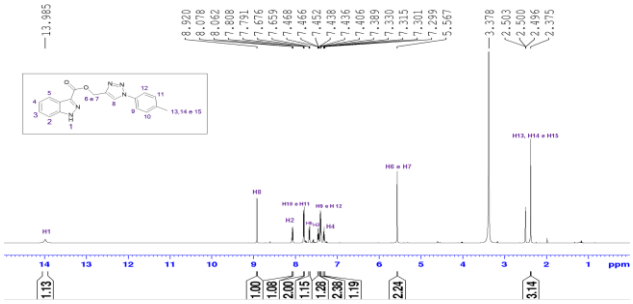


Figure 4. Characterization of the final compound with *p*-CH_3 substituent.

Conclusions

It was possible to synthesize 12 aromatic azides, the indazole alkyne and 9 final compounds with para and meta substitution, which were characterized by infrared and NMR ^{1}H and ^{13}C. All the final compounds were sent for pharmacological tests in order to evaluate their antitumor profile.

Acknowledgements

The authors would like to thank CNPq, Faperj and LABRMN.

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Machine learning-based QSAR modeling for benzamide derivatives with anti-*staphylococcal* activity

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Key words: Antimicrobial Resistance, Cell division FtsZ, Quantitative structure-activity relationship, Staphylococcus aureus

Introduction

The growing antimicrobial resistance (AMR) poses a global public health threat, requiring the identification of novel bacterial targets[1]. The filamenting temperature-sensitive protein Z (FtsZ) emerges as a promising target due to its essential role in the divisome complex and high conservation among bacterial species. *Staphylococcus aureus*, a major pathogen responsible for severe infections, has shown susceptibility to FtsZ inhibition. Several benzamide derivatives have shown significant antimicrobial activity against this pathogen and FtsZ protein inhibition[2]. However, the structure–activity relationships (SAR) within this class remain poorly understood, limiting rational drug design efforts. Therefore, to build predictive models and identify key molecular features driving anti-*staphylococcal* activity, we investigated the performance of multiple machine learning algorithms for QSAR modeling of benzamide-based inhibitors.

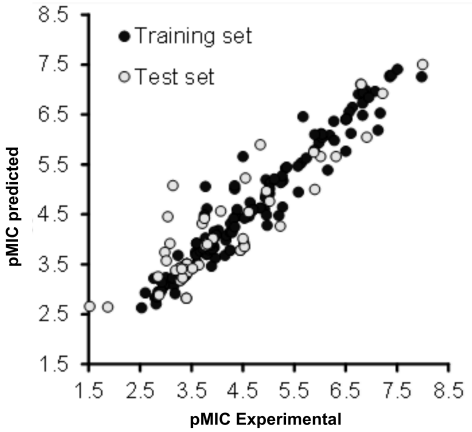
Results and Discussion

A dataset of 226 benzamide compounds with their respective activities (pMIC = 1.5 – 8.0) was obtained from the ChEMBL database (IDs 1157799, 3098047, 4002610, 4330069, and 4387753). Twenty-one physicochemical and topological descriptors were calculated using Dragon 4.5 software, previously selected in a multiple linear regression study[3]. Four machine learning algorithms were evaluated: Partial Least Squares (PLS), Support Vector Regression (SVR), Random Forest (RF), and XGBoost. Baseline models were generated from a data split (80% training/20% test, random_state=101) using autoscaled descriptors. All models met minimum validation criteria: k-fold cross-validation (k=5), external test set, and Y-scrambling. After hyperparameter optimization (Grid Search), all evaluated models successfully captured structure–activity relationships, showing robust statistical performance (Table 1). The SVR model (Figure 1) showed the best balance between robustness and predictive power, followed by PLS. RF and XGBoost models exhibited signs of overfitting, despite acceptable predictive power, possibly due to the limited dataset size or insufficient dimensionality. Variable importance analysis for PLS (VIP), SVR (Permutation Importance), and RF/XGBoost (SHAP) revealed significant convergence across methods. Results highlighted the key influence of lipophilicity (ALOGP), the number of benzene rings (nBnz), and molecular mass (BEHm3) on biological activity. The application of multiple ML algorithms combined with descriptor interpretation strategies and applicability domain (AD) calculation may establish a robust tool for virtual screening and the design of new compounds activity against *S. aureus*.

Table 1. Statistical values of the best ML models.

Algorithms	*R^2*	*Q^2*	*R^2test*	*Y-scramble*
PLS (5 PCs)	0.81	0.76	0.78	-0.32
SVR	**0.94**	**0.82**	**0.81**	**-0.42**
RF	0.95	0.74	0.73	-0.22
XGBoost	0.97	0.77	0.74	-0.44

Figure 1. Predicted vs. experimental pMIC of the optimized SVR model.



Conclusions

The developed models demonstrated good predictive performance and revealed key molecular features for anti-*staphylococcal* activity, offering valuable insights for the rational synthesis of novel benzamide analogues.

Acknowledgements

The authors acknowledge PIBIC/CNPq for financial support.

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Artificial Intelligence-Driven Design and Synthesis of α-Chloro-Keto-Pyrrole Covalent Inhibitors for SARS-CoV-2 Main Protease

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Key words: Mpro Inibitor, Phenylpyrrole, SARS-CoV-2, Covalent Inhibition.

Introduction

The COVID-19 global pandemic was one of the most terrifying times in the recent history, 7.1 M deaths until January of 2025[1]. Therefore, we must keep searching for antiviral compounds that could be used as an alternative treatment. In 2023, through an AI-driven approach, Saramago et al. identified the pyrrole compound (**1**) as a covalent inhibitor of the SARS-CoV-2 main protease (Mpro) that is active in Calu-3 cells infected by SARS-CoV-2. From these AI-based results, we have generated analogs of (**1**) to evaluate the influence of substituents in the phenyl ring.

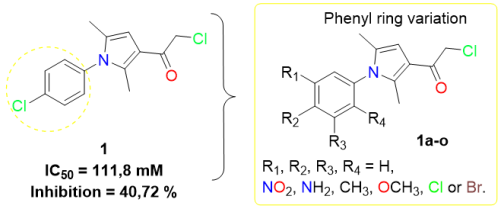
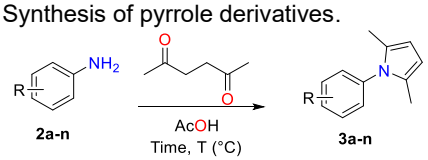


Figure 1: Mpro inhibitor (**1**) and the proposed compounds.

Results and Discussion

The pyrrole rings were accessed by a Paal Knorr reaction between 2,5 hexadienone and a series of anilines, in 8% to 89 % yield (Table 1).

Table 1. Synthesis of pyrrole derivatives.



R	Time	Temperature	Yield
4-Cl	1 h	rt	80%
4-Br	2 h 20 min	rt	72%
H	1 h	rt	89%
4-OCH_3	1 h	rt	85%
4-NO_2	2h 30 min	65 °C	60%
2,4-diCl	1h	65 °C	79%
3,4-diCl	2 h 30 min	rt	72%
2-OCF_3	1 h 30 min	rt	14%
3-OCF_3	1 h	rt	8%
3.5-diCl	48 h	65° C	76%
2-OCH_3,4-NO_2	ON	65 °C	66%
2-Cl,5-NO_2	72 h	65 °C	66%
3- NO_2,4-Cl	2 h	rt	82%
2-Cl,4-NO_2	24 h	65 °C	49%

The nature of the substituents exhibited a high effect on the time and temperature required to complete the reactions.

We also managed to obtain 5 of our targeted products (3 being isolated), by a Friedel Craft reaction between the pyrrole and chloro acetylchloride promoted by $AlCl_3$ in dry DCE at room temperature.

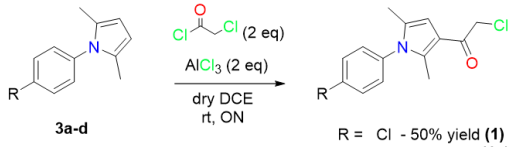


Figure 2: Synthesis of the α-Cl-keto-Pyrrole **1** and its two derivatives (**1a** and **1b**).

The low to moderate yields obtained are related to the degradation of the products and/or the formation of byproducts upon workup. This is especially true for the unsubstituted phenylpyrrole and the 4-methyl one, for which the formation of a more polar product has been observed. We're examining the structure of the latter to optimize the reaction conditions.

Conclusions

We were able to synthesize the Mpro inhibitor (**1**) in 2 steps and 45% overall yield; 15 pyrrole intermediates were obtained in good yields allowing the isolation of 2 targeted compounds (**1a-1b**). The synthesis of the others targeted products and the evaluation of the biological activities of compounds **1** and **1a-b** are currently in progress.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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Synthesis of SARS-CoV-2 Mpro Peptide-based Inhibitors Containing Substituted 1,2,3-triazoles

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Key words: peptidomimetic, antiviral, triazole, drug discovery, covalent inhibitors, bioisoster

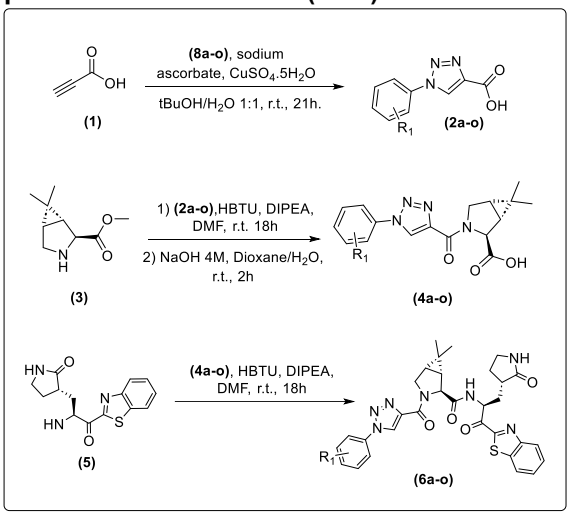
Introduction

SARS-CoV-2 is a β-coronavirus and the causative agent of the COVID-19 pandemic that started in 2020. Effective treatment and preventive measurements are currently available, like vaccines and the antiviral Paxlovid®, although with limited use[1]. Due to the imposed mutation pressure caused by these drugs and their respective limitations, research of novel drugs is still ongoing. The SARS-CoV-2 main protease (Mpro) is a cysteine protease responsible for the cleavage of the polyprotein during its replication cycle and a validated drug target[2]. Most known inhibitors are peptide-based or peptidomimetic, where enhanced properties can be achieved using bioisosters. In this context, 1,2,3-triazoles are a common surrogate for peptide bonds as they can exert similar ligand-protein interactions while also possessing enhanced physicochemical properties. Herein we present the synthesis of novel peptidomimetic inhibitors of SARS-CoV-2 Mpro containing substituted 1,2,3-triazole as a bioisoster surrogate for the amide bond.

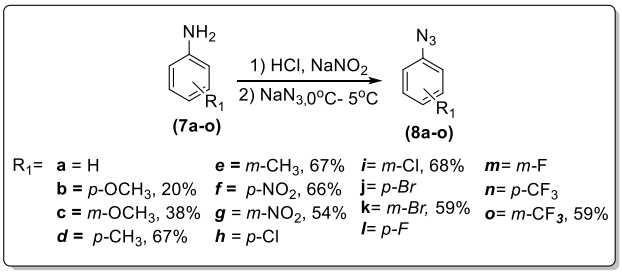
Results and Discussion

To obtain the substituted 1,2,3-triazoles **(2a-o)**, we performed a copper-catalyzed azide-alkyne cycloaddition (CuAAC) with propiolic acid **(1)**, followed by amide coupling reaction mediated by HBTU and DIPEA with the commercially available proline ester analogue **(3)** and hydrolysis**(Scheme 1)**. The resulting acids **(4a-o)** will then be coupled with the previously synthesized benzotiazolyl-ketone warhead **(5)** to yield the final compounds **(6a-o)**.
The corresponding azides **(8a-o)** for the synthesis of the 1,2,3-triazole moieties **(2a-o)** were obtained as shown in **Scheme 2**. Currently, compounds **(8a-o)** were obtained in moderate to good yields (20-68%), while compounds **(2d)** and **(4d)** were obtained with 43% and 83% yield, respectively. All compounds were characterized by NMR and IR. To obtain better insight on the possible binding mode, we are also performing molecular docking simulations with the target protein using GOLD through covalent docking protocols.

Scheme 1. Synthetic route for obtaining peptidomimetic inhibitors (6a-o).



Scheme 2. Reaction conditions for obtaining substituted azides



Conclusions

Synthesized compounds were obtained in moderate to good yields, while the remainder are currently being synthesized and expected to be tested *in vitro* shortly.

Acknowledgements

The authors gratefully acknowledge financial support from CAPES-PIPD, PIBIC, Fiocruz and UFRJ.

Allerton, C. M. N. *et al.* A Second-Generation Oral SARS-CoV-2 Main Protease Inhibitor Clinical Candidate for the Treatment of COVID-19. *J. Med. Chem.* (2024).

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Exploring of Lonidamine-Inspired Bis-1,2,3-Triazoles for their Antineoplastic Activity

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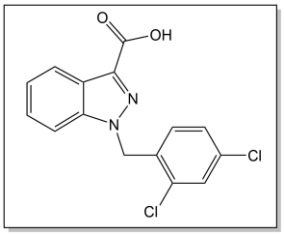
[1]Universidade Federal do Rio de Janeiro, Instituto de Química, Laboratório de Síntese Orgânica e Prospecção Biológica. Rio de Janeiro, Brasil.

Key words: Cancer, Antineoplastic agents, Nitrogen heterocycles, Lonidamine, Indazole ring, 1,2,3-Triazoles.

Introduction

Cancer is one of the four major chronic noncommunicable diseases and is the second leading cause of death worldwide, second only to cardiovascular disease. According to the National Cancer Institute[1] (INCA), prostate cancer is the second most common type of cancer among men. A study conducted by the Brazilian Medical Association (AMB) reports approximately 65,000 new cases and 15,000 deaths per year from prostate cancer in Brazil over the past 14 years. This type of cancer typically progresses silently, with few or no symptoms in the early stages, making early diagnosis and treatment challenging. In this context, lonidamine (*1-[(2,4-dichlorophenyl)methyl]-1H-indazole-3-carboxylic acid*) has been investigated for its potential in treating benign prostatic hyperplasia (BPH) and as a potentiator of conventional cancer therapies, including prostate cancer treatment. It works by targeting the energy metabolism of cancer cells[2,3], therefore, this work aims to synthesize novel compounds containing the 1,2,3-triazole nucleus, inspired by the structure of lonidamine, with the goal of achieving antineoplastic activity.

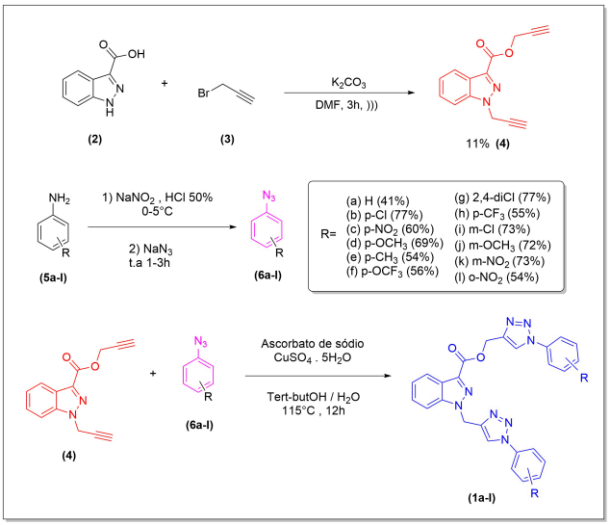
Figure 1: Lonidamine's structure.



Results and Discussion

The synthetic route of lonidamine-inspired bis-1,2,3-triazoles (**1a-l**) started with a nucleophilic substitution reaction, using 1H-Indazole-3-carboxylic acid (**2**), propargyl bromide (**3**) in alkaline conditions, with potassium carbonate, and DMF as solvent, forming the alkyne with an indazole ring (**4**). To purify this first product, a chromatographic column was used, resulting in an 11% yield. Therefore, this procedure will be reevaluated to increase yield. The second step consisted in a diazotization reaction, starting from 12 different commercial anilines (**5a-l**) using sodium nitrite, followed by an addition-elimination reaction with sodium azide to form aromatic azides (**6a-l**), which were obtained with yields between 41-77%. Lastly, the third step to be carried out is a copper-catalyzed 1,3-dipolar cycloaddition reaction in a copper(II) sulfate/sodium ascorbate system. Thus, this three-step approach is anticipated to generate a therapeutically relevant lonidamine analog, requiring subsequent biological validation.

Scheme 1: Synthetic route of lonidamine-inspired bis-1,2,3-triazoles.



Conclusions

The results obtained from the azide formation reaction were promising (yields between 41% and 77%). Although, the purification of the alkyne intermediate is currently under investigation to improve the overall yield, the target molecules were obtained and sent to biological assays, which, if successful, may contribute to the development of new therapeutic strategies for prostate cancer, aiming to improve treatment conditions for patients.

Acknowledgements

The authors would like to thank FAPERJ and LABRMN.

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Design and Synthesis of Novel Fluorescent Amino-Chalcones as Promising Anti-prion Agents and Probes for PrP^{Sc} Aggregates Detection

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Key words: prion protein, anti-prion, fluorescent probe, protein aggregation, amyloid, amino-chalcones.

Introduction

Prion diseases are fatal neurodegenerative disorders from the group of amyloidosis. They are characterized by the accumulation of pathogenic amyloid aggregates of the scrapie prion protein (PrP^{C}) in the brain. Our group previously identified novel anti-prion compounds, with the amino-chalcone **J8** as a standout candidate, despite its low solubility.[1] Given the fluorescent properties of chalcones bearing an amino substituent and its importance for anti-prion activity, **J8** was chosen as model for designing and evaluating novel structural *N*-aryl analogues with expected increased solubility (introduction of polar ionizable groups and higher Fsp^3) and efficacy as anti-prion and fluorescent probes for detecting PrP aggregates (**Fig. 1**).

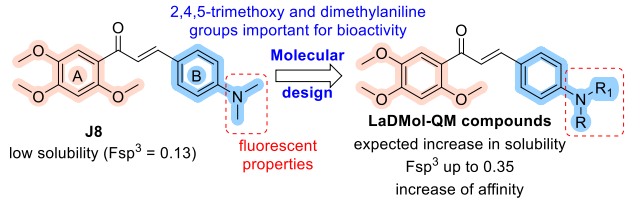


Fig. 1. Design of new *N*-arylated chalcones

Results and Discussion

The desired compounds were obtained by means of a new developed Buchwald-Hartwig (BH-CCR) *N*-arylation of chalcones in the last reactional step. This route simplified the task of obtaining many *N*-arylated derivatives (**1**) through a key intermediate (**6-Br**). A methodology optimization study was conducted, and the best and most universal condition is shown below (**Fig. 2**).

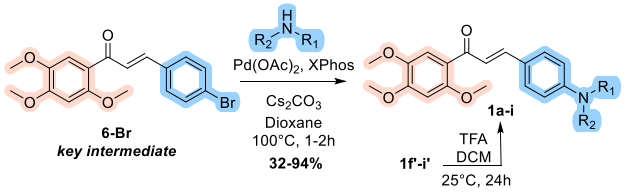


Fig. 2. General developed BH-CCR synthesis of new *N*-arylated chalcones

The effect of the compounds on PrP^{Res} accumulation in ScN2a-RML and ScN2a-22L cells was evaluated by dot blot assay.[2] All amino-chalcones (10 µM) inhibited at least 80% of PrP^{Res} accumulation in the RML strain (not shown). In 22L, only the diamino-chalcones (**1d-1g**) reduced PrP^{Res} levels, demonstrating the importance of the second ionizable nitrogen for the activity (**Fig. 3**).

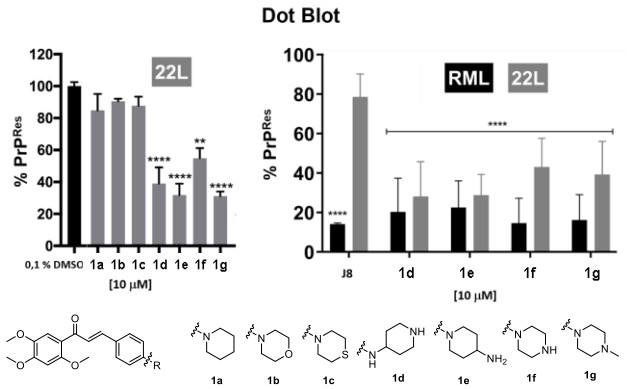


Fig. 3: Dot blot evaluation of PrP^{Res} content in ScN2a cells

Spectroscopic analyses showed polarity solvent-dependent characteristics (λ^{Em}_{max} = 516–565 nm) with large Stokes shifts (>100 nm) and negligible fluorescence emission in water. Turn-on mechanism, important for biological detections, was selectively triggered by aggregated PrP but not by their soluble monomeric forms in aqueous buffer solution, with at least a 6.2-fold fluorescence increase (**Fig. 4**).

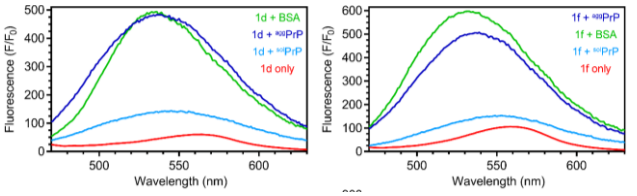


Fig. 4: Turn-on effect of **1d** and **1f** at 10 µM in aqueous buffer solution in the presence of 10 µM $^{agg}PrP^{23–231}$ (blue)

Conclusions

Our design and development of novel amino-chalcones as anti-prion and solvatochromic turn-on amyloid probes were highly successful, including an universal new BH-CCR protocol for modifying chalcones in the last step.

Acknowledgements

To CAPES code 001, FAPERJ and CNPq for scholarships and financial support.

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Accelerating Drug Screening: The Fully Automated Bioassays Facility at LNBio/CNPEM

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Key words: bioassays, drug screening, phenotypical assays, open-access facility.

Introduction

Biochemical and cell-based assays are crucial tools in the early stages of drug discovery, providing complementary information on compound efficacy, specificity, and safety. Through High-throughput (HT) and High-content (HC) screenings, bioassays efficiently enhance the identification and validation of new drug candidates by selecting compounds with both strong target affinity and efficient cellular activity. However, these assays usually require automated machines to improve reproducibility and decrease result variations, which makes the process more expensive and less accessible. Aiming to support academic researchers during their drug screening steps, the Laboratory of Bioassays (LBE) welcomes users to conduct biochemical and cell-based assays free of charge. The LBE team supports users during the entire workflow, from assay design to execution and data analysis, sharing more than access to advanced infrastructure, but also technical expertise and knowledge.

Results and Discussion

LBE receives users from all Latin America at no charge, in addition to providing financial support through CNPEM/MCTI resources. To access the LBE, users must submit a scientific proposal on the users' online platform "SAU", based on their interest in using the facility. The LBE staff will evaluate the technical aspects of the proposal and its compatibility with the equipment specifications. Once it meets the technical requirements, the proposal is considered feasible, and the user is contacted by the LBE team for an online meeting. All technical issues and expectations are aligned between both parties and, based on equipment availability, the user service is scheduled. During the user's visit, an LBE staff member is wholly dedicated to them, accompanying them throughout all experimental stages.

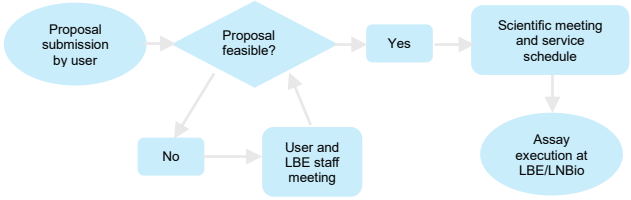


Figure 1: Steps to access LBE.

Users are invited to submit proposals for biochemical and/or cell assays aligned with the scientific goals of their projects. For biochemical assays, the LBE is equipped with ClarioStar multimode plate reader (BGM LabTech®), allowing measurements by absorbance, fluorescence, luminescence and anisotropy. An HCS Operetta fluorescent microscope (PerkinElmer®) is dedicated to phenotypical screening and morphological alteration analysis, using nuclear and other fluorescent markers across multiple excitation/emission wavelengths. Both assays are miniaturized to 384-well plates agreeing to high-throughput while saving samples and reagents by using a low volume for the system – typically around 30 µL. Reproducibility is ensured by Multridrop automated liquid dispenser (ThermoFisher®) able to dispense small volumes as low as 0.5 µL precisely. Regardless of the proposal type, users are guided and trained by the LBE staff to be autonomous throughout the workflow. The acquired data are processed and analyzed by using CDD.Vault platform, enabling fast and accurate automated data analysis.



Figure 2: Open-access LBE automated workflow.

Conclusions

Sharing the LBE's activities is key to expanding access for the scientific community in the field of drug discovery and medicinal chemistry to a modern and advanced infrastructure. With its free-access model and dedicated technical support, LBE/LNBio/CNPEM reinforces collaborations, promotes high-quality science by empowering researchers, and depolarizes cutting-edge science in Latin America.

Acknowledgements

We thank LNBio/CNPEM, from the Ministry of Science, Technology, and Innovation (MCTI), for the financial support.

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Design, Synthesis and Evaluation of the Antimalarial Activity of Chromenes Obtained by Hantzsch Multicomponent Reactions

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Key words: Malaria, Multicomponent Reaction, Hantzsch Reaction

Introduction

Malaria is a potentially serious infectious disease caused by protozoa of the genus Plasmodium, which is widely distributed throughout the world. In 2023, it's estimated that there were around 263 million cases and 597 thousand of deaths from Malaria in the World[1]. The need for new, less toxic antimalarial drugs is critical due to drug resistance.

This research focuses on chromenes, a class of molecules with promising antiprotozoal activity and low toxicity, obtained by the efficient and environmentally friendly one-pot multicomponent Hantzsch reaction. These chromenes were designed as antimalarial compounds.

Results and Discussion

In previous works from our group, dihydropyrimidinones (DHPMs) were obtained via the Biginelli reaction, which exhibited significant antiparasitic activity against the W2 strain, chloroquine-resistant, of *P. falciparum* (**figure 1**) and low toxicity[2]. Furthermore, the literature reports on antiparasitic activity in chromene derivatives[3]. Based on this, molecular design was carried out, modifying the dihydropyrimidinone cycle to the chromene cycle, as well as the cyclization of the side chain in order to obtain two distinct classes of compounds (**A** and **B**, **figure 1**), synthesized via a multicomponent reaction based on Hantzsch, as followed.

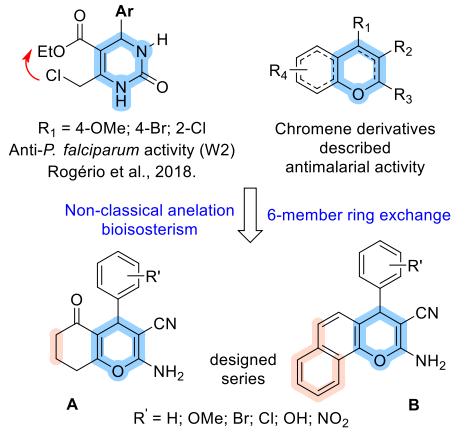


Figure 1: Molecular design of series **A** and **B**

The reactions were performed in a one-pot manner, with series **A** being constructed through the reaction of 1,3-cyclohexanedione (**1**) with the aldehydes of interest (**2**) and malononitrile (**3**) in ethanol at room temperature, using morpholine (**4**) as a catalyst for 24 h. For series **B** of compounds, α-naphthol (**5**), the aldehydes of interest (**2**), and malononitrile (**3**) were used as reagents, as well as morpholine (**4**) as a catalyst and ethanol as a solvent. For series **B**, an increase in temperature was used, with the reaction being carried out under reflux (**figure 2**). Series **A** (17 compounds) was obtained in yields from 12 to 86%, series **B** (3 compounds) in 61 – 81%. The purities for series **A** and **B** were analyzed by HPLC, being above 97% for all compounds.

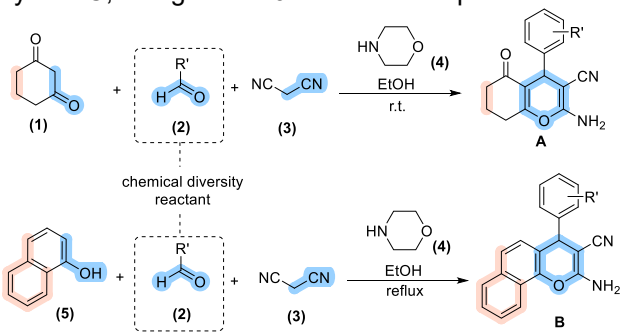


Figure 2: Synthesis of series **A** and **B**.

The compounds from both series are now under evaluation for their antimalarial activity

Conclusions

Through Hantzsch MCR, it was possible to obtain 20 different compounds whose structures were fully characterized, as well as their purity measured. The tests to evaluate potential biological activity as antimalarials are currently underway.

Acknowledgements

We thank CAPES code 001, FAPERJ, CNPq and FAPESP (CEPID grant 2013/07600-3, 2024/04805-8, 2024/04949-0) for scholarships and financial support.

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Enzymatic and phenotypic-guided evaluation of repurposed inhibitors against *Schistosoma mansoni* cathepsin D (*Sm*CD1)

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Key words: aspartyl protease, schistosomiasis

Introduction

Schistosomiasis is a neglected tropical disease caused by parasites of the genus *Schistosoma*. In 2022, more than 264 million people required preventive chemotherapy for this disease[1]. Currently, treatment relies solely on praziquantel (PZQ), a drug that is effective but has limited activity against the parasite's immature stages (larvae and juveniles)[2]. Several molecular targets have already been validated for the development of new antischistosomal drugs[3], including *Schistosoma mansoni* cathepsin D (*Sm*CD1), an aspartyl protease that plays an essential role in the parasite's nutrition and normal development[4]. In our group, the identification of *Sm*CD1 inhibitors has been carried out in recent years[5,6]. In this work, we present new *Sm*CD1 inhibitors, identified through a drug repositioning strategy. In partnership with Merck's Global Health Institute, we had access to a series of 76 acylguanidines, previously investigated as human cathepsin D inhibitors (*Hs*CD). The effects of these compounds on *Sm*CD1 activity and parasite motility were evaluated.

Results and Discussion

Enzymatic assays were carried out to evaluate the compounds as *Sm*CD1 inhibitors. A FRET peptide (Abz-AIAFFSRQ-EDDnp) served as the substrate, and the fluorescence of the cleaved product was monitored to assess enzyme activity. At 10 µM, 69 compounds reduced *Sm*CD1 activity by more than 50% (**Figure 1**).

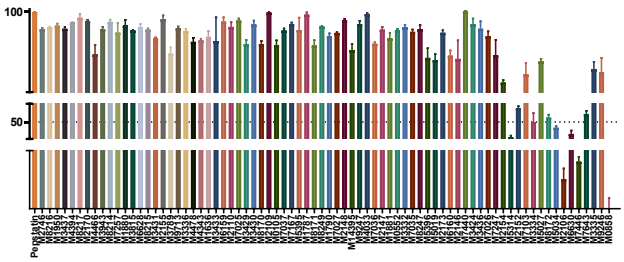


Figure 1: Inhibition of *Sm*CD1 activity by compounds at 10 µM. Data represents the percentage of inhibition relative to the negative control, expressed as the mean ± standard deviation of triplicates from two independent experiments (n = 2).

The effect of the compounds on parasites was evaluated by automated high-content microscopy (HCS)[7], and the images obtained were analyzed using the Cellprofiler software version 4.2.1[8]. A total of 31 compounds significantly reduced parasite motility at 10 µM, all of which were active against females and two also showed activity against males. Compounds M8247 and M2152 induced motility reduction comparable to PZQ (**Figure 2**).

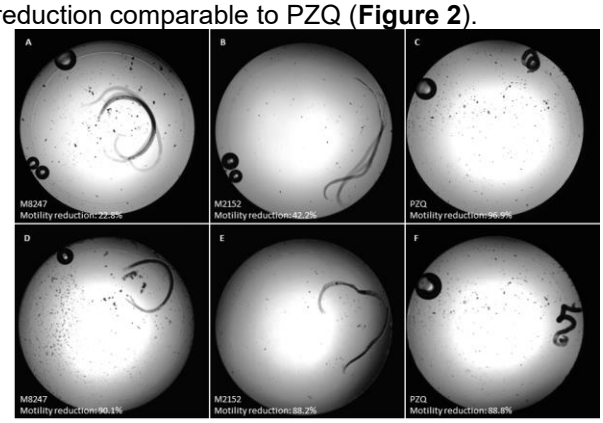


Figure 2: female *S. mansoni* immediately (time 0 h, A–C) and 72 hours (D–F) after treatment with M8247 (A, D), M2152 (B, E), and PZQ (C, F), and their respective percentages of motility reduction. Each panel consists of three consecutive images, acquired at 15-second intervals and overlaid to visualize the parasite movement.

Conclusions

Eleven compounds were considered hits, based on their ability to inhibit *Sm*CD1 and reduce *S. mansoni* motility. They are currently being evaluated for cytotoxicity, potency (IC_{50}) and the molecular basis of *Sm*CD1 inhibition. M8214 was more potent against *Sm*CD1 than it was for *Hs*CD.

Acknowledgements

CAPES, CNPq, FAPERJ, FIOCRUZ

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Multitarget drug design for Alzheimer's: insights from virtual screening.

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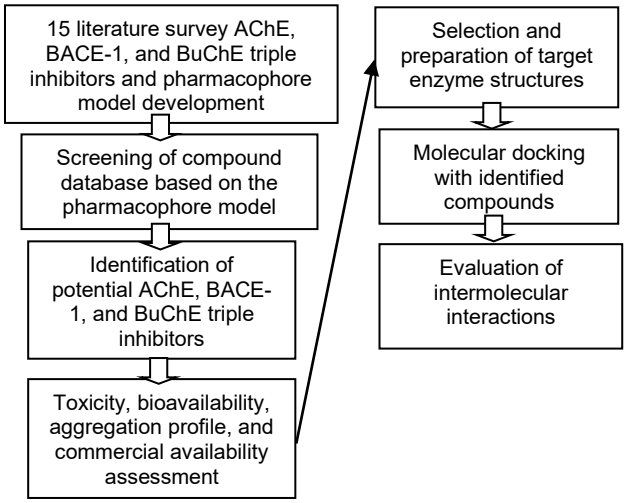
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Key words: Alzheimer's disease, virtual screening, multitarget inhibitors, drug design.

Introduction

Alzheimer's Disease (AD) is a progressive and irreversible condition of the central nervous system, responsible for about 70% of dementia cases and affecting approximately 55 million people worldwide—1.2 million in Brazil[1,2]. Current treatments, such as acetylcholinesterase (AChE) inhibitors, offer only symptomatic relief without altering disease progression, reinforcing the need for new therapeutic strategies. Given its multifactorial nature, AD drug development should target multiple enzymes like AChE, beta-secretase-1 (BACE-1), and butyrylcholinesterase (BuChE). AChE and BuChE are involved in acetylcholine regulation, while BACE-1 contributes to amyloid plaque formation[3,4]. In silico methods are valuable for virtual compound screening and identification of multi-target inhibitors[5]. This study aims to apply computational approaches to identify simultaneous inhibitors of AChE, BACE-1, and BuChE for more effective AD treatment.



Results and Discussion

In the search for AChE, BuChE, and BACE-1 inhibitors, 15 active compounds were used to generate 10 pharmacophore models via the Galahad module. The best-performing model (AUC = 0.95) guided a virtual screening of 214,446 Sigma-Aldrich molecules, identifying 309 hits with a QFIT value higher than the cut-off point (67.78). After predictive filtering, 21 compounds showed good oral bioavailability (with no more than one penalty to the Lipinski, Ghose, Veber, Egan, and Muegge models evaluated via the Swissadme server), no toxicity alerts (cardiotoxicity, hepatotoxicity, mutagenicity, and carcinogenicity via pkCSM server), and low aggregation risk (tanimoto index <0.7 via Aggregator advisor). Compounds Z13 and Z21 presented higher QFIT values and were selected for molecular docking. They showed interactions with key residues also targeted by known inhibitors of AChE, BACE-1, and BuChE, supporting their potential as multitarget inhibitors.

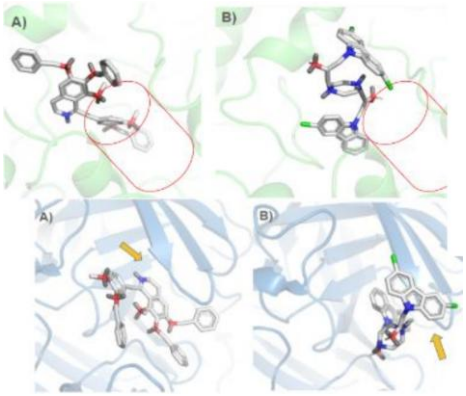


Figure 1: Interaction of compounds Z13 (A) and Z21 (B) with active sites of AChE (access tunnel to the site highlighted by the red cylinder) and BACE1 (flap loop indicated by yellow arrows).

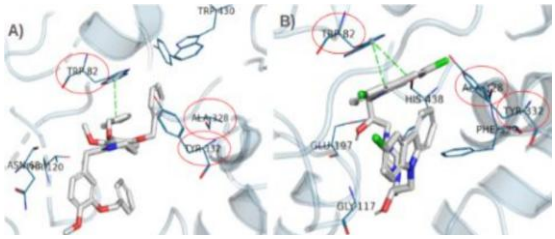


Figure 2: Interactions of compounds ZINC04576913 (A) and ZINC67997821 (B) with BuChE residues.

Conclusions

An integrated screening method based on ligand and receptor structures was developed and validated to identify potential ligands (e.g., Z13 and Z21) targeting three key pharmacological targets (AChE, BuChe, and BACE-1) in AD.

Acknowledgements

We thank FAPESB for financial support.

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Docking-Based Virtual Screening of Allosteric Ligands for Dengue Virus NS5: Rational Selection of Compounds with Favorable Oral Profile

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Key words: Dengue, NS5, Virtual Screening, Lipinski Rules.

Introduction

Dengue, an arboviral disease endemic to tropical and subtropical regions, is currently expanding its geographic range, mainly driven by global warming.[1] The disease has a major impact on public health, and no specific antiviral therapies are currently licensed. The NS5 protein, which plays a central role in viral RNA replication, stands out as a strategic target for pharmacological intervention due to its high conservation among serotypes and the absence of homologs in humans.[2] This work aims to identify compounds with potential interaction with the allosteric N-pocket of NS5 through virtual screening, using a unique, in-house chemical library developed by our group, containing presently 650 molecules from thesis and dissertations from the Post-Graduate Program in Chemistry at UFRRJ.

Results and Discussion

Chemical library compounds modeling was performed with Spartan'24 (Wavefunction), using the PM6 method. A molecular docking-based virtual screening was conducted with GOLD 2023.2.0 (CCDC), in the NS5 N-pocket allosteric site.[2] The ChemPLP scoring function was selected because of its performance in a redocking study with three NS5 crystallographic structures selected from PDB (5I3Q, 5JJS and 5K5M). In order to include the effect of conformational variations of the side chains of protein residues during the docking procedure, an ensemble docking approach was applied with these structures. The scores obtained were normalized by molecular mass, surface area, and √N (N = number of non-hydrogen atoms). This approach allowed comparisons of compounds of different sizes without bias related to molecular size. 10 molecules were selected for each normalization and a total of 20 molecules were selected for more optimizations. A group of 15 were fragments and were presented elsewhere.[3] The remaining 5 had MW greater than 300 Da and will be presented here. They showed favorable intermolecular interactions, including hydrogen bonds and cation-π interactions with key N-pocket residues. The corresponding interaction enthalpies were calculated from fully reoptimized structures (PM7 method, MOPAC2016) with inclusion of solvent effect by a dielectric continuum model, and they were quite favorable (Table 1). Ligands 1[4] and 2[5] (Fig. 1) fully meet the criteria established by Lipinski's rules (Fig. 2), calculated with SwissADME, suggesting them as candidates for oral administration.

	CHEMPLP Score	ΔH interaction (kcal/mol)
LIGAND 1	71,16	-86
LIGAND 2	82,30	-97
LIGAND 3	88,25	-130
LIGAND 4	91,15	-143
LIGAND 5	99,15	-135

Table 1: ChemPLP scores and predicted ΔH of selected ligands at the NS5 allosteric site.

Figure 1: Representation of ligand 2 interaction with the allosteric site of NS5. Carbons of the ligand in purple, carbons of key residues in orange. 2D structure of the ligand is show on top left.

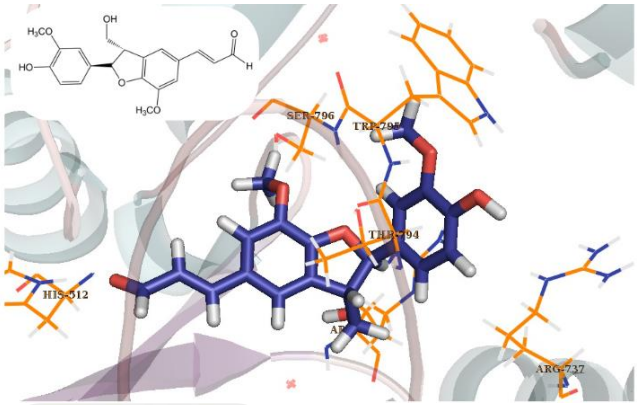




Figure 2: Radar plot of physicochemical properties for Ligand 2, generated with SwissADME.

Conclusions

Two selected compounds by virtual screening showed strong intermolecular interactions at the allosteric site of NS5 and favorable interaction enthalpy values, in addition to satisfying Lipinski's criteria. These results suggest that they are promising candidates for further development as orally available allosteric inhibitors of the dengue virus polymerase.

Acknowledgements

CAPES; CNPq; FAPERJ; PPGQ-UFRRJ.

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Structure-guided de Novo Design of *Tc*CYP51 Inhibitors.

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Keywords: Molecular docking, virtual screening, QSAR

Introduction

Chagas disease, caused by the *Trypanosoma cruzi* parasite, is a significant public health concern, especially in Latin America.[1] Although the current treatments are effective mainly in the acute phase, they have significant side effects and limited success in the chronic stages.[2] A promising strategy for new drug development targets a crucial enzyme in the parasite's ergosterol biosynthesis (*Tc*CYP51).[3] This work focuses on designing new compounds specifically aimed at inhibiting *Tc*CYP51 to develop more effective treatments against the *T. cruzi* parasite.

Results and Discussion

We designed 39 new compounds by assembling molecular fragments from known *Tc*CYP51 inhibitors using de novo generation via AutoGrow 4.0.2. The newly designed compounds were subjected to a virtual screening protocol,[4] which comprised molecular docking and QSAR modeling. Molecular docking was performed in the GOLD software. Validation through redocking employing the Chemscore function confirmed the reliability of our docking protocol. Subsequently, a QSAR model was applied to estimate biological activity (pEC_{50}), demonstrating good predictive performance (R^2 = 0.70, Q^2 = 0.58, RMSEE = 0.68).

Virtual screening identified two promising compounds with the pyrazolo-pyridine core, yielding predicted pEC_{50} values of 5.43 and 6.85. These scaffolds served as templates for subsequent structural modifications focused on nitrogen-containing heterocycles to enhance binding to the Fe-heme group, a key element in *Tc*CYP51 inhibition.

Among the optimized derivatives, three compounds (Figure 1) exhibited superior predicted interactions with the Fe-heme group compared to the original de novo-designed molecules. Docking analyses revealed that compounds 1 and 2 occupied binding poses near the Fe-heme group, while compound 3 additionally formed hydrogen bonds with the Tyr83 residue. The 3D-QSAR model predicted their pEC_{50} values higher than 8.0, indicating a higher potential for anti-*T. cruzi* agents.

Furthermore, the in silico ADMET profile indicated that although the first-generation compounds could exhibit undesirable effects, such as hepatotoxicity and broad inhibition of metabolism enzymes, compounds 1, 2, and 3 exhibited improved predicted pharmacokinetic and toxicity profiles, prompting the continuation of this work with these candidate compounds against *T. cruzi*.

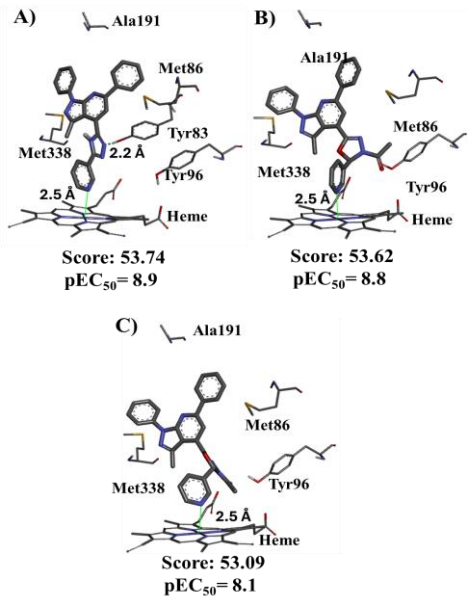


Figure 1: Docking poses and intermolecular interactions of the top 3 design *Tc*CYP51 inhibitors: 1 (A), 2 (B), and 3 (C).

Conclusions

Our results demonstrate the effectiveness of fragment-based design, combined with de novo approaches and virtual screening, in planning promising *Tc*CYP51 inhibitors. The newly designed compounds (1–3) showed favorable predicted activity and key molecular interactions with the target, supporting their potential as lead candidates for the development of new treatments against *T. cruzi.*

Acknowledgements

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Computational Screening of Compounds from the Northeastern Brazilian Semiarid Region as Potential Tyrosinase Inhibitors

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Key words: Tyrosinase; MITF; α-MSH Receptor; Inhibitors; Molecular Docking.

Introduction

Melanin is the primary pigment responsible for human skin color and plays a crucial role in protecting the skin from the harmful effects of ultraviolet radiation (UVR) and oxidative stress. However, the overproduction of melanin can lead to hyperpigmentation disorders such as melasma, freckles, solar lentigines, and post-inflammatory hyperpigmentation.[1, 2]

Tyrosinase is a metalloenzyme responsible for the pigmentation of skin, eyes, and hair in mammals due to its essential role in melanogenesis. Tyrosinase inhibition plays a central role in suppressing melanin production and is a key strategy in the treatment of pigmentation disorders.[4,5]

Results and Discussion

The crystal structure of tyrosinase retrieved from the Protein Data Bank (PDB ID: 3NQ1) was selected for molecular docking studies, along with an AlphaFold2-predicted structure of human tyrosinase available in UniProt (ID: P14679), for comparison and homology analysis.[6] Considering the two amino acids in the active site[7] that differ between the structures, a new tyrosinase structure was generated based on the bacterial structure. The amino acids Pro201 and Arg209 from the bacterial tyrosinase structure were replaced by serine and isoleucine, respectively, thus creating a new mutated tyrosinase structure.

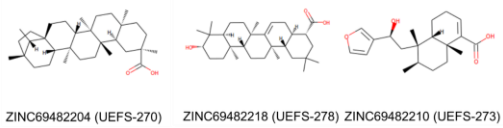
The computational molecular docking study evaluated the binding conformations of known tyrosinase inhibitors—kojic acid, hydroquinone, arbutin, and ascorbic acid—and subsequently with 503 three-dimensional structures of compounds from the Brazilian semiarid region, sourced from the natural molecules catalog of the State University of Feira de Santana (UEFS) within the ZINC 20 database, obtaining binding energy results shown in Table 1.

Table 1. Molecular Docking Results Between Tyrosinase/Positive Controls and Tyrosinase/Top-Scoring Molecules from the UEFS/ZINC Database

UEFS CODE	ZINC CODE	Binding Energy (kcal/mol)
Ascorbic Acid	-	-3,23
Kojic Acid	-	-3,67
Arbutin	-	-4,18
Hydroquinone	-	-3,46
UEFS-270	ZINC69482204	-11.08
UEFS-278	ZINC69482218	-11.08
UEFS-273	ZINC69482210	-10.76

The three compounds with the best results belong to the terpenoid group, which are among the main bioactive compounds found in essential oils, as shown in Figure 1.

Figure 1. 2D structures of ZINC compounds with the best molecular docking results against tyrosinase enzyme.



ZINC69482204 (UEFS-270) ZINC69482218 (UEFS-278) ZINC69482210 (UEFS-273)

Molecular dynamics simulations were performed using GROMACS for 100 nanoseconds (ns) with the CHARMM36m force field, for both the positive controls and the three top-ranked ZINC compounds. The results showed that the tested compounds formed stable complexes with tyrosinase, exhibiting RMSD values ranging from 0.15 to 0.25 nm and RMSF values below 1 nm.

Conclusions

The selected compounds exhibited significant molecular interactions with tyrosinase, demonstrating potential inhibitory effects on melanogenesis. Further in vitro and in vivo studies could help validate and enhance the understanding of the antimelanogenic activity of the studied compounds.

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Use of Pharmacophore-Based Virtual Screening (PBVS) to Select Compounds from the Northeastern Brazilian Semiarid Region as Potential Tyrosinase Inhibitors

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Key words: Tyrosinase; MITF; α-MSH Receptor; Inhibitors; Molecular Docking.

Introduction

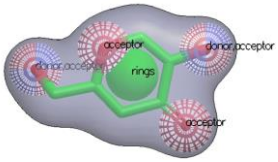
Tyrosinase is an oxidase enzyme fundamental to melanogenesis through its role in hydroxylating tyrosine to dihydroxyphenylalanine (DOPA) and subsequently oxidizing DOPA to DOPA quinone. Therefore, the discovery of tyrosinase inhibitors is important for the development of antimelanogenic products aimed at treating skin disorders caused by melanin hyperpigmentation, such as melasma and dark spots.[1,2,3]

Results and Discussion

The crystal structure of tyrosinase (PDB ID: 3NQ1), which includes co-crystallized kojic acid and two copper atoms interacting with the active site in high resolution, was selected for docking studies, as no human tyrosinase structure with these features was found at the time. To improve biological relevance, its active site was mutated based on the AlphaFold2-predicted human tyrosinase structure (UniProt ID: P14679).[4,5]

The pharmacophore model used in the virtual screening, shown in Figure 1, was based on the three-dimensional superposition of known tyrosinase inhibitors (kojic acid, hydroquinone, arbutin, and ascorbic acid), using the ROCS v3.3.1.2 software (OpenEye Scientific). This model illustrates potential interactions between the inhibitors and amino acids of the active site.

Figure 1: Pharmacophore model used in the virtual screening.



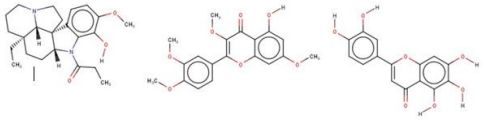
The molecular docking study, performed using AutoDock4, evaluated the binding conformations of known tyrosinase inhibitors and subsequently of 50 three-dimensional structures of compounds from the Brazilian semiarid region, selected by pharmacophore-based virtual screening (PBVS) from the natural molecules catalog of the State University of Feira de Santana (UEFS) within the ZINC 20 database, obtaining binding energy values shown in Table 1.

Table 1. Docking Results Between Tyrosinase/Positive Controls and Tyrosinase/Top-Scoring Molecules from the UEFS/ZINC Database

UEFS CODE	ZINC CODE	Binding Energy (kcal/mol)
Ascorbic acid	-	-3,23
Kojic acid	-	-3,67
Arbutin	-	-4,18
Hydroquinone	-	-3,46
UEFS-311	ZINC28538823	-6,23
UEFS-151	ZINC5733766	-5,92
UEFS-206	ZINC6092198	-5,91

Compound UEFS-311 is an alkaloid commonly known as Aspidoline; UEFS-151 is a flavonoid known as Retusin; and UEFS-206 is another flavonoid, 6-hydroxyluteolin, as shown in Figure 2.

Figure 2: 2D Structures of ZINC Compounds with the Best Molecular Docking Results Against Tyrosinase Enzyme.



Conclusions

The compounds selected by PBVS exhibited significant molecular interactions with tyrosinase, demonstrating potential inhibitory effects on melanogenesis. In vitro and in vivo studies are needed to further elucidate the antimelanogenic activity of these compounds.

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Design and *In Vitro* Characterization of Thioredoxin Glutathione Reductase Inhibitors from *Schistosoma mansoni* (*Sm*TGR)

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Key words: Schistosoma mansoni, Thioredoxin Glutathione Reductase (SmTGR), inhibitor, fragments, drug design

Introduction

Schistosomiasis, caused by Schistosoma parasites, affects over 200 million people worldwide. Treatment relies almost exclusively on praziquantel (PZQ), which is ineffective against immature forms and shows emerging resistance. The redox enzyme *Sm*TGR is essential for *S. mansoni* survival and a validated drug target. Our group screened 768 molecular fragments using protein crystallography as part of the Diamond/XChem project, identifying ligands at the S3, S8, and S9 sites. Structural data were manually curated to design compound combinations shown in Figure 1a. These compounds, synthesized in collaboration with LASOPB at IQ-UFRJ, link fragments via various spacers and are being explored as competitive *Sm*TGR inhibitors. We report their activity at 100 µM in enzymatic assays.

Results and Discussion

Among the tested compounds were the precursors (66a–h) of fragment x2457 and its analogs (67a–h), two analogs of x2058 (58 and 248), x2442 analogs (70a–f), a brominated analog of x2265 (157), and its BOC-protected derivative (174). From the set of 15 initial combinations (Figure 1a), one compound derived from combination 10 (Figure 1h), along with its BOC-protected precursor (202), was assessed. Additionally, two compounds (58 and 248), generated through the AutoGrow algorithm based on fragment x2058, were included in the analysis (Figure 1d).

The reference inhibitor 8VP192, previously reported by Petukhova et al. (2023) as a *Sm*TGR inhibitor (IC_{50} = 2.5 µM), was also tested, together with selected analogs (104) and precursors (62, 103, 105, 64, and 108). None of the compounds related to x2457 (Figure 1c), combination 6 (Figure 1e), combination 10 (Figure 1f), or those generated via AutoGrow (Figure 1d) demonstrated inhibitory activity. Among the compounds related to 8VP192, compound 64 exhibited the highest inhibition (16.87% at 100 µM), followed by 8VP192 itself (11.30%) (Figure 1g). These results suggest limitations of the current assay using truncated *Sm*TGR, the only difference from previous studies. Low aqueous solubility of several compounds may also have reduced their apparent activity.

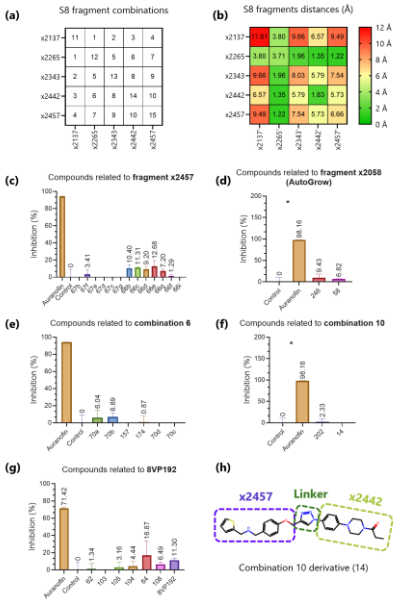


Figure 1 | Design and evaluation of compounds from S8 fragments. (a) Possible fragment combinations from co-crystal structures. (b) Distances between fragments in *Sm*TGR dimers. (c–g) Inhibition of *Sm*TGR by compounds from (c) x2457, (d) x2058 (AutoGrow), (e) combination 6, (f) combination 10, and (g) reference 8VP192 and analogs. Auranofin served as positive control. (h) Structure of compound 14 from combination 10 (x2457 + x2442) with linker.

Conclusions

A total of thirty compounds were evaluated for inhibitory activity against *Sm*TGR, among which two (14 and 248) represent final products derived from our initial design strategy. None of the compounds exhibited significant inhibition. Further investigations will be conducted to confirm these findings.

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Computational Investigation of Compounds from the PeQuiM Chemical Library as CDK5 Inhibitors

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Key words: molecular docking, CDK5, kinases, PeQuiM library.

Introduction

Cyclin-dependent kinase 5 (CDK5) differs from other kinases in that it does not rely on cyclins for activation, but instead requires specific regulatory subunits, such as p35 and p39. Although predominantly expressed in the nervous system — where it plays key roles in neuronal migration, NMDA receptor modulation, and synaptic plasticity — CDK5 has also been identified in other tissues, including pancreatic β-cells, corneal epithelial cells, and immune-related pathways. In these contexts, it contributes to inflammatory processes by promoting the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Dysregulation of CDK5 has been associated with various pathological conditions, including neurodegenerative diseases, cancer, chronic inflammation, and neuropathic pain, highlighting its potential as a therapeutic target. In this context, the present study aims to investigate, through computational approaches, the inhibitory potential of compounds from the chemical library of the Laboratory of Medicinal Chemistry Research (PeQuiM), which has been previously evaluated for their neuroprotective, anti-inflammatory, and antiviral activities.

Methodology

Molecular docking studies of the entire PeQuiM library were conducted using the DockThor platform [REF] (freely accessible at www.dockthor.lncc.br) against CDK5 to find potential inhibitors. Since many of the PeQuiM synthesized compounds already exhibit biological activities for a broad range of targets, it is expected that some compounds with CDK5 inhibitioncan act as promising multi-target candidates for the treatment of multifactorial diseases with neurodegenerative and inflammatory components. In this study, the compounds will be evaluated as type I inhibitors of CDK5, since this class of inhibitors binds to the active conformation of the kinase, occupying the ATP-binding site. The compounds were prepared using LigPrep, a tool available in the Maestro Schrödinger platform, while the protein was prepared using the interactive Protein Preparation Wizard within the same software. Due to the high conformational flexibility of kinases, studying different conformations is necessary to better understand the ATP-binding site, which can provide valuable insights into how inhibitors should be designed..

Results and Discussion

Analysis of the predicted binding modes of CDK5 with co-crystallized ligands from PDB structures 4AU8, 7VDP, 3O0G, 1UNL, 1UNG, and 1UNH allowed the identification of key residues involved in ligand binding and potential inhibition.Notably, the gatekeeper residue Phe80 plays a crucial role by forming π-π stacking interactions with aromatic rings of some inhibitors, enhancing binding affinity. Due to the structural similarity of some compound series to the co-crystallized inhibitors in the target structures, it is expected that at least 30% of the compound library will yield promising results, with binding affinities greater than -10.000 kcal/mol.

Conclusions

Based on the results, it will be possible to identify the best compound series exhibiting CDK5 inhibition, as well as to highlight individual compounds with promising binding affinity and interaction with key CDK5 residues.

Acknowledgements

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Effect of mutations in the RBD of the SARS-CoV-2 *Spike* protein on the interaction with hACE2-derived peptides, inhibitors of viral infection

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Keywords: COVID-19, Spike, mutation, hACE2-derived peptides, PM7.

Introduction

COVID-19, the disease caused by the SARS-CoV-2 coronavirus, has already caused more than 7 million deaths worldwide[1]. The virus invasion of human cells is initiated by the binding of its *Spike* protein, through the receptor-binding domain (RBD), to the human angiotensin-converting enzyme (hACE2)[2].

A 2020 study demonstrated that hACE2-derived peptides (SAPs) are capable of inhibiting *Spike*-mediated viral infection[3]. However, during the pandemic, new mutations emerged, making it necessary to understand their impact on SAPs' ability to inhibit viral infection. In this work, a quantum-mechanical method was used to investigate these effects on the interaction enthalpy (ΔH_{int}) of RBD-SAP complexes, in order to obtain information for the rational design of new peptides as *Spike* protein ligands.

Results and Discussion

The crystallographic structure of the native *Spike* RBD, linked to hACE2, was obtained from PDB (PDB ID: 6M0J, resolution of 2.45 Å). 3D structures with mutations (E484K, F456L, K417N, N501Y) in the RBD were obtained from the Dockthor server (LNCC). The RBD and the hACE2-derived peptides SAP1 (27-TFLDKFNHEAEDLFYQ-42), SAP2 (37-EDLFYQSSL-45) and SAP6 (37-EDLFYQ-42) were selected with Pymol 0.99rc (Delano Scientific LLC). These peptides were chosen because they presented good *in vitro* infection inhibitory activity[3].

Since the complete RBD-peptide complexes contain thousands of atoms, the semiempirical approach was used for the quantum-mechanical calculations. The complexes and their isolated components were fully optimized with the PM7 method of MOPAC2016 (Stewart Comp. Chem.). The surroundings were simulated by a continuum model with chosen dielectric constants; two different constants were evaluated, a lower one representing a membrane-like environment (Ɛ=4.0), and a medium one (Ɛ=20.0) representing a transition between a membrane-like environment and the aqueous phase. Enthalpy of formation data were used to calculate the ΔH_{int} (Table 1) by the following equation:

$\Delta H_{int} = (\Delta H^0_f(\text{RBD-SAP}) - [(\Delta H^0_f(\text{RBD}) + (\Delta H^0_f(\text{SAP})])$

Table 1. PM7-calculated interaction enthalpy values

	ΔH_{int} (kcal/mol) | Ɛ=20.0				
	native	E484K	F456L	K417N	N501Y
SAP1	-214.3	-200.3	-183.8	-173.5	-179.2
SAP2	-79.1	-95.1	-95.9	-85.9	-95.6
SAP6	-38.4	-62.8	-75.8	-57.4	-61.8
	ΔH_{int} (kcal/mol) | Ɛ=4.0				
	native	E484K	F456L	K417N	N501Y
SAP1	-205.4	-206.6	-167.9	-96.5	-166.3
SAP2	-51.4	-105.5	-98.1	-59.8	-114.8
SAP6	-54.6	-88.2	-96.9	-43.8	-67.2

Overall, mutations resulted in conformational changes and rearrangements of interactions, as can be seen in Figure 1 for the N501Y mutant.

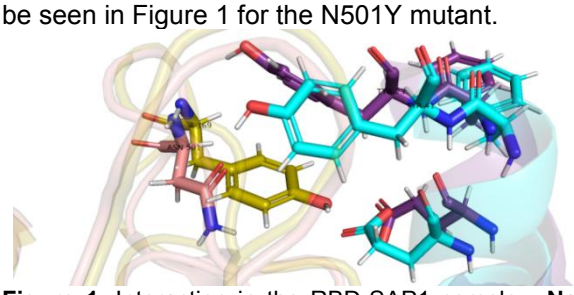


Figure 1: Interaction in the RBD-SAP1 complex. **Native**: hydrogen bond between RBD residue Asn501 (C, light pink) and SAP Tyr41 (C, cyan); **Mutant (N501Y)**: π-stacking between Tyr501 (C, yellow) and Tyr41 (C, purple).

Conclusions

As expected, the longer the SAP, the more favorable ΔH_{int} is, but mutations have different effects on RBD-SAP interactions depending on the peptide length. For the longer SAP, mutations weakened the interaction, while the opposite was observed for the shorter SAPs. Although SAP1 remains the best ligand for the RBD after all mutations, the results show that mutations favorably affected the interactions of SAP2 and SAP6 with the *Spike* RBD. These results demonstrate that the shorter peptides have the potential to maintain or even improve their infection-inhibitory activity after mutations. Because mutations frequently occur in *Spike*, they appear to be safer candidates for developing inhibitors of SARS-CoV-2 infection in the future.

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New Cannabidiol-based Terpene *N*-Acyl-Aryl-Hydrazones with Potent Antinociceptive and Anti-Inflammatory Activity

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Keywords: Cannabidiol; CBD analogues; antinociceptive activity; anti-inflammatory activity; N-acyl-hydrazones; CBD-based N-acylhydrazone analogues

Introduction

Inflammation is a protective mechanism of the organism in response to noxious stimuli (*e.g.*, viruses, bacteria, toxins, tissue injury), aiming to restore cellular homeostasis and tissue integrity. If the acute response fails, it may evolve into chronic inflammation, a key factor in the onset or progression of diseases like cardiovascular disorders, rheumatoid arthritis, diabetes, cancer, COVID-19, and neurodegenerative diseases. Common to these conditions is a systemic imbalance of cytokines (*e.g.*, IL-6, IL-1β, TNF-α) and reactive oxygen species (ROS) which are also overproduced during inflammation, contributing to cellular damage and further exacerbating the inflammatory response. The vanilloid receptor TRPV1 has emerged as a key player in pain modulation and is a potential target for cannabidiol (CBD) effects. CBD has been studied as a promising ligand of the central nervous system (CNS), exhibiting many properties of clinical interest, including antioxidant and anti-inflammatory, antiepileptic, anxiolytic, and antidepressant effects. In this context, we synthesized and pharmacologically evaluated eight novel *N*-acyl-aryl terpene hydrazone derivatives, designed as CBD-inspired molecules.

Results and Discussion

A series of eight new terpene *N*-acylaryl hydrazone analogues PQM-242 to PQM-249 (5**a**-5**h**), rationally designed as simplified structural CBD-based analogues, was synthesized in 10-57% global yields by a three-step synthetic route and evaluated for their *in vivo* potential antinociceptive and anti-inflammatory profile. Preliminary studies revealed compounds PQM-244 (IC50 = 14.15 ± 0.62 μM), PQM-245 (>200), and PQM-247 (IC50 = 4.76 ± 0.22) as showing the best radical scavenging activity (DPPH), with potencies comparable to those of ascorbic acid (IC50 = 14.92 ± 0.17) and quercetin (IC50 = 5.62 ± 1.82). Pharmacological evaluation stood out compound PQM-242 (10 μmol/kg) for its best antinociceptive effect in the inflammatory phase of the formalin-induced licking test, where it significantly reduced the licking time during the neurogenic phase but did not produce a significant effect in the inflammatory phase, and a central antinociceptive effect similar to morphine in the hot plate model (PQM-242 exhibited a potent central antinociceptive effect). In contrast, PQM-243 and PQM-248 exhibited the best antinociceptive properties in the capsaicin-induced nociception assay. Finally, compound PQM-244 seems to have the most promising peripheral antinociceptive profile, showing significant effects on both neurogenic and inflammatory phases of the formalin-induced licking test and potential antioxidant activity.

Conclusions

Our experimental results indicate that the new CBD-based architecture was capable of reproducing antinociceptive profile as observed for CBD, with adequate predicted ADME properties and no apparent toxicity. Our group is currently conducting additional studies aimed at elucidating the possible mechanisms of action underlying the antinociceptive profile and characterizing the potentialities of compounds PQM-242, PQM-243, PQM-244 (5a-c), and PQM-248 (5g) as new CBD-based drug candidate prototypes.

Acknowledgements

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Design, Synthesis, and Evaluation of New ROCK Inhibitors for the Treatment of Cerebral Cavernous Malformations

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Key words: ROCK, Indazole, Cerebral Cavernous Malformations

Introduction

Cerebral cavernous malformations (CCMs) are lesions affecting the capillaries of the central nervous system, characterized by the presence of dilated vascular channels, and represent the second most common intracranial vascular malformation in humans. One of the pathological features of CCMs is microbleeding, resulting in leakage within the vascular channels. Hemorrhage is a complication evidenced by hemosiderin deposits, which, along with CCM growth, may be associated with seizures and neurological deficits. Additionally, low blood flow and endothelial dysfunction may contribute to thrombus formation[1]. Currently, although there are pharmacological options available, CCMs still lack curative treatment options. In this context, Rho-kinase (ROCK) inhibitors exhibit therapeutic potential by reducing vascular inflammation and stabilizing the vascular wall, thereby preventing hemorrhages—processes directly related to CCM progression[2,3]. This work aims to design, synthesize, and pharmacologically and *in silico* evaluate novel indazole derivatives as ROCK1/2 inhibitors. To this end, a structure-based *de novo* design approach was employed, focusing on the indazole hinge-binding moiety and the identification of suitable linkers to enable exploration of the affinity pocket of ROCK1/2[4].

Results and Discussion

The proposed synthetic methodology for obtaining the target products involves an acyl nucleophilic substitution on chloroacetyl chloride, characterizing a Schotten–Baumann reaction, followed by a nucleophilic substitution of the intermediates obtained in the first step with the corresponding anilines, allowing the synthesis of the planned compounds. The application of this synthetic strategy enabled the preparation of an initial compound, and both the intermediate and the final product were characterized through the acquisition of nuclear magnetic resonance (NMR) and infrared (IR) spectra. The planned compound was subjected to HPLC using 70% acetonitrile and 30% water as the mobile phase, showing 95.174% purity and a retention time of 3.863 minutes, after which the enzymatic activity assay against ROCK was performed, showing activity in ROCK1 IC_{50}= 4.461 µM and ROCK2 IC_{50}= 3.954 µM. The designed derivatives will be evaluated *in silico* through molecular docking using the CSD software suite, via GOLD, and with crystallographic structures of ROCK being obtained from the Protein Data Bank. Complementarily, geometry optimization and binding enthalpy (ΔH) calculations will be performed using the semi-empirical method with the MOPAC software. Additionally, a molecular dynamics simulation step will be carried out using the Flare software to assess interaction modes and interaction distances over time. Image generation and data processing will be performed using the PyMOL and Prisma programs.

Conclusions

The synthetic route was followed until the first final product was obtained, with the characterization of both the intermediate and the final product. The evaluated planned compound exhibited activity in the micromolar range. Future perspectives include continuing the synthetic route to obtain the remaining planned indazole compounds, followed by enzymatic inhibition assays, as well as the advancement of molecular modeling studies involving molecular docking, semi-empirical calculations, and molecular dynamics simulations.

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Discovery of PQM-208: A New Synthetic Hydrazide-Triazole Prototype with Multi-Target Pharmacological Profile Against SARS-CoV-2.

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Key words: hydrazone-triazole hybrids, PQM-208, SARS-CoV-2, ACE2, Antiviral activity, Anti-inflammatory activity.

Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, highlighted the urgent need for the development of more effective antiviral drugs. Although treatments such as remdesivir and nirmatrelvir have been used throughout the course of the disease, they present limitations, including low efficacy at certain stages of infection and the risk of the emergence of resistant viral variants. Therefore, the continuous search for new multitarget therapeutic options is of great importance, especially those capable of acting at different stages of the viral cycle as well as modulating the host's inflammatory response. In addition, chemical structures such as triazole rings and hydrazone groups have shown promise in previous studies due to their ability to interact with viral proteins and their anti-inflammatory properties. In this context, we report the discovery of compound PQM-208 (INPI Patent: BR1020240229223), a new synthetic 1,2,3-triazole–acylhydrazone hybrid. The compound was obtained through a rationally designed synthetic route and evaluated for its antiviral activity against SARS-CoV-2, cellular toxicity, and its ability to counteract respiratory inflammation in vivo.

Results and Discussion

The synthesis of compound PQM-208 was carried out in six steps, as represented in Figure 1. Cytotoxicity was evaluated in Vero CCL-81 and bovine HEK293 cells, demonstrating that PQM-208 was non-toxic up to a concentration of 250 µM (CC_{50} > 250 µM), as presented in Figure 2. In in vitro antiviral assays against SARS-CoV-2, PQM-208 exhibited remarkable potency (IC_{50} = 0.573 nM) when compared to reference antivirals such as remdesivir (IC_{50} = 11.41 µM) and nirmatrelvir (IC_{50} = 8.08 µM), as illustrated in Figure 3. The selectivity index (SI) was calculated to be greater than 432,000, further reinforcing its favorable safety and efficacy profile (Figure 4).

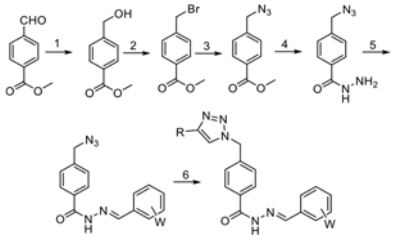


Figura 1: Rota sintética de obtenção do composto PQM-208.

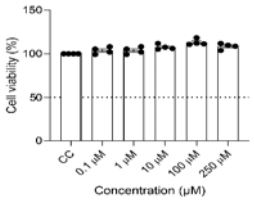


Figura 2: Viabilidade celular de células Vero CCL-81 frente a diferentes concentrações do composto PQM-208.

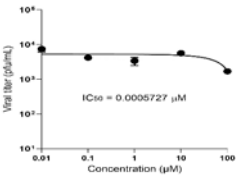


Figura 3: Atividade antiviral do composto PQM-208 contra o SARS-CoV-2 em células Vero CCL-8.

Compound	CC_{50} (µM)	IC_{50} (µM)	Selectivity Index (SI)
PQM 208	> 250	0.0005727	> 432,528.72
PQM 262	> 250	1	> 250
Nirmatrelvir	> 100	0.179	589

Figura 4: Índice de seletividade do composto PQM-208

Conclusions

Overall, our results demonstrate that the hydrazide-triazole derivative (E)-4-((-4-phenyltriazol-1-yl)methyl)-N'(4-butoxybenzylidene)benzohydrazide (PQM-208) is a promising compound with strong potential for the development of new multifunctional antivirals. Its hybrid structure, composed of triazole and hydrazone units, showed excellent performance as a multifunctional ligand due to its ability to exert antiviral activity against SARS-CoV-2 and its low cytotoxicity. These findings indicate that PQM-208 may be developed as a multitarget drug prototype, and in vivo studies are currently underway to evaluate its anti-inflammatory activity in a model of acute lung inflammation induced by SARS-CoV-2.

Acknowledgements

The authors are grateful to CNPq, CAPES, FAPEMIG, FAPERJ, FINEP, and INCT-INOFAR for financial support and fellowships.

Hydrazide-triazole compounds with potential antiviral activity. Patent application BR 10 2024 022922 3, June 6, 2024.

Design, Synthesis, and Evaluation of Antinociceptive Properties of Novel CBD-Based Terpene-Cinnamoyl-Acyl-Hydrazone Analogues

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Key words: *CBD analogues, antinociceptive activity, cannabidiol-based analogues, acute pain, bioactive acyl-hydrazone derivatives,*

Introduction

Cannabidiol (CBD) has been reported for its antinociceptive, anti-inflammatory, and neuroprotective activities. However, several legal restrictions on its medicinal uses and even research have contributed to the development of synthetic analogues. Therefore, the aim of this study was the design and synthesis of a novel series of CBD-based structural analogues, and the *in vivo* evaluation of their potential antinociceptive activity.

Results and Discussion

Using a two-step synthetic route, 26 new terpene cinnamoyl-acyl-hydrazone CBD analogues were obtained and were submitted to *in vivo* screening in the classical formalin-induced paw edema (Figure 1) and hot plate (Figure 2) assays. Compounds (10 µmol/kg) **PQM-292**, **PQM-293**, **PQM-295**, **PQM-307**, **PQM-308**, and **PQM-309** exhibited the best results in the neurogenic phase (First phase, Figure 1) of the formalin-induced licking response, showing comparable results to morphine (5 µmol/kg). Notably, in the inflammatory phase (Second phase, Figure 2), compound **PQM-292** exhibited the best anti-inflammatory activity. Interestingly, in the hot plate model, six other compounds (**PQM-274**, **PQM-291**, **PQM-294**, **PQM-304**, **PQM-305**, and **PQM-378**) showed the best antinociceptive activity in comparison to morphine (9 µmol/kg), especially **PQM-274**, which exhibited an antinociceptive effect comparable to the reference drug (relative latency approximately 33% greater than that of morphine). Interestingly, these findings suggested that these bioactive compounds, despite their structural similarity, act through different mechanisms, which were investigated by molecular docking with CB1, CB2, and TRPV1 receptors. *In silico* results indicated that the most active compounds should act through different mechanisms, probably involving interactions with TRPA1, which is currently under investigation.

Figure 1. Formalin-induced paw edema assay.

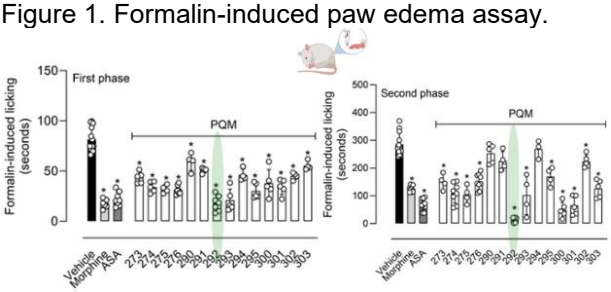
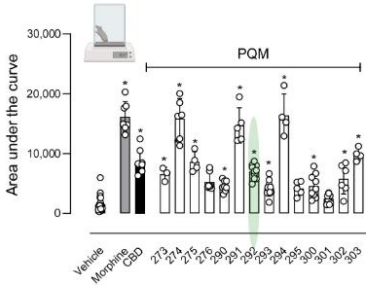


Figure 2. Hot plate assay.



Conclusions

Therefore, due to the promising antinociceptive activity observed for these highlighted compounds, particularly for **PQM-292** and **PQM-274**, without apparent toxicity and psychoactive effects, and the possible involvement of diverse mechanisms of action, these compounds could be considered as promising starting points to the development of new drug candidate prototypes of clinical interest.

Acknowledgements

National Council for Scientific and Technological Development (CNPq); Minas Gerais State Research Support Foundation (FAPEMIG); Funding Authority for Studies and Projects (FINEP); Coordination for the Improvement of Higher Education Personnel (CAPES); National Institutes of Science and Technology (INCT).

de Souza, M.L.; de Paiva, J.P.B.; et al. P*harmaceuticals,* **2025**, *18*, 755.

PQM-262: Discovery of a New Triazolyl-Hydrazone Molecular Hybrid as Multitarget Antiviral Agent Against SARS-COV-2

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Key words: SARS-CoV-2, Antiviral, COVID-19, hidrazones, triazole derivatives.

Introduction

Considering the emergence of new variants and the limited therapeutic approaches, the search for new strategies to combat the SARS-CoV-2 virus remains a global priority. In 2019, SARS-CoV-2 was identified as the causative agent of severe pneumonia, progressing to multiple organ failure and death, especially in high-risk patients. The coronavirus demonstrated its pandemic potential and high transmissibility, being spread through respiratory microdroplets from infected patients, whether symptomatic or asymptomatic, causing COVID-19. Thus, several public health agencies and private initiatives have been dedicated to developing new effective therapeutic alternatives against SARS-CoV-2 both in Brazil and worldwide. Remdesivir was the first drug approved by the Food and Drug Administration (FDA, USA) and the Brazilian Health Regulatory Agency (ANVISA), followed by Molnupiravir and Paxlovid; however, all of them presented high costs and low efficacy in severe cases of COVID-19, making them inaccessible to the broader population of developing countries. In this context, among the strategies for developing potentially effective drugs against COVID-19, hydrazides and hydrazones are compounds that have shown promise, especially when combined with 1,2,3-triazole rings, known for their antimicrobial potential. From this perspective, the present work addresses a new use for the molecular hybrid 1-(3-chloro-4-methoxyphenyl)-4-[(E)-[2-(2,5-dichlorophenyl)hydrazin-1-ylidene]methyl]-1H-1,2,3-triazole, named PQM-262.

Results and Discussion

PQM-262 was obtained from functionalized aromatic azides via click reactions and subsequently condensed with hydrazines. This new molecular hybrid stands out for exhibiting a multitarget antiviral activity profile, acting by suppressing SARS-CoV-2 replication, as an ACE-2 antagonist, and with anti-inflammatory effects in in vivo models of pneumonia induced by inactivated virus, representing an authentic innovation and therapeutic potential superior to the anti-COVID-19 drugs currently available on the market, already having a registered patent (BR 10 2024 022941 0).

Conclusions

Therefore, with the imminent need for innovative therapeutic approaches for the COVID-19 treatment, PQM-262 stands out with a powerfull antiviral potential, acting against the viral replication and showing anti-inflammatory effects, besides your multitargerts activity in *in vitro* models. This indicates its superiority to traditional anti-COVID-19 drugs and demonstrates the university's capacity to contribute to public health by producing an authentic molecule potentially capable of treating COVID-19.

Acknowledgements

The authors are grateful to CNPq, FAPEMIG, FAPERJ, CAPES, FINEP, and INCT-INOFAR for financial support and fellowships.

RIVA, L. et al. Discovery of SARS-CoV-2 Antivirals through Large-scale Drug Repositioning. [s.d.]. SAID, M. A. et al. New 1,2,3-Triazole Scaffold Schiff Bases as Potential Anti-COVID-19: Design, Synthesis, DFT- Molecular Docking, and Cytotoxicity Aspects. **Vaccines**, v. 9, n. 9, p. 1012, 11 set. 2021.

A LC-MS/MS Method for the Simultaneous Determination of Nicotine and Cotinine in Mice's Tissue Samples after Exposure to Vaping Compared to Cigarette Smoking

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Key words: nicotine, cotinine, electronic cigarette, smoke exposure, LC-MS/MS.

Introduction

Increased e-cigarette use raises intake of harmful, fast-absorbed nicotine. This study developed and applied LC-MS/MS to quantify nicotine in mouse tissues of mice exposed to electronic and conventional cigarette smoke.

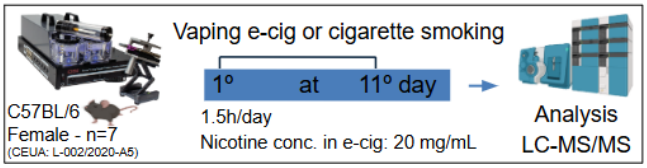


Figure 1. Schematic representation of experimental methodology.

Results and Discussion

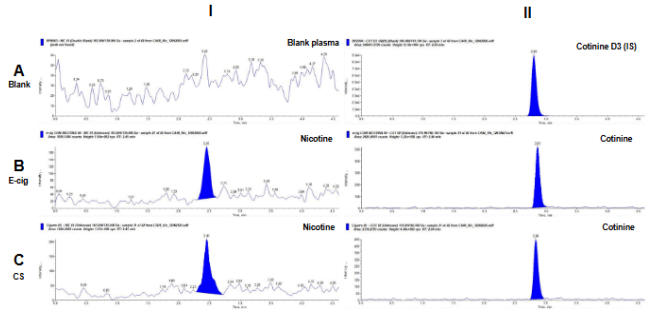
Bioanalytical Method

Table 1. Parameters of calibration curves for quantitative analysis of nicotine and cotinine in plasma, brain and lung.

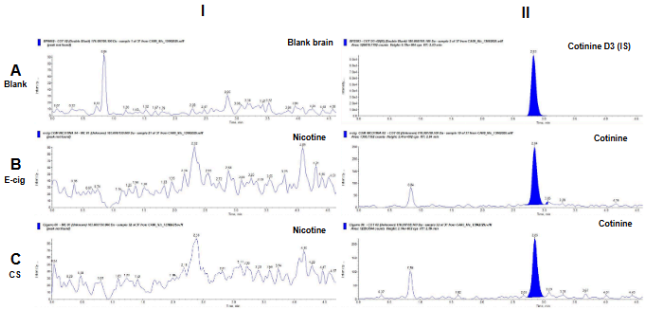
Compounds	Sample Type	Range	Regression Equation	r	Weighting Factor
Nicotine	Plasma	2.5 – 500 ng/mL	y= 0.135x + 0.00102	0.993	$1/x^2$
	Brain		y= 0.144x + 0.00034	0.995	
	Lung	5.0 – 500 ng/mL	y= 0.146x + 0.00067	0.995	
Cotinine	Plasma	0.5 – 500 ng/mL	y= 0.998x + 0.00136	0.999	$1/x^2$
	Brain		y= 0.978x + 0.00146	0.998	
	Lung		y= 0.987x + 0.00155	0.999	

Application of the Bioanalytical Method

2.1 Plasma



2.2 Brain



2.3 Lung

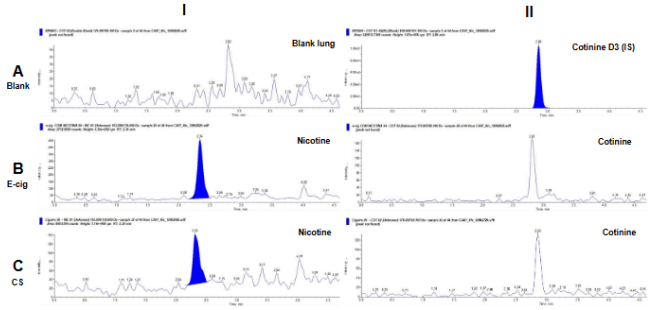


Figure 2: 2.1, 2.2 and 2.3 panels represent plasma, brain homogenate and lung homogenate chromatograms, respectively. (AI) Representative chromatograms of blank and (AII) internal standard. (BI) Representative chromatograms nicotine and (BII) cotinine after exposure to electronic cigarette smoke (E-cig). (CI) Representative chromatograms nicotine and (CII) cotinine after exposure to conventional cigarette smoke (CS).

Tissue Nicotine and Cotinine Levels

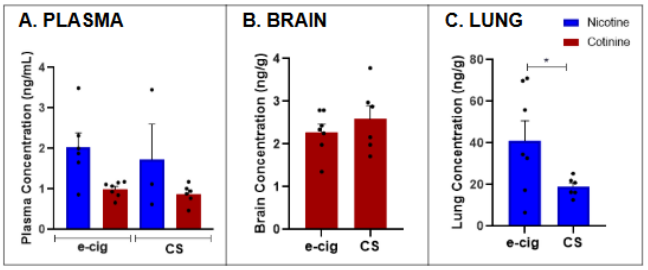


Figure 3: (A) Plasma, (B) brain and (C) lung levels of nicotine and cotinine in mice after exposure to vaping and cigarette smoking (* $p < 0.001$).

Conclusions

The LC-MS/MS method reliably quantified nicotine and cotinine in mouse tissues with high sensitivity, robustness, and reproducibility. After 11 days of exposure to electronic or conventional cigarette smoke, systemic nicotine and cotinine levels were similar across groups. In brain tissue, only cotinine was detected, with no significant differences among groups. In lung tissue, only nicotine was found, with significantly higher levels in mice exposed to vaping.

Acknowledgements

FIOCRUZ, CAPES and FAPERJ.

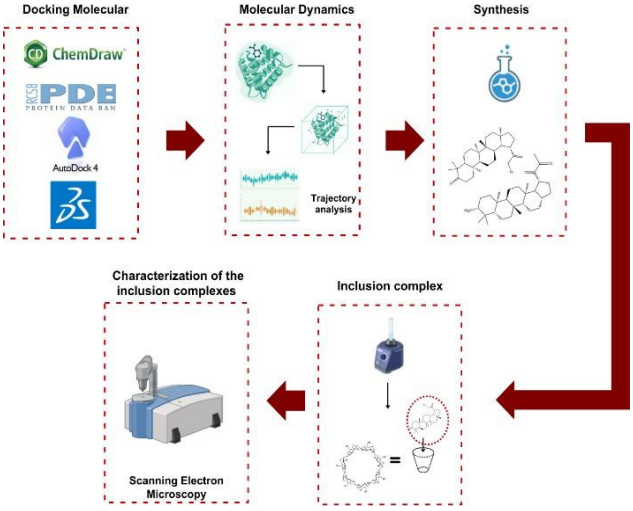
Mallock, N. *J. Chromatogr. B* **2021**, *1171*, 122736. doi.org/10.1016/j.jchromb.2021.122736.

Physicochemical assessment of the inclusion complex of Lupeol and its derivatives in β-Cyclodextrin

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E-mail address of the corresponding author: guilhon_simplicio@ufam.edu.br
Key words: Lupeol, derivatives, complexation, beta-cyclodextrin, physicochemical

Introduction

Cyclodextrins (CDs) are used in the pharmaceutical industry to improve the physicochemical and pharmacological properties of compounds. Souza (2023) carried out the synthesis of lupeol derivatives that showed high lipophilicity, leading to the proposal of *in silico* studies and characterization aiming to reduce this limitation.



methodology used in the complexation process.

Results and Discussion

	Binding energy (Kcal/mol)		
Lupeol	**αCD (-4.9)**	**βCD (- 6.4)**	**γCD (- 7.2)**
DEMS01	**αCD (-5.1)**	**βCD (-7.6)**	**γCD (-7.8)**
DEMS02	**αCD (- 4.9)**	**βCD (-7.9)**	**γCD (-8.2)**

Table 1. Binding energy of α-, β-, and γ-CD with Lupeol, DEMS01, and DEMS02.

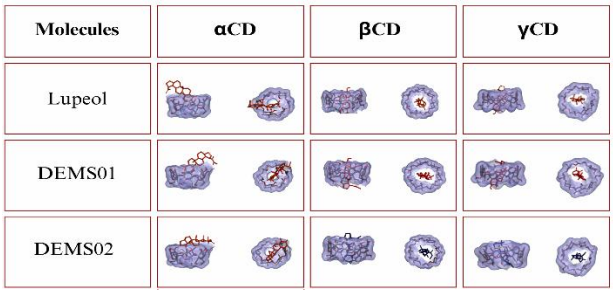


Figure 2. Representation of the cavities of the three CDs, showing the insertion of lupeol and its derivatives.

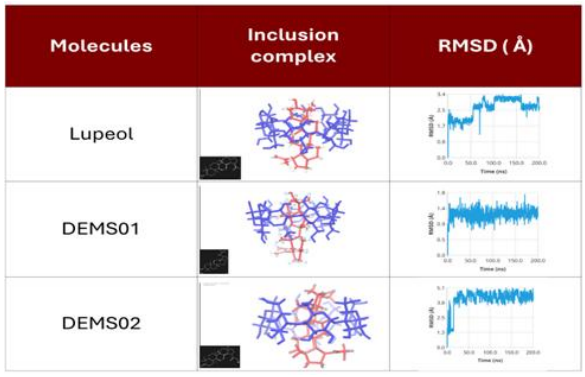


Table 3. RMSD analyses of the inclusion complexes performed in the molecular dynamics simulations.

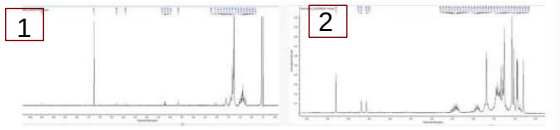


Figure 3: NMR analysis of the syntheses. (1) DEMS01 and (2) DEMS02.

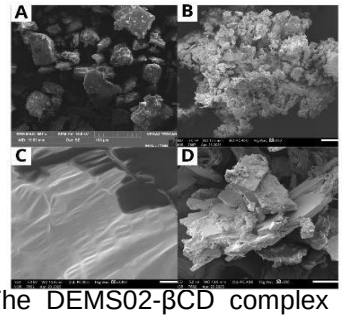


Figure 4: Micrographs of βCD at a 10µm scale (A); Complex-Lupeol at 10µm (B); DEMS01/ βCD at 10µm (C); DESM02/ βCD at 10µm (D).

The DEMS02-βCD complex (ethanol 1:1, 30 min) reveals structural changes in βCD; the structure shows fragmentation and flattening of the particles, with less organized surfaces, which supports the hypothesis of disruption of the original crystalline structure of β-CD.

Conclusions

These analyses are promising and indicate the potential of the DEMS02 derivative, especially in complexation with β-CD. Further analyses, such as DSC and FTIR, are necessary for confirmation before conducting *in vitro* tests with the complex.

Acknowledgements

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[1]SOUZA, Douglas Emanuel Mota de. In silico design and synthesis of lupeol derivatives with anti-inflammatory activity. 2023. 120 p. Dissertation (Master's in Pharmaceutical Sciences) - Universidade Federal do Amazonas, Manaus, 2023.

Investigation of novel antitrypanosomal compounds through experimental and in silico medicinal chemistry approaches

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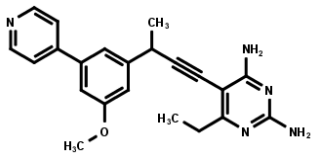
Key words: NTDs, Chagas Disease, Drug Discovery.

Introduction

Neglected Tropical Diseases (NTDs) refer to a group of 21 diseases prevalent in tropical and subtropical regions. Among the various NTDs, Chagas disease stands out as a significant public health concern. Caused by the protozoan *Trypanosoma cruzi*, it is endemic in Brazil, affecting approximately 7 million people worldwide and being responsible for around 12,000 deaths annually[1]. Despite being discovered and characterized over 100 years ago by the Brazilian scientist Carlos Chagas, therapeutic options remain extremely limited, with severe side effects that often lead to treatment discontinuation[2]. This scenario highlights the urgent need for research and development of new drugs for this disease. In this context, this study aimed to identify and characterize novel active compounds against Chagas disease.

Results and Discussion

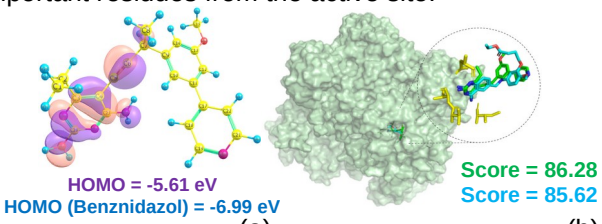
A series of 10 compounds provided by the non-governmental organization Medicines for Malaria Venture was tested against the amastigote intracellular form of *T. cruzi* to identify potential candidates for Chagas disease. Compound **1** was the most promising of the series, exhibiting an IC_{50} of 0.73 µM and a selectivity index (SI) > 85 (**Figure 1**).



IC_{50} = 0.73 ± 0.18 µM
CC_{50} > 64 µM

Figure 1: Compound **1**, a potent and selective canti-*Trypanosoma cruzi* agent.

Computational studies were carried to further investigate this series of bioactive compounds. The density functional theory (DFT) characterized the electronic properties, highlighting its high absolute electron-donating capacity. A target prediction search using the compound's structure on PubChem identified CID: 71525312, which is known to have activity against dihydrofolate reductase (DHFR). Since the DHFR activity of *T. cruzi* is essential for the parasite, it represents a potential target for Chagas disease[3]. Molecular docking assays (**Figure 2**) stated that compound **1** has binding affinity to the protein active site, forming hydrogen bonds with important residues from the active site.



(a) (b)

Figure 2: Energy values of Highest Occupied Molecular Orbital (HOMO) for **1** and benznidazol (a); DHFR (PDB: 3KJS) with the co-crystalized ligand (blue) and compound **1** (green) (b).

Given that poor pharmacokinetics properties are one of the most common causes for drug failure, we carried out early *in vitro* ADME studies[4]. The results (**Table 1**) showed the profile of compound **1** for important pharmacokinetic properties as a guide for further optimization studies.

Table 1. Pharmacokinetics properties.

LogD	KS	PAMPA	Cl_{int}	$T_{1/2}$	HepG2
3,83	14,22	3,88	124,00	22,36	10,175

LogD - distribution coefficient at pH 7.4; KS - kinetics solubility at pH 7.4; PAMPA - parallel artificial membrane permeability assay; $T_{1/2}$ – half-life time (min); HepG2 - Half-maximal inhibitory concentration of hepatocellular carcinoma cell line (µM).

Conclusions

The phenotypic screenings led to the identification of promising compounds. The results indicate compound **1** as a candidate for structural modifications and further *in vitro* studies, aiming to identify novel agents for Chagas disease therapy.

Acknowledgements

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Synthesis and Anti-*Mycobacterium tuberculosis* activity of Bortezomib derivatives planned as Clp modulators

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Key words: *Mycobacterium tuberculosis*, Clp modulators, bortezomib, antitubercular drugs.

Introduction

Mycobacterium tuberculosis (MTB), the primary causative agent of tuberculosis (TB), is responsible for the annual death of approximately 1.5 million people worldwide. One of the major global concerns today is the increasing number of multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) cases, due to their high mortality rates[1]. Therefore, the search for new, safe, and effective agents is urgent.

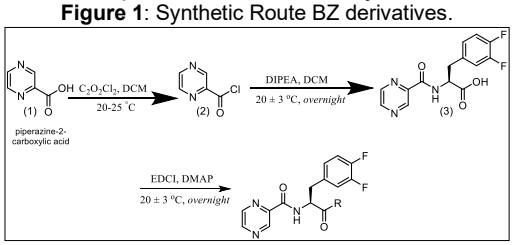
Through drug repurposing strategies, some authors have begun investigating the effects of bortezomib BZ as a potential agent against mycobacteria[2–4]. In these studies, it was observed that both BZ and its derivatives may modulate the Clp complex[3,5], which is considered an essential organelle for mycobacterial survival and has been explored as a promising therapeutic target[3].

Considering that BZ derivatives can act as Clp modulators and aim to evaluate the potential activity of BZ analogues against mycobacteria, the BZ-derived compounds presented in this work were designed, synthesized, characterized and biologically evaluated in $H_{37}Rv$ strains.

Results and Discussion

The synthesis began with the chlorination of (1) using oxalyl chloride in DCM under cooling, forming an intermediate used without purification. This intermediate was then coupled with (2) in DCM at room temperature overnight. After basic extraction and pH adjustment, the crude was isolated with ethyl acetate, dried, and concentrated to yield intermediate (3). In the final step, the correspondent amines were reacted with (3), DMAP, and EDCI in DCM at room temperature overnight. The reaction mixture was washed, dried, and concentrated to afford the final products in 60–70% yield.

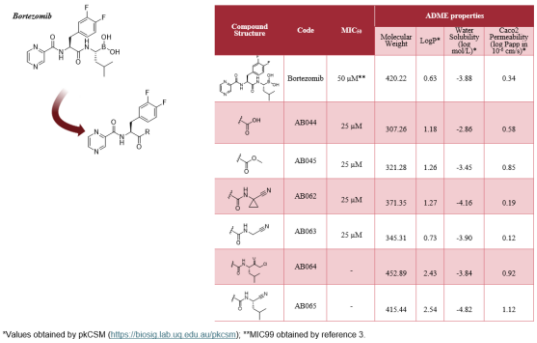
Figure 1: Synthetic Route BZ derivatives.



[1]

(2)

Figure 2. Chemical Structures and MIC_{50} values.



*Values obtained by pkCSM (https://biosig.lab.uq.edu.au/pkcsm); **MIC99 obtained by reference 3.

A total of six compounds were designed, synthesized, and characterized using analytical techniques such as NMR and mass spectrometry, which supported the formation of the desired structures. In addition, the compounds were evaluated for their biological activity *in vitro* against the *Mtb* $H_{37}Rv$ strain, showing phenotypic activity in the range of approximately 25 μM. Furthermore, *in silico* studies revealed predicted Log P values between 0.73 to 2.54, solubility ranging from –2.86 to –4.82 log mol/L, and permeability values between 0.12 and 1.12 log Papp (in 10^{-6} cm/s), all of which are comparable to the predicted ADME properties of the selected lead compound in the series.

Conclusions

Despite the efforts in design, synthesis, and characterization, three derivatives showed activity only at relatively high concentrations. Still, the results offer useful insights and guide future structural optimization to enhance potency and selectivity. Based on these findings, future studies can focus on refining both the compounds and the strategies employed, aiming to improve potency and selectivity.

Acknowledgements

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PBPK Modeling of Oral Benznidazole: A Whole-Body Approach

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Key words: PBPK modelling,neglected desease,Chagas desease.

Introduction

Given the increasing demand for New Approach Methodologies (NAMs) to replace, reduce, or complement traditional animal testing in pharmacokinetic and toxicological studies, this work uses benznidazole as a model compound, particularly relevant in the context of neglected tropical diseases. In addition to physiologically based pharmacokinetic (PBPK) modeling, which predicts organ-plasma partitioning, permeability-surface area products, and gastrointestinal absorption, dose-response curve prediction was also applied to support quantitative risk assessment[1,2].

Benznidazole's pharmacokinetic profile includes high oral bioavailability, a plasma half-life of 2–2.5 hours, ~50% plasma protein binding, no CYP450 modulation, and activity as a P-glycoprotein substrate and OAT3 inhibitor[2-4]. The PBPK model was developed in PK-Sim®, calibrated with human data[5] (CI 95%), and used alongside the U.S. EPA's Benchmark Dose Software (BMDS) to estimate a benchmark dose lower confidence limit (BMDL5), which served as the point of departure (PoD)[1]. The Permitted Daily Exposure (PDE) was then calculated in accordance with the ICH Q3D(R2) guideline (2022)[6] and by the equation:

$$PDE = \frac{PoD\ x\ BW}{F1xF2xF3}$$

where BW is average human body weight, assumed to be 70 kg; F1 (=10) is the factor to account for extrapolation between species; F2 (=10) is the factor accounting for interindividual variability; F3 (=3) is the factor to account for toxicity studies of short-term exposure**.**

Results and Discussion

The PBPK modelling of Benznidazole demonstrated that the simulated plasma concentration-time curve (Figure 1A, red line) shows good agreement with the *in vivo* observed data (Figure 1A, blue dots), successfully capturing the overall pharmacokinetic behavior of benznidazole over time, particularly the initial plateau phase near Cmax (3.0 ± 0.8 µg/mL)[5] and validated statistically by the strong correlation between simulated and observed values (R^2 = 0.96; 95% confidence interval), as shown in Figure 1B. The peak concentration occurs within the first few hours post-administration, followed by a prolonged elimination phase. However, simulated curve shows a slower elimination, a prolonged apparent half-life or lower interindividual variability, appearing to have higher drug exposure, which may impact benznidazole's efficacy and toxicity, especially with prolonged use.

The correlation between simulated and observed concentrations (Figure 1B (y=x; R^2=0,96), demonstrates that the most data points fall within the limits and are closely aligned with the identity line, indicating a strong linear relationship and minimal bias across a wide range of concentrations. This demonstrates the model's consistent predictive capability and reliability in estimating systemic exposure across multiple timepoints.

The combined use of PBPK modeling and dose-response analysis tools provide a mechanistic, quantitative basis for deriving toxicological values, strengthening risk assessment. Using the U.S. EPA's BMDS software, a BMCL5 value of 1.903 µg/kg/day (95% confidence interval) was derived, associated with a toxic response. This value was used as the Point of Departure (PoD) for risk assessment, serving as the basis for calculating a Permitted Daily Exposure (PDE) of 0.444 µg/day.

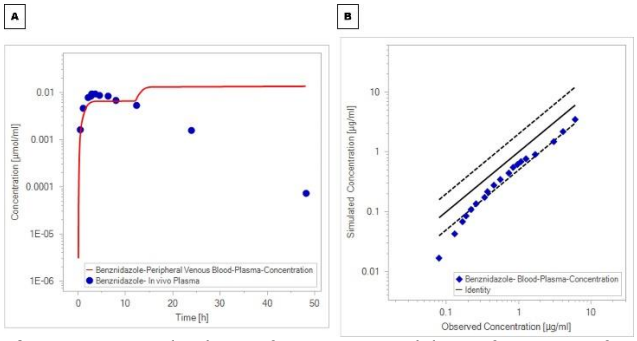


Figure 1: Evaluation of PBPK model performance for benznidazole; A) Simulated plasma concentration–time profile (red line) versus observed *in vivo* data (blue dots); B) Correlation between simulated and observed concentrations. Most points align closely with the identity line (y = x), indicating strong predictive accuracy within a two-fold deviation range (R^2=0,96).

Conclusions

In this context, the PBPK model for benznidazole shows potential to support interspecies extrapolation, define safe exposure thresholds, and contribute to the development of New Approach Methodologies (NAMs), particularly as a starting point for studies of new anti-*trypanosomatids* drug candidates.

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A molecular modeling study on the interaction between bradykinin B2 receptor (B2R) and the Ts14 toxin from the scorpion *Tityus serrulatus*.

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Key words: Tityus serrulatus, Neglected Tropical Diseases, Bradykinin, Hipotensive Toxin.

Introduction

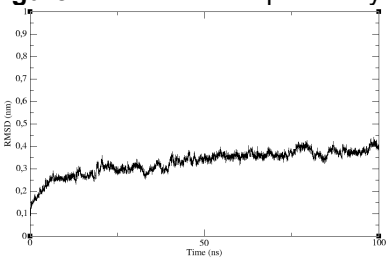
According to the WHO (World Health Organization) classification, neglected tropical diseases (NTDs) are endemic illnesses that persist in the most economically disadvantaged and marginalized populations in tropical countries which cover accidents caused by venomous animals and, particularly, scorpions. In Brazil, *Tityus serrulatus*, the yellow scorpion, is responsible for most envenomation cases, which can lead to severe complications such as pulmonary edema and cardiogenic shock. The primary treatment, anti-scorpion serum (ASS), faces challenges in production and storage due to stringent temperature requirements, underscoring the need for alternative prophylactic drugs to mitigate venom effects. In parallel, protein-protein interactions (PPIs) regulate intra- and extracellular processes, and in scorpion envenomation, ligand-receptor interactions are critical. Given that hypertension is a key effect of scorpion venom, identifying proteins with hypotensive properties could offer prophylactic solutions. In this sense, this work studies the Ts14 toxin (also known as TsHpt-I), a 24 amino acid hypotensive peptide present in the venom of *Tityus serrulatus*, aiming to model its bradykinin (BK) potentiation mechanism and evaluate its potential as a prophylactic drug.

Results and Discussion

Firstly, AlphaFold 2.0 was used to construct the peptide model and elucidate its agonist mechanism, providing insights for further analysis. The BK-B2 receptor (B2R) crystal structure (PDB code: 7F6H) was used for docking methodology validation. Redocking on HPEPDock and HDock servers, with energy minimization of BK from the crystal structure, yielded precise results (respectively, RMSD 0.695 Å, energy score -206.305 and RMSD 0.503 Å, energy score 394,740). Then, docking was carried out between Ts14 and B2R, focusing on the C-terminal portion, which contains the proline doublet (similarly the BK), finding agonist-like poses (for HPEPDock, RMSD: 0.091 Å with an energy score of -143.445 and, for HDock, RMSD: 0.295 Å with an energy score of -281.77).

A molecular dynamics simulation was performed on the complex formed by the 8 C-terminal amino acids of the Ts14 peptide and the B2R receptor, in the presence of a POPC phospholipid membrane. The simulation was carried out using the GROMACS 2024.2 software package, employing the CHARMM36M force field, with the system solvated in a cubic box. The simulation was conducted under physiological conditions, specifically at 310 K and neutral pH. The analysis assessed the trajectory of the complex over a 100 ns simulation, as shown in Figure 1.

Figure 1: RMSD of the protein system



Finally, a cluster analysis was performed on the receptor's trajectory to identify distinct conformational states, revealing four clusters. The most populated cluster had its centroid at 28.3 ns.

Conclusions

Conformational stability of the Ts14 peptide, especially its C-terminal proline duet, was assessed in solution and membrane, validating its agonist capacity with B2R. Molecular dynamics confirmed complex stability and the proposed agonist mechanism via proline, highlighting computational modeling's role in drug development. Future *in vitro* studies are crucial to validate Ts14's therapeutic potential.

Acknowledgements

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In silico study and optimization of inhibitors and mapping of allosteric binding sites of *Schistosoma mansoni* Thioredoxin Glutathione Reductase (*Sm*TGR)

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Key words: Schitosomiasis, Praziquantel, Schistosoma mansoni Thioredoxin Glutathione Reductase (SmTGR), allosterics binding sites, docking.

Introduction

Schistosomiasis affects more than 250 million people worldwide, and the treatment recommended by the World Health Organization is Praziquantel (PZQ)[1], but it has low efficacy against immature forms of the parasite, and it is associated with resistance cases and other complications[2]. Using X-ray crystallography, our research group screened 768 molecular fragments on *Sm*TGR, identifying 35 ligands in 16 sites, with emphasis on S3, S8 and S9. The presence of an inhibitor on S3 site, known as the "doorstop", prevents the binding of NADPH by modifying the conformation of Tyr296[3]. The S3 and S9 sites overlap with the NADPH binding region, enabling the development of competitive inhibitors. The S8 site, found at the dimer interface, is promising for small molecule binding[3]. Based on these analyses, 54 compounds were designed. For docking on sites S3, S8 and S9, nine of our 36 deposited structures were selected (**Table 1**).[3] They were subjected to redocking with the MOE software version 2024.0601, based on the score and E_conf results relation between them. The MOE version used adopts score as the estimated energy of the ligand–receptor complex after docking and E_conf which calculates efficiency by size vs estimated affinity.

This project aims to further study *Sm*TGR molecular dynamics to map its allosteric binding sites and characterize the designed inhibitors *in silico*.

Table 1: PDB structures selected to represent sites S3, S8 and S9 along with their bound fragments. *

1A

Site	Fragments	PDB ID	SCORE	E_conf
	X2053	8PL0	-6,3281	-20,288
	X2077	8PL3	-6,1768	-1,1889 ←
S3	X2361	8PLS	-6,2786	-109,624
	X2343	8PLN	-5,7694	-43,414
	X2442	8PLW	-6,1849	-24,7746 ←
S8	X2457	8PLY	-5,9458	-38,6096
	X2058	8PL1	-4,8917	-70,067
	X2137	8PL8	-5,1798	-88,4556
S9	X2265	8PLH	-5,4591	-25,2676 ←

*The black arrows indicate the reference structure selected for each site.

Results and Discussion

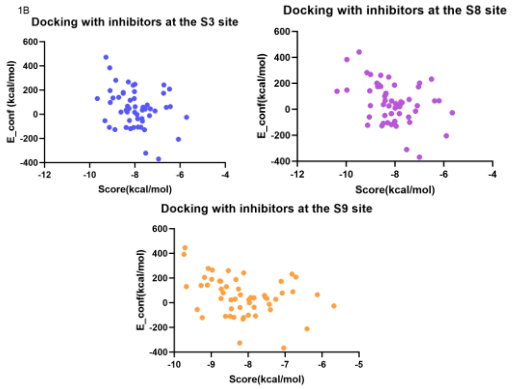


Figure 1. Scatter plots of docking results of the designed compounds, grouped by binding site.

Among the results for site s8, compound 9 (Figure 2) stood out, having the highest E_conf (142.8266kcal/mol) and lowest score (–9.0332 kcal/mol).

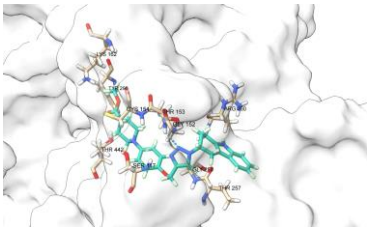


Figure 2. Compound 9 docked at S8 site.

Conclusions

Based on the docking results, the molecules with the lowest score values associated with the highest E_conf values at each trained site were identified and ranked. These molecules will be subjected to characterization steps, including analysis of protein-ligand interactions, evaluation of normal modes of vibration, and molecular dynamics simulations.

Acknowledgements

FIOCRUZ; CNPq, CAPES, FAPERJ.

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A novel N-acylhydrazone derivative targeting the RhoA/ROCK pathway attenuates dexamethasone-refractory inflammatory response.

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Key words: RhoA Kinase inhibitor, lung acute distress syndrome, glucocorticoids refractory.

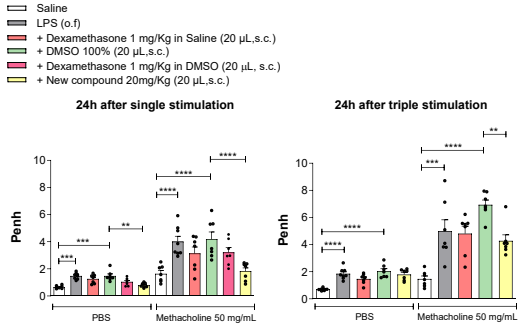
Introduction

The RhoA/ROCK pathway regulates key processes in the pathogenesis of acute respiratory distress syndrome (ARDS)[1,2]. This study investigated the anti-inflammatory effect of a novel N-acylhydrazone derivative in a murine model of LPS-induced acute lung inflammation that is refractory to glucocorticoids. Due to the ongoing intellectual property protection process, its name and chemical structure will be kept confidential.

Results and Discussion

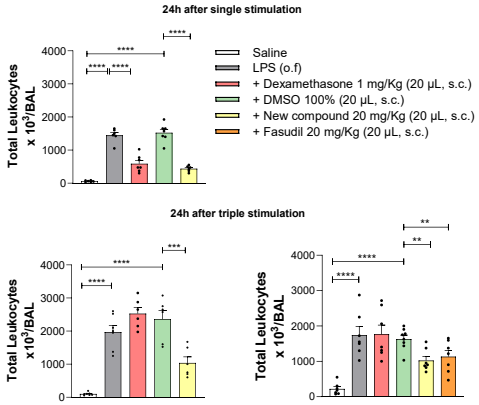
A/J mice were pre-treated with the new compound (20mg/mL), fasudil (20mg/mL) or dexamethasone (1mg/Kg) subcutaneously 1 h before stimulation with a single (25µg/40µL) or triple (10µg/40µL) LPS instillation by oropharyngeal aspiration. Twenty-four hours after the last stimulation, we evaluated the effect of the new compound on bronchoconstriction and airway hyperreactivity. We observed that animals subjected to single or triple stimulation with LPS presented increased Penh values even in the absence of methacholine (PBS aerosol). In the presence of the spasmogenic agent, the response was exacerbated, demonstrating airway hyperreactivity. Dexamethasone and 100% DMSO (treatment vehicle) did not alter this response under conditions in which the new compound significantly inhibited it (Figure 1).

Figure 1: Effect of subcutaneous treatment with the new compound and dexamethasone on airway hyperreactivity.



Treatment with the new compound significantly reduced total leukocyte counts in bronchoalveolar lavage (BAL) effluent after single or triple LPS stimulation. We compared the efficacy of the new compound with the reference ROCK inhibitor Fasudil, and a comparable protective effect was evident after triple LPS stimulation (Figure 2).

Figure 2: Effect of subcutaneous treatment with the new compound, fasudil and dexamethasone on total leukocyte count in BAL.



Furthermore, treatment with the new compound significantly reduced total protein levels in the BAL effluent and levels of malondialdehyde (MDA), a product of lipid peroxidation and an important marker of oxidative damage in lung tissue after triple stimulation with LPS.

Conclusions

Our findings showed that Rho/ROCK signalling is an essential target in the molecular mechanism that responds to glucocorticoid insensitivity, and that this new N-acylhydrazone derivative and ROCK inhibitor may be a promising therapeutic alternative for treating GC-resistant lung inflammation.

Acknowledgements

FIOCRUZ, FAPERJ, CAPES, CNPq and LASSBio, for their essential role in this study.

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Antiviral activity of novel eugenol–1,2,3-triazole derivatives targeting Mayaro virus

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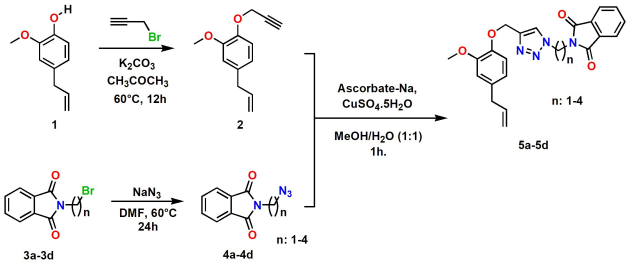
Key words: Mayaro virus, Antiviral agents, Eugenol derivatives 1,2,3-triazole, Click chemistry reaction.

Introduction

Mayaro virus (MAYV) is an emerging arbovirus in South America for which no specific antiviral treatment is currently available.[1] Efforts to identify antiviral agents have increasingly focused on bioactive natural products, which have historically served as valuable scaffolds in drug discovery.[2] Eugenol, a phenolic constituent of Eugenia caryophyllata essential oil exhibits broad pharmacological potential, including antiviral.[3] In this study, we report the synthesis and biological evaluation of four novel 1,2,3-triazole-isoindole-1,3-dione derivatives (**5a–d**) as potential antiviral agents against MAYV.

Results and Discussion

Following our synthetic strategy, the target compounds were obtained via copper (I)-catalyzed azide–alkyne cycloaddition (CuAAC) between azido phthalimide intermediates (**4a–4d**) and a terminal alkyne-functionalized eugenol derivative (**2**). (**Scheme 1**).



Scheme 1. General route of synthesis for 1,2,3-triazole-isoindole-1,3-dione derivatives (**5a-5d**).

Structural characterization of all derivatives was confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Antiviral activity was assessed *in vitro* using VERO cells infected with MAYV. Among the series, compounds **5a** and **5c** showed potent dose-dependent inhibition of MAYV replication, achieving > 99% inhibition at 20 µM, with EC_{50} values of 1.2 ± 0.08 and 0.62 ± 0.03 µM, respectively. Both compounds outperformed the reference antiviral ribavirin (EC_{50} of >100 µM). Cytotoxicity studies indicated low toxicity, with CC_{50} values ranging from 701 to 930 µM, and cell viability maintained at ~90% at effective concentrations. Additionally, virucidal assays confirmed that **5a** and **5c** significantly reduced viral replication by over 60% and 80%, respectively (**Figure 1**).

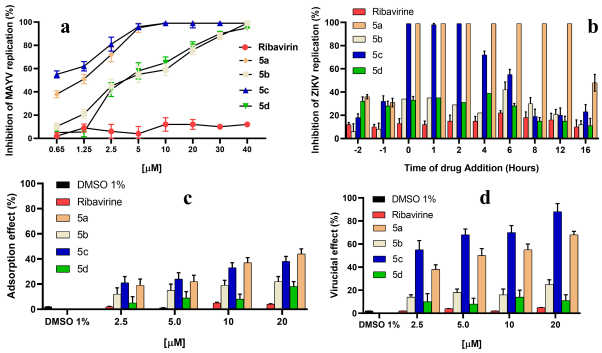


Figure 1. A. Evaluation of the anti- MAYV activity, **B**. virucidal assay, **C**. assessment of adsorption activity at an early stage of the viral life cycle, **D.** Inhibition by the time of addition of the compounds.

Conclusions

Four novel eugenol-1,2,3-triazole derivatives (**5a–5d**) were synthesized and evaluated against Mayaro virus (MAYV). Compounds **5a** and **5c** exhibited potent antiviral activity (EC_{50}: 1.2 µM and 0.62 µM, respectively), high selectivity, and strong virucidal effects, outperforming ribavirin with low cytotoxicity. These findings identify **5a** and **5c** as promising candidates for further in vivo studies.

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CAPES
PGQ Universidade Federal Fluminense UFF
CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico
LAME LAMATE

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[3] Y. Wang, et. al. *Fish Shellfish Immunol.* **2024**, *151,* 109748.

Synthesis and anticancer activities of novel 1,2,3-Triazole analogues of Lonidamine

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Key words: Lonidamine, 1,2,3-triazole, molecular hybridization, anticancer activities.

Introduction

Cancer is the 2nd cause of deaths in the world, only losing to cardiovascular conditions. Besides the benign or malign forms, there are more than one hundred types of cancer, which may be defined as an agglomerate of abnormal cells with uncontrollable growth, causing issues to the organism. Lonidamine is an anticancer active drug developed in 1976 initially for other biological targets. This substance had shown an improvement in chemotherapies been used as a combination with other compounds such as doxorubicin, against ovarian cancer (e.g.). Even so, lonidamine production was stopped in 2006 for economic reasons. Despite this, its potential anticancer properties are still promising, and besides the not high potency observed for itself, structural optimizations can be led. This work proposes some modifications: indazole hydrogenation, esterification of its carboxylic acid site, and hybridization of it by the introduction of 1,2,3-triazole ring. Changing the position of connection terminal triazole and benzyl coupled scaffolds was also a strategy, and it has shown good results after biological tests. All those possibilities were tested, isolated and combined to identify the best possible synergic improvements. A relevant observed result was the modifications of the substituents in the phenyl ring, replacing the chlorine original atoms. The study has shown very promising results in selecting some samples as significant hits that will be explored next in vivo and clinical trials. **Figure 1** below shows structural molecule changes planned and made over lonidamine original drug.

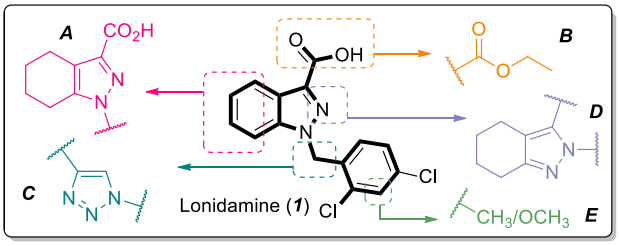
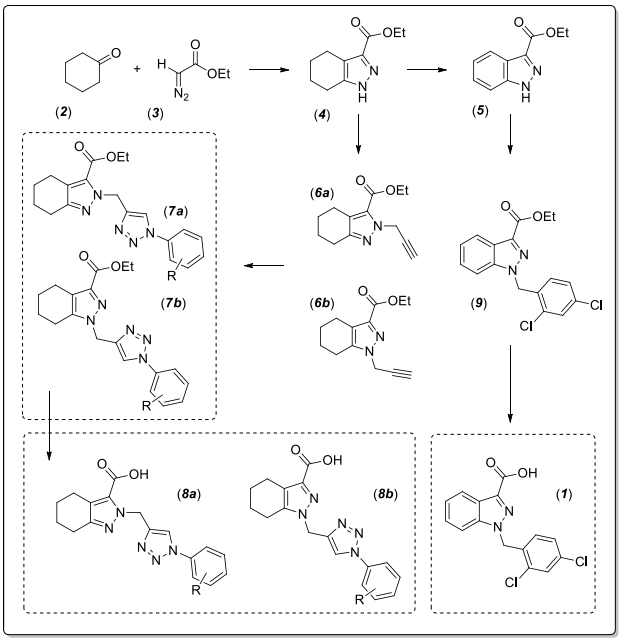


Figure 1. Lonidamine (**1**) in bold highlighting and modifications: (**A**) hydrogenation; (**B**) esterification; (**C**) hybridization with triazole; (**D**) isomerization; (**E**) substituents modifying the terminal benzene.

Results and Discussion

The insertion of hydrogen, ester, and triazole ring, have shown synergic improvement to anticancer activities, even better with bottom electron donor groups. A new route to lonidamine was established.



Scheme 1. Synthesis of lonidamine and analogues.

Table 1. Inhibitory relation against tumor lines.

Compound	(**1**) LND	(**7a**) *o*-OCH_3	(**7b**) *m*-OCH_3
A549: EC_{50} (μM)	222,8	64,2	52,4
22Rv1: EC_{50} (μM)	>500	82,8	73,2

Conclusions

Combined structural changes increased anticancer potency against A549 (lung) and 22Rv1 (prostate) and selectivity against WSS-1 (healthy kidney cells) by up to fivefold, outperforming doxorubicin (control).

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Design, Synthesis, and Biological Evaluation of Aryldiazepane Derivatives Against *Trypanosoma cruzi*

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Key words: Hit-to-lead optimization, Structure–activity relationship, pharmacokinetics.

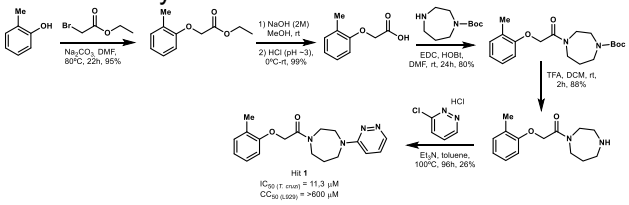
Introduction

Chagas disease is a neglected tropical illness caused by the protozoan parasite *Trypanosoma cruzi*, transmitted mainly by triatomine insects. Current treatments, such as benznidazole, have limitations related to efficacy and safety, especially in the chronic phase[1]. In this context, this study aims to contribute to the discovery of new anti-*T. cruzi* drug candidates through structural optimization of hit **1** (Scheme 1), identified via HTS and selected based on its *in vitro* potency ($IC_{50}^{T.cruzi}$ = 0,6 μM).

Results and Discussion

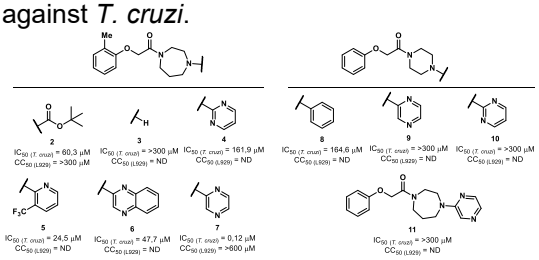
Hit **1** was synthesized via linear synthetic route (Scheme 1). Its *in vitro* activity against *T. cruzi* (amastigote) was confirmed through IC_{50} determination in biological assays.

Scheme 1. Synthetic route of the hit **1**.



Subsequently, structural modifications were carried out on all fragments of hit **1** (Figure 1).

Figure 1. Derivatives of hit **1** and their *in vitro* activity against *T. cruzi*.



All synthesized compounds were fully characterized by HRMS, as well as by ^{1}H and ^{13}C NMR spectroscopy. *In vitro* IC_{50} assays against amastigote of *T. cruzi* enabled structure-activity relationship exploration, revealing valuable insights into the series' biological profile. Cytotoxicity assays against L929 fibroblast cells indicated low cytotoxicity across the series, supporting a favorable selectivity index for the parasites. Among the synthesized derivatives, the pyrazine analogue stood out for its high *in vitro* potency, exhibiting low IC_{50} values and minimal cytotoxicity. The *in vitro* ADME evaluation of the pyrazine derivative (**7**) demonstrated excellent permeability, high kinetic solubility (at pH 2.0 and 7) and ideal eLogD values at pH 7.4, indicating strong potential for passive diffusion and favorable oral absorption (Table 1). Also, metabolic stability was assessed in mouse and human liver microsomes. Although the pyrazine derivative exhibited high clearance and a short half-life in human liver microsomes, its metabolic profile in mouse microsomes was more balanced, with moderate clearance and half-life values. These findings support a moderate pharmacokinetic profile and suggest potential for prolonged *in vivo* efficacy.

Table 1. Pharmacokinetic profile of derivative **7**.

Compound 7	eLogD (pH 7.4)	KS[a]	Permeability TGI (10^{-6} cm/s)	MLM[b]	HLM
	2.79	>84,41 μM (pH 2.0) >89,79 μM (pH 7.4)	41,61 High permeability	**Cl** = 98,80 μL/min/mg Moderate clearance $t_{1/2}$ = 28,06 min	**Cl** = 370,80 μL/min/mg High clearance $t_{1/2}$ = 7,48 min

a: Kinetic solubility (KS), b: metabolic stability in mouse liver microsomes (MLM), and human liver microsomes (HLM), expressed as intrinsic clearance (Cl, μL/min/mg) and half-life ($t_{1/2}$, min).

Conclusions

The synthetic strategy proved to be efficient, enabling the successful synthesis of all derivatives. Structural modifications provided key insights into the SAR of the series. Notably, derivative **7** showed a low IC_{50} value and a moderate pharmacokinetic profile in mouse liver microsomes. Future efforts will focus on further structural optimization to improve its pharmacokinetic properties.

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Antiplasmodial activity assessment of novel pyrrole-based compounds as lead candidates for malaria.

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Key words: malaria, pyrrole, medicinal chemistry, P. falciparum.

Introduction

Malaria, caused by protozoa of the genus *Plasmodium*, remains a leading cause of morbidity and mortality in endemic regions, disproportionately affecting children under five and pregnant women. Approximately 3.2 billion people worldwide are at risk of infection, with 263 million cases and over 597.000 deaths reported in 2023, predominantly in Africa[1]. The rapid emergence and dissemination of drug-resistant *Plasmodium* strains highlight the urgent need for new chemotherapeutic agents. Here, we report the antiplasmodial evaluation of 14 novel pyrrole-based compounds rationally designed by the research group of Prof. Arlene G. Corrêa (Department of Chemistry, Federal University of São Carlos, Brazil). ACS Omega 2025, 10, 17, 18017–18028.

Results and Discussion

The antiplasmodial potential of the compounds was evaluated against the chloroquine-sensitive *Plasmodium falciparum* 3D7 strain, in parallel with cytotoxicity and selectivity profiling in HepG2 cells. Of the fourteen compounds screened, four exhibited potent low-micromolar activity and were identified as the most promising candidates. These compounds showed low cytotoxic activity on HepG2 cells and achieved selectivity indexes greater than 10 (Table 1). Nitrogen-containing heterocycles are structural motifs frequently found in a wide range of pharmaceuticals[2], underscoring their relevance in rational drug design. The pyrrole nucleus is considered a privileged scaffold in medicinal chemistry, as it occurs in numerous bioactive molecules with diverse therapeutic profiles, including antimalarial, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, and antiviral properties. In this context, pyrrole-based compounds emerge as promising candidates, offering greater stability, bioavailability, and target specificity[3]. Based on these results, complementary assays will be conducted to further characterize the bioactive profile of the most promising compounds and investigate the possible mechanisms of action responsible for the observed antiplasmodial activity.

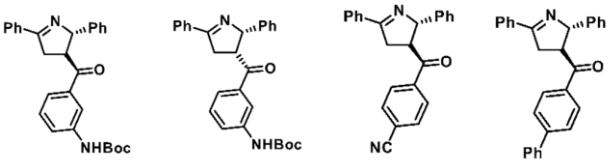


Figure 1. Molecular structures of the most promising compounds.

Table 1. Evaluation of the antiplasmodial activity against *P. falciparum* (IC_{50}^{3D7}), cytotoxicity in human hepatocarcinoma cells (CC_{50}^{HepG2}), and selectivity indices for the evaluated chemical series.

Code	IC_{50}^{3D7} (μM)	CC_{50}^{HepG2}	SI
LSPN994	4.87 ± 0.05	> 100	> 20
LSPN995	2.9 ± 0.6	> 100	> 34
LSPN996	9.1 ± 0.3	> 100	> 11
LSPN998	6 ± 1	> 100	> 16
artesunate	0.013 ± 0001	ND	ND

ND: not determined

Conclusions

Phenotypic screening of the fourteen chemical compounds identified four samples with significant antiplasmodial potency (IC_{50} ≤ 10 μM) and adequate selectivity for *P. falciparum* over mammalian HepG2 cells, exhibiting selectivity indices (SI) greater than 10. These findings suggest that the identified compounds are strong candidates for further investigation.

Acknowledgements

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