BOOK OF ABSTRACTS

We are excited to invite you to join us for the **OneHealth*drugs* Marathon**, a series of non-stop online presentations and discussions taking place from **December 2-5, 2025**. The One Health approach recognizes the fundamental interconnectedness of human, animal, and ecosystem health. By fostering collaboration across diverse sectors, we can more effectively address global health challenges, ranging from zoonotic diseases to critical environmental issues. This global initiative underscores the synergy required to achieve optimal health outcomes for all.

Through the **EU COST Action OneHealthdrugs (CA21111)**, we are committed to revolutionizing drug development. Our focus is on innovating drug discovery to ensure precise actions and the early assessment of environmental impact, ultimately leading to optimal drug profiles that align with the One Health paradigm.

The OneHealthdrugs Marathon offers a unique platform to:

Share Research: All network participants are invited to present their latest research, enriching our collective understanding of the diverse research landscape within the network.

Attract New Talent: Provide an engaging environment to attract and welcome new applicants to our growing network.

Building Community: Foster a positive atmosphere of open collaboration and knowledge exchange among peers.

Integrate Innovation: Facilitate the integration of new concepts and innovative technologies into ongoing research programs through potential collaborations, training schools, and STSM-COST Action fellowships.

This inspiring 4-day marathon of insightful presentations, expert opinions, and invaluable opportunities to connect with fellow researchers and innovators who are passionate about health and sustainability. Together, we can drive significant advancements and make a tangible difference in our communities and beyond.

December 2025
Tuesday, 02 of December 2025
Structural biology and Medicinal chemistry (including Natural products)

Moderators: Ulrike Wittig and Theodora Calogeropolou
14.30: Welcome (Theodora Calogeropolou, Ulrike Wittig)
Brief introduction MPCosti

14.40: Biljana Arsić, University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Niš, Republic of Serbia.
From Existing Medicines to New Applications: Drug Repurposing Approach for Dengue Feve.

15.00: Hamed Eid A. Alkhalaf, School of Infection and Immunity, University of Glasgow, Glasgow UK.
Metabolism of anti-kinetoplastid nucleoside leads in Leishmania and Trypanosoma species.

15.20: Ivan Bassanini, SCITEC, National Research Council of Italy, Italy.
From Plasmepsin Inhibitors to a Novel Highly Potent Antiplasmodial Chemotype with an Unidentified Target.

15.40: Lori Doko, Universita' degli studi eCampus.
High-Throughput In Vitro Screening of Antiparasitic Compounds for Neglected Tropical Diseases.

16.00: Break

16.20: David C. Magri Department of Chemistry, Faculty of Science, University of Malta, Malta
Cinchona Alkaloid Copolymer Logic Gates as Fluorescent Sensing Tools

16.40: Closing remarks

Wednesday, 03 of December 2025
Integration of Ecotoxicology in drug discovery

Moderators: Harry de Koning and Lorenzo Raffellini
Welcome (Harry de Koning and Lorenzo Raffellini)
9.30: Harry de Koning – Updates on the activity ongoing in the veterinary drugs studies

9.50: Lorenzo Raffellini, University of Pisa, Department of Pharmacy, Italy.
Nature-Driven Drug Discovery: Antimicrobial Potential of Pomegranate Constituents.

10.10: **Maria Paola Costi** – University of Modena and Reggio Emilia, Italy.
Fragment-Based Drug Design As A Working Strategy For The Discovery Of Novel And Safer Antiparasitic Agents?

10.30: Eleni Chontzopoulou, Cloudpharm, Athens, Greece.
G.AI.A: An Integrated Machine-Learning Platform for Predicting Bioaccumulation and Ecotoxicity of Pharmaceuticals

10.50: Sandra Gemma, Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy.
Oxyprenyl-Chalcones as Emerging Antinfective Candidates: From DoE-Driven Synthesis to Drug-Like Profiling and Biodegradability Prediction

11.10: Break

11.30: Bianca Martinengo, Department of Pharmacy and Biotechnology, Alma Mater Studiorum – University of Bologna.
Sustainable antiparasitic agents from an agro-industrial waste: mitochondria-targeting agents from cashew nutshell liquid

11.50: Jirgensons Aigars, Latvian Institute of Organic Synthesis, Latvia.
Inhibitors of Plasmodium serine protease SUB1 as anti-malarial drug leads.

12.10: Daniele Aiello, Department of Life Sciences, University of Modena and Reggio Emilia, Italy.
Integrating Ecotoxicological Constraints into Early Antiparasitic Drug Discovery: A Machine-Learning and GreenDrugScore Framework.

12.30: Closing remarks

Thursday, 04 of December 2025
YRI presentations

Moderators: Elisa Uliassi and Gülşah Bayraktar

14.30: Welcome (Elisa Uliassi and Gülşah Bayraktar)

Elisa Uliassi Updates about OHD COST Action Young Researchers and Innovators activity

14.40: Morgane Picard, INSERM U1124, Paris University, Paris, France**.**
Persistent myeloid cell reprogramming despite miltefosine treatment in leishmania-infected macaques.

15.00: Filippo Piazza, University of Bologna, Bologna, Italy.
Powering New Antiviral Frontiers: PROTAC-Based Approach for Emerging Flavivirus.

15.20: Gian Marco Elisi, Department of Biomolecular Sciences, University of Urbino Carlo Bo, Italy.
Integrated pharmacophore screening workflow for target identification and selection of antileishmanial compounds with an improved chemical profile.

15.40: Aurora Gaza, Department of Pharmacy and Biotechnology, Alma Mater Studiorum – University of Bologna.
Hydrophobic Tag Degraders (HyT) Targeting Trypanothione Reductase as a Potential Strategy Against Leishmaniasis.

16.00: Break

16.20: Huseyin Kosker, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Izmir Kâtip Celebi University, Izmir, Turkey
Synthesis, Structure Elucidation, Antimycobacterial and Antiparasitic Activities of New Piperidinhydrazide-hydrazone Derivatives

16.40: Cecilia Pozzi, University of Siena, Italy.
High throughput cloning, production, and crystallization of Leishmania infantum calpain, a new promising target against Leishmaniasis.

17.00: Closing remarks

Friday, 05 of December 2025
Parasitology and pharmacology and One Health

Moderators: Guy Caljon and Nuno Santarem
10.00: Welcome (Guy Caljon and Nuno Santarem)

Guy Caljon Updates about OHD COST Action activities in preclinical drug development

10.10: Ehab Kotb Elmahallawy*, University of Glasgow, UK.
Unraveling the Mechanisms of Action of Antikinetoplastid Nucleoside Prodrugs: Bridging the Gap in Drug Efficacy and Mechanisms

10.30: Calvo-Alvarez Estefania, Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy.
Exploring cryptic reservoirs and neuroimmune modulation by visceral Leishmania in the brain

10.50: Van den Broeck Lauren, Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp.
In vivo selection of a Leishmania cosmid library reveals candidate genes involved in sand fly transmission.

11.10: André Lopes, Centre of Chemistry of University of Minho, Campus de Gualtar, Universidade do Minho, 4710-057 Braga, Portugal.
Antileishmanials of 8-(Haloaryl)-Substituted Pyrimidopyrimidines: New insights into Structure-Activity Relationship.

11.30: Break

11.50: Sérgio Araujo, Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, Belgium.
Unveiling glial cells in the nasal mucosa as host cells for Leishmania with potential implications in disease outcomes

12.10: YRI award communication

12.30: Closing remarks

Medicinal Chemistry and Structural Biology (including Natural products)

2025-12-02

DAY 1 (02/12): https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1762122603736?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

From Existing Medicines to New Applications: Drug Repurposing Approach for Dengue Fever

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Dengue fever is a disease caused by the dengue virus. Every year, 100–400 million people are infected, most often through the bite of mosquitoes, specifically the *Aedes aegypti* species, and less commonly by *Aedes albopictus*, *Aedes polynesiensis*, and *Aedes scutellaris* [1]. According to the World Health Organization, a cure for the dengue virus has not yet been found. Currently, pain-relieving medications are used to treat infected individuals [2]. Drug repurposing analysis was conducted *via* the Drugst.One platform using the Drug Search module, and Network Proximity algorithm [3]. Found candidates originate from diverse therapeutic fields, including oncology, dermatology, infectious disease treatment, cardiovascular therapy, respiratory medicine, and neurology. Their presence aligns with published findings linking several chronic conditions to worse dengue outcomes. Neurological and psychiatric effects have been reported as well, including increased risks of anxiety, depression, and temporary sleep disorders after infection. It is necessary to determine the predictive ability of the applied theoretical approach and to further test the compounds identified in this research both *in vitro* and *in vivo*. The obtained candidates suggest that a potential drug or therapeutic contribution for dengue fever may be found across a wide range of medications. Examining the synergistic effects of these compounds, or their use in combination therapy, is desirable, as it could enhance the existing treatment options for dengue fever.

References

[1] Nanaware, N.; Banerjee, A.; Bagchi, S.M.; Bagchi, P.; Mukherjee, A. Dengue virus infection: a tale of viral exploitations and host responses. *Viruses* **2021**, *13*, 1967.

[2] WHO, 2025, https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

[3] Maier, A.; Hartung, M.; Abovsky, M.; Adamowicz, K.; Bader, G.D.; Baier, S.; Blumenthal, D.B.; Chen, J.; Elkjaer, M.L.; GarciaHernandez, C.; et al. Drugst.One-a plug-and-play solution for online systems medicine and network-based drug repurposing. *Nucleic Acids Res.* **2024**, *52*, W481–W488.

Acknowledgment

Authors want to thank for the financial support for this study to the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (project nos. 451-03-66/2024-03/200124 and 451-03-136/2025-03/200124 (Denis Mitov, Biljana Arsić), 451-03-137/2025-03/200113 (Budimir S. Ilić)). Also, thanks go to CA21111-One Health drugs against parasitic vector borne diseases in Europe and beyond (OneHealthdrugs).

Metabolism of anti-kinetoplastid nucleoside leads in *Leishmania* and *Trypanosoma* species.

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The aim of this Short-Term Scientific Mission (STSM) was to examine the enzymatic activation and intracellular metabolism of selected adenosine analogues in species of *Trypanosoma* and *Leishmania* in order to understand the profound differences in their drug sensitivities. *T. brucei* adenosine kinase (TbrAK) was first created but inactive and replaced with that from *T. vivax* (TvxAK).
TvxAK and *Leishmania mexicana* Adenosine Kinase (LmxAK) were expressed as His-Tagged proteins in *Escherichia coli* and purified by affinity chromatography. The data were collected through enzyme activity assay with selective adenosine analogues (50 µM – 1 mM), incubated 30 min at 37 °C. K_m and V_{max} values were determined for each kinase substrate pair. The analysis of reaction products and metabolites were done by high performance liquid chromatography (HPLC).
After the most efficiently phosphorylated substrates had been identified, mono, di, and triphosphate levels of these substrates were then examined in extracts of *L. mexicana* and *T. brucei* incubated with the analogues. Further analyses explored stability of the analogues and the degree of deribosylation by nucleoside hydrolases, nucleoside phosphatases or the *T. brucei* MTAP enzyme. These HPLC peaks represented all of the metabolites produced. IC_{50} values of selected analogues were determined using a scintillation counter.

References

1-Hulpia F, Campagnaro GD, Scortichini M, Van Hecke K, Maes L, De Koning HP, Caljon G, Van Calenbergh S (2019) Revisiting tubercidin against kinetoplastid parasites: aromatic substitutions at position 7 improve activity and reduce toxicity. Eur J Med Chem 164:689–705
2-Hulpia F, Mabille D, Campagnaro GD, Schumann G, Maes L, Roditi I, Hofer A, De Koning HP, Galjon G, Van Calenbergh S (2019) Combining tubercidin and cordycepin scaffolds

results in highly active candidates to treat late-stage sleeping sickness. Nat Commun 10:5564
3-Ranjbarian F, Vodnala M, Alzahrani KJH, Ebiloma GU, De Koning HP, Hofer A (2017) 9-(2′ -Deoxy-2′-Fluoro-β-D Arabinofuranosyl) Adenine Is a Potent Antitrypanosomal Adenosine Analogue That Circumvents Transport-Related Drug Resistance. Antimicrob Agents Chemother 61: e02719-16

Acknowledgment
The gratefully acknowledges the financial and organizational support provided by COST Action CA21111.

From Plasmepsin Inhibitors to a Novel Highly Potent Antiplasmodial Chemotype with an Unidentified Target

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In the quest for novel antimalarial agents, our research initially focused on the inhibition of plasmepsins (PLMs), aspartic proteases involved in haemoglobin digestion within the Plasmodium falciparum food vacuole. A series of statine-based peptides combining a PLM-inhibiting core and the 4-aminoquinoline scaffold of chloroquine was synthesized and evaluated. Unexpectedly, a weak correlation between PLM inhibition and parasite growth suppression suggested a distinct mechanism of action. [1, 2]
This observation led to intensive SAR-campaigns and the identification of a new lead compound, **DC18**, characterized by a chemotype, featuring a 4,4'-oxybisbenzoyl amide core, endowed with potent nanomolar activity against both chloroquine-sensitive and -resistant *P. falciparum* strains, despite lacking any known antimalarial pharmacophore and acting through an unidentified target. [3]
To improve **DC18**'s druggability, we then designed and synthesized structurally simplified analogues through a rational, *in silico*-guided approach. Peptide substituents were replaced with metabolically stable non-proteinogenic amino acids (2-aminoisobutyric acid, *tert*-butyl glycine, cyclohexyl glycine) or with chiral amino-alcohols to enhance stability and bioavailability. Among the new derivatives, **MR07**—bearing a *tert*-butyl glycine and a (*1S,2S*)-aminocyclohexanol moiety—displayed remarkable *in vitro* potency and an improved metabolic profile in both murine and human microsomal assays. [4]
Overall, this work defines the 4,4'-oxybisbenzoyl amide scaffold as a promising and unprecedented antiplasmodial chemotype, representing a potential starting point for the development of next-generation antimalarial agents acting through an as-yet unidentified mechanism whose elucidation is the current focus of our research efforts.

References

[1] S. Romeo *et al.* Bioorg Med Chem Lett. 2004, 14(11):2931-4.

[2] A. Pancotti, Med. Chem. Commun. 2015, 6:1173 – 1177.
[3] I. Bassanini *et al.* ChemMedChem. 2019,14(23):1982-1994
[4] I. Bassanini *et al.* ChemMedChem. 2022, 17(21):e202200355

High-Throughput In Vitro Screening of Antiparasitic Compounds for Neglected Tropical Diseases

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Background: Neglected tropical diseases (NTDs) such as human African trypanosomiasis, Chagas disease, and leishmaniasis lack sufficient therapeutic options, prompting high-throughput in vitro screening to discover new leads.
The screening follows a **standardised 96-well plate format** for each pathogen. Parasites are cultured under defined conditions (T. brucei in HMI-9 medium with 10 % FCS; T. cruzi Tulahuen β-galactosidase strain in MRC-5SV2 medium; L. infantum and L. donovani amastigotes harvested from infected hamster spleens and co-cultured with primary peritoneal mouse macrophages) at 37 °C, 5 % CO_2.
Compounds are prepared in **4-fold serial dilutions** (five or ten concentrations ranging from 64 µM down to 0.00024 µM) and added (10 µL) to each well containing 190 µL of parasite-cell inoculum. After incubation (3 days for T. brucei, 7 days for T. cruzi, 5 days for Leishmania), parasite growth is quantified: resazurin fluorescence for trypanosomes, CPRG-based colorimetric read-out for T. cruzi, and microscopic counting of amastigotes per macrophage for Leishmania.
IC_{50} values are calculated from dose-response curves; activity scores >3 trigger confirmatory testing. **Reference drugs** (Suramin or Melarsoprol for sleeping sickness, Nifurtimox for Chagas, Amphotericin B or Miltefosine for Leishmania) provide assay controls.
Parallel **cytotoxicity** is assessed on human MRC-5 fibroblasts using the same concentration series; cells are exposed to compounds in MEM with 5 % FCS, incubated 3 days, and viability measured by resazurin fluorescence. Compounds with MRC-5 IC_{50} > 30 µM are deemed non-toxic.
Selectivity is inferred when a compound shows potent antiparasitic IC_{50} (≤10 µM for most assays) **and** no cytotoxicity (MRC-5 IC_{50} > 30 µM).
Results & Conclusions: The SOPs enable rapid generation of reliable IC_{50} datasets, facilitating the prioritization of compounds that combine potent antiparasitic activity

with low mammalian toxicity. This workflow supports an efficient drug-discovery pipeline for NTDs and can accelerate the identification of candidates for downstream in vivo validation and clinical development.

References

Caljon, G. (2023). Standard operating procedures for DNDi in vitro screening against sleeping
sickness, Chagas disease, and leishmaniasis.
Buckner, F. S., Verlinde, C. L., La Flamme, A. C., & Van Voorhis, W. C. (1996). Efficient technique
for screening drugs for activity against Trypanosoma cruzi using parasites expressing β-galactosidase. Antimicrobial Agents and Chemotherapy, 40(11), 2592-2597.
Stauber, L. A. (1966). Characterization of strains of Leishmania donovani. Experimental Parasitology, 18, 1-11.

Acknowledgment

I thank Prof. Dr Guy Caljon and the entire lab team for their scientific support, availability, and professionalism throughout the project. A special thanks to COST Action CA21111 – One Health Drugs for enabling this international collaboration and for fostering a stimulating and multidisciplinary environment. Finally, I thank eCampus University for the constant academic support and for providing the theoretical and methodological foundation necessary to undertake this research experience.

Cinchona Alkaloid Copolymer Logic Gates as Fluorescent Sensing Tools

Medicinal Chemistry including Natural Products

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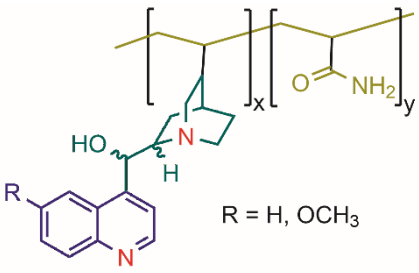
The field of molecular logic began with a molecule synthesized in an organic laboratory,[1] and ever since, so have many thousands of molecular logic gates. Nearly 3000 papers later,[2] solving real-life problems is at the forefront of molecular logic-based computation.[3] We are exploring fluorescent natural products (FNPs) as naturally sourced intelligent logic gates requiring little or no synthetic effort. Although FNPs have been known to chemists long before the birth of molecular logic,[1] and even before the dawn of mathematical logic by George Boole,[4] they have rarely featured as intelligent computing elements.[2] In this presentation, we will demonstrate that the *cinchona* alkaloids,[5] including the antimalarial quinine, and *cinchona* copolymers,[6,7] are fluorescent combinatorial INHIBIT logic gates.[5] Small molecule medicines and polymers with fluorescence and computing capabilities could be tools as theranostics for vector-borne infectious diseases.



References

[1] A. P. de Silva, H. Q. N. Gunaratne, C. P. McCoy, *Nature* **1993**, *364*, 42-44.
[2] N. Agius, D. C. Magri, *Natural Prod. Commun*. **2024**, *19*, 1-11.
[3] A. P. de Silva, *Molecular Logic-based Computation*, RSC, Cambridge, **2013**.
[4] G. Boole, An Investigation of the Laws of Thought, on Which are Founded the

Mathematical Theories of Logic and Probabilities, Walton & Maberly, **1854**.
[5] N. Agius, D. C. Magri, *RSC Adv*. **2023**, *13*, 13505-13510.
[6] N. Agius, C. J. Ashton, H. Willcock, D. C. Magri, *RSC Adv*. **2025**, *15*, 11121-11127.
[7] N. Agius, D. C. Magri, *New J. Chem.* **2025**, *49*, 10522-10529.

Acknowledgment

The Authors acknowledge the FUSION R&I: Research Excellence Programme, grant agreement no. REP-2023-023, administered by Xjenza Malta; and the University of Malta for the financial support.

Integration of Ecotoxicology in drug discovery

2025-12-03

DAY 2 (03/12): https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1762122757395?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

Meeting ID:

Nature-Driven Drug Discovery: Antimicrobial Potential of Pomegranate Constituents

Lorenzo Raffellini, [a] Oliver Keminer, [b] Reagon Karki, [b] Sheraz Gul, [b] Clementina Manera, [a] Simona Rapposelli [a]

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Ellagic Acid (EA) is a polyphenolic compound found in pomegranates, known for its noteworthy biological properties. Indeed, EA demonstrated antioxidant, anti-inflammatory, and anti-infective effects[1,2]. In our project, we utilized discarded pomegranate peels to create an EA-rich extract, which has potential applications in personal care products, dietary supplements, and agricultural antimicrobials. The extraction process we employed is sustainable, as it avoids the use of chemical solvents and operates under low-temperature conditions, thus minimizing environmental impact while preserving the bioactive compounds' integrity. We also assessed the ecotoxicity of the extracts, and studies on their antileishmanial activity are currently underway. From a medicinal chemistry standpoint, we have focused on synthesizing novel molecules derived from urolithins—metabolites of EA produced by the gut microbiota. Urolithins are known to have higher bioavailability than EA and offer enhanced systemic health benefits[3]. As part of this project, we designed and synthesized a small library of urolithin analogues, aiming to develop bioactive compounds with potential anti-infective properties. This presentation will outline the techniques used to produce the extract, the synthesis of the urolithin derivatives, and will present preliminary biological findings.

References

1. Dell'Agli, M. *et al.* Antiplasmodial activity of Punica granatum L. fruit rind. *Journal of Ethnopharmacology* **125**, 279–285 (2009).
2. Acquadro, S. *et al.* Punica granatum Leaf Ethanolic Extract and Ellagic Acid as Inhibitors of Zika Virus Infection. *Planta Med* **86**, 1363–1374 (2020).
3. Tomás-Barberán, F. A., García-Villalba, R., González-Sarrías, A., Selma, M. V. & Espín, J. C. Ellagic Acid Metabolism by Human Gut Microbiota: Consistent Observation

of Three Urolithin Phenotypes in Intervention Trials, Independent of Food Source, Age, and Health Status. *J. Agric. Food Chem.* **62**, 6535–6538 (2014).

Acknowledgment
The Authors acknowledge OHD (Cost Action 21111) and Floratek Pharma for the financial support.

Fragment-Based Drug Design as A Working Strategy for the Discovery of Novel and Safer Antiparasitic Agents?

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Parasitic diseases like trypanosomiasis and leishmaniasis affect billions globally, necessitating urgent new drug development. Trypanosomatids, critical for these diseases, rely on salvaged folates for survival. While they lack *de novo*folate synthesis, typical antifolates are ineffective due to the compensatory role of pteridine reductase-1 (PTR1). Consequently, PTR1 represents a prime drug target which we have been working for several years to exploit as a preclinical drug target. We have developed first a classical FBDD approach [1]. Recently we reviewed the results and developed a GreenDrugScore to evaluate the potential environmental impact of the translational compounds [2]. We exploited the contribution of the modular fragment with respect to the final compounds after the FBDD elaboration. The workflow of the study is developed through ì. a fragment-based drug design (FBDD) approach, beginning with crystallographic screening to identify pteridine-like fragments binding to PTR1 (Ki: 10^{-3}-10^{-4} M). ìì. Subsequent structure-based design, employing fragment growth and linking strategies, yielded two series of high-affinity, potent compounds. ìii Biological evaluation. Comprehensive enzymatic assays (PTR1, TS, DHFR) and early toxicity profiling were performed via HTS. iV. Early prediction of the compounds ADME-TOX/EcoTOX. Seven new crystal structures of *Tb*PTR1-inhibitor complexes confirmed design hypotheses and revealed additional binding sites. *In vitro* testing showed promising antiparasitic activity, selectivity, and synergy with methotrexate against *T. brucei*. This pipeline successfully identified lead candidates with reduced liabilities, suitable for advanced *in vivo*pharmacokinetic and efficacy studies in mouse models, demonstrating FBDD as a powerful strategy for developing high-quality potent and safer antiparasitic drug leads.

References
[1] JoannaPanecka-Hofman et al. J. Med. Chem. 2025, 68, 20595−20618
[2] Daniele Aiello et al. Submitted

Acknowledgment
The Authors acknowledge NMTRYPI (Grant agreement ID: 603240) and COST ACTION CA21111 for the financial support.

G.AI.A: An Integrated Machine-Learning Platform for Predicting Bioaccumulation and Ecotoxicity of Pharmaceuticals

: Eleni Chontzopoulou [a]

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Pharmaceutical pollution in aquatic environments poses a significant ecological threat due to the accumulation of bioactive compounds from human and veterinary sources. In support of the EU Green Deal's Chemicals Strategy for Sustainability, this study presents a computational framework for predicting two key environmental risk indicators in fish: bioconcentration and ecotoxicity. Bioconcentration, quantified by the bioconcentration factor (BCF), reflects a chemical's tendency to accumulate in organisms, while ecotoxicity is assessed via the median lethal concentration (LC50) over defined exposure periods. We developed two high-performing machine learning (ML) models, achieving ROC AUC scores of 94.60% for bioconcentration and 96.06% for ecotoxicity, validated across both internal and external datasets. To expand the scope of risk evaluation, we incorporated metabolite prediction using the SyGMa tool, selected after benchmarking multiple alternatives. This enables the assessment of both parent compounds and their potentially toxic metabolites. Model interpretability was enhanced through molecular fingerprint analysis, which identified structural features associated with toxicity and accumulation—informing the early stages of drug design. To support practical implementation, we introduced G.AI.A (https://gaiatox.eu/), an intuitive web platform that allows users to input Simplified Molecular Input Line Entry System (SMILES) strings for rapid prediction of environmental risk endpoints. The application domain of G.AI.A lies in predictive toxicology, enabling researchers and regulatory bodies to assess the toxicological profiles of small organic compounds—excluding those containing heavy metals—by analyzing their chemical structures. The platform supports batch processing and offers interactive visualizations, facilitating compound screening and early-stage environmental risk assessment. By integrating predictive modeling with interpretability and usability, our framework advances green-by-design pharmaceutical development and contributes to sustainable chemical management.

Oxyprenyl-Chalcones as Emerging Antinfective Candidates: From DoE-Driven Synthesis to Drug-Like Profiling and Biodegradability Prediction

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The rising threat of antimicrobial resistance underscores the need for new anti-infective agents that are both effective and environmentally sustainable, in line with One Health principles. Oxyprenylated chalcones offer a modular scaffold with broad bioactivity, and in this work we developed and functionalized a series of derivatives— including analogues bearing protonatable side chains—to improve membrane affinity and solubility. A key step of the synthetic route was optimized through a Design of Experiments (DoE) strategy, increasing efficiency and reducing resource use. The compounds were evaluated for antibacterial activity against Gram-positive and Gram-negative pathogens, alongside predictive toxicity and biodegradability profiling, which highlighted candidates with promising environmental fate. Preliminary screening also revealed encouraging antileishmanial activity within the series. Overall, these multifunctional chalcones, particularly the benzofuran derivative, emerge as potential One Health-aligned anti-infective leads warranting further optimization.

Acknowledgment
This research was supported by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT). L.M. was supported by Programma Operativo Nazionale

Ricerca e Innovazione 2014-2020, risorse FSE REACT-EU Azione IV.4 “Dottorati e contratti di ricerca su tematiche dell’innovazione” e Azione IV.5 “Dottorati su tematiche Green”.

Sustainable antiparasitic agents from an agro-industrial waste: mitochondria-targeting agents from cashew nutshell liquid

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Innovative, sustainable therapies are urgently needed for neglected vector-borne parasitic diseases.[1] In this study,[2] we leveraged cashew nutshell liquid (CNSL), an agro-industrial byproduct, to develop biobased phosphonium and ammonium salts (**5**–**25**) targeting parasite mitochondria. By combining CNSL-derived C8 alkyl chains with lipophilic cations, we synthesized novel compounds exhibiting highly potent in vitro and ex vivo activity against *Trypanosoma* and *Leishmania* spp., including veterinary-relevant strains like *T. b. evansi* and *T. b. equiperdum*. Compounds **5** and **7** outperformed reference drugs, demonstrating subnanomolar efficacy against *Trypanosoma brucei* spp., high selectivity indices (>1000), and no cross-resistance with current therapies, underscoring their potential as next generation antitrypanosomal agents. Reduced activity against *T. brucei* overexpressing alternative oxidase and against *Trypanosoma congolense* supports a mitochondrial mechanism. Preliminary bioassays in zebrafish and *Daphnia magna* indicated ecotoxicity lower than antiparasitic activity. These CNSL-derived agents represent promising, environmentally safer antiparasitic candidates aligned with One Health and Green Chemistry principles (Figure 1).[1]

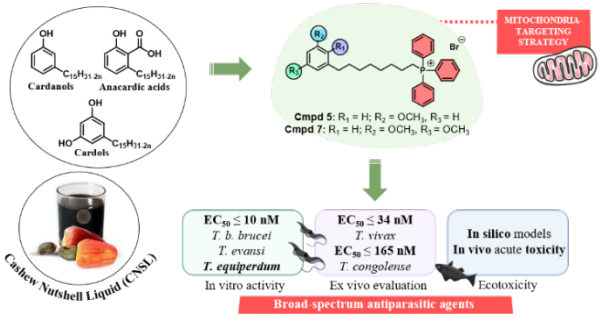

Figure 1: CNSL biobased phosphonium salts exhibiting broad-spectrum antiparasitic activity.

References

[1] B. Martinengo, et. al. *ACS Infect. Dis.* **2024**, 10, 1856−1870
[2] B. Martinengo, et al. *J. Med. Chem.* **2025**, 68, 19438−19462

Acknowledgment

The Authors acknowledge PON ‘‘Ricerca e Innovazione’’ 2014–2020 program (CUP J35F21003320006) for the financial support. This work was developed within the COST Action OneHealth drugs against parasitic vector-borne diseases in Europe and beyond (OneHealthdrugs) CA21111.

Inhibitors of plasmodium serine protease SUB1 as anti-malarial drug leads

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Malaria is caused by plasmodium parasites transmitted by the bit of a mosquito. It is a devastating disease which can lead to disability or even lethal outcome. Out of five species of plasmodium parasites that infect human, the most dangerous is *Plasmodium falciparum*. Resistant plasmodium strains have been registered to all currently used anti-malarial drugs which has urged the search for novel drugs preferentially targeting the parasite by unexplored mechanisms of action. Malarial serine protease (PfSUB1) is known to be involved in the parasite invasion and egress from human red blood cells and therefore PfSUB1 has a potential to be exploited as anti-malarial drug target.[1]

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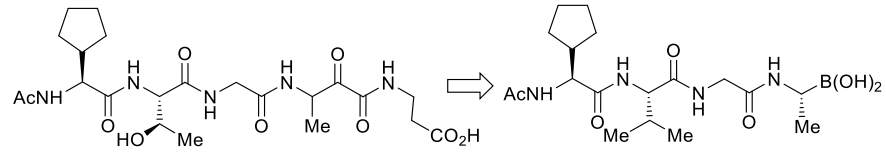
substrate specificity



peptidic ketoamide

IC_{50} (PfSUB1) ≥0.4 uM
no activity in cell-based assay

peptidic boronic acid

IC_{50} (PfSUB1) = 5.7 nM
Pf growth EC_{50} = 0.26 uM

Starting from the substrate specificity studies we first developed peptidic ketoamide PfSUB1 inhibitors and explored the SAR of the peptidic part.[2] Replacement of serine binding group with boronic acid resulted in the next generation inhibitors with low nanomolar PfSUB1 inhibition potency and submicromolar anti-malarial potency in red blood cell assay.[3,4] Representatives of boronic acid based inhibitors showed inhibition of merozoite egress from infected red blood cells.

References

[1] Lidumniece, E.; Withers-Martinez, C.; Hackett, F.; Blackman, M. J.; Jirgensons, A., J. Med. Chem. 2022, 65, 12535.
[2] Kher, S. S.; Penzo M.; Fulle, S.; Finn, P. W.; Blackman, M. J.; Jirgensons, A. Bioorg. Med. Chem. Lett., 2014, 24, 4486 .

[3] Lidumniece, E.; Withers-Martinez, C.; Hackett, F.; Collins, C. R.; Perrin, A. J.; Koussis, K.; Bisson, C.; Blackman, M. J.; Jirgensons, A. Proc. Natl. Acad. Sci. U.S.A., 2021, 118, e2022696118.
[4] Withers-Martinez, C.; Lidumniece, E.; Collins, C. R.; Taha, Z.; Blackman, M. J.; Jirgensons, A. J. Med. Chem. 2024, 67, 13033.

Acknowledgment
The Authors acknowledge grant No. lzp-2020/1-0327 for the financial support.

Integrating Ecotoxicological Constraints into Early Antiparasitic Drug Discovery: A Machine-Learning and GreenDrugScore Framework

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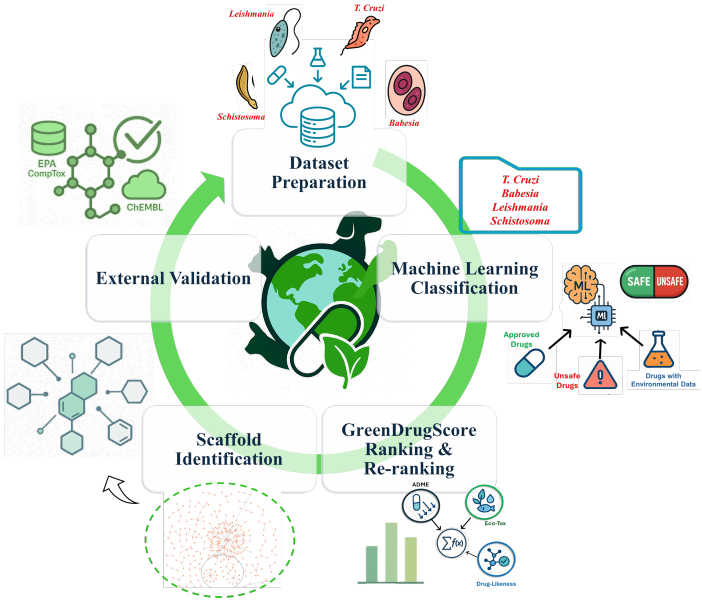
The increasing awareness of the ecological impact of pharmaceutical residues in aquatic environments is driving a transition toward sustainability-focused drug-discovery strategies. Parasitic diseases continue to represent a major global health burden, yet current antiparasitic pipelines largely prioritize potency and ADMET features alone, without adequately considering the potential environmental footprint of future chemotherapeutic agents.[1,2] Within the COST Action CA21111 “OneHealthdrugs”, we sought to address this gap by developing an integrated computational workflow that brings together medicinal chemistry, machine learning, and ecotoxicological prediction to identify antiparasitic scaffolds with reduced environmental impact. As the foundation of this effort, an extensive data-mining campaign was conducted across the 2019–2024 literature to gather all compounds reported with phenotypic activity below 10 µM against five major parasites: *Trypanosoma brucei*, *Leishmania spp.*, *Babesia spp.*, *Schistosoma spp.*, and *Trypanosoma cruzi*. This unified multi-parasite chemical

collection provided the structural diversity needed to design a predictive and sustainability-oriented prioritization framework. To characterize human and ecological liabilities, we developed a machine-learning classifier trained on three representative categories of chemical matter: marketed drugs, toxic or withdrawn compounds, and molecules with experimentally determined environmental toxicity. In parallel, we introduced the **GreenDrugScore (GDS)**, a modular scoring function that integrates ADMET properties, drug-likeness descriptors, and a dedicated ecotoxicological module (ECOscore).[3] Environmental impact was assessed through four ecotoxicological descriptors predicted via ADMETlab 3.0[4]: **bio-concentration factor (BCF)**, **growth inhibition in *Tetrahymena pyriformis* (IGC_{50})**, and the **LC_{50} values for *Daphnia magna* and Fathead minnow**. Together, these metrics capture bioaccumulation potential and aquatic toxicity across multiple trophic levels. *Trypanosoma brucei* served as the prototype dataset for constructing and calibrating the workflow. After validation, the full pipeline was extended to the remaining parasite libraries. Across *Leishmania*, *Babesia*, *Schistosoma*, and *T. cruzi*, introducing ecotoxicological constraints produced a consistent reshaping of scaffold priorities. Chemotypes with lower predicted bioaccumulation and milder ecotoxicological profiles were preferentially ranked, revealing structural regions more compatible with environmentally sustainable drug design. Overall, this study shows that integrating ML-based toxicity prediction with ecotoxicological scoring provides a robust strategy for identifying **green antiparasitic scaffolds** suitable for sustainable hit-to-lead development. The GDS-guided approach marks a concrete step toward environmentally conscious medicinal chemistry aligned with One Health principles.



REFERENCES

[1] N. Singh, P. Vayer, S. Tanwar, J.-L. Poyet, K. Tsaioun, B.O. Villoutreix, Drug discovery and development: introduction to the general public and patient groups, Front. Drug Discov. 3 (2023). https://doi.org/10.3389/fddsv.2023.1201419.

[2] J.C. Semenza, S. Paz, Climate change and infectious disease in Europe: Impact, projection and adaptation, Lancet Reg. Health Eur. 9 (2021) 100230. https://doi.org/10.1016/j.lanepe.2021.100230.

[3] Aiello, D.; Bertarini, L.; Karki, R.; Gul, S.; Pellati, F.; Tonelli, M.; Costi, M. P. Leveraging Ecotoxicity Parameters and Machine Learning to Redefine the Drug Discovery Pipeline. J. Pharm. Anal. 2025, under review.

[4] L. Fu, S. Shi, J. Yi, N. Wang, Y. He, Z. Wu, J. Peng, Y. Deng, W. Wang, C. Wu, A. Lyu, X. Zeng, W. Zhao, T. Hou, D. Cao, ADMETlab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support, Nucleic Acids Res. 52 (2024) W422–W431. https://doi.org/10.1093/nar/gkae236.

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DAY3 (12/04): https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1762122862735?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

Meeting ID:

Persistent myeloid cell reprogramming despite miltefosine treatment in leishmania-infected macaques

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Visceral leishmaniasis (VL) is a neglected tropical disease caused by protozoan parasites. An inflammatory immune response, associated with tissue injury, occurs shortly after infection. Using a rhesus macaque model of VL, we evaluated the impact of miltefosine therapy administered during the acute phase of infection. Despite therapy, parasites persist in multiple tissues, including the spleen, bone marrow, peripheral, and mesenteric lymph nodes. Parasite burden inversely correlates with cellular miltefosine levels. Notably, L. infantum remains detectable three months post-treatment. Single-

cell transcriptomic analysis reveals cellular heterogeneity and reprogramming of splenic myeloid cells post-treatment, including inflammatory macrophages, immature plasmacytoid dendritic cells, and type 2 dendritic cells (DCs). Flow cytometric sorting of splenic neutrophils, macrophages, and DCs confirms the presence of L. infantum post-treatment, highlighting the challenge of parasite clearance. Our findings reveal a disrupted innate immune landscape postinfection that persists after treatment, indicating myeloid cell reprogramming may sustain chronic infection and parasite persistence.

Powering New Antiviral Frontiers: PROTAC-Based Approach for Emerging Flavivirus

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In recent years, West Nile (WNV), Zika (ZIKV) and Dengue (DENV) have begun to expand rapidly to Europe due to global megatrends, uncontrolled urbanization, climate change and increased intercontinental travel[1]. Specifically, the Italian Emilia-Romagna region has been called a "virus-hotspot in Europe" due to its lagoon territory and humid climate where the vector is able to replicate very rapidly[1]. Currently, there are no treatments, and Flaviviruses can cause severe diseases like encephalitis or meningitis. In view of that, and with the aim to limit the likelihood of new epidemics, there is an urgent need to develop new selective drugs. Thus, we envisaged the use of PROteolysis TArgeting Chimeras (PROTACs), an innovative technology in medicinal chemistry[2]. This new approach is based on the use of the endogenous ubiquitin-proteasome system to induce protein degradation. PROTACs are heterobifunctional small molecules consisting of two ligands, one responsible for recruiting the E3-ligase, the other one binding the protein of interest (POI), joined by a suitable linker. Here, we aim to develop PROTACs targeting the allosteric site of the viral serine protease NS2B-NS3[3], essential for viral replication. This target is absent in mammalian host, conserved in DENV, WNV, and ZIKV, supports pan-degrader design, and is genetically and structurally validated. So far, a multi-step synthesis of the POI ligand has been optimized and three final PROTACs have been synthetized all of them with different E3-binders and linkers. A FRET assay has been used to evaluate the bio-enzymatic activity against our viral target. We were pleased to note that the POI inhibits both the NS2B-NS3 WNV and ZIKV viruses in a similar manner, with respective IC_{50} values of 16.2±3.67 and 19.6±3.81 µM. To evaluate the degradative profile of the PROTACs, western blot assay is up-running while, toxicity against HEK293 did not show any red flag (CC_{50}>30 µM).

Figure 1: Generic structure of PROTACs and mechanism of action.

References

[1] *Lancet Planet Health* **2025**; 9: 101291-101325.
[2] Sakamoto, K. M. et al. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8554-8559.
[3] Yao Y. et al. *J Am Chem Soc*. **2019**,141 (17), 6832-6836.

Acknowledgment

The Authors acknowledge Flaminia Foundation (Ravenna) for the financial support.

Integrated pharmacophore screening workflow for target identification and selection of antileishmanial compounds with an improved chemical profile

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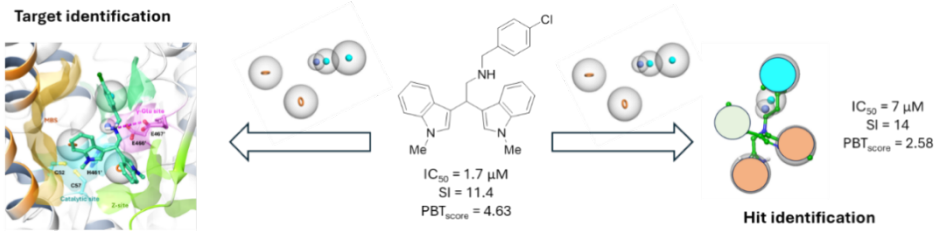
The broader consequences of globalization, together with the ongoing tropicalization of the Mediterranean region driven by global warming, are contributing to the emergence of new public health threats, including the progressive endemicization of leishmaniasis.[1] Recently, a class of bisindole derivatives has been reported with promising *in vitro* activity in phenotypic screening against *Leishmania infantum*, with favorable selectivity profiles toward human cells,[2,3] but no clear rationale has been provided for the development of novel compounds.

In this work, structure-activity relationship studies enabled the development of a pharmacophore model, based on conformational analyses of representative molecules belonging to the bisindole class. To explore potential molecular mechanisms, the ChEMBL database was queried for Leishmania target-specific inhibitors. Compounds with the strongest pharmacophore alignment[4] suggested trypanothione reductase as a plausible target. Docking studies supported this hypothesis and provided a preliminary model for bisindole binding within the enzyme's catalytic site. The activity was subsequently confirmed with enzymatic assays, resulting in the identification of a micromolar hit, amenable to future optimization studies aiming at higher enzyme inhibition of a target commonly considered undruggable.[5]

The pharmacophore model of 2,2'-di(indol-3-yl)ethanamines was additionally employed in a ligand-based virtual screening privileging enhanced solubility relative to starting compounds. Biological tests of the newly identified compounds permitted the identification of a new antileishmanial agent showing activity against promastigotes and

intracellular amastigotes with relatively low toxicity on human cells, as well as a lower predicted ecotoxicity through QSPR and deep-learning pipelines.[6]



References

1. Todeschini et al., *Euro Surveill*. **2023**, 29 (4), 2300190.
2. Diotallevi et al., *ACS Omega* **2021**, 6, 51, 35699-35710.
3. Centanni et al., *PLoS One* **2024**, 19(6), e0301901.
4. Chauhan et al., *MedChemComm* **2015**, 6(3), 351-356.
5. Exertier et al., *J. Med. Chem.* **2024**, 67, 1, 402–419.
6. Evangelista et al., *J. Chem. Inf. Model.* **2025**, 65, 7, 3248-3261.

Acknowledgement

This work has been funded by the European Union_NextGenerationEU, Mission 4, Component 2, under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041_VITALITY_CUP H33C22000430006.

Hydrophobic Tag Degraders (HyT) Targeting Trypanothione Reductase as a Potential Strategy Against Leishmaniasis

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Leishmaniasis is a vector-borne infectious disease caused by protozoa of the Leishmania genus and is recognized by the World Health Organization as an emerging and uncontrolled neglected tropical disease. Climate change and migratory flows have increased the risk of transmission, contributing to the appearance of cases outside traditional endemic regions. Despite decades of research, current therapies remain inadequate due to high toxicity, limited efficacy, increasing drug resistance, prohibitive cost, and restricted accessibility. These shortcomings underscore the urgent need for innovative and more effective treatments.1 Targeted Protein Degradation (TPD), an event-driven pharmacological strategy, offers a potential alternative to conventional small molecule inhibitors (Fig. 1). Rather than relying on target occupancy, TPD employs small molecule degraders, including hydrophobic tag-based degraders (HyTs), 3 that induce the elimination of disease-relevant proteins through the activation of the cellular degradation machinery.2 This project focuses on the design and synthesis of eight HyT degraders directed against trypanothione reductase (TR), an essential enzyme in the parasite's redox metabolism that is absent in the human host. Although TR is a validated target for leishmaniasis, its broad and featureless active site has made it difficult to modulate with conventional small molecule inhibitors.

4 HyTs can potentially overcome this limitation by linking a TR-binding ligand to a hydrophobic moiety that mimics misfolded proteins, thereby recruiting the parasite's protein quality control (PQC) machinery to selectively promote TR removal in Leishmania. To combine the TR recognition scaffold with diverse linker architectures and different hydrophobic moieties, we have optimized a convergent synthetic procedure centered on a key intermediate enabling rapid functionalization with five different hydrophobic moieties. The synthesized HyTs will be evaluated for anti Leishmania activity, and TR degradation. HyT-mediated TR degradation could contribute to next-

generation antiparasitic therapeutics, providing a new avenue for drug discovery against leishmaniasis and supporting the development of more effective antiparasitic agents.

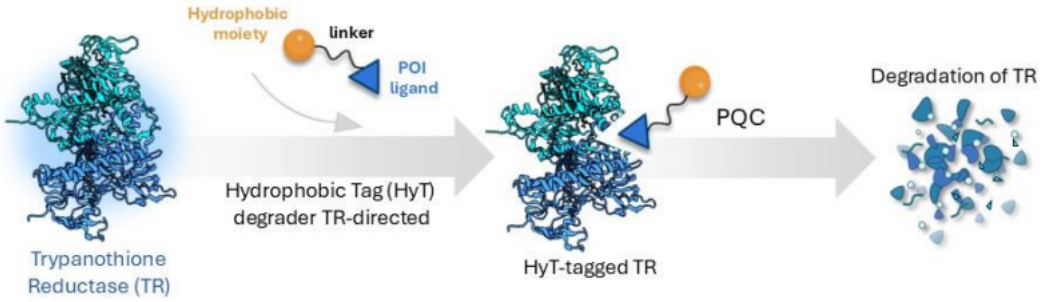

Figure 1 - A schematic of protein degradations induced by Hydrophobic Tag Degrader. POI represents the protein of interest and PQC represents protein quality control machinery in cells to degrade the proteins.

REFERENCES

[1] Pinheiro, A. C. et al. RSC Med. Chem. 2022, 13, 1029–1043.
[2] Espinoza-Chávez RM. et al. ACS Bio Med Chem Au. 2022, 3, 32-45.
[3] He Q, Zhao X, Wu D. et al. Eur J Med Chem. 2023, 260, 115741.
[4] Battista, T. et al. Molecules 2020, 25, 1924.

Synthesis, Structure Elucidation, Antimycobacterial and Antiparasitic Activities of New Piperidinhydrazide-hydrazone Derivatives

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Benzyl-N'-benzylidenepiperidine-4-carbohydrazide derivatives are compounds with important bioactivities in medicinal chemistry. These activities include antimycobacterial and antiparasitic activities[1-2], as well as acetylcholinesterase and butyrylcholinesterase enzyme inhibitory activities against Alzheimer's disease [3]. In the view of this reported literature information, new benzylidene piperidine-4-carbohydrazide derivatives were designed and synthesized in four compounds (Figure). Starting from the ethyl piperidine-4-carboxylate structure, piperidine-4-carbohydrazide-hydrazone structures with substituted benzyl and substituted aldehyde derivatives were synthesized in three steps. Spectroscopic methods were used to confirm the expected chemical structures and their purity. The antimycobacterial activities of the compounds were determined using the broth microdilution method on Mycobacterium smegmatis ATCC 14468 strain with levofloxacin as a positive control [4]. The antiparasitic activity was tested on promastigotes of Leishmania tropica promastigote strain [5].
The antiparasitic activity of four compounds ranged from 0.5 to 4 mM. The antimycobacterial MIC values ranged between 250 µM and 100 µM. We are continuing to work on more active and selective antimycobacterial/antiparasitic compounds. In silico studies are also underway to elucidate the mechanism of action of the compounds.

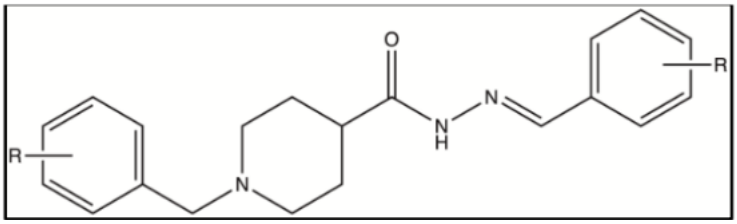

Figure. General structure of synthesized compounds

References

1. Briffotaux, J.; Xu, Y. A Hydrazine-Hydrazone Adamantine Compound Shows Antimycobacterial
Activity and Is a Probable Inhibitor of MmpL3. Molecules. 2022, 27(20), 7130.
2. Coa, J.C.; Castrillón, W. Synthesis, leishmanicidal, trypanocidal and cytotoxic activity of quinoline- hydrazone hybrids. Eur J Med Chem. 2015, 101:746-753.
3. Parlar, S.; Sayar, G. Synthesis, bioactivity and molecular modeling studies on potential anti-Alzheimer piperidinehydrazide-hydrazones. Bioorg Chem. 2019, 87:888-900. doi:10.1016/j.bioorg.2018.11.051.
4. Ramis, I. B.; Cnockaert, M. Antimicrobial susceptibility of rapidly growing mycobacteria using the rapid colorimetric method. Eur J Clin Microbiol Infect Dis. 2015, 34(7), 1403–1413.
5. Istanbullu, H.; Bayraktar, G. Design, synthesis, and in vitro biological evaluation of novel thiazolopyrimidine derivatives as antileishmanial compounds. Arch Pharm. 2020, 353(8):e1900325.

High throughput cloning, production, and crystallization of *Leishmania infantum* calpain, a new promising target against Leishmaniasis

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Leishmania infantum is one of the causative agents of visceral leishmaniasis (VL) [1–3]. VL is fatal (if untreated) and the currently available drugs are limited and often responsible for severe side-effects, limiting their use [1–4]. This STSM project focused on the investigation of a previously uncharacterized enzyme, *L. infantum* calpain (*Li*Clp), to expand the targets exploitable for drug development campaigns. Although no information is available on the parasite protein, Ca^{2+}-activated neutral proteases such as calpains are valuable markers of environmentally induced alterations in the protein metabolism of an organism [5,6]. Indeed, dose-responses in calpain and cathepsin activity were previously described in vertebrate and invertebrate animals exposed to chemicals and other abiotic factors. Thus, this target has dual importance both as drug target and as biomarker of environmentally induced alterations of an organism. Preliminarily to the STSM, we purchased the synthetic gene encoding for full-length *Li*Clp and attempted the production in *E. coli*, resulting in almost negligible yields due to the limited protein solubility. Although various conditions were tested, no significant improvements were obtained in the amount of isolated target (< 0.5 mg/L of culture). Therefore, in collaboration with Dr. Marco Mazzorana, high throughput (HT) methods were applied for gene cloning, protein production, and characterization, with the aim of improving yield and obtaining enough protein for structural, biochemical, and biophysical studies [7]. Using HT methods, we were able to obtain a library of plasmids with different combinations of constructs/tags. The results clearly demonstrated the role of the SUMO tag in improving the solubility profiles of the targets. Unfortunately, the poor solubility of the mature targets significantly compromised their purification, causing significant aggregation/precipitation after SUMO tag cleavage. Despite their poor solubility profile, we were able to significantly improve production yields, obtaining enough protein to perform a robotics-based crystallization campaign, screening over 500 different conditions.

References

[1] S. Sundar, M. Rai, *Expert Opinion on Pharmacotherapy* **2005**, *6*, 2821.

[2] B. Monge-Maillo, R. López-Vélez, *Drugs* **2013**, *73*, 1863.
[3] J. Q. Reimão, D. P. Pita Pedro, A. C. Coelho, *Expert Opinion on Drug Discovery* **2020**, *15*, 647.
[4] C. Maia, L. Campino, *Front. Cell. Infect. Microbiol.* **2018**, *8*, DOI 10.3389/fcimb.2018.00302.
[5] Y. Ono, T. C. Saido, H. Sorimachi, *Nat Rev Drug Discov* **2016**, *15*, 854.
[6] I. Shapovalov, D. Harper, P. A. Greer, *Expert Opinion on Therapeutic Targets* **2022**, *26*, 217.
[7] M. Sun, A. X. Gao, X. Liu, Y. Yang, R. Ledesma-Amaro, Z. Bai, *Microb Cell Fact* **2023**, *22*, 182.

Acknowledgment
The Authors acknowledge the STSM program of the CA21111 for financial support.

Parasitology and pharmacology and One Health

2025-12-05

DAY4 (12/05):
https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1762122949003?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

Unraveling the Mechanisms of Action of Antikinetoplastid Nucleoside Prodrugs: Bridging the Gap in Drug Efficacy and Mechanisms

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Abstract

Trypanosomatid parasites are responsible for major neglected diseases, transmitted by vectors and affecting humans, livestock, and wildlife, yet their metabolic vulnerabilities remain incompletely understood, limiting the development of targeted therapeutics. Nucleoside analogues represent a promising class of compounds because they depend on parasite specific transporters and metabolic enzymes. This study systematically evaluated a panel of nucleoside analogues, including tubercidin derivatives, against *Trypanosoma brucei* strains with distinct transporter profiles, including TbAT1 knockout, TbB48, and P2 transporter over-expressor, as well as *Leishmania mexicana* lines differing in adenosine kinase expression, including AK knockout and AK knockout expressing TbrAK. Drug susceptibility assays were performed in triplicate, and dose response analyses were used to determine EC50 values, revealing transporter and enzyme dependent activity. Several compounds exhibited potent trypanocidal effects, with lead analogues achieving submicromolar EC50 values and selective activity across mutant lines, indicating specific uptake routes and metabolic activation requirements. Fluorescence microscopy at four, twelve, and twenty-four hours after exposure revealed pronounced morphological alterations, including kinetoplast and nuclear deformation, cytoplasmic swelling, and flagellar disorganization. These changes reflect rapid intracellular stress and provide mechanistic insight into compound induced cytotoxicity, highlighting candidates with fast acting and disruptive effects. Preparatory transcriptomic workflows were established to assess potential impacts on RNA chain length, stability, and overall transcriptome integrity, laying the foundation for future molecular level mechanistic studies. Overall, this integrated approach combining quantitative pharmacology, phenotypic analysis, and preparatory molecular investigation identifies highly active nucleoside analogues, clarifies determinants of uptake and activation, and establishes a robust framework for mechanistic studies and structure activity optimization of next generation antitrypanosomatid therapeutics. By

addressing vector borne parasites that impact multiple host species, these findings are fully consistent with the One Health approach promoted by the COST Action.

References

1. Hulpia F, Campagnaro GD, Scortichini M, Van Hecke K, Maes L, De Koning HP, Caljon G, Van Calenbergh S (2019) Revisiting tubercidin against kinetoplastid parasites: aromatic substitutions at position 7 improve activity and reduce toxicity. Eur J Med Chem 164:689–705
2. Hulpia, F, Bouton J, Campagnaro GD, Alfayez IA, Mabille D, Maes L, De Koning HP, Caljon G, Van Calenbergh S. (2020) C6-*O*-Alkylated 7-deazainosine nucleoside analogues: Discovery of potent and selective anti-sleeping sickness agents. Eur J Med Chem 188:112018
3. Hulpia F, Campagnaro GD, Alzahrani KJ, Alfayez IA, Ungogo MA, Mabille D, Maes L, De Koning HP, Caljon G, Van Calenbergh S (2020) Structure-activity relationship exploration of 3'-deoxy-7-deazapurine nucleoside analogues as anti-*Trypanosoma brucei* agents. ACS Infect Dis 6:2045–2056.
4. Mabille D, Ilbeigi K, Hendrickx S, Ungogo MA, Hulpia F, Lin C, Maes L, De Koning HP, Van Calenbergh, S, Caljon G. (2022) Nucleoside analogues for the treatment of animal African trypanosomiasis. Int J Parasitol Drugs Drug Resist 19:21–30
5. Munday JC, Tagoe DNA, Eze AA, Krezdorn JA, Rojas López KE, Alkhaldi AAM, McDonald F, Still J, Alzahrani KJ, Settimo L and De Koning HP (2015) Functional analysis of drug resistance-associated mutations in the *Trypanosoma brucei* adenosine transporter 1 (TbAT1) and the proposal of a structural model for the protein. Mol Microbiol 96:887–900
6. Ward CP, Wong PE, Burchmore RJ, De Koning HP, and Barrett MP (2011) Trypanocidal furamidine analogues: influence of pyridine nitrogens on trypanocidal activity, transport kinetics and resistance patterns. Antimicrob Agents Chemother 55:2352–2361.
7. Glover, L. and D. Horn (2012) Trypanosomal histone gammaH2A and the DNA damage response. Mol Biochem Parasitol 183: 78–83.
8. Munday JC, Settimo L, and De Koning HP (2015) Transport proteins determine drug sensitivity and resistance in a protozoan parasite, *Trypanosoma brucei*. Frontiers Pharmacol 6:32

Acknowledgment

The Authors acknowledge Cost Action CA21111 for the financial support.

Exploring cryptic reservoirs and neuroimmune modulation by visceral Leishmania in the brain

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Visceral leishmaniasis (VL), caused by Leishmania spp. with tropism for internal organs, remains a major health concern in endemic regions1 . While classical target sites such as the spleen, liver and bone marrow have been extensively studied, accumulating evidence suggests that Leishmania may also reach the central nervous system (CNS), with the brain potentially serving as a cryptic parasite reservoir2. However, the dynamics and functional implications of this cerebral tropism remain largely unexplored. To address this gap, we engineered visceral Leishmania parasites to express the ultra-sensitive bioluminescent reporter Akaluc, enabling real-time imaging of parasite dissemination. Using in vivo bioluminescence imaging and ex vivo validation, we uncovered for the first time the spatiotemporal dynamics of cerebral invasion, the presence of viable parasites in brain tissue and their correlation with parasite loads in classical visceral organs. These findings point to the brain as an underappreciated reservoir that may contribute to long-term parasite persistence and dissemination. Given that Leishmania parasites reach the CNS, we hypothesized that their presence within the brain may lead to direct interactions with resident immune cells. Preferentially infecting macrophages, we focused on microglia (the brain's resident macrophages), to investigate the cellular and immunological dynamics of this potential host-parasite interface. Our studies revealed that visceral Leishmania actively subvert microglial immune functions by suppressing activation of the NLRP3 inflammasome, a key innate immune sensor responsible for pro-inflammatory cytokine production3. This immune evasion may promote an anti-inflammatory environment within the CNS. Notably, NLRP3 is also implicated in several neuroinflammatory diseases, including

Alzheimer's disease4. In this context, our findings raise the provocative hypothesis that cerebral Leishmania persistence may exert unexpected neuroprotective effects5 . Together, our data uncover the brain as a novel niche for visceral Leishmania, illuminate new aspects of host-parasite immune interactions and open avenues for investigating parasite-driven modulation of neuroinflammation in CNS disease.

References

[1] Burza, S. et al. Leishmaniasis. Lancet, 2018, 392. doi.org/10.1016/S0140-6736(18)31204-2

[2] Maia, C.S. et al. Neurological disease in human and canine leishmanisis-clinical features and immunipathogenesis. Parasite Immunology, 2015, 37, 385-393. doi.org/10.1111/pim.12203

[3] De Carvalho, R. & Zamboni, D. S. Inflammasome Activation in Response to Intracellular Protozoan Parasites. Trends in Parasitology, 2020, 36, 459-472. doi.org/10.1016/j.pt.2020.02.006

[4] Heneka, M. T. et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature, 2013, 493, 674-678. doi.org/10.1038/nature11729

[5] Trumble, B. C. et al. Apolipoprotein E4 is associated with improved cognitive function in Amazonian forager-horticulturalists with a high parasite burden. FASEB Journal, 2017, 31, 1508- 1515. doi.org/10.1096/fj.201601084R

Acknowledgment

The Authors acknowledge Fondazione Cariplo for the financial support (grant number 2022-0294).

IN VIVO SELECTION OF A LEISHMANIA COSMID LIBRARY REVEALS CANDIDATE GENES INVOLVED IN SAND FLY TRANSMISSION

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INTRODUCTION: Due to the absence of a protective human vaccine and the various challenges related to vector control, the management of leishmaniasis currently relies heavily on early and effective diagnosis and treatment. Despite compelling evidence of the importance of the vector, research has predominantly focused on parasite interactions with the vertebrate host. Studies in the invertebrate host have been limited and mostly based on loss-of-function approaches. The development of *Leishmania* promastigotes inside the sand fly vector is a complex, yet critical step in the parasite life cycle, shaped by intricate parasite-vector interactions that may offer interesting novel targets for disease management strategies.

METHODOLOGY: A genome-wide cosmid library of *Leishmania donovani* was subjected to *in vivo* selection through *Lutzomyia longipalpis* sand flies and subsequent mouse macrophage infection. This approach was used to identify candidate genes that provide a gain-of-function and enhance the parasite transmission potential.

RESULTS: Preliminary screening identified several genomic regions potentially conferring an advantage to parasite survival, metacyclogenesis and transmission.

CONCLUSIONS: This unique approach provides a genome-wide strategy for identifying parasite genes essential for vector transmission, deepening our understanding of parasite-vector interactions and offering potential targets for transmission-blocking interventions.

Current efforts focus on narrowing down candidate genes and validating their contribution to sand fly infection establishment, differentiation and macrophage infection through targeted mutagenesis

Antileishmanials of 8-(Haloaryl)-Substituted Pyrimidopyrimidines: New insights into Structure-Activity Relationship

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Leishmaniasis is a neglected tropical disease caused by vector-borne protozoan parasites and remains a growing health problem in many developing regions[1]. Existing treatments often show limited effectiveness, significant side effects, and rising drug resistance, highlighting the need for new therapeutic options[2]. Recent studies have identified pyrimidopyrimidine-based compounds as promising anti-Leishmania agents 3,4. The SAR analysis showed that substituents at C8 and C4 of the core nucleus affect activity. Aiming to deepen SAR study and considering that the target is unknown, new haloaryl groups were introduced at C8, and different substituents were introduced at C4 of the heterocycle to explore the chemical space at those positions. The activity of the new compounds was assessed in vitro against L. infantum promastigotes and intracellular amastigotes, while cytotoxicity was assessed in THP-1 cells. Hight potent and selective compounds were identified with IC${50}$ (promastigotes) =0.39 µM, IC${50}$ (amastigotes) =1.53 µM, and a selective index higher than 256 and 66, respectively. All the biological results and SAR analysis will be presented and discussed. The new insights allowed us to identify promising substitution patterns that can be further optimised to improve the efficacy and safety of this new class of antileishmanials.

References

[1] https://www.who.int/leishmaniasis/burden/en/, (accessed 16/11/2025).
[2] a) R. Balaña-Fouce, R. Álvarez-Velilla, C. Fernández-Prada, C. García-Estrada, R. M. Reguera, Int. J. Parasitology: Drugs and Drug Resistance 4, 2014, 326-337. b) S.L. Croft, P. Olliaro, Clin. Microbiol. Infect. 17, 2011, 1478-1483.

[3] A. Lopes, N. Santarém, A. Cordeiro-da-Silva, M. A. Carvalho, ACS Med. Chem. Lett. 2022, 13 (9), 1427-1433.
[4] A. Lopes, N. Santarém, A. Greco, A. Pagliaro, O. Keminer, S. Gul, A. Cordeiro-da-Silva, M. A. Carvalho ACS Med. Chem. Lett. 2024, 15, 9, 1541-1548.

Acknowledgment
This work received financial support from Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the projects UID/00686 -Centre of Chemistry of University of Minho (CQ-UM), from Rede Nacional de RMN (PINFRA/22161/2016), from PT-OPENSCREEN (NORTE-01-0145-FEDER-085468), and from COMPETE2030-FEDER-00715600, n.° 16016. AL thanks FCT for the PhD scholarship Ref. 2023.01262.BD (https://doi.org/10.54499/2023.01262.BD).

UNVEILING GLIAL CELLS IN THE NASAL MUCOSA AS HOST CELLS FOR *LEISHMANIA* WITH POTENTIAL IMPLICATIONS IN DISEASE OUTCOMES

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Abstract

Neurological involvement in leishmaniasis remains underexplored, despite reports of meningoencephalitis, peripheral neuropathy, and neuroinflammation in visceral and mucocutaneous manifestations. While macrophages are recognised as the primary host cells, emerging evidence suggests that *Leishmania* can exploit immune-privileged niches beyond classical phagocytes. Here, we investigate whether olfactory ensheathing cells (OECs), specialised glia in the nasal mucosa, constitute a previously unrecognised host cell population. These cells form a neuroimmune interface that bridges the peripheral and central nervous systems, potentially offering a sanctuary for parasite persistence.

Using an in vitro infection model, we compared OECs to PMA-differentiated U937 macrophage-like cells upon exposure to *L. braziliensis*, *L. major*, and *L. infantum*. Transcriptional profiling was performed by RT-qPCR, following MIQE 2.0 guidelines, which included efficiency-corrected quantification with prediction intervals, validated normalisation using multiple reference transcripts, and full QC reporting. Parasite viability was confirmed by SL-RNA quantification, supporting the hypothesis that OECs can harbour *Leishmania* under conditions mimicking mucosal infection.

Distinct immune signatures were observed in OECs, with modulation of NFκB-driven inflammatory pathways, interferon axis transcripts, and regulatory/metabolic mediators, suggesting a unique immunological phenotype compared to macrophages. These findings introduce OECs as a candidate reservoir that may contribute to subclinical persistence, relapse, and treatment failure, particularly in mucocutaneous disease. Beyond expanding the cellular tropism of *Leishmania*, this work highlights the importance of considering neuroimmune interactions in disease pathogenesis and therapeutic strategies. Recognising non-traditional host cells could reshape our understanding of chronic parasitic infections and open new avenues for targeted interventions, aligning with the objectives of EU COST Action OneHealthdrugs

(CA21111) to foster integrated strategies for drug development under the One Health framework.

Acknowledgements
This work was supported by Horizon Europe funding [HORIZON-MSCA-2023-PF-01 Project 101152054 -RespiriCO, S.A.], the Fonds Wetenschappelijk Onderzoek [www.fwo.be; grant numbers G033618N and G013118N (G.C.)], and the University of Antwerp [www.uantwerpen.be; Bijzonder Onderzoeksfonds (BOF) support of S.A. and G.C.]. LMPH is a partner of the Excellence Centre 'Infla-Med' (www.uantwerpen.be/infla-med) and participates in COST (European Cooperation in Science and Technology) Action CA21111.

Declaration of interests
The authors declare no competing interests.


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