













One Health drugs against parasitic vector borne diseases in Europe and beyond Strategies for breaking the chain between the parasite reservoir in animals and infections in humans.

4-6 June 2025

OneHealth*drugs*

The OneHealthdrugs COST Action network brings together experts to advance the discovery of innovative drugs and therapeutic targets aimed at combating vectorborne infections in both human and veterinary medicine. By adhering to the principles of an optimal drug profile, our approach enhances treatment effectiveness while minimizing environmental impact.

BOOK OF ABSTRACTS





Meeting Venue

University of Antwerpen – Grauwzusters, Lange Sint-Annastraat 7, Antwerp, Belgium

City campus



For Conference: s.S - Grauwzusters - Lange Sint-Annastraat 7 For Dinner: s.HvL - Hof van Liere - Prinsstraat 13

Organizing committee

Scientific committee

Prof. Jose Maria Alunda Prof. Mirco Bundschuh Prof. Guy Caljon Prof. Anabela Cordeiro Silva Prof. Elisabeth Davioud-Charvet Prof. Louis Maes Prof. Maria Paola Costi Prof. Joana Tavares Prof. Elisa Uliassi

Local supporting team

Yentl Jacobs Cassandra Present Lauren Van den Broeck Sin-Ting Wong Ting Yu







OneHealth*drugs*

Cost Action CA21111

Meeting Program

| Wednesday, June 4, 2025 | | | | | | |
|--|--|---|----------|--|--|--|
| 11:30 | Registration | | | | | |
| 12:30 | Welcome address (Guy Caljon and Maria Paola Costi) | | | | | |
| Session 1: Industry stakeholders – interface academia/industry (Translation) | | | | | | |
| Chair: Louis | Maes | | | | | |
| 12:45 | Thomas Geurden | Keynote lecture 1: How is the Animal Health industry driving innovation? | Page: 1 | | | |
| 13:20 | Serge Van Calenbergh | Purine nucleoside analogues against protozoan pathogens in humans and livestock: from bench to barn | Page: 2 | | | |
| 13:40 | Paul M. Selzer | Antiparasitic Research: An Animal Health Perspective | Page: 3 | | | |
| 14:00 | Guy Caljon | Strategic roadmap towards high-quality leads and drug development candidates: in vitro and in vivo laboratory approaches for kinetoplastid diseases | Page: 4 | | | |
| 14:20 | Anabel Olías Molero | Regulatory Framework for Veterinary Medicines in the European Union | Page: 5 | | | |
| 14:40 Co | ffee break | | | | | |
| Session 2 | : Roadmap and assays | for target and off-target effects (Pharmacology) | | | | |
| Chair: Sarah | Hendrickx | Г | | | | |
| 15:10 | Sheraz Gul | In vitro and in silico methods to assess ADME-Tox and ecotoxicological profiles of compounds in drug discovery | Page: 6 | | | |
| 15:30 | José María Alunda | Academic research ecosystem: does it fit the antiparasitic drug discovery process? | Page: 7 | | | |
| 15:50 | Maria Alice Carvalho | New fused-pyrimidine derivatives as promising broad-spectrum antiparasitic agents | Page: 8 | | | |
| 16:10 | De Koning Harry | A new class of minor groove binders active against animal trypanosomiasis in vivo | Page: 9 | | | |
| 16:30 | Ioannis P. Papanastasiou | Synthesis and Evaluation of Nitroheterocyclic Aromatic Adamantanohydrazones with Trypanocidal Activity | Page: 10 | | | |
| 16:40 | Črtomir Podlipnik | OCART: A Simple Chemoinformatics Toolkit in Orange3 for Drug Discovery in Vector-Borne Diseases | Page: 11 | | | |
| 16:50 | Margarida Duarte | Metabolic Rewiring in Leishmania Lacking PRX1/2 Reveals Hidden Vulnerabilities for Drug Targeting | Page: 12 | | | |
| 17:00 Poster session | | | | | | |
| 18:00 Social event 1 – local drink tasting | | | | | | |







OneHealth*drugs*

Cost Action CA21111

| Thursday, June 5, 2025 | | | | | | |
|--|--|---|----------|--|--|--|
| Session 3: Unintended side effects of pharmaceuticals on the ecosystem level (Environment) | | | | | | |
| Chair: Kayhan Ilbeigi | | | | | | |
| 9:00 | Tomas Brodin | Keynote lecture 2: The potential trade-off between human health and ecosystem health – ecological effects of pharmaceutical contamination | Page: 13 | | | |
| 9:30 | Mirco Bundschuh | Pharmaceuticals and their potential for ecological surprises | Page: 14 | | | |
| 9:50 | Johannes Charlier | Piloting sustainable worm control approaches in grazing livestock to minimize the environmental impact of anthelmintics in livestock | Page: 15 | | | |
| 10:10 | Maarten Vanhove | Monogenean flatworms: petite parasites, mighty models, innovative indicators? | Page: 16 | | | |
| 10:30 C | offee break + poster vie | ewing | | | | |
| Session 4 | : Parasite and host tar | gets: identification and structural characterization | | | | |
| Chair: Elisa | Uliassi | | | | | |
| 10:50 | Cecilia Pozzi | CA21111 OHD1 - Target database project: the BioTarget DataBase (BioT-DB) | Page: 17 | | | |
| 11:10 | Joana Tavares | Contributions to drug discovery against Leishmania infantum: from target-based strategies to insights into the biology of the infection | Page: 18 | | | |
| 11:30 | Benoit Stijlemans | Q586B2 is a crucial virulence factor during the early stages of Trypanosoma brucei infection that is conserved amongst trypanosomatids | Page: 19 | | | |
| 11:50 | Alvaro Baeza Garcia | Targeting host and Plasmodium immunomodulators for new malaria therapeutics | Page: 20 | | | |
| 12:10 lu | nch | | | | | |
| <u>13:30 CC</u> | ST general assembly n | neeting | | | | |
| <u>13:30 Br</u> | eakout workshop: You | ng innovators and career | | | | |
| 13:30 | Elsa Abranches | My Career Tale: one step at a time | Page: 21 | | | |
| 14:00- 17:00 | Corinne Herrijgers; Marjolijn De Clercq | Coaching session | | | | |
| 15:30 C | offee break + poster vie | ewing | | | | |
| Chair: Bend | ît Stijlemans | | | | | |
| 16:00 | Daniel Sojka | Proteases driving the apical complex of Babesia during egress and invasion of host red blood cells | Page: 22 | | | |
| 16:20 | Malgorzata Domagalska | Multi-drug tolerance in Leishmania persister-like cells | Page: 23 | | | |
| 16:40 | Ana M Tomás | Metal Transporters in Leishmania: Exploring Their Potential as Drug Targets | Page: 24 | | | |
| 16:50 | Jovana Ajdukovic | Heterocyclic steroid derivatives with antitrypanosomal activity and in silico binding affinity to glucose-6-phosphate dehydrogenase | Page: 25 | | | |
| 17:30: Social event 2 – Chocolate museum | | | | | | |
| 19:30: Congress dinner: Hof van Liere University Club | | | | | | |
| | | | | | | |







OneHealth*drugs*

Cost Action CA21111

| Friday June 6, 2025 | | | | | | | |
|--|---------------------------------|--|----------|--|--|--|--|
| Session 5: Promising synthetic and natural compounds for sustainable control | | | | | | | |
| Chair: Ioan | Chair: Ioannis P. Papanastasiou | | | | | | |
| 9:00 | Elisa Uliassi | Building Bridges Between Medicinal Chemistry and Sustainability: Parasitic Diseases in the One Health Context | Page: 26 | | | | |
| 9:20 | Maria Paola Costi | High-Throughput Screening Selection and Deselection Process for the Identification of High-Affinity PTR1 Inhibitors | Page: 27 | | | | |
| 09:40 | Elisabeth Davioud- Charvet | Preparation of a library of polysubstituted 3-benzylmenadiones and design of plasmodione prodrugs | Page: 28 | | | | |
| 10:00 | Orazio Taglialatela- Scafati | The potential of natural products against vector-borne diseases | Page: 29 | | | | |
| | Marco Persico | Computer-aided design of new nature-inspired antimalarials | Page: 30 | | | | |
| 10:30 Coffee break | | | | | | | |
| Chair: Mar | garida Duarte | | | | | | |
| 11:00 | Roland Hellinger | Discovery, characterization and application of nature-derived cystine- knot peptides with antimicrobial activity | Page: 31 | | | | |
| 11:20 | Theodora Calogeropoulou | Sustainable development of novel antiparasitic agents valorising by- products of the cashew industry | Page: 32 | | | | |
| 11:40 | Godwin U. Ebiloma | Development of a novel Trypanosome Alternative Oxidase inhibitor with a broad-spectrum activity against the causative agents of animal Trypanosomiasis | Page: 33 | | | | |
| 11:50 Concluding remarks (Guy Caljon and Maria Paola Costi) | | | | | | | |
| 12:00 Adjourn – city walk | | | | | | | |







OneHealth*drugs*

Cost Action CA21111

Poster Presentations

| Hafidh Akkari | Bioassay guided isolation and identification of Amoebicidal and leishmanicidal compounds from Tunisian natural sources | Page: 35 |
|--------------------------|--|----------|
| Gülşah Bayraktar | Studies on Thiazolopyrimidine Derivatives within One Health Perspective | Page: 36 |
| Dijana Blazhekovikj | Fish-borne parasitic zoonoses: One Health perspectives | Page: 37 |
| Aleksandar Cvetkovski | Role of crystal engineering in repurposing drugs for therapy of antiparasitic vector-born disease: structure-property relationship | Page: 38 |
| Roncareggi Davide | On the road to unravel the interactome of Leishmania molecular heat shock protein 90 | Page: 39 |
| Dilek Ömer Gürkan | Bridging Veterinary and Human Health: Drug Targets for Zoonotic Parasite Reservoirs | Page: 40 |
| Jasmina Đorđević | TIME-CHANGE LÈVY PROCESS IIN MODELLING OF EPIDEMICS | Page: 41 |
| Lori Doko | Pharmacological Advances in Vector-Borne Parasitic Diseases: Assays, Targets, and Environmental Impact | Page: 42 |
| Abazaj Erjona | Risk factors, mode of transmission and potential for acute toxoplasmosis serological diagnosis: A case series of this infection occurring during pregnancy | Page: 43 |
| David C. Magri | Cinchona Alkaloid Polymeric Fluorescent Logic Gates | Page: 44 |
| Miguel Marín Folgado | Assessment of dyes and new strategies in the design of anthelmintic drugs | Page: 45 |
| Sébastien Pomel | PEGylated liposomes encapsulating amphotericin B: an innovative formulation for the treatment of cutaneous leishmaniasis | Page: 46 |
| Ricardo Monteiro | Evaluation of Novel 4-Thiazolidinone Bioisosteres of Alkylphosphocholines as anti-Trypanosomal agents | Page: 47 |
| Essia Sebai | Chemical analysis and and in vitro acaricidal activity of Mentha pulegium and Thymus capitatus essential oils against Hyalomma dromedarii | Page: 48 |
| Asghar Talbalaghi | Leishmaniasis and Vector Control An Emerging Threat and New Prevention Strategies | Page: 49 |
| Theo Zacharis | Stakeholder Engagement for Sustainable Drug Development under the OneHealthDrugs Action | Page: 50 |





Funded by the European Union

One Health drugs against parasitic vector borne diseases in Europe and beyond OneHealth*drugs* Cost Action CA21111

Abstracts Oral presentations







How is the Animal Health industry driving innovation?

Authors: Geurden T.

Zoetis Mercuriusstraat 20, 1930 Zaventem, Belgium

thomas.geurden@ugent.be

The animal health market has experienced consistent growth, averaging an annual rate of 5% or more over the past decade. This expansion is driven by the introduction of innovative products and the development of new therapeutic areas, alongside heightened public concern regarding vector-borne and zoonotic diseases. In response to the threat of vector-borne diseases, new preventative measures, including vaccines and solutions to reduce disease transmission from vectors to hosts, have been developed. To support these innovations, substantial investments in fundamental research on the biology and epidemiology of vector-borne diseases have been necessary, along with the validation of new disease models and the establishment of scientific and regulatory guidelines. Despite significant advancements, gaps remain in both scientific knowledge and technical solutions for preventing or mitigating vector-borne diseases. Close collaboration among academia, public stakeholders, funding bodies, regulatory authorities, and industry is crucial for developing sustainable solutions and to discuss the benefit of every prevention or treatment option against the risk for the patient and the environment. An informed consensus on the benefit-risk ratio, both generally and for specific products, facilitates the adoption of new solutions by consumers. Early collaboration among stakeholders is also vital to bridge the funding and resource gap between fundamental and clinical research.







Purine nucleoside analogues against protozoan pathogens in humans and livestock: from bench

to barn.

Authors: Hulpia F,^a Bouton J,^aLin C,^a Van de Velde E,^a Traen J, Ilbeigi K,^b Caljon G,^b

Van Calenbergh S,^a

^a Ghent University, Belgium; ^b University of Antwerp, Belgium.

serge.vancalenbergh@ugent.be

While the therapeutic utility of nucleoside analogues was well established for viral infections and cancers, their potential for combating protozoan diseases remained largely underexplored. Unlike mammals, most parasitic protozoa are purine auxotrophs, depending on the uptake and processing of pre-formed purines from their hosts. To enable this, these organisms have developed specialized transporters and enzymatic machinery for salvaging and interconverting nucleosides and nucleotides, essential for DNA and RNA synthesis.¹

Inspired by these biochemical differences, in 2016 our research groups started with the synthesis of a library of (mainly purine-like) nucleoside analogues and assessed their activity and selectivity against a representative panel of Trypanosoma and Leishmania species. Structural modifications of tubercidin led to the discovery of several promising antiparasitic candidates, some of which demonstrated efficacy in relevant animal models for human parasitic diseases.^{2,3,4}

Additionally our library also allowed the discovery of a therapeutic candidate for animal trypanosomiasis,⁵ as well as other leads for protozoan infections that afflict animal health, such as histomoniasis and cryptosporidiosis.

This presentation will provide an overview of our most significant findings and examples and considerations for further developments.

References

[1] Hofer, A. FEMS *Microbiol. Rev.* 2023, 47: fuad020. [2] Hulpia, F. et al. *Nat. Commun.* 2019, 10: 5564. [3] Hulpia, F. et al. *J. Med. Chem.* 2018, 61, 9287. [4] Bouton, J. et al. *J. Med Chem.* 2021, 64, 4206. [5] Ilbeigi, K. et al. *ACS Inf. Dis.* 2024, 11, 131.







Antiparasitic Research: An Animal Health Perspective.

Authors: Paul M. Selzer^a

^a Boehringer Ingelheim Vetmedica GmbH,

Binger Str. 173, 55216 Ingelheim am Rhein, Germany

paul.selzer@boehringer-ingelheim.com

Antiparasitics acting on endo- or ectoparasites represent the second largest segment of the global animal health market, accounting for approximately 27% of market share. However, relatively few novel antiparasitic agents have been introduced into the market during recent decades. One exception, and a groundbreaking 21st century success story, are the isoxazolines, whose full potential have not yet been entirely explored. Other than that example, this scarcity is particularly evident for parasitic diseases caused by helminths where macrocyclic lactones continue to be the primary compound class employed for prevention and treatment. Moreover, resistance issues across a broad spectrum of parasitic diseases underscores a pressing market need for innovative, resistance-breaking antiparasitics that operate through novel modes of action. Advances in science and technologies strongly suggest that the answer lies in the synergistic integration of phenotypic screening with mechanism-based strategies to discover novel antiparasitics for the use in both animals and humans.







Strategic roadmap towards high-quality leads and drug development candidates: in vitro and in

vivo laboratory approaches for kinetoplastid diseases

Sarah Hendrickx¹, Kayhan Ilbeigi¹, Eli S.J. Thoré^{2,3}, Michael G. Bertram^{2,4,5}, Estefanía Calvo-Alvarez⁶, Sener Cintesun⁷, Ana Isabel Olías-Molero⁸, María Jesús Corral⁸, Marta Mateo-Barrientos⁹, Jérôme Estaquier^{10,11}, Sébastien Pomel¹², José María Alunda⁸, Sheraz Gul^{13,14}, Katrien Van Bocxlaer¹⁵, Frédéric Frézard¹⁶, Joana Tavares^{17,18,19}, Anabela Cordeiro da Silva^{17,18,20}, Maria Paola Costi²¹, Louis Maes^{1,*}, <u>Guy Caljon^{1,*}</u>

¹ Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, Belgium, ² Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Sweden, ³ Laboratory of Adaptive Biodynamics, Research Unit of Environmental and Evolutionary Biology, Institute of Life, Earth, and Environment, University of Namur, Belgium, ⁴ Department of Zoology, Stockholm University, Sweden, ⁵ School of Biological Sciences, Monash University, Australia, ⁶ Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy, ⁷ Department of Molecular Biology and Genetics, Yildiz Technical University, Turkey, ⁸ Department of Animal Health, Complutense University Madrid, Spain, ⁹ Department of Microbiology & Parasitology, Complutense University Madrid, Spain, ¹⁰ INSERM U1124, Université Paris Cité, France, ¹¹ Centre de Recherche du CHU de Québec, Université Laval, Canada, ¹² Université Paris-Saclay, CNRS BioCIS, France, ¹³ Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Germany, ¹⁴ Fraunhofer Cluster of Excellence for Immune-Mediated Diseases CIMD, Germany, ¹⁵ Skin Research Centre, University of York, UK, ¹⁶ Department of Physiology and Biophysics, Universidade Federal de Minas Gerais, Brazil, ¹⁷ i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal, ¹⁸ IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal, ¹⁹ ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal, ²⁰ Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, Portugal, ²¹ Department of Life Sciences, University of Modena and Reggio Emilia, Italy. * Authors share senior authorship

*Guy.Caljon@uantwerpen.be

Given the impact of kinetoplastid diseases in man and animals, the limited therapeutic options and risks of treatment failure, continued research efforts to discover novel chemical entities and drug targets are required. The ambition to deliver drug development candidates has mainly been taken on board by academia but remains highly challenging because of the lack of adequate funding, inefficient roadmap and non-standardized approaches leading to different laboratory procedures and 'endpoints' which largely influence 'go/no-go' decisions.

Within the aim to deliver more compelling proof-of-concept readouts, a multidisciplinary team within Onehealthdrugs proposes a systematic flow-chart of laboratory experiments and decision criteria to attain highquality leads and drug candidates with lower risk profiles, focusing on African trypanosomiasis, Chagas disease, and visceral and cutaneous leishmaniasis. Next to precision experimental design and reporting, an overview is provided of various complementary laboratory models reproducing kinetoplastid infection and disease. Technical aspects of conventional *in vitro* and *in vivo* approaches and more recently, *in silico* methods with 'endpoints' are presented with reference to specific preclinical R&D stages: (*i*) hit finding, (*ii*) hit profiling, (*iii*) lead definition, and (*iv*) drug development candidate, covering the expertise areas of chemistry, primary pharmacology, pharmacokinetics and -dynamics, (eco)toxicology and pharmaceutics.

This systematic overview intends to provide a pragmatic framework for decision-making within academic research networks bridging the translation from *in vitro* studies, whole-organism and *in vivo* discovery research and support a more focused early development path. Although specifically focusing on kinetoplastid diseases, the basic roadmap principles are also applicable to other microbial diseases.

Acknowledgement

This article is based upon work from COST Action OneHealthdrugs (CA21111). The Action is supported by COST (European Cooperation in Science and Technology).







Regulatory Framework for Veterinary Medicines in the Eupean Union

Authors: Olías Molero, Anabel^a

^a The Spanish Agency of Medicines and Medical Devices (AEMPS), Spain.

aolias@aemps.es

The regulation of veterinary medicines in the European Union (EU) ensures that medicines used in animals are safe, effective and of high quality, while protecting animal health, public health and the environment. To fulfill this mission, the European Medicines Agency (EMA) works closely with the national competent authorities in EU, in a partnership known as the European medicines regulatory network.

The main legal framework is the Regulation (EU) 2019/6 on veterinary medicinal products and repealing Directive 2001/82/EC, applicable from 28 January 2022, whose key objectives are to: harmonize and simplify marketing authorization processes, improve availability of veterinary medicines across EU countries, combat antimicrobial resistance, support innovation and new technologies and enhance pharmacovigilance and transparency.

Under this Regulation, veterinary medicinal products in the EU are classified into three main categories based on their composition and mechanism of action: Non-biological, Biological Non-immunological and Biological immunological veterinary medicinal products. Each type has specific regulatory requirements regarding quality, safety and efficacy.

For the development of new veterinary medicines of any of these types, the EMA and the national regulatory authorities in EU Member States provide guidance and support to researchers and developers. This includes scientific and regulatory information on how to design and run clinical trials, compliance standards, how to establish maximum residue limits for medicines and biocides, support to innovation, and support the development of veterinary medicines for limited markets, among others.

References

- [1] European Medicines Agency (EMA)
- [2] Regulation (EU) 2019/6 on veterinary medicinal products and repealing Directive 2001/82/EC







In vitro and in silico methods to assess ADME-Tox and ecotoxicological profiles of compounds in

drug discovery

Karki R^{a,b}, Greco A^{a,b,c}, Gadiya Y^{a,b}, Zaliani A^{a,b}, Raffellini L^{a,b,c}, Pagliaro A^{a,b,c} Rapposelli S^c & Gul S^{a,b}

^aFraunhofer Institute for Translational Medicine and Pharmacology ITMP, Hamburg, Germany; ^bFraunhofer Cluster of Excellence for Immune-Mediated Diseases CIMD, Hamburg, Germany; ^cDepartment of Pharmacy, University of Pisa, Pisa, Italy.

Sheraz.Gul@itmp.fraunhofer.de

We have developed an high throughput *in vitro* assay panel which comprises off-target liability, ADME-Tox and safety assays. These have been deployed over the past decade to profile compounds and select those for progression in the drug discovery value chain which meet industry standard Lead criteria [1-2]. Drug discovery now also has an *in silico* component which is the data-driven approach to use biological, chemical, and clinical data to streamline the identification of potential Lead and Candidate compounds. Knowledge graphs play a crucial role by representing complex relationships among genes, proteins, diseases, and compounds, allowing researchers to uncover novel drug targets and explore drug repurposing opportunities.

Another important aspect of our work has been to develop user-friendly dashboards and interfaces for non-programmers. The Fraunhofer Edge Cloud which is state-of-the-art technology for cloud computing and data storage (>600 Cores, 13 TB, RAM and >300 TB Storage) has enabled the development of several Machine Learning/Artificial Intelligence based ADMET models including gastrointestinal absorption, BBB/Caco2 permeability and PGP/CYP inhibition. These models have been trained with comprehensive and large datasets consolidated from public databases and in-house data and used to predict activity and property of compound libraries with high precision and accuracy [**3-5**]. This is currently being further enhanced by incorporating ecotoxicology information into the framework thus enhancing the drug safety and environmental impact of compounds. By analyzing the ecological effects of drug candidates within the knowledge graph, researchers can identify compounds with favorable profiles that minimize harm to ecosystems. This integrated approach promotes the development of effective therapeutics while safeguarding environmental health. Case studies from the above will be presented.

References

- [1] Corfu AI, Santarem N, Luelmo S, et al. Discovery of 1,3,4-Oxadiazole Derivatives as Broad-Spectrum Antiparasitic Agents. ACS Infect Dis. 2024, 10, 2222-2238. DOI: 10.1021/acsinfecdis.4c00181.
- [2] Linciano P, Quotadamo A, Luciani R, et al. High-Throughput Phenotypic Screening and Machine Learning Methods Enabled the Selection of Broad-Spectrum Low-Toxicity Antitrypanosomatidic Agents. J Med Chem. 2023, 66, 15230-15255. DOI: 10.1021/acs.jmedchem.3c01322.
- [3] Greco G, Karki R, Gadiya Y, Deecke C, Zaliani A, Gul s. Pharmacological profiles of neglected tropical disease drugs. Al in the Life Sciences. 2024, 6, 100116. DOI: 10.1016/j.ailsci.2024.100116.
- [4] Karki R, Gadiya Y, Zaliani A, Gribbon P. Mpox Knowledge Graph: a comprehensive representation embedding chemical entities and associated biology of Mpox. Bioinform Adv. 2023, 3, vbad045. DOI: 10.1093/bioadv/vbad045.
- [5] Krix S, DeLong LN, Madan S, et al. MultiGML: Multimodal graph machine learning for prediction of adverse drug events. Heliyon. 2023, 9, e19441. DOI: 10.1016/j.heliyon.2023.e19441.

Acknowledgment

The Authors acknowledge the OHD Cost Action (CA21111) including an STSM and the Erasmus+ programme of the European Union for the financial support to perform experimental work related to the work presented.







Academic research ecosystem: does it fit the antiparasitic drug discovery process?

Torrado JJ^a, <u>Alunda JM</u>^b

^a Dpt. of Galenic Pharmacy and Food Technology, Faculty of Pharmacy, University Complutense Madrid, Spain; ^b Dpt. of Animal Health, Faculty of Veterinary Medicine, University Complutense Madrid, Spain.

jmalunda@ucm.es

Parasitic infections affect billions of people and are a major constraint on animal production and welfare worldwide. *Toxoplasma gondii* affects over one third of the total human population [1] and *Plasmodium* will still cause an estimated 249 million malaria cases and 600,000 deaths in 2022, mainly in Africa and especially among children [2]. There are other human diseases associated with the poorest regions of the world, known globally as NTDs (Neglected Tropical Diseases). Twelve of the 21 NTDs are caused by parasites [3]. In addition, parasitic diseases of domestic animals have a huge economic impact in both hemispheres and some have zoonotic potential.

Knowledge of the life cycle of parasites and the epidemiology of the diseases they cause is essential to identify bottlenecks in the transmission process and to design non-medicinal control measures. Unfortunately, although some of these methods are effective and easy to implement, most require long-term efforts, are expensive and do not produce results in the short term. To make matters worse, vaccination against parasitic diseases is an elusive goal. So far, the most favored system has been chemical control of infections. However, chemotherapy for parasitic diseases affecting both humans and livestock is still inadequate.

Lack of industrial interest and scarcity of druggable new chemical entities, stricter safety regulations by regulatory authorities and new societal demands are clearly acting as barriers to the introduction of new antiparasitic drugs. These aspects have a notable impact on the drug discovery process, although there is no precise measure of their relative impact on successful drug discovery and development. However, in our view, even if all the above factors were addressed, the current structure and practices of research institutions are not conducive to the discovery of new and better antiparasitic drugs. The current situation and possible solutions are presented.

References

- [1] https://pmc.ncbi.nlm.nih.gov/articles/PMC7034049/].
- [2] https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023
- [3] https://pmc.ncbi.nlm.nih.gov/articles/PMC9832497/

Acknowledgment

The Authors acknowledge COST Action CA21111 for partial financial support.







New fused-pyrimidine derivatives as promising broad-spectrum antiparasitic agents

André Lopes^{1,2}, Sofia Teixeira¹, Nuno Santarém², Sofia Meirinho³, Alessandro Greco⁴, Oliver Keminer^{5,6}, Sheraz Gul^{5,6}, Pedro Ferreira³, Anabela Cordeiro-da-Silva², <u>Maria Alice Carvalho¹</u>

¹ Chemistry Centre of University of Minho (CQ-UM), Campus de Gualtar, 4710-057 Braga, Portugal and Departamento de Química, Escola de Ciências da Universidade do Minho, Braga, Portugal; ² Instituto de Investigação e Inovação em Saúde, Universidade do Porto and Institute for Molecular and Cell Biology, University of Porto, 4150-180, Porto, Portugal. Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto (FFUP), Porto, Portugal; ³ Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, and PT Government Associate Laboratory ICVS/3B's; ⁴ Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy; ⁵ Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Hamburg, Germany; ⁶ Fraunhofer Cluster of Excellence for Immune-Mediated Diseases CIMD, Hamburg, Germany.

mac@quimica.uminho.pt

Malaria, leishmaniasis, and sleeping sickness are vector-borne infections that together threaten the health of over 260 million people, mainly in tropical and subtropical regions. These diseases particularly affect low-income populations in underdeveloped and developing countries. In 2023, they were responsible for more than 600,000 deaths worldwide ^[1].

Currently, available treatments for these diseases are limited in their effectiveness due to several challenges, such as restricted access to medications in remote and underserved areas, high treatment costs, severe side effects, increasing drug resistance, inconsistent efficacy, and high toxicity levels ^[2,3]. These limitations highlight the urgent need for new, safer, and more effective therapeutic options. Following a phenotypic approach, our research group identified a new class of compounds with high *in vitro* activity against *P. falciparum*, *L. infantum* intramacrophage amastigote form, and *T. brucei* ^[4,5]. The most active compounds present nanomolar IC₅₀ values against *P. falciparum* (101 nM) and *T. brucei* (190 nM), and submicromolar IC₅₀ values against *L. infantum* (1.53 µM). The *in vitro* cytotoxicity was determined using the THP-1 cell line, and early *in vitro* ADME-Tox was carried out using *in vitro* assays for cytotoxicity (A549 and HEK293 cell lines) and CYP3A4 and *h*ERG cardiotoxicity liabilities. Remarkably, the most active derivatives displayed selectivity indexes higher than 500 against *T. brucei*, 58 against *L. infantum* intramacrophage amastigotes and 900 against *P. falciparum*, and most of the derivatives present an acceptable safety profile in the early *in vitro* ADME-Tox assays. All the results will be presented together with the SAR analysis.

References

- [1]. World Health Organization. <u>https://www.who.int/health-topics/</u> (accessed 19 April 2025).
- [2]. Abirami, M.; Kumar, B.K.; Faheem, Dey, S., et.al. Molecular-level strategic goals and repressors in Leishmaniasis Integrated data to accelerate target-based heterocyclic scaffolds. *Eur. J. Med. Chem.* **2023**, 257, 115471-115495.
- [3]. González-Sanz, M., et.al. Updates on malaria epidemiology and prevention strategies. Curr. Infect. Dis. Rep. 2023, 25, 131-139.
- [4]. Lopes, A.; Santarém, N.; Cordeiro-da-Silva, A.; Carvalho, M.A. Pyrimido[5,4-*d*]pyrimidine-based compounds as a novel class of antitrypanosomal and antileishmanial agentes, ACS Med. Chem. Lett. **2022**, 13, 1427-1433.
- [5]. Carvalho, M.A.; Ferreira, P.; Meirinho, S.; Cordeiro-da-Silva, A.; Tavares, J.; Lopes, A. Pyrimido[5,4-*d*]pyrimidine-based compounds, methods and uses thereof. WO/2023/209250 published 02/11/2023.

Acknowledgment

The Authors acknowledge the Portuguese Foundation for Science and Technology (FCT) the financial support the research unit 4293, the projects UID/00686 – Chemistry Centre of University of Minho (CQUM), and PTDC/SAU-PAR/2766/2021.

NS is funded by national funds through FCT and co-funded through the European Social Fund within the Human Potential Operating Programme 2021.04285.CEECIND.







A new class of minor groove binders active against animal trypanosomiasis in vivo

De Koning HP, ^a Ungogo M, ^a Sarode A, ^{b,a} Giordani F, ^a Barrett MP, ^a Gillingwater K, ^c Suckling CJ, ^b Scott

FK ^b

^a University of Glasgow, Glasgow, UK; ^b University of Strathclyde, Glasgow, UK; ^c Swiss Tropical and Public Health Institute, Basel 4051, Switzerland

Harry.de-Koning@glasgow.ac.uk

Minor groove binders such as pentamidine and diminazene have a long and successful history against both human and animal trypanosomiasis. However, the diamidines display uneven potency against different trypanosome species and, after decades of use, suffer from drug resistance. Strathclyde Minor Groove Binders (S-MGBs) are highly potent trypanocides, structurally different from classical diamidines. A very substantial SAR has been built giving rise to lead compounds with broad activity and no cross-resistance to existing trypanocides ^[1]. Lead compounds were shown to be curative in mouse models of both T. congolense and T. vivax; the compounds were stable with mouse liver microsomes and an elimination half-life of 6 h. Unlike diamidines and phenanthridines including isometamidium, S-MGBs are equally active against akinetoplastic cell lines. Moreover, it is proving impossible to induce resistance to them. Mechanism of action studies show they cause cell cycle anomalies including fragmented nuclei and cytokinesis defects, consistent with their effects being irreversible after limited exposure times. DNA binding was confirmed using thermal melt analysis and mass spectrometry of DNA complexes. Metabolomics analysis saw predominantly disturbances in nucleotide metabolism, consistent with effects on nucleic acid synthesis. Transcriptomics revealed large disturbances in mRNA levels, mostly downwards, with no clear functional pattern. Genomewide screening with an RNAi library (RITseq) to identify specific targets or resistance mechanisms identified 3 genes for follow-up. Upon individual RNAi targeting and cas9-directed mutagenesis, alterations of (the expression of) gene Tb927.9.12450, annotated as class I transcription factor A subunit 2, was found to be associated with a minor loss of susceptibility to the lead compound, S-MGB360. Our results are compatible with S-MGB360 acting as a minor groove binder against trypanosomes and not dependent on the known transporters for diamidine MGBs and hence not crossresistant and more broadly active against animal trypanosomiasis species.

References

[1] Giordani F, Khalaf AI, Gillingwater K, Munday JC, de Koning HP, Suckling CJ, Barrett MP, Scott FJ. 2019. Novel Minor Groove Binders Cure Animal African Trypanosomiasis in an in Vivo Mouse Model. Journal of Medicinal Chemistry 62:3021–3035.

Acknowledgment

This research was in part funded by the MSD Scottish Life Sciences fund. Funding was also provided by BBSRC grant BB/N007638/1 and by North-West Biosciences/BBSRC.







Synthesis and Evaluation of Nitroheterocyclic Aromatic Adamantanohydrazones with

Trypanocidal Activity. Part III

Angeliki-Sofia Foscolos,^{a,b} Konstantina Stavropoulou,^a Nuno Santarém,^c Anabela Cordeiro da Silva,^c Martin C. Taylor,^d John M. Kelly,^d Susan Wyllie,^e Theodora Calogeropoulou,^f Andrew Tsotinis, ^a and <u>Ioannis P. Papanastasiou</u>^a

^aNational and Kapodistrian University of Athens, Greece; ^b NCSR "Demokritos", Greece; ^cUniversity of Porto, Portugal; ^dLondon School of Hygiene and Tropical Medicine, UK; ^eUniversity of Dundee, Scotland; ^fNational Hellenic Research Foundation, Greece.

papanastasiou@pharm.uoa.gr

The protozoan parasites *Leishmania* spp., *Trypanosoma cruzi*, and *Trypanosoma brucei*, collectively known as the Tritryps, are the causative agents of the neglected tropical diseases (NTDs) leishmaniasis, Chagas disease (CD), and human African trypanosomiasis (HAT), respectively. Collectively, these diseases contribute to the highest mortality rates among all NTDs. Many of the currently available and newest trypanocidal agents are nitroaromatic drugs: Nifurtimox and benznidazole are the main treatments for CD, while nifurtimox is also used in combination therapy for the second stage of HAT.[1] In this work, the synthesis and the evaluation of a new series of nitroheterocyclic aromatic adamantanohydrazones against trypanosomes, is presented. These probes bear in their skeleton the adamantane cage, which is substituted at C1 and C2, by side chains of varying spacer lengths and diverse terminal nitroheterocyclic functionalities. The adamantane nitrofurans were in general more potent and less toxic than their congeners nitrothiophenes and nitroimidazole and benznidazole. The most potent phenylacetoxy hydrazone derivative (EC₅₀=11±0.9 nM, SI_{Tb}=770) is confirmed to be specifically activated by NTR 1 and is currently under *in vivo* investigation.



References

 M. C. Field, D. Horn, A. H. Fairlamb, M. A. J. Ferguson, D. W. Gray, K. D. Read, M. De Rycker, L. S. Torrie, P. G. Wyatt, S. Wyllie, I. H. Gilbert, Nat Rev Microbiol 2017, 15, 217–231







OCART: A Simple Chemoinformatics Toolkit in Orange3 for Drug Discovery in Vector-Borne Diseases

Authors: Podlipnik Črtomir^a

^a Faculty of Chemisry and Chemical Technology, University of Ljubljana , Slovenia

crtomir.podlipnik@fkkt.uni-lj.si

Abstract: OCART (Orange3 for Chemistry: Advanced Research Toolkit) is an easy-to-use chemoinformatics toolkit within Orange3¹, a visual data mining platform. OCART is designed to help researchers from academia and industry perform everyday chemistry tasks using drag-and-drop blocks without the need for programming. With these tools, users can calculate molecular properties, compare compounds, group similar molecules, evaluate drug similarity and ADMET properties, create biological activity prediction models (QSAR/QSPR) and much more.

Here we show some examples of how OCART supports early drug discovery for vector-borne diseases (VBDs). The toolkit helps to identify and prioritise promising compounds by assessing their impact on the target and potential side effects. It also shows functional patterns and relationships through easy-to-understand visualisations. OCART connects directly to free chemical databases so that users can quickly access and analyse real-world data.

This work supports the One Health approach - developing medicines that are effective and safe for humans, animals and the environment. OCART helps researchers select better compounds while avoiding those that could harm ecosystems.

By facilitating access to and analysis of chemical data, OCART accelerates the search for new treatments against VBDs. It also promotes collaboration by helping scientists and professionals from different backgrounds to work together through a common, user-friendly platform.

References

 Demsar J, Curk T, Erjavec A, Gorup C, Hocevar T, Milutinovic M, Mozina M, Polajnar M, Toplak M, Staric A, Stajdohar M, Umek L, Zagar L, Zbontar J, Zitnik M, Zupan B (2013). Orange: Data Mining Toolbox in Python, Journal of Machine Learning Research 14(Aug): 2349–2353.

Acknowledgment

The Authors acknowledge Slovenian Research Agency (ARRS) programme grant P1-0201.







Metabolic Rewiring in Leishmania Lacking PRX1/2 Reveals Hidden Vulnerabilities for Drug Targeting

^a Duarte M, ^b Rodrigues JA, ^a Lubbers C, ^a De Boeck V, ^b Bispo DSC, ^b Gil AM and ^{a,c} Tomás AM

^a i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208 4200-135 Porto, Portugal; ^b CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; ^c ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal.

mduarte@i3s.up.pt

Leishmaniasis remains a neglected tropical disease with limited treatment options and increasing rates of drug resistance. Therapeutic failure is often linked to the parasite's remarkable ability to adapt and survive under oxidative and pharmacological stress. While redox metabolism has emerged as a promising target for intervention, the complexity of the Leishmania antioxidant network and its integration with core metabolic pathways remain incompletely understood. Elucidating how Leishmania adapts to redox imbalance could reveal novel mechanisms that sustain parasite fitness and offer alternative targets for therapy.

Peroxiredoxins (PRXs) are central players in redox homeostasis, with Leishmania uniquely expressing two cytosolic isoforms, PRX1 and PRX2. These enzymes are capable of sensing hydrogen peroxide and oxidizing protein targets, suggesting roles in both detoxification and redox signaling. Surprisingly, a double knockout mutant lacking both PRX1 and PRX2 remains viable and shows no overt fitness defect in vitro, despite increased sensitivity to hydrogen peroxide [1]. Using this genetically engineered mutant as a tool and a combination of redox-sensitive probes, phenotypic assays, and metabolic profiling techniques-including proteomics and metabolomics-we aim to uncover the compensatory mechanisms underlying this unexpected resilience. Our findings suggest that Leishmania can activate alternative pathways to maintain redox balance and drug tolerance, in the absence of canonical antioxidant enzymes PRX1 and PRX2. Indeed, ablation of PRXs does not affect parasite survival or infectivity. Aside from ascorbate/cytochrome c peroxidase, a mitochondrial located enzyme, other peroxidases do not appear to change expression in the mutant. PRX-KO parasites rewire metabolism, relying more on the respiratory chain than glycolysis, yet maintain similar ROS levels to wild type, even upon short exposure to a lethal dose of miltefosine. We propose that these adaptations represent novel metabolic vulnerabilities that can be exploited for therapeutic intervention, either alone or in combination with existing anti-leishmanial drugs.

References

[1] The cytosolic hyperoxidation-sensitive and -robust Leishmania peroxiredoxins cPRX1 and cPRX2 are both dispensable for parasite infectivity. Castro H et al. Redox Biol. doi.org/10.1016/j.redox.2024.103122. 2024.

Acknowledgment

The Authors acknowledge FCT - Fundação para a Ciência e a Tecnologia, I.P. through National Funds, under the project UIDB/04293/2020 for the financial support.







The potential trade-off between human health and ecosystem health – ecological effects of pharmaceutical contamination

Brodin, T. ª

^a Swedish University of Agricultural Sciences

tomas.brodin@slu.se

Pharmaceuticals is a group of "good chemicals" containing a wide range of compounds that are used, or sometimes even designed, to have a biological effect in the human body. Because of their direct positive effect to human health their environmental impact was long overlooked. Over several decades there has been a steady increase in human consumption of pharmaceuticals, and consequently aquatic organisms are increasingly exposed to novel pharmaceutical pollutants with limited knowledge of the ecological and evolutionary consequences. Here I will present some of our work on ecological effects of behaviourally modifying pharmaceuticals in aquatic environments, bridging the gaps between human health, organismal effects and environmental impact. All though we still have limited understanding of the impacts of pharmaceutical pollution in the wild the fact of the matter is: even "good chemicals" can have bad effects.







Pharmaceuticals and their potential for ecological surprises

Authors: Mirco Bundschuh

^a University of Kaiserslautern-Landau (RPTU), Germany

mirco.bundschuh@rptu.de

Pharmaceuticals represent a group of chemicals that are applied with an intended biological response supporting human and animal welfare. In a OneHealth framework, the increasing development of multiple resistance in bacteria is probably one prime example. Less obvious is the impact of pharmaceutical in the environment which can reach from changes in behavior (see talk by T Brodin), over interruption of reproduction to impacts in ecosystem level processes. In this presentation I will primarily address recent examples pharmaceutical induced alterations in nutrient and carbon processing or cycling, thought to stimulate a critical reflection on current procedures in the development and use of pharmaceuticals application against the OneHealth framework with special emphasis on environmental hazards and risks.







Piloting sustainable worm control approaches in grazing livestock to minimize the

environmental impact of anthelmintics in livestock

<u>Authors: Johannes Charlier</u>^a, Laura Rinaldi^b, Eric R. Morgan^c, Edwin Claerebout^d, Dave J. Bartley^e, Smaragda Sotiraki^f, Marcin Mickiewicz^{g,h}, Maria Martinez-Valladaresⁱ, Natascha Meunier^j, Tong Wang^a, Alistair Antonopoulos^a, Fanny Baudoin^k, Leen Lietaer^k

^a Kreavet, Belgium; ^b University of Napoli Federico II, Italy; ^c University Belfast, Northen Ireland; ^d Ghent University, Belgium; ^e Moredun Research Institute, UK; ^f Veterinary Research Institute, Ellinikos Georgikos Organismos (HAO)-DIMITRA, Greece; ^g Toinen Pro Art Fundacja, Zduny, Poland; ^h Warsaw University of Life Sciences-SGGW, Warsaw, Poland; ⁱ Instituto de Ganadería de Montaña (CSIC-Universidad de León), Spain; ^j Animal Health Ireland, Ireland; ^k Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium

jcharlier@kreavet.com

Anthelmintic resistance is an escalating problem in Europe and the environmental consequences (soil and aquatic health) related to anthelmintic use are an increasing matter of concern. Several sustainable worm control practices are available now. These include the increased use of diagnostics and decision support enabling a targeted use of anthelmintics. Complementary control measures, referred to as the 'Basket of Options', include plant-based control, grazing management, nematode destroying fungi and selective breeding and can also reduce the need for anthelmintic use. Their use is more complex than the simple use of anthelmintics and their uptake has remained relatively low. Equipped by recent studies on the barriers and drivers for the uptake of sustainable worm control by farmers, we are now building a Community of Practice across Europe, termed SPARC – Sustainable Parasite Control in grazing ruminants, involving all relevant stakeholders at local, national and European level to achieve sustainable worm control together. We will present recent insights and activities from this effort.

References

Johannes Charlier, Laura Rinaldi, Eric R Morgan, Edwin Claerebout, Dave J Bartley, Smaragda Sotiraki, Marcin Mickiewicz, Maria Martinez-Valladares, Natascha Meunier, Tong Wang, Alistair Antonopoulos, Helena C de Carvalho Ferreira, Sustainable worm control in ruminants in Europe: current perspectives, *Animal Frontiers*, Volume 14, Issue 5, October 2024, Pages 13–23, <u>https://doi.org/10.1093/af/vfae033</u>

Acknowledgement

This publication conference abstract is based upon work from the Horizon Europe SPARC project [101134842], funded by the European Union (<u>www.wormsparc.com</u>) and COST Action ENVIRANT [CA23154], supported by COST (European Cooperation in Science and Technology) www.cost.eu.







Monogenean flatworms: petite parasites, mighty models, innovative indicators?

Vanhove Maarten P.M.

^a Hasselt University, Belgium; ^b IUCN SSC Parasite Specialist Group, Belgium; ^c University of Liège, Belgium

maarten.vanhove@uhasselt.be

Monogeneans are an often overlooked, understudied group of helminths. Even their mere existence has regularly come under scientific scrutiny! Often tiny, and mostly infesting external surfaces of fishes, they receive less research and policy attention than the better known flukes and tapeworms that are often of severe human and veterinary health concern. This is unfortunate, as their direct (one-host) lifecycle gives them a methodological advantage for scientists: the (history of) interactions with their hosts can be studied with less confounding factors than is the case for parasites with a complex lifecycle involving vectors or other intermediate hosts. With over 5700 known species and a cosmopolitan distribution covering a wide range of hosts and infection sites, monogeneans, therefore, present a diverse assemblage of candidate models in various subfields of parasitology [1]. For example, in the African Great Lakes, textbook playgrounds for generations of biologists, we studied them to investigate how host evolution [2] and ecology [3] influence parasite communities. Moreover, especially in captivity or when co-introduced with non-native species, monogeneans can wreak havoc to host populations.

Despite an evident negative connotation, parasites provide numerous ecosystem services. For example, they may act as tags for the history of their hosts. This can be applied in invasion biology, where monogenean parasites can help identify fish stocks [4] and infer routes and mechanisms of introduction [5]. The indicator potential of monogeneans also makes them sentinels for ecosystem-level changes resulting from human intervention [6]. The fact that anthropogenic impacts threaten parasites at least as much as they do their hosts and ecosystems, may even make our favourite flatworms interesting to conservation efforts [7]. They also hold promise in environmental parasitology, to help assess effects of pollution, and even for relatively well-studied systems, this field of parasitology is often still in its infancy [8].

References

- [1] Vanhove M.P.M., Pariselle A. & Kmentová N. (2024) Monogenean parasitic flatworms. *Current Biology* 34(22): R1122-R1124. https://doi.org/10.1016/j.cub.2024.10.033
- [2] Vanhove M.P.M., Kmentová N., Faes C., Fernandes J.M.O., Hahn C., Hens N., Pariselle A. & Koblmüller S. (2025) Understanding the influence of host radiation on symbiont speciation through parasites of species flocks. *Cold Spring Harbor Perspectives in Biology* 17(1): a041450. <u>https://doi.org/10.1101/cshperspect.a041450</u>
- [3] Kmentová N., Cruz-Laufer A.J., Milec L., Moons T., Heeren S., van den Hoorn E., Thys K.J.M., Makasa L., Chocha Manda A., Masilya Mulungula P., Van Steenberge M., Jorissen M.W.P. & Vanhove M.P.M. (2024) Host lifestyle and parasite interspecific facilitation mediate co-infection in a species-poor host-parasite system. *Oikos* 2024(10): e10360 <u>https://doi.org/10.1111/oik.10360</u>
- [4] Jorissen M.W.P., Vanschoenwinkel B., Pariselle A., Artois T., Danadu C., Huyse T., Kapepula Kasembele G., Muterezi Bukinga F., Šimková A., Snoeks J., Van Sever W., Vreven E., Wamuini Lunkayilakio S. & Vanhove M.P.M. (2025) Do parasite communities differ between invasive and native fish hosts? A case of monogeneans infecting the gills of Nile tilapia. *Hydrobiologia* <u>https://doi.org/10.1007/s10750-024-05789-8</u>
- [5] Kmentová N., Van Steenberge M., Thys van den Audenaerde D.F.E., Nhiwatiwa T., Muterezi Bukinga F., Mulimbwa N'sibula T., Masilya Mulungula P., Gelnar M. & Vanhove M.P.M. (2019) Co-introduction success of monogeneans infecting the fisheries target *Limnothrissa miodon* differs between two non-native areas: the potential of parasites as a tag for introduction pathway. *Biological Invasions* 21: 757-773. https://doi.org/10.1007/s10530-018-1856-3
- [6] Gobbin T.P., Van Steenberge M., Vranken N. & Vanhove M.P.M. (2024) Worms of change: anthropogenic disturbance changes the ectoparasite community structure of Lake Victoria cichlids. *bioRxiv* <u>https://doi.org/10.1101/2024.04.14.589059</u>
- [7] Vanhove M.P.M., Auwerx J., Kmentová N., Martel A., Nelson A., Terriere N., Van Wichelen J., & Gobbin T.P. (2025) Captive breeding of the critically endangered European weatherfish: a refuge for the conservation-sensitive parasite *Gyrodactylus fossilis*? *The Biologist (Lima)* 23(S1): S123-S124
- [8] Shigoley M.I., Antoine-Moussiaux N., Jauniaux T. & Vanhove M.P.M. (2024) Parasitology of one of the world's foremost fisheries target species lacks a One Health approach. *Hydrobiologia* <u>https://doi.org/10.1007/s10750-024-05695-z</u>

Acknowledgment

The Authors acknowledge the Czech Science Foundation (GAČR), the Research Foundation – Flanders (FWO-Vlaanderen), VLIR-UOS, the Special Research Fund (BOF) of Hasselt University, the Belgian Federal Science Policy Office (BELSPO), EU SYNTHESYS, and the Swiss National Science Foundation for the financial support.







CA21111 OHD1 - Target database project: the BioTarget DataBase (BioT-DB)

Ulrike Wittig^a, Andrea Ilari^b, Javier Santamaría^c, Alfonso T. Garcia-Sosa^d, Michael Bertram^e, Eli Thoré^e, Guy Caljon^f, Annette Ives^g, Emilio Parisini^h, Theodora Calogeropoulouⁱ, Marco Mazzorana^j, Marko Jukić^k, Anabela Cordeiro da Silva^l, Maria Paola Costi^m, Cecilia Pozziⁿ

^a Heidelberg Institute for Theoretical Studies, Germany; ^b Italian National Research Council, Italy; ^c Universidad de Cantabria, Spain; ^d University of Tartu, Estonia; ^e Swedish University of Agricultural Sciences, Sweden; ^f University of Antwerp, Belgium; ^g AC Bioscience, Switzerland; ^h Latvian Institute of Organic Synthesis, Latvia; ⁱ National Hellenic Research Foundation, Greece; ^j Diamond Light Source Ltd., United Kingdom; k University of Maribor, Slovenia; ¹ University of Porto and Institute for Molecular and Cell Biology, Portugal; ^m University of Modena and Reggio Emilia, Italy; ⁿ University of Siena, Italy

cecilia.pozzi@unisi.it

The *OHD1* - *Target database* project aims at developing the BioTarget DataBase (BioT-DB), collecting valuable information on the biological targets currently under investigation by the CA21111 members. The BioT-DB is structured in ten main sections, each focused on different macromolecular properties related to the OneHeathDrugs theme. Following the first section, reporting general information (e.g., name and acronym, sequence, IDs for UniprotKB [1] and BRENDA [2] databases), seven sections are dedicated to report useful information on the target function, production, biochemical, and biophysical characterization, omics, medium and high throughput screening, and the selectivity/specificity profile. A specific section is dedicated to collect available structural information on the targets, investigated by different techniques (e.g., X-ray crystallography, CryoEM, BioSAXS, and NMR). Useful links to the main structural databases, PDB [3], EMDB [4], SASBDB [5], and BMRB [6], are reported together with available, yet unpublished, structural characterizations. The last section is focused on the ecotoxicological impact of target molecules, evaluated by SeqAPASS [7] or other tools.

The BioT-DB is specifically designed to promote collaborations within the CA21111 members, reporting direct contact details in each section. Furthermore, the database aims to highlight key properties of the investigated biological macromolecules combined with their selectivity and specificity profiles and the evaluation of their ecotoxicological impact. The integration of all this information allows a widen perspective for biological target selection and investigation. At a later stage, the target data, collected within the BioT-DB, will be crossed with the compound data, included in CA21111compound databases.

References

- [1] <u>https://www.uniprot.org/</u>,
- [2] https://www.brenda-enzymes.org/
- [3] Velankar S, et al. Methods Mol Biol. (2021), 2305:3-21.
- [4] wwPDB Consortium Nucleic Acids Res. (2024), 52(D1):D456-D465.
- [5] Kikhney AG, et al. Protein Sci. (2020), 29(1):66-75
- [6] Hoch JC, et al. Nucleic Acids Res. (2023) 51:D1.
- [7] Doering JA, at al. Toxicol Sci. (2018), 166(1):131-145.







Contributions to drug discovery against *Leishmania infantum*: from target-based strategies to insights into the biology of the infection

Ricardo Monteiro^{a;b}, Niels Smits^c, W. Wouters^c, Helma Rutjes^c, Stuart McElroy^d, Ritika Sethi^e, Anabela Cordeiro da Silva ^{a;b*} and Joana Tavares^{a;f*}

^a Institute for Research and Innovation in Health (i3S), University of Porto, Porto, Portugal; ^b Departmento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; ^cPivot Park Screening Centre, The Netherlands; ^dUniversity of Dundee, UK; ^e University of Oxford, UK; ^f Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal.

jtavares@i3s.up.pt

Target-led drug discovery has focused on identifying parasite enzymes that catalyze essential biochemical processes markedly different from their human counterparts, offering exploitable therapeutic windows. Our research has led to the genetic validation of two promising targets. The first is ribose 5-phosphate isomerase B (RpiB), an enzyme involved in the non-oxidative branch of the pentose phosphate pathway [1–3]. Fragment library screening against *Leishmania infantum* RpiB (LiRpiB), combined with thermal shift analysis, identified hit fragments that inhibited enzyme activity and selectively suppressed parasite growth *in vitro* [4]. The second target is *L. infantum* silent information regulator 2-related protein 1 (LiSIR2rp1), a member of the sirtuin family known to deacetylate lysine residues in histones and non-histone proteins using NAD⁺ as a cofactor [5–8]. In collaboration with the European Lead Factory, we screened approximately 440,000 compounds in an enzymatic assay targeting LiSIR2rp1. From this, 1,997 compounds were selected for dose-response and orthogonal testing, yielding 397 promising candidates. Further prioritization based on activity, selectivity, structural characteristics, purity, and physicochemical properties identified 55 compounds for follow-up. Of these, 9 compounds demonstrated promising activity against *L. infantum* intracellular amastigotes. These hits now require their pharmacokinetics to be evaluated, followed by efficacy studies in mouse models.

Our lab has also contributed to drug development by using mouse models to assess efficacy and study infection mechanisms. In a model that presents clinical features of visceral leishmaniasis, such as hepatosplenomegaly [9], we observed that *L. infantum* spreads through the bloodstream and accumulates in the lungs, liver, and spleen. Ex vivo imaging revealed that the lungs contain approximately 90% of the total bioluminescent signal across multiple organs—including spleen, liver, heart, and kidneys—even after trans-cardiac perfusion. Limiting dilution assays confirmed comparable parasite loads in the lungs, liver, and spleen during early infection. Notably, while parasites persist in the spleen, liver, and bone marrow, most are cleared from the lungs, although some persist for severeal weeks. Understanding the mechanisms that control lung infection by *L. infantum* could inform the development of therapeutic strategies. These findings also highlight the importance of assessing pulmonary parasite burden during drug efficacy testing.

References

- Loureiro I, Faria J, Clayton C, Macedo-Ribeiro S, Santarém N, Roy N, Cordeiro-da-Siva A, Tavares J. Ribose 5-phosphate isomerase B knockdown compromises *Trypanosoma brucei* bloodstream form infectivity. *PLoS Negl Trop Dis* 2015, 9:e3430-11.
- [2] Faria J, Loureiro I, Santarem N, Cecilio P, Macedo-Ribeiro S, Tavares J, Cordeiro-da-Silva A. Disclosing the essentiality of ribose-5-phosphate isomerase B in Trypanosomatids. Sci Rep 2016, 6:26937.
- [3] Loureiro I, Faria J, Santarem N, Smith TK, Tavares J, Cordeiro-da-Silva A. 2018. Potential drug targets in the pentose phosphate pathway of trypanosomatids. Curr Med Chem 2018, 25:5239–5265.
- [4] Dickie EA, Ronin C, Sá M, Ciesielski F, Trouche N, Tavares J, Santarem N, Major LL, Pemberton IK, MacDougall J, Smith TK, Cordeiro-da-Silva A, Ciapetti P. Toward Chemical Validation of Leishmania infantum Ribose 5-Phosphate Isomerase as a Drug Target. Antimicrob Agents Chemother 2021, 65: e0189220.

[5] Vergnes, B.; Sereno, D.; Tavares, Cordeiro-da-Silva, A.; Vanhille, L.; Madjidian-Sereno, N.; Depoix, D.; Monte-Alegre, A.; Ouaissi, A. Targeted disruption of cytosolic SIR2 deacetylase discloses its essential role in Leishmania survival and proliferation. Gene 2005, 363, 85-96.

[6] Tavares, J.; Ouaissi, A.; Santarem, N.; Sereno, D.; Vergnes, B.; Sampaio, P.; Cordeiro-da-Silva, A. The Leishmania infantum cytosolic SIR2-related protein 1 (LiSIR2RP1) is an NAD+ -dependent deacetylase and ADP-ribosyltransferase. Biochem J 2008, 415, 377-386.

- [7] Tavares, J.; Ouaissi, A.; Kong Thoo Lin, P.; Loureiro, I.; Kaur, S.; Roy, N.; Cordeiro-da-Silva, A. Bisnaphthalimidopropyl derivatives as inhibitors of Leishmania SIR2 related protein 1. ChemMedChem 2010, 5, 140-147.
- [8] Ronin C, Costa DM, Tavares J, Faria J, Ciesielski F, Ciapetti P, Smith TK, MacDougall J, Cordeiro-da-Silva A, Pemberton IK. The crystal structure of the *Leishmania infantum* Silent Information Regulator 2 related protein 1: Implications to protein function and drug design. PLoS One 2018, 13, e0193602.
- [9] Mendes Costa D, Cecílio P, Santarém N, Cordeiro-da-Silva A, Tavares J. Murine infection with bioluminescent Leishmania infantum axenic amastigotes applied to drug discovery. Sci Rep 2019, 9:18989.

Acknowledgment

This project has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contribution. This work was continued in the context of the COST Action CA21111: One Health Drugs against Parasitic Vector-Borne Diseases in Europe and Beyond (OneHealthDrugs) and was also funded by National Funds through FCT-Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04293/2020. Ricardo Monteiro is funded by a FCT individual fellowship 2021.09486.BD.







Q586B2 is a crucial virulence factor during the early stages of Trypanosoma brucei infection that

is conserved amongst trypanosomatids

Stijlemans B.^{a,b}, De Baetselier P.^{a,b}, Van Molle I.^{c,d}, Lecordier L.^e, Hendrickx E.^f, Romão E.^g, Vincke C^{a,b}, Baetens W.^{a,b}, Schoonooghe S. ^g, Hassanzadeh-Ghassabeh G.^g, Korf H.^h, Wallays M.^h, Pinto Torres J.E.^a, Perez-Morga D.^{f,i}, Brys L.^{a,b}, Campetella O.^j, Leguizamón M.S.^j, Claes M.^k, Hendrickx S.^k, Mabille D.^k, Caljon G.^k, Remaut H.^{c,d}, Roelants K.^l, Magez S.^{a,m}, Van Ginderachter J.A.^{a,b,#}, De Trez C.^{a,#}

^a Brussels Center for Immunology, Vrije Universiteit Brussel, Brussels, Belgium; ^b Myeloid Cell Immunology Laboratory, VIB Center for Inflammation Research, Brussels, Belgium; ^c Structural Biology Brussels, Vrije Universiteit Brussel, Brussels, Belgium; ^d VIB-VUB Center for Structural Biology, Brussels, Belgium; ^e Biology of Membrane Transport Laboratory, Université Libre de Bruxelles, Gosselies, Belgium; ^f Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, Gosselies, Belgium; ^g VIB Nanobody Core, Vrije Universiteit Brussel, Brussels, Belgium; ^h Laboratory of Hepatology, Department of Chronic Diseases and Metabolism, KU Leuven, Leuven, Belgium; ⁱ Center for Microscopy and Molecular Imaging (CMMI), Université Libre de Bruxelles, Gosselies, Belgium; ^j Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín-CONICET, Buenos Aires, Argentina; ^k Laboratory of Microbiology, Parasitology, and Hygiene (LMPH), Infla-Med Centre of Excellence, University of Antwerp, Antwerp, Belgium; ¹ Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel, Brussels, Belgium; ^m Laboratory of Biomedical Research, Ghent University Global Campus, Incheon, South Korea. # Contributed equally.

benoit.stijlemans@vub.be

African trypanosomes are extracellular, flagellated protozoan parasites that cause debilitating diseases of both medical and veterinary importance across sub-Saharan Africa. Transmitted by the tsetse fly vector (*Glossina* spp.), they lead to sleeping sickness in humans (HAT) and Nagana in livestock (AAT)¹. The World Health Organization classifies HAT as a neglected tropical disease with high morbidity and mortality rates, limited treatment options, and no effective vaccine, primarily affecting impoverished populations^{2,3}. In addition, control of AAT is considered part of the One Health approach established by the FAO program against African Trypanosomiasis.

HAT, caused by the protozoan parasite *Trypanosoma brucei*, is characterized by the manipulation of the host's immune response to ensure parasite invasion and persistence⁴. Uncovering key molecules that support parasite establishment is a prerequisite to interfere with this process. We identified Q586B2 as a *T. brucei* protein that induces IL-10 in myeloid cells and which promotes parasite infection invasiveness. Q586B2 is expressed during all *T. brucei* life stages and is conserved in all Trypanosomatidae. Deleting the Q586B2-encoding *Tb927.6.4140* gene in *T. brucei* results in a decreased peak parasitemia and prolonged survival, without affecting parasite fitness *in vitro*, yet promoting short stumpy differentiation *in vivo*. Accordingly, neutralization of Q586B2 with newly generated nanobodies, *i.e.* single-domain antibody fragments derived from camelids, could hamper myeloid-derived IL-10 production and reduce parasitemia. In addition, immunization with Q586B2 induces cross-protection against heterologous parasites as well as against different trypanosomatids, including Trypanosoma cruzi.

Collectively, we uncovered a conserved protein playing an important regulatory role in Trypanosomatid infection establishment. Hence, our findings could pave the way to develop a broad-spectrum intervention strategy for human diseases such as HAT, leishmaniasis, and Chagas disease as well as veterinary and economically relevant diseases such as Nagana.

References

[1] Radwanska, M., Vereecke, N., Deleeuw, V., Pinto, J. & Magez, S. Salivarian trypanosomosis: a review of parasites involved, their global distribution and their interaction with the innate and adaptive mammalian host immune system. Front. Immunol.10.3389/fimmu.2018.02253 (2018).

[2] Lejon V, Lindner AK, Franco JR. Human African trypanosomiasis. Lancet. 2025 Mar 15;405(10482):937-950. doi: 10.1016/S0140-6736(25)00107-2.

- [3] Venturelli A, Tagliazucchi L, Lima C, Venuti F, Malpezzi G, Magoulas GE, Santarem N, Calogeropoulou T, Cordeiro-da-Silva A, Costi MP. Current Treatments to Control African Trypanosomiasis and One Health Perspective. Microorganisms. 2022 Jun 27;10(7):1298. doi: 10.3390/microorganisms10071298.
- [4] Stijlemans, B. et al. Immune evasion strategies of Trypanosoma brucei within the mammalian host: progression to pathogenicity. Front. Immunol.10.3389/fimmu.2016.00233 (2016).

Acknowledgment

The Authors acknowledge the Strategic Research Program (SRP3, SRP47 and SRP63, VUB), the FWO (FWO G015016N, KAN 1515813 N), the National Institute of Health (NIH) (R21CA259345), "Action de Recherches Concertées" of the University of Brussels (ULB) (ARC ADV), and the Fonds de la Recherche Scientifique (F.R.S.-FNRS) (PDR T.0159.13). BS was supported by the Strategic Research Program (SRP3 and SRP47, VUB) for their financial support. GC is supported by FWO and the University of Antwerp (www.uantwerpen.be; grant number TT-ZAPBOF 33049) and is member of the Centre of Excellence 'Infla-Med' (www.uantwerpen.be/infla-med) and EU-COST Action OneHealthDrugs (CA21111). HK is supported by internal funding from KU Leuven (C14/23/135). JAVG is supported by the FWO and is a member of the EU-COST action Mye-EUNITER.







Targeting host and *Plasmodium* immunomodulators for new malaria therapeutics

Alvaro Baeza Garcia

University of Antwerp, Laboratory of Microbiology, Parasitology and Hygiene, Belgium

alvaro.baezagarcia@uantwerpen.be

Plasmodium-expressed cytokine Macrophage Migration Inhibitory Factor (PMIF) prevents the acquisition of immunologic memory by activating the host MIF receptor CD74, which establishes severe malaria and promotes parasite replication. Immunoneutralization or genetic deletion of PMIF, which is strictly conserved in all *Plasmodium* species, enhances malaria immunologic memory, reduces parasite burden, and protects against severe malaria¹.

High expression of host MIF is associated with adequate cellular and humoral responses. However, low-expression MIF alleles occur more commonly in malaria endemic areas, probably due to the necessity of equilibrium between host and parasite MIF. Published and unpublished results in the context of vaccination demonstrate that host MIF deficiency² or low expression is associated with a reduced humoral antibody response to vaccination.

MIF molecules possess unique and conserved enzymatic activity. Although a physiological substrate for MIF molecules has not been identified, natural (p-hydroxyphenylpyruvate) and unnatural ligands (D-dopachrome) are used as "model" tautomerase substrates and for structure-based inhibitor designs. Additionally, host and Plasmodium MIF bind to the host receptor CD74 through the amino acid residues responsible for MIF tautomerase activity. Using these unique features, we have designed a PMIF antagonist³ and a host MIF agonist⁴.

We hypothesize that the PMIF antagonist provides a unique approach for interfering with a parasitespecific mechanism for suppressing host immunity, and that the host MIF agonist could counteract the adverse effect of low MIF expression and enhance response to vaccination against malaria.

References

[1] Baeza Garcia, A., et al. Nat Comm, 2018. 9(1): p. 2714.

[2] Mizue, Y., et al. Proc Natl Acad Sci U S A, 2005. 102(40): p. 14410-14415.

[3] Baeza Garcia, A., et al. Faseb j, 2021. 35(12): p. e21997.

[4] Jorgensen, W.L., et al. Bioorg Med Chem Lett, 2010. 20(23): p. 7033-6.

Acknowledgment

The Author acknowledges the University of Antwerp [www.uantwerpen.be; Bijzonder Onderzoeksfonds (BOF).







My Career Tale: one step at a time

Authors: Abranches A, a

^a ViSync Technologies SA, Portugal

eabranches@visync.com

This session will explore career themes in cell therapy development, drawing on personal, academia, regulatory and industry insights. It will highlight the personal and professional challenges of navigating innovative fields, from early education and research to clinical application. Topics will include approaches to translational technology, the role of interdisciplinary collaboration, and the importance of early engagement with relevant key players in advancing advanced therapies. Attendees will gain a deeper understanding of the evolving landscape of cell therapy manufacturing, the skills needed to navigate such complex environments, and the importance of having a good work-life balance while trying to bridge scientific research with clinical delivery.

Acknowledgment

The Author acknowledges all the grants, salaries and, most importantly, people's support throughout the journey.







Proteases driving the apical complex of Babesia during egress and invasion of host red blood cells

Pavla Šnebergerová^{a,b}, Gaelle Lentini^c, Oscar Vadas^c, Rémy Visentin^c, Luise Robbertse^a, Eliana F. G. Cubillos^b, Alper Dede^{a,b}, Viktoriya Levytska^a, Marie Jalovecká^{a,b}, Masahito Asada^d, Kayode K.Ojo^e, Dominique Soldati-Favre^c and <u>Daniel Sojka^a</u>*

^a Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 1160/31, 37005, České Budějovice, Czech Republic; ^bFaculty of Science, University of South Bohemia in České Budějovice, Branišovská 1760c, CZ-37005 České Budějovice, Czech Republic; ^cDepartment of Microbiology and Molecular Medicine - Faculty of Medicine, University of Geneva, rue Michel-Servet CH - 1211 Geneva 4, Switzerland; ^dNational Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, 〒080-8555 Hokkaido, Obihiro, Inadacho, Nishi 2 Sen− 1 1, Japan. ^e Department of Medicine, Division of Allergy and Infectious Disease, Center for Emerging and Reemerging Infectious Disease (CERID), University of Washington, Seattle, Washington, USA

sojkadan@gmail.com

Babesia divergens, a tick-borne apicomplexan, causes economically damaging bovine babesiosis and severe zoonotic infections in humans. With limited therapeutic options and growing concern over drug resistance, a One Health approach is needed to develop targeted, cross-species chemotherapies. Our research identifies two parasite-specific enzyme families—clade-C aspartyl proteases (BdASP3a/b) and calcium-dependent protein kinases (BdCDPKs)—as central regulators of parasite development and promising drug targets.

BdASP3a/b, homologous to *Plasmodium* plasmepsins IX/X and *Toxoplasma gondii* ASP3, are expressed during erythrocytic stages and localize to the apical complex. Recombinant BdASP3s exhibit proteolytic activity, while only BdASP3b appears to be inhibited by the PfPMX-targeting compound 49C, causing accumulation of extracellular merozoites and revealing BdASP3b role in invasion rather than egress. Functional genomics and cross-species complementation support their role in secretory protein maturation.

In parallel, we characterized four putative *B. divergens* CDPKs (BdCDPK1–4), all possessing small gatekeeper residues in the ATP-binding site—a structural feature enabling selective inhibition by bumped kinase inhibitors (BKIs). BKIs target apicomplexan kinases without affecting mammalian counterparts, offering a highly specific therapeutic strategy. Among six BKIs tested in vitro, BKI-1294 was most effective against *B. divergens*, showing specific inhibition of BdCDPK4. This treatment blocked egress while allowing continued intracellular replication, resulting in multinucleated parasite complexes. BdCDPK4, a phylogenetic ortholog of *T. gondii* CDPK1 and *P. falciparum* CDPK4, appears to regulate parasite egress. The inhibition phenotype closely resembles that seen in *T. gondii*, despite *B. divergens* relying solely on extracellular calcium signaling for egress.

Together, our findings highlight BdASP3s and BdCDPKs—particularly BdCDPK4—as essential, druggable enzymes. These molecular targets support the development of selective chemotherapy for babesiosis, aligning with One Health goals by addressing human, animal, and vector stages of *Babesia* infection.

Acknowledgment

This work was supported by projects 23-07850S and 21-11299S of the Czech Science Foundation (GAČR)







Multi-drug tolerance in Leishmania persister-like cells

Aroni-Soto A.^{a,b,e}, Monsieurs P.^{a,e}, Goovaerts O.^c, Choukri K.^{a,e}, Khanal B.^d, Adriaensen W.^c, Dujardin JC^{b, e}, Barrett MP^f, <u>Domagalska MA^{a,e*}</u>

^a Institute of Tropical Medicine, Experimental Parasitology Unit, Antwerp, Belgium;^b Department of Biomedical Sciences, University of Antwerp, Belgium; ^c Institute of Tropical Medicine, Clinical Immunology Unit, Antwerp, Belgium; ^d Department of Microbiology, BP Koirala Institute of Health Sciences, Dharan, Nepal; ^e Institute of Tropical Medicine, Molecular Parasitology Unit, Antwerp, Belgium; ^f School of Infection & Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

mdomagalska@itg.be

The ability of microorganisms to resist the killing action of antimicrobial agents is emerging as one of the most serious public health threats and has been observed for antibiotics, antifungals, and also antiparasitic compounds. Understanding the mechanisms that allow them to survive chemotherapy is essential. Several molecular mechanisms can underlie this phenomenon, but the most extensively studied one is drug resistance caused by acquired, heritable genetic changes, usually point mutations or insertion/deletion within genes in which these mutations enable cells to prevent the drug's toxic capacity. However, microorganisms can also adopt a physiological state of transient quiescence, manifested by non-proliferation and reduced metabolism.

Persisters are a small fraction of non-proliferative cells with reduced metabolism that are adapted to withstand a variety of environmental assaults, including lethal doses of antibiotics. Here, we present evidence of existence of persister-like cells in protozoan parasite *Leishmania*, induced upon exposure to normally lethal doses of antimonials. We show that *Leishmania* promastigotes survive lethal doses of antimonials by adopting a quiescence phenotype characterised by reduced proliferation, diminished metabolism and a reduced mitochondrial membrane potential. What is more, these cells demonstrate cross-tolerance to other anti-leishmanial drugs. Depending on the presence of genomic pre-adaption to antimonial resistance, two types of persister-like cells were observed for *L. donovani* field isolates. In wild-type lines, persister-like cells were transiently tolerant to antimonials and returned to the sensitive state upon removal of drug pressure. Surprisingly, isolates with genetic changes associated with antimony-resistance, acquired a level of hyper-resistance after transient passage through the quiescent state, without further genetic changes. Our results demonstrate extreme versatility of this eukaryotic pathogen in adaptation to drug pressure and highlight the need for the development of new antileishmanials targeting non-proliferative forms.

Acknowledgment

This study received financial support from the Flemish Fund for Scientific Research grant G075121N. Isolation and characterization of clinical isolates was financed by the European Commission through Leishnatdrug-R project, grant ICA4-CT-2001-10076. M.A.D. acknowledges support from Flemish Ministry of Science and Innovation







Metal Transporters in Leishmania: Exploring Their Potential as Drug Targets

Ana M Tomás^{1,2}, Teresa Leão¹, Sandra Carvalho¹, Maria Vieira¹, Margarida Duarte¹,

¹i3S, Instituto de Investigação e Inovação em Saúde, ²ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

atomas@ibmc.up.pt

Mammals commonly deploy nutritional immunity strategies to limit pathogen survival, either by restricting access to essential nutrients or, in the case of metals, by inducing metal toxicity. Consequently, proteins involved in micronutrient acquisition and regulation are often critical for parasite survival and represent potential targets for therapeutic intervention.

In this study, we explored the mechanisms by which *Leishmania infantum* competes with their hosts for zinc, with the aim of identifying the key components essential for viability. We demonstrate that several transporters contribute to the zinc acquisition machinery in these parasites. The first to be identified was ZIP3, a member of the ZIP family, which functions as a high-affinity zinc importer. Using CRISPR-Cas9-mediated gene ablation, we show that ZIP3 is essential under zinc-limiting conditions but dispensable when zinc is abundant, suggesting the existence of an alternative zinc uptake system, whose identity is currently under investigation. Although we assessed the potential role of the *L. infantum* homologue of LIT1 (reported as a *Leishmania* iron transporter) in this process, our results indicate that it does not fulfill this function. A third component of the zinc acquisition system is CDF1, a cation diffusion facilitator localized to acidocalcisomes. CDF1 appears to play a protective role against zinc toxicity by sequestering excess intracellular zinc into acidocalcisomes. Consistent with this function, deletion of CDF1 was only achievable under metal-limiting conditions, highlighting its importance during zinc overload.

We are currently investigating the roles of these zinc transporters during infection using in vivo models of leishmaniasis. The data gathered, which will be discussed during the conference, provide new insights into the role of zinc in *Leishmania*-host interactions and inform the potential for targeting metal acquisition pathways as a novel therapeutic strategy.

Acknowledgment

The Authors acknowledge project UIDB/04293/2020 (National Funds through FCT—Fundação para a Ciência e a Tecnologia, I.P.) for the financial support.







Heterocyclic steroid derivatives with antitrypanosomal activity and *in silico* binding affinity to

glucose-6-phosphate dehydrogenase

<u>Ajduković J.</u>^a, Matheeussen A.^b, Van Pelt N.^b, Marinović M.^c, Savić M.^a, Caljon G.^b

^aUniversity of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Serbia; ^bAntwerp University, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Laboratory of Microbiology, Parasitology and Hygiene, Belgium; ^cUniversity of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Serbia.

jovana.ajdukovic@dh.uns.ac.rs

Infectious diseases are an ongoing threat to human and animal health and the cause of significant economic losses. According to the WHO, human trypanosomiasis is one of the 13 most neglected tropical diseases. While treating bacterial infections continually progresses through the discovery of new antibiotics, parasitic infections remain more challenging to treat due to their high diversity. *Trypanosoma cruzi* relies heavily on glucose metabolism for its survival and proliferation in the host, so glucose-6-phosphate dehydrogenase (G6PD) could be an important target for antiparasitic drug discovery. This motivated the scientific community to focus on the discovery of safe, effective, and affordable antiparasitic drugs.

Steroids, particularly those with heterocycles, are **promising** drug candidates as they can easily penetrate cell membranes and bind to nuclear receptors. It is known that they possess anticancer, antiinflammatory, antimicrobial, and antiviral activity, but they are also recognized as antiparasitic agents ^[1]. Therefore, our main objective was the development of steroid-based compounds with different heterocyclic rings in their skeleton, as efficient antitrypanosomal agents. The A- and B-ring modified androstane derivatives were combined with lactone or pyridine moieties in multistep synthetic procedures, which were then tested against several parasitic species. Interestingly, only derivatives with pyridin-2-ylmethylidene function showed significant and selective activity against *T. cruzi*, consistent with the results obtained for some non-steroidal pyridinyl substituted pyrazoles ^[2]. The most promising compounds were evaluated *in silico* for their binding affinity to G6PD. Molecular docking analyses suggest strong binding of our derivatives to the enzyme, so they may act as inhibitors through steric hindrance, physically preventing the substrate from entering the active site.

References

I. Z. Kuzminac et al., Curr. Topics Med. Chem. 23 (2023), 791.
 T. Winge et al., ChemMedChem 19 (2024), e202400220.

Acknowledgment

The Authors acknowledge COST Action CA21111: One Health Drugs against Parasitic Vector-Borne Diseases in Europe and Beyond (OneHealthDrugs) and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/ 200125) for the financial support.







Building Bridges Between Medicinal Chemistry and Sustainability: Parasitic Diseases in the One

Health Context

Elisa Uliassi^a

^a Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy

elisa.uliassi3@unibo.it

The development of antiparasitic drugs presents a critical opportunity to align medicinal chemistry with the principles of sustainability, health equity, and environmental responsibility. In this context, we highlight the intersection between green chemistry and the Sustainable Development Goals (SDGs), advocating for a unified One Health approach for parasitic diseases.

We underscore the value of applying the 12 principles of green chemistry to antiparasitic drug design, aiming to reduce environmental toxicity, improve synthetic efficiency, and promote biodegradability without compromising therapeutic efficacy.¹ By integrating these principles, researchers can minimize the ecological footprint of pharmaceuticals, reduce the risk of antimicrobial resistance, and safeguard both human and animal health.

We emphasize the role of medicinal chemistry as a central pillar in achieving the United Nations' SDGs, particularly those related to health (SDG 3), clean water (SDG 6), and responsible consumption and production (SDG 12).² This is especially critical in addressing poverty-related and neglected diseases, which disproportionately affect vulnerable populations and demand sustainable, accessible therapeutic solutions. This calls for a paradigm shift in drug discovery and development, urging scientists to consider broader societal and planetary impacts alongside pharmacological performance. Together, these contributions argue for a transformative strategy in medicinal chemistry that embeds sustainability at every stage—from molecular design to manufacturing and disposal. By integrating the One Health concept with sustainable medicinal chemistry, this unified vision supports the creation of safer, more effective antiparasitic drugs that meet global health challenges, and sustainable development.

References

- [1] Martinengo B, Diamanti E, Uliassi E, Bolognesi ML. Harnessing the 12 Green Chemistry Principles for Sustainable Antiparasitic Drugs: Toward the One Health Approach. ACS Infect Dis. 2024 Jun 14;10(6):1856-1870. doi: 10.1021/acsinfecdis.4c00172.
- [2] Martinengo B, Diamanti E, Uliassi E, Bolognesi ML. Medicinal Chemistry: A Key Driver in Achieving the Global Sustainable Development Goals. J Med Chem. 2025 Apr 10;68(7):6916-6931. doi: 10.1021/acs.jmedchem.4c03016.

Acknowledgment

This work was supported by the COST Action CA2111 OneHealthDrugs and the University of Bologna.







High-Throughput Screening Selection and Deselection Process for the Identification of High-Affinity PTR1 Inhibitors

<u>Costi M.P.</u>^a, Cordeiro da Silva A^b.; Pozzi C.^c, Santarem N^b, Aiello D^a., Gul S.^d ^a University of Modena and Reggio Emilia, Italy; ^b i3S, Portugual;^c Univeristy of Siena, Italy, ^d Fraunhofer ITMP, Germany, mariapaola.costi@unimore.it

Current therapeutics for trypanosomatid infections are limited by toxicity, poor efficacy, and parasite resistance, thus underlining the urgent need for new targets and chemotherapies and^{1,2}. The folate pathway enzyme dihydrofolate reductase (DHFR) provides reduced folates, which are crucial for biological processes such as DNA, protein, and amino acid synthesis, or one-carbon transfer. In Trypanosomatids, DHFR inhibition was ineffective due to a metabolic bypass via the biopterin-reducing pteridine reductase 1 (PTR1). When DHFR is inhibited, PTR1, which can also reduce folates, is overexpressed and sustains sufficient metabolite levels to ensure parasite survival. Thus, when targeting the folate pathway, both DHFR and PTR1 need to be evaluated, and their inhibition properly balanced. We have discovered different compound libraries that were designed with the dual-targeting concept, therefore aiming to inhibit both enzymes with one individual compound³.



These compounds often show structural similarity with the pteridine core structure. Combining two independent compounds with high affinity towards DHFR and PTR1 could be a successful strategy. With this aim we initiated a hit-finding screening program with the European Lead Factory (ELF)³. Non-folate analogs, strongly inhibiting PTR1 could be combined (in various modes) with high-affinity inhibitors of DHFR. Large compound libraries were screened and selected/deselected following specific

criteria. Program progression criteria were based on the deselection of DHFR inhibition and structural folate similarity; selection was based on the highest selectivity index for DHFR/PTR1 activity, which was set at 10³. The program was conducted to a high-quality standard. Fifty compounds were finally selected, and eleven of them were prioritized for their QHL quality. ADME-(eco)Tox properties were also evaluated. Subsequently, *anti-Leishmania infantum* activity was measured, and the compounds showing the best biological activity were prioritized for the next step of lead optimization. We were able to identify four confirmed hits ready for development to the lead phase.

References

- 1. https://www.ecdc.europa.eu/en, https://www.malacards.org/card/trypanosomiasis
- 2. Domagalska, Malgorzata Anna et al. Trends in Parasitology, 2023, 39, Issue 4, 251 259
- 3. Ina Pöhner, et al. Journal of Medicinal Chemistry 2022 65, 9011-9033

Acknowledgment This research was funded by Innovative Medicines Initiative Joint Undertaking under grant agreement no. 115489, resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contributions.







Preparation of a library of polysubstituted 3-benzylmenadiones and design of plasmodione prodrugs

Davioud-Charvet E,^a Blandin SA,^b Meunier B,^c Keiser J.,^d Wittlin S,^d Rottmann M,^d Mäser P^{d,e}

^a Chimie Bio(IN)organique et Médicinale, UMR7042 CNRS-Université de Strasbourg-Université Haute-Alsace, Laboratoire d'Innovation Moléculaire et Applications (LIMA), F-67087 Strasbourg UMR 7042, 67087, Strasbourg, France ; ^b Institut de Biologie Moléculaire et Cellulaire, INSERM U1257 – CNRS UPR9022 – Université de Strasbourg, F-67084, Strasbourg, France ; ^c Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198 Gif-Sur-Yvette Cedex, France ; ^d Swiss Tropical and Public Health Institute, CH-4123 Allschwil, Switzerland; ^e University of Basel, CH-4001 Basel, Switzerland.

elisabeth.davioud@unistra.fr

Redox-active 3-benzylmenadione (bMD) have shown promising antiparasitic activities against two blood-feeding parasites, *P. falciparum* and *S. mansoni* worms. Plasmodione (PD) is the early lead bMD derivative[1], displaying fast-acting antima]arial activity and potential transmission-blocking properties [2], without any sign of toxicity in mice and even in G6PD-deficient red blood cells [3]. Following investigations on synthetic methodologies [4,5], we optimized the early lead PD and identified several drug metabolites [6], and drug targets in yeast [7].

In order to improve the solubility of our compounds, we prepared a library of bMDs by introduction of various groups on both west and east parts of the 3-benzylmenadione core [4,8]. More than 300 bMDs were tested in parasitic drug assays with two blood-feeding parasites, *P. falciparum* and *S. mansoni*. Finally, we discovered a superior bMD drug-candidate, MD40, decreasing the parasitemia following oral administration at 50 mg/kg, either when taken in monotherapy, by 96% in the *P. berghei*-infected murine model, and by 100 % (total oral cure) in combination with a drug partner, in a humanized *P. falciparum*-infected mouse model (SCID). In parallel, we also broke the aromaticity of the menadione core by introducing an angular methyl group susceptible to be oxidized and eliminated as a formyl group, in the oxidative milieu of hemoglobin digestion [9]. In the presence of a redox-cycling assay generating a steady flux of reactive oxygen species in the presence of a NADPH-dependent *P. falciparum* flavoenzyme and hematin, both prodrugs, the 4a- and the 8a-y-plasmodiones, were demonstrated to release plasmodione after LC-MS analysis. The 4a-y-plasmodione revealed to exert a high antischistosomal action in the NTS and adult *S. mansoni* worm assays.

References

[1] Müller T. et al. J. Am. Chem. Soc. 2011, 133, 11557-71. [2] Ehrhardt, K. et al., Antimicrob. Agents Chemother. 2013, 57, 2114-2120 and 2016, 60, 5146-58. [3] Bielitza, M. et al., Antioxid. Redox Signal. 2015, 22, 1337-51. [4] Donzel, M., et al., J. Org. Chem. 2021, 86, 10055-10066. [5] Dupouy B. et al. ACS Infect. Dis. 2024, 10, 3553-3576. [6] Feng L. et al., Org. Biomol. Chem. 2018, 16, 2647 - 2665.
[7] Mounkoro P., et al. Free Radic Biol Med. 2019, 141, 269–278 and 2021, 162, 533-541. [8] Rodo E. C. et al., Eur. J. Org. Chem. 2016, 2016(11), 1982–1993. [9] Cesar-Rodo E., et al. Molecules 2024, 29(22), 5268.

Acknowledgment

The Authors acknowledge the Agence Nationale de la Recherche for the grant to support the ROSaction project [ANR-22-CE93-0005-01] developed by E.D-C., S.A.B., M.R., P.M., and to the Laboratoire d'Excellence "LabEx ParaFrap" [ANR-11-LABX-0024] in which E. D.-C., S. A. B. are co-PI.







The potential of natural products against vector-borne diseases

Taglialatela-Scafati O.

University of Naples Federico II; School of Medicine and Surgery; Department of Pharmacy, Italy

scatagli@unina.it

Vector-borne diseases continue to be a major cause of morbidity and mortality all around the world. Malaria, trypanosomiasis, leishmaniasis continue to affect poor tropical and subtropical countries, while new burden of disease is being caused by arboviruses, e.g. dengue, even in middle-income nations.

This presentation will highlight the potential application of marine and terrestrial natural products, or of their strictly related semisynthetic derivatives, both in vector control and in disease prevention and management. The potential of natural products is well testified by the two major and historical breakthroughs in the fight against malaria: both quinine and artemisinin are natural products derived from plants (*Cinchona* and *Artemisia*, respectively).

The strong point in favor of natural products is their intrinsic chemical diversity, resulting from thousands of years of evolution, while challenges can be: a) discovery (often they are present in the natural source in small amounts); b) optimization (often natural products structures are not ready to be used as drugs and they need a structural optimization); c) preclinical/clinical development (sometimes pharmaceutical companies are not interested in developing natural products as drugs, while they prefer synthetic compounds).

These points will be discussed by the Author, both using literature case-studies and results from his own research.

Acknowledgment

The Author acknowledges EU-COST OneHealthdrugs (COST Action CA21111) for financial support.







Computer-aided design of new nature-inspired antimalarials

Authors: Persico M, ^a Tumbarello G, ^a Tkachuk O, ^a and Fattorusso C^a

^a Department of Pharmacy, University of Naples "Federico II", Via D. Montesano 49, 80131 Napoli, Italy

m.persico@unina.it

Malaria is a life-threatening disease and the development of drug resistance establishes one of the greatest threats to malaria control resulting in increased malaria morbidity and mortality [1]. Accordingly, there is an urgent need of new therapeutic tools, and, historically, natural products provided the greatest source of inspiration for the development of antimalarial drugs [2]. Computeraided drug design (CADD) plays a strategic role in the discovery of new potential therapeutic agents as well as in the identification of new therapeutic targets. However, while the extraordinary advances in computational resources have fuelled the development of CADD methods, they still present some limitations. This prompted us to develop an integrated approach combining different CADD techniques with experimental studies in the investigation of new antimalarial scaffolds [3]. Based on this approach, a new class of redox-active antiplasmodial thiazinoquinones was developed using the quinone scaffold of marine secondary metabolites as a chemical starting point [4]. By applying a similar integrated computational/experimental approach, a new class of PfGAPDH covalent inhibitors was developed inspired by acivicin, a fermentation product of Streptomyces sviceus [5]. The analysis of the structure-activity relationships of these compounds provided useful insights into the structural requirements needed for their antiplasmodial activity and on their putative mechanism of action, leading to the development of 3D pharmacophore models. These results disclosed important information for the rational development of new antimalarial leads highlighting the applicability of these integrated CADD approaches on new scaffolds and biological targets.

References

- [1] Assefa A, et Al. Malar J. 2024, 23(1), 34.
- [2] Tajuddeen N, et Al. Malar J. 2019, 18, 404.
- [3] Persico M, et Al. Mol Inform. 2016, 35(8-9),309.
- [4] Gimmelli R, et Al. ACS Infect Dis. 2020, 6(1), 124 and references therein.
- [5] Galbiati A, et Al. Eur J Med Chem. 2023, 254, 115286 and references therein.







Discovery, characterization and application of nature-derived cystine-knot peptides with

antimicrobial activity

Authors: Hellinger R,^a

^a Center for Pharmacology and Physiology, Medical University of Vienna, Schwarzspanierstrasse 17A, 1090 Vienna, Austria

*roland.hellinger@meduniwien.ac.at

Nature is a rich-source for bioactive peptides, amongst them antimicrobial and antiparasitic peptides, such as mellitin, cecropins or magainins (toxin-derived) as well as defensins, snakines or cystine-knottins (plant-derived). (1-3) A common mode of action for these antiparasitic peptides are membrane-, immune modulatory-activity as well as enzyme inhibition or a combination of them. Here, I report on the discovery, chemical and bioactivity characterization as well as the application of antiparasitic cystine-knot peptides.

My laboratory has developed peptidomic workflows for the discovery of ribosomally-synthesized and post-translationally modified peptides allowing insights into the diversity of these natural products. (4, 5) We further identify bioactive molecules, isolate them for proof-of-concept experiment and work on chemical engineering to prepare peptide therapeutics for biological evaluation.

We have studied the peptidome of various plants, amongst them of the Psychotria tribe (Rubiaceae) and of Cucurbitaceae species. Prototypic bioactive peptides were identified, isolated and the chemical synthesis established to allow further experiments. We show that membrane-activity of these peptides contribute to their anti-parasitic activity, leading to parasite lysis or apoptosis induction. The peptide psysol 2 from *Psychotria solitudinum* was obtained as a first in class inhibitor of prolyl endopeptidase (POP or PREP, Merops S9 family) with a mixed type inhibition modality. We conducted protease inhibition experiments with human, and parasite POP from *Trypanosoma brucei*, T. cruzei, *Leishmania infantum* and L. major as well as evaluated a parasite POP-dependent mechanism for parasite entry to mammalian host cells. Further, my laboratory studied the immunomodulatory activity of these peptide family towards T-lymphocytes and natural killer cells in the past. (6) Hence, we propose a multimodal mode of action for the peptide family, making them interesting candidates for therapeutic applications in the future. In conclusion, my study highlights natural peptides as bioactive molecules which are of interest for developmental work of new anti-parasitic therapeutics.

References

[1] J. P. Tam, S. Wang, K. H. Wong, W. L. Tan, Antimicrobial Peptides from Plants. Pharmaceuticals (Basel) 8, 711-757 (2015).

- [2] A. A. Robles-Loaiza et al., Peptides to Tackle Leishmaniasis: Current Status and Future Directions. Int J Mol Sci 22, (2021).
- [3] R. El-Dirany et al., Activity of Anti-Microbial Peptides (AMPs) against Leishmania and Other Parasites: An Overview. Biomolecules 11, (2021).
- [4] R. Hellinger et al., Peptidomics of Circular Cysteine-Rich Plant Peptides: Analysis of the Diversity of Cyclotides from Viola tricolor by Transcriptome and Proteome Mining. J Proteome Res 14, 4851-4862 (2015).
- [5] R. Hellinger et al., Inhibition of Human Prolyl Oligopeptidase Activity by the Cyclotide Psysol 2 Isolated from Psychotria solitudinum. J Nat Prod 78, 1073-1082 (2015).
- [6] J. List et al., Boosting the anti-tumor activity of natural killer cells by caripe 8 A Carapichea ipecacuanha isolated cyclotide. Biomed Pharmacother 177, 117057 (2024).

Acknowledgment

The Authors acknowledge the Austrian Science Fund (ZK81-B, P36736-B) and the Austrian Exchange Service (Oead) for the financial support.







Sustainable development of novel antiparasitic agents valorising by-products of the cashew industry

Nunes Lemes L.M.^a, Magoulas G.E, ^b, Alonso L.,^c, Souza de Oliveira A.,^a de Lucena Costa B.,^a Gomes R.S.,^c Dorta M.L.,^c Barrias E.,^d, de Camargo Nascente L.,^a, Granado R.,^e Teixeira de Macedo Silva S,^d de Souza W.,^d Alonso A.,^c Bolognesi **M.L.**,^f Soares Romeiro L.A.,^a <u>Calogeropoulou</u> **T.**^b

^a University of Brasília, Brasil; ^bNational Hellenic Research Foundation, Greece; ^cUniversidade Federal de Goiás, Brasil; ^dUniversidade Federal do Rio de Janeiro, Brasil; ^eNational Institute of Metrology, Quality and Technology - Inmetro, Brasil; University of Bologna, Italy

tcalog@eie.gr

Neglected tropical diseases (NTDs) represent a group of 20 pathologies, affecting primarily people living in developing countries, where access to the most effective medication remains either unavailable or financially prohibitive. Protozoan parasites of the Trypanosomatidae family are the etiological agents for several NTDs including Leishmaniasis,¹ and American Trypanosomiasis (Chagas Disease).² Even though these diseases have severe health and socioeconomic impact the available drugs are old and not efficient. Thus, new treatment options are desirable. In this context, Bolognesi and Romeiro started a drug discovery project aimed to develop new molecules that could address the sustainability requirements of the NTD field embracing the emerging waste-to-pharma concepts,³ exploring the possibility of developing new bioactive compounds starting from cashew nutshell liquid (CNSL). Capitalising on these efforts we incorporated the phenolic consituents of CNSL in the lipid portion of alkylphosphocholines. The new compounds were evaluated against T. cruzi different developmental stages. Two derivatives were the most potent exhibiting selectivity indices against trypomastigotes and intracellular amastigotes 32 fold and 7 fold higher than the current drug benznidazole.⁴ In addition three derivatives were equipotent to miltefosine against Leishmania amazonensis intracellular amastigotes while, their cytotoxicity was very low possessing selectivty indexes 8-12 fold higher than the current drug miltefosine.⁵

References

[1] Santarém, N. et al. *Microorganisms* **2023**, *11* (3), 16–19. <u>https://doi.org/10.3390/microorganisms11030696</u>.

[3] Espro, C. et al Chem Soc. Rev. 2021, 50, 11191-11207. https://doi.org/10.1039/D1CS00524C

[4] Nunes Lemes LF et al ACS Infect Dis. 2023, 9, 1334-1345. doi: 10.1021/acsinfecdis.3c00076.

[5] Alonso L. et al Mem Inst Oswaldo Cruz. 2025, 120: e240219, DOI: 10.1590/0074-02760240219.

Acknowledgment

The Authors acknowledge "OPENSCREEN-GR" (MIS) 5002691, CNPq #401864/2013-8 (PVE), #312709/2017-0 (DT-2), and #308486/2020-0 (DT-2)), Faperj (#E-26/010.002419/2019), CNPq (#46.6607/2019-8) and COST Action CA21111 "OneHealthDrugs" for the financial support.







Development of a novel Trypanosome Alternative Oxidase inhibitor with a broad-spectrum

activity against the causative agents of animal Trypanosomiasis.

<u>Godwin U. Ebiloma</u>^a, Emmanuel O. Balogun^b, Tomoo Shiba^c, Christophe Dardonville^d, and Harry P. De Koning^e

^aSchool of Science, Engineering & Environment, University of Salford, Manchester, United Kingdom; ^bDepartment of Biochemistry, Ahmadu Bello University, Zaria, Nigeria; ^cGraduate School of Science and Technology, Department of Applied Biology, Kyoto Institute of Technology, Kyoto, Japan; ^dInstituto de Química Médica, IQM–CSIC, Madrid, Spain: ^eSchool of Infection and Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom.

g.u.ebiloma@salford.ac.uk

Animal African Trypanosomiasis (AAT), a wasting disease caused by trypanosome parasite affects wildlife, and domestic animals across sub-Saharan Africa where it is a major constraint to livestock production and draught power for farmers. Isometamedium and diminazene are used to treat AAT however, these drugs are sometimes ineffective due to increasing drug resistance and the presence of a cheaper counterfeit versions. Hence, new effective and affordable drugs are needed to control the disease. Targeting parasite specific metabolism with chemical compounds that are not cross resistant with existing drugs is a useful strategy to curb AAT. The mitochondrion-based Trypanosome Alternative Oxidase (TAO) has been validated as essential for respiration and survival of bloodstream forms of trypanosomes. Since TAO is absent in mammals and it is conserved among Trypanosoma species, it offers a promising target for chemotherapy. But previous TAO inhibitors were unable to cross the parasite's membranes. We present here, a novel approach that involves boosting the trypanocidal efficacy of a mitochondria targeted 2,4-dihydroxybenzoate by conjugating the inhibitor with lipophilic cation (LC) that crosses lipid bilayers by non-carrier mediated transport, and thus accumulate specifically in the parasite's mitochondrion, driven by the transmembrane potentials of trypanosomes. This design provided an LC-TAO inhibitor conjugate that is active in the low nanomolar range against wild-type and resistant strains of trypanosomes (T. b. brucei, T. evansi, T. equiperdum, and T. congolense), with promising selectivity over human cells. In vitro biochemical assessments confirm TAO inhibition. Kinetic assay of the inhibitor against recombinant TAO revealed a noncompetitive inhibition mode, while X-ray diffraction analysis of the crystal structure of rTAO-inhibitor complex gave molecular insights into the mode of inhibition and shows that the inhibitor occupies an allosteric binding site distant from the active site. Thus, the LC- inhibitor conjugate strategy is useful for developing new potent class of trypanocides.







Abstracts poster presentations







Bioassay guided isolation and identification of Amoebicidal and leishmanicidal compounds from

Tunisian natural sources.

Hafidh AKKARI

Laboratory of Parasitology, National Veterinary School of Sidithabet, Tunisia

akkari_hafidh@yahoo.fr

Acanthamoeba genus includes opportunistic pathogens which are distributed worldwide and are causative agents of a fatal encephalitis and severe keratitis in humans and other animals. Leishmaniasis is considered as a major neglected tropical disease causing an enormous impact on global public health. Until present there are not fully effective therapeutic agents against these pathogens and thus the need to search for novel anti-amoebic compounds is urgent.

In the present study, an in vitro evaluation of the leishmanicidal ^[1] and anti-Acanthamoeba activity of the essential oil of Tunisian chamomile (*Matricaria recutita* L.) was carried out. Chamomile essential oil exhibits a good activity on promastigotes forms of *L. amazonensis* and *L. infantum* with a low inhibitory concentration at 50% (IC₅₀) (10.8 ± 1.4 and 10.4 ± 0.6 µg/mL, respectively). Bioguided fractionation was developed and led to the identification of (-)- α -bisabolol as the most active molecule with low IC₅₀ (16.0 ± 1.2 and 9.5 ± 0.1 µg/mL for L. amazonensis and L. infantum, respectively). This isolated sesquiterpene alcohol was studied for its activity on amastigotes forms (IC₅₀ = 5.9 ± 1.2 and 4.8 ± 1.3 µg/mL, respectively) and its cytotoxicity (selectivity indexes (SI) were 5.4 and 6.6, respectively). The obtained results showed that (-)- α -bisabolol was able to activate a programmed cell death process in the promastigote stage of the parasite. It causes phosphatidylserine externalization and membrane damage. Moreover, it decreases the mitochondrial membrane potential and total ATP levels.

After evaluation of the activity and toxicity of α -bisabolol against the *Acanthamoeba castellani Neff* strain , IC50 values of 20.839 \pm 2.015 for treated amoebae as well as low citotoxicty levels in a murine macrophage cell line was observed. Moreover, in order to elucidate mechanism of action of this molecule, changes in chromatin condensation levels, permeability of the plasmatic membrane, the mitochondrial membrane potential and the ATP levels in the treated amoebic strains were checked.

These results highlight the potential use of $(-)-\alpha$ -bisabolol against both Leishmania and Acanthamoeba genus, and further studies should be undertaken to establish it as novel therapeutic agents.

References

[1].Inacio JD, Gervazoni L, Canto-Cavalheiro MM, Almeida-Amaral EE (2014). The effect of epigallocatechin 3-O-gallate *in vitro* and *in vivo* in *Leishmania braziliensis*: involvement of reactive oxygen species as a mechanism of action. PLoS Negl Trop Dis 8(8):e3093

Acknowledgment

This work received financial support by "Laboratoired'Epidémiologie d'Infections Enzootiques des Herbivores en Tunisie" (Ministère de l'enseignement supérieur, Tunisia).







Studies on Thiazolopyrimidine Derivatives within One Health Perspective

<u>Gülşah Bayraktar</u>^a, Yamaç Tekintaş^b, Hüseyin Istanbullu ^c

^a Ege University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Izmir, Turkey; ^b Izmir Kâtip Celebi University, Faculty of Pharmacy, Department of Microbiology, Izmir, Turkey ^c Izmir Kâtip Celebi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Izmir, Turkey

gulsah.bayraktar@ege.edu.tr, bayraktargulsah@ege.edu.tr

One Health is a multidisciplinary collaborative effort that aims to ensure optimal outcomes for humans, animals and the environment.

Leishmaniasis, a parasitic disease, is classified as 'Neglected Tropical Diseases (NTD)' by WHO. The most common forms of leishmaniasis are cutaneous leishmaniasis, visceral leishmaniasis, and mucocutaneous leishmaniasis [1,2].

Developing antiparasitic drugs that address vector-borne diseases affecting both humans and animals is a key component of One Health framework. On the other hand, mycobacteria species cause diseases in both humans and animals therefore it is important to consider the zoonotic potential of these infections within One Health concept to help combat the public and animal health threats [3].

We have synthesized a series of thiazolopyrimidine derivatives possessing anti-leishmanial activities against L. tropica, L. infantum and L. major parasites [1,2]. Additionally, the antimicrobial activities of the compounds were determined using the broth microdilution method on *Mycobacterium smegmatis* ATCC 14468 strain with levofloxacin as a positive control [4]. *Mycobacterium smegmatis*, a nonpathogenic and fast growing species, is a good model for mycobacterium research. All the tested compounds exhibited anti-mycobacterial activity as well as their anti-leishmanial potency. Further investigations are currently ongoing in our laboratory.

References

- [1] Istanbullu, H., Bayraktar, G., Akbaba, H., Cavus, I., Coban, G., Debelec Butuner, B., ... & Erciyas, E. (2020). Design, synthesis, and *in vitro* biological evaluation of novel thiazolopyrimidine derivatives as antileishmanial compounds. *Arch der Pharm.*, 353(8), 1900325.
- [2] Istanbullu, H., Bayraktar, G., Karakaya, G., Akbaba, H., Perk, N. E., Cavus, I., ... & Alptuzun, V. (2023). Design, synthesis, in vitro–In vivo biological evaluation of novel thiazolopyrimidine compounds as antileishmanial agent with PTR1 inhibition. *E J Med Chem.*, 247, 115049.
- [3] Thirunavukkarasu, S., Plain, K. M., De Silva, K., Marais, B. J., & Whittington, R. J. (2017). Applying the one health concept to mycobacterial research–overcoming parochialism. *Zoonoses Public Health*, 64(6), 401-422.
- [4] Ramis, I. B.; Cnockaert, M. (2015). Antimicrobial susceptibility of rapidly growing mycobacteria using the rapid colorimetric method. *Eur J Clin Microbiol Infect Dis.*, 34(7), 1403–1413.

Acknowledgment

The Authors acknowledge TUBITAK for the financial support.







Fish-borne parasitic zoonoses: One Health perspectives

Author: Dijana Blazhekovikj - Dimovska^a

^aUniversity "St. Kliment Ohridski", Faculty of Biotechnical Sciences, Bitola, Macedonia

dijana.blazekovic@uklo.edu.mk

The world is encountering a dynamic environment of infectious diseases impacting both humans and animals, many of which can present serious risks to health and well-being. Zoonoses refer to diseases that can be naturally passed from animals to humans, which adds layers of complexity to creating lasting and effective control measures, and requires an approach that encompasses One Health. The transmission of pathogens among animal reservoirs and between animals and humans can lead to significant ecological and evolutionary impacts, influencing the development and persistence of drug resistance (Webster et al. 2016).

Threats posed by both established and emerging parasites and pathogens continue to arise, driven by alterations in the environment, shifts in agriculture and food production, and changes in the demographics and connectivity of today's 'global' village. Clarifying disease ecology is crucial, which involves recognizing important hosts and adjusting control measures appropriately, as well as comprehending parasite evolution, including how infectious agents might react and adapt to human-induced changes. (Gibbs 2005; Johnson et al. 2015).

The worldwide demand for seafood is continuously rising, along with the diversity of seafood options, such as meals featuring raw or lightly cooked fish, which heightens the chance of developing seafood-related parasitic illnesses. To meet the challenges, we face today in comprehending the biology and ecology of these parasites within a constantly evolving environment and to address their pathogenic potential, a multidisciplinary approach to research is essential. Furthermore, it is crucial to close the gap between researchers and stakeholders to mitigate the threat these parasites present to public health. A "One-Health" methodology in research is essential to evaluate consumer health, the well-being of aquatic animals, and environmental health issues in a coordinated and holistic way, leading to a deeper comprehension of the challenges related to seafood-borne parasitic diseases and possible solutions. To maintain their global standing as a source of healthy seafood, it is increasingly crucial to actively evaluate the risks linked to both local and imported products and to implement effective control and preventive measures.

Consequently, it is essential to adopt a "One-Health" strategy that involves several stakeholders and research. To ensure the ongoing supply of safe seafood, various professionals from different fields need to stay informed about the latest developments regarding parasites that affect seafood and the diseases they can cause, and they should collaborate to promote "One Health" strategies. The public health impact of parasites has often been undervalued in comparison to other food sectors, highlighting a critical requirement for the establishment of seafood safety regulations and guidelines in numerous countries. Substantial gaps still exist in our understanding of essential biological and ecological aspects of various new and emerging seafood-borne parasites, the diseases they produce, their associated public health importance, and the most effective strategies for preventing and controlling the transmission, infection, and disease caused by these parasites (Shamsi, 2019).

References

- Webster, J.P., Gower, C.M., Knowles, S.C., Molyneux, D.H., Fenton, A. (2016). One health an ecological and evolutionary framework for tackling Neglected Zoonotic Diseases. Evol Appl. 8; 9(2): 313-33.
- [2] Gibbs, E. P. (2005). Emerging zoonotic epidemics in the interconnected global community. Veterinary Record 157:673-679.
- [3] Johnson, P. T. J., de Roode J. C., and Fenton A. (2015). Why infectious disease research needs community ecology. Science 349:1259504.
- [4] Shamsi, S. (2019). Seafood-Borne Parasitic Diseases: A "One-Health" Approach Is Needed. Fishes, 4(1), 9.







Role of crystal engineering in repurposing drugs for therapy of antiparasitic vector-born disease:

structure-property relationship

Aleksandar Cvetkovski a Elena Drakalska Sersemova a

Faculty of medical science, Goce Delcev University, Krste Misirkov b.b. P. fax. 201, 2000 Stip, Republic of N. Macedonia

aleksandar.cvetkovski@ugd.edu.mk

Drug repurposing becomes an emerging approach for leveraging the utilization of the approved drugs or that what previously are investigated toward phases of clinical trail for treatment of the original diseases redirecting their well-establish pharmacokinetics and safety in validation the new therapies compared to their initial pharmacological treatments.

The identification of new opportunities for use of old drugs requires a holistic approach of innovative computational method in combination with biological testing on experimental cell/ animal models. Thus, customizing and optimizing screening potential of hit or lead compounds from existing databases using in silico calculations and subsequently confirming their activity through a series of biological experiments promise effective overcoming bottlenecks and expedite an cost-effective discovery of alternative therapeutic agents and their applications ^[1]. Drug repositioning accounts for approximately 30% of the newly US Food and Drug Administration (FDA)-approved drugs and vaccines in recent years.

The purpose of the presented crystal structures searched in the Cambridge Structural Database (CSD) among the insecticide class of compounds with isooxazoline molecular scaffold ^[2] as well Dibucaine, Phenylephrine, Acebutolol, Prilocaine, Albendazole, Ethacrynic acid, Ganciclovir, Benzthiazide, Ethionamide ^[3] and Praziquantel [^{4]} repurposed for treatment of Leishmania is to reveal the opportunities for engineering new crystal phased of multicomponent crystals combining these drug compounds with ligands selected based on the best proton donor/acceptor groups and molecular moieties that guide to non-covalent interactions, mainly Hydrogen-bonds which geometry may be utilized in crystal structure prediction with enhanced water solubility ^[5].

References

- [1] Hu, S.S. et al., Exploration of innovative drug repurposing strategies for combating human protozoan diseases: Advances, challenges, and opportunities *J.Pharm. Anal.* **2025**, 15, 101084
- [2] Miglianico, M., et al., Repurposing isoxazoline veterinary drugs for control of vector-borne human diseases, Proc. Natl. Acad. Sci. U.S.A. 2018, 115 (29) E6920-E6926
- [3] Oualha, R. *et al.*, Approved drugs successfully repurposed against Leishmania based on machine learning predictions. *Front. Cell. Infect. Microbiol.* **2024** 14:1403589.
- [4] Devogelaer, J-J., et al., Cocrystals of Praziquantel: Discovery by Network-Based Link Prediction Crystal Growth & Design 2021 21
 (6), 3428-3437
- [5] Ma, C.Y. et al. Exploring the CSD Drug Subset: An Analysis of Lattice Energies and Constituent Intermolecular Interactions for the Crystal Structures of Pharmaceuticals *J. Pharm. Sci.* **2023**, 112 435–445







On the road to unravel the interactome of *Leishmania* molecular heat shock protein 90

Roncareggi Davide^a, Sergio Romeo^{b, c}, Erica E. Ferrandi^c, Laura Sola^c, Silvia Parapini^a, Gaia Mazza

^d, Ivan Bassanini^c

^a Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano (Italy); ^b Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Milano (Italy); ^c Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", Consiglio Nazionale delle Ricerche (CNR), Milano (Italy); ^d Dipartimento di scienze biomediche chirurgiche e odontoiatriche, Università degli Studi di Milano, Milano, Milano (Italy)

davide.roncareggi@unimi.it

The human development of the life-threatening parasitosis Leishmaniasis -an endemic zoonosis in the Mediterranean basin- is strictly related to the ability of protozoan parasites of the genus *Leishmania* to undergo a sequence of morphological transformations, in response to rapid changes in host cells temperature and pH, during the human stages of their life cycle. Specifically, *Leishmania* differentiation from the vector-transmitted promastigote forms to the pathogenic intra-macrophage amastigotes is regulated, among other key factors, by the molecular chaperone Hsp90.

Recently, in the framework of the target-oriented development of novel antiprotozoal drugs, we have prepared and tested a panel of celastrol derivatives. Celastrol, is a natural allosteric inhibitor of human Hsp90 which also shows a modest antiprotozoal activity and modulatory effects against protozoan Hsp90 variants. 1

In vitro screening identified a sub-family of basic celastrol carboxiamides as potent leishmanicidals agents against *Leishmania* promastigotes cultures able to strongly inhibit *Leishmania* Hsp90 by allosterically targeting its middle-domain. In accordance with previous findings, these compounds also demonstrated potent growth inhibitory activity against cultures of *Leishmania* amastigotes, despite Hps90's theoretical limited role in their homeostasis. 1

In the light of these findings, our research project focuses on understanding, at a molecular level, the origin of the high leishmanicidal activity demonstrated by celastrol-based Hsp90 inhibitors towards *Leishmania* amastigotes.

The implementation of standard affinity-based protein fishing strategies coupled with an innovative method of non-covalent fluorous proteomics 2 aims to identify and compare the interactome of Hsp90 in the human amastigote and promastigote stages of *Leishmania*. Collected data will enable the determination of potential down-stream events of chaperone inhibition in *Leishmania* amastigotes discovering Hsp90-related off-target effects and potentially-druggable protein targets among the isolated Hsp90-interacting clients and co-chaperones.

References

- [1] Bassanini, I.; Parapini, S.; Ferrandi, E.E.; Gabriele, E.; Basilico, N.; Taramelli, D.; Sparatore, A. Design, Synthesis and In Vitro Investigation of Novel Basic Celastrol Carboxamides as Bio-Inspired Leishmanicidal Agents Endowed with Inhibitory Activity against Leishmania Hsp90. Biomolecules **2021**, 11, 56.
- [2] Bassanini, I.; Galli, C.; Ferrandi, E. E.; Vallone, F.; Andolfo, A.; Romeo, S. Perfluorinated Probes for Noncovalent Protein Recognition and Isolation. Bioconjug. Chem. 2020, 31 (3), 513–519.

Acknowledgment

The Authors acknowledge European Union – Next Generation EU, Mission 4 CUP G53D23000590006 for the financial support.







Bridging Veterinary and Human Health: Drug Targets for Zoonotic Parasite Reservoirs

*Dilek, O.G.

¹University of Mehmet Akif Ersoy Faculty of Veterinary Medicine, Burdur, Türkiye,

ogdilek@gmail.com

Parasitic diseases with zoonotic potential continue to pose a significant global health challenge, particularly in regions where close interactions between humans, animals, and the environment facilitate cross-species transmission. The One Health approach-integrating human, animal, and environmental health—has become vital for addressing these complex disease dynamics. Novel drug development and targeted therapeutic strategies are essential to interrupt the parasite life cycle at the animal reservoir level, thereby reducing the risk of human infection. Promising targets include vector microbiota modulation, stage-specific parasite enzymes, and host-parasite interface molecules such as proteases and kinases involved in parasite survival and transmission (Geary et al., 2012; Molyneux et al., 2016). Recent advances in transcriptomics and proteomics have enhanced the identification of conserved drug targets across species, enabling the design of broad-spectrum antiparasitics with efficacy in both animals and humans (Tian et al., 2020). Moreover, integrated treatment strategies combining antiparasitic drugs with vaccination or vector control have shown synergistic effects in breaking transmission chains (WHO, 2021). Ensuring drug accessibility and appropriate use in veterinary settings is equally critical, as resistance development in animal reservoirs can undermine human treatment efforts. By aligning drug development with ecological and epidemiological insights from One Health research, it is possible to create sustainable, cross-sectoral interventions that protect both animal and human populations. Continued investment in cross-disciplinary research and collaborative policy frameworks will be pivotal in achieving long-term success in parasite control.

References

- [1] Geary, T. G., Sakanari, J. A., & Caffrey, C. R. (2012). Antiparasitic drug discovery: into the future. *The Journal of Parasitology*, 98(6), 1155–1165.
- [2] Molyneux, D. H., Savioli, L., & Engels, D. (2016). Neglected tropical diseases: progress towards addressing the chronic pandemic. *The Lancet*, 389(10066), 312–325.
- [3] Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2020). CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Research*, 48(W1), W45–W50.
- [4] World Health Organization. (2021). Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030.

Acknowledgment

The Author acknowledge there is no financial support.







TIME-CHANGE LEVY PROCESS IN MODELLING OF EPIDEMICS

Jasmina Đorđević ^a

^a Department of Mathema3cs, University of Niš, Serbia

jasmina.djordjevic@pmf.edu.rs (joint work with Giulia Di Nunno and Nenad Šuvak)

We introduce time-changed Lèvy noises when the time-change is independent of the Lèvy process in compartment SIRV model. It is proposed that transmission rate is described with mean reverting process via time changed diffusion process with jumps. Existence and uniqueness of positive solution of SIRV system with time changed Lèvy process is proved. Furthermore the problems of persistence and extinction are analyzed.

References

- M. Ishikawa, OpNmal Strategies for VaccinaNon using the StochasNc SIRV Model, Special Issue on the 43rd ISCIE InternaNonal Symposium on StochasNc Systems Theory and Its Applica Nons—II, Vol. 25, No. 12, pp. 343–348, 2012.
- [2] J. Djordjevic, Silva, Torres, A stochasNc SICA epidemic model for HIV transmission, Appl. Math. Le^ers, 84 (2018), 168–175
- [3] Di Nunno, S. Sjursen, On chaos representaNon and orthogonal polynomials for the doubly stochasNc Poisson process, Robert C. Dalang, Marco Dozzi, Francesco Russo (Eds.), Seminar on StochasNc Analysis, Random Fields and ApplicaNons VII, in: Progress in Probability 67, Springer, Basel, 2013, 23–54.
- [4] G. Di Nunno, S. Sjursen, BSDEs driven by Nme-changed L'evy noises and opNmal control, StochasNc Processes and their ApplicaNons 124, 2014, 1679-1709.







Pharmacological Advances in Vector-Borne Parasitic Diseases: Assays, Targets, and

Environmental Impact.

Lori Doko

Universita degli studi eCampus , Albania, Italy.

dokolori6@gmail.com; lori.doko@studenti.uniecampus.it

The human and protective health burden of vector-borne parasitic diseases is considerable and urges the need for new types of treatment. This project revolves around developing and perfecting the procedures of target validation, drug synthesis screening, and the examination of natural products for their antiparasitic properties. Drug development is being transformed for the better in the context of disease control by the use of high-throughput screening technologies and bioinformatics- guided target identification. Moreover, the environmental impacts of drug side effects are becoming of increasing concern for public health which makes the need for impact assessment urgent. Drug residue in the environment can disrupt microbial populations, cause adverse effects on other organisms, and lead to increased resistance. This paper seeks to examine the nature of ecotoxicological assessments, achieving balance between the degree of needed pharmaceutical efficacy and the social sustainability. The goal of this research is to reconcile ecological safety and pharmacological change by providing a sustainable approach to the control of vector borne parasitic diseases.

References

- [1] Human Health Burden of Vector-Borne Diseases : Vector-borne diseases account for more than 17% of all infectious diseases, causing over 700,000 deaths annually. <u>World Health Organization (WHO)</u>.
- [2] High-Throughput Screening in Drug Discovery : High-throughput screening technologies have been employed to identify potential anthelmintic compounds against gastrointestinal nematode parasites. <u>PubMed</u>
- [3] Natural Products in Antiparasitic Research : Natural products have been a significant source of new drugs, with many anti-infective agents derived from natural sources. <u>PubMed+2ACS Publications+2ACS Publications+2</u>
- [4] Environmental Impact of Pharmaceutical Residues : Pharmaceuticals and personal care products in the environment raise concerns about their effects on human and ecological health. <u>EHP Publishing+4EHP Publishing+4PubMed+4</u>
- [5] Ecotoxicological Assessments for Sustainable Drug Development : Designing pharmaceuticals with environmental biodegradability in mind is crucial for reducing their ecological impact.

Acknowledgment

The author sincerely thank the **University of Antwerp**, the **LPHM laboratory**, **COST Action CA21111**, and **eCampus University** for their support in facilitating this research. Their contributions to advancing innovative therapeutic strategies for vector-borne diseases are greatly appreciated.







Risk factors, mode of transmission and potential for acute toxoplasmosis serological diagnosis: A

case series of this infection occurring during pregnancy

Abazaj Erjona^a; Puca Edmond^b; Bylykbashi Edlira^c; Qyra Shpetim^a; Bino Silva^a

^aInstitute of Public Health, Tirana, Albania; ^b "Mother Teresa" University Hospital Center, Tirana, Albania; ^c Bylykbashi Clinic, Tirana, Albania

abazajerjona@gmail.com

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii*. It remains a major worry among congenital infections because its effects can result in a myriad of severe medical issues in babies. Considering the rate of occurrence and the dire consequences on fetal well-being, it is warranted to study deeply six specific cases of acute congenital toxoplasmosis with emphasis on the delay in the diagnosis of the illness and the role of cats as plausible sources of infection. From November 2024 to January 2025 (three months), six women considered to be pregnant and suspected of having toxoplasmosis infection came to the Laboratory of the Institute of Public Health, and they were all participants of the case study. All participants underwent testing for the presence of specific antibodies: anti-IgM, IgG, and IgG avidity, employing the Enzyme-Linked Immunosorbent Assay (ELISA) method. Study participants had a mean age of 28.3 years (\pm 2.11 Standard Deviation), and the dominant age category was between 25-30 years. Out of the participants, 4 women were on their second pregnancy and 2 were on their first pregnancy, all participants being in their first trimester. Interestingly, only one woman was living in an apartment, while the other five participants were residing in private houses. Serological tests confirmed that all patients were positive for IgM antibodies, and two women had IgG levels that were demonstrably four-fold higher than the norm. Results from the IgG avidity assay showed a higher avidity in just 2/6 (33.3%), suggesting acute infections for the remaining 4/6 (66.7%), which heightens the risk of congenital transmission. An analysis of suggestive risks pointed out that having a cat in the household, combined with not fully cooking food from fast food places, might be infection sources. Through this case series, we documented acute congenital toxoplasmosis while highlighting the lack of awareness due to feline exposure and undercooked meals as the primary contributors to the problem in this patient cohort. Timely treatment and frequent testing of pregnant women are essential to improve health outcomes. There is insufficient awareness about congenital toxoplasmosis, and routine screening is needed to manage such cases effectively during pregnancy.

Keywords: acute toxoplasmosis; pregnancy; serology test

References

- [1] Abazaj E, Ferataj Deliu E, Petri O, Shundi L, Hysaj B, Ali E, Xinxo S, Tanka M, Qyra S. 2016. IgG avidity test for diagnosis of acute *Toxoplasma gondii* in pregnant women. J Environ Prot Ecol 17:681–686.
- [2] Deganich M, Boudreaux C, Benmerzouga I. Toxoplasmosis Infection during Pregnancy. Trop Med Infect Dis. 2022 Dec 21;8(1):3. doi: 10.3390/tropicalmed8010003. PMID: 36668910; PMCID: PMC9862191.
- [3] Vueba AN, Faria CP, Almendra R, Santana P, Sousa MdC (2020) Serological prevalence of toxoplasmosis in pregnant women in Luanda (Angola): Geospatial distribution and its association with socio-demographic and clinical-obstetric determinants. PLOS ONE 15(11): e0241908. <u>https://doi.org/10.1371/journal.pone.0241908</u>







Cinchona Alkaloid Polymeric Fluorescent Logic Gates

Nicola' Agius,^a Catherine J. Ashton,^b Helen Willcock,^b David C. Magri^a

^a Department of Chemistry, Faculty of Science, University of Malta, Malta ^b Department of Materials, Loughborough University, Leicestershire, England, UK

david.magri@um.edu.mt

Given the availability of three hundred or so known fluorescent natural products [1], our aim was to design intelligent fluorescent natural product copolymers with potential anti-parasitic applications. We reasoned this unexploited approach could open new research directions in multiple disciplines from material science, environmental protection and medicine [2,3]. We previously demonstrated that the *cinchona* alkaloids, quinine, quinidine, cinchonine and cinchonidine, have remarkable intrinsic Boolean logic properties using chemicals as inputs and fluorescence as the output [4]. In our latest findings [5], we report the extraordinary properties of *cinchona* alkaloids copolymers, notably with the antimalarial quinine, as logic-based copolymers as potential theranostic agents combining diagnostic imaging and therapy.



References

[1] R. Duval and C. Duplais, Nat. Prod. Rep., 2017, 34, 161.

- [2] N. Agius and D. C. Magri, Nat. Prod. Commun., 2024, 19, 1.
- [3] N. Agius and D. C. Magri, New J. Chem., 2021, 45, 14360.
- [4] N. Agius and D. C. Magri, RSC Adv., 2023, 13, 13505.
- [5] N. Agius, C. J. Ashton, H. Willcock and D. C. Magri, RSC Adv., 2025, 15, 11121.

Acknowledgment

The authors acknowledge Xjenza Malta for a FUSION R&I: Research Excellence grant, REP-2023-023 and the Engineering and Physical Sciences Research Council (EP/R513088/1) for financial support.







Assessment of dyes and new strategies in the design of anthelmintic drugs

Miguel Marín Folgado^{a,b}, Javier Sanchez Montejo^b, Sergio Ramos Varela^a, Antonio Muro Alvarez^b,

Julio Lopez Abán^b, Rafael Pelaez Lamamie De Clairac Arroyo^a

^aInstituto de Investigación Biomédica de Salamanca (IBSAL), University of Salamanca, Spain; ^bCentro de Investigación de Enfermedades Tropicales de la Universidad de Salamanca (CIETUS), University of Salamanca.

Spainmmarin@usal.es

Strongyloidiasis is a parasitic disease that affects over 600,000 individuals globally. The development of effective pharmaceuticals against this disease has yielded no results in decades, and its treatment continues to rely on ivermectin and benzimidazoles, which, due to mass administration, are at risk of inducing resistance. The lack of understanding of the parasite's biology increases the challenges in designing effective drugs against it. In this study, we utilized commonly used laboratory dyes, such as methylene blue and crystal violet, to investigate how *Strongyloides* absorbs compounds from its environment. Our findings indicate that this uptake is selective and appears to be facilitated by structural characteristics of the molecule, such as the presence of positive charges. As a proof of concept, we introduced a positive charge to an inactive compound from one of our chemical libraries and reassessed its activity. The results of this study underscore the need to employ innovative techniques in drug design for neglected diseases. Results of the in vitro experiments against L3s and adult stage as well as an in vivo experiment will be presented ^[11].

References

[1] Marín, M.; Sánchez-Montejo, J.; Ramos, S.; Muro, A.; López-Abán, J.; Peláez, R. Deciphering Chemical Rules for Drug Penetration into Strongyloides. Pharmaceutics 2024, 16, 1224. https://doi.org/10.3390/pharmaceutics16091224

Acknowledgment

The Authors acknowledge financial support from Grant PID2021-1274710B-I00, funded by MCIN/AEI/10.13039/501100011033, Junta de Castilla y León (SA0116P20), and "ERDF A way of making Europe" by the "European Union". M.M. thanks Junta de Castilla y León for a predoctoral fellowship 2020 cofounded by the EU Social Funds (ORDEN EDU/601/2020) and an EMBO Scientific Exchange Grant (SEG number: 10384). S.R. acknowledges a predoctoral fellowship from the Universidad de Salamanca Programa III 2018, cofounded by Banco de Santander. J.S.M. acknowledges the predoctoral fellowship program of Junta de Castilla y León, cofunded by "Fondo Social Europeo" (Orden EDU875/2021).







PEGylated liposomes encapsulating amphotericin B: an innovative formulation for the treatment of cutaneous leishmaniasis

Thais T. Santos^{a,b}, Guilherme Ramos^a, Eduardo Burgarelli^c, Tiago Ricotta^c, Marta Aguiar^c, <u>Sébastien</u>

Pomel^b, Frédéric Frézard^a

^a University Federal de Minas Gerais, Institute of Biological Sciences, Department of Physiology and Biophysics, Belo Horizonte, Brazil; ^b Université Paris-Saclay, Faculty of Pharmacy, UMR CNRS 8076, BioCIS, Orsay, France; ^c University Federal de Minas Gerais, Faculty of Pharmacy, Belo Horizonte, Brazil.

sebastien.pomel@universite-paris-saclay.fr

Leishmaniasis, a group of neglected tropical diseases, predominantly affects socially vulnerable populations with limited access to healthcare, often involving immunocompromised individuals. The disease, caused by various Leishmania species, manifests primarily as cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). In 2022, there were 205,986 and 12,842 new cases of CL and VL, respectively (1). Among the few drugs available, amphotericin B (AmB) is considered the most potent antileishmanial. Despite its effectiveness, AmB faces challenges such as low solubility, high molecular weight, and self-aggregation, leading to reduced bioavailability and increased toxicity (2). Liposomal AmB, or AmBisome®, is considered highly effective, yet limitations exist, especially in complicated CL cases and HIV/VL coinfected patients. This study introduces innovative AmB-PEGylated liposomes (LAmB) for CL therapy, demonstrating favorable characteristics, including a hydrodynamic diameter of 128.4 \pm 4.8 nm, low polydispersity index of 0.10 \pm 0.02, and slightly negative surface charge -3.6 ± 0.6 mV, suitable for *in vivo* administration. A high encapsulation rate $(94.8 \pm 5.2\%)$ was also obtained. The physicochemical characteristics of LAmB remained stable over 30 days in the refrigerator without the need for lyophilization. Furthermore, LAmB demonstrates lower hemolytic activity compared to the commercial formulation Anforicin B®. In a murine model infected with L. (L.) amazonensis, LAmB treatment leads to a marked reduction in lesion size (8.77 \pm 1.60 mm) in comparison to the untreated control (11.32 \pm 1.70 mm) at the end of treatment. Notably, the LAmB group had smaller lesion size than AmBisome® (9.87 ± 1.85 mm). This novel formulation emerges as a promising therapeutic approach for CL.

References

Acknowledgment

The Authors acknowledge CAPES, CNPq and FAPEMIG for the financial support.

^[1] World Health Organization. Global leishmaniasis surveillance, 2022: assessing trends over the past 10 years. n. 40, p. 471–487, 2023.

^[2] Lanza, J. S. et al. Recent advances in amphotericin B delivery strategies for the treatment of leishmaniases. Expert opinion on drug delivery, v. 16, n. 10, p. 1063-1079, 2019.







Evaluation of Novel 4-Thiazolidinone Bioisosteres of Alkylphosphocholines as anti-Trypanosomal

agents

Evanthia Chazapi^{a, #}, Nuno Santarém^{b,c, #}, Theano Fotopoulou^a, <u>Ricardo Monteiro^{b,c,\$}</u>, Vivi Bafiti^a, George E. Magoulas^a, Theodora Katsila^a, Kyriakos C. Prousis^a, Joana Tavares^{b,d}, Anabela Cordeiro da Silva^{b,c,*}, Theodora Calogeropoulou^{a,*}

^a Institute of Chemical Biology, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, 1135, Athens, Greece; ^b Institute for Research and Innovation in Health (i3S), University of Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal; ^c Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal; ^d Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, 4200-135 Porto, Portugal. [#] Equally contributed. * Corresponding authors.

^{\$} ricardopsm@i3s.up.pt.

Infections caused by protozoan parasites, including those from *Leishmania* and *Trypanosoma* genera, constitute a major public health problem and contribute to a substantial global burden of illness and death (1). Given the increasing importance of One Health approaches to tackle vector-borne diseases, new environmentally friendly drug therapies are required for the treatment of both human and animal parasitic infections (2). Miltefosine, firstly proposed as an anticancer drug, was considered by the WHO an essential medicine for the treatment of cutaneous and visceral leishmaniasis (3). Despite its efficacy, challenges related to toxicity have been described (4). Over the last years, miltefosine has been a lead molecule for the development of new derivatives (5). In the present study, we conducted a phenotypical screening of novel 4-thiazolidinone bioisosteres of alkylphosphocholines by assessing their activity in vitro against Trypanosoma brucei and Leishmania infantum and their cytotoxicity using THP-1 derived macrophages. SNAP-Shot pharmacokinetic profiles in mice were then determined for the most promising molecules. Overall, excellent pharmacokinetic profiles were obtained, and efficacy against visceral leishmaniasis was then evaluated in a well-established murine model (6). Treatment efficacy was monitored using whole-body bioluminescence imaging, and unexpectedly, no differences, except for miltefosine, were found in the bioluminescent parasite burden throughout treatment when compared to animals receiving vehicle only. The lack of antileishmanial activity was further confirmed by quantifications of parasite burdens in target organs using a limiting dilution assay. Given the favorable pharmacokinetic profile in the blood, activity against bioluminescent blood-circulating T. b. brucei GVR35 parasites is ongoing. Nonetheless, these compounds produced using a sustainable synthetic process (7) demonstrated high potency against protozoan parasites and low toxicity against mammalian cells. Further optimization is required to generate new derivatives with in vivo efficacy.

References

- [1] Filardy AA, Guimarães-Pinto K, Nunes MP, Zukeram K, Fliess L, Pereira L, et al. Human Kinetoplastid Protozoan Infections: Where Are We Going Next? Front Immunol [Internet]. 2018;Volume 9-2018. Available from: https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2018.01493
- [2] Ilbeigi K, Barata C, Barbosa J, Bertram MG, Caljon G, Costi MP, et al. Assessing Environmental Risks during the Drug Development Process for Parasitic Vector-Borne Diseases: A Critical Reflection. ACS Infect Dis. 2024 Apr;10(4):1026–33.
- [3] Croft SL, Engel J. Miltefosine discovery of the antileishmanial activity of phospholipid derivatives. Trans R Soc Trop Med Hyg [Internet]. 2006 Dec 1;100(Supplement_1):S4–8. Available from: https://doi.org/10.1016/j.trstmh.2006.03.009

[4] Dorlo TPC, Balasegaram M, Beijnen JH, de Vries PJ. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. J Antimicrob Chemother [Internet]. 2012 Nov 1;67(11):2576–97. Available from: https://doi.org/10.1093/jac/dks275

[5] Chazapi E, Magoulas GE, Prousis KC, Calogeropoulou T. Phospholipid Analogues as Chemotherapeutic Agents Against Trypanosomatids [Internet]. Vol. 27, Current Pharmaceutical Design. 2021. p. 1790–806. Available from: http://www.eurekaselect.com/article/112290

[6] Mendes Costa D, Cecílio P, Santarém N, Cordeiro-da-Silva A, Tavares J. Murine infection with bioluminescent Leishmania infantum axenic amastigotes applied to drug discovery. Sci Rep [Internet]. 2019;9(1):18989. Available from: https://doi.org/10.1038/s41598-019-55474-3

[7] Cioc RC, Ruijter E, Orru RVA. Multicomponent reactions: advanced tools for sustainable organic synthesis. Green Chem [Internet]. 2014;16(6):2958–75. Available from: http://dx.doi.org/10.1039/C4GC00013G

Acknowledgments

This work was funded by the Operational Program "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund). NS is funded by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P. and co-funded through the European Social Fund within the Human Potential Operating Program 2021.04285.CEECIND. This work was supported by National Funds through FCT under the project UIDB/04293/2020, and co-funded by FEDER through the COMPETE 2020 - Operational Program for Competitiveness and Internationalization POCI-01-0145-FEDER-031013. RM is funded by a FCT individual fellowship 2021.09486.BD. This work was produced in the context of the COST Action CA21111: One Health Drugs against Parasitic Vector-Borne Diseases in Europe and Beyond (OneHealthDrugs).







Chemical analysis and and in vitro acaricidal activity of Mentha pulegium and Thymus capitatus

essential oils against Hyalomma dromedarii,

Essia Sebai^a, Safa Amairia^a, Oumayma Ardhaoui^b, Hafidh Akkari^a, Mohamed Aziz Darghouth^a.

^a University of Manouba, National School of Veterinary Medicine of Sidi Thabet, 2020 Ariana, Tunisia. ^b University of Manouba, ISBST, BVBGR-LR11ES31, Biotechpole SidiThabet, 2020, Ariana, Tunisia.

essia.sebai@fst.utm.tn

Ticks exhibit an impressive ability to infest various vertebrate hosts and transmit a wide range of pathogenic bacteria, viruses, protozoa, and helminthic parasites through their hematophagy. The excessive and improper use of synthetic acaricides and repellents has led to the emergence and development of resistance in tick populations, reducing the efficacy of chemical products¹. Various alternative approaches, more coherent with the EcoHealth principles, have gained attention as potential tick control strategies. Among these, the utilization of plant-derived products and essential oils has garnered significant interest lower incidence of resistance, and fewer eco-systemic side effects. The objective of this study is to determine the acaracidal potential of Thymus capitatus and Mentha pulegium essential oils against larvae of Hyalomma dromedarii using larval immersion test $(LIT)^2$. M. pulegium. EO showed total tick mortality at 5 µl/mL while T. capitatus EO showed 100% of tick mortality at 0.8 μ l/mL. In addition, we noticed an *in vitro* chitinase activity and a neurotoxic activity by a significant decrease of acetylcholinesterase ACHE biomarker³. The chemical analysis of *M. pulegium* EO performed by GC-MS allowed the identification of 21 substances. Pulegone is the main compound (62.98%) followed by menthone (28%), neo-menthol (1.82%) and isopulegone (1.08%). For T. capitatus essential oil, GC-MS analysis revealed carvacrol (58.46%) and β -cymene (11.37%) as the two major compounds.

References

- [1] Alota, S.L., Edquiban, T., Galay, R.L. et al. Determination of resistance status to amitraz in the cattle tick *Rhipicephalus (Boophilus) microplus* from Luzon, Philippines, through bioassay and molecular analysis. Exp Appl Acarol , 2021, 83, 399–409.
- [2] Klafke, Guilherme M., Thomas, Donald B., Miller, Robert J.. Efficacy of a water-based botanical acaricide formulation applied in portable spray box against the southern cattle tick, *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae), infesting cattle. *Ticks and Tick-borne Diseases*, 2021, vol. 12, no 4, p. 101721.
- [3] Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M. A New and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961, 7, 88–95.

Acknowledgment

The Authors acknowledge the Laboratory of Epidemiology of Enzootic Infections in Herbivores in Tunisia: Application to Control" (LR16AGR01) funded by the Ministry of Higher Education and Scientific Research, Tunisia.for the financial support.







Leishmaniasis and Vector Control

An Emerging Threat and New Prevention Strategies

Authors: Talbalaghi A^{1,2*}., Hassndoust S.¹

¹Italian Mosquito Control Association ² Free lance Medical Cconsultant, Medical Entomologist

> imca@zanzare.eu talbalaghi@libero.it

Leishmaniasis, a vector-borne disease transmitted by sand flies (phlebotomines), is emerging as a growing public health concern in several regions of Italy. Traditionally associated with rural environments, sand flies are now expanding into urban and peri-urban areas due to changing climatic and ecological conditions. These vectors feed on a wide range of animal reservoirs, particularly rodents and domestic animals, facilitating the persistence and spread of the Leishmania parasite.

Current control strategies, which often focus on larval breeding sites, are inadequate for managing sand fly populations, as these insects require targeted interventions at the adult stage. The World Health Organization emphasizes the importance of personal protective measures, such as repellents, in reducing transmission risk. Recent advancements in repellent technologies, especially the development of repellent-treated fabrics, represent a promising, passive, and innovative tool for disease prevention.

These innovative repellent-based measures have been the focus of interdisciplinary efforts during a coordinated action concluded a few years ago, which emphasized the potential of this approach. However, its effective implementation still requires more robust technical and scientific contributions to ensure practical application and integration into public health strategies.

This contribution underscores the importance of adopting integrated, interdisciplinary approaches within a One Health framework, encouraging collaboration among entomologists, chemists, engineers, and public health professionals. Such strategies are essential to address the increasing threat of leishmaniasis and enhance vector control efforts in both rural and urban settings

Reference

Overview of Personal Protection Measures Through the Innovative Use of Repellent-Textiles as Avant-Garde Disease Control Via Arthropods Nano-Tech-Repellents

Bio-mathematics, Statistics and Nano-Technologies: Mosquito Control Strategies DOI: 10.1201/9781003035992-3







Stakeholder Engagement for Sustainable Drug Development under the OneHealthDrugs Action

Authors: Costi M.P.^a, Zacharis T^b

^a University of Modena and Reggio Emilia, Italy; ^bbioGLOT Venture Greek Scientific Society

theo@greek-scientists-society.org

Pharmaceutical pollution has emerged as a silent but significant threat to environmental and public health, particularly in the context of vector-borne and parasitic diseases. With over 4,000 active pharmaceutical ingredients (APIs) circulating globally—many detected in water bodies and soils—current regulatory frameworks fail to address the long-term ecological impact of drug residues. The OneHealthDrugs (OHD) Action recognises this pressing challenge and is actively pursuing stakeholder engagement to catalyse regulatory and industry change across Europe.

Our engagement strategy focuses on three core objectives: integrating early-stage ecotoxicity screening into drug approval processes, ensuring the long-term sustainability of the OHD initiative, and building a cross-sector coalition involving policymakers, pharmaceutical companies, regulators, veterinarians, and environmental experts. We have initiated a structured roadmap leading to a high-level stakeholder meeting in Brussels, targeting key actors from the European Commission and leading pharmaceutical firms such as GSK, Novartis, and Bayer.

So far, we have developed a stakeholder matrix, initiated personalised outreach, and prepared engagement materials tailored to policy and industry interests. This process includes curated briefing notes, one-pagers, and policy Q&A sessions to align agendas and secure commitment. Our ultimate goal is to transition from voluntary practices to a mandatory, harmonised framework for ecotoxicity assessments, guided by AI-enabled predictive tools and green chemistry.

Looking ahead, we envision establishing a permanent OHD Policy Forum and releasing a white paper co-authored by our coalition. This will serve as a springboard for regulatory reform, funding mobilisation, and the mainstreaming of environmental responsibility in drug design. The OHD stakeholder initiative represents a model for science-policy-industry integration under the One Health paradigm.

References

- European Environment Agency (EEA, 2022) Pharmaceuticals in Europe's environment: Occurrence, impacts and policy options. <u>https://www.eea.europa.eu/en</u>
- [2] University of York (2022) Pharmaceutical pollution of the world's rivers: a global survey. <u>https://www.york.ac.uk/healthsciences/research/trials/ytutrialsandstudies/trials-pharmaceutical/</u>. WHO (2022) – Pharmaceuticals in drinking-water (updated global status report). <u>https://www.who.int/publications/i/item/9789240054987</u>
- [3] COST Action OneHealthdrugs, https://onehealthdrugs.com











Miltenyi Biotec

