**BOOK OF ABSTRACTS**

**Medicinal Chemistry and Structural Biology**

**2024-11-25**

DAY 1 (25/11): <https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1729135211346?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d>

Meeting ID: 315 238 710 568 Passcode: V5uNn8

**Exploring the Ubiquitin-Proteasome System for Structure-Based Design of Effective PROTACs targeting Trypanothione Reductase**

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Leishmaniasis is a vector-borne neglected disease with high morbidity and mortality rates, considered an emergent global health concern. The disease is endemic in tropical and subtropical areas but also in southern Europe, comprising up to 1.2 million cases each year. Currently used drugs possess several drawbacks, such as limited efficacy, toxic side-effects, and resistance development. All in all, this alarming scenario advocates for the development of new drugs. Trypanothione reductase (TR), a validated antileishmanial target, is a parasite-specific enzyme critical for antioxidant defense. The absence of TR in the host, its vital role for the parasite and the development of numerous TR inhibitors make this enzyme an attractive target for antileishmanial drugs. However, among the TR-inhibiting compounds reported so far, only few molecules possess adequate antiparasitic/inhibitory activity, mainly due to two aspects: i) survival of the parasites is affected when TR activity is reduced by more than 90%; ii) a large and featureless TR active site hampers efforts to develop effective inhibitors.

Recently, targeted protein degradation has emerged as an alternative strategy to traditional drug design. This methodology, which has proven its efficiency against viral infection and tumors, is based on the utilization of bifunctional molecules, namely PROTACs (PROteolysis Targeting Chimeras) that force the ubiquitination of a target protein yielding its proteasome-dependent degradation (1). We investigated the Ubiquitin Proteosome System in Leishmania to apply this new approach to tackle Leishmaniasis infection by engaging TR towards degradation. In this context, we rationally designed bifunctional PROTACs, we characterized the inhibition carried out on *Leishmania infantum* TR by a promising one, namely AP41, using X-ray crystallography and spectroscopic methods and we tested AP41 against *L. infantum* parasite.

**Reference:**

[1] Espinoza-Chávez RM et al. ACS Bio Med Chem Au. 2022 Dec 15;3(1):32-45.

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**Natural Products against Neglected Disesases**

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Research in my group, for the last 20 years or more, has had a major focus on the search for and characterization of new natural products (NPs) against neglected diseases. Some examples of interesting hits with antiprotozoal activity from various biosynthetic classes of NPs (e.g. sesquiterpenes, polyacetylenes, chromene derivatives, neolignans as well as, most recently, aminosteroids and aminotriterpenes) will be presented. Following our initial discovery [1], that certain sesquiterpene lactones (STLs) from plants of the family Asteraceae show very strong activity against protozoans, especially trypanosomatid parasites, we have tested, over the years, more than 130 chemically diverse STLs and studied their antitrypanosomal structure-activity relationships in detail [2]. The latest development in this long-term work is the discovery that STLs from *Arnica montana*, as constituents of Arnica tincture, can be used with very good efficacy to treat cutaneous Leishmaniasis in an animal model [3] and in human patients: The preparation is currently undergoing clinical studies in Colombia where it shows very promising results [4].

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**Acknowledgment**

The author acknowledges the many collaboration partners in the Research Network Natural Products against Neglected Diseases (ResNet NPND, see [www.resnetnpnd.org](http://www.resnetnpnd.org)). Particular thanks go to Swiss TPH, Allschwil, Switzerland, for the intense and fruitful cooperation over more than two decades and to Sara M. Robledo, Univ. of Antioquia, Medellin, Colombia, for the excellent collaboration with very successful in vivo and clinical work on Arnica. Many thanks to countless doctoral and master students who dedicated their work to the various projects. Thanks are due to various sponsors, particularly the Apothekerstiftung Westfalen-Lippe and Wilhelm-Doerenkamp-Foundation, for financial support.

**Novel scaffolds from African medicinal plants for antiparasitic drug discovery**

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Parasitic diseases have continued to be a significant global health burden, especially in Africa. As the search for effective drugs redirects towards natural products as a source of inspiration, African medicinal plants present a unique reservoir of novel scaffolds with antiparasitic potential due to their rich biodiversity. Integrating ethnobotanical knowledge, advanced analytical techniques, and computational modeling has helped to identify and evaluate promising natural scaffolds for drug development. It is essential to secure funding, encourage cross-disciplinary collaboration, and address challenges such as limited access to natural product resources, regulatory hurdles, and gaps in knowledge. Leveraging ethnobotanical insights, and integrating semi-synthetic approaches, and AI technologies can enhance the discovery process. Although natural product drug discovery presents challenges for the pharmaceutical industry and investors, its growing recognition as a cornerstone of healthcare underscores its importance. Africa's rich medicinal plant resources present an opportunity to explore new plants and sources, reposition natural products, and significantly contribute to developing novel treatments for diseases that continue to impact the continent.

**Investigation of bipyrazole derivatives**

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Cancer is difficult to cure and one of the most common causes of demise [1]. Nowadays, there are several ways to treat cancer, but more effective ways to do it are being sought [2]. Different scientific articles have widely described pyrazole derivatives, and their biological activity has been investigated. However, there is an aim to find more and more new pyrazole derivatives with better activity [3]. Pyrazoles are appealing to scientists due to their diverse biological properties: antiviral, anti-inflammatory, anticancer, and other biological properties [4, 5].

In the beginning, the original pyrazole derivative was converted to carbaldehyde according to the already known synthesis methodology, from which the hydrazone derivative was obtained. After synthesis conditions optimization, 3,4-bipyrazole was synthesized. Based on the diversity of biological properties of pyrazole and bipyrazole, it was decided to perform the functionalization of new bipyrazole compounds. For this, the very widely used palladiumcatalyzed Suzuki-Miyaura methodology was chosen for the functionalization of the pyrazole derivative. The resulting functionalized bipyrazole derivatives were investigated for their anticancer activity. Based on the obtained results, it can be stated that functionalized bipyrazole derivatives have good anticancer activity against different cancer cell lines.

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**Synthesis of substituted oxadiazoles and evaluation of anthelmintic properties**

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Heterocycles are a large and unique class of organic compounds with a wide variety of properties. Belonging to the same class, oxadiazole and its derivatives exhibit favorable physical, chemical, and pharmacokinetic properties such as an increased stability, relatively easy operation and broad biological application. [1,2] This knowledge encourages the search and creation of more analogous compounds preferably characterized by better cost and/ or atom economy, greater specificity, efficiency, and new exposure possibilities. [3]

The aim of the work is to synthesize a group of new oxadiazole compounds with different aromatic structural bases by applying the principles of alkylation reactions in the hope of obtaining compounds with biological properties directed to antihelmintic activity.

The research started with the preparation of oxadiazole thiol according to the known reaction conditions [4]. The further synthesized oxadiazole thiol was treated with benzyl halides and the target alkylated oxadiazole derivatives were obtained in good to excelent yields. Detailed spectroscopic studies confirmed the structures and high purity of the target compounds.

Afterwards, it was decided to evaluate the antihelmintic properties of the synthesized compounds for research using model nematodes *C. elegans*. Using the chitinase test, it was determined that two synthesized compounds had a greater influence on the development of the model nematode *C. elegans*, while others showed moderate activity.

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**Molecular Logic Gates Derived from Fluorescent Natural Products**

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Fluorescent natural products, numbering more than 300 known compounds, have been a source of fascination for centuries [1]. They are old molecules, which we believe are an untapped source for new concepts in the field of molecular logic-based computation [2]. The first molecular logic gate was designed and engineered in an organic laboratory [3], and ever since, so have many thousands of molecular logic gates. We will explain and highlight, however, that naturally occurring fluorescent molecular logic gates have existed long before the birth of molecular logic in 1993, and even long before the introduction of mathematical logic by George Boole in 1854 [4]. In fact, even though examples were in plain sight to the fluorescence chemical sensing community, they were overlooked [5]. In this presentation, we will highlight examples of natural products and polymeric natural product derivatives as optical logic-based molecules [6]. The *cinchona* alkaloids, including the classic anti-malarial drug quinine, are demonstrated as fluorescent INHIBIT logic gates and colorimetric AND logic gates [7,8].

A structure of a chemical formula

Description automatically generated

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**Acknowledgment**

The Authors acknowledge Xjenza Malta for the financial support, grant no. REP-2023-023.**High Throughput screening deselection of *Leishmania infantum* dihydrofolate reductase for highly selective pteridine reductase inhibitors**

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Pteridine reductase (PTR1) is a well-known target in antiparasitic drug discovery emerged from drug resistance studies in *Leishmania infantum* parasite [1]. As one of the important enzymes in the folate metabolic pathway, it is present only in the Trypanosmatidic parasites therefore it is considered a species-specific drug target. Medicinal chemistry approaches consider the parasitic dihydrofolate reductase (DHFR) enzyme as an optimal partner for PTR1 to achieve efficient antiparasitic activity. A combination of inhibitors targeting the two enzymes or a single inhibitor with dual inhibition activity of the same were considered as the best strategy to follow [2]. However, the mechanistic basis of the combined inhibition is still unclear because the interplay of PTR1-DHFR both of which use the same dihydrofolic acid (DHF) substrate. In order to address this, we proposed to identify PTR1 inhibitors inactive towards the parasitic DHFR to be used as a drug/probe for detailed molecular studies and/or a novel drug discovery program. To this end, a collaborative project with the European Lead Factory (ELF) supported by Innovative Medicine Initiative (IMI) was undertaken with the aim of developing *on target* high throughput screening led discovery of low nanomolar, *Leishmania infantum* PTR1 inhibitors with 103-104 selectivity index against DHFR [3]. This programme was performed following the ELF development track by including a progressive assays/molecular properties/full profile testing, validation and final re-synthesis of the selected hits. We successfully obtained compounds with the preferred profile. These are currently under evaluation for their antiparasitic activity.

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**Acknowledgment**

The Authors acknowledge the European Lead Factory and Lygature for the financial support of the programme. Individual ELF scientists have not been included as co-authors at this stage, but their contributions are acknowledged.

**Opportunities and challenges in the discovery of PROTACS for Vector-borne Parasitic Diseases**

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The development of innovative drugs is crucial to complement existing therapies and achieve control and elimination of vector-borne parasitic diseases. Targeted protein degradation (TPD) through small-molecule degraders is a promising new chemical modality for the development of novel anti-parasitic drugs. The most established approach to date is the design of small molecule proteolysis-targeting chimeras (PROTACs). PROTACs are bifunctional small molecules constituted by a protein of interest (POI) ligand and an E3 ligase ligand joined via a linker. As such, PROTACs harness the endogenous ubiquitin-proteasome system to induce ubiquitination and subsequent degradation of the protein of interest (POI) in contrast to conventional inhibitors.

TPD might offer several advantages, including the ability to target previously "undruggable" proteins and a catalytic mechanism that holds potential to overcome resistance in drug-resistant parasite strains. Additionally, PROTACs provide opportunities to "recycle" inhibitors from previous drug discovery efforts [1]. Despite these advantages, the field remains nascent and faces significant challenges. These include a limited understanding of parasite-specific protein degradation machinery, poor cell permeability of PROTACs, and potential host-side effects. Consequently, no PROTACs have yet been developed for parasitic diseases.

Nonetheless, we believe that innovative and unconventional approaches are vital to addressing these challenges. In this presentantion, we will share our research efforts aimed at developing the first PROTACs for leishmaniasis, which could represent the next-generation of anti-parasitic drugs.

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**Exploring the chemical space of small molecules targeting trypanothione reductase for parasitic diseases**

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Trypanothione reductase (TR), a key enzyme in parasite redox homeostasis, has emerged as a validated drug target for parasitic diseases. However, the development of effective small molecules targeting TR is challenging due to the enzyme's broad and featureless binding site.

In this presentation, we will give you an overview of different medicinal chemistry strategies that we have pursued over the years to develop novel TR small molecules modulators. These efforts have focused on designing compounds with diverse chemotypes and distinct modes of action, showcasing innovative approaches to target TR. We have applied the multi-target drug design strategy to design TR inhibitors endowed with an additional activity to obtain potentially more effective small molecules against *Trypanosoma* strains [1]. We have also exploited a fragment-based drug discovery approach to identify novel TR inhibitors targeting *Leishmania*. Using fragment merging, linking, and growing strategies, we successfully developed potent TR inhibitors [2]. Recently, we have harnessed the hydrophobic tag-based protein degradation modality [3], to develop potential TR-directed bifunctional degraders beyond PROTACs.

By exploiting the unique biology of trypanosomes and their reliance on trypanothione system, small molecules targeting this enzyme offer a promising strategy for addressing parasitic diseases caused by these parasites.

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**Acknowledgment**

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**Integration of Ecotoxicology in drug discovery**

**2024-11-26**

DAY 2 (26/11): <https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1729135292941?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d>

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**Characterisation of persistence, bioaccumulation and toxicity of biologically active compounds with deep learning-based methods**

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Assessing Persistence, Bioaccumulation, and Toxicity (PBT) is crucial for understanding the potential risk associated to the release of chemicals in the environment. While PBT screening remains challenging due to limited and low-quality data, it can be expressed through the PBT index using traditional QSPR models based on structural molecular descriptors.1 However, these models' accuracy decreases with large, heterogeneous datasets. This project aims to predict the PBT index of pharmaceutically relevant molecules using advanced deep learning (DL), specifically Chemprop, a message passing neural network (MPNN).2 We assembled a comprehensive dataset from public agencies, resulting in 5,129 molecules clearly labeled as PBT or non-PBT after removing ambiguous and duplicate compounds. The training set was used to train a binary classification model that assigns each compound a PBT or non-PBT label based on features extracted from its structure. Once trained, the model was used to make predictions on the test set. We further enhanced the robustness of our PBT prediction model by increasing structural dissimilarity between training set and test set molecules by means of a clustering strategy. When applied to the test set, the figures of merit show how the model achieved an accuracy of 0.90, and a recall rate of 0.92. Overall, this study highlights the potential of DL models for predicting PBT properties, thereby focusing on enhancing model’s generalizability. Moreover, using Chemprop’s built-in ‘interpret’ function,2 we are able to extract PBT-related significant substructures that are responsible for the PBT prediction in order to provide guarantees of model explainability for the PBT assessment. Lastly, a dataset of compounds of pharmaceutical interest will be tested to the proposed model in order to identify potential PBTs among pharmaceuticals defined as contaminants of emerging concern.3

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**Integrating Ecotoxicological Profiling and Computational Approaches for Sustainable**

**Drug Discovery Against Neglected Parasitic Diseases**

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Compounds prioritization in library from different sources represents one of the most important steps of a medicinal chemistry program. Selection & deselection of certain molecules may change the entire program result. This is so important that a huge work of predictive tools and assays have been proposed and used to this aim. Recently, additional parameters were included in the compound selection process, following the greener approaches. This allows more selective and predictively safer compounds to be identified. With this aim, at OneHealthdrugs we have started a metadata search of antiparasitic compounds highly activity against Trypanosoma, Leishmania, Schistosoma, and Babesia. The collected data included information on the presence or absence of specific molecular targets, IC50 and Ki values for these targets, phenotypic IC50, and selectivity against human cell lines. Structural information were also collected. These data were organized into a database developed within the framework of OHD, intended for future studies promoting the One Health concept and environmentally sustainable approaches. The primary objective was to identify scaffolds with antiparasitic activity and favorable ecotoxicological properties. One Health drugs against parasitic vector borne diseases in Europe and beyond OneHealthdrugs Cost Action CA21111 Two case studies were conducted to evaluate the integration of ecotoxicological parameters in compound selection. In the first case, a dataset of compounds derived from virtual screening study towards Leishmania infantum calpain1-4 was analyzed. ADMETLab 3.05 was used to calculate ADME and ecotoxicological properties, including bioconcentration factor (BCF) and hERG inhibition. These parameters, combined with molecular docking scores and MMGBSA ΔG Bind calculations, guided the selection of the most promising compounds. A similar workflow was applied to a second dataset of natural compounds active against Trypanosoma brucei identified during the data collection phase. Both datasets were further analyzed through fingerprint generation, Tanimoto similarity clustering, and scaffold decomposition to identify common structural features. This systematic approach underscores the value of integrating computational tools, ecotoxicological profiling, and structural analysis to advance sustainable drug discovery for neglected parasitic diseases.

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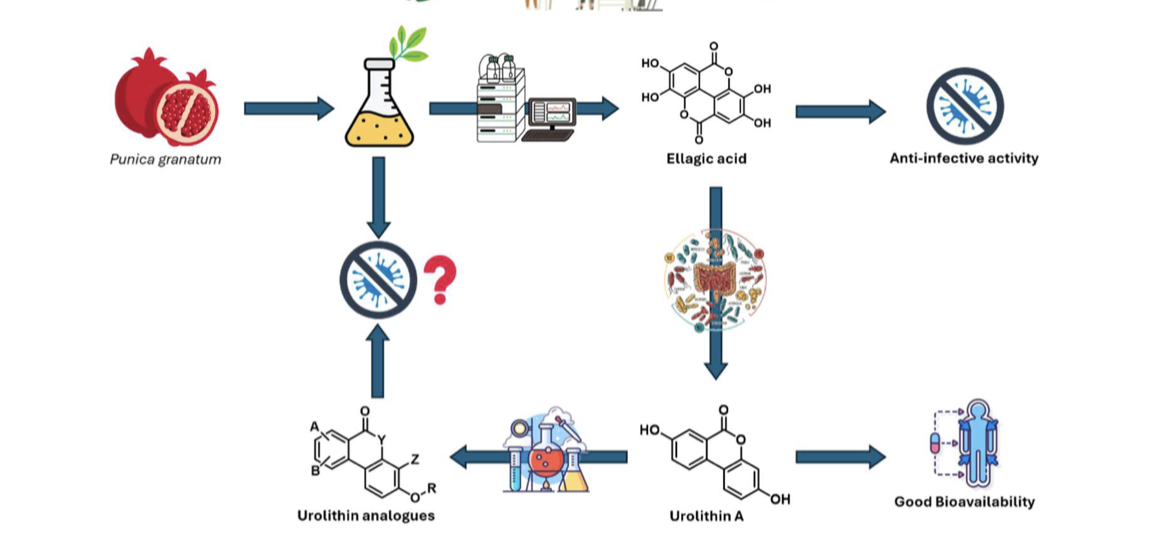
**Waste of Punica Granatum: a source of bioactive components to develop new antiinfective treatments**

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Recently, plant-sourced substances are gaining attention in drug discovery for many pathologies. In this landscape, Punica granatum proved beneficial effects against neglected infectious diseases (NIDs) such as L. donovani and L. infantum and Zika Virus (ZV) infection without evident toxicity1,2 . Ellagic Acid (EA), one of the major constituent of pomegranate extracts, showed antileishmanial activity on in vitro and in vivo models, suggesting that it could be accountable for such activity3 . Moreover, docking and molecular dynamics studies reveal that EA is able to potently inhibit ZV NS3-helicase, demonstrating the pomegranate antiviral effect4 . Based on this evidence, we decided to exploit eco-friendly and sustainable extraction methods to obtain extracts enriched with EA starting from pomegranate waste materials. Moreover, since metabolites of EA, namely urolithins, demonstrated to inhibit Leishmania proliferation5 , we decided to synthesise novel urolithin-derivatives in order to develop potent anti-infective agents. Herein we will discuss the chemical characterization of the extracts and the preliminary results as antibacterial.



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**Machine learning models for assessing toxicity of aquatic model organisms**

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Predictive models based on machine learning offer a promising framework for assessing the toxicity of chemical compounds for model organisms such as *Daphnia magna* and zebrafish (*Danio rerio*). These computational approaches address the limitations of traditional in vivo testing, which is time-consuming, resource-intensive, and often relies on animal testing, raising ethical concerns.

This study utilizes chemoinformatics to construct robust models that use molecular descriptors and toxicity datasets to predict the toxicity of compounds expressed as pLC50 or pEC50 values. Comprehensive datasets for *Daphnia magna* and zebrafish were curated from various sources covering pesticides, pharmaceuticals, and industrial chemicals. Molecular descriptors, including 1D, 2D, and 3D traits, were calculated using tools such as RDKit, Dragon, and ISIDA fragments. Feature selection methods identified the most relevant descriptors, while hierarchical clustering enhanced dataset diversity for *Daphnia magna*. Random Forest (RF) models were developed and validated for regression and classification tasks. Zebrafish developmental toxicity was assessed over 48- and 120-hour intervals, with additional classification into toxic and non-toxic categories.

The models demonstrated moderate to high predictive power, with regression R² values ranging from 0.394 to 0.850 and classification metrics achieving balanced accuracy above 0.75 in external validations. This study underscores the utility of combining diverse molecular descriptors and machine learning techniques to predict toxicity across structurally varied compounds. It also highlights the need for refining descriptor sets and exploring alternative machine learning algorithms to further improve performance.

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**Green chemistry strategies for vector-borne parasitic disease drug discovery: design, synthesis and biological evaluation of cashew nut shell liquid derivatives**

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The use of biowaste in medicinal chemistry for developing new drug candidates has gained significant attention, particularly in the context of vector-borne parasitic diseases (VBPD). Cashew nutshell liquid (CNSL), derived as a by-product during the processing of cashew nuts (*Anacardium occidentale*), is an inedible oil rich in phenolic compounds such as cardanols, cardols, 2-methylcardols, and anacardic acids. These compounds share a pentadecyl alkyl side chain with varying degrees of unsaturation. Despite CNSL’s potential as a source of drug precursors, its transformation into value-added small molecules remains underexplored. While CNSL components exhibit intrinsic biological activity, they are not potent enough to serve as standalone drug candidates.

Interestingly, CNSL and its derivatives have demonstrated potential in the treatment of VBPDs, which are a major global health concern due to their prevalence and lethality in regions such as Asia, Africa, the Americas, and the Mediterranean basin. The need for novel and effective treatments is critical, as current pharmacological options are inadequate.

In response to this challenge, our research group has investigated the use of CNSL as a sustainable starting material for developing antiparasitic compounds. Employing environmentally friendly synthetic approaches, we synthesized a new series of semi-synthetic CNSL derivatives using green chemistry principles. These derivatives were further modified by incorporating lipophilic cations, such as triphenylphosphonium (TPP) salts, leveraging a mitochondrion-targeting strategy known to enhance drug selectivity and cidal activity against parasites.

The synthesized compounds underwent rigorous screening against major human and animal VBPD pathogens, including *Trypanosoma spp.* and *Leishmania spp.*, demonstrating promising in vitro and ex vivo activity. Notably, the phosphonium salt derivatives exhibited significant antiparasitic effects with low in vitro toxicity (selectivity index, SI > 1000). Ongoing studies are focused on elucidating the compounds’ modes of action. These findings highlight the potential of CNSL derivatives as sustainable and effective candidates for addressing the urgent need for new VBPD treatments.

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**PARTICIPATORY AND MOLECULAR SURVEILLANCE OF TICK-**

**BORNE THEILERIOSIS, AND ANAPLASMOSIS IN DISTRICT BAHAWALPUR, PUNJAB, PAKISTAN**

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Ticks (Acari: Ixodidae) are vicious –blood-sucking ectoparasites of livestock extensively found around the globe and cause significant economic losses. They vector a higher range of important pathogenic agents including Theileria spp. and Anaplasma spp. The aim of present study is to specify the molecular epidemiology and its associated risk factors of two well-distributed tick- borne protozoa and bacteria Theileria spp. and Anaplasma spp. in cattle population from Bahawalpur, Punjab, Pakistan. Blood and ticks samples were collected from local breed cattle,crossbreed, cholistani and from exotic breed with total of 384 blood samples and four species of ticks. Genus-specific PCR assays were performed to detect the presence of Anaplasma spp. And Theileria spp. based on 16S rRNA and MPSP makers. PCR results showed that Anaplasma was 17% prevalent and Theileria was 25.9 % prevalent in the study area with comorbidities prevalence rate was 25% in the tested blood samples. Univarable analysis of risk factors showed that only breed and acaricidal treatment were significant determinants (P &lt; 0.05) for these comorbidities, however, in case of Theileria spp. and Anaplasma spp. ,breed, age, gender,grazing practice, season, presence of poultry on farm and acaricidal treatment were potential determinants (P &lt; 0.05). Multivariable analysis specified that breed and acaricidal treatment were considered as significant risk factors for these infections (P &lt; 0.05) whereas acaricidal treatment was found to be a significant determinant for comorbidities (P &lt; 0.05). Phylogenetic analysis indicated that Anaplasma spp. and Theileria spp. isolates showed similarities and shared phylogeny with same isolates reported from Asia, China, Europe and Japan. This is the first molecular report on the epidemiology and risk factors analysis of Anaplasma spp. and Theileria spp. infections in cattle population from Bahawalpur, Pakistan. Further large-scale study is required to investigate molecular, epidemiological and genotypic aspects as well as potential risk factors analysis from the country to facilitate designing strategies to control tick-borne pathogen and reduce losses to cattle industry.

Key Words: PCR (Polymerase Chain Reaction), Comorbidities, Phylogenetic, Epidemiology

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and Ixodid ticks”

**ASP3a/b - the promising druggable plasmepsin IX/X analogues from Babesia parasites**

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Apicomplexan parasites use a cascade of specific proteolytic enzymes driving the function of the apical complex during egress and invasion of host cells. These processes are critical for propagation of *Babesia* through the asexual cycle in host erythrocytes causing for babesiosis. Analysis of *Babesia* omics datasets, leveraging knowledge from *Toxoplasma* and *Plasmodium*, identified two clade-C *Babesia divergens* aspartyl proteases, homologous to *Plasmodium falciparum* plasmepsins IX/X (*Pf*PMIX/X) and *Toxoplasma gondii* *Tg*ASP3, designated *Bd*ASP3a/b. These are considered key drivers of apical complex processing associated with egress and invasion of host erythrocytes. Expression profiling across the *B. divergens* lifecycle revealed *Bd*ASP3a/b presence in blood stages but, in contrast with malarial *Pf*PMX, not in tick/vector stages. This indicates unique coevolutionary adaptations of piroplasms to the tick bloodfeeding behavior. *Bd*ASP3s were expressed as active enzymes in bacterial and baculovirus-infected insect cells. Immunomicroscopy utilizing gained polyclonal antibodies localized BdASP3a to apical complex associated organelles of intraerythrocytic *B. divergens*. Active recombinant BdASP3s were used to determine enzyme kinetics with *P. falciparum*-derived fluorescent substrates, and to confirm enzyme inhibition by 49C, the hydroxyethylamine inhibitor *of Pf*PMX/IX. Its addition to RBC-cultured *B. divergens* hinted *Bd*ASP3a/b roles in invasion rather than egress evidenced by the accumulation of free merozoites. Trans-genera complementation with iKD-*Tg*ASP3 *T. gondii* strain suggested *Bd*ASP3s' involvement in protein maturation, mirroring *Tg*ASP3's secretory pathway but not its deleterious phenotype, indicating species-specific functions. Importantly, our use of conventional and conditional gene KO/KD is instrumental in deciphering the molecular phenotypes by proteomic analyses. These strategies are crucial for confirming the indispensable roles of BdASP3a/b and for their validation as therapeutical targets. In conclusion, in line with their malarial orthologues, the two *Bd*ASP3enzymesplay analogous yet not completely identical role in *Babesia* propagation and represent druggable proteolytic targets for the development of the yet missing *Babesia*-specific chemotherapy.

Obsah obrázku venku, obloha, osoba, Lidská tvář

Popis byl vytvořen automaticky

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**Parasitology and pharmacology**

**2024-11-27**

DAY3: <https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1729135378158?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d>

Meeting ID: 389 260 837 739 Passcode: 72oEXE

**Recent Structural Optimizations of Pyrimido[5,4-d]pyrimidines Exhibiting Antileishmanial Activity**

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Leishmaniasis, a neglected tropical disease caused by vector-borne protozoan parasites1, represents an increasing health challenge in developing nations. Current treatments are hindered by poor efficacy, severe side effects, and increasing drug resistance2, underscoring the urgent need for new therapeutic options. Recently, pyrimidopyrimidine-based compounds have emerged as promising candidates against *Leishmania* parasites3,4.

In this study, we optimised new pyrimidopyrimidine-based derivatives for activity against *L. infantum* promastigotes and intracellular amastigotes. Cytotoxicity was assessed using the THP1 cell line, and early ADME-Tox studies were performed on selected compounds. The two most potent derivatives demonstrated no toxicity against THP1 cell line, exhibited anti-amastigote IC50 values below 5 µM and selective indices greater than 32. Early *in vitro* ADME-Tox evaluations revealed a favourable safety profile for these compounds.

The biological results and structure-activity relationship (SAR) analyses will be presented and discussed. Our findings highlight a promising hit compound that can be further optimized to enhance solubility and improve the selectivity index, paving the way for more effective and safer treatments for leishmaniasis.

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**Investigation of the bifunctional enzyme dihydrofolate reductase-thymidylate synthase (DHFR-TS) from *Leishmania major***

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Leishmaniasis is a tropical disease caused by a vector-borne, obligate intracellular protozoan parasite of the genus *Leishmania* [1]. Dihydrofolate reductase thymidylate synthase (DHFR-TS) is a bifunctional enzyme that exists as a single unit in *Leishmania* parasites and is vital in folate biosynthesis. The folate cycle is a vital process that enables the parasite to produce nucleotides and folate necessary for DNA and RNA synthesis, and for cellular growth [2]. Thus the activity of DHFR-TS is necessary for parasite survival, making this enzyme a promising target for new treatment approaches. The objective of the present study is to produce, purify and characterize the bifunctional enzyme DHFR-TS of Leishmania major and to perform functional studies on its isolated domains. For this purpose the pET-15b-TEV-*Lm*DHFR-TS and pET-15b-TEV-*Lm*DHFR plasmids were employed for the production of wild-type enzymes in *E. coli* ArcticExpress (DE3) cells. The enzymes were purified using nickel-affinity and size exclusion chromatography. Five different mutants were generated by site-directed mutagenesis in the *DHFR* active site of both the bifunctional enzyme and the isolated *Lm*DHFRdomain to investigate their functional role in the catalyzed reaction. The production and kinetic characterization of the point mutant enzymes will be performed in accordance with the protocols established for the parent wild-type proteins. This study will facilitate a deeper understanding of the functional roles of mutations in the DHFR active site in the catalysis reaction and provide insights for designing more effective selective inhibitors.

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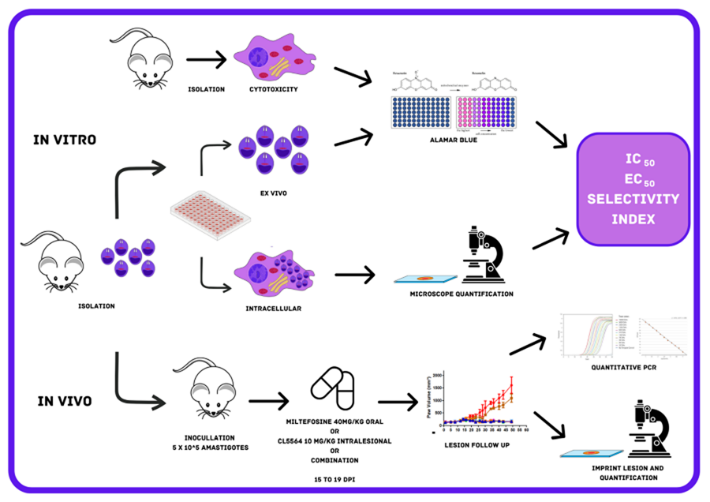
**A drug discovery journey: exploring N6-Methyltubercidin as an alternative antileishmanial drug treatment in a cutaneous Leishmania amazonensis mouse model**

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Cutaneous leishmaniasis is a neglected tropical disease caused by the intracellular protozoan parasite *Leishmania*. It is transmitted when female sand flies bite mammalian hosts (e.g. humans), to obtain a bloodmeal. The disease mainly occurs in (sub)tropical areas but is spreading to previously unaffected areas. Currently, there are a few antileishmanial drug treatments available, e.g. antimonials, amphotericin B, miltefosine and paromomycin. However, these drugs have multiple practical shortcomings, their mode of action is unknown or poorly understood, or they face the development of drug resistance. Therefore, it is essential to develop new antileishmanial drug treatments. Nucleoside analogues are considered promising alternatives. Since *Leishmania* parasites are unable to synthesise their own purines *de novo*, they rely completely on salvaging purines from the host. This process provides interesting targets for drug discovery of new antileishmanial compounds. In this project, we explored the *in vitro* and *in vivo* activity of N6-methyltubercidin (CL5564), against *L. amazonensis*, after it demonstrated potent activity against *Trypanosoma cruzi* and *L. infantum*. With selectivity indices of 278 and 43, respectively, CL5564 was 6.5 -fold (p = 0.0002) more potent than miltefosine against intracellular amastigote forms in peritoneal mouse macrophages. Combination treatment of CL5564 and miltefosine on *ex vivo* amastigotes resulted in an additive effect. These results stimulated us to study the activity of CL5564 in a mouse model of cutaneous *Leishmania* infection: BALB/c female and male mice infected by *L. amazonensis* and treated with CL5564 (10 mg/kg, intralesional, five days) presented a >93% reduction of paw lesion size, similar to MilteforanTM given orally at 40 mg/kg, while the combination (10 + 40 mg/kg of CL5564 and MilteforanTM, respectively) caused >96% reduction. The qPCR data confirmed the suppression of parasite load after treatment, but only the combination approach reached 66% of parasitological cure. These results warrant additional studies with nucleoside derivatives.



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**Robotic Workflow for Antiparasitic Screening: Streamlined Cell Culture and Data Analysis in Pharmacology**

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The critical need for new antiparasitic medications is highlighted by the rising incidence of parasitic infections and the growing resistance to current treatments. In pharmacology, high-throughput screening (HTS) has become essential for quickly identifying viable drug candidates, and robotics advancements have made this field possible with previously unheard-of efficiency. This work introduces a completely automated robotic approach that combines high-throughput plate manufacture, accurate medication administration, and thorough data analysis to expedite cell culture procedures and antiparasitic screening. The method lowers human error and resource consumption by enabling quick, repeatable cell culture preparation and guaranteeing constant conditions across hundreds of assay plates.

Our method improves the accuracy of dose-response studies by automating crucial steps in antiparasitic screening, such as chemical dispensing, media change, and sample processing. Following drug exposure, substances with high antiparasitic activity are identified through real-time data gathering and analysis using machine learning algorithms. Through the prediction of structure-activity relationships (SAR) and the optimisation of compounds for future development, these data-driven insights expedite the identification of possible leads. Furthermore, this method gives researchers the consistency and accuracy to monitor parasite inhibition rates and cell survival that would be difficult to accomplish by hand.

The results show that robotic automation greatly enhances throughput and quality control in antiparasitic pharmacology. This makes it possible to evaluate a larger chemical library more quickly than with manual methods.

This study creates a scalable paradigm for antiparasitic drug discovery by fusing robotic accuracy with sophisticated data analytics, which could hasten the creation of efficient medicines for parasitic illnesses. Our results highlight how crucial it is to combine robotics and computer analysis in pharmacology to satisfy the increasing need for innovative antiparasitic treatments in the field of global health.

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**The role of NOD2 in macrophage activation and its therapeutic targeting in *Leishmania* infections**

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Effective pathogen recognition by innate immune cells, particularly macrophages, is vital for initiating inflammatory responses and microbicidal activities1. Among pattern recognition receptors (PRRs), the intracellular NOD-like receptor (NLR) NOD2 is pivotal in detecting muramyl dipeptide (MDP) from bacterial peptidoglycan2. Emerging evidence suggests that NOD2 also plays a critical role in responses to non-bacterial pathogens, including protozoan parasites3,4. *Leishmania* species, such as *Leishmania infantum* and *L. tropica*, which cause visceral and cutaneous leishmaniasis, respectively, interact with host PRRs to manipulate immune responses5. However, the role of NOD2 in recognizing *Leishmania* spp. and mediating inflammatory responses to control these infections remains underexplored. In this study, we used bone marrow-derived macrophages (BMDMs) from NOD2 knockout (KO) mice, as well as BMDMs deficient in RIP2 and CARD9 (two key adaptor proteins in the NOD2 signaling pathway), to assess the role of NOD2 in macrophage activation during *Leishmania* infection. We found that genetic ablation of NOD2, RIP2, or CARD9 significantly impaired nitric oxide (NO) production, inducible nitric oxide synthase (iNOS) expression, and the release of pro-inflammatory cytokine TNF-α in response to infection. Additionally, we employed GSK7176, a specific pharmacological inhibitor of NOD2, to complement our genetic approach. Inhibition of NOD2 in wild-type BMDMs led to an increase in intracellular amastigote burden and a reduction in both NO production and iNOS expression, corroborating the findings from the NOD2 KO models. This pharmacological validation highlights the therapeutic potential of targeting NOD2 in enhancing macrophage activity against *Leishmania* infections. Moreover, MDP exhibited immunomodulatory properties by enhancing NOD2-mediated responses, significantly boosting TNF-α and iNOS levels when combined with *L. infantum*. Our findings suggest that NOD2 represents a promising therapeutic target against these parasites, and the use of MDP to augment anti-*Leishmania* immune responses could become a novel immunomodulatory strategy.



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**Investigation of the bifunctional enzyme dihydrofolate reductase-thymidylate synthase (DHFR-TS) from *Leishmania major***

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Leishmaniasis is a tropical disease caused by a vector-borne, obligate intracellular protozoan parasite of the genus *Leishmania* [1]. Dihydrofolate reductase thymidylate synthase (DHFR-TS) is a bifunctional enzyme that exists as a single unit in *Leishmania* parasites and is vital in folate biosynthesis. The folate cycle is a vital process that enables the parasite to produce nucleotides and folate necessary for DNA and RNA synthesis, and for cellular growth [2]. Thus the activity of DHFR-TS is necessary for parasite survival, making this enzyme a promising target for new treatment approaches. The objective of the present study is to produce, purify and characterize the bifunctional enzyme DHFR-TS of Leishmania major and to perform functional studies on its isolated domains. For this purpose the pET-15b-TEV-*Lm*DHFR-TS and pET-15b-TEV-*Lm*DHFR plasmids were employed for the production of wild-type enzymes in *E. coli* ArcticExpress (DE3) cells. The enzymes were purified using nickel-affinity and size exclusion chromatography. Five different mutants were generated by site-directed mutagenesis in the *DHFR* active site of both the bifunctional enzyme and the isolated *Lm*DHFRdomain to investigate their functional role in the catalyzed reaction. The production and kinetic characterization of the point mutant enzymes will be performed in accordance with the protocols established for the parent wild-type proteins. This study will facilitate a deeper understanding of the functional roles of mutations in the DHFR active site in the catalysis reaction and provide insights for designing more effective selective inhibitors.

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**Synergistic inhibition of Plasmodium’s vital proteins: A multi-target strategy using Buxus sempervirens**

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The emergence of antimalarial resistance in Plasmodium falciparum poses a critical global health challenge, necessitating the development of novel therapeutic approaches. This study employs an integrated approach combining structure-based drug design and machine learning to identify potential antimalarial agents from Buxus sempervirens. A total of 82 bioactive compounds were screened against essential parasitic proteins including Falcipain-2, Plasmepsin II, and Farnesyl Transferase, with their inhibitory activities predicted through machine learning models. Molecular docking analysis revealed Bebeerine as a potent inhibitor of Farnesyl Transferase, displaying a docking score of -9.5 and IC50 of 386.47 nM (pIC50 6.41). (+)-Buxaquamarine demonstrated strong binding to Plasmepsin II (docking score -5.6), while Buxenone showed significant inhibition (IC50 3425.74 nM, pIC50 5.47). Against Falcipain 2, (+)-Buxoxybenzamine exhibited promising inhibition (IC50 3579.35 nM, pIC50 5.45). ADMET profiling indicated favorable drug-like properties across all compounds, including high intestinal absorption (>87%) and strong plasma protein binding, with Bebeerine and Isochondrodendrine specifically showing no hepatotoxicity. All compounds satisfied Lipinski's Rule of Five, supporting their potential as drug candidates. These findings highlight Bebeerine and (+)-Buxoxybenzamine as promising leads for antimalarial drug development, warranting further experimental validation against resistant P. falciparum strains.

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**TOWARDS KNOWING YOUR TARGET IN LEISHMANIA: ESTABLISHMENT OF VALIDATED IN SITU ASSAYS FOR ANTILEISHMANIAL DRUG DISCOVERY**

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Antileishmanial drug discovery has witnessed significant progress over the last two decades empowered by advanced functional genomic technologies. However, the high attrition rate of compounds in clinical development emphasizes the need to explore new target-validated antileishmanial leads. To meet the “ Know your Target” principle of drug discovery, we engineered *Leishmania* lines to investigate the mode-of-action and activity of new antileishmanial compounds. In *Leishmania*, like any eukaryotic cell, spatiotemporal molecule trafficking between subcellular organelles is maintained by endosomes, while active shuffling of molecules between the cytoplasm and nucleus relies on dynamic nucleocytoplasmic transport.

First, by elaborate mode-of-action studies of antileishmanial aminopyrazoles, we discovered that disrupting the assembly of endosomes is an exploitable and druggable pathway. MUT-SEQ and CRISPR-Cas9 gene editing has independently confirmed an association between 10-30-fold aminopyrazole resistance and multiple independent heterozygous mutations in the FYVE containing protein LINF\_180011100. Next, genetic fusion with an N-terminal green fluorescent protein (GFP) tag demonstrated that the protein primarily localizes in cytoplasmic/endocytic vesicles and an impact of treatment with aminopyrazoles. Proteomic analysis of co-immunoprecipitates with the GFP tagged LINF\_180011100, confirmed the interaction with recycling endosomes that are associated with the ribosomal translation machinery and mitochondria.

Our second initiative was to visualize and target the Nuclear Protein Import (NPI). NPI relies on interactions between cargo molecules carrying a nuclear localization signal (NLS) and their specific transport receptors, regulated in turn by a Ran GTPase cycle. Using genetic engineering, a pLEXSY vector encoding the mCherry fluorescent protein with a C-terminal NLS, was introduced into the *L. infantum* genome. Confocal fluorescence microscopy demonstrated parasites with a nuclear localisation of mCherry. As a specific inhibitor of importin-β, importazole exhibited the anticipated dose-dependent inhibition of NPI as well as a broad antiprotozoal activity. Next, assay specificity was demonstrated by evaluating the effect of lead compounds of the Drugs for Neglected Diseases initiative with different modes-of-action, revealing cell death without NPI impairment.

Collectively, our transgenic *Leishmania* lines can provide an *in situ* read-out of endosomal assembly or nuclear protein transport. In addition to offering opportunities for mechanistically informed drug discovery, this also provides an innovative research tool for understanding the *Leishmania* cell biology.

**Immunomodulatory effects of *Helicobacter pylori* Neutrophil Activating Protein (HP-NAP) on human and canine macrophages infected with *Leishmania infantum***

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Macrophages play a central role in *Leishmania* infection, serving as the primary host cells for the parasites while also acting as key effector cells in their elimination through the production of mediators such as nitric oxide and reactive oxygen species. *Helicobacter pylori* Neutrophil-Activating Protein (HP-NAP) is a bacterial protein with pro-inflammatory and immunomodulatory properties. HP-NAP has been shown to stimulate neutrophils to produce reactive oxygen species and inflammatory cytokines (1). However, its role in macrophage activation against *Leishmania* remains unexplored. This study aimed to evaluate the in vitro effects of HP-NAP on *Leishmania infantum*-infected macrophages.

Human and canine monocytes were isolated from blood samples collected from healthy donors. Monocytes were differentiated into macrophages as described (2,3). Macrophages were then infected with stationary-phase promastigotes of *L. infantum* at a promastigote-to-cell ratio of 10:1 for 24 hours. Following infection, macrophages were treated with HP-NAP (from 20 to 1.25 µg/mL) for 72 hours. At the end of the incubation, the percentage of infected macrophages and the mean number of amastigotes per cell were determined. Cytokine production by *L. infantum*-infected macrophages treated with HP-NAP was assessed in the supernatants using ELISA.

Both canine and human macrophages efficiently phagocytized *L. infantum* promastigotes. After 24 hours of infection, canine macrophages exhibited an infection rate of 82±16% and a mean of 7.4±2 amastigotes per cell. Treatment with 20 µg/mL HP-NAP reduced these values to 68±14% and 4.8±0.9, respectively. A similar trend was observed in human macrophages, with initial values of 68.6±2.1% infected cells and 16±3.1 amastigotes per cell, which decreased to 53.3±0.4% and 9.9±0.02 following HP-NAP treatment (20 µg/mL). HP-NAP induced IL-12, a key cytokine for anti-leishmanial Th1 responses, in infected canine macrophages, while IL-10 remained undetectable under all conditions.

These findings indicate that HP-NAP can inhibit parasite growth and modulate cytokine production, supporting its potential as an immunomodulatory strategy.

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**Plasmodione antimalarial activity is partially mediated by the NADH dehydrogenase PfNDH2**

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Plasmodione and several derivatives of the 3-benzylmenadione (bMD) family, have strong antimalarial activity at low nanomolar concentrations, against both asexual and sexual forms of *Plasmodium* parasites. Hence, they have both curative and transmission-blocking properties. Its mode of action is based on the redox cycling of bMD metabolites with flavoenzymes in infected red blood cells (iRBCs), depleting parasites from NA(D)PH and producing oxidative stress, and ultimately leading to parasite death. Using drug-adapted parasites, we identified three independent mutations in the same domain of the NADH dehydrogenase PfNDH2. We validated the role of PfNDH2 in bMD mode of action using both genetically modified *Plasmodium* parasites and yeast as a model. These data will be discussed during the presentation.

**Π-Π stacking stabilized polymeric micelles for hydrophobic drug delivery in**

**the treatment of leishmaniasis**

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Current strategies to control leishmaniases – a group of diseases caused by *Leishmania* parasites – rely on chemotherapy, which is associated with significant drawbacks, including severe side effects and limited efficacy. The public health impact of these diseases underscores the urgent need for new therapeutic approaches. A family of compounds, the quinoline-(1*H*)-imines, have emerged as promising lead compounds for antileishmanial drug development [1]. However, their poor aqueous solubility poses a significant challenge. In the present study, quinoline-(1*H*)-imines were encapsulated into polymeric micelles (PMs) composed of block copolymer of methoxy poly(ethylene glycol)-*b*-poly(N-2-benzoyloxypropyl methacrylamide) [mPEG-*b*-p(HPMAm-Bz)]. These PMs are known to establish noncovalent π-π stacking interactions between the aromatic rings present in the p(HPMAm-Bz) block and the drug molecules, to enhance solubility. Encapsulation of quinoline-(1*H*)-imines resulted in comparable efficacy to the free drugs against *Leishmania* parasites, while significantly reducing toxicity toward primary macrophages *in vitro*. In a murine model of visceral leishmaniasis, the micellar formulation facilitated targeted drug accumulation in organs typically affected by the disease, as the liver, spleen and bone marrow. In contrast, the free drug was undetectable or present in markedly lower concentrations after 24 hours. These findings highlight the potential of mPEG-*b*-p(HPMAm-Bz) micelles as effective delivery systems for quinoline-(1*H*)-imines and potentially other antileishmanial agents. Nevertheless, further studies are needed to optimize micellar stability and dosing regimens to fully harness their therapeutic potential.

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**YRI presentations + STSM presentations & @13:00 Awards**

**2024-11-28**

DAY4: <https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1729135479241?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d>

Meeting ID: 343 891 093 619 Passcode: ELxcww

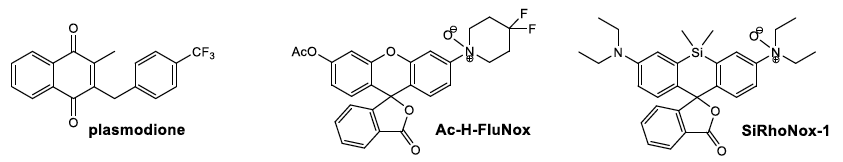
**Imaging of Fe2+ gradients as a ferroptosis marker in malaria parasites using a fluorogenic labile heme reporters**

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Plasmodione (Figure 1), is an early lead antimalarial redox-active agent acting as a prodrug. Its selectivity against malaria is due to a specific bioactivation process that generates a 3-benzoylmenadione metabolite (plasmodione oxide). This metabolite acts as a substrate for flavoenzymes, through a cascade of redox reactions that produces harmful oxidative metabolites, ultimately leading to parasite death, likely through ferroptosis. Ferroptosis is a form of programmed cell death dependent on labile heme-bound iron (Fe²⁺) accumulation and lipid peroxidation. The aim of this study was to investigate whether plasmodione and other antimalarial analogues induce ferroptosis. The fluorogenic probes Ac-H-FluNox to detect ferrous labile heme (LH) [1] and SiRhoNox-1 for total Fe²⁺ species [2] (Figure 1), were specifically detected in infected red blood cells (iRBCs), and not in uninfected red blood cells (niRBCs). Si-RhoNox produced a stronger signal in treated iRBCs as compared to Ac-FluNox, upon plasmodione treatment producing a stronger signal than erastin. These findings suggest that Si-RhoNox may serve as a useful tool for detecting Fe²⁺ changes upon drug treatment in iRBCs. Further experiments with Si-RhoNox and using other ferroptosis reporter are necessary to validate this hypothesis.



**Figure 1**. Chemical structure of plasmodione (left) and of the two fluorogenic Fe2+ reporters (Ac-H-FluNox for labile heme (Fe2+) and SiRhoNox-1 for total labile Fe2+)

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**Revealing the Mechanism of Action of the innovative antileishmanial agent H80 through fractionated MS Proteomics, untargeted metabolomics, and fluorescence imaging**

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*Leishmania infantum* is a parasite responsible for zoonotic leishmaniases, affecting humans and dogs, particularly in the Mediterranean Basin, the Middle East, and Central Asia. The disease manifests as cutaneous leishmaniasis (CL) or visceral leishmaniasis (VL), with VL potentially being fatal if untreated, often due to complications like hemorrhage or infections, especially in HIV co-infected patients. Current treatments include amphotericin B, miltefosine, and antimonials, though these options are limited by toxicity, cost, and increasing drug resistance [1]. To this aim, MS based omics technologies, in particular proteomics and metabolomics, represent key tools to be integrated in the modern Medicinal Chemistry tools to speed up the development of innovative antimicrobial agents able to overcome drug resistance issues.

Drug resistance in *L. infantum* arises through mechanisms such as reduced drug uptake, enhanced efflux pumps, mutations in drug targets, and metabolic adaptations, which compromise the efficacy of existing treatments. To address these challenges, a novel benzothiophene-flavonol derivative, **H80**, has been identified [2]. This compound exhibits broad-spectrum anti-leishmanial activity, comparable to miltefosine but with lower toxicity [3]. By combining fluorescence spectroscopy, MS proteomics and untargeted metabolomics, researchers have explored H80's subcellular effects and its potential mechanism of action, aiming to clarify its biochemical impact and accelerate drug discovery efforts. By whole cell, fractionated MS-Proteomics, we gained information about the differentially expressed proteins induced bt H80 administration, including the main cell compartments (cytosol, mitochondria, and membranes) in which H80 action and metabolic response take place. As matter of facts, while Miltefosine proved that its main action is expleted in membranaceuos compartment, which is consistent with its lipidic mechanism of action, **H80** mainly acts at the cytosolic level, with a specific affection of the energetic metabolism and TCA cycle. Fluorescence internalization assays were also conducted on THP-1 cells infected with *L. infantum* promastigotes, and revealed that captation mechanism takes place with endocytosis, and its accumulation in the cytosolic compartment. Moreover, untargeted MS Metabolomics data currently under analysis will help to better understand H80 mechanism of action and off-target effect, to provide a complete target engagement analysis to be exploited in lead optimization studies.

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**Molecular docking study on antiparasitic nucleoside drugs**

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New drugs against trypanosomiasis and leishmaniasis are urgently needed, including for veterinary applications, and any new drugs will have OneHealth implications. Adenosine analogues have shown great promise against both human and veterinary *Trypanosoma* species, but have generally displayed less activity against *Leishmania* species. Screening with a *Leishmania mexicana* cell line defective in adenosine kinase (ADK) shows that the activity of the most potent analogues is dependent on this enzyme, and that activity can be restored and even improved by the experession of *T. brucei* ADK. Thus, differences in the ADKs of the two species may help explain the differences in antiparasitic activity against the different species, and potentially the high level of specificity regarding mammalian cells as well, Leishmania ADK being substantially different from the equivalent in other eukaryotes [1].

Here, we present results from *in silico* molecular modeling based on docking for predicting binding poses of antiparasitic nucleoside drugs. The tested hypothesis is whether nucleoside analogues are phosphorylated more easily by *Tb*ADK than by *Leishmania mexicana* (*Lmx*ADK). Differences in geometry of active site and ligands conformation were observed, which impact differences in predicted score. The best docking score and the most efficient adenosine analogue in vitro, tubercidin, exerts different patterns of molecular interaction with ligand and amino acid residues in the binding sites of *Lmx*ADK and *Tb*ADK. Optimising SAR for efficient recognition by the ADKs and adenosine transporters of both *Leishmania* and *Trypanosoma* have potential to develop drugs with broad anti-trypanosomatid activity.

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**Synthesis and biological evaluation of new antiparasitic 4-thiazolidinone bioisosters of alkylphosphocholines**

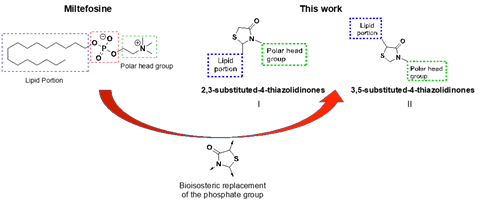
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Leishmaniasis and Human African Trypanosomiasis (HAT) are neglected tropical diseases (NTDs) impacting the world’s poorest populations in the old and the new world [1]. Current treatments are unsatisfactory, presenting several limitations, including high toxicity, lack of efficacy, and emerging drug resistance [2].

Aiming to enrich the drug pipeline for NTDs with novel chemical entities in this work, we designed and synthesized 41 novel miltefosine bioisosters using a multicomponent reaction methodology in which the key phosphate moiety was replaced by its bioisoster heterocycle 4-thiazolidinone, while, a trimethylammonioethyl or N-methylpiperidiniumethyl or N-methylmorpholiniumethyl group was attached at N3, mimicking the choline head group (Fig. 1).



**Figure 1.** Design of the phosphate bioisosteres of the present study.

The compounds were then evaluated for their *in vitro* antiparasitic activity against promastigotes and intracellular amastigotes of *Leishmania infantum*, *Trypanosoma brucei* bloodstream forms and for toxicity against THP1 cells. Six compounds were potent antileishmanial agents, 5 derivatives possessed potent antitrypanosomal activity against *T. brucei* and 2 analogues possessed broad spectrum antiparasitic activity. Importantly, the active compounds retained their antileishmanial activity against miltefosine resistant strains. 5 thiazolidinone derivatives with the most promising antiparasitic results were further evaluated for their ADME-Tox and pharmacokinetic (PK) profile. The early *in vitro* ADME-Tox didn’t show any biodegradation or liver metabolism issues, while Isozyme-speciﬁc CYP450 experiments revealed that the majority of the active compounds were weak or moderate inhibitors of the CYP450 system except. Finally, the SNAP-PK studies showed favorable levels of the active thiazolidinone analogues and their *in vivo* evaluation is in progress.

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**Addressing the drug resistance issue by targeting the folate cycle proteins in *Trypanosoma brucei* and *Leishmania major* parasites**

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Human African Trypanosomiasis (HAT), also known as sleeping sickness, remains a neglected tropical disease despite control programs having significantly reduced its prevalence. The WHO’s 2030 eradication target highlights the need for innovative and collaborative efforts, such as the One Health approach. [1]The disease, caused by *Trypanosoma brucei*, is treated with a limited set of six drugs, including the recently introduced fexinidazole. However, issues related to toxicity, resistance, and costs complicate treatment, making the development of safer and more effective therapies essential. Research into enzymes involved in folate metabolism, particularly dihydrofolate reductase (DHFR) and pteridine reductase 1 (PTR1), represents a promising avenue. Addressing folate metabolism in *Trypanosoma* and related parasites such as *Leishmania* offers dual potential to combat other parasitic diseases, such as leishmaniasis. Continued research is vital to support global eradication efforts for HAT and related diseases.[2] The present study aims to demonstrate the activity of new 1,3,5-triazine derivatives against the dihydrofolate reductase (DHFR) of *Trypanosoma brucei* and *Leishmania major*. The synthesized 1,3,5-triazine derivatives were also tested against two additional targets: pteridine reductase 1 of *Trypanosoma brucei* and human DHFR, to evaluate toxicity and species specificity. Moreover, for both targets, production and purification protocols were optimized to achieve higher yields. The 1,3,5-triazine derivatives exhibited activity (IC50) in the low micromolar range (1–20 µM) against the DHFR of *Trypanosoma brucei* and *Leishmania major*, while showing activity in the high micromolar range (100–400 µM) against the pteridine reductase 1 of *Trypanosoma brucei*. However, the compounds also demonstrated activity in the low micromolar range (10–30 µM) against human DHFR, indicating a lack of specificity toward parasitic proteins. In conclusion, it can be stated that some of these compounds could serve as promising inhibitors for the treatment of trypanosomiasis, provided they are further developed.

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**Participatory and molecular surveillance of tick-borne theileriosis, and anaplasmosis in district Bahawalpur, Punjab,Pakistan**

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Ticks (Acari: Ixodidae) are vicious –blood-sucking ectoparasites of livestock extensively found around the globe and cause significant economic losses. They vector a higher range of important pathogenic agents including Theileria spp. and Anaplasma spp. The aim of present study is to specify the molecular epidemiology and its associated risk factors of two well-distributed tick- borne protozoa and bacteria Theileria spp. and Anaplasma spp. in cattle population from Bahawalpur, Punjab, Pakistan. Blood and ticks samples were collected from local breed cattle,crossbreed, cholistani and from exotic breed with total of 384 blood samples and four species of ticks. Genus-specific PCR assays were performed to detect the presence of Anaplasma spp. And Theileria spp. based on 16S rRNA and MPSP makers. PCR results showed that Anaplasma was 17% prevalent and Theileria was 25.9 % prevalent in the study area with comorbidities prevalence rate was 25% in the tested blood samples. Univarable analysis of risk factors showed that only breed and acaricidal treatment were significant determinants (P &lt; 0.05) for these comorbidities, however, in case of Theileria spp. and Anaplasma spp. ,breed, age, gender,grazing practice, season, presence of poultry on farm and acaricidal treatment were potential determinants (P &lt; 0.05). Multivariable analysis specified that breed and acaricidal treatment were considered as significant risk factors for these infections (P &lt; 0.05) whereas acaricidal treatment was found to be a significant determinant for comorbidities (P &lt; 0.05). Phylogenetic analysis indicated that Anaplasma spp. and Theileria spp. isolates showed similarities and shared phylogeny with same isolates reported from Asia, China, Europe and Japan. This is the first molecular report on the epidemiology and risk factors analysis of Anaplasma spp. and Theileria spp. infections in cattle population from Bahawalpur, Pakistan. Further large-scale study is required to investigate molecular, epidemiological and genotypic aspects as well as potential risk factors analysis from the country to facilitate designing strategies to control tick-borne pathogen and reduce losses to cattle industry.

Key Words: PCR (Polymerase Chain Reaction), Comorbidities, Phylogenetic, Epidemiology

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**Libraries of analogues of Eucalyptus G-endoperoxides, antiparasitic activities, mechanisms of action**

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Malaria remains a major world health problem owing to the spread of *P. falciparum*, the most dangerous of the *Plasmodium* parasites, that is resistant to the most antimalarial drugs. It affects approximately 200 million humans every year, causing almost half a million deaths among infected individuals, according to WHO (World Health Organization).



**Figure 1**

For many years now, we have been interested in the naturally occurring phytormones known as G- factors (**G1**, **G2**, **G3**) synthesized from syncarpic acid and incorporating an endocyclic endoperoxide frame. Among them, the methylated compound G3Me and its derivatives have been shown to be potent antimalarial agents[1]. In addition, other O-alkylated peroxyhemiketalic endoperoxide derivatives show good antimalarial activity[2]. Therefore, we decided to incorporate alkyne and azide probes into the **R** position. This could still provide active compounds but in addition these functional groups could be used for click modifications: i) by incorporating another pharmacophore or ii) by applying to chemoproteomics studies.

So, we report here the synthesis of nine key-compounds, appropriate for the study of the O- alkylation.

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**Towards the discovery of novel Sortase A inhibitors as potential antibiofilm agents**

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Antimicrobial resistance (AMR) is a critical threat to public health and the sustainability of healthcare systems, with superbug bacteria accounting for approximately 25% of infections and nearly 30% of AMR-related deaths. Among bacterial virulence factors, biofilm formation plays a central role in chronic and persistent infections[1]. Biofilm is a highly organized bacterial community embedded in a self-produced extracellular polymeric matrix, inherently resistant to antibiotics. Targeting bacterial adhesion, a crucial step for biofilm development, represents a promising strategy in this contest. One key target is the enzyme Sortase A (SrtA), which mediates bacterial adhesion to host tissues [2]. The project aims to develop and validate novel inhibitors of SrtA able to interfere with bacterial adhesion to the host tissue.

Starting from the PDB structure of SrtA (PDB code: 2KID) [3], computational studies, including molecular dynamics (MD) and molecular docking, were employed to identify novel potential inhibitors of bacterial SrtA enzyme. Protein preparation and MD simulations were performed using the Schrödinger suite [4]. The ZINC20 database [5] was downloaded and prepared [4] for the subsequent docking studies, which were carried out by means of Glide v.8.9 SP algorithm[4].

Finally, to prioritize compounds with structural diversity, seven compounds were purchased as potential SrtA ligands. Biological assays with recombinant SrtA, combined with bacterial adhesion studies on Staphylococcus aureus to immobilized fibrinogen, confirmed the promising activity of two natural compounds as effective SrtA inhibitors. This integrative approach demonstrates the potential of combining computational screening with experimental validation to identify novel anti-adhesion compounds, offering valuable insights into the development of strategies against AMR and biofilm-associated infections.

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**Preparedness of veterinarians to face emergency of animal leishmaniosis in urban settings of Morocco**

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Leishmaniosis caused by *Leishmania infantum*, *L. major* and *L. tropica* is endemic in Morocco. Growing evidence of both human and canine *Leishmania* infections in urban centres has been reported. Since many forms of the disease are zoonotic, veterinarians play an important role in leishmaniosis control by intervening at the parasite host level. This study aimed to bring together One Health principles to connect canine and feline leishmaniosis epidemiology within urban centres of Morocco (Rabat and Fez) and assess the level of awareness of Moroccan veterinarians about facing this threat.

As a proof of concept, a serological and molecular epidemiological survey was developed in the cities of Rabat and Fez to confirm the presence of *Leishmania-*infected animals. For the purpose, blood samples were collected from owned and sheltered dogs (n=159) and cats (n=32) between February 2023 and December 2023. Total anti-*Leishmania* IgG was detected using an ELISA assay based on soluble *L. infantum* promastigote antigen (SPLA); recombinant KDDR (rKDDR) and *Leishmania* cytosolic peroxiredoxin (CPX) antigens. In parallel, molecular detection of *Leishmania* kDNA was performed by primers RV1 and RV2, further complemented with ITS-1 sequencing, whenever it was possible to amplify the latter. Alongside, a survey on local veterinarians’ knowledge, perceptions, and practices (KPP) regarding animal leishmaniosis was conducted on Google Forms and implemented as an online questionnaire. Potential respondents (n=220) were contacted by email and social media with a request to participate through a link provided to assess the questionnaire.

A considerable prevalence of infection was identified in dogs from urban centres of Morocco. Additionally, this is the first report of feline infection with *Leishmania* spp. in this country and in urban settings. Moroccan veterinarians are aware that animal leishmaniosis is endemic in Morocco, representing a public health threat, and are knowledgeable about canine leishmaniosis diagnosis and treatment.

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