"Novel leads and drugs for vector borne diseases: Targets and off targets (toxicity and ecotoxicity) and mechanism of action"

19-20 September 2024

Program

Thursday 19th September 2024	
08:30	*Arrival & registration*
09:00	*Welcome*
Session 1: **Targets and Mechanism of Action Studies for Vector Borne Diseases** *(Chairing:……..)*	
09:15	*"Innovative approaches to target trypanothione reductase an essential enzyme for the survival of trypanosomatids in the host"* *Andrea Ilari, Università Sapienza, Italy*
09:35	*"Cyclic AMP signaling and nucleoside activated Protein Kinase A in Trypanosoma and Leishmania: genetic target validation and structure-guided inhibitor design"* *Michael Boshart, University of Munich (LMU), Germany*
09:55	*"Chemical tools to decipher the modes of action of antiplasmodial redox-active 3-benzylmenadiones"* *Elisabeth Davioud-Charvet, CNRS-Université de Strasbourg-Université Haute-Alsace, France*
10:15	*"Unravelling the mechanism of action of highly potent Pteridine Reductase 1 inhibitors: new insights into dual targeting of PTR1 and DHFR* *Nuno Santarém, Universidade do Porto, Portugal.*
10:35	*Coffee Break*
11:05	Promoters: Cecilia Pozzi, Ulrike Wittig and the Team **OHD1 - Target database project: the BioTarget DataBase (BioT-DB)** *Round table discussion:* **Structural biology approaches to understand and fight VBDs.**

Session 2: **Novel leads and drugs for Vector Borne Diseases** *(Chairing.....)*	
12:00	*"Phenotypic and target-based screening of nucleoside analogues as antitrypanosomal agents"* *Ewout Van de Velde, Ghent University, Belgium.* *Recipient of the "Best presentation on vector-borne diseases" from the COST action (CA21111) at the Paul Ehrlich meeting held in Rome on June 2024.*
12:20	*"Specialized pro-resolving mediators as leads for resolution pharmacology targeting vector borne diseases."* *Trond Vidar Hansen, University of Oslo, Norway*
12:40	*"Robenidine Derivatives As Potential Antischistosomal Drug Candidates"* *Christian N. Lotz, University of Basel, Switzerland*
13:00	*Break-Refreshments*
14:00	*"Synthesis and Anti-Parasitic Evaluation of Fused N,S-Heterocyclic Derivatives"* *Maria João Ribeiro Queiroz , Universidade of Minho, Portugal*
14:20	*"Discovery and preliminary preclinical in vivo evaluation of a dicationic candidate for the oral treatment of leishmaniasis"* *Christophe Dardonville, Medicinal Chemistry Institute, CSIC. C. Madrid, Spain.*
14:40	*"Arnica tincture is effective against cutaneous Leishmaniasis in human patients: A novel drug for this vector borne disease without toxic or ecotoxicological impact"* *Thomas J. Schmidt, University of Münster, Germany*
15:00	*Drug Discovery and Development for Human and Animal African Trypanosomiasis: A Comprehensive Database of Natural Compounds with Anti-trypanosomal Activity"* *Laura Bertarini, University of Modena and Reggio Emilia, Italy*
15:20	Promoters: Jose Maria Alunda, Anabela Cordeiro da Silva Guy Caljon and the Team **OHD3 project: Transition from in vitro to in vivo evaluation: recommendations for obtaining high-quality leads against kinetoplastids and round table discussion.**
16:00	*Coffee break*
16:30-18:40	*Poster Presentations. Oral presentations of posters (3 slides-7min).* *In the case of multiple accepted posters by the same presenting author they should select one to present.*
20:30	*Dinner*

Friday 20th September	
Session 3: **Medicinal chemistry and One Health principle integration: expanding the concepts of selectivity in drug discovery** (*Chairing .....*)	
09:00	*Nikolaos Thomaidis* *Title to be announced* *National and Kapodistrian University of Athens, Greece*
09:20	*CLOUDPHARM* *Title to be announced*
09:40	*Simone Brogi* *Title to be announced* *University of Pisa, Italy*
10.00	*"Optimizing Trypanothione Reductase Inhibitors for Leishmania Treatment: A Multiparametric Prediction Approach to Enhance Solubility and Biodegradability"* *Sandra Gemma, University of Siena, Italy*
10:20	*"Development of NMT-A004-loaded biodegradable nanocarriers"* *Theano Fotopoulou, National Hellenic Research Foundation, Greece*
10:40	Coffee Break
11:10	*"One Health Approach in Drug Discovery for Leishmaniasis by Targeting Calpain cys-protease"* *Daniele Aiello, University of Modena and Reggio Emilia, Italy*
11:30	*"Innovative Therapeutic Strategies for Vector-Borne Diseases: Exploring Novel Drug Targets and Addressing Off-Target Toxicity"* *Lori Doko, University of natural sciences and biological sciences, Albania*
11:50	"Bioethical and human security approaches on evolution of drugs for vector borne diseases Serghei Sprincean, Moldova State University, Moldova
12:10	Promoters: Sandra Gemma and Gulsah Bayraktar and the Team **OHD2 – Compound Database project: Antiparasitic drug discovery and emerging scaffolds with predictive low environmental impact.** *Round table discussion*
13:00	Break-Refreshments
14:00	*COST CA21111meeting (Planning Action activities and YRI)*
17.00	*Closing remarks*

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Oral communication

Poster

Acknowledgement
This publication is based upon work from COST Action CA2111 " OneHealthdrugs", supported by COST (European Cooperation in Science and Technology). COST (European Cooperation in Science and Technology) is a funding agency for research and innovation networks. Our Actions help connect research initiatives across Europe and enable scientists to grow their ideas by sharing them with their peers. This boosts their research, career and innovation.

1. Innovative approaches to target trypanothione reductase an essential enzyme for the survival of trypanosomatids in the host

Exertier C.[a], Antonelli L.[a], Fiorillo A.[a], Colotti G.[a], Liuzzi A.[a], Salerno A.[b], Fiorentino E.[c], Ocello R.[b], Seghetti F.[b], Caciolla J.[b], Masetti M.[b], Uliassi E.[b], Orsini S.[c], Di Muccio T.[c], Bolognesi M.L.[b],Ilari A.[a]

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Trypanothione reductase (TR) is a validated target, essential for trypanosomatid antioxidant defense. The absence of TR in the host, its essential role for the parasite, and the development of numerous TR inhibitors make this enzyme an appealing target for anti-trypanosomatid drugs. However, among the reported TR-inhibiting compounds, only a few exhibit sufficient antiparasitic/inhibitory activity, primarily due to two factors: i) parasite survival is significantly impacted when TR activity is reduced by more than 90%; ii) the large active site of TR complicates the development of effective inhibitors. Moreover, TR has a human homolog, glutathione reductase (GR), and despite the latter's substrate cavity having quite different characteristics, the design of drugs active only on TR still represents a challenge. To overcome these problems, we have chosen two innovative strategies: the first involves the design of TR potent inhibitors starting from fragment screening and the second TR elimination through the Ubiquitin Proteasome System. Concerning the first strategy an initial crystallographic fragment screening allowed us to identify 12 fragments able to bind TR sub-pockets [1]. Supported by docking studies, a small library of compounds was obtained by merging, linking and/or growing five selected fragments, which bind in the Z-sub-pocket. The synthetized molecules have been tested to evaluate TR inhibitory activity, followed by anti-leishmanial phenotypic assays of the most promising TR inhibitors and the crystal structure resolution of the complexes they form with TR [2]. The second strategy, alternative to traditional drug design also for infective diseases, 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 [3]. We propose to apply this new approach to tackle Leishmaniasis infection by engaging TR towards degradation. In this context, we rationally designed bifunctional PROTACs and characterized their ability to bind TR.

References

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Acknowledgment

The Authors acknowledge MIUR- FISR2019_03796 819 “Proteolysis targeting chimeras (PROTACs) to treat leishmaniasis”- PROLEISH for the financial support.

2. Cyclic AMP signaling and nucleoside activated Protein Kinase A in *Trypanosoma* and *Leishmania*: genetic target validation and structure-guided inhibitor design

Michael Boshart [a], Qingping Wu [a], Matjaz Brvar [b], Sabine Bachmaier [a], George Githure [a], Veronica Ober [a], Yuri Volpato [a], Frank Schwede [c], Oliver Plettenburg [b]

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Stage development and host adaptation of vector transmitted parasites require fast and reliable perception of signals from the host environments. *Kinetoplastida* share with model eukaryotes second messengers and signaling modules like protein kinases, but their connections, activation mechanisms and interaction in signaling pathways differ significantly from the host, providing opportunities for drug development. The second messenger cAMP is important for innate immunity subversion of trypanosomes in the mammalian host[1] and for transmission via colonization of the tsetse salivary glands[2]. We begin to understand the role of the large adenylate cyclase family and a new multi-cyclase regulator (CARP3) in these processes. The cAMP effector protein(s) in this pathway are still uncharacterized[3]. Notably, protein kinase A (PKA), the primary mammalian cAMP effector, has lost its regulation by cAMP[4]. Nucleoside ligands adopted this role. The structural basis and evolutionary origin of ligand and activation specificity of PKA orthologues and paralogues has been investigated by crystallography, binding assays and a large number of site-directed mutants *in vitro* and *in vivo*[5]. The repurposing of PKA for novel ligands and pathways other than cAMP evolved in the Euglenozoa. PKA is essential and was genetically validated as target of high specificity. A structure-guided strategy of dominant-negative allosteric inhibition was developed and explored by a series of >100 compound syntheses. Two orders of magnitude of separation of binding affinity and intrinsic activation potency were achieved so far. The results illustrate the complexities of conformational inhibition in drug design and the power of dominant-negative strategies to target a multigene family of kinases.

[1]Salmon, D. *et al.* Adenylate Cyclases of Trypanosoma brucei Inhibit the Innate Immune Response of the Host. *Science* **337**, 463-466 (2012). doi.org/10.1126/science.1222753
[2]Bachmaier, S. *et al.* A multi-adenylate cyclase regulator at the flagellar tip controls African trypanosome transmission. *Nat Commun* **13**, 5445 (2022). doi.org/10.1038/s41467-022-33108-z
[3]Bachmaier, S. *et al.* Novel kinetoplastid-specific cAMP binding proteins identified by RNAi screening for cAMP resistance in Trypanosoma brucei. *Frontiers in Cellular and Infection Microbiology* **13** (2023). doi.org/10.3389/fcimb.2023.1204707
[4]Bachmaier, S. *et al.* Nucleoside analogue activators of cyclic AMP-independent protein kinase A of Trypanosoma. *Nat Commun* **10**, 1421 (2019). doi.org/10.1038/s41467-019-09338-z
[5]Ober, V. T. *et al.* Purine nucleosides replace cAMP in allosteric regulation of PKA in trypanosomatid pathogens. *eLife* **12**, RP91040 (2024). doi.org/10.7554/eLife.91040

Funded by Federal Ministry of Education and Research, BMBF collaborative grant reference 16GW0281-3

3. Chemical tools to decipher the modes of action of antiplasmodial redox-active 3-benzylmenadiones

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One of the main research topic of the team is focused on the development of redox-active antiparasitic drug-candidates based on the 3-benzylmenadione (bMD) core [1]. Plasmodione (PD) is a new early lead bMD derivative identified, 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]. Our present investigations are aimed at: (i) the chemical optimization of the early lead PD and the identification of potential drug metabolites [4,5], (ii) the improvement of their pharmacokinetic properties [6], (iii) the identification of the protein targets to unravel their modes of action [7-8], and (iv) the validation of the antiparasitic properties of the optimized compounds in vivo.
For the identification/visualization of the biological targets of plasmodione, two strategies are currently being developed. The first strategy is based on PD activity-based protein profiling (ABPP). We synthesized new bMD alkyne probes using the reported synthetic route previously described[4]. The general ABPP method consists in several steps: 1) UV-irradiation of the photoreactive ABPP probe incubated with the cell lysate, 2) conjugation of biotin azide through the CuAAC reaction, 3) enrichment of the biotinylated protein adducts, 4) protein digestion and LC-MS analysis. Then, the whole procedure was applied to S. cerevisiae WT to validate the experimental workflow [9]. After method validation, it has been applied to P. falciparum cell extracts. The second strategy uses the CuAAC reaction between the alkyne probe and fluorophore azides to visualize the locus of the probe in bMD-treated *P. falciparum* trophozoites and *T. gondii* tachyzoites parasites [6].

References

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Acknowledgment

The Authors acknowledge the CA21111 for the financial support given to V.M. (STSM grant).

4. Unravelling the mechanism of action of highly potent Pteridine Reductase 1 inhibitors: new insights into dual targeting of PTR1 and DHFR

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Trypanosoma brucei, is the protozoan parasite responsible for Human African Trypanosomiasis. Trypanosomiasis is also a One Health problem, representing a significant concern in African countries with Animal African Trypanosomiasis having a large impact on livestock. As *T. brucei* are folate auxotroph's, antifolate therapy holds great potential for much needed Trypanosomiasis drug development. Initial antifolate drug development focused on DHFR, an essential enzyme for the folate pathway. However, parasites can bypass DHFR mediated folate reduction using pteridine reductase 1 (PTR1). This shifted the focus to PTR1, which reduces both unconjugated pterins and folate, being now a hope for a possible therapeutic approach either as a monotherapy or in combination with DHFR inhibitors [1]. It is still unclear how the inhibition mechanism of this binary system works. In a previous work a structure-based approach generated variously decorated pteridine derivatives for TbPTR1 inhibition [2]. Among them two picomolar inhibitors of TbPTR1, C113 and C131, were considered suitable to address the PTR1/DHFR interplay and evaluate their antiparasitic potential, using a combination of enzymatic, anti-parasitic, and structural biology characterization. The capacity of C113 and C131 to inhibit the PTR1-mediated reduction of folic acid or biopterines was confirmed through enzymatic assays, and X-ray crystallography. The antiparasitic activity of C113 and C131 in monotreatment was 14.9 and 3.70 µM, respectively. Both have synergetic activity when combined with Methotrexate. C113 being the most synergic. Both molecules are also more potent in low folate medium with an increase in potency of 10x for C113 and 39x for C131. Upon substrate supplementation with biopterin we confirmed that C113 and C131, in monotreatment, are acting mostly through the PTR1-mediated biopterin reduction axis. In combination with Methotrexate, the data suggest that the compounds are acting through the DHFR and PTR1-mediated reduction of folic acid. Overall, major strides in the exploitation of DHFR/PTR1 interplay as drug targets and new avenues towards the discovery of inhibitors capable of inhibiting DHFR/PTR1 binary system with a higher therapeutic efficacy can be foreseen.

References

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5. Phenotypic and target-based screening of nucleoside analogues as antitrypanosomal agents

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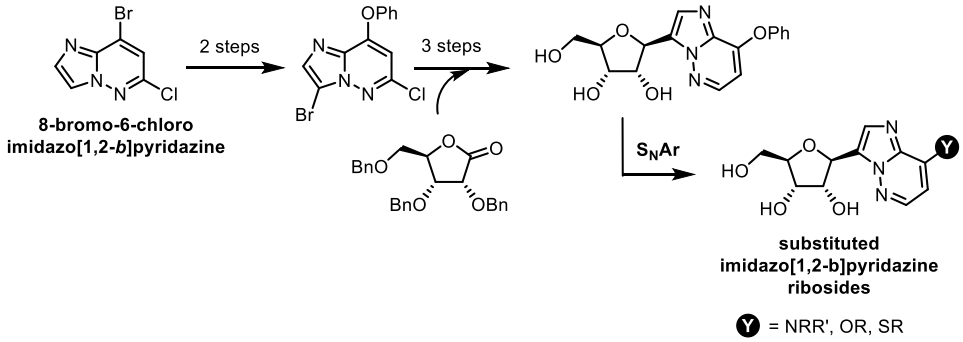
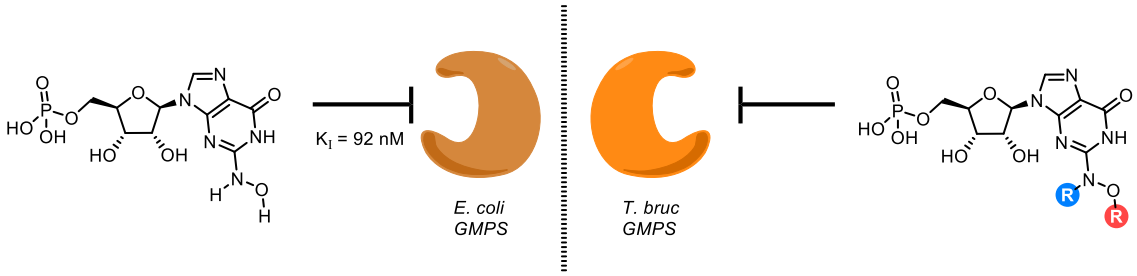
Over the past decade, our lab has been constructing a focused library of purine-like nucleoside analogues with the aim of targeting purine-auxotroph protozoan parasites, mainly trypanosomes, via phenotypic based screening. More recently, we have diverted our attention towards imidazo[1,2-b]pyridazine ribosides, another underexplored C-nucleoside scaffold. With a focus on robustness and late-stage diversification, we optimized a synthesis method for this class of compounds.



Simultaneously, we are investigating the inhibition of guanosine monophosphate synthase (GMPS) as a critically underexplored strategy to target trypanosomatids. GMPS was deemed essential in *Trypansoma brucei* via a knockout study.[1] Furthermore, N^2-hydroxy guanosine monophosphate displayed a time-dependent potent inhibition of *E. coli* GMPS with a K_I of 92 nM.[2] Inspired by these studies, we wish to explore the inhibition of *T. brucei* GMPS using appropriately functionalized N^2-hydroxy guanosine monophosphate analogues. At a later stage, the most potent analogues will be converted to their phosphate prodrugs to elicit a response in a cellular based assay against *T. brucei* promastigotes.



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6. Specialized pro-resolving mediators as leads for resolution pharmacology targeting vector borne diseases.

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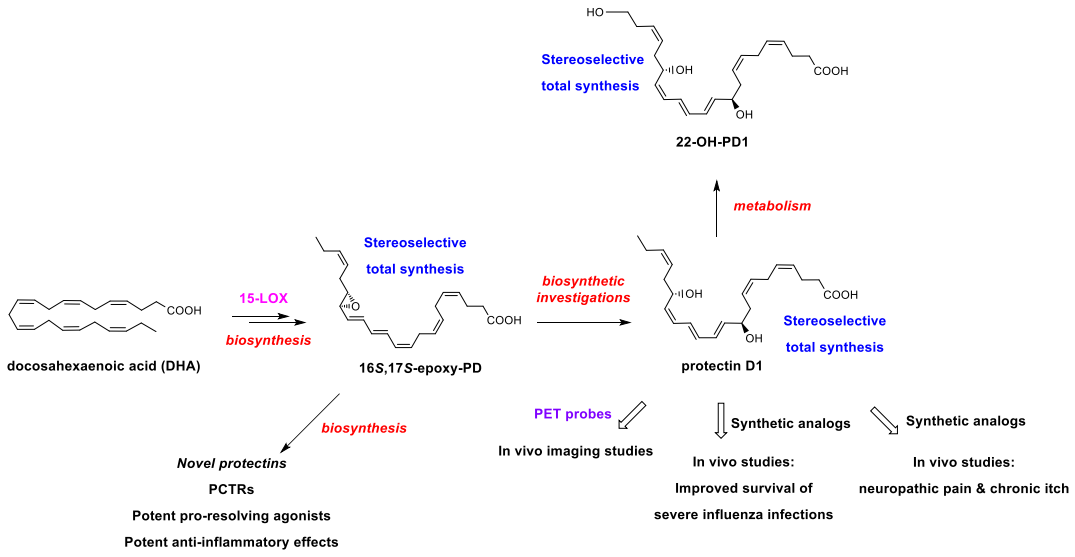
Specialized pro-resolving mediators (SPMs) are endogenous regulators of infection and inflammation, biosynthesized from omega-3 and omega-6 polyunsaturated fatty acids during the early events of infections. SPMs are potent resolution agonists towards individual G Protein Coupled Receptors (GPCRs). Due to their dual pro-resolving and anti-inflammatory bioactions demonstrated in several human disease models, SPMs are interesting leads in drug discovery. Based on collaborative studies, we have reported total synthesis, biosynthetic studies, medicinal chemistry projects and biological evaluations with several SPMs.[2]

In this presentation, some examples outlined in the figure below will be outlined as a starting point towards collaborations targeting vector borne diseases.



References

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7. Robenidine Derivatives As Potential Antischistosomal Drug Candidates

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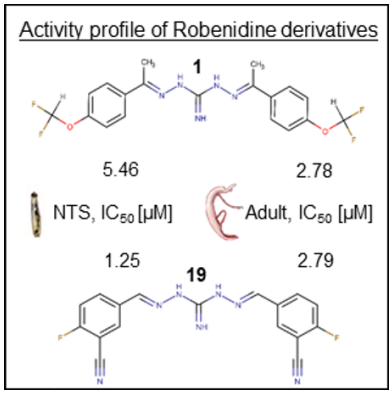

Schistosomiasis, caused by *Schistosoma* spp., imposes a significant health burden on millions worldwide. The limited availability of effective drugs and the increasing risk of resistance due to extensive usage underscore the urgent need for new antischistosomal treatments. Recent studies have indicated that robenidine derivatives, containing an aminoguanidine core, show promising activity against *Plasmodium falciparum*. This finding has prompted further investigation into their efficacy against *Schistosoma mansoni*, due to their similar habitat and related cellular mechanisms, such as the heme detoxification pathway. Phenotypic screening of robenidine and 80 derivatives against newly transformed schistosomula and adult *Schistosoma mansoni* identified 11 candidates with low EC50 values for newly transformed schistosomula (1.12–4.63 µM) and adults (2.78–9.47 µM). The structure-activity relationship revealed that electron-withdrawing groups at the phenyl moiety and the presence of methyl groups adjacent to the guanidine moiety enhanced the activity of derivatives against both stages of *Schistosoma mansoni*. Toxicity was assessed using HepG2 and L6 cell lines, as well as the VirtualToxLab tool, revealing that six derivatives bind to estrogen or mineralocorticoid receptors. Based on their potency, cytotoxicity, pharmacokinetic, and physicochemical properties, two compounds—2,2′-Bis[(3-cyano-4-fluorophenyl)methylene] carbonimidic dihydrazide hydrochloride (1) and 2,2′-Bis[(4-difluoromethoxyphenyl)ethylidene] carbonimidic dihydrazide hydrochloride (19)—were selected for an in vivo study in *Schistosoma mansoni*-infected mice. Although these compounds failed to significantly reduce the worm burden (worm burden reduction <20%), robenidine derivatives, given their easy and versatile synthesis and in vitro activity, remain promising agents in the fight against schistosomiasis. Thus, they require further refinement to achieve higher antischistosomal specificity and in vivo activity [1].

References

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8. Synthesis and Anti-Parasitic Evaluation of Fused *N,S*-Heterocyclic Derivatives

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New 7-arylthioetherthieno[3,2-*b*]pyridines bearing arylamides or (hetero)aryl-1,2,3-triazoles 1,4-disubstituted (Figure 1) were synthesized by reaction of 3-(thieno[3,2-*b*]pyridin-7- ylthio)aniline either with benzoyl chlorides to give the first or, in one pot procedure *via* the corresponding azide by a Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) to give the latter. The anti-parasitic activity of the compounds was evaluated at 10 µM against *Leishmaniainfantum, Trypanosoma brucei* and *Trypanosoma cruzi* and some SARs were established. The diarylamide with a NO_2 group in the *meta* position relative to the carbonyl presented high anti-parasitic activity against both *T. brucei* (102% inhibition) and *T. cruzi* (95% inhibition) and no activity for *L. infantum*. When a F atom is placed in the same position, there was a decrease of the activity for *T. brucei* and *T. cruzi* and similarly no activity against *L. infantum*. The change ofthe F atom to the *para* position gave a high activity for *T. brucei* (102% inhibition), and for *L. infantum* (81% inhibition), and only a moderate activity for *T. cruzi* (52% inhibition). When the amide linkage was replaced by a 1,2,3-triazole 1,4-disubstituted moiety, no activity was observed for *L. infantum* for the tested compounds. However, the compound with a phenyl group showed high activity for *T. cruzi* (97% inhibition) and only moderate for *T. brucei* (64% inhibition). The compound with a F atom in the *meta* position relative to the triazole moiety showed activity against both *T. brucei* and *T. cruzi* (98% and 81% inhibition, respectively). Substitution with heterocycles (pyridin-3-yl or thien-3-yl) in the triazole moiety, resulted in high activity for both *T. brucei* and *T. cruzi*, but only for the thiophene derivative (97% and 80%inhibition, respectively). At the tested concentration, the compounds did not exhibit toxicity in PMA-differentiated THP-1 cells, as determined by the MTT assay. However, CC50 estimation suggest that selectivity may be improved.

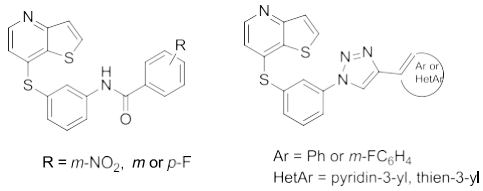

Figure 1- Sctrutures of the compounds tested

Acknowledgments
To the COST (European Cooperation in Science and Technology) Action 21111 OneHealthDrugs

9. Discovery and preliminary preclinical in vivo evaluation of a dicationic candidate for the oral treatment of leishmaniasis

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Leishmaniasis is a widely distributed group of vector-borne parasitic diseases caused by kinetoplastid parasites from the genus *Leishmania*. Clinical course relates to the *Leishmania* spp. involved and the functionality of the immune system of the hosts. Progression of the human infection is linked to non-effective immune system and therefore, the disease is more frequent in children, elderly, and in individuals with impaired immunity. Visceral leishmaniasis (VL), provoked by *Leishmania donovani* and *Leishmania infantum*, is the most severe condition, which is fatal unless treated; it is prevalent in Asia, South America and southern Europe, and its expansion to northern latitudes has been reported. The available therapeutic arsenal used for the treatment of human leishmaniasis remains scarce so new treatments are badly and urgently needed.[1]

Our group has discovered a new series of dicationic molecules that are very effective *in vitro* against the clinically-relevant forms of the kinetoplastid parasites *Trypanosoma brucei*, *T. cruzi*, and *Leishmania donovani*.[2] Importantly, this series was highly effective *in vitro* against intracellular amastigotes of *L. donovani*. In particular, compound **1** was nearly as effective as the reference drug amphotericin and more active than miltefosine. These new series of compounds belong to a family of DNA minor groove binders (MGBs) that target the kDNA of kinetoplastid parasites.[3] However, the *in vivo* potential of this series in rodents or higher animals has not been studied so far. In this work, we report the preliminary preclinical *in vivo* assessment of a lead compound from this new series of DNA MGBs in different animal models to collect information about efficacy, toxicity, PK/PD data, and pharmaceutical formulations.

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10. Arnica tincture is effective against cutaneous Leishmaniasis in human patients: A novel drug for this vector borne disease without toxic or ecotoxicological impact

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In continuation of our very successful animal studies proving that Arnica tincture (AT) is a possible new treatment for the vector-borne neglected tropical disease cutaneous Leishmaniasis (CL) [1, 2] with little or no toxic impact [3], we have now performed an initial observational study with human patients. In this study conducted in Colombia, 15 patients have so far been included. Males and females of age 18-60 years were eligible to participate in the study if they have no more than 4 CL lesions with a maximum size of 4 cm largest diameter, not located at complicated areas (ear, face, near mucous membranes, joints). All received topical AT treatment (dripping of tincture to cover the lesion) 3 x /day for 30 or 45 days. They were followed up for 180 post-treatment (PT) days. The progress of healing was assessed and photo-documented at various time points. Of the 15 patients enrolled so far, four had to be withdrawn during the study for different reasons. Of the other eleven patients, six have completely finished the study so far. All were definitely cured with no signs of relapse at the end of PT. Of the remaining patients still in the PT interval, three were cured and one displayed an improvement after the end of treatment. One patient is currently under treatment. The study is being continued to a total number of 16 patients. Based on these very promising results, a clinical study with more complex cases of CL, a higher number of patients and direct comparison with antimonial therapy is now in preparation.

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11. Drug Discovery and Development for Human and Animal African Trypanosomiasis: A Comprehensive Database of Natural Compounds with Anti-trypanosomal Activity

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Human African trypanosomiasis (HAT), also known as sleeping sickness, and Animal African trypanosomiasis (AAT) are life-threatening neglected tropical diseases generally caused by the *Trypanosoma brucei* subspecies. HAT represents a critical public health issue in 36 African countries, with approximately 65 million people at risk, predominantly affecting impoverished communities in sub-Saharan Africa, where it poses a significant health and economic burden[1]. AAT, similarly, affects livestock, leading to severe economic losses due to decreased productivity and increased mortality in affected animals. Despite advances in reducing HAT cases and improving treatments, AAT remains a reservoir of infection, making its control crucial as part of the One Health approach. Current treatments, including suramin, pentamidine, melarsoprol, eflornithine, and the recent nifurtimox-eflornithine combination therapy, are associated with several drawbacks, like toxicity, high cost, and emerging drug resistance[2]. Given these limitations, there is an urgent need for new, effective and safe trypanocidal drugs.
Natural products, with their unique chemical structures and bioactivity, represent a promising resource for drug discovery[3]. However, information on natural compounds with trypanocidal activity is often fragmented, with existing reviews typically focusing on a single class of molecules and often outdated. Establishing a comprehensive database of natural candidates with proven trypanocidal activity is crucial to identify promising scaffolds and accelerate the discovery of new hits/leads. The database built in this work includes relevant data on natural compounds active against *T. brucei*, incorporating literature data from 2019 to 2024, with their chemical structure, biological activity, cytotoxicity and, when described in the literature, molecular target/s. By systematically classifying natural products with anti-trypanosomal properties, this database, properly integrated with ADME and ECOTOX parameters, represents a first crucial step to disclose new effective treatments for both HAT and AAT, thus significantly contributing to the global effort to control and eradicate trypanosomiasis.

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12. G.AI.A. A computational platform for ecotoxicity predictions of chemical compounds

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Nowadays, significant amounts of chemical substances are detected in various environmental compartments. In particular, the aquatic environment has become a reservoir for drugs and fertilizers released through the discharge of effluent water [1,2]. The introduction of these emerging contaminants into the environment can negatively impact ecosystems[3]. It is thus critical to reduce the overall environmental impact of pharmaceuticals by developing novel computational tools towards green-by-design compounds.
For this purpose, Cloudpharm PC[4] has designed and develops a multimodal platform that offers state-of-the-art cheminformatics-based classification models to assess the potential effect of pharmaceuticals on the aquatic environment. Predicting the bioconcentration factor (BCF) and Lethal Concentration, 50% (LC50) of pharmaceuticals is critical for the relevant environmental risk assessment. Since the experimental determination of BCF and LC50 are of high experimental cost and requires a large number of vertebrate animals to perform the assays, in silico methods such as quantitative structure-activity relationships (QSARs) powered by machine learning (ML) models were performed to assess the environmental risk of pharmaceuticals[5,6]. Our effective models can help eliminate candidates in the earlier processes of the drug discovery pipeline and thus shape future compounds in terms of green-by-design ambitions. Accurate classification of compounds based on bioconcentration and toxicity is also predicted for the metabolites of parent compounds, as these derivatives can play a pivotal role in shaping the overall ecotoxicity profile of pharmaceutical compounds. Eventually, both parent compound and its metabolites are assessed for the fulfilment of the principles of green chemistry[7].

The platform aims to be accessible to scientists in the field of drug discovery and be a novel screening methodology as part of drug discovery pipelines, which can also serve as a regulatory checkpoint in the pharmaceutical industry.

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13. Optimizing Trypanothione Reductase Inhibitors for Leishmania Treatment: A Multiparametric Prediction Approach to Enhance Solubility and Biodegradability

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Leishmaniases are vector-borne diseases caused by protozoan parasites of the genus Leishmania categorized as zoonosis or anthroponosis according to the primary reservoir hosts of the human infection. Human leishmaniasis and canine leishmaniasis due to L. infantum represent good examples of how the animal and human health come together. L. infantum is the etiological agent of visceral leishmaniasis (VL) in South America, the Mediterranean basin, and West and Central Asia and is also the etiological agent of canine leishmaniasis (CanL) in Europe.
One promising target for the development of new drugs is the enzyme trypanothione reductase (TR), that is involved in the trypanothione metabolism and plays a key role in the survival of the parasites within macrophages. We previously developed, investigated the structure-activity relationships (SARs) and identified potent TR inhibitors endowed with anti-leishmania activity in vitro against *L. infantum* amastigotes with good selectivity index [1,2]. This previous series of compounds suffered from low solubility. Following the One Health concept, we decided to follow a multiparametric optimization of this class of compounds based on prediction of selected drug-like properties in parallel with selected environmental parameters. Accordingly, based on the bioactive conformation of the crystallized hit compound, we designed a library of compounds by varying the central portion of the scaffold connecting two important pharmacophoric elements. We then selected the compounds to be synthesized based on their 3D-conformation, predicted solubility and environmental biodegradability. A selection of compounds has been synthesized tested for their inhibition of TR.

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14. Development of NMT-A004-loaded biodegradable nanocarriers

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Neglected infectious diseases (NIDs) caused by protozoan parasites belonging to the genera *Leishmania* and *Trypanosoma* have high mortality and morbidity rates, affecting impoverished populations in the developing world [1]. Current therapies can have issues such as toxicity or teratogenicity, while clinical resistance is on the rise. Thus, the discovery of new, safer and inexpensive drugs for NIDs is an ongoing challenge.

Calogeropoulou's group has pioneered the development of improved ether phospholipid derivatives, possessing enhanced antileishmanial and/or antitrypanosomal activity and reduced toxicity. In particular, an alkyl phosphocholine-dinitroaniline hybrid **NMT-A004** [2] exhibited a broad spectrum antiparasitic activity against *Leishmania* intracellular amastigotes, *T. brucei* trypomastigotes blood form and *T. cruzi Y* strain epimastigotes. However, its SNAP-PK evaluation showed reduced oral bioavailability. To address this issue we developed and characterized biodegradable nanostructured vectors that increase its half-life.

The *in silico* studies showed the lipophilic character of the compound giving insights about the formulation design challenges. Three types of unloaded and **NMT-A004**-loaded nanocarriers were produced: **A) Lipid cubosomes**, **B) Polymeric cubosomes** and **C) Nanostructured Lipid Carriers (NLCs)** (Figure 1). The encapsulation of **NMT-A004** was performed in three concentrations (10, 100 and 1000 μM) by incubation or direct mixing methods and the corresponding encapsulation efficiency (EE%) and drug loading (DL%) were determined by the developed validated quantification method.

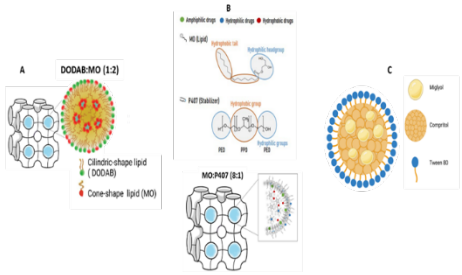

Figure 1. Schematic illustration of the biodegradable nanocarrier systems

Thus, **15** successful formulations were produced and characterized in terms of mean particle size, polydispersity index (PDI), surface charge (zeta potential) and colloidal-stability (4 weeks). The negative charge polymeric cubosomes seem more stable than the positive charge lipidic ones despite their bigger sizes. Moreover, the negative charge NLCs were also stable with very small sizes but higher PDI. Finally, all the formulations present EE% close to 100% and in terms of drug loading, lipid cubosomes had the higher drug content (>15 % for 1000 μM of **NMT-A004**). The *in vitro* release studies are *in progress*.

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15. One Health Approach in Drug Discovery for Leishmaniasis by Targeting Calpain cys-protease.

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Leishmaniasis plague millions in impoverished regions, causing disfiguring skin lesions and potentially fatal organ damage. Despite the urgent need, research and development for new treatments remain underfunded, leaving those affected with limited options. To protect human health, a One Health approach is critical, tackling the disease at its environmental and animal roots. MS proteomic study[1] revealed an up-regulation of a protein known as Calpain-like Cysteine Peptase (Uniprot: A0A6L0WUS2). We started this project with this sequence, which consists of 115 residues. Unfortunately, very few references regarding this protein are available. The lack of information and the absence of a catalytic site in this sequence forced us to focus on the full-length sequence of Calpain (GenBank: XP_001468282.2) for our virtual screening study. The cys-protease protein was already known as a drug target for antitrypanosomatidic drug discovery, but unclear aspects about the on target studies prompted our interest. Within the One Health framework, we started to study the protein and compared the sequence by alignment with calpain sequences from other species to ensure that this is an environmentally friendly target with low impact on other species. Bioinformatics tools[2-4] were then used to compare the calpain sequence with counterparts from various species, revealing significant homology with trypanosomatidic sequences and very low homology with other species, making calpain a suitable target for the development of new selective drugs against leishmaniasis. We constructed and validated a homology model for the calpain protein from *Leishmania infantum*[5], which laid the groundwork for a comprehensive virtual screening campaign. Using libraries of compounds from Enamine and ChemDiv, thorough screening methods found 4000 compounds that might be able inhibit calpain enzymatic activity. Further refinement prioritized 400 compounds based on ΔG binding calculations and selectivity against human calpain. SwissADME and ADMETlab 3.0 have screened the top 100 compounds to evaluate their pharmacokinetics and eco-tox profile, resulting in a more environmentally friendly selection of the top 50 candidates. These candidates will undergo in vitro tests to evaluate their efficacy, selectivity, and safety profiles. Furthermore, a collaborative work within structural biologists from Diamond Light Source and from the University of Siena, started to develop a plasmid that will be used to produce the catalytic domain of the protein for X-ray crystallographic studies and for the development of an in vitro assay with the recombinant protein, aiming to validate the data obtained from the virtual screening study and progress with the medicinal chemistry program. Advances of the project will be presented.

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16. Innovative Therapeutic Strategies for Vector-Borne Diseases: Exploring Novel Drug Targets and Addressing Off-Target Toxicity.

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Vector-borne diseases, such as malaria, dengue, and leishmaniasis, represent significant global health challenges, particularly in tropical and subtropical regions. These diseases are transmitted by vectors such as mosquitoes, sandflies, and ticks, which carry pathogens capable of causing severe illness in humans. Traditional therapeutic approaches have often been hampered by the development of drug resistance, necessitating the search for novel pharmacological leads and drugs with distinct mechanisms of action. Recent advances in high-throughput screening and computational drug design have facilitated the identification of new molecular entities targeting essential biological processes in both the vector and the pathogen. These novel leads show promise in disrupting key life cycle stages, such as transmission, replication, and host interaction, thereby offering potential therapeutic solutions where current treatments are failing. However, the challenge of off-target effects, including toxicity and ecotoxicity, remains a significant concern in drug development. Off-target toxicity can lead to severe side effects in patients, while ecotoxicity can disrupt local ecosystems by affecting non-target species. Therefore, a thorough understanding of the mechanisms of action and specificity of these novel compounds is essential for minimising adverse outcomes. This abstract provides an overview of recent advancements in the discovery and development of new drugs targeting vector-borne diseases. It discusses the importance of identifying and mitigating off-target effects through rigorous preclinical testing and highlights the role of interdisciplinary collaboration in accelerating the translation of these novel leads into safe and effective therapies. The focus on both efficacy and safety is crucial to ensuring that new treatments not only combat disease effectively but also do so without causing harm to patients or the environment.

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17. Bioethical and human security approaches on evolution of drugs for vector borne diseases

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Bioethics and human security as one of the newest and most important methodological perspectives, defend human dignity, social resilience and equity, human rights and personal safety, and advocate for the elimination of human risks in the context of health changes and individual transformations. Social, economic, ethical, methodological, cultural or environmental processes, phenomena and events are interdependent, inter-influencing each other.

Given the magnification of the adverse effects of the contemporary global crisis which is characterized by widening complexity and multidimensioning, with such local, regional or temporary manifestations, appear a number of new risks and threats to national, regional or global security.

In this context, the human security converges to as a common set of approaches and methodologies for identification and elaboration of reliable solutions to contemporary crisis in which evolution of drugs for vector borne diseases in accordance with bioethical principles and imperatives is a logical and expected outcome in conditions of an absolute necessity to overcome the results of global multidimensional crisis.

Patients taking drugs for vector borne diseases, being one of the most vulnerable citizens in a contemporary society concentrated on individualistic and liberal values and practices, also have to be treated from the bioethical and human security perspective as well.

Bioethics and human security as civilizationist perspectives, offer for patients dependent on drugs for vector borne diseases, a chance to be treated with dignity and respect in the social environments, including in those hostile.

The imperative necessity of counteracting contemporary threats at the level of protection of human person can be fulfilled through re-conceptualization at local, regional and global scales, in methodological and bioethical ways, of the perspectives of strengthening human security

The imperative necessity of counteracting contemporary threats at the level of protection of human person can be fulfilled through re-conceptualization at local, regional and global scales, in methodological and bioethical ways, of the perspectives of strengthening human security.

18. Antitrypanosomal activity of D-ring modified steroid derivatives

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The development of new therapeutic agents is essential for the treatment of parasitic diseases, as the incidence rate is still unacceptably high. Many of the current therapeutics are limited by significant side effects, acquired resistance, or high cost, which has enabled the rational development of new antiparasitic therapies. Compounds presenting antiparasitic activity can be of natural or synthetic origin and are structurally very diverse, with steroid compounds also recognized as important antiparasitic agents [1]. Namely, steroids belong to one of the main classes of natural compounds with an important role in biological functions in the human body but also represent an interesting structural motif for further modifications. In this respect, it was found that introducing heteroatoms into the steroid molecule improves the biological activity of the compounds and leads to the emergence of new biological and pharmacological properties.

Research strategies for new drugs involve a multidisciplinary approach, including chemistry and biology. Therefore, our principal aim was the development of D-modified steroid derivatives as safe and effective antitrypanosomal agents. Structural modifications consisted on the introduction of different heterocyclic moieties into the D-ring of the steroid nucleus, such as 17a-homo lactone, 17-picoline or 17-picolinylidene. These derivatives were tested against several parasitic species, where only derivatives with 17-picolinylidene function (Fig. 1) showed selective activity against *Trypanosoma cruzi*.

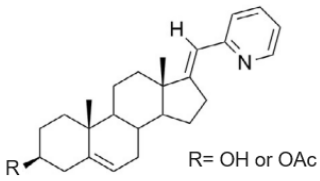

Fig. 1. 17-picolinylidene androstane compounds with antitrypanosomal activity.

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19. Toxicity, repellency, and anti-cholinesterase activities of essential oil and crude extracts of Ruta chalepensis, against vector of several major pathogenic diseases in livestock and poultry in Tunisia.

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Abstract

The current study assayed the toxicity of *Ruta chalepensis* essential oil and crude extracts obtained using solvents of increasing polarity (cyclohexane, acetone and ethanol), on two ectoparasites of veterinary importance, i.e., *Hyalomma scupense* and *Dermanyssus gallinae.*

Essential oil was extracted using hydrodistillation technique. Gas chromatography-mass spectrometry (GC-MS) was performed to identify the chemical composition of the tested plant. To evaluate the adulticidal, ovicidal, larvicidal and repellent proprieties of essential oi and ethanolic extract on *H. scupense* and *Dermanyssus gallinae, in vitro* assays were performed using the adult immersion test (AIT), the ovicidal test, the larval packet test (LPT), the filter paper test and anti-acetylcholinesterase (AChE) activity.

Determination of chemical composition revealed that leaves and flowers essential oils samples were dominated by ketone particularly 2-undecanone derivative accounting for 85.94 and 89.89% of leaves and flowers oils, respectively.

After treatment, 2-undecanone, the primary phytoconstituent of Ruta oil, which accounts for 90% of the whole oil, had 99.22% acaricide activity and inhibited egg hatching at a concentration of 10 mg/mL. 2-undecanone and Ruta essential oil showed potent adulticidal effect at high concentrations (10 mg/mL), achieving 100 and 93.76% mortality, respectively. The ethanolic extract exhibited moderate activity. At high concentration, the larvicidal activity of *Ruta* oil, 2-undecanone, and ethanolic extract were 100, 100, and 77.18%, respectively. In filter paper experiments, when tested at the concentration 5 mg/mL; 2-undecanone showed the longest repellent effect up to 6 h. We also found that 2-undecanone was the most active AChE inhibitor (IC_{50} = 0.178 mg/mL). Nevertheless, additional investigations are required to confirm the accurate mechanism and the relevance of Ruta in practical application.

Overall, our research indicated that, because its effectiveness as acaricide, *Ruta* essential oil and its phytoconstituent 2-undecanone may offer an alternative source for the control of tickborne diseases.

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Acknowledgment

This work received financial support from Laboratory of Bioactive Substances, Centre of Biotechnology of Borj Cedria, Tunisia.

20. Activity of novel heterocyclic compounds against promastigote and amastigote-like *Leishmania infantum* and *Leishmania tropica*

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Leishmaniasis is a neglected disease that affects 12 million people, primarily living in developing countries [1]. The disease occurs in various forms depending on the type of parasite: cutaneous (CL), visceral (VL) and mucocutaneous infections (MCL) [2]. Three primary Leishmania species are responsible for the most cases. While *Leishmania infantum (L. infantum)* causes VL, *Leishmania tropica (L. tropica)* and *Leishmania major (L. major)* cause CL [3].

The parasite exhibits two forms throughout its life cycle: Promastigotes with extracellular flagella in the vector and non-motile amastigotes in mammalian hosts after transmission [4]. As both forms can lead to initiation of the infections, targeting not only promastigote but also amastigote transition is one of the promising starting points for drug discovery studies [5].

Herein, based on the obtained data in our previous studies and literature survey we designed and synthesized novel chlorobenzamide derivatives thiazolo[5,4-*d*]pyrimidine ring. In the derivatization of the compounds, it was considered to determine the contribution of the number and the positions of chlorine atoms to the activity. Then, *in vitro* antileishmanial bioactivity of synthesized compounds on promastigote and amastigote-like forms of *L. tropica* and *L. infantum* parasites regarding the structure activity relationships. [6-8]. Additionally, we evaluated *in silico* physicochemical properties.

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21. Molecular modelling on antiparasitic nucleoside drugs: revealing mechanism of drug action

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Adenosine kinase (ADK; EC 2.7.1.20) is phosphotransferase type of enzyme that converts the purine ribonucleoside adenosine into 5'-adenosine-monophosphate.

$$\text{Adenosine} + \text{ATP} \xrightarrow{Mg^{2+}} \text{5'-adenosine-monophosphate} + \text{ADP}$$

ADK is an upstream regulator of adenosine, and thus affects its extremely short plasma half-life (<1 s). Inhibition of adenosine kinase results in increased intracellular adenosine which passes out of the cell via passive diffusion or via nucleoside transporter(s) to activate nearby cell-surface adenosine receptors. Consequently, ADK inhibition plays role as an alternative mechanism for activation of adenosine receptors and production of adenosine-associated potential inhibitors. Parasitic protozoa are incapable of *de novo* synthesis of the purine rings making themselves obliged to utilize a unique series of purine salvage enzymes to scavenge host and exogenous purines for their own growth and reproduction. Biophysical and biochemical characteristics of ADK isolated form *Leishmania Donovan* are different then ADK from other eukaryotic sources regarding to the mode of action.

Referring to the differences in purine salvage between *T. brucei* and *Leishmania* due to adenosine kinase is not subject to substrate inhibition, and it has a comparably low affinity for adenosine (K_m = 33 µM) making the major route of salvage to occur via cleavage to adenine, which is deaminated by adenine amidotransferase, a *Leishmania*-specific enzyme that does not exist in other studied trypanosomatids or in mammalian cells, we present results from *in silico* molecular modelling based on docking study for testing binding affinity of antiparasitic nucleoside drugs such tubercidin and other lead compounds testing the hypothesis whether ADK of Leishmania is less effective in phosphorylation reaction of nucleoside analogues then *Tb*ADK.

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22. Pathogens and vectors of zoonosis and vector borne diseases in Albania; a literature review

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In Albania data on anaplasmosis are registered only in animals, (goats, dogs) and circulating species are *Anaplasma phagocytophilum* and *A. Platys* (Mati 1987, Shukullari 2016). *Ehrichia canis* has also recorded in dogs and in transmited vector, the tick *Rhipicephalus sanguineus* (Shukullari 2016, Christova 2003). Anti-*Coxiella burnetti* antibodies have been found in animal farms; 9.8% in sheep and goats and 7.9% in cattle (Cekani, 2008). During the period 2003-2006, from the serological examination of 34 sera collected from patients in rural areas of Kukes and Has, 29.4% and 2,9% of the cases were positive for leptospirosis and rickettsiosis, respectively (Papa, 2008). *Leptospira grippotyphosa* and *L. Pomona* are confirmed in cattle (Alla, 2015) while the infection in humans circulates more in man where the range of the mortality varies from 3.84 – 8.58% (Puca, 2011; Puca, 2018). *Ehrlichia canis, E. Chaffeensis* and *Rickettsia conorii* are isolated in *Rhipicephalus sanguineus* during 2003. Meanwhile from *Rhipicephalus bursa* as the transmited tick, is isolated *Anaplasma phagocytophila*, *Ehrlichia chaffeensis*, *Rickettsia conorii* and *Rickettsia* sp. IRS3. *Hyaloma plumbeum* tick is a vector transmittion of *Rickettia helvetica* and *R. Conorii* (Christova, 2003).

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23. Analyzing High Throughput Malaria Rapid Diagnostic Test Effectiveness and Genetic Diversity in Dominican Republic: Considerations for Elimination Efforts

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Malaria is a significant health concern in tropical regions worldwide. Challenges include limited diagnostic tools, drug resistance, and the impact of COVID-19. We evaluated the accuracy of malaria Rapid Diagnostic Tests (RDTs) in the Dominican Republic (DR), and their limitations in assessing Plasmodium spp. genetic diversity, and explored factors contributing to the high prevalence of P. falciparum. Samples were collected from suspected malaria cases in DR, and underwent DNA extraction followed by quantitative polymerase chain reaction (qPCR) identified 2.3% of falsenegative RDTs with low-level parasitemia. Among the positive samples, all cases were P. falciparum except one P. vivax case. Additionally, we genotyped the Duffy domain rs2814778 mutation and found that 59% of samples exhibited the Duffy-negative mutation, indicating significant genetic diversity with potential implications for malaria susceptibility and transmission. The study highlights the limitations of RDTs in detecting low-density parasite infections, posing challenges for malaria elimination in the DR. The detection of P. vivax raises concerns about relapse, underlining the need for improved surveillance and disease management. These findings are crucial for shaping future malaria control strategies to achieve the 2025 elimination goal, emphasizing the importance of further investigations.

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24. 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).

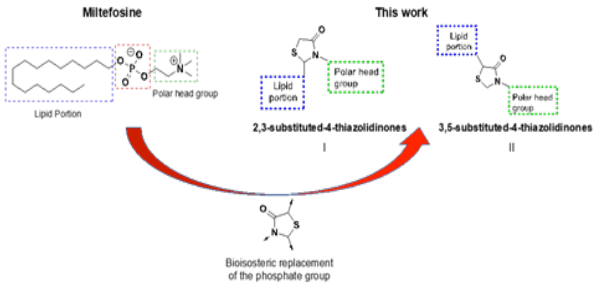

Figure 1. Design of the phosphate bioisosteres of the present study.

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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25. *Plasmodium falciparum* type-II NADH:ubiquinone:oxidoreductase as a possible target for bioreductively activated antiplasmodial agents

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Plasmodium falciparum type-II NADH:ubiquinone oxidoreductase (*Pf*NDH2) contains FAD in the active center and is attached to the matrix side of the mitochondrial inner membrane. It was supposed that *Pf*NDH2 inhibitors could be effective antiplasmodial agents, but this view later has become debatable [1]. On the other hand, another function of *Pf*NDH2, the reductive activation of potential antiplasmodial agents such as quinones (Q), aromatic nitrocompounds ($ArNO_2$) and *N*-oxides (ArN-O), remains insufficiently elucidated. Our data on the reactions of *Pf*NDH2 with these compounds (n = 40) can be summarized as follows: (i) the reactivity of Q, $ArNO_2$ and ArN-O (log k_{cat}/K_m) increases with increasing their single-electron reduction potential (E^1_7) following a common but strongly scattered parabolic relationship; (ii) the reactivity of quinones increases with their lipophilicity; (iii) *Pf*NDH2 reduces quinones in two-electron way with the formal features of 'ping-pong' mechanism; (iv) at close to saturating quinone concentrations, the reaction product NAD^+ (1.0-7.0 mM) acts as a linear competitive to NADH inhibitor; (v) at [NADH] >> K_m, NAD^+ acts as an incomplete competitive inhibitor to quinone with 60% maximal inhibition degree. NADH decreases the efficiency of NAD^+ inhibition, and the k_{cat}/K_m of quinone depends on the [NAD^+]/[NADH] ratio. This suggests that quinones, as has been shown for other NDH2, oxidize the complex of reduced FAD with NAD^+ and bind in a different domain than nicotinamide [2]. This implies a 'hybrid ping-pong' mechanism. A new finding is that a complex of reduced FAD and NADH may be involved in the reaction. The obtained data imply that *Pf*NDH2 may be responsible for the two-electron reduction of aziridinyl-substituted quinones which contributes to their enhanced antiplasmodial activity [3]. It may also generate the hydroquinone form of antiplasmodial agent 3-[4-(trifluoromethyl)benzyl]-menadione (plasmodione), which is a necessary intermediate in the formation of its active benzoyl metabolite [4].

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Acknowledgment

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26. Detection of *Strongyloides stercoralis* during the control of school-aged children in a rural area of Tirana.

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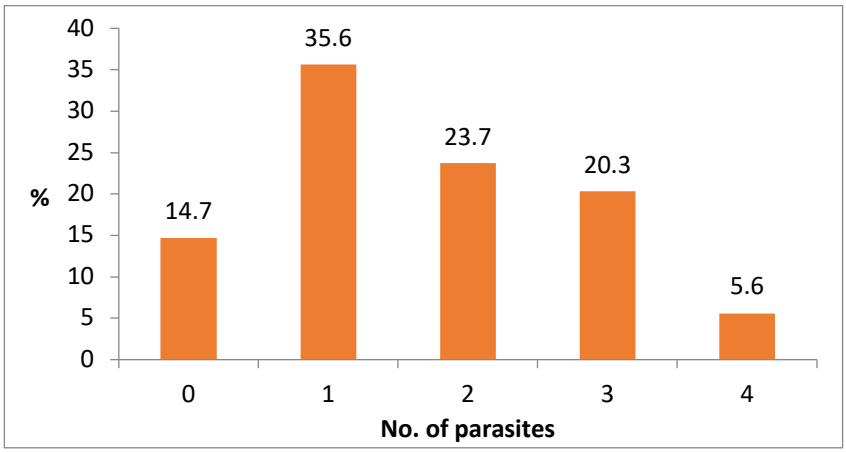
In retrospective, this study aims to evaluate the presence of the soil-transmitted helminths as neglected tropical diseases and other intestinal parasites of school-aged children, in a rural area of Tirana.
A cross-sectional study of 177 school-aged children, 102 (57.6%) female and 75 (42.4%) male, was conducted in the village of Larushk, a rural area of Tirana, during 2002, to assess the presence of soil-transmitted helminths and other intestinal parasites. The formalin-ether concentration technique (FECT) was performed to exam the collected formaldehyde-preserve stool samples.
A total of 151 (85.3%) 95%CI (72.9 – 90.2) out of 177 school- aged children resulted positive for intestinal parasites. Among them *Trichuris trichiura* is the most prevalent with 97 (54.8%) positive followed by *Blastocystis hominis* 68 (38.4%), *Entamoeba histolytica* 46 (26.0%), *Giardia lamblia* 41 (23.2%), *Ascaris lumbricoides* 23 (13.0%), *Hymenolepis nana* 18 (10.2%), and 1 (0.6%) *Enterobius vermicularis.* The *Strongyloides stercoralis was found in a 9 years old girl.* Most of the cases 63 (35.6%) are infected with only one parasite followed by two parasites 42 (23.7%), three 36 (20.3%) and with four parasites 10 (5.6%) of the total (Graph).
The results of our study reveal that soil-transmitted helminths and other intestinal parasites represents a public health problem for school-aged children, in rural areas where the sanitation and hygiene conditions are poor.



27. Vivax malaria cases imported from Greece during 2010-2016

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Albania is a malaria free country and since 1967 no autochthonous malaria cases are registered, only imported.
In retrospective four cases of imported *Plasmodium vivax* are registered in the period of 2010-2016 in Albanian citizens traveling to Greece.
A questionnaire completion was performed for each case of malaria. No blood transfusion and/or organ transplant is registered. All of them at the active age group of 17 - 35 years old as seasonal workers.
All cases have no travel history to malaria endemic countries, but traveled to Greece for seasonal working in areas of Chalkidiki, Tanagra, Viotia and Larissa known as well as areas where autochthonous cases are reported (Vakali, 2012; https://eody.gov.gr)
The first case, a 17 years old male is diagnosed in May 2010 followed by the second case diagnosed in September 2012, a 24 years old male. The third case was a 22 years old male, diagnosed in August 2015 and the last one a 35 years old male, diagnosed in August as well, of 2016.
Suspected cases are hospitalized in Infectious Diseases Service, University Hospital Center Mother Theresa in Tirana.
The diagnosis was confirmed following the EU case definition with demonstration of malaria parasites by light microscopy of Giemsa stained thick and thin blood smear (on separate slides each).
Malaria should be considered as differential diagnosis in travelers returning from Greece.

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28. Novel Napthyl Indolyl maleimides (NIM's) as inhibitors of kinase and new leads in the treatment of Leishmaniasis

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Leishmaniasis is a parasitic disease which affects about 6 million people globally. With one million new cases registered per year there is a pressing need for new medicines to treat this debilitating disease. Current pharmaceutical treatment options include drugs such as amphotericin B, antimony, fluconazole and miltefosine, neither designed nor optimized for parasitic disease. Recently, with the advent of kinase inhibitors, key stages of the parasitic life cycle can now be targeted with potentially dramatic effects on leishmania.[1]

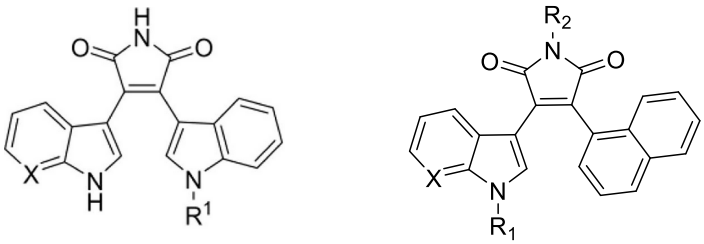

Figure 1 Structures of bisindolylmaleimides and naphthylindolyl maleimides (NIM's)

Work in our laboratory has previously disclosed the synthesis of substituted bisindolylmaleimides (BIM's) and related analogues with potent kinase inhibitory activity (Figure 1).[2] We hereby describe for the first time the synthesis of a series of naphthyl indolyl maleimides (NIM's) and report kinase inhibitory activity against both human and parasitic kinases. Variation of the maleimide headgroup and indole subunit reveal new lead compounds for drug discovery.

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29. Comparative analysis of activated cysteine residues in human and parasitic enzymes: potential for covalent inhibition

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The search for new therapeutic agents against parasitic infections requires an understanding of the active sites of enzymes, especially those with activated cysteine residues. This study identifies and characterizes these residues in enzymes of *Trypanosoma brucei*, *Leishmania* spp. and *Plasmodium falciparum*, and compares them with their human counterparts. We focused on enzymes such as GAPDH, ALDH, cysteine proteases, trypanothione reductase and glutamine amidotransferases, and identified activated cysteine residues involved in catalytic diads or triads with a p*K*a of about 6. These enzymes are crucial for parasite survival and replication, making them attractive drug targets. We have established a comprehensive library of these target enzymes and performed covalent docking studies with compounds from our in-house library[1] as well as novel derivatives. Using 3-bromo-4,5-dihydroisoxazole as a reactive warhead, we targeted cysteine residues via a nucleophilic substitution mechanism and displaced the bromine atom. Preliminary results indicate that several parasitic enzymes have conserved cysteine residues analogous to those in human ALDHs, suggesting a potential for selective inhibition. A comparative analysis reveals structural differences that could increase specificity and reduce off-target effects. This research lays the foundation for the development of covalent inhibitors that target parasitic enzymes and offer a promising avenue for therapeutic intervention. Further validation through biochemical assays and in vitro studies is essential to confirm these findings and progress towards optimized antiparasitic agents.

References

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30. Molecular Detection Of Filarial Nematode Parasites In Mosquitoes From Albania

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Key words: Parasitic mosquito-borne diseases, filarial nematodes, mosquitoes, Albania.

Mosquito-borne parasitic diseases are a real public health concern worldwide, while their burden from adult mosquitoes has not yet been studied in Albania. *Aedes* and *Anopheles* mosquitoes are the most competent vectors for transmission of filarial nematodes to human and animals (dogs). Studies in Albania have shown their presence in animals and humans in the country. We intended to test a collection of adult mosquitoes over a period of three years (2019-2021) in some regions of Albania. Adult collection was performed via different adult traps like BG-Sentinel+Lure+CO_2 and light traps augmented with CO_2. We monitored 12 stations/locations that were regularly sampled every two weeks during the three-year period. Collected adults were transported on dry ice to the lab and identified on dry ice to protect potentially parasite-infected mosquitoes from degradation. Females and males were placed separately in tubes together with two 3mm metals beads to allow for disruption and lysis of the tissue. A volume of 500µl of media solution was added to samples containing one individual, and 750µl to samples with 2-25 individuals of adult mosquitoes. We tested only *Aedes albopictus* and the Anophelinae (*Anopheles maculipennis* complex species and *Anopheles hyrcanus*) mosquitoes. Pools of lysed mosquitoes were extracted with the NucleoMag VET Kit in a KingFisher Flex. For detection of filarial nematode DNA, a filarioid-specific real-time PCR assay was performed with the QuantiTect SYBR Green PCR Kit (Czajka et al. 2012). We examined a total of 206 samples and a total of 1543 adult female mosquitoes of the three species mentioned above. The results of the PCR showed that no filarial nematodes were present in the collected adult mosquitoes. These mosquito species are the vector of parasitic diseases in Albania and pose a potential threat for the spread of these parasitic infectious diseases to humans and animals in Albania. Despite the absence of the filarial nematodes in adult mosquitoes in Albania, their presence in animals and humans indicates that adult mosquitoes play an important role as vectors between infected and non-infected animals. Thus, we encourage further studies to determine the burden of these filarial nematodes. In addition, programs and strategies to support mosquito control in the country are strongly recommended to prevent the spread of these parasitic diseases to human and animals.

31. Adamantane Nitro-Heterocyclic Derivatives with Activity against *L. infantum*

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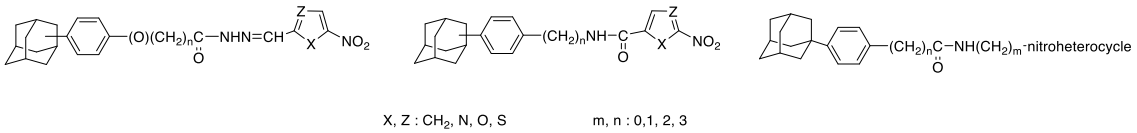
The available drugs against neglected tropical diseases (NTDs) are characterized by toxicity, limited efficacy and increasing resistance. This has led the World Health Organization (WHO) to coordinate public sector and private partnerships as part of a global effort to develop new and safer dugs [1].

Herein, we present the synthesis and pharmacological evaluation of 4-(adamant-1- or 2-yl)phenyl-5-nitrofuramides, 4-(adamant-1- or 2-yl)phenyl-2-nitroimidazole acetamides, 4-(adamant-1-yl)phenyl-2-nitroimidazole alkyl acetamides, 4-(adamant-1-yl)phenyl-3-nitrotriazole alkyl alkylamides, hydrazones of 5-nitro-1H-2-imidazolcarboxaldehyde with adamantanephenylalkanohydrazides, hydrazones of 5-nitro-2-thiophenecarboxaldehyde with adamantanephenylalkanohydrazides and hydrazones of 5-nitro-2-furaldehyde with adamantanephenylalkanohydrazides. We have previously noticed that the structural characteristics of the benzene-substituted adamantane scaffold and the 5-nitrofuran heterocycle appear to provide optimum potency against *T.brucei* and *T.cruzi* at the nanomolecular scale [2], and we expand the evaluation of the abovementioned derivatives against *L. infantum* promastigotes, as well. Most of the hydrazones and the amides show high anti-leishmanial activity. However, the type of bond and the length of the spacer affect the pharmacological activity as well as the cytotoxicity, with hydrazones clearly endowed with the highest therapeutic indices. The structural modification comprising of a phenyl ring insertion between the adamantane core and the hydrazone side chain has improved the pharmacological profile, in terms of activity and toxicity.



X, Z : CH_2, N, O, S m, n : 0,1, 2, 3

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