Advancements in Structural and Functional Drug Discovery of Vector-borne Diseases
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1. Assessment of giardiasis, patients' characteristics and risk factors

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Introduction: Giardia lamblia is an intestinal protozoan parasite that causes giardiasis, a serious infection with global implications for public health. It is one of the most important human intestinal parasites, infecting domestic and wild animals. This study was conducted to determine the prevalence, patient characteristics, and risk factors for G. lamblia infection.

Methods: This was year-long descriptive research. Stool samples were collected from 741 suspected participants and examined using formalin-ether sedimentation methods in the laboratory. We used a standard questionnaire to acquire information on their demographics, socioeconomic status, clinical symptoms, underlying morbidities, and disease risk factors. We calculated data using the statistical software SPSS, version 26.0. A p-value of less than 5% was thought to be statistically significant.

Results: The overall prevalence of Giardia infection among suspected patients was 21.45% (159/741). The average age was 7.4±3.8StD, ranging from 2.5 to 15 years old. The age distribution was 42.78% (317/741) between 2.5-5 years and 57.22% (427/741) between ≥6-15 years. In terms of gender, 57.9% (429/741) of the children were male and 42.1% (312) were female, whereas the positive rate was higher in males 55.3% (88/159) than females 44.7% (69/159), without statistical significance (p value=0.59; OR = 0.90; CI: 0.63-1.3). Some risk variables were shown to be significantly associated with Giardiasis infection. For instance, being 4-10 years old, attending kindergarten, living in a community with other children, having an animal (dog), having a brother or sister at home throughout pediatric age, etc. ($p<0.05$). The most common clinical complaints among children were diarrhoea (29%), abdominal pain (17%), anorexia (4%), bloating, and nausea (11%). After suffering recurring symptoms, around 19.5 of the patients who received particular therapy for Giardiasis infection returned to the laboratory to be re-diagnosed.

Conclusion: Giardiasis among children still is an important problem in public health, especially in our country. Predisposing factors influence the number of positive cases. Parents should pay attention when symptoms are evident, as these persons and their family members should be diagnosed immediately, this infection is contagious, and more cases are asymptomatic. Immediate treatment of positive cases, as well as follow-up until they become negative would be a crucial step toward disease prevention.

Keywords: Giardiasis, pediatric age, specific symptoms

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2. Discovery of novel drug action mechanisms by studying antileishmanial aminopyrazoles

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Substantial advancements have been made in the discovery of novel antileishmanial leads and clinical candidates by phenotypic evaluation on intramacrophage amastigotes of the visceral Leishmania species. Aminopyrazoles have emerged as a promising series and hit-to-lead optimization by the Drugs for Neglected Diseases initiative (DNDi) resulted in compounds with highly potent activity in animal models of leishmaniasis.

Molecular target deconvolution for the most potent aminopyrazoles has proven to be a major challenge because successive drug exposure failed to select for stably resistant phenotypes. Chemical mutagenesis with either ethyl methanesulfonate (EMS) or N-ethyl-N-nitrosourea (ENU) combined with drug selective pressure and whole genome sequencing was used as an alternative approach. From the obtained panel of 28 resistant lines an association between >10-fold resistance and multiple independent heterozygous mutations adjacent to the Zn2+ binding site of the zinc finger containing protein LINF_180011100 was discovered. Overexpression of the mutated gene increased resistance up to 10-fold, whereas susceptibility could be restored in mutant lines by transfection of a wildtype copy. Gene editing by CRISPR-Cas9 independently confirmed the contribution of the EMS and ENU mutations, resulting in H594Y and H594P substitutions respectively, to 10-32-fold resistance exhibited both at the extracellular promastigote and intracellular amastigote stage. The predicted protein structure reveals five Ran protein domains and a C- terminal FYVE domain with multiple Zn2+ binding sites. Prediction of the molecular function of LINF_180011100 suggests a role in nucleocytoplasmic transport, cell trafficking and cell cycle control. Genetic fusion of the gene with an N-terminal GFP tag demonstrated that the protein primarily localises in cytoplasmic /endocytic vesicles.
Collectively, our data provide a sequential validation of LINF_180011100 as a drug target for several aminopyrazole leads and gives unprecedented insights in the mechanism of action.

3. Natural product against *Cryptosporidium*: *In vitro* and *in vivo* assays

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(1) Background: This study investigated the toxic activity of *Artemisia judaica* ethanolic extract (ArEx) as well as its phenolic fraction (ArPh) and terpenoid fraction (ArT) against *Cryptosporidium parvum* (*C. parvum*) oocysts.

(2) Methods: Estimation of the total phenolic (TPC), total flavonoids (TFC), and total terpenoids contents (TTC) in ArEx; investigation of the *in vitro* antioxidant activity of ArEx, ArPh, and ArT; evaluation of ArEx, ArPh, and ArT toxic activity against *C. parvum* oocysts using MTT assay; on ArPh-treated *C. parvum* oocysts, parasitological analyses and comet assay were performed both *in vitro* and *in vivo* (infectivity).

(3) Results: The ArEx TPC, TFC, and TTC was 52.6±3.1 mgGAE/g, 64.5±3.1 mg QE/g, and 9.5±1.1 mg Linol/g respectively. Regarding the phytochemical *in vitro* antioxidant activity, the ArPh exhibited the highest antioxidant activity compared to the ArEx and ArT. The ArPh showed promising free radical scavenging activity of DPPH and ABTS•+ with IC50 values of 47.27±1.86 µg/mL and 66.89±1.94 µg/mL, respectively. Moreover, the FRAP of ArPh was 2.97±0.65 mMol Fe+2/g while its TAC was 46.23±3.15 mg GAE/g. The ArPh demonstrated toxic activity against *C. parvum* oocysts with a potent IC50 value of 31.6 µg/mL compared to ArT (promising) and ArEx (non-effective). ArPh parasitological analysis demonstrated MIC90 at 1000 µg / mL and effective oocysts destruction on count and morphology. ArPh fragmented oocysts nuclear DNA in the comet assay. Beginning at 200 µg/mL, ArPh-treated oocysts did not infect mice. (4) Conclusions: To combat *C. parvum* infection, the phenolic fraction of *A. judaica* L. shows promise as an adjuvant therapy or as a source of potentially helpful lead structure for drug discovery.

4. The use of medicinal plants against vector-borne diseases: Evaluation of in vitro leishmanicidal activity of the Chamomile essential oil

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According to the World Health Organization, leishmaniasis is considered as a major neglected tropical disease causing an enormous impact on global public health. Available treatments were complicated due to the high resistance, toxicity, and high cost. Therefore, the search for novel sources of anti-leishmania agents is an urgent need. In the present study, an in vitro evaluation of the leishmanicidal activity of the essential oil of Tunisian chamomile (Matricaria recutita L.) was carried out. Chamomile essential oil exhibits a good activity on promastigotes forms of L. amazonensis and L. infantum with a low inhibitory concentration at 50% (IC50) (10.8 ± 1.4 and 10.4 ± 0.6 µg/mL, respectively). Bio-guided fractionation was developed and led to the identification of (-)-α-bisabolol as the most active molecule with low IC50 (16.0 ± 1.2 and 9.5 ± 0.1 µg/mL for L. amazonensis and L. infantum, respectively). This isolated sesquiterpene alcohol was studied for its activity on amastigotes forms (IC50 = 5.9 ± 1.2 and 4.8 ± 1.3 µg/mL, respectively) and its cytotoxicity (selectivity indexes (SI) were 5.4 and 6.6, respectively). The obtained results showed that (-)-α-bisabolol was able to activate a programmed cell death process in the promastigote stage of the parasite (1). It causes phosphatidylserine externalisation and membrane damage. Moreover, it decreases the mitochondrial membrane potential and total ATP levels. These results highlight the potential use of (-)-α-bisabolol against both L. amazonensis and L. infantum, and further studies should be undertaken to establish it as novel leishmanicidal therapeutic agents. References (1).Inacio JD, Gervazoni L, Canto-Cavalheiro MM, Almeida-Amaral EE (2014). The effect of epigallocatechin 3-O-gallate in vitro and in vivo in Leishmania braziliensis: involvement of reactive oxygen species as a mechanism of action. PLoS Negl Trop Dis 8(8):e3093

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5. Drug formulation, bridging the gap from in vitro experiments to in vivo results

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Therapeutic arsenal against Vector Borne Protozoal Diseases (VBPD) is scarce and the available drugs have important drawbacks and launching of new chemical entities (NEC) has slowed down. A key component of the "druggability" of a potentially useful molecule is its bioavailability. This aspect, frequently overlooked by researchers, is critical in the translation process from a molecule to a drug.

Most promising active molecules are poorly soluble products that should be dissolved to test their activity and toxicology. However, dissolution and potential degradation of these molecules after dissolution can compromise *in vivo* experiments. Before starting *in vivo* trials, it is important to study the dissolution and stability characteristics of these potentially active molecules. HPLC assay methods are the most efficient way to chemically characterise these two critical properties, solubility and stability. Chemical testing can be used to determine the best dissolution conditions, such as pH, choice of cosolvents and other solubilising excipients, and to check stability. These dissolution conditions depend on the route of administration to be used in the *in vivo* experiments. For example, pH requirements are not the same for oral and intravenous administration (e.g., hydroxypropyl ß-cyclodextrin is a suitable solubiliser for oral administration but not for parenteral administration). Excipient concentration is also an important issue and sometimes too high a concentration of solubilisers can be associated with toxicity and poor bioavailability results. All these aspects are studied in formulation studies, including aggregation state of the molecules, and should be considered during drug development, especially prior to the animal testing phases.

6. Biological Properties and In Silico Studies of Thiazolopyrimidine Derivatives Active Against Visceral and Cutaneous Leishmania spp. Amastigote Forms

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Visceral leishmaniasis, the most severe form, is caused by *L. donovani* and *L. infantum*; cutaneous leishmaniasis is the most common endemic form of leishmaniasis and mainly caused by *L. tropica* and*L. major*. A series of thiazolopyrimidine derivatives were reported possessing anti-promastigote activities against *L. tropica*, *L. infantum* and *L. major* parasites in our previous studies (1,2). Here, we investigated their activities against *L. donovani* and *L. major* axenic amastigote, intra-macrophage amastigote forms and evaluated their cytotoxicity on macrophages to assess selectivity.

Most of the tested compounds found non-cytotoxicity on the RAW264.7 macrophage cell line and more importantly, their anti-amastigote activity was as potent as positive control, miltefosine. Last but not least, several ligand- and structure-based *in silico* studies was performed to elucidate the mechanism of action.

Taken together, these results confirm the antileishmanial activity of the previously reported novel class of thiazolopyrimidine scaffold and demonstrate the promising results for the generation of new lead compounds for treating visceral leishmanias and cutaneous leishmaniasis.

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7. Possibilities for treating parasites in fish with essential oils and plant extracts

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Aquaculture has overgrown in recent years, representing a good source of food supply, improving the health of the population and the planet, as long as it is done in a manner that is environmentally friendly, socially responsible, and considers food safety and animal welfare. However, infectious disease outbreaks, especially parasite diseases, have also increased in aquaculture, causing a serious reduction of fish population which leads to huge economic losses. Fish farmers have applied conventional treatments such as anti-parasitics and chemical compounds to control fish parasites. Still, previous studies have shown an accumulation of these chemical residues in fish tissues, and a negative environmental impact on aquatic organisms, especially in aquaculture in open waters where drugs are not easily controlled. Nowadays, scientists and practices are developing an alternative to conventional methods, in which many plant-derived compounds such as essential oils and plant extracts from selected plant species have been used as an efficient treatment to control parasites in freshwater and marine aquaculture systems.

Terpenes, terpenoids, alkaloids, flavonoids, saponins, coumarins, and phenolics, as well as their synergistic relationship represent bioactive compounds that are part of essential oils, as secondary metabolites of medicinal plants. The hydrophobic compounds of essential oils can penetrate the parasitic cells causing cell deformities and organelles dysfunctions. On the other hand, the using of essential oils in the fish diet can also modulate growth, immunity, and resistance to infectious diseases in fish.

Many compounds isolated from plant extracts have a great potential to prevent and control fish parasites in aquaculture, especially against protozoans, myxozoans, and monogeneans. However, serious research is necessary to determine the sufficient concentration for the administration, seems that oral administration through the feed has been the most suitable way in aquaculture.

The use of plant-derived compounds, as well as the potential for discovering new essential oils, plant extracts, and bioactive compounds have continuously increasing recent years, in terms of phytotherapy, but there is a need for further examinations to prove the efficiency of these plant-derived compounds and their pharmacological activities for controlling fish parasitic diseases.

8. Novel insights from animal models into uncharted Leishmania biology

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For a plethora of infectious diseases, host sanctuary and pathogen quiescence are increasingly understood to determine treatment outcomes. Infection of rodents with bioluminescent and fluorescent transgenic parasites revealed that stem cells in the bone marrow act as a source of relapse in visceral leishmaniasis [1]. We discovered that these cells harbour exceptionally high numbers of parasites, a feature that was also confirmed for human stem cells. Dissecting the underlying mechanisms by immunophenotyping, transcriptomics and reverse genetics identified a unique transcriptional 'StemLeish' signature and coordinated pathways involved in regulating Leishmania infection.

We also identified these stem cells as a unique cellular niche where parasites enter into quiescence [2]. Transcriptional studies were conducted to compare the quiescent and non-quiescent metabolic states. Evidence was found for quiescence resulting from a rapid evolutionary adaptation response that confers enhanced resistance to treatment. Exploiting the asset of a sand fly colony at LMPH, one of the few facilities worldwide, the fitness of parasites that transitioned through a quiescent state was also assessed. The observation of an increased cellular infectivity and efficient transmissibility by sand flies highlights that quiescence-associated traits pose a significant threat and can efficiently propagate.

As the current R&D pipeline does not yet specifically explore aspects of sanctuary and quiescence, initiatives will be presented for the discovery of novel assays, targets and drugs to reduce the risk of relapse and subsequent propagation.

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9. Antiparasitic drug discovery and emerging scaffolds with predictive low environmental impact

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Vector-borne diseases (**VBDs**) are caused by parasites, bacteria, or viruses and account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually (www.who.int/news-room/fact-sheets/detail/vector-borne-diseases). Leishmaniasis, trypanosomiases, schistosomiasis and malaria, are the most popular and debilitating VBDs worldwide, affecting more than one billion of the poorest people in the globe, while Babesia

infections are exponentially increasing. These infectious diseases can be transmitted via vectors among humans, among animals, or from animals to humans or may have animals as reservoir.
The existing medications for these parasitic VBDs suffer from a variety of problems including serious side effects, the requirement of long-term and expensive treatment, few therapeutic options and the development of parasite resistance other than reduction of the therapeutic efficacy due to environmental factors.
The COST Action CA21111 "OneHealthdrugs" aims at coordinating the discovery of drugs halting human and animal VBDs keeping with the principles of optimal profile for both organisms. In particular, the project OHD2: Antiparasitic drug discovery and emerging scaffold with predictive low environmental impact aims to foster the collection of antiparasitic compounds from different sources and associate the
chemotypes with in silico prediction of their molecular properties and ecotoxicological profile.

To achieve this the following strategy 2 steps are proposed. Regarding step 1, the following is proposed. A) Collection of published compounds during the period 2019-2024 with activity against Leishmaniasis, Human African Trypanosomiasis, Chagas Disease, Malaria, Babesia and Schistosomiasis. The selection was based on Target Product Profiles (TPP) set for hit compounds for each of the mentioned diseases, ensuring a diversity of chemotypes; B) Selection of the most diverse scaffolds based on computational analysis combining structural features, biological results (on target/phenotypic) and pre-clinical data and C) Evaluation of in silico molecular and ecotoxicological properties for the identified scaffolds of interest (COMPoI).

A second step of the OHD2 compounds database project is the collection of in-house compounds from the COST Action participants and external interested collaborators in a structured Database OHD tool. The OHD database will include chemical-specific information for antiparasitic drugs enriched with target specific and off-target effects including ecotox properties (https://doi.org/10.1093/bioadv/vbad045).
This approach will enable to identify at an early stage the most appropriate scaffolds for further medicinal chemistry development in the VBPD field showing a low environmental impact in the early phase of the drug discovery process. The project OHD2 is aligned with the objectives of Working Group 1 (WG1) (Compound libraries coordination and integration of compound design) and Working Group 2 (WG2) (Integration of early phase studies and low environmental impact actions). OHD2 aims at increasing the number of compounds available for the drug screening campaigns adopting virtual or phenotypic approaches thus, fostering innovation in antiparasitic drug discovery, promoting safer and more environmentally friendly drugs not only for VBPDs but for other diseases as well broadening the scope of this coordinated activity within "OneHealthdrugs".

10. Leishmania infantum ribose 5-phosphate isomerase a validated drug target

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Neglected tropical diseases caused by kinetoplastid parasites place a significant health and economic burden on developing nations worldwide. Current therapies are largely outdated and face mounting drug resistance from the causative parasites. Thus, there is an urgent need for drug discovery and development. Target-led drug discovery approaches have focused on the identification of parasite enzymes catalysing essential biochemical processes, which significantly differ from equivalent proteins found in humans, thereby providing potentially exploitable therapeutic windows. One such target is ribose 5-phosphate isomerase B (RpiB), an enzyme involved in the nonoxidative branch of the pentose phosphate pathway, which catalyses the interconversion of d-ribose 5-phosphate and d-ribulose 5-phosphate [1-3]. Although protozoan RpiB has been the focus of numerous targeted studies, compounds capable of selectively inhibiting this parasite enzyme have not been identified. In this study, we present the results of a fragment library screening against Leishmania infantum RpiB (LiRpiB), performed using thermal shift analysis. The hit fragments were shown to be effective inhibitors of LiRpiB in activity assays, and several fragments were capable of selectively inhibiting parasite growth in vitro.
These results support the identification of LiRpiB as a validated therapeutic target. The X-ray crystal structure of apo LiRpiB was also solved, permitting docking studies to assess how hit fragments might interact with LiRpiB to inhibit its activity [4].

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11. Survey on repurposing of anti-parasitic drugs in babasia treatment

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Drug repurposing (DR)/ repositioning is an effective strategy in discovering or developing new pharmacological/therapeutic indications utilizing already approved, conventional drugs or that ones in pipeline. Though many anti-malaria drugs, such as artesunate, artemether, dihydroartemisinin, and chloroquine, lumefantrine, have been repurposed in treatment of Babesia, their efficacy against parasite infection at the selected dose has been decreased [1].

We present the review of case studies of anti-malaria drugs with potent anti-babnesia activities. Inefficiency in inhibition of early-stage of parasite development that exerts chloroquione in babesia therapy is due to lacking of hemozoin in parasite specimen Babesia comparing to Naphtoquine phosphate salt which mechanism of action has been assessed to inhibition of hemozoin bio-crystallization in the digestive vacuole at the late-stage of parasite and disruption of the membrane system. Case study on modelling of *B. bovis* Thrombospondin-Related.

Anonymous Protein, TRAP1 and TRAP4 tertiary structure reveals proteins folded the metal-ion-dependent adhesion site (MIDAS) domain structure of *Plasmodium* TRAP proteins may serve as potential vaccine targets to prevent infection of bovine and ticks with *B. bovis* essential for controlling the spread of bovine babesiosis. [2]

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

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One of the main research topic of the CBM team is focused on the development of redox-active antiparasitic drug-candidates based on the 3-benzylmenadione (bMD) core (1). Two strategies are currently being developed for the identification/visualisation of the biological targets of plasmodione (PD), an antiplasmodial bMD. So far, the cellular target(s) of PD have been essentially investigated through the phylogenetically distant yeast model (2), or by hypothesis-driven targeted experiments with *P. falciparum* proteins (1,3).

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. After method validation, it has been applied to *P. falciparum* cell extracts (5).

Although *P. falciparum* can be cultured in vitro and manipulated genetically, the blood stage ring stage is one of the smallest eukaryotic cells known (~1.5 µm), which is a considerable hurdle for microscopic studies (6). On the other hand, the closely-related apicomplexan parasite *Toxoplasma gondii* is much more amenable to cell biology studies because of the availability of many cell markers and the relative ease with which the parasite can be studied with various microscopic techniques. *T. gondii* and *P. falciparum* share much of their underlying biology, making *T. gondii* an attractive model to decipher key features more generally related to the Apicomplexa phylum (7).
The second strategy uses the CuAAC reaction between the alkyne probe and a fluorophore azide to visualise the locus of the probe in bMD-treated *P. falciparum trophozoites* and *T. gondii tachyzoites* parasites (8).

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13. New amides of shikimic acid as powerful antimicrobial agents – synthesis, in vitro and in silico studies

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Background
The wide spread of bacterial infections caused by a variety of pathogenic bacteria with proven resistance is an ongoing worldwide health problem. The treatment of patients infected with resistant strains is ineffective, time consuming and expensive. To overcome these unfavorable trends, it is necessary to develop new antibacterial agents. The use of natural products is already spreading. Novel compounds designed were attack shikimate kinase, which is vital for the metabolism of plants, bacteria and fungi, but is not present at all in mammals and humans. Here, we performed synthesis of new compounds and tested their efficiency on different pathogenic bacteria.

Methods
Twelve amides of triacetyl shikimic acid were synthesised in two steps. First step includes preparation of triacetyl shikimic acid from readily available natural shikimic acid. Desired amides were prepared in the second step by implementation of coupling reactions between triacetyl shikimic acid and different amines. All the compounds were subjected to ADME predictions by a computational method. The in vitro antimicrobial activity of was determined by broth microdilution method according to ISO 20776-1:2006 against *Escherichia coli, Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*.

Results
The amides were obtained in pure form and fully characterised by NMR and analytical methods. Promising docking scores were obtained for all compounds in different protein targets, confirming the results from in vitro experiments. The interactions in the proteins binding sites were thoroughly analysed. Many of the compounds showed very good antibacterial and antifungal activities, compared to gentamicin and amphotericin B. One was the most active against all bacteria and fungi tested, with MIC values, comparable to those of gentamicin. Two of them are significantly active against S.aureus and MRSA. Only one possessed strong activity against E. coli. Four showed moderate activity against P. aeruginosa comparable to gentamicin. Among the individual compounds, one showed the weakest antibacterial effect against all bacteria tested.

Conclusions
More of the compounds demonstrated very good antibacterial activity against different pathogenic bacteria, compared to the reference antibiotics and can be used as a starting point

for further in-depth studies of their pharmacokinetics and other biological in vitro and in vivo properties.

Acknowledgements
We gratefully acknowledge support from Bulgarian Science Foundation (Grant KP-06-H39/7).
Keywords: new synthesised natural shikimic acid compounds, antibacterial activity, molecular docking

14. Mitochondrial NADH/NAD+ balance in Leishmania survival and its interest for chemotherapy

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Regulation of the mitochondrial NADH/NAD+ ratio is crucial for maintaining a metabolic state compatible with cell survival. During their life cycle, Leishmania spp. adapt to different environments in insect and mammalian hosts, undergoing several morphological and metabolic changes. Consequently, Leishmania parasites possess several enzymes involved in mitochondrial NADH oxidation, including the canonical complex I, fumarate reductase (FDR), and type II NADH dehydrogenase (NDH2). The expression and relative contribution of these systems in maintaining mitochondrial redox balance across different environments remain unclear as is their value as drug targets.

We evaluated the expression of these enzymes in Leishmania infantum (a visceralising species, Li) and L. major (a cutaneous species, Lm) through western blot analysis and oxygen consumption assays with intact parasites. Subcellular localisation was assessed using immunofluorescence studies and western blot analysis. Gene deletion was performed using CRISPR-Cas9 techniques, and the mutants' ability to thrive in animal models of infection was evaluated in mice.

NDH2 protein is expressed in both the promastigote and amastigote stages of L. infantum, whereas complex I activity was not detected. Overexpression of LiNDH2 increased basal oxygen consumption of intact parasites, confirming its role as a component of the respiratory chain. Moreover, we found that LiNDH2 is essential in L. infantum, including in the disease-causing stage. NDH2 is also essential in L. major, a species that expresses active complex I.

Complex I is dispensable in L. major provided NDH2 is sufficiently expressed. L. infantum promastigotes tolerate complex I disruption, which aligns with the negligible expression of the enzyme in this species. However, these parasites are less virulent in mice, suggesting a potential role for complex I in intracellular amastigotes. FRD is expressed in promastigotes of L. major at higher levels than in L. infantum. This protein is not required for parasite survival, even in the context of in vivo infections, however, Lifrd-/- mutants are less virulent than wild type in mice.

From a drug perspective point of view, our findings i) unravel NDH2, a protein without counterparts in mammals, as a promising target for developing leishmanicidal drugs, and ii) cast doubts on the value of both complex I and fumarate reductase.

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15. Exploring new frontiers in fighting animal trypanosomiasis: assessing antitrypanosomal and (eco)toxicological characteristics of novel nucleoside-based leads

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Animal trypanosomiasis (AT) is a widespread disease caused by *Trypanosoma spp.* and has a devastating effect on animal husbandry all over the world due to the scarcity of efficient drugs and development of drug resistance, hence emphasizing the need for novel treatment options. Following previous identification of 3'-deoxytubercidin as a highly potent trypanocide with curative activity in mouse models of both stage-1 and stage-2 Human African Trypanosomiasis (HAT), we now present a comprehensive preclinical evaluation of new 6-amino substituted tubercidin analogues with promising activity against a broad range of AT species. Potent hits were identified in vitro across all important AT species, i.e. *Trypanosoma brucei brucei, isometamidium (ISM)-resistant* and -susceptible *Trypanosoma congolense, Trypanosoma vivax, Trypanosoma evansi (type A and B)* and *Trypanosoma equiperdum*. Selected 'hits' were further tested for in vitro metabolic stability (using bovine, horse and piglet liver microsomes), in vivo mouse models for each AT species, genotoxicity assays and mode-of-action studies (i.e. genome-wide RNA interference library screening, metabolomics). Analogue 3 was highly active in *T. vivax, T. congolense, T. equiperdum, T. evansi and T. brucei* curative mouse models. Furthermore, there was no indication of in vivo toxicity or in vitro genotoxicity in Vitotox®, micronucleus and comet assays. Mode-of-action studies for 3 revealed that the P1 nucleoside transporter and adenosine kinase are involved in drug uptake and activation, respectively. Ecotoxicological assessments on *Daphnia* and green alga *Desmodesmus* revealed that the compound has an acceptable ecotoxicological footprint. Given the preferred target product profile for a broad-spectrum drug against AT, analogue 3 represents an advanced lead candidate for treatment of animal trypanosomiasis, regardless of the causative species.
Keywords: Animal trypanosomiasis, drug discovery, nucleoside analogues, RNA

16. Review of veterinary pharmaceuticals against Parasitic Vector-Borne Diseases and their environmental impacts

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Parasites are organisms that require a dependent mode of existence to complete their lifecycle, living at least part of their lives *in* or *on* (endoparasites or ectoparasites, respectively) a host organism. The parasite exploits its host, whether it's a human, animal, arthropod, or plant, by feeding off it. Consequently, the host may suffer from parasitic diseases (PDs), which may represent severe life-threatening conditions (Goater et al, 2013; Taylor et al. 2016), compromising livelihoods and life expectancy. Yet, these conditions remain overlooked in public health agendas, as they are often included in the broader complex of syndemic diseases associated with poverty in tropical and subtropical regions. Animals can host a broad spectrum of PDs that have notable zoonotic implications. Livestock parasites are responsible for significant production and economical losses and transmission of food-borne parasitoses, while companion animals are considerably affected by numerous PDs. Indeed, the impact of parasite control in animals is mirrored in the 23% of the global Animal Health market currently represented by antiparasitic drugs (Selzer et al. 2021).
Parasitic diseases of vector-borne origin (PVBDs) are particularly challenging to tackle, as control measures should be orchestrated in a multifactorial dimension, targeting vectors,

parasites, and hosts. Such approaches rely on mass administration of drug combinations, including repellent and/or parasiticidal compounds, characterised by insufficient safety profiles for both hosts and environment (Boxal et al. 2002; Boxal et al. 2004; Kaczala and Blum. 2016).

More recently, evidence of decreased parasite susceptibility and increased resistance is being reported, besides toxicity to non-target organisms.
It is expected that the development of parasiticides follows scientific progress to overcome epidemiological variations in parasite distribution and infection patterns, while safeguarding public, animal, and environmental health, according to the OneHealth principles. We seek to evaluate the chemical and pharmacological properties of the current drugs against PVBDs of animals and predict their environmental fate and ecological risks, with the ultimate goal to guide the development of greener anti-PVBDs drugs. For this purpose, our multidisciplinary team of medicinal chemists, ecotoxicologists, parasitologists and veterinarians is conducting a comprehensive literature review and discussion paper, with insights to be shared at the meeting for additional input. The final document will lay out the vision and strategy of CA21111 for the development of new anti-parasite drugs compliant with the OneHealth prerequisites, including the reduction of environmental impacts limiting drug resistance of zoonotic PDs threatening the treatability of PVBD.

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17. Ferrocene conjugates as potential antiparasitic vector-borne lead compounds

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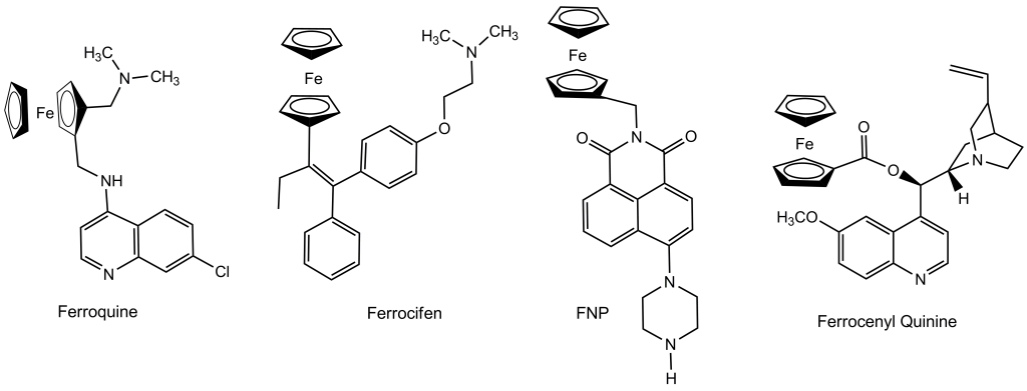
Ferrocene derivatives have attracted significant interest as anticancer and antiparasitic lead candidates. [1, 2] The poster examples are Ferroquine and Ferrocifen, which advanced to clinical trials for the treatment of malarial and cancer, respectively. Ferroquine is a chloroquine derivative with ferrocene incorporated within the alkyl side chain. Ferrocifen is a derivative of the breast cancer drug, Tamoxifen, with ferrocene substituted for a phenyl moiety. The incorporation of ferrocene into existing drugs can function as a bioisostere for a phenyl or heteroatom unit, while the three-dimensional shape of the metallocene offers the possibility of filling a hydrophobic cavity in a way that a phenyl or heteroaromatic moiety cannot. Our group has developed ferrocenyl 4-amino-1,8-naphthalimides as anti-cancer and cellular imaging agents. [3] In vitro studies against MCF-7 and K562 cancer cell lines indicated those compounds with a secondary amine (i.e. piperazine, FNP) [4] are more cytotoxic than those with a tertiary amine. Given the historic precedence for piperazine and piperazine derivatives (i.e. Praziquantel) as antiparasitic agents, [5] we propose ferrocenyl 4-amino-1,8-naphthalimides as potential antiparasitic vector-borne lead compounds. Our recent efforts have been towards the study [6] and development of cinchona alkaloid analogues (i.e. Ferrocenyl Quinine). This presentation will highlight our library of ferrocenyl compounds available for study as antiparasitic agents.



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18. Babesiosis; the disease with the great impact not explored yet

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Our country has recorded zoonotic and vector-borne diseases with data on babesiosis since 1928. The species of *B. bovis, B. bigeminum, B. divergens* and *B. major* were found in farm animals of the country (Dodbiba 1965, Mati 1987). The disease is spread mainly in the southern part of Albania in Lushnjë, Fier, Gjirokastra, Tepelena, Sarandë. Also, a larger number of cases were identified in 1955 with significant damage to the country's economy of that time (Dodbiba 1965). Based on studies in the field of veterinary medicine, the infection has an enzootic character with the period of May - October.

Data on babesiosis in dogs have been recorded only in recent years. The range of the infection in dogs is 7.3% - 23% mostly in Tirana and Elbasan. The species circulating are *Babesia canis* and *B. vogeli* (Dhamo 2006, Hamel 2009).

The first and the only positive case of babesiosis in humans was recorded in a boy from Patosi, Fier during 2004 (Sallabanda 2004).

Entomological studies show the presence of the tick as a transmited vector of *Ixodes ricinus, Hyalomma sp, Boophilus calcaratus, Rhipicephalus bursa, Rhipicephalus sanguineus, Haemaphysalis punctata* (Mati 1987)

To have the real situation of this disease, it is important the sero-epidemiological studies, declaration of tick bites and especially when travel to high-risk areas, confirmation of human cases (only one case since now). Mild and moderate form of the disease usually remain undiagnosed and follow the evolution of spontaneous recovery.

19. Structural investigation of Trypanosoma folate enzymes for the development of mew antiparasitic agents

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The WHO has identified 17 neglected tropical diseases (NTDs) posing health burden to over 1.4 billion people. Trypanosomatid parasites are responsible for threatening insect-vector borne NTDs, such as human African trypanosomiasis (HAT, sleeping sickness), caused by *Trypanosoma brucei*, Chagas disease, caused by *Trypanosoma cruzi*, and leishmaniasis, caused by *Leishmania* spp [1]. Current therapeutics are limited by toxicity, poor efficacy, and parasite resistance, thus underlining the need for novel antiparasitic agents [2,3]. Dihydrofolate reductase (DHFR), a known anticancer, antibacterial, and antimalarial target, provides reduced folates crucial to biological processes like DNA, protein, and amino acid synthesis or one-carbon transfer. In trypanosomatids, DHFR inhibition is ineffective due to the metabolic bypass provided by pteridine reductase 1 (PTR1) [2,3]. When DHFR is inhibited, PTR1 is overexpressed and sustains sufficient reduced folate levels to ensure parasite survival. To effectively inhibit the folate pathway in *Trypanosoma* parasite, DHFR and PTR1 should be both targeted. Within the EU-FP7 project New Medicines for Trypanosomatidic Infections (NMTrypI), we investigated the structures of PTR1 and DHFR-TS enzymes from different *Trypanosoma* parasites, defining the main targetable pockets within their active sites [3-8]. Furthermore, we performed mechanistic studies on PTR1 and comparative analysis with DHFR to map their main active site features [3-6]. At variance with PTR1, which is lacking in the human host, DHFR and TS enzymes are shared by parasites and humans. Their structures were compared to identify targetable areas for designing selective inhibitors, that can be exploited, in combination with PTR1 blockers, for developing new antitrypanosomatidic agents [3-8].

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20. Advancing sustainable drug development: comparative preclinical study of H80 and Miltefosine using imaging and proteomics

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Leishmaniasis treatment demands safer, orally available drugs with shorter treatment durations than current options. Although Miltefosine (MIL) is effective, it comes with severe side effects. Our research aims to surpass MIL's limitations by discovering compounds like H80, which has shown potent activity against various Leishmania strains with minimal drug resistance. Our study focuses on understanding H80's mechanism of action and molecular targets, crucial for rational drug design. Using fluorescence imaging and mass spectrometry-based proteomics, we analysed the protein expression profiles of Leishmania parasites treated with H80 and MIL. Our findings revealed significant overlap in differentially expressed proteins (DEPs) between H80 and MIL treatment, particularly those involved in membrane transport and biosynthesis. This convergence suggests shared pathways impacted by both compounds, offering insights into their mechanism of action. Furthermore, fluorescence imaging shows H80's cellular uptake mechanism, indicating endocytosis-mediated internalisation and cytoplasmic localisation within parasites. Our research will investigate deeper into the biochemical pathways modulated by H80 compared to MIL, aiming to identify specific protein interactions impacted by H80 using proteomic techniques like LC-MS/MS analysis on promastigote *(L.infantum*). We also compared two treatments of H80 (EC10 and EC50) to further explore the mechanisms of cell death in the parasite. These comparative studies of H80 treatments showed that Leishmania parasites responded in a dose-dependent manner, providing a more sophisticated understanding of how different H80 concentrations affect various cellular pathways and death mechanisms.

In conclusion, imaging studies demonstrated that THP-1 cells internalise H80 via endocytosis, leading to the colocalisation of the compound and parasite in the cytoplasm of the macrophages. According to proteomics, H80 influences cytosolic proteins in *L. infantum*, while miltefosine modifies membrane components. Our results indicate that the mechanism of parasite death involves a decrease in vacuolar acidity, with pH playing a central role in the developmental switch between promastigote and amastigote forms, crucial for the parasite's cell cycle.

In the future, SeqAPASS (Sequence Alignment to Predict Across Species Susceptibility) will be utilised to analyse target proteins across diverse species, predicting ecotoxicological risks and

identifying susceptible non-target organisms. This method aims to improve the safety of drug design by evaluating possible side effects and reducing environmental impact while maximising therapeutic efficacy.

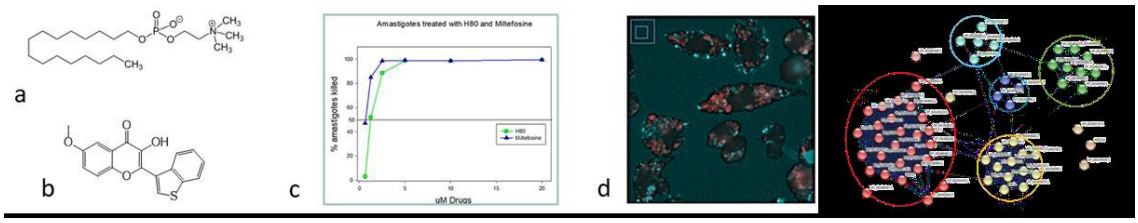

Figure 1. (a) Miltefosine structure. (b) H80 structure. (c) EC50 of Miltefosine and H80 in L infantum amastigotes. (d) fluorescence-based immunoassay for internalisation study of H80 (e) MS samples were analysed with Progenesis (Waters) with a label free approach and the main pathways were studied with String software (STRING Consortium 2023)

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21. Adamantane imidazolines with trypanocidal activity

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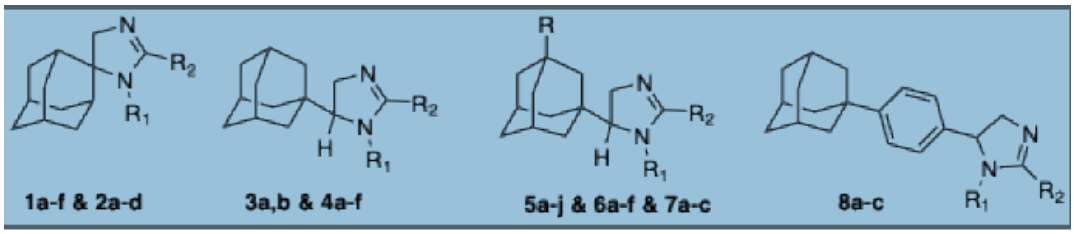
The current drugs used against HAT and CD are suboptimal. The approved regimen presents many restrictions, such as serious adverse side effects, excessive toxicity, limited efficacy and increasing resistance. This has led the World Health Organization (WHO) to coordinate public sector and private partnerships as part of a global effort to develop new and safer dugs. [1]

We have been exploring the chemical space of structurally different adamantane derivatives and their biological role against trypanosomes, over the last decade. In this work, the adamantane skeleton is attached into the imidazoline ring, in derivatives 1-4. Adduct 5-8 are enriched with more lipophilic features The incorporated amidine moiety is well known as trypanocidal. [2,4]

The introduction of a cyclopentyl or cyclohexylgroup on C3 of adamantane seems to increase the trypanocidal activity in comparison to the unsubstituted adamantane analogues. The introduction of phenyl substitution either on C3 of adamantane or in N1 of imi dazoline causes a drastic reduction of trypanocidal action. On the other hand, the insertion of the phenyl ring between the adamantane core at C1 position and the imidazoline ring has improved the pharmacological characteristics, in terms of activity and toxicity, as well.



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22. Intestinal parasite infections' prevalence in the Tirana district

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Introduction: Intestinal parasitic infections (IPIs) pose a global health problem affecting over one billion people worldwide. It is important to understand the prevalence and effects of infection based on the parasite species to implement therapeutic interventions and prevention controls. The stool test is the primary way of diagnosing IPIs. The objective is to determine the prevalence, diversity, and distribution of parasitic infections in Tirana.

Material and method: This is a cross-sectional study conducted in ALNET during the period 2021-2023. Data about the detection and identification of intestinal parasites were obtained from laboratory examination of stool specimens by using wet mount and Zinc Sulphate Floatation techniques. As subjects were taken many patients showed clinical manifestations with orienting values for a parasitic disease.

Results: A total of 863 patients with an age range of 3-34 years. A total of 20% of kids had positive parasite tests (95% CI 13.2 – 15.0). The age group of 6 to 10 years old showed the highest levels of optimism, and there was a statistically significant trend showing that the proportion of positivity decreased with age. In terms of the distribution of parasite types in positive children, Taenia spp. account for 1% of the total parasites, *A.lumbricoides* for 3.9% of the children, *Enterobius vermicularis* for 13.6%, *Hymenolepis nana* for 2.8%, *Entamoeba histolytica* for 0.6%, and *Trichiura trichiuris* for 0.1% of the children are in the lead. Giardia lamblia is significantly more common in each age range compared to other parasite species, with statistically significant differences observed. In comparison to other parasite species, Giardia lamblia has a statistically significant higher percentage across all months. Furthermore, the *Giardia lamblia & E. vermicularis* combination predominates with statistically significant differences with the other combinations. In individuals infected with parasites, the mean percentage of eosinophils is 9.3% (±2.6). The patients with Giardia lamblia (13.6 ±3.1) and *A. lumbricoides* (11.5 ±3.1) had the highest values of eosinophils. 12.2% of cases from the urban area and 7.8% from the rural area were positive based on the resident's place of residence. Parasites have a greater impact on urban regions than on rural ones.
Conclusions: The highest susceptibility to parasites was observed in the age group of 6-10 years. Giardia lamblia is the most frequently detected parasite. Among the children screened, men are more affected.

Keywords: children, intestinal parasites, infection, prevention

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

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

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

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24. Malaria, a parasitic mosquito-borne disease; from imported cases re-emerge, to the presence of primarily Anophelinae vectors population in Albania

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Malaria was hyper endemic in Albania and a real public health concern in the beginning of 19th century and its control in Albania was achieved in 1967. Further studies on malaria *Anopheles* vectors showed a gained insecticide resistance, except malathion. The increased of malaria imported cases in the recent years, raised awareness of reemerging potential in the country. First records of mosquitoes in Albania dated in 1918-1919, by the French and Austro-Hungarian antimalarial military forces. Bates described the present of 10 *Anopheles* mosquitoes during 1936-1939. We intend to have a new update on the mosquito fauna of the country, comparing distribution, taxonomy and potential of the malaria mosquito-borne parasitic diseases in the country. A field study was undertaken throughout to gather new records on the mosquito fauna, preferences for habitats, as well as to determine the control measures if needed. Mosquito collection was performed by sampling of larvae and eggs in stagnant water, and adults' trapping with CDC light traps, resting and human landing catch. The collected material was identified to species level. Our study showed that *Anopheles* comprised 13 species, and a high species diversity on mosquito fauna in Albania with a higher density along the coast. This study strengthens the need to undertake mosquito control programs to minimise the population of the *Anophelinae* mosquito species. Due to the fact of the imported cases of Malaria in Albania in the recent years; there is an increased risk for local infection, where 4 primary vectors of malaria are present, 3 secondary vectors and several other *Anopheles* species, which might have capacity to transmit Malaria in humans. There is a high risk of the re-emerge and emerge of the parasitic mosquito-borne diseases carried by *Anophelinae* mosquitoes in Albania.

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25. Unprecedented high-resolution chemical imaging of proteins, surfaces of microbes & histological tissue using mid-IR photo-induced force microscopy

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The field of high-resolution chemical imaging of biological cells and histological tissue is in a phase of transition: technological advances have triggered the development of nanoscale spectroscopic imaging methods and their application in the Life Sciences and in Environmental Research [1-4]. Within this emerging field, mid-IR photo-induced force microscopy (IR PiFM, PiF-IR) is highly promising due to its exceptional spatial resolution combined with its high spectral resolution [1,4-6] as illustrated in Fig. 1. The demonstrated ability of PiF-IR to provide submolecular chemical imaging of proteins [1] in combination with its unprecedented spatial resolution of surfaces of microbes [5,6] and histological tissue [1] has the potential to revolutionise chemical nano-imaging in the Life Sciences and beyond. Its access to localised submolecular chemical characterisation is a potential key to understand relevant chemical processes involved in parasitic infection, immune response, cell death and localised molecular actions during therapy.

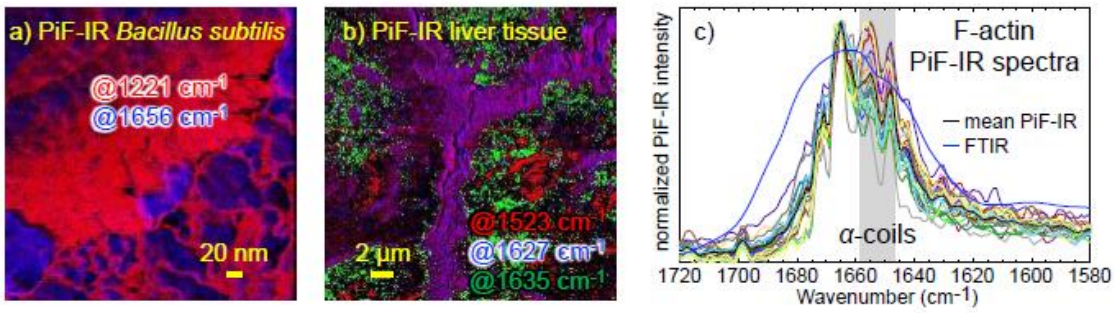

Figure 1. a) Unprecedented chemical resolution of Bacillus subtilis cell wall: Merged PiF-IR contrasts scanned at @1221 cm-1 (red, glycans) and @1656 cm-1 (blue, amides) [6]. b) Merged PiF-IR contrasts of histological mouse liver tissue scanned @1523 cm-1 (red), @1627 cm-1 (blue, bundled F-actin), and @1635 cm-1 (green, monomeric actin)[1]. c) Spectral sensitivity of PiF-IR to secondary protein structure observed in the amide I-band in normalized PiF-IR spectra of F-actin[1].

The BioPOLIM team has investigated and applied PiF-IR and tapping atomic force microscopy – infrared (tapping AFM-IR) to a variety of materials including histological tissue [1], organic monolayers on various substrates, aligned polymer films for organic photo-electronics [7],

nanoparticles [8], natural and engineered biopolymer compositions [1], single bacteria cells [6] and human retina which we receive from or collaborators. In a recent study, we applied PiF-IR to F-Actin prepared from Actin Binding Protein Biochem Kit. In our PiF-IR hyperspectra obtained from a 60 nm x 60 nm area of a dried droplet of arbitrarily polymerised F-Actin we found local variations in absorption bands @1655 cm-1 and @1630 cm-1 related to α-helices and β-sheets, respectively [1]. Preliminary results from the investigation of a dipeptide revealed submolecular vibrational components in the PiF-IR hyperspectra. Nanoscale local variations are also found in high-resolution PiF-IR scans and hyperspectra of Bacillus subtilis [5,6] and histological liver tissue from a mouse model [1] demonstrating the high potential of the method for revolutionising our understanding of chemical compositions in the surface structure of biological cells, microbes and histological tissue. This potential of PiF-IR is highly suited to contribute to the structural and functional drug development in vector-borne diseases.

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26. Identification of novel Leishmania infantum SIR2RP1 inhibitors

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Neglected tropical diseases caused by kinetoplastid parasites place a significant health and economic burden on developing nations worldwide. Current therapies are largely outdated and face mounting drug resistance from the causative parasites. Thus, there is an urgent need for drug discovery and development. The proteins belonging to the Silent Information Regulator 2 (SIR2) family, also known as Sirtuins, deacetylate lysine residues of histones and non-histone substrates using NAD+ as a cofactor (1). Unlike humans who express seven different sirtuins, there are only three sirtuins homologs in trypanosomatids. Trypanosoma brucei and Leishmania spp both have three sirtuins namely, Sir2 related protein 1 (Sir2rp1), Sir2rp2, and Sir2rp3; whereas Trypanosma cruzi possesses only Sir2rp1 and Sir2rp3. Genetic approaches through the disruption of the sir2rp1 gene in Leishmania infantum and Trypanosoma cruzi indicated that this protein was determinant to parasite survival due to the impossibility of generating null chromosomal mutants without episomal rescue (2,3). Our search for inhibitors of LiSIR2RP1 has led to the identification of the antiparasitic and anticancer bisnaphthalimidopropyl (BNIP) alkyl di- and triamines with an IC50 in the single micromolar range for the most active compounds (3,4). To further identify novel LiSir2rp1 specific inhibitors we have established a collaborative project with the European Lead Factory. A total of approximately 440,000 compounds were first tested in a primary enzymatic screening assay. About 1% of the compounds gave ≥35% effect in the primary screen. The selected compounds were combined with 108 additional compounds derived from Bayesian model, deployed to rescue false negatives. In total 1997 compounds were delivered and were tested in dose-response experiments, orthogonal as well as deselection assays, which ultimately resulted in a promising hit list of 397 compounds. Among these 55 compounds were further prioritised based on their activity, selectivity, structures, purity of the sample, and physicochemical properties. Antiparasitic activity against L. infantum intracellular amastigotes was found promising for 9 hits. The resolution of the LiSir2rp1 crystal structure will be important for future optimisation studies (5).

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27. Swimming in medicated waters: Understanding and mitigating the impacts of pharmaceutical pollutants on aquatic wildlife

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The widespread use of pharmaceuticals leads to their eventual introduction into natural environments worldwide. While some pharmaceuticals cause acute toxic effects in wildlife, such as mass mortality or reproductive failure, the majority are present in concentrations too low to be overtly toxic. However, these low concentrations can induce subtle behavioural changes in wildlife, potentially leading to significant ecological consequences. The last few decades have seen a surge in behavioural ecotoxicology research, driven by methodological and technological innovations that allow for the collection of high-resolution behavioural data in both laboratory and field settings. Despite these advancements and our increased understanding of the ecological risks posed by pharmaceuticals, this knowledge is seldom integrated into chemical regulation frameworks. This presentation highlights the necessity of incorporating behavioural ecotoxicology findings into regulatory practices to better protect wildlife and their ecosystems.

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28. The Peroxiredoxins of Leishmania revisited

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In trypanosomatids, peroxidases from the 2-Cys Peroxiredoxin family (PRX) are the final components of sophisticated enzymatic cascades in which the reducing power from NADPH is successively transferred to trypanothione reductase, trypanothione, tryparedoxin, and PRX, which ultimately reduce peroxides. These trypanothione-dependent cascades are central for redox balance in trypanosomatids, and their enzymatic components are considered promising therapeutic targets.

Here, we present biochemical, structural, and functional studies conducted by our group to characterise the four PRXs of Leishmania and establish their therapeutic value. Contrary to the prevailing dogma in the trypanosomatid community, our data reveal that some of these enzymes are not strictly essential. Indeed, Leishmania can adapt to the simultaneous loss of three PRXs: the two cytosolic PRXs (cPRX1 and cPRX2, the parasite's primary antioxidant defences!), and the glycosomal PRX (gPRX). This adaptation appears to involve a metabolic shift towards increased dependence on oxidative phosphorylation.

Of the four PRXs of Leishmania, only the mitochondrial PRX (mPRX) is indispensable to the cell. Interestingly, this necessity is unrelated to its peroxidase activity; rather, it is the capacity of this PRX to function as a molecular chaperone that is essential to parasites. Chaperone activation and client binding studies have been performed as an attempt to understand mPRX mode of action.

On the whole, these studies highlight previously unanticipated aspects of trypanosomatid PRXs, and collectively provide a new understanding of the antioxidant machinery of Leishmania.

29. Plant extracts applied against parasitic diseases in Greece: an overview

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Medicinal plants promote sustainable development, preserve the environment, and enhance public health. Plants offer a unique source for discovering new antipathogenic compounds. Parasitic diseases remain a significant health concern affecting a considerable number of populations, especially in developing nations of the world. The rise of parasites resistant to current chemotherapies and the side effects associated with currently available synthetic medications underscores the significance of different plant extracts as potential antiparasitic agents. A wide range of herbal-based metabolites, including alkaloids, phenolic compounds, quinones, terpenes, saponins, lignans, toxoids, and anthranoids, have been thoroughly examined for their potential in this regard. As a biodiversity hotspot, Greece hosts a remarkable diversity of plants, including 6,760 taxa belonging to 1,087 genera and 184 families. The Greeks have made significant contributions to the systematic advancement of herbal medicine utilisation. This presentation aims to review various plant extracts and their compounds for treating parasitic diseases studied in Greece.

There were seventeen reports on the *in vitro/in vivo* antiparasitic efficacy of different medicinal plants and their components against parasitic diseases, including malaria, leishmaniasis, trypanosomiasis, coccidiosis and helminthic infections. A total of 21 genera and 25 species of plants belonging to 16 families have been indicated with antiparasitic effect. Most studies (71%) were conducted *in vitro* with extra virgin olive oil as the most studied plants extracts. Based on the type of parasites, Greek studies recorded *Leishmania infantum, L. major, L. donovani, Plasmodium falciparum, Trypanosoma brucei rhodesiense, T. cruzi, Eimeria tenella, E. acervulina, E. maxima, Haemonchus contortus,* and *Trichostrongylus colubriformis.*

With its favourable climate, Greece is a prime location for the sustainable utilisation of natural resources, particularly medicinal and aromatic plants. Our study highlights the potential of Greek flora as a rich source of novel therapeutic compounds against parasitic infections. Despite the limited number of reports, our overview illuminates the promising future of plant-based remedies for parasitic diseases and advocates for more comprehensive future investigations on plant extracts, which are currently underestimated in this regard.

Keywords: plant-based medical products, plant extract, parasitic diseases, Greece

30. OHD3: Transition from *in vitro* to *in vivo* evaluation: recommendations for obtaining high-quality leads against kinetoplastids

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Given the impact of kinetoplastid diseases, the limited therapeutic options and the risk of treatment failure, the discovery of novel chemical entities and innovative drug targets remains an urgent medical and veterinary need. Considering the 3R guiding principle for the use of animals in research, stringent decision criteria need to be formulated and implemented during the translation from the *in vitro* identification of hit compounds to a minimal/optimised *in vivo* evaluation in infection models to identify high-quality leads that are de-risked for various liabilities. Besides potency and selectivity, experimental and *in silico* data such as physicochemical properties, metabolic stability, (eco)toxicity, formulation, and snapshot pharmacokinetics, can serve as tools in the decision-making process. In addition, the respective target product profiles and proper design and reporting of the experiments are key in an animal-friendly approach to achieve high quality leads. A range of first-line standard and more refined animal models (e.g. bioluminescent infection models) exist that represent human infection and disease and have proven essential for selecting clinical candidates and eventually bringing novel drugs to market. Nevertheless, the limitations and complementarities of the various models need to be well understood, while emerging challenges and opportunities based on clinical observations stimulate the development of advanced, second-line models. We are preparing recommendations about the transition from *in vitro* to *in vivo* evaluation for strengthening the proof-of-concept of high-quality leads.

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