TB – 005 - **Targeting the unique decapping enzyme ALPH1 for trypanosomatid drug discovery**

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Trypanosomatids encode no homologues of canonical decapping enzymes, but employ the ApaH-like phosphatase ALPH1, belonging to a phosphatase family absent in mammalian systems, for the essential process of mRNA decapping. ALPH1 is meeting key criteria of a robust drug target: (i) Essentiality for *T. brucei* viability (ii) Absence from mammalian systems holds promise for inhibition with high selectivity (iii) Tractability, given by the availability of active enzyme in recombinant form that is assayable in a robust high-throughput manner and (iv) Druggability, as based on protein family precedence. With this target in hand, we developed a screening pipeline to identify inhibitors of ALPH1 activity. Our primary assay relies on the quantification of ADP liberated from a dinucleotide cap analog by a coupled enzymatic reaction, where we identified 13 dose-dependent ALPH1 inhibitors with IC_{50} < 10 uM. These compounds were validated with a secondary orthogonal m^7GTP-C_4-pyrene assay and biophysically characterized using isothermal calorimetry. They displayed a robust binding affinity with dissociation constants ranging from 0.3 – 11.7 uM. Testing in *T. brucei* bloodstream form and *L. major* axenic amastigotes revealed 8 and 3 compounds impacting the viability of the respective parasite. Here, we report 3 dose-dependent inhibitors of ALPH1 activity, *T. brucei* and *L. major* viability from our screen encompassing 12760 small molecules. **Supported by:** DFG, GAČR (23-07890L) and NCN
Keywords: ALPH1; Trypanosomes; Inhibitors.

TB – 006 - **Trypanosoma alternative metabolic enzymes as tools to dissect human mitochondrial energy metabolism**

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Parasites possess sophisticated mechanisms to adapt their energy metabolism to different host environments. *Trypanosoma brucei* (*T. brucei*), the causative agent of human African trypanosomiasis, a neglected tropical disease, undergoes a remarkable metabolic transition during its life cycle between the tsetse fly vector and the mammalian host. In the insect stage, *T. brucei* relies on mitochondrial respiratory-chain complexes and oxidative phosphorylation (OXPHOS) for energy production. In contrast, bloodstream form parasites lose most respiratory chain activities and switch to an OXPHOS independent energy metabolism. Two mitochondrial enzymes, trypanosome alternative oxidase (TAO) and acetate: succinate CoA-transferase (ASCT), play key roles in this alternative energy metabolism. TAO is a single polypeptide terminal oxidase that oxidizes quinol and functionally bypasses mammalian respiratory complexes III and IV, whereas ASCT, together with succinyl-CoA synthetase (SCS), forms the ASCT/SCS cycle, enabling ATP production independently of OXPHOS. Here, we aimed to establish an experimental system for dissecting the respective contributions of mitochondrial electron transport and ATP synthesis in human cells by exploiting TAO and ASCT as independent tools for quinol oxidation and ATP production. We generated human cell lines individually expressing TAO or ASCT and examined their responses to specific inhibitors of mitochondrial energy metabolism. TAO expression conferred resistance to antimycin A, a complex III inhibitor, whereas ASCT expression increased resistance to oligomycin A, an ATP synthase inhibitor. Extracellular flux analysis further demonstrated that these parasite derived enzymes function in human cells, as assessed by oxygen consumption rate (OCR) and ATP production. These results demonstrate that TAO and ASCT can independently complement distinct components of mitochondrial energy metabolism and provide a unique approach to functionally dissect electron transport from ATP synthesis in human cells. In future studies, we will extend this system to experiments using complex I inhibitors and cells in which genes known to cause mitochondrial diseases are knocked down. By examining whether TAO- or ASCT-mediated alternative metabolic pathways can compensate for specific mitochondrial defects, we aim to establish these parasite-derived enzymes as experimental tools for dissecting the molecular mechanisms underlying mitochondrial diseases. **Keywords:** Trypanosoma; Mitochondria; OXPHOS.

TB – 007 - **Repurposing Cabazitaxel Against *Trypanosoma cruzi*: Antitrypanosomal Activity and Ultrastructural Studies**

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Chagas disease is an infectious parasitic disease caused by the trypanosomatid protozoan *Trypanosoma cruzi*, which has unique features when compared to other eukaryotes, such as a single mitochondrion and a characteristic cytoskeletal organization. This structure consists of subpellicular microtubules associated with the plasma membrane and distributed throughout the cell body. Cytoskeletal disorganization has been reported following treatment with several chemotherapeutic agents. Previous studies by our group demonstrated that the histone deacetylase inhibitors Trichostatin A and Tubastatin A induced cell cycle arrest and distinct morphological alterations in *T. cruzi* epimastigotes. Likewise, Taxol, which stabilizes microtubules by binding to β-tubulin, induces cell cycle arrest and changes in parasite morphology, flagellar length, and motility. Cabazitaxel is a clinically used, second-generation semisynthetic taxane that also stabilizes microtubules, but its effects on trypanosomatids remain poorly understood. Here, we investigated the activity of Cabazitaxel against *T. cruzi*. Epimastigotes and LLC-MK_2 cells were treated for up to 96 h. Cabazitaxel exhibited an IC_{50} of 50 nM against epimastigotes after 72 h. The CC_{50} in LLC-MK_2 cells was higher than 100 µM after 96 h. ADME analysis indicated high molecular weight and flexibility, lack of blood-brain barrier permeability, and few hydrogen-bond donors and acceptors. Ultrastructural analyses, activity on amastigotes and trypomastigotes, and molecular docking are in progress. These findings support further investigation of Cabazitaxel as a potential antitrypanosomal compound and as a tool to better understand *T. cruzi* cell biology. Supported by CNPq and FAPERJ. **Supported by:** CNPq e FAPERJ (E-26/203.069/2026) **Keywords:** Cabazitaxel; Chemotherapy; *Trypanosoma cruzi*.

TB – 008 - **Genes involved in lipid biosynthesis and metabolism as potential markers of miltefosine resistance in *L. amazonensis***

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Cutaneous leishmaniasis is a neglected tropical disease that affects around 20,000 people per year in Brazil, which is mainly caused by Leishmania braziliensis, L. guyanensis and L. amazonensis. The latter species is responsible for localized cutaneous leishmaniasis, and may eventually progress to diffuse cutaneous leishmaniasis. The disease treatment is a public health challenge due to the limited number of drugs available with severe side effects, as well as the emergence of resistant parasites, due to their high genome plasticity. Miltefosine is currently the only oral drug available for treatment, and the mechanism of action and resistance, particularly in endemic species in Brazil, is not completely understood. Recently, we sequenced the genome of a L. amazonensis resistant line to miltefosine (MF150.3) and identified mutations in genes involved in lipid biosynthesis and metabolism. Among these mutations, heterozygous mutations were detected in the genes encoding a lanosterol synthase (LS) and a Fatty Acid Elongase (FAE). The LS enzyme is responsible for the formation of lanosterol, the precursor of the most abundant sterol in the parasite's plasma membrane: ergosterol. The FAE enzyme participates in the synthesis and elongation of long-chain fatty acids, providing essential lipids for the plasma membranes. To functionally validate the role of these enzymes in miltefosine resistance, we used genetic tools to knockout and overexpress these enzymes to evaluate the impact of these mutations in the resistance phenotype in L. amazonensis. The LS and FAE genes were amplified and cloned in an expression vector, and consequently transfected into both wild-type and MF150.3 resistant line. Additionally, using CRISPR/Cas9 technology, a single knockout line for the LS gene has been generated, while for the FAE gene, we could not generate a knockout line in the wild-type strain of this species. All transgenic lines were characterized for their susceptibility to miltefosine at promastigote and intracellular amastigote stages. Our findings showed that the single knockout line for the LS gene is resistant to the drug at the amastigote stage. For the FAE gene, the overexpression of this gene increased MF susceptibility in promastigotes of the wild-type strain and of the MF150.3 resistant line. Altogether, these findings indicate the potential role of LS and FAE enzymes in the mechanism of action and resistance to miltefosine in L. amazonensis. **Supported by:** FAPESP (2024/11197-4) , CNPq- PIBIC (Processo 122000/2023-4) e FAPESP 2024/00360-1. **Keywords:** Leishmania amazonensis; drug resistance; miltefosine.

TB – 009 - **Functional analysis of *Leishmania amazonensis* genes DUSP and EF2K in paromomycin resistance**

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Leishmaniasis is a group of neglected diseases caused by protozoa of the genus *Leishmania*. *Leishmania amazonensis* is endemic in Brazil and responsible for cutaneous and diffuse cutaneous leishmaniasis. Current antileishmanial drugs include pentavalent antimonial, pentamidine, miltefosine and amphotericin B. Besides the restricted number of drugs, therapies face significant limitations such as severe side effects, high cost, parenteral administration and the emergence of resistance parasites. Paromomycin (PM) is an alternative drug used in the treatment of visceral and cutaneous leishmaniasis in Southeast Asia and Africa. In addition, cases of clinical resistance associated with PM administration have not been reported, which demonstrates its potential for use in the treatment of the disease in Brazil. Understanding the molecular mechanisms underlying resistance and identifying molecular markers is crucial for the surveillance of resistance parasites and for the establishment of new interventions. In this context, this study aims to functionally analyze previously identified genes potentially involved in resistance to PM in *L. amazonensis*. Genome analysis of PM-resistant lines selected in vitro and susceptible clinical isolates indicated that distinct genes are potentially involved in resistance and susceptibility phenotypes. Candidate genes were selected for functional analyses using gene knockout and overexpression approaches. We selected two genes for functional analysis: *LAMA_270029300*, which encodes the enzyme Dual specificity protein phosphatase (*DUSP*) and *LAMA_200055200*, which encodes the enzyme Elongation factor-2 kinase-like protein (*EF2K*). Phosphatases such as DUSP are known to regulate critical signal pathways by dephosphorylating its substrates, while EF2K is a kinase involved in the regulation of protein synthesis. We have successfully generated both overexpressing and knockout lines for DUSP and EF2K in *L. amazonensis*. Upcoming susceptibility assays with the transgenic lines will determine the impact of these proteins on PM resistance phenotype. **Supported by:** FAPESP 2026/02363-3 e FAPESP 2024/00360-1 **Keywords:** Leishmania amazonensis; Paromomycin; Drug resistance.

TB – 010 - **Taxonomy and Phylogeny of Three Novel Freshwater Ciliates of the Genus Loxodes (Ciliophora, Karyorelictea) from Southern Brazil**

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Despite the fundamental roles that karyorelictid ciliates play in benthic freshwater food webs, Neotropical ecosystems remain substantially undersampled. Furthermore, historical descriptions within the family Loxodidae often lack quantitative silver-impregnation morphometry and DNA sequence data, complicating species identification. Using an integrative taxonomic protocol combining bright-field in vivo observation, silver staining, and SSU rDNA phylogeny, we investigated ciliates in the genus Loxodes of a lentic wetland in Porto Alegre, Brazil, resulting in the discovery of three novel species. Loxodes sp. 1 represents a morphological giant among bi-macronucleate congeners, measuring 646–788.8 µm in vivo and possessing 29–34 right somatic kineties. Loxodes sp. 2 (409–660 µm in vivo) is diagnosed by a unique oral-to-body size ratio, featuring a markedly reduced buccal field that occupies only 5% of the cell length (mean 27.3 µm). Loxodes sp. 3 is a small-sized species (159–253.1 µm in vivo) displaying a transparent cytoplasm, reduced cellular flexibility during swimming, and a proportionally small buccal field spanning approximately 8% of the total body length (mean 17.1 µm). Maximum Likelihood and Bayesian Inference analyses recover Loxodes as a monophyletic sister group to Remanella and place the three new species into well-separated topological branches. Notably, Loxodes sp. 3 occupies a more basal position within the tree (48% ML / 0.78 BI). Overall, our results confirm the monophyly of Loxodes (96% ML / 1.00 BI) and demonstrate that combining quantitative oral proportions with molecular phylogenetics is indispensable for uncovering cryptic ciliate diversity in undersampled South American wetlands. **Supported by:** Cnpq **Keywords:** Protozoa; Neotropics; Morphometry.

TB – 011 - **Phenotypic Screening and HT-RoK Profiling for Efficient Hit Prioritization for Chagas Disease Drug Discovery**

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Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a neglected tropical disease estimated to affect 8 million people worldwide and cause over 10,000 deaths annually (WHO, 2026). Current CD treatments—nifurtimox and benznidazole—remain limited by high toxicity, prolonged treatment regimens, and poor efficacy in the chronic stage of the disease, highlighting the need for safer and more effective drugs with distinct mechanisms of action (MoAs). Phenotypic screening was performed against the intracellular amastigote stage of *T. cruzi* using two chemical libraries: the Osaka University library (63,680 compounds) and the Chemical Library of the Institute of Transformative Bio-Molecules at Nagoya University (36,160 compounds), totaling 99,840 compounds. Hits were subsequently prioritized using a pipeline combining cytotoxicity assessment, chemical clustering, and high-throughput rate-of-kill (HT-RoK) profiling to identify non-cytotoxic trypanocidal compounds. HT-RoK profiling distinguished trypanocidal from trypanostatic compounds, enabling prioritization based on parasite-killing activity. For the Osaka University Library, activity against four *T. cruzi* strains and luciferase-2 inhibition were also assessed to exclude strain-specific compounds and luciferase reporter-related false positives. Phenotypic screening followed by cytotoxicity assessment yielded 708 prioritized hits. Chemical clustering further refined the selection to 34 compounds representing 13 distinct chemical clusters (seven series and six singletons). The integrated prioritization pipeline ultimately identified four non-cytotoxic compounds with intracellular trypanocidal activity from nearly 100,000 screened compounds. This study demonstrates that HT-RoK profiling enables efficient prioritization of compounds with parasite-killing activity against intracellular amastigotes, addressing a key challenge in Chagas disease drug discovery. **Supported by:** Research Support Project for Life Science and Drug Discovery (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)) from AMED under Grant Number JP25ama121054
Keywords: Chagas Disease; Drug Discovery; Phenotypic screening.

TB – 012 - **Identification and Experimental Validation of Repurposed Inhibitors of *Trypanosoma cruzi* Phosphodiesterases**

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Trypansoma cruzi phosphodiesterases (TcPDEs) play key roles in cyclic nucleotide signaling and regulate biological processes that are critical for parasite survival and infectivity. In previous studies, we demonstrated, using CRISPR/Cas9-mediated gene editing, that knockdown of TcPDEC and TcPDB1/B2 markedly impaired parasite viability, validating these enzymes as promising therapeutic targets for Chagas disease. Building upon these findings, we implemented a hybrid virtual screening workflow to identify drug repurposing opportunities targeting TcPDEC and/or TcPDB1. Compounds from the DrugBank and Drug Repurposing Hub databases were prioritized through the integration of machine learning models and molecular docking. Selected candidates were subsequently evaluated in phenotypic assays against bloodstream trypomastigotes (K98 strain) and intracellular amastigotes (K98 and Tulahuen strains). Compounds displaying antiparasitic activity comparable to that of benznidazole were further assessed in biochemical assays to confirm direct modulation of the proposed targets. The most promising candidates were then subjected to preliminary pharmacokinetic studies in mice and evaluated in an acute murine model of Chagas disease, using vehicle and benznidazole (100 mg/kg/day for five consecutive days) as negative and positive controls, respectively. Biochemical assays confirmed inhibition of T. cruzi phosphodiesterases for several candidates, with some compounds exhibiting submicromolar IC_{50} values. Although none of the evaluated compounds achieved an in vivo efficacy comparable to benznidazole, several candidates produced improved control of parasitemia and/or increased survival relative to vehicle-treated animals. These findings provide additional evidence supporting TcPDEC and TcPDB1/B2 as attractive therapeutic targets in T. cruzi and demonstrate the value of combining hybrid computational approaches with drug repurposing strategies to accelerate the identification of novel anti-T. cruzi agents. Furthermore, the results highlight promising chemical starting points for future lead optimization. **Supported by:** GHIT T2020-154 **Keywords:** CHAGAS DISEASE; DRUG REPURPOSING; PHOSPHODIESTERASES.

TB – 013 - **ISOLATION, CHARACTERIZATION AND ANTI-*TRYPANOSOMA CRUZI* ACTIVITY OF A METABOLITE FROM THE EXTREMOPHILIC BACTERIUM *CYCLOBACTERIUM PLANTARUM***

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Chagas disease is a neglected tropical disease caused by *Trypanosoma cruzi* that affects approximately 8 million people worldwide. Despite decades of treatment, current chemotherapy remains limited by toxicity and efficacy, highlighting the urgent need for new therapeutic alternatives. Microorganisms represent an important source of bioactive scaffolds for drug discovery. Among them, extremophilic bacteria biosynthesize specialized metabolites as adaptations to extreme environmental conditions, resulting in chemical diversity and the production of bioactive compounds with pharmaceutical potential. In this context, we investigated the anti-*Trypanosoma cruzi* potential of metabolites produced by *Cyclobacterium plantarum*, an extremophilic bacterium isolated from a sediment collected in Laguna Amarga, Chilean Patagonia. The bacterial metabolites were extracted with organic solvents, yielding a crude extract that achieved 100% parasite killing at the highest tested concentration, with an EC_{50} of 18 µg/mL. Subsequent bioassay-guided fractionation led to the isolation of VAS8, a phosphatidylethanolamine identified by NMR and LC-MS/MS. VAS8 exhibited activity against both clinically relevant forms of *T. cruzi,* the trypomastigotes and intracellular amastigotes, with EC_{50} values of 49 µM and 19 µM, respectively, while displaying low cytotoxicity for mammalian cells (CC_{50}>150 µM). To investigate its lethal action in *T. cruzi*, spectrofluorimetric and fluorescence microscopy studies were performed using SYTOX Green. VAS8 induced concentration- and time-dependent alterations in parasite plasma membrane permeability, followed by morphological alterations toward a rounded shape, ultimately leading to parasite death within 1 h. These findings highlight microorganisms from extremophilic environments as promising sources of antitrypanosomal prototypes. **Supported by:** FAPESP 2026/19813-1 **Keywords:** *Trypanosoma cruzi*; Treatment; Extremophiles.

TB – 014 - **Genomic Characterization of a Clinical Isolate (*Leishmania amazonensis*) Resistant to Amphotericin B**

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Cutaneous leishmaniasis is a neglected tropical disease endemic in Brazil caused mainly by *Leishmania braziliensis, L. guyanensis* and *L. amazonensis*. The parasite is transmitted through the blood meal of infected female sand flies. The infection by *L. amazonensis* may evolve to either the localized cutaneous leishmaniasis (LCL) or to the more complex to treat diffuse cutaneous leishmaniasis (DCL). In Brazil, the treatment of leishmaniasis remains a major challenge due to the limited number of approved drugs, which are highly toxic and restricted to pentavalent antimonials, amphotericin B (AmB), pentamidine and miltefosine. Furthermore, unresponsiveness to treatment may be linked to the emergence of drug resistance that is due to the high genetic plasticity of the parasite. Recently, our research group reported the first clinical isolate of *L. amazonensis* resistant to AmB. This clinical isolate was obtained from a patient from Maranhão, Brazil, diagnosed with diffuse cutaneous leishmaniasis and co-infected with HIV, whose patient was refractory to AmB treatment. In this study, bioinformatic analyses of the genome this clinical isolate identified 47 single-nucleotide polymorphisms (SNPs), of which 13 were selected for validation by Sanger sequencing. All selected variants were confirmed and classified as either heterozygous or homozygous. Among the confirmed variants, a homozygous mutation (P175L) was identified in the gene encoding a subunit of succinate dehydrogenase (*SDH*), an essential mitochondrial enzyme involved in the parasite's energy metabolism. This enzyme catalyses the oxidation of succinate to fumarate in the tricarboxylic acid cycle (TCA), and acts in the complex II of the mitochondrial electron transport chain. This mutation is in a highly conserved residue among different *Leishmania* species. Based on this, the *SDH* gene will undergo functional validation through genetic manipulation in the parasite, such as gene overexpression and knockout, followed by AmB susceptibility assays at promastigote and intracellular amastigote stages. These findings will contribute to a better understanding of the biological role of SDH in *Leishmania*, as well as its role in the AmB resistance phenotype. **Supported by:** FAPESP, 2025/20756-0; 2024/00360-1 **Keywords:** Cutaneous leishmaniasis; Drug resistance; Amphotericin B.

TB – 015 - **Investigating Amphotericin B resistance in *Leishmania amazonensis* clinical isolates**

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Amphotericin B (AmB) is one of the main drugs used to treat leishmaniasis and is recommended in Brazil for severe cases of disease and for unresponsive patients to first-line therapies. Although reports of clinical resistance remain rare, therapeutic failure is usually associated with HIV coinfection, whose incidence has increased in the country in the past years. Our group recently characterized a *Leishmania amazonensis* clinical isolate recovered from a patient with diffuse cutaneous leishmaniasis co-infected with HIV and refractory to multiple treatment regimens, including AmB. This isolate displayed reduced susceptibility to AmB both *in vitro* and *in vivo*. Therefore, this study aims to identify the molecular basis associated with AmB resistance in this clinical isolate through comparative analyses with other clinical isolates and strain of *L. amazonensis* displaying differential susceptibility to the drug. In parallel, laboratory-selected AmB-resistant lines are being generated *in vitro* by stepwise selection in promastigote and intracellular amastigote stages, as well as after chemical mutagenesis. Whole-genome sequencing of clinical isolates, strains and resistant lines is currently ongoing, and comparative genomic analyses will be employed to identify candidate genes associated with the resistance phenotype. Candidate genes will subsequently be functionally validated using reverse genetics. This study will contribute to a better understanding of AmB activity against *Leishmania*, and may identify clinically relevant molecular markers of resistance. **Supported by:** FAPESP (Processos 2025/20984-2 e 2024/00360-1) **Keywords:** Leishmania amazonensis; Drug resistance; Amphotericin B.

TB – 016 - **Insights into Class Karyorelictea (Alveolata, Ciliophora) phylogeny with focus on Trachelocercidae.**

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The class Karyorelictea comprises a distinctive and often overlooked lineage of ciliates characterized by simplified ciliary patterns and the absence of somatic nuclear division. Despite these features, the group has received limited attention, and molecular data are strongly biased toward the Northern Hemisphere. In South America, particularly in Brazil, records are scarce and mostly incidental, with no previous studies focusing on karyorelicteans from the coast of Rio Grande do Sul. As a result, knowledge of their diversity, distribution, and ecological roles along the Brazilian coast remains limited, constraining our understanding of benthic microbial assemblages in the South Atlantic. In this study, we analyzed SSU rDNA sequences from six karyorelictean species collected along the southern Brazilian coast, generating novel molecular data from a geographically underrepresented region and helping to fill a significant taxonomic and biogeographic gap. Phylogenetic analyses based on maximum likelihood and Bayesian inference recovered five of the six recognized families of Karyorelictea, most as monophyletic. All newly generated sequences were placed within Trachelocercidae and were morphologically assigned to Tracheloraphis and Trachelocerca. Four species attributed to Tracheloraphis formed a divergent, well-supported clade distantly related to other congeners, despite morphological similarity to described taxa. Additionally, both Tracheloraphis and Trachelocerca were recovered as non-monophyletic. These results reveal underestimated diversity within Karyorelictea, expand knowledge of their distribution in the South Atlantic, and provide a basis for future integrative taxonomic and biogeographic studies of this poorly known ciliate lineage. **Supported by:** CNPq **Keywords:** Taxonomic; Interstitial Environment; Uncultured Ciliates.

TB – 017 - **DISCOVERY OF SMALL-MOLECULE ANTI-*TRYPANOSOMA CRUZI* COMPOUNDS FROM MARINE CORALS**

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Trypanosoma cruzi is the etiologic agent of Chagas disease, a Neglected Tropical Disease that affects 6-8 million people worldwide. Current treatment relies on two toxic and low effective drugs, associated with significant adverse effects. In this context, marine natural products represent a promising source for drug discovery, as more than 50% of FDA-approved drugs are derived from natural products. This study aimed to evaluate the anti-*T. cruzi* activity of extracts from corals of the genera *Palythoa*, *Mussismilia*, and *Madracis*, with focus on *Tubastraea coccinea* for the isolation and characterization of secondary metabolites with therapeutic potential. Coral metabolites were extracted with methanol, followed by filtration and concentration under reduced pressure using a rotary evaporator. Antiparasitic activity was evaluated against the trypomastigote forms of *T. cruzi* (Y strain) using light microscopy for visualization of the morphology and motility and the fluorimetric assay of resazurin for cell viability. Our results demonstrated that all crude extracts achieved 100% parasite elimination at the highest tested concentration of 200 µg/mL. *Madracis decactis* exhibited the highest potency (IC50 13.7 µg/mL), followed by *Palythoa caribaeorum* (IC50 62.8 µg/mL), *Mussismilia hispida* (IC50 68.7 µg/mL), and *Tubastraea coccinea* (IC50 70.7 µg/mL). The *T. coccinea* extract was further subjected to liquid–liquid partitioning using butanol, dichloromethane, and hexane, yielding three fractions. The hexane fraction showed the highest activity (IC50 61.0 µg/mL), followed by the dichloromethane (IC50 111.7 µg/mL) and the butanol (IC50 138.8 µg/mL), suggesting the presence of different active compounds with different chemical polarities. These findings demonstrate that marine corals are a promising source of bioactive metabolites with anti-*T. cruzi* activity, reinforcing the importance of marine natural product bioprospecting for the discovery of new drug candidates for the treatment of Chagas disease. **Supported by:** Fundação Butantan, 001/0708/000. 238/2026 **Keywords:** *Trypanosoma cruzi*; Marine natural products; Chagas disease.

TB – 018 - **Guanidine-derived compounds as drug candidates for cutaneous leishmaniasis: *in vitro* and mechanism of action studies**

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Leishmaniasis remains a major public health issue due to a limited number of effective drugs available for treatment, their toxicity, and the emergence of resistant *Leishmania* parasites. Guanidine derivatives represent a promising source of novel antileishmanial compounds. To identify guanidine derivatives with leishmanicide activity against *Leishmania amazonensis*, a library of 75 guanidine derivatives was screened against promastigotes. Compounds showing >50% parasite viability at 1 µM were excluded. The half-maximal effective concentration (EC_{50}) values were determined, and compounds with EC_{50} >10 µM were also discarded. The remaining candidates were evaluated for cytotoxicity in murine macrophages, and compounds with a selectivity index (SI) <10 were excluded. Selected compounds were further tested against intracellular amastigotes, and only those displaying EC_{50} <2 µM and SI >2 were retained. This sequential screening strategy identified ten guanidine derivatives with potent activity against intracellular amastigotes and favorable selectivity, highlighting their potential as lead compounds for the treatment of cutaneous leishmaniasis. To further elucidate their mechanisms of action and identify potential molecular targets, L. amazonensis resistant lines are currently being generated through chemical mutagenesis with N-methyl-N′-nitro-N-nitrosoguanidine and stepwise selection, using the lead compounds: RCP-A39 (promastigote $EC_{50} \approx 0.45$ µM; intracellular amastigote $EC_{50} \approx 0.09$ µM) and RCP-D26-Mono (promastigote $EC_{50} \approx 0.5$ µM; intracellular amastigote $EC_{50} \approx 0.03$ µM). These studies will provide valuable insights into the molecular basis of compound activity and support the preclinical development of this new series of antileishmanial candidates. **Supported by:** CAPES, CNPQ, FAPESP 2024/00360-1 **Keywords:** Cutaneous leishmaniasis; Leishmania amazonensis; Drug discovery.

TB – 019 - **A Putative Cullin-1 RING Ubiquitin Ligase Interactor Is Implicated in Genome Stability in *Leishmania***

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Leishmaniases are a group of neglected tropical diseases caused by protozoan parasites of the genus *Leishmania*. Intracellular proteolysis is essential for host adaptation throughout the parasite life cycle and, consequently, for the success of parasitism. In eukaryotes, the ubiquitin–proteasome system (UPS) is the primary regulator of proteolysis. Despite its importance in cell biology, the UPS in *Leishmania spp.* remains largely unknown. We aimed to characterize the *Leishmania* orthologs of the human genes SKP1, RBX1, and CUL1, which are components of Cullin-RING Ligases (CRLs/SCF). The Skp1 and Cul1 interactomes revealed the presence of a CRL1 complex in *L. infantum*, as well as six F-box proteins. To further investigate CRL1 function, we used CRISPR/Cas9 to generate knockout lines, each containing a unique barcode, for all *L. mexicana* orthologs of Skp1 and Cul1 interactors previously identified. Viable knockouts were pooled into a single culture and subjected to proliferation and differentiation assays, followed by fitness assessment using Bar-seq. Among the targets, we identified an incomplete knockout of *LmxM.29.2660*, which displayed a proliferation defect in both promastigote and amastigote forms. Further characterization of this mutant revealed aberrant DNA content, increased DNA damage signaling, and genome-wide gains and losses of chromosome copy numbers. An interactome of *LmxM.29.2660* and confocal analyses in cells treated or not with hydroxyurea are being analyzed to elucidate the function of this protein in genome maintenance. Co-immunoprecipitation assays in HEK293T cells are being developed to validate the interaction between LmxM.29.2660 protein and Skp1. We identified the first CRL1 interactor involved in *Leishmania* genome maintenance. Given the unusual extent of *Leishmania* genome plasticity and its implications for adaptation and drug resistance, these findings provide insights into how ubiquitination contributes to genome variability in this parasite. **Supported by:** FAPESP 2021/10971-0 **Keywords:** Leishmania; Ubiquitin proteasome system (UPS); Cullin-RING ligase.

TB – 020 - **Exploring the Chemical Diversity of Marine Sponge-Associated Bacteria from Fernando de Noronha Archipelago Against *Trypanosoma cruzi***

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Affecting between 6-8 million people worldwide, Chagas disease (CD) is a Neglected Tropical Disease (NTD) caused by the protozoan *Trypanosoma cruzi.* Current treatment options are limited and present several drawbacks, highlighting the urgent need to develop new drugs. In this context, marine-derived natural compounds have demonstrated enormous value, serving as prototypes for FDA-approved drugs. In this work, we evaluated the anti- *Trypanosoma cruzi* potential of marine bacterial metabolites that were isolated from marine sponges (and sediments) collected in the Fernando de Noronha Archipelago. Bacterial isolates were identified by 16S rRNA gene sequencing. Low-molecular-weight metabolites were obtained through ethyl acetate/water biphasic system. Anti-*Trypanosoma cruzi* activity was evaluated against trypomastigotes (Y strain) using resazurin. The most active extract was further characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Among the six bacterial isolates investigated, four were identified as *Aureivirga marina* and *Vibrio hyugaensis*, both isolated from *Xestospongia muta*; *Pseudovibrio denitrificans*, isolated from *Aiolochroia crassa*; and *Vibrio alginolyticus*, isolated from marine sediment. All bacterial extracts exhibited potent antitrypanosomal activity, resulting in 100% parasite death at the highest tested concentration (200 µg/mL). After 24 h of incubation, the extracts displayed EC 50 values ranging from 3.1 to 13 µg/mL, resulting in a trypanocidal effect. The *V. hyugaensis* extract was the most active, with an EC 50 value of 3.1 µg/mL. ^{1}H and ^{13}C NMR analyses indicated that unsaturated fatty acids were the major constituents of the extract, together with minor signals attributable to aromatic protons, suggesting the presence of phenolic compounds and other aromatic metabolites. Marine microorganisms are promising sources of bioactive compounds and could be used for the development of new antiparasitic agents. **Supported by:** FAPESP 2025/22452-8, PROASA 2025/19173-0 **Keywords:** *Trypanosoma cruzi*; Therapy; Secondary metabolites.

TB – 021 - **MARINE ALKALOID-INSPIRED DRUG DISCOVERY IDENTIFIES A POTENT AND SELECTIVE COMPOUND AGAINST *TRYPANOSOMA CRUZI* INTRACELLULAR AMASTIGOTES**

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Among the Neglected Tropical Diseases, Chagas disease represents an important health problem in developing countries, affecting 6-8 million people. Limitations of the available treatment, demonstrate the urgent need for new therapeutic agents. Marine natural products represent a promising source of compounds with several biological activities. Ascididemin is a pentacyclic pyridoacridine alkaloid originally isolated from marine ascidians and reported to display antitumor and antiparasitic activities. In this work, we explored this scaffold to synthesize an ascididemin-derivative named LVO-01, and evaluated its anti-*Trypanosoma cruzi* activity. After synthesis, LVO-01 was purified by semi-preparative UHPLC using a C18 column. Trypomastigotes of Y strain were incubated with LVO-01 and the viability was determined by light microscopy and by the fluorimetric assay with resazurin. Using peritoneal macrophages as host cells, intracellular amastigotes were exposed to LVO-01 and the infectivity index determined by counting using a light microscope. Benznidazole (BNZ) was used as standard drug. Our results showed that LVO-01 was a selective and potent compound against the amastigotes, with an IC_{50} of 0.092 µM, showing 40-fold higher potency than BNZ. LVO-01 also showed potent activity against the trypomastigotes, eliminating 100% of the parasites at 0.15 µM. Light microscopy analysis (motility and morphology of trypomastigotes compared to untreated parasites) and mitochondrial activity studies (rezasurin assay) confirmed a trypanocidal activity of the compound. These preliminary results demonstrate the potential of LVO-01 as an antiparasitic candidate for further mechanism of action studies and reinforce the importance of marine derivatives as promising scaffolds for Chagas disease. **Supported by:** 001/0708/000.263/2026 **Keywords:** *Trypanosoma cruzi*; LVO-01; intracellular amastigotes.

TB – 022 - **FROM EXTREME ENVIRONMENTS TO DRUG DISCOVERY: INVESTIGATING PSEUDARTHROBACTER HUMI FOR ANTI-*TRYPANOSOMA CRUZI* COMPOUNDS**

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Neglected Tropical Diseases (NTD) affect billions of people worldwide. Chagas disease, is a NTD caused by *Trypanosoma cruzi*, characterized by a chronic and a severe disease with limited treatment options. Due to the high toxicity of the drugs and a limited efficacy, there is an urgent need for new therapeutics. Natural products have been served as scaffolds for FDA-approved drugs, and in this context, secondary metabolites derived from extremophilic bacteria may be useful pharmaceutical prototypes. Therefore, this study aimed to evaluate the anti-*Trypanosoma cruzi* activity of microbial small molecules from an extremophilic bacterial isolate. The bacterium was isolated from sediments collected from Grey Lake, in Chilean Patagonia, and was subsequently identified by 16S rRNA gene sequencing. After growth on Petri dishes containing tryptic soy agar, the molecules from the bacterial biomass were extracted using ethyl acetate/aqueous system. The organic extract was then incubated with *Trypanosoma cruzi* (Y strain) and subjected to a resazurin-based fluorimetric assay to evaluate antiparasitic activity. Bio-guided studies using solid-phase extraction was performed using C18 cartridge and chemical characterization was carried out using 1 H and 13 C Nuclear Magnetic Resonance spectroscopy. Our 16S rRNA gene sequencing revealed the bacterium *Pseudarthrobacter humi*. The organic extract with the metabolites showed potent antiparasitic activity, killing 100% of trypomastigotes at the highest tested concentration of 150 µg/mL, resulting in an IC 50 value of 20 µg/mL, with a trypanocidal effect confirmed by the lack of morphology and motility as well as the lack of mitochondrial activity. Chemical characterization of the extract is under evaluation. In conclusion, metabolites produced by *Pseudarthrobacter humi* demonstrate considerable potential for future fractionation studies aiming the isolation of active metabolites against *Trypanosoma cruzi.* **Supported by:** FAPESP 2024/16243-4 and CNPq 407899/2024-3 **Keywords:** Chagas disease; Pseudarthrobacter humi; Drug Discovery.

TB – 023 - **Drug repurposing for cutaneous leishmaniasis reveals CLK1/CLK2 as targets of kinase inhibitors**

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Leishmaniasis is a neglected tropical disease for which current treatments are limited by toxicity, prolonged treatment regimens, and emerging drug resistance, highlighting the need for new therapeutic options. Drug repurposing offers a promising strategy to accelerate antileishmanial drug discovery, while protein kinases represent attractive therapeutic targets due to their essential roles in cellular signaling and the availability of numerous clinically approved kinase inhibitors. Here, we investigated 121 clinically approved kinase inhibitors against *Leishmania amazonensis* promastigotes. Thirteen compounds reduced parasite viability by > 50% at 1 µM, and two showed potent activity against intracellular amastigotes with favorable selectivity toward host macrophages. Previous studies reported the anti-trypanosomatid activity of the covalent kinase inhibitors AB1 and WZ8040 and identified the kinetochore-associated kinases CLK1/CLK2 as their molecular targets. To investigate the compound´s mechanisms of action, resistant parasites were generated through chemical mutagenesis and stepwise drug selection. DNA sequencing of resistant clones revealed mutations in CLK1 and CLK2 genes. Biochemical assays and intact mass spectrometry analysis with recombinant proteins confirmed that both compounds covalently inhibit CLK1/CLK2, and that resistance-associated mutations reduce drug sensitivity. Genetic and chemoproteomic approaches are being employed to further investigate the role of these kinases in drug activity. Both compounds are currently being evaluated in a murine model of cutaneous leishmaniasis. Together, these findings reinforce CLK1/CLK2 as promising targets for antileishmanial drug development and demonstrate the potential of kinase inhibitor repurposing as a strategy for discovering new treatments for leishmaniasis. **Supported by:** FINEP, FAPESP, Eurofarma Laboratórios S/A. **Keywords:** Leishmania; Reurposing; Drug discovery.

TB – 024 - **In vivo efficacy and biochemical target engagement of a chromene-based inhibitor of *Trypanosoma cruzi* lysyl-tRNA synthetase**

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Chagas disease remains a neglected parasitic disease with limited therapeutic options. Aminoacyl-tRNA synthetases have emerged as promising antiparasitic targets, but *in vivo* support for *Trypanosoma cruzi* lysyl-tRNA synthetase (TcLysRS) has so far relied mainly on quinazoline inhibitors. Here, we evaluated compound 5, a chromene-based LysRS inhibitor previously developed for *Plasmodium falciparum* and *Cryptosporidium parvum*, as an independent chemotype to probe TcLysRS tractability in *T. cruzi*. Compound 5 showed potent activity against intracellular amastigotes (EC_{50} = 0.9 µM), low host-cell toxicity, and favorable oral exposure, with high bioavailability and delayed absorption. In an acute murine model of Chagas disease, oral treatment suppressed blood parasitemia and significantly reduced parasite burden in heart, spleen, and colon. Cardiac histopathology further showed reduced inflammatory foci and absence of detectable amastigote nests in treated animals. To assess target engagement, recombinant cytosolic TcLysRS was kinetically characterized and inhibited by compound 5 in a dose-dependent manner (IC_{50} = 2.7 µM). Differential scanning fluorimetry and X-ray crystallography supported ligand binding, and the first crystal structures of TcLysRS revealed compound 5 occupying the ATP-binding pocket in the presence of lysine. Structural comparisons with apicomplexan and human LysRS orthologs showed conserved ATP-site mimicry, while highlighting adjacent regions that may be explored for selectivity. Collectively, these data provide cellular, biochemical, structural, pharmacokinetic, and in vivo evidence that TcLysRS is a tractable antichagasic target. This work expands LysRS validation beyond quinazolines and identifies a chromene scaffold as a starting point for future studies on Chagas disease and other kinetoplastid infections. **Supported by:** This study was funded in part by the São Paulo Research Foundation (FAPESP; grants 2021/14741-9 and 2022/03873-4) and FINEP (01.22.0473.00). **Keywords:** *Trypanosoma cruzi*; lysyl-tRNA synthetase; target engagement.

TB – 025 - **Exploring Starfish-Associated Marine Bacteria as a Source of Anti-*Trypanosoma cruzi* Metabolites**

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Chagas disease, caused by *Trypanosoma cruzi*, is a Neglected Tropical Disease affecting 6 to 8 million people worldwide. Current treatment relies on two drugs with limited efficacy and considerable toxicity, highlighting the need for new therapeutics. Oceans harbor microorganisms adapted to unique environmental conditions, driving the biosynthesis of structurally diverse secondary metabolites with pharmaceutical potential. In this work, we investigated the anti-*Trypanosoma cruzi* activity of marine microbial metabolites from bacteria isolated from the starfish *Echinaster brasiliensis*, collected in São Sebastião, São Paulo. Bacterial isolates were obtained from the internal tissue of *E. brasiliensis* and cultivated in Marine Agar plates. Strains were identified by 16S rRNA sequencing and Gram staining. Metabolites were extracted using an ethyl acetate/water biphasic system and dried in rotary evaporator. Crude extracts were incubated with *T. cruzi* trypomastigotes (Y strain) for 24 h at 37°C / 5% CO_2. The morphology and motility were analyzed by light microscopy and the viability determined by the resazurin assay. The isolates were identified as *Pseudoalteromonas piscicida, Pseudoalteromonas phenolica* and *Pseudoalteromonas* sp. and one isolate is under investigation. The extracts showed potent antitrypanosomal activity, with IC_{50} values of 3.1 µg/mL, 1.1 µg/mL, 5.6 µg/mL and 1.0 µg/mL, respectively. All extracts eliminated 100% of the parasites at the highest tested concentration (150 µg/mL), showing a trypanocidal effect. Chemical characterization of *P. phenolica* extract is under investigation by ^{1}H and ^{13}C Nuclear Magnetic Ressonance. These preliminary results indicate that bacteria isolated from *E. brasiliensis* produce metabolites with pharmacological potential against *T. cruzi*, supporting further fractionation and structural elucidation of active compounds. **Supported by:** Supported by CNPq 407899/2024-3 and FAPESP PROASA 2025/19173-0. **Keywords:** *Trypanosoma cruzi*; *Echinaster brasiliensis*; anti *Trypanosoma cruzi* activity.

TB – 026 - **Functional characterization of *Trypanosoma cruzi* serine hydroxymethyltransferase and evaluation of pyrazolopyran inhibitors as potential drug candidates.**

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Chagas disease is a neglected tropical infection affecting millions under social vulnerability and current treatments face high dropout rates due to adverse effects. In this scenario, the enzyme Serine Hydroxymethyltransferase stands out as a strategic target due to its central role in one-carbon metabolism, which is essential for nucleotide biosynthesis and epigenetic regulation. This study aims to functionally characterize recombinant TcSHMT and evaluate its inhibition profile against pyrazolopyran compounds. Catalytic activity and kinetic parameters were determined via retro-aldol cleavage of β-phenylserine, and six pyrazolopyrans were evaluated through enzymatic inhibition assays. Additionally, cell-based phenotypic assays were performed with epimastigote and amastigote forms of the parasite to evaluate the biological activity of the compounds. Kinetic characterization against β-phenylserine revealed Michaelis-Menten behavior (Km = 17.50 mM; Vmax = 0.05 mM/s; Kcat = 5 s^{-1}; R^2 = 0.9992). The evaluation of the inhibitory potential with a series of six reference pyrazolopyrans demonstrated enzymatic inhibition for all tested compounds, notably the enantiomer (+)SHIN2, SHIN1 and SHMT-IN-2. In phenotypic assays, all inhibitors proved to be cytotoxic against the intracellular amastigote form, whereas against the epimastigote form, only two demonstrated cell death potential. These results validate the potential of TcSHMT as a promising therapeutic target, showing that the recombinant enzyme is catalytically active and efficiently inhibited by pyrazolopyrans. Furthermore, the cytotoxic activity observed in cell assays, especially against the clinically relevant amastigote form, reinforces the biological relevance of these compounds. Future steps involve expanding to chemical libraries with greater diversity to optimize selectivity and potency against *Trypanosoma cruzi*. **Supported by:** CAPES - 88887.203496/2025-00 **Keywords:** Chagas diseases; Drug discovery; One-carbon metabolism.

TB – 027 - **In vivo anti-*Leishmania amazonensis* effects of topical treatment with a biocurative containing the cobalt metal complex A3310 in combination with systemic treatment**

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Cutaneous leishmaniasis is a neglected disease caused mainly by *Leishmania amazonensis*, principal causative agent in Brazil. Conventional treatment relies on pentavalent antimonials and amphotericin B (AmB), limited by high toxicity, highlighting the need for new therapeutic strategies. Metal complexes have demonstrated *in vitro* efficacy against *L. amazonensis*, emerging as promising alternatives. The *in vivo* efficacy of the cobalt metal complex A3310 was evaluated in BALB/c mice infected in the ear pinna with amastigotes of *L. amazonensis* (Josefa strain). Animals were treated topically and systemically for 40 days and divided into five groups: Control; Hydrogel + A3310 (100 µg/kg); Hydrogel + A3310 (200 µg/kg); Hydrogel + AmB (200 µg/kg); and Hydrogel alone. The same concentrations were administered intraperitoneally without hydrogel. Wound healing, lesion progression, parasite load, and ultrastructure were evaluated by calipers, histopathology, transmission electron microscopy, and pro- and anti-inflammatory gene expression by RT-qPCR. Treatment with Hydrogel + A3310 progressively reduced lesion size, with greater effect and tissue regeneration at 200 µg/kg compared with the AmB group. Histopathological analysis of the Hydrogel + A3310 (200 µg/kg) group showed reduced parasite load, tissue recovery, mild inflammatory infiltrate, and dermal restoration. Amastigotes exhibited ultrastructural alterations suggestive of mitochondrial impairment. In contrast, the Control and AmB groups showed high parasite load, tissue disorganization, and preserved ultrastructure of parasites. Although both treatments activated cytokine-related pathways (*Ifng, Il10ra, Il2rg*), the Hydrogel + A3310 (200 µg/kg) group stood out by overcoming the cell migration limitation induced by AmB (200 µg/kg), characterized by *Itgb2* underexpression, restoring the adhesion machinery essential for tissue effector response. Results indicate remarkable systemic and topical therapeutic potential for A3310. **Keywords:** Cutaneous leishmaniasis; Metal complexes; *Leishmania amazonensis*.

TB – 028 - **DEVELOPMENT OF THE PEP01-NH2-R8 BIOCONJUGATE: A NOVEL ANTILEISHMANIAL STRATEGY**

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Given the limitations and high toxicity of current treatments for cutaneous leishmaniasis, the development of novel therapeutic approaches is urgently needed. In this context, cysteine protease B (CPB) from Leishmania amazonensis has emerged as a promising therapeutic target. Although the nitrile-based dipeptide, PEP01, demonstrates CPB inhibition and activity against amastigotes, its high hydrophilicity restricts cell penetration. To overcome this limitation, conjugation to the cationic cell-penetrating peptide (CPP) R8 represents a promising strategy to enhance cellular uptake and intracellular delivery. Therefore, this project aims to synthesize, purify, and characterize the PEP01-NH2-R8 bioconjugate and evaluate its antileishmanial activity and ability to retain CPB inhibitory activity. The R8 carrier was independently synthesized by Solid-Phase Peptide Synthesis (Fmoc-SPPS), purified by reversed-phase HPLC (RP-HPLC), and characterized by mass spectrometry (MS). Before conjugation, R8 was evaluated for cytotoxicity (CC50) in murine macrophages using the MTT assay, inhibitory activity (EC50) against promastigotes (MTT assay), and inhibitory activity (IC50) against CPB using a fluorometric assay. Preliminary results demonstrate the successful synthesis of R8 with 100% purity, as determined by RP-HPLC. Mass spectrometry confirmed its identity, revealing a peak corresponding to the triply charged ion, consistent with the theoretical molecular mass of 1266.52 Da. In biological assays, isolated R8 exhibited a favorable in vitro safety profile, characterized by low cellular toxicity, no intrinsic activity against promastigotes, and non inhibition of CPB. In conclusion, these results support R8 as a suitable carrier and provide a methodological foundation for the synthesis and subsequent biological evaluation of the final PEP01-NH2-R8 bioconjugate. **Supported by:** FAPESP: 2025/27074-1; FAPESP 2025/17976-8; FAPESP: 2024/20579-8. **Keywords:** *L. amazonensis*; bioconjugation; cell-penetrating peptide.

TB – 029 - **Amphotericin B-loading superparamagnetic iron oxide nanoparticles as a promising therapeutic strategy for cutaneous leishmaniasis**

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Leishmaniasis is a neglected tropical disease with a major global impact. Because pharmaceutical investment in new treatments remains limited, current therapies are often highly toxic and associated with serious drawbacks, including adverse effects, high costs, the need for hospitalization, and parasite resistance. Amphotericin B (AmB) is widely used to treat leishmaniasis worldwide, including cutaneous leishmaniasis (CL). However, its use is sometimes limited by serious adverse effects and high cost. Therefore, this study aimed to develop a drug delivery system composed of superparamagnetic iron oxide nanoparticles (SPIONs) loaded with AmB to provide a new therapeutic alternative for treating CL caused by *Leishmania amazonensis*. SPIONs-AmB showed anti-*Leishmania* effects against intracellular amastigote and promastigote forms, which were concentration- and time-dependent. Cytotoxicity assays demonstrated that SPIONs-AmB significantly reduced AmB cytotoxicity toward macrophages in a concentration- and time-dependent manner. Compared with free AmB (SI: 112), SPIONs-AmB displayed a markedly improved selectivity index (SI: 7593), indicating preferential activity against protozoan cells over host cells. The effects on the morphology and ultrastructure of promastigotes treated with SPIONs-AmB were analyzed by optical and electron microscopy. Microscopy analyses showed treatment-induced changes in promastigote morphology: immunofluorescence revealed altered cell shape without cytoskeletal disruption; SEM showed surface morphological changes; and TEM confirmed SPIONs-AmB internalization by the parasites. Taken together, SPIONs-AmB represent a promising local therapeutic alternative for CL, offering reduced cytotoxicity toward host macrophages and high efficacy against both parasite developmental stages. **Supported by:** FAPERJ (E-26/201.191/2022) **Keywords:** Cutaneous leishmaniasis; SPIONs; Amphotericin B.

TB – 030 - **Biophysical Characterization of the recombinant *Leishmania donovani* Adenylosuccinate Lyase (*Ld*ASL) as a Target for Drug Discovery**

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Visceral Leishmaniasis (VL), caused by *Leishmania donovani*, is a severe tropical disease with limited therapies due to high toxicity and resistance. Since trypanosomatids rely exclusively on the purine salvage pathway for nucleotide biosynthesis, the enzyme Adenylosuccinate Lyase from *Leishmania donovani* (*Ld*ASL) emerges as a promising therapeutic target, presenting exploitable structural differences compared to the human enzyme (HsASL). This study aims to express, purify, and characterize *Ld*ASL to enable the screening of 400 compounds from the Pandemic Response Box library. Recombinant *Ld*ASL was expressed in *Escherichia coli* BL21(DE3) at 25°C. Cells were centrifuged and lysed by sonication, and the soluble fraction was purified via affinity chromatography (Ni-NTA). Biophysical evaluation was conducted using Dynamic Light Scattering (DLS), Differential Scanning Fluorimetry (DSF), and Circular Dichroism (CD). Catalytic activity was spectrophotometrically monitored by quantifying the consumption of the adenylosuccinate substrate (SAMP). Purification of *Ld*ASL achieved an optimal yield of 3 mg/mL, confirmed by a 53.4 kDa band on SDS-PAGE. DLS analysis indicated high monodispersity (99.5% by volume), a main hydrodynamic diameter of 11.22 nm, and an estimated mass of 190.6 ± 73.1 kDa, suggesting a tetrameric state in solution. CD validated the expected folding with a secondary structure rich in alpha-helices (minima at 208 and 222 nm), while DSF determined a melting temperature of 44°C under optimized buffer conditions. Finally, enzymatic assays validated the in vitro catalytic function, demonstrating the immediate and linear consumption of 150 µM SAMP over 70 seconds. In summary, *Ld*ASL was obtained with preserved conformational integrity and activity. These parameters establish a robust foundation for subsequent enzymatic inhibition and co-crystallization assays, which are essential for the rational design of novel drugs against leishmaniasis. **Supported by:** CNPq - 144372/2025-8 **Keywords:** Visceral Leishmaniasis; Adenylosuccinate Lyase; Drug Discovery.

TB – 031 - **Essential oils from Piper spp. in anti-*Trypanosoma cruzi* drug discovery: antiparasitic activity and cytotoxicity assessment**

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Chagas disease, caused by *Trypanosoma cruzi*, affects millions of people and can progress to chronic cardiac and gastrointestinal complications. Current treatment relies mainly on benznidazole and nifurtimox, which are associated with adverse effects, long treatment regimens, and limited efficacy in the chronic phase, highlighting the need for new therapeutic alternatives. Natural products are a well-established source of bioactive molecules for drug discovery. This study evaluated the trypanocidal activity of essential oils (EOs) from *Piper* spp., Tween 80-based nanoemulsions (NEs), and selected commercial plant-derived compounds. Activity against epimastigotes was assessed by resazurin reduction and optical density, while cytotoxicity and activity against intracellular amastigotes were evaluated in infected H9c2 cells by Hoechst nuclear staining and high-content confocal microscopy using the Operetta system. Most formulations showed activity against epimastigotes, with all EOs inhibiting parasite growth. *P. aduncum* and *P. baccans* EOs displayed the lowest IC50 values, followed by *P. hispidum* and *P. marginatum*, respectively. Among NEs, *P. marginatum* showed the lowest IC50, although nanoemulsification generally reduced activity compared with the corresponding EOs, possibly due to Tween 80-related formulation effects. In infected H9c2 cells, the tested concentration range did not allow reliable IC50 or selectivity index determination; however, *P. aduncum* EO and piperine showed trends toward selectivity, with piperine reaching approximately 50% inhibition at 40 µM, whereas *P. hispidum*, *P. baccans*, and *P. marginatum* showed higher cytotoxicity. These findings highlight *Piper*-derived essential oils and constituents as promising starting points for anti-*T. cruzi* drug discovery. Future studies should investigate their mechanisms of action and expand amastigote assays across broader concentration ranges to better define potency and selectivity. **Keywords:** *Trypanosoma cruzi*; Natural products; Drug discovery.

TB – 032 - **Identification of novel protein synthesis inhibitors for *Trypanosoma cruzi***

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Chagas disease causes 12,000 fatalities annually and has restricted treatment options. It is a neglected disease that has been spread through non endemic areas due to environmental changes, migration and expansion of the vector range. The disease is caused by the protozoan parasite *Trypanosoma cruzi*, that has an uncommon translation process, characterized by enlarged ribosomes and a unique mRNA cap attached to the 5’ end of a 39-nucleotide spliced leader sequence at the 5’ end of all its mRNAs. Those significant differences between the *T. cruzi* protein synthesis machinery and that of its mammalian host could be explored for new therapeutic options, similar to many antimicrobial agents that are protein synthesis inhibitors. Here, we used an assay consisting of *T. cruzi* extracts from a strain that accumulates less hemin to translate a capped and polyadenylated Renilla luciferase mRNA. We tested 128 chemicals that are already known to suppress *T. cruzi* growth *in vitro* and then identified quinazoline compounds that inhibit translation of epimastigotes and proliferation of wild-type amastigotes inside mammalian cells at a low micromolar range and a selectivity index higher than 200. Future target identification and medicinal chemistry based on the identified hits will help to develop new treatments for Chagas disease. **Supported by:** (Grant 5R21AI154196, NIH), (FAPESP, Processes 2020/07870-4 and 2021/10599-3), (Grant 408273/2024-0 INCTV), (CNPq, 303788/2020-8), (CAPES, 8887.920638/2023-0), (CNPq, 409540/2023-4 and 308033/2025-6), (CAPES, 88887.176598/2025-00), FAPESP (2022/11551-7), DNDi is grateful to its donors, public and private, who have provided funding.
Keywords: *Trypanosoma cruzi*; Protein synthesis inhibitors; Chagas disease.

TB – 033 - **Evaluation of novel triazolopyrimidine derivatives against *Leishmania amazonensis* using *in vitro* and *ex vivo* models.**

GOOSSENS, G.M.S.[1]; AZEVEDO, R.[1]; BATISTA, M.M.[1]; CASCABULHO, C.[1]; FEITOSA, L.D.M.[2]; FRANÇA, R.R.F.[2]; PINHEIRO, L.C.D.S.[3]; BOECHAT, N.[2]; CALJON, G.[4]; SOEIRO, M.D.N.C.[1].
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Leishmaniasis remains a Neglected Tropical Disease with limited therapeutic options, highlighting the urgent need for novel treatments. Triazolopyrimidines have previously demonstrated antiplasmodial activity, supporting their potential for drug repurposing. This study aimed to evaluate the antileishmanial activity, cellular targets, and cytotoxic profiles of a series of triazolopyrimidine derivatives. Ultrastructural and functional alterations in the parasite were further investigated using electron microscopy and flow cytometry. The findings indicate that four compounds were active against the intracellular amastigote stage in mouse peritoneal macrophages and one compound (**4**) exhibited an EC_{50} = 1.28 μM (with selectivity index = 37.19) and an indifferent interaction when evaluated in combination with miltefosine. Electron microscopy and flow cytometry of *ex vivo* treated amastigotes showed plasma membrane disruption and mitochondrial dysfunction. These results highlight the potential of triazolopyrimidines as a promising starting point for the development of new therapeutic strategies against leishmaniasis. **Supported by:** Fundação Oswaldo Cruz (Fiocruz), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (Faperj), Fundação Oswaldo Cruz. MNCS and NB are researchers of CNPq and CNE/FAPERJ. **Keywords:** Leishmania amazonensis; drug repurposing; triazolopyrimidines.

TB – 034 - **A POTENT COMPOUND AGAINST *TRYPANOSOMA CRUZI* EPIMASTIGOTES: MOLECULAR INSIGHTS INTO TCBDF3 RECOGNITION**

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Chemical optimization of bioactive scaffolds provides an opportunity to enhance antiparasitic potency while exploring their molecular recognition profiles in *Trypanosoma cruzi*. A1B4, a hydrazone with activity against epimastigotes (IC_{50} = 23 ± 2.04 μM), also binds TcBDF3 (IC_{50} = 2.4 ± 0.3 μM), an atypical bromodomain associated with acetylated α-tubulin and parasite differentiation. Based on this scaffold, compound 81 was investigated to determine whether structural modification could improve antiparasitic activity while retaining molecular interactions with TcBDF3. Dm28c/pLacZ epimastigotes (1×10^4 parasites/well) were treated with compound 81 (0.1–10 μM) for 72 h at 28 °C. Antiparasitic activity was determined using the β-galactosidase/CPRG colorimetric assay at 595 nm, and IC_{50} was determined by nonlinear regression. For molecular docking, an AlphaFold2-predicted TcBDF3 model was refined following 50 ns of molecular dynamics. Docking was performed using GOLD v5.8, generating 100 poses per ligand, and ChemScore was selected for best reproducing the interaction pattern of A1B4. Compound 81 showed potent activity against epimastigotes, with an IC_{50} of 0.4673 μM (95% CI: 0.42–0.52 μM), being approximately 49-fold more potent than A1B4. Docking revealed interactions with Val70, Leu75, Val77, Asn119, Cys120, and Asn124, including hydrogen bonds with Asn119 and Asn124. Interactions with Val70, Leu75, Val77, Asn119, and Cys120 were also observed for A1B4, suggesting a partially conserved interaction profile within the TcBDF3 binding site. Together, the marked antiparasitic activity of compound 81 and the conservation of key interactions within the TcBDF3 cavity highlight this derivative as a promising anti-*T. cruzi* compound and support further investigation of its interaction with TcBDF3 and validation in clinically relevant parasite stages. **Supported by:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil, Grants No. 2025/07619-3 and 2025/03878-4; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 **Keywords:** Chagas disease; Drug discovery; Molecular docking.

TB – 035 - **Expanding TcBDF3 Pharmacological Modulation: Development of a PROTAC for *Trypanosoma cruzi***

SCARIM, C.B.[1]; GINI, A.L.R.[1]; DA CUNHA, P.S.T.[1]; RUIZ, D.A.G.[2]; DO NASCIMENTO JUNIOR, N.M.[2]; DOS SANTOS, J.L.[1]; SERRA, E.C.[3].

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The search for new therapeutic strategies for Chagas disease has encouraged the exploration of molecular targets in *Trypanosoma cruzi*. Bromodomain factor 3 (TcBDF3) recognizes acetylated proteins and is involved in parasite differentiation, cytoskeletal dynamics, motility, and infectivity, supporting its relevance as a therapeutic target. A TcBDF3-binding ligand with micromolar activity (IC_{50} = 2.4 μM) was used as a starting point for the design of functionalized derivatives for bifunctional molecules. Here, we synthesized a PROTAC containing a functionalized TcBDF3-binding ligand and investigated whether functionalization preserved target recognition. The PROTAC was synthesized in four steps, including condensation with vanillin, introduction of a glutaric linker, and coupling to a VHL ligand. The parent ligand and its functionalized derivative were evaluated by molecular docking against an AlphaFold2 TcBDF3 model refined by molecular dynamics. The PROTAC was obtained in 8.28% overall yield. ^1H and ^13C NMR supported its structure, with diagnostic signals for the azomethine proton (δ 8.06 ppm), amide NHs (δ 11.70, 8.57 and 7.96 ppm), glutaric linker (δ 2.58–1.86 ppm), VHL moiety (δ 4.57–4.22 and 0.96 ppm), and carbonyl carbons (δC 160.45–171.96 ppm). Docking showed preserved contacts with Val70, Leu75, Val77, Tyr80, Val84, Asn119, Cys120, and Tyr123. Interactions observed for the parent ligand with Met88, Arg96, Tyr81, and Pro87 were not retained, while the functionalized derivative formed a new hydrogen bond with Leu80. Thus, functionalization partially reorganized the binding mode while preserving relevant TcBDF3 contacts. These findings support the functionalized ligand as a TcBDF3-binding moiety and provide a structural basis for future functional studies of TcBDF3 degradation, expanding its pharmacological modulation beyond conventional inhibition and supporting targeted protein degradation as a potential therapeutic strategy for Chagas disease. **Supported by:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil, Grants No. 2025/07619-3 and 2025/03878-4; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. **Keywords:** Targeted protein degradation; Bromodomains; Drug discovery.

TB – 036 - **EVALUATION OF THE TRYPANOCIDAL EFFECT OF TRYPAGE-2 IN COMBINATION WITH A REPURPOSED DRUG: A POSSIBLE THERAPEUTIC ALTERNATIVE FOR CHAGAS DISEASE**

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1. FUNDAÇÃO OSWALDO CRUZ, RIO DE JANEIRO - RJ - BRAZIL; 2. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO - RJ - BRAZIL.

Chagas disease remains a public health challenge, with benznidazole and nifurtimox as the only drugs available for its treatment. However, limited efficacy in the chronic phase and treatment-related adverse effects reinforce the need for new therapeutic strategies. This study evaluated the trypanocidal efficacy of the repurposed metal-chelating drug POD-1 combined with the established antiparasitic agent TrypAge-2 against trypomastigote and axenic amastigote forms of *Trypanosoma cruzi* (Y strain), and investigated the mechanisms. Activity was determined by cell viability assays, fractional inhibitory concentration index (FICI), and isobologram. Mechanisms were investigated by flow cytometry using Annexin V and Propidium Iodide, TBARS, nitrite determination in VERO cells infected or not with *T. cruzi* by the Griess reaction, scanning electron microscopy (SEM), and in silico prediction of pharmacokinetic properties using SwissADME. POD-1 showed trypanocidal activity against trypomastigote and axenic amastigote forms of *T. cruzi*, and its association with TrypAge-2 increased antiparasitic activity, with a synergistic interaction at a POD-1 ratio of 4:1. The treatments increased lipid peroxidation products and nitrite concentration, indicating alterations in redox and nitrosative balance, although these parameters did not explain synergism. Flow cytometry showed a predominance of AV+/PI+ parasites, while SEM revealed blebs, surface irregularities, and plasma membrane discontinuities, evidencing impaired cellular integrity. In silico analyses indicated that POD-1 has pharmacokinetic properties compatible with a candidate for therapeutic repurposing. Overall, POD-1 showed trypanocidal activity, and its association with TrypAge-2 potentiated antiparasitic activity in a ratio-dependent manner, supporting the involvement of multiple processes related to parasite cellular homeostasis and providing an experimental basis for further investigations into its mechanisms of action. **Supported by:** Capes - código 0001 **Keywords:** Chagas disease; Drug repositioning; Combined therapy.

TB – 037 - **Antileishmanial strategy targeting sterol metabolism and intracellular cholesterol transport in *Leishmania amazonensis***

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The treatment of leishmaniasis remains challenging due to the limited number of available drugs, their toxicity, and therapeutic resistance. Sterol metabolism is a promising target for antileishmanial drug development, as *Leishmania* spp. depend on endogenous sterol biosynthesis and can acquire cholesterol from the host. Previous studies have shown that inhibition of sterol biosynthesis may be compensated by increased uptake of exogenous cholesterol, suggesting a mechanism associated with therapeutic failure. This study aims to investigate cholesterol acquisition by intracellular amastigotes of *Leishmania amazonensis* and establish a strategy combining inhibitors of sterol biosynthesis and intracellular cholesterol transport. As a first step, an experimental model was standardized for amastigote isolation and enrichment. Macrophages were infected with *L. amazonensis* expressing green or red fluorescent protein, followed by mechanical lysis and parasite recovery by imaging flow cytometry and cell sorting. Different macrophage models, infection ratios, lysis conditions, and fluorescent markers were evaluated. Mechanical lysis released intracellular amastigotes, but generated samples with considerable cellular debris and residual macrophages. Percoll density-gradient purification was ineffective under the conditions tested. Imaging flow cytometry and cell sorting enabled parasite identification and separation according to size and fluorescence; however, macrophage autofluorescence impaired GFP-expressing parasite discrimination and purification. Preliminary analyses using RFP-expressing *L. amazonensis* showed higher fluorescence and reduced spectral overlap with host-cell autofluorescence, indicating that RFP is a promising marker for parasite detection and purification. These results establish parameters for obtaining enriched amastigote populations and support subsequent metabolic studies. Future experiments will assess cholesterol incorporation using ^13C-labeled substrates and gas chromatography coupled to mass spectrometry, as well as cholesterol transport inhibitors combined with sterol biosynthesis inhibitors. This approach may clarify cholesterol acquisition and support new strategies targeting parasite sterol metabolism.
Keywords: Leishmania amazonensis; Sterol metabolism; Cholesterol transport.

TB – 038 - **Análise de citotoxicidade frente a formas promastigotas e células THP-1 diferenciadas em macrófagos de compostos da *Pandemic Response Box***

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Leishmaniasis comprises diseases caused by flagellated unicellular parasites of the genus Leishmania and transmitted by phlebotomine sand flies. It manifests mainly as visceral leishmaniasis (VL) and tegumentary leishmaniasis (TL). VL has a high case-fatality rate and may cause death in over 90% of untreated individuals. Brazil plays a prominent role in its global epidemiology, reporting the highest incidence in the Americas. Although TL has low mortality, it can cause ulcerative cutaneous and mucosal lesions, with significant social impacts and impaired quality of life due to difficulties in speech, breathing, and swallowing. Current treatments for VL and TL are limited to pentavalent antimonials, amphotericin B, including its liposomal formulation, and miltefosine, which are associated with high toxicity, high costs, and therapeutic challenges. Given this scenario, high-throughput screening was conducted on Leishmania infantum amastigotes using 400 repurposing compounds from the DNDi Pandemic Response Box library. Based on this analysis, four promising compounds A1, A2, B1, and B2 were selected. Following this selection, MTT screening assays were performed to determine the cytotoxic concentration for 50% of macrophages (CC_{50}) and the inhibitory concentration for 50% of promastigotes (IC_{50}). Differentiated THP-1 cells and parasites were incubated with varying compound concentrations for 24 hours in 96-well plates. Viability was assessed by adding MTT solution and measuring absorbance. Values were normalized against the untreated control and analyzed using GraphPad Prism® to determine concentration-response curves and CC_{50} and IC_{50} values. The selectivity index (SI) was calculated as the CC_{50}/IC_{50} ratio. Compounds A1, A2, B1, and B2 exhibited SI values of 0.71, 4.13, 7.55, and 33.23, respectively. Although A1 had the lowest SI, it showed the highest CC_{50} and IC_{50} values, 7.71 mM and 10.82 mM, respectively. **Keywords:** Drug-repurposing; Leishmaniasis; Screening.

TB – 039 - **From Environmental Contaminants to Drug Resistance: Assessing the Potential of Pesticides to Induce Benznidazole Cross-Resistance in *Trypanosoma cruzi***

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Chagas disease (CD) is an endemic zoonosis in Latin America, placing over 100 million individuals at risk of infection. These numbers are increasing due to climate change, which facilitates the territorial expansion of triatomine vectors. Current CD treatment relies on two drugs with limited efficacy against *Trypanosoma cruzi*, and resistant strains have emerged. The World Health Organization identifies antimicrobial resistance (AMR) as a major threat to human health, contributing to 4.7 million deaths globally in 2021. Part of this problem is caused by cross-resistance, in which resistance to different compounds arises from shared or overlapping modes of action. Given the widespread presence of pesticides, herbicides, and veterinary drugs in Latin American soils and riverbeds, along with the indirect exposure of *T. cruzi* to these compounds via their vectors, this study evaluates the resistance-inducing potential of veterinary and agro-industrial compounds (VAiCs) reported as environmental contaminants against benznidazole. A pesticide library comprising 465 compounds is being screened against epimastigotes of the DM28c strain using a resazurin assay. To date, 9 of 110 VAiCs demonstrate EC_{50} values consistent with those detected in environmental or veterinary samples (furazolidone, nitrofurazone, chlorhexidine, berberine hemisulfate, fluazinam, quinoclamine, trifloxystrobin, DDAC, and bronopol). Some of them are extensively used in Brazilian rice, soybean, and fruit crops. The nitroheterocyclic compounds furazolidone (EC_{50} 2.71 ± 2.05 µM) and nitrofurazone (EC_{50} 7.27 ± 1.20 µM) were selected to induce resistance through intermittent and continuous prolonged exposure in DM28c (DTU I), ARMA13 cl1 (DTU III), and ERA cl2 (DTU IV). VAiC-resistant phenotypes will be characterized against the Colombiana strain (a naturally BZ-resistant strain) to provide a molecular profile. Within a One Health framework, agrochemical exposure may shape parasite resistance prior to host infection. Therefore, forecasting cross-resistance mechanisms in *T. cruzi* offers a valuable approach to understanding treatment failure in CD and to guide the development of more effective antiparasitic drugs and next-generation strategies targeting AMR. **Supported by:** FAPESP (Processo 2021/10599-3) **Keywords:** antimicrobial resistance; One Health approach; Cross-Resistance.

TB – 040 - **Functional analysis of methionine sulfoxide reductase in antimony resistance in *Leishmania amazonensis***

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Leishmaniasis is a neglected tropical disease endemic in Brazil, which is caused by protozoa of the genus *Leishmania*. Treatment is limited to a few options of drugs that present several issues such as parenteral administration, severe side effects and reduced clinical responsiveness. Pentavalent antimonials (SbV) are the first-line drugs for cutaneous leishmaniasis, despite having only a 40-60% efficacy in the treatment of the disease. Antimonials induce oxidative stress in the parasite, and genes involved in the response to oxidative damage may contribute to its survival under drug pressure. Among the clinical isolates studied by our group, an isolate of *Leishmania amazonensis* obtained from a patient co-infected with HIV and refractory to treatment with pentavalent antimonial (Glucantime) and liposomal amphotericin B was recently characterized. In vitro susceptibility assays confirmed the resistance phenotype to SbV and amphotericin B for this isolate. Analysis of the genome of this isolate identified two mutations in the gene encoding methionine sulfoxide reductase (MSR), which is involved in the response to oxidative damage, and was selected for investigation regarding its possible role in resistance to antimonials. Functional validation of MSR is being conducted through the generation of CRISPR-Cas9 gene knockout and overexpression approaches to investigate the role of MSR in antimony resistance. These studies may shed light on the role of MSR in the response of *L. amazonensis* to antimonials and assess its potential involvement in the resistance phenotype. **Supported by:** CNPq (2024/00360-1) and FAPESP (2026/02239-0). **Keywords:** *Leishmania amazonensis*; antimonials; CRISPR-cas9.

TB – 041 - **Novel Antileishmanial Prototypes: from Hit-to-Lead optimization to mechanistic investigation**

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Leishmaniases are neglected tropical diseases whose treatment remains limited by toxicity, parasite resistance, and prolonged therapeutic regimens, reinforcing the need for safer and more effective drugs. Here, a *hit-to-lead* strategy was applied to identify and optimize antileishmanial prototypes from five synthetic series: N-acylhydrazones (PHID), eugenol-derived triazoles (HPO), 4-quinolone-3-carboxamides (CR), triazole-quinolone hybrids (CT), and amidoximes (OSC). Compounds were evaluated for antileishmanial activity, cytotoxicity, structure–activity relationships, and mechanisms of action. CT-H and OSC166 emerged as highly potent compounds against intracellular *Leishmania amazonensis* amastigotes, with IC_{50} values of 0.25 and 0.3 μM and selectivity indices (SI) >512 and >666, respectively. OSC075 was also highly active (IC_{50} = 1.3 μM; SI = 87.8) and served as a scaffold for new nitro derivatives. Among them, OSC254 showed a 2.15-fold increase in sensitivity in NTR1-overexpressing parasites, supporting nitroreductase-mediated bioactivation. Stereochemical analysis of OSC166 revealed enantioselective activity against promastigotes, with the S enantiomer (IC_{50} = 17 μM) more potent than the R enantiomer (IC_{50} = 27 μM). Mechanistic profiling distinguished the series: nitro-substituted N-acylhydrazones were not bioactivated by NTR1, whereas OSC254 showed NTR1-dependent bioactivation. Eugenol-derived triazoles did not reproduce the canonical CYP51 inhibition profile, while CT-H induced distinct sterol alterations consistent with interference in late sterol biosynthesis. CR derivatives also caused mitochondrial membrane depolarization. Overall, these findings identify potent and selective antileishmanial prototypes and show that pharmacophoric motifs such as nitro or triazole groups do not, by themselves, define mechanism of action; rather, biological activity and mechanism depend on the broader molecular architecture. **Keywords:** Leishmaniasis; Drug Discovery; Mechanism of action.

TB – 042 - **Cross-sectional survey on community knowledge, attitudes, and practices toward cutaneous leishmaniasis in three endemic cantons in Pichincha, Ecuador**

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Cutaneous leishmaniasis (CL) remains a relevant public health problem in Ecuador. This study assessed community knowledge, attitudes, and practices related to CL in three endemic cantons of Pichincha, Ecuador. A cross-sectional household survey was conducted from September 2023-July 2024 among 500 adult community members. Descriptive analyses were performed, KAP scores of correct responses were calculated, and bivariate and multivariable analyses were used to explore factors associated with KAP scores. Among the participants, 61.4% were women, 50.2% lived in urban areas, and 41.8% reported that they or someone in their household had previously been diagnosed with CL. Although 87.8% had heard of CL and 75.6% had seen a lesion compatible with the disease, only 9.6% correctly identified the sand fly vector and 8.0% selected skin ulcers as a primary disease symptom. The majority (84.4%) believed they at risk for CL, 69.0% believed disease could be prevented, and 89.8% reported that they would seek medical care if affected. Regarding personal preventive practices, most respondents reported sleeping indoors (98.8%), remaining indoors during evening hours (80.4%), and using window barriers (73.6%), although few reported using environmental cleaning (9.2%) or hygiene-based prevention measures (16.2%) as a prevention strategy. In multivariable analysis, having a household history of leishmaniasis was associated with higher knowledge scores, while urban residence and higher educational attainment were associated with higher attitude scores. Communities in these endemic cantons showed familiarity with CL, but important knowledge gaps existed regarding vector awareness and other risk factors, symptom recognition, and some prevention-related concepts. These findings support the development of locally tailored educational interventions and community-based prevention strategies to strengthen leishmaniasis control in endemic areas of Ecuador. **Supported by:** Project funding assistance provided by the Cornell–USFQ Global Strategic Collaboration Awards **Keywords:** KAP survey; Leishmaniasis; Ecuador.