TB-12 - **Assessing the efficacy of MMV-Covid Box drugs on tachyzoites of Toxoplasma gondii**

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Despite the medical relevance of toxoplasmosis, only a few drugs are available for its treatment. Additionally, some parasite strains are already resistant to the current chemotherapy. Besides, all available drugs only act on the acute phase of the disease and are associated with several side effects, often leading to treatment interruption. Thus, the discovery of new treatments for toxoplasmosis is imperative. In the current study, an initial in vitro screening was conducted using drugs from the initiative Medicines for Malaria Venture (MMV)'s Covid Box, which has 160 drugs that can potentially be repositioned for the treatment of neglected infectious diseases. Screening of compounds was done using 6-well plates seeded with NHDF cells in supplemented RPMI-1640 medium and maintained at 37 ºC and 5% CO2. Cells were infected with 600 tachyzoites of RH strain. Each infected well was treated with 1 µM of one of the 160 drugs for 7 days. Antiproliferative activity was assessed by analyzing plaque size using ImageJ. Out of the 160 drugs, 18 were able to inhibit tachyzoite proliferation in more than 70%. Assays to evaluate the cytotoxicity and IC50 of the best drugs in inhibiting tachyzoite proliferation are being performed. **Supported by:**CNPq, CAPES, FAPEMIG. **Keywords:**Toxoplasmosis;drug and compounds repositioning;treatment.

TB-13- **Unravelling the resistance mechanisms of paromomycin in *Leishmania amazonensis***

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Paromomycin (PM) is an aminoglycoside antibiotic used in the treatment of visceral leishmaniasis in Southeast Asia. Due to the limitations of the treatment and the potential of PM, our aim is to identify potential genes associated with PM susceptibility and resistance in *L. amazonensis*, using clinical isolates with differential susceptibility to PM and PM resistant lines selected *in vitro*. The selection of PM resistant lines was done through three strategies: *in vitro* mutagenesis and stepwise selection in promastigotes and amastigotes. After confirmation of the resistance phenotype through drug susceptibility assays, we performed whole genome sequencing of clinical isolates and PM resistant lines selected *in vitro*. Potential genes involved in PM resistance are being functionally validated by gene knockout and/or gene overexpression. *CDPK1*, which is involved in PM resistance in *L. infantum*, was mutated in 3 of 5 PM resistant lines selected by *in vitro* mutagenesis, but not in a clinical isolate that is intrinsically resistant to PM. We inactivated *CDPK1* gene by CRISPR/Cas9 and confirmed its role in PM resistance in *L. amazonensis. L23a* is a ribosomal protein involved in translation that interacts with CDPK1 and was previously involved in PM and antimony resistance in *Leishmania* spp. We generated knockout lines for this gene in *L. amazonensis* and the transgenic lines were resistant only in the promastigote form. Moreover, the PM accumulation in isolates with differential susceptibility was evaluated by fluorescence microscopy and flow cytometry, using a fluorescent analog of PM. We found a direct correlation between PM susceptibility and accumulation of this drug in this species, indicating that a transporter may be involved in the resistance phenotype. This study will contribute for the identification of genes involved in resistance and susceptibility to PM in *Leishmania* that can be potentially useful as markers of resistance in endemic areas where PM is used. **Supported by:**FAPESP (Processo 2019/22175-3) **Keywords:**Paromomycin;Drug Resistance;Whole Genome Sequencing.

TB-14 - **Evaluation of in vivo susceptibility of atypical strains of *Toxoplasma gondii* with a profile of resistance to SDZ and PYR to the alternative drugs used for the treatment of toxoplasmosis**

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Toxoplasma gondii is an obligate intracellular protozoan responsible for causing toxoplasmosis. In Brazil, toxoplasmosis has a seroprevalence ranging from 40 to 80% among adult individuals. Currently, the gold-standard treatment for toxoplasmosis consists of the combination of sulfadiazine (SDZ) and pyrimethamine (PYR), and alternative drugs such as sulfamethoxazole (SMX), trimethoprim (TMP), clindamycin (CLN), and atovaquone (ATV) may also be used. There are reports of treatment failures due to parasite resistance, especially in Brazil. Genetic diversity among strains from South America may be the reason for the differences in the pattern of susceptibility to treatment. Previous studies by part of this group reported four atypical strains of *T. gondii* with decreased susceptibility to SDZ and PYR. Therefore, the objective of this study was to study the in vivo susceptibility of these atypical strains (CTBr4, CTBr11, CTBr17, and CTBr23) against the alternative drugs used for the treatment of toxoplasmosis. In vivo assays were performed using female Swiss mice i.p.-infected with 10000 tachyzoites of the four different strains. Mice were then distributed in groups administrated for 10 days with different dosages and associations of SDZ, PYR, SMX, TMP, CLN, and ATV. Mice mortality was monitored daily for 42-days. Survival curve analysis showed that treatment with SDZ and PYR showed good responses only in CTBr11. Different dosages of SMX and its association with TMP tended to increase the survival rate in all studied strains. CLN treatment led to a mice survival rate higher than 80% in CTBr11 and CTBr23 groups but a high rate of failure was seen in mice infected with CTBr4 and CTBr17. No survival differences were seen among groups treated with ATV. Some strains showed resistance characteristics to the drugs studied as in the ATV, SDZ, PYR and CLN groups. Additional assays can be performed to better characterize susceptibility in vitro. **Supported by:**Conselho Nacional de Pesquisa (CNPq); Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). **Keywords:**Treatment of toxoplasmosis;Resistance;Alternative drugs.

TB-15 - **Genomic characterization and functional analysis of miltefosine transporter gene of *Leishmania braziliensis***

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Cutaneous leishmaniasis in Brazil is caused mainly by *Leishmania braziliensis*. There are no vaccines for leishmaniasis and the treatment has a limited number of drugs that are toxic and induce several side effects. Miltefosine is an alternative drug that was recently approved for the treatment of cutaneous leishmaniasis in Brazil. Recent studies have shown a significant variation in *in vitro* susceptibility to miltefosine in *L. braziliensis* isolates that were never exposed to miltefosine, suggesting that intrinsic resistance may occur in this species. The uptake of miltefosine in *Leishmania* is mainly performed by the miltefosine transporter (MT) in association with its subunit Ros3. In this study, our aim is to understand the role of MT in miltefosine susceptibility in *L. braziliensis* through strategies like overexpression and knockout by CRISPR/Cas9 technology. We also performed whole genome sequencing of *L. braziliensis* (M2903 strain) and our previous analysis indicated that there are three copies of *MT* gene, differently for what is reported at TriTrypDB, where only two copies are annotated. Sequence analyses of these three copies indicated some SNPs, but it still is unknown if these changes may affect miltefosine susceptibility in this species. The episomal overexpression of one of these *MT* gene copies in a heterologous species, *L. amazonensis*, indicated that this gene is functional and transgenic parasites were more susceptible to miltefosine than parasites transfected with the empty vector. Furthermore, we have already generated a *L. braziliensis* line with at least two copies of *MT* gene inactivated by CRISPR/Cas9. Miltefosine susceptibility assays with these transgenic lines is in progress. Our findings will be helpful to predict whether MT may also affect the effectiveness of this drug against this species, and whether treatment with alternative drugs is need when MT is not functional, for example. **Supported by:**FAPESP (Processo 2021/00171-6) **Keywords:** Leishmania braziliensis;Miltefosine;Miltefosine Transporter.

TB-16 - **A methodology to search specific protein synthesis inhibitors for *Trypanosoma cruzi***

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Chagas disease (CD) is a public health issue and due to emigration of infected people to non-endemic, CD has been affecting the global population. It is estimated that over 6 to 7 million people worldwide are infected and about 75 million are at risk of contamination. The disease is caused by *Trypanosoma cruzi*, and its protein synthesis is distinct from other eukaryotic organisms because of significant differences in their ribosomes. Blocking protein synthesis is the mechanism of many employed antimicrobials nowadays. Therefore, our main goal in this project was to establish a *T. cruzi* competent cellular extract assay that can translate a messenger RNA codifying the luciferase enzymes, which can be detected by light production as the purpose of finding new inhibitors to be used as therapeutic treatments for CD. In this work we obtained translation competent extracts from cultured epimastigotes from a non-virulent clone derived from DM28c strain. The extracts were prepared by nitrogen cavitation lysis followed by centrifugation and stored frozen at -80 C. The extracts were able to translate capped and polyadenylated RNA synthesized in vitro. The resulting mRNA containing either Renilla or Firefly luciferase and a 3' UTR region of tubulin was incubated with the cell extract in a multiwell format and generated luminescence that was abolished by protein synthesis inhibitors. This assay is therefore useful to select compounds able to inhibit specifically *T. cruzi* protein synthesis that might be used in treatment of Chagas disease. **Supported by:**CNPq-Pibic
Keywords: Trypanosoma cruzi;Translation;Protein synthesis inhibitors.

TB-17 - **CHARACTERIZATION OF TRYPANOSOMA CRUZI eIF2α PROTEIN KINASE 3**

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Trypanosoma cruzi, the protozoan that causes Chagas disease, is exposed during its life cycle to changes in temperature, pH, nutrients, osmotic or ionic variations and to oxidizing agents. Thus, these organisms need to adapt to different environments, controlling changes in their metabolism, followed by changes in gene expression and morphology. Adaptive stress responses are in part regulated by protein kinases that phosphorylate translation initiation factor 2 (eIF2) inhibiting general protein synthesis and promoting the expression of stress responsive genes. One of the candidate protein kinases predicted to phosphorylate eIF2 in *T. cruzi* is called K3, based on its sequence similarity to eIF2 protein kinases in other organisms. To characterize the enzyme, we cloned and expressed its kinase domain in *E. coli.* We obtained a recombinant protein that generates ADP as detected by ADP-Glo assay, suggesting it could be autophosphorylated, but it could not phosphorylate Creb, p70-S6, or histone H3, while the first two substrates were phosphorylated by other *T. cruzi* eIF2 kinases. We also generated specific antibodies by immunizing rabbits with the recombinant protein and generated a mNeonGreen-Myc tag in the parasite employing Crispr/Cas9. Experiments to identify the enzyme substrates in vitro and to localize the protein in normal conditions or upon stress are underway.
Keywords: Trypanosoma cruzi;Kinase 3;eIF2.

TB-18 - **Complete assembly, annotation of virulence genes and CRISPR editing of the genome of *Leishmania amazonensis* PH8 strain**

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Remoto Poster Session

Leishmania amazonensis is one of the etiological agents of cutaneous leishmaniasis. Genome studies focused on parasite genes encoding virulence factors that play crucial roles in the establishment of the infection constitute an essential step towards the molecular characterization of a protozoan parasite that is being increasingly recognized as a significant human pathogen. Here, we report the sequencing and assembly of the *L. amazonensis* PH8-strain combining data from long PacBio reads, short Illumina reads and synteny with the *Leishmania mexicana* genome. The final assembly, composed of 34 chromosomes, represents a genome of ~ 32 Mb with 8225 annotated genes. Several multigene families encoding virulence factors, such as A2, amastins, metalloproteins GP63 and cysteine proteases, were identified and compared to their annotation in the genome of other Leishmania species. The *L. amazonensis* PH8-strain genome has 27 genes encoding all four sub-classes of amastins (α, β, γ and δ), 5 genes genes encoding A2 antigens, 8 genes encoding the metalloprotease GP63 and 48 genes encoding cysteine proteases. As they have been recently recognized as virulence factors essential for disease establishment and progression of the infection, we have also identified 13 genes encoding proteins involved in the parasite iron and heme metabolism and compared to this gene repertoire in other Trypanosomatids. To follow these studies with a genetic approach that would allow to directly address the role of these virulence factors, we tested two CRISPR-Cas9 protocols to generate *L. amazonensis* knockout cell lines. Using the Miltefosine Transporter gene as a proof of concept, we transfected promastigotes expressing the *Streptococcus pyogenes* Cas9 with in vitro transcribed sgRNA targeting this gene. Alternatively, promastigotes were transfected with recombinant *Staphylococcus aureus* Cas9 complexed with sgRNAs. With both strategies we were able to disrupt the TM gene with high efficiency. **Supported by:**CAPES, INCTV
Keywords: Leishmania amazonensis;virulence factors;CRISPR editing.

TB-19 - **P2X7 receptor participating in intestinal infection control in mice infected with *T. gondii* – EGS strain**

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The classic immune response against *Toxoplasma* involves secretion of IL-12 by innate immune cells, with the consequent activation of other innate or adaptive immune cells that secrete IFN-γ, which activated microbicidal mechanisms in infected cells (YAROVINSKY, 2014). The P2X7 receptor (P2X7R) belongs to the purinergic receptor family, with ATP as its activator molecule. P2X7R activation has been shown to be an important mechanism of control of *T. gondii* infection. The activation of this receptor triggers several intracellular pathways involved in production of inflammatory mediators such as cytokines, chemokines, reactive oxygen species (ROS), lysosomal fusion, and IL-1β secretion (MOREIRA-SOUZA AND COUTINHO-SILVA R., 2021) in the context of Toxoplasma infection. In this work, we investigated the P2X7 receptor contribution during intestinal inflammation induced by the EGS strain of *T. gondii.* The EGS strain was isolated in 1998 from the aminiotic fluid of a patient in Minas Gerais – Brazil and is a recombinant genotype (I/III) (VIDIGAL et al., 2002; FERREIRA et al., 2004). C57black/6 wild-type mice (WT), and P2X7R knockout ($P2X7^{-/-}$) mice were analyzed after 8 days post-infection with the EGS strain. The infection induced an increase in morbidity in all infected animals. When we observed the small intestine of infected animals, we found a reduction in tissue length, indicative of inflammation. The RT-qPCR assay showed reduction in expression of IL-12, and an increase in IFN-γ expression in $P2X7^{-/-}$ mice in comparison with WT. The parasite load was higher in $P2X7^{-/-}$ mice than in WT, when analyzed by the expression of the *Toxoplasma* B1 gene by qPCR. We conclude that the presence of P2X7 receptor is important in the controlling of parasite load, contributing to the classic immune response against *T. gondii*, during EGS strain infection. **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**Purinergic signaling;Immune response;Toxoplasma Brazilian strain.

TB-20 - **Effect of histone deacetylase inhibitors as an alternative for the treatment of ocular toxoplasmosis**

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Toxoplasmosis is a cosmopolitan zoonosis caused by the obligate intracellular protozoan *Toxoplasma gondii*. Ocular toxoplasmosis can be acquired post-birth or by congenital infection. The main symptoms include coriorretinitis with loss of visual acuity or blindness. In Brazil, there is a high seroprevalence of toxoplasmosis, which varies according to the region, with a high frequency of ocular toxoplasmosis related to the presence of atypical genotype strains. The treatment of toxoplasmosis is restricted to antifolates, which present several adverse effects. The search for new alternatives is necessary to increase the therapeutic arsenal. In recent study of our group, histone deacetylase inhibitor Tubastatin A (TST) was pointed out as potential anti-*Toxoplasma* chemotherapy. In this work, we evaluated the *in vivo* effect of TST, via intravitreal administration, in an acute ocular toxoplasmosis model. Males C57Black/6 of 8-12 weeks-old were inoculated with 10^4 tachyzoites of ME49 strain, intraperitoneally. The mice were treated with a single dose of 20µg/2µl in both eyes on the tenth day of infection. The eyes were dissected and processed after 72 and 120h of treatment. Morphological and ultrascructural analysis of the retina was carried out by optical microscopy and transmission electron microscopy, respectively. The untreated group showed a large displacement of retinal pigment cells of the sensory layer, suggesting photoreceptors degeneration and swelling of choroid due to vessels dilation. In treated groups we observed preservation of retinal layers organization and choroid. TST treatment led to preservation of the retinal structure and likely the interruption of inflammatory process. These results point out histone deacetylase as targets to be explored for the development of new therapeutical protocol for local treatment of posterior uveitis caused by *T. gondii* and encourage further investigation. **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**in vivo tests;ocular lesion ;intravitreal treatment.

TB-21 - **PARASITE DETECTION IN VISCERAL LEISHMANIASIS SAMPLES BY A SYBR GREEN-BASED REAL-TIME PCR USING NEW GENOMIC DNA TARGETS OF TRYPANOSOMATIDS**

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In Brazil, Visceral Leishmaniasis (VL) is caused by *Leishmania infantum* parasites transmitted by sandflies. Previously, we identified a non-*Leishmania* parasite isolated from an atypical and fatal case of VL. Phylogenomics of two clinical isolates revealed that both are more closely related to the monoxenous *Crithidia fasciculata* (called *Crithidia-like*). Thus, our objective was to test two new species-specific parasite genes (LinJ31_X for *L. infantum* and LVH60_12060 for *Crithidia-like*) in a SYBR Green qPCR based-assay to evaluate their potential for species identification and for quantifying parasite load in biological and experimental samples. Samples from patients and domestic animals diagnosed with VL, as well as samples from experimental infection in hamsters and in vitro infections using THP-1 cell line were used for genomic DNA isolation. DNA samples from other *Leishmania* species and *C. fasciculata* were also used as control reactions. In this work, we demonstrate that by means of calculations based on the genome of these parasites, it is possible to quantify the parasite load and detect traces of infections caused by *L. infantum* in clinical, animal, vector and experimental samples with the primer LinJ31_X. The LVH60_12060 target amplified for *Crithidia-like* and also for *C. fasciculata*, however, the specificity of the material with this target was observed through the differences in the melting curve temperature, indicating the distinction between them. In the standard curve the points varied between 1 equivalent number of parasite DNA/reaction with Cq 37.2±0.2 to 10e5 equivalent number/reaction of parasite DNA (Cq 18.5±0.1). There was the detection of parasite DNA in biological samples representing 1 equivalent number of parasite DNA (Cq 34.34± 1.8). Therefore, the LinJ31_X and LVH60_12060 primers will help in molecular diagnosis, as it was possible to determine the sensitivity in different types of samples, can be used for further investigation and screening. **Supported by:**Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP JP 2016/20258-0, and grant 2021/12464-8); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq scholarship 133661/2020-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES Finance Code 001). **Keywords:**Molecular Diagnosis;Leishmania infantum;Crithidia-like.

TB-22 - **Novel histone deacetylase inhibitors as an alternative for the treatment of toxoplasmosis- *in vitro* analysis**

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Toxoplasmosis is a cosmopolitan zoonosis, caused by the obligate intracellular protozoan *Toxoplasma gondii*. *T. gondii* infection can cause uveitis, congenital sequels, including chorioretinitis and hydrocephalus, and encephalitis in immunocompromised individuals. The treatment of toxoplasmosis is restricted to antifolate administration and has several side effects. The search for new alternatives and the understanding of the *T. gondii* biology is necessary to expand the therapeutic arsenal. In recent study of our group, histone deacetylase inhibitors, as Tubastatin A and Suberoylanilide Hydroxamic Acid were pointed out as potential anti-parasitic chemotherapy. In this work we evaluated the *in vitro* effects of novel synthesized compounds, selective inhibitors for HDAC6 derivatives of Tubastatin A (TST). LLC-MK_2 monolayers infected with *T. gondii* RH strain tachyzoites were treated with the compounds 1a, KV30 and KV24 for 48h. All the three compounds showed IC_{50} in the nanomolar range: 1a (330 nM), KV30 (320nM) and KV24 (230nM). Cytotoxicity analysis performed by the MTS essay against LLC-MK_2 showed 1a, KV30 and KV24 have high selectivity for *T. gondii*. Immunofluorescence using RH-ACP-YFP (green apicoplast) tachyzoites, α-αtubulin, α-IMC-1 (for the parasite inner membrane complex), α-Centrin-1, α-SAG-1(parasite surface antigen) and Mitotracker showed treatment of the cells with 1μM of any of the 3 compounds, for 24h, impaired parasite endodyogeny. The appearance of masses of non-individualized parasites and compromised localization of organelles, as apicoplast and the centrosomes were observed. The effects of the compounds analyzed by transmission electron microscopy confirmed the irreversible damage in the formation of new daughter cells, in parasites treated for 48h. These results corroborate HDACs as pharmacological targets for the development of new parasitic chemotherapy and indicate these compounds as potential drugs for in vivo tests with *T. gondii.* **Supported by:**CNPq, CAPES e FAPERJ **Keywords:**Chemotherapy;Histone deacetylase inhibitors;Endodyogeny.

TB-23 - **Evaluation of the leishmanicidal activity of the drug combination ezetimibe and posaconazole in *Leishmania infantum.***

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Leishmaniases are part of the group of neglected tropical diseases. They are considered a series of parasitic diseases caused by different species of protozoa of the genus *Leishmania*. The treatment is based on pentavalent antimonials, miltefosine (MT), amphotericin B, and pentamidine. This group of drugs has limitations, such as high cost, toxicity, difficult route of administration, and resistance. Therefore, there is a need to develop new therapeutic strategies for the treatment of leishmaniasis. Drug repositioning is a strategy that can be used to search for more effective drugs for treatment. This work evaluated the efficacy of the combination of posaconazole (POSA) and ezetimibe (EZE) against intracellular amastigotes of *Leishmania infantum*. Miltefosine (MT) was used as a reference drug. Cytotoxicity assays of the drugs (POSA and EZE) on macrophage cultures revealed that the compounds alone and in combination and MT are not toxic to uninfected peritoneal macrophages at the concentrations tested. Intracellular amastigotes were incubated with different concentrations of the drugs alone or in combination for 72 hours. The IC_{50} in intracellular amastigotes of *L. infantum* were (POSA) 0.79 ± 1.0 μM and (EZE) 9.5 ± 1.04 μM and (MT) 0.5 ± 0.4. Finally, the combinations (POSA and EZE) at the proportions 3:2 and 1:4 in intracellular amastigotes of *L. infantum* showed a synergistic effect, indicating a promising alternative for treating visceral leishmaniasis. **Supported by:**FAPERJ E-26/200.028/2021 **Keywords:**Leishmania infantum ;Ezetimibe;Posaconazole .

TB-24 - **Evaluation of almitrine for inhibition of *Toxoplasma gondii* growth *in silico* and *in vitro***

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Toxoplasma gondii is an obligate intracellular protozoan parasite that belongs to Apicomplexa Phylum and is the etiological agent of toxoplasmosis. The parasite diverged from closer species due to its capability to infect a wide range of hosts, re-enforced by flexible transmission pathways. Despite the importance of toxoplasmosis to public health, considering its high prevalence in the human population and the severe clinical manifestations mainly in immunocompromised patients and in cases of congenital infection, there are still few available therapeutic options, which are effective only against the acute form of the disease. Aiming to find new uses for already known compounds, the international organization Medicines for Malaria Venture (MMV) created the COVID Box, a collection of 160 structurally diverse active compounds, set for trial against infectious diseases. Amongst them, we selected almitrine (MMV1804175) based on a previous virtual screening that indicated the existence of a *T. gondii* protein (Sodium/potassium-transporting ATPase alpha-1 chain) as its possible target. Therefore, the goal of this work was to evaluate the anti-*T. gondii* activity of amlitrine in a cell-based assay. We used a 96-well plate assays based on b-galactosidase expression to estimate the viability of *T. gondii* tachyzoites (RH strain). The cytotoxicity against mammalian cells was evaluated using human foreskin fibroblasts (HFF) cultures through the MTT assay. Almitrine presented a Half Effective Concentration (EC_{50}) value of 0.424 µM against the parasite and a Half Cytotoxic Concentration (CC_{50}) value higher than 20 µM, the top concentration evaluated. The ratio between the CC_{50} against HFF and the EC_{50} against the parasite resulted in a selectivity index greater than 47. Almitrine showed interactions with the Na+/K+ ATPase transporter for *Homo sapiens* and *Mus musculus*, indicating a possible mechanism of action of this compound. **Supported by:**FAPESP (grant number 2018/18954-4 and 2020/03399-5). **Keywords:**Toxoplasmosis;drug repurposing;drug discovery.

TB-25 - **Anti-*Toxoplasma gondii* activity of the antitumor compound milciclib and its interference in the integrity of the plasma membrane of the parasite**

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Toxoplasmosis, one of the most important parasitic diseases worldwide, is caused by the protozoan *Toxoplasma gondii* and affects approximately 25 to 30% of the world population. New and improved medicines are greatly needed to cure *Toxoplasma* infection, as no medicine eliminates the chronic, encysted form of the parasite. Milciclib is a heterocyclic compound that is under investigation in patients with malignant Thymoma. This compound is part of the “Pathogen Box” collection provided by the “Medicine for Malaria Venture” foundation. The present work aims to characterize the anti-*T. gondii* activity of milciclib and its cytotoxicity against mammalian cells, and to investigate its possible mechanism of action against *T. gondii*. The values of Half Effective Concentration (EC_{50}), Half Cytotoxic Concentration (CC_{50}) and Selectivity Index (SI) of milciclib were determined, using the drug pyrimethamine as positive control. Assays to evaluate the antiparasitic activity of milciclib were performed using *T. gondii* tachyzoites (RH strain) encoding a transgenic copy of β-galactosidase, which were maintained in confluent monolayers of human foreskin fibroblasts (HFF). Cytotoxicity assays were performed using HFF monolayers and the SI was given by the ratio between EC_{50} and CC_{50}. The integrity of the plasma membrane of milciclib-treated parasites was evaluated through the incorporation of propidium iodide, using 1% triton as positive control. Milciclib presented EC_{50} and CC_{50} values of 0.06 and 7.64 µM, respectively, resulting in an SI of 127. The compound induced immediate damage to the plasma membrane of the parasite in a dose-dependent manner. The damage to the plasma membrane of the parasite induced by milciclib may be related to cell death by necrosis. The evaluation of the efficacy of milciclib in *T. gondii*-infected animals is promising, considering its selective activity against the parasite and its good oral availability. **Supported by:**FAPESP (grant number 2018/18954-4 and 2020/03399-5). **Keywords:**Toxoplasmosis;drug repurposing;drug discovery.

TB-26 - **Establishment and validation of a medium-throughput screening method to discover new hits and leads for the treatment of leishmaniasis**

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Leishmaniasis is a serious public health problem, and it is necessary to identify new compounds, with different mechanisms of action, that can become drug candidates for the treatment of this disease. Several kinds of screening tests are performed to search for hits in chemical libraries, each one with pros and cons. Conventional phenotypic screening assays are expensive and labor-intensive, as they depend on exhaustive counting under an optical microscope and on previous experience for data analysis, making the process unfeasible to analyze a large number of compounds. To overcome these gaps in antiparasitic screening, this work proposes the establishment and validation of a medium-throughput screening methodology using intracellular *L. amazonensis*-GFP amastigotes. It is known that several reporter genes are useful in quantification methods for phenotypic assays. Then, an extensive revision was performed and a comparative table was created analyzing the advantages and disadvantages of each method and which could serve a small laboratory. In addition, the first assays for high content screening (HCS) analysis using promastigotes and intracellular amastigotes of GFP-transfected parasites and DAPI staining yielded good images, suggesting the feasibility of the technique. The next step is setting up the software to proceed with automatized quantification. **Supported by:**PIBITI - 161191/2021-5 - **Keywords:**Leishmania amazonensis ;High Content Screening (HCS);Green Fluorescent Protein (GFP).

TB-27 - **AMPHO-DEPOT®: SINGLE-DOSE INTRALESIONAL FORMULATION WITH AMPHOTERICIN B FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS**

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Cutaneous leishmaniasis (CL) treatment remains unsatisfactory due to requirement of repeated parenteral injections that cause severe systemic side effects. Local intralesional injections with antimonials also demand repetition due to their high hydrophilicity and blood absorption that can also lead to reported systemic effects with an increase in the resistance to antimoniate. Amphotericin B (AmB) is the most active antileishmanial drug clinically available, but development of an effective topical formulation is challenging due to poor absorption through the skin. The aim of this work is to describe the development and therapeutic use of a novel AmB formulation based on sustained release delivery system for a single intralesional injection (Ampho-Depot®, mark deposited in 2021). For that, poly(lactide-co-glycolide acid) (PLGA) microparticles loaded with AmB were prepared by the emulsion solvent evaporation method and sterilized by gamma irradiation. AmphoDepot® was then characterized according to sterility, particle size distribution, zeta potential, scanning electron microscopy and encapsulation efficiency. The microparticles exhibited zeta potential of -25.3 mV, spherical shape, and mean diameter of 2.8 ± 0.4 µm (span=1.55). AmB entrapment efficiency was 73.5 ± 2.6%. No chemical or physical changes were produced by 25 kGy gamma irradiation that effectively prevented contaminated bacteria and yeast growth. *In vivo*, a single i.l. injection into *Leishmania amazonensis*-infected mouse ears revealed that Ampho-Depot® was much more effective in reducing lesion growth than the same dose of deoxycholate AmB formulation Anforicin®. Measurement of parasite burden on day 56 of infection revealed 86% parasite burden reduction as compared with vehicle controls. Anforicin® reduction was only 32%. Ampho-Depot® has strong potential as a new safe therapy, and is pharmaceutically ready for Phase IIb clinical trial in patients with CL. **Supported by:**Instituto Tecnológico Vale (ITV), CNPq and FAPERJ **Keywords:** CUTANEOUS LEISHMANIASIS; AMPHOTERICIN B;POLYMERIC MICROPARTICLES.

TB-28 - **Employment of computer-aided drug discovery to select natural compounds for leishmaniasis treatment by structure-based virtual screening**

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Leishmaniasis treatment remains a serious public health problem, and new oral and safe therapeutic alternatives are necessary. Over the years, a computational approach has been used to aid this search. Thus, the goal of this study was to select a natural compound using a structure-based virtual screening approach, and trypanothione reductase from *Leishmania infantum* (TrLi) was used as a target. NC2 demonstrated ΔG and estimated Ki values of -7.45 and -7.32 and 3.44 μM and 4.34 μM for 2JK6 and 4ADW, respectively. Additionally, NC2 interacts with residues participating in the catalytic site of the enzyme, supporting the hypothesis that this molecule acts as a competitive inhibitor of TrLi. To confirm their antileishmanial effect, L. infantum promastigotes were incubated with different concentrations of NC2 (0.98 – 500 μM) for 72 hours, and the viable cells were estimated fluorometrically by resazurin. The compound inhibited cellular proliferation in a concentration-dependent manner, reaching 98% inhibition at the highest concentration (500 μM), demonstrating an IC_{50} of 5.5 μM. Taken together, computational approaches can be an excellent ally to drug discovery, and our data suggest that NC2 is a safe compound administered by the oral route and inhibits *L. infantum* promastigotes, acting as a competitive inhibitor. **Supported by:**CAPES; CNPq; FAPERJ; IOC/FIOCRUZ **Keywords :**Computer-Aided Drug Discovery; Trypanothione reductase; Leishmaniasis.

TB-29 - **Use of computational approaches in a rational strategy to select natural compounds for leishmaniasis treatment by structure-based virtual screening**

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The treatment for leishmaniasis still presents a serious public health problem, and it is necessary to search for compounds that are less toxic, more effective, and able to be orally administered. Thus, the aim of this work was to select natural compounds for a new chemical entity using trypanothione reductase from *Leishmania infantum* (TrLi) in a structure-based virtual screening (SBVS) approach. Libraries with 65 natural compounds were obtained, and computational approaches were used to select the most promising compounds, study their molecular mechanism of action (MMA) and, therefore, characterize their antileishmanial effects. After *in silico* analyses, 5 compounds demonstrated ADMET properties compatible with oral administration and predicative low toxicity parameters. Virtual screening showed that all of the compounds interact in the active site of the enzyme, suggesting a possible interaction between the enzyme-compound complex. However, the compound NC3 was selected for presenting lower ΔG values. The MMA study calculated ΔG and estimated Ki values of -7.88 and -8.31 and 1.68 μM and 805 nM for 2JK6 and 4ADW, respectively. Additionally, the compound interacts with residues participating in the catalytic site of the enzyme, suggesting a competitive inhibition mode. To characterize its activity, an antipromastigote assay was performed. *L. infantum* promastigotes were incubated with increasing concentrations of the compound (0.98 – 1000 μM) for 72 hours, and cell viability was quantified using resazurina. NC3 inhibited 81% of parasite proliferation (1000 μM) in a concentration-dependent manner and demonstrated an IC_{50} of 331.9 μM. Together, our data demonstrated that SBVS could be an excellent alternative to select promising compounds able to be a competitive inhibitor of TRLi, encouraging us to continue to investigate their effects in *L. infantum* and suggesting that these compounds are potential candidates for oral leishmaniasis treatment. **Supported by:**CAPES; CNPq; FAPERJ; IOC/FIOCRUZ **Keywords:**Structure-based virtual screening;Trypanothione reductase;natural products.

TB-30 - **Effect of Isatin and naphtoquinones derivatives against *L. amazonensis***

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Leishmaniases, neglected tropical and subtropical diseases caused by parasites of the genus Leishmania, occur in 98 countries worldwide and are the second leading cause of parasite-related death. The disease manifests itself as tegumentary and visceral forms, depending on the species and the host response. There are a few available treatment options for leishmaniasis, like pentavalent antimonials, amphotericin B and its lipid formulations, miltefosine, paromomycin, and pentamidine. However, all these drugs present adverse effects, most are administered parenterally (except for miltefosine), and the emergence of resistant strains and treatment failures have been reported. Therefore, it is essential to develop less toxic drugs, more effective, and preferably for oral administration. In this study, we evaluated the effect of compounds derived from isatin and/or naphthoquinones against Leishmania amazonensis. In previous work, we demonstrated that BrMLP and BrPLP are effective against L. amazonensis promastigotes and intracellular amastigotes and are not toxic to macrophages. Aiming to enhance their effect, we modified these molecules by replacing bromine with chlorine. Here we tested the effect of ClMLP and ClPLP, analogs of BrMLP and BrPLP, respectively. These new analogues do not show toxicity to macrophages (CC50 > 200 µM) and to neutrophils (CC50 > 100 µM) and did not induce extracellular neutrophil traps (NETs) release. Our findings reveal that ClPLP has a better effect on parasite growth than the BrPLP analog, since at 5 µM, ClPLP reduced 48% of parasite viability, while BrPLP reduced 27%. They had similar effects at higher concentrations, both being able to reduce promastigote survival by 80%. On the other hand, the ClMLP analog did not improve the leishmanicidal effect compared to its analog BrMLP. BrMLP reduced parasite growth at 5 µM, and ClMLP have no effect at this concentration. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:**Leishmania;natural produtcs;naphthoquinones.

TB-31 - **Development of prodrugs bioactivated by nitroreductases of *Leishmania infantum***

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The therapeutic arsenal for leishmaniasis has become obsolete, with unacceptable side effects and increasing cases of resistance nowadays. Therefore, we aim to search for new compounds that can be used as a therapeutic alternative. Several studies have shown that n-acyl hydrazones have leishmanicidal activity. Derivatives of N-acyl hydrazones were designed and synthesized to have different functional groups, such as nitroaromatic, pyrrole, furan and others, generating the PHID series. Among the molecules of the series, PHID40 and PHID121 stand out as the most potent, with IC_{50} of 2.8 µM and 1.8 µM in *L. infantum* promastigotes. After analyzing the structural differences, it was observed that the PHID121 molecule has a nitro group in its structure. Nitroderivatives have significant potential for drug development, and the foundation that made these advances possible was the identification of nitroreductases in trypanosomatids that are not homologous to humans, offering a differential for drug candidates that would be selectively bioactivated, presenting selective toxicity to the parasite, which led us to investigate its role as a prodrug and activation mechanism. Nitroderivatives work as prodrugs being metabolized by NTR I of *Leishmania* spp. and the overexpression of this enzyme makes the parasites more sensitive to the action of these compounds. To test our hypothesis, *Leishmania infantum* promastigotes wild type (*Li*WT) and overexpressing a type 1 nitroreductase (*Li*NTR1) were incubated in the presence of various concentrations (0-128µM). It was possible to observe that the promastigotes transfected with the *Li*NTR1 were about four times more sensitive to the nitroderivative PHID121 than those transfected with the plasmid without the gene, a fact not observed for PHID40. Together, these results suggest that the nitroderivative PHID121 is a prodrug bioactivated by nitroreductases. **Supported by:**CIENTISTA DO NOSSO ESTADO - FAPERJ E-26/202.918/2018 **Keywords:**nitroreductases;nitroderivative;leishmanicidal activity.

TB-32 - **Antileishmanial activity of original amidoxime derivatives**

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The therapeutic arsenal against leishmaniasis is restrict and has several limitations. Our project concerns the synthesis of amidoxime derivatives presenting a 2,3-dihydrofuran heterocyclic scaffold that could offer a new option for the treatment of leishmaniasis. In this context, 2,3-dihydrofuran scaffold of the original structure bearing the amidoxime group was synthesized by a three-step procedure with different strategies: manganese (III) acetate radical oxidative cyclization by microwave irradiation, transition metal-catalyzed coupling reactions, amide bond formation and β-ketosulfone formation leading to heterocyclic derivatives. The amidoximes derivatives were subjected to *in vitro* evaluation of the cytotoxicity on murine peritoneal macrophages and antileishmanial activity on *Leishmania amazonensis.* We found two principal HITs, the 4-(5-benzyl-3-(4-fluorophenylsulfonyl)-5-methyl-4,5-dihydrofuran-2-yl)-*N'*-hydroxy benzimidamide (*L. amazonensis* promastigotes IC_{50}= 5.4 ± 1.0 µM, amastigotes IC_{50}= 7.9 ± 1.1 µM; CC_{50}= 102.0 ± 2.8 µM), and its methylate derivative in the benzyl group (*L. amazonensis* promastigotes IC_{50} : 5.6 ± 0.9 µM, amastigotes IC_{50}= 6.7 ± 1.2 µM; CC_{50}: 111.5 ± 7.3 µM) with a selectivity index (SI) of 12.9 and 16.6 respectively. Both with a better selectivity index than pentamidine which is 4.5 (IC_{50}= 1.9 ± 0.12 and CC_{50}= 8.5 ± 1.3 µM). From our ongoing work, we have previously reported that the presence of the dihydrofurane and amidoxime groups is necessary for the antileishmanial effect. Moreover, the influence of benzyl group substitution has been demonstrated. In conclusion, amidoxime derivatives presenting a 2,3-dihydrofuran heterocyclic scaffold are promising candidates for further biological evaluation. **Supported by:**FAPERJ - E-26/200.028/2021 **Keywords:** amidoximes; dihydrofuran; Leishmania amazonensis.

TB-33 - **Sterilization of PLGA-Amphotericin B microparticles by gamma irradiation**

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Cutaneous leishmaniasis (CL) is a neglected infectious disease caused by species of the genus Leishmania spp. and transmitted to humans by sandflies, causing ulcerated lesions. Although CL is not lethal, it generates morbidity, and the treatment is still based on multiple injections of toxic drugs, such as Amphotericin B (AMB), which is currently the most effective drug. Our group has demonstrated the local and single-dose efficacy of subcutaneous implants containing PLGA particles with AmB. The ultimate goal is to produce easily scalable particles that can enter the market to impact CL treatment. According to the Brazilian Pharmacopoeia, injectable formulations need to be sterile, so the objective of this work is to verify the sterility and physicochemical stability of the particles submitted to gamma irradiation. For this, the AmB particles were irradiated at a dose of 25 kGy with a Cobalt-60 gamma-ray source and then incubated in soy casein broth and TSA solid medium (Tryptic Soy Agar) for 14 days at 32º C. Analyzed size distribution (DLS), zeta potential, morphology (SEM) and thermal (DSC) and chemical (FTIR) stability. We did not observe microbial growth in the irradiated particles. The size and zeta potential were similar for irradiated and non-irradiated particles, being 2.5 µm and -24.6 mV; and 2.8 µm and -23.7 mV, respectively. In SEM, no damage was found on the surface of the particle. In DSC, no changes were observed in the thermal properties. In FTIR, no changes in chemical structure were observed either. It was seen that there were no changes in the efficacy and safety of amphotericin B. Thus, we conclude that there were no significant changes in the physicochemical characteristics of the particles. Gamma irradiation at a dose of 25 kGy proved to be effective for sterilization of the particles, without generating any morphological or physicochemical changes. **Supported by:**Vale -I.BIOF-22307 **Keywords:**PLGA;amphotericin B ;gamma irradiation.

TB-34 - **Evaluation of Antileishmanial Activity and Cytotoxicity of Chalcones-Thiosemicarbazones Hybrids Against *Leishmania infantum***

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Visceral leishmaniasis (VL) has the most severe clinical manifestations among leishmaniasis. The drugs used in its therapy have drawbacks such as high toxicity and resistance, which justifies the search for new drugs for the treatment. Recently, our research group evaluated hybrid compounds between chalcones and thiosemicarbazones against Leishmania amazonensis parasites. The results were promising and motivated us to prepare new hybrids and assess them against L. infantum, the species that causes VL. Antileishmanial activity and cytotoxicity of 13 hybrids were determined using the methodologies described by Andrade-Neto et al. All compounds evaluated against the L. infantum promastigote were active, presenting IC_{50} values in the range of 3.20-23.32 μM. The less active compounds have a strong electron-withdrawing group (IC_{50}: CT6 = 23,32 ± 3,56 μM; CT7 = 17,37 ± 2,61 μM; CT14 = 12,64 ± 3,75 μM), indicating that this electronic characteristic may be detrimental to the antileishmanial activity of the series evaluated. The other substances exhibited IC_{50} below 8 μM. Preliminary cytotoxicity assays revealed that the most active compounds against the parasite (CT1-5 and CT9-12) were also more harmful to isolated macrophages when tested at a concentration of 50 μM, with lethality above 50%. Interestingly, CT8, the most active hybrid against promastigotes (IC_{50} = 3,20 ± 0,87 μM), showed one of the lowest macrophage lethalities. Thus, the results obtained so far suggest that the presence of a fluorine atom in the meta position of the benzene ring possibly results in the best balance between antiparasitic activity and cytotoxicity. **Supported by:**CIENTISTA DO NOSSO ESTADO - FAPERJ E-26/202.918/2018 **Keywords:**Leishmania infantum;Chalcones-Thiosemicarbazones Hybrids;Visceral Leishmaniasis.

TB-35 - **Effect of Fluoroamodiaquine derivatives against *Leishmania amazonensis.***

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Leishmaniasis is treated with a small arsenal of drugs, all of which have disadvantages in terms of toxicity, efficacy, price, or treatment regimen. Efforts have been made in the search for new and more effective leishmanicidal drugs. In this work, we evaluated in silico the physicochemical properties and ADMET profile of new fluoroamodiaquine (FAQ) analogs and their effect on *Leishmania amazonensis* and their toxicity on murine macrophages. Five FAQ analogs were designed and synthesized, using the tertiary amine to introduce a piperazine moiety plus a substituent (MR94: butyl, MR100: phenyl, MR102: 3-chlorobenzyl, MR104: 3,4-methylenedioxybenzyl, and MR106: tetrahydrofuran-2-carbonyl). The *in silico* evaluation was performed on the pkCSM platform. The best global result was obtained with MR106, as it does not infringe the Ro5. All of them have a high probability of intestinal absorption, with MR106 with the highest probability (92.7%). All show interaction as a substrate or an inhibitor of cytochrome P 450 enzymes, such as CYP3A4. The derivatives MR100, 102 and 104 are likely to have a positive AMES. All compounds showed a probability of hepatotoxicity, which is already known for amodiaquine derivatives; however, studies have shown that this characteristic does not remain in the analogs of the FAQ. The *in vitro* activity was evaluated by incubating promastigotes of *L. amazonensis* for 72h. The parasite viability was evaluated by the resazurin assay. For comparative purposes, we evaluated the activity of amodiaquine (AMQ) and miltefosine. Like AMQ (IC_{50} 11.6μM), all FAQ analogues were active, with the following IC_{50}s: 7.2μM (MR94), 4.2μM (MR100), 5.3μM (MR102), 2.8μM (MR104), and 44.7μM (MR106). All compounds and AMQ demonstrated similar cytotoxicity on murine macrophages, with CC_{50} around 35 μM. The derivatives with an aromatic ring showed greater potency on promastigotes, and despite a better in silico profile, tetrahydrofuran-2-carbonyl indicated a loss of potency. **Supported by:**FAPERJ **Keywords:** Drug Discovery; Leishmania; Fluoroamodiaquine.

TB-36 - **High-content screening of FDA-approved drugs in combination with benznidazole reveals potential anti - Trypanosoma cruzi agents.**

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Drug combinations and drug repurposing have emerged as promising strategies to discover novel therapeutics for Chagas diseases. We performed a fluorescence microscopy-based screening for anti-*T. cruzi* activity, in which a library of 640 FDA-approved drugs was tested isolated or combined with the reference drug benznidazole (BZN). From the primary screening, 30 drugs and 25 drug combinations were selected for further analysis. Among them, 3 drugs (oxcarbazepine, conduritol B and manidipine) and 2 drug combinations (clarithromycin + BZN and granisetron + BZN) were prioritized due to their superior antiparasitic activity and selectivity. The drug manidipine (MAN), a calcium channel blocker, was highly potent and efficacious (EC_{50} = 0.9 µM; maximum activity = 100%) and presented a selectivity index > 10. MAN efficacy was further confirmed in different host cells (U2OS, THP-1 and AC-16) and across distinct *T. cruzi* strains (Y, CL Brener and Colombiana). MAN displayed inhibitory effect on trypomastigote (EC_{50} = 14.5 µM) and epimastigote forms (EC_{50} = 4.8 µM), demonstrating a broad-spectrum profile. The drug also presented a fast-killing action, eliminating the infection after 24 hours of treatment. Furthermore, after removal of the drug, no intracellular amastigotes were observed, suggesting that MAN has a strong trypanocidal effect.
Regarding MAN+BZN combination, the fixed concentration of MAN at 1 µM potentiated the activity of BZN by 23-fold, allowing a significant reduction in the dose of the reference drug needed to achieve an effective response. Isobolographic studies demonstrated that BZN and MAN have an additive interaction (Σ FIC of 1.01).
In summary, our results demonstrate that MAN represents a promising drug candidate for Chagas disease treatment. Furthermore, our work reinforces the value of strategies based on drug combinations and repurposing to develop novel therapeutic strategies against Chagas disease and/or fuel targeted drug discovery efforts. **Supported by:**CAPES/FCT (Edital nº 28/2017 - 88887.156341/2017-00) **Keywords:**Chagas disease drug discovery;drug combination;drug repurposing/repositioning.