Quimioterapia - Chemotherapy

QT01 - THE AZOLES ITRACONAZOLE AND FLUCONAZOLE AS PROMISING DRUGS AGAINST TOXOPLASMOSIS

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Toxoplasma gondii is an important oportunistic pathogen affecting imunocompromissed patients with AIDS. The toxoplasmic encephalitis in those patients is responsible for great morbidity and mortality. Besides *T. gondii*, AIDS patients are either susceptible to opportunistic fungal infections such as cryptococcal meningitis and candidiasis. Although the coexistence of both *T. gondii* and fungal infections in AIDS patients is commum, the evaluation of the associative effect of the drugs administrated for the treatment of both infections has never been tested against *T. gondii*. In this study we investigated the activity of the antifungic azoles fluconazol and itraconazole and the association with sulfadiazine (SDZ) and pyrimethamine (PYR) - the first choice therapy for toxoplasmosis - *in vitro* and *in vivo.* Monolayers of LLC-MK2 epithelial cells infected with tachyzoites of RH strain were incubated with different concentrations of itraconazole (ITZ) and fluconazole (FLZ) for 24h and 48h. The IC_{50} values were 114nM and 53.6nM for ITZ and 4.6µM and 1.5µM for FLZ after 24h and 48h of interaction, respectively, demonstrating a selective effect of this drug against *T. gondii in vitro*. When the azoles were associated to sulfadiazine and pyrimethamine *in vitro*, only an additive effect was observed. FCZ was more efficient *in vivo* than ITZ, although *in vitro*, ITZ demonstrated to be the most potent. Swiss mice infected with 1000 tachyzoites of the virulent RH strain had a survival rate of 25% when 20mg/kg of FLZ were orally administrated for ten days associated with 20mg/kg SDZ + 0.5mg/kg PYR. On the order hand those that were treated with 20mg of ITZ associated with 20mg/kg SDZ + 0.5mg/kg PYR showed the same survival rate found for the mice that only received 20mg/kgSDZ + 0.5mg/kg PYR for ten days. This work is supported by CNPq, FAPERJ and PRONEX.

QT02 - TREATMENT OF THE *Toxoplasma gondii* WITH LLCMK2 INTERACTION USING β-LAPACHOL DERIVED (LQB-118)

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Toxoplasma gondii, the agent of toxoplasmosis, is an obligate intracellular protozoan able to infect a wide range of vertebrate cells including nonprofessional and professional phagocytes. For this, the drugs to control this parasite must be active intracellular. In previous studies, quinines show different biological activity as microbicide and trypanossomicide action. For example, the oxidative burst, that produces oxygen derivatives (O_2, OH, O_2 -, H_2O_2). Inside the natural naftoquinines, lapachol is an important representative. The principal importance of lapachol is that this drug can generate oxidative stress inside cells and cause damage in intracellular parasites, such as *T. gondii*. The LQB-118 is a structure synthetic analog of β-lapachol. Our group tested this derivate of β-lapachona in interactions of *T. gondii* with nonprofessional phagocytes. For this, LLCMK2 were cultured with RPMi 1640 supplemented 10 % Fetal Bovine Serum. The cells were cultured in 24 weel plates. The interaction was realized in the presence or absence of LQB-118. We shown by Scanning Electron microscopy that LQB-118 cause damages in parasite without affect the host cells. The LQB-118 was capable to reduce the infection index during interaction with LLCMK2, relative to the control of interactions without drugs. The use concentrations of LQB-118 in interactions alternate of 1 to 2,5 μM. These results suggest that LQB-118 can be a potential chemotherapy for Toxoplasmosis, but more tests, principally “in vivo”, are necessary.

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QT03 - ANTIMALARIAL ACTIVITY OF EXTRACTS FROM *ASPIDOSPERMA* PLANTS TESTED IN VITRO AGAINST *PLASMODIUM FALCIPARUM* DRUG RESISTANT PARASITES AND IN ANIMAL MODELS

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Several factors at present make the malaria control difficult, for instance: (i) most *P. falciparum* are multiresistant; (ii) there are no prophylactic drugs against this human parasite; (iii) treatment requires drug combinations; (iv) resistance emerges easily under drug pressure, including to artesunate. Thus, the search of new antimalarials is an urgent need. The fact that first line treatment drugs (quinine and artemisinine derivates) are originated from medicinal plants reinforces the ethnopharmacology importance in antimalarial research. Species of *Aspidosperma* (Apocynaceae) are commonly used in the Amazon region as medical plants. This family is chemically characterized by the frequent occurrence of alkaloids. Experimentally, *A. ramiflorum* extracts have been shown to have bactericide and anti-*Leishmania* activities; *A. macrocarpon* have anti-*Plasmodium* activity with high parasite specificity (Mesquita *et al.*, J Ethno 2007). We now evaluated the antimalarial activity of crude and alkaloid extracts of two *Aspidosperma* species. Both types of extracts of *A. ramiflorum*, a native tree of southeast Brazilian Mata Atlântica, had partial activity against chloroquine resistant *P. falciparum* parasites (W2) (IC_{50} 11-40μg/ml); in vivo tests with *P. berghei* in mice showed that the crude extract was partially active at 500mg/kg; and the alkaloid extract at 250mg/kg. Extracts of *A. nitidum* were also active in vitro and in vivo and upon fractionation several alkaloids were detected. Further studies are now being performed to determine the structure responsible to such antimalarial activities and its specificity. We conclude that this genus is promising in the search of new antimalarials and may lead to new prototypes of importance. Financial support: FIOCRUZ and CNPq.

QT04 - CLOROQUINE TREATMENT DID NOT PREVENT COGNITIVE DAMAGE IN MICE WITH CEREBRAL MALARIA

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Cerebral malaria (CM) is the most severe neurological complication of infection with *Plasmodium falciparum* and is the major cause of acute non-traumatic encephalopathy in tropical countries. Persistent neurological impairments are common and severe in Sub-Saharan children. To investigated possible persistent cognitive damage male C57Bl/6 and Balb/c mice (20-28 g, n=10/ group) were infected on day 0 (200 □L, i.p.) with *Plasmodium berghei* Anka parasited erythrocytes (PRBC 10^6). The animals were orally treated (200 □L) with chloroquine 25 mg/kg during 7 days, starting on day 6 post-infection. As a control groups infected with 10^6 non parasited erythrocytes were treated with the standard drug or received physiologic saline. On day 15 mice were submitted to behavioural task. Animals were placed on the open field in the left quadrant, and were allowed to explore the arena for 5 min (training session) and 24 hrs later submitted again to a similar session (test session) and the number of quadrants were recorded. No differences in the number of crossings was observed in the habituation to the open field in C57Bl/6 mice groups during test session, but animals from group infected PRBC treated with chloroquine explored more them the group RBC on test session, showing a lack of memory ($p<0,05$ Student's T test in relation to saline and chloroquine groups). In Balb/c mice was observed a significant reduction in crossings in all tested groups in relation to training and test session ($p<0.05$ Student's T test). The deficit demonstrated in Black/6 mice, a cerebral malaria susceptible strain, is similar, at least in part, to the cognitive alterations observed in patients surviving from cerebral malaria, particularly on memory impairment. The murine model of cerebral malaria will help to investigate the biological mechanisms involved in the cognitive deficits and suggest new therapeutics approaches. Supported by FIOCRUZ, CNPq and FAPERJ.

QT05 - *IN VIVO* ANTIMALARIAL EFFECT OF THE CHLOROQUINE DERIVATIVE PQUI08001/06

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Malaria remains the most important parasitic disease, causing 2–3 million deaths every year. Multi-drug resistance is one of the most important problems in malaria control. The aim of this work was to test new antimalarial compounds that could potentially be developed into new therapeutics. The compound PQUI08001/06 was obtained by mixing 7-chloro-4-(2-chloroethylamine) quinoline with 1-ethanolamine and triethylamine at 120°C. The reaction was added to distillated water and extracted with chloroform. The organic phase was dried with sodium sulphate, filtered and evaporated at reduced pressure. The solid product was washed with ethylic ether (yield 70% in mass). The *in vivo* antiplasmodial activity was evaluated by the 4 day test. Male SW mice (25-32 g, n=10/group) were infected on day 0 (200 □L, i.p.) with *Plasmodium berghei* Anka parasited erythrocytes (10^7). Two hours later the animals were orally treated (200 □L) with different dose of PQUI08001/06. The control group received DMSO 1% (vehicle) or chloroquine 25 mg/kg (p.o.). The treatments were done from the first day (D0) to the fourth day (D3). On day 4, tail blood smears were prepared, stained and parasitaemia (%) was recorded. The treatment with dose of 10, 25 and 50 mg/kg b.w. of PQUI08001/06 significantly reduced the parasitaemia (53, 83,5 and 95,2% respectively, $p<0.05$ or less, Newman-Keuls Multiply Comparison Test) as well as the standard drug chloroquine (95,8%). This date suggest a possible new drug to malaria treatment.

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QT06 - EFFECTS OF METHANOLIC EXTRACT OF *ZANTHOXYLUM CHILOPERONE* AGAINST *PLASMODIUM BERGHEI*

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Introduction: Malaria is one of the most important diseases nowadays. Therefore, much effort has been done to obtain new drugs from different sources and plants have shown promising results in several reports. Some plant species belonging to the *Zanthoxylum* genus are widely used as antimalarials or antipyretics in traditional medicine. A decoction of *Z. chiloperone* root bark is used traditionally in Paraguay for its antimalaric, emmenagogue and antirheumatic properties. **Objectives:** Besides, because of its also known antiparasitic properties, we decided to assess the bioactivity of methanolic extracts of *Zanthoxylum chiloperone* on *Plasmodium berghei*-infected mice. It was tested *in vivo* in the concentrations of 50mg/Kg, 100mg/Kg and 200mg/Kg, and using the 4-day suppressive test. **Results:** For the concentration of 50mg/Kg, the inhibition of parasite multiplication (ipm) was 15.47% on day 5, 0% on day 7 and 21.21% on day 9. The concentration of 100mg/Kg presented ipm of 0% on days 5 and 7, and 57.51% on day 9. For the 200mg/Kg group we had an ipm of 0% on days 5 and 7, and 20.31% on day 9. Besides, mice survived for a few days longer than the untreated group. The control treated group (chloroquine 200mg/kg) presented an ipm of 100% on days 5, 7 and 9. **Conclusion:** These results may be explored for the study of new antimalarial drugs, since the ipm for 100mg/Kg group was almost 60%. This inhibition takes place mostly on day 9, suggesting a matter of drug bioavailability.

QT07 - EFFECTS OF A NEW AMINOQUINOLINE DERIVED DRUG AGAINST *PLASMODIUM BERGHEI*

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Introduction and objectives: Aminoquinoline derived drugs constitute a big group of antimalarial drugs, in use and under research. To assess antimalarial activity, a new aminoquinoline derived drug 4-(6-thiopurine)-7-chloroquinoline (MPQUI) was tested *in vivo* using the 4-day suppressive test in 2 moments: in the concentrations of 100mg/kg, 50mg/kg and 25mg/kg. **Results:** all the mice of 100mg/Kg group died before the first blood smear be made (before day 5 after infection); most of mice of 50mg/Kg and 25mg/Kg groups died before the untreated group and had an initial parasitaemia higher than the last one. These data suggest immunosuppression and/or toxicity of those drugs concentrations and we have looked forward to performing new researches to elucidate it. We tested these concentrations again without infecting the mice and no one died. Cytotoxicity in mice peritoneal macrophage was 57.87% for 100mg/Kg, 32.60% for 50mg/Kg and 6.23% for 25mg/Kg. In a second moment, we tested the concentrations: 10mg/kg, 5mg/kg and 1mg/kg. The concentration of 10mg/Kg presented an inhibition of parasite multiplication (ipm) of 0% on day 5 and 7 and 7,9% on day 9. The concentration of 5mg/Kg presented activity in a lower concentration than chloroquine, demonstrating an ipm of 66.28% on day 5, 47.34% on day 7 and 36.81% on day 9. The concentration of 1mg/Kg presented an ipm of 26.61% on day 5, 2.4% on day 7 and 0% on day 9. The control treated group (chloroquine 200mg/kg) presented an ipm of 100% on days 5, 7 and 9. Besides, for all concentrations, the surviving time was longer than the untreated group and the parasitaemia in the 5mg/Kg group started lower (2%) than the untreated group (6.1%). **Conclusion:** Therefore, this aminoquinoline derived (MPQUI) may be object of researches for a new antimalarial drug. Supported by Fapemig, CNPq.

QT08 - PROPHYLACTIC ACTIVITY OF MEFAS – A NEW HYBRID MOLECULE OF ARTESUNATE AND MEFLOQUINE – AGAINST *Plasmodium berghei*

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Malaria caused by *Plasmodium falciparum* requires drug combinations for treatment due to the spread of multi-drug resistance parasites worldwide. At present, the WHO recommends the use of artesunate (AS) derivatives in combination therapy with other antimalarials, especially mefloquine (MQ). We have now evaluated the antimalarial activity of a new molecule, MEFAS, a hybrid salt from MQ and AS synthesized at FARMANGUINHOS-FIOCRUZ (Patent International application WO 2005/100370 A1). MEFAS showed more intense activity in vitro against *P.falciparum* and in vivo in mice infected with *P. berghei* than the AS and MQ combination used in parallel in similar mass proportions (1:1). MEFAS significantly reduced parasitemia at 5mg/kg in mice treated orally for three consecutive days and it was curative at 10 mg/kg, a data further confirmed by sub-inoculation tests and PCR results. Citotoxicity tests in vitro and in vivo showed that MEFAS is less toxic than AS+MQ combination (submitted). The prophylactic activity of MEFAS was also evaluated after a single oral dose given to normal mice 1h or 6h before *P. berghei* inoculation. Controls untreated and those treated with AS+MQ (1:1) and MEFAS were followed during 15 consecutive days for determination of the malaria pre-patent period, parasitemia curves and cumulative mortality. MEFAS or AS+MQ combination at 40mg/kg cured all the animals. At lower doses MEFAS and AS+MQ were protective when administred 1h or 6h before inoculation, decreasing parasitemia and mortality in mice. The results indicate that MEFAS is as active as the AS+MQ combination and less toxic. MEFAS is a promising drug to treat chloroquine-resistant malaria as a potential alternative compared to the combination AS+MQ. Further tests based on pharmacokinetics and pharmacodynamics are still needed to validate its prophylactic use against human malaria. Financial support: CNPq, PDTIS-FIOCRUZ

QT09 - BIOLOGICAL EVALUATION OF NEW BENZYL AND CYCLOHEXYL AMINOQUINOLINES IN PROMASTIGOTES AND INTRACELLULAR AMASTIGOTES OF *LEISHMANIA* SP.

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Introduction and objective: Protozoan parasites in the genus *Leishmania* are obligate intracellular pathogens that can cause a wide spectrum of diseases. *Leishmania* exhibit a dimorphic life cycle, the forms flagellated promastigotes and amastigotes that reside within mammalian macrophages. The current treatment for leishmaniasis is unsatisfactory. Therefore, the search for novel, effective and safe therapeutic compounds has become a priority. Aminoquinolines have recently re-emerged as an important class of compounds, which have shown great promise for development of future antiprotozoals drugs. So, in this study, we analyzed the anti-*Leishmania* activity of novel synthetic aminoquinoline analogs. The *in vitro* cytotoxic effects of these derivatives on the host cells were also determined. **Methods:** The compounds benzylaminoquinoline and cyclohexylaminoquinoline were assayed against promastigote and amastigote forms of *L. amazonensis* and *L. chagasi*. The viability of promastigotes was checked using the tetrazoliumdye (MTT) colorimetric method. For anti-amastigote activity, were used cells J774A.1 infected with promastigotes of *L. amazonensis*. The antiparasitic effect of the substances was evaluated by counting the intracellular amastigotes after 24, 48 and 72 hours of treatment. **Results:** benzylaminoquinoline and cyclohexylaminoquinoline showed antiproliferative activity against promastigote forms of *L. amazonenis* (IC_{50} of 14 µM and 60 µM, respectively) and *L. chagasi* (IC_{50} of 28 µM and 12 µM, respectively). Both aminoquinoline derivatives led to a decrease of the amastigote proliferation (28-36%) only 24 hours after treatment. None of the substances was found to have significant toxicity effect on macrophages. Preliminary results indicated that the drugs inhibited NO production in promastigotes, macrophages and infected macrophages. **Conclusions:** Our results showed antileishmanial activity of aminoquinoline derivatives and it encourages us for the development of new analogs for better *in vitro* and *in vivo* studies. Supported by FAPEMIG, CNPq and UFJF.

QT10 - *IN VITRO* ANTI-LEISHMANIAL EFFECTS OF SYNTHETIC COUMARINS

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Introduction – Alvim et al., (2005) reported the potential effect of some synthetic coumarins against enzyme GAPDH from *Trypanosoma cruzi* and consequently inhibition of growth of these parasites. Since the ability of some synthetics coumarins have anti microbial activity, we evaluate the effect of these compounds against *Leishmania (leishmania) amazonensis* promastigotes forms culture. **Objectives** – The aim of this work was evaluate the anti-leishmanial activity of three synthetic coumarins. **Methods** – Coumarin 01, coumarin 05 and coumarin JB were assayed against promastigotes forms of *L. (L.) amazonensis* in a range of 6,0 to 100,0µg/well and after 24h the survival index were determinated by Neubauer chamber couting. Murine peritoneal machrophages were used in citotoxicity assay. **Results** – From these compounds assayed, only Coumarin 01 presented an inhibitory concentration 50% (IC50) of 30µg/well after 24h. It also presented a high citotoxicity activity with a IC50 of 6,0µg/well. Coumarin 02 and coumarin 03 presented no leishmanicidal activity and they were not evaluated over murine macrophages. **Conclusion** – Despite of potential inhibitory effect of these coumarins on growth of *Trypanosoma*, and the positive effect of the coumarin 01 to inhibit the development of *Leishmania (Leishmania) amazonensis,* we conclude that these compound assayed here as well as other is not good candidates for potential therapeutic use since the component presented citotoxicity against host cell. Supported by FAPESP and LIM-50 HC-FMUSP.

QT11 - ANTILEISHMANIAL ACTIVITY OF NEW DIAMINOALKYLQUINOLINE DERIVATIVES

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Introduction and objective: *Leishmania* sp. cause a spectrum of diseases, ranging from a cutaneous ulceration to a progressive and fatal visceral disease. The disease is transmitted by a sand fly vector to humans, dogs and some vertebrates. The drugs of choice for the treatment of leishmaniasis are pentavalent antimonials, but toxic side effects, limited efficacy to control parasite proliferation and drug resistance are frequently encountered. In rational trials we analyzed the antileishmanial activity of new quinoline derivatives including 4,7-dichloroquinoline; 4-(1,2-diaminethyl)-7-chloroquinoline; 4-(1,3-diaminpropyl)-7-chloroquinoline and 4-(1,4-diaminbuthyl)-7-chloroquinoline . **Material and methods:** All the compounds were assayed against *L. amazonensis*, *L. major* and *L. chagasi* promastigote forms. Each concentration was screened in duplicate and it was performed in flat-bottomed 96-well plastic tissue-culture plates. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The results are expressed as the concentrations inhibiting parasite growth by 50 percent (IC50) after three days incubation period. **Results and conclusions:** Among all the species assayed, the drugs showed the higher activity against *L. chagasi* promastigote forms (IC50 values of 7.09 µM, 2.92 µM, 0.16 µM for 4-(1,2-diaminethyl)-7-chloroquinoline; 4-(1,3-diaminpropyl)-7-chloroquinoline and 4-(1,4-diaminbuthyl)-7-chloroquinoline, respectively). The compound 4,7-dichloroquinoline, the starting material, not showed antileishmanial activity. It is interesting to emphasize that for these drugs there was structure-activity relation, in which the increase in carbons number increases the leishmanicidal activity. The compound 4-(1,4-diaminbuthyl)-7-chloroquinoline was 10 times more active than the reference drug amphotericin B against *L. chagasi* promastigotes. These results show a good in vitro activity of quinoline derivatives and reinforce the need to investigate their activity against intracellular amastigotes. Supported by FAPEMIG, CNPq and UFJF.

QT12 - Antileishmanial activity of HIV protease inhibitors

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The introduction of the Highly Active Antiretroviral Therapy (HAART) in the treatment of AIDS showed a strong reduction of opportunistic infections including those caused by parasites. The objective of this study was to analyze the effect of HIV aspartic protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir and saquinavir) on Leishmania amazonensis growth, ultrastructure, differentiation and macrophage interaction. The promastigotes were collected from late log phase of growth and incubated in microplates with the compounds at final concentrations ranging from 10 to 500 µM and the rate of multiplication was assessed by counting the parasites daily using a haemocytometer chamber. Our results showed that amprenavir, nelfinavir and lopinavir inhibited the parasite growth by about 50% in the 250 µM, 50 µM, and 30 µM range, respectively. The differentiation of the parasite in vitro was not inhibited by the drugs at the IC dose. On the other hand electronic microscopy of the treated parasites revealed important ultrastructural alterations and the pre-treatment of promatigote with protease inhibitors was able to reduce macrophage interaction drastically. These results suggest that HIV aspartic protease inhibitors could have a direct effect on Leishmania viability, as already observed in other parasitic protozoa.

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QT13 - ANTILEISHMANIAL ACTIVITY OF ESSENTIAL OIL OBTAINED FROM LEAVES AND FLOWERS OF *Achillea millefolium*

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Leishmaniasis is one of the major global public-health problems that affect approximately 12 million people, accounting for cause severe cases of disease and mortality in several countries. The classic forms of leishmaniasis, cutanea and visceral forms still face specific difficulties in terms of diagnosis and treatment. Due to the characteristics undesirable to drugs, as the toxic effect and its ineffective activity against parasite resistant is very important the search for new compounds, through medicinal plants. In this study, we tested the essential oil isolated from leaves and flowers of *Achillea millefolium,* to determine the activity in vitro on the growth of *Leishmania amazonensis*. The antileishmanial activity was determined to promastigote forms of *L. amazonensis* com different concentrations of the essencial oil (4, 8, 15, 20 µg/ml). The concentration that inhibited 50% parasite growth (IC_{50}) was determined by direct counting of the cells in a Neubauer chamber. The cytotoxic effect was assessed on macrophage strain J774G8 (5 x 10^5 macrophages per ml). The macrophages were incubated for 48 h with different concentrations of essential oil. Thereafter, absorbance was read in a 96-well plate reader. The essential oil showed significant activity against *L. amazonensis* promastigote form, with 50% inhibition concentration on cell growth at 8 µg/ml. The cytotoxic effect on macrophages line J774G8 was of 60 µg/ml. The promastigote form was treated with concentrations of 8, 15, 20 µg/ml of essential oil. The scanning electron microscopy and optical microscopy showed significant morphological alterations: a rounded, extremely swollen appearance and two or more flagella and extensive damage. The present results suggest that essential oil of *Achillea millefolium* will provide a promising approach for the development of new drugs to Leishmaniasis.

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QT14 - β-CARBOLINE DERIVATIVES AS PROMISING LEISHMANICIDAL AGENTS

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Leishmaniasis is caused by species of the genus *Leishmania* and has an overwhelming impact on global public health especially in tropical and subtropical countries. Infection by various strains of leishmania causes a wide spectrum of diseases in humans, with many clinical manifestations (cutaneus, mucocutaneus and visceral). The currently available leishmanial drugs depend on pentavalent antimony compounds like sodium stibogluconate (Pentostan) and meglumine which have serious side effects and low efficacy. A great number of natural and synthetic compounds have been tested in the past few years against leishmaniasis and the importance of β-carboline class of compounds, including natural and synthetic β-carbolines alkaloids is well established in antiparasitic chemotherapy and for several other biological activities. In this work, we evaluate the leishmanicidal activity of twenty eight 1,3-disubstituted-β-carbolines through growth inhibition of promastigote forms of *L. amazonensis*. The 1-(substituted phenyl)-β-carboline-3-carboxylic acid (**5a-g**) were prepared through Pictet-Spengler condensation of L-tryptophan methyl ester (**2**) with appropriate aromatic aldehydes, oxidation of methyl tetrahydro-β-carboline-3-carboxylates (**3a-g**) and subsequent hydrolyzation of the compound methyl β-carboline-3-carboxylates (**4a-g**). Conversion of the derivatives **5a-g** to the corresponding 1-(substituted phenyl)-3-*N*-isopropylcarboxamide (**6a-g**), -3-*N*-benzylcarboxamide (**7a-g**) and -3-*N*-cycloexylcarboxamide β-carbolines (**8a-h**) was carried out by reaction of **5a-g** with isopropylamine, benzylamine and cycloexylamine, respectively. The IC_{50} values for the compounds **5a-g** (>10.0 µg/ml), **6a-g** (<1.0 to 2.9 µg/ml), **7a-g** (1.0 to 3.6

µg/ml) and **8a-g** (1.5 to 4.8 µg/ml) demonstrated that they have pronounced activity against *L. amazonensis*. The series of β-carbolines containing the *N*-carboxamide group in position-3 demonstrated increased activity when compared to the β-carbolines 3-carboxylic acid derivatives and the series of compounds **6a-g** were the most active of all synthesized compounds. For this, more studies are being done to accomplish that these β-carbolines 1-(substituted phenyl)-3-*N*-isopropylcarboxamide can be promising antileishmanial agents.

Acknowledgements: This study was supported through grants from CNPq, FINEP, and PRONEX/Fundação Araucária.

QT15 - EVALUATION OF LEISHMANICIDAL PROPERTIES OF A NEW NAPHTHOQUINONE OF *CIPURA PALUDOSA*

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Although considerable advances have been made in recent years, the chemotherapy of leishmaniasis is still based on pentavalent antimonials, diaminas and antifungal polyene. Most of these agents generate resistance, require long-term treatment and present a considerable toxicity in humans. Plants are very rich sources of new compounds that can be developed into new drugs for various diseases. Naphtoquinones are known for their significant microbicidal activity. This research aimed to evaluate the inhibitory activity of a naphthoquinone unprecedented in the literature, named CPBC-1, on the in vitro growth and intracellular development of *Leishmania amazonensis*. The promastigotes parasites were treated in the absence or in the presence of several concentrations of CPBC-1 for 5 days at 23°C. The parasite numbers were daily scored at Neubauer chamber with erythrosin B. The pentamidine was used as a negative control. For statistical analysis of data was used Probit. The CPBC-1 showed a potent leishmanicidal activity against promastigotes with IC_{50}=0.92µg/ml (6.64 µM) and IC_{90}=8.58µg/ml. To evaluate the effect of this compound on intracellular development of *L. amazonensis*, promastigotes from early stationary phase were used to infect mouse peritoneal macrophages and after that, the cultures of infected macrophages were treated with this drug for 24 and 48h. This substance did not present cytotoxicity in peritoneal macrophages of BALB/c mice. The infection rate and the number of intracellular parasites decreased in infected macrophages treated with CPBC-1. It was observed that after 48 hours of treatment the rate of infected macrophages reduced by 50%. This research suggests that naphthoquinones can be important tools in the development of new chemotherapy for the treatment of leishmaniasis.
Financial Support: CNPq, PIBIC.

QT16 - Anti-leishmanial activity of a NaATPase inhibitor furosemide

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In previous studies, we showed that specific inhibition of Na+-ATPase of *L. amazonensis* promastigotes by furosemide led to parasite killing (De Almeida-Amaral, E.E *et al*.,2008). In this work, we extended those studies by investigating the cytotoxicity of the Na+-ATPase inhibitor furosemide against intracellular amastigotes and the macrophage host cell. Murine peritoneal macrophages (10^6 cells / well) were infected with promastigotes of *L. amazonensis* (Josefa strain) transfected with Green Fluorescence Protein - GFP (1:10), and then cultivated in the absence or presence of different concentrations of furosemide for 48h. After that time, the parasite load in culture was measured by the intensity of fluorescence according to our routine protocol. We found that the Na+-ATPase inhibitor was also active against the intracellular parasites (IC 50 = 2.0 ± 0.4 mM = 661.5 ± 132.3 ug/ml). The IC50 for the control drug Pentostan was 50 microg / ml in the same conditions. To test for cytotoxicity against macrophages, after 48 hours of cultivation with different furosemide concentrations, the supernantants of infected cells were harvested for the determination of cytoplasmic lactate dehydrogenase (LDH) enzyme. The Na+-ATPase

inhibitor was not toxic to macrophages up to the highest inhibitor concentration tested (2,0 mM, 661.5 ug / ml), indicative of drug selectivity against the parasites. In vivo experiments are in progress to determine furosemide efficacy against the disease.

De Almeida-Amaral, E.E; Caruso-Neves, C; Pires, V.M.P.; Meyer-Fernandes, J.R. (2008). *Experimental Parasitology,* 118: 165-171.

Suporte Financeiro: CNPq, CAPES

QT17 - ANTILEISHMANIAL ACTIVITY AND MURINE MACROPHAGES ALTERATIONS CAUSED BY A SECUNDARY METABOLITE OBTAINED FROM *ASPERGILLUS* FUNGI

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Parasites of the genus *Leishmania* are transmitted by the sandflies and infected cells of the mononuclear phagocyte lineage of their vertebrate hosts. The chemotherapy is one of the most effective treatments for this disease. Although a number of antileishmanial drugs are available, these drugs are in general toxic, expensive and require long-term treatment. The secondary metabolite is a transparent crystalline particulate substance highly soluble in water, ethanol, acetone and is produced by some species of *Aspergillus*, *Penicillium* and *Acetobacter.* This metabolite has bacteriostatic activity and it effectively inhibits the formation of DOPA from tyrosine in the process of melanin biosynthesis. However antileishmanial activity and effects on host immune cells are not well known. The present work was designed to determinate morphological alterations on macrophages and the effect of this metabolite on *L. amazonensis in vitro* infection. Firstly, treated cells (50µg/mL) showing increased cytoplasm and spreading ability, following of actin filaments polymerization, a high number of cytoplasmatic projections and vacuoles besides a higher superoxide production, detected by histochemistry assay with Nitro Blue Tetrazolium (NBT), when compared with control cells. Moreover those alterations weren't due to unspecific citotoxic effect, as observed through MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). Another aspect observed was the inhibition of 68% and 79% in promastigotes and amastigotes *in vitro* infection, respectively. These results demonstrated that secondary metabolite is involved with antileishmanial activity, mainly by the activation of host cell microbicidal response.

Acknowledgements:
CNPq/ UFPa.

QT18 - A COMPARATIVE STUDY OF THE PYRAZOLES DERIVATIVES AGAINST LEISHMANIA SPP.

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In a previous work searching for new and highly effective antileishmanial drugs, we reported the *in vitro* activity and the low toxicity of a series of pyrazoles derivatives against *Leishmania* (*L.*) *amazonensis*. As a part of our research program on chemotherapy against *Leishmania* spp., we decided to assay those amidine-1-aryl-4- (4,5-dihidro-1H-imidazole-2-yl) pyrazoles derivatives against other two different species of *Leishmania*, which were associated to the others clinical forms of disease. *Leishmania (V.*) *braziliensis* that usually caused mucocutaneous disease and is endemic in the State of Rio de Janeiro and *Leishmania* (*L.*) *chagasi* that is the causal agent of visceral leishmaniasis. These compounds were assayed against promastigotes forms of *L. braziliensis* (MCAN/BR/98/R619 strain) and *L. chagasi* (MCAN/BR/97/P142 strain) and using Pentamidine Isethionate as reference drug. After 24 hours incubation, the anti-*Leishmania* activity was determined by addition of tetrazolium bromide (MTT) and was evaluated in spectrophotometer with wavelength of 490 nm. As a result, comparing the effect of this pyrazole derivative (MSS73) against the three species, it was observed the

significant difference of the sensitivity of *L. braziliensis* and *L. chagasi* (IC_{50} > 320 µg/mL; IC_{50}= 267,83 ± 0,0 µg/mL, respectively), while against *L.amazonensis* (IC_{50}=8,7±1.6 µg/mL) it was more effective. Pentamidine Isethionate, the drug used as reference, showed effectiveness similar to this pyrazole derivative (MSS73) against all *Leishmania* species.

Supported by FAPERJ/CNPq/PDTIS/IOC-FIOCRUZ

QT19 - THE ANTICANCER DRUG TAMOXIFEN REDUCES *LEISHMANIA (L.) CHAGASI* INFECTION IN GOLDEN HAMSTERS

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We have previously demonstrated the *in vitro* activity of tamoxifen against *Leishmania* (*L.*) *amazonensis*, *L.* (*L.*) *major*, *L.* (*V.*) *braziliensis*, *L.* (*L.*) *chagasi* and *L.* (*L.*) *donovani* promastigotes with IC50% ranging from 9.0 to 20.2 µM. Moreover, we have shown that 50% of intracellular *L.* (*L.*) *amazonensis* amastigotes are killed with 5.4 µM tamoxifen (Miguel et al., J Antimicrob Chemother. 60(3):526-34, 2007). Recently, our group has established the in vivo efficacy of tamoxifen in the treatment of *L.* (*L.*) *amazonensis*–infected BALB/c mice, a highly susceptible model of infection (Miguel et al., PLoS Negl Trop Dis. 2(6):e249, 2008). Our purpose in the present study was to evaluate the activity of tamoxifen in a visceral leishmaniasis experimental model. Golden hamsters were infected with 1 x 10^8 *Leishmania* (*L.*) *chagasi* amastigotes. After 4 weeks hamsters were treated with saline, 20 mg/kg/d tamoxifen or 20 mg/kg/d meglumine antimoniate (Glucantime) intraperitoneally for 15 days. Immediately at the end of treatment, spleen parasite burden and liver histopathological analysis were assessed. Tamoxifen-treated animals presented a significant decrease in parasite burden when compared to hamsters that received saline. The efficacy of tamoxifen was very similar to that of Glucantime with 95 - 98% reduction in parasite load. Taking into consideration the difficulties in the current scenario of leishmaniasis chemotherapy, tamoxifen can be considered an alternative option for treatment of visceral leishmaniasis. Validation of this application for tamoxifen depends on further tests on other experimental models and in natural infections. Supported by FAPESP and CNPq.

QT20 - REDUCED TISSUE PARASITIC LOAD IN DOGS NATURALLY INFECTED WITH *Leishmania (Leishmania) chagasi* TREATED WITH AMPHOTERICIN B AND ALLOPURINOL IN A VETERINARY CLINIC OF BELO HORIZONTE, MINAS GERAIS, BRAZIL

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Visceral leishmaniasis in Brazil is a zoonotic disease, caused by *Leishmania chagasi* and transmitted by *Lutzomyia longipalpis*. Dogs are the most important reservoir of the parasite. The treatment of canine visceral leishmaniasis (CVL) in Brazil is controversial, but practiced since the 1990s. Authors suggest that treatment of CVL reduces the parasitic burden on the skin and the infectivity of treated dogs to sand flies. Because of this, become necessary to evaluate the effect of treatment of CVL on parasitic load in dogs treated in Brazil. This study aimed to compare the parasite load in skin of dogs naturally infected by *L. chagasi* before and after treatment in a veterinary clinic of Belo Horizonte. Thirty one dogs with CVL (various races, both sexes) were treated with amphotericin B (0.6mg/kg/EV/72h) for 48 days and allopurinol (20mg/kg/PO/12h) in continuous use. Prior to treatment and 15.96 ± 7.0 months after started, skin samples of ear were obtained by biopsy and submitted to immunohistochemistry (IHC) and PCR (specific for *L. chagasi k*DNA) tests. Prior to treatment, the frequency of animals that were IHC-negative for the presence of *Leishmania* in the skin was 43.3%, whilst after treatment the frequency increased to 93.5%. Similarly, a significantly larger proportion of study animals (80.6%) were *Leishmania*-negative, as determined by PCR, after treatment than prior to treatment (29.0%). The number of dogs with negative results in search of the parasite by IHC and PCR, after treatment was significantly higher than the number of animals with negative results prior to treatment ($p<0001$). Our results suggest that the treatment of CVL with

amphotericin B and allopurinol allows significant reduction of parasite load in the skin of study dogs, but is necessary more studies to check the ability of these animals to infect sand flies. Financial support: Laboratório Sorologia de *Leishmania* (ICB-UFMG); CAPES

QT21 - EFFICACY OF THE DIFFERENT PREPARATIONS OF MEGLUMINE ANTIMONIATE-CONTAINING LIPOSOME WITH PHOSPHATIDYLSERINE IN THE TREATMENT OF *LEISHMANIA (L.) CHAGASI* INFECTED MACROPHAGE

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Leishmaniasis, a vector-borne disease produced by invasion of the reticuloendothelial system of a mammalian host by parasite protozoa *Leishmania,* is endemic in large areas of the tropics, subtropics and the Mediterranean basin. This disease is characterized by both diversity and complexity, and causes high morbidity and mortality. The pentavalent antimonial has been the first-line treatment for all forms of leishmaniasis, but high toxicity and failures have been reported. An alternative to conventional treatment is delivery antileishmanial agents using colloidal carriers systems, as liposomes, which improve drug activity by decreasing the required dose and increasing the efficacy of entrapped drug at the intracellular disease involving the mononuclear phagocyte system (MPS). The aim of the present study was to evaluate the *in vitro* efficacy of the meglumine antimoniate-containing liposome with phosphatidylserine. Liposomes formulations were prepared from phosphatidylserine, cholesterol and phosphatidylcholine in the molar ratio 1:4:5. Meglumine antimoniate was encapsulated in liposomes by two different methods: freezed-dried empty liposome (FDEL-MA) or in multilamellar vesicles by filter extrusion (FEL-MA). Physical characterization of liposome was visualized by negative staining electron microscopy. The efficacy of FDEL and FEL was evaluated in *Leishmania (L.) chagasi* infected macrophages. FDEL-MA and FEL-MA showed an EC50% of the 2.65 µg/mL (IC95% 1.75-4.00) and 0.95 µg/mL (IC95% 0.80-1.12), respectively. The liposome formulations were about 20 to 54 times more effective that the free meglumine antimoniate in treating of infected macrophages. The FDEL-MA and FEL-MA showed a mean vesicle size smaller than 200 nm, and the FDEL-MA showed more homogeneous mean vesicle size than FEL-MA. These results suggest that liposome-encapsulated meglumine antimoniate is more efficacious than the free drug against *Leishmania (L.) chagasi* in this model of study. Drug delivery system as liposome may be useful as carriers of drugs to treat infectious diseases involving MPS, especially leishmaniasis. This research was supported by Brazilian agency CNPq/MCT.

QT22 - SENSITIVITY OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* ISOLATES TO ANTIMONIALS, AMPHOTERICIN B AND TAMOXIFEN

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Antimonials and amphotericin B are the drugs currently used in the treatment of leishmaniasis. These compounds are toxic and there are reports of therapy failure and resistance to antimonials. Cutaneous leishmaniasis in Brazil is mainly caused by *Leishmania (Viannia) braziliensis*. In this study, we assessed the in vitro sensitivity of 11 isolates of *L. (V.) braziliensis* from Leishbank-IPTSP/UFG/GO to meglumine antimoniate and amphotericin B. These isolates were obtained from patients attending at Tropical Disease Hospital (Anuar Auad, Goiânia, Goiás, Brazil). All these patients were submitted to chemotherapy with Glucantime and 36% (4/11) showed clinical response with healing of the lesions. One patient was not cured after 2 courses of treatment and 6 patients were still in evaluation. Sensitivity to antimonial was tested in vitro in bone marrow derived macrophages infected with promastigotes and cultured in the presence of increasing concentrations of the drug for 5 days. Antimonial EC_{50} for the 11 isolates ranged between 69.0 and 145.0 μg/mL, comparable therefore with the EC_{50} of 85.0 μg/mL for the type strain MHOM/BR/1975/M2903. Sensitivity to amphotericin B was tested against promastigotes. The EC_{50} of the isolates for amphotericin B varied from 0.04 to 0.12 μM, again comparable with the EC_{50} for the type strain of 0.09 μM. Additionally, we

also tested the sensitivity of these isolates to tamoxifen, a drug recently shown to be effective in vitro against *Leishmania* sp and in vivo for the treatment of *L. amazonensis* infected BALB/c mice (Miguel et al. PLoS Negl Trop Dis. 2008 Jun 11;2:e249). Sensitivity to tamoxifen was uniform amongst the isolates with EC_{50} varying from 6.0 to 13.7 μM. In conclusion, this study shows that the *L. (V.) braziliensis* isolates tested were similarly susceptible to antimonials, amphotericin B and tamoxifen. Financial support: CNPq and FAPESP.

QT23 - Antileishmanial activity of synthetic quinones on *Leishmania braziliensis*

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The treatment for Leishmaniasis in Brazil has been done basically with pentavalent antimonials, which have prolonged therapy and side effects. Therapeutic failure is a relevant problem in several coutries, mainly due to the emergence of drug resistant parasites. In this aspect, new chemotherapeutic agents have been tested. Quinones have been largely studied for antitumoral, antiparasitc, anti-inflammatory and antimicrobial activities. In previous studies, the synthetic naphthoquinone LBQ 118 presented strong *in vitro* and *in vivo* activities on *Leishmania amazonensis*. Our purpose is to investigate the effect of two synthetic naphthoquinones (LBQ 118 and 144) on *Leishmania braziliensis* (Thor strain) growth. Promastigotes forms were cultivated with testing substances for 96h /28°C and controls were parasites cultivated with medium or 0,06% DMSO. The parasite numbers were daily counted at Neubauer chamber. The activity on amastigotes was tested by infecting mouse peritoneal macrophages with promastigote (1:5 ratio, during 4 h/37°C) and counting the number of intracellular parasites after 48h in the presence of quinones. The naftoquinona synthetic LBQ 118 inhibited completely (100%) growth of promastigotes on the 4th day of culture and LBQ 144 inhibited 60%, both at 20 μM. The LBQ 118 showed a dose dependent activity, inhibited 30% to 5 μM, 78% to 10 μM and 98% to 20 μM. A reduction of 77% of the number of intracellular amastigotes was observed with 20μM of LBQ 118. These results point new perspectivities by leishmaniasis treatment. To assess the mode of action of the quinones biochemical analysis of the parasite is being currently held. Supported by CNPq.

QT24 - EVALUATION OF THE LEISHMANICIDAL ACTIVITY OF *LANTANA CAMARA* METHANOLIC EXTRACT IN *LEISHMANIA AMAZONENSIS*

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Introduction and objective: The drugs currently in use for the treatment of leishmaniasis display high liver and heart toxicities and develop clinical resistance after a few weeks of treatment. So, there is an urgent need for effective drugs to replace or supplement those in current use. The plant kingdom is undoubtedly valuable as a source of new medicinal agents. So, in this work we tested the methanolic extract of *Lantana camara* to evaluate its *in vitro* antiparasitic effect against promastigote and amastigote forms of *L. amazonensis* and the NO production of the promastigote forms. **Materials and methods:** The viability of promastigotes was checked using the tetrazoliumdye (MTT) colorimetric method. The result expressed as the concentration inhibiting parasite growth by 50% (IC50) after 24, 48 and 72 hours of treatment. The antiamastigote activity was evaluated using macrophages J774A.1 infected with promastigotes. The result for this test was expressed as the number of parasite by macrophage after 24 and 48 hours in the presence of 14 μg/ml or 28 μg/ml of extract. NO production was investigated by measuring nitrite, a by-product of nitric oxide released into culture supernatants. **Results and conclusions:** The methanolic extract of *L. camara* demonstrated activity against promastigote forms of *L. amazonensis* in a time dependent manner with great inhibition after 72 hours of treatment (14 μg/ml). However, against amastigote forms the extract showed between 20-26% of inhibition in the concentration of 14 μg/ml after 24 and 48 hours of treatment. Preliminary results indicated that *L. camara* methanolic extract reduced the production of NO in promastigote forms and it was not dose dependent. These results suggest the antileishmanial activity of *L. camara* methanolic extract and further studies with

purified fractions and isolated compounds will be done. Supported by FAPEMIG, UFJF.

QT25 - PLANTS USED IN FOLK MEDICINE VS ACTIVITY AGAINST AMASTISGOTE FORMS OF LEISHMANIA AMAZONENSIS

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Introduction and objective: Plants have been traditionally used for the treatment of diseases of different aethiology. Especially in the last decade, phytotherapy has received considerable attention in the search for alternatives to chemotherapy in parasitic diseases control, such as leishmaniasis. So, in this work we tested the methanolic extracts of *Cordia verbenacea, Rosmarinus officinalis* and *Schinus terebinthifolius* to evaluate *in vitro* effect against amastigote forms of *Leishmania amazonensis*. Previously, these extracts demonstrated a significant activity against promastigote forms of *L. amazonensis*. **Methods:** The antiamastigote activity was evaluated using macrophages J774A.1 infected with promastigotes. The result for this test was expressed as the number of parasite by macrophage after 24 and 48 hours in the presence of extracts. The concentrations used were 1- and 2-fold IC_{50} values for *L. amazonensis* promastigotes. **Results and conclusions:** The methanolic extracts showed better antiamastigote activity at 24 hours of treatment and it was not dose-dependent. *C. verbenacea* demonstrated the highest antileishmanial activity of the tested extracts, reducing the number of intracellular amastigotes (44.2%) at 120 µg/mL.The other extracts tested *R. officinalis* and *S. terebinthifolius* demonstrated inhibition under 30% after 24 and 48 hours of treatment. Furthermore, none of extracts was found to have significant toxicity effect on macrophages. These results suggest that these extracts have promising antileishmanial potential and contribute to the advance in the research for the new chemotherapy for leishmanisis. Supported by FAPEMIG, UFJF.

QT26 - Antileishmanial activity of constituents of plants from the Mato Grosso State flora against intracellular amastigotes of *Leishmania amazonensis.*

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Currently, chemotherapy for treatment of leishmaniasis is restricted to a few drugs, which presented limited efficacy and shows undesirable side effects. The Brazilian plant biodiversity represents an enormous source of new potential antiparasitic compounds. Plant extracts from Mato Grosso State flora have been demonstrated to display activity against *Leishmania* promastigotes *in vitro*. In this study, we evaluated the antileishmanial activity against intracellular amastigotes of these natural products previously screened. Four hexanic crude extracts *(Xilopia aromatica, Bowdichia virgiloides, Aspidosperma cuspa* and *Acosmium dasycarpum)*, two fractions (ethyl acetate and dichloromethane fractions of *Zanthoxylum riedelianum*) and one isolated compound (coumarin, isolated from *Spiranthera odoratissima*) were solubilized in DMSO (50mg/mL) and maintained at 4°C until use. J774.A1 cells were infected with 10:1 axenic amastigotes of *Leishmania amazonensis* (575 strain) in suspension at 34ºC overnight. After the infection the cells were seeded in 96-well plates in the presence of different concentration of natural products (200, 40, 8 and 1.6 µg/mL) for 48h at 34°C/5% CO_2 in RPMI medium + 20% FBS. Amphoterecin B (1µg/mL) and DMSO 1% were used as controls. After treatment, cell monolayers were fixed with methanol and stained with Giemsa. Antileishmanial activity was evaluated by counting the number of infected cells in 200 randomly chosen cells as well as the number of intracellular amastigotes per infected cell. The citotoxicity for J774-A1 was evaluated using the MTT method. Only the hexanic crude extract of *A. dasycarpum* did not presented antileishmanial activity. Crude extracts from *B. virgiloides and A. cuspa,* fractions from *Z. riedelianum* and the coumarin showed rates of inhibition amastigotes growth ranging from 55 to 64.5% at 1.6µg/mL concentration. None of the active extract, fractions or compound was

citotoxic up to 90µg/mL. Studies with purified compounds are under progress to identify the active leishmanicidal compounds.
Supported by CNPq/UFSC/UFMT and CPP.

QT27 - *The Effects of MDL 28170 in Leishmania amazonensis Infection In Vitro*

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Infections by protozoans are a major worldwide health problem, with high endemicity in developing countries. The drugs of choice for the treatment of leishmaniasis are the pentavalent antimonials, which show renal, cardiac toxicity and induction of parasite resistance. These facts have given rise to the development of new approaches for leishmaniasis therapy. As part of the search for new drugs against leishmaniasis, we evaluated the in vitro anti-leishmanial activity of MDL 28170, a potent calpain inhibitor, as well as its toxicity for vertebrate cells. A culture of infected macrophages was maintained for 24, 48 and 72h at 37°C in 5%CO_2 in the presence or absence of different concentrations (15, 20 and 30 µM) of the drug. The coverslips were fixed in methanol and stained for 1 hour with Giemsa 36%. The percentage of infected macrophages was determined by counting at least 200 cells in duplicate cultures. The results demonstrate that the drug presents anti-leishmanial activity in infected macrophages and inhibits the replication of *L. amazonensis* amastigotes in vertebrate cells. The MDL 28170 inhibits parasite survival in a dose-dependent manner. In addition, the calpain inhibitor showed no cytotoxicity against murine peritoneal macrophages in culture, as judged by trypan blue assay. These researches provided new perspectives on the development of novel drugs with leishmanicidal activity.

Supported by MCT/CNPq, CEPG/UFRJ, FAPERJ and FIOCRUZ.

QT28 - MORPHOLOGICAL AND ULTRASTRUCTURAL ALTERATIONS OF *Leishmania amazonensis* TREATED WITH *Cymbopogon citratus* AND ITS MAJOR COMPONENT (CITRAL)

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Leishmaniasis is a disease caused by a number of species of protozoa of the genus *Leishmania*. The classic forms of leishmaniasis still pose particular difficulties in terms of diagnosis and treatment. *Cymbopogon citratus* is commonly used in folk medicine in Brazil for treatment of nervous and gastrointestinal disturbances. Several investigations have demonstrated its antibacterial, antifungal, and antiprotozoal properties. In the present study, we report morphological and ultrastructural alterations on *L. amazonensis* treated with citral-rich essential oil (EO) obtained by steam distillation of fresh leaves of *C. citratus* and citral was obtained commercially from Sigma-Aldrich. To evaluate the morphological and ultrastrutural changes induced by the EO from *C. citratus* or citral in promastigote forms of *L. amazonensis,* the parasites were treated with *C. citratus* or citral. For scanning electron microscopy (SEM), parasites were placed on a support, dehydrated in ethanol, critical-point dried in CO_2, coated with gold, and observed in a Shimadzu SS-550 SEM. For transmission electron microscopy (TEM), cells were post-fixed in osmium tetroxide, dehydrated in acetone, and embedded in Epon. Ultrathin sections were observed in Zeiss CEM-900. SEM of promastigote forms revealed that the parasites exposed to EO from *C. citratus* or citral had aberrant morphology. These alterations were progressive with increasing drug concentrations, and eventually included the disintegration of the parasite. The cells treated with EO when observed by TEM showed mitochondrial swelling, appearance of two or more flagella. In addition, protozoa treated with citral showed more drastic alterations in their morphology, such as the presence of exocytic projections in the flagellar

pocket, and swollen mitochondria, with plasma membrane blebs seeming to detach from the parasite surface. The present study revealed that citral-rich essential oil from *C. citratus* has promising antileishmanial properties.

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QT29 - INITIAL STUDIES OF AMIODARONE AND POSACONAZOL ON *Leishmania amazonensis*

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Leishmaniasis is a parasitosis caused by organisms of the *Leishmania* genus which are associated with significant rates of morbidity and mortality throughout the world. The mainstay of chemotherapy employed is based on pentavalent antimonials as first line of compounds, and in special cases, on miltefosine, amphotericin B and pentamidine. However, they are very unsatisfactory and there is an urgent need for safer and more efficacious anti-*Leishmania* agents. Posaconazole and Amiodarone are two novel compounds with potent effects in *Trypanosoma cruzi,* interfering directly with the ergosterol metabolism. Amiodarone also acts in the Ca^{2+} homeostasis leading to several effects on the mitochondrion physiology. We report here preliminary results on the primary effects of Posaconazole and Amiodarone on *Leishmania amazonensis*. The IC_{50} values found for promastigote forms were about 100 nM and 5 µM to Posaconazole and Amiodarone, respectively. Cells incubated in the presence of both compounds displayed an intense alteration in the morphology of the promastigotes which showed a rounded shape and became swollen. Transmission electron microscopy demonstrated the presence of several alterations. The mitochondrion was the main organelle affected with the treatment, presenting an intense swelling, lost of the matrix content and some alterations in the mitochondrial membranes. In addition, we also observed the presence of several large vacuoles containing part of the cytoplasm and membrane profiles ressembling to autophagic structures. Taken together, these results indicate that Amiodarone and Posaconazole are promising compounds against *Leishmania*-parasites. Further studies are in progress to evaluate the effect of these drugs in intracellular amastigotes, the clinical relevant stage in leishmaniasis, and their mechanisms of action.
Financial support: CNPq-MCT-DECIT, FAPERJ, PRONEX.

QT30 - EVALUATION OF THE IN VITRO ACTIVITY OF THE CRUDE ETHANOLIC EXTRACT AND ELUATES OF $CHCl_3$ AND EtOAc FROM *CIPURA PALUDOSA* AGAINST *LEISHMNIA AMAZONENSIS* PROMASTIGOTES

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Leishmania amazonensis causes human diseases that range from self-healing cutaneous lesions to diffusion cutaneous leishmaniasis. The chemotherapeutic of this disease require long-term treatment and have been based on the use of pentavalent antimonials. *Cipura paludosa* Aubl. (Iridaceae), which is known as “batatinha roxa”, is used in traditional medicine by the population of Rondônia as effective in tea form, against affections of renal tract, amoeba, diarrhea, inflammations and for menstrual flow regulation. This research aimed to assess the potential therapeutic of *C. paludosa* against leishmaniasis, investigating the activity of crude ethanolic extract (EE), acetate eluate (EtOAc) and chloroform eluate ($CHCl_3$) against promastigotes forms of *Leishmania amazonensis*. Briefly, the parasites were incubated with various concentrations of *C. paludosa* fractions and its growth was accompanied by 5 days at a temperature of 24°C. Daily, number of promastigotes was counted in optical microscope with an increase of 400X in presence of erythrosin B. Pentamidine was used as negative control (1µg/ml). For statistical analysis of data was used Probit. For EE the

concentration of 100µg/ml promoted the death of 100% of parasites and presented IC_{50}=9.12µg/ml and IC_{90}=128.88µg/ml (P<0,01). For EtOAc and $CHCl_3$ eluates all concentrations tested greater than 6µg/ml promoted the death of 100% of parasites. The IC_{50} and IC_{90} were 0.84 and 4.75µg/ml for the EtOAc eluate, and 0.31 and 1.71µg/ml for the $CHCl_3$ eluate. The fractions tested showed no cytotoxic effect when tested in murine macrophages. The leishmanicidal activity of *C. paludosa* has never been described, but pharmacological activities of EE have been evaluated, with regard to antinociceptive, anti-inflammatory and antioxidant properties. These results show that *C. paludosa* might be a good choice for the analysis of a new potentially leishmanicidal substances.

Financial Support: CNPq, PIBIC.

QT31 - ANTI-LEISHMANIAL ACTIVITY OF PIPERINE AND THEIR ANALOGUES ON *Leishmanai amazonensis*.

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Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania* and is a significant cause of morbidity and mortality in several countries. The current treatment presents high toxicity and is not fully effective. Piperine, the major amide of *Piper* species, have some biological activities such as lipid oxidation, anti-parasitic, anti-tumor. In this work, we study the effect of piperine and some analogues on *Leishmania amazonensis*, the causative agent of cutaneous and diffuse cutaneous leishmaniasis in New World. Piperine and analogues were tested on promastigotes culture and on macrophages infected with amastigotes forms. Our results showed that piperine, and its derivatives, tetrahydropiperine and phenyl amide inhibit promastigotes growth *in vitro*, with IC_{50} of 14.3 µM, 24.5 µM and 29.6 µM, respectively. Piperine and phenyl amide, at 50µM, also affect amastigotes, with 53% and 73% of survival inhibition, respectively. Tetrahydropiperine showed an anti-amastigotes effect lower than piperine. At 50µM, piperine and analogues were not cytotoxic to peritoneal macrophages as evaluated by the XTT test, after 24 hour of treatment. In order to identify the possible mechanism involved on anti-amastigotes and promastigotes activities we analyzed the phagocytic capacity of macrophages treated with piperine, tetrahydropiperine and phenyl amide. Our results showed that at 50 □M none of the compounds affected the phagocytic capacity of treated macrophages. Thin-layer chromatography of lipids extracted from promastigotes after 48 hr treatment with piperine, demonstrated a reduction around 80% in the cholesterol and triacylglycerol was not detected. Morphological analysis by optical microscopy showed that after 24 and 48 hr of piperine treatment at 50 µM, parasites presented irregular number of nucleus and flagellum, and abnormal morphologies in comparison to untreated cells. Our results point piperine and phenyl amide as efficient compounds against *Leishmania amazonensis* and provide new perspectives for novel compounds for leishmaniasis treatment.

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QT32 - 10-FORMYL-THF IS AN ESSENTIAL METABOLITE FOR *LEISHMANIA MAJOR*

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Our laboratory is interested in the role of pteridines in *Leishmania* growth, differentiation and virulence, and these metabolic pathways have great potential for selective chemotherapy. In other organisms, 10-CHO-THF is used in *de novo* purine biosynthesis and the formylation of the mitochondrial initiator tRNA (fMet-tRNA). However, most protozoans are purine auxotrophs and there is some debate about the essentiality of fMet-tRNA in prokaryotes and eukaryotes. In mammals, 10-CHO-THF is synthesized in the cytoplasm through

the action of a trifunctional methylene tetrahydrofolate dehydrogenase-cyclohydrolase/formyl tetrahydrofolate ligase (*DHCH-FTL*) or in the mitochondrion by a bifunctional *DHCH*. Database mining revealed single separate *DHCH* and *FTL* genes in *Leishmania major*, and characterization of recombinant DHCH and FTL showed these proteins had typical properties and enzymatic activities. Antisera raised against these proteins confirmed that they were exclusively cytosolic. *ftl*$^-$ null mutants were viable and exhibited normal growth, metacyclogenesis and virulence in animal infections. While *DHCH* heterozygotes were readily obtained attempts to generate *dhch*$^-$ KOs yielded only aneuploids, bearing both planned replacement but retaining a *DHCH* copy, a typical sign of essential genes. Chromosomal KOs could be obtained following introduction of an episomal *DHCH* or *FTL*. The latter result implies rescue by 10-CHO-THF arising from overexpressed FTL. Notably, the pXNG vectors enabled a 'forced segregation' test of gene function employing negative selection using TK or GFP/FACS sorting; in these studies, neither *DHCH* nor *FTL* could be lost despite analysis of >1000 events. These data argue first that 10-CHO-THF is an essential metabolite, secondly that WT *FTL* levels are insufficient to maintain needed levels, and thirdly that DHCH and 10-CHO-THF metabolism has potential as a drug target. This was confirmed as we synthesized and tested a broadly-acting DHCH inhibitor, and found this to inhibit both parasite DHCH and growth with good potency (100 nM and 1 uM respectively).

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QT33 - *IN VITRO* SCREENING FOR ANTILEISHMANIAL AND TRYPANOCIDAL ACTIVITY OF CONSTITUENTS FROM SOFT CORAL AND SPONGE EXTRACTS

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Currently, the drugs used for treatment of Leishmaniasis and Chagas disease are highly toxic and present limited efficacy. Screening natural products provide the chance to discover new promising molecules. Thus, in the present study we evaluated the *in vitro* leishmanicidal and trypanocidal effect of twenty-eight chloroform-methanol (1:1) crude extracts from corals (Photidae, Plexauridae and Gorgoniidae) and sponges (Agelasidade, Clionaidae, Axinellidae, Spirastrellidae, Anthothelidae, Niphatidae, Microcionidae, Spirastellidae, Oceanopiidae, Zoanthidea, Nepheliospongiidae, Desmacellidae and Aplysinellidae. Culture forms of *Trypanosoma cruzi* (Y strain), *Leishmania amazonensis* (575 strain) and *L. chagasi* (PP75 strain) were grown in LIT medium (10% FBS) and Schneider's medium (5% FBS) and had their concentration adjusted to $5x10^6$ parasites/mL. The parasites were incubated in a 96 well plates with serial dilutions of the compounds at 27ºC for 48h. As controls, parasites were incubated with 1% DMSO or 100µM of benznidazole (*T. cruzi*) or 10µM amphotericine B (*Leishmania* spp.). Citotoxicity was evaluated against VERO cells. Both antiprotozoal and citotoxicity assays were observed in an inverted microscopy Olympus followed by the MTT method. The obtained data were compared by ANOVA and CC_{50} and IC_{50} values were calculated by linear regression analysis. Although neither of extracts showed trypanocidal effect, leishmanicidal activity was significantly increased, mainly against *L. chagasi.* Forty-three percent of the tested extracts showed some leishmanicidal effect, particularly those of the families Gorgoniidae (*Pseudopterogorgia elisabethae*), Agelasidae (*Agelas conifera*) and Clionaidae (*Cliona tenuis*) with IC_{50} values between 13.1µg/mL to 178.2µg/mL. The antileishmanial effect of these extracts was at least 3-4 times higher than the citotoxicity. The most promissory extract was from de family Aplysinidae (*Aplysina insularis*) with IC_{50} value of 9.4µg/mL, with a selective index (SI) of 48. Fractionations of active extracts are under progress to identify the leishmanicidal compounds.

Supported by: CNPq, UFSC and Colciencias.

QT34 - ACTIVITY OF A TRITERPENE FROM *Croton Cajucara* AMAZON PLANT ON DIFFERENT STRAINS OF *Trypanosoma cruzi*

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American trypanosomiasis (Chagas disease) is caused by the kinetoplastid protozoan *Trypanosoma* (Schizotrypanum) *cruzi* affecting about 16 -/18 million people in Latin America. The available chemotherapy presents limited efficacy in the chronic phase and undesirable side effects, encouraging the research for alternative drugs. In the last decades occurred a movement known as "back to Nature", reinforced by the success of taxol for cancer chemotherapy and artemisinin for malaria. Terpenes are hydrocarbons which the basic unit is composed of 5 carbon atoms named isoprene or hemiterpene. It has already been reported the trypanocidal activity of different terpenes isolated from different sources. In the present work we investigate the effects of the triterpene acetyl aleuritolic acid (AAA) obtained from the stem bark of the Amazon plant *Croton cajucara* Benth (Euphorbiaceae) on *T. cruzi* epimastigote and tripomastigote forms from the main phylogenetic lineages: TCI (DM28c strain and C45 isolate derived from *Philander frenatus*) and TCII (Y strain and 291 isolate derived from *Leontopithecus* rosalia). The ED_{50}/24, 48, 72, 96h and the ED_{50}/24h of AAA on *T. cruzi* epimastigotes and trypomastigotes respectively, were determined by cell counting in a Neubauer chamber. It was observed a dose-dependent effect of AAA on epimastigote proliferation, being the clone Dm28C the most susceptible to AAA with IC_{50} values of 579.1 $\pm$ 75.8 and 686.6 $\pm$ 30 for, respectively, 3 and 4 days of treatment. For trypomastigotes, the most susceptible isolates were 291 (IC_{50}/24h = 156.92 $\pm$ 18.46 μM) and C45 (IC_{50}/24h = 491.9 $\pm$ 57.5 μM). The results have shown trypanocidal activity of AAA on *T. cruzi*, being tripomastigotes the most susceptible. Moreover, no correlation between drug sensibility and *T. cruzi* phylogenetic lineages were observed. Work supported by PAPES/Fiocruz, CNPq and FAPERJ.

QT35 - THE EFFECT OF TOPOISOMERASE INHIBITORS AND DNA BINDING DRUGS ON *TRYPANOSOMA CRUZI* PROLIFERATION AND ULTRASTRUCTURE

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Trypanosomatids present a typical structure, termed kinetoplast, which corresponds to an enlarged portion of the single mitochondrion that is composed by catenated DNA (kDNA). DNA topoisomerases are essential for cellular functions, as replication, transcription and repair, constituting the main chemotherapy targets in the kinetoplast. The kDNA network is also sensitive to intercalating drugs that interfere with kDNA replication. In this work, we analyzed the effects of topoisomerase I and II inhibitors, as Camptothecin and Norfloxacin and also the impact of DNA intercalating drugs, in the proliferation and ultrastructure of *Trypanosoma cruzi* epimastigote——forms. After 24 hours of growth, part of the cells was cultivated (or not – control cells) in medium containing drugs at the following concentrations: 1, 5, 10 and 50uM to Camptothecin; 50, 150, 300 and 500ug/mL to Norfloxacin and 2, 10, 20 and 50uM to Berenil. Samples were collected after each 24 hours for counting on Neubauer's chamber or for processing to transmission electron microscopy until 72 hours of cultivation. Our results showed that drug treated cells presented a dose dependent decrease on cell proliferation. Camptothecin promoted the higher growth impairment when compared to Norfloxacin and Berenil. Ultrastructural analysis revealed that camptothecin did not promote alterations on kDNA arrangement. However the nuclear DNA was affected, since the condensed chromatin, which is usually found close to the nuclear envelope and around the nucleolus, disappears after drug treatment. Furthermore, most cells present the G1/S phenotype, indicating that the nuclear DNA replication was inhibited. On the other hand, Berenil promoted mitochondrial swelling and changes on kDNA compactation, similar to that described to Acriflavine, another DNA intercalating drug. These results emphasize the essential role of

topoisomerases in DNA replication and kDNA organization and reinforce the idea that the kinetoplast is a potential chemotherapeutic target for trypanosomatid protozoa.
Supported by CNPq and Faperj.

QT36 - BIOLOGICAL ACTIVITY OF PICOLINIC ACID AGAINST *Trypanosoma cruzi*

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Chagas' disease remains a substantial health problem in Latin America. Benznidazole, the only available drug in Brazil for its treatment, has low efficiency in the chronic phase and it causes serious side effects in patients, which can lead to discontinuation of the treatment. An approach used today is the study of compounds extracted from natural sources such as plants, or obtained from chemical synthesis, to replace the drugs used for the treatment of this disease. Picolinic acid is a naturally occurring end-product of L-tryptophan catabolism. Several functions have been attributed to picolinic acid, including cell cycle control, metal-chelation, antitumor activity in mice, and modulation of macrophage and neutrophil functions. Antimicrobial activities have also been observed, such as inhibition of *Escherichia coli* and *Mycobacterium avium* complex organisms' growth, and *Bacillus subtilis* sporulation. In addition, picolinic acid is a potent costimulus in the induction of macrophage or neutrophil-mediated microbicidal activity against *Candida albicans* and *M. avium*. In a previous work, our group showed the inhibitory activity of picolinic acid against trypanosomes of the subgenus *Schizotrypanum* isolated from the bat *Phyllostomus hastatus,* a non-pathogenic trypanosomatid. Here, the *in vitro* effects of picolinic acid on growth of replicative forms of *Trypanosoma cruzi Dm*28c were investigated. The IC_{50}/96 h of picolinic acid was 0.68 mM, and compared to HEp-2 cell line, epimastigote forms of *T. cruzi* are 8.5 times more sensitive to this compound. Parasites with altered cell shape, presenting two or more flagella were observed by scanning electron microscopy after the treatment with the compound. Ultrastructural observations showed alterations in Golgi apparatus as well as the presence of great cytoplasmic vacuoles and myelin figures. Actually, the effect of picolinic acid on the host-parasite interaction is under investigation.

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QT37 - Trypanocidal activity of a DB1362 against *Trypanosoma cruzi: in vivo* studies

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Chagas disease is an important medical and social problem in endemic areas of Latin America, however, the available therapeutic options are considered unsatisfactory. Aromatic diamidinas are DNA minor groove-binding ligands displaying excellent anti-microbial activity. Our previous data have demonstrated good activity of diamidines and analogs against *T. cruzi in vitro* and *in vivo*. Then, due these evidences, our aim was evaluate the effects of a diarylthiophene diamidina (DB1362) during the infection of Swiss Webster mice with 10^4 bloodstream forms of *T. cruzi* (Y strain) using different drug concentrations (25 and 50mg/kg) and treatment schemes (two-day or a ten-day regime), starting the treatment at the parasitemia onset. The best results were obtained when the acutely infected mice were treated with the lower concentration, providing 100% of survival compared to the infected and untreated mice group. Although not displaying higher efficacy as compared to benznidazole, DB1362 was able to largely reduce both cardiac parasitism and inflammation, in addition to protecting against cardiac alterations, as reflected by ECG measurements, related to *T. cruzi*-infection. The present in vitro and in vivo results support further investigation of diamidines and related compounds, as potential agents for Chagas disease.

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QT38 - Activity of naphtoquinones and derivatives on *Trypanosoma cruzi*

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Currently, the treatment of Chagas' disease is unsatisfactory, being limited to two nitroheterocycles, benznidazole and nifurtimox. In this context, our group is involved in the synthesis and the evaluation of the trypanocidal activity of new naphthoquinones prepared from nor-lapachones and lapachones.

Stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO), with the final concentration of the solvent never exceeding 0.1%. The assays were performed with trypomastigote forms from the Y strain (10^7 cells/ml) in the presence of 5% of mouse blood. Compounds **15** and **20** presented values of IC_{50}/24 h of 86.3 ± 4.6 and 88.2 ± 6.7 μM, respectively, both being more active than benznidazole (Bz). Compounds **7**, **10** and **14** showed activity similar to Bz, in the range of 140 to 180 μM. For **13**, **17**, **18**, **19** and **22**, this parameter was situated between 300 and 1000 μM, and the other derivatives were considered inactive.

The 1,2- and 1,4-naphthoquinones are considered privileged structures in medicinal chemistry, being the easiness of reduction-oxidation of the quinoidal moiety the basis for their participation in electron transport and oxidative-phosphorylation processes. Ours results showed that the most of the derivatives assayed were more active than the original quinones, suggesting that this type of compounds are a good starting point for a medicinal chemistry investigation aiming the chemotherapy of Chagas' disease.

QT39 - EVALUATION OF RECENTLY SINTHETISED MANNICH BASES AGAINST EPIMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is the causative agent of Chagas'disease, which still affects millions of people in Latin America, keeping high rates of mortality and morbility. The only available medication is Benznidazol, but due to its low effectiveness and lots of side effets, researches on the development of new drugs are necessary. Mannich bases are very versatile compounds, with various farmacological activities described, sometimes even more effective and less toxic than their parent compounds. In this work, we describe the activity of some recently sinthetized Mannich bases against epimastigote forms of *T. cruzi*. Materials and Methods: *T.cruzi* Dm28c epimastigotes were raised in BHI-medium. Stock solutions of R401, R411, R416 and R420 were prepared in DMSO and their effect at 50μM were determined after quantification of alive parasites on 72h and 144h of culturing, by counting in a Neubauer chamber using optical microscopy (Olympus Bx41). DMSO 1.0% was used as negative control. Results and Conclusion: Among the four substances tested, R416 was the one that presented best results, killing 98,29% of the parasites in 72h and 98,41% in 144h. Further tests will be made in order to determine the concentrations that kill 100% of the parasites and to evaluate the citotoxicity of these substances to mammalian cells *in vitro*. Financial Support: CNPq, PROPP/UFF, FAPERJ

QT40 - EFFECTS OF THREE GLUTAMATE ANALOGS ON DIFFERENT STAGES OF *TRYPANOSOMA CRUZI*

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It is well established that amino acids are involved in several biological processes in trypanosomatids. In particular, *Trypanosoma cruzi* metacyclogenesis is promoted by amino acids such as proline,

glutamate and aspartate, and the transformation of epimasigotes-like to trypomastigotes inside the host cells is promoted by proline. These facts led us to evaluate these amino acid metabolic pathways as possible targets for new therapeutic drugs. In the present work, we analyzed the effects of three glutamate analogs, methionine sulfoximine (MS), methionine sulfone (MSO) and methionine sulfoxide (MSE) on the epimastigote growth and the trypomastigote infection of CHO-K1 cells. The epimastigotes cultures were supplemented with different concentrations of drugs. The IC_{50} obtained for epimastigotes growth were: MS=17.0±0.3 mM, MSO=32.4±4.8 mM and MSE=38.7±11.9 mM. The interaction of the three drugs with stress conditions such as high temperature, nutrients starvation and oxidative stress, were also evaluated. The three analogs showed synergistic effects with temperature, oxidative and nutritional stresses. The IC_{50} obtained at 37 ºC were lower than at 28 ºC (MS=9.4±1.5 mM, MSO=9.7±0.4 mM and MSE=14.0±0.3 mM), and the parasite viability diminished more of 50 % in conditions of nutritional and oxidative stress. To evaluate the efficiency of the compounds on the infected host cells, their toxicity on the not-infected cells were previously tested at different concentrations (MS = 10, 15 and 20 mM, MSO and MSE = 30, 40 and 50 mM). At those concentrations, no inhibition was observed on the trypomastigote burst. Combination of drugs inducing oxidative stresses will be evaluated to optimize the treatment of the infected cells. These results remark that metabolic differences among the different life-cycle stages should be taken into account when proposing a new drug for therapy, particularly when it is based on trypanocidal activities obtained from *T. cruzi* epimastigotes.
Supported by USP-FAPESP.

QT41 - EVALUATION OF THE ANTI-*TRYPANOSOMA CRUZI* ACTIVITY OF THREE SYNTHETIC DNA BINDERS.

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Trypanosoma cruzi is the etiological agent for Chagas' disease which affects 16-18 million people in the Americas. This parasite shows a complex life cycle, characterized by several forms both in the insect vector and in mammalian host. The therapy against this infection relies mainly on two therapeutic drugs, with low efficiency and undesirable side effects. Due to these facts the search of new leader compounds with trypanocidal activity is considered as a relevant goal. In the present work, we studied three synthetic DNA binders: MB-11($C_{22}H_{18}N_8O_2S.2HCl.5H_2O$), MB-12 ($C_{28}H_{30}N_8O_2S.2,5HCl.4H_2O$) and MB-13 ($C_{26}H_{22}N_8O_2S.2HCl.4H_2O$). Epimastigotes were cultured in LIT medium in usual temperature and pH conditions (pH=7.5 and 28 ºC) and supplemented with different concentrations of drugs. MB-11 and MB-13 did not show any effect on epimastigotes growth. However, the IC_{50} obtained for epimastigotes by MB-12 were 5.4±1.3 µM. The interaction of this compound with stress conditions was also analyzed by submitting the cells to high temperature (33 and 37 ºC) or low pH stresses (pH=5.5 and 6.5). The IC_{50} was reduced at 33 and 37 ºC, being 2.2±0.08 µM and 3.1±0.7 µM, respectively. At pH=5.5 the epimastigotes growth was diminished both in the control and in drug supplemented cultures, whereas the IC_{50} at pH=6.5 was 9.7±5.1 µM. Concentrations up to 50 µM were not toxic to mammalian CHO-K1 cells and there were no inhibition on the trypomastigotes bursting at concentrations of 20, 35 and 50 µM, showing that this compound does not affect the *T. cruzi* infective stage. In further studies the possible synergistic effects between MB-12 and compounds causing oxidative stress will be analyzed on the infected host cells.
Supported by USP-FAPESP (Brazil) and Ministry of Science, Education and Sports (Republic of Croatia).

QT42 - ACCURATE REAL-TIME PCR STRATEGY FOR MONITORING BLOODSTREAM PARASITIC LOADS IN CHAGAS DISEASE PATIENTS UNDER ETIOLOGICAL TREATMENT

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Introduction and Objectives:
We aimed to develop a sensitive Q-PCR strategy to accurately quantify *T.cruzi* loads in peripheral blood samples for monitoring Chagas disease patients under etiological treatment.
Results:
The Q-PCR detection limit is 0.1 and 0.01 parasites/mL, with a dynamic range of 10^6 and 10^7 for Silvio X10 cl1 (*T.cruzi* I) and Cl-Brener stocks (*T.cruzi* IIe) respectively, with an efficiency of 99% and a coefficient of determination (R^2) of 0.998. In order to express accurately the parasitic loads: 1) we adapted a commercial kit based on silica-membrane technology to enable efficient processing of Guanidine Hydrochloride- EDTA treated blood samples and minimize PCR inhibition; 2) results were normalized incorporating a linearized recombinant plasmid as an internal standard of the whole procedure and 3) a correction factor according to the representativity of satellite sequences in each parasite lineage group was determined using a modified real-time PCR protocol (Lg-PCR).
The Q-PCR strategy was applied to estimate basal parasite loads and treatment follow-up of pediatric Chagas disease patients, as well as to monitor chronic Chagas heart disease patients who underwent heart-transplantation and displayed events of clinical reactivation due to immunosupression.
Conclusions:
All together, the high analytical sensitivity of the Q-PCR strategy, the low levels of intra- and inter-assay variations, as well as the accuracy provided by the Lg-PCR based correction factor, support this methodology as a key laboratory tool to complement current Chagas disease diagnosis, as well as for monitoring etiological treatment outcome and reactivation in immunosupressed patients.

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QT43 - TRYPANOCIDAL ACTIVITY OF MAJOR *Eugenia jambolana* Lam. CONSTITUENTS AGAINST DIFFERENT EVOLUTIVE FORMS OF TRYPANOSOMA CRUZI

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is an important endemic illness in Latin America. Although transmission has been reduced, an effective treatment for the infected population is lacking. Treatment of Chagas disease show chemotherapeutic failure and unpleasant side effects. We have demonstrated the trypanocidal effect of Eugenia jambolana essential oil on different evolutive forms of *T. cruzi*. Expression of NO and TNF-α production by infected and noninfected macrophages treated with the essential oil, indicates that the toxicity against the parasite is independent of cellular activation. Chemical profile of this oil shows several constituents, terpens as majority. Four *E. jambolana* derivatives showed a dose dependent trypanocidal effect when utilized against tripomastigotes forms on non-cytotoxic concentrations to mammalian cells. When tested on release of these forms by infected macrophages and development of amastigotes inside these cells, these compounds also show activity. Our work is currently focused on studies of associations between the derivatives and parasitical effect in experimental model of Chagas disease.

QT44 - THE INHIBITORY EFFECTS OF THE CALPAIN INHIBITOR MDL28170 AGAINST *TRYPANOSOMA CRUZI*

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Peptidases are involved in several physiological processes, which makes this class of enzymes an obvious target for the development of therapeutic agents to treat infectious diseases. In this study, we report the growth alterations caused by the calpain inhibitor MDL28170 in *Trypanosoma cruzi* clone Dm28c epimastigote forms and the location of calpain homologues in the parasite. The calpain inhibitor at 70 μM promoted a powerful reduction on the cellular growth rate by approximately 80% after 24 h and 90% after 48 h. MDL28170 promoted alterations in the cell morphology, and the antitrypanosomal effect was reversible, since cells pre-treated for 72 h with the calpain inhibitor at 70 μM were able to grow when subcultured in fresh medium. In order to detect the presence of calpain homologues, flow cytometry analysis was performed employing the anti-calpain antibody raised against cytoskeleton-associated protein of *Trypanosoma brucei* (CAP 5.5) and the antibody raised against *Drosophila melanogaster* calpain (anti-Dm-calpain). The use of the latter showed calpain homologues on the cell surface of epimastigote forms. A significant raise in the fluorescence intensity was observed with both antibodies when cells were previously permeabilized, showing that calpain homologues were preferentially located in cytoplasmic compartments. The immunofluorescence microscopy showed labeling throughout the cell body, including the flagellum. In this context, we may conclude that calpain inhibitors may be useful against important human pathogens, including *T. cruzi*.

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QT45 - ACTIVITY OF HETEROCYCLIC CATIONS ON *Trypanosoma cruzi*

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Trypanosomes are unicellular protozoan that cause serious public health problems in Latin America, such as Chagas Disease. Their available therapies are acknowledged inadequate, justifying the development and screening of new compounds. Trypanosomes exhibit biochemical peculiarities, several of which of pharmacological importance. They present a single mitochondrion genome organized into a network, called Kinetoplast DNA (kDNA), which is considered a highly selective target for the design of new antitrypanosomal compounds. In fact, heterocyclic cations are DNA binders that display considerable biological activity with low toxicity against several microorganisms. Testing on clinically relevant forms of *Trypanosoma cruzi* (bloodstream forms and intracellular amastigotes), we are determining their *in vitro* tripanocidal activity and sub-cellular localization, besides evaluating the toxicity against mammalian cells, such as embryonic cardiomyocytes. Up to now, our results compiled from the activity of 11 different compounds upon bloodstream trypomastigotes (Y strain) and cardiac cells *in vitro* allowed subdivide these drugs in two major groups regarding their respective selectivity index profiles (IS). In the first group we have drugs with IS ranging from 30 to 180 and on the second, between <3 up to 6. Further *in vitro* analyses are under way and the early data suggest that at the first group behave as good candidates for further *in vivo* analysis. Due to their intrinsic fluorescence, we followed the uptake and accumulation of these compounds within the parasite and found that they accumulate in the nuclei and KDNA, the latter showing higher labeling, which is suggestive of drug activity upon the mitochondrion of the parasite. Further analyses are under way to determine the precise mechanism of action of heterocyclic dications to contribute to the rational design of new compound for Chagas Disease chemotherapy.

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QT46 - NITRIC OXIDE DONOR (*trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$) AND BENZNIDAZOLE: A NEW THERAPEUTIC PERSPECTIVE TO CHAGAS DISEASE

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Several NO donors have been used as therapeutic agents in many diseases, which release NO when activated by biological reducing agents. NO may be produced by activated macrophages and destroys intracellular parasites. Here, we investigated the *in vitro* and *in vivo* activity of *trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$ (NO donor) against *T. cruzi*. *Trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$ was incubated with a partially drug-resistant *T. cruzi* Y strain and the anti-proliferative and trypanocidal activities were evaluated. Mice were treated in the acute phase of Chagas disease and anti-*T. cruzi* activity was evaluated by the capacity of the compound to reduce parasitemia, survival rate, cardiac parasitism and inflammation, as well as cure rate. We observed similar or higher activity of the compound in vitro, when compared to benznidazole, against extra (epimastigotes and trypomastigotes) and intracellular (amastigotes) forms of the parasite, at 0.1 mM, 0.5 mM and 1.0 mM concentrations. Treatment of mice using *trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$ at 1.0 mM through 20 consecutive days suppressed the parasitemia, protected 100% of the animals from mortality, and reduced inflammation and cardiac parasitism. We observed that *trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$, benznidazole, and *trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$ added to benznidazole, presented cure rates of 20%, 40% and 80%, respectively. The treatment protocol with the association of benznidazole and *trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$ enhanced cure rate. In conclusion, our results demonstrate that *trans*-[RuCl([15]aneN$_4$)NO]$^{2+}$ has trypanocidal activity *in vitro* and *in vivo* and is a promissory drug for Chagas disease therapeutic. Strategies to enhance the period of NO release, as nanoparticules, associated with benznidazole treatment, may enhance the effectiveness of therapeutic schedule.

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QT47 - Effects of Actin and Tubulin Inhibitors on intracellular infection of *Trypanosoma cruzi*

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Trypanosoma cruzi, is the ethiological agent of Chagas disease and it is estimated that is afflicts 16-18 million people in the Americas. Microfilaments were never observed in the cytoplasm of *T. cruzi*. However, actin, the main component of microfilaments, was identified in *T. cruzi* by immunofluorescence microscopy and by biochemical studies. Microtubules, the most important citoskeletal component of trypanosomatids, are distributed along all the cell body immediately under the plasma membrane (subpellicular microtubules). We report here the effects of two inhibitors of cytoskeleton components, cytochalasin D (microfilament depolimerizer) and nocodazole (microtubule depolimerizer), on the intracellular infection of *T. cruzi* (Y strain). When treated with cythocalasin D, the intracellular infection is significantly reduced after 24 hours of treatment. During the whole treatment (96 hours – corresponding to 144 hours of infection), the infection is always smaller in the treated groups than in the control, however it does not seem to be dose dependent. With regards to the intracellular development, the drug affects the intracellular multiplication of the parasites only when the cells are treated with 2.0 µM cytochalasin D during the first 24 hours of treatment. When the cells were treated with nocodazole, the infection was reduced but not as much as with the first drug. The intracellular development of the amastigotes was not affected by this drug. The effect of cytochalasin D on the host cell has not been observed at the concentrations tested. Even though cytochalasin D had some effect on the intracellular parasites, higher concentrations of the drug were not tested yet. Therefore, further tests are still needed to observe its effects more thoroughly with the aid of ultrastructural analysis through the use of transmission electron microscopy techniques.

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QT48 - Effects of Protein Kinase Inhibitors on the Intracellular Development of Amastigotes of *Trypanosoma cruzi*

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Little is known about the function of PKs in parasites and the occurrence of changes in protein phosphorylation during parasite life cycles suggests that these enzymes play important roles in parasite virulence, differentiation and cell division. An increasing number of PKs of parasitic protozoa are being evaluated as drug targets. Some inhibitors of PKs have been shown to display antiproliferative effects on the protozoa. We report here the effects of staurosporin (STA) (inhibitor of serin/threonin kinase) and wortmannin (WOR) (phosphatidylinositol 3' (PI_3) kinase inhibitor) on the intracellular development of amastigotes of *Trypanosoma cruzi*. The effect of this drug was previously studied on epimastigotes and the IC50 was 6.43 uM. The rate of infection was reduced by both drugs, and most drastically by wortmannin. These drugs did not interfere with the division of intracellular amastigotes or with its differentiation to trypomastigotes. However as trypanosomes have kinomes that contain a large set of protein kinases and phosphatases, PKs should not be discarded as an important target for chemotherapy of Chagas disease.

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