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Biologist by the Faculty of Natural and Exact Sciences, National University of Asunción (Paraguay) and PhD in Microbiology and Parasitology, by the Faculty of Pharmacy, Complutense University of Madrid (Spain). After completing the doctoral thesis in Spain returned to Paraguay to implement the research line developed during the doctoral thesis, in Pharmacological Screening of chemical compounds on the parasite Trypanosoma cruzi, in the Center for Scientific Research Development (CEDIC) and at the National University of Asuncion. Currently the research focuses on determining in vitro *and* in vivo *leishmanicidal and trypanocidal activity of chemical or natural compounds, and the cytotoxicity on mammalian cell lines.*

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ISID Small Grant Report

In vitro assays of 2,5-dihidroxibencil derivatives on *Trypanosoma cruzi* and *Leishmania donovani*

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Introduction

The effort to find more effective and affordable forms of treatment for Chagas disease and Leishmaniasis is framed in the priority list of the WHO. Currently, drugs used to treat these diseases are seriously limited given the following reasons: they are expensive and efficaciously questionable; they require prolonged treatment and regular medical supervision; and, they have significant side effects. The search for new drugs is needed to help treatment efforts in Latin America. Conservative figures show that 8 million people are infected with Chagas disease, including the 50,000 people who die annually. Moreover, for leishmaniasis, an estimated 12 million people are infected worldwide, and 350 million people are at risk of acquiring Leishmaniasis (1). Leishmaniasis is a vector-borne disease that affects 72 countries in total; 13 of these are least developed countries. Visceral Leishmaniasis (VL) is the most severe form of Leishmania infections and it annual incidence is estimated 500,000 new cases and 60,000 deaths occur each year (2, 3).

For these reason, the main objective of this project was to establish a sequential and rationale screening of new compounds by using in vitro assays on *T. cruzi* and *L. donovani* to decrease the access time in the development of new antiprotozoan drugs. The potent *in vitro* and *in vivo* pharmacological activity showed for 2 out of 14 phenolic derivatives, with high activity on *T. cruzi* and *Leishmania sp* and low toxicity on mammalian cells (Grant BID/FAPEP 1691-OC/PR-CONACYT-Paraguay) motivated this proposal. These 2,5-dihydroxybibencyl derivatives compounds have served as trypanocidal and leishmanicidal lead compound and new twelve derivatives were synthesized for their evaluation to obtain a small library of active and non-toxic compounds.

Materials and Methods

1. *Chemistry.* In the library design, we selected the 2,5-dihydroxibenzyl unit as active structure for combinatorial derivatization (4).

2. Epimastigote susceptibility assay: The screening assay was performed in 96-well microplates with CL-B5 of *T. cruzi* epimastigotes cultures. Epimastigotes were seeded at 1 x 10^5 per milliliter in 200 µl. The plates were then incubated with the drugs at 28°C for 72 hours, at which time 50 µl of CPRG solution was added. The plates were incubated at 37°C for an additional 4 h and were then read at 595 nm (5).

3. Promastigote susceptibility assay: The assay was performed using *L. donovani* (MHOM/IN/80/DD8) promastigotes. Promastigotes (2.5x10^5 parasites/well) was cultured in 96-well plastic plates. Different dilutions of the compounds up to 200 µL final volume were added. After 72 h at 26°C, 20 µL of 2.5 mM resazurin solution was added and the oxidation-reduction was quantified at 570 and 595 nm (6).

4. *Cytotoxicity assays:* The cell lines used were NCTC clone 929 and murine J774 macrophages. The procedure for cell viability measurement is evaluated with resazurin (19) by a colorimetric method (7).

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Results and Discussion

Twelve new hydroxybenzyl derivatives compounds, called AP24, AP28, AP29, AP30, AP31, AP32, AP38, AP40, AP43, AP44, AP45, and AP47 were synthesized and tested on parasites of *T. cruzi* epimastigotes and *L. donovani* promastigotes and macrophages and fibroblast cell lines. These compounds are 2,5 dihidroxybibencyl derivatives and present different substituents, divided into five groups: AP24 and AP28 are hydroxyphenyl derivatives; AP29 and AP31 are alkyloxyphenyl derivatives; AP30, AP32, AP38, and AP47 are alkyl derivatives; AP43, AP44 and AP45 are halophenyl derivatives; and AP40 is a pyridyl derivative.

The new compounds present different substituents such as alkyl, hydroxyphenyl, alkyloxyphenyl, and halophenyl groups. The halophenyl derivatives were more active on *T. cruzi*, particulary AP44, a fluorophenyl derivative, with IC50=38 μM, CC50=351 μM and SI= 9. Only the halophenyl derivative AP45 showed a selective activity on *L. donovani* parasites with IC= 26 μM, CC50= 91 μM and SI= 4. All compounds were less toxic on J774 macrophage and NCTC 929 cells, except the alkyl derivative AP47 that presented higher toxicity than both reference drugs. Six out of twelve compounds assayed showed trypanocidal activity and one of them also has leishmanicidal activity.

Conclusions

Trypanocidal and leishmanicidal activity of twelve new dihidroxybencyl derivatives were tested *in vitro*, six of them showed trypanocidal activity and one also presented a leishmanicidal activity. Three of these compounds are halophenyl derivative (AP43, AP44, AP45), and the other are alkyloxyphenil (AP31), alkyl (AP38) and pyridyl (AP40) derivatives.

In view of the results obtained, the tested compounds are very promising and should advance to the next stage of pharmacological screening in order to assess their potential activity against *in vitro* intracellular amastigotes.

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