The benzodiazepine class of compounds as a potential for the treatment of cutaneous leishmaniasis

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Introduction

Cutaneous leishmaniasis is a parasitic disease caused by a vector-borne intramacrophage parasite belonging to the Kinetoplastid order of protozoan parasites. The disease endemicity extends to over 88 countries including regions in the tropics and neotropics. Parasites are transmitted to humans through a bite if an infected sandfly. Causative agents of the cutaneous form of the disease include Old and New world species based on transmission by their vector hosts, Phlebotomus and Lutzomyia, respectively. Different *Leishmania spp.* are known to infect macrophages and dendritic cells of the host immune system, resulting in symptoms that include disfiguring cutaneous and mucocutaneous lesions, widespread destruction of mucous membranes and visceral disease affecting haemopoietic organs.

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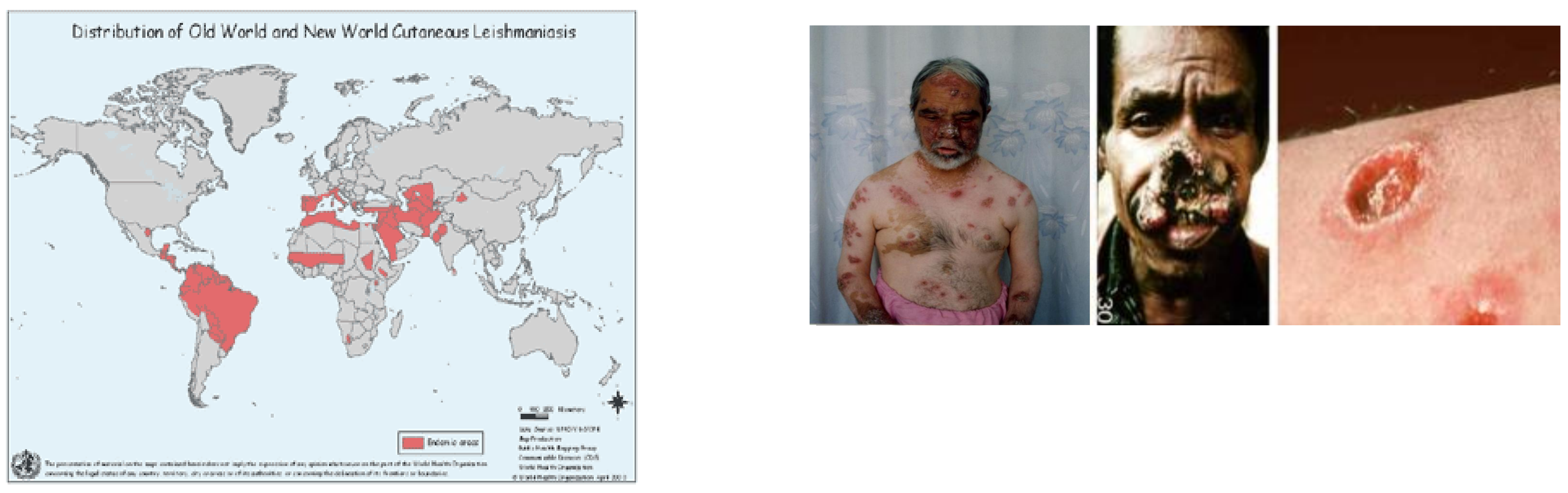

Figure 1. (A) Distribution of Old World and New World cutaneous leishmaniasis . Taken from www.who.int/entity/leishmaniasis/burden/geo_d...
(B) **Mucocutaneous and cutaneous leishmaniasis.** Taken from wiz2.pharm.wayne.edu/module/leishmania.jpg

Current chemotherapeutic options are limited as a result of increased widespread resistance to current mainstays and the requirement of long periods of treatment resulting in serious side effects, including cardiac and renal toxicity. Moreover, most classes of antileishmanial drugs (sodium stibogluconate, *N*-methylglucamine antimoniate, pentamidine and amphotericin B) are not effective when administered orally. The above has prompted the need for the development of new classes of effective antileishmanial drugs.

The current study explores the benzodiazepine class of inhibitors as a potential for the development of antileishmanial drugs. This class of drugs are psychoactive drugs known to have tranquilising effects and are used to treat anxiety, epileptic seizures, mania, alcohol withdrawals and insomnia. Benzodiazepines exert their neurotransmitter activity by binding to the gamma sub-unit of the GABA-A receptor, inducing an allosteric change that results in an increase in receptor activity leading to an increase in chloride ion conductance and inhibition of action potential in nerve cells.

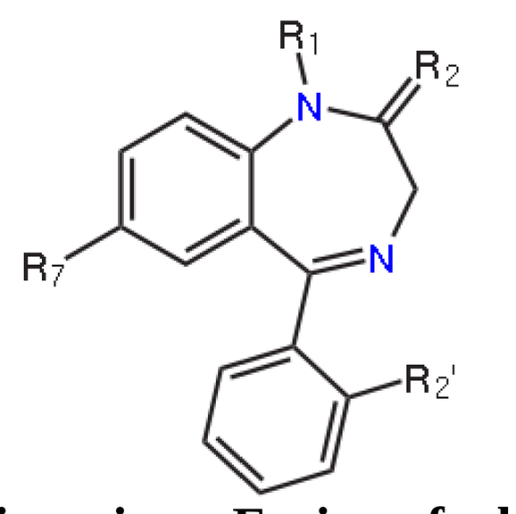

Figure 2. Core structure of Benzodiazepines: Fusion of a benzene and a diazepine ring.
"R" labels denote common locations of side chains, which give differentbenzodiazepines their unique properties.

Methods

Promastigotes: Inhibition studies were performed on *L. aethiopica* (MHOM/ET/72/L100), *L. tropica* (MHOM/SU/58/OD). *L. mexicana* and *L. major* (MHOM/SU/73/5ASKH). Promastigotes of all 4 species were cultured at 24°C in complete Schneider medium supplemented with 10% fetal calf serum and 1x Penicillin/Streptomycin/ Glutamate, till late stationary phase.

Leishmanicidal activity assay: Inhibitors were solubilised in 100% DMSO, diluted in liquid culture medium with final DMSO concentration maintained at 0.05% w/v. Parasites were plated at a density of $1x10^6$/ml in triplicate in a 96-well late at a final volume of 200 µl inclusive of plant-based inhibitors. Plates were incubated for 24 hrs at 24 °C. Inhibition of parasite growth was detrmined using the MTS assay.

MTS assay: MTS (2mg/ml) solubilised in PBS, pH 7.2, was combined with PMS (Phenazine methosulphate,0.92mg/ml) solubilised in the same buffer, in a 5:1 ratio in the dark[1]. Approximately, 20 µl ,of this mixture was added to each well containing 200 µl of treated and untreated *Leishmania spp*. Medium containing drug alone (no parasites) were included as negative control to normalise for background activity. Cultures were incubated for 3 hrs at 37 °C. The absorbance was measured at 490nm and the percentage of viability was calculated as described[1].

Results

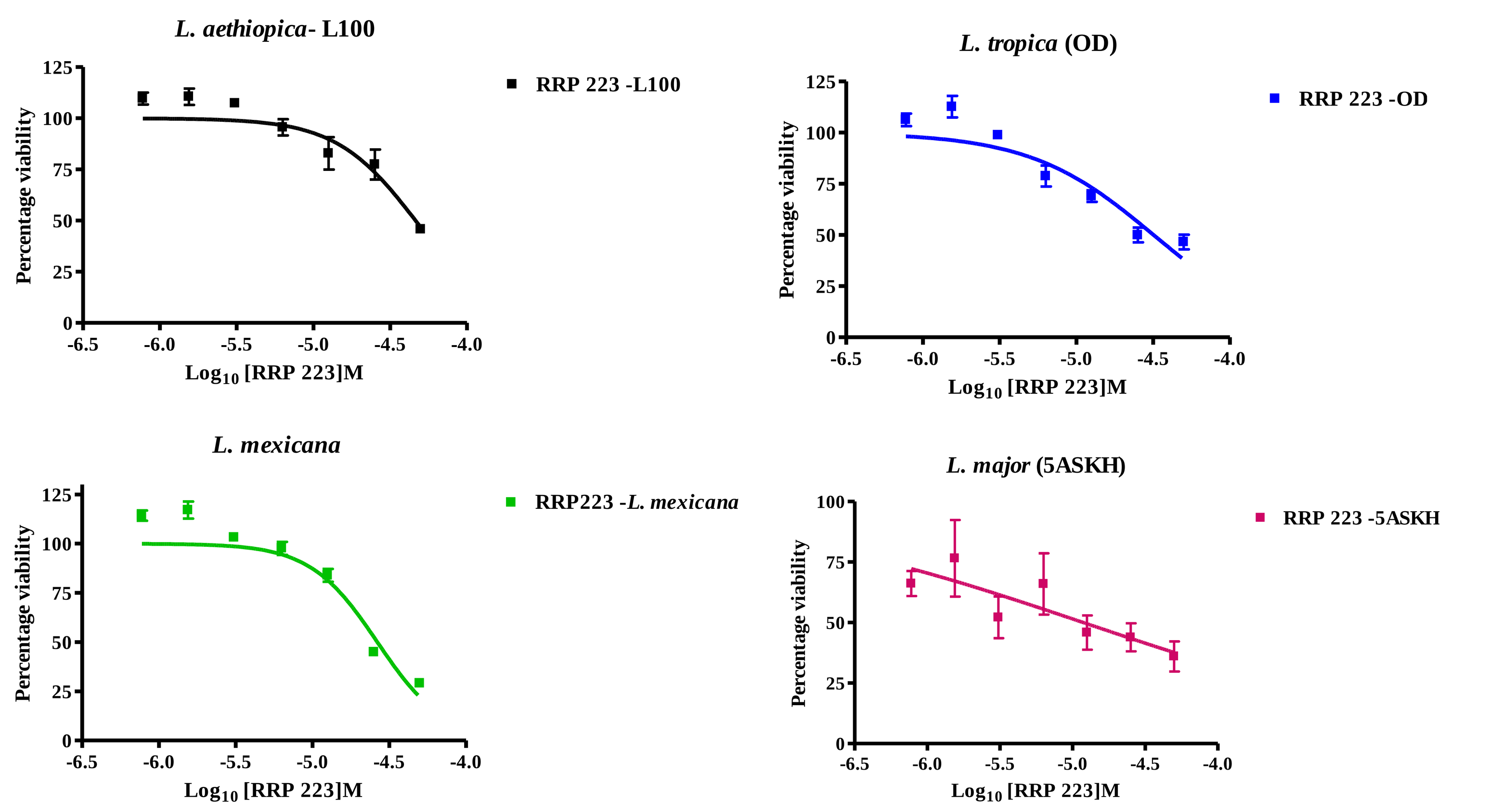

Figure 3. Dose response curves of RRP 223 on *Leishmania spp*.
Parasites were assayed over a range (0.78-50µM) of RRP 223 concentrations. Results for each species shown are a representation of a single experiment repeated in triplicate. Results are expressed as a mean for triplicate wells for each concentration and error bars represent standard deviation. Inhibitor RRP 223 is shown as an example of seven other inhibitors tested this way.

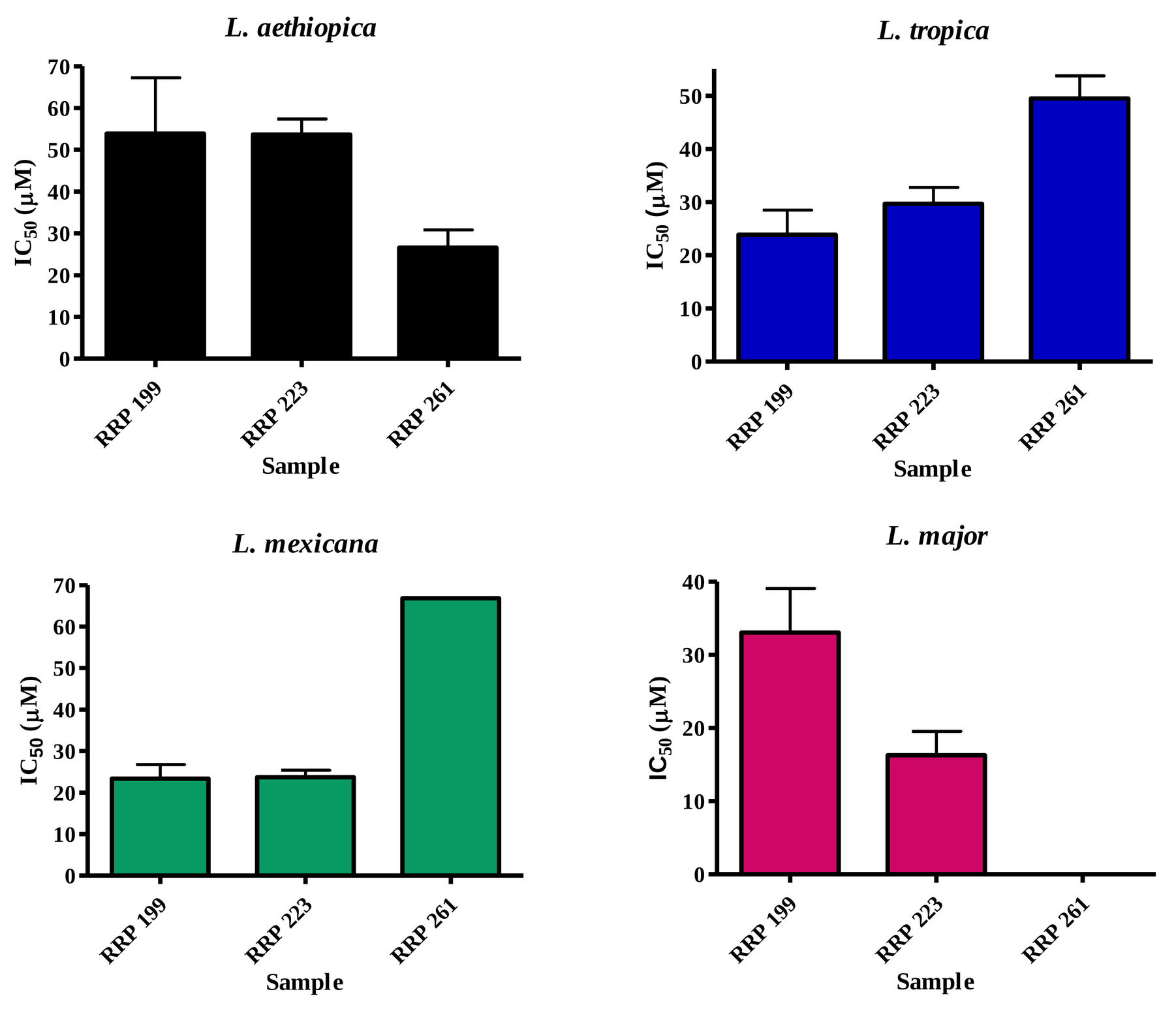

Figure 4. IC_{50} data of RRP series of inhibitors. Standard error bars of the mean(SEM) shown for average of three IC_{50} values expressed in µM. Where error bars are not shown, IC_{50} values are shown from individual experiments.

Conclusion

Three inhibitors (RRP 199, RRP 223 and RRP 261) out of seven displayed antileishmanial activity on promastigote stages of *Leishmania spp*.

RRP 223 exhibited potent activity on *L. major*.

RRP 261 is not inhibitory to *L. major* promastigote growth in the micromolar range.

Levels of antiparasitic activity of the benzodiazepine class of compounds are species specific.

Acknowledgements

References

1. Getti, G., Durgadoss P., Dominguez-Carmona, D., Martin-Quintal, Z., Peraza-Sanchez, S., Pena-Rodriguez, L. M., and Humber, D. 2009. Leishmanicidal activity of Yucatecan medicinal plants on Leishmania species responsible for cutaneous leishmaniasis.J. Parasitol. 95(2): 456-460