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In vitro evaluation and in vivo efficacy of nitroimidazole-sulfanyl ethyl derivatives against *Leismania* (*V.*) *braziliensis* and *Leishmania* (*L.*) *mexicana*

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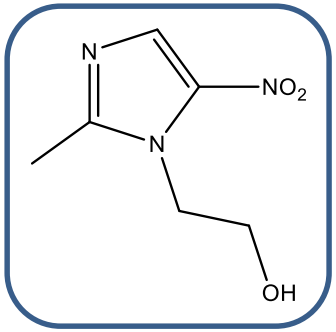
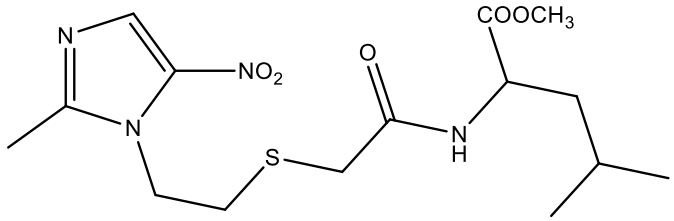
Graphical Abstract

Metronidazole: Drug Repositioning





Optimization against Leishmania spp.
L. (V) braziliensis DL50 21 ug/mL
L. (L) mexicana DL50 18 ug/mL

Mice infected with Leishmania species:
- ***Reduced toxicity***
- ***Less size of lesions***
- ***Longer survival time***

Abstract:
The aim of this study was the synthesis of several small molecules of the type nitroimidazole-sulfanyl, and the evaluation of the biological properties against the main species that cause cutaneus leishmaniasis in Venezuela. Final compounds (**4**-**7**) were generated through simple nucleophilic substitution of 1-(2- chloroethyl)-2-methyl-5-nitroimidazole **3** with 2-mercaptoethanol, 1-methyl-2-mercaptoethanol and 2-thiolacetic acid derivative. Compound **8** was synthesized via a coupling reaction between **7** and an amino acid. The inhibitory concentrations (IC_{50}) of (**1-8**) against *Leismania* (*V*.) *braziliensis* and (*L*.) *mexicana* in promastigotes and macrophages were determined by *in vitro* activity assays. For the *in vivo* evaluation of the more active compounds **7** and **8**. Balb/c mice were infected with promastigotes of the two species and divided into four groups of ten (10) animals**,** and a control group. Two ways were used to the treatment intramuscular and intralesional. The parasitological diagnosis was determinate by PCR. Considering the defined parameters, compounds **7** and **8** showed *in vitro* and *in vivo* activity against *L*. (*V*.) *braziliensis* and *L*. (*L*.) *mexicana*. These compounds may represent an alternative treatment for this two species, which are the most important, from the epidemiological point of view, to produce cutaneous leishmaniasis in Venezuela.

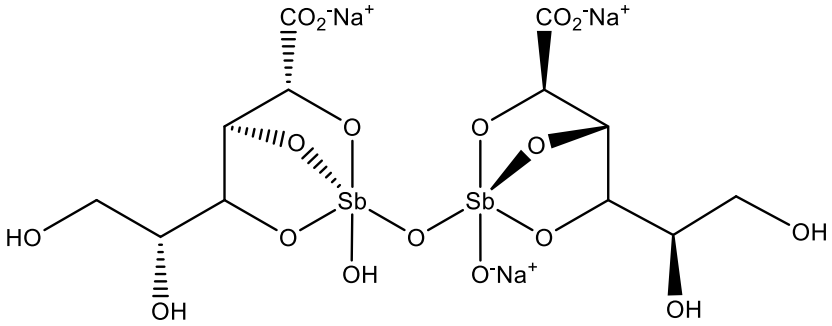
Keywords: *L. braziliensis*; *L. mexicana*; nitroimidazole; synthesis

Introduction

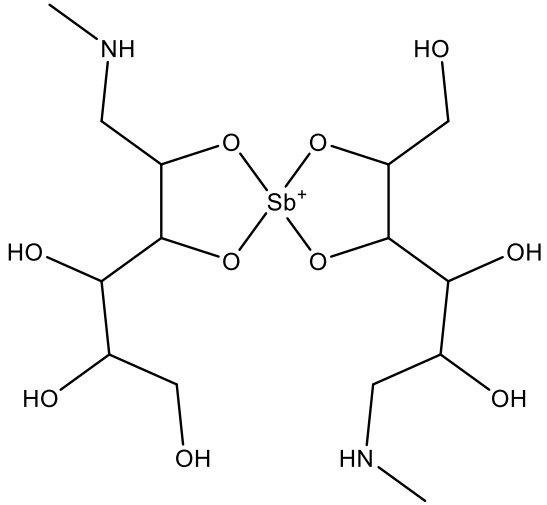
- Leishmaniasis is a neglected tropical disease (NTD) caused by protozoa of the genus *Leishmania.*
- The disease is endemic in 98 countries with an overall prevalence of 12 million cases and an annual mortality rate of more 59.000 deaths.
- Over 20 *Leishmania* species known to be infective to humans are transmitted by the bite of infected female phlebotomine sandflies.
- Three main types of leishmaniasis: visceral (VL), cutaneous (CL), and mucocutaneous (MCL).
- It is estimated that approximately 0.7 to 1.2 million of new CL cases occur each year. [1]

Introduction

➢ The recommended first-line therapies: include pentavalent antimony compounds, such as sodium stibogluconate® and meglutamine antimoniate®. Disadvantages, such as toxicity, high costs.[2-4]



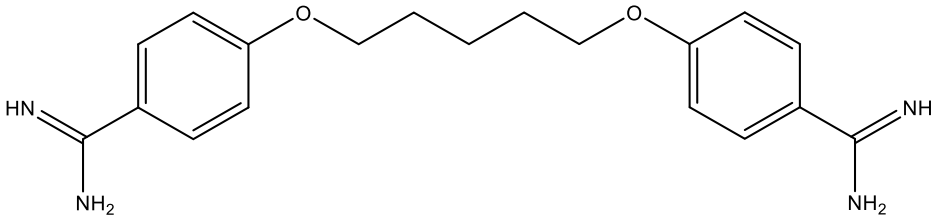
Sodium Stibogluconate



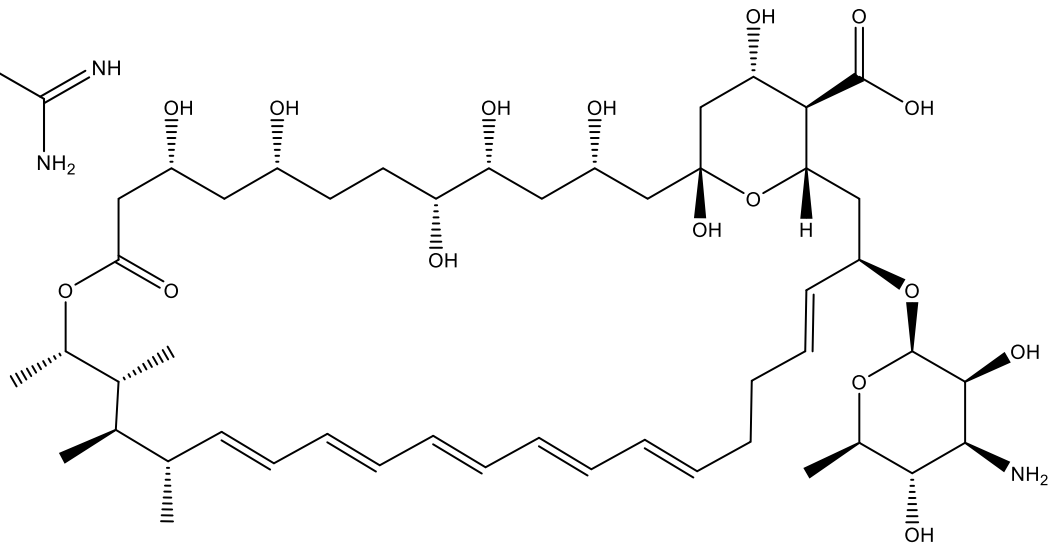
Meglutamine Antimoniate

Introduction

➢ The second-line treatments include pentamidine and amphotericin B, but their use is limited because of toxicity and cost.[2-4]



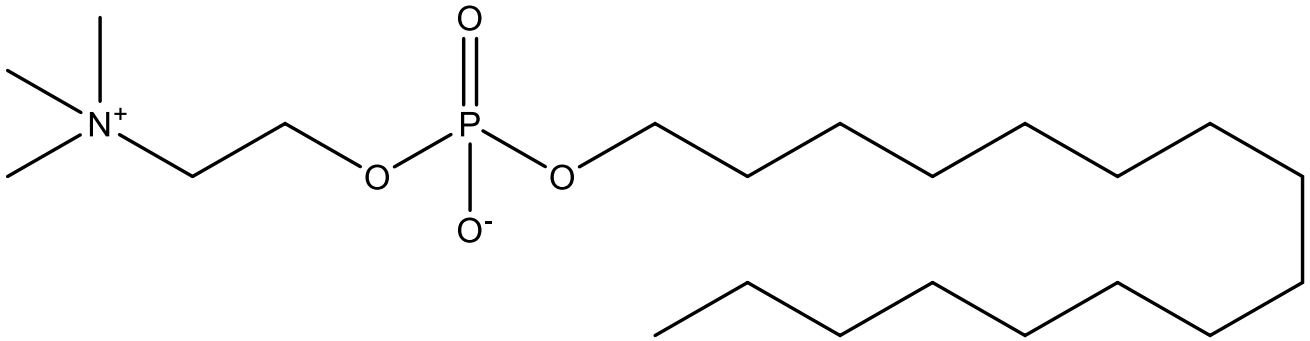
Pentamidine



Amphotericin B

Introduction

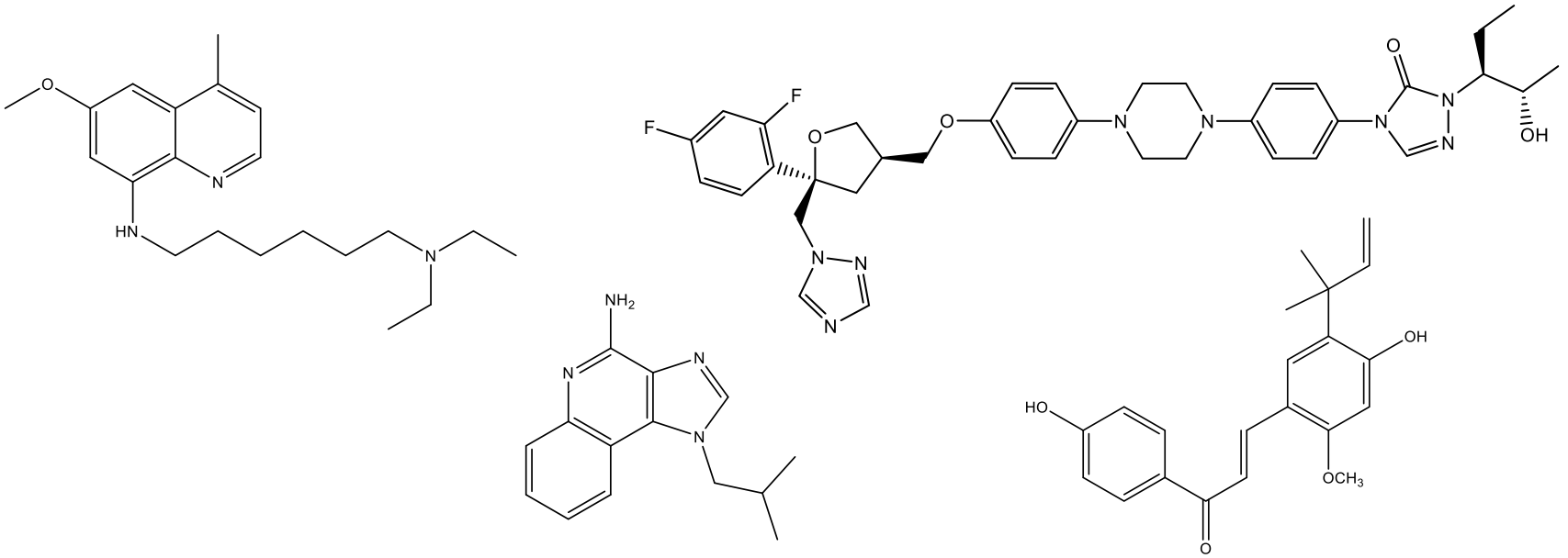
➢ Recently, the oral administration of miltefosine has been used for the treatment of VL in some countries, but despite its great efficacy, miltefosine is not free either from toxicity as it shows teratogenic potential.[2-4]



Miltefosine

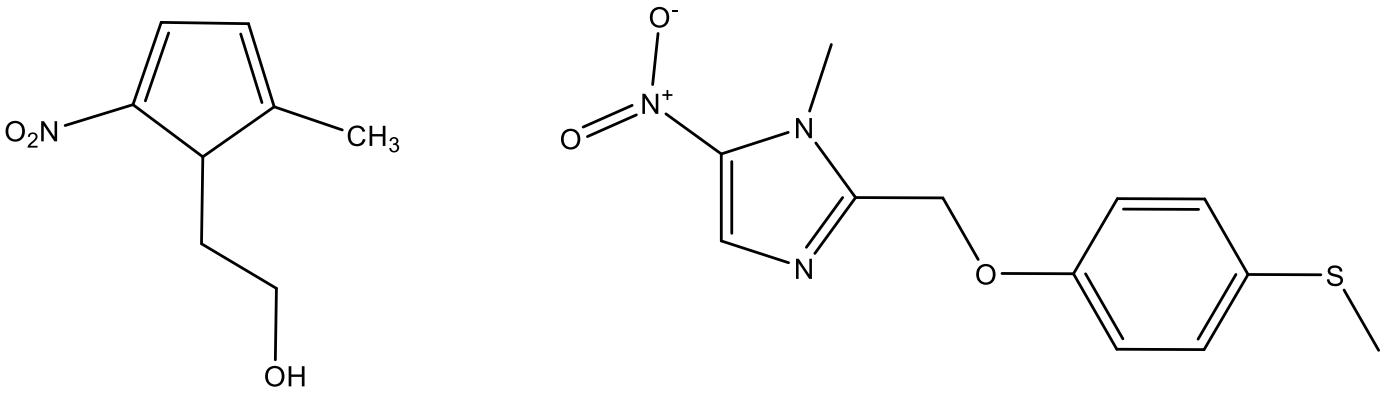
Introduction

➢ Several compounds that show leishmanicidal activity are currently in different stages of development. Among them, a few classes of compounds, such as the sitamaquine,[5] the imiquimod,[6] the posaconazole,[7] as well as some natural product derivatives, such as licochalcone A.[8]



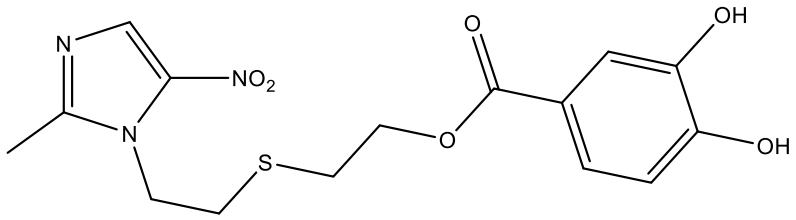
Introduction

➢ In addition, the drawbacks associated with the currently available treatments have led to the development of new strategies aiming at leishmaniasis control. In this context, special attention has been given nitroheteroaromatic scaffolds. In particular, nitrocontaining imidazol like, metronidazole or fexnidazole and its sulfonic metabolite now in clinical trial were shown to have effective antileishmanial efficacy.[9,10]

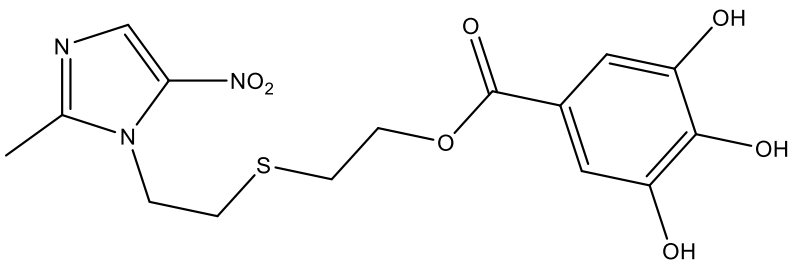


Introduction

➢ The catecholic and pyrogallolic benzoate derivatives **9a** and **9b** were synthesized by our group, the biological results revealed that these two compounds constitute promising candidates in the search for improved therapies against *L. (V.) braziliensis* and *L. (L.) mexicana*.[11]

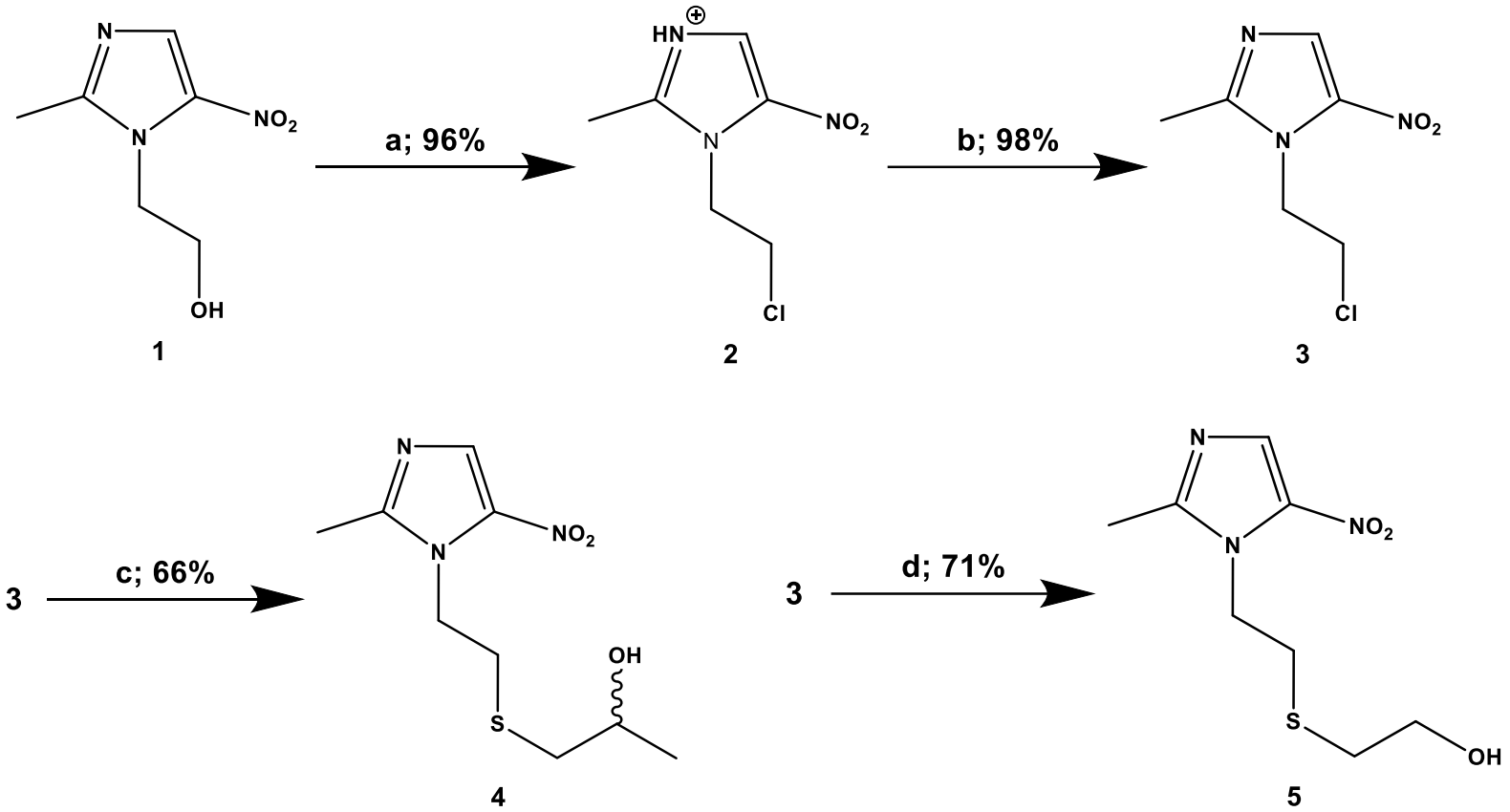


9a



9b

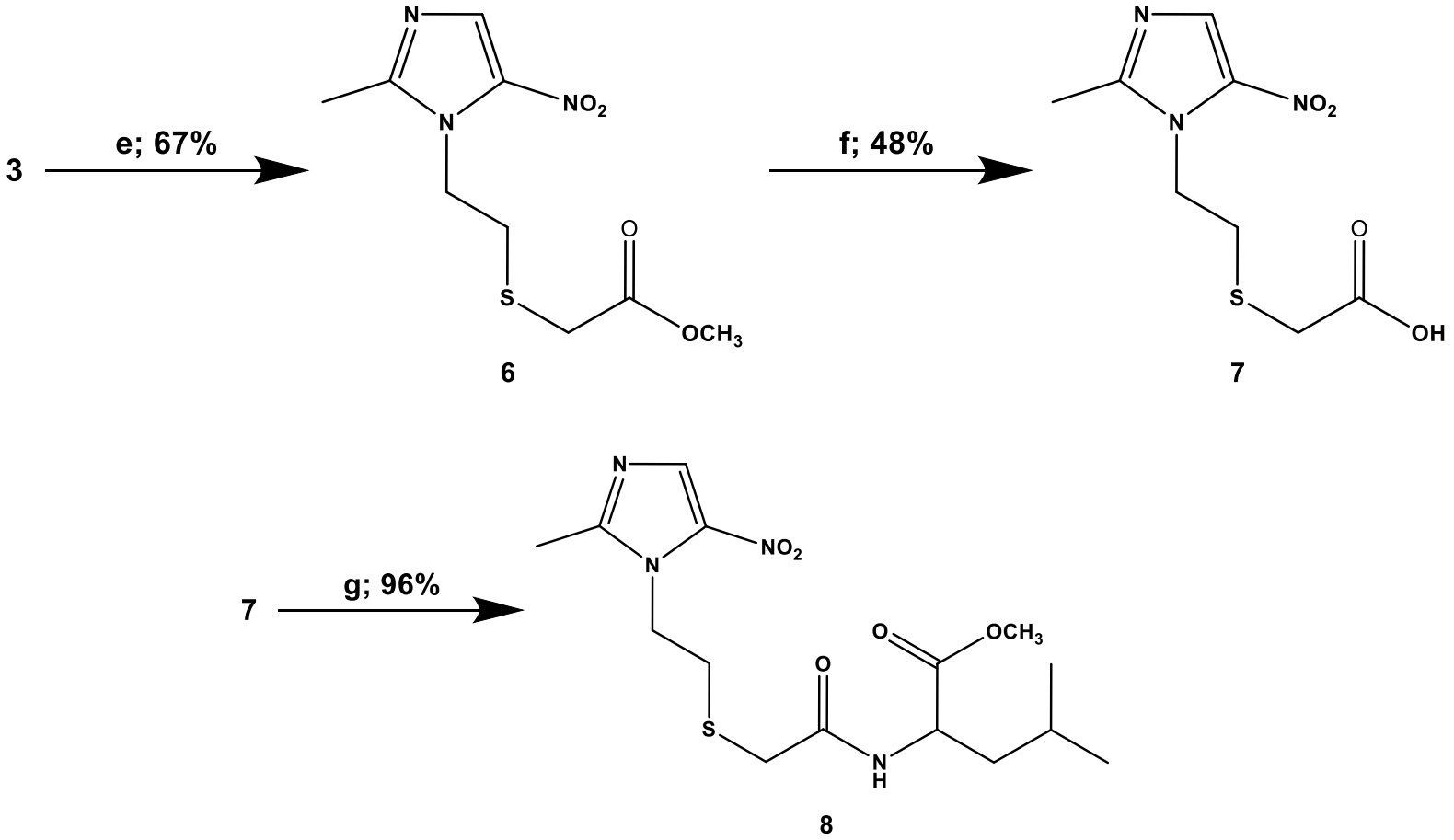
Results and discussion. *Chemistry*



Conditions: a) SOCl2 (5Eq), CH2Cl2, rt, 6h; **b)** H2O, Et3N until pH 8.5, rt; **c)** 2-mercapto-1-propanol (1.2Eq), K2CO3 (3Eq), ACN, reflux, 6h; **d)** 2-mercaptoethanol (1.2Eq), K2CO3 (3Eq), ACN, reflux, 6h.

Results and discussion

Chemistry



Conditions:; **e)** methyl thioglycolate (1.2Eq), K2CO3 (3Eq), ACN, reflux, 4h; **f)** LiOH (1Eq), THF:MeOH:H2O (3:3:1), 0°C, 30 min; **g)** (S)-Methyl 2-amino-4-methylpentanoate hydrochloride (1.2Eq), EDC (1.2Eq), DMAP (0.2Eq), DCM, 0°C, 12h.

Results and discussion. *Biological*

- The synthesized compounds were evaluated for their antileishmanial activity against *in vitro* forms of *L. (V.) braziliensis* and *L. (L.) mexicana* (promastigotes) strains. In order to calculate the LC_{50} two reported methods were used.[11]
- Female Balb/c mice inoculated in the foot pad with promastigotes of *L.(L.)mexicana* or *L.(V.)braziliensis* were treated with compound **7** and **8**.[12]
- The evolution of the lesion size in animals infected after of the treatment was determined as a function of time.
- The PCR methodology was used for parasitologic diagnosis.

Female Balb/c mice, weigth 18-22 g, were maintained on a commercial pellet diet at libitum and under conditions approved by Ethics Committee of the Institute of Biomedicine, Faculty of Medicine, Central University of Venezuela. Glucantime was used as control.

Results and discussion. *Biological*

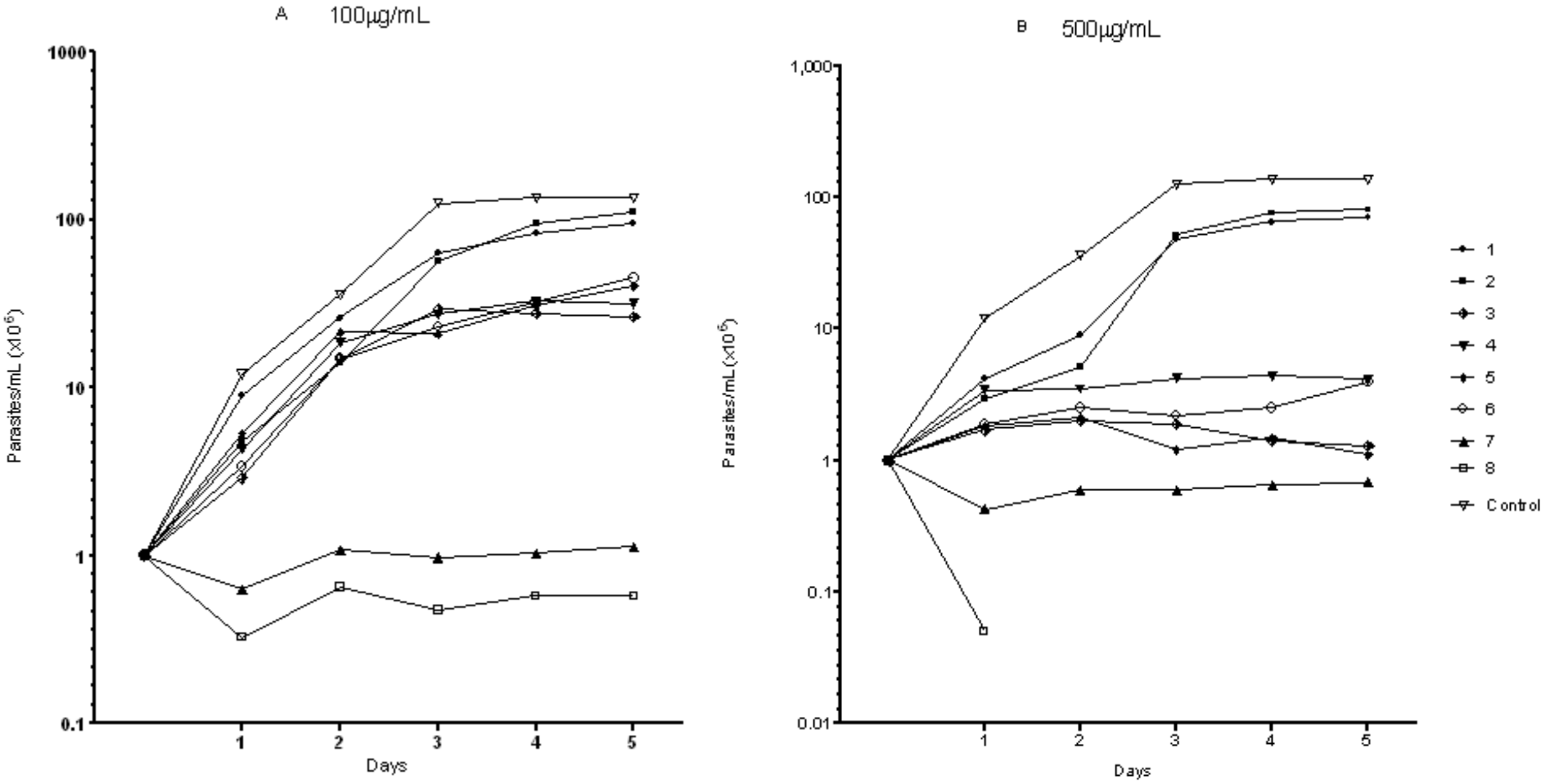


Fig 1.- Promastigotes of ***L.(L.)mexicana*** treated whit compounds 3,4,7,and 8. A (100µg/mL) B (500µg/mL). Parasites were counted every day during 5 days.

Results and discussion. *Biological*

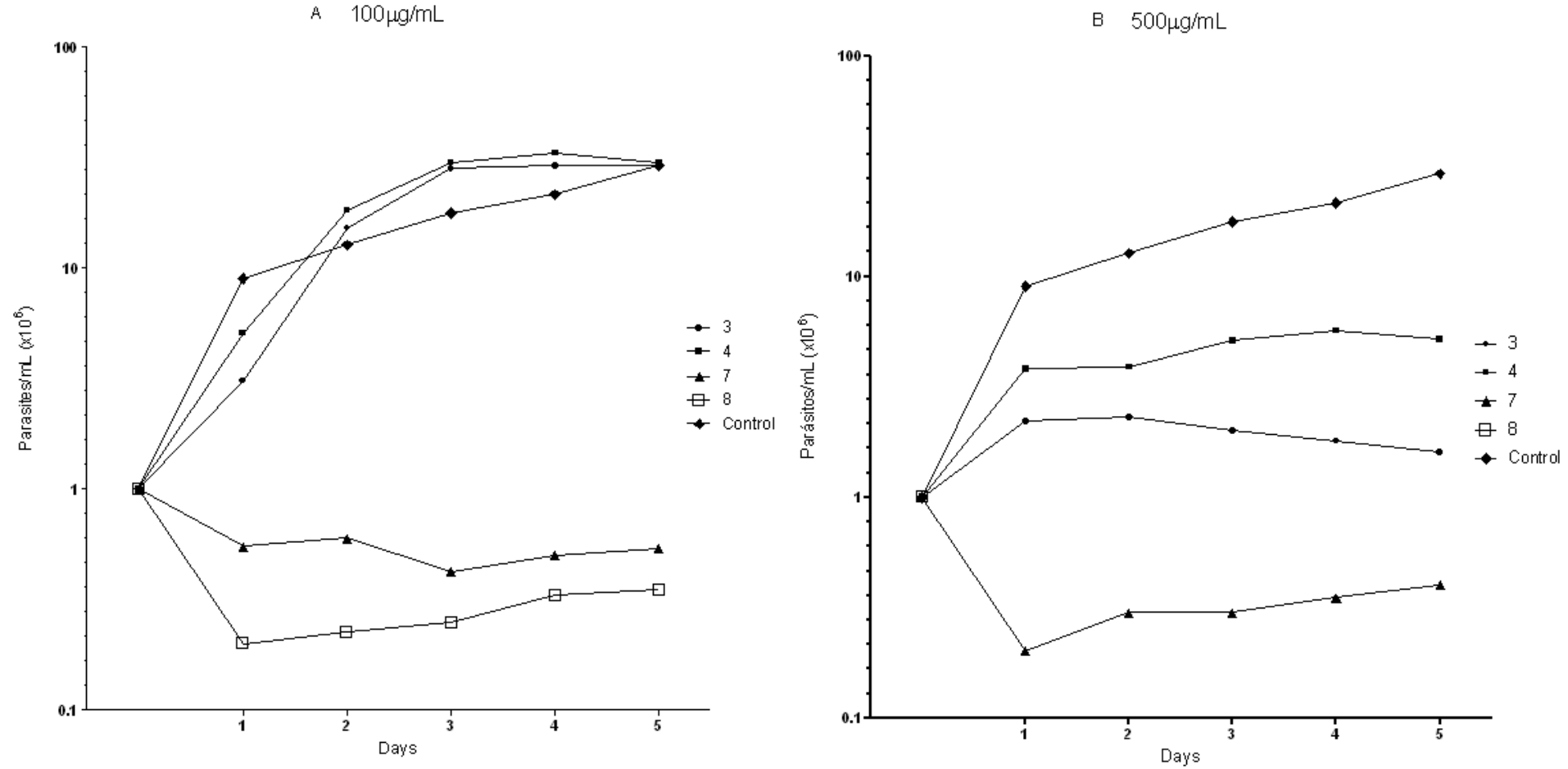


Fig 2.- Promastigotes of ***L.(V.)braziliensis*** treated whit compounds 3,4,7,and 8. A (100µg/mL) B (500µg/mL). Parasites were counted every day during 5 days.

Results and discussion. *Biological*

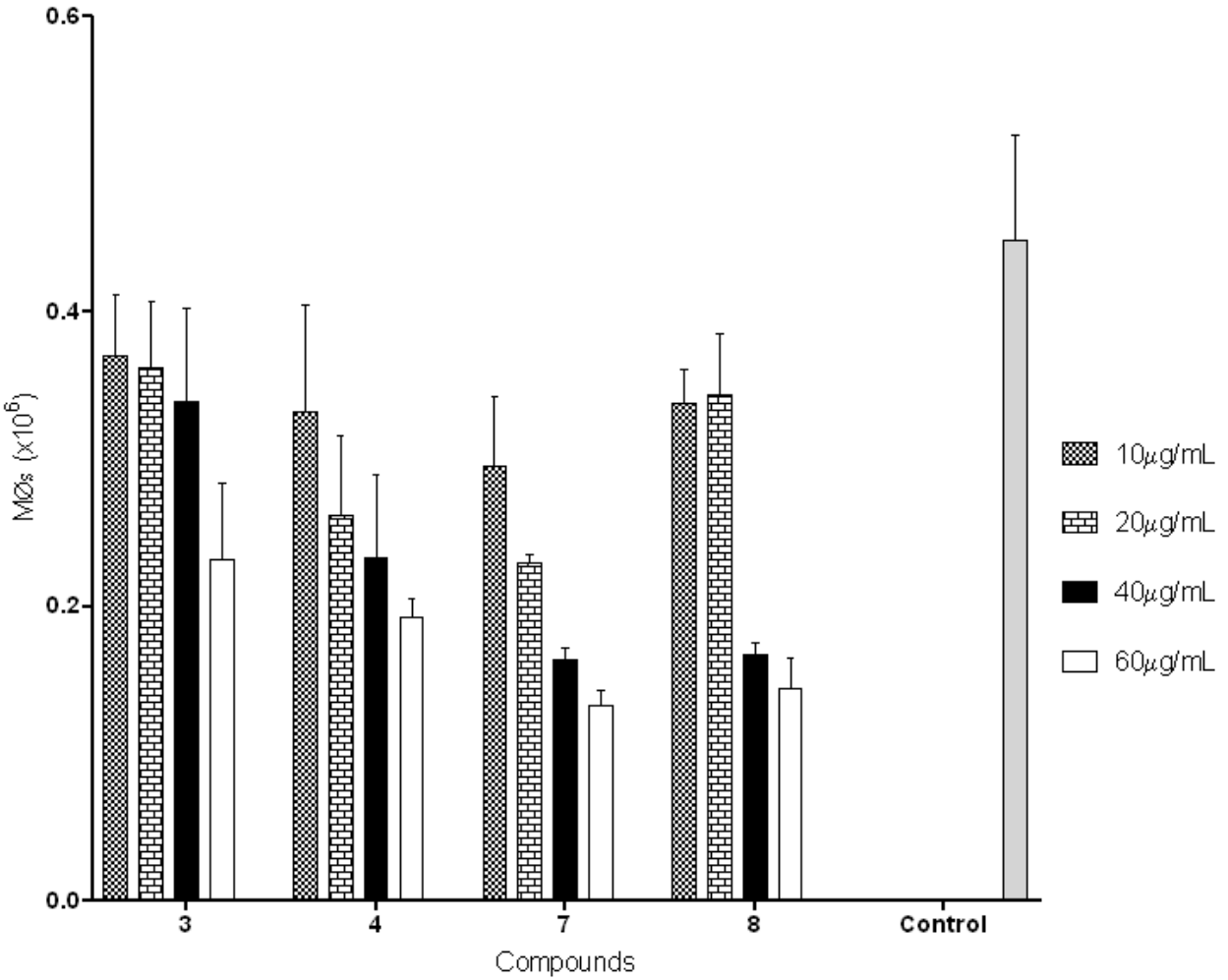


Fig 3.- Macrophages J774-G8 treated with differents concentrations of compounds 3,4,7 and 8.

Results and discussion. *Biological*

A

MØs infected *L.(L.)mexicana*

Compuesto	0 µg/mL DO (570nm)	15 µg/mL DO (570nm)	20 µg/mL DO (570nm)	25 µg/mL DO (570nm)	30 µg/mL DO (570nm)	LC50 µg/mL
3	0,448	0,370	0,363	0,338	0,231	66
4	0,448	0,332	0,264	0,232	0,192	40
7	0,448	0,295	0,230	0,134	0,132	22
8	0,448	0,338	0,343	0,168	0,115	18

B

MØs infected *L.(V.)braziliensis*

Compuesto	0 µg/mL DO (570nm)	15 µg/mL DO (570nm)	20 µg/mL DO (570nm)	25 µg/mL DO (570nm)	30 µg/mL DO (570nm)	LC50 µg/mL
3	0,443	0,390	0,357	0,292	0,240	75
4	0,443	0,350	0,290	0,232	0,201	47
7	0,443	0,310	0,236	0,164	0,145	26
8	0,443	0,285	0,226	0,151	0,130	21

Fig 4.- Macrophages infected with *L.(L.)mexicana* (A) and *L.(V.)braziliensis* (B) and treated with differents concentrations of the compounds. LD_{50} was calculated.

Results and discussion. *Biological*

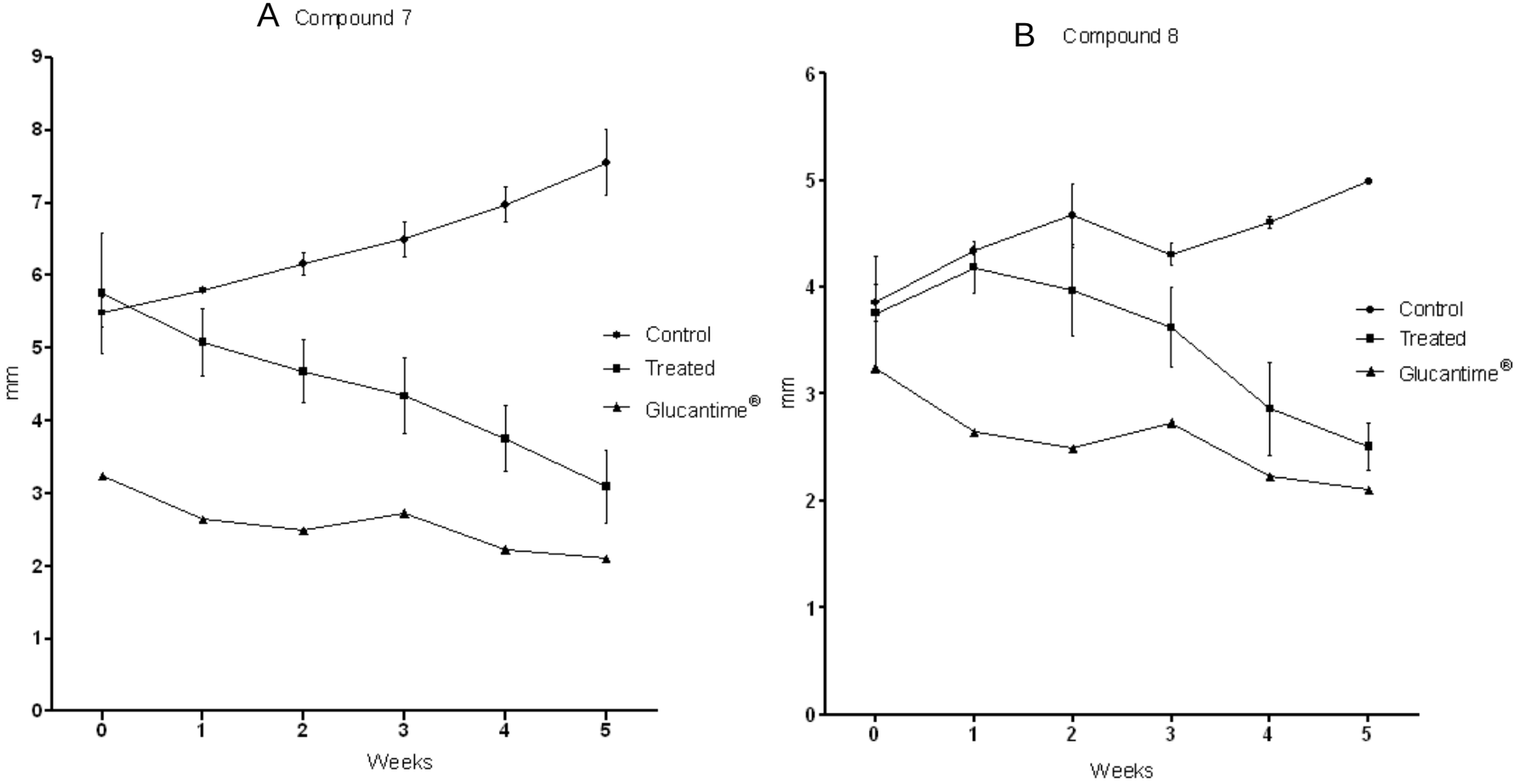


Fig 5.- Balb/c mice inoculated in the foot pad iwith promastigotes of ***L.(L.)mexicana*** and treated with compound 7 (Fig. A) and 8 (Fig. B). Size of the lesion was measured every week during five weeks.

Results and discussion

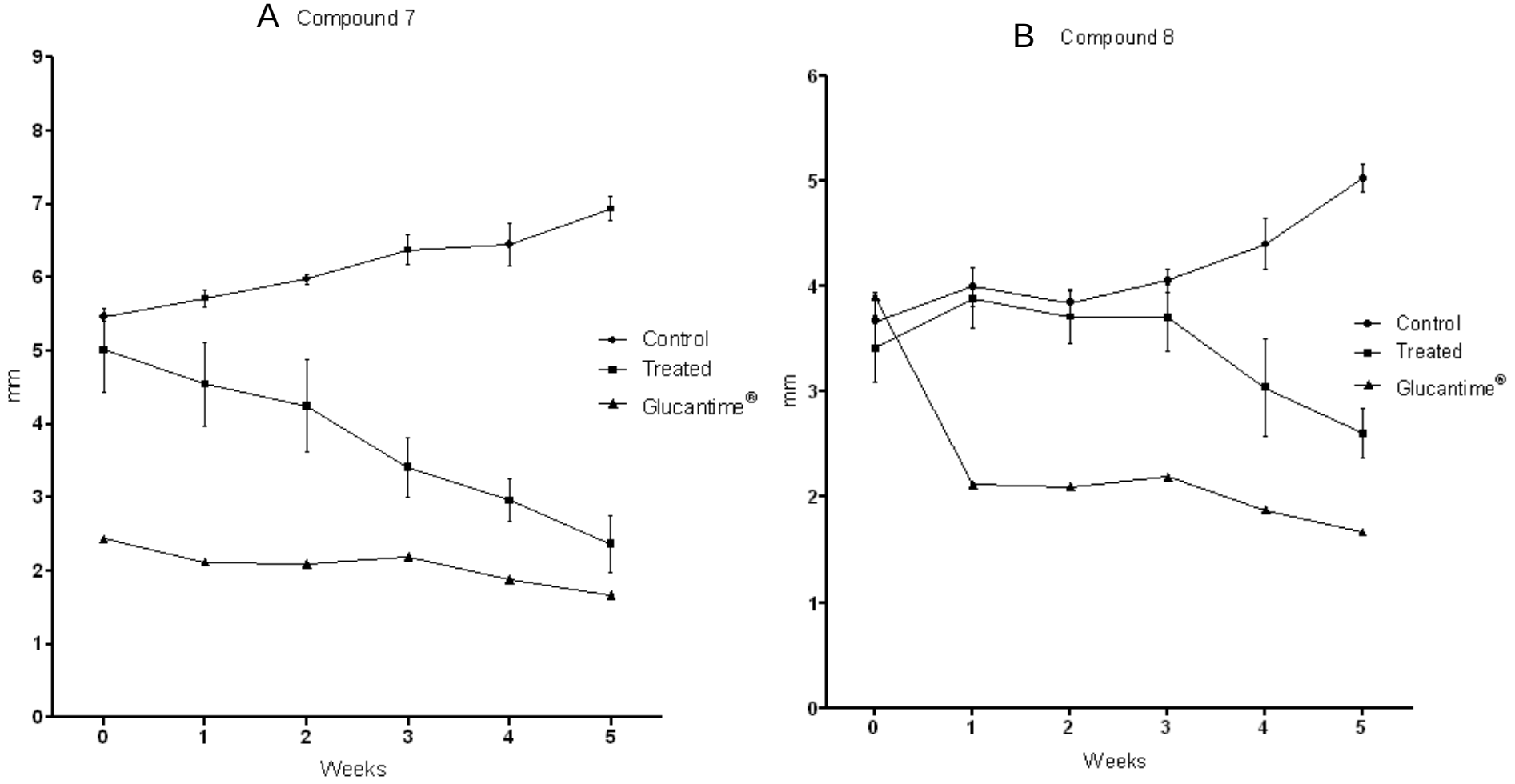


Fig 6.- Balb/c mice inoculated in the foot pad with promastigotes of ***L.(V.)braziliensis*** and treated with compound 7 (Fig. A) and 8 (Fig. B). Size of the lesion was measured every week during five weeks.

Results and discussion

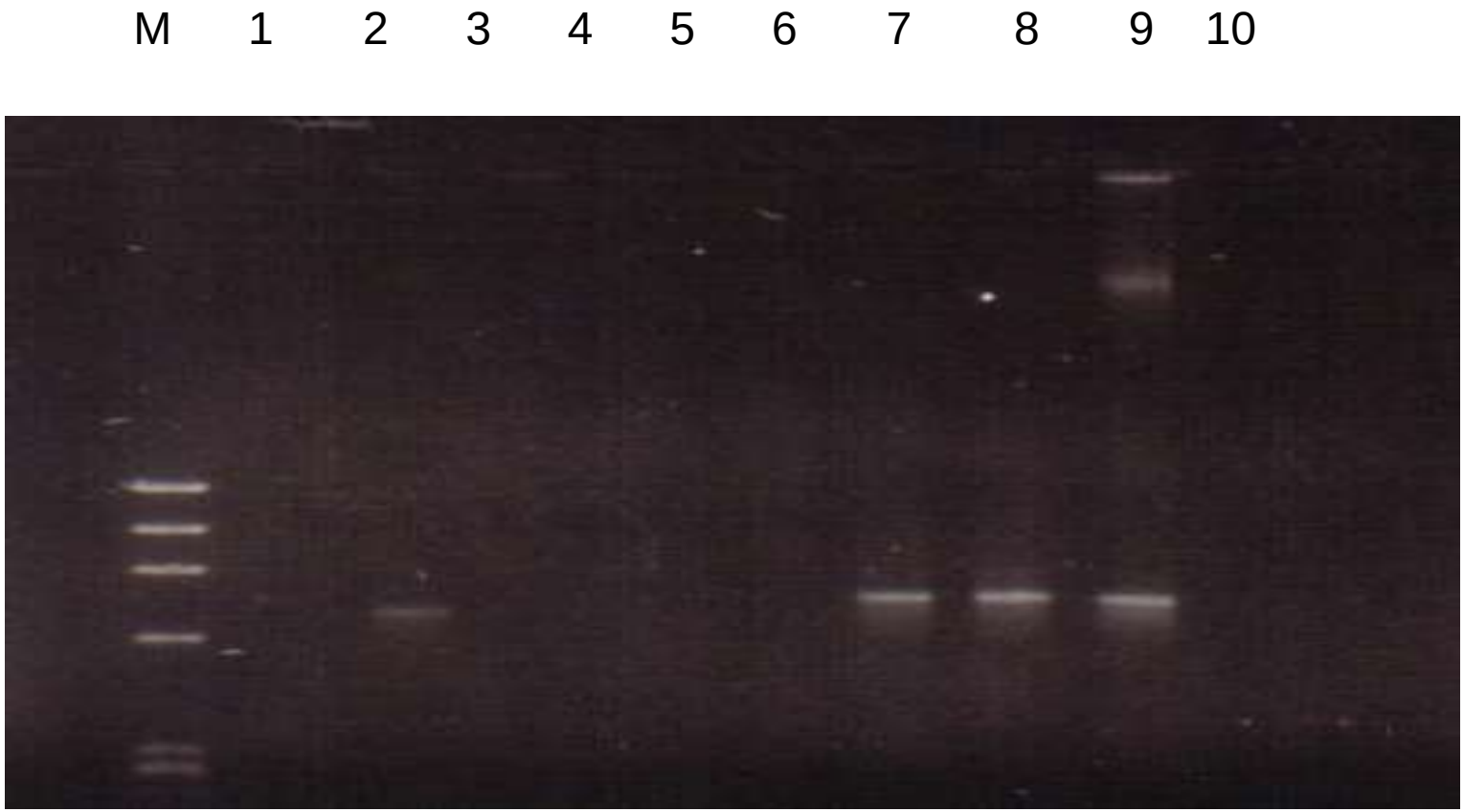


Fig 7.- Pasitological diagnosis using PCR and samples obteined from the food pad of the treated mice. Lanes 2,7,and 8 resulted positives for *L.(V.) braziliensis* and 1,3,4,5 and 6 were negatives with no parasites. Lane 9.- positive control; lane 10 negative control.. M.- Molecular weight marker.

Conclusions

- New metronidazole derivatives endowed with a sulfanyl dridge were designed, synthesized and screened for their activities against the main species that cause cutaneus leishmaniasis in Venezuela.
- The biological results revealed that these two compounds **7** and **8**, showed better activity than metronidazole and the activity was substituent dependent.
- These findings provide us lead and encourage us to continue the efforts towards the optimization of the efficacy profile of this structural moiety for treatment of cutaneus leishmaniasis.
- The combination of these two compounds with other leishmanicidal drugs may lead to dose reduction and an enhancement in their efficacy.

Acknowledgments

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