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Nitazoxanide, tizoxanide and a new analogue [4-nitro-N-(5-nitro-1,3-thiazol-2-yl)benzamide; NTB] inhibit the growth of kinetoplastid parasites (*Trypanosoma cruzi* and *Leishmania mexicana*) *in vitro*

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Sir,
Parasitic diseases such as leishmaniasis and trypanosomiasis, both caused by protozoan parasites of the *Kinetoplastida* order, represent a serious problem to the health and the economy of many developing countries. The *Leishmania* species cause a variety of diseases, from self-healing cutaneous lesions to life-threatening visceral infections. Clinical manifestations depend on the species of the infecting parasites. There are an estimated annual 1.5–2.0 million new cases of leishmaniasis, of which approximately 500000 belong to the visceral form, which is potentially fatal. American trypanosomiasis, or Chagas' disease, is still one of the major causes of morbidity and mortality due to cardiovascular diseases in Latin America.¹

Common chemotherapeutic agents currently used against both diseases are often inadequate since they require long courses of parenteral administration, may have toxic side effects or become less effective due to the emergence of resistant

strains. Therefore, new, effective and inexpensive drugs that can be used to treat these diseases are urgently required.

Nitazoxanide [2-(5-nitrothiazol-2-ylcarbamoyl)phenyl acetate; Alinia[®]] is a broad-spectrum antiparasitic compound belonging to a nitroheterocyclic class named thiazolides.² In humans, nitazoxanide is rapidly metabolized to tizoxanide, which is a compound that is as effective as the parent drug (Figure 1).³ Detailed *in vitro* and *in vivo* studies have been conducted regarding the efficacy of nitazoxanide and other nitroheterocyclic drugs against helminths, extracellular anaerobic protozoa and bacteria, intracellular parasites and viruses.^{4–6}

Due to the limited information regarding the efficacy of nitazoxanide against kinetoplastid parasites such as *Trypanosoma cruzi* and *Leishmania mexicana*, the goal of this work was to show the potential of nitazoxanide and its major metabolite, tizoxanide, as antileishmanial and trypanocidal drugs.

In this work, we evaluated the *in vitro* activity of nitazoxanide, tizoxanide and the newly synthesized analogue 4-nitro-N-(5-nitro-1,3-thiazol-2-yl)benzamide (NTB) (Figure 1) against *T. cruzi* and *L. mexicana*. The activity was compared with the activities of benznidazole and pentamidine, well-known drugs that act against *T. cruzi* and *L. mexicana*, respectively.

Nitazoxanide was synthesized in our laboratory starting from the acylation of 2-amino-5-nitrothiazole with acetylsalicyloyl chloride and triethylamine in methylene chloride as solvent.² An alkaline hydrolysis of nitazoxanide produced tizoxanide in good yields. NTB was synthesized by means of the same procedure, using 4-nitrobenzoyl chloride instead of acetylsalicyloyl chloride. All compounds were characterized by spectroscopic and spectrometric techniques and all data agreed with reported literature values.

The growth inhibition test was performed on promastigotes of *L. mexicana* (MHOM/MX/84/ISSETGS; clinical strain originally isolated from a patient with diffuse cutaneous leishmaniasis) and epimastigotes of *T. cruzi* (MHOM/MX/1994/Ninoa; clinical strain originally isolated from a patient with Chagas' disease in the acute phase). Parasites were cultivated at 26°C in Schneider's *Drosophila* medium, supplemented with 10% fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 µg/mL). Biological assays were performed in 96-well plates and all compounds were evaluated in duplicate. Compounds were solubilized in dimethylsulphoxide and diluted in a liquid medium. Aliquots of 100 µL of compound solution and 100 µL of culture medium containing 10000 *Leishmania* promastigotes or 20000 *T. cruzi*

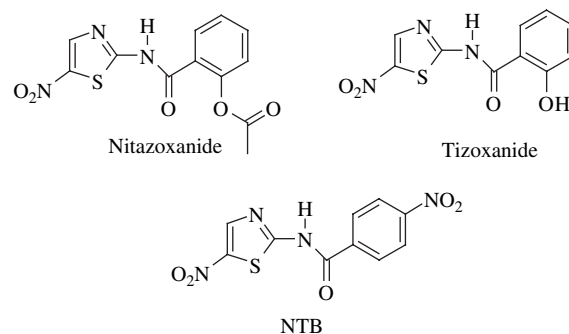


Figure 1. Chemical structures of nitazoxanide, tizoxanide and the analogue NTB.

Table 1. Comparative median inhibitory concentration (IC₅₀) of investigated compounds against kinetoplastid parasites

Antiprotozoal drug	IC ₅₀ (µg/mL)	
	<i>L. mexicana</i> (MHOM/MX/84/ ISETGS)	<i>T. cruzi</i> (MHOM/MX/94/ Ninoa)
Nitazoxanide	1.899 (6.179 µM)	5.757 (18.735 µM)
Tizoxanide	1.641 (6.188 µM)	4.636 (17.478 µM)
NTB	0.745 (2.533 µM)	4.421 (15.021 µM)
Pentamidine	4.532 (13.321 µM)	NA
Benznidazole	NA	8.942 (34.384 µM)

NA, not active.

epimastigotes were added to obtain concentrations of 10, 5, 2.5 and 1.25 µg/mL. Benznidazole (first-line antichagasic drug) and pentamidine (second-line antileishmanial drug) were used as positive controls. Cultures containing parasites without compound solution were also included. Plates were incubated at 26°C for 72 h and the leishmanicidal activity of compounds was determined by direct count of parasites in a Neubauer chamber. The concentrations required to inhibit 50% of parasite growth (IC₅₀) were calculated by probit analysis and are summarized in Table 1.

It is clear from Table 1 that nitazoxanide and tizoxanide reduced the growth of the kinetoplastid parasites *L. mexicana* and *T. cruzi* *in vitro* with IC₅₀s 2-fold lower than those of pentamidine and benznidazole, respectively.

The newly synthesized analogue NTB was 2-fold more potent than nitazoxanide and tizoxanide and 6-fold more active than pentamidine against *L. mexicana*. The *in vitro* bioactivity against *T. cruzi* was similar to that of nitazoxanide and tizoxanide, being 2-fold more potent than that of benznidazole (Table 1).

It is worth noting that nitazoxanide and its major metabolite had similar effects against the protozoa tested. However, the *in vivo* administration of nitazoxanide seems to be more feasible due to its more lipophilic character compared with its hydrolysed product. Also, the phenol moiety in tizoxanide could be an irritant to the gastrointestinal mucous membrane. The analogue NTB was more active than the parent compounds against *L. mexicana*. To the best of our knowledge, this is the first study reporting the *in vitro* activities of nitazoxanide, tizoxanide and the newly synthesized analogue NTB against *L. mexicana* and *T. cruzi*. The recent identification of an unusual NADH-dependent, mitochondrially localized, bacterial-like type I nitroreductase enzyme in trypanosomatids might explain why these parasites are susceptible to these drugs.⁷

Our results suggest that the three structures are a promising source of potentially new anti-kinetoplastid-parasite compounds. Nitazoxanide and tizoxanide could be useful drugs for the treatment of leishmaniasis and trypanosomiasis infections. Additional *in vivo* studies are required to confirm these findings. The chemical synthesis of closely related analogues of nitazoxanide, tizoxanide and NTB, as well as the determination of the corresponding broad antiparasitic activities *in vitro* and *in vivo*, are in progress in our laboratory.

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Transparency declarations

None to declare.

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Evaluation of anti-sleeping-sickness drugs and topoisomerase inhibitors in combination on *Trypanosoma brucei*

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