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Synthesis of benzologues of Nitazoxanide and Tizoxanide: A comparative study of their in vitro broad-spectrum antiprotozoal activity

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ABSTRACT

We have synthesized two new benzologues of Nitazoxanide (NIT) and Tizoxanide (TIZ), using a short synthetic route. Both compounds were tested in vitro against six protozoa (*Giardia intestinalis*, *Trichomonas vaginalis*, *Entamoeba histolytica*, *Plasmodium berghei*, *Leishmania mexicana* and *Trypanosoma cruzi*). Compound **1** (benzologue of NIT) showed broad antiprotozoal effect against all parasites tested, showing IC_{50}'s <5μM. This compound was five-times more active than NIT, and 18-times more potent than metronidazole against *G. intestinalis*. It was 10-times more active than pentamidine against *L. mexicana*, and it was sevenfold more potent than benznidazole versus *T. cruzi*. This compound could be considered as a new broad spectrum antiprotozoal agent.

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Protozoan diseases are a main problem worldwide, affecting hundreds of millions people and animals.[1] The chemotherapy against diseases such as giardiasis, amoebiasis, trichomoniasis, leishmaniasis and trypanosomiasis is limited by the existence of only a few drugs in the market, most of which are of low efficacy, showing toxic side effects, and frequently lead to the appearance of resistant strains.[2] This reflects the need to continue searching for new and better antiprotozoal drugs.[3]

Nitazoxanide (NIT, Alinia®), is a broad-spectrum antiparasitic compound belonging to a nitroheterocyclic class named thiazolides.[4] In humans, NIT is rapidly metabolized to tizoxanide (TIZ), which is a compound equally effective as the parent drug (Fig. 1).[5]

Detailed in vitro and in vivo studies have currently been conducted on the efficacy of NIT and other thiazolide drugs against helminthes, extracellular anaerobic protozoa and bacteria, intracellular parasites and viruses, such hepatitis C and AH_1N_1.[6–9]

As a part of our search for basic information about the structural requirements for new antiprotozoal molecules, we have synthesized two benzologues of NIT and TIZ (Fig. 2). The in vitro antiparasitic activities of these compounds on intestinal unicellular parasites (*Giardia intestinalis* and *Entamoeba histolytica*), a urogen-

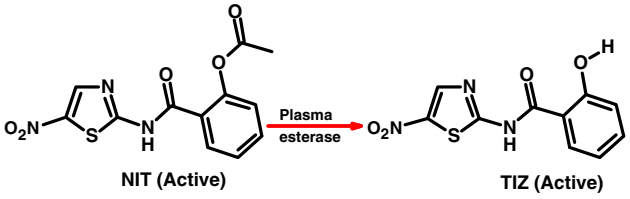

Figure 1. Metabolism of nitazoxanide (NIT).

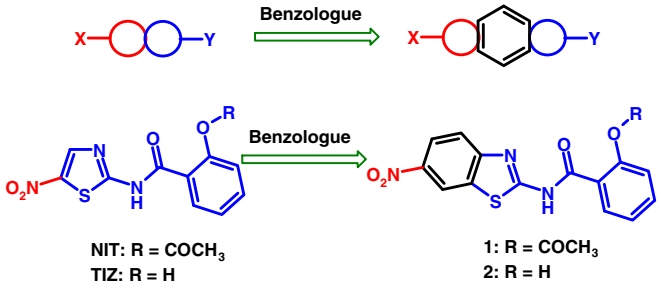

Figure 2. Thiazolides used as leads and drug design of compounds **1** and **2** using vinylogy principle (benzologue).

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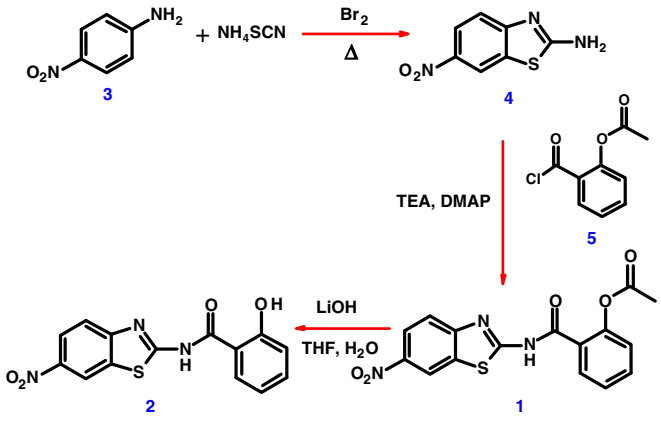
ital tract parasite (*Trichomonas vaginalis*), a red blood cell parasite (*Plasmodium berghei*), and kinetoplastid parasites such as *Trypanosoma cruzi* and *Leishmania mexicana* are reported in this letter.

Compounds **1** and **2** were designed on the basis of the structure of antiprotozoal drug Nitazoxanide (NIT), and its active metabolite, Tizoxanide (TIZ). The vinylogy principle was used for the drug design. According to this principle, two substituents X and Y, linked to aromatic rings in position *ortho* and *para*, one in relation to the other, or separated by a chain of conjugated double bonds or arenes (benzologue), usually function as being attached directly one to another (Fig. 2). It implies that biological activity could be conserved if the electronic communication between X and Y is also retained.[10]

Compounds **1** and **2** were prepared starting from 4-nitroaniline (**3**), which was reacted with ammonium thiocyanate and bromine under reflux, to give 2-amino-6-nitrobenzothiazole (**4**). This compound was acylated with acetylsalyciloyl chloride, in presence of triethylamine and catalytic amounts of DMAP, to get compound **1**, which was hydrolyzed with lithium hydroxide in a mixture of THF–H_2O 9:1, to obtain compound **2** (Scheme 1). Compounds were purified by recrystallization. The chemical structures of the synthesized compounds were confirmed on the basis of their spectral data.[11]

In the nuclear magnetic resonance spectra (^{1}H NMR; δ ppm), the signals of the respective protons of the compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants.



Scheme 1. Synthesis of benzologues **1** and **2**.

The benzamide region of the ^{1}H NMR spectrum contained displacements of the four phenyl protons (H-3′ to H-6′), ranging from 6.95 to 7.98 ppm, in compounds **1** and **2**.

We also observed in both compounds a characteristic ABX pattern for 6-nitrobenzothiazole core: a doublet signal ranging in δ 7.87–7.88 ppm, attributable to H-4, with *ortho* coupling constant (J_o = 8.9–9.1 Hz); a doublet of doublets signal ranging from 8.28 to 8.31 ppm, assigned to H-5, with *ortho* and *meta* coupling constants (J_m = 2.4 and J_o = 9.1 Hz). A second doublet signal in 9.07–9.10 ppm, with J_m = 2.4 Hz, was assigned to H-7.

The new benzologues **1** and **2** were tested in vitro as antiprotozoal agents. Biological assays results against the six protozoa tested are summarized in Table 1. Comparison was made among new compounds and the antiprotozoal drugs of choice: metronidazole and NIT, against *G. intestinalis*, *E. histolytica* and *T. vaginalis*. In order to compare bioactivities, TIZ, pentamidine and benznidazole were also tested. In vitro susceptibility assays were performed using a method previously described.[12–14]

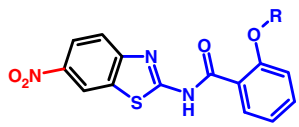
G. intestinalis (syn. *duodenalis, lamblia*) is an intestinal protozoan parasite infecting humans and various other mammalian hosts. It is one of the most commonly diagnosed protozoal causes of diarrhea worldwide. Clinical resistance has been reported for current chemotherapeutics (metronidazole and albendazole).[15] It is interesting to note that compound **1** (benzologue of NIT) was more potent than metronidazole against *G. intestinalis*, being 18-times more active than this drug of choice. Compound **1** was also fourfold more potent than NIT and 2.4-times more active than TIZ. On the other hand, compound **2** was twofold less active than compound **1**, but it was 9-fold more potent than metronidazole, two-times more active than NIT, and as active as TIZ against *G. intestinalis*. Pentamidine (an anti-*Pneumocystis*, trypanocidal and leishmanicidal drug) was as active as metronidazole against this protozoan.

T. vaginalis is the causative agent of trichomoniasis, a common sexually-transmitted disease in humans.[16] Compound **1** showed nanomolar trichomonicidal potency (IC_{50} = 842 nM). However, it was 12-times less active than NIT (IC_{50} = 68 nM), and three-times less active than metronidazole and TIZ. Compound **2** was also less active than the three drugs of choice.

The protozoan parasite *E. histolytica* causes amebic colitis and amebic liver abscess, diseases that afflict millions of individuals in developing countries.[17] Compounds **1** and **2** showed activity against this protozoa in the low micromolar order (IC_{50}'s <9 μM). However none of them showed more amoebicidal activity compared than NIT, TIZ and metronidazole.

The *Leishmania* species causes a variety of diseases from self-healing cutaneous lesions to life-threatening visceral infections. Clinical manifestations depend on the infecting parasites species. There is an estimated annual 1.5–2.0 million new cases of leish-

Table 1
In vitro antiprotozoal and cytotoxic activities of NIT and TIZ benzologues



Compd	R	IC_{50} (μM)						CC_{50} (μM)
		G. intestinalis	*T. vaginalis*	*E. histolytica*	*L. mexicana*	*T. cruzi*	*P. berghei*	VERO
1	$-COCH_3$	0.297	0.842	3.515	1.350	4.890	2.420	683
2	–H	0.590	2.147	8.021	>50	>50	2.370	607
NIT		1.214	0.068	0.504	6.180	18.730	3.890	833
TIZ		0.716	0.211	1.229	6.190	17.470	5.240	388
Metronidazole		5.36	0.290	0.770	>50	>50	>50	387
Pentamidine		4.079	3.815	11.800	13.320	>50	2.942	47
Benznidazole		22.58	18.620	4.270	>50	34.380	4.070	14

maniasis, from which approximately 500,000 belong to the visceral form, which is potentially fatal.[18]

In vitro antileishmanial assay was carried out using a method previously described.[12,18] Compound **1** had an excellent activity against this kinetoplastid protozoa (IC_{50} = 1.350 μM). It was 4.5-times more active than NIT and TIZ. The last two drugs were previously reported by our group as potential new antikinetoplastid parasite compounds.[18] Pentamidine (second-line antileishmanial drug) was used as positive control. In this research, compound **1** was almost 10-times more potent than pentamidine against promastigotes of *L. mexicana*. Compound **2** was inactive against this protozoan.

American trypanosomiasis or Chagas' disease, caused by its etiological agent *T. cruzi*, is still one of the major causes of morbidity and mortality due to cardiovascular diseases in Latin America.[18]

In vitro trypanocidal assay was performed using a technique previously reported.[12,18] Compound **1** was four-times more active than NIT and TIZ, which were twofold more potent than benznidazole (first-line antichagasic drug). Compound **1** was seven-times more active than benznidazole against epimastigotes of *T. cruzi*. Compound **2** did not show activity in this assay.

Malaria continues to be a major health challenge in most tropical and many subtropical regions. It is caused by protozoan parasites of the genus *Plasmodium*. The rodent malaria parasite, *P. berghei*, is a useful model to screen new antimalarial drugs.[19] Cultured schizonts of *P. berghei* were used to assess antimalarial activity of compounds and were prepared following the protocol described previously.[12,20,21] We evaluated the antiplasmodial activity of compounds **1** and **2**, which were as active as pentamidine (a known plasmocidal drug). NIT and TIZ showed micromolar activity (IC_{50} = 3.890 and 5.240 μM) against schizonts development of *P. berghei*. Benznidazole (trypanocidal drug) also showed good potency (IC_{50} = 4.070 μM) in this assay. To the best of our knowledge, this is the first study reporting the in vitro activity of NIT, TIZ and benznidazole, against *P. berghei*.

Compounds **1** and **2** were evaluated for their cytotoxicity against mammalian VERO cell line,[12,22] showing a median cytotoxic concentration (CC_{50}) of 683 and 607 μM, respectively (Table 1). NIT showed also low cytotoxicity, meanwhile TIZ and metronidazole showed moderated toxicity against VERO cell line, compared with **1**, **2** and NIT. It is interesting to note that the most cytotoxic compounds were pentamidine and benznidazole, with CC_{50} of 47 and 14 μM, respectively.

The selectivity index (SI) of the compounds, defined as the ratio of cytotoxicity to biological activity (SI = CC_{50}VERO cells/IC_{50} parasites) was calculated (Table 2).

It is generally considered that biological efficacy is not due to in vitro cytotoxicity when SI $\geqslant$ 10.[22] Compound **1** showed a nanomolar giardicidal effect, having a selectivity index of 2300. The SI calculated for this compound versus all protozoa was >140, this fact implies that exist a real selective toxicity of **1** against the protozoa, over the mammalian cells.

Although nitro compound-containing drugs and analogues are frequently avoid in a drug discovery program due to mutagenic and potentially toxic side effects, the metabolic stability of nitazoxanide and the lack of nitro reduction as part of its mechanism of action, make it an important exception.[23,24]

Table 2
Selectivity indexes of **1**, **2**, NIT and TIZ

Compd	*G. intestinalis*	*T. vaginalis*	*E. histolytica*	*L. mexicana*	*T. cruzi*	*P. berghei*
Selectivity index (SI = CC_{50}/IC_{50})						
1	2300	811	194	506	140	282
2	1029	283	76	ND	ND	256
NIT	686	12250	1653	135	44	214
TIZ	542	1839	316	63	22	74

In conclusion, we have synthesized and screened the in vitro antiprotozoal activities of two new NIT and TIZ benzologues. This study demonstrated that the insertion of benzene ring between the NIT pharmacophore region (nitro and thiazole), have generated a new antiprotozoal scaffold. Compound **1** (benzologue of NIT), showed high bioactivity against all parasites tested (IC_{50}'s <5 μM) and low cytotoxic effect. The obtained results are very promising since compound **1** exhibited higher bioactivity than NIT, TIZ, benznidazole and pentamidine especially towards *G. intestinalis*, *L. mexicana*, *T. cruzi* and *P. berghei*. Compound **1** could be considered as a new broad spectrum antiprotozoal agent. Further optimization of new series and pharmacokinetic characterization of both compounds are in progress in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.100.

References and notes

1. Pink, R.; Hudson, A.; Marie-Annick, M.; Bendig, M. *Nat. Rev. Drug Disc.* **2005**, *4*, 727.
2. Argüello-García, R.; Cruz-Soto, M.; Romero-Montoya, L.; Ortega-Pierres, G. *Infect. Genet. Evol.* **2009**, *9*, 1057.
3. Soares, M. B. *Curr. Drug Targets* **2009**, *10*, 176.
4. Rossignol, J. F.; Cavier, R. *Chem. Abstr.* **1975**, *83*, 28216.
5. Broekhuysen, J.; Stockis, A.; Lins, R. L.; De Graeve, J.; Rossignol, J. F. *Int. J. Clin. Pharmacol. Ther.* **2000**, *38*, 387.
6. Ballard, T. E.; Wang, X.; Olekhnovich, I.; Koerner, T.; Seymour, C.; Hoffman, P. S.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3537.
7. Tchouaffi-Nana, F.; Ballard, T. E.; Cary, C. H.; Macdonald, T. L.; Sifri, C. D.; Hoffman, P. S. *Antimicrob. Agents Chemother.* **2010**, *54*, 2767.
8. Rossignol, J.-F.; Elfert, A.; El-Gohary, Y.; Keeffe, E. B. *Gastroenterology* **2009**, *136*, 856.
9. Rossignol, J. F. O.; La Frazia, S.; Chiappa, L.; Ciucci, A.; Santoro, M. G. *J. Biol. Chem.* **2009**, *284*, 29798.
10. , 3rd*The Practice of Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press: London, 2008; pp 283–287.
11. *2-{[(6-Nitro-1,3-benzothiazol-2-yl)amino]carbonyl}phenyl acetate* (**1**). Recrystallized from ethanol. Yield: 73%, mp: 300–303.5 °C. ^{1}H NMR (400 MHz, DMSO) δ: 2.25 (s, 3H, CH_3), 7.03 (t, 1H, H-5, J = 7.5 Hz), 7.08 (d, 1H, H-3′, J = 8.2 Hz), 7.52 (td, 1H, H-4′, J = 1.7, J = 8.2 Hz), 7.88 (d, 1H, H-4, J = 8.9 Hz), 7.98 (dd, 1H, H-6′, J = 1.7, J = 8.2 Hz), 8.31 (dd, 1H, H-5, J = 2.4, J = 8.9 Hz), 9.10 (d, 1H, H-7, J = 2.4 Hz), 12.76 (br s, 1H, NH) ppm. ^{13}C NMR (100 MHz, DMSO) δ: 22.9 (CH_3), 117.3 (C-3′), 119.1 (C-7), 120.6 (C-4), 121.8 (C-5), 122.0 (C-1′), 130.6 (C-6), 132.2 (C-4′, 135.0 (C-6′), 135.0 (C-7a), 142.9 (C-5′), 143.1 (C-2′), 143.1 (C-3a), 153.5 (C-2), 163.5 (NHC=O), 170.2 ((OC=O) ppm; MS/FAB$^+$: m/z 358 (M+H$^+$). HRMS (FAB$^+$): m/z 358.0497 [M+H]$^+$ (calcd for $C_{16}H_{11}N_3O_5SH^+$ 358.0498). 2-Hydroxy-*N*-(6-nitro-1,3-benzothiazol-2-yl) benzamide (**2**). Recrystallized from ethanol. Yield: 81%, mp: 317–320.9 °C. ^{1}H NMR (400 MHz, DMSO) δ: 6.95 (t, 1H, H-5, J = 7.9 Hz), 7.08 (d, 1H, H-3′, J = 7.9 Hz), 7.51 (td, 1H, H-4′, J = 1.7, J = 8.5 Hz), 7.87 (d, 1H, H-4, J = 9.2 Hz), 7.97 (dd, 1H, H-6′, J = 1.7, J = 7.9 Hz), 8.28 (dd, 1H, H-5, J = 2.5, J = 8.9 Hz), 9.07 (d, 1H, H-7, J = 2.4 Hz), 12.76 (br s, 1H, NH) ppm. ^{13}C NMR (100 MHz, DMSO) δ: 117.3 (C-3′), 119.0 (C-7), 120.6 (C-4), 121.8 (C-5), 122.0 (C-1′), 130.6 (C-6), 132.2 (C-4′), 135.0 (C-6′), 135.0 (C-7a), 142.9 (C-5′), 143.1 (C-3a), 153.5 (C-2), 157.5 (C-2′), 163.5 (NHC=O) ppm; MS/FAB$^+$: m/z 316 (M+H$^+$). HRMS (FAB$^+$): m/z 316.0356 [M+H]$^+$ (calcd for $C_{14}H_{12}N_3O_4SH^+$ 316.0392).
12. For more details, see Supplementary data.
13. Cedillo-Rivera, R.; Chávez, B.; González-Robles, A.; Tapia, A.; Yépez-Mulia, L. *J. Eukaryot. Microbiol.* **2002**, *49*, 201.
14. Hernandez-Nuñez, E.; Tlahuext, H.; Moo-Puc, R.; Torres-Gomez, H.; Reyes-Martınez, R.; Cedillo-Rivera, R.; Nava-Zuazo, C.; Navarrete-Vazquez, G. *Eur. J. Med. Chem.* **2009**, *44*, 2975.
15. Suk, D. H.; Bonnac, L.; Dykstra, C. C.; Pankiewicz, K. W.; Patterson, S. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2064.

16. Dornbush, P. J.; Vazquez-Anaya, G.; Shokar, A.; Benson, S.; Rapp, M.; Wnuk, S. F.; Wrischnik, L. A.; Land, K. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7466.
17. Abid, M.; Azam, A. *Bioorg. Med. Chem.* **2005**, *13*, 2213.
18. Chan-Bacab, M. J.; Hernández-Núñez, E.; Navarrete-Vázquez, G. *J. Antimicrob. Chemother.* **2009**, *63*, 1292.
19. Ikegami-Kawai, M.; Arai, C.; Ogawa, Y.; Yanoshita, R.; Ihara, M. *Bioorg. Med. Chem.* **2010**, *18*, 7804.
20. Thathy, V.; Menard, R. *Methods Mol. Med.* **2002**, *72*, 317.
21. Torres-Gómez, H.; Hernández-Núñez, E.; León-Rivera, I.; Guerrero-Alvarez, J.; Cedillo-Rivera, R.; Moo-Puc, R.; Argotte-Ramos, R.; Rodríguez-Gutiérrez, M. C.; Chan-Bacab, M. J.; Navarrete-Vázquez, G. *Bioorg. Med. Chem.* **2008**, *18*, 3147.
22. Nava-Zuazo, C.; Estrada-Soto, S.; Guerrero-Álvarez, J.; León-Rivera, I.; Molina-Salinas, G. M.; Said-Fernández, S.; Chan-Bacab, M. J.; Cedillo-Rivera, R.; Moo-Puc, R.; Mirón-López, G.; Navarrete-Vazquez, G. *Bioorg. Med. Chem.* **2010**, *18*, 6398.
23. Ballard, T. E.; Wang, X.; Olekhnovich, I.; Koerner, T.; Seymour, C.; Salamoun, J.; Warthan, M.; Hoffman, P. S.; Macdonald, T. L. *ChemMedChem* **2011**, *6*, 362.
24. Hoffman, P. S.; Sisson, G.; Croxen, M. A.; Welch, K.; Harman, W. D.; Cremades, N.; Morash, M. G. *Antimicrob. Agents Chemother.* **2007**, *51*, 868.