Bioorganic & Medicinal Chemistry Letters 21 (2011) 3168–3171

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Gabriel Navarrete-Vazquez [a,*], Fabiola Chávez-Silva [a], Rocío Argotte-Ramos [b], María del Carmen Rodríguez-Gutiérrez [b], Manuel Jesús Chan-Bacab [c], Roberto Cedillo-Rivera [d], Rosa Moo-Puc [d], Emanuel Hernández-Nuñez [a,e]

[a] Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos 62209, Mexico
[b] Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos 62508, Mexico
[c] Departamento de Microbiología Ambiental y Biotecnología, Universidad Autónoma de Campeche, Campeche 24030, Mexico
[d] Unidad de Investigación Médica Yucatán, IMSS Mérida, Yucatán 97000, Mexico
[e] Facultad de Química, Universidad Autónoma de Yucatán, Mérida, Yucatán 97000, Mexico

ARTICLE INFO

Article history:
Received 19 January 2011
Revised 17 February 2011
Accepted 22 February 2011
Available online 26 February 2011

Keywords:
Nitazoxanide
Giardia intestinalis
Leishmania mexicana
Benzologue

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.

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-

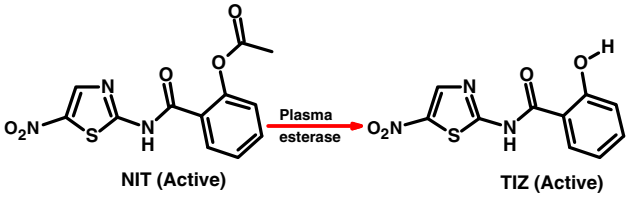


Figure 1. Metabolism of nitazoxanide (NIT).

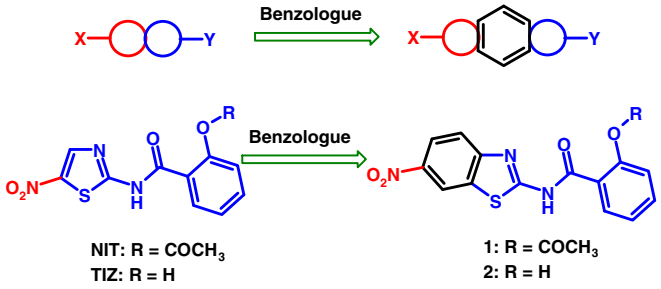


Figure 2. Thiazolides used as leads and drug design of compounds **1** and **2** using vinylogy principle (benzologue).

* Corresponding author. Tel./fax: +52 777 3297089.
E-mail address: gabriel_navarrete@uaem.mx (G. Navarrete-Vazquez).

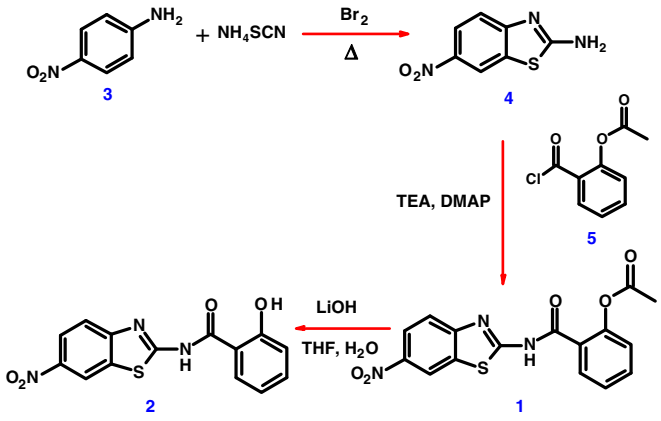
0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.02.100

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]

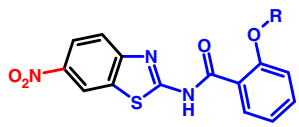
G. intestinalis (syn. *duodenalis, lamblia*) is an intestinal protozoan parasite infecting humans and various other mammalian hosts. It is one of the most commonly diagnosed protozoal causes of diarrhea worldwide. Clinical resistance has been reported for current chemotherapeutics (metronidazole and 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