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Original Article

Antigiardiais Effects of New Metronidazole Derivatives on Trophozoites in TYI-S-33

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Abstract

Giardia is a flagellate protozoan with worldwide distribution that causes significant gastrointestinal diseases. The life cycle of the organism alternates between the active, proliferating trophozoite and the dormant cyst. 5-Nitroimidazoles have been used extensively in the treatment of amoebiasis, *giardia*sis, and trichomoniasis. We have previously synthesized some new analogues of metronidazole containing a phenyl or cyclohexanol ring in the side chain of the imidazole ring, and evaluated their anti*giardia*sis activity on *giardia* cyst. In the present study, we evaluated their activity against trophozoites of the parasite. For this purpose TYI-S-33 media was used and their MIC were compared with metronidazole and DMSO as positive and negative controls, respectively. The results showed that the new compounds had desirable anti*giardia*sis activities. Analogues which contain phenyl group in their structure are more active than those which contain cyclohexanol moiety. Although all the new compounds had higher MIC than metronidazole but their anti*giardia*sis activity were comparable to metronidazole and they may prove good alternatives for metronidazole.

Keywords: *Giardia*sis; Metronidazole; Trophozoite.
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1. Introduction

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem [1]. *Giardia* is a flagellate protozoan with worldwide distribution that causes significant gastrointestinal diseases [2]. The life cycle of *G. intestinalis* alternates between the active, proliferating trophozoite and the dormant cyst [3, 4]. The infectious cysts begin excysting in the acidic environment of the stomach and become trophozoites (the vegetative form). The trophozoites attach to the intestinal mucosa through the suction generated by a ventral disk [5].

Nitroimidazoles are a well established group of antiprotozoan and antibacterial agents. Metronidazole (MTZ) is a synthetic 5-nitroimidazole that is used in the treatment of infections caused by gram negative anaerobic

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bacteria like *Helicobacter pylori*, and protozoan such as *Giardia lamblia*, *Entomoeba histolytica* and *Trichomonas vaginalis* [6]. Resistance against metronidazole is common in protozoan organism. Also, *in vitro* trophozoites of *E. histolytica* are able to adapt to therapeutically relevant levels of the drug [7]. MTZ is fairly well tolerated, but gastrointestinal and central nervous system toxicity has been associated with its administration [2, 8]. Genotoxic activity of MTZ has been studied in different *in vivo* and *in vitro* assays. It is mutagenic for bacteria and induces gene mutations and recombination in fungi and classified in the 2B group as possibly carcinogenic to humans [6, 9]. Neurotoxicity is one of the most important adverse effects of MTZ therapy and other neurotoxicities such as encephalopathy, seizure, and mental confusion have also been documented [8]. However, MTZ and the related compounds (tinidazole) are the only drugs effective for the treatment of trichomoniasis and giardiasis, and in the event of overt clinical resistance, there is no alternative treatment for either trichomoniasis or invasive amoebiasis [7, 10].

Previously, we have reported the synthesis of some new analogues of MTZ including 2-(1*H*-1-imidazolyl)-1-phenyl-1-ethanol (**1a**), 2-(2-methyl-1*H*-1-imidazolyl)-1-phenyl-1-ethanol (**1b**), 2-(2-methyl-4-nitro-1*H*-1-imidazolyl)-1-phenyl-1-ethanol(**1c**),2-(1*H*-1-imidazolyl)-1-cyclohexanol (**2a**), and 2-(2-methyl-4-nitro-1*H*-1-imidazolyl)-1-cyclohexanol (**2b**) (Figure 1). In that study antigiardiasis effects of the above compounds were tested against giardia cysts by Bingham method. Our previous results showed that the compounds which contain both methyl and nitro groups in their structures were more active than others compounds [11]. The aim of this study is to evaluate the antigiardiasis effects of our new compounds on trophozoites in TYI-S-33 Media.

2. Materials and methods

Newly synthesized compounds were obtained from Department of Medicinal Chemistry, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Contaminated stool sample with giardiasis were obtained from Shahid Faghihi and Namazi Hospitals, Shiraz University of Medical Sciences.

The Giardia cysts were separated and purified from stool of patients and set into inducting solution. Flotation technique based on Bingham method was used for washing, purification and isolation of giardia cysts. Briefly, 5 to 10 grams of feces was diluted with 10 ml of saline, and then it was poured through gauze into another cup. The mixture spined for five min at 400 rpm. For further purification, the washing method was repeated two times and then the cyst were collected from the sediment. Purification continued by addition of 10 ml (4×2.5 ml) flotation solution (Sucrose, 0.5, 0.75, 1 and 1.5 M). Tubes were spined for 30 min. at 1000 rpm. Cysts were collected from the top of tube and then washed again with 1/10 distilled water. Cysts were collected and maintained at 4 ºC.

Eosin solution (0.001 M) was used for determination of live cysts. The number of viable cysts (negative staining by eosin) in 1 µl were determined by hemocytometer method.

G. lamblia cultivation purified cysts were also used for initiation of giardia growth in

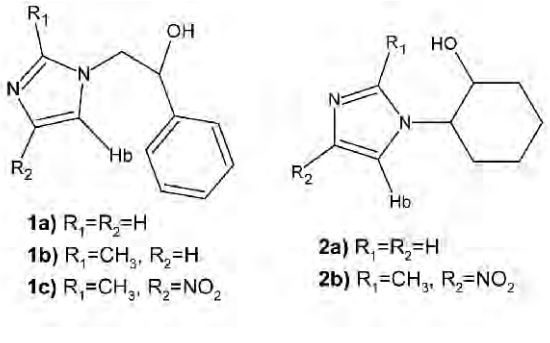

Figure 1. Chemical structures of the newly synthesized compounds.

TYIS-33 medium supplemented with bile (1 mg/ml; Sigma). Trophozoites for drug assays were harvested in the mid-logarithmic phase of growth and were distributed with the same amount among 5-ml TYIS-33 media containing different concentrations of compounds. Tubes were incubated at 37 ºC and after 48 h, the effect of compounds were determined by centrifugation of tubes for 5 min at 400 rpm, following incubation on ice for 5 min. The viability of trophozoites was determined using eosin solution (0.001 M) and microscopical examination of trophozoites. These exams were repeated for three times. Effects of DMSO and metronidazole on Giardia cysts were determined as negative and positive control, respectively.

A solution of different concentrations of metronidazole and tested compounds (4 to 15 mg/ml) in DMSO were added to a 100 µl suspension of cysts in Eppendorf tubes and the tubes were maintained at 37 ºC for 30 min. Antigiardiasis effects of the compounds were determined again by hoemocytometer lamel (Table 1). Each compound in different concentrations were examined against 8 giardia trophozoites samples isolated from different patients.

3. Results

Biological assays showed that Eosin and DMSO had 4.66% and 7.11% mortality, respectively. MTZ had about 100% efficacy at 2 mg/ml concentration. Compounds 1c, 1b and 2b showed 100% efficacy at 6, 8 and 9 mg/ml, respectively. Compound 1a was moderately active and compound 2a was less active than other compounds (Table 1).

A comparison of antigiardiasis activity of the tested compounds showed that phenyl ethanol and also cyclohexanol derivatives with nitro group (1c and 2b) are more active than those without nitro substitution (1a, 1b and 2a). Substitution of both methyl and nitro groups on the imidazole ring increased the antigiardiasis potency.

Table 1. Antigiardiasis effects of metronidazole derivatives

Compds.	Concentration (µg/ml)	Mean of mortality (%)
Eosin	0.001	4.66
DMSO	0.001	7.11
MTZ	1	0
MTZ	2	100
1a	8	62.5
1a	9	75
1a	10	100
1a	11	100
1b	6	75
1b	7	87.5
1b	8	100
1b	9	100
1c	4	75
1c	5	87.5
1c	6	100
1c	7	100
2a	12	62.5
2a	13	75
2a	14	100
2a	15	100
2b	7	62.5
2b	8	87.5
2b	9	100
2b	10	100

4. Discussion

G. lamblia is one of the most common eukaryotic pathogens and is classified as a category B agent of bioterrorism [12]. The parasite has two stages: the vegetative trophozoite that inhabits the small intestine of the host which is responsible for causing disease, and the environmentally resistant cyst which is responsible for the transmission of the parasite among susceptible hosts [13]. Giardia is commonly ingested in the form of cysts but *in vitro* studies suggest that giardia passage through the stomach of its host effects transformation into the trophozoite. The trophozoite then replicates within the lumen of the small intestine, resisting expulsion by adherence to the intestinal epithelium [14]. Trophozoite multiplication presents many features not seen in a typical eukaryotic mitotic cycle, such as the presence of two nuclei and the alternation between tetraploid and octaploid sets of chromosomes [12]. In our previous study, we reported the property

of the newly synthesized compounds against giardia cysts [11]. In this study, we evaluated the potency of these compounds against the giardia trophozoites. Our previous results showed that compounds 1a, 1b and 1c were more potent and had similar activities on cycts but for trophozoites, compound 1c was more active than 1a and 1b. For cycts and also for trophozoites, compound 2a was the least active compound. Substitution of nitro group increases the potency in both cycts and trophozoites.

On the basis of the preliminary studies and good antigiardiasis activities of the tested compounds, investigations on their toxicities and pharmacokinetics for development of compounds with higher activity and less toxicity are justified.

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