Synthesis of metronidazole derivatives as anti-giardiasis agents

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ABSTRACT

Metronidazole (MTZ) and its derivatives have been extensively used to treat infections caused by protozoa and anaerobic bacteria. In this investigation several novel imidazole and nitroimidazol derivatives namely; 2-(1H-1-imidazolyl)-1-phenyl-1-ethanol 1a, 2-(2-methyl-1H-1-imidazolyl)-1-phenyl-1-ethanol 1b, 2-(2-methyl-4-nitro-1H-1-imidazolyl)-1-phenyl-1-ethanol 1c, 2-(1H-1-imidazolyl)-1-cyclohexanol 2d and 2-(2-methyl-4-nitro-1H-1-imidazolyl)-1-cyclohexanol 2e were prepared by the reaction of the corresponding imidazoles with styrene oxide or cyclohexene oxide respectively and their biological activity against Giardia lamblia cyst in compareion with MTZ were determined by flotation technique based on Bingham method. These compounds were less active than metronidazole but showed significant anti-giardiasis activity.

Keywords: Metronidazole, Giardiasis, Imidazole, Epoxide

INTRODUCTION

Giardia is a flagellate protozoan with worldwide distribution that causes significant gastrointestinal diseases in a wide variety of vertebrates including cats and human (1). The protozoan parasite Entamoeba histolytica is the cause of amoebic dysentery and liver abscesses and is responsible for 100,000 deaths per year in a number of countries (2). Nitroimidazoles are a well established group of compounds which their therapeutic success are due to selective uptake by anaerobic gram negative bacteria and on the basis of their cytotoxic action on these microorganisms have been used as an antiprotozoan and antibacterial (3,4).

Metronidazole (MTZ) is a synthetic compound that is used in the treatment of infections caused by Gram negative anaerobic bacteria like Helicobacter pylori, and protozoans such as Giardia lamblia, Entamoeba histolytica, Trichomonas vaginalis. MTZ is fairly well tolerated, but it can produces several adverse effects (5) such as critic lateral effects, neurologic alterations impairment of cardiac rhythm due to the chelation of MTZ with calcium ions, induction of some tumors in rodents and it is classified in the 2B group as possibly carcinogen in humans (6). However MTZ and the related compounds (tinidazole) are the only drugs effective for 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).

The aim of this work is to synthesize some new analogues of MTZ and to evaluate their anti-giardiasis effects.

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Chemistry

The classical method for the preparation of β-amino alcohol involves heating an epoxide with excess of an amine in a protic solvent (8,9). Although this non-catalytic reaction is satisfactory in many cases, it has a number of limitations. For example, the reactions with aromatic amines like imidazole take places with difficulties (9). The epoxide opening reaction with certain nucleophiles is generally performed by acid or base catalysis, and in the absence of such a catalyst, the reaction is moderately slow. These methods require large amounts of reagents, long reaction times, and results in undesirable side products (10). In general the epoxide-based alkylation of heterocycles requires rather drastic conditions, i.e. prolonged heating at high temperature with the concomitant use of strong base (8).

In this study, the reaction of imidazole, 2-methylimidazole or 2-methyl-4-nitroimidazole as the azole part of the molecule with styrene oxide or cyclohexene oxide were used to make the hydroxylated side chain and by this method compounds 2 -(1H - 1- imidazolyl) -1 -phenyl-1-ethanol 1a, 2-(2-methyl -1H-1-imidazolyl)-1-phenyl-1-ethanol 1b, 2-(2-methyl-4-nitro-1H-1-imidazolyl) – 1 – phenyl - 1-ethanol 1c, 2-(1H-1-imidazolyl)-1-cyclohexanol 2d and 2-(2-methyl-4-nitro-1H-1-imidazolyl)-1-cyclohexanol 2b were prepared.
MATERIALS AND METHODS
A 250 MHz Brucker 9FT-NMR and a HP 6890 Mass spectroscopic instruments were used to obtain NMR and Mass spectra. Contaminated stool samples with giardiasis were obtained from Shahid Faghihi Hospital of Shiraz University of Medical Sciences.

General procedure
For the synthesis of compounds 1a, 1b and 1c, solutions of imidazole, 2-methylimidazole or 2-methyl-4-nitroimidazole (0.02 mol) with styrene oxide (0.02 mol) and TBAF (tetrabutyl ammonium fluoride, 0.002 mol) and for the synthesis of compounds 2d and 2e solutions of imidazole or 2-methyl-4-nitroimidazole (0.02 mol) with cyclohexene oxide (0.02 mol) and TBAB (tetrabutyl ammonium bromide, 0.002 mol) in acetonitrile (10-20 ml) were refluxed for 15-24 hours (Scheme1). The reaction mixtures were then diluted with water (15-20 ml) and extracted with chloroform (15-20 ml). The organic layer was dried over anhydrous sodium sulfate and the crude product was purified by flash chromatography using ethanol / dichloromethane (1:9) as eluting solvent.

2-(1H-1-imidazolyl)-1-phenyl-1-ethanol, 1a
M.P.=145°C, 75%
¹H-NMR (DMSO): δ = 7.51 (s, 1H, Ha), 7.30 (m, 5H, aryl), 7.13 (s, 1H, Hb), 7.11 (s, 1H, Hc), 5.91 (t, 1H, CH, J=7.5), 4.19 (t, 2H, CH₂, J=10), 3.50 (s, 1H, OH).
MS: m/z (%): 188 (M, 20), 107 (30), 82 (100), 54 (15).

2-(2-methyl-1H-1-imidazolyl)-1-phenyl-1-ethanol, 1b
M.P.=113°C, 75%
¹H-NMR (DMSO): δ = 7.44 (m, 1H, Ha), 7.22 (s, 1H, Hb), 6.68 (s, 1H, Hc), 4.97 (t, 1H, CH, J=13), 4.12 (t, 2H, CH₂, J=9), 3.31 (t, 1H, OH, J=10), 2.46 (s, 3H, CH₃).
MS: m/z (%): 202 (M, 42), 107 (35), 96 (100), 81 (25), 79 (45), 55 (20).

2-(2-methyl-4-nitro-1H-1-imidazolyl)-1-phenyl-1-ethanol, 1c
M.P.=138°C, 68%
¹H-NMR (DMSO): δ = 8.25 (s, 1H, Hb), 7.38 (m, 5H, aryl), 5.87 (t, 1H, CH, J=8.7), 4.17 (t, 1H, OH, J=8), 3.44 (d, 2H, CH₂), 2.20 (s, 3H, CH₃).
MS: m/z (%): 247 (M, 10), 141 (100), 107 (85), 81 (25), 79 (87), 53 (25).

2-(1H-1-imidazolyl)-1-cyclohexanol, 2a
M.P. =174°C, 75%
¹H-NMR (DMSO): δ = 7.52 (s, 1H, Ha), 6.84 (s, 1H, Hb), 6.51 (s, 1H, Hc), 5.83 (s, 1H, CH-OH), 3.65 (m, 1H, N-CH), 2.21 (t, 1H, OH, J=9.25), 2.01-1.19 (m, 8H, aliphatic).
MS: m/z (%): 166 (M, 20), 100 (45), 84 (87), 79 (87), 53 (25).

2-(2-methyl-4-nitro-1H-1-imidazolyl)-1-cyclohexanol, 2b
M.P. =124°C, 70%
¹H-NMR (DMSO): δ = 7.31 (s, 1H, Hb), 5.35 (t, 1H, N-CH, J=8.1), 3.65 (m, 1H, CH-OH), 2.35 (s, 3H, CH₃), 2.19 (t, 1H, OH, J=9.25), 1.82-1.01 (m, 8H, aliphatic).
MS: m/z (%): 225 (M, 20), 181 (13), 164 (87), 82 (73), 79 (87), 53 (25).

Scheme 1: Synthesis of metronidazole derivatives 1a-c and 2a-b
### Biological Assay

*Giardia cyst isolation:* Stool specimens from the infected patients with giardia were collected and flotation technique based on Bingham method was used for washing, purification and isolation of giardia cysts. Briefly 5 to 10 grams of feces were diluted with 10 ml of saline, and the mixture passed through gauze into another cup. The mixture spined for five min at 15000 rpm and after addition of the flotation solution (sucrose, 2M) it was spined for five min at 15000 rpm Cysts were collected from the top of tube and then washed again with distilled water, collected and maintained at 4°C. Eosin solution (0.001M) was used for staining live cysts. Number of viable cysts (negative staining by eosin) in 1µl were determined by hemocytometer method.

A solution of different concentration of metronidazole and tested compounds (0.002 to 0.006M) in DMSO (2-4 ml) were added to a 100µl suspension of cysts in eppendorf tubes and the tubes maintained at 37°C for 30min. Antigiardiasis effects of compounds were determined again by hemocytometer lamel (Table1). Each compound in different concentrations was examined against 14 giardia cysts samples which were isolated from different patients.

### RESULTS AND DISCUSSION

Biological assays showed that mortality by Eosin and DMSO had 4.66% and 7.11% respectively. MTZ at 0.002M had about 90% efficacy. In comparison to MTZ, compounds 1a, 1b and 1c showed the same efficacy at 0.006M. Compound 2b was moderately active and compound 2a was less active than other compounds (Table 1).

A comparison of antigiardiasis activity of the tested compounds show that both phenylethanol and cyclohexanol and 2b derivatives containing methyl and nitro substitutions were more active than compounds having only methyl group (1b) which in turn were less active than compounds without methyl group (1a and 2a).

On the basis of preliminary antigiardiasis activity of the tested compounds, investigations of their toxicities and further development of these compounds are justified.

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**Table 1. Antigiardiasis effects of metronidazole derivatives 1a-c and 2a,b**

<table>
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<tr>
<th>Compounds</th>
<th>Conc. (M)</th>
<th>Number of cysts</th>
<th>Mortality (%)</th>
<th>Compounds</th>
<th>Conc. (M)</th>
<th>Number of cysts</th>
<th>Mortality (%)</th>
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<td>4.66</td>
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<tr>
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