Synthesis of metronidazole derivatives as antigiardiasis agents

*[1]Khabnadideh S., [1]Rezaei Z.,. [2]Khalafi Nezhad A , [3]Motazedian M.H., [1]Eskandari M.

[1]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, [2]Department of Chemistry, College of Science, Shiraz University, [3]Department of Parasitology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Received 17 Jan 2006; Revised 17 April 2006; Accepted 18 Apr 2006

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-(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 **2d** and 2-(2-methyl-4-nitro-1*H*-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 compareison with MTZ were determined by flotation technique based on Bingham method. These compounds were less active than metronidazole but showed significant antigiardiasis 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 abscess 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 anerobic 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, Entomoeba 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 antigiardiasis effects.

Chemistry

The classical method for the preparation of *β*-amino alcohol involves heating an epoxide with excess of an amine in a protonic 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 heteroc-ycles 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 cyclohexen oxide were used to make the hydroxylated side chain and by this method compounds 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 **2d** and 2-(2-methyl-4-nitro-1*H*-1-imidazolyl)-1-cyclohexanol **2b** were prepared.

Correspondence: khabns@sums.ac.ir

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-(1*H*-1-imidazolyl)-1-phenyl-1-ethanol, 1a

M.P.=145°C, 75%

^{1}H-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, CH_2, J=10), 3.50 (s, 1H, OH).

MS: m/z (%): 188 (M, 20), 107 (30), 82 (100), 54 (15).

2 - (2 – methyl – 1 *H*-1 - imidazolyl) –1- phenyl-1- ethanol, 1b

M.P.=113°C, 75%

^{1}H-NMR (DMSO): δ= 7.44 (m, 5H, aryl), 7.22 (s, 1H, Hb), 6.68 (s, 1H, Hc), 4.97 (t, 1H, CH, J=13), 4.12 (t, 2H, CH_2, J=9), 3.31 (t, 1H, OH, J=10), 2.46 (s, 3H, CH_3).

MS: m/z (%): 202 (M, 42), 107 (35), 96 (100), 81 (25), 79 (45), 55 (20).

2-(2-methyl-4-nitro-1*H*-1-imidazolyl)-1-phenyl-1-ethanol, 1c

M.P.=138°C, 68%

^{1}H-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, CH_2), 2.20 (s, 3H, CH_3).

MS: m/z (%): 247 (M, 10), 141 (100), 107 (85), 81 (13), 79 (87), 53 (25).

2-(1*H*-1-imidazolyl)-1-cyclohexanol, 2a

M.P. =174°C, 75%

^{1}H-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).

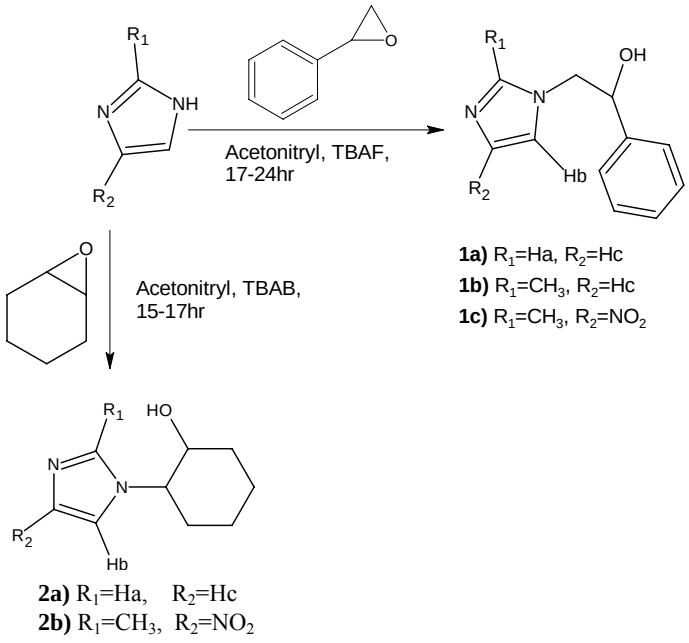
MS: m/z (%): 166 (M, 20), 100 (45), 84 (87), 79 (87), 53 (25).

2 - (2 – methyl – 4 – nitro -1*H* - 1- imidazolyl)-1-cyclohexanol, 2b

M.P.=124°C, 70%

^{1}H-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, CH_3), 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**

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

Comps	Conc. (M)	Number of cysts	Mortality (%)	Comps	Conc. (M)	Number of cysts	Mortality (%)
Eosin	0.001	118	4.66	**1c**	0.002	123	40.5
DMSO	0.001	120	7.11	**1c**	0.003	121	53.29
MTZ	0.001	108	51.17	**1c**	0.004	131	61.32
MTZ	0.002	112	89.9	**1c**	0.005	131	70.07
1a	0.002	104	25.37	**1c**	0.006	133	91.36
1a	0.003	106	25.57	**2a**	0.004	89	21.1
1a	0.004	115	41.72	**2a**	0.005	89	33.2
1a	0.005	112	52.02	**2a**	0.006	89	50.1
1a	0.006	112	90.82	**2b**	0.004	89	40.4
1b	0.002	112	43.5	**2b**	0.005	89	53.07
1b	0.003	120	50.8	**2b**	0.006	89	85.1
1b	0.004	121	52.87				
1b	0.005	109	66.4				
1b	0.006	105	90.42				

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 mixuture 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 hoemocytometer 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.

REFERENCES

1. Scorza AV, Lappin M R. Metronidazole for the treatment of feline giardiasis. J Feline Medicine & Surgery 2004; 6: 157-160.
2. Bharti N, Athar F, Maurya MR, Azam A. Synthesis, characterization and in vitro anti-amoebic activity of new palladium(II) complexes with 5-nitrothiophene-2-carboxaldehyde N(4)-substituted thiosemicarbazones. Bioorganic & Medicinal Chemistry 2004; 12: 4679-4684.
3. Cava T, Gozukara SE. Genotoxicity evaluation of metronidazole using the piscine micronucleus test by acridine orange fluorescent staining. Environmental Toxicology and Pharmacology 2005; 19: 107-111.
4. Lindmark DG, Müller M. Antitrichomonad action, mutagenicity, and reduction of metronidazole and other nitroimidazoles**.** Antimicrobial Agents and Chemotherapy 1976; 10: 476–482.
5. Kim DW, Park JM, Yoon BW, Baek MJ, Kim JE, Kim SYJ. Metronidazole-induced encephalopathy. J Neurological Sciences 2004; 224: 107-111.
6. Arroyo SG, Castro SM, Pietrini RV, Camargo EM, Zamora HS, Aldrete MEC. Cytogenetic study of metronidazole and three metronidazole analogues in cultured human lymphocytes with and without metabolic activation. Toxicology in Vitro 2004; 18: 319-324.
7. Upcroft JA, Campbell RW, Benakli K, Upcroft P, Vanelle P. Efficacy of New 5-Nitroimidazoles against Metronidazole-Susceptible and -Resistant *Giardia*, *Trichomonas*, and *Entamoeba* spp. Antimicrobial Agents and Chemotherapy 1999; 43: 73–76.
8. Carre MC, Houmounou JP, Caubere P. A convenient preparation of β-amino alcohols from epoxides and halomagnesium alkylamides. Tetrahedron Lett 1985; 26: 3107-3110.
9. Fu XL, Wu SH. A regio-and stereoselective synthesis of β-amino alcohols. Synthetic Communications. 1997; 27: 1677-1683.
10. Kotsuki H, Hayashida K, Shimanouchi T, Nishizawa H. High pressure organic chemistry. 19. high pressure-promoted, silica gel-catalyzed reaction of epoxides with nitrogen heterocycles. J. Org. Chem 1996; 61: 984-990.
11. Harrison TR. Principles of Internal Medicine. 14th ed. McGraw-Hill; 1998.