

Synthesis and Biological Evaluation of Metronidazole Derivatives as Anti-Giardia Agents

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Abstract

Nitroimidazole derivatives such as Metronidazole (MTZ) have been used as anti- protozoa and anti-anaerobic bacteria. In this study several derivatives of MTZ were synthesized and evaluated against *Giardia lamblia* cyst. MTZ were reacted with several alkyl halide to obtain o-alkyl MTZ derivatives, then products were purified and their chemical structures were confirmed by spectral analysis (1HNMR and Mass). In order to assess biological evaluation, all compounds were investigated against 25 *Giardia* samples isolated from *Giardia*-infected patients. Results showed that compound 2 had the most activity on cyst of *Giardia* in comparison with MTZ.

Keywords: Nitromidazole, Metronidazole derivatives, Antigiardida.

1. Introduction

Giardia is a flagellate protozoan that causes significant gastrointestinal diseases in a wide variety of vertebrates, including human (1). The parasite is responsible for significant morbidity and mortality in a number of countries. The life cycle of *Giardia* alternates between the active, proliferating trophozoite and the dormant cyst (2, 3). 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 (4).

Several drugs are used for the therapy and prophylaxis of parasitic diseases, both in humans and domestic animals (2). Nitroimidazoles are a very important class of pharmacophores with wide

range of biological activities (5). Nitroimidazoles are a well-established group of antiprotozoan and antibacterial agents that are effective against infections due to anaerobic protozoa and bacteria (6).

Metronidazole (MTZ) is a nitroimidazole derivative that is used in the treatment of infections caused by gram negative anaerobic bacteria like *Helicobacter pylori*, and protozoan such as *Giardia lamblia*, *Entamoeba histolytica* and *Trichomonas vaginalis* (7). Due to its vital role in defense against *Helicobacter pylori* infections, MTZ has been included in the "essential medicines" list by the WHO (8-10). Several adverse effects of MTZ are reported that neurotoxicity is one of the most important of them (11). Also, impairment of cardiac rhythm due to the chelation of MTZ with calcium ions is an important adverse effect of MTZ. It also induces some tumors in rodents, and it is mutagenic in bacteria (12). MTZ is classified in the 2B group as possibly carcinogenic to humans and proved carcinogenic to animals.

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In order to improve pharmacokinetic and pharmacodynamic properties of MTZ, many modifications have been done on MTZ structure (13-18). Biological activities of many synthesizedazole compounds having diphenylmethyl or trityl moiety were reported in our previous studies (19-21). Due to reasonable activities of those compounds, in this study a new series of 2-methyl, 4-nitro imidazole derivatives with diphenylmethyl or trityl moiety was synthesized and their anti-Giardia effects were investigated against samples isolated from giardiasis patients.

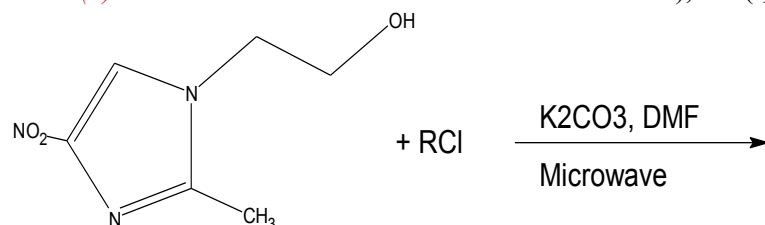
2. Material and methods

All solvents and reagents were purchased from Sigma or Merck Chemical Companies. The reactions were done under microwave condition in Sharp instrument. The products were purified by column chromatography techniques. NMR spectra were recorded on a Bruker Avance DPX 250 MHz instrument. Mass spectra were recorded on a Hewlett-Packard GC-MS.

2.1. General procedures for the synthesis of compounds 2-6

Five mmol of appreciated alkyl chloride were added to five mmol of MTZ in 10 ml dimethylformamide (DMF) in the presence of 40 mg K_2CO_3 . The reaction was done under microwave condition for maximum 5 times for 20 seconds. The progress of the reaction was monitored with TLC. After completion of the reaction, the suspension was filtered and then washed with water and ethyl acetate. The organic phase was extracted, dried over anhydrous Na_2SO_4 and evaporated by rotary evaporator. The product was purified by column chromatography using ethyl acetate/petroleum ether or ethyl acetate/petroleum benzene (90/10) as eluent to get the final compounds (Scheme 1).

2.2. 1-(2-chloroethyl)-2methyl-5-nitro-1-H-imidazole (1)



Scheme 1. Synthesis of MTZ derivatives (2-6).

Five mmol (0.59 g, 0.36 ml) thionyl chloride and five mmol (0.855 g) of MTZ were added to 20 ml benzene at reflux condition for 8 h. After completion of reaction, the solvent was evaporated under vacuum and then the product was extracted with ethyl acetate. The product was purified by column chromatography with ethyl acetate/petroleum benzene (70/30) (90%, mp=98 °C).

1H -NMR(250MHz, $CDCl_3$), δ (ppm)=7.84(s, 1H, Imidazole), 4.51(t, 2H, N-CH₂), 3.76(t, 2H, CH₂-Cl), 2.45(s, 3H, CH₃).

^{13}C -NMR (125 MHz): 14.62, 30.79, 42.68, 47.54, 133.34, 138.20, 151.49.

MS, M/Z (%): 189(54), 143(100), 63(77), 154(8).

2.3. 1-[2-(diphenylmethoxy) ethyl]-2-methyl-5-nitroimidazole (2)

Five mmol (0.855 g) of MTZ and 5 mmol (1.013 g) of chlorodiphenyl methane were added to 10 ml of DMF in the presence of anhydrous potassium carbonate (0.40 g) and then the reaction was done under microwave radiation (11.3%, mp=148 °C). 1H -NMR (250 MHz, $CDCl_3$), δ (ppm)=7.87(s, 1H, Imidazole), 7.14–7.33(m, 10H, phenyl), 5.25(s, 1H, H_b), 4.55(t, 2H, N-CH₂), 3.84(t, 2H, O-CH₂), 2.65(s, 3H, CH₃).

^{13}C -NMR(125 MHz): 14.63, 46.57, 67.11, 84.23, 126.55, 127.78, 128.52.

MS, M/Z(%): 336

(M-1, 75), 165(100), 290(50), 154(25), 273(8),

182(8), 124(6).

2.4. 1-[2-(4chlorophenyl)-(phenylmethoxy) ethyl]-2-methyl-5-nitro-2-H-imidazole (3)

Five mmol (0.855 g) of MTZ and 5 mmol (1.185 g) of 4-chlorobenzhydryl chloride were added to 10 ml of DMF in the presence of anhydrous potassium carbonate (0.40 g) and then the reaction was done under microwave radiation condition (12.5%, mp=112 °C).

1H -NMR(250MHz, $CDCl_3$), δ (ppm)=7.89(s, 1H, Imidazole), 6.98–7.26(m, 9H, phenyl), 5.15(s, 1H, H_b), 4.59(t, 2H, N-CH₂), 3.69(t, 2H, O-CH₂), 2.40(s, 3H

,CH₃).
¹³C-NMR(125M
 Hz):55.24,113.20,127.14,127.83,127.87,127.92,
 128.06, 129.20, 130.07.
 MS,M
 /Z(%):371.5(17),165(100),201(84),109(50),
 325(19),125(17), 217(13).

2.5. 1-[2-(trityloxy)ethyl]-2-Methyl-5-nitro-1-H-imidazole (4)

Five mmol of MTZ (0.855 g) and 5 mmol (1.395 g) of chlorotriphenyl methane were added to 10 ml of DMF in the presence of anhydrous potassium carbonate (0.40 g) and then the reaction was done under microwave radiation condition (12.8%, mp=170 °C). ¹H-NMR (250 MHz, CDCl₃), δ(ppm)=7.95(s,1H,Imidazole),6.9–7.3(m,15H, phenyl), 4.48 (t, 2H, N-CH₂), 3.5 (t, 2H, O-CH₂), 2.5(s,3H,CH₃).
¹³C-NMR(125M
 Hz):14.67,46.54,62.14,127.28,127.70,127.93,
 128.35,132.95, 143.
 MS,M/Z(%):412
 (M-1,17),241(100),153(25),257(8),124(4),77(3).

2.6. 1-[(4-methoxyphenyl)-(diphenylmethoxy)ethyl]-2-methyl-5-nitro-1-H-imidazole (5)

Five mmol (0.855 g) of MTZ and 5 mmol (1.544 g) of 4-methoxy-chlorotriphenyl methane were added to 10ml of DMF in the presence of anhydrous potassium carbonate (0.40 g) and then the reaction was done under microwave radiation condition (6%, mp=124 °C). ¹H-NMR (250 MHz, CDCl₃), δ(ppm)=7.96(s,1H,Imidazole),6.98–7.19(m,14H,phenyl),4.44(m,2H,N-CH₂),4.02(m,1H,O-CH₂),3.38(s,3H,O-CH₃),3.79(m,2H,O-CH₂),2.64(s,3H,CH₃).
¹³C-NMR(125M
 Hz):55.24,113.20,127.14,127.83,127.87,127.92,
 128.06, 129.20,130.07.
 MS,M/Z(%):440(M-3,12.5),271(100),152(8).

2.7. 1-[2-bis(4-methoxyphenyl)-(phenylmethoxy)ethyl]-2-methyl-5-nitro-1-H-Imidazole (6)

Five mmol (0.855 g) of MTZ and 5 mmol (1.690 g) of 4,4'-dimethoxy-chlorotriphenyl methane were added to 10ml of DMF in the presence of anhydrous potassium carbonate (0.40 g) and then the reaction was done under microwave radiation condition (10.3%, mp=118 °C). ¹H-NMR(250MHz,CDCl₃), δ(ppm)=7.86(S,1H-

,Imidazole),6.74–7.22(m,13H,Ar),4.37(t,2H,N-CH₂),3.8(s,6H,O-CH₃),3.7(t,2H,O-CH₂),2.64(s,3H,CH₃)
¹³C-NMR (125 MHz): 14.66, 46.64, 55.22, 61.92, 113.19, 127.76, 127.90, 129.12, 129.76
 MS, M/Z (%): 473(8), 303(100), 154(8).

2.8. Isolation of Giardia lamblia cysts

Giardia lamblia cysts were isolated from faecal samples of giardiasis patients. Twenty samples which had no contamination with other parasites were selected. *Giardia* cysts were purified from the stool samples, using 1.2 gradients of sucrose. Briefly, 5-10 g of stool sample was added to 25 ml of distilled water mixed thoroughly. The samples was passed through a four-fold gauze and centrifuged at 4000 g for 10 min. Supernatant was discarded and the pellet was washed. In a 15 ml Falcon tube, 5 ml of 1.2 molar sucrose and 6 ml of parasite suspension was slowly added to the tube and centrifuged at 800 g for 7 min. The upper layer was removed and centrifuged at 1400 g. Pellet which were contained the purified cysts was washed with normal saline and stored at in PBS at 4 °C until use. Viability of the cysts was assessed by using 1% eosin. Numbers of viable cysts (negative staining by eosin) were determined by hemocytometer.

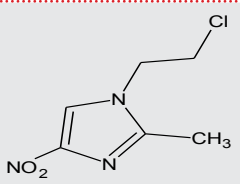
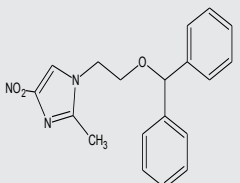
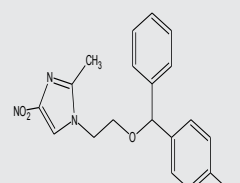
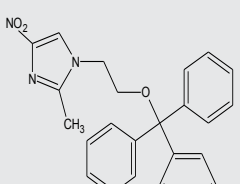
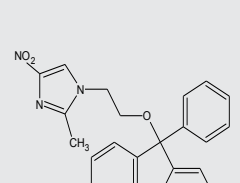
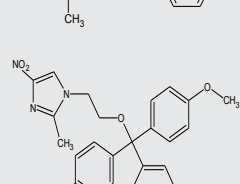
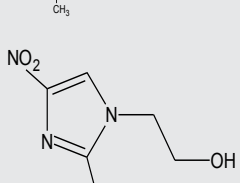
2.9. Assessment of anti-Giardia activities

In this study, four concentrations of each compound, 1, 2, 4 and 8 mM, were used. First, for each compound a stock solution of 20 mM in 2% DMSO was prepared and final dilution of compounds were prepared immediately before test. *Giardia* cysts suspension, 100µl, was exposed to different concentration of the compounds in a one ml Eppendorf tube in one ml volume solution. Tubes were kept at room temperature for 30 min and viability of the cysts was evaluated by 1% Eosin method. As a positive control, metronidazole powder was purchased from Sigma-Aldrich and was used at same concentration as compounds. For each isolate negative control was also considered where the cysts were exposed to DMSO solution (same concentration as used as diluent of the compounds). All experiments were performed in duplicate.

3. Results and discussion

3.1. Chemistry

Table 1. Anti-Giardia effects of metronidazole derivatives at different concentrations.

Compound	Mortality (%)			
	1 mM	2 mM	4 mM	8 mM
1 	25	36.47	38	37.57
2 	58.8	60.06	58.8	64.26
3 	29.72	39.13	48.95	47.9
4 	42.42	52.42	52.1	44
5 	32.17	37.76	46.15	44.52
6 	23.53	28.66	32.8	32.6
MTZ 	84.4	82.56	77.88	69.24

In this study, six MTZ derivatives were synthesized at microwave radiation and their chemical structures were confirmed by NMR and

Mass spectroscopy. However, the yield of reactions were less than 20% but the time of reactions were very fast and it is nature friendly and sup-

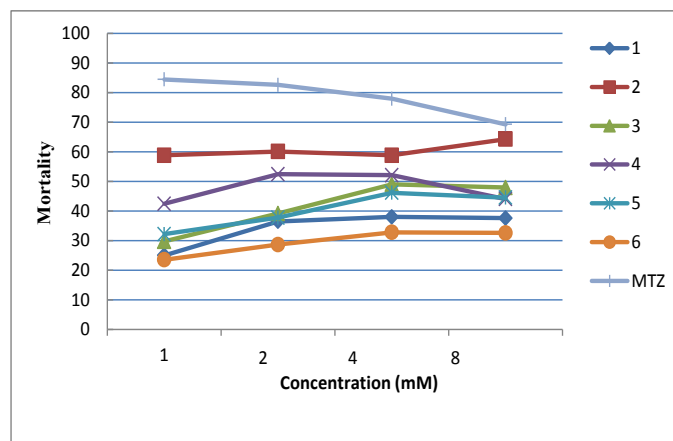


Figure 1. Mean of mortality rate of metronidazole derivatives (compounds 1-6) in comparison with MTZ con *Giardia* cysts.

3.2. Pharmacological assay

Mortality rate of controls, Eosin and DMSO, were less than 5%. MTZ at concentration of 1 to 8 mM had mortality rate of 84.4 to 69.24 percent. Statistical analysis of the results, using multivariate analysis, revealed that the efficacy of the studied compounds on *Giardia* cyst are different and these differences were statistically significant ($p < 0.05$). Using post HOC test, Tukey HSD, it was found that some of the compounds have a better efficacy against *Giardia* cysts. Compound 2 had the highest activity, compound 4 was the second most effective and compound 6 had the lowest effects against *Giardia* cysts. Compound 1 and 3 had the highest effect at 4 mM concentration while compound 2 had the highest efficacy at 8 mM concentration. The effect of compound 4 and 5 on *Giardia* cysts were not dose dependent.

Table 1 shows the anti-*Giardia* activities of the studied compounds and Fig. 1 shows the

mortality effect of the compounds on *Giardia* cysts in comparison with MTZ.

MTZ and other nitroimidazole derivatives like tinidazole are used in giardiasis infections. Our previous studies showed that MTZ derivatives with a bulky group in position 1 had anti-giardiasis effect (18), and also azole derivatives with diphenyl or triphenyl moiety showed antifungal and antimicrobial activity (19-23). In order to increase antimicrobial activity as well as improve physicochemical properties, here some derivatives of MTZ were synthesized with a flexible chain and a diphenyl or triphenyl group. Our results showed that compound 2 with diphenyl moiety had the best anti-giardiasis effect and compound 6 with dimethoxy-trityl moiety had the lowest activity. In conclusion increasing the lipophilicity and size of the substitutions decreased the activity.

Conflict of Interest:

None declared.

4. References

- Scorza AV, Lappin MR. Metronidazole for the treatment of feline giardiasis. *J Feline Med Surg.* 2004;6:157-60.
- Turner ML, Cockerell EJ, Brereton HM, Badenoch PR, Tea M, Coster DJ, Williams KA. Antigens of selected *Acanthamoeba* species detected with monoclonal antibodies. *Int J Parasitol.* 2005;35:981-90.
- Karabay O, Tamer A, Gunduz H, Kayas D, Arinc H, Celebi H. Albendazole versus metronidazole treatment of adult giardiasis: an open randomized clinical study. *World J Gastroenterol.* 2004;10:1215-7.

- Adam RD. The biology of *Giardia* spp. *Microbiol Rev.* 1991;55:706-32.
- Anthwal Amit, Rawat DS, Rawat MSM. 5-Nitroimidazole derivatives: a scope of modification for medicinal chemists. *Res J Chem Sci.* 2013;3:104-13.
- Cava T, Gozukara SE. Genotoxicity evaluation of metronidazole using the piscine micronucleus test by acridine orange fluorescent staining. *Environ Toxicol Pharmacol.* 2005;19:107-11.
- 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)-sub-

stituted thiosemicarbazones. *Bioorg Med Chem.* 2004;12:4679-84.

8. Sisson G, Goodwin A, Raudonikiene A, Hughes NJ, Mukhopadhyay AK, Berg DE, Hoffman PS. Enzymes associated with reductive activation and action of nitazoxanide, nitrofurans and metronidazole in *Helicobacter pylori*. *Antimicrob Agents Chemother.* 2002;46:2116-23.

9. Hoffman JS, Caves DR. Treatment of *Helicobacter pylori*. *Curr Opin Gastroenterol.* 2001;17:30-4.

10. World Health Organization, WHO model list of essential medicines, 15th edition, Available: <http://www.who.int/medicines/publications/Es-sMedList15.pdf>. (2013).

11. Kim DW, Park JM, Yoon BW, Baek MJ, Kim JE, Kim SY. Metronidazole-induced encephalopathy. *J Neurol Sci.* 2004;224:107-11.

12. 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. *Antimicrob Agents Chemother.* 1999;43:73-6.

13. Beena, Kumar Nitin, Rohilla Rajesh K., N. Roy, Rawat Diwan S. Synthesis and antibacterial activity evaluation of metronidazole-triazole conjugates. *Bioorg Med Chem Lett.* 2009;19:1396-8.

14. Bertinaria M, Galli U, Brenciaglia MI, Sorba, Fruttero GR, Maddalena Scaltrito M, Gasco A, Dubini F. Synthesis and anti-*Helicobacter pylori* properties of NO-donor/metronidazole hybrids and related compounds. *Drug Dev Res.* 2003;60:225-39.

15. Demirayak S, Karaburun AÇ, Kiraz N. Synthesis and antibacterial activities of some 1-[2-(substituted pyrrol-1-yl)ethyl]-2-methyl-5-nitroimidazole derivatives. *Eur J Med Chem.*

1999;34:275-8.

16. Kumar L, Sarswat A, Lal N, Sharma VL, Jain A, Kumar R, Verma V, Maikhuri JP, Kumar A, Shukla PK, Gupta G. Imidazole derivatives as possible microbicides with dual protection. *Eur J Med Chem.* 2010;45:817-24.

17. Saadeh HA, Mosleh IM, El-Abadelah MM. New Synthesis and antiparasitic activity of model 5-aryl-1methyl-4-nitroimidazoles. *Molecules.* 2009;14:2758-67.

18. Khabnadideh S, Rezaei Z, Khalafi Nezhad A, Motazedian MH, Eskandari M. Synthesis of metronidazole derivatives as anti-giardiasis agents. *DARU.* 2007;15:17-20.

19. Rezaei Z, Khabnadideh S, Zomorodian K, Pakshir K, Kashi G, Sanagoei N, Gholami S. Design, synthesis and antifungal activity of some new imidazole and triazole derivatives. *Arch Pharm life scie.* 2011;344:658-65.

20. Khabnadideh S, Rezaei Z, Ghasemi Y, Montazeri-Najafabadi N. Antibacterial activity of some new zole compounds. *Antiinfect Agents Med Chem.* 2012;10:26-33.

21. Rezaei Z, Khabnadideh S, Pakshir K, Hos-saini Z, Amiri F, Assadpour E. Design, synthesis, and antifungal activity of triazole and benzotriazole derivatives. *Eur J Med Chem.* 2009;44:30647.

22. Khabnadideh S, Rezaei Z, Khalafi Nezhad A, Pakshir K, Roosta A, Baratzadeh Z. Design and synthesis of imidazole and benzimidazol derivatives az antifungal agents. *Antiinfect Agents Med Chem.* 2008; 7: 215-8.

23. Khabnadideh S, Rezaei Z, Khalafi Nezhad A, Pakshir K, Heirran MJ, Shobeiri H. Design and synthesis of 2-Methyl and 2-Methyl-4-Nitro imidazole derivatives as antifungal agents. *IJPS.* 2009; 5: 37-44.