Bioorganic & Medicinal Chemistry Letters 25 (2015) 3545–3549

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Metronidazole hydrazone conjugates: Design, synthesis, antiamoebic and molecular docking studies

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ARTICLE INFO

*Article history:*
Received 11 February 2015
Revised 31 May 2015
Accepted 24 June 2015
Available online 3 July 2015

*Keywords:*
Metronidazole
Amoebiasis
*Entamoeba histolytica*
MTT-assay
Thioredoxin reductase

ABSTRACT

Metronidazole hydrazone conjugates (**2–13**) were synthesized and screened in vitro for antiamoebic activity against HM1: IMSS strain of *Entamoeba histolytica*. Six compounds were found to be better inhibitors of *E. histolytica* than the reference drug metronidazole. These compounds showed greater than 50–60% viability against HeLa cervical cancer cell line after 72 h treatment. Also, molecular docking study was undertaken on *E. histolytica* thioredoxin reductase (EhTHRase) protein which showed significant binding affinity in the active site. Out of the six actives, some of the compounds showed lipophilic characteristics.



Amoebiasis, a contagious disease of the human gastrointestinal tract caused by parasitic protozoa *Entamoeba histolytica* (*E. histolytica*).[1,2] The parasite causes invasive infections and induces tissue destruction, producing amoebic colitis, dysentery and liver abscesses that affects 50 million people and causes 100,000 death per annum worldwide.[3,4] Moreover, relapses of intestinal and hepatic amoebiasis have been reported.[5] Metronidazole (MNZ), tinidazole (TZ) and ornidazole (OZ) (Fig. 1) are the widely used medicament for the treatment of protozoal infections, in which MNZ is the drug of choice for the treatment of amoebiasis, but long term use causes several side effects.[6] Although, it is mutagenic in bacteria, carcinogenic to rodents and genotoxic to human cells.[7] However, due to inadequate epidemiological evidence, it is not considered as a risk factor of cancer in humans.

The side chains attached to MNZ provide an opportunity to carry out modifications to derive novel molecules which might exhibit better antiamoebic activity and lesser toxicity for the host. In our previous studies some metronidazole conjugates have been found to exhibit promising antiamoebic activities[8–10]

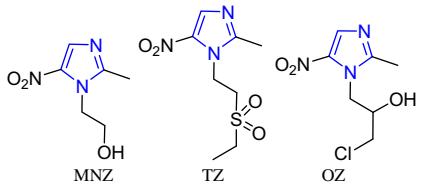


**Figure 1.** Antiamoebic drugs having imidazole ring.

(Fig. 2). Also, hydrazone derivatives are one of the widely studied pharmacophores showing a vast range of biological activities.[11] Hydrazone bearing pyridyl[12] or quinoline moiety[13] showing promising antiamoebic activity have been reported by us (Fig. 3). Considering this perspective, it was envisaged to modify the metronidazole framework to synthesize novel metronidazole hydrazone conjugates. In this paper, we herein report the synthesis, antiamoebic activity, molecular docking and lipophilic studies of metronidazole hydrazone conjugates (Fig. 4).

The synthetic pathway leading to target compounds (**2–13**) is depicted in Scheme 1. The key intermediate 4-[2-(2-methyl-5-nitro-1*H*-imidazole-1-yl) ethoxy]benzaldehyde (**1**), was synthesized by a reported method.[14] Further, the condensation reaction

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http://dx.doi.org/10.1016/j.bmcl.2015.06.091
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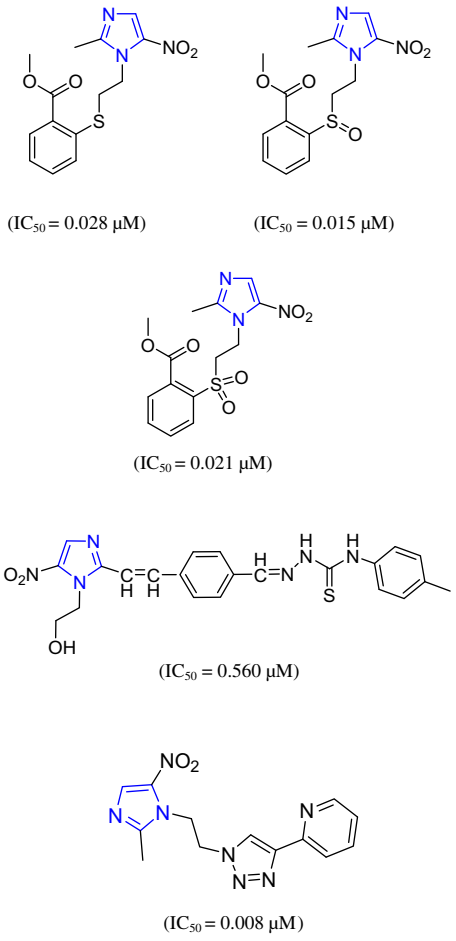



**Figure 2.** Metronidazole based compounds having antiamoebic activity.

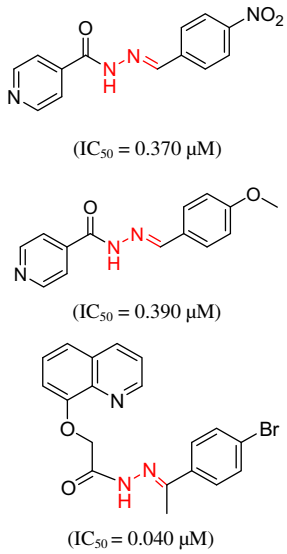



**Figure 3.** Hydrazones with antiamoebic activity.

of intermediate (**1**) with various aryl hydrazides furnished the final compounds (**2**–**13**). The structures of all the compounds were elucidated on the basis of FT-IR, $^{1}$H NMR, $^{13}$C NMR and ESI-MS.

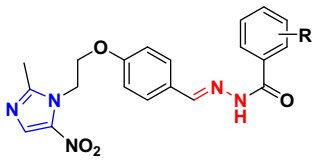
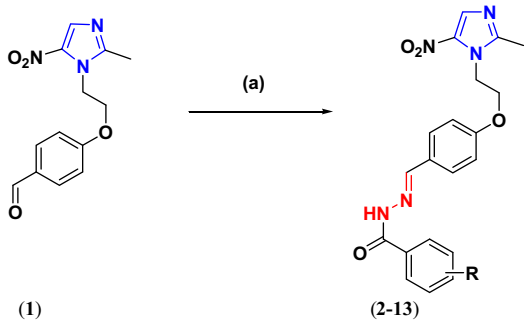


**Figure 4.** General structure of metronidazole hydrazone conjugates (blue and red colour depicts imidazole ring and hydrazone linkage respectively).




**Scheme 1.** Synthesis of Metronidazole hydrazone conjugates (**2**–**13**): Reagents and condition: (a) Different aryl hydrazides, ethanol, reflux.

The purity of the compounds was confirmed by the elemental analyses.

In order to explore the possible antiamoebic potential of newly synthesized metronidazole-hydrazone conjugates (**2**–**13**), all the compounds were screened against HM1: IMSS strain of *E. histolytica* by microdilution method[15] and the results were compared with the most widely used antiamoebic drug MNZ that had 50% inhibitory concentration ($IC_{50}$) 1.81 μM in our experiments. All the title compounds (**2**–**13**) exhibited better $IC_{50}$ values (0.20–7.12 μM) than the compound **1** ($IC_{50}$ = 11.48 μM). Compound **3** having chloro group at *para* position of phenyl ring exhibited most promising antiamoebic activity ($IC_{50}$ = 0.20 μM) followed by compound **5** ($IC_{50}$ = 0.36 μM) with nitro group at *para* position which can be attributed to electron withdrawing effect. Incorporation of hydroxy (**4**, $IC_{50}$ = 0.38 μM), amino (**8**, $IC_{50}$ = 0.43 μM) and methyl (**7**, $IC_{50}$ = 0.49 μM) group at *para* position, exerted significant inhibitory effect whereas the introduction of methoxy (**6**, $IC_{50}$ = 7.12 μM) and tertiary butyl group (**9**, $IC_{50}$ = 1.98 μM) at the same position did not affect the antiamoebic activity. Compounds **4** and **12** had mono-hydroxy and di-hydroxy substitution on the phenyl group respectively but their antiamoebic activities were almost same. However compounds having mono-methoxy (**6**), di-methoxy (**11**) and tri-methoxy (**13**) group vary in their antiamoebic activities with the increase in number of methoxy groups. Therefore, it can be concluded that the antiamoebic activity varied with the nature as well as the position of the substituents. However, a comparison between precursor (**1**) and final compounds revealed that the better antiamoebic activities of metronidazole–hydrazone conjugates are due to presence of hydrazone moiety.

Therefore, it can be concluded that the combination of 5-nitroimidazole, hydrazone and the nature as well as the position of substitution on the phenyl group was responsible for antiamoebic activity (Table 1).

Further to assess the effect of the compounds (**3**, **4**, **5**, **7**, **8** and **12**) on cervical cancer cell line, HeLa, cells (4000 cells/well) were plated in 96 well plate in triplicate. Cells were treated with compounds (**3**, **4**, **5**, **7**, **8** and **12**) as indicated in the Figure 5, the value