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European Journal of Medicinal Chemistry 43 (2008) 2035–2039
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and antiamoebic activity of metronidazole thiosemicarbazone analogues

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Received 23 June 2007; received in revised form 1 December 2007; accepted 6 December 2007
Available online 23 December 2007

Abstract

Repeated treatment of *Entamoeba histolytica* infection with commonly used antiamoebic drugs results in not only increasing the toxicity potential but also leads to the development of clinical resistance. Thus new effective agents with less toxicity against amoebiasis are urgently required. With this view, metronidazole thiosemicarbazone analogues **1**–**11** were synthesized wherein thioamide moiety was substituted by different cyclic and aromatic amines. These compounds were screened against *HM1:IMSS* strain of *E. histolytica* parasite cultured *in vitro* and the sensitivity of the parasite to the metronidazole thiosemicarbazones was evaluated using the microdilution method. Eight compounds (**1**–**4**, **7**–**9** and **11**) were found better inhibitors of *E. histolytica* growth since IC_{50} values elicited by these compounds were much lower than metronidazole with compound **4** showing the most promising antiamoebic activity ($IC_{50} = 0.56$ μM). The study suggests the beneficial potential of these leads that need to be further explored in order to discover and develop better and yet safer therapeutic agents for amoebiasis.

Keywords: Thiosemicarbazones; Antiamoebic activity; Metronidazole; *Entamoeba histolytica*

1. Introduction

Protozoan parasites including *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum* and other spore forming protozoa that parasitize human intestine are among the most common pathogens in the world responsible for affecting approximately 25% of the world population [1]. Among protozoal infections, amoebiasis is the most aggressive human disease next only to malaria [2]. According to current estimates it infects nearly 50 million people worldwide resulting in about 40,000–100,000 deaths annually mainly in tropical and subtropical countries [3]. Infection is primarily treated by instituting antiamoebic therapy. Antiamoebic drugs such as metronidazole, tinidazole, ornidazole, emetine kill amoeba in host tissue and organs (tissue amoebicides) whereas drugs like iodoquinol, diloxanide furoate, paromomycin act on large intestine (luminal amoebicides) are used for treatment. Particularly metronidazole is the most preferred treatment choice as 90% of patients respond to the therapy [4]. Also in the last several years a large number of new compounds have been isolated and/or synthesized of which a few have shown *in vitro* activity against *E. histolytica*. However, resistances to metronidazole in many pathogenic bacteria and protozoa as well as several side effects are also well documented [5]. Therefore it is desirable to search for new lead compounds.

Thiosemicarbazones are a class of small molecules that have been evaluated against *Plasmodium falciparum*, *Trypanosoma brucei* and *Trypanosoma cruzi* for various diseases. The effectiveness of thiosemicarbazone analogues in treating these diseases is reported due to their activity against cysteine proteinases including rhodesain [6–9]. In addition various thiosemicarbazone derivatives have been shown to possess anticancer, antiproliferative, antioxidant and many other biological properties [10–14]. Therefore, in view of these considerations we synthesized several metronidazole thiosemicarbazone

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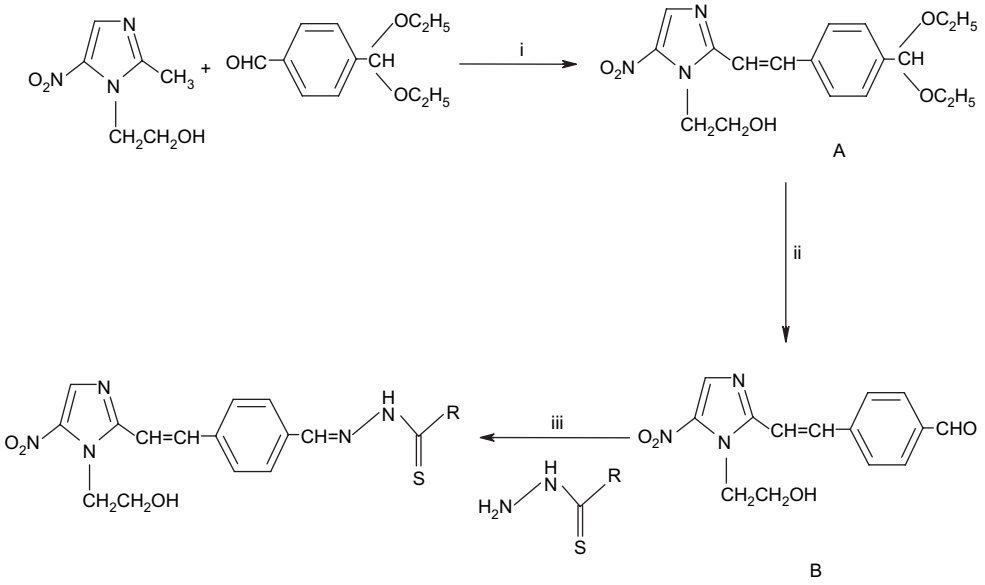
doi:10.1016/j.ejmech.2007.12.007

analogues and evaluated their antiamoebic activity *in vitro* against *HM1:IMSS* strain of *E. histolytica* to assess their ability to inhibit the growth of parasites.

2. Synthesis

All the thiosemicarbazone analogues of metronidazole **1–11** were synthesized by the general route (Scheme 1), followed by the modification of the N^4 substituent in appropriate cases. Reaction of metronidazole (12 mmol) with terephthaldialdehyde-monodiethylacetal (16 mmol) in 6 ml of DMSO by adding rapidly a stirred solution of sodium methoxide (12.8 mmol) in methanol at room temperature resulted in the formation of reaction intermediate **A**. The product obtained gave good yield (62%), and was confirmed by IR and ^{1}H NMR. The deprotection of the aldehyde group of **A** (7.4 mmol) was done by dissolving in tetrahydrofuran (12.5 ml) with stirring and warming to 50 °C. Water (0.25 ml) and conc. HCl (0.1 ml) were added and stirring was continued overnight. After cooling and standing at room temperature for 1 h, the yellow crystalline solid was collected in high amount and further recrystallized with ethanol to give 2-(4-carboxaldehyde-styryl)-1-(β-hydroxy ethyl)-5-nitro-imidazole **B**. The condensation of **B** (0.5 mmol) was done with various N^4-substituted thiosemicarbazides (0.5 mmol) in ethanol (5 ml). The reaction mixture was refluxed at 80 °C for 12–13 h and left overnight at room temperature. After cooling, the solid was filtered and recrystallized from appropriate solvent to give the desired metronidazole thiosemicarbazone analogues **1–11**. All the compounds could be isolated in good yield and were stable both in the solid and solution state. Analytical and spectral data (IR, electronic, ^{1}H and ^{13}C NMR) are in good agreement with the composition of the compounds [15]. Other analytical and physiochemical data of the compounds are presented in Table 1. The purity of the compounds was established by thin layer chromatography (TLC) and elemental analyses. Silica gel 60F254 was used to purify the compounds using chloroform and methanol (9.5:0.5) as the solvent system. The interest in the IR spectra of thiosemicarbazone analogues of metronidazole **1–11** lies mainly in the bands due to (NH–C=S) and (C=N) groups. All the compounds may exist in thione–thiol tautomerization since they contain a thioamide (NH–C=S) functional group. IR spectra of all the compounds indicate that all thiosemicarbazones retain their thione form in the solid state. This is further confirmed by the presence of a strong band at 1187–1061 cm^{-1} due to ν(C=S). A strong band appearing in the region 1629–1663 cm^{-1} is assigned to ν(C=N) stretch. The conjugated C=N of the imidazole ring was also observed but at low frequency at 1529–1590 cm^{-1}. The band due to (NH–C=S) was observed at 3210–3314 cm^{-1}. The OH group at the side chain of the imidazole ring was observed as a broad band at 3408–3492 cm^{-1}. The electronic spectra of all the thiosemicarbazones studied in the UV region in methanol exhibited three absorption bands at 395.7–382, 383–240 and 237–203 nm assignable to n → π*, π → π* and n → σ* transitions, respectively. The band at 395.7–382 nm is assigned to the n → π* transition involving the thione portion (C=S) of thiocarboxamide group. The two other absorption bands at 383–240 and 237–203 nm were due to π → π* transition of imidazole/phenyl ring and n → σ* transition of azomethine nitrogen, respectively. The ^{1}H NMR spectra recorded using $CDCl_3$ and DMSO-d_6 as the solvents clearly support the proposed structures of the compounds. The OH proton at imidazole ring was observed as a singlet at 8.15–9.96 ppm. The NH proton of the thioamide functionality appeared as a singlet at 7.85–8.87 and 7.82–8.62 ppm. Imidazole ring proton was also appeared as singlet at 8.01–8.32 ppm. The styryl protons were observed as two doublets at 7.61–7.92 and 7.23–7.82 ppm, respectively. The coupling



Scheme 1. (i) Sodium methoxide, DMSO, methanol, room temperature; (ii) HCl, THF, 50 °C; (iii) ethanol, 80 °C, reflux.