Bioorganic & Medicinal Chemistry Letters 22 (2012) 5694–5699

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Metronidazole thiosalicylate conjugates: Synthesis, crystal structure, docking studies and antiamoebic activity

Attar Salahuddin [a], Subhash M. Agarwal [b], Fernando Avecilla [c], Amir Azam [a,*]

[a] Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India
[b] Institute of Cytology and Preventive Oncology (ICPO) I-7, Sector—39, Noida 201301, Uttar Pradesh, India
[c] Departamento de Química Fundamental, Universidade da Coruña, Campus da Zapateira s/n, 15071 A Coruña, Spain

ARTICLE INFO

Article history:
Received 7 June 2012
Revised 23 June 2012
Accepted 26 June 2012
Available online 3 July 2012

Keywords:
Metronidazole
Amoebiasis
Entamoeba histolytica
MTT assay
Thioredoxin reductase

ABSTRACT

Metronidazole thiosalicylate conjugates were synthesized and crystallised in order to discover new molecules having better efficacy than therapeutically administered drug metronidazole, used against *Entamoeba histolytica*. The three compounds (**4**–**6**) showed lower IC_{50} values than metronidazole on HM1:IMSS strain of *E. histolytica* and displayed low cytotoxicity on MCF-7 cell line. In order to get an insight into the mechanisms of action of these compounds, a homology model of *E. histolytica* thioredoxin reductase (EhTHRase) was constructed and molecular docking was performed into the binding pocket to identify the nature of interactions. The docking studies suggest that the improved inhibitory activity of the newly synthesised metronidazole analogues could be due to involvement of the additional hydrophobic interactions in the binding mode. The result of the present study indicates the molecular fragments that play an essential role in improving the antiamoebic activity.

Invasive amoebiasis is an emerging problem in the developed East Asian countries men who had sex with men having co-infection of amoeba and HIV.[1] It is well known that amoebeasis caused by *E. histolytica* is transmitted by the ingestion of food or water containing the cyst form of *E. histolytica* which is prevalent in travelers and immigrants from endemic areas[2] causing significant morbidity and mortality.[3] Incidence of this infection is further increasing and now more than 50 million people are getting affected causing 100,000 fatalities worldwide annually.[4] Additionally, the infection is not limited to amoebic dysentery but also causes abscess in other body organs viz. liver[4] and brain[5] making it more dreadful and life threatening.

5-Nitroimidazole based drugs have been a boon to human beings for the treatment of infections caused by bacteria and a range of pathogenic protozoan parasites since 60 years. Presently, Metronidazole, Tinidazole and Ornidazole (Fig. 1) are the highly recommended drugs for the treatment of anaerobic protozoan infections.[6] The activity of 5-nitroimidazole drugs is ascribed to the reduction at the nitro group that results either in the formation of single-electron transfer reduction products, nitro radical anions or further reduced highly reactive intermediates, nitrosoimidazole or hydroxylamineimiadazoles.[7]

This reduction can either occur by reduced ferrodoxin[8] or by thioredoxin reductase.[9] The damage to the cells mainly occurs in two ways either by oxidative stress[10] or by the formation of adducts of non-protein thiol or protein with the intermediate metabolites (Fig. 2). Recently, in *E. histolytica* it has been proved that thioredoxin reductase is a target for nitroimidazole bearing drugs.[9]

In addition to accepted therapeutic efficacy clinical resistance to nitroimidazole based drugs has been observed and documented.[11] Besides, in many cases concern regarding carcinogenicity of metronidazole has also been raised.[12] To combat this neglected disease and minimize clinical resistance there is continuous need of designing and developing new compounds endowed with better activity and low toxicity. In recent years discovery of the novel hybrid molecules against protozoal diseases have displayed enhanced biological activities.[13] In view of this we have designed and synthesized some novel thiosalicylate analogs of metronidazole having different oxidation states of sulfur. Our interest in metronidazole analogs as an alternative to antiamoebic treatment is facilitated by the fact that not only metronidazole is effective but also side

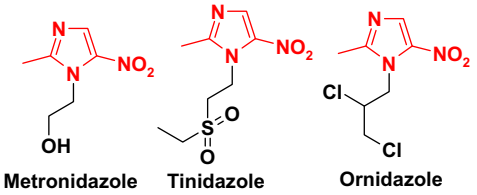


Figure 1. Antiprotozoal drugs Nitroimidazole core ring (red).

* Corresponding author. Tel.: +91 11 26981717/3254; fax: +91 11 26980229.
E-mail address: amir_sumbul@yahoo.co.in (A. Azam).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.06.083

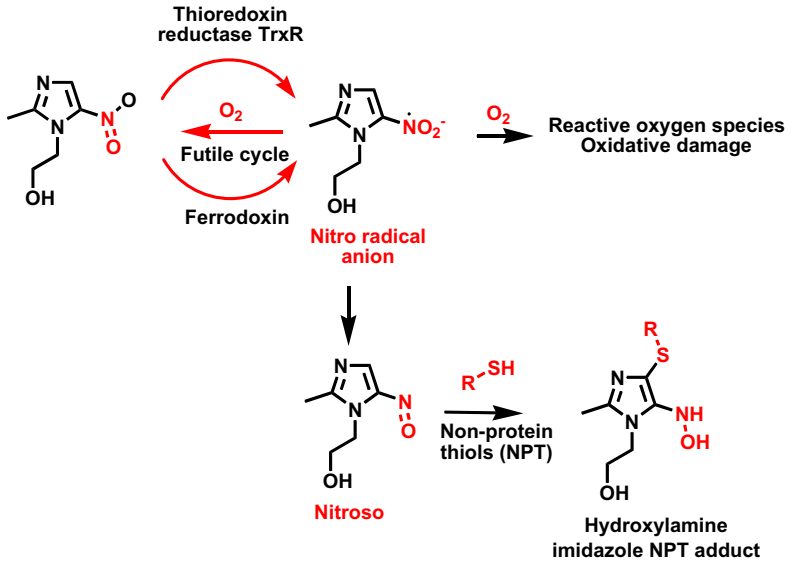


Figure 2. Role of thioredoxin reductase in Nitroimidazole activation[8–10].

chains attached to the imidazole ring structure provides an opportunity to carry out various modifications. Moreover, previously we have reported that different metronidazole analogues exhibit significant antiamoebic activity.[14]

The primary alcoholic group of metronidazole (**1**) was converted to the chloro group with thionyl chloride giving the hydrochloride salt of 1-(2-chloroethyl)-2-methyl-5-nitro-imidazole (**2**). Nucleophilic substitution of methyl thiosalicylate (**3**) produced the thioether linked metronidazole analogue (**4**). The thioether linkage was easily regioselectively oxidised to sulfoxide (**5**) by sodium metaperiodate and to sulfone (**6**) by metachloroperbenzoic acid (Scheme 1).[15]

The compound **4** crystallizes in the orthorhombic system, with $P2_12_12_1$ space group, and the compound **5** in the triclinic system, with P $\bar{1}$ space group (Fig. 3, Fig. 4 and Table 1 Supplementary data).[16] The angles C(1)–S(1)–C(9) were found to be 102.88(15)° in **4** and 95.79(7)° in **5,** and the angles O(3)–S(1)–C(1) and O(3)–S(1)–C(9) were found to be 105.93(7)° and 103.63(7)°, respectively,

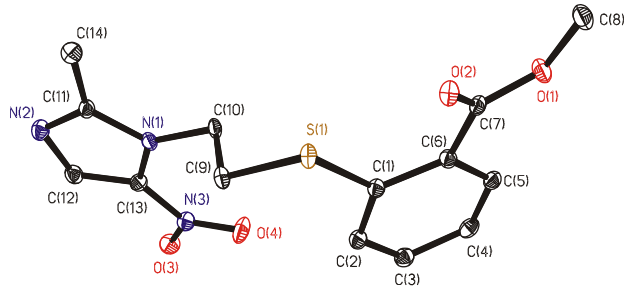
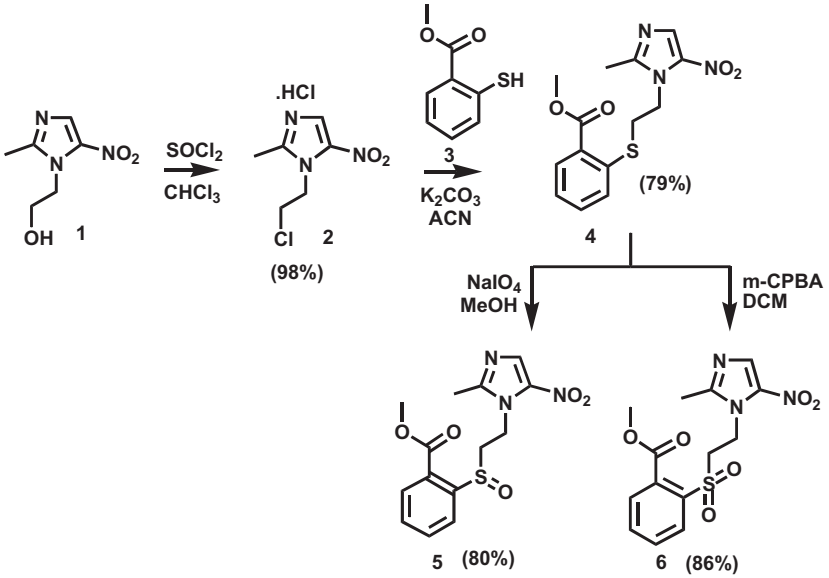


Figure 3. Molecular structure of 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethylthio) benzoate (4), showing the atomic numbering scheme. The ORTEP plot is at 30% probability level.

in **5**, all of them closer than S(sp^3). These angles can have an explanation for the presence of intramolecular non-bonded S···O interaction which appear in the crystalline structures of **4** and **5.** The



Scheme 1. Synthesis of metronidazole-thiosalicylate conjugates. The percentage in bracket shows yield.

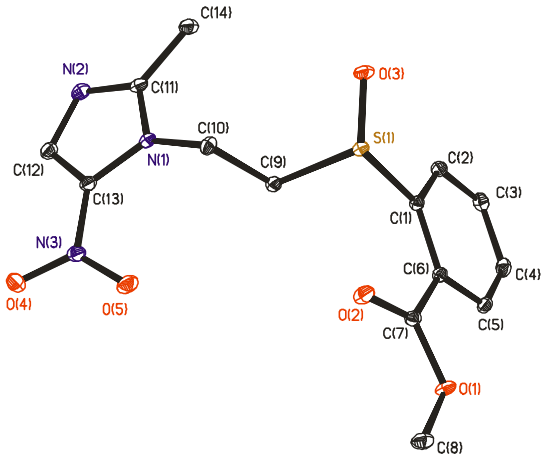


Figure 4. Molecular structure of 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethylsulfinyl) benzoate (**5**), showing the atomic numbering scheme. The ORTEP plot is at 30% probability level.

Table 1
In vitro antiamoebic activity and cytotoxicity of compounds **4**, **5**, **6** and metronidazole (MNZ)

Compound No.	Antiamoebic activity HM1:IMSS		Cytotoxicity MCF-7	
	IC_{50} (μM)	S.D.	IC50 (μM)	S.D.
4	0.028	0.002	>250	0.27
5	0.015	0.002	>250	0.24
6	0.021	0.003	>250	0.21
MNZ	1.46	0.011	>250	0.32

sum of the van der Waals radii for S–O is 3.25 Å. The non-bonded S···O distances were found to be 2.760 Å, and 2.749 Å, respectively. The quasi-rings adopt planar, conjugated five-membered structures, closed by the non-bonded sulfur and oxygen atoms. In **5**, s-*cis*–s-*trans* (synperiplanar-antiperiplanar) arrangement was found around the C–C and C–S single bonds.[17] The planarity of the S(1), O(2), C(1), C(6) and C(7) moiety is better recognized in the molecule of **5** than in **4** (mean deviations from planarity were found to be, 0.1002 Å in **4**, and 0.0532 Å in **5**). Torsion angles for S(1)–C(1)–C(6)–C(7) and C(1)–C(6)–C(7)–O(2) were found to be 7.3(4)° and 15.3(4)° in **4,** and 6.2(2)° and 5.5(2)° in **5**. This fairly stable S···O close contact might play an important role in the low cytotoxicity of these compounds.[18] The planes containing benzyl and imidazole rings form a dihedral angles of 29.47(14)° in **4**, and 72.07(6)° in **5**. In the crystal packing of **5**, the distance between the centers of imidazole rings is 3.633 Å, which indicate the presence of π–π stacking interactions between them. In **4**, π–π stacking interactions appear between imidazole and benzene rings and the distance between the centers of the rings is 3.585 Å.

Experiments were carried out to determine the in vitro antiamoebic activity of the three compounds (**4**–**6**) and MNZ by microdilution method using HM1:IMSS strain of *E. histolytica*.[19,20] All the experiments were carried out in triplicate at each concentration level and repeated thrice. The data is presented in terms of percent growth inhibition relative to untreated controls and plotted as probit values as a function of drug concentration. The antiamoebic effect was compared with the most widely used antiamoebic medication metronidazole which had a 50% inhibitory concentration (IC_{50}) of 1.46 μM in our experiments. The Sulfoxide compound (**5**) was most active with IC_{50} = 0.015 μM. The sulfone compound (**6**) showed less activity than the Sulfoxide with IC_{50} = 0.021 μM. Among the three compounds the sulfide (**4**) was least active with IC_{50} = 0.028 μM. The results are summarized in Table 1.

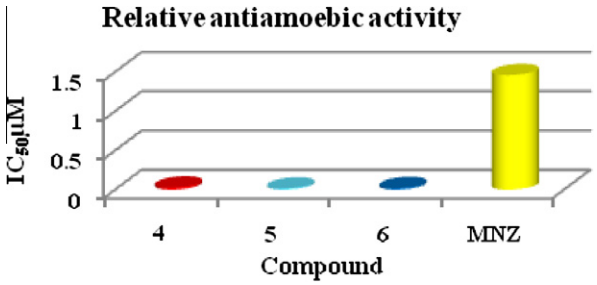


Figure 5. Relative in vitro antiamoebic activity.

All the thiosalicylate analogs evaluated for their antiamoebic activity were relatively found more active than the standard drug metronidazole. (Fig. 5)

To examine the effect of compounds **4**, **5** and **6** as well as metronidazole on cell proliferation, we studied their cytotoxicity on human breast cancer MCF-7 cell line by MTT assay.[21,22], A subconfluent population of MCF-7 cells was treated with increasing concentrations of compounds and the number of viable cells was measured after 48 h by MTT cell viability assay based on mitochondrial reduction of the yellow MTT tetrazolium dye to a highly blue colored formazan product. This assay usually shows high correlation with number of living cells and cell proliferation.

The concentration range for all the compounds (**4**, **5**, **6** & MNZ) is mentioned in Figure 6, which illustrates that all the compounds and metronidazole were non toxic in the concentration range of 2.5–250 μM.

It was recently demonstrated by applying two-dimensional gel electrophoresis and mass spectrometry that thioredoxin reductase reduces metronidazole and other nitro compounds suggesting a central role for this enzyme in the treatment of infections caused by microaerophilic parasites, including *E. histolytica*.[9] Therefore, in order to offer insight into the mechanisms of action of the metronidazole based analogs synthesized and crystallized (**4**, **5**) we undertook homology modeling of the amoebic enzyme and identified the interactions of inhibitors with the model.[23,24] To build the 3D model of EhTHRase, a BLAST search was performed against the Protein Data Bank and Yeast THRase (PDB code 3D8X) was selected as the starting scaffold for model construction as it showed maximum homology (75%). The sequence alignment of the 3D8X with EhTHRase sequence that was used for model construction is shown in Figure 7.

Homology modeling was then carried out through Modeller 9v9.[25] The final predicted structure was checked for main chain conformation using PROCHECK,[26] which showed that 92.3% of the residues were in the 'most favoured region' and 7.7% in the combined 'allowed region' as compared to 84.5% and 15.5%, respectively for the template. No residue was found in the

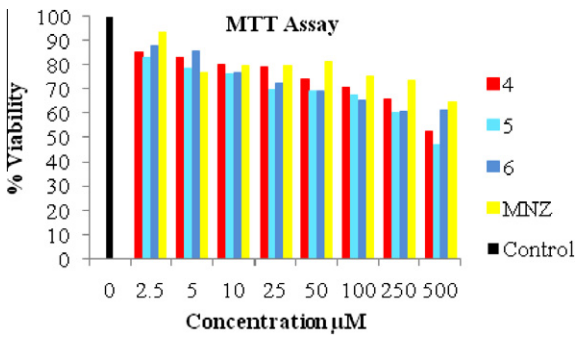


Figure 6. Cytotoxicity assessment by MTT Assay.

```
EhTHR   5    HDVVIIGSGPAAHTAAIYLGRSSLKPVMYEGFMAGGVAAGGQLTTTTIIENFPGFPNGID  64
             + V IIGSGPAAHTAAIYL R+ +KP++YEG MA G+AAGGQLTTTT IENFPGFP+G+
3D8X    11   NKVTIIGSGPAAHTAAIYLARAEIKPILYEGMMANGIAAGGQLTTTTEIENFPGFPDGLT  70

EhTHR   65   GNELMMNMRTQSEKYGTTIITETIDHVDFSTQPFKL---FTEEGKEVLTKSVIIATGATA  121
             G+ELM  MR QS K+GT IITET+  VD S++PFKL   F E+ + V T ++I+ATGA+A
3D8X    71   GSELMDRMREQSTKFGTEIITETVSKVDLSSKPFKLWTEFNEDAEPVTTDAIILATGASA  130

EhTHR   122  KRMHVPGEDKYWQNGVSACAICDGAVPIFRNKVLMVVGGGDAAMEEALHLTKYGSKVIIL  181
             KRMH+PGE+ YWQ G+SACA+CDGAVPIFRNK L V+GGGD+A EEA  LTKYGSKV +L
3D8X    131  KRMHLPGEETYWQKGISACAVCDGAVPIFRNKPLAVIGGGDSACEEAQFLTKYGSKVFML  190

EhTHR   182  HRRDAFRASKTMQERVLNHPKIEVIWNSELVELEGDGDLLNGAKIHNLVSGEYKVVPVAG  241
              R+D  RAS  MQ+R   + KIE+++N+  +E +GDG LLN  +I N    E   +PV+G
3D8X    191  VRKDHLRASTIMQKRAEKNEKIEILYNTVALEAKGDGKLLNALRIKNTKKNEETDLPVSG  250

EhTHR   242  LFYAIGHSPNSKFLGGQVKTADDGYILT--EGPKTSVDGVFACGDVCDRVYRQAIVAAGS  299
             LFYAIGH+P +K + GQV T + GYI T      TSV G FA GDV D  YRQAI +AGS
3D8X    251  LFYAIGHTPATKIVAGQVDTDEAGYIKTVPGSSLTSVPGFFAAGDVQDSKYRQAITSAGS  310

EhTHR   300  GCMAALSCEKWLQT  313
             GCMAAL  EK+L +
3D8X    311  GCMAALDAEKYLTS  324
```

Figure 7. Sequence alignment of EhTHRase with the template (Pdb id: 3D8X).

disallowed region. The PROCHECK result summary also showed only 5 of 307 residues labeled while the torsion angles of the side chain designated by $\chi1-\chi2$ plots showed only 6 labeled residues out of 167. Also, it is established that the score for G-factors should be above −0.50 for a reliable model. We observed that the G-factor scores of the model was 0.02 for dihedral bonds, −0.14 for covalent bonds and −0.14 overall. The distribution of the main chain bond lengths and bond angles was 99.5% and 94.2% within limits. The high quality of the structure is further evident by the fact that according to VERIFY 3D[27] 98.39% of the residues have a score of greater than 0.2, which indicates a good quality model. Further, the root-mean-square deviation (RMSD) between the backbone atoms of the template and the homology model was observed to be 0.302 Å indicating reasonably good structural parameters of the predicted structure (Fig. 8).

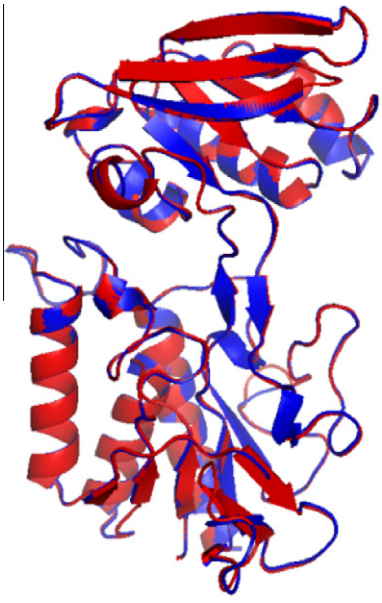


Figure 8. Structural superimposition of Cα trace of EhTHRase model (represented in blue color) with known crystal structure (represented in red color). The root-mean-square deviation (RMSD) between the template and the homology model was 0.302 Å.

After the final model was built, the active site information was obtained through superimposing 3-D structure of the EhTHRase model with that of template protein 3D8X. Active site of modeled thioredoxin reductase was constituted by amino acid residues Met 124, Gly 159, Gly 161, Ala 163, Arg 183, Arg 184 Arg 188 and Ile 246 corresponding to Met 126, Gly 161, Gly 163, Ser 165, Arg 185, Lys 186, Arg 190 and Ile 248 of template protein. Thus, the active site forming residues were found to be mostly conserved (Fig. 9).

Further, in order to decipher the possible interactions of the crystal resolved inhibitors (**4** and **5**) with EhTHRase, the inhibitors were docked in the binding pocket of the modeled protein using GOLD v3.1.1 program.[28] It was noted that GOLD score of **4** and **5** was 59.29 and 59.70 respectively, which is greater than metronidazole (antiamoebic drug in use) score value 39.33. This is in accordance with our activity profile data, which indicated that both compounds (**4**) and (**5**) have IC_{50} value lower than the standard drug metronidazole. Further it is evident from the ligplot[29] analysis of docked complexes that the inhibitors place themselves nicely into the active site of the enzyme and are mainly stabilized by the hydrophobic interactions (Fig. 10a and 10b). The higher activity of the synthesized metronidazole analogs compared to MNZ could be partially due to involvement of these additional hydrophobic interactions in the binding mode.

Specifically, the residues that interact with compound **4** and contribute to the hydrophobic interactions are Met 124, Val 158, Gly 159, Leu 181, His 182, Arg 184, Glu 210 and Ile 246, while

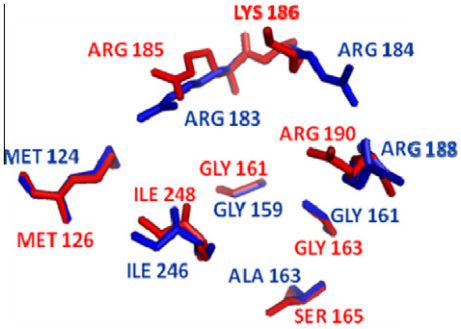


Figure 9. Overlay of the active site residues of the homology model with the template 3D8X. Blue and red color sticks represents modeled and template proteins respectively.

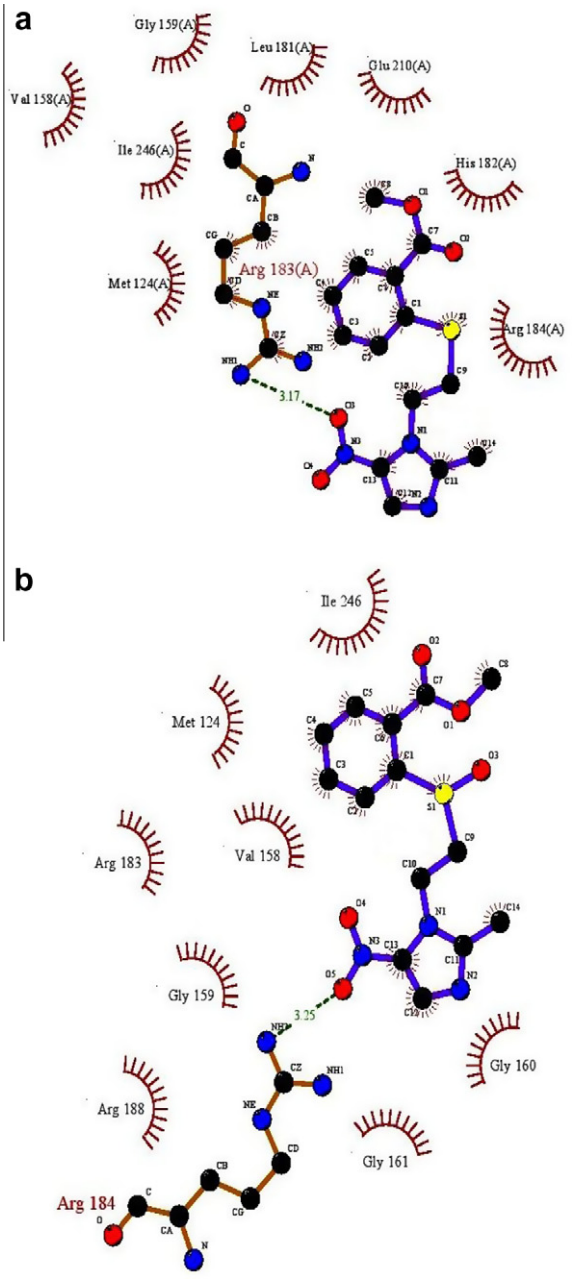


Figure 10. Schematic 2D representation of interactions of (a) Compound **4** and (b) Compound **5**. Hydrogen bonds are shown with green dashed lines and hydrophobic contacts by red arcs with radiating lines.

the NH1 atom of Arg 183 of the active site of the receptor forms hydrogen bond with O3 of compound **4** (Fig. 10a). In case of Compound **5**, it interacts predominantly with amino acid residue Arg 184 through hydrogen bond and other non ligand residues that shows involvement in hydrophobic interactions are Met 124, Val 158, Gly 159, Gly 160, Gly 161, Arg 183, Arg 188 and Ile 246. (Fig. 10b). Previously too, studies from others[30] as well as from our group[14b] have demonstrated that 5-nitroimidazole analogues having hydrophobic side chains show better antiamoebic activity probably because these modifications enhance solubility and membrane permeability of the compounds. Thus, the docking simulations corroborate the observed activity profile and confirm the basis that has led to the development of newer molecules.

To conclude, the present study describes the synthesis of metronidazole thiosalicylate analogs (**4**–**6**) and their evaluation as antiamoebic agents. It was found that 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethylsulfinyl) benzoate (**5**) shows promising in vitro antiamoebic activity as well as low cytotoxicity. Further, docking simulations indicated that the enhanced antiamoebic activity could be due to additional hydrophobic interactions in the binding site of *E. histolytica* thioredoxin reductase. These findings provide us lead and encourage us to continue the efforts towards the optimization of the efficacy profile of this structural moiety for treatment of amoebiasis.

Acknowledgments

This work was supported by Council of Scientific and Industrial Research (CSIR Grant # 01(2278)/08/EMR-II New Delhi, India). One of us (A.S) acknowledges the Senior Research Fellowship from the CSIR.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.083.

References and notes

1. Watanabe, K.; Gatanaga, H.; Cadiz, E. A.; Tanuma, J.; Nozaki, T.; Oka, S. *PLoS Negl. Trop. Dis.* **2011**, *5*, e1318.
2. Haque, R.; Huston, C. D.; Hughes, M.; Houpt, E.; Petri, W. A., Jr *N. Engl. J. Med.* **2003**, *348*, 1565.
3. Li, E.; Stanley, S. L., Jr *Gastroenterol. Clin. North Am.* **1996**, *25*, 471.
4. Sharma, N.; Sharma, A.; Varma, S.; Lal, A.; Singh, V. *BMC Res Notes* **2010**, *3*, 21.
5. Ozüm, U.; Karadağ, O.; Eğilmez, R.; Engin, A.; Oztoprak, I.; Ozçelik, S. *Br. J. Neurosurg.* **2008**, *22*, 596.
6. Azam, A.; Agarwal, S. M. *Curr. Bioact. Compd.* **2007**, *3*, 121.
7. Moreno, S. N.; Docampo, R. *Environ. Health Perspect.* **1985**, *64*, 199.
8. (a) Yarlett, N.; Yarlet, N. C.; Lloyd, D. *Biochem. Pharmacol.* **1986**, *35*, 1703; (b) Vanacova, S.; Liston, D. R.; Tachezy, J.; Johnson, P. J. *Int. J. Parasitol.* **2003**, *33*, 235.
9. Leitsch, D.; Kolarich, D.; Wilson, I. B.; Altmann, F.; Duchene, M. *PLoS Biol.* **2007**, *5*, e211.
10. (a) Mason, R. P.; Holtzman, J. L. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 1267–1274; (b) Zeller, T.; Klug, G. *Naturwissenschaften* **2006**, *93*, 259.
11. (a) Johnson, P. J. *Parasitol. Today* **1993**, *9*, 183; (b) Upcroft, J. A.; Upcroft, P. *Parasitol. Today* **1993**, *9*, 187; (c) Voolmann, T.; Boreham, P. F. L. *Med. J. Aust.* **1993**, *159*, 490.
12. (a) Legator, MS.; Connor, TH.; Stoeckel, M. *Science* **1975**, *188*, 1118; (b) Bendesky, A.; Menéndez, D.; Ostrosky-Wegman, P. *Mut. Res.* **2002**, *511*, 133; (c) Lindmark, D. G.; Muller, M. *Lindmark and Miklós Müller Antimicrob. Agents Chemother.* **1976**, *10*, 476.
13. (a) Meunier, B. *Acc. Chem. Res.* **2008**, *41*, 69; (b) Chavain, N.; Davioud-Charvet, E.; Trivelli, X.; Mbeki, L.; Rottmann, M.; Brun, R.; Biot, C. *Bioorg. Med. Chem.* **2009**, *17*, 8048; (c) Muregi, F. W.; Ishih, A. *Drug Dev. Res.* **2010**, *71*, 20.
14. (a) Athar, F.; Husain, K.; Abid, M.; Agarwal, S. M.; Coles, S. J.; Hursthouse, M. B.; Maurya, M. R.; Azam, A. *Chem. Biodivers.* **2005**, *2*, 1320; (b) Abid, M.; Agarwal, S. M.; Azam, A. *Eur. J. Med. Chem.* **2008**, *43*, 2035.
15. Synthesis Procedure
(a) *Synthesis of 1-(2-chloroethyl)-2-methyl-5-nitro-1H-imidazole Hydrochloride* (**2**): To a stirred solution of Metronidazole (1) (5 g, 15.57 mmol) in 100 mL of chloroform at room temperature was added thionyl chloride 10 mL. The resulting mixture was stirred for 12 h and concentrated under vacuo to yield hydrochloride salt of compound (2) Yield 98%; mp: 92–93 °C; Anal. calcd for $C_6H_{10}ClN_3O_2$: C 37.61, H 18.50, N 21.93% found: C 37.89, H 18.22, N 22.2%; ^{1}H NMR ($CDCl_3$) δ (ppm): 8.0 (s,1H), 8.24 (4.64 t, 2H, J = 5.7 Hz), 3.90 (t, 2H, J = 5.7 Hz), 2.58 (s, 3H), FAB-MS (m/z): [M$^+$+1] 192.
(b) *Synthesis of methyl 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethylthio) benzoate* (**4**): To a stirred solution of methyl thiosalicylate (5 g, 29.72 mmol) in 100 mL of acetonitrile was added potassium carbonate (12.3 g, 89.16 mmol), the reaction mixture was then stirred for 15 min. at room temperature and portion wise compound 2 (6.78 g, 29.72 mmol) was added. The reaction mixture was then refluxed for 24 h and concentrated. The residue was partitioned between ethylacetate and water, the organic layer was separated and washed with brine, dried over anhydrous sodium sulphate and concentrated to yield crude product which was crystallized in ethanol to give yellow crystals of compound 4. Yield 79.8%; mp: 148–150 °C; Anal. calcd for $C_{14}H_{15}N_3O_4S$: C, 52.33; H, 4.70; N, 13.08; S, 9.98, found: C, 52.11; H, 4.51; N, 13.2; S, 9.9%; IR ν_{max} (cm^{-1}): 1735 (C=O), (C–S–C), 1537 (NO_2); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.96 (d, 1H, J = 7.8 Hz), 7.91 (s,1H), 7.53-7.43 (m, 2H), 7.23 (d, 1H, J = 7.5 Hz) 4.55 (t, 2H, J = 6.9 Hz), 3.92 (s, 3H), 3.37 (t, 2H, J = 7.2 Hz), 2.44 (s,

3H), ^{13}C NMR ($CDCl_3$) δ (ppm): 166.7, 150.69, 138.32, 137.25, 133.31, 132.64, 131.57, 128.71, 126.13, 125.16, 52.32, 45.45, 31.64, 14.42. FAB-MS (m/z): [M^+] 322.1 (100.0%) [M^++1]323.09 (16.1%).

(c) *Synthesis of methyl 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethylsulfinyl) benzoate* (**5**): To a stirred solution of compound 4 (4 g, 12.4 mmol) in methanol 30 mL was added sodium metaperiodate (3.98 g, 18.61 mmol) the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated and the residue was diluted with water and extracted with dichloromethane (DCM), the combined organic extracts were washed with brine and concentrated to yield crude compound 5 which was purified by column (DCM/MeOH) chromatography to give pure product which was crystallized in 30% DCM:hexane. Yield 80%; mp: 178–180 °C; Anal. calcd for $C_{14}H_{15}N_3O_5S$: C, 49.84; H, 4.48; N, 12.46; O, 23.71; S, 9.50; found: C, 49.8; H, 5.2; N, 12.59; S, 9.47%; IR ν_{max} (cm^{-1}): 1743 (C=O), 1052 (S=O), 1533 (NO2); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.21–8.23 (dd, 1H, *J* = 7.5 & 0.6 Hz), 8.09-8.12 (dd,1H *J* = 7.8 & 1.2 Hz), 7.99 (s, 1H), 7.80–7.86 (dt, 1H *J* = 7.8 &1.2 Hz), 7.57–7.63 (dt, 1H *J* = 7.8 &1.2 Hz), 4.73–4.76 (m, 2H), 3.87 (s, 3H), 3.61–3.71 (m, 1H), 3.01–3.09 (m, 1H), 2.68 (s, 1H) ^{13}C NMR ($CDCl_3$) δ (ppm): 165.75, 150.94, 146.76, 138.81, 134.01, 133.43, 131.22, 130.78, 126.52, 124.62, 56.52, 52.89, 41.03, 14.54. FAB-MS (*m*/*z*): [M^+] 338.02, [M^++1] 339.08 (17.1%).

(d) *Synthesis of methyl 2-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethylsulfonyl) benzoate (6)*: To a stirred solution of compound 4 (2 g, 6.2 mmol) in 25 mL of DCM was added slowly m-Chloro perbenzoic acid (2.24 g, 13.02 mmol), the reaction mixture was then stirred for 4–5 h and precipitate was obtained and filtered. The filtrate was then washed once with saturated sodium bicarbonate and then with brine, dried over anhydrous sodium sulphate and concentrated to yield crude compound 6 which was purified with column chromatography (DCM/MeOH) Yield 86.5%; mp: 115–116 °C; Anal. calcd for $C_{14}H_{15}N_3O_6S$: C, 47.59; H, 4.28; N, 11.89; S, 9.07 found: C, 47.53; H, 4.55; N, 11.7; S, 9.1;%; IR ν_{max} (cm^{-1}): 1752 (C=O), 1315 1150 (SO2), 1530 (NO2); ^{1}H NMR ($CDCl_3$) δ (ppm): 7.98 (d, 2H, *J* = 7.2 Hz), 7.93 (s,1H), 7.46-7.54 (m, 2H), 7.25 (d, 1H *J* = 7.4 Hz), 4.82 (t, 2H *J* = 8.8 Hz), 4.07 (t, 2H, *J* = 8.8 Hz), 3.94 (s, 3H), 2.64 (s, 3H) ^{13}C NMR ($CDCl_3$) δ (ppm): 167.09, 151.1, 138.32, 137.3, 134.3, 133.23, 132.99, 131.56, 130.38, 130.24, 55.13, 53.37, 39.71, 14.34. FAB-MS (*m*/*z*): [M^+] 354 (100.0%), [M^++1] 355.08 (16.3%), [M^++2] 356.07 (4.2%).

16. *X-ray crystal structure determination*: Three-dimensional X-ray data were collected on a Bruker SMART Apex CCD diffractometer at 100(2) K, using a graphite monochromator and Mo-K_α radiation (λ = 0.71073 Å) by the ϕ-ω scan method. Reflections were measured from a hemisphere of data collected of frames each covering 0.3 ° in ω. Of the 13972 in 4 and 18584 in 5 reflections measured, all of which were corrected for Lorentz and polarization effects, and for absorption by semi-empirical methods based on symmetry-equivalent and repeated reflections, 2152 in 4 and 2720 in 5 independent reflections exceeded the significance level $|F|/\sigma(|F|)>4.0$. Complex scattering factors were taken from the program package SHELXTL.[16] The structures were solved by direct methods and refined by full-matrix least-squares methods on F^2. The non-hydrogen atoms were refined with anisotropic thermal parameters in all cases. All hydrogen atoms were left to refine freely. A final difference Fourier map showed no residual density outside: 0.197 and −0.341 in 4 and 0.554 and −0.419 in compound 5 e.Å^{-3}. CCDC No. 872750 for compound 4 and 872751 for 5 contain the supplementary crystallographic data for this Letter. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.
17. Ángyán, J. G.; Poirier, R. A.; Kucsman, Á.; Csizmadia, I. G. *J. Am. Chem. Soc.* **1987**, *109*, 2237.
18. Nagao, Y.; Hirata, T.; Goto, S.; Sano, S.; Kakehi, A.; Iizuka, K.; Shiro, M. *J. Am. Chem. Soc.* **1998**, *120*, 3104.
19. Wright, C. W.; O_Neill, M. J.; Phillipson, J. D.; Warhurst, D. C. *Antimicrob. Agents Chemother.* **1988**, *32*, 1725; (b) Diamond, L. S.; Harlow, D. R.; Cunnick, C. C. *Trans. R Soc. Trop. Med. Hyg.* **1978**, *72*, 431; (c) Gillin, F. D.; Reiner, D. S.; Suffness, M. *Antimicrob. Agents Chemother.* **1982**, *22*, 342; (d) Keene, A. T.; Harris, A.; Phillipson, J. D.; Warhurst, D. C. *Plant. Med.* **1986**, 278.
20. *In vitro antiamoebic assay: Compounds 4, 5 and 6 were screened in vitro for antiamoebic activity against HM1*:IMSS strain of *E. histolytica* by microdilution method. *E. histolytica* trophozoites were cultured in culture tubes by using Diamond TYIS-33 growth medium. The test compounds (1 mg) were dissolved in DMSO (40 µL, level at which level no inhibition of amoeba occurs). The stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/mL. Twofold serial dilutions were made in the wells of 96-well microtiter plate (costar). Each test includes metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) and a blank (culture medium only). All the experiments were carried out in triplicate at each concentration level and repeated thrice. The amoeba suspension was prepared from a confluent culture by pouring off the medium at 37 °C and adding 5 mL of fresh medium, chilling the culture tube on ice to detach the organisms from the side of flask. The number of amoeba/mL was estimated with the help of a heamocytometer, using trypan blue exclusion to confirm the viability. The suspension was diluted to 10^5 organism/ml by adding fresh medium and 170 µL of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 µL). An inoculum of 1.7×10^4 organisms/well was chosen so that confluent, but not excessive growth, took place in control wells. Plate was sealed and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h. After incubation, the growth of amoeba in the plate was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed with sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly and the plate was not allowed to cool in order to prevent the detachment of amoeba. The plate was allowed to dry at room temperature and the amoebae were fixed with methanol and when dried, stained with (0.5%) aqueous eosin for 15 min. The stained plate was washed once with tap water, then twice with distilled water and then allowed to dry. 200 µl portions of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a micro plate reader. The% inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best fitting line from which the IC_{50} value was found.
21. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
22. *Cytotoxicity*: MCF-7 cells were cultured and maintained as a monolayer in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% of fetal calf serum (Sigma) and 1% antibiotics penicillin/streptomycin/Neomycin. All cells were cultured at 37 °C in a 100% humidity atmosphere and 5% CO_2. The effect of compounds 4, 5, 6 and the standard drug Metronidazole on cell proliferation was measured by using an MTT based assay.[18] Exponentially growing viable cells were plated at 1.2×10^4 cells per well into 96-well plates and incubated for 48 h before the addition of the compounds/metronidazole. Stock solutions of compounds were initially dissolved in 20% (v/v) DMSO and further diluted with fresh complete medium. The growth-inhibitory effects of the compounds were measured using standard tetrazolium MTT assay. After 48 h of incubation at 37 °C and 5% CO_2, the medium was removed and 25 µL of MTT (5 mg/mL) in serum free medium was added to each well. The plates were incubated at 37 °C for 4 h. At the end of the incubation period, the medium was removed and 100 µL DMSO added to all wells. The metabolized MTT product dissolved in DMSO was quantified by reading the absorbance at 570 nm with a reference wavelength of 655 nm in an ELISA plate reader (Labsystems Multiskan RC, Helsinki, Finland. All assays were performed in triplicate. Percent viability was defined as the relative absorbance of treated versus untreated control cells.
23. *Homology modeling of Entamoeba histolytica Thioredoxin reductase (EhTHRase)*: *E. histolytica* Thioredoxin reductase (EhTHRase) full amino acid sequence of 314 residues was retrieved from Entrez database (Accession: EAL50345.1) and identification of homologues was carried out by performing PDB database search using BLAST (http://blast.ncbi.nlm.nih.gov). An appropriate template, PDB id: 3D8X (Thioredoxin reductase from Yeast) was identified based on the e-value and sequence identity. The template and the target sequences were then aligned. The alignment contains residues numbered 5 to 313 of the target protein. The first 4 and last 1 amino acids are not present in the model, as the X-ray crystal structure 3D8X does not contain the equivalent amino acids. Thus the model is made up of residues 5–313. Modeller9v9[25] which models protein tertiary structure by satisfaction of spatial restraints was used for protein structure modeling. The modeled structures were ranked on the basis of molpdf scores generated by modeler and the one with least score was selected for model validation. Thereafter, the goodness of predicted EhTHRase model was assessed using PROCHECK[26], which checks the stereochemical quality of a protein structure by analyzing overall and residue by residue geometry. The protein was further subjected to VERIFY3D[27], which derives a '3D-1D' profile based on the local environment of each residue, described by the statistical preferences for: the area of residue which is buried, the fraction of side-chain area that is covered by polar atoms (oxygen and nitrogen) and the local secondary structure (alpha, beta, loop). Further, in order to assess the reliability of the modeled structure of EhTHRase, we calculated the root mean square deviation (RMSD) by superimposing it on the known template structure.
24. *Molecular docking*: The binding mode and biomolecular interactions between the crystals resolved inhibitors (4 and 5) and modeled EhTHRase was analyzed using GOLD v3.1.1 program.[28] The GOLD 3.1.1 (Genetic Optimization for Ligand Docking) from Cambridge Crystallographic Data Centre, UK, uses genetic algorithm to explore the full range of ligand conformational flexibility with partial flexibility of the protein. Active site radiuses were taken as equivalent positions in the template structure obtained by structural superimposition of template-target structure. Standard default settings, consisting of population size-100, number of islands-5, selection pressure-1.1, niche size-2, migrate-2, cross over-95, number of operations-100,000 were adopted for docking of each molecule to the protein. Also, 100 docking conformations (poses) were generated and the best docked conformation was selected based on the Goldscore ranking, for further analysis. Finally, Ligplot[29] was used to map the hydrogen and hydrophobic interaction of the docked inhibitor to the modeled structure.
25. Sali, A.; Blundell, T. L. *J. Mol. Biol.* **1993**, *234*, 779.
26. Laskowski, R. A.; MacArthur, M. W.; Moss, D. S.; Thornton, J. M. *J Appl. Cryst.* **1993**, *26*, 283.
27. Lüthy, R.; Bowie, J. U.; Eisenberg, D. *Nature* **1992**, *5*, 83.
28. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727.
29. Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. *Protein Eng.* **1995**, *8*, 127.
30. (a) Upcroft, J. A.; Campbell, R. W.; Benakli, K.; Upcroft, P.; Vanelle, P. *Antimicrob. Agents Chemother.* **1999**, *43*, 73; (b) Upcroft, J. A.; Dunn, L. A.; Wright, J. M.; Benakli, K.; Upcroft, P.; Vanelle, P. *Antimicrob. Agents Chemother.* **2006**, *50*, 344; (c) Busatti, H. G.; Vieira, A. E.; Viana, J. C.; Silva, H. E.; Souza-Fagundes, E. M.; Martins-Filho, O. A.; Alves, R. J.; Gomes, M. A. *Parasitol. Res.* **2007**, *102*, 145.