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ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 2008

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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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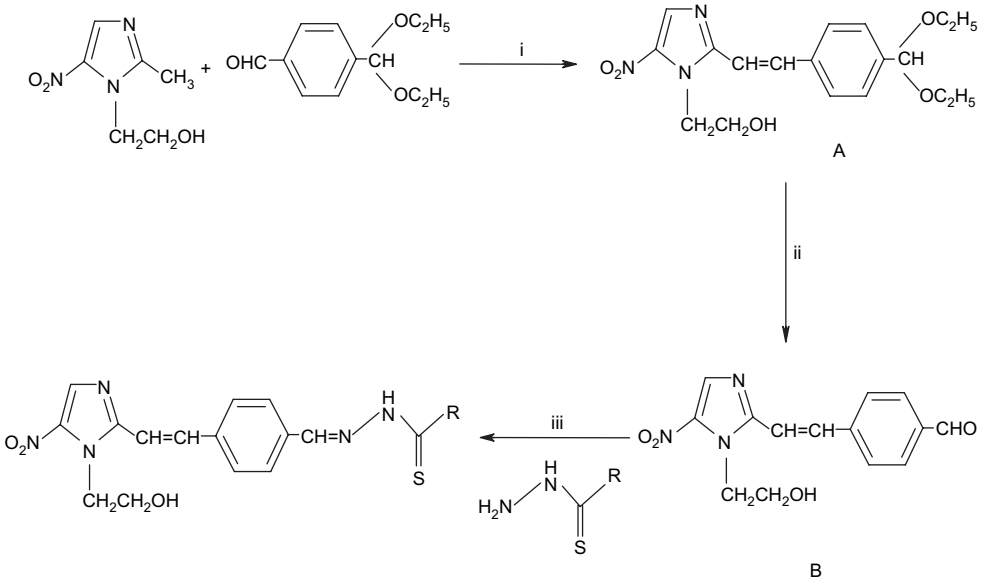
doi:10.1016/j.ejmech.2007.12.007

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.

Table 1
Analytical and physicochemical data of metronidazole thiosemicarabzone analogues (**1**–**11**)

S. no.	Compound/stoichiometry	Colour	Yield (%)	M.pt. (°C)	Found (Calc.)		
					C	H	N
1	MNZ–CHA–TSC $C_{21}H_{26}N_6O_3Sl$	Pale yellow	56	235	57.09 (57.01)	5.96 (5.89)	18.89 (19.00)
2	MNZ–COA–TSC $C_{23}H_{30}N_6O_3S$	Yellow	28	203	58.79 (58.72)	6.45 (6.38)	17.85 (17.87)
3	MNZ–*o*-TOL–TSC $C_{22}H_{22}N_6O_3S$	Dark yellow	21	221	58.65 (58.67)	4.92 (4.89)	18.78 (18.67)
4	MNZ–*p*-TOL–TSC $C_{22}H_{22}N_6O_3S$	Orangish yellow	65	215	58.77 (58.67)	4.96 (4.89)	18.69 (18.67)
5	MNZ–2-ClBz–TSC $C_{22}H_{21}N_6O_3SCl$	Orange	37	225	54.53 (54.49)	4.29 (4.33)	17.37 (17.34)
6	MNZ–PYRR–TSC $C_{19}H_{22}N_6O_3S$	Pale yellow	53	209	55.11 (55.07)	5.26 (5.31)	20.18 (20.29)
7	MNZ–HMI–TSC $C_{21}H_{26}N_6O_3S$	Yellow	27	282	57.11 (57.01)	5.87 (5.89)	18.95 (19.00)
8	MNZ–4-MePIP–TSC $C_{21}H_{26}N_6O_3S$	Yellow	29	245	57.09 (57.01)	5.96 (5.89)	18.89 (19.00)
9	MNZ–*N*-PhPIP–TSC $C_{25}H_{27}N_7O_3S$	Yellow	19	220	59.38 (59.41)	5.36 (5.35)	19.39 (19.41)
10	MNZ–*N*-MeBz–TSC $C_{21}H_{26}N_6O_3S$	Yellow	35	235	57.09 (57.01)	5.96 (5.89)	18.89 (19.00)
11	MNZ–1,2,3,4-THQ–TSC $C_{24}H_{24}N_6O_3S$	Yellow	57	240	60.44 (60.50)	5.16 (5.04)	17.58 (17.65)

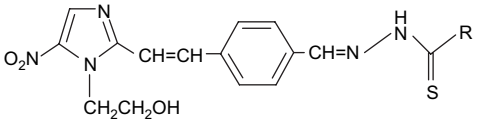
constant was found to be in the range of $J \sim 15.23$–16.51 Hz confirming the presence of *E* isomers in all the compounds. The CH_2 protons of β-hydroxy ethyl group at imidazole ring showed two triplets in the region 4.36–4.94 and 3.41–3.93 ppm, respectively. A singlet was also observed at 7.69–8.08 ppm for azomethine proton. The protons belonging to the aromatic ring and the other cyclic groups were observed with the expected chemical shift and integral values. The ^{13}C NMR spectra of all the compounds showed a signal at 159.2–166.2 ppm were assigned due to the azomethine carbon. Thiocarboxamide carbon (C=S) displayed a signal at 174.5–178.5 ppm in all the compounds. The signals from 140.1 to 159.6 ppm were assumed due to the imidazole ring carbons. The styryl carbons appeared at 136.3–139.6 and 132.8–136.1 ppm, respectively. The carbons at β-hydroxy ethyl group in imidazole ring showed signal at 72.5–78.2 and 36.5–41.7 ppm, respectively. The carbons at 1-*N*-substituted cyclic and aromatic groups resonate at their usual positions and are shown in the data [15].

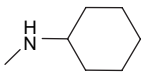
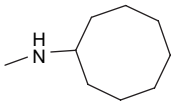
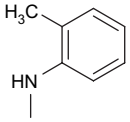
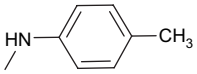
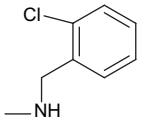
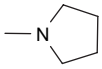
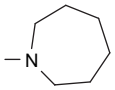
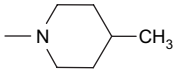
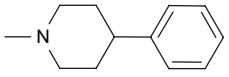
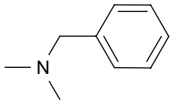
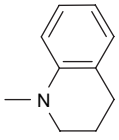
3. In vitro antiamoebic activity

All the 11 synthesized thiosemicarbazone analogues of metronidazole **1**–**11** were screened for antiamoebic activity against *HM1:IMSS* strain of *E. histolytica* using a microdilution method [16–18]. Briefly, *E. histolytica* trophozoites were cultured in TYIS-33 growth medium in 96 well microtiter plate. All the compounds tested were dissolved in DMSO (40 μl) followed by enough culture medium to obtain a concentration of 1 mg/ml. Two fold serial dilutions of the synthesized compounds were prepared in 170 μl of medium. Metronidazole was taken as reference drug. Cell suspension (170 μl) was then added to the test and control wells so that the wells are adequately filled. The plates were sealed with expanded polystyrene, secured with tape, placed in a modular incubation chamber and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h. After incubation, the medium was removed and subsequently washed once with sodium chloride solution (0.9%) at 37 °C. The plate was dried at room temperature, and the amoebae were fixed with methanol followed by staining with aqueous eosin (0.5%) for 15 min. Stained plate was washed and 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 microplate reader and the results were estimated as the percentage of growth inhibition compared with the untreated controls and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best-fitting straight line from which the IC_{50} value was calculated. The IC_{50} values were calculated as μM and are given in Table 2. The thiosemicarbazones presented in this study showed IC_{50} in the range of 0.56–3.67 μM while metronidazole the reference drug had a 50% inhibitory concentration of 1.69–1.81 μM in our experiments. Further, the results were statistically evaluated by the analysis of variance and the null hypothesis was tested using *t*-test [19]. The difference between the IC_{50} mean values of metronidazole and the most active compounds (**2**, **4**, **8**) was found to be significantly different at 5% level, thus concluding that the character under study was appreciably influenced.

Our interest in the metronidazole analogues as an alternative to antiamoebic treatment was facilitated by the fact that not only metronidazole is effective but also side chain attached to the imidazole ring structure provides an opportunity to carry out various modifications. Also, we have reported during previous years that different thiosemicarbazone derivatives do have very promising antiamoebic activity [4]. Considering the substitution at N^4 position of thiosemicarbazones, the better antiamoebic activity was shown by those compounds, which had cyclohexyl amine (**1**, $IC_{50} = 1.50$ μM), cyclooctyl amine (**2**, $IC_{50} = 0.80$ μM), *o*-toluidine (**3**, $IC_{50} = 1.57$ μM), *p*-toluidine (**4**, $IC_{50} = 0.56$ μM), hexamethylineimine (**7**, $IC_{50} = 1.60$ μM), 4-methyl piperidine (**8**, $IC_{50} = 1.13$ μM), *N*-phenyl piperizine (**9**, $IC_{50} = 1.25$ μM) and 1,2,3,4-tetrahydroquinoline (**11**, $IC_{50} = 1.18$ μM) as N^4 substitution. Thiosemicarbazone with pyrrolidine as the N^4 substitution showed comparable antiamoebic activity (**6**, $IC_{50} = 1.92$ μM versus $IC_{50} = 1.82$ μM of metronidazole), while the compounds with *N*-methyl benzyl amine (**5**) and 2-chloro-benzyl amine (**10**) showed moderate antiamoebic activity. The significance of the data presented in Table 2 is the low *in vitro* activity for eight metronidazole thiosemicarbazone analogues. Previously too, we have observed

Table 2
In vitro antiamoebic activity of MNZ thiosemicarbazone derivatives



Compound	R	IC_{50} (μM)	S.D.
1		1.50	0.07
2		0.80[a]	0.12
3		1.57	0.14
4		0.56[a]	0.16
5		3.67	0.20
6		1.92	0.12
7		1.62	0.17
8		1.13[a]	0.14
9		1.25	0.13
10		2.34	0.31
11		1.18	0.06
Metronidazole (MNZ)		1.81	0.14

All the experiments were carried out in triplicate at each concentration level and were repeated thrice.
[a] Compounds on which *t*-test was applied.

that metronidazole complexes of palladium(II), platinum(II), copper(II), gold(I) and ruthenium(II) are significantly more effective antiamoebic agents than metronidazole [20,21]. Particularly compounds **2** and **4** provide precedence for the design of new metronidazole thiosemicarbazone antiamoebic drugs. Based on these results it was concluded that the presence of bulky groups at position N^4 of thiosemicarbazide group greatly enhances antiamoebic activity. Also studies of several other groups have shown that 5-nitroimidazole analogues having more hydrophobic side chain show better antiamoebic activity probably because these modifications enhance solubility and membrane permeability of these compounds [22–25]. The present study again demonstrates the importance of metronidazole analogues in antiparasitic activity. Therefore continued designing and biological assessment of new metronidazole analogues will be extremely worthwhile. Furthermore, the identification of such compounds may be helpful in overcoming cross-resistance of *E. histolytica* to metronidazole and will provide basis for the development of new leads against clinically resistant strains.

Acknowledgement

This work was supported from a grant received from Department of Science and Technology [Grant no. VII-PRDSF/44/2004-05/TT], New Delhi, India. SM Agarwal acknowledges Senior Research Fellowship from University Grants Commission, New Delhi, India. The authors are thankful to Prof. Alok Bhattacharya and Prof. Sudha Bhattacharya, School of Life Sciences and School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, respectively, for providing laboratory facilities for conducting antiamoebic activities.

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[15] All the new compounds (**1**–**11**) gave satisfactory spectral data consistent with their proposed structures. Selected spectral data for compounds **1**–**11**. Compound **1**: λ_{max} (cm^{-1}): 387, 383, 237, 205.8; IR: ν_{max} (cm^{-1}) 3432 (O–H), 3221 (NH), 1642 (C=N), 1583 (C=N), 1206 (C–N), 1185s (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.28 (s, 1H, O–H), 8.15

(s, 1H, imidazole ring proton), 7.82 (s, 1H, CH=N), 8.08 (s, 1H, NH), 7.85 (s, 1H, NH), 7.68 (d, $J = 15.23$, 1H, CH), 7.57 (d, $J = 15.23$, 1H, CH), 7.11–7.32 (m, 4H, Ar–H), 4.62 (t, 2H, CH_2), 3.57 (t, 2H, CH_2), 4.25 (m, 1H, CH), 1.12–2.57 (m, 10H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.5 (C=S), 163.0 (C=N), 158.8, 149.4, 140.2 (imidazole–C), 137.3 (CH), 135.5 (CH), 122.4–133.1 (aryl–C), 76.6 (CH_2), 46.4 (CH), 39.1 (CH_2), 26.7 ($2CH_2$), 23.4 (CH_2), 13.4 (CH_2). Compound **2**: λ_{max} (cm^{-1}): 388.5, 383, 237.3; IR: ν_{max} (cm^{-1}) 3415 (O–H), 3314 (NH), 1630 (C=N), 1590 (C=N), 1182 (C–N), 1092 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.19 (s, 1H, O–H), 8.01 (s, 1H, imidazole ring proton), 7.94 (s, 1H, CH=N), 8.87 (s, 1H, NH), 8.10 (s, 1H, NH), 7.87 (d, $J = 15.73$, 1H, CH), 7.23 (d, $J = 15.73$, 1H, CH), 7.28–7.65 (m, 4H, Ar–H), 4.53 (t, 2H, CH_2), 3.93 (t, 2H, CH_2), 4.63 (m, 1H, CH), 1.24–2.84 (m, 14H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 175.6 (C=S), 166.2 (C=N), 157.4, 148.3, 142.2 (imidazole–C), 138.6 (CH), 136.1 (CH), 122.8–133.1 (aryl–C), 76.5 (CH_2), 51.08 (CH), 39.1 (CH_2), 31.06 ($2CH_2$), 25.6 (CH_2), 21.7 ($2CH_2$), 13.4 (CH_2). Compound **3**: λ_{max} (cm^{-1}): 389, 383, 204.5; IR: ν_{max} (cm^{-1}) 3408 (O–H), 3232 (NH), 1629 (C=N), 1588 (C=N), 1181 (C–N), 1095 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.61 (s, 1H, O–H), 8.25 (s, 1H, imidazole ring proton), 7.96 (s, 1H, CH=N), 8.64 (s, 1H, NH), 8.42 (s, 1H, NH), 7.71 (d, $J = 16.50$, 1H, CH), 7.63 (d, $J = 16.50$, 1H, CH), 7.06–7.55 (m, 8H, Ar–H), 4.46 (t, 2H, CH_2), 3.41 (t, 2H, CH_2), 2.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 175.5 (C=S), 164.2 (C=N), 157.4, 147.2, 144.2 (imidazole–C), 139.1 (CH), 133.2 (CH), 120.4–131.6 (aryl–C), 78.2 (CH_2), 36.5 (CH_2), 19.9 (CH_2). Compound **4**: λ_{max} (cm^{-1}): 390.7, 383, 240, 203.3; IR: ν_{max} (cm^{-1}) 3431 (O–H), 3275 (NH), 1663 (C=N), 1584 (C=N), 1262 (C–N), 1187 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.39 (s, 1H, O–H), 8.10 (s, 1H, imidazole ring proton), 7.87 (s, 1H, CH=N), 8.43 (s, 1H, NH), 8.13 (s, 1H, NH), 7.63 (d, $J = 16.10$, 1H, CH), 7.49 (d, $J = 16.10$, 1H, CH), 7.17–7.29 (m, 4H, Ar–H), 4.86 (t, 2H, CH_2), 3.91 (t, 2H, CH_2), 2.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.3 (C=S), 163.7 (C=N), 158.1, 149.2, 140.9 (imidazole–C), 136.3 (CH), 134.5 (CH), 121.4–133.6 (aryl–C), 76.0 (CH_2), 41.3 (CH_2), 18.6 (CH_2). Compound **5**: λ_{max} (cm^{-1}): 384, 235, 204.9; IR: ν_{max} (cm^{-1}) 3412 (O–H), 3224 (NH), 1630 (C=N), 1586 (C=N), 1181 (C–N), 1092 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.96 (s, 1H, O–H), 8.03 (s, 1H, imidazole ring proton), 7.99 (s, 1H, CH=N), 8.79 (s, 1H, NH), 8.62 (s, 1H, NH), 7.92 (d, $J = 16.21$, 1H, CH), 7.82 (d, $J = 16.21$, 1H, CH), 7.27–7.77 (m, 8H, Ar–H), 4.36 (t, 2H, CH_2), 3.84 (t, 2H, CH_2), 4.25 (d, 2H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 175.3 (C=S), 161.8 (C=N), 157.8, 147.2, 142.2 (imidazole–C), 137.3 (CH), 134.3 (CH), 122.6–132.1 (aryl–C), 74.3 (CH_2), 53.4 (CH_2), 39.4 (CH_2). Compound **6**: λ_{max} (cm^{-1}): 386, 382, 341, 240, 208; IR: ν_{max} (cm^{-1}) 3434 (O–H), 3217 (NH), 1663 (C=N), 1565 (C=N), 1270 (C–N), 1183 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.15 (s, 1H, O–H), 8.07 (s, 1H, imidazole ring proton), 7.74 (s, 1H, CH=N), 7.97 (s, 1H, NH), 7.85 (s, 1H, NH), 7.68 (d, $J = 15.38$, 1H, CH), 7.55 (d, $J = 15.38$, 1H, CH), 6.96–7.38 (m, 4H, Ar–H), 4.37 (t, 2H, CH_2), 3.65 (t, 2H, CH_2), 1.29–3.36 (m, 8H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.5 (C=S), 166.2 (C=N), 159.6, 151.4, 142.2 (imidazole–C), 139.6 (CH), 133.5 (CH), 120.2–131.4 (aryl–C), 72.5 (CH_2), 37.1 (CH_2), 34.7 ($2CH_2$), 25.8 ($2CH_2$). Compound **7**: λ_{max} (cm^{-1}): 395.6, 327, 208; IR: ν_{max} (cm^{-1}) 3431 (O–H), 3265 (NH), 1630 (C=N), 1583 (C=N), 1183 (C–N), 1061 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.56 (s, 1H, O–H), 8.32 (s, 1H, imidazole ring proton), 7.88 (s, 1H, CH=N), 8.07 (s, 1H, NH), 7.93 (s, 1H, NH), 7.61 (d, $J = 15.81$, 1H, CH), 7.52 (d, $J = 15.81$, 1H, CH), 7.15–7.46 (m, 4H, Ar–H), 4.62 (t, 2H, CH_2), 3.76 (t, 2H, CH_2), 1.25–2.57 (m, 12H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.1 (C=S), 165.4 (C=N), 157.4, 149.1, 141.7 (imidazole–C), 139.3 (CH), 134.2 (CH), 122.2–133.8 (aryl–C), 74.3 (CH_2), 41.7 (CH_2), 36.6 ($2CH_2$), 22.4 ($2CH_2$), 14.4 ($2CH_2$). Compound **8**: λ_{max} (cm^{-1}): 395.7, 326.9, 205.6; IR: ν_{max} (cm^{-1}) 3429 (O–H), 3312 (NH), 1659 (C=N), 1529 (C=N), 1183 (C–N), 1082 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.69 (s, 1H, O–H), 8.10 (s, 1H, imidazole ring proton), 7.93 (s, 1H, CH=N), 8.32 (s, 1H, NH), 8.19 (s, 1H, NH), 7.89 (d, $J = 15.75$, 1H, CH), 7.77 (d, $J = 15.75$, 1H, CH), 7.21–7.56 (m, 4H, Ar–H), 4.66 (t, 2H, CH_2), 3.84 (t, 2H, CH_2), 1.13–3.28 (m, 9H, CH_2), 0.98 (d, 3H, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 176.8 (C=S), 165.3 (C=N), 153.8, 148.9, 142.4 (imidazole–C), 139.1 (CH), 135.1 (CH), 121.2–134.6 (aryl–C), 74.2 (CH_2), 38.6 (CH_2), 42.6 ($2CH_2$), 27.4 ($2CH_2$), 29.4 (CH), 11.2 (CH_3). Compound **9**: λ_{max} (cm^{-1}): 389.1, 384, 242; IR: ν_{max} (cm^{-1}) 3433 (O–H), 3265 (NH), 1641 (C=N), 1584 (C=N), 1267 (C–N), 1184 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.68 (s, 1H, O–H), 8.28 (s, 1H, imidazole ring proton), 7.88 (s, 1H, CH=N), 8.11 (s, 1H, NH), 7.90 (s, 1H, NH), 7.84 (d, $J = 15.44$, 1H, CH), 7.74 (d, $J = 15.44$, 1H, CH), 7.31–7.65 (m, 9H, Ar–H), 4.94 (t, 2H, CH_2), 3.93 (t, 2H, CH_2), 3.14–3.73 (m, 8H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.5 (C=S), 162.2 (C=N), 157.3, 148.3, 140.1 (imidazole–C), 138.1 (CH), 134.5 (CH), 121.5–132.1 (aryl–C), 75.6 (CH_2), 46.4 ($2CH_2$), 49.7 ($2CH_2$), 36.4 (CH_2). Compound **10**: λ_{max} (cm^{-1}): 387, 383, 237, 205.8; IR: ν_{max} (cm^{-1}) 3432 (O–H), 3221 (NH), 1642 (C=N), 1583 (C=N), 1206 (C–N), 1185 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.28 (s, 1H, O–H), 8.15 (s, 1H, imidazole ring proton), 8.08 (s, 1H, CH=N), 7.85 (s, 1H, NH), 7.82 (s, 1H, NH), 7.68 (d, $J = 16.05$, 1H, CH), 7.57 (d, $J = 16.05$, 1H, CH), 7.11–7.32 (m, 4H, Ar–H), 4.62 (t, 2H, CH_2), 3.57 (t, 2H, CH_2), 4.25 (m, 1H, CH), 1.12–2.57 (m, 10H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.5 (C=S), 163.0 (C=N), 158.8, 149.4, 140.2 (imidazole–C), 137.3 (CH), 135.5 (CH), 122.4–133.1 (aryl–C), 76.6 (CH_2), 46.4 (CH), 39.1 (CH_2), 26.7 ($2CH_2$), 23.4 (CH_2), 13.4 (CH_2). Compound **11**: λ_{max} (cm^{-1}): 387.9, 382, 203; IR: ν_{max} (cm^{-1}) 3492 (O–H), 3210 (NH), 1642 (C=N), 1584 (C=N), 1265 (C–N), 1129 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.76 (s, 1H, O–H), 8.11 (s, 1H, imidazole ring proton), 7.69 (s, 1H, CH=N), 8.47 (s, 1H, NH), 8.34 (s, 1H, NH), 7.77 (d, $J = 15.87$, 1H, CH), 7.57 (d, $J = 15.87$, 1H, CH), 7.13–7.45 (m, 8H, Ar–H), 4.66 (t, 2H, CH_2), 3.58 (t, 2H, CH_2), 2.27–3.29 (m, 6H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.5 (C=S), 163.0 (C=N), 158.8, 149.4, 140.2 (imidazole–C), 137.3 (CH), 135.5 (CH), 122.4–133.1 (aryl–C), 76.6 (CH_2), 46.4 (CH), 39.1 (CH_2), 26.7 ($2CH_2$), 23.4 (CH_2), 13.4 (CH_2).

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