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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Mannich base derivatives of 1,3,4-oxadiazole: synthesis and screening against *Entamoeba histolytica*

Shadab Miyan Siddiqui · Attar Salahuddin · Amir Azam

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Abstract Mannich base derivatives of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione with substituted piperazine were synthesized and characterized. In vitro antiamoebic activity was performed against HM1: IMSS strain of *Entamoeba histolytica* and the cytotoxicity of the compounds having IC_{50} value less than metronidazole was assessed by MTT assay on human breast cancer MCF-7 cell line. The results revealed that compounds **2**, **3** and **5** were found to be better inhibitors of *E. histolytica* than the reference drug metronidazole and found low cytotoxic in the concentration range of 2.5–250 μM. This study suggests the possibility of developing 1,3,4-oxadiazole analogues as potential drug candidates.

Keywords 1,3,4-Oxadiazole · Mannich base · Antiamoebic activity · *E. histolytica* · MTT assay

Introduction

During the last few decades, the number of life threatening infections caused by pathogenic protozoa has reached an alarming level in the hospitals and community. Amoebiasis, a contagious disease of the human gastrointestinal tract caused by parasitic protozoa *Entamoeba histolytica*, is one of them (Tengku and Norhayati, 2011; Watanabe *et al*., 2011). In general, the infection occurs by the ingestion of cysts of *E. histolytica* in contaminated food and water, but it has been reported that the parasite can be transmitted by homosexual as well as heterosexual activity (Salit *et al*., 2009). Amoebiasis is emerging parasitic complication in the human immunodeficiency virus (HIV)-infected patients (Watanabe *et al*., 2011; Wu *et al*., 2008) and is responsible for approximately 100,000 fatalities annually, making it the second leading cause of death due to parasitic disease (Ralston and Petri, 2011).

The treatment for amoebiasis is the administration of nitroimidazoles such as metronidazole and tinidazole (Fig. 1) but long-term uses of these medicaments produce several side effects in patients (Ordaz-Pichardo *et al*., 2005). Moreover, resistance of *E. histolytica* to metronidazole has been reported (Becker *et al*., 2011) and the treatment failure may emerge as major public health issue. Therefore, new effective antiamoebic agents are required.

Some compounds having oxadiazole ring have been reported to exhibit remarkable antiamoebic activities. For instance, BTI 2286^{E} (±)-E-3-(4-methylsulphinylstyryl)-1,2,4-oxadiazole has been found to show potent amoebicidal activity in a single-dose treatment against *E. histolytica* infection in the liver of golden hamsters and the caeca of mice, hamsters and rats (Bhopale *et al*., 1993). Apart from this, 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione, a prominent heterocyclic scaffold, also have been found to be endowed with antiamoebic activity (Siddiqui *et al*., 2012a). Pyridine and piperazine rings are important components of several anti-amoebic compounds (Hayat *et al*., 2010; Bhat *et al*., 2009; Singh *et al*., 2006). Some *N*-phenyl piperazine containing thiocarbamoyl pyrazolines (Abid and Azam, 2005; Budakoti *et al*., 2007) and their metal complexes (Husain *et al*., 2008) have been reported to show excellent antiamoebic activities. Considering these perspectives, we decided to design compounds derived from active core of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione containing substituted piperazine (Fig. 2). The final compounds were considered to be synthetic equivalents of thiosemicarbazone fragment

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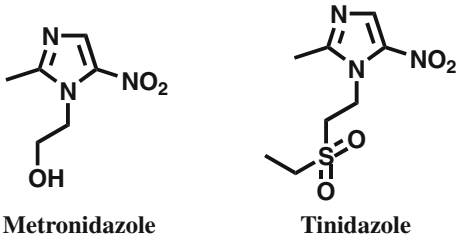


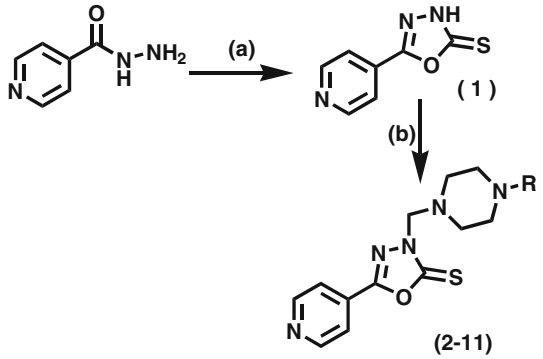
Fig. 1 Standard drugs for amoebiasis treatment

embedded within the 1,2,4-triazole ring (Siddiqui *et al.*, 2012b). Combination of these active components within a single molecular framework was expected to show remarkable antiamoebic activity. Although some of the Mannich base derivatives of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione were synthesized and evaluated for antimicrobial and antimycobacterial activities (Bayrak *et al.*, 2009; Mamolo *et al.*, 2005; Oza and Patel, 2010; Singh *et al.*, 1986), it was important to evaluate these compounds against amoebiasis. In this view a series of Mannich base derivatives of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione incorporating piperazine ring (**2**–**11**) was synthesized and evaluated in vitro against HM1: IMSS strain of *E. histolytica*.

Results and discussion

Chemistry

Synthesis of Mannich base derivatives of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione (**2**–**11**) is out lined in Scheme 1. The precursor 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione (1) was prepared by the reported method (Reid and Heindel, 1976). The NH proton at position-3 of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione (1) is adequately acidic to obtain the target compounds (**2**–**11**) by Mannich



Scheme 1 Synthesis of Mannich base derivatives of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H) thione (**2**–**11**). Reagents and conditions: **a** CS_2, KOH/H^+, reflux 10 h **b** different substituted piperazines, 37 % HCHO, EtOH, rt, 2 h

reaction. Mannich reaction is a three-component condensation reaction involving active hydrogen containing compound, formaldehyde and a primary or secondary amine and has considerable importance for the synthesis and modification of biologically active compounds (Reddy *et al.*, 2008). The structure of all the desired compounds (**2**–**11**) was elucidated by spectral studies and their purity was confirmed by elemental analyses.

Antiamoebic activity

Preliminary experiments were carried out to determine the in vitro antiamoebic activity of all the compounds (**2**–**11**) by microdilution method using HM1: IMSS strain of *E.* histolytica (Wright *et al.*, 1988). All the experiments were carried out in triplicate at each concentration level and repeated thrice. The results are summarized in Table 1. The data are present in terms of percent growth inhibition relative to untreated controls, and plotted as probit values as a function of drug concentration. The antiamoebic effect

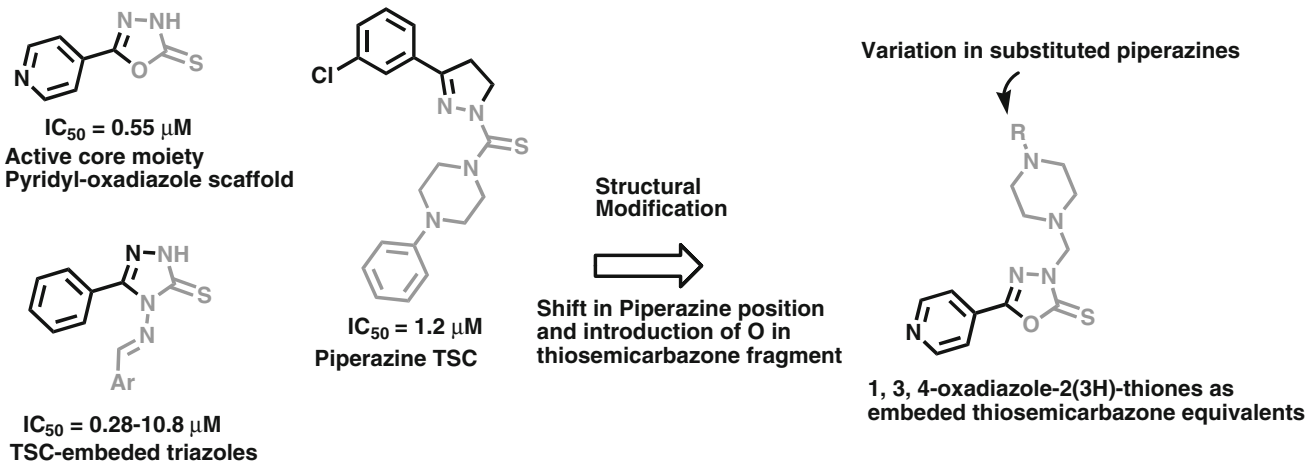
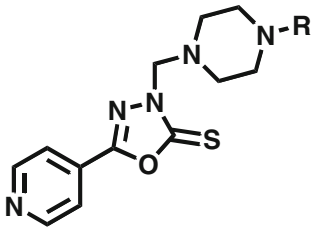


Fig. 2 Structural modification and rational designing

Table 1 In vitro antiamoebic activity of Mannich base derivatives of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione (**2–11**) against HM1: IMSS strain of *E. histolytica* and cytotoxicity profile of compounds **2**, **3**, **5** and metronidazole



(2-11)

(**2–11**)

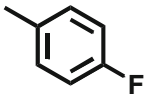
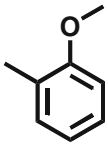
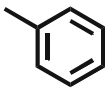
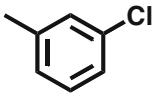
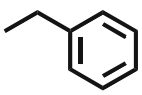
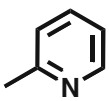
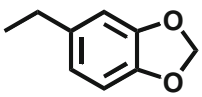
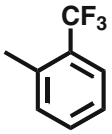
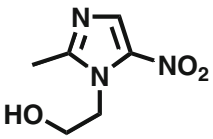
Compound no.	*R*	Antiamoebic activity		Cytotoxicity profile	
		IC_{50} (μM)	SD[a]	IC_{50} (μM)	SD[a]
2		0.245	0.009	>250	0.24
3		1.06	0.015	>250	0.27
4		3.47	0.018	N.D.	N.D.
5	C_2H_5	0.327	0.005	>250	0.21
6	CH_3	5.11	0.001	N.D.	N.D.
7		2.57	0.007	N.D.	N.D.
8		3.40	0.013	N.D.	N.D.
9		2.82	0.014	N.D.	N.D.
10		3.62	0.006	N.D.	N.D.

Table 1 continued

Compound no.	*R*	Antiamoebic activity		Cytotoxicity profile	
		IC_{50} (μM)	SD[a]	IC_{50} (μM)	SD[a]
11		2.87	0.013	N.D.	N.D.
		1.81	0.011	>250	0.89

N.D. not done

[a] Standard deviation

was compared with the most widely used antiamoebic medication metronidazole which had a 50 % inhibitory concentration (IC_{50}) 1.81 μM in our experiments. The results revealed that the oxadiazole derivatives (**2**–**11**) (Fig. 3) showed IC_{50} values in the range 0.25–5.11 μM. Among all the screened compounds (**2**–**11**), compounds **2**, **3**, and **5** exhibited IC_{50} values less than the standard drug metronidazole, while the remaining compounds showed IC_{50} values more than metronidazole and were considered to be inactive. Structure activity relationship (SAR) showed that the maximum activity was shown by compound **2** (IC_{50} = 0.245 μM), having 4-fluorophenyl group attached with piperazine ring. The replacement of the 4-fluorophenyl group with ethyl group (5, IC_{50} =0.327 μM)) did not reduce the antiamoebic activity significantly, but the replacement of the 4-fluoro phenyl group with 2-methoxyphenyl group (3, IC_{50} = 1.06 μM) drastically reduced the antiamoebic activity. The biological data showed that the antiamoebic activity is dependent on substituent (R).

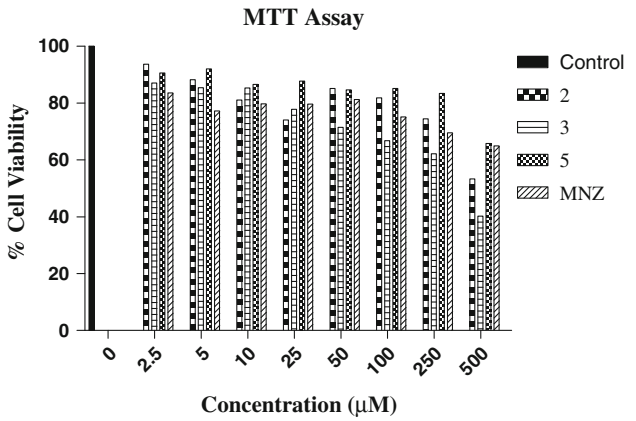


Fig. 3 Percentage of viable cells after 48 h pre-treatment of human breast cancer MCF-7 cells with compounds **2**, **3**, **5**, and metronidazole (MNZ), evaluated by the MTT assay

Cytotoxicity profile

The compounds (**2**, **3** and **5**) having less IC_{50} value than metronidazole were screened against human breast cancer MCF-7 cell line. A sub-confluent 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 coloured blue formazone product. This assay usually shows high correlation with number of living cells and cell proliferation. The % cell viability shown by the compounds (**2**, **3**, **5** and metronidazole) at concentration range of 2.5–500 μM is given in Fig. 3. These results showed that all the compounds and metronidazole were low cytotoxic in the concentration range of 2.5–250 μM.

Experimental

Synthesis

Melting points (mp) were recorded on a KSW apparatus and are uncorrected. Elemental analysis was carried out on CHNS Elemental Analyzer vario MICRO, Elementar Analysensysteme GmbH, Germany. IR spectra were recorded on Perkine-Elmer model 1600 FT-IR RX1 spectrophotometer as KBr discs. The ^{1}H NMR spectra were recorded on Bruker Spectrospin DPX 300 MHz spectrometer using DMSO and $CDCl_3$ as a solvent and trimethylsilane (TMS) as an internal standard. Chemical shift

values are given in part per million (ppm) with respect to TMS. Mass spectra were recorded by GC–MS (Perkin-Elmer Clarus 500 GC).

*Preparation of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione (**1**)*

It was prepared by the reported method (Reid and Heindel, 1976).

*General procedure for the synthesis of Mannich bases of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-(3H)-thione (**2**–**11**)*

A mixture of 5-(pyridine-4-yl)-1,3,4-oxadiazole-2 (3H)-thione (0.01 mol), substituted piperazine (0.01 mol) and 37 % formaldehyde solution (1.5 mL), in ethanol (15 mL), was stirred at room temperature for 2 h and allowed to stand overnight. The precipitated crude product was filtered, washed with water, dried, and recrystallized from ethanol.

*3-{[4-(4-Fluorophenyl)piperazin-1-yl]methyl}-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**2**)* Yield: 68 %; m.p.: 172 °C; Anal. calc. for $C_{18}H_{18}FN_5OS$: C 58.21, H 4.88, N 18.86, S 8.63 %; found: C 58.19, H 4.91, N 18.83, S 8.59 %; IR υ_{max} (cm^{-1}): 3052 (Ar–CH), 2852 (CH_2), 1612, 1446 (C=N), 1358 (C=S), 1239 (C–O–C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.82 (d, 2H, J = 3.0 Hz, Ar–H), 7.79 (d, 2H, J = 6.0 Hz, Ar–H), 6.98–6.82 (m, 4H, Ar–H), 5.17 (s, 2H, N–CH_2–N), 3.14–3.00 (m, 8H, piperazine); MS (EI, 70 eV): *m/z* = 371.43 [M^+].

*3-{[4-(2-Methoxyphenyl)piperazin-1-yl]methyl}-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**3**)* Yield: 72 %; m.p.: 110 °C; Anal. calc. for $C_{19}H_{21}N_5O_2S$: C 59.51, H 5.52, N 18.26, S 8.36 %; found: C 59.55, H 5.50, N 18.23, S 8.36 %; IR υ_{max} (cm^{-1}): 3032 (Ar–CH), 2941 (CH_3), 2827 (CH_2), 1618, 1439 (C=N), 1352 (C=S), 1233 (C–O–C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.82 (d, 2H, J = 6.0 Hz, Ar–H), 7.78 (d, 2H, J = 6.0 Hz, Ar–H), 7.00–6.82 (m, 4H, Ar–H), 5.18 (s, 2H, N–CH_2–N), 3.82 (s, 3H, OCH_3), 3.17–2.91 (m, 8H, piperazine); MS (EI, 70 eV): *m/z* = 383.46 [M^+].

*3-[(4-Phenylpiperazin-1-yl)methyl]-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**4**)* Yield: 67 %; mp: 188 °C; Anal. calc. for $C_{18}H_{19}N_5OS$: C 61.17, H 5.42, N 19.81, S 9.07 %; found: C 61.19, H 5.38, N 19.80, S 9.03 %; IR υ_{max} (cm^{-1}): 3042 (Ar–CH), 2854 (CH_2), 1609, 1436 (C=N), 1354 (C=S), 1243 (C–O–C). ^{1}H NMR ($CDCl_3$) δ (ppm): 8.82–7.25 (m, 6H, Ar–H), 6.90 (d, 2H, J = 6.0 Hz, Ar–H), 5.17 (s, 2H, N–CH_2–N), 3.20–3.02 (m, 8H, piperazine); MS (EI, 70 eV): *m/z* = 353.44 [M^+].

*3-[(4-Ethylpiperazin-1-yl)methyl]-5-pyridin-4-yl-1,3,4-oxadiazole-2(3H)-thione (**5**)* Yield: 66 %; mp.160 °C; Anal. calc. for $C_{14}H_{19}N_5OS$: C 55.06, H 6.27, N 22.93, S 10.50 %; found: C 55.01, H 6.23, N 22.89, S 10.54 %; IR υ_{max} (cm^{-1}): 2910 (CH_3), 2849 (CH_2), 1651, 1437 (C=N), 1355 (C=S), 1243 (C–O–C); ^{1}H NMR ($CDCl_3$) δ (ppm): ^{1}H NMR ($CDCl_3$) δ (ppm): 8.71 (d, 2H, J = 3.0 Hz, Ar–H), 8.65 (d, 2H, J = 3.0 Hz, Ar–H), 7.76–7.62 (m, 4H, Ar–H), 5.14 (s, 2H, N–CH_2–N), 3.55–2.96 (m, 10H, piperazine and CH_2) 1.42–1.37 (t, 3H, CH_3); MS (EI, 70 eV): *m/z* = 305.39 [M^+].

*3-[(4-Methylpiperazin-1-yl)methyl]-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**6**)* Yield: 65 %; m.p.: 240 °C; Anal. calc. for $C_{13}H_{17}N_5OS$: C 53.59, H 5.88, N 24.04, S 11.00 %; found: C 53.63, H 5.91, N 24.01, S 11.04 %; IR υ_{max}(cm^{-1}): 2930 (CH_3), 2852 (CH_2), 1658, 1449 (C=N), 1365 (C=S),1263 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.67 (d, 2H, J = 6.0 Hz, Ar–H), 7.68 (d, 2H, J = 6.0 Hz, Ar–H), 5.19 (s, 2H, N–CH_2–N), 3.28–2.80 (m, 8H, piperazine), 2.37 (s, 3H, CH_3); MS (EI, 70 eV): *m/z* = 291.37 [M^+].

*3-{[4-(3-Chlorophenyl)piperazin-1-yl]methyl}-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**7**)* Yield: 62 %; m.p.: 166 °C; Anal. calc. for $C_{18}H_{18}ClN_5OS$: C 55.74, H 4.68, N 18.06, S 8.27 %; found: C 55.71, H 4.63, N 18.09, S 8.31 %; IR υ_{max} (cm^{-1}): 3063 (Ar–CH), 2876 (CH_2), 1636, 1458 (C=N), 1352 (C=S), 1263 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.78 (d, 2H, J = 6.0 Hz, Ar–H), 7.78 (d, 2H, J = 6.0 Hz, Ar–H), 7.13–6.90 (m, 4H, Ar–H), 5.13 (s, 2H, N–CH_2–N), 3.27–2.92 (m, 8H, piperazine); MS (EI, 70 eV): *m/z* = 387.88 [M^+].

*3-[(4-Benzylpiperazin-1-yl)methyl]-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**8**)* Yield: 58 %; mp: 180 °C; Anal. calc. for $C_{19}H_{21}N_5OS$: C 62.10, H 5.76, N 19.06, S 8.73 %; found: C 62.14, H 5.73, N 19.07, S 8.75 %; IR υ_{max} (cm^{-1}): 3046 (Ar–CH), 2851 (CH_2), 1608, 1440 (C=N), 1359 (C=S), 1234 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.69 (d, 2H, J = 3.0 Hz, Ar–H), 7.70 (d, 2H, J = 3.0 Hz, Ar–H), 7.22–7.18 (m, 5H, Ar–H), 4.97 (s, 2H, N–CH_2–N), 3.59–2.99 (m, 10H, CH_2Ph and piperazine); MS (EI, 70 eV): *m/z* = 367.46 [M^+].

*5-(Pyridin-4-yl)-3-{[4-(pyridin-2-yl)piperazin-1-yl]methyl}-1,3,4-oxadiazole-2(3H)-thione (**9**)* Yield: 61 %; m.p.: 154 °C; Anal. calc. for $C_{17}H_{18}N_6OS$: C 57.61, H 5.12, N 23.17, S 9.05 %; found: C 57.66, H 5.15, N 23.13, S 9.04 %; IR υ_{max} (cm^{-1}): 3051 (Ar–CH), 2823 (CH_2), 1648, 1430 (C=N), 1363 (C=S), 1248 (C–O–C);^{1}H NMR (DMSO-d_6) δ (ppm): 8.57 (d, 2H, J = 6.0 Hz, Ar–H), 8.05–8.01 (m, 2H, Ar–H), 7.59 (d, 2H, J = 6.0 Hz, Ar–H),

7.52–7.39 (m, 2H, Ar–H), 4.05 (s, 2H, N–CH_2–N), 3.58–3.06 (m, 8H, piperazine); MS (EI, 70 eV): $m/z = 354.42$ [M^+].

*3-((4-((Benzo[d][1,3]dioxol-5-yl)methyl)piperazine-1-yl)methyl)-5(pyridine-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**10**)* Yield: 64 %; mp: 146 °C; Anal. calc. for $C_{20}H_{21}N_5O_3S$: C 58.38, H 5.14, N 17.02, S 7.79 %; found: C 58.41, H 5.11, N 17.06, S 7.81 %; IR υ_{max} (cm^{-1}): 3024 (Ar–CH), 2878 (CH_2), 1608, 1438 (C=N), 1363 (C=S), 1248 (C–O–C); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.67 (d, 2H, J = 6.0 Hz, Ar–H), 7.69 (d, 2H, J = 6.0 Hz, Ar–H), 6.86–6.72 (m, 3H, Ar–H), 6.03–5.98 (m, 4H, N–CH_2–N and O–CH_2–O), 3.45–2.90 (m, 4H, piperazine), 2.44–2.34 (m, 4H, piperazine); MS (EI, 70 eV): $m/z = 411.47$ [M^+].

*3-(2-(Trifluoromethyl)benzyl)-5-(pyridine-4-yl)-1,3,4-oxadiazole-2(3H)-thione (**11**)* Yield: 62 %; mp: 152 °C; Anal. calc. for $C_{20}H_{20}N_5F_3OS$: C 55.16, H 4.63, N 16.08, S 7.36 %; found: C 55.12, H 4.67, N 16.12, S 7.39 %; IR υ_{max} (cm^{-1}): 3030 (Ar–CH), 2832 (CH_2), 1649, 1433 (C=N), 1365 (C=S), 1249 (C–O–C); ^{1}H NMR ($CDCl_3$) δ (ppm): 8.69 (d, 2H, J = 6.0 Hz, Ar–H), 7.65 (d, 2H, J = 6.0 Hz, Ar–H), 7.25–7.20 (t, 1H, Ar–H), 7.02–6.94 (m, 3H, Ar–H), 5.05 (s, 2H, N–CH_2–N), 3.14–2.50 (m, 8H, piperazine); MS (EI, 70 eV): $m/z = 435.46$ [M^+].

In vitro antiamoebic assay

All the title compounds were screened for in vitro antiamoebic activity against HM1: IMSS strain of *E. histolytica* by microdilution method (Wright *et al*., 1988). *Entamoeba histolytica* trophozoites were cultured in culture tubes using Diamond TYIS-33 growth medium. The test compounds (1 mg) were dissolved in DMSO (40 mL, level at which no inhibition of amoeba occurs) (Gillin *et al*., 1982). The stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/mL. Two-fold 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 haemocytometer, 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 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. A 200 µL portion 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 microplate 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. The IC_{50} values are reported in Table (1).

MTT assay

The human breast cancer MCF-7 cells were obtained from NCCS (Pune, India). The cells were cultured in DMEM (Sigma) with 10 % foetal bovine serum and 1 % penicillin/streptomycin/neomycin. The effect of compounds **2**, **3**, **5** and the standard drug metronidazole on cell proliferation was measured using an MTT-based assay (Mosmann, 1983). In brief, the cells (10,000 $well^{-1}$) were incubated in triplicate in a 96-well plate in the presence of various concentrations of compounds **2**, **3**, **5** as well as metronidazole or vehicle (DMSO) alone in a final volume of 200 µL at 37 °C and 5 % CO_2 in and humidified chamber for 48 h. At the end of this time period, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the cells were incubated at 37 °C in a humidified chamber for 4 h. After 4 h, the supernatant was removed from each well. The coloured formazan crystal produced from MTT was dissolved in 200 µL of DMSO, and then the absorbance (*A*) value was measured at 570 nm by a multiscanner autoreader. The following formula was used for the calculation of the percentage of cell viability (CV): CV (%) = (*A* of the experimental samples/*A* of the control) × 100.

Conclusion

It was concluded that the compounds **2**, **3** and **5** showed better antiamoebic activity than the reference drug metronidazole and found low cytotoxic against human breast cancer MCF-7 cell line. This study suggests the possibility of developing 1,3,4-oxadiazole analogues as potential drug candidates.

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