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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Pyrazolo[3,4-d]pyrimidine analogues: synthesis, characterization and their in vitro antiamoebic activity

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Abstract Pyrazolo[3,4-d]pyrimidine analogues were synthesized by treating 3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidine-4-amine with different sulfonyl chlorides and triethylamine in dry dichloromethane. The structure of all the compounds was elucidated by spectral data and their purity was confirmed by elemental analysis. In vitro antiamoebic activity was performed against HM1:IMSS strain of *Entamoeba histolytica* and two compounds, **4** ($IC_{50} = 0.57$) and **6** ($IC_{50} = 0.68$), were found better inhibitors than the reference drug metronidazole ($IC_{50} = 1.80$). Further, both the compounds (**4** and **6**) were low cytotoxic against the human breast cancer MCF-7 cell line in the concentration range of 2.5–250 μM. These preliminary results reveal that pyrazolo[3,4-d]pyrimidine analogues could help in designing better molecules with enhanced antiamoebic activity.

Keywords Pyrazolo[3,4-d]pyrimidine · HM1:IMSS · *Entamoeba histolytica* · Antiamoebic activity · MTT assay

Introduction

Amoebiasis can be considered the most aggressive disease of the human intestine (Salles *et al*., 2003). It is the second leading cause of mortality due to a protozoan infection (Lejeune *et al*., 2009). *Entamoeba histolytica* is the causative agent in humans and is responsible for 100,000 fatalities per annum (Ralston and Petri, 2011). Metronidazole (MNZ), the first line medicament against amoebiasis, is potentially carcinogenic to humans because it is genotoxic to human cells (Bendesky *et al*., 2002). Furthermore, resistance of *E. histolytica* to MNZ and relapses of intestinal and hepatic amoebiasis have been reported (Becker *et al*., 2011; Hwang *et al*., 2011). Therefore, new effective antiamoebic agents are required.

Antiamoebic drugs, e.g. MNZ, tinidazole and ornidazole (Fig. 1) have a basic core as imidazole ring having nitrogen atoms at 1, 3 position. Structurally, the imidazoles and pyrimidines are similar in having 1,3-dinitrogen system (Fig. 2). In our earlier studies, the reduced forms of pyrazole that are known as pyrazoline and some pyrimidine derivatives (Fig. 3) have been found to be endowed with antiamoebic activities (Hayat *et al*., 2010, 2011; Abid *et al*., 2009; Budakoti *et al*., 2007; Parveen *et al*., 2010, 2011). In this context, pyrazole and pyrimidine are crucial nitrogen-containing heterocyclic systems, which can provide privileged scaffolds for the development of antiamoebic compounds. Considering this perspective, it was planned to integrate pyrazole and pyrimidine rings together in a single molecular frame to form pyrazolo[3,4-d]pyrimidine pharmacophore. Literature survey revealed that the replacement of 1H of pyrazolo[3,4-d]pyrimidine system by some other biologically active molecule drastically alters their pharmacological properties (El-Sayed Ali, 2009). The introduction of sulphonamide group is a provocative tactic in drug designing for increasing pharmacological potency and/or the absorption, distribution, metabolism, and excretion (ADME) attributes of the lead chemical matter (Dai *et al*., 2011). Recently, we have demonstrated that some azole-based derivatives showed better antiamoebic activity than MNZ (Siddiqui *et al*., 2012; Irfan *et al*., 2010). In this paper, we herein report the synthesis of pyrazolo[3,4-d]pyrimidine analogues having a sulphonamide linkages and their in vitro antiamoebic activity against HM1:IMSS strain of *E. histolytica*.

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Results and discussion

Chemistry

All the desired compounds were prepared by multistep reaction starting from the synthesis of 2-(methoxy(phenyl)-methylene)malononitrile (**1**), which was synthesized by reacting benzoyl chloride with malononitrile in the presence of sodium hydride and dimethyl sulphate. Treatment of (**1**) with hydrazine hydrate yielded 5-amino-3-phenyl-1*H* pyrazole-4-carbonitrile (**2**). Then the reaction of compound (**2**) with formamide gave 3-phenyl 1*H*-pyrazolo[3,4-d]pyrimidine-4-amine (**3**). Finally, the reaction of compound **3** (1 eq.), triethylamine (3 eq.) and alkyl or aryl sulfonyl chlorides (1.2 eq.) in dry dichloromethane at 0 °C for about 2 h afforded the sulphonamide derivatives of pyrazolo [3,4-d]-pyrimidine (**4**–**11**). The structure of all the compounds was elucidated by spectral studies and their purity was confirmed by elemental analyses (Scheme 1).

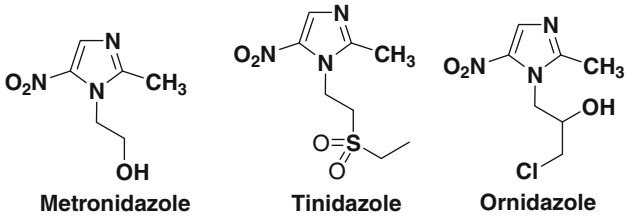


Fig. 1 Commercially available antiamoebic drugs bearing nitrogen-containing heterocyclic ring

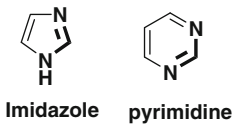


Fig. 2 Structural similarity imidazole and pyrimidine rings having 1,3-dinitrogen

Antiamoebic activity and cytotoxicity profile

Preliminary experiments were carried out to determine the in vitro antiamoebic activity of the target compounds by microdilution method against the 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 antiamoebic effect was compared with the most widely used antiamoebic medication MNZ, which had 50 % inhibitory concentration (IC_{50}) 1.80 μM in our experiments. All compounds showed IC_{50} values in the range 0.57–5.15 μM. In terms of IC_{50}, it can be concluded that compound (**4**) was endowed with the maximum activity (IC_{50} = 0.57 μM) followed by compound **6** (IC_{50} = 0.68 μM),

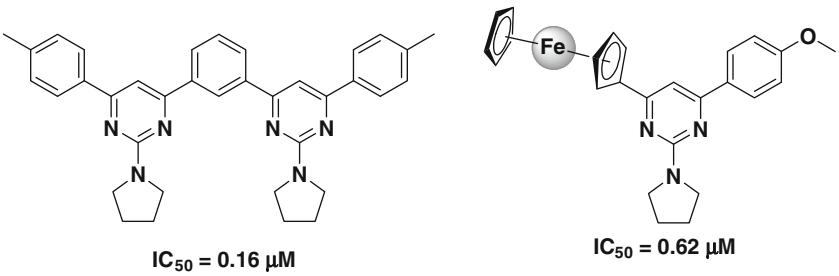
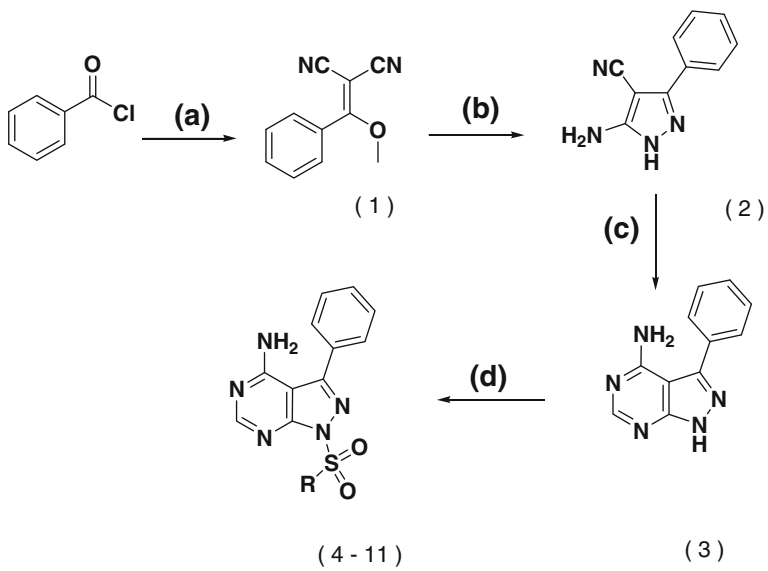


Fig. 3 Antiamoebic pyrimidine compounds



Scheme 1 Synthesis of pyrazolo[3,4-d]pyrimidine and its analogues. Reagents and conditions: **a** $CNCH_2CN$, NaH, THF, 0 °C/Me_2SO_4, 90 °C, 12 h, **b** $N_2H_4.H_2O/C_2H_5OH$, reflux 2 h, **c** $HCONH_2$, 180 °C, 5 h, **d** RSO_2Cl, Et_3N, DCM, rt, 7 h

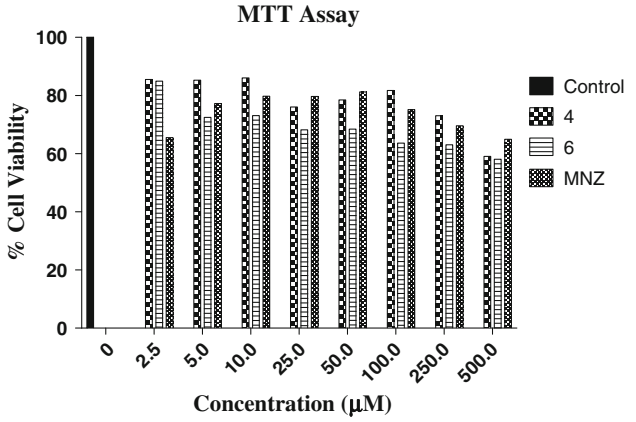


Fig. 4 Percentage of viable cells after 48 h pre-treatment of human breast cancer MCF-7 cells with compounds **4**, **6** and MNZ, evaluated by the MTT assay

while the remaining compounds showed IC_{50} value more than that of the reference drug MNZ, and therefore were considered to be inactive. Further, cytotoxicity of the compounds having IC_{50} value less than MNZ was assessed by MTT assay on human breast cancer MCF-7 cell line. A 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 (CV) assay based on mitochondrial reduction of the yellow MTT tetrazolium dye to a highly coloured blue formazan product. This assay usually shows high correlation with number of living cells and cell proliferation. The % CV shown by the compounds (**4**, **6** and MNZ) at concentration range of 2.5–500 μM is given in Fig. 4. The results showed that all the compounds and MNZ were low cytotoxic in the concentration range of 2.5–250 μM.

Experimental protocol

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 using DMSO and $CDCl_3$ as solvent and trimethylsilane (TMS) as an internal standard. Chemical shift values are given in parts per million (ppm) with respect to TMS. Mass spectra were recorded by GC–MS (Perkin-Elmer Clarus 500 GC).

*Synthesis of 2-(methoxy(phenyl)methylene)-malononitrile (**1**)*

To a stirred suspension of 60 % NaH (438.21 mmol) in 150 mL tetrahydrofuran (THF), a solution of malononitrile (219.1 mmol) in 100 mL THF was added dropwise at 0 °C. The reaction mixture was then stirred for 15 min at 0 °C then a solution of benzoyl chloride (219.1 mmol) in 200 mL THF was added dropwise to the reaction mixture. The solution was stirred at room temperature for 45 min and then a solution of dimethyl sulfate (262.92 mmol) in 50 mL of THF was added dropwise. The reaction mixture was then allowed to reflux at 90 °C overnight. The reaction mixture was quenched with saturated ammonium chloride solution (120 mL) and concentrated to remove THF. The black residue was dissolved in ethyl acetate (500 mL) and extracted with water. The separated organic extract was washed with 100 mL brine and dried over anhydrous sodium sulphate and concentrated to get crude product which was carried to next step without purification.

*Synthesis of 5-amino-3-phenyl-1H-pyrazole-4-carbonitrile (**2**)*

A solution of compound (**1**) (219.1 mmol) and hydrazine hydrate (876.4 mmol) in 500 mL ethanol was allowed to reflux for 2 h. The mixture was concentrated and the residue was diluted with ethyl acetate and washed with water. The separated organic extract was dried over anhydrous sodium sulphate and concentrated to get crude product which was taken to next step without purification.

*5-Amino-3-phenyl-1H-pyrazole-4-carbonitrile (**2**)*

Yield: 90 %; m.p. 202 °C; Anal. calc. for $C_{10}H_{10}N_4$: C 64.50, H 5.41, N 30.09 %; found: C 64.54, H 5.39, N 30.11 %. IR ν_{max} (cm^{-1}): 3442, 3303 (NH_2), 3218 (NH), 2210 (CN); ^{1}H NMR (DMSO-d_6) δ (ppm): 12.07 (s, 1H, NH), 7.74–7.31 (m, 5H, Ar–H), 6.33 (s, 2H, NH_2); MS (EI, 70 eV): $m/z = 186.21$ [M^+].

*Synthesis of 3-phenyl-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**3**)*

A solution of compound (**2**) (63.54 mmol) in 300 mL formamide was heated to 180 °C for 5 h. The reaction mixture was cooled to room temperature then poured over ice to get the crude dark brown precipitates of the product. The solid was filtered and then kept for stirring in 150 mL methanol for 15 min and again filtered. The solid precipitates were again washed with 100 mL diethyl ether and then dried under vacuum to get pure product.

*3-Phenyl-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**3**)*

Yield: 50 %; m.p. 185 °C; Anal. calc. for $C_{11}H_9N_5$: C 62.55, H 4.29, N 33.16 %; found: C 62.59, H 4.33,

N 33.11 %; IR ν_{max} (cm^{-1}): 3470, 3308 (NH_2), 3103 (NH); ^1H NMR (DMSO-d_6) δ (ppm): 13.58 (s, 1H, NH), 8.21 (s, 1H, Ar–H), 7.68–7.44 (m, 5H, Ar–H), 6.71 (s, 2H, NH_2); MS (EI, 70 eV): $m/z = 211.22$ [M^+].

*Synthesis of sulphonamide derivatives of 3-phenyl-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**4–11**)*

To a solution of compound (**3**) (1 eq) and triethylamine (3 eq) in dry dichloromethane at 0 °C, alkyl or aryl sulfonyl chlorides (1.2 eq) were added. The reaction mixture was stirred at 0 °C for about 2 h and the stirring was continued at room temperature for about 5 h. After the completion of the reaction, the reaction was quenched with distilled water and extracted with dichloromethane (3 × 15 mL). Finally, the separated organic layer was washed with distilled water again and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by recrystallization from ethanol.

*3-Phenyl-1-tosyl-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**4**)*

Yield 74 %; m.p. 212 °C; Anal. calc. for $C_{18}H_{15}N_5O_2S$: C 59.16, H 4.14, N 19.17, S 8.78 %; found: C 59.13, H 4.17, N 19.16, S 8.80 %; IR ν_{max} (cm^{-1}): 3441, 3302 (NH_2), 1629 (C=N), 1363 (SO_2); ^1H NMR ($CDCl_3$) δ (ppm): 7.94–7.80 (m, 4H, Ar–H), 7.42–7.26 (m, 6H, Ar–H), 6.01 (s, 2H, NH_2), 2.43 (s, 3H, CH_3); MS (EI, 70 eV): $m/z = 365.40$ [M^+].

*1-[(4-Nitrophenyl)sulfonyl]-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**5**)*

Yield: 61 %; mp: 240 °C; Anal. calc. for $C_{17}H_{12}N_6O_4S$: C 51.51, H 3.05, N 21.20, S 8.09 %; found: C 51.54, H 3.01, N 21.19, S 8.11 %; IR ν_{max} (cm^{-1}): 3469, 3306 (NH_2), 1643 (C=N), 1391 (SO_2); ^1H NMR ($CDCl_3$) δ (ppm): 8.54–8.37 (m, 3H, Ar–H), 7.95–7.39 (m, 7H, Ar–H), 5.85 (s, 2H, NH_2); MS (EI, 70 eV): $m/z = 396.37$ [M^+].

*N-{4-[(4-Amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)sulfonyl]phenyl}acetamide (**6**)*

Yield: 54 %; mp. 220 °C; Anal. calc. for $C_{19}H_{16}N_6O_3S$: C 55.87, H 3.95, N 20.58, S 7.85 %; found: C 55.84, H 3.96, N 20.61, S 7.88 %; IR ν_{max} (cm^{-1}): 3470, 3302 (NH_2), 1644 (C=N), 1397 (SO_2); ^1H NMR (DMSO): 13.77 (s, 1H, NH), 8.96–8.78 (m, 1H, Ar–H), 8.44–8.00 (m, 2H, Ar–H), 7.88–7.24 (m, 9H, Ar–H and NH_2), 3.67 (s, 3H, $COCH_3$); MS (EI, 70 eV): $m/z = 408.43$ [M^+].

*1-[(4-Chlorophenyl)sulfonyl]-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**7**)*

Yield: 66 %; mp: 224 °C; Anal. calc. for $C_{17}H_{12}N_5O_2ClS$: C 52.92, H 3.13, N 18.15, S 8.31 %; found: C 52.88, H 3.16, N 18.16, S 8.33 %; IR ν_{max} (cm^{-1}): 3443, 3306 (NH_2), 1630 (C=N), 1365 (SO_2); ^1H NMR ($CDCl_3$) δ (ppm): 8.60 (s, 1H, Ar–H), 8.22 (d, 2H, Ar–H, $J = 9.0$ Hz), 7.97–7.44 (m, 7H, Ar–H), 5.59 (s, 2H, NH_2); MS (EI, 70 eV): $m/z = 385.82$ [M^+].

*1-[(2,5-Dichlorophenyl)sulfonyl]-3-phenyl-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**8**)*

Yield: 58 %; m.p: 210 °C; Anal. calc. for $C_{17}H_{11}N_5O_2Cl_2S$: C 48.58, H 2.64, N 16.66, S 7.63 %; found: C 48.62, H 2.67, N 16.61, S 7.59 %; IR ν_{max} (cm^{-1}): 3442, 3303 (NH_2), 1630 (C=N), 1364 (SO_2); ^1H NMR (DMSO-d_6) δ (ppm): 8.53 (s, 1H, Ar–H), 7.79–7.54 (m, 8H, Ar–H), 7.49 (s, 2H, NH_2); MS (EI, 70 eV): $m/z = 420.27$ [M^+].

*1-(2-Naphthylsulfonyl)-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**9**)*

Yield: 56 %; m.p. 238 °C; Anal. calc. for $C_{21}H_{15}N_5O_2S$: C 62.83, H 3.77, N 17.45, S 7.99 %; found: C 62.81, H 3.80, N 17.44, S 7.96 %; IR ν_{max} (cm^{-1}): 3467, 3306 (NH_2), 1643 (C=N) 1389 (SO_2); ^1H NMR (DMSO): 8.21 (s, 1H, Ar–H,), 8.05–7.95 (m, 3H, Ar–H), 7.86–7.60 (m, 9H, Ar–H), 7.49 (s, 2H, NH_2); MS (EI, 70 eV): $m/z = 401.44$ [M^+].

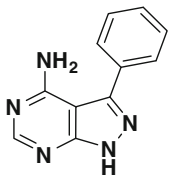
*3-Phenyl-1-(phenylsulfonyl)-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**10**)*

Yield: 66 %; mp. 226 °C; Anal. calc. for $C_{17}H_{13}N_5O_2S$: C 58.11, H 3.73, N 19.93, S 9.13 %; found: C 58.13, H 3.71, N 19.96, S 9.17 %; IR ν_{max} (cm^{-1}): 3463, 3308 (NH_2), 1641 (C=N), 1387 (SO_2); ^1H NMR (DMSO-d_6): 8.08–8.06 (m, 1H, Ar–H), 7.86–7.69 (m, 6H, Ar–H), 7.50 (s, 2H, NH_2); MS (EI, 70 eV): $m/z = 351.38$ [M^+].

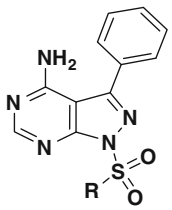
*3-Phenyl-1-(methyl sulfonyl)-1H-pyrazolo[3,4-d]pyrimidine-4-amine (**11**)*

Yield: 65 %; mp: 202 °C; Anal. calc. for $C_{12}H_{11}N_5O_2S$: C 49.82, H 3.83, N 24.21, S 11.08 %; found: C 49.81, H 3.87, N 24.19, S 11.12 %. IR ν_{max} (cm^{-1}): 3466, 3306 (NH_2), 1642 (C=N), 1390 (SO_2); ^1H NMR (DMSO-d_6): 8.51 (s, 1H, Ar–H), 7.57–7.35 (m, 5H, Ar–H), 7.65 (s, 2H, NH_2), 2.43 (s, 3H CH_3); MS (EI, 70 eV): $m/z = 289.31$ [M^+].

Table 1 In vitro antiamoebic activity of 3-phenyl-1*H*-pyrazolo[3,4-d]pyrimidine-4-amine and its derivatives against HM1:IMSS strain of *E. histolytica* and cytotoxicity profile of compounds **4**, **6** and MNZ



(3)



(4 - 11)

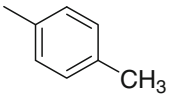
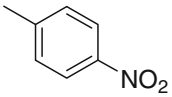
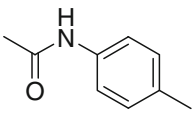
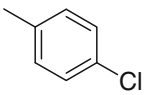
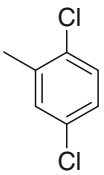
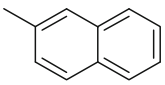
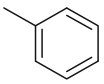
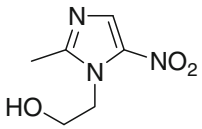
Compound no.	*R*	Antiamoebic activity		Cytotoxicity profile	
		IC_{50} (μM)	SD[a]	IC_{50} (μM)	SD[a]
3	–	4.73	0.019	N.D.	N.D.
4		0.57	0.016	>250	0.026
5		2.52	0.013	N.D.	N.D.
6		0.68	0.017	>250	0.021
7		3.23	0.008	N.D.	N.D.
8		2.37	0.021	N.D.	N.D.
9		3.11	0.024	N.D.	N.D.
10		4.24	0.011	N.D.	N.D.

Table 1 continued

Compound no.	*R*	Antiamoebic activity		Cytotoxicity profile	
		IC_{50} (μM)	SD[a]	IC_{50} (μM)	SD[a]
11	CH_3	5.15	0.027	N.D.	N.D.
		1.80	0.029	>250	0.022

N.D. not done

[a] Standard deviation

In vitro antiamoebic assay

All the desired compounds were screened for in vitro antiamoebic activity against HM1:IMSS strain of *E. histolytica* by microdilution method (Wright *et al.*, 1988). *E. 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. Twofold serial dilutions were made in the wells of 96-well microtiter plate (costar). Each test includes MNZ 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 kept under nitrogen for 10 min 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. 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 (**4**) and (**6**) and the standard drug MNZ on cell proliferation was measured using an MTT-based assay (Mosmann, 1983). Briefly, the cells (10,000/well) were incubated in triplicate in a 96-well plate in the presence of various concentrations of compounds (**4**), (**6**) as well as MNZ 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 CV: CV (%) = (*A* of the experimental samples/*A* of the control) × 100.

Conclusion

It was concluded that the compounds **4** and **6** showed better antiamoebic activity than the reference drug MNZ and found low cytotoxic against human breast cancer MCF-7 cell line. It is hoped that these preliminary results could help in designing better molecules with enhanced antiamoebic activity.

References

Abid M, Bhat AR, Athar F, Azam A (2009) Synthesis, spectral studies and antiamoebic activity of new 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines. Eur J Med Chem 44:417–425

Becker S, Hoffman P, Houpt ER (2011) Efficacy of antiamoebic drugs in a mouse model. Am J Trop Med Hyg 84:581–586

Bendesky A, Menendez D, Ostrosky-Wegman P (2002) Is metronidazole carcinogenic? Mutat Res 511:133–144

Budakoti A, Abid M, Azam A (2007) Synthesis characterization and in vitro antiamoebic activity of new Pd(II) complexes with 1-*N*-substituted thiocarbamoyl-3,5-diphenyl-2-pyrazoline derivatives. Eur J Med Chem 42:544–551

Dai HX, Stepan AF, Plummer MS, Zhang YH, Yu JQ (2011) Divergent C–H functionalizations directed by sulfonamide pharmacophores: last stage diversification as a tool for drug discovery. J Am Chem Soc 133:7222–7228

El-Sayed Ali T (2009) Synthesis of some novel pyrazolo[3,4-b]pyridine and pyrazolo[3,4-d]pyrimidine derivatives bearing 5,6-diphenyl-1,2,4-triazine moiety as potential antimicrobial agents. Eur J Med Chem 44:4385–4392

Gillin FD, Reiner DS, Suffness M (1982) Bruceantin, a potent amoebicide from a plant, *Brucea antidysenterica*. Antimicrob Agents Chemother 22:342–345

Hayat F, Salahuddin A, Umar S, Azam A (2010) Synthesis, characterization, antiamoebic activity and cytotoxicity of novel series of pyrazoline derivatives bearing quinoline tail. Eur J Med Chem 45:4669–4675

Hayat F, Salahuddin A, Azam A (2011) Synthesis, characterization, antiamoebic activity and cytotoxicity of new pyrazolo[3,4-d]pyrimidine-6-one derivatives. J Enzyme Inhib Med Chem 26:472–479

Hwang EW, Cheung L, Mojtahed A, Cartwright CA (2011) Relapse of intestinal and hepatic amoebiasis after treatment. Dig Dis Sci 56:677–680

Irfan I, Sawangjaroen N, Bhat AR, Azam A (2010) New dioxazole derivatives: synthesis and effects on growth of *Entamoeba histolytica* and *Giardia intestinalis*. Eur J Med Chem 45:1648–1653

Lejeune M, Rybicka JM, Chadee K (2009) Recent discoveries in the pathogenesis and immune response towards *Entamoeba histolytica*. Future Microbiol 4:105–118

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Parveen H, Hayat F, Salahuddin A, Azam A (2010) Synthesis, characterization and biological evaluation of novel 6-ferrocenyl-4-aryl-2-substituted pyrimidine derivatives. Eur J Med Chem 45:3497–3503

Parveen H, Hayat F, Mukhtar S, Salahuddin A, Khan A, Islam F, Azam A (2011) Synthesis, characterization and biological evaluation of novel 2,4,6-trisubstituted bis-pyrimidine derivatives. Eur J Med Chem 46:4669–4675

Ralston KS, Petri WA (2011) The ways of a killer: how does *Entamoeba histolytica* elicit host cell death? Essays Biochem 51:193–210

Salles JM, Moraes LA, Salles MC (2003) Hepatic amebiasis. Braz J Infect Dis 7:96–110

Siddiqui SM, Salahuddin A, Azam A (2012) Synthesis, characterization and antiamoebic activity of some hydrazone and azole derivatives bearing pyridyl moiety as a promising heterocyclic scaffold. Eur J Med Chem 49:411–416

Wright CW, O'Neill MJ, Phillipson JD, Warhurst DC (1988) Use of microdilution to assess in vitro antiamoebic activities of *Brucea javanica* fruits, *Simarouba amara* stem, and a number of quassinoids. Antimicrob Agents Chemother 32:1725–1729