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Short Communication

1-*N*-Substituted Thiocarbamoyl-3-Phenyl-2-Pyrazolines: Synthesis and In Vitro Antiamoebic Activities

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Abstract

The title compounds were prepared by reaction of Mannich bases with various *N*-4 substituted thiosemicarbazides. The chemical structures of the compounds were proved by means of their UV, IR, ^{1}H NMR, ^{13}C NMR spectroscopic data and elemental analyses. The in vitro antiamoebic activities of these compounds were evaluated by microdilution method against *HM1:IMSS* strain of *Entamoeba histolytica* and compared with the standard drug, metronidazole. It was concluded that 3-chloro and 3-bromo substituents on the phenyl ring at position 3 of the pyrazoline ring enhanced the antiamoebic activity. Compounds **9, 17, 18, 20** and **21** showed less IC_{50} value than metronidazole.

Keywords: Pyrazoline; Mannich base; Thiocarbamoyl; Thiosemicarbazide; *Entamoeba histolytica*

1. Introduction

Parasitic protozoa continue to beleaguer and kill millions of people in the subtropical and tropical regions of the world. The amitochondrial protist *E. histolytica* is estimated to infect up to 10% of the world's population. Fifty million cases of amoebic dysentery and liver abscess are reported each year [1]. These infections result in approximately 50,000 to 100,000 deaths annually [2]. Amoebiasis is primarily treated with the drug metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], even though significant side effects, such as neurological complications, and the possible selection of a resistant *E. histolytica* strain have been reported [3–6]. In addition, treatment failures among patients with amoebiasis often raise the possibility of drug resistance. Therefore it is desirable to search for new leads for amoebicidal drugs.

Pyrazoles and their reduced forms pyrazolines are well known nitrogen containing heterocyclic compounds and various procedures have been developed for their syntheses [7]. As a result, a wide variety of pyrazoles and pyrazolines have hitherto been described in the literature [7,8]. The interest of scientists in such compounds has been stimulated by their various promising pharmacological properties [9]. As evident from the literature, it was noted that very little research has been carried out on 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines [10], but no work has been done on screening of these compounds against *E. histolytica*. Earlier we have reported different heterocyclic thiosemicarbazones, their transition metal complexes and in vitro screening against *E. histolytica* [11–13] and their in vivo and cytotoxicity studies are in progress. As literature survey reveals the pharmacological importance of pyrazolines and their derivatives, this prompted us to synthesize new 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazoline derivatives **1-21** (Table 1) and their in vitro screening against *HM1:IMSS* strain of *E. histolytica*. To the best of our knowledge this is the first report against *E. histolytica*.

2. Chemistry

The Mannich reaction of various ketones with formaldehyde and dimethylamine hydrochloride generates the Mannich base precursor [14]. The reaction is sensitive to both the amount of hydrochloric acid and ethanol present. The reaction works best when a minimum amount of ethanol and 2 μL of acid/mmol ketone is added. The methyl phenyl ketone gave high yields above 80%, while the yields for 3-bromo

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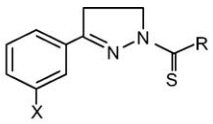
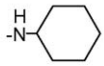
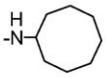
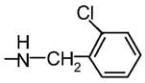
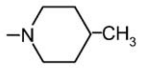
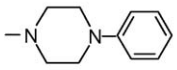
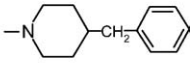
Table 1
Structure of 1-N-substituted thiocarbamoyl-3-phenyl-2-pyrazolines (**1-21**)



Compound	X	R
1.	H	
2.	Br	
3.	Cl	
4.	H	
5.	Br	
6.	Cl	
7.	H	
8.	Br	
9.	Cl	
10.	H	
11.	Br	
12.	Cl	
13.	H	
14.	Br	
15.	Cl	
16.	H	
17.	Br	
18.	Cl	
19.	H	
20.	Br	
21.	Cl	

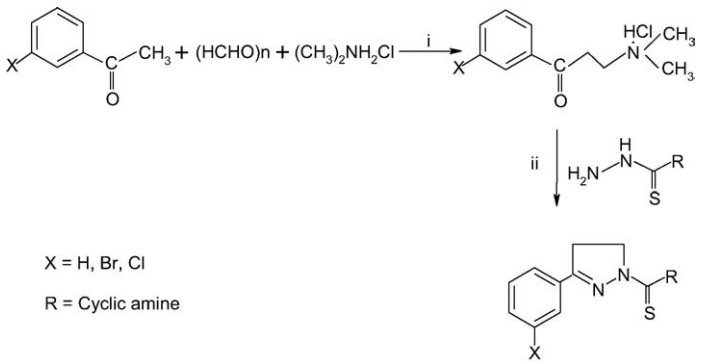
and 3-chloro acetophenones in the Mannich reaction were lower in the range of 40-60%. All the thiosemicarbazides were prepared by the method reported by O'Sullivan [15]. The condensation of Mannich reaction product with *N*-4 substituted thiosemicarbazides by different cyclic amines lead to the formation of 1-*N*-substituted thiocarbamoyl 3-phenyl-2-pyrazolines **1-21** (Scheme 1). According to the currently accepted mechanism, the formation of 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines is favored via thiosemicarbazone formation, which undergo cyclization under basic conditions to form desired pyrazoline ring in all the compounds [16,17]. All the compounds were purified by column chromatography to give crystalline solid compounds, but in low yield (9-25%). The compounds are stable in the solid as well as in the solution state. The structure of the compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR, electronic spectra and elemental analysis. The compounds are insoluble in water but soluble in methanol, ethanol and DMSO.

3. Pharmacology

All the 1-*N*-substituted pyrazoline derivatives **1-21** were screened in vitro for antiamoebic activity against *HM1:IMSS* strain of *E. histolytica* by microdilution method [18]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously [19] in wells of 96-well microtiter plate. Each compound tested was serially diluted and added to the growing trophozoites in microtiter plate. Effect on growth of trophozoites was monitored microscopically at regular interval and quantitative estimation of the drug action was made by protein estimation. The % inhibition of amoeba was calculated from the optical densities of the control and tested wells and was plotted against the logarithm of concentration of the drug tested. Linear regression analysis was used to determine the best fitting straight line from which IC_{50} value was found.

4. Results and Discussion

The synthesis of 1-*N*-substituted 3-phenyl-2-pyrazolines (**1-21**) was done by cyclization of Mannich bases with various *N*-4 substituted thiosemicarbazides in methanol under basic conditions. The product mixture contained only unreacted Mannich base and the cyclization product, which was purified by column chromatography using silica gel $60F_{254}$ eluted with dichloromethane: methanol (98:2). The yield of cyclised product in unsubstituted thiosemicarbazide was in the range of 50-70%; and 9-25% in the case of substituted thiosemicarbazides. Analytical data of the compounds are in good agreement with their composition. The structure of the compounds were established by means of their spectral data (IR, UV, ^{1}H NMR, ^{13}C NMR) and elemental analyses and are presented in Table 1.



Scheme 1. (i) Ethanol, hydrochloric acid, reflux; (ii) Methanol, NaOH, reflux.

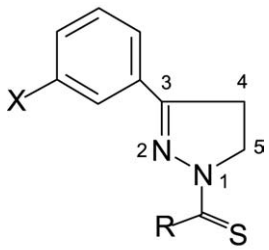
4.1. IR and electronic spectral studies

Selected diagnostic bands of the IR spectra of the 1-*N*-substituted 3-phenyl-2-pyrazolines (**1-21**) showed useful information about the structure of the compounds. All the compounds showed intense bands in the region 1001-1113 cm^{-1} due to the ν(C=S) stretch of the thiocarbamoyl group. The IR spectra of all the compounds showed ν(C=N) stretch at 1516-1599 cm^{-1} because of the ring closure. In addition, the absorption bands at 1115-1261 cm^{-1} were attributed to the ν(C-N) stretch vibrations, which also confirm the formation of desired pyrazoline ring in all the compounds. The compounds (**1-12**) showed additional sharp bands in the region 3260-3375 cm^{-1} due to the ν(NH) stretch.

The electronic spectra of all the compounds studied in the UV region in methanol, exhibited three absorption bands at 380.5-294 cm^{-1}, 288.5-233 cm^{-1} and 220-205.4 cm^{-1} assignable to n→π*, π→π* and n→σ* transitions respectively. The band at 380.5-294 cm^{-1} assigned to the n→π* transition involving the thione portion (C=S) of thiocarbamoyl group. The two other absorption bands at 288.5-233 cm^{-1} and 220-205.4 cm^{-1} were due to π→π* transition of phenyl ring and n→σ* transition of azomethine nitrogen, respectively.

4.2. Nuclear Magnetic Resonance spectral studies

The ^1H NMR spectra were recorded using $CDCl_3$ as the solvent clearly support the proposed structures of the compounds [8a]. The pyrazoline protons at C_4 and C_5 carbons appeared as broad triplets at 3.12-3.56 (J = 7.9-11.9 Hz) and 4.03-4.67 (J = 7.9-11.8 Hz) ppm respectively. The strong deshielding of the C_5 protons compared with the C_4 protons of the pyrazoline ring can be assumed due to its conformation A [14].



A

The NH proton of thiocarbamoyl group of the compounds (**1-12**) showed an additional singlet at 7.89-8.15 ppm. 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 were taken in $CDCl_3$ and the signals obtained are in good agreement with the proposed structures. The C_4 and C_5 carbons of the pyrazoline ring resonate at 43.8-49.6 and 73.5-78.5 ppm respectively. All the compounds showed a signal at 154.5-159.3 ppm due to the azomethine carbon of pyrazoline ring. Thiocarbamoyl carbon (C=S) displayed a signal at 174.1-182.5 ppm in all the compounds. The signals from 136.5 to 120.1 ppm were assumed due to the aromatic carbons. The carbons at *N*-1 substituted cyclic groups resonate at their usual positions and are shown in the data given in the experimental section.

4.3. Anti-amoebic activity

Two considerations governed the selection of compounds to be prepared as thiosemicarbazone analogues for this study. It was considered to have representatives of the three classes of 1-*N*-substituted 3-phenyl-2-pyrazolines with 3-bromo and 3-chloro substituents on the phenyl ring at position 3 of pyrazoline ring and 1-*N*-substituted with different cyclic groups. These include (1) 1-*N*- substituted derivatives, represented by compounds **1-12**; and (2) compounds where 1-*N* is contained in a medium ring, which includes all remaining compounds **13-21**. The choices were made to correspond to thiosemicarbazones with these bulky groups that displayed a high level of antiamoebic activity [11–13]. All the compounds were evaluated for antiamoebic activity in vitro using *HM1:IMSS* strain of *E. histolytica* to investigate the influence of the substitution. All the experiments were carried out in triplicate at each concentration level and repeated thrice. The IC_{50} values in μ M are shown in Table 2. The results were estimated as the percentage of growth inhibition compared with the untreated controls and plotted as probit values as a function of the drug concentration. The IC_{50} and 95%

Table 2
In vitro antiamoebic activities of 1-*N*-Substituted Thiocarbamoyl-3-Phenyl-2-Pyrazolines against (*HM1:IMSS*) strain of *E. histolytica*

Compound	IC_{50}(μM)	S.D.[a]
1.	6.5	0.5
2.	4.2	0.3
3.	3.7	0.3
4.	5.2	0.1
5.	3.1	0.1
6.	2.4	0.2
7.	2.5	0.3
8.	2.0	0.1
9.	1.5	0.2
10.	7.6	0.1
11.	2.6	0.2
12.	2.2	0.2
13.	4.8	0.1
14.	3.6	0.1
15.	2.8	0.3
16.	3.1	0.2
17.	1.6	0.1
18.	1.2	0.3
19.	2.3	0.3
20.	1.4	0.1
21.	1.0	0.1
Metronidazole	1.8	0.1

[a] Standard deviation.

confidence limits were interpolated in the corresponding dose-response curve. Metronidazole had a 50% inhibitory concentration (IC_{50} 1.7-1.8 μ M) in our experiments. All the 3-bromo and 3- chloro substituted pyrazoline derivatives were found to be more active than their respective unsubstituted analogues indicated that the substitution of bromine or chlorine on the phenyl ring at position 3 of pyrazoline ring enhances the antiamoebic activity. Among all the bromo and chloro derivatives, the most active compounds in this class were those 1-*N*-substituted 3-phenyl-2-pyrazoline derivatives, which have cyclooctyl amine (**9**, IC_{50} = 1.5 μ M), *N*-phenyl piperizine (**17**, IC_{50} = 1.6 μ M), (**18**, IC_{50} = 1.2 μ M), *N*-benzyl piperidine (**20**, IC_{50} = 1.4 μ M), (**21**, IC_{50} = 1.0 μ M) as 1-*N*-substitution. The results were statistically evaluated by analysis of variance. The null hypothesis was tested using *t*-test. The significativity of the difference between the IC_{50} values of metronidazole and the compounds **9**, **17**, **18**, **20** and **21** was evaluated by *t*-test. The values of the calculated *T* were found higher than the Table value of *T* at 5% level, thus concluding that the character under study is said to be significantly influenced by the treatment. It is concluded that the presence of these bulky groups at position 1-*N* of thiocarbamoyl group and substitution on the phenyl ring at position 3 of pyrazoline ring with chlorine or bromine greatly enhanced antiamoebic activity. Also important to note that the substitution of chlorine at 1-*N* thiocarbamoyl group does not effect the antiamoebic activity. Detailed studies of the toxicity of these compounds, mechanism of action as well as in vivo studies are in progress.

5. Conclusion

Although this class of compound is known since many years, and even the synthetic procedure for obtaining them has been recently published [10], very little is known about their anti-amoebic activity. This research examined the antiamoebic activity of the new 1-*N*-substituted 3-phenyl-2-pyrazolines (**1-21**) prepared by reaction of Mannich bases with thiosemicarbazides substituted by different cyclic amines. In vitro antiamoebic activity of the compounds was carried out against *HM1:IMSS* strain of *E. histolytica*. The biological behavior revealed that 3-chloro and 3-bromo substituents on the phenyl ring at position 3 of the pyrazoline ring increases the antiamoebic activity. All the bromo and chloro substituted pyrazoline derivatives show better antiamoebic activity than their respective unsubstituted analogues. Moreover, the compounds **9**, **17**, **18**, **20** and **21** were found to be more active than the reference drug, metronidazole.

6. Experimental protocols

6.1. Chemistry

Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} precoated thin layer plates. All the chemicals were purchased from Aldrich chemical company (USA). Elemental analyses (C,H,N) were carried out by Central Drug Research Institute, Lucknow, India, and the results were within 0.4% of the theoretical values. Melting points were recorded on a KSW melting point apparatus and were uncorrected. Electronic spectra were recorded in methanol on a Shimadzu UV- 1601 PC UV-Visible spectrophotometer. IR spectra on KBr disks were recorded on a Perkin Elmer model 1620 FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were obtained at ambient temperature using a Bruker Spectrospin DPX-300 MHz spectrophotometer in $CDCl_3$ using tetramethylsilane as an internal standard. Splitting patterns are designated as follows; s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values are given in (ppm) and coupling constants (*J*) in hertz. The ESI mass spectra of a few representative compounds were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer.

6.2. Synthesis of Mannich bases: A general method

A suspension of ketone (0.2 mol), dimethyl amine hydrochloride (0.26 mol) and paraformaldehyde (0.26 mol) in a mixture of 35 mL of ethanol and 0.5 mL of conc. HCl was refluxed for 2 hrs. After cooling, 200 mL of acetone was added .The crystals formed were collected, washed with acetone and dried in vacuo.

6.2.1. Acetophenone Mannich base (1)

White solid (chloroform). Yield: 87%; m.p.: 153 °C; IR: ν_{max} (cm^{-1}) 2952 (aliph. C-H), 1679 (C=O), 1468 (arom. C=C), 1224 (C-N); ^1H NMR ($CDCl_3$): δ 7.21-7.86 (m, 5H, Aryl), 3.29 (t, 2H, $-CH_2$), 2.97 (t, 2H, $-CH_2$), 2.53 (s, 6H, $-CH_3$).

6.2.2. 3-Bromo acetophenone Mannich base (2)

White solid (chloroform). Yield: 55%; m.p.: 176 °C; IR: ν_{max} (cm^{-1}) 2968 (aliph. C-H), 1689 (C=O), 1470 (arom. C=C), 1214 (C-N); ^1H NMR ($CDCl_3$): δ 7.12-7.95 (m, 4H, Aryl), 3.31 (t, 2H, $-CH_2$), 2.95 (t, 2H, $-CH_2$), 2.65 (s, 6H, $-CH_3$).

6.2.3. 3-Chloro acetophenone Mannich base (3)

White solid (chloroform). Yield: 42%; m.p.: 189 °C; IR: ν_{max} (cm^{-1}) 2967 (aliph. C-H), 1689 (C=O), 1480 (arom. C=C), 1218 (C-N); ^1H NMR ($CDCl_3$): δ 7.17-7.76 (m, 4H, Aryl), 3.33 (t, 2H, $-CH_2$), 2.92 (t, 2H, $-CH_2$), 2.71 (s, 6H, $-CH_3$).

6.3. Synthesis of 1-N-substituted thiocarbamoyl-3-phenyl-2-pyrazoline: A general method

Thiosemicarbazide (0.5 mmol) was dissolved in methanol (5 mL) upon refluxing under nitrogen. NaOH/H_2O (0.18 mL, 1:2 w/v) was added to the reaction mixture .The Mannich base (0.5 mmol) in methanol (5 mL) was added drop wise to

the reaction mixture and refluxed for 48-72 hrs. The methanol was removed in vacuo. The residue was dissolved in dichloromethane, washed with water and dried over anhydrous Na_2SO_4. The residual oil was purified via column chromatography on silica gel $60F_{254}$ eluted with 98:2 dichloromethane/methanol and crystallized using appropriate solvent.

6.3.1. N-cyclopentyl-3-phenyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (1)

Brown solid (methanol). Yield: 24%; m.p.: 160 °C; *Anal*. calc. ($C_{15}H_{19}N_3S$): C, 65.93; H, 6.96; N, 15.38; found: C, 65.89; H, 6.91; N, 15.41%; UV: λ_{max} (nm): 367, 324.1, 242.5, 206.2; IR: ν_{max} (cm^{-1}) 3309 (NH), 1560 (C=N), 1261 (C-N), 1098 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.19-7.64 (m, 5H, Aryl), 7.89 (s, 1H, -NH), 4.64 (t, 2H, -CH_2, J = 9.8 Hz), 3.29-3.60 (m, 1H, -CH), 3.17 (t, 2H, -CH_2, J = 9.0 Hz), 1.18-2.53 (m, 8H, -CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.2 (C=S), 157.4 (C=N), 131.7, 130.4, 129.1, 127.9, 125.3, 122.5 (Aryl-C), 74.2 (CH_2), 52.9 (CH), 45.9 (CH_2), 31.9 ($2CH_2$), 26.2 ($2CH_2$).

6.3.2. 3-(3-Bromophenyl)-N-cyclopentyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide(2)

Yellow solid (chloroform: methanol). Yield: 18%; m.p.: 172 °C; *Anal*. calc. ($C_{15}H_{18}N_3SBr$): C, 51.14; H, 5.11; N, 11.93; found: C, 51.07; H, 5.15; N, 11.91%; UV: λ_{max} (nm): 368, 241, 220; IR: ν_{max} (cm^{-1}) 3375 (NH), 1522 (C=N), 1192 (C-N), 1070 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.21-7.88 (m, 4H, Aryl), 8.10 (s, 1H, -NH), 4.43 (t, 2H, -CH_2, J = 8.7 Hz), 3.63-3.98 (m, 1H, -CH), 3.53 (t, 2H, -CH_2, J = 9.1 Hz), 1.27-2.17 (m, 8H, -CH_2); ^{13}C NMR ($CDCl_3$) : (δ, ppm) 180.3 (C=S), 158.7 (C=N), 133.2, 131.9, 129.4, 127.1, 125.8, 122.4 (Aryl-C), 73.8 (CH_2), 55.5 (CH), 46.3 (CH_2), 31.3 ($2CH_2$), 25.7 ($2CH_2$).

6.3.3. 3-(3-Chlorophenyl)-N-cyclopentyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (3)

Light yellow solid (chloroform). Yield: 15%; m.p.: 149 °C; *Anal*. calc. ($C_{15}H_{18}N_3SCl$): C, 58.54; H, 5.85; N, 13.66; found: C, 58.47; H, 5.78; N, 13.69; UV: λ_{max} (nm): 371, 351, 288, 249; IR: ν_{max} (cm^{-1}) 3375 (NH), 1516 (C=N), 1196 (C-N), 1080 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.22-7.99 (m, 4H, Aryl), 8.10 (s, 1H, -NH), 4.19 (t, 2H, -CH_2, J = 8.0 Hz), 4.55-4.67 (m, 1H, -CH), 3.29 (t, 2H, -CH_2, J = 8.2 Hz), 1.57-2.30 (m, 8H, -CH_2); ^{13}C NMR($CDCl_3$): (δ, ppm) 180.8 (C=S), 159.2 (C=N), 135.2, 133.9, 128.2, 126.8, 123.7, 121.5 (Aryl-C) 78.5 (CH_2), 53.9 (CH), 46.5 (CH_2), 29.7 ($2CH_2$), 25.3 ($2CH_2$).

6.3.4. N-cyclohexyl-3-phenyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (4)

Cream solid (chloroform: methanol). Yield: 20%; m.p.: 158 °C; *Anal*. calc. ($C_{16}H_{21}N_3S$): C, 66.90; H, 7.32; N, 14.63; found: C, 66.81; H, 7.19; N, 14.65; UV: λ_{max} (nm): 367, 325.5, 219.1, 205.5; IR: ν_{max} (cm^{-1}) 3296 (NH), 1526 (C=N), 1137 (C-N), 1089 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.20-7.72 (m, 5H, Aryl), 8.06 (s, 1H, -NH), 4.40 (t, 2H, -CH_2, J = 10.1 Hz), 3.47-3.63 (m, 1H, -CH), 3.25 (t, 2H, -CH_2, J = 10.7 Hz), 1.16-2.26 (m, 10H, -CH_2); ^{13}C NMR($CDCl_3$): (δ, ppm) 179.1 (C=S), 155.8 (C=N), 132.7, 130.5, 128.7, 126.2, 123.7, 121.4 (Aryl-C), 77.4 (CH_2), 54.2 (CH), 48.2 (CH_2), 33.2 ($2CH_2$), 25.5 ($2CH_2$), 23.8 (CH_2); ESI-MS: 288 (M+1).

6.3.5. 3-(3-Bromophenyl)-N-cyclohexyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (5)

White solid (chloroform). Yield: 16%; m.p.: 179 °C; *Anal*. calc. ($C_{16}H_{20}N_3SBr$): C, 52.46; H, 5.46; N, 11.48; found: C, 52.49; H, 5.40; N, 11.45; UV: λ_{max} (nm): 377.4, 240, 220; IR: ν_{max} (cm^{-1}) 3297 (NH), 1553 (C=N), 1227 (C-N), 1111 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.23-7.88 (m, 4H, Aryl), 8.15 (s, 1H, -NH), 4.35 (t, 2H, -CH_2, J = 9.0 Hz), 3.36-3.68 (m, 1H, -CH), 3.24 (t, 2H, -CH_2, J = 9.1 Hz), 1.22-2.01 (m, 10H, -CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.3 (C=S), 157.3 (C=N), 136.3, 132.7, 129.7, 126.2, 124.4, 122.2 (Aryl-C), 78.1 (CH_2), 54.5 (CH), 47.4 (CH_2), 32.8 ($2CH_2$), 25.5 ($2CH_2$), 23.8 (CH_2); ESI-MS: 367 (M+1).

6.3.6. 3-(3-Chlorophenyl)-N-cyclohexyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (6)

Light yellow solid (chloroform). Yield: 14%; m.p.: 161 °C; *Anal*. calc. for ($C_{16}H_{20}N_3SCl$): C, 59.72; H, 6.22; N, 13.06; found: C, 59.69; H, 6.31; N, 13.17; UV: λ_{max} (nm): 370.5, 349.5, 288.5, 249; IR: ν_{max} (cm^{-1}) 3350 (NH), 1521 (C=N), 1135 (C-N), 1060 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.21-7.97 (m, 4H, Aryl), 8.09 (s, 1H, -NH), 4.37 (t, 2H, -CH_2, J = 8.1 Hz), 3.60-3.82 (m, 1H, -CH), 3.29 (t, 2H, -CH_2, J = 8.7 Hz), 1.37-2.11 (m, 10H, -CH_2); ^{13}C NMR($CDCl_3$): (δ, ppm) 179.5 (C=S), 156.4 (C=N), 135.7, 133.7, 131.4, 129.7, 125.8, 120.8 (Aryl), 77.5 (CH_2), 54.5 (CH), 46.9 (CH_2), 31.6 ($2CH_2$), 24.1 ($2CH_2$), 22.2 (CH_2).

6.3.7. N-cyclooctyl-3-phenyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (7)

Yellow solid (chloroform). Yield: 17%; m.p.: 128 °C; *Anal*. calc. ($C_{18}H_{25}N_3S$): C 68.57, H 7.94, N 13.33; found: C 68.45, H, 7.92, N, 13.41; λ_{max} (nm): 377.4, 324.7, 245, 207; IR: ν_{max} (cm^{-1}) 3375 (NH), 1523 (C=N), 1259 (C-N), 1113 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.16-7.67 (m, 5H, Aryl), 7.92 (s, 1H, -NH), 4.04 (t, 2H, -CH_2, J = 11.8 Hz), 3.39-3.60 (m, 1H, -CH), 3.24 (t, 2H, -CH_2, J = 11.8 Hz), 1.25-2.16 (m, 14H, -CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 180.4 (C=S), 155.5 (C=N), 132.1, 129.4, 127.4, 126.1, 124.5, 122.4 (Aryl-C), 76.6 (CH_2), 52.9 (CH), 47.7 (CH_2), 33.9 ($2CH_2$), 30.3 ($2CH_2$), 24.8 ($2CH_2$), 22.4 (CH_2).

6.3.8. 3-(3-Bromophenyl)-N-cyclooctyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (8)

Yellow solid (chloroform: methanol). Yield: 13%; m.p.: 177 °C; *Anal*. calc. for ($C_{18}H_{24}N_3SBr$): C, 54.82, H, 6.09, N, 10.66; found: C, 54.79, H, 6.12, N, 10.55; λ_{max} (nm): 377.4, 328.4, 241.2, 210.6; IR: ν_{max} (cm^{-1}) 3349 (NH), 1526 (C=N), 1155 (C-N), 1068 (C=S); ^{1}H NMR($CDCl_3$): δ 7.17-7.86 (m, 4H, Aryl), 8.06 (s, 1H, -NH), 4.41 (t, 2H, -CH_2, J = 8.5 Hz),

4.55-4.68 (m, 1H, -CH), 3.24 (t, 2H, -CH_2, *J* = 7.9 Hz), 1.26-2.20 (m, 14H, -CH_2); ^{13}C NMR($CDCl_3$): (δ, ppm) 182.5 (C=S), 158.1 (C=N), 133.5, 131.2, 129.5, 126.1, 124.5, 122.8 (Aryl-C), 76.1 (CH_2), 58.7 (CH), 45.3 (CH_2), 33.2 ($2CH_2$), 30.7 ($2CH_2$), 26.3 ($2CH_2$), 24.1 (CH_2).

6.3.9. 3-(3-Chlorophenyl)-N-cyclooctyl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (9)

creamish yellow solid (chloroform). Yield: 15%; m.p.: 124 °C; *Anal*. calc. for ($C_{18}H_{24}N_3SCl$) : C, 61.80, H, 6.87, N, 12.02; found: C, 61.84, H, 6.79, N, 12.12; λ_{max} (nm): 378, 320, 247.4, 207; IR: ν_{max} (cm^{-1}) 3346 (NH), 1539 (C=N), 1115 (C-N), 1059 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.18-7.92 (m, 4H, Aryl), 8.14 (s, 1H, -NH), 4.56 (t, 2H, -CH_2, *J* = 8.7 Hz), 3.67-3.73 (m, 1H, -CH), 3.33 (t, 2H, -CH_2, *J* = 9.1 Hz), 1.57-1.99 (m, 14H, -CH_2); ^{13}C NMR($CDCl_3$): (δ, ppm) 181.6 (C=S), 156.9 (C=N), 133.4, 131.7, 129.4, 126.1, 124.5, 121.2 (Aryl-C), 75.2 (CH_2), 52.9 (CH), 47.7 (CH_2), 33.9 ($2CH_2$), 30.3 ($2CH_2$), 24.8 ($2CH_2$), 22.4 (CH_2).

6.3.10. N-[(2-chlorophenyl)methyl]-3-phenyl-4,5-dihydro-1H-pyrazole-1-thiocarboxam-ide (10)

White solid (chloroform). Yield: 14%; m.p.: 189 °C; *Anal*. calc. for ($C_{17}H_{16}N_3SCl$): C, 61.91, H, 4.85, N, 12.75; found: C, 61.89, H, 4.84, N, 12.77; λ_{max} (nm): 313, 244, 211.4; IR: ν_{max} (cm^{-1}) 3262 (NH), 1587 (C=N), 1130 (C-N), 1038 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.18-7.85 (m, 9H, Aryl), 8.14 (s, 1H, -NH), 4.47 (t, 2H, -CH_2, *J* = 8.0 Hz), 4.98 (d, 2H, -CH_2, *J* = 5.4 Hz), 3.27 (t, 2H, -CH_2, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.4 (C=S), 158.1 (C=N), 134.5, 133.3, 131.4, 130.5, 127.9, 125.4 – 121.6 (Aryl-C), 77.4 (CH_2), 52.6 (CH_2), 49.6 (CH_2); ESI-MS: 331 (M+1).

6.3.11. 3-(3-Bromophenyl)-N-[(2-chlorophenyl)methyl]-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (11)

Yellow solid (chloroform: methanol). Yield: 11%; m.p.: 177 °C; *Anal*. calc. for ($C_{17}H_{15}N_3SBrCl$): C, 49.94, H, 3.67, N, 10.28; found: C, 49.95, H, 3.89, N, 10.31; λ_{max} (nm): 377.4, 296, 243.5, 210.2; IR: ν_{max} (cm^{-1}) 3260 (NH), 1568 (C=N), 1129 (C-N), 1036 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.24-7.94 (m, 8H, Aryl), 8.14 (s, 1H, -NH), 4.46 (t, 2H, -CH_2, *J* = 7.9 Hz), 4.78 (d, 2H, -CH_2, *J* = 5.1 Hz), 3.27 (t, 2H, -CH_2, *J* = 8.1 Hz); ^{13}C NMR($CDCl_3$): (δ, ppm) 179.8 (C=S), 159.2 (C=N), 135.3, 132.6, 130.3, 128.4, 125.7-122.8 (Aryl-C), 78.3 (CH_2), 56.3 (CH_2), 48.9 (CH_2).

6.3.12. 3-(3-Chlorophenyl)-N-[(2-chlorophenyl)methyl]-4,5-dihydro-1H-pyrazole-1-thiocarboxamide (12)

Dark yellow solid (Chloroform: methanol). Yield: 9**%**; m.p.: 189 °C; *Anal*. calc. for ($C_{17}H_{15}N_3SCl_2$): C, 56.04, H, 4.12, N, 11.54; found: C, 56.11, H, 4.15, N, 11.58; λ_{max} (nm): 323.5, 245, 216; IR: ν_{max} (cm^{-1}) 3295 (NH), 1561 (C=N), 1185 (C-N), 1070 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.19-7.83 (m, 8H, Aryl), 8.11 (s, 1H, -NH), 4.36 (t, 2H, -CH_2, *J* = 9.1 Hz), 4.82 (d, 2H, -CH_2, *J* = 5.2 Hz), 3.29 (t, 2H, -CH_2, *J* = 9.6 Hz); ^{13}C NMR($CDCl_3$): (δ,ppm) 177.4 (C=S), 155.7 (C=N), 136.2, 134.5, 131.9, 127.9, 125.7-120.9 (Aryl-C), 76.1 (CH_2), 51.8 (CH_2), 47.2 (CH_2).

6.3.13. 4-Methyl-1-[(3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)thiocarbamoyl]piperidine (13)

Dark Brown solid (methanol). Yield: 18%; m.p.: 104 °C; *Anal*. calc. for ($C_{16}H_{21}N_3S$): C, 66.90, H, 7.32, N, 14.63; found: C, 66.92, H, 7.29, N, 14.65; λ_{max} (nm): 360.5, 321.1, 234, 205.4; IR: ν_{max} (cm^{-1}) 1596 (C=N), 1145 (C-N), 1101 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.18-7.69 (m, 5H, Aryl), 4.67 (t, 2H, -CH_2, *J* = 11.3 Hz), 3.13 (t, 2H, -CH_2, *J* = 11.1 Hz), 3.23-3.57 (m, 4H, -NCH_2), 1.89-2.21 (m, 1H, -CH), 1.19-1.69 (m, 4H, -CH_2), 1.01 (d, 3H, -CH_3, *J* = 5.6 Hz); ^{13}C NMR ($CDCl_3$): (δ,ppm) 178.5 (C=S), 157.5 (C=N), 132.3, 129.4, 127.6, 125.1, 122.4, 121.5 (Aryl-C), 77.0 (CH_2), 52.7 ($2CH_2$), 46.9 (CH_2), 36.2 (CH), 32.8 ($2CH_2$), 13.3 (CH_3).

6.3.14. 1-{[3-(3-Bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl]thiocarbamoyl}-4-methyl piperidine (14)

Brown solid (methanol). Yield: 12%; m.p.: 118 °C; *Anal*. calc. for ($C_{16}H_{20}N_3SBr$): C, 52.46, H, 5.46, N, 11.48; found: C, 52.47, H, 5.49, N, 11.46; λ_{max} (nm): 324.1, 295.5, 244, 217.4; IR: ν_{max} (cm^{-1}) 1564 (C=N), 1248 (C-N), 1069 (C=S); ^{1}H-NMR ($CDCl_3$): δ 7.22-7.88 (m, 4H, Aryl), 4.43 (t, 2H, -CH_2, *J* = 9.2 Hz), 3.16 (t, 2H, -CH_2, *J* = 9.2 Hz), 3.36-3.77 (m, 4H, -NCH_2), 2.21-2.59 (m, 1H, -CH), 1.75-2.16 (m, 4H, -CH_2), 1.02 (d, 3H, -CH_3, *J* = 4.6 Hz); ^{13}C NMR($CDCl_3$): (δ, ppm) 177.2 (C=S), 156.4 (C=N), 135.4, 133.8, 131.2, 128.9, 125.2, 122.7 (Aryl-C), 77.2 (CH_2), 52.4 ($2CH_2$), 48.3 (CH_2), 40.2 (CH), 33.7 ($2CH_2$), 13.1 (CH_3).

6.3.15. 1-{[3-(3-Chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]thiocarbamoyl}-4-methyl piperidine (15)

Yellow solid (Chloroform: methanol). Yield: 15%; m.p.: 147 °C; *Anal*. calc. for ($C_{16}H_{20}N_3SCl$): C, 59.72, H, 6.22, N, 13.06**;** found: C, 59.89, H, 6.27, N, 13.03; λ_{max} (nm): 380.5, 347.5, 293.5, 287, 246; IR: ν_{max} (cm^{-1}) 1549 (C=N), 1189 (C-N), 1060 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.21-7.87 (m, 4H, Aryl), 4.52 (t, 2H, -CH_2 , *J* = 8.2 Hz), 3.36 (t, 2H, -CH_2, *J* = 8.7 Hz), 3.47-3.69 (m, 4H, -NCH_2), 2.13-2.46 (m, 1H, -CH), 1.79-2.08 (m, 4H, -CH_2), 1.11 (d, 3H, -CH_3, *J* = 5.0 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.9 (C=S), 158.9 (C=N), 135.2, 131.8, 129.7, 126.2, 124.1, 121.3 (Aryl-C), 75.3 (CH_2), 53.7 ($2CH_2$), 47.9 (CH_2), 38.4 (CH), 32.8 ($2CH_2$), 13.7 (CH_3).

6.3.16. 4-Phenyl-1-[(3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)thiocarbamoyl]piperazine (16)

Golden yellow solid (chloroform: methanol). Yield: 15%; m.p.: 167 °C; *Anal*. calc. for ($C_{20}H_{22}N_4S$): C, 68.57, H, 6.29, N, 16.00; found: C, 68.59, H, 6.27, N, 15.98; λ_{max} (nm): 371, 295.4, 233, 206; IR: ν_{max} (cm^{-1}) 1597 (C=N), 1156 (C-N), 1001 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.20-7.71 (m, 10H, Aryl), 4.03 (t, 2H, -CH_2, *J* = 11.1 Hz), 3.36 (t, 2H, -CH_2, *J* = 11.8 Hz), 3.65 (t, 4H, -NCH_2), 3.19 (t, 4H, -NCH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.1 (C=S), 156.3 (C=N), 131.4,

130.8, 127.6, 125.1-121.2 (Aryl-C), 73.5 (CH_2), 56.2 ($2CH_2$), 46.7 (CH_2), 42.9 ($2CH_2$).

6.3.17. 1-{[3-(3-Bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl]thiocarbamoyl}-4-phenyl piperazine (17)

Yellow solid (chloroform: methanol). Yield: 12%; m.p.: 88 °C; *Anal*. calc. for ($C_{20}H_{21}N_4SBr$): C, 55.94, H, 4.89, N, 13.05; found: C, 56.01, H, 4.91, N, 13.06; λ_{max} (nm): 377.4, 289.2, 245.3, 216.3; IR: ν_{max} (cm^{-1}) 1564 (C=N), 1188 (C-N), 1068 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.18-7.85 (m, 9H, Aryl), 4.25 (t, 2H, -CH_2, *J* = 9.8 Hz), 3.56 (t, 2H, -CH_2, *J* = 10.0 Hz), 3.63-3.76 (m, 4H, -NCH_2), 3.19-3.47 (m, 4H, -NCH_2); ^{13}C NMR($CDCl_3$): (δ, ppm) 176.3 (C=S), 157.1 (C=N), 134.1, 132.2, 130.9, 129.4, 127.5, 126.3-122.9 (Aryl-C), 74.7 (CH_2), 56.7 ($2CH_2$), 45.8 ($2CH_2$), 48.9 (CH_2).

6.3.18. 1-{[3-(3-Chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]thiocarbamoyl}-4-phenyl piperazine (18)

Brown solid (methanol), Yield: 10%; m.p.: 117 °C; *Anal*. calc. for ($C_{20}H_{21}N_4SCl$): C, 62.42, H, 5.46, N, 14.56; found: C, 62.45, H, 5.41, N, 14.59; λ_{max} (nm): 370.5, 294, 249.5; IR: ν_{max} (cm^{-1}) 1599 (C=N), 1148 (C-N), 1015 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.23-7.87 (m, 9H, Aryl), 4.41 (t, 2H, -CH_2, *J* = 8.7 Hz), 3.35 (t, 2H, -CH_2, *J* = 8.2 Hz), 3.59-3.79 (m, 4H, -NCH_2), 3.17-3.30 (m, 4H, -NCH_2); ^{13}C NMR($CDCl_3$): (δ, ppm) 178.5 (C=S), 156.8 (C=N), 136.2, 134.9, 132.7, 130.4, 125.5-120.1 (Aryl-C), 77.1 (CH_2), 54.3 ($2CH_2$), 44.2 ($2CH_2$), 46.8 (CH_2).

6.3.19. 1-[(3-Phenyl-4,5-dihydro-1H-pyrazol-1-yl)thiocarbamoyl]-4-(phenylmethyl)- piperazine (19)

Yellow solid (chloroform: methanol). Yield: 12%; m.p.: 143 °C; *Anal*. calc. for ($C_{22}H_{25}N_3S$): C, 72.73, H, 6.89, N, 11.57; found: C, 72.74, H, 6.87, N, 11.54; λ_{max} (nm): 319.2, 280.1, 246.4, 210.5; IR: ν_{max} (cm^{-1}) 1597 (C=N), 1216 (C-N), 1056 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.16-7.75 (m, 10H, Aryl), 4.42 (t, 2H, -CH_2, *J* = 9.2 Hz), 2.59 (d, 2H, -CH_2), 3.36 (t, 2H, -CH_2, *J* = 8.9 Hz), 2.26-2.43 (m, 1H, -CH), 3.62-3.97 (m, 4H, -NCH_2), 1.50-1.86 (m, 4H, -CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.3 (C=S), 154.5 (C=N), 131.0, 129.3, 128.7, 125.3-122.4 (Aryl-C), 76.7 (CH_2), 56.4 ($2CH_2$), 45.1 (CH_2), 38.6 (CH_2), 29.4 ($2CH_2$), 34.5 (CH).

6.3.20. 1-{[3-(3-Bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl]thiocarbamoyl}-4-(phenyl methyl)piperazine (20)

Dark brown solid (methanol). Yield: 9%; m.p.: 133 °C; *Anal*. calc. for ($C_{22}H_{24}N_3SBr$): C, 59.73, H, 5.43, N, 9.50; found: C, 59.81, H, 5.47, N, 9.49; λ_{max} (nm): 377.4, 247.4, 209.2; IR: ν_{max} (cm^{-1}) 1559 (C=N), 1187 (C-N), 1097 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.22-7.90 (m, 9H, Aryl), 4.41 (t, 2H, -CH_2, *J* = 11.2 Hz), 2.64 (d, 2H, -CH_2, *J* = 7.1 Hz), 3.12 (t, 2H, -CH_2, *J* = 11.9 Hz), 2.29-2.57 (m, 1H, -CH, *J* = 4.8 Hz), 3.37-3.69 (m, 4H, -NCH_2), 1.59-2.13 (m, 4H, -CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.4 (C=S), 157.2 (C=N), 134.3, 133.7, 131.4, 129.8, 125.1-121.6 (Aryl-C), 76.3 (CH_2), 56.6 ($2CH_2$), 48.4 (CH_2), 35.7 (CH_2), 28.9 ($2CH_2$), 33.6 (CH).

6.3.21. 1-{[3-(3-Chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl]thiocarbamoyl}-4-(phenyl methyl)piperazine (21)

Cream solid (Chloroform), Yield: 10%; m.p.: 154 °C; *Anal*. calc. for ($C_{22}H_{24}N_3SCl$): C, 66.42, H, 6.04, N, 10.57; found: C, 66.49, H, 6.11, N, 10.54; λ_{max} (nm): 325.3, 233, 216; IR: ν_{max} (cm^{-1}) 1585 (C=N), 1169 (C-N), 1065 (C=S); ^{1}H NMR ($CDCl_3$): δ 7.21-7.85 (m, 9H, Aryl), 4.45 (t, 2H, -CH_2, *J* = 9.2 Hz), 2.63 (d, 2H, -CH_2, *J* = 5.8 Hz), 3.32 (t, 2H, -CH_2, *J* = 8.9 Hz), 2.19-2.46 (m, 1H, -CH), 3.39-3.76 (m, 4H, -NCH_2), 1.32-1.97 (m, 4H, -CH_2); ^{13}C NMR($CDCl_3$):(δ,ppm) 175.3 (C=S), 159.3 (C=N), 136.5, 133.8, 130.2, 128.1, 125.7-120.7 (Aryl-C), 75.9 (CH_2), 58.1 ($2CH_2$), 43.8 (CH_2), 36.9 (CH_2), 30.5 ($2CH_2$), 33.9 (CH).

6.4. In vitro testing against E. histolytica

All the 1-*N*-substituted 3-phenyl-2-pyrazoline derivatives (**1-21**) were screened in vitro for antiamoebic activity against (*HM1:IMSS*) strain of *E. histolytica* by using a microplate method [18]. DMSO (40 μ L) [20,21] was added to all the samples (~1 mg) followed by enough culture medium to obtain concentration of 1 mg/mL. Samples were dissolved or suspended by mild sonication in a sonicleaner bath for a few minutes and then further diluted with medium to concentrations of 0.1 mg/mL. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (Costar) in 170 μ L of medium. Each test included metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) was prepared from a confluent culture by pouring off the medium, adding 2 m L of medium and chilling the culture on ice to detach the organisms from the side of the flask. The number of amoeba per m L was estimated with a heamocytometer and trypan blue exclusion was used to confirm viability. The cell suspension used was diluted to 10^5 organism/mL by adding fresh medium and 170 μ L of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 μ L). An inoculum of 1.7 X 10^4 organisms/well was chosen so that confluent, but not excessive growth took place in control wells. Plates were sealed with expanded polystyrene (0.5 thick). Secured with tape, placed in a modular incubating chamber (flow laboratories, High wycombe, UK), and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h.

6.5. Assessment of antiamoebic activity

After incubation, the growth of amoebae 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 once in 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 amoebae. The plate was allowed to dry at room temperature, and the amoebae were fixed with methanol, when dry, stained with (0.5%) aqueous eosin for 15 min. Stained plate was washed once with tape water and then twice with

distilled water and allowed to dry. A 200 μ L 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 straight line from which the IC_{50} value was found.

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