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Short communication

Synthesis, spectral studies and antiamoebic activity of new 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines

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Abstract

Thirty new pyrazoline derivatives were synthesized by cyclization of Mannich bases with thiosemicarbazides being substituted by different cyclic and aromatic amines. The structures of the compounds were elucidated by elemental analyses, UV, IR, ^{1}H and ^{13}C NMR and ESI-MS spectral data. The *in vitro* antiamoebic activity was evaluated against *Entamoeba histolytica* in comparison with metronidazole used as reference substance. Out of the 30 compounds screened for antiamoebic activity, 10 (**5**, **6**, **15**, **18**, **25–30**) were found to be better inhibitors of *E. histolytica* since they showed lesser IC_{50} values than metronidazole. The preliminary results indicated that the presence of 3-chloro or 3-bromo substituent on the phenyl ring at position 3 of the pyrazoline ring enhanced the antiamoebic activity as compared to unsubstituted phenyl ring. The study suggests that the preliminary activity of these compounds may further be explored for the development of new targets for amoebiasis.

Keywords: Pyrazolines; Mannich bases; Thiocarbamoyl; *Entamoeba histolytica*

1. Introduction

Amoebiasis is a protozoan infection caused by intestinal parasite *Entamoeba histolytica*. The prevalence of amoebic colitis and liver abscess is greater in developing regions such as Central and South America than in the industrialized world. It is the third most common cause of death from parasitic diseases after malaria and schistosomiasis. It is estimated that 40–50 million cases of amoebic colitis and liver abscess due to *E. histolytica* occur worldwide and result in 100 000 deaths [1–3]. Amoebic abscesses of the brain are a dreadful complication of *E. histolytica* infection [4]. The cornerstones of amoebic liver abscess treatment are nitro imidazoles such as metronidazole. However, metronidazole is mutagenic and has been associated with serious side effects and some *E. histolytica* strains resistant to this drug have also begun to appear [5–8]. Therefore it is desirable to search for new lead molecules, which can be effectively used against amoebiasis.

In drug designing programs an essential component of the search for new leads is the synthesis of molecules, which is novel yet resembles known biologically active molecules by virtue of the presence of critical structural features. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules [9,10]. Pyrazoles and their reduced forms, pyrazolines, are well known nitrogen containing heterocyclic compounds, and various procedures have been developed for their syntheses [10]. The interest of scientists in such compounds has been stimulated by their various promising pharmacological properties [11]. 2-Pyrazoline derivatives have been reported to exhibit various pharmacological activities such as antibacterial, antifungal, antimicrobial and antidepressant [12–16]. In continuation to our ongoing research work on pyrazoline derivatives [17], we describe herein the synthesis, spectral studies and *in vitro* antiamoebic activity of new 1-*N*-substituted-2-pyrazoline derivatives against *HM1:IMSS* strain of *E. histolytica*.

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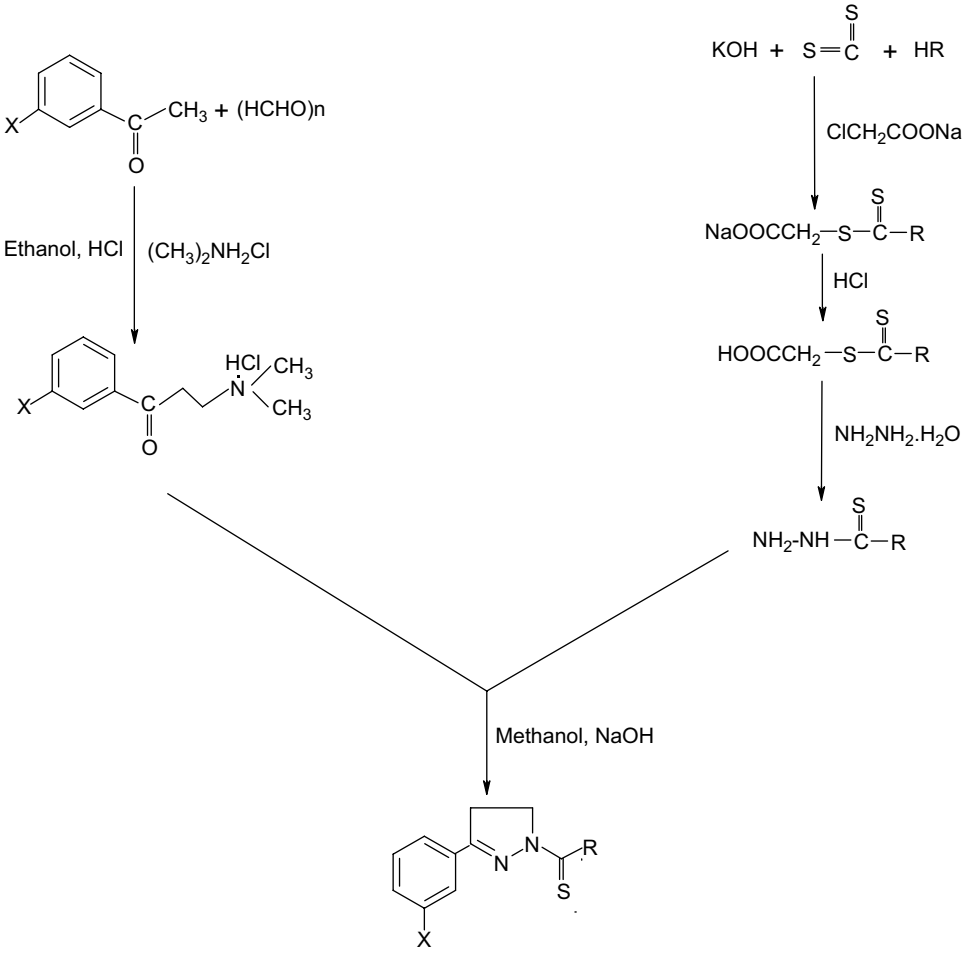
2. Chemistry

The aforementioned compounds were prepared according to the synthetic sequences illustrated in Scheme 1. The Mannich bases were synthesized by the reaction of respective ketones with paraformaldehyde and dimethylamine hydrochloride in a mixture of ethanol and conc. HCl [18]. High yields were obtained when a minimum amount of ethanol and 2 μL of acid/mmol of ketone are added in the Mannich reaction. The methyl phenyl ketone gave high yields of above 80%, while the yields for substituted acetophenone in the Mannich reaction were lower in the range of 40–60%. The synthesis of 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines (**1–30**) was carried out by reacting Mannich bases with *N*4-substituted thiosemicarbazides [19]. The formation of pyrazoline derivatives is favored via thiosemicarbazone formation, which undergoes cyclization under basic conditions to form the desired pyrazoline ring in all the compounds [20,21]. The purity of the compounds was established by thin layer chromatography (TLC) and elemental analyses. The compounds were stable in the solid as well as in the solution state. Elemental analysis and spectral data were in agreement with the structure of the synthesized compounds. The compounds were insoluble in water but soluble in chloroform, methanol and most of the organic solvents.

The interest in the IR spectra of the compounds lies mainly in the bands due to NH–C=S, C=N and C–N functional groups. The compounds **16–30** may exist in thione–thiol tautomerism since they contain a thioamide (–NH–C=S) functional group. The IR spectra of these compounds indicate that they retain their thione form in the solid state, as they showed a strong band at 1023–1100 cm^{-1} due to ν(C=S) stretching vibrations. A strong band appeared at 1516–1605 cm^{-1} was assigned to ν(C=N) because of ring closure [22]. In addition, the absorption bands at 1112–1262 cm^{-1} were attributed to the ν(C–N) stretching vibrations, which also confirm the formation of desired pyrazoline ring in all the compounds. The compounds **16–30** showed an additional sharp band in the region 3214–3446 cm^{-1} due to the ν(NH) stretch. The electronic spectra of all the compounds studied



Scheme 1.

in the UV region, exhibited three absorption bands at 382–284 cm^{-1}, 267–243 cm^{-1} and 237–203 cm^{-1} assignable to $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, respectively. The band at 382–284 cm^{-1} is assigned to the $n \rightarrow \pi^*$ transition involving the thione portion (C=S) of thiocarbamoyl group. The two other absorption bands at 267–243 cm^{-1} and 237–203 cm^{-1} were due to $\pi \rightarrow \pi^*$ transition of phenyl ring and $n \rightarrow \sigma^*$ transition of azomethine nitrogen, respectively. The ^1H NMR spectra showed two broad triplets at 3.13–3.93 ppm ($J = 6.66–10.71$ Hz) and 4.29–4.61 ppm ($J = 6.23–12.00$ Hz) due to pyrazoline protons at C_4 and C_5 carbons, 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 structure (Fig. 1).

The compounds **16–30** showed an additional singlet at 8.04–9.12 ppm due to NH proton of thiocarbamoyl group. The protons belonging to the aromatic ring and the other cyclic groups were observed with the expected chemical shift and integral values. The C_4 and C_5 carbons of the pyrazoline ring in the ^{13}C NMR spectra showed two signals at 44.9–52.1 ppm and 72.1–78.7 ppm. All the compounds showed a signal at 154.2–159.7 ppm, which was assigned to the azomethine carbon of pyrazoline ring. Thiocarbamoyl carbon (C=S) displayed a signal at 174.9–183.6 ppm in all the compounds. The signals from 138.6 ppm to 120.1 ppm were assumed to be due to the aromatic carbons. The carbons at 1-*N*-substituted cyclic groups resonate at their usual positions and are shown in the data given in Section 4. The characteristic peaks were observed in the mass spectra of pyrazoline derivatives. Their mass spectra exhibit molecular ion peaks and contain fragments that confirm the pyrazoline ring structure in all the compounds. The spectra of all the compounds suggest that the pyrazoline derivatives remain in thione–thiol tautomerization. The fragmentation of compound **17** showed an M − SH ion at *m/z* 342 due to the removal of thiol (SH) functionality of the compound. This is followed by elimination of CH_3 radical to give substituted pyrazoline ion at *m/z* 329 as the base peak. The fragmentation of the molecular ion may also occur by elimination of CH_3 radical followed by removal of SH radical to give ion peak at *m/z* 329. The removal of CN radical produced disubstituted pyrazoline ion at *m/z* 303. The removal of phenyl radical produces ion peak at *m/z* 226. The peak at *m/z* 182 was also observed due to *m*-bromo phenyl cyanide ion after the removal of azirinium radical (*m/z* 42).

The fragmentation of compound **18** also shows the same fragmentation pattern. It shows an M − SH ion at *m/z* 299 due to the removal of thiol (SH) functionality of the compound. This is followed by elimination of CH_3 radical to give substituted pyrazoline ion at *m/z* 284 as the base peak. The fragmentation of the molecular ion may also occur by elimination of CH_3 radical followed by removal of SH radical to give ion peak at *m/z* 284. The removal of CN radical produced disubstituted pyrazoline ion at *m/z* 261. The removal of phenyl radical produces ion peak at *m/z* 185. The peak at *m/z* 141 was also observed due to *m*-chloro phenyl cyanide ion.

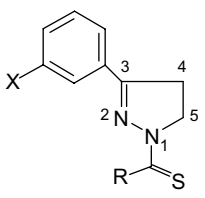
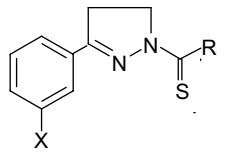


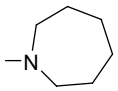
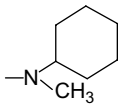
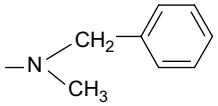
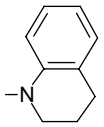
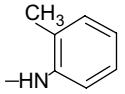
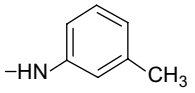
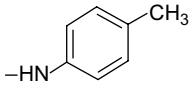
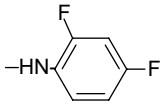
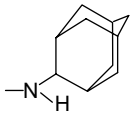
Fig. 1.

3. Antiamoebic activity

All new pyrazoline derivatives were screened for antiamoebic activity against *HM1:IMSS* strain of *E. histolytica* using a microdilution method [23,24]. Three classes of 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines were synthesized, the compounds having unsubstituted phenyl ring, 3-bromo or 3-chloro substituents on the phenyl ring at position 3 of pyrazoline ring and 1-*N*-thiocarbamoyl group substituted with different cyclic and aromatic amines to establish the structure–activity relationship (SAR). The IC_{50} values in micromolar are shown in Table 1. 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% confidence limits were interpolated in the corresponding dose–response curve. Metronidazole had a 50% inhibitory concentration (IC_{50} 1.68–1.81 μM) in our experiments. The pyrazoline derivatives with unsubstituted phenyl ring showed IC_{50} in the range of 9.56–1.76 μM. The compounds **25** and **28** in this series showed better antiamoebic activity ($IC_{50} = 1.76$ μM for **25**, $IC_{50} = 1.79$ μM for **28**, versus $IC_{50} = 1.81$ μM for metronidazole). The cyclized pyrazolines with bromo substituted phenyl ring showed IC_{50} in the range of 5.33–0.58 μM. The compounds **5**, **26** and **29** in this series were found to be better inhibitors of *E. histolytica* ($IC_{50} = 1.09$ μM for **5**, $IC_{50} = 0.67$ μM for **26**, $IC_{50} = 0.58$ μM for **29** versus $IC_{50} = 1.68$ μM for metronidazole). Among all the chloro derivatives, the most active compounds in this class were those pyrazoline derivatives, which have hexamethyline imine (**6**, $IC_{50} = 0.89$ μM), tetrahydro quinoline (**15**, $IC_{50} = 1.77$ μM), *o*-toluidine (**18**, $IC_{50} = 1.68$ μM), 2,4-difluoro aniline (**27**, $IC_{50} = 0.51$ μM) and adamantyl amine (**30**, $IC_{50} = 0.47$ μM) as 1-*N*-substitution. All the 3-bromo and 3-chloro substituted cyclized pyrazoline derivatives were found to be more active than their respective unsubstituted analogues. It was concluded that the presence of bulky groups at position 1-*N* of thiocarbamoyl group and 3-bromo or 3-chloro substituents on the phenyl ring at position 3 of pyrazoline ring greatly enhanced the antiamoebic activity. The results were statistically evaluated by analysis of variance. The null hypothesis was tested using *t*-test. The significance of the difference between the IC_{50} values of metronidazole and the most active compounds was evaluated by *t*-test. The values of the calculated *t* were found to be 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.

Table 1
In vitro antiamoebic activities of 1-*N*-substituted thiocarbamoyl-3-phenyl-2-pyrazolines (**1–30**) against (*HM1:IMSS*) strain of *E. histolytica*



Compound	X	R	IC_{50} (μM)	S.D.[a]
1	H		3.73	0.15
2	Br		2.82	0.25
3	Cl		2.31	0.10
4	H		4.39	0.55
5	Br		1.09	0.23
6	Cl		0.89	0.17
7	H		5.94	0.31
8	Br		5.25	0.25
9	Cl		3.71	0.19
10	H		7.31	0.89
11	Br		4.44	0.57
12	Cl		2.91	0.15
13	H		6.19	0.90
14	Br		2.78	0.33
15	Cl		1.77	0.53
16	H		4.78	0.53
17	Br		3.82	0.76
18	Cl		1.68	0.27
19	H		5.01	0.35
20	Br		3.35	0.23
21	Cl		2.81	0.56
22	H		9.56	1.10
23	Br		5.33	0.75
24	Cl		2.40	0.45
25	H		1.76	0.24
26	Br		0.67	0.11
27	Cl		0.51	0.10
28	H		1.79	0.57
29	Br		0.58	0.12
30	Cl		0.47	0.11
Metronidazole			1.80	0.27

[a] Standard deviation.

4. Experimental

The Mannich base and thiosemicarbazides substituted by different cyclic and aromatic amines were prepared using a two-step synthetic route as reported earlier [18,19]. All the chemicals were purchased from Aldrich Chemical Company (USA). Melting points were recorded on a KSW melting point apparatus and are uncorrected. The ESI mass spectra of a few representative compounds were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. 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 (300 MHz) spectra were obtained at ambient temperature using a Bruker spectrospin DPX-300 MHz spectrophotometer in $CDCl_3$ using tetramethylsilane as an internal standard. Chemical shifts are expressed as parts per million from tetramethylsilane. Splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Coupling constants (*J* values) are given in hertz (Hz). The reactions were monitored on Merck pre-coated aluminium TLC plates $60F_{254}$ and the products were visualized by exposure to UV light. Merck silica gel (230–400 mesh) was used for column chromatography. Elemental analysis (C, H, N) was carried out by Central Drug Research Institute, Lucknow, India, and the results were within 0.4% of the theoretical values.

4.1. Synthesis of Mannich base: a general method

A suspension of ketone (0.2 mol), dimethylamine 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 h. After cooling, 200 mL of acetone was added. The crystals formed were collected, washed with acetone and dried *in vacuo*.

4.2. Synthesis of 1-N-substituted thiocarbamoyl-3-phenyl-2-pyrazolines (*1–30*): a general method

Thiosemicarbazide (0.5 mmol) was dissolved in methanol (5 mL) by 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 h. The reflux time was dependent upon the thiosemicarbazide taken. 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 dichloromethane:-methanol (98:2) and crystallized using appropriate solvent (chloroform or methanol).

4.2.1. (3-Phenyl-4,5-dihydro-1H-pyrazol-1-yl) (pyrrolidin-1-yl)methanethione (*1*)

Cream solid. Yield: 22%; mp: 105 °C. Anal. Calcd. for $C_{14}H_{17}N_3S$: C, 64.86; H, 6.56; N, 16.22; found: C, 64.89; H, 6.54; N, 16.24%; λ_{max} (MeOH)/(nm): 364, 321, 236, 204; ν_{max} (cm^{-1}): 1541 (C=N), 1193 (C–N), 1033 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.18–7.63 (5H, m, Ph), 4.47 (2H, t, $J = 8.70$ Hz, CH_2), 3.34 (2H, t, $J = 9.32$ Hz, CH_2), 2.67–3.02 (4H, m, CH_2), 1.53–2.05 (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.2 (C=S), 156.1 (C=N), 133.7, 131.1, 128.4, 126.1, 123.3, 122.4 (Aryl-C), 78.4

(CH_2), 48.9 (CH_2), 42.2 ($2CH_2$), 31.3 ($2CH_2$); *m/z* (ESI): 260.5 (M + 1).

4.2.2. (3-(3-Bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyrrolidin-1-yl)methanethione (**2**)

Yellow solid. Yield: 19%; mp: 119 °C. Anal. Calcd. for $C_{14}H_{16}N_3SBr$: C, 49.70; H, 4.73; N, 12.43; found: C, 49.68; H, 4.71; N, 12.46%; λ_{max} (MeOH)/(nm): 371, 292, 243, 233, 221, 214; ν_{max} (cm^{-1}): 1561 (C=N), 1189 (C−N), 1069 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.18−7.97 (4H, m, Ar), 4.45 (2H, t, $J = 10.4$ Hz, CH_2), 3.37 (2H, t, $J = 7.5$ Hz, CH_2), 2.49−2.88 (4H, m, CH_2), 1.58−2.17 (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.2 (C=S), 155.7 (C=N), 135.1, 133.3, 129.2, 126.1, 124.8, 121.1 (Aryl-C), 78.3 (CH_2), 48.4 (CH_2), 39.7 ($2CH_2$), 29.5 ($2CH_2$); *m/z* (ESI): 339.5 (M + 1).

4.2.3. (3-(3-Chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyrrolidin-1-yl)methanethione (**3**)

Brown solid. Yield: 15%; mp: 129 °C. Anal. Calcd. for $C_{14}H_{16}N_3SBr$: C, 57.24; H, 5.45; N, 14.31; found: C, 57.29; H, 5.39; N, 14.35%; λ_{max} (MeOH)/(nm): 371, 291.5, 249, 234, 224; ν_{max} (cm^{-1}): 1569 (C=N), 1191 (C−N), 1077 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.22−7.91 (4H, m, Ar), 4.41 (2H, t, $J = 8.13$ Hz, CH_2), 3.33 (2H, t, $J = 9.50$ Hz, CH_2), 2.41−2.97 (4H, m, CH_2), 1.61−2.25 (4H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.4 (C=S), 154.5 (C=N), 134.2, 133.8, 131.7, 129.4, 126.2, 122.2 (Aryl-C), 76.1 (CH_2), 48.1 (CH_2), 40.2 ($2CH_2$), 32.4 ($2CH_2$).

4.2.4. Azepan-1-yl(3-phenyl-4,5-dihydro-1H-pyrazol-1-yl) methanethione (**4**)

Brown solid. Yield: 18%; mp: 106 °C. Anal. Calcd. for $C_{16}H_{21}N_3S$: C, 66.89; H, 7.32; N, 14.63; found: C, 66.95; H, 7.31; N, 14.59%; λ_{max} (MeOH)/(nm): 382, 377, 322, 243, 203; ν_{max} (cm^{-1}): 1540 (C=N), 1112 (C−N), 1076 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.21−7.78 (5H, m, Ph), 4.43 (2H, t, $J = 8.75$ Hz, CH_2), 3.93 (2H, t, $J = 7.50$ Hz, CH_2), 1.57−3.15 (12H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.7 (C=S), 156.9 (C=N), 133.3, 130.5, 127.1, 125.3, 123.5, 121.2 (Aryl-C), 76.6 (CH_2), 48.3 (CH_2), 42.7 ($2CH_2$), 33.1 ($2CH_2$), 24.8 ($2CH_2$); *m/z* (ESI): 288 (M + 1).

4.2.5. Azepan-1-yl(3-(3-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)methanethione (**5**)

Yellow solid. Yield: 13%; mp: 169 °C. Anal. Calcd. for $C_{16}H_{20}N_3SBr$: C, 52.46; H, 5.46; N, 11.48; found: C, 52.44; H, 5.49; N, 11.45%; λ_{max} (MeOH)/(nm): 371, 288, 247, 234, 224, 214; ν_{max} (cm^{-1}): 1562 (C=N), 1140 (C−N), 1067 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.26−7.95 (4H, m, Ar), 4.40 (2H, t, $J = 8.03$ Hz, CH_2), 3.27 (2H, t, $J = 10.71$ Hz, CH_2), 1.56−2.92 (12H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 180.2 (C=S), 157.7 (C=N), 137.2, 135.7, 131.8, 129.4, 126.1, 124.7 (Aryl-C), 75.4 (CH_2), 52.1 (CH_2), 45.5 ($2CH_2$), 35.7 ($2CH_2$), 27.8 ($2CH_2$); *m/z* (ESI): 366.3 (M^+).

4.2.6. Azepan-1-yl(3-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)methanethione (**6**)

Dark brown solid. Yield: 16%; mp: 110 °C. Anal. Calcd. for $C_{16}H_{20}N_3SCl$: C, 59.72; H, 6.22; N, 13.06; found: C, 59.79; H, 6.24; N, 13.1%; λ_{max} (MeOH)/(cm^{-1}): 328.6, 246.1, 206.4; ν_{max} (cm^{-1}): 1572 (C=N), 1169 (C−N), 1065 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.19−7.87 (4H, m, Ar), 4.43 (2H, t, $J = 6.23$ Hz, CH_2), 3.33 (2H, t, 2H, $J = 8.57$ Hz, CH_2), 1.49−2.97 (12H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 182.4 (C=S), 155.2 (C=N), 133.1, 131.4, 129.7, 127.3, 125.5, 121.8 (Aryl-C), 77.2 (CH_2), 51.4 (CH_2), 44.8 ($2CH_2$), 33.3 ($2CH_2$), 25.4 ($2CH_2$).

4.2.7. N-cyclohexyl-N-methyl-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**7**)

Bright yellow solid. Yield: 15%; mp: 127 °C. Anal. Calcd. for $C_{17}H_{23}N_3S$: C, 67.77; H, 7.64; N, 13.95; found: C, 67.79; H, 7.62; N, 13.97%; λ_{max} (MeOH)/(nm): 374, 321, 206, 204; ν_{max} (cm^{-1}): 1568 (C=N), 1128 (C−N), 1069 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.21−7.68 (5H, m, Ph), 4.41 (2H, t, $J = 10.0$ Hz, CH_2), 3.13 (2H, t, $J = 8.33$ Hz, CH_2), 2.90−3.07 (1H, m, CH), 1.62−2.04 (10H, m, CH_2), 1.56 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.3 (C=S), 156.2 (C=N), 133.2, 131.9, 128.9, 126.5, 124.4, 121.1 (Aryl-C), 74.5 (CH_2), 54.2 (CH), 47.9 (CH_2), 34.5 ($2CH_2$), 26.3 ($2CH_2$), 22.6 (CH_2), 20.7 (CH_3); *m/z* (ESI): 301.6 (M + 1).

4.2.8. 3-(3-Bromophenyl)-N-cyclohexyl-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**8**)

Dark brown solid. Yield: 13%; mp: 151 °C. Anal. Calcd. for $C_{17}H_{22}N_3SBr$: C, 53.68; H, 5.79; N, 11.05; found: C, 53.45; H, 5.81; N, 11.11%; λ_{max} (MeOH)/(nm): 382, 371, 351, 290, 251; ν_{max} (cm^{-1}): 1560 (C=N), 1190 (C−N), 1037 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.19−7.97 (4H, m, Ar), 4.33 (2H, t, $J = 8.57$ Hz, CH_2), 3.16 (2H, t, $J = 8.57$ Hz, CH_2), 3.36−3.56 (1H, m, CH), 1.68−2.12 (10H, m, CH_2), 1.57 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.2 (C=S), 159.4 (C=N), 136.7, 133.2, 129.7, 127.4, 125.2, 122.4 (Aryl-C), 76.3 (CH_2), 55.3 (CH), 47.3 (CH_2), 33.2 ($2CH_2$), 27.3 ($2CH_2$), 23.2 (CH_2), 20.9 (CH_3); *m/z* (ESI): 380.7 (M^+).

4.2.9. 3-(3-Chlorophenyl)-N-cyclohexyl-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**9**)

Brown solid. Yield: 12%; mp: 131 °C. Anal. Calcd. for $C_{17}H_{22}N_3SCl$: C, 60.80; H, 6.56; N, 12.52; found: C, 60.75; H, 6.49; N, 12.61%; λ_{max} (MeOH)/(cm^{-1}): 372.5, 318.2, 245.6, 213.3; ν_{max} (cm^{-1}): 1584 (C=N), 1179 (C−N), 1067 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.19−7.91 (4H, m, Ar), 4.47 (2H, t, $J = 8.07$ Hz, CH_2), 3.31 (2H, t, $J = 9.23$ Hz, CH_2), 3.47−3.54 (1H, m, CH), 1.43−2.09 (10H, m, CH_2), 1.63 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 182.3 (C=S), 158.4 (C=N), 136.3, 133.8, 131.2, 128.9, 125.2, 121.4 (Aryl-C), 77.2 (CH_2), 52.4 (CH), 48.3 (CH_2), 32.2 ($2CH_2$), 26.6 ($2CH_2$), 22.4 (CH_2), 19.3 (CH_3); *m/z* (ESI): 336.5 (M + 1).

*4.2.10. N-Benzyl-N-methyl-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**10**)*

Creamish yellow solid. Yield: 17%; mp: 96 °C. Anal. Calcd. for $C_{18}H_{19}N_3S$: C, 69.90; H, 6.15; N, 13.59; found: C, 69.85; H, 6.19; N, 13.54%; λ_{max} (MeOH)/(nm): 374, 231, 208; ν_{max} (cm^{-1}): 1560 (C=N), 1146 (C–N), 1074 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.21–7.79 (10H, m, Ph), 4.47 (2H, t, $J = 6.8$ Hz, CH_2), 3.13 (2H, t, $J = 6.8$ Hz, CH_2), 2.61 (2H, s, CH_2), 1.58 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.4 (C=S), 155.3 (C=N), 133.3, 131.7, 130.4, 129.6, 125.2–120.8 (Aryl-C), 76.9 (CH_2), 46.7 (CH_2), 38.4 (CH_2), 19.6 (CH_3).

*4.2.11. N-Benzyl-3-(3-bromophenyl)-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**11**)*

Dark brown solid. Yield: 15%; mp: 179 °C. Anal. Calcd. for $C_{18}H_{18}N_3SBr$: C, 55.67; H, 4.64; N, 10.82; found: C, 55.59; H, 4.61; N, 10.85%; λ_{max} (MeOH)/(nm): 371, 291, 250, 230, 221; ν_{max} (cm^{-1}): 1549 (C=N), 1169 (C–N), 1078 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.19–8.01 (9H, m, Ar), 4.37 (2H, t, $J = 7.79$ Hz, CH_2), 3.25 (2H, t, $J = 8.57$ Hz, CH_2), 2.93 (2H, s, CH_2), 1.96 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.6 (C=S), 159.3 (C=N), 135.4, 133.9, 131.2, 127.6, 125.8–121.1(Aryl-C), 72.1 (CH_2), 49.7 (CH_2), 32.6 (CH_2), 18.5 (CH_3).

*4.2.12. N-Benzyl-3-(3-chlorophenyl)-N-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**12**)*

Light brown solid. Yield: 14%; mp: 148 °C. Anal. Calcd. for $C_{18}H_{18}N_3SCl$: C, 62.88; H, 5.24; N, 12.23; found: C, 62.85; H, 5.22; N, 12.25%; λ_{max} (MeOH)/(nm): 371, 290, 249.5, 230, 221, 207; ν_{max} (cm^{-1}) 1541 (C=N), 1151 (C–N), 1077 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.24–7.97 (9H, m, Ar), 4.29 (2H, t, $J = 8.04$ Hz, CH_2), 3.24 (2H, t, $J = 8.17$ Hz, CH_2), 2.98 (2H, s, CH_2), 1.91 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 176.2 (C=S), 157.8 (C=N), 136.9, 134.1, 132.2, 129.7, 126.8–120.6 (Aryl-C), 74.3 (CH_2), 48.6 (CH_2), 33.6 (CH_2), 19.1 (CH_3).

*4.2.13. (3,4-Dihydroquinolin-1(2H)-yl)(3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)methanethione (**13**)*

Brown solid. Yield: 12%; mp: 181 °C. Anal. Calcd. for $C_{19}H_{19}N_3S$: C, 71.03; H, 5.92; N, 13.08; found: C, 71.11; H, 5.97; N, 13.06%; λ_{max} (MeOH)/(nm): 372, 367, 267, 293, 254, 214.5; ν_{max} (cm^{-1}): 1605 (C=N), 1181 (C–N), 1095 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.16–7.83 (9H, m, Ph), 4.34 (2H, t, $J = 8.17$ Hz, CH_2), 3.32 (2H, t, $J = 8.97$ Hz, CH_2), 1.76–3.12 (6H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.1 (C=S), 154.2 (C=N), 133.5, 131.9, 129.5, 127.6, 125.5–120.7 (Aryl-C), 78.7 (CH_2), 53.2 (CH_2), 44.9 (CH_2), 34.5 (CH_2), 31.7 (CH_2).

*4.2.14. (3-(3-Bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(3,4-dihydroquinolin-1(2H)-yl)methanethione (**14**)*

Light yellow solid. Yield: 10%; mp: 191 °C. Anal. Calcd. for $C_{19}H_{18}N_3SBr$: C, 57.00; H, 4.50; N, 10.50; found: C, 56.95; H, 4.49; N, 10.52%; λ_{max} (MeOH)/(nm): 371, 284, 249, 243, 237, 223; ν_{max} (cm^{-1}): 1582 (C=N), 1172 (C–N), 1045 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.22–7.99 (8H, m, Aryl), 4.33 (2H, t, $J = 7.79$ Hz, CH_2), 3.16 (2H, t, $J = 8.17$ Hz, CH_2), 1.68–3.19 (6H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.2 (C=S), 159.4 (C=N), 135.2, 133.8, 131.2, 129.7, 125.2–122.4 (Aryl-C), 76.3 (CH_2), 55.3 (CH_2), 47.3 (CH_2), 33.2 (CH_2), 27.3 (CH_2).

*4.2.15. (3-(3-Chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(3,4-dihydroquinolin-1(2H)-yl)methanethione (**15**)*

Yellow solid. Yield: 12%; mp: 184 °C. Anal. Calcd. for $C_{19}H_{18}N_3SCl$: C, 64.13; H, 5.06; N, 11.81; found: C, 64.21; H, 5.01; N, 11.85%; λ_{max} (MeOH)/(nm): 371, 293.5, 246, 232, 215; ν_{max} (cm^{-1}): 1541 (C=N), 1123 (C–N), 1079 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.19–7.91 (8H, m, Aryl), 4.47 (2H, t, $J = 9.14$ Hz, CH_2), 3.31 (2H, t, $J = 8.79$ Hz, CH_2), 1.89–3.23 (6H, m, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.3 (C=S), 156.4 (C=N), 136.3, 133.8, 128.9, 125.2–121.4 (Aryl-C), 77.2 (CH_2), 52.4 (CH_2), 48.3 (CH_2), 33.7 (CH_2), 29.1 (CH_2); *m/z* (ESI): 355.2 (M^+).

*4.2.16. 3-Phenyl-N-o-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**16**)*

Reddish brown solid. Yield: 18%; mp: 124 °C. Anal. Calcd. for $C_{17}H_{17}N_3S$: C, 69.15; H, 5.76; N, 14.24; found: C, 69.11; H, 5.79; N, 14.21%; λ_{max} (MeOH)/(nm): 374, 325, 246, 213.5; ν_{max} (cm^{-1}): 3322 (NH), 1574 (C=N), 1169 (C–N), 1078 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.84 (1H, s, N*H*), 7.16–7.99 (9H, m, Ph), 4.50 (2H, t, $J = 6.66$ Hz, CH_2), 3.39 (2H, t, $J = 6.66$ Hz, CH_2), 2.36 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.1 (C=S), 155.9 (C=N), 133.3, 130.5, 128.7, 126.2–122.6 (Aryl-C), 77.4 (CH_2), 48.2 (CH_2), 24.9 (CH_3).

*4.2.17. 3-(3-Bromophenyl)-N-o-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**17**)*

Brown solid. Yield: 14%; mp: 160 °C. Anal. Calcd. for $C_{17}H_{16}N_3SBr$: C, 54.55; H, 4.28; N, 11.23; found: C, 54.61; H, 4.29; N, 11.21%; λ_{max} (MeOH)/(nm): 374, 362, 213, 220; ν_{max} (cm^{-1}): 3214 (NH), 1538 (C=N), 1132 (C–N), 1072 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.79 (1H, s, N*H*), 7.19–7.95 (8H, m, Ar), 4.51 (2H, t, $J = 10.71$ Hz, CH_2), 3.33 (2H, t, $J = 10.71$ Hz, CH_2), 2.46 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.4 (C=S), 156.7 (C=N), 136.6, 133.9, 131.2, 130.8, 129.6, 125.4–121.7 (Aryl-C), 78.2 (CH_2), 46.8 (CH_2), 22.6 (CH_3); *m/z* (ESI): 373, 359, 342, 329, 303, 225, 182.

*4.2.18. 3-(3-Chlorophenyl)-N-o-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**18**)*

White solid. Yield: 11%; mp: 124 °C. Anal. Calcd. 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} (MeOH)/(nm): 329, 235.4, 209; ν_{max} (cm^{-1}): 3446 (NH), 1541 (C=N), 1169 (C–N), 1025 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.80 (1H, s, N*H*), 7.21–7.98 (8H, m, Ar), 4.52 (2H, t, $J = 6.87$ Hz, CH_2), 3.36 (2H, t, $J = 7.04$ Hz, CH_2), 2.36 (3H, s, CH_3); ^{13}C NMR

($CDCl_3$): (δ, ppm) 177.8 (C=S), 156.1 (C=N), 134.6, 132.1, 130.8, 127.6, 125.4–120.1 (Aryl-C), 76.5 (CH_2), 47.6 (CH_2), 21.9 (CH_3); *m/z* (ESI): 332, 317, 299, 284, 261, 185, 141.

*4.2.19. 3-Phenyl-N-m-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**19**)*

Brown solid. Yield: 20%; mp: 112 °C. Anal. Calcd. for $C_{17}H_{17}N_3S$: C, 69.15; H, 5.76; N, 14.24; found: C, 69.09; H, 5.71; N, 14.22%; λ_{max} (MeOH)/(nm): 373, 293, 289, 249, 209; ν_{max} (cm^{-1}): 3320 (NH), 1541 (C=N), 1179 (C–N), 1075 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.97 (1H, s, N*H*), 7.19–7.87 (9H, m, Ph), 4.43 (2H, t, $J = 7.07$ Hz, CH_2), 3.23 (2H, t, $J = 9.82$ Hz, CH_2), 2.38 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.3 (C=S), 155.2 (C=N), 133.7, 129.2, 127.6, 125.8–120.5 (Aryl-C), 76.4 (CH_2), 48.1 (CH_2), 27.3 (CH_3).

*4.2.20. 3-(3-Bromophenyl)-N-m-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**20**)*

Brown solid. Yield: 17%; mp: 135 °C. Anal. Calcd. for $C_{17}H_{16}N_3SBr$: C, 54.55; H, 4.28; N, 11.23; found: C, 54.49; H, 4.26; N, 11.25%; λ_{max} (MeOH)/(nm): 371, 351, 292.5, 249; ν_{max} (cm^{-1}): 3328 (NH), 1543 (C=N), 1179 (C–N), 1094 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 9.01 (1H, s, N*H*), 7.19–7.96 (8H, m, Ar), 4.51 (2H, t, $J = 12.00$ Hz, CH_2), 3.36 (2H, t, $J = 10.5$ Hz, CH_2), 2.38 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 182.6 (C=S), 158.3 (C=N), 136.2, 134.8, 133.3, 129.1, 127.3, 125.6–122.7 (Aryl-C), 77.2 (CH_2), 46.3 (CH_2), 29.5 (CH_3); *m/z* (ESI): 374.2 (M^+).

*4.2.21. 3-(3-Chlorophenyl)-N-m-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**21**)*

Yellow solid. Yield: 13%; mp: 163 °C. Anal. Calcd. for $C_{17}H_{16}N_3SCl$: C, 61.91; H, 4.85; N, 12.75; found: C, 61.87; H, 4.79; N, 12.72%; λ_{max} (MeOH)/(nm): 371, 352, 292, 249.5, 243.5; ν_{max} (cm^{-1}): 3314 (NH), 1539 (C=N), 1261 (C–N), 1100 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.04 (1H, s, N*H*), 7.19–7.72 (8H, m, Ar), 4.41 (2H, t, $J = 9.87$ Hz, CH_2), 3.38 (2H, t, $J = 8.53$ Hz, CH_2), 2.13 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 176.2 (C=S), 157.9 (C=N), 136.2, 133.8, 130.5, 127.6, 125.4–122.7 (Aryl-C), 77.4 (CH_2), 49.3 (CH_2), 28.8 (CH_3).

*4.2.22. 3-Phenyl-N-p-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**22**)*

Dark yellow solid. Yield: 24%; mp: 121 °C. Anal. Calcd. for $C_{17}H_{17}N_3S$: C, 69.15; H, 5.76; N, 14.24; found: C, 69.04; H, 5.69; N, 14.22%; λ_{max} (MeOH)/(nm): 367.5, 321.4, 229, 225, 220, 207; ν_{max} (cm^{-1}): 3344 (NH), 1531 (C=N), 1126 (C–N), 1037 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 9.00 (1H, s, N*H*), 7.17–7.77 (8H, m, Ar), 4.49 (2H, t, $J = 6.82$ Hz, CH_2), 3.50 (2H, t, $J = 10.23$ Hz, CH_2), 2.35 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.6 (C=S), 156.9 (C=N), 136.4, 134.6, 132.9, 129.7, 126.3–123.8 (Aryl-C), 76.1 (CH_2), 47.4 (CH_2), 27.3 (CH_3); *m/z* (ESI): 296.4 (M + 1).

*4.2.23. 3-(3-Bromophenyl)-N-p-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**23**)*

Light yellow solid. Yield: 12%; mp: 134 °C. Anal. Calcd. for $C_{17}H_{16}N_3SBr$: C, 54.55; H, 4.28; N, 11.23; found: C, 54.48; H, 4.24; N, 11.25%; λ_{max+}(MeOH)/(nm): 371, 294, 250, 229.5, 220; ν_{max} (cm^{-1}): 3365 (NH), 1521 (C=N), 1221 (C–N), 1070 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.97 (1H, s, N*H*), 7.20–8.06 (8H, m, Ar), 4.40 (2H, t, $J = 11.04$ Hz, CH_2), 3.33 (2H, t, $J = 9.78$ Hz, CH_2), 2.45 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.4 (C=S), 156.3 (C=N), 135.4, 133.3, 131.7, 129.6, 126.2–122.5 (Aryl-C), 78.4 (CH_2), 46.1 (CH_2), 28.6 (CH_3); *m/z* (ESI): 374.3 (M^+).

*4.2.24. 3-(3-Chlorophenyl)-N-p-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**24**)*

Brown solid. Yield: 12%; mp: 161 °C. Anal. Calcd. for $C_{17}H_{16}N_3SCl$: C, 61.91; H, 4.86; N, 12.75; found: C, 61.95, H, 4.81; N, 12.71%; λ_{max} (MeOH)/(nm): 371, 351, 291, 248.5; ν_{max} (cm^{-1}): 3369 (NH), 1516 (C=N), 1196 (C–N), 1080 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.10 (1H, s, N*H*), 7.22–7.99 (8H, m, Ar), 4.39 (2H, t, $J = 9.13$ Hz, CH_2), 3.29 (2H, t, $J = 10.07$ Hz, CH_2), 2.39 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$): (δ, ppm) 180.3 (C=S), 159.7 (C=N), 136.3, 135.2, 131.9, 127.8, 125.8–122.5 (Aryl-C), 78.6 (CH_2), 46.5 (CH_2), 29.7 (CH_3); *m/z* (ESI): 331.4 (M + 1).

*4.2.25. N-(2,4-Difluorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**25**)*

Yellow solid. Yield: 14%; mp: 176 °C. Anal. Calcd. for $C_{16}H_{13}N_3SF_2$: C, 60.56; H, 4.10; N, 13.25; found: C, 60.62; H, 4.14; N, 13.24%; λ_{max} (MeOH)/(cm^{-1}): 345.6, 306.8, 234.6; ν_{max} (cm^{-1}): 3336 (NH), 1554 (C=N), 1184 (C–N), 1086 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.95 (1H, s, N*H*), 7.17–8.20 (8H, m, Ar), 4.49 (2H, t, $J = 11.32$ Hz, CH_2), 3.40 (2H, t, $J = 9.90$ Hz, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.4 (C=S), 156.3 (C=N), 137.3, 136.9, 134.5, 130.7, 127.6, 125.4–123.8 (Aryl-C), 76.9 (CH_2), 48.7 (CH_2).

*4.2.26. 3-(3-Bromophenyl)-N-(2,4-difluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**26**)*

Yellow solid. Yield: 10%; mp: 192 °C. Anal. Calcd. for $C_{16}H_{12}N_3SF_2Br$: C, 48.48; H, 3.03; N, 10.61; found: C, 48.39; H, 3.02; N, 10.56%; λ_{max} (MeOH)/(nm): 371, 352, 291, 246; ν_{max} (cm^{-1}): 3345 (NH), 1560 (C=N), 1170 (C–N), 1045 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 9.31 (1H, s, N*H*), 7.27–8.27 (7H, m, Ar), 4.61 (2H, t, $J = 7.17$ Hz, CH_2,), 3.38 (2H, t, $J = 8.63$ Hz, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 182.6 (C=S), 158.2 (C=N), 138.6, 136.5, 133.9, 131.8, 129.7, 126.4–123.6 (Aryl-C), 77.2 (CH_2), 46.3 (CH_2); *m/z* (ESI): 396.5 (M^+).

*4.2.27. 3-(3-Chlorophenyl)-N-(2,4-difluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**27**)*

Dark brown solid. Yield: 9%; mp: 140 °C. Anal. Calcd. for $C_{16}H_{12}N_3SF_2Cl$: C, 54.62; H, 3.41; N, 11.95; found: C, 54.67; H, 3.44; N, 11.91%; λ_{max} (MeOH)/(nm): 382, 371, 347, 292;

ν_{max} (cm^{-1}): 3445 (NH), 1541 (C=N), 1262 (C–N), 1023 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.12 (1H, s, N*H*), 7.24–8.14 (7H, m, Ar), 4.55 (2H, t, $J = 8.17$ Hz, CH_2), 3.41 (2H, t, $J = 9.89$ Hz, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 183.6 (C=S), 157.9 (C=N), 136.1, 135.4, 133.9, 130.6, 127.8, 126.1–124.9 (Aryl-C), 76.2 (CH_2), 48.3 (CH_2).

4.2.28. N-(2-Adamantyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**28**)

Dark yellow solid. Yield: 11%; mp: 163 °C. Anal. Calcd. for $C_{20}H_{25}N_3S$: C, 70.79; H, 7.37; N, 12.39; found: C, 70.81; H, 7.35; N, 12.41%; λ_{max} (MeOH)/(nm): 374, 368, 248, 228, 207; ν_{max} (cm^{-1}): 3446 (NH), 1540 (C=N), 111.4 (C–N), 1077 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.06 (1H, s, N*H*), 7.21–7.89 (5H, m, Ar), 4.67 (1H, t, $J = 6.45$ Hz, C*H*), 4.39 (2H, t, $J = 9.12$ Hz, CH_2), 3.26 (2H, t, $J = 10.13$ Hz, CH_2), 1.45–2.07 (14H, m, adaman. ring); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.8 (C=S), 156.2 (C=N), 133.2, 131.3, 129.8, 127.1, 125.7, 121.4 (Aryl-C), 74.4 (CH_2), 44.3 (CH_2), 55.30, 52.24, 38.55, 34.21, 32.98, 32.14, 30.86, 31.11, 31.02, 26.111 (adaman. ring).

4.2.29. N-(2-Adamantyl)-3-(3-bromophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**29**)

Creamish yellow solid. Yield: 13%; mp: 181 °C. Anal. Calcd. for $C_{20}H_{24}N_3SBr$: C, 57.42; H, 5.74; N, 10.05; found: C, 57.45; H, 5.77; N, 10.01%; λ_{max} (MeOH)/(nm): 381, 368, 328, 288, 249, 237, 224, 216; ν_{max} (cm^{-1}): 3369 (NH), 1569 (C=N), 1142 (C–N), 1076 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.14 (1H, s, N*H*), 7.23–7.89 (4H, m, Ar), 5.02 (1H, t, $J = 5.16$ Hz, C*H*), 4.39 (2H, t, $J = 8.07$ Hz, CH_2), 3.21 (2H, t, $J = 9.14$ Hz, CH_2), 1.36–2.17 (14H, m, adaman. ring); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.3 (C=S), 156.3 (C=N), 136.5, 133.7, 129.2, 126.3, 124.7, 122.9 (Aryl-C), 73.3 (CH_2), 49.4 (CH_2), 54.13, 52.24, 39.22, 35.18, 32.32, 32.87, 32.22, 31.45, 31.02, 26.11 (adaman. ring).

4.2.30. N-(2-Adamantyl)-3-(3-chlorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**30**)

Dark yellow solid. Yield: 9%; mp: 161 °C. Anal. Calcd. for $C_{20}H_{24}N_3SCl$: C, 64.26; H, 6.43; N, 11.24; found: C, 64.24; H, 6.45; N, 11.27%; λ_{max} (MeOH)/(nm): 366, 334, 306, 249, 230; ν_{max} (cm^{-1}): 3340 (NH), 1565 (C=N), 1179 (C–N), 1070 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.11 (1H, s, N*H*), 7.19–7.87 (4H, m, Ar), 5.12 (1H, t, $J = 5.92$ Hz, C*H*), 4.33 (2H, t, $J = 9.23$ Hz, CH_2), 3.29 (2H, t, $J = 9.87$ Hz, CH_2), 1.42–2.34 (15H, m, adaman. ring); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.9 (C=S), 155.2 (C=N), 137.0, 134.9, 132.7, 127.4, 125.2, 123.8 (Aryl-C), 77.4 (CH_2), 48.6 (CH_2), 56.32, 51.40, 39.75, 35.80, 33.83, 32.57, 31.86, 31.07, 30.09, 27.19 (adaman. ring).

4.3. Organism culture and assessment of antiamoebic activity

All the pyrazoline derivatives were screened *in vitro* for antiamoebic activity against *HM1:IMSS* strain of *E. histolytica* by microdilution method. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously in wells of 96-well microtiter plate [23,24]. All the compounds (ca. 1 mg) were dissolved in DMSO (40 μL) and the stock solutions of the compounds were prepared freshly before use by adding enough culture medium to obtain a concentration of 1 mg/mL. Dissolution was facilitated by mild sonication in a sonicleaner bath for a few minutes. As evidenced by a comparison of UV–vis spectra of the compounds dissolved in DMSO on one hand, and DMSO + TYIS-33 medium on the other hand, the compounds preserve their integrity under biological conditions. Under these conditions, the compounds are stable and no inhibition of amoeba occurs [25,26]. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (Costar) in 170 μL of medium. Each test included metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) and a blank (culture medium only). The control wells were prepared from a confluent culture by pouring off the medium, adding 2 mL of fresh medium and chilling the culture on ice to detach the organism from the flask wall. The number of amoeba per millilitre 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 culture medium and 170 μL of this suspension was added to the test and control wells so that the wells are 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. 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 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. The 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 and, when dry, stained with (0.5%) aqueous eosin for 15 min. Stained plate was washed once with tap water and then twice with distilled water and 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 percentage 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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