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Bioorganic & Medicinal Chemistry 13 (2005) 2213–2220
Bioorganic & Medicinal Chemistry

Synthesis and antiamoebic activities of 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones

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Received 24 November 2004; revised 24 December 2004; accepted 24 December 2004
Available online 21 January 2005

Abstract—A series of 21 new 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones were synthesised by cyclisation of Mannich bases with thiosemicarbazides of variegated nature. The chemical structures of the compounds were proved by UV, IR, ^{1}H NMR, ^{13}C NMR spectroscopic data and elemental analyses. The antiamoebic activities of these compounds were evaluated by microdilution method against *HM1:1MSS* strain of *Entamoeba histolytica*. It was found that 3-chloro and 3-bromo substituents on the phenyl ring at position 3 of the pyrazoline ring enhanced the antiamoebic activity as compared to unsubstituted phenyl ring. Compounds **15**, **17**, **18**, **20** and **21** showed less IC_{50} value than metronidazole. Moreover, compound **21** have shown the most promising antiamoebic activity (IC_{50} = 0.6 μM vs IC_{50} = 1.8 μM of metronidazole).

1. Introduction

The diverse parasitic protozoa such as *Entamoeba histolytica*, *Giardia deudenalis* and *Trichomonas vaginalis* have significant impact on the mucosal health of humans. Infection with *E. histolytica* may result in massive destruction of host tissue and life-threatening disease. The protozoan parasite *E. histolytica* causes amebic colitis and amebic liver abscess, diseases that afflict millions of individuals in developing countries. More than 50 million people worldwide are infected and up to 110,000 of these die every year.[1] Metronidazole is one of the most widely used medications against amoebiasis. More importantly, the drug is known to have common side effects[2] including nausea. It is mutagenic in bacteria[3] and higher doses may cause cancer in rodents.[4] Due to its toxic effects on DNA, metronidazole is considered a potential carcinogenic chemical by the International Agency for Research on Cancer.[5] To date, the ideal treatment for amoebiasis does not exist. It is important to search for new amoebicidal.

Considerable attention has been focused on substituted pyrazoline derivatives due to their interesting biological activity. Compounds with a pyrazole structure are known to possess tranquilizing, muscle relaxant, psychoanaleptic, hypnotic, ulcerogenic, antidepressant, antibacterial–antifungal and analgesic–anti-inflammatory properties,[6–19] which extends to recent reports on cerebroprotective effects.[20] As evident from the literature, it was noted that very little research has been carried out on pyrazoline analogues of thio-semicarbazones[21] but no work has been done on the screening of these derivatives on *E. histolytica*. Earlier we have reported different heterocyclic thiosemicarbazones and their transition metal complexes and in vitro screening against *E. histolytica*.[22–25] The compounds found active, 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 synthesise new 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones **1**–**21** and their in vitro screening against *HM1:1MSS* strain of *E. histolytica*. To the best of our knowledge this is the first report of cyclised pyrazoline analogues having a thiosemicarbazide moiety and report very encouraging results of in vitro activity against *E. histolytica*.

2. Results and discussion

The Mannich reaction of various ketones with formaldehyde and dimethylamine hydrochloride generates the

Keywords: Pyrazoline analogues; Mannich bases; Thiosemicarbazides; *Entamoeba histolytica*.
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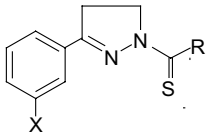
doi:10.1016/j.bmc.2004.12.050

Mannich base precursor. 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 and 3-chloro acetophenones in the Mannich reaction were lower in the range of 40–60%. The condensation of Mannich reaction product with *N*-4 substituted thiosemicarbazides by different aliphatic amines leads to the formation of 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones (Scheme 1). According to the currently accepted mechanism[26,27] the formation of the cyclised pyrazoline analogues is favoured via thiosemicarbazones formation, which undergo cyclisation under basic conditions to form desired pyrazoline ring in all the compounds. The product mixture contained only unreacted starting material and the cyclisation product, which was purified by column chromatography using silica gel $60F_{254}$ eluted with dichloromethane–methanol (98:2) to give crystalline solid compounds but in low yield. The yield of cyclised product in unsubstituted thiosemicarbazide was in the range of 50–70%; and 10–30% in the case of substituted thiosemicarbazides. The compounds obtained are stable in the solid as well as in the solution state. Analytical data of these compounds are in good agreement with their composition (see Experimental). The compounds are insoluble in water but soluble in most of the organic solvents. The structures 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.

Table 1. Structure of cyclised pyrazoline analogues (**1**–**21**)

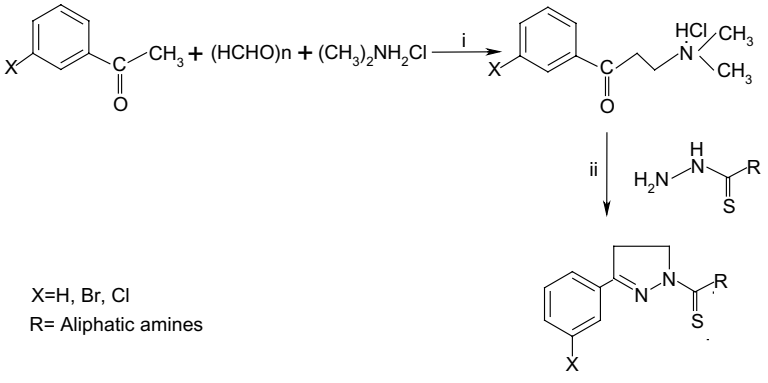


Compound	X	R
1	H	
2	Br	-NH-CH_2-CH_2-CH_3
3	Cl	
4	H	
5	Br	-NH-CH(CH_3)-CH_3
6	Cl	
7	H	
8	Br	-NH-CH_2-CH_2-CH_2-CH_3
9	Cl	
10	H	
11	Br	-NH-CH_2-CH(CH_3)-CH_3
12	Cl	
13	H	
14	Br	-N(CH_2-CH_2-CH_2-CH_3)(CH_3)
15	Cl	
16	H	
17	Br	-N(CH_2-CH_3)(CH_2-CH_3)
18	Cl	
19	H	
20	Br	-N(CH_2-CH_2-CH_3)(CH_2-CH_2-CH_3)
21	Cl	

2.1. IR and electronic spectral studies

Selected diagnostic bands of the IR spectra of the cyclised pyrazoline analogues of thiosemicarbazones (**1**–**21**) showed useful information about the structure of the compounds. All the compounds showed intense bands in the region 1022–1080 cm^{-1} due to the *v* (C=S) stretch of the thiocarboxamide group.[22] The IR spectra of all the compounds showed *v* (C=N) stretch at 1522–1585 cm^{-1} because of the ring closure.[28] In addition, the absorption bands at 1124–1211 cm^{-1} were attributed to the *v* (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 3221–3386 cm^{-1} due to the *v* (NH) stretch.

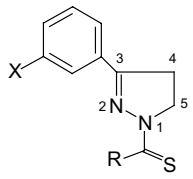
The electronic spectra of the cyclised pyrazoline analogues studied in the UV region in methanol, exhibited three absorption bands at 377.6–296, 267–232.4 and 233–204.3 nm assignable to $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, respectively. The band at 377.6–296 nm assigned to the $n \rightarrow \pi^*$ transition involving the thione portion (C=S) of thiocarboxamide group. The two other absorption bands at 267–232.4 and 233–204.3 nm were due to $\pi \rightarrow \pi^*$ transition of phenyl ring and $n \rightarrow \sigma^*$ transition of azomethine nitrogen, respectively.



Scheme 1. Reagents and conditions: (i) ethanol, hydrochloric acid, reflux; (ii) methanol, NaOH, reflux.

2.2. Nuclear magnetic resonance spectral studies

The ^{1}H NMR spectra were recorded using $CDCl_3$ as the solvent clearly supports the proposed structures of the compounds. The pyrazoline protons at C_4 and C_5 carbons appeared as broad triplets at 3.02–3.47 (J = 7.18–12.76 Hz) and 4.29–4.63 (J = 7.69–12.50 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.[29]



A

The NH proton of thiocarboxamide group of the compounds (**1**–**12**) showed a singlet at 7.96–8.14 ppm. The protons belonging to the aromatic ring and the other aliphatic 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 46.3–49.7 and 75.3–78.7 ppm, respectively. All the compounds showed a signal at 154.5–161.1 ppm was assigned due to the azomethine carbon of pyrazoline ring. Thiocarboxamide carbon (C=S) displayed a signal at 175.3–183.7 ppm in all the compounds. The signals from 137.1 to 120.5 ppm were assumed due to the aromatic carbons. The carbons at 1-*N*-substituted aliphatic groups resonate at their usual positions and are shown in the data given in the Experimental section.

2.3. In vitro antiamoebic 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 cyclised pyrazoline analogues of thiosemicarbazones; the compounds having phenyl ring, 3-bromo and 3-chloro substituents on the phenyl ring at position 3 of pyrazoline ring and 1-*N*-substituted with different aliphatic groups. These include (1) 1-*N*-substituted derivatives, represented by compounds **1**–**12**; and (2) 1-*N*, 1-*N*-dialkyl derivatives, which includes all remaining compounds **13**–**21**. In our previous communications it was found that the activity of the resulting thiosemicarbazones enhanced due to the substitution of bulky group at N^4 position of thiosemicarbazides.[22–25] All the compounds were evaluated for their antiamoebic activity in vitro using *HM1*:*1MSS* strain of *E. histolytica* to investigate the influence of the substitution. The IC_{50} values in μM 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% confidence limits were interpolated in the corresponding dose–response curve.

Table 2. In vitro antiamoebic activities of cyclised pyrazoline analogues of thiosemicarbazones against (*HM1*:*1MSS*) strain of *E. histolytica*

S. No.	Compound	IC_{50} (μM)	SD*
1	3-Ph-2-Pz-1-*N*-Pr-TC	14.0	2.1
2	3-3-BrPh-2-Pz-1-*N*-Pr-TC	8.5	1.8
3	3-3-ClPh-2-Pz-1-*N*-Pr-TC	8.0	1.3
4	3-Ph-2-Pz-1-*N*-*iso*-Pr-TC	23.0	2.7
5	3-3-BrPh-2-Pz-1-*N*-*iso*-Pr-TC	15.2	1.5
6	3-3-ClPh-2-Pz-1-*N*-*iso*-Pr-TC	12.2	1.4
7	3-Ph-2-Pz-1-*N* Bu-TC	23.3	2.2
8	3-3-BrPh-2-Pz-1-*N* Bu-TC	14.2	1.1
9	3-3-ClPh-2-Pz-1-*N* Bu-TC	12.3	1.6
10	3-Ph-2-Pz-1-*N*-*iso*-Bu-TC	11.2	0.9
11	3-3-BrPh-2-Pz-1-*N*-*iso*-Bu-TC	6.1	1.1
12	3-3-ClPh-2-Pz-1-*N*-*iso*-Bu-TC	5.0	0.8
13	3-Ph-2-Pz-1-*N* MBu-TC	5.7	1.5
14	3-3-BrPh-2-Pz-1-*N* MBu-TC	2.4	0.7
15	3-3-ClPh-2-Pz-1-*N* MBu-TC	0.7	0.4
16	3-Ph-2-Pz-1-*N*,*N*-diEt-TC	4.2	0.7
17	3-3-BrPh-2-Pz-1-*N*,*N*-diEt-TC	1.2	0.3
18	3-3-ClPh-2-Pz-1-*N*,*N*-diEt-TC	1.0	0.3
19	3-Ph-2-Pz-1-*N*,*N*-diPr-TC	2.0	0.9
20	3-3-BrPh-2-Pz-1-*N*,*N*-diPr-TC	0.8	0.4
21	3-3ClPh-2-Pz-1-*N*,*N*-diPr-TC	0.6	0.3
	Metronidazole	1.8	0.3

* Standard deviation.

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 **15**, **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. Metronidazole had a 50% inhibitory concentration (IC_{50} = 1.6–1.8 μM) in our experiments. All the 3-bromo and 3-chloro substituted cyclised pyrazoline derivatives were found to be more active than their respective unsubstituted analogues. The cyclised pyrazoline analogues with unsubstituted phenyl ring showed IC_{50} in the range of 23.3–2.0 μM. The compound **19** in this series showed comparable antiamoebic activity with the reference drug, metronidazole (IC_{50} = 2.0 μM versus IC_{50} = 1.8 μM of metronidazole). It was interesting to note that the compounds (**1**, **2**, **3**) with *N*-propyl amine showed lesser IC_{50} values than the compounds (**7**, **8**, **9**) with *N*-butyl amine as 1-*N* substitution. In rest of the compounds, the better antiamoebic activity was found in those derivatives, substituted with bulkier groups. Among all the bromo and chloro derivatives, the most active compounds in this class were those cyclised pyrazoline analogues of thiosemicarbazones, which have *N*-methyl butyl amine (**15**, IC_{50} = 0.7 μM), *N*,*N*-diethyl amine (**17**, IC_{50} = 1.2 μM), (**18**, IC_{50} = 1.0 μM) and *N*,*N*-dipropyl amine (**20**, IC_{50} = 0.8 μM), (**21**, IC_{50} = 0.6 μM) as 1-*N* substitution. It was concluded that the presence of these bulky groups at position 1-*N* of thiocarboxamide group and 3-bromo or 3-chloro substituents on the phenyl ring at position 3 of pyrazoline ring greatly enhanced antiamoebic activity. Detailed studies of the toxicity, in vivo and mechanism of action of these compounds are in progress.

3. Conclusion

This research examined the antiamoebic activities of new cyclised pyrazoline analogues of thiosemicarbazones prepared by the reaction of Mannich bases with thiosemicarbazides substituted by different aliphatic amines. In vitro antiamoebic activity of these compounds was carried out against *HM1:1MSS* strain of *E. histolytica*. The biological behaviour of the compounds revealed that 3-chloro and 3-bromo substituents on the phenyl ring at position 3 of the pyrazoline ring increased the antiamoebic activity. All the bromo and chloro substituted pyrazoline derivatives showed better antiamoebic activity than their respective unsubstituted analogues. Moreover, compounds **15**, **17**, **18**, **20** and **21** showed less IC_{50} value than metronidazole.

4. Experimental

4.1. Materials and methods

Reactions were monitored by TLC analysis using Merck silica gel 60F-254 thin layer plates. All the chemicals were purchased from Aldrich chemical company (USA). All the thiosemicarbazides were prepared as reported earlier.[30] Elemental analysis (C, H, N) was carried out by Central Drug Research Institute, Lucknow, India, and the results were within 0.4% of calculated 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. ^{1}H NMR 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; q, quartet; m, multiplet. Coupling constants (*J*) are given in Hertz. The ESI mass spectra of a few representative compounds were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer.

4.2. Synthesis of Mannich base: 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 concd 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. The compounds have been reported earlier,[21] we have characterised these compounds by IR and ^{1}H NMR to confirm the isolation of the products.

4.2.1. Acetophenone Mannich base. Yield: 87%; white solid: mp 153 °C; IR: ν_{max} (cm^{-1}) 3033 (arom. C–H), 2952 (aliph. C–H), 1679 (C=O), 1224 (C–N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.21–7.86 (5H, m, aryl), 3.29 (2H, t, $-CH_2$), 2.97 (2H, t, $-CH_2$), 2.53 (6H, s, $-CH_3$).

4.2.2. 3-Bromo acetophenone Mannich base. Yield: 56%; white solid: mp 176 °C; IR: ν_{max} (cm^{-1}) 3049 (arom. C–H), 2968 (aliph. C–H), 1689 (C=O), 1214 (C–N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.12–7.95 (4H, m, aryl), 3.31 (2H, t, $-CH_2$), 2.95 (2H, t, $-CH_2$), 2.65 (6H, s, $-CH_3$).

4.2.3. 3-Chloro acetophenone Mannich base. Yield: 42%; white solid: mp 189 °C; IR: ν_{max} (cm^{-1}) 3019 (arom. C–H), 2967 (aliph. C–H), 1689 (C=O), 1218 (C–N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.17–7.76 (4H, m, aryl), 3.33 (2H, t, $-CH_2$), 2.92 (2H, t, $-CH_2$), 2.71 (6H, s, $-CH_3$).

4.3. Cyclised pyrazoline analogue of thiosemicarbazones: 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 dropwise to the reaction mixture and refluxed for 48–72 h. The reflux time was dependent upon the thiosemicarbazide taken. N^4-substituted thiosemicarbazides were cyclised with Mannich base in 48 h to give compounds **1**–**12**; while N^4,N^4-disubstituted thiosemicarbazides refluxed for 72 h to give compounds **13**–**21**. 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 dichloromethane–methanol (98:2) and crystallised using appropriate solvent (chloroform or methanol).

4.3.1. 3-Phenyl-2-pyrazoline-1-(*N*-propyl)thiocarboxamide (1). Yield: 28%; brown solid; R_f 0.72; mp 106 °C; Anal. Calcd ($C_{13}H_{17}N_3S$): C, 63.16; H, 6.88; N, 17.00. Found: C, 63.25; H, 6.82; N, 16.95%; UV: λ_{max} (nm): 376, 324, 233, 206; IR: ν_{max}/cm^{-1}: 3375 (NH), 3033 (arom. C–H), 2910 (aliph. C–H), 1565 (C=N), 1187 (C–N), 1078 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.07 (1H, s, –NH), 7.19–7.72 (5H, m, aryl), 4.41 (2H, t, $-CH_2$, J = 9.78 Hz), 3.69 (2H, q, $-CH_2$, J = 6.52 Hz), 3.29 (2H, t, $-CH_2$, J = 11.41 Hz), 1.64–1.72 (2H, m, $-CH_2$), 1.03 (3H, t, $-CH_3$, J = 6.52 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.4 (C=S), 155.7 (C=N), 132.8, 131.7, 129.8, 127.1, 125.8, 124.4 (aryl–C), 76.2 (CH_2), 56.8 (CH_2), 48.9 (CH_2), 30.4 (CH_2), 15.5 (CH_3).

4.3.2. 3-(3-Bromophenyl)-2-pyrazoline-1-(*N*-propyl)thiocarboxamide (2). Yield: 21%; dark brown solid; R_f 0.62; mp 112 °C; Anal. Calcd ($C_{13}H_{16}N_3SBr$): C, 47.85; H, 4.91; N, 12.88. Found: C, 47.89; H, 4.92; N, 12.87%; UV: λ_{max} (nm): 329, 296, 234, 211; IR: ν_{max}/cm^{-1}: 3342 (NH), 3069 (arom. C–H), 2914 (aliph. C–H), 1549 (C=N), 1165 (C–N), 1070 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.05 (1H, s, –NH), 7.21–7.93 (4H, m, aryl), 4.42 (2H, t, $-CH_2$, J = 8.97 Hz), 3.55 (2H, q, $-CH_2$, J = 6.39 Hz), 3.30 (2H, t, $-CH_2$, J = 9.83 Hz), 1.59–1.87 (2H, m, $-CH_2$, J = 6.07 Hz), 1.10 (3H, t, $-CH_3$, J = 5.36 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 182.3 (C=S), 157.4 (C=N), 135.4, 133.5, 131.8, 129.4, 127.6, 125.7 (aryl–C), 78.2 (CH_2), 56.3 (CH_2), 48.1 (CH_2), 30.8 (CH_2), 15.6 (CH_3).

4.3.3. 3-(3-Chlorophenyl)-2-pyrazoline-1-(*N*-propyl)thiocarboxamide (3). Yield: 22%; dark yellow solid; R_f 0.80; mp 119 °C; Anal. Calcd ($C_{13}H_{16}N_3SCl$): C, 55.42; H, 5.68; N, 14.92. Found: C, 55.55; H, 5.69; N, 14.91%; UV: λ_{max} (nm): 324, 237, 210; IR: ν_{max}/cm^{-1}: 3353 (NH), 3021 (arom. C–H), 2923 (aliph. C–H), 1525 (C=N), 1124 (C–N), 1078 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.07 (1H, s, –NH), 7.18–7.89 (4H, m, aryl), 4.47 (2H, t, $–CH_2$, J = 8.97 Hz), 3.54 (2H, q, $–CH_2$, J = 6.45 Hz), 3.29 (2H, t, $–CH_2$, J = 10.03 Hz), 1.37–1.67 (2H, m, $–CH_2$, J = 5.89 Hz), 1.13 (3H, t, $–CH_3$, J = 6.01 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.2 (C=S), 155.4 (C=N), 136.2, 133.2, 131.4, 129.2, 125.4, 122.8 (aryl–C), 75.3 (CH_2), 55.5 (CH_2), 46.9 (CH_2), 31.9 (CH_2), 15.1 (CH_3).

4.3.4. 3-Phenyl-2-pyrazoline-1-(*N*-isopropyl)thiocarboxamide (4). Yield: 27%; cream solid; R_f 0.73; mp 132 °C; Anal. Calcd ($C_{13}H_{17}N_3S$): C, 63.16; H, 6.88; N, 17.00. Found: C, 63.12; H, 6.85; N, 17.02%; UV: λ_{max} (nm): 373, 323.3, 242, 212; IR: ν_{max}/cm^{-1}: 3325 (NH), 3029 (arom. C–H), 2933 (aliph. C–H), 1559 (C=N), 1166 (C–N), 1061 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.96 (1H, s, –NH), 7.13–7.64 (5H, m, aryl), 4.51–4.66 (1H, m, –CH, J = 7.21 Hz), 4.40 (2H, t, $–CH_2$, J = 8.04 Hz), 3.28 (2H, t, $–CH_2$, J = 10.71 Hz), 1.32 (6H, d, $–CH_3$, J = 5.36 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 176.7 (C=S), 155.2 (C=N), 133.4, 130.5, 127.9, 126.3, 124.4, 121.5 (aryl–C), 77.2 (CH_2), 57.3 (CH), 46.3 (CH_2), 18.5 ($2CH_3$); MS (ESI) m/z: 246 (M−1).

4.3.5. 3-(3-Bromophenyl)-2-pyrazoline-1-(*N*-isopropyl)-thiocarboxamide (5). Yield: 21%; light yellow solid; R_f 0.75; mp 155 °C; Anal. Calcd ($C_{13}H_{16}N_3SBr$): C, 47.85; H, 4.91; N, 12.88. Found: C, 47.87; H, 4.89; N, 12.91%; UV: λ_{max} (nm): 376, 318, 237.2, 215.3; IR: ν_{max}/cm^{-1}: 3326 (NH), 3019 (arom. C–H), 2914 (aliph. C–H), 1541 (C=N), 1164 (C–N), 1061 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.06 (1H, s, –NH), 7.11–7.88 (4H, m, aryl), 4.58–4.65 (1H, m, –CH, J = 6.25 Hz), 4.42 (2H, t, $–CH_2$, J = 12.5 Hz), 3.25 (2H, t, $–CH_2$, J = 12.5 Hz), 1.32 (6H, d, $–CH_3$, J = 4.69 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.3 (C=S), 155.3 (C=N), 132.6, 131.1, 129.7, 127.8, 125.3, 122.6 (aryl–C), 75.3 (CH_2), 55.6 (CH), 47.2 (CH_2), 15.6 ($2CH_3$); MS (ESI) m/z: 326 (M^+).

4.3.6. 3-(3-Chlorophenyl)-2-pyrazoline-1-(*N*-isopropyl)-thiocarboxamide (6). Yield: 18%; brown solid; R_f 0.57; mp 145 °C; Anal. Calcd ($C_{13}H_{16}N_3SCl$): C, 55.42; H, 5.68; N, 14.92. Found: C, 55.49; H, 5.65; N, 14.89%; UV: λ_{max} (nm): 361, 307.3, 235, 206.2; IR: ν_{max}/cm^{-1}: 3221 (NH), 3018 (arom. C–H), 2976 (aliph. C–H), 1561 (C=N), 1168 (C–N), 1052 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 7.98 (1H, s, –NH), 7.19–7.87 (4H, m, aryl), 4.55–4.66 (1H, m, –CH, J = 6.38 Hz), 4.42 (2H, t, $–CH_2$, J = 9.57 Hz), 3.25 (2H, t, $–CH_2$, J = 7.98 Hz), 1.34 (6H, d, $–CH_3$, J = 6.14 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.2 (C=S), 156.4 (C=N), 137.1, 134.9, 132.7, 130.6, 127.4, 126.3 (aryl–C), 77.4 (CH_2), 57.7 (CH_2), 48.4 (CH_2), 14.8 ($2CH_3$); MS (ESI) m/z: 282 (M^+).

4.3.7. 3-Phenyl-2-pyrazoline-1-(*N*-butyl)thiocarboxamide (7). Yield: 24%; creamish yellow solid; R_f 0.65; mp 94 °C; Anal. Calcd ($C_{14}H_{19}N_3S$): C, 64.37; H, 7.28; N, 16.09. Found: C, 64.41; H, 7.29; N, 16.11%; UV: λ_{max} (nm): 363.3, 325.5, 214.5, 204.3; IR: ν_{max}/cm^{-1}: 3368 (NH), 3069 (arom. C–H), 2995 (aliph. C–H), 1540 (C=N), 1168 (C–N), 1034 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.01 (1H, s, –NH), 7.16–7.67 (5H, m, aryl), 4.41 (2H, t, $–CH_2$, J = 7.69 Hz), 3.72 (2H, q, $–CH_2$, J = 5.77 Hz), 3.29 (2H, t, $–CH_2$, J = 9.61 Hz), 1.61–1.71 (2H, m, $–CH_2$, J = 7.95 Hz), 1.37–1.49 (2H, m, $–CH_2$, J = 6.81 Hz), 1.00 (3H, t, CH_3, J = 7.95 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.4 (C=S), 154.8 (C=N), 132.2, 130.3, 129.6, 126.6, 124.7, 121.8 (aryl–C), 77.0 (CH_2), 56.2 (CH_2), 46.6 (CH_2), 32.9 (CH_2), 25.5 (CH_2), 14.3 (CH_3).

4.3.8. 3-(3-Bromophenyl)-2-pyrazoline-1-(*N*-butyl)thiocarboxamide (8). Yield: 18%; brown solid; R_f 0.56; mp 96 °C; Anal. Calcd ($C_{14}H_{18}N_3SBr$): C, 49.41; H, 5.29; N, 12.35. Found: C, 49.39; H, 5.19; N, 12.37%; UV: λ_{max} (nm): 329, 295.4, 242.4, 208.5; IR: ν_{max}/cm^{-1}: 3365 (NH), 3025 (arom. C–H), 2975 (aliph. C–H), 1541 (C=N), 1211 (C–N), 1078 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.06 (1H, s, –NH), 7.19–7.88 (4H, m, aryl), 4.43 (2H, t, $–CH_2$, J = 8.33 Hz), 3.73 (2H, q, $–CH_2$, J = 6.66 Hz), 3.26 (2H, t, $–CH_2$, J = 9.00 Hz), 1.62–1.72 (2H, m, $–CH_2$, J = 8.74 Hz), 1.33–1.51 (2H, m, $–CH_2$, J = 7.28 Hz), 1.01 (3H, t, $–CH_3$, J = 5.82 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 183.7 (C=S), 161.1 (C=N), 136.4, 135.2, 131.8, 127.3, 125.1, 122.3 (aryl–C), 78.7 (CH_2), 58.2 (CH_2), 49.7 (CH_2), 31.6 (CH_2), 23.2 (CH_2), 15.1 (CH_3).

4.3.9. 3-(3-Chlorophenyl)-2-pyrazoline-1-(*N*-butyl)thiocarboxamide (9). Yield: 14%; dark yellow solid; R_f 0.51; mp 108 °C; Anal. Calcd ($C_{14}H_{18}N_3SCl$): C, 56.85; H, 6.09; N, 14.21; found: C, 56.81; H, 6.07; N, 14.24%; UV: λ_{max} (nm): 376.4, 304.4, 233.3, 204.3; IR: ν_{max}/cm^{-1}: 3345 (NH), 3033 (arom. C–H), 2927 (aliph. C–H), 1581 (C=N), 1178 (C–N), 1042 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.12 (1H, s, –NH), 7.21–7.89 (4H, m, aryl), 4.39 (2H, t, $–CH_2$, J = 8.96 Hz), 3.73 (2H, q, $–CH_2$, J = 6.87 Hz), 3.23 (2H, t, $–CH_2$, J = 9.61 Hz), 1.70–1.59 (2H, m, $–CH_2$, J = 7.71 Hz), 1.34–1.47 (2H, m, $–CH_2$, J = 6.39 Hz), 1.01 (3H, t, $–CH_3$, J = 5.89 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.3 (C=S), 155.2 (C=N), 137.0, 134.2, 132.9, 127.3, 124.8, 122.7 (aryl–C), 76.6 (CH_2), 56.3 (CH_2), 48.6 (CH_2), 31.4 (CH_2), 24.5 (CH_2), 14.1 (CH_3); MS (ESI) m/z: 296 (M^+).

4.3.10. 3-Phenyl-2-pyrazoline-1-(*N*-isobutyl)thiocarboxamide (10). Yield: 21%; cream solid; R_f 0.69; mp 115 °C; Anal. Calcd ($C_{14}H_{19}N_3S$): C, 64.36; H, 7.28; N, 16.09. Found: C, 64.35; H, 7.26; N, 16.12%; UV: λ_{max} (nm): 322, 222.1, 216.3; IR: ν_{max}/cm^{-1}: 3365 (NH), 3060 (arom. C–H), 2975 (aliph. C–H), 1532 (C=N), 1126 (C–N), 1056 (C=S); ^1H NMR ($CDCl_3$): (δ, ppm) 8.00 (1H, s, –NH), 7.16–7.65 (5H, m, aryl), 4.42 (2H, t, $–CH_2$, J = 10.13 Hz), 3.55 (2H, t, $–CH_2$, J = 6.08 Hz), 3.30 (2H, t, $–CH_2$, J = 10.13 Hz), 1.96–2.05 (1H, m, –CH, J = 7.26 Hz), 1.01 (6H, d, $–CH_3$, J = 6.05 Hz);

^{13}C NMR ($CDCl_3$): (δ, ppm) 178.3 (C=S), 155.7 (C=N), 131.3, 130.7, 128.6, 127.5, 124.4, 121.3 (aryl–C), 76.4 (CH_2), 57.3 (CH_2), 48.3 (CH_2), 38.4 (CH), 14.3 ($2CH_3$).

4.3.11. 3-(3-Bromophenyl)-2-pyrazoline-1-(*N*-isobutyl)-thiocarboxamide (11). Yield: 18%; yellow solid; R_f 0.71; mp 102 °C; Anal. Calcd ($C_{14}H_{18}N_3SBr$): C, 49.41; H, 5.29; N, 12.35. Found: C, 49.40; H, 5.13; N, 12.45%; UV: λ_{max} (nm): 368, 246, 210; IR: ν_{max}/cm^{-1}: 3385 (NH), 3027 (arom. C–H), 2995 (aliph. C–H), 1522 (C=N), 1181 (C–N), 1022 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.14 (1H, s, –NH), 7.21–7.86 (4H, m, aryl), 4.43 (2H, t, $-CH_2$, J = 11.29 Hz), 3.56 (2H, t, $-CH_2$, J = 6.45 Hz), 3.26 (2H, t, $-CH_2$, J = 9.67 Hz), 1.93–2.07 (1H, m, –CH, J = 7.89 Hz), 1.02 (6H, d, $-CH_3$, J = 6.90 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.3 (C=S), 159.7 (C=N), 134.3, 131.2, 129.8, 127.6, 125.2, 124.3 (aryl–C), 76.4 (CH_2), 55.2 (CH_2), 47.1 (CH_2), 38.3 (CH), 13.5 ($2CH_3$).

4.3.12. 3-(3-Chlorophenyl)-2-pyrazoline-1-(*N*-isobutyl)-thiocarboxamide (12). Yield: 14%; light brown solid; R_f 0.70; mp 117 °C; Anal. Calcd ($C_{14}H_{18}N_3SCl$): C, 56.85; H, 6.09; N, 14.21. Found: C, 56.82; H, 6.11; N, 14.26%; UV: λ_{max} (nm): 324.5, 296, 234.2, 204.3; IR: ν_{max}/cm^{-1}: 3386 (NH), 3019 (arom. C–H), 2935 (aliph. C–H), 1528 (C=N), 1127 (C–N), 1025 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.04 (1H, s, –NH), 7.19–7.97 (4H, m, aryl), 4.43 (2H, t, $-CH_2$, J = 9.83 Hz), 3.54 (2H, t, $-CH_2$, J = 7.13 Hz), 3.37 (2H, t, $-CH_2$, J = 7.87 Hz), 1.87–2.11 (1H, m, –CH), 1.07 (6H, d, $-CH_3$, J = 6.13 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.2 (C=S), 156.4 (C=N), 137.2, 135.5, 131.8, 130.3, 127.1, 125.8 (aryl–C), 75.4 (CH_2), 55.2 (CH_2), 47.3 (CH_2), 38.9 (CH), 13.9 ($2CH_3$).

4.3.13. 3-Phenyl-2-pyrazoline-1-(*N*-methyl butyl)thiocarboxamide (13). Yield: 18%; white solid; R_f 0.75; mp 121 °C; Anal. Calcd ($C_{15}H_{21}N_3S$): C, 65.45; H, 7.64; N, 15.27. Found: C, 65.46; H, 7.68; N, 15.29%; UV: λ_{max} (nm): 322, 267, 249, 205; IR: ν_{max}/cm^{-1}: 3024 (arom. C–H), 2928 (aliph. C–H), 1522 (C=N), 1133 (C–N), 1065 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.11–7.65 (5H, m, aryl), 4.39 (2H, t, $-CH_2$, J = 7.98 Hz), 3.45 (2H, t, $-CH_2$, J = 9.61 Hz), 3.23 (2H, t, $-CH_2$, J = 9.92 Hz), 1.59–1.78 (2H, m, $-CH_2$, J = 6.32 Hz), 1.51 (3H, s, $-CH_3$), 1.18–1.33 (2H, m, $-CH_2$, J = 5.83 Hz), 1.04 (3H, t, $-CH_3$, J = 6.45 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.2 (C=S), 154.5 (C=N), 131.8, 130.2, 129.1, 126.2, 124.7, 122.3 (aryl–C), 76.2 (CH_2), 55.3 (CH_2), 48.3 (CH_2), 31.4 (CH_2), 25.1 (CH_2), 13.3 (CH_3), 32.9 (CH_3).

4.3.14. 3-(3-Bromophenyl)-2-pyrazoline-1-(*N*-methyl butyl)thiocarboxamide (14). Yield: 14%; brown solid; R_f 0.83; mp 98 °C; Anal. Calcd ($C_{15}H_{20}N_3SBr$): C, 50.85; H, 5.65; N, 11.86. Found: C, 50.81; H, 5.68; N, 11.89%; UV: λ_{max} (nm): 377.6, 305, 233, 207; IR: ν_{max}/cm^{-1}: 3018 (arom. C–H), 2969 (aliph. C–H), 1560 (C=N), 1187 (C–N), 1069 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.22–7.93 (4H, m, aryl), 4.29 (2H, t, $-CH_2$, J = 10.0 Hz), 3.50 (2H, t, $-CH_2$, J = 7.66 Hz), 3.02 (2H, t, $-CH_2$, J = 8.33 Hz), 1.62–1.81 (2H, m, CH_2, J = 6.67 Hz), 1.21–1.34 (2H, m, $-CH_2$, J = 6.37 Hz), 1.50 (3H, s, $-CH_3$), 0.94 (3H, t, CH_3, J = 3.33 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.1 (C=S), 156.2 (C=N), 133.4, 131.5, 129.7, 126.2, 124.7, 121.6 (aryl–C), 76.6 (CH_2), 56.3 (CH_2), 48.4 (CH_2), 32.2 (CH_2), 22.6 (CH_2), 13.7 (CH_3), 30.6 (CH_3).

4.3.15. 3-(3-Chloropenyl)-2-pyrazoline-1-(*N*-methyl butyl)thiocarboxamide (15). Yield: 12%; light brown solid; R_f 0.87; mp 143 °C; Anal. Calcd ($C_{15}H_{20}N_3SCl$): C, 58.16; H, 6.46; N, 13.57. Found: C, 58.19; H, 6.39; N, 13.55%; UV: λ_{max} (nm): 322, 234, 216.3; IR: ν_{max}/cm^{-1}: 3039 (arom. C–H), 2988 (aliph. C–H), 1585 (C=N), 1169 (C–N), 1080 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.22–7.97 (4H, m, aryl), 4.63 (2H, t, $-CH_2$, J = 10.0 Hz), 3.65 (2H, t, $-CH_2$, J = 9.14 Hz), 3.19 (2H, t, $-CH_2$, J = 8.07 Hz), 1.67–1.82 (2H, m, $-CH_2$, J = 6.67 Hz), 1.61 (3H, s, $-CH_3$), 1.19–1.47 (2H, m, $-CH_2$, J = 6.01 Hz), 0.99 (3H, t, $-CH_3$, J = 5.97 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.2 (C=S), 155.4 (C=N), 133.5, 131.6, 130.1, 127.8, 125.2, 124.8 (aryl–C), 76.6 (CH_2), 55.9 (CH_2), 46.3 (CH_2), 34.5 (CH_2), 25.3 (CH_2), 13.8 (CH_3), 32.4 (CH_3).

4.3.16. 3-Phenyl-2-pyrazoline-1-(*N*,*N*-diethyl)thiocarboxamide (16). Yield: 18%; cream solid; R_f 0.54; mp 84 °C; Anal. Calcd ($C_{14}H_{19}N_3S$): C, 64.37; H, 7.28; N, 16.09. Found: C, 64.35; H, 7.26; N, 16.12%; UV: λ_{max} (nm): 362, 232.4, 206; IR: ν_{max}/cm^{-1}: 3021 (arom. C–H), 2914 (aliph. C–H), 1540 (C=N), 1137 (C–N), 1064 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.21–7.73 (5H, m, aryl), 4.45 (2H, t, $-CH_2$, J = 9.57 Hz), 3.82 (4H, q, CH_2, J = 6.38 Hz), 3.14 (2H, t, $-CH_2$, J = 12.76 Hz), 1.40 (6H, t, $-CH_3$, J = 7.83 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.5 (C=S), 157.2 (C=N), 131.3, 129.4, 127.2, 125.5, 124.8, 120.7 (aryl–C), 78.6 (CH_2), 55.4 ($2CH_2$), 49.7 (CH_2), 13.6 ($2CH_3$); MS (ESI) *m*/*z*: 262 (M+1).

4.3.17. 3-(3-Bromophenyl)-2-pyrazoline-1-(*N*,*N*-diethyl)-thiocarboxamide (17). Yield: 12%; reddish yellow solid; R_f 0.57; mp 132 °C; Anal. Calcd ($C_{14}H_{18}N_3SBr$): C, 49.41; H, 5.29; N, 12.35. Found: C, 49.44; H, 5.28; N, 12.34%; UV: λ_{max} (nm): 330.5, 227.5, 210.3; IR: ν_{max}/cm^{-1}: 3028 (arom C–H), 2935 (aliph. C–H), 1560 (C=N), 1179 (C–N), 1064 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.26–7.95 (4H, m, aryl), 4.45 (2H, t, $-CH_2$, J = 9.37 Hz), 3.75 (4H, q, $-CH_2$, J = 7.81 Hz), 3.23 (2H, t, $-CH_2$, J = 7.18 Hz), 1.39 (6H, t, $-CH_3$, J = 6.25 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.2 (C=S), 156.1 (C=N), 133.6, 132.7, 130.3, 129.5, 125.3, 122.9 (aryl–C), 77.0 (CH_2), 55.2 ($2CH_2$), 48.4 (CH_2), 16.3 ($2CH_3$).

4.3.18. 3-(3-Chlorophenyl)-2-pyrazoline-1-(*N*,*N*-diethyl)-thiocarboxamide (18). Yield: 12%; brown solid; R_f 0.78; mp 149 °C; Anal. Calcd ($C_{14}H_{18}N_3SCl$): C, 56.85; H, 6.09; N, 14.21. Found: C, 56.79; H, 6.11; N, 14.24%; UV: λ_{max} (nm): 377, 324.4, 244.2, 216; IR: ν_{max}/cm^{-1}: 3051 (arom. C–H), 2933 (aliph. C–H), 1562 (C=N), 1161 (C–N), 1022 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.21–7.83 (4H, m, aryl), 4.53 (2H, t, $-CH_2$,

J = 9.13 Hz), 3.81 (4H, q, –CH_2, J = 6.13 Hz), 3.14 (2H, t, –CH_2, J = 10.01 Hz), 1.43 (6H, t, –CH_3, J = 6.45 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 175.3 (C=S), 155.4 (C=N), 136.9, 135.2, 133.3, 131.8, 127.1, 124.4 (aryl–C), 76.5 (CH_2), 53.9 ($2CH_2$), 48.7 (CH_2), 15.6 ($2CH_3$).

4.3.19. 3-Phenyl-2-pyrazoline-1-(*N*,*N*-dipropyl)thiocarboxamide (19). Yield: 16%; yellow solid; R_f 0.68; mp 86 °C; Anal. Calcd ($C_{16}H_{23}N_3S$): C, 66.43; H, 7.96; N, 14.53. Found: C, 66.45; H, 7.89; N, 14.57%; UV: λ_{max} (nm): 362.2, 306, 233, 209; IR: ν_{max}/cm^{-1}: 3049 (arom. C–H), 2975 (aliph. C–H), 1540 (C=N), 1139 (C–N), 1049 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.19–7.58 (5H, m, aryl), 4.45 (2H, t, –CH_2, J = 8.70 Hz), 3.67 (4H, t, –CH_2, J = 5.36 Hz), 3.11 (2H, t, –CH_2, J = 10.71 Hz), 1.77–1.90 (4H, m, –CH_2, J = 7.95 Hz), 0.96 (6H, t, –CH_3, J = 6.82 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 176.1 (C=S), 156.9 (C=N), 130.2, 128.7, 126.4, 124.9, 123.3, 120.5 (aryl–C), 77.4 (CH_2), 52.7 ($2CH_2$), 46.3 (CH_2), 32.8 ($2CH_2$), 13.6 ($2CH_3$); MS (ESI) m/z: 290 (M+1).

4.3.20. 3-(3-Bromophenyl)-2-pyrazoline-1-(*N*,*N*-dipropyl)thiocarboxamide (20). Yield: 11%; light yellow solid; R_f 0.8; mp 149 °C; Anal. Calcd ($C_{16}H_{22}N_3SBr$): C, 52.17; H, 5.98; N, 11.41. Found: C, 52.21; H, 5.99; N, 11.42%; UV: λ_{max} (nm): 341, 306.4, 245, 211.2; IR: ν_{max}/cm^{-1}: 3044 (arom. C–H), 2994 (aliph. C–H), 1549 (C=N), 1172 (C–N), 1078 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.21–7.89 (4H, m, aryl), 4.32 (2H, t, –CH_2, J = 8.07 Hz), 3.65 (4H, t, –CH_2, J = 6.41 Hz), 3.23 (2H, t, –CH_2, J = 9.32 Hz), 1.81–2.09 (4H, m, CH_2, J = 6.87 Hz), 1.04 (6H, t, –CH_3, J = 6.12 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.4 (C=S), 155.7 (C=N), 135.3, 133.3, 131.8, 129.1, 127.8, 125.4 (aryl–C), 77.3 (CH_2), 56.2 ($2CH_2$), 47.5 (CH_2), 32.2 ($2CH_2$), 14.3 ($2CH_3$).

4.3.21. 3-(3-Chlorophenyl)-2-pyrazoline-1-(*N*,*N*-dipropyl)thiocarboxamide (21). Yield: 13%; dark brown solid; R_f 0.79; mp 127 °C; Anal. Calcd ($C_{16}H_{22}N_3SCl$): C, 59.35; H, 6.80; N, 12.98. Found: C, 59.39; H, 6.39; N, 12.97%; UV: λ_{max} (nm): 328.2, 233, 206; IR: ν_{max}/cm^{-1}: 3041 (arom. C–H), 2972 (aliph. C–H), 1564 (C=N), 1156 (C–N), 1076 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.21–7.90 (4H, m, aryl), 4.46 (2H, t, –CH_2, J = 7.89 Hz), 3.59 (4H, t, –CH_2, J = 6.02 Hz), 3.47 (2H, t, –CH_2, J = 9.31 Hz), 1.60–1.82 (4H, m, –CH_2, J = 6.45 Hz), 1.01 (6H, t, –CH_3, J = 5.63 Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 176.4 (C=S), 157.9 (C=N), 137.1, 135.5, 133.8, 129.4, 127.6, 124.4 (aryl–C), 77.6 (CH_2), 56.3 ($2CH_2$), 47.2 (CH_2), 33.5 ($2CH_2$), 13.8 ($2CH_3$).

4.4. In vitro testing against *E. histolytica*

All the cyclised pyrazoline analogues were screened in vitro for antiamoebic activity against (*HM1:1MSS*) strain of *E. histolytica* by microdilution method.[31] *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously in wells of 96 well microtiter plate.[32] All the compounds were dissolved in DMSO (40 μL) at which level no inhibition of amoeba occurs[33,34] and the stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/mL. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (Costar). Each test includes metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) and a blank culture medium only). The number of amoeba per mL 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 μL of this suspension was added to the test and control wells in the plate. An inoculum of 1.7×10^4 organisms/well was chosen so that confluent, but not excessive growth took place in control wells. Plates were sealed and gassed for 10 min. with nitrogen before incubation at 37 °C for 72 h.

4.5. 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 and, 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 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 straight line from which the IC_{50} value was found. The results are reported in Table 2.

Acknowledgements

Mohammad Abid acknowledges a Junior Research Fellowship from Council of Scientific and Industrial Research, New Delhi, India. The authors are thankful to Prof. Alok Bhattacharya and Dr. Sudha Bhattacharya, School of Life Sciences and School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, respectively, for providing Laboratory facilities for biological activities.

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