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Short communication

Syntheses and evaluation of 3-(3-bromo phenyl)-5-phenyl-1-(thiazolo [4,5-*b*] quinoxaline-2-yl)-2-pyrazoline derivatives

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Abstract

A variety of 3-(3-bromo phenyl)-5-phenyl-1-(thiazolo [4,5-*b*] quinoxaline-2-yl)-2-pyrazoline were obtained by the refluxing of 1-*N*-thiocarbamoyl 3,5-diphenyl-2-pyrazoline with 2,3-dichloroquinoxaline. The chemical structures of the compounds were elucidated by UV, IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. The purity of the compounds was confirmed by their elemental analysis. The antiamoebic activity of these compounds was evaluated by microdilution method against *HMI:IMSS* strain of *Entamoeba histolytica* and the IC_{50} values were compared with the standard drug metronidazole. Some of the quinoxaline derivatives showed less IC_{50} values than metronidazole. To elucidate the toxic effect, MTT assay was performed using kidney epithelial cell line. The results showed that all the compounds are non-toxic.

Keywords: Chalcone; Pyrazolines; Thiocarbamoyl; Quinoxaline: Antiamoebic activity

1. Introduction

Pathogenic intestinal protozoa are responsible for clinically important infections in both the developed and the developing world [1]. Amoebiasis is an infection of the mucous membrane of the large intestine where *Entamoeba histolytica* is the causative organism. This organism occurs in the intestine in the form of trophozoites and cysts. It may be characterized by abdominal pain, anorexia, fever, weight loss and hepatomegaly [2]. Metronidazole is an orally active drug in the treatment of several types of illness caused by protozoa, as well as anaerobic bacteria [3–5]. Side effects are, however, common and include a disulfiram-like reaction (predominantly nausea and vomiting) when taken with alcohol, in addition to a dry mouth and headache [6]. Entamoebas' strain resistant to this drug has also begun to appear [7]. It is therefore necessary to search for new and effective amoebicidals.

Literature survey revealed that the quinoxaline compounds showed better antiprotozoal, antimicrobial, and monamine oxidase inhibitors' activity [8–11]. In our earlier report, we have reported the synthesis of quinoxaline derivatives and it was found that 3-bromo and 3-chloro substituents on the phenyl ring and 4-methyl group in pyrazoline ring greatly effect antiamoebic activity [8]. These results increase as to synthesize some new derivatives of quinoxalines. We report in this paper the synthesis and antiamoebic activity of bromo substituted derivatives of quinoxalines and compared the antiamoebic activity of chalcones and pyrazolines.

2. Results and discussion

The Claisen-Schmidt reaction of various ketones and aldehyde generates different types of chalcones (**1**–**8**). The reaction is sensitive to amount of NaOH. Different chalcones with thiosemicarbazide lead to the formation of thiocarbamoyl-3,5-diphenyl-2-pyrazolines (**9**–**16**) (Scheme 1) which after refluxing with 2,3-dichloroquinoxaline in THF gave the corresponding fused 3,5-diphenyl-2-(thiazolo [4,5-*b*] quinoxaline-2-yl)-2*H*-pyrazole (**17**–**24**). Analytical and spectral data

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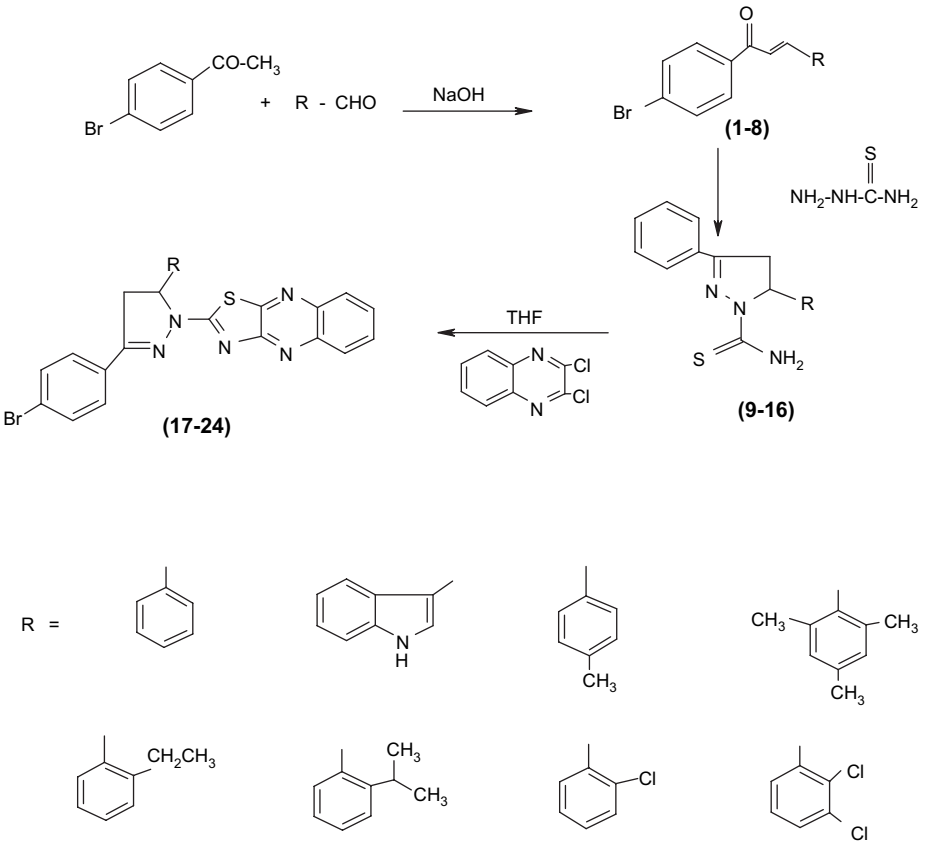


Scheme 1. Synthesis of quinoxaline derivatives.

(IR, UV, ^{1}H NMR, ^{13}C NMR) of all the synthesized compounds were in full agreement with the proposed structures. All the compounds were obtained in good yields and are stable in the solid as well as in the solution state.

2.1. IR and electronic spectral studies

The electronic spectra of the cyclised pyrazoline analogues (**9**–**16**) studied in the UV region in methanol exhibited three absorption bands at 371–290, 270–236 and 232–205 nm assignable to n → π*, π → π* and n → σ* transitions, respectively. The band at 371–290 nm was assigned to the transition involving the thione portion (C═S) of thiocarbamoyl group. The two other absorption bands at 270–236 and 232–205 nm were due to π → π* transition of phenyl ring and n → σ* transition of azomethine nitrogen, respectively. The UV spectral data of quinoxaline derivatives (**17**–**24**) were also studied which showed the same type of transitions as observed in compounds **9**–**16**. It showed three spectral bands at 388.3–299, 287–248 and 239–204 nm assigned to n → π*, π → π* and n → σ* transitions of thiocarbamoyl group (C–S–C), aromatic ring and azomethine nitrogen, respectively.

2.2. Nuclear Magnetic Resonance spectral studies

Further evidence for the formation of pyrazoline compounds and their quinoxaline derivatives was obtained from the ^{1}H NMR and ^{13}C NMR spectra, which provide diagnostic tools for the positional elucidation of the protons. Assignments of the signals are based on the chemical shifts and intensity patterns. The pyrazoline protons H_A and H_B (Fig. 1) are geminal protons at C_4 carbon, appears in the region 3.90–3.09 and 3.32–3.98 ppm as doublet of doublets in all compounds. The CH proton also appeared as doublet of doublets in the region of 6.32–5.40 ppm due to vicinal coupling with two non-equivalent geminal protons of C_4 carbon. These protons were slightly shifted in case of quinoxaline compounds. H_A and H_B protons at C_4 carbon, appears in the region 3.99–3.11 and 3.80–2.78 ppm as doublet of doublets in all quinoxaline compounds (**17**–**24**). The CH proton also appeared as doublet of doublets in the region of 6.58–5.11 ppm due to vicinal coupling with two non-equivalent geminal protons of C_4 carbon.

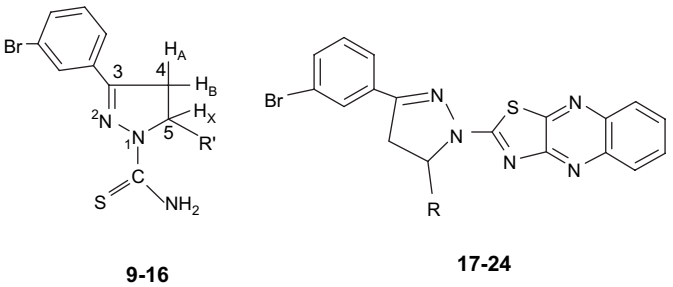


Fig. 1.

The NH proton of different substituted thiocarbamoyl pyrazoline compounds showed a doublet at 9.24–7.17 ppm. In the ^{13}C NMR spectra, the C_4 and C_5 carbons of the pyrazoline ring in compounds **9–16** resonate at 37.43–37.06 and 62.98–60.49 ppm, respectively. The phenyl–C resonates at 149.93–132.71 ppm. All the pyrazoline compounds showed a signal at 189.23–170.16 ppm, which was assigned to azomethine carbon of pyrazoline ring. Thiocarbamoyl carbon (C=S) displayed a signal at 204.23–169.42 ppm. The quinoxaline compounds **17–24** showed two signals at 151.66–152.36 and 143.35–140.11 ppm due to azomethine carbon of the pyrazoline ring and quinoxaline ring, respectively. Thiocarbamoyl carbon (C–S–C) displayed a signal at 143.07–139.31 ppm in all the compounds.

2.3. In vitro antiamoebic activity

The antiamoebic activity of all the compounds was determined against *HM1:IMSS* strain of *E. histolytica* and the results are summarized in Tables 1 and 2. A comparative study of IC_{50} values of chalcones (**1–8**), pyrazolines (**9–16**), quinoxaline analogues (**17–24**) and reference compound metronidazole is done. The results were estimated as the percentage of the 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. The different degree of inhibition of the growth of *E. histolytica* by three series may reflect sound differences in the ability of the activities of the three types of compounds. Chalcone (**1–8**) exhibited antiamoebic activity with IC_{50} of 3.48–1.41 μM. All the pyrazoline derivatives (**9–16**) showed $IC_{50} = 0.23$–4.37 μM. In case of quinoxaline derivatives activities varying in the range $IC_{50} = 0.08$–2.49 μM. All the quinoxaline compounds were more potent than their corresponding chalcone and pyrazoline analogues. The introduction of quinoxaline moiety enhanced activity by many folds in all the compounds. In the quinoxaline series, out of eight compounds six had better activity than metronidazole. If we further take into account the substituent effect, in general all the compounds substituted with methyl phenyl groups had shown less potency than the phenyl, indolyl and chlorophenyl substituents. The compound **18** with $IC_{50} = 0.17$ μM was found most active among all the compounds which has indolyl group along with quinoxaline. The results were statistically evaluated by analysis of variance. The null hypothesis was tested using the *t*-test and the significance of the differences between the IC_{50} value(s) of metronidazole versus **1**, **2**, **8**, **9**, **12**, **16–20**, **23** and **24** was evaluated. The calculated *t*-values were higher than the table values at the 4% level. Hence, the character under study was influenced by the treatment.

2.4. Toxicity profile

The *in vitro* toxic activity of newly synthesized compounds was studied on human kidney epithelial cell line. All the quinoxaline compounds showed significant antiamoebic activity than their corresponding chalcone and pyrazoline analogues. The better antiamoebic quinoxaline derivatives of pyrazolines (**17–24**) were subjected to MTT assay. At a concentration of 60 μM (Table 2), these compounds did not show any inhibitory activity. To investigate the selectivity of the compounds, the ''safety index'' (SI) was calculated and defined as: Toxicity IC_{50}/Protozoal IC_{50}; where toxicity IC_{50} is defined as the concentration of compound that kills 50% of the human (kidney epithelial) cell line and protozoal IC_{50} is the concentration that kills 50% of amoeba protozoa. This allows an estimate of which compounds might be efficacious or toxic against human cells and potentially *in vivo*. The numerical results for each compound are given in Table 2. It is interesting to note that the compound **18** has lowest toxicity and highest antiamoebic activity and overall compounds show more favorable safety profile along with the most promising antiamoebic activity.

3. Conclusion

This research involved the syntheses of chalcones (**1–8**), the cyclization of these chalcones by thiosemicarbazide and synthesis of pyrazoline derivatives (**9–16**) and their quinoxaline derivatives (**17–24**). We have further examined the antiamoebic activities of all the compounds. The biological behavior revealed that the most of the compounds showed better activity. The toxicity studies of quinoxaline derivatives (**17–24**) showed that these compounds will remain toxic to the parasite, whilst in the human host there will be a decreased likelihood of toxicity.

4. Experimental

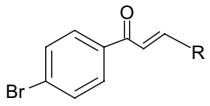
4.1. Materials and methods

All the chemicals were purchased from Aldrich Chemical Company (U.S.A.) and were used without further purification. The reactions were monitored by pre-coated aluminium silica gel $60F_{254}$ thin layer plates procured from Merck (Germany). All thiosemicarbazides were prepared by a reported method [12] and their purity was confirmed by C, H and N analyses carried out at Central Drug Research Institute Lucknow, India. Chlorine was estimated by decomposing the complexes with Na_2O_2/NaOH and precipitated as AgCl with $AgNO_3$ after dissolving in dil. HNO_3. Melting points were recorded on KSW melting point apparatus and are 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 brucker spectroscopin DPX-300 MHZ spectrophotometer in $CDCl_3$ using tetramethylsilane as an internal standard.

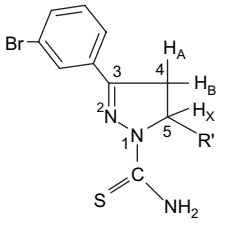
4.2. Synthesis of bromo acetophenone chalcones: general procedure

A solution of bromo acetophenones (50 mmol) and appropriate aldehyde (50 mmol) in methanolic NaOH was stirred

Table 1
In vitro antiamoebic activity of substituted chalcones (**1**–**8**) and pyrazolines (**9**–**16**)



1-8



9-16

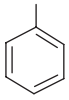
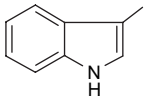
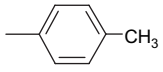
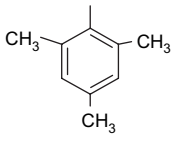
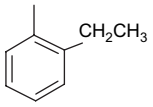
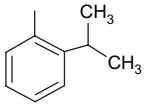
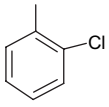
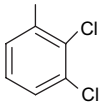
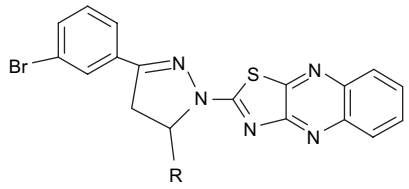
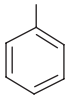
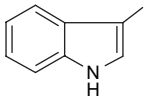
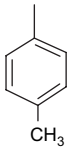
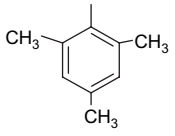
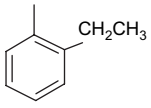
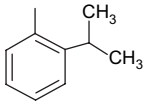
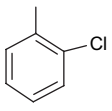
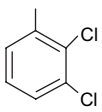
R group	Compound	Antiamoebic activity		Compound	Antiamoebic activity	
		IC_{50} (μM)	S.D.		IC_{50} (μM)	S.D.
	**1**	1.39	0.05	**9**	0.19	0.04
	**2**	1.05	0.48	**10**	2.28	0.23
	**3**	2.49	0.98	**11**	4.38	0.02
	**4**	1.80	0.34	**12**	1.71	0.18
	**5**	3.63	0.48	**13**	2.61	0.54
	**6**	1.92	0.23	**14**	2.34	0.34
	**7**	2.23	0.18	**15**	1.90	0.09
	**8**	1.11	0.03	**16**	0.56	0.03
Metronidazole		1.8	0.05			

Table 2
In vitro antiamoebic activity and toxicity of substituted quinoxaline derivatives of pyrazolines



17-24

Compound	R group	Antiamoebic activity		Toxicity profile, IC_{50} (μM)	Safety Index (SI)
		IC_{50} (μM)	S.D.		
17		0.14	0.09	>60	>428
18		0.08	0.03	>60	>750
19		0.10	0.06	>60	>600
20		1.29	0.56	>60	>46.51
21		2.49	1.90	>60	>24.09
22		1.87	0.05	>60	>32.08
23		0.68	0.30	>60	>88.23
24		0.70	0.09	>60	>85.71
	Metronidazole	1.8	0.05	>100	>55.55

for 18 h at 5–28 °C. Solid obtained, washed with ice-cold water and then rectified spirit, dried and recrystallized by ethanol [12,13].

*4.2.1. 1-3-Br-ph-3-ph-prop-2-ene-1-one (**1**)*

Pale yellow crystals (chloroform); yield: 93%; m.p.: 121 °C. Anal. calc. for ($C_{15}H_{11}OBr$): C 62.93, H 3.84; found: C 62.82, H 3.81%. UV/vis λ_{max} (nm): 371, 291, 236, 217; IR ν_{max} (cm^{-1}): 1656 (C=O), 1622 (CH=CH), 1580 (C=C); ^1H NMR ($CDCl_3$) (δ, ppm): 7.8 (m, 10H, Ar), 7.74 (d, 1H, 1H, $J = 15$ Hz), 7.63 (d, 1H, H_B, $J = 15$ Hz).

*4.2.2. 1-(3-Br-ph)-3-indolyl prop-2-ene-1-one (**2**)*

Pale yellow crystals (chloroform); yield: 55%; m.p.: 123 °C. Anal. calc. for ($C_{17}H_{12}NOBr$): C 62.76, H 3.69, N 4.30; found: C 60.05, H 3.11, N 4.34%. UV/vis λ_{max} (nm): 371, 291, 236, 217; IR ν_{max} (cm^{-1}): 1664 (C=O), 1620 (CH=CH), 1568 (C=C); ^1H NMR ($CDCl_3$) (δ, ppm): 5.82 (d, 1H, CH), 6.86 (1H, d, CH–Ar), 7.11–7.70 (9H, m, Ar–H), 9.01 (1H, s, NH of indole).

*4.2.3. 1-(3-Br-ph)-3-(4-me-ph)-prop-2-ene-1-one (**3**)*

Pale yellow crystals (chloroform); yield: 73%; m.p.: 144 °C. Anal. calc. for ($C_{16}H_{13}OBr$): C 64, H 4.33; found: C 64.05, H 4.16%. UV/vis λ_{max} (nm): 371, 291, 236, 217; IR ν_{max} (cm^{-1}): 1710 (C=O), 1630 (CH=CH), 1550 (C=C); ^1H NMR ($CDCl_3$) (δ, ppm): 7.8 (m, 10H, Ar), 7.74 (d, H_A, $J = 15$ Hz, 1H), 7.63 (d, H_B, $J = 15$ Hz, 1H), 2.5 (s, 3H, CH_3).

*4.2.4. 1-(3-Br-ph)-3-(2,4,6 tri-me-ph)-prop-2-ene-1-one (**4**)*

Pale yellow crystals (chloroform); yield: 62%; m.p.: 175 °C. Anal. calc. for ($C_{18}H_{17}OBr$): C 65.85, H 5.18; found: C 65.78, H 5.16%. UV/vis λ_{max} (nm): 299, 236, 217; IR ν_{max} (cm^{-1}): 1710 (C=O), 1630 (CH=CH), 1550 (C=C); ^1H NMR ($CDCl_3$) (δ, ppm): 7.8 (m, 10H, Ar), 7.01 (d, H_A, $J = 15$ Hz, 1H), 7.23 (d, H_B, $J = 15$ Hz, 1H), 3.01 (s, 3H, CH_3).

*4.2.5. 1-(3-Br-ph)-3-(3-isopropyl ph)-prop-2-ene-1-one (**5**)*

Pale yellow crystals (chloroform); yield: 44%; m.p.: 113 °C. Anal. calc. for ($C_{17}H_{15}OBr$): C 64.96, H 4.77; found: C 63.98, H 4.68%. UV/vis λ_{max} (nm): 291, 258, 236; IR ν_{max} (cm^{-1}): 1722 (C=O), 1623 (CH=CH), 1554 (C=C); ^1H NMR ($CDCl_3$) (δ, ppm): 7.7 (m, 10H, Ar), 7.4 (d, H_A, $J = 13.6$ Hz, 1H), 7.6 (d, H_B, $J = 15$ Hz, 1H), 1.2–2.1 (m, 2H, CH_2).

*4.2.6. 1-(3-Br-ph)-3-[3-(me-ethyl)-ph] prop-2-ene-1-one (**6**)*

Pale yellow crystals (chloroform); yield: 56%; m.p.: 129 °C. Anal. calc. for ($C_{18}H_{17}OBr$): C 65.85, H 5.18; found: C 65.85, H 5.12%. UV/vis λ_{max} (nm): 371, 291, 236, 217; IR ν_{max} (cm^{-1}): 1698 (C=O), 1643 (CH=CH), 1555 (C=C); ^1H NMR ($CDCl_3$) (δ, ppm): 7.8 (m, 10H, Ar), 7.5 (d, H_A, $J = 14.8$ Hz, 1H), 7.2 (d, H_B, $J = 15$ Hz, 1H), 2.5 (s, 3H, CH_3).

*4.2.7. 1-(3-Br-ph)-3-(2-Cl-ph) prop-2-ene-1-one (**7**)*

Pale yellow crystals (chloroform); yield: 54%; m.p.: 175 °C. Anal. calc. for ($C_{17}H_{10}OBrCl$): C 56.25, H 3.12, Cl 10.9; found: C 56.51, H 3.12, Cl 1.93%. UV/vis λ_{max} (nm): 371, 291, 236, 217; IR ν_{max} (cm^{-1}): 1710 (C=O), 1630 (CH=CH), 1550 (C=C); ^1H NMR ($CDCl_3$) (δ, ppm): 7.66 (m, 10H, Ar), 7.52 (d, H_A, $J = 15$ Hz, 1H), 7.63 (d, H_B, $J = 15$ Hz, 1H).

*4.2.8. 1-(3-Br-ph)-3-(2,4 diCl-ph) prop-2-ene-1-one (**8**)*

Pale yellow crystals (chloroform); yield: 54%; m.p.: 175 °C. Anal. calc. for ($C_{15}H_9OBrCl_2$): C 50.70, H 2.53, Cl 20; found: C 50.65, H 2.57%. UV/vis λ_{max} (nm): 371, 291, 236, 217; IR ν_{max} (cm^{-1}): 1710 (C=O), 1630 (CH=CH), 1550 (C=C); ^1H NMR ($CDCl_3$) (δ, ppm): 7.89 (m, 10H, Ar), 7.2 (d, H_A, $J = 15$ Hz, 1H), 7.51 (d, H_B, $J = 15$ Hz, 1H).

4.3. Synthesis of pyrazoline derivatives

A mixture of chalcone (10 mmol), thiosemicarbazide (10 mmol) and NaOH (25 mmol) was refluxed in ethanol (25 ml) for 8 h. The solution was poured into ice water. The precipitate was filtered and recrystallized from methanol [14].

*4.3.1. 1-N-tc-3-(3-br-ph)-5-ph-2-pz (**9**)*

Pale yellow powder (chloroform); yield: 24%; m.p.: 175 °C. Anal. calc. ($C_{16}H_{14}N_3SBr$): C 53.48, H 3.89, N 11.69; found: C 53.45, H 3.84, N 11.63%. UV/vis λ_{max} (nm): 371, 290, 236, 217; IR ν_{max} (cm^{-1}): 3435 (N–H), 2924 (C–H), 1558 (C=N), 1357 (C=S), 1122 (C–N); ^1H NMR ($CDCl_3$) (δ, ppm): 6.5–7.5 (m, 10H, Ar), 3.48 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.49 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.3 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25), 9.2 (d, 2H, NH_2); ^{13}C NMR ($CDCl3$) (δ, ppm): 176.19 (C=S), 149.57 (C=N), 144.52–125.72 (Phenyl–C), 62.86 (C–H), 37.06 (CH_2).

*4.3.2. 1-N-tc-3-(3-br-ph)-5-(1H-3-indolyl)-2-pz (**10**)*

Pale yellow crystal (chloroform); yield: 21%; m.p.: 185 °C. Anal. calc. for ($C_{17}H_{15}N_4SBr$): C 52.85, H 3.88, N 14.50; found: C 52.76, H 3.82, N 14.50%. UV/vis λ_{max} (nm); 371, 270, 236; IR ν_{max} (cm^{-1}): 3233 (N–H), 2928 (C–H), 1590 (C=N), 1336 (C=S), 1052 (C–N); ^1H NMR ($CDCl_3$) (δ, ppm): 6.61–7.17 (m, 15H, Ar), 3.31 (dd, 1H, H_A, J_{AB} 17.33, J_{Ax} 9.33), 3.35 (dd, 1H, H_B, J_{AB} 16.6, J_{Ax} 9.52), 6.0 (dd, 1H, H_x, J_{Ax} 9.33, J_{Bx} 8.75), 7.81 (d, 2H, NH_2); ^{13}C NMR ($CDCl_3$) (δ, ppm): 170.16 (C=S), 149.57 (C=N), 149.93–126.07 (Phenyl–C), 38.28 (CH), 33.86 (CH_2).

*4.3.3. 1-N-tc-3-(3-br-ph)-5-(4-me-ph)-2-pz (**11**)*

Pale yellow crystal (chloroform); yield: 21%; m.p.: 185 °C. Anal. calc. for ($C_{17}H_{16}N_3SBr$): C 54.69, H 4.28, N 11.26; found: C 54.69, H 4.22, N 11.34%. UV/vis λ_{max} (nm); 371, 270, 236; IR ν_{max} (cm^{-1}): 3242 (N–H) 2911 (C–H), 1567 (C=N), 1354 (C=S), 1078 (C–N); ^1H NMR ($CDCl_3$) (δ, ppm): 6.43–7.10 (m, 15H, Ar), 3.31 (dd, 1H, H_A; J_{AB} 17.33, J_{Ax} 9.33), 3.20 (dd, 1H, H_B, J_{AB} 16.6, J_{Ax} 9.52), 6.02 (dd, 1H, H_x, J_{Ax} 9.33, J_{Bx} 8.75), 7.89 (d, 2H, NH_2), ^{13}C NMR ($CDCl_3$) (δ, ppm): 170.16 (C=S), 149.57 (C=N), 149.93–126.07 (Phenyl–C), 38.28 (CH), 33.86 (CH_2).

*4.3.4. 1-N-tc-3-(3-br-ph)-5-(2,4,6 tri-me-ph)-2-pz (**12**)*

Pale yellow powder (chloroform); yield: 24%; m.p.: 175 °C. Anal. calc. ($C_{19}H_{20}N_3S$): C 56.85, H 4.98, N 10.47 found: C 56.85, H 4.96, N 10.43%. UV/vis λ_{max} (nm): 371, 290, 236, 217; IR ν_{max} (cm^{-1}): 3444 (N–H), 2971 (C–H), 1543 (C=N), 1321 (C=S), 1127 (C–N); ^1H NMR ($CDCl_3$) (δ, ppm): 6.51–7.50 (m, 10H, Ar), 3.48 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.49 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.32 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25) 2.13 (s, 3H, CH_3), 9.24 (d, 2H, NH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm): 176.19 (C=S), 149.57 (C=N), 144.52–125.72 (Phenyl–C), 62.86 (C–H), 37.06 (CH_2).

*4.3.5. 1-N-tc-3-(3-br-ph)-5-(2-ethyl ph)-2-pz (**13**)*

Pale yellow powder (chloroform); yield: 24%; m.p.: 175 °C. Anal. calc. ($C_{18}H_{18}N_3SBr$): C 55.81, H 4.65, N 10.85; found: C 55.78, H 4.61, N 10.85%. UV/vis λ_{max} (nm): 371, 290, 236, 217; IR ν_{max} (cm^{-1}): 3226 (N–H), 2924 (C–H), 1505 (C=N), 1357 (C=S), 1105 (C–N); ^1H NMR ($CDCl_3$) (δ, ppm): 6.4–7.5 (m, 10H, Ar), 3.48 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.49 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.32 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25) 2.13 (s, 3H, CH_3), 8.10 (d, 2H, NH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm): 176.19 (C=S), 149.57 (C=N), 144.52–125.72 (Phenyl–C), 62.86 (C–H), 37.06 (CH_2).

*4.3.6. 1-N-tc-3-(3-br-ph)-5-[(2-isoprop)-ph]-2-pz (**14**)*

Pale yellow powder (chloroform); yield: 24%; m.p.: 175 °C. Anal. calc. ($C_{19}H_{20}N_3SBr$): C 56.85, H 4.98, N 10.47; found: C 56.43, H 4.88, N 10.48%. UV/vis λ_{max} (nm): 371, 290, 236, 217; IR ν_{max} (cm^{-1}): 3362 (N–H), 2995 (C–H), 1489 (C=N), 1377 (C=S), 1038 (C–N); ^1H NMR ($CDCl_3$) (δ, ppm): 6.5–7.5 (m, 10H, Ar), 3.48 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.49 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.32 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25) 2.13 (s, 3H, CH_3), 9.24 (d, 2H, NH_2); ^{13}C NMR ($CDCl_3$) (δ, ppm): 176.19 (C=S), 149.57 (C=N), 144.52–125.72 (Phenyl–C), 62.86 (C–H), 37.06 (CH_2).

*4.3.7. 1-N-tc-3-(3-br-ph)-5-(4-Cl-ph)-2-pz (**15**)*

Pale yellow powder (chloroform); yield: 24%; m.p.: 175 °C. Anal. calc. ($C_{16}H_{15}N_3SClBr$): C 48.79, H 3.30, N 10.68, Cl 9.03; found: C 48.56, H 3.33, N 10.81, Cl 9.12%. UV/vis λ_{max} (nm): 371, 290, 236, 217; IR ν_{max} (cm^{-1}): 3367 (N–H), 2939 (C–H), 1533 (C=N), 1357 (C=S), 1079 (C–N); ^1H NMR ($CDCl_3$) (δ, ppm): 6.51–7.50 (m, 10H, Ar), 3.48 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.49 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.32 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25) 2.13 (s, 3H, CH_3), 9.43 (d, 2H, NH_2); ^{13}C NMR (CDCl3): (δ, ppm): 176.19 (C=S), 149.57 (C=N), 144.52–125.72 (Phenyl–C), 62.86 (C–H), 37.06 (CH_2).

*4.3.8. 1-N-tc-3-(3-br-5-(2,3 di-Cl-ph)-2-pz (**16**)*

Pale yellow powder (chloroform); yield: 24%; m.p.: 175 °C. Anal. calc. ($C_{16}H_{14}N_3SCl_2Br$): C 44.65, H 3.25, N 9.76, Cl 16.51; found: C 44.78, H 3.22, N 9.66, Cl 16.40%. UV/vis λ_{max} (nm): 371, 290, 236, 217; IR ν_{max} (cm^{-1}): 3283 (N–H), 2924 (C–H), 1498 (C=N), 1357 (C=S), 1152 (C–N); ^1H NMR ($CDCl_3$) (δ, ppm): 6.51–7.50 (m, 10H, Ar), 3.48 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.49 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.32 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25) 2.13 (s, 3H, CH_3), 9.33 (d, 2H, NH_2); ^{13}C NMR ($CDCl_3$) (δ, ppm): 176.19 (C=S), 149.57 (C=N), 144.52–125.72 (Phenyl–C), 62.86 (C–H), 37.06 (CH_2).

4.4. Synthesis of 3,5 substituted diphenyl-1-(thiazolo [4,5-b] quinoxaline-2-yl)-2-pyrazole derivatives; general procedure

A mixture of 1-*N*-thiocarbamoyl 3,5 substituted diphenyl pyrazoline compound (3.47 g, 0.01 mol) and 2,3-dichloroquinoxaline (1.99 g, 0.01 mol) in THF (10 ml) was refluxed for 24 h. The solvent was evaporated under reduced pressure. The residue was recrystallized from ethanol [15].

*4.4.1. 3-(3-Br-ph)-5-ph-1-(tz [4,5-b] qx-2-yl)-2-pz (**17**)*

Yellow solid (chloroform); yield: 18%; m.p.: 162 °C. Anal. calc. ($C_{24}H_{16}N_5SBr$): C 59.38, H 3.29, N 14.43; found: C 59.89, H 3.89, N 14.97%. UV/vis λ_{max} (nm): 371, 298, 266, 236, 212; IR ν_{max} (cm^{-1}): 2892 (C–H), 1542 (C=N), 1539 (C=N), 1085 (C–N), 885 (C–S); ^1H NMR ($CDCl_3$) (δ, ppm): 6.72–7.50 (14H, m, Ar), 3.48 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.49 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.32 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25).

*4.4.2. 3-(3-Br-ph) 5-(3-indolyl)-1-(tz [4,5-b] qx-2-yl) 2-pz (**18**)*

Yellow solid (chloroform); yield: 34%; m.p.: 180 °C. Anal. calc. ($C_{26}H_{18}N_6S$ Br): C 59.38, H 3.29, N 14.43; found: C 59.89, H 3.89, N 14.97%. UV/vis λ_{max} (nm): 371, 290, 236, 217; IR ν_{max} (cm^{-1}): 2992 (C–H), 1489 (C=N), 1566 (C=N), 1108 (C–N), 881 (C–S); ^1H NMR ($CDCl_3$) (δ, ppm): 6.61–7.71 (14H, m, Ar), 3.32 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.47 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 5.81 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25).

*4.4.3. 3-(3-Br-ph)-5-(4-me-ph)-1-(tz [4,5-b] qx-2-yl)-2-pz (**19**)*

Pale yellow crystal (chloroform); yield: 16%; m.p.: 155 °C. Anal. calc. ($C_{25}H_{18}N_5SBr$): C 60.12, H 3.60, N 14.02; found: C 60.45, H 3.89, N 14.21%. UV/vis λ_{max} (nm): 367, 299, 256, 236, 217; IR ν_{max} (cm^{-1}): 2924 (C–H), 1558 (C=N), 1516 (C=N), 1142 (C–N), 912 (C–S); ^1H NMR ($CDCl_3$) (δ, ppm): 7.12–7.14 (14H, m, Ar), 3.11 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.39 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.18 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25).

*4.4.4. 3-(3-Br-ph) 5-(4-ethyl ph)-1-(tz [4,5-b] qx-2-yl)-2-pz (**20**)*

Pale yellow crystal (chloroform); yield: 26%; m.p.: 188 °C. Anal. calc. ($C_{26}H_{20}N_5SBrCl$): C 60.81, H 3.89, N 13.64; found: C 60.78, H 3.89, N 13.56%. UV/vis λ_{max} (nm): 373, 288, 236, 217; IR ν_{max} (cm^{-1}): 2910 (C–H), 1498 (C=N), 1558 (C=N), 1116 (C–N), 869 (C–S); ^1H NMR ($CDCl_3$)

(δ, ppm): 6.72–7.11 (14H, m, Ar), 3.44 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.49 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25).

4.4.5. 3-(3-Br-ph) 5-[2-(isopropyl) ph]-1-(tz [4,5-b] qx-2-yl)-2-pz (**21**)

Pale yellow crystal (chloroform); yield: 18%; m.p.: 158 °C. Anal. calc. ($C_{27}H_{19}N_5SBr$): C 61.83, H 3.62, N 13.35; found: C 61.34, H 3.89, N 13.29%. UV/vis λ_{max} (nm): 371, 298, 266, 236, 209; IR ν_{max} (cm^{-1}): 2969 (C–H), 1538 (C=N), 1161 (C–N), 876 (C–S); ^1H NMR ($CDCl_3$) (δ, ppm): 7.23–7.44 (14H, m, Ar), 3.11 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.76 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 5.89 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.20), 6.28 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25).

4.4.6. 3-(3-Br-ph) 5-(2,4,6 tri-me-ph)-1-(tz [4,5-b] qx-2-yl)-2-pz (**22**)

Orange crystal (chloroform); yield: 22%; m.p.: 192 °C. Anal. calc. ($C_{27}H_{22}N_5SBr$): C 61.48, H 4.17, N 13.28; found: C 61.04, H 4.89, N 13.98%. UV/vis λ_{max} (nm): 367, 290, 236, 221; IR ν_{max} (cm^{-1}): 2924 (C–H), 1485 (C=N), 1522 (C=N), 1122 (C–N), 892 (C–S); ^1H NMR ($CDCl_3$) (δ, ppm): 6.7–7.4 (14H, m, Ar), 3.48 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.51 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 6.32 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25).

4.4.7. 3 (3-Br-ph) 5-(2-Cl-ph)-1-(tz [4,5-b] qx-2-yl)-2-pz (**23**)

Pale yellow crystal (chloroform); yield: 18%; m.p.: 177 °C. Anal. calc. ($C_{24}H_{15}N_5SClBr$): C 55.49, H 3.09, N 13.48; found: C 55.64, H 3.89, N 13.61%. UV/vis λ_{max} (nm): 371, 290, 231, 217; IR ν_{max} (cm^{-1}): 2916 (C–H), 1576 (C=N), 1558 (C=N), 1051 (C–N), 947 (C–S); ^1H NMR ($CDCl_3$) (δ, ppm): 6.72–7.50 (14H, m, Ar), 3.38 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.56 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 5.44 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25).

4.4.8. 3 (3-Br-ph) 5-(2,3diCl-ph)-1-(tz [4,5-b] qx-2-yl)-2-pz (**24**)

Pale yellow crystal (chloroform); yield: 34%; m.p.: 175 °C. Anal. calc. ($C_{24}H_{14}N_5SCl_2Br$): C 62.07, H 2.53, N 12.65; found: C 62.33, H 2.22, N 12.34%. UV/vis λ_{max} (nm): 371, 290, 236, 217; IR ν_{max} (cm^{-1}): 2924 (C–H), 1558 (C=N), 1593 (C=N), 1122 (C–N), 920 (C–S); ^1H NMR ($CDCl_3$) (δ, ppm): 6.72–7.50 (14H, m, Ar), 3.71 (dd, 1H, H_A, J_{AB} 18.5, J_{Ax} 9.25), 3.86 (dd, 1H, H_B, J_{AB} 18.5, J_{Bx} 9.25), 5.79 (dd, 1H, H_x, J_{Ax} 11.10, J_{Bx} 9.25).

4.5. 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 [16]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously in wells of 96 well microtiter plate [17]. All the compounds were dissolved in DMSO (40 μl) at which level no inhibition of amoeba occurs [18,19] and the solution was further diluted to 1 ml by adding freshly prepared medium to make a concentration of 1 mg/ml. Twofold serial dilutions were made in the wells of 96-well microtiter plate (Costar) in 170 μl of culture medium. 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 haemocytometer 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.

4.6. Assessment of antiamoebic activity

After incubation the growth of the amoeba 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 Tables 1 and 2.

4.7. MTT toxicity assay

The quinoxaline derivatives of pyrazoline (**17–24**) which showed the better antiamoebic results than their parent compounds i.e.; chalcones (**1–8**) and pyrazolines (**9–16**) were assessed for toxic profile. For the toxicity assay, transformed human kidney epithelium (Graham) cells were continuously maintained in culture at 37 °C in 5% CO_2. The MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide, USB) cellular viability assay was used to determine the toxicity profile of these compounds [20]. The trypsinized cell suspension was adjusted to 0.5 million cells/mL and plated out with the compounds (**17–24**). After 44 h of incubation, 2 mM MTT was added to the plates and incubated for a further 4 h. DMSO was then added to stop the reaction and dissolve the formazan crystals. The absorbance was read at the test wavelength of 540 nm and reference wavelength of 690 nm and the percentage cellular viability calculated with appropriate controls

taken into account. The mean ± S.D. values of IC_{50} values in (Table 2) are from three independent experiments.

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