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Short communication

Novel Pd(II) complexes of 1-*N*-substituted 3-phenyl-2-pyrazoline derivatives and evaluation of antiamoebic activity

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Abstract

Cyclization of Mannich base with N^4-substituted thiosemicarbazides by different aliphatic, aromatic and cyclic amines afforded a series of new 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones (PYZ-TSC) **1**–**10**. Reaction of $[Pd(DMSO)_2Cl_2]$ with pyrazoline derivatives led to new palladium(II) complexes $[Pd(PYZ\text{-}TSC)Cl_2]$ **1a**–**10a**. The structures of all the compounds were characterized by spectroscopic methods. It was concluded that the pyrazoline thiosemicarbazone derivatives have two chelating arms, one attached at the 2-position of the pyrazole ring (that is, N donor) and other (S donor) linked to the thiosemicarbazone branch. The determination of antiamoebic activity of all the compounds was done using *HM1:IMSS* strain of *Entamoeba histolytica*, among all the complexes, **8a** showed the most promising $IC_{50} = \mathbf{0.37}$ μM vs. $IC_{50} = \mathbf{1.81}$ μM of metronidazole, the reference drug. MTT assay showed that the compounds are non-toxic to human kidney epithelial cell line.

Keywords: Palladium(II) complexes; Cyclised pyrazoline analogues; Thiosemicarbazones; *Entamoeba histolytica*

1. Introduction

Amoebiasis, caused by *Entamoeba histolytica*, an early branching eukaryote, is one of the major threats to public health in much of the developing world. More than 50 million people worldwide are infected and up to 110,000 die every year due to amoebiasis [1]. It is the third most common parasitic disease in humans after malaria and schistosomiasis [2,3]. The highest incidence is observed in children below the age of 5 years [4]. Trophozoites of the extracellular protozoan parasite *E. histolytica* colonize the human gut, occasionally invade the intestinal mucosa, and sometimes metastasize to other organs [5]. Recently it is found that amoebic abscesses of the brain are a dreadful complication of this disease [6]. The effective treatment for amoebiasis is metronidazole; however, lengthy treatment or high doses often cause side effects such as headache, nausea, vomiting, dry mouth, metallic taste, dizziness, vertigo, stomatitis, glossitis and neurological complications [7–9]. Therefore, it is necessary to search for new alternative medications that can eliminate the parasite without generating carcinogenic effects.

The chemistry of cyclised heterocyclic systems especially containing pyrazole moiety has been largely investigated due to their effective use in pharmacological areas [10–21]. In particular, the pyrazolate coordination chemistry has recently received a major appraisal of structurally unprecedented and exciting compounds of great pharmacological importance and thus has attracted considerable attention in recent years as versatile ligands in coordination chemistry [22–26]. In the recent years, pyrazole-containing complexes have been reported to possess antitumor activity comparable to that of cisplatin [27–29].

Pioneering studies in our laboratory [30–32] have led to synthesize a new series of 1-*N*-substituted cyclised pyrazoline thiosemicarbazones and their palladium(II) complexes. The

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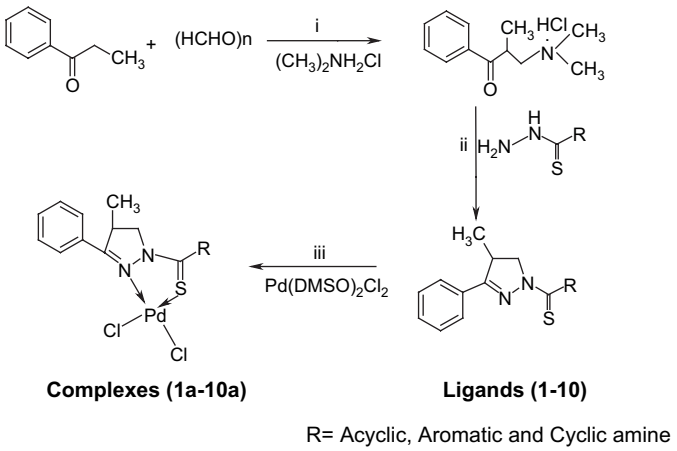
antiamoebic activity and toxicity study of these compounds were performed against *E. histolytica* and KB cell lines, respectively.

2. Results and discussion

2.1. Chemistry

The Mannich reaction of propiophenone with formaldehyde and dimethylamine hydrochloride gives the Mannich base precursor. The reaction works best when a minimum amount of ethanol and 2 μL of acid/mmol ketone is added. Propiophenone gave satisfying yield around 90% in the Mannich reaction. The condensation of this Mannich reaction product with N^4-substituted thiosemicarbazides by different amines leads to the formation of 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones (Scheme 1). The product was generally crystalline in case of aliphatic substituted thiosemicarbazides and oil in rest of the compounds. According to the currently accepted mechanism [33,34] the formation of the cyclised pyrazoline analogues is favored via thiosemicarbazones formation, which undergoes cyclization under basic conditions to form the desired pyrazoline ring. The product mixture contained only unreacted starting material and the cyclization product, which was purified by column chromatography using silica gel $60F_{254}$ eluted with chloroform:methanol (98:2). The yield of cyclised products was assorted from low to high. The compounds obtained are stable. $[Pd(DMSO)_2Cl_2]$ serves as a good starting material to prepare complexes reported here thereby giving novel palladium(II) complexes of 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones (**1a**–**10a**). The precursor $[Pd(DMSO)_2Cl_2]$ used for the synthesis of palladium complexes was synthesized by the literature procedure [35]. Reaction of 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones and $[Pd(DMSO)_2Cl_2]$ in equimolar ratio in refluxing methanol gave amorphous complexes with satisfactory yields. Crystallization was done in suitable solvents. All the complexes are soluble in DMF and DMSO,



Scheme 1. Reagents and conditions: (i) methanol, NaOH (11%), 15–28 °C; (ii) ethanol, reflux, NaOH; (iii) dry MeOH, 70 °C.

and sparingly soluble in methanol, ethanol and insoluble in water. The complexes do not undergo any weight loss up to 230 °C, which suggests their fair thermal stability. The structures of all the ligands and Pd(II) complexes were established by means of elemental analysis, IR, UV, ^{1}H NMR, ^{13}C NMR, thermogravimetric analysis and FAB MS (Scheme 2).

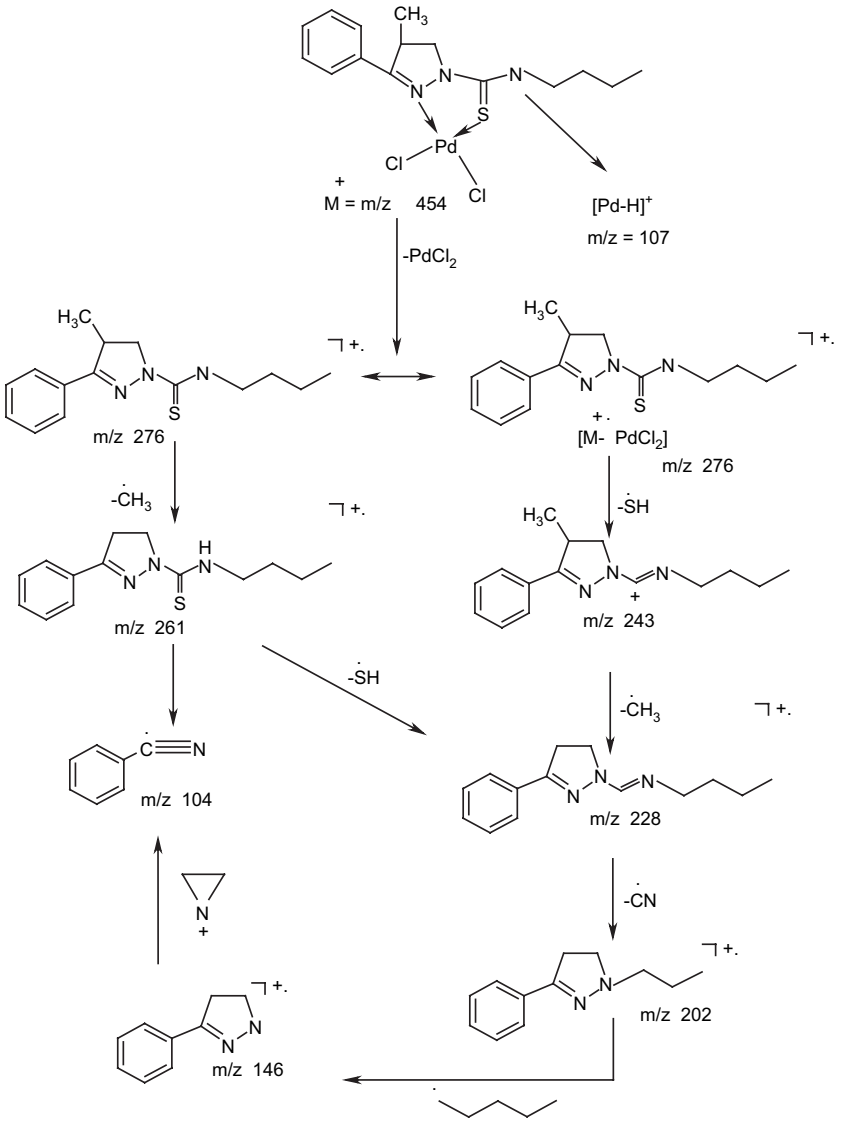
2.1.1. Electronic spectral studies

The electronic spectra of the cyclised pyrazoline analogues **1**–**10** exhibited three absorption bands at 260–278, 218–230 and 202–212 nm assignable to $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, respectively. The band at 260–278 nm assigned to the $n \rightarrow \pi^*$ transition was due to the thione portion (C=S) of thiocarboxamide group. The two other absorption bands at 218–230 and 202–212 nm were due to $\pi \rightarrow \pi^*$ transition of phenyl ring and $n \rightarrow \sigma^*$ transition of azomethine nitrogen, respectively. The UV/vis spectrum of all the palladium(II) complexes exhibits similar pattern, representing three bands in the region 289–225, 237–245 and 212–221 nm. A careful study of electronic spectral bands of complexes showed that there was change in the energy of these bands due to extended conjugation of ligands. Complexes **1a**–**10a** show a single band in the region 212–221 nm due to charge-transfer transition, which is in a higher-energy region with respect to ligands. Bands at higher energies 289–225 nm are attributed to the $\pi \rightarrow \pi^*$ transitions of the aromatic ring. In complexes, the $\pi \rightarrow \pi^*$ transition of azomethine chromophore shifted to 237–245 nm indicating that the imino *N*-atom is involved in the coordination. An intense charge-transfer band is observed for the complexes in the 371–373 nm region which is reasonably assignable to a combination of ligand to metal charge transfer and metal d–d band transitions. Such observations have also been noticed earlier in other palladium(II) complexes of similar ligand systems [36].

2.1.2. IR spectral analysis

Assignments of selected characteristic IR band positions provide significant indication for the formation of the cyclised pyrazoline analogues of thiosemicarbazones **1**–**10**. All the compounds showed intense bands in the region 1021–1109 cm^{-1} due to the ν(C=S) stretch of the thiocarboxamide group along with a weak band appeared in the region 2500–2600 cm^{-1} due to ν(C–SH) [36, 37] suggesting that all the cyclised pyrazoline analogues exhibit thione ↔ thiole tautomerism as depicted in Fig. 1. The IR spectra of all the compounds showed ν(C=N) stretch at 1525–1598 cm^{-1} because of the ring closure.

In addition, the absorption bands at 1102–1198 cm^{-1} were attributed to the ν(C–N) stretch vibrations, which also confirm the formation of the desired pyrazoline ring in all the compounds. The mono-substituted amines showed additional sharp bands in the region 3275–3389 cm^{-1} due to the ν(NH) stretch. The IR spectra in the range 40,000–400 cm^{-1} show that the ligands are coordinated to the Pd(II) metal ion giving **1a**–**10a**. The most striking bands of the ligands (ν(C=N), ν(N–H), and ν(C–N)) increase their frequency when they are part of the complexes. Moreover, the band appearing in the region

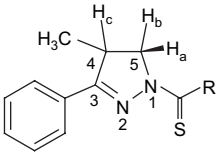


Scheme 2. Proposed mass fragmentation pattern for complex.

1001–1050 cm^{-1} ν(C=S) in the spectrum is shifted to lower wave number by ca. 15–30 cm^{-1} after complexation, indicating that the thione sulphur participates as a coordinating site. The far-IR spectra of the complexes exhibit bands in the region 443–489 cm^{-1} tentatively attributed to ν(Pd–N) and ν(Pd–S) stretches, further indicating the involvement of (–C=N) nitrogen and (–C=S) sulphur in these complexes. This confirms the fact that in this case cyclised pyrazolyl thiosemicarbazone ligands behave as neutral NS donor bidentate upon complexation.

2.1.3. Nuclear magnetic resonance spectral studies

Further evidence for the formation of pyrazoline compounds was obtained by the ^{1}H NMR spectra. The pyrazoline proton Hc, at C_4 carbon appears downfield in the region 3.16–3.87 as multiplets in all compounds and the geminal protons Ha and Hb, at C_5 carbon exhibit two signals, one as triplet in the range 3.60–4.76 ppm ($J = 3.6–5.1$, 10.3–14.3 Hz) and the other as a doublet of doublets ranging from 3.96 to 4.96 ppm ($J = 10.3–14.3$ Hz) which is due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 and the geminal proton at position 5 of the pyrazoline ring which may be due to its conformation A given below.



A

The CH_3 protons attached to the pyrazoline nucleus at C_4 appear as a doublet in δ 1.30–1.36 ppm region. The NH proton of different substituted thiocarboxamide group of the compounds **1–10** showed a singlet at 8.37–8.96 ppm. The protons belonging to the aromatic ring and the other aliphatic groups were observed with the expected chemical shift and

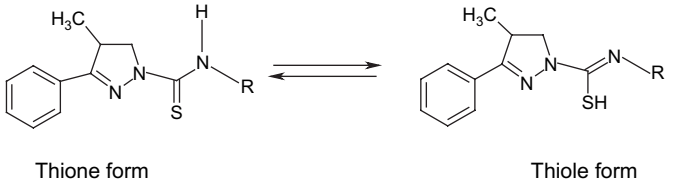


Fig. 1. Structure of pyrazoline thiosemicarbazones exhibiting thione ↔ thiole tautomerism.

integral values. Comparing the ^{1}H NMR spectra of pyrazolic ligands and complexes, a slight downfield shift of thioamide (NH–C=S) proton signal in the region 8.54–9.18 ppm for complexes was observed which indicates the involvement of thione sulphur in complex formation. The complexes **1a–10a** do not show any resonance at ca. 4.0 ppm, attributed to –SH proton resonance, indicating that even in a polar solvent such as DMSO they remain in the thione form. This information suggests the adjustment of electronic current upon coordination of >C=S group to the metal ion. As a result, establishing the direct involvement of =N–N–C=S group in coordination. Other protons, namely viz. CH_3 protons, CH_2 protons and aryl protons in complexes **1a–10a** resonate nearly at the same region as that of free ligands.

The ^{13}C NMR spectra of all the cyclised pyrazoline analogues of thiosemicarbazones **1–10** were taken in $CDCl_3$ and the signals obtained further confirm the proposed structures. The C_4 and C_5 carbons of the pyrazoline ring resonate at 46.3–51.1 and 50.8–54.1 ppm, respectively. All the compounds showed a signal at 150.6–166.3 ppm which was assigned to the azomethine carbon of pyrazoline ring. Thiocarboxamide carbon (C=S) displayed a signal at 173.9–184.3 ppm in all the compounds. The signals in the range 139.1–125.0 ppm were due to the aromatic carbons. The carbons at 1-*N*-substituted amino groups resonate at their usual positions as is shown in the data given in Section 4.

2.1.4. TGA analysis

Thermograms of Pd(II) complexes of 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones **1a–10a** were recorded under nitrogen with a heating rate of 10 °C/min between room temperature and 800 °C. These complexes did not lose weight up to 230 °C. Further increments of temperature caused decomposition of the complexes in two steps, the temperature range for the first step being 230–290 °C for the palladium(II) complexes where losses of mixed fragments were observed. The second step starts immediately after the first one and continues until complete decomposition of the ligand and formation of PdS as the end product were observed. Although decomposed fragments of the ligands could not be approximated due to continuous weight loss, the total percentage weight loss of the complexes corresponds to the loss of the respective ligand after considering the transfer of one sulphur atom to the metal ion and residues correspond to the palladium sulphide.

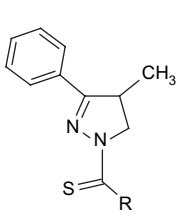
2.1.5. FAB MS analysis

The structure of the pyrazolyl palladium complexes **1a–10a** was additionally confirmed from its mass spectral fragments and gave the molecular ion peak as $(M + 1)^+$ ion. It is assumed that the fragmentation pathway is initiated by loss of the two chlorine ions simultaneously, giving $(M - Cl_2)$ ion peaks at implicit positions. Elimination of Pd metal from cyclised pyrazoline thiosemicarbazone complexes and its further fragmentation follows a similar pathway in all the complexes which occurs via cleavage of [SH] and [CH_3] moieties giving ideal peaks in all the compounds which correspond to the $[M - SH]^+$ or $[M - CH_3]^+$ ion. This is followed by elimination of CN to produce a disubstituted pyrazoline ion. Further elimination of alkyl radical of the substituted amines leads to the formation of mono-substituted pyrazoline ion at *m/z* 146 as a base peak in all the complexes. The major fragmentation pathway involved the split of pyrazoline ring giving fragment at *m/z* 104. The azirinium ions are barely detected and the ions at *m/z* 13, 91, 77 arise from further decomposition in the usual way. The FAB MS fragmentation pattern of compound **3** is depicted in Scheme 2.

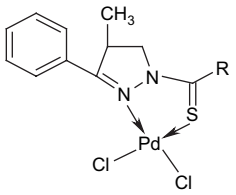
2.1.6. In vitro antiamoebic activity

All the 1-*N*-substituted thiocarboxamide-4-methyl-3-phenyl 2-pyrazolines with thiosemicarbazides of variegated nature and their Pd(II) complexes were screened for antiamoebic activity against *HM1:IMSS* strain of *E. histolytica*. The compounds under study are 1-*N*-substituted with different aliphatic, alicyclic and aromatic groups to investigate the influence of the substitution on the antiamoebic activity. The IC_{50} values in μM are indexed 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 was used as the reference drug and had a 50% inhibitory concentration (IC_{50} 1.6–1.8 μM) in our experiments. All the compounds showed IC_{50} values in the range 0.37–15.25 μM. Among all the cyclised ligands, the *N-cyclo*-octyl amine **7** in this series showed a close value for antiamoebic activity with the reference drug, metronidazole ($IC_{50} = 2.3$ μM vs. $IC_{50} = 1.8$ μM of metronidazole). The complexation of cyclised pyrazoline thiosemicarbazones **1–10** with palladium(II) results in complexes **1a–10a**, which showed $IC_{50} = 0.37–5.65$ μM. All the metal complexes were found more active than their respective ligands indicating that the complexation to metal enhances the activity of the ligand. Fig. 2 represents the graph of pyrazoline thiosemicarbazones and Pd(II) complexes exhibiting comparison of IC_{50} values in μM. This may be explained by Tweedy's theory [38], according to which chelation reduces the polarity of the central metal atom because of partial sharing of its positive charge with the ligand, which favors permeation of the complexes through the lipid layer of cell membrane. Moreover, the complexes **2a** and **4a–10a** displayed the most promising *in vitro* amoebicidal activity and were found better inhibitors of the parasite than metronidazole. The Pd-complex precursor

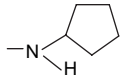
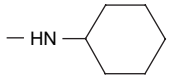
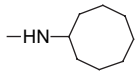
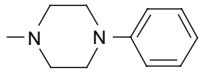
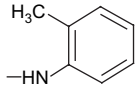
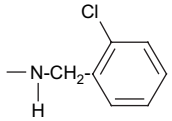
Table 1
In vitro antiamoebic activity of compounds against *E. histolytica* and cytotoxic profile



(1-10)



(1a-10a)

Compound	R	Antiamoebic activity IC_{50} (μM)	S.D.[a]	Toxicity profile IC_{50} (μM)	Safety index (SI)
1	$-NH_2$	10.32	0.11	>100	>9.68
1a		5.65	0.01	>100	>17.69
2	$-HN-CH(CH_3)-CH_3$	15.25	0.20	>100	>6.55
2a		1.68	0.19	>100	>59.52
3	$-HN-CH_2-CH_2-CH_2-CH_3$	4.32	0.09	>100	>23.14
3a		3.57	0.03	>100	>28.01
4	$-HN-CH_2-CH(CH_3)-CH_3$	9.13	0.06	>100	>10.95
4a		1.76	0.02	>100	>56.81
5		8.75	0.24	>100	>11.42
5a		1.81	0.02	>100	>55.24
6		6.63	0.10	>100	>15.08
6a.		1.49	0.18	>100	>67.11
7		2.32	0.08	>100	>43.10
7a		1.39	0.05	>100	>71.94
8		8.69	0.07	>100	>11.50
8a		0.37	0.03	>100	>270.27
9		4.57	0.24	>100	>21.88
9a		0.58	0.01	>100	>172.40
10		4.62	0.17	>100	>21.64
10a		0.82	0.02	>100	>121.95
$[Pd(DMSO)_2Cl_2]$		8.00	0.34	=100	>12.50
METRONIDAZOLE (MNZ)		1.81	0.03	>100	>55.24

[a] Standard deviation.

$[Pd(DMSO)_2Cl_2]$ was also evaluated for antiameobic activity and compared with Pd(II) complexes and metronidazole, which showed no activity against *E. histolytica*. It was concluded that the presence of bulky groups at position N^4 of the thiosemicarbazone moiety greatly enhanced the antiamoebic activity. The IC_{50} values indicate that all the Pd(II) complexes cause a marked inhibition, while the parent ligands are less active than complexes. 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 compounds was evaluated by *T*-test. The values of the calculated *T* were found higher than the tabulated *T* at 5% level, thus concluding that the character under study is significantly influenced by the treatment. Detailed studies of the toxicity, *in vivo* and mechanism of action of these complexes are in progress.

2.1.7. Toxicity profile

The pyrazoline ligands and their palladium complexes were studied against human kidney epithelial cell line to ensure that compounds were not toxic to human cells. None of the compounds inhibited cell growth at a concentration of 100 μM. 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)

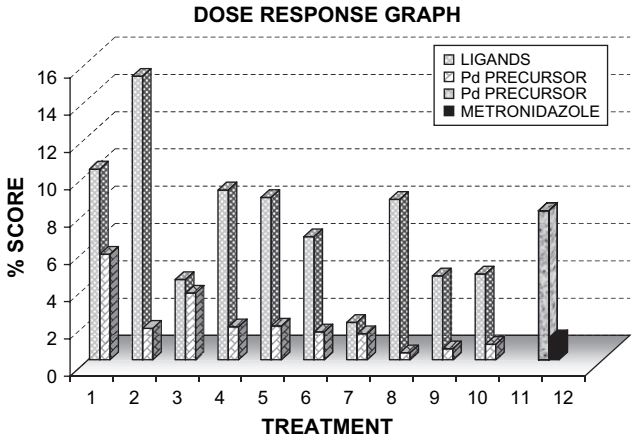


Fig. 2. Pyrazoline thiosemicarbazones and Pd(II) complexes exhibiting comparison of IC_{50} values (μM).

cell line and protozoal IC_{50} is the concentration that kills 50% of amoeba protozoa. This allows to estimate which compounds might be efficacious or toxic against human cells and potentially in vivo. The numerical results for each compound are given in Table 1. These safety indices were then plotted against antiamoebic IC_{50} values, obtaining the results depicted in Fig. 3. These results show that none of the pyrazoline ligands show significant values of SI. In contrast, complex **8a** has lowest cytotoxicity and highest antiamoebic activity and in an overall result, complexes **2a** and **4a**−**10a** show the more favorable safety profile along with the most promising antiamoebic activity.

3. Conclusion

This research examined the antiamoebic activities of novel palladium(II) complexes of 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones. It was found that the pyrazoline thiosemicarbazone derivatives have two chelating arms and act as neutral NS bidentate chelators. Moreover, the substituents did not have any influence on the coordination pattern of compounds. *In vitro* antiamoebic evaluation of the ligands and metal complexes was carried out against *E. histolytica* using *HM1:IMSS* strain. The biological behavior revealed that among all the complexes, **2a** and **4a**−**10a** were found to possess remarkable amoebistatic to amoebicidal values ranging from 0.37 to 1.81 μM i.e., equal to or less than metronidazole. It was found that upon complexation, the activity of ligand was considerably increased. The complexes of ligands having aromatic moieties showed most promising antiamoebic activity as compared to complexes of ligands having aliphatic and alicyclic moieties that can be attributed to the presence of bulky aryl groups such as *N*-phenyl piperizine, *N*-*o*-toluidine, *N*-2-chlorobenzyl. MTT assay showed that the compounds are non-toxic to human kidney epithelial cell line.

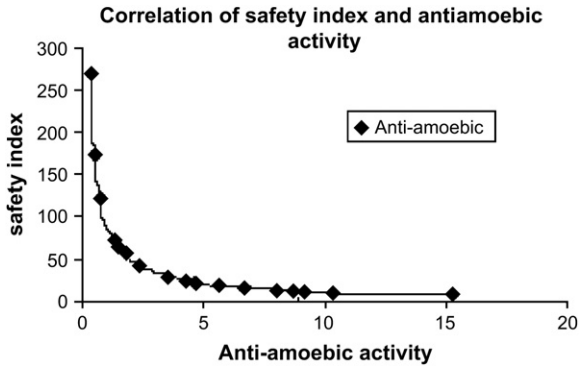


Fig. 3.

4. Experimental

All the chemicals were purchased from Aldrich chemical company (USA). Elemental analyses (C, H, N) were performed by Central Drug Research Institute, Lucknow, India, and the results were within 0.4% of the theoretical values. Chlorine was estimated by standard method. 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/vis 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; m, multiplet. Chemical shift values are given in (parts per million) and coupling constants (J) in hertz. The FAB mass spectra of all the complexes were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer/Data System using argon/xenon 6 kV, 10 mA as the FAB gas and *m*-nitro benzyl alcohol (NBA) was used as the matrix. Thermogravimetric analysis of the complexes was performed on a TG 51 thermogravimetric analyzer under nitrogen atmosphere with the heating rate of 10 °C/min. Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} precoated thin-layer plates.

4.1. Synthesis of Mannich base

A suspension of propiophenone (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, the solvent was removed *in vacuo*. A few drops of HCl were added, and the mixture was worked up with dichloromethane and water. The aqueous layer was adjusted to basic and extracted with dichloromethane (3×). The dichloromethane layer was combined and dried. The product was obtained as colorless oil. The compound has been reported earlier [39], we have characterized this compound by IR and ^{1}H NMR spectra. Yield: 91%; colorless oil; IR: ν_{max} (cm^{-1}) 3025 (arom. C−H), 2950 (aliph. C−H), 1677 (C=O), 1221 (C−N); ^{1}H NMR ($CDCl_3$): (δ, ppm) 7.33−7.49 (5H, m, aryl), 3.68 (H, m, −C*H*), 2.71−2.55 (2H, dd, −CH_2, $J = 5.7$, 10.7 Hz), 2.17 (6H, s, −CH_3), 1.12 (3H, d, −CH_3, $J = 7.09$ Hz).

4.2. 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 thiosemicarbazides taken. N^4-Substituted thiosemicarbazides were cyclised with Mannich base within 48 h while for N^4,N^4-disubstituted thiosemicarbazides the refluxing time was 48–72 h. The methanol was removed *in vacuo*. The residue was dissolved in dichloromethane, washed with water and dried over anhydrous Na_2SO_4. In some cases precipitate was formed and the product was obtained by filtration in ice-cold ethanol. If no precipitate was formed then 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-methyl-2-pyrazoline-1-thiocarboxamide (**1**)

Yield 51%. R_f 0.72; pale yellow solid; m.p. 97 °C. Anal. calc. ($C_{11}H_{13}N_3S$): C 60.27, H 5.93, N 19.20; found: C 60.54, H 5.88, N 18.97; λ_{max} (nm): 262, 222, 202; IR: ν_{max}/cm^{-1} 3289 (NH), 2921 (C–H), 1530 (C=N), 1457 (C=C), 1125 (C–N), 1021 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.54 (s, 2H, NH_2), 7.26–7.74 (m, 5H, Ar–H), 4.43 (t, 1Ha, CH, $J = 11.4$ Hz), 4.19 (dd, 1Hb, CH, $J = 4.5$, 11.4 Hz), 3.82 (m, 1Hc, CH), 1.36 (d, 3H, CH_3, $J = 6.4$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 175.3 (C=S), 156.0 (C=N), 135.9, 129.3, 129.2, 128.7, 126.4 (aryl-C), 50.8 (CH_2), 48.8 (CH_2), 14.6 (CH_3).

4.2.2. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-isopropyl)thiocarboxamide (**2**)

Yield 57%. R_f 0.63; cream white crystals ($CHCl_3$); m.p. 120 °C. Anal. calc. for $C_{14}H_{19}N_3S$: C 64.36, H 7.27, N 16.09; found: C 63.70, H 7.21, N 16.04; λ_{max} (cm^{-1}): 267, 224, 205; IR: ν_{max} (cm^{-1}) 3344 (NH), 2924 (CH), 1536 (C=N), 1459 (C=C), 1108 (C–N), 1064 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.69 (s, 1H, NH), 7.13–7.74 (m, 5H, Ar–H), 4.61 (m, 1Hc, CH), 4.41 (t, 1Ha, CH, $J = 11.3$ Hz), 4.19 (dd, 1Hb, CH, $J = 5.1$, 10.3 Hz), 3.71 (m, 1Hc, CH), 1.30 (d, 6H, CH_3, $J = 6.6$ Hz), 1.32 (d, 3H, CH_3, $J = 6.39$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 175.4 (C=S), 157.1 (C=N), 135.8, 129.5, 129.3, 128.7, 128.6, 126.4 (aryl-C), 51.5 (CH_2), 51.1 (CH), 41.8 (CH_2), 14.6 (CH_3).

4.2.3. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-butyl)thiocarboxamide (**3**)

Yield 67%. R_f 0.71; pale yellow crystals ($CHCl_3$); m.p. 180 °C. Anal. calc. for $C_{15}H_{21}N_3S$: C 65.45, H 7.63, N 15.27; found: C 65.41, H 7.29, N 15.45; λ_{max} (cm^{-1}): 270, 228, 208; IR: ν_{max} (cm^{-1}) 3338 (NH), 2962 (CH), 1545 (C=N), 1456 (C=C), 1142 (C–N), 1081 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.69 (s, 1H, NH), 7.20–7.74 (m, 5H, Ar–H), 4.41 (t, 1Ha, CH, $J = 11.5$ Hz), 4.20 (dd, 1Hb, CH, $J = 4.3$, 11.5 Hz), 3.71 (m, 1Hc, CH), 3.54 (m, 2H, CH_2), 1.76 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 1.03 (t, 3H, CH_3, $J = 7.2$ Hz), 1.32 (d, 3H, CH_3, $J = 6.39$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.5 (C=S), 157.1 (C=N), 135.8, 129.5, 129.3, 128.6, 128.3, 126.7 (aryl-C), 52.5 (CH_2), 51.5 (CH_2), 51.0 (CH), 44.9 (CH_2), 30.6 (CH_2), 19.9 (CH_2), 14.6 (CH_3), 13.5 (CH_3).

4.2.4. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-isobutyl)thiocarboxamide (**4**)

Yield 36%. R_f 0.69; pale yellow crystals ($CHCl_3$); m.p. 105 °C. Anal. calc. for $C_{15}H_{21}N_3S$: C 65.45, H 7.63, N 15.27; found: C 65.37, H 7.21, N 15.25; λ_{max} (cm^{-1}): 268, 230, 204; IR: ν_{max} (cm^{-1}) 3361 (NH), 2956 (CH), 1541 (C=N), 1457 (C=C), 1102 (C–N), 1057 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.68 (s, 1H, NH), 6.93–8.08 (m, 5H, Ar–H), 4.43 (t, 1Ha, CH, $J = 11.1$ Hz), 4.22 (dd, 1Hb, CH, $J = 4.2$, 11.1 Hz), 3.75 (m, 1Hc, CH), 3.56 (t, 2H, CH_2, $J = 6.4$ Hz), 1.35 (d, 6H, CH_3, $J = 6.6$ Hz), 1.32 (d, 3H, CH_3, $J = 7.2$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.5 (C=S), 157.0 (C=N), 136.0, 129.0, 129.0, 128.7, 128.3, 126.9 (aryl-C), 52.5 (CH_2), 51.5 (CH_2), 51.0 (CH), 30.3 (CH), 20.4 (2CH_3), 14.6 (CH_3).

4.2.5. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-cyclopentyl)thiocarboxamide (**5**)

Yield 27%. R_f 0.74; yellow crystals ($CHCl_3$); m.p. 158 °C. Anal. calc. for $C_{16}H_{21}N_3S$: C 66.89, H 7.31, N 14.63; found: C 66.56, H 6.92, N 14.15; λ_{max} (cm^{-1}): 272, 230, 208; IR: ν_{max} (cm^{-1}) 3301 (NH), 2925 (CH), 1525 (C=N), 1457 (C=C), 1171 (C–N), 1054 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.68 (s, 1H, NH), 7.21–7.38 (m, 5H, Ar–H), 4.41 (t, 1Ha, CH, $J = 11.3$ Hz), 4.19 (dd, 1Hb, CH, $J = 4.3$, 11.3 Hz), 3.71 (m, 1Hc, CH), 1.35 (d, 3H, CH_3, $J = 7.1$ Hz), 1.30–1.74 (m, 8H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.3 (C=S), 158.0 (C=N), 138.8, 129.5, 129.3, 128.7, 128.3, 126.9 (aryl-C), 51.5 (CH_2), 51.1 (CH_2), 51.0 (CH_2), 32.3 (2CH_2), 22.9 (2CH_2), 14.6 (CH_3).

4.2.6. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-cyclohexyl)thiocarboxamide (**6**)

Yield 41%. R_f 0.76; pale yellow crystals ($CHCl_3$); m.p. 140 °C. Anal. calc. for $C_{17}H_{23}N_3S$: C 67.77, H 7.64, N 13.95; found: C 67.04, H 7.11, N 14.05; λ_{max} (cm^{-1}): 272, 226, 210; IR: ν_{max} (cm^{-1}) 3303 (NH), 2925 (CH), 1527 (C=N), 1457 (C=C), 1140 (C–N), 1056 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.67 (s, 1H, NH), 7.24–7.35 (m, 5H, Ar–H), 4.40 (t, 1Ha, CH, $J = 11.5$ Hz), 4.19 (dd, 1Hb, CH, $J = 4.2$, 11.5 Hz), 3.71 (m, 1Hc, CH), 1.34 (d, 3H, CH_3, $J = 7.1$ Hz), 1.27–1.85 (m, 10H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.9 (C=S), 159.1 (C=N), 135.9, 129.7, 129.5, 128.9, 128.5, 125.0 (aryl-C), 51.5 (CH_2), 51.0 (CH_2), 50.4 (CH_2), 32.2 (2CH_2), 25.6 (2CH_2), 25.1 (CH_2), 14.6 (CH_3).

*4.2.7. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-cyclooctyl)thiocarboxamide (**7**)*

Yield 77%, R_f 0.72; pale yellow crystals ($CHCl_3$); m.p. 160 °C. Anal. calc. for $C_{19}H_{27}N_3S$: C 69.72, H 7.64, N 12.84; found: C 69.70, H 7.12, N 12.67; λ_{max} (cm^{-1}): 278, 224, 212; IR: ν_{max} (cm^{-1}) 3299 (NH), 2926 (CH), 1526 (C=N), 1454 (C=C), 1129 (C–N), 1023 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.67 (s, 1H, N*H*), 7.26–7.73 (m, 5H, Ar–*H*), 4.40 (t, 1Ha, C*H*, $J = 11.1$ Hz), 4.19 (dd, 1Hb, C*H*, $J = 4.1$, 11.4 Hz), 3.71 (m, 1Hc, C*H*), 1.32 (d, 3H, CH_3, $J = 7.1$ Hz), 1.27–2.00 (m, 14H, CH_2); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.3 (C=S), 157.0 (*C*=N), 135.8, 129.3, 128.7, 128.5, 126.4 (aryl-*C*), 51.5 (*C*H_2), 51.1 (*C*H), 51.0 (*C*H_2), 31.7 (*C*H_2), 26.6 (2*C*H_2), 25.2 (*C*H_2), 21.9 (2*C*H_2), 14.6 (*C*H_3).

*4.2.8. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-phenylpiperizenyl)thiocarboxamide (**8**)*

Yield 13%, R_f 0.78; creamish yellow solid ($CHCl_3$); m.p. 160 °C. Anal. calc. for $C_{21}H_{24}N_4S$: C 69.23, H 6.59, N 15.38; found: C 68.99, H 6.59, N 15.06; λ_{max} (cm^{-1}): 269, 228, 208; IR: ν_{max} (cm^{-1}) 2923 (CH), 1598 (C=N), 1457 (C=C), 1146 (C–N), 1024 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 6.85–7.97 (m, 10H, Ar–*H*), 4.81 (t, 1Ha, C*H*, $J = 11.4$ Hz), 4.17 (dd, 1Hb, C*H*, $J = 4.3$, 11.4 Hz), 3.43 (m, 1Hc, C*H*), 3.12–3.41 (m, 8H, CH_2), 1.34 (d, 3H, CH_3, $J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 186.6 (*C*=S), 157.2 (*C*=N), 151.1, 136.1, 129.7, 129.3, 129.2, 129.0, 128.8, 126.3, 120.3, 117.2 (aryl-*C*), 53.8 (2*C*H_2), 52.1 (*C*H_2), 47.6 (2*C*H_2), 46.7 (*C*H), 14.6 (*C*H_3).

*4.2.9. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-o-toluidine)thiocarboxamide (**9**)*

Yield 62%; R_f 0.62; yellow crystals ($CHCl_3$); m.p. 157 °C. Anal. calc. for $C_{18}H_{19}N_3S$: C 69.90, H 6.14, N 13.59; found: C 69.48, H 6.13, N 13.60 %; UV/vis: λ_{max} (nm) 270, 220, 204; IR: ν_{max} (cm^{-1}) 3339 (NH), 2962 (CH), 1545 (C=N), 1458 (C=C), 1142 (C–N), 1081 (C=S); ^{1}H NMR (DMSO): (δ, ppm) 8.86 (1H, s, –N*H*), 7.17–8.08 (m, 9H, Ar–*H*), 4.52 (t, 1Ha, C*H*, $J = 14.3$ Hz), 4.28 (dd, 1Hb, C*H*, $J = 3.6$, 14.3 Hz), 3.75 (m, 1Hc, C*H*), 1.59 (s, 3H, CH_3), 1.33 (d, 3H, CH_3, $J = 7.8$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.5 (*C*=S), 150.6 (*C*=N), 144.0, 139.1, 136.4, 133.9, 129.4, 129.3, 128.9, 128.7, 126.4, 126.3, 123.2, 116.9, 116.4 (aryl-*C*), 51.8 (*C*H_2), 46.9 (*C*H), 17.9 (*C*H_3), 14.6 (*C*H_3).

*4.2.10. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-2-chlorobenzyl)thiocarboxamide (**10**)*

Yield 18%, R_f 0.65; yellow crystals ($CHCl_3$); m.p. 120 °C. Anal. calc. for $C_{18}H_{18}N_3SCl$: C 62.88, H 5.24, N 12.22; found: C 63.01, H 5.89, N 12.21; λ_{max} (cm^{-1}): 264, 228, 202; IR: ν_{max} (cm^{-1}) 3316 (NH), 2920 (CH), 1560 (C=N), 1457 (C=C), 1154 (C–N), 1037 (C=S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.90 (s, 1H, N*H*), 6.27–8.05 (m, 9H, Ar–*H*), 4.96 (t, 1Ha, C*H*, $J = 11.6$ Hz), 4.76 (dd, 1Hb, C*H*, $J = 4.6$, 11.6 Hz), 3.62 (m, 1Hc, C*H*), 3.46 (d, 2H, CH_2), 1.33 (d, 3H, CH_3, $J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 176.5 (*C*=S), 163.7 (*C*=N), 139.1, 134.4, 133.5, 130.7, 129.5, 129.4, 129.3, 129.2, 129.1, 128.9, 127.2, 126.4 (aryl-*C*), 51.5 (*C*H_2), 51.1 (*C*H), 50.0 (*C*H_2), 14.6 (*C*H_3).

4.3. Pd(II) complexes of cyclised pyrazoline analogues of thiosemicarbazones

General procedure: to a hot solution of the appropriate ligand (2 mmol) in dry methanol (10 mL) was added a solution of [Pd(DMSO)$_2$Cl$_2$] (2 mmol) dissolved in minimum quantity of dry methanol and the reaction mixture was heated under reflux for 1–3 h. After keeping the solution at 0 °C overnight the colored solid separated out. This was filtered off and washed with ice-cold ethanol followed by small quantity of methanol and dried to give amorphous solids.

*4.3.1. 3-Phenyl-4-methyl-2-pyrazoline-1-thiocarboxamide palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-TC}Cl$_2$] (**1a**)*

Yield: 48%; dark brown solid (DMSO). Anal. calc. for $C_{11}H_{13}N_3SCl_2Pd$: C 33.30, H 3.28, N 10.59, Cl 17.91; found: C 33.52, H 3.19, N 10.50, Cl 17.80; UV/vis: λ_{max} (nm) 371, 295, 242, 216; IR: ν_{max} (cm^{-1}) 3255 (NH), 2925 (CH), 1547 (C=N), 1134 (C–N), 1001 (C=S), 487, 446 (Pd–N, Pd–S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.95 (s, 2H, NH_2), 7.14–7.79 (m, 5H, Ar–*H*), 4.41 (t, 1H, C*H*, $J = 11.5$ Hz), 4.20 (dd, 1H, C*H*, $J = 4.5$, 11.5 Hz), 3.81 (m, 1H, C*H*), 1.36 (d, 3H, CH_3, $J = 6.3$ Hz); FAB MS: *m/z* 398 [M + 1], 362.5, 327, 220, 205, 187, 172, 145, 103.

*4.3.2. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-isopropyl)thiocarboxamide palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-isoPrTC}Cl$_2$] (**2a**)*

Yield 94%; orangish brown solid (DMSO). Anal. calc. for $C_{14}H_{19}N_3SCl_2Pd$: C 38.32, H 4.33, N 9.58, Cl 16.19; found: C 38.55, H 4.35, N 9.04, Cl 15.20; UV/vis: λ_{max} (nm) 371, 289, 244, 221; IR: ν_{max} (cm^{-1}) 3245 (NH), 2977 (CH), 1580 (C=N), 1134 (C–N), 1012 (C=S), 487, 445 (Pd–N, Pd–S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.97 (s, 1H, N*H*), 7.47–7.90 (m, 5H, Ar–*H*), 4.53 (m, 1H, C*H*), 4.34 (t, 1H, C*H*, $J = 11.2$ Hz), 4.18 (dd, 1H, C*H*, $J = 5.1$, 10.5 Hz), 3.71 (m, 1H, C*H*), 1.28 (d, 6H, CH_3, $J = 6.7$ Hz), 1.32 (d, 3H, CH_3, $J = 6.39$ Hz); FAB MS: *m/z* 440 [M + 1], 404.5, 369, 262, 247, 229, 214, 188, 145, 103.

*4.3.3. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-butyl)thiocarboxamide palladium(II) chloride[Pd{3-Ph-4-Me-2-Pz-1-N-BuTC}Cl$_2$] (**3a**)*

Yield 83%; mud brown solid (DMSO). Anal. calc. for $C_{15}H_{21}N_3SCl_2Pd$: C 39.80, H 4.45, N 9.28, Cl 15.69; found: C 39.79, H 4.64, N 9.28, Cl 15.70; UV/vis: λ_{max} (nm) 373, 289, 241, 221; IR: ν_{max} (cm^{-1}) 3202 (NH), 2967 (CH), 1584 (C=N), 1142 (C–N), 1021 (C=S), 488, 446 (Pd–N, Pd–S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.16 (s, 1H, N*H*), 7.47–7.96 (m, 5H, Ar–*H*), 4.51 (t, 1H, C*H*, $J = 11.5$ Hz), 4.19 (dd, 1H, C*H*, $J = 4.3$, 11.5 Hz), 3.81 (m, 1H, C*H*), 3.66 (m, 2H, CH_2),1.68 (m, 2H, CH_2), 1.38 (m, 2H, CH_2), 1.03 (t, 3H, CH_3, $J = 7.3$ Hz), 1.34 (d, 3H, CH_3, $J = 6.39$ Hz); FAB

MS: *m/z* 454 [M + 1], 418.5, 383, 276, 261, 243, 228, 202, 145, 103.

*4.3.4. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-isobutyl)thiocarboxamide palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-isoBuTC}Cl_2] (**4a**)*

Yield 36%; brown solid (DMSO). Anal. calc. for $C_{15}H_{21}N_3SCl_2Pd$: C 39.79, H 4.64, N 9.28, Cl 15.69; found: C 39.25, H 4.46, N 9.24, Cl 15.29; UV/vis: λ_{max} (nm) 371, 291, 244, 220; IR: ν_{max} (cm^{-1}) 3211 (NH), 2962 (CH), 1582 (C═N), 1156 (C−N), 1045 (C═S), 487, 446 (Pd−N, Pd−S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 9.18 (s, 1H, N*H*), 7.41−8.32 (m, 5H, Ar−*H*), 4.40 (t, 1H, C*H*, $J = 11.3$ Hz), 4.29 (dd, 1H, C*H*, $J = 4.2$, 11.1 Hz), 3.67 (m, 1H, C*H*), 3.54 (t, 2H, CH_2, $J = 6.3$ Hz), 1.34 (d, 6H, CH_3, $J = 6.5$ Hz), 1.32 (d, 3H, CH_3, $J = 7.2$ Hz); FAB MS; *m/z* 454 [M + 1], 418.5, 383, 276, 261, 243, 228, 202, 145, 103.

*4.3.5. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-cyclopentyl)thiocarboxamide palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-CPTC}Cl_2] (**5a**)*

Yield 27%; brownish yellow solid (DMSO). Anal. calc. for $C_{16}H_{21}N_3SCl_2Pd$: C 34.12, H 4.52, N 9.04, Cl 15.29; found: C 41.07, H 4.69, N 9.00, Cl 15.23; UV/vis: λ_{max} (nm): 371, 290, 242, 212; IR: ν_{max} (cm^{-1}) 3231 (NH), 2936 (CH), 1569 (C═N), 1184 (C−N), 1047 (C═S), 487, 443 (Pd−N, Pd−S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.86 (s, 1H, N*H*), 7.47−7.99 (m, 5H, Ar−*H*), 4.57 (t,1H, C*H*, $J = 11.1$ Hz), 4.17 (dd, 1H, C*H*, $J = 4.3$, 11.1 Hz), 3.71 (m, 1H, C*H*), 1.36 (d, 3H, CH_3, $J = 7.3$ Hz), 1.31−1.77 (m, 8H, CH_2); FAB MS: *m/z* 466 [M + 1], 430.5, 395, 288, 273, 255, 240, 214, 145, 103.

*4.3.6. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-cyclohexyl)thiocarboxamide palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-CHTC}Cl_2] (**6a**)*

Yield 50%; orangish yellow crystals (DMSO). Anal. calc. for $C_{17}H_{23}N_3SCl_2Pd$: C 43.00, H 4.85, N 8.85, Cl 14.96; found: C 42.87, H 4.52, N 8.27, Cl 14.23; UV/vis: λ_{max} (nm) 373, 293, 239, 217; IR: ν_{max} (cm^{-1}) 3230 (NH), 2936 (CH), 1569 (C═N), 1157 (C−N), 1050 (C═S), 483, 444 (Pd−N, Pd−S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.86 (s, 1H, N*H*), 7.47−7.99 (m, 5H, Ar−*H*), 4.31 (t, 1H, C*H*, $J = 11.5$ Hz), 4.17 (dd, 1H, C*H*, $J = 4.1$, 11.4 Hz), 3.68 (m, 1H, C*H*), 1.35 (d, 3H, CH_3, $J = 7.3$ Hz), 1.27−1.87 (m, 10H, CH_2); FAB MS: *m/z* 479 [M + 1], 443.5, 409, 302, 287, 269, 254, 228, 145, 103.

*4.3.7. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-cyclooctyl)thiocarboxamide palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-COTC}Cl_2] (**7a**)*

Yield 88%; orangish yellow crystals (DMSO). Anal. calc. for $C_{19}H_{27}N_3SCl_2Pd$: C 45.03, H 5.33, N 8.29, Cl 14.02; found: C 44.87, H 5.07, N 8.27, Cl 14.01; UV/vis: λ_{max} (nm): 371, 292, 245, 217; IR: ν_{max} (cm^{-1}) 3300 (NH), 2923 (CH), 1565 (C═N), 1134 (C−N), 1016 (C═S), 482, 445 (Pd−N, Pd−S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.91 (s, 1H, N*H*), 7.47−7.99 (m, 5H, Ar−*H*), 4.38 (t, 1H, C*H*, $J = 11.4$ Hz), 4.17 (dd, 1H, C*H*, $J = 4.3$, 11.4 Hz), 3.71 (m, 1H, C*H*), 1.32 (d, 3H, CH_3, $J = 7.1$ Hz), 1.27−2.00 (m, 14H, CH_2); FAB MS: *m/z* 508 [M + 1], 472.5, 437, 330, 315, 297, 282, 256, 145, 103.

*4.3.8. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-phenylpiperizenyl)thiocarboxamide palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-PPTC}Cl_2] (**8a**)*

Yield 24%; brown solid (DMSO). Anal. calc. for $C_{21}H_{24}N_4SCl_2Pd$: C 46.55, H 4.43, N 10.34, Cl 13.11; found: C 46.27, H 4.52, N 10.27, Cl 13.23; UV/vis: λ_{max} (nm) 371, 290, 237, 213; IR: ν_{max} (cm^{-1}) 2920 (CH), 1599 (C═N), 1157 (C−N), 1017 (C═S), 481, 446 (Pd−N, Pd−S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 6.84−7.97 (m, 10H, Ar−*H*), 4.79 (t, 1H, C*H*, $J = 11.3$ Hz), 4.17 (dd, 1H, C*H*, $J = 4.3$, 11.4 Hz), 3.42 (m, 1H, C*H*), 3.12−3.42 (m, 8H, CH_2), 1.34 (d, 3H, CH_3, $J = 6.7$ Hz); FAB MS: *m/z* 543 [M + 1], 507.5, 472, 365, 350, 332, 317, 291, 145, 103.

*4.3.9. [3-Phenyl-4-methyl-2-pyrazoline-1-(N-o-toluidine)thiocarboxamide] palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-o-tolTC}Cl_2] (**9a**)*

Yield 64%; black solid (DMSO). Anal. calc. for $C_{18}H_{18}N_3SCl_2Pd$: C 44.50, H 3.70, N 8.65, Cl 21.94; found: C 44.37, H 3.70, N 8.66, Cl 21.90; UV/vis: λ_{max} (nm) 371, 292, 242, 221; IR: ν_{max} (cm^{-1}) 3257 (NH), 2918 (CH), 1587 (C═N), 1167 (C−N), 1025 (C═S), 478, 443 (Pd−N, Pd−S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 8.98 (s, 1H, N*H*), 7.21−7.78 (m, 9H, Ar−*H*), 4.78 (t, 1H, C*H*, $J = 11.4$ Hz), 4.18 (dd, 1H, C*H*, $J = 4.4$, 11.4 Hz), 3.62 (m, 1H, C*H*), 3.46 (d, 2H, CH_2), 1.33 (d, 3H, CH_3, $J = 7.1$ Hz); FAB MS: *m/z* 489 [M + 1], 453.5, 418, 310, 295, 277, 262, 236, 145, 103.

*4.3.10. 3-Phenyl-4-methyl-2-pyrazoline-1-(N-2-chlorobenzyl)thiocarboxamide palladium(II) chloride [Pd{3-Ph-4-Me-2-Pz-1-N-2-ClBzTC}Cl_2] (**10a**)*

Yield 70%; mud brown solid (DMSO). Anal. calc. for $C_{18}H_{19}N_3SCl_3Pd$: C 41.40, H 3.45, N 8.06, Cl 20.41; found: C 41.27, H 3.52, N 8.09, Cl 20.43; UV/vis: λ_{max} (nm) 371, 291, 242, 216; IR: ν_{max} (cm^{-1}) 3209 (NH), 2961 (CH), 1567 (C═N), 1157 (C−N), 1023 (C═S), 489, 447 (Pd−N, Pd−S); ^{1}H NMR (DMSO): (δ, ppm) 8.97 (1H, s, −N*H*), 7.33−8.09 (m, 9H, Ar−*H*), 4.49 (t, 1H, C*H*, $J = 14.1$ Hz), 4.27 (dd, 1H, C*H*, $J = 3.5$, 14.1 Hz), 3.79 (m, 1H, C*H*), 1.58 (s, 3H, CH_3), 1.35 (d, 3H, CH_3, $J = 7.8$ Hz); FAB MS: *m/z* 533.5 [M + 1], 488, 452.5, 345.5, 330.5, 312.5, 297.5, 271.5, 145, 103.

4.4. In vitro testing against E. histolytica

All the 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones **1−10** and their palladium complexes **1a−10a** were screened *in vitro* for antiamoebic activity against *HM1:IMSS* strain of *E. histolytica* by using a microplate method [40]. DMSO (40 μL) was added to all the samples (∼1 mg) followed by enough culture medium to obtain

concentration of 1 mg/mL [41,42]. Samples were dissolved or suspended by mild sonication in a sonicleaner bath for a few minutes and then further diluted with medium to concentrations of 0.1 mg/mL. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (Costar) in 170 μL of medium. Each test included metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) was prepared from a confluent culture by pouring off the medium, adding 2 mL of medium and chilling the culture on ice to detach the organisms from the side of the flask. The number of amoeba per mL was estimated with a heamocytometer and trypan blue exclusion was used to confirm viability. The cell suspension was diluted to 10^5 organism/mL by adding fresh medium and 170 μL of this suspension was added to the test and control wells in the plate so that the wells were completely filled (total volume, 340 μL). An inoculum of 1.7×10^4 organisms/well was chosen so that confluent, but not excessive growth took place in control wells. Plates were sealed with expanded polystyrene (0.5 thick). Secured with tape, placed in a modular incubating chamber (Flow Laboratories, High Wycombe, UK), and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h.

After incubation, the growth of amoebae in the plate was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed once in sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly, and the plate was not allowed to cool in order to prevent the detachment of amoebae. The plate was allowed to dry at room temperature, and the amoebae were fixed with methanol, when dry, stained with (0.5%) aqueous eosin for 15 min. Stained plate was washed once with 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 (Labsystem Multiskane Bichromatic, UK). 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.

4.5. MTT toxicity assay

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 the compounds [43]. The trypsinized cell suspension was adjusted to 0.5 million cells/mL and plated out with the various compounds. After 44 h of incubation, 2 mM MTT was added to the plates and incubated for 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 was taken into account. All assays were performed in triplicate.

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