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Short communication

Synthesis, characterization and antiamoebic activity of new indole-3-carboxaldehyde thiosemicarbazones and their Pd(II) complexes

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Abstract

In continuation of our research on thiosemicarbazones and their metal complexes as antiamoebic agents, a new series of indole-3-carboxaldehyde thiosemicarbazones (TSC) **1–7** were prepared by condensing indole-3-carboxaldehyde with cycloalkylaminothiocarbonyl hydrazines. Their palladium(II) complexes of the $[Pd(TSC)Cl_2]$ type, were synthesized upon coordination with $[Pd(DMSO)_2Cl_2]$. The chemical structures of all the compounds were established by elemental analyses, electronic, IR, ^1H NMR and ^{13}C NMR spectral data. The structure of the complexes was further established by thermogravimetric analysis and FAB MS. Spectroscopic data revealed that thiosemicarbazones act as bidentate ligands, making use of thione sulphur and azomethine nitrogen atom for coordination to the Pd(II) ion. Among all the compounds evaluated for antiamoebic activity using *HM1:IMSS* strain of *Entamoeba histolytica*, all palladium complexes were found to be more active than their respective ligands. Moreover, ligand **5** and complexes **1a–3a, 5a** and **7a** showed antiamoebic activity, at lower IC_{50} doses when compared to the reference drug metronidazole with $IC_{50} = 1.81$ μM.

Keywords: Palladium(II) complexes; Indole-3-carboxaldehyde; Thiosemicarbazones; *Entamoeba histolytica*

1. Introduction

Amitochondrial protist, *E. histolytica* parasitize the human intestine and causes amoebiasis, which is a significant source of morbidity and mortality in developing countries. Amoebic abscesses of the brain are dreadful complications of *E. histolytica* infection [1]. WHO in its recent estimates has placed the death toll from amoebiasis at 40 000–100 000 lives annually [2]. Nitroimidazole derivatives such as metronidazole are used in the treatment of anaerobic protozoan and bacterial infection [3–8]. However, the drug is known to have common side effects such as nausea, vomiting, headache, metallic taste, glossitis and in few cases convulsions [9,10]; therefore search for new and efficient leads as amoebicidal drugs is required in amoebiasis therapy.

Thiosemicarbazones have aroused considerable interest in chemistry and biology due to their antibacterial, antimalarial, antineoplastic and antiviral activities [11–14]. The biological activities of thiosemicarbazones are considered to be related to their ability to form chelates with metals. Biological activities of metal complexes differ from those of either free ligands or metal ions, and increased or decreased activities in relation to the uncomplexed thiosemicarbazones have been reported for several transition metal complexes [15,16]. Some promising results have been obtained with palladium complexes, such as *trans*-palladium complexed to a bulky amine ligand, which showed higher activity against the L 929 cell line [17].

Abbreviations: 3-ICA-CPTSC, indole-3-carboxaldehyde-*N*(4)cyclopentyl thiosemicarbazone (**1**); 3-ICA-COTSC, indole-3-carboxaldehyde-*N*(4)cyclooctyl thiosemicarbazone (**2**); 3-ICA-4MPTSC, indole-3-carboxaldehyde-*N*(4)4-methylpiperidine thiosemicarbazone (**3**); 3-ICA-PYRTSC, indole-3-carboxaldehyde-*N*(4)-pyrrolidine thiosemicarbazone (**4**); 3-ICA-ADMTSC, indole-3-carboxaldehyde-*N*(4)adamentamine thiosemicarbazone (**5**); 3-ICA-NPPTSC, indole-3-carboxaldehyde-*N*(4)phenylpiperizine thiosemicarbazone (**6**); 3-ICA-THQTSC, indole-3-carboxaldehyde-*N*(4)1,2,3,4-tetrahydroquinoline thiosemicarbazone (**7**).

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Moreover palladium(II) complexes with nitrogen containing ligands are the subject of intensive biological evaluation in the search for less toxic and more selective anticancer therapies [18,19].

In addition, indole molecules possessing functionality in the 3-position are attractive targets for the pharmaceutical industry [20,21]. Continuing with our research program on thiosemicarbazones and their complexes [22–24], we report here the synthesis and spectroscopic characterization of indole-3-aldehyde thiosemicarbazones and their Pd(II) complexes in order to study their coordination behavior as well as their *in vitro* against the *HM1:IMSS* strain of *E. histolytica*.

2. Chemistry

All the thioglycolic acids were prepared by the method reported by O'Sullivan et al. [25]. Cycloalkylaminothiocarbonylhydrazines were prepared by refluxing the alkaline solution of thioglycolic acid with hydrazine hydrate and their thiosemicarbazones were synthesized by refluxing aqueous solutions of cycloalkylaminothiocarbonylhydrazines and ethanolic solution of indole-3-carboxaldehyde in equimolar ratio. The precursor $[Pd(DMSO)_2Cl_2]$ used for the synthesis of Pd(II) complexes was synthesized by the literature procedure [26,27]. The complexes were prepared by mixing the appropriate ligand with $[Pd(DMSO)_2Cl_2]$ in 1:1 ligand to metal molar ratio in refluxing methanol as shown in Eqs. (1) and (2).

$$PdCl_2 + 2DMSO \rightarrow Pd(DMSO)_2Cl_2 \quad (1)$$

$$Pd(DMSO)_2Cl_2 + TSC \xrightarrow[\text{Reflux}]{CH_3OH} Pd(TSC)Cl_2 + 2DMSO \quad (2)$$

where TSC = thiosemicarbazone.

The reactions were monitored by TLC (aluminium sheets, silica gel 60 F_{254}, Merck) using methylene chloride–methanol (4.8:0.2) as eluent. The products thus obtained were separated from the solution by filtering at room temperature and drying *in vacuo* over silica gel. The complexes gave high yields around 80%, while the yields for thiosemicarbazones were in the range of 20%–60%. The compounds were obtained as crystalline solids, in case of thiosemicarbazones but as amorphous solids for complexes. The compounds are stable in the solid state as well as in solution. All complexes are soluble in DMF and DMSO, sparingly soluble in methanol, ethanol and insoluble in water. The chemical structures of all compounds were confirmed by means of elemental analysis and electronic, IR, ^{1}H NMR spectral studies. The structures of the ligands were further established by ^{13}C NMR and that of complexes by thermo gravimetric analysis and FAB MS.

3. Pharmacology

Indole-3-carboxaldehyde thiosemicarbazones and their Pd(II) complexes were screened *in vitro* for antiamoebic activity against *HM1:IMSS* strain of *E. histolytica* by microdilution method [28]. Trophozoites of *E. histolytica* isolate *HM1:IMSS* were cultured axenically in TYIS-33 medium supplemented with 10% heat-inactivated bovine sera and 3% complete vitamin mixture at 35 °C [29]. Each tested compound was serially diluted and added to the growing trophozoites in 96-well microtiter plate. Effect on growth of trophozoites was monitored microscopically at regular interval and quantitative evaluation of the drug action was made by protein estimation. The standard drug used was metronidazole, which presented *in vitro* activity with $IC_{50} = 1.81$ μM concentrations. The % inhibition of amoeba growth was calculated from the optical densities of the control and tested wells and was plotted against the logarithm of concentration of the tested drug. Linear regression analysis was used to determine the best fitting straight line from which IC_{50} value was found. The results are reported in Table 1.

4. Results and discussion

Indole-3-carboxaldehyde thiosemicarbazones (**1**–**7**) were readily prepared and compounds were isolated in satisfactory yield. The structure of the ligands was established using various spectroscopic studies and shown in Fig. 1. Treatment of these ligands with $[Pd(DMSO)_2Cl_2]$ gave amorphous solid complexes. The analytical data of these compounds are in good agreement with their composition. The complexes do not undergo any weight loss up to 240 °C, which suggests their fair thermal stability. The structure of all complexes presented in Fig. 2 was established by comparing spectral data (IR, UV–visible and ^{1}H NMR) with their respective ligands and was further supported by their FAB MS and thermogravimetric analysis.

4.1. Electronic spectral studies

The electronic spectra of all thiosemicarbazones show similar pattern, exhibiting three bands in 343–332, 269–258, and 208–204 nm regions. The $n \rightarrow \pi^*$ transitions of thioamide and the azomethine functions [30] are overlapped at 451–332 nm in the spectra of thiosemicarbazones. The probable

Table 1
In vitro antiamoebic activities of indole-3-carboxaldehyde thiosemicarbazones and their new Pd(II) complexes against *HM1:IMSS* strain of *E. histolytica*

Compound	IC_{50} (μM)	S.D.[a]
1	13.9	0.12
1a	0.98	0.02
2	6.08	0.10
2a	0.62	0.02
3	8.37	0.08
3a	1.18	0.08
4	14.64	0.02
4a	2.32	0.02
5	1.08	0.03
5a	0.47	0.06
6	3.09	0.09
6a	1.85	0.03
7	1.68	0.01
7a	0.78	0.08
$Pd(DMSO)_2Cl_2$	8.00	0.34
Metronidazole (MNZ)	1.81	0.03

[a] Standard deviation.

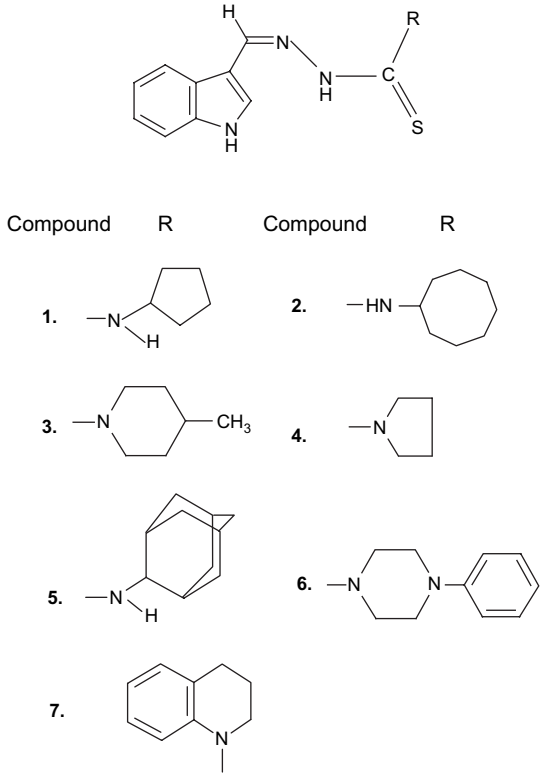


Fig. 1. Structure of thiosemicarbazones of indole-3-carboxaldehyde.

assignment for these bands are due to the n → π* (thiosemicarbazones), n → π* (thiophene) and π → π* (thiophene) transitions. A careful comparison of electronic spectra shows that the absorptions shift to lower energies (384, 277 and 212 nm) in the complexes due to extended conjugation. A very intense band at ca. 450 nm in the electronic spectra of the complexes 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 [31].

4.2. IR spectral analysis

The thiosemicarbazones **1–7** exhibit thione and thiol tautomerism. The thiosemicarbazones **1–7** show intense strong bands in the region 790–820 cm^{-1} due to ν(C=S) but no band appeared due to ν(C–SH) in the region 2500–2600 cm^{-1} suggesting that in the solid state thiosemicarbazones remain as the thione tautomer. The band appearing at 790–820 cm^{-1} ascribed to ν(C=S) [32] of ligands is shifted to lower wave number by ca. 15–30 cm^{-1}, indicating that the thione sulphur participates as a coordinating site. This coordination is confirmed by the presence of two new bands at around 550 and 440 cm^{-1} ν(Pd–N, Pd–S) [33]. The preferential coordination of thionic sulphur over nitrogen of indole is due to more nucleophilic character of the former. The band due to ν(C–N–C) (ring) of indole moiety remains unaltered in **1a–7a**, indicating non-participation of ring nitrogen in coordination. The negative shift of 13–33 cm^{-1} of ν(C=N) stretch in the complexes indicates the involvement of azomethine nitrogen in complexation [34]. This was supported by the shift of N–N band of ligand on coordination to higher frequency (Table 2). The occurrence of the ν(N–N) band at higher frequency in the IR spectra of the complexes compared to that in the ligand is confirmation of the coordination through the azomethine nitrogen atom. The broad band observed in the region 3300 cm^{-1} due to ν(N–H) stretch is slightly shifted in the complex due to probably adjustment of current arising due to coordination of thionic sulphur. Thus, confirming the fact that ligands behave as neutral NS donor bidentate in these complexes.

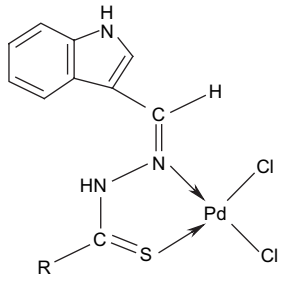


Fig. 2. Structure of palladium(II) complexes (**1a**, R = $-NHC_5H_9$; **2a**, R = $-NHC_8H_{15}$; **3a**, R = $-NC_4H_{12}$; **4a**, R = $-NC_4H_8$; **5a**, R = $-NHC_{10}H_{14}$; **6a**, R = $-NC_{10}H_{13}N$; **7a**, R = $-NC_9H_{10}$).

4.3. Nuclear magnetic resonance spectral studies

The ^1H NMR spectra recorded using DMSO-d_6 as the solvent further supports the coordinating mode of thiosemicarbazones **1–7**. Ligands **1–7** do not show any resonance at ca. 4.0 ppm, attributed to the –SH proton resonance, while the appearance of a broad peak at 11.11–11.86 ppm due to the –NH proton of the thioamide group indicates that even in a polar solvent such as DMSO they remain in the thione form. The –NH proton signal of the thiosemicarbazones usually shifts 2.29–3.22 ppm upfield in their respective complexes. However, in the complexes, we are unable to calculate the coupling constant for aromatic region due to the merging of peaks upon coordination. This information suggests the adjustment of electronic current upon coordination of >C=S group to the metal ion. The protons belonging to the aromatic ring and the other cyclic groups were observed with the expected chemical shift and integral values in the same region as that of free ligands.

Table 2
IR frequencies for thiosemicarbazone derivatives **1**, **2**, **3** and **4** and their palladium(II) complexes

	ν (cm^{-1})						
	NH	–N=C–	–C=S	–C–N	–N–N=	M–N	M–S
1	3149	1610	748	1239	1060	–	–
2	3151	1612	756	1215	1040	–	–
3	3106	1621	760	1244	1048	–	–
4	3167	1604	762	1236	1052	–	–
1a	3144	1594	722	1231	1102	587	456
2a	3145	1580	735	1232	1110	504	457
3a	3105	1527	731	1242	1082	502	453
4a	3166	1598	732	1230	1103	514	478

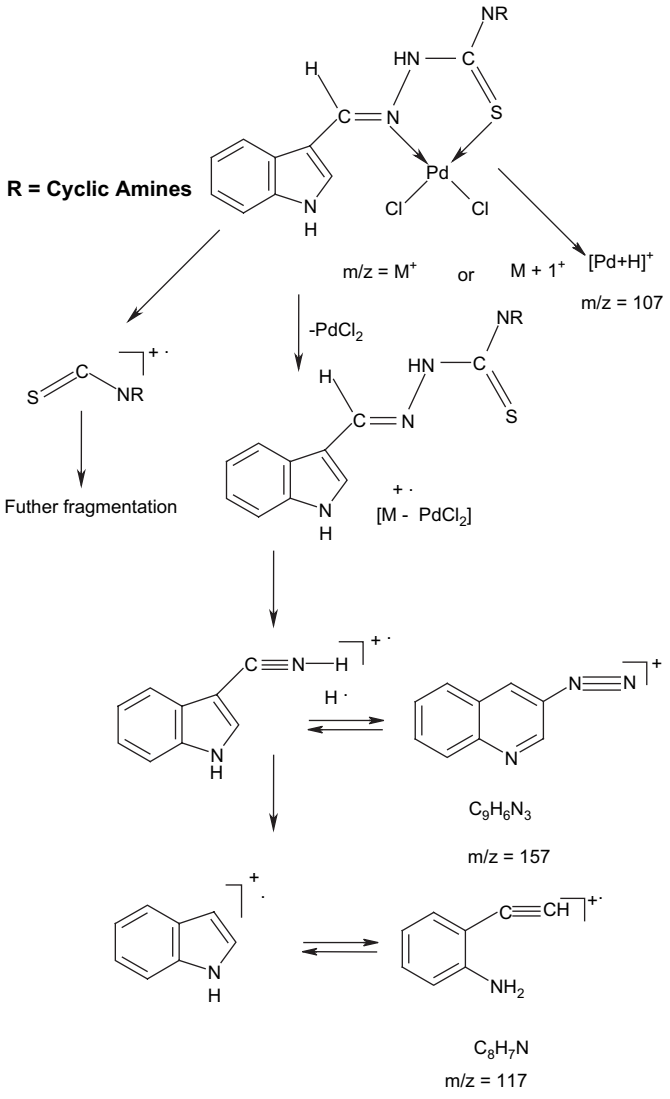
The ^{13}C NMR spectra of all ligands were recorded in DMSO and the spectral signals are in good agreement with the probable structures. The ligands showed two signals at 136.71–182.54 and 127.68–136.70 ppm assigned to thioamide (C=S) and azomethine carbon (C=N), respectively. The signals from 111.6 to 132.71 ppm were due to the indole ring carbons. The signal at around 140 ppm is attributed to the C–H part of azomethine group. The carbons at 1-*N*-substituted cyclic groups in ligands resonate at their usual positions and are shown in the data given in the experimental section. ^{13}C NMR spectra also provide diagnostic tools for the elucidation of the coordinating mode of the ligands in complexes. Assignments of the signals are based on the chemical shifts and intensity patterns and coordination induced shift (CIS), $\Delta\delta$ [$\Delta\delta = \delta$ (complex) $- \delta$ (free ligand)], of the signals for carbon atom in the vicinity of the coordinating functions. Thus the C=S carbon in ligands experiences CIS value of 5–7 ppm in complexes **1a**–**7a**, indicating the coordination of thione sulphur. As a result of variation of electron density on coordination, azomethine carbon signal is shifted downfield by 2–4 ppm in their respective complexes, which indicates coordination of nitrogen lone pair to metal. Other carbons (CH_3, CH_2 and aryl carbons) in these complexes resonate nearly at the same region as that of free ligands.

4.4. TGA analysis

The thermogravimetric analysis profiles of complexes **la**–**7a** along with the % weight at different temperatures are recorded. These complexes do not lose weight up to 240 °C. Further increment of temperature causes decomposition of the complexes in two steps, the temperature range for the first step being 302–371 °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 MS {where, M = Pd(II)} as the end product. Although decomposed fragments of the ligands could not be approximated due to continuous weight loss, the total % 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 corresponds to the palladium sulphide.

4.5. FAB MS analysis

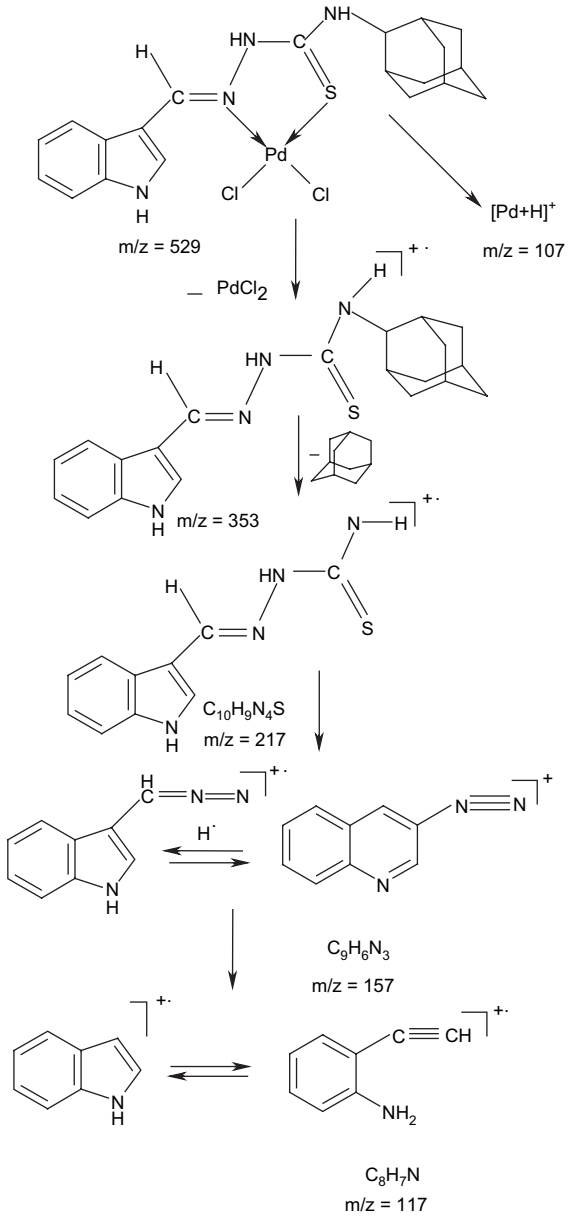
The FAB mass spectra of complexes **1a**–**7a** were recorded using *m*-nitrobenzyl alcohol (NBA) as the matrix. The spectra of pd(II) complexes showed a number of informative fragment ions of different intensity confirming their molecular weights. A general splitting pathway followed by the complexes is shown in Scheme 1. The result presented here is interpreted in terms of simple bond cleavages and ligand losses. The molecular ion peaks were observed as $[M]^{\bullet+}$ or $[M + 1]^{\bullet+}$ and the major fragmentation pathway involved the cleavage of thioamide group (CS–NH) giving fragment at *m/z* 157 which is the highest mass ion. A peak at *m/z* 117 corresponds to the indole ring fragment. The FAB MS fragmentation pattern of compound **5a** is depicted in Scheme 2.



Scheme 1. Proposed mass fragmentation pattern for complexes.

4.6. In vitro antiamoebic activity

All thiosemicarbazones and their Pd(II) complexes were screened for *in vitro* antiamoebic activity by using *HM1:1MSS* strain of *E. histolytica* and the results were compared with the standard amoebicidal drug, metronidazole ($IC_{50} = 1.81$ μM). The free ligands **1**–**7** exhibited antiamoebic activity with $IC_{50} = 1.08$–13.90 μM. Considering the substitution at N^4 position of thiosemicarbazones, the better antiamoebic activity was observed in those compounds, which have adamentamine (**5**, $IC_{50} = 1.08$), and 1,2,3,4-tetrahydroquinoline (**7**, $IC_{50} = 1.68$ μM). The complexation of thiosemicarbazones **1**–**7** with palladium(II) results in complexes **1a**–**7a**, which showed $IC_{50} = 0.47$–2.32 μM. All metal complexes were found more active than their respective ligands indicating that complexation enhances the activity of the ligand. This may be explained by



Scheme 2. Proposed mass fragmentation pattern for compound **5a**.

Tweedy's theory [35], 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. The literature also reports that interaction of Pd complexes with DNA results in enhancement of activity [36]. Moreover, complexes **1a**–**3a**, **5a** and **7a** displayed the most promising *in vitro* amoebicidal activity and were found to be better inhibitor of the parasite growth than metronidazole. The $[Pd(DMSO)_2Cl_2]$ precursor was also evaluated and showed low activity against *E. histolytica*. It was concluded that the presence of bulky groups at position N^4 of the thiosemicarbazone moiety greatly enhanced antiamoebic activity. The IC_{50} values indicate that all Pd(II) complexes cause a marked inhibition, while the parent ligands are less active than the complexes. The activity of the precursor and derived Pd containing complexes indicate that complexation to Pd increases the antiamoebic activity of the parent ligand. The results were statistically evaluated by analysis of variance. The null hypothesis was tested using *T*-test. The significance of the differences between the IC_{50} values of metronidazole and compounds **5**, **7**, **1a**–**3a**, **5a** and **7a** 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 of these complexes, mechanism of action as well as *in vivo* studies are in progress.

5. Conclusion

The antiamoebic activities of new thiosemicarbazones having different cyclic amines as N^4 substitution and their novel palladium(II) complexes were examined. All the thiosemicarbazones act as NS bidentate chelators and 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 *HM1:IMSS* strain of *E. histolytica*. The complexes of indole-3-carboxaldehyde thiosemicarbazones were more active than their respective ligands. Among all the thiosemicarbazones, **5** and **7** showed noteworthy antiamoebic activity, while complexes **1a**–**3a**, **5a** and **7a** have shown significantly less IC_{50} value than metronidazole.

6. Experimental protocols

6.1. Chemistry

Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} precoated thin-layer plates. All the chemicals were purchased from Aldrich chemical company (USA). All the cycloalkylaminothiocarbonylhydrazines were prepared as reported earlier [21]. 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 decomposing the complexes with Na_2O_2/NaOH and precipitating as $AgCl_2$ with $AgNO_3$ after dissolving in dilute HNO_3. Melting points were recorded on a KSW melting point apparatus and were uncorrected. Electronic spectra were recorded in methanol on a Shimadzu UV-1601 PC UV–visible spectrophotometer. IR spectra on KBr disks were recorded on a Perkin Elmer model 1620 FT-IR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were obtained at ambient temperature using a Bruker Spectrospin DPX-300 MHz spectrophotometer in DMSO and $CDCl_3$ using tetramethylsilane as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values are given in (ppm) and coupling constants (J) in hertz. The 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*-nitrobenzyl alcohol (NBA) was used as the matrix. Thermogravimetric analysis of the complexes were performed on a TG 51 thermogravimetric

analyzer under nitrogen atmosphere with the heating rate of 10 °C/min.

6.2. Synthesis of indole-3-carboxaldehyde thiosemicarbazones: a general method

All thiosemicarbazones were synthesized by refluxing an ethanolic solution of cycloalkylaminothiocarbonylhydrazines (3 mmol) and indole-3-carboxaldehyde (3 mmol) at 80 °C for 3 h with continuous stirring. After cooling at ca. 10 °C for 24 h, the precipitated compound was filtered and recrystallized from appropriate solvent.

6.2.1. Indole-3-carboxaldehyde-N(4)cyclopentyl thiosemicarbazone (**1**)

Brownish grey solid (methanol:chloroform). Yield: 26%; m.p.: 218 °C. Anal. calc. for ($C_{15}H_{18}N_4S$): C, 62.93; H, 6.29; N, 19.58; found: C, 62.80; H, 5.37; N, 19.90%; UV: λ_{max} (nm) 332, 261.9, 205; IR: ν_{max} (cm^{-1}) 3295, 3149 (N*H*), 1610 (C═N), 1552 (C═C), 1239 (C–N), 752 (C═S), 1060 (N–N); ^{1}H NMR (DMSO): (δ, ppm) 12.16 (1H, s, –N*H*), 11.64 (1H, s, –N*H*), 11.27 (1H, s, –N*H*), 8.01 (1H, s, –C*H*═N), 8.15 (1H, d, indole C_4–*H*, $J = 8.6$ Hz), 7.84 (1H, s, indole C_2–*H*), 7.46 (1H, d, indole C_7–*H*, $J = 8.6$ Hz), 7.11–7.24 (2H, m, indole C_5–*H* and C_6–*H*), 4.65 (1H, m, –C*H*), 1.60–2.08 (8H, m, –CH_2). ^{13}C NMR ($CDCl_3$): (δ, ppm) 136.71 (C═S), 127.68 (C═N), 140.4 (CH) 132.71, 122.6, 121.8, 120.28,116.51, 111.63 (Aryl-C), 51.37 (CH), 32.28 ($2CH_2$), 22.97 ($2CH_2$).

6.2.2. Indole-3-carboxaldehyde-N(4)cyclooctyl thiosemicarbazone (**2**)

Yellow solid (methanol:chloroform). Yield: 57%; m.p.: 198 °C. Anal. calc. for ($C_{18}H_{24}N_4S$): C, 65.80; H, 7.32; N, 17.07; found: C, 65.15; H, 7.36; N, 17.05%; UV: λ_{max} (nm) 332.1, 261.9, 204.9; IR: ν_{max} (cm^{-1}) 3288, 3151 (N*H*), 1612 (C═N), 1547 (C═C), 1215 (C–N), 756 (C═S), 1040 (N–N); ^{1}H NMR (DMSO): (δ, ppm) 12.19 (1H, s, –N*H*), 11.67 (1H, s, –N*H*), 11.27 (1H, s, –N*H*), 8.29 (1H, s, –C*H*═N), 8.02 (1H, d, indole C_4–*H*, $J = 7.5$ Hz), 7.84 (1H, s, indole C_2–*H*), 7.47 (1H, d, indole C_7–*H*, $J = 7.5$ Hz), 7.09–7.23 (2H, m, indole C_5–*H* and C_6–*H*), 4.44 (1H, m, –C*H*), 1.57–1.91 (14H, m, –CH_2).). ^{13}C NMR ($CDCl_3$): (δ, ppm) 136.70 (C═S), 127.68 (C═N), 140.41 (CH) 132.71, 127.68, 122.6, 121.7, 120.28, 116.51, 116.3 (Aryl-C), 49.74 (CH), 31.78 ($2CH_2$), 26.67 (CH_2), 25.2 (CH_2), 21.94 ($2CH_2$).

6.2.3. Indole-3-carboxaldehyde-N(4)4-methylpiperidine thiosemicarbazone (**3**)

Yellow solid (methanol:chloroform). Yield: 38%; m.p.: 220 °C. Anal. calc. for ($C_{16}H_{20}N_4S$): C, 64.00; H, 6.66; N, 18.66; found: C, 63.77; H, 6.52; N, 17.94%; UV: λ_{max} (nm) 332, 269, 204; IR: ν_{max} (cm^{-1}) 3300, 3106 (N*H*), 1621 (C═N), 1502 (C═C), 1244 (C–N), 760 (C═S), 1048 (N–N); ^{1}H NMR (DMSO): (δ, ppm) 12.13 (1H, s, –N*H*), 11.63 (1H, s, –N*H*), 8.90 (1H, s, –C*H*═N), 8.12 (1H, d, indole C_4–*H*, $J = 7.3$ Hz), 7.87(1H, s, indole C_2–*H*), 7.47 (1H, d, indole C_7–*H*, $J = 7.3$ Hz), 7.07–7.22 (2H, m, indole C_5–*H* and C_6–*H*), 1.64–3.18 (8H, m, –CH_2), 1.23 (1H, m, –C*H*), 0.95 (3H, d, $J = 5.8$ Hz, –CH_3), ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.22 (C═S), 136.88 (C═N), 140.59 (CH) 132.71, 127.94, 122.6, 121.6,121.8, 120.28, 116.51, 116.3 (Aryl-C), 52.17 ($2CH_2$), 34.45 (CH), 33.25 ($2CH_2$), 22.01 (CH_3).

6.2.4. Indole-3-carboxaldehyde-N(4)pyrrolidine thiosemicarbazone (**4**)

Brownish black solid (methanol:chloroform). Yield: 49%; m.p.: 208 °C. Anal. calc. for ($C_{14}H_{16}N_4S$): C, 61.76; H, 5.88; N, 20.58; found: C, 61.22; H, 5.98; N, 20.27%; UV: λ_{max} (nm) 342, 266.7, 205.1; IR: ν_{max} (cm^{-1}) 3301, 3167 (N*H*), 1604 (C═N), 1530 (C═C), 1236 (C–N), 762 (C═S), 1052 (N–N);; ^{1}H NMR (DMSO): (δ, ppm) 12.89 (1H, s, –N*H*), 11.50 (1H, s, –N*H*), 8.40 (1H, s, –C*H*═N), 8.27 (1H, d, indole C_4–*H*, $J = 7.7$ Hz), 7.73 (1H, s, indole C_2–*H*), 7.43 (1H, d, indole C_7–*H*, $J = 7.7$ Hz), 7.09–7.24 (2H, m, indole C_5–*H* and C_6–*H*), 2.08–3.72 (8H, m, –CH_2). ^{13}C NMR ($CDCl_3$): (δ, ppm) 182.54 (C═S), 136.7 (C═N), 140.31 (CH) 132.71, 127.94, 125.6, 121.8, 120.28, 116.51, 116.28 (Aryl-C), 50.59 ($2CH_2$), 25.30 ($2CH_2$).

6.2.5. Indole-3-carboxaldehyde-N(4)adamentamine thiosemicarbazone (**5**)

Brownish yellow solid (methanol:chloroform). Yield: 26%; m.p.: 170 °C. Anal. calc. for ($C_{21}H_{26}N_4S$): C, 68.85; H, 7.10; N, 15.30; found: C, 68.13; H, 7.06; N, 15.09%; UV: λ_{max} (nm) 337, 261, 208; IR: ν_{max} (cm^{-1}) 3349, 3154 (N*H*), 1613 (C═N), 1531 (C═C), 1243 (C–N), 765 (C═S), 1049 (N–N); ^{1}H NMR (DMSO): (δ, ppm) 12.54 (1H, s, –N*H*), 11.86 (1H, s, –N*H*), 11.11 (1H, s, –N*H*), 8.90 (1H, s, –C*H*═N), 1.03–2.31 (18H, m, adamentyl ring), 8.06 (1H, d, indole C_4–*H*, $J = 7.3$ Hz), 7.84 (1H,s, indole C_2–*H*), 7.46 (1H, d, indole C_7–*H*, $J = 7.3$ Hz), 7.14–7.28 (2H, m, indole C_5–*H* and C_6–*H*). ^{13}C NMR ($CDCl_3$): (δ, ppm) 136.7 (C═S), 128.68 (C═N), 140.40 (CH), 120.68, 122.60, 121.8, 120.28, 116.51, 111.63 (Aryl-C), 55.0 (CH), 38.2 (CH_2), 34.8 ($4CH_2$), 30.93 (2CH), 27.95 (2CH).

6.2.6. Indole-3-carboxaldehyde-N(4)phenylpiperizine thiosemicarbazone (**6**)

Green solid (methanol:chloroform). Yield: 19%; m.p.: 180 °C. Anal. calc. for ($C_{20}H_{21}N_4S$): C, 66.11; H, 5.78; N, 19.28; found: C, 66.86; H, 5.77; N, 18.93%; UV: λ_{max} (nm) 343, 258, 208; IR: ν_{max} (cm^{-1}) 3300, 3145 (N*H*), 1600 (C═N), 1529 (C═C), 1232 (C–N), 762 (C═S), 1048 (N–N); ^{1}H NMR (DMSO): (δ, ppm) 12.61 (1H, s, –N*H*), 11.58 (1H, s, –N*H*), 8.38 (1H, s, –C*H*═N), 8.33 (1H, d, indole C_4–*H*, $J = 7.7$ Hz), 7.85 (1H, s, indole C_2–*H*), 7.43 (1H, d, indole C_7–*H*, $J = 7.7$ Hz), 7.15–7.24 (2H, m, indole C_5–*H* and C_6–*H*), 6.79–7.13 (5H, m, phenyl-*H*), 4.11 (8H, m, –CH_2), ^{13}C NMR ($CDCl_3$): (δ, ppm) 151.83 (C═S), 135.87 (C═N), 140.59 (CH), 132.71, 129.1, 129.0, 127.94, 122.6, 120.28, 120.2, 117.3, 117.2, 116.5, 111.6 (Aryl-C), 52.93 ($2CH_2$), 47.57 ($2CH_2$).

*6.2.7. Indole-3-carboxaldehyde-N(4)1,2,3, 4-tetrahydroquinoline thiosemicarbazone (**7**)*

Grey solid (methanol:chloroform). Yield: 39%; m.p.: 215 °C. Anal. calc. for ($C_{19}H_{18}N_4S$): C, 68.26; H, 5.38; N, 16.76; found: C, 69.13; H, 5.40; N, 16.07%; UV: λ_{max} (nm) 342, 268.4, 205.8; IR: ν_{max} (cm^{-1}) 3280, 3187 (N*H*), 1610 (C=N), 1538 (C=C), 1225 (C–N), 763 (C=S), 1052 (N–N); ^{1}H NMR (DMSO): (δ, ppm) 12.78 (1H, s, –N*H*), 11.78(1H, s, –N*H*), 8.89 (1H, s, –C*H*=N), 8.33(1H, d, indole C_4–*H*, $J = 7.5$ Hz), 7.86(1H, s, indole C_2–*H*), 7.45(1H, d, indole C_7–*H*, $J = 7.5$ Hz), 7.11–7.24 (2H, m, indole C_5–*H* and C_6–*H*), 6.99–7.10 (4H, m, quinoline-*H*), 3.17–5.29 (6H, m, –CH_2), ^{13}C NMR ($CDCl_3$): (δ, ppm) 146.43 (C=S), 136.7 (C=N), 140.59 (CH), 127.94, 126.36,122.6, 121.8, 120.28, 117.68, 116.51, 114.86, 111.63 (Aryl-C), 43.72 (CH_2), 27.47 (CH_2), 21.72 (CH_2).

6.3. Synthesis of Pd(II) complexes of thiosemicarbazones: a general method

To a hot solution of the appropriate ligand (2 mmol) in methanol (10 mL) was added a solution of [Pd(DMSO)$_2$Cl$_2$] (2 mmol) dissolved in minimum quantity of 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 hot water followed by small quantity of methanol and dried to give amorphous solids.

*6.3.1. [Pd(3-ICA-CPTSC)$_2$Cl$_2$] (**1a**)*

Maroon solid (methanol:DMSO). Yield: 82%; m.p.: 241 °C. Anal. calc. for ($C_{15}H_{18}N_4SCl_2Pd$): C, 38.81; H, 3.88; N, 11.08; Cl, 15.32; found: C, 38.40; H, 3.98; N, 11.25; Cl, 15.68%; UV: λ_{max} (nm) 475.3, 373, 287, 228; IR: ν_{max} (cm^{-1}) 3292, 3144 (N*H*), 1594 (C=N), 1231 (C–N), 772 (C=S), 1102 (N–N), 587, 456 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.09 (1H, s, –N*H*), 8.64 (1H, s, –N*H*), 8.50 (1H, s, –N*H*), 7.65 (1H, s, –C*H*=N), 1.56–1.93 (8H, m, –CH_2), 4.01 (1H, m, –C*H*), 7.24–7.85 (5H, m, aryl); FAB MS; *m/z* 463 [M], 463, 410, 286, 157, 117, 107. ^{13}C NMR (DMSO-d_6): (δ, ppm) 135.56 (C=S), 121.13 (C=N), 140.9 (CH) 132.71, 122.6, 121.8, 120.28,116.51, 111.63 (Aryl-C), 51.37 (CH), 32.28 (2CH_2), 22.97 (2CH_2).

*6.3.2. [Pd(3-ICA-COTSC)$_2$Cl$_2$] (**2a**)*

Reddish brown solid (methanol:DMSO). Yield: 82%; m.p.: 275 °C. Anal. calc. for ($C_{18}H_{24}N_4SCl_2Pd$): C, 42.70; H, 4.74; N, 11.07; Cl, 14.05; found: C, 42.73; H, 4.68; N, 10.89; Cl, 13.94; UV: λ_{max} (nm) 466, 371, 291, 219; IR: ν_{max} (cm^{-1}) 3287, 3145 (N*H*), 1580 (C=N), 1232 (C–N), 783 (C=S), 1110 (N–N), 504, 457 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.19 (1H, s, –N*H*), 8.64 (1H, s, –N*H*), 8.48 (1H, s, –N*H*), 7.65 (1H, s, –C*H*=N), 1.23–1.87 (14H, m, –CH_2), 3.74 (1H, m, –C*H*), 7.24–7.63 (5H, m, aryl); FAB MS; *m/z* 505 [M], 427, 328, 296, 202,170, 157, 117, 107. ^{13}C NMR (DMSO-d_6): (δ, ppm) 135.58 (C=S), 123.22 (C=N), 140.97 (CH) 132.71, 127.68, 122.6, 121.7, 120.28, 116.51, 116.3 (Aryl-C), 49.74 (CH), 31.78 (2CH_2), 26.67 (CH_2), 25.2 (CH_2), 21.94 (2CH_2).

*6.3.3. [Pd(3-ICA-4-MPTSC)$_2$Cl$_2$] (**3a**)*

Brown solid (methanol:DMSO). Yield: 43%; m.p.: 307 °C. Anal. calc. for ($C_{16}H_{20}N_4SCl_2Pd$): C, 40.22; H, 4.19; N, 11.73; Cl, 14.87; found: C, 40.73; H, 4.18; N, 11.53; Cl, 14.94; UV: λ_{max} (nm) 475.3, 371, 294, 217; IR: ν_{max} (cm^{-1}) 3300, 3105 (N*H*), 1527 (C=N), 1242 (C–N), 785 (C=S), 1112 (N–N), 502, 453 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.13 (1H, s, –N*H*), 9.34 (1H, s, –N*H*), 8.32 (1H, s, –C*H*=N), 1.69–3.86 (8H, m, –CH_2), 1.27 (1H, m, –C*H*), 7.16–8.07 (5H, m, aryl); FAB MS; *m/z* 478 [M], 460, 302, 157, 117, 107. ^{13}C NMR (DMSO-d_6): (δ, ppm) 180.62 (C=S), 131.24 (C=N), 140.87 (CH) 132.71, 127.94, 122.6, 121.6,121.8, 120.28, 116.51, 116.3 (Aryl-C), 52.17 (2CH_2), 34.45 (CH), 33.25 (2CH_2), 22.01 (CH_3).

*6.3.4. [Pd(3-ICA-PYRTSC)$_2$Cl$_2$] (**4a**)*

Brownish black solid (methanol:DMSO). Yield: 97%; m.p.: 331 °C. Anal. calc. for ($C_{14}H_{16}N_4SCl_2Pd$): C, 37.36; H, 3.55; N, 12.45; Cl, 15.80; found: C, 37.95; H, 3.69; N, 12.70; Cl, 15.96; UV: λ_{max} (nm) 477.2, 371, 291, 212; IR: ν_{max} (cm^{-1}) 3300, 3166 (N*H*), 1572 (C=N), 1230 (C–N), 789 (C=S), 1103 (N–N), 514, 478 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.87 (1H, s, –N*H*), 8.37 (1H, s, –N*H*), 8.30 (1H, s, –C*H*=N), 3.25–3.66 (4H, m, –CH_2), 1.76–2.50 (4H, m, –CH_2), 6.86–7.53 (5H, m, aryl); FAB MS; *m/z* 449 [M], 301, 377, 273, 239, 157, 117, 107. ^{13}C NMR (DMSO-d_6): (δ, ppm) 181.07 (C=S), 132.90 (C=N), 140.72 (CH) 132.71, 127.94, 125.6, 121.8, 120.28, 116.51, 116.28 (Aryl-C), 50.59 (2CH_2), 25.30 (2CH_2).

*6.3.5. [Pd(3-ICA-ADMTSC)$_2$Cl$_2$] (**5a**)*

Brown solid (methanol:DMSO). Yield: 53%; m.p.: 250 °C. Anal. calc. for ($C_{21}H_{26}N_4SCl_2Pd$): C, 45.34; H, 4.53; N, 10.57; Cl, 13.41; found: C, 45.73; H, 4.18; N, 10.53; Cl, 13.40; UV: λ_{max} (nm) 442.3, 371, 291, 212; IR: ν_{max} (cm^{-1}) 3347, 3153 (N*H*), 1598 (C=N), 1241 (C–N), 790 (C=S), 1111 (N–N), 516, 458 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.54 (1H, s, –N*H*), 8.77 (1H, s, –N*H*), 8.05 (1H, s, –C*H*=N), 1.67–2.16 (18H, m, –CH_2), 3.36 (1H, m, –C*H*), 7.09–8.29 (5H, m, aryl); FAB MS; *m/z* 529 [M], 457, 353, 217, 157, 117, 107. ^{13}C NMR (DMSO-d_6): (δ, ppm) 135.89 (C=S), 129.25 (C=N), 140.91 (CH), 120.68, 122.60, 121.8, 120.28, 116.51, 111.63 (Aryl-C), 55.0 (CH), 38.2 (CH_2), 34.8 (4CH_2), 30.93 (2CH), 27.95 (2CH).

*6.3.6. [Pd(3-ICA-NPPTSC)$_2$Cl$_2$] (**6a**)*

Brown solid (methanol:DMSO). Yield: 86%; m.p.: 269 °C. Anal. calc. for ($C_{20}H_{21}N_5SCl_2Pd$): C, 44.37; H, 3.88; N, 12.94; Cl, 13.14; found: C, 44.73; H, 3.18; N, 12.53; Cl, 13.94; UV: λ_{max} (nm) 431, 372, 293, 223; IR: ν_{max} (cm^{-1}) 3298, 3142 (N*H*), 1587 (C=N), 1228 (C–N), 786 (C=S), 1104 (N–N), 512, 457 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.60 (1H, s, –N*H*), 8.73 (1H, s, –N*H*), 8.37 (1H, s, –C*H*=N), 3.21–3.28 (4H, m, –CH_2), 3.65–4.02 (4H, m,

–CH_2), 6.82–7.67 (5H, m, aryl); FAB MS; *m/z* 540 [M], 380, 364, 331, 202, 157, 117, 107. ^{13}C NMR (DMSO-d_6): (δ, ppm) 151.02 (C=S), 130.98 (C=N), 140.59 (CH), 132.71, 129.1, 129.0, 127.94, 122.6, 120.28, 120.2, 117.3, 117.2, 116.5, 111.6 (Aryl-C), 52.93 ($2CH_2$), 47.57 ($2CH_2$).

*6.3.7. [Pd(3-ICA-4-THQTSC)$_2$Cl$_2$] (**7a**)*

Brown solid (methanol:DMSO). Yield: 68%; m.p.: 258 °C. Anal. calc. for ($C_{19}H_{18}N_4SCl_2Pd$): C, 43.69; H, 3.44; N, 10.73; Cl, 13.88; found: C, 43.59; H, 3.48; N, 10.53; Cl, 13.94; UV: λ_{max} (nm) 431, 371,271,221; IR: ν_{max} (cm^{-1}) 3276, 3186 (N*H*), 1577 (C=N), 1222 (C–N), 788 (C=S), 1102 (N–N), 514, 459 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.77 (1H, s, –N*H*), 8.56 (1H, s, –N*H*), 8.37 (1H, s, –C*H*=N), 2.06–3.38 (6H, m, –CH_2), 6.80–7.65 (5H, m, aryl); FAB MS; *m/z* 511 [M], 442, 335,184, 157, 117,107. ^{13}C NMR (DMSO-d_6): (δ, ppm) 145.77 (C=S), 131.72 (C=N), 140.79 (CH), 127.94, 126.36,122.6, 121.8, 120.28, 117.68, 116.51, 114.86, 111.63 (Aryl-C), 43.72 (CH_2), 27.47 (CH_2), 21.72 (CH_2).

6.4. In vitro testing against E. histolytica

All the *N*(4)-substituted indole thiosemicarbazones (**1**–**7**) and their palladium complexes (**1a**–**7a**) were screened *in vitro* for antiamoebic activity against (*HM1:IMSS*) strain of *E. histolytica* by using a microplate method [28]. The biological test was carried out using DMSO as solvent (40 μL) in which the compounds are stable. The maximum concentration of DMSO in the test did not exceed 0.1%, at which level no inhibition of amoebial growth occurred [37,38]. 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 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 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. The plate was then immediately washed once in sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly, and the plate was not allowed to cool in order to prevent the detachment of amoebae. The plate was allowed to dry at room temperature, and the amoebae were fixed with methanol, 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 % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best-fitting straight line from which the IC_{50} value was found.

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