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Short communication

New Pd(II) complexes of the synthesized 1-*N*-substituted thiosemicarbazones of 3-indole carboxaldehyde: Characterization and antiamoebic assessment against *E. histolytica*

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Abstract

Reaction of 3-indole carboxaldehyde with aminothiocarbonyl hydrazines resulted in the formation of 3-indole carboxaldehyde thiosemicarbazones (TSCs) **1**–**13**. The synthesized thiosemicarbazones were used as ligands in the formation of $[Pd(TSC)Cl_2]$ complexes with palladium(II) metal ion precursor, $[Pd(DMSO)_2Cl_2]$. The chemical structures of all the compounds were established by electronic, IR, ^{1}H NMR and ^{13}C NMR spectral data. The structure of the complexes was further established by FABMS and DTA. It is concluded that the thione sulphur and the azomethine nitrogen atom of the ligands are bonded to the metal ion. The testing of the antiamoebic activity of these compounds against the protozoan parasite *Entamoeba histolytica* suggests that compounds **5**, **3a**, **5a** and **8a**–**13a** might be endowed with important antiamoebic properties since they showed less IC_{50} values than metronidazole. Moreover, compound **12a** displays remarkable antiamoebic activity than metronidazole (IC_{50} values of **0.29** vs **1.81** μM, respectively). MTT assay showed that the compounds are non-toxic to human kidney epithelial cell line.

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

1. Introduction

Amoebiasis is the second leading cause of death among parasitic diseases worldwide. The causative protozoan parasite, *Entamoeba histolytica*, is a potent pathogen. Secreting proteinases that dissolve host tissues, killing host cells on contact, and engulfing red blood cells, *E. histolytica* trophozoites invade the intestinal mucosa, causing amoebic colitis [1]. Metronidazole (MTZ, 1-[2-hydroxyethyl]-2-methyl-5-nitroimidazole) is an antibacterial and antiprotozoal drug that has been in use for over 35 years. In an event of overt clinical resistance to metronidazole in the anaerobic protozoa, there is no alternative treatment for invasive amoebiasis, keeping in mind the documented cross-resistance between currently used nitroimidazole drugs [2] and worldwide availability. Treatment with metronidazole has several side effects that include nausea, vomiting, dry mouth, metallic taste, abdominal pain and headache. In some cases additional side effects viz. dizziness, vertigo, paresthesias and rarely encephalopathy or convulsions have been reported leading to even discontinuation of the drug [3]. Due to long-term treatment toxicity and clinical resistance to drugs commonly used, new effective agents are urgently needed. On the positive side, a great deal of flexibility is offered by the replacement of the imidazole ring structure by the heterocyclic moiety.

Of the various heterocyclic systems, the indole nucleus has been reported as a common dominator of psychotropism and is of great value in the field of medicine and biochemistry [4,5]. This nitrogen heterocycle and its derivatives have occupied a unique place in the chemistry because of its varied biodynamic properties such as anticancer [6–9], antidepressant [10], antihypertensive [11], psychomimetic [12], antimicrobial [13,14], antidiabetic [15], antimalarial [16] and anti-inflammatory [17,18]. Moreover, thiosemicarbazones are a class of

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compounds very promising in the treatment of many diseases and their development is still in progress [19–21]. In addition, metal ions are known to accelerate drug action, and the efficacy of a therapeutic agent may be enhanced upon coordination with a metal ion [22]. In particular, neutral palladium(II) and (IV) complexes exhibit potential antitumour activity and apoptosis [23,24]. The literature reports that palladium(II) and platinum(II) complexes of phenylacetaldehyde thiosemicarbazone are able to *in vitro* bind to DNA, and present enhanced capacity to form inter-strand crosslinks by comparison with cisplatinum [22,25].

We have previously reported the structural and spectral studies of several transition metal complexes of *N*4-substituted thiosemicarbazones with the aim to correlate the structural features and chelating ability to the antiprotozoal properties [26,27]. Here the synthesis of 3-indole carboxaldehyde thiosemicarbazones **1–13** and their subsequent bidentate Pd(II) complexes **1a–13a** were screened for their antiamoebic activities against *HM1:IMSS* strain of *E. histolytica in vitro* experiments and it was found that the coordination of palladium to thiosemicarbazone enhances the activity. The toxicity study of these compounds was performed against KB cell lines.

2. Results and discussion

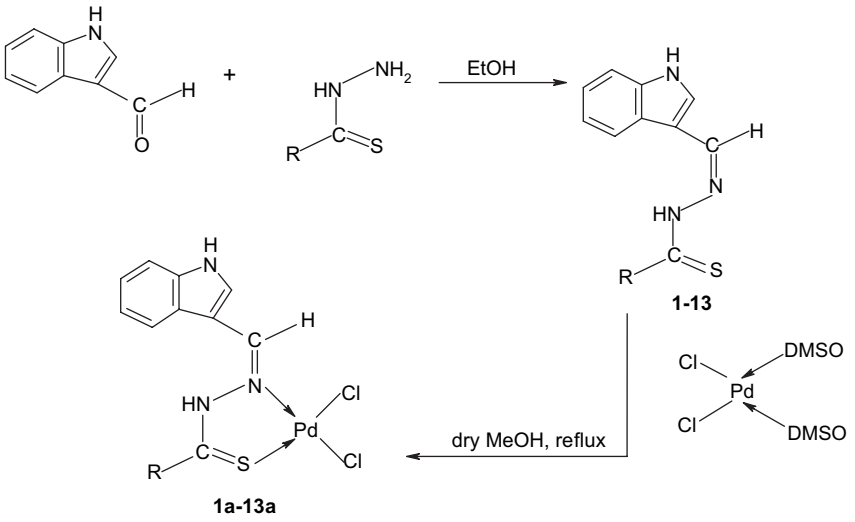
The synthesis of indole-3-carboxaldehyde thiosemicarbazones and their Pd(II) complexes is represented in Scheme 1. All the thioglycolic acids were prepared by the reported O'Sullivan method [28]. Thiocarbonylhydrazines were prepared by refluxing the alkaline solution of thioglycolic acid with hydrazine hydrate and their thiosemicarbazones were synthesized by refluxing aqueous solution of thiocarbonylhydrazines and ethanolic solution of 3-indole-carboxaldehyde in equimolar ratio at 8 °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. The precursor $[Pd(DMSO)_2Cl_2]$ used for the synthesis of Pd(II) complexes was synthesized by the literature procedure [29,30]. The complexes were prepared by mixing 1:1 ratio of the appropriate ligand and $[Pd(DMSO)_2Cl_2]$ 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 *in vacuo* over silica gel to give amorphous solids. The complexes gave high yields around 80%, while the yields for thiosemicarbazones showed variable results. The chemical structures of all the compounds were confirmed by means of elemental analysis and electronic, IR, ^{1}H NMR and ^{13}C NMR spectral studies. The structures of complexes were further established by thermogravimetric analysis and FABMS. The analytical data of these compounds are in good agreement with their composition (Table 1).

Selected significant IR bands revealed the structural characteristics of the compounds. All the thiosemicarbazones **1–13** may exhibit in thione–thiol tautomerization since they contain thione group (C=S) and a proton adjacent to thione group. The absence of ν (S–H) stretch at 2500–2600 cm^{-1} and the presence of ν (N–H) stretch at 3100–3300 cm^{-1} in the spectra of the ligands suggest that all the ligands remain in the thione form in the solid state. The band appearing at 790–820 cm^{-1} ascribed to ν (C=S) [31] 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 ν (Pd–N, Pd–S) [32]. Thione form reveals 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–13a**, indicating non-participation of ring nitrogen in coordination. The negative shift of 25–57 cm^{-1} of ν (C=N) stretch in the complexes indicates the involvement of azomethine nitrogen in complexation [33]. The broad band observed in region 3300 cm^{-1} due to ν (N–H) stretch is slightly shifted in complex probably due to the 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.

The electronic spectra of these complexes exhibited bands at expected position as cited in the literature for the similar system [34]. A perusal of these spectra revealed that they were dominated by intense intra-ligand and charge transfer bands. Thus a band appearing at 47 500–49 000 cm^{-1} in the spectra of complexes was assigned to π–π^* transition of indole ring. Another band at 38 000–40 000 cm^{-1} was attributed to n–π^* transitions of indole ring. This band, followed by a shoulder band at 29 000–31 000 cm^{-1}, appears due to the thiosemicarbazones' moiety. A comparison of these three bands with free ligands revealed that there is increase in intensity and decrease in the frequency that is attributed to extended conjugation in the ligand moieties after complexation. The complexes show absorption peaks in the visible region due to the d–d transition of the single d electron of the palladium(II) ion.

Further evidence for the formation of compounds was obtained from the ^{1}H 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 ligands **1–13** do not show any resonance at ca. 4.0 ppm, attributed to –SH proton resonance, while the appearance of a broad peak at 11.51–11.69 ppm due to the –NH proton of 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 0.86–2.83 ppm upfield in their respective complexes. However, in complexes, we are unable to calculate the coupling constant values for aromatic region. This might be assumed 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 those of free ligands.

Moreover, the ^{13}C NMR spectra of all the ligands taken in DMSO gave the spectral signals in good agreement with the



1/1a —NH_2

2/2a —HN—CH_2—CH_2—CH_3

3/3a —HN—CH(—CH_3)—CH_3

4/4a —HN—CH_2—CH_2—CH_2—CH_3

5/5a —HN—CH_2—CH(—CH_3)—CH_3

6/6a —N(—CH_2—CH_3)—CH_2—CH_3

7/7a —HN(—CH_3)—CH_2—CH_2—CH_2—CH_3

8/8a —HN— (2-amino-5-methylphenyl)

9/9a —HN— (2-amino-3-methylphenyl)

10/10a —HN— (2-amino-4-methylphenyl)

11/11a —N(—CH_3)—CH_2— (N-methyl-N-benzylamine)

12/12a —HN—H_2C— (2-chlorobenzylamine)

13/13a —HN— (2,6-difluoroaniline)

Scheme 1.

Table 1
Analytical and physicochemical data of thiosemicarbazones and their Pd(II) complexes

Compound/stoichiometry	Yield (%)	Mp/dec temp (°C)	Found (calcd)			
			C	H	N	Cl
1/3-ICA-TSC $C_{10}H_{10}N_4S$	76	220	54.71 (55.04)	4.35 (4.58)	25.66 (25.68)	–
1a/[Pd(3-ICA-TSC)$_2$Cl$_2$] $C_{10}H_{10}N_4S$	64	160	30.10 (30.35)	2.50 (2.52)	14.25 (14.16)	17.92 (17.96)
2/3-ICA-PrTSC $C_{13}H_{16}N_4S$	13	226	59.55 (60.00)	6.01 (6.15)	22.00 (21.53)	–
2a/[Pd(3-ICA-PTSC)$_2$Cl$_2$] $C_{13}H_{16}N_4SCl_2Pd$	50	267	32.81 (32.95)	3.97 (3.65)	12.76 (12.80)	16.21 (16.23)
3/3-ICA-*iso*-PrTSC $C_{13}H_{16}N_4S$	62	200	59.87 (60.00)	6.23 (6.15)	21.54 (21.53)	–
3a/[Pd(3-ICA-IPTSC)$_2$Cl$_2$] $C_{13}H_{16}N_4SCl_2Pd$	45	160	35.95 (35.64)	3.31 (3.65)	12.76 (12.75)	16.07 (16.23)
4/3-ICA-BuTSC $C_{14}H_{18}N_4S$	61	170	61.02 (61.31)	6.42 (6.56)	20.77 (20.43)	–
4a/[Pd(3-ICA-BTSC)$_2$Cl$_2$] $C_{14}H_{18}N_4SCl_2Pd$	83	160	37.20 (37.22)	3.98 (3.98)	12.65 (12.40)	15.70 (15.73)
5/3-ICA-*iso*-BuTSC $C_{14}H_{18}N_4S$	67	145	61.29 (61.31)	6.54 (6.56)	20.41 (20.43)	–
5a/[Pd(3-ICA-IBTSC)$_2$Cl$_2$] $C_{14}H_{18}N_4SCl_2Pd$	84	295	37.70 (37.90)	3.83 (3.98)	12.72 (12.39)	15.54 (15.71)
6/3-ICA-diETSC $C_{14}H_{18}N_4S$	61	140	61.70 (61.31)	6.54 (6.56)	20.39 (20.43)	–
6a/[Pd(3-ICA-DETSC)$_2$Cl$_2$] $C_{14}H_{18}N_4SCl_2Pd$	27	285	37.37 (37.22)	3.87 (3.98)	12.47 (12.40)	15.39 (15.73)
7/3-ICA-NMButTSC $C_{15}H_{20}N_4S$	62	140	62.39 (62.50)	6.85 (6.94)	19.90 (19.44)	–
7a/[Pd(3-ICA-NMButTSC)$_2$Cl$_2$] $C_{15}H_{20}N_4SCl_2Pd$	62	160	38.83 (38.84)	4.43 (4.29)	12.31 (12.02)	12.04 (12.06)
8/3-ICA-*p*-TolTSC $C_{17}H_{16}N_4S$	20	200	66.31 (66.23)	4.99 (5.19)	17.97 (18.18)	–
8a/[Pd(3-ICA-*p*-TolTSC)$_2$Cl$_2$] $C_{17}H_{16}N_4SCl_2Pd$	87	235	42.12 (42.03)	3.43 (3.29)	11.31 (11.52)	14.04 (14.62)
9/3-ICA-*o*-TolTSC $C_{17}H_{16}N_4S$	21	198	66.00 (66.23)	5.60 (5.19)	18.05 (18.18)	–
9a/[Pd(3-ICA-*o*-TolTSC)$_2$Cl$_2$] $C_{17}H_{16}N_4SCl_2Pd$	86	344	42.52 (42.03)	3.08 (3.29)	11.68 (11.52)	14.64 (14.64)
10/3-ICA-*m*-TolTSC $C_{17}H_{16}N_4S$	62	192	66.46 (66.23)	5.03 (5.19)	18.15 (18.18)	–
10a/[Pd(3-ICA-*m*-TolTSC)$_2$Cl$_2$] $C_{17}H_{16}N_4SCl_2Pd$	43	354	42.10 (42.03)	3.42 (3.29)	11.67 (11.52)	14.59 (14.62)
11/3-ICA-NMBzTSC $C_{18}H_{18}N_4S$	19	180	67.00 (67.08)	5.24 (5.59)	17.27 (17.39)	–
11a/[Pd(3-ICA-NMBzlTSC)$_2$Cl$_2$] $C_{18}H_{18}N_4SCl_2Pd$	41	267	43.37 (43.25)	3.35 (3.60)	11.22 (11.21)	14.39 (14.21)
12/3-ICA-2-CBzTSC ($C_{17}H_{15}N_4SCl$)	57	130	59.21 (59.56)	4.47 (4.37)	16.39 (16.35)	–
12a/[Pd(3-ICA-2-CBTSC)$_2$Cl$_2$] $C_{17}H_{15}N_4SCl_3Pd$	65	257	39.12 (39.24)	2.93 (2.88)	10.31 (10.77)	20.64 (20.48)
13/3-ICA-2,4-diFATSC $C_{16}H_{12}N_4SF$	20	160	58.28 (58.18)	3.96 (3.63)	16.97 (16.96)	–
13a/[Pd(3-ICA-2,4-DFATSC)$_2$Cl$_2$] $C_{16}H_{12}N_4SF_2Cl_2Pd$	49	215	37.80 (37.84)	2.38 (2.36)	11.15 (11.03)	14.01 (13.99)

probable structures. All the ligands showed two signals at 172.49–179.12 and 134.11–141.73 ppm assigned due to the thioamide (C=S) and azomethine carbon (C=N), respectively. The signals from 111.6 to 138.8 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 resonate at their usual positions and are shown in the data given in Section 3. ^{13}C NMR spectra also provide diagnostic tools for the elucidation of the coordination 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**–**13a**, 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 those of free ligands.

Thermal behavior of all complexes (**1a**–**13a**) was studied in the temperature range 25–900 °C. Fig. 1 provides the TGA and DTA plots of a representative complex **8a**. All the dehydrated complexes are stable up to ca.110 °C and then decompose in three major steps. The temperature range for the first endothermic step being 110–180 °C which is followed by exothermic second step covering the temperature range 180–240 °C. DTA plots for complexes suggest their exothermic nature of decomposition in the subsequent stages. Third stage of decomposition starts immediately after second one and continues until complete decomposition of the ligands and formation of PdS as the end product. The final temperature at which PdS obtained varied from complex to complex but in any case the formation of final stable product does not go beyond 900 °C. 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 ligands after considering the transfer of one sulphur atom to the metal ion and residues corresponds to the palladium sulphide.

Additionally, a general splitting pathway with the characteristic peaks was observed by the thiosemicarbazone Pd(II) complexes **1a**–**13a**. The positive ion FAB mass spectrum of all the complexes was recorded using *m*-nitro benzyl alcohol (NBA) as the matrix. The spectra of Pd(II) complexes showed a number of informative fragment ions of different intensities confirming their molecular weights. 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 azirinium ions are barely detected and the ions at *m*/*z* 13, 91, 77 arise from further decomposition in the usual way. The FABMS fragmentation pattern of compound **9a** is depicted in Scheme 2.

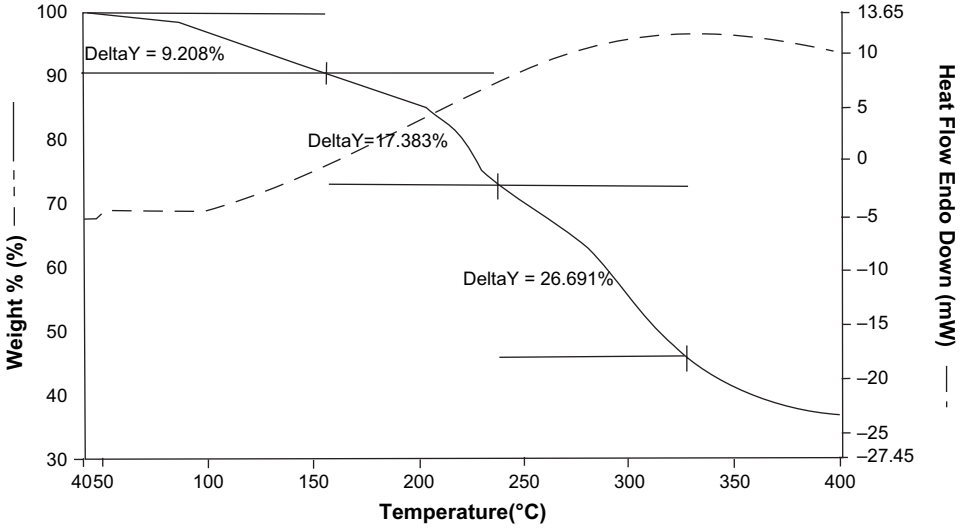
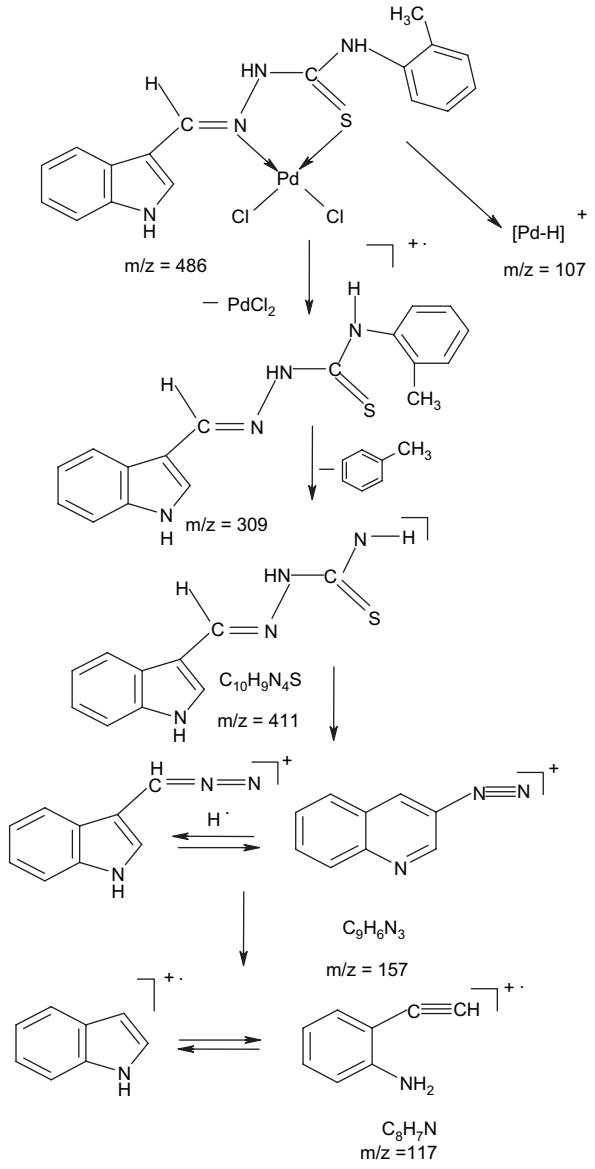


Fig. 1. DTA plot for thermal behavior of Pd(II) complex **8a**.

2.1. In vitro antiamoebic activity

Two considerations governed the selection of compounds to be prepared as thiosemicarbazone analogues for this study. It was considered to have representatives of the two classes of 1-*N*-substituted 3-indole carboxaldehyde thiosemicarbazones, the compounds having 1-*N*-substituted with different aliphatic groups. These include: (1) 1-*N*-substituted derivatives with different aliphatic groups represented by compounds **1**–**7**; and (2) with different aromatic groups represented by compounds **8**–**13**. In our previous communications it was found that the activity of the resulting thiosemicarbazones enhanced due to the substitution of bulky group at N^4 position of thiosemicarbazides [22,26]. All the compounds were evaluated for their antiamoebic activity *in vitro* using *HM1:IMSS* strain of *E. histolytica* to investigate the influence of the substitution. The IC_{50} values in μM are shown in Table 2 and a relative graph has been plotted in Fig. 2. Metronidazole had a 50% inhibitory concentration (IC_{50} 1.6–1.8 μM) in our experiments. 3-Indole carboxaldehyde thiosemicarbazones taken as ligands showed IC_{50} in the range of 0.29–11.52 μM. Compound **12** in this series showed notable antiamoebic activity in comparison with the reference drug, metronidazole ($IC_{50} = 0.29$ μM vs $IC_{50} = 1.8$ μM of metronidazole). The complexation of thiosemicarbazones **1**–**13** with palladium(II) results in complexes **1a**–**13a**, which showed $IC_{50} = 0.29$–11.52 μM. All the metal complexes were found more active than their respective ligands indicating that the complexation to metal enhances the activity of the ligands. This may be explained by 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. Moreover, the complexes **3a**, **5a** and **8a**–**13a** displayed the most promising *in vitro* antiamoebic activity and were found to be better inhibitors of the parasite than metronidazole. The Pd-complex precursor $[Pd(DMSO)_2Cl_2]$ was also evaluated for antiamoebic 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 antiamoebic activity. Moreover, it was found that aliphatic compounds and their complexes were less active than aromatic compounds and their complexes. 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 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. 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 **5**, **3a**, **5a** and **8a**–**13a** was evaluated by *T*-test. The values of the calculated *T* were found higher than the table value of *T* at 5% level, thus concluding that the character under study is thus significantly influenced by the treatment. The results indicate that the complexation to Pd increases the activity of parent ligands.

The thiosemicarbazone 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) cell line and protozoal IC_{50} is the concentration that kills 50% of amoeba protozoa. It gives an idea about the efficacy or toxicity of compounds against human cells and potentially active *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



Scheme 2. Proposed mass fragmentation pattern for compound **9a**.

of the thiosemicarbazone ligands show significant values of SI. In contrast, compound **12a** has lowest cytotoxicity and highest antiamoebic activity and in an overall result, complexes **3a**, **5a** and **8a**–**13a** show the more favorable safety profile along with the most promising antiamoebic activity. The result indicates that MTT assay showed that the compounds are non-toxic to human kidney epithelial cell line. Detailed studies on the mechanism of action of these compounds as well as further modifications of these and other related thiosemicarbazones are in progress.

3. Experimental

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 [28]. 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–visible spectrophotometer. IR spectra on KBr disks were recorded on a Perkin Elmer model 1620 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were obtained at ambient temperature using a Bruker Spectrospin DPX-300 MHz spectrophotometer in $CDCl_3$ using tetramethylsilane as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; 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) 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.

3.1. 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.

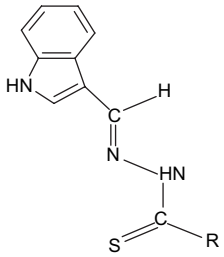
*3.1.1. Indole-3-carboxaldehyde-N(4) thiosemicarbazone, 3-ICA-TSC (**1**)*

Light brown solid (methanol–chloroform); UV/vis: ν (cm^{-1}) 30 303, 37 313, 48 780; IR: ν_{max} (cm^{-1}) 3378, 3232 (NH), 1614 (C=N), 1538 (C=C), 1251 (C–N), 752 (C=S); ^{1}H NMR (DMSO): (δ, ppm) 12.16 (1H, s, –NH), 11.60 (1H, s, –NH), 11.18 (2H, s, $-NH_2$), 8.29 (1H, s, –CH=N), 8.23 (1H, d, indole C_4–H, $J = 7.14$ Hz), 8.03 (1H, s, indole C_2–H), 7.43 (1H, d, indole C_7–H, $J = 7.14$ Hz), 7.10–7.22 (2H, m, indole C_5–H and C_6–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 173.10 (C=S), 139.96 (C=N), 136.70, 128.35, 122.6, 121.8, 120.29, 116.51, 111.63 (aryl–C).

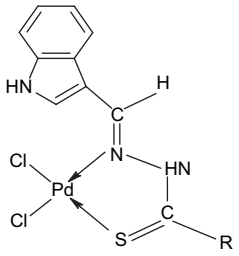
*3.1.2. Indole-3-carboxaldehyde-N(4)propyl thiosemicarbazone, 3-ICA-PrTSC (**2**)*

Greenish black solid (methanol–chloroform); UV/vis: ν (cm^{-1}) 28 818, 38 610, 48 309; IR: ν_{max} (cm^{-1}) 3265, 3120 (NH), 1614 (C=N), 1550 (C=C), 1243 (C–N), 758 (C=S); ^{1}H NMR (DMSO): (δ, ppm) 12.14 (1H, s, N–NH), 11.68 (1H, s, N–NH), 11.18 (1H, t, CS–NH), 8.90 (1H, s, –CH=N), 8.29 (1H, d, indole C_4–H, $J = 8$ Hz), 7.81 (1H, s, indole C_2–H), 7.43 (1H, d, indole C_7–H, $J = 8$ Hz), 7.12–7.24 (2H, m, indole C_5–H and C_6–H), 1.69 (2H, m, $-CH_2$), 3.6 (2H, t, $-CH_2$, $J = 6.6$ Hz), 0.94 (3H, t, $-CH_3$, $J = 6.97$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 175.86 (C=S),

Table 2
In vitro antiamoebic activity of compounds against *E. histolytica* and cytotoxic profile



(1-13)



(1a-13a)

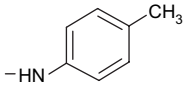
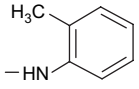
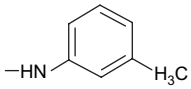
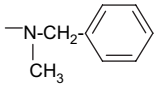
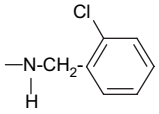
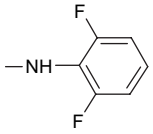
Compound no.	R	Antiamoebic activity		Toxicity profile IC_{50} (μM)	Safety index S.I.
		IC_{50} (μM)	S.D.[a]		
1	$-NH_2$	11.52	0.01	>100	>8.68
1a		3.57	0.02	>100	>28.01
2	$-HN-CH(CH_3)_2$	6.84	0.11	>100	>14.61
2a		4.06	0.02	>100	>24.63
3	$-NH-CH_2-CH_2-CH_2-CH_3$	7.64	0.01	>100	>13.08
3a		**1.44**	0.11	>100	>69.44
4	$-NH-CH_2-CH_2-CH_3$	4.09	0.03	>100	>24.44
4a		2.78	0.08	>100	>35.97
5	$-HN-CH_2-CH(CH_3)_2$	5.76	0.02	>100	>17.36
5a		**1.24**	0.13	>100	>80.64
6	$-N(CH_2-CH_3)_2$	2.58	0.04	>100	>38.75
6a		2.22	0.08	>100	>45.04
7	$-N(CH_3)(CH_2-CH_2-CH_2-CH_3)$	5.50	0.03	>100	>18.18
7a		2.14	0.08	>100	>46.72
8		3.25	0.01	>100	>30.76
8a		**1.30**	0.09	>100	>76.92
9		2.05	0.02	>100	>48.78
9a		**0.58**	0.02	>100	>172.41
10		**1.29**	0.09	>100	>77.51
10a		**0.52**	0.07	>100	>192.30
11		2.46	0.10	>100	>40.65
11a		**0.74**	0.02	>100	>135.13
12		3.28	0.04	>100	>30.48
12a		**0.29**	0.03	>100	>344.82

Table 2 (*continued*)

Compound no.	R	Antiamoebic activity		Toxicity profile	Safety
		IC_{50} (μM)	S.D.[a]	IC_{50} (μM)	index S.I.
13		6.05	0.04	>100	>16.52
13a		**0.62**	0.01	>100	>161.29
$Pd(DMSO)_2Cl_2$		8.00	0.34	=100	>12.50
Metronidazole (MNZ)		1.81	0.03	>100	>55.24

[a] Standard deviation.

140.55 (C═N), 136.50, 128.38, 122.4, 121.7, 120.25, 116.51, 111.63 (aryl−C), 21.94 (CH_2), 11.41 (CH_3).

*3.1.3. Indole-3-carboxaldehyde-N(4)isopropyl thiosemicarbazone, 3-ICA-iso-PrTSC (**3**)*

Light brown solid (methanol−chloroform); UV/vis: ν (cm^{-1}) 29 940, 37 453, 47 846; IR: ν_{max} (cm^{-1}) 3360, 3131 (NH), 1615 (C═N), 1540 (C═C), 1232 (C−N), 760 (C═S); ^{1}H NMR (DMSO): (δ, ppm) 12.53 (1H, s, −NH), 11.63 (1H, s, −NH), 11.22 (1H, s, −NH), 8.31 (1H, s, −CH═N), 8.15 (1H, d, indole C_4−H, $J = 7.2$ Hz), 7.83 (1H, s, indole C_2−H), 7.45 (1H, d, indole C_7−H, $J = 7.2$ Hz), 7.11−7.23 (2H, m, indole C_5−H and C_6−H), 3.58 (1H, m, −CH), 1.14 (6H, d, $-CH_3$, $J = 6$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 173.80 (C═S), 141.26 (C═N), 136.58, 128.13, 122.12 121.74, 120.48, 117.11, 111.74 (aryl−C), 41.41 (CH), 21.30 ($2CH_3$).

*3.1.4. Indole-3-carboxaldehyde-N(4)butyl thiosemicarbazone, 3-ICA-BuTSC (**4**)*

Light brown solid (methanol−chloroform); UV/vis: ν (cm^{-1}) 28 985, 37 037, 48 780; IR: ν_{max} (cm^{-1}) 3365, 3139 (NH), 1612 (C═N), 1540 (C═C), 1233 (C−N), 762 (C═S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 12.14 (1H, s, −NH), 11.61 (1H, s, −NH), 11.16 (1H, s, −NH), 8.29 (1H, s, −CH═N), 8.19 (1H, d, indole C_4−H, $J = 8.1$ Hz), 7.82 (1H, s, indole C_2−H), 7.44 (1H, d, indole C_7−H, $J = 8.1$ Hz), 7.12−7.23 (2H, m, indole C_5−H and C_6−H), 1.63 (2H, m, $-CH_2$), 1.42 (2H, m, $-CH_2$), 1.05 (3H, t, $-CH_3$, $J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 173.22 (C═S), 140.55 (C═N), 136.74, 128.38, 122.67, 121.98, 121.45, 117.89, 112.03 (aryl−C), 44.61 (CH_2), 30.64 (CH_2), 19.90 (CH_2), 13.59 (CH_3).

*3.1.5. Indole-3-carboxaldehyde-N(4)isobutyl thiosemicarbazone, 3-ICA-iso-BuTSC (**5**)*

Brown solid (methanol−chloroform); UV/vis: ν (cm^{-1}) 29 411, 37 878, 48 780; IR: ν_{max} (cm^{-1}) 3360, 3131 (NH), 1614 (C═N), 1545 (C═C), 1232 (C−N), 761 (C═S); ^{1}H NMR ($CDCl_3$): (δ, ppm) 11.63 (1H, s, −NH), 12.15 (1H, s, −NH), 11.22 (1H, s, −NH), 8.31 (1H, s, −CH═N), 8.28 (1H, d, indole C_4−H, $J = 8.5$ Hz), 7.81 (1H, s, indole C_2−H), 7.42 (1H, d, indole C_7−H, $J = 8.5$ Hz), 7.10−7.21 (2H, m, indole C_5−H and C_6−H), 3.49 (2H, t, $-CH_2$, $J = 6.4$ Hz), 2.02 (1H, m, −CH), 0.94 (6H, d, $-CH_3$, $J = 6.6$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 173.90 (C═S), 141.73 (C═N), 137.84, 127.08, 123.36, 122.08, 121.45, 117.89, 111.78 (aryl−C), 51.78 (CH_2), 28.10 (CH), 20.42 ($2CH_3$).

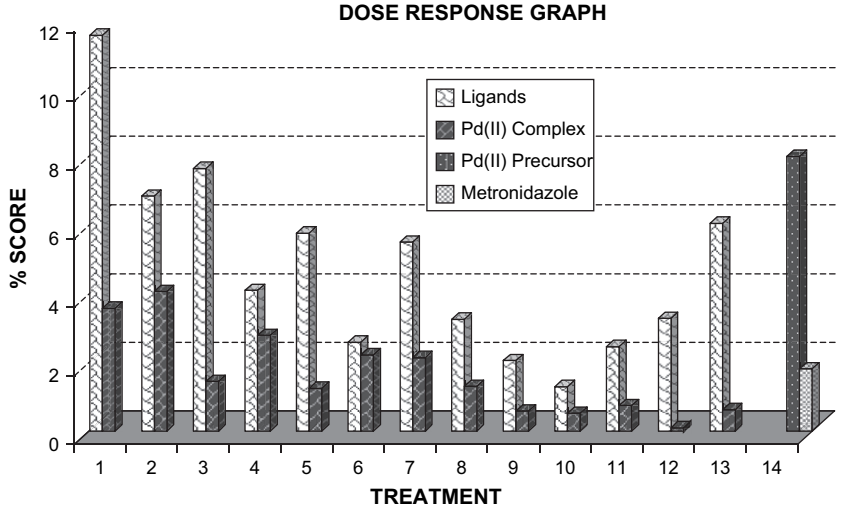


Fig. 2. *In vitro* activity of indole thiosemicarbazones and their Pd(II) complexes exhibiting comparison of IC_{50} values (μM).

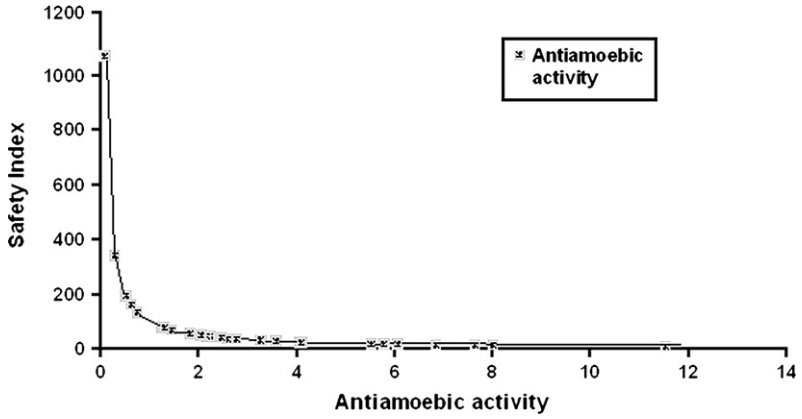


Fig. 3. Correlation of safety index and antiamoebic activity.

*3.1.6. Indole-3-carboxaldehyde-N(4,4)diethyl thiosemicarbazone, 3-ICA-diETSC (**6**)*

Yellow solid (methanol–chloroform); UV/vis: ν (cm^{-1}) 30 395, 37 037, 47 846; IR: ν_{max} (cm^{-1}) 3363, 3170 (NH), 1616 (C=N), 1518 (C=C), 1243 (C–N), 759 (C=S); ^1H NMR (DMSO): (δ, ppm) 12.15 (1H, s, –NH), 11.51 (1H, s, –NH), 8.90 (1H, s, –CH=N), 8.32 (1H, d, indole C_4–H, $J = 7.9$ Hz), 7.85 (1H, s, indole C_2–H), 7.43 (1H, d, indole C_7–H, $J = 7.9$ Hz), 7.11–7.31 (2H, m, indole C_5–H and C_6–H), 3.82 (6H, m, $-CH_3$), 1.21 (4H, t, $-CH_2$, $J = 6$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.90 (C=S), 140.63 (C=N), 138.87, 128.08, 123.67, 122.56, 121.74, 117.43, 111.33 (aryl–C), 41.21 ($2CH_2$), 11.80 ($2CH_3$).

*3.1.7. Indole-3-carboxaldehyde-N(4,4)methyl butyl thiosemicarbazone, 3-ICA-NMButTSC (**7**)*

Light brown solid (chloroform); UV/vis: ν (cm^{-1}) 29 239, 38 167, 48 076; IR: ν_{max} (cm^{-1}) 3215, 3112 (NH), 1621 (C=N), 1578 (C=C), 1244 (C–N), 765 (C=S); ^1H NMR (DMSO): (δ, ppm) 12.14 (1H, s, –NH), 11.71 (1H, s, –NH), 8.90 (1H, s, –CH=N), 0.91 (3H, t, $-CH_3$, $J = 6.7$ Hz), 8.34 (1H, d, indole C_4–H, $J = 7.1$ Hz), 7.91 (1H, s, indole C_2–H), 7.48 (1H, d, indole C_7–H, $J = 7.1$ Hz), 7.15–7.29 (2H, m, indole C_5–H and C_6–H), 3.96 (2H, t, $-CH_2$, $J = 6.7$ Hz), 3.67 (3H, s, $-CH_3$), 2.27 (2H, m, $-CH_2$), 1.59 (2H, m, $-CH_2$); ^{13}C NMR ($CDCl_3$): (δ, ppm) 177.76 (C=S), 140.73 (C=N), 137.00, 128.07, 123.37, 122.45, 121.67, 117.34, 112.56 (aryl–C), 37.40 (CH_3), 28.71 (CH_2), 14.03 (CH_3).

*3.1.8. Indole-3-carboxaldehyde-N(4)p-toluidine thiosemicarbazone, 3-ICA-p-TolTSC (**8**)*

Light yellow solid (DMSO); UV/vis: ν (cm^{-1}) 29 411, 38 759, 47 393; IR: ν_{max} (cm^{-1}) 3256, 3154 (NH), 1610 (C=N), 1544 (C=C), 1213 (C–N), 763 (C=S); ^1H NMR (DMSO): (δ, ppm) 12.17 (1H, s, –NH), 11.69 (1H, s, –NH), 11.55 (1H, s, –NH), 8.40 (1H, s, –CH=N), 8.23 (1H, d, indole C_4–H, $J = 7.6$ Hz), 7.91 (1H, s, indole C_2–H), 7.48 (1H, d, indole C_7–H, $J = 7.6$ Hz), 7.50 (2H, d, phenyl-H, $J = 7.8$ Hz), 7.12–7.23 (2H, m, indole C_5–H and C_6–H), 7.07 (2H, d, phenyl-H, $J = 7.8$ Hz), 2.32 (3H, s, $-CH_3$); ^{13}C NMR ($CDCl_3$): (δ, ppm) 173.08 (C=S), 134.11 (C=N), 137.19, 136.01, 134.1, 132.71, 130.74, 127.99, 126.7, 122.6, 121.8, 120.96, 120.28, 111.45 (aryl–C), 21.00 (CH_3).

*3.1.9. Indole-3-carboxaldehyde-N(4)o-toluidine thiosemicarbazone, 3-ICA-o-TolTSC (**9**)*

Light brown solid (DMSO); UV/vis: ν (cm^{-1}) 29 498, 38 759, 47 393; IR: ν_{max} (cm^{-1}) 3272, 3149 (NH), 1607 (C=N), 1547 (C=C), 1249 (C–N), 760 (C=S); ^1H NMR (DMSO): (δ, ppm) 12.17 (1H, s, –NH), 11.65 (2H, s, –NH), 11.52 (2H, s, –NH), 8.40 (1H, s, –CH=N), 8.30 (1H, d, indole C_4–H, $J = 7.8$ Hz), 7.87 (1H, s, indole C_2–H), 7.50 (2H, d, phenyl-H, $J = 7.8$ Hz), 7.42 (1H, d, indole C_7–H, $J = 7.8$ Hz), 7.15–7.28 (2H, m, indole C_5–H and C_6–H), 7.07–7.13 (2H, m, phenyl-H), 2.28 (3H, s, $-CH_3$); ^{13}C NMR ($CDCl_3$): (δ, ppm) 172.82 (C=S), 134.17 (C=N), 144.9, 136.02, 133.02, 127.99, 126.90, 124.68, 123.74, 122.6, 121.8, 120.80, 120.30, 116.05, 111.67 (aryl–C), 17.90 (CH_3).

*3.1.10. Indole-3-carboxaldehyde-N(4)m-toluidine thiosemicarbazone, 3-ICA-m-TolTSC (**10**)*

Green solid (DMSO); UV/vis: ν (cm^{-1}) 29 585, 37 735, 47 393; IR: ν_{max} (cm^{-1}) 3267, 3182 (NH), 1609 (C=N), 1550 (C=C), 1244 (C–N), 758 (C=S); ^1H NMR (DMSO): (δ, ppm) 12.18 (1H, s, –NH), 11.69 (1H, s, –NH), 11.58 (1H, s, –NH), 8.40 (1H, s, –CH=N), 8.41 (1H, s, phenyl-H), 8.35–8.82 (3H, m, phenyl-H), 8.33 (1H, d, indole C_4–H, $J = 7.8$ Hz), 7.91 (1H, s, indole C_2–H), 7.45 (1H, d, indole C_7–H, $J = 7.8$ Hz), 7.00–7.20 (2H, m, indole C_5–H and C_6–H), 2.33 (3H, s, $-CH_3$); ^{13}C NMR ($CDCl_3$): (δ, ppm) 174.23 (C=S), 134.16 (C=N), 137.20, 136.21, 136.01, 134.10, 132.56, 130.78, 128.01, 126.45, 122.76, 121.89, 120.96, 120.28, 111.67 (aryl–C), 21.13 (CH_3).

*3.1.11. Indole-3-carboxaldehyde-N(4,4)methylbenzyl thiosemicarbazone, 3-ICA-NMBzTSC (**11**)*

Dark yellow solid (DMSO); UV/vis: ν (cm^{-1}) 29 498, 37 735, 47 846; IR: ν_{max} (cm^{-1}) 3388, 3161 (NH), 1613

(C=N), 1540 (C=C), 1243 (C–N), 756 (C=S); ^{1}H NMR (DMSO): (δ, ppm) 12.13 (1H, s, –NH), 11.53 (1H, s, –NH), 8.46 (1H, s, –CH=N), 8.26 (1H, d, indole C_4–H, $J = 7.5$ Hz), 7.88 (1H, s, indole C_2–H), 7.74 (1H, d, indole C_7–H, $J = 7.5$ Hz), 7.26–7.46 (2H, m, indole C_5–H and C_6–H), 7.04–7.24 (5H, m, phenyl-H), 5.21 (2H, s, $-CH_2$), 3.18 (3H, s, $-CH_3$); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.12 (C=S), 140.73 (C=N), 139.75, 136.71, 132.71, 129.62, 129.59, 127.94, 127.73, 122.6, 121.80, 120.96, 120.28, 111.98 (aryl–C), 53.91 (CH_3), 38.90 (CH_3).

3.1.12. Indole-3-carboxaldehyde-N(4)2-chlorobenzyl thiosemicarbazone, 3-ICA-2-CBzTSC (**12**)

Dark yellow solid (DMSO); UV/vis: ν (cm^{-1}) 29 411, 38 759, 48 076; IR: ν_{max} (cm^{-1}) 3289, 3166 (NH), 1611 (C=N), 1547 (C=C), 1243 (C–N), 757 (C=S); ^{1}H NMR (DMSO): (δ, ppm) 12.15 (1H, s, –NH), 11.65 (1H, s, –NH), 11.46 (1H, t, –NH), 8.44 (1H, s, –CH=N), 8.28 (1H, d, indole C_4–H, $J = 8$ Hz), 7.85 (1H, s, indole C_2–H), 8.08 (1H, d, indole C_7–H, $J = 8$ Hz), 7.42–7.52 (2H, m, indole C_5–H and C_6–H), 7.08–7.33 (4H, m, phenyl-H), 4.75 (2H, d, $-CH_2$, $J = 6.5$ Hz); ^{13}C NMR ($CDCl_3$): (δ, ppm) 175.02 (C=S), 140.55 (C=N), 139.63, 136.70, 134.57, 132.71, 130.87, 130.21, 128.99, 127.91, 127.68, 122.60, 121.80, 120.96, 120.28, 111.63 (aryl–C), 49.31 (CH_2).

3.1.13. Indole-3-carboxaldehyde-N(4)2,4-difluoroaniline thiosemicarbazone, 3-ICA-2,4-diFATSC (**13**)

Grayish brown solid (DMSO); UV/vis: ν (cm^{-1}) 29 239, 38 461, 48 076; IR: ν_{max} (cm^{-1}) 3291, 3184 (NH), 1610 (C=N), 1540 (C=C), 1244 (C–N), 758 (C=S); ^{1}H NMR (DMSO): (δ, ppm) 12.14 (1H, s, –NH), 11.62 (1H, s, –NH), 11.18 (1H, s, –NH), 9.07 (1H, br s, –CH=N), 8.60 (1H, s, phenyl-H), 8.27 (2H, d, phenyl-H, $J = 8.8$ Hz), 8.15 (1H, d, indole C_4–H, $J = 9.5$ Hz), 7.81 (1H, s, indole C_2–H), 7.40 (1H, d, indole C_7–H, $J = 9.5$ Hz), 7.11–7.23 (2H, m, indole C_5–H and C_6–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 172.49 (C=S), 134.34 (C=N), 166.82 (C–F), 158.38 (C–F), 136.87, 132.67, 129.16, 127.99, 125.86, 122.6, 121.8, 120.96, 120.42, 120.28, 112.09 (aryl–C).

3.2. 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 palladium chloride (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.

3.2.1. Dichloro(indole-3-carboxaldehyde-N(4) thiosemicarbazone) palladium(II), [Pd(3-ICA-TSC)$_2$Cl$_2$] (**1a**)

Reddish brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 24 390, 26 954, 34 602, 45 248; IR: ν_{max} (cm^{-1}) 3376, 3229 (NH), 1587 (C=N), 1218 (C–N), 723 (C=S), 514, 457 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.16 (1H, s, –NH), 8.68 (2H, s, –NH), 8.56 (1H, s, –NH), 8.13 (1H, s, –CH=N), 7.09–7.64 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.21 (C=S), 142.92 (C=N), 136.70, 128.35, 122.6, 121.8, 120.29, 116.51, 111.63 (aryl–C); FABMS: *m/z* 396 [M], 367, 323, 157, 117, 107.

3.2.2. Dichloro(indole-3-carboxaldehyde-N(4)propyl thiosemicarbazone) palladium(II), [Pd(3-ICA-PTSC)$_2$Cl$_2$] (**2a**)

Dark brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 21 097, 36 954, 34 013, 45 248; IR: ν_{max} (cm^{-1}) 3263, 3118 (NH), 1590 (C=N), 1221 (C–N), 725 (C=S), 514, 459 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.14 (1H, s, –NH), 10.53 (1H, s, –NH), 8.47 (1H, s, –NH), 8.02 (1H, s, –CH=N), 1.63 (2H, m, $-CH_2$), 3.61 (2H, t, $-CH_2$, $J = 6.6$ Hz), 0.96 (3H, t, $-CH_3$, $J = 6.98$ Hz), 7.09–8.25 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 183.01 (C=S), 143.03 (C=N), 136.50, 128.38, 122.4, 121.7, 120.25, 116.51, 111.63 (aryl–C), 21.94 (CH_2), 11.41 (CH_3); FABMS: *m/z* 437 [M], 383, 209, 157, 117, 107.

3.2.3. Dichloro(indole-3-carboxaldehyde-N(4)isopropyl thiosemicarbazone) palladium(II), [Pd(3-ICA-IPTSC)$_2$Cl$_2$] (**3a**)

Dark brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 23 584, 26 666, 34 246, 45 248; IR: ν_{max} (cm^{-1}) 3357, 3128 (NH), 1590 (C=N), 1233 (C–N), 728 (C=S), 512, 457 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.52 (1H, s, –NH), 10.23 (1H, s, –NH), 8.15 (1H, s, –CH=N), 3.56 (1H, m, –CH), 1.15 (6H, d, $-CH_3$, $J = 6$ Hz), 6.95–8.46 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.08 (C=S), 144.08 (C=N), 136.58, 128.13, 122.12, 121.74, 120.48, 117.11, 111.74 (aryl–C), 41.41 (CH), 21.30 ($2CH_3$); FABMS: *m/z* 437 [M], 366, 214, 157, 117, 107.

3.2.4. Dichloro(indole-3-carboxaldehyde-N(4)butyl thiosemicarbazone) palladium(II), [Pd(3-ICA-BTSC)$_2$Cl$_2$] (**4a**)

Deep red solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 23 584, 26 954, 34 364, 46 512; IR: ν_{max} (cm^{-1}) 3363, 3135 (NH), 1595 (C=N), 1233 (C–N), 1099 (C=S), 528, 453 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.14 (1H, s, –NH), 8.65 (1H, s, –NH), 8.15 (1H, s, –CH=N), 1.63 (2H, m, $-CH_2$), 1.42 (2H, m, $-CH_2$), 1.04 (3H, t, $-CH_3$, $J = 7.1$ Hz), 7.22–7.73 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 180.56 (C=S), 142.76 (C=N), 136.74, 128.38, 122.67, 121.98, 121.45, 117.89, 112.03 (aryl–C), 44.61 (CH_2), 30.64 (CH_2), 19.90 (CH_2), 13.59 (CH_3); FABMS: *m/z* 452 [M], 273, 323, 209, 157, 117, 107.

3.2.5. Dichloro(indole-3-carboxaldehyde-N(4)isobutyl thiosemicarbazone) palladium(II), [Pd(3-ICA-IBTSC)$_2$Cl$_2$] (**5a**)

Deep red solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 23 697, 26 954, 34 247, 45 872; IR: ν_{max} (cm^{-1}) 3357, 3129 (NH), 1586 (C=N), 1232 (C–N), 731 (C=S), 517, 423

(Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.14 (1H, s, –NH), 8.45 (1H, s, –CH=N), 3.26 (2H, t, $-CH_2$, $J = 6.4$ Hz), 2.02 (1H, m, –CH), 0.94 (6H, d, $-CH_3$, $J = 6.6$ Hz), 7.23–8.64 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 178.80 (C=S), 143.84 (C=N), 137.84, 127.08, 123.36, 122.08, 121.45, 117.89, 111.78 (aryl–C), 51.78 (CH_2), 28.10 (CH), 20.42 ($2CH_3$); FABMS: *m/z* 452 [M], 273, 241, 157, 117, 107.

3.2.6. Dichloro(indole-3-carboxaldehyde-N(4,4)diethyl thiosemicarbazone) palladium(II), [Pd(3-ICA-DETSC)$_2$Cl$_2$] (**6a**)

Brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 24 155, 31 546, 34 364, 44 643; IR: ν_{max} (cm^{-1}) 3361, 3167 (NH), 1587 (C=N), 1243 (C–N), 735 (C=S), 548, 437 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.15 (1H, s, –NH), 10.37 (1H, s, –NH), 8.64 (1H, s, –CH=N), 3.71 (6H, m, $-CH_3$), 1.21 (4H, t, $-CH_2$, $J = 6$ Hz), 7.00–8.75 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 182.11 (C=S), 144.37 (C=N), 138.87, 128.08, 123.67, 122.56, 121.74, 117.43, 111.33 (aryl–C), 41.21 ($2CH_2$), 11.80 ($2CH_3$); FABMS: *m/z* 465 [M], 379, 273, 157, 117, 107.

3.2.7. Dichloro(indole-3-carboxaldehyde-N(4,4)methyl butyl thiosemicarbazone) palladium(II), [Pd(3-ICA-NMButTSC)$_2$Cl$_2$] (**7a**)

Light brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 20 619, 26 810, 34 130, 45 249; IR: ν_{max} (cm^{-1}) 3213, 3110 (NH), 1550 (C=N), 1231 (C–N), 737 (C=S), 537, 446 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.14 (1H, s, –NH), 9.51 (1H, s, –NH), 8.86 (1H, s, –NH), 8.74 (1H, s, –CH=N), 3.91 (2H, t, $-CH_2$, $J = 6.7$ Hz), 3.65 (3H, s, $-CH_3$), 2.27 (2H, m, $-CH_2$), 1.57 (2H, m, $-CH_2$), 7.02–7.69 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 181.96 (C=S), 145.01 (C=N), 137.00, 128.07, 123.37, 122.45, 121.67, 117.34, 112.56 (aryl–C), 37.40 (CH_3), 28.71 (CH_2), 14.03 (CH_3); FABMS: *m/z* 465 [M], 258, 157, 117, 107.

3.2.8. Dichloro(indole-3-carboxaldehyde-N(4)p-toluidine thiosemicarbazone) palladium(II), [Pd(3-ICA-p-TolTSC)$_2$Cl$_2$] (**8a**)

Orange solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 20 747, 26 954, 34 602, 47 393; IR: ν_{max} (cm^{-1}) 3254, 3150 (NH), 1586 (C=N), 1231 (C–N), 734 (C=S), 548, 457 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.17 (1H, s, –NH), 9.25 (1H, s, –NH), 8.79 (1H, s, –NH), 8.48 (1H, s, –CH=N), 2.32 (3H, s, $-CH_3$), 7.16–7.69 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.01 (C=S), 138.09 (C=N), 137.19, 136.01, 134.1, 132.71, 130.74, 127.99, 126.7, 122.6, 121.8, 120.96, 120.28, 111.45 (aryl–C), 21.00 (CH_3); FABMS: *m/z* 486 [M], 391, 379, 157, 117, 107, 77.

3.2.9. Dichloro(indole-3-carboxaldehyde-N(4)o-toluidine thiosemicarbazone) palladium(II), [Pd(3-ICA-o-TolTSC)$_2$Cl$_2$] (**9a**)

Maroon solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 20 161, 26 954, 34 483, 46 512; IR: ν_{max} (cm^{-1}) 3265, 3181 (NH), 1594 (C=N), 1231 (C–N), 1108 (C=S), 549, 447 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.17 (1H, s, –NH), 9.09 (1H, s, –NH), 8.64 (1H, s, –NH), 8.59 (1H, s, –CH=N), 2.23 (3H, s, $-CH_3$), 7.19–7.59 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 176.17 (C=S), 137.96 (C=N), 144.9, 136.02, 133.02, 127.99, 126.90, 124.68, 123.74, 122.6, 121.8, 120.80, 120.30, 116.05, 111.67 (aryl–C), 17.90 (CH_3); FABMS: *m/z* 486 [M], 451, 411, 397, 309, 157, 117, 107, 89.

3.2.10. Dichloro(indole-3-carboxaldehyde-N(4)m-toluidine thiosemicarbazone) palladium(II), [Pd(3-ICA-m-TolTSC)$_2$Cl$_2$] (**10a**)

Brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 20 747, 26 954, 34 843, 45 872; IR: ν_{max} (cm^{-1}) 3265, 3181 (NH), 1595 (C=N), 1231 (C–N), 739 (C=S), 535, 447 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.49 (1H, s, –NH), 9.25 (1H, s, –NH), 8.79 (1H, s, –NH), 8.48 (1H, s, –CH=N), 2.34 (3H, s, $-CH_3$), 6.77–8.19 (5H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.04 (C=S), 138.21 (C=N), 137.20, 136.21, 136.01, 134.10, 132.56, 130.78, 128.01, 126.45, 122.76, 121.89, 120.96, 120.28, 111.67 (aryl–C), 21.13 (CH_3). FABMS: *m/z* 486 [M], 470, 411, 309, 279, 209, 157, 117, 107, 88.

3.2.11. Dichloro(indole-3-carboxaldehyde-N(4,4)methylbenzyl thiosemicarbazone) palladium(II), [Pd(3-ICA-NMBzlTSC)$_2$Cl$_2$] (**11a**)

Dark brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 21 978, 26 810, 34 130, 46 512; IR: ν_{max} (cm^{-1}) 3386, 3159 (NH), 1588 (C=N), 1230 (C–N), 732 (C=S), 512, 437 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.12 (1H, s, –NH), 11.83 (1H, s, –NH), 8.72 (1H, s, –CH=N), 4.83 (2H, s, $-CH_2$), 3.18 (3H, s, $-CH_3$), 7.08–8.79 (10H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 185.56 (C=S), 143.76 (C=N), 139.75, 136.71, 132.71, 129.62, 129.59, 127.94, 127.73, 122.6, 121.80, 120.96, 120.28, 111.98 (aryl–C), 53.91 (CH_3), 38.90 (CH_3); FABMS: *m/z* 499 [M], 289, 214, 209, 157, 117, 107, 105.

3.2.12. Dichloro(indole-3-carboxaldehyde-N(4)2-chlorobenzyl thiosemicarbazone) palladium(II), [Pd(3-ICA-2-CBTSC)$_2$Cl$_2$] (**12a**)

Dark brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 22 831, 26 954, 34 364, 45 872; IR: ν_{max} (cm^{-1}) 3289, 3164 (NH), 1596 (C=N), 1232 (C–N), 736 (C=S), 544, 446 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.07 (1H, s, –NH), 11.59 (1H, s, –NH), 8.71 (1H, s, –NH), 8.62 (1H, s, –CH=N), 4.74 (2H, d, $-CH_2$, $J = 6.6$ Hz), 7.12–8.13 (9H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 183.72 (C=S), 144.07 (C=N), 139.63, 136.70, 134.57, 132.71, 130.87, 130.21, 128.99, 127.91, 127.68, 122.60, 121.80, 120.96, 120.28, 111.63 (aryl–C), 49.31 (CH_2); FABMS: *m/z* 520 [M], 504, 368, 260, 214, 157, 117, 107.

*3.2.13. Dichloro(indole-3-carboxaldehyde-N(4)2,4-difluoroaniline thiosemicarbazone) palladium(II), [Pd(3-ICA-2,4-DFATSC)$_2$Cl$_2$] (**13a**)*

Blackish brown solid (methanol–DMSO); UV/vis: ν (cm^{-1}) 21 277, 36 810, 34 483, 47 619; IR: ν_{max} (cm^{-1}) 3289, 3183 (NH), 1590 (C=N), 1221 (C–N), 730 (C=S), 545, 461 (Pd–N, Pd–S); ^{1}H NMR (DMSO-d_6): (δ, ppm) 12.14 (1H, s, –NH), 11.05 (1H, s, –NH), 10.48 (1H, s, –NH), 8.96 (1H, s, –CH=N), 7.08–8.89 (8H, m, aryl–H); ^{13}C NMR ($CDCl_3$): (δ, ppm) 179.06 (C=S), 139.96 (C=N), 166.82 (C–F), 158.38 (C–F), 136.87, 132.67, 129.16, 127.99, 125.86, 122.6, 121.8, 120.96, 120.42, 120.28, 112.09 (aryl–C). FABMS: *m/z* 543 [M], 442, 184, 157, 117, 107, 91.

3.3. In vitro testing against E. histolytica

All the *N*(4)-substituted indole thiosemicarbazones (**1**–**13**) and their palladium complexes (**1a**–**13a**) were screened *in vitro* for antiamoebic activity against (*HM1:IMSS*) strain of *E. histolytica* by using a microplate method [36,37]. DMSO (40 μL) [38,39] was added to all the samples (~1 mg) followed by enough culture medium to obtain a concentration of 1 mg/mL. 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. Twofold 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 test was used to confirm viability. The cell suspension used was diluted to 10^5 organisms/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 cm 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 % 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.

3.4. 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 [40]. 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 μM 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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References

[1] S.L. Stanley Jr., Lancet 361 (2003) 1025–1034.
[2] S.M. Townson, H. Laqua, P.F.L. Boreman, P. Upcroft, J.A. Upcroft, Trans. R. Soc. Trop. Med. Hyg. 86 (1922) 521–522.
[3] A. Azam, S.M. Agarwal, Current Bioactive Compounds 3 (2007) 121–133.
[4] K.C. Joshi, P. Chand, Pharmazie 37 (1982) 1–12.
[5] K.C. Joshi, P. Chand, Heterocycles 23 (1985) 957–968.
[6] T. Owa, T. Okauchi, K. Yoshimatsu, N.H. Sugi, Y. Ozawa, T. Nagasu, N. Koyanagi, T. Okabe, K. Kitoh, H. Yoshino, Bioorg. Med. Chem. Lett. 10 (2000) 1223–1226.
[7] E.B. Skibo, C. Xing, T. Robert, J. Med. Chem. 44 (2001) 3545–3562.
[8] T.P. Zabawa, S.R. Chemler, Org. Lett. 9 (2007) 2035–2038.
[9] A.N. Acharya, J.M. Ostresh, R.A. Houghten, J. Org. Chem. 66 (2001) 8673–8676.
[10] L. Matzen, C. van Amsterdam, W. Rautenberg, H.E. Greiner, J. Harting, C.A. Seyfried, H. Bottcher, J. Med. Chem. 43 (2000) 1149–1157.
[11] C.P. Chow, K.J. Shea, S.M. Sparks, Org. Lett. 4 (2002) 2637–2640.
[12] S. Maayani, H. Weistein, S. Cohen, M. Sokolovsky, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 3103–3107.
[13] R.H. Khan, R.C. Rastogi, J. Agric. Food Chem. 39 (1991) 2300–2303.
[14] C. Gil, A. Schwogler, S. Brase, J. Comb. Chem. 6 (2004) 38–42.
[15] Z. Wang, Y. Yuan, Y. Chen, G. Sun, X. Wu, S. Zhang, C. Han, G. Wang, L. Li, G. Liu, J. Comb. Chem. 9 (2007) 652–660.
[16] S. Tu, C. Li, G. Li, L. Cao, Q. Shao, D. Zhou, B. Jiang, J. Zhou, M. Xia, J. Comb. Chem. 9 (2007) 1144–1148.

[17] T.Y. Shen, T.B. Windholz, A. Rosegay, B.E. Witzel, A.N. Wilson, J.D. Willett, W.J. Holtz, R.L. Ellis, A.R. Matzuk, S. Lucas, C.H. Stammer, F.W. Holly, L.H. Sarett, E.A. Risley, G.W. Nuss, C.A. Winter, J. Am. Chem. Soc. 85 (1963) 488–489.
[18] G.S. Gadaginamath, S.A. Patil, Indian J. Chem. 35B (1996) 681–684.
[19] D.R. Richardson, Crit. Rev. Oncol. Hematol. 42 (2002) 267–281.
[20] D.B. Lovejoy, D.R. Richardson, Blood 100 (2002) 666–676.
[21] P. Jutten, W. Schumann, A. Hartl, L. Heinisch, U. Grafe, W. Werner, H. Ulbricht, Bioorg. Med. Chem. Lett. 12 (2002) 1339–1342.
[22] A.G. Quiroga, C.N. Ranninger, Coord. Chem. Rev. 248 (2004) 119–133.
[23] A.I. Matesanz, J.M. Perez, P. Navarro, J.M. Moreno, E. Colacio, P. Souza, J. Inorg. Biochem. 76 (1999) 29–37.
[24] L. Szűčová, Z. Trávníček, M. Zatloukal, I. Popa, Bioorg. Med. Chem. 14 (2006) 479–491.
[25] A.G. Quiroga, J.M. Perez, E.I. Montero, J.R. Masaguer, C. Alonso, C.N. Ranninger, J. Inorg. Biochem. 70 (1998) 117–123.
[26] S. Singh, N. Bharti, N. Naqvi, A. Azam, Eur. J. Med. Chem. 39 (2004) 459–465.
[27] N. Bharti, S. Singh, S. Sharma, F. Naqvi, A. Azam, Bioorg. Med. Chem. 11 (2003) 2923–2929.
[28] D.G. O'Sullivan, P.W. Sadler, C. Webley, Chemotherapia 7 (1963) 17–24.
[29] J.H. Price, A.N. Williamson, R.F. Schramm, B.B. Wayland, Inorg. Chem. 116 (1972) 1280–1284.
[30] M.O. Albers, T.V. Ashworth, H.E. Oosthuizen, E. Singleton, Inorg. Synth. 26 (1989) 68–77.
[31] S.A.J. Chandra, Sangeetika, Rathi, J. Saudi Chem. Soc. 5 (2001) 175–182.
[32] S. Chandra, U. Kumar, Spectrochim. Acta, Part A 60 (2004) 2825–2829.
[33] B. Singh, H. Mishra, J. Indian Chem. Soc. 63 (1986) 692–695.
[34] Shailendra, N. Bharti, F. Naqvi, A. Azam, Bioorg. Med. Chem. Lett. 13 (2003) 689–692.
[35] B.G. Tweedy, Phytopathology 55 (1964) 910.
[36] C.W. Wright, M.J. O'Neill, J.D. Phillipson, D.C. Warhurst, Antimicrob. Agents Chemother. 32 (1988) 1725–1729.
[37] L.S. Diamond, D.R. Harlow, C.C. Cunnick, Trans. R. Soc. Trop. Med. Hyg. 72 (1978) 431–432.
[38] F.D. Gillin, D.S. Reiner, M. Suffness, Antimicrob. Agents Chemother. 22 (1982) 342–345.
[39] A.T. Keen, A. Harris, J.D. Phillipson, D.C. Warhurst, Planta Med. (1986) 278–284.
[40] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.