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ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY · AUGUST 2003

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New Palladium(II) Complexes of 5-Nitrothiophene-2-carboxaldehyde Thiosemicarbazones: Synthesis, Spectral Studies and In Vitro Anti-Amoebic Activity

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Received 11 November 2002; accepted 24 March 2003

Abstract—Thiosemicarbazones (**1**–**7**) and their palladium(II) complexes (**1a**–**7a**) of the type $[Pd(TSCN)Cl_2]$ (where TSCN = thiosemicarbazone) were prepared from 5-nitro thiophene-2-carboxaldehyde and $[Pd(DMSO)_2Cl_2]$, respectively. Coordination via the thionic sulphur and the azomethine nitrogen atom of the thiosemicarbazones to the metal ion were confirmed by spectral data. These compounds were screened in vitro against (*HK-9*) strain of *Entamoeba histolytica* possess amoebicidal properties. Enhancement of antiamoebic activity resulted due to the introduction of palladium metal in the thiosemicarbazone moiety. The most promising of the group tested are $[Pd(5\text{-N-2-TCA-COTSCN})Cl_2]$ and $[Pd(5\text{-N-2-TCA-AdmTSCN})Cl_2]$ comparable to that of metronidazole.

Introduction

Entamoeba histolytica, the causative agent for human amoebiasis, is an enteric protozoan parasite capable of invading the mucosa of the colon and entering the blood circulation to reach various extraintestinal organs.[1] Fifty million people suffer from amoeba induced colitis or extra intestinal abscesses resulting in 100,000 deaths annually.[2] The nitroimidazoles are the principal drugs of choice in the treatment of amoebiasis since they are effective against extra intestinal and intestinal wall infection. Metronidazole, the leading drug, has been shown to be both mutagenic and carcinogenic in animals.[3,4] A new amoebicide with at least equivalent efficacy to metronidazole, well tolerated, with no carcinogenic potential, would provide a new dimension in therapy.

Thiosemicarbazones are of considerable interest because of their chemistry and potentially beneficial biological activities, such as antitumor, antibacterial, antiviral and antimalarial activities.[5–12] The biological activities of thiosemicarbazones are considered to be due to their ability to form chelates with heavy metals.[13–15] Biological activities of metal complexes differ from those of either ligands or the metal ions and increased and/or decreased biological activities are reported for several transition metal complexes, such as copper(II) and nickel(II).[10–12] Thiosemicarbazones are versatile compounds: structural isomers (*E*, *E*′, *Z*) are reported[15] and they coordinate to the metal either as a neutral ligand or as a deprotonated ligand through the S, N, N atoms or through the O, N, S atoms.[16] Thiosemicarbazones containing a pyridine ring give rise to NNS tridentate systems have been extensively investigated due to their biological properties.[17,18] However, thiosemicarbazones with non-pyridine heterocyclic rings have not been exhaustively studied.[19–21]

Uses of metal ions in therapeutic agents are known to accelerate drug action and their efficacy enhanced upon the coordination with a metal ion.[22,23] The classical coordination complex, *cis*-DDP or cisplatin (*cis*-diammine dichloroplatinum),[24] has been the subject of much recent attention towards the metal-based chemotherapy, because of its beneficial effects in the treatment of cancer.[25,26] The significant anti-trypanosomal activity[27] of semicarbazones of 5-nitrothiophene-2-carboxaldehyde led us to study the screening of thiosemicarbazones and their palladium(II) complexes as antiamoebic agents. Continuing our efforts to

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doi:10.1016/S0968-0896(03)00213-X

develop improved antiamoebic drugs, we have recently reported Pd(II) and Ru(II) complexes of NS donor ligands, which showed promising activity against *E. histolytica*.[28–30]

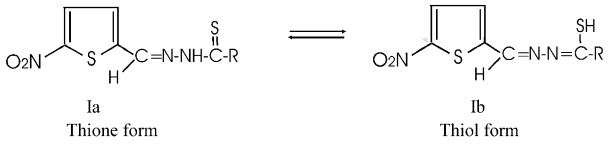
The present work deals with the synthesis of new thiosemicarbazones (Fig. 1) and their Pd(II) complexes (Fig. 2). The structures of all the compounds were elucidated by IR, UV, ^{1}H NMR and elemental analysis. These compounds were tested in vitro against *HK-9* strain of *E. histolytica* for their ability to inhibit the growth of parasite, resulted that the chelation induce significant changes in the antiamoebic activity.

Results and Discussion

Reaction of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones with $[Pd(DMSO)_2Cl_2]$ gave amorphous solid compounds. All the compounds were isolated in good yield and are stable both in the solid and solution state. Analytical data of these compounds are in good agreement with their composition (Table 1). The complexes are insoluble in water, methanol and ethanol, soluble in DMF and DMSO. The complexes do not undergo any weight loss up to 240 °C, which suggest their fair thermal stability. The structure of the complexes presented in Figure 2 was established by comparing spectral data (IR, UV–visible and ^{1}H NMR) with the free ligand along with their thermo gravimetric analysis.

IR and electronic spectral studies of the complexes

The thiosemicarbazones **1**–**7** can exist thione and thiol tautomeric form **Ia** and **Ib**, respectively.



Ia
Thione form

Ib
Thiol form

However, the existence of a strong band in the region 1028–1082 cm^{-1} due to ν(C=S) and absence of any band in the region 2500–2600 cm^{-1} due to ν(C–SH) suggesting that all the thiosemicarbazones remain in

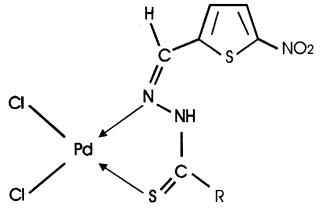
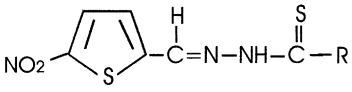


Figure 2. Structure of palladium(II) complexes: (1a) R = –NHC_7H_7; (2a) R = –NHC_7H_7; (3a) R = –NHC_7H_7; (4a) R = –NC_5H_{12}; (5a) R = –NC_8H_{10}; (6a) R = –NHC_8H_{15}; (7a) R = –$NHC_{10}H_{15}$).



Thiosemicarbazones (1-7)

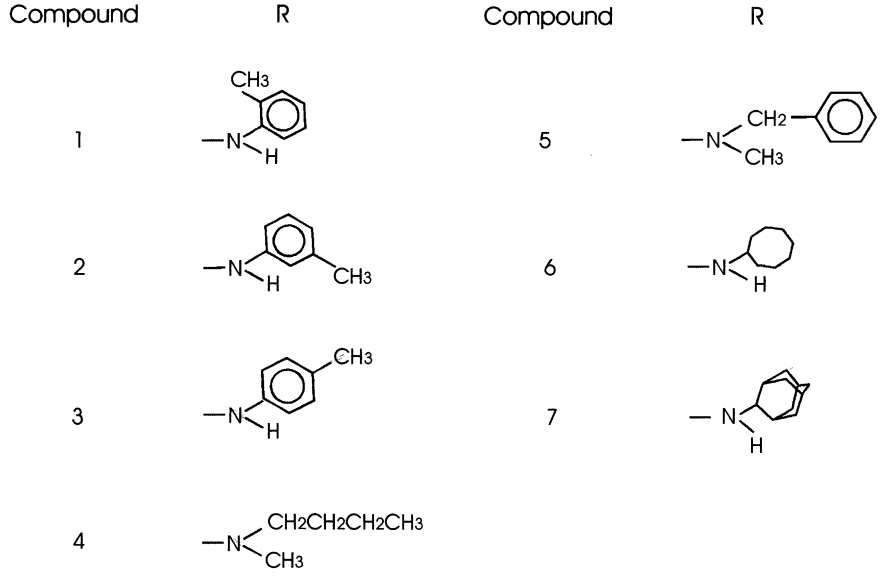


Figure 1. 1. 5-N-2-TCA-*o*-TolTSCN, 5-nitrothiophene-2-carboxaldehyde-*o*-toludinethiosemicarbazone; 2. 5-N-2-TCA-*m*-TolTSCN, 5-nitrothiophene-2-carboxaldehyde-*m*-toludinethiosemicarbazone; 3. 5-N-2-TCA-*p*-TolTSCN, 5-nitrothiophene-2-carboxaldehyde-*p*-toludinethiosemicarbazone; 4. 5-N-2-TCA-NMBuTSCN, 5-nitrothiophene-2-carboxaldehyde-*n*-methylbutylthiosemicarbazone; 5. 5-N-2-TCA-NMBzTSCN, 5-nitrothiophene-2-carboxaldehyde-n-methylbenzylthiosemicarbazone; 6. 5-N-2-TCA-COTSCN, 5-nitrothiophene-2-carboxaldehyde-cyclooctylthiosemicarbazone; 7. 5-N-2-TCA-AdmTSCN, 5-nitrothiophene-2-carboxaldehyde-admantaminethiosemicarbazone.

Table 1. Analytical and physicochemical data of thiosemicarbazones and their new palladium(II) complexes

S. no.	Compd/stoichiometry[a]	Colour	Yield (%)	Mp/Dec. temp (°C)	Found (calcd)			
					C	H	N	Cl
1.	5-N-2-TCA-*o*-TolTSCN $C_{13}H_{12}N_4S_2O_2$	Yellow	49	180	48.92 (48.75)	3.56 (3.75)	17.43 (17.50)	
1a	[Pd(5-N-2-TCA-*o*-TolTSCN)Cl_2] $C_{13}H_{12}N_4S_2O_2Cl_2Pd$	Brick red	51	197	31.10 (31.45)	2.56 (2.42)	11.43 (11.29)	14.20 (14.31)
2.	5-N-2-TCA-*m*-TolTSCN $C_{13}H_{12}N_4S_2O_2$	Reddish yellow	53	195	48.90 (48.75)	3.58 (3.75)	17.47 (17.50)	
2a	[Pd(5-N-2-TCA-*m*-TolTSCN)Cl_2] $C_{13}H_{12}N_4S_2O_2Cl_2Pd$	Brick red	43	205	31.15 (31.45)	2.54 (2.42)	11.56 (11.29)	14.59 (14.31)
3.	5-N-2-TCA-*p*-TolTSCN $C_{13}H_{12}N_4S_2O_2$	Yellow	51	200	48.91 (48.75)	3.60 (3.75)	17.53 (17.50)	
3a.	[Pd(5-N-2-TCA-*p*-TolTSCN)Cl_2] $C_{13}H_{12}N_4S_2O_2Cl_2Pd$	Brick red	47	207	31.29 (31.45)	2.56 (2.42)	11.43 (11.29)	14.20 (14.31)
4.	5-N-2-TCA-NMBuTSCN $C_{11}H_{16}N_4S_2O_2$	Dark brown	72	188	44.34 (44.00)	5.39 (5.33)	18.92 (18.67)	
4a.	[Pd(5-N-2-TCA-NMBuTSCN)Cl_2] $C_{11}H_{16}N_4S_2O_2Cl_2Pd$	Brick red	67	222	27.34 (27.73)	3.49 (3.36)	11.92 (11.74)	14.67 (14.92)
5.	5-N-2-TCA-NMBzTSCN $C_{14}H_{14}N_4S_2O_2$	Red	87	184	50.05 (50.30)	4.40 (4.19)	16.44 (16.77)	
5a.	[Pd(5-N-2-TCA-NMBzTSCN)Cl_2] $C_{14}H_{14}N_4S_2O_2Cl_2Pd$	Brick red	56	235	32.72 (32.94)	2.40 (2.74)	11.24 (10.98)	14.04 (13.92)
6.	5-N-2-TCA-COTSCN $C_{14}H_{20}N_4S_2O_2$	Light yellow	52	183	49.57 (49.41)	6.01 (5.88)	16.24 (16.47)	
6a.	[Pd(5-N-2-TCA-COTSCN)Cl_2] $C_{14}H_{20}N_4S_2O_2Cl_2Pd$	Brick red	63	214	32.40 (32.56)	3.54 (3.88)	10.41 (10.85)	13.59 (13.76)
7.	5-N-2-TCA-AdmTSCN $C_{16}H_{24}N_4S_2O_2$	Brownish yellow	67	154	51.85 (52.17)	6.89 (6.52)	15.50 (15.22)	
7a.	[Pd(5-N-2-TCA-AdmTSCN)Cl_2] $C_{16}H_{24}N_4S_2O_2Cl_2Pd$	Brick red	73	218	35.64 (35.55)	3.56 (3.70)	10.43 (10.37)	13.40 (13.15)

[a]For abbreviations, see Figure 1.

their thione form. The band due to ν(C–S–C) (ring) of thiophene moiety remains unaltered thereby indicating the non-involvement of ring sulphur in coordination. The negative shift (14–39 cm^{-1}) of ν(C=N) band observed in all complexes indicates the involvement of azomethine nitrogen upon complexation.[31] This was further supported by the upward shift of N–N band of ligand on coordination. The strong band at 1028–1082 cm^{-1} ascribed to ν(C=S) of ligands is shifted to lower frequency (13–35 cm^{-1}) indicating the bonding of metal through thionic sulphur. The broad band observed in region 3159–3251 cm^{-1} due to ν(N–H) stretch is only slightly affected on the coordination. The preferential coordination of thionic sulphur over sulphur of thiophene is due to more nucleophilic character of the former.

The electronic spectra of the ligands exhibit three bands in the region 24,691–25,752, 35,461–37,879 and 47,846–48,780 cm^{-1}. The probable assignment for these bands are due to the $n\rightarrow\pi^*$, $\pi\rightarrow\pi^*$ and $\varphi\rightarrow\varphi^*$ transitions, respectively. In the spectra of complexes, these bands appeared at ca. 21,500, 37,000 and 49,500 cm^{-1} respectively with little change in the energy of these bands. The band appeared at ca. 37,000 and 49,500 cm^{-1} are assigned to $\pi\rightarrow\pi^*$ and $\varphi\rightarrow\varphi^*$ transitions, respectively. As intensity of the band appearing at ca. 21500 cm^{-1} is comparable with other ligand bands, this is assigned due to $n\rightarrow\pi^*$ transition. The band due to ligand to metal charge transfer transition is probably appearing underneath to $n\rightarrow\pi^*$ transition and is usually not seen in the present complexes. Such observations have also been noticed earlier in other palladium(II) complexes of similar ligands systems.[32,33]

^{1}H NMR spectral analysis

Further evidence for the coordinating mode of the thiosemicarbazones **1**–**7** was obtained from ^{1}H NMR spectra. The ^{1}H NMR spectra of thiosemicarbazones **1**–**7** recorded in DMSO-d_6 exhibit a broad peak at 9.82–10.98 ppm due to –NH proton, which indicate that even in a polar solvent they remain in the thione form. The –NH proton signal of the thiosemicarbazones usually shifts to up field and appear at 3.32–3.71 ppm in their respective complexes. In complexes **4a** and **6a**, we are unable to locate –NH proton signal. This either merges with aromatic protons or resonates beyond 15 ppm. This information suggests the adjustment of electronic current upon coordination of >C=S group to the metal ion. Other protons, namely viz. CH_3 protons, CH_2 protons and aryl carbons in complexes **1a**–**7a** resonate nearly at the same region as that of free ligands.

TGA analysis

The TGA (under nitrogen, rate 10 °/min) profiles of complexes along with the % weight at different temperatures were recorded. These complexes do not lose weight up to 245 °C. Further increment of temperature causes decomposition of the complexes in two steps. The temperature range for the first step being 245–395 °C where loss of mixed fragments was observed. The second

step starts immediately after first one and continues until the complete decomposition of the ligand and formation of MS [M = Pd(II)] as the end product. The total% weight loss corresponds to the loss of the respective ligand after considering the transfer of one sulphur atom to the metal ion and residue corresponds to the metal sulphide.

Biological activity

All the compounds were evaluated for anti-amoebic activity in vitro using HK-9 strain of *E. histolytica*. The IC_{50} values in micro molar are shown in Table 2. The free ligands **1**–**7** exhibited anti-amoebic activity with IC_{50} of 1.73–7.70 μ M. Considering the substitutions at N^4 position in thiosemicarbazones with cyclooctyl amine (**6**) and adamantamine (**7**) turned out to be potent anti-amoebic agents (IC_{50} = 1.71–1.73 μM) as compared to standard anti-amoebic drug, metronidazole (IC_{50} = 2.05 μM). Complexation of the thiosemicarbazones with Pd(II) results in compounds (**1a**–**7a**), which showed a significant improvement in anti-amoebic activity towards HK-9 strain of *E. histolytica* (IC_{50} = 0.73–4.06 μM). All the complexes are more active than their respective ligands indicate that the complexation to metal enhances the activity of the ligand. This may be explained by Tweedy's theory,[34] 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 most active compounds in this class were again those thiosemicarbazone Pd(II) complexes, which have cyclooctyl amine (**6a**, IC_{50} = 0.81 μ M) and adamantamine (**7a**, IC_{50} = 0.73 μ M) as N^4 substitution. It is concluded that the presence of these bulky groups at position N^4 of the thiosemicarbazone moiety enhanced anti-amoebic activity. It has also been observed by the other researchers.[35] The Pd-complex precursor $[Pd(DMSO)_2Cl_2]$ was also evaluated for anti-amoebic activity and compared with Pd(II) complexes and metronidazole, which showed no activity against *E. histolytica*. In our earlier studies, it was found that the transition metal complexes of NS donor ligands showed good anti-amoebic activity against the same strain of *E. histolytica*.[28–30] It was noted that antiparasitic activity was limited to those compounds in which the alkylidene group is attached to the 2-position, rather than 3- or 4-position of the heterocyclic ring and also to those in which a thiocarbonyl, rather than a carbonyl group, is present.[36]

Table 2. In vitro anti-amoebic activities of thiosemicarbazones and their Pd(II) complexes against (HK-9) strain of *E. histolytica*

S. no.	Compd	IC_{50} (μM)	SD[a]
1.	5-N-2-TCA-*o*-TolTSCN	4.59	0.87
1a.	[Pd(5-N-2-TCA-*o*-TolTSCN)Cl_2]	1.95	0.26
2.	5-N-2-TCA-m-TolTSCN	4.78	0.74
2a.	[Pd(5-N-2-TCA-*m*-TolTSCN)Cl_2]	2.05	0.33
3.	5-N-2-TCA-*p*-TolTSCN	4.65	0.82
3a.	[Pd(5-N-2-TCA-*p*-TolTSCN)Cl_2]	1.99	0.35
4.	5-N-2-TCA-NMBuTSCN	7.70	1.69
4a.	[Pd(5-N-2-TCA-NMBuTSCN)Cl_2]	4.06	1.78
5.	5-N-2-TCA-NMBzTSCN	3.71	0.54
5a.	[Pd(5-N-2-TCA-NMBzTSCN)Cl_2]	1.70	0.36
6.	5-N-2-TCA-COTSCN	1.73	0.37
6a.	[Pd(5-N-2-TCA-COTSCN)Cl_2]	0.81	0.22
7.	5-N-2-TCA-AdmTSCN	1.71	0.41
7a.	[Pd(5-N-2-TCA-AdmTSCN)Cl_2]	0.73	0.18
	$[Pd(DMSO)_2Cl_2]$	8.15	1.73
	Metronidazole	2.05	0.33

[a]SD, standard deviation.

The importance of such work lies in the possibility that the new complexes might be more efficacious drugs against amoebiasis for which a thorough investigation regarding the structure–activity of the complexes and their stability is required in order to understand the variation in their biological effects, which could be helpful in designing more potent anti-amoebic agents therapeutic use.

Conclusion

Although the synthesis of thiosemicarbazones and their metal complexes were reported several years ago, very little is known about their anti-amoebic activity. This research examined the biological activities of the new thiosemicarbazones prepared from 5-nitrothiophene-2-carboxaldehyde and their Pd(II) complexes. The substituents did not have any influence on the coordination and chelation of compounds that showed the same SN bidentate behavior. HK-9 strain of E. histolytica was employed for in vitro anti-amoebic evaluation. The biological behavior revealed that most of the ligands show a weak activity against *E. histolytica*. The chelation induced significant changes in the biological activity of the ligands and the palladium complexes **6a** and **7a** have shown greater activity, whereas complexes **1a**–**3a** and **5a** showed similar activity as metronidazole in vitro.

Experimental

Materials and methods

Palladium chloride was purchased from Aldrich chemical company (USA). All the Cycloalkylaminothiocarbonylhydrazines were prepared as reported earlier.[37] The metal precursor $[Pd(DMSO)_2Cl_2]$ was prepared by the literature method.[38] Elemental analysis (C, H, N) was carried out by Central Drug Research Institute, Lucknow, India. Chlorine was estimated by standard method. Melting points were recorded on a KSW melting point apparatus and were uncorrected. Electronic spectra were recorded in DMF on a Shimadzu UV-1601 PC UV-Visible spectro-photometer. IR spectra on KBr disks were recorded on a Perkin-Elmer model 1620 FT-IR spectrophotometer. ^1H NMR spectra were obtained at ambient temperature using a Bruker spectrospin DPX-300 MHz spectrophotometer in $CDCl_3$ and DMSO-d_6 using tetramethylsilane as an internal standard. Thermograms of the complexes were recorded under nitrogen on a TG 51 thermo gravimetric analyzer with increasing the temperature at 10 °C per minute.

Synthesis of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones

All thiosemicarbazones were synthesized by mixing an aqueous solution of cycloalkylaminothiocarbonylhydrazines (0.003 mol in 10 mL) and ethanolic solution of 5-nitrothiophene-2-carboxaldehyde (0.003 mol in 10 mL) at 25 °C for 3 h with continuous stirring. After cooling, the precipitated compound was filtered and recrystallized from appropriate solvent.

5-N-2-TCA-*o*-TolTSCN (1). Yellow solid (methanol); λ_{max}/cm^{-1}: 25,752, 35,722, 48,490; IR: ν_{max}/cm^{-1}: 3236 (NH), 1587 (C=N), 1502 (C=C), 1113 (C–N), 1035 (C=S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 8.42 (1H, s, –CH=N), 10.61 (2H, s, –NH), 1.98 (3H, s, $–CH_3$), 7.20–8.04 (6H, m, aryl).

5-N-2-TCA-*m*-TolTSCN (2). Reddish yellow solid (methanol); λ_{max}/cm^{-1}: 24,752, 35,842, 48,780; IR: ν_{max}/cm^{-1}: 3217 (NH), 1600 (C=N), 1504 (C=C), 1143 (C–N), 1034 (C=S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 8.04 (1H, s, –CH=N), 9.87 (2H, s, –NH), 2.53 (3H, s, $–CH_3$), 7.03–8.42 (6H, m, aryl).

5-N-2-TCA-*p*-TolTSCN (3). Yellow solid (methanol); λ_{max}/cm^{-1}: 24,938, 37,879, 48,309; IR: ν_{max}/cm^{-1}: 3239 (NH), 1599 (C=N), 1531 (C=C), 1142 (C–N), 1035 (C=S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 8.36 (1H, s, –CH=N), 9.82 (2H, s, –NH), 2.40 (3H, s, $–CH_3$), 6.98–7.85 (6H, m, aryl).

5-N-2-TCA-NMBuTSCN (4). Dark brown solid (acetone); λ_{max}/cm^{-1}: 24,846, 37,879, 48,537; IR: ν_{max}/cm^{-1}: 3221 (NH), 1525 (C=N), 1487 (C=C), 1123 (C–N), 1047 (C=S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 8.37 (1H, s, CH=N), 10.98 (1H, s, –NH), 4.43 (6H, m, $–CH_2$), 2.69 (3H, s, $–CH_3$), 2.47 (3H, t, $–CH_3$), 7.09–7.94 (2H, m, aryl).

5-N-2-TCA-NMBzTSCN (5). Red solid (chloroform); λ_{max}/cm^{-1}: 24,938, 37,313, 47,846; IR: ν_{max}/cm^{-1}: 3186 (NH), 1524 (C=N), 1493 (C=C), 1105 (C–N), 1028 (C=S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 8.46 (1H, s, –CH=N), 10.79 (1H, s, –NH), 4.63 (2H, s, $–CH_2$), 2.89 (3H, s, $–CH_3$), 7.21–8.06 (7H, m, aryl).

5-N-2-TCA-COTSCN (6). Light yellow solid (methanol); λ_{max}/cm^{-1}: 24,691, 35,461, 48,020; IR: ν_{max}/cm^{-1}: 3159 (NH), 1580 (C=N), 1525 (C=C), 1136 (C–N), 1082 (C=S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 8.33 (1H, s, –CH=N), 9.87 (1H, s, –NH), 8.19 (1H, d, –NH), 4.45 (14H, m, $–CH_2$), 7.02–7.67 (2H, m, aryl).

5-N-2-TCA-AdmTSCN (7). Brownish yellow solid (methanol); λ_{max}/cm^{-1}: 24,938, 37,879, 48,309; IR: ν_{max}/cm^{-1}: 3251 (NH), 1584 (C=N), 1518 (C=C), 1132 (C–N), 1074 (C=S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 8.29 (1H, s, –CH=N), 10.04 (1H, s, –NH), 8.45 (1H, d, –NH), 4.53 (18H, m, $–CH_2$), 7.21–8.03 (2H, m, aryl).

Preparation of palladium(II) complexes

All Pd(II) complexes were prepared by mixing the equimolar ratio of ligand and $[Pd(DMSO)_2Cl_2]$ in refluxing methanol. The solution was kept at 0 °C overnight, the product was separated by filtration and finally washed with methanol. Recrystallization was effected in methanol/DMF (8:2).

$$[Pd(DMSO)_2Cl_2] + TSCN \xrightarrow[5\ h,\ reflux]{CH_3OH} [Pd(TSCN)Cl_2] + 2\ DMSO$$

(where TSCN = thiosemicarbazones **1**–**7**).

[Pd(5-N-2-TCA-oTolTSCN)Cl_2] (1a). Brick red solid; λ_{max}/cm^{-1}: 21,453, 36,418, 49,513; IR: ν_{max}/cm^{-1}: 3435 (NH), 1563 (C=N), 1500 (C=C), 1018 (C=S), 495, 428 (M–N, M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm.: 7.96 (1H, s, –CH=N), 3.68 (1H, s, –NH), 10.38 (1H, s, –NH), 2.03 (3H, s, $–CH_3$), 7.23–8.09 (6H, m, Aryl).

[Pd(5-N-2-TCA-mTolTSCN)Cl_2] (2a). Brick red solid; λ_{max}/cm^{-1}: 21,945, 36,315, 49,419; IR: ν_{max}/cm^{-1}: 3437 (NH), 1582 (C=N), 1499 (C=C), 1012 (C=S), 498, 441 (M–N, M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 7.34 (1H, s, –CH=N), 3.71 (1H, s, –NH), 9.96 (1H, s, –NH), 2.06 (3H, s, $–CH_3$), 7.20–8.02 (6H, m, Aryl).

[Pd(5-N-2-TCA-p-TolTSCN)Cl_2] (3a). Brick red solid; λ_{max}/cm^{-1}: 22,340, 38,112, 49,415; IR: ν_{max}/cm^{-1}: 3435 (NH), 1585 (C=N), 1529 (C=C), 1022 (C=S), 517, 429 (M–N, M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 7.72 (1H, s, –CH=N), 3.69 (1H, s, –NH), 9.68 (1H, s, –NH), 2.05 (3H, s, $–CH_3$), 7.18–8.03 (6H, m, Aryl).

[Pd(5-N-2-TCA-NMBuTSCN)Cl_2] (4a). Brick red solid; λ_{max}/cm^{-1}: 22,010, 38,011, 49,229; IR: ν_{max}/cm^{-1}: 3446 (NH), 1510 (C=N), 1485 (C=C), 1012 (C=S), 510, 435 (M–N, M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 7.86 (1H, s, –CH=N), 2.41 (3H, s, $–CH_3$), 2.23 (3H, m, $–CH_3$), 4.41 (6H, m, $–CH_2$), 7.13–7.99 (2H, m, Aryl).

[Pd(5-N-2-TCA-NMBzTSCN)Cl_2] (5a). Brick red solid; λ_{max}/cm^{-1}: 21,445, 36,454, 49,750; IR: ν_{max}/cm^{-1}: 3491 (NH), 1505 (C=N), 1487 (C=C), 1007 (C=S), 490, 423 (M–N, M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 8.07 (1H, s, –CH=N), 3.32 (1H, s, –NH), 2.92 (3H, s, $–CH_3$), 4.69 (2H, s, $–CH_2$), 7.16–8.01 (7H, m, Aryl).

[Pd(5-N-2-TCA-COTSCN)Cl_2] (6a). Brick red solid; λ_{max}/cm^{-1}: 21670, 37109, 49654; IR: ν_{max}/cm^{-1}: 3460 (N–H), 1541 (C=N), 1516 (C=C), 1054 (C=S), 502, 453 (M–N, M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 7.78 (1H, s, –CH=N), 8.07 (1H, d, –NH), 4.48 (14H, m, $–CH_2$), 6.97–7.69 (2H, m, Aryl).

[Pd(5-N-2-TCA-AdmTSCN)Cl_2] (7a). Brick red solid; λ_{max}/cm^{-1}: 21,431, 37,552, 49,112; IR: ν_{max}/cm^{-1}: 3435 (NH), 1565 (C=N), 1504 (C=C), 1052 (C=S), 516, 458 (M–N, M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 7.65 (1H, s, –CH=N), 3.42 (1H, s, –NH), 8.39 (1H, d, –NH), 4.32 (18H, m, $–CH_2$), 7.17–8.09 (2H, m, Aryl).

In vitro testing against *E. histolytica*

The thiosemicarbazones and their Pd(II) complexes were screened in vitro for antiamoebic activity against (*HK-9*) strain of *E. histolytica* by microdilution method.[39] *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously[40] in wells of 96-well microtiter plate. All the compounds were dissolved in DMSO (40 μL) at which level no inhibition of amoeba occurs[41,42] and the stock solutions of the compounds were prepared freshly before use at a concentration of 1 mg/mL. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (Costar). Each test includes metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) and a blank (culture medium only). The number of amoeba per mL was estimated with a heamocytometer and trypan blue exclusion was used to confirm viability. The cell suspension used was diluted to 10^5 organism/mL by adding fresh medium and 170 μL of this suspension was added to the test and control wells in the plate. An inoculum of 1.7×10^4 organisms/well was chosen so that confluent, but not excessive growth took place in control wells. Plates were sealed and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h.

After incubation, the growth of amoebae in the plate was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed once in sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly, and the plate was not allowed to cool in order to prevent the detachment of amoebae. The plate was allowed to dry at room temperature, and the amoebae were fixed with methanol and, when dry, stained with (0.5%) aqueous eosin for 15 min. Stained plate was washed once with tape water and then twice with distilled water and allowed to dry. A 200-μL portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The% inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best-fitting straight line from which the IC_{50} value was found. The results are reported in Table 2.

Acknowledgements

This work was supported from Council of Scientific and Industrial Research [Grant no. 27(0117)/02/EMR-ll], New Delhi, India. Shailendra acknowledges the senior research fellowship from CSIR, New Delhi, India. The authors are thankful to Prof. Alok Bhattacharya and Dr. Sudha Bhattacharya, School of Life Sciences and School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, respectively, for providing Laboratory facilities for biological activities.

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