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Synthesis, characterization and in vitro anti-amoebic activity of new palladium(II) complexes with 5-nitrothiophene-2-carboxaldehyde N(4)-substituted thiosemicarbazones

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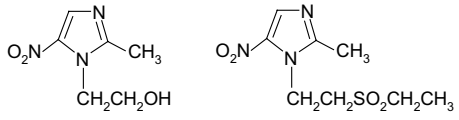
Abstract—Reaction between $[Pd(DMSO)_2Cl_2]$ (DMSO = dimethylsulfoxide) and N^4-substituted thiosemicarbazones derived from 5-nitrothiophene-2-carboxaldehyde (L) afforded the complexes $[Pd(L)Cl_2]$. These new complexes have been characterized by elemental analyses and spectroscopic studies. Spectroscopic studies reveal that thionic sulfur and azomethine nitrogen atom of thiosemicarbazones are coordinated to metal ion. The testing of the anti-amoebic activity of these complexes against the protozoan parasite *Entamoeba histolytica* suggests that compound **9**, **10**, and **11** might be endowed with important anti-amoebic properties since they showed less IC_{50} values than metronidazole. Moreover, compound **11** displays notable amoebicidal activity than metronidazole (IC_{50} values of 0.79 μM vs 1.93 μM, respectively).

1. Introduction

There is a wide variation in the availability and efficacy of drugs for the therapy and prophylaxis of parasitic diseases, both in humans and domestic animals. The 5-nitroimidazoles, for example, metronidazole and tinidazole have been used extensively in the treatment of amoebiasis, giardiasis, and trichomoniasis.[1] However, there remain major deficiencies in anti-parasitic chemotherapy.



Metronidazole Tinidazole

The protozoan parasite *Entamoeba histolytica* (*E. histolytica*) is the cause of amoebic dysentery and liver abscess. It is, therefore, responsible for significant morbidity and mortality in a number of countries. Infection with *E. histolytica* is treated with nitroimidazoles,

Abbreviations: [Pd(5-NT-PrTSC)Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-propyl thiosemicarbazones) palladium(II); [Pd(5-NT-isoPrTSC)Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-isopropyl thiosemicarbazones) palladium(II); [Pd(5-NT-BuTSC)Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-butyl thiosemicarbazones) palladium(II); [Pd(5-NT-isoBuTSC)Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-isobutyl thiosemicarbazones) palladium(II); [Pd(5-NT-DiEtTSC)Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-diethyl thiosemicarbazones) palladium(II); [Pd(5-NT-DiPrTSC)Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-dipropyl thiosemicarbazones) palladium(II); [Pd(5-NT-DiisoBuTSC)Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-diisobutyl thiosemicarbazones) palladium(II); [Pd(5-NT-NMCHTSC)Cl_2], dichloro(5-nitrothiophene-2-carboxaldehyde-n-methylcyclohexylthiosemicarbazones) palladium(II); [Pd(5-NT-CPTSC)Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-cyclopentyl thiosemicarbazones) palladium(II); [Pd(5-NT–CHTSC)-Cl_2], Dichloro (5-nitrothiophene-2-carboxaldehyde-cyclohexyl thiosemicarbazones) palladium(II); [Pd(5-NT-HMINTSC)Cl_2] Dichloro (5-nitrothiophene-2-carboxaldehyde-hexamethyleneimine thiosemicarbazones) palladium(II).

Keywords: 5-Nitrothiophene-2-carboxaldehyde; Palladium(II) complexes; Anti-amoebic activity; In vitro.

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primarily with metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole]. Though failures of the treatment of liver abscess and dysentery with metronidazole have been reported,[2] it has common side effects[3] including nausea. It is mutagenic in bacteria[4] and high doses may cause cancer in rodents.[5] However, resistance against metronidazole is common in bacteria and other protozoan organism and in vitro trophozoites of *E. histolytica* are able to adapt to therapeutically relevant levels of the drug.[6,7] As cross-resistance exists among the nitroimidazoles,[1] there is no equally effective and tolerated class of drug available in the case of rising resistance to metronidazole. There is a lack of useful alternative classes of substances for the treatment of invasive amoebiasis. Infection with this protozoan occurs in many countries of the world, resulting an estimated 42 million cases occur annually, which are responsible for 100,000 deaths each year.[8]

Heterocyclic thiosemicarbazones and their metal complexes have been reported due to their potential for medicinal use.[9–11] Introduction of metal ion into thiosemicarbazones some times enhances their activity to highest. For instance, 3-ethoxy-2-oxobutyraldehyde bis (thiosemicarbazonato) copper(II) has proved to be an efficient anti-tumor agent.[12] Several palladium(II) and platinum(II) complexes of thiosemicarbazone complexes having potential biological activity have also been reported recently.[13–15] We have initiated an investigation on complexes of palladium with derivatives of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones in an effort to investigate whether thiosemicarbazones act synergically in new complexes containing both the parent ligand and Pd(II), and also to correlate the structure with biological activity.[16,17] Continuing our efforts to develop improved anti-amoebic drugs, we have recently reported Pd(II) and Ru(II) complexes of NS donor ligands, which showed promising activity against *E. histolytica*.[18–21]

This report includes synthesis and spectral characterization of the newly prepared palladium(II) complexes (Fig. 1) derived from 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones (Fig. 2) and their screening for in vitro anti-amoebic activity.

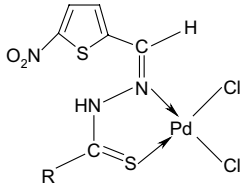


Figure 1. Structure of Pd(II) complex.

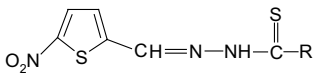


Figure 2. Structure of thiosemicarbazone (legends: (1) R = $-NC_3H_8$; (2) R = $-NC_3H_8$; (3) R = $-NC_4H_{10}$; (4) R = $-NC_4H_{10}$; (5) R = $-NC_4H_{10}$; (6) R = $-NC_6H_{14}$; (7) R = $-NC_8H_{18}$; (8) R = $-NC_7H_{14}$; (9) R = $-NC_5H_{10}$; (10) R = $-NC_6H_{12}$; (11) R = $-NC_6H_{12}$).

2. Results and discussion

All the thiosemicarbazones were prepared by the method described earlier.[22] Their purity were checked by TLC and melting point determination and structures were confirmed by IR, ^{1}H NMR and electronic spectra. The precursor used for the synthesis of Pd(II) complexes that is $[Pd(DMSO)_2Cl_2]$ was synthesized by the literature procedure.[23] The complexes were prepared by mixing an equimolar ratio of the appropriate ligand and $[Pd(DMSO)_2Cl_2]$ refluxing in methanol with constant stirring as shown by Eq. 1

$$[Pd(DMSO)_2Cl_2] + L \xrightarrow[4\,h]{CH_3OH} [Pd(L)Cl_2] + 2DMSO, \quad (1)$$

where L = thiosemicarbazone.

The complexes were separated from the solution by filtering at room temperature and dried in vacuo over silica gel to constant weight. These complexes were characterized by elemental analysis, IR, ^{1}H NMR and electronic spectra. All the compounds were highly soluble in DMF and DMSO while slightly soluble in methanol and ethanol. Analytical and spectral data are in good agreement with the proposed structure for metal complexes.

2.1. IR and electronic spectral studies of the complexes

Selected diagnostic bands of the infrared spectra of the thiosemicarbazones and their complexes showed useful information for determining the mode of coordination in the metal complexes. Thiosemicarbazones showed intense bands in the region 1028–1087 cm^{-1} due to the ν(C=S) stretch but no band appeared near 2570 cm^{-1} ascribed to ν(C–SH), suggesting that the ligands remain in the thione form in the solid state that is ligands exhibit no thione–thiol tautomerization. This is further supported by the absence of a peak assignable to (C–SH) proton in the ^{1}H NMR spectra of the ligands. The downward shift of ν(C=S) band by 5–30 cm^{-1} in the complexes suggested the coordination of thione sulfur. The spectra of all the thiosemicarbazones exhibit a strong band at 1492–1664 cm^{-1} region due to the ν(C=N) mode of the azomethine linkage. In the complexes, this band shifted to lower frequency by 10–23 cm^{-1} suggesting that the unsaturated nitrogen atom of the azomethine linkage is coordinated to the metal ion. The bands in the region 447–539 cm^{-1} and 419–457 cm^{-1} are assigned to the ν(M–N) and ν(M–S) bands, respectively, that further supported the coordination of the azomethine nitrogen and thionic sulfur. The two strong bands observed at 716–680 and 605–597 cm^{-1} regions are attributed to the thiophene ring deformation modes in the ligands.[22] These thiophene ring deformation vibrations are not affected in the complexes indicating the nonparticipation of ring sulfur atom in coordination.

Interest in the electronic spectra of thione particularly in thioamides arose due to their similarity with the oxygen

analogues, besides the thione transitions giving rise to absorption bands at longer wavelengths and thus in more accessible regions. Three bands were observed in the spectra of thiosemicarbazones in the regions 24630–25000, 34965–37879 and 45045–49020 cm^{-1} assignable to $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$ and $\varphi \rightarrow \varphi^*$ transitions, respectively. In the spectra of complexes, these bands appeared at ca. 21310, 36000 and 47000 cm^{-1}, respectively, with little change in the energy of these bands. The band appeared at ca. 36,000 and 47,000 cm^{-1} are assigned to $\pi \rightarrow \pi^*$ and $\varphi \rightarrow \varphi^*$ transitions, respectively. As intensity of the band appearing at ca. 21310 cm^{-1} is comparable with other ligand bands, and thus 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 ligand systems.[24,25]

2.2. ^{1}H NMR spectral studies

The ^{1}H NMR spectra of the thiosemicarbazones in DMSO-d_6 and $CDCl_3$, exhibit a broad band due to the –NH proton in the region 9.81–11.40 ppm, which indicate that even in a polar solvent they remain in the thione form. This signal usually shifts to downfield and appears at 13.47–13.87 ppm in the complexes (**1**–**11**). This information suggests the adjustment of electronic current upon coordination of >C═S group to the metal ion. Aromatic as well as other protons resonate nearly at the same region with slight shielding as that of the free thiosemicarbazone.

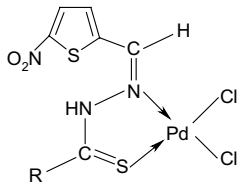
2.3. Thermogravimetric analysis (TGA)

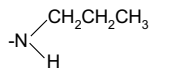
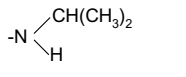
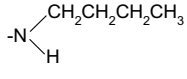
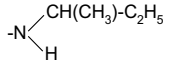
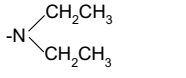
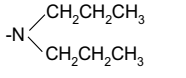
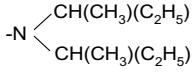
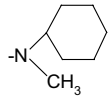
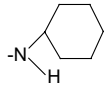
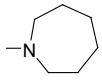
Thermograms of complexes were recorded under nitrogen with a heating rate of 10 °C/min between room temperature and 800 °C. All the complexes were stable up to 200 °C. Further increment of temperature causes decomposition of the complexes in two steps. The temperature range for the first step being 245–395 °C where loss of mixed fragments was observed, which made it difficult to predict the loss of any particular group at any step. The second step starts immediately after first step and continues until the complete decomposition of the ligand and formation of end product as palladium sulfide (PdS). The total weight loss of the complexes corresponds to the loss of the respective ligand after considering the transfer of one sulfur atom to the metal ion and residues correspond to the palladium sulfide.

2.4. Biological activity

All the complexes were screened for anti-amoebic activity in vitro against HK-9 strain of *E. histolytica*. The IC_{50} values in μM are shown in Table 1. Complexation of the thiosemicarbazones with palladium(II) results complexes (**1**–**11**). The complexes with less IC_{50} value than metronidazole in this class were those Pd(II) complexes of thiosemicarbazone, which have cyclopentylamine (**9**, $IC_{50} = 1.45\,\mu M$) cyclohexylamine (**10**, $IC_{50} = 0.87\,\mu M$) and hexamethyleneimine (**11**, $IC_{50} = 0.79\,\mu M$) as N^4 substitution. It is concluded that the presence of these bulky groups at N^4 position of the thiosemicarbazone moiety enhanced anti-amoebic activity. The Pd-complex precursor $[Pd(DMSO)_2Cl_2]$ showed no activity against *E. histolytica* when evaluated for anti-amoebic activity and compared with Pd(II) complexes and metronidazole. We have observed that the transition metal complexes of NS donor ligands showed good anti-amoebic activity against the same strain of *E. histolytica*.[18–21] It was reported that chelation reduces the polarity of the central metal atom due to partial sharing of its positive charge with the ligand, which favors permeation of the complexes through the lipid layer of cell membranes

Table 1. In vitro anti-amoebic activity of Pd(II) complexes against *E. histolytica* (HK-9)



Compound	R	IC_{50} (μM)	SD[a]
1		4.76	1.4
2		4.07	0.75
3		4.70	0.99
4		4.25	0.84
5		3.21	0.62
6		3.03	0.59
7		7.66	1.61
8		3.71	0.93
9		1.45	0.29
10		0.87	0.42
11		0.79	0.26
Metronidazole		1.93	0.40

[a] SD, standard deviation.

indicating that complexation to metals enhances the activity of the ligand.[26] It was noted that anti-parasitic 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.[27]

Our interest in the thiosemicarbazone metal complexes was stimulated by the fact that the new complexes might be more efficacious drugs against amoebiasis. 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 for therapeutic use.

3. Conclusion

We report herein, the synthesis and biological activity of the new palladium(II) complexes of thiosemicarbazones derived from 5-nitrothiophene-2-carboxaldehyde. In vitro anti-amoebic evaluation of the metal complexes was carried out against HK-9 strain of *E. histolytica*. The biological behavior revealed that most of the complexes show a weak activity against *E. histolytica*. The chelation induced significant changes in the biological activity of the palladium complexes **9**–**11** have shown less IC_{50} value than metronidazole.

4. Experimental

4.1. Materials and methods

Palladium(II) chloride was purchased from Aldrich chemical company (USA). All the Cycloalkylaminothiocarbonylhydrazines were prepared as reported earlier.[22] The metal precursor $[Pd(DMSO)_2Cl_2]$ was prepared by the literature method.[23] Elemental analyses (C, H, and 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–vis spectro-photometer. IR spectra on KBr disks were recorded on a Perkin–Elmer model 1620 FT-IR spectrophotometer. ^{1}H 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 thermogravimetric analyzer with increasing the temperature at 10 °C per minute.

4.2. Preparation of palladium(II) complexes

All Pd(II) complexes were prepared by a general method. An equimolar ratio of the ligand and a suspension of $[Pd(DMSO)_2Cl_2]$ were mixed in hot dry methanol with constant stirring. The reaction mixture was then refluxed for 4 h. After keeping the reaction mixture at 0 °C for 12 h, the solid was separated by filtration, washed with cold methanol and dried in vacuo. Details of the isolated complexes are given below:

4.2.1. [Pd(5-NT-PrTSC)Cl$_2$] (1). Brick red solid (methanol: DMSO). Yield: 63%; decomp. temp: 285 °C; Anal Calcd ($C_9H_{12}N_4S_2O_2Cl_2Pd$): C, 24.12; H, 2.68; N, 12.54; Cl, 15.85. Found: C, 24.04; H, 2.51; N, 12.30; Cl, 15.62. λ_{max}/cm^{-1}: 22413, 36543, 49503; IR: ν_{max}/cm^{-1} 3325 ν(NH), 1537 ν(C=N), 1525 (C=C), 1015 (C=S), 515, 425 (M–N, M–S); ^{1}H NMR($(CD_3)_2SO$)/ppm: 7.62 (1H, s, H–C=N), 13.65 (1H, s, –NH), 4.23 (4H, m, –CH_2), 2.32 (3H, t, –CH_3), 7.07–8.15 (2H, m, Aryl).

4.2.2. [Pd(5-NT-isoPrTSC)Cl$_2$] (2). Brick red solid (methanol:chloroform). Yield: 67%; decomp. temp: 324 °C; Anal Calcd ($C_9H_{12}N_4S_2O_2Cl_2Pd$): C, 24.12; H, 2.68; N, 12.50; Cl, 15.85. Found: C, 24.23; H, 2.47; N, 12.69; Cl, 15.67. λ_{max}/cm^{-1}: 22315, 37542, 49915; IR: ν_{max}/cm^{-1}: 3429 ν(NH), 1583 ν(C=N), 1537 (C=C), 1020 (C=S), 529, 447 (M–N, M–S); ^{1}H NMR($(CD_3)_2SO$)/ppm: 7.46 (1H, s, H–C=N), 13.47 (1H, s, –NH), 2.97 (6H, d, –CH_3), 6.87–7.73 (2H, m, Aryl).

4.2.3. [Pd(5-NT-BuTSC)Cl$_2$] (3). Brick red solid (methanol:chloroform). Yield: 69%; decomp. temp: 246 °C; Anal Calcd ($C_{10}H_{14}N_4S_2O_2Cl_2Pd$): C, 25.97; H, 3.03; N, 12.12; Cl, 15.37. Found: C, 25.64; H, 2.87; N, 12.01; Cl, 15.25. λ_{max}/cm^{-1}: 21975, 37755, 49216; IR: ν_{max}/cm^{-1}: 3434 ν(NH), 1567 ν(C=N), 1517 (C=C), 1023 (C=S), 504, 456 (M–N, M–S); ^{1}H NMR($(CD_3)_2SO$)/ppm: 7.78 (1H, s, H–C=N), 13.53 (1H, s, –NH), 4.15 (6H, m, –CH_2), 2.16 (3H, t, –CH_3), 7.10–7.98 (2H, m, Aryl).

4.2.4. [Pd(5-NT-isoBuTSC)Cl$_2$] (4). Brick red solid (methanol:chloroform). Yield: 63%; decomp. temp: 225 °C; Anal Calcd ($C_{10}H_{14}N_4S_2O_2Cl_2Pd$): C, 25.97; H, 3.03; N, 12.12; Cl, 15.37. Found: C, 25.64; H, 2.87; N, 12.01; Cl, 15.53. λ_{max}/cm^{-1}: 22517, 37859, 49423; IR: ν_{max}/cm^{-1}: 3435 ν(NH), 1585 ν(C=N), 1534 (C=C), 1022 (C=S), 525, 446 (M–N, M–S); ^{1}H NMR($(CD_3)_2$ SO)/ppm: 7.59 (1H, s, H–C=N), 13.87 (1H, s, –NH), 4.34 (2H, m, –CH_2), 2.04 (6H, m, –CH_3), 7.19–7.98 (2H, m, Aryl).

4.2.5. [Pd(5-NT-DiEtTSC)Cl$_2$] (5). Brick red solid (methanol:DMSO). Yield: 74%; decomp. temp: 249 °C; Anal Calcd ($C_{10}H_{14}N_4S_2O_2Cl_2Pd$): C, 29.70; H, 3.47; N, 13.86; Cl, 17.57. Found: C, 29.81; H, 3.10; N, 13.89; Cl, 17.34. λ_{max}/cm^{-1}: 22870, 36995, 47003; IR: ν_{max}/cm^{-1}: 3382 ν(NH), 1565 ν(C=N), 1492 (C=C), 1021 (C=S), 506, 453 (M–N, M–S); ^{1}H NMR ($(CD_3)_2SO$)/ppm: 7.85 (1H, s, H–C=N), 13.53 (1H, s, –NH), 2.51 (6H, m, –CH_3), 4.25 (4H, m, –CH_2), 7.37–8.06 (2H, m, Aryl).

4.2.6. [Pd(5-NT-DiPrTSC)Cl$_2$] (6). Brick red solid (methanol:chloroform). Yield: 51%; decomp. temp: 314 °C; Anal Calcd ($C_{12}H_{18}N_4S_2O_2Cl_2Pd$): C, 29.39; H, 3.67; N, 11.43; Cl, 14.49. Found: C, 29.70; H, 3.79; N, 11.13; Cl, 14.63. λ_{max}/cm^{-1}: 21890, 38319, 49519; IR: ν_{max}/cm^{-1}: 3491 ν(NH), 1570 ν(C=N), 1455

(C=C), 1045 (C=S), 524, 457 (M–N, M–S); ^{1}H NMR((CD$_3$)$_2$SO)/ppm: 7.64 (1H, s, H–C=N), 13.57 (1H, s, –NH), 2.23 (6H, m, –CH$_3$), 4.25 (8H, m, –CH$_2$), 7.04–8.97 (2H, m, Aryl).

4.2.7. [Pd(5-NT-DiisoBuTSC)Cl$_2$] (7). Brick red solid (methanol:chloroform). Yield: 67%; decomp. temp: 237 °C; Anal Calcd ($C_{14}H_{22}N_4S_2O_2Cl_2Pd$): C, 32.43; H, 4.25; N, 10.81; Cl, 13.71. Found: C, 32.49; H, 4.51; N, 10.59; Cl, 13.93. λ_{max}/cm^{-1}: 21946, 35998, 48814; IR: ν_{max}/cm^{-1}: 3420 ν(NH), 1613 ν(C=N), 1523 (C=C), 1012 (C=S), 519, 443 (M–N, M–S); ^{1}H NMR((CD$_3$)$_2$SO)/ppm: 7.79 (1H, s, H–C=N), 13.67 (1H, s, –NH), 2.11 (12H, m, –CH$_3$), 4.05 (4H, m, –CH$_2$), 6.85–7.63 (2H, m, Aryl).

4.2.8. [Pd(5-NT-NMCHTSC)Cl$_2$] (8). Brick red solid (methanol:chloroform). Yield: 63%; decomp. temp: 249 °C; Anal Calcd ($C_{13}H_{18}N_4S_2O_2Cl_2Pd$): C, 31.08; H, 3.59; N, 11.15; Cl, 14.14. Found: C, 31.35; H, 3.95; N, 11.44; Cl, 14.37. λ_{max}/cm^{-1}: 21998, 38413, 48843; IR: ν_{max}/cm^{-1}: 3475 ν(NH), 1555 ν(C=N), 1537 (C=C), 1063 (C=S), 507, 439 (M–N, M–S); ^{1}H NMR((CD$_3$)$_2$SO)/ppm: 7.53 (1H, s, H–C=N), 13.87 (1H, s, –NH), 2.68 (3H, s, –CH$_3$), 4.64 (10H, m, –CH$_2$), 7.37–7.97 (2H, m, Aryl).

4.2.9. [Pd(5-NT-CPTSC)Cl$_2$] (9). Brick red solid (methanol:chloroform). Yield: 54%; decomp. temp: 246 °C; Anal Calcd ($C_{11}H_{14}N_4S_2O_2Cl_2Pd$): C, 27.84; H, 2.95; N, 11.81; Cl, 14.98. Found: C, 27.64; H, 2.80; N, 11.51; Cl, 14.83. λ_{max}/cm^{-1}: 21312, 36311, 49217; IR: ν_{max}/cm^{-1}: 3466 ν(NH), 1654 ν(C=N), 1539 (C=C), 1021 (C=S), 487, 419 (M–N, M–S); ^{1}H NMR((CD$_3$)$_2$SO)/ppm: 7.93 (1H, s, H–C=N), 13.73 (1H, s, –NH), 4.68 (8H, m, –CH$_2$), 6.98–7.72 (2H, m, Aryl).

4.2.10. [Pd(5-NT–CHTSC)Cl$_2$] (10). Brick red solid (methanol:chloroform). Yield: 69%; decomp. temp: 159 °C; Anal Calcd ($C_{12}H_{16}N_4S_2O_2Cl_2Pd$): C, 29.51; H, 3.28; N, 11.48; Cl, 14.55. Found: C, 29.33; H, 3.34; N, 11.62; Cl, 14.49. λ_{max}/cm^{-1}: 21,975, 36,140, 49,298; IR: ν_{max}/cm^{-1}: 3470 ν(NH), 1517 ν(C=N), 1440 (C=C), 1018 (C=S), 515, 443 (M–N, M–S); ^{1}H NMR ((CD$_3$)$_2$SO)/ppm: 7.71 (1H, s, H–C=N), 13.74 (1H, s, –NH), 4.43 (10H, m, –CH$_2$), 6.90–7.63 (2H, m, Aryl).

4.2.11. [Pd(5-NT-HMINTSC)Cl$_2$] (11). Brick red solid (methanol:chloroform). Yield: 67%; decomp. temp: 167 °C; Anal Calcd ($C_{12}H_{16}N_4S_2O_2Cl_2Pd$): C, 29.51; H, 3.28; N, 11.48; Cl, 14.55. Found: C, 29.35; H, 3.28; N, 11.61; Cl, 14.82. λ_{max}/cm^{-1}: 22017, 36852, 48913; IR: ν_{max}/cm^{-1}: 3402 ν(NH), 1565 ν(C=N), 1515 (C=C), 1019 (C=S), 539, 457 (M–N, M–S); ^{1}H NMR((CD$_3$)$_2$SO)/ppm: 7.58 (1H, s, H–C=N), 13.67 (1H, s, –NH), 4.34 (12H, m, –CH$_2$), 7.02–7.86 (2H, m, Aryl).

4.3. In vitro testing against *E. histolytica*

E. histolytica trophozoites were cultured in TYlS-33 growth medium as described previously in wells of 96-well microtiter plate.[28] Activity against *E. histolytica* (strain HK-9) in vitro was assessed using a microdilution method.[29] DMSO (40 μL) was added to all the complexes (~1 mg) at which level no inhibition of amoeba occurs,[30,31] followed by enough culture medium to obtain 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. Two-fold serial dilutions were made in the wells of 96-well microtiter plate 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 milliliter was estimated with a heamocytometer and trypan blue exclusion was used to confirm viability. Fresh culture medium was added to dilute the suspension to 10^5 organism/mL, 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. Plate was sealed and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h.

4.4. Assessment of anti-amoebic activity

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. The percentage inhibition of amoebal growth was calculated from the optical densities of the control and the 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 1.

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