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Short communication

Synthesis, characterization and in vitro Antiamoebic Activity of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones and their Palladium (II) and Ruthenium (II) Complexes

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Abstract

Synthesis of new Palladium(II) and Ruthenium(II) complexes of the type, $[Pd(L)Cl_2]$ and $[Ru(\eta^4\text{-}C_8H_{12})(L)Cl_2]$ {where, L = thiosemicarbazones derived from 5-nitrothiophene-2-carboxaldehyde and cycloalkylaminothiocarbonyl hydrazines} have been isolated by the reaction of $[Pd(DMSO)_2Cl_2]$ and $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$ with 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones. The spectral data revealed that the thiosemicarbazones act as bidentate ligands, making use of thionic sulphur and the azomethine nitrogen atom for coordination to the central metal ion. Microdilution method was used for the assessment of antiamoebic activity of all the compounds against *HK-9* strain of *Entamoeba histolytica*. Among all the thiosemicarbazones, 5-NT-4-BPTSCN (**3**) showed significant antiamoebic activity (IC_{50} – 2.56 μM). Enhancement of antiamoebic activity resulted by introducing palladium and ruthenium metals in the thiosemicarbazone moiety. All the Pd(II) and Ru(II) complexes of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones were found more active then their respective ligands. The complexes **1a-4a, 1b** and **3b** showed antiamoebic activity.

Keywords: Thiosemicarbazones; 5-Nitrothiophene-2-carboxaldehyde; Palladium (II) complexes; Ruthenium (II) complexes; In vitro antiamoebic activity

1. Introduction

Parasitic diseases cause huge suffering in many parts of the world. However, comparatively little research is done in the field [1,2]. Amoebiasis has become resurgent at a time when current chemotherapy is failing [3]. It is estimated that about 50 million symptomatic cases of intestinal amoebiasis occurs annually resulting in 100,000 deaths being caused by *Entamoeba histolytica* [3]. 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 effect in bacteria and carcinogenic to rodents [4]. Severe side effects (nausea, metallic taste, dizziness, hypertension etc) as well as resistance have been reported [5]. The ideal treatment for this disease does not, therefore, exist and new agents are required [6].

The heterocyclic thiosemicarbazones and their metal complexes are among the most widely studied compounds for their potential therapeutic uses, such as antibacterial [7,8], antimalarial [9], antineoplastic [10,11] and antiviral [12] activities. $[RuCl_2(DMSO)_2(4\text{-nitroimidazole})_2]$ has been used as radiosensitizer successfully. Earlier studies on Ru complexes such as, *cis*-$RuCl_2(DMSO)_2$, as antineoplastic agents, have suggested a DNA binding mechanism [13]. Several palladium(ll) and platinum(ll) thiosemicarbazone complexes having potential antitumor activity [14,15] have been recently reported. However, the potential of palladium and ruthenium complexes of thiosemicarbazones as antiamoebic agents has so far been very little explored [16–20]. The relationship between structural and biological properties of metal complexes of different stoichiometries has been explored which provide diversity and unique scaffolds for potential exploitation of therapeutic effect [21].The significant trypanosomal activity of semicarbazones of 5-nitrothiophene-2-carboxaldehyde [22] led us to study the

Abbreviations: DMF, Dimethyl formamide; DMSO, Dimethyl sulfoxide; *E. histolytica*, *Entamoeba histolytica*.

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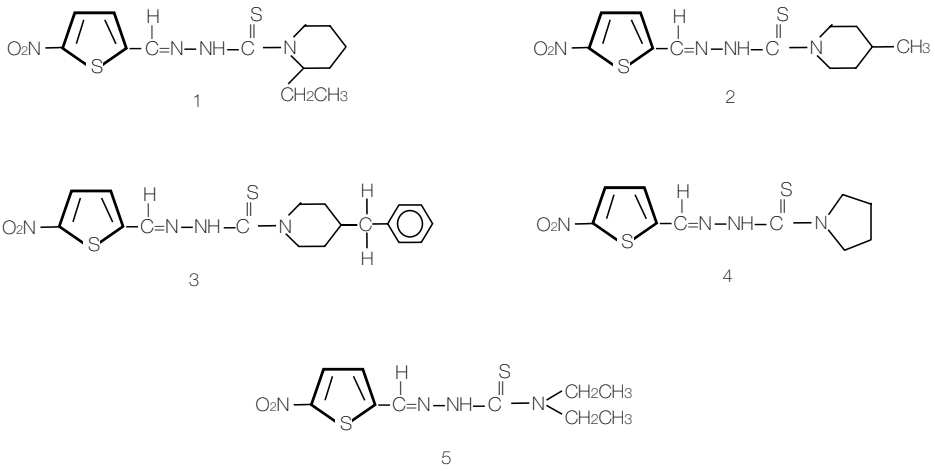


Fig. 1. 1. 5-NT-2-EPTSCN, 5-Nitrothiophene-2-carboxaldehyde-2-ethylpiperidinethiosemicarbazone
2. 5-NT-4-MPTSCN, 5-Nitrothiophene-2-carboxaldehyde-4-methylpiperidinethiosemicarbazone
3. 5-NT-4-BPTSCN, 5-Nitrothiophene-2-carboxaldehyde-4-benzylpiperidinethiosemicarbazone
4. 5-NT-PYRTSCN, 5-Nitrothiophene-2-carboxaldehyde-pyrrolidinethiosemicarbazone
5. 5-NT-diEtTSCN, 5-Nitrothiophene-2-carboxaldehyde-diethylthiosemicarbazone

synthesis, antiamoebic screening of thiosemicarbazones and their palladium and ruthenium complexes.

We now describe the synthesis of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones (Fig. 1) and their new Pd(II) and Ru(II) complexes (Fig. 2). The structures of all these compounds were elucidated by elemental analysis, ^{1}H-NMR, IR, UV-visible spectroscopy and thermogravimetric analysis. These compounds were screened in vitro against *HK-9* strain of *E. histolytica* for their ability to inhibit the growth of parasite, which showed that the chelation induces significant changes in the antiamoebic activity.

2. Chemistry

Palladium chloride (99%), Ruthenium trichloride (99%) and 5-nitrothiophene-2-carboxaldehyde (99%) were purchased from Aldrich Chemical Company (U.S.A.). All the thioglycolic acids were prepared by the method reported by O'Sullivan [23]. Cycloalkylaminothiocarbonylhydrazines were prepared by refluxing the alkaline solution of thioglycolic acid with hydrazine hydrate and their thiosemicarbazones were synthesized by stirring the solution of cycloalkylaminothiocarbonylhydrazines in water and the solution of thiophene-2-carboxaldehyde in ethanol in equimolar ratio. The purity of the thiosemicarbazones was checked by TLC. The precursor used for the synthesis of Pd(II) and Ru(II) complexes $[Pd(DMSO)_2Cl_2]$ and $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$, respectively was synthesized by the literature procedure [24,25]. All the complexes were obtained by general method. The complexes were prepared by mixing equimolar ratio of the appropriate ligand and $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$ / $[Pd(DMSO)_2Cl_2]$ in refluxing methanol as shown by Equations 1 and 2,

$$\left[\mathrm{Ru}\left(\eta^4-\mathrm{C_8H_{12}}\right)\left(\mathrm{CH_3CN}\right)_2\mathrm{Cl_2}\right]+\mathrm{L}\xrightarrow[\text{reflux}]{\mathrm{CH_3OH}}\mathrm{Ru}\left(\eta^4-\mathrm{C_8H_{12}}\right)\left(\mathrm{L}\right)\mathrm{Cl_2}]]+2\mathrm{CH_3CN}- \quad (1)$$

$$\left[\mathrm{Ru}\left(\mathrm{DMSO}\right)_2\mathrm{Cl_2}\right]+\mathrm{L}\xrightarrow[\text{reflux}]{\mathrm{CH_3OH}}\left[\mathrm{Pd}\left(\mathrm{L}\right)\mathrm{Cl_2}\right]+2\mathrm{DMSO}-- \quad (2)$$

(Where L = thiosemicarbazones)

The products thus obtained were separated from the solution by filtering at room temperature and drying in *vacuo* over silica gel. All the complexes are soluble in DMF and DMSO, sparingly soluble in methanol, ethanol and insoluble in water. The structure of all the ligands and their metal complexes was confirmed by elemental analysis, IR, ^{1}H-NMR, electronic spectra and thermogravimetric analysis.

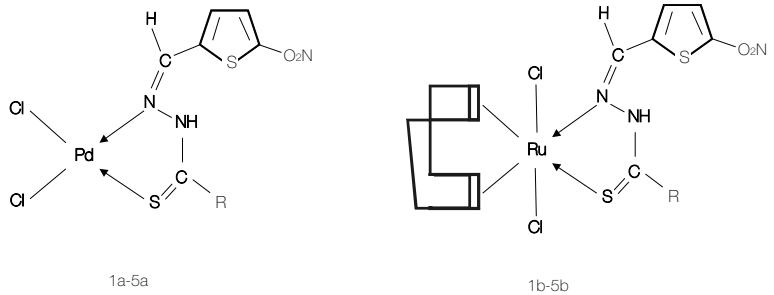


Fig. 2. Structure of Palladium (II) and Ruthenium (II) Complexes (1a,1b. R= -NC_7H_{14} ; 2a,2b. R= -NC_6H_{12} ; 3a,3b. R= -$NC_{12}H_{16}$; 4a,4b. R= -NC_4H_8 ; 5a,5b. R= -NC_4H_{10}).

Table 1
In vitro antiamoebic activities of thiosemicarbazones and their Pd (II) and Ru (II) complexes against (*HK-9*) strain of *E. histolytica*.

S.No.	Compound	IC_{50}(μM)	S.D.*
1	5-NT-2-EPTSCN	3.05	0.65
1a.	[Pd(5-NT-2-EPTSCN)Cl_2]	0.96	0.26
1b.	[Ru(η^4-C_8H_{12})(5-NT-2-EPTSCN)Cl_2]	1.81	0.42
2	5-NT-4-MPTSCN	3.72	0.69
2a.	[Pd(5-NT-4-MPTSCN)Cl_2]	1.56	0.34
2b.	[Ru(η^4-C_8H_{12})(5-NT-4-MPTSCN)Cl_2]	2.4	0.51
3	5-NT-4-BPTSCN	2.56	0.61
3a.	[Pd(5-NT-4-BPTSCN)Cl_2]	0.84	0.19
3b.	[Ru(η^4-C_8H_{12})(5-NT-4-BPTSCN)Cl_2]	1.75	0.4
4	5-NT-PYRTSCN	2.91	0.38
4a.	[Pd(5-NT-PYRTSCN)Cl_2]	1.23	0.25
4b.	[Ru(η^4-C_8H_{12})(5-NT-PYRTSCN)Cl_2]	2.04	0.31
5	5-NT-diEtTSCN	9.96	1.97
5a.	[Pd(5-NT-diEtTSCN)Cl_2]	2.56	0.49
5b.	[Ru(η^4-C_8H_{12})(5-NT-diEtTSCN)Cl_2]	4.19	0.87
	Metronidazole	1.93	0.32

* Standard Deviation

These complexes were high melting solid and decompose before their melting temperatures.

3. Pharmacology

Thiosemicarbazones and their new palladium(II) and ruthenium(II) complexes were tested in vitro for antiamoebic activity against *HK-9* strain of *E. histolytica* by microdilution method [26]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously [27] in wells of 96-well microtiter plate. Each compound tested was serially diluted and added to the growing trophozoites in microtiter plate. Effect on growth of trophozoites was monitored microscopically at regular interval and quantitative estimation of the drug action was made by protein estimation. The % inhibition of amoeba was calculated from the optical densities of the control and tested wells and was plotted against the logarithm of concentration of the drug tested. Linear regression analysis was used to determine the best fitting straight line from which IC_{50} value was found. The results are reported in Table 1.

4. Results and Discussion

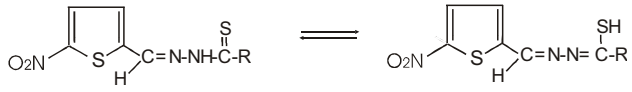
The synthesis of 5-nitrothiophene-2-carboxaldehyde thiosemicarbazones are straight forward and the compounds were isolated in good yield. Reaction of these ligands with [Pd$(DMSO)_2Cl_2$] and [Ru(η^4-C_8H_{12})$(CH_3CN)_2Cl_2$] gave amorphous solid complexes. The analytical data of these compounds are in good agreement with their composition. The complexes do not undergo any weight loss up to 235° C, which suggest their fair thermal stability. The structure of all the complexes presented in Fig. 2 was established by comparing spectral data (IR, UV-visible and ^{1}H-NMR) with their respective ligands and was further supported by their thermogravimetric analysis.

4.1. Electronic spectra of the complexes

The electronic spectra of all the thiosemicarbazones shows similar pattern, exhibiting three bands in the region 28752 - 30120, 37945 - 38840 and 47619 - 48490 cm^{-1}. The probable assignment for these bands are due to the $n \rightarrow \pi^*$ (thiosemicarbazones), $n \rightarrow \pi^*$ (thiophene) and $\pi \rightarrow \pi^*$ (thiophene) transitions. A careful comparison of the bands of electronic spectral bands of complexes with those of the free ligands showed that there was little change in the energy of these bands due to extended conjugation of ligands after complexation. In the spectra of complexes, these bands appeared at ca. 25000, 37000 and 49000 cm^{-1} respectively. A very intense band at ca. 21500 cm^{-1} in the electronic spectra of the complexes is reasonably assignable to a combination of ligand to metal charge transfer and metal d-d band transitions. Such observations have also been noticed earlier in other palladium (II) and ruthenium (II) complexes of similar ligands systems [28,29].

4.2. IR spectral analysis

The thiosemicarbazones **1-5** exhibit thione and thiol tautomerism,



Thione form Thiol form

The thiosemicarbazones **1-5** show intense strong bands in the region 1031-1052 cm^{-1} due to ν (C=S) but no band appeared due to ν (C-SH) in the region 2500-2600 cm^{-1} suggesting that all the thiosemicarbazones remain in the thione form. The strong band at 1031-1052 cm^{-1} ascribed to ν (C=S) of ligands is shifted to lower frequency (14-32 cm^{-1})

indicating the bonding of metal through thionic sulphur. The preferential coordination of thionic sulphur over sulphur of thiophene is due to more nucleophilic character of the former. The band due to ν (C-S-C) (ring) of thiophene moiety remains unaltered in **1a-5b**, indicating non-participation of ring sulphur in coordination. The negative shift of (16-39 cm^{-1}) of ν (C=N) stretch in the complexes indicates the involvement of azomethine nitrogen in complexation [30]. This was supported by the shift of N-N band of ligand on coordination. The broad band observed in region 3300 cm^{-1} due to ν (N-H) stretch is slightly shifted in complex due to probably adjustment of current arising due to coordination of thionic sulphur. Thus, ligands behave as neutral NS donor bidentate in these complexes.

4.3. ^{1}H-NMR analysis

Further evidence for the coordinating mode of the thiosemicarbazones **1-5** was obtained by ^{1}H NMR spectra. In the ^{1}H-NMR spectra in DMSO-d_6, all the thiosemicarbazones **1-5** do not show any resonance at ca. 4.0 ppm, attributed to –SH proton resonance, while the appearance of a broad peak at 9.35-11.02 ppm due to the –NH proton 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 to up field and appears at 3.49-4.32 ppm in their respective complexes. However, in some complexes, we are unable to locate the –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 viz. CH_3 protons, CH_2 protons and aryl carbons in complexes resonate nearly in the same region as that of free ligands.

4.4. TGA analysis

The thermogravimetric analysis profiles of complexes **la-5b** along with the % weight at different temperatures are recorded. These complexes do not lose weight up to 235°C. Further increment of temperature causes decomposition of the complexes in two steps, the temperature range for the first step being 235–410°C for the palladium and ruthenium (II) complexes where losses of mixed fragments were observed. The second step starts immediately after the first one and continues until complete decomposition of the ligand and formation of MS {where, M = Ru (II) and Pd (II)} as the end product. Although decomposed fragments of the ligands could not be approximated due to continuous weight loss, the total % weight loss of the complexes corresponds to the loss of the respective ligand after considering the transfer of one sulphur atom to the metal ion and residues corresponds to the palladium or ruthenium sulphide.

4.5. Biological Activity

Metronidazole had a 50% inhibitory concentration (IC_{50} 1.93 μM) in our experiment, which is close to the previously reported (IC_{50} 2.01 μM) obtained against the same strain of *E. histolytica* [31]. All the compounds were evaluated for antiamoebic activity in vitro using *HK-9* strain of *E. histolytica*. The IC_{50} values in micro molar are shown in table 1. The free ligands **1-5** exhibited antiamoebic activity with IC_{50} of 2.56-9.96 μM. Substitutions at N^4 position in thiosemicarbazones with 4-benzylpiperidine (**3**) associated with better IC_{50} value (IC_{50} = 2.56 μM) among all the ligands. Complexation of the thiosemicarbazones with palladium (II) and ruthenium (II) results in compounds (**1a-5b**), which showed less (IC_{50} = 0.84 - 4.19 μM) than their respective ligands indicating that the complexation to metal enhances the activity of the ligand. This may be explained by Tweedy's theory [32], 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. It is concluded that the presence of these bulky groups at position N^4 of the thiosemicarbazone moiety enhanced antiamoebic activity. In our earlier studies, it was found that the transition metal complexes of NS donor ligands showed good antiamoebic activity against the same strain of *E. histolytica* [16–20]. 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 [33]. As shown in Table 1, all the Ru (II) and Pd (II) complexes cause a marked inhibition, while the parent compounds are less active than metronidazole. These activities indicate that the complexation to Pd not only increases the activity of the parental drug but also modifies it from amoebostatic to amoebicidal. The complexes **1a-4a, 1b** and **3b** display amoebicidal activity. Detailed studies of the toxicity of these complexes, mechanism of action as well as in vivo studies are in progress.

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 antiamoebic agents for therapeutic use.

5. Conclusion

This research examined the biological activities of the new thiosemicarbazones prepared from 5-nitrothiophene-2-carboxaldehyde and their ruthenium (II) and palladium (II) complexes.. All the thiosemicarbazone ligands act as NS bidentate chelators and the substituents did not have any influence on the coordination of compounds. In vitro antiamoebic evaluation of the ligands and metal complexes was carried out against HK-9 strain of *E. histolytica*. The biological behavior revealed that most of the ligands show a weak activity against *E. histolytica*. The chelation induced signifi-

cant changes in the biological activity of the ligands and their palladium and ruthenium complexes **1a-4a ,1b** and **3b** have shown less IC_{50} value than metronidazole.

6. Experimental protocols

6.1. Chemistry

The carbon, hydrogen and nitrogen analyses were performed by the Central Drug Research Institute, Lucknow, India. Chlorine was estimated by standard method. Melting points were recorded on a KSW melting point apparatus and are uncorrected. UV-visible spectra were recorded in DMF on a Shimadzu UV-1601 PC UV-visible spectrophotometer. IR spectra were run as potassium bromide pellets on Perkin-Elmer model 1620 FT-IR spectrophotometer. ^{1}H-NMR spectra were obtained at ambient temperature using Bruker Spectrospin DPX-300 MHz spectrometer in DMSO-d_6 using tetramethylsilane as an internal standard and chemical shifts were reported in ppm. Thermograms of the complexes were recorded under nitrogen on a TG 51 thermogravimetric analyzer with increasing the temperature at 10°C per minute.

6.2. Synthesis of thiosemicarbazones: A general method

All the thiosemicarbazones were synthesized by refluxing the solution of cycloalkylaminothiocarbonylhydrazines (0.003 mole) in water (10 mL) and the solution of 5-nitrothiophene-2-carboxaldehyde (0.003 mole) in ethanol (10 mL) below 25°C for 3 h with continuous stirring. After cooling, the compound was filtered and recrystallized from appropriate solvent.

*6.2.1. 5-Nitrothiophene-2-carbaldehyde-*N*(4)2-ethylpiperidinethiosemicarbazone(1)*

Yellowish grey solid (methanol). Yield: 59%; m.p.: 182°C; *Anal* calc. ($C_{13}H_{18}N_4S_2O_2$): C, 47.85; H, 5.52; N, 17.18; found: C, 47.92; H, 5.56; N, 17.43%; UV/VIS: λ_{max}/cm^{-1}: 28752, 37945, 47619; IR: ν_{max}/cm^{-1}: 3336 (NH), 1601 (C=N), 1529 (C=C), 1128 (C-N), 1034 (C=S); ^{1}H-NMR($(CD_3)_2SO$)/ppm: 8.28 (1H, s, -CH=N), 10.46 (1H, s, -NH), 4.67 (10H, m, -CH_2), 2.31 (3H, t, -CH_3), 6.98-7.59 (2H, m, aryl).

*6.2.2. 5-Nitrothiophene-2-carbaldehyde-*N*(4)4-methylpiperidinethiosemicarbazone(2)*

Yellow solid (methanol). Yield: 88%; m.p.: 191°C; *Anal* calc. ($C_{12}H_{16}N_4S_2O_2$): C, 46.15; H, 5.13; N, 17.95; found: C, 46.25; H, 5.04; N, 17.63 %; UV/VIS: λ_{max}/cm^{-1}: 29624, 38610, 48309; IR: ν_{max}/cm^{-1}: 3328 (NH), 1571 (C=N), 1507 (C=C), 1128 (C-N), 1031 (C=S); ^{1}H-NMR($(CD_3)_2SO$)/ppm: 8.25 (1H, s, -CH=N), 10.21 (1H, s, -NH), 4.41 (8H, m, -CH_2), 2.61 (3H, d, -CH_3), 7.12-7.79 (2H, m, aryl).

*6.2.3. 5-Nitrothiophene-2-carbaldehyde-*N*(4)4-benzylpiperidinethiosemicarbazone(3)*

Brown solid (methanol). Yield: 82%; m.p.: 205°C; *Anal* calc. ($C_{18}H_{20}N_4S_2O_2$): C, 55.67; H, 5.15; N, 14.43; found: C, 55.82; H, 5.46; N, 14.24 %; UV/VIS: λ_{max}/cm^{-1}: 30120, 38610, 48490; IR: ν_{max}/cm^{-1}: 3343 (NH), 1584 (C=N), 1523 (C=C), 1132 (C-N), 1052 (C=S); ^{1}H-NMR($(CD_3)_2SO$)/ppm: 8.31 (1H, s, -CH=N), 11.02 (1H, s, -NH), 4.53 (10H, m, -CH_2), 7.09-7.83 (7H, m, aryl).

*6.2.4. 5-Nitrothiophene-2-carbaldehyde-*N*(4)pyrolidine thiosemicarbazone (4)*

Light yellow solid (methanol). Yield: 71%; m.p.: 183°C; *Anal* calc. ($C_{10}H_{12}N_4S_2O_2$): C, 42.25; H, 4.22; N, 19.69; found: C, 42.37; H, 4.05; N, 19.82 %; UV/VIS: λ_{max}/cm^{-1}: 29256, 38840, 47925; IR: ν_{max}/cm^{-1}: 3321 (NH), 1634 (C=N), 1518 (C=C), 1127 (C-N), 1039 (C=S); ^{1}H-NMR($(CD_3)_2SO$)/ppm: 8.21 (1H, s, -CH=N), 9.35 (1H, s, -NH), 4.12 (8H, m, -CH_2), 7.15-7.79 (2H, m, aryl).

*6.2.5. 5-Nitrothiophene-2-carbaldehyde-*N*(4,4)diethyl thiosemicarbazone (5)*

Brick red solid (methanol). Yield: 89%; m.p.: 150°C; *Anal* calc. ($C_{10}H_{14}N_4S_2O_2$): C, 41.95; H, 4.89; N, 19.58; found: C, 41.92; H, 4.56; N, 19.43%; UV/VIS: λ_{max}/cm^{-1}: 28752, 38712, 48045; IR: ν_{max}/cm^{-1}: 3384 (NH), 1585 (C=N), 1490 (C=C), 1106 (C-N), 1036 (C=S); ^{1}H-NMR($(CD_3)_2SO$)/ppm: 8.39 (1H, s, -CH=N), 10.02 (1H, s, -NH), 2.53 (6H, m, -CH_3), 4.23 (4H, m, -CH_2), 7.40-8.12 (2H, m, aryl).

6.3. Preparation of complexes: A general method

A solution of appropriate thiosemicarbazone (0.001 mole) in 10 mL of methanol was added with stirring to a stirred suspension of $[Pd(DMSO)_2Cl_2]$ / $[Ru(\eta^4\text{-}C_8H_{12})(CH_3CN)_2Cl_2]$ (0.001 mole) in 10 mL of hot methanol. The obtained mixture was refluxed on a water bath for 4 h during which starting material dissolved and orange complex started to separate. After keeping the reaction flask at room temperature for 2 h, the brown solid was filtered, washed with methanol and dried in *vacuo* over silica gel.

*6.3.1. [Pd(5-*N*-2-TCA-2-EPTSCN)Cl_2] (1a)*

Orange solid (methanol:DMSO). Yield: 69%; dec. temp.-297°C. *Anal*. calc. for ($C_{13}H_{18}N_4S_2O_2Cl_2Pd$): C 31.00, H 3.60, N 11.12, Cl 14.08; found: C 31.12, H 3.43, N 11.31, Cl 14.04 %; UV/VIS: λ_{max} (cm^{-1}) 21615, 24630, 36410, 48814; IR: ν_{max} (cm^{-1}) 3437 (NH), 1617 (C=N), 1524 (C=C), 1017 (C=S), 487, 446 (Pd-N, Pd-S); ^{1}H-NMR (DMSO-d_6): (δ, ppm) 3.49 (1H, s, -NH), 7.93 (1H, s, -CH=N), 2.93 (10H, m, -CH_2), 3.43 (1H, m, -CH), 2.03 (3H, t, -CH_3), 6.90-7.21 (2H, m, aryl).

*6.3.2. [Ru(COD)(5-*N*-2-TCA-2-EPTSCN)Cl_2] (1b)*

Brick red solid (methanol). Yield: 73%; dec. temp.: 173°C; *Anal c*alc. ($C_{21}H_{30}N_4S_2O_2Cl_2Ru$): C, 41.58; H, 4.95; N, 9.24; Cl, 11.72; found: C, 41.92; H, 4.56; N, 9.43; Cl, 11.83%; UV/VIS: λ_{max}/cm^{-1}: 21889, 24635, 37144, 48998; IR: ν_{max}/cm^{-1}: 3437 (NH), 1593 (C=N), 1524 (C=C), 1056 (C=S), 520, 465 (M-N, M-S); ^{1}H-NMR ($(CD_3)_2SO$)/ppm:

7.76 (1H, s, H-C=N), 2.37 (3H, t, -CH_3), 4.39 (10H, m, -CH_2), 2.65 (4H, m, *exo* CH_2), 2.05 (4H, m, *endo* CH_2), 7.12-7.87 (2H, m, Aryl).

6.3.3. [Pd(5-N-2-TCA-4-MPTSCN)Cl_2] (2a)

Orange solid (methanol:DMSO). Yield: 75%; dec. temp.- 283°C. *Anal*. calc. for ($C_{12}H_{16}N_4S_2O_2Cl_2Pd$): C 29.43, H 3.29, N 11.44, Cl 14.48; found: C 29.37, H 3.35, N 11.47, Cl 14.39 %; UV/VIS: λ_{max} (cm^{-1}) 21978, 24635, 37525, 49519; IR: ν_{max} (cm^{-1}) 3385 (NH), 1571 (C=N), 1498 (C=C), 1012 (C=S), 512, 481, 437 (Pd-N, Pd-S); ^{1}H-NMR (DMSO-d_6): (δ, ppm) 4.01 (1H, s, -NH), 8.03 (1H, s, -CH=N), 3.61 (8H, m, -CH_2), 3.91 (1H, m, -CH), 2.03 (3H, d, -CH_3), 6.98-7.32 (2H, m, aryl).

6.3.4. [Ru(COD)(5-N-2-TCA-4-MPTSCN)Cl_2] (2b)

Brick red solid (methanol). Yield: 54%; dec. temp.: 150°C; *Anal* calc. ($C_{20}H_{28}N_4S_2O_2Cl_2Ru$): C, 40.54; H, 4.73; N, 9.46; Cl, 11.99 found: C, 40.82; H, 4.56; N, 19.43; Cl, 12.21%; UV/VIS: λ_{max}/cm^{-1}: 21797, 24658, 37257, 49223; IR: ν_{max}/cm^{-1}: 3435 (NH), 1535 (C=N), 1504 (C=C), 1022 (C=S), 526, 448 (M-N, M-S); ^{1}H-NMR (($CD_3)_2$SO)/ppm: 7.80 (1H, s, H-C=N), 2.64 (3H, d, -CH_3), 4.37 (8H, m, -CH_2), 2.49 (4H, m, *exo* CH_2), 2.12 (4H, m, *endo* CH_2), 7.17-8.02 (2H, m, Aryl).

6.3.5. [Pd(5-N-2-TCA-4-BPTSCN)Cl_2] (3a)

Orange solid (methanol:DMSO). Yield: 71%; dec. temp.- 258°C. *Anal*. calc. for ($C_{18}H_{20}N_4S_2O_2Cl_2Pd$): C 38.21, H 3.56, N 9.90, Cl 12.53; found: C 38.40, H 3.38, N 9.85, Cl 12.68 %; UV/VIS: λ_{max} (cm^{-1}) 21535, 24938, 36995, 48843; IR: ν_{max} (cm^{-1}) 3422 (NH), 1584 (C=N), 1527 (C=C), 1026 (C=S), 515, 494, 461 (Pd-N, Pd-S); ^{1}H-NMR (DMSO-d_6): (δ, ppm) 4.10 (1H, s, -NH), 8.19 (1H, s, -CH=N), 3.81 (10H, m, -CH_2), 3.94 (1H, m, -CH), 7.06-7.49 (7H, m, aryl).

6.3.6. [Ru(COD)(5-N-2-TCA-4-BPTSCN)Cl_2] (3b)

Brick red solid (methanol). Yield: 56%; dec. temp.: 166°C; *Anal* calc. ($C_{26}H_{32}N_4S_2O_2Cl_2Ru$): C, 46.71; H, 4.79; N, 8.38; Cl, 10.63; found: C, 46.92; H, 4.56; N, 8.43; Cl, 10.49%; UV/VIS: λ_{max}/cm^{-1}: 21905, 24752, 37514, 48758; IR: ν_{max}/cm^{-1}: 3435 (NH), 1531 (C=N), 1514 (C=C), 1022 (C=S), 535, 463 (M-N, M-S); ^{1}H-NMR (($CD_3)_2$SO)/ppm: 7.86 (1H, s, H-C=N), 4.38 (10H, m, -CH_2), 2.57 (4H, m, *exo* CH_2), 2.16 (4H, m, *endo* CH_2), 7.14-8.04 (7H, m, Aryl).

6.3.7. [Pd(5-N-2-TCA-PYRTSCN)Cl_2] (4a)

Orange solid (methanol:DMSO). Yield: 65%; dec. temp.- 249°C. *Anal*. calc. for ($C_{10}H_{12}N_4S_2O_2Cl_2Pd$): C 26.02, H 2.62, N 12.14, Cl 15.36; found: C 25.94, H 2.47, N 12.26, Cl 15.57%; UV/VIS: λ_{max} (cm^{-1}) 21658, 24752, 37314, 48229; IR: ν_{max} (cm^{-1}) 3176 (NH), 1589 (C=N), 1510 (C=C), 1022 (C=S), 512, 481, 435 (Pd-N, Pd-S); ^{1}H-NMR (DMSO-d_6): (δ, ppm) 4.32 (1H, s, -NH), 8.33 (1H, s, -CH=N), 3.41 (8H, m, -CH_2), 6.98-7.24 (2H, m, aryl).

6.3.8. [Ru(COD)(5-N-2-TCA-PYRTSCN)Cl_2] (4b)

Dark brown solid (methanol:DMSO). Yield: 53%; dec. temp.- 286°C. *Anal*. calc. for ($C_{18}H_{24}N_4S_2O_2Cl_2Ru$): C 38.30, H 4.29, N 9.92, Cl 12.56; found: C 38.35, H 4.03, N 9.87, Cl 12.75 %; UV/VIS: λ_{max} (cm^{-1}) 21670, 24938, 35998, 49750; IR: ν_{max} (cm^{-1}) 3194 (NH), 1585 (C=N), 1507 (C=C), 1019 (C=S), 509, 475, 441 (Ru-N, Ru-S); ^{1}H-NMR (DMSO-d_6): (δ, ppm) 3.92 (1H, s, -NH), 8.19 (1H, s, -CH=N), 3.99 (8H, s, -CH_2), 2.53 (4H, m, *exo* CH_2), 2.19 (4H, m, *endo* CH_2), 7.05-7.31 (2H, m, aryl).

6.3.9. [Pd(5-N-2-TCA-DETSCN)Cl_2] (5a)

Brick red solid (methanol:DMSO). Yield: 74%; dec. temp.: 249°C; *Anal* calc. ($C_{10}H_{14}N_4S_2O_2Cl_2Pd$): 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%; UV/VIS: λ_{max}/cm^{-1}: 21870, 24638, 36995, 49003; IR: ν_{max}/cm^{-1}: 3382 (NH), 1565 (C=N), 1492 (C=C), 1021 (C=S), 506, 453 (M-N, M-S); ^{1}H-NMR (($CD_3)_2$SO)/ppm: 7.85 (1H, s, H-C=N), 3.53 (1H, s, -NH), 2.51 (6H, m, -CH_3), 4.25 (4H, m, -CH_2), 7.37-8.06 (2H, m, Aryl).

6.3.10. [Ru(COD)(5-N-2-TCA-DETSCN)Cl_2] (5b)

Brick red solid (methanol:chloroform). Yield: 74%; dec. temp.: 257°C; *Anal* calc. ($C_{18}H_{26}N_4S_2O_2Cl_2Ru$): C, 42.52; H, 5.12; N, 11.02; Cl, 13.98; found: C, 42.81; H, 5.10; N, 10.89; Cl, 14.11%; UV/VIS: λ_{max}/cm^{-1}: 21924, 24938, 36410, 48314; IR: ν_{max}/ cm^{-1}: 3382 (NH), 1556 (C=N), 1487 (C=C), 1025 (C=S), 518, 454 (M-N, M-S); ^{1}H-NMR(($CD_3)_2$SO)/ppm: 7.92 (1H, s, H-C=N), 2.50 (6H, m, -CH_3), 4.27 (4H, m, -CH_2), 2.61 (4H, m, *exo* CH_2), 2.03 (4H, m, *endo* CH_2), 7.35-8.07 (2H, m, Aryl).

6.4. In vitro testing against E. histolytica

Activity against *E. histolytica* (strain *HK-9*) in vitro was assessed using a microplate method [26]. DMSO (40 μL) [34,35] was added to sample of ligands or complexes (~1 mg) 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 (Nunc) 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 haemocytometer 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 X 10^4 organisms/well was chosen so that confluent, but

not excessive growth took place in control wells. Plate was sealed with expanded polystyrene (0.5 thick). Secured with tape, placed in a modular incubating chamber (flow laboratories, High Wycombe, UK), and gassed for 10 min. with nitrogen before incubation at 37 °C for 72 h.

6.5. Assessment of antiamoebic 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 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 (Labsystem Multiskane Bichromatic, UK). The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best-fitting straight line from which the IC_{50} value was found.

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