Synthesis, Characterization, and Screening for Antiamoebic Activity of Palladium(II), Platinum(II), and Ruthenium(II) Complexes with NS-Donor Ligands

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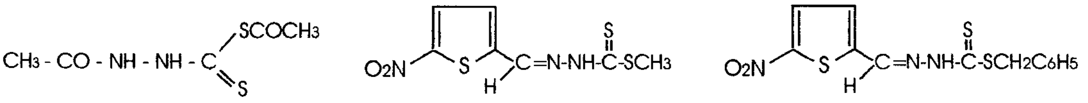
Reaction of [$PdCl_2(DMSO)_2$], [$PtCl_2(DMSO)_2$], and [$RuCl_2(\eta^4\text{-}C_8H_{12})(MeCN)_2$] with *S*-acetyl N^β-acetyldithiocarbazate (=2-acetylhydrazinecarbodithioic acid anhydrosulfide with ethanethioic acid; aadt; **1**), *S*-methyl N^β-[(5-nitrothiophene-2-yl)methylene]dithiocarbazate (=*S*-methyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate; mntdt; **2**), and *S*-benzyl N^β-[(5-nitrothiophene-2-yl)methylene]dithiocarbazate (=*S*-benzyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate; bntdt; **3**) led to new complexes [$PdCl_2$(L)], [$PtCl_2$(L)], and [$RuCl_2(\eta^4\text{-}C_8H_{12})$(L)] (L = ligands **1**–**3**). All these compounds were characterized by elemental analysis, IR, ^{1}H- and ^{13}C-NMR and UV/VIS spectra and thermogravimetric analysis. Ligand **1** coordinates through the thioxo S-atom and the carbazate N(β) atom, whereas in ligands **2** and **3** the thioxo S-atom and the azomethine N-atom are coordinated to the metal ion. Screening of antiamoebic activity of these compounds was performed *in vitro* against the *HK-9* strain of *E. histolytica*. All the complexes were more active than their respective ligands; compound **3a** showed the most promising activity.

Introduction. – Amoebiasis is the infection of the human gastrointestinal tract by *Entamoeba histolytica* (*E. histolytica*). Invasive amoebiasis is more common in developing countries and remains a significant threat to health in large parts of the world. Approximately 50 million people develop intestinal disease or the major extra-intestinal complication of amoebic liver abscess [1], which are responsible for up to 100000 deaths per year [2]. Nitroimidazole drugs such as metronidazole (=2-methyl-5-nitro-1*H*-imidazole-1-ethanol) is highly effective against the acute disease, but has common side effects [3]. It is poorly tolerated, and mutagenic effects of metronidazole in bacteria has raised fear that the drug may be carcinogenic in man [4]. There have been a few reports of resistance of *E. histolytica* to metronidazole [5]. Therefore, it is desirable to search new amoebicidal agents better than the actually available medication. Metals have been used in medicine for centuries, the success of *cis*-[$PtCl_2(NH_3)_2$] (cisplatin) as an anticancer drug [6–8] has stimulated a renewed interest in metal-based chemotherapies. The transition-metal complexes of *Schiff* bases derived from dithiocarbazates are widely studied because of their potential for therapeutic uses [9–12] and have applications in health and skin care [13]. The antitumor activity of transition-metal chelates of *Schiff* base ligands derived from *S*-methyl dithiocarbazate have been reported [14]. Carcinostatic activities also have been found for some metal complexes of dithiocarbazoic acid and the *Schiff* bases derived from its *S*-methyl ester [15]. However, the potential of *Schiff* bases derived from *S*-alkyl dithiocarbazate and their Pd^{II} complexes as antiamoebic agents has so far been very little explored [16]. As part of our ongoing research work on antiamoebic compounds [17][18], we report herein the synthesis, characterization, and *in vitro* screening for amoebicidal activity of new Pd^{II}, Pt^{II} and Ru^{II} complexes with *S*-acetyl *N*$^{\beta}$-acetyldithiocarbazate (=2-acetylhydrazinecarbodithioic acid anhydrosulfide with ethanethioic acid; **1**), *S*-methyl *N*$^{\beta}$-[(5-nitrothiophene-2-yl)methylene]dithiocarbazate (=*S*-methyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate; **2**), and *S*-benzyl *N*$^{\beta}$-[(5-nitrothiophene-2-yl)methylene]dithiocarbazate (=*S*-benzyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate; **3**) (*Fig. 1*) against the *HK-9* strain of *E. histolytica.*



1 **2** **3**

Fig. 1. *Structure of ligands* S-*acetyl* N$^{\beta}$-*acetyldithiocarbazate* (=aadt; **1**), S-*methyl* N$^{\beta}$-*[(5-nitrothiophene-2-yl)methylene]dithiocarbazate* (mntdt; **2**), *and* S-*benzyl* N$^{\beta}$-*[(5-nitrothiophene-2-yl)methylene]dithiocarbazate* (bntdt; **3**)

Experimental. – 1. *General.* Palladium chloride, platinum chloride, and ruthenium chloride were purchased from *Aldrich* chemical company (USA). *S*-Acetyl *N*$^{\beta}$-acetyldithiocarbazate, *S*-methyl dithiocarbazate, and *S*-benzyl dithiocarbazate were prepared by the method described previously [19–21]. The complex precursors [$PdCl_2(DMSO_2)$)], [$PtCl_2(DMSO)_2$] and [$RuCl_2(\eta^4-C_8H_{12})(MeCN)_2$] were prepared by literature procedures [22][23]. Melting points: *KSW* melting-point apparatus; uncorrected. UV/VIS Spectra: DMF solns.; *Shimadzu UV-1601-PC*-UV-VIS spectrophotometer; λ_{max} in cm^{-1}. IR Spectra: KBr disks; *Perkin-Elmer 1620*-FT-IR spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker Spectrospin-DPX-300-MHz* spectrophotometer; at r.t. in $CDCl_3$ and (D_6)DMSO; δ in ppm rel. to $SiMe_4$ as internal standard. Thermograms of the complexes were recorded under N_2 with a *TG-51* thermogravimetric analyser by 10° temp. increase/min. Elemental analyses (C, H, N) were carried out by the Central Drug Research Institute, Lucknow, India; the Cl-content was determined by the standard method.

2. *Ligands.* S-*Acetyl* N$^{\beta}$-*Acetyldithiocarbazate* (aadt; **1**). Ligand **1** was prepared according to [19]. Yield 64%. Yellow solid. M.p. 215°. UV/VIS: 23095, 29154. IR: 3310 (NH), 1620 (C=O), 1053 (C=S). ^{1}H-NMR ($CDCl_3$): 3.34 (*s*, 2 Me); 10.53 (*d*, 2 NH). ^{13}C-NMR ((D_6)DMSO): 202.47 (C=S); 162.63 (C=O); 27.49 (Me). Anal. calc. for $C_5H_8N_2O_2S_2$: C 31.21, H 4.20, N 14.40; found: C 31.25, H 4.16, N 14.58.

S-*Methyl* N$^{\beta}$-*[(5-Nitrothiophene-2-yl)methylene]dithiocarbazate* (mntdt; **2**) *and* S-*Benzyl* N$^{\beta}$-*[(5-Nitrothiophene-2-yl)methylene]dithiocarbazate* (bntdt; **3**). A soln. of *S*-methyl dithiocarbazate or *S*-benzyl dithiocarbazate (3 mmol) in abs. EtOH (15 ml) was mixed with a soln. of 5-nitrothiophene-2-carboxaldehyde (3 mmol) in EtOH (15 ml). The mixture was heated on a water bath for 4 h and then allowed to stand for 2 h at r.t. The solid that separated out was filtered, washed with cold EtOH and dried *in vacuo* over silica gel.

Data of **2**. Yield 54%. Orange solid. M.p. 155°. UV/VIS: 24093, 33223. IR: 3180 (NH), 1520 (C=N), 1064 (C=S). ^{1}H-NMR ($CDCl_3$): 8.24 (*s*, CH=N); 2.53 (*s*, Me); 11.01 (*s*, NH); 7.38–7.97 (*m*, 2 arom. H). ^{13}C-NMR ((D_6)DMSO): 199.53 (C=S); 17.84 (Me); 140.45 (C=N); 125.95–129.47, 142.26 (arom. C). Anal. calc. for $C_7H_7N_3O_2S_3$: C 32.18, H 2.68, N 16.09; found: C 32.04, H 2.85, N 15.79.

Data of **3**. Yield 79%. Orange solid. M.p. 176°. UV/VIS: 24272, 33445. IR: 3495 (NH), 1515 (C=N), 1045 (C=S). ^{1}H-NMR ($CDCl_3$): 7.91 (*s*, CH=N); 4.54 (*s*, PhC*H*$_2$); 10.18 (*s*, NH); 7.21–7.53 (*m*, 7 arom. H). ^{13}C-NMR ((D_6)DMSO): 197.47 (C=S); 37.64 (Ph*C*H$_2$); 151.27 (C=N); 127.32–130.95, 139.34, 145.20 (arom. C). Anal. calc. for $C_{13}H_{11}N_3O_2S_3$: C 46.29, H 3.26, N 12.46; found: C 46.54, H 3.14, N 12.22.

3. *Metal Complexes.* A soln. of ligand **1**, **2**, or **3** (1 mmol) in MeOH (10 ml) was added with stirring to a suspension of the appropriate metal precursor (1 mmol) in hot MeOH (10 ml). The mixture obtained was refluxed on a water bath for 4 h. On keeping the mixture overnight at 0°, a colored compound precipitated; it was filtered, washed with cold EtOH, and dried *in vacuo* over silica gel.

*(2-Acetylhydrazinecarbodithioic acid-κ*N^2,*κ*S′ *anhydrosulfide with ethanethioic acid)dichloropalladium* ([$PdCl_2$(aadt)]; **1a**): Yield 67%. Dark brown solid. M.p. 280°. UV/VIS: 37453. IR: 3190 (NH), 1624 (C=O), 1031 (C=S). ^{1}H-NMR ((D_6)DMSO): 3.21 (*s*, 2 Me); 3.56 (*d*, 2 NH). ^{13}C-NMR ((D_6)DMSO): 185.35 (C=S); 163.27 (C=O); 29.16 (Me). Anal. calc. for $C_5H_8N_2S_2O_2Cl_2Pd$: C 16.41, H 2.04, N 7.76, Cl 19.39; found: C 16.26, H 2.16, N 7.58, Cl 19.24.

*(2-Acetylhydrazinecarbodithioic acid-κ*N^2,*κ*S′ *anhydrosulfide with ethanethioic acid)dichloroplatinum* [$PtCl_2$(aadt)]; **1b**): Yield 48%. Brown solid. M.p. 250°. UV/VIS: 37313. IR: 3175 (NH), 1627 (C=O), 1033 (C=S). ^{1}H-NMR ((D_6)DMSO): 3.27 (*s*, 2 Me); 3.63 (*d*, 2 NH). ^{13}C-NMR ((D_6)DMSO): 192.56 (C=S); 162.95 (C=O); 27.85 (Me). Anal. calc. for $C_5H_8Cl_2N_2O_2PtS_2$: C 13.02, H 1.86, N 6.30, Cl 15.33; found: C 13.10, H 1.74, N 6.11, Cl 15.50.

*(2-Acetylhydrazinecarbodithioic acid-κ*N^2,*κ*S′ *anhydrosulfide with ethanethioic acid)dichloro[(1,2,5,6-η)-cycloocta-1,5-diene]ruthenium* ([$RuCl_2$(aadt)(η^4-C_8H_{12})]; **1c**): Yield 52%. Dark brown solid. M.p. 270°. UV/VIS: 37736. IR: 3150 (NH), 1625 (C=O), 1035 (C=S). ^{1}H-NMR ((D_6)DMSO): 3.19 (*s*, 2 Me); 3.49 (*d*, 2 NH); 2.59 (*m*, 4 '*exo*' H, CH_2); 2.07 (*m*, 4 '*endo*' H, CH_2). ^{13}C-NMR ((D_6)DMSO): 196.72 (C=S); 164.18 (C=O); 28.23 (Me). Anal. calc. for $C_{13}H_{20}Cl_2N_2O_2RuS_2$: C 33.22, H 4.08, Cl 14.91, N 5.70; found: C 33.05, H 4.23, Cl 15.04, N 5.93.

*Dichloro[*S*-methyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate-κ*N^2,*κ*S′*]palladium* ([$PdCl_2$(mntdt)]; **2a**): Yield 72%. Orange solid. M.p. 278°. UV/VIS: 23256, 25974, 36630. IR: 3395 (NH), 1498 (C=N), 1041 (C=S). ^{1}H-NMR ((D_6)DMSO): 8.17 (*s*, CH=N); 2.56 (*s*, Me); 7.23–7.95 (*m*, 2 arom. H). ^{13}C-NMR ((D_6)DMSO): 187.85 (C=S); 17.65 (Me); 145.27 (C=N); 127.73–131.52, 138.41 (arom. C). Anal. calc. for $C_7H_7Cl_2N_3O_2PdS_3$: C 19.22, H 1.60, Cl 16.24, N 9.61; found: C 18.97, H 1.75, Cl 16.27, N 9.47.

*Dichloro[*S*-methyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate-κ*N^2,*κ*S′*]platinum* ([$PtCl_2$(mntdt)]; **2b**): Yield 68%. Brown solid. M.p. 237°. UV/VIS: 23310, 26178, 36496. IR: 3378 (NH), 1502 (C=N), 1047 (C=S). ^{1}H-NMR ((D_6)DMSO): 8.13 (*s*, CH=N); 2.61 (*s*, Me); 3.47 (*s*, NH), 7.51–8.04 (*m*, 2 arom. H). ^{13}C-NMR ((D_6)DMSO): 195.78 (C=S); 18.03 (Me); 148.63 (C=N); 124.62–132.78 (arom. C). Anal. calc. for $C_7H_7Cl_2N_3O_2PtS_3$: C 15.94, H 1.32, Cl 13.47, N 7.97; found: C 16.08, H 1.15, Cl 13.81, N 7.78.

*Dichloro[(1,2,5,6-η)-cycloocta-1,5-diene][*S*-methyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazine carbodithioate-κ*N^2,*κ*S′*]ruthenium* [$RuCl_2$(η^4-C_8H_{12})(mntdt)]; **2c**): Yield 54%. Black solid. M.p. 216°. UV/VIS: 23474, 26110, 36101. IR: 3364 (NH), 1489 (C=N), 1052 (C=S). ^{1}H-NMR ((D_6)DMSO): 8.35 (*s*, CH=N); 2.49 (*s*, Me), 3.65 (*s*, NH); 7.26–7.79 (*m*, 2 arom. H); 2.49 (*m*, 4 '*exo*' H, CH_2); 1.97 (*m*, 4 '*endo*' H, CH_2). ^{13}C-NMR ((D_6)DMSO): 185.64 (C=S); 17.18 (Me); 146.71 (C=N); 127.53–130.81, 141.69 (arom. C). Anal. calc. for $C_{15}H_{19}Cl_2N_3O_2RuS_3$: C 40.91, H 3.37, Cl 11.53, N 6.82; found: C 41.04, H 3.26, Cl 11.48, N 6.93.

*[*S*-Benzyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate-κ*N^2,*κ*S′*]dichloropalladium* ([$PdCl_2$(bntdt)]; **3a**): Yield 75%. Orange solid. M.p. 234°. UV/VIS: 22988, 26316, 37037. IR: 3515 (NH), 1497 (C=N), 1020 (C=S). ^{1}H-NMR ((D_6)DMSO): 8.06 (*s*, CH=N); 4.59 (*s*, PhC*H*$_2$); 3.71 (*s*, NH); 7.37–7.82 (*m*, 7 arom. H). ^{13}C-NMR ((D_6)DMSO): 178.34 (C=S); 36.45 (Ph*C*H$_2$); 159.18 (C=N); 126.45–130.17, 137.59, 151.80 (arom. C). Anal. calc. for $C_{13}H_{11}Cl_2N_3O_2PdS_3$: C 30.41, H 2.14, Cl 13.84, N 8.19; found: C 30.23, H 2.31, Cl 13.69, N 8.25.

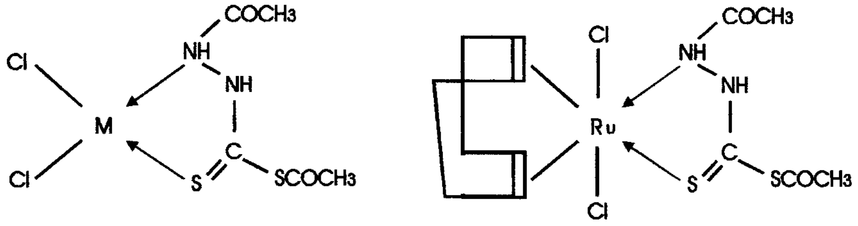
*[*S*-Benzyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate-κ*N^2,*κ*S′*]dichloroplatinum* ([$PtCl_2$(bntdt)]; **3b**): Yield 64%. Brown solid. M.p. 265°. UV/VIS: 23095, 26040, 36767. IR: 3482 (NH), 1503 (C=N), 1025 (C=S). ^{1}H-NMR ((D_6)DMSO): 7.96 (*s*, CH=N); 4.42 (*s*, PhC*H*$_2$); 7.15–7.68 (*m*, 7 arom. H). ^{13}C-NMR ((D_6)DMSO): 192.05 (C=S); 37.27 (Ph*C*H$_2$); 154.92 (C=N); 125.73–129.46, 135.42, 148.67 (arom. C). Anal. calc. for $C_{13}H_{11}Cl_2N_3O_2PtS_3$: C 25.87, H 1.82, Cl 13.84, N 6.97; found: C 25.96, H 1.65, Cl 13.81, N 7.05.

*[*S*-Benzyl 2-[(5-nitrothiophene-2-yl)methylene]hydrazinecarbodithioate-κ*N^2,*κ*S′*]dichloro[(1,2,5,6-η)-cycloocta-1,5-diene]ruthenium* ([$RuCl_2$(bntdt)(η^4-C_8H_{12})]; **3c**): Yield 58%. Dark brown solid. M.p. 229°. UV/VIS: 22831, 26385, 37313. IR: 3499 (NH), 1493 (C=N), 1014 (C=S). ^{1}H-NMR ((D_6)DMSO): 8.02 (*s*, CH=N); 4.47 (*s*, PhC*H*$_2$); 3.57 (*s*, NH); 7.34–7.85 (*m*, 7 arom. H); 2.55 (*m*, 4 '*exo*' H, CH_2); 1.94 (*m*, 4 '*endo*' H, CH_2). ^{13}C-NMR ((D_6)DMSO): 187.81 (C=S); 37.48 (Ph*C*H$_2$); 155.67 (C=N); 126.61–134.54, 147.93 (arom. C). Anal. calc. for $C_{21}H_{23}Cl_2N_3O_2RuS_3$: C 40.91, H 3.37, Cl 11.53, N 6.82; found: C 41.04, H 3.26, Cl 11.37, N 6.93.

4. In vitro *Testing against* E. histolytica. The ligands **1**–**3** and their Pd^{II}, Pt^{II}, and Ru^{II} complexes were screened *in vitro* for antiamoebic activity against the *HK-9* strain of *E. histolytica* by the microdilution method [24]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously [25] in wells of 96-well microtiter plate. All the compounds were dissolved in DMSO (40 μl) [26][27], and the stock solns. 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 plates (*Costar*). Each test included metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae), and a blank (culture medium only). 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. Plates were sealed and gassed for 10 min with N_2 before incubation at 37° for 72 h. After incubation, the growth of amoebae in the plate was checked with a low-power microscope, and the optical density of the soln. in each well was determined at 490 nm with a micro-plate reader. The % inhibition of amoebal growth was calculated [24] from the optical densities of the control and tested well 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 the IC_{50} value was found. The results are reported in *Table 1*.

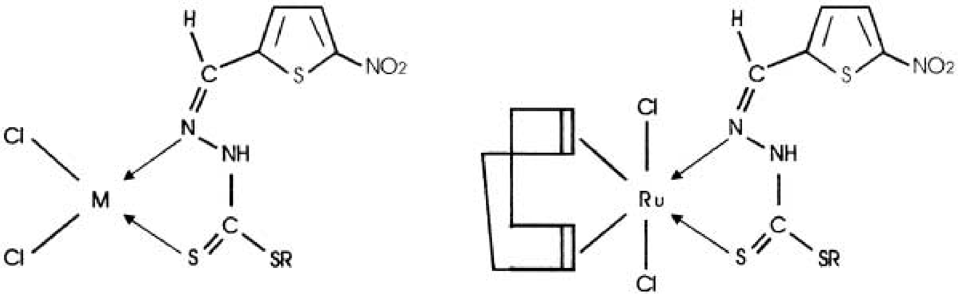
Results and Discussion. – Reaction of ligands **1**–**3** (*Fig. 1*) with $[PdCl_2(DMSO)_2]$, $[PtCl_2(DMSO)_2]$, and $[Ru(cod)(MeCN)_2]Cl_2$ (cod = cycloocta-1,5-diene) gave amorphous solid compounds **1a**–**c**, **2a**–**c**, and **3a**–**c**, respectively, in good yield. All these compounds are stable in both the solid and solution states, and their analytical data are in good agreement with their composition. The complexes are insoluble in H_2O, MeOH, and EtOH, but soluble in DMF and DMSO. They do not undergo any weight loss up to 250°, which suggests a fair thermal stability. The structures of the complexes **1a**–**c**, **2a**–**c**, and **3a**–**c** were established by comparing their spectral data (IR, UV/VIS, ^{1}H- and ^{13}C-NMR) with those of the corresponding free ligand and were further supported by their thermogravimetric analysis (*Figs. 2* and *3*).



1a M = Pd,
b M = Pt

1c

Fig. 2. *Proposed structures of the complexes* **1a**, **1b**, *and* **1c**



2a R = Me, M = Pd
b R = Me, M = Pt
3a R = $PhCH_2$, M = Pd
b R = $PhCH_2$, M = Pt

2c R = Me,
3c R = $PhCH_2$

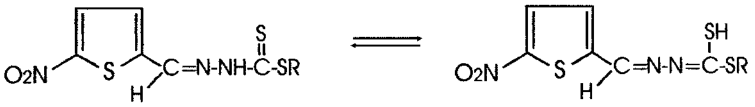
Fig. 3. *Proposed structures of the complexes* **2a**–**c** *and* **3a**–**c**

UV/VIS Spectral Studies. The UV/VIS spectrum of ligand **1** exhibits a broad band at 29150 cm^{-1} and a sharp band at 23095 cm^{-1} due to intraligand transitions. Complexes **1a**–**c** show a single band in the region 37736–37313 cm^{-1} due to a charge-transfer transition, which is in a higher-energy region with respect to ligand **1**. The ligands **2** and **3** exhibit broad bands at 24272–24093 cm^{-1}, which is assigned to the π-π^* transition of the azomethine moiety [28]. Bands at higher energies (33223–33445 cm^{-1}) are attributed to the π-π^* transition of the thiophene ring. In complexes **2a–c** and **3a–c**, the π-π^* transition due to the azomethine chromophore is shifted to 25974–26385 cm^{-1} indicating that the imino N-atom is involved in the coordination. An intense charge-transfer band is observed for the complexes in the 22831–23474 cm^{-1} region.

IR Spectral Studies. The IR spectrum of ligand **1** shows characteristic bands at 1053 and 3310 cm^{-1} attributed to the stretching vibrations $\tilde{\nu}$(C=S) and $\tilde{\nu}$(N–H), respectively. A significant shift of $\tilde{\nu}$(C=S) to lower wave numbers indicates the coordination of the thioxo S-atom. The coordination of N(β) of the hydrazine moiety is evident from the shift of $\tilde{\nu}$(N–H) of the free ligand (*ca.* 3310 cm^{-1}) to lower wave numbers (3150–3190 cm^{-1}) in the complexes. The $\tilde{\nu}$(C=O) mode of the free ligand remains unchanged in complexes **1a–c**, which excludes the possibility of the coordination of the oxo O-atom to the metal ion. The far-IR spectra of the complexes exhibit bands in the region 480–415 cm^{-1}, which are tentatively attributed to $\tilde{\nu}$(M–N) and $\tilde{\nu}$(M–S), respectively.

The ligands **2** and **3** can exhibit thione ⇄ thiol tautomerism (*Scheme*). However, the existence of a strong band in the region 1045–1064 cm^{-1} due to $\tilde{\nu}$(C=S) and no band due to $\tilde{\nu}$(C–SH) near 2570 cm^{-1} suggests their existence only in the thione form. The downward shift of $\tilde{\nu}$(C=S) in the corresponding metal complexes suggests coordination of ligands **2** and **3** to the metal ion through the C=S group. The ligands **2** and **3** also exhibit a strong band in the 1515–1520 cm^{-1} region due to $\tilde{\nu}$(C=N) of the azomethine moiety. In the corresponding metal complexes, this band is shifted to lower frequency, thereby suggesting that the unsaturated N-atom of the azomethine moiety is coordinated to the metal. The far-IR spectra of the complexes exhibit bands in the region 497–430 cm^{-1} tentatively attributed to $\tilde{\nu}$(M–N) and $\tilde{\nu}$(M–S), respectively.

Scheme



¹H-NMR Spectral Studies. Further evidence for the coordinating mode of the ligands **1–3** was obtained from the ¹H-NMR spectra. The ¹H-NMR spectra of ligands **2** and **3** in (D_6)DMSO do not show any SH resonance (expected at *ca.* 4.0 ppm). The appearance of a broad peak for NH (10.18–11.01 ppm) indicates that even in a polar solvent, **2** and **3** remain in their thione form. The NH signal of **1–3** is usually shifted upfield in the corresponding complexes (3.47–3.71 ppm). In complexes **2a** and **3b**, we were unable to locate the NH signal. This either merges with aromatic protons or resonates beyond 15 ppm, suggesting the coordination of the thioxo S-atom. A negligible shift of the MeS or CH_2S resonance on complexation indicates that the S-atom of MeS or CH_2S is not involved in coordination and that the group is still present in the complexes. Aromatic protons of the complexes resonate nearly in the same region as those of the free ligands.

¹³C-NMR Spectral Studies. ¹³C-NMR Spectra also provide diagnostic tools for the elucidation of the coordinating mode of the ligands in complexes. Assignments of the signals are based on the chemical shifts and intensity patterns and on the coordination-induced shift (CIS) $\Delta\delta$ ($\Delta\delta = \delta$(complex) – δ(free ligand)) of the signals for C-atoms in the vicinity of the coordinating functions [29]. Thus, the C=S signal of ligand **1** (202.47 ppm) experiences a $\Delta\delta$ of 5 – 17 ppm in complexes **1a** – **c**, indicating the coordination of the thioxo S-atom. The position of the C=O signal remains unchanged and thus excludes the coordination of the C=O group to the metal. Also the δ(C) of the Me group appears in the same region as that of free ligand. In the ^{13}C-NMR spectra of ligands **2** and **3**, the proposed assignments are based on those suggested by *Giuliani et al.* [30].

The signals at 197.47 – 199.53 ppm of **2** and **3** are assigned to the C=S group, while the signals at 140.45 – 151.27 ppm are assigned to the azomethine C-atom. The latter signal is shifted downfield by 3 – 8 ppm in the corresponding complexes, which indicates the coordination of the N-atom lone pair to the metal as a result of complex formation. Further, an upfield shift of the C=S signal by 4 – 19 ppm for the complexes **2a** – **c** and **3a** – **c** as compared to the free ligands also suggests the coordination of the thioxo S-atom to the metal. Other C-atoms (Me, CH_2, and arom. C-atoms) of these complexes resonate in nearly the same region as those of the free ligands.

Thermogravimetric Analysis. The thermogravimetric analysis (under N_2, rate 10°/min) profiles of the Pd^{II}, Pt^{II}, and Ru^{II} complexes along with the % weight at different temperatures were recorded. These complexes did not lose weight up to 250°. Further increments of temperature caused decomposition of the complexes in two steps. The first step occurred at 250 – 405° implying loss of mixed fragments, the second step started immediately after the first one and continued until the complete decomposition of the ligand and formation of MS ($M = Pd^{II}$, Pt^{II}, and Ru^{II}) as the end product. The total % weight loss corresponds to the loss of the respective ligand after consideration of the transfer of one S-atom to the metal ion, and the residue corresponds to the respective metal sulfide.

Biological Activity. Metronidazole had a 50% inhibitory concentration (IC_{50}) of 0.33 μg/ml in our experiment, which is within the range (0.17 – 0.37 μg/ml) of previously reported IC_{50}s obtained against the same strain of *E. histolytica* [31]. As shown in *Table 1*, complexes **1a** – **c**, **2a** – **c**, and **3a** – **c** cause a marked inhibition as compared to their respective ligands **1**, **2**, and **3**, respectively. Compound **2a** was as active as metronidazole, while **3a** had a better IC_{50} value. The IC_{50} values of Pd-, Pt-, and Ru-complex precursors were also determined establishing that these metal-complex precursors have no activity against *E. histolytica* (*Table 2*). These activities indicate that the complexation to Pd not only increases the activity of the parent drug but also modifies it from amoebostatic to amoebicidal. Detailed studies on the mechanism of action of these complexes as well as further modifications of these and other related metal derivatives are in progress.

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Table 1. In vitro *Screening for Amoebicidal Activity of Ligands and Their* Pd^{II}, Pt^{II}, *and* Ru^{II} *Complexes against the* HK-9 *Strain of* E. histolytica

	Compound	*IC*$_{50}$ [μg/ml][a])
1	aadt	0.94 ± 0.15
1a	$[PdCl_2(aadt)]$	0.53 ± 0.09
1b	$[PtCl_2(aadt)]$	0.61 ± 0.08
1c	$[RuCl_2(aadt)(\eta^4\text{-}C_8H_{12})$	0.70 ± 0.10
2	mntdt	0.59 ± 0.07
2a	$[PdCl_2(mntdt)]$	0.32 ± 0.08
2b	$[PtCl_2(mntdt)]$	0.39 ± 0.06
2c	$[RuCl_2(\eta^4\text{-}C_8H_{12})(mntdt)]$	0.43 ± 0.06
3	bntdt	0.57 ± 0.13
3a	$[PdCl_2(bntdt)]$	0.28 ± 0.07
3b	$[PtCl_2(bntdt)]$	0.37 ± 0.06
3c	$[RuCl_2(bntdt)(\eta^4\text{-}C_8H_{12})]$	0.40 ± 0.09
	metronidazole	0.33 ± 0.06

[a]) Mean ± 2 s.d.

Table 2. IC$_{50}$ *Values Obtained for Metal-Complex Precursors and Metronidazole Against the* HK-9 *Strain of* E. histolytica

	IC$_{50}$ [μg/ml][a])
$[PdCl_2(DMSO)_2]$	2.66 ± 0.70
$[PtCl_2(DMSO)_2]$	3.90 ± 0.80
$[RuCl_2(\eta^4\text{-}C_8H_{12})(MeCN)_2]$	5.17 ± 1.25
Metronidazole	0.31 ± 0.06

[a]) Mean ± 2 s.d.

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