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Original article

Palladium(II) complexes of NS donor ligands derived from S-methyl-dithiocarbazate, S-benzyldithiocarbazate and thiosemicarbazide as antiamoebic agents

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Abstract – Synthesis of palladium(II) complexes of the type [$PdLCl_2$] (where L = Schiff bases derived from 2-acetylpyridine and S-methyldithiocarbazate, S-benzyldithiocarbazate or thiosemicarbazide) have been isolated by the reaction of [$Pd(DMSO)_2C1_2$] and respective ligands. The complexes have been characterized by elemental analyses, IR, ^{1}H-NMR, electronic spectra and thermogravimetric analysis. It is concluded that the thionic sulphur and the azomethine nitrogen atoms of the ligands are bonded to the metal ion. Assessment of antiamoebic activity against *Entamoeba histolytica* (strain HK-9) was done by using a microdilution method. [Pd(2-Acpy-SMDT)$C1_2$] and [Pd(2-Acpy-SBDT)$C1_2$] have shown greater activity, whereas [Pd(2-Acpy-TSC)$C1_2$] showed similar activity as metronidazole in vitro.

palladium complexes / 2-acetylpyridine-S-methyldithiocarbazate / 2-acetylpyridine-S-benzyldithiocarbazate / 2-acetylpyridinethiosemicarbazide / antiamoebic evaluation

1. Introduction

Amoebiasis is the result of infection by the protozoan parasite *Entamoeba histolytica* which is responsible for up to 100 000 deaths per annum, placing it second only to malaria in mortality due to a protozoan parasite. Untreated disease may progress to hepatic amoebiasis and other complications [1]. The metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) is a highly effective amoebicide and is considered by many clinicians to be the drug of choice for treating acute amoebiasis. However, it has mutagenic effects in bacteria and is carcinogenic to rodents [2]. The drug is relatively ineffective against asymptomatic infection in the intestinal lumen (cyst-passers) [2] and adverse effects include especially severe nausea and interaction with alcohol may reduce the level of patient compliance. To date there have been few reports of resistance of *E. histolytica* to metronidazole but the possibility of this developing must be born in mind [3, 4], especially as emetine resistant mutants have been isolated in the laboratory [5]. The ideal treatment for this disease does not, therefore, exist and new agents are required [6].

Coordination metal complexes are gaining increasing importance in recent years particularly in the design of repository, slow release or long acting drugs in nutrition and in the study of metabolism [7–10]. Metal ions are also known to accelerate drug action. The efficacy of a therapeutic agent is known to be enhanced upon coordination with a metal ion [11, 12]. Metal complexes of heterocyclic thiosemicarbazones are important due to

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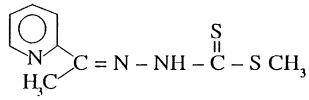
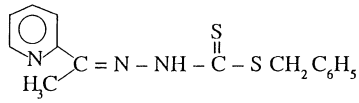
Abbreviations: 2-acpy-SBDT: 2-acetylpyridine-S-benzyldithiocarbazate; 2-acpy-SMDT: 2-acetylpyridine-S-methyldithiocarbazate; 2-acpy-TSC: 2-acetylpyridine-thiosemicarbazide; DMF: dimethyl formamide; DMSO: dimethyl sulfoxide; *E. histolytica: Entamoeba histolytica.*

[1](2-Acpy-SMDT)



[2](2-Acpy-SBDT)



[3](2-Acpy-TSC)

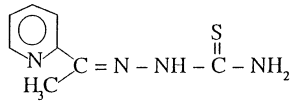


Figure 1. Structures of Schiff bases 1, 2 and 3.

their biological activity [13–16]. Thiosemicarbazone derivatives have raised considerable interest in chemistry and biology due to their antibacterial [17, 18], antimalarial [19, 20], antineoplastic [21, 22] and antiviral [23, 24] activities. A number of transition metal complexes of methyl-3-[1-(2- pyridyl) ethylidene] carbodithioate have been reported for antitumor activity [25].

In view of these considerations we decided to prepare some palladium(II) complexes of the following Schiff bases **1–3** (*figure 1*) and study their antiamoebic activity against *E. histolytica* (strain HK-9). To our knowledge this is the first report of palladium complexes having a dithiocarbazate moiety and report very encouraging results of in vitro activity against *E. histolytica*.

2. Chemistry

Palladium chloride (99%) and 2-acetylpyridine (99%) were purchased from Aldrich Chemical Company (USA). Thiosemicarbazide was procured from E. Merck (Germany). S-methyldithiocarbazate [26] S-benzyldithiocarbazate [27] and $[Pd(DMSO)_2C1_2]$ [28] were prepared by the methods described previously. All ligands were prepared by the literature methods [29, 30]. Their purities were checked by melting point determination and structures were confirmed by IR, ^{1}H-NMR and electronic spectra. All the complexes were obtained by a general method. The complexes were prepared by mixing an equimolar ratio of the appropriate ligand and $[Pd(DMSO)_2C1_2]$ in refluxing methanol as shown by equation 1.

$$[Pd(DMSO)_2C1_2] + L \xrightarrow[\text{reflux}]{CH_3OH} [PdLC1_2] + 2DMSO \qquad (1)$$

(where L = ligands **1**, **2** and **3**).

The products thus obtained were separated from the solution by filtering at room temperature and drying in vacuo over silica gel to constant weight. All the complexes were insoluble in water, methanol and ethanol and soluble in DMF and DMSO. The complexes were characterized by their elemental analysis. The structure of the compounds was confirmed by thermogravimetric analysis, IR, UV-visible and ^{1}H-NMR spectroscopy. These complexes are high melting solids and decompose before their melting temperatures. The analytical and physicochemical data are collected in *table I*.

3. Pharmacology

The synthesized complexes **1a**, **2a** and **3a** (*table I*) and their respective ligands (*figure 1*) were tested for antiamoebic activity against strain (HK-9) of *E. histolytica* by a microdilution method [31]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium as described previously [32] in 96-well microtitre plates. Each compound tested was serially diluted and added to the

Table I. Analytical and physico-chemical data of complexes.

S. No.	Compound/Stoichiometry	Colour	Yield %	Dec. temp. (°C)	Found (calcd.) C	H	N	Cl
1a	$[Pd(2\text{-Acpy-SMDT})Cl_2]$ $(C_9H_{11}N_3S_2Cl_2Pd)$	orange	62	286	26.64 (26.93)	3.10 (2.74)	10.66 (10.47)	17.44 (17.70)
2a	$[Pd(2\text{-Acpy-SBDT})Cl_2]$ $(C_{15}H_{15}N_3S_2Cl_2Pd)$	orange	69	263	37.25 (37.73)	3.32 (3.14)	8.54 (8.80)	14.65 (14.88)
3a	$[Pd(2\text{-Acpy-TSC})Cl_2]$ $(C_8H_{10}N_3SCl_2Pd)$	orange	47	320	26.69 (26.96)	3.18 (2.81)	11.86 (11.79)	20.26 (19.94)

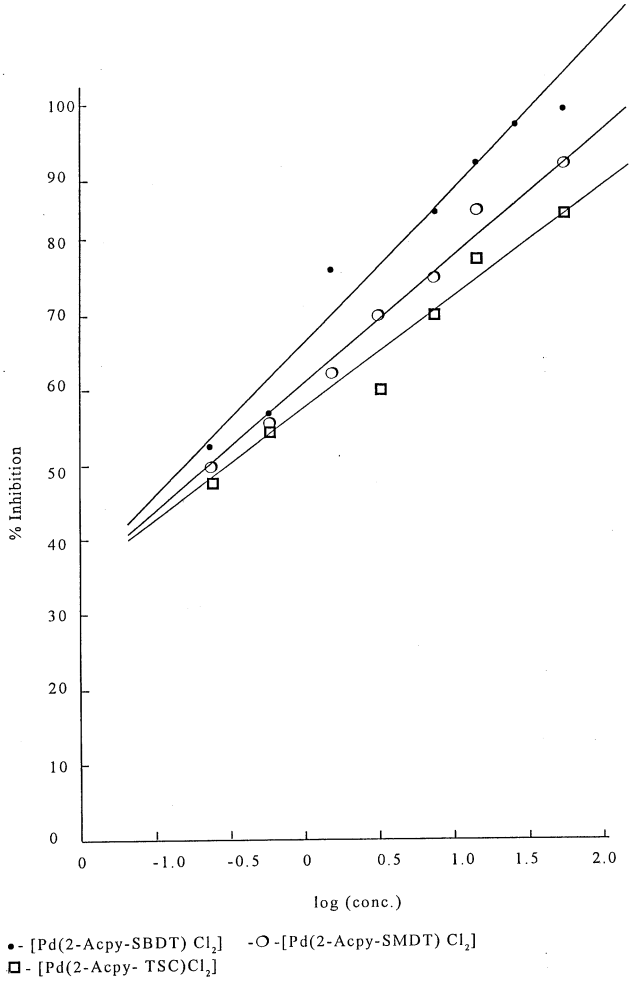


Figure 2. Antiamoebic activity of complexes against *Entamoeba histolytica* (HK-9).

growing trophozoites in the microtitre plate. The effect on growth of trophozoites was monitored microscopically at regular intervals and a quantitative estimation of the drug action was made by protein determination. The % inhibition of amoeba was calculated from the optical densities of the control and test well and was plotted against the logarithm of the 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 (*figure 2). The results are reported in table II.*

4. Results and discussion

$[Pd(DMSO)_2C1_2]$ serves as a good starting material to prepare the complexes reported here. The analytical data

Table II. In vitro activities of ligand and their complexes against *Entamoeba histolytica* (HK-9).

S. No.	Compound	Antiamoebic activity (IC_{50} µg.mL^{-1})
1	2-Acpy-SMDT	0.39
1a	$[Pd(2\text{-Acpy-SMDT})Cl_2]$	0.19
2	2-Acpy-SBDT	0.38
2a	$[Pd(2\text{-Acpy-SBDT})Cl_2]$	0.16
3	2-Acpy-TSC	0.33
3a	$[Pd(2\text{-Acpy-TSC})Cl_2]$	0.33
	Metronidazole	0.33

are in good agreement with the composition $[PdLC1_2]$. The complexes do not undergo any weight loss up to 260 °C (wide infra) which suggest their fair thermal stability. The palladium(II) complex with ligand **3** has earlier been reported [30] but the complex reported here is different from that. The structures of the complexes were established by comparing spectral data of the complexes with those of the free ligands and was further supported by their thermogravimetric analysis.

4.1. Electronic spectra of the complexes

The electronic spectral data of the ligands and their complexes are summarized in *table III*. The spectra of the ligands exhibit a broad band at 29 800 cm^{-1} (at 31 400 cm^{-1} in 2-acpy-TSC). This broadness of band is probably due to n $\rightarrow$ π transition of pyridine as well as azomethine nitrogen of the thiocarbazate/thiosemicarbazide residues [30]. In the metal complexes these two bands are separated well and appeared at 28 650–29 400 cm^{-1} and 31 400–32 600 cm^{-1}, where the former was due to the azomethine nitrogen and the latter one due to the pyridyl residue. In addition, all these complexes exhibit one or two additional bands between 21 280 and 27 700 cm^{-1} due to combination of sulphur $\rightarrow$ metal charge transfer (LMCT) and d–d transitions.

4.2. IR spectral analysis

A partial listing of IR spectra of the ligands and their complexes is given in *table IV*. The IR spectra of the ligands showed characteristic bands at 1 060–1 083 cm^{-1} and 3 150–3 230 cm^{-1}. The sharp band at ~ 1 060 cm^{-1} can be attributed to the stretching vibrations of the C=S group, while the broad band observed in the region of 3 200 cm^{-1} may be due to the ν_{N-H} stretch. These bands indicate the thione nature of the ligands. These two bands remain present in the complexes also. However, a significant shift of the $\nu_{C=S}$ stretch to lower wave number indicates coordination of the thionic sulphur as thioeno-

Table III. Electronic spectral data (in cm^{-1}) of ligands and complexes.

S. No.	Compound	λ_{max}
1	2-Acpy-SMDT	29 850
1a	[Pd(2-Acpy-SMDT)Cl_2]	21 277
		29 412
		32 573
2	2-Acpy-SBDT	29 762
2a	[Pd(2-Acpy-SBDT)Cl_2]	21 277
		28 653
		32 680
3	2-Acpy-TSC	31 397
3a	[Pd(2-Acpy-TSC)Cl_2]	23 419
		27 700
		29 112
		32 468

lization and consequent deprotonation on coordination would show the disappearance of both peaks [33]. A strong band in the region 1 579–1 600 cm^{-1} is assigned to the $\nu_{C=N}$ (azomethine) stretch and this band undergoes a positive shift (5–20 cm^{-1}) in the complexes indicating the participation of the azomethine nitrogen in coordination [30]. Thus, IR data suggest the neutral NS bidentate behaviour of the ligands.

4.3. ^{1}H-NMR analysis

The ^{1}H-NMR spectra of the ligands and complexes are recorded using $CDCl_3$ or DMSO-d_6 as solvent. The typical chemical shifts are shown in *table V*. The Schiff bases exhibit signal due to the –NH proton at 9.00–9.90 ppm. This signal usually shifts up field and appears at 3.50–3.55 ppm. However, in [Pd(2-acpy-TSC)$C1_2$], we were unable to locate the –NH proton signal. This either merges with aromatic protons or resonates beyond 15 ppm. Other protons viz. CH_3 protons of the acetylpyridine and of S-methyldithiocarbazate residues, CH_2 protons of S-benzyldithiocarbazate residue and aromatic protons resonate nearly at the same region as that of the free ligands.

Table IV. IR spectral data (in cm^{-1}) of ligands and complexes.

S. No.	Compound	$\nu_{C=N}$	$\nu_{C=S}$	ν_{N-H}
1	2-Acpy-SMDT	1 579	1 062	3 150
1a	[Pd(2-Acpy-SMDT)Cl_2]	1 598	1 040	3 400
2	2-Acpy-SBDT	1 580	1 064	3 200
2a	[Pd(2-Acpy-SBDT)Cl_2]	1 598	1 037	3 450
3	2-Acpy-TSC	1 600	1 083	3 230
3a	[Pd(2-Acpy-TSC)Cl_2]	1 605	1 037	3 250

4.4. TGA analysis

The TGA profiles of complexes **1a** and **2a** along with the % weight at different temperatures are given in *figure 3*. These complexes do not lose weight up to 260 °C. Further increment of temperature causes decomposition of the complexes in two steps. The temperature range for the first step being 285–377 °C (complex **1a**), 260–385 °C (complex **2a**) or 320–385 °C (complex **3a**) where loss of mixed fragments are observed. The second step starts immediately after the first one and continues until the complete decomposition of the ligand and formation of PdS as the end product. Although decomposed fragments of the ligands could not be approximated due to continuous weight loss, the total % weight loss corresponds to the loss of the respective ligand after considering the transfer of one sulphur atom to the palladium and residue corresponds to the respective metal sulphide.

5. Conclusion

Metronidazol had a 50% inhibitory concentration (IC_{50}) 0.33 µg.mL^{-1} in our experiment which is close to the previously reported (IC_{50}) 0.35 µg.mL^{-1} obtained against the same strain of *E. histolytica* [34]. As shown in *table II*, complexes **1a** and **2a** cause a marked inhibition, while the parent compounds are less active than metron-

Table V. ^{1}H-NMR spectral data (in ppm) of ligands and complexes[a].

S. No.	Compound	CH_3–C=N	S–CH_3	–CH_2	Aromatic proton	–NH
1	2-Acpy-SMDT	2.20 (s)	2.60 (s)	–	7.30–7.50 (m)	9.90 (s)
1a	[Pd(2-Acpy-SMDT)Cl_2]	2.46 (s)	2.63 (s)	–	6.90–7.40 (m)	3.50 (s)
2	2-Acpy-SBDT	2.55 (s)	–	4.57 (s)	7.26–8.95 (m)	9.95 (s)
2a	[Pd(2-Acpy-SBDT)Cl_2]	2.46 (s)	–	4.40 (s)	7.80–8.00 (m)	3.55 (s)
3	2-Acpy-TSC	2.44 (s)	–	–	7.28–8.90 (m)	7.55 (s)
3a	[Pd(2-Acpy-TSC)Cl_2]	3.38 (s)	–	–	7.57–8.55 (m)	–

[a] Letters in parentheses indicate the type of signal: s = singlet, m = multiplet.

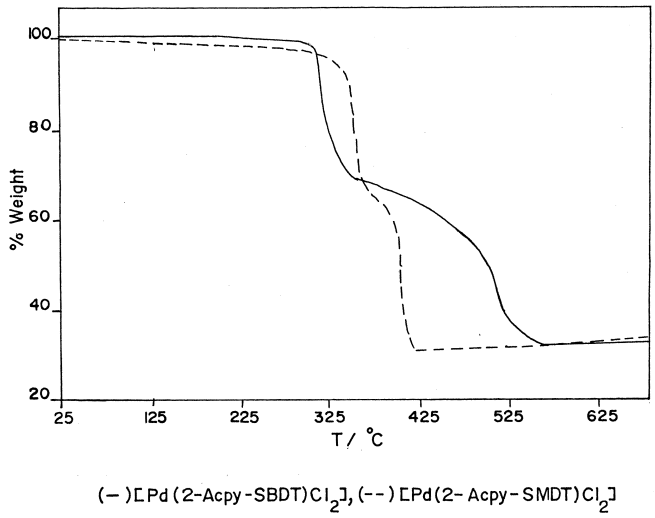


Figure 3. TGA profile of complexes.

idazole. Compounds **3** and **3a** are as active as 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** and **2a** display a high amoebicidal activity. 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. It is therapeutically more relevant to test potential new drugs on *E. histolytica*.

6. Experimental protocols

6.1. Chemistry

The carbon, hydrogen and nitrogen analyses were performed by the micro analytical section of the National Chemical Laboratory, Pune, 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 a Perkin-Elmer model 1620 FT-IR spectrophotometer. ^{1}H-NMR spectra were obtained at ambient temperature using a Bruker Spectrospin DPX-300 MHz spectrometer in DMSO-d_6 using tetramethylsilane as an internal standard and chemical shifts (δ) are reported in ppm. Thermograms of the complexes were recorded under nitrogen on a TG 51 thermogravimetric analyser.

6.2. Preparation of complexes: a general method

A solution of appropriate ligand (0.001 mol) in 10 mL of methanol was added with stirring to a suspension of $[Pd(DMSO)_2C1_2]$ (0.001 mol) in 10 mL of hot methanol. The obtained mixture was refluxed on a water bath for 4 h, during which starting material dissolved and an orange complex started to separate. After keeping the reaction flask at room temperature for 2 h the orange solid was filtered, washed with methanol and dried in vacuo over silica gel.

6.3. In vitro testing against E. Histolytica

Activity against *E. histolytica* (strain HK-9) in vitro was assessed using a microplate method [31]. DMSO (40 µL) [35, 36] was added to sample of ligands or complexes (~ 1 mg) followed by enough culture medium to obtain a concentration of 1 mg/mL. Samples were dissolved or suspended by mild sonication in a sonicleaner bath (Julabo, USRI, West Germany) 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 microtitre plates (Nunc) in 170 µL of medium. Each test included metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) were 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 1×10^5 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. Plates were sealed with expanded polystyrene (0.5 cm thick). Secured with tape, placed in a modular incubating chamber (flow laboratories, High Wycombe, UK), and gassed for 10 min with nitrogen before incubation at 37 °C for 72 h.

6.4. 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. The 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 plates were washed once with tap water and then twice with distilled water and allowed to dry. A 200 µL portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader (Labsystem Multiskane Bichromatic, UK). The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best fitting straight line from which the IC_{50} value was found.

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