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In vitro antitumour and antibacterial studies of some Pt(IV) dithiocarbamate complexes

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Abstract

A few Pt(IV) complexes of the type $[Pt(L)_2Cl_2]$ [where L = morpholine dithiocarbamate (L^1), aniline dithiocarbamate (L^2), *N*-(methyl, cyclohexyl) dithiocarbamate (L^3) and *N*-(ethyl, cyclohexyl) dithiocarbamate (L^4)] were synthesized. The complexes have been characterized by elemental analysis, molar conductance, IR, electronic, ^1H and ^{13}C NMR spectroscopic studies. The ligands found to act in monobasic bidentate fashion. Cyclicvoltammetric studies, antibacterial and in vitro antitumour studies were also carried out.

Keywords: Synthesis; Characterisation; Spectral; Antibacterial; Antitumour

1. Introduction

The clinical utility of platinum anticancer agents is well proved [1–5]. However, the clinical usefulness of cisplatin has been frequently limited by its severe side effects such as nephrotoxicity, nausea, ototoxicity, neurotoxicity and myelotoxicity [6] development of acquired resistance [7] low activity against breast and colon cancer. Therefore, it is desirable to develop a new platinum-based anticancer drug with broader spectrum of activity, improved clinical efficacy and reduced toxicity, better than cisplatin. The interest in dithiocarbamate complexes of platinum has been stimulated because it was reported that dialkyl dithiocarbamate when coadministered with cisplatin reduces its nephrotoxicity [8–10]. A broad spectrum of preclinical antitumour activity of platinum(IV) complexes have been reported having a different mechanism from platinum(II) and remain active against general cancer cell lines as well as other cell lines resistant to cisplatin [11]. Moreover, metal dithiocarbamates have diversified applications in the field of rubber chemistry as vulcanization accelerator and antioxidants [12]. These have gained acceptance as fungicides, insecticides and rodent repellents [13–15]. The present study deals with synthesis, characterization of platinum(IV) dithiocarbamates complexes including in vitro antitumour and antibacterial study.

2. Experimental

2.1. Materials and methods

All the reagents used were of AR grade. IR and far IR spectra were recorded on Perkin-Elmer spectrum 2000 FT-IR spectrometer. Electronic spectra were recorded on Beckman DU-64 UV–vis spectrophotometer. Conductance measurements were carried out on Elico conductivity Bridge Model CM-102, India. ^1H and ^{13}C NMR were recorded on Bruker spectrospin advance 300 spectrometer and BAS-CV 50 W voltammetric analyser was used for recording the voltammograms of complexes.

2.2. Preparation of dithiocarbamates

The dithiocarbamates were prepared by the method described by Gilman and Blatt [16] with some modifications.

2.2.1. Preparation of KL^1 and KL^2

0.4 mol of corresponding amine was dissolved in the 14 ml methanol and chilled to this a chilled solution of 2.24 g (0.04 mol) potassium hydroxide in aqueous methanol was mixed with constant stirring. The mixed solution was treated with an

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ice-cold solution of 2.5 mL (0.04 mol) carbon disulphide (density 1.266) in 4 mL methanol keeping the temperature of the reaction mixture below 10 °C. During the process, desired white crystalline precipitates separated. It was filtered and washed with ice-cold aqueous methanol and recrystallized from hot water.

2.2.2. Preparation of NaL^3 and NaL^4

A three necked flask was cooled in a freezing mixture of common salt and ice. First of all 2 g (0.05 mol) of sodium hydroxide dissolved in minimum quantity of distilled water was added followed by the addition of 3 mL (0.05 mL) of pure carbon disulphide (density 1.2) through the separating funnel and to the stirred mixture (0.05 mol) of distilled corresponding amine was added. The mixture was stirred mechanically for two hours keeping temperature below 5 °C when a yellowish-white crystalline product separated out which was filtered, washed with small portion of ether and finally recrystallized from acetone–petroleum ether and dried under vacuum over $CaCl_2$ at room temperature.

2.3. Preparation of complexes

2.3.1. Preparation of dithiocarbamate complexes $[Pt(L)_2Cl_2]$ where $L = L^1, L^2, L^3$ and L^4

The corresponding ligand L [where $L = L^1$ (0.101 g, 0.50 mmol), L^2 (0.104 g, 0.50 mmol), L^3 (0.106 g, 0.50 mmol), L^4 (0.113 g, 0.50 mmol)] in distilled water was added to aqueous solution of K_2PtCl_6 (0.104 g, 0.25 m mol). The solution was stirred and heated for two hours. The colour of the solution changed to dark yellow (mustard) and yellowish orange coloured complex separated. It was centrifuged and washed with distilled water several times.

3. Results and discussion

Elemental analysis (Table 1) reveals that complexes are of good purity. All the complexes are soluble in DMSO and insoluble in most of the organic solvents. The molar conductance values of the isolated complexes measured in d_6-DMSO are found to be less than 15 Ω^{-1} cm^2 mol^{-1} suggesting their non-electrolytic nature.

3.1. Electronic spectra

The electronic spectra of dithiocarbamates show high intensity absorption due to chromophore NCS_2. Two bands are expected because of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. In UV spectra of ligand L^1 two bands at 273 nm ($\log \varepsilon = 3.268$) and 295 nm ($\log \varepsilon = 3.304$) appear. Two absorption bands are observed for ligand L^2 at 270 nm ($\log \varepsilon = 3.134$) and 300 nm ($\log \varepsilon = 3.279$). Ligand L^3 absorbs at 272 nm ($\log \varepsilon = 3.027$) and 295 nm ($\log \varepsilon = 3.184$). Ligand L^4 absorbs at 273 nm ($\log \varepsilon = 2.989$) and 299 nm ($\log \varepsilon = 3.162$). On complexation these bands are shifted. All the complexes are found to be diamagnetic. So the Pt(IV) complexes must be octahedral. Pt (IV) is d^6 system and four bands are expected corresponding to $^1A_{1g} \rightarrow {}^3T_{1g}$, $^1A_{1g} \rightarrow {}^3T_{2g}$, $^1A_{1g} \rightarrow {}^1T_{1g}$ and $^1A_{1g} \rightarrow {}^1T_{2g}$ transitions [16]. Strong charge transfer transitions may interfere and prevent the observation of all the expected bands [17–21]. Strong bands ~350 nm is assignable to a combination of metal ligand charge transfer (M → LCT) and d–d band. The very intense band ~420 is assignable to combination of sulphur → metal charge transfer (Lπ → MCT) and d–d bands. Electronic spectra of the complexes are depicted in Table 2.

Table 2
Electronic spectra of dithiocarbamate complexes of Pt(IV)

	λ_{max} (nm)	log (ε)
$[Pt(L^1)_2Cl_2]$	289	4.32
	325	4.08
	350	4.06
$[Pt(L^2)_2Cl_2]$	304	4.10
	350	3.79
$[Pt(L^3)_2Cl_2]$	299	4.27
	343	4.05
$[Pt(L^4)_2Cl_2]$	300	4.16
	325	3.72
	355	3.56
	415	2.25

3.2. Infrared spectra

Three regions in IR spectra of dithiocarbamate complexes are of particular importance. The stretching frequency region 1440–1550 cm^{-1} can be assigned to polar C≡≡N partial double bond of $S_2C{\equiv\equiv}NR_2$ as reported by Chatt et al. [22].

The $\nu_{(C{\equiv\equiv}S)}$stretching frequency has been used to distinguish between the monodentate and bidentate behaviour of the dithiocarbamate group. As reported by Bonati and Ugo [23] and others [24], in case of monodentate dithiocarbamate ligand a doublet arises around 1000 cm^{-1} separated by ≥20 cm^{-1} which is due to non-equivalence of two C–S stretching vibrations. On the other hand in caser of bidentate dithiocarbamate a strong singlet is observed in ~1000 cm^{-1} region, which is indicative of

Table 1
Elemental analysis of dithiocarbamate complexes

Complexes	Found (calculated) (%)					
	C	H	N	S	Cl	Metal
$[Pt(L^1)_2Cl_2]$	20.35 (20.30)	2.65 (2.71)	4.98 (4.73)	21.80 (21.66)	12.70 (12.01)	33.80 (33.16)
$[Pt(L^2)_2Cl_2]$	28.13 (27.86)	1.78 (1.99)	4.23 (4.64)	21.95 (21.27)	11.43 (11.77)	33.14 (32.50)
$[Pt(L^3)_2Cl_2]$	28.19 (28.28)	4.78 (4.71)	4.32 (4.12)	18.50 (18.85)	11.32 (10.46)	30.13 (28.86)
$[Pt(L^4)_2Cl_2]$	32.94 (32.19)	4.68 (4.76)	4.49 (4.17)	19.73 (19.08)	10.94 (10.58)	29.90 (29.21)

Table 3
IR frequencies (cm^{-1}) of dithiocarbamates and their Pt(IV) complex (in KBr pallets)

	$\nu_{(C=N)}$	$\nu_{(C=S)}$	$\nu_{(M-S)}$	$\nu_{(M-Cl)}$
KL^1	1462	991, 1019	–	–
$[Pt(L^1)_2Cl_2]$	1494	1004	390	293
KL^2	1463	990, 1015	–	–
$[Pt(L^2)_2Cl_2]$	1500	1000	390	293
NaL^3	1464	991, 1027	–	–
$[Pt(L^3)_2Cl_2]$	1508	1009	385	294
NaL^4	1465	987, 1013	–	–
$[Pt(L^4)_2Cl_2]$	1500	1002	387	301

symmetrically, bound dithiocarbamate moiety. In present series of dithiocarbamate complexes we observed only one strong band around 1000 cm^{-1}, which indicates that all the dithiocarbamate ligands are bidentate and symmetrically bonded.

Far IR spectra contain the M–S stretching frequencies [25] in the region 390–320 cm^{-1}. The band at ∼280 is assigned to $\nu_{(Pt-Cl)}$ vibrations. The important IR and far IR bands of dithiocarbamate complexes are depicted in Table 3.

3.3. NMR spectra

3.3.1. ^1H NMR spectra

^1H NMR spectra of ligands and complexes were recorded in d_6-DMSO taking TMS as internal standards.

L^1: δ (ppm) 4.26 (t, 4H, $-CH_2-N$), 3.46 (t, 4H, $-CH_2-O$); $[Pt(L^1)_2Cl_2]$: δ (ppm) 3.35–3.93 (m, 16H, $-CH_2$).

L^2: δ (ppm) 6.6–7.2 (m, 5H, $-C_6H_5$), 4.95 (s, br, 1H, –NH); $[Pt(L^2)_2Cl_2]$: δ (ppm) 6.5–7.13 (m, 10H, $-C_6H_5$), 5.03 (s, br, 2H, –NH).

L^3: δ (ppm) 2.06 (s, 3H, $-CH_3$), 1.1–1.9 (m, 11H, –cyclohexyl); $[Pt(L^3)_2Cl_2]$: δ (ppm) 2.10 (s, 6H, $-CH_3$), 1.2–1.97 (m, 22H, –cyclohexyl).

L^4: δ (ppm) 2.5 (q, 2H, $-CH_2$), 2.1 (t, 3H, $-CH_3$), 1.1–1.9 (m, 11H, –cyclohexyl); $[Pt(L^4)_2Cl_2]$: δ (ppm) 2.54 (q, 4H, $-CH_2$), 2.08 (t, 6H, $-CH_3$), 1.18–1.83 (m, 22H, –cyclohexyl).

As reported by Tsipis and Manoussakis [26], in case of metal dithiocarbamate complexes, sharp ^1H NMR spectra without being split indicate the non-coexistance of mono and bidentate dithiocarbamate ligands.

3.3.2. ^{13}C NMR

^{13}C NMR of the compounds was recorded in d_6-DMSO 10–20 ppm upfield shifts are observed for all the complexes for NCS_2 carbon in comparison to free ligands. This further supports the coordination via CS_2^-.

3.4. Cyclicvoltametric studies

Over the last 20 years and so, electrochemical and chemical studies of the redox properties of dithiocarbamate compounds have demonstrated the ability of this ligand to stabilize a wide range of oxidation states. In the case of platinum, well characterized $Pt(R_2dtc)_2$ and $[Pt(R_2dtc)_3]^+$ compounds exist and conventional reductive elimination and oxidative addition reaction occur [27,28]. Voltametric oxidation of $Pt(R_2dtc)_2$ is less well characterized, although a small yield of $[Pt(R_2dtc)_3]^+$ has been observed in bulk electrolysis experiments [29], but no evidence for any Pt(III) intermediates was reported in these studies.

Cyclic voltammetric reduction of all the Platinum complexes gave irreversible peak for overall two electron process. Electron transfer is assumed to be two electron only because Pt(III) if formed is highly unstable oxidation state and is not detectable with in the instrumental time limit. The next electron is then quickly accepted by it to give Pt(II):

$$[Pt(L_2)Cl_2] + 2e^- \rightarrow [PtL_2] + 2Cl^-$$

The electron transfer is irreversible since no peak corresponding to oxidation was observed on reversing the scan direction.

In some complexes, two irreversible reduction peaks (83.8 and 1037.8 mV) are arising which may be due to the reduction of the second geometrical isomer formed in solution only (*cis–trans* isomerisation) during the CV measurements. This is not due to two couples Pt(IV)/Pt(III) and Pt(III)/Pt(II) again because Pt(III) is too short lived to be sensed by the electrode.

3.5. Antibacterial activity

Some of the samples were tested against *Zymomonas mobilis*, *Bacillus subtilis*, *Klebseilla aeroginosa*, *Escherichia coli* and *Pseudomonas aeruginosa*. The dithiocarbamate complexes were less active against these bacteria strains. The antibacterial activity of the complexes is presented in Table 4.

3.6. In Vitro antitumour activity

In vitro activity against human colour adenocarcinoma cell line, was cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 μg/mL streptomycin. A 100 μg/mL penicillin and 50 μg/mL gentamycine. For the assay, 10,000 cells were plated per well of a 96 well tissue culture plate and allowed to adhere overnight at 37 °C in a 5% CO_2 incubator. Next day, the medium was changed to RPMI 1640 containing 5% FCS. The compound to be tested was dissolved in DMSO and subsequent serial dilutions were made in

Table 4
Antibacterial activity of the complexes

Complex	*Zymomonas mobilis*	*Bacillus subtilis*	*Klebseilla aeroginosa*	*Escherichia coli*	*Pseudomonas aeruginosa*
$[Pt(L^1)_2Cl_2]$	+	++	–	–	+
$[Pt(L^3)_2Cl_2]$	++	–	–	–	+
$[Pt(L^4)_2Cl_2]$	–	++	–	+	+

RPMI 1640 medium. The cells were treated with various concentrations of the compounds (10 μM–10 pM) and allowed to incubate at 37 °C in a CO_2 incubator. A second dose of the compound was added to the cells after 24 h and thereafter the cells were incubated for a further 24 h. Appropriate controls for assessing cytotoxicity due to DMSO were included in the assay. Cytotoxicity was assessed using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) [30].

One complex was tested for the antitumour activity. The complex was tested on primary adenocarcinoma (colour). Appropriate controls for DMSO (solvent) were included in the final values.

4. Conclusion

The dithiocarbamate complexes were found less active against *Z. mobilis*, *B. subtilis*, *K. aeroginosa*, *E. coli* and *P. aeruginosa* bacteria strains. In in vitro study, the complex showed good activity at 100 and 10 nM solutions but proliferation started in complexes on further dilution. It was further tested for and showed no adverse effect on the various organs like liver, kidneys, brain and bladder of rabbits.

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