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Synthesis, characterization, antibacterial and cytotoxic study of platinum (IV) complexes

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Abstract—Platinum (IV) complexes [Pt $(L)_2Cl_2$] [where, L = benzyl-*N*-thiohydrazide (L^1), (benzyl-*N*-thio)-1,3-propanediamine (L^2), benzaldehyde-benzyl-*N*-thiohydrazone (L^3) and salicylaldehyde-benzyl-*N*-thiohydrazone (L^4)] have been synthesized. The thiohydrazide, thiodiamine and thiohydrazones can exist as thione-thiol tautomer and coordinate as a bidentate N–S ligand. The ligands were found to act in monobasic bidentate fashion. Analytical data reveal that metal to ligand stoichiometry is 1:2. The complexes have been characterized by elemental analysis, IR, mass, electronic and ^{1}H NMR spectroscopic studies. In vitro antibacterial and cytotoxic studies have been carried out for some complexes. Various kinetic and thermodynamic parameters like order of reaction (n), activation energy (E_a), apparent activation entropy ($S^{\#}$) and heat of reaction (ΔH) have also been carried out for some complexes.

1. Introduction

Platinum (IV) complexes are widely applied in the treatment of various types of cancers such as testicular, ovarian and bladder carcinomas.[1–5] Cisplatin is used in the treatment of head and neck cancer, lung carcinoma, stomach carcinoma, and so on.[6,7] However, the clinical usefulness of cisplatin has been frequently limited by its severe side effects such as nephrotoxicity, nausea, ototoxicity, neurotoxicity and myelotoxicity.[8–10] Besides, there is development of acquired resistance low activity against breast and colon cancer. Therefore, it is desirable to develop new platinum-based drugs with broader spectrum of activity, improved clinical efficacy and reduced toxicity, better than cisplatin.[11]

Platinum (IV) complexes have revealed significantly greater activity in human than that of cisplatin.[12,13] The high activity was ascribed to high cellular uptake, but in vivo reduction alters the pharmacological properties and thus the effectiveness of the drug. However, platinum (IV) complexes have enormous potential as anticancer agents in terms of both high activity and low toxicity, but this potential has not been realized by the drugs investigated to date, probably because they are reduced too readily in the bloodstream. The potential advantages of platinum (IV) complexes that remain in the higher oxidation state in the bloodstream are low reactivity that would diminish loss of active drugs and also lowers the incidence of unwanted side reactions that lead to side effects.[14]

Platinum complexes suitable for oral administration have been known to be water-soluble, lipophilic, and robust enough to survive the gastric environment. For the platinum (IV) complexes, ligand substitution reactions are slow as compared with their platinum (II) analogues. The platinum (IV) complexes may be required to be reduced to the kinetically more labile and reactive platinum (II) derivatives in vivo.[14,15] Nowadays attention is focused on platinum (IV) complexes with bioactive ligands, because of the lower toxicity of platinum (IV) and the possibility of oral administration of some potent platinum (IV) compounds as well as the fact that they can coordinate to DNA. In view of number of applications of the thiohydrazides and thiohydrazones[16–18] and the well-proven clinical utility of the platinum-metal complexes, we have prepared platinum (IV)

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complexes of the thiohydrazide, thiodiamine and thiohydrazones. These complexes were characterized and screened for antibacterial and cytotoxic activity. Thermodynamic parameters such as activation energy (E_a), apparent activation entropy ($S^{\#}$) and enthalpy change (ΔH) for the dehydration and decomposition reactions of the complexes have also been evaluated.

2. Results and discussion

2.1. Elemental analysis

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

2.2. Electronic spectra

The electronic spectra (Table 2) of the thiohydrazides (L^1), thiodiamines (L^2) and thiohydrazones (L^3 and L^4) show spectral bands because of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition. On complexation these bands are shifted. Strong charge transfer transitions may interfere and prevent the observation of all the expected bands.[19,20] Strong bands ~340 nm are assignable to a combination of metal ligand charge transfer (M → LCT) and d–d band. The very intense band ~390 is assignable to combination of sulfur → metal charge transfer (Lπ → MCT) and d–d bands.

2.3. IR spectra

The IR spectra (Table 3) of the thiohydrazides, thiodiamines and thiohydrazones contain groups –NH–C=S as a potential bond forming site. The IR bands are shifted on complex formation due to increased double bond character of C=N group on complexation. The band due to ν(C=S) 750–900 cm^{-1} is a major contributor and ν(C=N) as minor. This is shifted to lower frequency on complexation indicating the coordination to metal ion is through thioamide sulfur C=S. This shift is ~80–140 cm^{-1}, if coordination is through thiol sulfur [21] and 30–40 cm^{-1}, if coordination is through the thione sulfur.[22] In all the complexes of the thiohydrazide, thiodiamine and thiohydrazone ligand, no band for ν(S–H) in the region 2600–2800 cm^{-1} is observed which shows the absence of any thiol (–SH) tautomer in the solid state. However, in solution and in the presence of certain metal ion, the ligands may exist in equilibrium with the tautomeric thiol form.

In all the platinum (IV) complexes the metal nitrogen vibration, ν(M–N), is assigned to the new bands [23] in the far IR between 460 and 490 cm^{-1}, while in the region between 350 and 390 cm^{-1} gives metal-sulfur, ν(M–S) band stretching.[24] The band at ~330–270 cm^{-1} is assigned due to $\nu_{(Pt-Cl)}$ stretching vibrations.

2.4. NMR spectra

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

$\delta_{(ppm)}$ 2.56 (s, 2H^a, –CH_2), 7.74–6.9 (m,5H, Ar-H), 8.9 (br s, 1H^b, –NH), 3.67 (br s, 2H^c, –NH_2).
[$Pt(L^1)_2Cl_2$] $\delta_{(ppm)}$ 3.32 (s, 2H^a, –CH_2), 8.5–7.3 (m, 10H, Ar-H), 9.07 (br s, 2H^b, NH), 4.67 (br s, 4H^c, –NH_2).

Table 2. Electronic spectra of the complexes

Complex	λ_{max} (nm)	log(ε)
$Pt(L^1)_2Cl_2$	284	3.88
	341	2.79
	401	2.18
$Pt(L^2)_2Cl_2$	300	3.77
	343	2.78
	401	2.32
$Pt(L^3)_2Cl_2$	286	4.15
	379	3.57
	416	2.66
$Pt(L^4)_2Cl_2$	295	4.27
	307	3.86
	339	3.52
	402	2.41

Table 3. IR spectra of the complexes

Complex	$\nu_{C=N}$	ν_{N-N}	$\nu_{C=S}$	ν_{M-N}	ν_{M-S}	ν_{M-Cl}
L^1	—	1027	882	—	—	—
$Pt(L^1)_2Cl_2$	—	1029	858	462	377	275
L^2	—	1028	881	—	—	—
$Pt(L^2)_2Cl_2$	—	1024	801	477	375	295
L^3	1595	1030	854	—	—	—
$Pt(L^3)_2Cl_2$	1617	1024	818	480	379	308
L^4	1601	1028	890	—	—	—
$Pt(L^4)_2Cl_2$	1618	1032	763	477	375	309

Table 1. Elemental analysis of the complexes

Complex	Found (calculated)					
	C	H	N	S	Cl	Metal
L^1	53.10 (53.04)	6.02 (6.08)	23.32 (23.20)	17.59 (17.68)	—	—
$Pt(L^1)_2Cl_2$	30.48 (30.57)	3.47 (3.50)	13.26 (13.37)	10.23 (10.19)	11.26 (11.31)	31.55 (31.05)
L^2	59.23 (59.19)	7.56 (7.62)	18.91 (18.83)	14.28 (14.35)	—	—
$Pt(L^2)_2Cl_2$	37.18 (37.08)	4.72 (4.77)	11.22 (11.79)	9.09 (8.98)	10.55 (9.97)	27.55 (27.39)
L^3	66.95 (66.91)	5.56 (5.58)	15.62 (15.61)	11.86 (11.89)	—	—
$Pt(L^3)_2Cl_2$	45.29 (44.78)	3.67 (3.73)	10.33 (10.44)	8.12 (7.96)	8.89 (8.83)	24.45 (24.25)
L^4	63.12 (63.15)	5.22 (5.26)	14.72 (14.73)	11.20 (11.22)	—	—
$Pt(L^4)_2Cl_2$	43.11 (43.06)	3.55 (3.59)	10.19 (10.05)	7.89 (7.66)	8.47 (8.49)	23.40 (23.33)

$\delta_{(ppm)}$ 2.55 (s, 2H^a, –CH_2), 7.85–6.45 (m, 5H, Ar-H), 9.0 (br s, 1H^b, –NH), 3.3 (t, 4H^c, –CH_2), 1.5 (m, 4H^d, –CH_2), 3.92 (br s, 2H^e, –NH_2).
$[Pt(L^2)_2Cl_2]$ $\delta_{(ppm)}$ 3.4 (s, 4H^a, –CH_2), 8.45–7.5 (m, 10H, Ar-H), 9.14 (br s, 2H^b, –NH), 3.45 (t, 8H^c, –CH_2), 1.71 (m, 4H^d, –CH_2), 4.62 (br s, 2H^e, –NH_2)
$\delta_{(ppm)}$2.4 (s, 2H^a, –CH_2), 7.5–6.6 (m, 10H, Ar-H), 8.97 (br s, 1H^b, –NH), 10.4 (br s, 1H^c, –NH), 8.33 (s, 1H^d, –CH).
$[Pt(L^3)_2Cl_2]$ $\delta_{(ppm)}$ 2.68 (s, 4H^a, –CH_2), 7.75–7.1 (m, 20H, Ar-H), 9.09 (br s, 2H^b, –NH), 10.21 (br s, 2H^c, –NH), 8.41 (s, 2H^d, –CH).
$\delta_{(ppm)}$ 2.54 (s, 2H^a, –CH_2), 7.41–6.7 (m, 9H, Ar-H), 8.88 (br s, 1H^b, –NH), 10.6 (br s, 1H^c, –NH), 8.35 (s, 1H^d, –CH), 11.52 (s, 1H^e, –OH).
$[Pt(L^4)_2Cl_2]$ $\delta_{(ppm)}$ 3.33 (s, 4H^a, –CH_2), 8.08–6.96 (m, 18H, Ar-H), 8.97 (br s, 2H^b, –NH), 10.45 (br s, 2H^c, –NH), 8.21 (s, 2H^d, –CH), 11.47 (s, 1H^e, –OH).

The ^{1}H NMR spectrum of thiohydrazides, thiodiamines and thiohydrazones[25,26] shows two signals at $\delta \sim 9.0$–10.3 and $\delta \sim 4.0$ ppm, due to the presence of NH protons which are lost on D_2O exchange. This is observable in the complexes also suggesting that hydrogen bonding to the solvent occurs in the complexes as well as free ligands. The resonance assigned to aldehyde –CH is generally shifted upfield, indicating coordination of azomethine nitrogen.[27]

2.5. Antibacterial study

In the current study, some synthesized complexes were tested against pathogenic bacterial strains such as *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) using the disc diffusion method. Gentamycin was used as reference drug for bacteria. The bacterial strains with the zone of inhibition were observed, 8–9 mm at minimum inhibitory concentration (MIC) of 60.0 μg/disc.

2.6. Cytotoxic study

In the present studies, the cytotoxic study of one metal complex has been determined. The study was used to test the growth inhibition by MTT assay. Data are expressed in terms of percentage (%) cytotoxicity. The metal complexes caused ~40% inhibition. The results of the antitumour activity of the metal complexes suggested that the complexes are an effective inhibitor at moderate concentrations. The importance of such work lies in the possibility that the new complexes might be more efficacious drug against tumours 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 antitumour agents for therapeutic use (Tables 4 and 5).

2.7. Thermal study

The TG and DTA study in air atmosphere has been carried out for one complex. Thermal studies were utilized to elucidate the number of kinetic and thermodynamic parameters. From TG curve, order of reaction (n), activation energy (E_a) and apparent activation entropy ($S^{\#}$) were enumerated by the Coats–Redfern method.[28] From the DTA curves, the heat of reaction was calculated. Kinetic parameters of each step by Coats–Redfern method are shown in Figures 3–5 and the thermal data are tabulated in Tables 6–8.

Table 4. Antibacterial study of the complex

S. no.	Complex	Zone of inhibition (mm)		MIC (μg/disc)	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
1	$Pt(L^1)_2Cl_2$	—	—	—	—
2	$Pt(L^2)_2Cl_2$	—	—	—	—
3	$Pt(L^3)_2Cl_2$	9	9	60.0	60.0
4	$Pt(L^4)_2Cl_2$	8	8	60.0	60.0
Gentamycin		16	16	1.0	1.0

Table 5. Cytotoxicity study of the complex

S. no.	Complex	Concentrations	
		100 μg/mL	10 μg/mL
1	$Pt(L^1)_2Cl_2$	—	—
2	$Pt(L^2)_2Cl_2$	42.5	—
3	$Pt(L^3)_2Cl_2$	—	—
4	$Pt(L^4)_2Cl_2$	40.9	11.1
Cisplatin		69.7	73.5

2.8. $[Pt(L^4)_2Cl_2]$ complex

TG curve (Fig. 1) shows two-step decomposition. The first decomposition step (302–670 K) corresponds to the loss of all organic moieties for which observed and calculated weight losses are 67.60% and 68.18%, respectively. The second step starts at (670–1149 K) corresponding to the platinum metal residue for which the observed and calculated weight losses are 77.37% and 76.67%.

The DTA profile (Fig. 2) shows one exotherm at 598 K corresponding to the fusion of the compounds and one exotherm at 691 K corresponding to the oxidation of organic moieties.

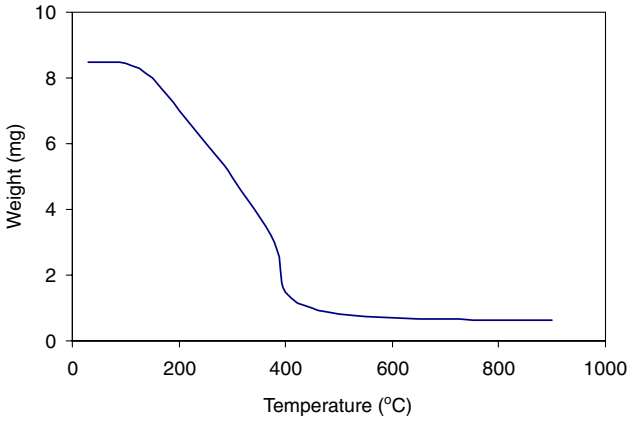


Figure 1. TG curve of $[Pt(L^4)_2Cl_2]$ complex.

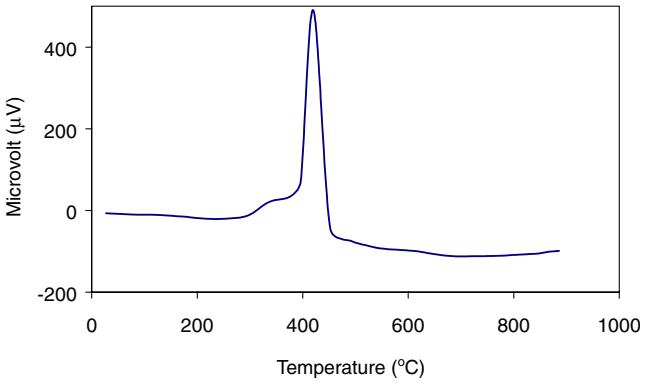


Figure 2. DTA curve of $[Pt(L^4)_2Cl_2]$ complex.

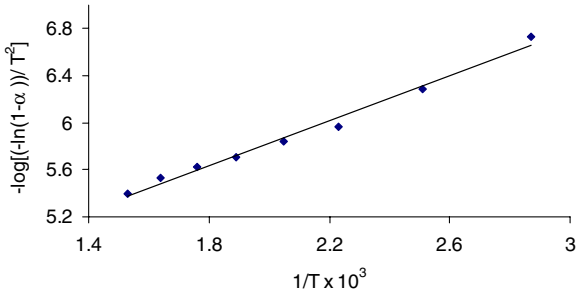


Figure 3. The curve plotted by Coats–Redfern method for$[Pt(L^4)_2Cl_2]$ for step I.

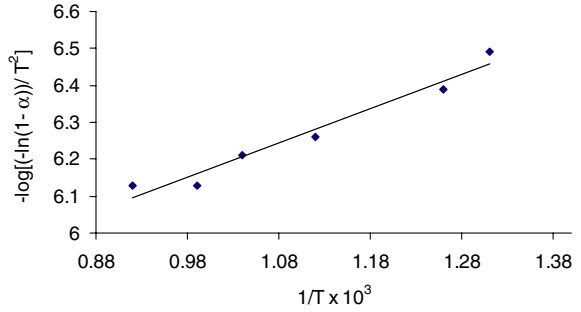


Figure 4. The curve plotted by Coats–Redfern method for $[Pt(L^4)_2Cl_2]$ for step II.

3. Conclusion

All the complexes are found to be diamagnetic, so the platinum (IV) complexes must be octahedral. Platinum (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. The shift towards lower frequency on complexation indicates the coordination to metal ion is through thioamide sulfur. The antibacterial study of the complexes shows significant activity. The bacterial strains with the zone of inhibition were observed, 8 mm. One complex was tested for the cytotoxic activity. The complex was tested on primary adenocarcinoma (colour). The complex showed good activity at 100 and 10 μM solutions. On dilution activity decreases, which shows that, the complex is an effective inhibitor at moderate concentrations. The thermal data (TG/DTA) of the complex indicate that for all the three steps the reaction order is found to be one and activation energy, apparent activation entropy and heat of reaction are found to be significant. On the basis of these spectroscopic studies the probable structure of the complexes is as follows (Figure 5).

4. Experimental

4.1. General

Materials and chemicals: All the reagents used were of AR grade. The analysis of CHNS/O contents of ligands and metal complexes was done on Elementar Analysensysteme GmbH Vario El-III. IR and far IR were recorded on Perkin-Elmer spectrum 2000 FTIR spectrometer. Electronic spectra were recorded on Shimadzu UV–vis spectrophotometer Model 1601. Conductance measurements were carried out on Digital Conductometer Model PT-827, India. Model Jeol SX102/DA-600 (KV 10MA) was used for recording Mass spectra of the ligands. ^1H NMR was recorded on Brucker spectrospin 300 spectrometer. TG/DTA curves for the complexes were recorded on Shimadzu, model 60 WS Thermal analyzer, in static air at a heating rate of 10 °C min^{-1}. The platinum crucible was used with alumina as the reference material.

4.2. Preparation of thiohydrazide, thiodiamine and thiohydrazones

Benzyl-*N*-thiohydrazide, (benzyl-*N*-thio)-1,3-propanediamine, benzaldehyde-benzyl-*N*-thiohydrazone and salicylaldehyde-benzyl-*N*-thiohydrazone were prepared by modification of literature method.[16–18,29]

4.3. Preparation of benzyl-*N*-thiohydrazide (L^1)

In a three-necked round-bottomed flask 5.45 mL (0.05 mol) of benzyl amine (density 0.983) was dissolved in 25 mL methanol and chilled it. To this, a chilled solution of 2.8 g (0.05 mol) potassium hydroxide in 1 mL water and 10 mL methanol was mixed with constant stirring. The mixed solution was treated with an ice-cold solution of 3.02 mL (0.05 mol) carbon disulfide (density 1.26) in 3 mL methanol. The temperature of the reaction mixture was maintained below 10 °C by keeping the flask in a freezing mixture of common salt and ice. During the process, a yellowish-white crystalline precipitate of *N*-benzyl dithiocarbamate separated. It was filtered, washed with ice-cold aqueous methanol. The product was then suspended in 10 mL methanol and treated with freshly prepared potassium chloroacetate [(0.05 mol){potassium chloroacetate was obtained by dissolving 4.73 g chloroacetic acid in 3 mL ice-cold water and mixing it in 5 mL aqueous solution of 2.8 g potassium hydroxide}]. The temperature of the reaction mixture was kept at about 40 °C for an hour and the contents were left overnight at room temperature. After 24 h, methanolic

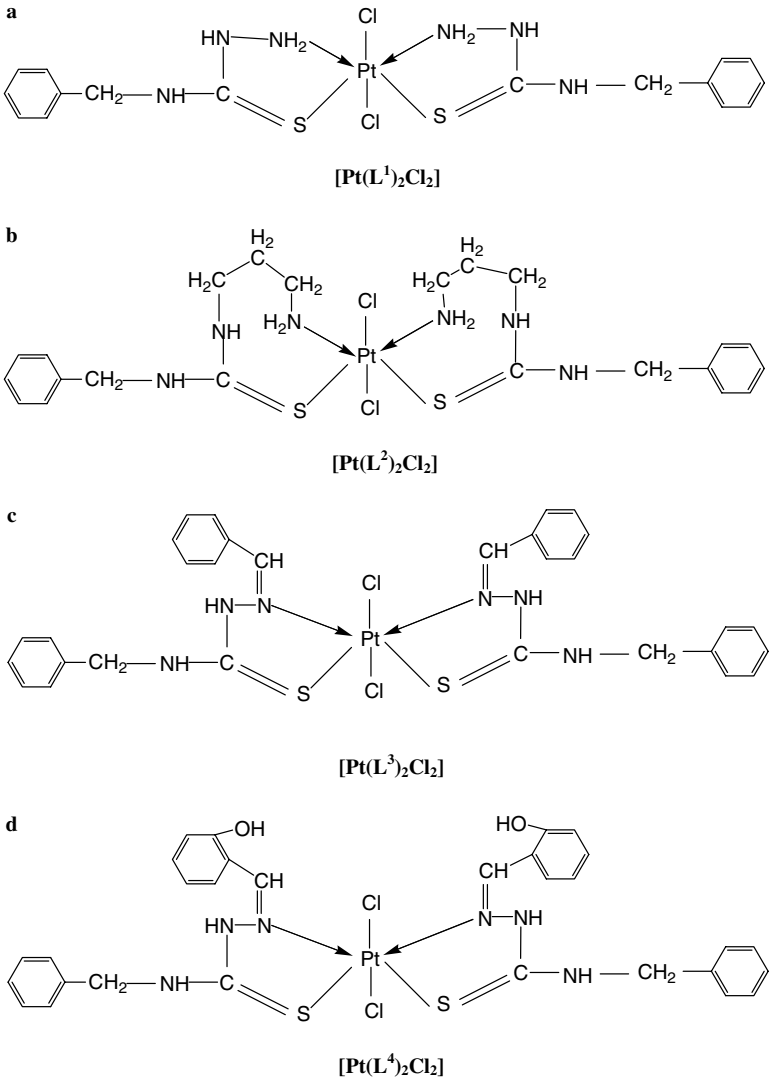


Figure 5. Proposed structure of the complexes.

solution of 2.44 mL (0.05 mol) hydrazine hydrate (density 1.026) was added to the reaction mixture. The content was then heated on a water bath for about 45 min when the desired product began to separate out. It was cooled in ice for 24 h and filtered. Benzyl-*N*-thiohydrazide thus obtained was recrystallized from methanol and dried under vacuum over $CaCl_2$ at room temperature.

The reactions taking place in the preparation are shown below

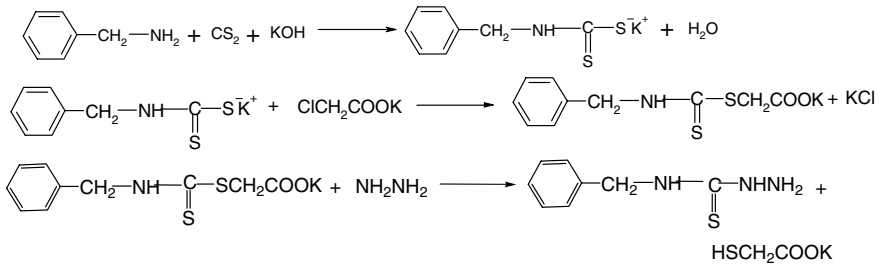


Table 6. Kinetic parameters from TG for $[Pt(L^4)_2Cl_2]$ complex by Coats–Redfern method (step I)

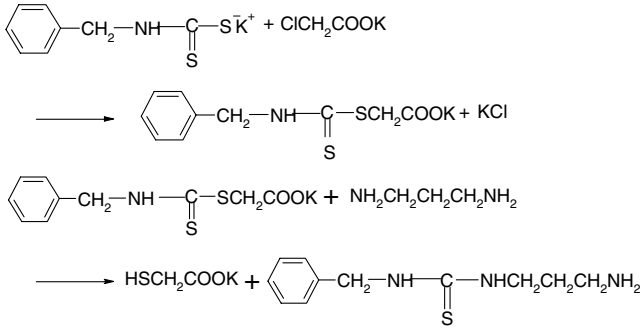
α	$1-\alpha$	T (K)	T^2	$1/T\times10^{-3}$	$-\log\left(\frac{-\ln(1-\alpha)}{T^2}\right)$
0.05	0.95	348	121,104	2.87	6.73
0.17	0.83	398	158,404	2.51	6.29
0.39	0.61	448	200,704	2.23	5.97
0.55	0.45	488	238,144	2.05	5.84
0.71	0.29	528	278,784	1.89	5.71
0.83	0.17	569	323,761	1.76	5.62
0.92	0.08	609	370,881	1.64	5.52
0.98	0.02	653	426,409	1.53	5.40

CHNS analysis. Found (calculated): C, 53.10 (53.04); H, 6.02 (6.08); N, 23.32 (23.20); S, 1.59 (17.68). Mass spectra; *m*/*z*: 181.82.

4.4. Preparation of (benzyl-*N*-thio)-1,3-propanediamine (L^2)

11.05 g (0.05 mol) of *N*-benzyl dithiocarbamate prepared as earlier was suspended in 15 mL methanol and treated with freshly prepared potassium chloroacetate [(0.05 mol){potassium chloroacetate was obtained by dissolving 4.73 g chloroacetic acid in 3 mL ice-cold water and mixing it in 5 mL aqueous solution of 2.8 g potassium hydroxide}]. The temperature of the reaction mixture was kept at about 40 °C for an hour and the contents were left overnight at room temperature. After 24 h, methanolic solution of 4.36 mL (0.05 mol) 1,3-propanediamine (density 0.85) was added to the reaction mixture. The content was then heated on a water bath for about 45 min when the desired product began to separate out. It was cooled in ice for 24 h and filtered. (Benzyl-*N*-thio)-1,3-propanediamine thus obtained was recrystallized from methanol and dried under vacuum over $CaCl_2$ at room temperature.

The reactions taking place in the preparation are shown below



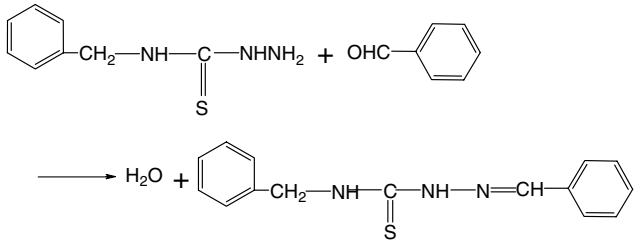
CHNS analysis. Found (calculated): C, 59.23 (59.19); H, 7.56 (7.62); N, 18.91 (18.83); S, 14.28 (14.35). Mass spectra; *m*/*z*: 223.69.

4.5. Preparation of thiohydrazones

The thiohydrazones were prepared by refluxing the thiohydrazide with corresponding aldehydes in methanol.

4.6. Preparation of benzaldehyde benzyl-*N*-thiohydrazone (L^3)

5.43 g (0.03 mol) of benzyl-*N*-thiohydrazide and 3.05 mL (0.03 mol) of benzaldehyde (density 1.044) were



refluxed in methanol for 3 h. On cooling yellowish mass obtained was filtered and washed with cold methanol. It was recrystallized from hot methanol.

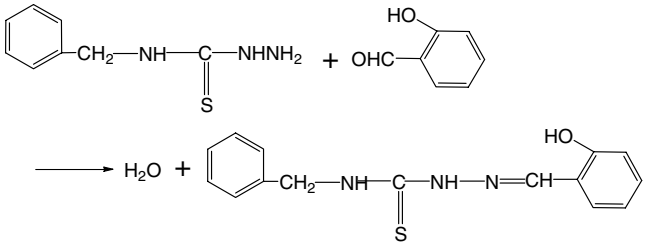
Table 7. Kinetic parameters from TG for $[Pt(L^4)_2Cl_2]$ complex by Coats–Redfern method (step II)

α	$1-\alpha$	T (K)	T^2	$1/T\times10^{-3}$	$-\log\left(\frac{-\ln(1-\alpha)}{T^2}\right)$
0.35	0.65	764	583,696	1.31	6.49
0.44	0.56	793	628,849	1.26	6.40
0.63	0.37	890	792,100	1.12	6.26
0.73	0.27	958	917,764	1.04	6.21
0.82	0.18	1006	1,012,036	0.99	6.13
0.86	0.14	1082	1,170,724	0.92	6.14

CHNS analysis. Found (calculated): C, 66.95 (66.91); H, 5.56 (5.58); N, 15.62 (15.61); S, 11.86 (11.89). Mass spectra; *m*/*z*: 269.23.

4.7. Preparation of salicylaldehyde benzyl-*N*-thiohydrazone (L^4)

5.43 g (0.03 mol) of benzyl-*N*-thiohydrazide and 3.15 mL (0.03 mol) of salicylaldehyde (density 1.164) were refluxed in methanol for 3 h. On cooling yellowish mass obtained was filtered and washed with cold methanol. It was recrystallized from hot methanol.



CHNS analysis. Found (calculated): C, 63.12 (63.15); H, 5.22 (5.26); N, 14.72 (14.73); S, 11.20 (11.22). Mass spectra; *m*/*z*: 285.63.

4.8. Preparation of complexes

4.8.1. Preparation of thiohydrazide $[Pt(L)_2Cl_2]$ complexes where L = L^1, L^2, L^3 and L^4. The corresponding ligand L [where L = L^1 (0.091 g, 0.5 mmol), L^2 (0.112 g, 0.5 mmol), L^3 (0.135 g, 0.5 mmol) and L^4 (0.143 g, 0.5 mmol) in methanol was added to aqueous solution of H_2PtCl_6 (0.103 g, 0.25 mmol). The solution was stirred for 4–5 h. The colour of solution changed from yellow to yellowish orange. It was washed with double distilled water several times and dried in desiccator over $CaCl_2$ under vacuum.

4.9. In vitro antibacterial activity

Most of the compounds have been screened in vitro against *S. aureus* and *E. coli*. Various methods[30–33] are available for the evaluation of the antibacterial activity of different types of drugs. However, the most widely used method[33], which consists in determining the anti-

Table 8. Thermal data of the complexes

Complexes	Step no.	TG (Coats–Redfern method)				ΔH (kJ g^{-1})
		Temperature range (K)	n	E_a (kJ mol^{-1})	$S^{\#}$ (JK^{-1} mol^{-1})	
$[Pt(L^4)_2Cl_2]$	I	302–670	1	18.26	5.66	89.54
	II	670–1149	1	17.76	4.18	165.19

bacterial activity of the drug, is to add it in known concentrations to the cultures of the test organisms.

4.10. Disc diffusion assay

The disc diffusion assay (Rasoanaivo and Ratsimamanga-Urverg, 1993) was used to determine antibacterial activity of the drug using Gram-positive and Gram-negative strains of bacteria namely *S. aureus* and *E. coli*. Base plates were prepared by pouring 10 mL of autoclaved Muller–Hinton agar (Biolab) into sterile Petri dishes (9 cm) and allowing them to settle. Molten autoclaved Muller–Hinton agar that had been kept at 48 °C was inoculated with a broth culture (10^6–10^8 mL^{-1}) of the test organism and then poured over the base plate. The discs were air-dried and placed on the top of the agar layer. Four replicants of each drug were tested (four disc per plate) with a gentamycin disc (0.5 μg/disc) as a reference. The plates were then incubated for 18 h at room temperature. Antibacterial activity is expressed as a ratio of the inhibition zone produced by the drug to the inhibition zone produced by the gentamycin standard.

4.11. Micro dilution antibacterial assay

The serial dilution technique described by Eloff (1998), using 96-well micro plates, to determine the minimum inhibitory concentration (MIC) of the drugs for antibacterial activity was used. Two milliliter cultures of four bacterial strains of *S. aureus* and *E. coli* were prepared and placed in a water bath overnight at 37 °C. The overnight cultures were diluted with sterile Muller–Hinton broth. The drugs were resuspended to a concentration of 60 μg/disc (in DMSO) with sterile distilled water in a 96 well micro plate. A similar 2-fold serial dilution of gentamycin (Sigma) was used as positive control against each bacterium. One hundred microliters of each bacterial culture was added to each well. The plates were covered and incubated overnight at 37 °C. To indicate bacterial growth, *p*-iodonitrotetrazolium violet was added to each well and the plates were incubated at 37 °C for 30 min. Bacterial growth in the wells was indicated by a red colour, whereas clear wells indicated inhibition.

4.12. In vitro cell growth inhibition assay

Cells were seeded in 96-well plates at a concentration of 0.1–1.0×10^4 cells/well in 200 μL of complete media and incubated for 24 h at 37 °C in 5% CO_2 atmosphere to allow for cell adhesion. Stock solutions (4 mM) of the compounds made in DMSO were filter-sterilized, then diluted to 1 mM in incomplete media. The 1 mM solutions were further diluted to 500 and 50 μM incomplete media for treatment against HeLa cell lines, where 40–4 μL of compound solutions added to 160–196 μL, respectively, of fresh medium in wells to give final concentrations of 100–1 μM. All assays were performed in two independent sets of quadruplicate tests. Control group containing no drug as well as equivalent amounts of DMSO was run in each assay.

Following 48 h of exposure of cells to drug, each well was carefully rinsed with 200 μL PBS buffer. Cytotoxicity was assessed using MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide). MTT solutions 20 μL (5 mg mL^{-1} dd H_2O) along with 200 μL of fresh, complete media were added to each well and plates were incubated for 4 h. Following incubation, the medium was removed and the purple formazan precipitate in each well was sterilized in 200 μL DMSO. Absorbance was measured using Techman Magellan microplate reader (molecular device) at 570 nm and the percentage (%) cytotoxicity was calculated as

$$\%\ \text{Cytotoxicity} = 1 - \frac{\text{OD in sample well}}{\text{OD in control well}} \times 100$$

FCS, foetal calf serum; PBS, phosphate-buffered saline. (FCS was obtained from Genetix, DMSO from cell culture tested, MTT from SRL and DMEM was purchased from Sigma, USA.)

References and notes

1. Wheate, N. J.; Collins, J. G. *Coord. Chem. Rev.* **2003**, *133*, 241.
2. Mqano, C.; Treviasan, A.; Giovagnini, L.; Fregona, D. *Toxicol. In Vitro* **2002**, *16*, 413.
3. Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385.
4. Einhorn, L. H.; Donohue, J. *J. Am. Intern. Med.* **1977**, *87*, 293.
5. Ozols, R. F.; Young, R. C. *Semin. Oncol.* **1984**, *11*, 251.
6. Soloway, M. S. *J. Urol.* **1978**, *120*, 716.
7. Fiorentino, M. V.; Ghiotto, C. In *Platinum and Other Meatl Coordination Compounds in Cancer Chemotherapy*; Nicolini, M., Ed.; Martinus Nijhoff: Boston, 1988; p 415.
8. Wagener, D. J.; Yap, S. H.; Wobbes, T.; Burghouts, J. T.; van Dam, F. E.; Hillen, H. F.; Hogendoorn, G. J.; Scheerder, H.; van der, V. *Cancer Chemother. Pharmacol.* **1985**, *15*, 86.
9. Von Hoff, D. D.; Schilsky, R.; Reichert, C. M.; Reddick, R. L.; Rozencweig, M.; Young, R. C.; Muggia, F. M. *Cancer Treat. Rep.* **1979**, *63*, 1527.
10. Krakoff, I. H. *Cancer Treat. Rep.* **1979**, *63*, 1523.
11. Manav, N.; Mishra, A. K.; Kaushik, N. K. *Spectrochim. Acta, Part A* **2004**, *60*, 3087.
12. Hall, M. D.; Hambley, T. W. *Coord. Chem. Rev.* **2002**, *232*, 49.
13. Hambley, T. W.; Jones, A. R. *Coord. Chem. Rev.* **2001**, *212*, 35.

14. Kwon, Y.; Whang, K.; Park, Y.; Kim, K. H. *Bioorg. Med. Chem.* **2003**, *11*, 1669.
15. Kovala-Demertzi, D.; Demertzis, M.; Yadav, P. N.; Castineiras, A.; West, D. X. *Transition Met. Chem.* **1999**, *24*, 642.
16. Manav, N.; Kaushik, N. K. *Transition Met. Chem.* **2002**, *27*, 849.
17. Kaushik, N. K.; Mishra, A. K. *Indian J. Chem., Sect A* **2003**, *42*, 2762.
18. Mishra, A. K.; Manav, N.; Kaushik, N. K. *Spectrochim. Acta, Part A* **2005**, *61*, 3097.
19. Kovala-Demertzi, D.; Domopoulou, A.; Nicholls, D.; Michaelides, A.; Aubry, A. *J. Coord. Chem.* **1993**, *30*, 65.
20. West, D. X.; Lockwood, M. S.; Liberta, A. E.; Chen, X.; Willet, R. D. *Transition Met.Chem.* **1993**, *18*, 221.
21. Chaudhary, R. K.; Yadav, S. N.; Tiwari, H. N.; Mishra, L. K. *J. Indian Chem. Soc.* **1998**, *75*, 392.
22. West, D. X.; Carlson, C. S.; Galloway, C. P.; Liberta, A.; Daniel, C. R. *Transition Met. Chem.* **1990**, *15*, 19.
23. Durig, J. R.; Layton, R.; Sink, D. W.; Mitchell, B. R. *Spectrochim. Acta* **1965**, *21*, 1367.
24. Kaul, B. B.; Pandeya, K. B. *J. Inorg. Nucl. Chem.* **1978**, *40*, 171.
25. Singh, N. K.; Srivastava, A.; Sodhi, A.; Ranjan, P. *Transition Met. Chem.* **2000**, *25*, 133.
26. West, D. X.; Stark, A. M.; Bain, G. A.; Liberta, A. E. *Transition Met. Chem.* **1996**, *21*, 289.
27. Chaudhary, R. K.; Keshari, B. N.; Mishra, L. K. *J. Indian Chem. Soc.* **2000**, *77*, 29.
28. Coats, A. W.; Redfern, J. P. *Nature* **1964**, *201*, 68.
29. (a) Ya Kazakova, V.; Ya Partovskii, I. *Dokl. Akad. Nauk. SSR* **1960**, *134*, 824; (b) *Chem. Abstr*. **1961**, *55*, 6843a.
30. Blanc, D. S.; Wenger, A. *J. Bille, J. Clin. Microbiol.* **2003**, *8*, 3499.
31. Saha, D.; Pal, J. *Lett. Appl. Microbiol.* **2002**, *34*, 311.
32. Tiwari, R. K.; Singh, D.; Singh, J.; Yadav, V.; Pathak, A. K.; Dabur, R.; Chiller, A. K.; Singh, R.; Sharma, G. L.; Chandra, R.; Verma, A. K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 413.
33. Eldeen, I. M. S.; Elgorashi, E. E.; Stadan, J. V. *J. Ethnopharmacol.* **2005**, *102*, 457.