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Original article

Synthesis, characterization, cytotoxicity, antibacterial and antifungal evaluation of some new platinum (IV) and palladium (II) complexes of thiodiamines

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Abstract

Some new platinum (IV) and palladium (II) thiodiamine complexes of type $[Pt(L)_2Cl_2]$ and $[Pd(L)Cl_2]$, [where, L = (cyclohexyl-*N*-thio)-1,2-ethylenediamine (L^1) and (cyclohexyl-*N*-thio)-1,3-propanediamine (L^2)] have been synthesized. The thiodiamines coordinate as a bidentate N–S ligand. The synthesized platinum (IV) and palladium (II) complexes of the thiodiamines were characterized by elemental analysis, IR, mass, electronic and ^{1}H NMR spectroscopic studies. These complexes were also screened for cytotoxicity, in vitro antifungal and in vitro antibacterial activities. Thermodynamic parameters such as activation energy (E_a), apparent activation entropy ($S^{\#}$) and enthalpy change (ΔH) for the dehydration and decomposition reactions of one complex has also been evaluated.

Keywords: Synthesis; Spectral; Cytototoxicity; Antibacterial; Antifungal and thermal

1. Introduction

The development of potent and effective antineoplastic, antibacterial, antimalarial and antiviral drugs has considerable interest in chemistry and biology [1,2]. The chemistry of transition metal complexes of thiodiamines has been largely studied because of their pharmacological properties [3].

The interest in platinum based antitumour drugs has its origin with the serendipitous discovery by Rosenberg of the inhibition of the cell division by platinum complexes. *Cis*-diaminedichloro-platinum(II) (*cis*-$[PtCl_2(NH_3)_2]$) and *cis*-diamine-tetrachloro platinum (IV) (*cis*-$[PtCl_4(NH_3)_2]$) were identified as the platinum complexes deducing that platinum compounds would have uses in cancer treatment with Sarcoma 180 and Leukemia L1210 bearing mice. *Cis*platin are widely applied in the treatment of various types of cancer such as testicular, ovarian, bladder carcinomas, head and neck cancers, lung carcinoma and stomach carcinoma [3–8]. However, the clinical usefulness of *cis*platin has been frequently limited by its severe side effects such as nephrotoxicity, nausea, ototoxicity, neurotoxicity and myelotoxicity [9–11]. Besides, there is a development in 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 *cis*platin [12].

Platinum (IV) and palladium (II) complexes have revealed significantly greater activity in human than that of *cis*platin [13,14]. 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) and palladium (II) complexes have enormous potential as anticancer agents. The potential advantages of platinum (IV) complexes that remain in higher oxidation state in the bloodstream are their lower reactivity would diminish loss of active drug and lower the incidence of unwanted side reactions that lead to toxic side effects [15,16].

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Nowadays, attention is focused on platinum (IV) and palladium (II) complexes with bioactive ligands, because of the lower toxicity and the possibility of oral administration of some potent platinum (IV) and palladium (II) compounds, as well as the fact that they can coordinate to DNA.

From the studies of platinum complexes in differing cancer cell lines and DNA binding studies, some important structure activity rules have previously been summarized [17]. The three overriding factors in designing platinum drugs appear to be chain length and flexibility, hydrogen bonding capacity and charge of linking chain and the geometry of the chloro ligands to the linking chain. For the aliphatic chains used by some workers [17,18], the ideal length of the linker appears to be eight atoms (two amine and six methylene groups). This is evident in the dinuclear and trinuclear complexes. Both are much more active than the other analogues where the linking chain is either shorter or longer. For the polyamine platinum complexes, it has been proved that increasing the charge and the chain length, leads to complexes which are more active [17–20].

However, flexibility also appears to be a major factor. Studies show that both the dinuclear [19] and trinuclear [20] complexes were much less active than their aliphatic equivalents. It has been observed [20,21] that the complexes containing aromatic ligands show poorer cytotoxicity. This would indicate that straight chain aliphatic, or NH_3, ligands are preferable, over aromatic ones. The best linking ligands appear to contain both positive charges and hydrogen bonding capacity in the form of either a charged platinum centre and/or charged amine groups.

In view of number of applications of the thiodiamines [17–22] and the well proven clinical utility the platinum metal complexes, we have prepared, platinum (IV) and palladium (II) complexes of the thiodiamines. These complexes were characterized and screened for antibacterial and cytotoxic activities. 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. Materials and methods

All the reagents used were AR grade. The analysis of CHNS/O contents of ligands and metal complexes were done on Elementar Analysensysteme Gmbh Vario El-III. IR spectra were recorded on Perkin–Elmer spectrum 2000 FTIR spectrometer using KBr disc. Electronic spectra were recorded in DMSO solvent on Shimadzu UV-visible spectrophotometer Model 1601. Conductance measurements were carried out on Digital Conductometer Model PT-827, India using DMSO solution. Model Jeol SX102/DA-600 (KV 10MA) was used for recording mass spectra of the ligands in CH_3OH solution. ^1H NMR was recorded using d_6-DMSO on Bruker Spectrospin 300 spectrometer. Thermogravimetric analysis/differential thermal analysis 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.

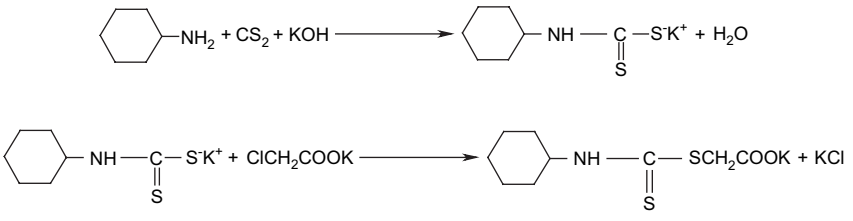
2.1. Preparation of thiodiamines

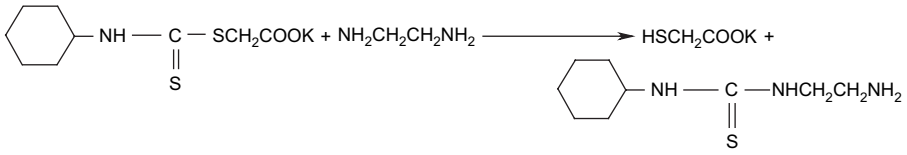
The (cyclohexyl-*N*-thio)-1,2-ethylenediamine and (cyclohexyl-*N*-thio)-1,3-propanediamine have been prepared by the modification of the reported method [21–23].

2.1.1. Preparation of (cyclohexyl-N-thio)-1,2-ethylenediamine (L^1)

In a three necked round bottle flask 5.72 mL (0.05 mol) of cyclohexylamine (density 0.867 g cm^{-3}) was dissolved in 25 mL methanol and chilled it. To this, solution 2.8 g (0.05 mol) of potassium hydroxide in 1 mL of water and 10 mL of methanol was added and mixed with constant stirring. An ice-cold solution of 3.02 mL (0.05 mol) carbon disulphide (density 1.26 g cm^{-3}) in 3 mL methanol was added to the above mixed solution. The temperature of the reaction mixture was maintained below 10 °C by keeping flask in a freezing mixture of common salt and ice. During the process, a white crystalline precipitate of *N*-cyclohexyl dithiocarbamate was separated. It was filtered, washed with ice-cold aqueous methanol (water: methanol; 2:8). The product was then suspended in 10 mL methanol and treated with freshly prepared potassium chloroacetate. The potassium chloroacetate was freshly prepared 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 in a water bath and the contents were left overnight at room temperature (25 °C). After 24 h methanolic solution of 3.37 mL (0.05 mol) ethylenediamine (density 0.897 g cm^{-3}) was added to the reaction mixture. The content was then heated at 40 °C 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. (Cyclohexyl-*N*-thio)-1,2-ethylenediamine thus obtained was recrystallized from methanol and dried under vacuum over $CaCl_2$ at room temperature (25 °C).

The reactions taking place in the preparation are shown below:





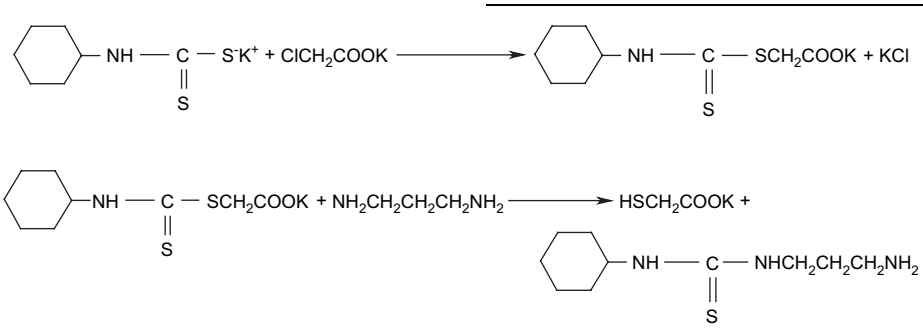
CHNS-analysis; Found (calculated) %: C; 52.91 (53.73), H; 8.69 (9.45), N; 20.17 (20.89), S; 15.92 (16.03).

Mass spectra (CH_3OH); *m/z*: 201.68.

2.1.2. Preparation of (cyclohexyl-N-thio)-1,3-propanediamine (L^2)

N-cyclohexyl dithiocarbamate [10.65 g (0.05 mol)] prepared as earlier was suspended in 18 mL methanol and treated with freshly prepared potassium chloroacetate. The potassium chloroacetate was freshly prepared 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 in a water bath and the contents were left overnight at room temperature (25 °C). After 24 h methanolic solution of 4.36 mL (0.05 mol) 1,3-propanediamine (density 0.85 g cm^{-3}) was added to the reaction mixture. The content was then heated on a water bath at 40 °C for about 45 min. When the desired product began to separate out, it was cooled in ice for 24 h and filtered. (Cyclohexyl-*N*-thio)-1,3-propanediamine thus obtained was recrystallized from methanol and dried under vacuum over $CaCl_2$ at room temperature (25 °C).

The reactions taking place in the preparation are shown below:



CHNS-analysis; Found (calculated) %: C; 55.23 (55.81), H; 9.89 (9.77), N; 18.73 (19.53), S; 14.88 (13.97).

Mass spectra (CH_3OH); *m/z*: 215.69.

2.2. Preparation of complexes

2.2.1. Preparation of thiohydrazide[$Pt(L)_2Cl_2$] complexes where $L = L^1$ and L^2

The corresponding ligand L [where L^1 (0.101 g, 0.5 mmol), L^2 (0.108 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. The resulting yellowish orange precipitate obtained was washed with double distilled water several times and dried in desiccator over $CaCl_2$ under vacuum.

2.2.2. Preparation of thiohydrazide [$Pd(L)Cl_2$] complexes where $L = L^1$ and L^2

The corresponding ligand L [where L^1 (0.101 g, 0.5 mmol), L^2 (0.108 g, 0.5 mmol)] in methanol and added with constant stirring to 1 N HCl solution of palladium chloride (0.089 g, 0.5 mmol). The solution was stirred for 4–5 h. The brownish precipitate appeared immediately, which separated, washed with double distilled water several times and dried in desiccator over $CaCl_2$ under vacuum.

2.3. In vitro antifungal activity

Most of the compounds have been screened in vitro against *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*. Among several methods [24] available, the one method [25,26] that is common in use in recent times has been adopted.

2.3.1. Microbroth dilution assay

The susceptibility of the fungi to various fractions of compounds was assayed by microbroth dilution method [27]. Sabouraud dextrose medium was dissolved in glass double distilled water and autoclaved at 10 psi for 15 min. A volume of 90 μL of medium was added to the wells of cell culture plates (Nunc Nunclon). The different concentrations in the range of 15–1000 μg/mL of various fractions were prepared in duplicate wells and then the wells were incubated with 10 μL of conidial suspension containing 1×10^4 conidia. The plates were incubated at 37 °C and examined macroscopically after 48 h for the growth of *Aspergillus* mycelia. The activity was represented as −ve if growth was there and +ve if medium appeared clear without any visible growth of *A. fumigatus*, *A. flavus* and *A. niger*.

2.3.2. Spore germination inhibition assay

The basic method for spore germination inhibition was modified and used to evaluate the activity of various test fractions against fungi. The *A. fumigatus*, *A. flavus* and *A. niger* were grown on Sabouraud dextrose agar plates and their homogeneous conidial suspension was prepared in the Sabouraud maltose broth. The conidia were counted and their number in the suspension was adjusted to 1×10^4 mL^{-1}. Various concentrations of the test samples in 90 μL of culture medium were prepared in 96-well flat bottom micro-culture plates (Nunc Nunclon) by double dilution method. The wells were prepared in duplicates for each concentration. The wells were inoculated with 10 μL of conidial suspension containing 100 ± 5 conidia. The plates were incubated at 37 °C for 10 h and then examined for spore germination under inverted microscope (Nikon Diphot). The number of germinated and non-germinated conidia was recorded. The percent spore germination inhibition (PSGI) was calculated using following formula:

$$\text{PSGI} = 100 - \frac{\text{No. of conidia germinated in drug treated well}}{\text{No.of conidia germinated in control well}} \times 100$$

2.4. In vitro antibacterial activity

Most of the compounds have been screened in vitro against *Escherichia coli* (*E. coli*). Various methods [28–31] are available for the evaluation of the antibacterial activity of different types of drugs. However, the most widely used method [31] consists in determining the antibacterial activity of the drug is to add in known concentrations to the cultures of the test organisms.

2.4.1. Disc diffusion assay

The disc diffusion assay was used to determine antibacterial activity of the drug using gram negative strains of bacteria *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 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 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.

2.4.2. Micro dilution antibacterial assay

The serial dilution technique was performed using 96-well microplates to determine the minimum inhibitory concentration (MIC) of the drugs for antibacterial activity was used. Two milliliter cultures of gram negative bacterial strain namely *E. coli* were prepared and placed in a water bath overnight at 37 °C. The overnight cultures were diluted with Muller–Hinton broth. The drugs were suspended to a concentration of 60 μg/disc (in DMSO) with sterile distilled water in a 96-well microplate. A similar two-fold serial dilution of gentamycin (Sigma) was used as positive control against each bacterium. Each bacterial culture of 100 μL 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 incubated at 37 °C for 30 min. Bacterial growth in the wells was indicated by a red colour, whereas clear wells indicate inhibition.

2.5. In vitro cell growth inhibition assay (cytotoxicity test)

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 μM and 50 μM incomplete media for treatment against HeLa cell lines, where 40–4 μL of compound solutions were 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-diphenyl-2H-tetrazolium bromide). MTT solutions 20 μL (5 mg/mL 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 precipitated in each well was solubilized in 200 μL DMSO. Absorbance was measured using Techman Magellan microplate reader (molecular device) at 570 nm and the percentage (%) cytotoxicity was calculated as:

$$\%\,\text{Cytotoxicity} = 1 - \frac{\text{O.D. in sample well}}{\text{O.D. in control well}} \times 100$$

FCS = Fetal calf serum, PBS = Phosphate buffered saline, O.D. = Optical density (FCS was obtained from Genetix, DMSO from cell culture tested, MTT from SRL, and DMEM were purchased from Sigma, USA).

3. Result and discussion

3.1. 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

Table 1
Elemental analysis of the complexes

Complexes	Found (calculated) %					
	C	H	N	S	Cl	Metal
$Pt(L^1)_2Cl_2$	32.37 (32.34)	5.70 (5.69)	12.55 (12.57)	9.60 (9.58)	10.61 (10.63)	29.22 (29.19)
$Pd(L^1)Cl_2$	28.55 (28.57)	5.03 (5.03)	11.15 (11.11)	8.45 (8.47)	18.77 (18.78)	28.00 (28.04)
$Pt(L^2)_2Cl_2$	34.49 (34.48)	6.02 (6.03)	11.99 (12.07)	9.20 (9.19)	10.25 (10.20)	28.02 (28.01)
$Pd(L^2)Cl_2$	30.60 (30.61)	5.36 (5.36)	10.75 (10.71)	8.15 (8.16)	18.12 (18.11)	27.05 (27.04)

DMSO are found to be less than 15 $ohm^{-1}\,cm^2\,mol^{-1}$ suggesting their non-electrolytic nature.

3.2. Electronic spectra

The electronic spectra (Table 2) of the thiodiamines (L^1 and L^2) show spectral bands because of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. On complexation these bands are shifted. Strong charge transfer transitions may interfere and prevent the observation of all the expected bands [32,33]. Strong bands ~340 nm is assignable to a combination of metal ligand charge transfer (M → LCT) and d–d band. The very intense band ~390 nm is assignable to combination of sulphur → metal charge transfer (Lπ → MCT) and d–d bands. One absorption band observed at ~285 nm is assigned to the $\pi \rightarrow \pi^*$ intraligand electronic transition, N=C=S. This band shifts on complexation revealing involvement of C=S group in complexation, in all the complexes. Additional bands appear in the complexes because of d–d and charge transfer transitions.

3.3. IR spectra

In the present studies, the IR spectra (Table 3) of the thiodiamine which contain groups, –NH–C=S as a potential bond forming site. The thioamide group displays prominent IR bands due to ν(N–H), ν(C–N) and ν(C=S). Coordination of azomethine nitrogen to the metal ions is supported by displacement of the ν(N–N) stretching band frequency. Significant changes in the ligand band upon complexation include a shift in ν(C=N). These data indicates coordination through azomethine nitrogen but no interaction between the terminal amine nitrogen and the metal ions. The thioamide band in the range of 750–900 cm^{-1} is due to ν(C=S) as major contributor and ν(C–N) as minor. This is shifted to lower frequency on complexation indicates the coordination to metal ion is through thioamide sulphur C=S. This shift is ~120–150 cm^{-1}, if coordination is through thiol sulphur [34] and 30–40 cm^{-1}, if coordination is through the thione sulphur [35].

In all the platinum (IV) and palladium (II) complexes the metal nitrogen vibrations, ν(M–N) are assigned to the new bands [36] in the far IR between 460 and 480 cm^{-1}, while in the region between 375 and 385 cm^{-1} gives metal–sulphur, ν(M–S) band stretching [37]. The band at ~285–300 cm^{-1} is assigned due to $\nu_{(Pt-Cl)}$ and $\nu_{(Pd-Cl)}$ stretching vibrations.

Table 2
Electronic spectra of the complexes

Complexes	λ_{max} (nm)	Log(ε)
L^1	212	3.95
	254	3.22
	285	2.70
$Pt(L^1)_2Cl_2$	272	3.89
	336	2.32
	392	2.06
$Pd(L^1)Cl_2$	292	3.71
	352	2.79
	411	2.26
L^2	213	4.33
	242	3.96
	287	2.56
$Pt(L^2)_2Cl_2$	274	3.75
	344	2.40
	402	2.23
$Pd(L^2)Cl_2$	276	3.23
	338	2.81
	397	2.42

3.4. NMR spectra

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

[L^1] $\delta_{(ppm)}$ 1.16–1.8 (m, 11H, –cyclohexyl), 2.42 (t, 2H, $-CH_2$), 3.8 (br s, 2H, $-NH_2$), 8.9 (br s, 1H, –NH).
[$Pt(L^1)_2Cl_2$] $\delta_{(ppm)}$ 1.22–1.73 (m, 22H, –cyclohexyl), 2.8 (t, 4H, $-CH_2$), 4.03 (br s, 4H, $-NH_2$), 8.79 (br s, 2H, -NH).
[$Pd(L^1)Cl_2$] $\delta_{(ppm)}$ 1.18–1.91 (m, 11H, –cyclohexyl), 2.85 (t, 4H, $-CH_2$), 4.1 (br s, 2H, $-NH_2$), 8.76 (br s, 1H, –NH).
[L^2] $\delta_{(ppm)}$ 1.16–1.77 (m, 11H, cyclohexyl–H), 1.4 (m, 2H, $-CH_2$), 2.2 (t, 4H, $-CH_2$), 4.3 (br s, 2H, $-NH_2$), 9.09 (br s, 1H, –NH).
[$Pt(L^2)_2Cl_2$] $\delta_{(ppm)}$ 1.2–1.8 (m, 22H, cyclohexyl–H), 1.92 (m, 4H, $-CH_2$), 2.45 (t, 8H, CH_2), 5.1 (br s, 4H, $-NH_2$), 9.11 (br s, 2H, –NH).

Table 3
IR spectra of the complexes

Complexes	ν_{N-N}	$\nu_{C=S}$	ν_{M-N}	ν_{M-S}	ν_{M-Cl}
L^1	1025	886	–	–	–
$Pt(L^1)_2Cl_2$	1028	802	461	385	295
$Pd(L^1)Cl_2$	1019	788	465	389	290
L^2	1028	889	–	–	–
$Pt(L^2)_2Cl_2$	1030	765	477	382	285
$Pd(L^2)Cl_2$	1026	803	480	375	297

Table 4
Antifungal studies of the complexes

S. No.	Complexes	*Aspergillus fumigatus*		*Aspergillus flavus*		*Aspergillus niger*	
		MDA MIC (μg/mL)	PSGI MIC (μg/mL)	MDA MIC (μg/mL)	PSGI MIC (μg/mL)	MDA MIC (μg/mL)	PSGI MIC (μg/mL)
1	$Pt(L^1)_2Cl_2$	250	250	250	250	500	500
2	$Pt(L^2)_2Cl_2$	125	125	250	250	500	500
Amphoterin B		5	5	5	5	5	5

MDA = Micro dilution activity and PSGI = Percent spore germination inhibition.

Table 5
Antibecterial studies of the complexes

S. No.	Complexes	Zone of inhibition (mm)	MIC (μg/disc)
		Escherichia coli	*Escherichia coli*
1	$Pt(L^1)_2Cl_2$	9	60.0
2	$Pt(L^2)_2Cl_2$	10	60.0
3	$Pd(L^2)Cl_2$	8	60.0
Gentamycin		16	1.0

$[Pd(L^2)Cl_2]$ $\delta_{(ppm)}$ 1.18–1.8 (m, 1H, cyclohexyl–H), 1.9 (m, 2H, $-CH_2$), 2.56 (t, 4H, $-CH_2$), 5.2 (br s, 2H, $-NH_2$), 9.13 (br s, 1H, –NH).

The ^{1}H NMR spectrum of thiodiamines [38,39] shows two signals at $\delta \sim 9.0$–10.3 ppm and $\delta \sim 4.0$ ppm, due to the presence of NH protons.

3.5. In vitro antifungal study

In the current study, (Table 4) some synthesized complexes were tested against pathogenic fungal strains such as *A. fumigatus*, *A. flavus* and *A. niger*. Amphotericin B was used as reference drug for fungi. The minimum inhibitory concentrations (MICs) by microbroth dilution assays (MDA) and percent spore germination inhibition assays (PSGIA) are 125–500 μg/mL. The complexes show significant activity at a higher concentration due to the fact that *Aspergillii* have hard chitinous outer wall and therefore, higher concentration of fungicidal compounds may be often required to kill the fungi.

3.6. Antibacterial study

In the current study, (Table 5) some synthesized complexes were tested against pathogenic bacterial strains such as *E. coli*

Table 6
Cytotoxicity studies of the complexes

S. No.	Complexes	Concentration (100 μg/mL)
1	$Pt(L^1)_2Cl_2$	54.5
2	$Pt(L^2)_2Cl_2$	60.9
3	$Pd(L^1)Cl_2$	–
*Cis*platin		72.5

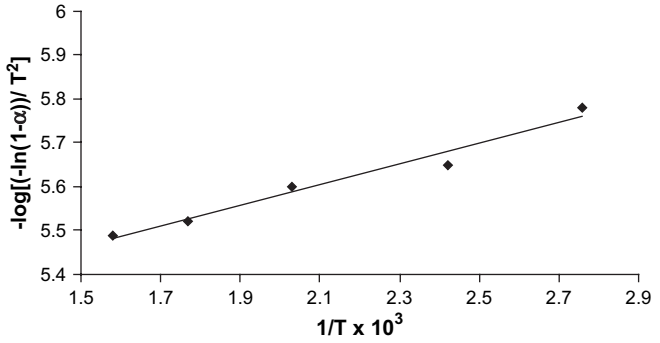


Fig. 1. The curve plotted by Coats–Redfern method for $[Pt(L^2)_2Cl_2]$ for step I.

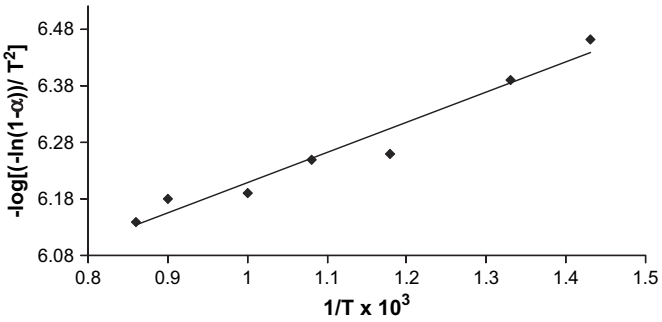


Fig. 2. The curve plotted by Coats–Redfern method for $[Pt(L^2)_2Cl_2]$ for step II.

Table 7
Kinetic parameters from TGA for $[Pt(L^2)_2Cl_2]$ complex by Coats–Redfern method: step I

α	$1-\alpha$	T (K)	T^2	$1/T \times 10^{-3}$	$-\log(-\ln(1(-\alpha))/T^2)$
0.39	0.61	362	131044	2.76	5.78
0.58	0.42	413	170569	2.42	5.65
0.75	0.25	492	242064	2.03	5.60
0.89	0.11	563	316969	1.78	5.52
0.95	0.05	633	400689	1.58	5.49

Table 8
Kinetic parameters from TG for $[Pt(L^2)_2Cl_2]$ complex by Coats–Redfern method: step II

α	$1-\alpha$	T (K)	T^2	$1/T \times 10^{-3}$	$-\log(-\ln(1(-\alpha))/T^2)$
0.32	0.68	698	487204	1.43	6.46
0.41	0.59	749	561001	1.33	6.39
0.59	0.41	845	714025	1.18	6.26
0.67	0.33	924	853776	1.08	6.25
0.77	0.23	994	988036	1.01	6.19
0.84	0.16	1108	1227664	0.90	6.18
0.89	0.11	1158	1340964	0.86	6.14

Table 9
Thermal data of the thiodiamine complexes

Complexes	Step no.	TG (Coats–Redfern Method)				ΔH (kJ/g)
		Temperature range (K)	n	E_a (kJ mol^{-1})	$S^{\#}$ (J K^{-1} mol^{-1})	
$[Pt(L^2)_2Cl_2]$	I	301–648	1	4.5	0.25	36.34
	II	648–1173	1	10.24	0.79	147.50

using the disc diffusion method. Gentamycin was used as reference drug for bacteria. The bacterial strains with the zone of inhibition were observed, 8–10 mm at minimum inhibitory concentration (MIC) of 60.0 μg/disc.

3.7. Cytototoxic study

In the present studies (Table 6) the cytotoxic study of three metal complexes have been determined. The study was used to test the growth inhibition by MTT assay. Data expressed in terms of percentage (%) cytotoxicity. The metal complexes caused ~60% inhibition. The result shows according to structure activity rule. It is found that by increasing chain length from $Pt(L^1)_2Cl_2$ to $Pt(L^2)_2Cl_2$, the cytotoxicity increases.

The importance of such work lays the possibility that the new complexes might more efficacious drug against tumors. 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. This could be helpful in designing more potent antitumour agents for therapeutic use.

4. Thermogravimetric analysis (TGA)/differential thermal analysis (DTA) study

4.1. Thiodiamine complexes

The TGA and DTA studies in air atmosphere have been carried out for one complex. Thermal studies were utilized to elucidate the number of kinetic and thermodynamic parameters. From TGA curve, order of reaction (n), activation energy (E_a), and apparent activation entropy ($S^{\#}$) were enumerated by the Coats–Redfern method [40]. From the DTA curves, the heat of reaction was calculated. Kinetic parameters of each step from Coats–Redfern method were shown in Figs. 1 and 2 and the thermal data were tabulated in Tables 7–9.

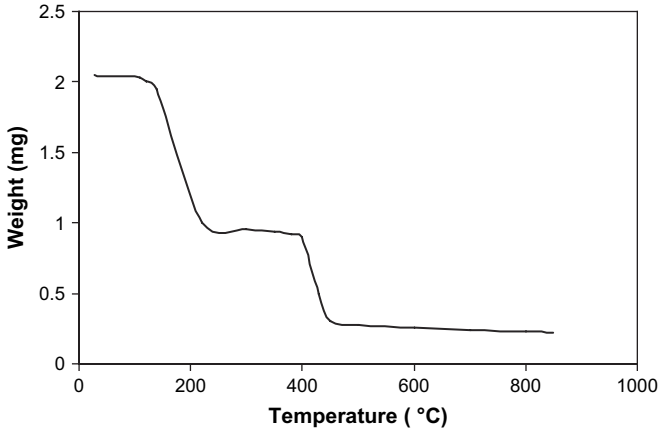


Fig. 3. TGA curve of $[Pt(L^2)_2Cl_2]$ Complex.

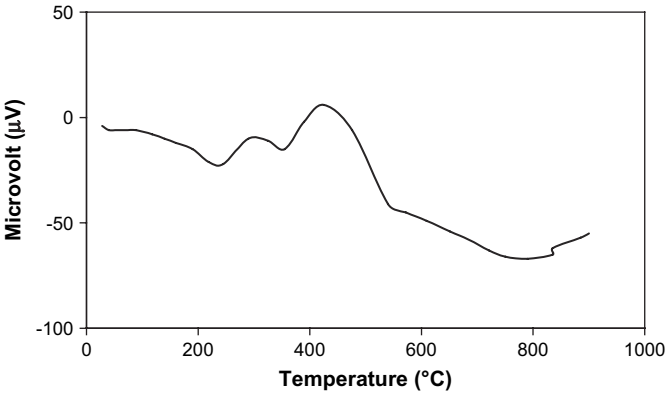


Fig. 4. DTA curve of $[Pt(L^2)_2Cl_2]$ Complex.

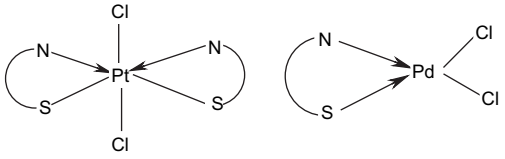


Fig. 5. Proposed structures of the complexes.

4.1.1. $[Pt(L^2)_2Cl_2]$ complex

TGA curve (Fig. 3) shows two steps decomposition. The first decomposition step (28–375 °C) corresponds to the loss of all organic moieties for which observed and calculated weight losses are 61.46% and 61.78%, respectively. The second step starts at 375–900 °C corresponds to the platinum metal residue for which the observed and calculated weight losses are 71.98% and 71.79%.

The DTA profile (Fig. 4) shows one exotherm at 360 °C corresponding to the fusion of the compounds and one exotherm at 430 °C corresponding to the oxidation of organic moieties.

5. Conclusion

The electronic spectra of the complexes suggest that all the complexes are found to be diamagnetic. The platinum (IV) complexes must be octahedral and palladium (II) complexes square planar. 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. Palladium (II) is a d^8 system and three predicted transitions are $^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1E_g$. The spectral studies indicate that the complexation takes place to the metal ion is through nitrogen and sulphur. The in vitro antifungal activity of complexes as compared with standard drug Amphotericin B shows significant activity. The minimum inhibitory concentrations (MICs) by microbroth dilution assays (MDA) and percent spore germination inhibition assays (PSGIA) are found to be 125–500 μg/mL. The in vitro antibacterial study of the complexes as compared with standard drug gentamycin shows significant activity. The bacterial strains with the zone of inhibition were observed, 8–10 mm. Three complexes were tested for the cytotoxic activity. The complex was tested on primary adenocarcinoma. The complex showed good activity at 100 μM solution as compared to standard drug *cis*platin. It is found that by increasing chain length from $Pt(L^1)_2Cl_2$ to $Pt(L^2)_2Cl_2$], the cytotoxicity increases. The thermal data (TGA/DTA) of the complex indicates that for all the three steps the reaction order are found to be one and activation energy, apparent activation entropy and heat of reaction are found to be significant. It is worth to mention that trials to get crystal suitable for X-ray structure determination went in vain due to amorphous character of the complexes. On the basis of spectroscopic studies the structures of the complexes are proposed (Fig. 5).

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