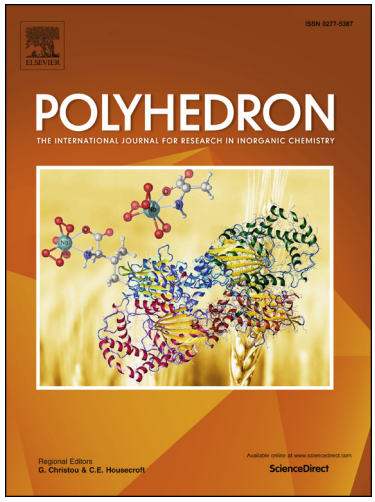

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Synthesis, characterization and anticancer evaluation of transplatin derivatives with heterocyclic thiones

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Abstract

Platinum(II) complexes of heterocyclic thiones (L) based on transplatin having the general formula, *trans*-$[Pt(NH_3)_2(Thione)_2](NO_3)_2$ have been synthesized and characterized using elemental analysis, IR, and NMR (^1H & ^{13}C) spectroscopy. The crystal structures of two of them, *trans*-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$ (**1**) and *trans*-$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$ (**3**) were determined by X-ray crystallography. The structures of **1** and **3** consist of *trans*-$[Pt(NH_3)_2L_2]^{2+}$ complex ions and nitrate counter ions. The platinum atom in both the complex ions adopts a distorted square planar geometry. The spectroscopic data indicated the coordination of thione ligands to platinum(II). The *in vitro* cytotoxicity of these compounds as well as of cisplatin and carboplatin was investigated using MTT assay against three human cancer cell lines, which are; A549 (lung carcinoma), MCF-7 (breast carcinoma) and HTC15 (colon cancer). The *in vitro* cytotoxicity in several cases is comparable or even higher than carboplatin and in two cases than cisplatin.

Key words: transplatin; cytotoxicity; heterocyclic thiones; anticancer evaluation; X-ray structure

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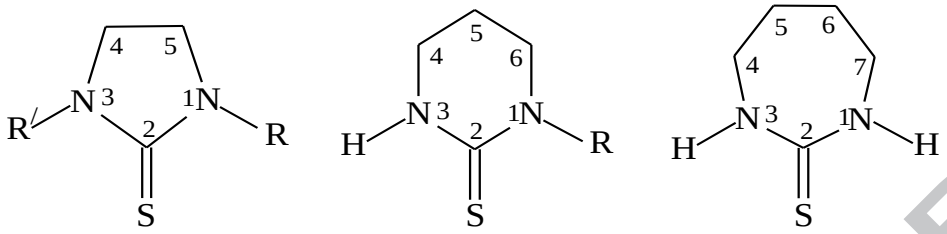
1. Introduction

The platinum anticancer compounds in clinical use such as, cisplatin, carboplatin and oxaliplatin generally exist as neutral molecules and contain two fairly labile cis ligands, *e.g.*, the two chlorido groups in cisplatin. The labile ligands are replaced by water through aquation reactions and the resulting cations form bifunctional adducts with DNA [1-12]. The platinum(II) complexes having two labile groups in a trans conformation, for example *trans*-$[Pt(NH_3)_2Cl_2]$ (transplatin) or monofunctional platinum(II) complexes, such as [PtCl(dien)]Cl (dien = diethylenetriamine) or $[Pt(NH_3)_3Cl]Cl$ were ineffective [11-13]. But later studies have shown that the replacement of one (or both) ammine ligand(s) of transplatin by aliphatic amines or heterocyclic ligands such as planar pyridine or non-planar piperazine greatly enhances the cytotoxicity of such species with respect to their corresponding cis isomers and also to cisplatin particularly, in cisplatin-resistant tumor cell lines [14-20].

Like *cis*-platinum-ammine complexes, DNA is also considered as the potential cellular target for the antitumor derivatives of transplatin [11-16]. However, the nature of Pt-DNA adducts is different for the two types of complexes. Cisplatin and its analogues mainly form 1,2-intrastrand cross-links [1-12], while transplatin is not able to form 1,2-intrastrand cross-links, because of the steric hindrance of the two ammine groups in *trans* position. Instead it mainly forms 1,3-interstrand cross-links [4,11,21]. Transplatin may form 1,3-intrastrand cross-links between two G residues, or between a G and a C residue, separated by at least one base [4-14,19,20]. The enhancement of activities in trans complexes was connected mainly to their enhanced accumulation in tumor cells and efficiency to form in DNA a markedly higher amount of more distorting cross-links than transplatin that forms preferentially less distorting and persisting monofunctional adducts [14].

Several structural studies of thione derivatives of transplatin have been reported [22-26], which describe a square-planar geometry around the metal center and the thione coordination in terminal S-bonded modes. But, the antitumor properties of these complexes were not reported, although many other transplatin analogues are known to exhibit anticancer activities [13-18]. Therefore, in the present study we have undertaken the synthesis, spectroscopic investigation and *in vitro* anticancer evaluation of a number of trans-platinum(II) complexes of heterocyclic thiones. Crystal structures of two of the complexes, *trans*-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$ (**1**) and *trans*-

$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$ (**3**) were also determined. The structures of the thiones used in this study are given in Scheme 1.



(a-g) (h-i) (j)

(a) R = R/ = H; Imidazolidine-2-thione (Imt), **(b)** R = H, R/ = CH_3; *N*-methylimidazolidine-2-thione (MeImt), **(c)** R = CH_3, R/ = CH_3; *N*,*N*/-dimethylimidazolidine-2-thione (Me_2Imt), **(d)** R = R/ = C_2H_5; *N*,*N*/-diethylimidazolidine-2-thione (Et_2Imt), **(e)** R = H, R/ = C_3H_7; *N*-propylimidazolidine-2-thione (PrImt), **(f)** R = H, R/ = *i*-C_3H_7; *N*-(*iso*propyl)imidazolidine-2-thione (*i*-PrImt), **(g)** R = R/ = *i*-C_3H_7; *N*,*N*/-(di-*iso*propyl)imidazolidine-2-thione (*i*Pr_2Imt), **(h)** R = C_2H_5; *N*-ethyl-1,3-Diazinane-2-thione (EtDiaz), **(i)** 1,3-Diazepane-2-thione (Diap).

Scheme 1. The structures of thiones used in this study

2. Experimental Section

2.1 Chemicals

Transplatin (*trans*-diamminedichloidoplatinum(II)) was obtained from Strem Chemical Company, USA. Dimethylsulfoxide-d_6 and D_2O were purchased from Fluka Chemical Co. The thione ligands were prepared according to the procedure mentioned in the literature [27, 28]. (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) was purchased from Sigma Chemical Co, St. Louis, MO, USA.

General procedure for the synthesis of *trans*-$[Pt(NH_3)_2(Thione)_2](NO_3)_2$ complexes (1 – 9)

All the compounds were prepared by adding (0.17 g, 1.0 mmol) of $AgNO_3$ to the solutions containing (0.15g, 0.5 mmol) of *trans*-diamminedichloridoplatinum(II) (transplatin) in 10 mL water and stirring the mixture for 2 hours in the dark at room temperature. The solution was filtered to remove silver chloride as solid. Then 1.0 mmol of thione ligand dissolved in 10 mL methanol were added to the filtrates drop wise. Mixing and stirring of the solutions resulted in a colored solution. The solution was filtered and kept at room temperature. Solid powder was obtained on slow evaporation of the solvent. The complexes, trans-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$ andtrans-$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$ were crystallized from a 1:1 mixture of water and methanol. Purity of the product was assessed through elemental analysis of C, H, N, and S. The CHNS data, melting/decomposition points, and % yield of the synthesized complexes are presented below:

***trans*-$[Pt(NH_3)_2(Imt)_2](NO_3)_2$ (1):** M. p. 156 - 158 ºC, Yield: 0.256g, 92%. C, H, N, and S% [Calculated C: 12.93%, H: 3.25%, N: 20.10, S: 11.50 %, Found: C: 12.23%, H: 3.28%, N: 20. 89%, S: 11.75%]. IR: ν_{max} = 3310 (s), 1042 (s), 501 (s), 836 (s), 272 (s).

***trans*-$[Pt(NH_3)_2(MeImt)_2](NO_3)_2$ (2):** M.p. 140 - 142 ºC, Yield: 0.234g, 80%. C, H, N, and S% [Calculated C: 16.41%, H: 3.79%, N: 19.14, S: 10.95 %, Found: C: 16.28%, H: 3.63%, N: 19.52%, S: 10.76%]. IR: ν_{max} = 3528 (s), 1112 (s), 837 (s), 503 (s), 274 (s).

***trans*-$[Pt(NH_3)_2(Me_2Imt)_2](NO_3)_2$ (3):** M.p. 130 - 132 ºC, Yield: 0.248g, 81%. C, H, N, and S% [Calculated C: 19.57%, H: 4.27%, N: 18.16, S: 10.45 %, Found: C: 19.22%, H: 4.25%, N: 18.87%, S: 10.57%]. IR: ν_{max} = 1118 (s), 825 (s), 491 (s), 265 (s).

***trans*-$[Pt(NH_3)_2(Et_2Imt)_2](NO_3)_2$ (4):** M.p. 124 - 126 ºC, Yield: 0.224g, 67%. C, H, N, and S %. [Calculated C: 25.11%, H: 5.12%, N: 16.73, S: 9.58 %, Found: C: 25.38%, H: 5.23%, N: 16.75%, S: 9.72%]. IR: ν_{max} = 1065 (s), 826 (s), 502 (s), 284 (s).

***trans*-$[Pt(NH_3)_2(PrImt)_2](NO_3)_2$ (5):** M.p. 106 - 108 ºC, Yield: 0.247g, 77%. C, H, N, and S % [Calculated C: 22.46%, H: 4.71%, N: 17.48, S: 9.99 %, Found: C: 22.87%, H: 4.83%, N: 17.21%, S: 10.05%]. IR: ν_{max} = 3373 (s), 1033 (s), 824 (s), 503 (s), 282 (s).

***trans*-$[Pt(NH_3)_2(i\text{PrImt})_2](NO_3)_2$ (6):** M. p. 83 - 85 ºC, Yield: 0.234g, 73%. C, H, N, and S% [calculated C: 22.46%, H: 4.71%, N: 17.48, S: 9.99 %, Found: C: 21.98%, H: 4.49%, N: 17.38%, S: 9.48%]. IR: ν_{max} = 3566 (s), 1064 (s), 867 (s), 495 (s), 279 (s).

***trans*-[Pt(NH$_3$)$_2$(*i*Pr$_2$Imt)$_2$](NO$_3$)$_2$ (7):** M. p. 132 – 134 ºC, Yield: 0.210g, 58%. C, H, N, and S% [Calculated C: 29.79%, H: 5.83%, N: 15.44, S: 8.84 %, Found: C: 29.89%, H: 5.87%, N: 15.27%, S: 8.58%]. IR: ν_{max} = 1106 (s), 856 (s), 497 (s), 283 (s).

***trans*-[Pt(NH$_3$)$_2$(EtDiaz)$_2$](NO$_3$)$_2$ (8):** M. p. 92 - 94 ºC, Yield: 0.227g, 71%. C, H, N, and S% [Calculated C: 22.46%, H: 4.71%, N: 17.48, S: 9.99 %, Found: C: 22.25%, H: 4.68%, N: 17.37%, S: 9.78%]. IR: ν_{max} = 3448 (s), 1074 (s), 835 (s), 501 (s), 279 (s).

***trans*-[Pt(NH$_3$)$_2$(Diap)$_2$](NO$_3$)$_2$ (9):** M. p. 164 - 166 ºC, Yield: 0.191g 62%. C, H, N, and S% [Calculated C: 19.57%, H: 4.27%, N: 18.26, S: 10.45 %, Found: C: 19.72%, H: 4.32%, N: 18.07%, S: 10.33%]. IR: ν_{max} = 3270 (s), 1053 (s), 822 (s), 510 (s), 269 (s).

2.2 Spectroscopic measurements

Elemental analysis of was performed on Perkin Elmer Series 11 (CHNS/O), Analyzer 2400. The solid state FTIR spectra of the ligands and their platinum(II) complexes were recorded on a Perkin Elmer FTIR180 spectrophotometer or NICOLET 6700 FTIR using KBr pellets over the range 4000-400 cm^{-1}.

The ^{1}H and ^{13}C NMR spectra in DMSO were carried out on a JEOL JNM-LA 500 NMR spectrometer at 500.00 MHz and 125.65 MHz operating frequency respectively. The ^{13}C NMR spectra were recorded with ^{1}H broadband decoupling at 297 K. The spectral conditions were; 32 K data points, 0.963 s acquisition time, 3.2 s pulse delay and a 5.75 µs pulse width for ^{1}H NMR, and 32 K data points, 0.963 s acquisition time, 2.5 s pulse delay and a 5.12 µs pulse width for ^{13}C NMR. The chemical shifts were measured relative to Tetramethylsilane (TMS).

2.3 X-ray diffraction analysis

The intensity data for **1** and **3** were collected at 173K (-100°C) on a Stoe Mark II-Image Plate Diffraction System [29]. Equipped with a two-circle goniometer and using MoKα graphite monochromated radiation (λ = 0.71073 Å). The structure was solved by direct methods with SHELXS-97 [30]. The refinement and all further calculations were carried with SHELXL-2014 [31].

The N- and C-bound H-atoms were included in calculated positions and treated as riding atoms with N–H = 0.91 Å, C–H = 0.99 and 0.98 Å for CH_2 and CH_3 H-atoms, respectively, and with $U_{iso}(H) = 1.5U_{eq}(C)$ for methyl H atoms and = $1.2U_{eq}$(N or C) for other H atoms. The non–H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. A semi-empirical absorption correction was applied using the MULABS routine in PLATON [32]. A summary of crystal data and refinement details for complexes **1** and **3** are given in Table 1.

Table1. Crystal data and refinement details for crystal structures of complexes **1** and **3**.

Parameter	**1**	**3**
Formula	$C_6H_{18}N_6PtS_2 \cdot 2(NO_3)$	$C_{10}H_{26}N_6PtS_2 \cdot 2(NO_3)$
Formula weight	557.49	613.60
Crystal size/mm	0.41 × 0.34 × 0.21	0.40 × 0.36 × 0.28
Wavelength/Å	0.71073	0.71073
Temperature/K	173	173
Crystal symmetry	Monoclinic	Monoclinic
Space group	*P* $2_1/n$	*P* $2_1/n$
a/Å	5.4002 (4)	7.0160 (5)
b/Å	23.4438 (15)	18.6235 (10)
c/Å	6.6485 (5)	8.0794 (6)
β/°	105.458 (6)	107.228 (6)
V/$Å^3$	811.26 (10)	1008.31 (12)
Z	2	2
ρ_{calc} /Mgm^{-3}	2.282	2.021
μ(Mo-Kα)/mm^{-1}	8.95	7.21
F(000)	536	600
θ value(°)	θ_{max} = 25.6, θ_{min} = 1.7	θ_{max} = 25.7, θ_{min} = 2.2
No. measured, independent and observed [$I > 2\sigma(I)$] reflections	10466, 1532, 1332	13070, 1900, 1550
R_{int}	0.087	0.046
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.023, 0.050, 1.04	0.015, 0.035, 0.98
T_{min}, T_{max}	0.297, 1.000	0.402, 1.000
Largest diff. peak, hole/e $Å^{-3}$	0.99, −1.86	0.76, −0.75

2.5 *In vitro* cytotoxic activity against A549, MCF7 and HTC15 human cancer cell lines

The *trans*-$[Pt(NH_3)_2(thione)_2](NO_3)_2$ complexes were evaluated for *in vitro* cytotoxic activity against A549 (human lung cancer), MCF-7 (human breast cancer) and HTC15 (human colon cancer) cell lines. The cells were seeded at 4 x 10^3 cells/well in 100 µL DMEM containing 10 % FBS in 96-wells tissue culture plate and incubated for 72 h at 37^{o}C, 5 % CO_2 in air and 90 % relative humidity in CO_2 incubator. After incubation, 100 µL of each sample solution (50, 25, 12.5 and 6.25 µM), prepared in DMEM, were added to cells and the cultures were incubated for 24 h. The medium of wells was discarded and 100 µL DMEM containing MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide) (5 mg/mL) was added to the wells and incubated in CO_2 incubator at 37^{o}C in dark for 4 h. After incubation, purple colored formazan (artificial chromogenic dye, product of the reduction of water insoluble tetrazolium salts e.g., MMT by dehydrogenases and reductases) in the cells is produced and appeared as dark crystals in the bottom of the wells. The resultant crystals were solublized by adding 100 µL of DMSO in each well. The solution was thoroughly mixed to dissolve the formazan crystals, producing a purple solution. The absorbance of the 96-wells plate was taken at 570 nm with Labsystems Multiskan EX-Enzyme-linked immunosorbent assay (EX-ELISA) reader against a reagent blank. The IC_{50} values were calculated from three independent experiments by generating an equation of logarithmic trend line of percentage cell viability against concentration of compounds in Microsoft excel.

3. Results and discussion

We prepared in the present work nine new analogues of transplatin in which chloride groups are replaced by heterocyclic thione ligands. The aqua derivatives of transplatin, $[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ were first prepared by addition of two equivalents of $AgNO_3$ to transplatin. The nitrate species were then reacted with thione ligands in a 1:2 molar ratio to produce the complexes of the general formula, *trans*-$[Pt(NH_3)_2(L)_2](NO_3)_2$. The purpose of preparing the nitrate complexes was to increase the solubility of the resulting compounds because the poor aqueous solubility is a problem for some platinum anticancer drugs. The characterization of these complexes was carried out by usual techniques: elemental analysis, IR,

^{1}H & ^{13}C NMR and X-ray crystallography. The elemental analysis data is consistent with the predicted composition. The compounds obtained are listed in experimental section. The *in vitro* cytotoxicity of these platinum compounds was evaluated by MTT colorimetric assay using A549, MCF-7 and HTC15 cancer cells with cisplatin and carboplatin as positive controls.

3.1 Crystal Structure Description of complexes 1 and 3

The molecular structures of complexes **1** and **3** are shown in Figures 1 and 2 respectively. The selected geometrical parameters are given in Table 2. The structure of both complexes consists of a complex cation, $[Pt(NH_3)_2(Imt)_2]^{2+}$ (in **1**) or $[Pt(NH_3)_2(Me_2Imt)_2]^{2+}$ (in **2**) and two nitrate ions. In the complex ions, Pt(II) atom is bound to two sulfur atoms of thione ligands and two N atoms of ammonia in a *trans* fashion. The platinum atom is located on the inversion center and adopts essentially a square-planar environment lying exactly within the plane defined by the two S and two N atoms. The *cis* angles around platinum vary between 87.50 (9)° and 92.50 (9)° in **1**, while in **2** they are 88.52 (7)° and 91.48 (7)°. The *trans* angles in both are 180°. The Pt–N and Pt–S bond distances are 2.046 (3) and 2.3260 (9) Å in **1** and, 2.054 (2) and 2.3199 (7) Å in **2**. These bond distance values are very close with the average values reported for similar complexes [22-26, 33-36]. The structures of compounds **1** and **3** are very similar to a related complex, *trans*-$[Pt(NH_3)_2(Diaz)_2]Cl_2{\cdot}2H_2O$ that also crystallizes in the monoclinic space group. However, **1** and **3** show slightly more distortion from the regular square planar geometry.

The complex cations, *trans*-$[Pt(NH_3)_2(Thione)_2]^{2+}$ and nitrate anions are associated to each other through formation of hydrogen bonds. In the crystal packing of complexes, the molecules are H-bonded *via* N-H of ammonia or C-H hydrogen of Imt and oxygen atoms of nitrate ion. In **1**, N-H hydrogen of Imt and in **3**, nitrogen atoms of NO_3^- are also involved in hydrogen bonding. The nitrate anion nitrogen shows the weakest contact. The hydrogen bonding interactions result in the formation of three-dimensional hydrogen bonded network as shown in Figures 3 and 4 for **1** and **3** respectively. The details of the hydrogen bonds are summarized in Tables S1 and S2 (Supporting information) for **1** and **3** respectively.

Table 2. Selected bond distances (Å) and bond angles (˚) for **1** and **3**

Bond Distance		Bond angles	
Complex 1			
Pt1—N3	2.046(3)	N3—Pt1—S1	92.50 (9)
Pt1—S1	2.3260(9)	$N3^{i}$—Pt1—S1	87.50 (9)
		$N3^{i}$—Pt1—N3	180
		S1—Pt1—$S1^{i}$	180
Complex 3			
Pt1—N3	2.054(2)	N3—Pt1—S1	88.52(7)
Pt1—S1	2.3199(7)	$N3^{i}$—Pt1—S1	91.48(7)
		$N3^{i}$—Pt1—N3	180
		S1—Pt1—$S1^{i}$	180

Symmetry codes: for **1**, (i) −x, −y, −z+1; for **3**, (i) −x+1, −y, −z+1.

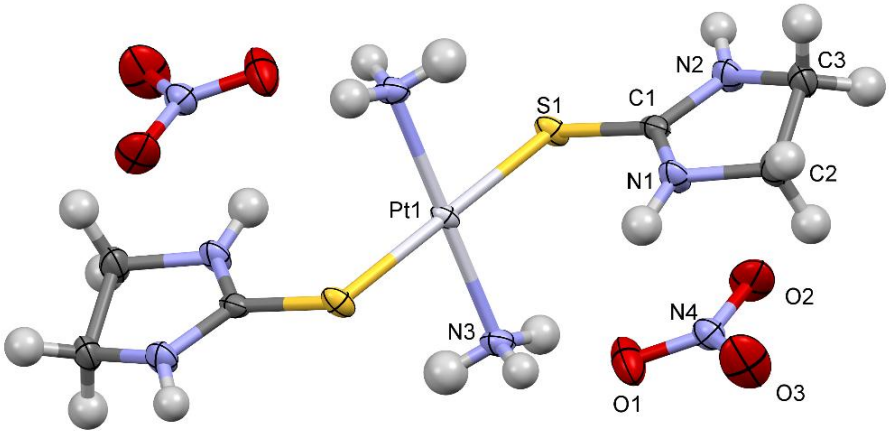


Figure 1 A view of the molecular structure of **1** with atomic labelling. The displacement ellipsoids are drawn at the 50% probability level. Atoms not labelled are related by inversion symmetry

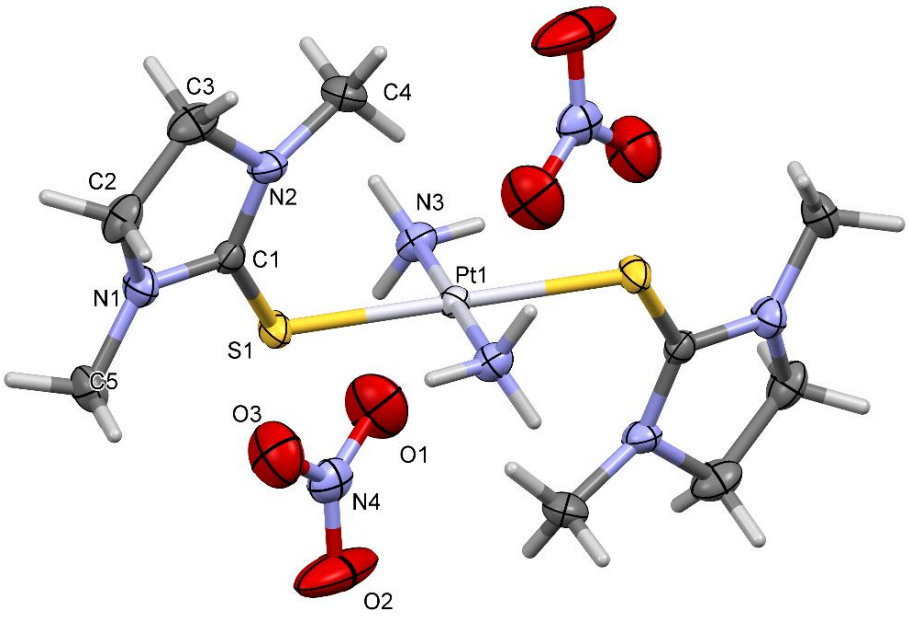


Figure 2 A view of the molecular structure of **3** with atomic labelling. The displacement ellipsoids are drawn at the 50% probability level. Atoms not labelled are related by inversion symmetry

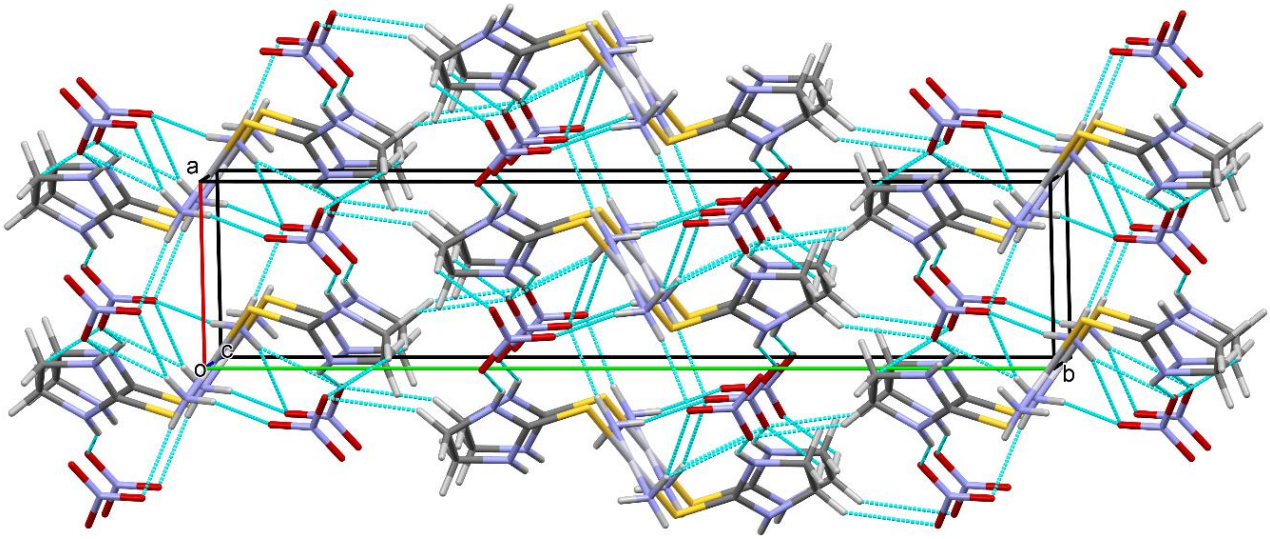


Figure 3 The crystal packing of **1**, viewed along the *c* axis. The N–H…O, N–H…N and C–H…O hydrogen bonds are shown as dashed lines and lead to the formation of a three-dimensional structure.

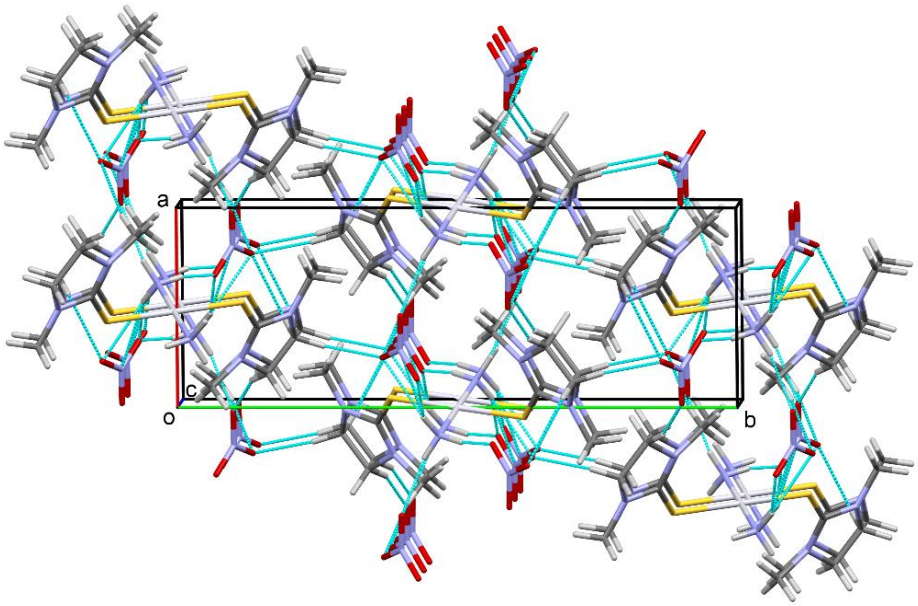


Figure 4 The crystal packing of **3**, viewed along the *c* axis. The N–H…O, N–H…N and C–H…O hydrogen bonds are shown as dashed lines and lead to the formation of a three-dimensional structure.

3.2 IR spectroscopy

Selected IR frequencies of free thiones and their platinum(II) compounds are given in the synthesis section and in Table S3 (Supplementary material). The characteristic vibrational bands in the IR spectra of thione complexes, are usually observed in three frequency regions; ν(C=S) vibration around 1200 and 600 cm^{-1}, the N–H stretching near 3200 cm^{-1} and M-sulfur stretching band below 400 cm^{-1}. The presence of ν(N–H) and ν(C=S) bands in all complexes proves that the thione ligands are coordinated to the metal atom. The spectra of free ligands display a band around 600 cm^{-1} as well as 1200 cm^{-1} that belongs to ν(C=S) stretching [37-39]. These bands shifted toward lower wave number upon complexation, while the N–H stretching vibrations shifted to higher frequency indicating the coordination of thiones through sulfur atom. In order to investigate the metal-sulfur stretching frequencies of the synthesized complexes, the spectra were recorded in far-infrared region below 400 cm^{-1}. This band lies in the range of about 300 cm^{-1} for the transition-metal complexes according to the literature [40]. In all complexes, we observed a

sharp peak around 280 cm^{-1} that was assigned to platinum- sulfur bond. A sharp band around 825 cm^{-1} for all *trans*-$[Pt(NH_3)_2(L)_2](NO_3)_2$ complexes and its absence in the free ligand spectra is attributed to the presence of non-coordinated nitrate ion [38].

3.3 ^{1}H and ^{13}C NMR Spectroscopy

The ^{1}H and ^{13}C chemical shifts of the ligands and their platinum(II) complexes in DMSO-d_6 are given in Tables 3 and 4 respectively. In ^{1}H NMR spectra of the complexes, the N–H signal of thiones became less intense upon coordination and shifted downfield from their positions in free ligands. The deshielding is related to an increase in π electron density in the C–N bond upon coordination.

In ^{13}C NMR, the C=S resonance of thiones in the complexes is shifted upfield by about 5.5 – 13.6 ppm as compared to that in free ligands in accordance with the data observed for other complexes of d^{10} metals with thiones [25,33,34,37,38]. The upfield shift is attributed to the lowering of C=S bond order upon coordination and a shift of N→C electron density, producing a partial double bond character in the C–N bond [37, 38]. As the shift difference of the C=S resonance may be related to the strength of metal-sulfur bond, Table 4 shows that the Me_2Imt complex would be the most stable among these complexes. A small deshielding effect is observed in other carbon atoms, which is due to an increase in π character of the C–N bond.

Table 3. ^{1}H NMR chemical shifts (ppm) of thiones and their Pt(II) complexes in DMSO

Species	**N–H**	**H-4**	**H-5**	**H-6**	**H-7/N-C3**	**N-C1**	**N-C2**
Imt	7.90	s,4H,3.59	s,4H,3.59	-	-	-	-
1	9.09	s,4H,3.69	s,4H,3.69	-	-	-	-
MeImt	7.93	t,2H,3.63	t,2H,3.43	-	-	s,3H,2.92	-
2	8.47	t,2H,3.71	t,2H,3.56	-	-	s,3H,2.96	-
Me_2Imt	-	s,4H,3.48	s, 4H,3.48	-	-	s,6H,2.91	-
3	-	s,4H,3.65	s, 4H,3.65	-	-	s,6H,3.29	-
Et_2Imt	-	s,4H,3.48	s,4H,3.48	-	-	q,4H,3.37	t,6H,0.97
4	-	s,4H,3.53	s,4H,3.53	-	-	q,4H,3.48	t,6H,1.12
PrImt	7.99	t,2H,3.58	t,2H,3.41	-	t,3H,0.73	t,2H,3.31	m,2H,1.45
5	8.81	t,2H,3.76	t,2H,3.62	-	t,3H,0.79	t,2H,3.35	m,2H,1.55
*i*PrImt	7.96	t,2H,3.53	t,2H,3.38	-	-	m,1H,4.35	d,6H,1.00
6	8.31	t,2H,3.70	t,2H, 3.54	-	-	m,1H,4.25	d,6H,1.07
iPr_2Imt	-	s,4H,3.22	s, 4H,3.22	-	-	m,1H,4.48	d,6H,0.99
7	-	s,4H,3.49	s, 4H,3.49	-	-	m,1H,5.10	d,6H,1.05
EtDiaz	7.89	t,2H,3.62	m,2H,1.83	t,2H,3.28	-	q, 2H,3.12	t, 3H,1.02
8	8.65	t,2H,3.59	m,2H,1.84	t,2H,3.33	-	q, 2H,3.21	t, 3H,1.09
Diap	7.70	t,4H,1.67	t,4H,3.18	t,4H,3.18	t,4H,1.67	-	-
9	8.70	t,4H,1.70	t,4H,3.24	t,4H,3.24	t,4H,1.70	-	-

s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet

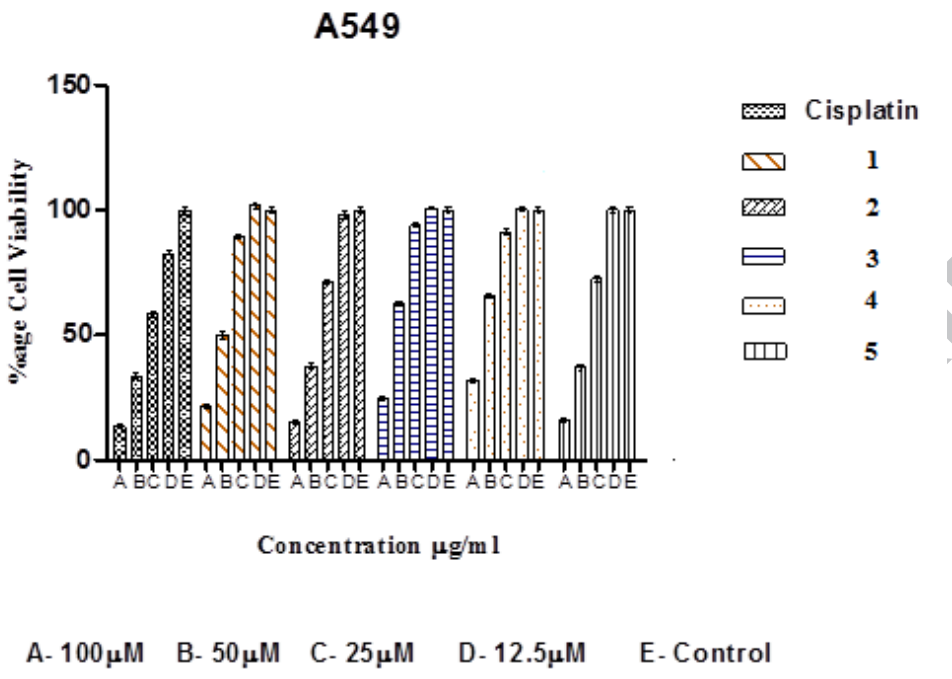
Table 4. ^{13}C NMR chemical shifts (ppm) of the ligands and their Pt(II) complexes in DMSO

Species	**C-2**	**C-4**	**C-5**	**C-6**	**C-7**	**N-C1**	**N-C2, N-C3**
Imt	182.11	45.38	45.38	-	-	-	-
1	175.74	45.76	45.76	-	-	-	-
MeImt	181.38	42.00	51.82	-	-	34.35	-
2	174.90	42.76	52.65	-	-	34.02	-
Me_2Imt	180.46	48.77	48.77	-	-	34.91	-
3	166.89	50.29	50.29	-	-	36.07	-
Et_2Imt	178.74	46.13	46.13	-	-	42.69	11.92
4	170.96	47.33	47.33	-	-	43.79	12.11
PrImt	180.87	49.14	48.86			42.11	20.65, 11.09
5	174.50	50.10	49.10			42.82	20.53, 10.95
*i*PrImt	179.70	43.73	42.21	-	-	48.18	19.24
6	174.17	44.65	42.71			48.78	19.18
iPr_2Imt	174.05	1.52	41.52	-	-	48.25	19.10
7	169.42	42.30	42.30			49.68	19.26
EtDiaz	173.26	46.48	20.83	41.04		49.41	12.33
8	167.43	47.32	20.27	41.09		49.67	12.19
Diap	183.99	45.86	26.99	26.99	45.86	-	-
9	176.53	46.35	26.51	26.51	46.35	-	-

3.4 Antitumor Activity

The cytotoxic activities of the synthesized complexes, cisplatin and carboplatin were evaluated *in vitro* against three human cancer lines, which are; A549 (human lung carcinoma), MCF7 (human breast cancer), and HCT15 (human colon adenocarcinoma). The cytotoxic effect was obtained by the stipulated increase in the concentration of the complexes, cisplatin and carboplatin against the fixed number of human cancer cells. The exposure of the cells to an increase in concentration of the complexes resulted in a dose dependent cytotoxic effect. The survival of the cells (A549, MCF7 and HCT15) as a function of concentration of compounds **1-9**

is explained in Figures 5-7. The IC_{50}values obtained from the curves of the concentration of the complexes and percentage viability of the cells are given in Table 5. The IC_{50} values of the complexes for A549 cell line range between 40 to 75 μM. Complexes **2** and **5** have *in vitro* cytotoxicity better than cisplatin with IC_{50} values 40 and 41 μM respectively, and almost two-fold better than that of carboplatin. Complexes **1, 6, 8** and **9** have *in vitro* cytotoxicity slightly lower than cisplatin but still higher than carboplatin. Complexes **3** and **7** have the same cytotoxicity as carboplatin. For MCF7 cell line, the IC_{50} values of the complexes are between 45 and 92 μM (Table 5). Only the complexes **5** and **8** were found to possess cytotoxicity against the MCF7 cell line between cisplatin and carboplatin. While the others displayed poor antiproliferative potency as indicated by their higher IC_{50} values. Against HCT15 cell line, the complexes **2, 5, 8** and **9** have activity comparable to cisplatin with IC_{50} values 40, 36, 43, and 41 μM respectively. The complexes **1** and **7** have almost the same cytotoxicity as carboplatin, while **3, 4** and **6** are less potent even than carboplatin. These results are consistent with a significant selective cytotoxicity of the complexes against particular cancer cell lines and its tendency to undergo ligand exchange with biomolecules like proteins and DNA. When the activity of the prepared complexes is compared with transplatin analogues of nitrogen donors, it is observed that the platinum amine complexes in comparison with thione derivatives, exhibit radically enhanced activity in tumor cell lines [41,42].



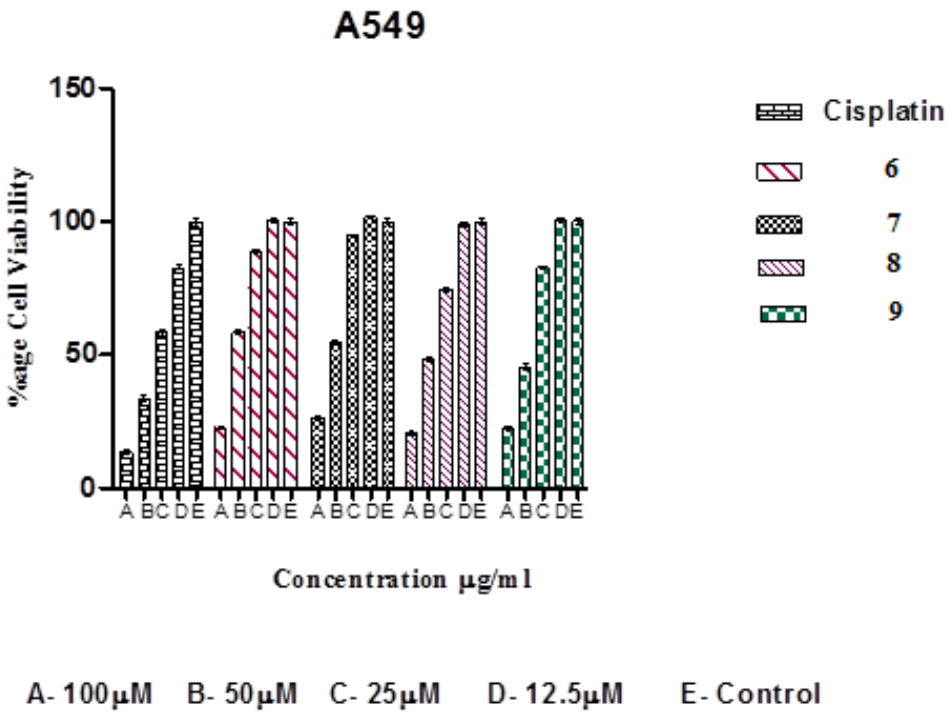
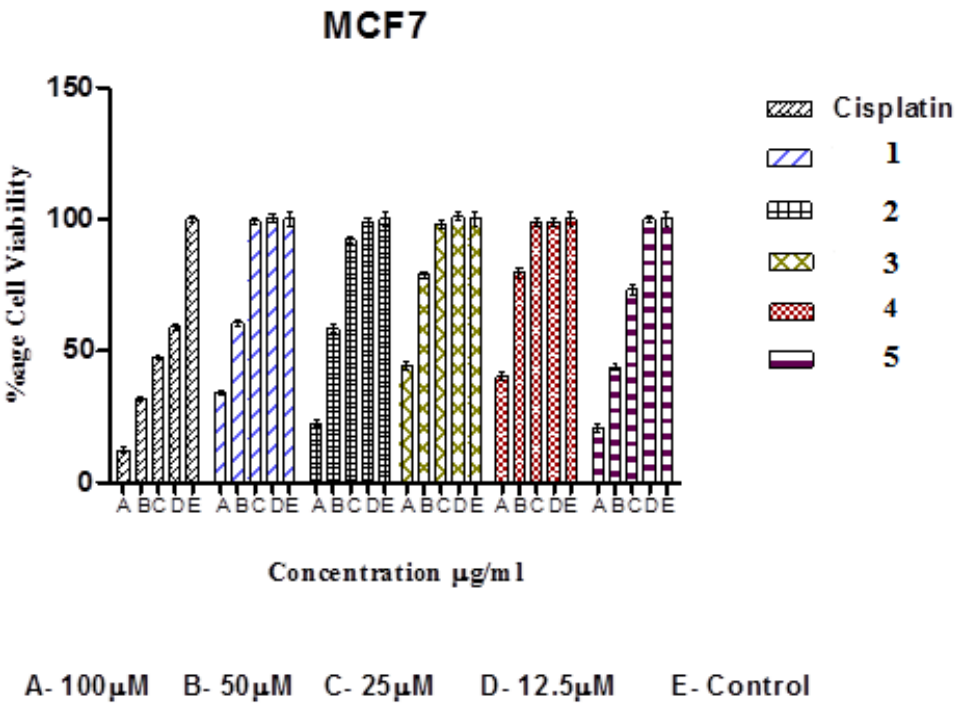


Figure 5 (a & b). Effect of concentration of complexes **1 – 9** on viability of **A549** cells.



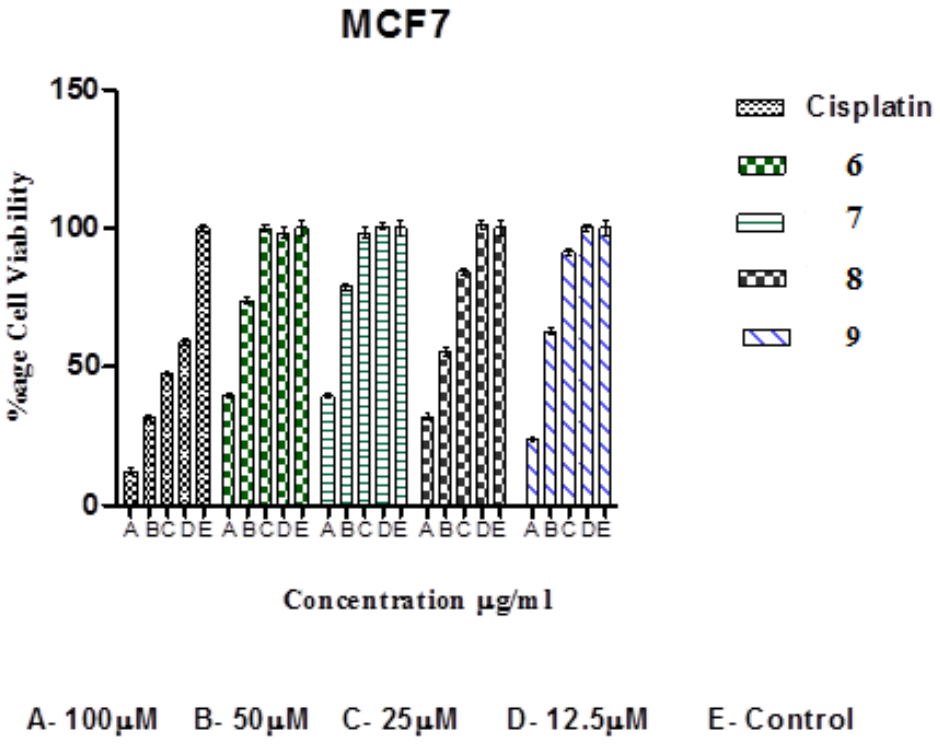
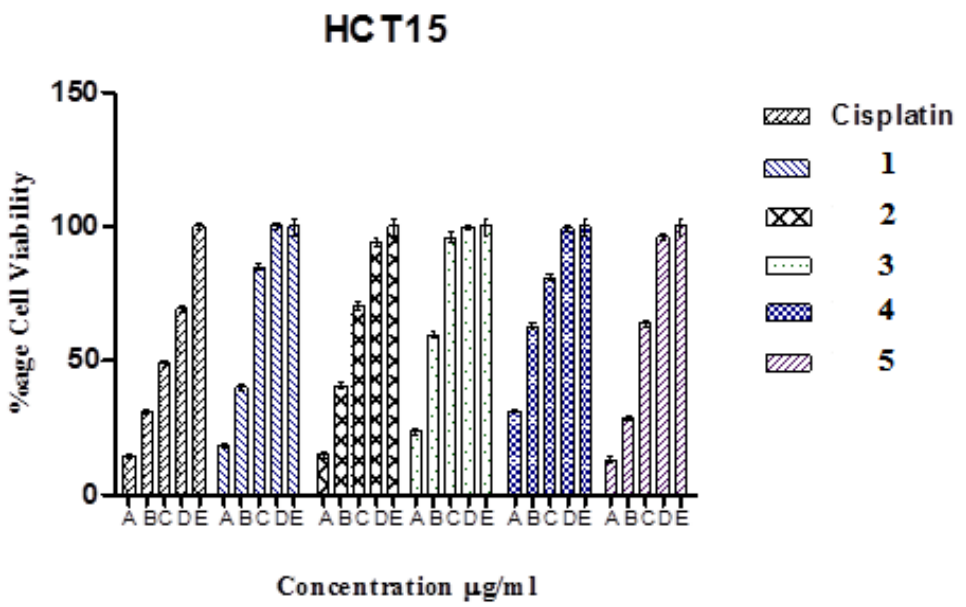


Figure 6 (a & b). Effect of concentration of complexes **1 – 9** on viability of **MCF7** cells.



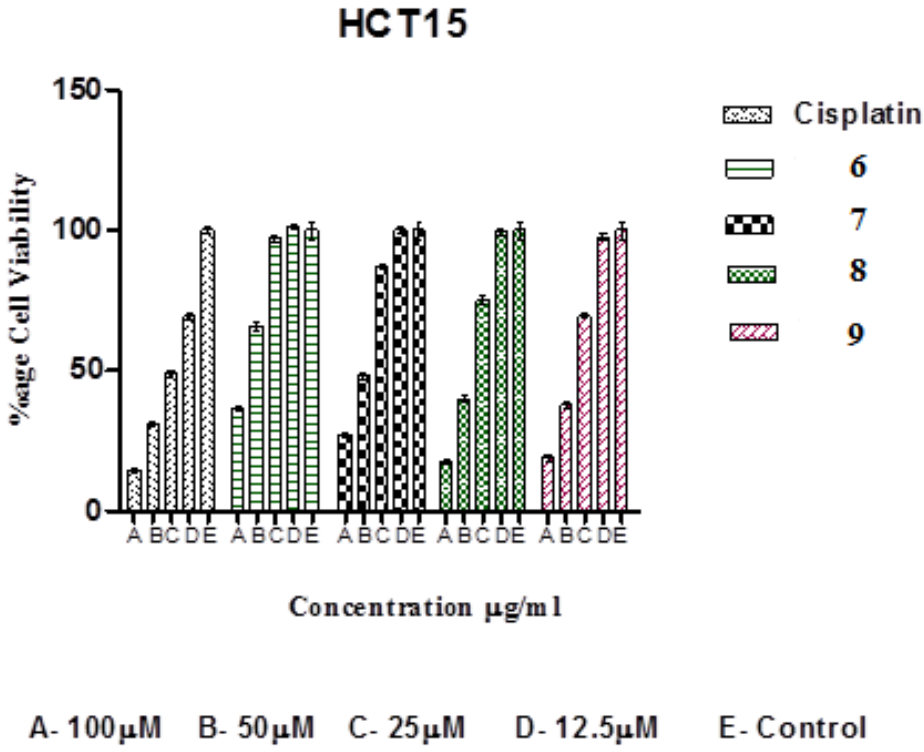


Figure 7 (a & b). Effect of concentration of complexes **1 – 9** on viability of **HCT15** cells.

Table 5. IC_{50} Values[a] (in μM) of Pt(II) compounds against three human tumor cell lines

Compounds	A549	MCF7	HCT15
Cisplatin	42 ± 2	23 ± 3	32 ± 2
Carboplatin	70 ± 2	63 ± 2	53 ± 2
1	52 ± 2	80 ± 1	50 ± 1
2	40 ± 1	70 ± 2	40 ± 2
3	70 ± 1	92 ± 2	69 ± 2
4	75 ± 1	89 ± 1	63 ± 1
5	41 ± 2	45 ± 1	36 ± 1
6	66 ± 1	86 ± 2	79 ± 1
7	69 ± 1	87 ± 2	53 ± 1
8	48 ± 1	60 ± 2	43 ± 1
9	49 ± 1	69 ± 1	41 ± 2

[a]Errors are standard deviations determined from at least three independent experiments.

4 Conclusion

In this study, transplatin-based complexes (**1**–**9**) with the general formulae, *trans*-$[Pt(NH_3)_2(Thione)_2](NO_3)_2$ have been synthesized and characterized using various analytical methods. The spectroscopic and crystallographic data strongly supported that the thione ligands coordinated to the Pt(II) center through the sulfur atom. The crystal structures of two complexes revealed a distorted square planar geometry around platinum. The prepared complexes were tested for *in vitro* antitumor activity against the human tumor cell lines. The complexes **2** and **5** exhibited a better cytotoxicity against A549 cancer cell line than cisplatin. This study extends the existing knowledge on the structure and mechanism of platinum based antitumor complexes possessing *trans* geometry.

Supplementary material

CCDC deposit numbers 1545440 and 1545441 have been assigned to the complexes **1** and **3** respectively. Crystallographic data in CIF or other electronic format can be obtained free of

charge via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

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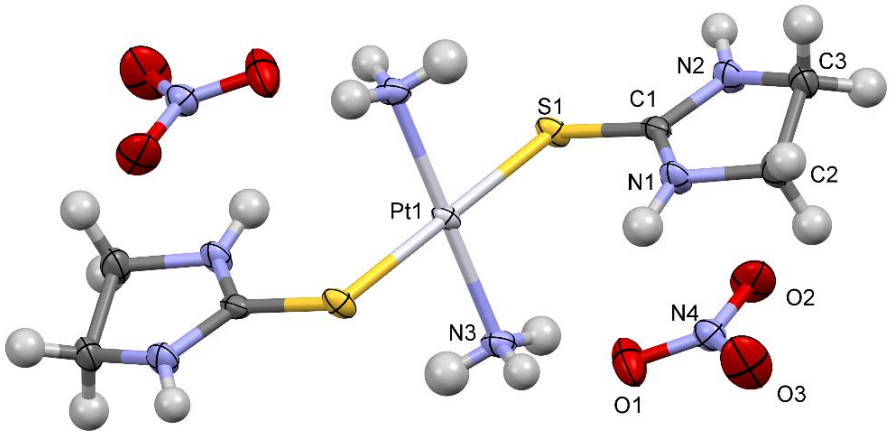
Graphical Abstract:



Synopsis

Platinum(II) complexes of heterocyclic thiones based on transplatin having the general formula, *trans*-$[Pt(NH_3)_2(Thione)_2].2NO_3$ have been synthesized and characterized using elemental analysis, IR, and NMR (^{1}H & ^{13}C) spectroscopy. The crystal structures of two of them, *trans*-$[Pt(NH_3)_2(Imt)_2].2NO_3$ and *trans*-$[Pt(NH_3)_2(Me_2Imt)_2].2NO_3$ were determined by X-ray crystallography.