Electronic Supplementary Information

In vitro model reaction of sulfur containing bio-relevant ligands with Pt(II) complex: Their kinetics, mechanism, bioactivity and computational studies

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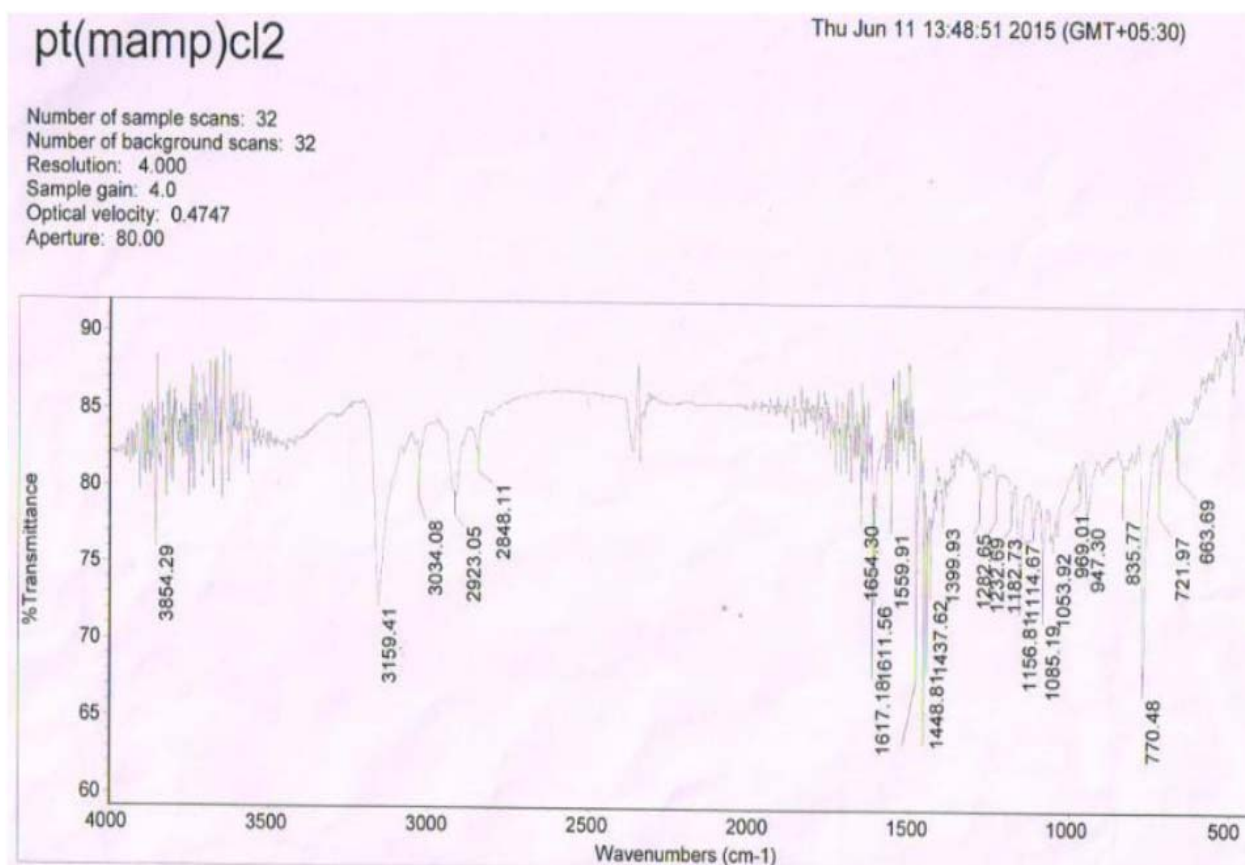
Author for correspondence, email: sankarmoi67@yahoo.com
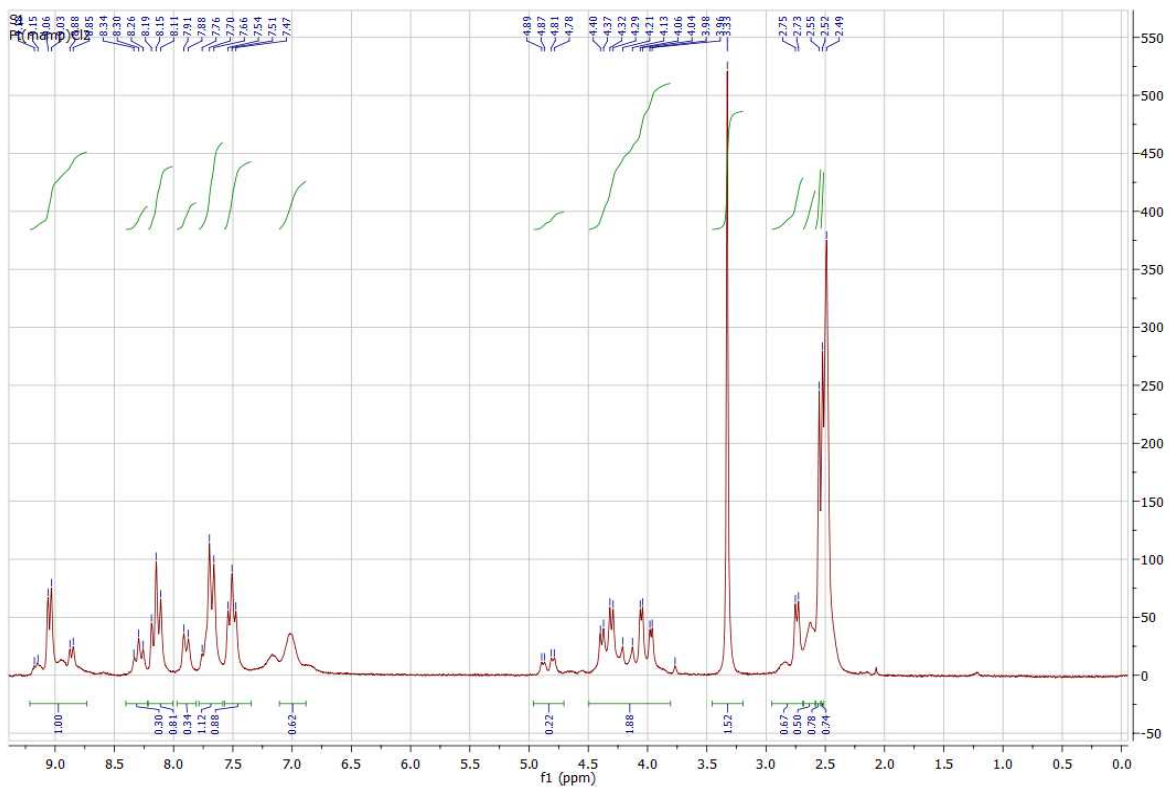
Fig S1 FTIR spectra of Complex **1**

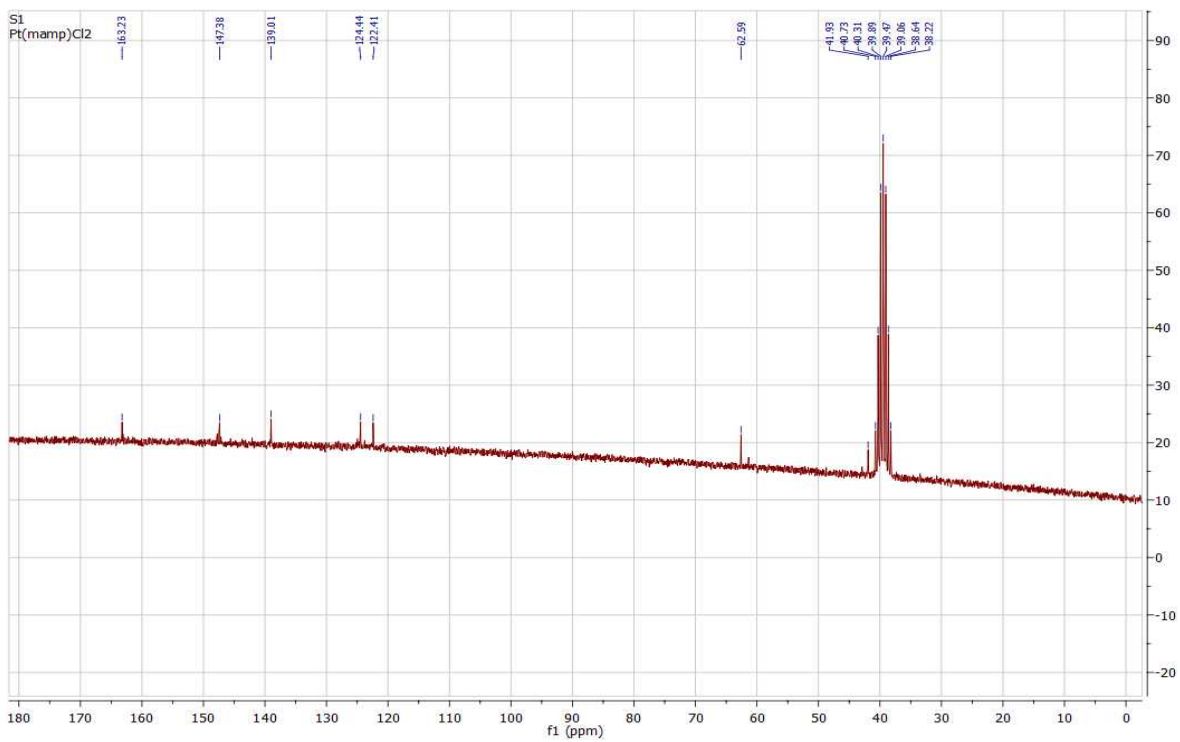**Fig S2** ^{1}H NMR spectra of complex **1** in DMSO-D6

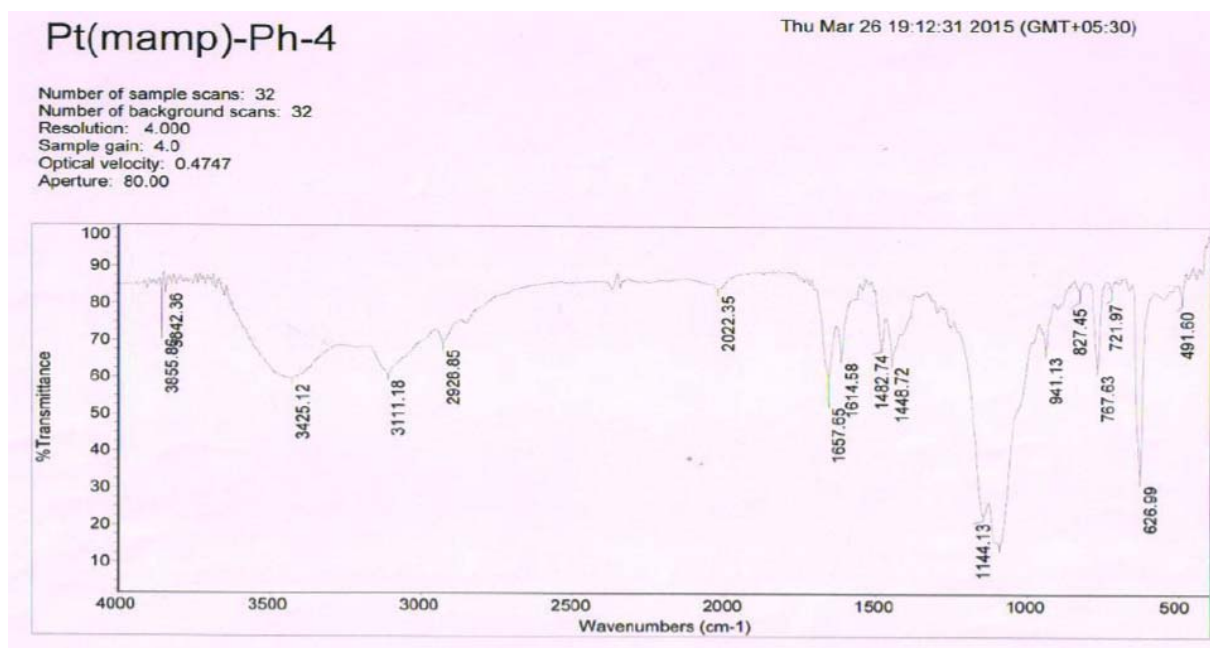**Fig S3** ^{13}C NMR spectra of complex **1** in DMSO-D6
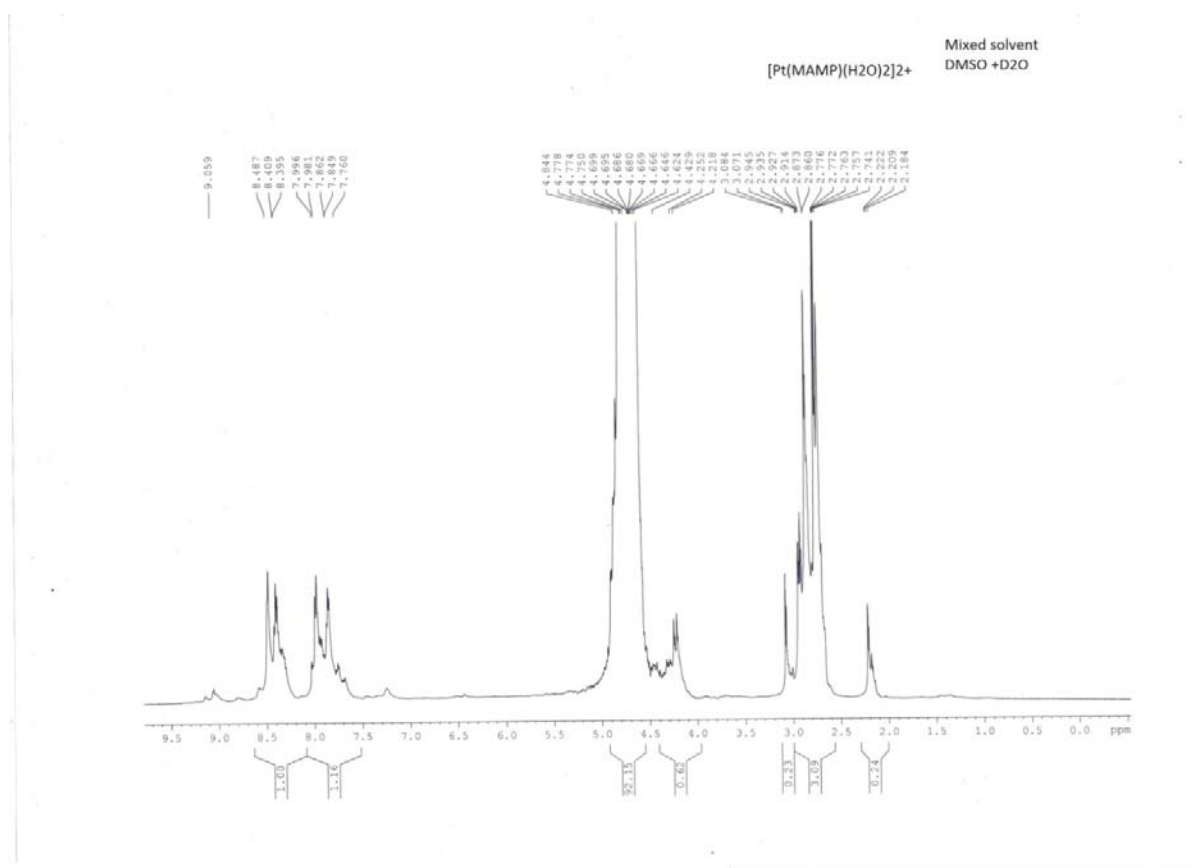
Fig S4 IR spectra of complex **2**

Fig S5 ¹H NMR spectra of complex **2** in mixed solvent DMSO-D6 and D₂O

Pt(mamp)+L-Cys-4

Number of sample scans: 32
Number of background scans: 32
Resolution: 4.000
Sample gain: 8.0
Optical velocity: 0.4747
Aperture: 80.00
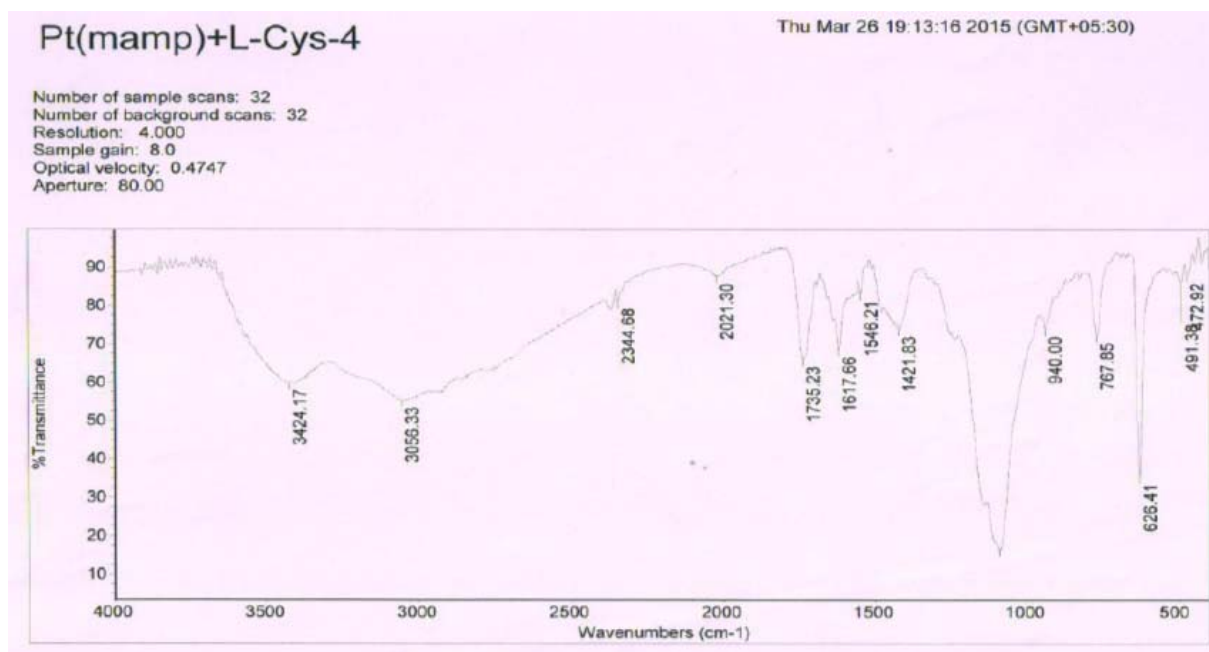
Fig S6 IR spectrum of complex **3**

A2-1H(SKD) 19may15.8

[Pt(MAMP)(L-Cysteine)]
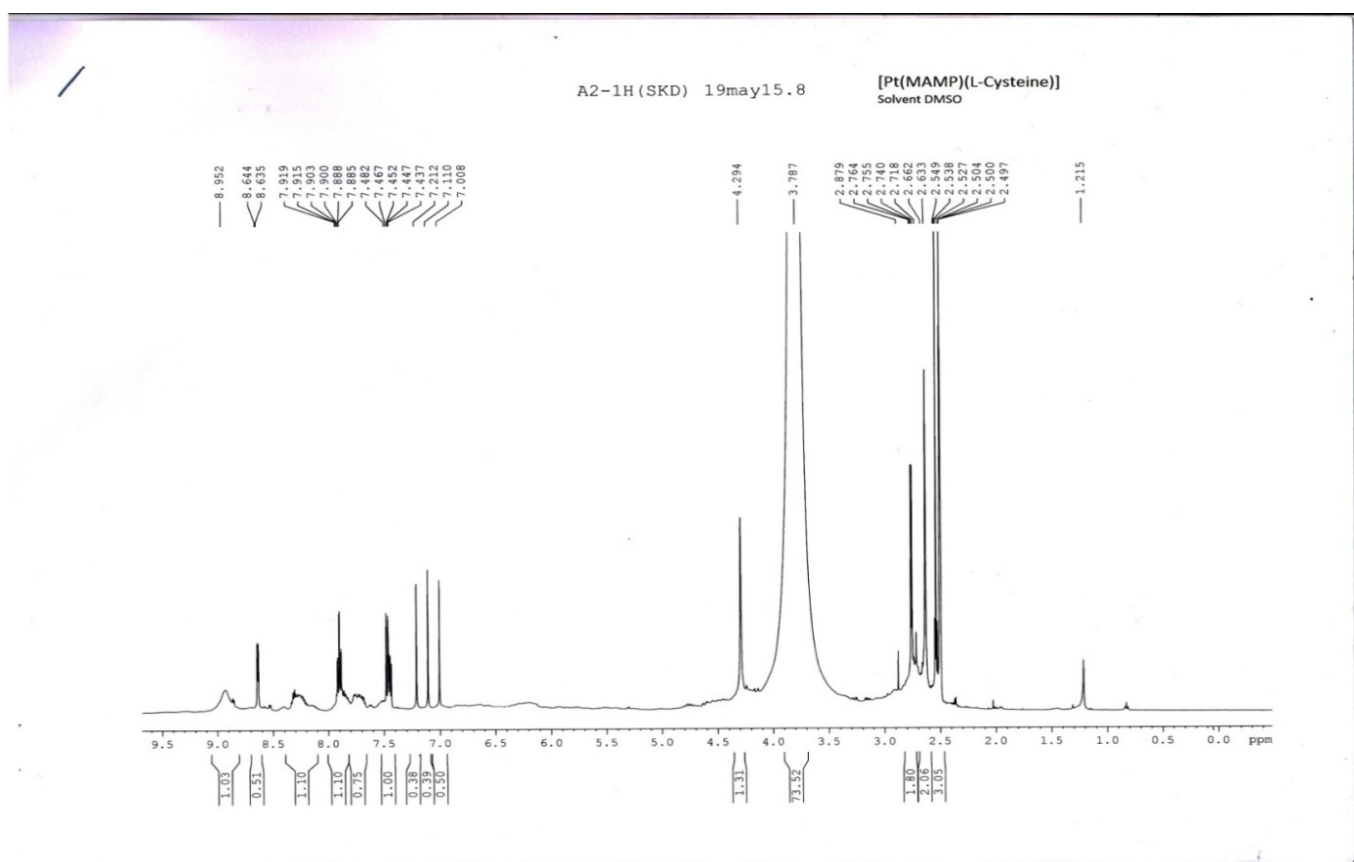Solvent DMSO

Fig S7 ^{1}H NMR spectra of complex **3** in DMSO-D6
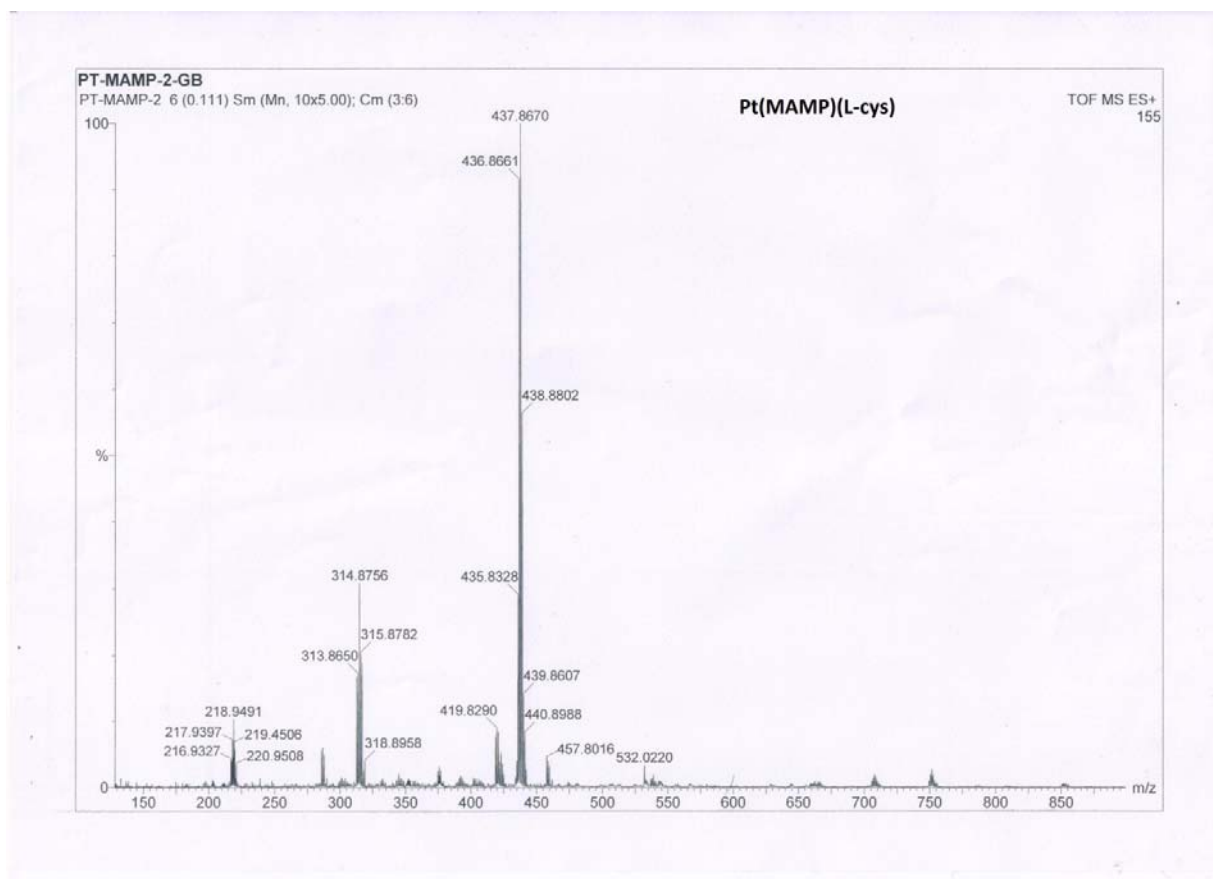
Fig S8 ESI Mass spectra of complex **3** in water
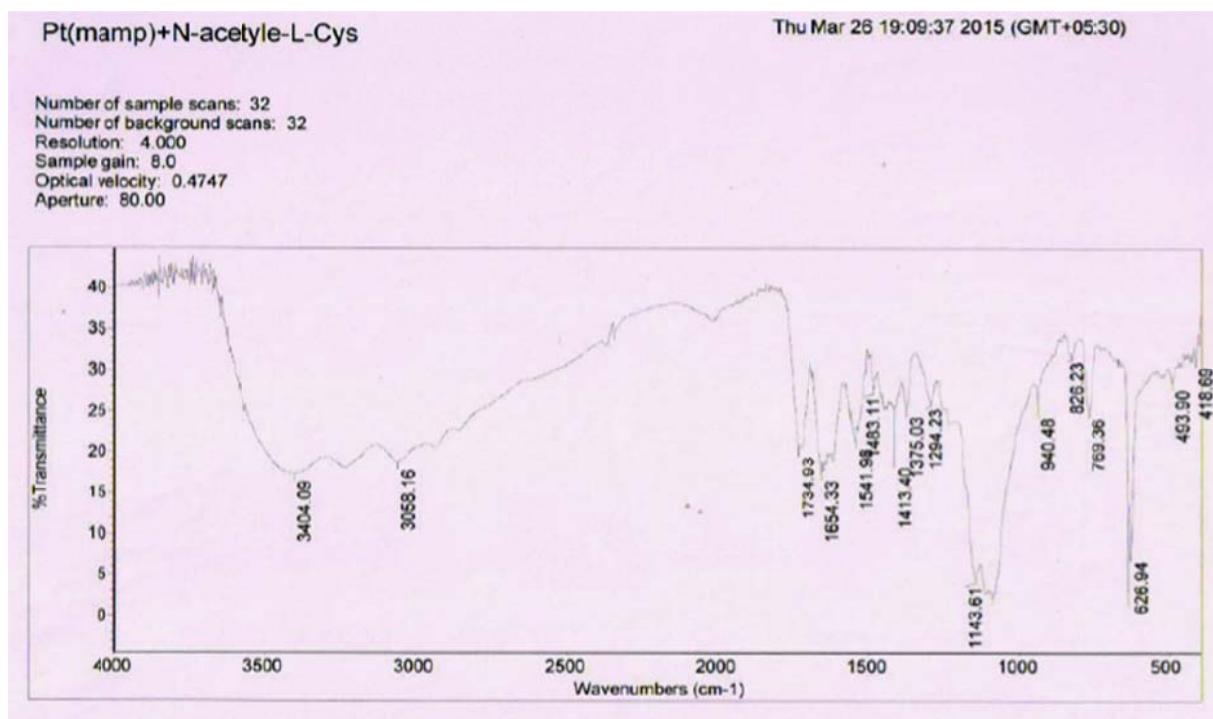
Fig S9 IR spectrum of complex $[Pt(MAMP)(OH_2)_2]^{2+}$,**4**.
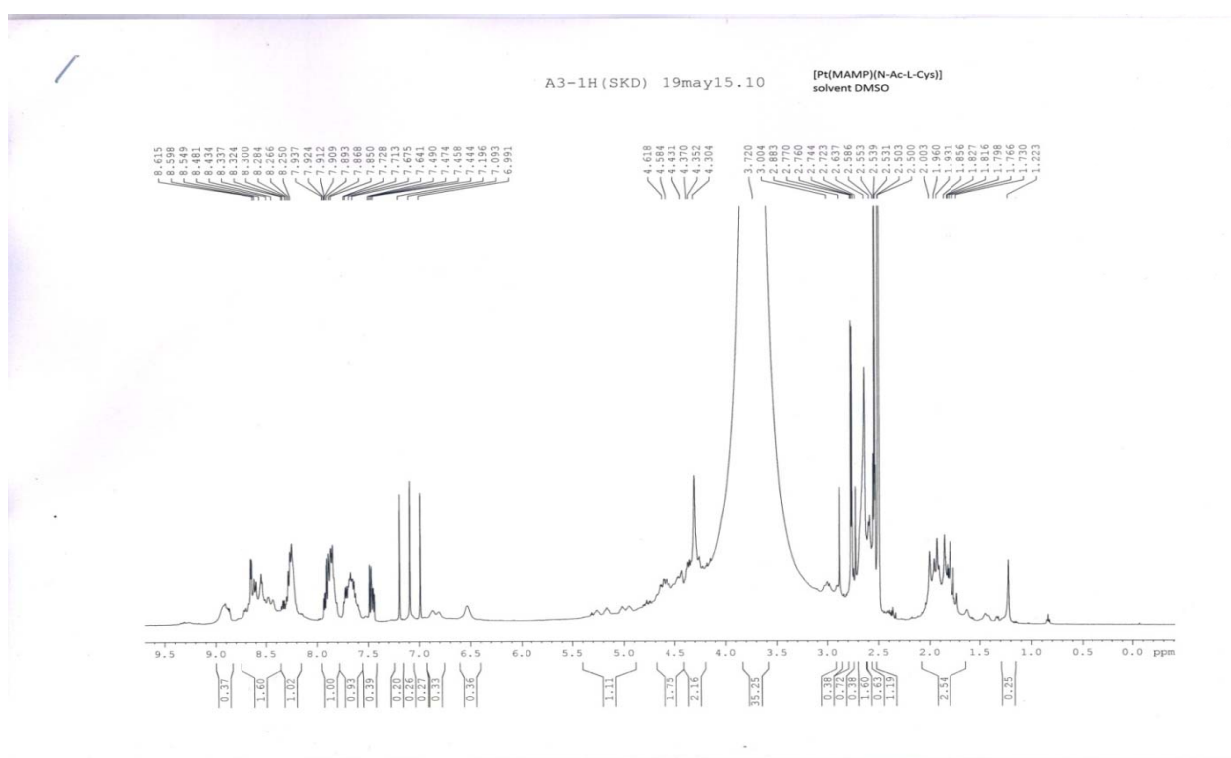
Fig S10 ^{1}H NMR spectra of complex $[Pt(MAMP)(N\text{-}ac\text{-}L\text{-}cys)]$,**4** in DMSO-D6.
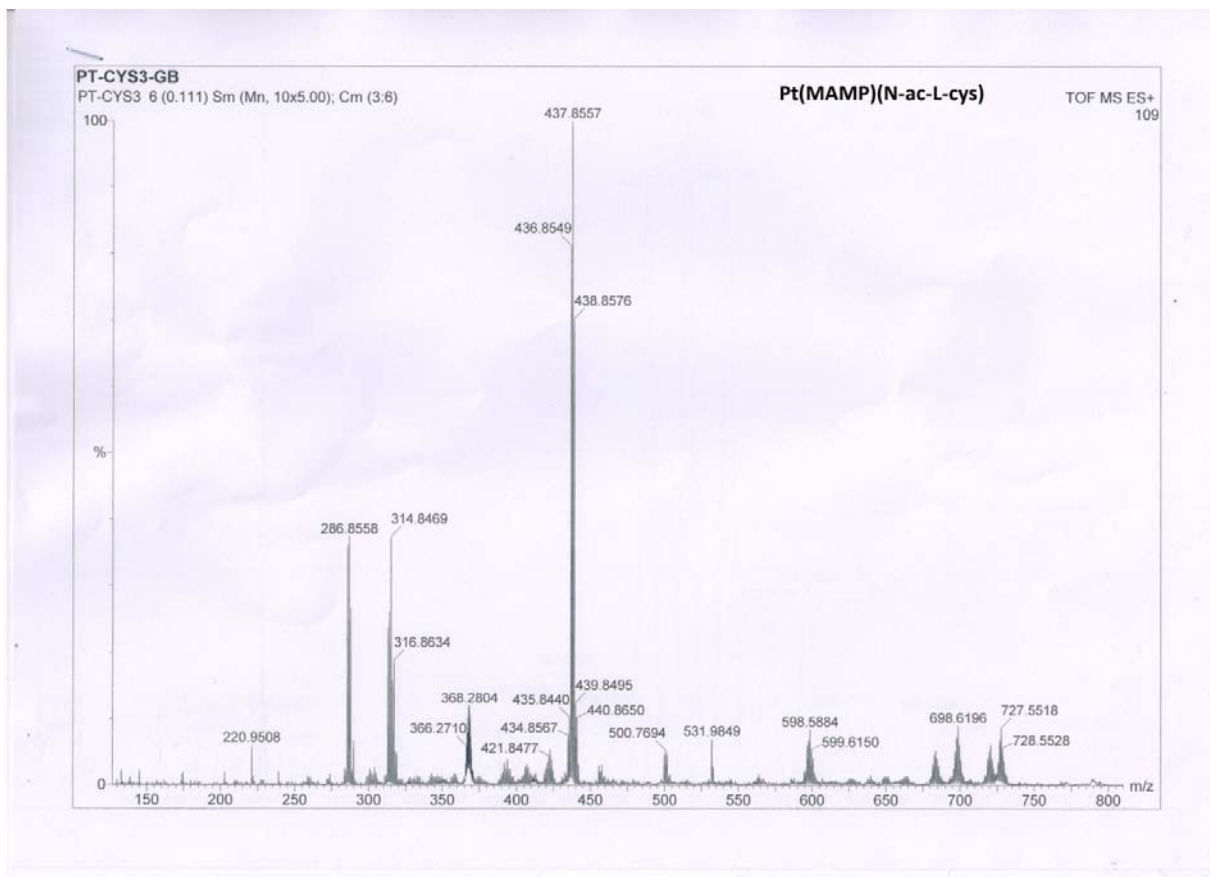
Fig S11 ESI Mass spectra of complex **4** in water
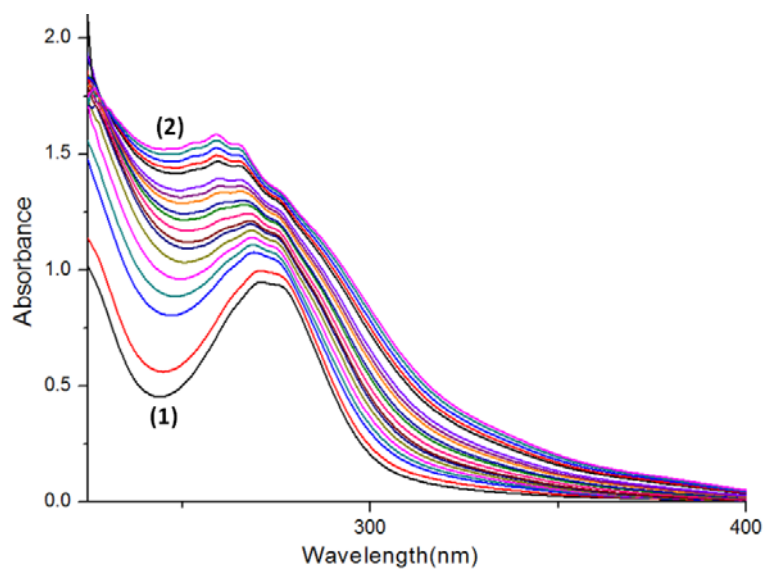
Fig S12 Spectral difference between reactant and product (Repetitive-scan spectra) (1) $[Pt(MAMP)(OH_2)_2]^{2+}= 3.04\times10^{-4}$ mol.dm^{-3}, (2) $[Pt(MAMP)(OH_2)_2]^{2+}=3.04\times10^{-4}$mol.dm^{-3}, [N-ac-L-cys]= 6.08×10^{-3} mol.dm^{-3}, pH 4.0, Cell used was 1cm quartz
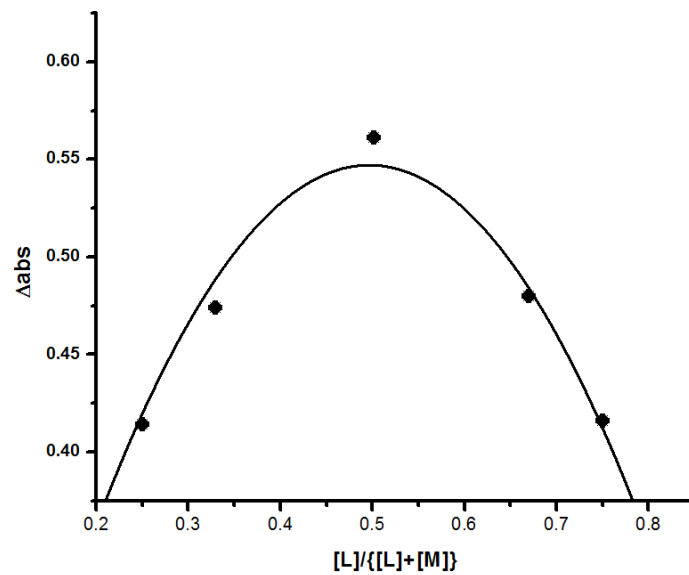
Fig S13 Job's plot of Abs versus [L]/*{[L]+[M]}* for the reaction of complex **2** with [L-cys] at pH = 4.0
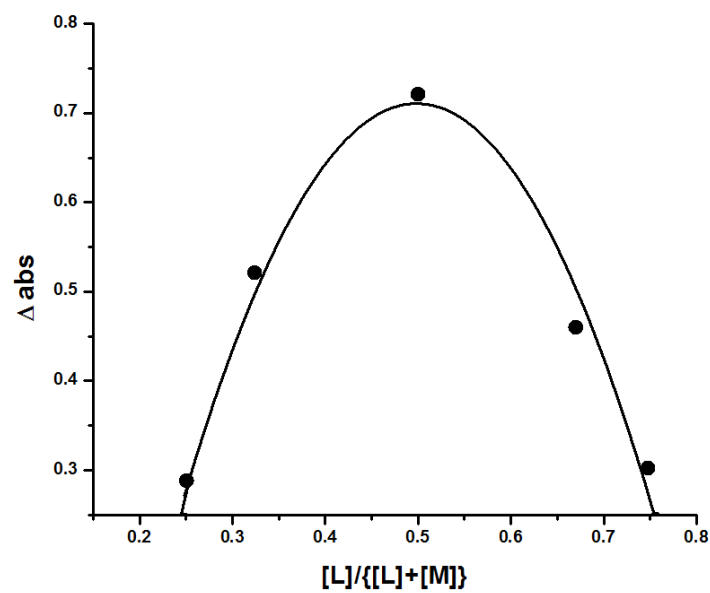
Fig S14 Job's Plot of Abs versus [L]/{[L]+[M]} for the reaction of complex 2 with [N-ac-L-Cys] at pH=4.0
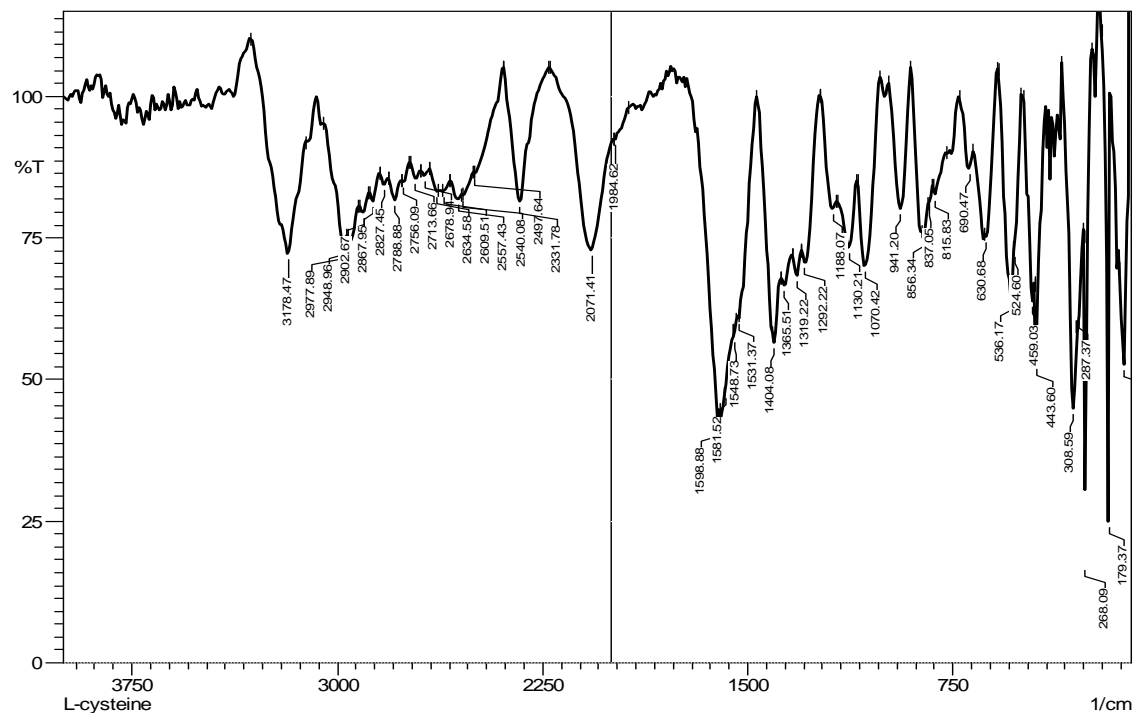
Fig S15 IR spectrum of [L-Cys] ligand
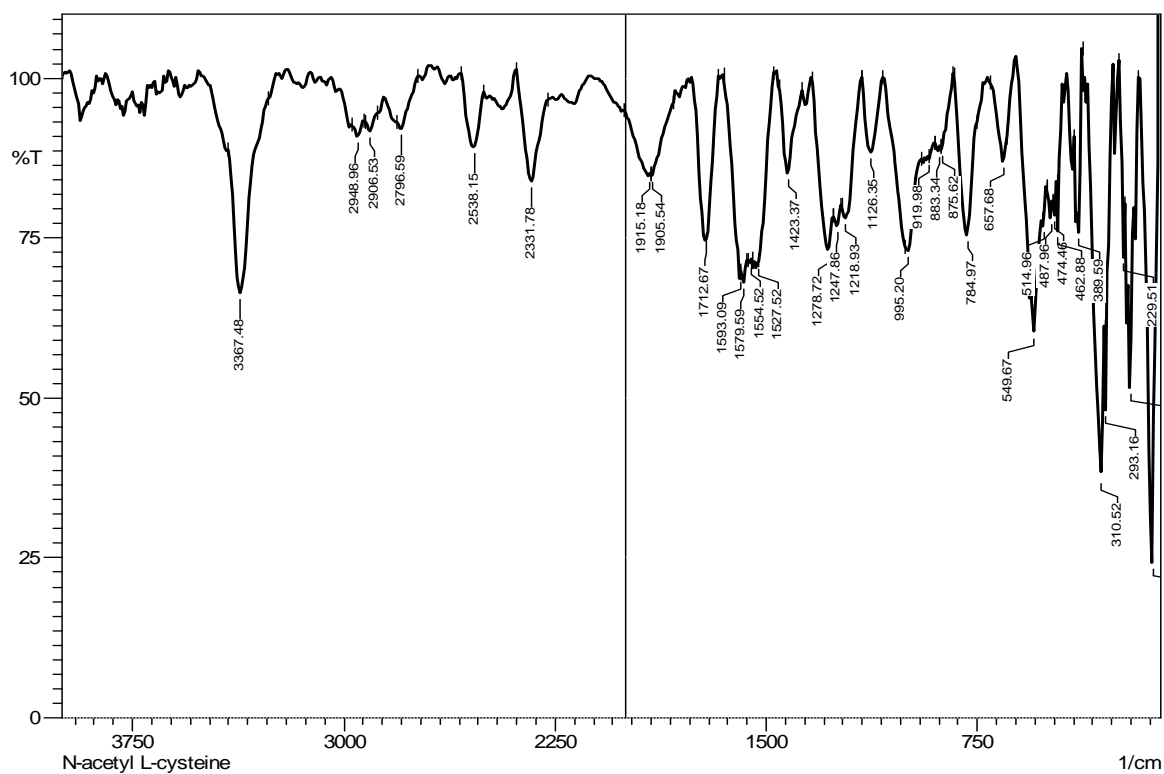
Fig S16 IR spectrum of [N-ac-L-cys] ligand.
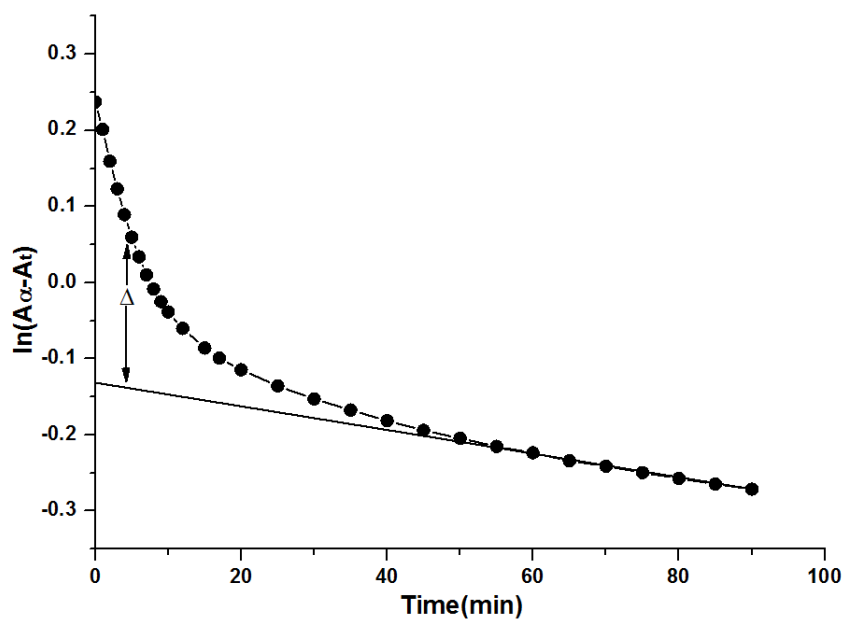
Fig S17 A typical plot of $\ln(A_\alpha-A_t)$ versus time (min), [complex **2**] = 3.04×10^{-4}mol.dm^{-3}, [N-ac-L-cys] = 6.08×10^{-3} mol.dm^{-3}, pH=4.0, t=25°C
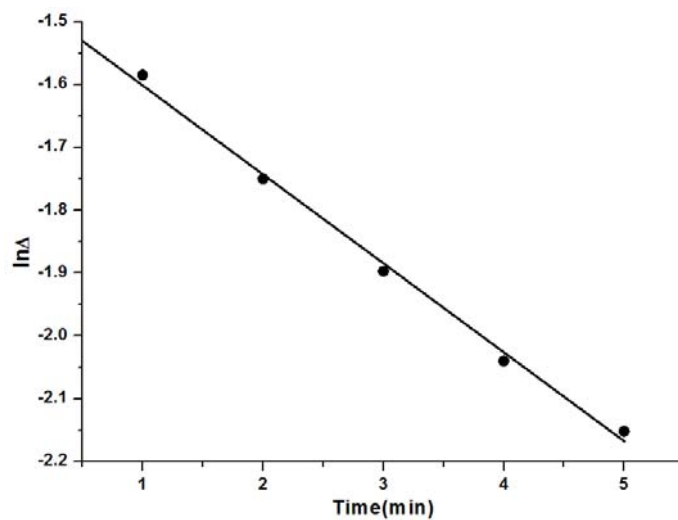
Fig. S18 A typical plot of $\ln \Delta$ versus time (min), [complex **2**] =3.04×10^{-4}mol.dm^{-3}, [N-ac-L-cys]=6.08×10^{-3}mol.dm^{-3}, pH=4.0, t=25 °C
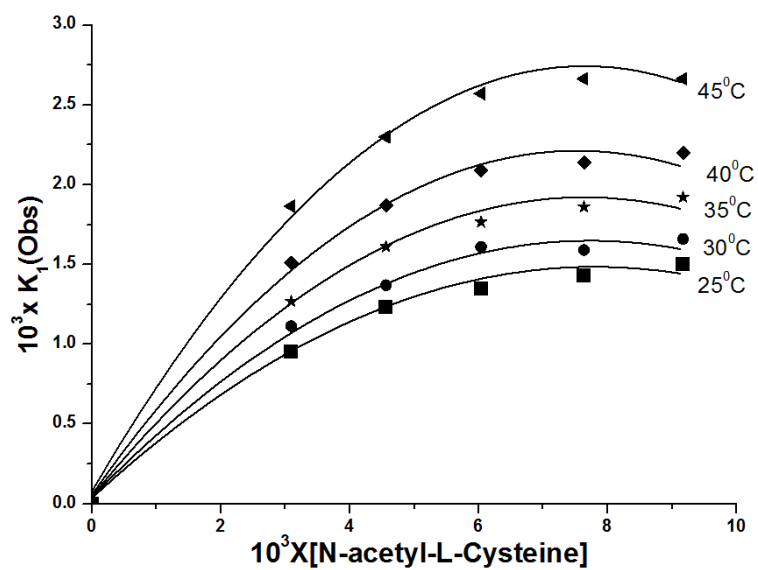
Fig. S19 Plot of 10^3 $k_{1(obs)}$ versus 10^3 [N-ac-L-cys] at different temperatures
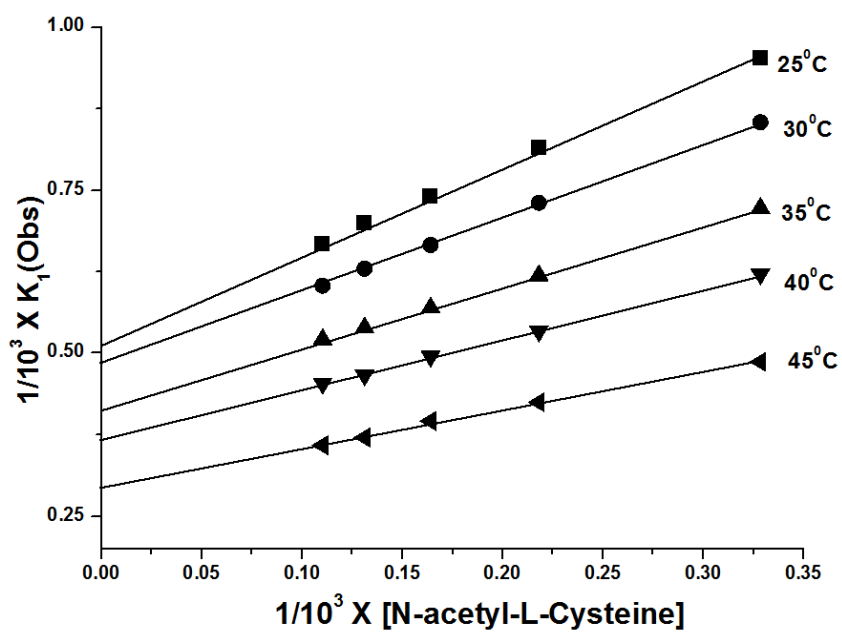
Fig. S20 Plot of $1/10^3$ $k_{1(obs)}$ versus $1/10^3$ [N-ac-L-cys] at different temperatures
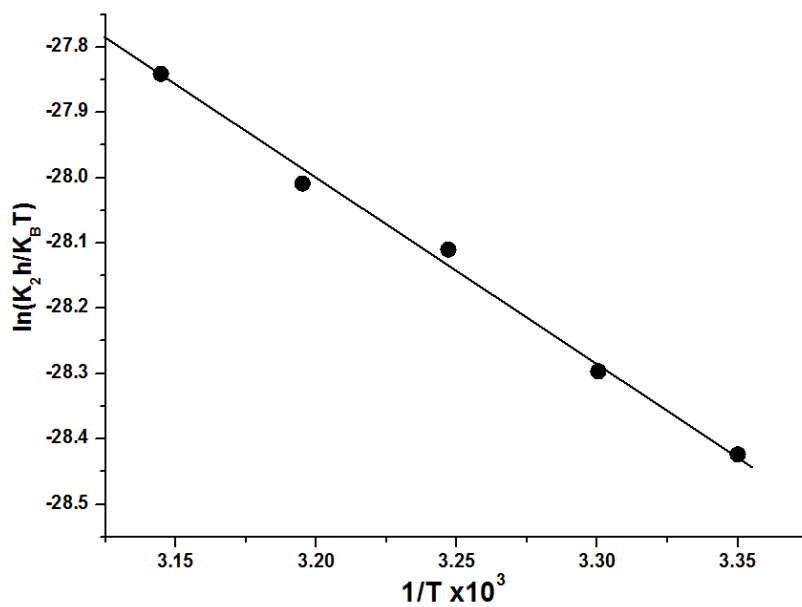
Fig. S21 Eyring plot of $(\ln k_2 h/K_B T$ vs. $10^3/T)$ for the reaction of L-cys with complex **2**
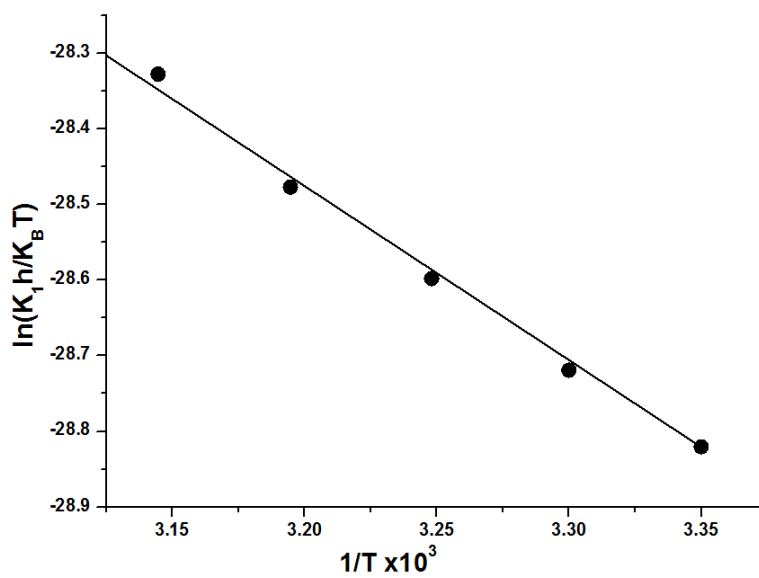
Fig S22 Eyring plot of $(\ln k_1 h/K_B T$ vs. $10^3/T)$ for the reaction of N-ac-L-cys with complex **2**
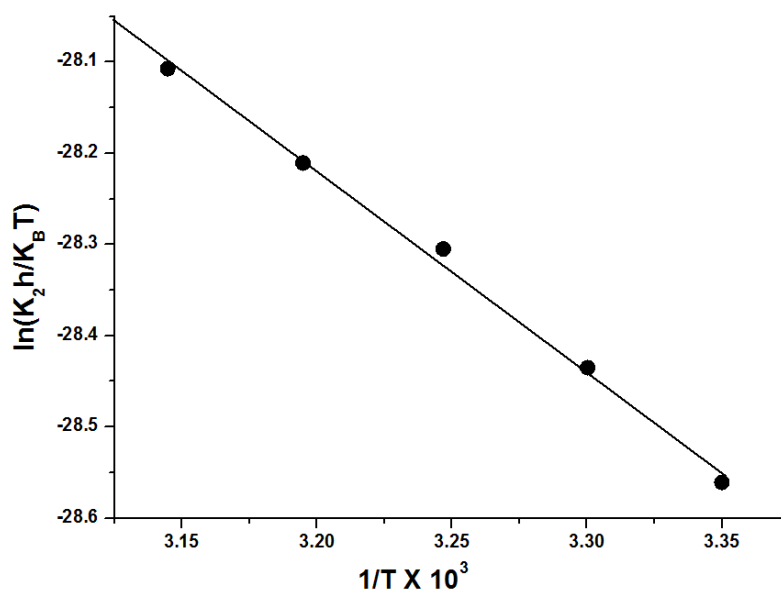
Fig S23 Eyring plot of ($\ln k_2 h / K_B T$ vs. $10^3/T$) for the reaction of N-ac-L-cys with complex **2**

In vitro antibacterial assay

Bacterial culture Suspension preparation: Bacterial culture suspensions were prepared by the direct colony method. The isolated single colonies were taken directly from the LB agar plate and sub-cultured in 100 ml of sterile LB broth (Himedia) and incubated at 37 ºC for 12 hours overnight. The turbidity of initial LB broth was adjusted to zero and this was constant control for the entire experiment. The cells were maintained and cultured in Luria-Bertani (LB) broth (Himedia). The main aim of the study is to find out the antibacterial activity of the compounds.

Determination of bacterial numbers: The standard plate count method consists of diluting a bacterial culture with sterile LB Broth until achievement of the bacteria with enough dilution to count accurately. The number of colonies should provide the number of bacterial cells that can grow under optimum incubation conditions employed. The ample series of dilutions (e.g., 10^{-4} to 10^{-10}) were normally plated for getting the exact number of bacteria. Greater accuracy is achieved by plating triplicates of each dilution.

Standard plate count: The isolated colonies of both the gram positive (Bacillus Subtilis) and gram negative (E.coli Dh5α) bacteria inoculated aseptically to 100 ml of freshly prepared LB broth, and incubated at 37 ºC for overnight. The initial dilutions made by transferring 1 ml of suspension culture to a 9 ml sterile LB Broth to make 1/10 or 10^{-1} dilution. This process was carried out to produce up to 10^{-10} dilution. From each dilution, 1 ml of the serially diluted broth was transferred into the corresponding Petri plates and LB agar poured onto it and incubated this

plate after solidification at 37 ^{o}C for 24 hours. At the end of the incubation period, all of the Petri plates containing the colonies between 30 and 300 colonies were selected. The colonies were counted on each plate by using a colony counter. The number of bacteria (CFU) per milliliter was calculated by dividing the number of colonies by the dilution factor multiplied by the amount of suspension added to liquefied agar (**Robert W. Finberg** et al., 2011).

Calculating the number of bacteria per ml

For calculating the number of bacteria per ml of serially diluted bacteria, the following equation was used

$$\frac{\text{Number of CFU}}{\text{Volume plated (ml) x total dilution used}} \longrightarrow \frac{\text{Number of CFU}}{\text{ml}}$$

Turbidometric determination of bacterial numbers

Bacterial count was also calculated by determining the optical density (OD) of the bacterial culture, based on turbidity developed, by Spectrophotometer at 600nm. The sterile LB broth was used as blank as the sample concentration equal to zero absorbance. 1ml of the serially diluted bacterial culture read the optical density at 600 nm against the blank. This step was carried out for all dilutions. The number of cells/ml in the culture was calculated by the standard population vs. absorbance relation of bacterial cells. (1 O.D of the culture $=1 \times 10^9$ cells/ml) (Jackie Reynolds et. al. 2005)

MTT ASSAY FOR ANTITUMOR PROPERTY DETERMINATION

Cells and culture conditions:

Human survical carcinoma cell lines (HeLa), which were obtained from Bose institute, Kolkata, were used in this study. The Cells were allowed to grown in culture flask possessing DMEM medium (Hi-Media) containing 10% FBS (Hi-Media) with antibiotic concentration 1% of penicillin/streptomycin (50 IU/ml and 500 μg/ml) respectively in CO_2 incubator with a humidified atmosphere (95% air /5% CO_2) at 37°C for 2 days. After obtaining the expansion of cells around 75% - 80% confluence, these cells maintained in the same cultural conditions. The cells were counted by haemocytometer and diluted to the appropriate concentration for further seeding. The appropriate volume of cell suspension added to a new flask containing medium (DMEM with antibiotic). This experiment was performed on cultures from passages 3 to 4.

Cell counting:

The number of cells, cultured in culture flasks was evaluated by detaching with trypsin solution(0.05% trypsin–0.02% sodium EDTA) and counting using Haemocytometer and trypan

blue solution (0.2% w/v final dye concentration).Viable HeLa and Hep G 2 cell number counts were obtained at 4, 15, and 24-h incubation.

MTT cell viability assay

Cell viability was investigated using the MTT colorimetric assay. MTT is taken up into cells by endocytosis or protein-facilitated mechanism and reduced, mainly by mitochondrial enzymes. MTT assay is based on a reaction between mitochondrial enzyme dehydrogenase from viable cells with the yellow tetrazolium rings of MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced to generate reducing equivalents such as NADH and NADPH by metabolically active cells, by producing dark blue formazan crystals. These crystals are impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The resulting intracellular purple formazan can be solubllized by adding a detergent, results in the liberation of the crystals. These were quantified by spectrophotometric means. The number of the surviving cells is correlated to the level of the formazan product which is generated. The colour can then be quantified by using the colorimetric assay on a multi-well scanning spectrophotometric method by ELISA reader (Stat Fax™® 2100Microplate Reader, USA).

The ability of cells to reduce MTT provides an indication of the mitochondrial integrity and activity which, In turn, may be interpreted as a measure of cell number or proliferation or viability or survival or toxicity.
MTT Solution: (5 mg/ml) 250 mg of MTT dissolved in 50 ml of 1X Phosphate Buffer Solution

MTT Solvent: 4 mM HCl, 0.1% Nondet P-40 (NP40) all in isopropanol

In vitro assays

In order to determine the cytotoxic effect of Pt(MAMP) complexes on Hela cell viability, a rapid colorimetric MTT assay protocol was used. The *in vitro* assays the experiments were divided into three main sections; each of them was used to determine: a) the correspondence between the quantity of viable cells and resultant absorbance at 540 nm for plotting standard curves. b) *In vitro* growth features of cell lines by plotting curves to correlate absorbencies of viable cells against times (0, 24, 48, 72 and 96 h), i.e. plotting growth curves. c) Finally, chemo sensitivity characteristics of cell lines against serially diluted concentrations of Pt(MAMP) complexes.

In vitro cytotoxicity assay

a) Assays were carried out in 96-well culture plates. The cells (1×10^5 per ml) were allowed to settle by incubating the plates for 24 hrs before addition of the compound solution. After 48 hrs and 72 hrs continuous exposures to the compounds at 37 $^\circ$C these plates were analyzed for cell viability using MTT assay. The effect of Pt(MAMP) compounds concentrations was assayed in 10 wells and within three independent experiments. The percentage of cell survival against

platinum complex concentration was calculated by the following equation for HeLa and Hep G 2 cell line.

$$\% \ of \ Survival \ = \frac{(At \ - \ Ab)}{(Ac \ - \ Ab)} x \ 100$$

Where;

At: Mean absorbance of the test compound

Ab: Mean absorbance of the blank

Ac: Mean absorbance of the negative control

b) HeLa cells were detached with trypsin EDTA (0.25%). The treated cells were counted and resuspended to a final concentration of 1×10^5 cells per ml. From cell lines, 100 µl of cell suspensions was added to each well of a 96-well plate. After 24 h of incubation, when cells were in the early exponential growth, the cells were treated with desired concentration of Pt(MAMP) complexes by keeping blank and control. Cis-platin used in the same concentrations as positive control. The plates were incubated for 48 and72 hrs. After that 20 µl MTT (5 mg/ml in PBS) was added to each well and incubated for another 3 hrs. Then 150 µl of DMSO was added to each well to dissolve the blue formazan product. The absorbance of this product was measured at 540 nm, using ELISA plate reader (Stat Fax™® 2100Microplate Reader, USA).
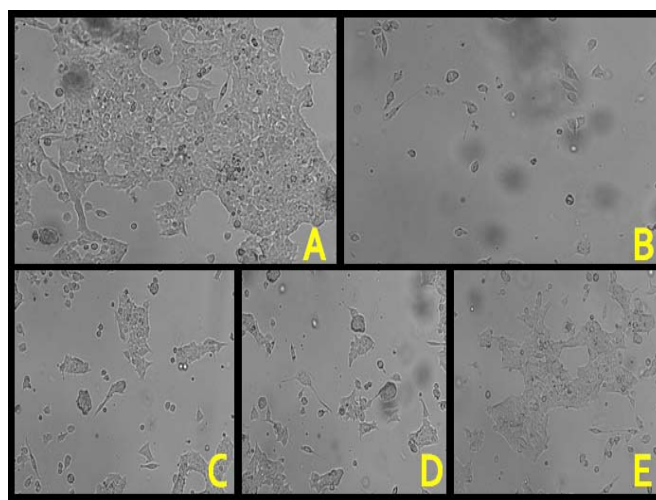

Fig. S 24 Morphological changes of cell death for Hep G2 : (A) cells treated without any complex; (B) with 10 µM of cisplatin; (C) treated with 10 µM of complex 2; (D) 10 µM complex 3 and (E) 10 µM complex 4 for 12 h.
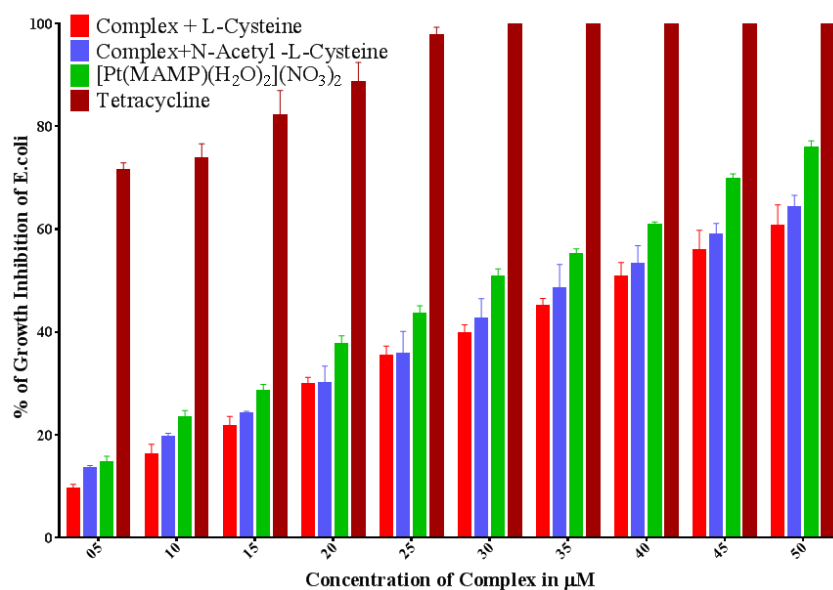
Fig. S 25 % of Inhibition growth of E. coli in presence of $[Pt(MAMP)(H_2O)_2](NO_3)_2$ and its L-cys & N-ac-L-cys substituted complexes from 5 µM to 50 µM concentration
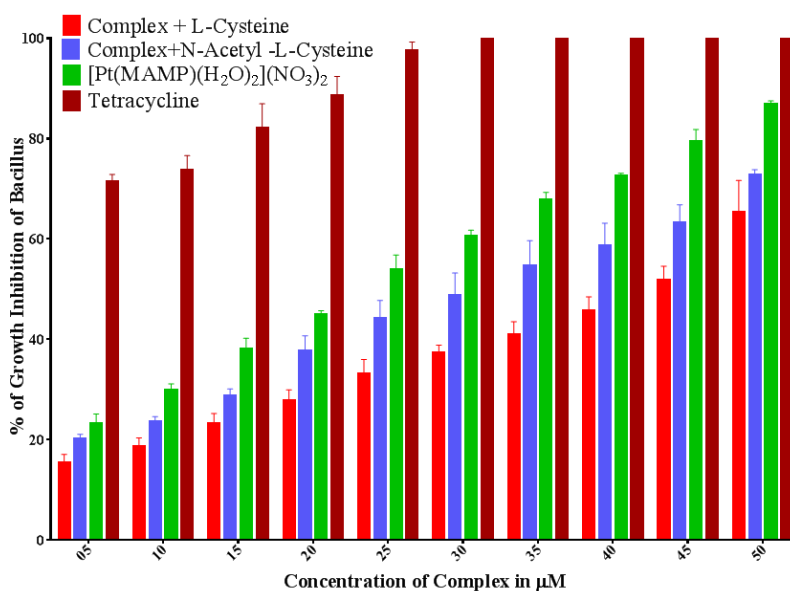

Fig. S26 % of Inhibition growth of Bacillus.subtilis in presence of $[Pt(MAMP)(H_2O)_2](NO_3)_2$ and its L-cys & N-ac-L-cys substituted complexes from 5µM to 50 µM concentration
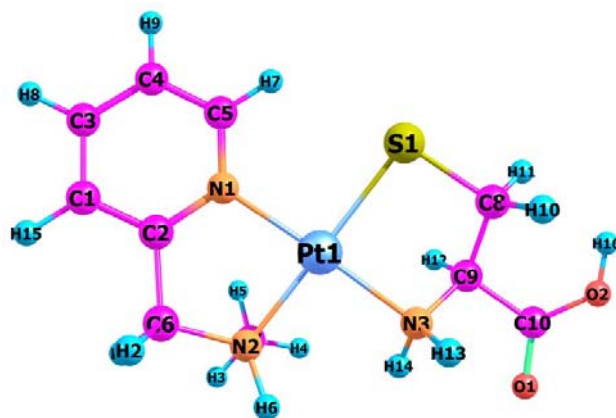
Fig. S27 optimised structure for probable Complex **3′**(S,N)
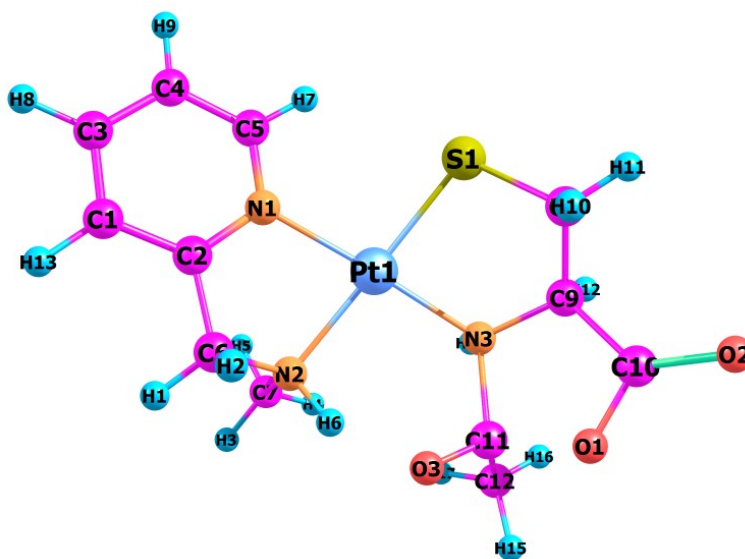

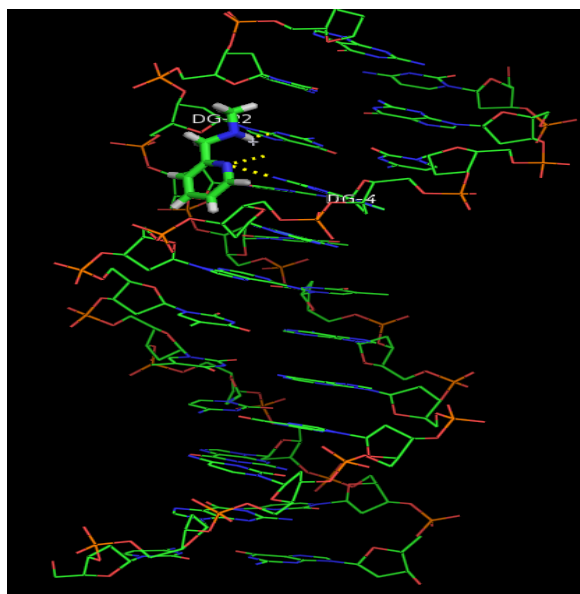**Fig. S28** optimised structure for probable Complex **4′**(S,N)

Fig. S29 Computational docking model (using HEX Software) of interaction between **2** and B-DNA (PDB ID: 1BNA)

Docking methodology

In order to follow a docking study using Hex software, first we have to load a receptor(B-DNA) and complex **2** in pdb file format structure using the File pull-down menu. Then docking was carried out using the options, Controls → Docking → Activate. To save the Docking Results: The current docking orientation was written in a single pdb file by selecting the manu File → Save → Both.

Various steps involved in metal complex Docking are, initially Structure of complex 2 was optimised using Gausian 09 and optimised structure was converted into pdb file using 'Open Babel' software. The structure of the receptor (B-DNA) was obtained from Protein Data Bank. Docking menu was clicked to carryout docking process. The results obtained after the completion of docking are shown in Fig 8. The E-Total values of metal complex against B- DNA were calculated using Hex 8.0.0 docking software, and it can be concluded from the energy value Pt complex **2** stably binds with the B-DNA.

Table S1 The parameters used in the docking process

correlation type	shape only
FFT Mode	3D
Grid Dimension	0.7
Receptor range	180
ligand range	180
twist range	360
distance range	40

Table S2 Charges (a.u) and electron configuration for the comlexes 4(S,O) and probable complex 4′ (S,N)

	Complex **4** (S,O)			Complex 4′(S,N)	
Atom	Charge	Natural electronic configuration	Charge	Natural electronic configuration	
Pt	0.32357	[core]6S(0.58)5d(8.74)6p(0.34)6d(0.01)7p(0.01)	0.30262	[core]6S(0.59)5d(8.80)6p(0.29)6d(0.02)7p(0.01)	
N6	-0.43481	[core]2S(1.28)2p(4.14)3p(0.01)	-0.43857	[core]2S(1.28)2p(4.14)3p(0.01)	
N8	-0.65501	[core]2S(1.34)2p(4.30)3p(0.01)	-0.67927	[core]2S(1.33)2p(4.33)3p(0.01)	
S20	-0.12610	[core]3S(1.71)3p(4.38)3d(0.01)4p(0.01)	-0.14583	[core]3S(1.73)3p(4.39)3d(0.01)4p(0.01)	
O27	-0.67158	[core]2S(1.66)2p(5.00)3p(0.01)	-0.6000	[core]2S(1.70)2p(4.88)3d(0.02)	
N28	--0.66023	[core]2S(1.26)2p(4.39)3p(0.01)	-0.70362	[core]2S(1.41)2p(4.28)3p(0.01)	