Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Ligand Substitution Reaction On Platinum(II) Complex With Bio-relevant Thiols: Their Kinetics, Mechanism And Bioactivity In Aqueous medium

Avradeep Samanta[a], Goutam Kr. Ghosh[a], Ishani Mitra[a], Subhajit Mukherjee[a], Jagadeesh C Bose.[b], Sudit Mukhopadhyay[b], Wolfgang Linert[c] and Sankar Ch. Moi*[a]

a. Department of Chemistry, National Institute of Technology, Durgapur-713209, W.B. India

b. Department of Bio-Tech, National Institute of Technology, Durgapur-713209, W.B. India

c. Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9/163- AC, 1060, Vienna, Austria

∗Author for correspondence, email: sankarmoi67@yahoo.com

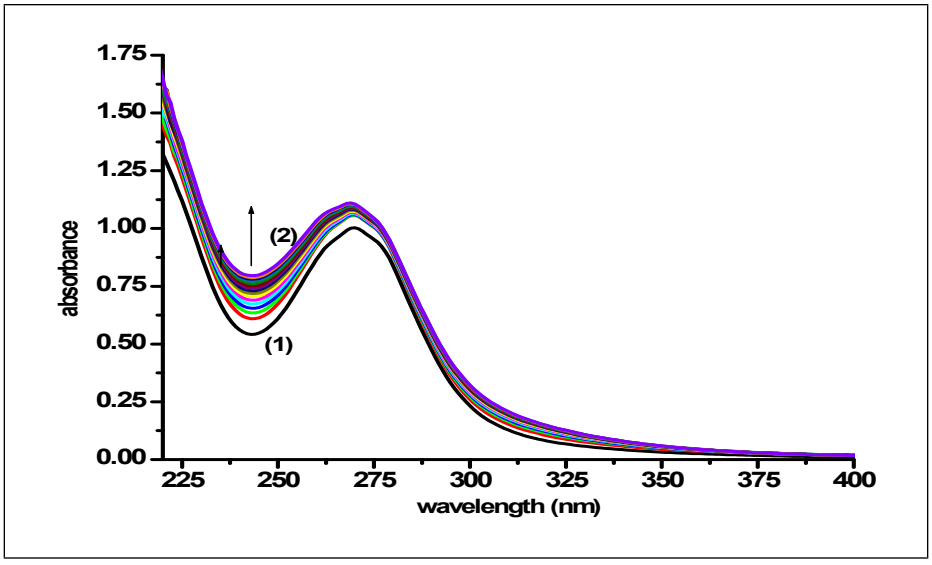

Fig S1: Spectral difference between the reactant and product (Repetitive-scan spectra) (1 cm quartz cell) (1): $[Pt(pic))_2(OH_2)_2]^{2+}$ = 2.43×10^{-4} mol.dm^{-3}, (2) $[Pt(pic)_2(OH_2)_2]^{2+}$ = 2.43×10^{-4} mol.dm^{-3}, [L-cysteine] = 2.43×10^{-3} mol.dm^{-3}, pH = 4.0

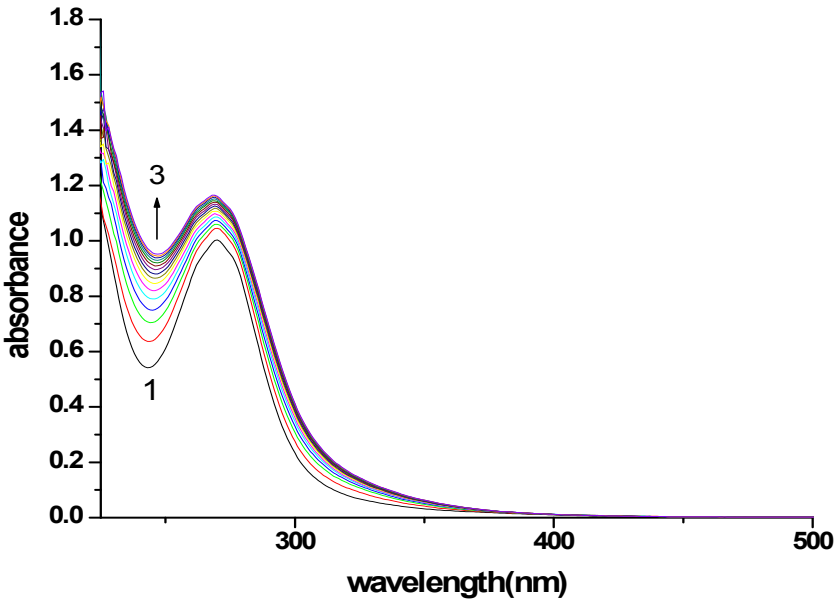

Fig S2: Spectral difference between reactant and product (Repetitive-scan spectra) (1) $[Pt(pic)(OH_2)_2]^{2+}$= 2.43×10^{-4} mol.dm^{-3}, (3) $[Pt(pic)(OH_2)_2]^{2+}$=2.43×10^{-4}mol.dm^{-3}, [N-acetyl-L-Cysteine]= 4.86×10^{-3} mol.dm^{-3}, pH 4.0, Cell used was 1cm quartz

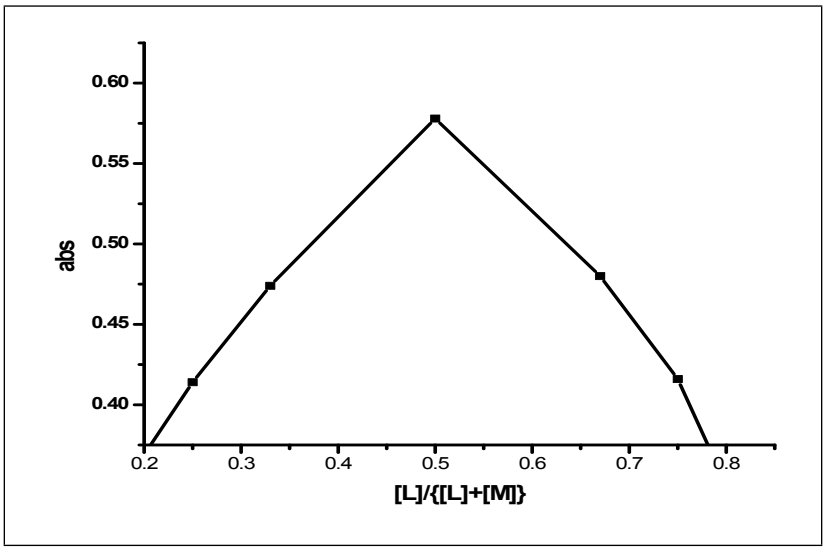

Fig S3: Job's plot of Abs versus [L]/{[L]+[M]} for the reaction of complex (1) with [L-cysteine] = 2.43×10^{-3} mol.dm^{-3}, pH = 4.0

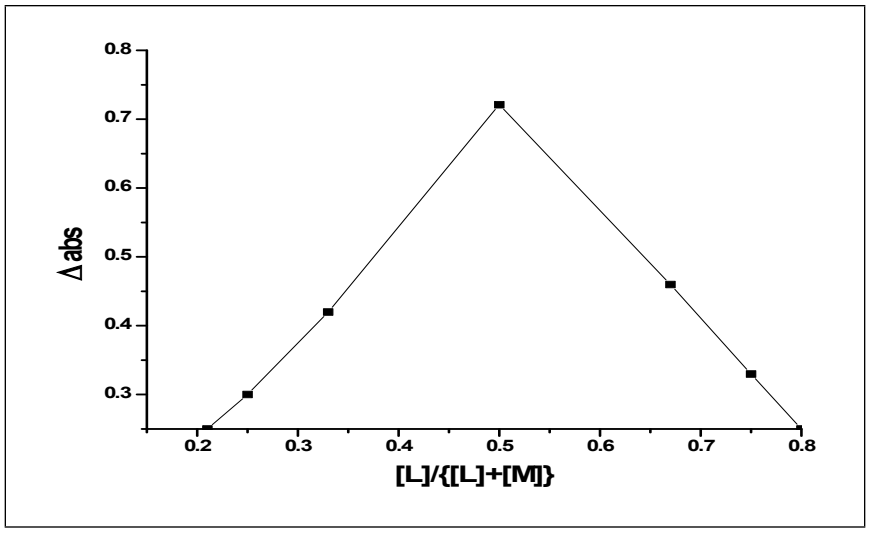

Fig S4: Job's Plot of Abs versus [L]/{[L]+[M]} for the reaction of $[Pt(pic)(OH_2)_2]^{2+}$ complex with [N-Acetyl-L-Cysteine]=2.43×10^{-3}mol.dm^{-3}

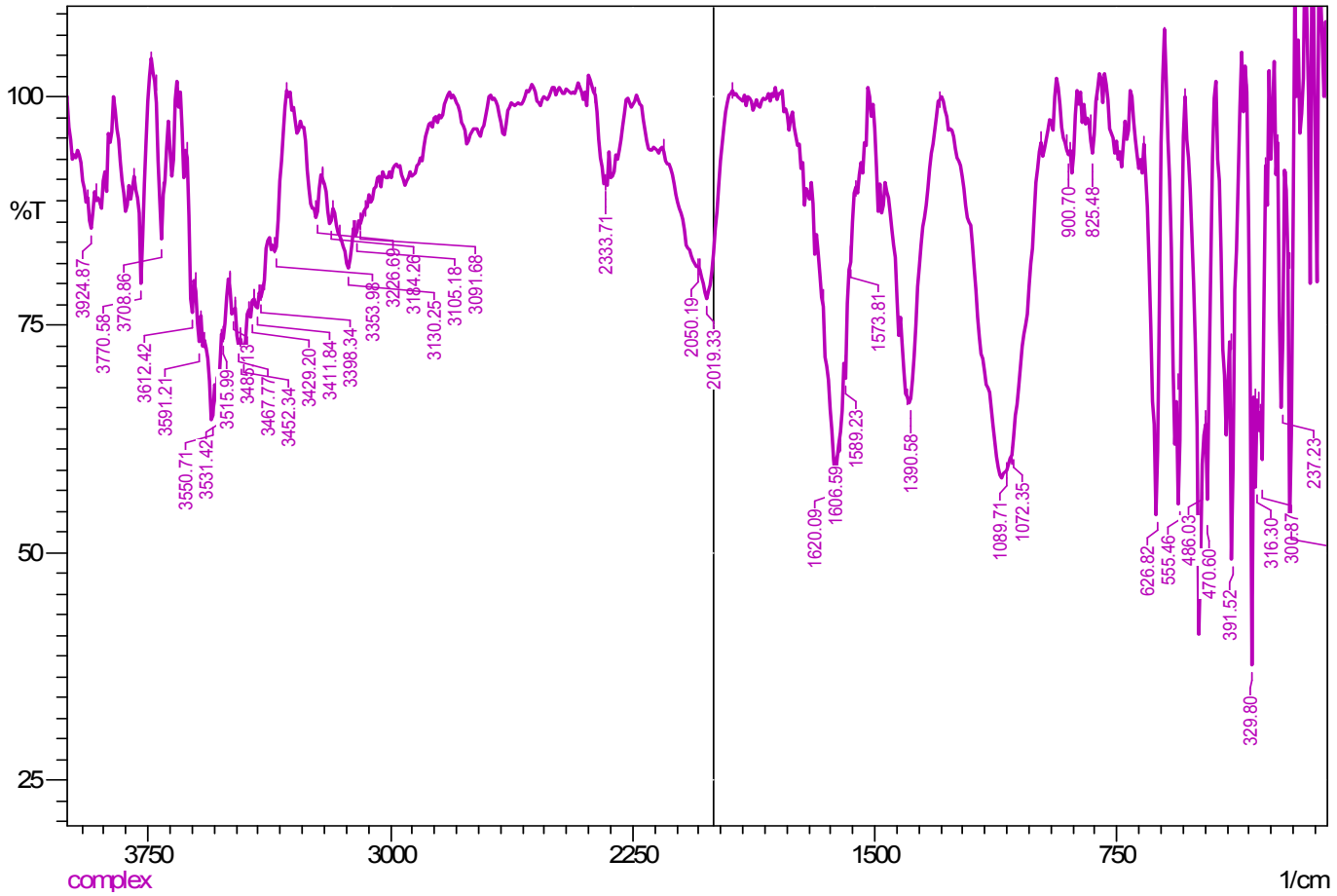

Fig S5: IR spectrum of $[Pt(pic)(OH_2)_2]^{2+}$ complex.

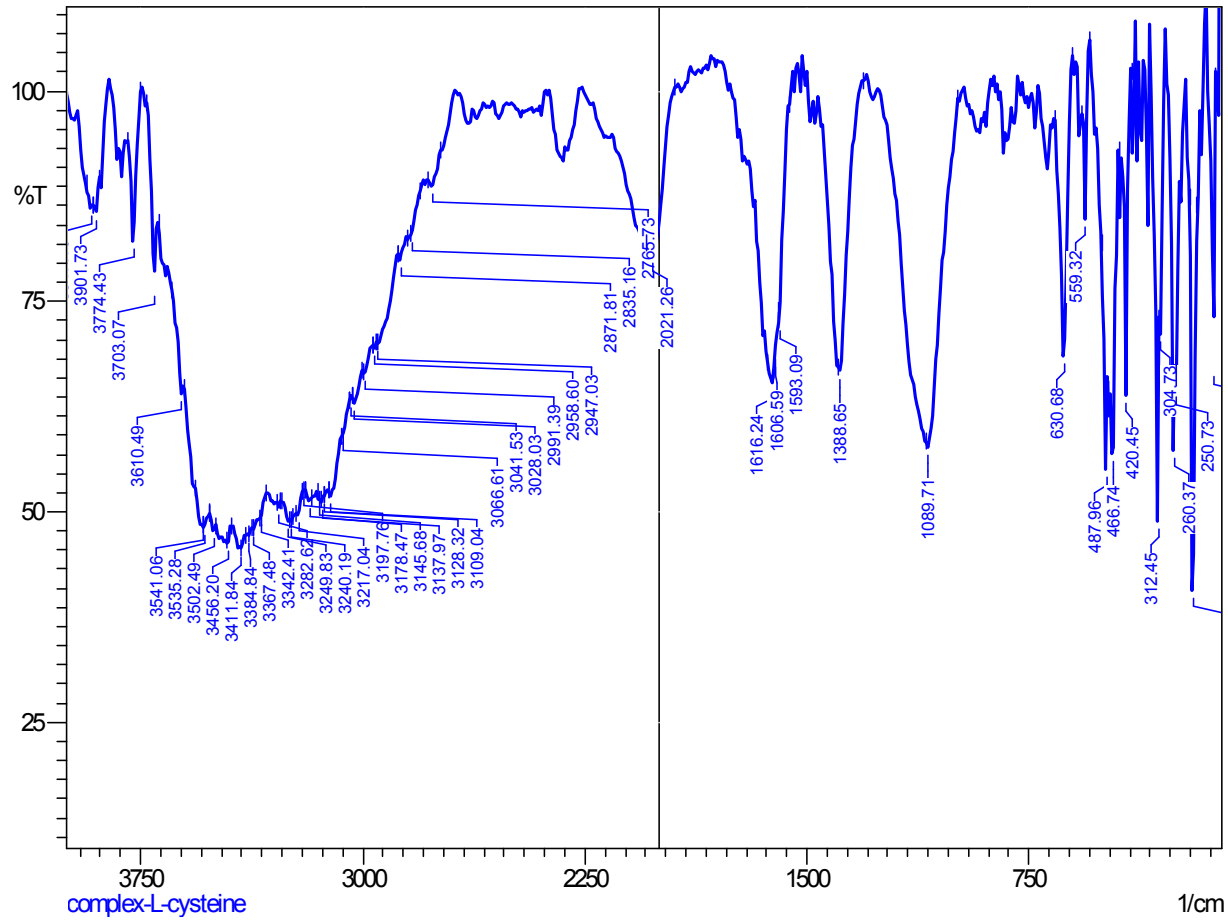

Fig S6: IR spectrum of $[Pt(pic)(OH_2)_2]^{2+}$ complex + [L-Cysteine] ligand.

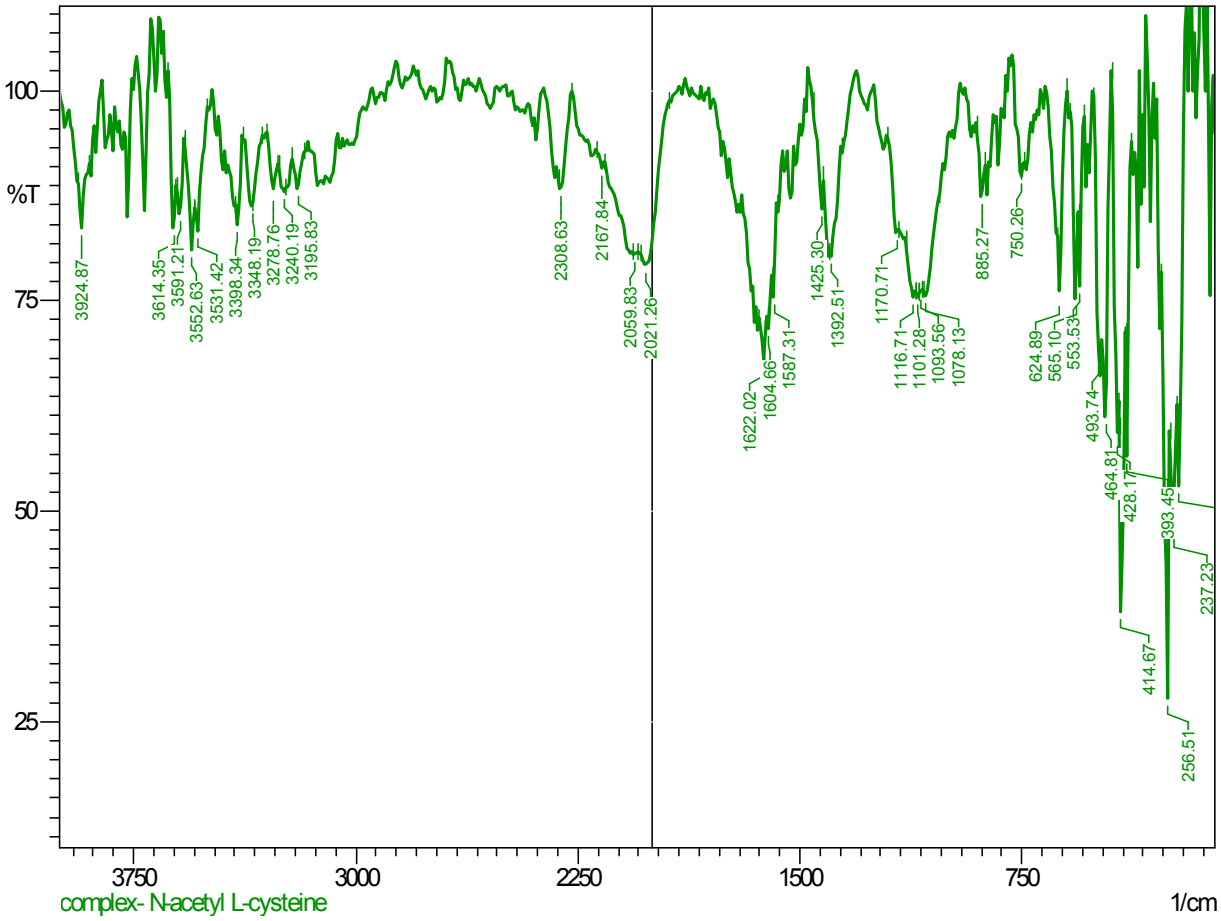

Fig S7: IR spectrum of $[Pt(pic)(OH_2)_2]^{2+}$ complex + [N-acetyl-L-Cysteine] ligand.

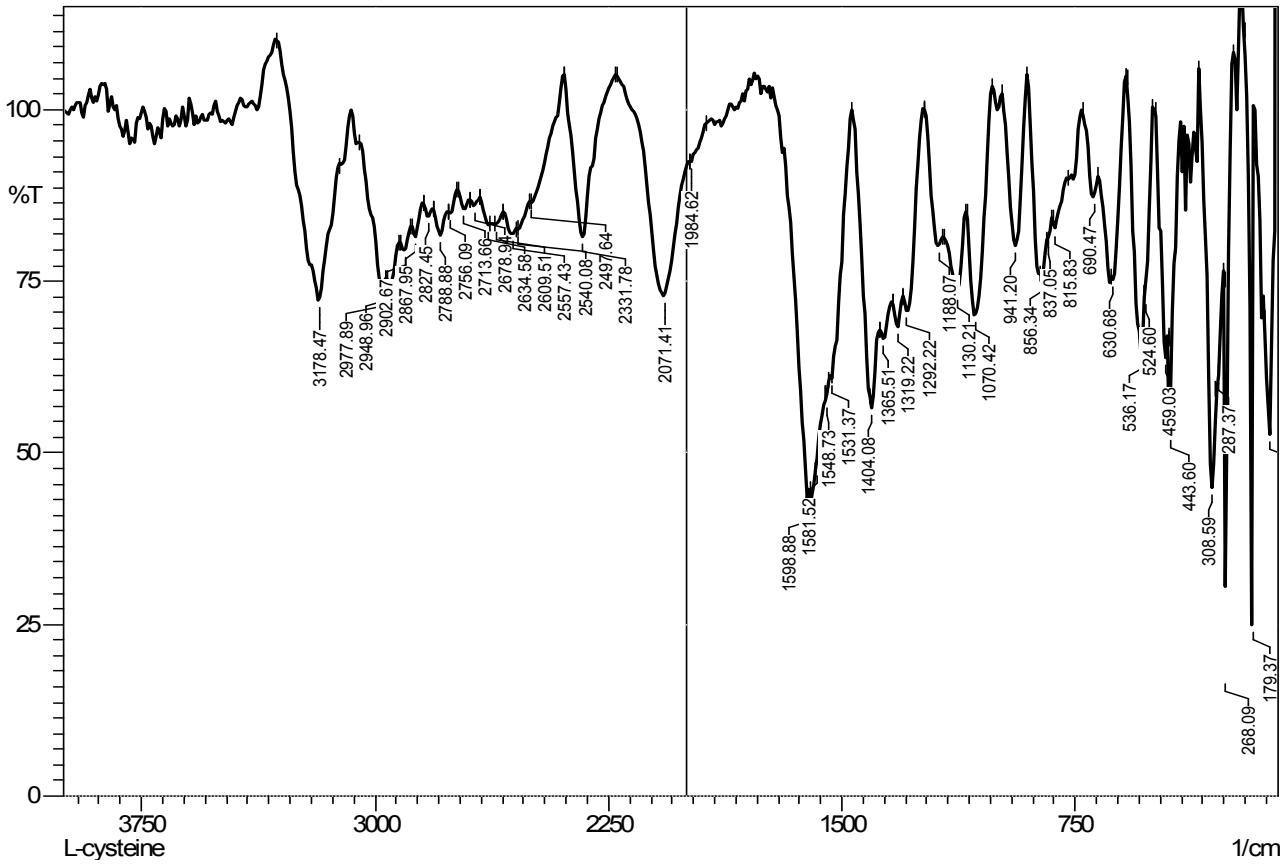

Fig S8: IR spectrum of [L-Cysteine] ligand.

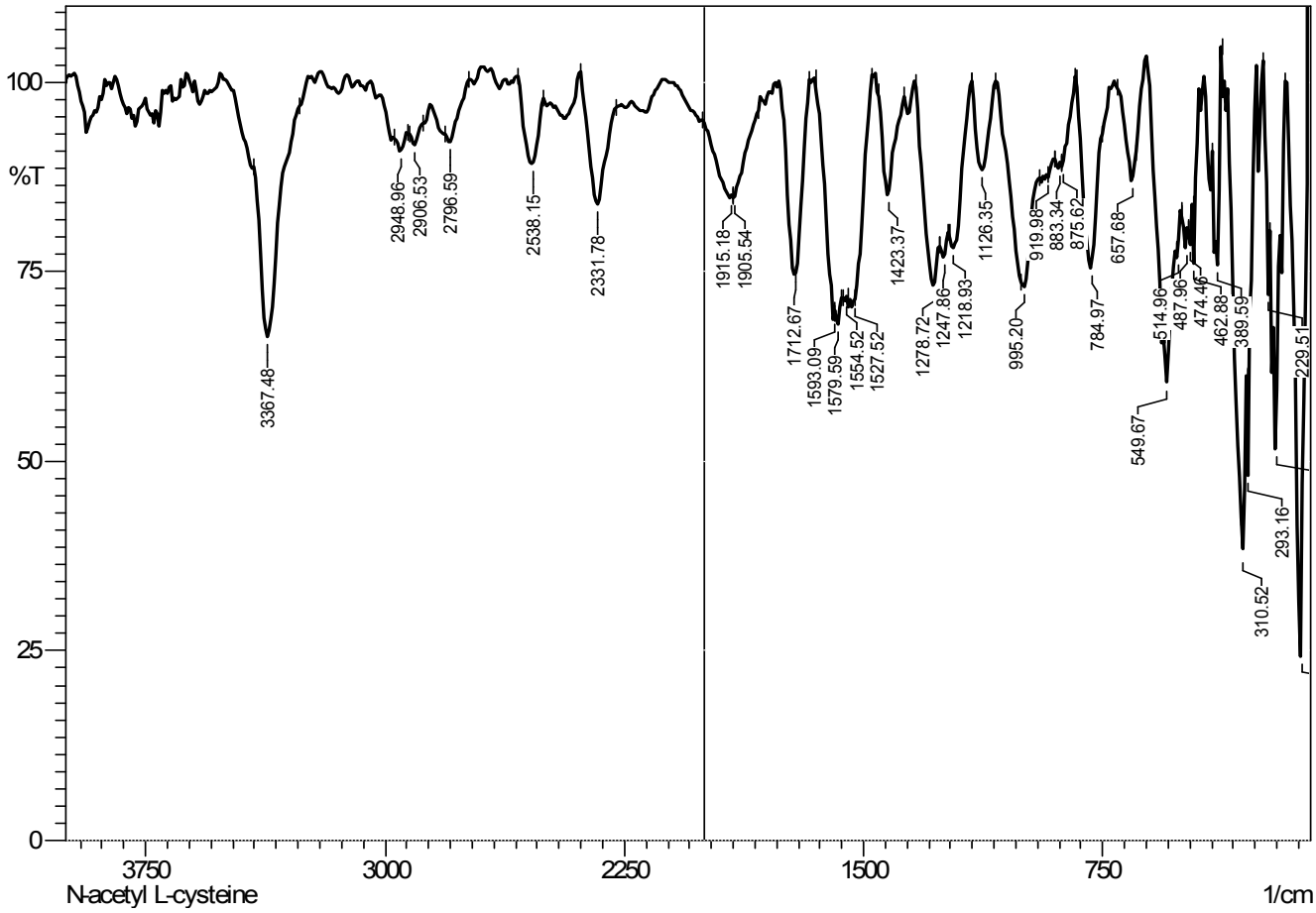

Fig S9: IR spectrum of [N-Acetyl-L-Cysteine] ligand.

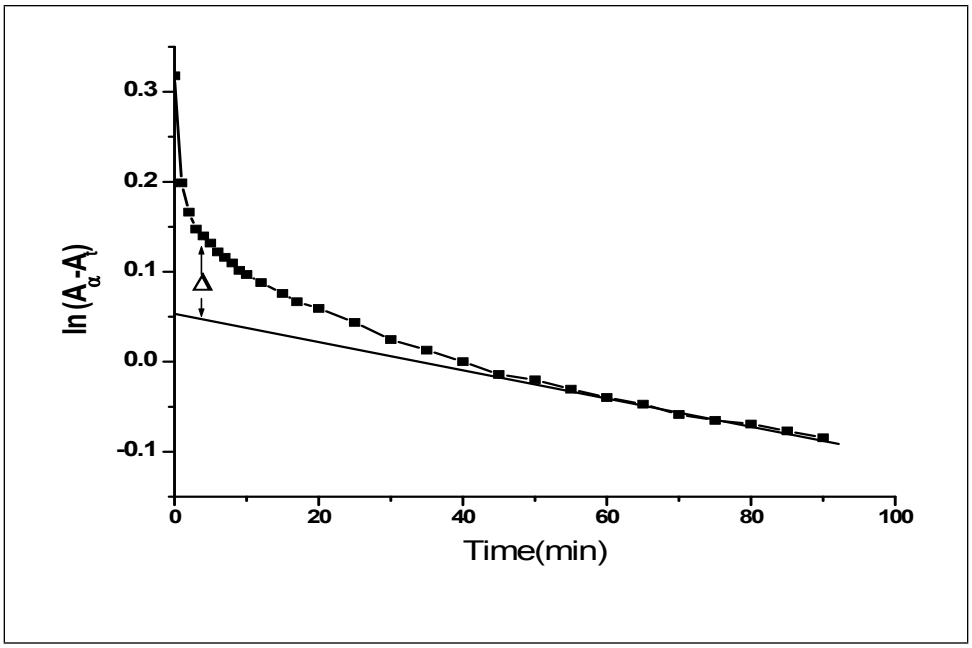

Fig S10: A typical plot of $\ln(A_\alpha - A_t)$ versus time (min), $[Pt(pic)(OH_2)_2]^{2+}=2.43\times10^{-4}$mol.dm^{-3}, [N-acetyl-L-Cysteine] =4.86 × 10^{-3} mol.dm^{-3},pH=4.0,t=25^{o}C

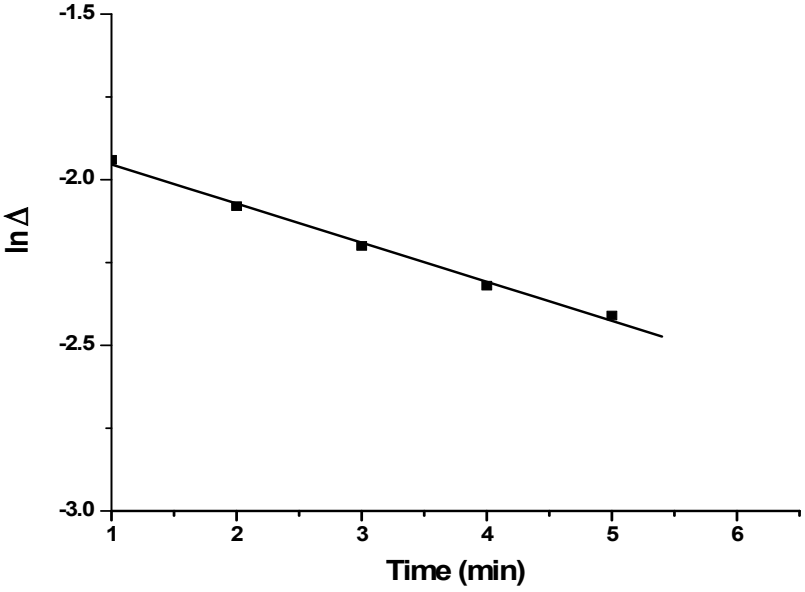

Fig S11: A typical plot of ln Δ versus time (min), $[Pt(pic)(OH_2)_2]^{2+}$ =2.43×10^{-4}mol.dm^{-3}, [N-Acetyl-L-Cysteine]=4.86×10^{-3}mol.dm^{-3}, pH=4.0, t=25^{o}C

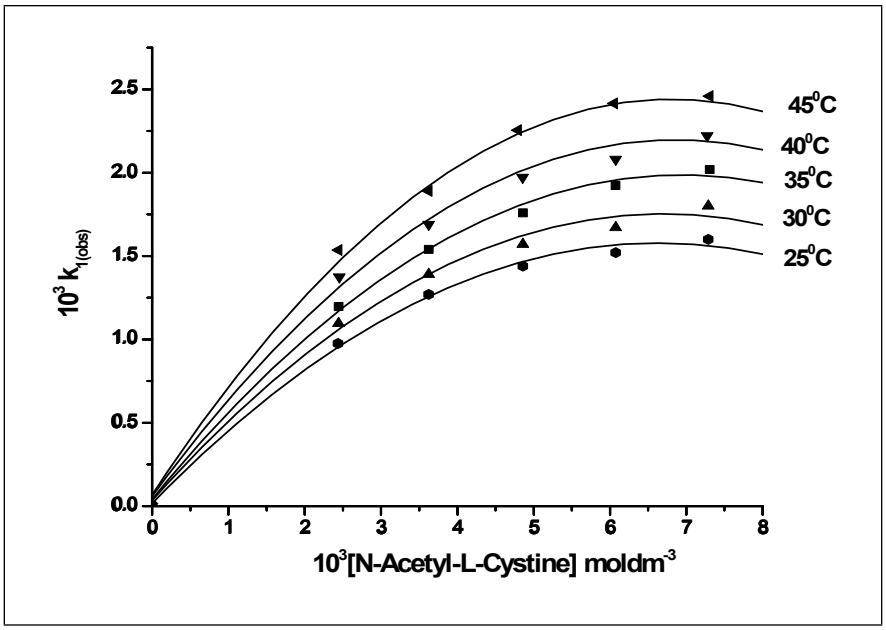

Fig S12: Plot of 10^3 $k_{1(obs)}$ versus 10^3 [N-acetyl-L-Cysteine] at different temperatures.

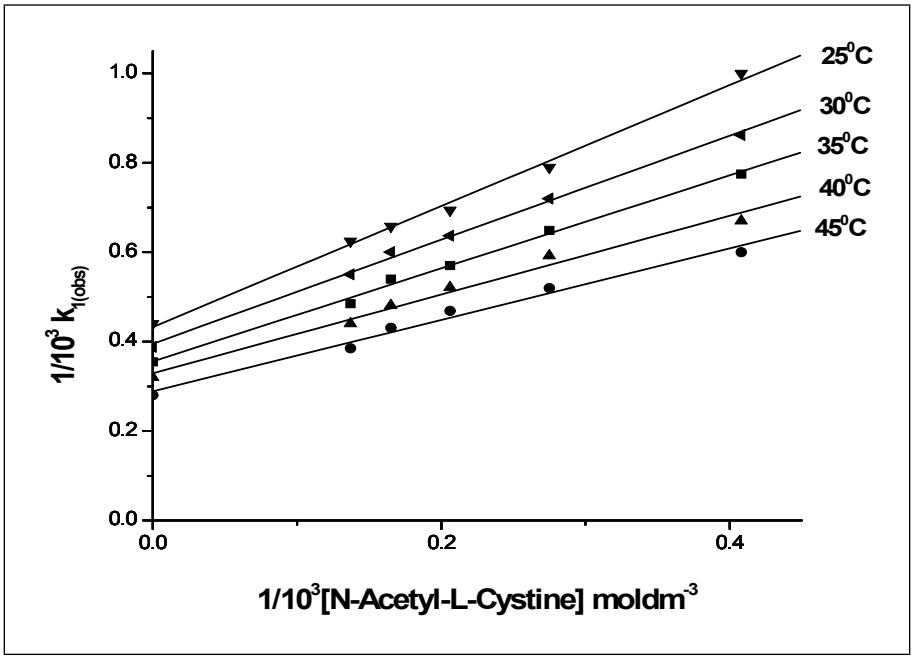

Fig S13: Plot of $1/10^3$ $k_{1(obs)}$ versus $1/10^3$ [N-acetyl-L-Cysteine] at different temperatures

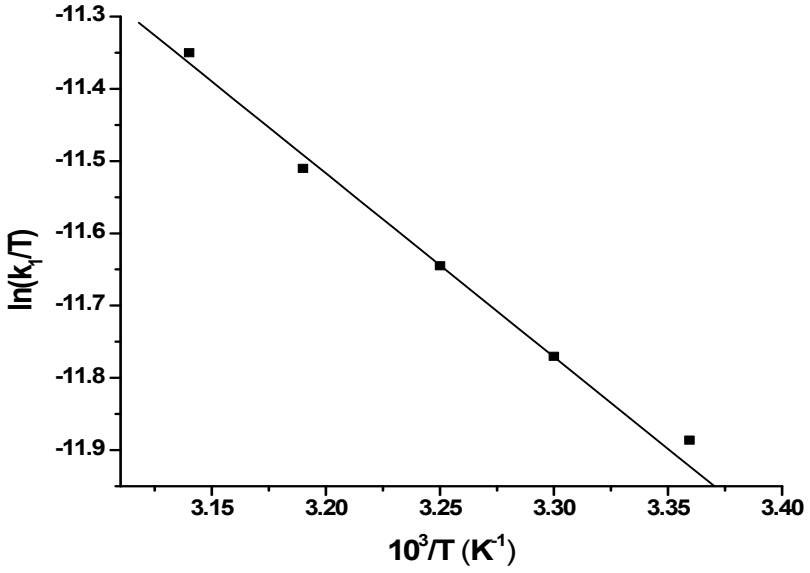

Fig S14 : Eyring plot of ($\ln k_1/T$ vs. $10^3/T$) for N-Acet-L-Cysteine.

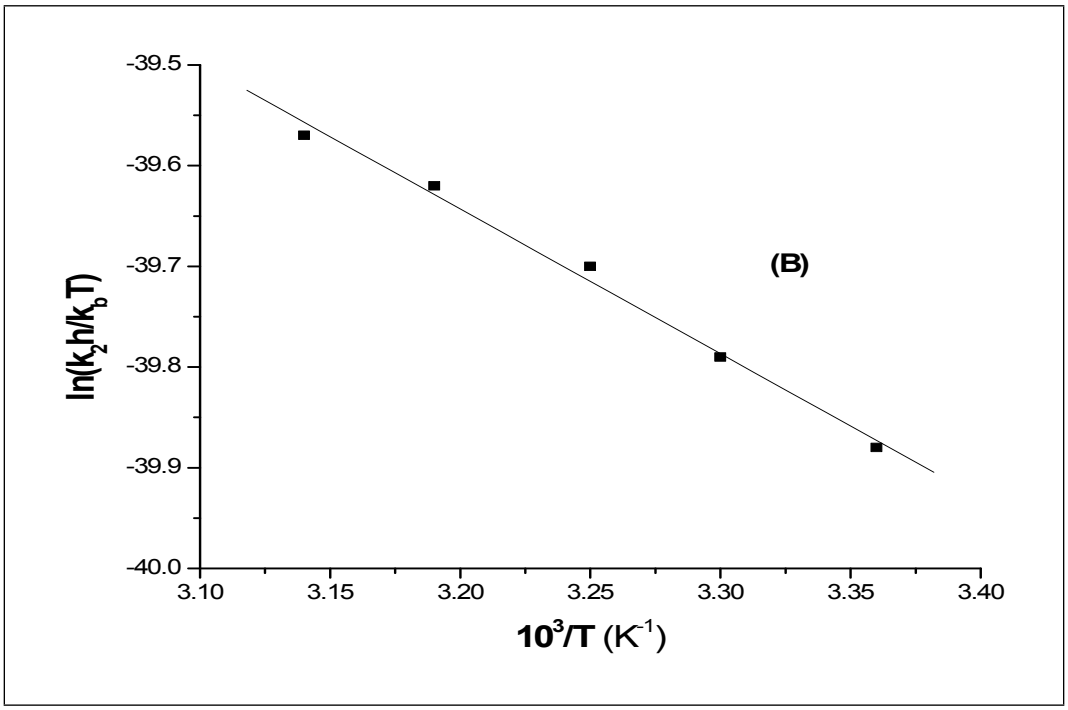

Fig S15: Eyring plot of ($\ln k_2/T$ vs. $10^3/T$) for N-Acetyl-L-Cysteine.

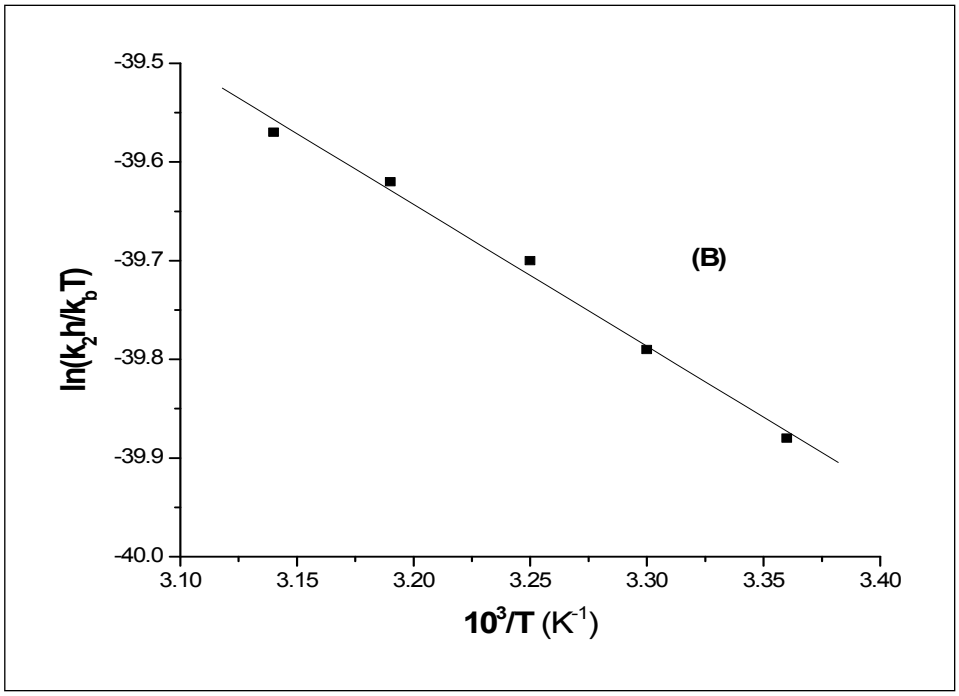

Fig S16. Eyring plot of ($\ln k_2/T$ vs. $10^3/T$) for L-Cysteine.

In vitro antibacterial assay

Test substances

The tested compounds Pt(pic) were dissolved in sterile double distilled water to achieve a concentration of 1mM.

Test microorganisms

Antibacterial activity of Pt(pic) complexes were studied on both the gram positive (Bacillus Subtilis) and gram negative (E.coli Dh5α) model organisms.

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

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

Turbidimetry 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 x 10^{9} cells/ml) (Jackie Reynolds et. al. 2005)

MTT ASSAY FOR ANTITUMORPROPERTY 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 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 cell number counts were obtained at 1-, 2-, 4-,15-, and 24-h incubation.

MTT cell viability assay

Cell viability was investigated by 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 solublelized 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(pic) 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(pic) 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 ^oC these plates were analyzed for cell viability using MTT assay. The effect of Pt(pic) 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 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(pic) 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 the media was carefully removed and 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).

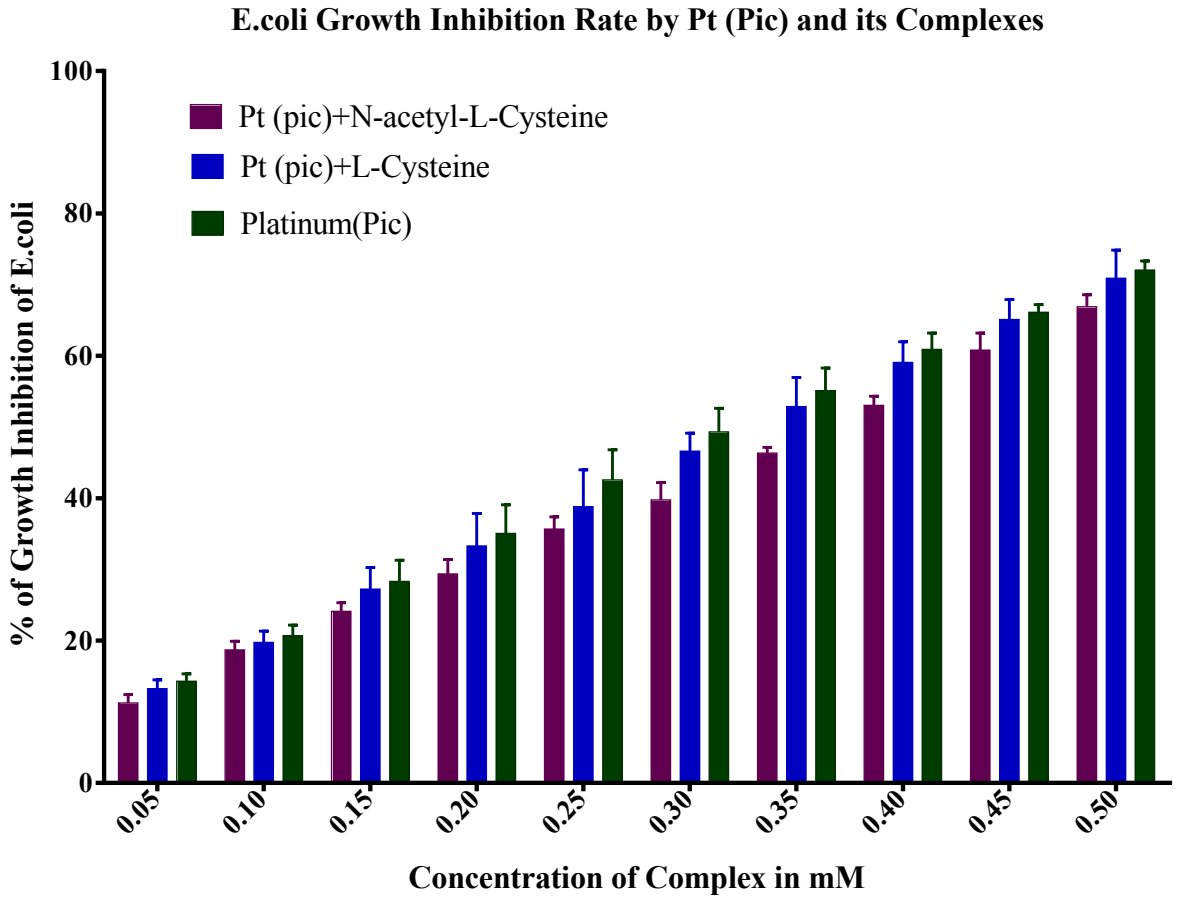

Fig. S17. % of Inhibition growth of E. coli in presence of $[Pt(pic)(H_2O)_2](ClO_4)_2$ and its L-cysteine & N-acetyl-L-cysteine substituted complexes from 0.05 µM to 0.5 µM concentration

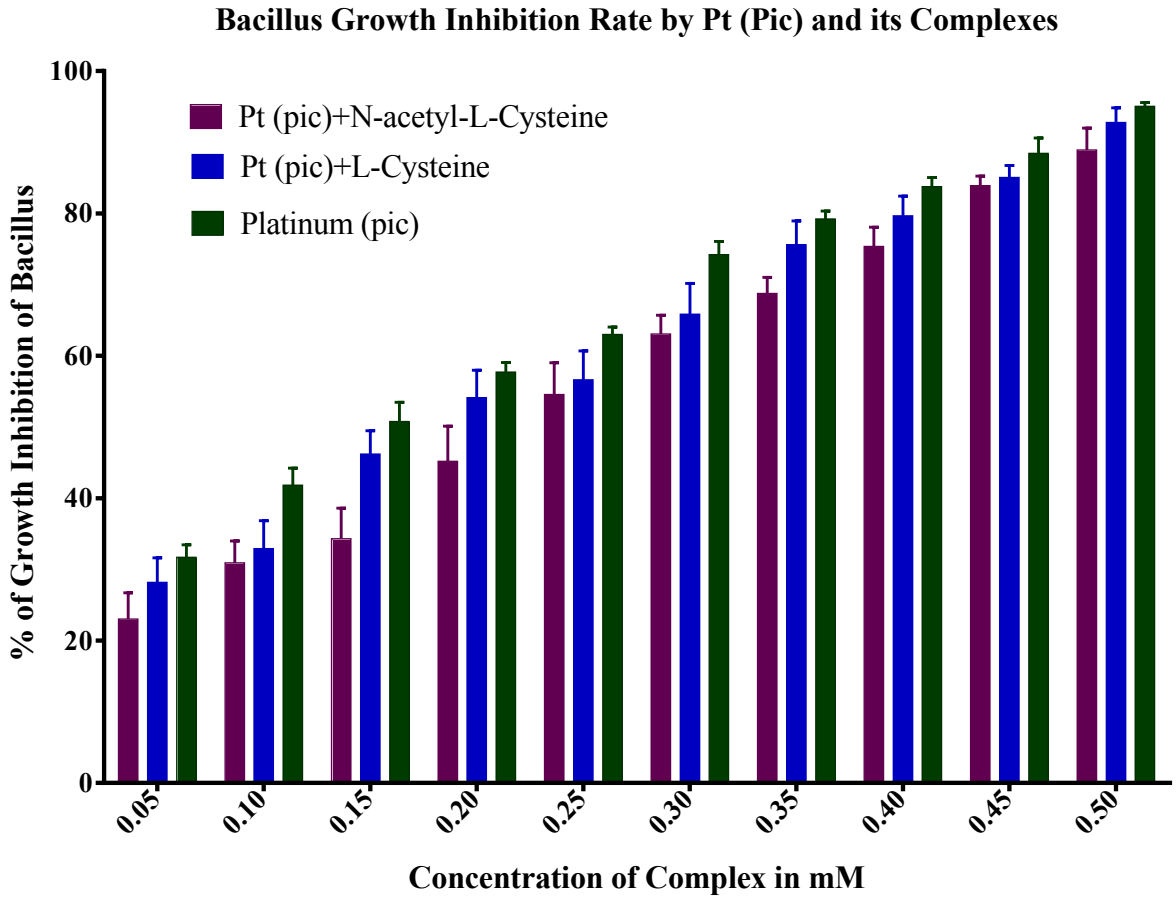

Fig. S18. % of Inhibition growth of Bacillus.subtilis in presence of $[Pt(pic)(H_2O)_2](ClO_4)_2$ and its L-cysteine & N-acetyl-L-cysteine substituted complexes from 0.05µM to 0.5 µM concentration