Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Interaction of bio-relevant thio-ether and thiols with dinuclear Pd(II) complex: Their kinetics, mechanism, bioactivity in aqueous medium and molecular docking

Koyel Misra[a], Goutam Kr. Ghosh[a], Ishani Mitra[a], Subhajit Mukerjee[a], Venkata P. Reddy[a] , Wolfgang Linert[b] , Bashkim Misini[b], Jagadeesh C Bose. K[c] , Sudit Mukhopadhyay[c] and Sankar Ch. Moi[a]*

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

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

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

*Corresponding author's *E*. mail: sankarmoi67@yahoo.com

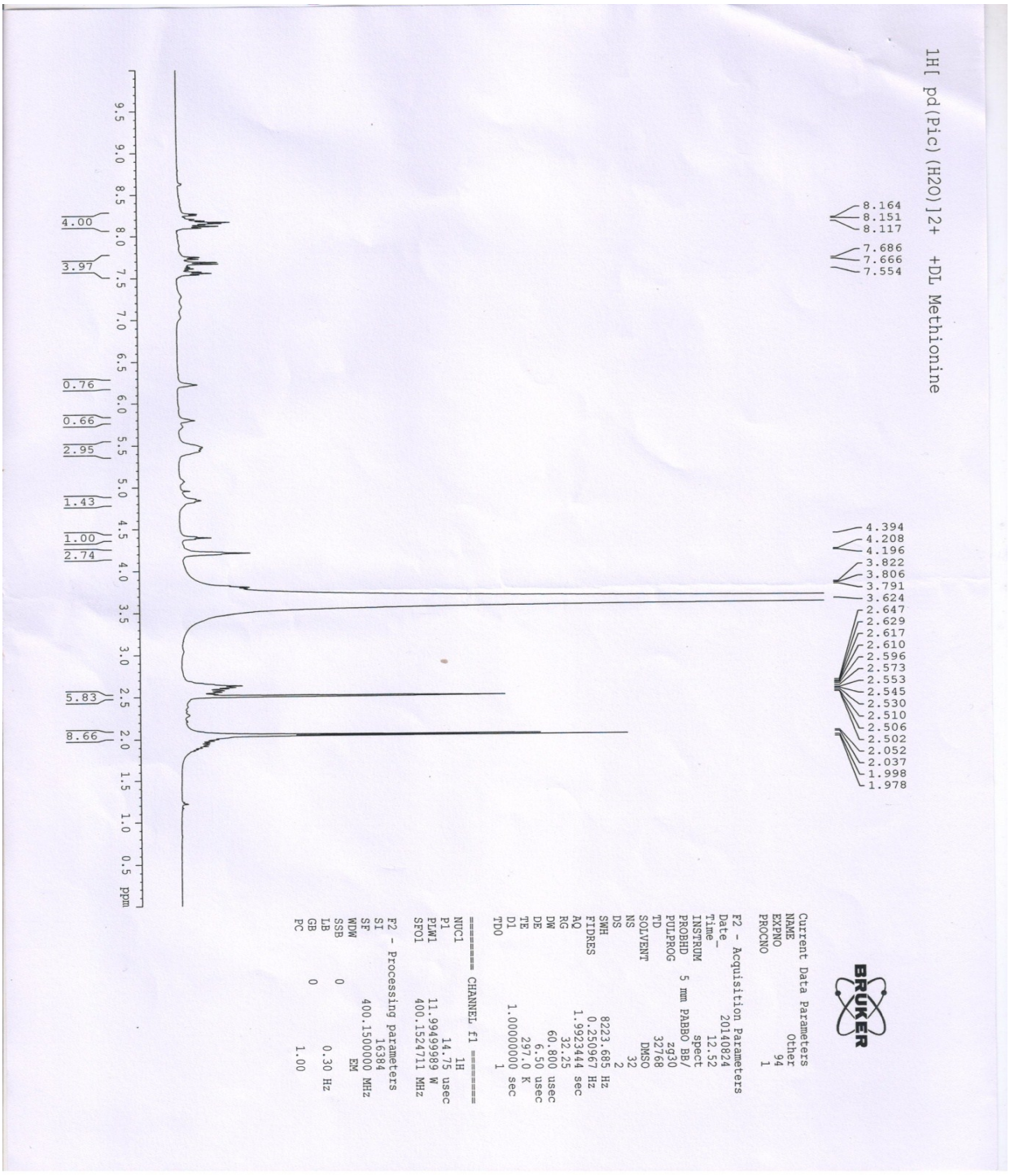
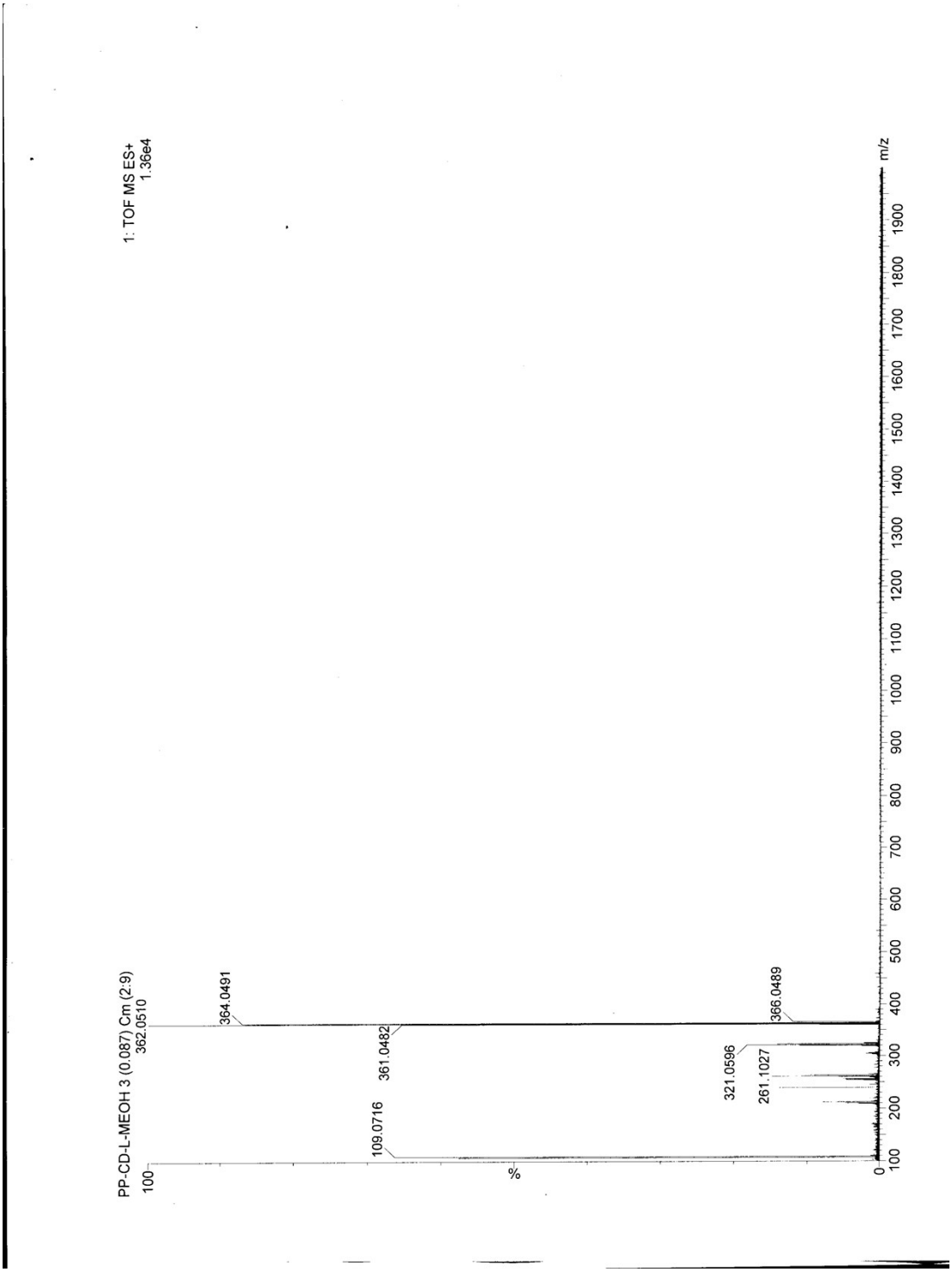


Fig. S1 : ^{1}H NMR spectra of $[Pd(pic)(H_2O)_2]^{2+}$+ DL-meth



SI Fig. S2: ESI mass spectra of complex 3 $[Pd(pic)(OH_2)_2]^{2+}$ + DL-meth-1H$^+$)

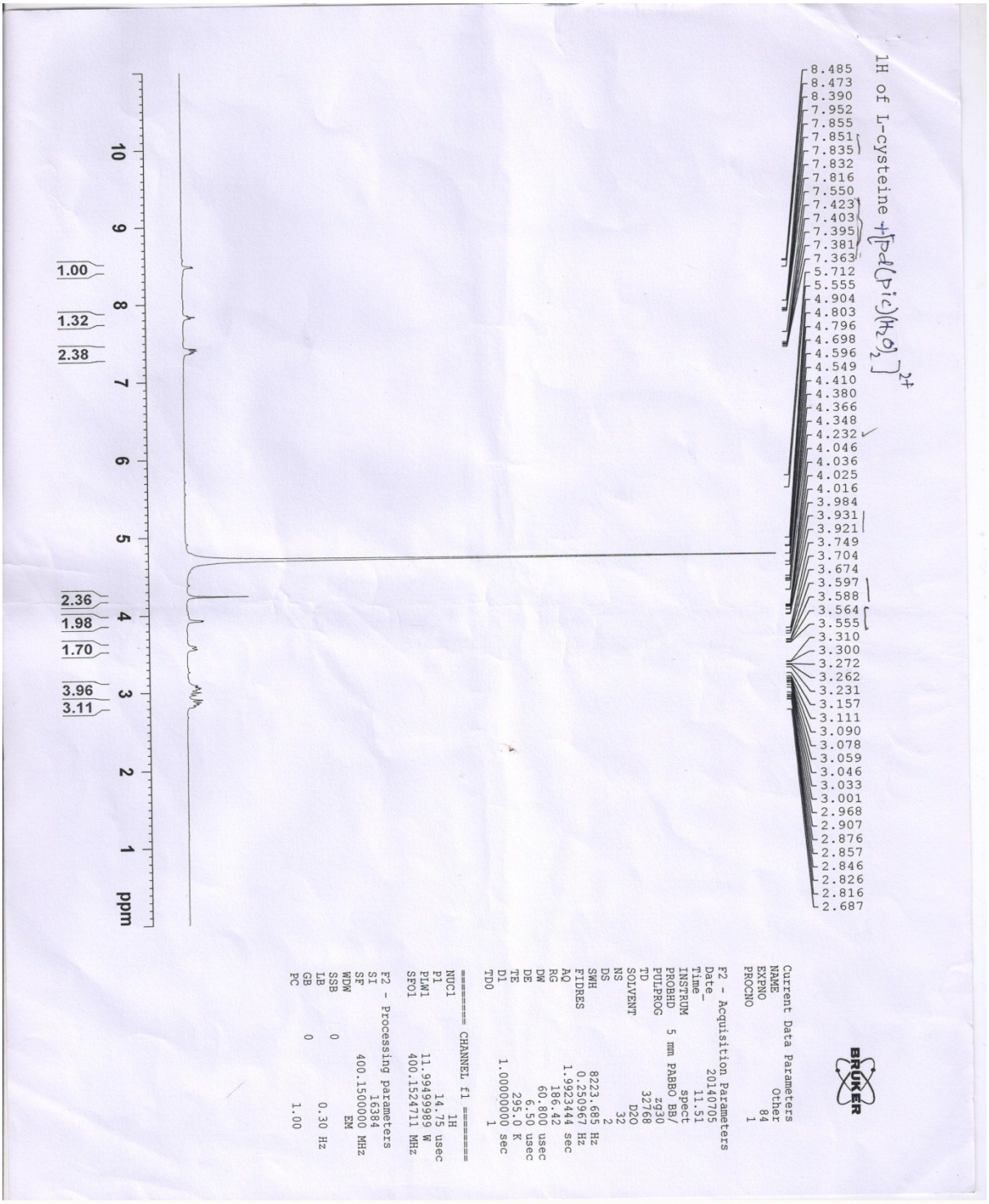


Fig. S3: ^{1}H NMR spectra of $[Pd(pic)(H_2O)_2]^{2+}$+ L-cys

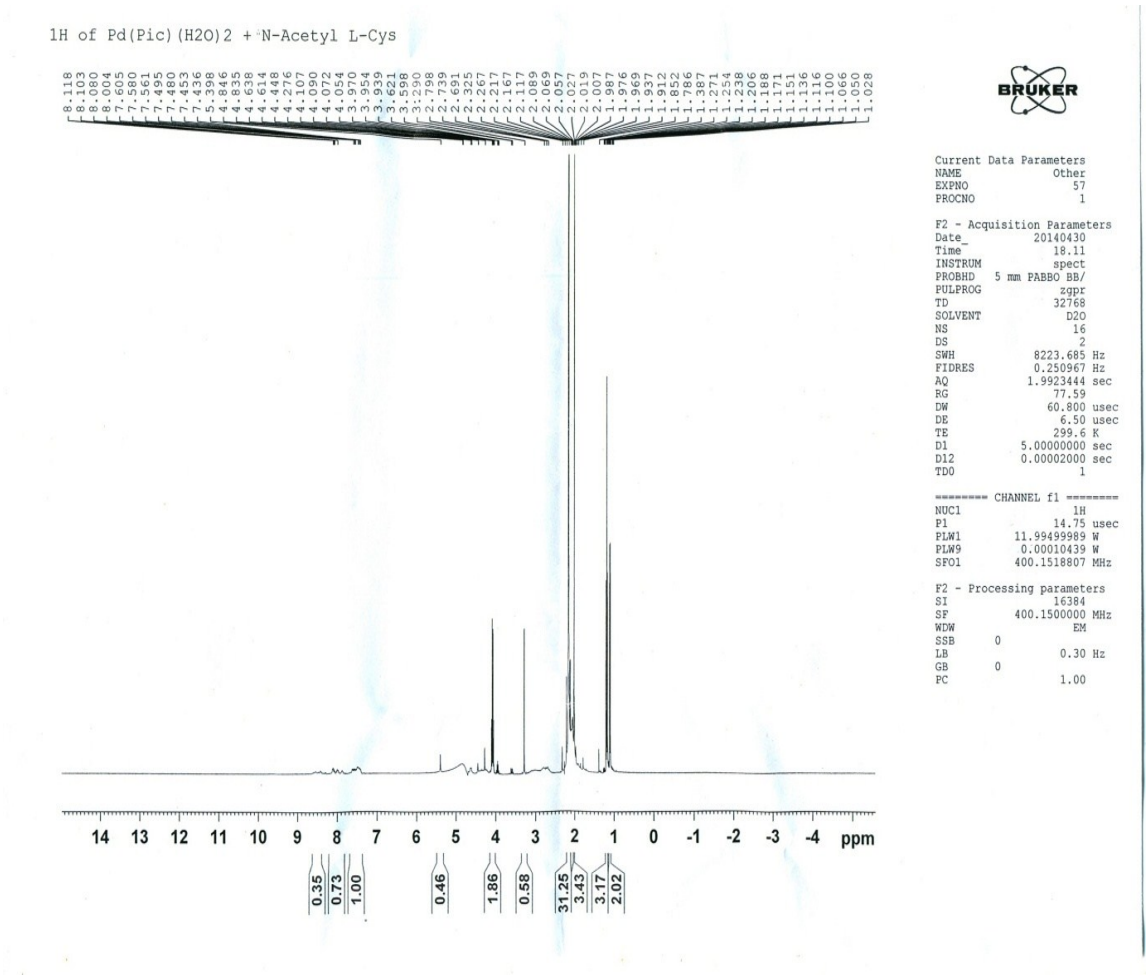


Fig. S4: ^{1}H NMR spectra of $[Pd(pic)(H_2O)_2]^{2+}$ + N-ac-L-cys

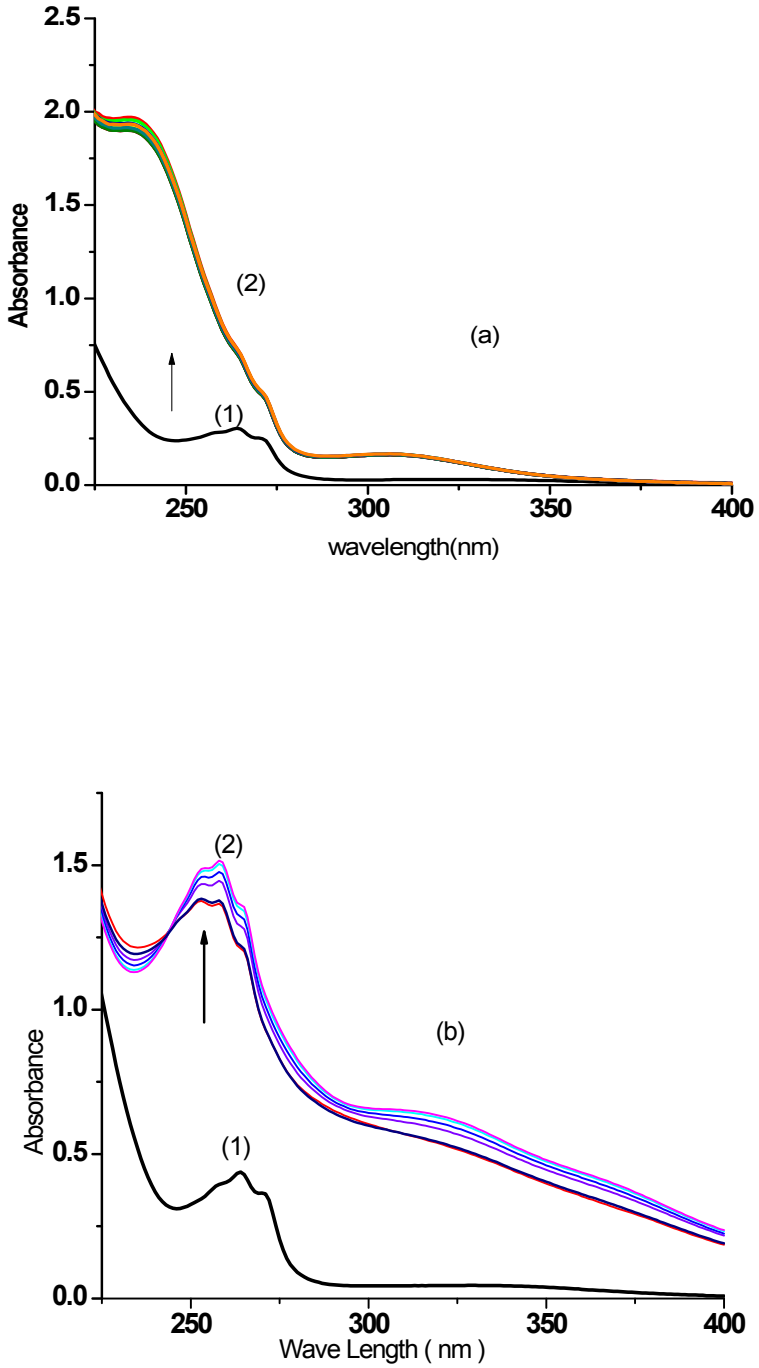


Fig. S5. Spectral difference between reactant and product (1)$[Pd_2(pic)_2(OH)_2]^{2+}$=2.62×10^{-4}M,(2)(a) $[Pd_2(pic)_2(OH)_2]^{2+}$=2.62×10^{-4}M, [DL-methionine = 2.62×10^{-3}M, (b) [N-ac-L-cysteine] = 3.93×10^{-3}M and (pH = 6.5, cell used 1cm. quartz

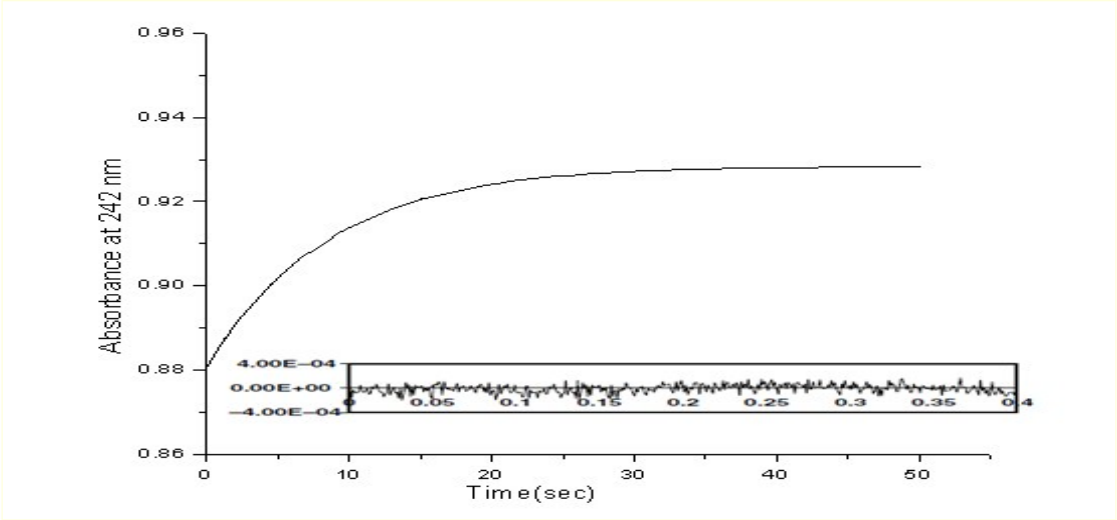


Fig.S6: Typical absorbance vs time plot at 242 nm to demonstrate the kinetic analysis for the two consecutive reactions. The upper trace represents the double exponential fitting of the experimental curve

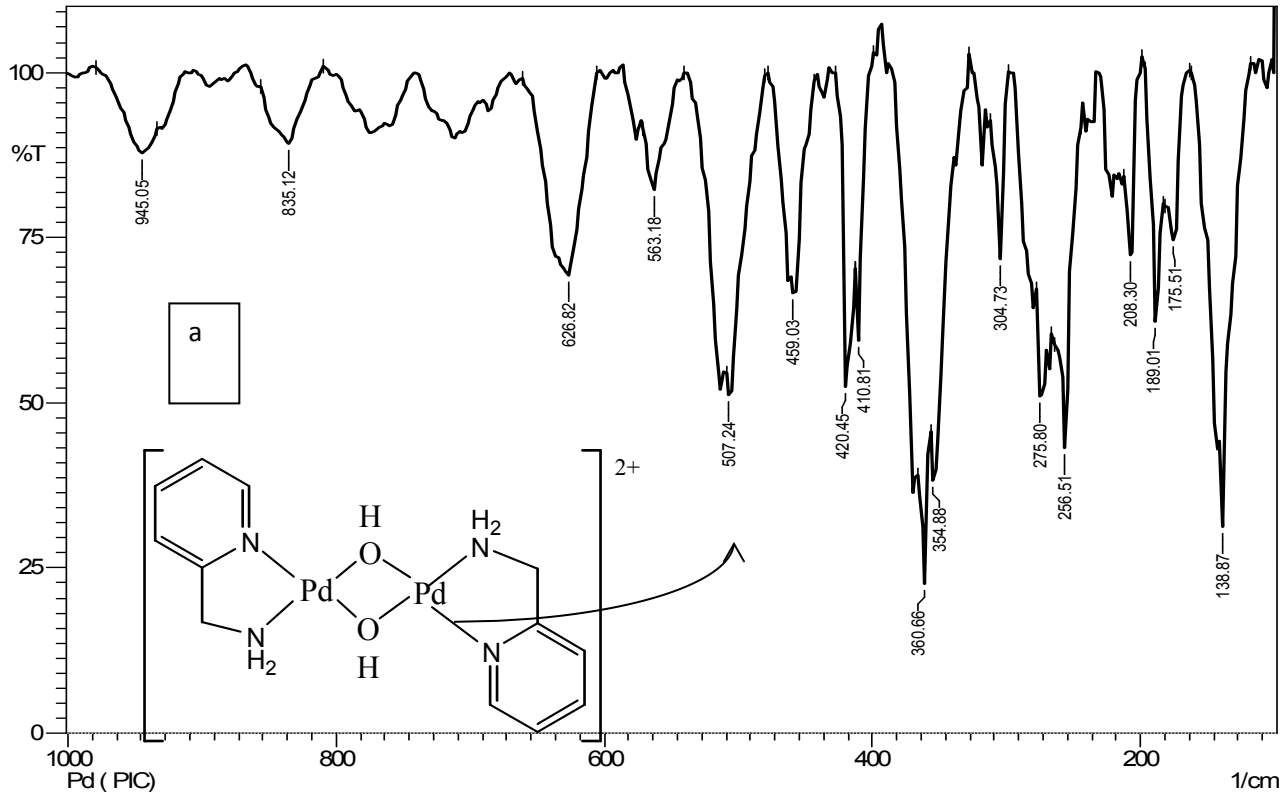


Fig. S7: IR spectra of $[Pd(pic)(OH)]_2^{2+}$ with the range of (a) 1000-100 cm^{-1}.

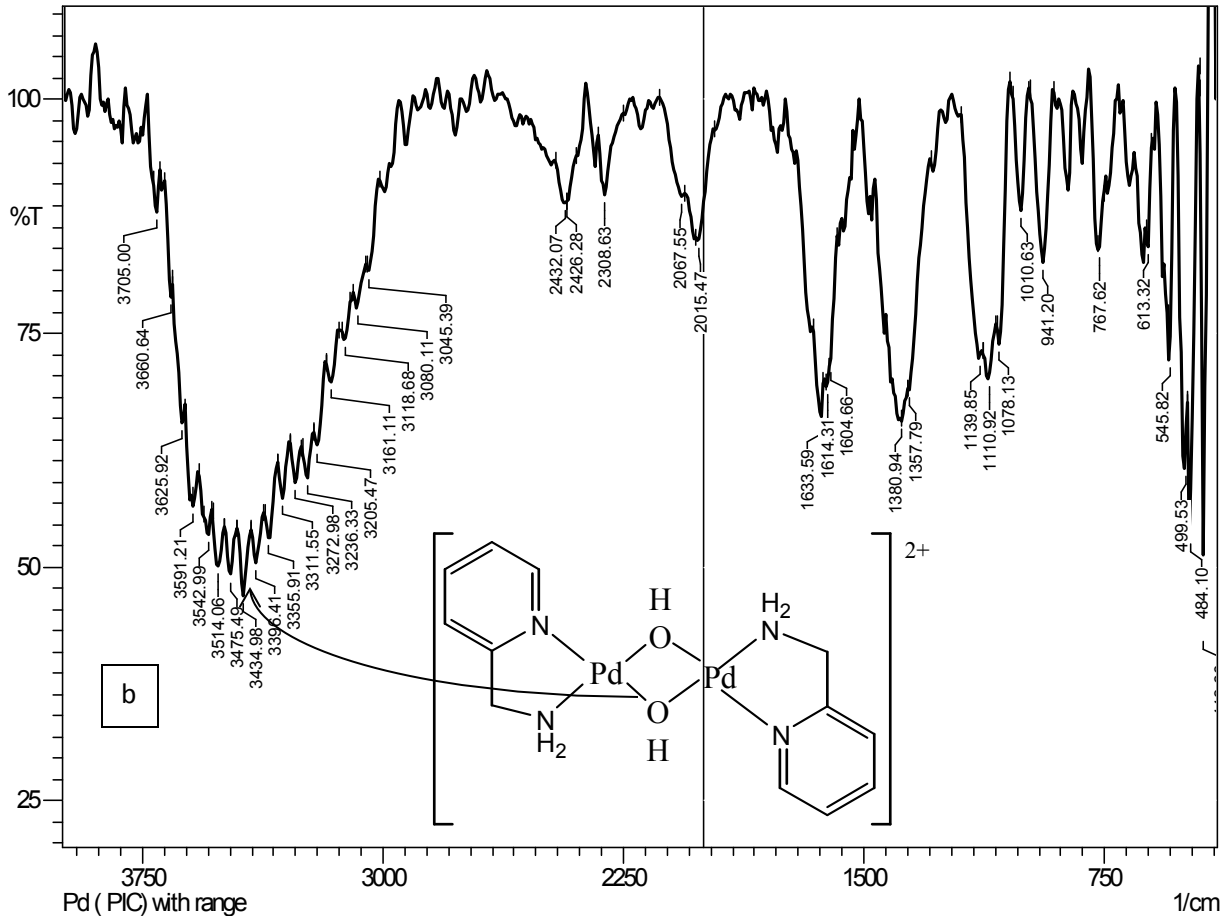


Fig. S8: IR spectra of $[Pd(pic)(OH)]_2^{2+}$ with the range of (b) 4000-400 cm^{-1}

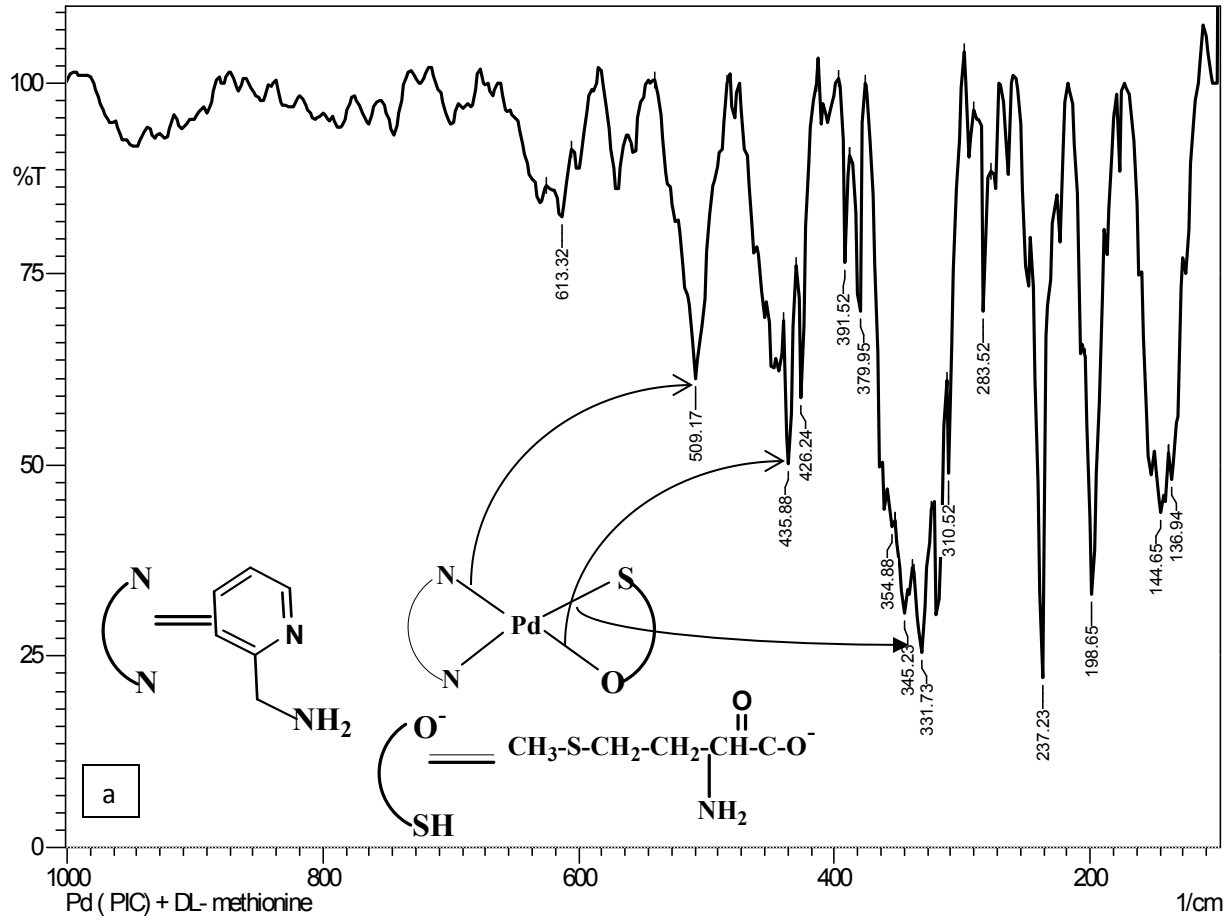


Fig. S9: IR spectra of substituted product [Pd(pic)L], where L=DL-methionine within the range of (a) 1000-100 cm^{-1}

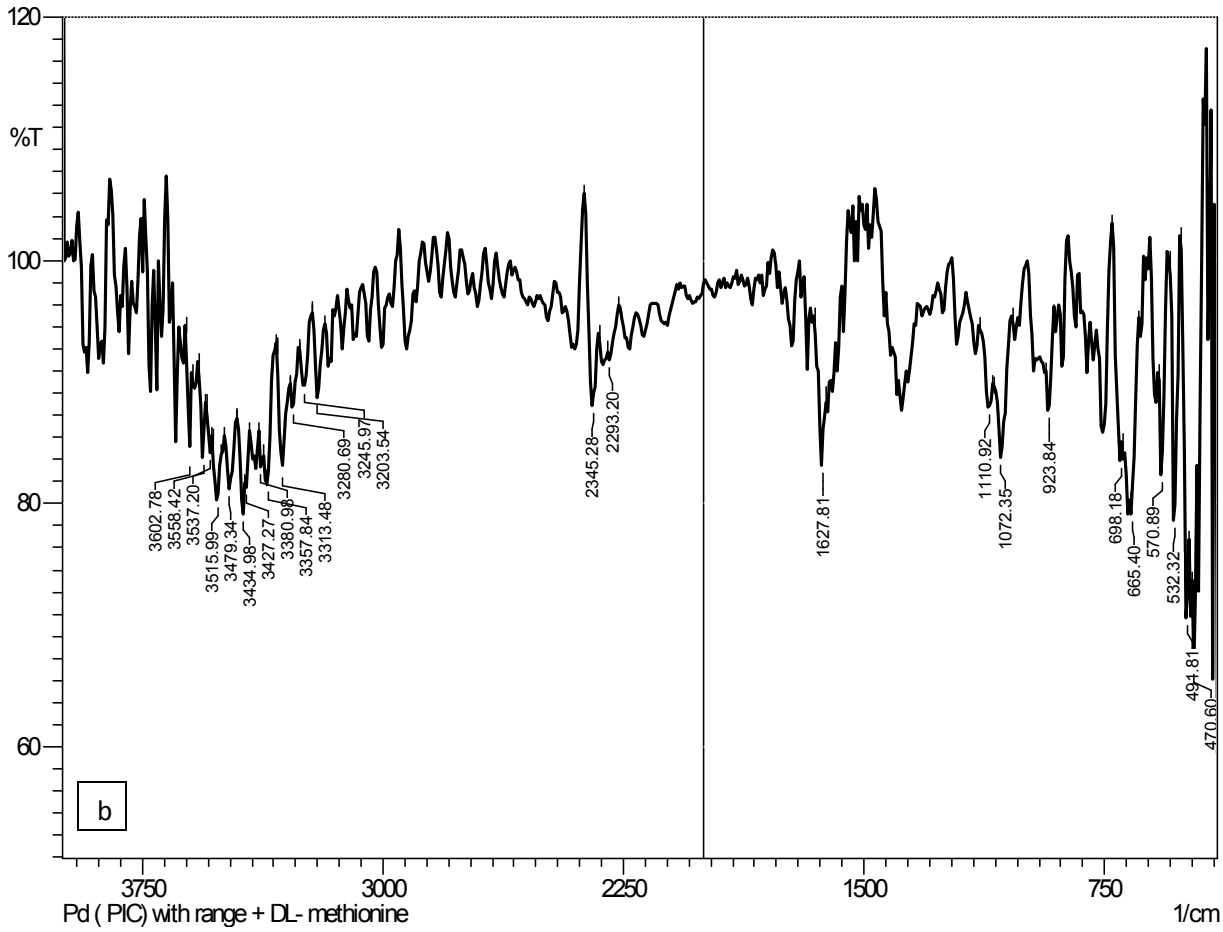


Fig. S10. IR spectra of substituted product [Pd(pic)L], where L=DL-methionine within the range of (b) 4000-400 cm^{-1}

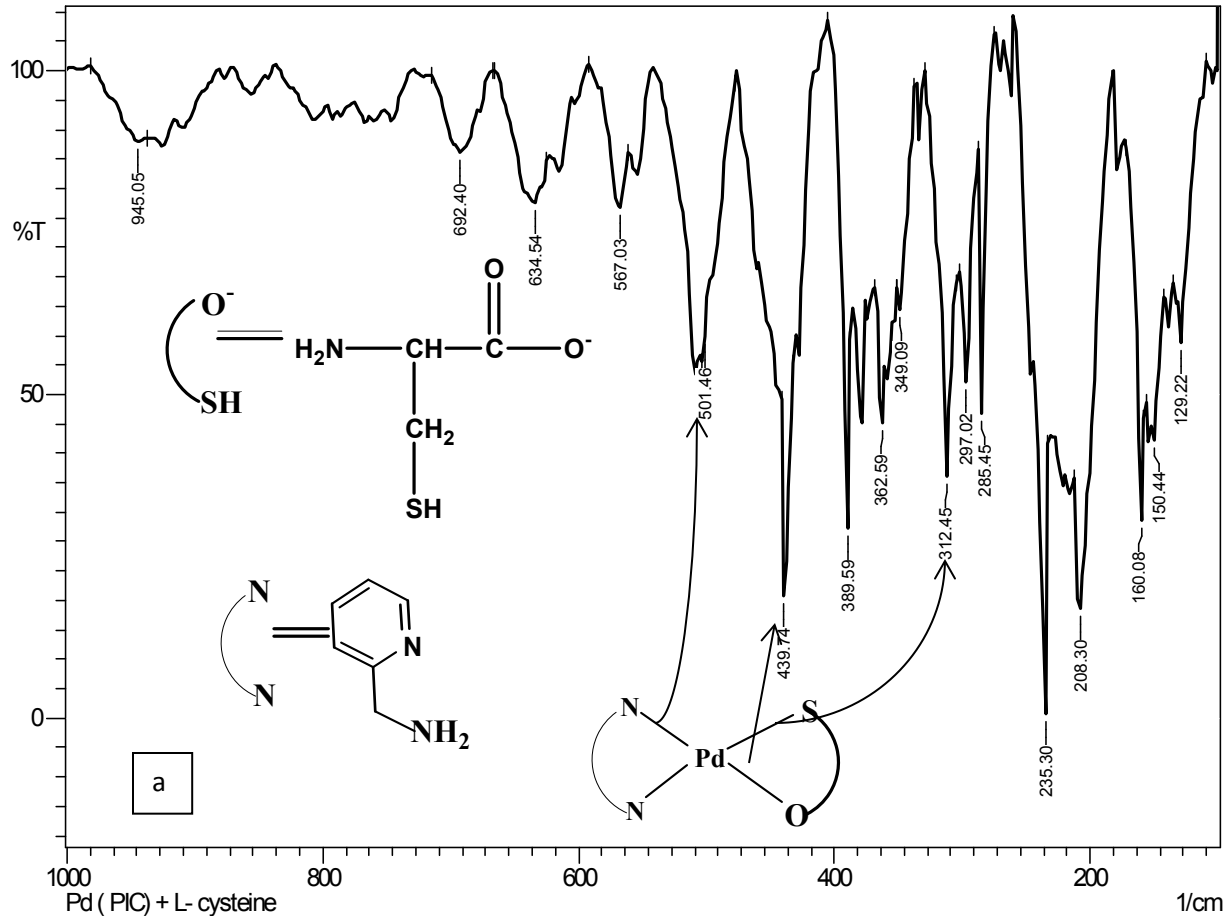


Fig. S11. IR spectra of substituted product [Pd(pic)L], where L=L-cysteine within the range of (a) 1000-100 cm^{-1}

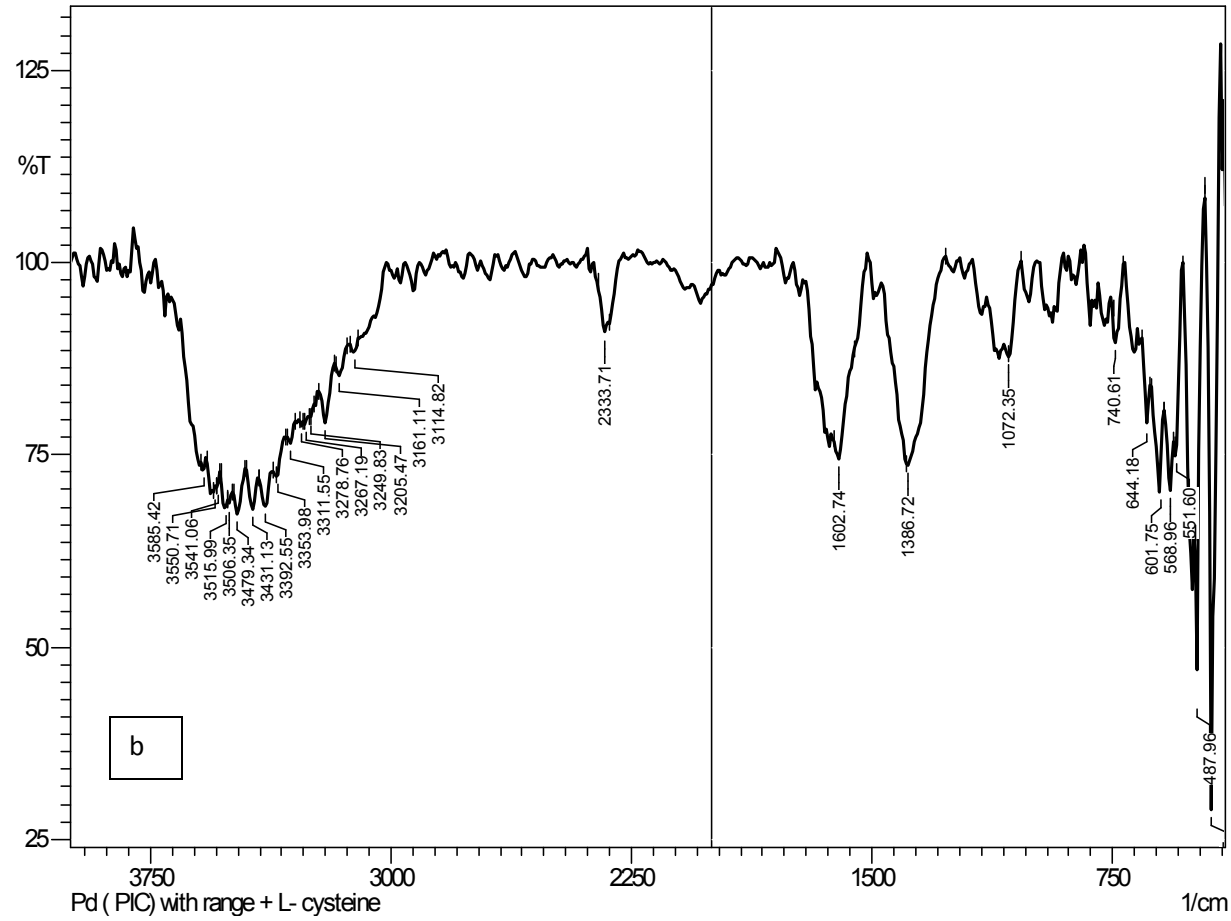


Fig. S12: IR spectra of substituted product [Pd(pic)L], where L=L-cysteine within the range of (b) 4000-400 cm^{-1}

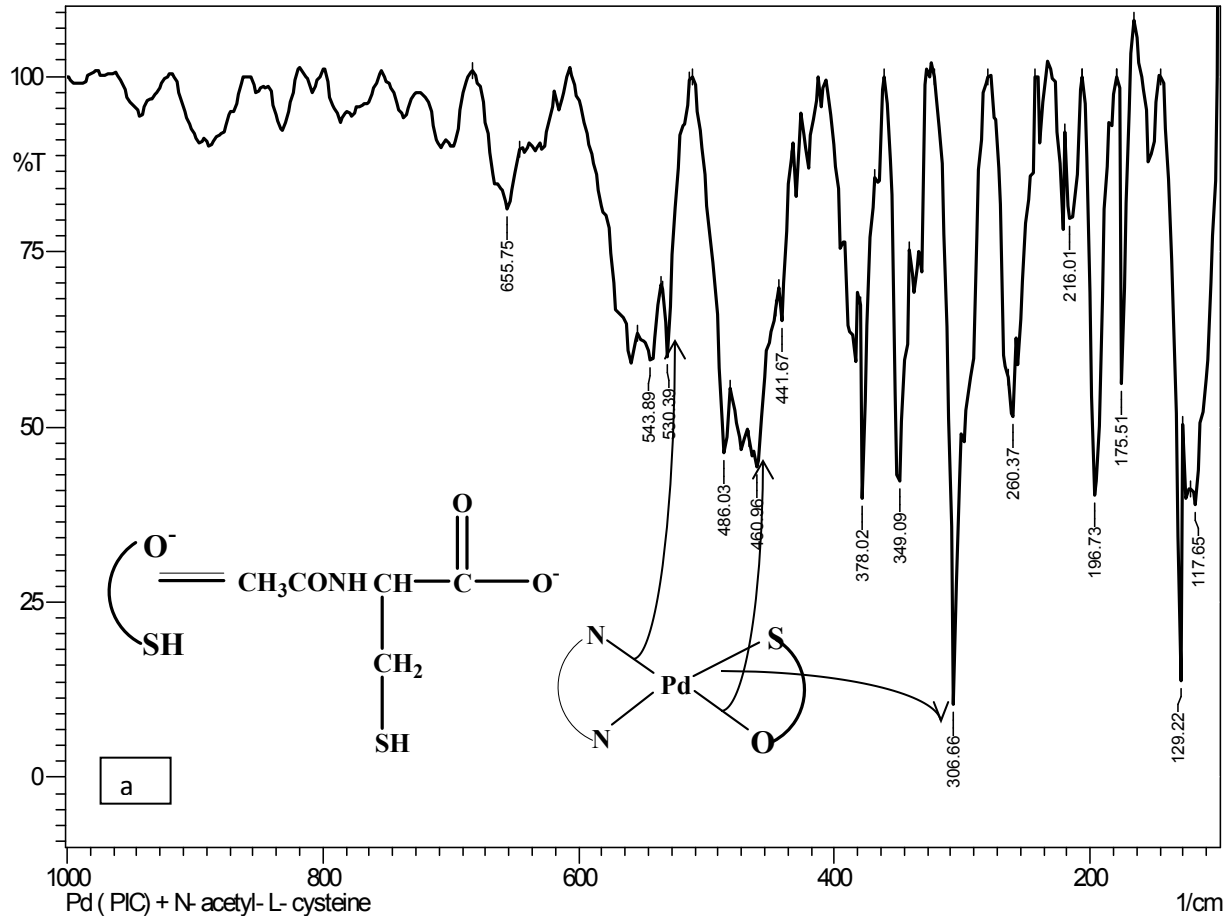


Fig. S13: IR spectra of substituted product [Pd(pic)L], where L=N-Acetyl-L-cysteine within the range of (a) 1000-100 cm^{-1}

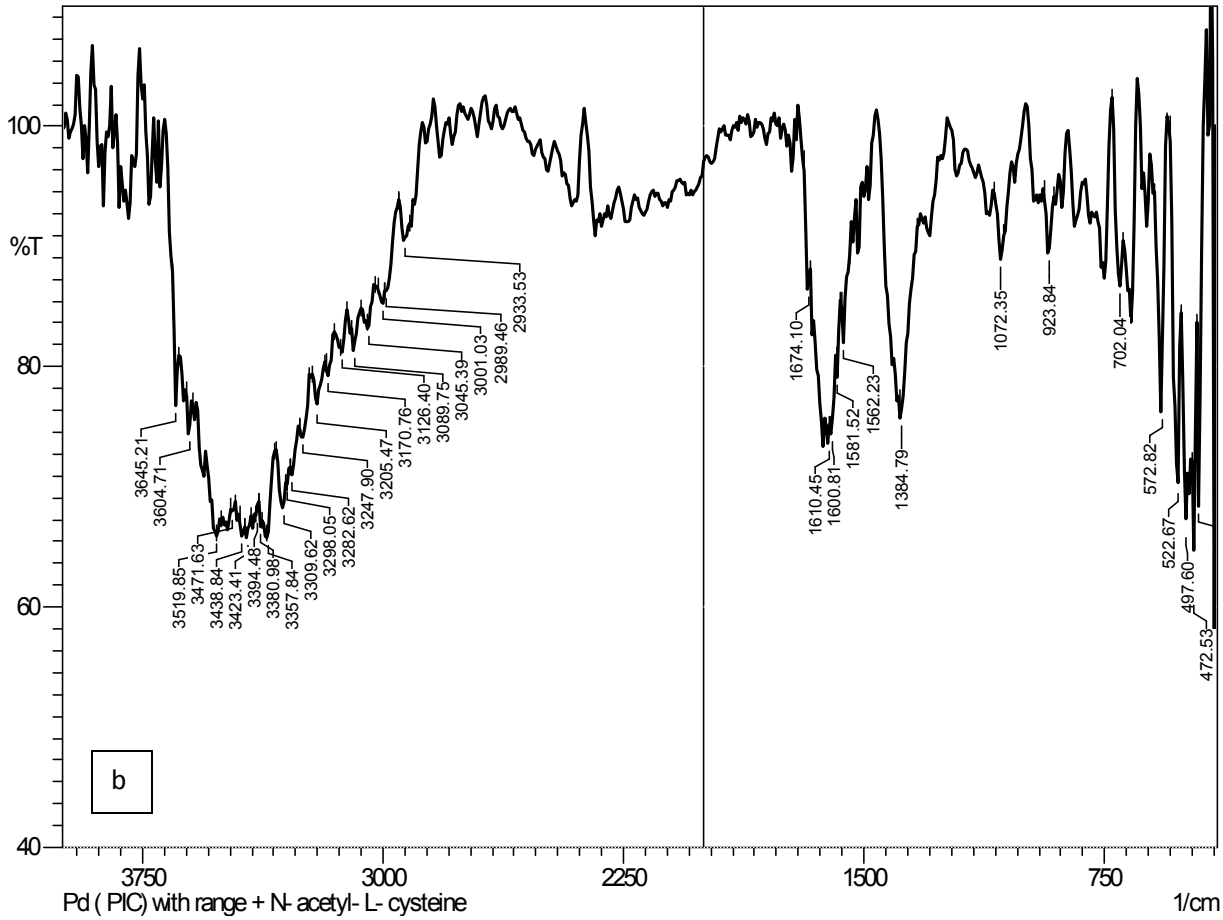


Fig. S14: IR spectra of substituted product [Pd(pic)L], where L=N-Acetyl-L-cysteine within the range of (b) 4000-400 cm^{-1}

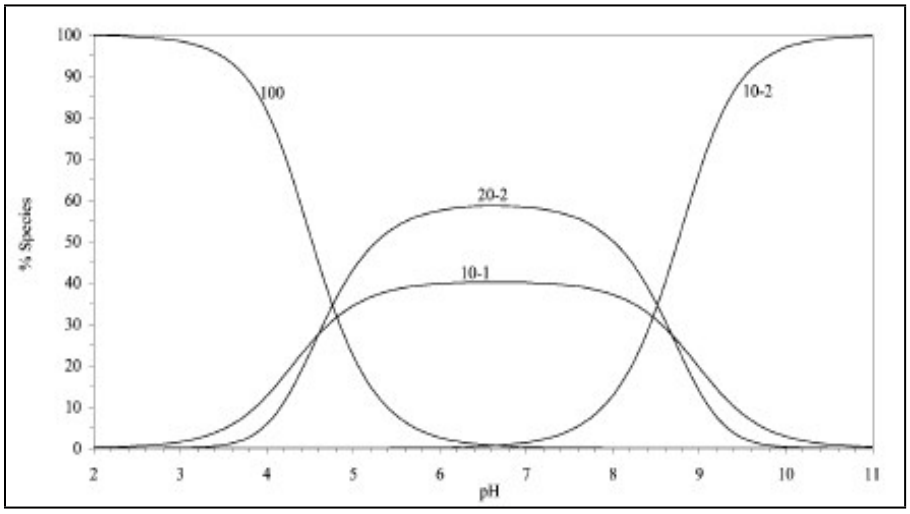


Fig. S15: Species distribution on various species as a function of pH in $[Pd(pic)(H_2O)_2]^{2+}$ system

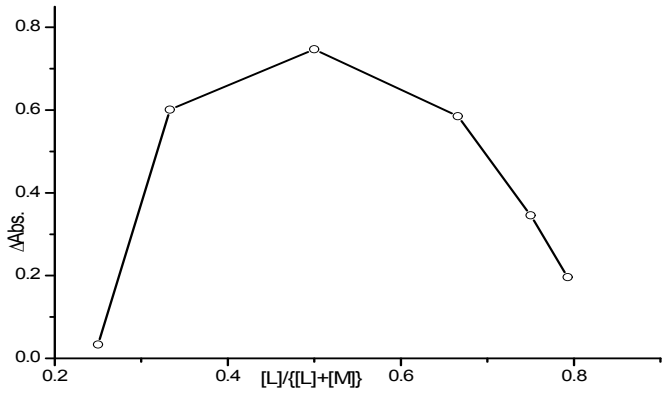


Fig. S16. Job's Plot of DL-methionine

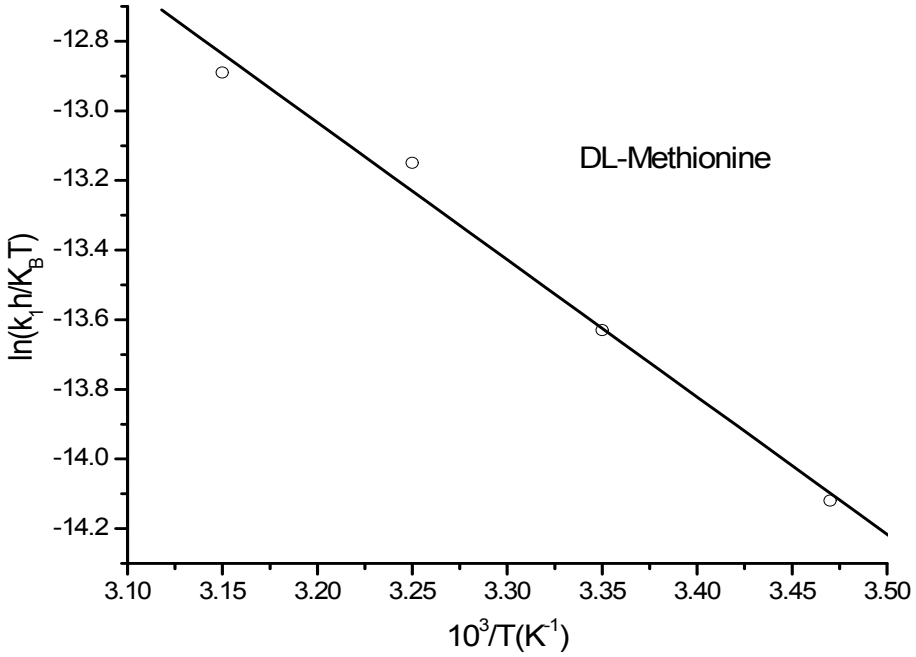


Fig. S17. Eyring plot of DL-methionine

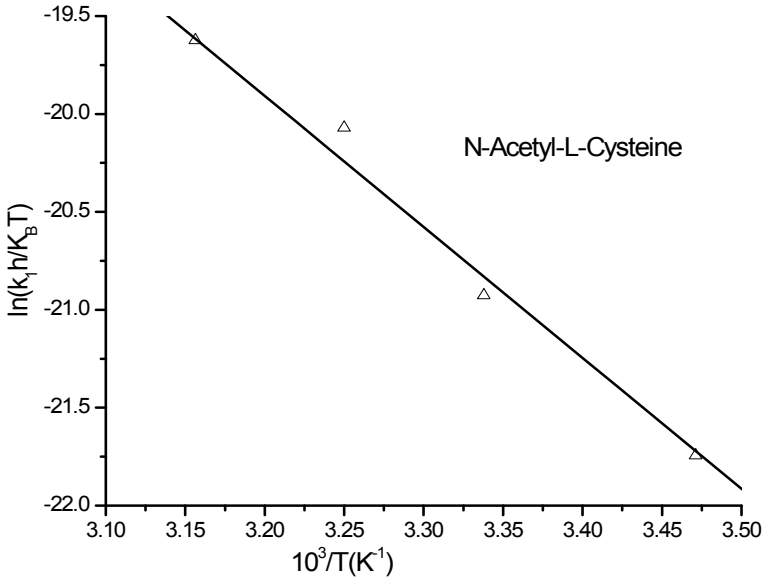


Fig. S18. Eyring plot of N-acetyl -L-cysteine

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 the 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 9ml 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˚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 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 solubilised 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 MTT assay gives an objective for quantification of cellular growth, since the formazan a blue purple colored product is only formed in viable and active cells. Practically, the growth rate for HeLa cells (Human cervical carcinoma cell) was observed using a linear regression analysis. In order to determine the cytotoxic effect of Pd(pic) complexes on HeLa cell viability, a rapid colorimetric MTT assay protocol was used. In the MTT assay, 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 absorbances 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 Pd (pic) complexes.
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 cytotoxicity assay

a) Assays were carried out in 96-well culture plates. The cells ($1x10^5$ 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^{o}C these plates were analyzed for cell viability using MTT assay. The effect of Pd (Pic) and Pt (Pic) compounds concentrations was assayed in 10 wells and within three independent experiments. The percentage of cell survival against Pd(II) 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 Pd (Pic) complex by keeping Blank and control. Cisplatin 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).

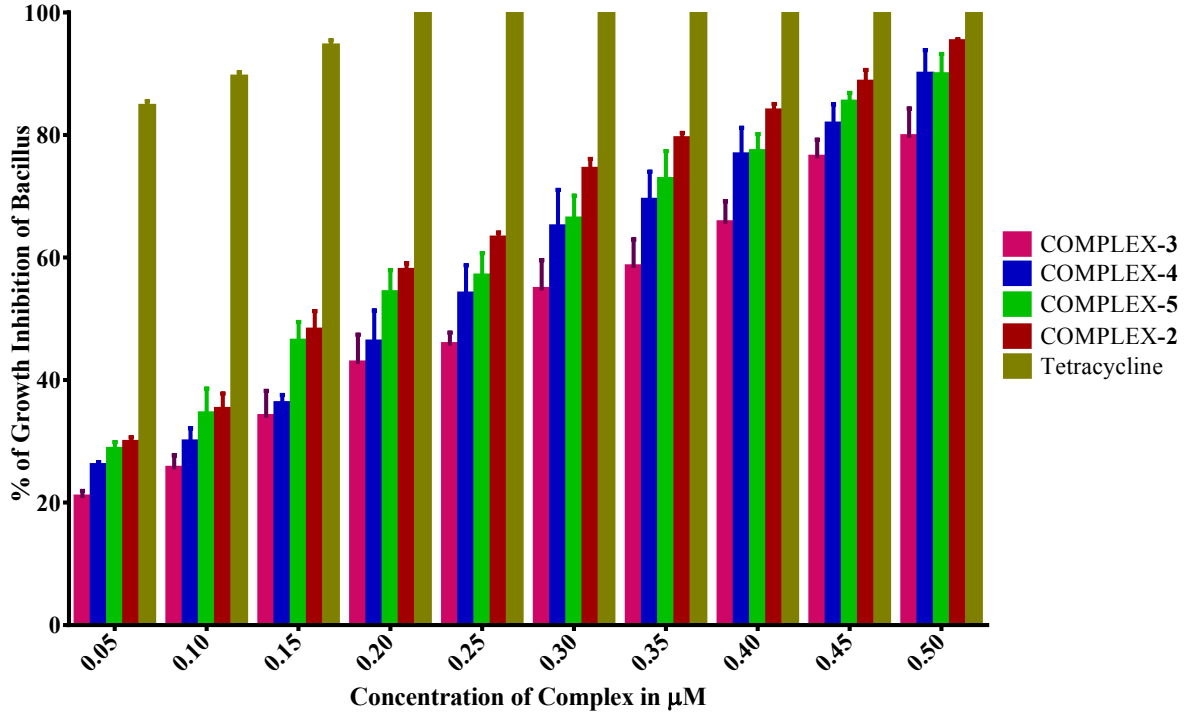


Fig. S19 % of Inhibition growth of Bacillus.subtilis in presence of $[Pd(pic)(HO)]_2(NO_3)_2$ and its DL- meth, L-cys & N-ac-L-cys substituted complexes from 0.05µM to 0.5 µM concentration

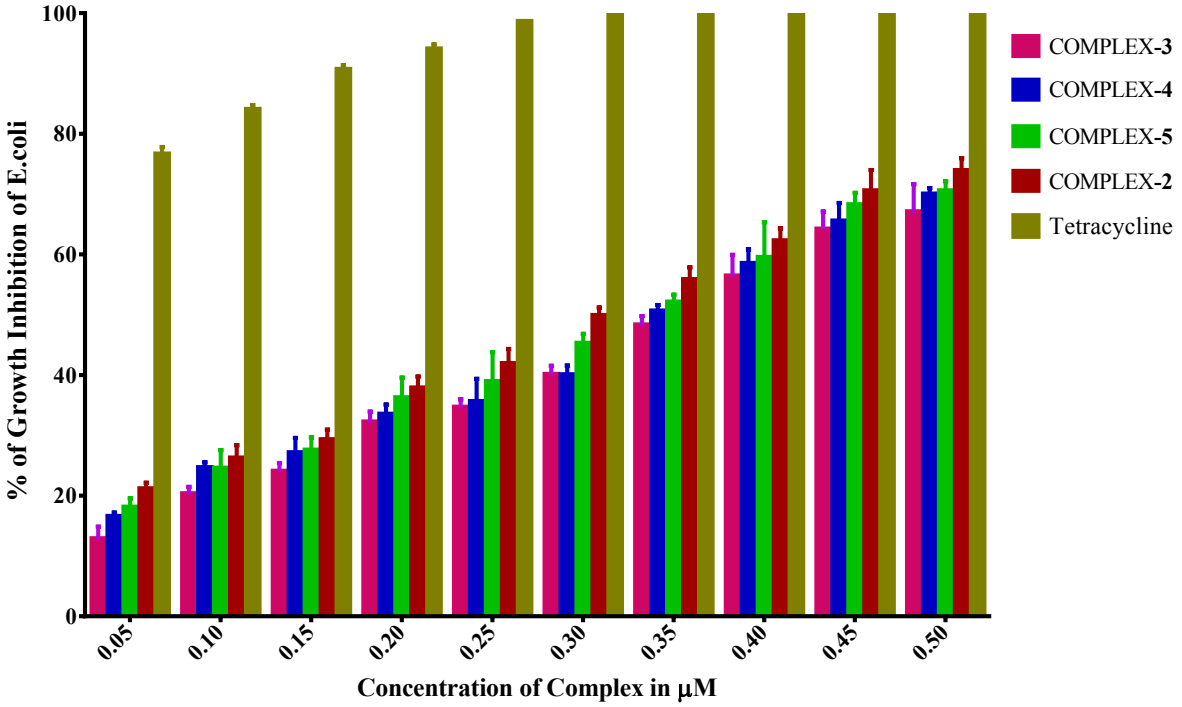


Fig. S20. % of Inhibition growth of E. coli in presence of $[Pd(pic)(HO)]_2(NO_3)_2$ and its substituted complexes from 0.05µM to 0.5 µM concentration

Table S1(A) : 10^3 $k_{1(obs)}$ (s^{-1}) values at different [DL-methionine] and at different temperatures: [complex(**2**)] = 2.62×10^{-4} mol.dm^{-3}, pH = 6.5 and ionic strength = 0.1 mol.dm^{-3} $NaClO_4$

Conc.	Temp(° C)			
	15	25	35	45
2.62×10^{-3}	6.3 ± 0.13	7.44 ± 0.19	9.12 ± 0.14	12.11 ± 0.18
3.93×10^{-3}	7.9 ± 0.12	11.7 ± 0.16	14.32 ± 0.18	17.67 ± 0.22
5.24×10^{-3}	9.5 ± 0.14	16.11 ± 0.19	17.16 ± 0.14	22.5 ± 0.18
6.55×10^{-3}	11.7 ± 0.14	19.01 ± 0.15	22.41 ± 0.19	26.44 ± 0.22
7.86×10^{-3}	15.5 ± 0.13	21.21 ± 0.18	26.22 ± 0.22	33.33 ± 0.23
13.1×10^{-3}	18.20 ± 0.13	23.78 ± 0.18	28.56 ± 0.21	39.66 ± 0.22

Table S1(B): 10^4 $k_{2(obs)}$ (s^{-1}) values at different [DL-methionine] and at different temperatures: [complex(**2**)] = 2.62×10^{-4} mol.dm^{-3}, pH = 6.5 and ionic strength = 0.1 mol.dm^{-3} $NaClO_4$

Conc.	Temp(º C)			
	15	25	35	45
2.62×10^{-3}	7.90 ± 0.11	10.81± 0.17	15.15 ± 0.15	17.82 ± 0.18
3.93×10^{-3}	8.10 ± 0.12	10.62 ± 0.16	14.92 ± 0.15	17.67 ± 0.17
5.24×10^{-3}	8.06 ± 0.11	11.11 ± 0.16	15.76 ± 0.14	18.42 ± 0.18
6.55×10^{-3}	7.80 ± 0.14	10.83 ± 0.14	15.43 ± 0.16	18.24 ± 0.15
7.86×10^{-3}	8.10 ± 0.15	11.21 ± 0.17	15.37 ± 0.15	18.16 ± 0.17
13.1×10^{-3}	8.20 ± 0.13	11.14 ± 0.18	15.14 ± 0.18	18.25 ± 0.17

Table S2(A): $k_{1(obs)}$ (s^{-1}) values at different [L-cysteine] and at different temperatures: [complex(**2**)] = 2.62×10^{-4} mol.dm^{-3}, pH = 6.5 and ionic strength = 0.1 mol.dm^{-3} $NaClO_4$

Conc.	Temp(º C)			
	15	25	35	45
2.62×10^{-3}	0.117 ± 0.02	0.18 ± 0.02	0.596 ± 0.03	0.688 ± 0.02
3.93×10^{-3}	0.225 ± 0.02	0.467 ± 0.01	0.675 ± 0.02	0.829 ± 0.03
5.24×10^{-3}	0.297 ± 0.02	0.562 ± 0.01	0.804 ± 0.02	0.972 ± 0.03
6.55×10^{-3}	0.379 ± 0.02	0.705 ± 0.02	0.942 ± 0.03	1.14 ± 0.02
7.86×10^{-3}	0.509 ± 0.02	0.832 ± 0.01	1.08 ± 0.02	1.33 ± 0.03
13.1×10^{-3}	1.35 ± 0.02	1.70 ± 0.01	1.87 ± 0.03	2.34 ± 0.02

Table S2(B): 10^4 $k_{2(obs)}$ (s^{-1}) values at different [L-cysteine] and at different temperatures: [complex(2)] = 2.62×10^{-4} mol.dm^{-3}, pH = 6.5 and ionic strength = 0.1 mol.dm^{-3} $NaClO_4$

Conc.	Temp(° C)			
	15	25	35	45
2.62×10^{-3}	12.40 ± 0.21	15.01 ± 0.20	18.20 ± 0.24	19.01 ± 0.21
3.93×10^{-3}	12.80 ± 0.22	15,63 ± 0.19	18.10 ± 0.23	19.62 ± 0.22
5.24×10^{-3}	13.11 ± 0.21	15.98 ± 0.19	18.93 ± 0.24	19.98 ± 0.21
6.55×10^{-3}	13.60 ± 0.22	16.40 ± 0.21	19.10 ± 0.24	19.52 ± 0.20
7.86×10^{-3}	13.87 ± 0.22	16.56 ± 0.17	19.23 ± 0.24	20.14 ± 0.24
13.1×10^{-3}	13.12 ± 0.22	16.24 ± 0.19	19.20 ± 0.22	19.86 ± 0.25

Table S3(A): 10^3 $k_{1(obs)}$ (s^{-1}) values at different [N-ac- L-cys] and at different temperatures: [complex(2)] = 2.62×10^{-4} mol.dm^{-3}, pH = 6.5 and ionic strength = 0.1 mol.dm^{-3} $NaClO_4$

Conc.	Temp(° C)			
	15	25	35	45
2.62×10^{-3}	2.6 ± 0.16	5.6 ± 0.18	10.01 ± 0.19	13.01 ± 0.17
3.93×10^{-3}	3.0 ± 0.18	4.26 ± 0.15	11.02 ± 0.11	16.11 ± 0.18
5.24×10^{-3}	3.7 ± 0.17	6.7 ± 0.19	12.8 ± 0.21	18.12 ± 0.23
6.55×10^{-3}	4.8 ± 0.18	7.2 ± 0.16	14.01 ± 0.22	20.7 ± 0.22
7.86×10^{-3}	6.7 ± 0.19	10.01 ± 0.13	17.11 ± 0.24	24.11 ± 0.21
13.1×10^{-3}	8.21 ± 0.13	12.87 ± 0.19	19.56 ± 0.21	30.12 ± 0.25

Table S3(B): $10^4\ k_{2(obs)}\ (s^{-1})$ values at different [N-ac- L-cys] and at different temperatures: [complex(2)] = 2.62×10^{-4} mol.dm^{-3}, pH = 6.5 and ionic strength = 0.1 mol.dm^{-3} $NaClO_4$

Conc.	Temp(° C)			
	15	25	35	45
2.62×10^{-3}	8.01 ± 0.18	8.51 ± 0.16	8.90 ± 0.15	9.17 ± 0.18
3.93×10^{-3}	8.56 ± 0.18	9.24 ± 0.16	8.57 ± 0.18	9.56 ± 0.18
5.24×10^{-3}	8.90 ± 0.14	9.40 ± 0.17	9.60 ± 0.17	9.70 ± 0.17
6.55×10^{-3}	8.84 ± 0.17	9.62 ± 0.16	9.52 ± 0.16	9.98 ± 0.18
7.86×10^{-3}	9.30 ± 0.17	9.70 ± 0.17	10.10 ± 0.16	10.20 ± 0.17
13.1×10^{-3}	9.21 ± 0.15	9.86 ± 0.14	10.22 ± 0.16	10.32 ± 0.18

Table S4. Analogous Activation parameters

System	$\Delta H_1^{\ddagger}$ (kJmol^{-1})	$\Delta S_1^{\ddagger}$ (JKmol^{-1})	$\Delta H_2^{\ddagger}$(kJmol^{-1})	$\Delta S_2^{\ddagger}$ (JK^{-}mol^{-1})	Ref.
$[Pd(pic)(OH)]_2^{2+}$ /					This work
DL-methionine	32.75 ±1.98	-45.51 ± 6.22	4.73 ± 1.10	-205.65 ± 5.21	"
/L-cysteine	21.94 ± 1.51	-89.19 ± 5.1	5.40 ± 1.10	-195.44 ± 8.22	,,
/N-Ace. L-cyst	35.53 ± 2.62	-54.52 ± 3.6	1.66 ± 0.56	-197.31± 5.26	
$[Pd_2(phen)_2(OH)_2]^{2+}$/					[1]
DL-methionine	73.16 ± 2.5	-93.03 ±4.5	66.5 ± 3.2	-63.01±4.2	
$[Pd_2(bipy)_2(OH)_2]^{2+}$/					[2]
Thiourea	24.2±1.6	-210 ± 5.0	7.5 ± 1.0	-306 ± 6.0	
$[Pd(Me_4dien)(OH_2)_2]^{2+}$ /					
Cl^-	---	---	40.0	-49.0	[3]
Br^-	---	---	39.0	-47.0	
I^-	---	---	34.0	-55.0	
$[Pd(Et_4dien(OH_2)_2]^{2+}$/					

Cl^-	---	---	56.0	-45.0	
HCO_3^-	---	---	68.0	-32.0	[4]
$[Pd(OH_2)_4]^{2+}$ /					
Me_2SO	----	---	58.0	-44.0	
Water exchange	----	----	49.0	-26.0	[5]
$[Pd(hzpy)(H_2O)_2]^{2+}$/					
5'-AMP	32 ± 2	-168 ± 7	28 ± 1	-126 ± 5	[6]

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