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Supporting Information

A Safe and Efficacious Pt(II) Anticancer Prodrug: Design, Synthesis, In-vitro Efficacy, Role of Carrier Ligand and In-vivo Tumour Growth Inhibition

Pradip Dutta[1‡], Rupali Sharma[1‡], Smita Kumari[1‡], Ravindra Dhar Dubey[1], Sujit Sarkar[1], Justin Paulraj[1], Gonela Vijaykumar[3], Manoj Pandey[1], L. Sravanti[2], Mallik Samarla[1], Harisankar Das[3], Yashpal[1], Heeralal B[1], Ravinder Goyal[1], Nimish Gupta[1,2], Swadhin K. Mandal[3], Aniruddha Sengupta[1,2*], Arindam Sarkar[1,2*]

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1. EXPERIMENTAL

a. General:

All reagents were procured from Sigma or Spectrochem and were used without further purification unless otherwise stated. All the reactions were carried out with dried and freshly distilled solvents under anhydrous atmosphere. Starting material (dichloro)cyclohexyldiammine platinum was prepared from K_2PtCl_4 according to the literature procedure.[1] (1R, 2R)-1,2 Diaminocyclohexane was purchased from sigma and K_2PtCl_4 was purchased from Arora Matthey. L-Glutathione reduced (GSH) purchased from Sigam-Aldrich and Guanosine 5'-monophosphate disodium salt hydrate (5'-GMP) was pursued from HIMEDIA. For melting point determination, Buchi B-540 melting point apparatus was used. ^{1}H, ^{13}C, ^{195}Pt NMR were recorded in Bruker Avance III 500 MHz or JEOL 400 MHz spectrometer. ^{195}Pt NMR was recorded with K_2PtCl_4 as external reference (dissolved in D_2O and the reference value was set at -1616 ppm). HRMS was obtained by Agilent QTof instrument. IR spectra were obtained by Perkin Elmer FT-IR Spectrometer Spectrum RXI. The data of crystals were collected at 100.00(10) K on SuperNova, Dual, Cu at zero, Eos diffractometer. Liposome was prepared using thin film hydration method.[2,3]

Particle size and zeta potential were measured using a Dynamic Light Scattering method using Zetasizer Nano ZS90 (Malvern, UK). Pt equivalents in drug concentrations were quantified using atomic absorption spectrometer (AAS) (PinAAcle 900Z, US). For the stability and reactivity study experiments of 1 and 2, analytical HPLC was carried out using a Waters e2695 system with PDA (2998) and fluorescence (2475) detector inline . Equisil C8 (250 mm x 4.6 mm, 5 µm; Dr. Maisch, Germany) column was used as stationary phase for the experiment with a gradient of 95 % water (0.008 % TFA) /5 % (0.008 % TFA) MeOH to 100% (0.008% TFA) MeOH as mobile phase. For the stability and reactivity study experiments of Oxaliplatin and Carboplatin, analytical HPLC was carried out using a Waters e2695 system with a PDA (2998) detector inline. Reprosil C18 (250 mm x 4.6 mm, 5 µm; Dr. Maisch, Germany) column was used as stationary phase for the experiment with a gradient of 99 % water/1 % MeOH to 100% MeOH as mobile phase.

b. Reactivity studies of 1 and 2 in presence of Glutathione and 5'-GMP:

2 mM solution of 1 or 2 was prepared by dissolving1 or 2 in 1 mL (500 µl DMSO /500 µl DPBS, pH 7.2 buffer) of solution. 2 mM solutions of Oxaliplatin (AK Scientific) and Carboplatin (AK Scientific) were similarly prepared in 1:1 mixture of water/DPBS, pH 7.2. L-Glutathione reduced (Sigma) was added to a final concentration of 10 mM to each of the reactions and pH was adjusted to 7.2. The mixtures were incubated at 37 °C with shaking. The reactions were monitored for 3 days and samples were analysed by analytical HPLC.

pH 3 Buffer preparation for 5'-GMP reaction: Stock solution of 0.1 M solution of citric acid (21.01 g in 1 liter) and 0.1 M solution sodium citrate (29.41 g in 1 liter) were made. 20.5 mL of 0.1 M solution of citric acid and 29.5 ml of 0.1 M sodium citrate were taken from the stock solution and make up to 100 mL with distilled water. 1N HCl solution used to adjust to pH 3. To obtain 2mM solution of comp 2 with 10 mM of 5'-Gmp, 1.15 mg (2mM) of comp 2 and 3.63 mg (10 mM) of 5'-GMP dissolved in 1mL (200 µl-DMSO/800 µl pH3 buffer) of solution. Retention time of comp 2, its carrier ligand with 5'-GMP at pH3, checked up to 72h. 10% release of carrier ligand is observed.

c. **Preparation of liposomal formulations of 1 and 2**:

L-α-phosphatidylcholine, hydrogenated (soy) (HSPC) (25.5 mol%), 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) (58 mol%), compound **1** or **2** (8.5 mol%), free cholesterol (3 mol%) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG) (5 mol%) were dissolved in dichloromethane:methanol (1:1) mixture. Solvent was evaporated into a thin and uniform lipid-drug film under vacuum using a rotary evaporator at 45 °C. The dry lipid-drug film was then hydrated with required volume of 5 % lactose solution and allowed to rotate on rotary evaporator at 65°C (above the lipid phase transition temperature) for 90 min. Hydrated MLVs (multilamellar vesicles) were sequentially extruded through 400 nm, 200 nm and 100 nm pore size membrane (Whatman® filters) supported by filter support (Avanti No: 230600) for 10 times each using LIPEXTM extruder (Northern Lipids, Canada) under nitrogen pressure connected to circulating water bath at 65 °C.[2] Finally, the glass vial containing formulation were cooled at 4 °C for 30 min, then -20 °C for 2 h and at -80 °C for 12 h and finally freeze dried overnight at -80 °C.

d. Characterization of the liposome:

The mean particle size and polydispersity index (PDI) of the formulation was measured by Dynamic Light Scattering method using Zetasizer Nano ZS90 (Malvern, UK). 50 µL of formulation was diluted to 1 mL using DI water and measurement was performed at 90 degree scattering angle at 25 °C to get the average particle size distribution. The zeta potential was estimated based on electrophoretic mobility under an electric field using same instrument a Zetasizer Nano ZS90.

e. **Fluorescence measurement of complexes and ligands**

Fluorescence measurements were carried out using a FluoroMax-4 spectrofluorometer from HORIBA Jobin Yvon. The concentration of the ligands as well as their metal complexes is $2x10^{-5}$ M in $CHCl_3$ for ligands and MeOH for their metal complexes. In case of the ligand **1a**, excitation at 415 nm shows emission maxima at 445 and 470 nm.

Corresponding metal complex **1** shows no emission on excitation at 470 nm. Similarly, we found the emission band at 488 and 510 nm for the ligand **2a** on excitation at 445 nm and its corresponding metal complex **2** shows no emission on excitation at 505 nm.

f. In vitro release kinetics studies

The in vitro release of **1** and **2** from its freeze dried liposomal formulation was carried out by dialysis membrane tube method. For the release kinetics analysis, specified quantity of lyophilized comp 2 liposome was reconstitution with 2 mL MilliQ water and placed into the pretreated dialysis bags. Citrate buffer (pH 5.5) and phosphate buffer saline (pH 7.4) with 0.2%, Tween 80 was used as release medium. The dialysis bag poured into the 100 mL of each medium at 37 °C with continuous shaking at 100 rpm simulate the infinite sink tank condition. 100 µL of the aliquot was collected from the dialysis tube at predetermined time intervals and replaced with equal volume of fresh medium, and the released Pt equivalent was quantified by AAS.

g. Cell lines and reagents

The in vitro testing of platinum drugs was carried out in A549, HCT-116, MCF-7 and LLC cell lines. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100µM streptomycin in a fully humidified incubator at 37°C in an atmosphere containing 5% CO2. Clinical grade platinum drugs were used for these studies and all platinum solutions were freshly diluted before usage.

h. Compound-DNA interaction

To evaluate interaction of Compound 2 with DNA, genomic DNA (gDNA) isolated from mammalian cells was quantified using the spectrophotometer and treated with various concentrations of Compound 2 in a final reaction volume of 20µl in Tris-EDTA buffer (pH-8.3). For each reaction, 180µg gDNA was treated with 0, 10µM, 50µM, 100µM, 250µM, 500µM concentration of Compound 2 (dissolved in DMSO) for 40 min at 42°C. A control reaction was set up with gDNA and equivalent volume of DMSO to determine the effect of solvent on DNA. Following the reactions, DNA loading dye was added to the samples to stop the reaction and samples were loaded on a 0.8% agarose gel to evaluate gDNA migration.

i. Cytotoxicity assay and intracellular accumulation of platinum compounds

Cells grown in culture plates were counted and adjusted to required concentrations before seeding in 96-well plates 24 h before cytotoxicity assay. Cells were treated with the platinum compounds (0-50 µM) and grown for 72 h at 37°C and cell viability was quantified using colorimetric MTT assay. Wells containing media alone was considered as blank to measure the background. Absorbance of the plates was recorded in an ELISA-plate reader at 550 nm, with background correction at 655 nm. Cell survival was plotted and IC50 values calculated using the GraphPad Prism software package.

For intracellular Pt(II) accumulation, cells (5 × 105/plate) were seeded in 6-well plates, grown for 24 hrs and then incubated with 10 µM Pt-compounds for 5 h at 37°C. Cells were washed twice with PBS, harvested by trypsinization, resuspended in PBS and counted. The

cell pellet was digested with 70% HNO_3 and diluted to 1 ml with 0.05% HCl. The Pt concentration was determined by flameless graphite-furnace atomic absorption spectroscopy (AAS). External standards of Pt were prepared in the 10-100 ppb range and the lower limit of quantification was 10 ng/ml. The cellular accumulation of platinum was expressed as ng of platinum per 105 cells.

j. Cellular internalization of Compound 2

Cellular internalization of Compound **2** in tumor cells was assessed using epifluorescence microscopy. A549 cells were plated on coverslips in 12-well plates and allowed to adhere overnight at 37°C in a humidified atmosphere of 5% CO_2. Cells were then treated with 5μM Compound **2** and incubated for 30min, 2hrs, 4hrs, 6hrs, 24hrs and 48hrs at 37°C. Post-incubation, cells were fixed using 4% formaldehyde and counterstained with the nuclear stain DAPI for 5 minutes. The coverslips were mounted with ProLongTM Gold antifade mounting media (ThermoFisher Scientific) and analyzed under Nikon epifluorescence microscope.

k. TUNEL assay

A549 cells were seeded at an appropriate confluency (60-70%) on coverslips in 12-well plates and allowed to adhere overnight at 37°C in a humidified atmosphere of 5% CO_2. Cells were then treated with 10 μM Compound 2 or Oxaliplatin, diluted in complete media and incubated at 37°C for 24 hrs. Post-incubation, the media was removed, and cells washed thrice with PBS at 37°C. Cells were fixed for 5 min with freshly prepared 4% paraformaldehyde at room temperature, followed by PBS wash. Cells were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. Coverslip were processed for apoptotic cell death in cells by TUNEL assay using the In Situ Cell Death Detection Kit, TMR red® (Roche, Germany), according to the manufacturer's protocol. The nuclei were stained with DAPI and coverslip mounted with prolonged antifade mounting media. The label incorporated at the damaged sites of DNA in treated cells was visualized by fluorescence microscopy.

l. Antitumor activity of Compound 2 in LLC tumor model

The in vivo efficacy of Compound **2** was compared with Oxaliplatin in LLC, a syngeneic non-small cell lung cancer tumor model. Tumors were propagated in C57/BL6 mice by subcutaneous injection of 1 x 10^6 LLC cells in the lower abdomen. Mice were examined for the presence of tumor and then randomized into treatment groups (Group-I: untreated control, Group-II: Oxaliplatin, Group-III: Compound **2**). Tumors were allowed to grow to an average size of 125 mm3 before initiation of treatment. The treatment consisted of i.v. injections of 5 mgPt/kg dose of Oxaliplatin or Compound **2**. Oxaliplatin was administered once every week, while Compound **2** was dosed continuously for 5 days. Animals were examined daily for signs of treatment related toxicity or mortality. Tumor volume and body weight were regularly measured during the study and animals were taken off the study and sacrificed when their body weight loss was greater than 20 % compared to day 0 or when tumor volume reached 2000 mm3 or tumors became ulcerated or necrotic. All animal experiments were conducted under Institutional Animal Care and Use Committee guidelines and approval.

m. Tissue distribution of Compound 2 in C57/BL6 mice:

Tissue distribution was evaluated after dosing Compound **2** and Oxaliplatin intravenously at a dose of 5 mgPt/kg in female Balb/c Mice. Blood, Plasma, Spleen, Kidney and Lungs were collected 24hr post dose in both the groups. Platinum concentrations were measured using Atomic Absorption Spectroscopy.

n. DNA-Platination

A549 cells were seeded in 75 cm2 flasks and treated with oxaliplatin or Compound 2 (cells were 80-90% confluent) for 24 h at a Pt-equivalent concentration of 30 μM. Following incubation, flasks were rinsed thrice with PBS, trypsinized and cell pellets were collected in 1.5ml microfuge tubes by centrifugation. Cellular DNA was isolated using DNAzol® (ThermoFisher Scientific) according to the manufacturer's instructions. Following determination of concentration, the DNA in solution was digested with 70% HNO3 and diluted to 1 ml with 0.05% HCl. The Pt concentration was determined by flameless graphite-furnace atomic absorption spectroscopy (AAS). External standards of Pt were prepared in the 2-20 ppb range. The DNA-Pt adduct was represented as ng of platinum per μg of DNA.

o. Statistical methods

Results are expressed as mean ± standard deviation. A Student t-test was used to compare variables. Differences at * $p \leq 0.05$, ** $p \leq 0.005$ and *** $p \leq 0.0005$ have been considered statistically significant. For cell culture experiments, at least three independent experiments were performed.

2. Computational Method,

a. Aquation kinetics

All the quantum mechanical calculations were carried out with Gaussian 09 program to search minima and transition state structures located on the potential energy surface of the aquation reaction of different molecules which are studied. Density functional theory (DFT) was used with B3LYP hybrid exchange correlation functional in these calculations. B3LYP functional consists of 3 parameters of exchange functional developed by Becke in combination with Lee, Yang and Parrs correlation functional. The square-planar Pt moieties are common among all the three compounds (**1**, **2** and **3**) subjected to aquation kinetics study. Pt atom was treated with effective core potential (ECP) basis such as LANL2DZ with MWB60. ECP is a pseudo-potential which effectively uses core potential without considering explicitly all electrons of heavy atoms which speeds up computation. 6-311 g (d, p) basis set is used for the atoms other non-metal atoms. The convergence to equilibrium and transition state geometries was verified from vibrational frequency calculation by diagonalizing of the mass-weighted Hessian matrix using same level of theory. The single point energy calculations were performed on geometry optimized and transition state structures with 6-311 g++(2df, 2pd) basis set to obtain more accurate energy. 2 sets of d-type function and 1 set of f-type of polarisation function were added on non-hydrogen atoms, whereas for hydrogen atoms 2 set of p-type function and one set of d-type were used for more accurate calculation of electronic energy, zero-point energy, thermal enthalpies. All the geometry optimization and transition state search in neutral and acidic conditions was performed in presence of water environment using conductor-like polarisable continuum model (CPCM).[4] Zero-point energies and enthalpy corrections at 298.15 K were incorporated in the reported energies. The potential energy profiles for aquation of 1, 2, and 3 under neutral and acidic condition are given in the Figure S7.

b. logP calculation:

Log P evaluates the octanol/water partition coefficient which is a measure of the molar hydrophobicity. log P values of **1**, **2**, and **3** molecules are calculated using MarvinSketch software[5] The log P calculation method derived from the work of Viswanadhan et al.[6] The standard log P values for oxaliplatin and carboplatin using these structures (shown in table) are 1.73 and 1.07, respectively.[7] The log P values of **1**, **2**, and **3** are found to be 3.47, 3.40, and 3.32 respectively which indicates that Pt-PLY complexes are more hydrophobic than oxaliplatin and the order of hydrophobicity of **1**> **2**> **3**.

3. Synthesis and Characterization:

a. Synthesis and characterization of Compound 1:

(1,2-Diaminocyclohexane)platinum (II) chloride (600mg,1.5 mmol) was taken in 60 mL water. To the above solution, silver nitrate (530 mg, 3.1 mmol) was added. The resulting solution was stirred at rt. After 24 h, the reaction mixture was centrifuged and filtered using 0.2 micron filter to remove AgCl. The filtrate was lyophilized to reduce the volume to 5 mL (aquated DACH Platinum) (1.5 mmol). To this solution, 30 mL ethanol, 9-hydroxy phenalenone 1a (306 mg, 1.56 mmol) was added along with KOH (86 mg, 1.56 mmol solution in ethanol). The resulting solution was stirred for 24 h. at 90º C. After completion, reaction mixture was concentrated to remove ethanol and washed with water (30 mL). The precipitate obtained was collected through centrifugation and washed with dichloromethane. The pure compound was obtained as crystals from methanol/toluene solvent mixture. The yield of the compound 1 is (0.63g) 80.15%. M.P. above 300(°C) (decomposed), IR (KBr): 3436, 3193, 3097, 2935, 1627, 1581, 1508, 1384, 1325, 1237, 1178, 851; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.43-8.45 (m, 4 H), 7.69 (t, J = 8 Hz, 1 H), 7.23 (d, J = 8 Hz, 2 H), 6.53 (d, J = 4 Hz, 2 H), 6.02 (t, J = 8 Hz, 2 H), 2.35 (m, 2 H), 1.95 (d, J = 8 Hz, 2 H), 1.53 (d, J = 4 Hz, 2 H), 1.35 (d, J = 4 Hz, 2 H), 1.07-1.11 (m, 2 H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ: 170.54, 138.96, 132.33, 126.67, 126.26, 125.87, 124.88, 114.42, 62.28, 31.49, 23.97 ppm. ^{195}Pt (107 MHz, DMSO-d6) -1575 ppm. HRMS (ESI) m/z calcd for $C_{19}H_{21}N_2O_2Pt$ $(M-NO_3)^+$, 504.1251; found 504.1213. Anal. Cal. (For $C_{19}H_{21}N_3O_5Pt$) C, 40.29; H, 3.74; N, 7.42; Found: C, 39.71; H, 3.84; N, 7.29)

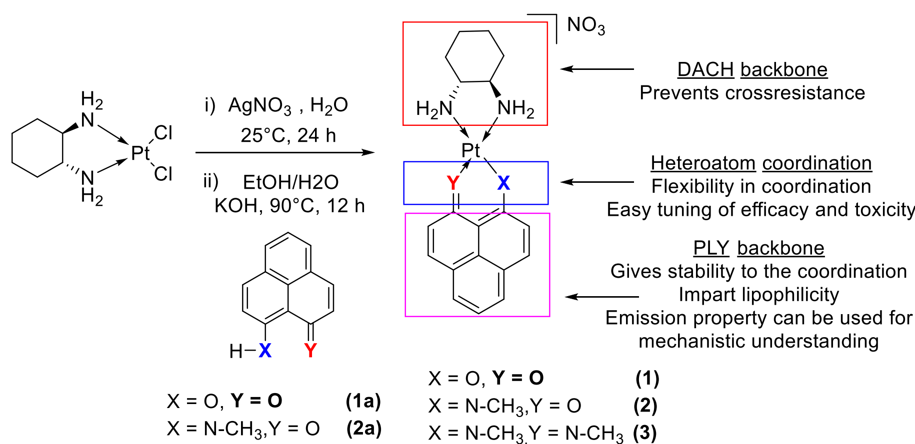
b. Synthesis and characterization of Compound 2:

(1,2-Diaminocyclohexane) platinum(II) chloride (600mg,1.5 mmol) was taken in 60 mL water. To the above solution, silver nitrate (530 mg, 3.1 mmol) was added. The resulting solution was stirred at rt. After 24 h, the reaction mixture was centrifuged and filtered using 0.2 micron filter to remove AgCl. The filtrate was lyophilized to reduce the volume to 5 ml. To this solution, 30 mL ethanol, **2a** (328 mg, 1.56 mmol) was added along with KOH (86 mg,1.56 mmol solution in ethanol). The resulting solution was stirred for 24 h at 90º C. After completion, reaction mixture was concentrated to remove ethanol and washed with water (30 mL). The precipitate obtained was collected through centrifugation and washed with dichloromethane. The pure compound was obtained as crystals from methanol/diethyl ether solvent mixture.

The yield of the compound 2 is 0.78 g (96 %). M.P. above 300(° C) (decomposed), IR (KBr): 3436, 3226, 3119, 2925, 2858, 2421, 1646, 1585, 1506, 1388, 1330, 1190, 837; ^{1}H NMR (500 MHz, DMSO-d6) δ: 8.15-8.20 (m, 2 H), 8.10 (d, J = 8 Hz, 1 H), 8.04 (d, J = 8 Hz, 1 H), 7.45-7.50 (m, 2 H), 7.17 (d, J = 4 Hz, 1 H), 6.12 (d, J = 4 Hz, 1 H), 6.02 (d, J = 4 Hz, 1 H), 5.74 (t, J = 8 Hz, 1 H), 5.62 (t, J = 8 Hz, 1 H), 3.75 (s, 3 H), 2.37-2.39 (m, 1 H), 2.26-2.28 (m,

1 H), 1.95-1.97 (m, 2 H), 1.54-1.55 (m, 2 H), 1.33-1.35 (m, 2 H), 1.10-1.11 (m, 2 H) ppm.^{13}C NMR (125 MHz, DMSO-d6) δ: 163.54, 151.71, 135.37, 134.04, 130.40, 129.39, 127.39, 126.36, 125.04, 123.43, 119.54, 113.26, 62.51, 60.35, 46.17, 31.92, 31.88, 24.05, 24.03 ppm. ^{195}Pt (107 MHz, DMSO-d6) -1844.11 ppm. HRMS (ESI) m/z calcd for $C_{20}H_{25}N_3OPt$ $(M-NO_3)^+$, 517.1567 ; found 517.1605. Anal. Cald. (For $C_{20}H_{25}N_4O_4Pt$) C, 41.45; H, 4.13; N, 9.07; Found: C, 41.00; H, 4.13; N, 9.07.

4. Schemes and Figures:



Scheme S1. Design and synthesis of Pt-PLY compounds from RR-DACH Pt Cl_2

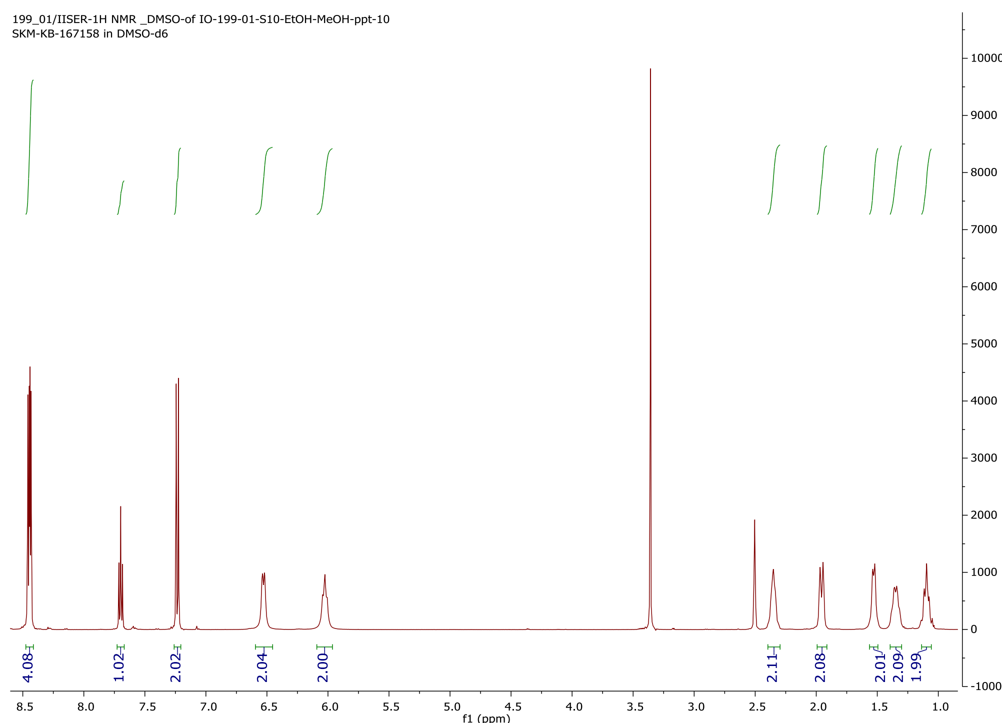


Figure S1: ^{1}H NMR (DMSO-*d6*) of compound **1**.

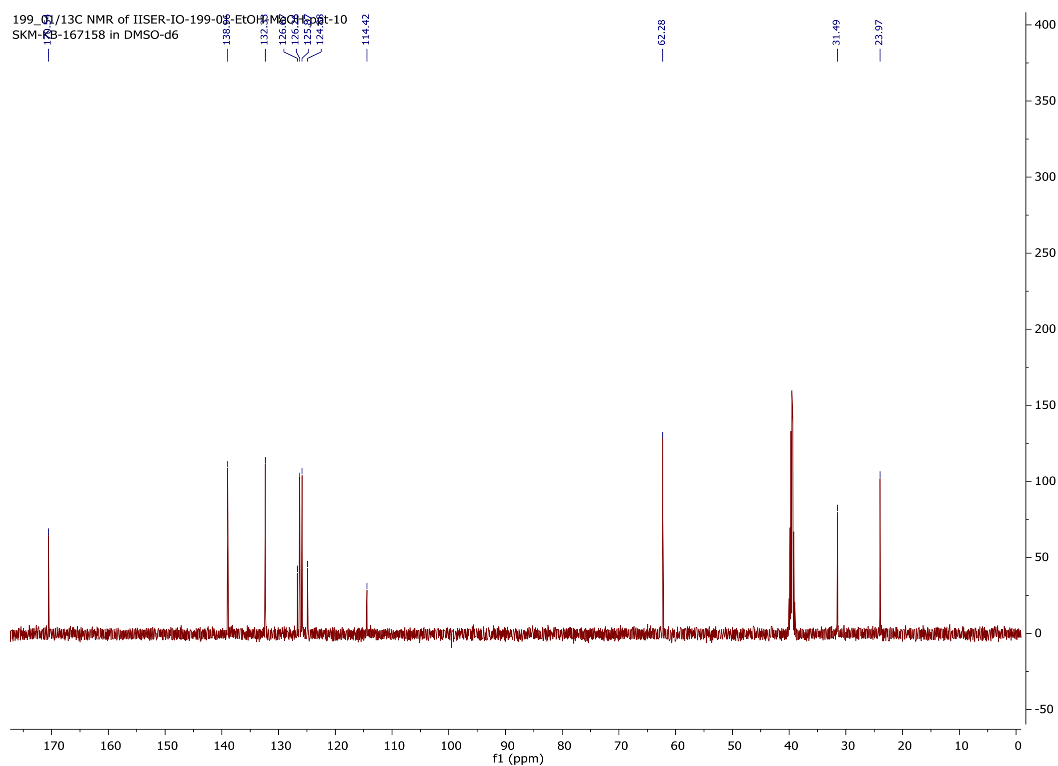


Figure S2: ^{13}C NMR (DMSO-*d*6) of compound **1**.

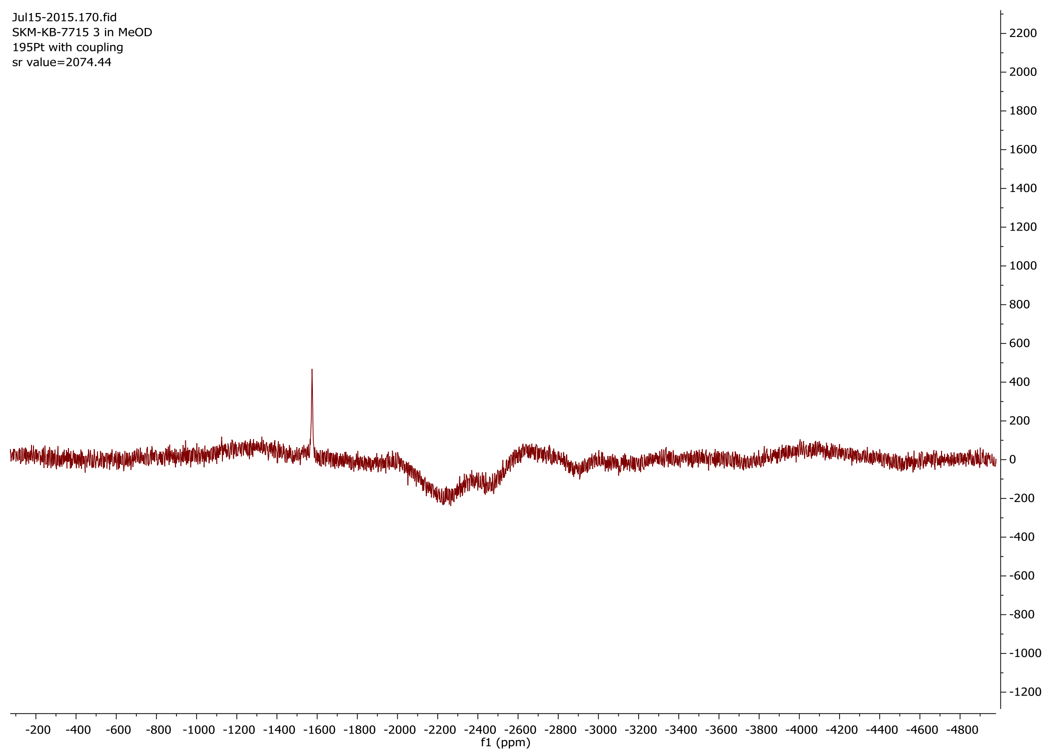


Figure S3: ^{195}Pt NMR (DMSO-*d*6) of compound **1**.

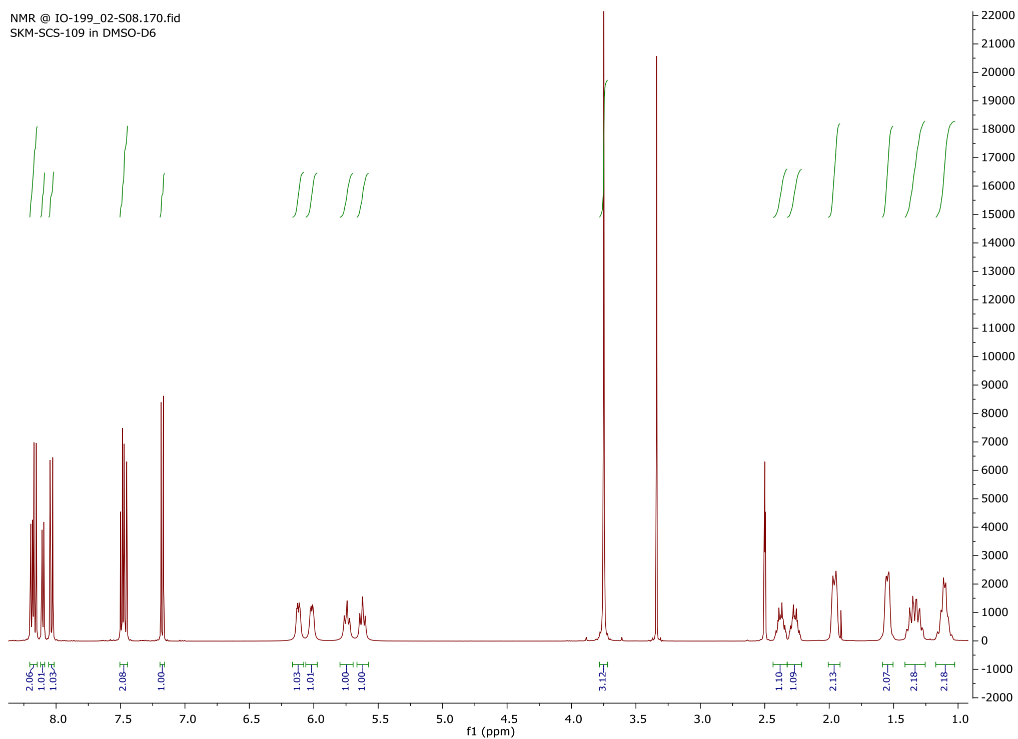


Figure S4: ^{1}H NMR (DMSO-*d*6) of compound **2.**

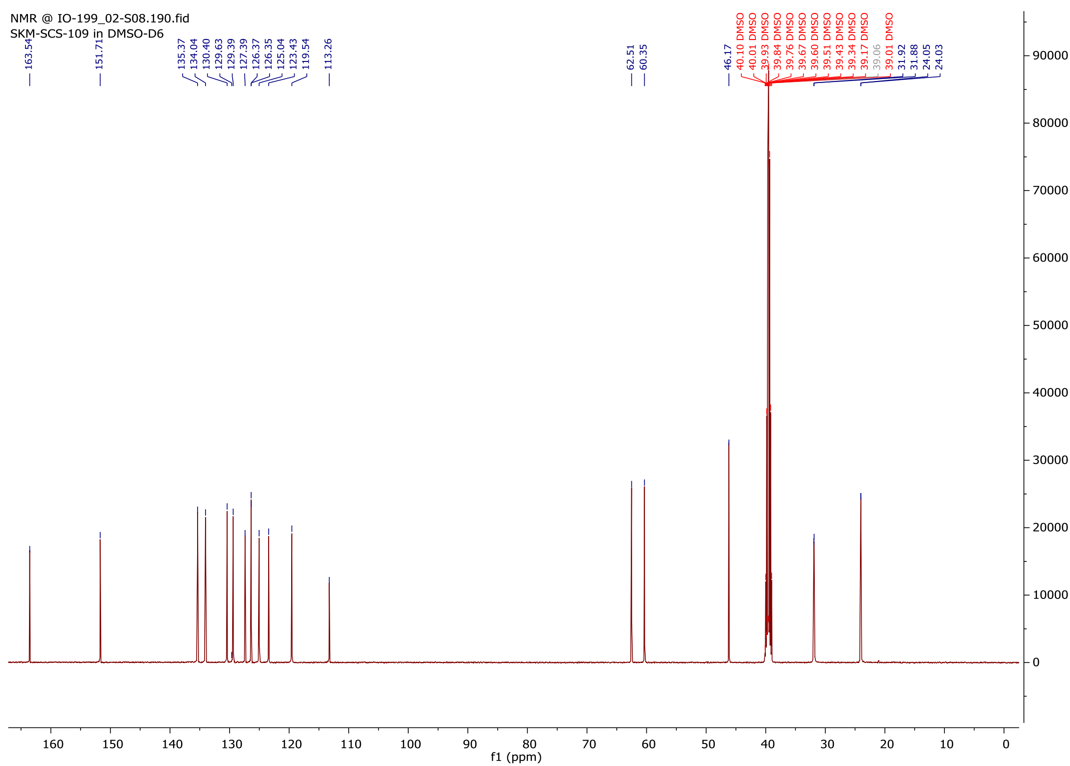


Figure S5: ^{13}C NMR (DMSO-*d*6) of compound **2.**

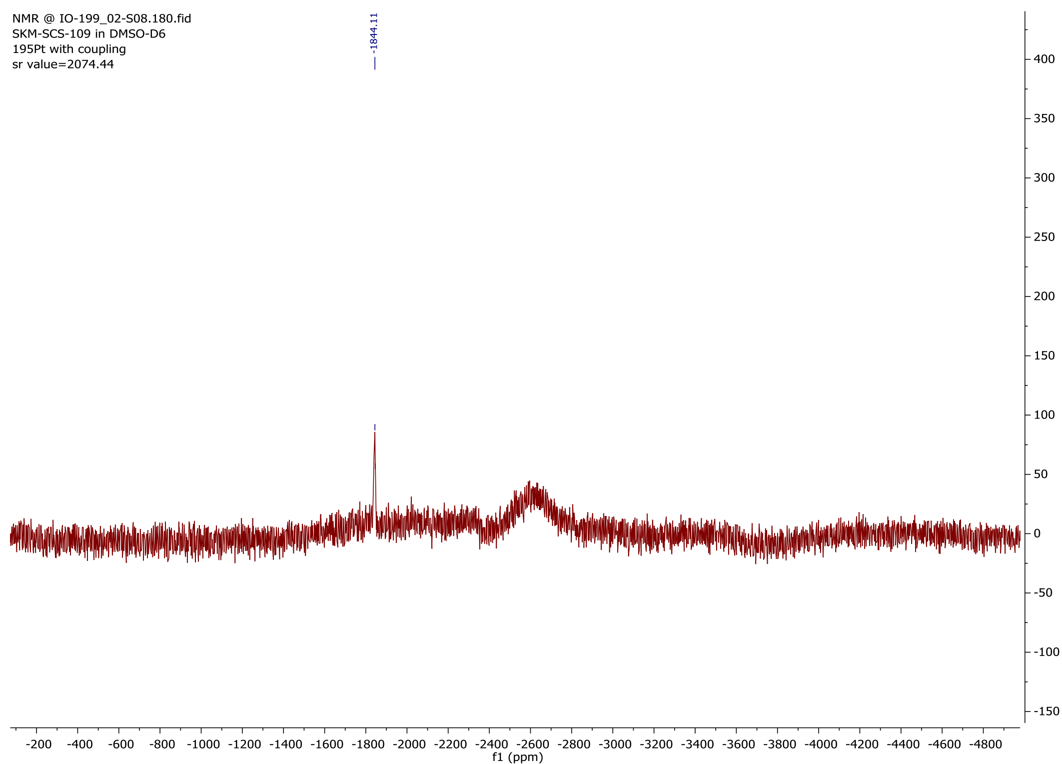
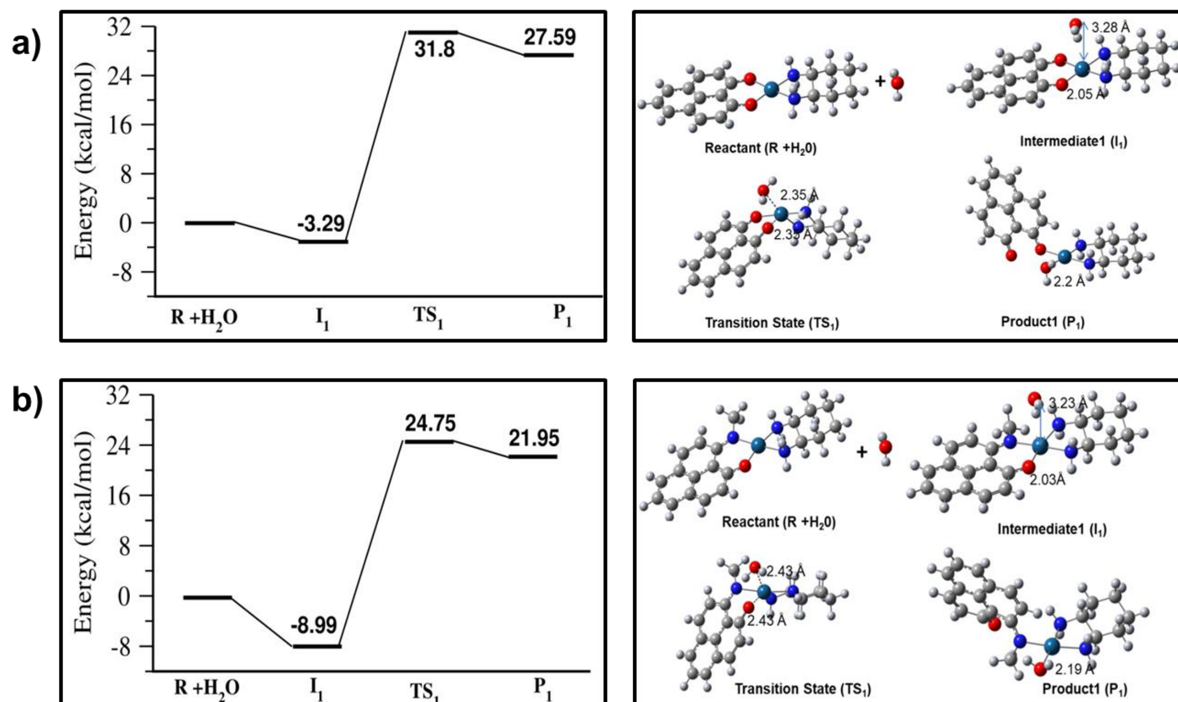
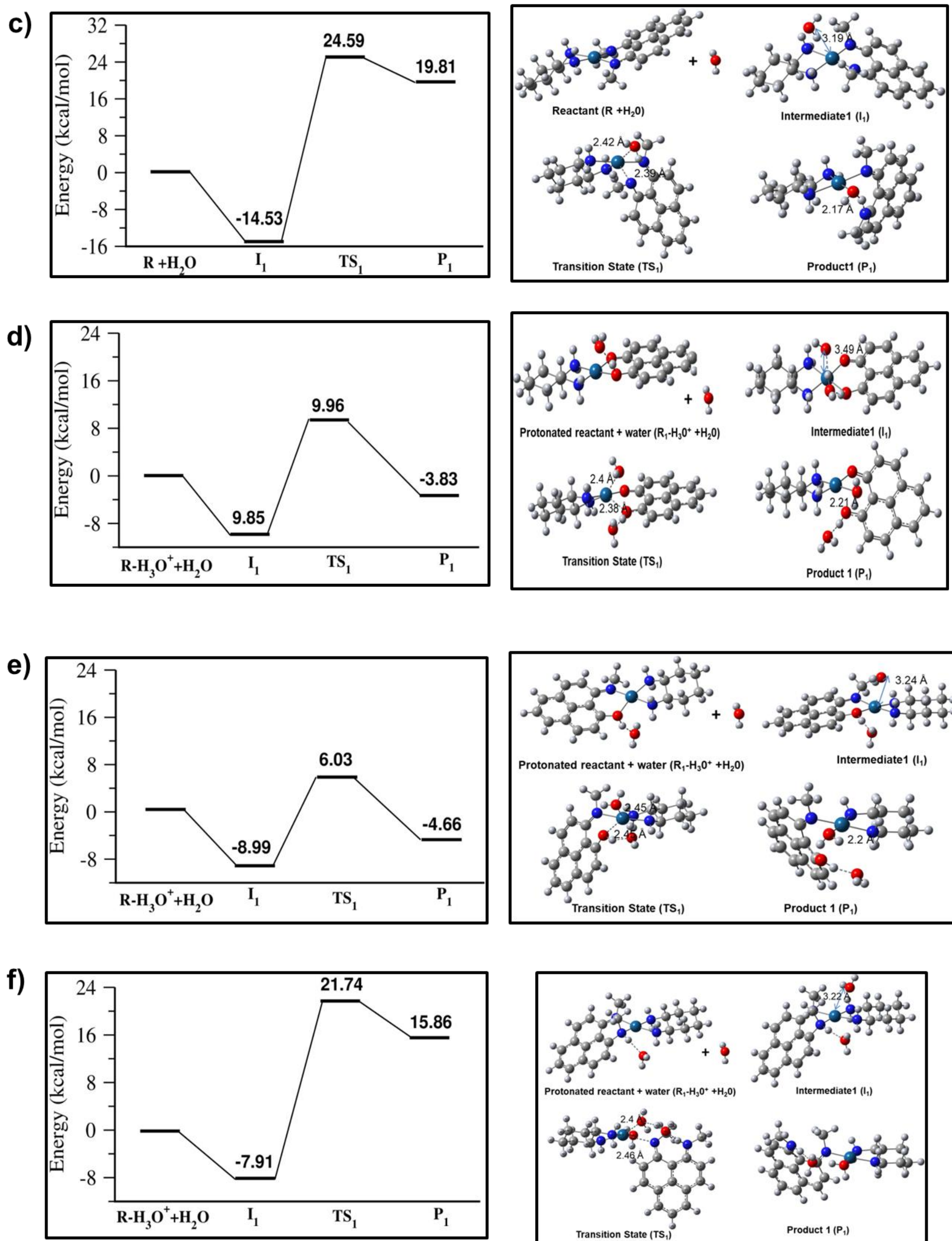
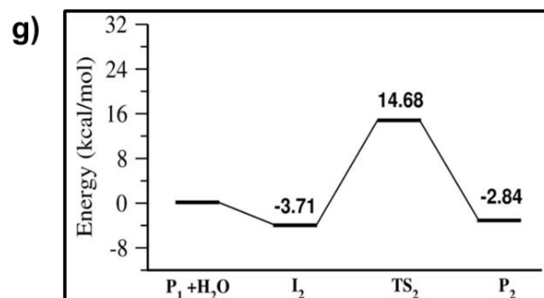
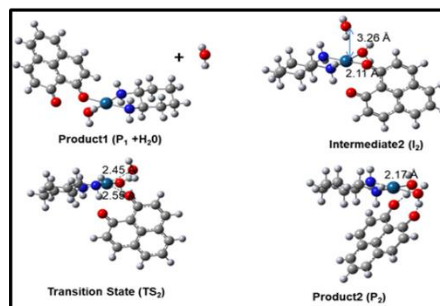


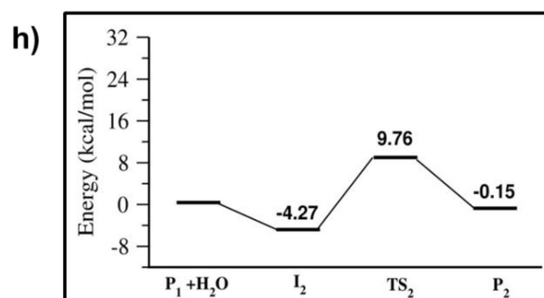
Figure S6: ^{195}Pt NMR (DMSO-*d*6) of compound **2**.

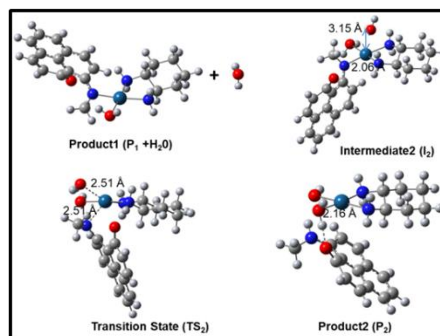


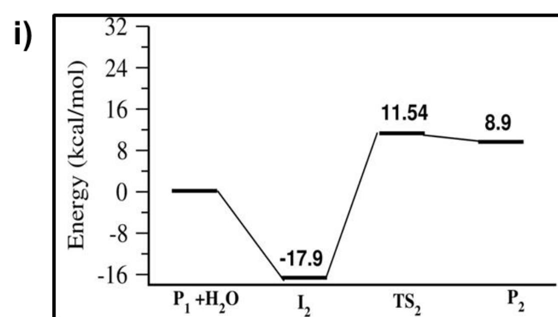

c)
Energy (kcal/mol)
24.59
19.81
-14.53
R +H2O
I1
TS1
P1
Reactant (R +H20)
Intermediate1 (I1)
Transition State (TS1)
Product1 (P1)
3.19 Å
2.42 Å
2.39 Å
2.17 Å
d)
9.96
-3.83
9.85
R-H3O+ +H2O
Protonated reactant + water (R1-H30+ +H20)
Intermediate1 (I1)
Transition State (TS1)
Product 1 (P1)
3.49 Å
2.4 Å
2.38
2.21 Å
e)
6.03
-4.66
-8.99
3.24 Å
2.2 Å
f)
21.74
15.86
-7.91
2.46 Å

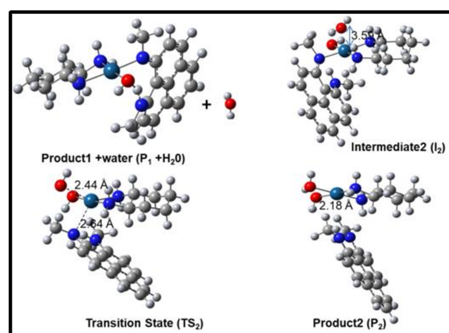
g) 

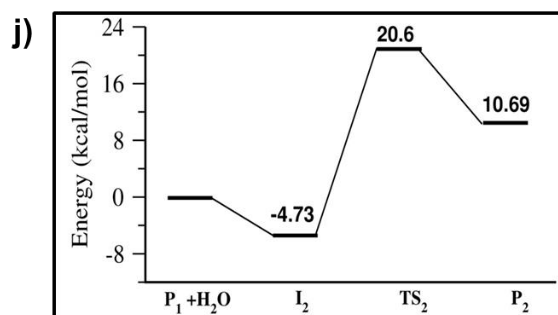


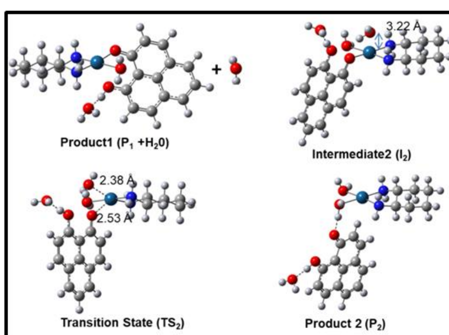
h) 



i) 



j) 



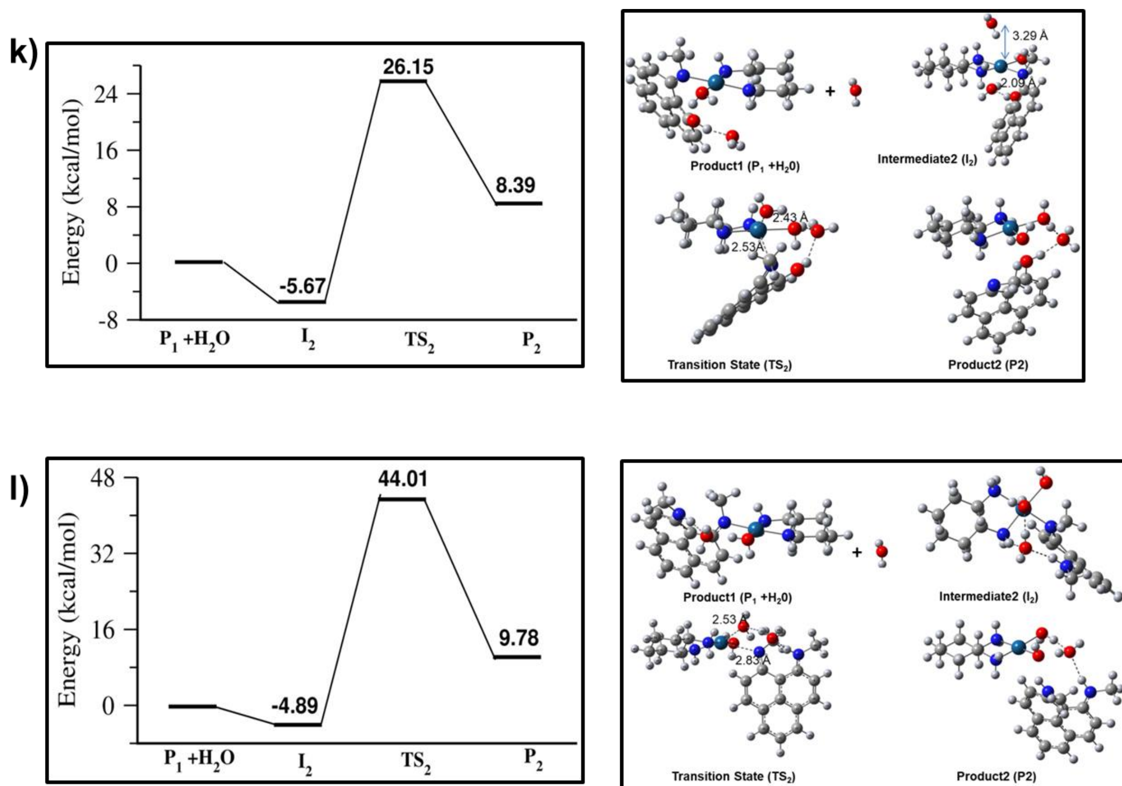


Figure S7: Potential energy profiles and intermediate structures; for the first phase of hydrolysis involving reactant (R), water (R+H_2O), intermediate (I_1), transition state (TS_1), and product (P_1) are shown for (a) Compound 1 in neutral condition, (b) Compound 2 in neutral condition, (c) Compound 3 in neutral condition. The extra proton (H_3O^+) involved during the first phase of hydrolysis in acidic condition which are shown for 1, 2 and 3 molecules in plot d, e, and f respectively. Potential energy profiles of second phase of hydrolysis involves second attacking water and product which obtained after first hydrolysis (P_1+H_2O), intermediate (I_2), transition state (TS_2), and final product (P_2). They are shown in plot g, h, i for 1, 2, 3 respectively under neutral conditions, whereas plot j, k, and l show the same for compounds **1**, **2** and **3**
respectively in acidic conditions.

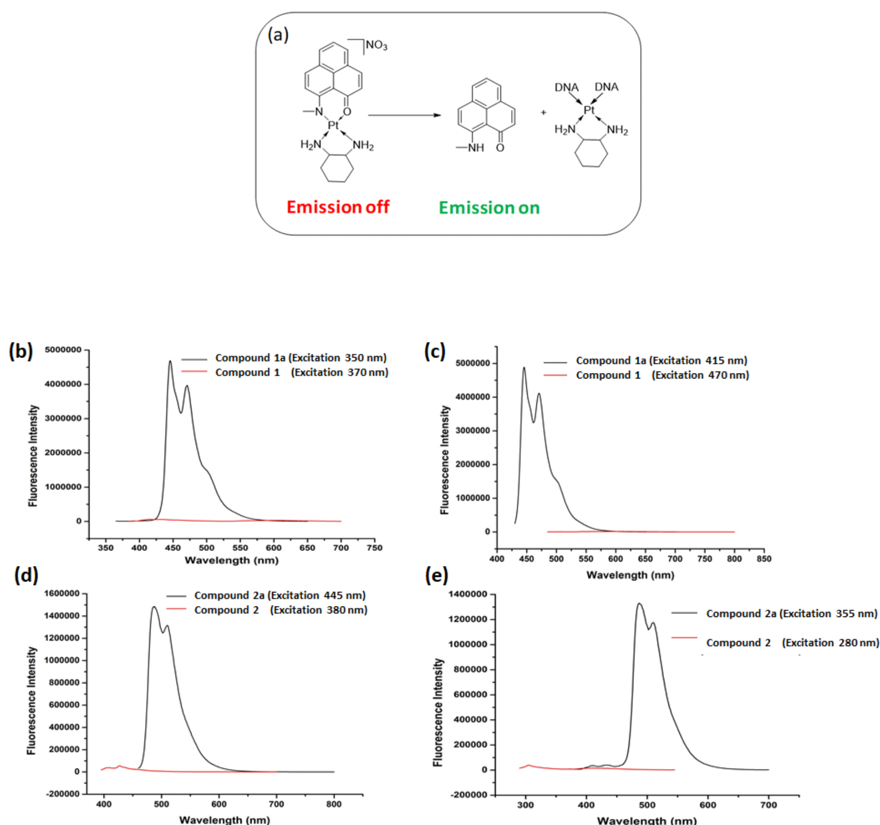


Figure S8: (**a**) Schematic representation of the emission on-off characteristic of the designed compounds in un-complexed and complexed forms; (**b-c**) Emission spectra of compound **1a** and **1** in different excitations; (**d-e**) Emission spectra of compound **2a** and **2** in different excitations.

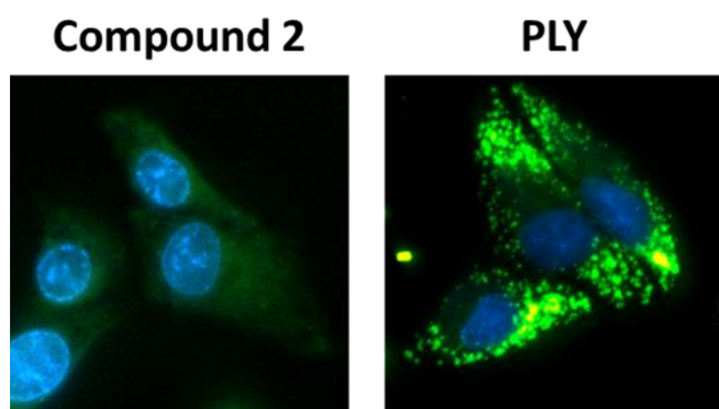


Figure S9: Representative images depicting cellular internalization of Compound 2 and PLY in A549 cells. Both the compounds were dissolved in organic solvents (DMA or DMSO) and molar equivalent of compounds were added to A549 cells. Cells were observed under fluorescent microscope 16 hours post treatment and cellular internalization was noted with respect to PLY release. The staining was diffuse, with low fluorescence intensity for released PLY in samples treated with Compound 2. Samples treated with PLY showed punctate dots with higher fluorescence intensity. Scale bar represents 10µm.

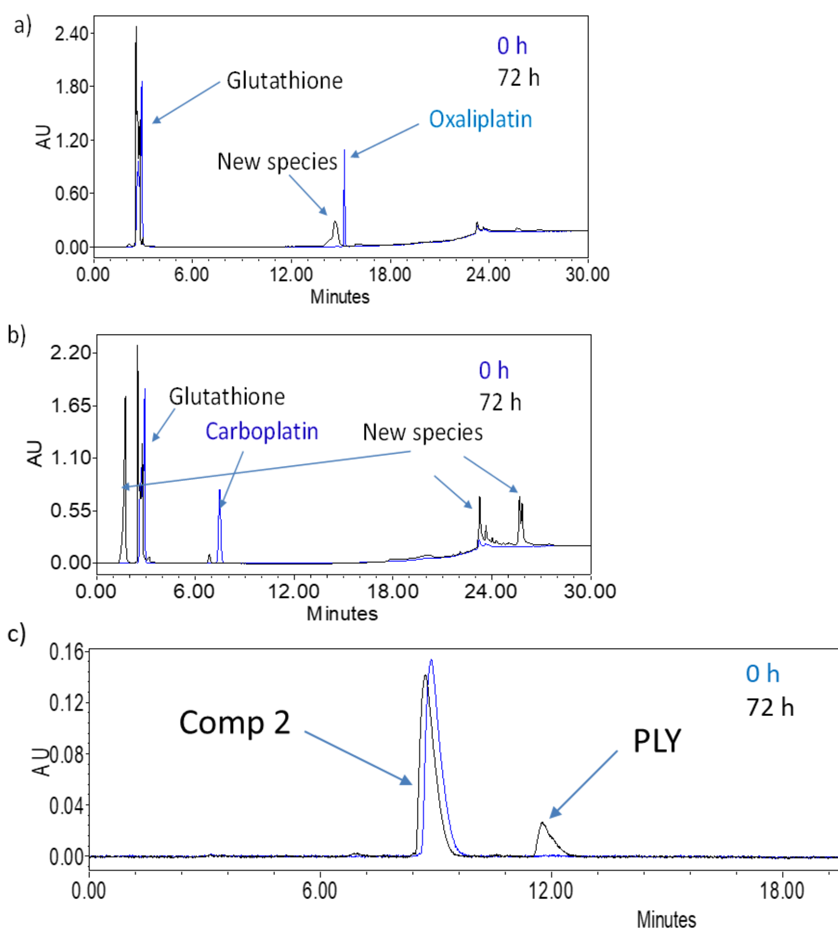


Figure S10: a) Reactivity of Oxaliplatin with glutathione in PBS shows no oxaliplatin remain unreacted after 72 h; b) Reactivity of Carboplatin with glutathione in PBS shows no carboplatin remain unreacted after 72 h; c) Reactivity of 2 with 5'-GMP shows 10% release of carrier ligand in 72 h; all data were recorded at 440 nm for ligand or complex 2, so that peaks associated with phenalenyl framework are only visible in the chromatogram. Oxpt and carboplatin data were recorded at 210 nm.

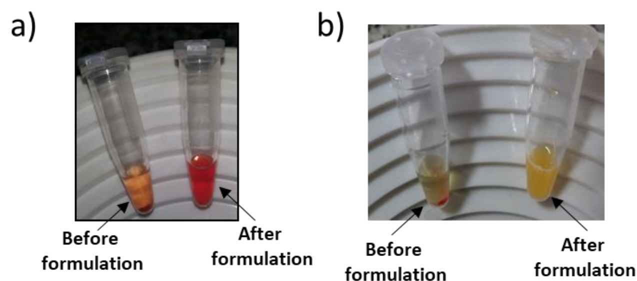
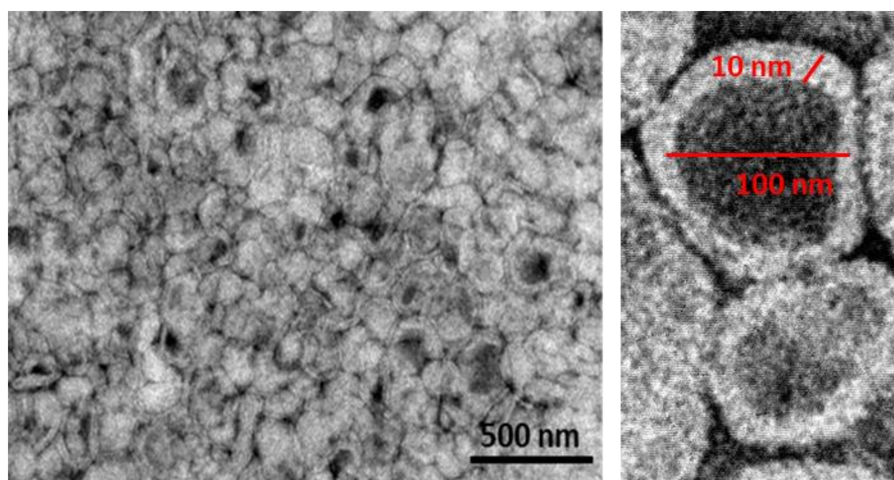


Figure S11: Significant enhancement of solubility of a) **2** and b)**1** after formulation.

a)



b)

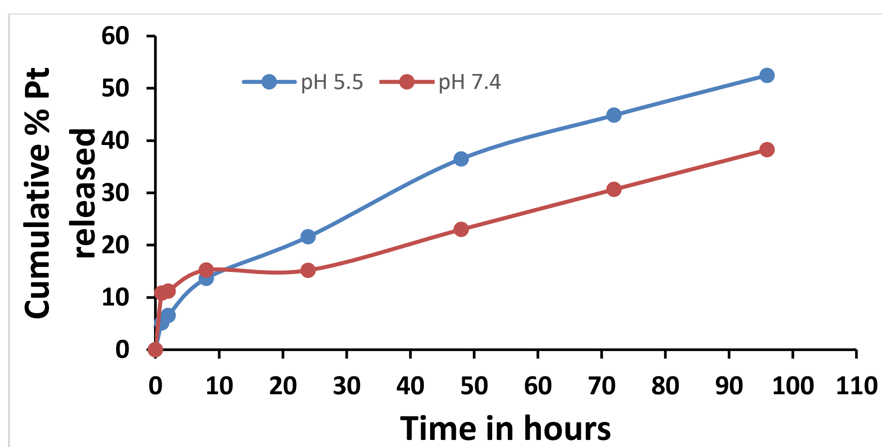


Fig. S12. a) HR-TEM data of compound **2**, Inset: magnified particle is shown; b) *In vitro* release of comp 2 from its liposomal formulation in citrate buffer (pH 5.5) and phosphate buffer saline (pH 7.4) at 37 °C.

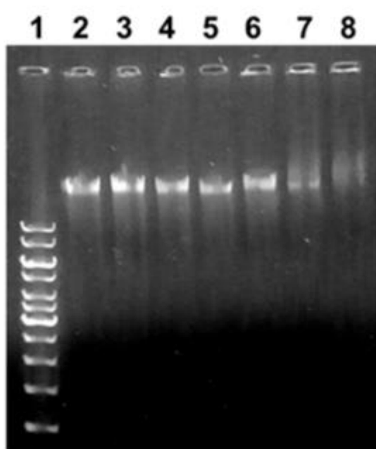


Figure S13. DNA interaction of compound 2. Agarose gel electrophoresis of genomic DNA treated with various concentrations of 2; Lane 1, DNA marker; lane 2, untreated; lane 3, DMSO; lane 4, 10μM; lane 5, 50μM; lane 6, 100μM; lane 7, 250μM; lane 8, 500μM.

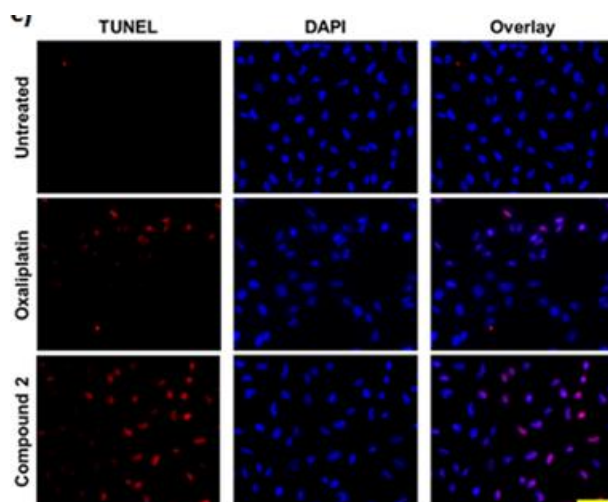


Figure S14. Compound 2 induces higher apoptosis in A549 cells. A549 cells treated with 10μM platinum equivalence of compounds were examined for induction of apoptosis through TUNEL assay (TUNEL, TMR-Red; Nucleus, DAPI). Scale bar represents 50 μm.

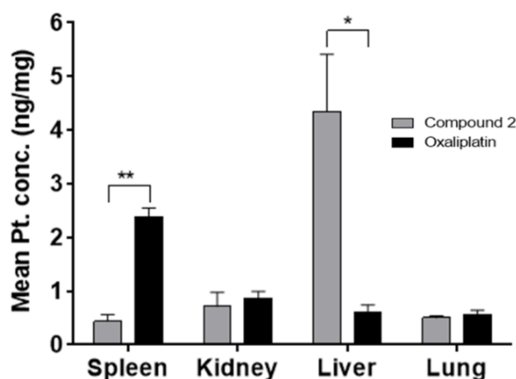


Figure S15: Biodistribution of Compound 2 and Oxaliplatin was evaluated in syngeneic LLC tumour model. The mice were implanted with 1 × 10^6 LLC cells and treated with Oxaliplatin and Compound 2. Tissues were harvested from Oxaliplatin treated group on day 14, while those from Compound 2 treated group were harvested on day 18. The biodistribution of the tested compounds in tissues was recorded by Pt-AAS. (*, $p \leq 0.05$; **, $p \leq 0.005$).

5. Tables

Table S1: Crystallographic data parameters for 1 and 2

Identification code	**PLYNOPt (2)**	**PLYOOPt (1)**
Empirical formula	$C_{40}H_{48}N_8O_8Pt_2$	$C_{38}H_{42}N_6O_{10}Pt_2$
Formula weight	1159.04	1132.96
Temperature/K	99.98(10)	100.01(10)
Crystal system	monoclinic	triclinic
Space group	$P2_1$	P1
a/Å	8.5924(5)	8.5488(5)
b/Å	17.7287(9)	9.3389(6)
c/Å	13.3203(7)	12.6348(6)
α/°	90.00	110.339(5)
β/°	107.246(7)	97.169(4)
γ/°	90.00	98.871(5)
Volume/Å^3	1937.87(19)	917.03(8)
Z	2	1
ρ_{calc}g/cm^3	1.986	2.052
μ/mm^{-1}	13.855	7.689
F(000)	1128.0	548.0
Crystal size/mm^3	0.56 × 0.41 × 0.25	0.56 × 0.38 × 0.21
Radiation	CuKα (λ = 1.54184)	MoKα (λ = 0.71073)
2Θ range for data collection/°	6.94 to 132.52	4.74 to 55.88
Index ranges	$-10 \leq h \leq 10$, $-19 \leq k \leq 21$, $-15 \leq l \leq 15$	$-9 \leq h \leq 11$, $-11 \leq k \leq 11$, $-12 \leq l \leq 16$
Reflections collected	10161	6777
Independent reflections	4917 [R_{int} = 0.0869, R_{sigma} = 0.0698]	4935 [R_{int} = 0.0420, R_{sigma} = 0.0726]

Data/restraints/parameters	4917/2/441	4935/3/217
Goodness-of-fit on F^2	1.044	1.042
Final R indexes [I>=2σ (I)]	R_1 = 0.0810, wR_2 = 0.2074	R_1 = 0.0456, wR_2 = 0.1113
Final R indexes [all data]	R_1 = 0.0870, wR_2 = 0.2135	R_1 = 0.0487, wR_2 = 0.1164
Largest diff. peak/hole / e $Å^{-3}$	4.42/-4.42	2.81/-2.73
Flack parameter	0.03(3)	0.06(2)

Table S2. Selected bond distances and angles for compounds **1** and **2**

Compound 1				Compound 2			
Bond	Length	Angle		Bond	Length	Angle	
Pt1-O1	1.97(3)	O1-Pt1-O2	93.9(10)	Pt1-O1	1.97(1)	O1-Pt1-N1	91.9(8)
Pt1-O2	1.93(2)	N1-Pt1-N2	83.7(11)	Pt1-N1	1.98(1)	N2-Pt1-N3	83.5(7)
Pt1-N1	2.03(2)			Pt1-N2	2.04(1)		
Pt1-N2	2.04(3)			Pt1-N3	2.06(2)		
O1-C1	1.31(3)			O1-C1	1.30(2)		
O2-C11	1.36(3)			N1-C11	1.30(2)		

Table S3. Activation enthalpies (Kcal/mol) for 1st and 2nd aquation of compounds 1, 2 and 3.

Comp. No.	Activation enthalpies in neutral condition for 1st aquation	Activation enthalpies in neutral condition for 2nd aquation	Activation enthalpies in acidic condition for 1st aquation	Activation enthalpies in acidic condition for 2nd aquation
1	31.8	14.7	10.0	20.6
2	24.7	9.8	6.0	26.1
3	24.6	11.5	21.7	44.0

Table S4. Composition and DLS data of compound **1** encapsulated liposome

Liposome composition (mol %)					DLS data		
Comp **1**	HSPC	POPC	Chol.	DSP-PEG	Zavg (nm)	PDI	Zpot (mV)
8.0	25.0	59	3	5	117.63±17.25	0.114±0.03	-16.26±2.25

Drug loading 2 mmol, determined by Pt-AAS

Table S5. Composition and DLS data of compound **2** encapsulated liposome

Liposome composition (mol %)					DLS data		
Comp **2**	HSPC	POPC	Chol.	DSP-PEG	Zavg (nm)	PDI	Zpot (mV)
6.0	25.0	61.0	3.0	5.0	121.26±9.11	0.113±0.02	-12.93±1.69

Drug loading 3 mmol, determined by Pt-AAS

Table S6. The IC_{50} (µm) values obtained for the formulated and DMSO solution of compounds in various cell lines tested. The data represents mean ± SD for at least 3 independent experiments.

Comp. No.	A549	HCT-116	MCF-7
Comp. 1(Liposome)	10.34 ± 1.1	12.93 ± 2.4	13.84 ± 1.6
Comp. 2 (Liposome)	3.48 ± 1.0	1.39 ± 0.3	1.28 ± 0.3
Comp.2(DMSO)	7.37 ± 0.78	17.42 ± 2.48	15.73 ± 3.11
Oxaliplatin	6.49 ± 0.9	3.52 ± 0.6	34.91 ± 3.8
Carboplatin	64.84 ± 3.6	81.68± 12.1	67.57± 19.2
PLY(DMSO)	> 100		

Table S7. logP values of the complexes **1-3**, Oxaliplatin and Carboplatin.

Compound	logP
1	3.47
2	3.40
3	3.32
Oxaliplatin	1.73
Carboplatin	1.06

References:

1. Vollano. J. F.; Al-Baker S.; Dabrowiak. J. C., Schurig J. E. *J. Med. Chem*. **1987**, *30*, 716.
2. Szoka F. J.; Papahadjopoulos D. *Ann. Rev. Biophys. Bioeng*. **1980**, *9*, 467-508.
3. Frisken B. J.; Asman C., Patty P. J. *Langmuir* **2000**, *16*, 928-933
4. (a) Klamt, A.; Schüürmann, G. *J. Chem. Soc., Perkin Trans. 2* **1993,** 799-805 (b) Andzelm, J.; Kölmel, C. *J. Chem. Phys.* **1995**, *103*, 9312–9320 (c) Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995–2001 (d) Cossi, M; Rega, N.; Scalmani, G.; Barone, V. *J. Comput. Chem.* **2003**, *24*, 669–681 (e) Takano, Y.; Houk, K. N. *J. Chem. Theory Comput*., **2005**, *1,* 70–77.
5. MarvinSketch (version 17.14 (2017), calculation module developed by ChemAxon, http://www.chemaxon.com/products/marvin/marvinsketch.
6. Viswanadhan, V. N.; Ghose, A. K.; Revankar, G. R.; Robins, R. K., J. Chem. Inf. Comput. Sci., 1989, 29, 163-172.
7. https://www.drugbank.ca/drugs/DB00958, http://www.hmdb.ca/metabolites/HMDB14667