Electronic Supplementary Information

Dynamic Supramolecular Self-Assembly of Platinum(II) Complexes Perturbs an Autophagy-Lysosomal System and Triggers Cancer Cell Death

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Experimental Details

Materials and reagents

$K_2[PtCl_4]$, tpy (2,2';6',2"-terpyridine) and $^tBu_3$tpy (4,4',4''-tri-*tert*-butyl-2,2':6',2''-terpyridine) were purchased from Aldrich. β-D-glucose pentaacetate, 4-iodophenol, trimethylsilyl acetylene, tetrabutylammonium fluoride (1.0 M solution in THF), copper(I) iodide, triethylamine, sucrose, nocodazole were obtained from TCI chemicals. The solvents used for synthesis were of analytical grade unless stated otherwise. Calf thymus DNA solution and bovine serum albumin were obtained from Thermo Fisher Scientific. GC-rich CC mismatched and telomeric G-quadruplex (HTelo) DNAs were purchased from Integrated DNA Technologies with sequences shown as below. β-glucosidase (from almonds) and human serum albumin were purchased from Sigma Aldrich. Carbonylcyanide m-chlorophenylhydrazone was purchased from Cayman chemical. Acridine orange, LysoTracker deep red, pepstatin A–BODIPY FL conjugate, annexin V-FITC conjugate, propidium iodide, JC-1 dye, 2'-7'dichlorofluorescin diacetate (DCFH-DA), FxCycle PI/RNase staining solution and mitochondria isolation kit with extraction reagents were purchased from Thermo Fisher Scientific. Primary antibodies of LC3B, γ-H2AX, GAPDH and Ki-67, as well as MitoTracker Green were purchased from Cell Signaling Technology.

Nucleic acids	DNA Sequence
GC-rich DNA	5′-CGCCGGCCCGGGCCCCGGGGCC-3'
	5′-GGCCCCGGGGCCCGGGCCGGCG-3'
CC mismatched DNA	5′-GCGCCGTCGTCCATGTG-3'
	5′-CACATGCACGACGGCGC-3'
Telomeric G-quadruplex (HTelo)	5'-TAGGGTTAGGGTTAGGGTTAGGG-3'

The purity of the platinum(II) terpyridyl acetylide complexes (**1a**, **1b**, **1c**, **1d** and **2**) was determined to be ≥ 98 %, as examined by UPLC/MS analysis (Fig. S10). The experimental and instrumental conditions are described in later part. All platinum(II) complexes for *in vitro* cell-based studies were reconstituted in dimethyl sulfoxide (DMSO) and then diluted with cell culture medium. The final concentration of DMSO in medium was at most 0.5 %. For *in vivo* animal experiments, phosphate buffered saline was used as a solvent vehicle. Cisplatin was freshly prepared in saline solution (NaCl, 0.9 % w/v) and immediately diluted in culture medium for *in vitro* experiments.

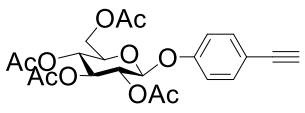
Instrumentations

^{1}H, ^{13}C and ^{195}Pt NMR spectra were recorded on Bruker AVANCE 400, 500 and 600

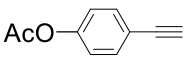
FT-NMR spectrometer at 298 K with chemical shift (in ppm) relative to residual non-deuterated solvent. UV–vis absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer or on a Perkin-Lambda 19 UV-vis spectrophotometer. Positive ion electrospray (ESI) mass spectra and liquid chromatograms were recorded by ultra-performance liquid chromatographic system coupled to quadrupole-time-of-flight tandem mass spectrometer (UPLC/QTOF-MS; Waters Micromass QTOF Premier). Elemental analyses were performed at the Institute of Chemistry of the Chinese Academy of Sciences, Beijing. TEM samples were prepared by drop casting a few drops of sample dispersion onto a formvar-coated copper grid on the specimen mount, followed by air-drying in the open atmosphere. TEM images were acquired using a transmission electron microscope (Philips CM100). Selected area electron diffraction (SAED) experiment was performed on a FEI Tecnai G2 20 S-TWIN transmission electron microscope with an accelerating voltage of 200 kV. Steady-state emission spectra of the binding study between the platinum complexes and human serum albumin (HSA) at 298 K were obtained on a SPEX Fluorolog-3 spectrofluorometer. The lipophilicity (Log *P*) of the platinum complexes was determined through measuring the Pt content partitioned in n-octanol and saline solution by using inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies 7500A, CA, U.S.A.). The UV–vis absorbance for the NBB and protein assays were recorded using microplate reader (Thermo Scientific Varioskan LUX). Binding affinities of the platinum(II) complexes towards DNAs were determined using isothermal titration calorimeter (MicroCal iTC200). Hydrodynamic particle size distribution studies were analyzed by Nanoparticle Tracking Analyzer (ZetaView). Flow cytometric analysis was performed on BD LSR Fortessa Analyzer. Cell imaging studies were performed using confocal laser scanning microscopes (Carl Zeiss LSM700 and 880).

Synthesis and Characterizations

Synthesis of arylacetylide ligands and platinum(II) terpyridyl acetylide complexes

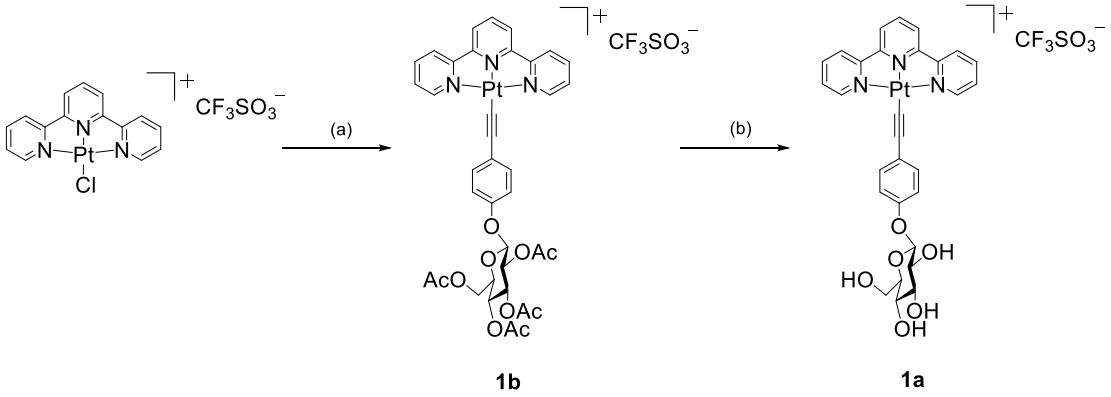


L1



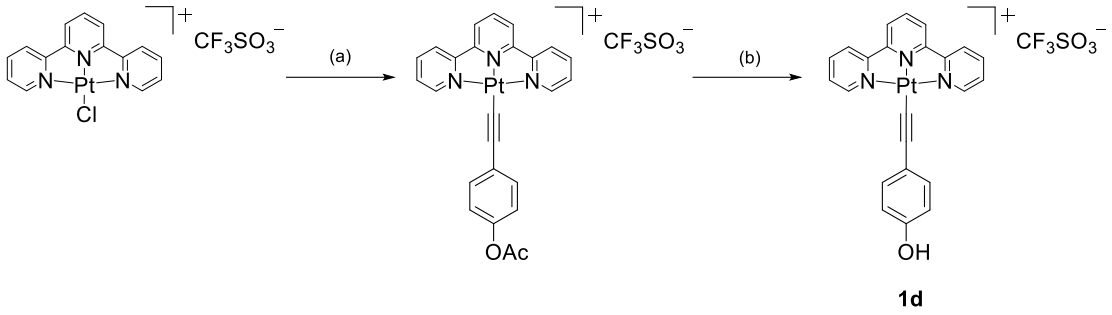
L2

All reactions were carried out under an inert atmosphere of nitrogen. Ligands **L1**[1, 2] and **L2**[3] as well as complexes $[Pt^{II}(tpy)Cl](CF_3SO_3)$[4], $[Pt^{II}(^tBu_3tpy)Cl](CF_3SO_3)$[5], **1b**[1] and **1c**[6] were synthesized according to the previously reported literature.



Scheme S1. Synthetic route for complex **1a**. (a) **L1**, CuI, Et_3N, degassed DMF, r.t., 18 h. (b) NaOMe (cat.), anhydrous MeOH, r.t., 2 h.

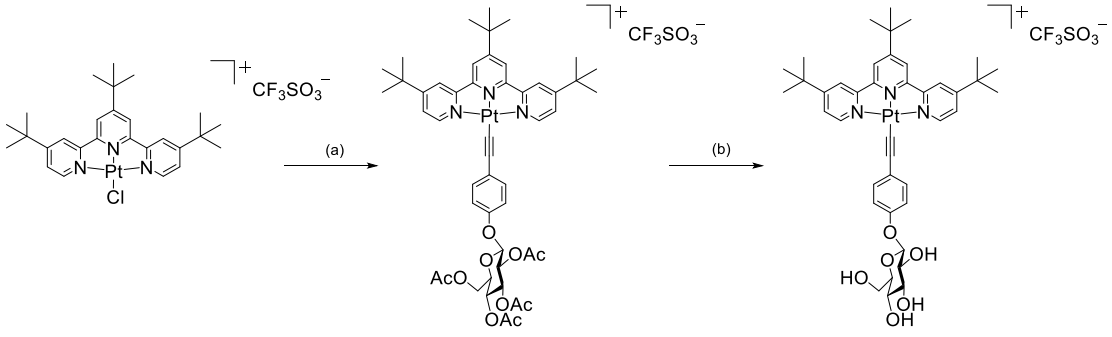
1a: To a solution of $[Pt^{II}(tpy)Cl](CF_3SO_3)$ (100 mg, 0.163 mmol) and **L1** (88 mg, 0.196 mmol) in degassed dimethylformamide (30 ml) and triethylamine (2 mL) was added a catalytic amount of copper(I) iodide. The solution mixture was stirred overnight at ambient temperature. After filtration and removing the dimethylformamide, the crude product was dissolved in dichloromethane and purified by flash chromatography on silica gel using dichloromethane-methanol (95:5 v/v) as eluent to afford **1b** as dark brown solid. Yield: 80 mg (48 %). **1b** (1.0 equiv.) was further dissolved in a solution of sodium methoxide (0.8 mg, 0.016 mmol, 0.2 equiv.) in anhydrous methanol (10 mL) and stirred for 2 h at ambient temperature. The solution was then neutralized with Amberlite® IR-120 (H^+) ion-exchange resin and filtered. The filtrate was concentrated. Recrystallization through a slow diffusion of diethyl ether into a concentrated dimethyl sulfoxide solution yield **1a** as a dark red solid. Yield: 54 mg (80 %). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.13-9.12 (d, *J* = 5.28 Hz, 2H), 8.65-8.62 (m, 4H), 8.58-8.55 (m, 1H), 8.50-8.48 (m, 2H), 7.92-7.90 (m, 2H), 7.44-7.42 (d, *J* = 8.68 Hz, 2H), 7.02-7.01 (d, *J* = 8.68 Hz, 2H), 5.36-5.35 (d, *J* = 5.08 Hz, 1H), 5.13-5.12 (d, *J* = 4.84 Hz, 1H), 5.07-5.06 (d, *J* = 5.38 Hz, 1H), 4.90-4.89 (d, *J* = 7.54 Hz, 1H), 4.61-4.59 (t, *J* = 5.68 Hz, 1H), 3.73-3.70 (m, 1H), 3.50-3.46 (m, 1H), 3.36-3.35 (m, 1H), 3.30-3.27 (m, 1H), 3.26-3.24 (m, 1H), 3.19-3.15 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 158.56, 155.83, 154.07, 153.75, 142.02, 141.89, 132.86, 129.63, 125.81, 124.15, 120.17, 116.82, 103.21, 97.86, 96.76, 73.82, 73.09, 71.56, 70.02, 60.82. ^{195}Pt NMR (107 MHz, DMSO-d_6): δ = –3129.59. HRMS (ESI): *m*/*z* for $[M–OTf]^+$ calcd: 707.1466; found: 707.1432. Elemental analysis calcd (%) for $C_{30}H_{26}F_3N_3O_9SPt·2.5H_2O$: C, 39.96; H, 3.47; N, 4.66; found: C, 40.09; H, 3.48; N, 4.66.



1d

Scheme S2. Synthetic route for complex **1d**. (a) **L2**, CuI, Et_3N, degassed DMF, r.t., 18 h. (b) K_2CO_3, ethanol, r.t., 3 h.

1d: To a solution of $[Pt^{II}(tpy)Cl](CF_3SO_3)$ (43 mg, 0.07 mmol) and **L2** (23 mg, 0.14 mmol) in degassed dimethylformamide (10 ml) and triethylamine was added a catalytic amount of copper(I) iodide. The solution mixture was stirred overnight at ambient temperature. After removing the dimethylformamide, the crude product was further dissolved in ethanol (10 mL). Potassium carbonate (48 mg, 0.35 mmol) was added and the resultant solution was stirred for 3 h at ambient temperature. The crude product was neutralized with 1M HCl solution, washed with deionized water and methanol, and recrystallized by slow diffusion of diethyl ether vapor into a concentrated acetonitrile solution to afford **1d** as dark brown solid. Yield: 27 mg (56 %). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.59 (s, 1H), 8.86-8.85 (m, 2H), 8.50-8.42 (m, 5H), 8.40-8.36 (t, *J* = 7.8 Hz, 2H), 7.81-7.78 (t, *J* = 6.54 Hz, 2H), 7.23-7.21 (d, *J* = 8.58 Hz, 2H), 6.77-6.75 (d, *J* = 8.58 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 158.45, 156.30, 153.73, 153.59, 141.86, 141.65, 133.04, 129.47, 125.73, 124.08, 117.12, 115.14, 103.53, 95.15. ^{195}Pt NMR (107 MHz, DMSO-d_6): δ = –3129.36. HRMS (ESI): *m*/*z* for $[M–OTf]^+$ calcd: 545.0937; found: 545.0911. Elemental analysis calcd (%) for $C_{24}H_{16}F_3N_3O_4SPt$: C, 41.50; H, 2.32; N, 6.05; found: C, 41.35; H, 2.48; N, 6.02.



2

Scheme S3. Synthetic route for complex **2**. (a) **L1**, CuI, Et_3N, degassed CH_2Cl_2, r.t.,

18 h. (b) NaOMe (cat.), anhydrous MeOH, r.t., 2 h.

2: The procedure was similar to that for **1a**, except that [Pt^{II}(^{t}Bu$_3$tpy)Cl](CF_3SO_3) (30 mg, 0.038 mmol) was used as the precursor. The resulting crude product was purified using flash chromatography on silica gel using dichloromethane-methanol (9:1 v/v) as eluent to afford **2** as an orange solid. Yield: 24 mg (61 %). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.03-9.02 (d, *J* = 5.96 Hz, 2H), 8.73 (s, 2H), 8.72-8.71 (m, 2H), 7.92-7.90 (m, 2H), 7.42-7.41 (d, *J* = 8.72 Hz, 2H), 7.02-7.00 (d, *J* = 8.72 Hz, 2H), 5.34-5.33 (d, *J* = 5.02 Hz, 1H), 5.12-5.11 (d, *J* = 4.84 Hz, 1H), 5.06-5.05 (d, *J* = 5.38 Hz, 1H), 4.89-4.87 (d, *J* = 7.54 Hz, 1H), 4.59-4.58 (t, *J* = 5.68 Hz, 1H), 3.73-3.70 (m, 1H), 3.49-3.45 (m, 1H), 3.36-3.35 (m, 1H), 3.30-3.23 (m, 2H), 3.18-3.15 (m, 1H), 1.53 (s, 9H), 1.44 (s, 18H). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 166.85, 166.26, 158.54, 156.06, 153.65, 132.71, 125.89, 123.56, 121.48, 120.35, 119.61, 116.13, 102.99, 100.35, 97.53, 77.13, 76.64, 73.23, 69.76, 60.75, 37.23, 36.21, 30.28, 29.80. ^{195}Pt NMR (107 MHz, DMSO-d_6): δ = –3132.16. HRMS (ESI): *m*/*z* for $[M–OTf]^+$ calcd: 875.3345; found: 875.3312. Elemental analysis calcd (%) for $C_{42}H_{50}F_3N_3O_9SPt·H_2O·0.5CH_2Cl_2$: C, 47.03; H, 4.92; N, 3.87; found: C, 47.23; H, 4.81; N, 3.80.

Stability Studies

The platinum(II) complex **1a** (10 μM) were freshly prepared in 10 mM ammonium bicarbonate buffer containing 5 % MeOH, and/or co-incubated with glutathione (1 mM) at 37 ℃ for 24 h. An aliquot of each solution mixture at the indicated time were diluted 10-fold with methanol for QTOF mass spectrometric analysis. The QTOF mass spectrometer was operated in positive mode and the conditions were optimized as follows: source temperature, 120 ℃; desolvation temperature, 400 ℃; nebulization gas flow rate, 800 L/h; cone gas flow rate, 20 L/h; capillary voltage, 3.0 kV; sampling cone voltage, 25 V. The TOF *m*/*z* scan range was set from 250 to 1100 Da. The stability of **1a** in RPMI 1640 culture medium was examined according to the reported procedure with modification.[7] Briefly, complex **1a** (10 μM) was incubated in culture medium containing 0.5 % DMSO at 37 ℃ for 24 h. Followed by protein precipitation with three volumes of ice-cold acetonitrile and solvent extraction by methanol, the extracts were subjected to UPLC/MS analysis. For the liquid chromatographic conditions, a BEH C18 analytical column (1.7 μm, 50 × 2.1 mm; Waters ACQUITY) in combination with a BEH C18 guard column (1.7 μm, 5 × 2.1 mm) was used. The mobile phase was a mixture of 0.1 % formic acid in water (eluent A) and 0.1 % formic acid in acetonitrile (eluent B). Separation was achieved by using 5–100% eluent B from 0 to 20 min. The flow rare was 0.4 mL/min and the injection volume was 3 μL.

Biological Studies

Cell Culture

NCI-H460, A2780, PLC, HCT116, MDA-MB-231 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing fetal bovine serum (FBS, 10 %) and penicillin/streptomycin (100 U/mL, 1 %) and maintained at 37 ℃ in a humidified environment with 5 % CO_2. For normal lung fibroblast cell line (CCD-19Lu), the cells were maintained in minimum essential medium (MEM) supplemented with FBS (10 %) and penicillin/streptomycin (100 U/mL, 1 %). NCI-H460 cells expressing mRFP-GFP-tagged LC3 were obtained by stable transfection of the cells with tfLC3 plasmid (Addgene plasmid #21074, a gift from Dr. Tamotsu Yoshimori) using Lipofectamine 3000 (Thermo Fisher).

***In Vitro* Anti-proliferative Assay**

Different cancer (4–6 × 10^3 cells/well) and normal cells (1 × 10^4 cells/well) were seeded in flat-bottomed 96-well plate, respectively for 24 h. Cells were then incubated with varying concentration of complexes **1a**–**1d** or cisplatin, respectively for 72 h. After removing the culture medium, the cells were fixed with formaldehyde (3 %; 50 µL) in PBS and stained with naphthol blue black reagent (0.05 %, 0.1 M sodium acetate, 9 % acetic acid; 50 µL) for overnight. The stained cells in each well were washed thrice gently with deionized water after removing the staining reagent and solubilized in sodium hydroxide solution (50 mM; 100 µL). The cell viability was determined by measuring the absorbance of 620 nm by multiplate reader (Varioskan LUX, Thermal Scientific). The experiments were repeated in triplicate. The IC_{50} values are presented as the mean ± standard deviation. For the study of time-dependent anti-proliferative activity, the experimental procedures are similar, except that the cells were exposed to complexes **1a**, **1b** and cisplatin for 24 and 48 h, respectively.

Absorption Titration with DNAs

A solution of platinum(II) complex (30 µM) in Tris-HCl buffer (5 mM, pH 7.4) was titrated against increasing concentrations of DNAs (ctDNA or telomeric G-quadruplex (HTelo) DNA, 0–90 µM). The absorption spectra of samples were recorded 1 min after each addition of the DNA aliquots.

Measurement of DNA Binding Affinity by Isothermal Titration Calorimetry

For the determination of the binding constant for the interactions between the platinum(II) complexes and DNAs (ctDNA, CC mismatched DNA and GC-rich DNA), DNAs (50 µM) in aqueous buffer (Tris-HCl, 5 mM and NaCl, 25 mM, pH 7.4) containing 5 % DMSO was titrated against increasing concentrations of platinum(II)

complexes (0–100 μM). Heat released from each addition was recorded and the data of heat changes throughout the titration were fitted by using a non-linear least square method. The binding association constant (*K*) of the biomolecular interactions was obtained from the best-fitted graph. The apparent binding dissociation constant, $K_{D,}$ is the inverse of *K*.

Electron Microscopic Characterization of Superstructures

A dimethyl sulfoxide solution of platinum(II) complexes (**1a**–**1d** and **2**; 10 mM) was added into aqueous solution to afford solution mixture with final organic solvent content in 1 %. A drop of different complexes in the solution mixture was then deposited on glow-discharged copper grids and air-dried for 10 min. The TEM images were taken on a transmission electron microscope (Philips CM100). For the physiological buffered conditions, **1a** in aqueous buffer solutions were freshly prepared by adding an aliquot of DMSO stock solution into phosphate buffered saline at pH 7.4 and 5.8, respectively, with final organic solvent content in 1 %. For the enzymatic condition, **1a** was incubated with β-glucosidase (0.5 unit, 0.49 μM) in phosphate buffered saline at pH 5.8 for 24 h. (1 unit of β-glucosidase corresponds to the amount of enzyme which generate 1 μmol of glucose per minute at pH 5.0 and 37 ℃ using salicin as substrate). For the buffer solution with the presence of serum protein, an aliquot of DMSO stock solution of **1a** was added into phosphate buffered saline (pH 7.4) containing 10 % fetal bovine serum with final organic solvent content in 1 %.

^{1}H NMR Measurement

The aggregation property of platinum(II) complex **1a** was monitored by ^{1}H NMR measurement. Complex **1a** was freshly prepared in deuterated dimethyl sulfoxide solution and with increasing percentage of D_2O (0–98 %) with final concentration of 200 μM, respectively. ^{1}H NMR spectra of different solution mixtures were recorded at 298 K and the spectra were compared using TopSpin software.

Particle Size Distribution Analysis

Complex **1a** (100 μM) in aqueous solution containing 1 % DMSO, phosphate buffer saline containing 10 % fetal bovine serum and 1 % DMSO, or RPMI 1640 culture medium containing 10 % fetal bovine serum and 0.5 % DMSO were prepared for the particle size and concentration measurements using ZetaView PMX-120 Nanoparticle Tracking Analyzer.

LC/MS Analysis of *In Vitro* Enzymatic Cleavage Reaction

To a 10 μM solution of **1a** in phosphate buffered saline at pH 5.8 containing 0.5 %

DMSO, 0.5 unit (0.49 μM) of β-glucosidase was added, and the reaction mixture was incubated at 37 ℃ for 24 h. The solution mixture without the addition of β-glucosidase was prepared as control. An aliquot of each solution mixture was mixed with 10 volumes of methanol to stop the enzymatic reactions, vacuum-dried. The pellets were resuspended in methanol for UPLC/MS analysis. The instrumental conditions were similar as that of stability analysis, except that the separation was achieved by using 5–100 % eluent B from 0 to 10 min. For the cleavage study of **1a** *in cellulo*, the experimental procedures were similar, except that NCI-H460 cells were treated with **1a** (10 μM) or DMSO vehicle for 24 h.

Cellular Uptake and Distribution Study

NCI-H460 cells (1×10^5 cells/well) were seeded in 12-well plate for 24 h. Then, the cells were treated with different platinum(II) complexes (10 μM) in RPMI culture medium in time-dependent manner. Cells were then washed thrice with PBS. Deionized water (130 μL) was added onto the cell monolayer for the cell lysis and the cell lysate (100 μL) were digested with conc. HNO_3 (200 μL) at 70 ℃ for 18 h. The amount of platinum (μg/L) in cell lysate was determined by ICP-MS analysis and normalized with the protein content as examined by Bradford protein assay. For the comparative study in cellular uptake between NCI-H460 lung cancer cells and CCD-19Lu normal lung fibroblasts, the experimental procedures were similar as that of time-dependent study. For the energy- or endocytosis-dependent cellular uptake studies, the experimental procedures were similar, except that the cells were pre-treated at 4 ℃ or with endocytosis inhibitors (sucrose, 450 mM or nocodazole, 50 μM) for 30 min, followed by the treatment of complexes **1a** or **1d** for 2 h. For quantifying the cellular distribution of platinum content, the mitochondrial, nuclear and cytosolic fractions of **1a**- or **1d**-treated cells after 24h-treatment were separated by using mitochondria isolation kit (Thermo Scientific) and digested with concentrated HNO_3 (200 μL) at 70 ℃ for 18 h.

Lipophilicity Measurement (Log *P*)

Platinum complexes **1a**–**1d** (10 μM) in 500 μL *n*-octanol (saturated with sodium chloride) was mixed with equal volume of aqueous sodium chloride solution (0.9 %, w/v). The mixture was shaken for 1 h at 298 K to allow partitioning. Following centrifugation of the sample at 1000 × g for 10 min to separate two different layers, an aliquot (10 μL) of each phase were digested with conc. HNO_3 (290 μL) at 70 ℃ for 18 h. The amount of platinum (μg/L) in two different phases was determined by ICP-MS analysis. The log *P* was determined as the logarithmic ratio of the concentration of platinum in the *n*-octanol and aqueous phases.

Acridine Orange (AO) Staining

NCI-H460 cells (1×10^5 cells/well) were seeded in 3.5 cm glass-bottom confocal dishes for 24 h. The cells were exposed to complex **1a**, **1d**, cisplatin (10 μM), DMSO vehicle or saline vehicle for 24 h. The cells were then stained with acridine orange (10 μM) at 37 ℃ for 0.5 h. Following washing the cells twice with PBS, the cells were imaged with a confocal laser scanning microscope (LSM 880, Carl Zeiss; excitation at 488 nm, and emission from 515 to 545 nm and from 610 to 640 nm).

Cell Imaging with Organelle Trackers

NCI-H460 cells (1×10^5 cells/well) were seeded in 3.5 cm glass-bottom confocal dishes for 24 h. The cells were exposed to complex **1a**, **1d**, cisplatin (10 μM), DMSO vehicle or saline vehicle for 24 h. The cells were then stained with LysoTracker™ Deep Red (50 nM) and MitoTracker Green (50 nM) at 37 ℃ for 15 min. The cells were then washed twice with PBS and then imaged with a confocal laser scanning microscope (LSM 880, Carl Zeiss) using green (excitation at 488 nm, and emission from 515 to 545 nm) and red (excitation at 633 nm, and emission from 650 to 740 nm) fluorescence channels.

Pepstatin A–BODIPY FL Conjugate and LysoTracker Costaining

NCI-H460 cells (1×10^5 cells/well) were seeded in 3.5 cm glass-bottom confocal dishes for 24 h. The cells were exposed to complex **1a**, **1d**, cisplatin (10 μM), DMSO vehicle or saline vehicle for 24 h. The cells were then stained with pepstatin A–BODIPY FL conjugate (1 μM) and LysoTracker™ Deep Red (50 nM) at 37 ℃ for 0.5 h. Following washing the cells twice with PBS, the cells were imaged with a confocal laser scanning microscope (LSM 880, Carl Zeiss) using green (excitation at 488 nm, and emission from 500 to 545 nm) and red (excitation at 633 nm, and emission from 650 to 740 nm) fluorescence channels.

Immunofluorescence Staining

NCI-H460 cells (1×10^5 cells) were seeded in 3.5 cm glass-bottom confocal dishes for 24 h. The cells were treated with **1a**, cisplatin (10 μM) or DMSO vehicle for 24 h, respectively. Treated cells were fixed with paraformaldehyde (4 %) in PBS for 10 min, permeabilized with Triton X-100 (0.1 %) solution for 5 min and blocked with BSA (3 %, w/v) in PBST solution for 1 h. The cells were incubated with primary rabbit monoclonal anti-γ-H2AX (1:400) or rabbit monoclonal anti-LC3B (1:1600) overnight at 4℃, followed by labeled with anti-rabbit Alexa Fluor 488-conjugated secondary antibodies. The cells were washed twice with PBS and the nuclei were counterstained with DAPI (1 μg/mL) for 5 min. The cells were imaged with a confocal laser scanning microscope (LSM 880, Carl Zeiss)

Live Cell Imaging of mRFP-GFP-tagged LC3

NCI-H460 cells stably transfected with mRFP-GFP-tagged LC3 (1×10^5 cells) were seeded in 3.5 cm glass-bottom confocal dishes for 24 h. The cells were treated with **1a** (10 μM) or DMSO vehicle for 24 h. Treated cells were washed twice with PBS and imaged with a confocal laser scanning microscope (LSM 880, Carl Zeiss). For mRFP-tagged LC3: excitation at 555 nm and emission from 560 to 700 nm; for GFP-tagged LC3: excitation at 488 nm and emission from 500 to 530 nm.

Bio-transmission Electron Microscopic (Bio-TEM) Study

NCI-H460 cells (1×10^5 cells/well) were seeded in a 6-well plate for 24 h. The cells were treated with **1a** or **1d** (10 μM) for 24 h. The cells were fixed by ice-cold glutaraldehyde (2.5 % in 0.1 M cacodylate buffer, pH 7.4) at 4 ℃ overnight. After washing the cells twice with cacodylate buffer (100 mM, pH 7.4), cells were post-fixed in 1 % OsO_4 (w/v, pH 7.2) for 15 min at room temperature and dehydrated gradually with increasing concentration of ethanol. Cells were embedded into epoxy resin. Ultrathin sections with 100 nm of thickness were cut by ultramicrotome (Leica), and doubly stained with uranyl acetate and lead citrate. Following the deposition of samples on copper grids, the samples were washed with deionized water and air-dried, the images were taken on a transmission electron microscope (CM100, Philips).

Immunoblotting

NCI-H460 cells (1×10^5 cells/well) were seeded in a 6-well plate for 24 h. The cells were then dose-dependently exposed to complex **1a** (0, 1, 5, 10, 30 μM) or cisplatin (0, 10, 20 μM) for 6 h or time-dependently (6, 12, 24 h) treated with **1a** (10 μM). After washing the cells thrice with ice-cold PBS, cells were harvested and lysed using lysis buffer (100 μL; 50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 1 % NP-40, 0.5 % sodium deoxycholate, 0.1 % SDS, 10 mM NaF, 5 mM sodium pyrophosphate, 5 mM sodium orthovanadate) with protease inhibitor cocktail on ice. Following centrifugation for the collection of cellular lysate, the concentration of cellular protein was quantified by the Bradford Protein Assay (Bio-Rad). Total protein samples (20–25 μg/lane) were separated on a SDS–PAGE in a Tris-Glycine running buffer and blotted on polyvinylidenefluoride (PVDF) membranes. The PVDF membranes were then blocked in TBST (20 mM Tris-HCl, pH 7.6, 0.1 % Tween 20; v/v) containing BSA (3 %, w/v) at room temperature for 1 h, followed by incubation with the primary antibody (LC3 (1:2000) or GAPDH (1:2000)) at 4 ℃ overnight and the respective secondary antibody conjugated with horseradish peroxidase (1:5000) for 2 h. Detection was performed by using the chemiluminescence procedure (ECL, GE healthcare). Equal loading of the proteins in each lane was verified by the intensity of

GAPDH.

Mitochondrial Depolarization Assay

NCI-H460 cells (5×10^4 cells/well) were seeded in a 24-well plate for 24 h. The cells were treated with **1a**, **1d**, cisplatin (10 μM), DMSO vehicle or saline vehicle for 24 h. JC-1 (5 μM) staining solution was then added to the cells and incubation was continued for 20 min in dark. The cells were then washed with PBS for twice, trypsinized and subjected to flow cytometric analysis (BD LSR Fortessa) by measuring the fluorescence with excitation at 488 nm, and emission filters at 515–545 nm and 580–595 nm. For positive control, carbonylcyanide m-chlorophenylhydrazone (CCCP, 50 μM) and JC-1 (5 μM) were co-incubated with the cells for 15 min and the fluorescence intensity was measured under the similar conditions. Data were analyzed by FlowJo software.

Intracellular Detection and Measurement of ROS Generation

NCI-H460 cells (5×10^4 cells/well) were seeded in a 24-well plate for 24 h. The cells were treated with **1a**, **1d** (10 μM) or DMSO vehicle for 24 h. Following the treatment, cells were then incubated with DCFH-DA (10 μM) for 15 min, washed twice in PBS, trypsinized and subjected to flow cytometric analysis (BD LSR Fortessa) by measuring the fluorescence with excitation at 488 nm, and emission filters at 515–545 nm.

Cell Cycle Analysis

NCI-H460 cells (1×10^5 cells/well) were seeded in a 6-well plate for 24 h. The cells were treated with **1a**, **1d**, cisplatin (10 μM) or DMSO vehicle for 24 and 48 h. Following the treatment, cells were then washed twice in PBS, trypsinized, fixed in cold ethanol (70 %) for 30 min and re-suspended in FxCycle PI/RNase staining solution (300 μL; Thermo Scientific) for 15 min. Samples were then subjected to flow cytometric analysis (BD LSR Fortessa) using Phycoerythrin (PE) channel (excitation at 561 nm and emission from 580–595 nm).

Annexin V-FITC/PI Double Staining Assay

NCI-H460 cells (1×10^5 cells/well) were seeded in a 6-well plate for 24 h. The cells were treated with **1a**, **1d**, cisplatin (20 μM), DMSO vehicle or saline vehicle in RPMI culture medium for 72 h. Following washing the cells twice with PBS and trypsinization, the cells were re-suspended in binding buffer (10 mM Hepes, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4) for staining with annexin V-FITC and propidium iodide (1 μg/mL) for 20 min at room temperature. Samples were then subjected to flow cytometric analysis (BD LSR Fortessa) using FITC (excitation at 488 nm,

emission from 515–545 nm) and PE channels (excitation at 561 nm, emission from 580–595 nm). Data were analyzed by FlowJo software.

Thermal Proteome Profiling Study[8]

NCI-H460 cells (5×10^6 cells/dish) were seeded in 10-cm dishes for 24 h. The cells were treated with **1a** (20 μM) or DMSO vehicle for 2 h. Following washing the cells twice with PBS, trypsinization and evenly distributing into ten 0.2 mL PCR tubes, aliquots of the **1a**- and DMSO-treated cells were heated in parallel at designated temperature (37, 41, 44, 47, 50, 53, 56, 59, 62 and 65 ℃) in a thermocycler for 3 min and kept at room temperature for another 3 min. The cells were then snap-frozen in liquid nitrogen and lyzed with three freeze-thaw cycles. Following centrifugation of the cell lysates at 100,000 × g at 4 ℃ for 20 min, the soluble proteins in the supernatant were collected and the concentrations of which at 37 ℃ were measured and normalized in the amount of proteins used for trypsin digestion.

Proteins were denatured in urea buffer (6 M urea in 100 mM Tris-HCl, pH 8.0) at 60 ℃ for 10 min, reduced with dithiothreitol and carboxyamidomethylated by iodoacetamide. The concentration of urea was diluted to 1 M with 100 mM Tris buffer (pH 8.0) before trypsin digestion at 37 ℃ for 16 h. The proteolysis was halted by adding formic acid (5%) and the digests were subjected to tandem mass tag (TMT) labeling.[9]

Peptides were labeled with 10-plex TMT (TMT10, Thermo Fisher Scientific) according to the manufacturer's protocol, enabling relative quantification of ten different temperature points from 37 ℃ to 65 ℃. The labeling reactions were performed in 50 mM triethylammonium bicarbonate (TEAB) buffer for 1 h at room temperature and quenched with hydroxylamine. The TMT-labeled peptides of **1a**- or DMSO vehicle treatments were combined into a single sample, desalted using stop-and-go-extraction tips (StageTips) and subjected to LC-MS/MS analysis.

Mass spectrometric data were collected on a FAIMS Pro™ equipped Orbitrap Exploris™ 480 mass spectrometer coupled to an UltiMate™ 3000 RSLCnano system connected with an with EASY-Spray™ PepMap™ RSLC C18 column (2 μm, 100 Å, 75 μm x 75 cm) (Thermo Scientific). TMT-labeled samples were resuspended in buffer A (LC-MS grade water with 0.1% formic acid (v/v)) and subjected for injection. Peptides were separated by a 260-min organic solvent gradient at a flow rate of 250 nL/min. The Orbitrap was operated with a positive ion voltage of 2600 V and a transfer tube temperature of 250 ℃. The full scans from 375 to 1575 *m*/*z* were recorded at a resolution of 60000. The HCD MS/MS scans were recorded at a resolution of 45000 with isolation window of 0.7 Da, collision energy (%) of 35 and the first mass of 110. For data acquisition including FAIMS, the dispersion voltage (DV) was set at −5000 V, the compensation voltages (CVs) were set at −45, −60, −75

and –90 V.

Proteins were identified by searching from the Uniprot Human Database (downloaded: Aug 10, 2021) using Protein Discoverer (version 2.5, Thermo Scientific). The search type was set to Reporter ion MS2 with 10-plex TMT. The protein abundance at 37 ℃ was set to 1 and relative protein quantification at each temperature was calculated. TPP R package was used to determine the melting curves and significant T_m shifts of the proteins.[10]

In Vivo Anti-tumor Study

All animal experiments were conducted under the guidelines approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. Balb/cAnN-nu nude mice (ca. 4–5 weeks old) were obtained from Charles River Laboratory (Wilmington, MA). To generate the NCI-H460 tumors, 4×10^6 NCI-H460 cells suspended in PBS (100 μL) were subcutaneously injected into the right back flank of each mouse. The mice were started to treat when the tumor volumes reached about 50 mm^3; the mice were randomly divided into two groups with six mice per group for (A) PBS vehicle and (B) complex **1a** (25 mg/kg). Mice were treated with vehicle control or **1a** according to the body weight thrice weekly by intravenous injection until the mice were sacrificed after 14 days of treatment. Treatment of mice with cisplatin (1.5 mg/kg) acting as a positive control was performed under similar administration regimen for 14 days. Tumor sizes were measured thrice weekly and the tumor volumes were calculated based on the formula ($V = ab^2 \times 0.52$; where a and b represent the length and width of the tumor). The tumor weight of mice in different treatments was also measured based on the tumor tissues harvested at the end of the experiment.

In Vivo Histopathological and Immunohistochemical Studies

At the end of the *in vivo* experiments, tumor tissues and major organs (liver, spleen, kidney, heart, lung) were harvested, fixed in 4 % formalin for overnight, embedded in paraffin and sectioned at 4 μm thickness. Following the standard protocol of haematoxylin and eosin (H&E) staining,[11] the slices were examined under an optical microscope (Olympus). For immunohistochemical staining, the paraffin-embedded tumor sections were de-waxed in xylene and rehydrated through graded alcohols. Antigen retrieval was performed with EDTA buffer (pH 8.0) in a pressure cooker for 5 min at 200 ℃, followed by inhibition of endogenous peroxidases using 3 % aqueous hydrogen peroxide for 10 min at room temperature. After incubation with primary anti-Ki-67 rabbit monoclonal antibody (1:400; Cell Signaling Technology) overnight at 4℃, sections were incubated with Primary Antibody Amplifier Quanto (UltraVision Quanto Detection system; Thermo Scientific) for 20 min and

horseradish-peroxidase (HRP) Polymer Quanto for 30 min. The expression of Ki-67 was developed with liquid 3,3'-diaminobenzidine (DAB) substrate (Thermo Scientific) and the nuclei were counterstained with Mayer's haematoxylin. Following the dehydration in graded alcohol, the stained sections were mounted with coverslip for examination under optical microscope.

***In Vivo* Blood Biochemistry Analysis**

The blood samples of tumor-bearing mice after treatment with PBS vehicle or **1a** (25 mg/kg) were collected at the end of experiment (day 14). The samples were then subjected to the determination of the biochemical indexes of alanine aminotransferase (ALT) and creatinine (CREA) to evaluate the functions of liver and kidney, respectively, using the commercial assay kits (BioVision) according to the manufacturer's protocol.

NMR Spectra

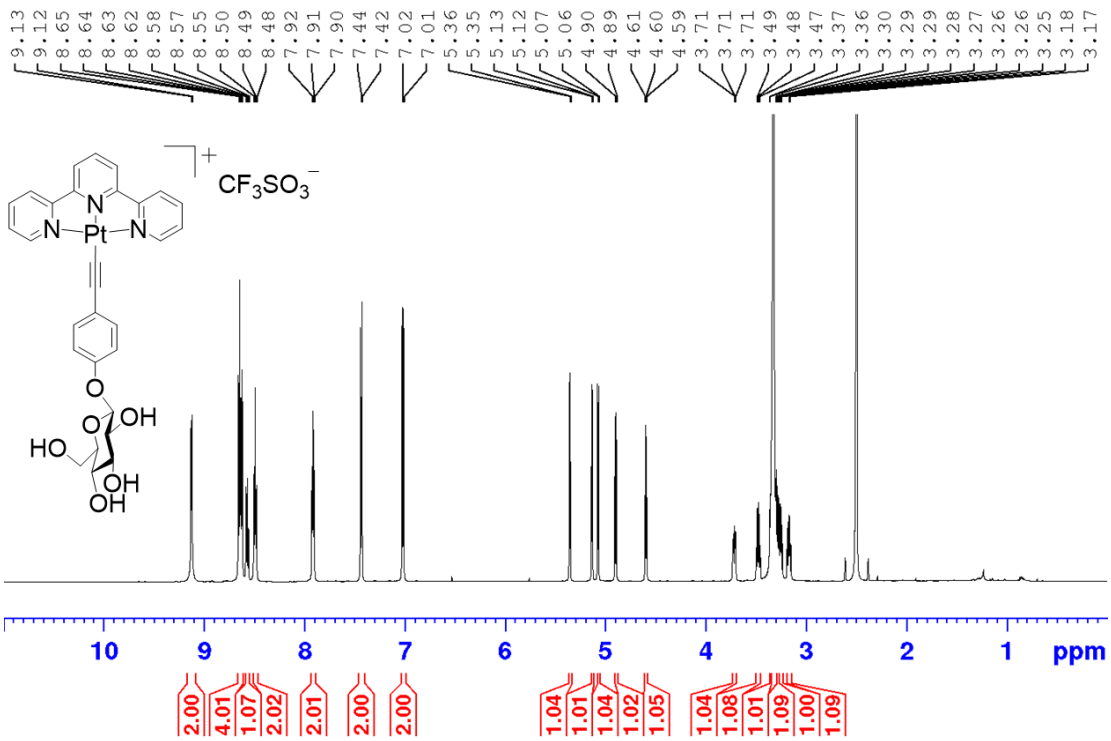


Fig. S1 ^{1}H NMR spectrum of **1a** in DMSO-d_6 at 298 K.

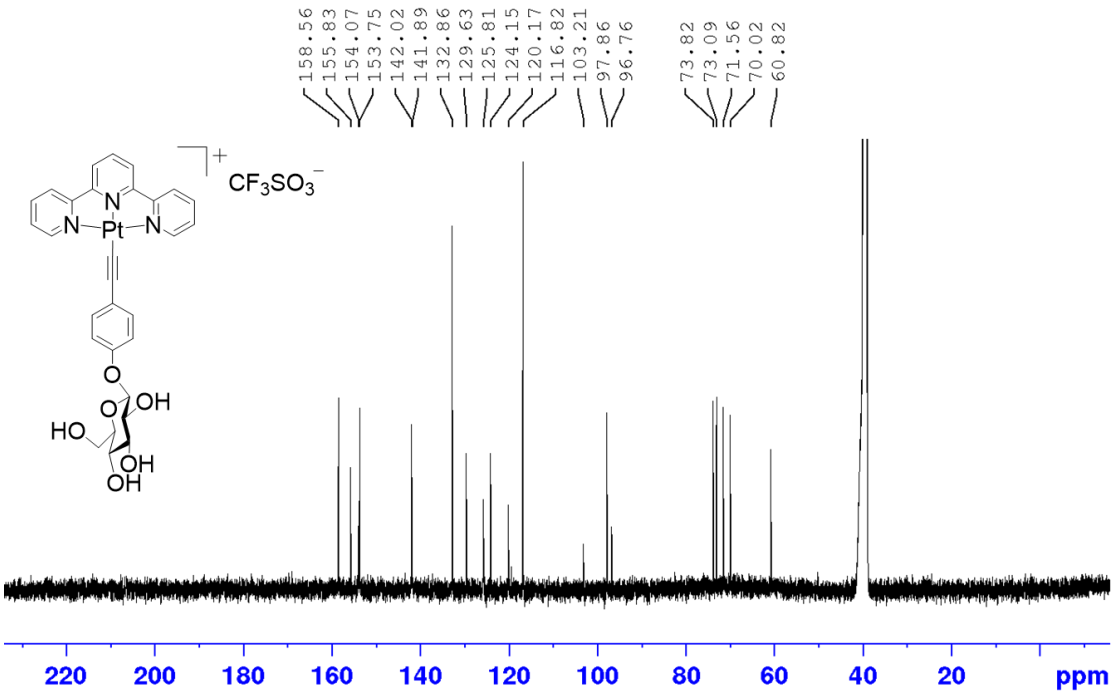


Fig. S2 ^{13}C NMR spectrum of **1a** in DMSO-d_6 at 298 K.

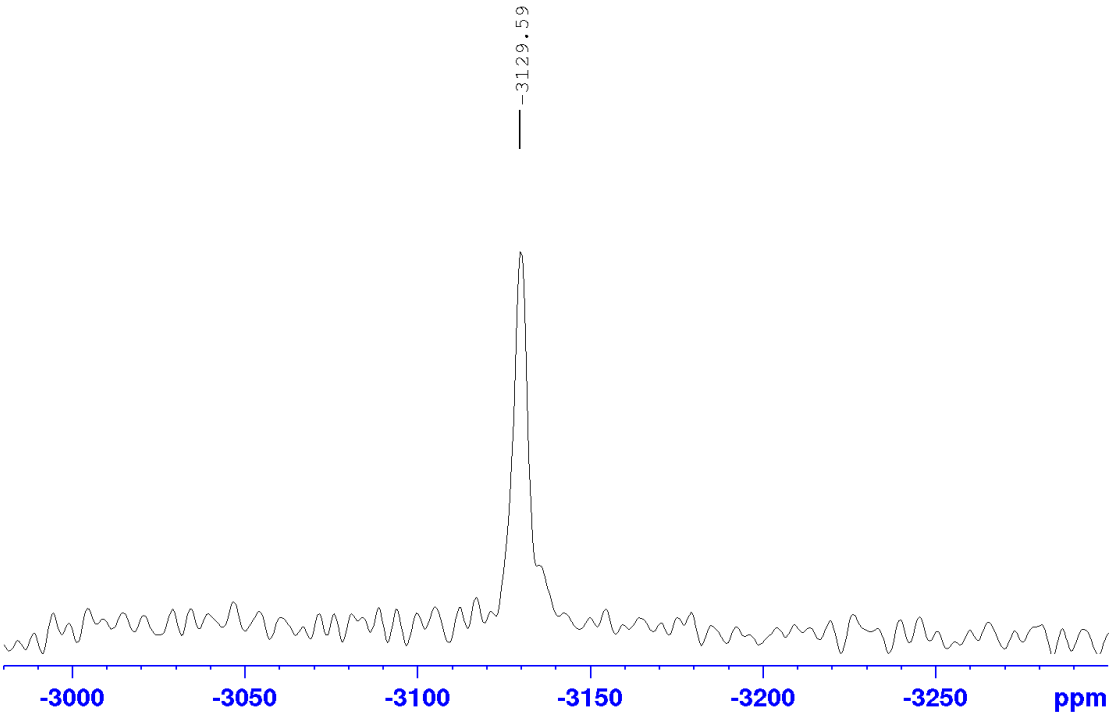


Fig. S3 ^{195}Pt NMR spectrum of **1a** in DMSO-d_6 at 298 K.

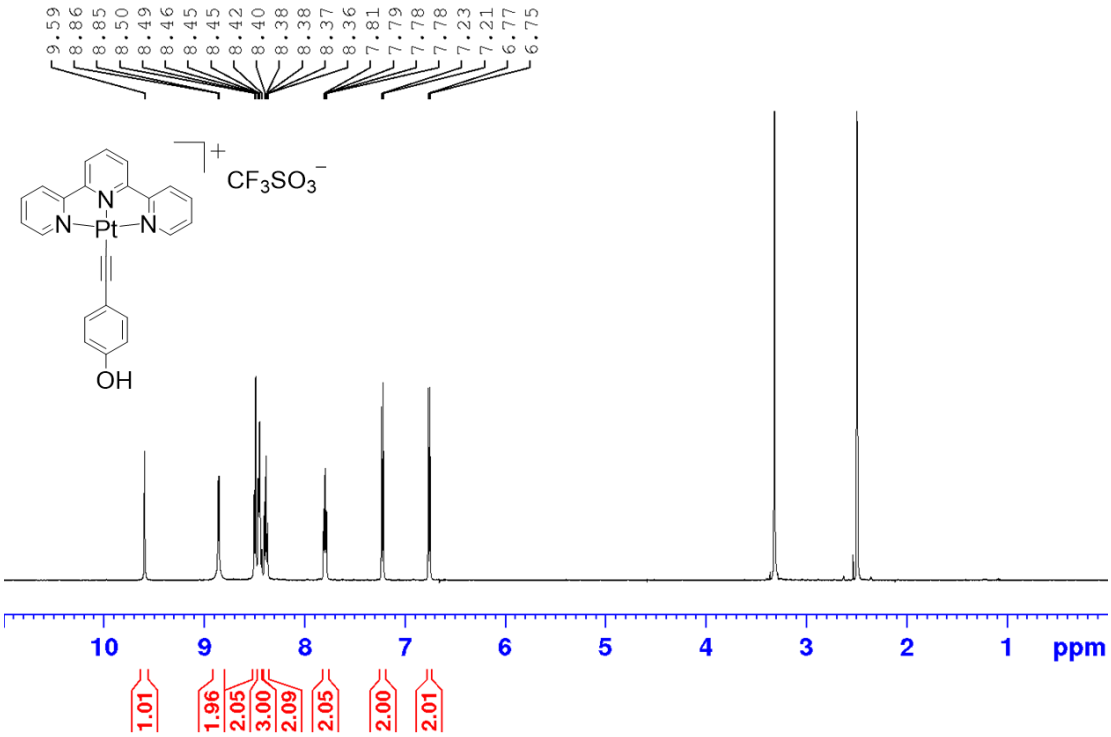


Fig. S4 ^{1}H NMR spectrum of **1d** in DMSO-d_6 at 298 K.

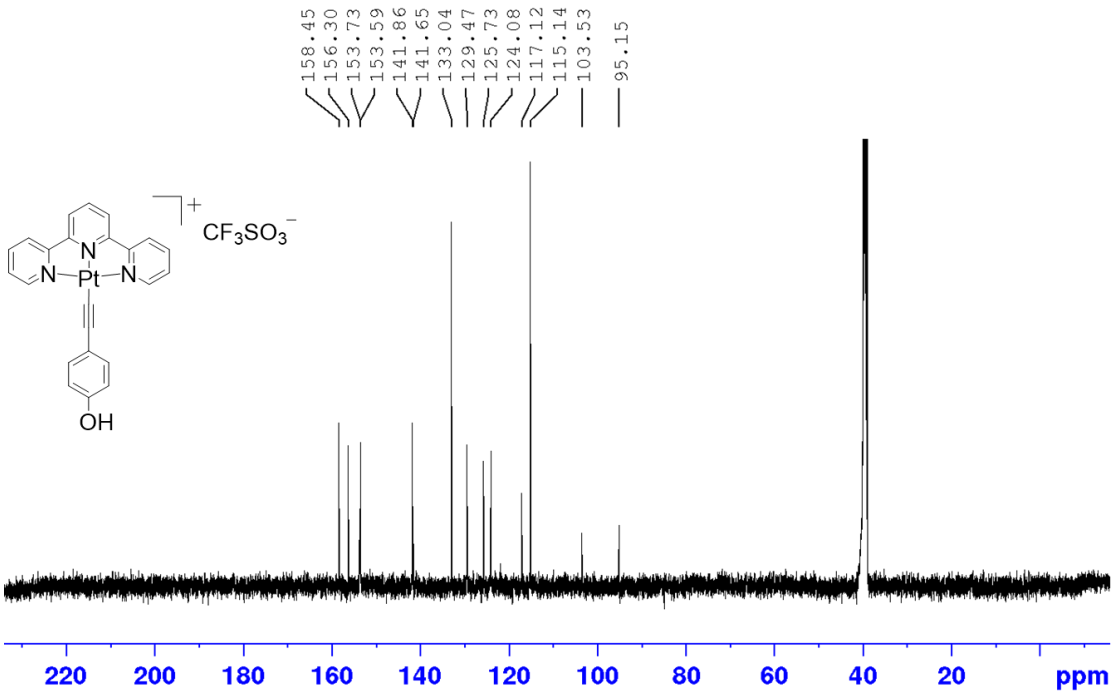


Fig. S5 ^{13}C NMR spectrum of **1d** in DMSO-d_6 at 298 K.

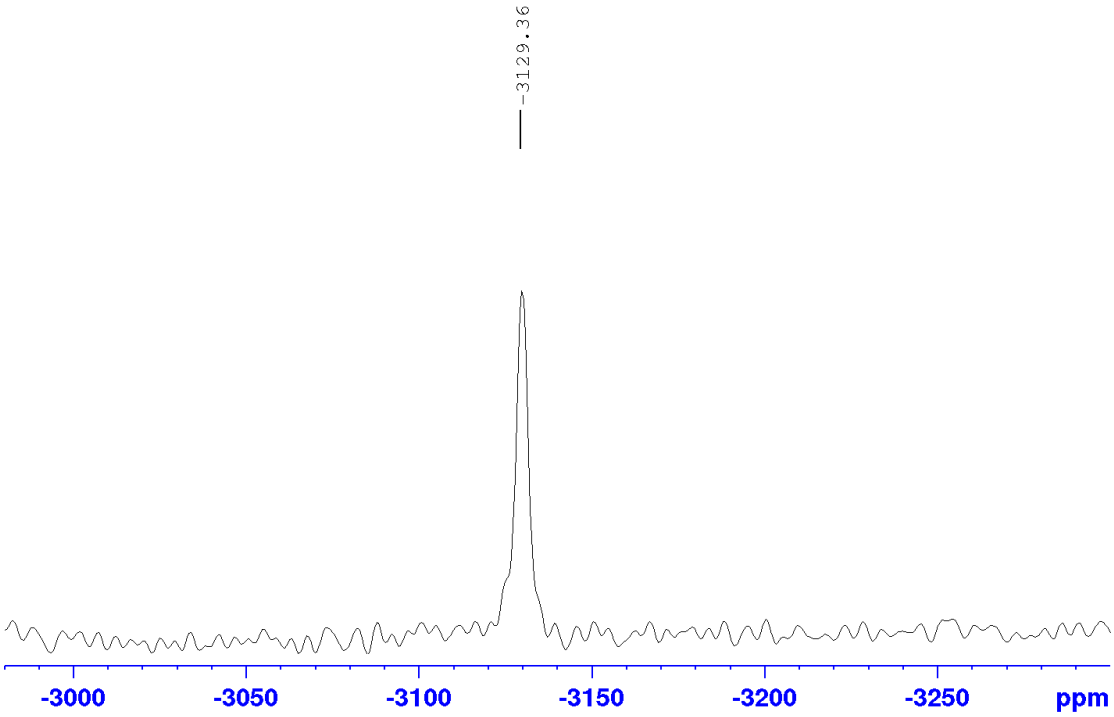


Fig. S6 ^{195}Pt NMR spectrum of **1d** in DMSO-d_6 at 298 K.

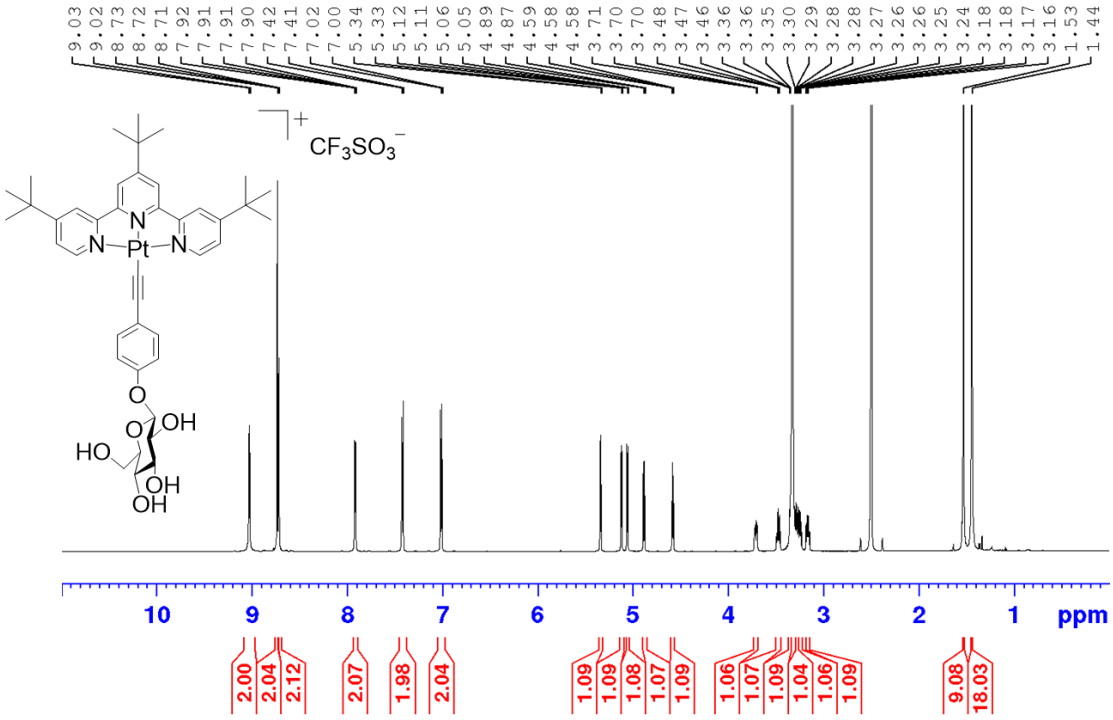


Fig. S7 ^{1}H NMR spectrum of **2** in DMSO-d_6 at 298 K.

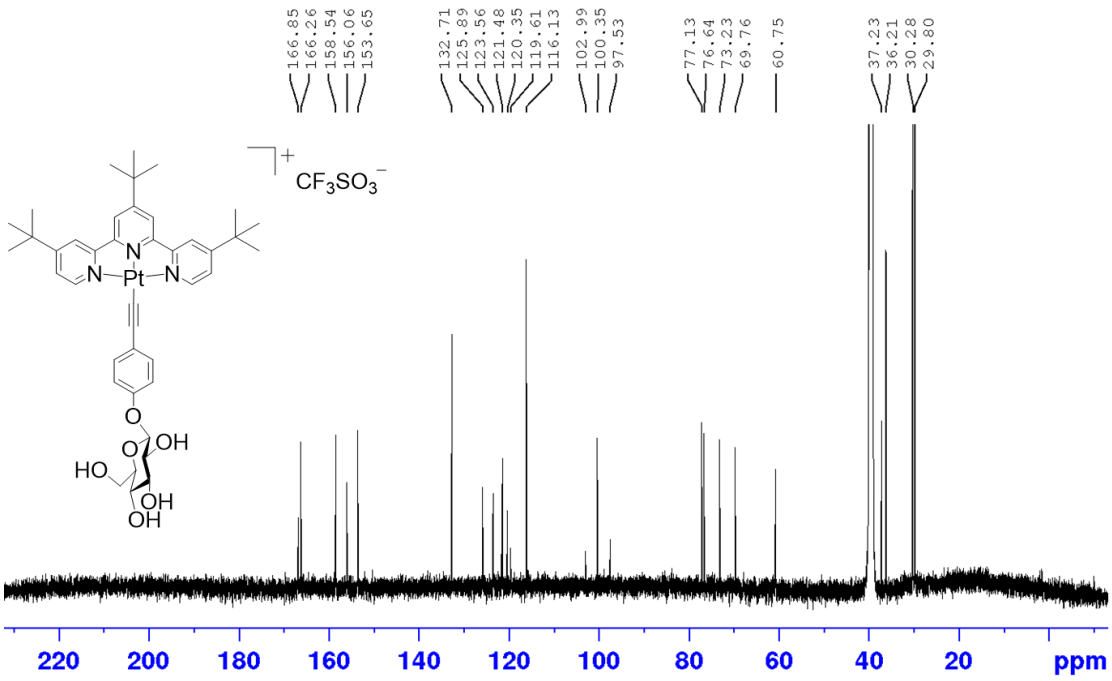


Fig. S8 ^{13}C NMR spectrum of **2** in DMSO-d_6 at 298 K.

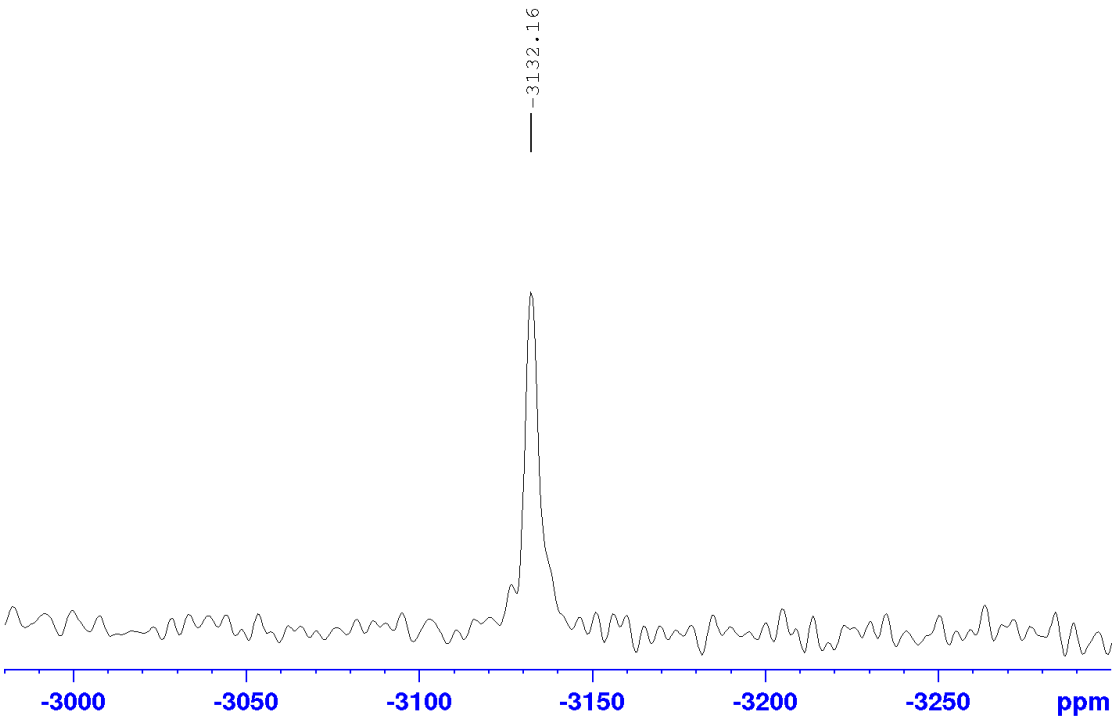
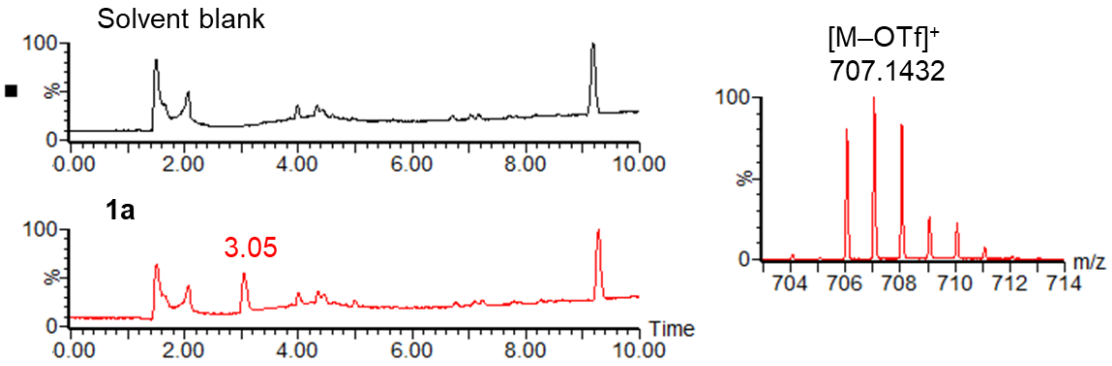


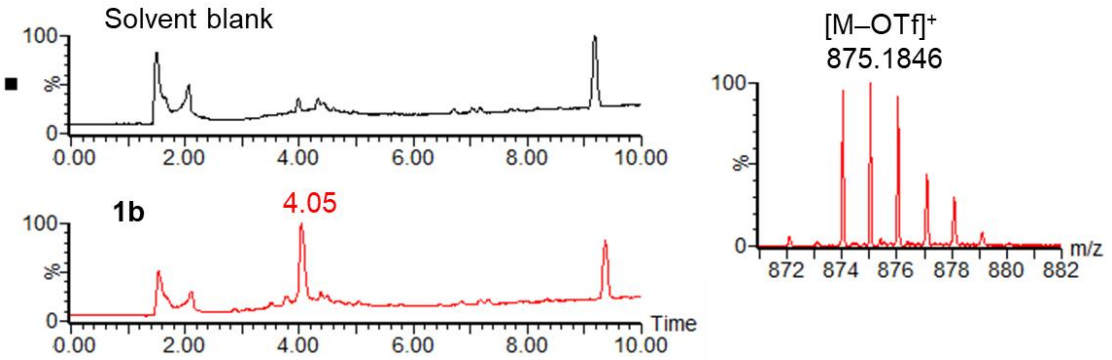
Fig. S9 ^{195}Pt NMR spectrum of **2** in DMSO-d_6 at 298 K.

Liquid Chromatographic Analysis

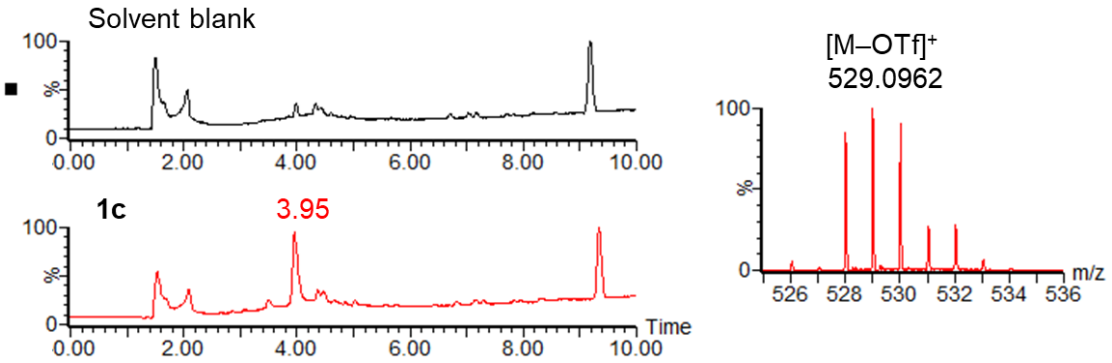
a)



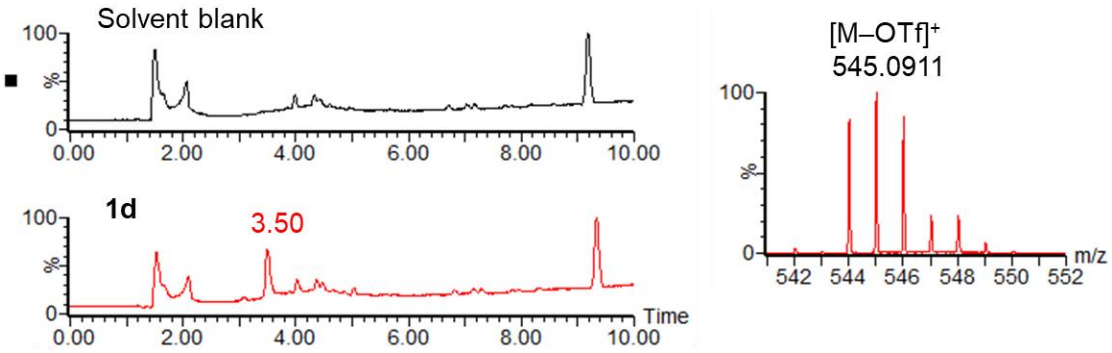
b)



c)



d)



e)

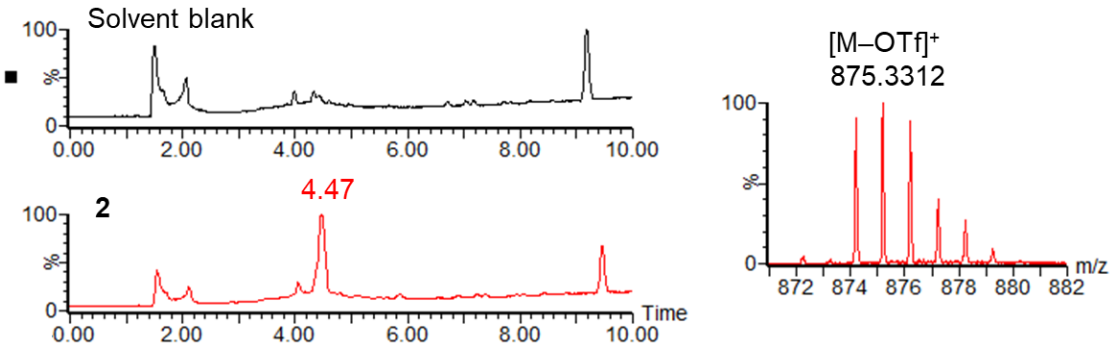


Fig. S10 UPLC/MS chromatograms of complexes (a) **1a**, (b) **1b**, (c) **1c**, (d) **1d** and (e) **2** (20 µM) in dimethyl sulfoxide (left: red-labeled retention time). The isotopic pattern of the molecular ion of the platinum(II) complex for the indicated eluting peak (right).

Supplementary Figures and Tables

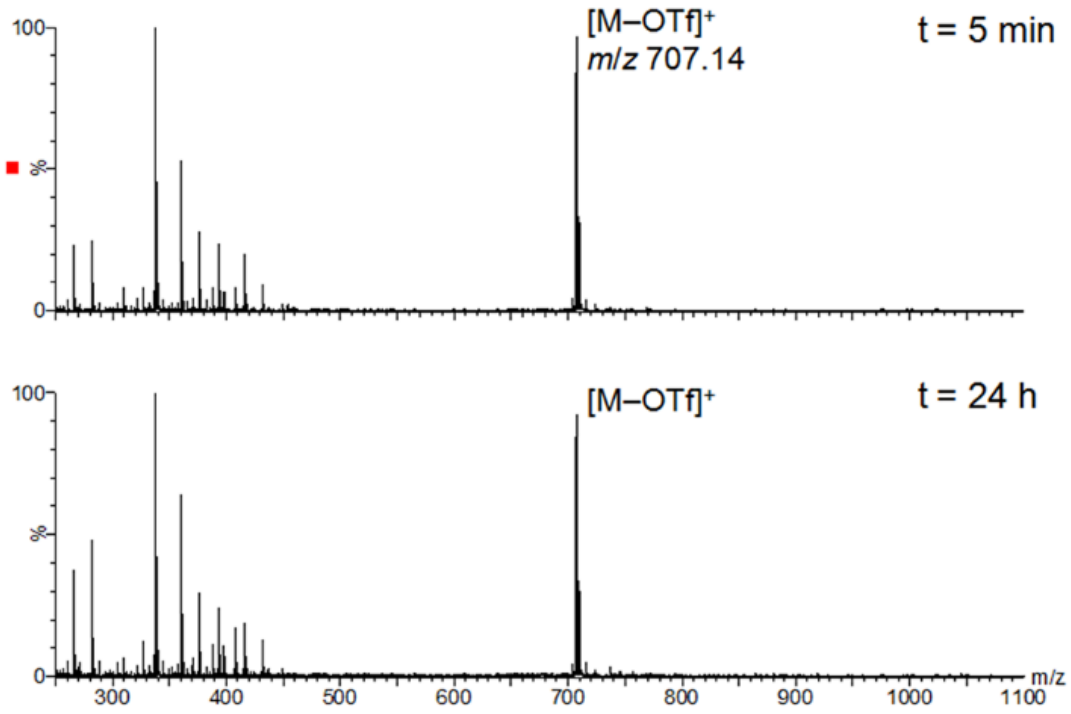


Fig. S11 ESI–MS spectra of **1a** (10 μM) in aqueous buffer solution (NH_4HCO_3:MeOH, 95:5) at 37 °C in 24 h.

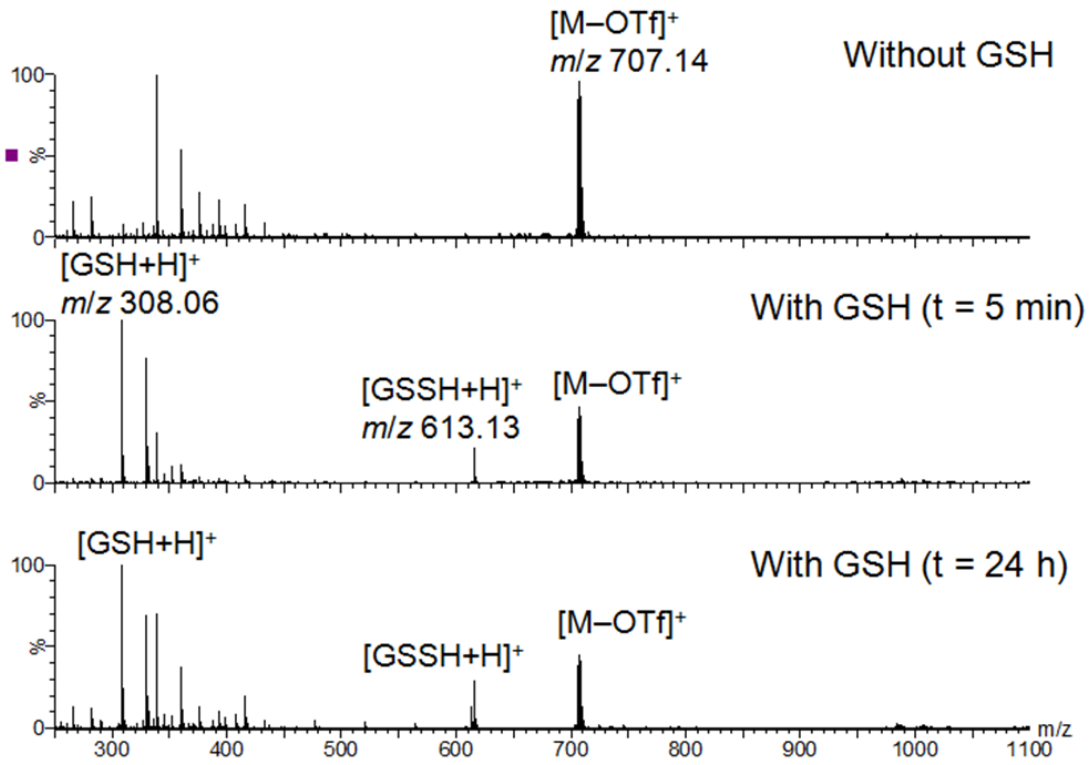


Fig. S12 ESI–MS spectra of **1a** (10 μM) in aqueous buffer solution (NH_4HCO_3:MeOH, 95:5) before and after the incubation with excess glutathione (GSH, 1 mM) at 37 ℃ in 24 h.

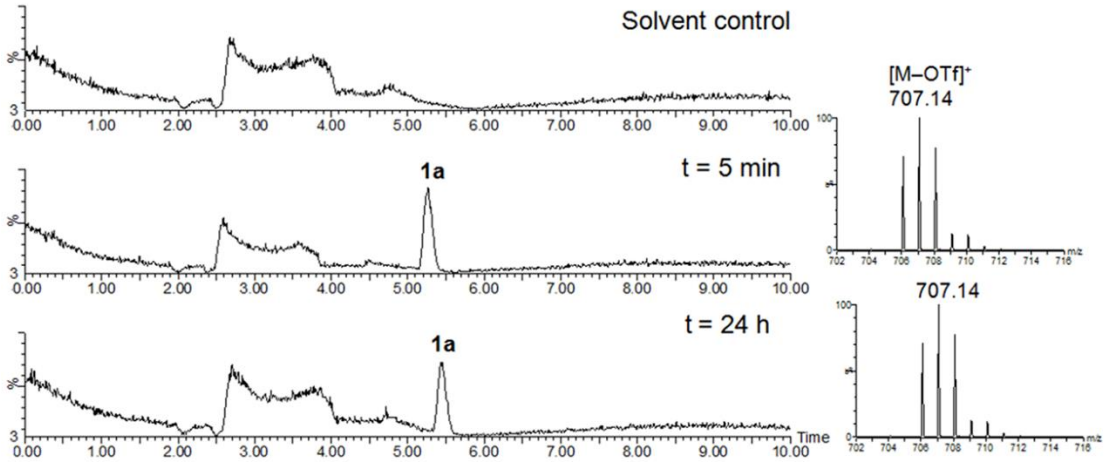


Fig. S13 Total ion chromatograms of **1a** (10 µM) after incubation in RPMI 1640 culture medium containing 0.5 % DMSO for 24 h.

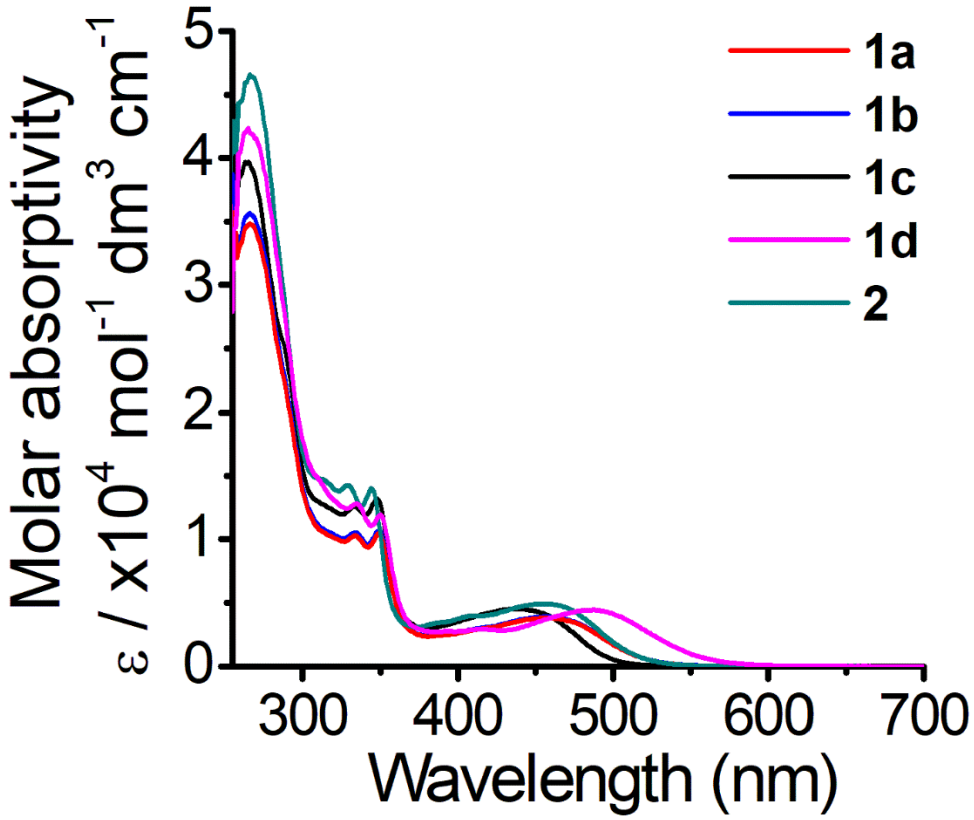


Fig. S14 UV-vis absorption spectra of **1a**–**1d** and **2** in DMSO at 298 K (4×10^{-5} M).

Table S1. UV–vis absorption data of **1a**–**1d** and **2** in DMSO at 298 K (4×10^{-5} M).

Complex	λ_{abs} [nm] (ε [10^3 mol^{-1} dm^3 cm^{-1}])
1a	266 (34.83), 292 (19.66), 318 (10.26), 334 (10.30), 349 (10.44), 414 (2.93), 457 (3.81)
1b	266 (35.66), 291 (20.89), 317 (10.56), 334 (10.55), 349 (10.70), 415 (3.02), 458 (3.89)
1c	263 (39.70), 290 (23.97), 313 (12.77), 333 (12.64), 348 (13.19), 436 (4.52)
1d	265 (42.38), 289 (26.98), 311 (14.65), 334 (12.82), 350 (11.93), 416 (2.93), 488 (4.45)
2	266 (46.55), 287 (30.19), 314 (14.67), 329 (14.27), 344 (14.01), 409 (3.97), 454 (4.92)

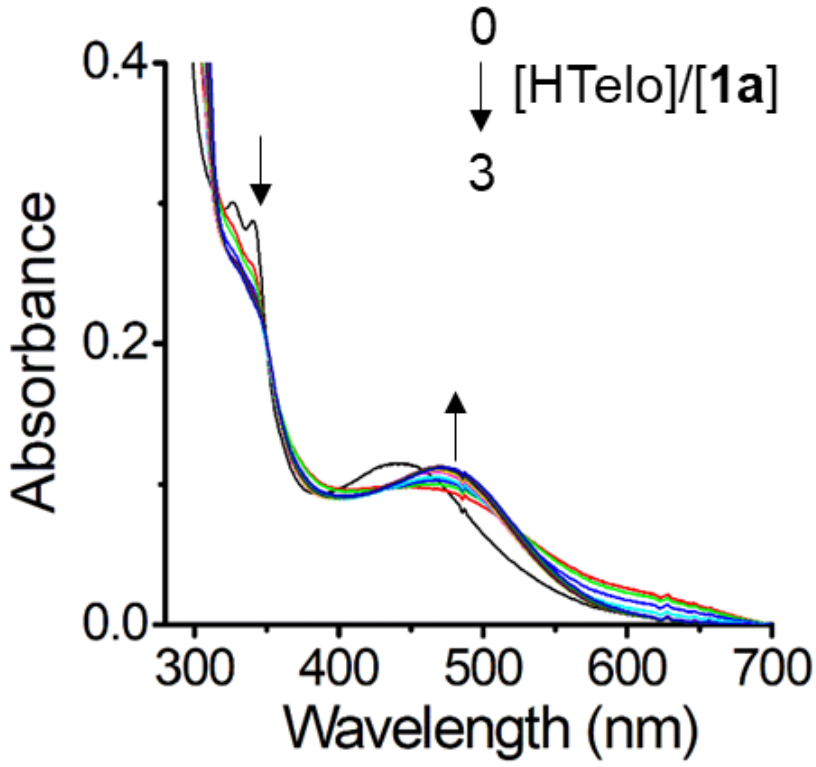


Fig. S15 Plot of the UV–vis absorption titration of complex **1a** (30 μM) with increasing concentration of telomeric G-quadruplex (HTelo) DNA.

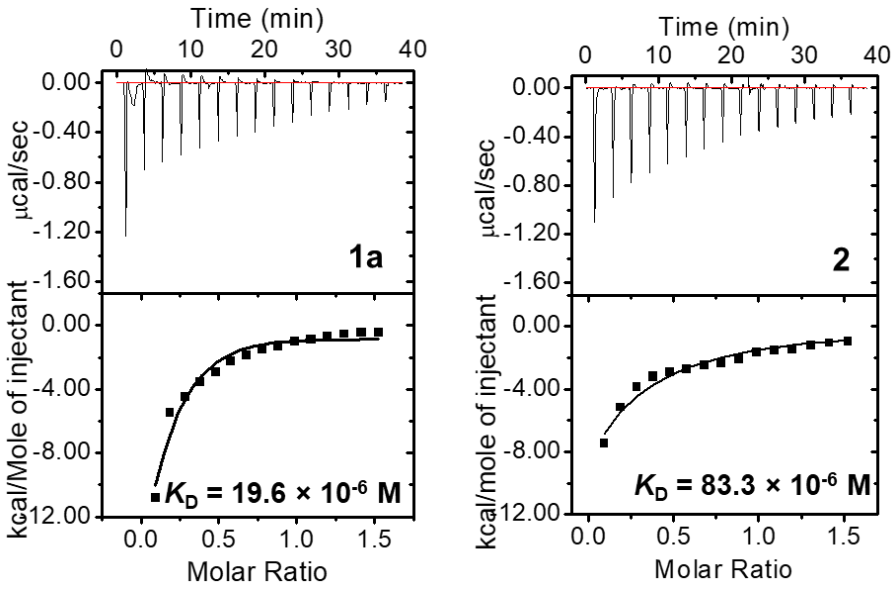
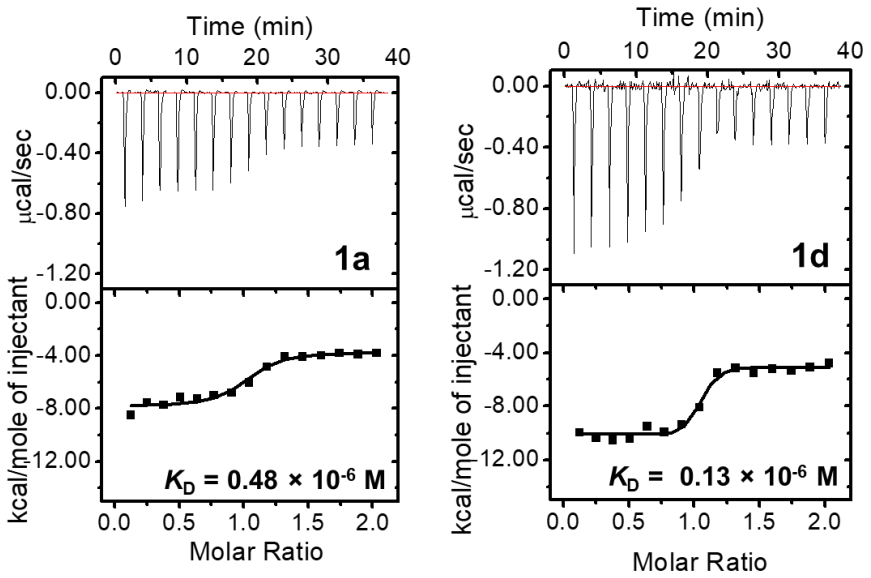


Fig. S16 ITC trace and binding curve of the binding of **1a** or **2** to cytosine-cytosine (CC) mismatched DNA. Data were fitted using a one-site binding model.

a)



b)

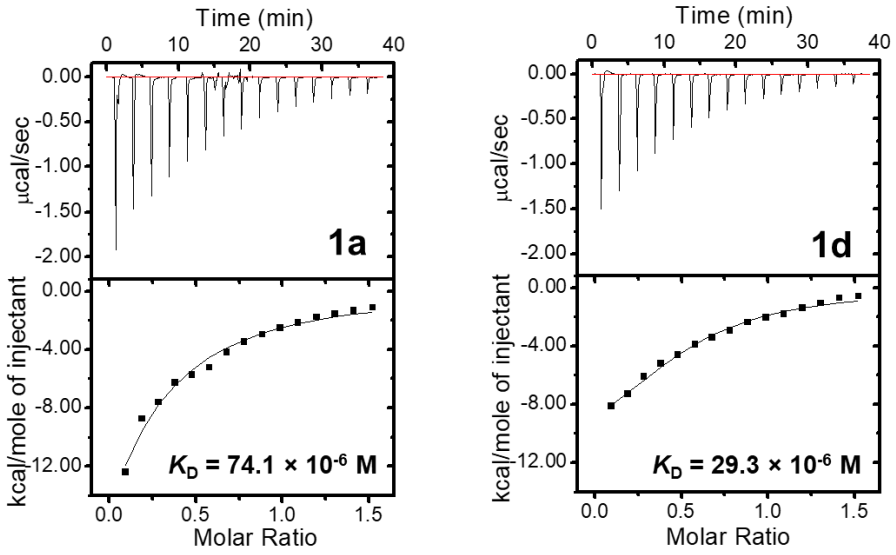
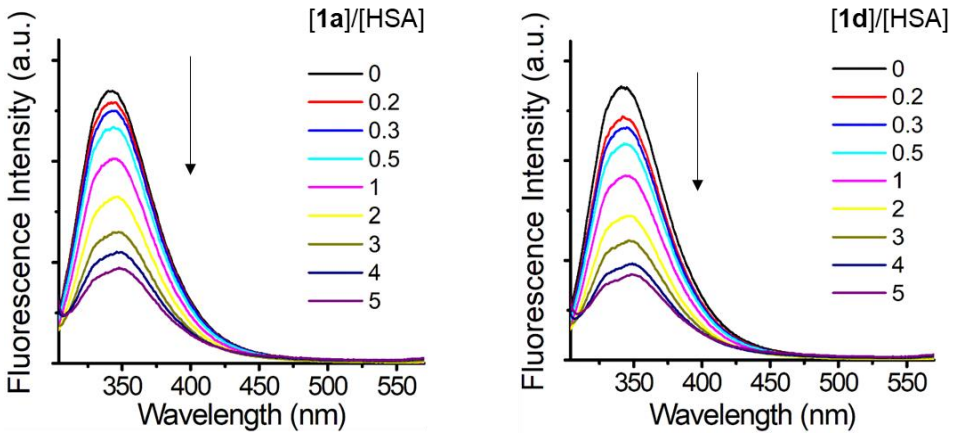


Fig. S17 ITC trace and binding curve of the binding of **1a** or **1d** to a) calf thymus or b) GC-rich DNA. Data were fitted using a one-site binding model.

a)



b)

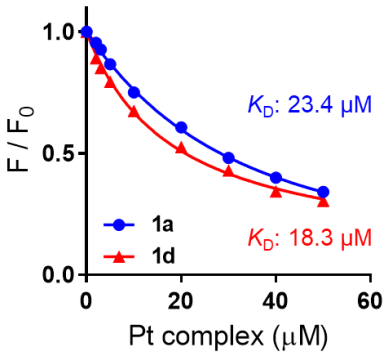


Fig. S18 a) Quenching of HSA (10 μM) fluorescence from tryptophan residue upon titration with complex **1a** or **1d** from 0 to 50 μM. (b) Plot of fluorescence intensity ratio F/F_0 versus concentration of the platinum complex on fluorescence quenching of tryptophan residue of HSA.

a) b)

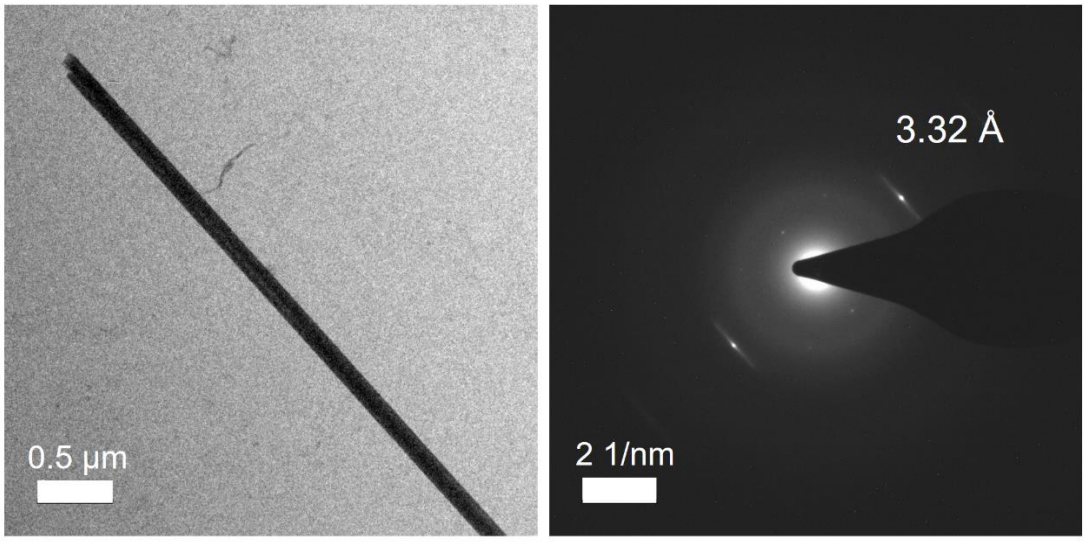


Fig. S19 a) TEM image of wire-like structures formed by complex **1d** (1×10^{-4} M) in DMSO/H_2O (1:99 v/v) and b) its corresponding selected area electron diffraction (SAED) pattern.

a) b)

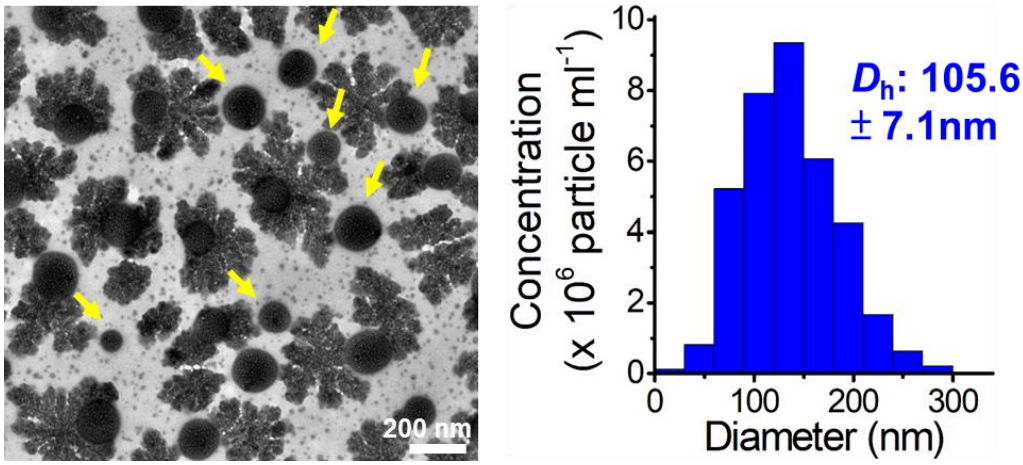


Fig. S20 a) TEM image and b) nanoparticle tracking analysis of particle-like structures formed by complex **1a** (1×10^{-4} M) in phosphate-buffered saline containing 10% fetal bovine serum and 1% DMSO.

Table S2. *In vitro* anti-proliferative activity (IC_{50}, μM; 24 and 48 h) of complexes **1a**, **1b** and cisplatin against human cancerous and normal cell lines.

Complex	Incubation time (h)	IC_{50} (μM)				
		NCI-H460	PLC	HCT116	MDA-MB-231	CCD-19Lu
1a	24	> 100	> 100	> 100	> 100	> 100
	48	54.62 ± 4.8	74.53 ± 8.1	63.43 ± 7.2	> 100	> 100
1b	24	> 100	> 100	>100	>100	> 100
	48	97.38 ± 8.7	> 100	90.65 ± 7.8	>100	> 100
Cisplatin	24	50.46 ± 4.3	63.91 ± 5.6	35.64 ± 4.5	68.37 ± 6.2	> 100
	48	21.61 ± 2.3	47.23 ± 3.9	16.27 ± 1.9	27.51 ± 3.1	84.37 ± 6.5

NCI-H460, non-small cell lung carcinoma; PLC, primary liver carcinoma; HCT116, colorectal carcinoma; MDA-MB-231, triple-negative breast cancer carcinoma; CCD-19Lu, normal lung fibroblast.

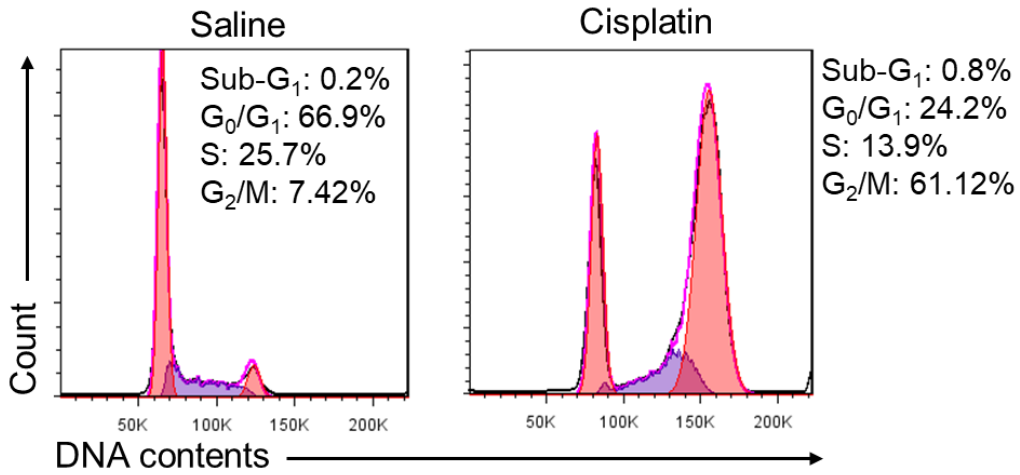


Fig. S21 Flow cytometric analysis of cell cycle of NCI-H460 cells treated with cisplatin (10 μM) or saline vehicle for 24 h.

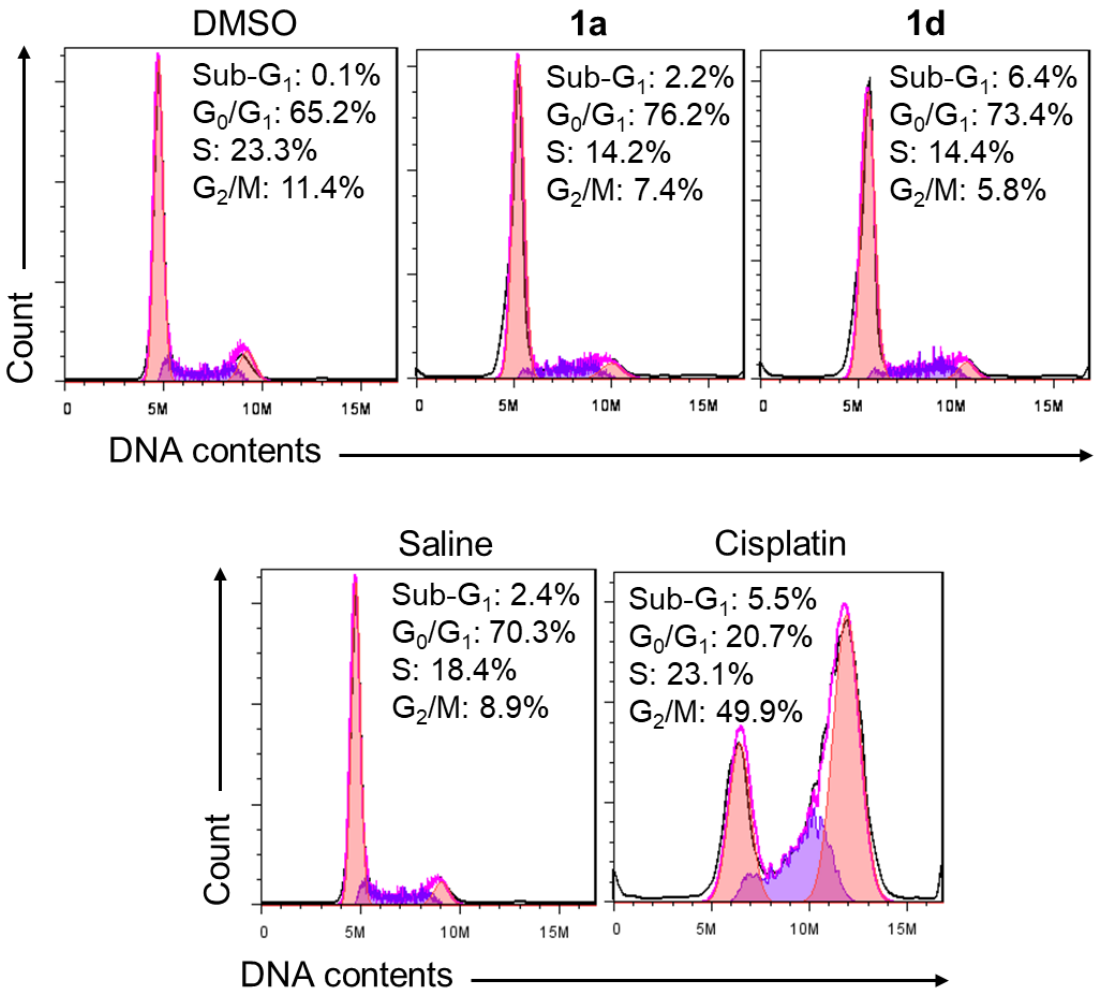


Fig. S22 Flow cytometric analysis of cell cycle progression in NCI-H460 cells treated with complexes **1a**, **1d**, cisplatin (10 μM), DMSO or saline vehicle for 48 h.

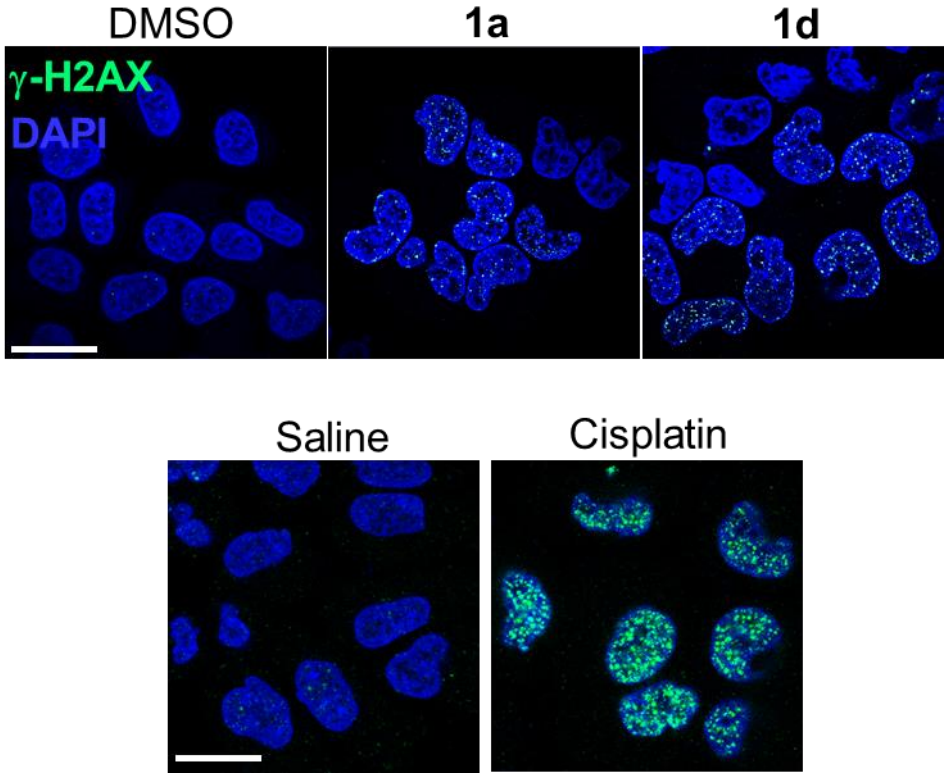


Fig. S23 Immunofluorescence staining of γ-H2AX (green; λ_{ex} = 488 nm, λ_{em} = 495–540 nm) in NCI-H460 cells treated with **1a**, **1d** or cisplatin (10 μM) for 24 h. Nuclei were stained with DAPI (blue; λ_{ex} = 405 nm, λ_{em} = 450–480 nm). Scale bar: 20 μm.

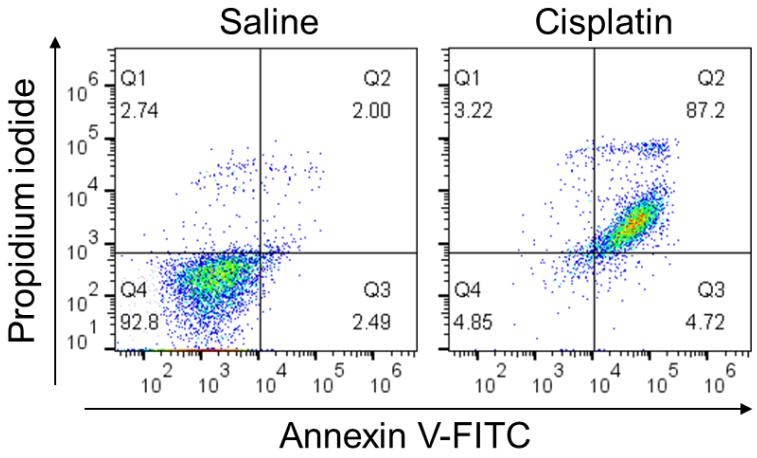


Fig. S24 Flow cytometric analysis of cell death of NCI-H460 cells treated with cisplatin (20 μM) for 72 h.

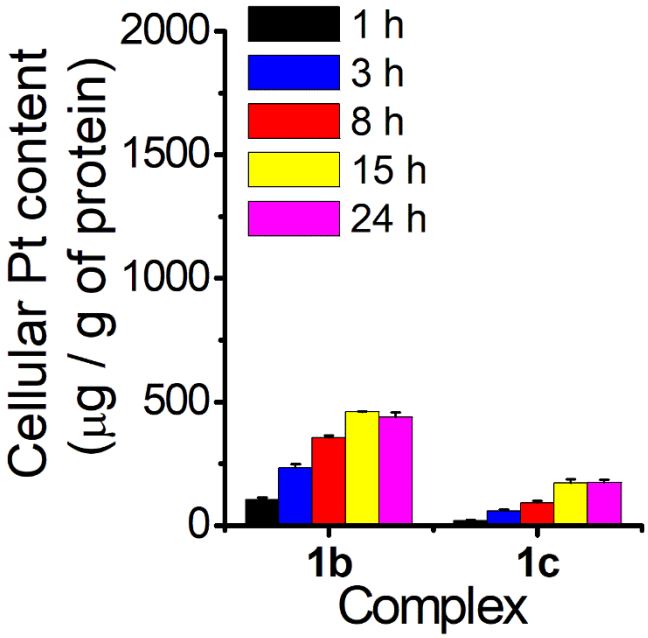
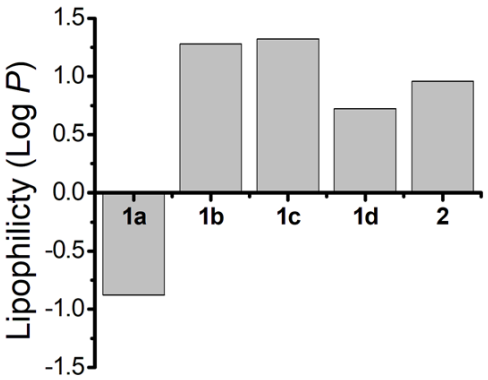


Fig. S25 Cellular platinum content in NCI-H460 cells after time-dependent treatments with complex **1b** or **1c** (10 µM) as determined by ICP-MS.



Complex	Log *P* [a]
1a	–0.88
1b	1.28
1c	1.32
1d	0.72
2	0.96

[a] Log *P* of each complex in terms of Pt content partitioned in n-octanol and water containing sodium chloride (0.9%, w/v)

Fig. S26 Lipophilicity (Log *P*) of different platinum(II) complexes (10 μM) as determined by ICP-MS.

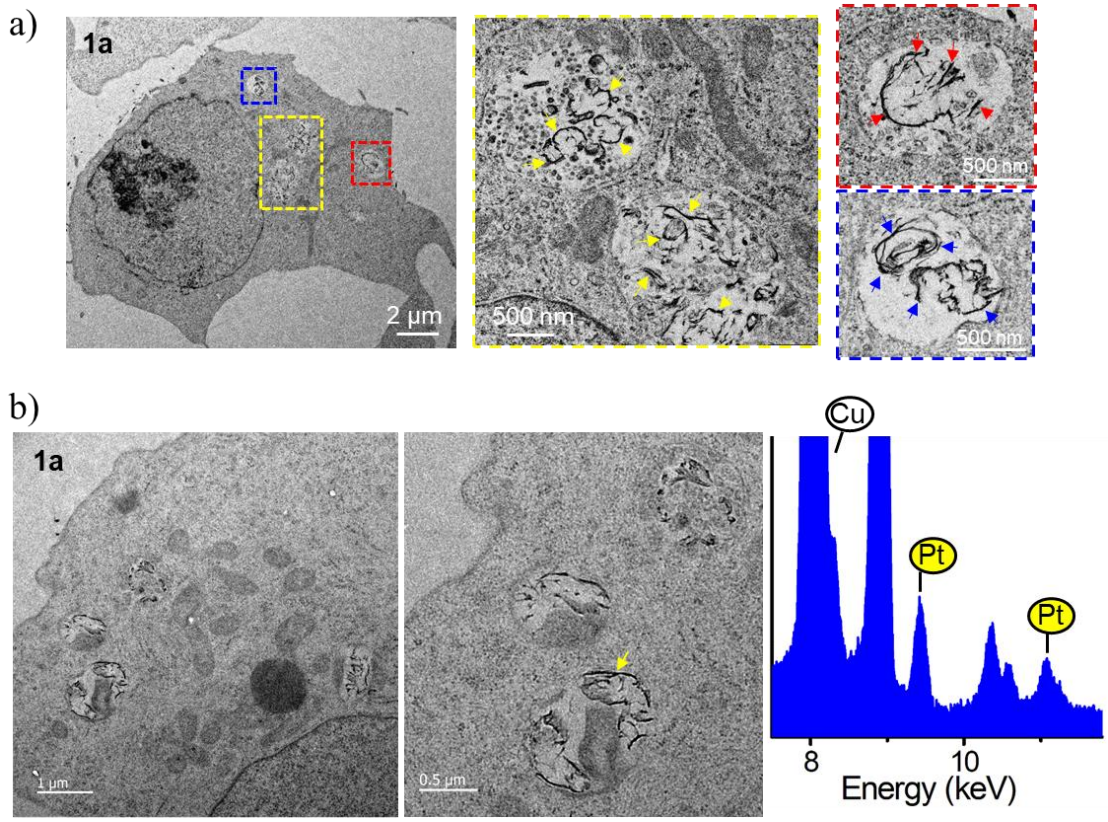
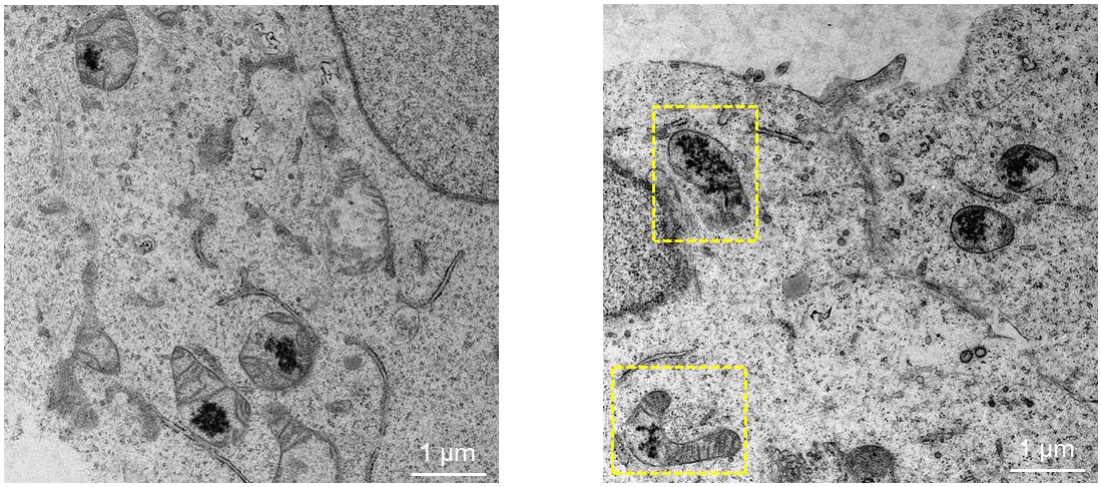
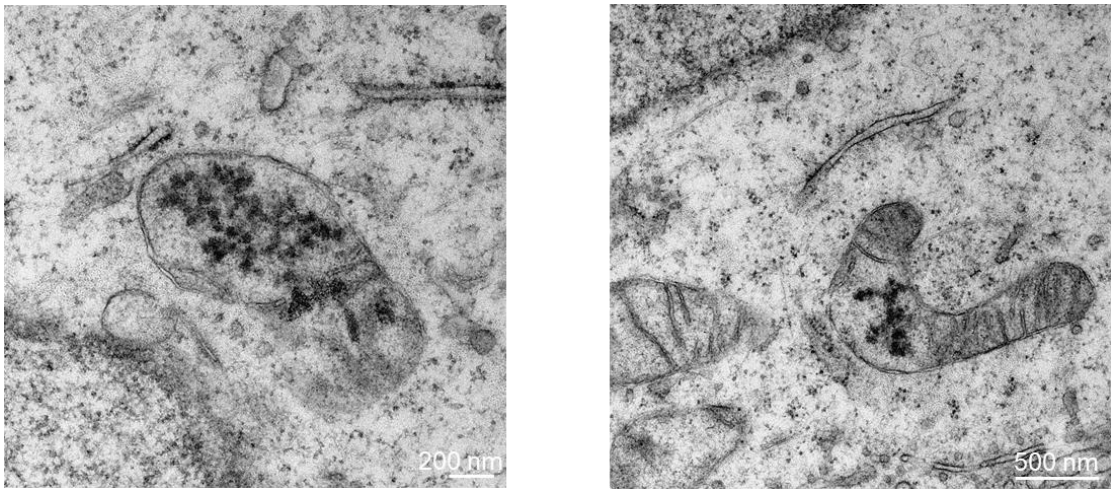


Fig. S27 Bio-TEM images of MDA-MB-231 breast cancer cells after treatment with **1a** (10 µM) for 24 h. a) The arrows in the dashed region highlight the electron-dense superstructures inside the cells. b) The presence of elemental platinum in the superstructures was confirmed by EDX analysis.

a)



b)



c)

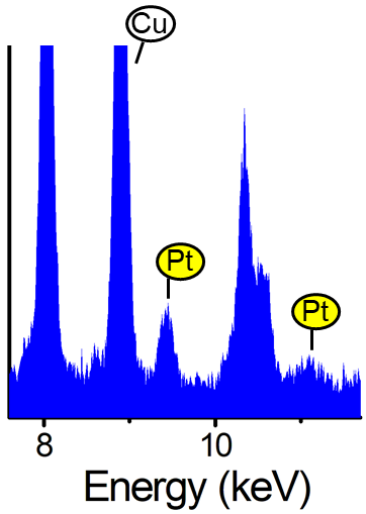
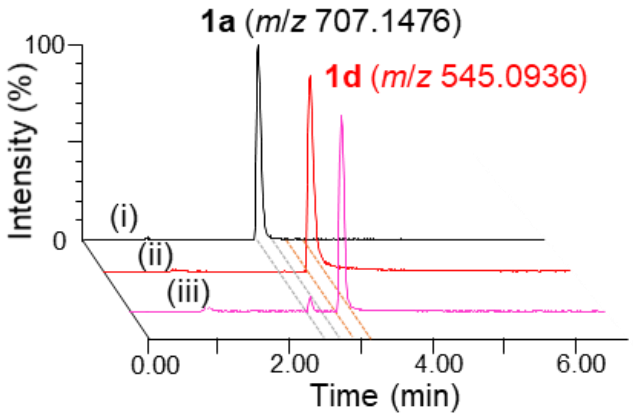


Fig. S28 a) Bio-TEM images of the electron-dense aggregates in the mitochondria of **1a**-treated NCI-H460 lung cancer cells. b) Enlarged images of the dashed region in (a). c) EDX spectrum showing the presence of elemental platinum in the electron-dense aggregates in the mitochondria.

a)



b)

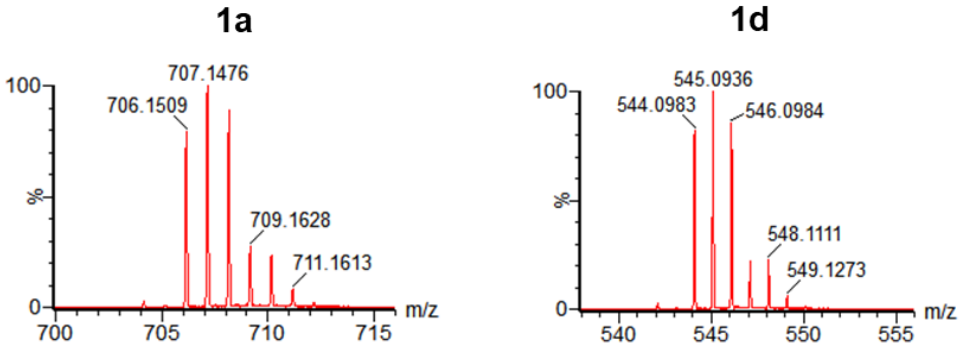


Fig. S29 a) UPLC/MS analysis of (i) complex **1a**, (ii) **1d** and (iii) extract from **1a**-treated NCI-H460 lung cancer cells after treatment for 24 h. b) ESI–MS spectra of the molecular ions with the isotopic patterns for the eluted peak in (a, iii).

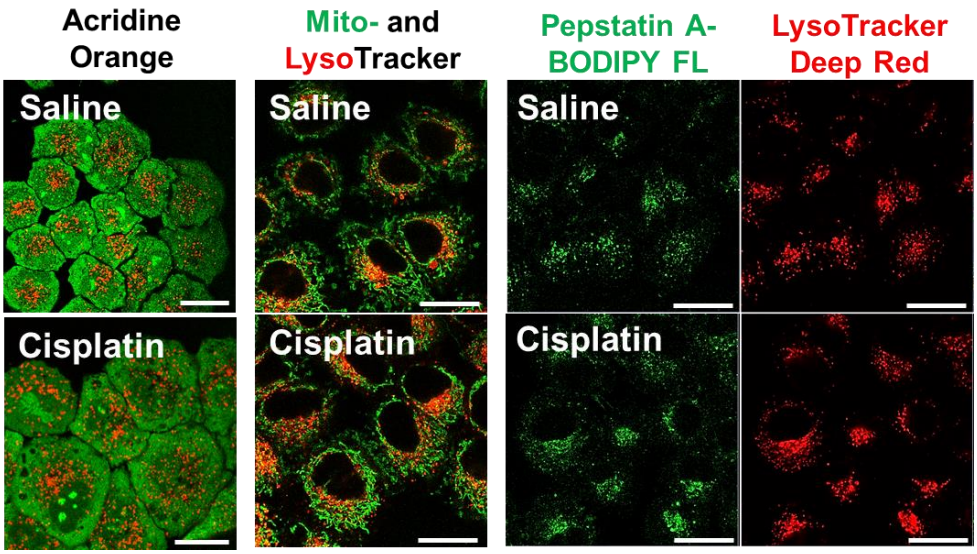


Fig. S30 Lysosomal integrity in NCI-H460 cells after 24h-treatment with cisplatin (10 µM) as determined by acridine orange (10 µM), LysoTracker Deep Red and MitoTracker Green (50 nM), or pepstatin A–BODIPY FL conjugate (1 µM) and LysoTracker Deep Red staining using confocal imaging microscopy. Scale bar = 20 µm.

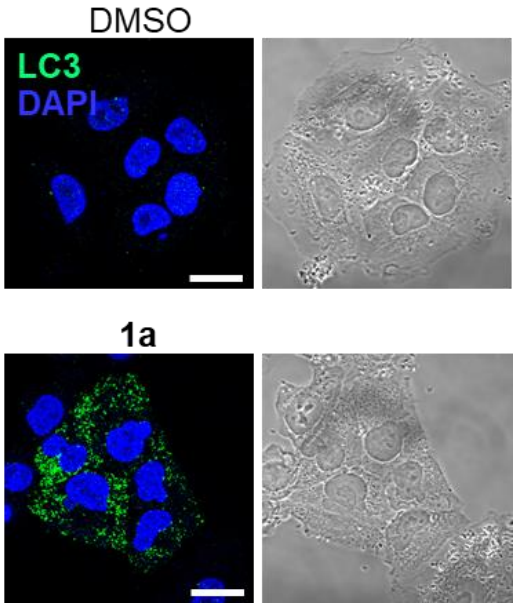


Fig. S31 Immunofluorescence staining of LC3 (green; λ_{ex} = 488 nm, λ_{em} = 495–540 nm) in NCI-H460 cells treated with **1a** (10 μM) or DMSO vehicle for 24 h. Nuclei were stained with DAPI (blue; λ_{ex} = 405 nm, λ_{em} = 450–480 nm). Scale bar: 20 μm.

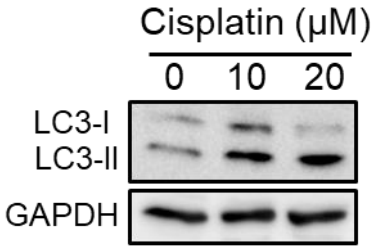


Fig. S32 Immunoblotting analysis of autophagy protein markers (LC3) in NCI-H460 lung cancer cells after cisplatin treatment in dose-dependent manner for 6 h.

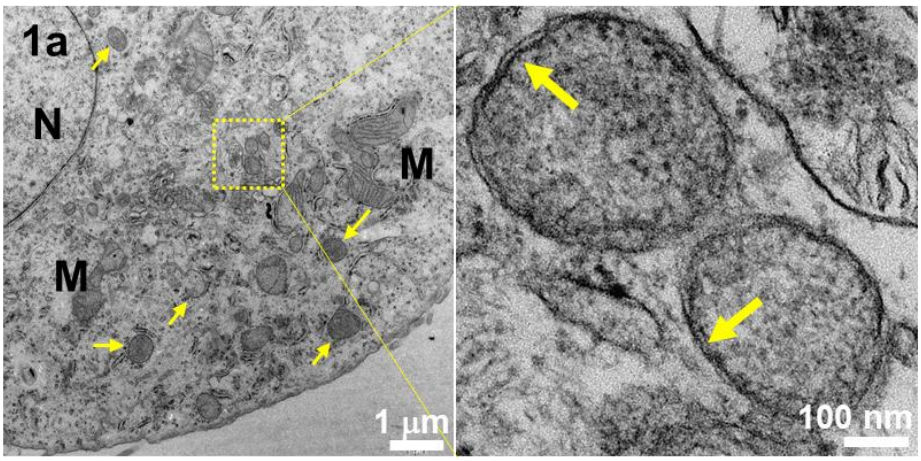
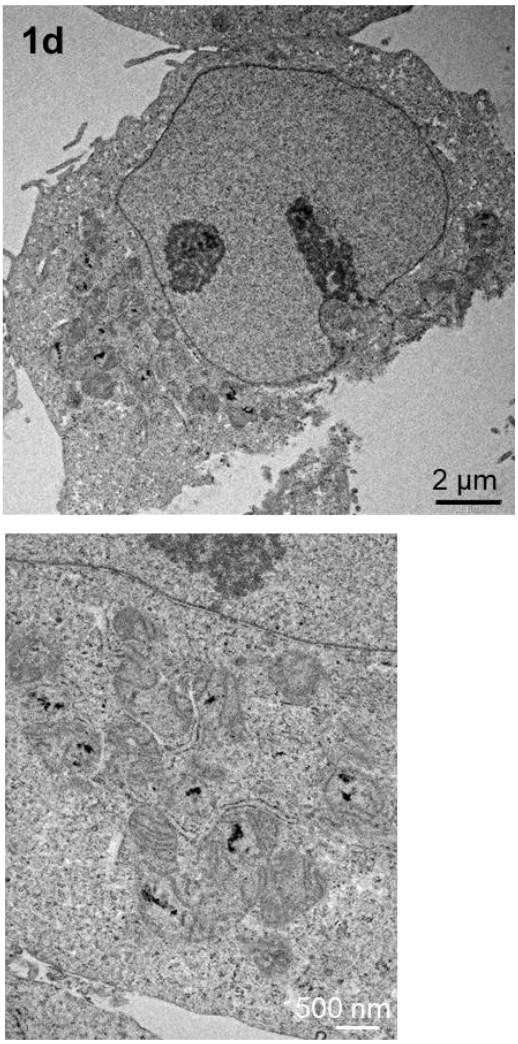


Fig. S33 Bio-TEM images of the accumulation of cytosolic vesicles in **1a**-treated NCI-H460 cells at 24 h. (g) Magnified view of the yellow box region shows the formation of double-membraned vesicles. (M: mitochondria; N: nucleus).

Table S3. List of cellular proteins showing thermal stabilization upon the treatment with **1a** in thermal proteome profiling analysis. The information on localization and functions are adapted from Uniprot.

Gene Symbol	Protein Name	Localization	ΔT_m	Functions
RPL17	60S ribosomal protein L17	Cytoplasm	3.51	Component of the large ribosomal subunit
RAP1B	Ras-related protein Rap-1b	Plasma membrane, endosomes	3.29	Involves in the control of vesicular trafficking
PPT1	Palmitoyl-protein thioesterase 1	Lysosomes	3.25	Involves in the catabolism of lipid-modified proteins during lysosomal degradation
HEL_S_100n	T-complex protein 1 subunit beta	Cytoplasm	3.24	Assists the folding of proteins
CIT	Citron Rho-interacting kinase	Cytoplasm	2.64	Plays a role in cytokinesis
SEPTIN2	Septin-2	Cytoskeleton	2.46	Required for normal organization of the actin cytoskeleton
CENPF	Centromere protein F	Cytoskeleton	2.28	Recycling of the plasma membrane involving recycling vesicles and the microtubule network
ADD1	Alpha-adducin	Cytoskeleton	2.26	Promotes the assembly of the spectrin-actin network
CRKL	Crk-like protein	Cytoplasm	2.23	Mediates the transduction of intracellular signals
SUPV3L1	ATP-dependent RNA helicase	Mitochondria	2.20	Mitochondrial RNA metabolism
TFRC	Transferrin receptor protein 1	Plasma membrane, endosomes	1.13	Cellular uptake of iron occurs via receptor-mediated endocytosis
NUMB	Protein numb homolog	Plasma membrane, endosomes	1.12	Regulates clathrin-mediated receptor endocytosis

a)



b)

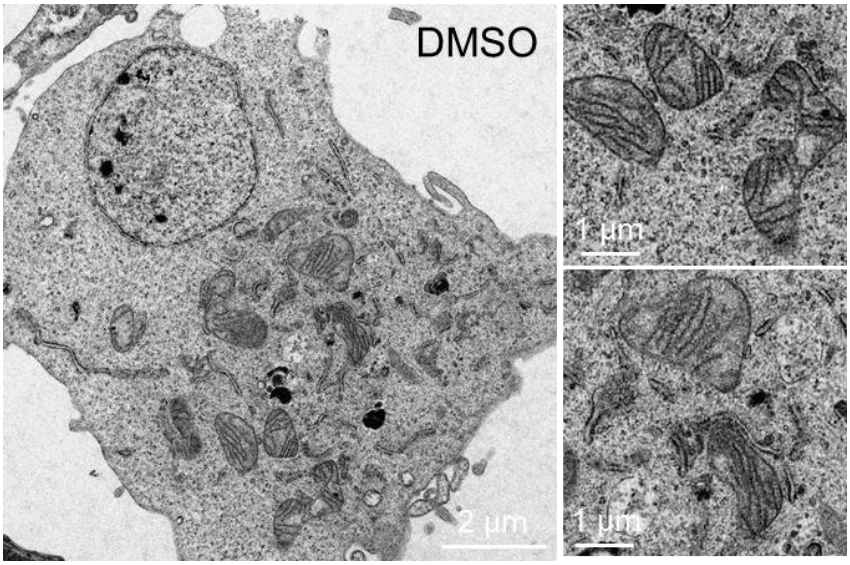


Fig. S34 Bio-TEM images of the mitochondria in NCI-H460 lung cancer cells after treatment with a) complex **1d** or b) DMSO vehicle for 24 h.

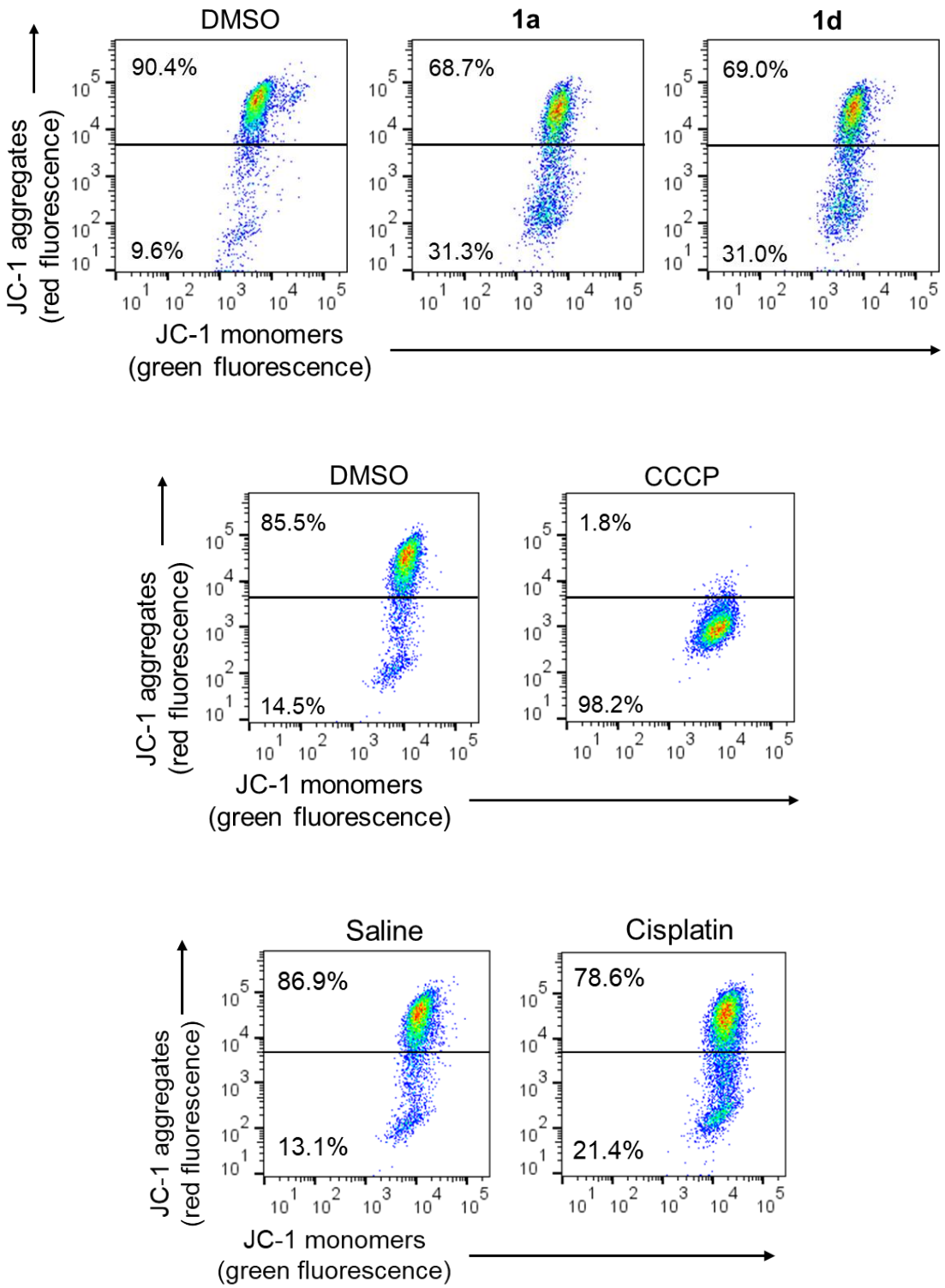


Fig. S35 Flow cytometric analysis of the mitochondrial membrane potential in NCI-H460 lung cancer cells treated with DMSO vehicle, complex **1a** or **1d**, saline vehicle or cisplatin for 24 h, using JC-1 fluorescence staining. Treatment of cells with carbonylcyanide m-chlorophenylhydrazone (CCCP, 50 μM) for 15 min was served as positive control.

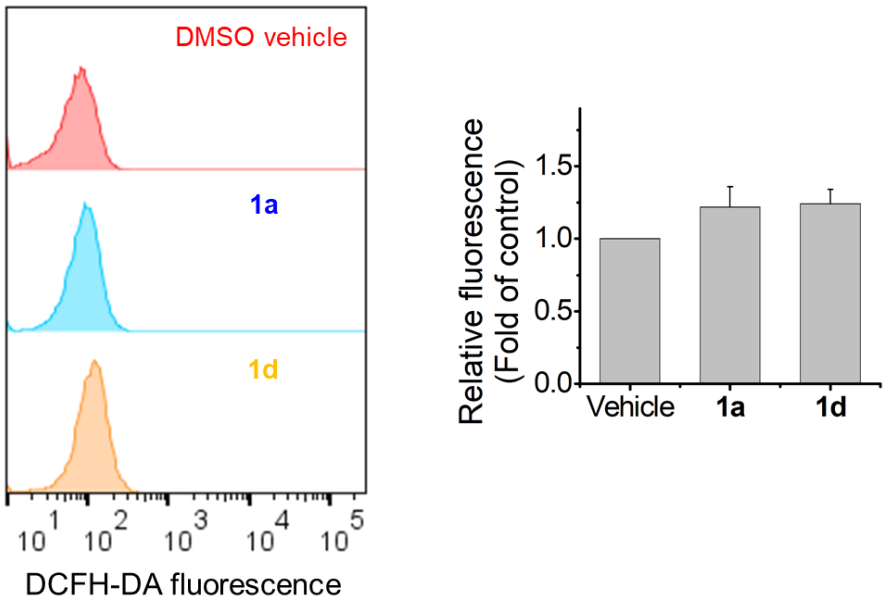


Fig. S36 Flow cytometric analysis of intracellular oxidant in NCI-H460 lung cancer cells treated with DMSO vehicle, complex **1a** or **1d** for 24 h, using DCFH-DA fluorescence staining. Bar chart illustrates the relative fluorescence of DCFH-DA in different treatments compared with vehicle control.

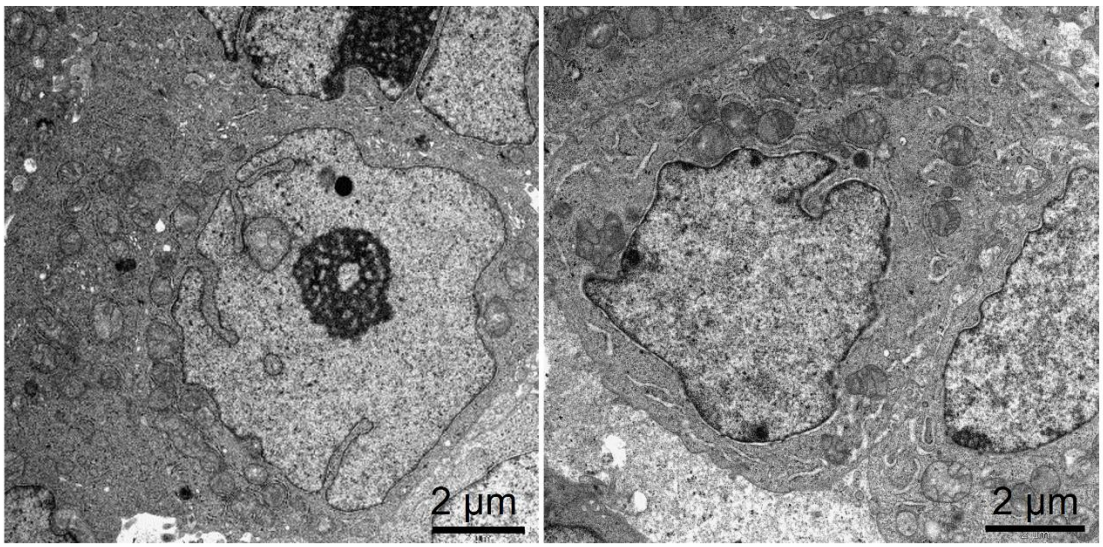


Fig. S37 TEM images of the ultrastructure of NCI-H460 tumors harvested from PBS vehicle-treated mice.

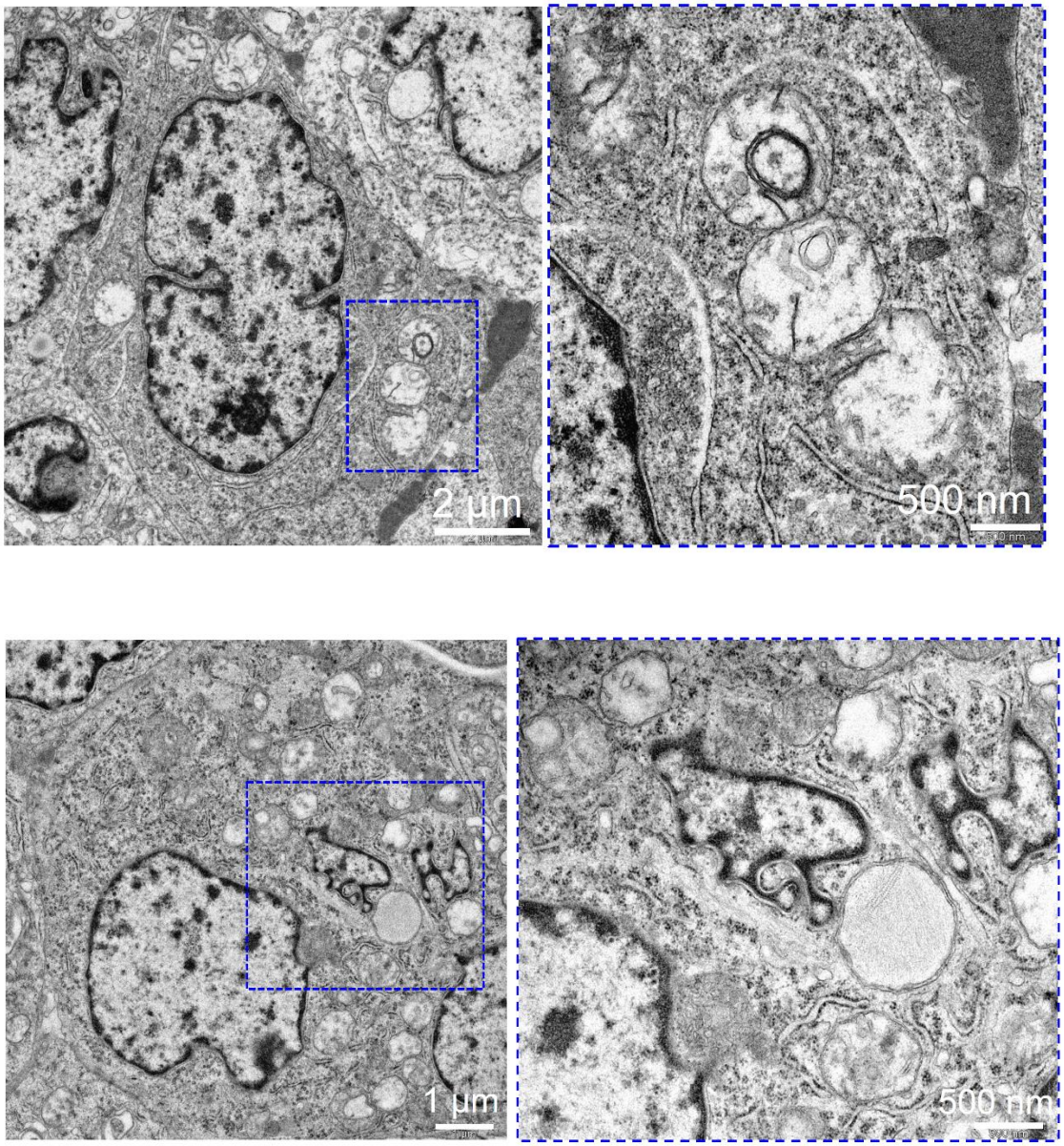


Fig. S38 TEM images of the ultrastructure of NCI-H460 tumors harvested from **1a**-treated mice.

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