Supporting Information

for

Glutathione-scavenging Poly(disulfide amide) Nanoparticles for Effective Delivery of Pt(IV) Prodrugs and Reversal of Cisplatin Resistance

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METHODS

1. Materials

L-Cysteine dimethyl ester dihydrochloride ($(H\text{-}Cys\text{-}OMe)_2 \cdot 2HCl$), succinyl chloride, adipoyl chloride, suberoyl chloride, sebacoyl chloride, dodecanedioyl dichloride, cisplatin, acetic anhydride, butanoic anhydride, hexanoic anhydride, octanoic anhydride, decanoic anhydride, benzoic anhydride, 2,4,6-trimethylbenzoic anhydride, 4-tert-butyl-benzoic acid anhydride were purchased from Sigma-Aldrich and used without further purification. Fluorescence dyes (Dil, Nile red, Coumarin 6, and DID) were purchased from Thermo Fisher Scientific. 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-3000] (ammonium salt) (DSPE-PEG 3000) was purchased from Avanti Polar Lipids. Other chemicals if not otherwise specified were purchased from VWR International. RPMI-1640 medium, McCoy's 5A medium, F-12K medium, EMEM medium, trypsin, fetal bovine serum (FBS), penicillin-streptomycin solution, phosphate-buffered saline (PBS) and water were supplied by Gibco.

2. Synthesis of Cys-PDSA polymers

Cys-PDSA polymers were prepared as previously described:[1] $(H\text{-}Cys\text{-}OMe)_2 \cdot 2HCl$ (10.0 mmol) and triethylamine (15.0 mmol) were dissolved in DMSO (20.0 mL), then the fatty acid dichloride (10.0 mmol) in DMSO (10.0 mL) was added dropwise. The solution was stirred for 1 h to obtain a uniform mixture, precipitated twice with 250 mL of ice-cold diethyl ether, and dried under vacuum to obtain a yellow or brown-yellow powder.

3. Synthesis of *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OH)_2]$

30 w/v% H_2O_2 (4.0 mL) was added dropwise into a 10 w/v% cisplatin suspension (10.0 mL), the mixture was stirred under protection from light at 25 °C for 24 h. After recrystallization at 4 °C overnight, the product was collected and washed with ice-cold water, ethanol, and

diethyl ether, respectively. Residual solvent was then removed under vacuum to give a bright yellow powder (yield: 90.3%).[2]

4. Synthesis of Pt(IV) prodrugs

Pt(IV) prodrugs were prepared as previously reported:[3-5] *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OH)_2$] (0.6 mmol) was suspended in dry DMF (8.0 mL), followed by the addition of appropriate anhydrides (1.4 mmol). After stirring in the dark at 50 °C for 24 h, the resulting solution was filtered, concentrated to 1.0 mL, then slowly added to a rapidly stirring volume of diethyl ether (40.0 mL). The resultant precipitate was collected, washed with acetone, diethyl ether and dried under vacuum for 12 h.

5. Nuclear Magnetic Resonance (NMR) spectra

^{1}H NMR spectra of Cys-PDSA polymers were the same as those reported in literature.[1]

^{1}H NMR and ^{13}C NMR spectra of Pt(IV) prodrugs were recorded on a Mercury VX-300 spectrometer at 400 MHz (Varian, USA), using DMF-d_7 as a solvent and MestReNova software for data analysis.

Pt(IV) prodrug **1**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCCH_3)_2$]: Pale yellow powder (yield: 79.2 %); ^{1}H NMR δ = 1.88 (s, 6H), 6.65-7.04 (m, 6H); ^{13}C NMR δ = 22.19, 179.08.

Pt(IV) prodrug **2**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_2CH_3)_2$]: Off-white powder (yield: 78.6 %); ^{1}H NMR δ = 1.03 (t, 6H), 1.66 (sext, 4H), 2.35 (t, 4H), 6.82-7.21 (m, 6H); ^{13}C NMR δ = 13.32, 19.28, 38.06, 181.69.

Pt(IV) prodrug **3**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_4CH_3)_2$]: Off-white powder (yield: 77.9 %); ^{1}H NMR δ = 1.02 (t, 6H), 1.42 (m, 8H), 1.64 (quin, 4H), 2.37 (t, 4H), 6.83-7.21 (m, 6H); ^{13}C NMR δ = 13.82, 22. 46, 25.55, 31.35, 36.00, 181.66.

Pt(IV) prodrug **4**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_6CH_3)_2$]: Off-white powder (yield: 70.0 %); ^{1}H NMR δ = 1.03 (t, 6H), 1.41 (m, 16H), 1.64 (quin, 4H), 2.38 (t, 4H), 6.84-7.20 (m, 6H); ^{13}C NMR δ = 13.81, 22.47, 25.86, 29.21, 31.82, 36.26, 181.90.

Pt(IV) prodrug **5**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_8CH_3)_2$]: White powder (yield: 68.9 %); ^{1}H NMR δ = 1.03 (t, 6H), 1.42 (m, 24H), 1.64 (quin, 4H), 2.38 (t, 4H), 6.82-7.21 (m, 6H); ^{13}C NMR δ = 13.82, 22.47, 26.11, 29.21, 31.86, 36.01, 181.66.

Pt(IV) prodrug **6**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_5)_2$]: Light brown powder (yield: 56.5%); ^{1}H NMR δ = 7.56-7.73 (m, 6H), 8.07-8.17 (m, 4H); ^{13}C NMR δ = 128.15-128.41, 129.72, 131.83, 174.09.

Pt(IV) prodrug **7**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_2(CH_3)_3)_2$]: Brown powder (yield: 46.9%); ^{1}H NMR δ = 2.34-2.48 (m, 18H), 6.91-6.97 (d, 4H); ^{13}C NMR δ = 19.05, 20.38, 127.64, 133.63, 137.04, 175.15.

Pt(IV) prodrug **8**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_4C(CH_3)_3)_2$]: Dark brown powder (yield: 44.7%); ^{1}H NMR δ = 1.49 (s, 18H), 7.63-7.66 (d, 4H), 8.04-8.07 (d, 4H); ^{13}C NMR δ = 30.53, 124.78, 129.44, 130.80, 154.81, 173.84.

6. Preparation and characterization of nanoparticles (NPs)

6.1. Standard protocol for preparation of CP5 NPs

Pt(IV) prodrug-loaded Cys-PDSA NPs coated with lipid-PEG in PBS were prepared as follows: Cys-**8**E polymer (1.1 mg) and Pt(IV) prodrug **5** (0.9 mg) were dissolved in DMSO

(0.1 mL) to form a homogenous solution. Under vigorous stirring (1200 rpm), the mixture was dropwise added to the water (2.9 mL) with lipid-PEG (DSPE-PEG3000, 0.5 mg). The NP dispersion was then transferred to an Amicon Ultra-15 Centrifugal Filter (MWCO 100 KDa, Millipore) and purified by centrifugation. The retained CP**5** NPs were then washed with PBS (3 × 5 mL) before being dispersed in 1.0 mL of PBS for further use.

6.2. Preparation of fluorescent dye-loaded NPs

Dil-loaded CP**5** NPs, Nile red and Coumarin 6-coloaded CP**5** NPs, and DID-loaded CP**5** NPs were prepared by mixing predetermined amounts of Cys-**8**E polymer, Pt(IV) prodrug **5** and dye (3 w/w% Dil, 1.0 w/w% Nile red and 0.1 w/w% Coumarin 6, 3 w/w% DID) in DMSO, respectively, then following the nanoprecipitation procedure described above. Control NPs were prepared following the same protocol, but in the absence of the Pt(IV) prodrug **5**.

6.3. Characterization of NPs

The particle size and zeta potential of NPs were measured using a ZetaPALS Dynamic Light Scattering Detector (DLS, 15mW laser, incident beam 1/4 676 nm, Brookhaven Instruments, USA) at 25 °C and a scattering angle of 90°.

The Pt loading of NPs was determined by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) using a Perkin-Elmer AAnalyst 600 spectrometer fitted with an AS 800 autosampler (Perkin Elmer, USA). The Pt absorption was measured at 265.9 nm and a Zeeman background absorption correction was applied. Samples were prepared by diluting with water until the Pt concentration fell within the linear calibration range (50-200 μg Pt/L). All GFAAS measurements were carried out in triplicate and the data averaged.

7. Electrochemical measurement

Electrochemical measurements of Pt(IV) prodrug **5** and Cys-**8**E polymer were performed in DMF:PBS/0.1 M KCl (1:4) at 25 °C on a CHI600E Electrochemical Analyzer, using a 3-electrode set-up comprising a glassy carbon working electrode, a Pt wire auxiliary electrode, and a Ag/AgCl reference electrode. The electrochemical data were uncorrected for junction potentials.

8. Kinetic measurement

Redox reactions of Pt(IV) prodrug **5** and Cys-**8**E polymer were investigated in DMSO using an Applied Photophysics SX20 Stopped Flow Spectrometer. All kinetic measurements were performed three-times under pseudo-first-order conditions with excess dithiothreitol (DTT) and constant temperature (37 °C) by monitoring the absorbance decrease at 272 nm for Pt(IV) prodrug **5** and 257 nm for Cys-**8**E polymer. Single-exponential kinetic traces were collected in all cases and followed for at least four half-lives, pseudo-first-order rate constants (k_{obs}) were derived from an iterative nonlinear least squares fit of the absorbance-time data, second-order rate constants (k) were obtained by dividing the k_{obs} by the DTT concentration.

9. GSH consumption by Cys-8E polymer

Control NPs without Pt(IV) prodrug **5** were incubated with 1 mM reduced glutathione (GSH) in PBS at 37 °C with stirring at 100 rpm. All the experiments were performed under an

atmosphere of N_2. At designed time points, 0.1 mL of sample solution was withdrawn and GSH concentration was quantified using the Glutathione Assay Kit (BioVision).

10. TEM study

To test morphology changes after treatment with DTT, CP**5** NPs were dispersed in water or 10 mM DTT for 72 h. Samples were diluted with water, negatively stained with 1% uranyl acetate and dried under air. The NP morphology was visualized on a Tecnai G2 Spirit BioTWIN Transmission Electron Microscope (TEM, FET, USA).

11. *In vitro* redox-triggered Pt release

CP**5** NPs were transferred into a Float-A-Lyzer G2 dialysis device (MWCO 100 KDa, Spectrum) and drug release was conducted in PBS (pH 7.4) at 37 °C with stirring at 100 rpm. At designed time points, 1.0 mL of sample solution was withdrawn and Pt concentration was quantified by GFAAS. An equal volume of fresh PBS was immediately replenished. The same drug release procedure was carried out in triplicate in the presence of 1 mM and 10 mM DTT, respectively.

12. Cell culture

A2780 and A2780cis cells were purchased from European Collection of Authenticated Cell Cultures (ECACC). PC-3, MCF7, HCT116, A549 and H460 cells were purchased from American Type Culture Collection (ATCC). Cells were cultured using recommended manufacturer protocols. For example, A2780cis cisplatin-resistant cells were grown in RPMI 1640 medium, supplemented with 10% FBS and 1 μM cisplatin. Exponentially growing cultures were maintained at 37 °C under an atmosphere of 5% CO_2 and 90% relative humidity, and grown to 70% confluence before splitting or harvesting.

13. Cellular uptake of CP5 NPs

A2780 and A2780cis cells were seeded in 6-well plates (50,000 cells per well) and incubated with 1 mL of RPMI 1640 medium containing 10% FBS for 24 h. Subsequently, cells were exposed to Dil-loaded CP**5** NPs at 0.5 mg/L. At selected time intervals, cells were washed with PBS three-times, collected and analyzed by Flow Cytometry (BD FACSAriaTM III, USA).

14. Intracellular disintegration of CP5 NPs

Nile red (Ex/Em = 530/590 nm) and Coumarin 6 (Ex/Em = 410/520 nm) were coloaded as FRET (Förster resonance energy transfer) pairs to interrogate the intracellular behavior of the CP5 NPs. A2780 and A2780cis cells were seeded in dishes (20,000 cells per well) and incubated with 1 mL of RPMI 1640 medium containing 10% FBS for 24 h. Subsequently, cells were incubated with Opti-MEM (Thermo Fisher Scientific) for 30 minutes before adding Nile red and Coumarin 6-coloaded CP**5** NPs. At predetermined time points, cells were washed with PBS three-times and imaged using an FV1200 Confocal Laser Scanning Microscope (CLSM, Olympus, Japan) (Ex = 410 nm, Em = 520 and 590 nm).

To investigate the effect of intracellular GSH on the disintegration of NPs, A2780 and A2780cis cells were pretreated with 50 μM N-ethylmaleimide (NEM) for 1 h to consume

sulfhydryl, then all other procedure was carried out as described above.

15. Intracellular relative GSH/GSSG ratio

A2780 and A2780cis cells were seeded in in dishes (1,000,000 cells per dish) and incubated with 1 mL of RPMI 1640 medium containing 10% FBS for 24 h. After incubation with cisplatin or CP**5** NPs at 0, 1.56, 6.25 or 25 μmol Pt/L for 24 h, cells were collected, homogenized and centrifuged. The supernatant was used in the GSH and GSSH assay according to the manufacturer protocol (BioVision).

16. *In vitro* cell proliferation

A2780, A2780cis, PC-3, MCF7, HCT116, A549 and H460 cells were seeded in 96-well plates (5,000 cells per well) and incubated with 100 μL of medium containing 10% FBS for 24 h. Thereafter, cells were treated with cisplatin, CP**5** NPs or cisplatin+control NPs at different concentrations. 48 h or 72 h later, cytotoxicity was evaluated using the AlamarBlue Cell Viability Assay (Thermo Fisher Scientific) according to the manufacturer protocol using a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, USA).

17. *In vitro* apoptosis

A2780 and A2780cis cells were seeded in 6-well plates (50,000 cells per well) and incubated with 1 mL of RPMI 1640 medium containing 10% FBS for 24 h. Thereafter, cisplatin, CP**5** NPs, or cisplatin+control NPs at 1, 5 or 25 μmol Pt/L was added. 24 h later, apoptosis was evaluated using the Dead Cell Apoptosis Kit with Annexin V Alexa Fluor 488 & Propidium Iodide (Thermo Fisher Scientific) according to the manufacturer protocol.

18. Animals

Healthy BALB/c mice and athymic nude mice (4-5 weeks old) were purchased from Charles River Laboratories. All the animal experiments reported in this manuscript were performed in accordance with National Institutes of Health animal care guidelines and in strict pathogen-free conditions in the animal facility of Brigham and Women's Hospital. The animal protocol was approved by the Institutional Animal Care and Use Committees (Harvard Medical School).

19. A2780cis xenograft tumor model

The cisplatin-resistant ovarian carcinoma model was generated by subcutaneously injecting A2780cis cell suspension (2,000,000 cells in 100 μL of RPMI 1640 medium and 100 μL of Matrigel) into the flank of athymic nude mice. When the size of A2780cis xenograft tumor reached ~100 mm^3, mice were used for the following *in vivo* experiments.

20. Pharmacokinetics of CP5 NPs

BALB/c mice were randomly assigned to three groups (n = 5) and given an intravenous injection of either (i) PBS, (ii) DID or (iii) DID-loaded CP**5** NPs at 1.0 mg DID dose per kg mouse weight. At predetermined time intervals, blood was withdrawn from retro-orbital plexus, heparinized, centrifuged at 4000 rpm for 20 min at 4 °C. The supernatant was

separated and stored at -80 °C until analysis. Fluorescence intensity was determined using a Microplate Reader and pharmacokinetic parameters were calculated by non-compartment analysis using a Phoenix WinNonlin 6.3 Program (Pharsight Cooperation, St. Louis Missouri, USA).

The following parameters were obtained from the NP circulation profile: the area under the plasma concentration-time curve from time zero to infinity ($AUC_{0\rightarrow inf}$), the area under the plasma concentration-moment curve from time zero to infinity ($AUMC_{0\rightarrow inf}$), total body clearance (CL), volume of distribution at steady state (V_{ss}), mean residence time from time zero to infinity ($MRT_{0\rightarrow inf}$).

21. *In vivo* biodistribution of CP5 NPs

A2780cis tumor-bearing athymic nude mice were randomly assigned to two groups (n = 3) and given an intravenous injection of either (i) DID or (ii) DID-loaded CP**5** NPs at 1.0 mg DID dose per kg mouse weight. A series of near-infrared (NIR) images were collected from mice under isoflurane anesthesia using the Maestro 2 *In Vivo* Imaging System (CRi Inc, USA). Finally, mice were sacrificed, and their plasma, tumors and organs (heart, liver, spleen, lung and kidney) were collected for *ex vivo* imaging. To evaluate the accumulation of NPs in the tissues, fluorescence intensity was quantified using the Image-J software.

22. Immunofluorescence staining

A2780cis tumor-bearing athymic nude mice were randomly assigned to two groups (n = 3) and given an intravenous injection of either (i) DID or (ii) DID-loaded CP**5** NPs at 1.0 mg DID dose per kg mouse weight. 24 h later, mice were sacrificed, and their tumors and organs were harvested, fixed with 4% paraformaldehyde, and then embedded into paraffin and cut into sections. To image vasculatures, slices were heated at 60 °C for 1 h and washed with xylene, ethanol, and PBS three-times. After blocking with 10% FBS for 1.5 h, slices were incubated with rat anti-mouse CD31 antibodies (Abcam) at 4 °C for 1 h, washed with PBS/0.2% Triton X-100 three-times, and incubated with Alexa Fluor 488-conjugated secondary antibodies (Goat anti-rat IgG, Abcam) for 1 h. Thereafter, slides were washed with PBS three-times, stained with Hoechst 33342, and then viewed by CLSM.

23. *In vivo* antitumor evaluation of CP5 NPs

A2780cis tumor-bearing athymic nude mice were randomly assigned to four groups (n = 5) and intravenously injected with (i) PBS, (ii) cisplatin, (iii) CP**5** NPs or (iv) control NPs at 4 mg Pt dose per kg mouse weight. The mice were injected four times at seven-day intervals, with the first injection day designated as day 0.

Tumor volume was monitored every two days using a caliper and calculated according to the equation: $V = a^2 \times b \times 0.5$, where a and b are the shortest and longest perpendicular diameters. Mice body weight and their physical performance were recorded every two days as indicators of systemic toxicity.

After tumor monitoring, mice were euthanized. Tumors were collected, weighed and pictured using the Maestro 2 *In Vivo* Imaging System. Tumor inhibition rate (TIR) was calculated according to the equation: $TIR\ (\%) = (m\text{-}m_t)/m \times 100$. Where m is the average

weight of tumors in PBS group; m_t is the average weight of tumors in other groups. Tumors were fixed and stained with H&E for histopathological examination.

24. Western blot

A2780cis tumor-bearing athymic nude mice were randomly assigned to four groups (n = 5) and intravenously injected with (i) PBS, (ii) cisplatin, (iii) CP**5** NPs or (iv) control NPs at 4 mg Pt dose per kg mouse weight. The mice were injected four times at seven-day intervals. After that, mice were sacrificed, and the tumors were quickly collected and immediately stored in liquid nitrogen.

Proteins were extracted with radioimmunoprecipitation assay lysis buffer (Cell Signaling), supplemented with protease/phosphatase inhibitor cocktail (Cell Signaling) and phenylmethanesulfonyl fluoride (PMSF, Cell Signaling). Equal amounts of these proteins, as determined by Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific) according to the manufacturer instruction, were added to SDS-PAGE gels and separated by gel electrophoresis. After transferring proteins from the gel to the polyvinylidene difluoride (PVDF) membrane, the membrane was blocked with 3% BSA in TBST (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% Tween 20), then incubated with primary antibodies (p53, Caspase 3, PARP, Cleaved PARP, Cell Signaling) and β-actin rabbit antibodies (Cell Signaling). Subsequently, blots were incubated with HRP-conjugated secondary antibodies (Goat anti-rabbit IgG, Cell Signaling) and an Enhanced Chemiluminescence Detection System (ECL, Pierce, Thermo Fisher Scientific), before finally being exposed on blue autoradiography films (Amersham Biosciences, USA).

25. Immunohistochemistry (IHC) and TUNEL

A2780cis tumor-bearing athymic nude mice were randomly assigned to four groups (n = 5) and intravenously injected with (i) PBS, (ii) cisplatin, (iii) CP**5** NPs or (iv) control NPs at 4 mg Pt dose per kg mouse weight. The mice were injected four times at seven-day intervals. After that, mice were euthanized, and the tumors were quickly collected and immediately stored in 4% paraformaldehyde.

Tumors were used to evaluate the expression of Proliferating Cell Nuclear Antigen (PCNA), p53, B-cell lymphoma 2 (Bcl-2), and Caspase 3 by immunohistochemistry. Slices were incubated with primary antibodies (Cell Signaling), then HRP/DAB Detection IHC kit (Abcam) according to the manufacturer instruction.

Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling (TUNEL) was performed using an In Situ Cell Death Detection Kit (Roche) in accordance with the instruction provided by the manufacturer.

26. Blood Chemistry

BALB/c mice were randomly assigned to four groups (n = 5) and intravenously injected with (i) PBS, (ii) cisplatin, (iii) CP**5** NPs or (iv) control NPs at 4 mg Pt dose per kg mouse weight. 24 h later, blood was collected from the retro-orbital plexus, heparinized, centrifuged at 4000 rpm for 20 min at 4 °C to obtain plasma for measuring alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine (Scr) using an Automatic Biochemical Analyzer (UniCel DxC 800

Synchron Clinical System, Beckman Coulter, USA).

27. Hemolysis

Red blood cells (RBCs), collected from BALB/c mice, were diluted with PBS to 2 v/v%. Cisplatin, CP**5** NPs, control NPs and Tween 80 were dispersed in RBC suspensions at gradient Pt concentrations, then incubated at 37 °C for 3 h, and centrifuged at 1500 rpm for 15 min. The supernatant was measured using a Microplate Reader at 541 nm, and the hemolysis rate was determined using the following relationship: Hemolysis rate (%) = $(A_{sample}-A_0)/(A_{100}-A_0) \times 100$. Where A_{sample} is the absorbance of sample, A_{100} is the absorbance of lysed RBCs in water (positive control), A_0 is the absorbance of 0% hemolysis in PBS (negative control). The concentrations of control NPs and Tween 80 were equal in weight to those in CP**5** NPs.

28. Histopathology

BALB/c mice were randomly assigned to four groups (n = 5) and intravenously injected with (i) PBS, (ii) cisplatin, (iii) CP**5** NPs or (iv) control NPs at 4 mg Pt dose per kg mouse weight four times at seven-day intervals. The mice were injected four times at seven-day intervals, before being euthanized. Their organs (heart, liver, spleen, lung, kidney) were then harvested, fixed with 4% paraformaldehyde, embedded in paraffin, and stained with haematoxylin and eosin (H&E) before undergoing histopathological examination using an Upright Microscope (BX63, Olympus, Japan).

29. Statistical analysis

All the experiments were repeated at least three times and data were expressed as the mean ± standard deviation (SD) unless otherwise noticed. Unpaired student's t-test was used for between two-group comparison and one-way analysis of variance (ANOVA) with LSD and S-N-K tests was for multi-group analysis. Asterisk represented statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Data were analysed using SPSS 20.0 software.

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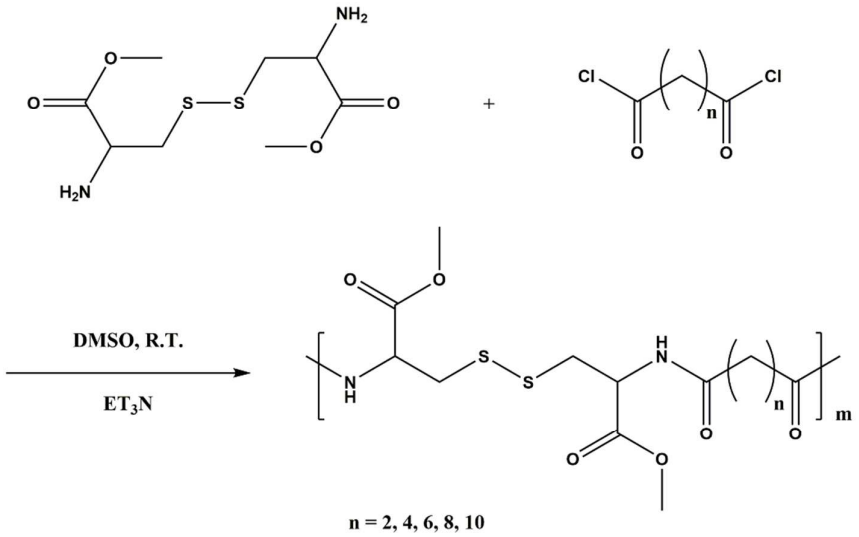

Figure S1. Synthetic routes of Cys-PDSA polymers.

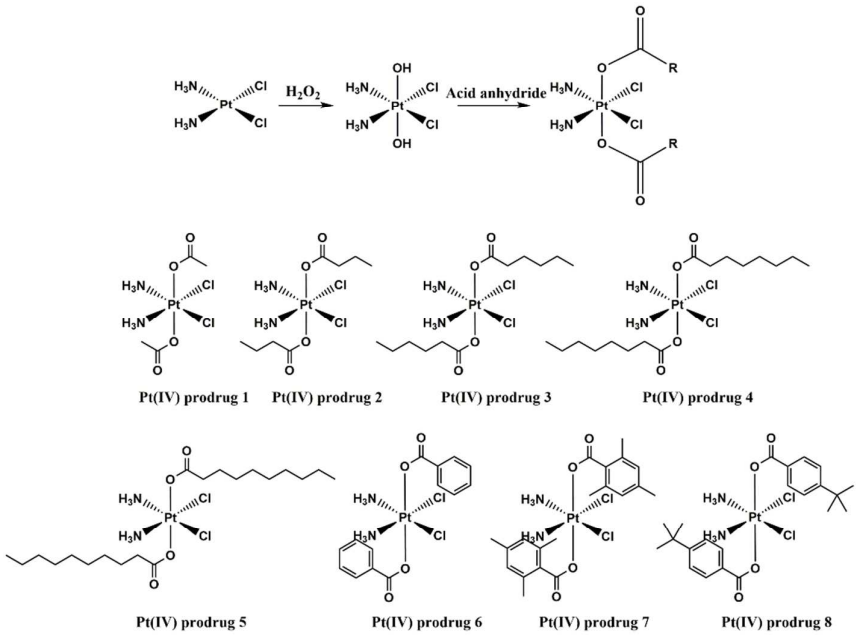

Figure S2. Synthetic routes of Pt(IV) prodrugs.

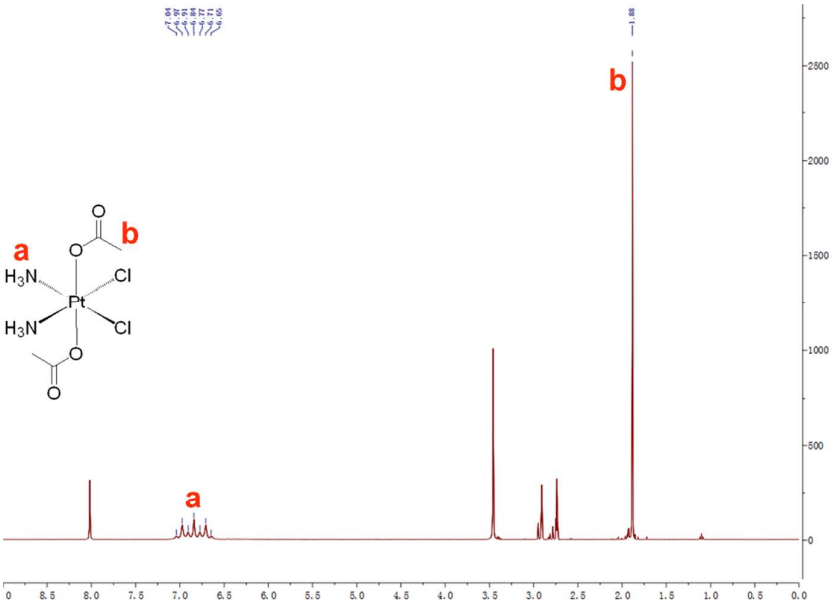

Figure S3. ^{1}H NMR spectrum of Pt(IV) prodrug **1**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCCH_3)_2$].

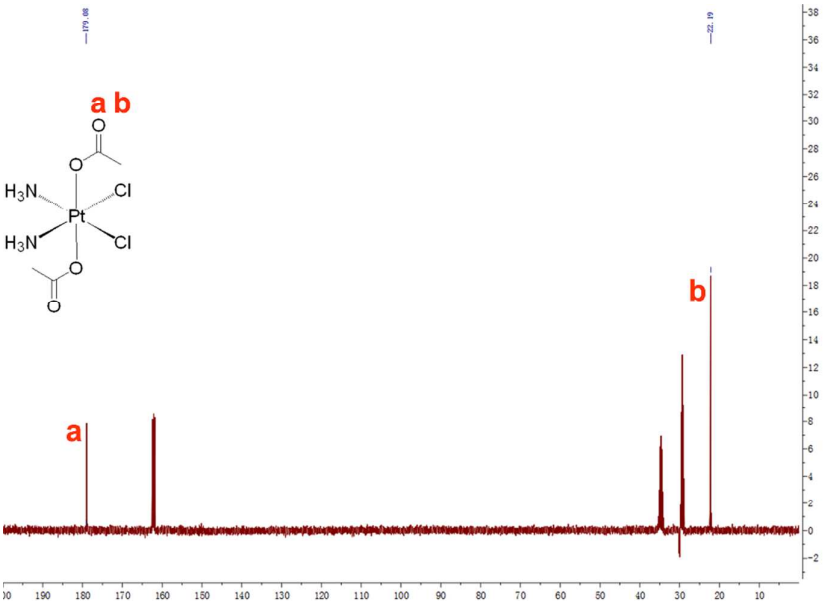

Figure S4. ^{13}C NMR spectrum of Pt(IV) prodrug **1**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCCH_3)_2$].

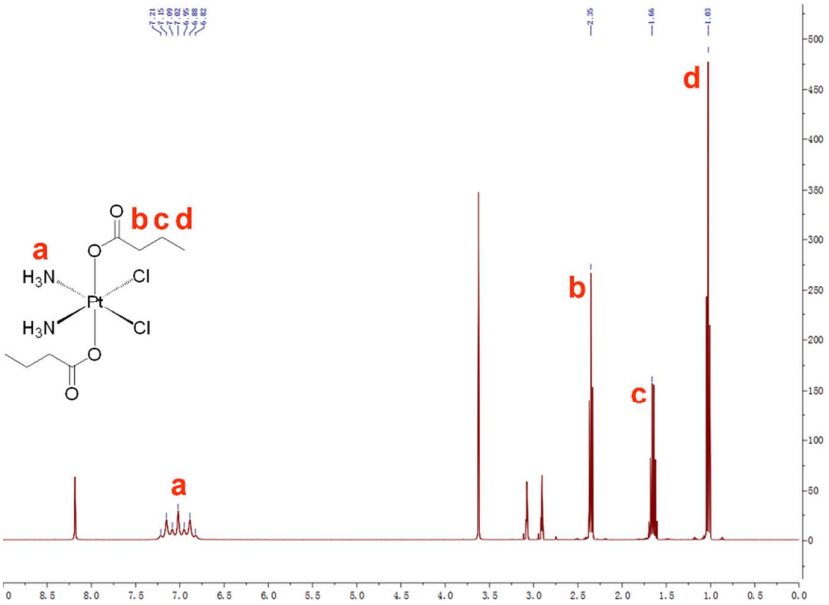

Figure S5. ^{1}H NMR spectrum of Pt(IV) prodrug **2**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_2CH_3)_2$].

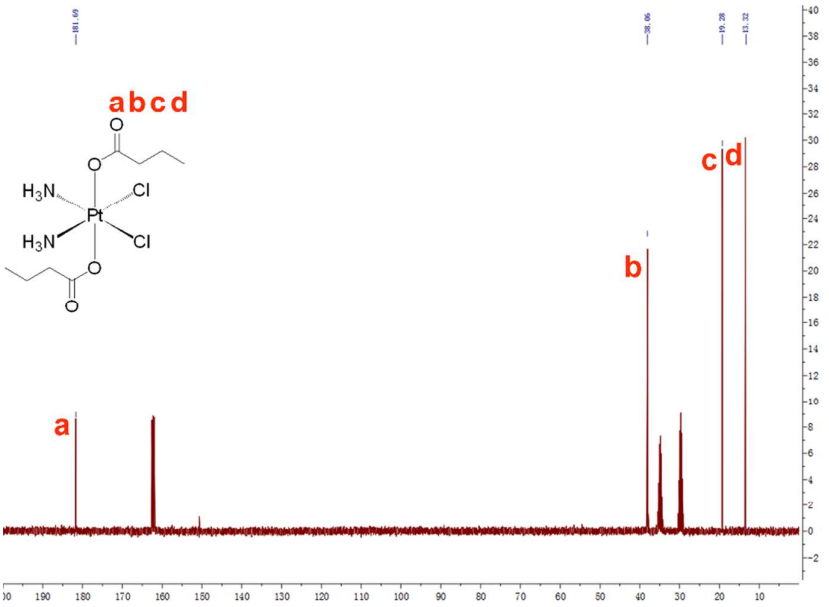

Figure S6. ^{13}C NMR spectrum of Pt(IV) prodrug **2**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_2CH_3)_2$].

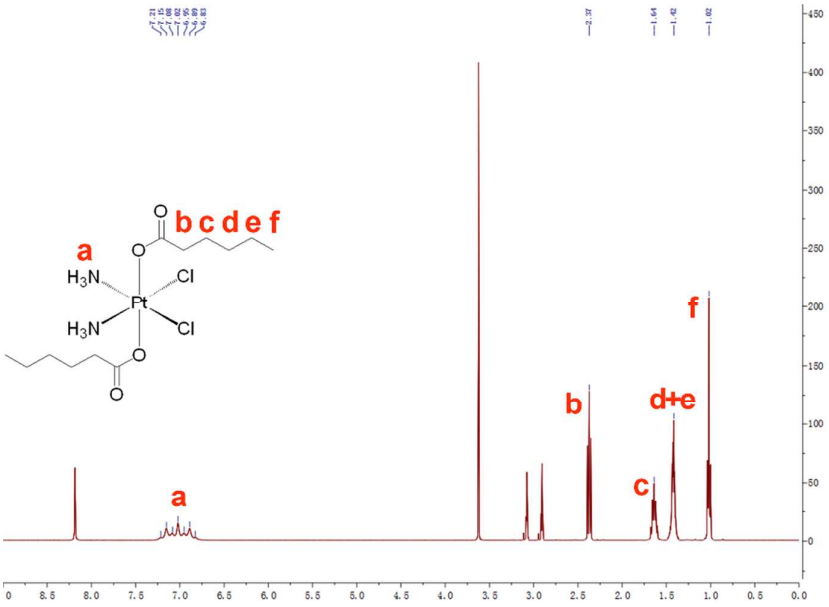

Figure S7. ^{1}H NMR spectrum of Pt(IV) prodrug **3**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_4CH_3)_2$].

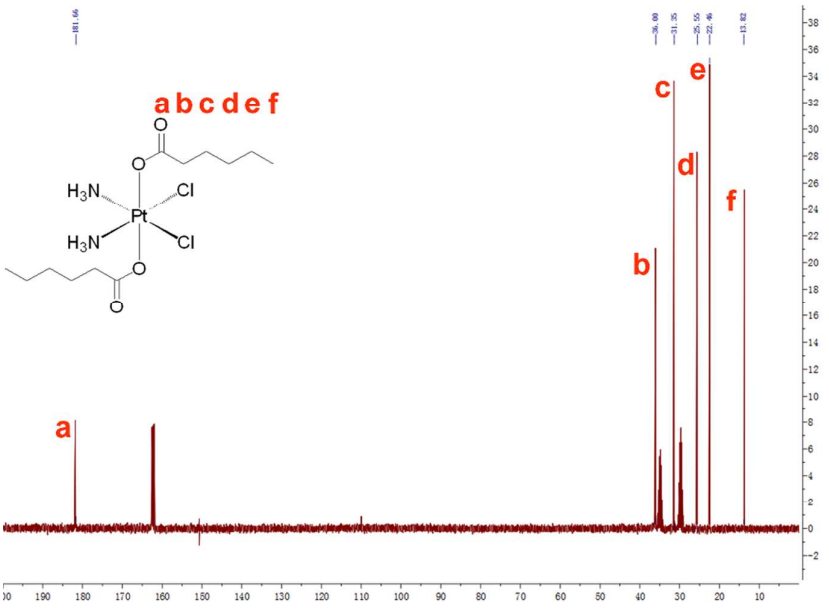

Figure S8. ^{13}C NMR spectrum of Pt(IV) prodrug **3**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_4CH_3)_2$].

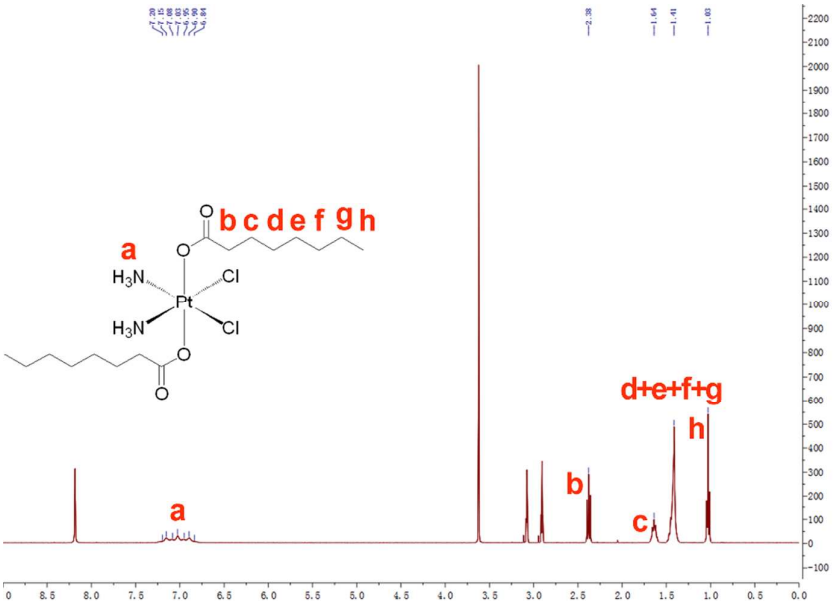

Figure S9. ^{1}H NMR spectrum of Pt(IV) prodrug **4**, *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OOC(CH_2)_6CH_3)_2]$.

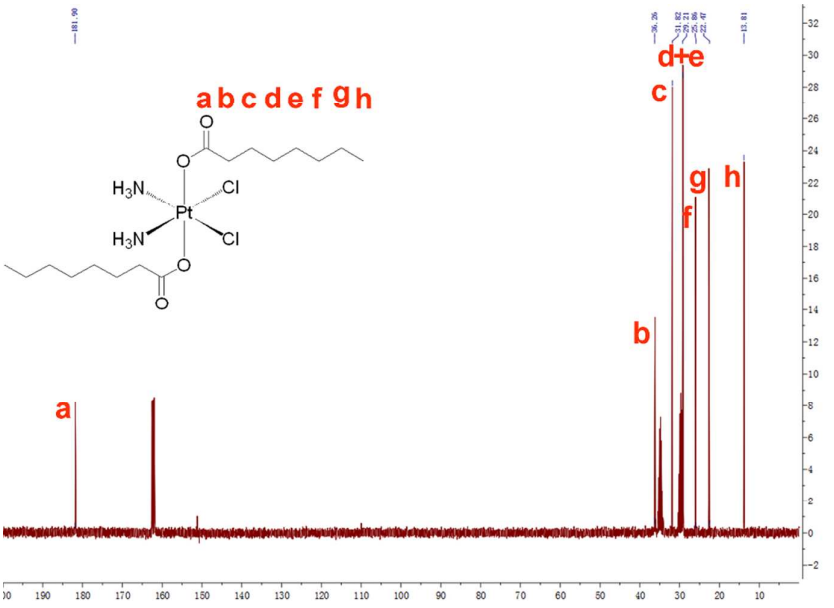

Figure S10. ^{13}C NMR spectrum of Pt(IV) prodrug **4**, *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OOC(CH_2)_6CH_3)_2]$.

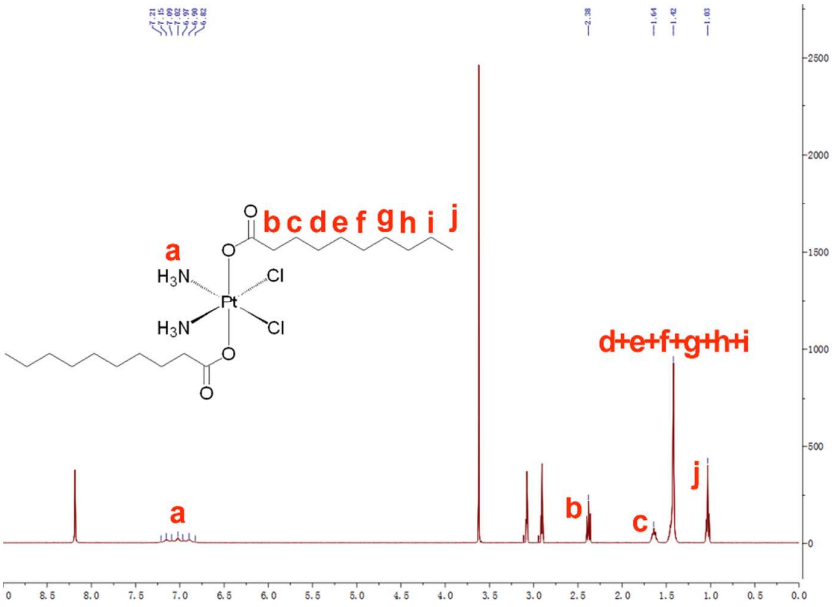

Figure S11. ^{1}H NMR spectrum of Pt(IV) prodrug **5**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_8CH_3)_2$].

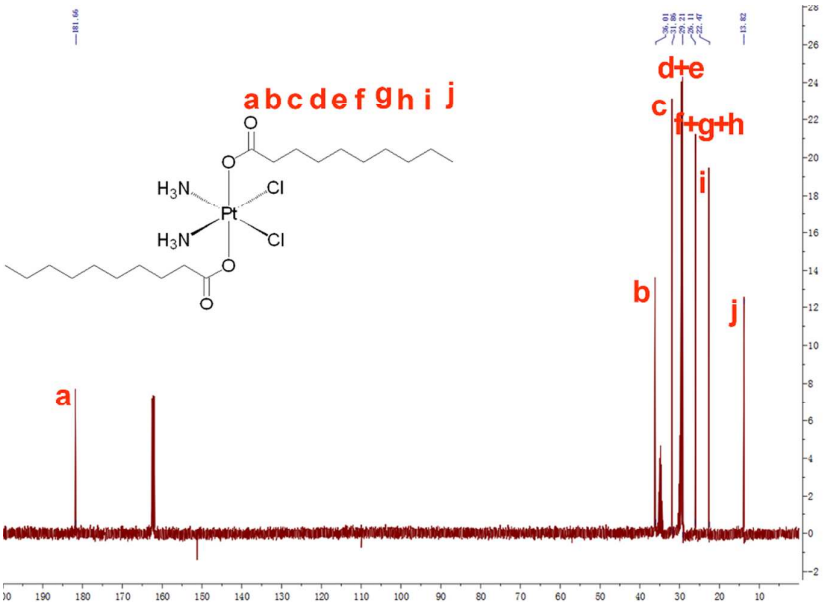

Figure S12. ^{13}C NMR spectrum of Pt(IV) prodrug **5**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOC(CH_2)_8CH_3)_2$].

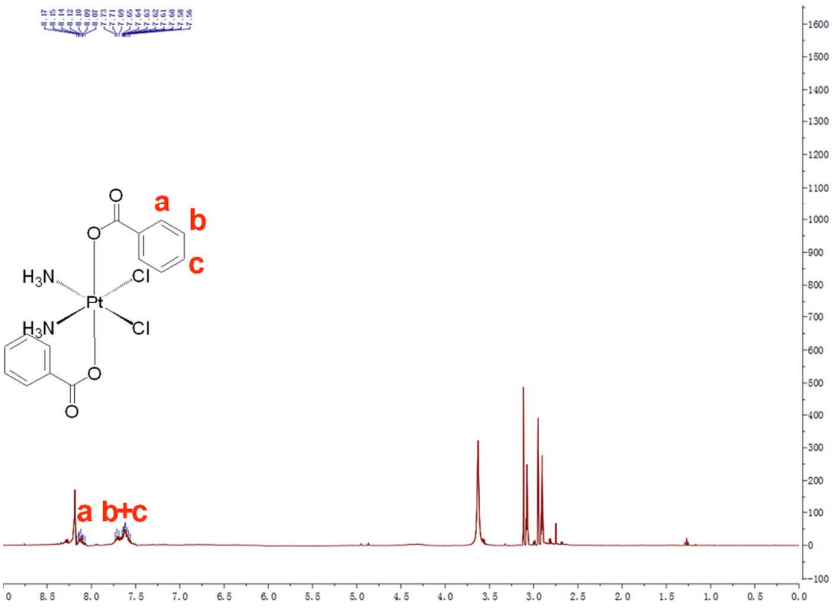

Figure S13. ^{1}H NMR spectrum of Pt(IV) prodrug **6**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_5)_2$].

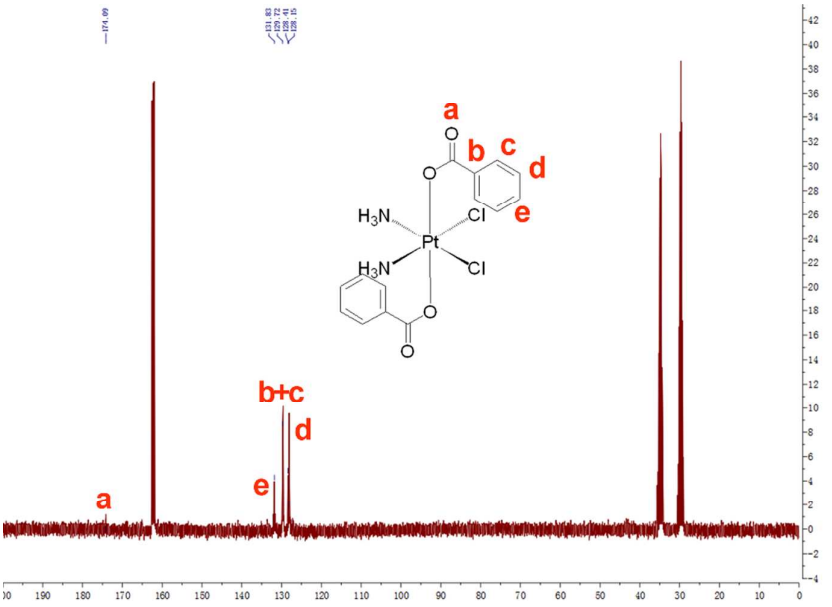

Figure S14. ^{13}C NMR spectrum of Pt(IV) prodrug **6**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_5)_2$].

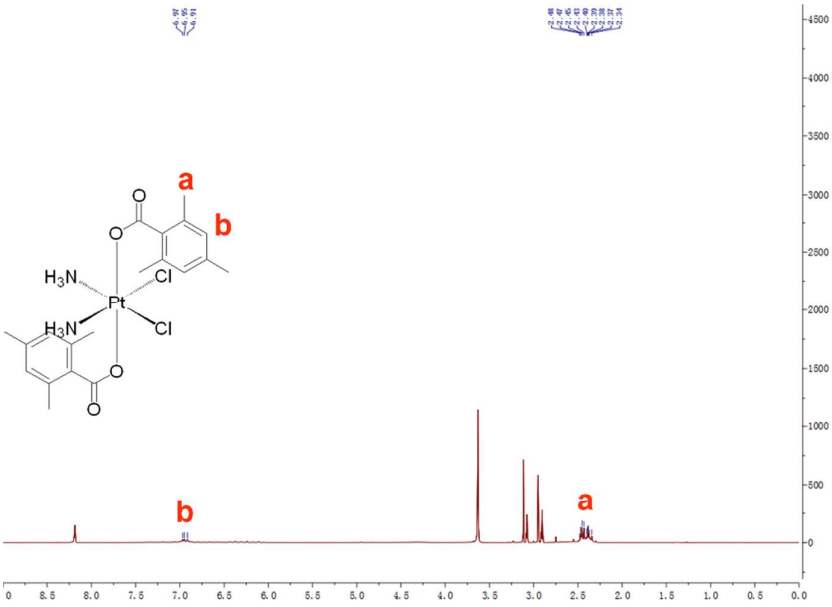

Figure S15. ^{1}H NMR spectrum of Pt(IV) prodrug **7**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_2(CH_3)_3)_2$].

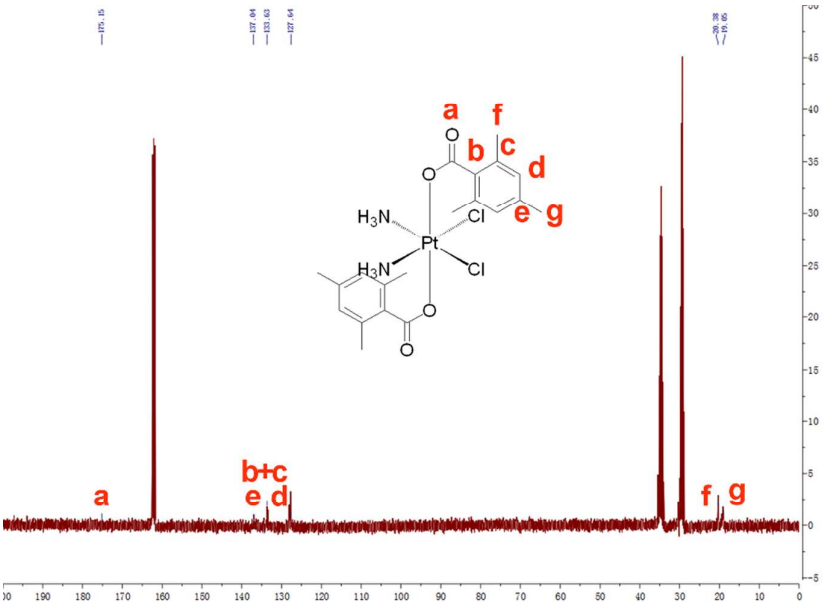

Figure S16. ^{13}C NMR spectrum of Pt(IV) prodrug **7**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_2(CH_3)_3)_2$].

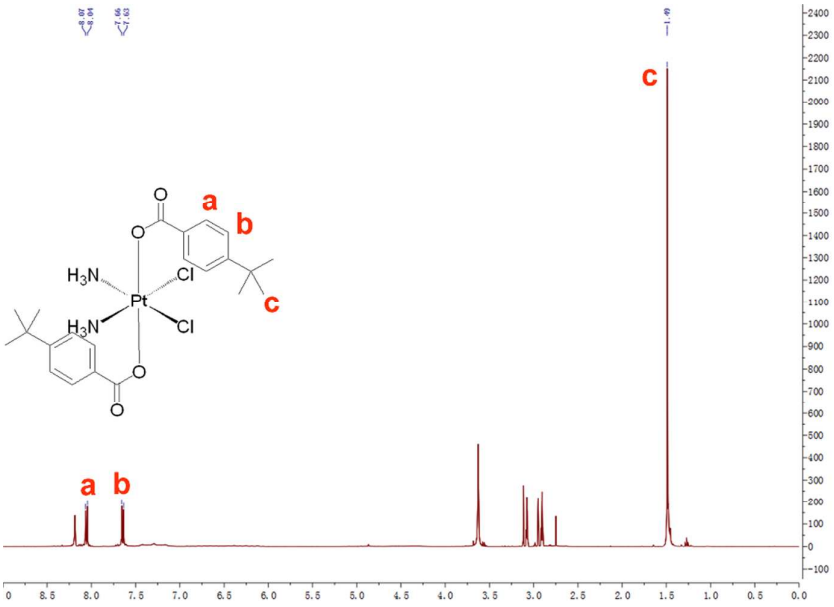

Figure S17. ^{1}H NMR spectrum of Pt(IV) prodrug **8**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_4C(CH_3)_3)_2$].

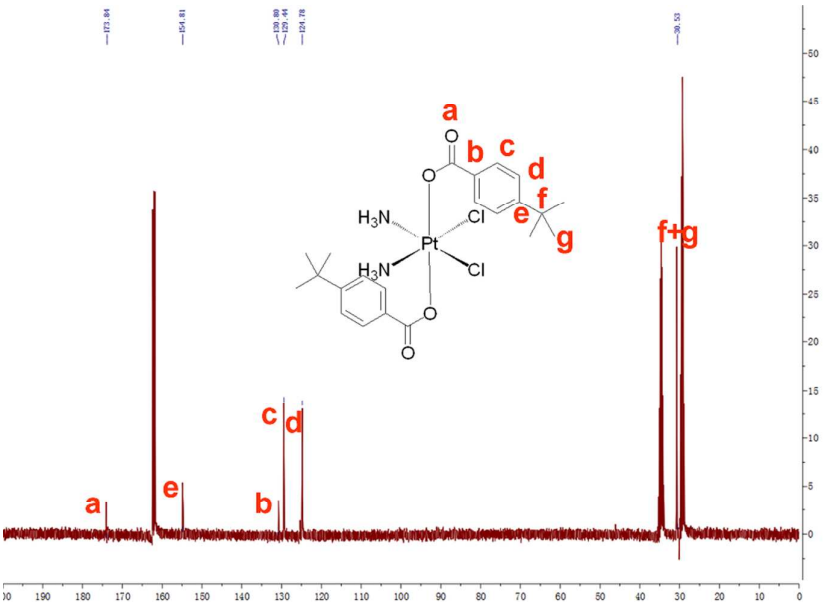

Figure S18. ^{13}C NMR spectrum of Pt(IV) prodrug **8**, *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCC_6H_4C(CH_3)_3)_2$].

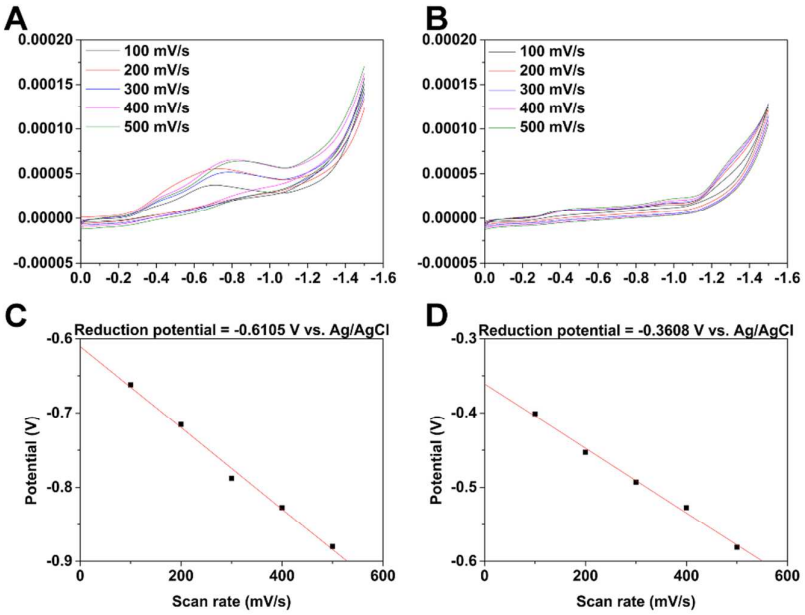

Figure S19. Cyclic voltammograms for (A) Pt(IV) prodrug **5** and (B) Cys-**8**E polymer. Plots of reduction peak potential maxima for (C) Pt(IV) prodrug **5** and (D) Cys-**8**E polymer from the voltammograms as a function of scan rates.

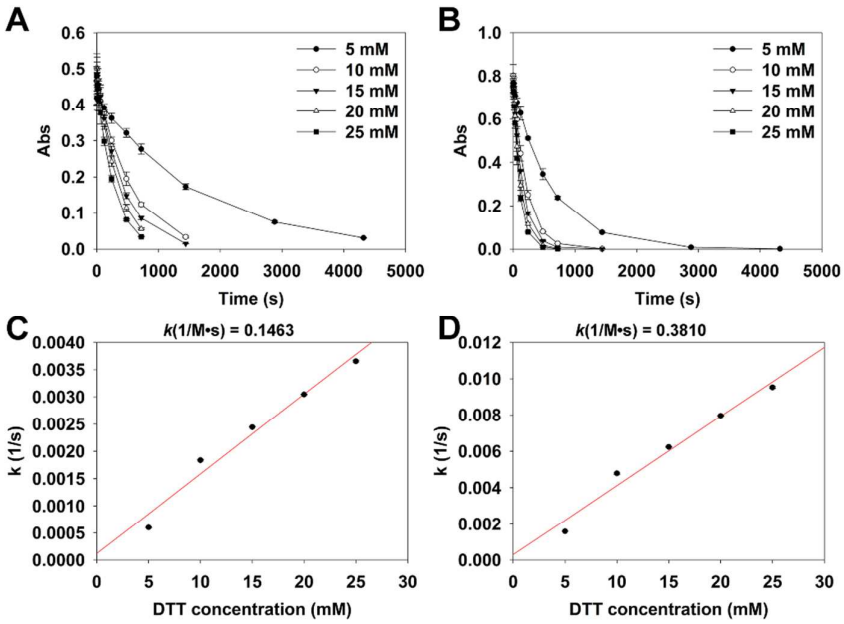

Figure S20. Plots of reduction kinetics for (A) Pt(IV) prodrug **5** and (B) Cys-**8**E polymer with different concentrations of DTT. Dependence of k_{obs} for reduction of (C) Pt(IV) prodrug **5** and (D) Cys-**8**E polymer by DTT.

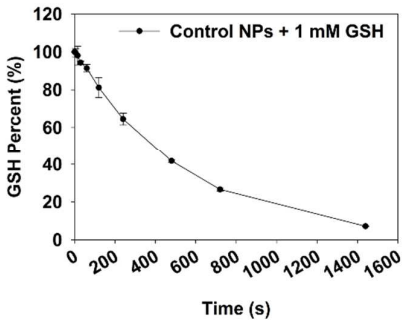

Figure S21. GSH consumption by Cys-**8**E polymer.

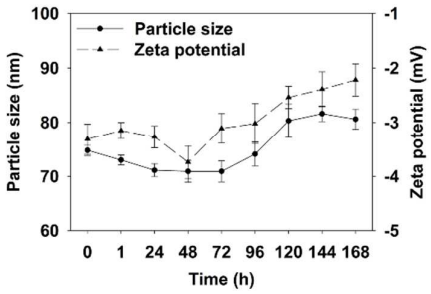

Figure S22. Particle size and zeta potential of CP**5** NPs monitored over the course of one week.

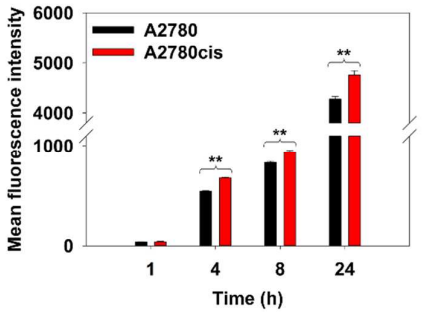

Figure S23. Mean fluorescence intensity of A2780 and A2780cis cells incubated with Dil-loaded CP**5** NPs for 1, 4, 8, and 24 h.

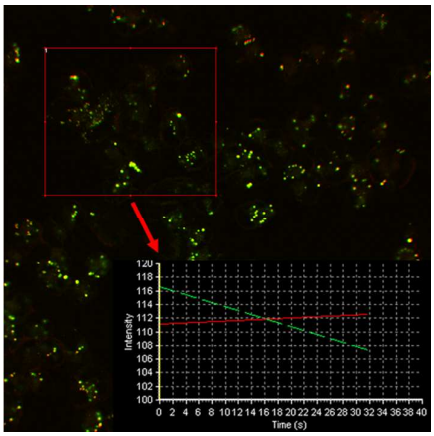

Figure S24. A2780cis cells incubated with Nile red and Coumarin 6-coloaded CP**5** NPs for 4 h (60× objective). After being excited at 410 nm, transfer of energy from Coumarin 6 (green) to Nile Red (red) due to their close proximity was observed.

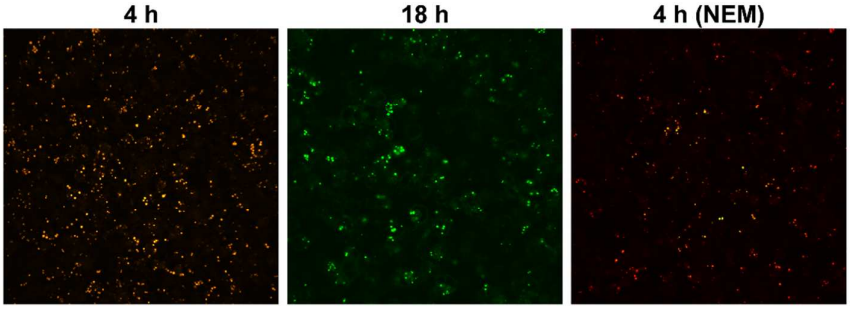

Figure S25. Confocal fluorescence images of A2780 cells incubated with Nile red and Coumarin 6-coloaded CP**5** NPs for 4 and 18 h (60× objective). Cells were also pretreated with NEM to consume intracellular GSH.

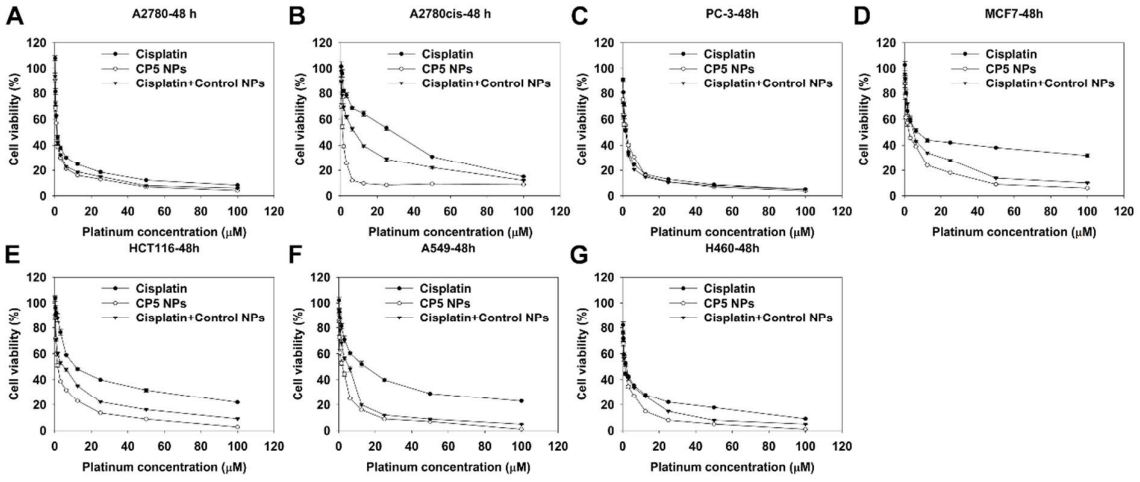

Figure S26. Cell viability of (A) A2780, (B) A2780cis, (C) PC-3, (D) MCF7, (E) HCT116, (F) A549 and (G) H460 cells incubated with cisplatin, CP**5** NPs or cisplatin+control NPs for 48 h.

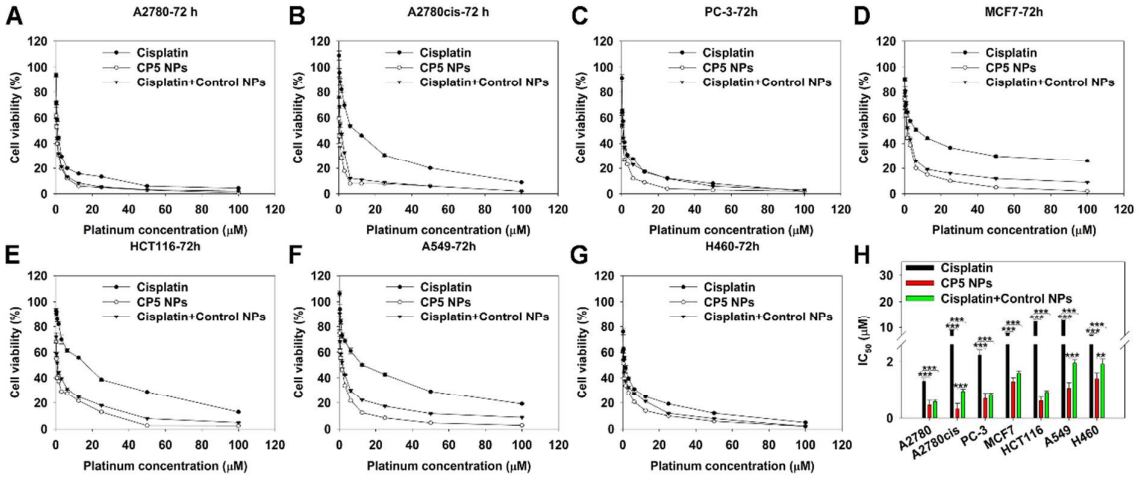

Figure S27. Cell viability of (A) A2780, (B) A2780cis, (C) PC-3, (D) MCF7, (E) HCT116, (F) A549 and (G) H460 cells incubated with cisplatin, CP**5** NPs or cisplatin+control NPs for 72 h. (H) IC_{50} values for above cell lines.

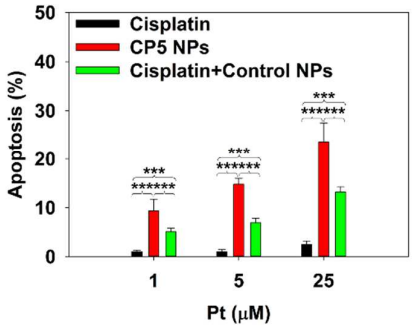

Figure S28. *In vitro* apoptosis of A2780 cells treated with cisplatin, CP**5** NPs or cisplatin+control NPs for 24 h.

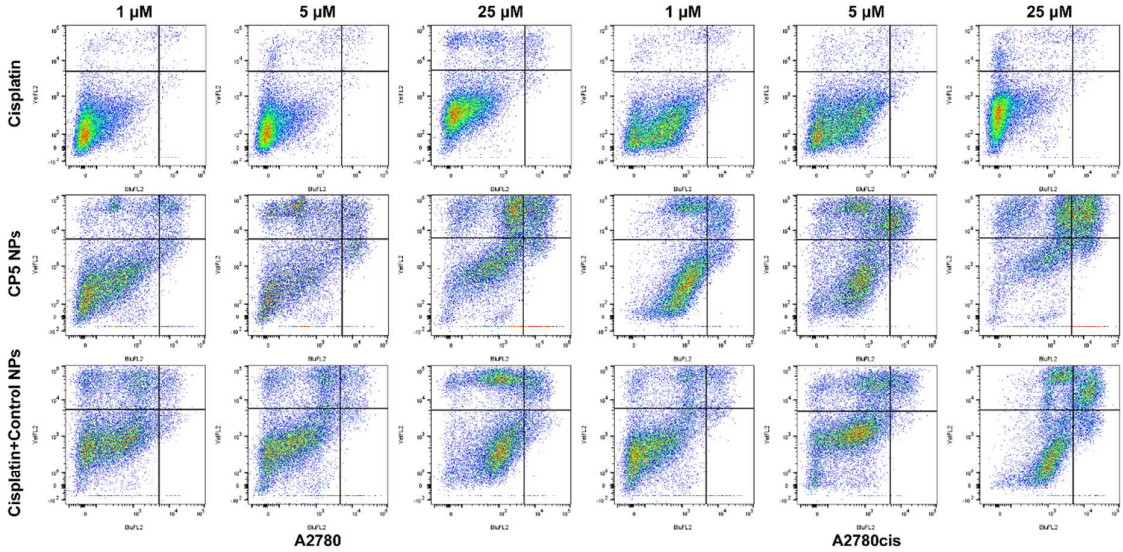

Figure S29. A2780 and A2780cis cells were incubated with cisplatin, CP**5** NPs or cisplatin+control NPs for 24 h to evaluate apoptosis. The BluFL2 channel and YelFL2 channel were used to detected AnnexinV-FITC and PI, respectively.

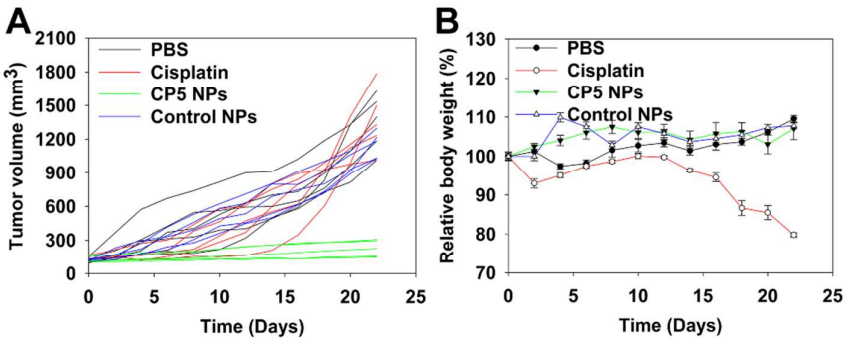

Figure S30. (A) Tumor growth curve of each mouse from the different groups of A2780cis tumor-bearing athymic nude mice treated with PBS, cisplatin, CP**5** NPs or control NPs. (B) Body weight changes of A2780cis tumor-bearing athymic nude mice treated with PBS, cisplatin, CP**5** NPs or control NPs.

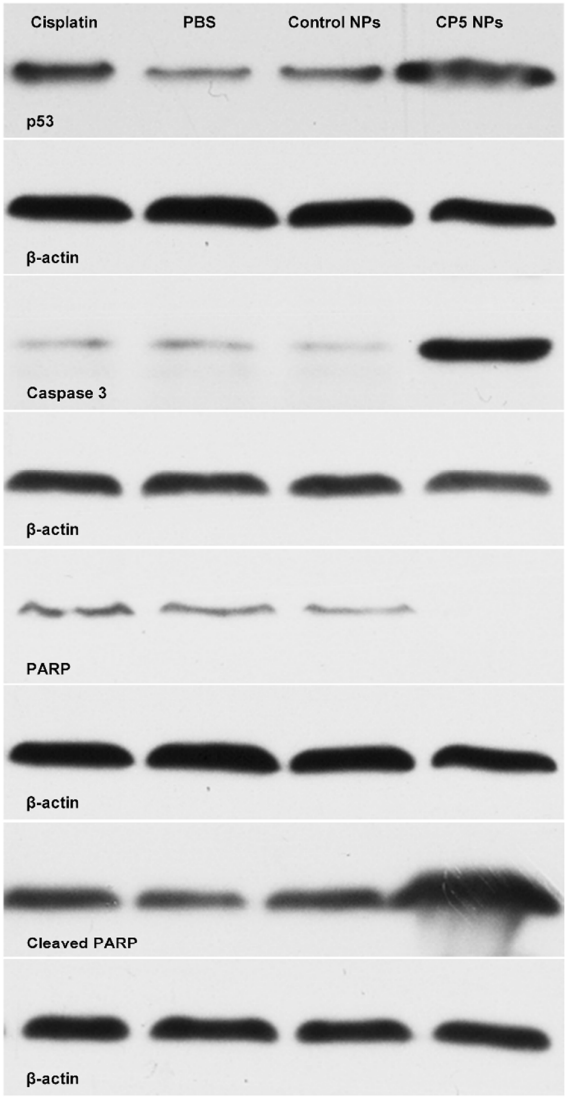

Figure S31. Western blot of p53, Caspase 3, PARP and Cleaved PARP.

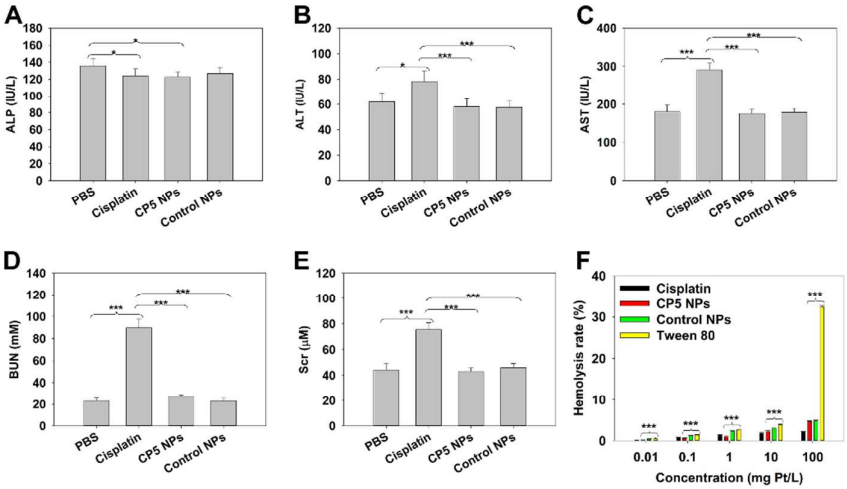

Figure S32. Activity changes of (A) ALP, (B) ALT, (C) AST, (D) BUN, and (E) Scr. (F) Hemolysis rate of cisplatin, CP**5** NPs, control NPs and Tween 80.

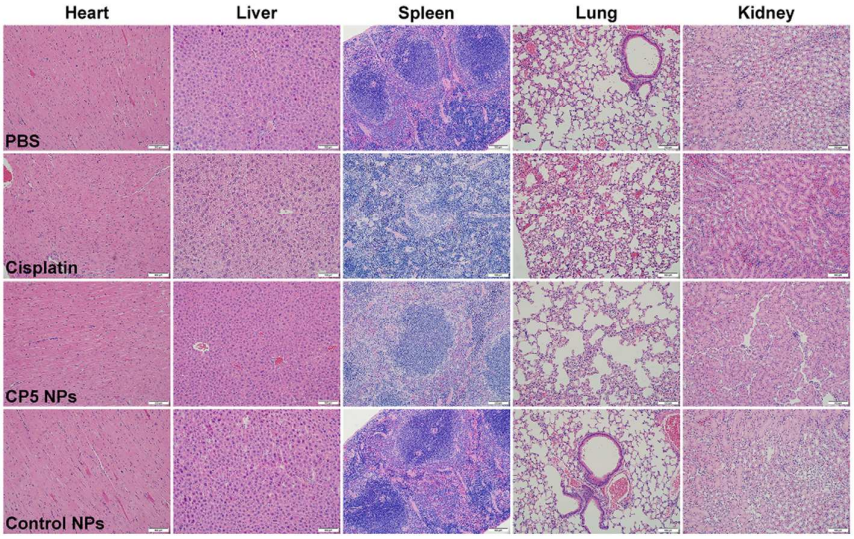

Figure S33. *Ex vivo* histological section of organs.

Table S1. Pt(IV) prodrugs and hydrophobic side chain (R) shown in Figure S2.

Pt(IV) prodrugs	Acid Anhydride	R
Pt(IV) prodrug **1**	Acetic anhydride	Methyl
Pt(IV) prodrug **2**	Butanoic anhydride	Propyl
Pt(IV) prodrug **3**	Hexanoic anhydride	Pentyl
Pt(IV) prodrug **4**	Octanoic anhydride	Heptyl
Pt(IV) prodrug **5**	Decanoic anhydride	Nonyl
Pt(IV) prodrug **6**	Benzoic anhydride	Phenyl
Pt(IV) prodrug **7**	2,4,6-trimethylbenzoic anhydride	2,4,6-trimethylphenyl
Pt(IV) prodrug **8**	4-tert-butyl-benzoic acid anhydride	4-tert-butyl-phenyl

Table S2. Particle size, zeta potential and Pt loading of NPs prepared from Cys-PDSA polymers and Pt(IV) prodrug **5**.

Cys-PDSA polymers	Obtained NPs	Particle size (nm)[a]	Polydispersity index[a]	Zeta potential (mV)	Pt loading (%)[b]
Cys-**2**E polymer	C**2**P NPs	31.2±0.4	0.216±0.011	-3.99±0.23	1.62±0.11
Cys-**4**E polymer	C**4**P NPs	41.5±0.7	0.223±0.008	-3.93±0.13	4.01±0.43
Cys-**6**E polymer	C**6**P NPs	52.2±0.9	0.252±0.004	-3.82±0.38	7.23±0.56
Cys-**8**E polymer	C**8**P NPs	76.2±0.9	0.265±0.010	-3.48±0.17	15.50±0.94
Cys-**10**E polymer	C**10**P NPs	120.3±1.2	0.241±0.007	-3.08±0.42	18.21±2.22

[a] Particle size and polydispersity index were determined DLS.

[b] Pt loading was calculated according to the equation: Pt loading (%) = Weight of charged Pt/ Weight of NPs × 100

Table S3. Particle size, zeta potential and Pt loading of NPs prepared from Cys-**8**E polymer and Pt(IV) prodrugs.

Pt(IV) prodrugs	Obtained NPs.	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Pt loading (%)
Pt(IV) prodrug **1**	CP**1** NPs	64.8±0.8	0.268±0.007	-4.77±0.65	1.44±0.04
Pt(IV) prodrug **2**	CP**2** NPs	66.6±2.1	0.266±0.005	-3.21±0.02	3.20±0.38
Pt(IV) prodrug **3**	CP**3** NPs	68.7±0.5	0.271±0.009	-4.82±0.28	3.88±0.44
Pt(IV) prodrug **4**	CP**4** NPs	79.3±0.7	0.228±0.006	-2.69±0.39	9.22±0.32
Pt(IV) prodrug **5**	CP**5** NPs	76.2±0.9	0.265±0.010	-3.48±0.17	15.50±0.94
Pt(IV) prodrug **6**	CP**6** NPs	116.4±1.6	0.219±0.090	-9.25±1.13	2.05±0.53
Pt(IV) prodrug **7**	CP**7** NPs	138.3±0.2	0.202±0.037	-13.83±0.38	3.13±0.22
Pt(IV) prodrug **8**	CP**8** NPs	249.0±10.6	0.260±0.011	-11.54±1.69	3.30±0.38

Table S4. Pharmacokinetics of BALB/c mice post intravenous injection of DID or DID-loaded CP**5** NPs.

Parameters (Non-compartment model)	Unit	DID	DID-loaded CP**5** NPs
$AUC_{0\rightarrow inf}$	(h·μg)/mL	56.55±1.97	248.16±13.74
$AUMC_{0\rightarrow inf}$	(h·h·μg)/mL	1231.85±147.04	5075.26±386.33
CL	mL/(h·kg)	31.86±1.10	0.01±0.00
V_{ss}	mL/kg	691.39±40.15	0.17±0.01
$MRT_{0\rightarrow inf}$	h	21.74±1.89	20.45±0.92