Supporting Information

Tumour microenvironment-responsive semiconducting polymer-based self-assembly nanotheranostics

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Experimental Section

1. Chemicals and Materials:

All solvents unless specified were used as received from Sigma-Aldrich and used as received without purification. *cis*-diamineplatinum(II) dichloride (cisplatin), gadolinium(III) chloride ($GdCl_3$), acetic acid, N,N-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), triethylamine (TEA), succinic anhydride, 5-hydroxydopamine hydrochloride (98%) were purchased from Sigma-Aldrich. 2-n-Octyl-1-dodecylamine was purchased from TCI AMERICA. Wheat germ agglutinin Alexa Fluor® 488 (WGA-488), phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), Tris buffer (1 M, pH 8.0), LIVE/DEAD Viability/Cytotoxicity Kit were purchased from Thermo Fisher Scientific. p-SCN-Bn-Deferoxamine was purchased from Macrocyclics. High-purity water (Milli-Q water) with a resistivity of 18.2 MΩ cm was obtained from a Millipore Milli-Q purification system (Millipore Corporation, U.S.A.).

2. General Characterization:

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were gained by a Bruker AV300 scanner using DMSO-d_6 and chloroform-*d* as the solvents. Transmission electron microscopy (TEM) images were obtained from a Tecnai TF30 transmission electron microscope (TEM) (FEI, Hillsboro, OR). The concentrations of platinum and gadolinium were detected by inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent 720-ES). Confocal microscopy images were acquired by a Zeiss LSM 780 microscope. Flow cytometry analysis was carried out with BD Beckman Coulter flow cytometer (Brea, CA) and FlowJo Software (TreeStar, Ashland, OR). PA coregistered images were acquired with a LAZR instrument (Visualsonics, 2100 High-Resolution Imaging System). PA tomography system (Endra Inc., Ann Arbor, Michigan) were used in this study.

3. Synthesis of poly(ethylene glycol)-perylene diimide-C_{20} (PEG-PDI-C_{20})

The methyl PEG-PDI-C_{20} and thiol based PDI-C_{20} was synthesis according to the previous report.[1]

4. Synthesis of poly(ethylene glycol)-perylene diimide-poly(diisopropanol amino ethyl methacrylate) (PEG-PDI-PDPA) (Scheme S1)

4.1 ***Synthesis of product 3:*** A mixture of dibromo-perylene diimide (PDI, 1 g, 1.7 mmol),[1] propionic acid (5 g, 8.33 mmol), ethanolamine (0.23 g, 3.8 mmol), N-Methyl-2-pyrrolidone (NMP, 100 mL) was stirred at 100 °C under N_2 for 8 h. After cooling down, the solution was dispensed into deionized water (2 L) and the red precipitate was separated by suction filtration with washing by deionized water. Then, two hydroxy group of the amide groups of PDI was protected by *Tert*-Butyldimethylsilyl chloride (TBDMSCl). Briefly, dissolved the two hydroxyl PDI in pyridine, and added excess TBDMSCl into the solution, stirring overnight. Removed the pyridine and by column chromatography to obtain the pure product 3, the structure was confirmed by ^{1}H NMR (Fig. S1) and ^{13}C NMR (Fig. S2). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 8.74-8.69 (s, 2H), 8.55-8.49 (s, 2H), 4.43-4.38 (s, 4H), 4.11-4.04 (s, 4H), 0.83-0.78 (s, 18H), 0.12-0.21 (s, 12H). ^{13}C NMR (75 MHz, chloroform-*d*, room temperature, ppm) δ: 163.4, 163.3, 136.2, 136.2, 132.8, 132.8, 130.7, 130.7, 130.9, 130.9, 129.2, 129.2, 129.1, 129.1, 128.5, 128.4, 127.7, 127.7, 124.1, 124.1, 123.8, 123.8, 61.8, 61.7, 42.7, 42.6, 25.9, 25.9, 25.9, 25.9, 25.9, 25.9, 17.5, 17.5, -4.8, -4.8, -4.8, -4.8. ESI-MS m/z: 866.13 $[M + H]^+$.

4.2 ***Synthesis of product 4:*** A mixture of 3 (100 mg) and 20 mL pyrrolidine was heated to 55 °C under N_2. The reaction mixture was kept at 55 °C. After 24 h. the solvents were evaporated using rotary evaporation and the crude product was purified by column chromatography on silica gel with $CHCl_3$ as eluent. The regioisomeric 1,7- dibromoperylene diimide was successful obtained by column chromatography at this step. After evaporation of the solvent, the product was collected as a green powder with yield 75%. To synthesize the asymmetry PDI structure 4, the one hand of amide group should be removed firstly. Dissolved the symmetry PDI in isopropanol and proper amount of sodium hydroxide was added. After 1h vigorous stirring, the solution was poured into acetic acid and the solution colour changed from blue to green. After precipitation by ether, precipitate was separated by suction filtration. The pure product 4 can be obtain by column chromatography. The confirmation of the structure can be seen by ^{1}H NMR (Fig. S3) and ^{13}C NMR (Fig. S4). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 8.54-8.43 (m, 4H), 7.75-7.52 (d, 2H), 4.50-4.43 (t, 2H), 4.02-4.00 (t, 2H), 3.76-3.67 (s, 4H), 3.02-2.73 (s, 4H), 2.25-2.01 (s, 8H), 0.93-0.79 (s, 9H), 0.12-0.21 (s, 6H). ^{13}C NMR (75 MHz, chloroform-*d*, room temperature, ppm) δ: 165.4, 162.3, 161.9, 147.4, 147.4, 147.3, 134.3, 132.6, 131.4, 130.8, 130.3, 129.1, 129,1, 128.9, 127.9, 127.7, 125.7, 123.8, 123.5, 120.3, 119.9, 118.2, 117.7, 61.0, 52.7, 52.7, 42.1, 30.6, 25.9, 25.9, 17.5, 17.5, -5.1, -5.1. ESI-MS m/z: 687.32 $[M + H]^+$.

4.3 ***Synthesis of product 5:*** product 4 was dissolved in NMP solution and reacted with equivalent PEG2000-NH_2 under 85°C for 2 h. Then the pure product 5 was obtained by precipitating the solution in ether, and drying for next steps. The confirmation of product 5 can be seen by ^{1}H NMR (Fig. S5). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 8.54-8.43 (m, 4H), 7.75-7.52 (d, 2H), 4.65-4.52 (t, 4H), 4.03-3.56 (m, 182H), 3.45-3.41 (s, 3H), 3.02-2.73 (s, 4H), 2.25-2.01 (s, 8H), 0.93-0.79 (s, 9H), 0.12-0.21 (s, 6H).

4.4 ***Synthesis of product 6:*** To remove the hydroxyl protection, product 5 (100 mg) was dissolved in 10 mL THF and reacted with equivalent TBAF under room temperature for 2 h. Then the pure product 6 was obtained by precipitating the solution in ether, and drying for next steps. The confirmation of product 6 can be seen by ^{1}H NMR (Fig. S6). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 8.54-8.43 (m, 4H), 7.75-7.52 (d, 2H), 4.65-4.52 (t, 4H), 4.03-3.56 (m, 182H), 3.45-3.41 (s, 3H), 3.02-2.73 (s, 4H), 2.25-2.01 (s, 8H).

4.5 ***Synthesis of product 7:*** To synthesize the initiator of the acid responding polymer, the product 6 (0.5 g) was dissolved in chloroform with triple equivalent dibromoisobutyryl bromide. The triethylamine can be used as a catalyst to promote the reaction. With overnight vigorous stirring, the solution can be obtained by rotary evaporator. Precipitation was used to purify the final PDI based initiator. The confirmation of product 7 can be found by ^{1}H NMR (Fig. S7). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 8.54-8.43 (m, 4H), 7.75-7.52 (d, 2H), 4.65-4.60 (s, 2H), 4.58-4.52 (t, 4H), 4.03-3.56 (m, 182H), 3.45-3.41 (s, 3H), 3.02-2.73 (s, 4H), 2.25-2.01 (s, 8H). 1.82-1.80 (s, 6H). Mn (GPC)= 2802, Mw/Mn (PDI)=1.01.

4.6 ***Synthesis of product 8:*** Atom Transfer Radical Polymerization (ATRP) was used for synthesis of acid PDI based sensitive polymer. Briefly, the product 7 (50 mg, 18 μM), 2-(Diisopropylamino)ethyl methacrylate (1.10 g, 5.2 mM), PMDETA were charged into a glass tube, Then anisole was added to dissolve the monomers and initiator. After three cycles of freeze-pump-thaw to remove the oxygen, CuBr (14 mg, 0.1 mmol) was added into the polymerization tube under nitrogen atmosphere, and the tube was sealed in vacuum. The polymerization was carried out at 90 °C for 8 h. After polymerization, the reaction mixture was diluted with 10 mL THF, and passed through a silica column to remove the catalyst. The THF solvent was removed by rotary evaporator. The residue was precipitated by methanol to give a green solid. After synthesis, the polymers were characterized by ^{1}H NMR (Fig. S8). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 8.54-8.43, 7.75-7.52, 4.65-4.60, 4.58-4.52, 4.03-3.75, 3.65-3.56, 3.45-3.41, 3.14-2.93, 2.67-2.57, 2.23-1.86, 1.35-0.7. Mn (GPC)=24868, Mw/Mn (PDI)=1.43.

5. Synthesis of the Pt(IV) prodrug polyphenols

The Pt(IV) prodrug polyphenols were prepared according to our previous method.[2] The structure was confirmed by ^{1}H NMR (Fig. S9). ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 6.21-6.20 (s, 4H), 3.22-3.20 (t, 4H), 2.46-2.42 (t, 8H), 2.27-2.23 (t, 4H), ESI-MS m/z: 835.01 $[M + H]^-$.

6. Fabrication of Gd/Pt prodrug PDPA/PDC nanoparticles (GPDPA NPs)

First, the Pt(IV) prodrug polyphenols (15 mg mL^{-1}, 100 μL in water) was mixed with $GdCl_3$ (3 mg mL^{-1}, 100 μL in water) and H_2O (500 μL). Then, a solution of Tetrahydrofuran (THF, 0.5 mL) with PEG-PDI-PDPA (5 mg mL^{-1}), and PEG-PDI-C20 (5 mg mL^{-1}) was added to the above mixture under stirring for 30 min. Then, Tris buffer (100 μL, 1 M, pH 8) was added to the solution. Afterwards, the above solution was stirred for 6 h with bubbling of N_2 (12 kDa) to evaporate THF. Finally, the GPDPA NPs solution was washed by H_2O and concentrated by Amicon Ultra centrifugal filters (10 kDa). The concentrations of Gd and Pt were determined by ICP-OES.

7. Photothermal heating procedure

GPDPA NPs aqueous solutions with various concentrations (0.2, 1.0, 2.5, 5 mg/mL) were irradiated with a 671 nm laser at a power of 0.5 W/cm^2 for 5 min. The PBS without GPDPA NPs was used as the control. To investigate the power-dependent photothermal property, GPDPA NPs solution with a concentration of 1 mg/mL was irradiated by a 671 nm laser under different powers (0.1, 0.2, 0.3, 0.5, 0.8 and 1.0 W/cm^2) for 5 min. A SC300 infrared camera was employed to record thermal images and measure the solutions temperature during the irradiation process.

8. *In vitro* cytotoxicity of cisplatin, prodrug and GPDPA NPs under different laser irradiation.

MTT assay was used to determine the cell viability by cisplatin, prodrug and GPDPA NPs under different concentration and laser irradiation. U87MG cells were cultured in a 96-well plate with a density of 8,000 cells per well at 37 °C, 5% CO_2 overnight. Then, the cells were treated by fresh media containing cisplatin, prodrug and GPDPA NPs with different concentrations. The cells were cultured for 8 h and irradiated with 671 nm laser for 5 min with a power of 0.3 W/cm^2. Then, the cells were incubated at 37 °C, 5% CO_2 for another 48 h. The media was replaced with MTT media solution (0.5 mg/mL) for 4 h. Then MTT solutions were replace with DMSO (100 μL) and the absorbance of each well was recorded at 570 nm by a plate reader.

9. Live and Dead Assay.

The LIVE/DEAD® Viability/Cytotoxicity Kit from ThermoFisher Sci was used to evaluate the toxicity. U87MG cells with a population of 30,000 were seeded in each well of an 8 well chamber and incubated overnight. The GPDPA NPs media solutions were added into each well and incubated for another 8 h. Then, the wells were irradiated with 671 nm laser with two powers (0.1 and 0.3 W/cm^2) for 5 min. After that, the cells were cultured for another 24 h. Finally, the cells were treated by the kit according to the provided protocol. The slides were observed under the confocal microscope.

10. Animal model.

All animal experiments were approved by the Animal Care and Use Committee of the National Institutes of Health Clinical Center (ACUC/ NIH CC), all experiments were performed in compliance with NIH Policy on Humane Care and Use of Laboratory Animals for PHS Supported Institutions. The U87MG xenograft tumour bearing mice were prepared by inoculating U87MG cells (4×10^6 cells/mouse) on the flank of the mice. The tumour volume was calculated through the formula: $V = (W^2 \times L)/2$ for caliper measurements. W and L are the tumour width and length, respectively. When the tumour reached a size between 200 and 300 mm^3, the PET, MR and PA imaging studies started. When the tumour size is around 150 mm^3, the tumour treatment experiment began on the other day. The relative tumour size was calculated by V/V_0, where V and V_0 are the tumour volume before and after treatment, respectively.

11. *In Vivo* MR and PA Imaging.

In vivo MRI was performed by a 7.0 T micro-MR scanner by using a mouse coil. The tumour bear mice were injected with GPDPA NPs (2 mg/mL) intravenously. All the parameters were given as follows: repetition time (TR) = 400 ms, echo time (TE) = 8 ms, flip angle = 180°, matrix size = 256 × 256. The PA imaging of tumours were recorded before and after injecting the GPDPA NPs (2 mg/mL) intravenously. PA images were acquired and quantified at various time points with an Endra Nexus 128 PA tomography system.

12. *In vivo* PET imaging

For the *in vivo* PET imaging experiment, 10 % of thiol based PEG-PDI-C_{20} was doped with PEG-PDI-C_{20} to form the GPDPA NPs, as described above. The thiol groups of GPDPA NPs could chelate with ^{64}Cu to obtain the stable labelling.[3] Then, 100 μCi of ^{64}Cu-labeled GPDPA NPs solution was intravenously injected into the xenograft U87MG tumour mice. An Inveon Micro PET scanner (Siemens Medical Solutions) was employed to conduct whole-body PET scans at different time points, and the data was calculated by 3-dimensional region of interests (ROIs). The % ID/g was then calculated according to the readings. After 48 h, the main organs were collected for γ-counting.

13. *In vivo* tumour treatment

The U87MG tumour-bearing mice were randomized into six groups. The mice were injected with GPDPA NPs (3 groups, Pt dose of 2 mg/kg), free cisplatin (one group, Pt dose of 2 mg/kg) and PBS (two groups) once. After 24 hours, two of GPDPA NPs groups were irradiated with 671 nm laser at 0.5 W/cm^2 and 0.3 W/cm^2 for 5 min, respectively. One of the PBS group was irradiated with 671 nm laser at 0.5 W/cm^2 as the negative control. Real-time thermal images of the above mice were recorded by an infrared camera. The body weight and tumour size were monitored every other day.

Equations:

$$SNR=SI_{mean}/SD_{noise} \tag{S1}$$

$$SNR=(SNR_{post}-SNR_{pre})/SNR_{pre} \times 100\% \tag{S2}$$

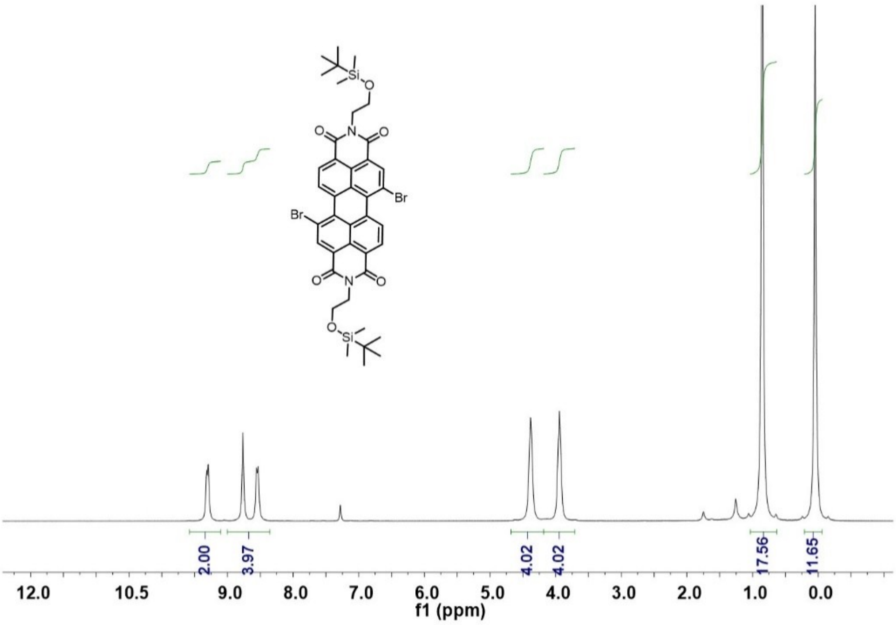

Fig. S1. ^{1}H NMR spectrum of 3 (chloroform-*d*, room temperature).

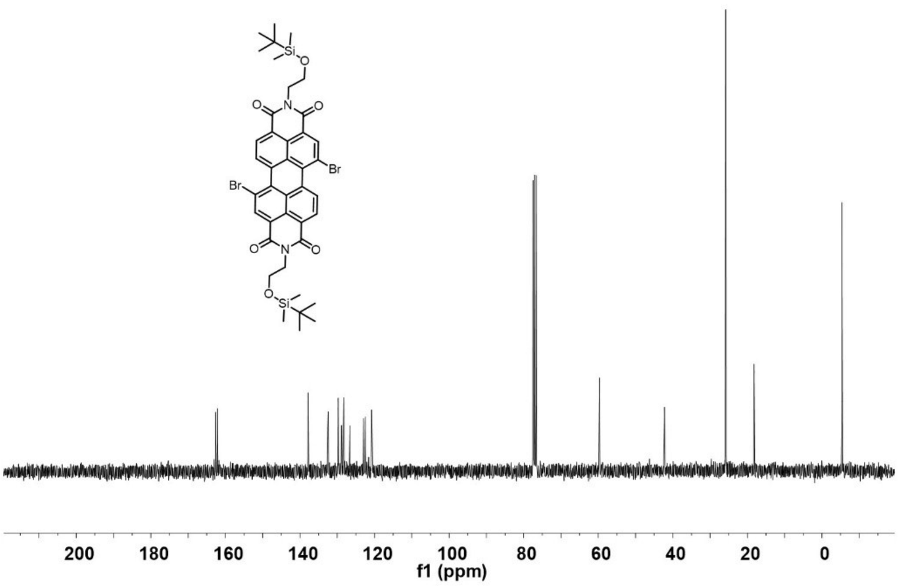

Fig. S2. ^{13}C NMR spectrum of 3 (chloroform-*d*, room temperature).

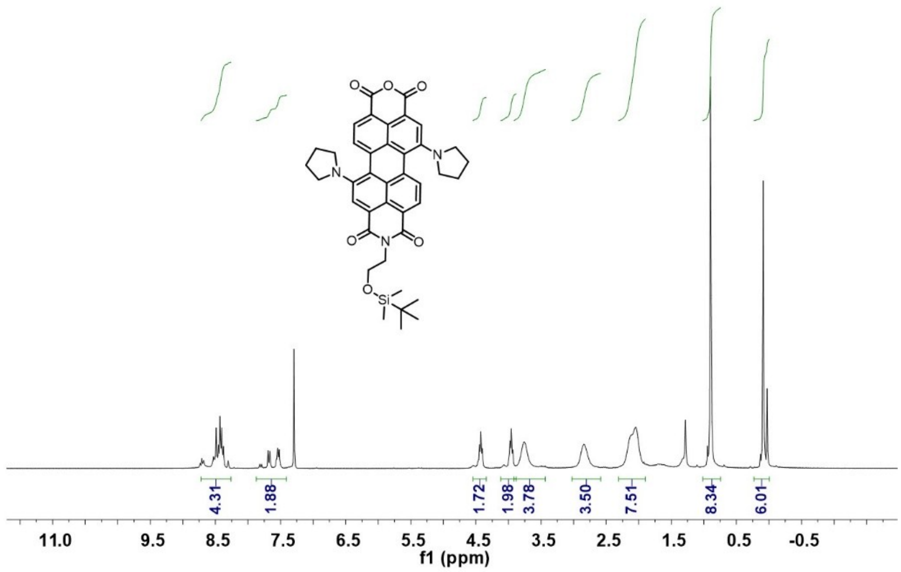

Fig. S3. ^{1}H NMR spectrum of 4 (chloroform-*d*, room temperature).

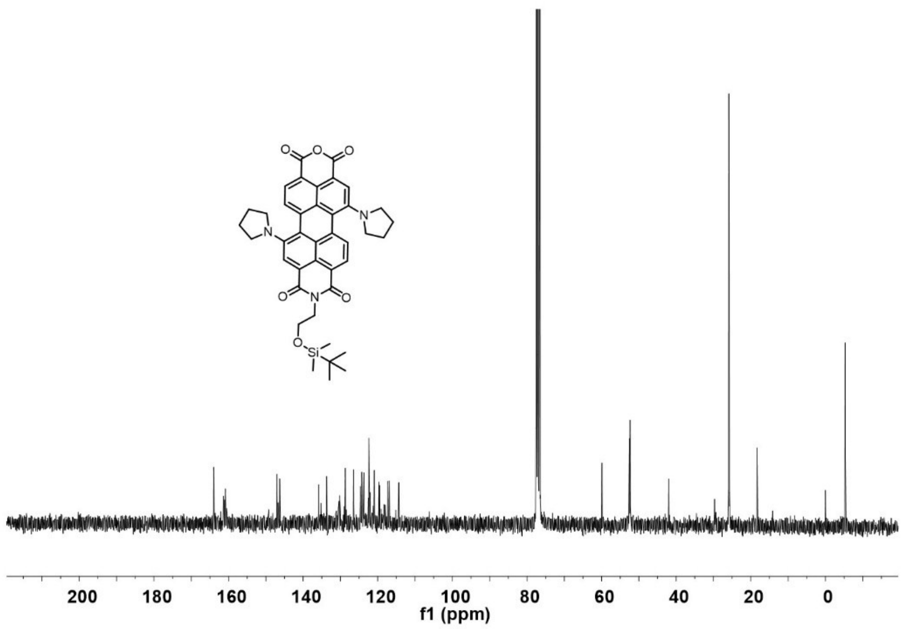

Fig. S4. ^{13}C NMR spectrum of 4 (chloroform-*d*, room temperature).

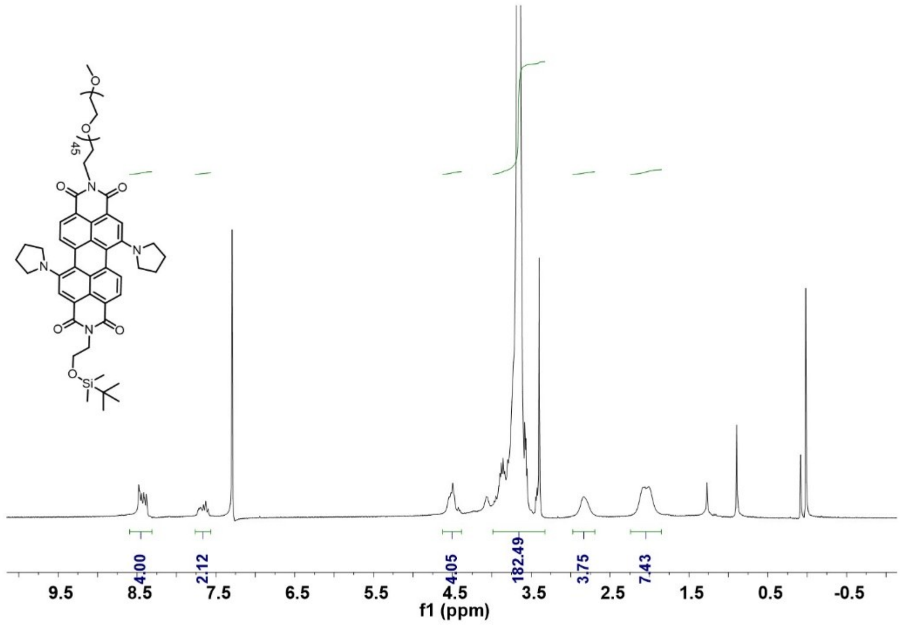

Fig. S5. ^{1}H NMR spectrum of 5 (chloroform-*d*, room temperature).

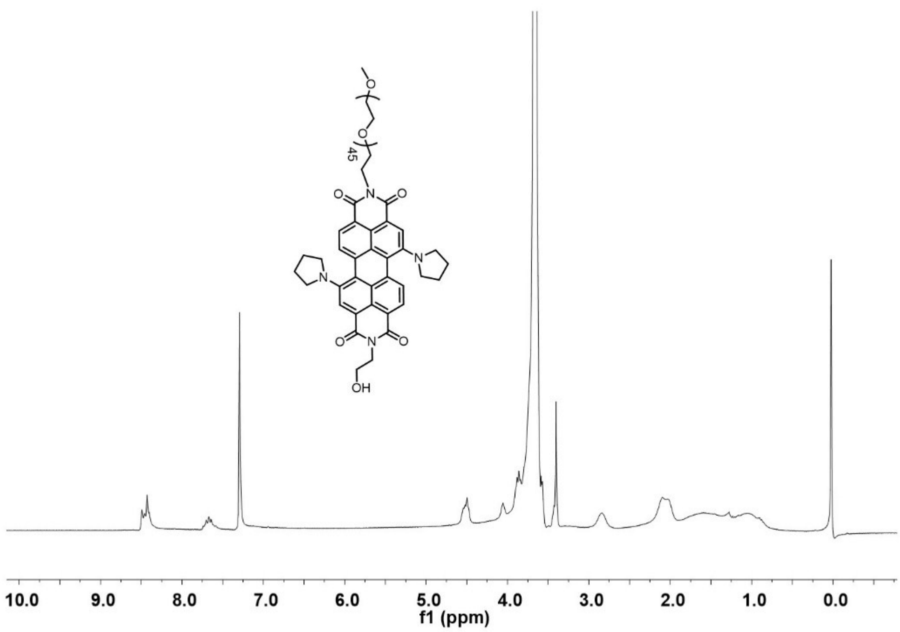

Fig. S6. ^{1}H NMR spectrum of 6 (chloroform-*d*, room temperature).

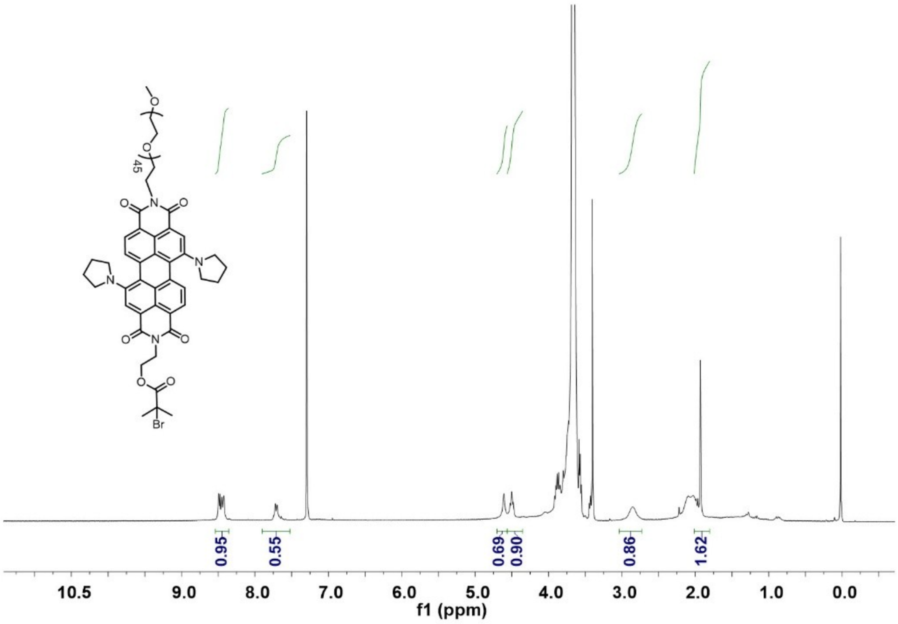

Fig. S7. ^{1}H NMR spectrum of 7 (chloroform-*d*, room temperature).

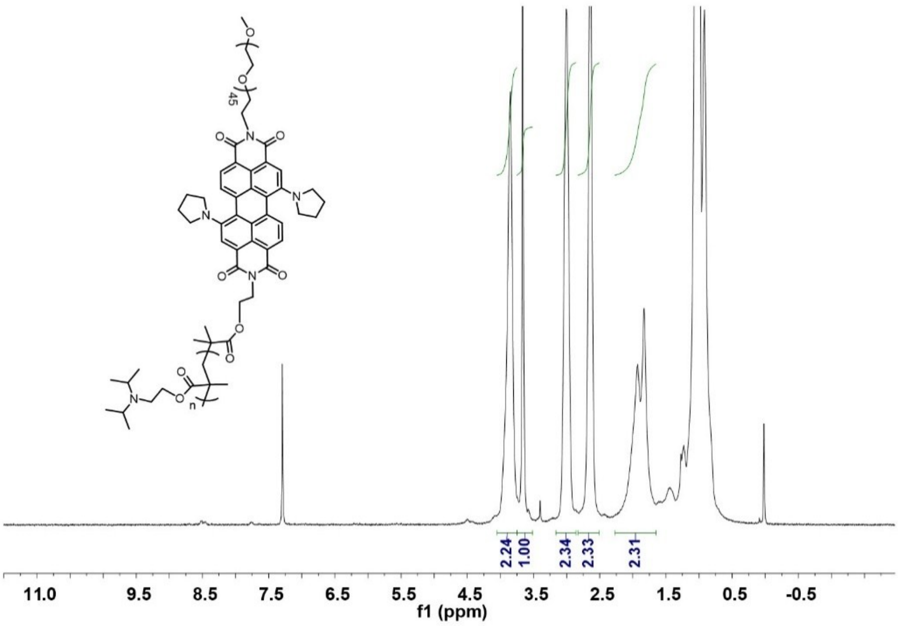

Fig. S8. ^{1}H NMR spectrum of 8 (chloroform-*d*, room temperature).

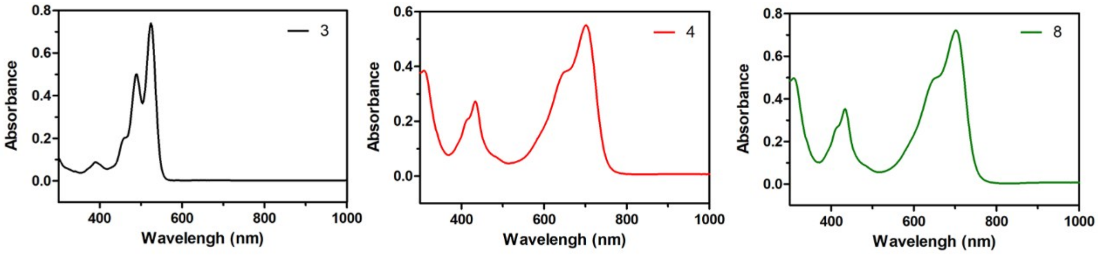

Fig. S9. The UV spectra of PDI intermediates in chloroform.

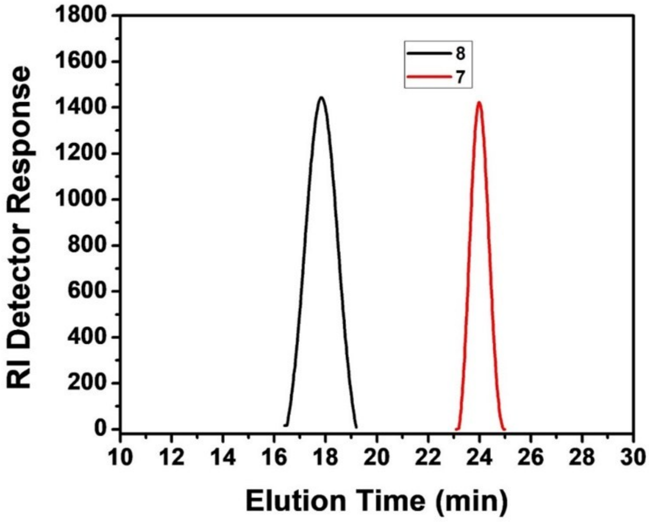

Fig. S10. The GPC curves of product 7 and 8.

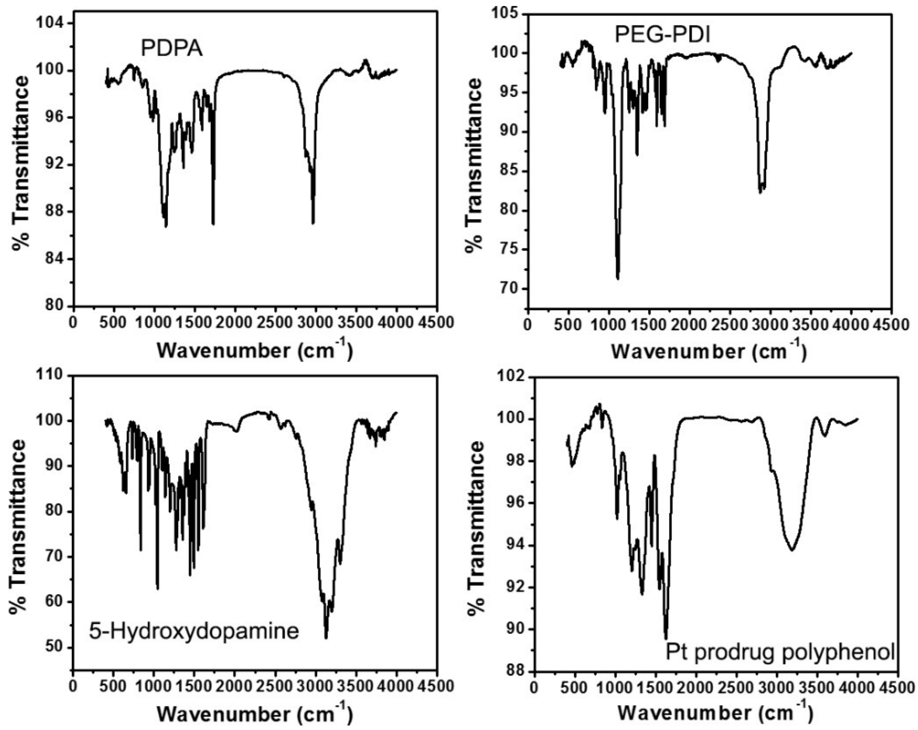

Fig. S11. The FT-IR spectra of PDPA, PEG-PDI, 5-hydroxydopamine and Pt prodrug polyphenols.

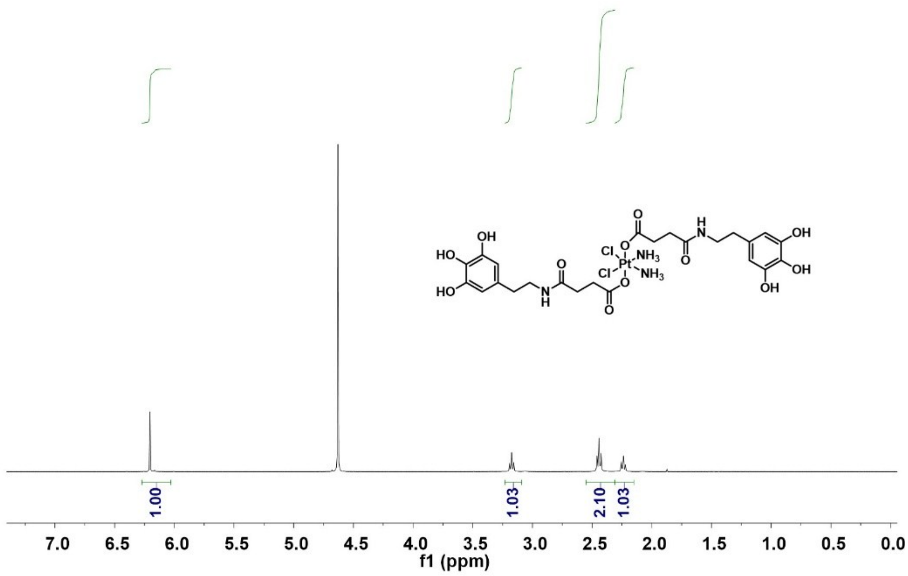

Fig. S12. [1]H NMR spectrum of Pt prodrug polyphenols (DMSO-d_6, room temperature).

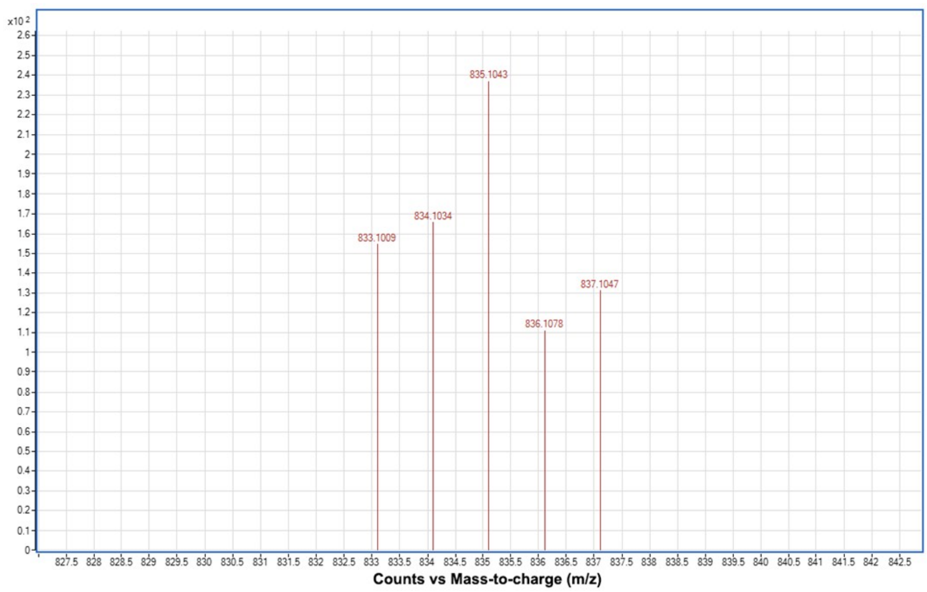

Fig S13. ESI-MS spectrum of the Pt(IV) prodrug polyphenol, the major peak m/z 835.1043 assigned to $C_{24}H_{33}Cl_2N_4O_{12}Pt$ [M-H].

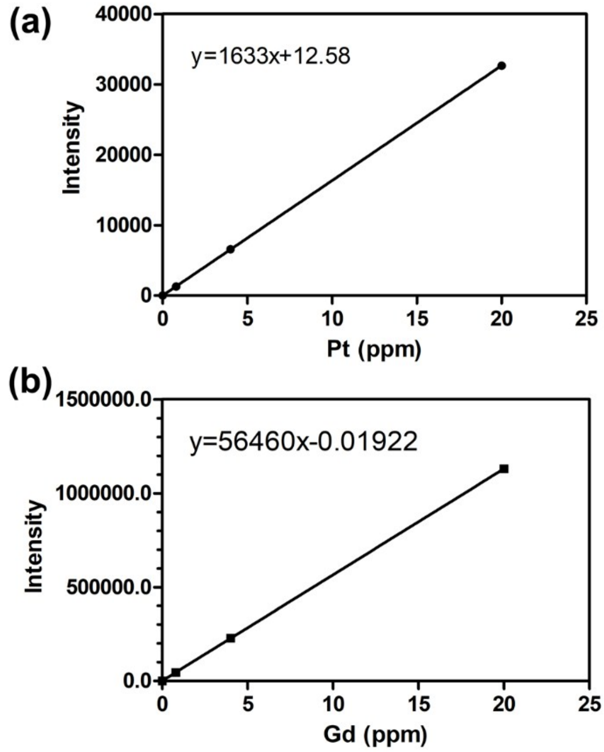

Fig. S14. Standard curve of ICP-OES intensity for quantitative analysis of Pt (a) and Gd (b) concentration.

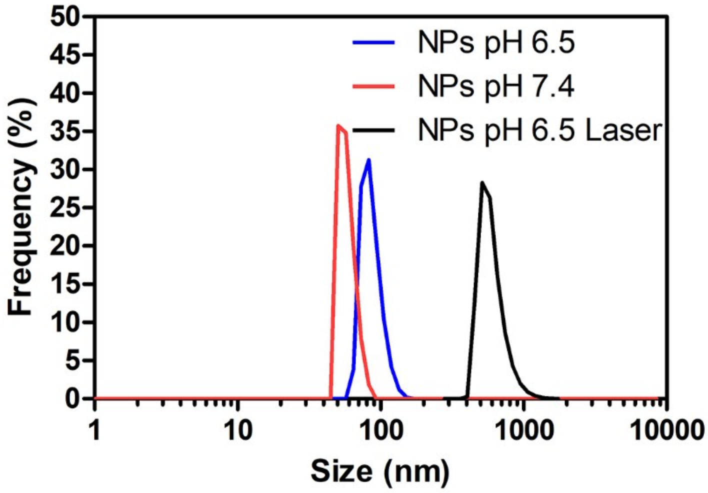

Fig. S15. The DLS of GPDPA NPs in PBS with various conditions.

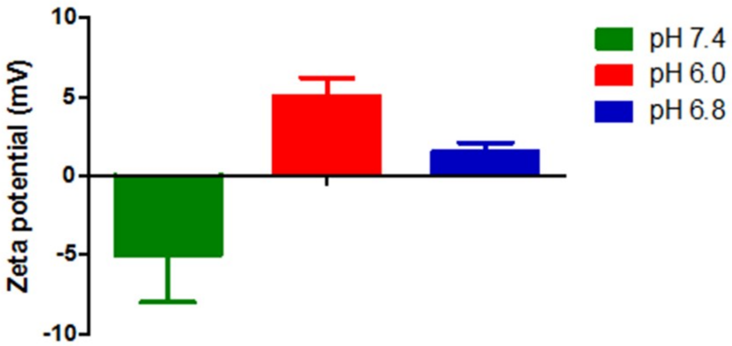

Fig. S16. The zeta potential of GPDPA NPs under various pH. Values are the mean ± s.d. for n=3.

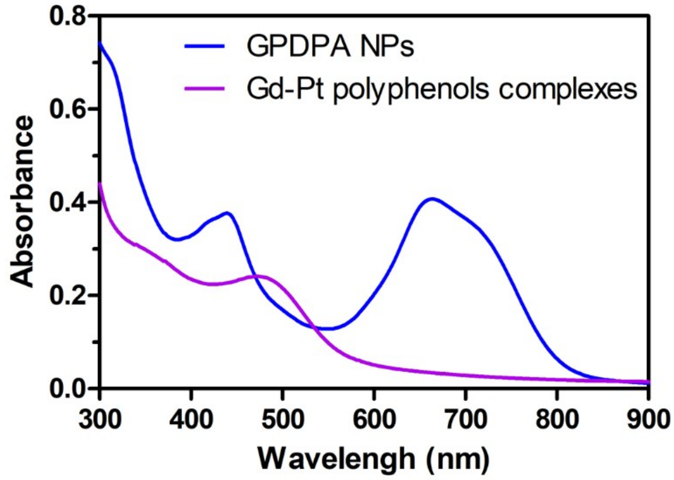

Fig. S17. The UV-vis spectrum of GPDPA NPs and Gd-Pt polyphenols complexes.

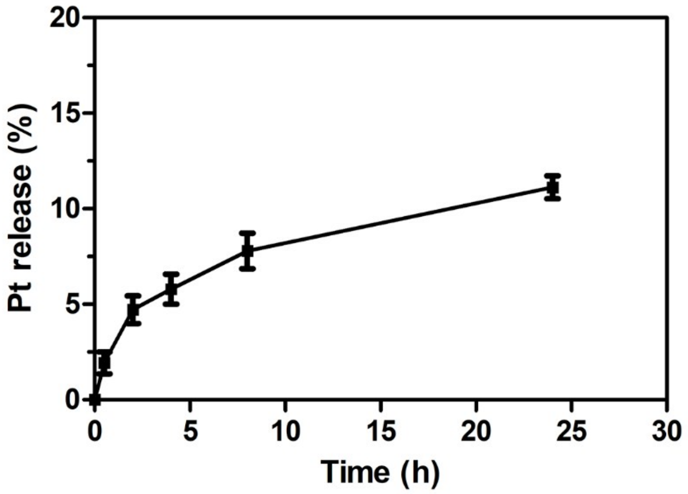

Fig. S18. The release of Pt from GPDPA NPs in the FBS, Values are the mean ± s.d. for n=3.

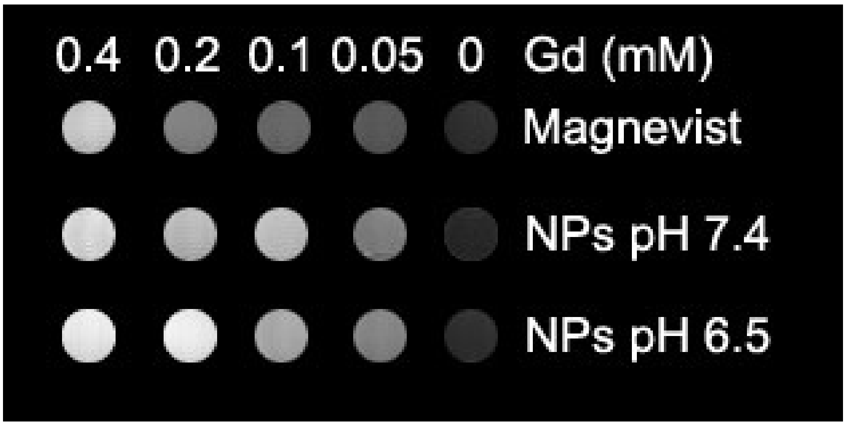

Fig. S19. T_1-weighted MR images of GPDPA NPs and Magnevist at a function of Gd concentrations.

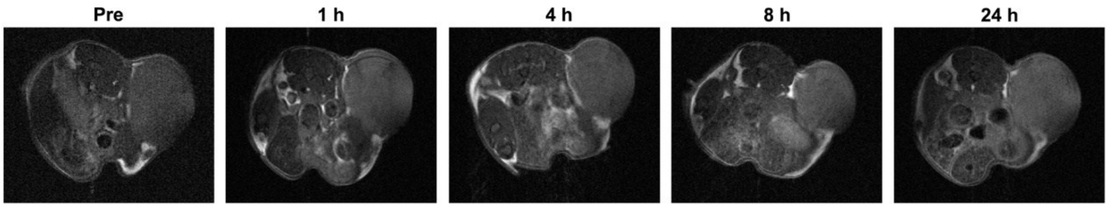

Fig. S20. Original T_1-weighted MR images of U87MG xenograft tumour mice with GPDPA NPs administration at various time points.

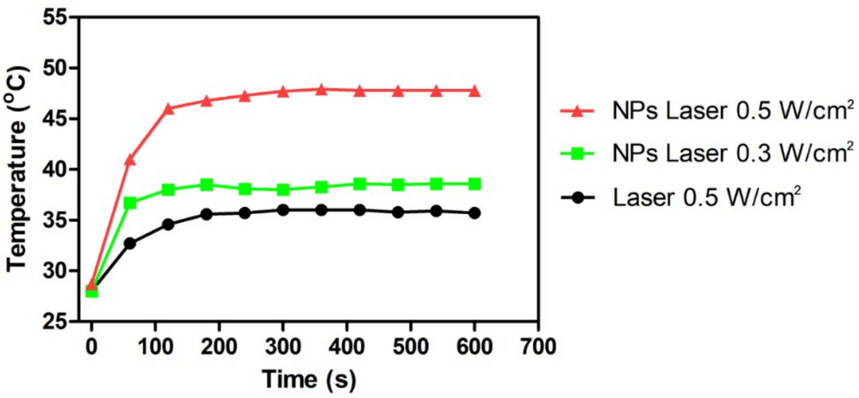

Fig. S21. The temperature curves at tumour regions with various treatments.

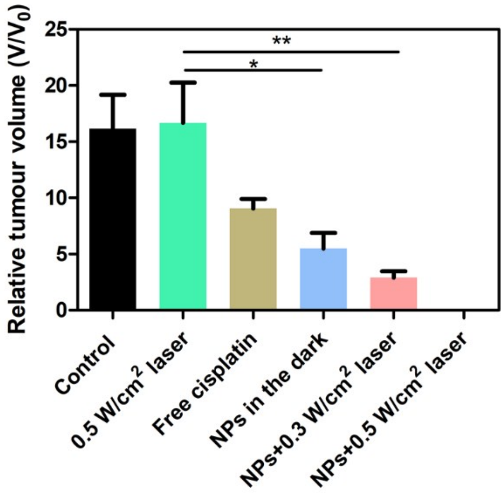

Fig. S22. The tumour size of various groups on the 19th day. (*P* values, **P* < 0.05, ***P* < 0.01, are calculated by *t*-test.). Values are the mean ± s.d. for n=4.

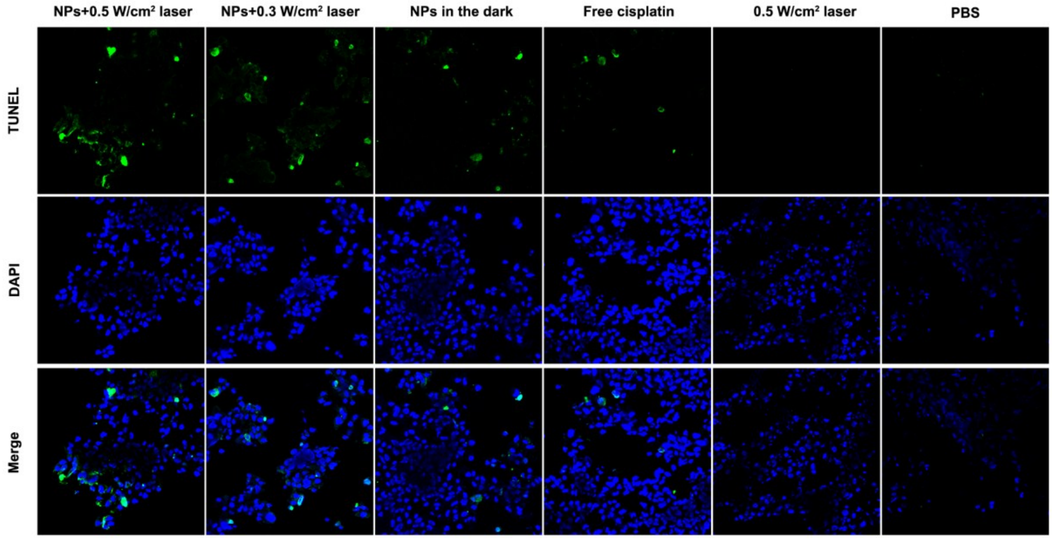

Fig. S23. The TUNEL assay of tumour cells from various groups acquired 24h after laser irradiation.

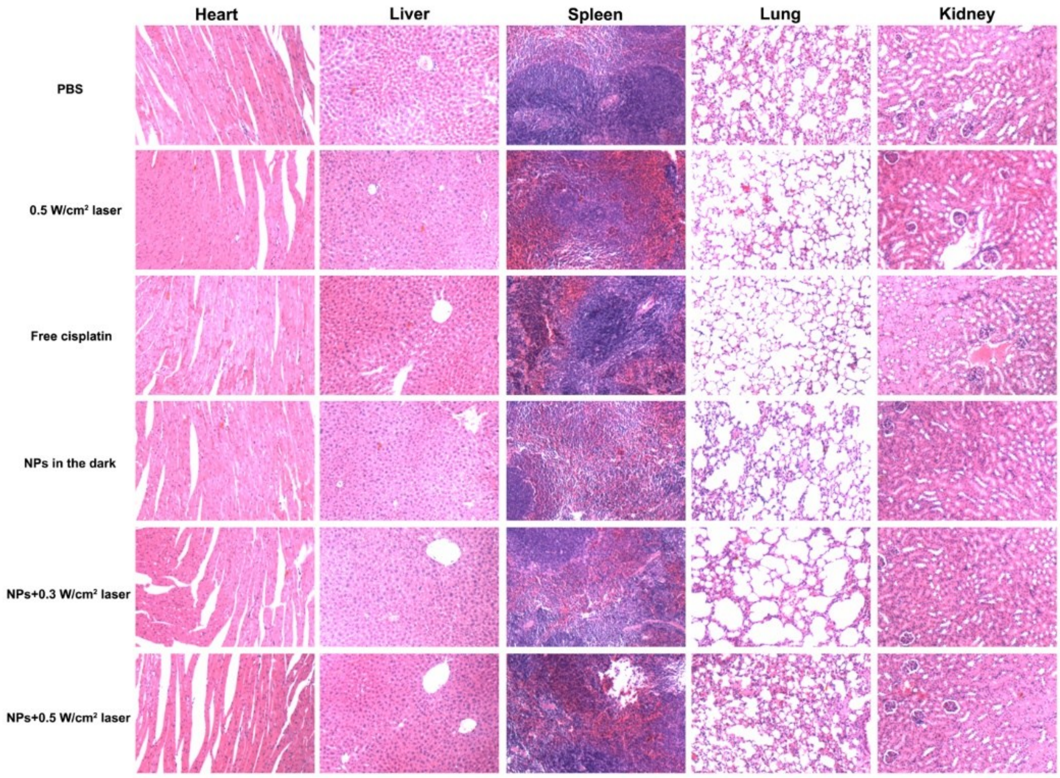

Fig. S24. H&E staining of main organs (heart, liver, spleen, lung, and kidney) from various groups on the 19th day of treatment.

Table S1. The composition of the nanoparticles.

Pt prodrug polyphenols	Gd^{3+}	PDI based polymer
20 %	3 %	77 %

Table S2. T_1 relaxivity of (r_1 value) of Magnevist and GPDPA NPs at different pH.

GPDPA NPs, pH 6.5	GPDPA NPs, pH 7.4	Magnevist
10.27 mM^{-1} s^{-1}	3.547 mM^{-1} s^{-1}	4.351 mM^{-1} s^{-1}

Table S3. Half-maximal inhibitory concentration (IC_{50}) of free cisplatin, GPDPA NPs and GPDPA NPs with laser irradiation (0.3 W/cm^2, 5 min) against U87MG cancer cells for 48 h.

cisplatin	GPDPA NPs	GPDPA NPs with laser
0.634 µM	1.865 µM	0.316 µM

References:

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