Supporting Information for

Multifunctional carbon dots with near-infrared absorption and emission for targeted delivery of anticancer drugs, tumor tissue imaging and chemo/photothermal synergistic therapy

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Supporting experimental section

Synthesis of the RCDs

Osmanthus seed skin (15 g) was shredded, added to 30 mL of an anhydrous ethanol solution, and transferred to a 50 mL round bottom flask. The flask was sealed with nitrogen protection and fixed to an electric heating sleeve heated to 100 °C. The content was stirred at this temperature and under a nitrogen atmosphere for 12 h. After the reaction product solution was naturally cooled to room temperature, the supernatant was filtered via a 0.22 μm filter membrane. Up to 30 mL of the filtrate was transferred to a 50 mL beaker to which 0.2 g of H_2N-PEG-NH_2 anhydrous ethanol solution was added. The mixture was heated to 120 °C and then agitated for 10 h under a nitrogen atmosphere. The resulting supernatant was then filtered via a 0.22 μm filter membrane. The filtrate was dialyzed with a dialysis bag (MWCO 1000) in pure water for 48 h to obtain pure RCDs. The purified product was ultimately removed from the solvent by rotary evaporation under vacuum when dried for 24 h at 65 °C and then stored in a refrigerator at 4 °C.

Preparation and Characterization of RCD-Pt(IV)/PEG-CS-DA

Synthesis of the Pt(IV) prodrugs: the synthesis of the Pt(IV) prodrugs is illustrated in Fig. S7. Approximately 300.0 mg of Cis-Pt$(NH_3)_2Cl_2$ was suspended in 7.5 mL water in a water bath set to 50 °C. Subsequently, 10.5 mL of H_2O_2 (wt: 30%) was added dropwise and then stirred for 2 h under this condition. The reaction product solution was cooled to room temperature. The solvent was then concentrated to about 2 mL by rotary evaporation, and the resulting solution was stored overnight at 0 °C. The pale yellow crystals were washed and filtered with cold water and diethyl ether solution, and the product was obtained, which was then stored in a vacuum dryer; 120.0 mg of the product and 36.0 mg of succinic anhydride were dissolved in 2.0 mL dimethylformamide and agitated for 24 h at room temperature. The reaction product was freeze-dried, and the

solid was washed with cold acetone and an ether solution to obtain a light yellow pure Pt(IV) prodrug: c,c,t-[$PtCl_2(OH)(NH_3)_2(O_2CCH_2CH_2CO_2H)$].

Synthesis of RCD-Pt(IV): Up to 20 mg of of the Pt(IV) prodrug, 3.5 mg N-hydroxysuccinimide (NHS), and 4.5 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride were dissolved in water and stirred for 30 min at room temperature. Subsequently, 40.0 mg of the RCDs were added to the aforementioned solution and stirred for 24 h. The reaction product was dialyzed with a dialysis bag (MWCO 1000) in ultrapure water for 48 h to remove unreacted compounds. After freeze-drying, the obtained product (RCD-Pt(IV)) was stored at room temperature.

Synthesis of PEG-CS-DA: Synthesis was conducted in accordance with a method reported in the literature. The synthesis route is presented in Fig. S8. First, 0.2 g of CS was dissolved in 20 mL of ultrapure water to which 1.0 g of PEG-NHS was added. CS was then reacted with PEG-NHS for 24 h under magnetic stirring to obtain PEG-CS after the dialysis with ultrapure water. Subsequently, 0.2 g of PEG-CS was dissolved in a PBS solution of 20, mL pH 8.0, 3-dimethyl maleic anhydride (DMMA) that is, 3 times more than that of PEG-CS was added. With a 0.2 M NaOH solution, the reaction solution was adjusted to about 8.5. PEG-CS-DA was obtained by stirring for 8 h at room temperature. The product was dialyzed with a PBS (pH 8.0) solution and lyophilized.

Synthesis of RCD-Pt(IV)/PEG-CS-DA: Up to 60 mL of the RCD-Pt(IV) (1 mg/mL) solution was added dropwise to 150 mL of PEG-CS-DA (1 mg/mL) solution. The aforementioned mixed solution was stirred overnight at room temperature, freeze-dried to obtain RCD-Pt(IV)/PEG-CS-DA, and stored in a refrigerator at -20 °C.

In vitro Drug Release Experiments

Approximately 15.0 mg of RCD-Pt(IV)/PEG-CS-DA was dissolved in 1 mL of PBS buffer solution (pH 7.4 or 6.8) containing GSH at 0, 5 μM, and 10 mM. The mixed solution was transferred to a dialysis tube (MWCO:1 kDa), and dialyzed at 37 °C for 1, 2, 4, 6, 8, and 10 h by using a PBS buffer solution containing 0, 5 μM and 5 mM GSH (69 mL, pH 7.4 or 6.8). The content of Pt in the dialysate was determined by inductively coupled plasma mass spectrometry (ICP-MS).

Photothermal Effects of RCD-Pt(IV)/PEG-CS-DA

Approximately 20 μg of RCDs, 105 μg of RCD-Pt(IV)/PEG-CS-DA, and 155 μg of RCD-Pt(IV)/PEG-CS-DA were dissolved in 1 mL of PBS (pH 6.8) solution, Pt(II), and PBS solution, which were used as blank controls. The aforementioned solution was placed in a 1.5 mL centrifuge tube and continuously irradiated with 680 nm laser (1.5 W/cm2) for 10 min. Changes in the temperature of the solution were recorded at the 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, and 10 min time points by using a temperature tester. Thermographic images were acquired using an Optris PI infrared camera (Optris Infrared Sensing, LLC., Portsmouth, NH, USA).

In Vitro Chemical–Photothermal Synergistic Therapy by RCD-Pt(IV)/PEG-CS-DA

Six experimental groups, including PBS, PBS+NIR laser irradiation, RCDs, RCDs+NIR laser irradiation, RCD-Pt(IV)/PEG-CS-DA, and RCD-Pt(IV)/PEG-CS-DA+NIR laser irradiation were established in this experiment. Human lung cancer cells (A549 cells) were first inoculated in a six-well plate at a density of 1×105 cells/holes. After incubation for 24 h, the original medium was replaced with a fresh medium at pH=6.8. An appropriate amount of PBS, 20 μg/mL of RCDs, 20 μg/mL of RCDs (NIR group), 105 μg/mL of RCD-Pt(IV)/PEG-CS-DA, and 105 μg/mL RCD-Pt(IV)/PEG-CS-DA (NIR laser irradiation group) were added to each orifice plate, sequentially. They were incubated for 24 h and then washed with the PBS solution three times. The same amount of fresh incubation medium was ultimately added. The cells in the PBS+NIR, RCDs+NIR, and RCD-Pt(IV)/PEG-CS-DA+NIR groups were irradiated with 680 min laser (1.5 W/cm2) for 3 min. After laser irradiation, all groups of A549 cells were placed in a cell incubator and further incubated for 4 h. The survival rate of the A549 cells was then determined by confocal microscopy using calcium yellow–green AM/propidium iodide double staining. In this technique, the surviving cells emit green fluorescence, whereas the dead cells emit red fluorescence.

Tumor-Bearing Mouse Models and Fluorescence/Thermal Imaging of Tumor In Vivo

BALB/c nude mice (aged 5–6 weeks) were purchased from Hunan Srek Jingda Experimental Animal Co., Ltd. and kept in the Animal Laboratory of Guangxi Normal University. The animal handling procedures applied were approved by the Animal Ethics Committee of Guangxi Normal University (No. 2019151-XC). The mice were kept under specific-pathogen-free conditions with ad libitum access to standard food and water. Tumor-bearing mouse models were established by injecting a T24 cell suspension into the ventral skin of the nude mice. T24 cells were first inoculated in a cell culture dish at a density of 1×106 cells/dishes. After the cells were incubated for 48 h, the cells were digested with trypsin, and the saline cell suspension with a concentration of 2×107 cells/mL was prepared by cell counting. The mice were anesthetized with isoflurane, and 100 μL of the aforementioned T24 cell suspension was injected into the ventral side of the mouse. After waking up, the mice were reared in the animal room until the tumor reached 60–70 mm3. About 200 μL of the RCD-Pt(IV)/PEG-CS-DA solution was administered to the tumor-bearing mice via tail vein injection or subcutaneous injection into the tumor tissue after isoflurane anesthesia. Fluorescence imaging was performed 0.5, 1, 4, 6, 10, and 48 h after injection of RCD-Pt(IV)/PEG-CS-DA (excitation: 630 nm; emission: 700 nm).

During in vivo thermal imaging experiments, 200 μL of the PBS, Pt(II), RCDs, and RCD-Pt(IV)/PEG-CS-DA solutions were injected into the tumor tissues of the mice. Tumor sites in the mice were then irradiated with a 680 nm laser (1.5 W/cm2). The infrared thermal imager (optical PI) was used to record infrared thermal imaging images at different times and analyze the change in temperature at tumor sites.

Chemo–Photothermal Synergistic Therapy for Tumor-Bearing Mice

In this experiment, the tumor-bearing mice were randomly divided into experimental groups: PBS, Pt(II), RCDs, and RCD-Pt(IV)/PEG-CS-DA. Each group underwent a control experiment with and without laser irradiation. Female nude mice (18–20 g) with 60–70 mm3 tumor volume were selected as an experimental object after T24 cells implantation for about 7 days. The 200 μL PBS, Pt(II), RCDs, and RCD-Pt(IV)/PEG-CS-DA solutions were administered to the mice via tail vein injection; each nude mouse had

the same Pt content (10 mg/kg). The tumor site in the mice in the laser irradiation group was irradiated with 680 nm laser (1.5 W/cm2) for 3 min after the treatment agent was injected for 10 h via the tail vein. The same treatment lasted for 14 days, and the tumor volume of the nude mice was measured daily during treatment. After the tumor treatment experiment was completed, the mice were anesthetized, and the tumor tissue was removed for H&E staining. Histopathological experiments were conducted in accordance with standard operating procedures. After H&E staining, the pathological sections were observed and photographed using an optical microscope.

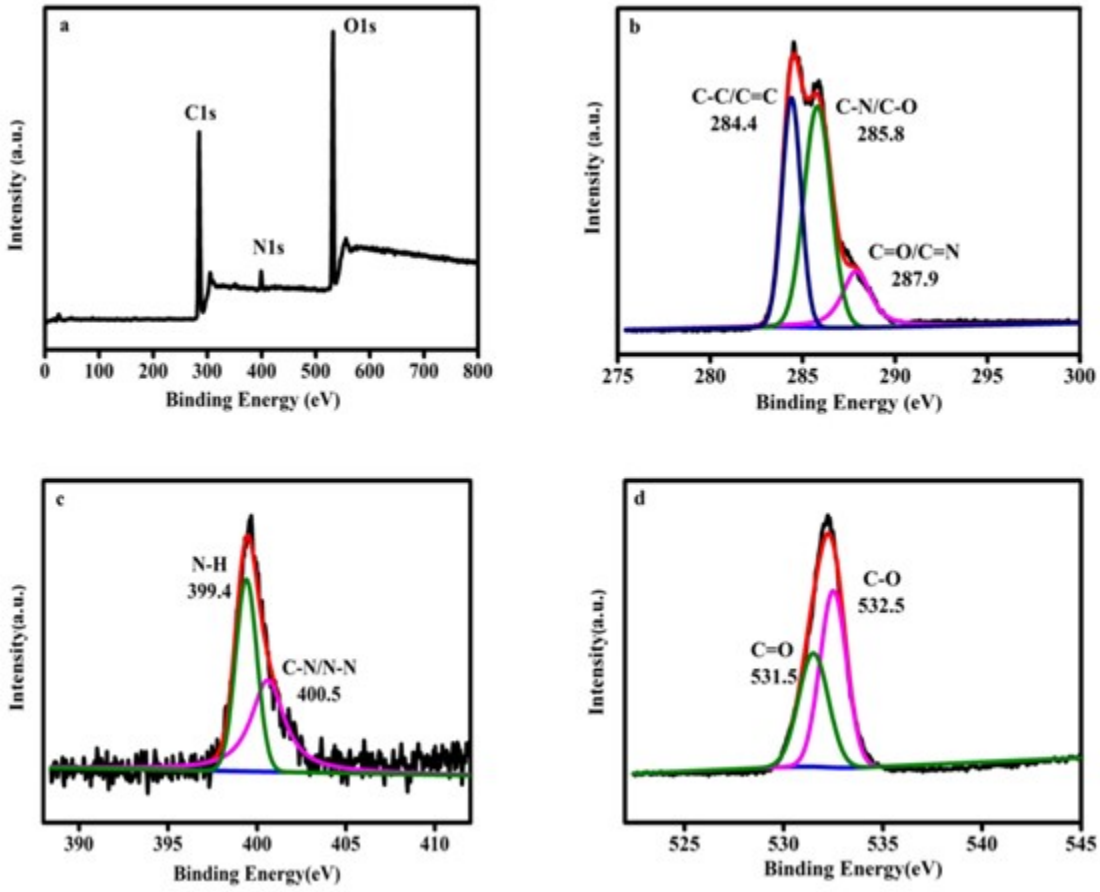

Figure S1. XPS spectrum of RCDs (a). High-resolution C1s (b), N1s (c), and O1s (d) spectra of RCDs.

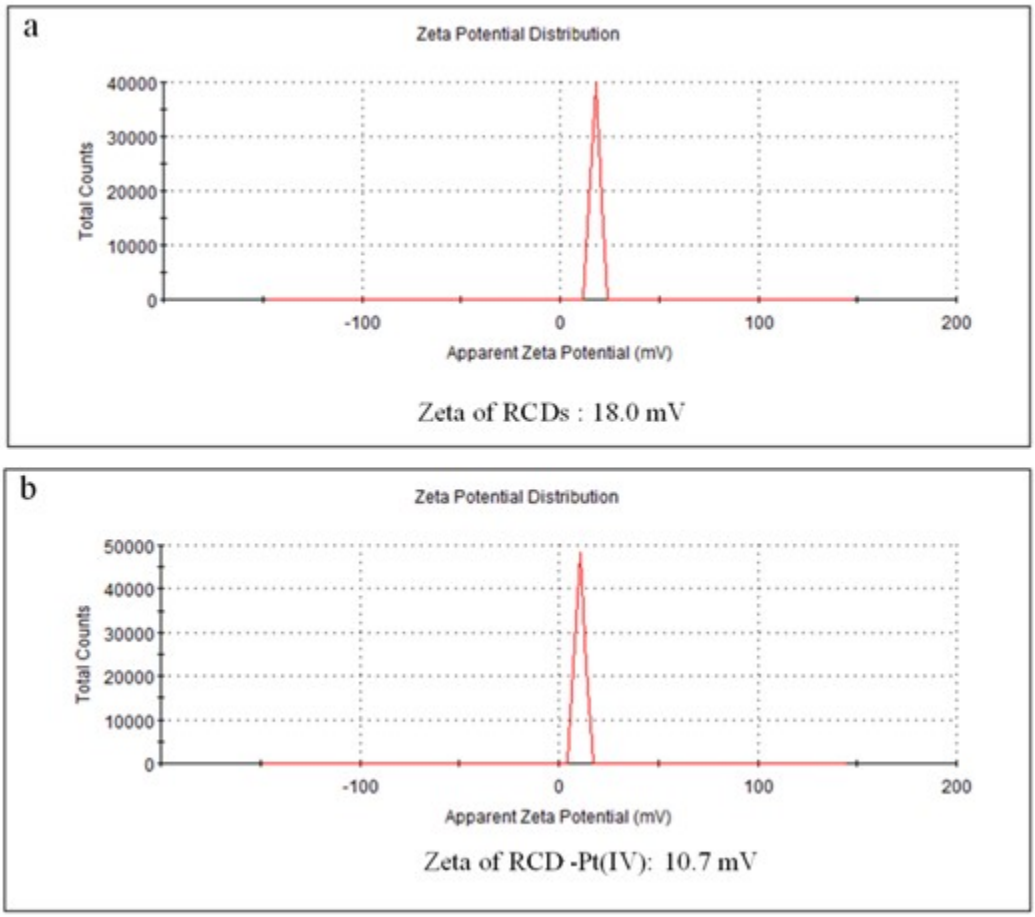

Figure S2. Zeta potential of RCDs (a) and RCD-Pt(IV) (b).

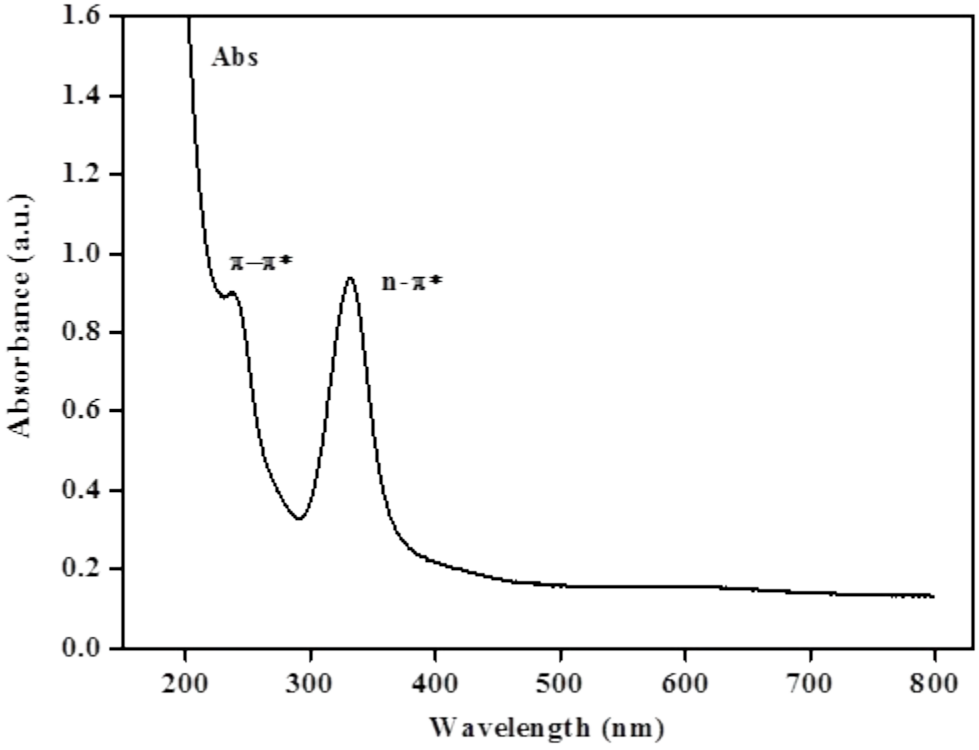

Figure S3. Absorption spectrum of RCD-Pt(IV).

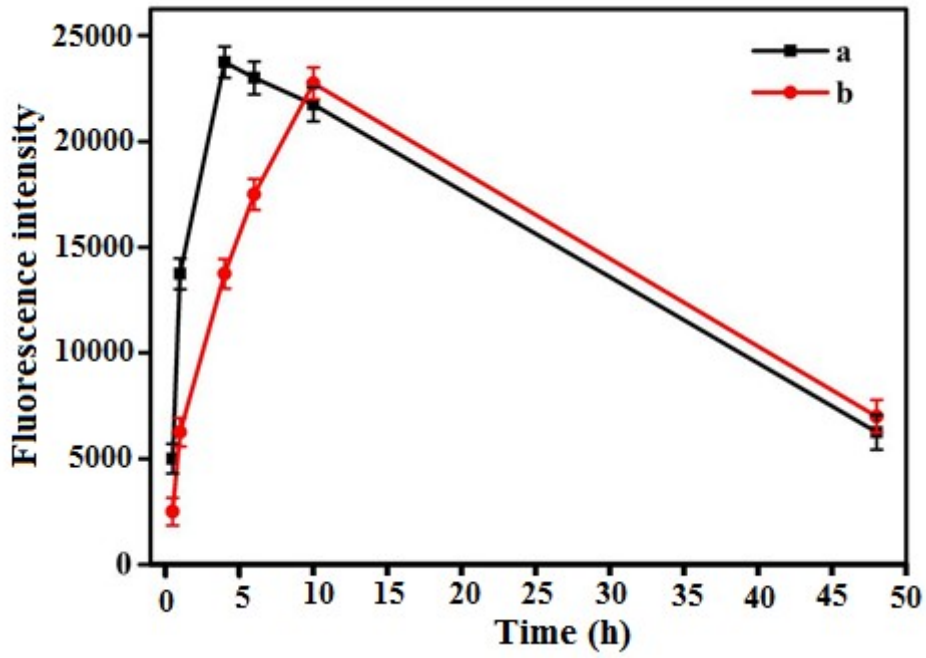

Figure S4. The mean fluorescence intensity of tumor tissue in different time points. The error bar represents the standard deviation (n=3).

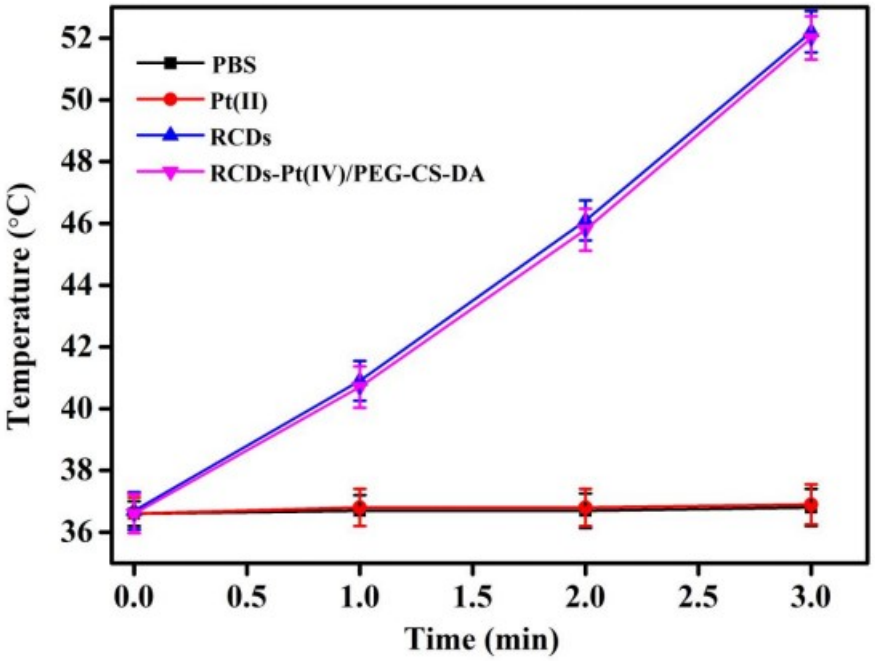

Figure S5. The mean temperature of tumor tissue in different time points. The error bar represents the standard deviation (n=3).

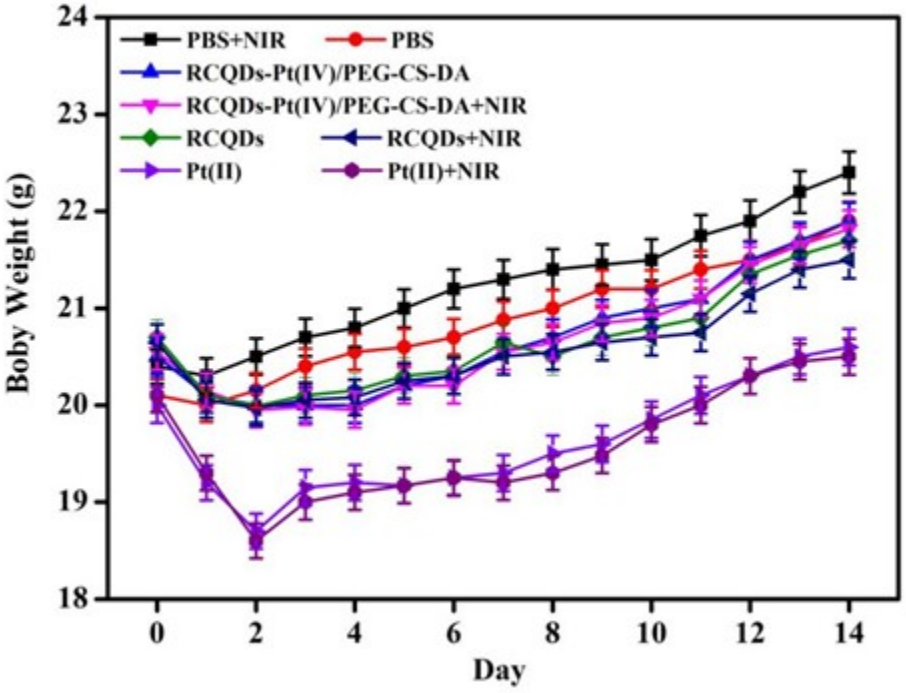

Figure S6. Changes of body weight of nude mice with time in different experimental groups. The error bar represents the standard deviation (n=3).

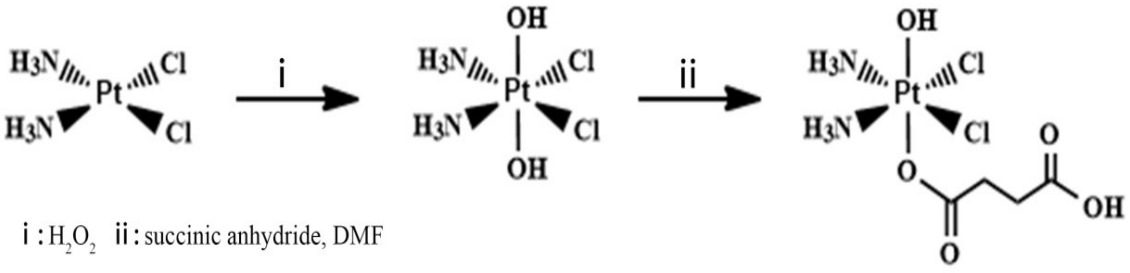

Figure S7. Synthesis of Pt(IV) prodrug.

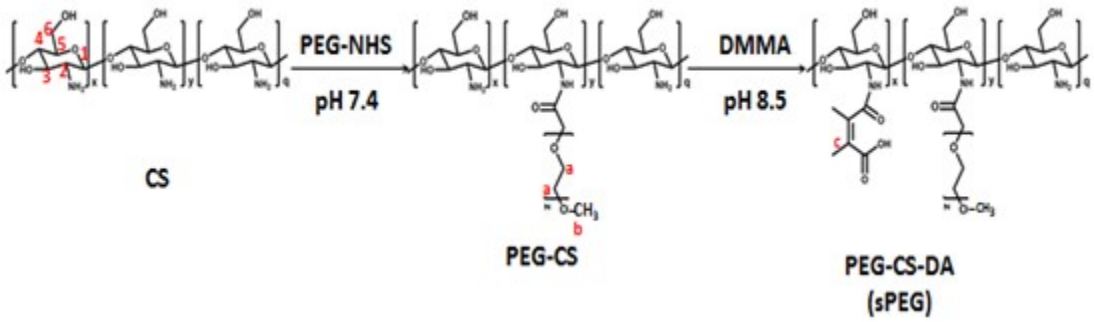

Figure S8. Synthesis of PEG-CS-DA.