Intelligent ZIF-8-Gated Polydopamine Nanoplatform for in vivo Cooperatively Enhanced Combinational Phototherapy

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Table of Contents

Experimental Section

Materials

Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, 98%), 2-methylimidazole (99%), sodium phosphate monobasic (NaH_2PO_4, 98%), sodium phosphate dibasic (Na_2HPO_4, 99%), catalase (from bovine liver), sodium acetate (NaAc, 99%), trizma hydrochloride (99%), trizma base (99%), L-glutathione reduced (GSH, 98%) and 1,3-diphenylisobenzofuran (DPBF, 97%) were purchased from Sigma-Aldrich. Methylene blue trihydrate (MB), ethanol absolute (AR) and acetic acid (HAc, AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. Dopamine hydrochloride (98%) were purchased from Aladdin. Thiazolyl blue (98%), Trypsin-EDTA (0.25%), Pen Strep (100×) (Pan 10000 U/mL, Str 10000 μg/mL, PBS 0.01 mol/L) and paraformaldehyde (4%) were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. DMEM/HIGH GLUCOSE (L-Glutomine 4.00 mM, Glucose 4500 mg/L) and phosphate buffer saline (1×) (0.0067 M, none of Calcium and Magnesium) were purchased from HyClone. 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) and reactive oxygen species detection kit (DCFH-DA) were purchased from Beyotime Biotechnology Co., Ltd. Tis (4,7-diphenyl-1,10-phenanthroline) ruthenium (II) dichloride $[Ru(dip)_3]Cl_2$ was purchased from Alfa Aesar (China) Chemicals, Ltd. Fetal bovine serum (Australian origin) was purchased from PAN-Biotech. Calcein-AM/PI double stain kit was purchased from Shanghai Yeasen Biotechnology Co., Ltd.

Characterization

Instruments for characterization of materials are Smart lab 9 kW X-ray diffractometer (Rigaku, Japan), Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific, USA), Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, USA), UV-2600 UV-Vis Spectrophotometer (Shimadzu, Suzhou), Zeta sizer Nano-ZS90 (Malvern, UK), Field Emission Scanning Electron Microscopy (SEM) (Zeiss Merlin Compact, Germany) and HT-7700 Transmission Electron Microscopy (TEM) (Hitachi, Japan). Instruments for cell experiments are Revolution XD spinning-disk confocal microscope (Andor, UK), Laser instrument (Hi-Tech Optoelectronics Co., Ltd. China), Multiskan GO (Thermo Fisher Scientific, USA) and BD FACS Verse flow cytometry (BD Medical

Technology, China).

Synthesis of polydopamine nanoparticles (PDAs). PDAs were synthesized from a mixture of $NH_3 \cdot H_2O$ (3.0 mL, 28%-30%), ethanol (40 mL), H_2O (90 mL) by a previously reported procedure with slight modification. The mixture was further mildly stirred for 30 min at 30 °C before dopamine hydrochloride (0.5 g DA, 10 mL H_2O aqueous solution) was injected into the mixture, and then stirred for 24 h. PDAs were collected by centrifugation at 10000 rpm for 20 min and washed several times with water. The precipitate was finally dispersed in water (4 mg/mL) for further used and stored at 4 °C.

Synthesis of PDAs-ZIF-8 (PZ). 2-Methylimidazole (2-MIM, 1 mL, 1.32 M) was added into PDAs (1.0 mL, 400 μg/mL) under stirred. One minute later, $Zn(NO_3)_2 \cdot 6H_2O$ (1 mL, 24 mM) was injected into the above mixture quickly, and stirred for 5 minutes, then placed for 3 h at room temperature. The product was collected by centrifugation at 12000 rpm for 30 min and washed with water for several times.

One-pot Synthesis of PDAs-MB-CAT-ZIF-8 (PMCZ). Firstly, 2-methylimidazole (2-MIM, 1 mL, 1.32M) and MB (1 mg) was mixed together in water, then the mixture was added into PDAs (1.0 mL, 4 00 μg/mL) under stirred. After 1 min, the mixture of $Zn(NO_3)_2 \cdot 6H_2O$ (1 mL, 24 mM) and 5 mg catalase was added quickly, continued stirring for 5 minutes. The reaction was placed for 3 h at room temperature. As a control group, PMZ was synthesized without catalase. The final product was collected by centrifugation and dried in vacuum.

BCA protein Assay. The concentration of catalase was determined by BCA reagents (purchased from Beyotime) in a standard method. The protocol was carried out based on the supplier.

Photothermal performances analysis. For measuring the photothermal conversion performance of PMCZ, 1 mL aqueous dispersion of PMCZ with different concentrations (0, 25, 50, 100, 200 μg/mL) were introduced in a quartz cuvette and irradiated with an 808 nm NIR laser at a power density of 2 W/cm^2 for 500 s, respectively. A thermocouple probe with an accuracy of 0.1 °C was inserted into the PMCZ aqueous solution perpendicular to the path of the laser. The temperature was

recorded every 10 s by a digital thermometer with a thermocouple probe.

Extracellular O_2 evolution in response to H_2O_2. For detection of extracellular O_2, the commercial chemical probe RDPP was used by detecting its fluorescence intensity at 455 nm excitation wavelength. In a total volume 500 μL, RDPP (25 μL, 10 mM) was added to PMCZ (25 μL, 4 mg/mL) in water solution (445 μL). After the addition of H_2O_2, fluorescence spectrum was recorded with fluorescence spectrophotometer. As the control, the groups of PDAs, PZ and PMZ were detected with the same method, respectively.

Intracellular O_2 evolution in response to H_2O_2. HeLa cells (1×10^5) were seeded in confocal dishes for 24 h. Then cells were washed with PBS for three times and incubated with RDPP (5 μM) for 4 h at 37 °C. Subsequently, cells incubated with PMCZ (50 μg/mL) for 4 h. The fluorescence images were obtained at 488 nm excitation using a confocal imaging system.

Extracellular $^1O_2$ detection. The extracellular $^1O_2$ was determined by DPBF, a $^1O_2$ probe whose absorption intensity can be strongly decreased by $^1O_2$. Briefly, DPBF (15 μL, 10 mM) and PMCZ (50 μg/mL) were mixed with PBS-ethanol (v/v = 4:6) mixed solution (pH 7.4). After added with H_2O_2, the sample was irradiated under 660 nm laser for 6 min. The loss of UV absorption intensity of DPBF at 410 nm was recorded at a certain time. As a control group, the solution without H_2O_2 at pH 7.4 was served. To further study the *in vitro* acidic H_2O_2-enhanced $^1O_2$ generation, DPBF (15 μL, 10 mM) and PMCZ (50 μg/mL) were mixed with PBS-ethanol (v/v = 4:6) mixed solution (pH 5.0) instead of pH 7.4.

Intracellular $^1O_2$ detection. HeLa cells were incubated with PMCZ (50 μg/mL) for 4 h and washed three times by PBS. Then DPBF (10 μM) was further incubated with cells for 30 min at 37 °C. Afterwards, cells were irradiated under 660 nm laser for 3 min. Finally, the fluorescence images were obtained at 488 nm excitation using a confocal imaging system. In order to study the intracellular acidic H_2O_2-enhanced $^1O_2$ generation, the group of PMZ was detected with the same method.

Cellular uptake study. To evaluate the cell uptake ability of PMCZ (50 μg/mL), HeLa cells were pre-seeded in confocal dishes for 24 h in CO_2 incubator at 37 °C. After 4 h

incubation, the loaded cells were washed several times with PBS to remove the residual materials. After fixed with 4% paraformaldehyde for 15 min at 4 °C and then washed with PBS for three times, cells were stained with DAPI for 15 min and finally imaged.

Photothermal and photodynamic therapy. HeLa cells were pre-plated in 96-well plates (1×10^4 cells per well) and cultured overnight. Cells were incubated with s series of concentrations of PMCZ under 37 °C within 5% CO_2. After 4 h, the PTT group was under 808 nm laser irradiation (2 W/cm^2) for 3 min, while the PDT group was under 660 nm laser irradiation (100 mW/cm^2) for 3 min. To verify the combined PDT and PTT effect at the cellular level, cells were irradiated by 660 nm laser and then 808 nm laser. After illumination, the cells were incubation for another 24 h, then cellular viability was evaluated by standard MTT assay.

The live/dead cell staining assay was carried out on HeLa cells using Calcein-AM/PI double stain kit. Cells were first incubated with PMCZ for 4 h and washed three times by PBS. Subsequently, the cells were exposed under laser and then medium was replaced with con-staining reagent. The fluorescence images were obtained at 488 nm excitation (for calcein AM) and 543 nm excitation (for PI) using a confocal imaging system.

The flow cytometry assay was carried out to evaluate the cytotoxicity of PDT and PTT. The cells were incubated with PMCZ for 4 h, then under 808 nm or 660 nm laser irradiation for 5 min. After incubated for another 24 h, the cells were containing with Annexin V-FITC/PI for 15 min and analyzed by flow cytometry.

Animal tumor xenograft models. All the animal experiments were performed based on the Principles of the Administration of Affairs Concerning Experimental Animals. The animal protocols used in this study were approved by The Institutional Animal Use and Care Committee of Wuhan University of China. HeLa tumor model was established by subcutaneous injection about 1×10^6 cells (dispersed in PBS) into the BABL/c-nude mice (4-6 weeks old). The tumor assays would be carried out when the tumor volumes reached to 80-100 mm^3. The tumor volume (V) was determined as the reported method, $V = (W^2\times L)/2$ (L, length; W, width).

***In vivo* Fluorescence imaging.** The fluorescence imaging *in vivo* was performed on

CRi maestro small animal imaging system. 100 μL PMCZ or MB was injected into the HeLa bearing-mice *via* tail vein at a consistent concentration of MB. After the last time points, all mice were sacrificed and the major organs were collected for fluorescence imaging.

***In vivo* photothermal imaging.** PMCZ (50 μL, 800 μg/mL) was injected into the mice. As the control, PBS was injected into another mice. The power density of 808 nm laser used was 1 W/cm^2. IR thermal camera was utilized to monitor the temperature change and quantified the temperature by the BM-IR software.

***In vivo* photothermal therapy and photodynamic therapy.** The mice were randomly divided into six groups (n≥4) and treated with: group1, PBS (50 μL); group 2, PMCZ (50μL, 800 μg/mL); group 3, PMCZ (50 μL, 800 μg/mL) with 660 nm laser irradiation, PMCZ+PDT; group 4, PMCZ (50 μL, 800 μg/mL) with 808 nm laser irradiation, PMCZ+PTT; group 5, PMZ (50 μL, 800 μg/mL) with 660 nm laser irradiation and 808 nm laser irradiation, PMZ+PDT+PTT; 6, PMCZ (50 μL, 800 μg/mL) with 660 nm laser irradiation and 808 nm laser irradiation, PMCZ+PDT+PTT. After 12 h treated, PDT was carried out on group 3 treated with 660 nm laser irradiation for 5 min at the laser power density of 1 W/cm^2. PTT was carried out on group 4 treated with 660 nm laser irradiation for 5 min at the laser power density of 1 W/cm^2. Combination of PDT and PTT were carried out on group 5 and group 6. The two groups were treated with 660 nm laser irradiation for 5 min at the laser power density of 1 W/cm^2 and then 808 nm laser irradiation for 5 min at the laser power density of 1 W/cm^2.The tumor volumes and the body weights were recorded every other day and for 14 days totally (day 0, 2, 4, 6, 10, 12 and 14). After the last day treatment, 6 mice (one mouse per group) were sacrificed and cancer tissues were got for histological analysis.

***In vivo* toxicity evaluation.** After 14-day treatment, all mice were sacrificed and their blood and organs were collected. The blood cells were used for Hemolysis Test. The blood cells were incubated with PMCZ at 37 °C for 30 min, and then the samples were centrifuged to detect the hemolysis. The organs were stained with H&E and detected as described above.

HeLa tumor-bearing mice tail vein injection with PMCZ (160 μL, 2 mg/mL).

Subsequently, all treated mice were sacrificed, followed the tissues were taken out and weighed. In order to digest these tissues, the mixture of HNO_3 and H_2O_2 (volume ratio=1:2) were added into the solution and kept the reaction temperature higher than 200 °C. Then ICP-Ms were carried out to analyze the Zn standard solutions with different concentrations (0, 0.5, 1, 5, 10, 50, and 100 μg/mL), and the content of Zn in the prepared solution. The amount of PMCZ was finally calculated by normalizing to percentages of injected dose per gram of tissue (%ID/g).

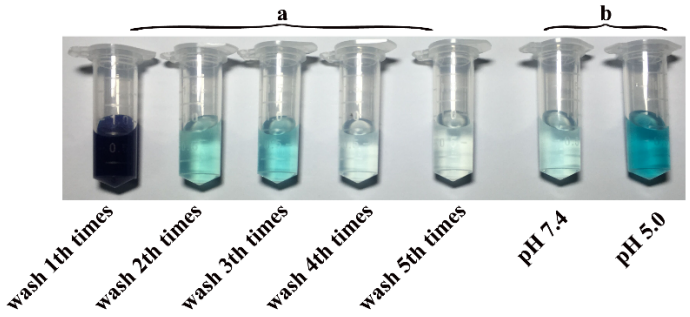

Fig. S1 The photo images of PDAs-MB-CAT-ZIF-8 (PMCZ, 50 μg/mL) after certain round of washing times (a). The corresponding photo images of the supernatant of PMCZ (PMCZ, 50 μg/mL) that was reacted in pH 7.4 and pH 5.0 (b).

The prepared PMCZ was dissolved in 1 mL of water solution. Subsequently, the supernatant obtained from centrifugation was collected for photograph. Repeated this procedure for five times until the supernatant color was clear and transparent. To study the drug release experiment under different pH conditions, PMCZ (50 μg/mL) was dispersed in 1 mL of PBS solution at pH 7.4 and 5.0, respectively. After reaction for 30 min, the supernatant was collected by centrifugation for photograph.

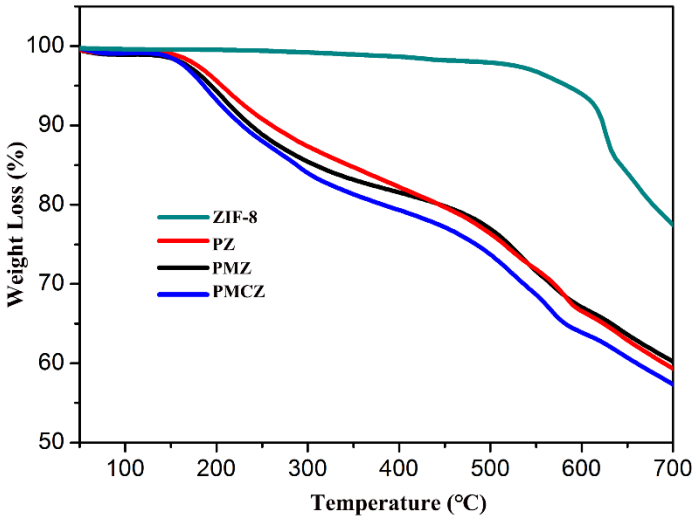

Fig. S2 TGA curves of ZIF-8, PDAs-ZIF-8 (PZ, 50 μg/mL), PDAs-MB-ZIF-8 (PMZ, 50 μg/mL) and PMCZ (50 μg/mL).

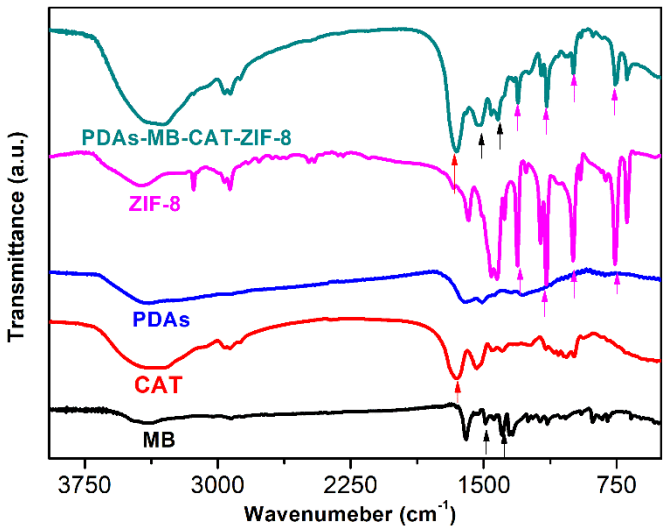

Fig. S3 FT-IR spectrum of free CAT, MB, PDAs, ZIF-8 and PMCZ.

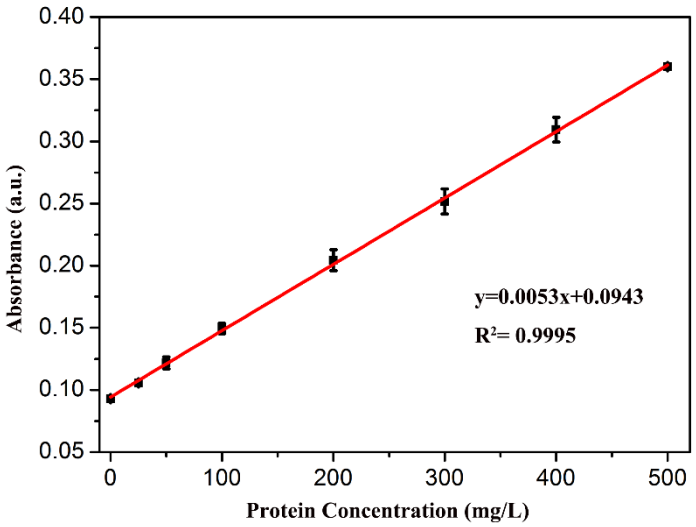

Fig. S4 Liner relationships between UV-vis absorbance and BSA concentration.

In detail, bovine serum albumin (BSA) standards was firstly prepared as manufacturer's instructions. Then a series of BCA working reagent (WR) with increasing BCA was prepared with varying concentrations of standards present in 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL. The volumes of all WR solutions were kept constant by adding pure water so that only the BCA concentration varied. Subsequently, the supernatant obtained from centrifugation and washed with H_2O in the above process of synthesis was collected, 20 μL of the supernatant was added into the WR solutions. After leave the reaction mixture at 37 °C for 30 minutes, the absorbance of wavelength A562 nm was measured by microplate reader. Finally, the protein concentration of the sample was calculated according to the standard curve and the volume of the used sample. The CAT loading capacity was about 3.4%.

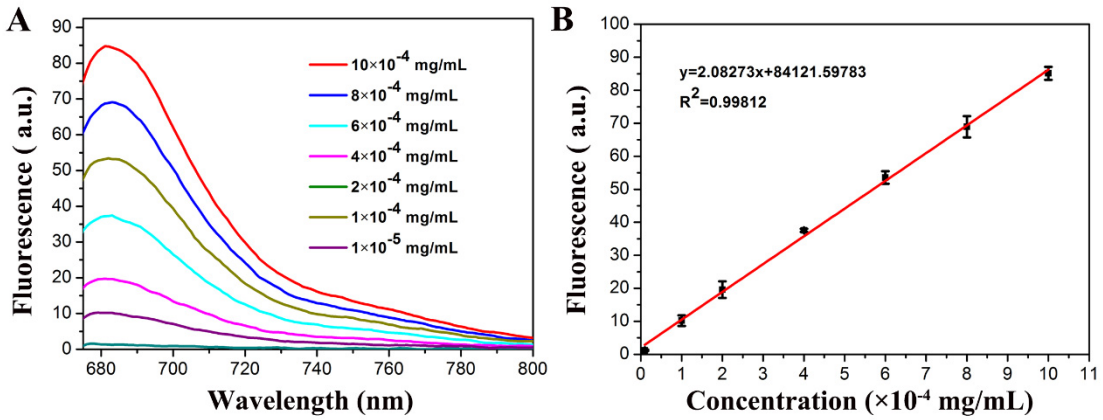

Fig. S5 (A) The fluorescence spectra of MB in water at various concentrations. (B) The calibration curve of MB at 681 nm. The loading amount of MB for PMCZ was quantified by reading characteristic emission peak of MB at 681 nm. According the following equation, the MB loading capacity was calculated: Loading capacity (%) = $(M-M_1)/M_2*100\%$, where M represents the initial feeding amount of MB, M_1 represents the MB content in the supernatant, and M_2 represents the mass of PMCZ. MB loading efficiency were calculation to 5%.

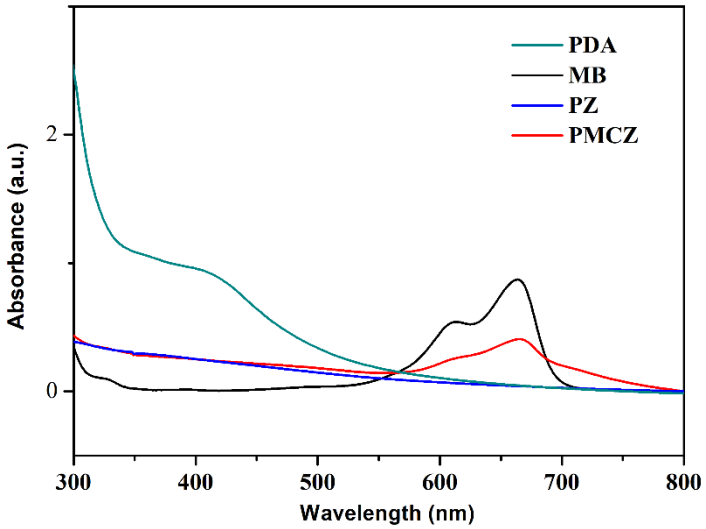

Fig. S6 UV-vis absorption spectra of PDA, MB, PZ and PMCZ. For measuring the UV-vis absorption spectra of different samples, the samples were firstly dispersed in water. Then the UV-vis spectra were recorded on a UV-2600 UV-Vis Spectrophotometer (Shimadzu, Suzhou). Excitation and emission were set to the same wavelength and scanned from 300 nm to 800 nm.

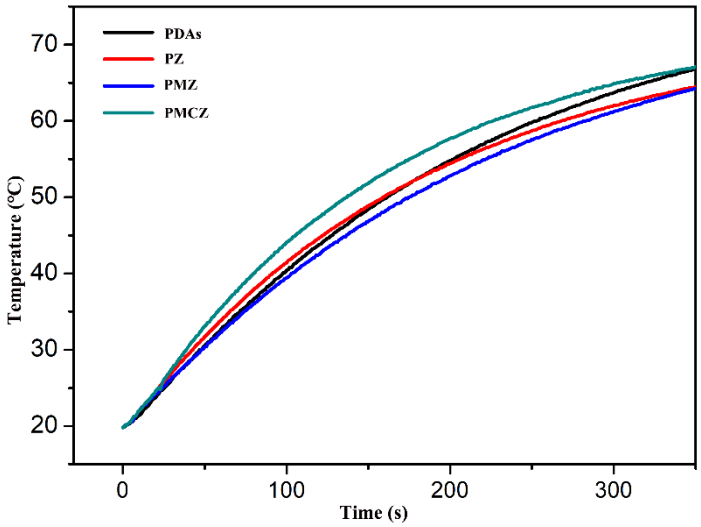

Fig. S7 Temperature rising curves of different samples (ZIF-8, PZ, PMZ and PMCZ, 200 μg/mL) under 808 nm irradiation (2 W/cm^2) for 300 s.

To analyze the photothermal performance of different samples, 1 mL aqueous dispersion of ZIF-8, PZ, PMZ and PMCZ with concentration of 50 μg/mL were introduced in a quartz cuvette and irradiated with an 808 nm NIR laser at a power density of 2 W/cm^2 for 350 s, respectively. A thermocouple probe with an accuracy of 0.1 °C was inserted into the above aqueous solution perpendicular to the path of the laser. The temperature was recorded every 10 s by a digital thermometer with a thermocouple probe. A similar temperature change was obtained for both of PMCZ and PDAs, indicating that ZIF-8 did not influence the photothermal effect of PDAs.

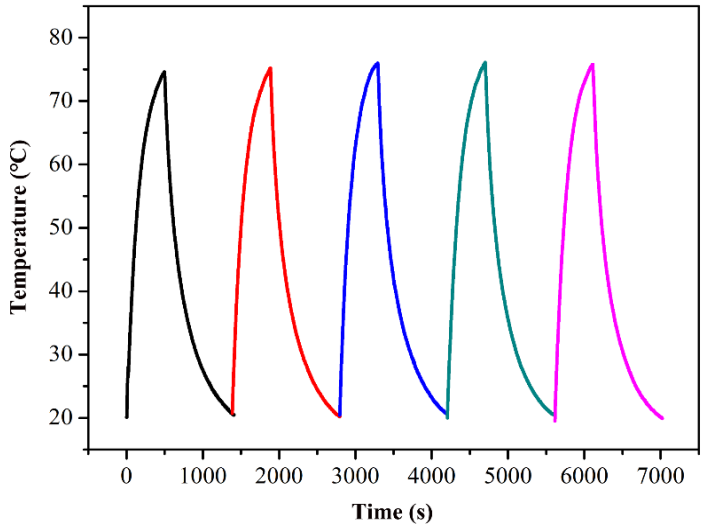

Fig. S8 Temperature change curves of PMCZ (200 μg/mL) during five cycles of 808 nm laser irradiation (2 W/cm^2) with on/off states. To evaluate the NIR stability of PMCZ, PMCZ (200 μg/mL) were irradiated for 500 s at a power density of 2 W/cm^2 and then the laser was turned off. This procedure was repeated for five times to assess the photostability of PMCZ.

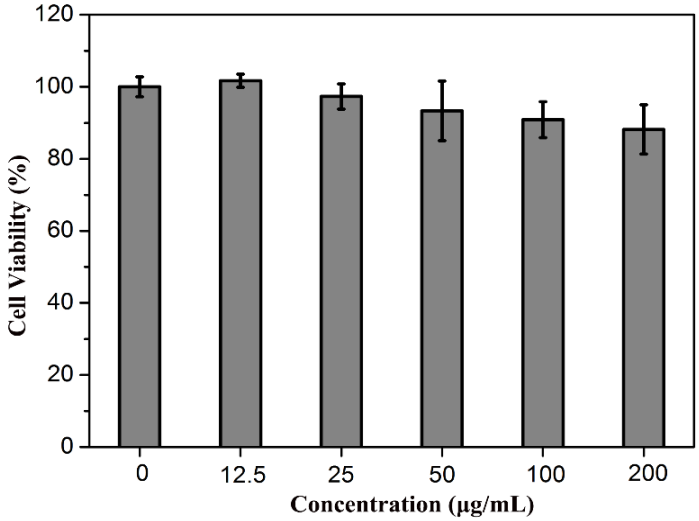

Fig. S9 Cell viability of PMCZ on HeLa cells. The HeLa cells were incubated with different concentrations of PMCZ (0, 12.5, 25, 50, 100, 200 μg/mL) for 4 h.

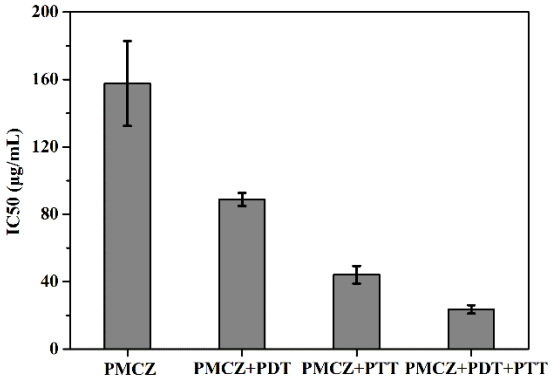

Fig. S10 IC50 values for the groups of the HeLa cells treated with various concentrations of nanomaterials. The plotting of the IC50 value for each group was calculated using SPSS software.

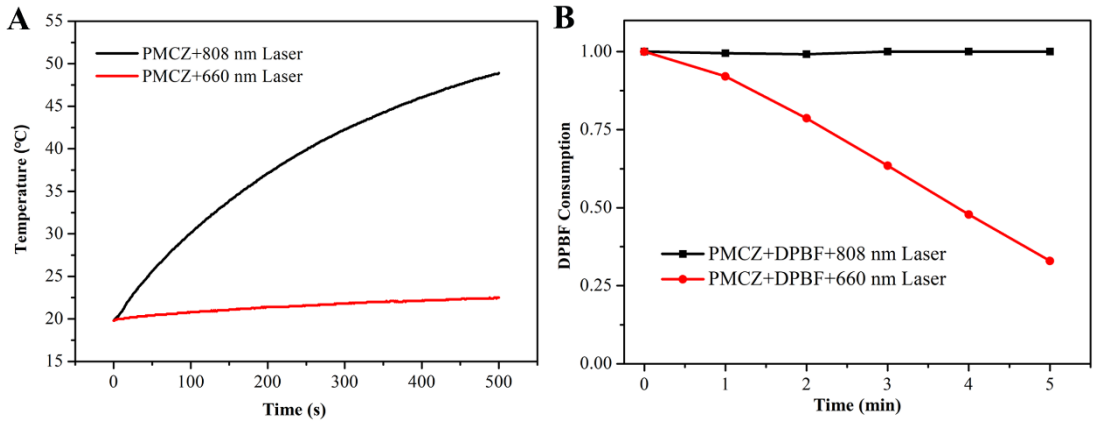

Fig. S11 (A) Temperature changes after irradiation (660 nm, 100 mW/cm^2 or 808 nm, 2 W/cm^2) for different time in 1 mL of PBS containing PMCZ (50 μg/mL). (B) $^1O_2$ production efficiency after irradiation (660 nm, 100 mW/cm^2 or 808 nm, 2 W/cm^2) for different time in 1 mL of PBS containing PMCZ (50 μg/mL).

To validate feasibility of the system under 660 nm laser irradiation for PTT, 50 μg/mL of PMCZ was exposed to irradiation using 660 nm laser with a power density of 100 mW/cm^2 for 5 min, and the temperature increases of the sample were monitored as a function of laser irradiation time. Figure S11A showed that the temperature increased only several degrees, much lower than the temperature of the sample under 808 nm irradiation with a power density of 2 W/cm^2 for 5 min. The result indicated that the photothermal effect of PMCZ was insignificant, and the current system was not suitable for PTT under 660 nm laser irradiation.

To validate feasibility of the system under 808 nm laser irradiation for PDT, 1,3-diphenylisobenzofuran (DPBF) was employed as a $^1O_2$ probe to evaluate the production of $^1O_2$. Upon $^1O_2$ oxidation, the intensity of the characteristic absorption of DPBF at around 410 nm would decrease. Under 808 nm laser with a power density of 2 W/cm^2 for 5 min, we found that 50 μg/mL of PMCZ induced negligible the generation of $^1O_2$ (Figure S11B). The results indicated that the current system was not suitable for PDT under 808 nm laser irradiation.

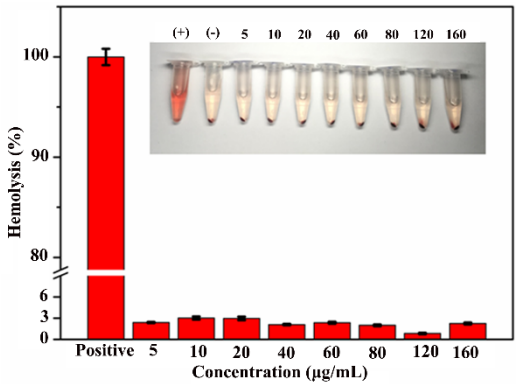

Fig. S12 Hemolytic activity of PMCZ as a function of concentration. Insert: photograph of hemolytic activity of PMCZ as a function of concentration.

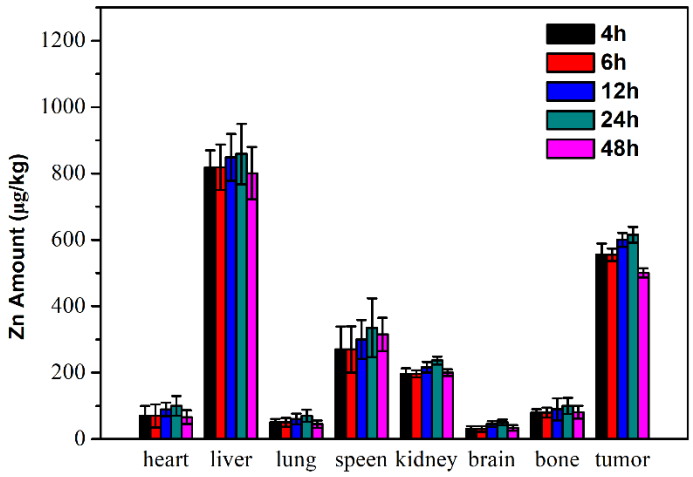

Fig. S13 Biodistribution of Zn after mice were injected with PMCZ (160 μL, 2 mg/mL) at different time points (4, 6, 12, 24, 48 h). The data were according to ICP-MS analysis of Zn contents from different organs.

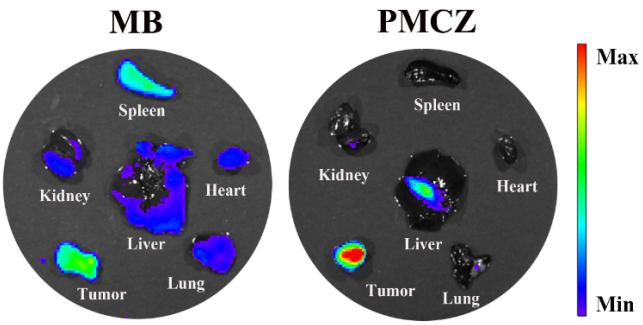

Fig. S14 *Ex vivo* fluorescence images of heart, liver, spleen, lung, kidney and tumor extracted from HeLa tumor-bearing mice treated with free MB and PMCZ (50 μL, 800 μg/mL). The tumor-bearing mice were sacrificed after intravenously injected with MB or PMCZ for 24 h, and the major organs were collected for imaging.

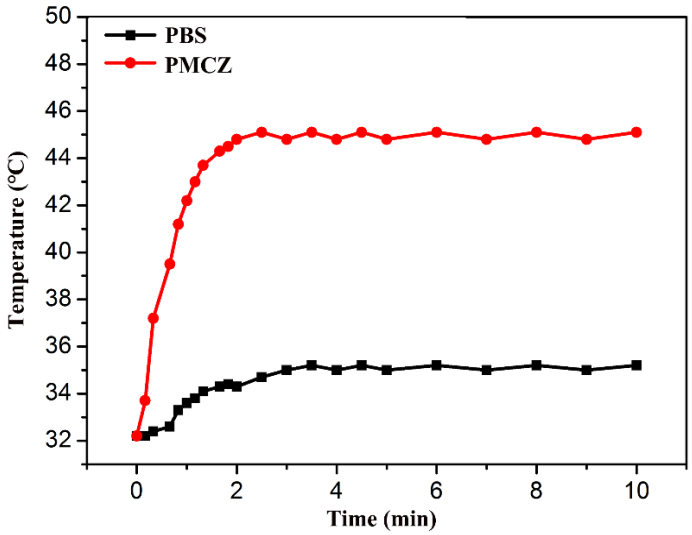

Fig. S15 Temprature curves of tumor sites in mice treated with PBS (50 μL) or PMCZ (50 μL, 800 μg/mL) followed 808 nm laser irradiation for 10 min (1 W/cm^2).

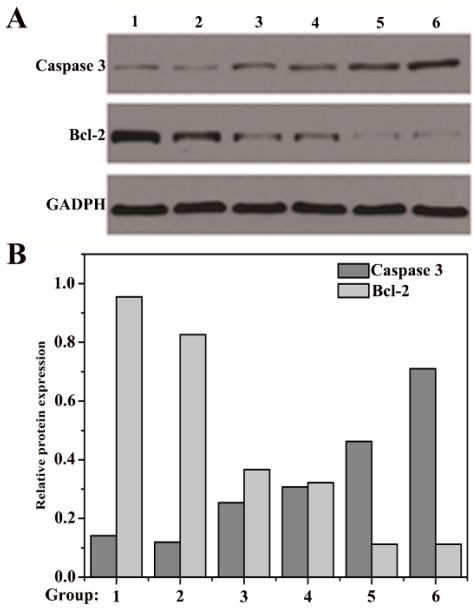

Fig. S16 (A) Western blot assay of caspase-3 and Bcl-2 protein expressions from tumors after different treatments. (B) The corresponding quantitative analysis of caspase-3 and Bcl-2 proteins in tumors after different treatments as determined by western blot assay.

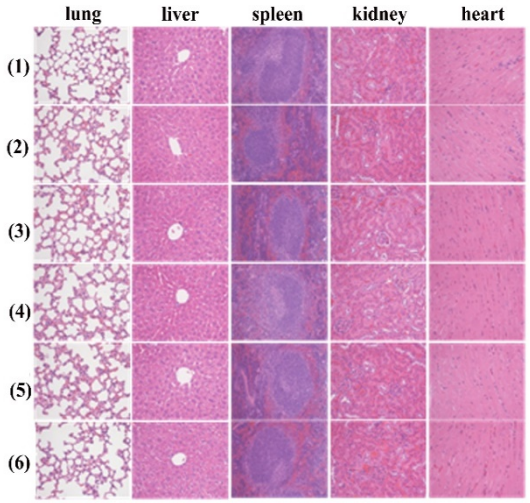

Fig. S17 Pathological H&E stained images of tissue sections from heart, liver, spleen, lung and kidney harvested from HeLa tumor-bearing mice on the 14th day treated with PBS (50 μL) (1), PMCZ (50 μL, 800 μg/mL) (2), PMCZ (50 μL, 800 μg/mL)+PDT (660 nm, 1 W/cm^2) (3), PMCZ (50 μL, 800 μg/mL)+PTT (808 nm, 1 W/cm^2) (4), PMZ (50 μL, 800 μg/mL)+PDT (660 nm, 1 W/cm^2)+PTT (808 nm, 1 W/cm^2) (5) and PMCZ (50 μL, 800 μg/mL)+PDT (660 nm, 1 W/cm^2)+PTT (808 nm, 1 W/cm^2) (6).

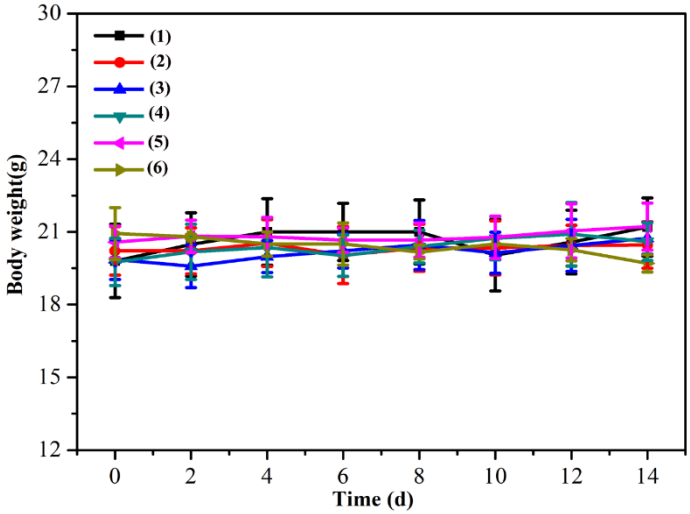

Fig. S18 Body weights of HeLa tumor-bearing mice after different treatments: PBS (50 μL) (1), PMCZ (50 μL, 800 μg/mL) (2), PMCZ (50 μL, 800 μg/mL)+PDT (660 nm, 1 W/cm^2) (3), PMCZ (50 μL, 800 μg/mL)+PTT (808 nm, 1 W/cm^2) (4), PMZ (50 μL, 800 μg/mL)+PDT (660 nm, 1 W/cm^2)+PTT (808 nm, 1 W/cm^2) (5) and PMCZ (50 μL, 800 μg/mL)+PDT (660 nm, 1 W/cm^2)+PTT (808 nm, 1 W/cm^2) (6).