Supporting Information

A cascade nanoplatform for intelligent response to tumor microenvironment and collaborative cancer therapy

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1. Materials and methods

1.1. Materials

Disulfiram(DSF), methylene blue (MB), 1,3-diphenylisobenzofuran (DPBF), glutathione (GSH), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), 2,2,6,6-tetramethylpiperidine (TEMP) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were purchased from Energy Chemical (China). Zinc nitrate hexahydrate ($ZnNO_3 \cdot 6H_2O$), copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), hydrogen peroxide (H_2O_2, 30%), potassium permanganate ($KMnO_4$) and sodium hydroxide (NaOH) were purchased from Xilong Science. 2-Methylimidazole (2-MIM) was purchased from Aladdin. Chlorin e6 (Ce6), Titanous sulfate ($Ti(SO_4)_2$) and hyaluronic acid (HA) were purchased from Macklin. Cell Counting Kit-8 (CCK-8) were purchased from APExBIO. Calcein acetoxymethyl ester and propidium iodide (calcein-AM/PI), 2',7'-dichlorofluorescein diacetate (DCFH-DA), mitochondrial membrane potential assay kit (JC-1), Glutathione Assay Ki, Hydrogen Peroxide Assay Kit, and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from Beyotime Biotechnology Co. Shanghai, China. All chemicals were used without further purification.

1.2. Characterization

Transmission electron microscope (TEM; Hitachi H 9000 NAR) images were obtained on a TECNAI G2 equipped with energy dispersive spectroscopic (EDS) at 200 kV. Scanning electron microscopy (SEM) images were collected on a field emission scanning electron microscope FE-SEM (XL30, FEG, Micro FEI PHILIPS). Hydrodynamic Powder X-ray diffraction (XRD) data was carried out on a Scintag XDS 2000 X-ray powder diffractometer, operating at 40 kV, 40 mA with Cu Kα radiation (λ = 1.5418 Å). X-ray photoelectron spectra (XPS) were collected by X-ray photoelectron spectroscopy (American Thermo Fisher-K-alpha, U.S.). Hydrodynamic diameter and zeta potential were collected on Zetasizer Nano ZS (Malvern Instruments Ltd., UK). UV-visible-NIR spectra were detected on a UV-visible-NIR spectrophotometer (VARIAN CARY 50). Fluorescence spectra were detected by JASCO F-6000 fluorescence spectrometer. Nitrogen adsorption-desorption analysis was measured using N_2 gas at 150 °C on Autosorb iQ Station 1. Fourier transform infrared (FT-IR) spectra were collected on a VERTEX 70 FTIR spectrometer (Bruker). Inductively coupled plasma mass spectrometry (ICP-MS) data were obtained on an ELAN 9000/DRC system. The dissolved oxygen content was tested on a portable dissolved oxygen meter (JPBJ-608, Shanghai instrument scientific instrument Co., LTD. Shanghai, China). Electron spin resonance (ESR) spectra were collected by an electron paramagnetic resonance spectrometer (EMXnano, Bruker). The fluorescence images of cells were measured by a confocal laser fluorescence scanning microscope (CLSM, Leica TCS SP2, Leica Microsystems, Mannheim, Germany).

1.3. Cell lines and animals

4T1 cells were cultured and subcultured in 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C under a 5% CO_2 atmosphere. Healthy female Balb/c mice (~18 g) were obtained from the Beijing Vital River Laboratory Animal Technology Co., LTD.

1.4. pH-responsive decomposition

The detection of Ce6, Cu^{2+} and CuET in degradation products. DCZCu (1 mg, without DSF) was dispersed in 1 mL of different PBS buffer (pH = 5.5, 7.4). Place the sample in a shaker at a speed of 120 rpm under 37°C. Samples were centrifuged at different time points to collect the supernatant and refilled with 1 mL of PBS. The release of Ce6 and Cu^{2+} were determined using UV-visible spectrophotometer and ICP-MS, respectively. All samples were performed in triplicate. The TEM assays were performed for the degradation test. The release of Ce6 molecules under different pH conditions after 24 h was also detected using fluorescence spectroscopy. The degradation products of DZ and DZCu were collected and dispersed in methanol, and the release of DSF and the generation of CuET were detected by UV-visible spectrophotometer.

1.5. H_2O_2 release assay

The H_2O_2 released from DCZCu was detected by $KMnO_4$. The $KMnO_4$ (50 μg mL^{-1}) was dissolved in PBS buffer (pH = 5.5). The mixture was treated with DCZ, DCZCu, CuO, or H_2O_2 for 10 min. The supernatant was collected by centrifugation and the UV-visible absorption spectra were measured from 400 to 700 nm. In vitro H_2O_2 generation was also measured with $Ti(SO_4)_2$. ZIF-8@CuO_2, H_2O, or H_2O_2 was dispersed in 1 mL of pH 5.5 and the mixture was incubated for 1 h at room temperature. Next, the supernates were collected by centrifugation and mixed with 1 mL of $Ti(SO_4)_2$ solution (1 mg/mL). After that, the UV-visible absorption spectra of the solutions were examined.

1.6. O_2 generation

The O_2 content of the solution was detected in real-time using a portable dissolved oxygen meter. DCZCu (1 mg mL^{-1} or 5 mg mL^{-1}) in PBS buffer (pH = 5.5) at 23.7 ℃. The dissolved O_2 concentration was monitored every 1 minute by a portable dissolved oxygen meter. The PBS buffer (pH = 5.5) was set as a control.

1.7. In vitro detection of •OH

The •OH production capacity of DZCu was assessed by UV-visible absorption spectra and ESR spectra. DZ and DZCu (1 mg mL^{-1}) containing MB (10 μg mL^{-1}) were mixed separately in PBS buffer at pH = 5.5. The supernatants were collected by centrifugation and their UV-visible absorption spectra were determined after incubation at 37 °C for 24 h. In addition, the same method was used to determine the in vitro •OH generation of different concentrations of DZCu and DZCu (1 mg mL^{-1}) at different pH and different incubation times. For ESR experiments, DZ or DZCu (45 μL, 0.5 mg mL^{-1}), DZCu + GSH (5mM) was mixed with DMPO (5 μL) and ESR spectra were collected by capillary sample preparation

method to detect •OH production. The ESR spectra of DZCu at different pH conditions were determined by the same method using the same approach. DMPO without sample was used as a control.

1.8. In vitro detection of $^1O_2$

The generation of $^1O_2$ was measured by DPBF. DPBF (20 μg mL^{-1}) was added to DZCu and DCZCu (50 μg mL^{-1}), respectively, and irradiated with a laser (650 nm, 0.5 W cm^{-2}). The absorption at 425 nm was measured in the dark at different irradiation times. For ESR experiments, DCZCu (90 μL, 0.5 mg mL^{-1}) dispersed in different pH was mixed with TEMP (10 μL) and then irradiated with a 650 nm laser (0.5 W cm^{-2}) for 1 min. ESR spectra were then collected to detect $^1O_2$ production. TEMP without sample was used as a control.

1.9. GSH depletion study

GSH depletion was detected by DTNB. DZCu (0.2 mg mL^{-1}) containing GSH (1 mM) was mixed into PBS buffer (pH = 5.5), and DTNB (20 μg mL^{-1}) was added to the above solution after incubation at 37 °C for different time points. The remaining GSH in the mixed solution was measured at 412 nm by UV-visible absorption spectra.

1.10. Cellular uptake study

4T1 cells were seeded in the 35 mm culture dish and incubated at 37 ℃ for 24 h. Subsequently, 2 mL fresh medium containing DCZCu (100 μg mL^{-1}) was used to incubate the cells. After 0.5, 1, 2, and 4h, the cells were washed with PBS, and the cell nucleus was stained by DAPI. Finally, the cells were imaged using CLSM after they were rinsed for three times.

1.11. Cell cytotoxicity

The cytotoxicity was determined by CCK-8 assay. Typically, 4T1 cells were cultured in 96-well plates for 24 h. The medium was replaced by nanoparticles (DCZ, DCZCu, or DCZCu+Laser) with different concentrations, respectively. After 12 h incubation, the laser irradiation groups were treated with 650 nm laser (0.5 W cm^{-2}) for 5 min. The cells were then incubated at 37 ℃ for another 12 h. For the laser-free groups, the plate was directly incubated for 24 h. Then, cells were washed with PBS and incubated with CCK-8 reagent for 1 h. Finally, the absorbance was measured with a microplate reader at 450 nm.

1.12. Intracellular H_2O_2 measurement

4T1 cells were seeded in 6-well plates and incubated at 37 °C for 24 h. Then, the cells were incubated with PBS, DZ, DCZ, DZCu, and DCZCu (50 μg mL^{-1}) for 4h. After that, the H_2O_2 content was measured using a hydrogen peroxide detection kit.

1.13. Intracellular GSH measurement

4T1 cells were seeded in 6-well plates and incubated at 37 °C for 24 h. Then, the cells were incubated with PBS, DZ, DCZ, DZCu, and DCZCu (50 μg mL^{-1}) for 12 h. After that, the GSH content was measured using a GSH detection kit.

1.14. Live/Dead cell staining assay

4T1 cells were seeded in 12-well plates and incubated at 37 °C for 24 h. Then, the cells were incubated with PBS, Laser, DCZ, DCZCu, and DCZCu+Laser (50 μg mL^{-1}) for 24 h. For the laser groups, each well was treated with 650 nm laser (0.5 W cm^{-2}) for 5 min. Then, cells were washed with PBS and incubated with calcein AM (1 μg mL^{-1}) and PI (2 μg mL^{-1}) for 30 min. Finally, fluorescence imaging of live/dead cells were performed with CLSM after washing.

1.15. Intracellular ROS generation

4T1 cells were seeded in 6-well plates and incubated at 37 °C for 24 h. Then, the cells were incubated with PBS, Laser, DCZ, DCZCu, and DCZCu+Laser (50 μg mL^{-1}) for 12 h. For the laser groups, the well was treated by 650 nm laser (0.5 W cm^{-2}) for 5 min. After washing with PBS, the cells were incubated with DCFH-DA for 20 min. Finally, fluorescence imaging were observed by CLSM after washing.

1.16. Mitochondrial membrane potential assay

4T1 cells were seeded in 6-well plates and incubated at 37 °C for 24 h. Then, the cells were incubated with PBS, Laser, DCZ, DCZCu, and DCZCu+Laser (50 μg mL^{-1}) for 12 h. For the laser groups, the well was treated by 650 nm laser (0.5 W cm^{-2}) for 5 min. After washing with PBS, the cells were incubated with JC-1 assay solution for 20 min. Finally, fluorescence imaging were observed by CLSM after washing.

1.17. Hemolysis test

Blood from normal Balb/c mice was taken for hemolysis test. Firstly, the blood was washed three times with PBS buffer, and then the lower red blood cells were collected. The red blood cells were diluted with PBS solution. Then, 20 μL of cell suspension was added to different concentrations of DCZCu (480 μL). Then, the mixed solution was incubated for 2 h at 37 ℃. After centrifugation at 13000 r min^{-1} for 5 min, the UV-visible absorption spectra were measured. Finally, the hemolysis ratio was calculated according to the following equation:

$$\text{Hemolysis (\%)} = (A_{sample} - A_{negative}) / (A_{positive} - A_{negative}) \times 100\ \%$$

A_{sample}: the absorbance of supernatant with different concentrations; $A_{negative}$: the absorbance of supernatant in PBS group; $A_{positive}$: the absorbance of supernatant in the pure water group.

1.18. In vivo antitumor efficacy evaluation

The tumor models were established by subcutaneous injection of 4T1 cells into the right back of Balb/c mice. When the tumor volumes reached 100 mm^3, the mice were randomly split into five groups (n = 6): (I) PBS; (II) Laser; (III) DCZ; (IV) DCZCu and (V) DCZCu+Laser. The samples were injected 20 mg kg^{-1} into the tumor of mice on days 0 and 7 and treated by 650 nm laser (0.5 W cm^{-2}) for 10 min after 4 h of injection. Subsequently, the body weights and tumor volumes of mice were monitored every other day after administration. The tumor volume of mice was determined: tumor volume = $width^2 \times length/2$.

At day 14, all the mice were euthanized. Mouse blood was collected for blood biochemistry analysis, and their tumors were weighed and used for H&E staining and HIF-1α staining. The main organs (heart, liver, spleen, lungs, and kidneys) of mice were collected for H&E staining.

1.19. Statistical analysis

Results were shown as mean ± SD. Asterisks were used to represent significant differences (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

1.20. Animal Ethics Statement

The animal experiments were approved by the Animal Ethics Committee of Changchun Institute of Applied Chemistry, Chinese Academy of Sciences (Approval No. 2023[0071], Changchun, China) and were carried out according to the relevant institutional guidelines and laws.

2. Supplementary Figures

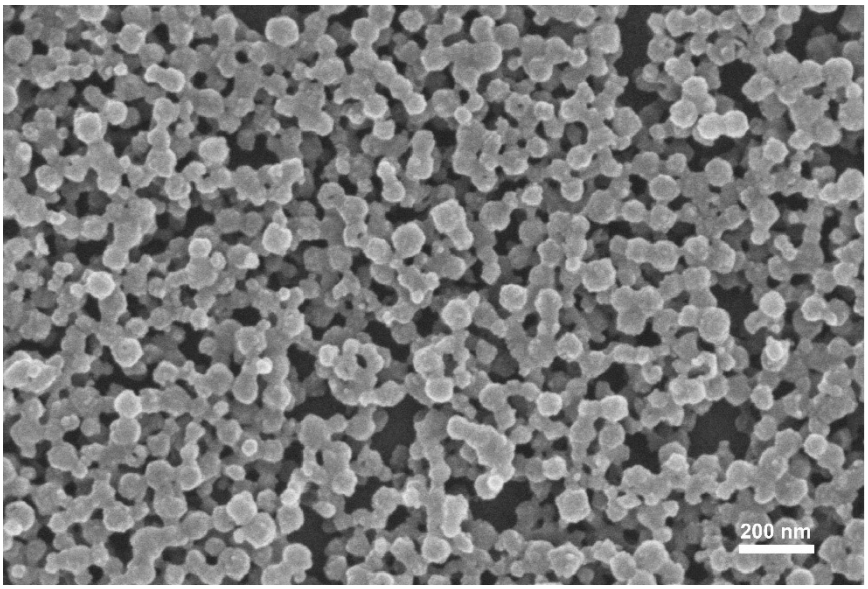

Fig. S1 SEM image of DCZ (scale bar = 200 nm).

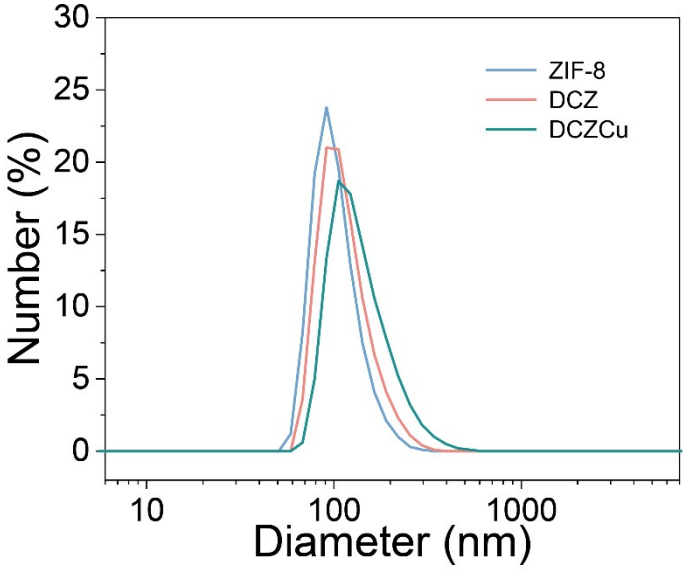

Fig. S2 DLS size distribution of ZIF-8, DCZ, and DCZCu.

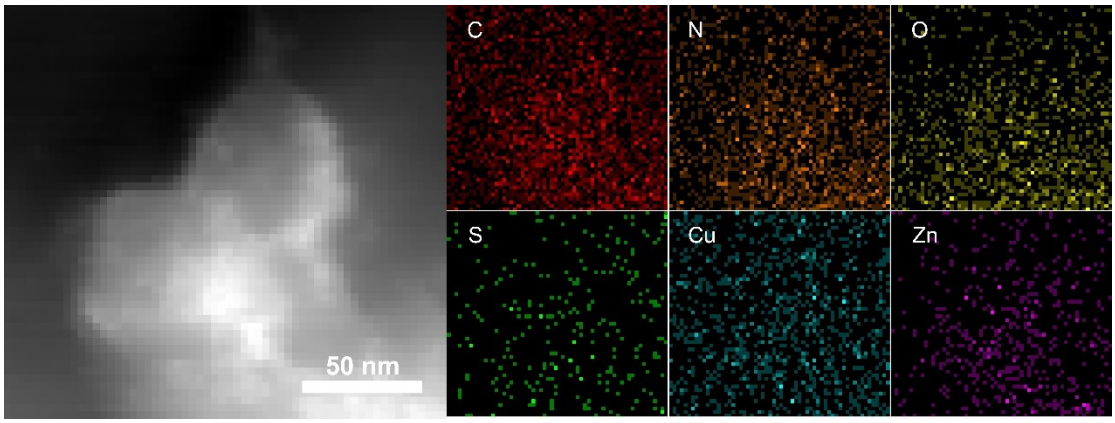

Fig. S3 Elemental mapping images of DCZCu (scale bar = 50 nm).

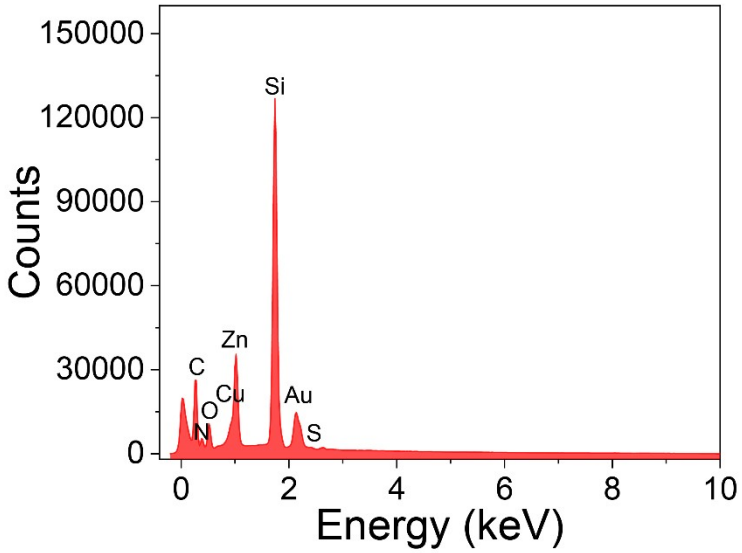

Fig. S4 EDS spectrum of DCZCu.

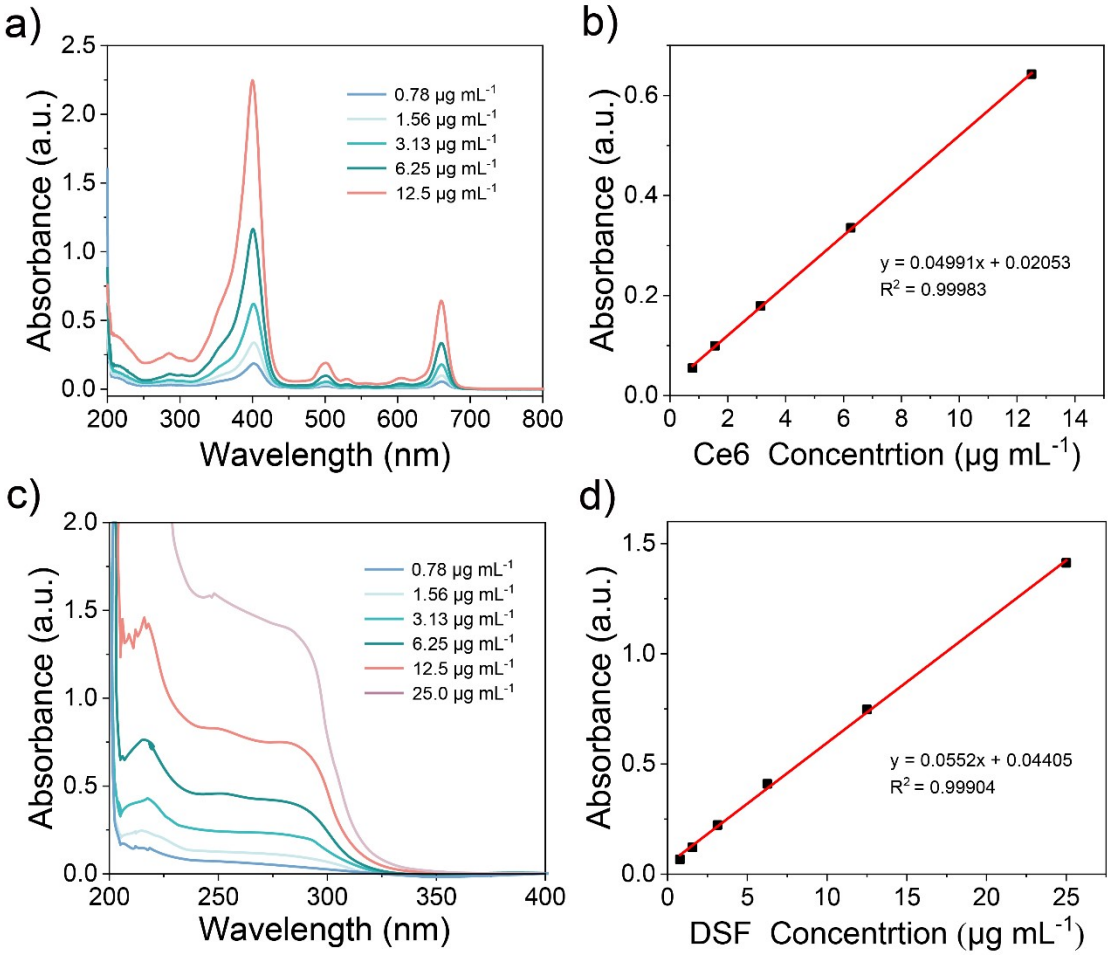

Fig. S5 (a) UV-visible absorption spectra of Ce6 at different concentrations. (b) The standard curve of Ce6 at 661 nm. (c) UV-visible absorption spectra of DSF at different concentrations. (d) The standard curve of DSF at 280 nm.

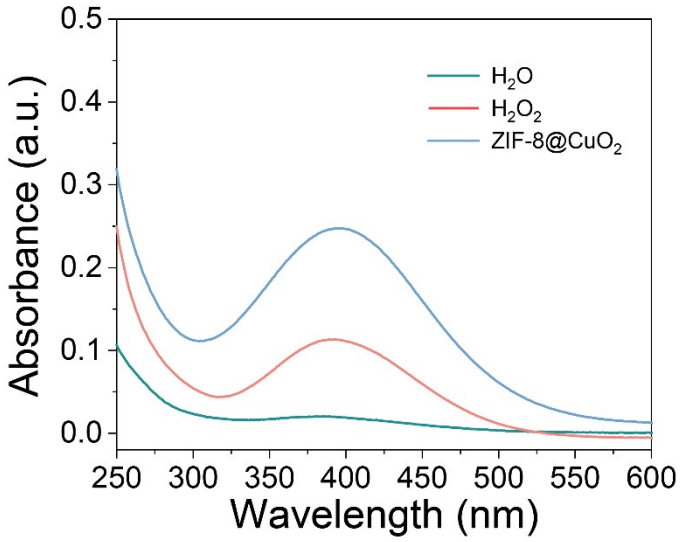

Fig. S6 UV-visible absorption spectra of $TiSO_4$ solutions after the addition of H_2O, H_2O_2 and ZIF-8@CuO_2.

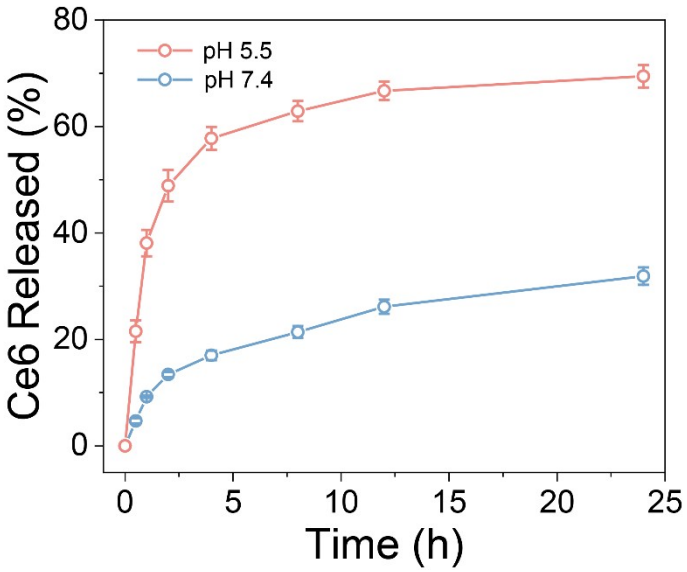

Fig. S7 Cumulative release of Ce6 at pH 7.4 and 5.5 (n = 3).

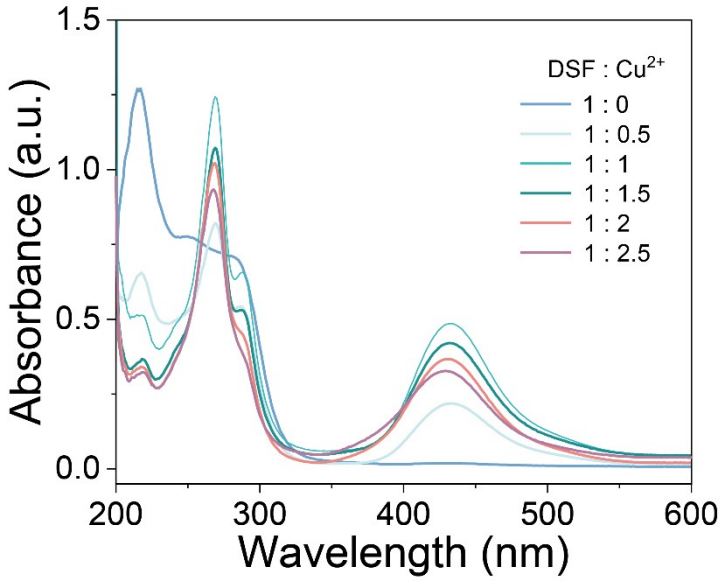

Fig. S8 UV-visible absorption spectra of the reaction between DSF and Cu^{2+}.

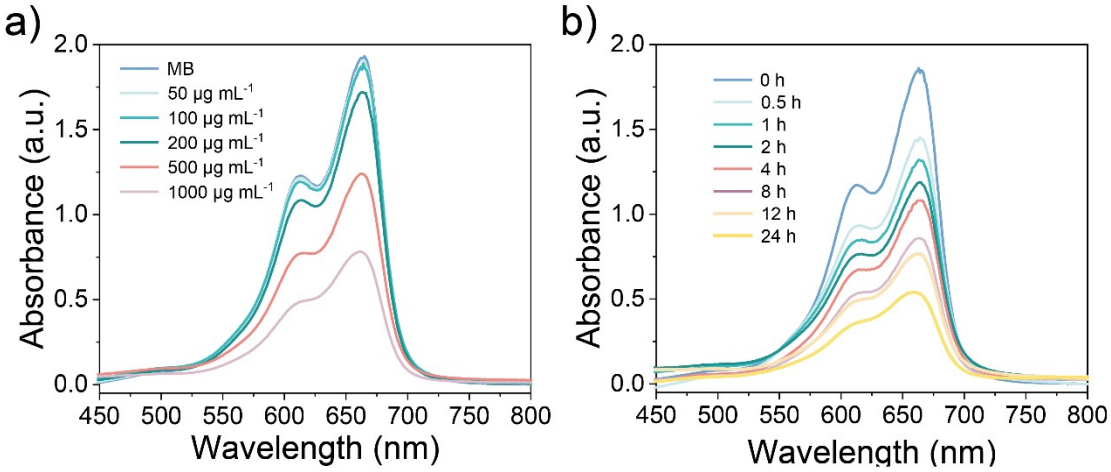

Fig. S9 (a) UV-visible absorption spectra of MB treated with different concentrations of DZCu. (b) Time-dependent UV-visible absorption spectra of MB contained DZCu.

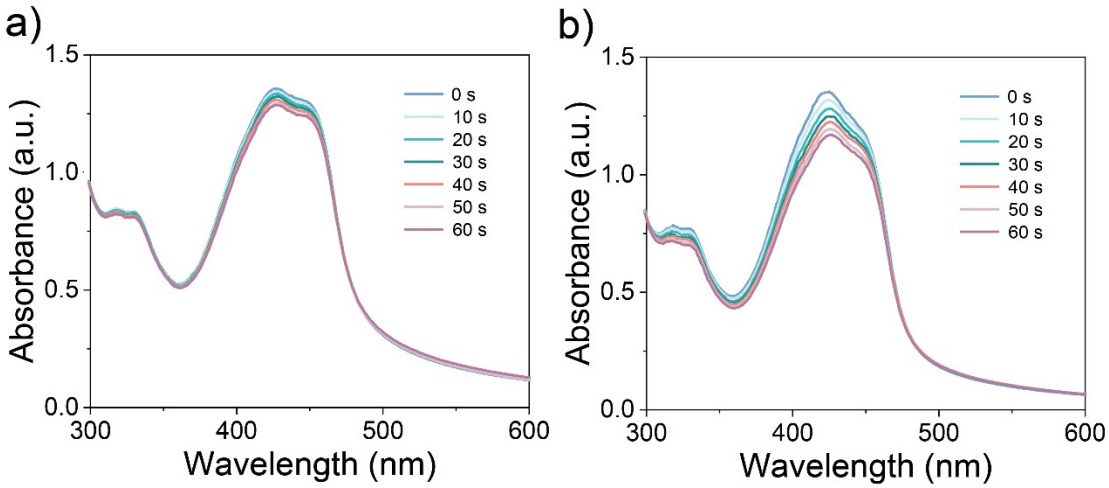

Fig. S10 Decrease in absorbance of DPBF under different treatments after 650 nm laser irradiation, as recorded every 10 s. (a)DPBF (b)DZCu. Laser irradiation was conducted at 650 nm (0.5 W cm^{-2}).

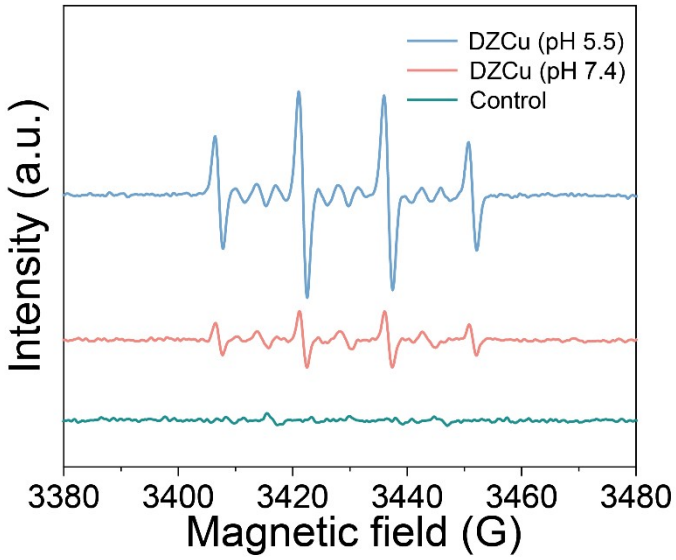

Fig. S11 ESR spectra of DZCu recorded using DMPO as the •OH-capture agent under laser at different pH (pH 7.4 and 5.5).

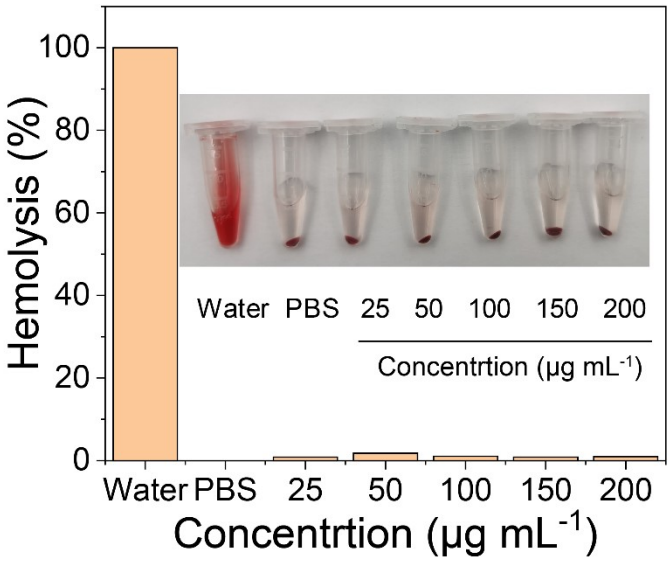

Fig. S12 Hemolysis test with different concentrations of DCZCu.

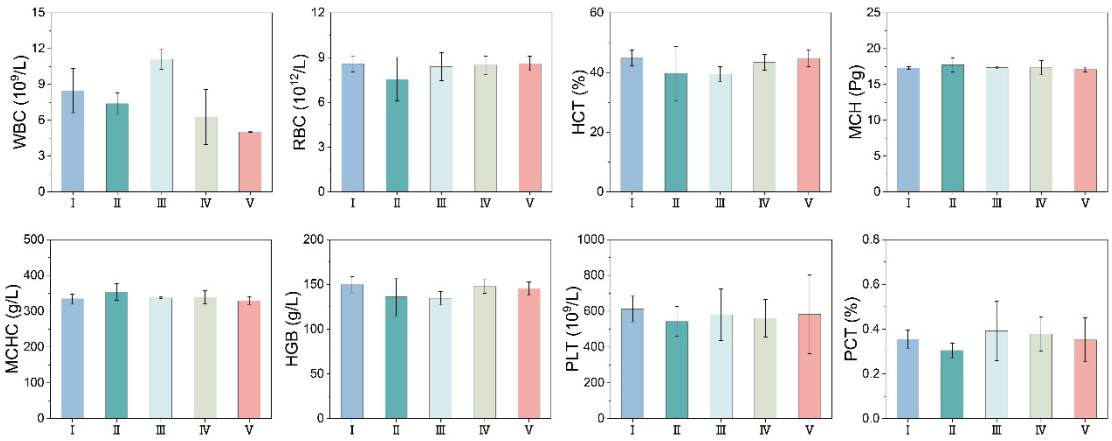

Fig. S13 Blood hematology analysis of mices after different treatments. (I) PBS; (II) Laser; (III) DCZ; (IV) DCZCu and (V) DCZCu+Laser (n = 3).

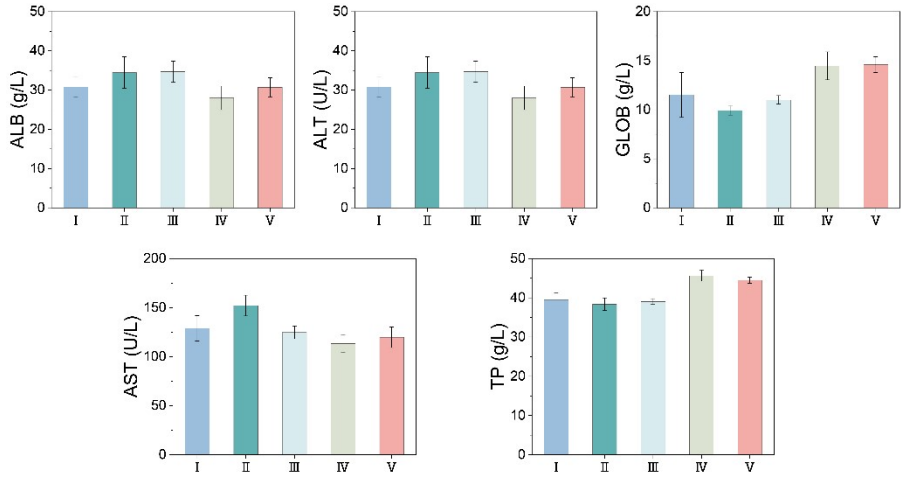

Fig. S14 The liver function indexes of mices after different treatments. (I) PBS; (II) Laser; (III) DCZ; (IV) DCZCu and (V) DCZCu+Laser (*n*=3).

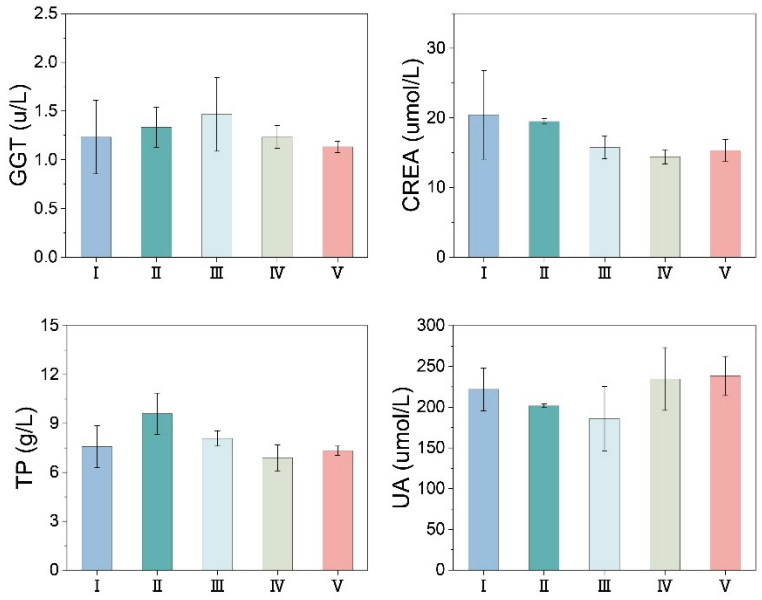

Fig. S15 The kidney function indexes of mices after different treatments. (I) PBS; (II) Laser; (III) DCZ; (IV) DCZCu and (V) DCZCu+Laser (*n*=3).

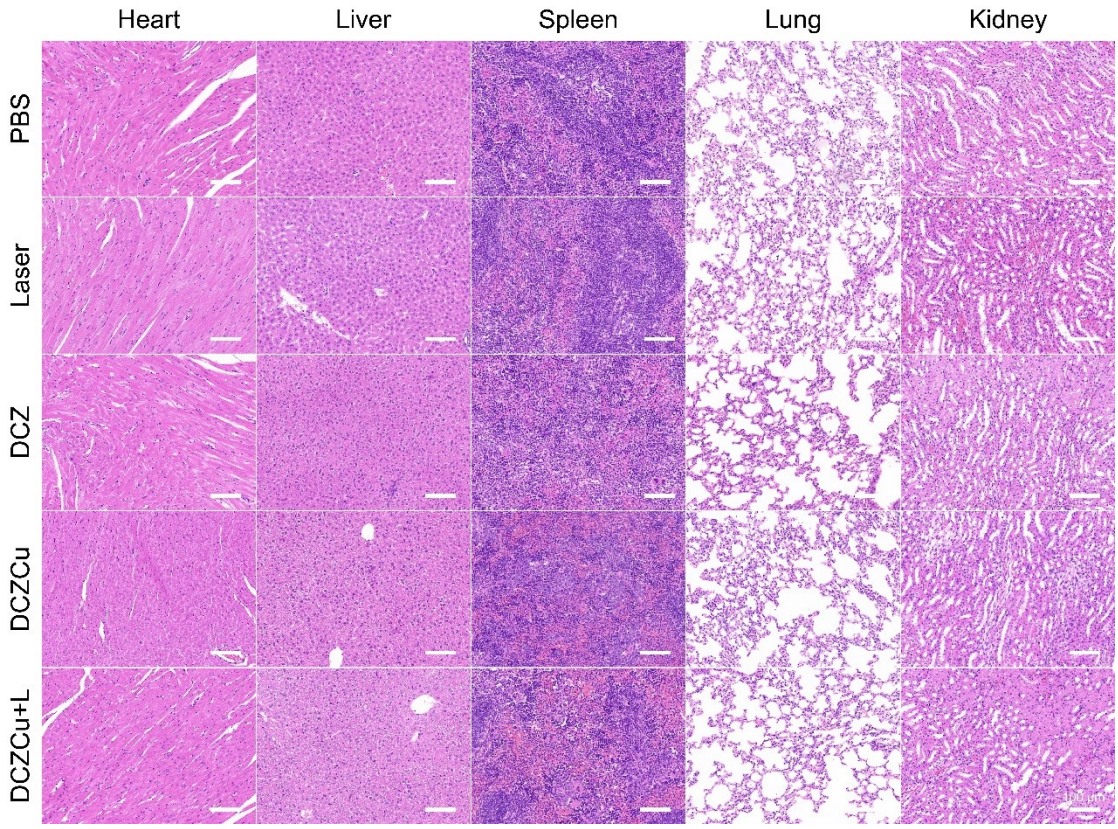

Fig. S16 H&E staining images of major organs (heart, liver, spleen, lung, and kidney) collected from 4T1 tumor-bearing mice after 14 days of treatment (scale bar = 100 μm). Laser irradiation was conducted at 650 nm (0.5 W cm^{-2}).