Supporting Information

Engineering of a double targeting nanoplatform to elevate ROS generation and DSF anticancer activity

Wenqiu Li[1], Haowu Huang[1], Shunyu Yao[1], Yiwang Zhao[1], Mingxing Liu[1], Xiaoqing Liu[2], Huiling Guo[1]*

[1] Key Laboratory of Fermentation Engineering (Ministry of Education), Key Laboratory of Industrial Microbiology in Hubei, National "111" Center for Cellular Regulation and Molecular Pharmaceutics, Cooperative Innovation Center of Industrial Fermentation (Ministry of Education & Hubei Province), School of Bioengineering and Food, Hubei University of Technology, Wuhan 430068, China,

[2] Center for Materials Research and Analysis Wuhan University of Technology, Wuhan 430070, PR China

*Corresponding author: guoguo0302@126.com

Contents

Materials

Copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), disulfiram (DSF), methanol, dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH) and potassium permanganate ($KMnO_4$) were purchased from Sinopdrug Group Chemical Reagents Co., LTD. Hydrogen peroxide (H_2O_2, 30%) was purchased from Tianjin Tianli Chemical Reagent Co., LTD. Polyvinylpyrrolidone (PVP, Mw=10000), titanium sulfate ($Ti(SO_4)_2$), methylene blue (MB), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), rhodamine B (RB), calcein, triphenylphosphine (TPP), agarose and α, ω-diamino polyethylene glycol (NH_2-PEG-NH_2) were purchased from Shanghai Macklin Co., LTD. Acridine orange (AO) and methyl thiazolyl tetrazolium (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluorescein diacetate (FDA), 2',7'-dichlorofluorescein diacetate (DCFH-DA), propidium iodide (PI), glutathione (GSH), 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl imidacloprid iodide (JC-1) kit, annexin V-fluorescein isothiocyanate/propidium iodide (Annexin V-FITC/PI) apoptosis and necrosis detection kit, and Mito-Tracker Green were purchased from Beyotime Biotechnology. Dibenzylamine hydrochloride H33342 (Hoechst 33342) was purchased from Solarbio Life Science. Hyaluronic acid (HA), 2-methylimidazole (99%), 3-aminotriazole (3-AT), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxythiosuccinimide (NHS) were purchased from Tianjin heown Reagent Co., LTD. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotics, trypsin, and all other cell culture related supplies were from Gibco (Carlsbad, CA, USA). All chemicals were commercially available and used without further purification. Ultrapure water (18.25 MΩ·cm) was used as a default solvent unless otherwise indicated.

Methods and instruments

Morphologies of samples were recorded using a transmission electron microscopy (HT7700, Hitachi, Japan) and scanning electron microscopy (JSM6390LV). The element mapping images of nanoparticles (NPs) were obtained using JEM 2100 (JEOL) transmission electron microscopy. The particle sizes and zeta potentials of samples were measured by dynamic light scattering method (DLS, Nano ZS 90, Malvern, England). Ultraviolet visible (UV-vis) absorption spectra were performed by a UV-1800 spectrophotometer (Hitachi, Japan). X-ray photoelectron spectroscopy (XPS) spectra were performed on PHI 5000 VersaProbe (UIVAC-PHI, Japan) spectrometer. Fourier transform infrared (FT-IR) spectra was recorded by an infrared spectrometer (Thermo Scientific Nicolet iS10). The nitrogen adsorption/desorption analysis was performed using the Brunner-EmmetTeller (BET) meter (Micromeritics ASAP 2020, USA). The drug loading of DSF was determined by high performance liquid chromatography (Shimadzu LC-20AD). Flow cytometric analysis was carried out on the Becton Dickinson FACSCalibur Flow Cytometer. Fluorescence microscopy of cells and tissue sections was performed with inverted fluorescence

microscopy (IX73, Olympus, Japan). Confocal laser scanning microscope (CLSM) images were taken on LEICA SP8 confocal laser scanning fluorescence microscope (LEICA Co., Ltd., Germany). An IVIS imaging system (Fusion FX7, Vilber, France) was adopted for living body fluorescence imaging.

Cell culture

Mouse fibroblasts L929 and mouse breast cancer cells (4T1) were obtained from China Center for Type Culture Collection (CCTCC) (Wuhan, China). Cells were cultured in DMEM containing fetal bovine serum (FBS, 10%), penicillin (100 $U \cdot mL^{-1}$), and streptomycin (100 $\mu g \cdot mL^{-1}$) at 37 ℃ in a 5% CO_2 atmosphere and 100% humidity.

Animals

Female BALB/c mice (7-8 weeks old, 16-20 g) were provided by the Hubei Provincial Center for Disease Control and Prevention (Wuhan, China). All animal experiments were conducted in accordance with the National Research Council's Guidelines for the Care and Use of Experimental Animals, and were approved by the Animal Ethics Committee of Hubei University of Technology (HBUT20230080).

To establish a tumor model, in vitro cultured 4T1 cells were injected into the left back leg of the mouse in a number of 1×10^6 cells in 100 μL. When the tumor volume of the mouse reached about 100 mm^3, the mouse was used for subsequent experiments.

Preparation of CuO_2 (CP)

PVP (0.5 g) was dissolved in 10 mL of ultrapure water containing $CuCl_2 \cdot 2H_2O$ (8.5 mg, 0.05 mmol). Then, NaOH (5 mL, 0.02 M) and H_2O_2 (100 μL) were added sequentially to the above mixture. After stirring for 30 min, the PVP-coated CuO_2 (CP) NPs were centrifugated for collection.

Stability of CP@MAF-DSF@PEG-TPP @HA (CPMDTH)

The size stability of CPMDTH NPs was determined by measuring the size change via DLS. In detail, the NPs were kept at room temperature and the average particle size was measured by DLS for one week.

Colorimetric assay of peroxo groups

$KMnO_4$ (50 $\mu g \cdot mL^{-1}$) was dissolved in an aqueous solution containing H_2SO_4 (1 mM). The mixture was treated with CP, CPMTH, or H_2O_2 for 10 min. Subsequently, the UV-vis spectra were measured from 400 to 650 nm by spectrophotometer.

Tumor penetration study via 3D tumor spheroids

3D tumor spheroid model of 4T1 cells (about 200 μm) was established in vitro. Tumor spheroids were incubated with RB, CPMR, CPMRH, CPMRT, and CPMRTH for 6 h respectively, and the penetration of RB fluorescence at different depths of tumor spheroids was observed with a confocal laser scanning microscope (CLSM).

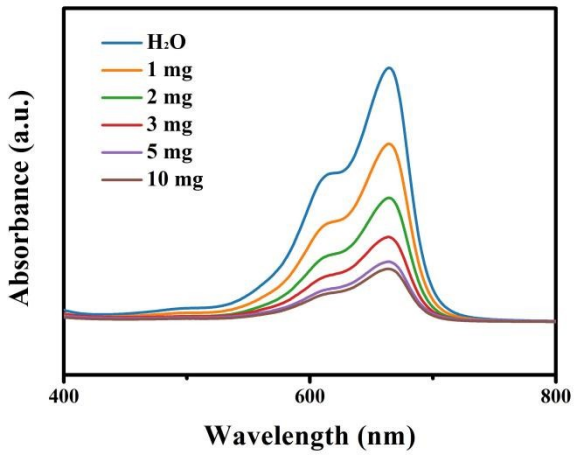

Fig. S1 Production of •OH in the presence of 3-AT of various amounts.

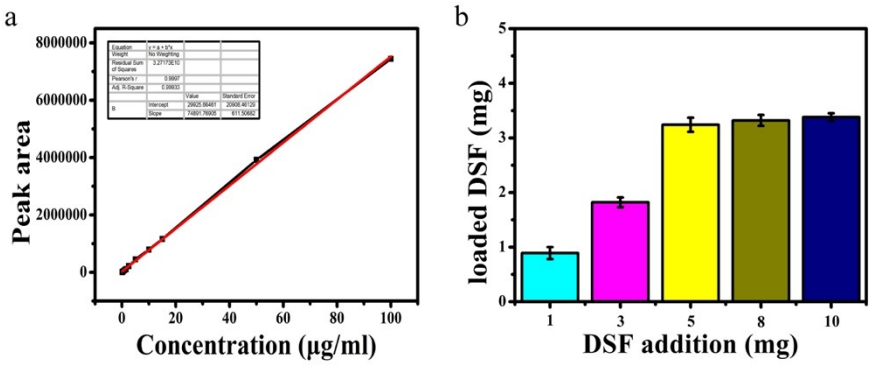

Fig. S2 Standard curve of DSF established by HPLC (a) and drug loading corresponding to different DSF addition (b).

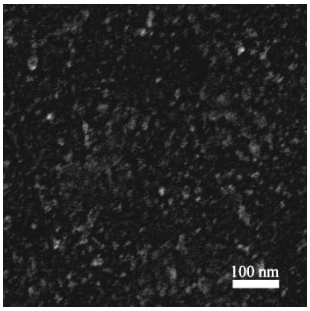

Fig. S3 SEM image of CP.

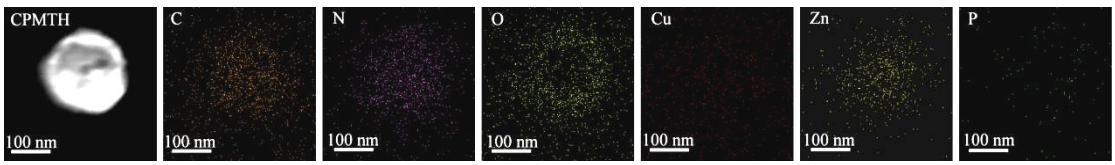

Fig. S4 TEM elemental mapping images of CPMTH.

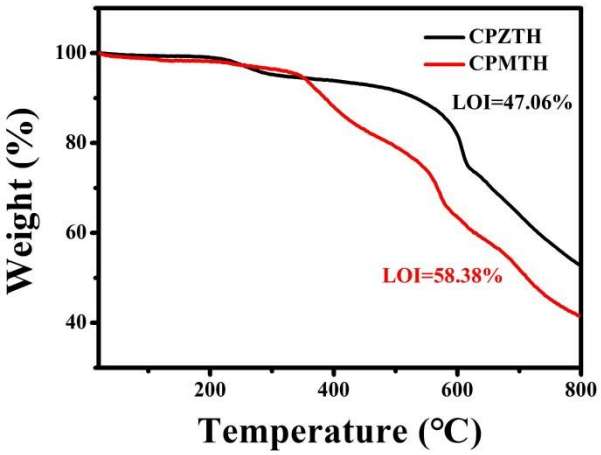

Fig. S5 TGA curves of various formulations.

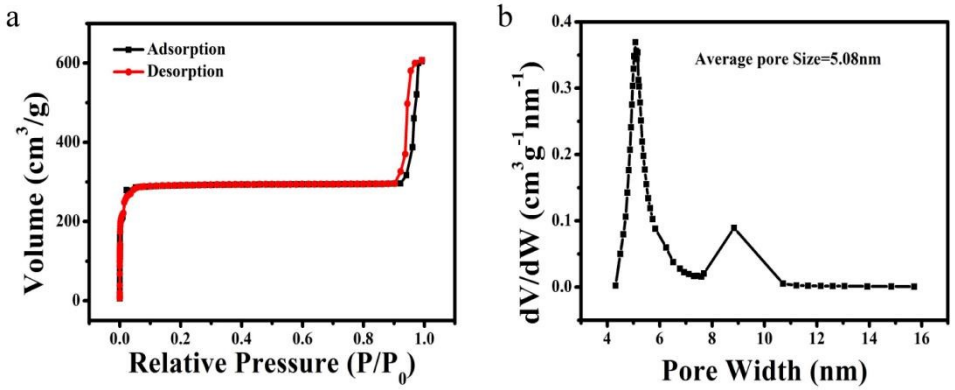

Fig. S6 Nitrogen adsorption-desorption isotherm (a) and pore size distribution (b) of CPM.

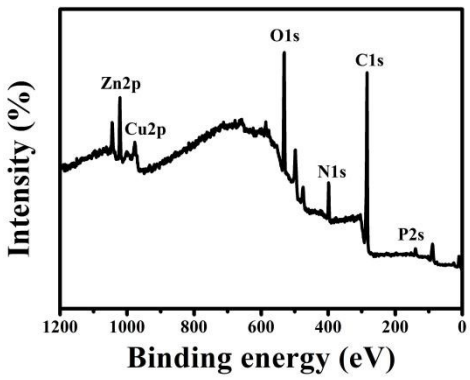

Fig. S7 XPS spectrum of CPMDTH.

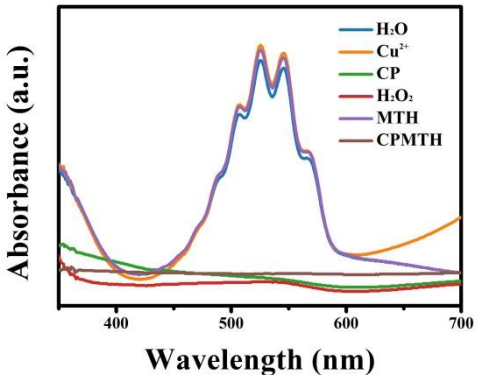

Fig. S8 Colorimetric assay of peroxo groups in CPMTH**.**

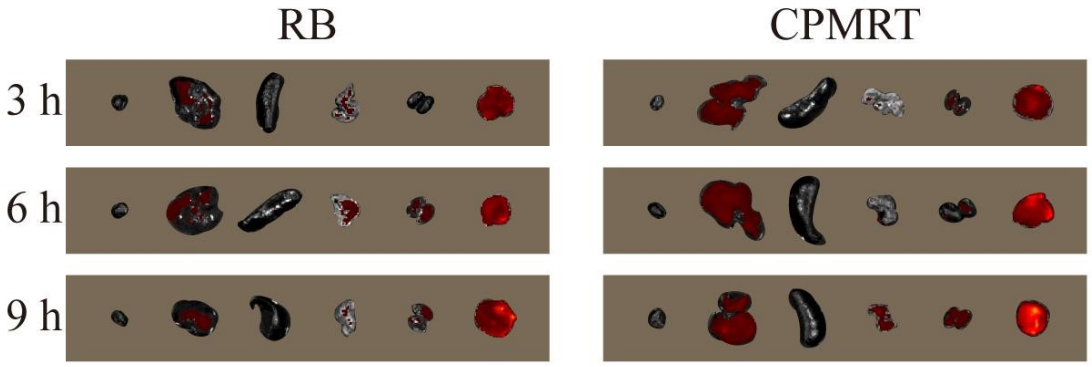

Fig. S9 The *ex vivo* fluorescence imaging of the major organs and tumors at 3, 6, and 9 h post-injection of free RB and CPMT.

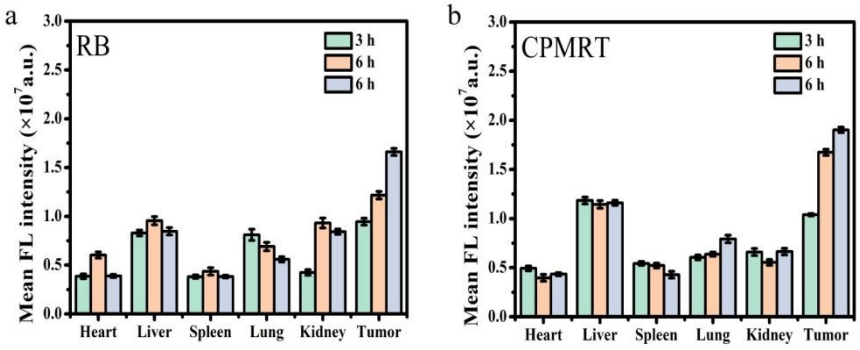

Fig. S10 The mean fluorescence intensity values of the major organs and tumors of free RB (a)and CPMRT(b).

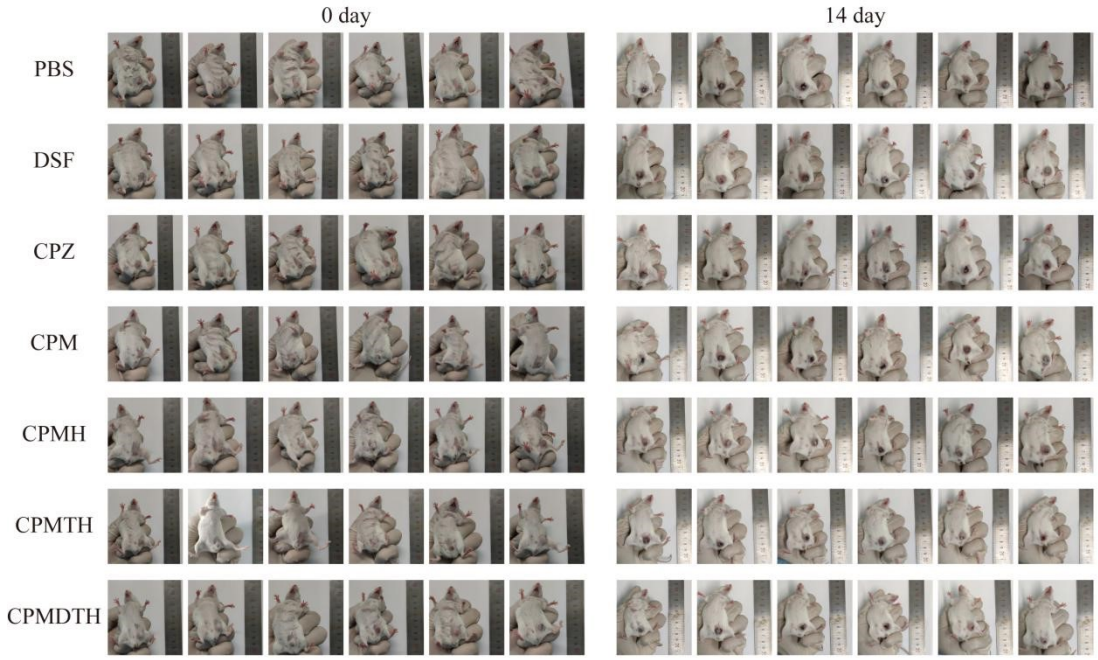

Fig. S11 Digital photographs of 4T1 tumor-bearing BALB/c mice (n =6) on day 0 and 14.