Supporting Information for

A Pt nanoenzyme- and BODIPY-loaded nanoscale covalent organic framework for relieving intratumoural hypoxia to enhance photodynamic therapy

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1. Materials and Instrumentations

1,3,5-Tris(4-aminophenyl)benzene, 2,5-bis(2-propyn-1-yloxy)terephthalaldehyde and 10-(4-chlorophenyl)-5,5-difluoro-2-formyl-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-c:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide (BODIPY) were purchased from Jilin Province Yanshen Technology Co., Ltd. H_2PtCl_6 (8 wt.% in H_2O) was purchased from Sahn Chemical Technology (Shanghai) Co., Ltd. 1,3-Diphenylisobenzofuran (DPBF) was purchased from TCI (Shanghai) Development Co., Ltd. Benzaldehyde was purchased from Aladdin Reagent Co., Ltd.All reactants were used as purchased without further purification. Acetonitrile, acetic acid, copper acetate monohydrate, thioacetamide, and ethylenediamine were purchased from Sinopharm Chemical Reagent Co., Ltd. Ultra-pure water was prepared with an Aquapro System (18 MΩ). CCK-8 assay kit was purchased from Dojindo (Shanghai, P.R. China). Phosphate-Buffered Saline (PBS), Dulbecco's Phosphate-Buffered Saline (DPBS), and Fetal bovine serum (FBS) were purchased from VivaCell (Shanghai, P. R. China). Dulbecco's Modified Eagle Medium (DMEM), Penicillin Streptomycin Mixtures (Pen-Strep), and Trypsin-EDTA Solution (0.25%) were purchased from HyClone Laboratories, Inc. Normocin was purchased from Invivogen (San Diego, CA, USA).

Fourier transform infrared (FT-IR) spectra were obtained in the 400-4000 cm^{-1} range using a Thermo Scientific Nicolet iS50 FT-IR Spectrometer equipped with a diamond attenuated total reflection (ATR) module. Each spectrum was an average of 16 scans. Ultraviolet-visible (UV−vis) absorption spectra were recorded on a Shimadzu UV-2700 Double Beam UV−vis Spectrophotometer. Scanning electron microscopy (SEM) micrographs were recorded on a Hitachi SU8010 Scanning Electron Microscope. Transmission electron microscope (TEM) micrographs were recorded on a Hitachi HT7700 120 kV Compact-Digital Transmission Electron Microscope. Powder X-ray diffraction (PXRD) patterns were obtained on a Rigaku SmartLab SE X-Ray Powder Diffractometer with Cu Kα line focused radiation (λ = 1.5405 Å) from 2θ = 2.00° up to 50.00° with 0.01° increment. Nitrogen adsorption isotherms were measured at 77 K with a Micromeritics ASAP2020 HD88 Surface Area and Porosity Analyser. Before measurement, the samples were degassed in a vacuum at 120°C for 12 h. The Brunauer–Emmett–Teller (BET) equation was utilized to calculate the specific surface areas. The pore size distribution was derived from the sorption curve by using the non-local density functional theory (NLDFT) model. Hydrodynamic particle size and zeta potential were measured using Malvern Zetasizer Nano ZS90 System. Laser scanning confocal fluorescence images were captured with a Leica TCS SP8 Confocal Laser Scanning Microscopy with an objective lens (×20). Microplate assays were carried out on a Molecular Devices SpectraMax i3x Multi-Mode Microplate Detection System. Laser scanning confocal fluorescence images of cells were captured with a Leica TCS SP8 Confocal Laser Scanning Microscopy equipped with 405, 458,

488, 514, 561, and 633 nm lasers. Glass bottom dishes and 4-well chamber slides (Cellvis, Mountain View, CA, USA) were used for cell culture to provide biological replicates of each experiment.

2. Cell Culture and Laboratory Animals

The MCF-7 (human breast adenocarcinoma cell line) was provided by Stem Cell Bank, Chinese Academy of Sciences (Shanghai, P. R. China), and cultured in DMEM supplemented with FBS (10%), Normocin (50 μg/mL), penicillin (100 U/mL) and streptomycin (100 μg/mL) in an atmosphere of CO_2 (5 vol%) and air (95 vol%) at 37°C.

Nude mice (BALB/c-nu, femina, aged 4 weeks, 15–20 g) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. Animal experiments were reviewed and approved by the Ethics Committee of Shandong Normal University, Jinan, P. R. China (approval number AEECSDNU 2022050). All the animal experiments complied with relevant guidelines of the Chinese government and regulations for the care and use of experimental animals.

3. Synthesis of materials

3.1 Synthesis of COF-Alkynyl (1)

1,3,5-Tris(4-aminophenyl)benzene (23.89 mg, 0.068 mmol), 2,5-bis(2-propyn-1-yloxy)terephthalaldehyde (24.70 mg, 0.102 mmol) were dissolved in acetonitrile (25 mL) and glacial acetic acid (2.7 mL) was added and stirred for 12 h at room temperature. The product was centrifuged and washed 3 times with acetonitrile, and washed with ethanol for 3 times. Finally, the solids were dried in air at 40°C. FT-IR (ATR, cm^{-1}): 3290 (m), 2971 (w), 1615 (m), 1592 (s), 1505 (s), 1485 (m), 1444 (w), 1423 (s), 1373 (w), 1290 (m), 1194 (s), 1148 (m), 1114 (w), 1030 (s), 916 (w), 878 (w), 830 (s), 669 (w), 632 (w), 538 (w).

3.2 Synthesis of Pt@COF (2)

COF-Alkynyl (**1**) (10 mg) was dispersed in H_2O (10 mL), and chloroplatinic acid (8 wt.% in H_2O, 50 μL) was added while stirring. Triethylamine (38 μL) was added to adjust the pH to neutral and stirred for 30 min while passing nitrogen gas. The mixture was irradiated with simulated sunlight under stirring conditions for 2 h. It was centrifuged, washed three times and dried to give **Pt@COF** (**2**) as a black-green powder. FT-IR (ATR, cm^{-1}): 3296 (m), 2971 (m), 1592 (s), 1507 (s), 1424 (m), 1393 (w), 1157 (w), 1117 (w), 1046 (w), 1013 (w), 880 (w), 829 (s), 736 (w), 536 (w).

3.3 Synthesis of Pt@COF -BDP (3)

A mixture of **2** (5 mg, 0.02 mmol), BODIPY (7.5 mg, 0.02 mmol) and glacial acetic acid (50 μL, 3 M) in 5 mL ethanol was stirred at 75°C for 24 h. The resulting solids were completely

washed with ethanol to generate **Pt@COF -BDP** (**3**) as brick red solids. ICP-OES analysis indicated that the loading amounts of Pt in **Pt@COF -BDP** (**3**) was 10.1 wt%. The standard curve method analysis indicated that the loading amounts of BODIPY in **Pt@COF -BDP** (**3**) was 8.6 wt%. FT-IR (ATR, cm^{-1}): 3289 (m), 2973(s), 2928 (w), 2867 (w), 1613 (m), 1592 (s), 1507 (s), 1444 (m), 1425 (m), 1381 (m), 1292 (w), 1195 (m), 1152 (m), 1116 (s), 1037 (s), 880 (w), 830 (s).

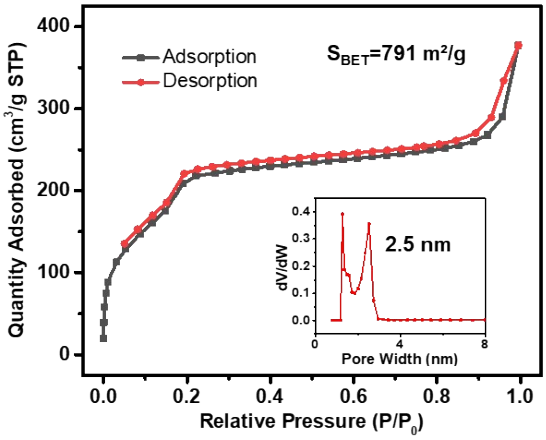

Fig. S1 N_2 adsorption isotherms and pore widths of **1**.

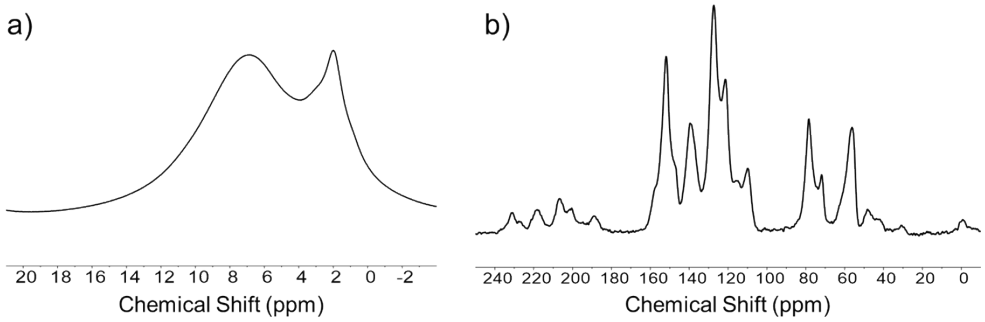

Fig. S2 ^{1}H (a) and ^{13}C (b) cross-polarization−magic angle spinning nuclear magnetic resonance (CP-MAS NMR) spectra of **1**. (The peaks at 157 ppm unambiguously identified the presence of the carbon atoms in the C=N group.)

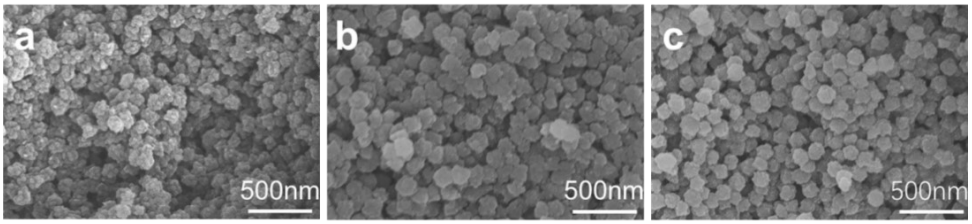

Fig. S3 SEM images of **1**, **2,** and **3**.

3.4 Synthesis of COF-BDP

A mixture of **COF-Alkynyl** (4.9 mg, 0.02 mmol), BODIPY (7.5 mg, 0.02 mmol) and glacial acetic acid (50 μL, 3 M) in 5 mL ethanol was stirred at 75°C for 24 h. The resulting solids were completely washed with ethanol to generate **COF-BDP** as brick red solids. The ion chromatography analysis indicated that the loading amounts of BODIPY in **COF-BDP** was 9.50 wt%. FT-IR (ATR, cm^{-1}): 3265 (w), 2951 (w), 2821 (w), 1617 (m), 1514 (s), 1351 (m), 1284 (m), 1203 (w), 1142 (m), 1051 (m), 972 (w), 830 (m).

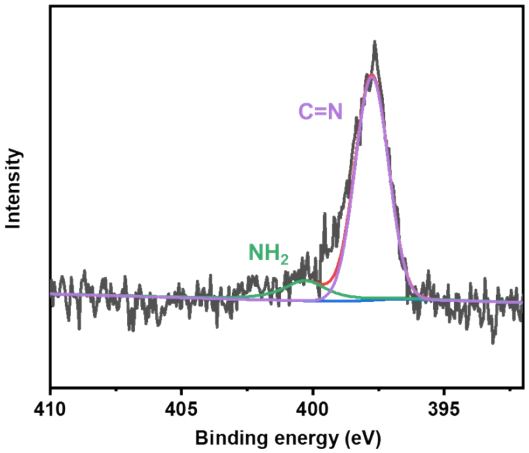

Fig. S4 High-resolution X-ray photoelectron spectroscopy data of **1** in the N 1s region.

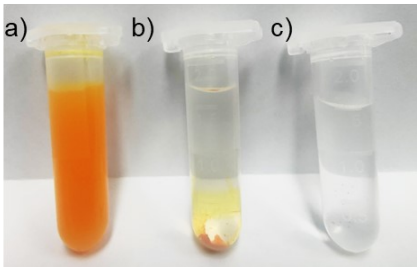

Fig. S5 The leaching test of **3** by immersion in acetonitrile solution for 8 hours (a: suspension, b: centrifugation, c: supernatant).

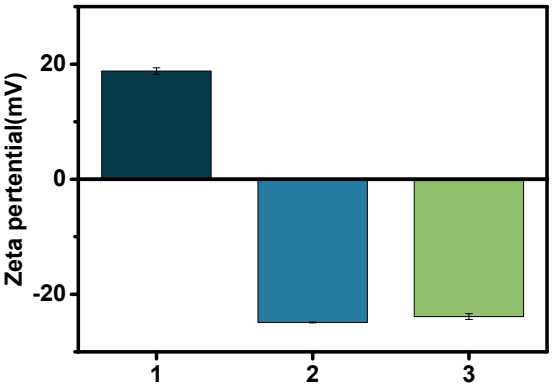

Fig. S6 Zeta potentials of **1**, **2** and **3**.

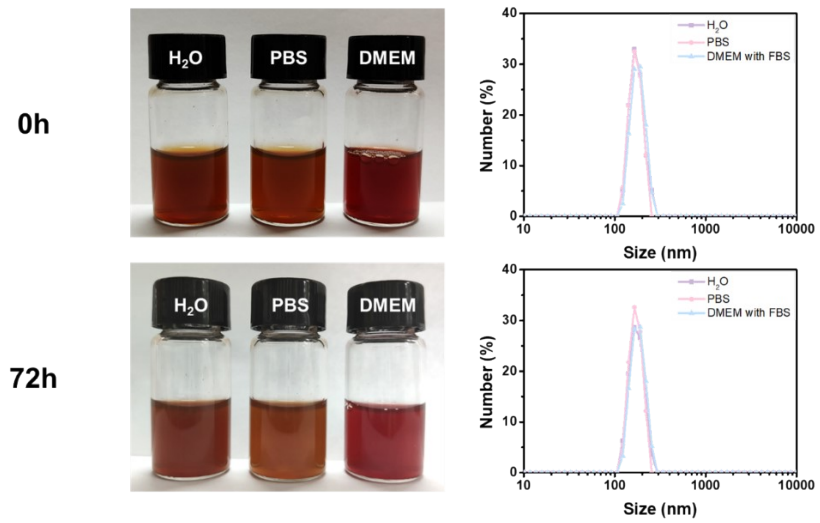

Fig. S7 The stability of **3** in different physiological solutions of H_2O, PBS, and DMEM with 10% fetal bovine serum.

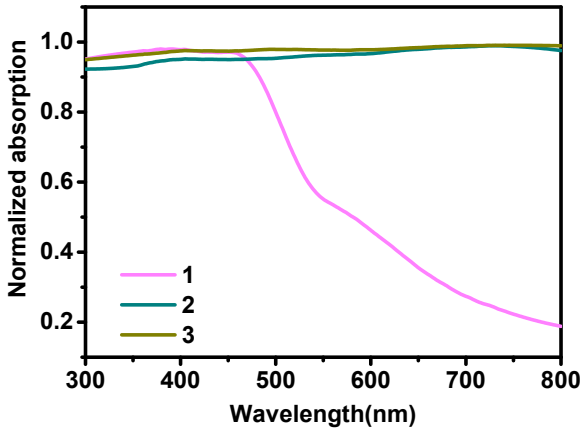

Fig. S8 Diffuse reflectance spectrums of **1**, **2**, and **3**.

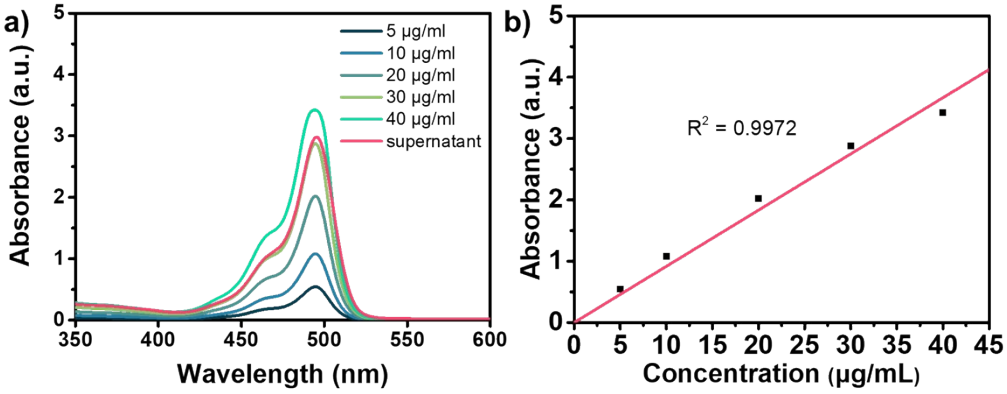

Fig. S9 (a) UV−vis spectra of BODIPY in ethanol. (b) Standard curve of BODIPY in ethanol.

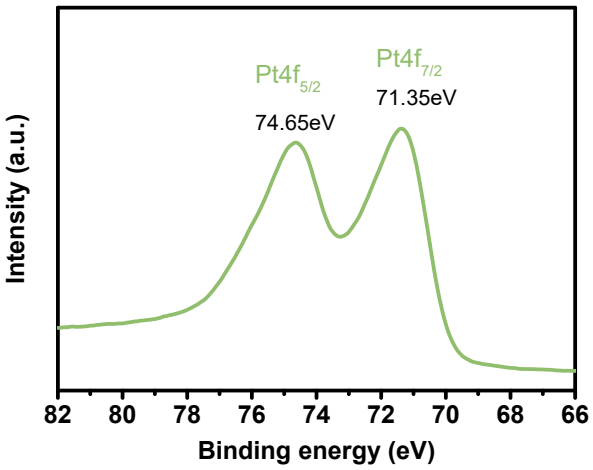

Fig. S10 High-resolution X-ray photoelectron spectroscopy data of **3** in the Pt 4f region.

4. Photodynamic Property

The dispersion of **1** (2 mL, 50 μg/mL), **2** (2 mL, 50 μg/mL), **3** (2 mL, 4.3 μg/mL for BODIPY), or **COF-BDP** (2 mL, 4.3 μg/mL for BODIPY) and DPBF DMF solution (200 μL, 1 mM) were added to a quartz dish and irradiated with a 520 nm laser (50 mW/cm^2) 8 min. The absorbance of DPBF at 414 nm in the mixture was recorded at 1 min intervals. The $^1O_2$ generation rate was determined from the reduced absorbance over time. To characterize the difference in the rate of $^1O_2$ introduced by different lasers, the absorbance of DPBF at 414 nm was calculated. The dispersion of **1**, **2**, **3**, or **COF-BDP** was used as the reference for this UV-vis measurement.

5. 3 Catalytic H_2O_2 decomposition

Mixed H_2O_2 (8 mM) and **3** (20 μg/mL) in PBS at 37 °C with shaking (solution A). Dissolved $Ti(SO_4)_2$ (50 mg) in H_2SO_4 (8.33 ml) and further diluted to a volume of 50 ml with deionised water (Solution B). Every 30 minutes, 1 mL of Solution A was centrifuged and 0.35 mL of supernatant was mixed with 2.65 mL of Solution B and its UV-vis absorbance at 405 nm was measured.

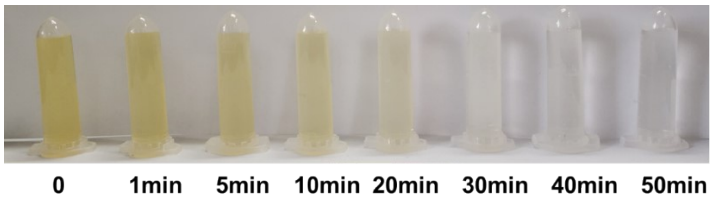

Fig. S11 Picture of the colour change over time of a $Ti(SO_4)_2$ solution treated with **3** at different times.

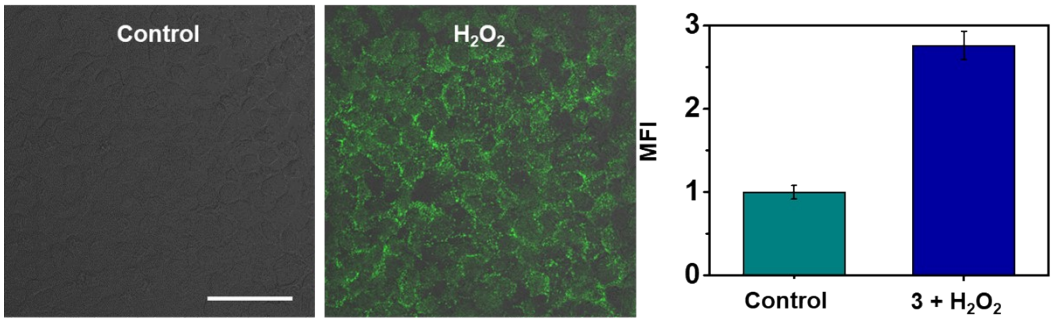

Fig. S12 Laser confocal microscopy for imaging intracellular H_2O_2 content in MCF-7.

6. 3 Catalytic O_2 generation from H_2O_2

Aqueous solutions of different concentrations of **3** (0, 10, 25, 50, 100 μg/mL) were prepared, sonicated and dispersed in water containing 3 mM of H_2O_2 and the oxygen content was measured over time by a Portable DO Meters

7. Cell uptake and subcellular localization

Cells were seeded into glass-bottom dishes and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **3** (500 μL, 100 μg/mL) for 2 h in a CO_2 incubator, and washed with DPBS twice carefully. After additional 4 h incubation, cells were incubated with Hoechst 33258 (200 μL, 10 μM) for an additional 10 min, and washed with DPBS twice. Finally, the laser scanning confocal fluorescence images were captured. The green images of **3** were excited by 488 nm light, and the emission wavelength range was collected at 525±20 nm. The blue images of the nucleus were excited by 405 nm light, and the emission wavelength range was collected at 460±20 nm. Controls were conducted to make sure images were free of crosstalk.

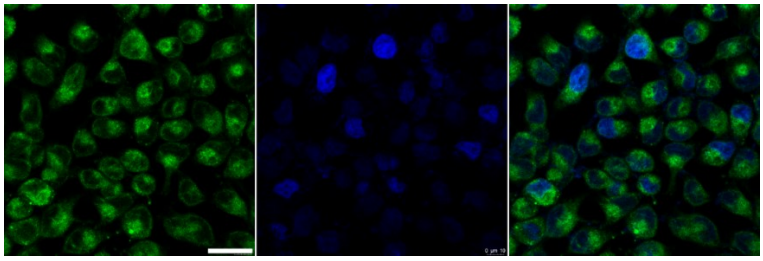

Fig. S13 Cell uptake and nuclei subcellular localization of **3** in MCF-7 cells. Scale bar = 20μm.

8. *In Vitro* Antitumor Therapy

We first evaluated the dark toxicity of high concentrations of **3**, **3** + H_2O_2 (100μM), and **COF-BDP**. Cells were seeded into 96-well plates with a cell number of ~5k cells/well and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **3** or **COF-BDP** (100 μL, 0-500 μg/mL) for 2 h in a CO_2 incubator, for **3**+H_2O_2, after incubation with **3** for 2h, remove **3** and continue incubating for 1 h with 100 μM of H_2O_2. After additional 24 h incubation, CCK-8 (10 μL) was added to each

well and incubated for an additional 4 h in a CO_2 incubator. Finally, followed by recording the absorbance at 450 nm.

Then, the phototoxicity of low concentrations of **3** and **COF-BDP** with or without H_2O_2 (100μM) was evaluated. The cells were incubated with DPBS dispersion of **3** and **COF-BDP** (100 μL, 0–400 μg/mL) for 2 h in a CO_2 incubator. For **3** + H_2O_2 (100μM) and **COF-BDP**+ H_2O_2 (100μM), after incubation with **3** or **COF-BDP** for 2h, remove **3** or **COF-BDP** and continue incubating for 1h with 100 μM of H_2O_2. Then cells were exposed to a 520 nm laser (25 mW/cm^2, 10 min). After additional 24 h incubation, CCK-8 (10 μL, 5 mg/mL) was added to each well and incubated for an additional 4 h in a CO_2 incubator. Finally, followed by recording the absorbance at 450 nm.

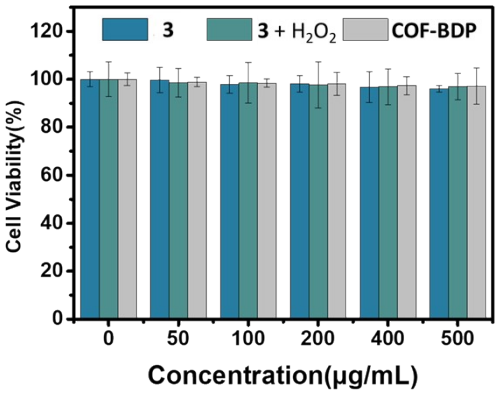

Fig. S14 CCK-8 assays of MCF-7 cancer cells incubated with different concentrations of **3**, **3** + H_2O_2 (100 μM), and **COF-BDP**.

9. Hemolysis analysis

First, fresh nude mouse blood samples (2 mL) were added to PBS solution (4 mL), and red blood cells (RBC) were separated by centrifugation at 3000 rpm for 10 minutes. After washing 5 times with 10 mL PBS solution, the purified red blood cells were diluted to the original solution with PBS (10 times). For hemolysis assay, 0.2 mL diluted RBCs suspension was mixed with 0.8 mL PBS as a negative control, 0.8 mL deionized water as a positive control, and 0.8 mL **3** suspension at a concentration range of 0 to 500 μg/mL. All mixtures were then allowed to stand at 37 °C for 5 h and centrifuged at 13300 rpm for 10 minutes. The absorbance of 541 nm supernatant was measured by a synergy HT multi-mode microplate reader. The hemolytic percentage of red blood cells was calculated by the following formula.

Hemolysis Rate = [(Dt – Dnc) / (Dpc – Dnc)] ×100%

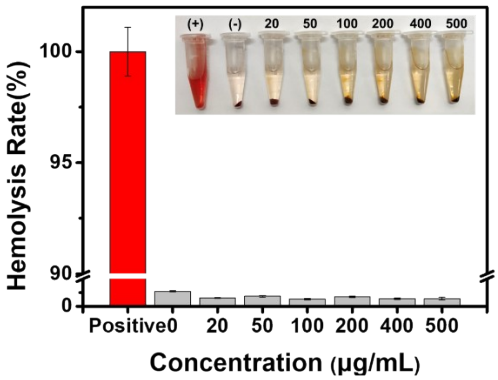

Fig. S15 Hemolytic assay using red blood cells incubated with control solvents and different concentrations of **3**.

10. *In Vivo* Antitumor Therapy

MCF-7 cancer cells (10^6 cells) suspended in DPBS (100 μL) were subcutaneously injected into the flanks of each mouse to establish the MCF-7 xenograft model. The length (L) and width (W) of the tumor were determined by a digital calliper. The tumor volume (V) was calculated by the formula $V = 1/2 \times L \times W^2$. When the tumor size reached ~100 mm^3, the nude mice bearing MCF-7 tumours (n = 20) were randomly distributed into 4 groups, were the control group, **3**, **COF-BDP** + light, **3** +light. After intratumoral injection PBS (100 μL), **2** or **3** (50 μL, 1 mg mL^{-1}), the nude mice were fed for 4 h, and for the treatment group, light treatment (520 nm laser, 100 mW cm^{-2}, 6 min) was performed on the tumor site. The mice continued to be fed for 14 days. The tumor volume and nude mouse body weight were recorded daily during the experimental period.

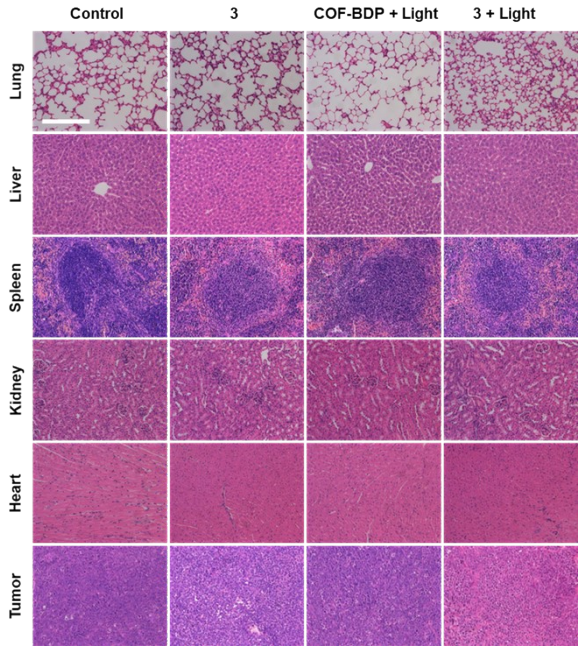

Fig. S16 H&E-stained tissue sections from the heart, liver, spleen, lung, and kidney of the nude mice at the end of the treatment. Scale bar, 100 μm.