Supporting Information for

Metal–organic cage-based nanoagent for enhanced photodynamic antitumor therapy

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1. Instruments and materials

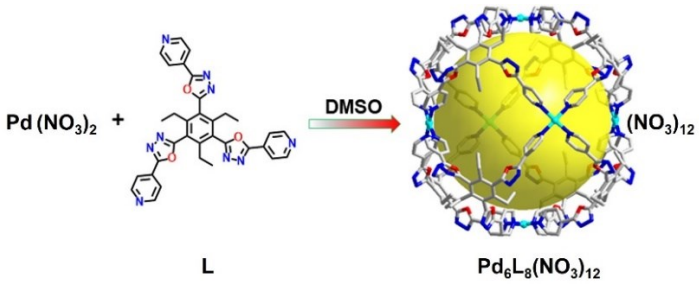
All the reactants were obtained from commercial sources and used without further purification. All organic solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. Ultra-pure water was prepared with an Aquapro System (18 MΩ). Phosphate-Buffered Saline (PBS), Dulbecco's Phosphate-Buffered Saline (DPBS), and Fetal Bovine Serum (FBS) were purchased from Biological Industries USA, Inc. 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). LysoTracker Green DND-99, MitoTracker Green FM, Cell Counting Kit-8 (CCK-8) were purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd. Singlet Oxygen Sensor Green (SOSG), Trypan Blue Solution and JC-1 were purchased from Thermo Fisher Scientific Inc. Acridine orange (AO) and formalin fixative were purchased from Beijing Solarbio Science & Technology Co., Ltd. Liquid-state ^{1}H nuclear magnetic resonance (NMR) spectra were recorded using a Bruker AVANCE III HD 400 MHz NMR Spectrometer. Chemical shifts were reported as δ values relative to tetramethylsilane (TMS) as internal reference. Ultraviolet–visible absorption spectra were recorded on a Shimadzu UV-2700 Double Beam UV-Vis Spectrophotometer. Transmission electron microscope (TEM) micrographs were recorded on a Hitachi HT7700 120kV Compact-Digital Transmission Electron Microscope. Atomic force microscopy (AFM) was conducted on Cypher VRS AFM (Oxford Instruments). Infrared (IR) spectrums were obtained in the 400-4000 cm^{-1} range using a Bruker ALPHA FT-IR Spectrometer. Elemental analyses were performed on a Perkin-Elmer model 2400 analyzer. MS spectra were obtained by Bruker maxis ultra-high resolution-TOF MS system. 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, ×10). Microplate assays were carried out on a Molecular Devices SpectraMax i3x Multi-Mode Microplate Detection System. The 808 nm laser (FC-808-2000-MM) was purchased from Shanghai Xilong Optoelectronics Technology Co., Ltd. In vivo and ex vivo fluorescence images of mice were captured with a PerkinElmer IVIS Spectrum In Vivo Imaging System. Thermal images of mice were captured with a FOTRIC 280 thermal imaging system.

The samples used for in vitro/in vivo studies were prepared by diluting the corresponding aqueous dispersions (1mg/mL) of **2-4** to the desired concentrations with H_2O, PBS, or DMEM.

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

Animal experiments were reviewed and approved by the Ethics Committee of Shandong Normal University, Jinan, P. R. China (approval number AEECSDNU 2021009). All the animal experiments complied with relevant guidelines of the Chinese government and regulations for the care and use of experimental animals.

2. Synthesis and characterization of 1



Scheme 1. Synthesis of $Pd_6L_8(NO_3)_{12}$ cage.

Synthesis of 1. The preparation of 1,3,5-tris(4'-pyridyloxadiazole)-2,4,6-triethylbenzene (L) is according to our previous report.[1] ^{1}H NMR (400 MHz, DMSO-d_6, 25°C, TMS): 8.89 (d, J = 7.9 Hz, 6H, C_5NH_4), 8.05 (d, J = 7.9 Hz, 6H, C_5NH_4), 2.42 (q, J = 7.4 Hz, 6H, C_2H_5), 1.11 (t, J = 7.4 Hz, 9H, C_2H_5). ^{13}C NMR (101 MHz, DMSO-d_6, 25°C, TMS): 164.26, 162.26, 151.52, 150.58, 130.77, 123.71, 120.93, 26.08, 16.41.IR (KBr pellet) [(cm^{-1})]: 3044 (w), 2980 (m), 2944 (w), 2880 (w), 1734 (w),1612 (s), 1587 (s), 1572 (s), 1539 (s), 1474 (m), 1413 (s), 1215 (m), 1101 (m), 1060 (m), 965 (m), 875 (s), 800 (w), 695 (s), 517 (w). ESI-MS: m/z 598.2312 ($C_{33}H_{27}N_9O_3$, calcd. 598.2310). A mixture of L (79.6 mg, 10 mmol) and $Pd(NO_3)_2$ (23.0 mg, 10 mmol) was dissolved in DMSO (4 mL). This mixture was heated at 70°C for 2 h to generate **1** in a quantitative yield.

Single crystals of 1: a solution of $Pd(NO_3)_2$ (3.1 mg, 0.0135 mmol) in MeCN (7 ml) was layered onto a solution of L (11 mg, 0.0183 mmol) in $CHCl_3$ (7 mL). The system was left for about 60 days at room temperature to afford slightly yellow crystals of **1**.

Single crystal data collection and refinement. Suitable single crystals were selected and mounted in air onto thin glass fibres. X-ray intensity data were measured at 100.01 K on an Agilent SuperNova CCD-based diffractometer (Cu Kα radiation, λ = 1.54184 Å). The raw frame data for the complexes were integrated into SHELX-format reflection files and corrected for Lorentz and polarization effects using SAINT.[2] Corrections for incident and diffracted beam absorption effects were applied using SADABS.[2] None of the crystals showed evidence of crystal decay during data collection. All structures were solved by a combination of direct methods and difference Fourier syntheses and refined against F^2 by full-matrix least-squares techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms attached to carbon atoms were placed in geometrically idealized positions and refined using a riding model with C-H = 0.93 (aromatic) and 0.96 Å (methyl) and Uiso(H) = 1.5Ueq (C) for methyl group and Uiso(H) = 1.2Ueq(C) for all other H atoms. A very large void space is present between the framework, which contain many significant electron density peaks. The species were too severely disordered to be modeled, and were treated with SQUEEZE/PLATON.[3] The program calculated a solvent-accessible void volume of 55549 Å^3 (59.5% of the total unit cell volume 93372 (3) Å^3) corresponding to 3818 e^-/cell. These species include nitrate ions and some DMSO, acetonitrile and H_2O molecules. The contribution of the disordered species was removed from the structure factor calculations. The tabulated F(000), MW and density reflect known cell contents only. Crystallographic data for **1** has been deposited at the Cambridge Crystallographic Data Centre with a CCDC reference number of 1840207.

Table S1. Crystallographic data of **1**.

compound	**1**
empirical formula	$C_{264}H_{224}N_{76}O_{36}Pd_6$
formula weight	5657.58
temp (K)	100.0(1)

crystal system	Tetragonal
space group	I 4/m
a (Å)	28.5568(17)
b (Å)	28.5568(17)
c (Å)	28.6245(15)
α (deg)	90.00
β (deg)	90.00
γ(deg)	90.00
V (Å^3)	23343(3)
Z	2
ρ_{calc} (g/cm^3)	0.807
F(000)	5808.0
data/restraints/params	11419/6/451
GOF on F^2	1.057
final R indices [I > 2sigma(I)]	R_1 = 0.1005 wR_2 = 0.3516

3. Synthesis and characterization of 2

A DMSO solution of **1** (150 μL) was combined with deionized water (100 μL), and the resulting mixture was stirred at room temperature for 4 h. After centrifugation, the light yellow nanoplates of **2** was obtained. After IR (KBr pellet) [(cm^{-1})]: 2981(m), 1624(m), 1574(m), 1540(m), 1428(m), 1375(m), 1219(w), 1101(w), 1058(m), 966(w), 832(s), 709(w), 526(w). Anal. Calcd for **2**: C, 51.44; H, 3.53; N, 19.09. Found: C, 51.37; H, 3.59; N, 19.21. ESI-MS for **2** in DMSO: m/z 965.1895 ($[Pd_6L_8(NO_3)_6]^{6+}$, calcd. 965.1912), 1170.6307 ($[Pd_6L_8(NO_3)_7]^{5+}$, calcd. 1170.6271), 1478.7755 ($[Pd_6L_8(NO_3)_8]^{4+}$, calcd. 1478.7810).

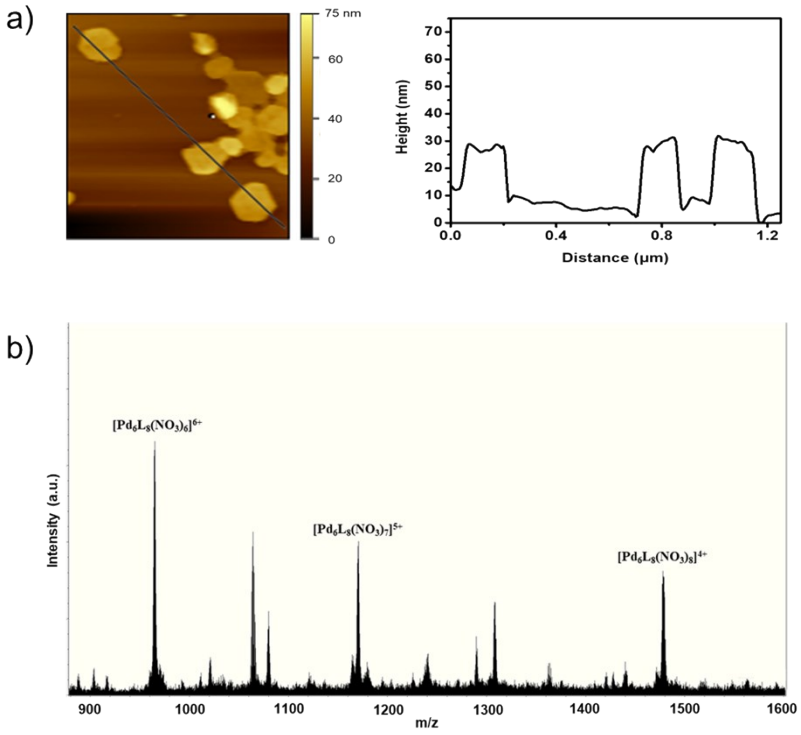


Fig. S1 Atomic force microscopy (AFM) image of **2** (a) and ESI-MS for **2** in DMSO (b).

4. Synthesis and characterization of 3 and 4

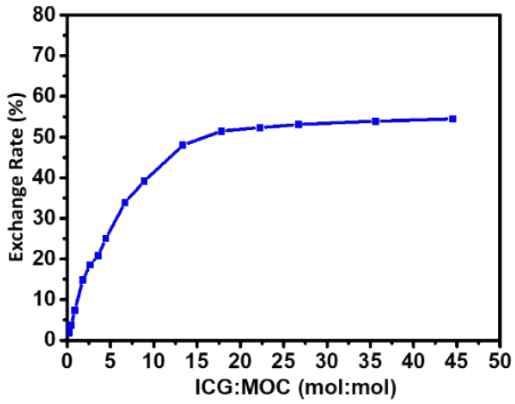


Fig. S2 Exchange rate of nitrate by ICG in **2**.

1 mL aqueous solution of ICG with different concentrations (5 - 1000 μg/mL) was added into an aqueous dispersion of **2** (300 μL, 0.6 mg/mL). The mixture was stirred in the dark for 24 h at room temperature. The solid was collected by the centrifugation and completely washed with water, and then the corresponding absorbance was measured by UV and the exchange capacity of ICG was calculated by standard curve method.

Based on the exchange rate of nitrate by ICG shown above, we prepared nanoplates of **3** by the combination of aqueous solution of **2** (1 mL, 400 μg/mL) with ICG aqueous solution (1 mL, 1 mg/mL) at room temperature for 24 h under stirring in dark. After centrifugation and completely washed with water, **3** was obtained as the green nanoplates. IR (KBr pellet) [(cm^{-1})]: 2980(m), 1624(m), 1574(m), 1539(m),

1508(m), 1472(m), 1416(s), 1375(w), 1357(m), 1218(w), 1092(s), 1058(m), 834(m), 710(w), 521(w). Anal. Calcd for **3**: C, 60.50; H, 4.84; N, 10.98; Found: C, 60.39; H, 4.93; N, 10.86.

Discrete molecular **4** was obtained by dissolving **3** in DMSO. ^{1}HNMR (400 MHz, DMSO-d_6, 25 °C, TMS, ppm): δ 9.76-9.75 (d, 48H), 8.38-8.36 (d, 48H), 8.27-8.24 (d, 11H), 8.07-8.03 (t, 34H), 7.78-7.75 (d, 11H), 7.66-7.63 (t, 13H), 7.51-7.47 (t, 11H), 7.62-7.49 (q, 22H), 4.23 (s, 22H), 2.61-2.63 (t, 22H), 2.17 (s, 48H), 1.92 (s, 62H), 1.85 (s, 44H), 1.12-1.08 (t, 11H), 0.87-0.83 (t, 72H). ESI-MS: m/z 1193.9640 ($[Pd_6L_8(NO_3)_4(ICG)_2]^{6+}$, calcd. 1193.9549), 1308.8544 ($[Pd_6L_8(NO_3)_3(ICG)_3]^{6+}$, calcd. 1308.8382), 1445.1548 ($[Pd_6L_8(NO_3)_5(ICG)_2]^{5+}$, calcd. 1445.1449), 1582.9686 ($[Pd_6L_8(NO_3)_4(ICG)_3]^{5+}$, calcd. 1583.0049).

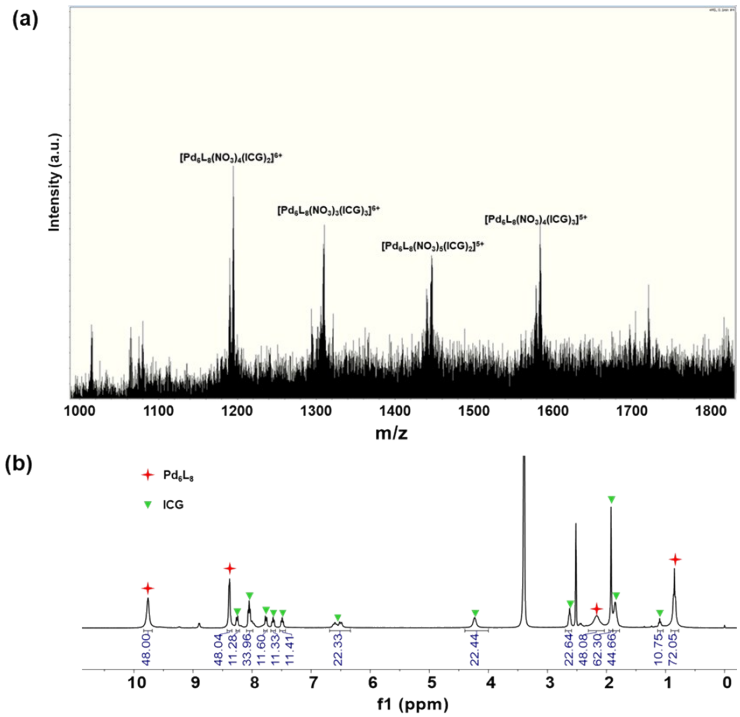


Fig. S3 ESI-MS and ^{1}H NMR spectra (400 MHz, DMSO-d^6, 298 K) of **4**.

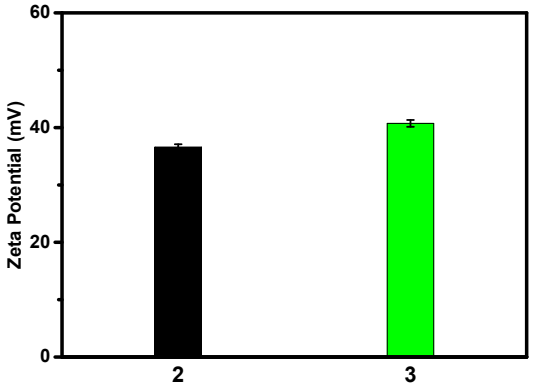


Fig. S4 Zeta potentials of **2** and **3** in PBS, data are presented as the mean ± SD (n = 3).

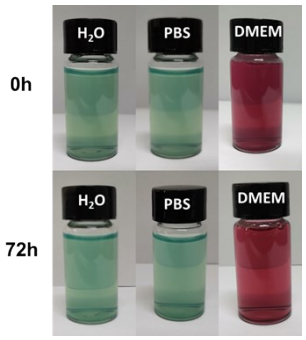


Fig. S5 Stability of **3** in di□erent physiological media within 3 days.

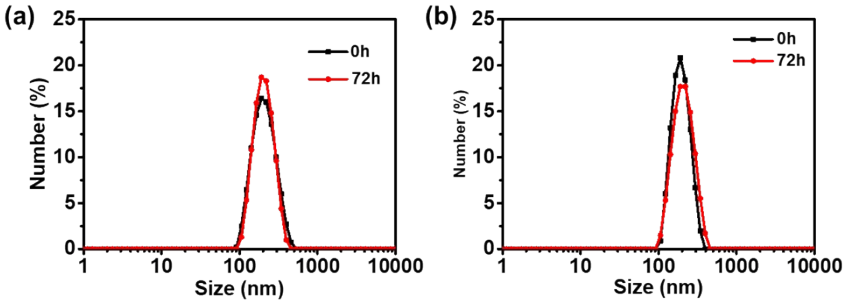


Fig. S6 The DLS of **2** (a) and **3** (b) in cell culture medium of DMEM with 10% fetal bovine serum within 3 days.

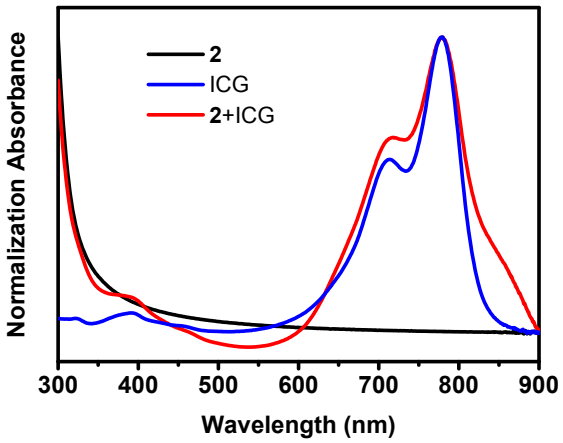


Fig. S7 UV–vis absorption spectra of **2**, ICG and **2**+ICG.

5. Photodynamic property

Pipetted the EtOH dispersions of **2**, **3** or **4** (2 mL, 10 μg/mL) into quartz cuvette, and DPBF solution of EtOH (200 μL, 1 mM) was added. Then the mixture was exposed to 808 nm laser (100 mW/cm^2) at room temperature for 5 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 the absorbance over time. To characterize the difference in the rate of $^1O_2$ produced by different samples, the absorbance of DPBF at 414 nm were calculated. EtOH dispersions of **2**, **3** or **4** (2 mL, 10 μg/mL) was used as the reference for this UV−vis measurement.

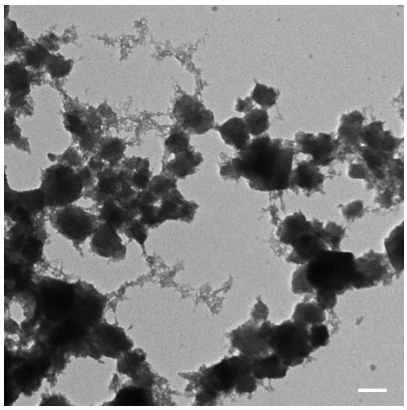


Fig. S8 TEM image of **4** (Scale bar, 200 nm) in EtOH.

6. Cell uptake of 3 and 4

MCF-7 cells were incubated with dispersion of **3** (20 μg/mL) in CO_2 incubator for 2 h, washed with DPBS twice. The cells were imaged with a laser scanning confocal microscope. The red images were excited by 561 nm light, and the emission wavelength range was collected at 630 ± 30 nm. **4** (20 μg/mL) was used instead of **3** to repeat the cell uptake experiment under the same conditions as shown above.

7. Subcellular localization of lysosomes

MCF-7 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** (200 μL, 10 μg/mL) for 2 h in a CO_2 incubator, and carefully washed with DPBS twice. After additional 4 h incubation, cells were incubated with LysoTracker Green DND-99 (200 μL, 50 nM) for an additional 10 min, and washed with DPBS twice. Finally, the laser scanning confocal fluorescence images were captured. The red images of **3** were excited by 561 nm light, and the emission wavelength range was collected at 630 ± 30 nm. The green images of lysosomes were excited by 488 nm light, and the emission wavelength range was collected at 511 ± 11 nm. Controls were conducted to make sure images were free of crosstalk. Colocalization was analyzed by ImageJ software.

8. Subcellular localization of mitochondria

MCF-7 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** (200 μL, 10 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 MitoTracker Green FM (200 μL, 50 nM) for an additional 10 min, and washed with DPBS twice. Finally, the laser scanning confocal fluorescence images were captured. The red images of 1-ICG-NP were excited by 561 nm light, and the emission wavelength range was collected at 630 ± 30 nm. The green images of mitochondria were excited by 488 nm light, and the emission wavelength range was collected at 516 ± 16 nm. Controls were conducted to make sure images were free of crosstalk. Colocalization was analyzed by ImageJ software.

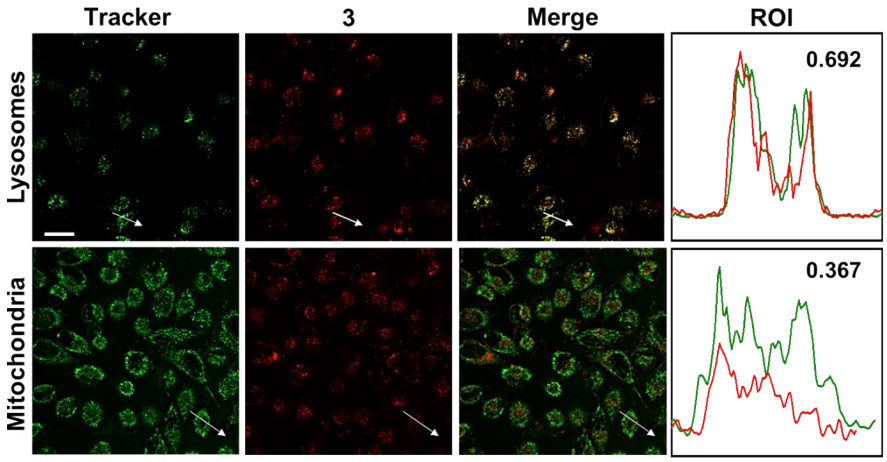


Fig. S9 Subcellular localization of **3** in MCF-7 cancer cells.

9. Mitochondrial membrane potential

MCF-7 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** (200 μL, 20 μg/mL) for 2 h in a CO_2 incubator. The cells were exposed to 808 nm laser (100 mW/cm^2, 8 min). After additional 4 h incubation, the cells were incubated with JC-1 (200 μL, 10 μg/mL) for 10 min, washed with DPBS twice, and the laser scanning confocal fluorescence images were captured. The green images of monomer were excited by 488 nm light, and the emission wavelength range was collected at 530 ± 15 nm. The red images of *J*-aggregate were excited by 514 nm light, and the emission wavelength range was collected at 590 ± 17 nm. Controls were conducted to make sure images were free of crosstalk. The MFI was analyzed by ImageJ software.

10. Lysosomal membrane permeabilization

MCF-7 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** (200 μL, 20 μg/mL) for 2 h in a CO_2 incubator. The cells were exposed to 808 nm laser (100 mW/cm^2, 8 min). After additional 4 h incubation, the cells were incubated with AO (200 μL, 5 μg/mL) for 10 min, washed with DPBS twice, and the laser scanning confocal fluorescence images were captured. The green images were excited by 488 nm light, and the emission wavelength range was collected at 530 ± 20 nm. The red images were excited by 488 nm light, and the emission wavelength range was collected at 640 ± 20 nm. Controls were conducted to make sure images were free of crosstalk.

11. *In vitro* antitumor therapy

We first evaluated the dark toxicity of **3** with a wide concentration range. 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** (100 μL, 0 – 500 μg/mL) for 2 h in a CO_2 incubator. After additional 24 h incubation, CCK-8 (10 μL, 5 mg/mL) was added to each well and incubated for additional 4 h in a CO_2 incubator. Finally, followed by recording the absorbance at 450 nm.

In addition, the phototoxicity and dark toxicity of **3** and **4** with low concentrations were evaluated. The cells were incubated with DPBS dispersion of **3** and **4** (100 μL, 0 – 30 μg/mL) for 2 h in a CO_2 incubator.

Then cells were exposed to 808 nm laser (100 mW/cm^2, 8 min). The other steps were consistent with the above. The preparation of **4** was the same as the cell uptake experiment.

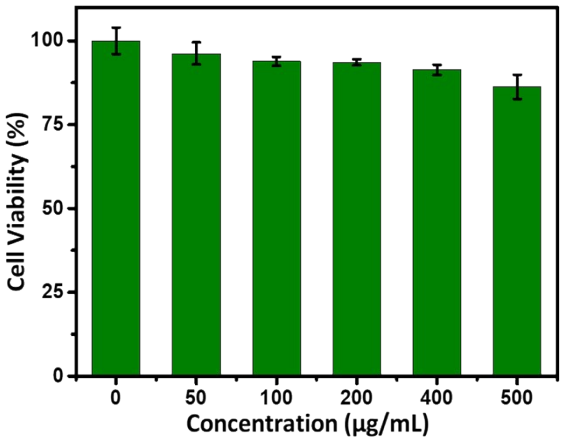


Fig. S10 CCK-8 assays of MCF-7 cancer cells incubated with **3** at different concentrations.

12. Intracellular singlet oxygen generation

MCF-7 cells were incubated with DPBS dispersion of **3** (2 mL, 10 μg/mL) in CO_2 incubator for 2 h, washed with DPBS twice, and further incubated with SOSG (5 μM, 200 μL) for 15 min, and washed with DPBS twice carefully. The cells were exposed to 808 nm laser (100 mW/cm^2) for 5 min and imaged with a laser scanning confocal microscope. The green images were excited by 488 nm light, and the emission wavelength range was collected at 525 ± 20 nm.

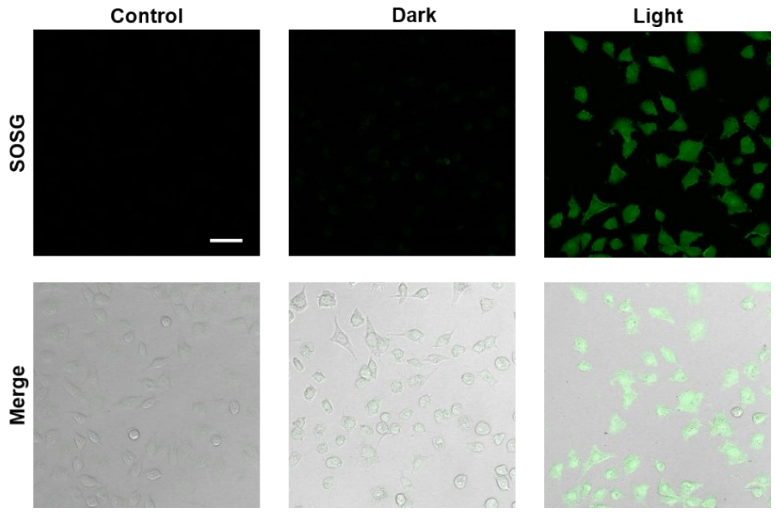


Fig. S11 MCF-7 cells laser scanning confocal images of intracellular $^1O_2$. Scale bar, 50 μm.

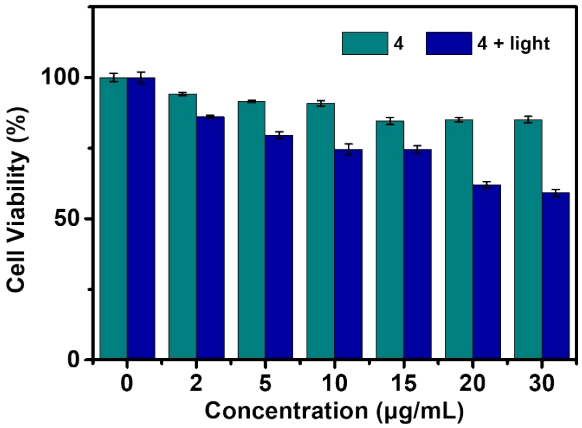


Fig. S12 CCK-8 assays of MCF-7 cancer cells incubated with **4** at different concentrations, and the phototoxicity of **4** upon 808 nm laser (100 mW/cm^2, 8 min) irradiation.

13. Hemolysis analysis

Nude mice were anesthetized. The blood was sampled from mice by eyeball extirpating and put into an EP tube with anticoagulants. 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 negative control, 0.8 mL deionized water as positive control, and 0.8 mL **3** suspension at a concentration range of 1 to 200 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 synergy SpectraMax i3x multi-mode microplate reader. The hemolytic percentage of red blood cells was calculated by the following formula: Hemolysis Rate = [(Dt – Dnc)/(Dpc – Dnc)] ×100%.

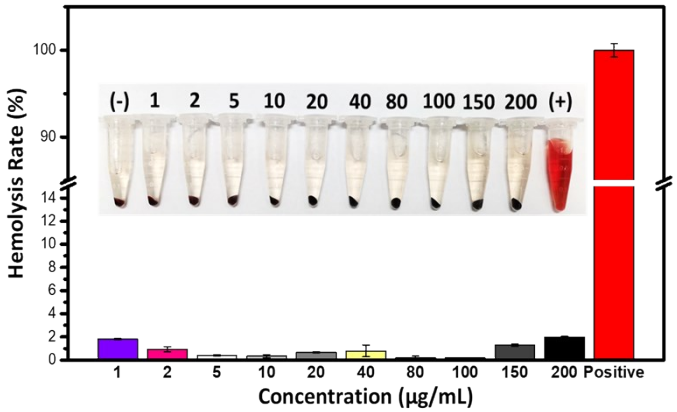


Fig. S13 Hemolytic assay using red blood cells incubated with control solvents and **3** with different concentrations.

14. *In vivo* antitumor therapy

MCF-7 cancer cells (10^6 cells) suspended in DPBS (100 μL) were subcutaneously injected into the flanks of each mice to establish MCF-7 xenograft model. Length (L) and width (W) of the tumor were determined by digital calipers. 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 tumors (n = 20) were randomly distributed into four groups, i.e., control, **2** + light, ICG + light, **3**, and **3** + light groups. After intratumoral

injection PBS (100 μL), **2** or **3** (100 μL, 100 μg mL^{-1}), the nude mice were feeding for 4 h, and for the treatment group, light treatment (808 nm laser, 100 mW cm^{-2}, 8 min) was performed on the tumor site. The mice continued to be fed for 15 days. The tumor volume and nude mouse body weight were recorded every other day during the experimental period. After the mice were sacrificed, the organs were harvested from each group, stored in 4 % paraformaldehyde, and then were processed routinely into paraffin. The obtained slices were stained separately for H&E assay. Images were observed by Fluorescent Inverted microscope for pathological analysis.

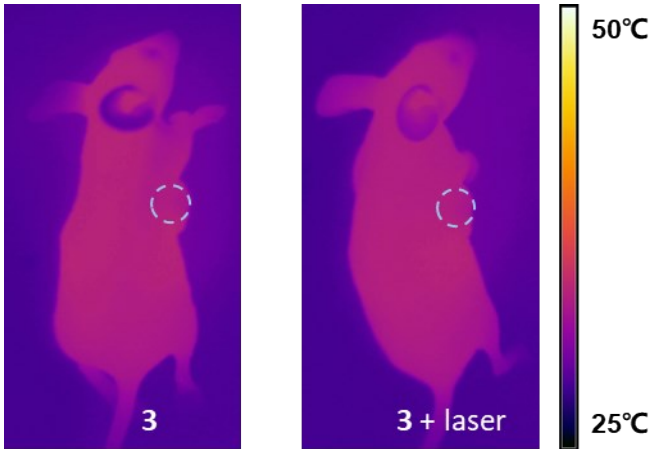


Fig. S14 Thermal images of tumor-bearing mice before and after laser irradiation.

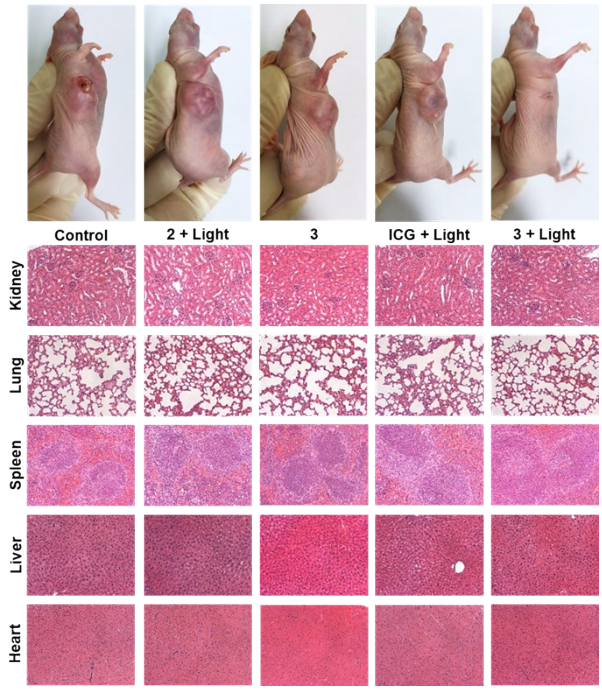


Fig. S15 Representative photographs of the nude mice at the end of the treatment. H&E stained tissue sections from the heart, liver, spleen, lung, and kidney of the nude mice at the end of the treatment.

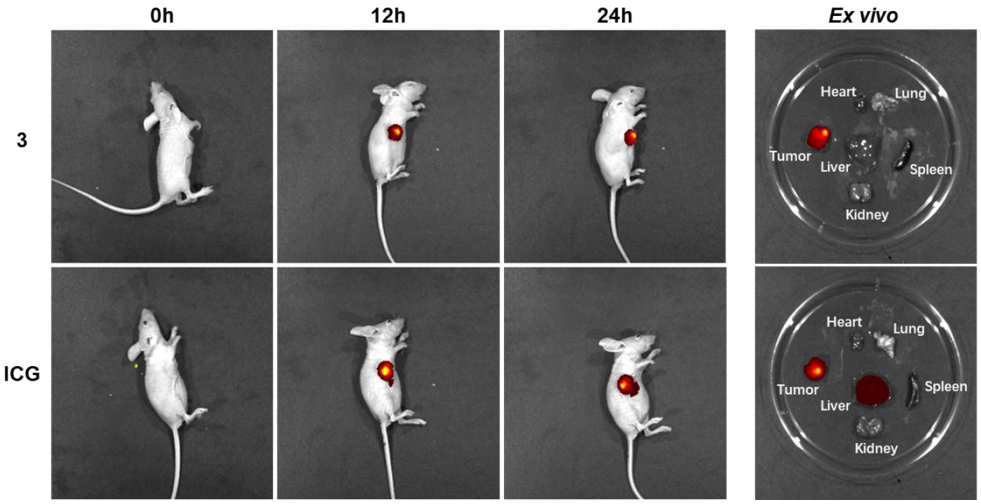


Fig. S16 Fluorescence imaging of the mice at different time points after intratumoral injection and *Ex vivo* fluorescence images of the internal organs of mice sacrificed at 24 h after injection.

15. References

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