

**Electronic Supplementary Information**

**Non-aggregated and tumour-associated macrophage-targeted photosensitiser for photodynamic therapy: A novel zinc(II) phthalocyanine containing octa-sulphonates**

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## Experimental Section

### 1. Synthesis

**General.** Dimethyl sulfoxide (DMSO) and *n*-pentanol were dried over molecular sieves and further distilled under reduced pressure before use. Potassium carbonate and zinc acetate were activated in muffle at 300 °C. Chromatographic purifications were performed on silica gel columns (100-200 mesh, Qingdao Haiyang Chemical Co., Ltd, China) with the indicated eluents. Ion-exchange and size-exclusion chromatography were carried out on a 732 cation exchange resin (Sinopharm Chemical Reagent Co., Ltd, China) and a Sepharose-Sephadex G25 column (0.3-1.2 mm, Beijing Weishibohui Chromatography Technology Co., Ltd, China), respectively, by using deionised water as the eluent. ZnPcS<sub>4</sub> was prepared as reported previously.<sup>1</sup> All other solvents and reagents were of reagent grade and used as received.

FTIR spectra were recorded at room temperature using the KBr pellet method on a SP2000 spectrometer in the 400-4000 cm<sup>-1</sup> with average of 50 scans. <sup>1</sup>H NMR spectra were determined on a Bruker-400 spectrometer (400 MHz) in DMSO-d<sub>6</sub>. Chemical shifts were relative to internal SiMe<sub>4</sub> ( $\delta = 0$  ppm). High-resolution mass spectra (HRMS) were recorded on a TripleTOF 4600 LC-MS/MS spectrometer or a Finnigan LCQ Deca XP MAX LC/MS spectrometer.

**3-[6,8-potassium disulfonate-2-naphthyloxy]phthalonitrile (3).** A mixture of potassium 2-naphthol-6,8-disulfonate (**1**) (1.91 g, 5.0 mmol), 3-nitrophthalonitrile (**2**) (0.87 g, 5.0 mmol) and anhydrous K<sub>2</sub>CO<sub>3</sub> (1.38 g, 10.0 mmol) in DMSO (10 mL) was stirred at room temperature for 48 h under an atmosphere of nitrogen. The reaction mixture was filtered, and the filtrate was poured into ice chloroform (150 mL) to give white precipitate, which was collected by filtration, washed with ethanol (20 mL) and acetone (20 mL), and then drying *in vacuo*. The product was afforded as a white solid (2.35 g, 93%). *R*<sub>f</sub> = 0.67 (ethanol). IR (KBr, cm<sup>-1</sup>): 3096.6 (Ar-H); 2239.6 (C≡N); 1622.2, 1572.0, 1504.0, 1455.7 (C=C); 1283.6 (Ar-O-Ar); 1200.2, 1042.6 (S=O); 905.1, 847.9, 805.1 (Ar-H). HRMS (ESI): *m/z* Calcd for C<sub>18</sub>H<sub>9</sub>N<sub>2</sub>O<sub>7</sub>S<sub>2</sub> [M-2K+1H]<sup>-</sup>

428.9857, found 428.9860.  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm):  $\delta$  8.54 (d,  $J = 2.0$  Hz, 1 H), 8.27 (s, 1 H), 8.19 (s, 1 H), 8.15 (d,  $J = 9.2$  Hz, 1 H), 7.81-7.87 (m, 2 H), 7.44 (dd,  $J = 8.8, 2.0$  Hz, 1 H), 7.28 (d,  $J = 8.0$  Hz, 1 H).

**1, 8(11), 15(18), 22(25)-tetrakis-[6,8-sodium disulfonate-2-naphthyloxy] phthalocyanine zinc(II) (ZnPcS<sub>8</sub>)**. A mixture of **3** (1.96 g, 3.87 mmol) and dodecyl trimethyl ammonium bromide (DTAB) (3.00 g, 9.73 mmol) was stirred in water (20 mL) at room temperature for 3 h. The mixture was extracted with ethyl acetate (50 mL  $\times$  3). The combined organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness *in vacuo* to give a sticky pale yellow solid of dodecyl trimethyl ammonium-ionised phthalonitrile with an equivalent amount. The obtained pale yellow solid (1.50 g, 1.69 mmol) in *n*-pentanol (30 mL) was stirred at 90 °C under an atmosphere of nitrogen for 10 min, and then zinc acetate (0.30 g, 1.64 mmol) and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (0.50 mL, 3.35 mmol) were added. The resulting mixture was stirred at 130 °C for 15 h. After removing the volatiles *in vacuo*, the residue was first purified by silica gel column chromatography by using ethanol/*N,N*-dimethyl formamide (DMF) (4:1, v/v) as eluent to give a dark-green crude, which was further treated with a strongly acidic cation exchange resin using deionised water as eluent, and the obtained fraction was neutralised with NaOH aqueous solution (1.0 M) to pH  $\sim$  7. After removing most of the water *in vacuo*, the phthalocyanine crude was further purified by size exclusion chromatography on a Sepharose-Sephadex G25 column using deionised water as eluent to give a dark-green solid ZnPcS<sub>8</sub> (86.2 mg, 10%).  $R_f = 0.55$  (methanol/ethanol = 3:2, v/v). IR (KBr,  $\text{cm}^{-1}$ ): 3402.9 (O-H); 1620, 1580, 1480, 1445 (C=C, C=N-); 1220 (Ar-O-Ar); 1190, 1040 (S=O). HRMS (ESI):  $m/z$  Calcd for C<sub>72</sub>H<sub>36</sub>N<sub>8</sub>O<sub>28</sub>S<sub>8</sub>Zn [M-8Na+4H]<sup>4+</sup> 445.4678, found: 445.4685.  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm):  $\delta$  8.888-9.42 (m, 5 H, Pc-H <sub>$\alpha$</sub>  and Ar-H); 8.72-8.83 (m, 3 H, Ar-H); 8.26-8.35 (m, 5 H, Pc-H <sub>$\beta$</sub> ); 7.01-8.15 (m, 7 H, Ar-H and Pc-H <sub>$\beta$</sub> ); 7.39-7.96 (m, 12 H, Ar-H). Anal. Calcd for ZnPcS<sub>8</sub>·12H<sub>2</sub>O: C, 39.69; H, 2.59; N, 5.14. Found: C, 39.35; H, 3.00; N, 5.08.

## 2. Photophysical and photochemical studies

Electronic absorption spectra were measured on a Shimadzu UV-2450 UV-vis spectrophotometer. Fluorescence spectra were taken on an Edinburgh FL900/FS900 spectrofluorometer. Fluorescence quantum yield ( $\Phi_F$ ) and singlet oxygen yield ( $\Phi_\Delta$ ) were determined as described in our previous manuscripts.<sup>2</sup>

The singlet oxygen generation efficiency was also tested in aqueous solution. 1,3-diphenylisobenzofuran (DPBF) was first dissolved in DMF to give a 50 mM of solution, which was formulated with Cremophor EL and then diluted with D<sub>2</sub>O to 100  $\mu$ M (containing 0.2% DMF and 0.1% Cremophor EL). The DPBF solution (1 mL) was then mixed with ZnPcS<sub>8</sub> (8  $\mu$ M, 1 mL) or MB (26  $\mu$ M, 1 mL) solution in D<sub>2</sub>O in the dark to give a mixture of DPBF and ZnPcS<sub>8</sub> (or MB) (containing [DPBF] = 50  $\mu$ M, [ZnPcS<sub>8</sub>] = 4  $\mu$ M, [MB] = 13  $\mu$ M, 0.1% DMF, and 0.05% Cremophor EL). The mixture was irradiated with red light immediately, and then DPBF degradation at 415 nm was monitored along with irradiated time.

### **3. *In vitro* test**

**Organisms and growth conditions.** J774A.1 mouse macrophages (from ATCC) and HepG2 human hepatocarcinoma cells (from ATCC) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (50 units·mL<sup>-1</sup>), and streptomycin (50  $\mu$ g·mL<sup>-1</sup>) at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere.

***In vitro* photocytotoxicity against HepG2 or J774A.1 cells.** The solution of ZnPcS<sub>8</sub> (1.0 mM) in water was diluted with the culture medium to appropriate concentrations. HepG2 or J774A.1 cells (about  $1 \times 10^4$  cells per well) were maintained in 96-well plates overnight at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere. The cells were then incubated with 100  $\mu$ L of the solutions of ZnPcS<sub>8</sub> in the dark for 2 h. After that, the cells were rinsed with phosphate buffered saline (PBS) and re-fed with 100  $\mu$ L of the culture medium before illumination at ambient temperature. The light source consisted of a 500 W halogen lamp, a water tank for cooling, and a colored glass filter cut-on 610 nm. The fluence rate ( $\lambda > 610$  nm) was 15 mW·cm<sup>-2</sup>. An illumination of 30 min led to a total fluence of 27 J·cm<sup>-2</sup>.

Cell viability was determined by the colorimetric 3-(4,5-dimethyl-2-thiazolyl) -

2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.<sup>3</sup> An MTT (Sigma) solution in PBS (20  $\mu\text{L}$ , 2.5  $\text{mg}\cdot\text{mL}^{-1}$ ) was added to each well followed by incubation for 4 h (HepG2 cells) or 5 h (J774A.1 cells) under the same condition. 150  $\mu\text{L}$  of DMSO was then added to each well. The 96-well plate was agitated on a microplate reader (Tecan M200Pro) at ambient temperature for 20 s before the absorbance at 490 nm at each well was taken. The average absorbance of the blank wells, which did not contain the cells, was subtracted from the readings of the other wells. The cell viability was then determined by the equation: Cell Viability (%) =  $[\Sigma(A_i/\bar{A}_{control} \times 100)]/n$ , where  $A_i$  is the absorbance of the  $i$ th data ( $I = 1, 2, \dots, n$ ),  $\bar{A}_{control}$  is the average absorbance of the control wells, in which the phthalocyanine was absent, and  $n$  ( $\geq 3$ ) is the number of the data points.

For the competitive assay on J774A.1 or HepG2 cells, the procedure was the same as that described above except for the following details, the cells was first incubated with polyinosinic acid (poly I) at the concentration of 50 or 100  $\mu\text{g}\cdot\text{mL}^{-1}$  for 0.5 h, and then ZnPcS<sub>8</sub> solutions at different concentrations were added to for further 2 h co-incubation.

**Cellular uptake.** About  $1 \times 10^5$  HepG2 or J774A.1 cells in RPMI 1640 medium were seeded in confocal dishes and incubated overnight at 37 °C under a humidified 5% CO<sub>2</sub> atmosphere. After removing the medium, the cells were incubated with the solution of ZnPcS<sub>8</sub> or ZnPcS<sub>4</sub> in the medium (1  $\mu\text{M}$  for ZnPcS<sub>8</sub> and 2  $\mu\text{M}$  for ZnPcS<sub>4</sub>, both at 400  $\mu\text{L}$ ) for 2 h under the same condition. The cells were then rinsed with PBS twice and imaged using a Leica laser fluorescent confocal microscope. The compounds were excited at 635 nm and monitored at 645-750 nm. The images were then digitised and analysed by using the SPE ROI Fluorescence Statistics software. The average intracellular fluorescence intensities (a total of 50 cells for each sample) were also determined.

For the competitive assay on J774A.1 or HepG2 cells, the procedure was the same as that described above except for the following details, the cells was first incubated with poly I at the concentration of 50 or 100  $\mu\text{g}\cdot\text{mL}^{-1}$  for 0.5 h, and then

ZnPcS<sub>8</sub> or ZnPcS<sub>4</sub> solutions was added to for further 2 h co-incubation.

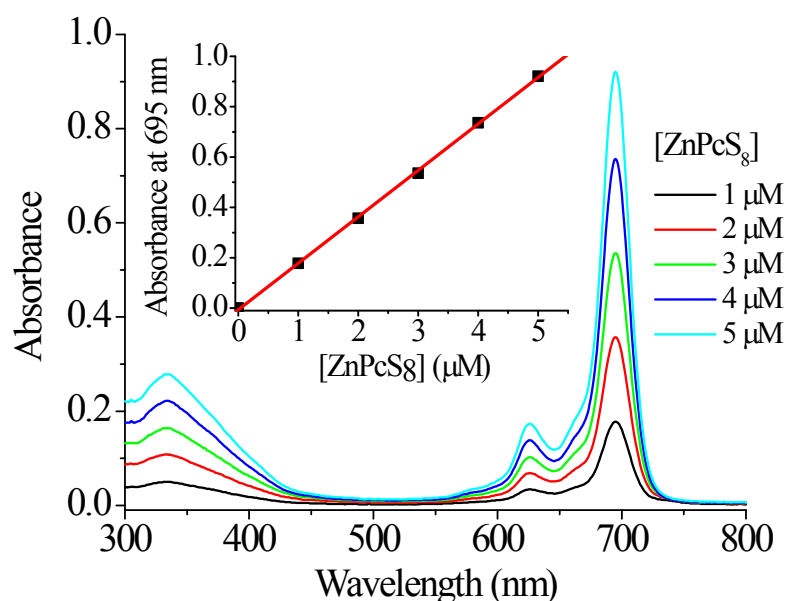
**Subcellular localisation.** About  $1 \times 10^4$  HepG2 or J774A.1 cells in the culture medium were seeded on a confocal dishes and incubated overnight at 37 °C with 5% CO<sub>2</sub>. After removing the medium, the cells were incubated with the solutions of ZnPcS<sub>8</sub> in the medium (5 μM, 400 μL) for 0.5 h under the same condition, and then Lyso-Tracker Red (5 μM, 20 μL) was added for further 60 min co-incubation, followed by Mito-Tracker Green (5 μM, 20 μL) for further 30 min co-incubation, leading to a total incubation time of 2 h for the photosensitiser, 1.5 h for Lyso-Tracker Red, and 0.5 h for Mito-Tracker Green. Subsequently, the cells were rinsed with PBS and viewed with a Leica laser fluorescent confocal microscope. The Mito-Tracker Green and Lyso-Tracker Red were excited at 488 and 543 nm, and monitored at 499-529 and 552-617 nm, respectively. The photosensitiser were excited at 635 nm and monitored at 645-750 nm. The subcellular localisation of ZnPcS<sub>8</sub> was revealed by comparing the intracellular fluorescence images caused by the Lyso-Tracker, Mito-Tracker, and the photosensitiser.

#### **4. *In vivo* test**

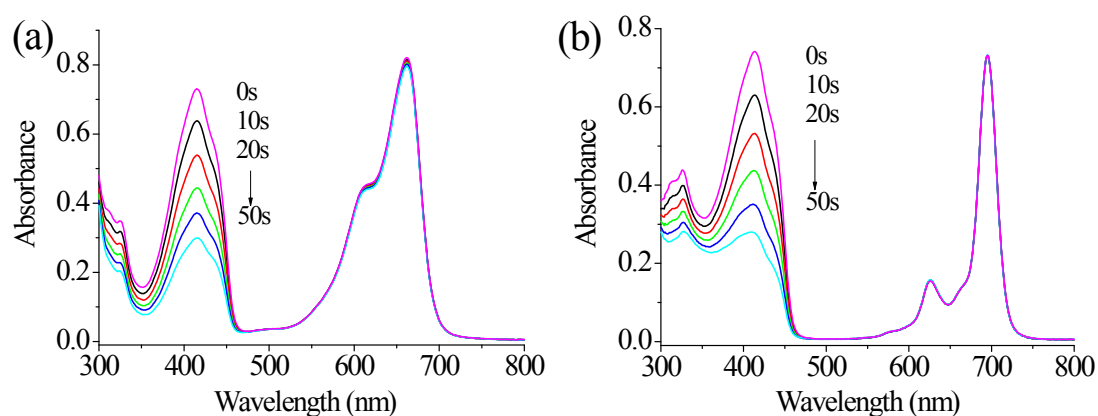
Nude mice were purchased from Vital River Co., Ltd, China. All animal studies were performed in compliance with guidelines of the Animal Care Committee of Fuzhou University. To build a subcutaneous tumor model, HepG2 cells ( $\sim 1 \times 10^7$  cells in 200 μL) were inoculated subcutaneously on the axilla of the nude mice (20-25 g). When the tumours had grown to 600-1000 mm<sup>3</sup>, a ZnPcS<sub>8</sub> aqueous solution (64 μmol·mL<sup>-1</sup>, 200 μL) was intravenously injected into the tail vein of the tumour-bearing mice. *In vivo* fluorescence imaging of nude mice was performed from 700 nm at different time points with LB983 NC100 imaging system (excited at 630 nm). After *in vivo* imaging studies, the nude mice were euthanised at 24 h post-injection. Tumours and other organs were harvested and their fluorescence imaging were measured.

#### **5. Reference**

1. X.-S. Li, M.-R. Ke, W. Huang, C.-H. Ye, and J.-D. Huang, *Chem. Eur. J.*, 2015, DOI: 10.1002/chem.201404514.
2. X. M. Shen, X. J. Jiang, C. C. Huang and J. D. Huang. *Tetrahedron*, 2010, **66**, 9041-9048.
3. H. Tada, O. Shiho, K. Kuroshima, M. Koyama and K. Tsukamoto. *J Immunol Method*, 1986, **93**, 157-165.



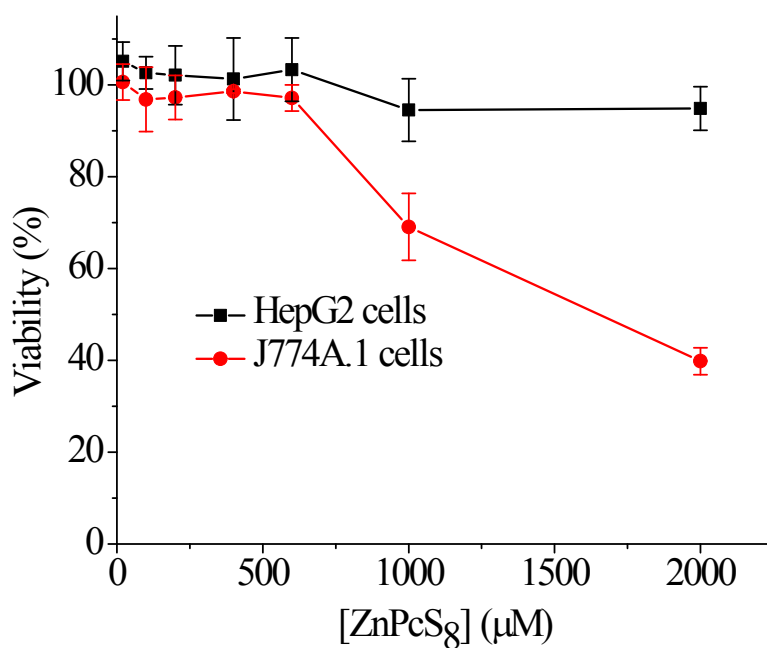
**Fig. S1** UV-vis spectra of ZnPcS<sub>8</sub> at different concentrations in H<sub>2</sub>O. The inset plots the Q-band absorbance vs. the concentration of ZnPcS<sub>8</sub>.



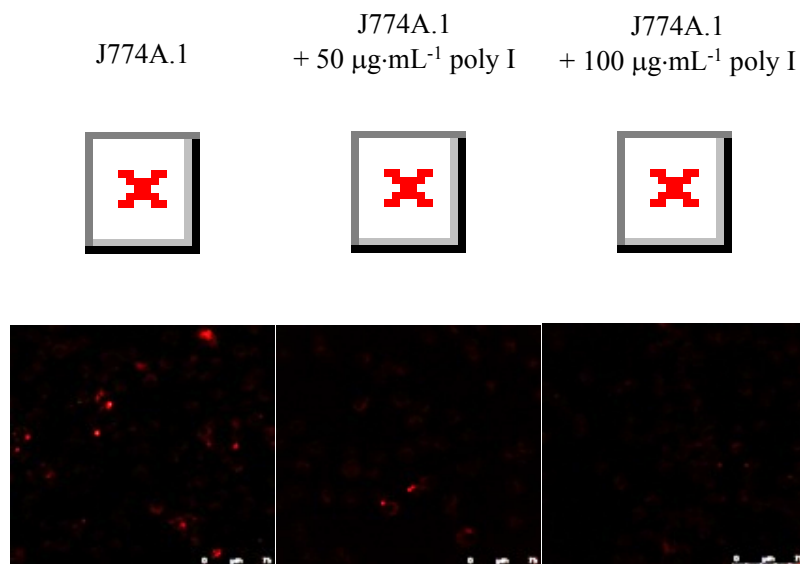
**Fig. S2** The singlet oxygen generation efficiency of ZnPcS<sub>8</sub> in D<sub>2</sub>O using MB as reference. The UV-vis spectra of (a) MB (13 μM) or (b) ZnPcS<sub>8</sub> (4 μM) in the presence of DPBF (50 μM) in D<sub>2</sub>O (containing 0.1% DMF and 0.05% Cremophor EL)



at different irradiation time.

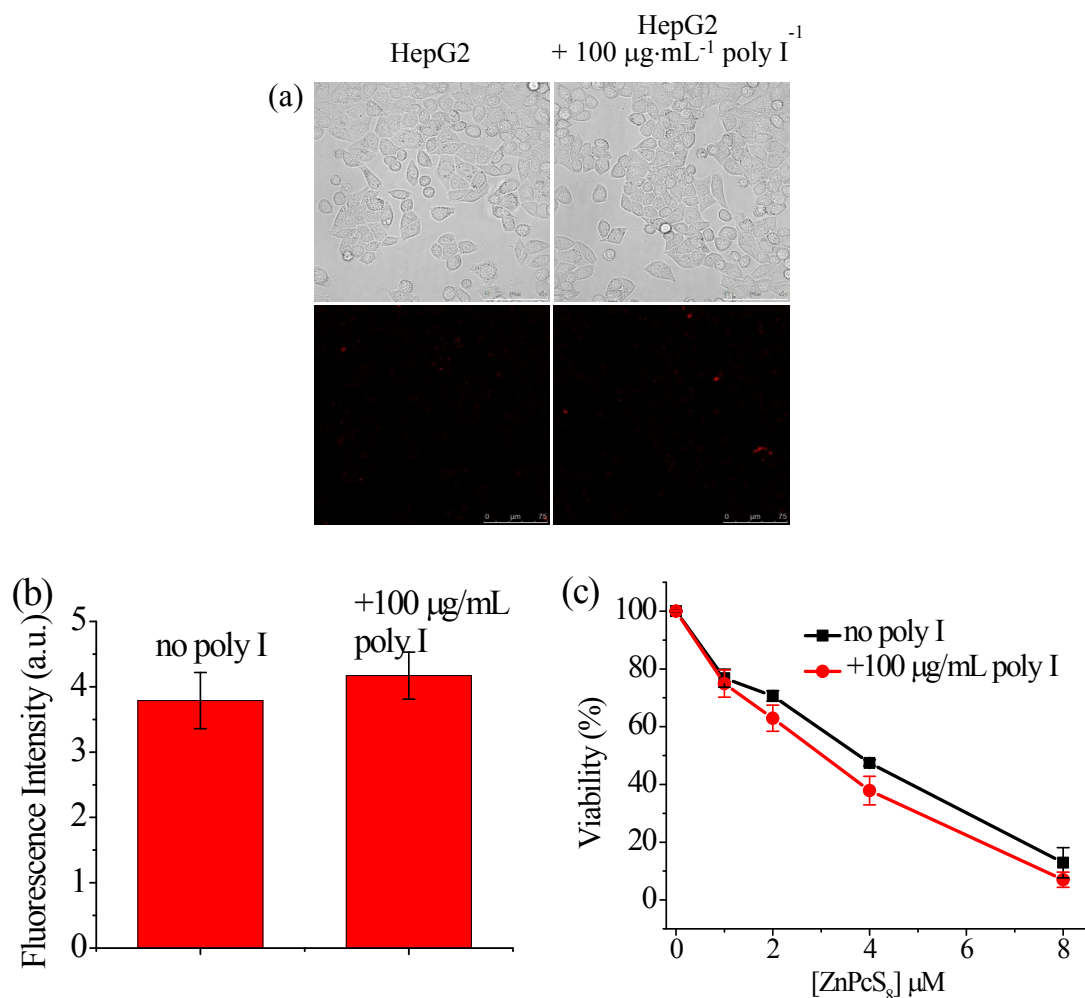


**Fig. S3** Cytotoxic effects of ZnPcS<sub>8</sub> on HepG2 and J774A.1 cells in the absence of light. Data are expressed as mean  $\pm$  standard deviation ( $n = 3$ ).

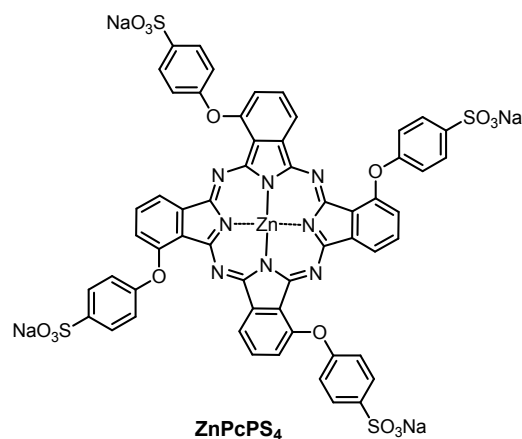


**Fig. S4** Bright field (up row) and intracellular fluorescence (down row) images of J774A.1 cells after incubation with ZnPcS<sub>8</sub> (1  $\mu\text{M}$ ) for 2 h, in the absence or presence of different concentrations of poly I (50  $\mu\text{g}\cdot\text{mL}^{-1}$  or 100  $\mu\text{g}\cdot\text{mL}^{-1}$ ) for a competitive

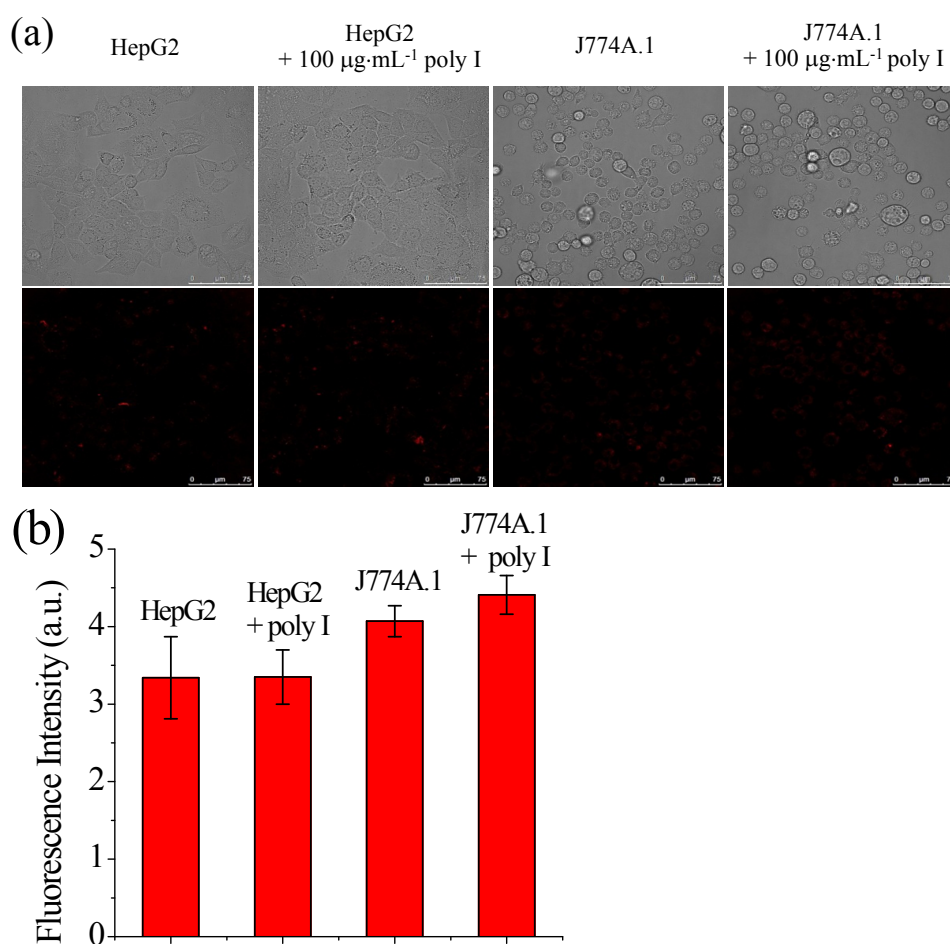
assay.



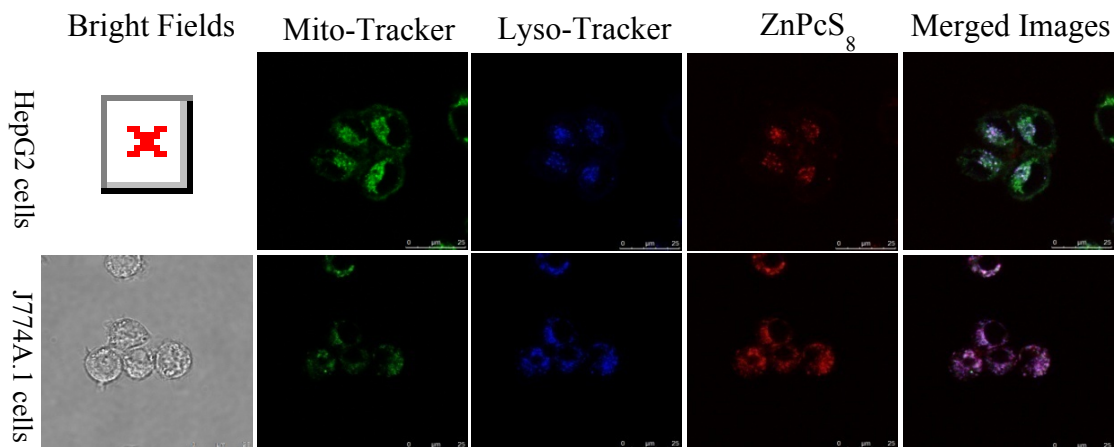
**Fig. S5** (a) Bright field (up row) and intracellular fluorescence (down row) images of HepG2 cells after incubation with ZnPcS<sub>8</sub> (2  $\mu\text{M}$ ) in the absence or presence of poly I (100  $\mu\text{g}\cdot\text{mL}^{-1}$ ). (b) The corresponding average intracellular fluorescence intensities. Data are expressed as the mean  $\pm$  SD (number of cells = 50). (c) The photocytotoxicities of ZnPcS<sub>8</sub> against HepG2 cells in the absence (■) and presence (●) of poly I (100  $\mu\text{g}\cdot\text{mL}^{-1}$ ). Data are expressed as mean  $\pm$  SD ( $n = 3$ ).



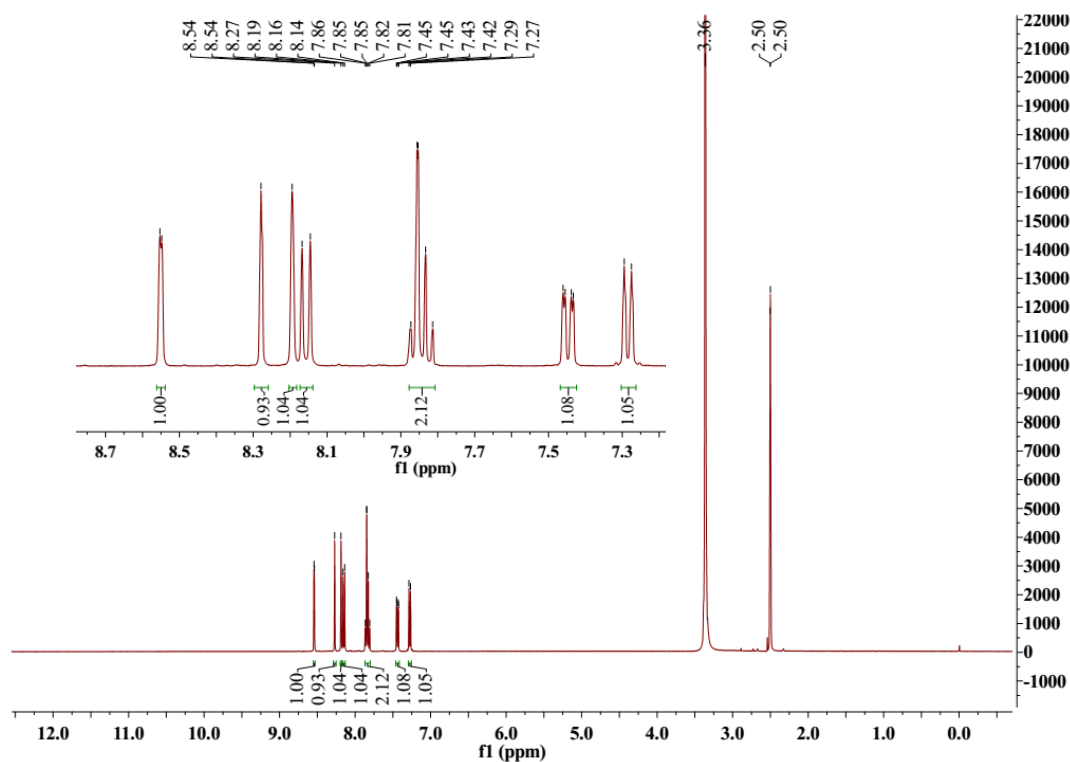
**Fig. S6** Structure of ZnPcS<sub>4</sub>. (Here only the major C<sub>4h</sub> isomers were showed for tetra-substituted phthalocyanines, which likely present the other isomers)



**Fig. S7** (a) Bright field (up row) and intracellular fluorescence (down row) images of HepG2 and J774A.1 cells after incubation with ZnPcS<sub>4</sub> (2  $\mu\text{M}$ ) in the absence or presence of poly I (100  $\mu\text{g}\cdot\text{mL}^{-1}$ ). (b) The corresponding average intracellular fluorescence intensities. Data are expressed as the mean  $\pm$  SD (number of cells = 50).



**Fig. S8** Subcellular localisation of ZnPcS<sub>8</sub> in HepG2 and J774A.1 cells. Visualisation of the intracellular fluorescence of HepG2 and J774A.1 cells by using filter sets specific for ZnPcS<sub>8</sub> (in red, column 4), Lyso-Tracker (in blue, column 3), or Mito-Tracker (in green, column 2). The corresponding superimposed images and the bright field images are given in column 5 and column 1, respectively.



**Fig. S9** <sup>1</sup>H NMR spectrum of **3** in DMSO-d<sub>6</sub>.

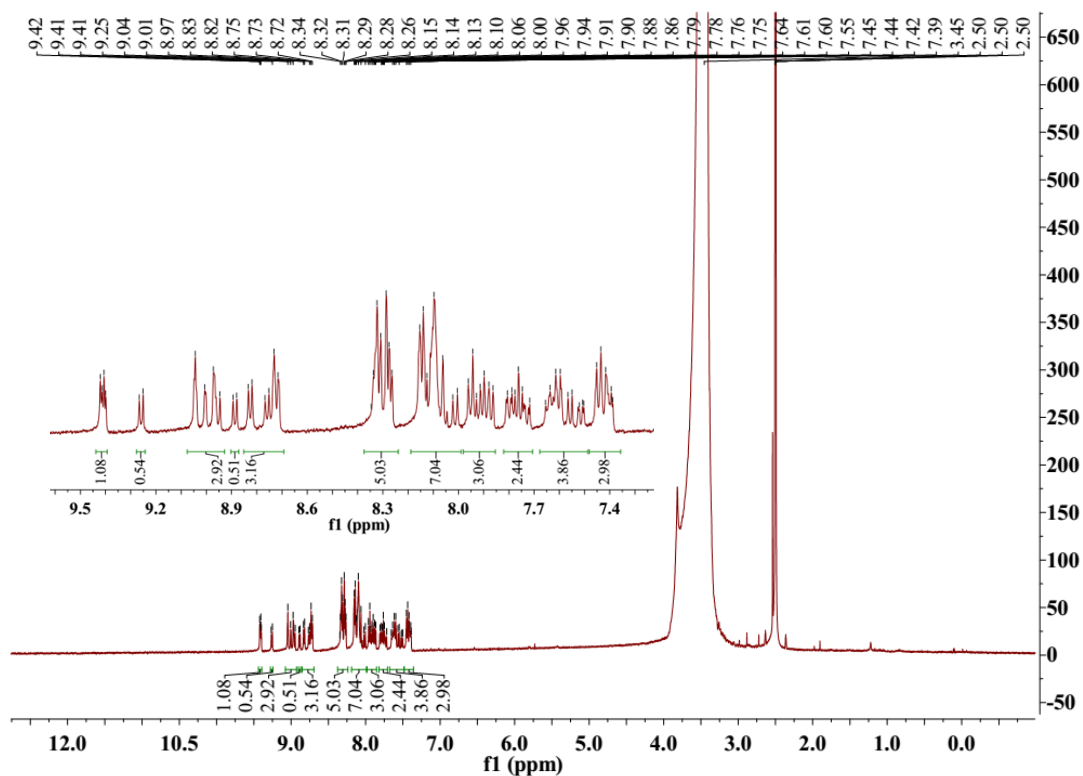


Fig. S10  $^1\text{H}$  NMR spectrum of  $\text{ZnPcS}_8$  in  $\text{DMSO-d}_6$ .

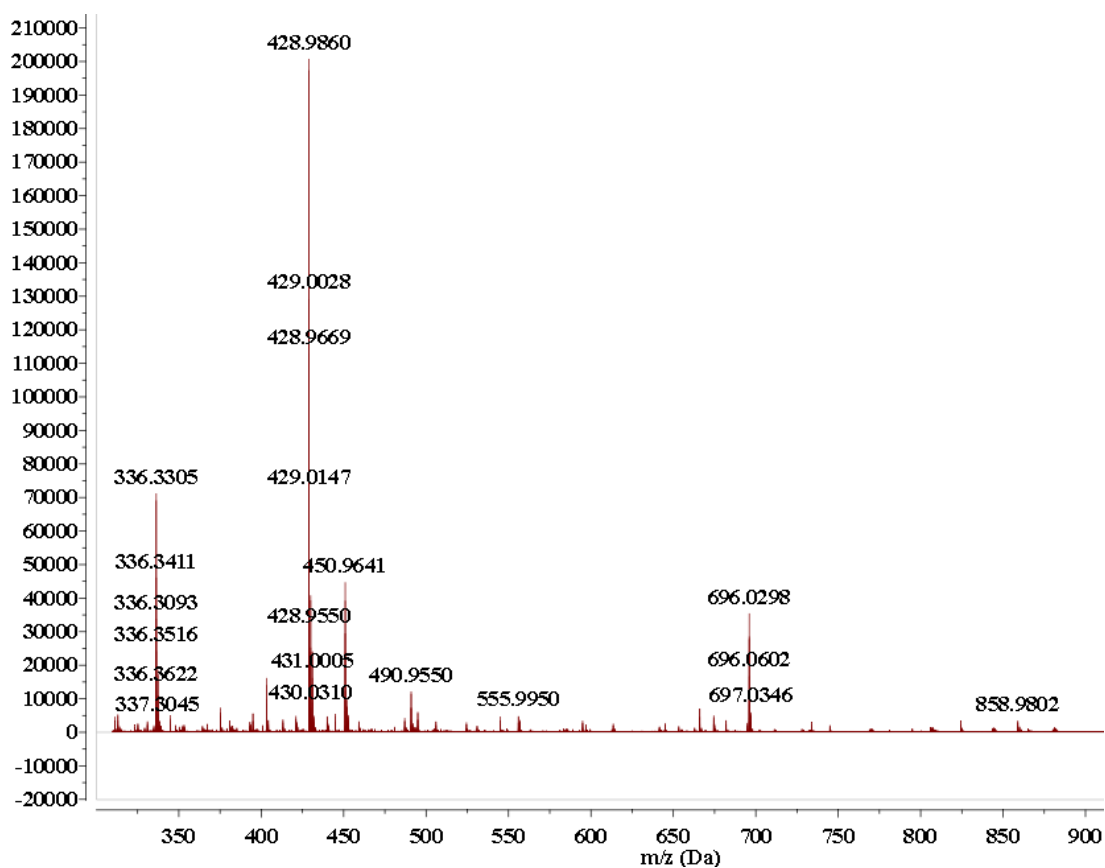


Fig. S11 HRMS spectrum of **3**.

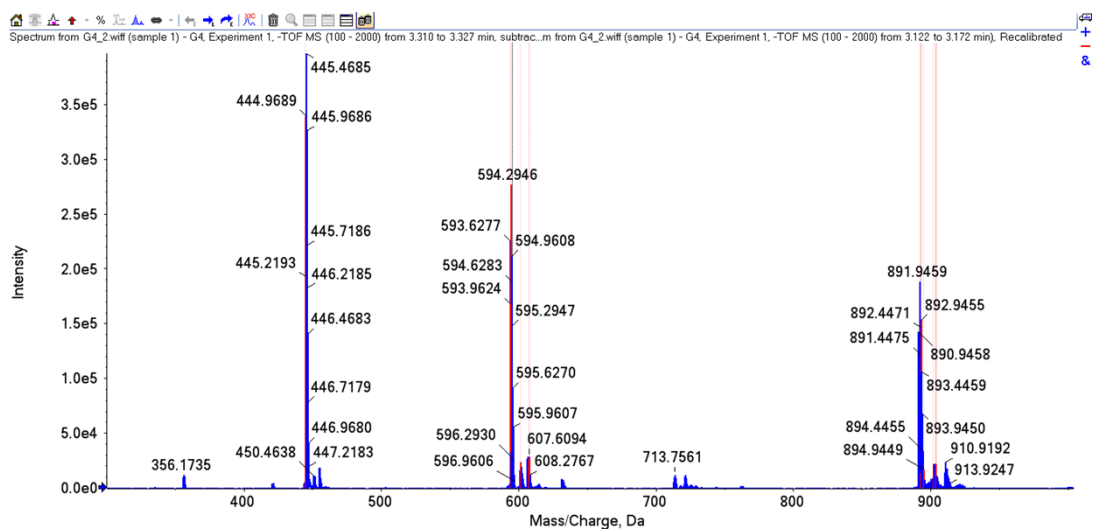


Fig. S12 HRMS spectrum of ZnPcS<sub>8</sub>.