

## Electronic Supplementary Information

### Novel Strategy for Targeting Photodynamic Therapy. Molecular Combo of Photodynamic Agent Zinc(II) Phthalocyanine and Small Molecule Target-based Anticancer Drug Erlotinib.

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## Experimental Section

All the reactions were performed under an atmosphere of nitrogen. Pentanol was distilled from sodium. Azide (oligoethylene glycol) was prepared as described.<sup>1</sup> All other solvents and reagents were of reagent grade and used as received; <sup>1</sup>H and <sup>13</sup>C{<sup>1</sup>H} NMR spectra were recorded on AVANCE III 400 (<sup>1</sup>H, 400; <sup>13</sup>C, 100.6 MHz) or 500 (<sup>1</sup>H, 500; <sup>13</sup>C, 125.4 MHz) spectrometer in CDCl<sub>3</sub> or DMSO-d<sub>6</sub>; chemical shifts were expressed in ppm relative to TMS (0 ppm); Electronic adsorption spectra were measured on a Beijing PuXi Tu-1901 spectrometer; and fluorescence spectra were obtained on a Varian carye clipse spectrometer; HRMS analyses were carried on a DECA-30000 LCQ Deca XP mass spectrometer; Subcellular location was carried on Olympus FV1000 confocal laser scanning microscope; In vivo fluorescence imaging was recorded on PE Fluorescence Molecular Tomography (FMT™ 2500LX); The purity of all the new compounds was determined by HPLC analysis and found to be ≥ 95%.

### 1. Synthesis

#### Synthesis of compound 1a and 1b

A mixture of azide (oligoethylene glycol) (3.29 mmol), Erlotinib (5.09 mmol), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.64 mmol), sodium ascorbate (1 mmol) in THF (10 ml), H<sub>2</sub>O (5 ml) and t-BuOH (10 ml) was stirred at 40 °C under an atmosphere of nitrogen for 5 h. The volatiles were evaporated under reduced pressure, and then the residue was mixed with water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The obtained organic extracts were dried over anhydrous MgSO<sub>4</sub>. After evaporation of solvent under reduced pressure, the residue was purified by silica gel column chromatography using CH<sub>2</sub>Cl<sub>2</sub>/ Ethyl acetate /CH<sub>3</sub>OH (20:1:1 V/V/V) as the eluent to afford the product (yield: 54% for **1a** and 63% for **1b**). **1a**: <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ = 3.37 (s, 3 H, CH<sub>3</sub>), 3.39 (s, 3 H, CH<sub>3</sub>), 3.39-3.42 (m, 2 H, CH<sub>2</sub>), 3.44-3.49 (m, 2 H, CH<sub>2</sub>), 3.50-3.54 (m, 2 H, CH<sub>2</sub>), 3.55-3.60 (m, 2 H, CH<sub>2</sub>), 3.74-3.78 (m, 2 H, CH<sub>2</sub>), 3.80 (vt, *J* = 4.8 Hz, 2 H, CH<sub>2</sub>), 3.90 (t, *J* = 5.2 Hz, 2 H, CH<sub>2</sub>), 4.30-4.35 (m, 4 H, CH<sub>2</sub>), 4.57-4.62 (m, 3 H, CH<sub>2</sub>+OH), 7.29 (br s, 1 H, Ar-H), 7.47 (t, *J* = 8.0 Hz, 1 H, Ar-H), 7.55 (d, *J* = 7.6 Hz, 1 H, Ar-H), 7.91 (d, *J* = 8.4 Hz, 1 H, Ar-H), 7.99 (s br, 1 H, Ar-H), 8.28 (s, 1 H, Ar-H), 8.56 (s, 1 H, triazole-H), 9.58 ppm (s, 1 H, pyrimidine-H); <sup>13</sup>C{<sup>1</sup>H} NMR (100.6 MHz, DMSO-d<sub>6</sub>): δ = 156.52, 153.89, 148.67, 146.72, 140.55, 131.58, 129.48, 128.56, 122.28, 122.21, 120.76, 119.20, 104.00, 72.81, 70.63, 70.56, 70.12, 69.16, 68.88, 68.55, 60.71, 58.86, 58.81, 50.10 ppm; HRMS (ESI): *m/z* calculated for C<sub>28</sub>H<sub>37</sub>N<sub>6</sub>O<sub>7</sub> [M+H]<sup>+</sup>, 569.2718; found, 569.2784. **1b**: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 3.47 (s, 6 H, CH<sub>3</sub>), 3.53-3.65 (m, 14 H, CH<sub>2</sub>), 3.68-3.72 (m, 3 H, CH<sub>2</sub>+OH), 3.81-3.92 (m, 6 H, CH<sub>2</sub>), 4.28 (t, *J* = 4.8 Hz, 2 H, CH<sub>2</sub>), 4.32 (t, *J* = 4.8 Hz, 2 H, CH<sub>2</sub>), 4.56 (t, *J* = 4.8 Hz, 2 H, CH<sub>2</sub>), 7.22 (s, 1 H, Ar-H), 7.44 (t, *J* = 7.6 Hz, 1 H, Ar-H), 7.50 (s, 1 H, Ar-H), 7.60 (d, *J* = 7.6 Hz, 1 H, Ar-H), 7.96 (d, *J* = 7.6 Hz, 1 H, Ar-H), 8.03 (s, 1 H, triazole-H), 8.06 (s, 1 H, Ar-H), 8.26 (br s, 1 H, N-H), 8.63 ppm (s, 1 H, pyrimidine-H); <sup>13</sup>C{<sup>1</sup>H} NMR (125.4 MHz, CDCl<sub>3</sub>): δ = 156.82, 154.06, 153.40, 148.52, 147.19, 146.98, 139.64, 131.09, 129.20, 121.98, 121.61, 121.02, 119.15, 109.51, 108.04, 102.98, 72.63, 70.71, 70.37, 70.31, 70.30, 70.15, 70.05, 69.31, 68.69, 68.12, 61.31, 59.09, 59.07, 50.19 ppm; HRMS (ESI): *m/z* calculated for C<sub>32</sub>H<sub>45</sub>N<sub>8</sub>O<sub>9</sub> [M+H]<sup>+</sup>, 657.3242; found, 657.3257.

#### Synthesis of 2a and 2b

**1a** or **1b** (1.76 mmol) and 2,3-Dicyano-1-nitrobenzene (3.23 mmol) were completely dissolved in DMF (10 ml) followed by addition of  $K_2CO_3$  (8.90 mmol) and stirred at 50 °C under an atmosphere of nitrogen for 5 h. DMF were evaporated under reduced pressure, and then the residue was mixed with saturated NaCl aqueous solution and extracted with  $CH_2Cl_2$ . The obtained organic extracts were dried over under reduced pressure. The residue was purified by silica gel column chromatography using  $CH_2Cl_2/CH_3OH$  (30:1 V/V) as the eluent to afford the product (yield: 64% for **2a** and 72% for **2b**). **2a**:  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  = 3.47 (s, 3 H,  $CH_3$ ), 3.48 (s, 3 H,  $CH_3$ ), 3.63-3.67 (m, 2 H,  $CH_2$ ), 3.69-3.73 (m, 2 H,  $CH_2$ ), 3.80 (t,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 3.83-3.89 (m, 4 H,  $CH_2$ ), 3.93 (t,  $J$  = 4.8 Hz, 2 H,  $CH_2$ ), 4.13 (t,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 4.28 (t,  $J$  = 4.8 Hz, 2 H,  $CH_2$ ), 4.32 (t,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 4.59 (t,  $J$  = 4.8 Hz, 2 H,  $CH_2$ ), 7.08 (d,  $J$  = 8.8 Hz, 1 H, Ar-H), 7.22 (s, 1 H, Ar-H), 7.26 (d,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.37 (d,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.39 (d,  $J$  = 7.2 Hz, 1 H, Ar-H), 7.51 (d,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.53 (d,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.86 (br s, 1 H, Ar-H), 7.88 (br s, 1 H, Ar-H), 8.01 (s, 1 H, triazole-H), 8.04 (s, 1 H, NH), 8.63 ppm (s, 1 H, pyrimidine-H);  $^{13}C\{^1H\}$  NMR (100.6 MHz,  $CDCl_3$ ):  $\delta$  = 161.12, 156.57, 154.32, 153.42, 148.84, 147.22, 147.19, 139.51, 134.62, 131.28, 129.35, 125.25, 121.71, 121.42, 121.04, 118.91, 117.07, 116.61, 115.41, 113.25, 109.39, 108.50, 104.60, 102.49, 70.81, 70.80, 70.58, 70.44, 69.47, 69.31, 68.94, 68.28, 59.21, 50.40 ppm; HRMS (ESI):  $m/z$  calculated for  $C_{36}H_{39}N_8O_7$   $[M+H]^+$ , 696.2936; found, 696.2993. **2b**:  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  = 3.47 (s, 3 H,  $CH_3$ ), 3.48 (s, 3 H,  $CH_3$ ), 3.57-3.73 (m, 12 H,  $CH_2$ ), 3.77 (t,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 3.83-3.92 (m, 6 H,  $CH_2$ ), 4.06 (t,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 4.27 (t,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 4.33 (t,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 4.59 (t,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 7.01 (d,  $J$  = 8.4 Hz, 1 H, Ar-H), 7.21 (s, 1 H, Ar-H), 7.27 (br s, 1 H, Ar-H), 7.41 (t,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.48-7.53 (m, 2 H, Ar-H), 7.60 (d,  $J$  = 7.6 Hz, 1 H, Ar-H), 7.96 (d,  $J$  = 8.0 Hz, 1 H, Ar-H), 8.04 (s, 1 H, triazole-H), 8.12 (s, 1 H, Ar-H), 8.23 (br s, 1 H, NH), 8.59 ppm (s, 1 H, pyrimidine-H);  $^{13}C\{^1H\}$  NMR (125.4 MHz,  $CDCl_3$ ):  $\delta$  = 161.06, 156.63, 154.33, 153.44, 148.78, 147.22, 147.13, 139.54, 134.58, 131.31, 129.32, 125.24, 121.70, 121.49, 121.02, 118.95, 116.99, 116.59, 115.39, 113.18, 109.42, 108.35, 104.55, 102.91, 71.15, 70.81, 70.45, 70.39, 70.35, 69.48, 69.36, 69.02, 68.98, 68.27, 59.22, 50.30 ppm; HRMS (ESI):  $m/z$  calculated for  $C_{40}H_{47}N_8O_9$   $[M+H]^+$ , 783.3460; found, 783.3490.

### Synthesis of **3a** and **3b**

**2a** or **2b** (0.35 mmol), 1,2-Dicyanobenzene (3.18 mmol) and  $Zn(OAc)_2 \cdot 2H_2O$  (1.76 mmol) in n-pentanol (10 ml) was heated to 100 °C under an atmosphere of nitrogen, then 0.8 ml DBU was added. The mixture was stirred at 150 °C for 5 h. The volatiles were evaporated under reduced pressure, and then the residue was mixed with water and extracted with  $CH_2Cl_2$ . The obtained organic extracts were dried over under reduced pressure. The residue was purified by silica gel column chromatography using  $CH_2Cl_2/CH_3OH$  (30:1 V/V) as the eluent to afford the product (yield: 23% for **3a** and 25% for **3b**). **3a**:  $^1H$  NMR (400 MHz,  $DMSO-d_6$ ):  $\delta$  = 3.30 (s, 3 H,  $CH_3$ ), 3.33 (s, 3 H,  $CH_3$ ), 3.65 (vt,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 3.66-3.70 (m, 2 H,  $CH_2$ ), 3.79 (br s, 2 H,  $CH_2$ ), 3.96 (vt,  $J$  = 4.4 Hz, 2 H,  $CH_2$ ), 4.03 (br s, 2 H,  $CH_2$ ), 4.11-4.18 (m, 4 H,  $CH_2$ ), 4.29 (br s, 2 H,  $CH_2$ ), 4.55 (vt,  $J$  = 4.8 Hz, 2 H,  $CH_2$ ), 4.69 (br s, 2 H,  $CH_2$ ), 6.90 (s, 1 H, NH), 7.31 (t,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.41 (br s, 1 H,  $Pc-H_\beta$ ), 7.47 (d,  $J$  = 7.6 Hz, 1 H, Ar-H), 7.70 (s, 1 H, Ar-H), 7.76 (s, 1 H, Ar-H), 7.86 (br s, 1 H, Ar-H), 8.04 (br s, 1 H,  $Pc-H_\beta$ ), 8.08-8.21 (m, 7 H,  $Pc-H_\beta$ +Ar-H), 8.50 (s, 1 H, triazole-H), 8.64 (br s, 1 H,  $Pc-H_\alpha$ ), 8.99 (br s, 1 H,  $Pc-H_\alpha$ ), 9.15 (br s, 2 H,  $Pc-H_\alpha$ ), 9.22 (br s, 3 H,  $Pc-H_\alpha$ ), 9.36 ppm (s, 1 H, pyrimidine-H);  $^{13}C\{^1H\}$  NMR (125.4 MHz,  $DMSO-d_6$ ):  $\delta$  = 156.60, 155.78, 153.82, 153.09, 152.93, 152.78, 152.68, 152.61, 148.32, 146.96, 146.67, 140.38, 138.47, 138.22, 138.18, 138.09, 131.42, 130.89, 129.58, 129.36, 124.91, 122.63, 122.50, 122.24, 121.92, 120.60, 119.01, 115.15, 113.62, 109.19, 108.13, 107.47, 103.41, 70.72, 70.47, 70.38, 69.22, 68.98,

68.67, 68.40, 67.10, 58.80, 50.1 ppm; HRMS (ESI):  $m/z$  calculated for  $C_{60}H_{55}N_{10}O_7Zn$   $[M+H]^+$ , 1143.3351; found, 1143.3308. **3b**:  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 3.31 (s, 3 H, CH<sub>3</sub>), 3.32 (s, 3 H, CH<sub>3</sub>), 3.39-3.52 (m, 8 H, CH<sub>2</sub>), 3.64-3.72 (m, 6 H, CH<sub>2</sub>), 3.80 (t,  $J$  = 4.8 Hz, 2 H, CH<sub>2</sub>), 3.98 (t,  $J$  = 4.8 Hz, 2 H, CH<sub>2</sub>), 4.15-4.20 (m, 4 H, CH<sub>2</sub>), 4.27-4.31 (m, 2 H, CH<sub>2</sub>), 4.52 (t,  $J$  = 4.8 Hz, 2 H, CH<sub>2</sub>), 4.74 (br s, 2 H, CH<sub>2</sub>), 6.93 (s, 1 H, NH), 7.40 (t,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.49 (d,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.51 (d,  $J$  = 8.0 Hz, 1 H, Pc-H $_{\beta}$ ), 7.74 (s, 1 H, Ar-H), 7.82 (d,  $J$  = 8.0 Hz, 1 H, Ar-H), 7.93 (t,  $J$  = 7.6 Hz, 1 H, Pc-H $_{\beta}$ ), 8.08-8.20 (m, 7 H, Pc-H $_{\beta}$ +Ar-H), 8.23 (s, 1 H, Ar-H), 8.45 (s, 1 H, triazole-H), 8.71 (d,  $J$  = 7.2 Hz, 1 H, Pc-H $_{\alpha}$ ), 9.04 (d,  $J$  = 7.2 Hz, 1 H, Pc-H $_{\alpha}$ ), 9.15-9.28 (m, 5 H, Pc-H $_{\alpha}$ ), 9.43 ppm (s, 1 H, pyrimidine-H);  $^{13}C\{^1H\}$  NMR (125.4 MHz, DMSO- $d_6$ ):  $\delta$  = 160.50, 156.72, 153.89, 153.13, 152.94, 152.65, 152.47, 152.27, 152.15, 152.09, 148.39, 147.02, 146.71, 140.44, 139.97, 138.17, 138.11, 137.99, 137.83, 131.54, 131.04, 129.49, 129.42, 129.30, 129.13, 123.40, 122.56, 122.52, 122.36, 122.25, 122.20, 120.79, 119.24, 118.02, 109.25, 108.23, 105.71, 103.60, 70.64, 70.54, 70.46, 70.45, 70.40, 70.34, 70.18, 69.67, 69.15, 68.76, 68.43, 68.31, 58.82, 58.79, 50.13 ppm; HRMS (ESI):  $m/z$  calculated for  $C_{64}H_{63}N_{10}O_9Zn$   $[M+H]^+$ , 1231.3875; found, 1231.3858.

## 2. Photophysical and photochemical studies

Fluorescence quantum yields ( $\Phi_F$ ) were measured according to the equation:  $\Phi_F$  (sample) =  $(F_{\text{sample}} / F_{\text{reference}}) (A_{\text{reference}} / A_{\text{sample}}) (\eta_{\text{sample}}^2 / \eta_{\text{reference}}^2) \Phi_F$  (reference), where  $F$ ,  $A$ , and  $\eta$  are the integrated fluorescence area ( $E_x$  = 610 nm, area under the emission peak), the absorbance at the excitation wavelength (610 nm), and the refractive index of the solvent, respectively. Unsubstituted zinc(II) phthalocyanine (ZnPc) in DMF was used as the reference with a value for  $\Phi_F$  = 0.28. The  $\Phi_F$  measurements were performed using diluted solutions (absorbances of PSs at 610 nm are 0.03-0.05). Singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) were measured using 1,3-Diphenylisobenzofuran (DPBF) as the scavenger. Light laser (670 nm) was used as the light source and ZnPc was selected as the reference ( $\Phi_{\Delta}$  = 0.56 in DMF).

## 3. In vitro test

### Cellular Uptake.

The HELF and HepG2 cells suspension were plated on a culture dish (10 000 : 10 000 cells) and incubated overnight at 37 °C under 5% CO<sub>2</sub>. Then the cells were exposed to 10  $\mu$ M phthalocyanine and incubated for 24 h. After incubation, the cells were rinsed with PBS for three times and the intracellular fluorescence caused by phthalocyanines was recorded and statistically analyzed by Confocal laser scanning microscope.

### Intracellular ROS Measurements

Reactive oxygen species (ROS) were measured on the basis of the intracellular peroxide-dependent oxidation of DCFH-DA to form the fluorescent compound 2',7'-dichlorofluorescein (DCF). Cells were seeded on to a cell culture dish (diameter = 35 mm) at a density of 50 000 cells per well and cultured overnight. Then fresh medium containing 10  $\mu$ M phthalocyanine was added and cells were incubated for 12 h in dark. After washing three times with PBS, 10  $\mu$ M DCFH-DA was added and cells were incubated for 20 min. The old medium was discarded and washed three times with PBS followed by illumination for 10 min. After another 10 min incubation, the cells were lysed with 1% SDS (1 mL) for 10 min at a table concentrator and then the DCF fluorescence was measured by fluorescence spectrometer (excitation/emission: 488/525 nm).

### Cytotoxicity assay.

HepG2 cells were seeded onto 96-well plates at 10000 cells per well and incubated overnight. Phthalocyanines were diluted to the needed concentration and added to six plicate wells. After another 24 h



incubation, the medium containing drugs were replaced by fresh medium and the cells were exposed to a light dose of  $1.5 \text{ J}\cdot\text{cm}^{-2}$ . The cells after irradiation were incubated again for 24 h and then a MTT solution in PBS (10  $\mu\text{l}$ ,  $5 \text{ mg}\cdot\text{ml}^{-1}$ ) was added to each well followed by incubation for 4 h. 100  $\mu\text{l}$  DMSO was then added into each well. The plate was incubated at room temperature for 30 min. The absorbance at 570 nm at each well was taken by a microplate reader. For the dark toxicity, the procedures are almost the same as above; expect that there is no irradiation. The survival curves plotted as a function of concentration of PSs and  $\text{IC}_{50}$  values were calculated.

#### **Intracellular localisation**

Approximately 10 0000 HepG2 cells were plated on a cell culture dish (diameter = 35 mm) and incubated overnight. Then the medium was replaced by fresh medium containing 10  $\mu\text{M}$  phthalocyanine and incubated for 24 h. After incubation, the cells were rinsed with PBS three times and incubated with MitoTracker® Green FM (invitrogen, 2  $\mu\text{M}$  in culture medium, Incubated for 60 min) or LysoTracker® Green DND-26 (invitrogen, 0.25  $\mu\text{M}$  in culture medium, Incubated for 30 min). Then the cells were rinsed with PBS three times again and the subcellular localisation of these dyes was revealed by comparing the intracellular fluorescence images caused by the fluorescent probe and phthalocyanines.

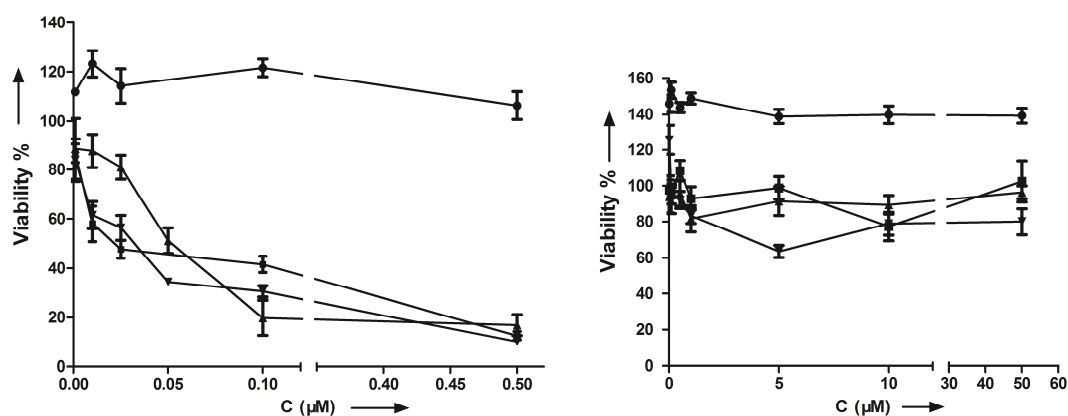
#### **4. In vivo test**

The in vivo studies were conducted in 4-6 week old male mice. Nude mice bearing A431 cells (weight  $\approx 20 \text{ g}$ , Model Animal Resource Center, Nan Jing) were intravenously injected with **3a** or **3b** through a tail vein at the drug dose of  $0.53 \text{ mM}\cdot\text{g}^{-1}$ . Then the mice were imaged by FMT at different time. The content of phthalocyanine in tumor tissues was recorded by FMT as pmol. The experiments were carried out in accordance with the guidelines issued by the Ethical Committee of Fuzhou University (Repubilc of China).

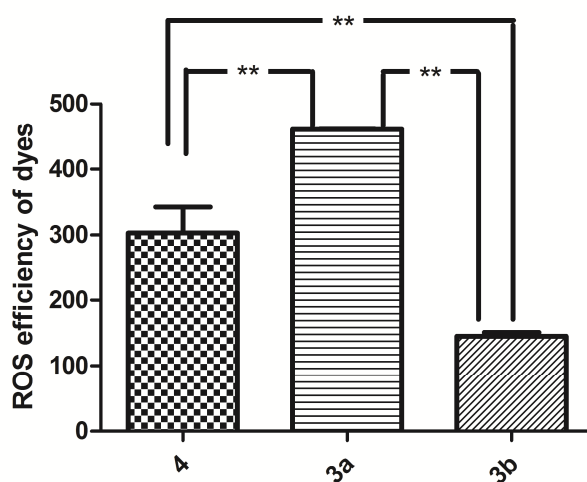
#### **Reference:**

1. G. Lu, K. Burgess, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3902.

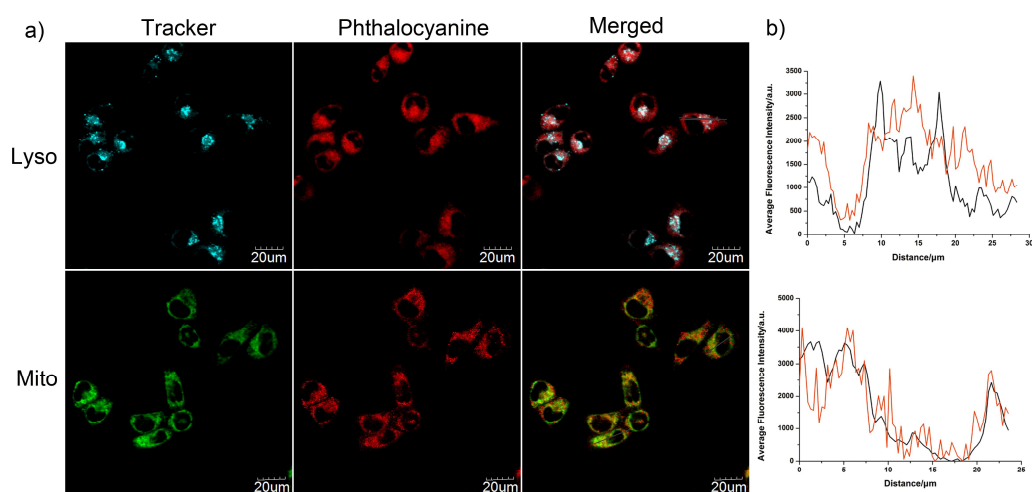




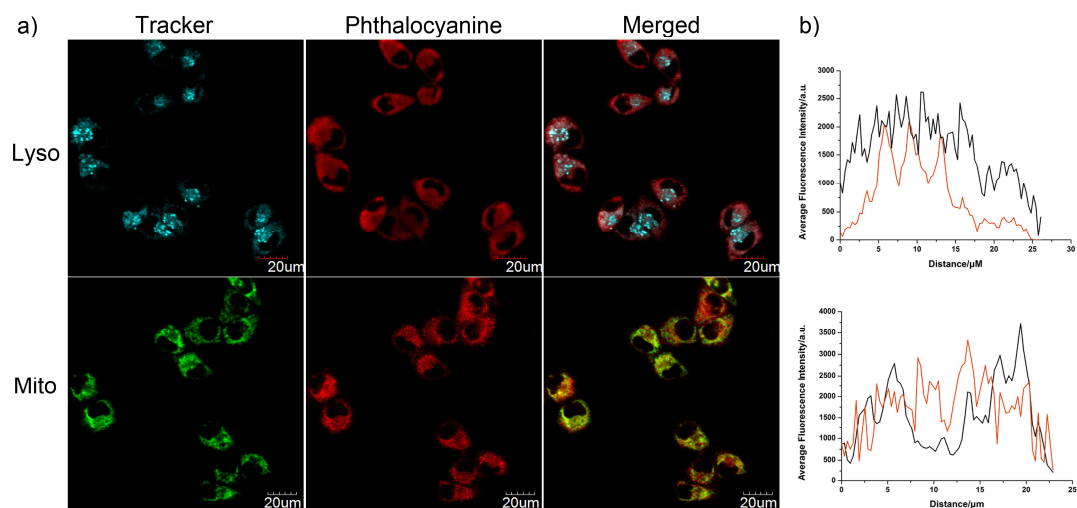
**Fig. S3.** Cytotoxic effects of phthalocyanines **3a** (■), **3b** (▲), **4** (▼) and Eriotinb (●) towards HepG2 cells in light ( $1.5 \text{ J} \cdot \text{cm}^{-2}$ ) (left) and dark (right) using the MTT assay. Data are expressed as mean values  $\pm$  standard error of the mean value (SEM) of three independent experiments, each performed in quadruplicate.



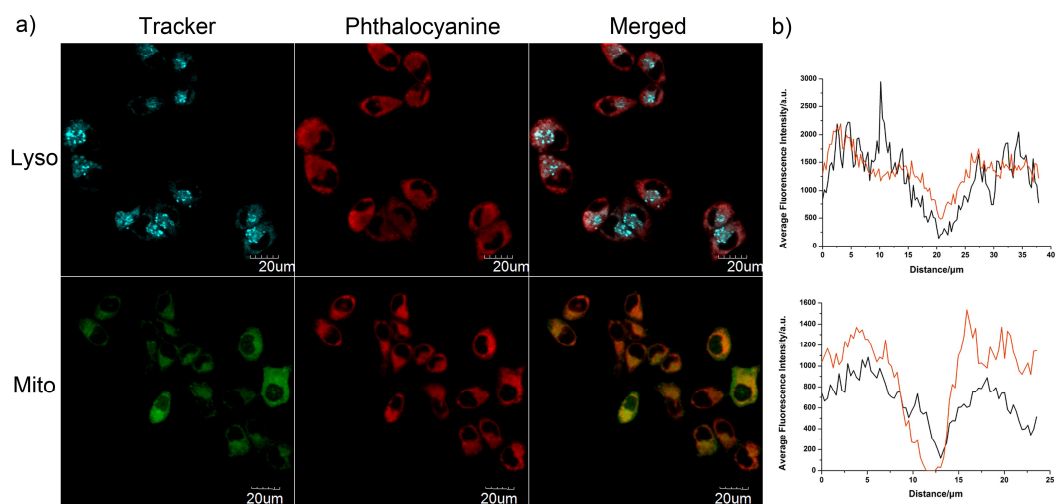
**Fig. S4.** Cellular ROS generation efficiency for **3a**, **3b** and **4** at the incubation concentration of  $10 \mu\text{M}$ . Values are means  $\pm$  SD. Statistical significant \*\* ( $P < 0.01$ ).



**Fig. S5.** a) Confocal fluorescence images of HepG2 cells after incubation with **3a** for 24 h (at  $10 \mu\text{M}$ ). b) Fluorescence intensity profiles of **3a** (red line) and Tracker (black line) traced along the white lines in the merged images.



**Fig. S6.** a) Confocal fluorescence images of HepG2 cells after incubation with **3b** for 24 h (at 10  $\mu\text{M}$ ). b) Fluorescence intensity profiles of **3b** (red line) and Tracker (black line) traced along the white lines in the merged images.



**Fig. S7.** a) Confocal fluorescence images of HepG2 cells after incubation with **4** for 24 h (at 10  $\mu\text{M}$ ). b) Fluorescence intensity profiles of **4** (red line) and Tracker (black line) traced along the white lines in the merged images.

