Electronic Supplementary Material (ESI) for Chemical Communications

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.

Feng-Ling Zhang[1], Qi Huang[1], Ke Zheng, Jun Li, Jian-Yong Liu, Jin-Ping Xue**

Experimental Section

Experimental Section

All the reactions were performed under an atmosphere of nitrogen. Pentanol was distilled from sodium. Azide (oligoethylene glycol) was prepared as described.[1] All other solvents and reagents were of reagent grade and used as received; ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on AVANCE III 400 (^{1}H, 400; ^{13}C, 100.6 MHz) or 500 (^{1}H, 500; ^{13}C, 125.4 MHz) spectrometer in $CDCl_3$ or DMSO-d_6; 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 DECAX-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^{TM} 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), $CuSO_4.5H_2O$ (0.64 mmol), sodium ascorbate (1 mmol) in THF (10 ml), H_2O (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_2Cl_2. The obtained organic extracts were dried over anhydrous $MgSO_4$. After evaporation of solvent under reduced pressure, the residue was purified by silica gel column chromatography using CH_2Cl_2/ Ethyl acetate /CH_3OH (20:1:1 V/V/V) as the eluent to afford the product (yield: 54% for **1a** and 63% for **1b**). **1a**: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.37 (s, 3 H, CH_3), 3.39 (s, 3 H, CH_3), 3.39-3.42 (m, 2 H, CH_2), 3.44-3.49 (m, 2 H, CH_2), 3.50-3.54 (m, 2 H, CH_2), 3.55-3.60 (m, 2 H, CH_2), 3.74-3.78 (m, 2 H, CH_2), 3.80 (vt, *J* = 4.8 Hz, 2 H, CH_2), 3.90 (t, *J* = 5.2 Hz, 2 H, CH_2), 4.30-4.35 (m, 4 H, CH_2), 4.57-4.62 (m, 3 H, CH_2+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); $^{13}C\{^{1}H\}$ NMR (100.6 MHz, DMSO-d_6): 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 $C_{28}H_{37}N_6O_7$ $[M+H]^+$, 569.2718; found, 569.2784. **1b**: ^{1}H NMR (400 MHz, $CDCl_3$.): δ = 3.47 (s, 6 H, CH_3), 3.53-3.65 (m, 14 H, CH_2), 3.68-3.72 (m, 3 H, CH_2+OH), 3.81-3.92 (m, 6 H, CH_2), 4.28 (t, *J* = 4.8 Hz, 2 H, CH_2), 4.32 (t, *J* = 4.8 Hz, 2 H, CH_2), 4.56 (t, *J* = 4.8 Hz, 2 H, CH_2), 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); $^{13}C\{^{1}H\}$ NMR (125.4 MHz, $CDCl_3$): 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 $C_{32}H_{45}N_8O_9$ $[M+H]^+$, 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**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 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{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ = 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**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 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{^{1}H} NMR (125.4 MHz, $CDCl_3$): δ = 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 $Zn(OAc)\cdot 2H_2O$ (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**. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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_β), 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_β), 8.08-8.21 (m, 7 H, Pc-H_β+Ar-H), 8.50 (s, 1 H, triazole-H), 8.64 (br s, 1 H, Pc-H_α), 8.99 (br s, 1 H, Pc-H_α), 9.15 (br s, 2 H, Pc-H_α), 9.22 (br s, 3 H, Pc-H_α), 9.36 ppm (s, 1 H, pyrimidine-H); ^{13}C{^{1}H} NMR (125.4 MHz, DMSO-d_6): δ = 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): δ = 3.31 (s, 3 H, CH_3), 3.32 (s, 3 H, CH_3), 3.39-3.52 (m, 8 H, CH_2), 3.64-3.72 (m, 6 H, CH_2), 3.80 (t, *J* = 4.8 Hz, 2 H, CH_2), 3.98 (t, *J* = 4.8 Hz, 2 H, CH_2), 4.15-4.20 (m, 4 H, CH_2), 4.27-4.31 (m, 2 H, CH_2), 4.52 (t, *J* = 4.8 Hz, 2 H, CH_2), 4.74 (br s, 2 H, CH_2), 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-$H_β$), 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-$H_β$), 8.08-8.20 (m, 7 H, Pc-$H_β$+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-$H_α$), 9.04 (d, *J* = 7.2 Hz, 1 H, Pc-$H_α$), 9.15-9.28 (m, 5 H, Pc-$H_α$), 9.43 ppm (s, 1 H, pyrimidine-H); $^{13}C\{^1H\}$ NMR (125.4 MHz, DMSO-d_6): δ = 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. Photophyscial and photochemical studies

Fluorescence quantum yields ($Φ_F$) were measured according to the equation: $Φ_{F\ (sample)} = (F_{sample} / F_{reference})(A_{reference} / A_{sample})(η_{sample}^2 / η_{reference}^2) Φ_{F\ (reference)}$, where F, A, and η 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 $Φ_F$ = 0.28. The $Φ_F$ measurements were performed using diluted solutions (absorbances of PSs at 610 nm are 0.03-0.05). Singlet oxygen quantum yields ($Φ_Δ$) 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 ($Φ_Δ$ = 0.56 in DMF).

3. In vitro test

Cellular Uptake.

The HELF and HepG2 cells suspension were plated on a culture dish (10 0000 : 10 0000 cells) and incubated overnight at 37 °C under 5% CO_2. Then the cells were exposed to 10 *μ*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 0000 cells per well and cultured overnight. Then fresh medium containing 10 μM phthalocyanine was added and cells were incubated for 12 h in dark. After washing three times with PBS, 10 μM 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 $J \cdot cm^{-2}$. The cells after irradiation were incubated again for 24 h and then a MTT solution in PBS (10 μl, 5 $mg \cdot ml^{-1}$) was added to each well followed by incubation for 4 h. 100 μl 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 IC_{50} values were calculated.

Intracellular localisation

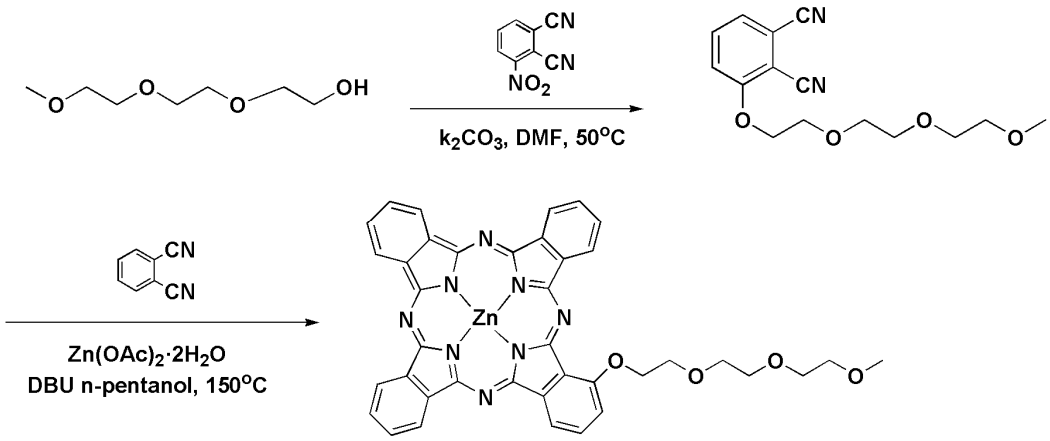
Approximately 10 0000 HepG2 cells were plated on a cell culture dish (diameter = 35 mm) and incubated overnight. Then the medium was replaced by fresh medium containing 10 μM phthalocyanine and incubated for 24 h. After incubation, the cells were rinsed with PBS three times and incubated with MitoTracker@ Green FM (invitrogen, 2 μM in culture medium, Incubated for 60 min) or LysoTracker@ Green DND-26 (invitrogen, 0.25 μM 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 ≈20 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 $mM.g^{-1}$. 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 (Republic of China).

Reference:

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



Scheme S1. Synthesis of phthalocyanine **4.**

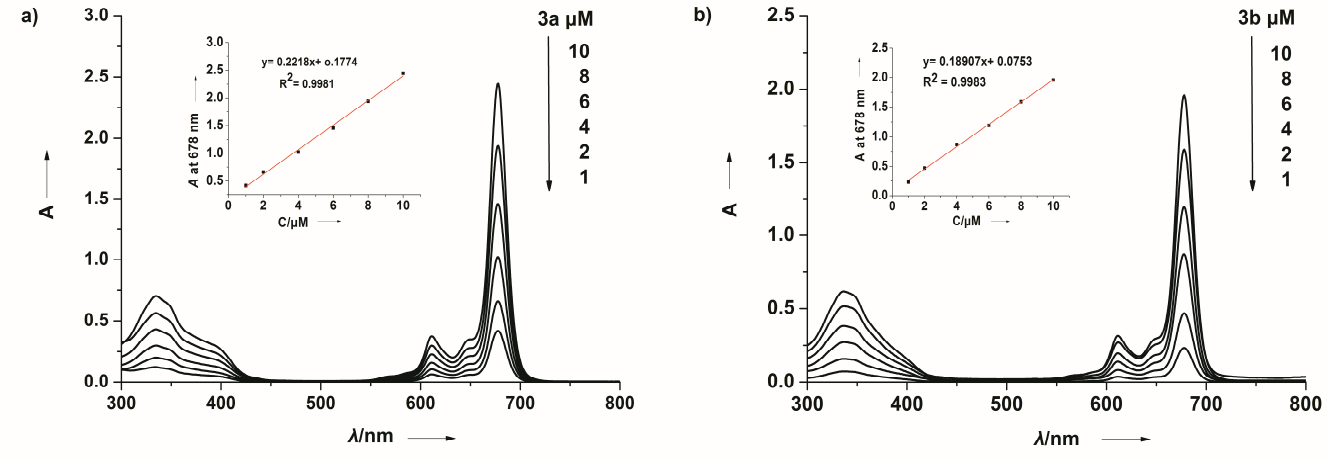


Fig. S1. Electronic absorption spectra of **3a** and **3b** in DMF at different concentrations. The inset plots the absorbance at 685 nm and 686 nm versus the concentration of **3a** and **3b**, respectively.

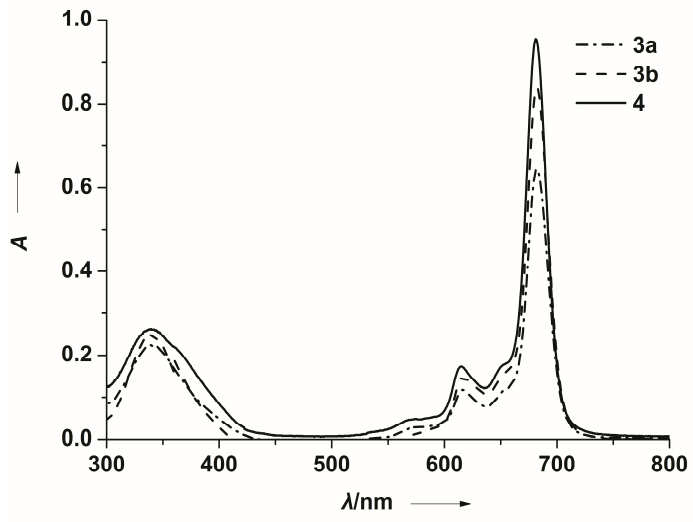


Fig. S2. Electronic absorption spectra of **3a**, **3b** and **4** in the RPMI 1640 culture medium (all at 3.5 μM).

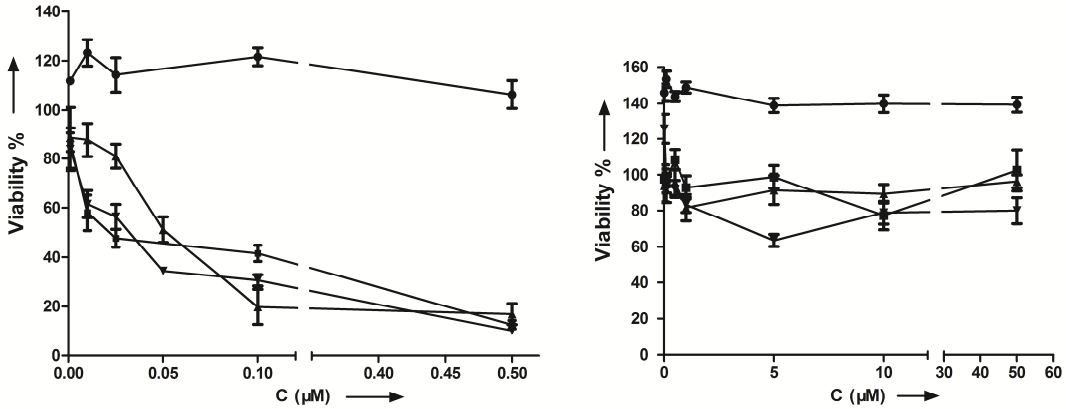


Fig. S3. Cytotoxic effects of phthalocyanines **3a** (■), **3b** (▲), **4** (▼) and Erotinb (●) towards HepG2 cells in light (1.5 J·cm^{-2}) (left) and dark (right) using the MTT assay. Data are expressed as mean values ± standard error of the mean value (SEM) of three independent experiments, each performed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

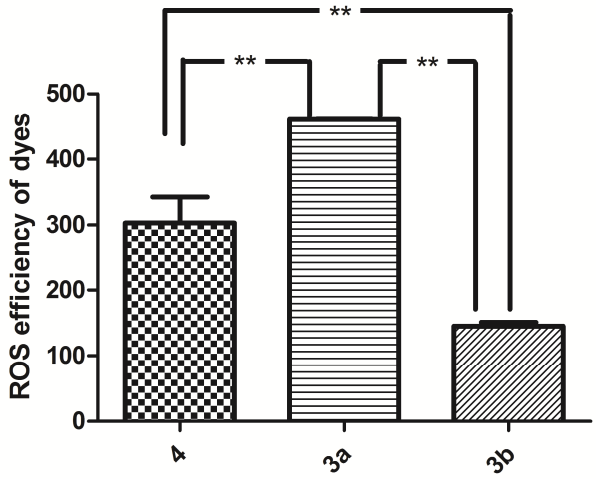


Fig. S4. Cellular ROS generation efficiency for **3a**, **3b** and **4** at the incubation concentration of 10 μM. Values are means ± SD. Statistical significant ** ($P < 0.01$).

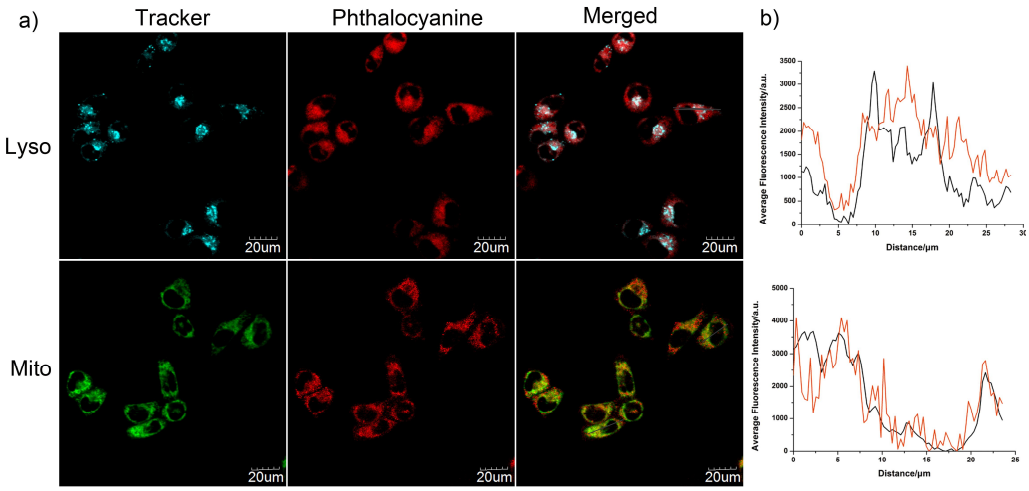


Fig. S5. a) Confocal fluorescence images of HepG2 cells after incubation with **3a** for 24 h (at 10 μM). b) Fluorescence intensity profiles of **3a** (red line) and Tracker (black line) traced along the white lines in the merged images.

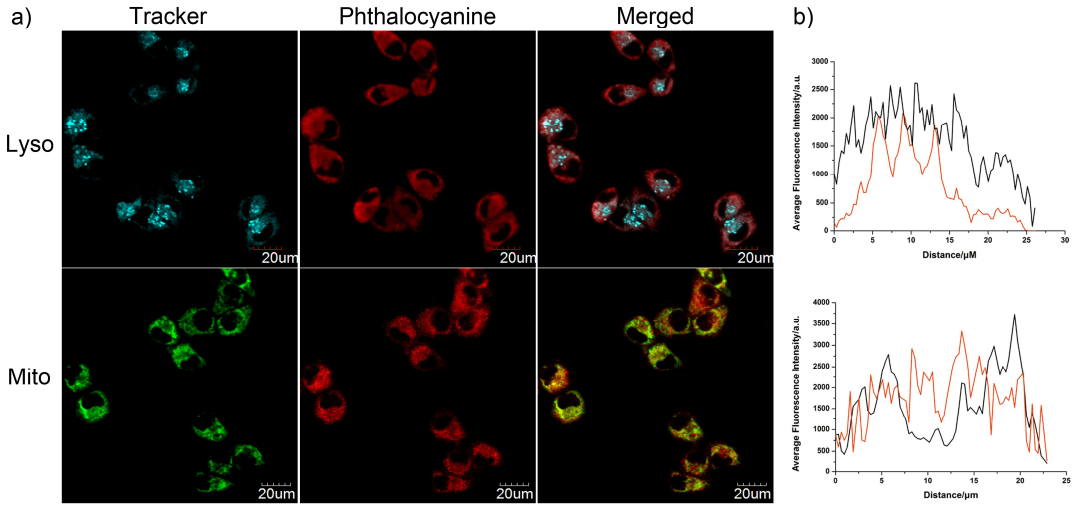


Fig. S6. a) Confocal fluorescence images of HepG2 cells after incubation with **3b** for 24 h (at 10 μM). b) Fluorescence intensity profiles of **3b** (red line) and Tracker (black line) traced along the white lines in the merged images.

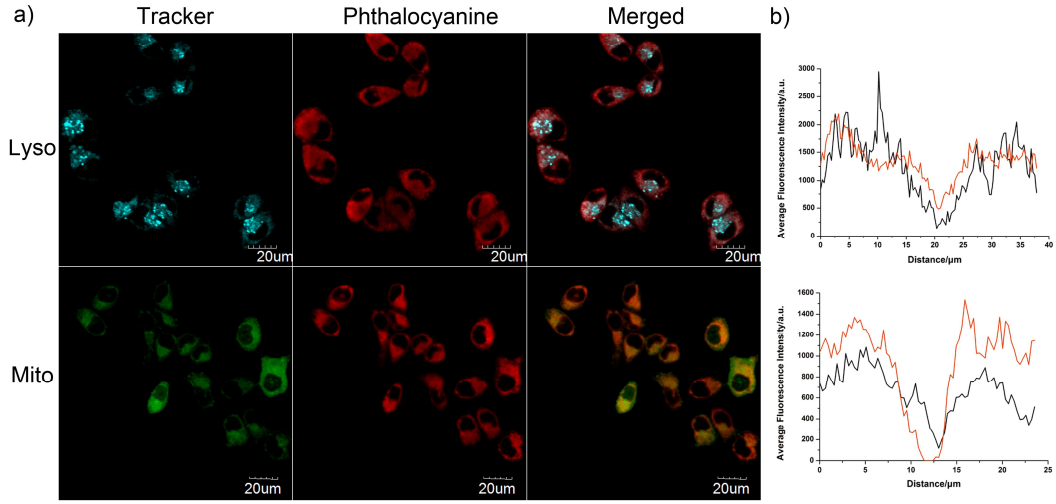


Fig. S7. a) Confocal fluorescence images of HepG2 cells after incubation with **4** for 24 h (at 10 μM). b) Fluorescence intensity profiles of **4** (red line) and Tracker (black line) traced along the white lines in the merged images.

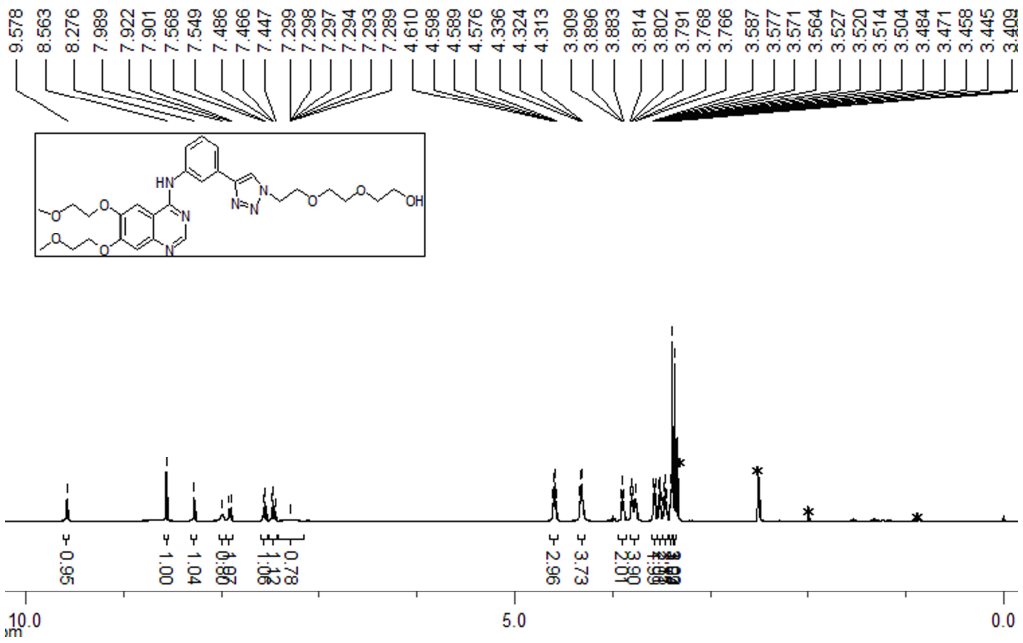


Fig. S8. ^{1}H NMR spectrum of **1a**

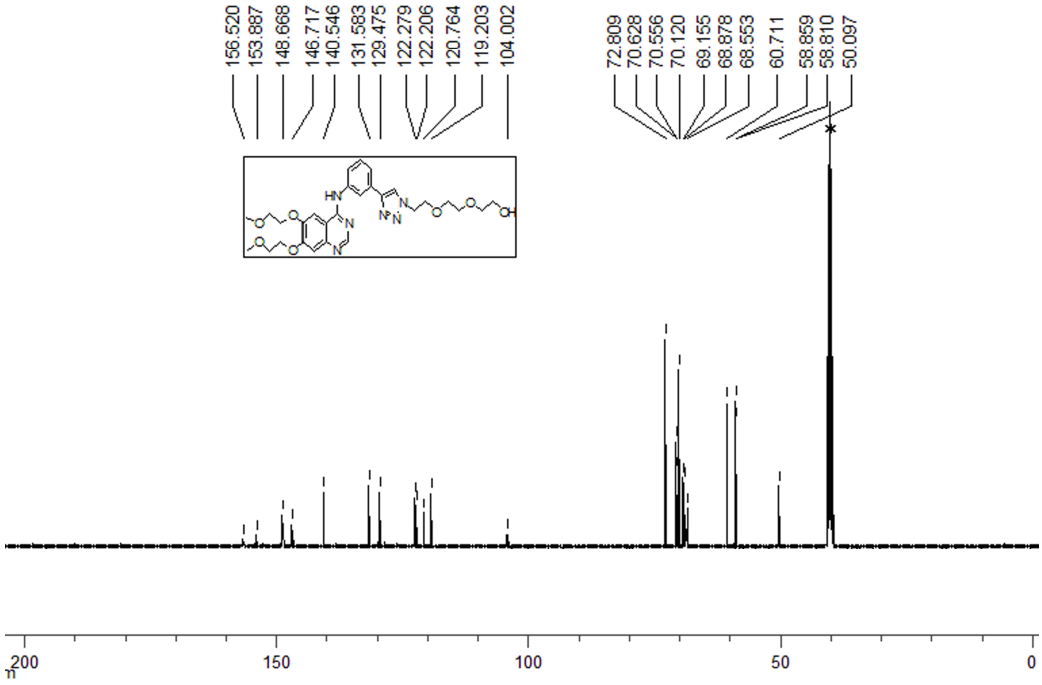


Fig. S9. $^{13}C\{^{1}H\}$ NMR spectrum of **1a**

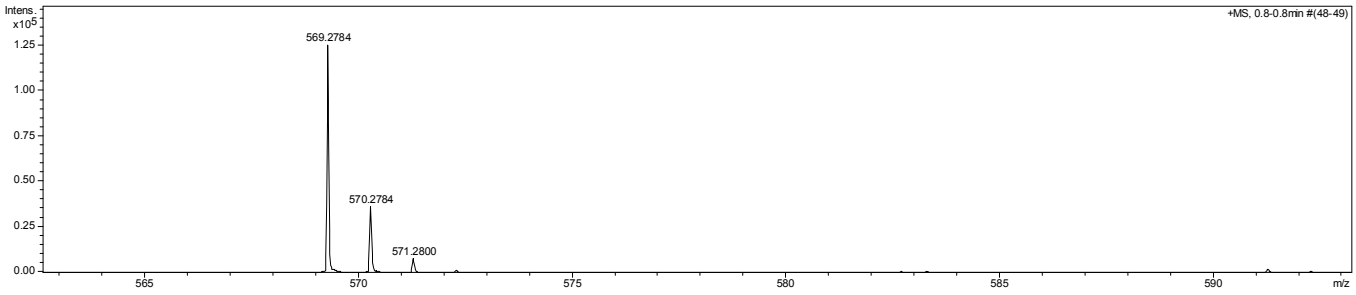


Fig. S10. HRMS spectrum of **1a**

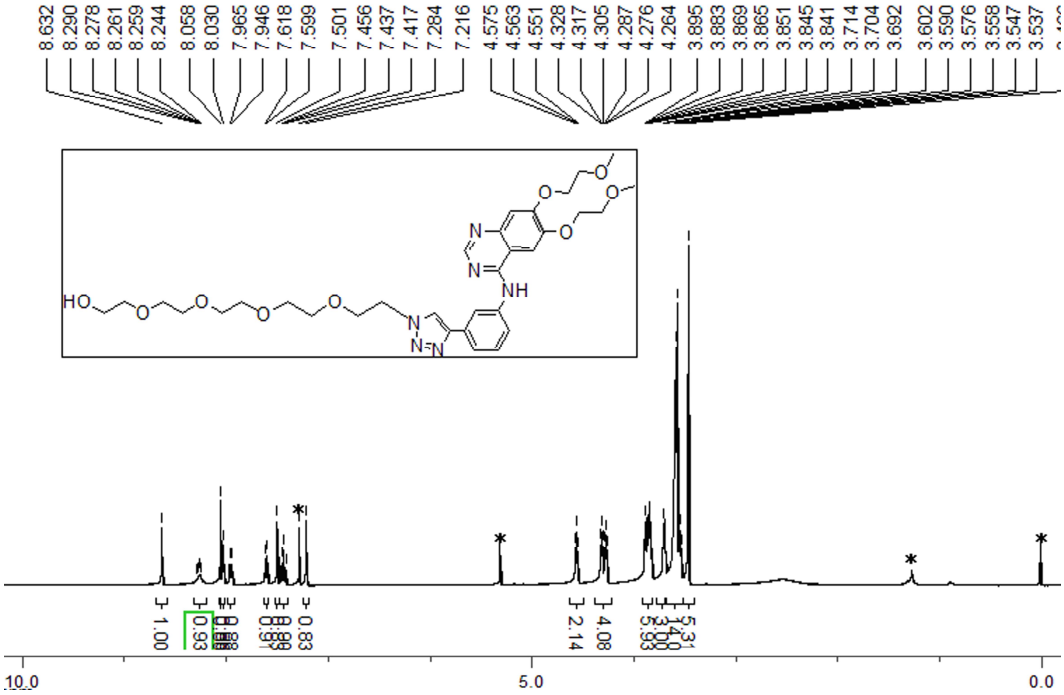


Fig. S11. ^{1}H NMR spectrum of **1b**

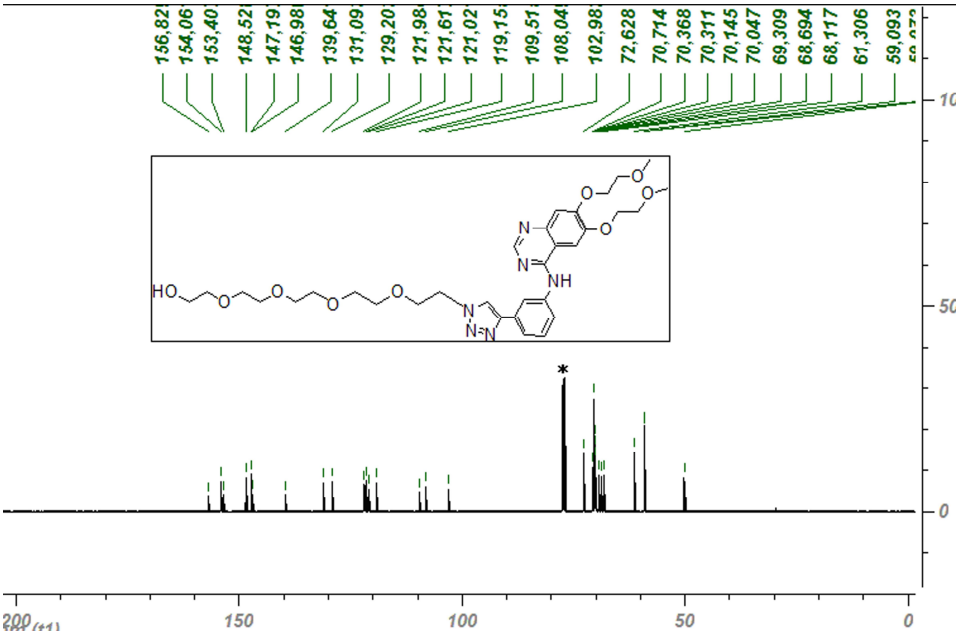


Fig. S12. ^{13}C{^{1}H} NMR spectrum of **1b**

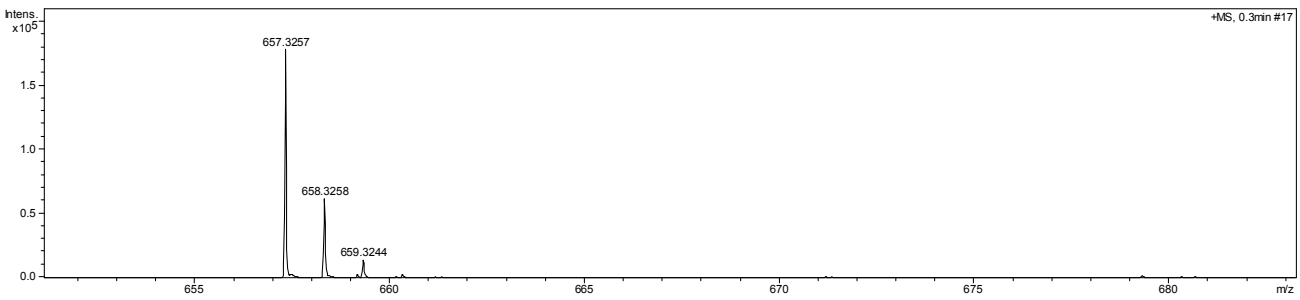


Fig. S13. HRMS spectrum of **1b**

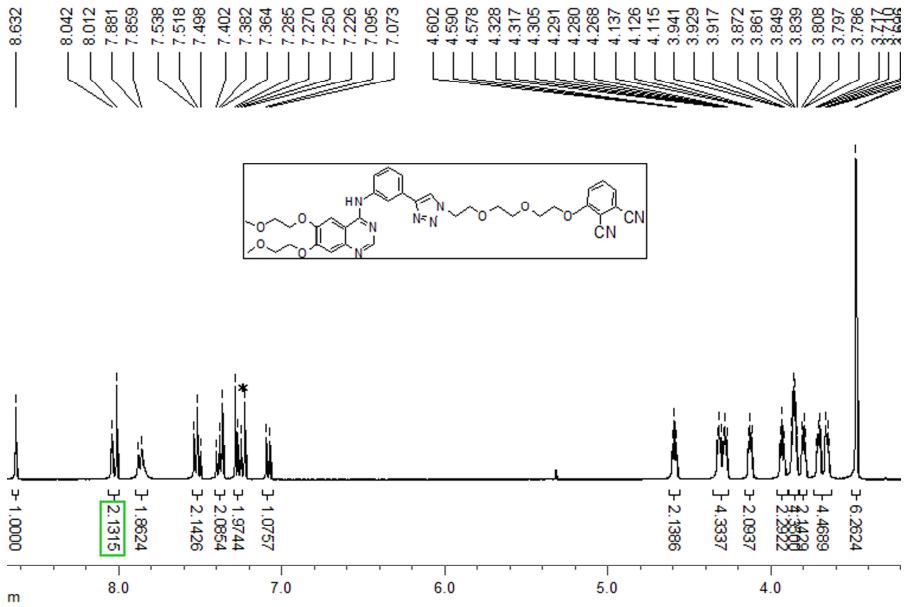


Fig. S14. ^{1}H NMR spectrum of **2a**

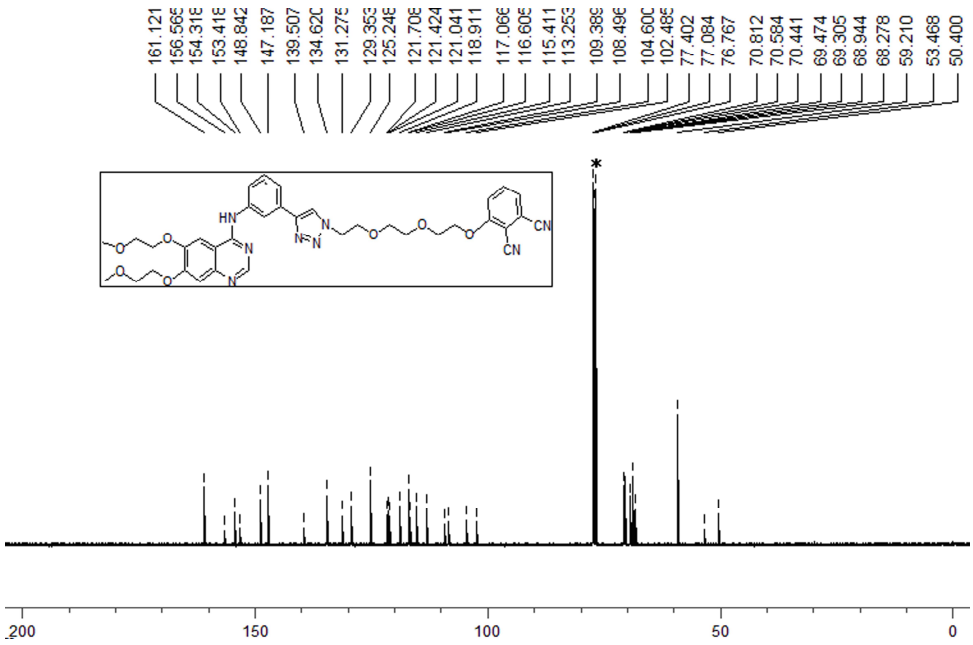


Fig. S15. ^{13}C{^{1}H} NMR spectrum of **2a**

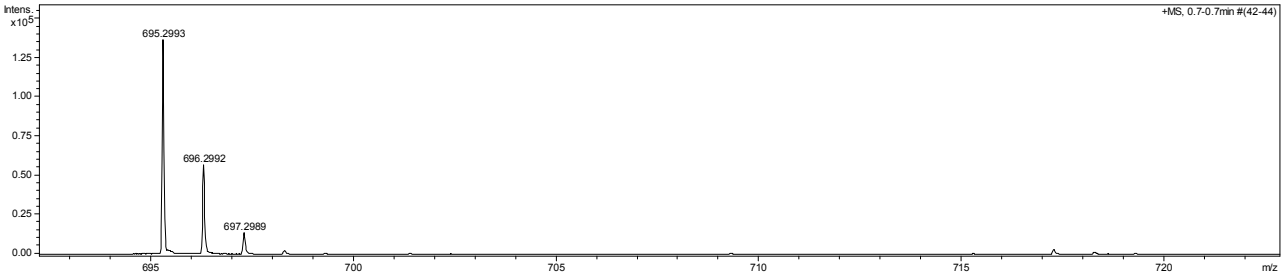


Fig. S16. HRMS spectrum of **2a**

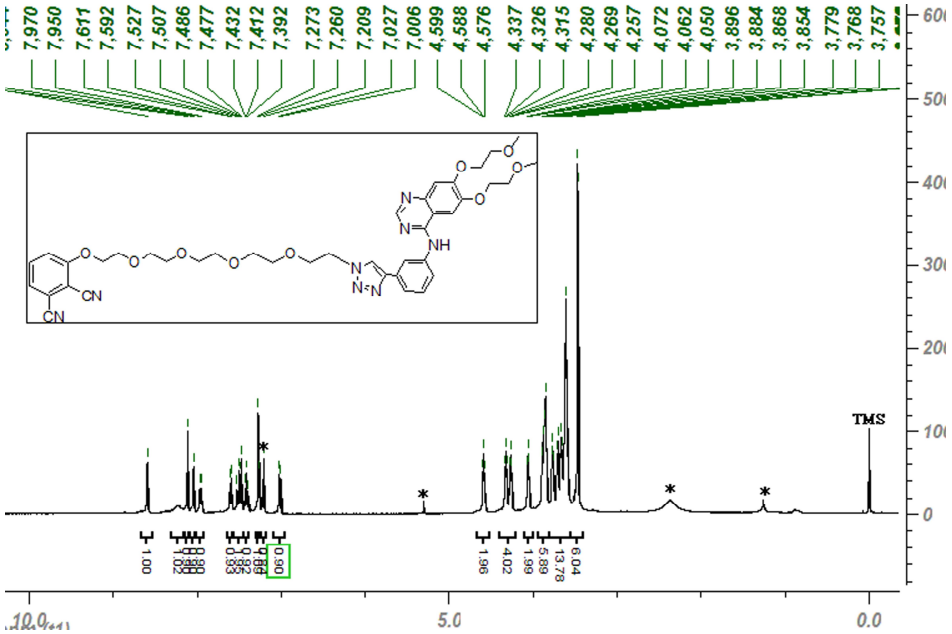


Fig. S17. ^{1}H NMR spectrum of **2b**

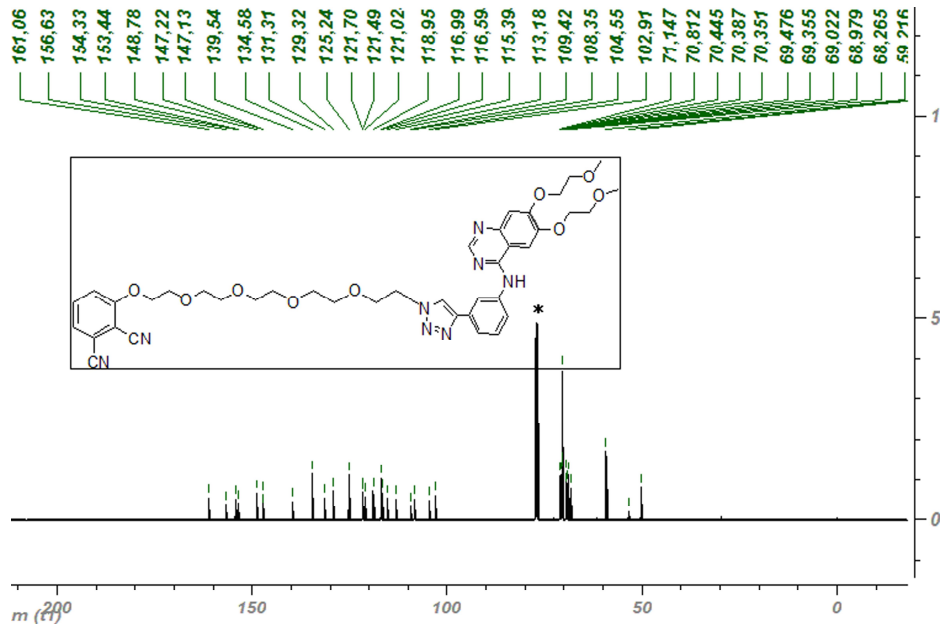


Fig. S18. ^{13}C{^{1}H} NMR spectrum of **2b**

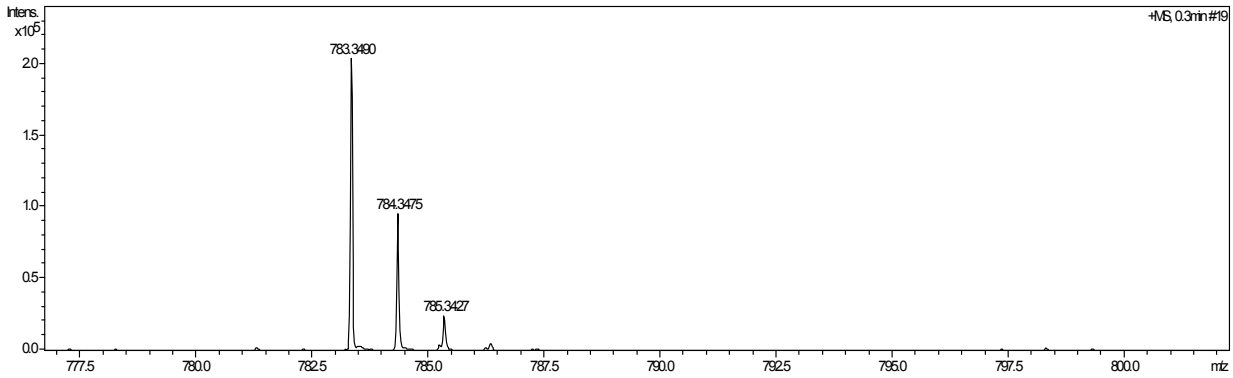


Fig. S19. HRMS spectrum of **2b**

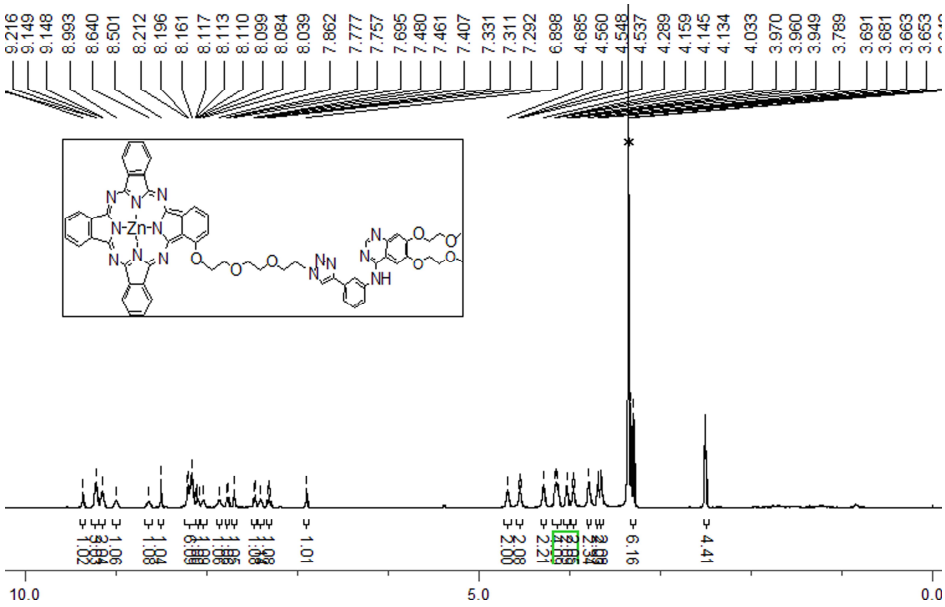


Fig. S20. ^{1}H NMR spectrum of **3a**

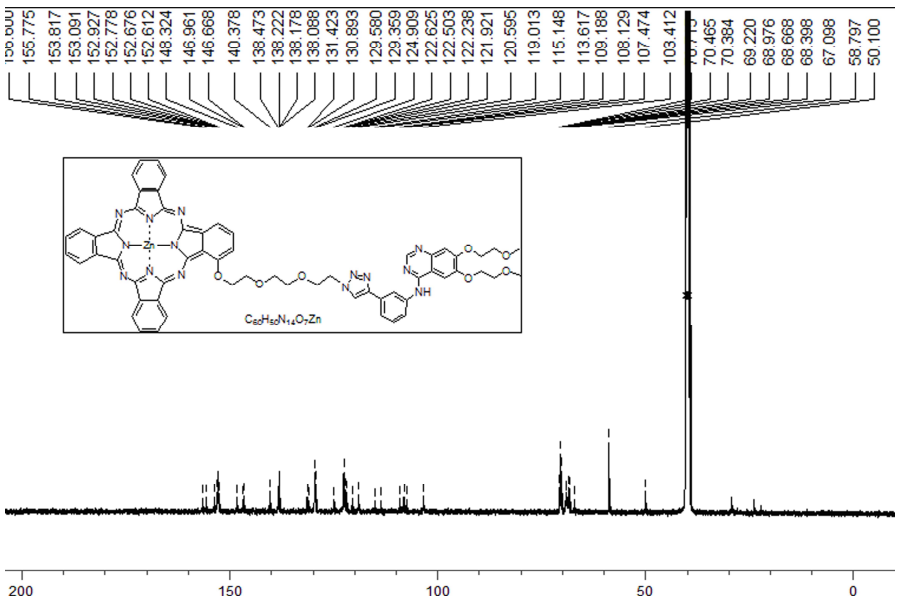


Fig. S21. $^{13}C\{^{1}H\}$ NMR spectrum of **3a**

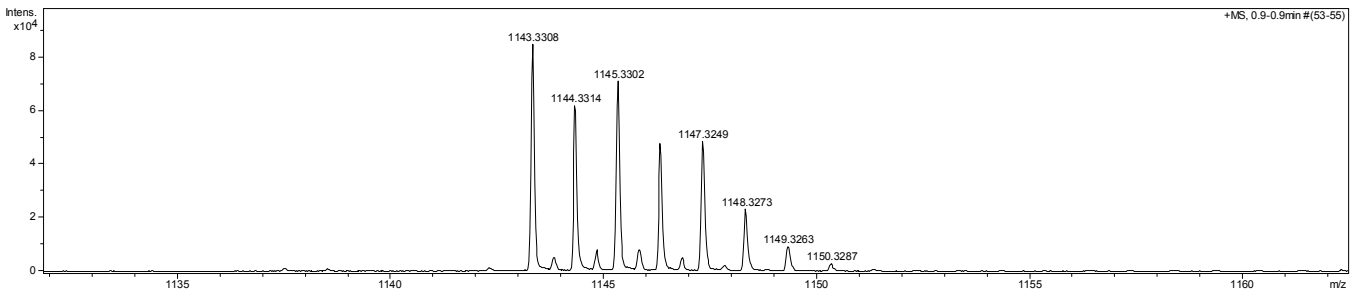


Fig. S22. HRMS spectrum of **3a**

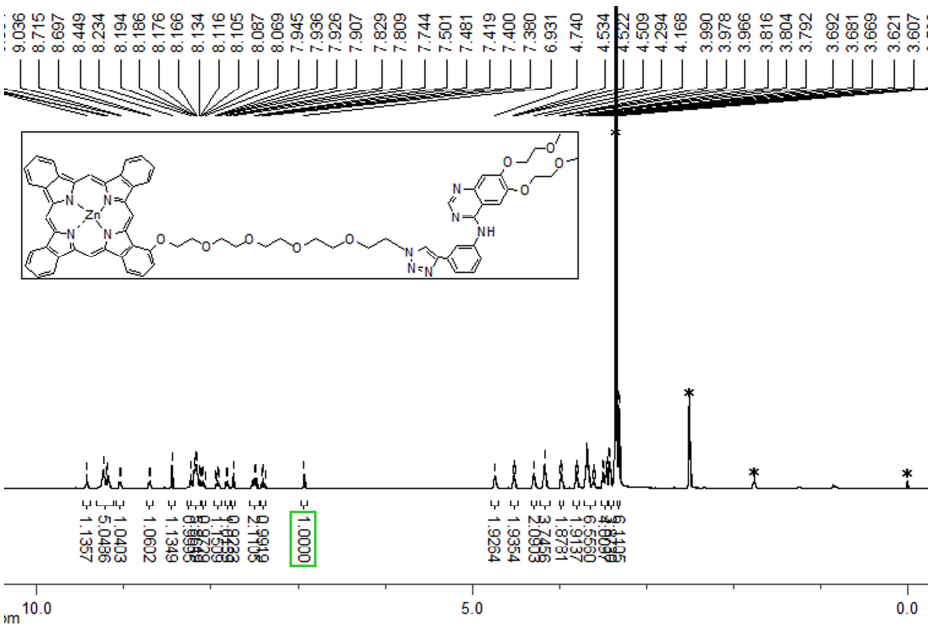


Fig. S23. ^{1}H NMR spectrum of **3b**

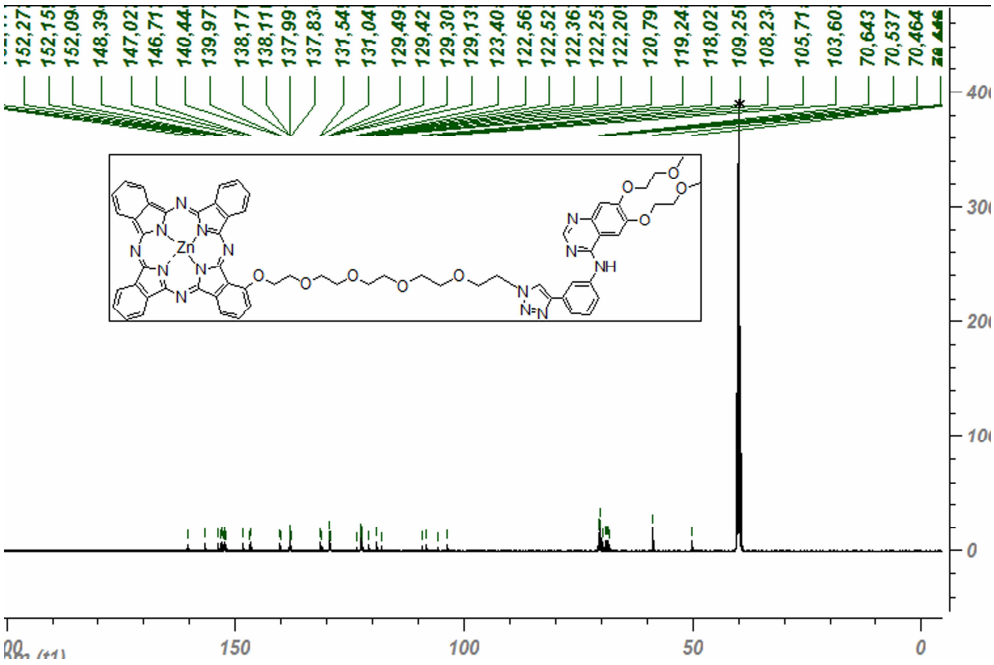


Fig. S24. ^{13}C{^{1}H} NMR spectrum of **3b**

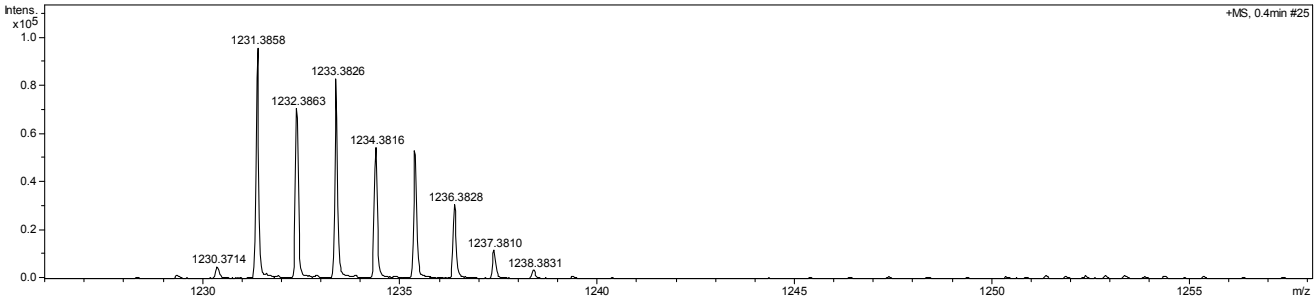


Fig. S25. HRMS spectrum of **3b**