

Electronic Supporting Information

**A pH-responsive fluorescence probe and photosensitizer based on a
tetraamino silicon(IV) phthalocyanine**

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Experimental Section

Materials and Methods. All the reactions were performed under an atmosphere of nitrogen. *N,N*-Dimethylformamide (DMF), pyridine and toluene were distilled from barium oxide, calcium hydride and sodium respectively. All other solvents and reagents were of reagent grade and used as received.

^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra were recorded on a Bruker DPX 300 spectrometer (^1H , 300; ^{13}C , 75.4 MHz) in CDCl_3 , CD_3OD or DMSO-d_6 . Spectra were referenced internally using the residual solvent (^1H : δ 3.31 for CD_3OD , δ 2.49 for DMSO-d_6 ; ^{13}C : δ 77.0 for CDCl_3 , δ 39.5 for DMSO-d_6) resonances relative to SiMe_4 . Electrospray ionisation (ESI) mass spectra were recorded on a Thermo Finnigan MAT 95 XL mass spectrometer. Elemental analyses were performed by the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China.

UV-Vis and steady-state fluorescence spectra were taken on a Cary 5G UV-Vis-NIR spectrophotometer and a Hitachi F-4500 spectrofluorometer respectively. The fluorescence quantum yields of the samples [$\Phi_{\text{F}(\text{sample})}$] were determined by the equation: $\Phi_{\text{F}(\text{sample})} = (F_{\text{sample}}/F_{\text{ref}})(A_{\text{ref}}/A_{\text{sample}})(n_{\text{sample}}^2/n_{\text{ref}}^2)\Phi_{\text{F}(\text{ref})}$,^{R1} where F , A and n are the measured fluorescence (area under the emission peak), the absorbance at the excitation position (610 nm) and the refractive index of the solvent respectively. The unsubstituted zinc(II) phthalocyanine (ZnPc) in DMF was used as the reference [$\Phi_{\text{F}(\text{ref})} = 0.28$].^{R2} To minimise re-absorption of radiation by the ground-state species, the emission spectra were obtained in very dilute solutions where the absorbance at 610 nm was about 0.03. The singlet oxygen quantum yields (Φ_{Δ}) were measured in DMF by the method of chemical quenching of 1,3-diphenylisobenzofuran (DPBF) using ZnPc as the reference ($\Phi_{\Delta} = 0.56$).^{R3}

The reactive oxygen species (ROS) generation efficiency of **1** was examined in citrate buffer solutions with pH at 5.0, 6.0, 7.4 and 8.0. A mixture of **1** (4 μM) and dihydroethidium (20 μM) in the buffer (3 mL) was prepared in a quartz cell. Its fluorescence spectra ($\lambda_{\text{ex}} = 465 \text{ nm}$, $\lambda_{\text{em}} = 500\text{-}700 \text{ nm}$ due to the oxidised product) were recorded immediately after irradiation with red light for every 5 seconds. The light source consisted of a 100 W halogen lamp and a colour glass filter (Newport) cut-on 610 nm. The rate of oxidation of dihydroethidium, which reflects the ROS generation efficiency of **1**, was monitored for a total of 30-second irradiation.

Preparation of Phthalocyanine 1. A mixture of 2-[[2-(dimethylamino)ethyl]methylamino]ethanol (0.42 g, 2.87 mmol), silicon(IV) phthalocyanine dichloride (0.22 g, 0.36 mmol) and pyridine (0.5 mL) in toluene (15 mL) was heated under reflux for 4 h. After cooling, the solvent was evaporated *in vacuo*. The residue was washed with water (500 mL) and hexane (250 mL), then dissolved in CH_2Cl_2 (100 mL) and dried over anhydrous Na_2SO_4 . After evaporation, the product was obtained as a blue solid (0.24 g, 81%). ^1H NMR (CD_3OD): δ 9.69-9.72 (m, 8 H, Pc- H_α), 8.45-8.48 (m, 8 H, Pc- H_β), 1.59 (s, 12 H, CH_3), 1.09 (virtual t, $J = 7.5 \text{ Hz}$, 4 H, CH_2), 0.69 (virtual t, $J = 7.5 \text{ Hz}$, 4 H, CH_2), 0.41 (s, 6 H, CH_3), -0.63 (t, $J = 6.0 \text{ Hz}$, 4 H, CH_2), -1.91 (t, $J = 6.0 \text{ Hz}$, 4 H, OCH_2). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 149.2, 136.0, 130.8, 123.6, 56.6, 56.3, 54.2, 53.1, 45.1, 41.3. HRMS (ESI): m/z calcd for $\text{C}_{46}\text{H}_{50}\text{N}_{12}\text{NaO}_2\text{Si}$ $[\text{M}+\text{Na}]^+$ 853.3841, found 853.3842. Anal. Calcd for $\text{C}_{46}\text{H}_{50}\text{N}_{12}\text{O}_2\text{Si}$: C, 66.48; H, 6.06; N, 20.22. Found: C, 66.23; H, 6.02; N, 20.16.

Preparation of Phthalocyanine 2. A mixture of **1** (0.16 g, 0.19 mmol) and iodomethane (0.29 g, 2.04 mmol) in CHCl_3 was stirred at room temp. for 5 min. The

resulting green precipitate was collected by filtration, washed thoroughly with CHCl_3 and THF, and dried *in vacuo* (0.17 g, 79%). ^1H NMR (DMSO-d_6): δ 9.67-9.70 (m, 8 H, Pc- H_α), 8.52-8.55 (m, 8 H, Pc- H_β), 2.18 (s, 18 H, CH_3), 2.14 (virtual t, $J = 6.9$ Hz, 4 H, CH_2), 0.95 (virtual t, $J = 6.9$ Hz, 4 H, CH_2), 0.11 (s, 6 H, CH_3), -0.74 (t, $J = 5.7$ Hz, 4 H, CH_2), -2.06 (t, $J = 5.7$ Hz, 4 H, OCH_2). $^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO-d_6): δ 148.8, 134.9, 132.2, 123.7, 60.4, 55.0, 52.5, 51.8, 49.7. HRMS (ESI): m/z calcd for $\text{C}_{48}\text{H}_{56}\text{N}_{12}\text{O}_2\text{Si}$ $[\text{M}-2\text{I}]^{2+}$ 430.2204, found 430.2208. Anal. Calcd for $\text{C}_{48}\text{H}_{58}\text{I}_2\text{N}_{12}\text{O}_3\text{Si}$ ($2 \cdot \text{H}_2\text{O}$): C, 50.89; H, 5.16; N, 14.84. Found: C, 50.51; H, 5.48; N, 14.50.

Preparation of Phthalocyanine 3. A mixture of **2** (0.11 g, 0.10 mmol) and iodomethane (2.84 g, 0.02 mol) in DMF (10 mL) was stirred at room temp. for 1 h. The mixture was then poured into diethyl ether (100 mL) to give a green precipitate, which was filtered, washed thoroughly with CHCl_3 , THF and diethyl ether, and dried *in vacuo* (0.13 g, 90%). ^1H NMR (DMSO-d_6): δ 9.73-9.76 (m, 8 H, Pc- H_α), 8.58-8.60 (m, 8 H, Pc- H_β), 2.60 (virtual t, $J = 7.5$ Hz, 4 H, CH_2), 2.45 (s, 18 H, CH_3), 2.06 (virtual t, $J = 7.5$ Hz, 4 H, CH_2), 0.76 (s, 12 H, CH_3), 0.56 (virtual s, 4 H, CH_2), -1.73 (virtual s, 4 H, OCH_2); $^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO-d_6): δ 148.9, 134.7, 132.5, 124.0, 62.6, 55.7, 55.5, 52.2, 49.2, 48.2. HRMS (ESI): m/z calcd for $\text{C}_{50}\text{H}_{62}\text{N}_{12}\text{O}_2\text{Si}$ $[\text{M}-4\text{I}]^{4+}$ 222.6217, found 222.6218. Anal. Calcd for $\text{C}_{50}\text{H}_{70}\text{I}_4\text{N}_{12}\text{O}_6\text{Si}$ ($3 \cdot 4\text{H}_2\text{O}$): C, 40.83; H, 4.80; N, 11.43. Found: C, 41.14; H, 4.93; N, 11.37.

Cell Lines and Culture Conditions. The HT29 human colorectal carcinoma cells (from ATCC, no. HTB-38) were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, no.10313-021) supplemented with fetal calf serum (10%), penicillin-streptomycin ($100 \text{ units mL}^{-1}$ and $100 \mu\text{g mL}^{-1}$ respectively), L-

glutamine (2 mM) and transferrin (10 $\mu\text{g mL}^{-1}$). The HepG2 human hepatocarcinoma cells (from ATCC, no. HB-8065) were maintained in RPMI medium 1640 (Invitrogen, no. 23400-021) supplemented with fetal calf serum (10%) and penicillin-streptomycin (100 units mL^{-1} and 100 $\mu\text{g mL}^{-1}$ respectively). Approximately 3×10^4 (for HT29) or 4×10^4 (for HepG2) cells per well in these media were inoculated in 96-multiwell plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere.

Cellular Uptake Studies. About 6.0×10^4 HT29 cells in the culture medium (2 mL) were seeded on a coverslip and incubated overnight at 37 °C under 5% CO_2 . The medium was then removed. The cells were incubated with a solution of **1-3** in the medium (2 μM for **1**, 8 μM for **2** and **3**, 2 mL) for 2 h under the same conditions. Then the cells were rinsed with phosphate buffered saline (PBS) and viewed with a Leica SP5 confocal microscope equipped with a 633 nm helium neon laser. Phthalocyanines **1-3** were excited at 633 nm and their emission was monitored at 640-700 nm. The images were digitised and analysed by Leica Application Suite Advanced Fluorescence.

pH-Dependent Intracellular Fluorescence Studies. About 1.2×10^5 HT29 cells in the growth medium (2 mL) were seeded on a coverslip and incubated overnight at 37 °C under 5% CO_2 . The medium was removed, then the cells were incubated with a solution of phthalocyanine **1** in the medium (0.5 μM , 2 mL) for 30 min under the same conditions. The cells were then rinsed with PBS and incubated with nigericin (Sigma) in PBS (25 μM , 2 mL) at different pH (5.0, 6.0 and 7.4) for

further 20 min. The cells were viewed with a Leica SP5 confocal microscope equipped with a 633 nm helium neon laser. Emission signals from 640-700 nm (gain = 750 V) were collected and the images were digitised and analysed by Leica Application Suite Advanced Fluorescence. The intracellular fluorescence intensities (totally 25 cells for each pH solution) were also determined.

Photocytotoxicity Assay. Phthalocyanines **1-3** were first dissolved in DMF to give 1.6 mM solutions, which were diluted to appropriate concentrations with the culture medium. The cells, after being rinsed with PBS, were incubated with 100 μ L of these phthalocyanine solutions for 2 h at 37 °C under 5% CO₂. The cells were then rinsed again with PBS and refed with 100 μ L of the culture medium before being illuminated at ambient temperature. The light source consisted of a 300 W halogen lamp, a water tank for cooling and a colour glass filter (Newport) cut-on 610 nm. The fluence rate ($\lambda > 610$ nm) was 40 mW cm⁻². An illumination of 20 min led to a total fluence of 48 J cm⁻².

Cell viability was determined by means of the colourimetric MTT assay.^{R5} After illumination, the cells were incubated at 37 °C under 5% CO₂ overnight. An MTT (Sigma) solution in PBS (3 mg mL⁻¹, 50 μ L) was added to each well followed by incubation for 2 h under the same environment. A solution of sodium dodecyl sulfate (Sigma; 10% by weight, 50 μ L) was then added to each well. The plate was incubated in an oven at 60 °C for 30 min, then 80 μ L of *iso*-propanol was added to each well. The plate was agitated on a Bio-Rad microplate reader at ambient temperature for 10 s before the absorbance at 540 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

following equation: % viability = $[\sum(A_i/A_{\text{control}} \times 100)]/n$, where A_i is the absorbance of the i th data ($i = 1, 2, \dots, n$), A_{control} is the average absorbance of the control wells in which the phthalocyanine was absent, and n ($= 4$) is the number of the data points.

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- R5 H. Tada, O. Shiho, K. Kuroshima, M. Koyama and K. Tsukamoto, *J. Immunol. Methods*, 1986, **93**, 157.

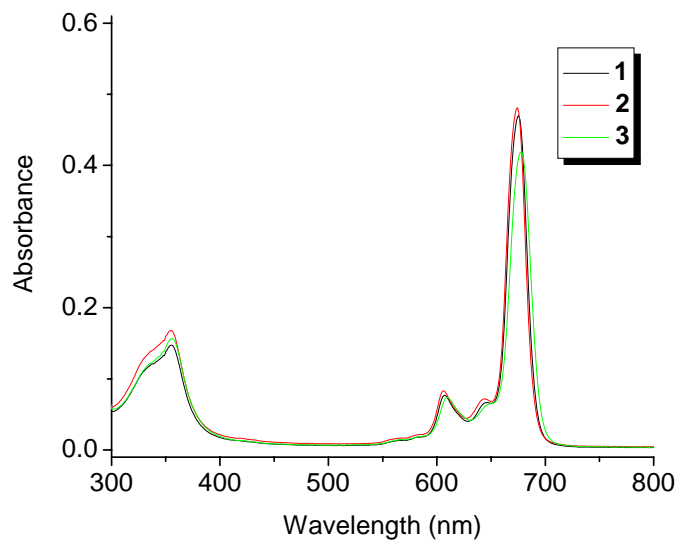


Fig. S1 UV-Vis spectra of **1-3** (2 μM) in DMF.

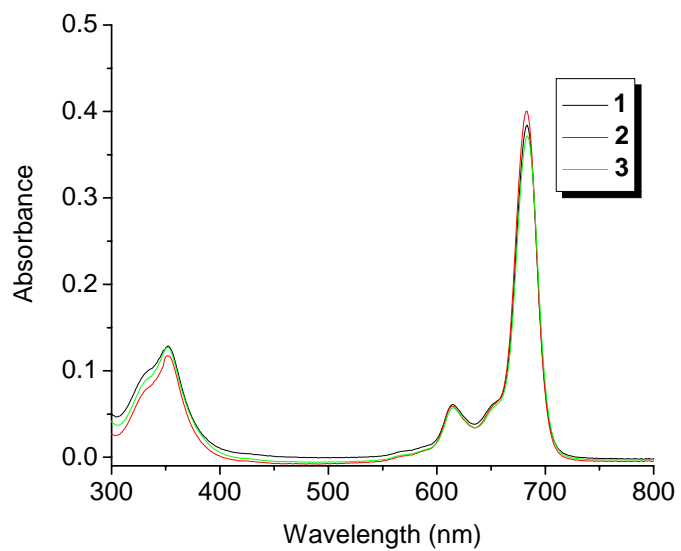


Fig. S2 UV-Vis spectra of **1-3** (2 μM) in water (with 0.1% DMF).

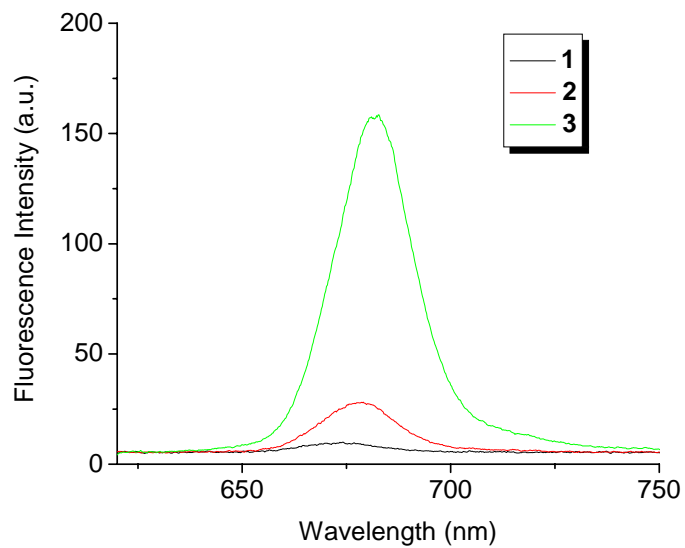


Fig. S3 Fluorescence spectra of **1-3** (1 μ M) in DMF (excited at 610 nm).

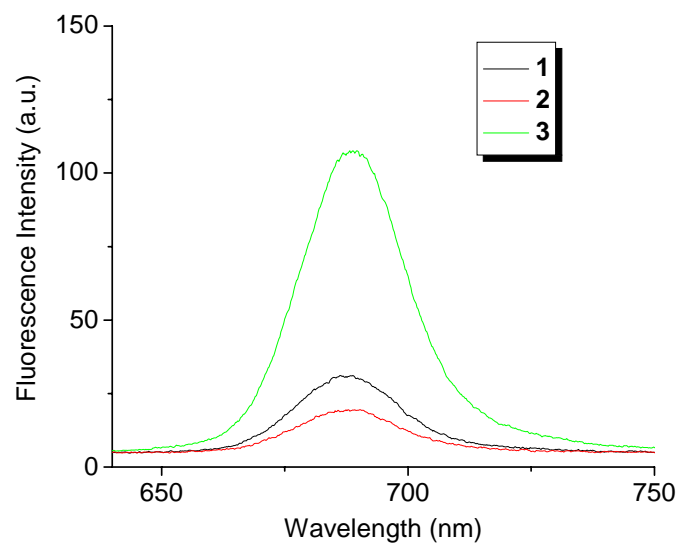


Fig. S4 Fluorescence spectra of **1-3** (1 μ M) in water (with 0.1% DMF) (excited at 610 nm).

Table S1 Electronic absorption and photophysical data for **1-3** in DMF.

Compound	λ_{\max} (nm) ($\log \epsilon$)	λ_{em} (nm) ^a	Φ_{F} ^b	Φ_{Δ} ^c
1	354 (4.87), 606 (4.57), 644 (4.51), 674 (5.36)	676	0.01	0.02
2	357 (4.90), 607 (4.58), 647 (4.50), 676 (5.36)	679	0.05	0.05
3	346 (4.85), 610 (4.53), 678 (5.28)	681	0.35	0.37

^a Excited at 610 nm. ^b Using ZnPc in DMF as the reference ($\Phi_{\text{F}} = 0.28$). ^c Using ZnPc as the reference ($\Phi_{\Delta} = 0.56$ in DMF).

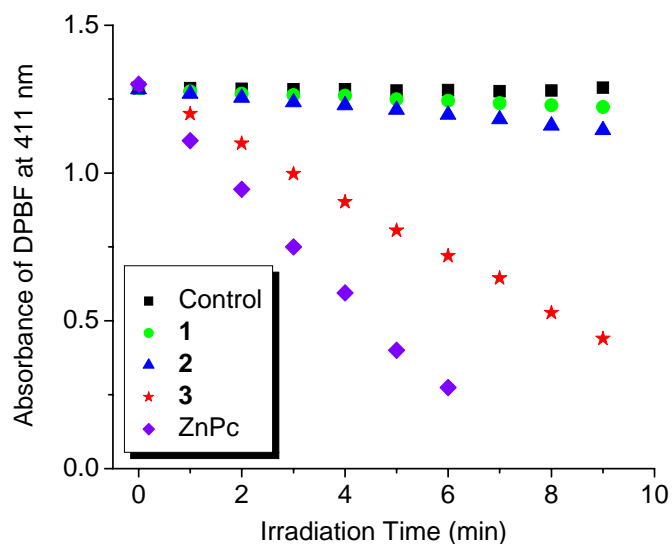


Fig. S5 Comparison of the rates of photodegradation of DPBF in DMF as monitored spectroscopically at 411 nm with time, using **1-3** and ZnPc (2 μM) as the photosensitisers. The data for null photosensitiser are also given as a control.

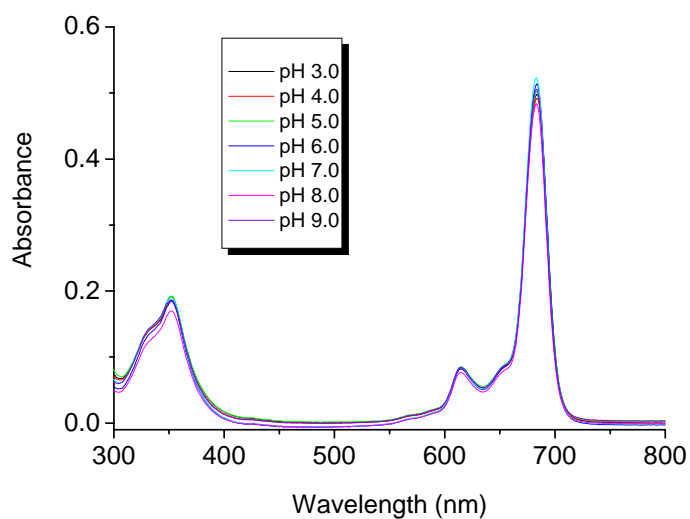


Fig. S6 UV-Vis spectra of **1** (2 μM) in citrate buffer solutions with different pH.

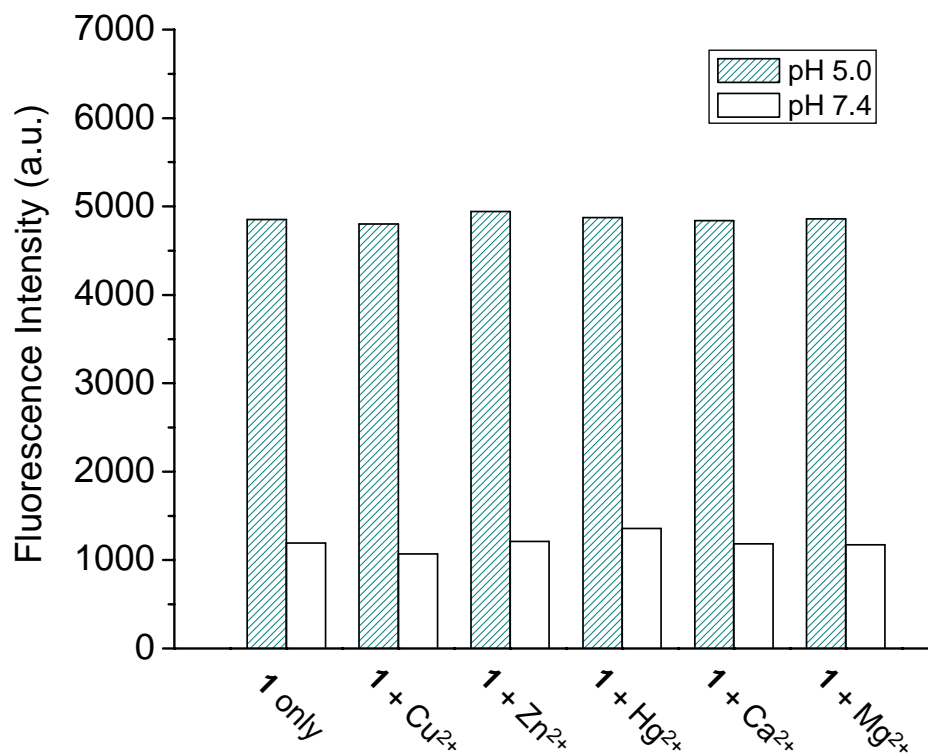


Fig. S7 Effects of metal ions (100 μM) on the fluorescence intensity of **1** (2 μM) in citrate buffer solution at pH 5.0 or 7.4.

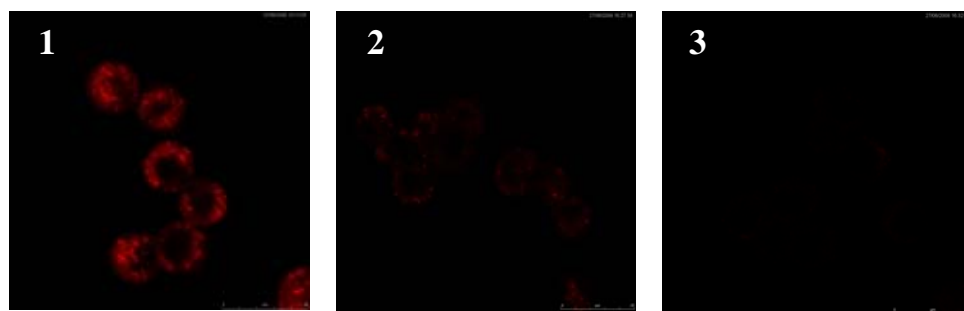


Fig. S8 Confocal fluorescence images of HT29 cells after incubation with **1** (2 μM), **2** (8 μM) and **3** (8 μM), respectively, for 2 h.

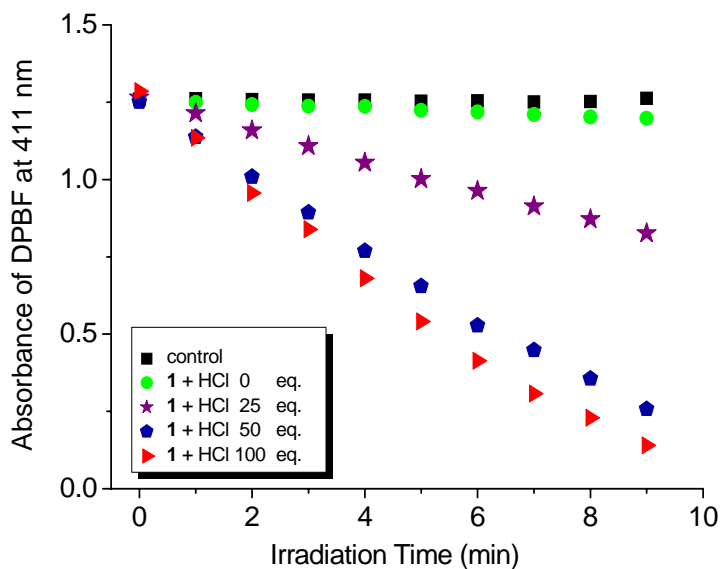


Fig. S9 Comparison of the rates of photodegradation of DPBF in DMF as monitored spectroscopically at 411 nm with time, using **1** (2 μ M) as the photosensitiser, both in the absence and presence of HCl. The data for null photosensitiser are also given as a control.

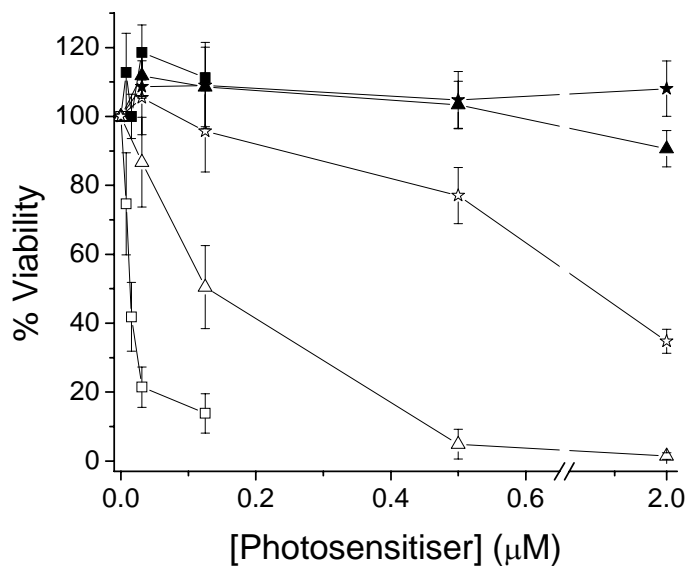


Fig. S10 Cytotoxic effects of **1** (squares), **2** (triangles) and **3** (stars) on HepG2 cells in the absence (closed symbols) and presence (open symbols) of light ($\lambda > 610$ nm, 40 mW cm⁻², 48 J cm⁻²). Data are expressed as mean values \pm standard error of the means of three independent experiments, each performed in quadruplicate.

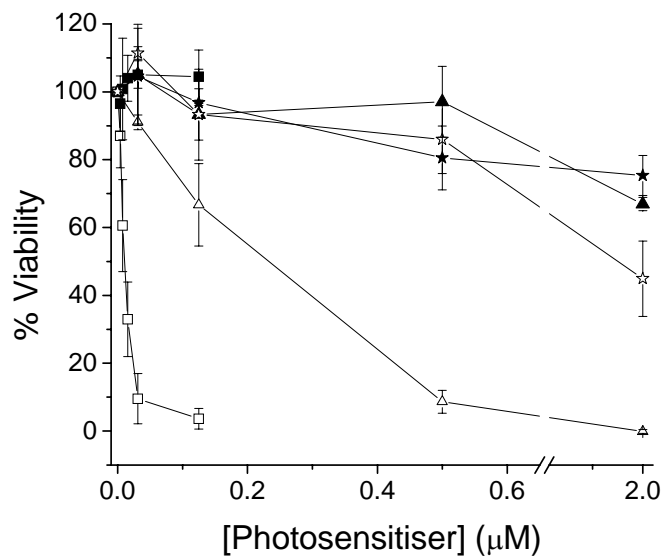
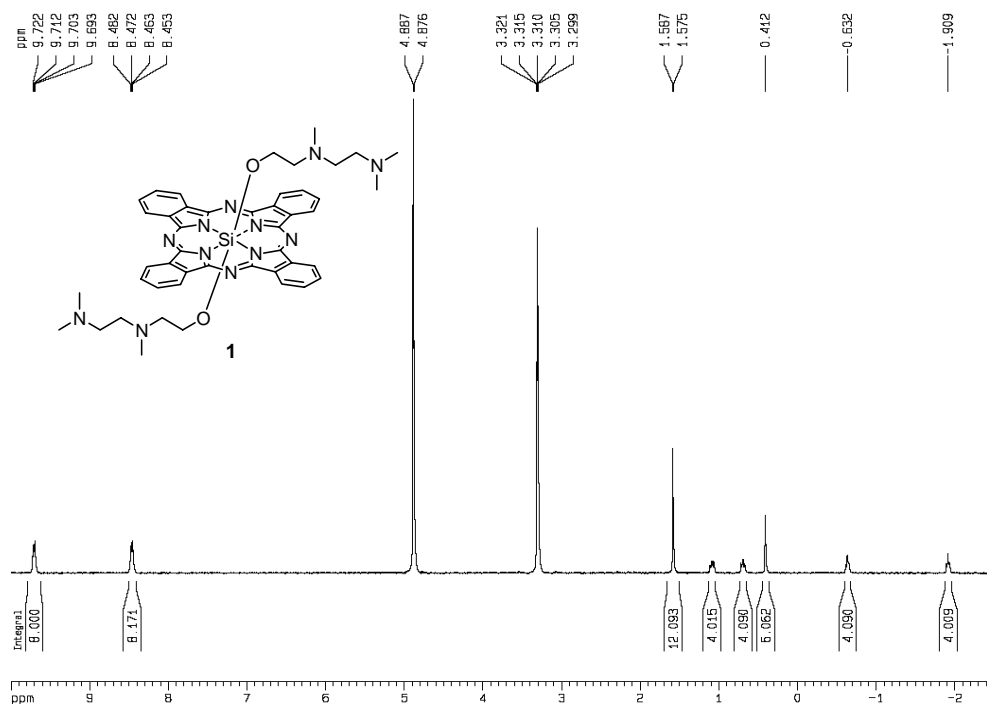
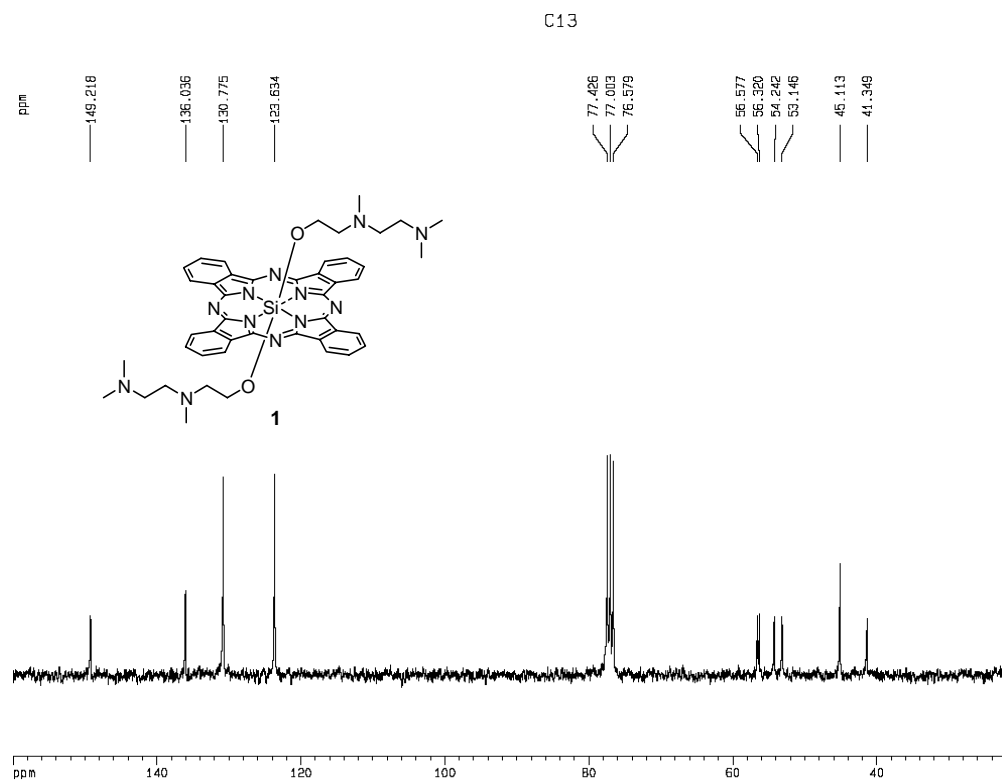


Fig. S11 Cytotoxic effects of **1** (squares), **2** (triangles) and **3** (stars) on HT29 cells in the absence (closed symbols) and presence (open symbols) of light ($\lambda > 610$ nm, 40 mW cm⁻², 48 J cm⁻²). Data are expressed as mean values \pm standard error of the means of three independent experiments, each performed in quadruplicate.

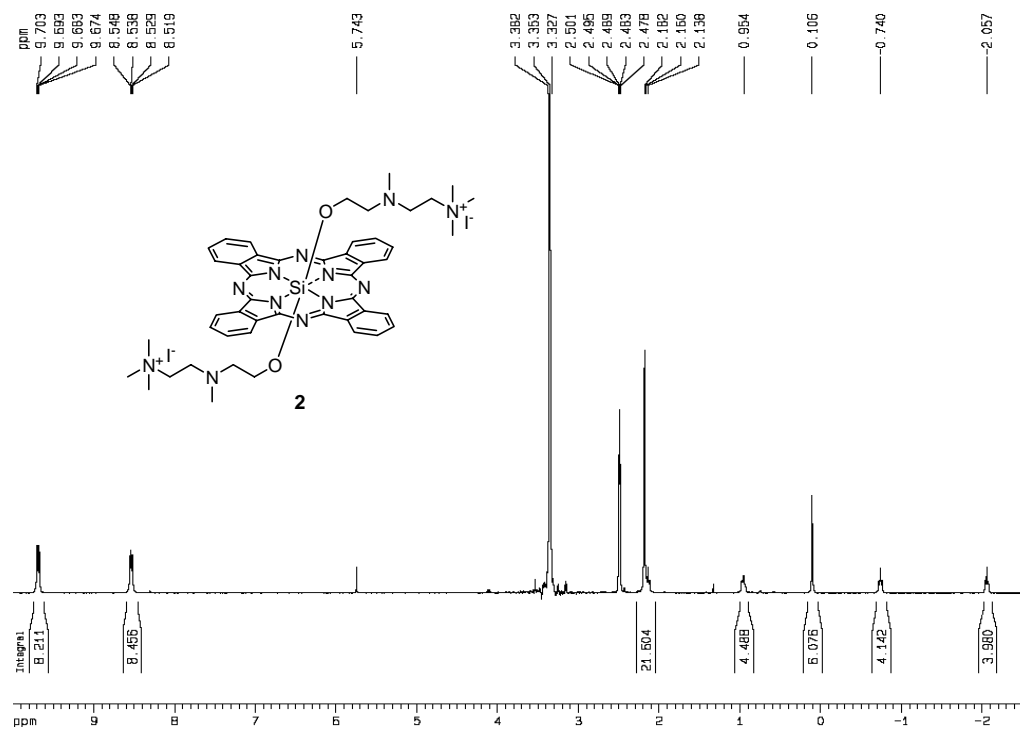
^1H NMR spectrum of **1** in CD_3OD



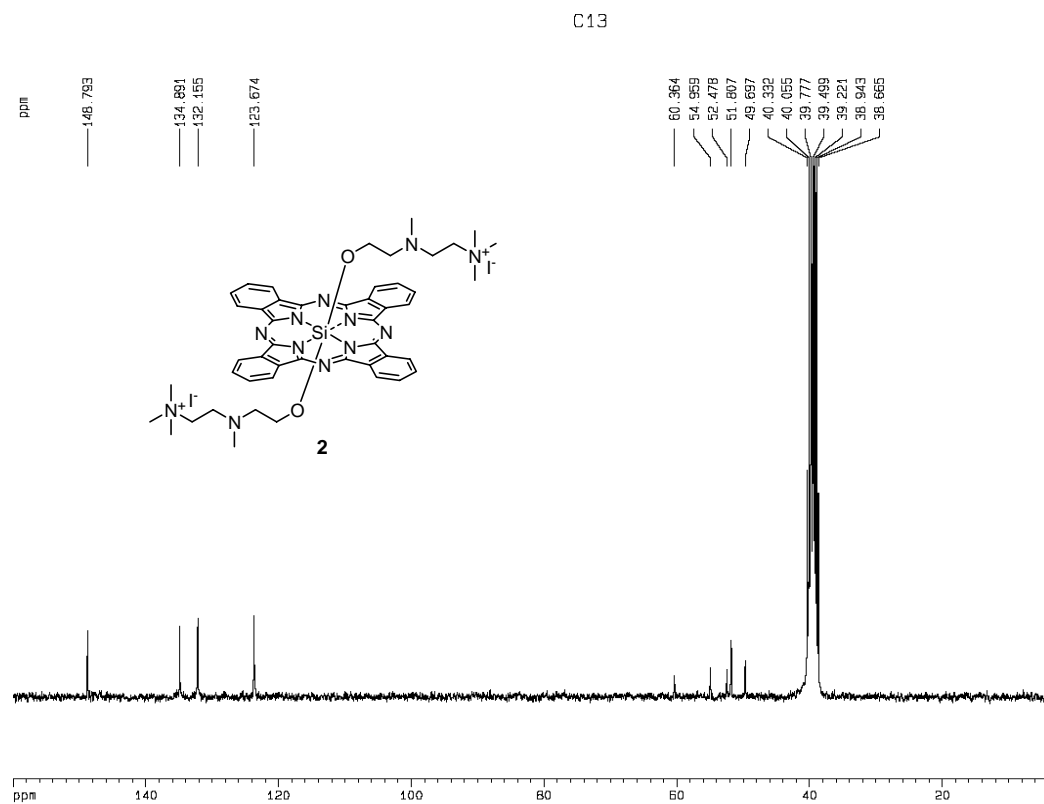
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **1** in CDCl_3



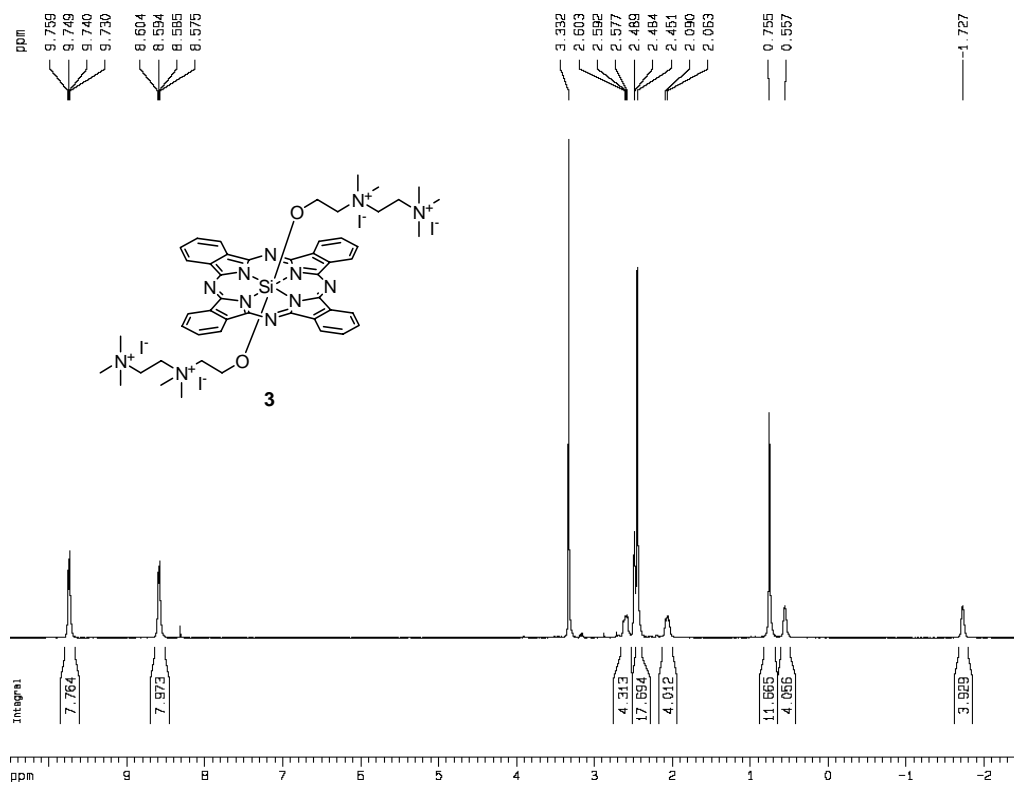
^1H NMR spectrum of **2** in DMSO-d_6



$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **2** in DMSO-d_6



^1H NMR spectrum of **3** in DMSO-d_6



$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **3** in DMSO-d_6

