

## Electronic Supplementary Information

### **A disulfide-linked conjugate of ferrocenyl chalcone and silicon(IV) phthalocyanine as an activatable photosensitiser**

Janet T. F. Lau, Xiong-Jie Jiang, Dennis K. P. Ng and Pui-Chi Lo\*

#### Contents

Experimental Section

**Scheme S1** Synthesis of phthalocyanine **7**.

**Table S1** Electronic absorption and photophysical data for **6a**, **6b** and **7** in DMF.

**Fig. S1** Electronic absorption spectra of **6a**, **6b**, **7** and **5a** in DMF in different concentrations.

**Fig. S2** Comparison of the rate of photodegradation of DPBF sensitised by **6a**, **6b**, **7** and **ZnPc** in DMF.

**Fig. S3** Changes in fluorescence spectra of **6a** and **6b** with time upon exposure to different concentrations of DTT in PBS with 0.5% Cremophor EL.

**Fig. S4** Changes in absorption spectra of **6a** and **6b** with time upon exposure to different concentrations of DTT in PBS with 0.5% Cremophor EL.

- Fig. S5** Comparison of the rate of photodegradation of DPBF sensitised by **6b** and **7** upon exposure to 0 or 10 mM of DTT for 24 h in PBS with 0.5% Cremophor EL.
- Fig. S6** Cytotoxic effects of **6a**, **6b**, **7** and **5a** on MCF-7 cells pre-treated with different concentrations of DTT for 1 h followed by drug incubation for 6 or 24 h in the absence and presence of light.
- Fig. S7** Confocal fluorescence images of MCF-7 cells pre-treated with different concentrations of DTT followed by incubation with **6b** for 24 h.
- Fig. S8** Fluorescence images of MCF-7 cells pre-treated with 4 mM of DTT using filter sets specific for ER-Tracker and **6a**.
- Fig. S9** Fluorescence images of MCF-7 cells pre-treated with 4 mM of DTT using filter sets specific for **6a** and LysoTracker or MitoTracker.

$^1\text{H}$  and  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of all the new compounds

## Experimental Section

All the reactions were performed under an atmosphere of nitrogen. *N,N*-dimethylformamide (DMF) was dried over barium oxide and distilled under reduced pressure. Tetrahydrofuran (THF), toluene and pyridine were distilled from sodium benzophenone ketyl, sodium and calcium hydride respectively. Chromatographic purifications were performed on silica gel (Macherey-Nagel, 230-400 mesh) columns with the indicated eluents. All other solvents and reagents were of reagent grade and used as received. Compound **1** was prepared as described.<sup>R1</sup>

<sup>1</sup>H and <sup>13</sup>C{<sup>1</sup>H} NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer (<sup>1</sup>H, 400; <sup>13</sup>C, 100.6 MHz) in deuterated solvents. Spectra were referenced internally by using the residual solvent (<sup>1</sup>H:  $\delta = 7.26$  for CDCl<sub>3</sub>,  $\delta = 2.49$  for DMSO-d<sub>6</sub> and  $\delta = 2.05$  for acetone-d<sub>6</sub>) or solvent (<sup>13</sup>C:  $\delta = 77.0$  for CDCl<sub>3</sub>,  $\delta = 39.5$  for DMSO-d<sub>6</sub> and  $\delta = 29.8$  for acetone-d<sub>6</sub>) resonances relative to SiMe<sub>4</sub>. 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-7000 spectrofluorometer respectively. The fluorescence

quantum yields ( $\Phi_F$ ) of the samples (in DMF) were determined by the equation:  $\Phi_{F(\text{sample})} = (F_{\text{sample}}/F_{\text{ref}})(A_{\text{ref}}/A_{\text{sample}})(n_{\text{sample}}^2/n_{\text{ref}}^2)\Phi_{F(\text{ref})}$ ,<sup>R2</sup> 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. Unsubstituted zinc(II) phthalocyanine (ZnPc) in DMF was used as the reference [ $\Phi_{F(\text{ref})} = 0.28$ ].<sup>R3</sup> The singlet oxygen quantum yields ( $\Phi_\Delta$ ) were measured in DMF by the method of chemical quenching of 1,3-diphenylisobenzofuran (DPBF) by using ZnPc as the reference ( $\Phi_\Delta = 0.56$ ).<sup>R4</sup>

To mimic the biological environment, phosphate buffered saline (PBS) was also used as the solvent. For fluorescence measurements, the phthalocyanines were dissolved in DMF to give 1 mM solutions, which were diluted to 4  $\mu\text{M}$  with PBS (with 0.5% Cremophor EL). DTT was dissolved in deionised water to give a 1 M solution. Mixtures of the phthalocyanines (4  $\mu\text{M}$ ) with DTT (2  $\mu\text{M}$ , 10 mM or 40 mM) or without DTT in PBS (72 mL) were prepared and stirred continuously. Their fluorescence spectra ( $\lambda_{\text{ex}} = 610 \text{ nm}$ ,  $\lambda_{\text{em}} = 630\text{-}800 \text{ nm}$ ) were recorded with time. For singlet oxygen measurements, DPBF was first dissolved in DMF to give a 10 mM solution. Mixtures of the phthalocyanines (4  $\mu\text{M}$ ) with DTT (2  $\mu\text{M}$ , 5 mM or 10 mM) or without DTT in PBS were stirred continuously for 24 h. These solutions (3 mL) were mixed with the DPBF solution (10 mM, 21  $\mu\text{L}$ ) followed by illumination with red light coming from a 100 W halogen lamp after passing through a water tank for cooling and a colour glass filter (Newport) cut-on 610 nm. The decay of DPBF at

411 nm was monitored with time.

**Compound 2.** A mixture of 3-ferrocenyl-1-(*p*-hydroxyphenyl)-prop-2-en-1-one (**1**) (7.10 g, 21.4 mmol), ethyl bromoacetate (7.14 g, 42.8 mmol) and anhydrous K<sub>2</sub>CO<sub>3</sub> (8.86 g, 64.1 mmol) was heated under reflux in acetone (100 mL). The solvent was evaporated under reduced pressure, then the residue was mixed with water (200 mL) and extracted with CHCl<sub>3</sub> (100 mL × 3). The combined organic layers were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The crude product was purified by silica-gel column chromatography using CHCl<sub>3</sub>/ethyl acetate (10:1) as the eluent. It was finally recrystallised from CHCl<sub>3</sub>/hexane to afford a reddish brown solid (7.86 g, 88%). R<sub>f</sub> [CHCl<sub>3</sub>/ethyl acetate (10:1)] = 0.90. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ = 8.00 (d, *J* = 8.8 Hz, 2 H, ArH), 7.74 (d, *J* = 15.2 Hz, 1 H, CH=CH), 7.12 (d, *J* = 15.2 Hz, 1 H, CH=CH), 6.98 (d, *J* = 8.8 Hz, 2 H, ArH), 4.70 (s, 2 H, CH<sub>2</sub>), 4.59 (t, *J* = 1.6 Hz, 2 H, Fc-H), 4.48 (t, *J* = 1.6 Hz, 2 H, Fc-H), 4.29 (q, *J* = 7.2 Hz, 2 H, CH<sub>2</sub>), 4.18 (s, 5 H, Fc-H), 1.31 (t, *J* = 7.2 Hz, 3 H, CH<sub>3</sub>). <sup>13</sup>C {<sup>1</sup>H} NMR (CDCl<sub>3</sub>): δ = 188.1, 168.3, 161.1, 146.1, 132.3, 130.6, 118.7, 114.3, 79.3, 71.3, 69.8, 68.9, 65.2, 61.6, 14.1. MS (ESI): an isotopic cluster peaking at *m/z* = 418 (100%, [M]<sup>+</sup>). HRMS (ESI): *m/z* calcd for C<sub>23</sub>H<sub>22</sub>FeO<sub>4</sub> [M]<sup>+</sup>: 418.0862; found: 418.0862. Anal. calcd for C<sub>23</sub>H<sub>22</sub>FeO<sub>4</sub>: C, 66.05; H, 5.30. Found: C, 65.94; H, 4.94.

**Compound 3.** A mixture of **2** (7.26 g, 17.4 mmol), 5 M NaOH (31 mL, 155.0 mmol) and acetone (50 mL) was heated under reflux for 1 h. The solvent was evaporated under reduced

pressure. The residue was mixed with water and acidified with 3 M HCl until pH = 4. The reddish orange precipitate formed was washed thoroughly with water and dried under reduced pressure at 60 °C (5.81 g, 86%). <sup>1</sup>H NMR (acetone-d<sub>6</sub>): δ = 8.08 (d, *J* = 8.8 Hz, 2 H, ArH), 7.69 (d, *J* = 15.2 Hz, 1 H, CH=CH), 7.41 (d, *J* = 15.2 Hz, 1 H, CH=CH), 7.08 (d, *J* = 8.8 Hz, 2 H, ArH), 4.85 (s, 2 H, CH<sub>2</sub>), 4.78 (t, *J* = 1.6 Hz, 2 H, Fc-H), 4.50 (t, *J* = 1.6 Hz, 2 H, Fc-H), 4.20 (s, 5 H, Fc-H). <sup>13</sup>C{<sup>1</sup>H} NMR (acetone-d<sub>6</sub>): δ = 187.5, 169.7, 162.5, 145.9, 133.0, 131.3, 119.8, 115.3, 80.6, 71.9, 70.5, 69.9, 65.3. MS (ESI): an isotopic cluster peaking at *m/z* = 390 (100%, [M]<sup>+</sup>). HRMS (ESI): *m/z* calcd for C<sub>21</sub>H<sub>18</sub>FeO<sub>4</sub> [M]<sup>+</sup>: 390.0549; found: 390.0550. Anal. calcd for C<sub>21</sub>H<sub>18</sub>FeO<sub>4</sub>: C, 64.64; H, 4.65. Found: C, 64.39; H, 4.34.

**Compound 5a.** A mixture of **3** (0.53 g, 1.36 mmol), 2-hydroxyethyl disulfide (**4a**) (0.42 g, 2.72 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC) (0.33 g, 1.60 mmol), 4-(dimethylamino)pyridine (DMAP) (0.05 g, 0.41 mmol) and 1-hydroxybenzotriazole (HOBt) (0.22 g, 1.63 mmol) in THF (20 mL) was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure. The residue was purified by silica-gel column chromatography using CHCl<sub>3</sub>/MeOH (4:1) as the eluent. The crude product was redissolved in a minimum amount of CHCl<sub>3</sub> and the white solid left was filtered off. This process was repeated several times until there was no white solid left. The product was isolated as a reddish brown oily liquid (0.37 g, 52%). R<sub>f</sub> [CHCl<sub>3</sub>/MeOH (4:1)] = 0.50. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ = 7.99 (d, *J* = 8.8 Hz, 2 H, ArH), 7.74 (d, *J* = 15.6 Hz, 1 H, CH=CH), 7.12 (d, *J* = 15.6 Hz, 1

H, CH=CH), 6.99 (d,  $J = 8.8$  Hz, 2 H, ArH), 4.74 (s, 2 H, CH<sub>2</sub>), 4.59 (t,  $J = 1.6$  Hz, 2 H, Fc-H), 4.51, (t,  $J = 6.4$  Hz, 2 H, CH<sub>2</sub>) 4.48 (t,  $J = 1.6$  Hz, 2 H, Fc-H), 4.18 (s, 5 H, Fc-H), 3.86-3.90 (m, 2 H, CH<sub>2</sub>), 2.96 (t,  $J = 6.4$  Hz, 2 H, CH<sub>2</sub>), 2.88 (t,  $J = 6.0$  Hz, 2 H, CH<sub>2</sub>).  
<sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>):  $\delta = 188.1, 168.2, 161.0, 146.3, 132.4, 130.6, 118.7, 114.3, 79.2, 71.3, 69.7, 68.9, 65.0, 63.2, 41.5, 36.7, 33.9$ . MS (ESI): an isotopic cluster peaking at  $m/z = 526$  (98%, [M]<sup>+</sup>). HRMS (ESI):  $m/z$  calcd for C<sub>25</sub>H<sub>26</sub>FeO<sub>5</sub>S<sub>2</sub> [M]<sup>+</sup>: 526.0566; found: 526.0578.

**Compound 5b.** According to the procedure for **5a**, **3** (0.51 g, 1.31 mmol) was treated with 1,6-hexanediol (**4b**) (0.31 g, 2.62 mmol), DCC (0.32 g, 1.55 mmol), DMAP (0.05 g, 0.41 mmol) and HOBT (0.21 g, 1.55 mmol) in THF (20 mL) to give the product as a reddish brown oily liquid (0.36 g, 56%). R<sub>f</sub> [CHCl<sub>3</sub>/MeOH (4:1)] = 0.49. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 7.99$  (d,  $J = 8.8$  Hz, 2 H, ArH), 7.74 (d,  $J = 15.2$  Hz, 1 H, CH=CH), 7.12 (d,  $J = 15.2$  Hz, 1 H, CH=CH), 6.98 (d,  $J = 8.8$  Hz, 2 H, ArH), 4.72 (s, 2 H, CH<sub>2</sub>), 4.59 (t,  $J = 1.6$  Hz, 2 H, Fc-H), 4.48 (t,  $J = 1.6$  Hz, 2 H, Fc-H), 4.22 (t,  $J = 6.4$  Hz, 2 H, CH<sub>2</sub>), 4.18 (s, 5 H, Fc-H), 3.59-3.64 (m, 2 H, CH<sub>2</sub>), 1.64-1.72 (m, 2 H, CH<sub>2</sub>), 1.51-1.57 (m, 2 H, CH<sub>2</sub>), 1.34-1.41 (m, 4 H, CH<sub>2</sub>).  
<sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>):  $\delta = 188.2, 168.5, 161.2, 146.3, 132.4, 130.6, 118.8, 114.3, 79.3, 71.3, 69.8, 69.0, 65.5, 65.2, 62.7, 32.6, 28.5, 25.6, 25.3$ . MS (ESI): an isotopic cluster peaking at  $m/z = 491$  (100%, [M+H]<sup>+</sup>). HRMS (ESI):  $m/z$  calcd for C<sub>27</sub>H<sub>31</sub>FeO<sub>5</sub> [M+H]<sup>+</sup>: 491.1515; found: 491.1507.

**Compound 6a.** A mixture of silicon phthalocyanine dichloride (SiPcCl<sub>2</sub>) (0.23 g, 0.38

mmol), **5a** (0.50 g, 0.95 mmol) and pyridine (1 mL, 12.4 mmol) in toluene (20 mL) was heated under reflux for 24 h. After evaporating the solvent *in vacuo*, the residue was chromatographed on a neutral alumina column with CHCl<sub>3</sub> as the eluent. The crude product was recrystallised from CHCl<sub>3</sub>/EtOH to afford a shiny dark blue solid (0.22 g, 37%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ = 9.63-9.66 (m, 8 H, Pc-H<sub>α</sub>), 8.33-8.36 (m, 8 H, Pc-H<sub>β</sub>), 7.92 (d, *J* = 8.8 Hz, 4 H, ArH), 7.73 (d, *J* = 15.2 Hz, 2 H, CH=CH), 7.09 (d, *J* = 15.2 Hz, 2 H, CH=CH), 6.82 (d, *J* = 8.8 Hz, 4 H, ArH), 4.58 (t, *J* = 1.6 Hz, 4 H, Fc-H), 4.48 (t, *J* = 1.6 Hz, 4 H, Fc-H), 4.40 (s, 4 H, CH<sub>2</sub>), 4.17 (s, 10 H, Fc-H), 3.58 (t, *J* = 6.4 Hz, 4 H, CH<sub>2</sub>), 1.59 (t, *J* = 6.4 Hz, 4 H, CH<sub>2</sub>), -0.38 (t, *J* = 6.4 Hz, 4 H, CH<sub>2</sub>), -1.77 (t, *J* = 6.4 Hz, 4 H, CH<sub>2</sub>). <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>): δ = 188.0, 167.7, 160.9, 149.4, 146.1, 135.9, 132.3, 131.0, 130.5, 123.7, 118.8, 114.3, 79.3, 71.3, 69.8, 68.9, 64.7, 62.5, 53.8, 38.8, 35.9. MS (ESI): an isotopic cluster peaking at *m/z* = 1592 (13%, [M+H]<sup>+</sup>), 1065 (28%, [M-C<sub>25</sub>H<sub>25</sub>FeO<sub>5</sub>S<sub>2</sub>]<sup>+</sup>). HRMS (ESI): *m/z* calcd for C<sub>82</sub>H<sub>66</sub>Fe<sub>2</sub>N<sub>8</sub>O<sub>10</sub>S<sub>4</sub>Si [M]<sup>+</sup>: 1591.2276; found: 1591.2266. Anal. calcd for C<sub>82</sub>H<sub>66</sub>Fe<sub>2</sub>N<sub>8</sub>O<sub>10</sub>S<sub>4</sub>Si: C, 61.89; H, 4.18; N, 7.04. Found: C, 62.22; H, 4.26; N, 7.29.

**Compound 6b.** According to the procedure for **6a**, SiPcCl<sub>2</sub> (0.33 g, 0.54 mmol) was treated with **5b** (0.66 g, 1.35 mmol) and pyridine (1 mL, 12.4 mmol) in toluene (20 mL) to give the product as a shiny dark blue solid (0.34 g, 41%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ = 9.62-9.64 (m, 8 H, Pc-H<sub>α</sub>), 8.32-8.34 (m, 8 H, Pc-H<sub>β</sub>), 7.90 (d, *J* = 8.8 Hz, 4 H, ArH), 7.70 (d, *J* = 15.2 Hz, 2 H, CH=CH), 7.04 (d, *J* = 15.2 Hz, 2 H, CH=CH), 6.85 (d, *J* = 8.8 Hz, 4 H, ArH), 4.55 (s, 4



H, CH<sub>2</sub>), 4.47 (s, 8 H, Fc-H), 4.14 (s, 10 H, Fc-H), 3.51 (t,  $J = 6.8$  Hz, 4 H, CH<sub>2</sub>), 0.54 (quintet,  $J = 7.2$  Hz, 4 H, CH<sub>2</sub>), -0.48 (quintet,  $J = 7.2$  Hz, 4 H, CH<sub>2</sub>), -1.44 (quintet,  $J = 7.2$  Hz, 4 H, CH<sub>2</sub>), -1.63 (quintet,  $J = 6.8$  Hz, 4 H, CH<sub>2</sub>), -2.10 (t,  $J = 6.0$  Hz, 4 H, CH<sub>2</sub>). <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>):  $\delta = 187.9, 168.0, 161.0, 149.2, 146.0, 135.9, 130.8, 130.5, 123.6, 118.7, 114.2, 79.2, 71.2, 70.0, 68.9, 68.4, 65.0, 64.9, 54.4, 28.7, 27.5, 23.6, 23.2$ . MS (ESI): an isotopic cluster peaking at  $m/z = 1029$  (30%, [M-C<sub>27</sub>H<sub>29</sub>FeO<sub>5</sub>]<sup>+</sup>). HRMS (ESI):  $m/z$  calcd for C<sub>59</sub>H<sub>45</sub>FeN<sub>8</sub>O<sub>5</sub>Si [M-C<sub>27</sub>H<sub>29</sub>FeO<sub>5</sub>]<sup>+</sup>: 1029.2626; found: 1029.2611. Anal. calcd for C<sub>86</sub>H<sub>74</sub>Fe<sub>2</sub>N<sub>8</sub>O<sub>10</sub>Si: C, 67.99; H, 4.91; N, 7.37. Found: C, 67.61; H, 4.59; N, 7.16.

**Compound 7.** A mixture of SiPcCl<sub>2</sub> (0.26 g, 0.43 mmol), 2-hydroxyethyl disulfide (**4a**) (0.33 g, 2.13 mmol) and pyridine (1 mL, 12.4 mmol) in toluene (20 mL) was heated under reflux for 24 h. The volatiles were evaporated *in vacuo*. The crude product was purified by silica-gel column chromatography using CHCl<sub>3</sub> followed by CHCl<sub>3</sub>/MeOH (10:1) as the eluents. The product was obtained as a blue solid (0.25 g, 69%). R<sub>f</sub> [CHCl<sub>3</sub>/MeOH (10:1)] = 0.24. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 9.65-9.67$  (m, 8 H, Pc-H <sub>$\alpha$</sub> ), 8.36-8.38 (m, 8 H, Pc-H <sub>$\beta$</sub> ), 2.88-2.93 (m, 4 H, CH<sub>2</sub>), 1.48 (t,  $J = 6.0$  Hz, 4 H, CH<sub>2</sub>), -0.34 (t,  $J = 6.0$  Hz, 4 H, CH<sub>2</sub>), -1.76 (t,  $J = 6.0$  Hz, 4 H, CH<sub>2</sub>). <sup>13</sup>C{<sup>1</sup>H} NMR (DMSO-d<sub>6</sub>):  $\delta = 149.3, 135.6, 132.3, 124.1, 60.0, 58.9, 54.6, 43.1$ . MS (ESI):  $m/z = 693$  (100%, [M-C<sub>4</sub>H<sub>9</sub>O<sub>2</sub>S<sub>2</sub>]<sup>+</sup>). HRMS (ESI):  $m/z$  calcd for C<sub>36</sub>H<sub>25</sub>N<sub>8</sub>O<sub>2</sub>S<sub>2</sub>Si [M-C<sub>4</sub>H<sub>9</sub>O<sub>2</sub>S<sub>2</sub>]<sup>+</sup>: 693.1306; found: 693.1309. Anal. calcd for C<sub>40</sub>H<sub>34</sub>N<sub>8</sub>O<sub>4</sub>S<sub>4</sub>Si: C, 56.72; H, 4.05; N, 13.22. Found: C, 56.32; H, 3.98; N, 12.92.

**Cell lines and culture conditions.** The MCF-7 human breast cancer cells (from ATCC, no. HTB-22) were maintained in RPMI medium 1640 (Invitrogen, no. 223400-21) supplemented with fetal calf serum (10%), sodium pyruvate (1 mM) and penicillin-streptomycin (100 units mL<sup>-1</sup> and 100 µg mL<sup>-1</sup> respectively). Approximately 3 × 10<sup>4</sup> cells per well in the medium were inoculated in 96-multiwell plates and incubated overnight at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere.

**Photocytotoxicity assay.** All the phthalocyanines were first dissolved in DMF to give 1.6 mM solutions, which were diluted to 80 µM with the culture medium (with 0.5% Cremophor EL). For compound **5a**, it was dissolved in DMF to give a 100 mM solution, which was diluted to 10 mM with the culture medium (with 0.5% Cremophor EL). These solutions were then further diluted with the culture medium for photocytotoxicity study.

DTT was dissolved in deionised water to give a 1 M stock solution, which was further diluted with the culture medium to give 2 µM and 4 mM DTT solutions. Approximately 3 × 10<sup>4</sup> cells per well in the culture medium were inoculated in 96-multi-well plates and incubated overnight at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere. The cells were rinsed with PBS and incubated with DTT solution (2 µM or 4 mM, 100 µL) or the culture medium (without DTT) (100 µL) for 1 h under the same conditions. The cells, after being rinsed with PBS twice, were incubated with 100 µL of the above drug solutions for 6 or 24 h at 37 °C under 5% CO<sub>2</sub>. The cells were then rinsed again with PBS and refilled with 100 µL 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  $40 \text{ mW cm}^{-2}$ . Illumination of 20 min led to a total fluence of  $48 \text{ J cm}^{-2}$ .

Cell viability was determined by means of the colourimetric MTT assay.<sup>R5</sup> After illumination, the cells were incubated at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$  overnight. An MTT (Sigma) solution in PBS ( $3 \text{ mg mL}^{-1}$ ,  $50 \mu\text{L}$ ) 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,  $50 \mu\text{L}$ ) was then added to each well. The plate was incubated in an oven at  $60^\circ\text{C}$  for 30 min, then  $80 \mu\text{L}$  of *iso*-propanol was added to each well. The plate was agitated on a Bio-Rad microplate reader at ambient temperature for 10 sec before the absorbance at 540 nm for 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 =  $[(A_i/A_{\text{control}} \times 100)]/n$ , where  $A_i$  is the absorbance of the  $i$ th data ( $i = 1, 2, \dots, n$ ),  $A_{\text{control}}$  is the average absorbance of the control wells in which the phthalocyanine was absent and  $n$  ( $= 4$ ) is the number of the data points.

**Intracellular fluorescence studies.** About  $6 \times 10^4$  MCF-7 cells were seeded on a coverslip and incubated overnight at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$ . The cells, after being rinsed with PBS, were incubated with DTT solution ( $2 \mu\text{M}$  or  $4 \text{ mM}$ ,  $2 \text{ mL}$ ) or the culture medium (without

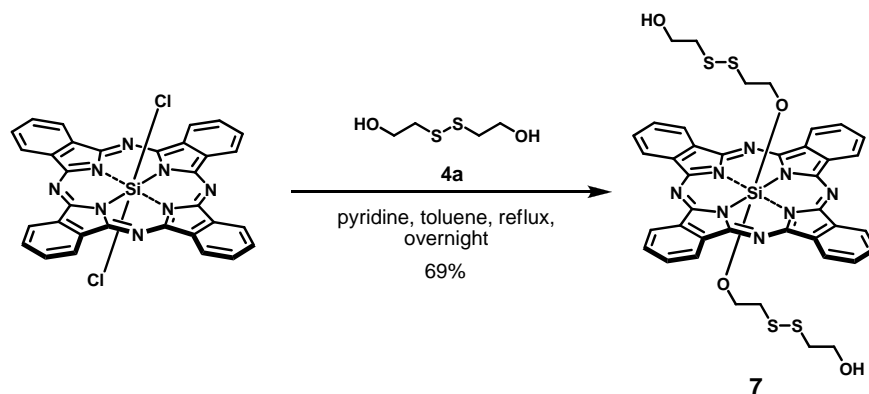
DTT) (2 mL) for 1 h under the same conditions. The cells were rinsed with PBS twice and were further incubated with **6a** or **6b** in the medium (1  $\mu$ M, 2 mL) for 24 h under the same conditions. The cells were rinsed with PBS and then viewed with a Leica SP5 confocal microscope equipped with a 633 helium neon laser. Emission signals from 650-760 nm (gain = 700 V) were collected and the images were digitised and analysed by Leica Application Suite Advanced Fluorescence. The intracellular fluorescence intensities (a total of 30 cells for each sample) were also determined.

**Subcellular localisation studies.** About  $6 \times 10^4$  MCF-7 cells were seeded on a coverslip and incubated overnight at 37 °C under 5% CO<sub>2</sub>. The cells, after being rinsed with PBS, were incubated with DTT solution (4 mM, 2 mL) for 1 h under the same conditions. The cells were rinsed with PBS twice and were further incubated with **6a** in the medium (1  $\mu$ M, 2 mL) for 24 h under the same conditions. For the study using ER-Tracker, the cells were incubated with ER-Tracker Green (Molecular Probe, 0.2  $\mu$ M in PBS) under the same conditions for a further 30 min. For the study using LysoTracker and MitoTracker, the cells were incubated with LysoTracker Green DND 26 (Molecular Probe, 2  $\mu$ M in the medium) or MitoTracker Green FM (Molecular Probe, 0.25  $\mu$ M in the medium) for a further 10 min. For all the cases, the cells were then rinsed with PBS and viewed with a Leica SP5 confocal microscope equipped with a 488 nm argon laser and a 633 helium neon laser. All the Trackers were excited at 488 nm and monitored at 510-560 nm, whereas **6a** was excited at 633 nm and monitored at

650-760 nm. The images were digitised and analysed using Leica Application Suite Advanced Fluorescence. The subcellular localisation of **6a** after disulfide cleavage was revealed by comparing the intracellular fluorescence images caused by the three Trackers and **6a** after reductive cleavage.

## References

- R1 V. Zsoldos-Mády, A. Csámpai, R. Szabó, E. Mészáros-Alapi, J. Pásztor, F. Hudecz and P. Sohár, *ChemMedChem*, 2006, **1**, 1119.
- R2 D. F. Eaton, *Pure Appl. Chem.*, 1988, **60**, 1107.
- R3 I. Scalise and E. N. Durantini, *Bioorg. Med. Chem.*, 2005, **13**, 3037.
- R4 M. D. Maree, N. Kuznetsova and T. Nyokong, *J. Photochem. Photobiol. A: Chem.*, 2001, **140**, 117.
- R5 H. Tada, O. Shiho, K. Kuroshima, M. Koyama and K. Tsukamoto, *J. Immunol. Methods*, 1986, **93**, 157.

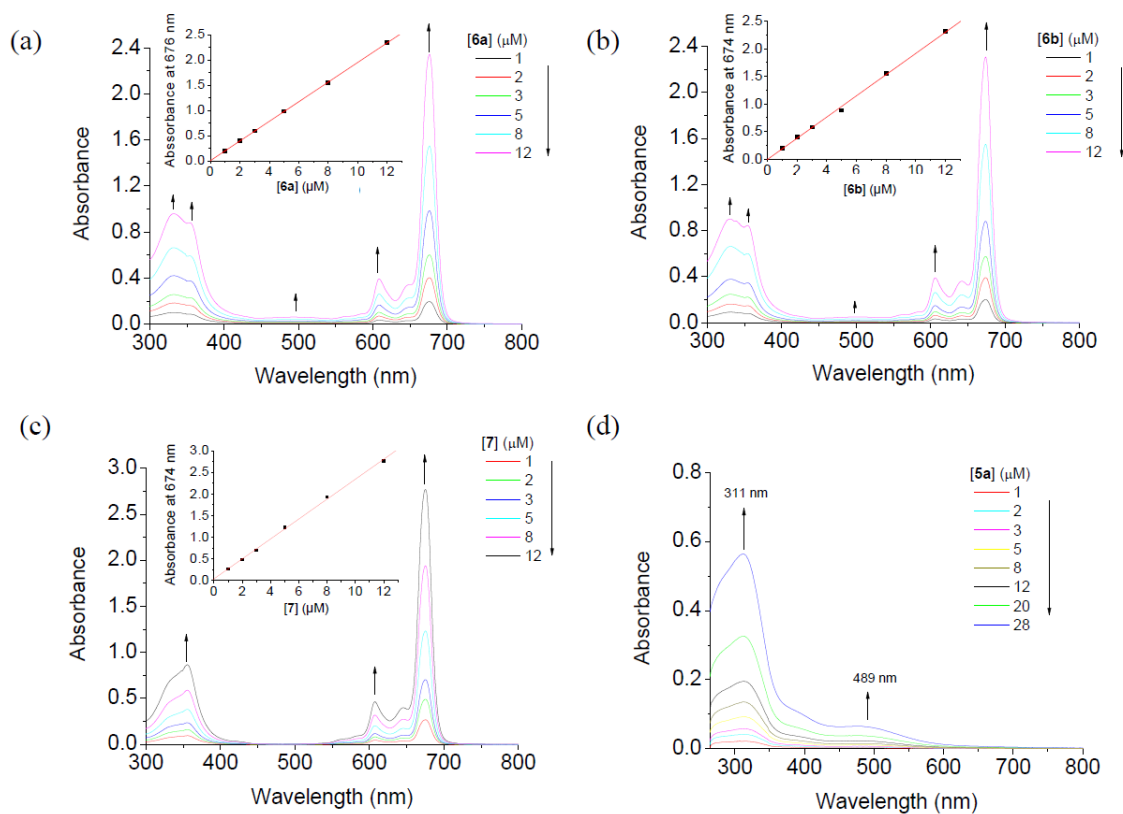


**Scheme S1** Synthesis of phthalocyanine 7.

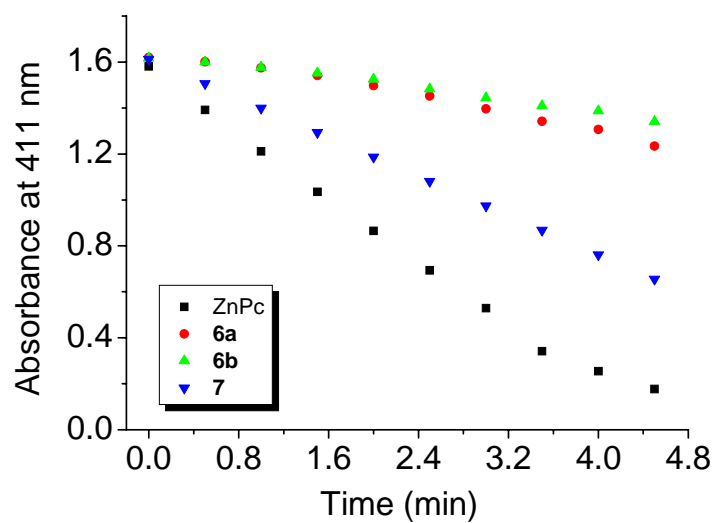
**Table S1** Electronic absorption and photophysical data for **6a**, **6b** and **7** in DMF.

Compound	$\lambda_{\max}$ (nm) ( $\log \epsilon$ )	$\lambda_{\text{em}}$ (nm) <sup>a</sup>	$\Phi_{\text{F}}$ <sup>b</sup>	$\Phi_{\Delta}$ <sup>c</sup>
<b>6a</b>	332 (4.90), 353 (4.86), 494 (3.67), 608 (4.51), 646 (4.44), 676 (5.29)	688	0.04	0.17
<b>6b</b>	330 (4.88), 353 (4.85), 498 (3.63), 608 (4.51), 642 (4.48), 674 (5.28)	684	0.05	0.13
<b>7</b>	355 (4.85), 608 (4.58), 645 (4.52), 674 (5.36)	687	0.28	0.34

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



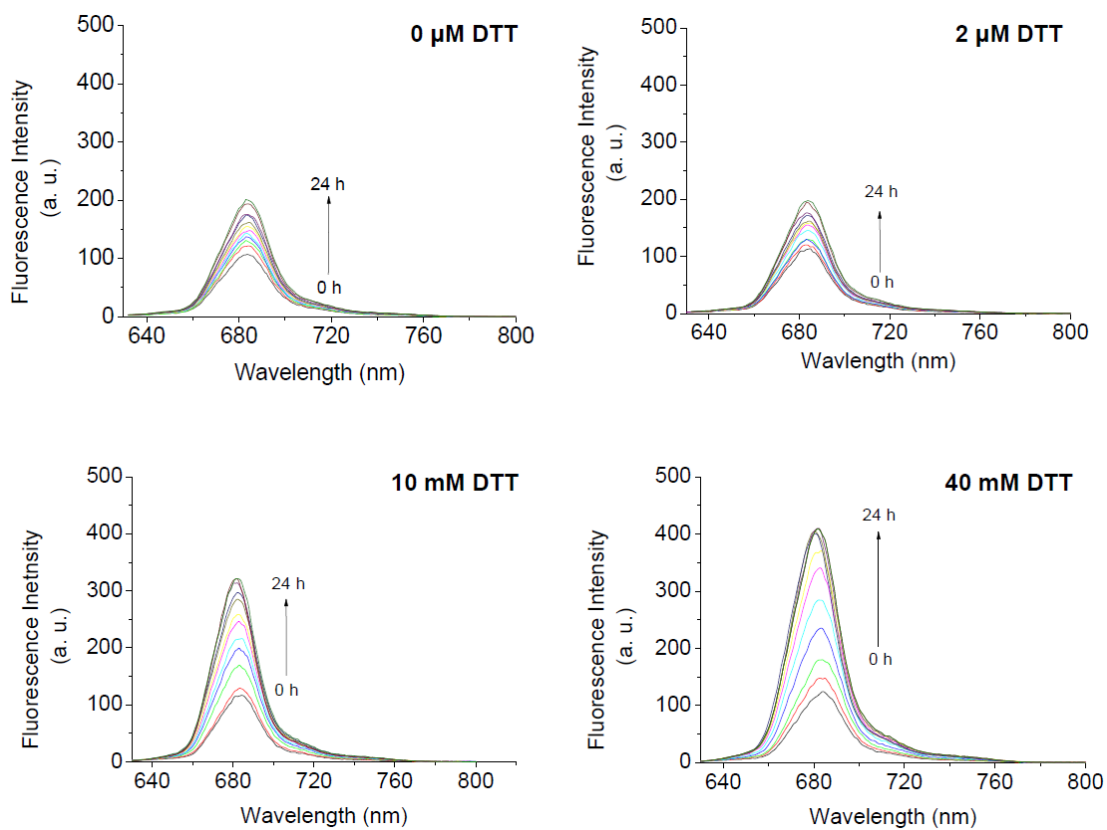
**Fig. S1** Electronic absorption spectra of (a) **6a**, (b) **6b**, (c) **7** and (d) **5a** in DMF in different concentrations. The insets of spectra (a) to (c) plot the Q-band absorbance versus the concentration of the phthalocyanine, and the line represents the best-fitted straight line.



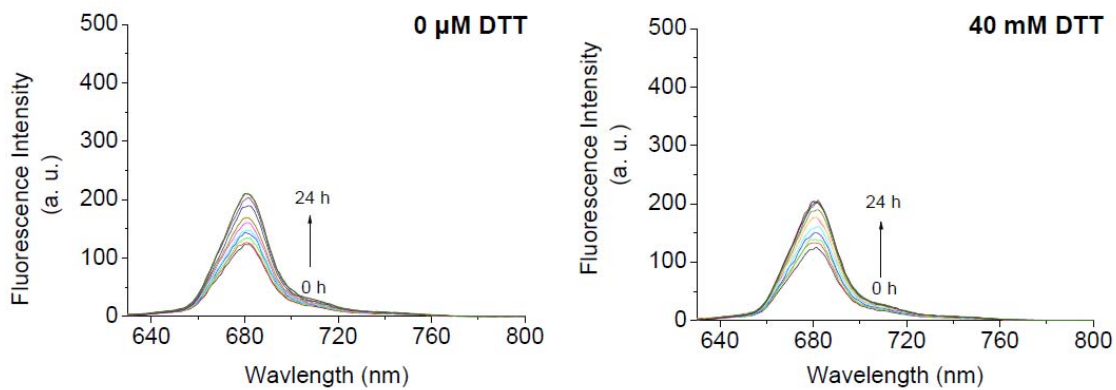
**Fig. S2** Comparison of the rate of photodegradation of DPBF (initial concentration = 70  $\mu\text{M}$ ) sensitised by **6a**, **6b**, **7** and **ZnPc** (all at 3  $\mu\text{M}$ ) in DMF as shown by the decrease in the absorbance at 411 nm along with the irradiation time.



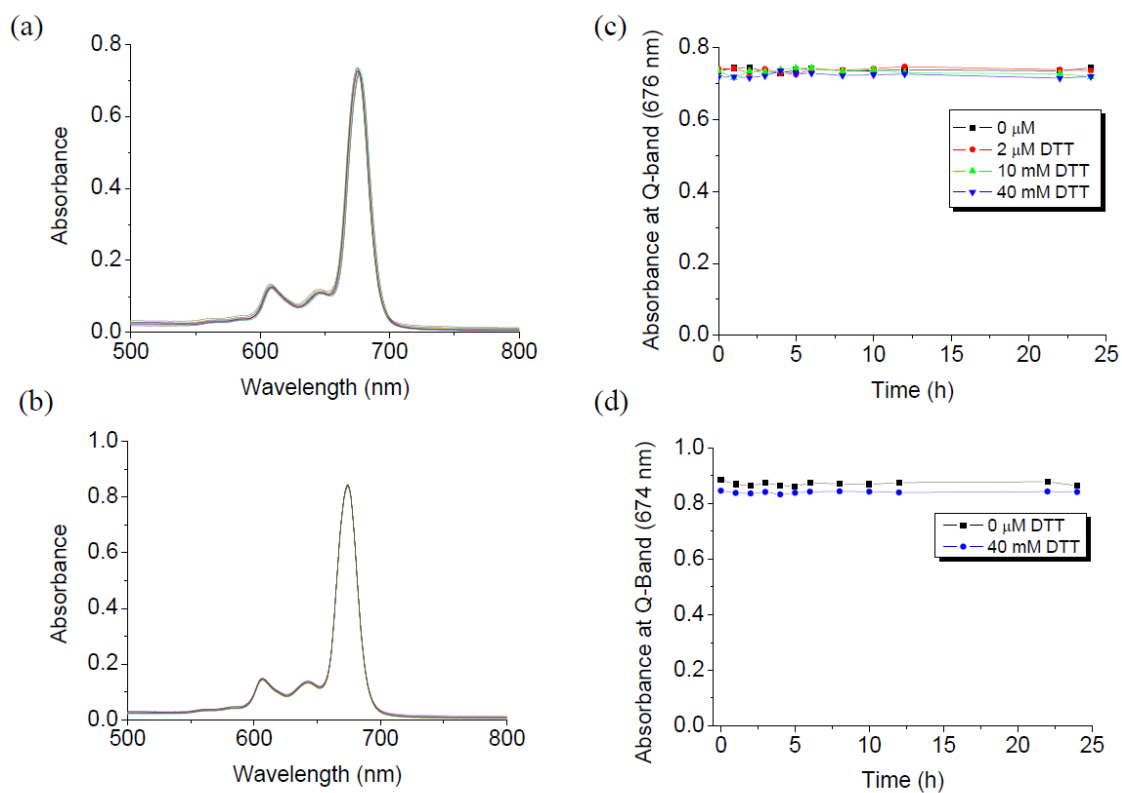
(a) For compound **6a**:



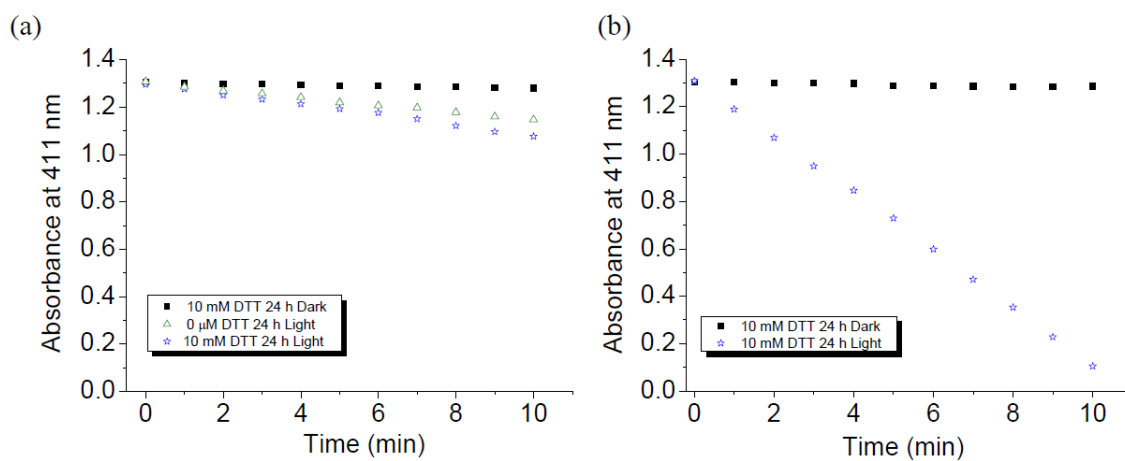
(b) For compound **6b**:



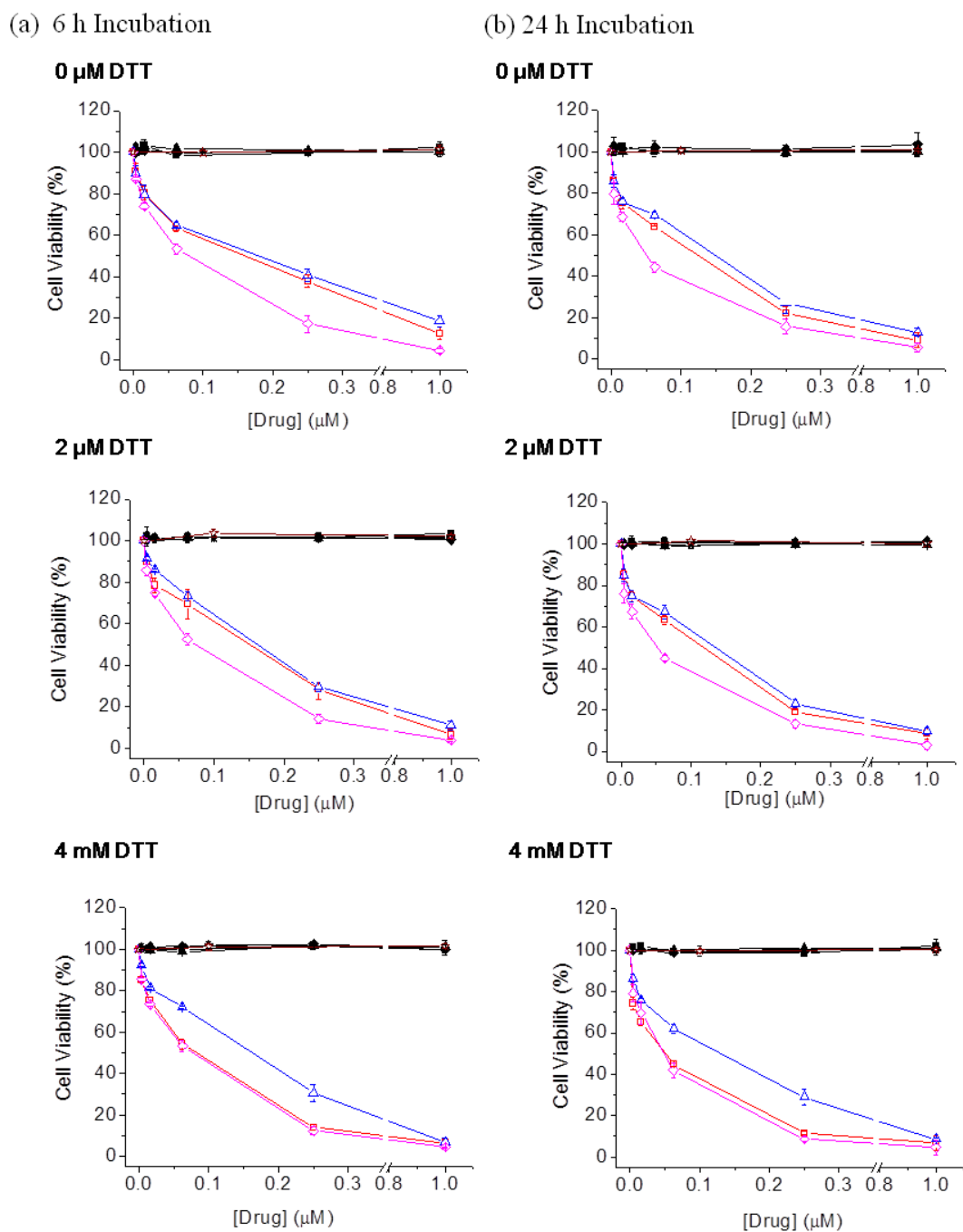
**Fig. S3** Changes in fluorescence spectra ( $\lambda_{\text{ex}} = 610 \text{ nm}$ ) of (a) **6a** and (b) **6b** (both at 4  $\mu\text{M}$ ) with time upon exposure to different concentrations of DTT in PBS with 0.5% Cremophor EL.



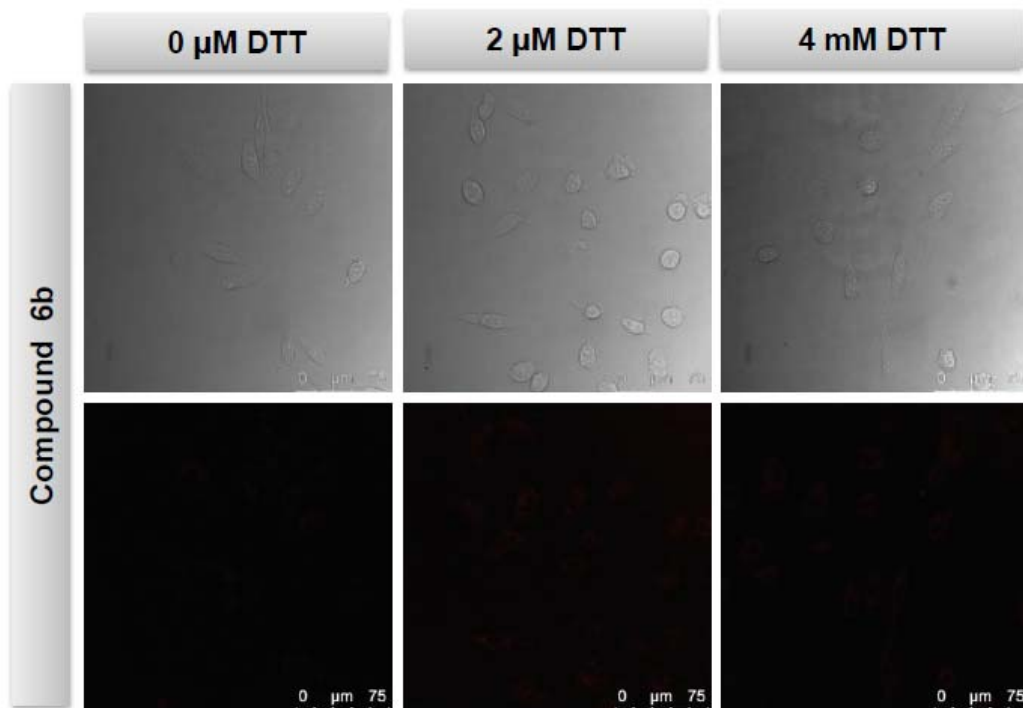
**Fig. S4** Changes in absorption spectra of (a) **6a** and (b) **6b** (both at 4 μM) with time upon exposure to different concentrations of DTT in PBS with 0.5% Cremophor EL. The corresponding changes in Q-band absorbance are shown in (c) and (d) respectively.



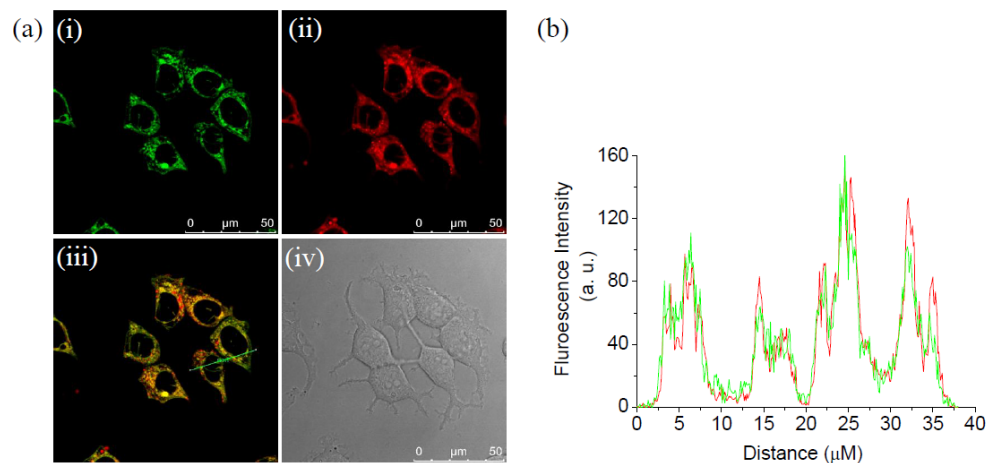
**Fig. S5** Comparison of the rate of photodegradation of DPBF (initial concentration = 70  $\mu$ M) sensitised by (a) **6b** and (b) **7** (both at 4  $\mu$ M) upon exposure to 0 or 10 mM of DTT for 24 h in PBS with 0.5% Cremophor EL. The dark control is shown with closed symbols.



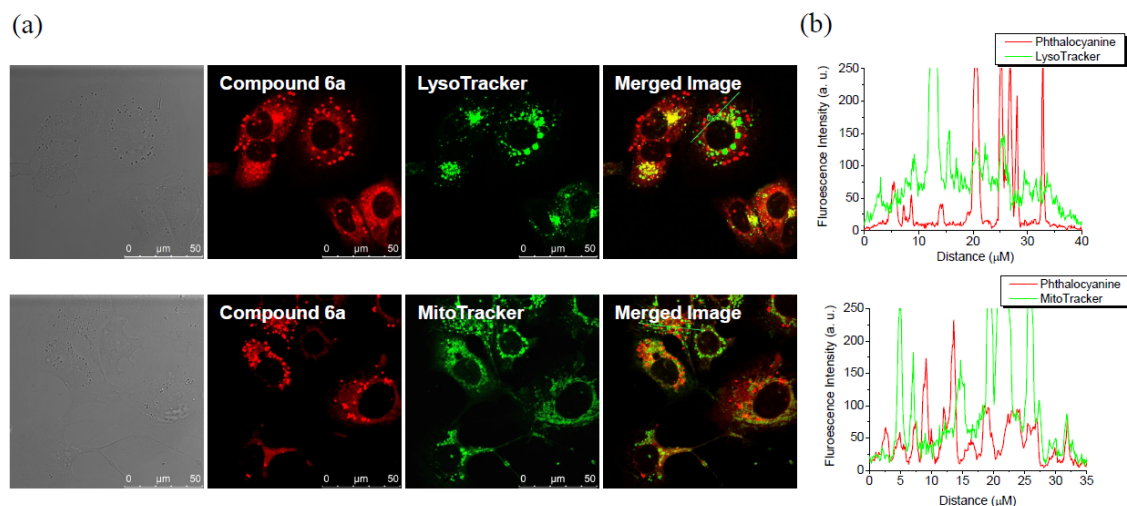
**Fig. S6** Cytotoxic effects of **6a** (squares), **6b** (triangles), **7** (rhombus) and **5a** (stars) on MCF-7 cells pre-treated with different concentrations of DTT for 1 h followed by drug incubation for (a) 6 h or (b) 24 h in the absence (closed symbols) and presence (open symbols) of light ( $\lambda > 610$  nm,  $40$  mW  $\text{cm}^{-2}$ ,  $48$  J  $\text{cm}^{-2}$ ). Data are expressed as mean  $\pm$  standard error of the mean of three independent experiments, each performed in quadruplicate.



**Fig. S7** Confocal fluorescence images of MCF-7 cells pre-treated with different concentrations of DTT followed by incubation with **6b** (1 μM) for 24 h (lower row). The corresponding bright field images are shown in the upper row.

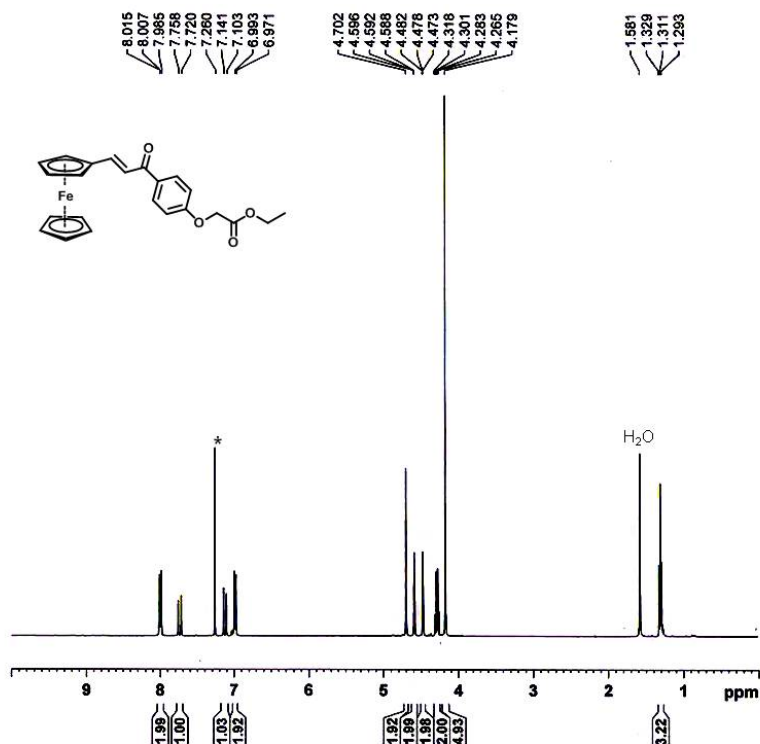


**Fig. S8** (a) Fluorescence images of MCF-7 cells pre-treated with 4 mM of DTT using filter sets specific for (i) ER-Tracker (in green) and (ii) **6a** (in red). The corresponding superimposed and bright field images are shown in (iii) and (iv) respectively. Figure (b) shows the fluorescence intensity profiles of **6a** (red) and ER-Tracker (green) traced along the green line in (a) (iii).

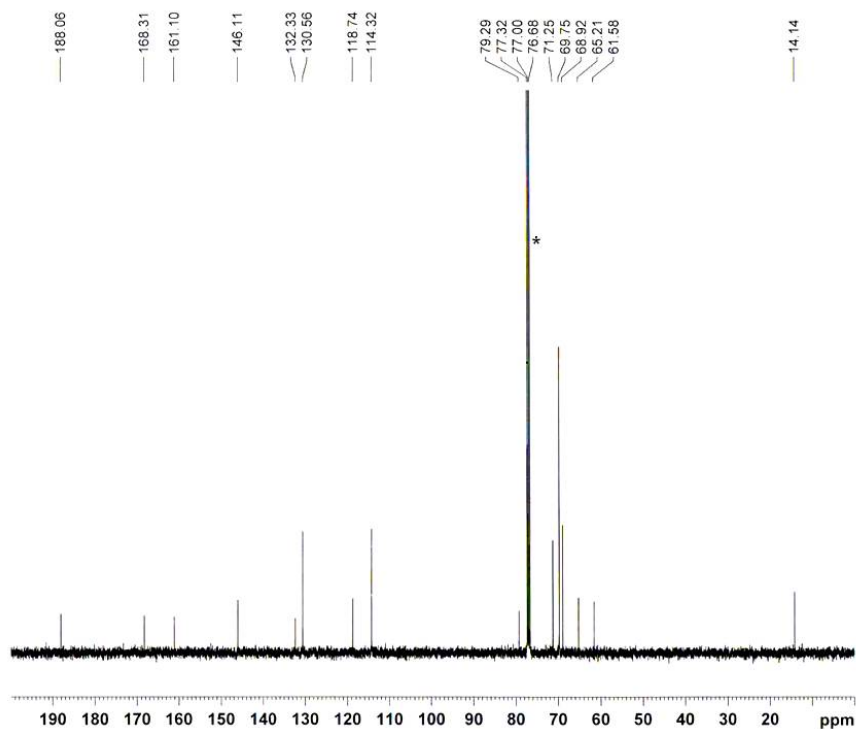


**Fig. S9** (a) Fluorescence images of MCF-7 cells pre-treated with 4 mM of DTT using filter sets specific for **6a** (in red, column 2) and LysoTracker or MitoTracker (in green, column 3). The corresponding superimposed and bright field images are given in columns 4 and 1 respectively. Figure (b) shows the fluorescence intensity profiles of **6a** (red) and LysoTracker or MitoTracker (green) traced along the green lines in the superimposed images.

In the following spectra, residual solvent signals are marked with asterisks.

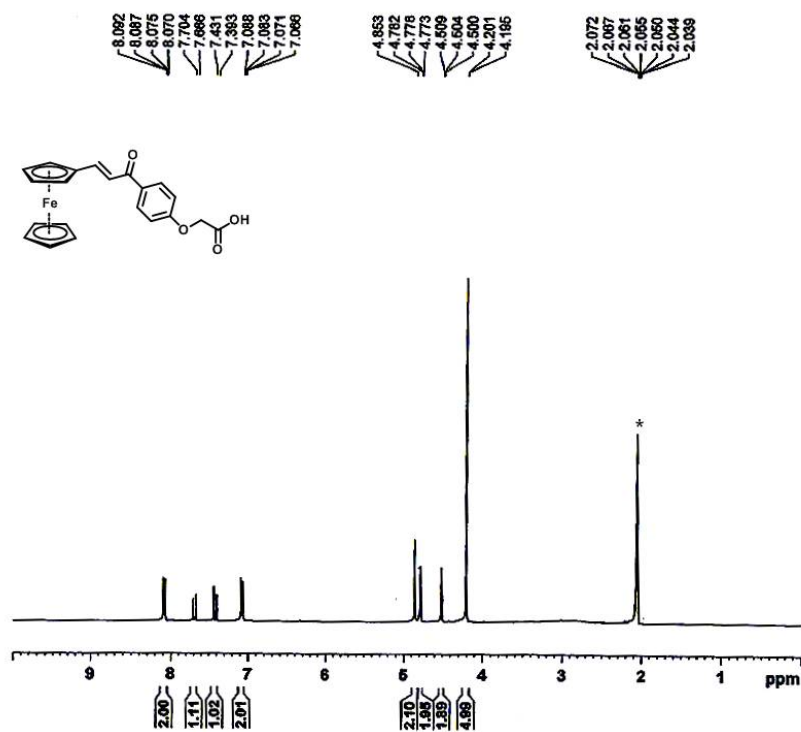


<sup>1</sup>H NMR spectrum of **2** in CDCl<sub>3</sub>.

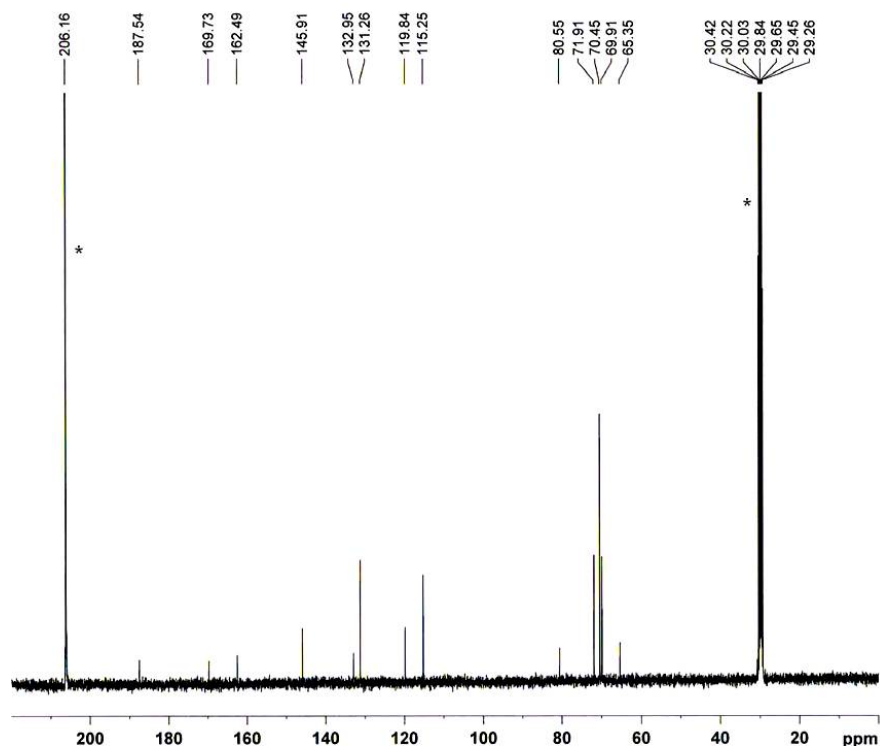


<sup>13</sup>C{<sup>1</sup>H} NMR spectrum of **2** in CDCl<sub>3</sub>.

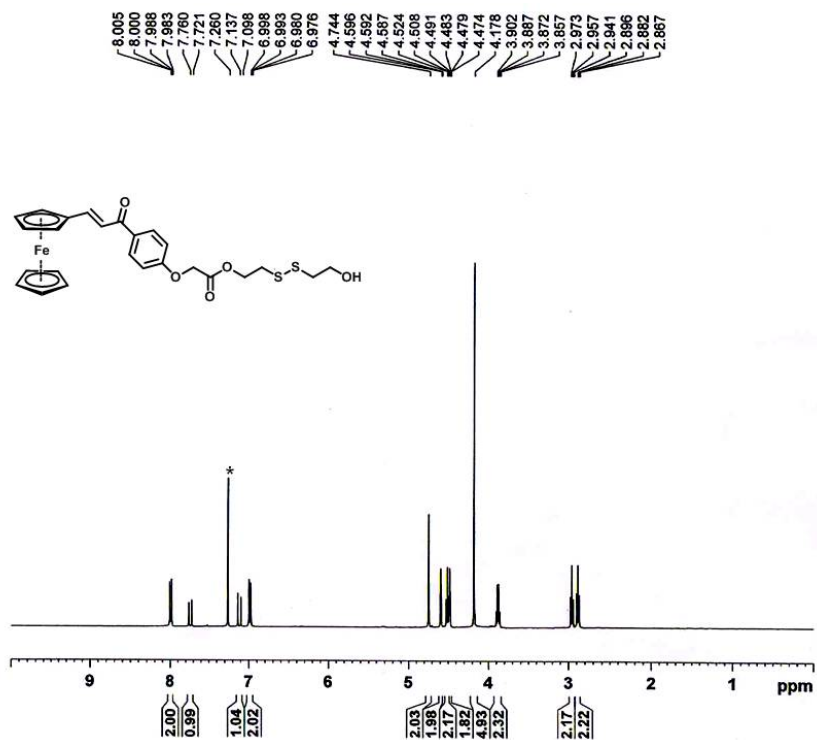




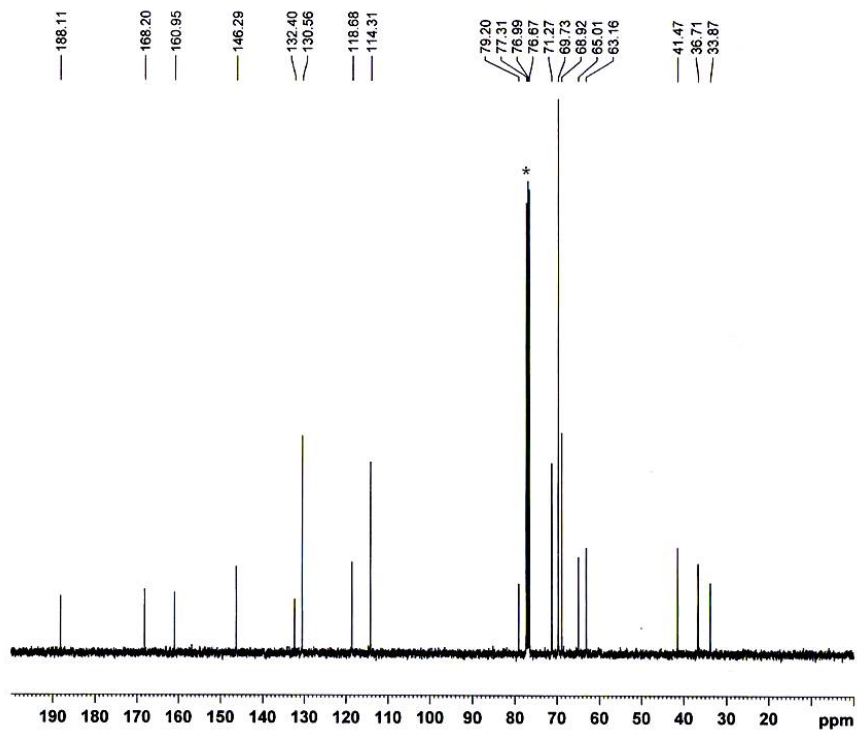
<sup>1</sup>H NMR spectrum of 3 in acetone-d<sub>6</sub>.



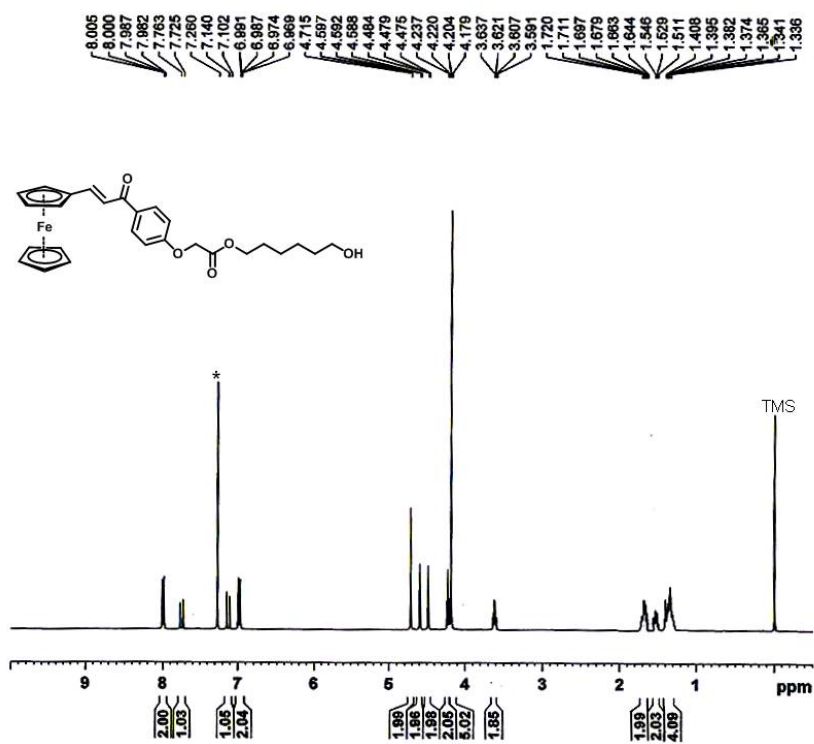
<sup>13</sup>C{<sup>1</sup>H} NMR spectrum of 3 in acetone-d<sub>6</sub>.



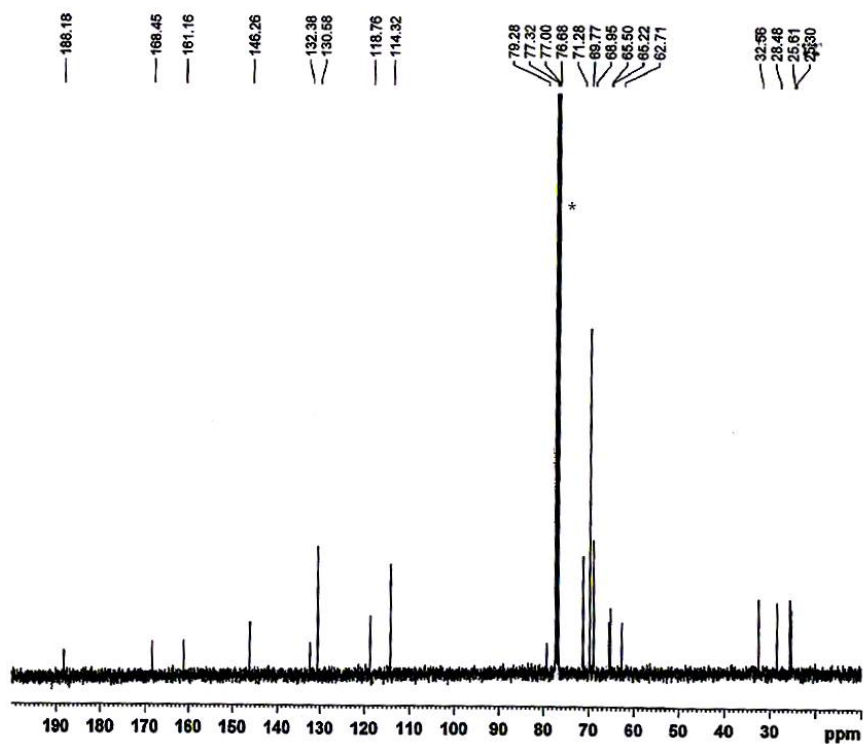
$^1\text{H}$  NMR spectrum of **5a** in  $\text{CDCl}_3$ .



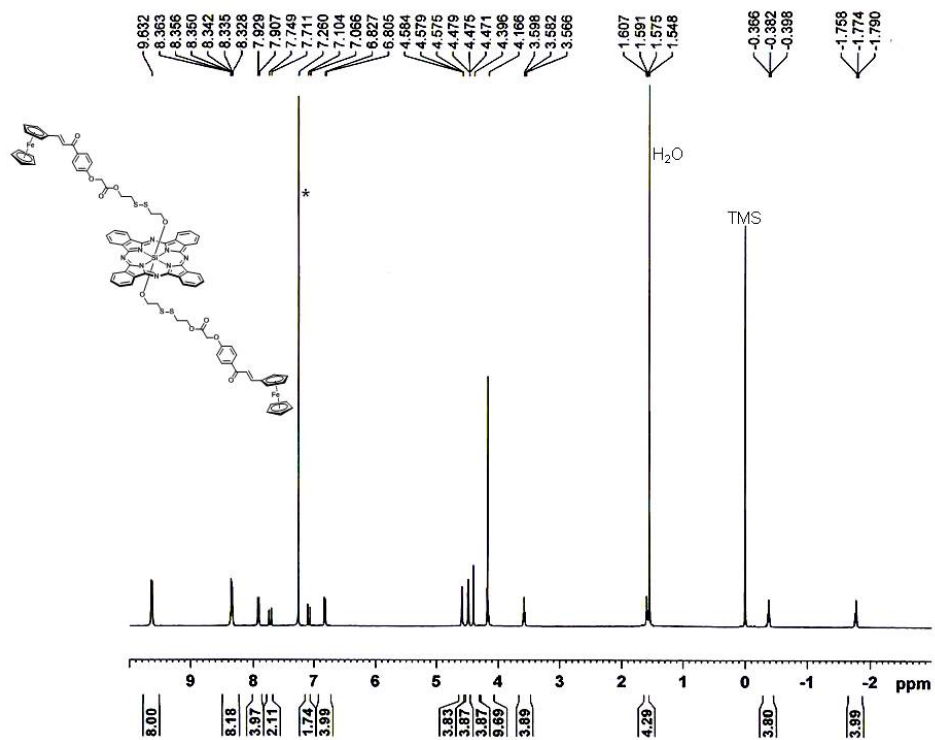
$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **5a** in  $\text{CDCl}_3$ .



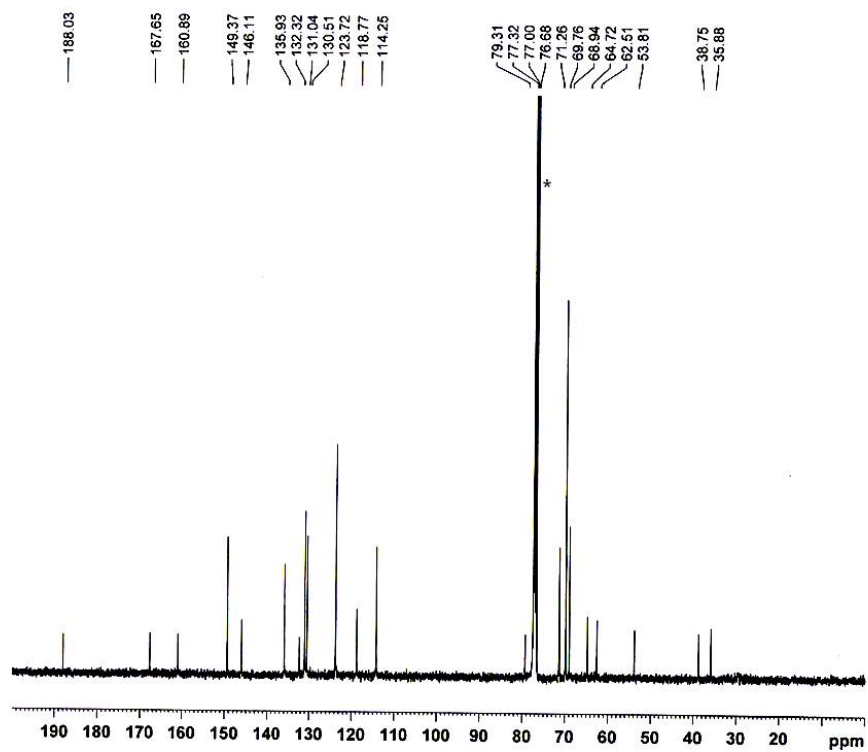
$^1\text{H}$  NMR spectrum of **5b** in  $\text{CDCl}_3$ .



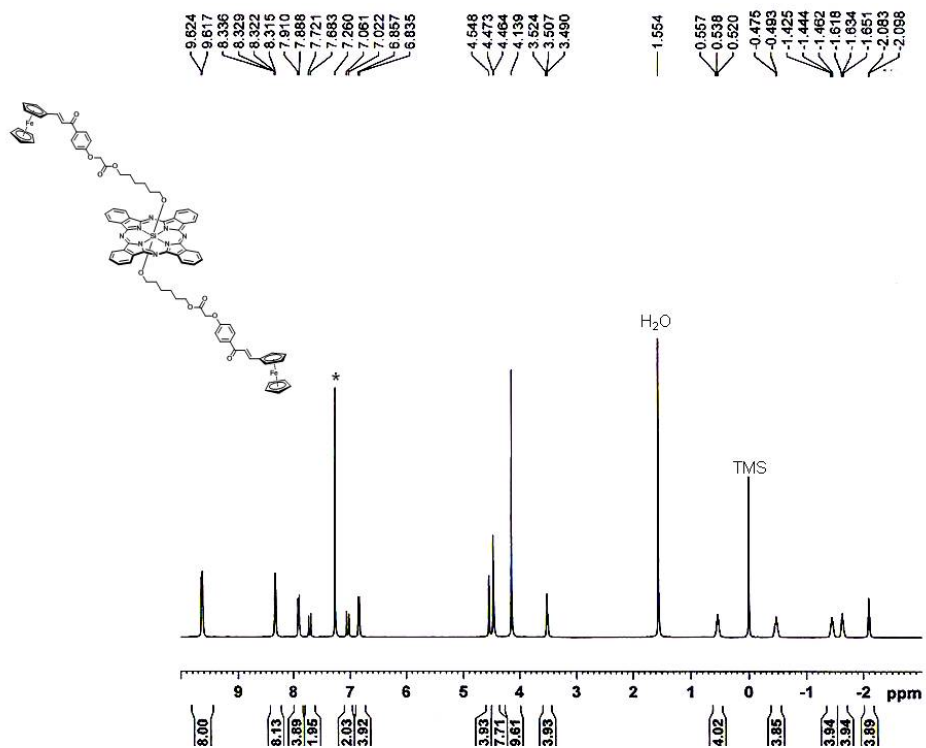
$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **5b** in  $\text{CDCl}_3$ .



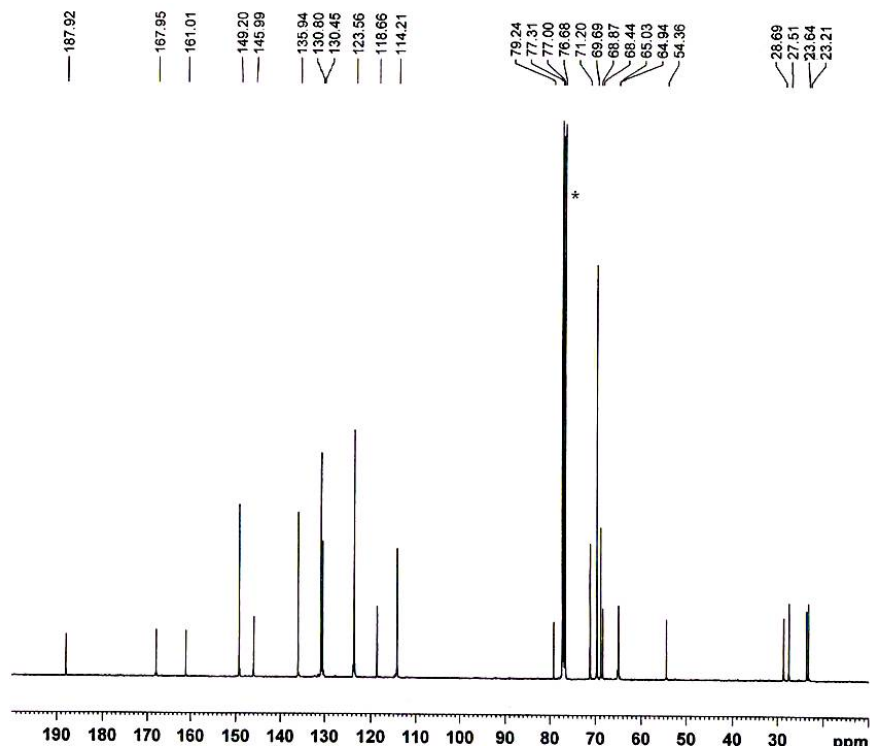
<sup>1</sup>H NMR spectrum of **6a** in CDCl<sub>3</sub>.



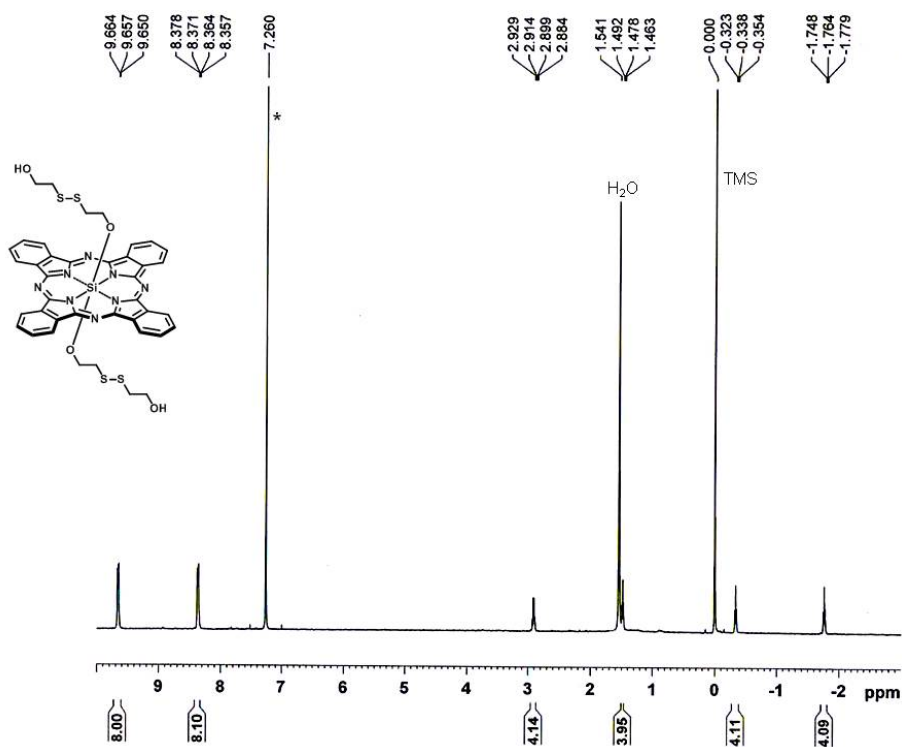
<sup>13</sup>C{<sup>1</sup>H} NMR spectrum of **6a** in CDCl<sub>3</sub>.



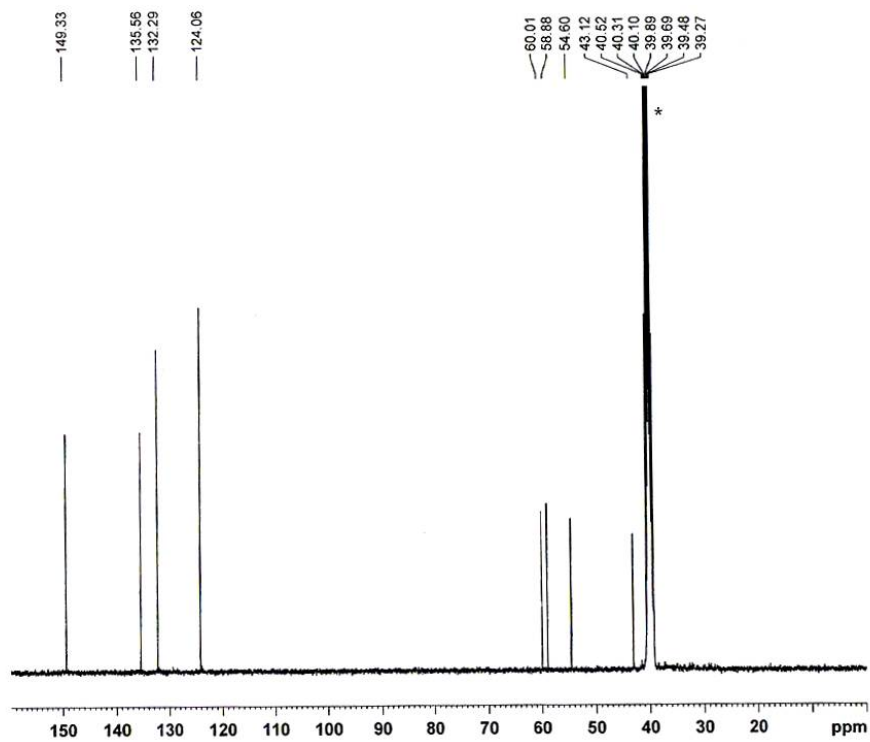
<sup>1</sup>H NMR spectrum of **6b** in CDCl<sub>3</sub>.



<sup>13</sup>C{<sup>1</sup>H} NMR spectrum of **6b** in CDCl<sub>3</sub>.



<sup>1</sup>H NMR spectrum of **7** in CDCl<sub>3</sub>.



<sup>13</sup>C{<sup>1</sup>H} NMR spectrum of **7** in DMSO-d<sub>6</sub>.