Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Modulation of Singlet Oxygen production in Photodynamic Therapy using a pH controlled Photoinduced Electron Transfer (PET) based sensitiser.

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1.0. Materials and Methods.

1.1 Equipment and Reagents: Chemicals were purchased from commercial sources at the highest possible purity and used as received. UV-Vis spectra were recorded with a Varian Cary spectrometer, using quartz cells with a path length of 1 cm. Emission spectra were recorded with a Varian Cary Eclipse spectrometer in aerated solutions with slits = 5 nm. NMR spectra were recorded on a Varian 500 MHz spectrometer. ESI-MS characterisation was achieved using a LCQTM quadrupole ion-trap mass spectrometer (Finnigan MAT, San Jose, California, USA) utilising electrospray ionisation (ESI).

*1.2 Synthesis of 1,8-dihydroxy-3-methoxy-6-methyl-anthracene-9,10-dione (**2**):* Methyl iodide (11.5 mL, 185.1 mmol) was added to a solution of emodin **1** (10.0 g, 37.0 mmol) and K_2CO_3 (10.2g, 74.0 mmol) in anhydrous acetone (120 mL) and the solution was refluxed for 18 hours. The reaction was monitored by TLC (Merck Silica 60, HF 254, chloroform as mobile phase). After cooling, the solvent was removed *in vacuo* and water (120 mL) was added. The resulting precipitate was collected via filtration and the crude solid mass was purified by silica gel (60-120 mesh) column chromatography using chloroform as eluting agent. The product **2** was isolated as an orange crystalline solid (4.7 g, Yield = 44%): mp 220-223 °C; ^{1}H NMR (500MHz, $CDCl_3$) δ 12.30 (s,1H, OH), 12.11 (s,1H, OH), 7.62 (s,1H,

H_{Ar}), 7.36 (s,1H, H_{Ar}), 7.07 (s,1H, H_{Ar}), 6.68 (s,1H, H_{Ar}), 3.94 (s,3H, OMe), 2.45 (s, 3H, Me), ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.8 (C=O), 182.0 (C=O), 166.6 (C), 165.2 (C), 162.5 (C), 148.5 (C), 135.3(C), 133.2(C), 124.5(C), 121.3(CH), 113.7(C), 110.3(CH), 108.2 (CH), 106.8 (CH), 56.1 (OCH_3), 22.2 (CH_3). ESMS $[M+H]^+$ found 285.2, calculated for $C_6H_{12}O_5$ = 284.3.

*1.3 Synthesis of 3-methoxy-6-methyl-9,10-dioxo-9,10-dihydroanthracene-1,8-diyl bis(4-methylbenzenesulfonate) (**3**)*: To a solution of **2** (4.3 g, 15.1 mmol) and K_2CO_3 (9.83g, 71.1 mmol) in anhydrous acetone (150 mL), tosyl chloride (13.56g, 71.1 mmol) was added and the solution was refluxed for 16 hours. The reaction mixture was then cooled and the solvent removed under reduced pressure. Water (150ml) was added to the mixture and the contents stirred for four hours before being filtered and washed with ether (40 mL) to yield **3** as a yellow solid (8.82g, Yield = 98%); mp 198-200 °C; ^{1}H NMR (500MHz, $CDCl_3$) δ 7.97 (s,1H, H_{Ar}), 7.94 (dd, J = 7.90, 7.57 Hz, 4H, H_{Ar}), 7.62 (s,1H, H_{Ar}), 7.49 (s,1H, H_{Ar}), 7.31 (dd, J = 6.95, 7.11 Hz, 4H, H_{Ar}), 7.15 (s,1H, H_{Ar}), 3.94 (s, 3H, OMe), 2.48 (s, 3H, Me), 2.42 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.8 (C=O), 177.6 (C=O), 163.3 (C), 149.2 (C), 147.2 (C), 145.6(C), 145.5(C), 145.4(C), 135.8(C), 134.2(C), 132.4(C), 130.7(CH), 129.7(CH), 129.0(CH), 128.9(CH),126.4(CH), 125.1(CH), 120.9(CH), 116.3(CH), 109.7(CH), 56.2 (OCH_3), 21.7(CH_3), 21.6 (CH_3). ESMS $[M+Na]^+$ found 615.1, calculated for $C_{30}H_{24}NaO_9S_2$ = 615.6.

1.4 Synthesis of 1,8-bis(2-dimethylaminoethylamino)-3-methoxy-6-methyl-anthracene-9,10-dione (**4**): *N,N*-dimethylethylenediamine (1 mL, 9.2 mmol) was added to a stirred solution of **3** (1.1 g, 1.8 mmol) in 2-methoxyethanol (20 mL) and the solution was stirred at 130°C for 4.5 hours. The reaction mixture was cooled and solvent was removed *in vacuo* before purification by column chromatography (MeOH: $CHCl_3$, 1 : 9 v/v) to give **4** as a dark purple semi-solid (0.38g, Yield = 49.6 **%**): ^{1}H NMR (500MHz, $CDCl_3$) δ 9.72 (s,1H, NH), 9.65 (s, 1H, NH), 7.36 (s, 1H, H_{Ar}), 7.13 (s, 1H, H_{Ar}), 6.81 (s, 1H, H_{Ar}), 6.40 (s, 1H, H_{Ar}), 3.91 (s, 3H, OMe), 3.38-3.42 (m, 4H, CH_2 X 2), 2.68 (t, J = 6.13 Hz, 4H, CH_2 X 2,), 2.38 (s, 15H, CH_3 X 5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.5 (C=O), 184.9 (C=O), 163.9 (C), 153.2(C), 151.1(C),

144.6(C), 136.4(C), 134.0(C), 117.6(CH), 116.4(CH), 112.6(CH), 110.2 (C), 101.4(C), 101.0 (CH), 58.0(CH_2), 55.5 (OCH_3), 45.6(CH_2), 22.2(CH_3). ESMS $[M+H]^+$ found 425.2, calculated for $C_{24}H_{32}N_4O_3$ = 424.5.

*1.5 pH-fluorescence titration of **4**:* A 70 μM solution of **4** was prepared in a H_2O : MeOH (1:1, 150 mL) solvent system. The solution pH was adjusted from acidic (pH 2.0) to basic (pH 10.6) using 5 M HCl and 5 M NaOH ensuring volume addition was as low as possible to avoid dilution. The fluorescence spectra were recorded approximately every 1.0 pH unit.

*1.6 Determination of singlet oxygen generation of **4** at different pH values:* Four solutions containing **4**(5 μM) and SOSG (2.5 μM) in 3 mL of H_2O were prepared. Two of the solutions were pH adjusted to pH 3.3 and the remaining two adjusted to pH 10.3. One of the pH 3.3 and one of the pH 10.3 solutions were treated with white light for 5 min (113 J/cm^2) while the remaining two solutions were kept in the dark. The fluorescence intensity of SOSG (λ_{EX} = 505 nm) was recorded at 525 nm at the beginning and end of the experiment.

*1.7 In vitro PDT effect of **4** in HeLa cells at pH 6.0 and pH 7.4:* HeLa cells were cultured in DMEM, supplemented with foetal bovine serum (10 % v/v) and penicillin (200 μg mL^{-1}). The cells were seeded in 96 well plates at a density of 5 x 10^3 cells per well, incubated for 24h at 37°C in a humidified CO_2 (5%) atmosphere and spiked with concentrations of **4** prepared in pH 6.0 or pH 7.4 PBS solution to allow for final concentrations of 3, 5 and 10 μM. The cells were incubated in the dark for a further 3 h, the medium was removed and each well was washed twice with PBS (pH 6.0 or pH 7.4). Fresh PBS (pH 6 or pH 7.4, 100 μL) was added to each well and selected wells were subjected to 30 seconds treatment with white light (11.4 J/cm^2). After irradiation the PBS was removed,replaced by fresh medium (100 μL) and cells were allowed to incubate in the dark for a further 24 hrs at 37°C in a humidified CO_2 (5%) atmosphere. Cell viability was then determined using a MTT assay.[1]

*1.8 In vivo PDT effect of **4** on mice bearing human xenograft ectopic BxPc-3 pancreatic tumours:* All animals employed in this study were treated humanely and in accordance with licensed procedures under the UK Animals (Scientific Procedures) Act 1986. BxPc-3 cells were maintained in RPMI-160 medium supplemented with 10% foetal calf serum. Cells were cultured at 37 °C under 5% CO_2 in air. BxPc-3 cells (1×10^6) were re-suspended in 100 μL of Matrigel® and implanted into the rear dorsum of male Balb/c SCID (C.B.-17/IcrHan®Hsd-*Prkdc*scid) mice. Tumour formation occurred approximately 2 weeks after implantation and the animals were randomly distributed into 3 groups (n = 3). Following induction of anaesthesia (intraperitoneal injection of Hypnorm/Hypnovel), a 100 μL aliquot of PBS containing **4** was injected directly into each tumour on day 0 (1.25 mg/kg) and day 3 (4.0 mg/kg). Where applicable, animals were then exposed to white light for 3 X 3 min treatments (205 J/cm^2) with a 1 minute interval between each treatment. After treatment, animals were allowed to recover from anaesthesia and tumours were measured daily using callipers. Tumour volume was calculated by the equation; tumour volume = $4\pi R^3/3$. The % increase in tumour volume was expressed relative to the pre-treatment measurements for each group.

2.0 Supplementary Figures

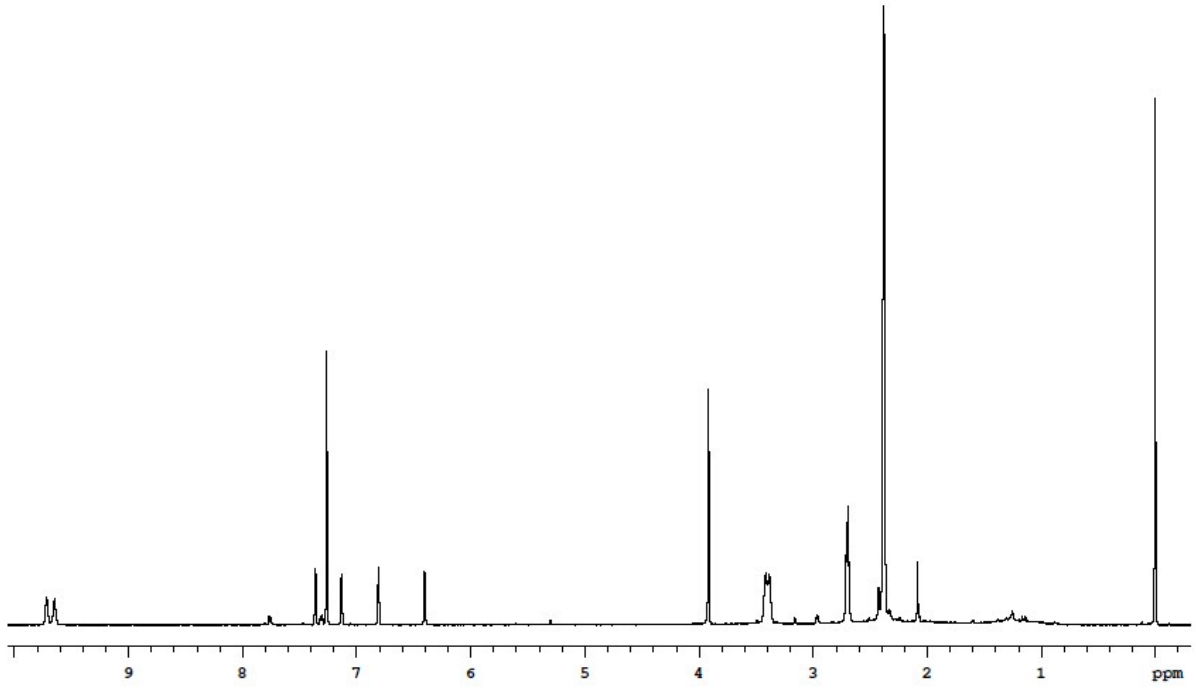

Figure S1 ^{1}H-NMR spectrum of **4** in $CDCl_3$

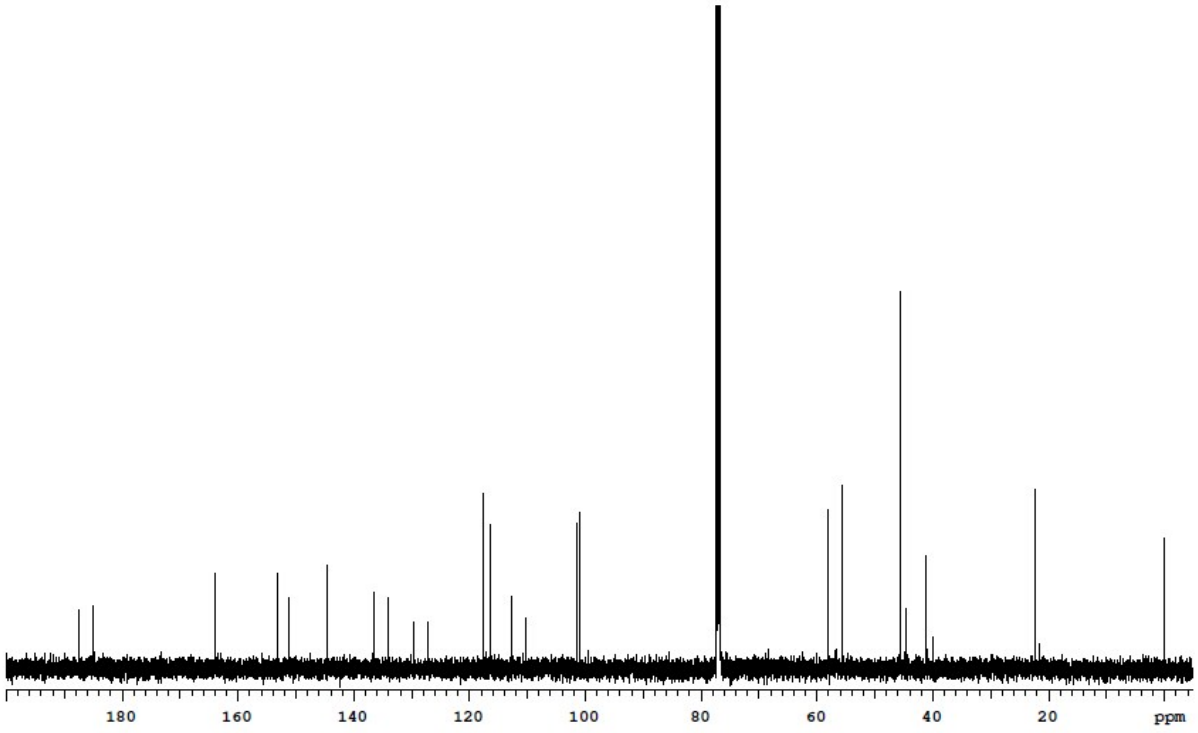

Figure S2 ^{13}C NMR spectrum of **4** recorded in $CDCl_3$.

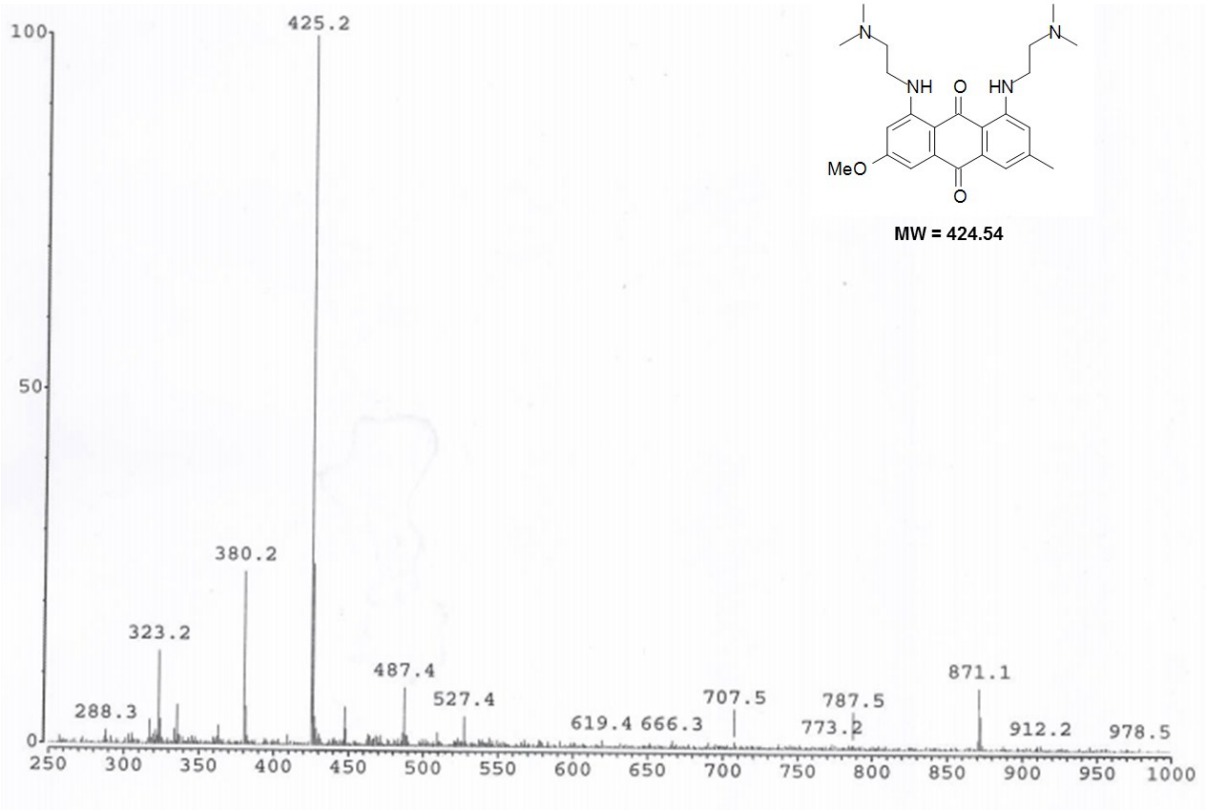

Figure S3 Positive mode ESMS of **4**.

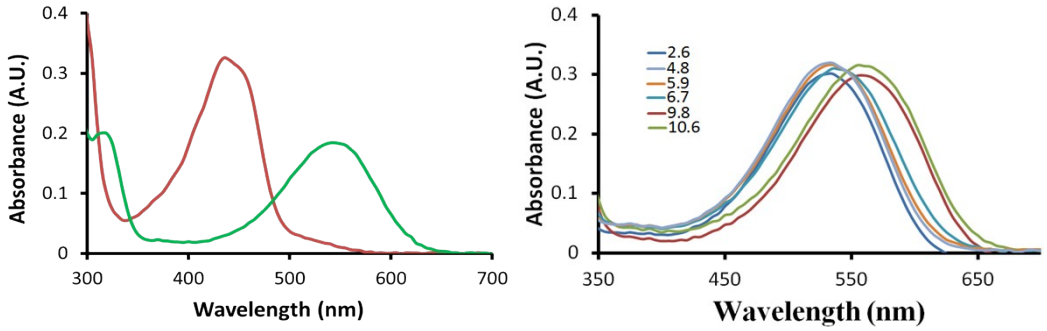

Figure S4 (a) UV-Vis spectrum of **1** (red) and **4** (green) recorded in MeOH. [**1**] = 30 μM; [**4**] = 20 μM. (b) UV-Vis spectra of **4** recorded over various pH values in H2O:MeOH (1:1) solvent.

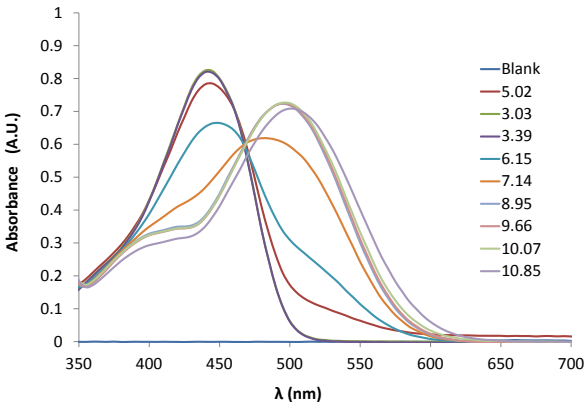

Figure S5 UV-Vis spectra of **1** recorded over various pH values in H_2O:MeOH (1:1) solvent.

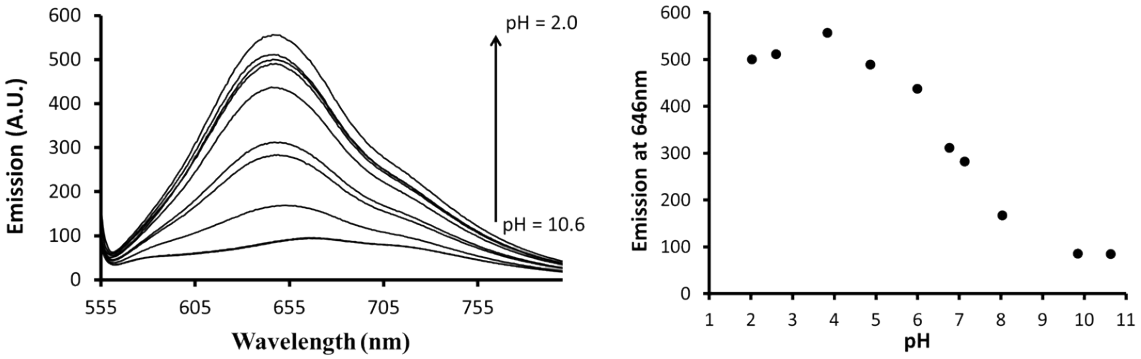

Figure S6 (a) Fluorescence spectra of **4** recorded as a function of pH and (b) plot of fluorescence intensity of **4** at 646 nm against pH. [**4**] = 70 μM, solvent = H_2O:MeOH (1:1).

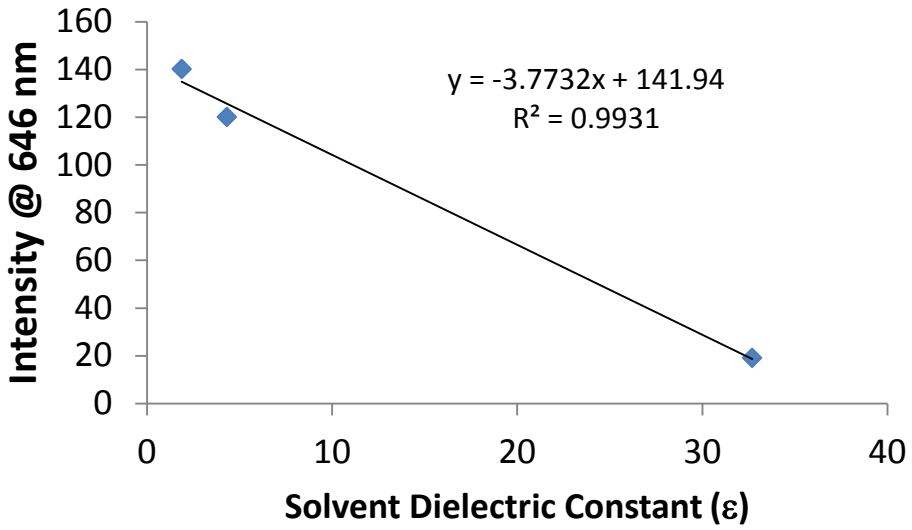

Figure S7 Plot of fluorescence intensity of **4** recorded as a function of solvent dielectric constant. Solvents use were: MeOH (ε 32.7), Et_2O (ε 4.33) and hexane (ε 1.88).

References:

1. McHale A.P.; McHale L., *Cancer Lett.*, 1988, **41**, 315-321