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A self-assembled metallomacrocycle singlet oxygen sensitizer for photodynamic therapy

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Experimental details

All chemicals and solvents were purchased from Sigma unless otherwise stated. Complexes **1** and **2** were synthesised as previously reported.[1,2] ^{1}H NMR and mass spectra data were in agreement with previously-reported results.

DNA photocleavage

Photoinduced cleavage of supercoiled (SC) pBR322 DNA by the complexes was studied by agarose gel electrophoresis. The reactions were carried out under illuminated conditions using 476 nm CW laser (100 mW). The sample was prepared in a dark room at room temperature using SC DNA (3 μL, ~3 μg) in 50 mM Tris-HCl buffer (pH 7.2) containing 50 mM NaCl and varying concentrations of the complex. The solution path length used for illumination in the sample vial was 6 mm. After photoexposure, the sample was incubated for 1 h at 37 °C, followed by the addition of the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol (2 μL), and the solution was finally loaded on an 0.9% agarose gel containing 1.0 μg/mL ethidium bromide. Electrophoresis was carried out for 1 h at 60 V in TAE (Tris-acetate-EDTA) buffer. Bands were visualized by UV light and photographed.

Singlet Oxygen generation

Singlet oxygen quantum yield (φΔ) in dichloromethane was determined as previously described employing 355 nm excitation and perinaphthenone as the standard (φΔ perinaphthenone = 95%).[3]

Light Irradiation Source Apparatus

The apparatus used to irradiate the samples was a custom made device featuring a broadband illumination source fully contained in an empty computer base unit, referred to as the Light Irradiation Source Apparatus (LISA). The technical specifications of the bulb contained within are as follows (**Table 1**).

Product Code	871691
International Model Number	HC01080i
Description	CFL 80W E40 Integrated
Watts	80W
Cap	E40
Operating Hours	15000
Colour Temp	4000K
Lumens	5400 lm
Dimming	No
Dimensions (length x diameter)	256mm x 80mm

Table 1 – Specification of the bulb contained within the irradiation apparatus

Determination of Fluence

In order to quantify the power output of this light source, an experiment was carried out with the running conditions replicated whilst being monitoring by a power meter. The setup utilised was a THORLABS *S175C Sensor* coupled to a *PM100USB Power and Energy Meter* with the readout displayed and stored on a connected netbook. Running the three timed periods of irradiation in succession as under experimental conditions (for three separate samples) determined an accurate power output that the cells were exposed to (**Table 2**).

Stage	Stabilisation	**5 minutes ON**	Sample change	**15 minutes ON**	Sample Change	**30 minutes ON**
Time (minutes)	2	5	2	15	2	30
Average Power (W)	0.000	0.075	0.035	0.121	0.040	0.126
Corrected Power (W)	-	0.075	-	0.085	-	0.086
Energy (J)	-	22.6	-	76.9	-	155.7
Fluence (J cm^{-2})	-	**8**	-	**24**	-	**48**

Table 2 – Table summarising the fluence calculations from timed irradiations

Cell Culture

A2780 and A2780CIS cell lines were cultured in RPMI-1640 medium. All growth medium was supplemented by 2mM L-Glutamine, 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 10% v/v fetal bovine serum (FBS). Cultures were grown within the appropriate range of confluence at 37°C in an atmosphere of 5 % CO_2 / 95% air. Cultures were routinely subcultured using trypsin (0.1% v/v in PBS) at 80 – 90% confluence. Cell lines were used between passage numbers 6 – 60.

Confocal microscopy

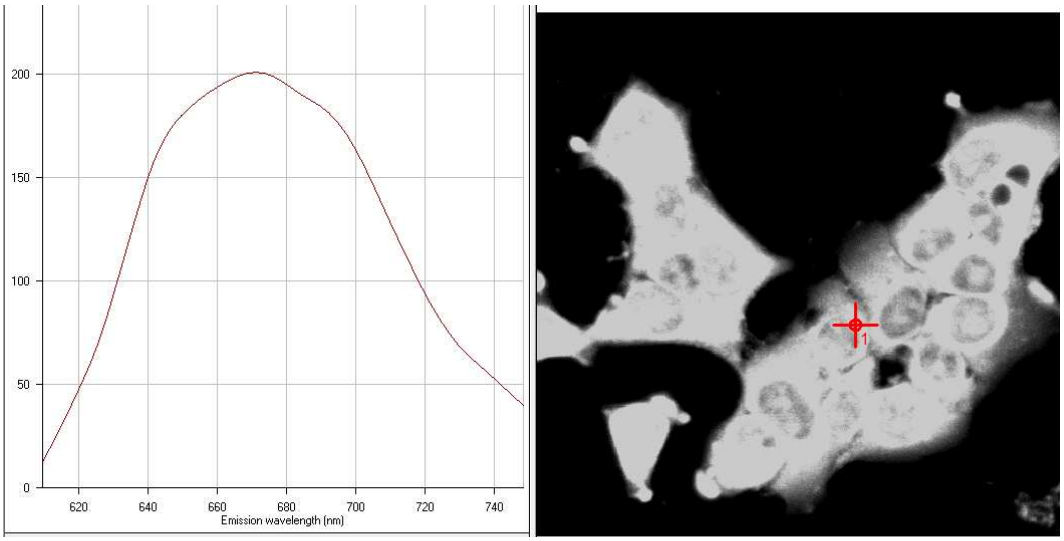
Cells were seeded in 6 well plates and luminescently imaged on a Zeiss LSM 510 META upright confocal laser microscope using 40x and 63x water immersion objectives. Where appropriate, cell fixation was achieved using 5% paraformaldehyde for ten minutes followed by permabilisation using 0.1 % Triton. **1** and **2** were excited with an Ar-ion laser at 458 nm and emission collected at 640-700 nm using a polychomric (META) detector. Live cell imaging experiments involved constant laser exposure 0 – 100 mins with images recorded every 5 min. Image data acquisition and processing was performed using Zeiss LSM Image Browser.

Photocytotoxicity (phototoxicity)

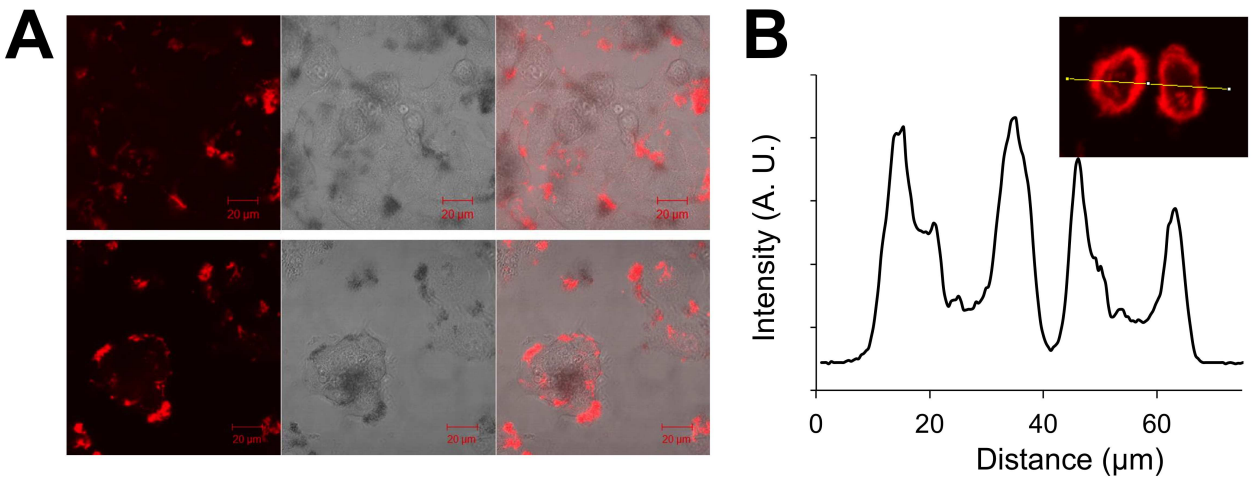
Cell cultures were grown on 48 well plates at a seeding density of 50,000 cells per well and allowed to grow for 24 hours. The cells were then treated with compound (solubilised in 10% PBS/water: 90% medium) of a 1 – 100 µM concentration range, in triplicate, and incubated for 24 hours. All compound solution (and control media) was removed from the cells and replaced with regular growth medium 30 minutes prior to irradiation. Of the four prepared well plates, one remained in the incubator whilst the other three were exposed to the LISA for the duration of 5, 15 or 30 minutes (corresponding to light doses of 8, 24 or 48 J cm^{-2}) before being incubated for a further 24 hours after culmination of light treatment. All media was then removed and cells incubated with MTT (0.5 mg ml^{-1} dissolved in PBS) for 30 – 40 minutes. The MTT was removed and formazan product eluted using 120 µl/well acidified isopropanol, 100 µl of which was transferred to a 96 well plate for the absorbance to be quantified by spectrophotometer (540 nm, referenced at 640 nm). An average absorbance for

each concentration was calculated and cell viability was determined as a percentage of the untreated negative control wells (10% PBS/water: 90% medium, average of triplicate). Data were plotted in a graph of concentration against cell viability to produce a curve from which the IC_{50} value (the concentration corresponding to a cell viability of 50%) could be derived by interpolation.

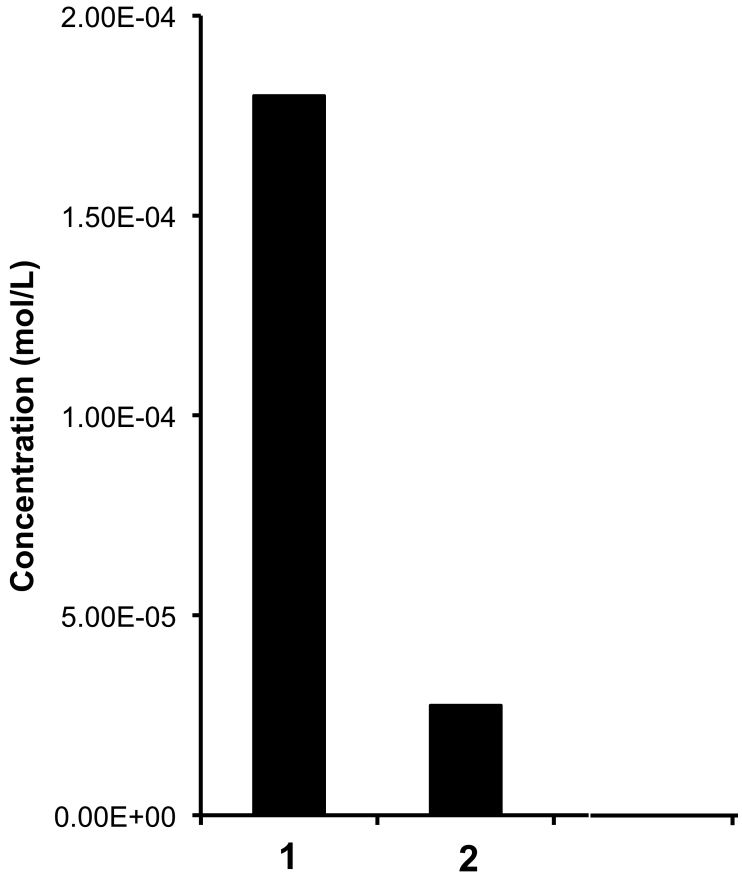
Supplementary Figures



Supplementary Figure 1. Emission spectra (left) of fixed MCF7 cells stained with **1** (right) (100 μM, 10 mins).



Supplementary Figure 2. (**A**) Live MCF7 cells stained with **1** ([**1**] = 200 μM) but not exposed to continuous laser light. Cells imaged 100 min after complex addition. (**B**) Emission profile of cells (across white line) illustrating that the majority of staining is restricted to the plasma membrane.



Supplementary Figure 3. cellular uptake levels of **1** and **2** in A2780CIS cells (50 μM, 12 h), as quantified by ICP-MS analysis of Ru content.

References

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