Supporting Information for

Positively charged styryl pyridine substituted Zn (II) Phthalocyanines for Photodynamic Therapy and Photoantimicrobial Chemotherapy: Effect of the number of charges

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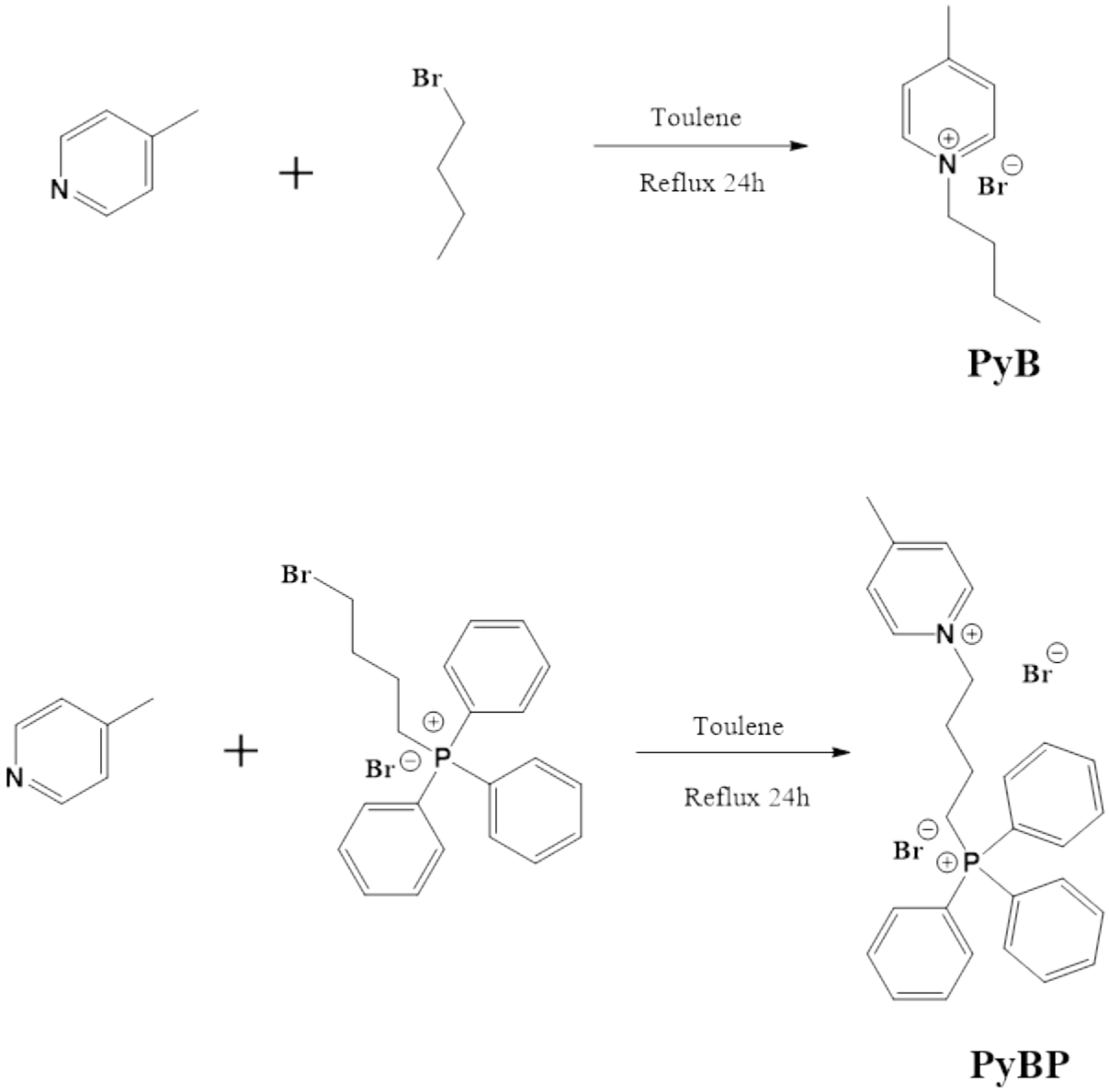
1. Synthesis of 1-butyl-4-methylpyridin-1-ium bromide (PyB) and 4-methyl-1-(4-(triphenylphosphonio)butyl)pyridin-1-ium bromide (PyBP)

The precursor **PyB** and **PyBP** were synthesised by refluxing 4-methyl pyridine (0.1 mol) with the corresponding alkyl halide (0.12 mol) in toluene for 24h **(Scheme S1**). The reaction mixture is cooled and the solidified product is washed with diethyl ether to give **PyB** and **PyBP** as pink solid [1, 2]. ^{1}H NMR spectra shown in Figs. S1 and S2

PyBP: (Yield: 79%);^{1}H NMR (400 MHz , DMSO-d_6): δ (ppm), (d, J = 6.0 Hz, 2H), 7.99 (d, J = 6.4 Hz, 2H), 4.63 (t, J = 8.0 Hz, 2H), 3.53 (s, 3H), 1.79 (t, J = 7.5 Hz, 2H), 1.17 (m, 2H), (d, J = 5.8 Hz, 3H).

PyBP: (Yield: 84%); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm), 9.02 (d, 2H), 8.03 (d, J = 6.4 Hz, 2H), 7.81 (m, 15H), 4.68 (t, J = 6.5 Hz, 2H), 3.83 (t, J = 12.7 Hz, 2H), 2.56 (m, 3H), 2.19 (m, 2H), 1.59 (m, 2H).



Scheme S1: Synthetic pathway of 1-butyl-4-methylpyridin-1-ium bromide **(PyB)** and 4-methyl-1-(4-(triphenylphosphonio)butyl)pyridin-1-ium bromide **(PyBP)**

2. Cancer cell studies

2.1 Cell culture preparation

Human breast carcinoma MCF-7 cell model was cultured using Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose with L-glutamine (0.11 g/L) and phenol red, supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), and 100 unit/mL penicillin streptomycin-amphotericin B. The cells were grown in 75 cm^2 vented flasks (Porvair®) and incubated in a humidified atmosphere at 37°C and 5 % CO_2. Once 90-100 % cell confluence was achieved, determined through microscopic examination, the cells were rinsed twice with Dulbecco's modified phosphate buffer saline (DPBS). Cells were passage through routine trypsinization and centrifugation. Routine viability and cell enumeration were performed using the trypan blue dye exclusion assay (0.4 % (v/v) trypan blue solution) using a haemocytometer.

2.2 PDT activity

Stock concentrations (drug formulation) of **1**, **2** and **3** were prepared in DMSO (1%) and made up to the volume with supplemented DMEM. The media only/placebo control consisted of cells incubated in supplemented DMEM. Cells were seeded at a density of 10,000 cell/well in supplemented DMEM containing phenol red in 96-well tissue culture plates (Porvair®) and incubated in a humidified atmosphere at 37 °C and 5 % CO_2 for 24 h to foster cell attachment to the wells. The attached cells were rinsed with 100 μl DPBS once, followed by administration of 100 μL supplemented DMEM containing gradient concentrations (1.6–100 μM) of **1, 2** and **3**.

After 24 h incubation with supplemented DMEM with phenol red, 5 mg/mL of MTT (20 μL) was added to each well and incubated for an additional 3 h. The cultured medium was discarded, and 200 μL of DMSO was added to dissolve the formazan crystals of reduced MTT. The absorbance at 540 nm was measured using a Molecular Devices Spectra Max M5 plate reader.

The percentage cell viability was determined using equation 1:

$$\%\ cell\ viability\ = \frac{Absorbance\ of\ sample\ at\ 540\ nm}{Absorbance\ of\ \ control\ at\ 540\ nm} \times 100 \qquad (1)$$

where the absorbance of the sample is the cells containing **1**, **2** and **3** alone while absorbance of control is the placebo cells containing only supplemented DMEM with phenol red. The IC_{50} values of the complexes were from percentage cell viability *vs* concentration curve.

2.3 Cellular uptake

The MCF-7 cells (1×10^5 cells/well) were seeded in 24-well cell culture plates and incubated for 24 h. The cells were exposed to the Pc complexes (20 μg/mL) for 24 h in the dark. After the incubation time, the cells were washed three times with PBS, lysed with 30 μL of Triton-X 100 and internalised complex is solubilized in 70 μL of DMSO. The cellular uptake was measured by determining the Q-band absorbance of the complexes with an ELISA reader.

3. Antimicrobial studies

The bacteria culture was prepared according to a procedure reported in the literature [3]. Briefly, aliquots of the culture were aseptically transferred to 4 mL of fresh broth and incubated at 37 °C to mid logarithmic phase (absorbance ~ 0.6 at 620 nm). The bacteria culture in the logarithmic phase of growth were harvested through the removal of broth culture by centrifugation (3000 RPM for 15 min), washed once with 10 mM of PBS and re-suspended in

4 mL of PBS. Then the bacteria culture was diluted to 1/1000 in PBS (working stock solution), corresponding to ~10^8 colony forming units (CFU)/mL.

The photoantimicrobial chemotherapy studies were performed by dissolving **1**, **2** and **3** in PBS. The bacterial suspensions were incubated in an oven equipped with a shaker for 30 min in the dark at 37 °C. Then, half (2.5 ml) of the incubated bacterial suspension were irradiated at the Q-band maximum of the photosensitizers in 24 well plates, while the other half kept in the dark. After irradiation, 100 µl samples were diluted with 900 µl PBS and were spotted on agar plates using a micropipette. The plates were incubated at 37 °C for 24 h. All the studies were conducted in triplicates.

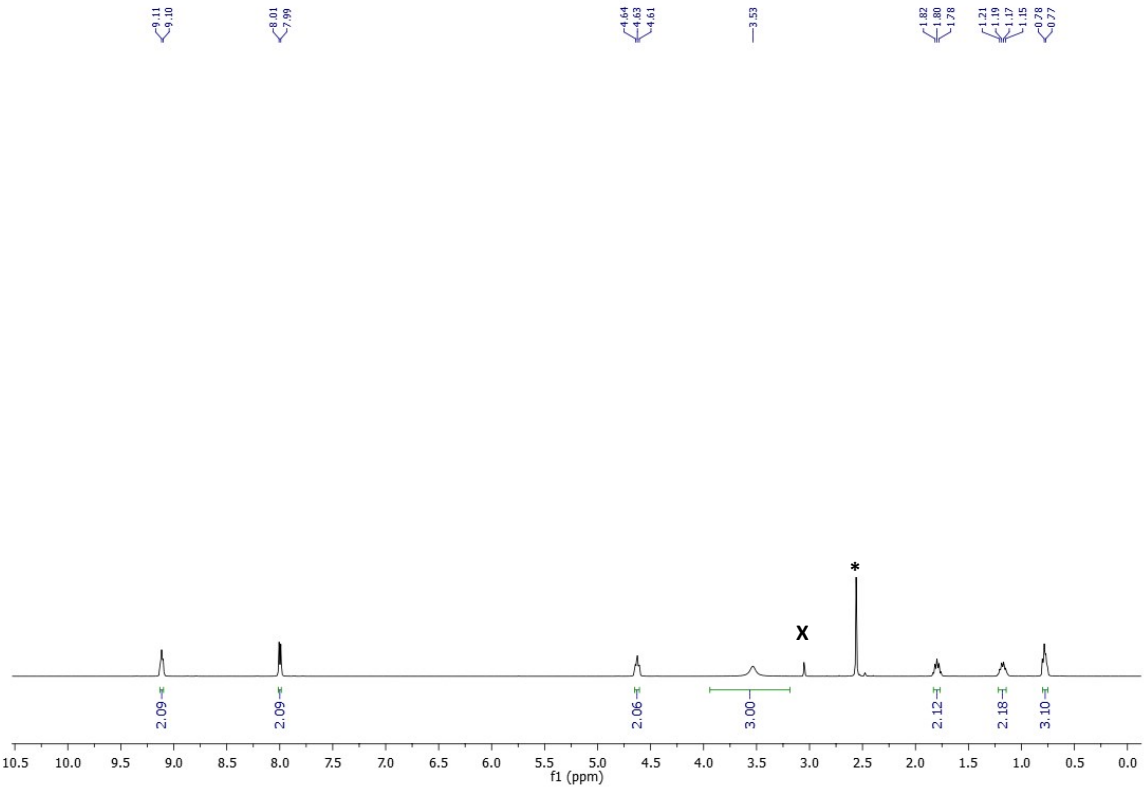


Fig. S1: ^{1}H-NMR spectrum of **PyB** at 400MHz in DMSO-d_6 (X-solvent, *- water).

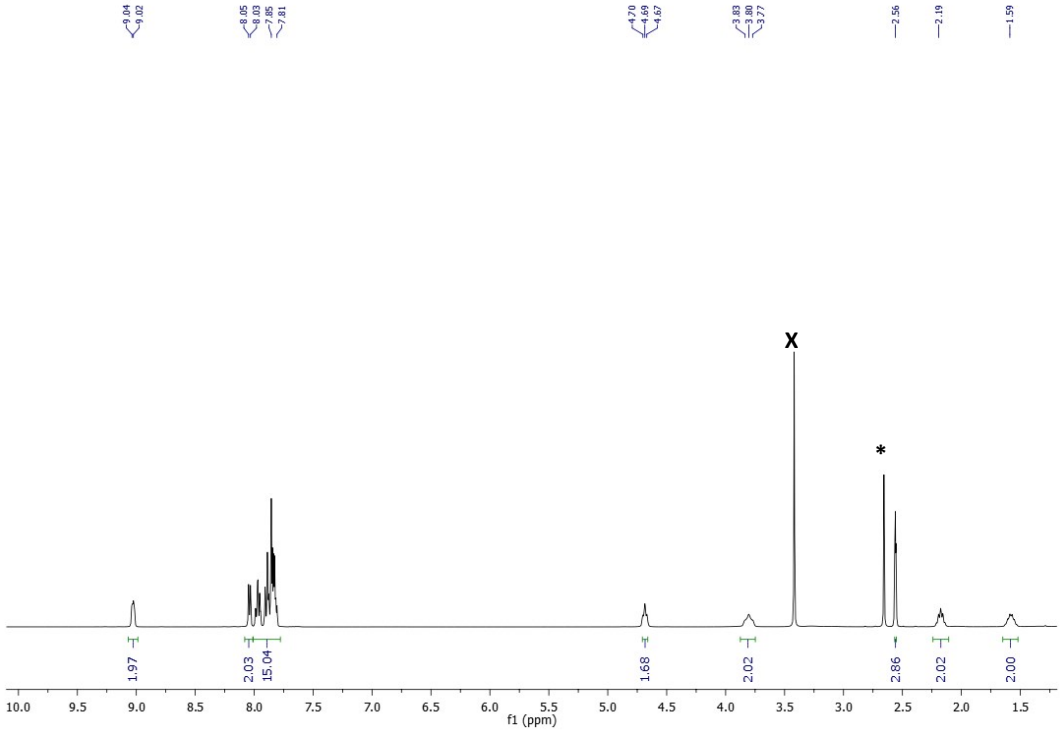


Fig. S2: ^{1}H-NMR spectrum of **PyBP** at 400MHz in DMSO-d_6 (X-solvent, *- water).

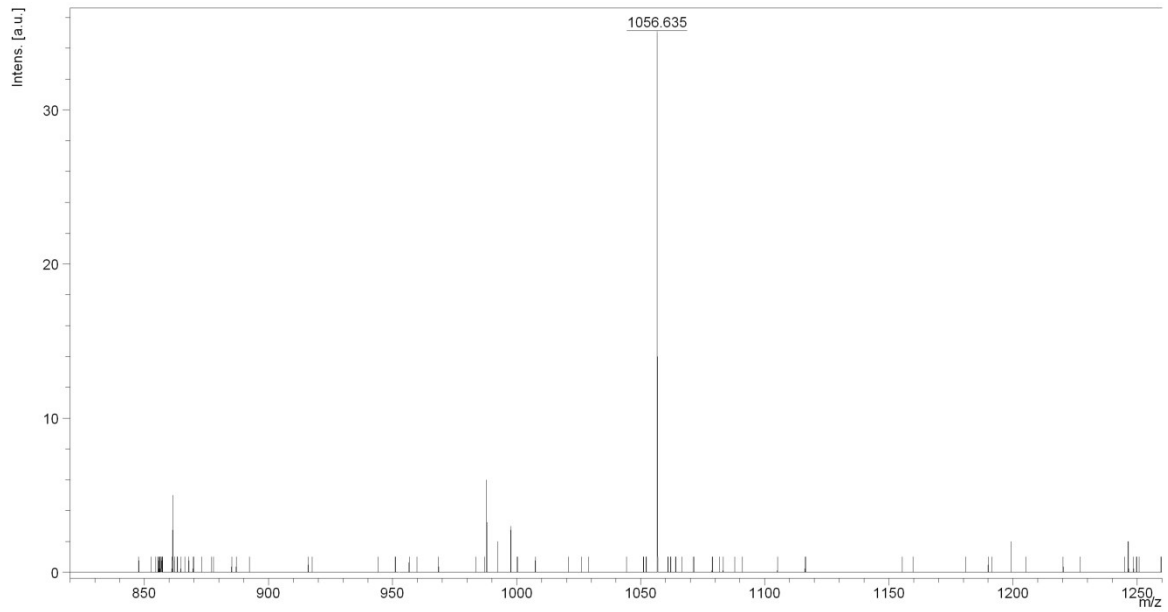


Fig. S3. MALDI-TOF MS data for **1**, showing $[M]^{+/}z$ peak.

Fig. S4. MALDI-TOF MS data for **2**, showing $[M]^{4+/}z$ peak.

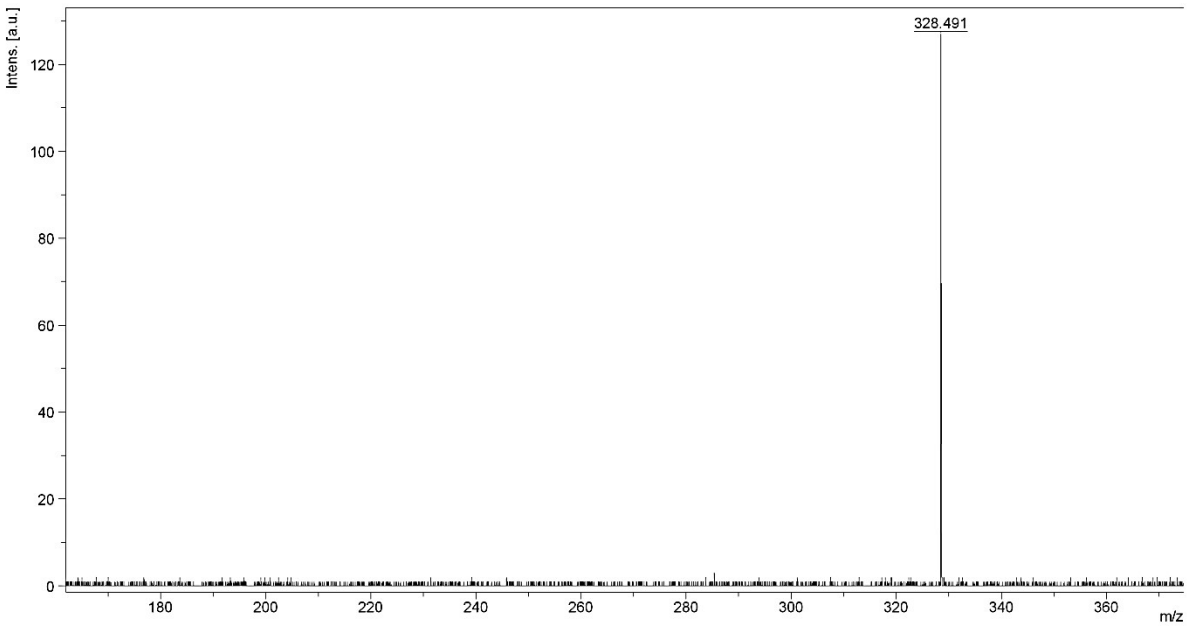


Fig. S5. MALDI-TOF MS data for **3**, showing $[M]^{8+/}z$ peak.

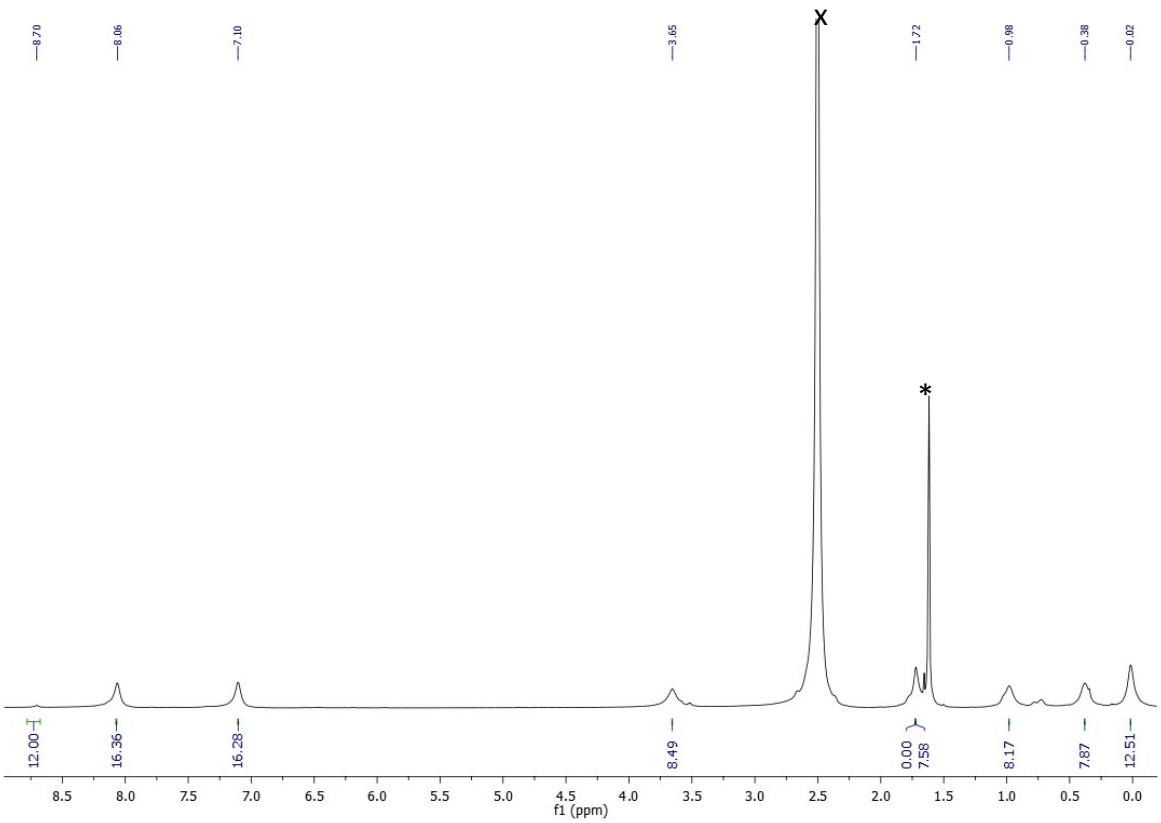


Fig. S6: [1]H-NMR spectrum of **2** at 600MHz in DMSO-d_6 (X-solvent, *- water).

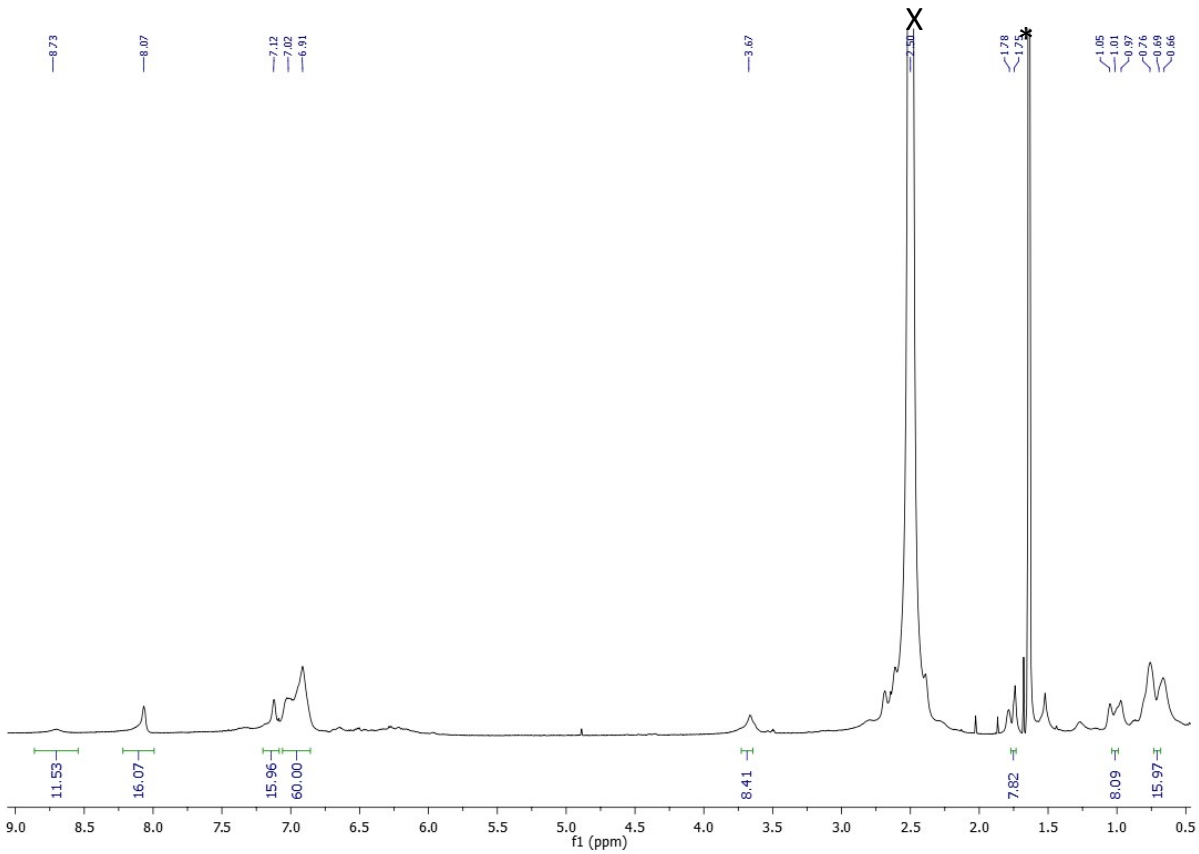


Fig. S7: ^{1}H-NMR spectrum of **3** at 600MHz in DMSO-d_6 (X-solvent, *- water).

Table S1. TD-DFT spectra of the B3LYP optimized geometries for **1** – **3** and ZnPc calculated with the CAM-B3LYP functional and 6-31G(d,p) basis sets [S4].

1					
Band	**#[a]**	**λ_{calc} [b]**	***f*** **[c]**	**λ_{exp} [d]**	**Wave Function[e] =**
Q	1	572	0.63	683	**96% a → -a/-s**; …
	2	570	0.64		**96% a → -a/-s**; …
B	16	306	0.91	353	41% H−4^{S} → -a/-s; **18% s → -a/-s;** 16% 1b1g^{N} → -a; …
	17	305	1.39		40% H−4^{S} → -a/-s; **26% s → -a/-s;** …
2					
Band	**#[a]**	**λ_{calc} [b]**	***f*** **[c]**	**λ_{exp} [d]**	**Wave Function[e] =**
Q	1	574	0.99	683	**87% a → -a/-s; 6% s → -a/-s;** …
	2	575	0.98		**88% a → -a/-s; 6% s → -a/-s;** …
CT	9	387	0.52	472	15% H−4^{S} → L+2^{S}; 12% H−1^{S,N} → L+1^{S}; …
	10	384	2.75		30% H−2^{S} → L+2^{S}; 13% H−5^{S,N} → L^{S}; 11% H−3^{S} → L^{S}; …
	11	383	3.30		35% H−3^{S} → L+2^{S}; 17% H−4^{S,N} → L+1^{S}; 12% H−1^{S,N} → L+1^{S}; …
	12	374	0.11		19% H−3^{S} → L+2^{S}; 15% H−2^{S} → L+2^{S}; 10% H−4^{S,N} → L+1^{S}; …
B	20	300	0.78	356	**39% s → -a/-s;** 21% H−4^{S,N} → -a/-s; 17% H−5^{S,N} → -a/-s; …
	21	300	0.81		**42% s → -a/-s;** 24% H−5^{S,N} → -a/-s; 10% H−4^{S,N} → -a/-s; …
3					
Band	**#[a]**	**λ_{calc} [b]**	***f*** **[c]**	**λ_{exp} [d]**	**Wave Function[e] =**
Q	1	583	1.17	684	**80% a → -a/-s;** 7% a → L+1^{S}; **5% s → -a/-s;** …
	2	582	1.12		**82% a → -a/-s;** 6% a → L^{S}; **5% s → -a/-s;** …
CT	9	406	1.03	476	30% H−2^{S} → L+1^{S}; 10% H−5^{S} → L^{S}; …
	10	402	2.99		14% H−2^{S} → L+1^{S}; 13% H−5^{S} → L^{S}; 10% H−3^{S} → L+2; 10% H−4^{S} → L^{S}; …
	11	401	3.27		19% H−3^{S} → L+2^{S}; 16% H−4^{S} → L+3^{S}; 10% H−3^{S} → L+3; 10% H−5^{S} → L+2^{S}; …
	12	390	0.07		18% H−3^{S} → L+2^{S}; 13% H−5^{S} → L+3^{S}; 12% H−2^{S} → L+1^{S}; 11% H−4^{S} → L+3^{S}; …
B	24	301	0.51	355	25% H−5^{S} → -a/-s; **23% s → -s;** 11% H−2^{S} → a; 10% H−2^{S} → L^{S}; …
	25	300	0.74		**36% s → -a/-s;** 34% H−5^{S} → -s; **7% s → -a/-s;** …
ZnPc					
Band	**#[a]**	**λ_{calc} [b]**	***f*** **[c]**	**λ_{exp} [d]**	**Wave Function[e] =**
Q	1	563	0.47	672	**100% a → -a;** …
	2	562	0.47		**100% a → -s;** …
B	12	292	1.00	344	**73% s → -a/-s;** 15% 1b$_{2u}$ → -a/-s; **7% s → -a/-s;** …
	13	292	1.00		**73% s → -a/-s;** 15% 1b$_{2u}$ → -a/-s; **7% s → -a/-s;** …

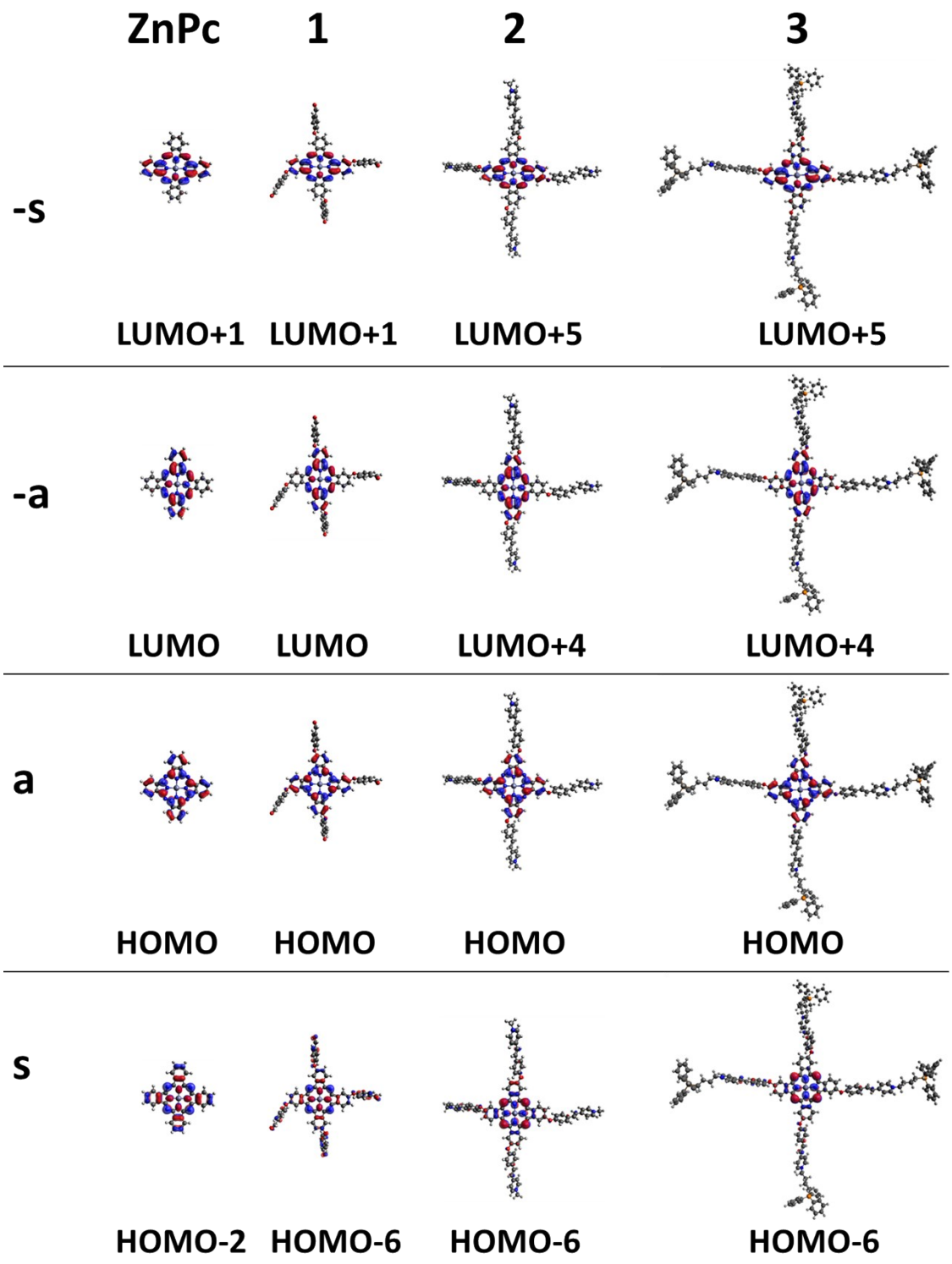


Fig. S8. The angular nodal patterns of the frontier orbitals of **1-3** and the unsubstituted parent **ZnPc** model complex that are associated with the Q and B transitions at an isosurface of 0.2 a.u.

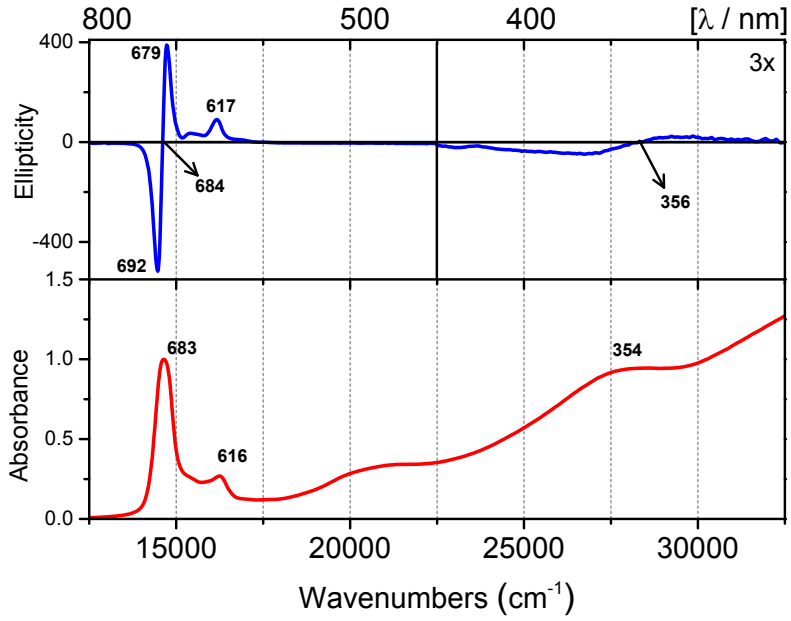


Fig. S9. Absorption (bottom) and MCD (top) spectra of **2** in DMSO.

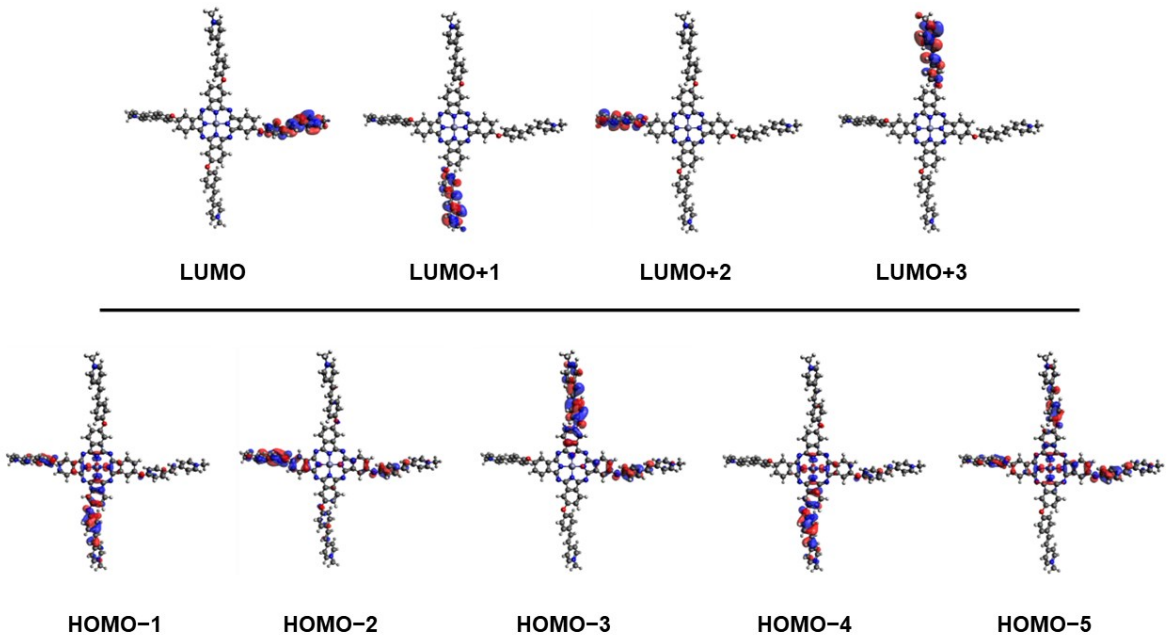


Fig. S10. The angular nodal patterns of the frontier orbitals of **2** that are associated with the CT band at an isosurface of 0.2 a.u.

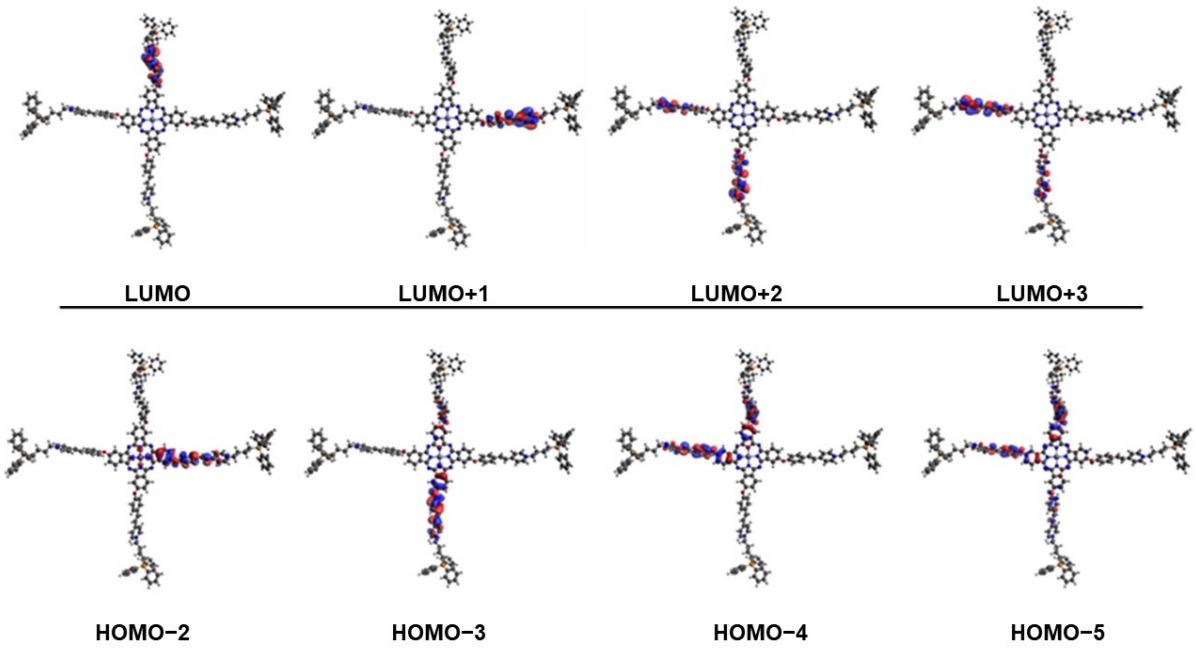


Fig. S11. The angular nodal patterns of the frontier orbitals of **3** that are associated with the CT band at an isosurface of 0.2 a.u.

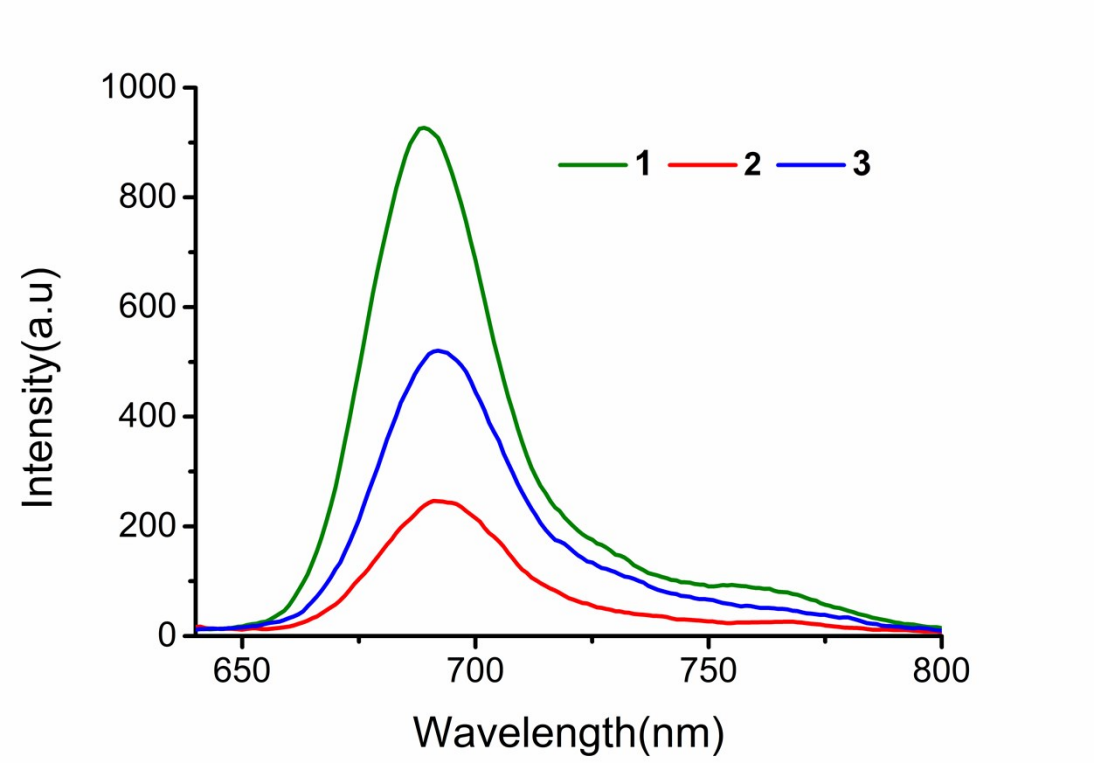


Fig. S12: Emission spectra of **1**, **2** and **3** in DMSO when excited at the respective Q-band (Absorbance of compounds were maintained ≤ 0.1).

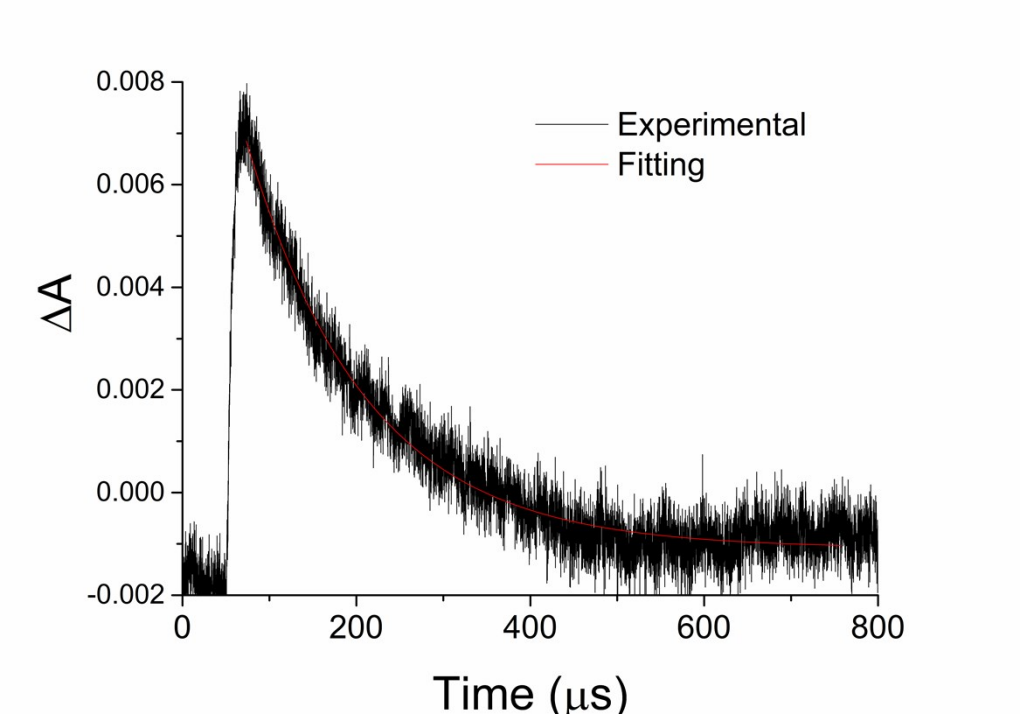


Fig. S13: Triplet excited state absorption decay curves of **2** in DMSO

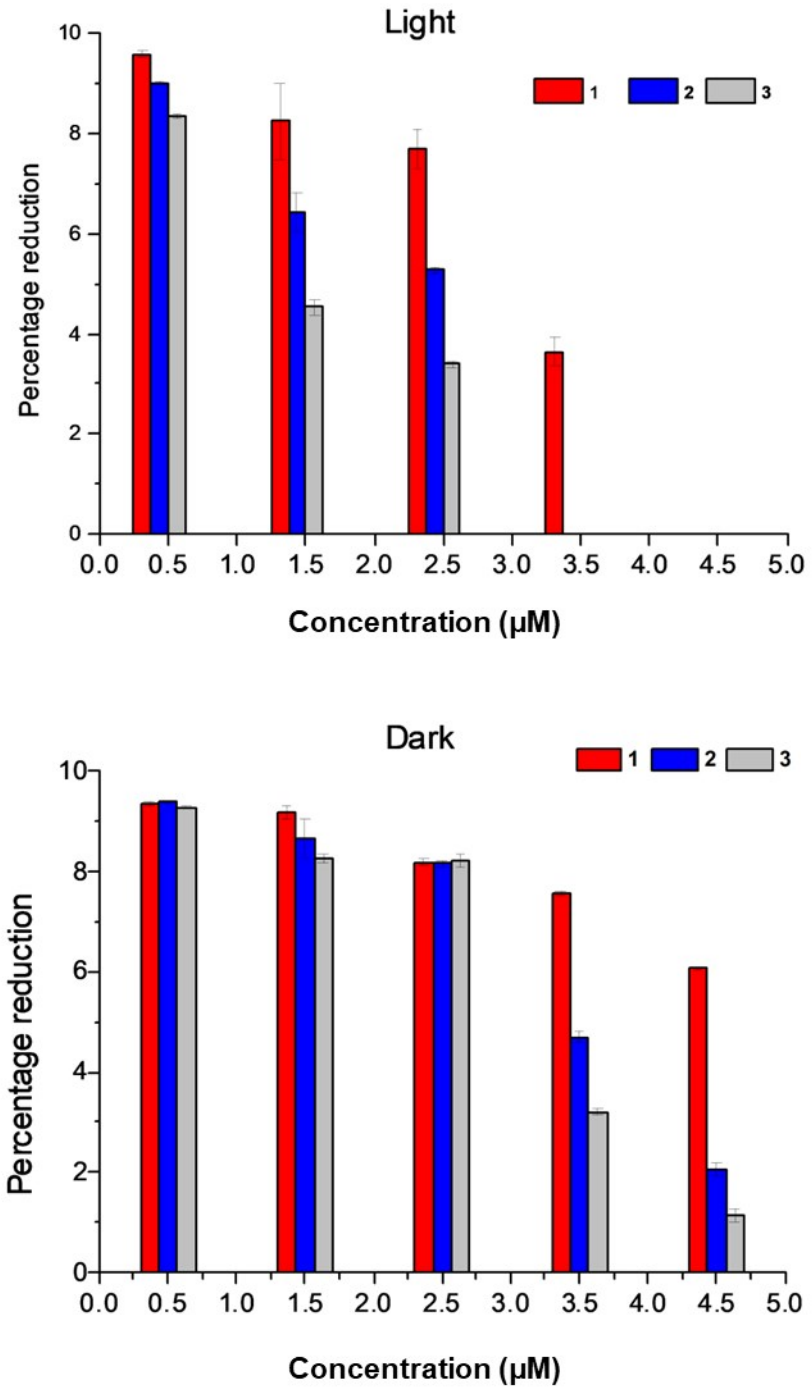


Fig. S14: Percentage reduction due to complexes **1**, **2** and **3** in the presence of light and dark against *E.*coli in PBS. Concentration = 0.5, 1.5, 2.5, 3.5 and 4.5 μM

References

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[2] W. Yang, P. Shan Chan, M. S. Chan, K. F. Li, P. K. Lo, N.K. Mak, K. W. Cheah, M. S. Wong, Chem. Commun., 2013, 49, 3428.

[3] O. L. Osifeko, M. Durmus T. NyokongJ. Photochem. Photobiol. A, 2015, 301, 47.

[4]. Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.