Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

A series of photosensitizers with incremental positive electric charges for photodynamic antitumor therapy

Shufeng Yan,[ab] Qingqing Huang,[a] Xiaorong Song,[b] Zhuo Chen,[b] Mingdong Huang[c] and Juncheng Zhang[a]*

[a] Medical Plant Exploitation and Utilization Engineering Research Center, Sanming University, Sanming, Fujian 365004, China.

[b] State Key Laboratory of Structural Chemistry, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou, Fujian 350002, China.

[c] College of Chemistry, Fuzhou University, Fuzhou, Fujian 350116, China.

E-mail: Juncheng Zhang, 19900204@fjsmu.edu.cn

Contents

1. Synthesis of ZnPc-4, ZnPc-8 and ZnPc-12

1.1. Synthesis of 4-(2,4,6-tris((dimethylamino)methyl)phenoxy)phthalonitrile

The compound 4-(2,4,6-tris((dimethylamino)methyl)phenoxy)phthalonitrile was synthesized according to the literature method with slight modification.[1] Briefly, 2, 4, 6-tris (*N, N'*-dimethylaminomethyl) phenol (2 g, 7.8 mmol) and K_2CO_3 (3.12 g, 22.6 mmol) were stirred in DMF (15 mL) for 1 h at room temperature (RT). To this mixture, a solution of 4-nitro-phthalonitrile (651.8 mg, 3.77 mmol) in DMF (15 mL) was added dropwise in 15 min at RT. The reaction mixture was then stirred for further 10 h until completion of the reaction, which was monitored by thin layer chromatography (TLC). The reaction mixture was filtered to remove the unreacted salt. The filtrate was added with water (30 mL), followed by concentrating under reduced pressure. Cold petroleum ether (15 mL at 3 ^oC) was added to the concentrate and stirred for 15 min to precipitate the product and wash out the impurities. The precipitates were collected and dried in vacuo to obtain 4-(2,4,6-tris((dimethylamino)methyl)phenoxy)phthalonitrile (750 mg, yield 50.8 %).

1.2. Synthesis of $ZnPc(TAP)_4$

$ZnPc(TAP)_4$ was synthesized by modifying the literature method as follows.[2-3] 5 drops of 1, 8-diazabicyclo-[5.4.0]undec-7-ene (DBU) were stirred at a solution of compound 4-(2,4,6-tris((dimethylamino)methyl)phenoxy)phthalonitrile (500 mg, 1.28 mmol), and anhydrous zinc acetate (200 mg, 1.10 mmol) and n-pentanol (40 ml) were added. The reaction mixture was quickly heated up to 145 ^oC and refluxed for 8 h. At the end of reaction, the solvent was evaporated under reduced pressure and the residue was dissolved in THF (3 mL) followed by extraction of desired product by petroleum ether (30 mL). Next, the product was taken out of petroleum ether solution by washing with 0.25 M HCl aqueous solution (50 mL). Then, the solution was added with solid NaCl to become brine solution, followed by extraction with THF to remove the unreacted diiminoisoindoline impurities. Final, we used 0.25 M NaOH aqueous solution (50 mL) to precipitate the product. The precipitates were collected and dried in vacuo to obtain the $ZnPc(TAP)_4$ (100 mg, yield 19.2 %). ^{1}HNMR (DMSO-d_6), (δ: ppm): 7.70-7.52 (t, 12H, Pc-H), 7.31-7.25 (s, 8H, Ar-H), 3.67-3.45 (t, 24H, -CH_2), 2.44-2.20 (m, 72H, -CH_3).

1.3. Synthesis of $ZnPc(TAP)_4^{12+}$ (ZnPc-12)

Methylation of all amino moieties in compound $ZnPc(TAP)_4$ was carried out to synthesize compound ZnPc-12.[4] $ZnPc(TAP)_4$ (83 mg, 0.051 mmol) dissolved in DMF (50 mL) was reacted with CH_3I (43 μL, 0.6732 mmol) for 6 h at RT. The reaction mixture was concentrated to ~5 mL by removing the solvent under reduced pressure. The product was precipitated by addition of diethyl ether to the residue. The precipitates collected by centrifugation were dissolved in methanol. The insoluble impurities were filtered off and the filtrate was precipitated again by diethyl ether. The product was centrifuged and dried in vacuo to yield the ZnPc-12 (157 mg, yield 92 %). ^{1}HNMR (DMSO-d_6), (δ: ppm): 9.64-8.96 (m, 12H, Pc-H), 8.39-8.26 (s, 8H, Ar-H), 5.92-4.54 (d, 24H, -CH_2), 3.4-3.34 (s, 108H, -CH_3).

1.4. Synthesis of ZnPc-4 and ZnPc-8

The synthesis of compounds ZnPc-4 and ZnPc-8 was accomplished according to the above mentioned procedure for ZnPc-12 except that exact stoichiometric amounts (1:4 for compound ZnPc-4 & 1:8 for compound ZnPc-8) of the reactants i.e. $ZnPc(TAP)_4$ and CH_3I, were used. To ensure the uniform mixing of reactants and to avoid the excess of methylation on a single Pc molecule, the reactants were mixed at low temperature (-40^oC). After addition, the reaction mixture was allowed to react at RT for 6 h followed by the work up as described for compound ZnPc-12. Yields of compounds ZnPc-4 and ZnPc-8 were 42 % and 60 % respectively. The compounds with a lower number of cationic charges, $ZnPc(TAP)_4^{n+}$ (n=4, 8), were only characterized by UV/Vis spectroscopy. The average number of cationic charges in the compounds was deduced from the amount of CH_3I used for the reactions.

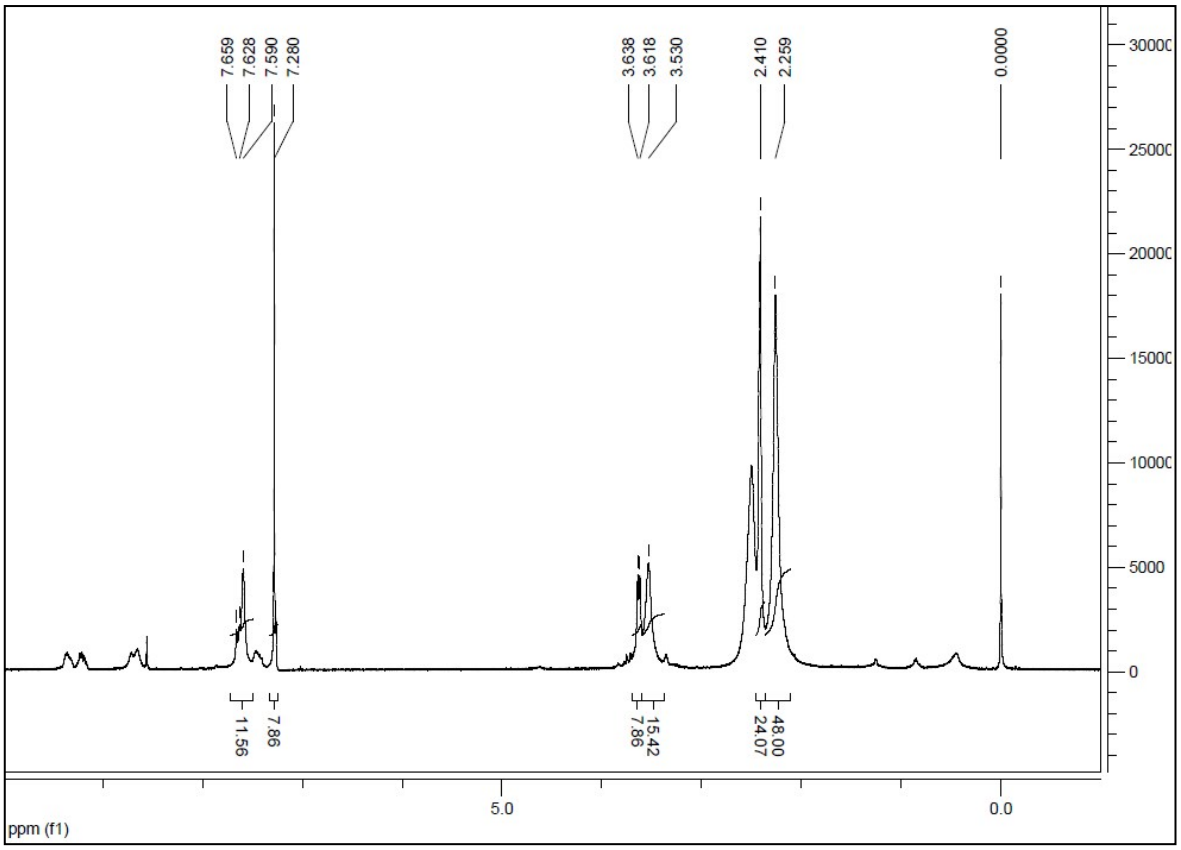

Figure S1. Proton NMR spectrum of non-charged precursor $ZnPc(TAP)_4$.[4]

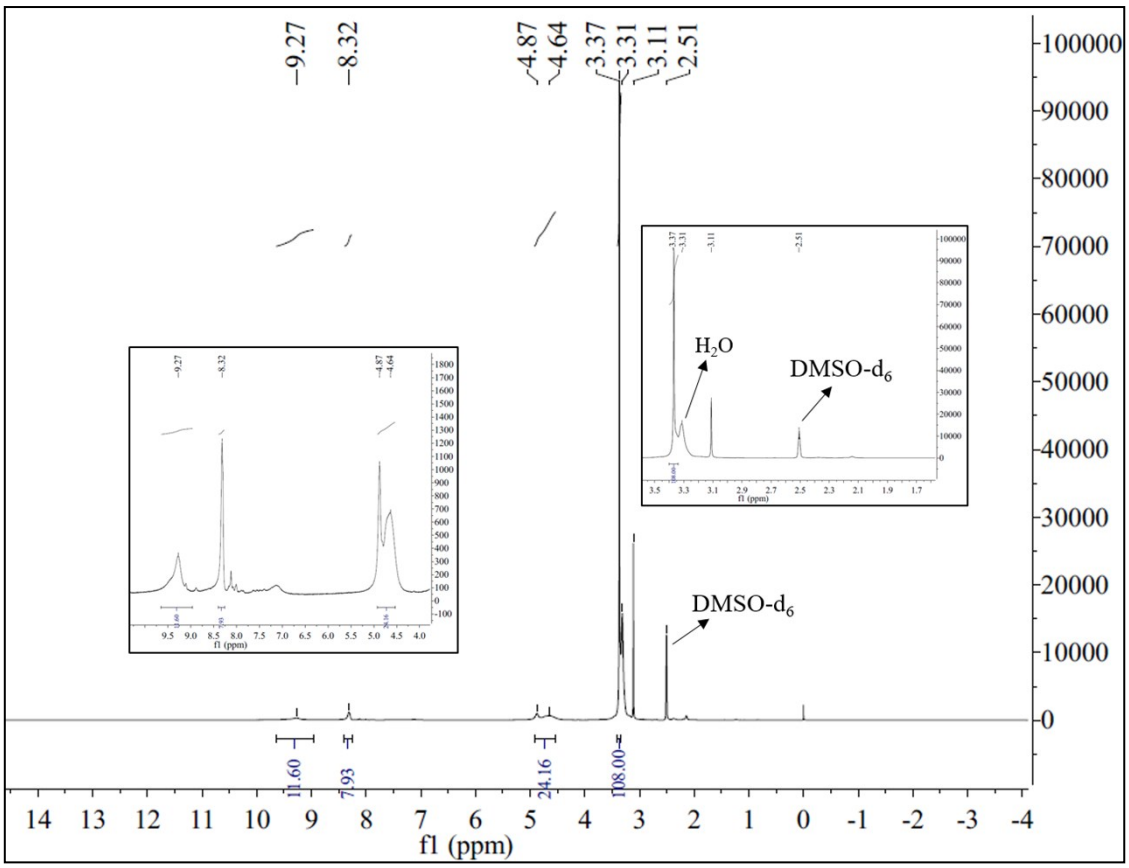

Figure S2. Proton NMR spectrum of $ZnPc(TAP)_4^{12+}$.[4]

pKa values of compounds

Methods	Compounds		
	ZnPc-4	ZnPc-8	ZnPc-12
Titration assay	8.34	8.46	8.52
Predicted by ACD-Labs 6.0	8.80	8.55	Not Applicable
Predicted by ChemDraw 16	8.58	8.64	Not Applicable

Figure S3. pKa values of ZnPc-4, ZnPc-8 and ZnPc-12.

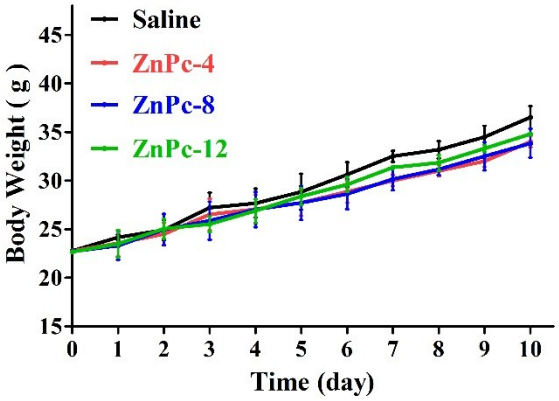

Figure S4. Body weights of mice throughout the *in vivo* experiments.

References

1. Sesalan, B. S.; Koca, A.; Gul, A., Water soluble novel phthalocyanines containing dodeca-amino groups. *Dyes Pigments* **2008,** *79* (3), 259-264.
2. Gumustas, M. K.; Sesalan, B. S.; Atukeren, P.; Yavuz, B.; Gul, A., The photodegradation of a zinc phthalocyanine. *J. Coord. Chem.* **2010,** *63* (24), 4319-4331.
3. Li, X. S.; Guo, J.; Zhuang, J. J.; Zheng, B. Y.; Ke, M. R.; Huang, J. D., Highly positive-charged zinc(II) phthalocyanine as non-aggregated and efficient antifungal photosensitizer. *Bioorg. Med. Chem. Lett.* **2015,** *25* (11), 2386-2389.
4. Zhang, Y. X.; Zheng, K.; Chen, Z.; Chen, J. C.; Hu, P.; Cai, L. R.; Iqbal, Z.; Huang, M. D., Rapid killing of bacteria by a new type of photosensitizer. *Appl. Microbiol. Biot.* **2017,** *101* (11), 4691-4700.