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Synthesis and *in vitro* photodynamic activity of new hexadeca-carboxy phthalocyanines†

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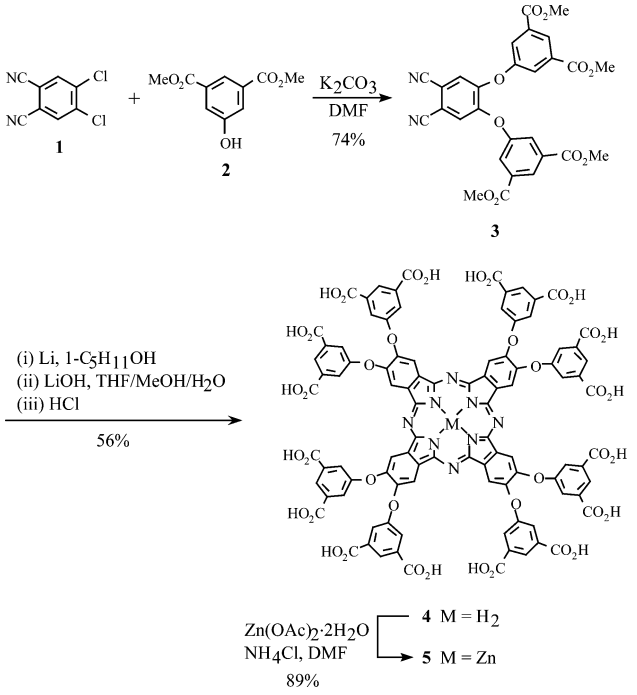
Two new hexadeca-carboxy phthalocyanines have been synthesised and evaluated for their photodynamic activities; the zinc(II) analogue exhibits a high class-A scavenger-receptor mediated photocytotoxicity towards the J774 murine macrophage cell line.

Photodynamic therapy (PDT) has been an experimental clinical modality for the treatment of a range of cancer and wet age-related macular degeneration for the past two decades.[1] It involves three individually non-toxic components, namely photosensitisers, light and oxygen, that are combined to cause cellular and tissue damage. The efficacy of PDT depends on several interdependent factors, among which photosensitisers certainly play a decisive role. Although Photofrin® remains the most widely used photosensitiser, it suffers from a high degree of chemical heterogeneity, poor absorption of tissue-penetrating red light and long-lasting cutaneous photosensitivity.[2] Significant efforts have therefore been put in the development of new photosensitisers which have better photophysical properties, greater tumour specificity and less skin photosensitivity.[3] Owing to their many desirable characteristics, phthalocyanines have emerged to be a promising class of second-generation photosensitisers.[3,4] As part of our continuing interest in the development of phthalocyanine-based photosensitisers,[5] we describe herein the preparation and *in vitro* photodynamic activities of two new phthalocyanines with 16 -COOH substituents.[6] In contrast to many previously described photosensitisers such as the sulfonated and hydroxy zinc(II) and aluminium(III) phthalocyanines, and the well-known silicon(IV) phthalocyanine Pc4,[3,4] these compounds do not have structural isomers and contain a large number of negatively charged carboxylate moieties upon deprotonation. These highly charged species can target tumour-associated macrophages *via* the scavenger receptor without the need of conjugation of any biological ligands.[7]

Scheme 1 shows the synthetic route to prepare these hexadeca-carboxy phthalocyanines. Treatment of the readily available 4,5-dichlorophthalonitrile (**1**)[6b] with dimethyl 5-hydroxyisophthalate (**2**) in the presence of K_2CO_3 gave the disubstituted product **3** in good yield. Cyclisation of **3** using lithium in 1-pentanol, followed by alkaline hydrolysis with LiOH in a mixture of THF–MeOH–H_2O and acidification with HCl resulted in the isolation of the metal-free hexadeca-carboxy phthalocyanine **4**. Attempts to prepare the zinc analogue by treating **4** with $Zn(OAc)_2{\cdot}2H_2O$ in DMF were not successful initially. The reaction led to precipitation which might be due to complexation between the peripheral COOH groups and the zinc(II) ions. This problem could be circumvented by the addition of NH_4Cl, which suppresses this complexation, allowing the zinc(II) ions to be incorporated into the phthalocyanine core. Phthalocyanine **5** was successfully prepared in this manner in 89% yield (see the characterization data for **3**–**5** in ESI†).[8]



Scheme 1

The UV–Vis spectra of **4** and **5** in THF were typical for non-aggregated phthalocyanines. In the presence of NaOH, these compounds were also soluble in water giving similar spectra properties (see their UV–Vis spectra in ESI†). The absorption spectrum of **5** showed the Q band at 673 nm. Under slightly acidic conditions (pH = 6.5–6.8), another broad band at *ca.* 630 nm was also seen, which can be attributed to the aggregated species.[8] At pH > 7, a sharp Q band was observed. This together with the strong fluorescence emission at 684 nm [quantum yield (Φ_f) = 0.26][9] suggests that this compound is relatively free from aggregation under alkaline conditions. The metal-free phthalocyanine **4** behaved similarly with a Φ_f value of 0.12. It is worth noting that phthalocyanines are usually highly aggregated in aqueous media due to the strong hydrophobic interactions.[8] The non-aggregated nature of **4** and **5** (in deprotonated form) can be ascribed to the inherent repulsion of the highly negatively charged molecules. This property is particularly important for photosensitising applications.

To evaluate the photosensitising efficiency of these two novel phthalocyanines, their singlet oxygen quantum yields (Φ_Δ) were determined by a steady-state method using 1,3-diphenylisobenzofuran as the scavenger.[10] It was found that both compounds are

† Electronic supplementary information (ESI) available: characterization data for **3**–**5**, UV–Vis spectra of **4** and **5** in the presence of NaOH in water, and photodynamic effect of **4** on HepG2 and J774. See http://www.rsc.org/suppdata/cc/b4/b405868b/

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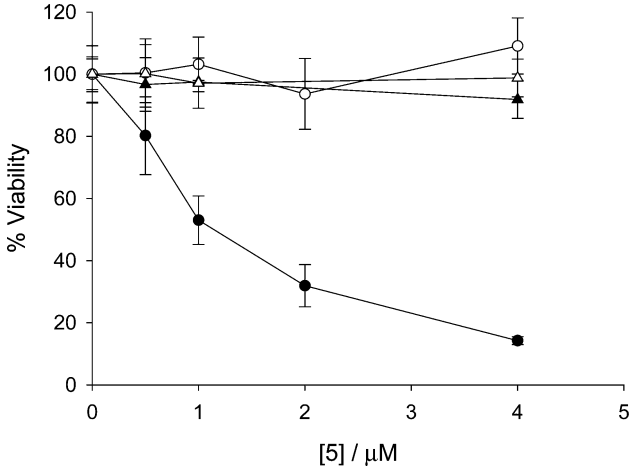

Fig. 1 Effect of **5** on HepG2 (triangles) and J774 (circles) in the absence (open symbols) and presence (closed symbols) of light. For the latter, the cells were illuminated with a red light ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean ± SD ($n = 5$).

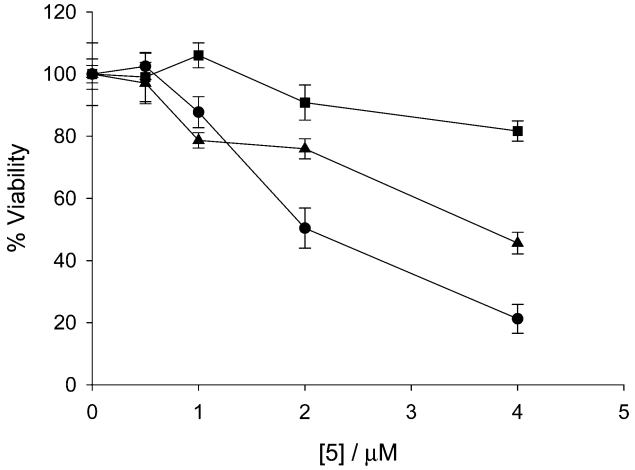

Fig. 2 Effect of poly I on the photodynamic activities of **5** on J774 cells. J774 macrophages were incubated for 2 hours with various concentrations of **5** in the absence (●) and presence of 25 μg cm^{-3} (▲) or 50 μg cm^{-3} poly I (■). Cells were illuminated and cytotoxicity was measured. Data are expressed as mean ± SD ($n = 5$).

singlet-oxygen generators. The Φ_Δ value of the zinc(II) phthalocyanine **5** (0.40 in DMF) is significantly higher than that for the metal-free counterpart **4** (0.27).[9]

The photodynamic activities of phthalocyanines **4** and **5** (in deprotonated form) were investigated against two different cell lines, namely murine macrophage J774 and human hepatocellular carcinoma HepG2.[11] The cells were incubated with different concentrations of **4** or **5** for 2 hours. Cell viability was determined after 20 hours by the colorimetric MTT assay.[5] In the absence of light, both compounds were not cytotoxic. The two cell lines differed, however, in their response after illumination. While HepG2 was resistant to the phototoxic effect of **4** and **5** at concentrations below 4 μM, J774 was very sensitive. As shown in Fig. 1, the percent viability of cells decreases gradually with increase in the concentration of **5**. Fifty percent cell death can be observed around 1 μM of **5**. The photodynamic activity of **4**, however, was somewhat lower. About 4 μM of **4** was needed to induce 50% of cell death (ESI†).

The J774 murine macrophage cell line has been used as a model system in the study of PDT, particularly in scavenger-receptor targeted PDT.[12] Covalent conjugation of photodynamic drugs to scavenger receptor ligands, for example, maleylated serum albumin, has been used successfully to increase their uptake into macrophage. J774 and HepG2 cells differ in the types of scavenger receptor they possess. For example, it is known that class-A scavenger receptor is expressed in J774 cells but not in HepG2 cells.[12,13] This, together with the observations that **4** and **5** act specifically on J774 but not HepG2, led to the speculation that class-A scavenger receptor was involved in mediating the cytotoxicity of the photosensitisers on J774. To show this, a competitive assay was performed in the presence of polyinosinic acid (poly I), a ligand of class-A scavenger receptor which is known for its high capacity to bind a broad range of polyanionic molecules, including modified lipoproteins, polyribonucleotides and polysaccharides.[14] As shown in Fig. 2, poly I inhibits the cytotoxicity of **5** in a dose-dependent manner. Co-incubation with 50 μg cm^{-3} poly I can increase the cell viability from *ca.* 20% to *ca.* 80% in the presence of 4 μM of **5**. These results show that the poly I-sensitive class-A scavenger receptor is responsible for the uptake of **5**, and subsequently, the cell death in J774 cells. Although **5** did not kill the tumour cell directly, the killing effect of macrophage is just as important as the tumour-associated macrophages can help the tumour to grow and spread,[12] for example, they produce mediators that increase the degree of angiogenesis.

In summary, we have prepared two novel hexadeca-carboxy phthalocyanines, which have many desirable characteristics as second-generation photosensitisers. The zinc(II) analogue exhibits a high and selective photocytotoxicity against J774 cells of macrophage origin *via* receptor-mediated endocytosis without the need of any bioconjugation.

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