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Original article

Photodynamic effects of isosteric water-soluble phthalocyanines on human nasopharynx KB carcinoma cells

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ABSTRACT

The photodynamic activity of water-soluble cationic zinc(II) phthalocyanines using human nasopharynx carcinoma (KB cells) was investigated. A sulfur-linked cationic dye, named: 2,9(10),16(17),23(24)-tetrakis [(2-trimethylammonium)ethylsulfanyl]phthalocyaninatozinc(II) tetraioidide (**13**) is the most active of four sensitizer assays and shows a singlet oxygen quantum yield of 0.58 and a higher bathochromic shift of 10 nm for the Q-band as compared with the oxygen-linked cationic aliphatic phthalocyanine: 2,9 (10),16(17),23(24)-tetrakis[(2-trimethylammonium)ethoxy]phthalocyaninatozinc(II) tetraioidide (**11**) and the best photo-stability in water in comparison with their tetra-α-substituted counterparts 1,8(11),15 (18),22(25)-tetrakis[(2-trimethylammonium)ethoxy]phthalocyaninatozinc(II) tetraioidide (**12**) and 1,8 (11),15(18),22(25)-tetrakis[(2-trimethylammonium)ethylsulfanyl]phthalocyaninatozinc(II) tetraioidide (**14**). Phthalocyanine **13**, partially localized in lysosomes, led to cell photoinactivation in a concentration- and light dose-dependent manner. After photodynamic treatment, compound **13** induced an apoptotic response – as indicated by morphological cell changes – an increase in the activity of caspase-3 and the cleavage of poly-ADP-ribose-polymerase substrate (PARP).

1. Introduction

Photodynamic therapy (PDT) is emerging as a promising method for the treatment of a variety of oncological, dermatological, cardiovascular and ophthalmic diseases [1–5]. PDT combines the intravenous or topical administration of a photosensitizer which preferentially localizes within the tumor, followed by illumination of the target tissue thus resulting in the formation of reactive oxygen species, believed to be responsible for the cascade of cellular and molecular events that finally lead to selective tumor destruction [1].

Phthalocyanines have been found to have applications as phototoxic drugs for PDT [4,6–8]. In addition to their well-known chemical stability, phthalocyanines possess characteristic absorption spectra [9], with a Soret band at approximately 350 nm and a usually narrow but very strong Q-band around 675 nm, with a molar absorption coefficient in the range of 10^5 M^{-1} cm^{-1}.

Zinc, aluminum, and silicon phthalocyanines that have been found to be useful photosensitizers for PDT, are efficient generators of singlet oxygen, the cytotoxic species capable of destroying malignant cells [10–14]. The silicon phthalocyanine Pc **4** is in various stages of clinical evaluation for cutaneous and subcutaneous lesions from diverse solid tumor origins [15].

The uptake and efficacy of photosensitizers such as phthalocyanines, porphyrins, and core-modified porphyrins are directly related to the number of hydrophilic groups [8,10] that the dye carries on its structure. Thus, in the aluminum sulfonate phthalocyanine series, those with two groups attached to the macrocycle show greater uptake and phototoxicity than phthalocyanines with three sulfonate groups which in turn show greater uptake and efficacy than phthalocyanines with four sulfonate groups. In BALB/c mice bearing EMT-6 tumors, aluminum sulfonate phthalocyanine substituted with two sulfonate groups is 10 times more phototoxic than aluminum phthalocyanine carrying four sulfonate groups [14]. Great efforts have been made to study the structure–activity relationship between the site [16,17] and kind of substitution [18] and the photodynamic activity. However, to our knowledge, studies directed to establish the structure–activity relationship between isosteric phthalocyanines are quite uncommon.

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We herein report the photobiological studies of four isosteric water-soluble cationic zinc(II) phthalocyanines using human nasopharynx KB carcinoma cells. The properties of these phthalocyanines are compared and discussed in connection with their photophysical and photochemical evaluation. Our results show the efficiency of one of them: 2,9(10),16(17),23(24)-tetrakis[(2-trimethylammonium)ethylsulfanyl]phthalocyaninatozinc(II) tetraioidide (**13**) for suitable PDT applications. To further elucidate the cellular mechanism of action, we examined the intracellular localization for the purpose of determining the primary photodamage site. In addition, the prevailing mechanism of cell death after treatment was evaluated.

2. Results and discussion

2.1. Chemistry

In order to evaluate the effect of substitution on the efficacy of phthalocyanines, we synthesized cationic dyes **11**–**14**. The synthetic route is shown in Scheme 1; precursors **3**–**6** and phthalocyanines **7**–**14** were prepared by methods similar to those described in the literature with minor modifications [19–23]. Taking into account that the above phthalocyanines are for photophysical and photobiological studies, the synthesis of all compounds was described under Experimental Section. Briefly, phthalonitriles **3**–**6** were prepared by reaction of the corresponding commercially available 4- and 3-nitrophthalonitrile with the appropriate nucleophiles in the presence of potassium carbonate [19,24,25] in dry N,N-dimethylformamide (DMF) that is more effective than dimethylsulfoxide (DMSO) as the reaction solvent because it resulted in improved yields of pure products.

Phthalocyanines **7**–**8** were readily prepared by cyclotetramerization of phthalonitriles **3**–**4** employing 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) and zinc acetate at 150 °C whereas phthalocyanines **9**–**10** were prepared by reacting **5**–**6** with DBU and zinc acetate in butanol. Cationic phthalocyanines **11**–**14** were obtained by treatment of **7**–**10** with methyl iodide in methylene chloride. Intermediates **3**–**6** and dyes **7**–**14** were characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopy while EI-MS spectroscopy at 70 eV was performed for the characterization of **3**–**4**, o-dinitriles **5**–**6** ESI-MS spectroscopy was determined with a Micromass Ultima triple QUAD spectrometer since EI-MS at 70 eV or 40 eV only afforded decomposition fragments. Besides, ESI-TOF mass spectroscopy was employed for the characterization of phthalocyaninates **7**–**14**.

With regard to the solubility of phthalocyanines **7**–**10** they are soluble in almost all organic solvents, while cationic derivatives **11**–**14** are fully soluble in water and DMF.

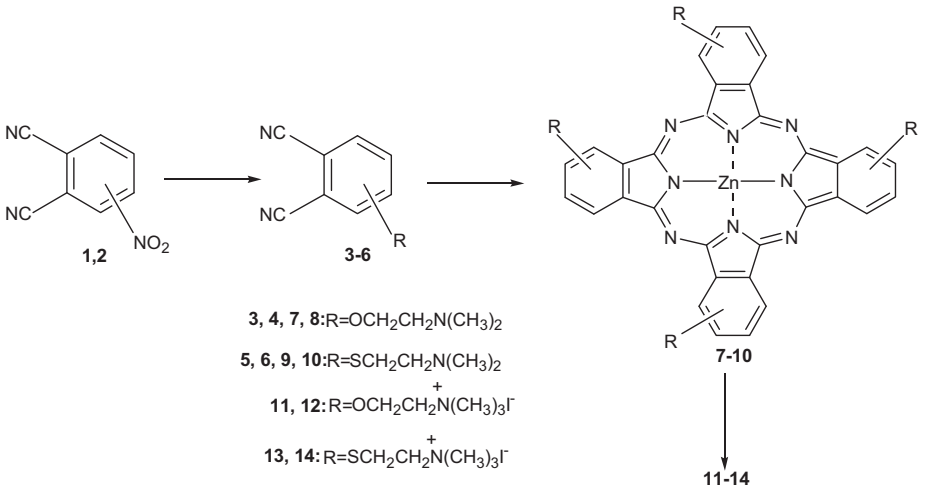
2.2. Photophysical studies

The absorption spectra of zinc(II) phthalocyanines **11**–**14** in the 400–800 nm range are similar to those previously reported for other analogs in homogeneous media [26,27] (Fig. 1). Phthalocyanines **11**–**14** are strongly aggregated in water; their absorption spectra show an important dimer band blue-shifted even at low concentrations as shown in Fig. 1. This situation is reverted when DMF is employed as a solvent: the monomer band at *c.a.* 680 nm is enhanced and the dimer absorption becomes negligible.

The same behavior is evidenced by deviations of the Lambert–Beer law, in water (data not shown). The monomer spectra are similar to that reported for different zinc(II) phthalocyanines. On the other hand dimers are blue-shifted and different from the corresponding spectra of other phthalocyanines of the same family previously studied in our laboratory [26]. This agrees with the fact that, while dimer spectra of metallated phthalocyanines are dependent on the peripheral substituents and solvent polarity, that of the monomer remains practically unchanged [28].

Dyes were characterized by their photophysical properties; the Q-band of substituted 1, 8(11), 15(18), 22(25) (α) zinc(II) phthalocyanines **8**, **10**, **12** and **14** lie at a longer wavelength (14–20 nm) than 2,9(10),16(17),23(24) (β) derivatives **7**, **9**, **11** and **13** (Table 1). This red spectral shift is consistent with previous reports for substituted zinc(II) phthalocyaninates with electron-releasing groups [29,30] which can be reasonably explained by considering the extent of the atomic orbital coefficients of the carbon atoms derived from molecular orbital calculations. According to these authors since the coefficient of the α carbon atoms is larger than that of β carbon atoms in the HOMO, the destabilization extent of this orbital by introducing electron-donating groups is larger when said groups are linked to the α-positions, a fact that makes the HOMO–LUMO gap smaller and thereby results in the Q-band shifting to a longer wavelength [30].

A bathochromic shift of 10 nm is observed for the Q-bands when oxygen and sulfur are alternatively present. This effect was observed for phthalocyanines **7**–**10** and cationic derivatives **11**–**14** respectively [31,32]. Such bathochromic shift into the therapeutic window is highly promising for a second-generation photosensitizer for biomedical purposes in photodynamic therapy. Cationic



Scheme 1. Synthetic route to phthalocyanines **11**–**14**.