Synthesis and photodynamic potential of tetra- and octa-triethyleneoxysulfonyl substituted zinc phthalocyanines

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Received 22 June 2006; received in revised form 31 July 2006; accepted 28 August 2006
Available online 1 September 2006

Abstract

Synthesis of the water soluble zinc phthalocyanines (3, 4) obtained from the phthalonitriles substituted with oligo(ethyleneoxy)thia groups are described. The new compounds have been characterized by elemental analysis, IR, \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopy, including HSQC, HMBC and COSY bidimensional correlation techniques, electronic spectroscopy and mass spectra. The aggregation behaviour of the phthalocyanine compounds (3, 4) was investigated using UV–vis spectroscopy in dimethylsulphoxide. Photochemical and photophysical measurements were conducted on oligo(ethyleneoxy)thia appended zinc phthalocyanines. General trends are described for quantum yields of photodegradation, fluorescence yields, triplet lifetimes and triplet quantum yields as well as singlet oxygen quantum yields of these compounds. The phototoxicity against cancer cells of the new compounds was investigated during several in vitro experiments. The dye-sensitized photooxidation of 1,3-diphenylisobenzofurane via \textsuperscript{1}O\textsubscript{2} was studied in dimethylsulphoxide.

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Keywords: Phthalocyanine; Quantum yields; Singlet oxygen; Photodynamic therapy; Phototoxicity

1. Introduction

For photodynamic therapy (PDT), a combination of a photosensitizing drug and light in the presence of molecular oxygen is used to obtain a therapeutic effect, and has been proposed as an alternative treatment to complement conventional protocols in the management of malignant tumours and many other non-oncologic diseases [1]. The use of photosensitizing agents for inactivation of several cancer cells has been widely studied [2].

The first photosensitizers were hematoporphyrin derivatives and have already been described in detail in several articles [3]. Second generation photosensitizers such as phthalocyanines (Pcs) have also been introduced for PDT in research and clinical trials [4]. Due to their high molar absorption coefficient in the red part of the spectrum, photostability, and long lifetimes of the photoexcited triplet states, Pcs are known to be useful photosensitizers [5,6]. Altering the peripheral substitution of the macrocyclic ring is one way of tailoring the solubility properties of the Pc material. The aggregation properties of Pcs are very important for the development of new photosensitizers [7]. The introduction of either long chains or bulky substitutents to the periphery of the macrocycle should prevent the aggregation [8].

Recently, zinc Pcs have found applications as photosensitizers in PDT since diamagnetic central metals, such as Zn or Mg enhance photocytotoxicity of Pc’s [9–12]. Thiol-derivatized metallophthalocyanine (MPC) complexes show rich spectroscopic and photochemical properties. For example, they are known to absorb at longer wavelengths (>700 nm) [13–16] than other MPC complexes. Therefore these complexes have a very useful feature...
for applications in optoelectronics, near-IR devices and PDT. The phototoxicity of Pcs is dependent on various factors such as subcellular localization (e.g. different partitioning in different compartments of cell membranes), physico-chemical structure, concentration, incubation time, exposure time, light energy and properties of cell lines [17].

In this work water soluble tetra and octa-triethyleneoxy-sulfonyl substituted zinc Pcs (3, 4) were synthesized. Aggregation behavior, photophysical (triplet state lifetimes and quantum yields, and fluorescence quantum yields) and photochemical (singlet oxygen and photodegradation quantum yields) properties, biological effects and possible phototoxicity of the Pcs compounds were investigated. Since PDT activity is mainly based on singlet oxygen, its production was determined by the dye-sensitised photooxidation of 1,3-diphenylisobenzofuran (DPBF), a specific scavenger of this toxic species [18]. Studies of the photostability of MPcs during photosensitized reactions is also of immense importance.

2. Experimental

2.1. Materials and equipment

4(4,7,10-Trioxaundecan-1-sulfonyl) phthalonitrile (1) and 4,5-bis(4,7,10-trioxadecan-1-sulfonyl) phthalonitrile (2) were prepared according to published procedures [19]. All other reagents and solvents were reagent-grade quality, were obtained from commercial suppliers, and were dried before use, as described by Perrin and Armarego [20].

Elemental analyses were obtained from Carlo Erba 1106 Instrument. Infrared spectra in KBr pellets were recorded on a Bio-Rad FTS 175C FT-IR spectrophotometer. Absorption spectra in UV–vis region were recorded with a Shimadzu 2001 UV–vis spectrophotometer and Varian 500 UV–vis–NIR spectrophotometer. Fluorescence excitation and emission spectra, were recorded on a Varian Eclipse spectroflurometer using 1 cm pathlength cuvettes at room temperature. Electrospray spectra were recorded on a Varian Eclipse spectroflurometer using 1 cm pathlength cuvettes at room temperature. Electrospray full scan spectra, in the range of m/z 50–2000 amu or m/z 2000–3000 amu, were obtained by infusion through fused silica tubing at 2–10 μl min⁻¹. The solutions were analyzed in a positive mode. The LCQ calibration (m/z 50–2000) was achieved according to the standard calibration procedure from the manufacturer (mixture of caffeine, MRFA and Ultramark 1621). An ES-Tuning Mix solution (Agilent) was used to calibrate the spectrometer between 2000 and 3000 amu. The temperature of the heated capillary of the LCQ was set to the range of 180–200°C, the ion spray voltage was in the range of 1–7 kV with an injection time of 5–200 ms. 1H and 13C NMR chemical shifts are given in Tables 1 and 2, respectively.

2.2. Synthesis

2.2.1. Tetrakis(4,7,10-trioxadecan-1-sulfanyl) phthalocyaninato zinc (3)

A mixture of 1 (0.50 g, 1.63 mmol), anhydrous Zn(O₂CMe)₂ (30.00 mg, 0.50 mmol), 0.07 ml (0.45 mmol) 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) and dried 1-hexanol (5 ml) were heated to reflux for 18 h under argon in a round-bottomed flask. The resulting green suspension was cooled and the crude product was precipitated by addition of hexane. The crude green product was purified by column chromatography (silica gel, CH₂Cl₂:MeOH 15:1). Yield: 174 mg (% 33). C₆₀H₇₂N₈O₁₂S₄Zn (1290); Found C, 56.85; H, 5.26; N, 8.53; requires C, 56.12; H, 5.65; N, 8.72; IR (KBr): νmax (cm⁻¹): 3055, 2926–2854(CH₂, CH₃), 1600 (C=O), 1350 (C–N), 1281 (C–O–C), 1200, 1160–1090. MS (ES–MS), m/z (%): 1291(1 0 0) [M + H]⁺. 1H and 13C NMR chemical shifts are given in Tables 1 and 2, respectively.

2.2.2. Octakis(4,7,10-trioxadecan-1-sulfanyl) phthalocyaninato zinc (4)

A mixture of 2 (0.75 g, 1.55 mmol), anhydrous Zn(O₂CMe)₂ (30.00 mg, 0.50 mmol), 0.07 ml (0.45 mmol) DBU and dried 1-hexanol (6 ml) were heated to reflux for 18 h under argon in a round-bottomed flask. The resulting green suspension was cooled and the crude product was precipitated by addition of hexane. After that 4 was isolated and purified by the same procedure as for 3. Yield: 108 mg (% 18). C₈₈H₁₂₈N₈O₂₄S₈Zn (2003); Found C, 53.00; H, 6.43; N, 5.18; requires C, 52.92; H, 6.46; N, 5.61; IR (KBr): v max (cm⁻¹): 3055, 2920–2840(CH₂, CH₃), 1600 (C=O, N), 1530, 1350 (C–N), 1290 (C–O–C), 1250, 1120–1070. MS (ES–MS), m/z (%): 2003(1 0 0) [M + H]⁺.

Table 1

<table>
<thead>
<tr>
<th>Proton</th>
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<tr>
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<td>3.12 (s)</td>
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<tr>
<td>H₂</td>
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<tr>
<td>H₃</td>
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<tr>
<td>H₂₁</td>
<td>4.02 (t)</td>
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</table>
Φ was employed for the calculations of quantum yields. The deaerated solutions of the respective octa and tetra substituted ZnPc complexes were introduced into a 1 cm pathlength 10 mm spectrophotometric cell and irradiated at the Q band maxima with the laser system described above. Triplet lifetimes were determined by exponential fitting of the kinetic curves using OriginPro 7.5 software.

2.4. Singlet oxygen and photodegradation quantum yields

Singlet oxygen (ΦΔ) and photodegradation (Φd) quantum yield determinations were carried out using the experimental set-up described above [24,26,27]. Typically, a 2 ml portion of the respective octa and tetra substituted the ZnPc (3 and 4) solutions (absorbance ~1 at the irradiation wavelength) containing the singlet oxygen quencher was irradiated in the Q band region with the photo-irradiation set-up described in the references [24,26,27]. ΦΔ values were determined in air using the relative method with 1,3-diphenylisobenzofuran (DPBF) as singlet oxygen chemical quencher in DMSO (Eq. (4))

ΦΔ = ΦΔstd R Iabs std Rstd Iabs

where ΦΔstd is the singlet oxygen quantum yield for the standard ZnPc (ΦΔstd = 0.67) in DMSO [28], R and Rstd are the DPBF photobleaching rates in the presence of the respective the Pcs complexes (3 or 4) and standard, respectively; Iabs and Iabs std are the rates of light absorption by the samples (3 or 4) and standard, respectively. The concentrations of DPBF in the solutions were calculated using the determined values of log ε = 4.36 at 417 nm (DPBF in DMSO). The light intensity used for ΦΔ determinations was found to be 8.36 × 1016 photons s⁻¹ cm⁻². The error in the determination of ΦΔ was ~10% (determined from several ΦΔ values). Photodegradation quantum yields were determined using the following equation:

Φd = (C0 − C t) VN A Iabs St

where C0 and C t are the samples (3 or 4) concentrations before and after irradiation respectively, V is the reaction volume, N A the Avogadro’s constant, S the irradiated cell area and t the irradiation time. Iabs is the overlap integral of the radiation source light intensity and the absorption of the sample (3 or 4). A light intensity of 2.86 × 10¹⁷ photons s⁻¹ cm⁻² was employed for Φd determinations.

2.5. Cell cultures

MCF-7 (human breast cancer) cells were grown in OptiMEM medium (Gibco) supplemented with 10% fetal calf serum, 25 IU/ml penicillin and 25 mg/ml streptomycin. Cells were at 37°C in an atmosphere containing 5% CO₂ at 100% humidity.
2.6. Cell treatments

Exponentially growing MCF-7 cells were seeded in 4 cm diameter Petri dishes (1000 cells/dish). After 24 h seeding, the Pcs 3 and 4 were added to final concentrations of 1, 5, 10, 20, 50 and 100 μM/ml, respectively.

2.7. Irradiation with laser

For cell irradiation, an Argon-pumped Ti:Sapphire laser (Coherent, CA) emitting at 693 and 709 nm were used. The output power was set to 5 mW/cm² and the cell dishes were placed onto the laser beam so that the cells were entirely irradiated. A total optical dose of 500 mJ was delivered to the Petri dish.

2.8. Cell proliferation assay (WST-1)

The tetrazolium compound reagent (WST-1, Roche) was used for the quantification of cell proliferation and cell viability. It is a colorimetric assay and based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in proliferating cells. For this purpose; cells were cultured in medium at a density of 4000 cells per well into 96-well microtiter plates and incubated. Various concentrations of phthalocyanines were added to exponentially growing cells and after 24 h the cells were irradiated with laser irradiation. After illumination the cells were incubated for 24 h and then the plates were measured by reading optical density at 450 nm by the Universal Microplate Reader. For dark toxicity of the Pcs, the cells suspension were treated with the Pcs at different concentrations in dark for 24 h. After incubation, the optical densities were measured as above mentioned.

2.9. Microscopy

RR1022 cells from the rat were seeded on microscope slides and incubated for 4 h with the Pc (3) and the Pc (4) at a concentration of 10 μM. The cellular fluorescence distribution was observed with a laser scanning microscope LSM510 Meta (Zeiss, Germany). The fluorescence was excited in all cases with 633 nm, from HeNe Laser. The following beam splitter was used: HFT UV/488/543/633. The fluorescence was observed between 650 and 710 nm, using the appropriate band-pass filter. A 63x/1.4 oil immersion objective was used and the pinhole was set to 2.67 airy units.

3. Results and discussion

3.1. Synthesis and characterization

The Pc derivatives (3 and 4) were obtained from the reaction of the dicyano compounds 1 and 2 in the presence of corresponding metal salt and DBU in 1-hexanol at reflux temperature. The synthetic pathways were shown in Scheme 1. Elemental-analysis results and the spectral data (1H NMR, 13C NMR, FT-IR, UV–vis and MS) for newly synthesized Pcs (3 and 4) were consistent with the assigned formulations.

NMR investigation of the Pcs have provided the characteristic chemical shifts for the structures as expected. The 1H and 13C NMR spectra of compounds 3 and 4 in DMSO-d6 were assigned based on the COSY (Fig. 1), HSQC (Fig. 2) and HMBC (Fig. 3) experiments and confirmed the proposed structure. 1H NMR and 13C NMR chemical shifts were given in Tables 1 and 2, respectively. The homonuclear bidimensional DQF-COSY spectrum of compound 4 are shown in Fig. 1 and the signals along the diagonal reflect the normal 1H spectrum. The cross peaks provide the information that we need. In general, each cross peak represents a correlation due to either two- or three-bond H–H coupling. Taken in order, the observed cross peaks indicate the following correlations: H10–H9, H8–H7, and H6–H5 in Fig. 1. As the aromatic proton H3 was easily determined and there was no correlation with any aliphatic proton of it, the DQF-COSY spectrum of compound 4 did not show the aromatic region in Fig. 1. Due to absence of any cross peak belonging to H11, the spectrum was given only between 3.2 and 4.1 ppm range. The contour plot of the C,H-HSQC spectrum of 4 is shown in Fig. 2 and we determined which hydrogen were connected to which carbon via one-bond C–H coupling. Although chemical shifts

Scheme 1. Synthetic pathway for the preparation of compound 3 and 4. (i) n-Hexanol, DBU, zincacetate, reflux.
of methylene carbons (C₆, C₇, C₈ and C₉) were very close to one another, chemical shifts of these carbons were easily determined by using HSQC spectrum. In addition, the chemical shifts of these carbons (C₆, C₇, C₈ and C₉) were verified by HMBC spectrum of compound 4 (Fig. 3). HMBC spectrum give the ¹H–¹³C correlation via two- or three bond and each correlation give a cross peak. In Fig. 3, the observed cross peaks indicate the following correlations: H₁₁–C₁₀; H₁₀–C₉, C₁₁; H₉–C₁₀, C₈; H₈–C₇, C₉; H₇–C₆, C₈ and H₆–C₇, C₈. The carbon atoms chemical shifts were exactly determined with these data.

A close investigation of the mass spectra of the Pc compounds confirmed the proposed structures. The mass spectra of the Pcs were obtained by Electron Spray technique and the molecular ion peaks at m/z: 1291 for 3 and m/z: 2003 for 4 were observed.

3.2. Absorption spectra

In the electronic spectra of 3 and 4 in chloroform, intense Q absorption bands were observed at 693 and 709 nm, respectively. B band absorptions of the Pcs (3 and 4) were observed at 363 and 372 nm, respectively (Fig. 4 for compound 4). For the Pc derivatives the effect of thia-substitution is a shift of these intense Q bands to higher wavelengths when compared with unsubstituted, alkyl- or alkoxy-substituted derivatives.

The optical absorption spectra of compounds 3 and 4 are dependent on the nature of the solvent used. Aggregation is not detectable in tetrahydrofuran (THF), chloroform or pyridine. Methanol and water solutions cause drastic changes in the Q band with lowering of intensity and wavelengths of the peaks as a result of the aggregation as seen in Fig. 4 for compound 4.
Aggregation is usually depicted as a coplanar association of rings progressing from monomer to dimer and higher order complexes. It is dependent on the concentration, nature of the solvent, peripheral substituents, complexed metal ions and temperature [29,30]. In the aggregated state the electronic structure of the complexed Pc rings is perturbed resulting in alternation of the ground and excited state electronic structure [31]. In this study, the aggregation behaviour of the Pcs are investigated at different concentrations in DMSO. Compounds 3 and 4 are readily soluble in DMSO with strong absorption. We studied the effect of concentration on the absorption spectra for the Pcs (3 and 4) in DMSO. Beer–Lambert law was obeyed for all of these complexes in the concentrations ranging from $8.97 \times 10^{-6}$ to $8.97 \times 10^{-7}$ mol$^{-1}$ at maximum absorption. As the concentra-

![Fig. 2. H$^1$–$^{13}$C HSQC spectrum of compound 4.](image1)

![Fig. 3. H$^1$–$^{13}$C HMBC spectrum of compound 4.](image2)
tion is increased, the absorption maxima of the Q band also increased and the blue shift of Q band absorptions was not observed. According to the reported literature [16,30–32] and the results, the Pcs derivatives are not aggregated in wide range of concentrations in DMSO.

3.3. Photophysical and photochemical properties

The fluorescence spectra showed only one peak for both complexes, as is typical of Pc complexes (Fig. 5), and the excitation spectra was similar to the absorption spectra (Fig. 5). The values of the Stokes shifts were 9 and 10 nm for complex 3 and 4, respectively. These values are within the range reported for phthalocyanine complexes and show that fluorescence proceeds with minor geometric relaxation in the first excited state. The values of fluorescence quantum yields ($\Phi_F$) of 0.20 and 0.13 (Table 3) are within the range for MPc complexes [24]. The lower value of $\Phi_F$ for 4 compared to 3, suggests that either the larger number of substituents in the former enhance intersystem crossing or there in increased quenching in complex 4. The $\Phi_{IC}$ values were low for 3 and 4, confirming that it is not the quenching of the singlet state which results in the decrease of the $\Phi_F$ value for 4. Triplet decay trace for the complexes is exemplified by Fig. 6. The value of the triplet quantum yield ($\Phi_T$) for 4 is larger than that for 3, Table 3, in agreement with increased intersystem crossing in the presence of the larger number of substituents in the former. The $\Phi_T$ values for 3 and 4 show improvement compared to unsubstituted ZnPc standard. The consequence of increased intersystem crossing should be the shortening of triplet life time for 4, however this is not the case in Table 3. The triplet life times (Table 3) are reasonable and in the same range as for ZnPc standard. The $\Phi_T$ values for 4 resulted in increased $\Phi/Delta_1$ values as observed in Table 3.

The faction of triplet state quenched by singlet oxygen, $S_{\Delta}$, was calculated for the complexes using the following equation:

$$S_{\Delta} = \frac{\Phi_{\Delta}}{\Phi_T}$$

The $S_{\Delta}$ values are close to unity (Table 3) implying efficient quenching of the triplet states by singlet oxygen.

The photodegradation quantum yield ($\Phi_d$) values for the complexes are shown in Table 3 and are of the order of $10^{-4}$. These values show that the molecules are of intermediate stability. Stable ZnPc molecules show values as low as $10^{-6}$ and for unstable molecules, values of the order of $10^{-3}$ have been reported [33]. It seems the long chains decreases the stability of complexes 3 and 4, but they are not as unstable as ZnPc complexes con-

<table>
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<tr>
<th>Compound</th>
<th>$\lambda_{Qa}$ (nm) (log $\varepsilon$)$^b$</th>
<th>$\lambda_F$ (nm)</th>
<th>$\tau_T$ (µs)</th>
<th>$\Phi_F$</th>
<th>$\Phi_T$</th>
<th>$\Phi_{IC}$</th>
<th>$\Phi_d (10^4)$</th>
<th>$\Phi_{\Delta}$</th>
<th>$S_{\Delta}$</th>
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<tr>
<td>3</td>
<td>694 (5.31)</td>
<td>703</td>
<td>230</td>
<td>0.20</td>
<td>0.77</td>
<td>0.03</td>
<td>1.62</td>
<td>0.64</td>
<td>0.83</td>
</tr>
<tr>
<td>4</td>
<td>710 (5.32)</td>
<td>720</td>
<td>280</td>
<td>0.13</td>
<td>0.85</td>
<td>0.02</td>
<td>2.20</td>
<td>0.72</td>
<td>0.84</td>
</tr>
</tbody>
</table>

$^a$ Q band maxima shown for the low energy band only where bands are split.

$^b$ The log $\varepsilon$ values are for the low energy band where there are more than two bands.
Fig. 7. Intracellular localization of the Pcs in RR1022 cells after incubation for 24 h. A: ZnPc (3) prescan, B: ZnPc (3) post scan, C: ZnPc (4) and D: control.

Fig. 8. Cytotoxicity of the compounds 3 (▲) and 4 (■) in the MCF-7 cells in dark using cell proliferation assay (WST-1) mean ± S.D. of three independent data sets.

Fig. 9. Dark (lined) and light (dotted) Cytotoxicity of the ZnPc (3) at different concentration. MCF-7 cells treated with ZnPc (3) only without irradiation (lined) were compared with the cells which received irradiation (dotted). The control was MCF-7 cells without ZnPc (3) irradiated (lined) or non-irradiated (dotted) at 0 µM concentration.

3.4. Cell studies

In recent years, metal complexes of Pcs have been widely investigated. Pcs exhibit effective cell penetration because of their chemical stability, and proper light absorption region. Due to their strong Q band in the red region in which the biological tissue are rather transparent and fluorescent, which provides an opportunity for the establishment of their localization in the tissue [34,35]. Intracellular localization relates to many cytoplasmic targets including plasma membranes, mitochondria, golgi apparatus, lysosomes and cytoskeletal structure and are major targets to the photo-induced oxidative process [36]. Laser scanning microscope observations of the Pcs (3 and 4) indicated that they were localized in the cytoplasm and not in the cell nucleus as shown in Fig. 7. The fluorescence of the Pc 3 increased during scanning. This is presumably due to the higher value of $\Phi_F$ for Pc 3 compared to Pc 4.

Dark toxicity of the Pcs was measured using cell proliferation reagent WST 1 and the optical density was detected. The cells which were treated for 24 h from 0 to 100 µM concentrations of two phthalocyanines did not show any toxic effects as shown in Fig. 8. Optical density was not changed for all concentrations in comparison with control cells at 0 µM concentration.

A combination with light and the Pcs 3 and 4 showed different effects on cell killing of MCF-7 cells. As shown in Figs. 9 and 10, octa-substituted Pc derivative (4) displays much lower cell killing-ability, although octa-substituted Pc (4) has higher values of the photophysical and photochemical parameters than tetra-substituted Pc (3). It is known that the photophys-
MCF-7 cells. The photophysical and photochemical properties of the sensitizers does not necessarily always provide on accurate indication of their phototoxic activity [37,38] and this is shown by Table 3, where the tetra-substituted Pc (4) display a much lower triplet quantum yield ($\Phi_T$) than the octa-substituted Pc (4), yet this difference is not reflected by their cytotoxicity inducing ability as shown in Figs. 9 and 10. The differences in the cell killing abilities of the Pc dyes could be related to several factors including (i) cell uptake, cell type and subcellular localization; (ii) the photochemical properties of the dyes which in turn are affected by the extend of aggregation of the sensitizers; (iii) the cell killing abilities of the difference tetra- and octa-phthalocyanine complexes. Consequently, the octasubstituted Pc 4 is inferior to its tetra-substituted analog 3, which possesses high photobiological activity. This is presumably due to the decrease uptake of 4 into the cells, either because of the greater substitution and consequent steric hindrance [39–41].

4. Conclusion

Novel zinc phthalocyanines 3 and 4, containing triethyleoxyxosulfonil group as substituents, which are soluble in polar organic solvents, were synthesized and evaluated in cells, using MCF-7 cells. The photophysical and photochemical properties of the Pcs 3 and 4 were investigated. The triplet state ($\Phi_T$) and singlet oxygen ($\Phi_A$) quantum yields values suggest that the molecules have intermediate stability for the production of singlet oxygen. The tetra- substituted Pc 3 displays better several in vitro characteristics that make them highly suitable for continued evaluation as PDT agents, namely dark toxicity, phototoxicity at low light dose (5 mW/cm²), substantial uptake by cells, and favourable intracellular sites of localization than octa-substituted Pc (4).

Our further study is investigation of the cell death at molecular level especially apoptosis or necrosis induced by PDT in different cancer cell lines.

Acknowledgements

This work was supported by the Research Fund of Gebze Institute of Technology, the University of Zürich, Switzerland, TUBITAK (Kariyer-104T217), the National Research Foundation (NRF GUN #2053657) and by Rhodes University. Special thanks to TUBITAK for supporting a visit of Prof. H. Walt.

References


