Available online at www.sciencedirect.com
ScienceDirect
Journal of Photochemistry and Photobiology A: Chemistry 186 (2007) 298–307
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

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

Devrim Atilla [a], Nil Saydan [b], Mahmut Durmuş [a,e], Ayşe Gül Gürek [a], Tania Khan [c], Angelika Rück [d], Heinrich Walt [c], Tebello Nyokong [e], Vefa Ahsen [a,f,*]

[a] *Gebze Institute of Technology, Department of Chemistry, PO Box 141, Gebze 41400, Turkey*
[b] *Gebze Institute of Technology, Department of Biology, PO Box 141, Gebze 41400, Turkey*
[c] *University Hospital Zurich, Frauenklinikstrasse10, CH-8091 Zürich, Switzerland*
[d] *Institut for Lasertechnologies in Medicine and Metrology (ILM), Helmholtzstrasse 12, D-89081 Ulm, Germany*
[e] *Department of Chemistry, Rhodes University, Grahamstown 6140, South Africa*
[f] *TUBITAK-Marmara Research Center, Materials Research Institute, PO Box 21, Gebze 41470, Turkey*

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, ^{1}H and ^{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 photodegredation, 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 $^{1}O_2$ was studied in dimethylsulphoxide.

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 photoexicited 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 phototoxicity 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

* Corresponding author at: Gebze Institute of Technology, Department of Chemistry, PO Box 141, Gebze 41400, Turkey. Tel.: +90 262 605 31 06; fax: +90 262 605 31 01.
E-mail address: ahsen@gyte.edu.tr (V. Ahsen).

1010-6030/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2006.08.022

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-triethyleneoxysulfonyl 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 Pc 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-trioxaundecan-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 an Shimadzu 2001 UV Pc spectrophotometer and Varian 500 UV–vis–NIR spectrophotometer. Fluorescence excitation and emission spectra, were recorded on a Varian Eclipse spectrofluoremeter 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. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 solutions on a Bruker and Varian 500 MHz spectrometers using TMS as an internal reference.

Photo-irradiations were done using a General electric Quartz line lamp (300 W). A 600 nm glass cut off filter (Schott) and a water filter were used to filter off ultraviolet and infrared radiations, respectively. An interference filter (Intor, 670 nm with a band width of 20 nm) was additionally placed in the light path before the sample. Light intensities were measured with a POWER MAX5100 (molelectron detector incorporated) power meter. Triplet absorption and decay kinetics were recorded on a laser flash photolysis system, the excitation pulses were produced by a Quanta-Ray Nd: YAG laser providing 400 mJ, 90 ns pulses of laser light at 10 Hz, pumping a Lambda-Physik FL3002 dye (Pyridine 1 dye in methanol). Single pulse energy was 2 mJ. The analyzing beam source was from a Thermo Oriel xenon arc lamp, and a photomultiplier tube was used as a detector. Signals were recorded with a two-channel digital real-time oscilloscope (Tektronix TDS 360); the kinetic curves were averaged over 16 laser pulses.

2.2. Synthesis

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

A mixture of **1** (0.50 g, 1.63 mmol), anhydrous $Zn(O_2CMe)_2$ (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, CH_2Cl_2:MeOH 15:1). Yield: 174 mg (% 33). $C_{60}H_{72}N_8O_{12}S_4Zn$ (1290); Found C, 56.85; H, 5.26; N, 8.53; requires C, 56.12; H, 5.65; N, 8.72; IR (KBr): ν_{max} (cm^{-1}) 3055, 2926–2854(CH_2, CH_3), 1600 (C=N), 1350 (C–N), 1281 (C–O–C), 1200, 1160–1090. MS (ES–MS), *m/z* (%): 1291(1 0 0) $[M+H]^+$. ^{1}H and ^{13}C NMR chemical shifts are given in Tables 1 and 2, respectively.

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

A mixture of **2** (0.75 g, 1.55 mmol), anhydrous $Zn(O_2CMe)_2$ (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). $C_{88}H_{128}N_8O_{24}S_8Zn$ (2002); Found C, 53.00; H, 6.43; N, 5.18; requires C, 52.92; H, 6.46; N, 5.61; IR (KBr): ν_{max} (cm^{-1}) 3055, 2920–2840(CH_2, CH_3), 1600 (C_{ar}=N), 1530, 1350 (C–N), 1290 (C–O–C), 1250, 1200, 1140–1070. MS (ES–MS), *m/z* (%): 2003(1 0 0) $[M+H]^+$,

Table 1
^{1}H chemical shifts (ppm) for compounds **3** and **4** in DMSO-d_6

Proton	**3**	**4**
H_3	8.81 (s)	9.10 (s)
$H_{3'}$	8.88 (d)	9.10 (s)
$H_{4'}$	8.04 (d)	–
H_5	3.72 (t)	3.66 (t)
H_6	4.02 (t)	3.98 (t)
H_7	3.78 (t)	3.74 (t)
H_8	3.68 (t)	3.62 (t)
H_9	3.58 (t)	3.51 (t)
H_{10}	3.44 (t)	3.35 (t)
H_{11}	3.20 (s)	3.12 (s)

Table 2
^{13}C chemical shifts (ppm) for compounds **3** and **4** in DMSO-d_6

Carbon	**3**	**4**
C_1	151.80	158.20
$C_{1'}$	152.20	158.20
C_2	134.80	138.30
$C_{2'}$	135.00	138.30
C_3	120.18	121.60
$C_{3'}$	121.00	121.60
C_4	137.97	139.20
$C_{4'}$	128.00	139.20
C_5	32.80	34.20
C_6	68.06	69.84
C_7	68.63	70.60
C_8	68.70	70.62
C_9	68.42	70.40
C_{10}	70.02	71.94
C_{11}	55.40	59.00

1823(10) [M−6(OCH_3)]. ^{1}H and ^{13}C NMR chemical shifts are given in Tables 1 and 2, respectively.

2.3. Photophysical parameters

2.3.1. Fluorescence quantum yields

Fluorescence quantum yields (Φ_F) were determined by the comparative method (Eq. (1)) [21,22]

$$\Phi_F = \Phi_F(\text{std})\frac{FA_{std}\eta^2}{F_{std}A\eta^2_{std}} \quad (1)$$

where F and F_{std} are the areas under the fluorescence curves of the samples (**3** or **4**) and the standard, respectively. A and A_{std} are the respective absorbances of the sample and standard at the excitation wavelengths (which was ∼0.05 in all solvents used), and η and η_{std} are the refractive indices of solvents used for the sample and standard, respectively. Unsubstituted zinc phthalocyanine (ZnPc) in DMSO (Φ_F = 0.18) [23] was employed as the standard. Both the sample and standard were excited at the same wavelength.

2.3.2. Triplet quantum yields and lifetimes

The deaerated solutions of the respective octa and tetra substituted ZnPc (**3** and **4**) 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 quantum yields (Φ_T) were determined by a comparative method using triplet decay [24]. A comparative method [24], Eq. (2), was employed for the calculations

$$\Phi_T^{sample} = \Phi_T^{std}\frac{\Delta A_T^{sample}\varepsilon_T^{std}}{\Delta A_T^{std}\varepsilon_T^{sample}} \quad (2)$$

where ΔA_T^{sample} and ΔA_T^{std} are the changes in the triplet state absorbance of the samples (**3** or **4**) and standard, respectively; ε_T^{sample} and ε_T^{std}, the triplet state extinction coefficients for the samples (**3** or **4**) and standard, respectively. The standard employed was ZnPc in DMSO. The triplet quantum yield (Φ_T^{std}) for the standard is Φ_T^{std} = 0.65 for ZnPc [25] in DMSO.

Quantum yields of internal conversion (Φ_{IC}) were obtained from Eq. (3), which assumes that only three processes (fluorescence, intersystem crossing and internal conversion), jointly deactivate the excited singlet state of octa and tetra substituted the ZnPc complexes (**3** and **4**)

$$\Phi_{IC} = 1 - (\Phi_F + \Phi_T) \quad (3)$$

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 (Φ_Δ) and photodegradation (Φ_d) 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]. Φ_Δ values were determined in air using the relative method with 1,3-diphenylisobenzofuran (DPBF) as singlet oxygen chemical quencher in DMSO (Eq. (4))

$$\Phi_\Delta = \Phi_\Delta^{std}\frac{RI_{abs}^{std}}{R^{std}I_{abs}} \quad (4)$$

where Φ_Δ^{std} is the singlet oxygen quantum yield for the standard ZnPc (Φ_Δ^{std} = 0.67) in DMSO [28], R and R^{std} are the DPBF photobleaching rates in the presence of the respective Pcs complexes (**3** or **4**) and standard, respectively; I_{abs} and I_{abs}^{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 Φ_Δ determinations was found to be 8.36×10^{16} photons s^{-1} cm^{-2}. The error in the determination of Φ_Δ was ∼10% (determined from several Φ_Δ values). Photodegradation quantum yields were determined using the following equation:

$$\Phi_d = \frac{(C_0 - C_t)VN_A}{I_{abs}St} \quad (5)$$

where C_0 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. I_{abs} is the overlap integral of the radiation source light intensity and the absorption of the sample (**3** or **4**). A light intensity of 2.86×10^{17} photons s^{-1} cm^{-2} was employed for Φ_d determinations.

2.5. Cell cultures

MCF-7 (human breast cancer) cells were grown in Opti-MEM 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% CO_2 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 out put power was set to 5 mW/cm^2 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

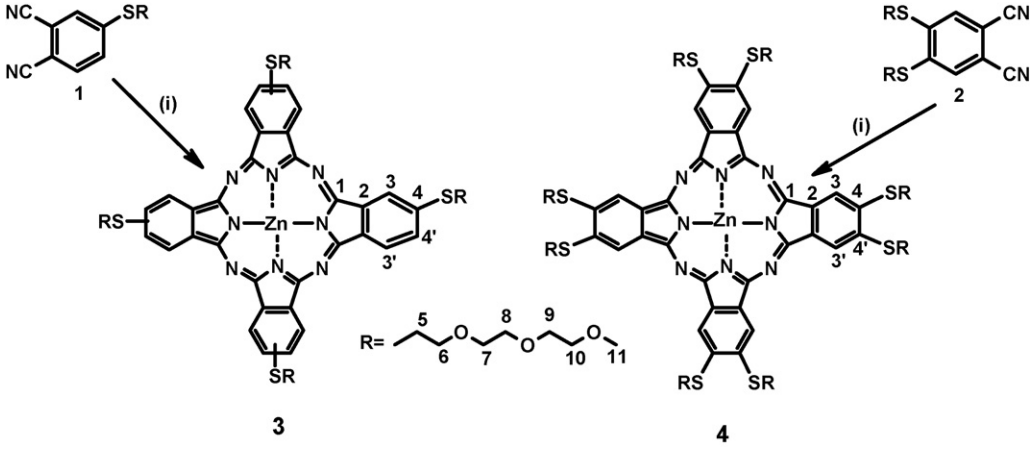
RR1022 cells from the rat were seeded on microscope slides and incubated for 4 h with the Pc (**3**) and the Pc (**4**) at a concentration of 10 μM. The cellular fluorescence distribution was observed with a laser scanning microscope LSM510 Meta (Zeiss, Germany). The fluorescence was excited in all cases 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 (^{1}H NMR, ^{13}C 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 ^{1}H and ^{13}C NMR spectra of compounds **3** and **4** in DMSO-d_6 were assigned based on the COSY (Fig. 1), HSQC (Fig. 2) and HMBC (Fig. 3) experiments and confirmed the proposed structure. ^{1}H NMR and ^{13}C 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 ^{1}H 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: H_{10}–H_9, H_8–H_7, and H_6–H_5 in Fig. 1. As the aromatic proton H_3 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 H_{11}, 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.

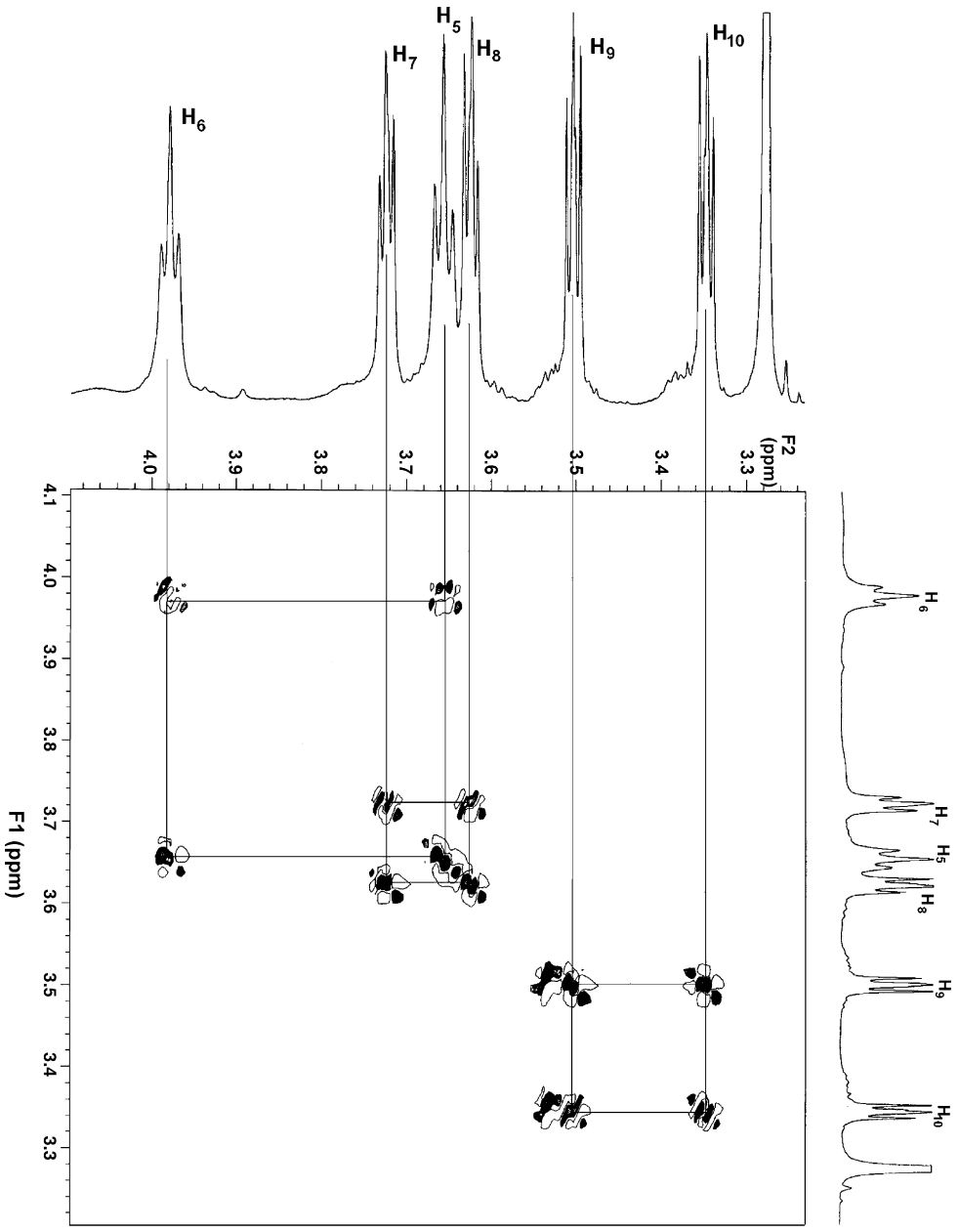


Fig. 1. DQF-COSY spectrum of compound **4**.

of methylene carbons (C_6, C_7, C_8 and C_9) 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 (C_6, C_7, C_8 and C_9) were verified by HMBC spectrum of compound **4** (Fig. 3). HMBC spectrum give the ^1H–^{13}C correlation via two- or three bond and each correlation give a cross peak. In Fig. 3, the observed cross peaks indicate the following correlations:H_{11}–C_{10}; H_{10}–C_9, C_{11}; H_9–C_{10}, C_8; H_8–C_7, C_9; H_5–C_6; H_7–C_6, C_8 and H_6–C_7, C_8. 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**.

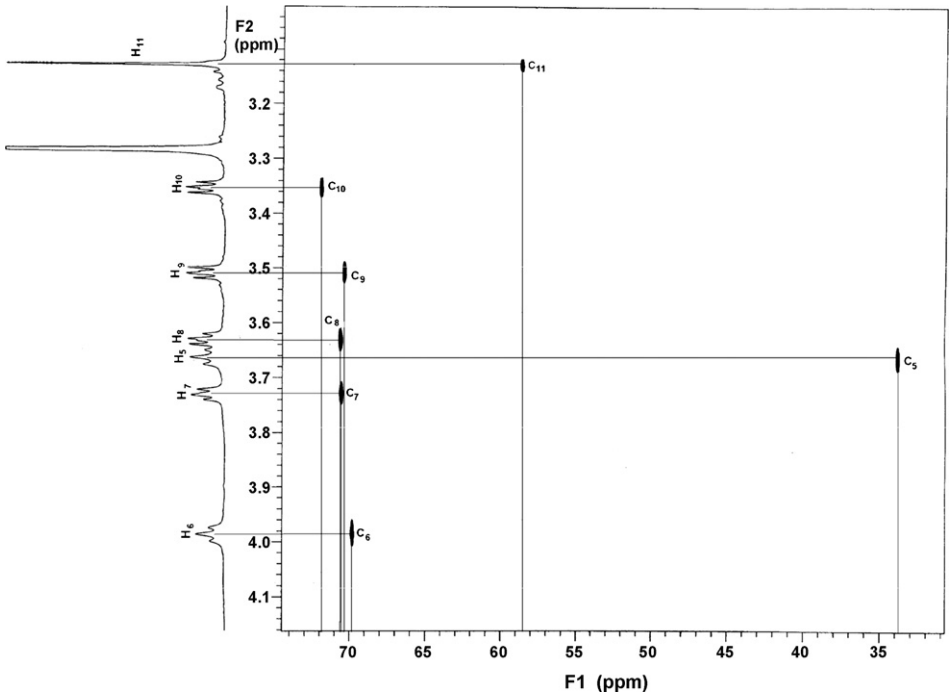


Fig. 2. H^1–^{13}C HSQC spectrum of compound **4**.

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 8.97×10^{-6} to 8.97×10^{-7} mol l^{-1} at maximum absorption. As the concentra-

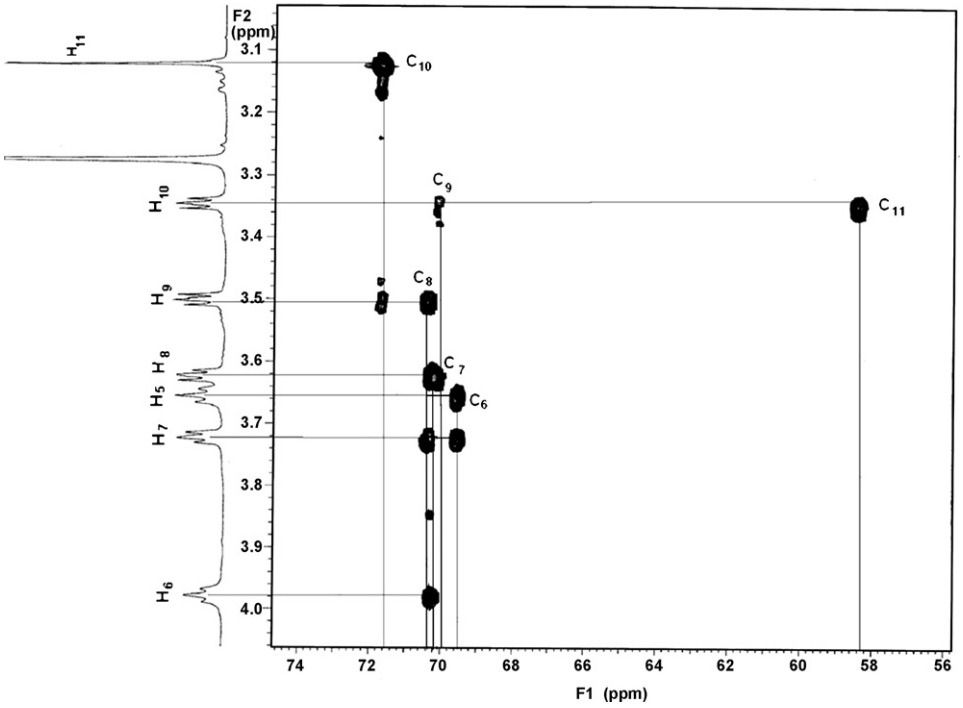


Fig. 3. H^1–^{13}C HMBC spectrum of compound **4**.

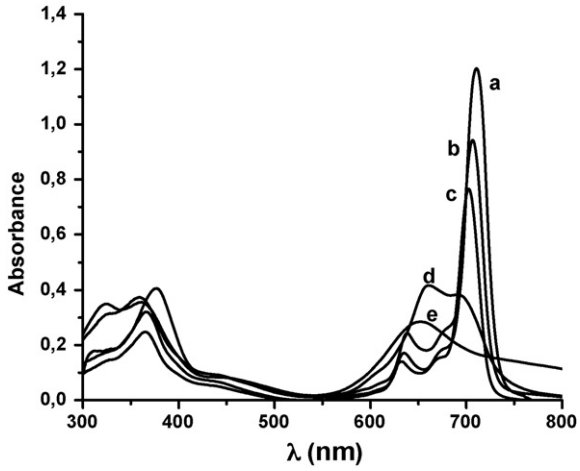


Fig. 4. UV–vis spectrum of **4** in different solvents: (a) Pyridine, 6×10^{-6} M, (b) chloroform, 4×10^{-6} M, (c) THF, 3×10^{-6} M, (d) MeOH, 4×10^{-6} M and (e) water, 2×10^{-6} M.

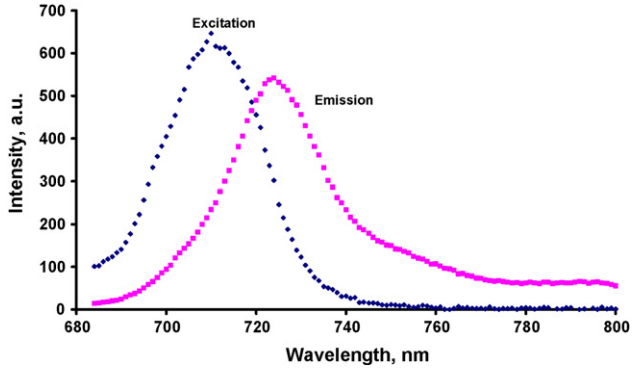


Fig. 5. Fluorescence excitation and emission spectra of **4** in DMSO. Excitation wavelength = 672 nm.

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

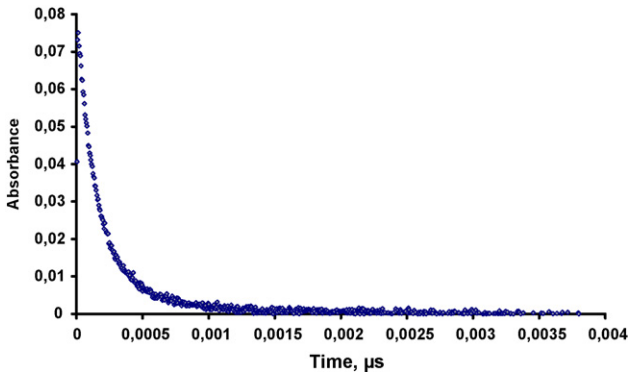


Fig. 6. Triplet decay curve of **3** in DMSO at 530 nm. Wavelength = 530 nm.

with minor geometric relaxation in the first excited state. The values of fluorescence quantum yields (Φ_F) of 0.20 and 0.13 (Table 3) are within the range for MPc complexes [24]. The lower value of Φ_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 Φ_{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 Φ_F value for **4**. Triplet decay trace for the complexes is exemplified by Fig. 6. The value of the triplet quantum yield (Φ_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 Φ_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 larger Φ_T for **4** resulted in increased Φ_Δ values as observed in Table 3.

The faction of triplet state quenched by singlet oxygen, S_Δ, was calculated for the complexes using the following equation:

$$S_\Delta = \frac{\Phi_\Delta}{\Phi_T} \quad (6)$$

The S_Δ values are close to unity (Table 3) implying efficient quenching of the triplet states by singlet oxygen.

The photodegradation quantum yield (Φ_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 3
Photophysical and photochemical parameters of tetra and octa substituted phthalocyanines in DMSO

Compound	λ_Q[a] (nm) (log ε)[b]	λ_F (nm)	τ_T (μs)	Φ_F	Φ_T	Φ_{IC}	Φ_d (10^4)	Φ_Δ	S_Δ
3	694 (5.31)	703	230	0.20	0.77	0.03	1.62	0.64	0.83
4	710 (5.32)	720	280	0.13	0.85	0.02	2.20	0.72	0.84

[a] Q band maxima shown for the low energy band only where bands are split.
[b] The log ε values are for the low energy band where there are more than two bands.

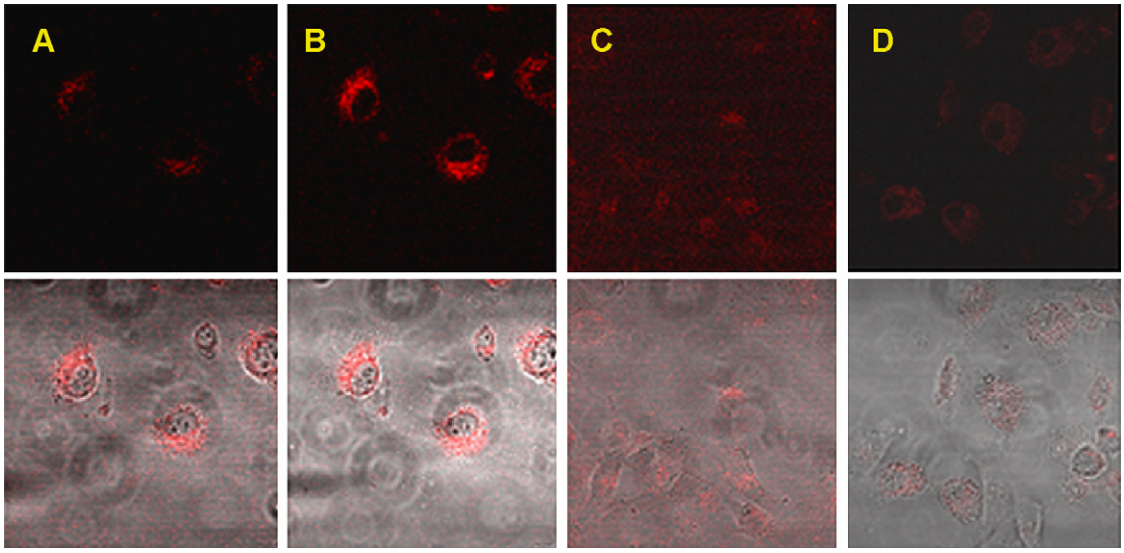


Fig. 7. Intracellular localization of the Pcs in RR1022 cells after incubation for 24 h. A: ZnPc (**3**) prescan, B: ZnPc (**3**) post scan, C: ZnPc (**4**) and D: control.

taining biological molecules such as cholesterol and estrone [33].

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 cytoskelatel 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 Φ_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-

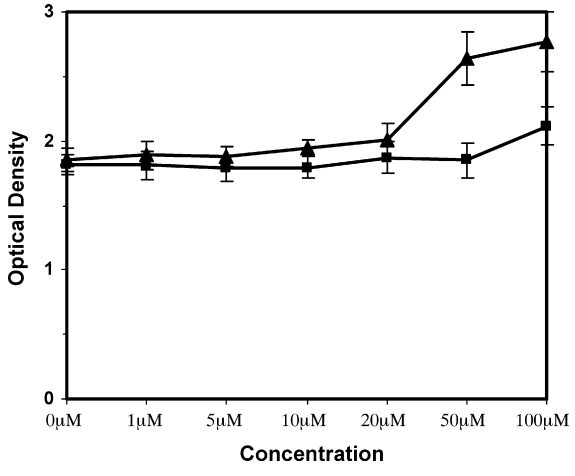


Fig. 8. Cytotoxicity of the compounds **3** (▲) and **4** (■) in the MCF-7 cells in dark using cell proliferation assay (WST-1) mean ± S.D. of three independent data sets.

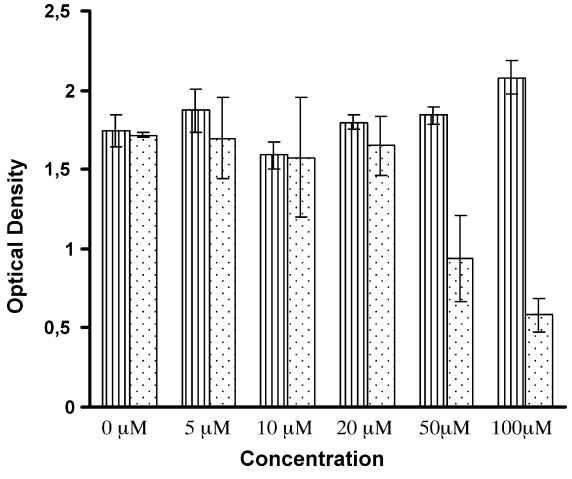


Fig. 9. Dark (lined) and light (dotted) Cytotoxicity of the ZnPc (**3**) at different concentration. MCF-7 cells treated with ZnPc (**3**) only without irradiation (lined) were compared with the cells which received irradiation (dotted). The control was MCF-7 cells without ZnPc (**3**) irradiated (lined)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

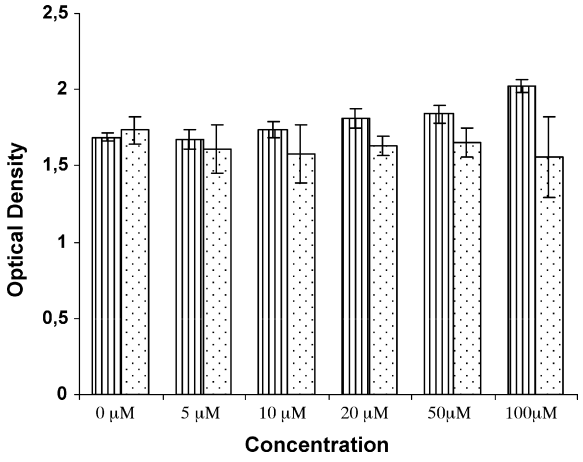


Fig. 10. Dark (lined) and light (dotted) cytotoxicity of the ZnPc (**4**) at different concentration. MCF-7 cells treated with ZnPc (**4**) only without irradiation (lined) were compared that received irradiation (dotted). The control was MCF-7 cells without ZnPc (**4**) irradiated (lined) or non-irradiated (dotted) at 0 μM concentration.

ical characteristic of the photosensitizers 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 (**3**) display a much lower triplet quantum yield (Φ_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 triethyleneoxysulfonyl 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 (Φ_T) and singlet oxygen (Φ_Δ) 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/cm^2), 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

[1] A.C. Tedesco, J.C.G. Rotta, C.N. Lunardi, Curr. Org. Chem. 7 (2003) 187–196.
[2] J. Moan, K. Berg, Photochem. Photobiol. 55 (1992) 931–948.
[3] T.J. Dougherty, J. Clin. Laser Med. Surg. 14 (1996) 219–222.
[4] E. Ben-Hur, I. Rosenthal, Photochem. Photobiol. 42 (1985) 129–133.
[5] S. Kudrevich, N. Brasseur, C. La Madeline, S. Gilbert, J.E. van Lier, J. Med. Chem. 40 (1997) 3897–3904.
[6] R. Bonnett, Chem. Soc. Rev. 24 (1995) 19–33.
[7] K. Tabata, K. Fukushima, K. Oda, I.J. Okura, J. Porphyrins Phthalocyanines. 4 (2000) 278–284.
[8] M.T.M. Choi, P.P.S. Li, D.K.P. Ng, Tetrahedron 56 (2000) 3881–3887.
[9] J.E. van Lier, in: D. Kessel (Ed.), Photodynamic Theraphy of Neoplastic, Diseases, vol. 1, CRC Press, Boca Raton, FL, 1990.
[10] M.I. Rosenthal, Photochem. Photobiol. 53 (1991) 859–870.
[11] M.I. Rosenthal, in: C.C. Leznoff, A.B.P. Lever (Eds.), Phthalocyanines: Properties and Applications, vol. 4, VCH, New York, 1996, pp. 481–514.
[12] M. Hu, N. Brasseur, S.Z. Yıldız, J.E. van Lier, C.C. Leznoff, J. Med. Chem. 41 (1998) 1789–1802.
[13] D. Wöhrle, M. Eskes, K. Shigehara, A. Yamada, Synthesis (1993) 194–196.
[14] A.G. Gürek, Ö. Bekaroğlu, J. Chem. Soc., Dalton Trans. (1994) 1419–1423.
[15] K. Takahashi, M. Kawashima, Y. Tomita, M. Itoh, Inorg. Chim. Acta 232 (1995) 69–73.
[16] K. Ozoemena, T. Nyokong, J. Chem. Soc., Dalton Trans. (2002) 1806–1811.
[17] G. Jori, C. Fabris, J. Photochem. Photobiol. B: Biol. 43 (1998) 181–185.
[18] U. Michelsen, H. Kliesch, G. Schnurpfeil, A.K. Sobbi, D. Wöhrle, Photochem. Photobiol. 64 (4) (1996) 694–701.
[19] S. Dabak, V. Ahsen, F. Heinemann, P. Zugenmaier, Mol. Cryst. Liq. Cryst. 348 (2000) 111–127.
[20] D.D. Perrin, W.L.F. Armarego, Purification of Laboratory Chemicals, 2nd ed., Pergamon, Oxford, 1989.
[21] S. Fery-Forgues, D. Lavabre, J. Chem. Ed. 76 (1999) 1260–1264.
[22] D. Maree, T. Nyokong, K. Suhling, D. Phillips, J. Porphyrins Phthalocyanines. 6 (2002) 373–376.
[23] P. Jacques, A.M. Braun, Helv. Chim. Acta 64 (1981) 1800–1806.
[24] J.H. Brannon, D. Magde, J. Am. Chem. Soc. 102 (1980) 62–65.
[25] T.H. Tran-Thi, C. Desforge, C. Thies, J. Phys. Chem. 93 (1989) 1226–1233.
[26] I. Seotsanyana-Mokhosi, N. Kuznetsova, T. Nyokong, J. Photochem. Photobiol. A: Chem. 140 (2001) 215–222.
[27] A. Ogunsipe, T. Nyokong, J. Mol. Struct. 689 (2004) 89–97.
[28] N. Kuznetsova, N. Gretsova, E. Kalmkova, E. Makarova, S. Dashkevich, V. Negrimovskii, O. Kaliya, E. Luk'yanets, Russ. J. Gen. Chem. 70 (2000) 133–140.
[29] J. Simon, P. Bassoul, in: C.C. Leznoff, A.B.P. Lever (Eds.), Phthalocyanines: Properties and Applications, vol. 2, VCH, New York, 1993, pp. 223–299.
[30] D.D. Dominquez, A.W. Snow, J.S. Shirk, R.G.S. Pong, J. Porphyrins Phthalocyanines. 5 (2001) 582–592.
[31] H.S. Nalwa, J.S. Shirk, in: C.C. Leznoff, A.B.P. Lever (Eds.), Phthalocyanines: Properties and Applications, vol. 4, VCH, New York, 1996, pp. 79–182.
[32] A.W. Snow, N.L. Jarvis, J. Am. Chem. Soc. 106 (1984) 4706–4711.
[33] S. Maree, T. Nyokong, J. Porphyrins Phthalocyanines. 5 (2001) 782–792.

[34] K. Wiktorowicz, J. Cofta, A. Dudkowiak, A. Waszkowiak, D. Frackowiak, Acta Biochim. Polon. 3 (51) (2004) 703–710.
[35] S.R. Wood, J.A. Holyrod, S.B. Brown, Photochem. Photobiol. 65 (1997) 397–402.
[36] N. Cauchon, H. Tian, R. Langlois, R. La Madeliene, S. Martin, H. Ali, D. Hunting, J.E. van Lier, Bioconjugate Chem. 16 (2005) 80–89.
[37] I. Seotsanyana-Mokhosi, T. Kresfelder, H. Abrahamse, T. Nyokong, J. Photochem. Photobiol. B: Biol. 83 (2006) 55–62.
[38] A.P. Castano, T.N. Demidova, M.R. Hamblin, Photodiag. Photodynam. Ther. 2 (2005) 1–23.
[39] I. Rosenthal, E. Ben-Hur, S. Greenberg, A. Concepcion-Lam, D.M. Drew, C.C. Leznoff, Photochem. Photobiol. 46 (1987) 959–963.
[40] C.C. Leznoff, S. Vigh, P.I. Svirskaya, S. Greenberg, D.M. Drew, E. Ben-Hur, I. Rosenthal, Photochem. Photobiol. 49 (1989) 279–284.
[41] C.N. Lunardi, J.C.G. Rotta, A.C. Tedesco, J. Porphyrins Phthalocyanines. 7 (2003) 493–499.