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# SUPPLEMENTARY INFORMATION

# Synthesis and photodynamic activities of novel silicon(IV) phthalocyanines axially substituted with water soluble groups against HeLa cancer cell line

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## Materials

3-Dimethylaminophenol, 3-diethylaminophenol, 5-chloro-1-propanol were purchased from Aldrich. Silicon phthalocyanine dichloride (**1**) [1] was prepared and purified according to the method giving in the literature. All reagents and solvents were of reagent grade quality and were obtained from commercial suppliers. All solvents were dried and purified as described by Perrin and Armarego [2]. 1,3-diphenylisobenzofuran (DPBF) and 9,10-antracenediyl-bis(methylene) dimalonic acid (ADMA) were obtained from Fluka. Zinc phthalocyanine used as standard for photophysical and photochemical measurements was purchased from Aldrich.

## Equipment

The FT-IR spectra were recorded on a Perkin Elmer 1600 FT-IR Spectrophotometer using KBr pellets. $^{1}H$ and $^{13}C$-NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometers in $CDCl_3$ and chemical shifts were reported ($\delta$) relative to $Me_4Si$ as internal standard. Mass spectra were measured on a Micromass Quatro LC/ULTIMA LC-MS/MS spectrometer. MALDI-MS of complexes were obtained in dihydroxybenzoic acid (DHB) as MALDI matrix using nitrogen laser accumulating 50 laser shots using Bruker Microflex LT MALDI-TOF mass spectrometer. Electronic absorption spectra were detected on a Shimadzu 2101 UV-Vis spectrophotometer. Fluorescence spectra were collected on an Varian Eclipse spectrofluorometer. Fluorescence lifetimes were measured using a time correlated single photon counting setup (TCSPC) (Horiba Fluorolog 3 equipment.) Fluorescence lifetimes were measured using a time correlated single photon counting setup (TCSPC) (Horiba Fluorolog 3 equipment.).

Photo-irradiations were done using a General Electric quartz line lamp (300W). 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 40 nm) was additionally placed in the light path before the sample. Light intensities were measured with a Power Max5100 (Molelectron detector incorporated) power meter.

***Photophysical parameters***

*Fluorescence quantum yields and lifetimes*

Fluorescence quantum yields ($\Phi_F$) are determined in DMSO by the comparative method using by equation 1 [3,4],

$$\Phi_F = \Phi_F(Std)\frac{F \,.\, A_{Std} \,.\, n^2}{F_{Std} \,.\, A \,.\, n^2_{Std}} \qquad (1)$$

where F and $F_{Std}$ are the areas under the fluorescence emission curves of the samples (**3,4-3a,4a**) and the standard, respectively. A and $A_{Std}$ are the respective absorbances of the samples and standard at the excitation wavelengths, respectively. $n^2$ and $n^2_{Std}$ are the refractive indices of solvents used for the sample and standard, respectively. Unsubstituted ZnPc ($\Phi_F$ = 0.20) [5] was employed as the standard in DMSO. The absorbance of the solutions at the excitation wavelength ranged between 0.04 and 0.05.

The fluorescence lifetime ($\tau_F$) values were directly determined by time correlated single photon counting **(**TCSPC) method. Natural radiative lifetimes ($\tau_0$) were evaluated using Equation 2.

$$\Phi_F = \frac{\tau_F}{\tau_0} \qquad (2)$$

***Photochemical parameters***

*Singlet oxygen quantum yields*

Singlet oxygen quantum yield ($\Phi_\Delta$) determinations are carried out using the experimental set-up described in literature [6] in both DMSO or aqueous solutions. Typically, a 3 mL portion of the respective unsubstituted or tetra-substituted zinc (II) phthalocyanine solutions (absorbance ~ 1.0 at the irradiation wavelength) containing the singlet oxygen quencher was irradiated in the Q band region with the photo-irradiation set-up described in references [7]. $\Phi_\Delta$ values were determined in air using the relative method with ZnPc (in DMSO) or $ZnPcS_{mix}$ (in aqueous media) as standards. DPBF and ADMA were used as chemical quenchers for singlet oxygen in DMSO and aqueous media, respectively. Equation 3 was employed for the determination of $\Phi_\Delta$ values:

$$\Phi_\Delta = \Phi_\Delta^{Std} \frac{R \cdot I_{abs}^{Std}}{R^{Std} \cdot I_{abs}} \qquad (3)$$

where $\Phi_\Delta^{Std}$ is the singlet oxygen quantum yields for the standards ZnPc ($\Phi_\Delta^{Std}$ = 0.67 in DMSO) [8] and $ZnPcS_{mix}$ ($\Phi_\Delta^{Std}$ = 0.45 in aqueous media) [7], R and $R_{Std}$ are the DPBF (or ADMA) photobleaching rates in the presence of the respective samples (**3,4-3a,4a**) and standards, respectively. $I_{abs}$ and $I_{abs}^{Std}$ are the rates of light absorption by the samples (**3,4-3a,4a**) and standards, respectively. To avoid chain reactions induced by quenchers (DPBF or ADMA) in the presence of singlet oxygen, the concentration of quenchers (DPBF or ADMA) was lowered to ~$3x10^{-5}$ M [9]. Solutions of sensitizer (absorbance = 1 at the irradiation wavelength) containing quencher (DPBF or ADMA) were prepared in the dark and irradiated

in the Q band region. DPBF degradation at 417 nm and ADMA degradation at 380 nm were monitored. The light intensity $6.21 \times 10^{15}$ photons $s^{-1}$ $cm^{-2}$ was used for $\Phi_\Delta$ determinations.

*Photodegradation quantum yields*

Photodegradation quantum yield ($\Phi_d$) determinations are carried out using the experimental set-up described in literature [7]. Photodegradation quantum yields were determined using equation 4 in both DMSO and water.

$$\Phi_d = \frac{(C_0 - C_t) \cdot V \cdot N_A}{I_{abs} \cdot S \cdot t} \qquad (4)$$

where $C_0$ and $C_t$ are the samples 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 samples. A light intensity of $2.17 \times 10^{16}$ photons $s^{-1}$ $cm^{-2}$ was employed for $\Phi_d$ determinations.

***In vitro PDT application***

*Cell culture*

Human HeLa cervical carcinoma cells were maintained in Dulbecco's modified Eagle medium (DMEM) (PANBiotech). The medium was supplemented with 10% FBS and antibiotics (penicillin 50 U/mL; streptomycin 50 lg/ml). Both cell lines were incubated at 37°C in a humidified incubator with 5% $CO_2$. Stock solutions of the studied SiPcs (all at 1 □M) were prepared in DMSO and stored at 4°C in the dark. The solution was further diluted with the cellular culture medium to appropriate concentrations.

*Measurement of photocytotoxicity*

Tumor cells in the exponential phase of growth were harvested and suspended in DMEM medium at a density of 4000 cells/well. Cells were cultured in 96 well plates and incubated for 24 h for cell adherence and growth. After 24 h, photosensitizers were added to each well with different concentrations (0, 0.25, 0.5, 1, 2, 3, 4, 5, and 10 □M). These plates were incubated for 24 h in the dark at 37°C in a humidified $CO_2$ incubator. Each well medium were refreshed with fresh DMEM medium before irradiation. For irradiation, cells were exposed to the light from the Lumacare (Model LC-122 fiber-optic probes) FOP systems with activation wavelength of 680 ± 10 nm. FOP tip and the cell plate surface was 20 cm. The light power of FOP systems on the exposure area was measured with a power meter that has silicon detector (Ophir).

The total light dose was approximately 2 J/cm$^2$. The exposure energy is controlled from the control panel by a timer. Dark control plates were left in dark. After illumination, the cells were incubated for 24 h at 37°C in DMEM medium under 5% $CO_2$ and cell viability was measured by colorimetric tetrazolium compound reagent (MTS; Promega; (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)- 2-(4-sulfophenyl)-2H-tetrazolium).40 The MTS solution (20 μL) was added to each well followed by incubation for 4 h under the same environment. MTS tetrazolium compound is metabolized by metabolically active cells into a colored formazan product that can be measured by reading the absorbance at 490 nm with a microplate reader. The average of the triplicate wells for each sample was calculated.

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