Journal of Photochemistry & Photobiology, B: Biology 159 (2016) 196–204

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Silicon(IV) phthalocyanines substituted axially with different nucleoside moieties. Effects of nucleoside type on the photosensitizing efficiencies and *in vitro* photodynamic activities

Bi-Yuan Zheng, Xiao-Min Shen, Dong-Mei Zhao, Yi-Bin Cai, Mei-Rong Ke, Jian-Dong Huang

College of Chemistry, State Key Laboratory of Photocatalysis on Energy and Environment, Fuzhou University, Fuzhou 350108, China

ARTICLE INFO

Article history:
Received 10 August 2015
Received in revised form 28 March 2016
Accepted 29 March 2016
Available online 8 April 2016

Keywords:
Silicon(IV) phthalocyanine
Photosensitizer
Photodynamic therapy
Nucleoside
Singlet oxygen quantum yields

ABSTRACT

A series of new silicon(IV) phthalocyanines (SiPcs) di-substituted axially with different nucleoside moieties have been synthesized and evaluated for their singlet oxygen quantum yields (Φ_Δ) and *in vitro* photodynamic activities. The adenosine-substituted SiPc shows a lower photosensitizing efficiency ($\Phi_\Delta = 0.35$) than the uridine- and cytidine-substituted analogs ($\Phi_\Delta = 0.42$–0.44), while the guanosine-substituted SiPc exhibits a weakest singlet oxygen generation efficiency with a Φ_Δ value down to 0.03. On the other hand, replacing axial adenosines with chloro-modified adenosines and purines can result in the increase of photogenerating singlet oxygen efficiencies of SiPcs. The formed SiPcs **1** and **2**, which contain monochloro-modified adenosines and dichloro-modified purines respectively, appear as efficient photosensitizers with Φ_Δ of 0.42–0.44. Both compounds **1** and **2** present high photocytotoxicities against HepG2 and BGC823 cancer cells with IC_{50} values ranging from 9 nM to 33 nM. The photocytotoxicities of these two compounds are remarkably higher than the well-known anticancer photosensitizer, chlorin e6 ($IC_{50} = 752$ nM against HepG2 cells) in the same condition. As revealed by confocal microscopy, for both cell lines, compound **1** can essentially bind to mitochondria, while compound **2** is just partially localized in mitochondria. In addition, the two compounds induce cell death of HepG2 cells likely through apoptosis.

1. Introduction

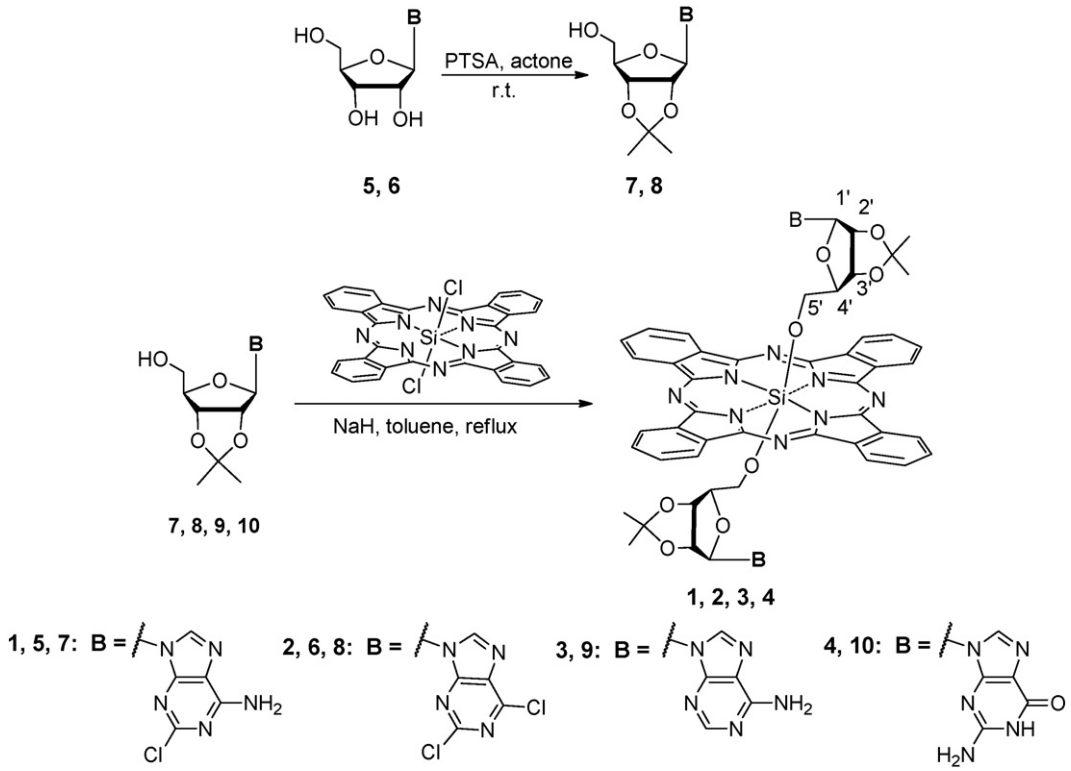
Photodynamic therapy (PDT) has attracted considerable attention as a promising minimal-invasive treatment for both neoplastic and non-neoplastic diseases [1–4]. One of the prominent advantages of PDT lies in its ability to eradicate malignant tissue without systemic toxicity, which is a major drawback of traditional therapeutic methods like chemotherapy and radiotherapy. PDT typically involves the systemic administration of a photosensitizer and the tumor-oriented illumination with a light of specific wavelength to generate reactive oxygen species (ROS), particularly singlet oxygen, leading to cell and tissue damage. The overall efficacy of the treatment depends greatly on the behavior of photosensitizers. As a result, various strategies have been developed to obtain a favorable photosensitizer [4–11]. Among these approaches, the conjugation of a photosensitizer with biological ligands (such as antibodies, peptides, carbohydrates, and folic acid), which are capable of improving the biocompatibility and tumor-targeting of the photosensitizers, has received a great deal of interest [6–13].

Having a number of advantageous characteristics, such as intense absorption in the "phototherapeutic window" (650–800 nm), low dark toxicity, and ease of chemical modification, phthalocyanines have been found to be highly promising as second-generation photosensitizers for PDT [2–4,12–16]. The introduction of biomolecules on the axial positions of phthalocyanines has appeared as an efficient and promising approach to obtain the desirable phthalocyanine-based photosensitizers [17–22]. Apart from aforementioned improvement of biocompatibility and tumor-targeting, the method could effectively decrease intrinsic aggregated propensity of phthalocyanines, which will lead to inefficient ROS generation and poor solubility in aqueous media. Among these biomolecules, we are particularly interested in nucleosides, which are important building blocks of DNA and RNA. Moreover, a number of nucleoside derivatives have been used as antiviral [23,24] and anticancer [25] drugs. However, the photobiological properties of phthalocyanines functionalized with nucleoside moieties remain hardly explored, although the synthesis and characterization of a few phthalocyanine-nucleoside conjugates have been reported [18,26–31].

Very recently, our group have reported the photodynamic activities of a series of silicon(IV) phthalocyanines (SiPcs) axially disubstituted with uridine and cytidine moieties in a preliminary communication [18]. As an extension of this study, we report herein four new analogs axially disubstituted with adenosine, purine, and guanosine derivatives, including their synthesis, spectroscopic properties, singlet oxygen generation efficiencies, and *in vitro* photodynamic activities against

E-mail address: jdhuang@fzu.edu.cn (J.-D. Huang).

http://dx.doi.org/10.1016/j.jphotobiol.2016.03.055
1011-1344/



Scheme 1. Preparation of SiPcs **1**–**4**.

HepG2 and BGC823 cells. With the goal of understanding the structure–activity relationship of this intriguing class of novel nucleoside-containing photosensitizer, the effects of nucleoside type on the photochemical properties and *in vitro* photodynamic activities have been discussed.

2. Experimental Section

2.1. General

All the reactions were performed under an atmosphere of nitrogen. Acetone and toluene were distilled from anhydrous calcium sulfate and sodium, respectively. 2′,3′-o-isopropyl-adenosin (**9**) and 2′,3′-o-isopropyl-guanosine (**10**) were purchased from Acros. Cremophor EL and ZnPc were obtained from Sigma Aldrich. Ce6 was obtained from J&K Scientific. Chromatographic purifications were performed on silica gel columns (100–200 mesh, Qingdao Haiyang Chemical Co., Ltd., China) with the indicated eluents. Size exclusion chromatography was performed on Bio-Rad Bio-Beads S-X3 beads with the indicated eluents. All other solvents and reagents were of reagent grade and used as received.

Table 1
Photophysical and photochemical data for SiPcs **1**–**4** and **11**–**12** in DMF.

SiPcs	λ_{max} (nm)	λ_{em} (nm)a	Stokes shift (nm)	$\varepsilon \times 10^5$ ($M^{-1}\cdot cm^{-1}$)	Φ_Fb	Φ_Δc
1	677	685	8	1.77	0.34	0.42
2	679	687	8	1.92	0.25	0.44
3	677	686	9	1.80	0.30	0.35
4	676	682	6	1.80	0.02	0.03
11 [18]	678	687	9	2.00	0.37	0.44
12 [18]	677	685	8	2.13	0.36	0.42

[a] Excited at 610 nm.
[b] Using ZnPc in DMF as the reference ($\Phi_F = 0.28$) [33].
[c] Determined by using DPBF as chemical quencher, and ZnPc in DMF as the reference ($\Phi_\Delta = 0.56$) [34].

1H NMR spectra were recorded on Bruker a AVANCE III 400 or 500 spectrometer in DMSO-d6 and $CDCl_3$. Chemical shifts were relative to internal $SiMe_4$ ($\delta = 0$ ppm). High resolution mass spectra were recorded on an Agilent ESI-Q-Tof 6520 mass spectrometer or a Thermo Fisher Scientific Exactive Plus Orbitrap LC–MS spectrometer. Electronic absorption spectra were measured on a Shimadzu UV-2450 UV–vis spectrophotometer. Fluorescence spectra were taken on an Edinburgh FL900/FS900 spectrofluorometer. Φ_F and Φ_Δ were determined as described in our previous manuscripts [18,32].

The purity of all the new phthalocyanines was determined by HPLC and was found to be ≥95%. The analytical HPLC experiments were performed on a Kromasir KR100-10C18 column (5 μm, 4.6 mm × 250 mm) by using a Shimadzu LC-10AT controller with a SPD-M10A diode array detector. The conditions were set as follows: mobile phase from 5% B changed to 95% B over 30 min, then maintained 95% B for a further 20 min (solvent A = distilled water; solvent B = DMF). The column temperature was set at 30 °C. The flow-rate was fixed at 0.5 ml min^{-1}.

Statistical analyses were performed by using the Student's *t*-test. *P* values of <0.05 were considered as statistically significant.

2.2. Synthesis

2′,3′-o-isopropyl-2-chloro-adenosin (7). A mixture of *p*-toluenesulfonic acid (PTSA) (1.72 g, 10 mmol) and dry acetone (20 ml) was added dropwise into a stirring mixture of 2-chloro-adenosin (309 mg, 1 mmol) and dry acetone (20 ml) in ice-water bath. The mixture was stirred at room temperature for 22 h and then was poured into ice sodium bicarbonate solution (4% w.t.) with stirring until the bubble disappeared. The liquid was then extracted with CH_2Cl_2 for three times. The organic layers were dried over anhydrous $MgSO_4$ and concentrated to afford a faint yellow solid **7** (283 mg, 83%). 1H NMR (400 MHz, DMSO-d6): $\delta = 8.37$ (s, 1H, imidazole-H), 7.89 (br., 2H, NH_2), 6.06 (d, $J = 2.4$ Hz, 1H, 1′-H), 5.28–5.30 (m, 1H, 2′-H), 5.10–5.12 (m, 1H, OH), 4.93–4.95 (m, 1H, 3′-H), 4.21–4.23 (m, 1H, 4′-H),