Tetrahedron 73 (2017) 378–384

Contents lists available at ScienceDirect

Tetrahedron

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

Comparison between amine-terminated phthalocyanines and their chlorambucil conjugates: Synthesis, spectroscopic properties, and *in vitro* anticancer activity

Xiao-Hui Peng, Shao-Fang Chen, Bi-Yuan Zheng, Bing-De Zheng, Qiao-Feng Zheng, Xing-Shu Li, 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 6 October 2016
Received in revised form
29 November 2016
Accepted 9 December 2016
Available online 11 December 2016

Keywords:
Photodynamic therapy
Phthalocyanine
Chlorambucil
Structure-activity relationship
Anticancer

ABSTRACT

Two amine-terminated phthalocyanines, including mono-α-substituted zinc phthalocyanine (ZnPc) **4** and axially di-substituted silicon phthalocyanine (SiPc) **6**, as well as their chlorambucil (CLB) conjugates (**5** and **7**) have been prepared. Both **4** and **6** show very high photocytotoxicities against HepG2 cells with IC_{50} values down to 31 nM and 9 nM, respectively. However, after conjugating with CLB, the anticancer activities of both conjugates, ZnPc-CLB **5** (IC_{50} = 0.20 μM) and SiPc-CLB **7** (IC_{50} = 17.47 μM), are greatly reduced as a result of their lower efficiency in fluorescence emission and singlet oxygen generation in aqueous solution. Moreover, both conjugates show significantly lower cellular uptake than their precursors, **4** and **6**. Synergetic chemo-photodynamic therapy could not be observed on the two conjugates.

1. Introduction

Photodynamic therapy (PDT) has received considerable attention as a novel promising method for the treatment of cancers.[1] Differing from conventional cancer therapies, PDT destroys the target tissues and cells through the combined action of photosensitizers and molecular oxygen which can be translated into reactive oxygen species (ROS) upon irradiation with light.[2] Owing to the desirable features, such as noninvasive nature, tolerance of repeated doses, reduction of side effects, and remission of drug-resistance problem, PDT has been extensively applied in various cancers including cervical cancer, ovarian cancer, gastric cancer, and lung cancer.[3] On the other hand, the desirable therapeutic outcome for cancers usually can't be achieved by an individual therapeutic modality. Recent studies have demonstrated that combination therapies including chemo-chemo, chemo-thermal, and chemo-photodynamic therapies can enhance the therapeutic efficacy.[4] Among these combination therapies, chemo-photodynamic therapy has been especially considered to be a promising strategy for cancer treatments, in which, chemotherapy drugs can act in concert with photosensitizers for tumor killing, and realize synergetic therapeutic efficiency.[4d–4g] To achieve a dual chemo-photodynamic therapy, the design and development of appropriate conjugates of photosensitizer and chemotherapeutic drug play a crucial role.

Recently, a few chemo-photodynamic conjugates have been reported, but they are mainly limited to the combination of porphyrin or pheophorbide-α with doxorubicin,[5a] paclitaxel,[5b] or cisplation.[6] To the best of our knowledge, using phthalocyanines as photosensitizer to construct chemo-photodynamic conjugates has rarely been studied.[7] In addition, the structure-activity relationship of chemo-photodynamic conjugates remains indistinct.

As the typical representative of the second-generation photosensitizers, phthalocyanines are favorable candidates for PDT on account of their several advantages including strong absorption in the red visible region (650–850 nm), low dark toxicity, excellent efficiency to generate single oxygen, and ease to be modified.[8,9] Among them, zinc(II) phthalocyanines (ZnPcs) and silicon(IV) phthalocyanines (SiPcs) are regarded as two primary types of phthalocyanines in cancer treatment. In general, a way of realizing functional modification of ZnPcs is to introduce substituents to the periphery of the macrocycle. The α-mono-substituted ZnPc is the

* Corresponding author.
E-mail addresses: jdhuang@fzu.edu.cn, 13805009843@163.com (J.-D. Huang).

http://dx.doi.org/10.1016/j.tet.2016.12.017
0040-4020/

most promising among ZnPcs modified with different number of the same substituent.[10] Differently, the derivative of SiPcs is to symmetrically introduce axial substitutions on the central cation.[11] However, it is still lack of direct comparison between α-mono-ZnPc and axial-di-SiPc that with the same substituent so far.

Recently, we have designed and synthesized a SiPc axially substituted with amine-terminated groups.[12] Herein, the same substituted group is introduced to periphery of zinc(II) phthalocyanine to obtain a mono-substituted ZnPc for comparing with the SiPc in photodynamic activities. Based on these compounds, two novel phthalocyanine-chlorambucil (CLB) conjugates were constructed by linking the terminal amine with CLB. As we know, CLB is a broad-spectrum chemotherapeutic drug, but its severe toxic and side effects, especially cardiotoxicity, restrict its further development.[13] A porphyrin-CLB conjugate has been reported,[14] but its anti-cancer effect is not satisfactory. Therefore, whether phthalocyanine-CLB conjugates possess more advantages in antitumor activity needs to be identified urgently. In this study, the photophysical and photochemical properties, cellular uptake, subcellular localization as well as *in vitro* anticancer activities of these compounds were investigated to study the structure-activity relationship.

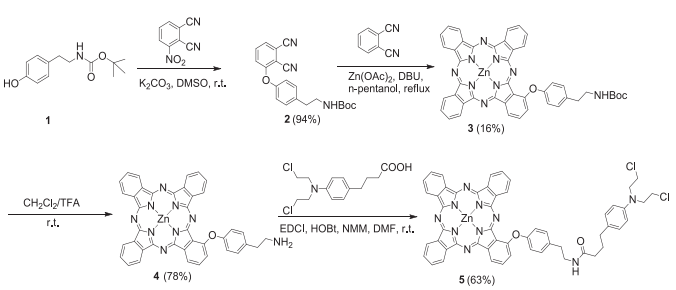
2. Result and discussion

Scheme 1 shows the synthetic route of the ZnPc-CLB conjugate **5**. Firstly, the amine group-protected tyramine (compound **1**) underwent nucleophilic substitution with 3-nitrophthalonitrile under alkaline condition to give the 3-substituted phthalonitrile **2**. A mixed cyclization of **2** and unsubstituted phthalonitrile in the presence of $Zn(OAc)_2$ and 1, 8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *n*-pentanol resulted in the formation of the monosubstituted ZnPc **3**. The ratio of phthalonitrile and substituted phthalonitrile was changed from theoretical 3:1 to 8:1 in the step of cyclization to reduce the generation of other multi-substituted ZnPcs. The relative low yield of **3** is probably due to the low efficiency of cyclization of ZnPc. **3** was further treated with trifluoroacetate (TFA) in CH_2Cl_2 to afford amino-mono-substituted ZnPc **4**. Finally, the amine group of **4** was coupled with the carboxyl group of CLB using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as activating agents, leading to the ZnPc-CLB conjugate **5**. Except the mixed cyclization, all the compounds were prepared in good yields (63%–94%). Similarly with **5**, the SiPc-CLB conjugate **7** was prepared by an amidation reaction of SiPc **6**[12] and CLB under EDCI, HOBt, and 4-methylmorpholine (NMM) condition in 70% yield (Scheme 2). The targeted phthalocyanines were highly soluble in common organic solvents and could be purified readily by column chromatography and size exclusion chromatography. All the new compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS.

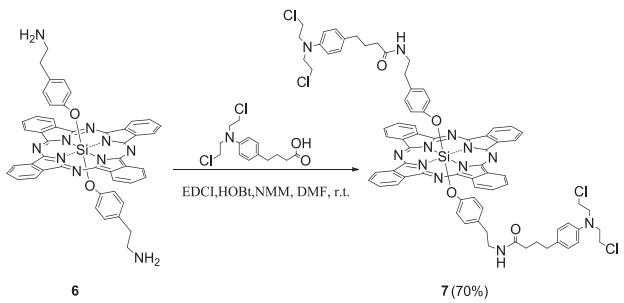
The electronic absorption spectra of compounds **4**, **5**, **6**, and **7** were measured in N, *N*-dimethylformamide (DMF) and the data is reported in Table 1. As shown in Fig. 1a, the UV–vis spectra of these phthalocyanines in DMF are similar and typical as the spectra of monomeric phthalocyanines with a broad B (or Soret)-band at 334–355 nm as well as an intense and sharp Q-band at 674–680 nm. The UV–vis spectra of these compounds at different concentrations in DMF have also been displayed in Fig. S1, and all of them strictly follows the Lambert-Beer law. Upon excitation at 610 nm, these compounds showed a fluorescence emission at 683–687 nm (Fig. 1b) with the Stokes shift of 7–11 nm, and their fluorescence quantum yields (Φ_F) relative to unsubstituted ZnPc ($\Phi_F = 0.28$)[15] are recorded in Table 1. To explore the photosensitizing efficiency of these phthalocyanines, the singlet oxygen quantum yields (Φ_Δ) in DMF were also determined by a steady-state method using 1, 3-diphenylisobenzofuran (DPBF) as the scavenger. The values of Φ_Δ relative to unsubstituted ZnPc ($\Phi_\Delta = 0.56$)[16] could be determined by spectroscopically monitoring the concentration of DPBF at 413 nm with time of irradiation (Fig. S2).

From Table 1, we can find that ZnPc **4** exhibits the same absorption position of the Q band (674 nm) and fluorescence emission (683 nm) with its corresponding conjugate **5** in DMF. This phenomenon also appears in SiPc **6** and its chlorambucil conjugate **7** with the Q band at 680 nm and fluorescence emission at 687 nm. Moreover, the Q band is red-shifted 6 nm for SiPcs (**6** and **7**) compared with ZnPcs (**4** and **5**). The Φ_F and Φ_Δ values of conjugate **5** (or **7**) are almost the same with that of ZnPc **4** (or SiPc **6**) in DMF, suggesting the CLB unit almost has no influence on Pc unit in the conjugates. Meanwhile, both values of Φ_F and Φ_Δ for SiPcs **6** ($\Phi_F = 0.04$, $\Phi_\Delta = 0.06$) and **7** ($\Phi_F = 0.04$, $\Phi_\Delta = 0.05$) were much lower than that of ZnPcs **4** ($\Phi_F = 0.20$, $\Phi_\Delta = 0.69$) and **5** ($\Phi_F = 0.22$, $\Phi_\Delta = 0.63$) under the same condition. These results may be attributed to the more obvious photoinduced electron transfer (PET) effect of the amino groups on SiPcs.[17]

The spectroscopic properties and singlet oxygen generation efficiency of **4**–**7** were also measured in aqueous solution to evaluate their photosensitzation abilities in a biological environment. Upon excitation at 610 nm, the fluorescence emission of SiPc **6** in phosphate-buffered saline (PBS) with 0.1% Cremophor EL is strong, and the intensity is even higher than ZnPc **4**. This observation may be explained by their excellent solubility in this aqueous solution. As shown in Fig. 2a, Pcs **4** and **6** remain nearly non-aggregated in water with sharp and strong Q-bands owing to the amino protonation. Moreover, the amino protonation of SiPc **6** is possibly stronger than the peripheral α-mono-ZnPc **4**. By contrast, conjugates **5** and **7** exhibit a much weaker fluorescence emission when compared to that in DMF (Fig. 2b), and the decreased magnitude for **5** is more significant than **7** owing to the different aggregation behavior. Conjugate **5** shows two broad and weak absorption bands peaking at 682 nm and 631 nm, indicating the co-existence of the



Scheme 1. Synthesis of ZnPc-CLB conjugate **5**.



Scheme 2. Synthesis of SiPc-CLB conjugate **7**.