Bioorganic & Medicinal Chemistry Letters 28 (2018) 2784–2788

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of 17^3-dicarboxylethyl-pyropheophorbide-a amide derivatives for photodynamic therapy

Wei Zhu [a], Lai-Xing Wang [b], Dan-Ye Chen [a], Ying-Hua Gao [a], Yi-Jia Yan [c], Xiao-Feng Wu [c], Mi Wang [a], Yi-Ping Han [b,*], Zhi-Long Chen [a,*]

[a] Department of Pharmaceutical Science & Technology, College of Chemistry and Biology, Donghua University, Shanghai 201620, China
[b] Shanghai Changhai Hospital, 200433, China
[c] Shanghai Xianhui Pharmaceutical Co. Ltd., Shanghai 200433, China

ARTICLE INFO

Article history:
Received 21 October 2017
Revised 12 December 2017
Accepted 14 December 2017
Available online 19 December 2017

Keywords:
Photosensitizer
Photodynamic therapy
Chlorophyll
Chlorin
Tumor

ABSTRACT

Three novel 17^3-dicarboxylethyl-pyropheophorbide-a amide derivatives as photosensitizers for photodynamic therapy (PDT) were synthesized from pyropheophorbide-a (Ppa). Their photophysical and photochemical properties, intracellular localization, photocytotoxicity *in vitro* and *in vivo* were investigated. All target compounds exhibited low cytotoxicity in the dark and remarkable photocytotoxicity against human esophageal cancer cells. Among them, **1a** showed highest singlet oxygen quantum yield. Upon light activation, **1a** exhibited significant photocytotoxicity. After PDT treatment, the growth of Eca-109 tumor in nude mice was significantly inhibited. Therefore, **1a** is a powerful and promising antitumor photosensitizer for PDT.

Photodynamic therapy (PDT) is a clinically approved, minimally invasive protocol for treatment that can be curative of early disease and palliative in advanced disease.[1–3] Compared with traditional cancer therapies such as surgery and chemotherapy, PDT damages the tumor cells selectively without destructing to surrounding normal tissues.[4,5] Due to the known limited diffusion of singlet oxygen through tissues, the PDT effects are largely localized to the photosensitizer-containing cells, thus reducing potential damage to normal cells in the vicinity of the tumor. An ideal photosensitizer should have absorption at long wavelengths.[6,7] In addition, an ideal photosensitizer should also have minimal toxicity in the dark, a high quantum yield of triplet state formation in the presence of light, high selectivity for tumor cells over normal cells, rapid clearance from normal tissues.[8,9]

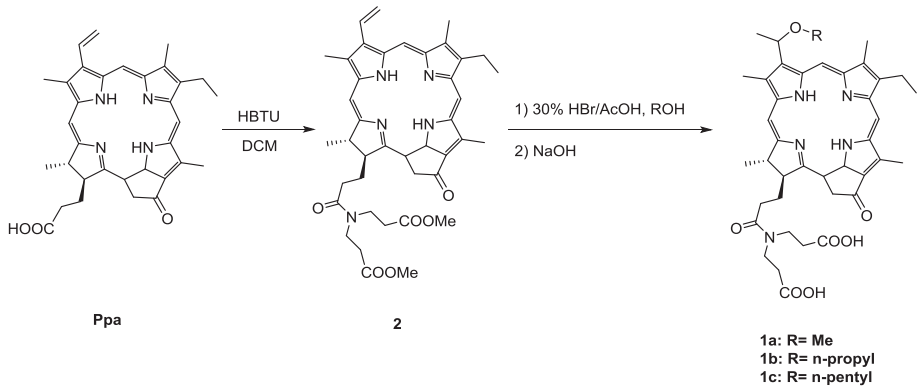
Porphyrin-based PS hematoporphyrin derivatives (HPD, Photofrin) were discovered by Lipson and Baldes in 1960s and is one of first generation PS to receive regulatory approval for the treatment of various tumors in more than 40 countries throughout the world.[10,11] But it also suffered from several drawbacks such as chemical heterogeneity, poor tissue penetration due to its limited maximum absorption wavelength, weak absorption at therapeutic wavelength (630 nm), and prolonged cutaneous photosensitivity caused by its slow elimination in normal tissue.[12] As mentioned by Wagner, chlorin-based photosensitizers have been found to have applications as phototoxic drugs for PDT.[13] Compared to porphyrins, chlorin-type PSs are receiving considerable attention owing to their intense absorption in near-infrared region (the PDT "therapeutic window" is 600–800 nm), which are relatively harmless and penetrate deeply in biological tissues.[14] In particular, chlorophyll derivatives are inherently amphiphilic macrocycles that have been extensively investigated. As compared to commercial photofrin, chlorophyll *a* derivatives generally showed low dark toxicities, rapid clearance from normal tissue, and low cutaneous phototoxicity.[15,16] However, poor water solubility is the main limitation of the chlorophyll *a* derivatives, which has hampered their PDT clinical application.[17]

In this work, the conjugates of 3,3′-azanediyldipropionate and pyropheophorbide-a were synthesized. Their photophysical properties, subcellular localization, photodynamic activities *in vitro* and *in vivo* are reported herein. Meanwhile, photodynamic antitumor assays highlighted **1a** as a promising PS for PDT treatment.

The synthetic route of the target compounds were shown in Scheme 1. 3,3′-Azanediyldipropionate was synthesized as the literature.[18] Pyropheophorbide-a is a derivative from plant chlorophyll.[19] It was reacted with 3,3′-azanediyldipropionate in the

* Corresponding authors.
E-mail addresses: greengarden2006@qq.com (Y.-P. Han), zlchen1967@qq.com (Z.-L. Chen).

https://doi.org/10.1016/j.bmcl.2017.12.034
0960-894X/



Scheme 1. The synthesis of 17[3]-dicarboxyethyl-pyropheophorbide-a amide derivatives.

Table 1
ClogP and LogP Values for compouds.[a]

Code	Formula	ClogP	LogP
1a	$C_{40}H_{47}N_5O_7$	4.24	2.3
1b	$C_{42}H_{51}N_5O_7$	4.97	2.7
1c	$C_{44}H_{55}N_5O_7$	5.82	3.1
2	$C_{33}H_{34}N_4O_3$	5.83	3.3

[a] ClogP values were calculated using Chemdraw 14.0 and logP values were measured by "shake-flask" method.

presence of *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluorophosphate (HBTU) and triethylamine to give *N*,*N*-dimethoxycarbonylethyl-pyropheophorbide-a amide (**2**).[20] Compound **2** was reacted with 30% hydrobromic acid/acetic acid to afford bromides and then reacted with methanol, *n*-propanol and *n*-pentanol.[19] After hydrolysis with NaOH solution in THF, the novel compounds **1a–c** was obtained. In order to simplify the synthesis procedure and improve the reaction yield, when the reaction was taken from compound **2** to compounds **1a–c,** the intermediate esters were directly hydrolyzed without further purification. The combination of ^{1}H NMR, ^{13}C NMR and HR-MS was used to elucidate the identities of **1a–c** and **2**. All spectra are provided in Supplementary information (Figs. S1–S4).

We have previously speculated that the introduction of two carboxyl groups could improve the water solubility of photosensitizers. To investigate whether the hydrophily of derivatives **1a–c** were improved, it was therefore necessary to establish the relative hydrophily of compounds under study. Theoretical values of logP (ClogP) were calculated using Chemdraw 14.0 and the experimentally determined LogP values obtained are reported in Table 1.

Because of the four conjugated pyrrole rings, porphyrins possess high rigidness and better coplanarity, exhibiting strong UV–visible absorption and fluorescence at room temperature.[21] The spectral characteristic in the presence of **1a–c** in DMSO were investigated.[3] As shown in Fig. 1a, **1a–c** had the characteristic Soret and Q band absorptions at 416 nm (Soret), 510 nm, 540 nm, 612 nm and 669 nm (Q band) in DMSO solution, respectively. When excited at 416 nm, **1a–c** showed strong emission peaks approximately at 664 nm and 721 nm (Fig. 1b).

The singlet oxygen quantum yield (Φ_Δ) value was determined by quenching the fluorescence intensity of DPBF.[22] The disappearance of DPBF spectra were monitored at 410 nm using UV–vis spectrophotometer, the absorption intensity of DPBF (λ – 410 nm) was continuously decreased with the irradiation time increasing (Fig. 2). After the data were plotted as $\ln[DPBF_0]/[DPBF_t]$ *versus* irradiation time *t*, straight lines were obtained for the sensitizers, and the slope for each compound was obtained after fitting with a linear function. Table 1 lists the quantum yield of singlet oxygen (Φ_Δ) values, the Φ_Δ values of **1a** and **Ppa** are almost the same, better than one of **1b–c** (Table 2).

The effect of **1a–c** on the viability of cultured Eca-109 cells was evaluated by MTT assay. As shown in Fig. 3a, there was almost no dark cytotoxicity observed when exposed up to 1 μM. When the concentration was increased to 5 μM, the viability of Eca-109 cells declined from approximately 80% to 50%. After irradiation with laser light (0–32 J/cm^2) at concentration 1 μM, a significant cytotoxicity was detected (Fig. 3b). Without light irradiation, the dark

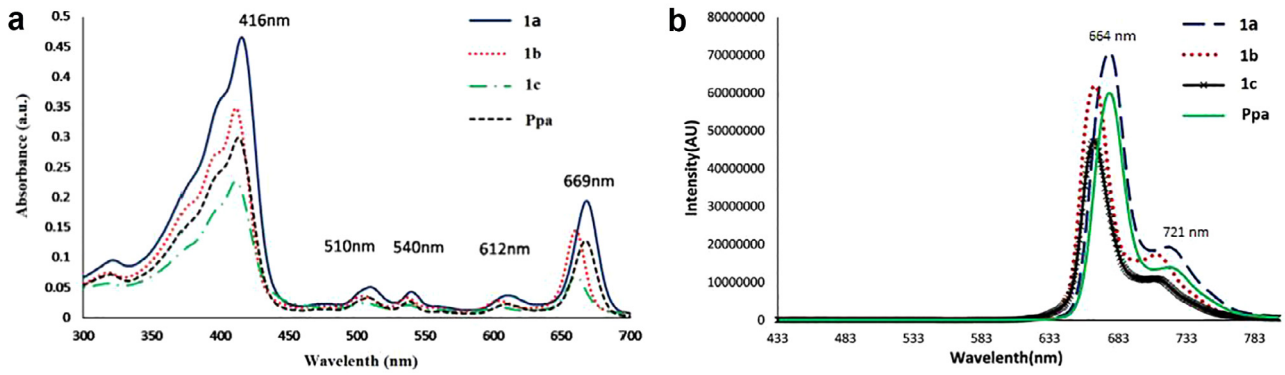


Fig. 1. UV–visible absorption and fluorescence spectra of **1a–c**. (a) Uv–vis absorption spectra of **1a–c** (10 μM in DMSO). (b) Fluorescence spectra of **1a–c**, excitation wavelength is 416 nm (10 μM in DMSO).