Chinese Chemical Letters xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Communication

In vitro and *in vivo* evaluation of improved EGFR targeting peptide-conjugated phthalocyanine photosensitizers for tumor photodynamic therapy

Qingle Chen[a,b,1], Yanhong Ma[b,1], Jisi Zhao[b], Mei Zhao[b], Wenjing Li[b], Qian Liu[b], Li Xiong[c,*], Wenjie Wu[a,*], Zhangyong Hong[b,*]

[a] College of Materials Science and Chemical Engineering, Tianjin University of Science and Technology, Tianjin 300457, China
[b] State Key Laboratory of Medicinal Chemical Biology, Tianjin Key Laboratory of Protein Sciences, College of Life Sciences, Nankai University, Tianjin 300071, China
[c] Department of General Surgery, Second Xiangya Hospital, Central South University, Changsha 410011, China

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Photodynamic therapy
Potosensitizer
Phthalocyanine
Peptide conjugate

ABSTRACT

Phthalocyanine (Pc) is one of the most promising photosensitizer candidates because of its strong absorption (extinction coefficient $\varepsilon > 10^5\,L\,mol^{-1}\,cm^{-1}$) at long wavelengths and strong singlet oxygen generation abilities (a singlet oxygen quantum yield of approximately 50%). However, low tumor targeting, low water solubility and a high tendency to aggregate appear to significantly restrict the compound's application in tumor treatment. Conjugating Pc with peptide ligands could be a useful strategy for alleviating these problems. Here, to further optimize the structures of peptide-conjugated zinc Pcs for PDT therapy, we finely tuned the hydrophilicity of the modified Pc aromatic macrocycle with varied length of polyethylene glycol (PEG) and added an extra PEG linker and an extra glutamic acid between the Pc ring and the peptide ligand to reduce the influence of the ligand on the Pc aromatic ring. Among the synthesized conjugates, **Pc-3** showed greatly improved targeting towards tumors and abolished inoculated tumors with only a single PDT treatment in a subcutaneous xenograft tumor model, making this approach a promising therapeutic agent for the treatment of cancer.

Photodynamic therapy (PDT) has emerged as an important non-invasive therapeutic modality in the management of a variety of premalignant and malignant diseases [1]. However, the limited availability of ideal photosensitizers with desired properties continues to be an important bottleneck in PDT therapy [2]. In recent decades, substantial effort has been made toward the development of new photosensitizers for PDT [3]. Among them, phthalocyanine (Pc) is one of the most promising candidates [4]. Pc has advantageous photophysical and photochemical properties, including strong absorption (extinction coefficient $\varepsilon > 10^5\,L\,mol^{-1}\,cm^{-1}$) at long wavelengths (>670 nm) and strong singlet oxygen generation abilities (a singlet oxygen quantum yield of approximately 50%) [5]. However, most Pcs exhibit extremely low solubility and strong tendency to aggregate in water due to the hydrophobic nature of the planar Pc macrocycle structure [6], which always renders Pcs photodynamically inactive in aqueous media [7] and significantly restricts their *in vivo* biological and medical applications. Many studies have reported on the chemical modification of Pcs through the attachment of hydrophilic substituents to peripheral positions on the macrocycle to increase the water solubility of Pcs [8,9]. However, hydrophilic modification greatly reduces the PDT activity of Pcs, possibly by reducing their cell permeability and membrane attachment [3b].

Conjugating Pcs to peptide ligands could be another practical and useful strategy for improving the physical properties of Pcs [10,11]. Conjugation with proper hydrophilic tumor-homing peptide ligands can increase water solubility and reduce the aggregation of Pcs. It is also possible to improve Pcs' ability to target tumors through interactions between receptors and the peptide ligands [12]. However, most studies have used non-modified or alkyl-group-modified Pcs with which to conjugate peptide ligands. In this context, the problems associated with the strong hydrophobicity of Pcs, such as aggregation and low water solubility, cannot be well resolved by peptide conjugation. In

* Corresponding authors.
E-mail addresses: lixionghn@163.com (L. Xiong), wwjie@tust.edu.cn (W. Wu), hongzy@nankai.edu.cn (Z. Hong).
[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.cclet.2018.04.025

addition, the strong hydrophobicity of Pcs enhances non-specific interactions with the cell membrane and destroys the binding function of the peptide ligands, increasing the non-specific background signal.

In our previous studies [13], using hydrophilic group-modified Pcs (*i.e.*, modification with triethyleneglycol monomethyl ether substitution or glycol chain substitution) to construct peptide-Pc conjugates, the conjugates were well-shielded from non-specific binding with cells and showed improved tumor selectivity in cell-based experiments. However, the PDT efficiency obtained in the cell-based experiments was appreciably reduced after making these modifications, particularly for the relatively highly hydrophilic modification (glycol chain substitution). Compared with the relatively less hydrophilic modification, the highly hydrophilic modification appears to increase tumor selectivity and reduce the background distribution *in vivo* but with relatively low PDT efficiency. This disparity may require us to finely tune the hydrophilicity of peptide-conjugated Pcs to obtain a better PDT outcome. Moreover, the PDT potential of such Pcs for *in vivo* tumor treatment has not been tested in animal models. Thus, we set out to further optimize the structure of peptide-conjugated Pcs by varying the hydrophilic substitutions on the Pc ring and by adding a hydrophilic linker to finely tune the hydrophilicity, with the aim of balancing the tumor selectivity and PDT activity of the conjugates.

We designed a solid-phase strategy [14] for the synthesis of the proposed conjugates. For comparison, four hydrophilic substitutions (ethylene glycol monomethyl ether group, diethylene glycol monomethyl ether group, triethylene glycol monomethyl ether group and hexaethylene glycol monomethyl ether group) were adopted to reduce the hydrophobicity of the Pc ring component (**Pc-1-4** in Fig. 1). Asymmetrically substituted A_3B-type Pcs with hydrophilic substitutions on the peripheral positions of the macrocycle and a single carboxylic acid were designed to facilitate convenient conjugation with amine-containing peptide ligands. An extra polyethylene glycol (PEG) chain [15] and an extra glutamic residue (**Pc-5** in Fig. 1) were incorporated between the Pc macrocycle component and peptide ligand to test their effect in further increasing water solubility of the conjugates and reducing the influence of the Pc aromatic macrocycle on the affinity of the peptide ligands. The short peptide Lys-Ala-Arg-Leu-Leu-Thr, a modified EGF receptor-targeting peptide [13b,16], was coupled to

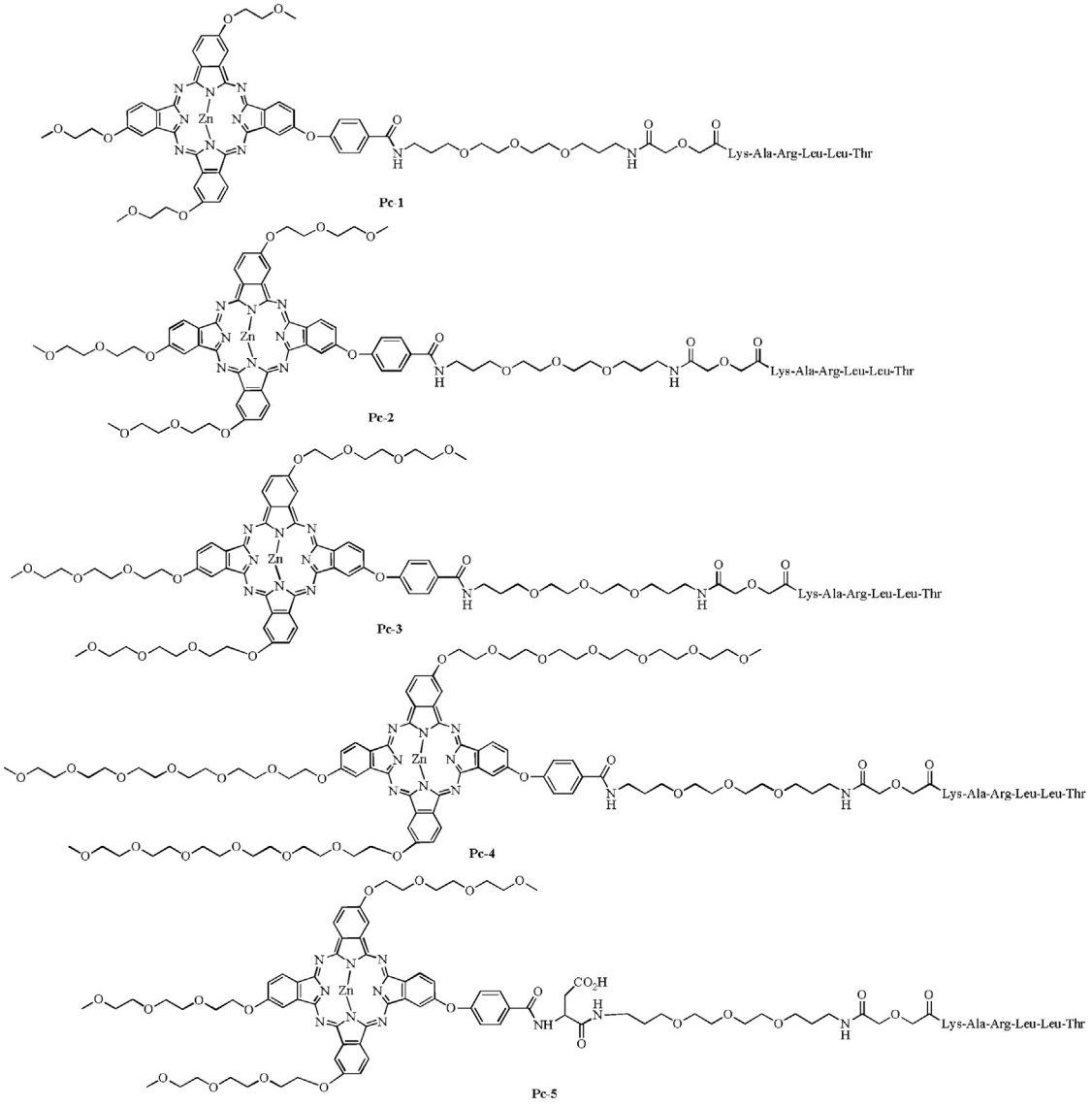

Fig. 1. Design of EGFR targeting peptide-conjugated Pcs. **Pc-1-4** feature ethylene glycol chains of varying lengths on the PC ring component. **Pc-5** features an extra aspartic acid residue on the linker compared with **Pc-3**.

Please cite this article in press as: Q. Chen, et al., *In vitro* and *in vivo* evaluation of improved EGFR targeting peptide-conjugated phthalocyanine photosensitizers for tumor photodynamic therapy, Chin. Chem. Lett. (2018), https://doi.org/10.1016/j.cclet.2018.04.025