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Tetraphenylporphyrin derivatives possessing piperidine group as potential agents for photodynamic therapy

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ABSTRACT

Photodynamic therapy (PDT) is a noninvasive therapeutic and promising procedure in cancer treatment and has attracted considerable attention in recent years. In the present paper, 2-piperidinetetraphenylporphyrin derivatives (**P1–P3**) conjugated with different substituents (Cl, Me, MeO group) at phenyl position were synthesized *via* nucleophilic substitution of 2-nitroporphyrin copper derivatives with piperidine by refluxing under a nitrogen atmosphere and then demetalization. The combination of ^{1}H NMR, ^{13}C NMR and HR-MS was used to elucidate the identities of them. Their photophysical and photochemical properties, intracellular localization, cytotoxicity *in vitro* and *in vivo* against QBC-939 cells were investigated. They have absorption at wavelength about 650 nm. All synthesized photosensitizers showed low dark cytotoxicity and comparable with that of hematoporphyrin monomethyl ether (**HMME**). And they were more phototoxic than **HMME** to QBC-939 cells *in vitro*. In bearing QBC-939 tumor BALB/c nude mice, when it treated with 5 mg/kg dose of PS and laser light (650 nm, 100 J/cm^2, 180 mW/cm^2), the growth of tumor was inhibited compared to the control group. Among them, **P3** exhibited better photodynamic antitumor efficacy on BALB/c nude mice at lower concentration. These results indicate that **P3** is a new potential antitumor photosensitizer in photodynamic therapy and deserves further investigation.

1. Introduction

Photodynamic therapy (PDT) is a noninvasive therapeutic procedure utilizing a photosensitizer (PS) activated by specific light to generate reactive oxygen species (ROS) [1–4], which can further trigger the tumor ablation [5]. The advantages of PDT as compared with other cancer treatment modalities, such as surgery, radiotherapy, or chemotherapy, lie in high selectivity to the irradiated cancer cells which reduces any damage to the non-irradiated normal cells [6–9]. PS can transfer electrons or protons to substrates in close proximity, giving rise to radicals, peroxides, superoxide radicals anions which are subsequently scavenged by oxygen to form reactive oxygen species (ROS, type I mechanism). As an alternative, PS can transfer its energy directly to the ground state of molecular triplet oxygen ($^{3}O_2$) to form singlet oxygen ($^{1}O_2$, type II mechanism), a very reactive and toxic form of oxygen [10,11]. Typical PSs undergo type II mechanism because $^{1}O_2$ is believed to be the major cytotoxic agent in PDT [12]. PDT also has the advantages of repeated dose tolerance and high specificity, which are achieved through the precise application of light [13].

Porphyrin-based PS hematoporphyrin derivatives (HPD, Photofrin®) developed in 1960s is one of first generation PS to receive regulatory approval for the treatment of various tumors in >40 countries all over the world [14,15]. However, HPD is a complex derivative and its selectivity and photocytotoxicity is insufficient for tumor therapy [16]. Hematoporphyrin monomethyl ether (**HMME**) is a second-generation porphyrin-related PS developed in China which has been used in clinic [17,18]. Compared with first generation PS, **HMME** has higher photoactivity, stronger photodynamic efficiency, lower toxicity and a faster clearance rate [19]. Moreover, **HMME** is less costly than other photoactive drugs [20]. Nowadays, the design and synthesis of novel PSs are central to the development of efficient PDT modalities, particularly the reduction of the doses of drug and photo irradiation by increasing photocytotoxicity, selective accumulation in the tumor, and rapid clearance after treatment to induce side effects such as prolonged skin sensitivity [21].

As the limited solubility of porphyrin in commonly used solvents affects their optical properties, many methods have been proposed to overcome these limitations [22]. One method which relies on the

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introduction to appropriate functional groups, such as amide, carboxylic, or sulfonic [23]. Other method relies on inducing steric isolation of the porphyrins' core through their substitution with bulky groups [24]. Synthesis of β-heteroatom-substituted porphyrin often utilize metal-catalyzed carbon-heteroatom formation. Many researchers have established a powerful new strategy for synthesis of heteroatom-substituted porphyrin from catalytic reactions of halogenated porphyrins with amines, amides and alcohols [25]. Another strategy for preparation of β-heteroatom-substituted porphyrin is palladium-catalyzed amination reactions of 2-aminoporphyrin nickel (II) with bromobenzene derivatives [26]. 2-nitroporphyrins or 2-nitroporphyrins copper have been seldom used as starting materials for the synthesis of other porphyrin derivatives by direct nucleophilic substitution of the 2-nitro group by alkoxides [27], 1,2-dicarbonyl compounds [28].

In the present study, the 2-piperidineporphyrins were previously synthesized *via* nucleophilic substitution of 2-nitroporphyrin copper derivatives with piperidine by refluxing under a nitrogen atmosphere and then demetalization. Their chemical characterizations, photophysical properities, subcellular localization, photodynamic activities *in vitro* and *in vivo* were studied.

2. Materials and Methods

2.1. Materials

All solvents and reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Melting points were obtained on a "Stuart" Bibby apparatus and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer. Chemical shifts were reported as δ values relative to the internal standard tetramethylsilane. MADLI-TOF mass spectra were recorded on an AB SCIEX 4800 Plus MALDI TOF/TOF™. HR-MS spectra were recorded on a Thermo Fisher Scientific LTQ FT Ultra Mass Spectrometer. Column chromatography was performed on silica gel H (300–400 mesh). UV–vis absorption spectra were recorded on an ultraviolet visible spectrophotometer (Model V-530, Japan). Fluorescence spectra were measured on a Fluorescence Spectrophotometer (FluoroMax-4, France).

2.2. Synthetic Procedures

2.2.1. General Procedure for the Synthesis of *5a–c*

The compound **4a–c** were synthesized according to literature procedure [29–31].

A solution of **4** (0.23 mmol) and K_2CO_3 (360 mg, 2.56 mmol) in dry piperidine (15 mL) was stirred at reflux for about 10 h under nitrogen, at which time no more starting material was detected by TLC. The mixture was cooled, diluted with dichloromethane (150 mL), washed with water (3 × 100 mL) and saturated brine (3 × 100 mL), dried over anhydrous Na_2SO_4, concentrated, and purified on a silica gel column using dichloromethane/petroleum ether as gradient eluent to yield product **5**.

2-Piperidine-5,10,15,20-tetra(4-chlorinephenyl)porphyrin copper (II) (5a)

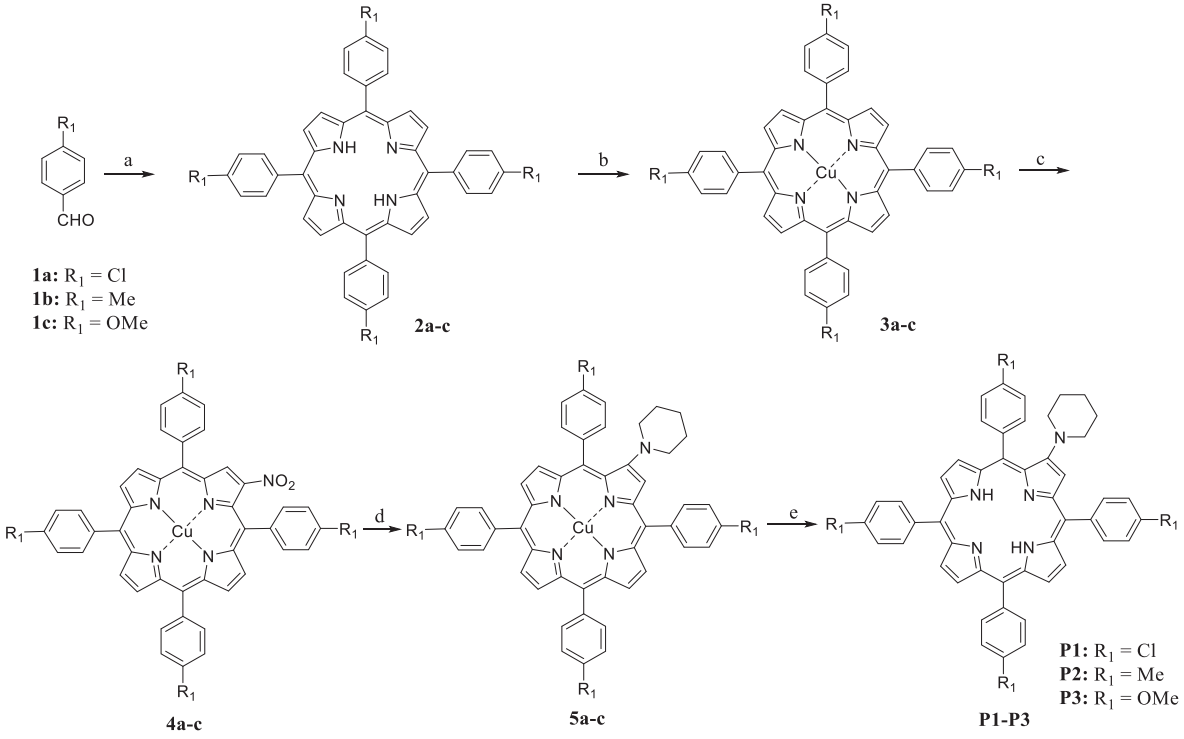
The crude porphyrin was purified on silica gel column using dichloromethane/petroleum ether (1:8) as gradient eluent to yield the dark green product **5a** (64 mg, 31%). Mp > 300 °C. MS (MALDI-TOF): *m/z* 895.1 $[M + H]^+$.

2-Piperidine-5,10,15,20-tetra(4-methylphenyl)porphyrin copper (II) (5b)

The crude porphyrin was purified on silica gel column using dichloromethane/petroleum ether (1:8) as gradient eluent to yield the dark green product **5b** (42.2 mg, 22.5%). Mp > 300 °C. MS (MALDI-TOF): *m/z* 815.3 $[M + H]^+$.

2-Piperidine-5,10,15,20-tetra(4-methoxylphenyl)porphyrin copper (II) (5c)

The crude porphyrin was purified on silica gel column using dichloromethane/petroleum ether (1:3) as gradient eluent to yield the desired



Scheme 1. Reagents and reaction conditions: (a) Pyrrole, propanoic acid, reflux 30 min; (b) $Cu(OAc)_2 \cdot H_2O$, CH_3COOH, CH_2Cl_2, RT, 15 min; (c) $Cu(NO_3)_2 \cdot 3H_2O$, Ac_2O, CH_3COOH, CH_2Cl_2, reflux 4 h; (d) K_2CO_3, piperidine, reflux; (e) H_2SO_4, TFA, 30 min.