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A novel nitroethylene-based porphyrin as a NIR fluorescence turn-on probe for biothiols based on the Michael addition reaction

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ABSTRACT

Biothiols, such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), play essential roles in many physiological and pathological processes. In this work, three porphyrin derivatives, **XQ1**, **XQ2** and **XQ3**, were synthesized by combining the porphyrin framework with nitroethylene, bis(methoxycarbonyl) ethylene and dicyanoethylene moieties, respectively. **XQ1** was almost nonfluorescent, which could be utilized as a good starting point for developing fluorescence turn-on probes. Thus, **XQ1** exhibits fast fluorescence enhancement and high selectivity towards the biothiols based on the Michael addition mechanism. It also exhibits high sensitivity towards the biothiols with the detection limits of 0.65 −1.1 μM. In addition, **XQ1** was successfully applied to cell imaging in living A549 cells for visualizing the biothiols. The results compose a good example of designing porphyrin derivatives for detecting biothiols, with the advantages of dramatic fluorescence enhancement in the NIR wavelength range, which exhibits good cell membrane permeability and practicability in detecting both exogenous and endogenous biothiols.

1. Introduction

Biothiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) play important roles in various physiological processes [1–5]. Abnormal levels of these species are closely related to certain diseases. Taking Cys as an example, its deficiency may result in syndromes like liver damage, slower development of children, and skin lesions [6–9]. Similarly, abnormal concentration of Hcy may be a sign for cardiovascular diseases [10,11]. On the other hand, GSH is a tripeptide and it is the most abundant free thiol in the cells [12,13]. Deficiency of GSH may change intracellular redox state, which is associated with certain diseases such as cancer, Alzheimer's disease and AIDS [14,15]. Thus, it is important to develop efficient methods for detection and quantification of biothiols for the early diagnosis and therapy of the related diseases. Because of the simplicity and convenience, fluorescent biothiol probes have attracted increasing interests in recent years [16–20]. Among various designing strategies, Michael addition type fluorescent probes have been intensively investigated. In this respect, a number of excellent receptors have been utilized, such as nitroolefin [21,22], quinone [23], acrylic acid [24], maleimide [25], α,β-unsaturated aldehydes [26], diesters [27] and malononitrile [28].

Among various fluorophores, porphyrins have large conjugated frameworks and long fluorescence emission wavelengths, and thus they have been used in developing ion chemosensors [29–31]. It is well known that fluorescent dyes emitting in the near infrared (NIR) regions are favorable for biological imaging applications because of the deep tissue penetration, minimum photo-damage to biological samples, and minimum background interference [32–34]. Furthermore, the hydrophobicity of porphyrins is quite similar to that of cholesterol, indicative of similar physical interactions of the two molecules in the lipid membrane [35]. Hence, porphyrins are promising for the applications in biological systems. Whereas, only a few porphyrin based probes for biothiols have been reported in this respect [36].

In this work, we aimed at developing novel probes based on porphyrin framework utilizing the well developed Michael addition

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mechanism. Thus, nitroethylene, bis(methoxycarbonyl) ethylene and dicyanoethylene were used as the receptor moiety to synthesize **XQ1**, **XQ2** and **XQ3**, respectively (Scheme 1). It was found that **XQ2** and **XQ3** exhibited strong fluorescence, only **XQ1** was demonstrated to be almost nonfluorescent, which could be utilized as a good starting point for developing fluorescence turn-on probes. Interestingly, the observed NIR fluorescence of **XQ1** was dramatically enhanced upon addition of the biothiols. Furthermore, **XQ1** was cell membrane permeable and it could be used for cell imaging and the detection of both exogenous and endogenous biothiols.

2. Experimental section

2.1. Materials and instrumentation

Commercially available solvents and reagents were used as received. Compounds **1** and **5** were synthesized according to our previously reported procedures [37], and the synthesis of **3** is described in the ESI. Water was used after redistillation. Deuterated solvents for NMR measurements were available from Aldrich. UV–Vis absorption spectra were recorded on a Varian Cary 100 spectrophotometer and fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer, with a quartz cuvette (path length = 1 cm). ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker AM 400 spectrometer with tetramethylsilane (TMS) used as the internal standard. High resolution mass spectra (HRMS) were measured on a Waters LCT Premier XE spectrometer or a Thermo Fisher Scientific LTQ FTICR mass spectrometer. Confocal laser scanning microscope (CLSM) images were taken on an inverted fluorescence microscope (Nikon A1R/A1).

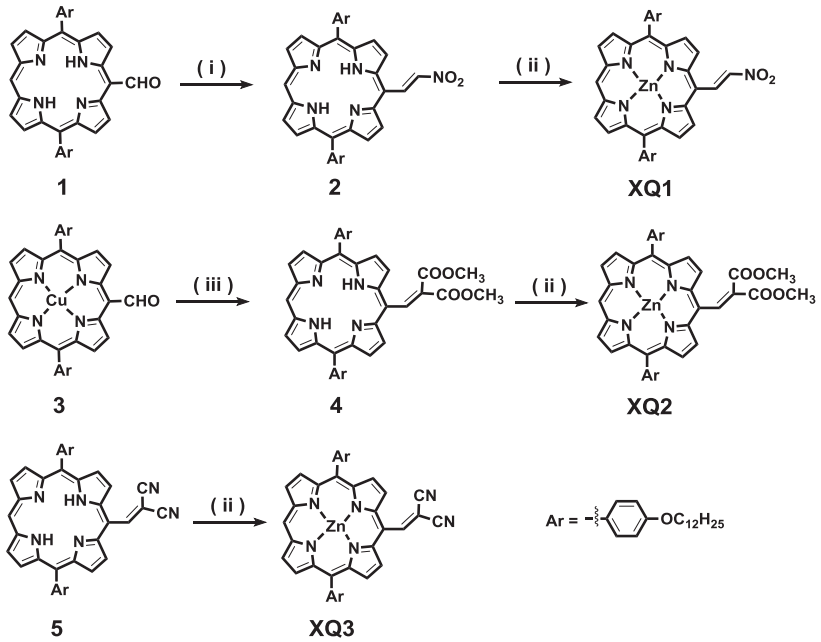
2.2. Syntheses

2.2.1. Synthesis of compound *2*

In a three-neck 100 mL flask, compound **1** (35 mg, 0.04 mmol), nitromethane (0.4 mL, 8 mmol), piperidine (0.8 mL, 8 mmol) and acetic acid (0.9 mL, 16 mmol) were mixed in toluene (30 mL) under nitrogen. After refluxing overnight, the reaction mixture was poured into water, washed with water, extracted with CH_2Cl_2 and dried over Na_2SO_4. Then the solvent was removed under reduced pressure and the residue was purified on a silica gel column using CH_2Cl_2/PE (3:1, v:v) as the eluent to afford a dark green solid of **2** (15.6 mg, yield 43.3%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 10.66 (d, J = 13.2 Hz, 1H, alkene-H), 10.22 (s, 1H, *meso*-H), 9.51 (d, J = 4.8 Hz, 2H, pyrrole), 9.30 (d, J = 4.8 Hz, 2H, pyrrole), 9.06 (d, J = 5.2 Hz, 2H, pyrrole), 8.99 (d, J = 4.4 Hz, 2H, pyrrole), 8.11 (d, J = 8.4 Hz, 4H, ph-H), 13.2 (d, J = 13.2 Hz, 1H, alkene-H), 7.33 (d, J = 8.4 Hz, 4H, ph-H), 4.28 (t, J_1 = 6.8 Hz, J_2 = 6.4 Hz, 4H, -OCH_2-), 2.01 (m, 4H, CH_2), 2.65 (m, 4H, CH_2), 1.31 (m, 32H, CH_2), 0.88 (t, J_1 = 8 Hz, J_2 = 8 Hz, 4H, CH_3-), −2.6 (s, 1H, -NH). HRMS (ESI, m/z): $[M+H]^+$ calcd for $C_{58}H_{72}N_5O_4$, 902.5584; Found, 902.5580.

2.2.2. Synthesis of compound *4*

In a three-neck 100 mL flask, **3** (55 mg, 0.06 mmol), dimethyl malonate (0.5 mL, 4.5 mmol), piperidine (0.2 mL) and acetic acid (40 μL) were mixed in toluene (30 mL) under nitrogen. After refluxing overnight, the reaction mixture was poured into water, washed with water, extracted with CH_2Cl_2 and dried over Na_2SO_4. Then the solvent was removed under reduced pressure and the residue was purified on a silica gel column using CH_2Cl_2/PE (3:2, v/v) as the eluent to give a dark red solid, which was dissolved in CH_2Cl_2 (20 mL), and 98% H_2SO_4 (1 mL) was added slowly at 0 °C. After stirring for 5 min, the reaction mixture was poured into water, extracted with CH_2Cl_2, washed with water and dried over Na_2SO_4. Then the solvent was removed under reduced pressure and the residue was purified on a silica gel column using CH_2Cl_2/PE (3:1, v:v) as the eluent to afford a dark red solid of **4** (28.2 mg, yield 95.9%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 10.43 (s, 1H, alkene-H), 10.19 (s, 1H, *meso*-H), 9.38 (d, J = 4.8 Hz, 2H, pyrrole), 9.30 (d, J = 4.8 Hz, 2H, pyrrole), 9.02 (d, J = 4.8 Hz, 4H, pyrrole), 8.12 (d,



(i) CH_3NO_2, AcOH, toluene, piperidine, reflux; (ii) $Zn(OAc)_2{\cdot}2H_2O$, CH_2Cl_2, methanol, reflux; (iii) (a) dimethyl malonate, piperidine, AcOH, toluene, reflux; (b) 98% H_2SO_4, CH_2Cl_2.

Scheme 1. Synthetic routes of **XQ1**, **XQ2** and **XQ3**.