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A colorimetric fluorescent chemodosimeter based on diketopyrrolopyrrole and 1,3-indanedione for cysteine detection and cellular imaging in living cells

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ABSTRACT

A novel colorimetric fluorescent chemodosimeter (**1**) based on diketopyrrolopyrrole (DPP) and indanedione for the selective detection of cysteine (Cys) over glutathione (GSH) was synthesized, which was involved by the conjugate addition of Cys to α,β-unsaturated ketones. The probe featured a fast response (a response time less than 2 min), excitation and emission in the visible region, dual-channel and high selectivity. Addition of Cys in PBS (pH = 7.4) to **1** in THF resulted in a rapid color change from purple to yellow together with appearance of a new absorption peak at 480 nm, while other amino acids did not induce any significant color change. Meanwhile, the Michael addition of Cys to **1** elicited 4.2-fold PL enhancement at 505 nm, which resulted in emission color change from deep red to yellow. Furthermore, **1** could be used as a fluorescent probe for detection Cys_{34} within BSA. In addition, the cellular imaging of human adult skin fibroblast cells indicated red fluorescence of **1** was present in the cytoplasm. The CCK-8 assay showed that the cytotoxicity of **1** was low.

1. Introduction

Among the twenty amino acids commonly found in proteins, cysteine (Cys) is special in that it is present more often than other residues in functionally important locations. Elevated level of Cys in plasma is a vascular disease risk factor and is associated with neurotoxicity. On the contrary, deficiency of Cys may result in some serious diseases such as hematopoiesis decrease, leukocyte loss, and psoriasis. Additionally, altered level of Cys has been implicated in hyperhomocysteinemia, which has been linked to the increased risks of Alzheimer's disease, neural tube defect, and osteoporosis. Thus, the detection of Cys continues to be of interest.

Up to now, numbers of thiol-specific probes have been developed on the basis of various strategies [1], such as cleavage reactions [2], Michael addition reactions [3], metal complexes/displaced coordination and others [4]. Among these strategies, Michael addition type probes attract many attentions. A number of excellent Michael acceptors have been exploited such as α,β-unsaturated aldehyde [5], ketone [6], diesters [7], malonitrile [8] and nitroolefin [9], etc. However, many of them are associated with some limitations, including a limited pH range, excitation and emission in the UV region (below 500 nm), especially a long response time. More importantly, only a few sensors able to detect Cys over GSH have been reported to date [10]. At the same time, the fluorophores used for thiol probes are limited to coumarin, fluorescein, rhodamine, or BODIPY [1]. Probes based on other fluorophores were rarely reported. On the other hand, diketopyrrolopyrrole (DPP) is a robust chromophore, but its application in thiol-specific fluorescent probes is very rare [8b]. So, much room is left to explore DPP-based probes to improve the sensing properties, such as achieving longer emission wavelength and rapid response time, *etc*. Furthermore, most of the thiol probes based on Michael addition are colorimetric or fluorescence probes [6,9,11], and a few thiol probes have been developed to combine the ratiometric and colorimetric fluorescence transductions.

Inspired by elegant development of the thiol probes based on Michael addition, herein we designed probe **1** (Scheme 1) preferably with excitation and emission in the visible region with the intention as follows. (1) The indandione-derived vinyl moiety could be more reactive and enhance the sensitivity of the nucleophilic addition reaction between the vinyl group and Cys. (2) Electron-deficient diketopyrrolopyrrole (DPP) is selected as a chromophore due to its many advantageous features including its enhanced

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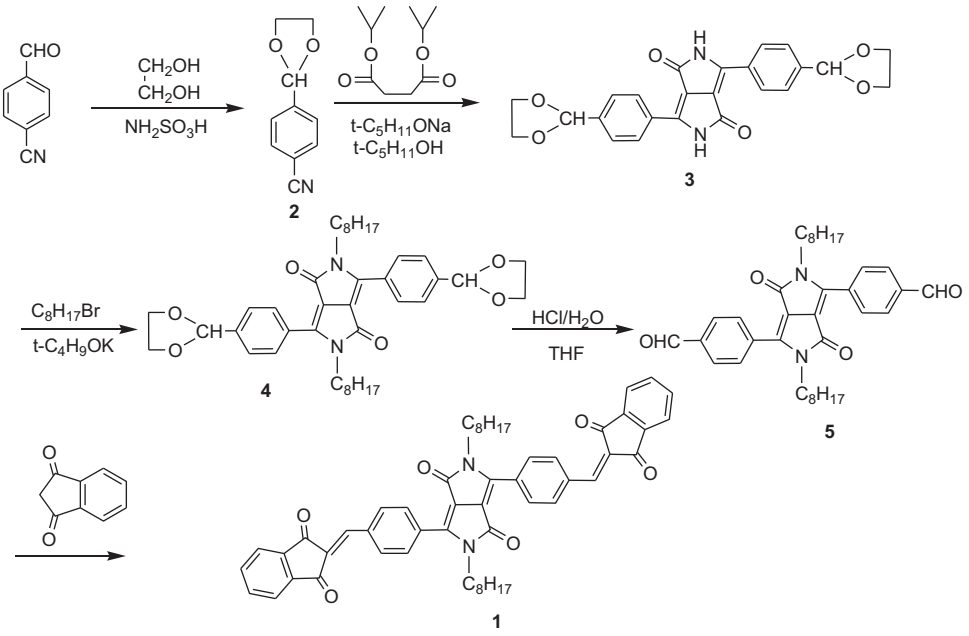

Scheme 1. Synthesis of probe **1**.

vinyl moiety reactivity with Cys, strong fluorescence as well as its distinct rigid ring structure. (3) Two vinyl moieties are introduced to probe **1**, which is favorable to accelerate reaction rate between probe **1** and Cys. Thus, a DPP–indanedione conjugate (**1**) as doubly activated Michael addition type probe for the colorimetric and fluorescent detection of Cys was developed, which was highly selective toward thiols over other amino acids with a fast response. Moreover, application of **1** in cellular imaging was demonstrated and discussed.

2. Experimental

2.1. Chemicals and instruments

Nuclear magnetic resonance spectra were recorded on Bruker Avance III 400 MHz and chemical shifts are expressed in ppm using TMS as an internal standard. The UV–vis absorption spectra were recorded using a Helios Alpha UV–Vis scanning spectrophotometer. Fluorescence spectra were obtained with a Hitachi F-4500 FL spectrophotometer with quartz cuvette (path length = 1 cm).

N-methyl-2-pyrrolidone (NMP) was dried with CaH_2 and distilled under nitrogen atmosphere. Other solvents were obtained from commercially available resources without further purification. Probe **1** was synthesized according to our published literature [12].

The recognition between **1** in THF and different amino acids in PBS (pH 7.4) was investigated by UV–vis and fluorescence spectroscopy at room temperature. The stock solution of **1** and amino acids was at a concentration of 10.0 mM. After the **1** and amino acids with desired concentrations were mixed, they were measured by UV–vis and fluorescence spectroscopy.

2.2. Cellular imaging

2.2.1. Cell culture

Human adult skin fibroblast cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (H-DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin streptomycin at 37 °C in a humidified environment containing 5% CO_2. Before the experiment, the cells were precultured until confluence was reached.

2.2.2. Cell imaging

The human adult skin fibroblast cells were seeded in the 12-well plate and cultured in H-DMEM with 10% FBS at 37 °C in a humidified environment containing 5% CO_2. After 80% confluence, the medium was removed and the adherent cells were rinsed twice with 1 × PBS. The **1** in DMEM medium with FBS at 10 μM was then added to the culture plate. After incubation for 2 h, the cells were washed three times with 1 × PBS buffer and fixed with 4% paraform for 2 h at room temperature. The nuclei were stained by 4′,6-diamidino-2-phenylindole for 10 min. The cell monolayer was then washed twice with 1 × PBS buffer and imaged using inverted phase contrast fluorescence microscope (Nikon Eclipsc Ti-U) with imaging software (Nikon NIS Elements).

2.2.3. Cytotoxicity

Cell viability was evaluated using the Cell Counting Kit-8 (CCK-8) assay. CCK-8 was just as WST-8 to produce formazan in the presence of an electron mediator, and the amount of the formazan generated in cells was directly proportional to the number of living cells. The human adult skin fibroblast cells were seeded in 96-well plates at a density of 3000 cells per well. **1** at different concentrations (1 and 10 μM, respectively) was added into a 96-well plate. Meanwhile, cells culture with complete medium (H-DME with 10% FBS) were evaluated as a control. The human adult skin fibroblast cells were incubated in the medium under 5% CO_2 in an incubator maintained at 37 °C for 1, 2, 4 and 7 days, respectively. Then, 10 μL of the CCK-8 was added to each well of a 96-well plate incubated for additional 2 h. The absorbance was measured at 450 nm using a microplate reader (Varioskan Flash, Thermo Scientific). The assay was repeated three times.

2.2.4. Statistical analysis

Statistical analysis was performed using a standard Student's *t*-test with a minimum confidence level of 0.05 for significant statistical difference. All experiments were performed in triplicate.