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A colorimetric and fluorescent probe for thiols based on 1, 8-naphthalimide and its application for bioimaging

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ABSTRACT

A new highly sensitive 1, 8-naphthalimide-based compound (probe **1**) has been designed and synthesized. The colorimetric and fluorescent properties of probe **1** towards various amino acids were investigated in detail. Specific colorimetry and fluorescence response of probe **1** for thiols were found in PBS buffer solution, which can be deduced to a reaction of thio-promoted conjugate addition/cyclization sequence between probe **1** and thiols. The probe **1** can selectively recognize cysteine rather than other natural amino acids in aqueous solution with a rapid response time. Con-focal fluorescence imaging of cells for detecting thiols in vivo was carried out successfully.

1. Introduction

Intracellular thiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), play essential roles in physiological matrices, including redox homeostasis and cellular growth [1–4]. A deficiency of Cys would cause many syndromes, like retarded growth, hair depigmentation, lethargy, liver damage, muscle and fat loss, skin lesions and weakness [5–8]. While an elevated level of Hcy in human plasma was proved to be involved in cardiovascular and Alzheimer's disease [9]. Therefore, it is of immense scientific and technological interest and importance in the area of biological chemistry to recognize and detect special mercapto biomolecules in biological samples.

Typical analytical methods for thiols include high performance liquid chromatography (HPLC) [10,11], mass spectrometry [12,13], electrochemical method [14,15], capillary electrophoresis [16,17] and colorimetric assays [18–26]. Most of these methods need complicated and expensive instruments and laborious pretreatment procedures such as isolation and purification before the instrumental analysis starts. In addition, few of them are convenient to apply in intracellular detection due to their limitations in vivo studies. Compared with those, the colorimetry and fluorescence method belonged to spectra field is more convenient and efficient for thiols detecting. Besides, the sensitivity of the chemosensor is also fairly noticeable among all the methods developed. As a result, colorimetric and fluorescent chemosensor for thiols has become a particularly important research field and is attracting growing interest. Till date, a wide variety of fluorescent probes for thiols based on different mechanisms have been developed, including cleavage reaction by thiols [27–32], cyclization reaction with aldehyde [33–40], Michael addition [41–53] and others [54–57]. Based on previous reports that 1, 4-addtion could be conducted in water at room temperature without the use of any catalyst [36,42], we sought to exploit this chemistry for biological thiol sensing. Herein, we report a highly selective and sensitive fluorescence turn-on probe for quantitative detection of biological thiols in aqueous solution and living cells with a rapid response. In addition, the property of visible light exciting makes probe **1** fit the preference of further application.

The probe was designed based on the Michael addition reaction mechanism, 4-hydroxy-N-butyl-1, 8-naphthalimide was selected as the fluorophore because of its desirable photophysical properties, such as a large Stokes' shift, long emission wavelength, and high fluorescence quantum yields. And α, β-unsaturated ketones moiety was chosen as the Michael acceptor, which serves as not only an electrophile but also a quencher of the 1, 8-naphthalimide fluorophore (Scheme 1).

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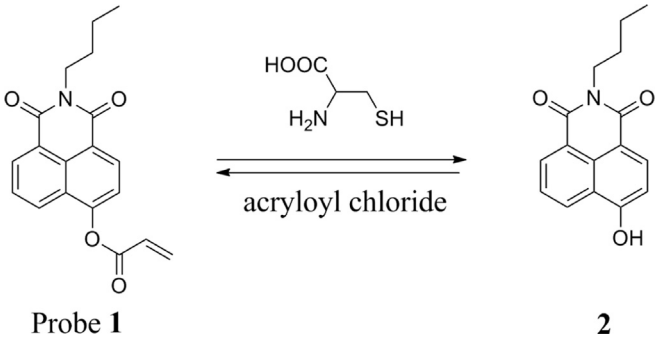
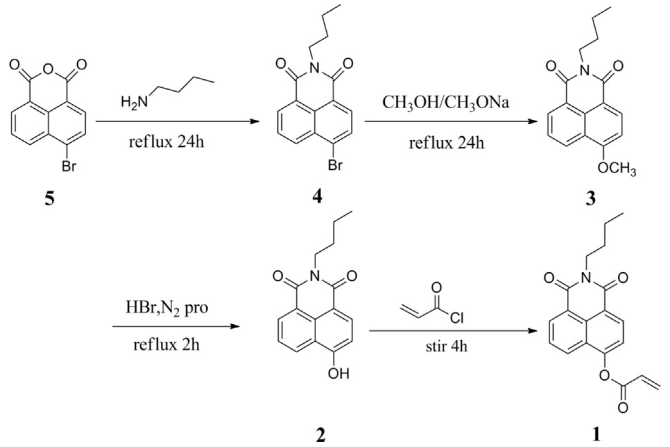


Scheme 1. Cleavage of probe **1** to form **2** and the preparation of Probe **1**.



Scheme 2. Synthesis of the target probe **1**.

2. Experimental details

2.1. Materials and instruments

All reagents and solvents were obtained commercially and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX400 spectrometer and referenced to the solvent signals. Mass spectra (ESI) were obtained on an LQC system (Finngan MAT, USA) and High Resolution Mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II mass spectrometer. UV–visible spectra were collected on a Varian Cary 100 spectrophotometer. Fluorescence spectra were performed on a Hitachi F-4500 luminescence spectrometer.

2.2. Preparation of amino acids solutions for fluorescent study

Stock solutions (10 mM) of amino acids including Cys, Hcy, GSH, Ala, Asn, Glu, Arg, Asp, Gln, Gly, His, Leu, Lyr, Lys, Met, Phe, Pro, Trp, Ser and Val in distilled water were prepared. Stock solution of probe **1** was prepared in dimethylsulfoxide. In a typical experiment, test solutions were prepared by placing 4 μL of the probe stock solution into the solution of 2 mL phosphate buffer. Fluorescence spectra were measured after addition of analytes for 5 min. An excitation and emission slit of 5.0 nm was used for the fluorescent measurements.

2.3. Fluorescence microscope experiment

Human promyelocytic leukemia cells line(HL60) were cultured in RPMI-1640 (Roswell Park Memorial Institute-1640) supplemented with 10% FBS (Fetal Bovine Serum), 2 mM glutamine, and 100 units mL^{-1} penicillin/streptomycin and maintained in an atmosphere of 5% CO_2 at 37 °C. Cells were plated in 12-well plates and incubated with 50 μM probe **1** for 15 min at 37 °C, washed with PBS buffer three times. Then fluorescence imaging was carried out with Zeiss Leica DM 4000B microscope (40 × objective lens).

2.4. Synthesis

Synthetic procedures of probe **1** (Scheme 2).

2.4.1. Synthesis of 4-hydroxy-N-butyl-1, 8-naphthalimide 2

4-hydroxy-N-butyl-1, 8-naphthalimide was synthesized according to a previous reported method [58]. m.p.236–238 °C. m/z 270.2 [M + H$^+$]. ^{1}H NMR (400 MHz, DMSO) δ (ppm) : 8.42 (d, J = 8.3 Hz, 1H), 8.34 (d, J = 7.2 Hz, 1H), 8.25 (d, J = 8.2 Hz, 1H), 7.69–7.60 (m, 1H), 7.08 (d, J = 8.2 Hz, 1H), 3.99–3.86 (m, 2H), 1.55 (dt, J = 14.9, 7.5 Hz, 2H), 1.30 (dq, J = 14.6, 7.3 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ (ppm): 168.46, 167.79, 165.06, 138.27, 135.83, 133.96, 133.62, 130.28, 127.18, 126.60, 117.46, 114.76, 44.03, 34.65, 24.76, 18.61.

2.4.2. Synthesis of 4-acrylate-N-butyl-1, 8-naphthalimide 1

4-hydroxy-N-butyl-1, 8-naphthalimide (0.269 g, 1 mmol) was dissolved in 5 ml anhydrous CH_2Cl_2, then the acryloyl chloride (2 eq) and Et_3N (2 eq) in anhydrous CH_2Cl_2 were added dropwise at 0 °C. After stirring at this temperature 90 min, the mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (30 mL), washed with H_2O and dried over anhydrous Na_2SO_4, after removal of the solvent, the residues were purified by silica column chromatography with DCM/hexane (3:2) to give as a white solid (232 mg, 72% yield). m.p.94.0–94.8 °C. HRMS (ESI): m/z 324.1233 [M + H$^+$], m/z 346.1037 [M + Na$^+$]. ^{1}H NMR (400 MHz, DMSO) δ (ppm) : 8.42 (d, J = 7.9 Hz, 2H), 8.21 (d, J = 7.8 Hz, 1H), 7.84–7.76 (m, 1H), 7.67 (d, J = 8.0 Hz, 1H), 6.76–6.67 (m, 1H), 6.59 (dd, J = 17.2 Hz, 10.2 Hz, 1H), 6.36–6.29 (m, 1H), 4.04–3.91(m, 2H), 1.64–1.52 (m, 2H), 1.34 (dd, J = 14.9 Hz, 7.4 Hz, 2H), 0.91 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ (ppm): 163.60, 163.08, 162.56, 150.86, 134.92, 131.21, 131.10, 128.45, 127.71, 127.55, 127.00, 124.58, 122.29, 119.94, 119.79, 39.81, 29.62, 19.82, 13.68.

3. Results and discussion

As a typical thiol-containing biomolecule, cysteine (Cys) was used to examine the spectroscopic properties of probe **1**. Spectroscopic studies of probe **1** were carried out in 0.1 M PBS buffer solutions (pH 7.40, containing 0.2% DMSO). As shown in Fig. 1, free probe **1** (20 μM) is weakly fluorescent (Φ = 0.25), due to photoinduced electron transfer (PET) effect caused by carbon–carbon double bond in the α, β-unsaturated ketone moiety. Upon addition of 20 equiv. Cys, the maximum absorption peak of probe **1** red shift 106 nm from 346 nm, a remarkable color changes ranged from colorless to yellow in the solution could be distinguished by the naked eyes (Fig. 1a). Additionally, the solution of probe **1** exhibited a strongly enhanced fluorescence emission centered at 547 nm, about 60-fold fluorescence enhancement could be observed (Fig. 1b). The changes in the absorption spectrum and fluorescence intensity should be attributed to the conjugate addition of thiols to carbon–carbon double bond, which blocked the PET process. Clearly, the results indicated that Cys could be detected through both fluorescence and colorimetric methods by probe **1**.

In order to better understand the sensing process, fluorescence and absorption (Figure. S1) spectra changes of probe **1** (20 mM) were measured with different concentrations of Cys. As illustrated in Fig. 2, a progressive increase in fluorescence intensity at 547 nm