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A fluorescence turn-on probe for cysteine and homocysteine based on thiol-triggered benzothiazolidine ring formation

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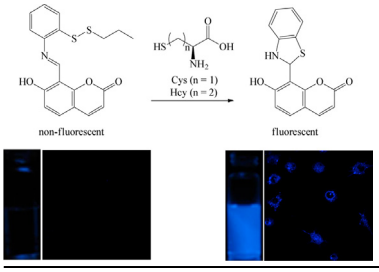
HIGHLIGHTS

- A coumarin-based blue fluorescent probe for sensing thiol was developed.
- The probe utilizes the thiol–disulfide exchange to produce a fluorescence response to the thiol.
- The probe might have application in the investigation of thiol roles in biological systems.

GRAPHICAL ABSTRACT



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ABSTRACT

We synthesized a new coumarin-based probe TP, containing a disulfide moiety, to detect biothiols in cells. A fluorescence turn-on response is induced by the thiol–disulfide exchange of the probe, with subsequent intramolecular benzothiazolidine ring formation giving rise to a fluorescent product. The probe exhibits an excellent selectivity for cysteine (Cys) and homocysteine (Hcy) over glutathione (GSH) and other amino acids. The fluorescent probe also exhibits a highly sensitive fluorescence turn-on response to Cys and Hcy with detection limits of 0.8 μM for Cys and 0.5 μM for Hcy. In addition, confocal fluorescence microscopy imaging using RAW264.7 macrophages demonstrates that the probe TP could be an efficient fluorescent detector for thiols in living cells.

1. Introduction

Cysteine (Cys) and homocysteine (Hcy) are two important biothiols in living organisms, and play crucial roles in biological systems. Cysteine is a semi-essential amino-acid, and its thiol side chain serves as a nucleophile in many enzymatic reactions [1,2]. The thiol in cysteine can be oxidized to become a disulfide bond, playing an important structural role in many proteins. Homocysteine, a homologue of cysteine, has an extra methylene bridge and can be converted into cysteine with the aid of vitamin B [3–5]. These cellular thiols influence the cellular redox environment; abnormal levels of these cellular thiols have been linked to several serious diseases, such as slowed growth, edema, liver damage, cardiovascular diseases, Alzheimer's disease, and Parkinson's disease [6,7]. Accordingly, the detection of important biothiols, including cysteine and homocysteine, has become an important research area.

Several methods for the quantitative measurement of thiols have been developed, such as capillary electrophoresis [8], electrochemical methods [9,10], high performance liquid chromatography (HPLC) [11,12], and mass spectrometry [13,14]. Recently, greater attention has been focused on the development of fluorescent probes for detecting biothiols, due to their high sensitivity and easy operation [15]. Various organic reactions have been used in the design of Cys/Hcy fluorescent probes, including Michael addition [16,17], the

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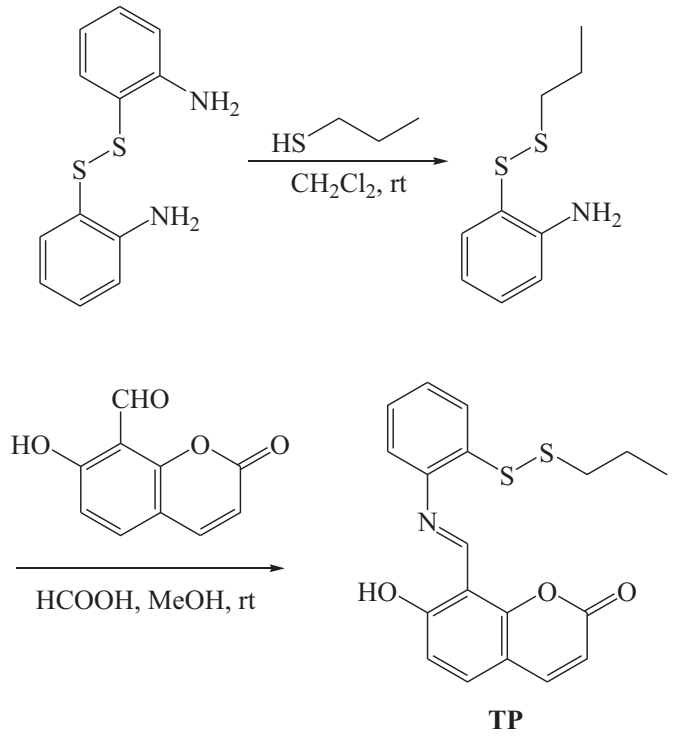

Scheme 1. Synthesis of the probe TP.

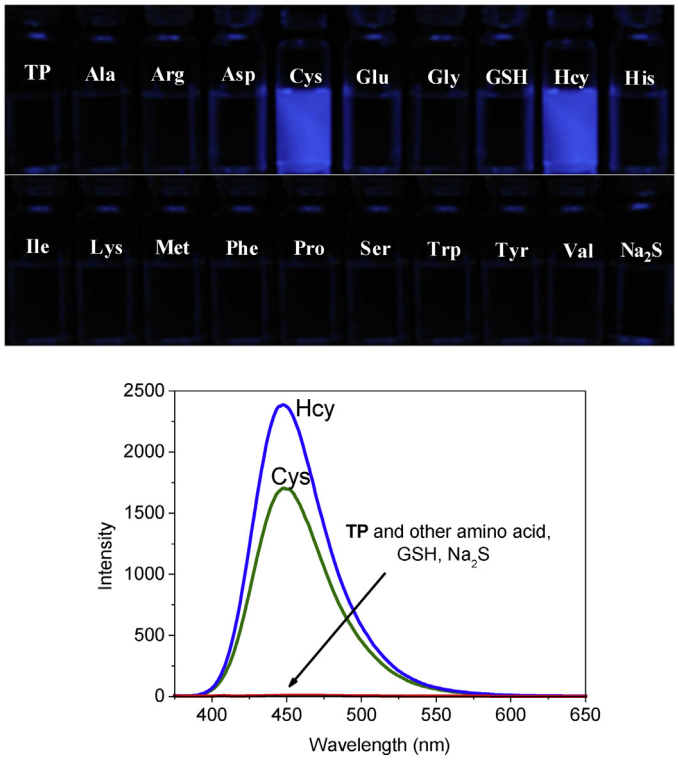

Fig. 1. Fluorescence changes of TP (10 μM) in response to 500 equivalent units of various amino acids, GSH, and Na_2S in a H_2O—CH_3OH (v/v = 99/1, 0.1 M PBS, pH 7.4) solution. The excitation wavelength was 355 nm.

cyclization reaction with an aldehyde [18–20], and the cleavage of sulfonamide, sulfonate esters [21,22], and disulfide bonds [23] by thiols. Here, a coumarin-based thiol probe relying on the specific cleavage of disulfide bonds by thiols has been studied.

In this work, a novel coumarin-based fluorescent probe TP, bearing a disulfide group, was designed for Cys and Hcy detection. Coumarin was used as the signal transduction unit in this study, while the disulfide unit served as a modulator to respond to the presence of Cys and Hcy. TP exhibits weak fluorescence with a quantum yield of $\Phi = 0.0008$ due to imine isomerization, which has been known to exhibit a non-radiative decay process in the excited state [24]. The strong blue fluorescence of coumarin is restored upon cleavage of the disulfide bond by Cys and Hcy with the subsequent intramolecular benzothiazolidine ring formation giving rise to a fluorescent product. This new probe displays high selectivity for Cys and Hcy over other amino acids, GSH, and Na_2S in aqueous solution. Most notably, TP shows good cell-membrane permeability and can be successfully applied to the imaging of biothiols in living cells.

2. Experimental

2.1. Materials and instrumentation

All reagents were obtained from commercial sources and used as received without further purification. UV/vis spectra were recorded on an Agilent 8453 UV/vis spectrometer. Fluorescence spectra measurements were performed on a Hitachi F-7000 fluorescence spectrophotometer. NMR spectra were obtained on a Bruker DRX-300 and Agilent Unity INOVA-500 NMR spectrometer. Fluorescent pictures were taken on a Leica TCS-SP5-X AOBS Confocal Fluorescence Microscope.

2.2. Synthesis of 2-(propyldisulfanyl) benzenamine [25]

1-Propanethiol (342 mg, 4.5 mmol, dissolved in 10 mL CH_2Cl_2) was added dropwise to a solution of 2-aminophenyl disulfide (744 mg, 3.0 mmol) in CH_2Cl_2 (20 mL) under N_2 atmosphere. The reaction mixture was stirred at room temperature for 6 h. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (hexane:ethyl acetate = 19:1) to give the compound a consistency of yellow oil. Yield: 143 mg (24%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (dd, J = 1.5, 7.8 Hz, 1H), 7.21–7.15 (m, 1H), 6.77–6.68 (m, 2H), 4.40 (brs, 2H), 2.74 (t, J = 7.2 Hz, 2H), 1.80–1.73 (m, 2H), 0.99 (t, J = 6.9 Hz, 3H).

2.3. Synthesis of TP

2-(Propyldisulfanyl) benzenamine (120 mg, 0.6 mmol, dissolved in 5 mL MeOH) and formic acid (0.5 mL) were added to a solution of 8-formyl-7-hydroxycoumarin [26] (95 mg, 0.5 mmol) in MeOH (20 mL). The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (hexane:ethyl acetate = 5:1) to make the compound appear an orange solid. Yield: 87 mg (47%). m.p. 112–113 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.32 (s, 1H), 9.25 (s, 1H), 8.03 (d, J = 10.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.59 (d, J = 7.5 Hz, 1H), 7.46–7.40 (m, 2H), 6.98 (d, J = 8.5 Hz, 1H), 6.35 (d, J = 10.0 Hz, 1H), 2.75 (t, J = 7.0 Hz, 2H), 1.66–1.59 (m, 2H), 0.89 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 164.3, 159.2, 156.8, 154.6, 144.8, 144.7, 133.8, 131.9, 128.4, 128.1, 127.1, 118.7, 114.1, 112.1, 110.7, 106.5, 40.0, 21.7, 12.7. MS (EI): m/z (%) = 371 (17), 297 (96), 296 (100), 210 (22), 136 (71). HRMS (EI): m/z calcd. for $C_{19}H_{17}NO_3S_2$ 371.0650; found 371.0651.

2.4. Cell culture for RAW264.7 macrophages

The cell line RAW264.7 was provided by the Food Industry Research and Development Institute (Taiwan). RAW264.7 cells