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A colorimetric and red emissive fluorescent probe for cysteine and its application in bioimaging

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ABSTRACT

Developing effective methods to detect and image Cys *in vivo* has become an increasingly important area of research. In this work, two colorimetric and fluorescent sensors **1** and **2** were presented for detecting Cys with different reactive rates in an intramolecular cyclization reaction between acrylate and thiols. Probe **2** is the isomer of the probe **1**, which has greater steric hindrance than probe **1**. The performed reaction kinetics experiments showed that the reaction rates of **1** with thiols were far more rapid than those of **2** with thiols. From the observed crystal structures of these two compounds, we speculate that the larger steric hindrance around the acrylate group in probe **2** outweighs the electronic effect, thus slowing down the reaction between the acrylate and thiols. Probe **1** was successfully applied with negligible cell toxicity toward the imaging of biothiols in living cells and zebrafish.

1. Introduction

Biothiols including glutathione (GSH), cysteine (Cys), and homocysteine (Hcy) are of essential importance in cellular processes such as the maintenance of the redox homeostasis of proteins, cells, and organisms [1]. GSH is the most abundant cellular thiol, and it is an essential endogenous antioxidant that plays a central role in cellular defense against toxins and free radicals [2]. As a precursor of GSH, Cys is an essential amino acid that plays an important role in detoxification, protein synthesis, and metabolism [3]. Cys deficiency is involved in many syndromes such as slowed growth rate, edema, lethargy, liver damage, and hair depigmentation [4]. On the other hand, elevated levels of Cys have been reported to be linked with neurotoxicity [5]. Hcy, an analog of Cys, has been associated with various types of vascular and renal diseases. For example, elevated levels of Hcy in the blood is a risk factor for neutral tube defects, cardiovascular disease, Alzheimer's disease, complications during pregnancy, inflammatory bowel disease, and osteoporosis [6]. Therefore, developing an effective method to detect and image biothiols is an urgent demand.

Compared with other analytical techniques such as electrochemical detection and high performance liquid chromatography (HPLC), fluorimetry is one of the most powerful detection methods due to its high sensitivity and high temporal and spatial resolution [7]. In the past few years, various fluorescent probes for thiols have been developed based on the mechanisms of Michael addition [8], cyclization with aldehyde [9], cleavage by thiols [10], and others [11]. However, the implementation of most of these probes have been met with some drawbacks such as poor selectivity and long reaction time.

In 2011, Strongin et al. used an acrylate moiety to develop an ingenious method to distinguish Cys and Hcy according to their different reaction rates in an intramolecular cyclization reaction between acrylate and thiols [12a]. Subsequently, several Cys-targeted probes were reported by introducing an acrylate group into a fluorophore [12b–f]. Among them, Yoon et al. constructed a highly selective tricarbocyanine-based ratiometric NIR fluorescent probe for Cys [12b], while Chen et al. found that double acrylate moieties could effectively enhance the selectivity of the probe to Cys compared with the single acrylate-containing analog [12c]. Disappointingly, most of the acrylate-based probes for Cys were studied in mixed solvents such as ethanol–PBS, and the reactivity and selectivity of these probes still have much room for improvement (Table 1). Moreover, detailed information about the reactivity and selectivity of the acrylate group has not yet been addressed in the literature. To solve the problem above, in this paper, we will describe two Cys-targeted probe isomers and discuss the effect of electronic characteristics and steric hindrance on their performance.

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Table 1
The comparisons of reported probes for Cys.

Fluorescence change	LOD (μM)	Testing media	Color change	Reaction time	Applications	Ref.
Ratiometric change from 377 nm to 487 nm	0.11	EtOH/phosphate buffer (20 mM, pH 7.4; 2:8, v/v)	NR	40 min	Detection of Cys in diluted (10%) deproteinized human plasma	[12a]
Ratiometric change from 740 nm to 560 nm	NR	EtOH: HEPES (1:9, pH 7.4, 0.01 M)	From light blue to red	30 min	Fluorescent imaging in MCF-7 cells	[12b]
Turn-on at 515 nm	0.077	EtOH/phosphate buffer (20 mM, pH 7.4, 2:8, v/v)	NR	15 min	Fluorescent imaging in PC-12 cells.	[12c]
Turn-on at 650 nm	0.0309	DMSO/HEPES buffer, 7:3	From red to hyacinthine	10 min	Detection of Cys in newborn-calf serum; fluorescent imaging in MCF-7 cells and mice.	[12d]
Ratiometric change from 430 nm to 547 nm	0.08	EtOH/PBS (20 mM, pH 7.4, 1:9, v/v)	From colorless to yellow	10 min	Fluorescent imaging in living RAW 264.7 macrophage cells.	[12e]
Turn-on at 610 nm	0.3	Triton X-100–PBS (pH = 7.4, 10 mM)	From yellow to purple	10 min	Fluorescent imaging in HeLa cells and zebrafish (without additional Triton X-100)	This study

NR: not reported.

2. Materials and methods

2.1. Materials

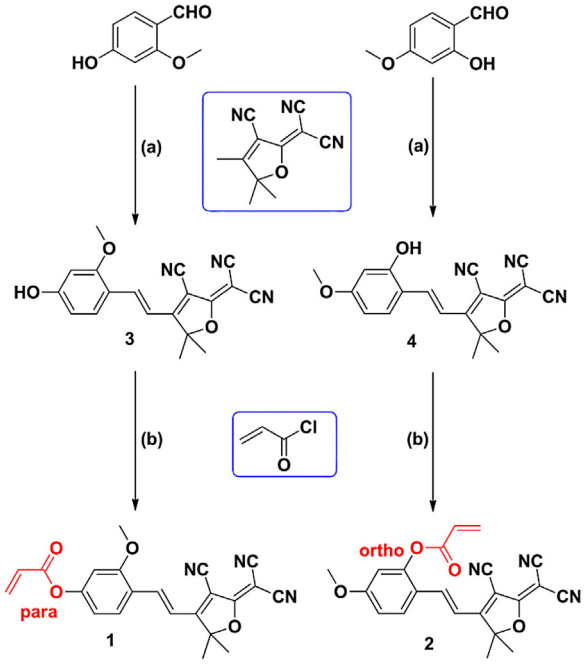
^{1}H NMR, ^{13}C NMR spectra were measured on a Bruker AM400 NMR spectrometer. Proton chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (1H, 0.00 ppm). ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics Bio TOF mass spectrometer, respectively. All pH measurements were performed with a digital pH-meter with a combined glass-calomel electrode. Fluorescence emission spectra were obtained at 298 K. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

2.2. Preparation and characterization of **3**

The synthesis route was depicted in Scheme 1. To a solution of 4-hydroxy-2-methoxybenzaldehyde (152 mg, 1.0 mmol) in a mixture of THF/EtOH (4 mL/1 mL) were added 2-(3-cyano-4,5,5-trimethylfuran-2(*5H*)-ylidene)-malononitrile (199 mg, 1.0 mmol) and ammonium acetate (77 mg, 1.0 mmol) at 25 °C. The mixture was stirred at 25 °C for 6 h and a brownish red precipitate was obtained. The precipitate was filtered and washed with cold EtOH for three times. Drying the precipitate in vacuum afforded 3 without further purification (270 mg, 69.6%). ^{1}H NMR (400 MHz, DMSO) δ 10.82 (s, 1H), 8.18 (d, J = 16.2 Hz, 1H), 7.84 (d, J = 9.1 Hz, 1H), 7.13 (d, J = 16.2 Hz, 1H), 6.53 (d, J = 7.5 Hz, 2H), 3.88 (s, 3H), 1.74 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ 177.9, 177.0, 165.0, 162.3, 144.3, 133.1, 115.3, 113.6, 112.7, 112.1, 109.9, 99.8, 99.1, 94.8, 56.5, 52.9, 25.8. HRMS calcd for $C_{19}H_{15}N_3O_3$ [M+Na]$^+$: 356.1006, found: 356.0999.

2.3. Preparation and characterization of **1**

To a solution of **3** (100 mg, 0.3 mmol) and 0.3 mL DIPEA in 15 mL CH_2Cl_2 at 0 °C, 0.4 mL acryloyl chloride (446.5 mg, 4.9 mmol) mixed with 5 mL CH_2Cl_2 was added dropwise. The reaction mixture was kept stirring at this temperature for 30 min. Then the mixture was warmed to room temperature and stirred for 4 h. 20 mL water was added to the solution and the mixture was extracted with CH_2Cl_2 (40 mL × 3). The combined organic layer was washed with saturated aqueous NaCl (50 mL) successively and dried over anhydrous Na_2SO_4. The solvent was removed under the reduced pressure and the residue was further purified by column chromatography



Scheme 1. Synthesis of probes **1** and **2**: (a) NH_4OAc, THF/EtOH = 4:1; (b) DIPEA, CH_2Cl_2.

over silica gel eluting with petroleum ether/CH_2Cl_2 to afford **1** (110 mg, 94.7%) as orange solid. ^{1}H NMR (400 MHz, DMSO) δ 8.18 (d, J = 16.5 Hz, 1H), 8.04 (d, J = 8.6 Hz, 1H), 7.33 (d, J = 16.5 Hz, 1H), 7.10 (s, 1H), 6.98 (dt, J = 8.6, 4.3 Hz, 1H), 6.62–6.54 (m, 1H), 6.47–6.40 (m, 1H), 6.21 (d, J = 10.3 Hz, 1H), 3.92 (s, 3H), 1.77 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ 40.50 (d, J = 21.0 Hz), 40.21 (s), 40.19 (s), 40.08 (d, J = 21.0 Hz), 39.77 (s), 39.56 (s), 39.35 (s), 25.41 (s). ^{13}C NMR (100 MHz, DMSO) δ 177.8, 176.2, 164.1, 160.4, 155.2, 142.1, 134.7, 131.5, 127.9, 121.1, 116.1, 114.3, 113.2, 112.4, 111.7, 106.9, 99.8, 98.4, 57.1, 54.6, 25.4. HRMS calcd for $C_{22}H_{17}N_3O_4$ [M+Na]$^+$: 410.1111, found: 410.1097.

2.4. Preparation and characterization of **4**

To a solution of 2-hydroxy-4-methoxybenzaldehyde (182.6 mg, 1.2 mmol) in a mixture of THF/EtOH (4 mL/1 mL) were added 2-(3-cyano-4,5,5-trimethylfuran-2(*5H*)-ylidene)-malononitrile (199 mg, 1.0 mmol) and ammonium acetate (92.4 mg, 1.2 mmol) at 25 °C. The mixture was stirred at 25 °C for 6 h and a red precipitate