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A sensitive fluorescent probe based on coumarin for detection of cysteine in living cells

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1. Introduction

In recent years, people pay much attention to small molecule thiols owing to their crucial roles in many physiological processes [1–8]. Compared with other amino acids, Cys plays numerous roles in maintaining biological systems because it can participate in reversible redox reactions and its cellular functions [9–13]. For example, Cys is related to detoxification, protein synthesis, and metabolism in live organisms [14–17]. The deficiency of Cys may lead to many syndromes, such as slowed growth, muscle and fat loss, liver damage, hematopoiesis reduction, hair depigmentation, skin lesions, weakness, lethargy, and edema protein synthesis, detoxification, and metabolism [18–20]. Therefore, to develop rapid and reliable methods for quantitative measurements of Cys is of great importance and has attracted much attention.

In the past few decades, large amount of effort have been made to find various methods for detecting of Cys quantitatively and selectively. These methods include electrochemical methods [21,22], high performance liquid chromatography [23–25], mass spectrometry [26,27], luminescent chemosensors [28,29], colorimetric detection [30], Gold Nanorods [31,32] and inductively coupled plasma emission spectrometry [33]. Among various detection techniques for Cys, fluorescent detection has been applied widely because of its high sensitivity, low cost, simplicity

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http://dx.doi.org/10.1016/j.jphotochem.2017.02.008 1010-6030/© 2017 Elsevier B.V. All rights reserved. ABSTRACT

Herein, we report a novel fluorescent probe based on coumarin to detect cysteine (Cys). The probe can react rapidly and selectively with Cys but not with the other amino acids. When the Cys was added to the solution of the probe, we could observe obvious color change from pale green to bright green. The detection limit of this probe for Cys was found to be $0.06 \,\mu$ M. Additionally, bioimaging of intracellular Cys by this probe was successfully applied in living cells, which indicating that this probe holds great potential for biological applications.

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and suitability for bioimaging analysis [34–36]. Up to now, several fluorescent probes have been reported for detection of amino acid [37,38], but most of them showed low selectivity for Cys. Thus we should focus on the study of the fluorescence probes that have high selectivity and sensitivity for Cys.

As traditional fluorescent dye, coumarin-based derivatives have strong fluorescence. Because they have high quantum stability and photoluminescence quantity, they are widely used in the detection of fluorescent probes. In this work, a coumarin-based fluorescent probe was designed and synthesized for the detection of Cys. This probe shows good selectivity and sensitivity for Cys. More importantly, it has used in fluorescence imaging of intracellular Cys in living cells. The synthetic route of probe **BTCA** is shown in Scheme 1.

2. Experimental

2.1. Materials and measurements

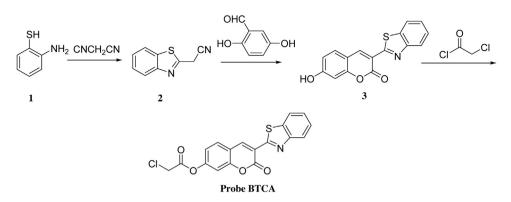
All reagents and solvents were purchased from commercial sources and used without further purification, unless otherwise stated. Double distilled water was used throughout the work. All reactions were monitored using thin-layer chromatography (TLC). ¹H NMR and ¹³C NMR spectra were recorded on a Brucker AN–400 MHz instrument for solutions in CDCl₃ or DMSO-*d*₆, using TMS as an internal reference. Electron impact mass spectra were conducted on MAT-212 spectrometer. Elemental analyses were done at Vario EL III. Ultraviolet-visible (UV–vis) absorption spectra were measured with a Varian Cary 50 spectrophotometer at 1 cm



Invited paper







Scheme 1. The synthetic routes of probe BTCA.

of the light path length. Fluorescence spectra were recorded on Varian cary Eclipse fluorescence spectrophotometer with an excitation wavelength of 404 nm.

2.2. Synthesis

2.2.1. Synthesis of compound 2

Compound **2** was synthesized according to a previous described procedure [39]. Acetic acid (0.4 mL) was added to an absolute ethanol (10 mL) solution of 2-aminothiophenol (510 mg, 4.1 mmol) and malononitrile (270 mg, 4.1 mmol). The mixture was refluxed at room temperature for 6 h until TLC indicated the end of the reaction. Then the solid product was filtered and crystallized from ethanol to afford a yellow product. Mp: 99–101 °C. ¹H NMR (400 MHz, CDCl₃) δ : 8.09–8.04 (m, 1H), 7.93–7.89 (m, 1H), 7.55 (ddd, *J* = 8.3, 7.3, 1.3 Hz, 1H), 7.49–7.44 (m, 1H), 4.26 (s, 2H).

2.2.2. Synthesis of compound 3

Compound **3** was synthesized according to a previous described procedure [40].Compound **2** (440 mg, 2.5 mmol) and 2,4-dihy-droxybenzaldehyde(350 mg, 2.5 mmol) were dissolved in 5 mL of absolute ethanol. Then the five drops of piperidine were added under stirring condition. The mixture was stirred at room temperature overnight. After filtration, the yellow solid was treated with 10% hydrochloric acid. The suspended solution was stirred at 100 °C overnight, and the resulting yellow residue was collected by filtration, washed with water, dried under reduced vacuum, and then crystallized from N,N-dimethylformamide to afford a yellow product. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 11.06 (s, 1H), 9.10 (s, 1H), 8.12 (d, *J* = 7.9 Hz, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 1H), 7.53 (t, *J* = 7.2 Hz, 1H), 7.42 (t, *J* = 7.3 Hz, 1H), 6.90 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.82 (d, *J* = 2.0 Hz, 1H).

2.2.3. Synthesis of probe 3-(benzo[d]thiazol-2-yl)-2-oxo-2Hchromen-7-yl 2-chloroacetate (**BTCA**)

To a solution of compound **3** (300 mg, 1 mmol) and triethylamine (303 mg, 3 mmol) in anhydrous CH_2Cl_2 (20 mL), 2-chloroacetyl chloride (282 mg, 2.5 mmol) mixed with anhydrous CH_2Cl_2 (10 mL) were added dropwise at -10 °C, and the resulting mixture was stirred at room temperature for 5 h until TLC indicated the end of the reaction. After removal of solvent, the residue was purified by silica gel column chromatography to afford the pure product. ¹H NMR (400 MHz, DMSO- d_6) δ : 9.27 (s, 1H), 8.18 (dd, J = 10.6, 8.4 Hz, 2H), 8.10 (d, J = 8.1 Hz, 1H), 7.59 (t, J = 7.2 Hz, 1H), 7.53–7.46 (m, 2H), 7.35 (dd, J = 8.5, 2.1 Hz, 1H), 4.77 (s, 2H). ¹³C NMR (100 MHz, DMSO d_6) ppm: 166.44, 160.11, 159.66, 154.40, 154.08, 152.38, 141.86, 136.40, 131.88, 127.19, 125.95, 123.00, 122.73, 119.59, 119.54, 117.65, 110.34, 55.38; HRMS (ESI) calcd. For $C_{18}H_{10}CINO_4S$ [M+Na]⁺: 393.9917; found: 393.9930

2.3. UV-vis and fluorescence measurements

A stock solution (50 μ M) of probe **BTCA** was prepared in DMSO. The stock solution of metal ions and amino acids was prepared in deionized water, and the concentration was 10 mM. Test solutions were prepared by placing 3 mL of the stock solution into a cuvette. All experiments were performed at room temperature.

2.4. Cells assay

Human Hela cells were cultured in DMEM (dulbecco's modified eagle medium) containing 10% fetal bovine serum in a humidified incubator at 37 °C and 5% CO₂. Cells were plated in 96-well plates and incubated with the probe **BTCA** concentration of 10 μ M for 24 h at 37 °C, washed with PBS 3 times. The fluorescence images were acquired by using a Nikon eclipse inverted fluorescence microscope.

3. Results and discussion

3.1. UV-vis and fluorescence and properties

In this work, the UV–vis absorption spectral of probe **BTCA** to Cys was researched in DMSO solution. As displayed in Fig. 1, probe **BTCA** exhibits an absorption peak with maximum at 370 nm and red shift to 394 nm after adding Cys into the solution of probe **BTCA**. Meanwhile, we can see the color of the complex solution changed from pale green to bright green. The obvious color change made it feasible to recognize of Cys in the naked-eye model.

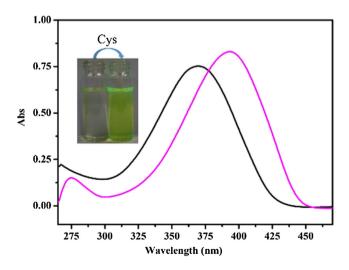


Fig. 1. (a) UV-vis spectra changes of probe BTCA upon the addition of Cys.

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