Dyes and Pigments 142 (2017) 167–174

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Reaction-based fluorescent turn-on probe for selective detection of thiophenols in aqueous solution and living cells

Xiao-Bo Wang, Xue Hao, Datong Zhang*, Yan Jiang

Shandong Provincial Key Laboratory of Fine Chemicals, School of Chemistry and Pharmaceutical Engineering, Qilu University of Technology, Jinan 250353, Shandong, PR China

ARTICLE INFO

Article history:
Received 27 December 2016
Received in revised form
15 March 2017
Accepted 15 March 2017
Available online 19 March 2017

Keywords:
Fluorescent probe
Coumarin 6H
2, 4-Dinitrophenlate
Thiophenols
Cell imaging

ABSTRACT

A novel turn-on florescent probe based on a nucleophilic substitution reaction was developed for the detection of thiophenols in aqueous solution and in living cells. In this probe, 10-hydroxyl derivative of coumarin 6H was selected as fluorophore, 2, 4-dinitrophenlate moiety acted simultaneously as recognition unit and fluorophore quencher. This probe features a remarkable large Stokes shift (128 nm) and shows a highly selective detection process for thiophenols with significant fluorescence turn-on response. Notably, biothiols, aliphatic thiols, amino acids and reducing anions do not interfere with the sensing of thiophenols. The probe shows good linearity ranges with low detection limit of 36 nM for thiophenols. More importantly, it was successfully applied for practical detection of thiophenols in real water samples with a good recovery and imaging of thiophenols in living cells, demonstrating its practical application in environmental samples and biological systems.

1. Introduction

Thiols including aliphatic thiols and thiophenols are an important class of organosulfur compounds in both the biological systems and chemical industry. Aliphatic thiols, such as cysteine (Cys), homocysteine (Hcy), glutathione (GSH) et al., play crucial roles in physiological processes [1–3]. Thiophenols have a broad synthetic utility in preparation of pharmaceuticals, pesticides and polymers [4,5]. However, as a class of highly toxic and pollutant compounds, thiophenols have been listed as one of the prioritized pollutants by the United States Environmental Protection Agency (USEPA waste code: P014) [6]. Due to their high toxicity and ease of entrance into human body by inhalation and skin absorption, the exposure to thiophenols liquid or vapor may induce grave systemic and central nervous injuries, even death [7–9]. Existence of thiophenols in soil and water are also reported to cause damage to natural surroundings [10]. Therefore, it is of great significance to establish a simple, highly sensitive and selective method to detect thiophenols in the fields of chemical, environmental and biological sciences. It is more important to develop efficient techniques to discriminate thiophenols from aliphatic thiols.

The field of sensing and imaging agents is growing fast, largely driven by the development of optical techniques. In particular, the method based on fluorescent probe has gained immense attention due to its high sensitivity, operational simplicity and non-destructive analysis et al. [11–15]. Considerable efforts have been devoted to develop fluorescent probes for thiophenols. Some of them can discriminate thiophenols from aliphatic thiols. However, among these reported fluorescent thiophenols probes, some still have several drawbacks including relatively weak fluorescence intensity and low sensitivity [16–22]. Furthermore, it can be seen from these reports that biothiols, such as Cys, GSH, and Hcy, which have similar physical and chemical properties with thiophenols, often interfere with the detection of thiophenols [23–25]. Due to the high level of biothiols in most cells and tissues (the intracellular concentration for Cys: 30–200 μM [26]; for GSH: 1–10 mM [27]), it is crucial to adjust the reactivity of the recognition unit in development of thiophenols selective probes for *in vivo* imaging.

A variety of thiophenols fluorescent probes have been developed by utilizing the high nucleophilic reactivity of thiol groups [28–35]. In many of these probes strongly electro-withdrawing 2, 4-dinitrobenzene-sulfonyl (DNBS) group has often been used as the recognition unit by getting attached with the fluorophore [32–35]. Aliphatic thiols are often capable of detaching the DNBS moiety partially from the fluorophore due to the high lability of DNBS toward -SH group, making discrimination of thiophenols from

* Corresponding author.
E-mail address: dtzhang@qlu.edu.cn (D. Zhang).

http://dx.doi.org/10.1016/j.dyepig.2017.03.033
0143-7208/

aliphatic thiols difficult. Protection of the 10-hydroxyl of coumarin 6H (HOCOU) with DNBS produced **DNBS-HOCOU** which showed poor selectivity between thiophenols and Cys ($I/I_{cys} = 1.33$) (Fig. 1). We envisioned that the introduction of 2, 4-dinitrophenyl (DNP) group with reduced reactivity would be more effective in obtaining higher selectivity towards thiophenols. By masking the HOCOU with DNP group, **DNCOU** was generated. As expected, there was almost no fluorescence in the solution of **DNCOU** after treatment with Cys. In contrast the solution of **DNCOU** had a marked fluorescence enhancement ($I/I_{cys} = 59$) after the addition of thiophenols (Fig. 1). Herein, **DNCOU** has a promising prospect for highly selective detection of thiophenols. Encouraged by this observation, we further investigated its optical response towards other aliphatic thiols, nucleophilic anions, amino acids as well as relevant species. The results suggested that the probe possesses impressively high selectivity towards thiophenols, with low detection limit of 36 nM. Furthermore, **DNCOU** has been successfully applied for the quantitative detection of thiophenols in real water samples and fluorescent imaging of thiophenols in living cells.

2. Experimental

2.1. Materials and apparatus

All analytical grade chemicals and reagents were purchased from commercial suppliers, and were used without further purification. Reaction was monitored by thin-layer chromatography (TLC) silica gel plates (GF-254). Column chromatography was performed on silica gel (200–300 mesh). Absorption spectra were detected on a T6 New century spectrometer. Fluorescent emission spectra were collected on a Lengguang F97Pro Fluorospectrophotometer equipped with a xenon lamp as the excitation source. The ^1H and ^{13}C NMR spectra were measured on a Bruker AVANCE II 400 spectrometer using tetramethylsilane (TMS; $\delta = 0$ ppm) as internal standard. The ESI mass spectra were measured on an Agilent-6510-Q-TOF spectrometer. All pH measurements were made with a Model PHS-3C pH meter (Shanghai, China). The fluorescence images were acquired through Olympus FV1000 laser scanning microscope.

2.2. Synthesis of intermediates and ***DNCOU***

The synthesis route of **DNCOU** is shown in Scheme 1. Compound 1, 2, 3 and 4 were synthesized according to the reported procedures [36,37], and the detailed data were presented in the Supporting information.

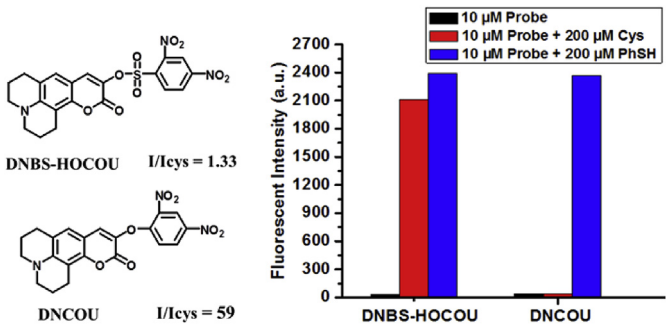

Fig. 1. Fluorescence intensity of **DNBS-HOCOU** (10 μM) and **DNCOU** (10 μM) after treatment with thiophenols (200 μM) or Cys (200 μM) for 50 min in PBS buffer (pH = 7.4, 10 mM) solutions containing 30% CH_3CN. I and I_{Cys} are the integrated fluorescence intensities after addition of thiophenols (200 μM) and Cys (200 μM).

2.2.1. Synthesis of ***HOCOU***

A suspension of compound 4 (300.0 mg, 1.17 mmol) in 5 mL of aqueous 1 M hydrochloric acid was refluxed under stirring for 4 h. After cooled and neutralized with ammonium hydroxide (25%), the reaction mixture was extracted with ethyl acetate. The organic layer was dried with anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel chromatography to give a yellow-green solid (263.6 mg, yield: 87.6%). ^1H NMR (400 MHz, $CDCl_3$): δ 6.92 (s, 1 H), 6.82 (s, 1 H), 5.76 (s, 1H), 3.21–3.25 (m, 4 H), 2.78–2.92 (m, 4 H), 2.01–2.05 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.79, 146.50, 142.92, 135.46, 123.16, 119.02, 115.78, 108.51, 107.15, 49.49, 49.11, 26.94, 21.15, 20.31, 20.02. HRMS (ESI) ($C_{15}H_{15}NO_3$) *m/z*: calculated for $[M+H]^+$: 258.1130. Found $[M+H]^+$: 258.1116.

2.2.2. Synthesis of ***DNCOU***

HOCOU (50.0 mg, 0.2 mmol) was dissolved in dry CH_3CN (2 mL). 1-Fluoro-2, 4-dinitrobenzene (55.8 mg, 0.3 mmol) and Et_3N (30 μL, 0.2 mmol) were added to the solution. The resultant solution was further stirred at room temperature for 6 h. After the reaction was completed, the mixture was washed by water and dried with anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residue was purified by column chromatography to give the title compound as a bright red solid (69.3 mg, yield: 80.7%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.86 (d, $J = 2.8$ Hz, 1 H), 8.32 (dd, $J = 2.4$ Hz, $J = 9.2$. Hz, 1 H), 7.53 (s, 1 H), 7.11 (d, $J = 9.2$ Hz, 1 H), 6.88 (s, 1 H), 3.29–3.33 (m, 4 H), 2.77–2.90 (m, 4 H), 1.99–2.09 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.13, 155.44, 150.25, 146.30, 141.65, 138.64, 133.16, 131.39, 128.69, 125.10, 122.25, 119.76, 117.64, 106.90, 50.05, 49.65, 27.53, 21.25, 20.36. HRMS (ESI) ($C_{21}H_{17}N_3O_7$) *m/z*: calculated for $[M+H]^+$: 424.1145. Found $[M+H]^+$: 424.1139.

2.3. Preparation of solutions of ***DNCOU*** and analytes

Stock solution of **DNCOU** (1 mM) was prepared in analytical grade CH_3CN. Stock solutions of PhSH, *p*-NH_2-PhSH, *p*-CH_3O-PhSH, CH_3CH_2SH, $HOOCCH_2SH$ and PhOH were prepared in CH_3CN (10 mM, respectively). Other analytes including phenylalanine (Phe), lysine (Lys), serine (Ser), proline (Pro), alanine (Ala), glycine (Gly), cysteine (Cys), glutathione (GSH), homocysteine (Hcy), KF, NaCl, KBr, KI, Na_2CO_3, AcONa, $NaNO_3$, Na_2S, Na_2SO_3, Na_2SO_4, KSCN, $Na_2S_2O_3$, $Na_2S_2O_4$, $NaNO_2$, $Co(NO_3)_3$, $Fe(NO_3)_3$, $Al(NO_3)_3$, $Zn(NO_3)_2$, $Ca(NO_3)_2$, $Mg(NO_3)_2$, and H_2O_2 were dissolved in deionized water to afford 10 mM aqueous solution. The stock solutions were used freshly and were diluted to desired concentrations when needed.

2.4. Measurements of fluorescence changes of ***DNCOU*** upon addition of various analytes

The hybrid solution of various analytes and **DNCOU** was prepared by adding 100 μL of **DNCOU** stock solution and an appropriate volume of each testing species to a 10 mL volumetric flask. PBS buffer (10 mM, pH 7.4) containing 30% CH_3CN was added to fill the volumetric flask. All spectra were recorded at room temperature ($\lambda_{ex} = 395$ nm). The incubation time was 50 min.

2.5. Cell viability study and fluorescence imaging

HepG2 cells were cultured in MEM (minimum essential medium) supplemented with 10% FBS (fetal bovine serum). The cells were digested with 0.25% Trypsin-EDTA when their confluence reached 90% and collected. After resuspended to 5×10^4 cells/mL, 100 μL of cells suspension was added to each well of 96-well plate and incubated overnight. Cells were treated with **DNCOU** (10 μM) for 4 h and 6 h, respectively. 100 μL of CCK8 was added to each well and incubated at 37 °C for 30 min. The optical absorbance was