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A novel cyanobiphenyl benzothiazole-based fluorescent probe for detection of biothiols with a large Stokes shift and its application in cell imaging

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ABSTRACT

An ESIPT-based fluorescent probe (Probe **1**) with a large Stokes shift (202 nm) for the sensing of biothiols has been developed based on cyanobiphenyl benzothiazole derivative exhibiting larger Stokes shift than 2-(benzothiazol-2-yl)phenol. This probe with 2,4-dinitrobenzenesulfonate (DNBS) as a highly thiol-selective group was constructed based on the combination of excited state intramolecular proton transfer (ESIPT) and photoinduced electron transfer (PET) mechanisms. Upon the treatment with biothiols, this probe produced a remarkable fluorescence enhancement at 482 nm. The detect limits for Cys, GSH and Hcy was calculated to be as low as 2.0×10^{-8} M, 1.7×10^{-7} M and 1.2×10^{-7} M, respectively (based on S/N = 3). Importantly, application of this probe was successfully demonstrated by imaging thiols in living NCI-H226 cells.

1. Introduction

Biological thiols, such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), have attracted much attention in recent years for their critical role in many biochemical pathways.[1–4] They participated in the process of reversible redox reactions to regulate the metabolism and cellular homeostasis. For example, the levels of Cys, Hcy and GSH in the plasmas were associated with various health problems such as hair depigmentation, lethargy, muscle loss, Alzheimer's, AIDS and Parkinson's diseases.[5–8] Thus, it is valuable to quantitatively assay these species in biological, medical, and clinical studies. Currently, the assay methods for biothiols detection include electrochemistry,[9] high-performance liquid chromatography,[10] spectrofluorimetry,[11] chemiluminescence.[12] However, expensive and sophisticated instrument or complicated procedures are generally associated with these detection methods. In contrast to these methods, fluorescence detection has become the dominant strategy for thiols sensing because of its high selectivity, low detection limit, operational simplicity and little damage to intact cells.[13–15]

Up to now, various fluorescent probes for thiols have been developed based on different strategies including cyclization reactions with aldehydes,[16–18] Michael additions, cleavage reactions,[19–21] and so on[22–24]. Among these approaches, the 2,4-dinitrobenzenesulfonate group (DNBS) as an efficient sensing moiety has been used to detect thiols with high selectivity against other thiol-free amino acids.[25–30] However, many of them still encounter some problems, such as low sensitivity, complicated synthesis and small Stokes shift. Thus, developing new fluorescent probes to monitor the existence of thiols in living cells remain to be a great challenge.

2-(Benzothiazol-2-yl)phenol and its derivatives were good candidates for the design of probes due to their intriguing optical properties such as good photostabilities, relatively high fluorescent quantum yield and good cell membrane permeability.[31–33] Importantly, upon excitation, these derivatives exhibited an excited state intramolecular proton transfer (ESIPT) process from the phenol form to the keto form, resulting in a large Stokes shift (138 nm).[34] It is known that fluorescent dyes with large Stokes shifts are more desirable for the application because they can improve the sensitivity by reducing the self-quenching and auto-fluorescence resulted from the minimal overlap of excitation and emission spectra.[35] Our research group investigated the optical properties of 3'-(benzothiazol-2-yl)-4'-hydroxy-4-biphenyl carbonitrile, **2**, and we found that dye **2** exhibited a 202 nm Stokes shift which was

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larger than that in 2-(benzothiazol-2-yl)phenol (Fig. 1). Furthermore, dye **2** exhibited cyan fluorescence and good photostability. These above traits suggested that dye **2** could serve as an ideal scaffold for the design of fluorescent probes.

In this work, based on our findings, we designed and synthesized probe **1** for the detection of biothiols based dye **2** using DNBS as sensing unit. The synthetic route of probe **1** is shown in Scheme 1. We speculated that the 2,4-dinitrobenzenesulfonate moiety acting as the electron acceptor in probe **1** would quench the fluorescent intensity of the fluorophore due to an effective photoinduced electron transfer (PET) process and the inhibition of the ESIPT process. When treated with biothiols, probe **1** would be converted into 4'-hydroxy-3'-(benzothiazol-2-yl)-4-biphenylcarbonitrile, dye **2**, which emits strong cyan fluorescence upon excitation via an ESIPT process (Scheme 2).

2. Experiment

2.1. Materials and equipment

Solvents were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Unless otherwise stated, all reagents were purchased from commercial suppliers and used as received. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from Qingdao Ocean Chemicals. NMR spectra were recorded on a BRUKER 600 spectrometer, HR-MS data were obtained with a MICROTOF-Q II mass spectrometer (BrukerDaltonik, Germany). UV–Vis absorption and fluorescence spectra were recorded on a Shimadzu UV-2450 spectrophotometer and a PerkinElmer LS45 fluorescence spectrophotometer with both the excitation and emission slit widths set at 2.5 nm, respectively. Cell imaging was performed with Axio observer A1.

2.2. Synthesis of 4'-hydroxy-3'-(benzothiazol-2-yl)-4-biphenylcarbonitrile *2*

In a 50 mL round-bottomed flask equipped with a magnetic stirrer, a solution of 3'-formyl-4'-hydroxy-4-biphenyl carbonitrile[36] (223.2 mg, 1.0 mmol), and 2-aminothiophenol (125.2 mg, 1.0 mmol) was prepared. Aq 37% HCl (55.0 mg, 1.0 mmol) and aq 30% H_2O_2 (102.0 mg, 1.0 mmol) were added and the mixture was stirred at room temperature for 3 h. The reaction was quenched with 100 mL of water. The resulting solid product was extracted twice with 60 mL portions of EtOAc and dried over anhydrous Na_2SO_4. After removing the solvent by distillation, the obtained residue was purified by flash chromatography on silica gel (6:1 hexane/ethyl acetate as eluent) to yield 4'-hydroxy-3'-(benzothiazol-2-yl)-4-biphenylcarbonitrile **2** as a yellow solid (206.9 mg, 63%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.17 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 2.9 Hz, 4H), 7.84 (d, J = 8.3 Hz, 1H), 7.57 (t, J = 7.4 Hz, 1H), 7.47 (t, J = 7.4 Hz, 1H), 7.24 (d, J = 8.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) 164.37, 156.79, 151.93, 144.38, 135.40, 131.43, 131.30, 129.96, 129.68, 127.48, 125.42, 123.11, 122.78, 119.94, 109.92. HRMS (EI) m/z calcd for $[C_{20}H_{12}N_2OS + H]^+$: 329.0749, Found: 329.0751.

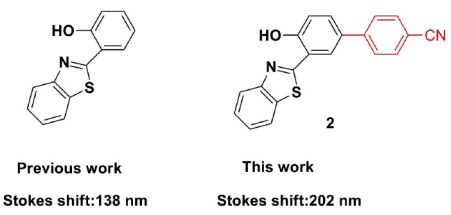
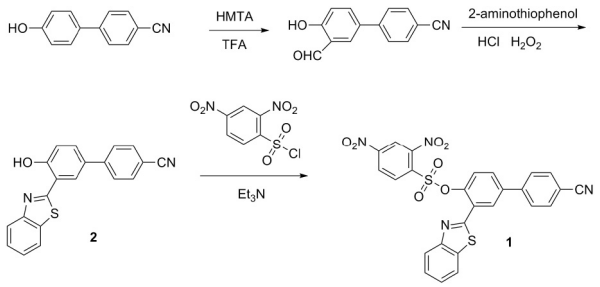
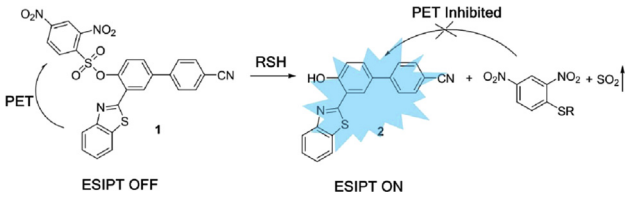


Fig. 1. Structure of 2-(Benzothiazol-2-yl)phenol and dye **2**.



Scheme 1. Synthetic route to probe **1**.



Scheme 2. Possible thiols-selective signaling mechanism.

2.3. Synthesis of probe *1*

To a stirred mixture of 4'-hydroxy-3'-(benzothiazol-2-yl)-4-biphenylcarbonitrile **2** (328.4 mg, 1.0 mmol) and Et_3N (31.0 mg, 1.3 mmol) in 20 mL dry CH_2Cl_2 under an argon atmosphere was added 2,4-dinitrobenzenesulfonyl chloride (347.0 mg, 1.3 mmol) at room temperature. The resulting reaction mixture was allowed to stir at room temperature for 3 h. The reaction was quenched with 50.0 mL of water and the resulting solution was extracted with twice with 50.0 mL portions of dichloromethane. After drying over anhydrous Na_2SO_4, the organic solvent was filtered and removed by distillation. The resulting residue was purified by silica gel column chromatography (3:1 hexane/ethyl acetate as eluent) to yield the desired product **1** (396.6 mg, 71%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.96 (d, J = 2.2 Hz, 1H), 8.42 (d, J = 2.4 Hz, 1H), 8.34 (dd, J = 8.7, 2.3 Hz, 1H), 8.20 (d, J = 8.7 Hz, 1H), 8.14 (d, J = 7.9 Hz, 1H), 8.06–7.98 (m, 6H), 7.59–7.51 (m, 2H), 7.51–7.45 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) 161.44, 152.69, 151.24, 148.03, 146.29, 142.63, 139.31, 135.77, 132.13, 132.02, 131.36, 128.49, 128.05, 127.62, 125.10, 122.64, 121.11, 111.50. HRMS (EI) m/z calcd for $[C_{26}H_{14}N_4O_7S_2 + H]^+$: 559.0374, Found: 559.0378.

3. Results and discussion

3.1. Spectroscopic studies

The spectral signaling behaviors of probe **1** and dye **2** (4'-hydroxy-3'-(benzothiazol-2-yl)-4-biphenylcarbonitrile) were investigated in HEPES-DMSO solution (50.0 mM, v/v = 4:1, pH = 7.4), respectively. Dye **2** displayed strong fluoresces (λ_{Abs} = 280 nm, λ_{em} = 482 nm) in cyan region (Φ = 0.20). However, due to an efficient PET process and prohibitions of ESIPT process produced by the 2, 4-dinitrobenzenesulfonate moiety, probe **1** was essentially non-fluorescent (Φ < 0.01) (Fig. 2 and Fig. S1).

In order to better understand the sensing process of probe **1**, the fluorescence spectra changes of probe **1** (10.0 μM) were measured with various concentrations of Cys in HEPES-DMSO solution