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A novel *p*-aminophenylthio- and cyano- substituted BODIPY as a fluorescence turn-on probe for distinguishing cysteine and homocysteine from glutathione

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ABSTRACT

Biothiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) play vital roles in various physiological and pathological processes. In this work, a BODIPY-based fluorescent probe **XCN** was synthesized from multi-step reactions. We first synthesized a BODIPY derivative with a cyano and a bromine moiety attached to the 8-diphenylaminophenyl substituent of BODIPY, followed by the reaction with *p*-aminothiophenol under basic condition. Interestingly, compound **XCN** was successfully obtained with the *p*-aminophenylthio moiety introduced into one of the α-positions of the pyrrolic units. This reaction may compose an efficient approach for synthesizing novel BODIPY derivatives with substituents attached to the pyrrolic unit without previously brominating it. **XCN** can be used as a fluorescence turn-on probe to selectively detect Cys and Hcy using the cyano group as the recognition site, with the *p*-aminophenylthio moiety left unreacted. **XCN** was found to be nearly nonfluorescent, and it exhibits only slight fluorescence enhancement when treated with GSH. However, upon interaction with Cys or Hcy, the fluorescence was enhanced by 1081 and 1126 folds, respectively. In addition, **XCN** exhibits good selectivity and sensitivity towards Cys and Hcy over GSH and other amino acids in a wide pH range from 2 to 10 in aqueous buffers. Furthermore, **XCN** was successfully used for imaging biothiols in living A549 lung cancer cells.

1. Introduction

In recent years, biothiols like cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) have attracted extensive interest because of their vital roles in a variety of physiological processes [1–5]. Abnormal levels of the three species may cause diseases. For example, Cys deficiency may result in syndromes like liver damage, slower development of children, detoxification weakening and skin lesions [6–8]. Abnormal concentration of Hcy may be a sign for cardiovascular diseases [9,10]. Lack of GSH may change intracellular redox state and lead to severe diseases such as cancer, and Alzheimer's [11–14]. Hence, it is of great importance to qualitatively and quantitatively monitor these biothiols. Among various techniques, fluorescent probes have been demonstrated to be powerful tools with the advantages of simplicity, high sensitivity and intracellular bioimaging capacity.

So far, a number of fluorescent probes have been designed and synthesized to detect the three biothiols. These probes are mostly reaction-based, utilizing mechanisms like nucleophilic substitution, Michael addition, and cyclization reactions with aldehydes and other functional groups [15–23]. However, it is still a great challenge to discriminate each of the three biothiols because of their similar structures and reactivity. Only a few reported sensors can be used to distinguish Cys, Hcy and GSH from one another. In this respect, Yang and coworkers reported a BODIPY-based ratiometric fluorescent sensor, which could selectively discriminate Cys and Hcy from GSH taking advantage of the nucleophilic attack of the thiol moiety followed by the displacement with the amino group to regenerate the thiol moiety, while the 2nd step was not observed for GSH [24]. Later the same group reported another probe for selectively detecting Cys over Hcy by means of different rates of the intramolecular displacement reactions [25]. Yoon and coworkers reported a biothiol probe based on

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nitrobenzothiadiazole substituted with a *p*-aminophenylthio moiety, which also could selectively detect Cys and Hcy based on their nucleophilicity [26]. Besides, Liang and coworkers have reported a fluorescent probe, utilizing a cyano group as the recognition moiety, which could distinguish Cys from the other two [27].

Inspired by the excellent studies mentioned above, we aimed to design and synthesize fluorescent probes to selectively detect the biothiols. Herein, we report the synthesis of a fluorescent probe **XCN** (Scheme 1) by introducing a *p*-aminophenylthio and a cyano group into a BODIPY moiety. Interestingly, **XCN** can be used as a fluorescence turn-on probe to selectively detect Cys and Hcy over GSH using the cyano group as the recognition site, with the *p*-aminophenylthio moiety left unreacted. Furthermore, **XCN** was successfully used for imaging biothiols in living A549 lung cancer cells.

2. Experimental section

2.1. Materials and instrumentation

Commercially available solvents and reagents were used as received. Water was used after redistillation. Deuterated solvents for NMR measurements were available from Aldrich. UV- vis absorption spectra were recorded on a Varian Cary 100 spectrophotometer and fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer, with a quartz cuvette (path length = 1 cm); both spectrophotometers were standardized. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker AM 400 spectrometer with tetramethylsilane (TMS) as the internal standard. High resolution mass spectra (HRMS) were measured on a Waters LCT Premier XE spectrometer. Confocal laser scanning microscope (CLSM) images were taken on an inverted fluorescence microscope (Nikon A1R/A1).

2.2. pH influence measurements

pH influence measurements were carried out in the mixtures of DMSO and the following buffers (2/1, v:v): Na_2HPO_4-citric acid buffer (20 mM, pH 2.0, 3.0, 4.0, 5.0), Na_2HPO_4-KH_2PO_4 buffer (20 mM, pH 6.0, 7.0, 7.4), glycine-NaOH buffer (50 mM, pH 9.0, 10.0), Na_2HPO_4-NaOH buffer (20 mM, pH 12.0).

2.3. Cell culture

Human lung adenocarcinoma A549 cells were supplied by the Institute of Cell Biology (Shanghai, China). The cell lines were cultured at 37 °C under a humidified 5% CO_2 atmosphere in the RPMI-1640 medium (GIBCO/Invitrogen, Camarillo, CA, USA) supplemented with 10% fetal bovine serum (FBS, Biological Industry, Kibbutz Beit Haemek, Israel) and 1% penicillin-streptomycin (10,000 U/mL penicillin and 10 mg/mL streptomycin, Solarbio life science, Beijing, China).

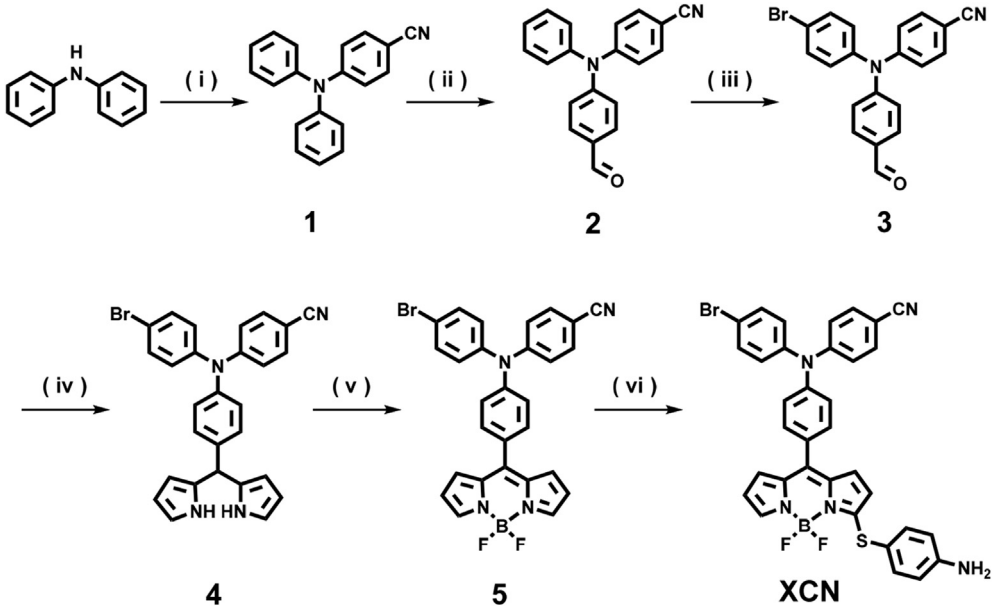
2.4. Syntheses of the compounds

2.4.1. Synthesis of compound ***1***

Diphenylamine (5.42, 32.0 mmol), 4-iodobenzonitrile (7.33, 32.0 mmol), $Pd_2(dba)_3$ (660 mg, 0.720 mmol), BINAP (678 m, 1.09 mmol), t-BuONa (10.8 g, 112 mmol) and xylene (240 mL) were added into a 500 mL three-neck flask. The mixture was stirred for 24 h at 120 °C under nitrogen. Xylene was removed under reduced pressure and the residue was purified on a silica gel column using CH_2Cl_2/PE (1/2, v:v) as the eluent to give a pale solid (5.23 g, yield 60.5%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.41 (d, J = 8.8 Hz, 2H), 7.33 (t, J = 8.0 Hz, 4H), 7.18–7.19 (m, 6H), 6.96 (d, J = 8.8 Hz, 2H).

2.4.2. Synthesis of compound ***2***

To the solution of compound **1** (5.00 g, 18.5 mmol) in dry 1,2-dichloroethane (300 mL), was added the Vilsmeier reagent freshly prepared from the reaction of *N,N*-dimethylformamide (DMF, 23.0 mL) with $POCl_3$ (14.0 mL, 185 mmol). The mixture was stirred at reflux for 24 h under nitrogen. The reaction mixture was cooled, washed with water and extracted with CH_2Cl_2. The organic solvent was evaporated to dryness and the residue was purified on a silica gel column using CH_2Cl_2/PE (1/2, v:v) as the eluent to give a canary yellow solid (1.66 g, yield 30.2%). ^{1}H NMR ($CDCl_3$, 400 MHz,



Scheme 1. Synthetic route of probe **XCN**. (i) 4-Iodobenzonitrile, $Pd_2(dba)_3$, BINAP, t-BuONa, xylene, 120 °C, 60.5%; (ii) $POCl_3$, DMF, $ClCH_2CH_2Cl$, 60 °C, 30.2%; (iii) NBS, CH_2Cl_2, 85.0%; (iv) pyrrole, TFA, 57.3%; (v) (a) DDQ; (b) Et_3N, $BF_3{\cdot}Et_2O$, 63.2%; (vi) *p*-aminothiophenol, Et_3N, THF, reflux, 23.6%.