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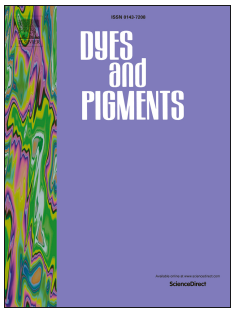

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PII: S0143-7208(17)31916-2

DOI: 10.1016/j.dyepig.2017.09.020

Reference: DYPI 6244

To appear in: *Dyes and Pigments*

Received Date: 14 August 2017

Revised Date: 8 September 2017

Accepted Date: 9 September 2017

Please cite this article as: Wang Q, Wei X, Li C, Xie Y, A novel *p*-aminophenylthio- and cyano-substituted BODIPY as a fluorescence turn-on probe for distinguishing cysteine and homocysteine from glutathione, *Dyes and Pigments* (2017), doi: 10.1016/j.dyepig.2017.09.020.

Graphical Abstract

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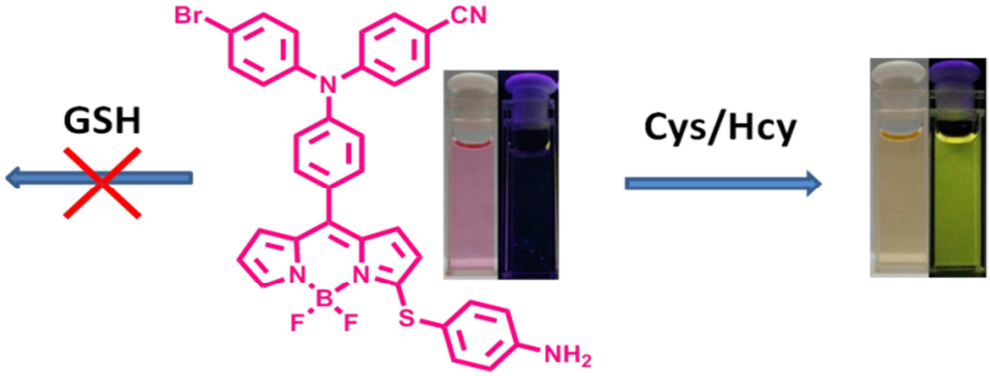

A novel *p*-aminophenylthio- and cyano- substituted BODIPY as a fluorescence turn-on probe for distinguishing cysteine and homocysteine from glutathione

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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.

Keywords: Fluorescent probes; Biothiols; BODIPY; Cell imaging.

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 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].

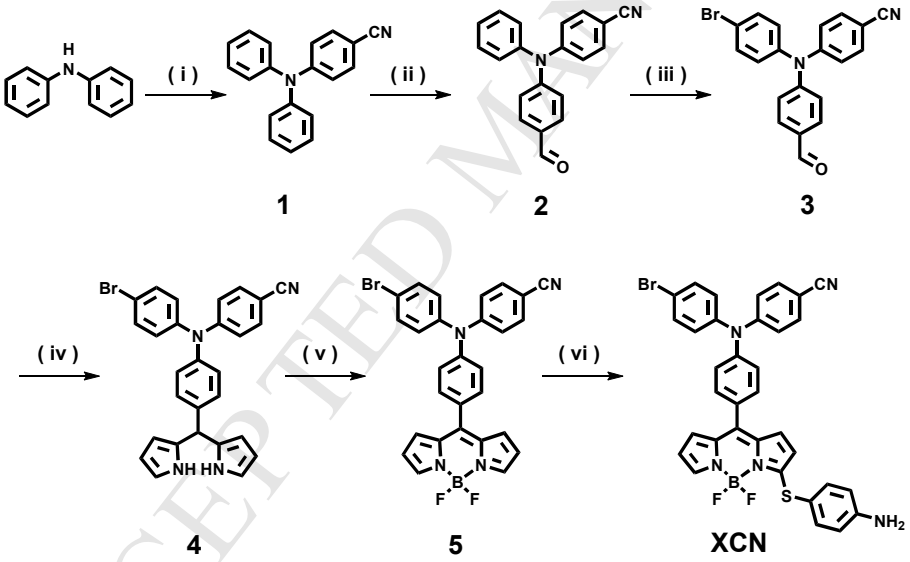
Inspired by the excellent studies mentioned above, we aimed to design and synthesize fluorescent probes to selectively detect the biothiols. Herein, we report the synthesis of a fluorescent probe **XCN** (Scheme 1) by introducing a *p*-aminophenylthio and a cyano group into a BODIPY moiety. Interestingly, **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).



(i) 4-Iodobenzonitrile, $Pd_2(dba)_3$, BINAP, t-BuONa, xylene, 120□, 60.5%; (ii) $POCl_3$, DMF, $ClCH_2CH_2Cl$, 60□, 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%.

Scheme 1 Synthetic route of probe **XCN**

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 ºC 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 hours at 120 □ 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 hours 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, ppm): δ 9.89 (s, 1H, -CHO), 7.77 (d, *J*=8.8 Hz, 2H, ph-H), 7.52 (d, *J*=8.8 Hz, 2H, ph-H), 7.40 (t, *J*=8.0 Hz, 2H, ph-H), 7.25 (t, *J*=7.6 Hz, 1H, ph-H), 7.18-7.13 (m, 6H, ph-H).

*2.4.3 Synthesis of compound **3***

To a solution of compound **2** (248 mg, 0.83 mmol) in dichloromethane (30 mL), *N*-bromosuccinimide (NBS, 236 mg, 1.33 mmol) was added gradually. The reaction mixture was stirred at room temperature for 4 hours. Then, the mixture was washed with water, extracted with dichloromethane and dried over Na_2SO_4. Then the solvent was removed under reduced pressure and the residue was purified on a silica gel column using CH_2Cl_2/PE (1/1, v:v) as the eluent to give a brown solid (266 mg, yield 85.0%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 9.89 (s, 1H, -CHO), 7.77 (d, *J*=8.4 Hz, 2H, ph-H), 7.53 (d, *J*=8.8 Hz, 2H, ph-H), 7.48 (d, *J*=8.4 Hz, 2H, ph-H), 7.14 (d, *J*=8.8 Hz, 2H, ph-H), 7.11 (d, *J*=8.8 Hz, 2H, ph-H), 7.01 (d, *J*=8.8 Hz, 2H, ph-H).

*2.4.4 Synthesis of compound **4***

Compound **3** (262 mg, 0.690 mmol) and pyrrole (5.00 mL, 34.8 mmol) were added into a 100 mL flask. The reaction mixture was stirred at room temperature for 30 min after trifluoroacetic acid (TFA, 156 μL, 2.00 mmol) was added. Then triethylamine (2.00 mL) was added into the flask to quench the reaction. Then the solvent was removed under reduced pressure and the residue was purified on a silica gel column using CH_2Cl_2/PE (2/3, v:v) as the

eluent to give a brown solid (195 mg, yield 57.3%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.0 (s, 2H, -NH), 7.43 (d, *J*=8.4 Hz, 4H, ph-H), 7.18 (d, *J*=8.0 Hz, 2H, ph-H), 7.06-6.95 (m, 6H, ph-H), 6.73 (s, 2H, pyrrolic), 6.17 (s, 2H, pyrrolic), 5.92 (s, 2H, pyrrolic), 5.46 (s, 1H, *meso*-H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 157.52, 150.76, 147.43, 145.74, 142.84, 138.77, 134.60, 132.61, 132.39, 131.06, 128.33, 126.37, 126.16, 119.41, 118.08, 116.72, 115.31, 55.56. HRMS (ESI, m/z): $[M+H]^+$ calcd for $C_{28}H_{20}BrN_4$: 491.0871, found: 491.0871.

*2.4.5 Synthesis of compound **5***

Compound **4** (195 mg, 0.390 mmol) was added into a 100 mL three-neck flask, followed by addition of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (108 mg, 0.470 mmol) under nitrogen. Then triethylamine (326 μL, 2.34 mmol) was added into the flask and stirred for 5 min at room temperature. Next, $BF_3 \cdot Et_2O$ (294 μL, 2.34 mmol) was added, and the reaction mixture was stirred for 6 hours. After that, the mixture was washed with sodium bicarbonate solution and extracted with dichloromethane for three times, followed by washing with water and extraction with dichloromethane. Then the solvent was removed under reduced pressure and the residue was purified on a silica gel column using CH_2Cl_2/PE (1/1, v:v) as the eluent to give an orange solid (133 mg, yield 63.2%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.94 (s, 2H, pyrrolic), 7.56-7.52 (m, 6H, ph-H), 7.22 (d, *J*=8.4 Hz, 2H, ph-H), 7.15 (d, *J*=8.4 Hz, 2H, ph-H), 7.09 (d, *J*=8.4Hz, 2H, ph-H), 7.01 (d, *J*=3.6 Hz, 2H, pyrrolic), 6.58 (s, 2H, pyrrolic). HRMS (ESI, m/z): $[M+H]^+$ calcd for $C_{28}H_{19}BBrF_2N_4$: 539.0854, found: 539.9557.

*2.4.6 Synthesis of probe **XCN***

p-Aminothiophenol (250 mg, 2.00 mmol), triethylamine (420 μL) and tetrahydrofuran (8.00 mL) were added into a 100 mL three-neck flask and stirred for 15 min. Then,

compound **5** (539 mg, 1.00 mmol) was added into the flask, and the mixture was refluxed for 16 hours under nitrogen. After that, the solvent was removed under reduced pressure and the residue was purified on a silica gel column using CH_2Cl_2/PE (1/1, v:v) as the eluent to give unreacted compound **5** (178 mg) and an amaranthine solid of **XCN** (92.0 mg, yield 23.6%). ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ 7.79 (s, 1H, pyrrolic), 7.70 (d, *J*=8.8 Hz, 2H, ph-H), 7.61 (d, *J*=8.8 Hz, 2H, ph-H), 7.58 (d, *J*=8.4 Hz, 2H, ph-H), 7.29 (d, *J*=8.8 Hz, 2H, ph-H), 7.22 (d, *J*=8.4 Hz, 2H, ph-H), 7.19 (d, *J*=8.8 Hz, 2H, ph-H), 7.11 (d, *J*=8.8 Hz, 2H, ph-H), 7.03 (d, *J*=4.4 Hz, 1H, pyrrolic), 6.82 (d, *J*=3.6 Hz, 2H, pyrrolic), 6.66 (d, *J*=8.8 Hz, 2H, ph-H), 6.55 (dd, J_1=4.0 Hz, J_2=2.4 Hz, 1H, pyrrolic), 5.92 (d, *J*=4.8 Hz, 1H, pyrrolic), 5.79 (s, 2H, -NH_2). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm): δ 165.22, 150.52, 148.66, 147.73, 144.72, 140.11, 138.98, 137.17, 137.04, 133.57, 133.41, 133.27, 132.02, 131.63, 131.23, 129.91, 128.04, 126.64, 123.85, 121.92, 119.49, 119.11, 118.90, 116.50, 115.88, 115.45, 115.30, 104.99. HRMS (ESI, m/z): $[M+H]^+$ calcd for $C_{34}H_{24}N_5SBrF_2B$: 662.0997, found: 662.1005.

3. Results and discussion

3.1 Design and syntheses

As mentioned above, BODIPY has advantages in developing fluorescent probes, and both the cyano and *p*-aminophenylthio groups may be utilized as the reaction site to selectively detect biothiols based on their specific reactions with biothiols. Inspired by the successful examples [26-27], we herein synthesized a new fluorescent probe for biothiols by introducing both of the reaction moieties into a BODIPY platform. Thus, we first synthesized a BODIPY derivative **5** with a cyano and a bromine moiety attached to the 8-diphenylaminophenyl

substituent of the BODIPY fluorophore (Scheme 1), followed by the reaction with *p*-aminothiophenol under basic condition. Interestingly, the *p*-aminophenylthio moiety was not attached to the 8-diphenylaminophenyl moiety by replacing the bromine atom. Instead, compound **XCN** was obtained with the *p*-aminophenylthio moiety attached to one of the α-positions of the pyrrolic units, which can be clearly evidenced by the molecular ion peak at 662.1005 and the isotope pattern characteristic for the presence of the bromine atom in the HRMS (Fig. S11). Consistent to this, one of the two pyrrolic α-protons disappeared in the ^{1}H NMR spectrum of **XCN**, as compared to that of compound **5** (Fig. 1). In addition, the remaining α-proton of **XCN** was up-field shifted to δ = 7.79 ppm because of the electron donating effect of the *p*-aminophenylthio moiety. The two protons of the amino group exhibit a singlet peak at 5.79 ppm, which disappears upon treatment with D_2O (Fig. S12). In addition, from the two-dimensional COSY and NOESY spectra of **XCN**, five pyrrolic protons can be identified, and the H1-H6, H6-H7 and H7-H8 couplings are clearly observed (Fig. S13, S14). All these observations unambiguously indicate that the *p*-aminophenylthio group was attach to the α-position of the pyrrole unit.

This reaction may compose an efficient approach for synthesizing novel BODIPY derivatives with substituents attached to the pyrrolic unit without previously brominating it. The synthesized compound **XCN** was found to be nearly nonfluorescent, which is a good starting point for developing fluorescence turn-on probes. Hence, we continued to check the biothiol sensing behavior of **XCN** and elucidate the respective roles of the *p*-aminophenylthio and the cyano moieties.

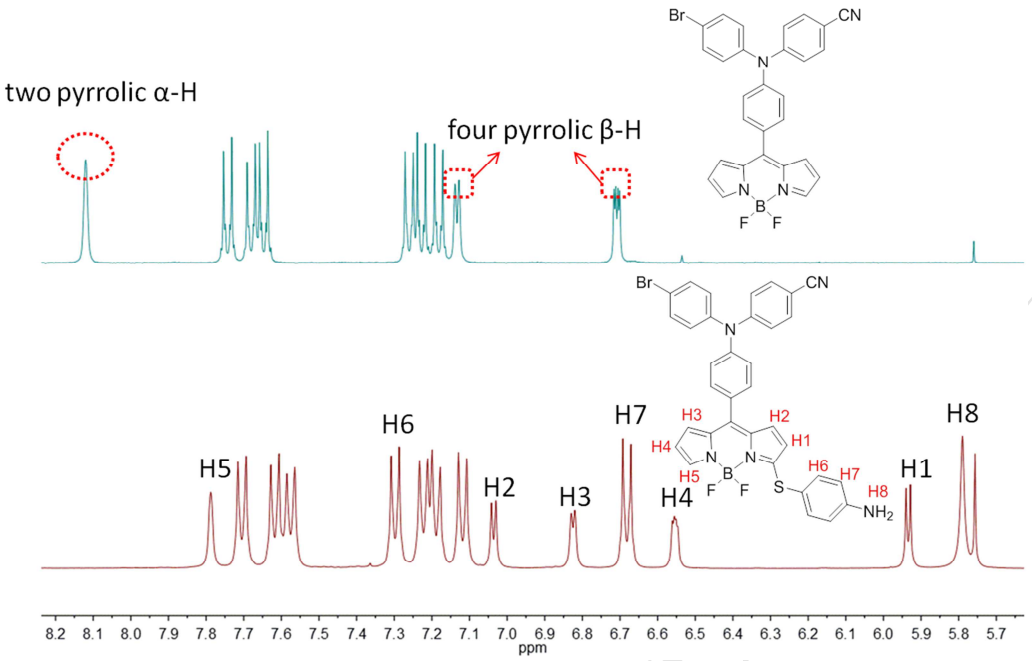


Fig. 1 The low field region of the ^{1}H NMR spectra of **5** and **XCN** in DMSO-d_6.

3.2 Spectroscopic Characteristics

After **XCN** was successfully synthesized, we firstly checked its spectral responses to Cys, Hcy and GSH. As shown in Fig. 2a, the UV-vis spectrum of free **XCN** showed an absorption peak at 546 nm. This band decreased sharply upon gradual addition of Cys, which was accompanied with the development of a new peak at 480 nm. As shown in Fig. 2b, **XCN** is nearly nonfluorescent. Upon adding Cys, a dramatic fluorescence enhancement was observed with an emission peak at 549 nm. The addition of Hcy to the solution of **XCN** induced absorption spectral changes and fluorescence enhancement similar to those observed for Cys (Fig. S15a, S15b). On the other hand, the treatment of **XCN** with GSH only caused slight fluorescence enhancement (Fig. S16). The results indicated that **XCN** can be used to discriminate Cys and Hcy from GSH. The kinetics study of **XCN** in the presence of Cys and Hcy was carried out at 37 □. As shown in Fig. 2c and S15c, upon addition of Cys and Hcy,

the fluorescence intensity approached their maxima within *ca*. 5 min, which indicated fast response of **XCN** towards Cys and Hcy [28-29]. When the fluorescence emission intensity of **XCN** was plotted as a function of Cys and Hcy concentration, good linear relationships were obtained in the ranges of 0.25-0.50 mM and 0-0.25 mM, respectively (Fig. S18, S19). Thus, the detection limits for Cys and Hcy were calculated to be 3.1 μM and 1.6 μM, respectively, according to the 3σ/k formula [30-33].

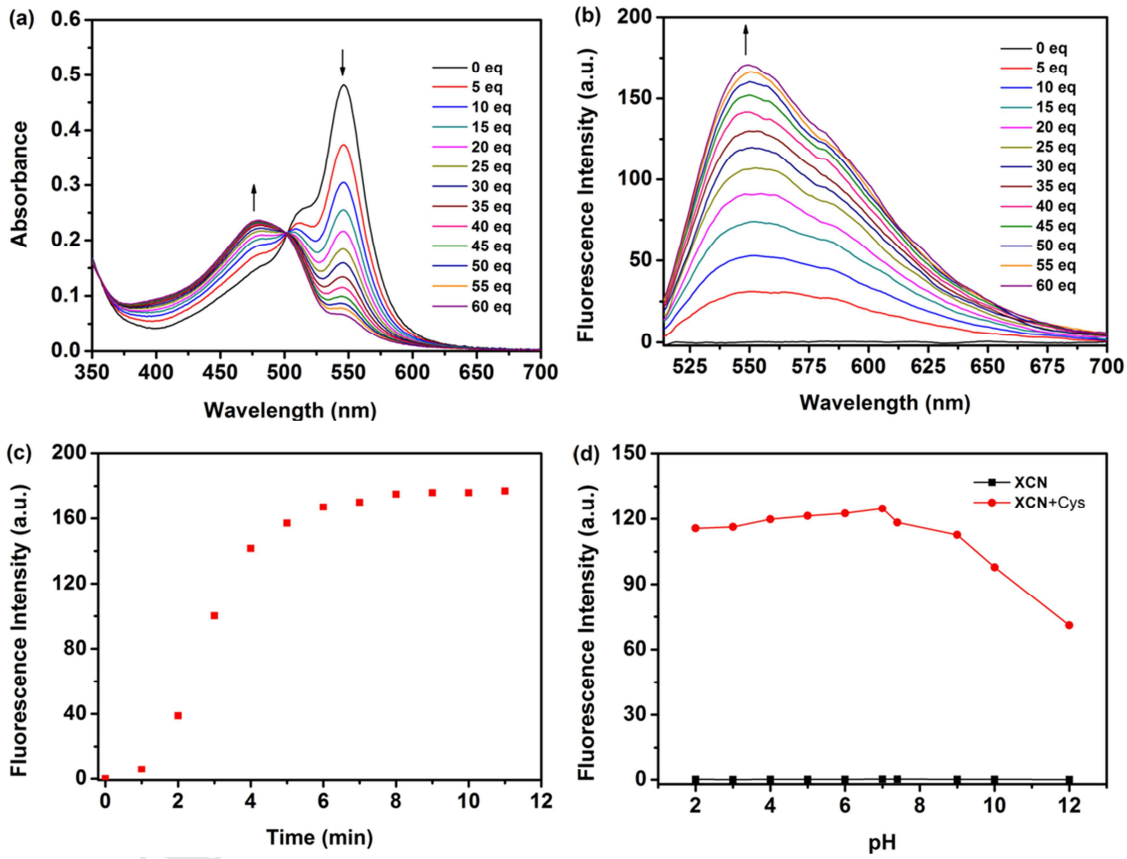


Fig. 2 (a) UV-vis absorption and (b) fluorescence emission spectra of **XCN** (10 μM) upon addition of Cys (0-60 eq) in DMSO. λ_{ex}= 501 nm (the isosbestic point); (c) Kinetics study of **XCN** (10 μM) upon interaction with Cys (100 eq) in DMSO. λ_{ex}=501 nm; (d) pH dependent fluorescence intensity at 549 nm of **XCN** (10 μM) in DMSO/buffers (2:1, v/v) upon addition of Cys (100 eq), λ_{ex}=501 nm.

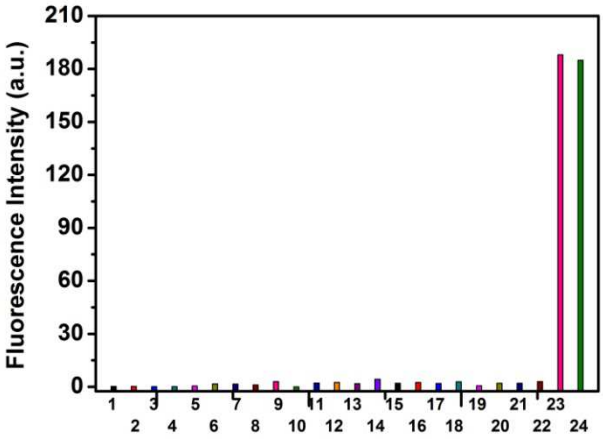


Fig. 3 Fluorescence responses of probe **XCN** (10 μM) at 550 nm towards various analytes (Cys and Hcy were used at 100 μM, other analytes were used at 200 μM). Analytes 1–24: 1-blank, 2-Thr, 3-Pro, 4-Ala, 5-Met, 6-Phe, 7-Tyr, 8-Gly, 9-Glu, 10-Val, 11-Gln, 12-Arg, 13-Ile, 14-His, 15-Trp, 16-Asp, 17-Leu, 18-Ser, 19-Asn, 20-Lys, 21-GSH, 22-S^{2-}, 23-Cys, 24-Hcy.

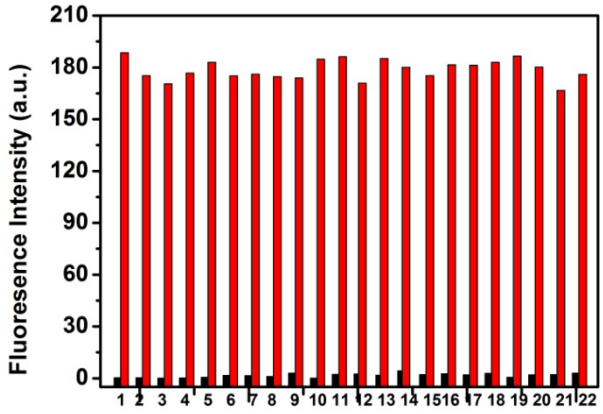


Fig. 4 Fluorescence intensity responses of probe **XCN** (10 μM) at 550 nm for Cys (100 μM) in the presence of various analytes (200 μM). Black bars represent the addition of a single analyte. Red bars represent the subsequent addition of Cys to the mixture. Analytes 1–22: 1-none, 2-Thr, 3-Pro, 4-Ala, 5-Met, 6-Phe, 7-Tyr, 8-Gly, 9-Glu, 10-Val, 11-Gln, 12-Arg, 13-Ile, 14-His, 15-Trp, 16-Asp, 17-Leu, 18-Ser, 19-Asn, 20-Lys, 21-GSH, 22-S^{2-}.

3.3 Selectivity test

Good selectivity is essential for practical applications of probes. To investigate the selectivity of **XCN** towards Cys and Hcy, we checked the fluorescence response of **XCN** in the presence of GSH, H_2S and various amino acids. As shown in Fig. 3, 4 and S20, only the addition of Cys and Hcy caused dramatic fluorescence enhancement, and the coexistence of

the interfering species did not seriously disturb the response of **XCN** towards Cys and Hcy. All these results indicated that probe **XCN** exhibited excellent selectivity towards Cys and Hcy.

3.4 pH dependence

To identify the pH influence on the sensing behavior, pH influence experiments were carried out at different pH within the range from 2 to 12. As shown in Fig. 2d and S15d, apparent fluorescence enhancement could be observed upon addition of Cys and Hcy within a wide pH range of 2−10, which is favorable for the practical applications of **XCN**.

3.5 Sensing mechanism study

Researches on the sensing mechanism of probe **XCN** were also carried out. Consistent with the reported results [26], the detection of Cys may be achieved by the nucleophilic addition of the thiol group at the cyano moiety followed by the formation of a five-membered heterocycle (Scheme S1), which may be evidenced by the absorption and fluorescence spectra of the main reaction product (Fig. S21), as well as the observed m/z of 779.0920, corresponding to the hypothesized product [**XCN-Cys**-H]$^-$ (calcd.779.0881 for $C_{37}H_{27}BBrF_2N_6O_2S_2$) (Fig. S22). Similarly, the m/z peak of the product [**XCN-Hcy**-H]$^-$ can also be observed in the HRMS (Fig. S23), indicative of a similar reaction mechanism. The reaction of the biothiols at the cyano moiety rather than the *p*-aminophenylthio moiety may be ascribed to the more electron deficient character of the cyano group as compared with the *p*-aminophenylthio moiety.

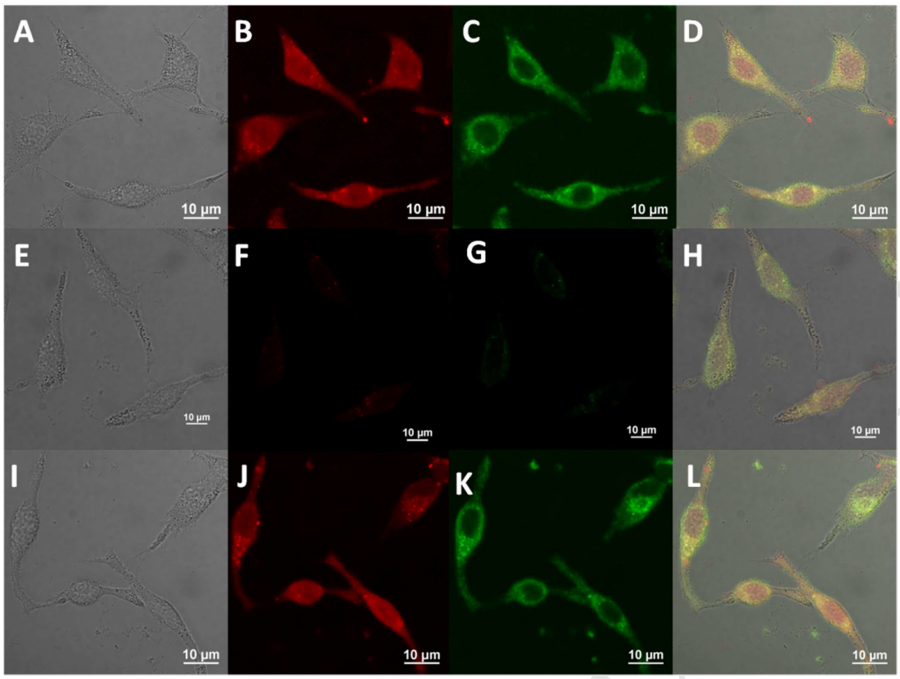


Fig. 5 (A-D) Confocal fluorescence and bright-field images of A549 cells incubated with probe **XCN** (5 μM) for 40 min after preincubation with Cys (200 μM) for 30 min. (E-H) Confocal fluorescence and bright-field images of A549 cells incubated with probe **XCN** (5 μM) for 40 min after preincubation with NEM (1 mM) for 40 min. (I-L) Confocal fluorescence and bright-field images of A549 cells incubated with probe **XCN** (5 μM) for 40 min. (A, E, I) bright-field images; (B, F, J) red channel images; (C, G, K) green channel images; (D, H, L) overlay.

3.6 Cell imaging

Encouraged by the above results, we continued to investigate the application of **XCN** in biosystems. Thus, the fluorescent imaging behavior was checked in living A549 lung cancer cells (Fig. 5). When A549 cells were incubated with **XCN** (5 μM) after preincubation with Cys (200 μM), bright fluorescence in green and red channels were observed simultaneously (Fig. 5B, 5C). However, when the cells were pretreated with 1 mM *N*-ethylmaleimide (NEM), a well-known thiol-blocking agent, before incubation with probe **XCN** (5 μM), only very weak fluorescence was observed (Fig. 5F, 5G). These results indicated the good membrane-permeability, biocompatibility and ability of **XCN** to detect exogenous biothiols.

Then, detection of endogenous biothiols was also checked. Apparent fluorescence was observed both in red and green channels upon incubation of probe **XCN** without preincubation of Cys (Fig. 5J, 5K). These results indicate that probe **XCN** can detect not only exogenous biothiols, but also endogenous biothiols. The excellent performance of **XCN** in cell imaging revealed that it may be practically used in bioimaging.

4. Conclusion

In summary, we herein report a new fluorescent probe **XCN** based on BODIPY. 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. Interestingly, **XCN** was obtained with the *p*-aminophenylthio moiety attached to one of the α-positions of the pyrrolic units. This reaction may compose an efficient approach for synthesizing novel BODIPY derivatives with functionalities 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 over GSH and other amino acids using the cyano group as the recognition site, with the *p*-aminophenylthio moiety left unreacted. The detection works well in a wide pH range from 2 to 10 in aqueous buffers. Furthermore, **XCN** was successfully used for imaging biothiols in living cells. These results provide further insight into developing novel fluorescent probes based on BODIPY.

Acknowledgment

This work was financially supported by NSFC (21472047, 21772041, 21702062), the Program for Professor of Special Appointment (Eastern Scholar, GZ2016006) at Shanghai

Institutions of Higher Learning, and the Fundamental Research Funds for the Central Universities (WK1616004, 222201717003).

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Highlights

1. A novel fluorescence turn-on probe based on a BODIPY moiety and a cyano group for discriminating Cys and Hcy from GSH

2. *p*-aminophenylthio moiety successfully introduced into one of the α-positions of BODIPY without previously brominating it

3. A novel biothiol probe based on the cyclization reaction between the cyano group and biothiols