Accepted Manuscript

A phthalimide-based fluorescent probe for thiophenol detection in water samples and living cells with a large Stokes shift

Xingjiang Liu, Liu Yang, Li Gao, Wenqiang Chen, Fengpei Qi, Xiangzhi Song

PII: S0040-4020(15)30028-4

DOI: 10.1016/j.tet.2015.08.074

Reference: TET 27096

To appear in: *Tetrahedron*

Received Date: 15 May 2015

Revised Date: 28 August 2015

Accepted Date: 31 August 2015

Please cite this article as: Liu X, Yang L, Gao L, Chen W, Qi F, Song X, A phthalimide-based fluorescent probe for thiophenol detection in water samples and living cells with a large Stokes shift, *Tetrahedron* (2015), doi: 10.1016/j.tet.2015.08.074.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Graphical Abstract

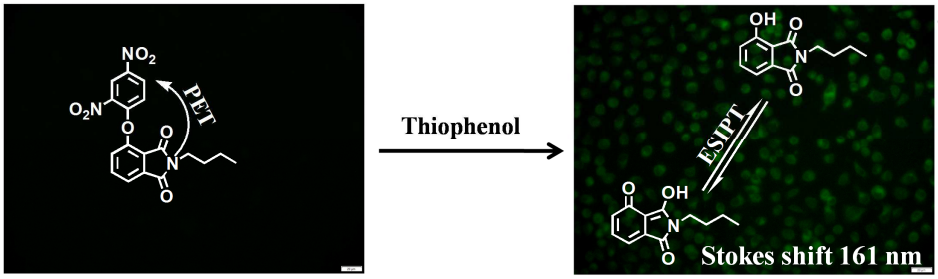
To create your abstract, type over the instructions in the template box below.

A phthalimide-based fluorescent probe for thiophenol detection in water samples and living cells with a large Stokes shift

Leave this area blank for abstract info.

Xingjiang Liu, Liu Yang, Li Gao,Wenqiang Chen, Fengpei Qi, and Xiangzhi Song*

College of Chemistry & Chemical Engineering, Central South University, Changsha, Hunan Province, P. R. China, 410083.



ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Tetrahedron
journal homepage: www.elsevier.com

A phthalimide-based fluorescent probe for thiophenol detection in water samples and living cells with a large Stokes shift

Xingjiang Liu [a], Liu Yang [a], Li Gao [a], Wenqiang Chen [a], Fengpei Qi [a], and Xiangzhi Song [abc, *]
[a] College of Chemistry & Chemical Engineering, Central South University, Changsha, Hunan Province, P. R. China, 410083.
[b] State Key Laboratory for Powder Metallurgy, Central South University, Changsha, Hunan province, P. R. China, 410083.
[c] State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116024, Liaoning Province, P. R. China, 116024.

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Fluorescent probe
Phthalimide
Photo-induced electron transfer (PET)
Excited-state intramolecular proton transfer (ESIPT)
Thiophenol

ABSTRACT

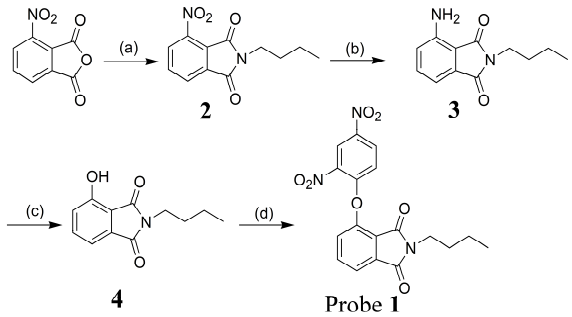
A phthalimide-based fluorescent probe for the detection of thiophenol was developed based on the combination of photo-induced electron transfer (PET) and excited-state intramolecular proton transfer (ESIPT) mechanisms. This probe displays high sensitivity and good selectivity toward thiophenol with a large Stokes shift (161 nm) and a low detection limit (3.5 nM, based on S/N = 3). Furthermore, the applications of this probe for quantitative detection of thiophenol in real water samples and imaging intracellular thiophenol in living cells were successfully demonstrated.

2009 Elsevier Ltd. All rights reserved.

1. Introduction

Thiophenols, the environmental pollutants with high toxicity, are widely used in production of pesticide, medicine and polymer materials.[1-3] Thiophenols can be readily autoxidized under physiological conditions to form aromatic disulphides, and generate the harmful superoxide radical and hydrogen peroxide in this process. On the other hand, aromatic diphenyl disulphides have been found to undergo a cyclic reduction/autoxidation reaction by virtue of glutathione (GSH) to generate 'active oxygen' species which can induce oxidative damage to erythrocytes cells.[4-8] Thus, it has been shown that thiophenol exposure not only induces grave central nervous system damage, but also causes other related systemic injuries including increased respiration, muscular weakness, hind limb paralysis, coma, and even death.[5, 9] As a consequence, considerable attention have been paid to techniques for the rapid, selective and sensitive detection of thiophenols in environmental and biological samples.[10-19] In this regard, fluorescent sensing is well suited to meet this need owning to its simplicity, high sensitivity, good selectivity, rapid response and non-invasiveness.[20-27]

3-Hydroxyphthalimide and its derivatives exhibit a large Stokes shift upon excited because of an excited-state intramolecular proton transfer (ESIPT) process in the excited state. Additionally, 3-hydroxyphthalimide possesses many favorable optical properties such as good photo stability, emission in green region, and relatively high fluorescent quantum. As a result, it would be an ideal candidate for the design of fluorescent probes.[28-31] We previously reported the first 3-hydroxyphthalimide-based fluorescent probe for biothiols.[32] It has been demonstrated that 2,4-dinitrophenlate moiety is an effective sensing group for the design of fluorescent probes to discriminate thiophenols from aliphatic thiols by taking advantage of their different pKa values (*Ca.* 6.5 and 8.5 for thiophenols and aliphatic thiols, respectively).[19, 33] Herein, we reported a 2,4-dinitrophenlate derivative of 3-hydroxyphthalimide, Probe **1**, that we designed as fluorescent probe for the selective detection of thiophenols with a large Stokes shift at a physiological condition. The synthetic route of Probe **1** in four steps was shown in Scheme 1. Due to a photo-



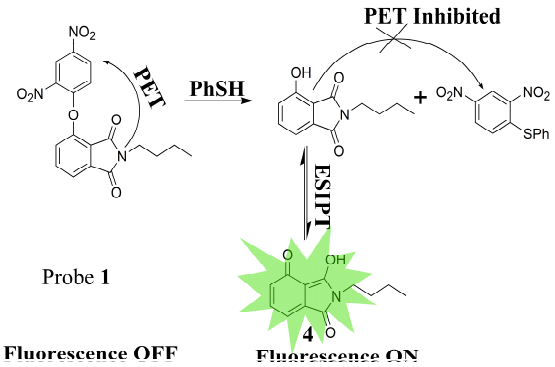
Scheme 1. Synthetic route of Probe **1**. (a) Butylamine, acetic acid, 120 °C, 2.5 h, yield 85.7%. (b) Palladium/C, H_2, CH_3OH, 65 °C, 12 h, 71.5%. (c) (1)

* Corresponding author. Tel.: +86-731-88836954; fax: +86-731-88836954; e-mail: xzsong@csu.edu.cn

ACCEPTED MANUSCRIPT

$NaNO_2$, 50% H_2SO_4, 0 °C, 0.5 h; (2) 90 °C, 1 h, yield 85.4%. (d) 1-Fluoro-2,4-dinitro-benzene, K_2CO_3, DMF, 90 °C, 10 h, yield 74.5%.

induced electron transfer (PET) process and inhibition of proton transfer (ESIPT) process induced by 2, 4-dinitrophenolate moiety,[34-35] we expected Probe **1** would be essentially non-fluorescent. Thiophenol rather than aliphatic thiols would selectively cleave the 2,4-dinitrophenolate moiety in Probe **1**, thus eliminating the possibility of PET-induced quenching and concomitantly allowing the occurrence of ESIPT process (shown in Scheme 2).



Scheme 2. The proposed sensing mechanism of Probe **1** with thiophenol (PhSH).

2. Results and discussion

*2.1. Sensing property of Probe **1** to thiophenol*

Dye **4** exhibits strong green fluorescence (Φ_F = 0.27) with a maximum at 516 nm. Notably, it displays a large Stokes shift (161 nm) which can reduce the interference from incident light. As expected, Probe **1** is essentially non-fluorescent (Φ_F < 0.001) in HEPES buffer solution (10.0 mM, pH = 7.4, containing 20% CH_3CN), which is attributed to the PET process resulted from the introduction of 2,4-dinitrobenzene moiety. The addition of thiophenol to the solution of Probe **1** triggered a strong green fluorescence (λ_{em}max = 516 nm) with an concomitant appearance of an absorption band at 350 nm, which are coincident with the spectral characteristic of dye **4** (Fig. 1 and Fig. S1). Concentration-dependent studies found that the fluorescence intensity at 516 nm increased with increasing thiophenol concentration. The fluorescence enhancement could be up to 64-fold when 5.0 equiv. of thiophenol was added to the solution of Probe **1**. A plot of fluorescence intensity vs the concentrations of thiophenol in the range of 0.0 – 10.0 μM showed good linearity (R = 0.99601) (Fig. 2). The detection limit was found to be 3.5 nM (based on S/N = 3), which was sufficiently low for the detection of thiophenol in environmental and biological samples. This result provided support for Probe **1** having high sensitivity to thiophenol.

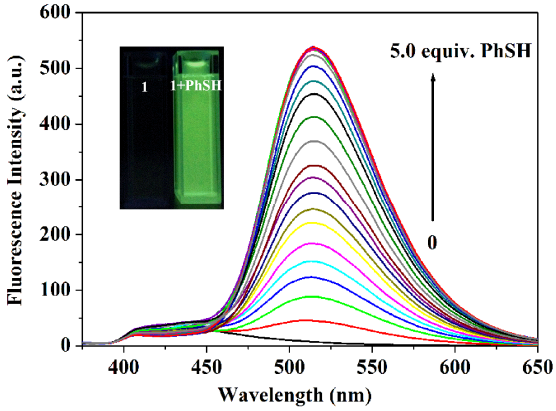


Fig. 1 Fluorescence spectra of Probe **1** (5.0 μM) in the presence of thiophenol (0.0 - 5.0 equiv.) in HEPES buffer (10.0 mM, pH = 7.4, containing 20% CH_3CN) with excitation wavelength at 358 nm. Inset: fluorescence images of Probe **1** (5.0 μM) before and after addition of thiophenol (5.0 equiv.) in HEPES buffer (10.0 mM, pH = 7.4 containing 20% CH_3CN) .

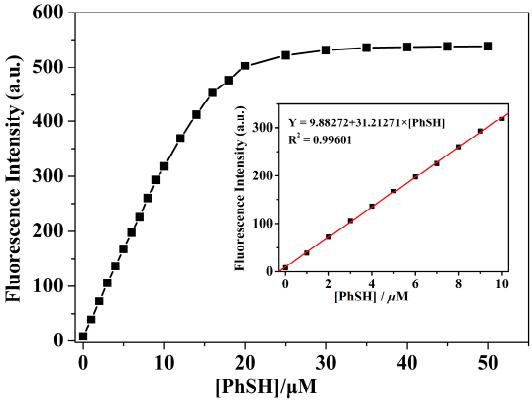


Fig. 2 Plot of the fluorescence intensity at 516 nm of Probe **1** (5.0 μM) as a function of the thiophenol concentration in HEPES buffer (10.0 mM, pH = 7.4, containing 20% CH_3CN). Inset: the linear relationship between the fluorescence intensity at 516 nm of Probe **1** and the concentration of thiophenol.

2.2. Mechanism studies

We reasoned that thiophenols could selectively cleave dinitrobenzene moiety in Probe **1** generating dye **4** which is highly fluorescent upon excited. To confirm the sensing mechanism, we carried out ^{1}H NMR spectral analysis on the reaction product of Probe **1** with thiophenol, as shown in Fig. 3. As expected, the ^{1}H NMR spectrum of the reaction product is very similar to that of dye **4**. Furthermore, the mass spectra of the reaction product of Probe **1** with thiophenol displayed a peak at m/z = 218.0229 (Fig. S11) which is nearly identical to the molar weight of dye **4** ([M-H] = 218.0817). Absorption and emission spectra, along with ^{1}H NMR and mass spectral analysis, confirm that dye **4** is the product generated from Probe **1** with thiophenol.

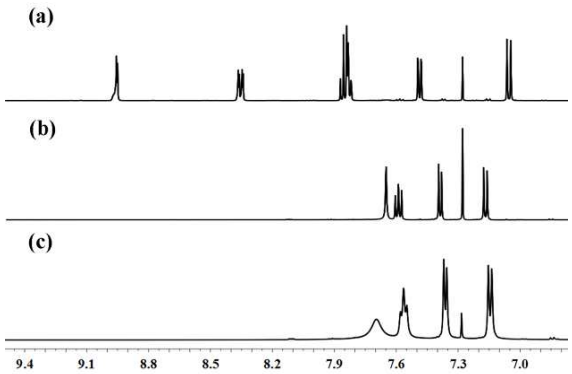


Fig. 3 Partial ^{1}H NMR spectra of Probe **1** (a), the isolated product of Probe **1** with thiophenol (b) and reference dye **4** (c) in $CDCl_3$.

2.3. Selectivity and competition studies

To evaluate the selectivity of Probe **1** (5.0 μM) towards thiophenol, we observed its fluorescence response to various analytes including aliphatic thiols (Cys, Hcy, mercaptoethanol, thiohydracrylic acid, 0.5 mM for each, and 1.0 mM GSH), some nucleophilic species (Ala, Gly, PhOH, $PhNH_2$, NaN_3, NaHS, 0.4 mM for each), and various anions and metal ions (NaF, NaBr, KI, $NaNO_2$, $Na_2S_2O_3$, Na_2SO_4, Na_3PO_4, Na_2SO_3, $ZnCl_2$, $CaCl_2$, $MgCl_2$, 0.4 mM for each; 10.0 mM NaCl and KCl). As shown in

ACCEPTED MANUSCRIPT

Fig. 4, only thiophenol resulted in a significant fluorescence enhancement whereas other interfering analytes did cause negligible fluoresce change. We were pleased that this probe showed excellent selectivity to thiophenol over aliphatic thiols (Cys, Hcy, and GSH) and NaHS. Moreover, the competition studies were also carried out in the same concentration of interfering analytes as mentioned above. The performance of Probe **1** for the detection of thiophenol was not obviously influenced by the co-existence of other interfering analytes except that the fluorescence intensity was weakened in the presence of Na_3PO_4. Therefore, it can be concluded that Probe **1** exhibits excellent selectivity for thiophenol.

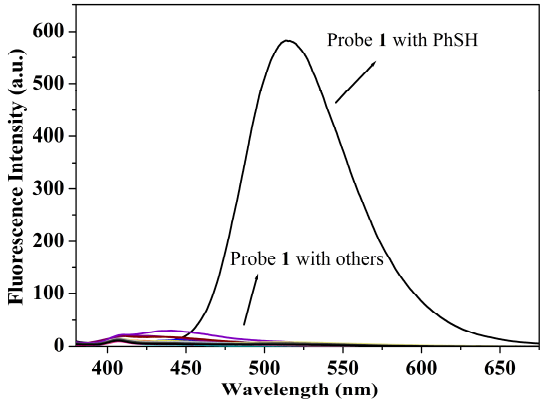


Fig. 4 Fluorescence spectra of Probe **1** (5.0 μM) with various tested analytes in HEPES buffer (10.0 mM, pH = 7.4, containing 20% CH_3CN). The analytes include thiophenol, Cys, Hcy, GSH, mercaptoethanol, thiohydracrylic acid, Ala, Gly, PhOH, $PhNH_2$, NaN_3, NaHS, NaF, NaCl, NaBr, KI, $NaNO_2$, $Na_2S_2O_3$, Na_2SO_4, Na_3PO_4, Na_2SO_3, $ZnCl_2$, $CaCl_2$, $MgCl_2$, KCl.

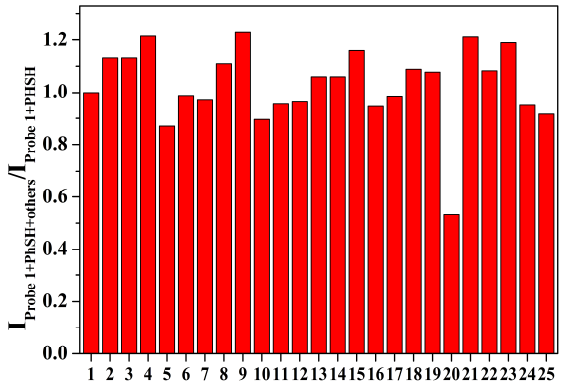


Fig. 5 The fluorescent responses of Probe **1** (5.0 μM) to thiophenol in the presence of various relevant species in HEPES buffer (10.0 mM, pH = 7.4, containing 20% CH_3CN). 1, thiophenol; 2, Cys; 3, Hcy; 4, GSH; 5, mercaptoethanol; 6, thiohydracrylic acid; 7, Ala; 8, Gly; 9, PhOH; 10, $PhNH_2$; 11, NaN_3; 12, NaHS; 13, NaF; 14, NaCl; 15, NaBr; 16, KI; 17, $NaNO_2$; 18, $Na_2S_2O_3$; 19, Na_2SO_4; 20, Na_3PO_4; 21, Na_2SO_3; 22, $ZnCl_2$; 23, $CaCl_2$; 24, $MgCl_2$; 25, KCl.

2.4. Kinetic studies

We next studied time-dependent fluorescence behavior of Probe **1** with thiophenol by monitoring changes in fluorescence intensity at 516 nm as a function of time (Fig. 6). Upon the addition of 5.0 equiv. of thiophenol to Probe **1**, a strong fluorescence signal was initiated within 3 min (7-fold enhancement) and reached a stable intensity plateau within 60 min. However, in the absence of thiophenol, no fluorescence signal was observed in the solution of Probe **1**. It's noted that GSH, Cys and Hcy didn't produce fluorescence enhancement when time-dependent fluorescence experiments were performed on Probe **1** under the same condition, which might be ascribed to the different pKa values of thiophenol and biothiols.

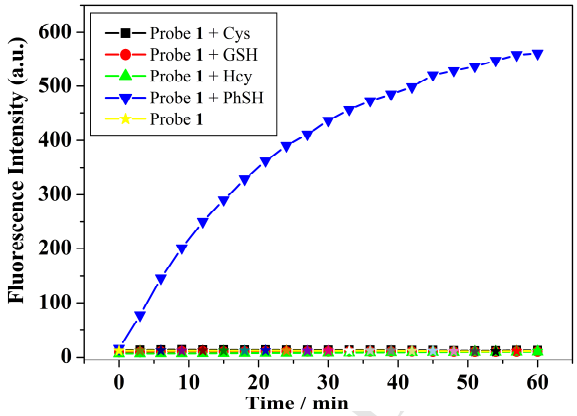


Fig. 6 Time-dependent fluorescence intensity at 516 nm of Probe **1** (5.0 μM) in response to thiophenol, Cys, Hcy, and GSH in HEPES buffer (10.0 mM, pH = 7.4, containing 20% CH_3CN).

2.5. pH effect studies

The potential pH effects on the fluorescence signal of Probe **1** with thiophenol was also investigated. As shown in Fig. 7, the fluorescence spectra of Probe **1** remained essentially unchanged within a wide pH range from 2 to 13, indicating that Probe **1** was very stable. In contrast, remarkable fluorescence enhancement was observed when this probe was treated with thiophenol within a pH range from 6.0 to 10.0. The data implied that Probe **1** was able to detect thiophenol in a relatively wide pH range.

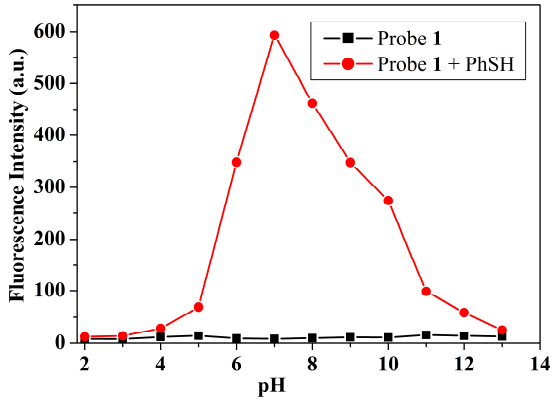


Fig. 7 Fluorescence intensity at 516 nm of Probe **1** (5.0 μM) in the absence (black line) and presence (red line) of thiophenol (25.0 μM) at different pH values.

*2.6. Preliminary application of Probe **1** in water samples and living cells*

Firstly, we applied Probe **1** to detect toxic thiophenol in real water samples to demonstrate its practical utility in environmental science. The water samples were collected from the Yangtze River and Tap water in Suzhou City, respectively. All the samples were filtrated before used. The fluorescence changes of Probe **1** (5.0 μM) were examined in all these samples which were spiked with thiophenol at different levels (0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 μM). From the results shown in Fig. 8a, we could see that fluorescence enhancements in real samples and in distilled water were coincident. As shown in Fig. 8b-c, a good linear relationship between the fluorescence

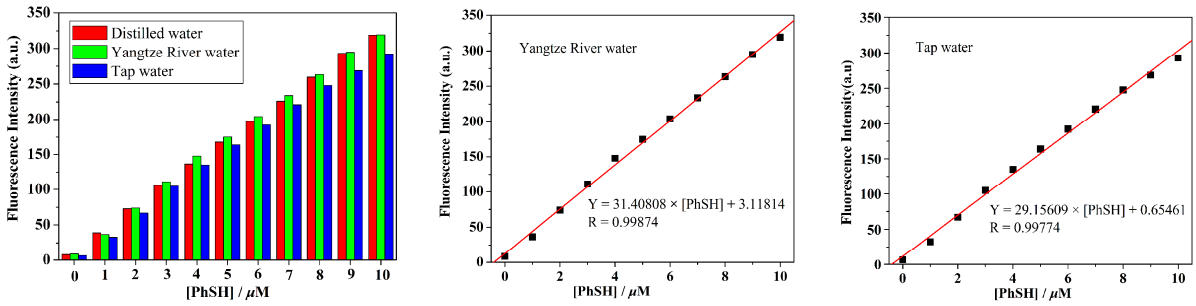


Fig. 8 (a) Fluorescent response of Probe **1** (5.0 μM) to different concentrations of thiophenol in different water samples (10.0 mM HEPES buffer, pH = 7.4, containing 20% CH_3CN). (b) and (c) Linear plots of fluorescent intensity of Probe **1** at 516 nm against the spiked concentrations of thiophenol from 0.0 to 10.0 μM in Yangtze River and tap water samples.

intensity at 516 nm and the spiked thiophenol concentrations was obtained for each real water sample. Moreover, the added thiophenol in these samples could be accurately measured with a good recovery (see Table S1). These results suggest that Probe **1** has potential application for quantitative detection of thiophenol in water samples.

Next, we evaluated the ability of Probe **1** to perform within living cells. Living HNE-2 cells, nasopharyngeal carcinoma cell lines, only incubated with Probe **1** showed little fluorescence, as depicted in Fig. 9a. However, cells pretreated with thiophenol and then stained with Probe **1** resulted in a strong green fluorescence (shown in Fig. 9b). These results indicate that Probe **1** is cell membrane permeable and can response to thiophenol in living cells.

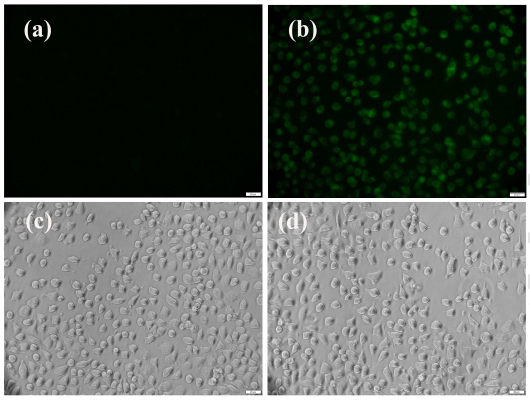


Fig. 9 Living-cell fluorescence images of thiophenol in living HNE-2 cells. (a) Fluorescence and (c) bright field images of cells incubated with Probe **1** (5.0 μM) for 30 min at 37 °C. (b) Fluorescence and (d) bright-field images of cells pretreated with thiophenol (25.0 μM) and then incubated with Probe **1** (5.0 μM) for 30 min at 37 °C. The scale bars mean 100 μm. The cells were magnified 20 times.

3. Conclusions

In conclusion, we presented the design, synthesis and preliminary evaluation of the 2,4-dinitrophenyl ether of 3-hydroxyphthalimide derivative, Probe **1**, as a turn-on fluorescent probe for the detection of thiophenol in both the real water sample and living cells. This probe exhibited a good selectivity toward thiophenol against aliphatic thiols and other biologically relevant anions and metal ions and high sensitivity with a 3.5 nM detection limit. This probe featured a 64-fold fluorescence enhancement with a 161 nm large Stokes shift in response to thiophenol.

4. Experimental section

4.1. Instruments and Materials

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker 500 NMR spectrometer using tetramethylsilane (TMS) as the internal standard for chemical shifts. Mass spectra were recorded on MICROTOF-Q II mass spectrometer (Bruker Daltonics, Germany). All spectral characterizations were carried out in HPLC-grade solvents at 20 °C within a 10 mm quartz cell. UV-vis absorption spectroscopy was measured with a UV-2450 spectrophotometer and fluorescence spectra was recorded on a Hitachi F-7000 spectrometer. The fluorescence quantum yields were measured at 20 °C with coumarin 151 as the reference (Φ_{fl} = 0.53 in ethanol).[36] Fluorescence imaging was performed with an Olympus IX83 inverted microscope. The pH measurement was carried out on a Leici PHS-3C meter. TLC silica gel plates and silica gel (mesh 200-300) for column chromatography were purchased from Qingdao Ocean Chemicals, China. All chemicals and solvents were obtained from commercial suppliers and used as received. HNE-2 cells were provided by Xiangya Third People's Hospital of Central South University (China).

4.2. Synthesis

*4.2.1. Synthesis of compound **2***

To a solution of 3-nitrophthalic anhydride (1.9361 g, 10.0 mmol) in acetic acid (20.0 mL) was slowly added butylamine (1.0951 g, 15.0 mmol) within 5 min. After stirring at room temperature for 10 min, the resulting mixture was refluxed at 120 °C for 2.5 h. Next, cool the reaction mixture to room temperature and pour into 50.0 mL cold water. The precipitated solid was collected and washed with water (3.0 mL × 3) to give compound **2** as a white solid (2.1252 g, 85.7% yield). ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ_H 8.27 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 7.5 Hz, 1H), 8.05 (t, J = 7.8 Hz, 1H), 3.57 (t, 2H), 1.63-1.51 (m, 2H), 1.38-1.24 (m, 2H), 0.90 (t, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 166.5, 163.8, 144.6, 136.5, 134.1, 128.6, 127.2, 123.5, 38.1, 30.2, 19.9, 13.9.

*4.2.2. Synthesis of compound **3***

Compound **2** (0.4964 g, 2.0 mmol) was hydrogenated in methanol (15.0 mL) under reflux at 65 °C for 12 h with 10% Pd/C (0.0496 g) as a catalyst. Then, the reaction mixture was filtered through celite to remove the catalyst. Next, the filtrate was concentrated in vacuum to give a residue. Pure compound **3** (0.6240 g, 71.5% yield) was obtained by silica gel column chromatography using petroleum ether/dichloromethane (v/v, 1:1) as eluent. ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ_H 7.42 (dd, J = 8.3, 7.1 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 6.86 (d, J = 8.8 Hz, 1H), 5.13 (s, 1H), 3.65 (t, 2H), 1.63-1.70 (m, 2H), 1.35-1.42 (m , 2H). 0.95 (t, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.4, 168.8, 145.2, 135.02, 132.9, 121, 112.6, 111.4, 37.4, 30.8, 20.1, 13.7.

*4.2.3. Synthesis of compound **4***

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

To a solution of compound **3** (0.2180 g, 1.0 mmol) in 50% sulfuric acid (10.0 mL) at 0 °C was added a solution of $NaNO_2$ (0.0692 g, 1.0 mmol) in 2.0 mL water dropwise. After stirring for 30 min at 0 °C, the mixture was heated to 90 °C and stirred for 1 h. The reaction mixture was diluted with 15.0 mL water, then extracted with ethyl acetate (20.0 mL × 3). The organic layer was combined, washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the solid was purified by silica gel flash chromatography using petroleum ether/dichloromethane (v/v, 1:1) as eluent to afford compound **4** (0.1862 g, 85.4% yield). ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ_H 7.70 (s, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.36 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 3.65 (t, 2H), 1.68-1.62 (m, 2H), 1.40-1.32 (m, 2H), 0.95 (t, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.5, 167.9, 154.6, 136.1, 132.1, 122.5, 115.8, 114.6, 37.5, 30.6, 19.9, 13.4.

*4.2.4. Synthesis of Probe **1***

To a solution of compound **4** (0.4406 g, 2.0 mmol) and 1-fluoro-2,4-dinitro-benzene (0.5582 g, 3.0 mmol) in DMF (10.0 mL) was added anhydrous K_2CO_3 (0.9484 g, 6.0 mmol). The mixture was heated to 90 °C and stirred for 10 h. After cooling to room temperature, the reaction mixture was filtered through celite to remove K_2CO_3. Next, the filtrate was diluted with 15 mL water, then extracted with dichloromethane (20.0 mL × 3). The organic layer was combined, washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the solid was purified by silica gel flash chromatography using petroleum ether/dichloromethane (v/v, 1:4) as eluent to afford Probe **1** (0.5741 g, 74.5% yield). HRMS (EI) m/z: calcd for $C_{18}H_{15}N_3O_7$ $[M + 1]^+$, 386.0910; found, 386.0983. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.96 (d, *J* = 2.7 Hz, 1H), 8.36 (dd, *J* = 9.2, 2.7 Hz, 1H), 8.06 – 7.68 (m, 2H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 9.2 Hz, 1H), 3.59 (t, 2H), 1.67 – 1.47 (m, 2H), 1.34 – 1.29 (m, 2H), 0.91 (t, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 167.0, 165.1, 155.2, 149.5, 142.5, 139.7, 136.7, 134.1, 128.9, 126.9, 122.5, 121.5, 121.3, 118.8, 38.1, 30.4, 20.0, 13.6.

*4.2.5. Synthesis of the reaction product of Probe **1** with thiophenol*

To a solution of Probe **1** (0.0385 g, 0.1 mmol) in a mixture of acetonitrile (2.00 mL) and distilled water (10.0 mL) was added thiophenol (0.0552 g, 0.5 mmol) at room temperature. Stir the reaction mixture for 4 h and extract with dichloromethane (20 mL × 3). The organic layer was combined and dried over anhydrous Na_2SO_4. After removal of the solvent, the solid was purified by silica gel flash chromatography using petroleum ether/dichloromethane (v/v, 2:1) as eluent to afford the target product (0.0142 g, 52.3% yield). ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ 7.66 (s, 1H), 7.59 (t, *J* = 7.6, 7.3 Hz, 1H), 7.39 (d, *J* = 7.2 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 3.67 (t, *J* = 7.3 Hz, 1H), 1.70-1.64 (m, 7.6 Hz, 1H), 1.35-1.41 (m, 2H), 0.97 (t, 3H).

4.3. Imaging of HNE-2 cells

HNE-2 cells were seeded in a 6-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin. The cells were incubated under an atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h. The cells were washed three times with PBS buffer before used. The cells were firstly incubated with thiophenol (25.0 μM) for 30 min at 37 °C, washed with PBS buffer and then incubated with Probe **1** (5.0 μM) for another 30 min. After washing with PBS buffer, cell imaging were performed. For a control experiment, HNE-2 cells were only incubated with Probe **1** (5.0 μM) for 30 min at 37 °C.

Acknowledgements

The research was supported by the State Key Laboratory of Fine Chemicals (KF1202) and Hunan Nonferrous Metals Holding Group Co. Ltd.

References and notes

† Electronic supplementary information (ESI) available. See DOI: 10.1016/j.tet.2015.xx.xxx.

1. Roy, K. M. In Ullmann's encyclopedia of industrial chemistry, 7th ed.; Wiley: New York, **2007**.
2. Shimada, K.; Mitamura, K. J. *Chromatogr. B: Biomed. Sci, Appl.*, **1994**, 659, 227.
3. Eychmuller, A.; Rogach, A. L. *Pure Appl. Chem.*, **2000**, *72*, 179.
4. Munday, R. *Free Radical Bio. Med.*, **1989**, *7*, 659.
5. Amrolia, P.; Sullivan, S. G.; Stern, A.; Munday, R. *J. Appl. Toxicol.*, **1989**, *9*, 113.
6. Munday, R. *J. Appl. Toxicol.*, **1985**, *5*, 402.
7. Munday, R. *J. Appl. Toxicol.*, **1985**, *5*, 409.
8. Munday, R.; Manns, E. *J. Appl. Toxicol.*, **1985**, 5, 414.
9. Juneja, T. R.; Gupta, R. L.; Samanta, S. *Toxicol. Lett.*, **1984**, 21, 185.
10. Jiang, W.; Cao, Y.; Liu, Y.; Wang, W. *Chem. Commun.*, **2010**, *46*, 1944.
11. Jiang, W.; Fu, Q.; Fan, H.; Ho, J.; Wang, W. *Angew. Chem. Int. Ed.*, **2007**, *46*, 8445.
12. Shao, X.; Kang, R.; Zhang, Y.; Huang, Z.; Peng, F.; Zhang, J.; Wang, Y.; Pan, F.; Zhang, W.; Zhao, W. *Anal. Chem.*, 2015, **87**, 399.
13. Yu, D.; Huang, F.; Ding, S.; Feng, G. *Anal. Chem.*, **2014**, *86*, 8835.
14. Li, J.; Zhang, C.-F.; Yang, S.-H.; Yang, W.-C.; Yang, G.-F. *Anal. Chem.*, **2014**, **86**, 3037.
15. Wang, Z.; Han, D.-M.; Jia, W.-P.; Zhou, Q.-Z.; Deng, W.-P. *Anal. Chem.*, **2012**, *84*, 4915.
16. Kand, D.; Mishra, P. K.; Saha, T.; Lahiri, M.; Talukdar, P. *Analyst*, **2012**, *137*, 3921.
17. Zhao, C.; Zhou, Y.; Lin, Q.; Zhu, L.; Feng, P.; Zhang, Y.; Cao, J. *J. Phys. Chem. B*, **2011**, *115*, 642.
18. Wang, X.; Cao, J.; Zhao, C. *Org. Biomol. Chem.*, **2012**, *10*, 4689.
19. Shiraishi, Y.; Yamamoto, K.; Sumiya, S.; Hirai, T. *Chem. Commun.*, **2013**, *49*, 11680.
20. Kim, G.-J.; Lee, K.; Kwon, H.; Kim, H.-J. *Org. Lett.*, **2011**, *13*, 2799.
21. Zhang, X.; Ren, X.; Xu, Q.-H.; Loh, K. P.; Chen, Z.-K. *Org. Lett.*, **2009**, *11*, 1257.
22. Li, H.; Fan, J.; Wang, J.; Tian, M.; Du, J.; Sun, S.; Sun, P.; Peng, X. *Chem. Commun.*, **2009**, 5904.
23. Wang, P.; Liu, J.; Lv, X.; Liu, Y.; Zhao, Y.; Guo, W. *Org. Lett.*, **2012**, *14*, 520.
24. Yi, L.; Li, H.; Sun, L.; Liu, L.; Zhang, C.; Xi, Z. *Angew. Chem. Int. Ed.*, **2009**, *48*, 4034.
25. Yang, X.; Guo, Y.; Strongin, R. M. *Angew. Chem. Int. Ed.*, **2011**, *50*, 10690.
26. Niu, L.-Y.; Guan, Y.-S.; Chen, Y.-Z.; Wu, L.-Z.; Tung, C.-H.; Yang, Q.-Z. *J. Am. Chem. Soc.*, **2012**, *134*, 18928.
27. Long, L.; Lin, W.; Chen, B.; Gao, W.; Yuan, L. *Chem. Commun.*, 2011, **47**, 893.
28. Nakazono, M.; Saita, K.; Kurihara, C.; Nanbu, S.; Zaitsu, K. J. *Photochem. Photobiol. A-Chem.*, **2009**, *208*, 21.
29. Perez-Ruiz, R.; Fichtler, R.; Miara, Y. D.; Nicoul, M.; Schaniel, D.; Neumann, H.; Beller, M.; Blunk, D.; Griesbeck, A. G.; Jacobi von Wangelin, A. *J. Fluoresc.*, 2010, **20**, 657.
30. Siretskii, Y. G.; Kalinovskaya, O. E. *Russ. J. Gen. Chem.*, **2000**, 70, 832.
31. Tan, A.; Bozkurt, E.; Kishali, N.; Kara, Y. *Helv. Chim. Acta*, **2014**, *97*, 1107.
32. Liu, X.; Gao, L.; Yang, L.; Zou, L.; Chen, W.; Song, X. *RSC Adv.*, **2015**, *5*, 18177.
33. Lin, W.; Long, L.; Tan, W. *Chem. Commun.*, **2010**, *46*, 1503.
34. Chen, S.; Hou, P.; Zhou, B.; Song, X.; Wu, J.; Zhang, H.; Foley, J. W. *RSC Adv.*, **2013**, *3*, 11543.
35. Lan, M.; Wu, J.; Liu, W.; Zhang, H.; Zhang, W.; Zhuang, X.; Wang, P. *Sens. Actuators B*, **2011**, *156*, 332.
36. Reynolds, G. A.; Drexhage, K. H. *Opt. Commun.*, **1975**, *13*, 222.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supporting Information

A phthalimide-based fluorescent probe for thiophenol detection in water samples and living cells with a large Stokes shift

Xingjiang Liu [a], Liu Yang [a], Li Gao [a], Wenqiang Chen [a], Fengpei Qi [a] and Xiangzhi Song [abc,*]

[a] College of Chemistry & Chemical Engineering, Central South University, Changsha, Hunan Province, P. R. China, 410083.

[b] State Key Laboratory for Powder Metallurgy, Central South University, Changsha, Hunan province, P. R. China, 410083.

[c] State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian, Liaoning Province, P. R. China, 116024.

* Corresponding author. Fax: +86-731-88836954; Tel: +86-731-88836954; Email: xzsong@csu.edu.cn (XZ Song).

Contents

ACCEPTED MANUSCRIPT

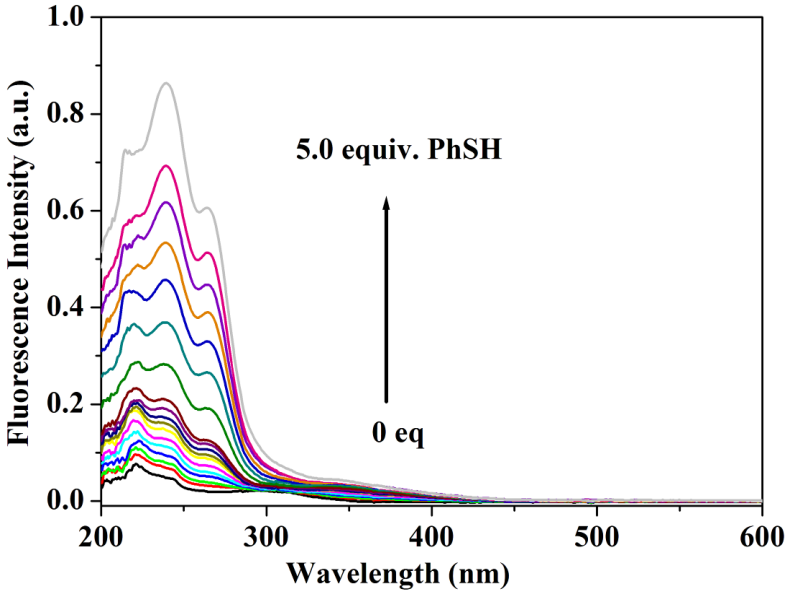


Fig. S1 Absorption spectra of Probe **1** (5.0 μM) upon the addition of thiophenol (0.0-5.0 equiv.) in HEPES buffer (10.0 mM, pH = 7.4, containing 20% CH_3CN).

Table S1 Determination of thiophenol concentration in water samples.

Sample	Thiophenol spiked (μM)	Thiophenol recovered (μM)	recovery (%)
Yangtze River water	0	not detected	
	1	0.99 ± 0.02	99
	2	2.06 ± 0.04	103
	3	3.24 ± 0.08	108
	4	4.10 ± 0.09	103
	5	5.29 ± 0.11	106
	6	6.23 ± 0.15	103
	7	7.18 ± 0.14	103
	8	8.12 ± 0.21	102
	9	9.13 ± 0.16	101
	10	9.91 ± 0.11	99
Tap water	0	not detected	
	1	0.92 ± 0.02	92
	2	1.83 ± 0.03	92
	3	3.04 ± 0.02	101
	4	4.01 ± 0.05	100
	5	4.94 ± 0.09	99
	6	5.84 ± 0.11	97
	7	6.76 ± 0.13	97
	8	7.63 ± 0.11	95
	9	8.29 ± 0.17	92
	10	9.07 ± 0.15	91

ACCEPTED MANUSCRIPT

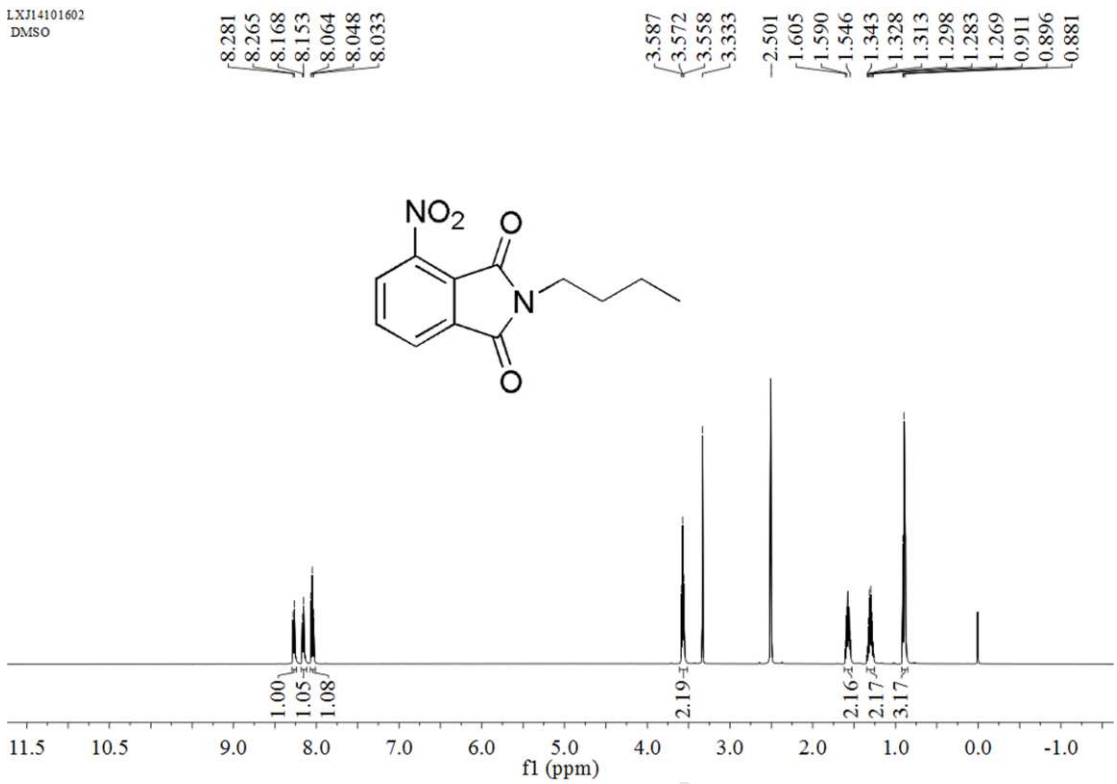


Fig. S2 ^{1}H NMR spectrum of compound **2**.

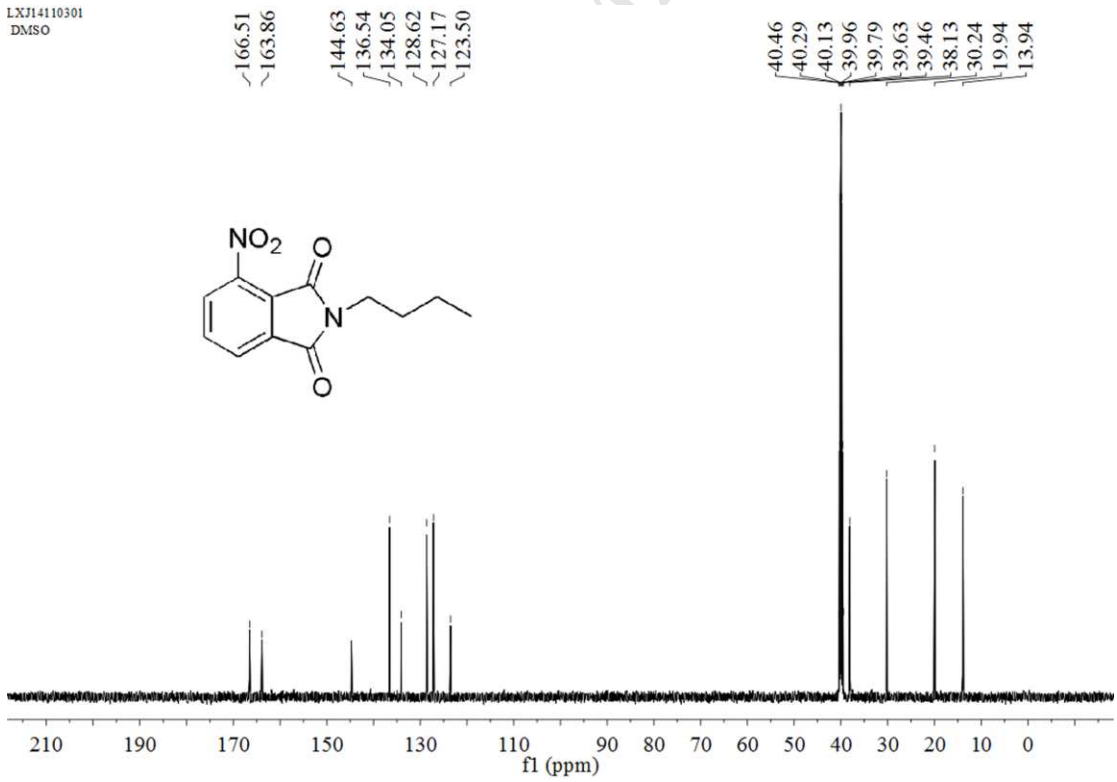


Fig. S3 ^{13}C NMR spectrum of compound **2**.

ACCEPTED MANUSCRIPT

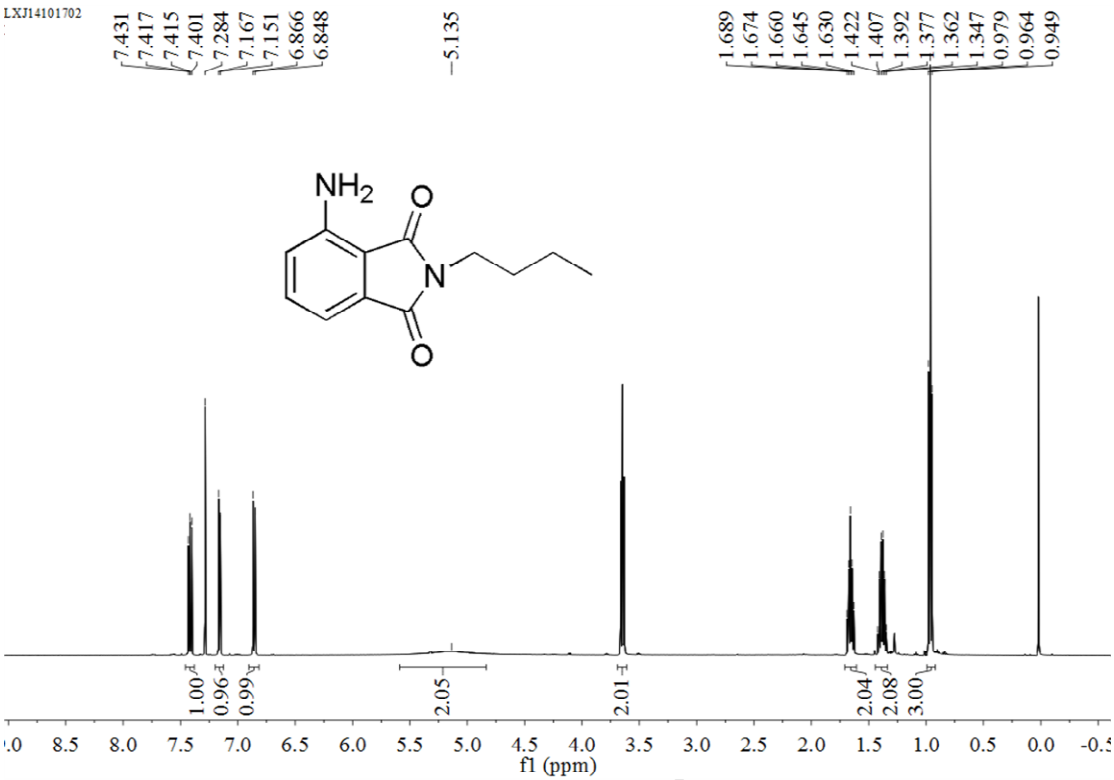


Fig. S4 ^{1}H NMR spectrum of compound **3**.

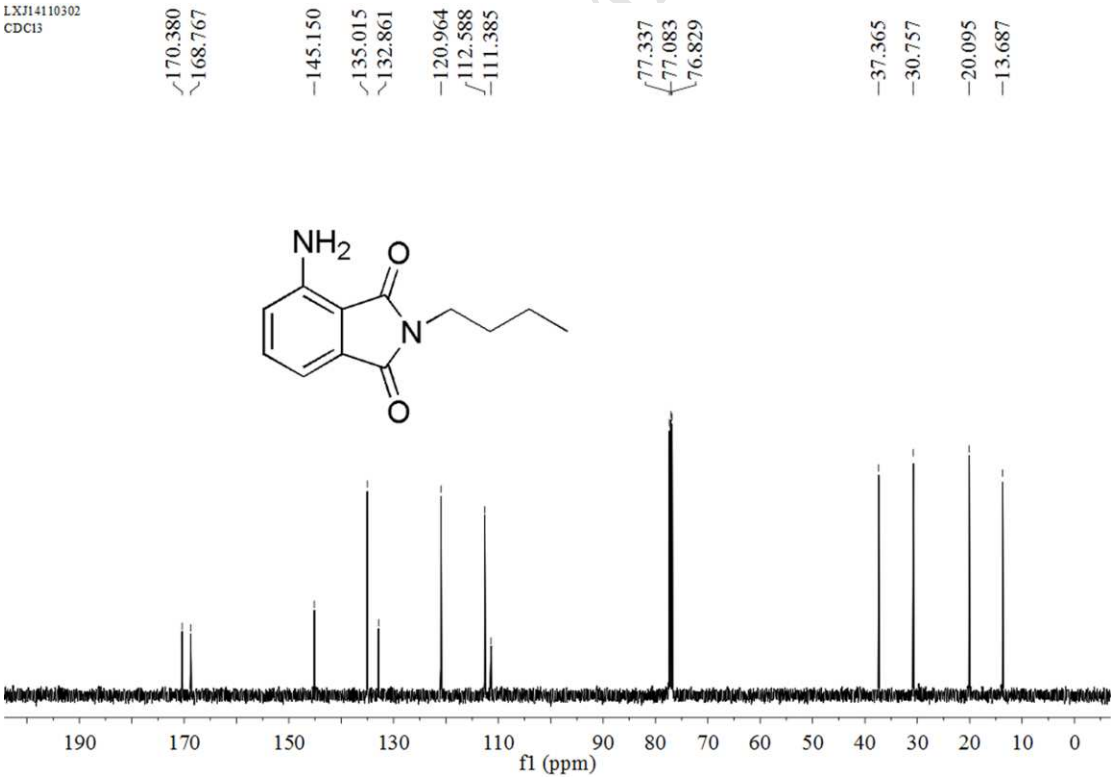


Fig. S5 ^{13}C NMR spectrum of compound **3**.

ACCEPTED MANUSCRIPT

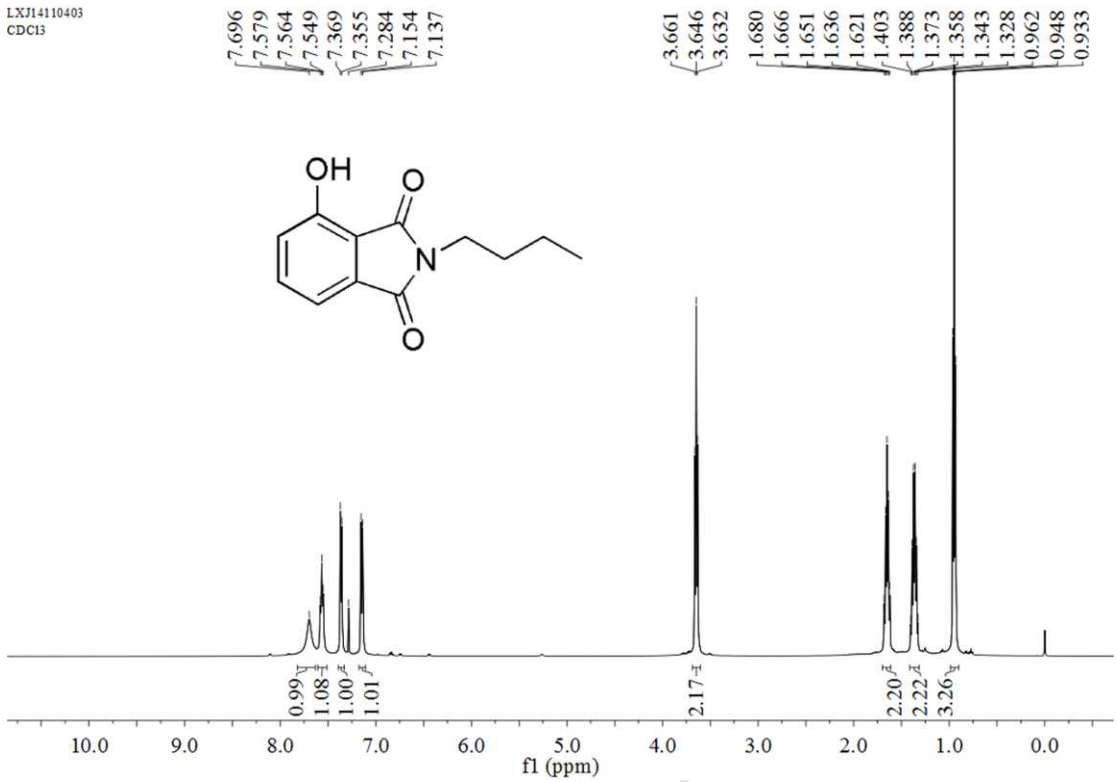


Fig. S6 ^{1}H NMR spectrum of compound **4**.

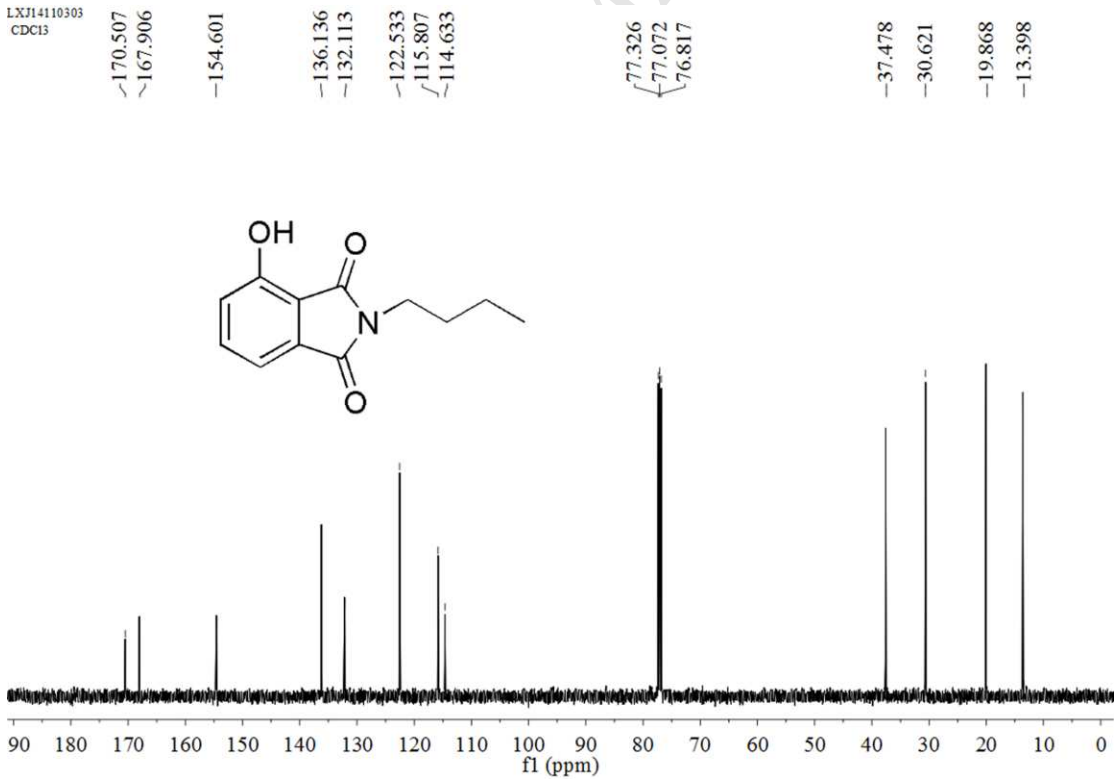


Fig. S7 ^{13}C NMR spectrum of compound 4.

ACCEPTED MANUSCRIPT

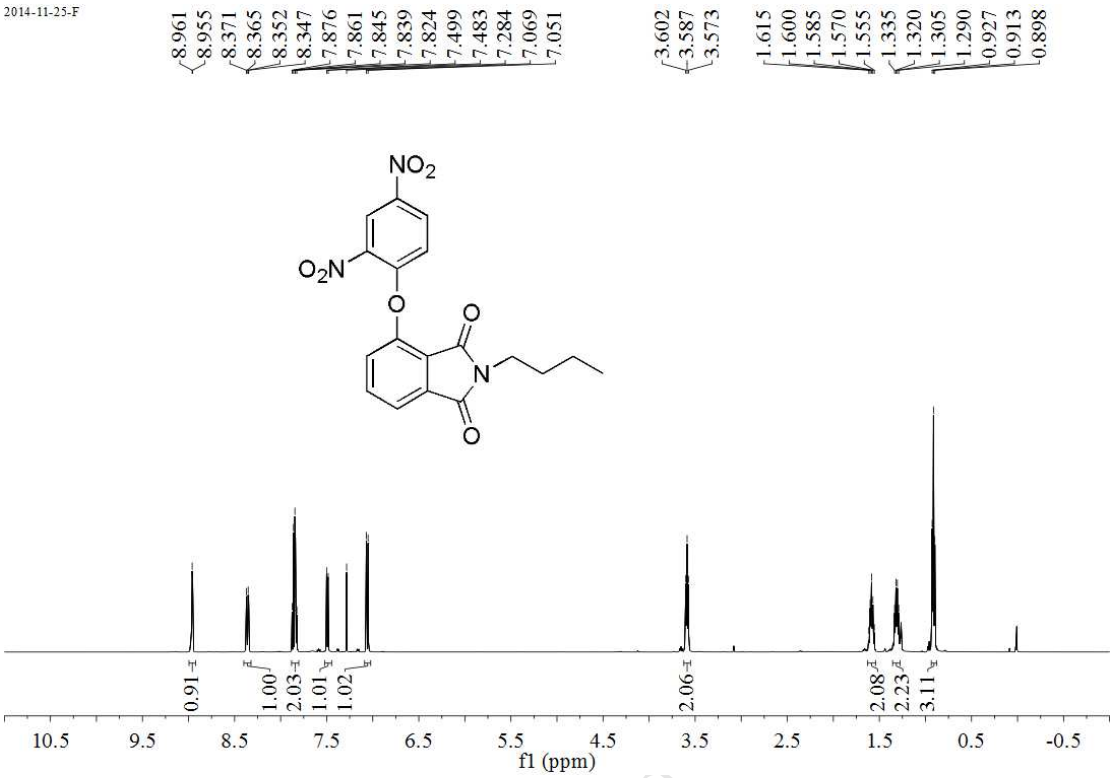


Fig. S8 ^{1}H NMR spectrum of Probe **1**.

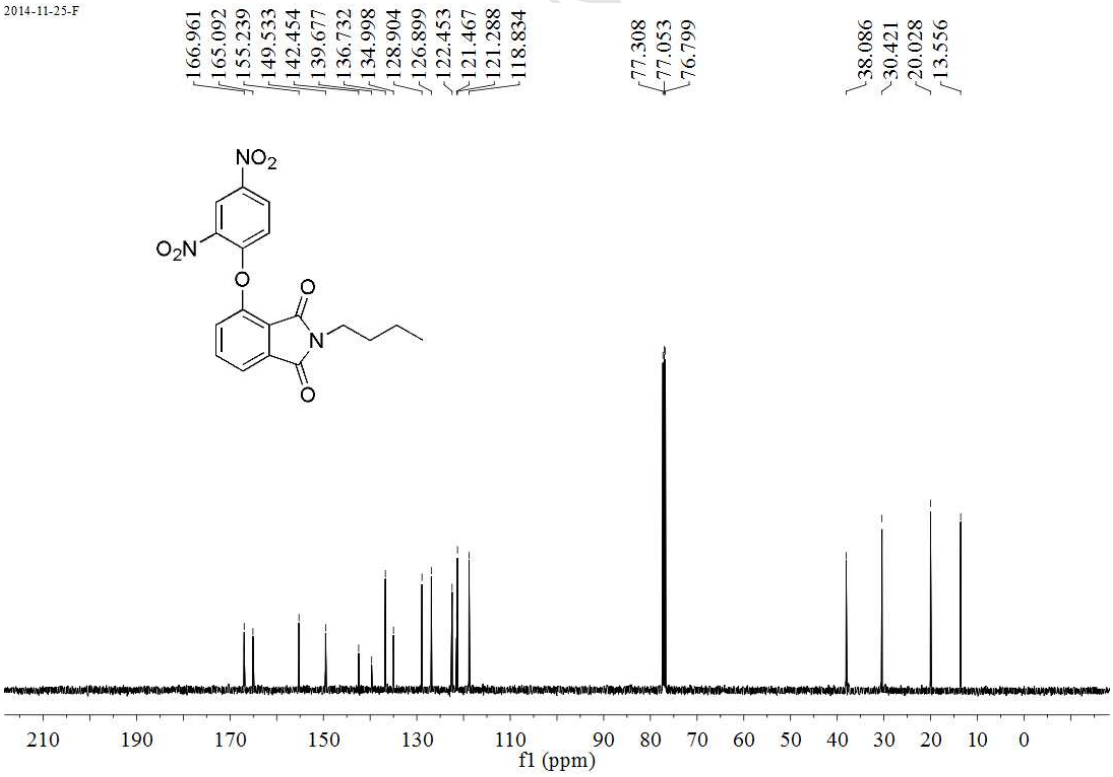


Fig. S9 ^{13}C NMR spectrum of Probe **1**.

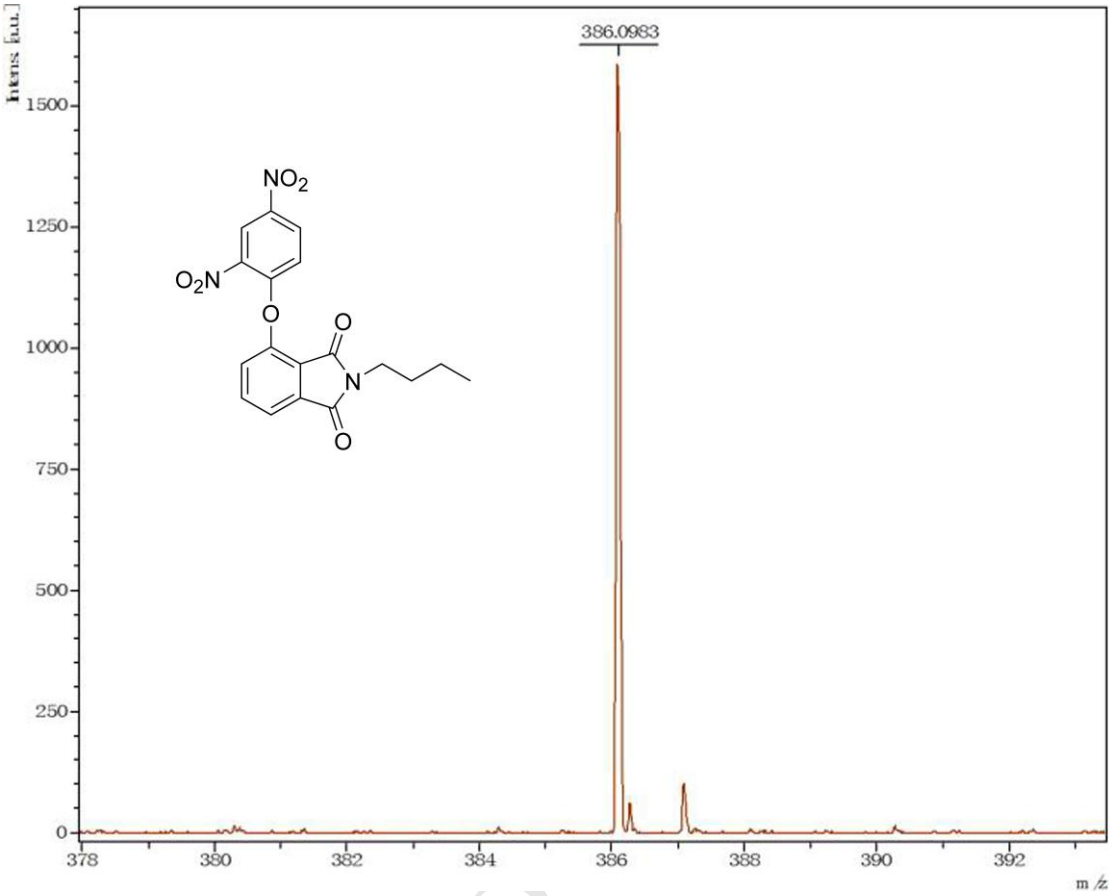


Fig. S10 HRMS spectrum of Probe **1**.

ACCEPTED

ACCEPTED MANUSCRIPT

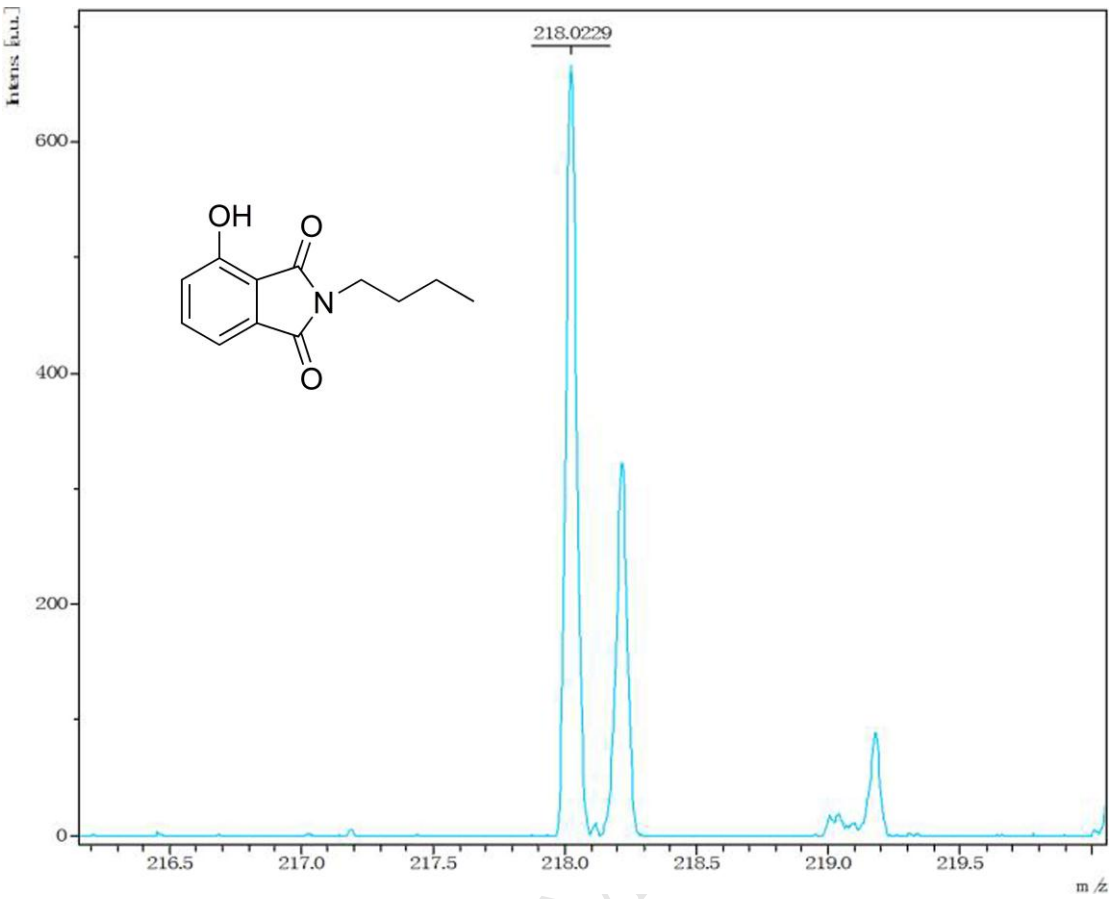


Fig. S11 HRMS spectrum of the reaction product of Probe **1** with thiophenol, dye **4.**

ACCEPTED MANUSCRIPT