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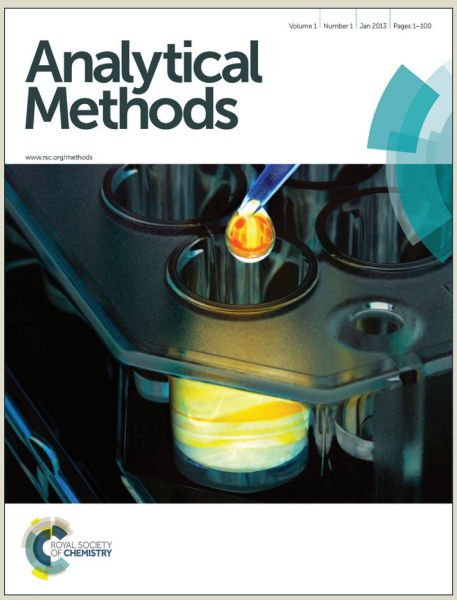

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A colorimetric and near-infrared fluorescent turn-on probe for in vitro and in vivo detection of thiophenols†

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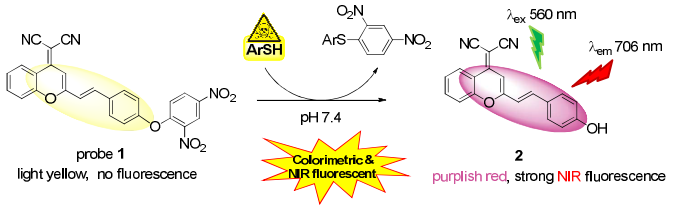
A colorimetric and near-infrared fluorescent probe for thiophenols was developed. This new probe features remarkable large Stokes shift, and shows a rapid, highly selective and sensitive detection process for thiophenols with obvious colorimetric and near-infrared fluorescent turn-on responses. The detection limit of this probe for thiophenol was found to be 70 nM. In addition, this probe shows great potential for *in vitro* and *in vivo* detection of thiophenols, which was exampled by the successful applications of this probe for quantitative detection of thiophenol in real water samples and fluorescent imaging of thiophenol in living cells and zebrafish.

Introduction

Thiophenols are highly toxic pollutants with the median lethal dose (LC50) ranging from 0.01 to 0.4 mM for fish.[1] Exposure to thiophenol liquid and vapor may cause a series of serious health problems to human body, including central nervous system damage, increased respiration, muscle weakness, hind limb paralysis, coma, and even death.[2] Despite this high toxicity, thiophenols are essential and widely used chemicals for preparation of agrochemicals, pharmaceuticals, and various industrial products.[3-4] Therefore, monitoring of thiophenols both in environmental and biological samples using simple, rapid and sensitive method is of great importance.

Fluorescent detection using fluorescent probes has been recognized as one of the most attractive method to monitor and visualize molecules due to its simplicity, convenience, and great potential for use in a wide range of chemical, biological, and environmental applications. Since Wang et al.[5] reported the first reaction-based fluorescent turn-on probe for selective detection of thiophenols, a number of fluorescent probes have been developed for thiophenols.[6-20] However, these probes are mostly excited and/or emit in the UV to visible region in the optical spectrum, which limited their biological applications. Compared to the fluorescence in the visible region, near-infrared (NIR, 650-900 nm) fluorescence is more suitable for bioimaging applications due to its increased ability for tissue penetration, reduced background autofluorescence and minimized photo-damage to biological samples.[21-22] However, NIR fluorescent probes for thiophenols are very rare to date. Very recently, we reported the first NIR fluorescent turn-on probe that can be used for rapid, highly selective and sensitive detection of thiophenols with a detection limit of 0.15 μM for PhSH.[23]

Herein, we report a new dicyanomethylene-benzopyran (DCMB)-based NIR fluorescent probe for thiophenols (probe **1** in Scheme 1). This probe uses a 2,4-dinitrophenyl-ether (DNP-ether) group as the thiophenol-responsive reaction site. Importantly, we found that this probe not only shows a rapid fluorescent turn-on sensing process for thiophenols with high selectivity and better sensitivity (DL = 70 nM), but also offers a convenient colorimetric detection process for thiophenols. It should be noted that a dual colorimetric and fluorescent probe is particularly valuable because it offers not only the sensitive detection by fluorescence, but also provides a convenient visual sensing by the "naked eye" without the need for advanced instrumentation. In addition, this probe can be used to detect thiophenol in real water samples and living cells, and moreover, we have also successfully applied it to detect thiophenol in zebrafish, which indicates that this probe has great potential for *in vitro* and *in vivo* applications.

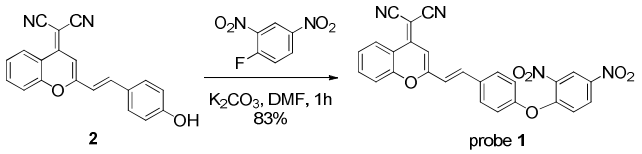


Scheme 1. Probe **1** for colorimetric and NIR fluorescent sensing of thiolphenols.

Results and discussion

1. Probe design and synthesis.

Probe **1** was designed to use compound **2** (Scheme 1) as the fluorophore and a DNP-ether unit as the reactive site for thiophenols. Compound **2** is a known NIR fluorophore with good photostability and large Stokes shift, and it was recently used by Zhu et al.[24] and us[25] to develop colorimetric and NIR fluorescent probes for biothiols. The DNP-ether unit is a well known fluorescent quencher but can be readily cleaved by thiophenols under physiological pH.[6,13,14] Therefore, we can expect that upon treatment with thiophenols, compound **2** will be released from the probe **1** solution, thus providing a convenient method to detect thiophenols with dual colorimetric and NIR fluorescence changes as shown in Scheme 1. With this expectation, probe **1** was synthesized.



Scheme 2. Synthesis of Probe **1**.

The synthesis of probe **1** is outlined in Scheme 2. Briefly, probe **1** can be readily prepared in 83% yield by the reaction of **2** with 1-fluoro-2, 4-dinitrobenzene under basic conditions at room temperature, and its structure and purity was confirmed by ^{1}H NMR, ^{13}C NMR, HR-MS and elemental analysis. The experimental details and structure characterizations are given in the experimental section and in the ESI†.

2. Colorimetric and NIR fluorescent sensing thiophenol by probe 1.

The sensing ability of probe **1** for thiophenol was investigated in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) with a probe concentration at 10 μM by absorption and fluorescence spectroscopy at 37°C. Under this condition, the probe **1** solution is light yellow with maximum absorption at 430 nm and as expected, it exhibits almost no fluorescence over 650 nm (Fig. 1). Upon addition of PhSH (50 μM) and after 5 min, the maximum absorption of the solution red-shifted 130 nm from 430 nm to 560 nm, accompanied by a distinct color change from light yellow to purplish red (Fig. 1a). Meanwhile, the probe **1** solution showed significant enhancement (~25 folds) of NIR fluorescence around 706 nm (Fig. 1b). It should be noted that the 146 nm of Stokes shift of probe **1** for detection of thiophenol is a big advantage as this can diminish the measurement error caused by the excitation light and scattered light effectively. In addition, fluorescence kinetics showed that the reaction between probe **1** and thiophenol can complete at about 5 min, and the reaction obeys a typical pseudo-first-order with rate constant k_{obs} determined to be about 0.542 min^{-1}, which corresponds to a half-life ($t_{1/2}$) of 1.28 min (Fig. 1, inserted). This indicates that probe **1** can be used for rapid detection of thiophenol. In addition, the fluorescence changes of probe **1** upon addition of thiophenol suggest that the reaction produced compound **2**. This was confirmed by TLC analysis of the reaction by comparing to the reference sample (Fig. S1, ESI†). We also investigated the effect of the amount of DMSO from 30-50% (v/v) and the reaction temperature at 37 and 25°C, and found that the reaction speed of probe **1** for sensing of thiophenol is more favorable if using 50% DMSO at 37°C (Fig. S2-S3, ESI†). In addition, experiments showed that probe **1** itself is not pH sensitive over a wide range of pH (4-10), however, in the presence of PhSH, a large enhancement in the absorbance intensity at 560 nm and fluorescence intensity at 706 nm can be observed over a wide pH range from 6 to 10 with maximum changes at pH over 8, indicating probe **1** can be used to detect PhSH over a wide pH range including physiological pH (Fig. S4, ESI†). Overall, all these results indicate that probe **1** can be used as a colorimetric and NIR fluorescent turn-on sensor for rapid detection of thiophenol in aqueous solution under mild conditions.

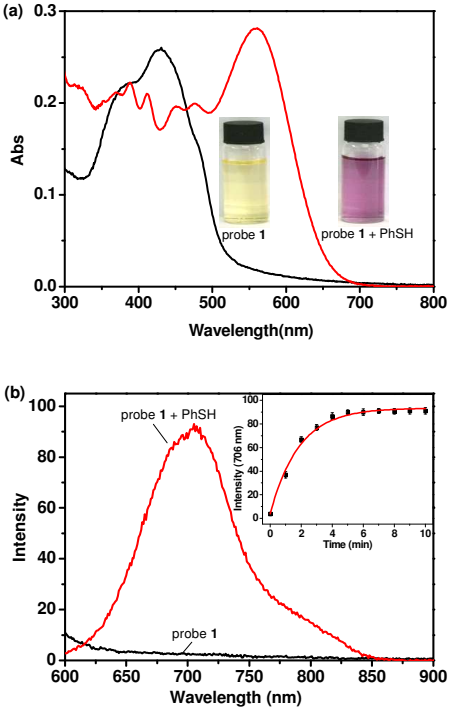


Fig. 1 (a) UV-Vis spectral changes and (b) Fluorescence spectral changes of probe **1** (10 μm) upon addition of PhSH (5eq) after 5 min in in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) at 37°C. For fluorescent measurement, λ_{ex} = 560 nm, slit width (10, 10). Insert in (b): Fluorescent kinetic curve of probe **1** (10 μM) at 706 nm with PhSH (50 μM) in DMSO-PBS buffer (10 mM, pH 7.4, 1:1, v/v) at 37°C, and the data were fitted (red line) by a first-order reaction scheme ($I = I_{max} + (I_0 - I_{max})\times e^{-k*T}$) to give rate constant k_{obs} = 0.542 min^{-1} (R^2 = 0.99817).

3. The sensitivity.

In order to explore the sensitivity of probe **1** for thiophenol, the absorbance and fluorescence changes of the probe **1** solution upon addition of different concentrations of PhSH were measured, respectively. As shown in Fig. S5-S6 (ESI†), with increasing concentrations of PhSH, the probe **1** solution

showed progressive increases in absorption at 560 nm and fluorescence intensity at 706 nm, respectively, and saturation occurred when PhSH was added more than 5 equiv. to the concentration of probe **1**. Moreover, as shown in Fig. 2, a good linear calibration curve can be found between the fluorescent intensity changes at 706 nm and the concentration of PhSH in the range of 0 to 5 μM (R^2 = 0.99763). Thus, the detection limit of probe **1** for PhSH was determined to be about 70 nM based on fluorescent detection with the signal to noise ratio (S/N) = 3. This indicates that probe **1** can be used to detect PhSH quantitatively with high sensitivity. In addition, if without any instrumentation, the discernible level of PhSH observed by the naked eye could be as low as 2 μM (Fig. S7, ESI†).

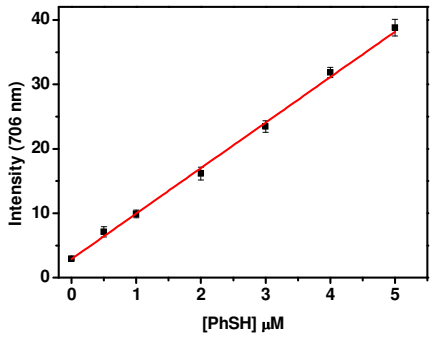


Fig. 2 Fluorescence intensity changes of probe **1** (10 μM) at 706 nm against concentration of PhSH from 1 to 5 μM. Each data was obtained 5 min after PhSH addition. λ_{ex} = 560 nm, slit width: (10, 10). The data were reported as the mean ± standard deviation of triplicate experiments.

4. The selectivity.

We next investigated the selectivity of probe **1** for thiophenols. To do this, various analytes including thiophenol derivatives (C_6H_5SH, *p*-CH_3-C_6H_4SH, *p*-NH_2-C_6H_4SH, *p*-CH_3O-C_6H_4SH and *p*-NO_2-C_6H_4SH), aliphatic thiols including cysteine (Cys), homocysteine (Hcy), glutathione (GSH), $(CH_3)_3CSH$ and $OHCH_2CH_2SH$, other potential interfering substances such as NaN_3, KBr, KI, KCN, NaSCN, $NaHSO_3$, C_6H_5OH, $C_6H_5NH_2$ and NaHS were tested. Since probe **1** is a colorimetric sensor, a simple colorimetric assay was firstly explored. As we can see in Fig. S8 (ESI†), among these analytes, only thiophenols (except *p*-NO_2-C_6H_4SH) induced obvious color changes to the probe **1** solution, indicating probe **1** is highly selective for other thiophenols. This high selectivity was further proved by the optical spectra changes of probe **1** towards these analytes. As shown in Fig. 3, obvious UV-vis spectral changes and fluorescence enhancement of the probe **1** solution were only observed upon addition of thiophenols. Since the detection process of probe **1** for thiophenol is based on the nucleophilic cleavage of the dinitrophenyl ether unit, the high selectivity for thiolphenols over aliphatic thiols can be explained by the much lower pK_a values of thiophenols (~6.5) than that of aliphatic thiols (~8.5),[5] which results in thiophenols much more reactive due to more thiolate forms existing as nucleophile under the test condition (pH 7.4). The exceptional results of *p*-NO_2-C_6H_4SH is probably because of its low nucleophilic activity towards dinitrophenyl ether owing to the strong electron-withdrawing NO_2 group,[14] which is consistent with our previous results[23] and other reports in the literature.[5,17] In addition, probe **1** is inert to many reactive nitrogen and oxygen species (ROS/RNS) such as ClO^-, H_2O_2, NO_2^-, $^{\bullet}OH$, $^{t}BuOO^{\bullet}$, $ROO^{\bullet}$, NO, and $O_2^{\bullet-}$, and detection of thiophenol using probe **1** in the presence of ROS/RNS and other analytes mentioned above is still effective (Fig. S9-S11, ESI†). Therefore, all these results clearly indicate that probe **1** has high selectivity to thiophenols (except *p*-NO_2-C_6H_4SH).

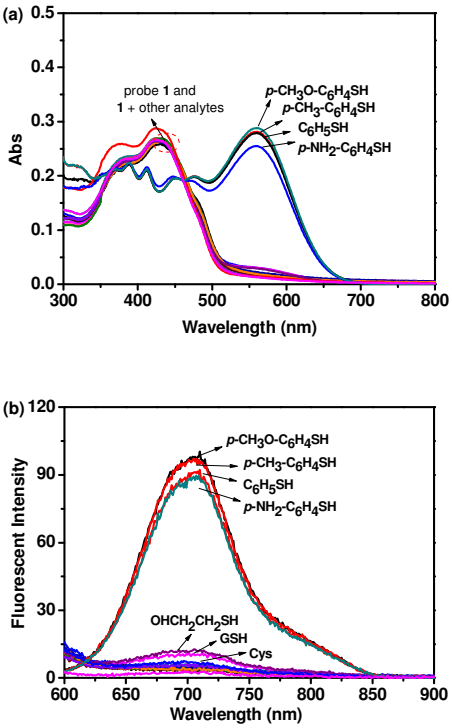


Fig. 3 (a) Absorption responses and (b) fluorescence responses of probe **1** (10 μM) upon addition of various analytes (blank, C_6H_5SH, Cys, Hcy, GSH, NaHS, $NaHSO_3$, NaN_3, NaCl, KBr, KI, KCN, NaSCN, C_6H_5OH, $C_6H_5NH_2$, $OHCH_2CH_2SH$, $(CH_3)_3CSH$, *p*-OCH_3-C_6H_4SH, *p*-CH_3-C_6H_4SH, *p*-NO_2-C_6H_4SH, and *p*-NH_2-C_6H_4SH. Concentration: thiophenols, 50 μM; others, 100 μM). All experiment was performed in PBS buffer (20 mM, pH 7.4) with 50% DMSO at 37°C and each spectrum was obtained 5 min after addition of analyte. λ_{ex} = 560 nm , slit width: (10,10).

5. The potential applications of probe 1.

After made the high selectivity and sensitivity of probe **1** for thiophenols clear, we employed probe **1** to determine thiophenol concentrations in real water samples to validate its practical utility in environmental science. The water samples were collected from the Yangtse River, East Lake and South Lake in Wuhan city. These water samples were directly analysed first, and then spiked with thiophenol at different levels (0.5 μM, 1 μM and 5 μM), and then the fluorescence responses of probe **1** at 706 nm towards all these samples were examined, respectively. After comparing the results with those

determined in distilled water, we are delight to see that the recoveries of thiophenol are fairly good (recovery ranged 94-108%, see Table 1), which indicates that the thiophenol in the water samples could be accurately measured with good recovery when probe **1** was applied as the probe. This result showed that probe **1** has potential application for quantitative detection of the highly toxic thiophenols in real water samples.

Table 1. Determination of PhSH concentration in water samples[a]

Water sample from	Thiophenol spiked (μM)	Thiophenol recovered (μM)	Recovery (%)
Yangtse River	0	not detected	-
	0.5	0.48 ± 0.01	96
	1	1.03 ± 0.06	103
	5	4.72 ± 0.20	94
East Lake	0	not detected	-
	0.5	0.51 ± 0.02	102
	1	0.99 ± 0.08	99
	5	5.42 ± 0.01	108
South Lake	0	not detected	-
	0.5	0.47 ± 0.02	94
	1	0.94 ± 0.02	94
	5	5.25 ± 0.23	105

[a]Note: The results shown in the Table 1 were reported as the mean ± standard deviation of triplicate experiments.

Fluorescent imaging of thiophenol in living cells was also investigated to explore the potential application of probe **1** in biological samples. As shown in Fig. 4, when HeLa cells were incubated with probe **1** (20 μM), no fluorescence can be observed (C). However, when HeLa cells were pre-incubated with PhSH (20 μM) for 30 min, and then incubated with probe **1** (20 μM) for another 30 min, strong red fluorescence were observed in the cells. These data clearly indicate that probe **1** can be applied to detect thiophenol in living cells.

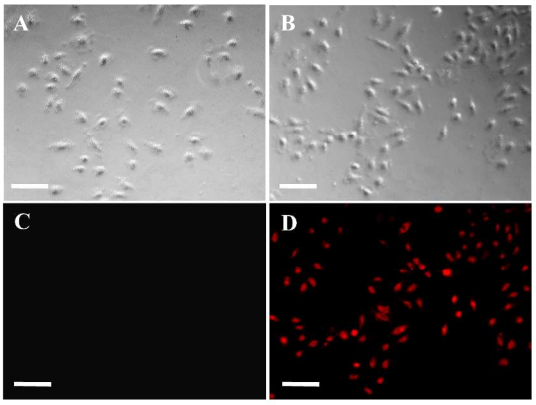


Fig. 4 Fluorescent imaging of thiophenol in HeLa Cells by probe **1**. Top: (A) and (B) are bright field images. (A) HeLa cells were treated with probe (20 μM) for 30 min; (B) HeLa cells were pre-incubated with 20 μM PhSH for 30 min and then treated with probe (20 μM) for 30 min. Bottom: (C) and (D) are fluorescence images of (A) and (B), respectively, with excitation wavelength at 515-560 nm. Scale bar = 100 μm.

In addition, fluorescent imaging of thiophenol in live zebrafish by probe **1** was also investigated. Initial experiments showed that zebrafish is still live even after 48 h in water containing 5-20 μM of probe **1**, indicating that probe **1** displays low toxicity to zebrafish under this condition. Based on this result, fluorescent imaging of thiophenol in live zebrafish was conducted by probe **1.** As shown in Fig. 5, when zebrafish were treated with probe **1** (5 μM) for 18 h, no fluorescence can be observed (A2 and B2). However, when zebrafish were pre-treated with probe **1** (5 μM) for 24 h and then treated with thiophenol (5 μM) for 2h, strong red fluorescence can be observed (C2 and D2). These data clearly show that probe **1** can be applied to detect thiophenol in zebrafish, indicating its great potential for *in vivo* imaging applications.

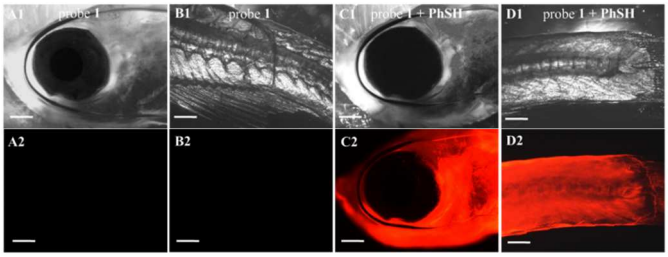


Fig. 5 Fluorescent imaging of thiophenol in live zebrafish by probe **1**. Top: A1-D1 are bright field images of the head part or the body part of Zebrafish. A1 and B1: Zebrafish was treated with probe (5 μM) for 18 h; C1 and D1: Zebrafish was pre-treated with probe (5 μM) for 18 h, washed with water, and then treated with PhSH (5 μM) for 2 h. Bottom: A2-D2 are fluorescence images of A1-D1, respectively, with excitation wavelength at 515-560 nm. Scale bar = 50 μm.

Experimentals

General

All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultrapurification system. TLC analysis was performed using precoated silica plates. IR spectra were recorded on a FT-IR spectrophotometer as KBr pellets and were reported in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 600 or 400 spectrometer. The low-resolution MS spectra were performed on an electron ionization mass spectrometer. HR-MS data were obtained with an LC/Q-TOF MS spectrometer. UV-Vis and fluorescence spectra were recorded on a UV-Vis spectrophotometer and a fluorescence spectrophotometer with a temperature controller, respectively. Standard quartz cuvettes with a 10 mm lightpath were used for all optical spectra measurements. Bioimaging was performed in an inverted fluorescence microscopy with a 20× objective lens for Hela cells and a 10× objective lens for Zebrafish, respectively.

Synthesis of compound 2[25]. 2-(2-methyl-4H-chromen-4-ylidene) malononitrile (208 mg, 1.0 mmol) and 4-hydroxybenzaldehyde (387.5 mg, 1.10 mmol) dissolved in 30 mL toluene. And then add 0.5 mL piperidine and 0.5 mL acetic acid. A dean-stark head was fitted and reaction mixture was heated under reflux for 12h. After the completion of the reaction, the mixture was allowed to cool to room temperature and then condensed under reduced pressure. A red solid was

obtained (205 mg, 41.0%). Mp: 256-258°C. TLC (silica plate): R_f 0.15 (hexane:ethyl acetate 3:1, v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 8.3 Hz, 1H), 7.91 (t, J = 7.8 Hz, 1H), 7.79 (d, J = 8.3 Hz, 1H), 7.70 (d, J = 16.0 Hz, 1H), 7.61 (dd, J = 21.2, 8.2 Hz, 3H), 7.27 (d, J = 15.9 Hz, 1H), 6.95 (s, 1H), 6.85 (d, J = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.1, 158.8, 152.7, 152.0, 139.2, 135.2, 130.3, 126.0, 124.6, 118.9, 117.4, 117.1 116.0, 115.8, 105.6, 59.0. IR (KBr, cm^{-1}): 3395, 2270, 2204, 1625, 1589, 1550, 1481, 1452, 1409, 1334, 1306, 1263, 1198, 1164, 1142, 975, 838, 811, 765, 742, 641; HR-MS (ESI): m/z, calcd for $C_{20}H_{13}N_2O_2^+$ (M + H$^+$) 313.0972; Found 313.0956. Elemental analysis calcd (%) for $C_{20}H_{12}N_2O_2$: C 76.91, H 3.87, N 8.97; found C 77.00, H 3.85, N 9.04.

Synthesis of probe 1. A mixture of compound **2** (312 mg, 1.0 mmol), 1-fluoro-2, 4-dinitrobenzene (205 mg, 1.1 mmol), and K_2CO_3 (274 mg, 2 mmol) in DMF (5 mL) was stirred at room temperature. After the reaction completes (about 1 h, monitored by TLC), the reaction mixture was poured into ice water. The precipitate was collected by filtration and washed with cold water to afford an yellow solid (394 mg, 83%). Mp: 277-280°C. TLC (silica plate): R_f 0.26 (hexane:ethyl acetate 3:1, v/v); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.93 (s, 1H), 8.75 (d, J = 8.2 Hz, 1H), 8.49 (d, J = 9.2 Hz, 1H), 8.00-7.89 (m, 2H), 7.86-7.74 (m, 2H), 7.64 (s, 1H), 7.57 (d, J = 16.2 Hz, 1H), 7.36 (dd, J = 14.0, 9.0 Hz, 2H), 7.07 (s, 1H), 5.75 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 157.9, 155.4, 154.1, 153.0, 152.0, 141.9, 139.8, 137.2, 135.5, 132.8, 130.5, 129.7, 126.3, 124.7, 121.9, 120.5, 120.4, 120.1, 119.1, 117.1, 115.8, 60.6. IR (KBr) νmax (cm^{-1}): 3441, 3088, 2207, 1629, 1599, 1556, 1534, 1498, 1456, 1408, 1348, 1269, 1199, 1166, 1140, 1016, 981, 835, 743; EI-MS: m/z found 478.30 (M$^+$); HR-MS (ESI): m/z, calcd for $C_{26}H_{15}N_4O_6^+$ (M + H$^+$) 479.0986, found 479.0993. Elemental analysis calcd (%) for $C_{26}H_{14}N_4O_6$ C 65.27, H 2.95, N 11.71; found C 65.37, H 2.82, N 11.51.

Preparation of solutions of probe 1 and analytes. Stock solution of probe **1** (1 mM) was prepared in HPLC grade DMSO. Stock solutions of C_6H_5SH, C_6H_5OH, $C_6H_5NH_2$, $OHCH_2CH_2SH$, $(CH_3)_3CSH$, p-CH_3-C_6H_4SH, p-NH_2-C_6H_4SH, p-CH_3O-C_6H_4SH, and p-NO_2-C_6H_4SH were prepared in DMSO (10 mM, respectively). Analytes including cysteine (Cys), homocysteine (Hcy), glutathione (GSH), NaN_3, NaCl, KBr, KI, KCN, KSCN, $NaHSO_3$, C_6H_5OH, $C_6H_5NH_2$ and NaHS were dissolved in distilled water to afford aqueous solutions of 2.5 mM for Cys and 10 mM for others. The stock solutions were used freshly and were diluted to desired concentrations with water when needed. The reactive nitrogen and oxygen species such as ClO^-, H_2O_2, NO_2^-, $^{\bullet}OH$, $^{t}BuOO^{\bullet}$, $ROO^{\bullet}$, NO, and $O_2^{\bullet-}$ were prepared according to our published procedure[23,25,26] and were used immediately after generated.

Determination of the fluorescence quantum yield： The fluorescence quantum yields of probe **1** (Φ = 0.0096) and compound **2** (Φ = 0.085) were determined in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v) at 37 °C, using rhodamine B (Φ = 0.89 in ethanol) as standard.[27-28]

Measurements of optical changes of probe 1 upon addition of analytes. A typical procedure: a solution of probe **1** (10 μM) was prepared in DMSO-H_2O solution (1:1, v/v, 20 mM PBS buffer), and then 3.0 mL of the probe **1** solution was placed in a quartz cell until the temperature reached at 37°C. The absorption or fluorescent spectra were then recorded upon addition of the specified analyte.

Thiophenol measurements in water samples. The crude water samples from the Yangtse River, East Lake and South Lake in Wuhan city were passed through a microfiltration membrane before use. The pH values of the water samples (50 mL) were adjusted using a sodium phosphate buffer (20 mM, pH 7.4), and aliquots of the water samples were then spiked with different concentrations of thiophenol (0.5, 1, 5 μM) that had been accurately prepared. The resulting samples were then treated with probe **1** in a phosphate buffer (20 mM, pH 7.4) to give the final mixtures (3.0 mL) containing probe **1** (10 μM) and thiophenol (0.5, 1 or 5 μM). The solutions were incubated for 5 min at 37°C, and then the fluorescence was measured at 706 nm. The results shown in Table 1 were reported as the mean ± standard deviation of triplicate experiments for thiophenol detection.

Cell Culture and Bioimaging. HeLa cells were cultured were cultured according to our published procedures.[27-31] For living cells imaging experiments, part of cells were incubated with 20 μM probe **1** (with 0.4% DMSO, v/v) for 30 min at 37 °C and washed three times with prewarmed PBS buffer, and then imaged. Meanwhile, another part of cells were pretreated with thiophenol (20 μM, 0.4% DMSO) for 30 min at 37 °C, washed three times with prewarmed PBS buffer, and then incubated with probe (20 μM in 0.4% DMSO) for 30 min. Cell imaging was then carried out after washing cells with prewarmed PBS buffer.

Imaging of zebrafish. The zebrafish (one month old) was incubated with probe **1** (5 μM) in water for 18 h at 28°C. After washing with water three times, the zebrafish was imaged or was further incubated with 5 μM of thiophenol in water for 2 h at 28°C, and then imaged after washing with water. These experiments were approved by the institutional committee and were carried out strictly in accordance with the relevant laws and guidelines issued by the Ethical Committee of Wuhan University of China.

Conclusions

In summary, we have developed a new reaction-based colorimetric and NIR fluorescent probe for the detection of thiophenols. This probe shows a remarkable large Stokes shift, displays a rapid response, high selectivity and sensitivity for thiophenols (except p-NO_2-C_6H_4SH) with distinct color and NIR fluorescence turn-on signal changes. In addition, this probe can be used to detect thiophenol in real water samples, and can be used for fluorescent bioimaging of thiophenol in living cells and zebrafish. All these results indicate that this new probe holds great potential for detection of the highly toxic thiophenols both in environmental and in biological science.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Structure characterizations for probe **1**, additional data and spectra]. See DOI: 10.1039/b000000x/

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