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A novel isophorone-based Red-emitting/NIR probe for thiophenol and its application in real water sample and vivo

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Abstract: Fast, highly selective and sensitive thiophenol probes are highly desirable in the field of bioimaging and environmental monitoring. For that, based on the mechanism that thiophenol can effectively cleave the sulfonamide bond selectively[1, 2], we herein report a dicyanoisophorone-based Red-emitting/NIR probe for thiophenol detection. This probe had some desirable properties such as rapid response, high selectivity and sensitivity, remarkable large Stokes shift (181 nm), Red-emitting/NIR fluorescence region and low LOD value (80 nM, according to 3σ/s). Moreover, this novel Red-emitting/NIR probe can potentially be applied to the detection of thiophenols in real water samples quantitatively and fluorescent imaging in living cells and zebrafishes.

Keywords: Fluorescence probe; Isophorone-based dye; Thiophenol; Red-emitting/NIR; real water sample; vivo

1. Introduction

As a kind of important intermediates widely used in fine chemicals such as pharmaceutical intermediate and pesticides, thiophenol is inevitably faced with problems of high toxicity and heavy pollution. It is reported that the median lethal dose (LC_{50}) of thiophenol for fish were range from 0.01 to 0.4 mM [3] and dose (LD_{50}) for mouse were from 2.15 to 46.2 $mg \cdot kg^{-1}$[4]. In biological system, excessive intake of thiophenols will have a serious adverse effect especially on the human body, such as muscular weakness, nervous system damage and so on, even lead to death[5].

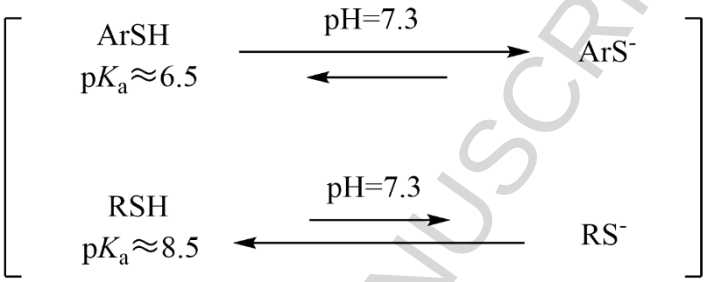
Therefore, considering its undesirable impact on organisms and environment. It has been important to develop a simple, rapid, sensitive, and selective method for detecting thiophenol in both environmental and biological fields. In the past few years, some methods have been developed to detect thiophenols such as

HPLC(high-performance liquid chromatography)[6], GC-MS(gas chromatography-mass spectrometry)[7] and UV–vis(Ultraviolet–visible spectroscopy) [8]. However, these methods have defects including high cost and complicated operation and so on. Compared with them, the fluorescent probe is considered to be a quite promising tool for its cheaper cost, lower detection limit, high sensitivity, quick response, and good biocompatibility, etc[9-11]. Due to the similar physical and chemical properties between thiophenol and bio-thiol, the development of fluorescent probes that can diff erentiate it is extremely challenging. Wang et al. [12] reported a fluorescent probe toward thiophenol with highly selectivity in 2007. It utilized the nucleophilic property of thiophenol that it is more efficiently dissociate to a thiolate species than thiols under neutral conditions (pH = 7.3) (Scheme 1)[13]. Then the nucleophilic thiolate ArS^- can cleave the sulfonamide and release the fluorophore to produce strong fluorescence. However, the existing fluorescent probes toward thiophenol still have many disadvantages. In addition to poor selectivity, low fluorescence intensity, sensitive to the environment, poor water solubility[14]and other disadvantages, most of them have an emission wavelength in range of 400 nm to 600 nm[15-28]. It is not desirable to the application of probes in bioimaging because of the high autofluorescence background, photobleaching and shallow tissue-penetration depth. In terms of Red-emitting/NIR fluorescent thiophenol probes especially that emission wavelength are over 650 nm, there are only three relevant reports up till now[29-31]. Therefore, there is an urgent need to develop fluorescent probe towards thiophenol with good performance especially in the Red-emitting/NIR region. Dicyanoisophorone-based dyes have been widely used in dye-sensitized solar cells [32, 33]and organic nonlinear optical crystals[34, 35] in a long time. But in recent years, it has been applied in fluorescence probe initially.

Now, we report a novel probe for detection of thiophenol which design dicyanoisophorone as NIR fluorophore due to its advantages of excellent membrane permeability, applicable fluorescence, and especially, the large Stokes shift owing to the ultrafast intramolecular charge transfer (ICT) as well as the Red-emitting/NIR

emission wavelength. 2,4-Dinitrobenzene-sulfonamide (DNBS) was designed as reaction site. As expected, thiophenol can make specific nucleophilic substitution reaction with the sulfonamide portion of **Probe 1** and release the fluorophore, inducing the fluorescence enhancement. Moreover, the **Probe 1** displayed high selectivity and sensitivity toward thiophenol in Red-emitting/NIR region.

Scheme 1. Illustration of the difference of nucleophilicity between thiophenols and thiols in Neutral Conditions.



2. Experimental section

2.1. Materials.

If not special specified，all the chemical and biological reagents were purchased from commercial suppliers and used directly. All the solvents (analytical grade and spectrophotometric grade) were purchased commercially and used as received unless otherwise mentioned. All the solutions were prepared by AR grade reagents and demineralized water. Silica gel (200−300 mesh) used in silica gel column chromatography was from Qingdao Haiyang Group Co., Ltd. in Qingdao city of China.

2.2. Instruments.

^{1}H and ^{13}C NMR spectra were obtained by Bruker Advance 600MHz at 25°C (using $CDCl_3$ and DMSO-*d*6 as solvent) and all chemical shifts were defined with ppm (δ). Fluorescence data were obtained through fluorescence spectrometer (Fluoromax-4, Horiba Jobin Yvon Inc). The quantum yields (Φ) were determined

based on an absolute method using an integrating sphere equipped with a fluorescence spectrometer (Fluoromax-4, Horiba Jobin Yvon Inc). UV–vis data were measured on UV-2600 (Shimadzu Corporation). Mass spectrometry data were measured on LCQAD-60000 (Thermo Electron Corporation). High-resolution mass spectra (HRMS) data were measured on HPLC-TOF (Agilent 1260-6224, Agilent Technologies). HPLC data were obtained from Ultimate 3000 (Thermofisher Scientific). Fluorescence imaging were obtained by Olympus FV3000 confocal microscope. The pH response was obtained from a Leici PHS-3C meter.

2.3. Synthesis.

Scheme 2 shows the synthetic routes and characterizations of each structure were shown in the Supplemental data.

2.3.1. Synthesis of compound **2**.

Isophorone (6.22 g, 45 mmol) was firstly dissolved in EtOH (90 mL), then malononitrile (3.30 g, 50 mmol), piperidine (0.4 mL, 4.0 mmol) and glacial acetic acid (0.24 g, 4.0 mmol) was added in turn. The mixture was refluxed for 6 h under N_2 atmosphere. After cooling to 25 ℃, the solution was slowly poured into water (150 mL) and the precipitated solid was obtained after filtration. Finally, recrystallized it from ethanol to obtain a yellow-white solid. Yield: 7.94 g (86%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.62 (s, 1H), 2.51 (s, 2H), 2.17 (s, 2H), 2.03 (s, 3H), 1.01 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.43, 158.88, 119.53, 112.19, 111.40, 77.13, 44.62, 41.60, 31.34, 26.78, 24.30.

2.3.2. Synthesis of compound **3**.

To a solution of compound **2** (1.87 g, 10 mmol), piperidine (0.20 mL) in acetonitrile (50 mL) was added 4-nitrobenzaldehyde (3.03 g, 20 mmol), the mixture was further stirred at 55 ℃ for 6 h. After the reaction, the precipitate was filtered, washed and recrystallized with ethanol to obtained compound **3** as light-yellow

crystal (2.49 g, 78%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.25 (d, J = 8.8 Hz, 2H), 7.65 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 16.2 Hz, 1H), 7.06 (d, J = 16.2 Hz, 1H), 6.94 (s, 1H), 2.64 (s, 2H), 2.49 (s, 2H), 1.10 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.76, 152.18, 147.87, 141.83, 133.73, 133.19, 127.93, 125.63, 124.34, 113.21, 112.37, 80.91, 42.78, 39.14, 32.07, 27.85.

2.3.3. Synthesis of compound **4**.

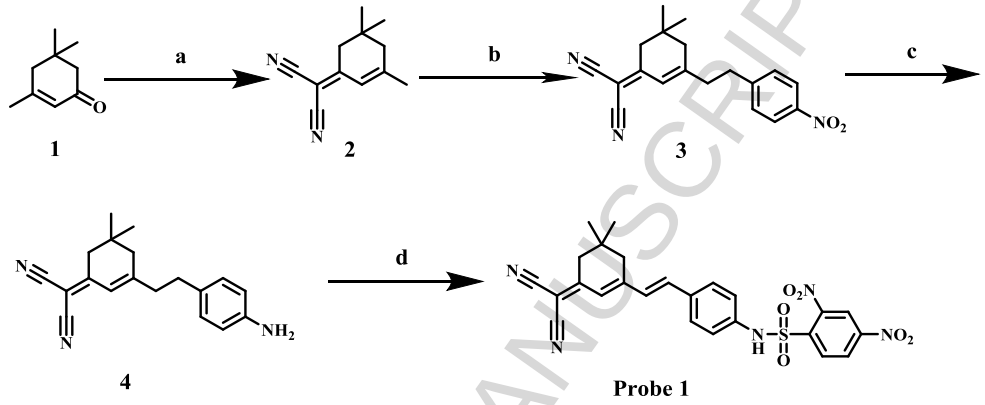
Hydrochloric acid (0.6 mL) was slowly added to a solution of compound **3** (2.55 g, 8 mmol) and $SnCl_2 \cdot 2H_2O$ (1.80 g, 8 mmol) in ethyl acetate (50 mL), the mixture was refluxed for 8 h, After the solvent was removed, the residue was washed with 15% sodium hydroxide and extracted with EA and dried over Na_2SO_4, purified by column chromatography over silica gel (eluent: PE/EA=6:1) to afford compound **4** as red powder (1.42 g, 70%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 16.0 Hz, 1H), 6.81 (d, J = 16.0 Hz, 1H), 6.76 (s, 1H), 6.67 (d, J = 8.5 Hz, 2H), 2.57 (s, 2H), 2.44 (s, 2H), 1.06 (s, 6H).^{13}C NMR (151 MHz, $CDCl_3$) δ 169.35, 154.92, 148.14, 137.70, 129.47, 126.19, 125.35, 122.00, 115.22, 113.92, 113.21, 43.00, 39.21, 32.01, 28.04.

2.3.4. Synthesis of **Probe 1**.

Compound **4** (231.5 mg, 0.8 mmol) was dissolved in dry CH_2Cl_2 (20 mL). Then K_2CO_3 (166 mg, 1.2 mmol) was added under N_2 atmosphere. Firstly, the mixture was cooled to 0 °C, then 2,4-dinitrobenzenesulfonyl chloride (266 mg, 1.0 mmol) dissolved in dry CH_2Cl_2 (10 mL) was added slowly. After 30 minutes of reaction, the mixture was stirred overnight at 25 °C. The mixture was then washed by 60 mL of water (3×20 mL) and brine (3×20 mL). Then dried by anhydrous Na_2SO_4. After removing the solvent, purified by column chromatography to get the **Probe 1** as a light-orange solid (150 mg, yield 36%). ^{1}H NMR (600 MHz, DMSO-*d*6) δ 11.35 (s, 1H), 11.35 (s, 1H), 8.92 (d, J = 2.3 Hz, 1H), 8.60 (dd, J = 8.7, 2.3 Hz, 1H), 8.25 (d, J = 8.7 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.32 (d, J = 16.1 Hz, 1H), 7.21 (d, J = 16.2 Hz,

1H), 7.16 (d, J = 8.7 Hz, 2H), 6.84 (s, 1H), 2.61 (s, 2H), 2.51 (s, 2H), 1.00 (s, 6H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 170.78, 156.23, 150.61, 148.33, 137.49, 137.03, 136.52, 133.20, 132.07, 129.72, 129.56, 127.78, 123.15, 120.93,120.88, 114.34, 113.54, 76.77, 42.82, 38.58, 32.15, 27.88. EI-MS: m/z found 518.1129 $(M\text{-}H^+)^-$. HR-MS calcd for $C_{25}H_{21}N_5O_6SNa^+(M + Na^+)^+$, 542.11049; found, 542.09829.

Scheme 2. Synthesis of **Probe 1**[a]



[a]Reagent and conditions: (a) piperidine, glacial acetic acid, 6 h, 86%; (b) piperidine, 4-nitrobenzaldehyde, 6 h, 78%; (c) Hydrochloric acid, $SnCl_2{\cdot}2H_2O$, 8 h, 70%; (d) K_2CO_3, CH_2Cl_2, piperidine, 2,4-dinitrobenzenesulfonyl chloride, r.t, 12 h, 36%.

2.4. Preparation of Solutions of **Probe 1** and Analytes.

Stock solution of **Probe 1** (100 μM) was prepared in CH_3CN (AR grade). Stock solutions of thiophenol derivatives, thiol derivatives and other water-insoluble nucleophiles (such as $C_6H_5NH_2$ and C_6H_5OH) were prepared in CH_3CN (2 mM). Amino acid nucleophile such as Cysteine (Cys), glutathione (GSH), homocysteine (Hcy) and alanine (Ala) were prepared in distilled water (10 mL) to obtain aqueous solution (2 mM). Metal ions and anions were also prepared in distilled water (50/100 mL) to obtain aqueous solution (2 mM) (All metal ions were derived from nitrate compounds, and the anions were all derived from sodium salts). The preparation of reactive nitrogen species (RNS) and reactive oxygen species (ROS) can be found in the Supplementary Material. All the stock solutions were used freshly.

2.5. Measurements of Spectral Response of **Probe 1** toward different Analytes.

Firstly, calculated and took different amounts of Stock solution of **Probe 1** (100 μM) and Stock solution of analytes (2 mM) then mixed them to obtain target solutions (5 mL) with different concentration of the analyte. Then, 4.0 mL of the mixed solution was placed in a quartz cell (10.0 mm width) for few minutes. The fluorescent spectra were recorded finally.

2.6. Detection of thiophenol in real water sample.

The real water samples were collected from the Yangtse River, the Xuanwu Lake, the Qinhuai River and the Jiulong Lake in Nanjing City and treated by microfiltration membrane before use. These water samples were analyzed after being spiked with different concentrations of thiophenol and finally gave the mixtures (5.0 mL) containing **Probe 1** (10 μM) and thiophenol (0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 20.0 μM). Each spectrum was obtained 10 minutes after addition of thiophenol, and the fluorescence spectra was obtained at 650 nm.

2.7. Cytotoxicity assays and Cell Imaging.

The cytotoxicity was determined by MTT assays[36]. A549 cells were firstly placed in a 96-well culture plate and were maintained in cell culture medium with 10% fetal bovine serum (FBS) and incubated in 5% CO_2 at 37 °C for 24 h. Then the A549 cells were interacted with different concentrations of **Probe 1**(0, 1, 2, 5, 10, 20, 50, 100, 200 μM) for 24 h, respectively. After incubated with probe, the cells were washed with PBS to remove remaining **Probe 1**, then added 0.1 mL serum-free medium containing 0.05% MTT each. After incubation for 4 h, removed the culture medium and dissolved the formazan with 0.15 mL DMSO and put them on the shaker for 10 min. Finally, the absorbance of treated cells was obtained at 469 nm and compared with the absorbance of the control group (100% viability) to get the cell viability.

A549 (human lung cancer line) were maintained in Roswell Park Memorial

Institute (RPMI)-1640 culture media (with 2 g/L D-Glucose, 0.3 g/L L-Glutamine, and 2 g/L Sodium Bicarbonate) with 10% FBS. Washed twice with PBS (pH 7.4) each time when use, the adherent cells were incubated with 10 μM **Probe 1** (with 0.2% CH_3CN, v/v) in CO_2 incubator (5% CO_2, 37 °C). After incubated for 30 min, the cells were washed with PBS (pH 7.4) three times. Then incubated with 20 μM thiophenol (with 0.2% CH_3CN, v/v) for 30 min. Fluorescence images were obtained by a confocal microscopy (Olympus, FV3000, Japan).

2.8. Imaging of zebrafish.

The 48h-old zebrafishes were kept in E3 embryo media at 28℃. Firstly, in the control group the 48h-old zebrafishes were treated with 10 μM **Probe 1** (E3 embryo media with 0.2% CH_3CN, v/v) for 30 min at 28 ℃. The zebrafishes in the other group was incubated with 10 μM **Probe 1** (E3 embryo media with 0.2% CH_3CN, v/v), then washed with PBS three times, the zebrafishes were interacted with 20 μM thiophenol (E3 embryo media with 0.2% CH_3CN, v/v) furtherly for 40 min at 28 ℃ and then imaged it by confocal microscope. All experiments were strictly in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals published by China Council on Animal Care.

3. RESULTS AND DISCUSSION

3.1. Properties of **Probe 1**.

Due to the predicted sensing mechanism when designing the probe, we expected that compound **4** was the expected product upon addition of thiophenol. Therefore, we first collected the UV and fluorescence spectrum of compound **4** and **Probe 1** respectively. As shown in Figure S1 (Supplementary Material). UV spectrum between compound **4** and probe had no obvious differences. However, the fluorescence spectrum showed that the probe had no obvious fluorescence when excited at 469 nm but compound **4** had a strong fluorescence. This tremendous difference in fluorescence intensity between the expected product compound **4 (**after addition of

thiophenol) and the probe itself was desirable in the design of sensitive probes. As shown in Figure S2 (Supplementary Material), The current **Probe 1** (10 μM) showed very low quantum yield (Φ=-0.61) under the excitation at 469 nm, which exhibited a larger Stokes shift and better quantum yield improvement (Φ=2.99) after addition of thiophenol (20 μM) in comparison with **Probe 1** (10 μM). The data was measured in PBS buffer (pH=7.4, containing 20% CH_3CN).

3.2. Response kinetics.

In order to explore the kinetics property of the probe upon addition of thiophenol, the response kinetics data was measured in PBS buffer (10 mM, pH=7.4, containing 20% CH_3CN). As shown in Fig. 1(a), after addition of thiophenol for a few minutes, the fluorescence of **Probe 1** (10 μM) showed an intense enhancement at 650 nm (λ_{ex}=469 nm). Kinetic curve demonstrated that the fluorescence intensity (at 650 nm) had a rapid response and levelled off within 7 min (Fig. 1(b)). This may be due to the probe had been fully reacted with thiophenol. After data processing, the kinetic curve conformed to the pseudo-first-order equation. The rate constant (k_{obs}) was about 0.70 min^{-1} ($t_{1/2} \approx 0.89$ min). These data fully demonstrated that **Probe 1** can detect thiophenol rapidly and distinctly in the Red-emitting/NIR region. In addition, the large Stokes shift exhibited by **Probe 1** is desirable for applications in the biological field.

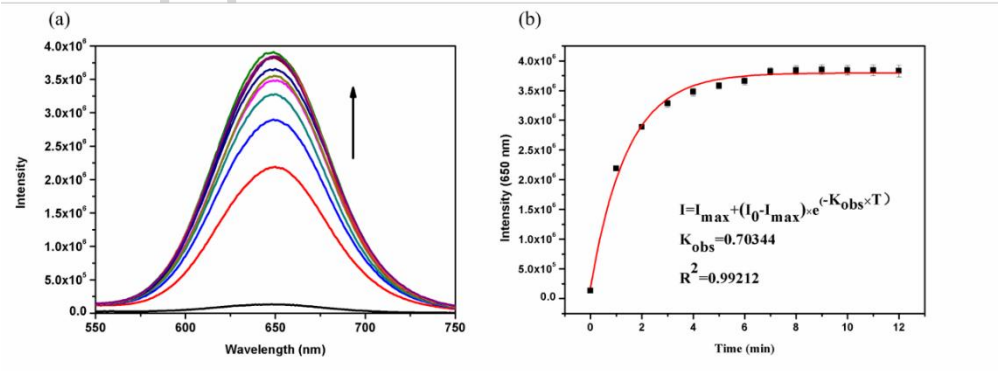


Fig. 1 (a) Fluorescence changes of **Probe 1** (10 μM) after addition of PhSH (100 μM) in PBS buffer (10 mM, pH=7.4, containing 20% CH_3CN). The spectra were obtained after adding thiophenol into solution of **Probe 1** at 1 min intervals. (b) Kinetic curve of **Probe 1** (10 μM) upon

addition of PhSH (100 μM) in PBS buffer (10 mM, pH=7.4, containing 20% CH_3CN).

3.3. Fluorescence titration.

Fig. 2 (a) shown the fluorescence spectral changes for probe upon addition of increasing concentrations of thiophenol (0-40 μM) and it reached a plateau upon addition of more than 2 equiv of thiophenol. It is notable that visible fluorescence can still be observed when the thiophenol concentration was lower than the stoichiometric conditions (1−10 μM). Fig. 2(b) displayed a good linearity (R=0.9907) with respect to thiophenol over the concentration range of 0–10 μM and detection limit of was 80 nM according to 3σ/s, which indicated that **Probe 1** had a potential employment in detecting thiophenol quantitatively. This result furtherly demonstrated that **Probe 1** has highly sensitivity toward thiophenol.

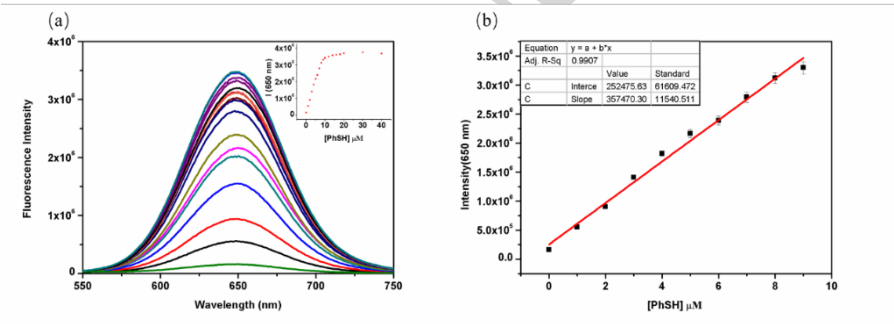


Fig. 2 (a) Fluorescence changes of **Probe 1** (10 μM) in PBS buffer (10 mM, pH=7.4, containing 20% CH_3CN) after addition of PhSH (different concentrations from 0 to 40 μM). Each spectrum was obtained after 10 min addition of PhSH. (b) Fluorescence intensity changes of **Probe 1**(10 μM) against thiophenol from 0 to 10 μM, which had good linearity.

3.4. Response of pH.

As shown in Figure S3 (Supplementary Material), we also investigated the pH effects on luminescent signal of **Probe 1** toward thiophenol. There was no obvious change in the fluorescence intensity of the probe itself as the pH changes and proved that the probe was stable over a wide pH range (from 4 to 10). However, after addition of thiophenol, fluorescence can be observed over the pH range from 4 to 10. It was noteworthy that the fluorescence intensity of the probe toward thiophenol

maintained a relative high plateau at a pH range from 7 to 11. This may due to the strong ionization of thiophenol (pK_a=6.5) at a condition that pH was over 6.5, which indicated that the probe can be applied over a relatively wide pH range.

3.5. Selectivity.

To investigate the selectivity of **Probe 1**(10 μM) toward thiophenol. We got the fluorescence change of **Probe 1** in response to various species including thiophenol analogues (C_6H_5SH, p-CH_3−C_6H_4SH, p-CH_3O−C_6H_4SH, p-NH_2−C_6H_4SH and p-NO_2−C_6H_4SH), aliphatic thiols (Cys, GSH, Hcy, $OHCH_2CH_2SH$, and $(CH_3)_3CSH$), some nucleophilic species (KI, NaN_3, C_6H_5OH, $C_6H_5NH_2$, Ala, and NaSH), some anions (HCO_3^-, $C_2O_4^{2-}$,$H_2PO_4^-$,HPO_4^{2-}, Cl^-, Br^-, I^-, PO_4^{3-}, F^-, CO_3^{2-}, Cl^-, NO_3^-, N_3^-, SO_4^{2-}, $S_2O_3^{2-}$) and some metal ions (Na^+, Mg^{2+}, Al^{3+}, K^+, Zn^{2+}, Ca^{2+}, Mn^{2+}, Ba^{2+}, Cu^{2+}, Cr^{3+}, Pb^{2+}, Co^{2+}, Fe^{3+}) as well as reactive oxygen and nitrogen species (ClO^-, H_2O_2, NO_2^-, $ONOO^-$, HNO, NO, ·OH, ROO·).

Probe 1 after addition of various major relevant species were shown in Fig. 3(a) and 3(b), only upon addition of thiophenol derivatives such as C_6H_5SH and p-CH_3−C_6H_4SH can induce an intense fluorescence enhancement at 650 nm, whereas other species resulted in no obvious change in the fluorescence emission spectra. However, p-NO_2−C_6H_4SH was an exception that only displayed a slight fluorescence enhancement. This may due to the low activity of sulfonamide cleavage owing to its relatively large S_{sulfur}^- value[37] and suggested that the detection of thiophenols which attached with an electron-withdrawing group was difficult and it was consisted with reports in the literature[15, 38].

In addition to above major specie, **Probe 1** solution had no obvious fluorescence change upon other mentioned species above including anions and metal ions (Fig. S4(a) and Fig. S4(b), Supplementary Material). Moreover, **Probe 1** still had superior selectivity against the interference of ROS (reactive oxygen species) and RNS (reactive nitrogen species) such as hypochlorite, superoxide, nitrite, etc. (Fig. S4(c) and Fig. S4(d), Supplementary Material).

All the spectra and data above indicated that **Probe 1** were highly selective for the detection of thiophenol over any other analytes we have mentioned.

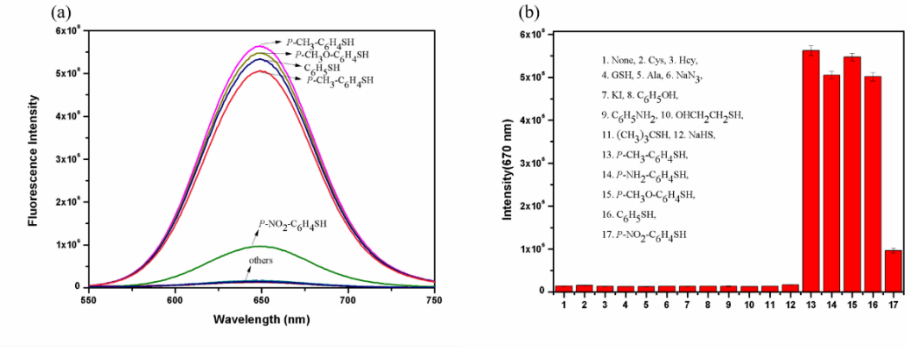
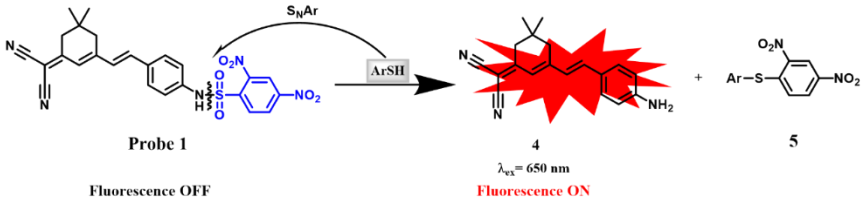


Fig. 3 (a) Fluorescence response of **Probe 1** (10 μM) upon addition of various analytes (100 μM). (b) Fluorescence responses of **Probe 1** (10 μM) upon addition of various analytes (100 μM) at 650 nm. All spectrum and data were obtained in PBS buffer (10 mM, pH=7.4, containing 20% CH_3CN), and each spectrum was measured upon addition of an analyte after 10 min. λ_{ex} = 469 nm; slit width: (5, 5).

3.6. Sensing mechanism.

A reasonable fluorescence sensing mechanism was shown in Scheme 3. Upon addition of thiophenol, sulfonamide bond was cut off by S_NAr process and fluorophore compound **4** was formed to displayed a strong fluorescence. This mechanism was confirmed by High-Resolution mass spectral analysis. After addition of thiophenol to the probe solution, a peak of 290.17213 found in the HRMS proved the formation of compound **5** and the other peak of 312.15545 further indicated the formation of [compound **4**+Na]$^+$ (Fig. S5). In addition, the HPLC spectrum can furtherly confirm this mechanism. According to Fig. S6, the retention time of compound **4** was 3.2 min, **Probe 1** was 2.6 min. In the spectrum part a, we can see that after addition of thiophenol, the peak of **Probe 1** ($t_{2.6}$) decreased and accompanied by the appearance of a new peak ($t_{3.2}$). This can further illustrate that fluorophore compound **4** was formed after S_NAr process.

Scheme 3. Diagram of the Probe Design for Thiophenol Detection.



3.7. Detection of Thiophenol in Water Samples.

To explore potential applications of **Probe 1** in real water sample. We collected samples from the Yangtze River, the Xuanwu Lake, the Qinhuai River and the Jiulong Lake in Nanjing and these water samples were analyzed by spiking with thiophenol at diff erent levels (0, 0.5, 1, 2, 4, 6, 8, 10 and 20 μM), and the fluorescence responses of **Probe 1** at 650 nm in all these real water samples were determined, respectively. As shown in Fig. 4(a), compared with those measured in distilled water, we can find good consistency and similarity between distilled water and real water sample. In addition, we can get good linearity with respect to thiophenol over the concentration range of 0–20 μM (Fig. 4(b)−(d)). And this was consistent with the linear relationship previously obtained in distilled water (Fig. 2(b)). Moreover, the recoveries of thiophenol (range from 86% to 109%, Table S1, Supplementary Material) indicated that the **Probe 1** can be used with good recovery. This result suggested that **Probe 1** had potential ability to detect thiophenols in water samples quantitatively.

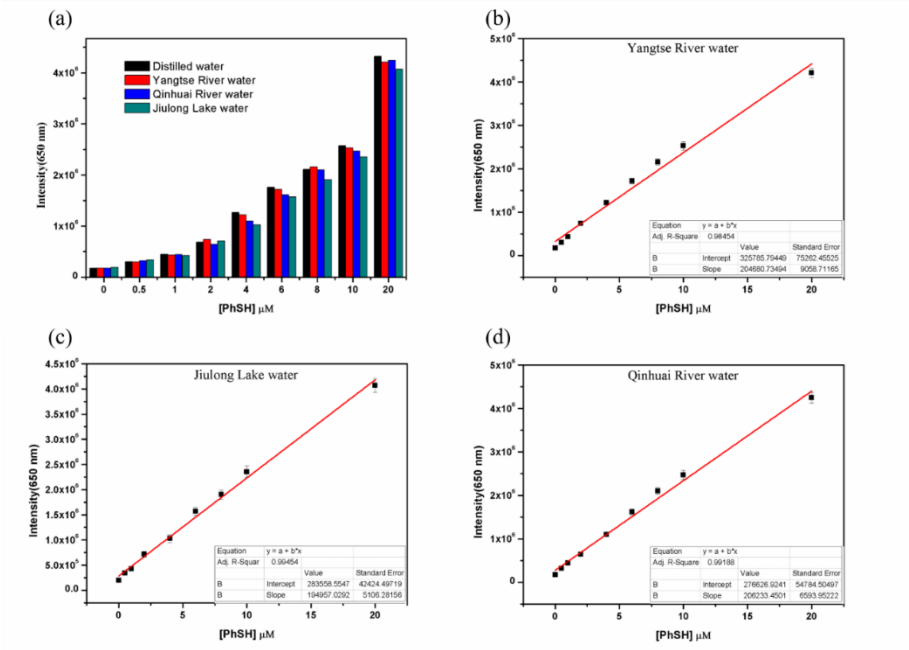


Fig. 4 (a) Fluorescent detection of **Probe 1** (10 μM) in "distilled water", "Yangtse River water", "Jiulong Lake water", and "Qinhuai River water" with 20% CH_3CN at 25 °C upon addition of different concentration of thiophenol. The data was obtained at 650 nm. Thiophenol was spiked in 0, 0.5, 1, 2, 4, 6, 8, 10 and 20 μM, respectively. λ_{ex} = 469 nm; slit width: (5, 5). (b−d) Linearity with respect to thiophenol over the concentration range of 0–20 μM in each sample.

3.8. Cell culture.

In order to evaluate the potential bio-applications of **Probe 1** for detection of thiophenol, cell imaging was carried out (Fig. 5). We firstly examined the cytotoxicity with A549 cells and the results showed that the viability of A549 cells was more than 85% when they were incubated with 0–100 μM of **Probe 1** for 24 h (Fig. S7, Supplementary Material), which indicated that **Probe 1** showed relatively low toxicity to A549 cells. As shown in Fig. 5, images of cells incubated only with **Probe 1** (10 μM) for 30 min (Fig. 5(a1)–(c1)), there were no fluorescence could be observed. As shown in Figures 5(a2) - (c2)), cells were first incubated with **Probe 1** (10 μM) for 30 minutes and then treated with thiophenol (20 μM) for 30 minutes. In the red channel, an intense red fluorescence could be clearly observed. The results demonstrated that **Probe 1** had low cytotoxicity and good cell permeability which can be used to

monitor thiophenol levels within living cells potentially.

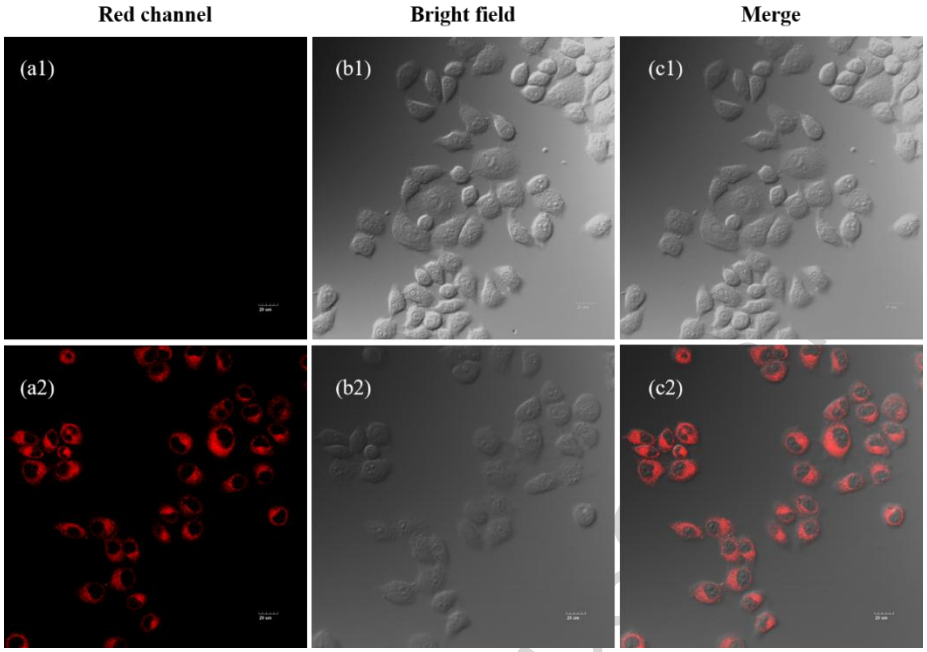


Fig. 5. Images of **Probe 1** responding to thiophenols in A549 cells. (a1–c1) A549 cells were interacted with **Probe 1** (10 μM) for 30 min; (a2–c2) A549 cells were interacted with **Probe 1** (10 μM) for 30 min, then interacted with thiophenol (20 μM) for 30 min; (a1) and (a2) were in red channel: λ_{em} =610–670 nm (λ_{ex} = 469 nm); (b1) and (b2) were in bright field; (c1) and (c2) were in merge. Scale bar: 20 μm.

3.9. Living imaging in zebrafish.

To further explore the application of **Probe 1** in vivo, fluorescence imaging of zebrafishes was obtained successfully. Firstly, in the control group, the 48h-old zebrafishes were interacted with **Probe 1**(10 μM) at 28 ℃ for 30 min. In the other group, the 48h-old zebrafishes were interacted with **Probe 1**(10 μM) for 30 min, then treated with thiophenol (10 μM) for 30 min. Finally, the fluorescence imaging was obtained through confocal microscopy. As shown in Fig. 6, the 48h-old zebrafishes that were incubated with thiophenol showed red fluorescence but those that were not incubated with thiophenol showed no obvious fluorescence. Therefore, this probe had the potential to become an ideal tool for monitoring thiophenol levels in vivo.

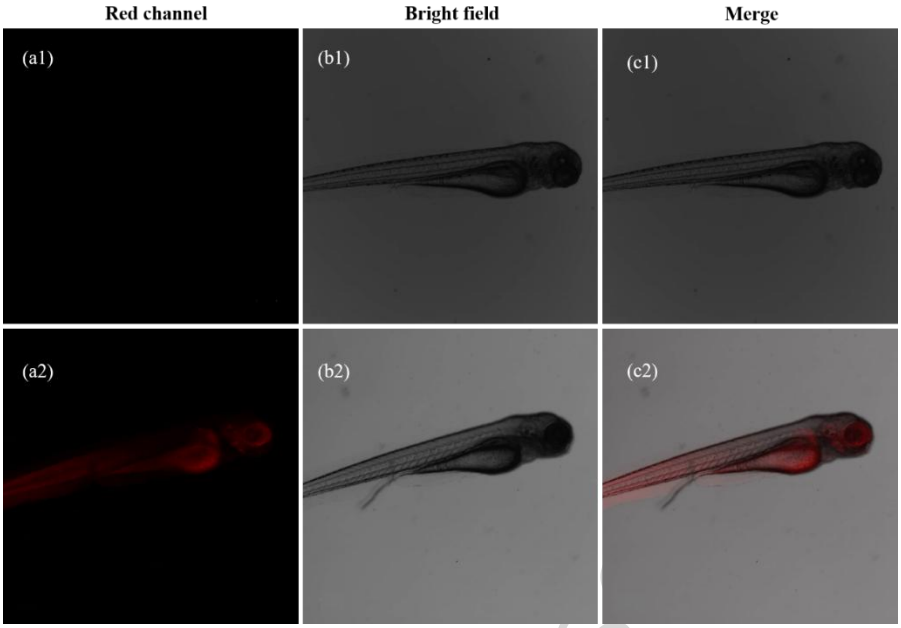


Fig. 6. Imaging of **Probe 1** toward thiophenols in zebrafishes. (a1–c1) Zebrafishes were interacted with **Probe 1**(10 μM) for 30 min; (a2–c2) zebrafishes were interacted with **Probe 1**(10 μM) for 30 min, then interacted with thiophenol (20 μM) for 30 min. (a1) and (a2) were in red channel: λ_{em} =610–670 nm (λ_{ex} = 469 nm); (b1) and (b2) were in bright field; (c1) and (c2) were in merge. Scale bar: 100 μm (a1–c1), 200 μm (a2–c2).

4. Conclusion

In conclusion, we have successfully developed a Red-emitting/NIR probe with sensitivity and selectivity for thiophenols by employing dicyanoisophorone derivatives as fluorophore and 2,4-dinitrobenzenesulfonamide as a response unit. After addition of thiophenols, the probe showed high selectivity and sensitivity response for thiophenols over other relevant species. In addition, the probe exhibited a relatively rapid response time, large Stokes shift (181 nm), and the LOD value was low to 80 nM. Furthermore, the probe was successfully applied for the quantitative detection of thiophenols in real water samples. Importantly, the application of this probe for detection of thiophenol in living cells and zebrafishes has proved that it would be a desirable tool in biological samples even in vivo.

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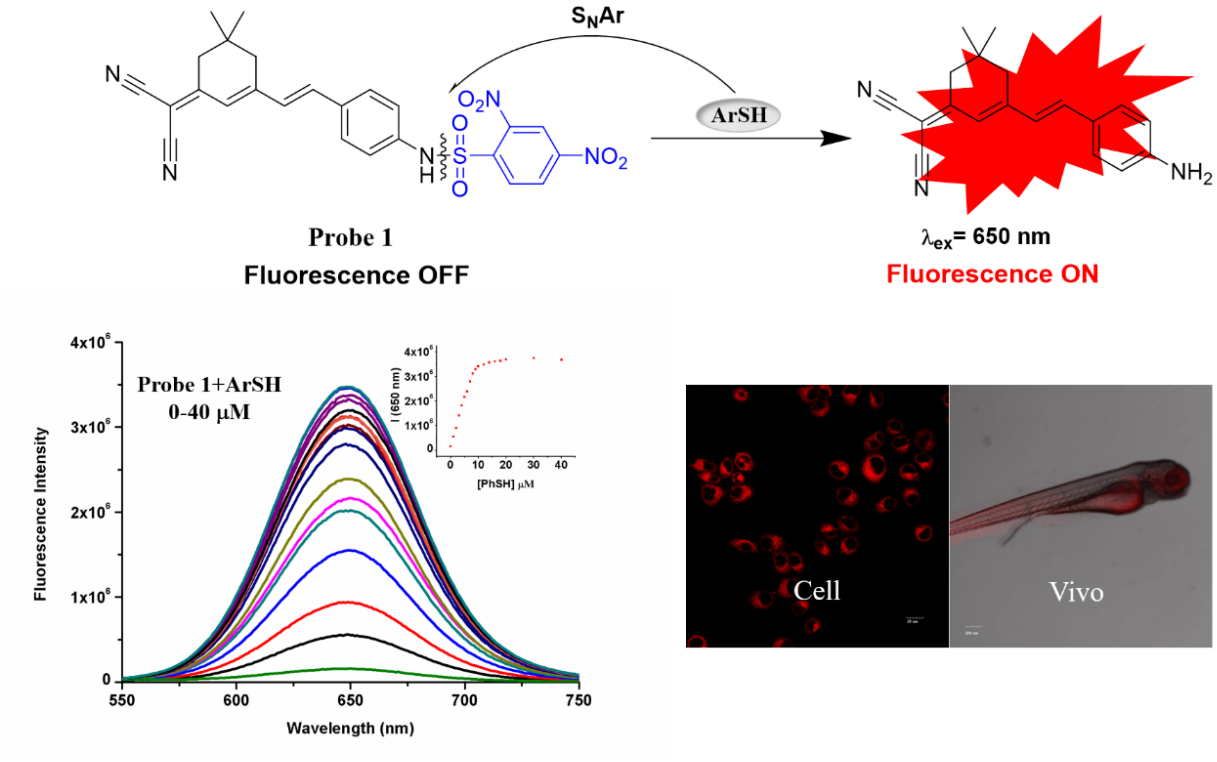
Graphical abstract



Highlights:

- Probe with dicyanoisophorone has good membrane permeability and large Stokes shift
- Ideal fluorescence intensity and Red-emitting/NIR emission (650 nm)
- Instantaneous response (few seconds) and intensity levelled off within 7 min
- Probe has High selectivity
- Application in real water sample and cell even in vivo(zebrafish)