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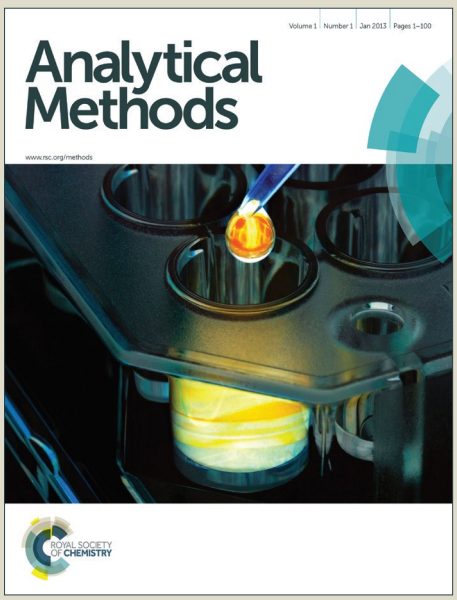

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An excited-state intramolecular proton transfer- based probe for discrimination of thiophenols over aliphatic thiols

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The development of probes for rapid, selective, and sensitive detection of thiophenols is of great importance, due to the toxicity of thiophenols and their derivatives in the environment. In this work, a new excited-state intramolecular proton transfer (ESIPT)-based probe for discrimination detection of thiophenols over aliphatic thiols was developed, in which 2-(1-(4-methoxyphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl)phenol (**DIP**) was acted as the fluorophore and the strongly electron-withdrawing 2,4-dinitrobenzene sulfonyl ester group was acted as the recognition unit. The non-fluorescent probe molecule can release the corresponding fluorescent fluorophore (**DIP**) through aromatic nucleophilic substitution (S_NAr) by thiolate anions from thiophenols. The fluorophore displayed a large Stokes shift (>170 nm) and the probe showed very fast response (within 1 min) to thiophenols. Quantitative detection of thiophenol with a linear response from 0.1 μM to 7 μM and detection limit of 0.189 μM was achieved. This probe shows highly selective to thiophenols over aliphatic thiols and other nucleophiles and it can be used to deteect thiophenol in real water samples with good recovery. The Probe is also a useful fluorescent probe for detecting thiophenols in living cells.

1. Introduction

Thiophenols are useful in organic synthesis and are widely used in preparing agrochemicals, pharmaceuticals, and various industrial products[1-3]. However, thiophenols are a class of highly toxic pollutant compounds with a median lethal dose (LC 50) of 0.01−0.4mM for fish[4]. Long-term exposure to thiophenols liquid and vapor can lead to serious health problems including central nervous system damage, increased respiration, muscle weakness, hind limb paralysis, coma, and even death[5-7]. Thus, thiophenols have been listed as one of the prioritized pollutants by the United States Environmental Protection Agency (USEPA). Simple, rapid, sensitive and selective detection of thiophenols is of great importance.

Traditionally, a number of analytical techniques such as high-performance liquid chromatography (HPLC)[8], gas chromatography (GC)[9] have been reported for the determination of thiophenols. However, most of these methods require either multiple experimental steps with tedious sample pretreatments or sophisticated instrumentation. The method based on fluorescent probe is more desirable due to their high sensitivity, low detection limit, and operational simplicity. Considerable efforts have been devoted to design thiols selective fluorescent probes during the past few decades. However, most of them are designed mainly for discrimination of aliphaticthiols such as cysteine, glutathione, and homocysteine from other amino acids and they exhibit poor selectivity for aliphatic thiols and thiophenols[10-13]. There is no fluorescent probe can clearly distinguishing aliphatic thiols and thiophenols until Wang et al. firstly developed the fluorescent probe for thiophenols[14]. This probe showed good water solubility and selectivity. Nevertheless, it still has some drawbacks including relatively weak fluorescence intensity, low quantum yield (Φ = 0.02) and low sensitivity (detection limit at 2 μM). From then on, different fluorescent probes with high sensitivity and selectivity have been developed to discriminate thiophenols from aliphatic thiols[15-25]. However, most of them are based on photoinduced electron transfer (PET) and Internal-molecular Charge Transfer mechanism (ICT) and suffered from interference of autofluorescence. Moreover, slow reactivity of these probes with thiophenols has resulted in relatively long response times (t_R). Recently, the fluorescent dyes based on excited-state intramolecular proton transfer (ESIPT) process have been used as an attractive fluorescent signal transducer in sensors[26]. ESIPT dyes generally have large Stokes shift (>150 nm), which could minimize the self-absorption and reduce the interference from autofluorescence for in vivo application[27]. There are many ESIPT-based probes for thiols so far[28-32]. However, to the best of our knowledge, no ESIPT-based probe for thiophenols has been reported. Therefore, a brighter and faster probe based on ESIPT for thiophenols is highly desirable.

Herein, we report a rational novel type of highly sensitive and selective fluorescent probe toward thiophenols. In this probe, a perfect ESIPT fluorescent dye 2-(1-(4-methoxyphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl)phenol (**DIP** in **Scheme 1**) as the fluorophore and the strongly electron-withdrawing 2,4-dinitrobenzenesulfonyl (DNS) group as the recognition unit were used. Indeed, comparing with some reported probes for thiophenols, probe 2-(1-(4-methoxyphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl) phenyl 2,4-dinitrobenzensulfonate (**DIPD**) has many advantages. Notably, this probe is able to detect thiophenols rapidly (within 1

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min) with significant fluorescent turn-on responses around 460 nm. To the best of our knowledge, this response times is the fastest among all reported fluorescent probes for thiophenol. In addition, **DIPD** is the first ESIPT-based fluorescent probe for discrimination of thiophenol over aliphatic thiols and the fluorophore of the probe shows a remarkable large Stokes shift (>170 nm), which is highly desirable for fluorescent probes with reliability and good sensitivity. This probe was successfully applied for the detection of thiophenol in water samples and living cells.

2 Experimental

2.1. Materials and instruments

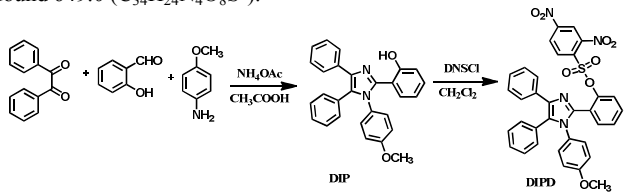
Saliylaldehyde, benzil, 4-methoxyaniline and 2,4-dinitrobenzenesulfonyl chloride were purchased from Sigma-Aldrich. Ammonium, acetic acid, dichloromethane, trimethuiamine and ethyl acetate were obtained from Sinopharm Chemical Reagent Company. All other chemicals used in this work were of analytical grade and used without further purification. Milli-Q ultrapure water (Millipore, ≥18 MΩ cm−1) was used throughout all experiments. Silica gel of 300–400 mesh (37–54 μm) was used for column chromatography. Thin-layer chromatography (TLC) was carried out on silica gel plates (60F-254) using UV-light to monitor the reaction. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVB-500 spectrometer using TMS as an internal standard. Electrospray mass spectrometry (ESI-MS) were acquired on a ZQ2000 mass spectrometer (Manchester, UK). UV-vis spectra were recorded on a UV2450 spectrophotometer (Shimadzu). Fluorescence spectra were recorded at room temperature using an F-7000 fluorescence spectrophotometer (HitachiCo, Japan) with the excitation and emission slit widths of 2.5 nm.

2.2. Synthesis of intermediates and probe

The synthetic route for probe **DIPD** is shown in **scheme 1**. **DIPD** was characterized by NMR spectroscopy and mass spectrometry (see **Fig. S3–S5**†).

Synthesis of DIP. Mixture of saliylaldehyde (321.6 mg, 2.4 mmol) benzil (166.4 mg, 0.8 mmol), 4-methoxyaniline, and ammonium acetate (1.23 g, 16 mmol) in glacial AcOH (10 mL) was heated to 100 °C for 30 min. The hot solution was cooled to room temperature, and the resulting white solid was collected by filtration and washed with acetate acid, dilute sodium hydrogen carbonate solution and water. The white solid was further dried under reduced vacuum and purified by silica gel column chromatography using acetone as eluent to afford the pure product (272.5 mg, 81.5%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.80 (s, 1H), 7.42 (d, J = 7.0 Hz, 2H), 7.33-7.31 (m, 4H), 7.30-7.27 (m, 5H), 7.23-7.16 (m, 2H), 6.95 (d, J = 8.5. Hz, 1H), 6.92 (d, J = 8.5. Hz, 2H), 6.70 (d, J = 8.5 Hz, 1H), 6.58 (t, J = 7.5. Hz, 1H) , 3.73 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 159.8, 157.9, 145.1, 134,6, 133.7, 131.8, 131.6, 130.6, 130.3, 130.2, 129.6, 129.2, 129.0, 128.9, 127.4, 127.0, 126.6, 118.7, 117.5, 115.0, 114.3, 55.8.

Synthesis of DIPD. Under N_2 atmosphere, DNSCl (305.6 mg, 0.8 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and Et_3N (1 mL).The mixture was cooled to 0 °C and a solution of **DIPD** (334.4mg 0.8 mmol) in dry CH_2Cl_2 (5 mL) was slowly added in. After stirred at 0 °C for 30 min, the mixture was then stirred at room temperature overnight. After the completion of the reaction, the solvent was evaporated in vacuo. The resulting residue was purified by silica gel plates (petroleum ether: ethyl acetate = 3:1) and the probe **DIPD** was obtained as a red solid (218 mg, 42%). ^{1}H NMR (500 MHz, $CDCl_3$) for major isomer: δ 8.14 (d, J = 8.5 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.99 (m, 1H), 7.50-7.45 (m, 2H), 7.29-7.24 (m, 5H), 7.16-7.14 (m, 7H), 7.08 (d, J = 9.0 Hz, 2H), 6.66 (d, J = 9.0 Hz, 2H), 3.70 (s 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.1, 150.0, 148.0, 147.6, 142.1, 136.8, 133.8, 133.7, 133.5, 132.6, 131.3, 131.0, 130.8, 130.0, 129.0, 128.6, 128.5, 128.3, 128.2, 127.7, 127.1, 126.6, 126.5, 125.2, 124.2, 120.4, 113.9, 55.2. ESI-MS: m/z found 649.0 ($C_{34}H_{24}N_4O_8S^+$).



Scheme 1 Synthetic route of **DIPD**

2.3. Sample pretreatment

Stock solution of probe **DIPD** (0.1 mM) was prepared in CH_3CN. Stock solutions of C_6H_5SH, 2-NH_2−C_6H_4SH, p−CH_3−C_6H_4SH, p−Cl−C_6H_4SH, $C_6H_5NH_2$, $CH_3(CH_2)_{10}CH_2SH$ and PhOH were dissolved in CH_3CN as well. Cysteine (Cys), homocysteine (Hcy), glutathione (GSH), Alanin (Ala), Proline (Pro), Arginine (Arg), NaN_3, NaI, were dissolved in double-distilled water. Test solutions were prepared by dissolving 200 μL of **DIPD** stock solutions and an appropriate amount of the analyte stock solution into a phosphate buffer (10 mM, pH = 7.4). The mixture (the final volume is 2 mL containing 50% CH_3CN as a co-solvent) was incubated at room temperature for 2 min. The fluorescence emission spectra of the resulting solutions were recorded at an excitation wavelength of 290 nm (unless otherwise noted, all fluorescent spectral data were measured according to this method). The water samples from the tap water and Xiangjiang River in Changsha city were filtered through a microfiltration membrane before use. The pH values of the water samples were adjusted using sodium phosphate buffer (10 mM, pH = 7.4).

2.4. Cell cultures and fluorescence imaging

HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (fetal bovine serum), 100 mg/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, water-saturated incubator at 37°C. Before cell imaging experiments, HeLa cells were seeded in 12-well culture plate for one night. For living cell imaging experiments, some of the cells were incubated with 10 μM probe **DIPD** for 30 min at 37 °C, washed three times with prewarmed PBS buffer, and then imaged. Meanwhile, another portion of the cells were pretreated with 1 μM thiophenol for 30 min at 37 °C, washed three times with prewarmed PBS buffer, and then incubated with 10 μM probe **DIPD**

for 30 min. Cell imaging was then carried out after washing cells with prewarmed PBS buffer.

3 Results and discussion

3.1. Probe Design and Synthesis

It was reported that 2-(2′-hydroxyphenyl)-9,10-phenanthroimidazole derivatives and their analogues show excellent photophysical and photochemical properties, such as typical ESIPT characteristic, high absorption coefficient and quantum yields, as well as the tunability of both absorption and emission bands. In addition, it can be easily prepared. Therefore, compound **DIP** was selected as the fluorophore to construct the ESIPT-based fluorescent probe for thiophenols in this pape. According to the reported strategy[14] developed by Wang et al, the protection of hydroxide group with strongly electron-withdrawing 2,4-dinitrobenzenesulfonyl (DNS) group could quench the fluorescence of compound **DIP** and the protecting group could be rapidly cleaved by thiophenols. Following this design concept, we designed and synthesized the DIPD as showed in Scheme 1, a new highly selective and sensitive ESIPT-based fluorescent probe for thiophenols.

3.2. Spectral characteristics of DIP and DIPD

The optical properties of **DIP** were characterized using UV-Vis absorbance and fluorescence spectroscopy. The optical properties of **DIPD** were characterized using fluorescence spectroscopy as shown in **Fig. 1**, **DIP** exhibited a maximum absorption peak at 280 nm (curve a), corresponding to the π–π* transition of the π-conjugated core. When excited at 290 nm (curve b), **DIP** displayed a maximum emission peak at 460 nm (curve c). So, The fluorophore displayed a large Stokes shift (170 nm). However, due to the strongly electron-withdrawing characteristic of DNS, the probe **DIPD** showed no fluorescence at 460 nm (curve d), corresponding to the photoinduced electron transfer (PET) pathway from the fluorophore to the DNS.

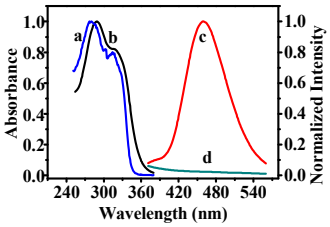
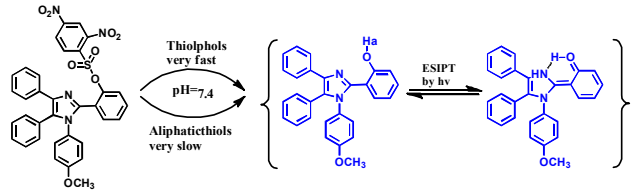


Fig. 1 (a) Absorption spectrum of, (b) excitation spectrum of, (c) fluorescence emission spectrum of **DIP**, (d) fluorescence emission spectrum of **DIPD**. The spectrum were recorded after incubation with 10 mM PBS buffer (50% CH_3CN, pH = 7.4).

3.3. Detection mechanism for thiophenols

In the new fluorescent probe **DIPD**, the protection of the hydroxide group with a DNS group results in quenching of the fluorescence. After the deprotection reaction promoted by thiophenols, the protecting group in the probe will be released and resulted in recovery of the fluorescence signal of **DIP**. To prove the mechanism (**Scheme 2**), the fluorescence of **DIPD** in the absence and presence of different concentrations thiophenol was investigated. As shown in **Fig. 2**, as expected, the probe **DIPD** (10 μM) exhibited almost no fluorescence in the absence of thiophenol (curve a). When thiophenol (10 μM) was added, a dramatic increase in fluorescence intensity (> 25 times) at 460 nm was observed (curve d), which should be attributed to the fluorescence emission of the reaction product. These results indicated that the probe reacted with thiophenol at room temperature to generate fluorophore **DIP**. The large Stokes shift (> 170 nm) of the reaction product with the typical characteristic of fluorophore **DIP** supports the mechanism (**Scheme 2**). Moreover, the reaction mechanism was also confirmed by **ESI-MS** analysis, after the reaction of probe with thiophenol, a peak at m/z 419.3 corresponding to $\mathbf{DIP}^+$ (**Fig. S6**†) was obtained. This observation could be looked as the direct evidence of the fluorescent response mechanism of **DIPD** toward thiophenol.



Scheme. 2 Proposed mechanism for the detection of thiophenol

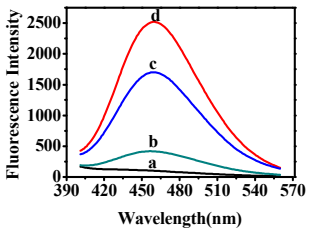


Fig. 2 Fluorescence spectrum of **DIPD** (10 μM) in the absence and presence of thiophenol in 10 mM PBS buffer (50% CH_3CN, pH = 7.4) λ_{ex} = 290 nm. silt: 2.5, 2.5. (a) probe only, (b) probe + 1 μM PhSH, (c) probe + 5 μM PhSH, (d) probe + 10 μM PhSH.

3.4. Optimization of the detection conditions

Various factors such as pH and incubation time may have effects on the experimental results. In order to obtain a highly sensitive response for thiophenols, the effects of the experimental parameters were investigated.

At first, the pH dependence of the fluorescent response of **DIPD** toward thiophenol was monitored. Fluorescence spectra of the probe **DIPD** (10 uM) were recorded (λ_{ex} = 290 nm) in 10 mM PBS buffer (50% CH_3CN pH = 4 - 10) in the absence and presence of thiophenol (10 uM). As shown in **Fig. S7**†, the probe did not exhibit

significant change in fluorescence response at 460 nm upon the variation of pH (pH = 4 - 10), which indicates that probe **DIPD** is stable in that pH range. In the presence of thiophenol, the observed fluorescence enhancement was low in the low pH range (pH = 4 - 6), which due to the low reactivity of the thiophenol (pKa = 6.5). At higher pH range (pH= 7 - 10), the fluorescence enhancement was very obviously. It may be ascribed to the high degree of the dissociation of thiophenol at relative higher pH value and resulted in the predominant generation of the corresponding thiolate which can effectively react with probe **DIPD**. These experiment results suggested that the compound **DIPD** is an effective probe for thiophenol sensing at physiological pH. Therefore, in order to obtain a better reactivity and to meet the physiological pH conditions, the further experiments were carried out at pH = 7.4.

Generally, Cys and GSH were the usual interferences on the detection of thiophenol with a reactive fluorescent probe. To evaluate the interference of Cys and GSH for thiophenol detection with fluorescent probe **DIPD**, the fluorescence change of the probe with the addition of Cys, GSH and thiophenol were measured under the same conditions. The results are shown in **Fig. 3**. The addition of Cys and GSH to the solution of probe **DIPD** did not result in any noticeable fluorescent response. In contrast, the addition of thiophenol to **DIPD** solution resulted in a rapidly increase in the fluorescence intensity at 460 nm (λ_{ex} = 290 nm), and the enhancement of fluorescence intensity reached a maximum after about 1 min. The time dependence on the fluorescent response of **DIPD** toward thiophenol was further investigated, which reflects the kinetics of the reaction of probe **DIPD** with thiophenol. A pseudo first order kinetics was observed with the rate constant k= 0.037 s^{-1} and half-life $t_{1/2}$ = 18.7 s. Compared to the reported fluorescent probes for thiophenol, probe **DIPD** provided a more excellent response time, t_R = 1 min. According to the reported strategy[33] for designing fast responsive thiophenol probes, there is a correlation of pK_{aH} values of the fluorophores of probes with t_R. The higher pK_{aH} values of the fluorophore of the probe, the faster the t_R is. As shown in **Fig. 4**, the pK_{aH} of **DIP** is the highest among **DIP**, **CM**, **EM**, **GM** and **JM** in **Fig. 4** and the t_R gradually decreased with the increasing pK_{aH} value.

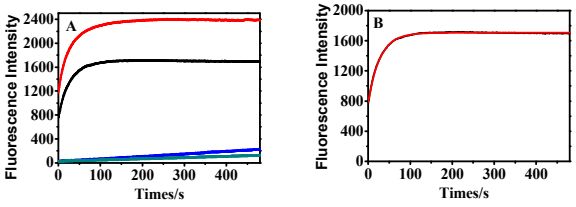


Fig. 3 (A) Time-dependent fluorescence changes of the probe **DIPD** (10 μM) upon interaction with 5 μM thiophenol (black), 7 uM thiophenol (red) , 50 μM Cys (blue) and 50 μM Hcy (green) in 10 mM PBS buffered solution (pH = 7.4) containing 50 percent acetonitrile. (B) Kinetic curve of probe **DIPD** (10 μM) interaction with PhSH (7 μM) in 10 mM PBS buffer (pH = 7.4) containing 50 percent acetonitrile. The data were reported as the mean ± standard deviation of triplicate experiments and fitted (red line) λ_{ex} = 290 nm, λ_{em} = 460 nm.Slit:2.5, 2.5 nm.

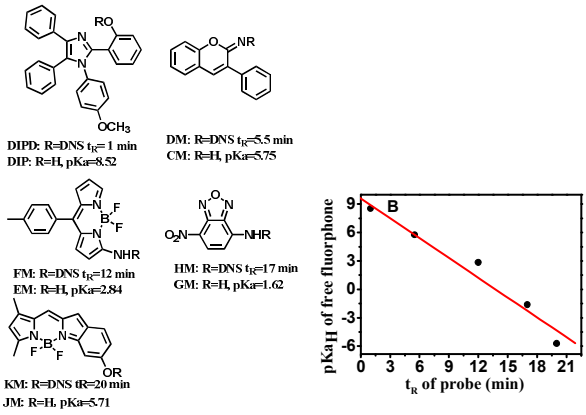


Fig. 4 (A) Structures of probes **DIPD**, **DM**, **FM**, **HM**, **KM** and corresponding free fluorophores **DIP**, **CM**, **EM**, **GM**, **JM** released during PhSH sensing. Response times t_R of these probes and pK_{aH} values of corresponding fluorophores are also provided. (B) Correlation diagram of pK_{aH} values of **DIP**, **CM**, **EM**, **GM**, **JM** with t_R values of probes **DIPD**, **DM**, **FM**, **HM**, **KM.**

3.5. Analytical performance

To evaluate the sensitivity of probe **DIPD**, the fluorescence changes of probe **DIPD** with addition of different concentrations of thiophenol were measured under the optimal conditions. As shown in **Fig. 5**, the fluorescence intensity gradually increased with the increasing thiophenol concentrations (**Fig. 5A**). A linear calibration curve between the fluorescent intensity change at 460 nm and the concentration of thiophenol in the range of 0 - 7 μM was obtained (**Fig. 5B**). Moreover, a linear regression equation [Y = 85.0202 + 323.0672 X (R=0.9991)] was obtained , where Y was the fluorescent intensity and X was the concentration of thiophenol. The detection limit of thiophenol was calculated to be 0.189 μM, based on the signal-to-noise ratio (S/N = 3) under the test conditions. These results revealed that the probe could be applied to quantitatively monitor thiophenol levels.

To evaluate the selectivity of probe **DIPD** for thiophenols, various analytes including thiophenol derivatives (C_6H_5SH, *p*-CH_3−C_6H_4SH, 2-NH_2−C_6H_4SH and *p*-Cl−C_6H_4SH), aliphatic thiols (Cys, Hcy, GSH) and ($CH_3(CH_2)_{10}CH_2SH$) and other potential interfering substances such as some nucleophilic species (Ala, Pro, Arg, NaN_3, KI, $PhNH_2$, PhOH, and NaSH) were tested. As shown in **Fig. 6** and **Fig. S8**†, there was no noticeable changes in the emission spectra were observed upon addition of analytes (60 μM), including Cys, Hcy, GSH, $CH_3(CH_2)_{10}CH_2SH$, Ala, Pro, Arg, NaN_3, KI, $PhNH_2$, PhOH and NaSH. However, the luminescence intensity was significantly enhanced at 460 nm in the presence of thiophenols (6 μM) such as 2-aminobenzenethiol, p-CH_3−C_6H_4SH, 4-chlorothiophenol, as well as thiophenol. These results clearly demonstrated that **DIPD** was a highly selective fluorescent probe for discrimination of thiophenols over aliphatic thiols and other nucleophiles. The excellent selectivity can be attributed to the distinct pKa values of benzenethiols (pKa = 6.5) and aliphatic thiols (pKa = 8.5).

3.6. Determination of thiophenol in water samples

To validate its practicality in environmental science, the probe **DIPD** was employed to determine thiophenol concentrations in water samples from both the tap water and Xiangjiang River in Changsha city using the standard addition method. When the probe **DIPD** was added directly to the water samples, no significant fluorescence enhancement was observed. When the water samples were spiked with different concentration thiophenols (0.8 μM and 5 μM), the recoveries were summarized in **Table 1**. These results showed that the thiophenol in the water samples could be quantified with good recovery when probe **DIPD** was applied as the probe. Thus, probe **DIPD** was capable of detecting thiophenol in water samples.

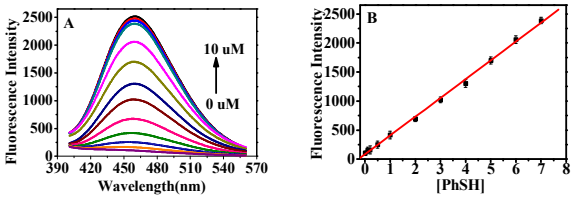


Fig. 5 (A) fluorescence spectra of probe **DIPD** (10 μM) in the presence of various concentrations of thiophenol (0, 0.1, 0. 2, 0.5, 1.0, 2. 0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 μM). (B) The fluorescence enhancement of probe **DIPD** toward thiophenol from 0.1 μM to 7 μM.

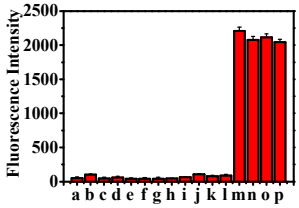


Fig. 6 Fluorescence responses of probe **DIPD** (10 μM) toward thiophenos (6 uM) and other substances (60 μM) in 10 mM PBS buffer at room temperature. After 2 min, the reaction solution was diluted and sampled for fluorescence measurement at λ_{ex} = 290 nm. The fluorescence intensity at λ_{em} = 460 nm was plotted versus substances: (a) probe only, (b) probe + NaN_3, (c) probe + KI, (d) probe + PhOH, (e) probe + $PhNH_2$, (f) probe + Ala, (g) probe + Pro, (h) probe + Arg, (i) probe + GSH, (j) probe + Hcy, (k) probe + Cys, (l) probe + $CH_3(CH_2)_{10}CH_2SH$, (m) probe + *p*-Toluenethiol, (n) probe + 2-aminobenzenethiol, (o) probe + PhSH, (p) probe + 4-chlorothiophenol.

Table 1 The results of determination of thiophenol in water samples

Sample	spiked (μM)	Recovered (μM)	Recovery (%)
Xiangjian river water	0	Not detected	—
	5	4.9 ± 0.05	98
	0.8	0.824 ± 0.015	103
tap water	0	Not detected	—
	5	4.85 ± 0.03	97
	0.8	0.816 ± 0.024	102

3.7. Detection of Thiophenol in Living Cells

To study the ability of probe to permeate cells and detect thiophenol in vivo, we performed a preliminary study in HeLa cells and observed the results with inverted fluorescence microscopy. As shown in **Fig. 6**, When HeLa Cells were incubated directly with probe **DIPD** (10 μM) at 37°C, no fluorescence was observed. However, when HeLa cells were pre-incubated with thiophenol (1 μM) and then incubated with probe **DIPD** (10 μM), strong blue fluorescence was observed. DIPD is a hydrophilic small molecule, it may enter Hela cells by diffusion. However, the detail mechanism of how DIPD enters the Hela cells is still remained to be further investigated at present in this work. These results indicate that probe **DIPD** can be applied to detect thiophenols in living cells.

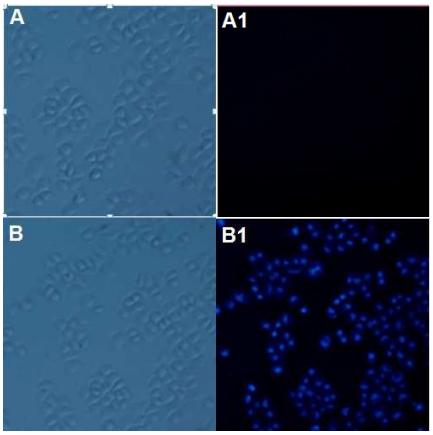


Fig. 7 Imaging of thiophenol in HeLa Cells by probe **DIPD** (10 μM) (A) Bright field images of HeLa cells after being treated with probe **DIPD** (10 μM) for 30 min. (B) Bright field images of HeLa cells preincubated with thiophenol (1 μM) and then incubated with probe **DIPD** (10 μM). A1 and B1 are fluorescence images of A and B, respectively.

4 Conclusions

In summary, an ESIPT-based off−on fluorescent probe **DIPD** was successfully designed and synthesized. The fluorophore in probe displayed a large Stokes shift (>170 nm) and the probe showed rapid response (1 min), good sensitivity, and satisfactory specificity for thiophenol detection. Furthermore, probe **DIPD** was successfully applied to thiophenol determinations in real water samples and living cells. This research indicated that probe **DIPD** holds promise for applications in thiophenol sensing in environmental and biological samples.

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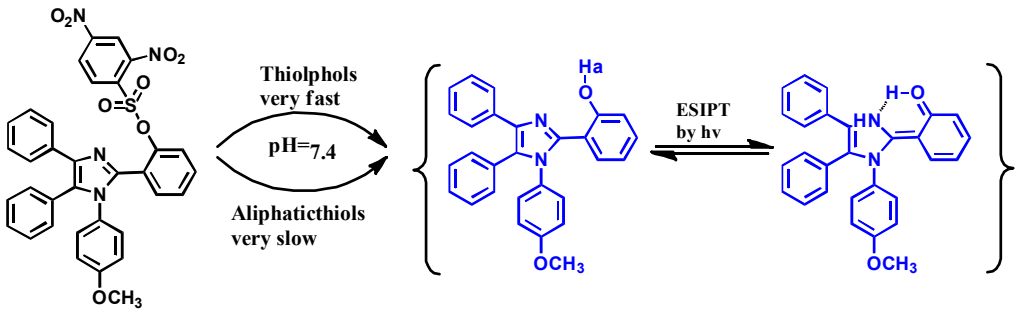
Graphical Abstract



Highlights

- A novel ESIPT based fluorescent probe for detection of thiophenols has been developed.
- The probe showed very fast response (within 1 min) to thiophenols.
- The probe can be used to monitor thiophenols level in water samples.
- The probe can be used to monitor thiophenols level in living cells