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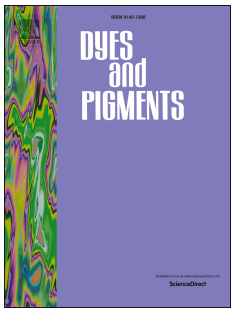

A rapid and highly sensitive fluorescent imaging materials for thiophenols

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PII: S0143-7208(16)30260-1

DOI: 10.1016/j.dyepig.2016.06.009

Reference: DYPI 5293

To appear in: *Dyes and Pigments*

Received Date: 24 April 2016

Revised Date: 1 June 2016

Accepted Date: 4 June 2016

Please cite this article as: Zhang W, Huo F, Liu T, Wen Y, Yin C, A rapid and highly sensitive fluorescent imaging materials for thiophenols, *Dyes and Pigments* (2016), doi: 10.1016/j.dyepig.2016.06.009.

Abstract Graphic

The Title:

A rapid and highly sensitive fluorescent imaging materials for thiophenols

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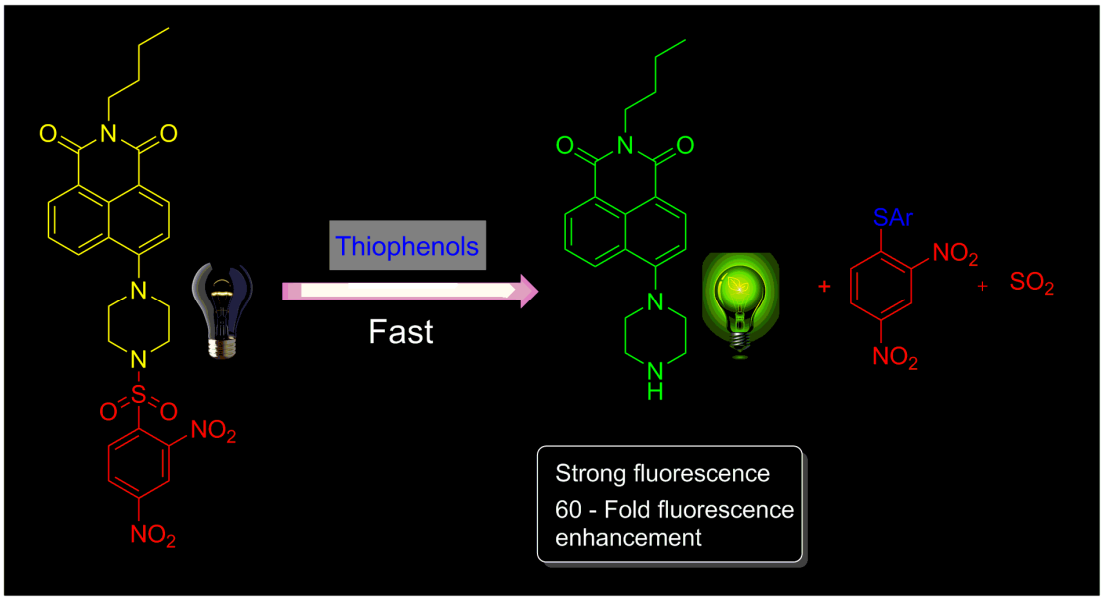

A novel highly sensitive and selective 'off-on' fluorescent probe for detecting thiophenols has been developed by an ICT mechanism through a rational design. It displays a large Stokes shift (140 nm), a good linearity range, a rapid signal response time (within 100 s) and the detection limit is as low as 13 nM. In addition, the ability of the probe to detect thiophenols in living cells (HepG2 cells) via an enhancement of the fluorescence has also been proved.

A rapid and highly sensitive fluorescent imaging materials for thiophenols

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Abstract: Thiophenols, a class of toxic and polluting compounds, are widely used in industrial production. Meanwhile, some aliphatic thiols play important roles in living organisms. Therefore, the development of probes for specific thiophenol detection is of great importance. Herein, a novel highly sensitive and selective 'off-on' fluorescent probe for detecting thiophenols has been developed by an ICT mechanism through a rational design. The probe displays a large Stokes shift (140 nm) and 60-fold fluorescence intensity enhancement. More importantly, the probe features a rapid signal response time (within 100 s), a good linearity range and the detection limit is as low as 13 nM. In addition, the ability of the probe to detect thiophenols in living cells (HepG2 cells) via an enhancement of the fluorescence has also been demonstrated.

Keywords: Thiophenols; Detection; Fluorescent probe; Bioimaging.

1. Introduction

Thiophenols, also named benzenethiols, are extensively used in organic synthesis for preparing various products in the agrochemical industry and pharmaceutical industry. However, thiophenols are poisonous to aquatic bio-organisms and animals, possessing a median lethal concentration (LC 50) of 0.01−0.4 mM in fish and a median lethal dose (LD 50) of 46.2 mg/kg in mouse. It was also reported that thiophenols can enter into the human body easily by inhalation and skin absorption to induce systemic injuries including shortness of breath, muscular weakness, nausea, vomiting, and even death [1-3]. Despite high toxicity, thiophenols are essential and widely used chemicals for the preparation of agrochemicals, pharmaceuticals, and various industrial products [4-6]. Considering their toxicity and the continuing environmental concerns, simple, rapid, sensitive, and selective detection of thiophenols is therefore of considerable interest in both environmental and biological science.

Fluorescence detection using fluorescent probes has been recognized as one of the most attractive methods 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. [7] reported the first reaction based fluorescent turn-on probe for selective detection of thiophenols, a number of fluorescent probes have been developed for these compounds [8-20]. Because of the similarity of chemical properties among aliphatic thiols and thiophenols, most of these probes are designed mainly for discrimination of aliphatic thiols such as cysteine and glutathione from other amino acids, and in general, they cannot clearly discriminate thiophenols

over aliphatic thiols [21-24]. 2,4-Dinitrobenzenesulfonyl (DNBS) was first used by Maeda et al. as a sulfonate ester of a fluorophore for the detection of thiols [25]. The high level of electron deficiency enables the DNBS moiety to act as an electron sink and incur intramolecular charge transfer (ICT), resulting in the quenching of the fluorescence. The DNBS ester can easily undergo desulfonylation in the presence of thiophenols through a nucleophilic aromatic substitution (SNAr) mechanism, liberating SO_2 and the attached fluorophore, thus resulting in an increase in fluorescence.

Herein, we report a novel type of highly sensitive and selective fluorescent probe toward thiophenols which operates through an ICT pathway by selecting *N*-butyl-4-amino-1,8-naphthalimide as a fluorophore, the 2,4-dinitrobenzene-sulfonamide group as a recognition unit, and a piperazine moiety as a linker to extend the fluorophore and reactive sulfonamide bond. The masked sulfonamide moiety can be easily removed by a highly nucleophilic thiolate anion through the S_NAr process (see Scheme 1)[26]. More importantly, we found that this probe not only shows high selectivity and sensitivity (DL = 13 nM) but also offers a rapid fluorescence turn-on sensing process (100s) for detecting thiophenols. Furthermore, this probe was successfully applied in fluorescent imaging in living cells.

2. Materials and methods

2.1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide solution (0.1 mol/L) was

added to aqueous HEPES (10 mmol/L) to adjust the pH to 7.4. Amino acids were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–visible (UV–vis) spectra were recorded on an Agilent 8453 UV-Visible spectrophotometer. Fluorescence spectra were measured on F-7000 FL Spectrophotometer. A PO-120 quartz cuvette (10 mm) was purchased from Shanghai Huamei Experiment Instrument Plants, China. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker AVANCE-600 MHz and 150 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). ESI determinations were carried out on AB Triple TOF 5600plus System (AB SCIEX, Framingham, USA). The ability of the probe to react with thiophenols in living cells was also evaluated by laser confocal fluorescence imaging using an Olympus FV1000 laser scanning microscope.

2.3. Preparation and characterization of the probe

2.3.1.Synthesis of N-butyl-4-Br-1,8-naphthalimide

The route employed to synthesise of the probe is summarized in scheme 2. 4-Bromo-1,8-naphthalic anhydride (2.76 g, 10 mmol) and *N*-butylamine (0.8 g, 11 mmol) was taken in EtOH (20 mL) and stirred at 55°C monitoring with TLC. After the reaction was complete, the reaction mixture was evaporated in vacuum and the residue was washed with water and dried to give a solid product. The solid obtained was purified by column chromatography (silica gel dichloromethane) to give a white

product in 88% yield. mp, 104℃-106℃. IR (KBr): 3389.40, 3340.32, 3308.66, 3069.14, 2954 45, 2930.41, 2871.51, 1700.09, 1658.69, 1616.26, 1590.03, 1569.12, 1502.94, 1460.23, 1435.22, 1402.59, 1380.89, 1359.43, 1342.57, 1259.15, 1229.36, 1191.63, 1151.33, 1116.74, 1098.79, 1075.34, 1042.95, 996.06, 940.45, 899.29, 873.13, 858.94, 848.36, 780.97, 748.66, 730.67, 711.70, 659.48, 567.65, 424.73. ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm): 8.54 (d, J = 7.2 Hz, 1H), 8.51 (d, J = 8.7 Hz, 1H), 8.30 (d, *J* = 7.6 Hz, 1H), 8.19 (d, *J* = 7.7 Hz, 1H), 7.98 (t, *J* = 7.8 Hz, 1H), 4.02 (t, *J* = 7.3 Hz, 1H), 1.61 (m, 1H), 1.35 (m, 1H), 0.93 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 163.22, 163.17, 132.95, 131.94, 131.74, 131.32, 130.14, 129.51, 129.17, 128.61, 123.10, 122.32, 30.02, 20.27, 14.17. ESI–MS m/z: $[B + H]^+$ Calcd. for $C_{16}H_{15}BrNO_2$ 332.0286, Found 332.0286 (Fig. S1).

2.3.2. Synthesis of N-butyl-4-(piperazin-1-yl)-1,8-naphthalimide

N-butyl-4-Br-1,8-naphthalimide (1.65 g, 5 mmol) and Piperazine(0.52 g, 6 mmol) was taken in 20 mL of 2-methoxyethanol. The mixture was stirred at 130℃ with monitoring by TLC. After the reaction was complete, the clear solution obtained was concentrated and left to cool. The brown solid was collected by filtration, washed with water and dried to give a solid product. After crystallization from MeCN, a solid was obtained to give a brown product in 82% yield. mp, 102℃-104℃. IR (KBr): 3374.44, 3324.79, 3291.76, 3066.96, 2956.74, 2927.13, 2862.04, 2837.13, 2748.11, 1691.30, 1652.99, 1613.22, 1588.15, 1512.96, 1456.36, 1427.33, 1384.64, 1355.30, 1287.47, 1243.90, 1181.73, 1164.87, 1137.39, 1118.77, 1089.48, 1070.96, 1024.50, 1001.53, 949.56, 929.37, 893.57, 873.89, 848.70, 835.18, 783.44, 758.58, 746.45,

671.98, 656.48, 412. 20. ^{1}H NMR (600 MHz, DMSO) δ 8.41 (dd, J = 11.9, 8.0 Hz, 2H), 8.35 (d, J = 7.9 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.27 (d, J = 7.9 Hz, 1H), 4.00 (t, J = 6.7 Hz, 2H), 3.13 (s, 4H), 3.00 (s, 4H), 1.58 (d, J = 6.4 Hz, 2H), 1.39 – 1.25 (m, 2H), 0.91 (t, J = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 163.98 (s), 163.45 (s), 156.74 (s), 132.69 (s), 131.16 – 130.91 (m), 129.59 (s), 126.30 (s), 125.74 (s), 122.98 (s), 115.70 (s), 115.32 (s), 54.57 (s), 46.14 (s), 40.48 (d, *J* = 16.2 Hz), 30.18 (s), 20.28 (s), 14.19 (s). ESI–MS m/z: $[C + H]^+$ Calcd. for $C_{20}H_{23}N_3O_2$ 337.179, Found 338.1839 (Fig. S2).

2.3.3.Synthesis of the probe

2,4-Dinitrobenzen-esulfonylchloride (0.662 g, 2.5 mmol) and N-butyl-4-(piperazin-1-yl)-1,8-naphthalimide (0.674 g, 2 mmol) in CH_2Cl_2 (15 mL) with 2 mmol (0.202 g) Et_3N. The mixture was stirred overnight at 0-5□ in ice-water with monitoring by TLC. After the reaction was completed, removing the solvent under reduced pressure gave a solid which was purified by column chromatography (silica gel dichloromethane) to give probe 1 in 77% yields. mp 218℃-220℃. IR (KBr): 3426.36, 3105.53, 2959.04, 2924.03, 2852.91, 2170.33, 1697.12, 1650.66, 1590.96, 1556.47, 1536.51, 1513.89, 1447.13, 1425.28, 1390.92, 1351.29, 1267.81, 1234.48, 1162.78, 1125.57, 1073.18, 956.88, 909.84, 845.95, 832.77, 782.04, 755.27, 703.44, 662.57, 620.28, 598.58, 571.76, 545.76, 515.76, 464.38. ^{1}H NMR (600 MHz, DMSO) δ 9.08 (s, 1H), 8.65 (d, *J* = 6.6 Hz, 1H), 8.48 (d, *J* = 7.2 Hz, 1H), 8.47 – 8.42 (m, 1H), 8.40 (t, *J* = 9.9 Hz, 1H), 8.37 (d, *J* = 8.7 Hz, 1H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 4.03 (s, 2H), 3.60 (s, 4H), 3.34 (s, 4H), 1.70 – 1.54 (m, 2H), 1.34 (dd, *J* =

14.8, 7.3 Hz, 2H), 0.90 (dd, J = 25.9, 18.6 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 163.97 (s), 163.46 (s), 155.16 (s), 150.78 (s), 148.40 (s), 134.50 (s), 132.88 (s), 132.48 (s), 131.23 (s), 130.84 (s), 129.45 (s), 127.51 (s), 126.86 (s), 125.96 (s), 123.10 (s), 120.59 (s), 117.01 (s), 116.41 (s), 52.56 (s), 46.37 (s), 40.52 (s), 30.16 (s), 20.26 (s), 14.20 (s). ESI–MS m/z: [Probe + H]$^+$ Calcd. for $C_{26}H_{25}N_5O_8S$ 567.14238, Found 568.1474 (Fig. S3).

<Inserted Scheme 2>

*2.4. Optical studies of probe **1***

Probe stock solutions were prepared in DMSO. Other analyte solutions were prepared by using deionized water. Fluorescence measurements were carried out with a slit width of 5 nm (λ_{ex} = 383 nm). UV–Vis and fluorescence spectra were obtained in DMSO–HEPES buffer (10 mmol/L, pH 7.4, 1 : 1, v/v).

2.5. General UV–vis and fluorescence spectra measurements

Thiophenol stock and Probe stock were prepared in DMSO. Other analytes were prepared by using deionized water. Fluorescence measurements were carried out with a slit width of 5 nm (λ_{ex} = 383 nm). UV–Vis and fluorescence spectra were obtained in HEPES buffer (10 mmol/L, pH 7.4)/DMSO (v/v, 1:1).

2.6. Detection wavelength range

Fluorescence spectra were measured from 450 to 650 nm with excitation at 383 nm (slit width: 5 nm/5 nm) in HEPES buffer (10 mmol/L, pH 7.4)/DMSO (v/v, 1:1). The detection threshold for thiophenol was 13 nM, and at this level the fluorescence color change was very obvious.

3. Results and discussion

3.1 The Selective response of probe to thiophenols

An important feature of the probe is that it has special selectivity for for one kind of analyte over other substances. In order to study its special recognition ability, we carried out the experiment by UV-Vis and fluorescence spectrometer. Figure 1 shows the fluorescence spectral changes upon addition of various analytes (10 equiv.) in HEPES buffer (10 mmol/L, pH 7.4)/DMSO (v/v, 1:1) solution, including PhOH, $PhNH_2$, $PhCH_3$, KCN, H_2O_2, Na_2S, Cl^-, Br^-, I^-, N_3^-, NO_3^-, HSO_3^-, SO_3^{2-}, ME, Cys, Hcy, GSH, p-Nitrothiophenol, thiosalicyclic acid they all triggered very minor changes. However, the probe displayed obvious fluorescence enhancement (Fig.1) (λ_{ex} = 383 nm, λ_{em} = 523 nm) in the presence of PhSH, p-NH_2-C_6H_4SH, p-CH_3-C_6H_4SH in HEPES buffer (10 mmol/L, pH 7.4)/DMSO (v/v, 1:1) solution, Similarly, the probe displayed obvious UV-Vis spectral (Fig. S4a) changes in the presence of PhSH, and p-NH_2-C_6H_4SH, p-CH_3-C_6H_4SH. These signal changes indicate that the probe can serve as a selective chemosensor for thiophenols .

<Inserted Figure 1>

3.2 The UV–vis and Fluorescence spectra titration for probe 1

A detailed spectral titration for thiophenol was carried out. Fig. 2 showed the fluorescence spectra change of probe 1 (2 μmol/L) upon addition of thiophenol (0-6 μmol/L) in HEPES (10 mmol/L, pH 7.4)/DMSO (v/v, 1:1). Initially, the probe (2 μmol/L) has no fluorescence (λ_{ex} = 383 nm). The addition of thiophenol caused a remarkable enhancement (60-fold) of emission intensity at 523 nm (Fig. 2b). The

sensitivity of probe 1 was then examined by the fluorescence response of the probe toward different concentrations of thiophenol under the same reaction condition as described above (Figure 3). The increase of fluorescence intensity was displayed in a concentration dependent manner. However, when more than 3 equiv of thiophenol were used, the enhancement of fluorescence intensity reached a maximum without further alteration.

The UV-Visible spectrum response of probe 1 (10 μmol/L) toward thiophenol (0-10 μmol/L) was studied in HEPES (10 mmol/L, pH 7.4)/DMSO (v/v, 1:1) solution. Fig. S4b shows the change of probe 1 (0.5 μmol/L) in the UV-Visible spectrum upon addition of thiophenol (0-10 μmol/L), the absorption peak of the probe at 375 nm is gradually increased.

<Inserted Figure 2>

To investigate the detection limit of probe 1 for thiophenol, probe 1 (2 μmol/L) was treated with various concentrations of thiophenol (0-6 μmol/L) and the relative emission intensity at 523 nm in HEPES (10 mmol/L, pH 7.4)/DMSO (v/v, 1:1) (λ_{ex} = 383 nm, slit: 5 nm/5 nm) was plotted as a function of the thiophenol concentration (Fig. 3). Subsequent data analysis revealed an excellent linear relationship (R^2 = 0.9999) between the relative fluorescence intensity at 523 nm and the thiophenol concentration (0-6 μmol/L). The detection limit for thiophenol, based on the definition by IUPAC (C_{DL}=3 Sb/m) [27-31], was found to be 13 nM, which was much lower than that of the ICT probe reported by Wang et al. A summary of the comparison of recently developed fluorescent probes for thiophenol is given in Table 1. The probe 1

showed its excellent analytical performance.

<Inserted Figure 3>

<Inserted Table 1>

3.3. pH dependence

As the pH value of system is often considered as a significant influencing factor on interactions. The effect of pH on the fluorescence intensity of the probe in the absence and presence of thiophenol were also investigated (Figure S5). Experimental results indicated that the probe was pH insensitive in the pH range from pH 2 to 13. As expected, due to the enhanced ionization of thiophenol to result in a faster S_NAr process, the fluorescence intensity increase with the pH ranging from pH 5 to 11, . Notably, the fluorescence changes of probe 1 to thiophenol reached a maximum and became almost constant in the pH range of 6−8, which is consistent with the pKa value of thiophenol (pK a ≈ 6.5). The weaker response at pH >8.0 was presumably attributed to diminished concentration of thiophenol, because of the disulfide formation under this conditions. Therefore, physiological pH (pH=7.4) could be selected as an appropriate working pH for the sensing of thiophenols with the designed probe.

3.4. Time-dependence in the detection process of thiophenol

Time-dependent modulations in the fluorescence spectra of probe 1 were monitored in the presence of 10 eq. of thiophenol. The kinetic study (Fig. 4) showed that the reaction was complete within 100 s for thiophenol, indicating that the sensor can achieve real-time detection of thiophenols.

<Inserted Figure 4>

3.5. Proposed mechanism

Similar to previously reported DNBS-based thiophenol probes [33, 34], the fluorescence enhancement response of probe 1 is attributed to a thiolate anion induced SNAr process which resulted in the cleavage of the DNBS group from the probe molecule (Scheme 1). Zhao etc has reported a new fluorescent probe for thiophenols based on 2,4-Dinitrosulfonyl functional group with 1-amino BODIPY. Under their experimental conditions, the probe displayed a selective response to thiophenols over aliphatic thiols and the detection limit could be improved to 34 nM. In our work, upon addition of thiophenol, the probe caused a remarkable enhancement (60-fold) of emission intensity at 523 nm and the detecting limit is as low as 13 nM, which was much lower compared with the literature reported. But, there are still room to improve in terms of detection limit and application in vivo. As mentioned before, after reacting with thiophenol, probe produced an obviously fluorescence enhancement due to a nucleophilic aromatic substitution (S_NAr) mechanism, releasing SO_2 gas and *N*-butyl-4-(piperazin-1-yl)-1,8-naphthalimide, thus resulting in an increase in fluorescence. Furthermore, The identification of the adduct in the ESI-MS analysis made it possible to propose the signaling mechanism: a peak at m/z 338.1852 corresponding to [probe-PhSH + H]$^+$ was clearly observed (Fig. S6). Further NMR spectroscopic analysis also provided the evidence for thiophenol nucleophilic substitution reactions with probe **1**. With addition of thiophenol to probe in DMSO-d_6, the resonance of the original proton at 9.08, 8.66, 8.64 ppm disappeared and new

peaks at 8.90, 7.58, 7.56 ppm appeared (Fig. S7). Thus, the sensing mechanism of probe **1** towards thiophenol, is based on the nucleophilic aromatic substitution (S_NAr) reactions as shown in Scheme 1.

3.6. Cellular Imaging

Ions play important roles in many industrial and biochemical processes[35, 36]. In order to evaluate the cell permeability and capability of probe 1 to selectively detect intracellular thiophenol, live-cell imaging studies were carried out (Fig. 5). As shown in Fig. 5a, HepG2 cells incubated with 10 μmol/L of probe for 30 min at 37 □ showed no fluorescence. In a further experiment it was found that HepG2 cells displayed yellow-green fluorescence when the cells were first incubated with 10 μmol/L of probe for 30 min at 37 □ and then incubated with 20 μmol/L thiolphenol (Fig. 5b). These data demonstrated the good cell-membrane permeability of probe and it can thus be used to mark thiolphenol within living cells.

4. Conclusions

In summary, we have developed a “turn-on” fluorescent probe for thiolphenols based on a 2,4-dinitrosulfonyl functional group with high selectivity and sensitivity in aqueous-DMSO media. Besides, owing the merits of a high selectivity and sensitivity, this probe shows a rapid detection process (100 s), high fluorescence enhancement (up to 60-fold), large Stokes shift and the detection limit is as low as 13 nM. Moreover, fluorescence scanning microscopic experiments demonstrated that the probe can readily be used to detect the intracellular thiophenol in living HepG2 cells.

Acknowledgments

The work was supported by the National Natural Science Foundation of China (No. 21472118), the Program for the Top Young and Middle-aged Innovative Talents of Higher Learning Institutions of Shanxi (TYMIT, No. 2013802), talents Support Program of Shanxi Province (No. 2014401), Shanxi Province Outstanding Youth Fund (No. 2014021002), and Shanxi University funds for study aboard 2014.

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Figure captions

Scheme 1 Proposed detection mechanism of the probe to thiophenol.

Scheme 2 The synthesis of the probe 1.

Fig. 1 (a) Fluorescence spectra spectra of the probe 1 (2 μmol/L) in DMSO–HEPES buffer (10 mmol/L, pH=7.4, 1 : 1, v/v) in the presence of 60 μmol/L PhOH, $PhNH_2$, $PhCH_3$, KCN, H_2O_2, Na_2S, ME, Cys, Hcy, GSH, and 6 μmol/L PhSH, p-NH_2-C_6H_4SH, p-CH_3-C_6H_4SH (b) Optical density two-dimensional graph of the probe at 523 nm upon the addition of various analytes.

Fig. 2 Fluorescence spectra of the probe 1 (2 μmol/L) in the presence of various concentrations of thiophenol (0-6 μmol/L) in DMSO–HEPES buffer (10 mmol/L, pH 7.4, 1 : 1, v/v) (λ_{ex} = 383 nm, slit: 5 nm/5 nm).

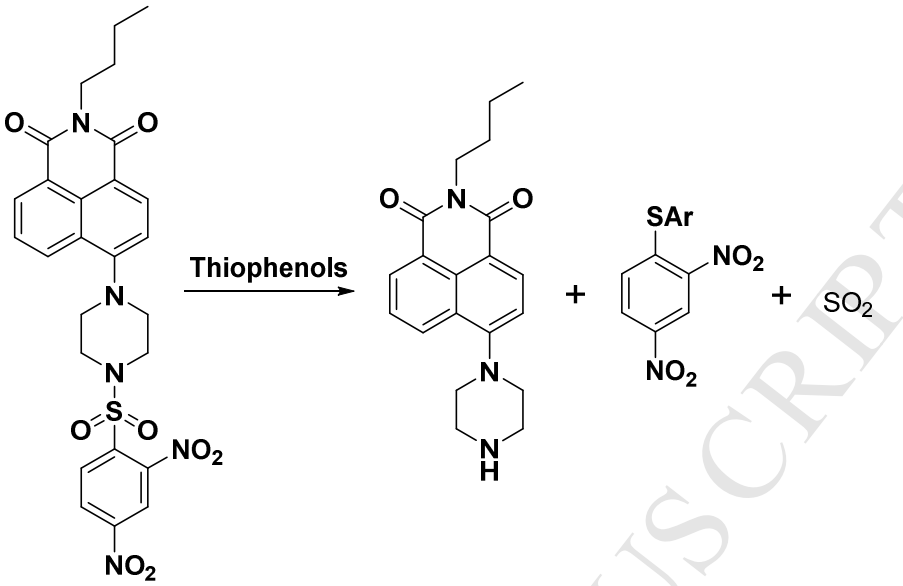
Fig. 3 The linearity of the relative fluorescence intensity versus thiophenol concentration.

Table. 1 Summary of fluorescent probes for thiophenols.

Fig. 4 Reaction time profiles of probe (2 μmol/L) with thiophenol (60 μmol/L).

Fig. 5 Confocal fluorescence images in HepG2 cells. (a) Fluorescence image of HepG2 cells with adding probe (10 μM) and its bright field image (c); (b) Fluorescence image of HepG2 cells incubated with 10 μM probe for 30 min at 37 °C, then incubated with 20 μM thiophenol for 30 min at 37 °C and its bright field image (d).

447 **Scheme 1**



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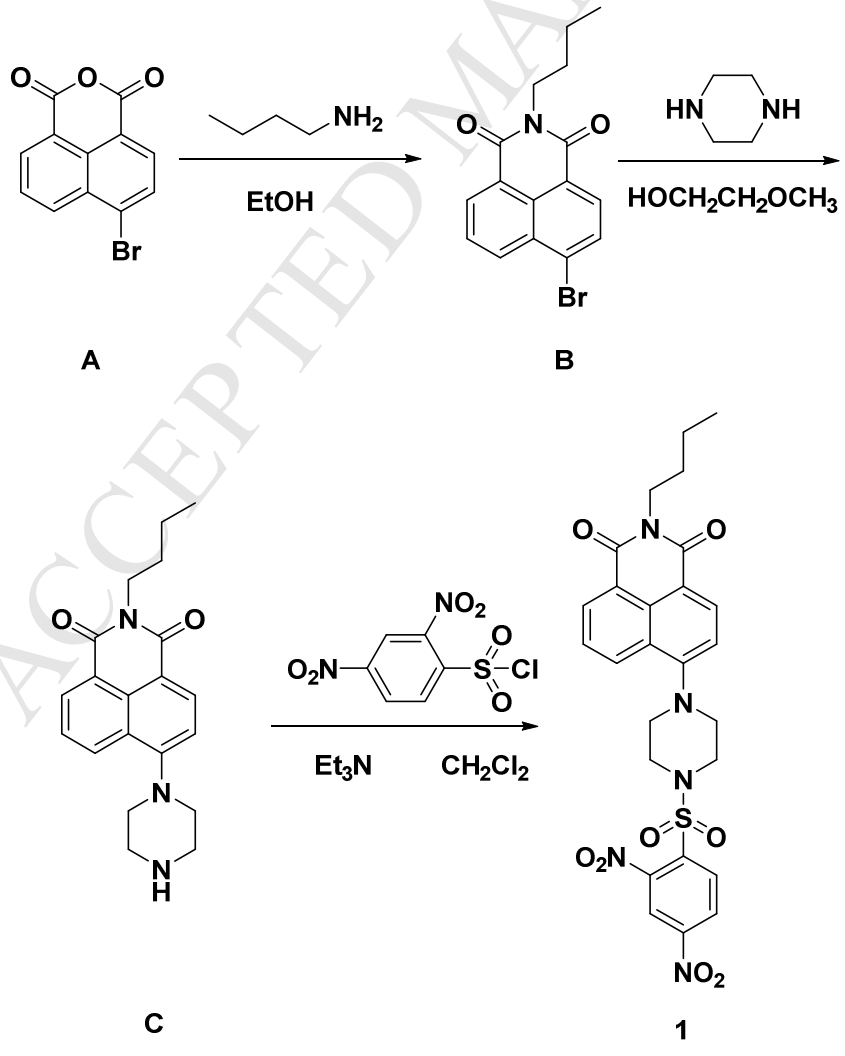
449 **Scheme 2**



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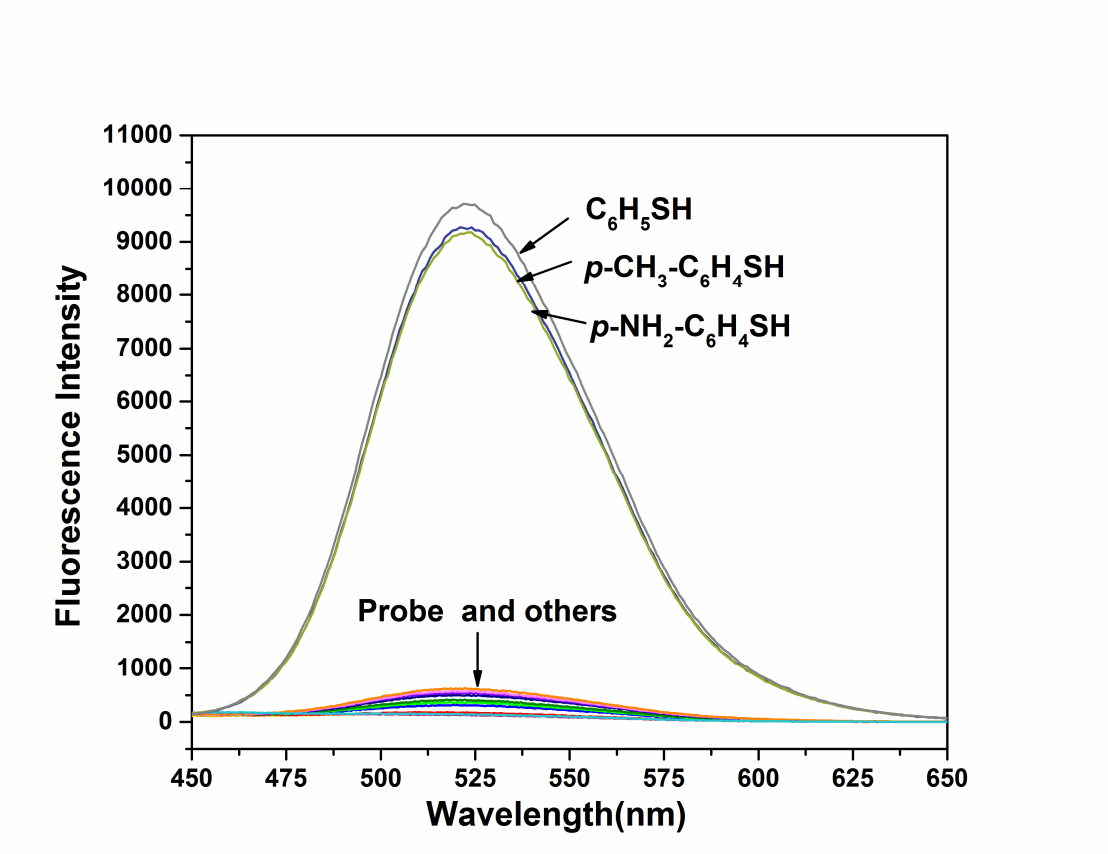
451 **Fig. 1 (a)**



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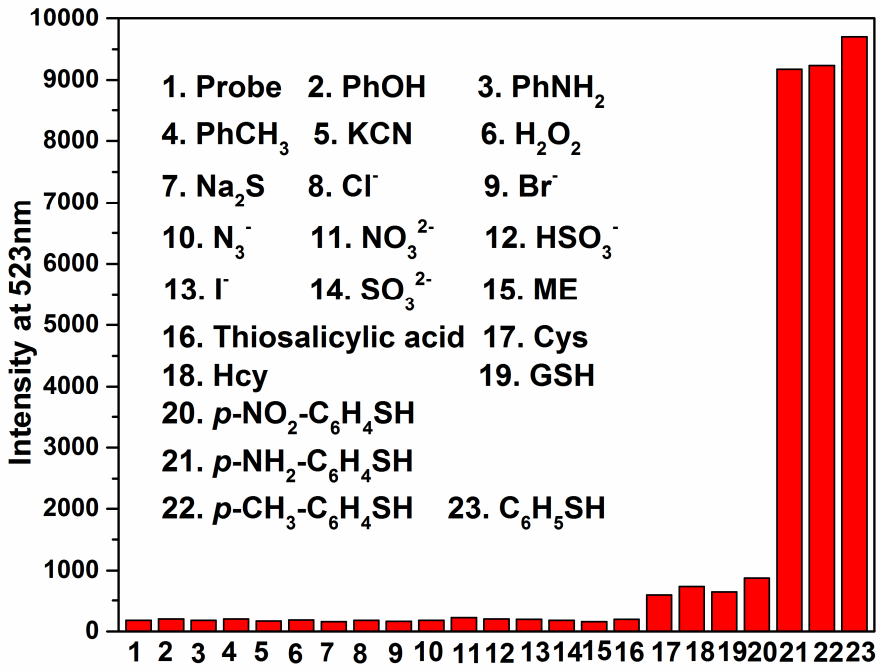
453 **(b)**



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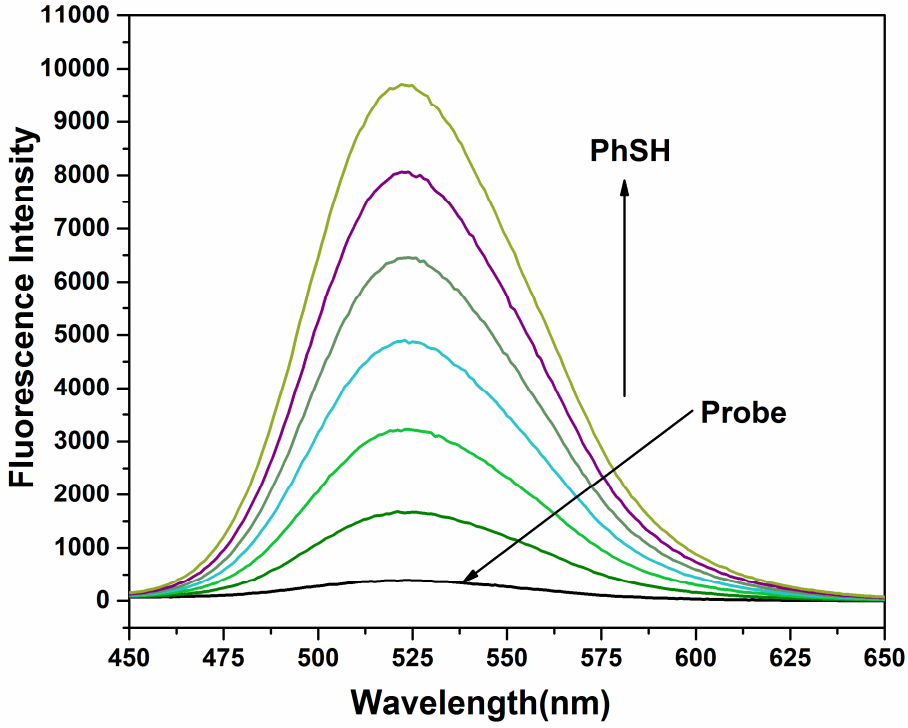
455 **Fig. 2**



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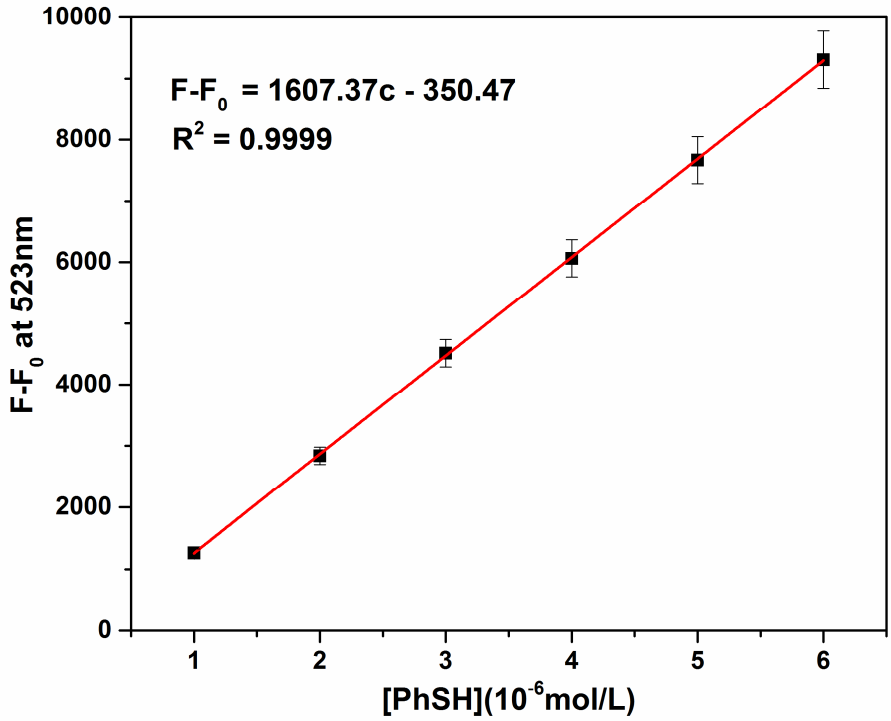
457 **Fig. 3**



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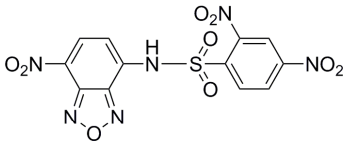
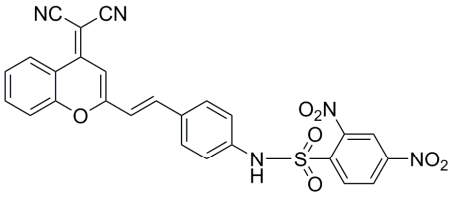
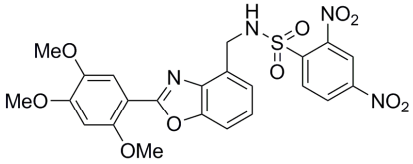
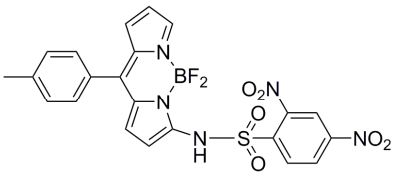
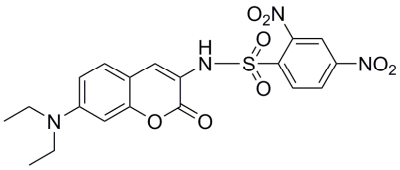
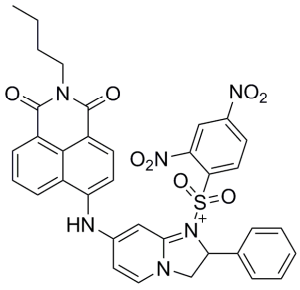
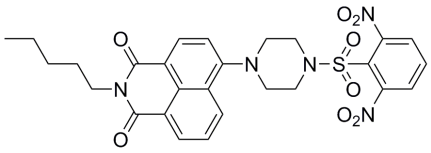
470 **Table 1**

Probe	$\lambda_{ex}/\lambda_{em}$ (nm)	Stokes shift (nm)	LOD	Ref.
	465/555	90	2.0×10^{-6} M	9
	490/670	180	1.5×10^{-7} M	32
	335/403	68	2.0×10^{-7} M	9
	444/521	77	3.44×10^{-8} M	14
	370/515	145	3.0×10^{-8} M	19
	481/590	109	2.0×10^{-8} M	13
	383/523	140	1.3×10^{-8} M	This work

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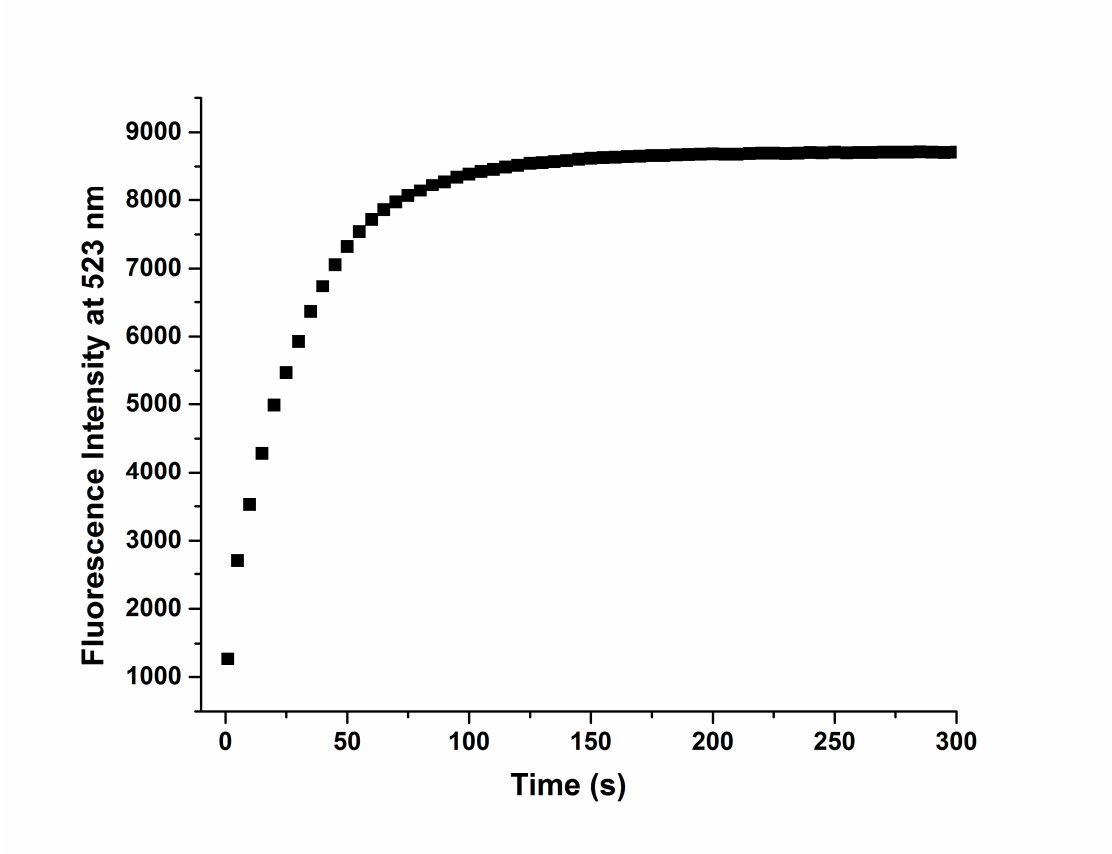
472 **Fig.4.**



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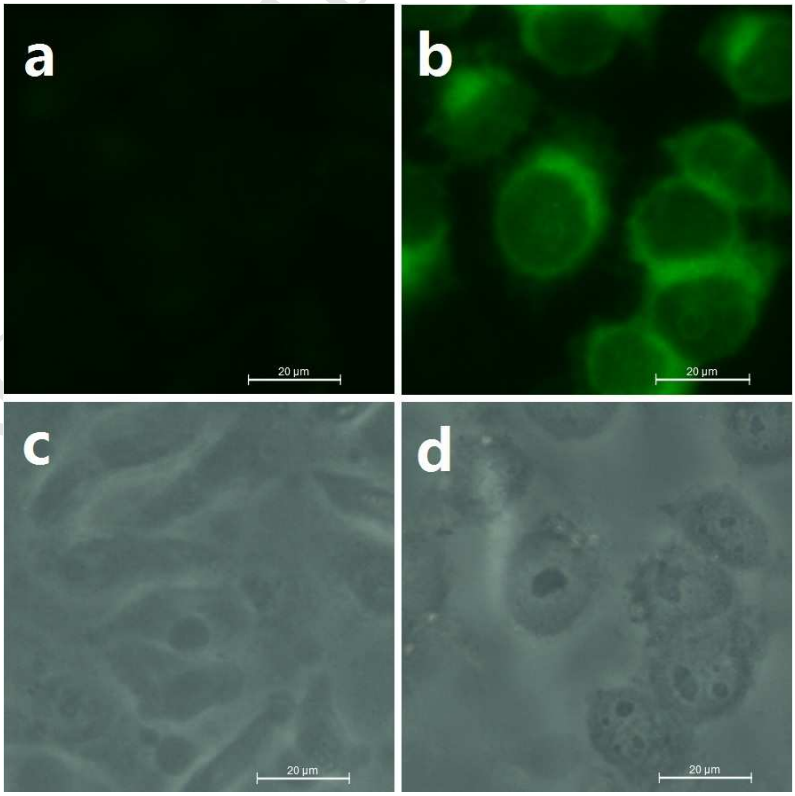
474 **Fig. 5.**



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Support Information

A rapid and highly sensitive fluorescent imaging materials for thiophenols

Figure S1: Structure characterization of compound B

Figure S2: Structure characterization of compound C

Figure S3: Structure characterization of the probe

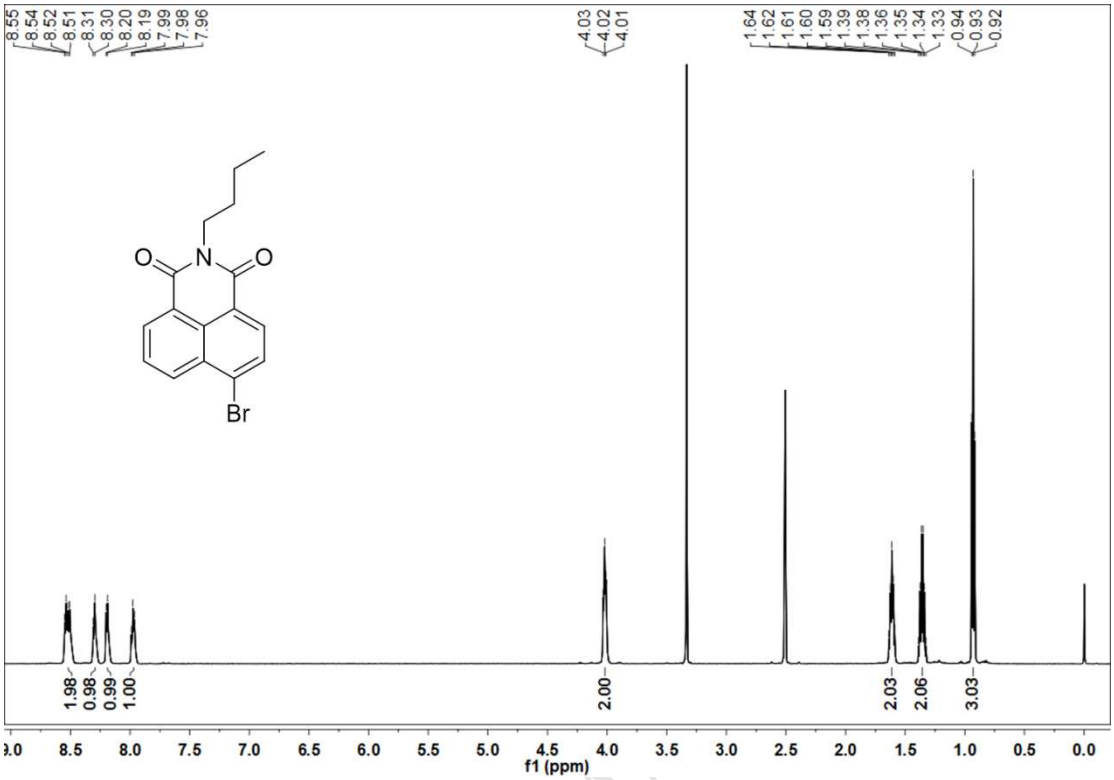
Figure S4: UV-Vis titration

Figure S5: PH interference to the detection

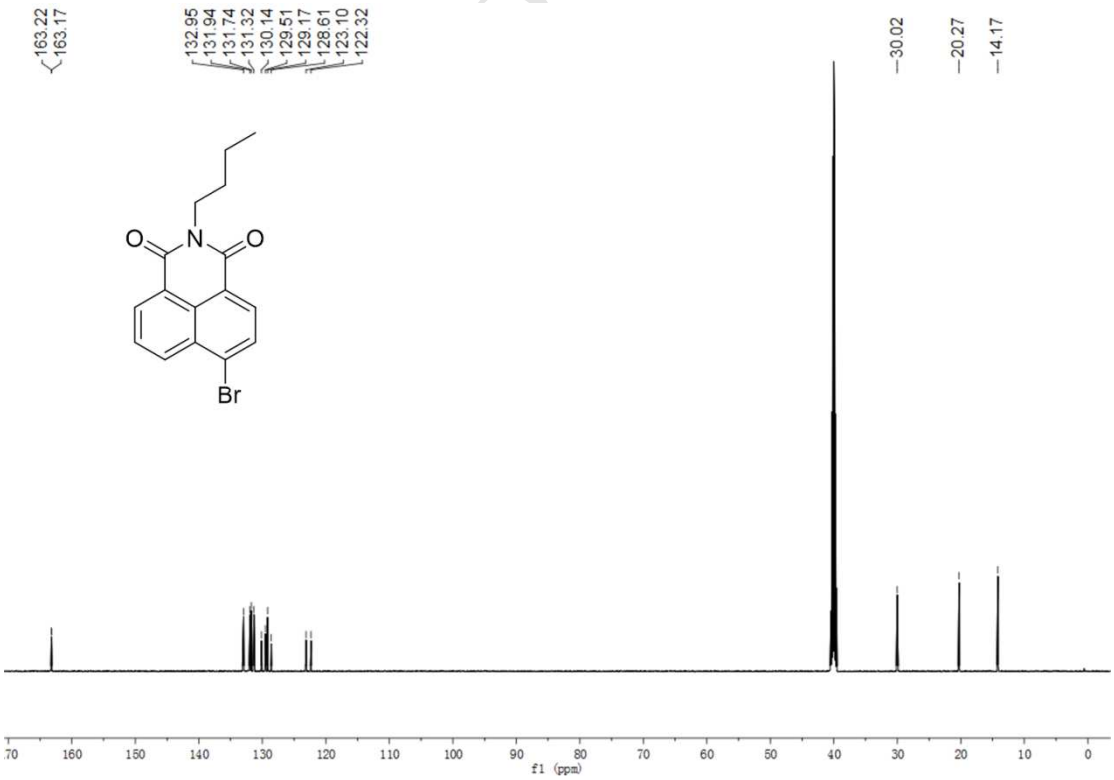
Figure S6: The ESI-MS of product obtained by reaction of probe and thiophenol

Figure S7: 1D ^{1}H NMR the probe and probe-thiophenol

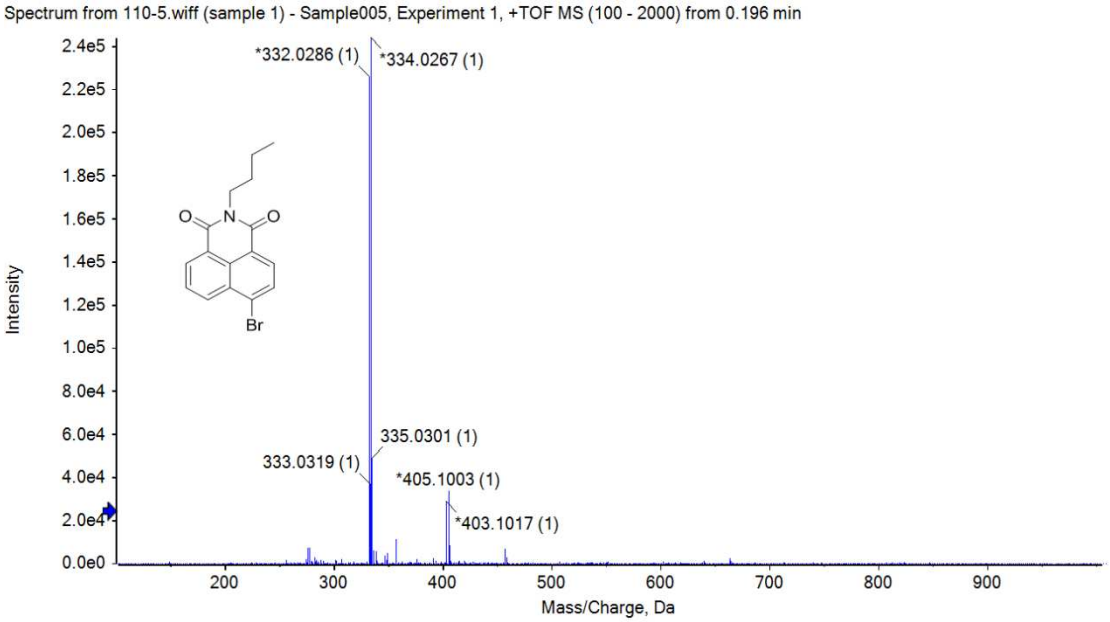
Figure S1: 1D ^{1}H NMR, ^{13}C NMR, ESI-MS and IR of compound B.



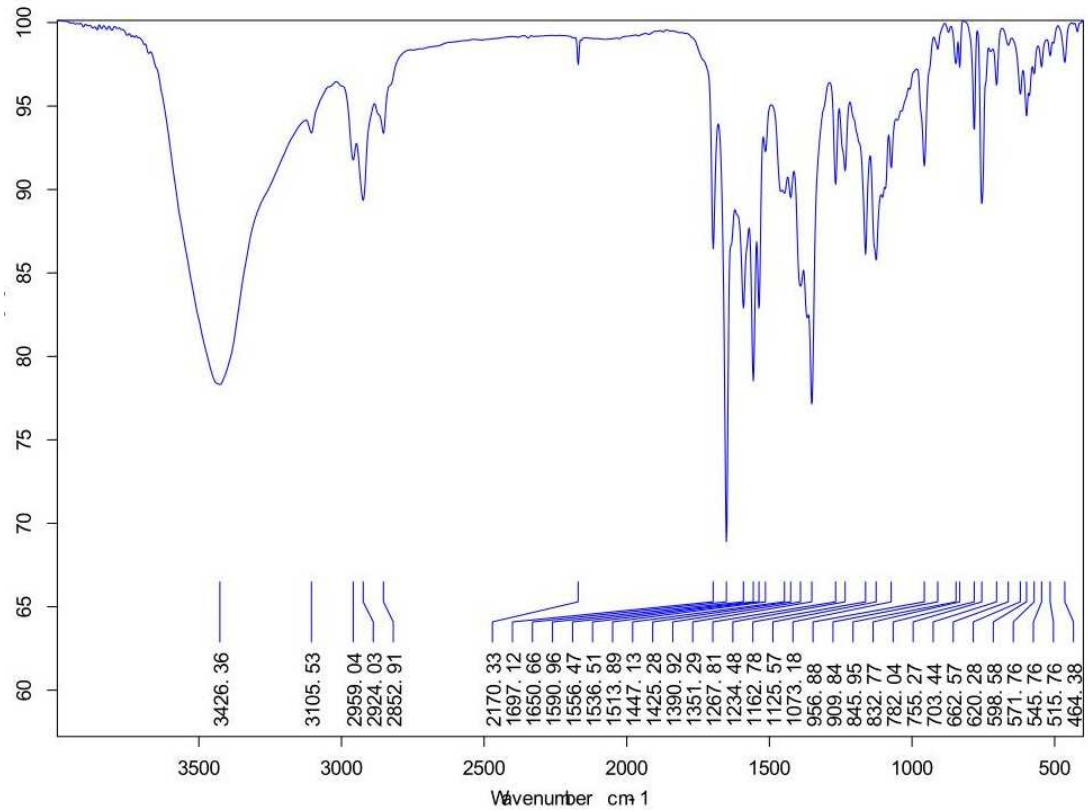
The ^{1}H NMR (600MHz) spectra of compound B in DMSO-d_6.



The ^{13}C NMR (150 MHz) spectra of compound B in DMSO-d_6.

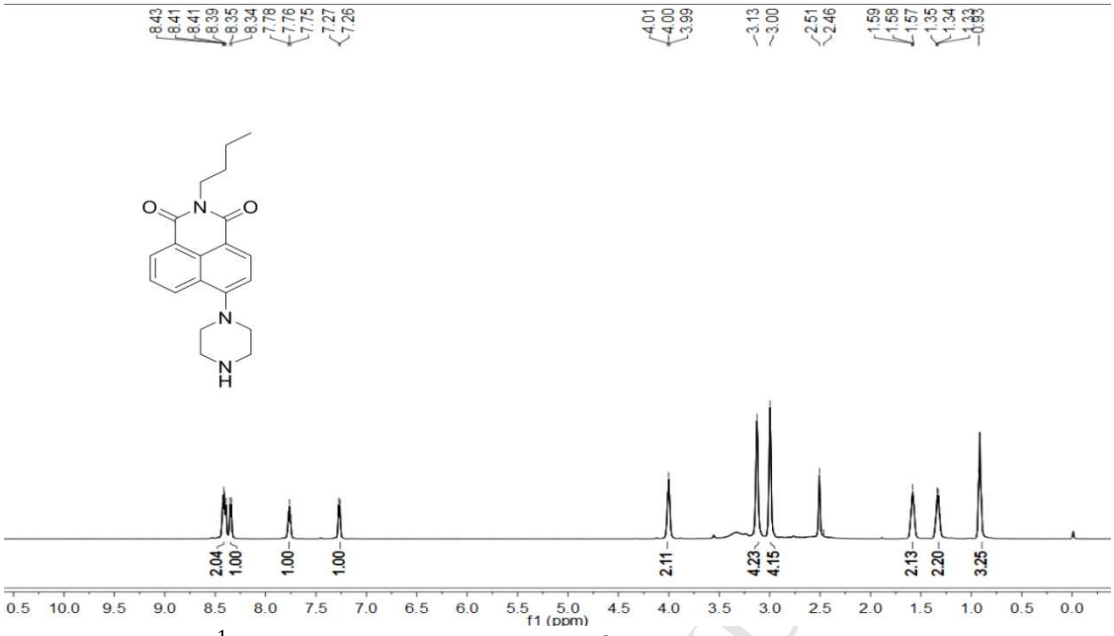


The ESI-MS of compound B**:** m/z: $[B + H]^+$ Calcd. for $C_{16}H_{15}BrNO_2$ 332.0286, Found 332.0286.

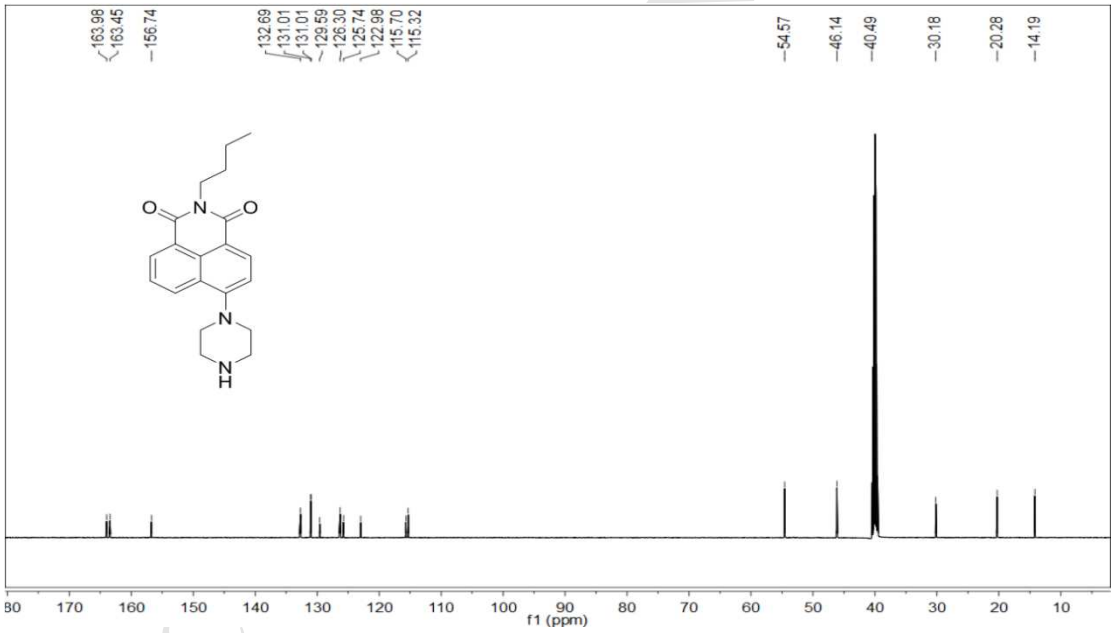


The IR spectra of compound B

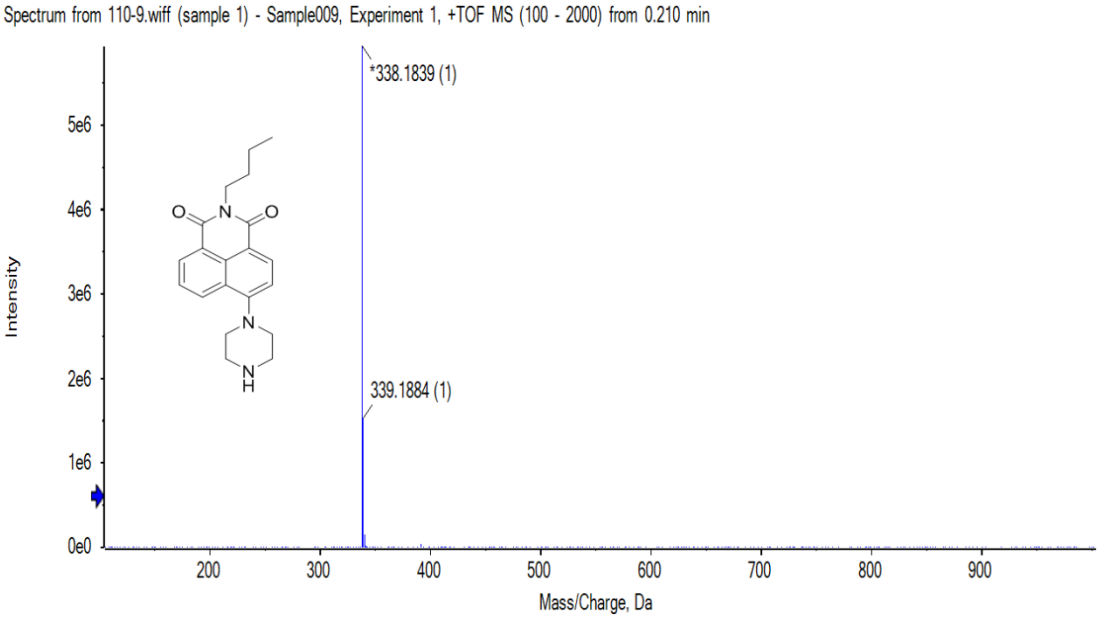
Figure S2 : 1D ^{1}H NMR, ^{13}C NMR, ESI-MS and IR of compound C.



The ^{1}H NMR (600MHz) spectra of compound C in DMSO-d_6.



The ^{13}C NMR (150 MHz) spectra of compound C in DMSO-d_6.



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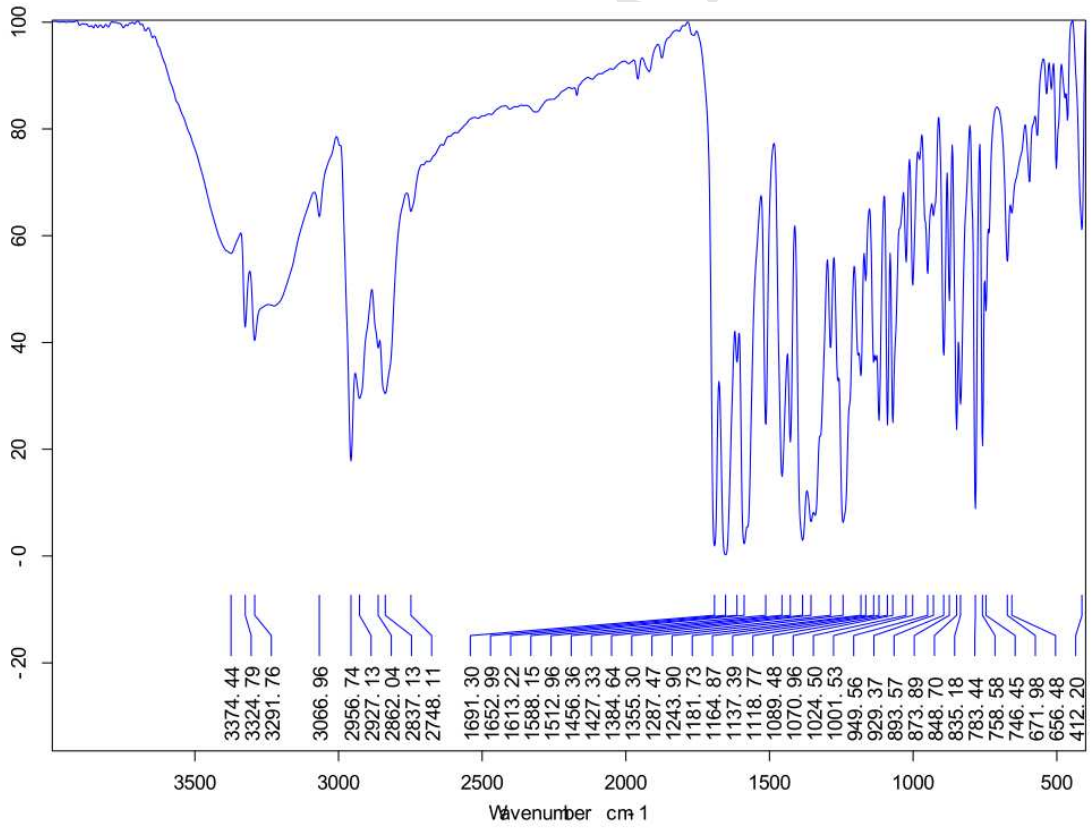
533 The ESI-MS of probe**:** m/z: [C + H] $^{+}$ Calcd. for $C_{20}H_{23}N_3O_2$ 337.17903, Found

534 338.1839.



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536 The IR spectra of compound C

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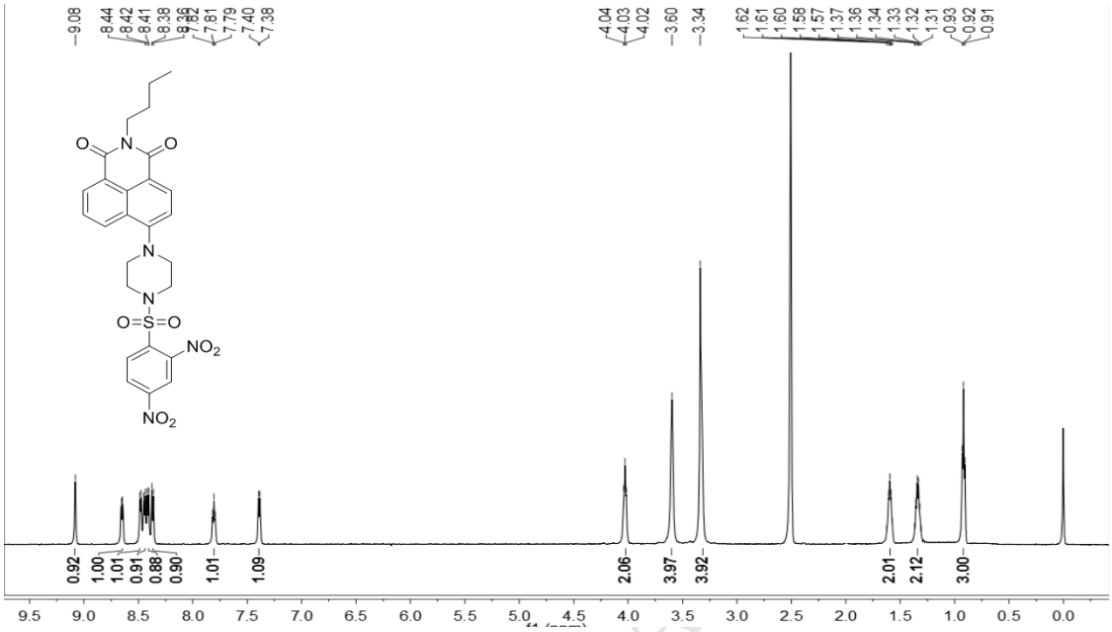
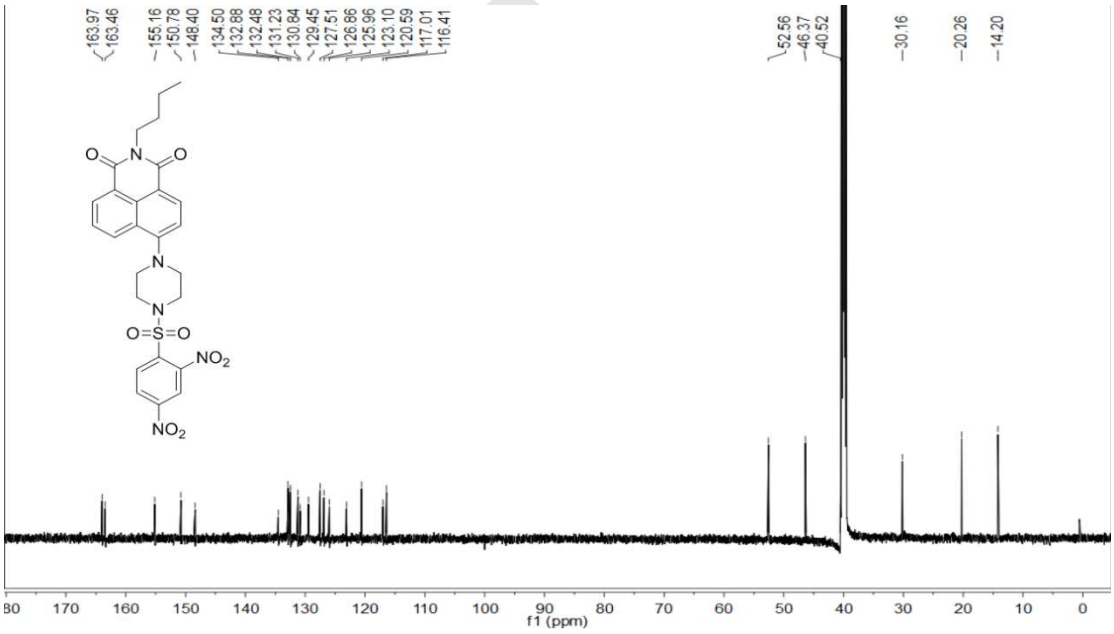
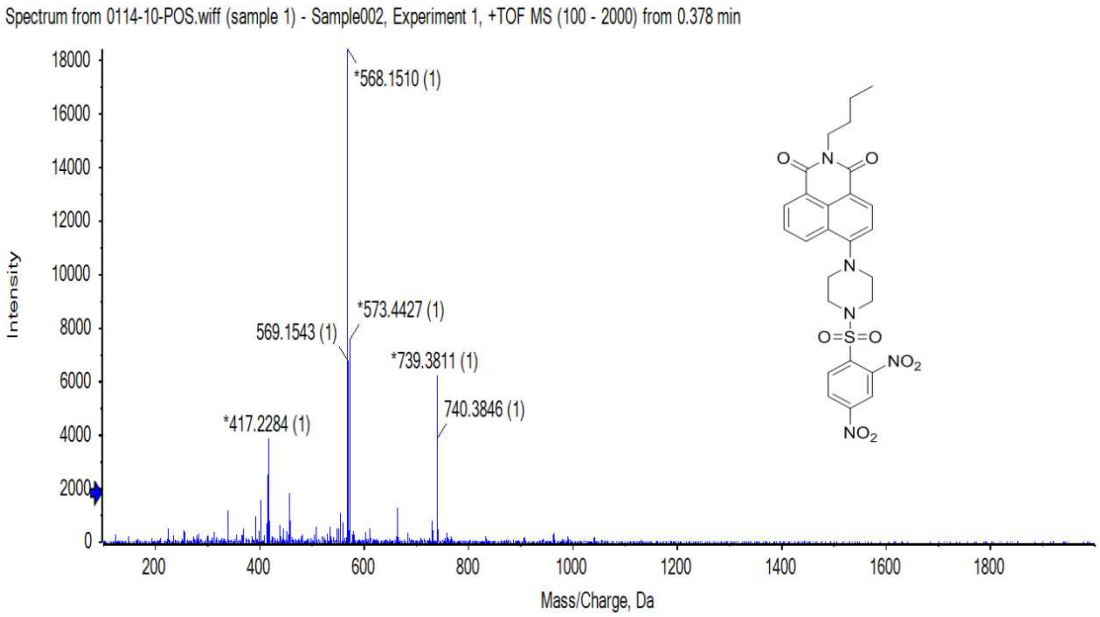
Figure S3: 1D ^{1}H NMR, ^{13}C NMR, ESI-MS and IR of the probe.



The ^{1}H NMR (600MHz) spectra of probe in DMSO-d_6.



The ^{13}C NMR (150 MHz) spectra of probe in DMSO-d_6.



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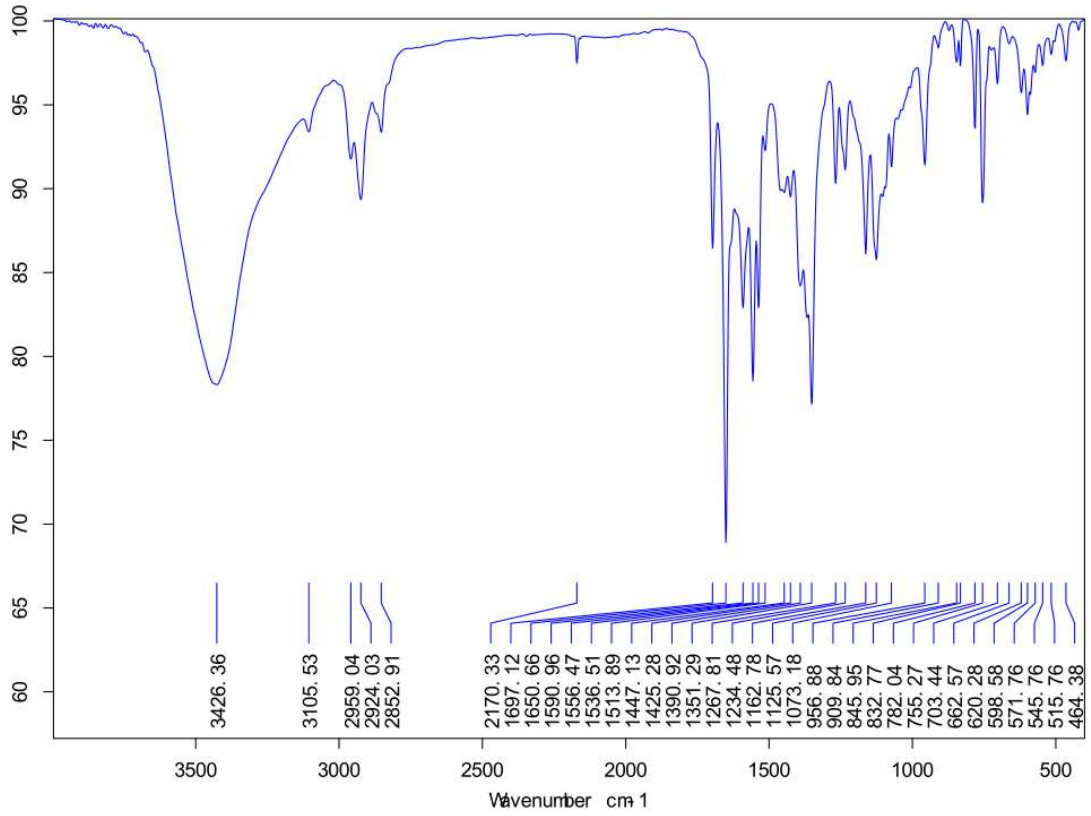
549 The ESI-MS of probe**:** m/z: [Probe + H] $^{+}$ Calcd. for $C_{26}H_{25}N_5O_8S$ 567.14238, Found

550 568.1474.



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552 The IR spectra of the probe

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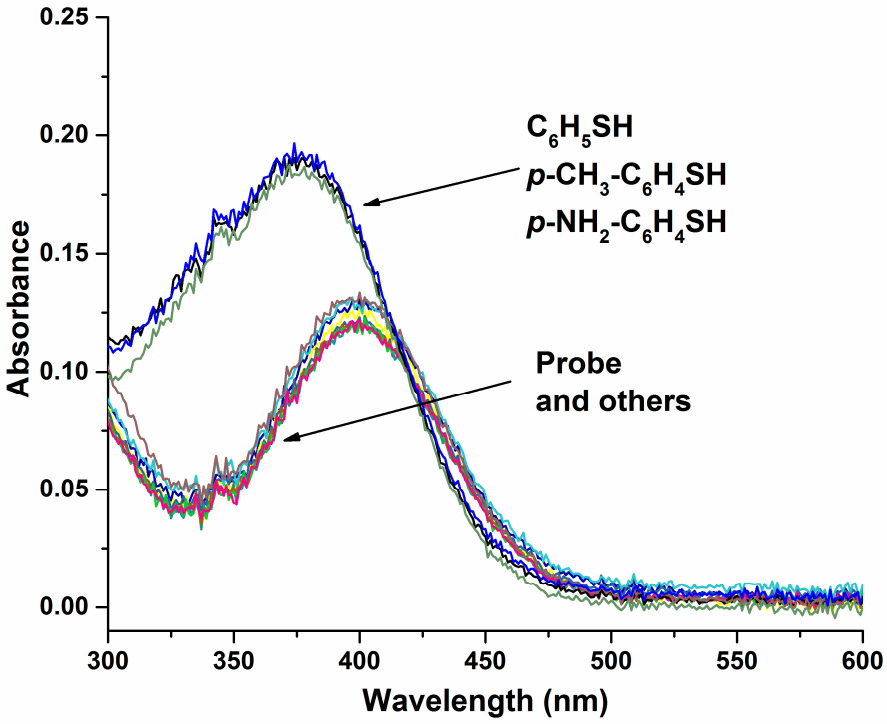
Figure S4: UV-Vis spectra

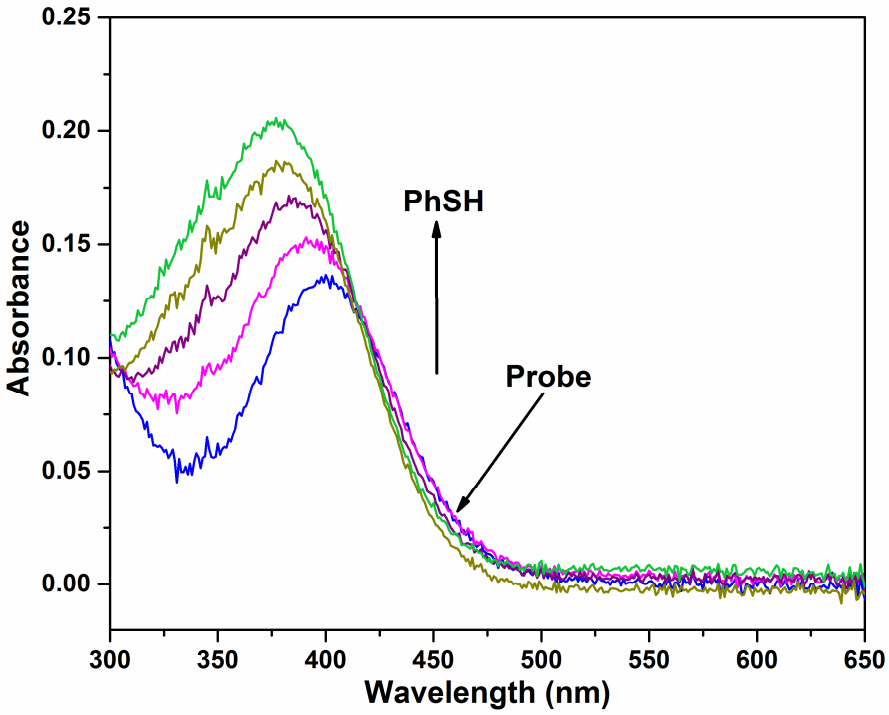
(a) UV–vis spectra of the probe 1 (10 μmol/L) in DMSO–HEPES buffer (10 mmol/L, pH=7.4, 1 : 1, v/v) in the presence of 60 μmol/L PhOH, $PhNH_2$, $PhCH_3$, KCN, H_2O_2, Na_2S, ME, Cys, Hcy, GSH, and 10 μmol/L PhSH, p-NH_2-C_6H_4SH, p-CH_3-C_6H_4SH

(b) UV-Visible spectrum of probe 1 (10 μmol/L) upon addition various concentrations of thiophenol (0-10 μmol/L) in DMSO–HEPES buffer (10 mmol/L, pH 7.4, 1 : 1, v/v).

(a)



570 (b)



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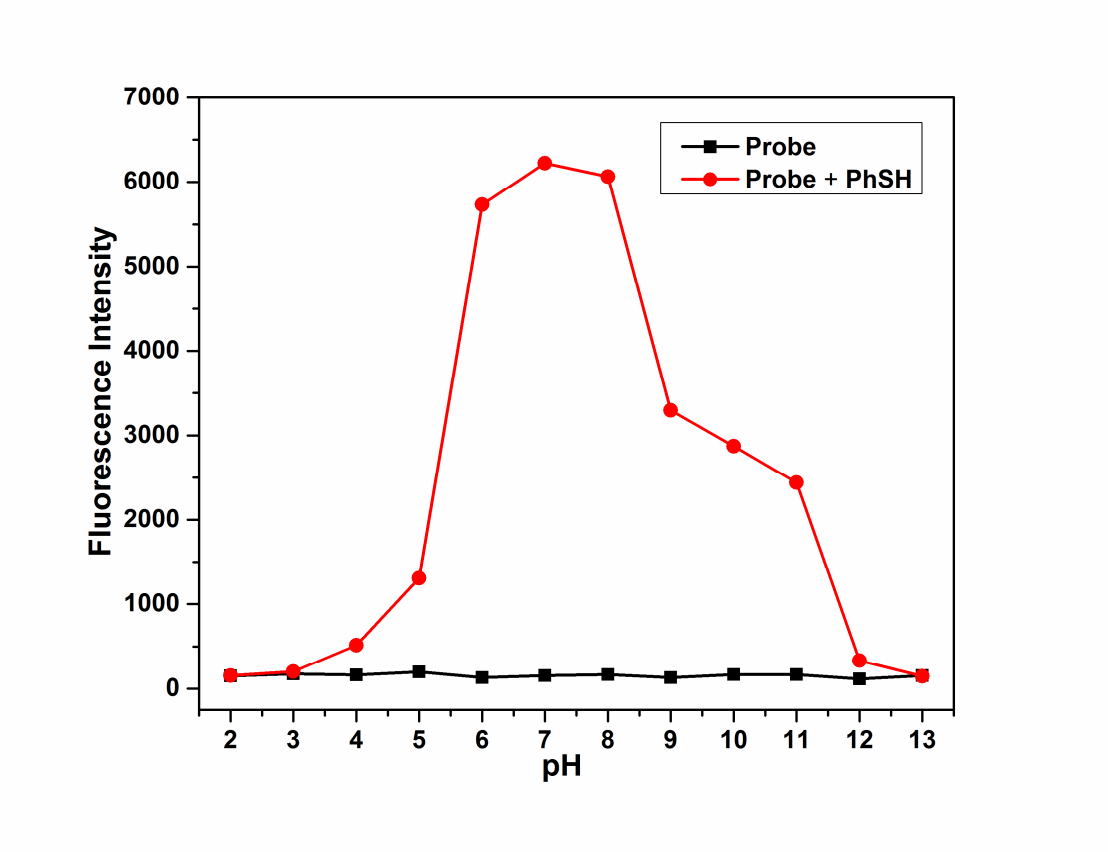
581 **Figure S5**: PH interference to the detection



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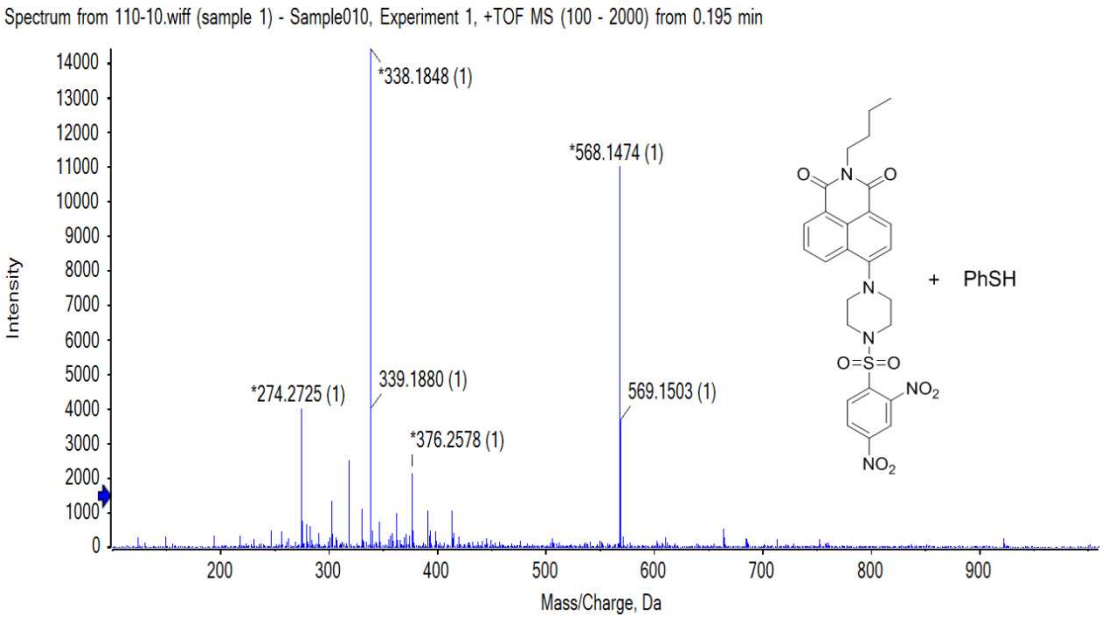
583 **Figure S6 :** The ESI-MS of product obtained by reaction of probe and thiophenol.

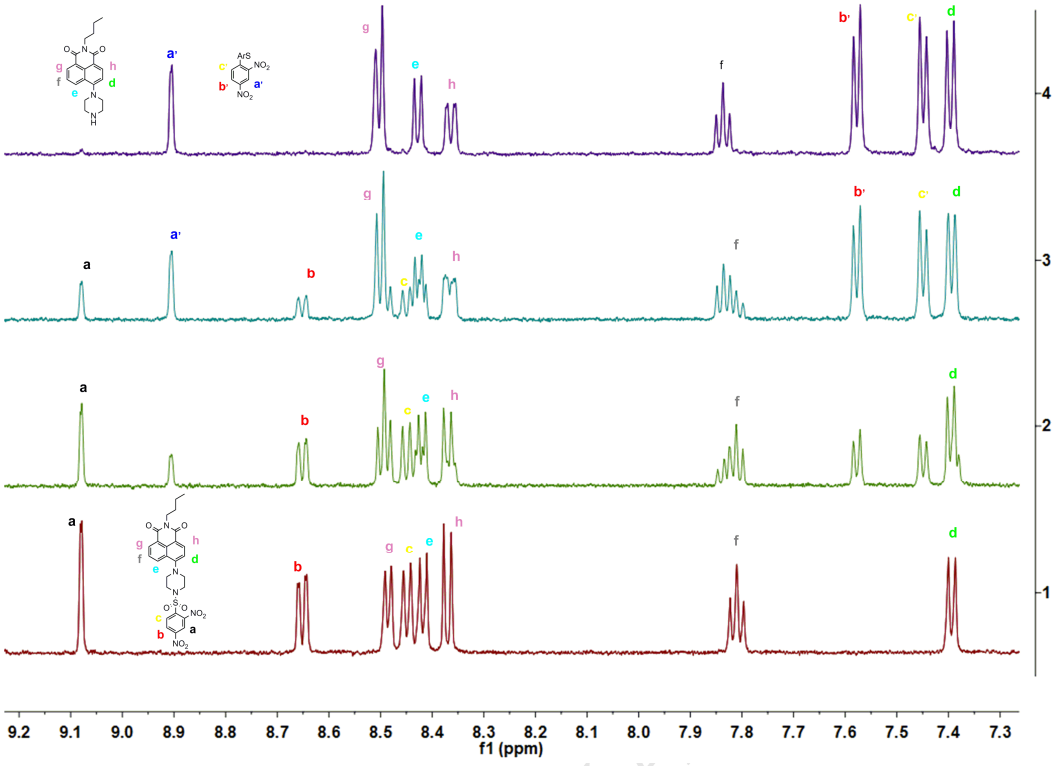


584

585 The ESI-MS of probe**:** m/z: [Probe + PhSH+H] $^{+}$ Calcd. for $C_{20}H_{23}N_3O_2$ 337.17903,

586 Found 338.1848.

Figure S7: 1D ^{1}H NMR the probe and probe-thiophenol.



^{1} H NMR spectra of 1 upon addition of thiophenol in DMSO-d6. (1) Only probe, (2) Probe with 0.5 equal of thiophenol, (3) Probe with 1 equal of thiophenol, (4) Probe with 1.5 equal of thiophenol.

Highlights

a) The interaction between probe 1 and thiophenol in solution was studied in detailed.

b) The probe can be applied in bioimaging.

c) The detection limit is as low as 13nM.