Supporting Information

A red emitting fluorescence probe for instantaneous sensing of thiophenol in both aqueous medium and living cells with a large Stokes shift

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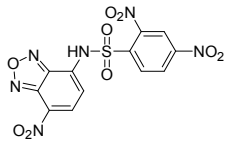
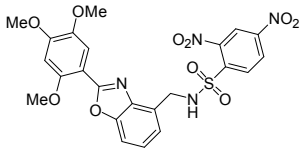
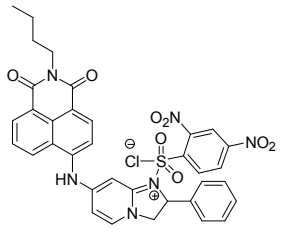
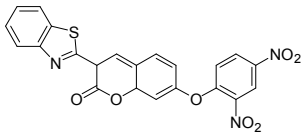
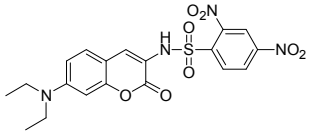
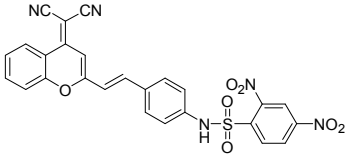
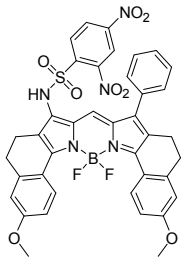
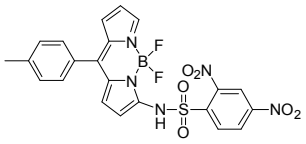
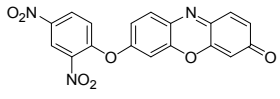
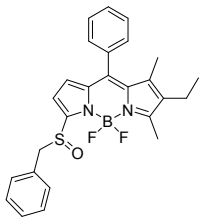
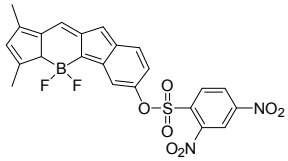
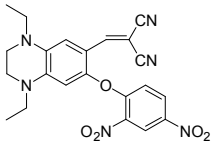
Contents

Table S1 Fluorescent probes for thiophenol.

Probe	$\lambda_{ex}/\lambda_{em}$	Stokes shift	Response time	Literature
	465 nm/555 nm	90 nm	10 min	Angew. Chem., Int. Ed. 2007, 46, 8445-8448
	335 nm/403 nm	68 nm	20 min	Chem. Commun. 2010, 46, 1944-1946.
	481 nm/590 nm	109 nm	15 min	Anal. Chem. 2012, 84, 4915-4920
	461 nm/494 nm	33 nm	40 min	Chem. Commun. 2010, 46, 1503-1505
	370 nm/515 nm	145 nm	30 min	Anal. Chem. 2014, 86, 3037-3042
	490 nm/670 nm	180 nm	10 min	Anal. Chem. 2014, 86, 8835-8841
	608 nm/633 nm	25 nm	90 min	Anal. Chem. 2015, 87, 399-405
	547 nm/568 nm	21 nm	70 min	Analyst, 2012, 137, 3921-3924
	545 nm/587 nm	42 nm	10 min	Chem. Commun. 2010, 46, 1503-1505

	484 nm/568 nm	84 nm	36 min	Dyes Pigm. 2013, 96, 328-332
	546 nm/565 nm	19 nm	20 min	J. Phys. Chem. B, 2011, 115, 642-647
	477 nm/606 nm	129 nm	2 min	This work

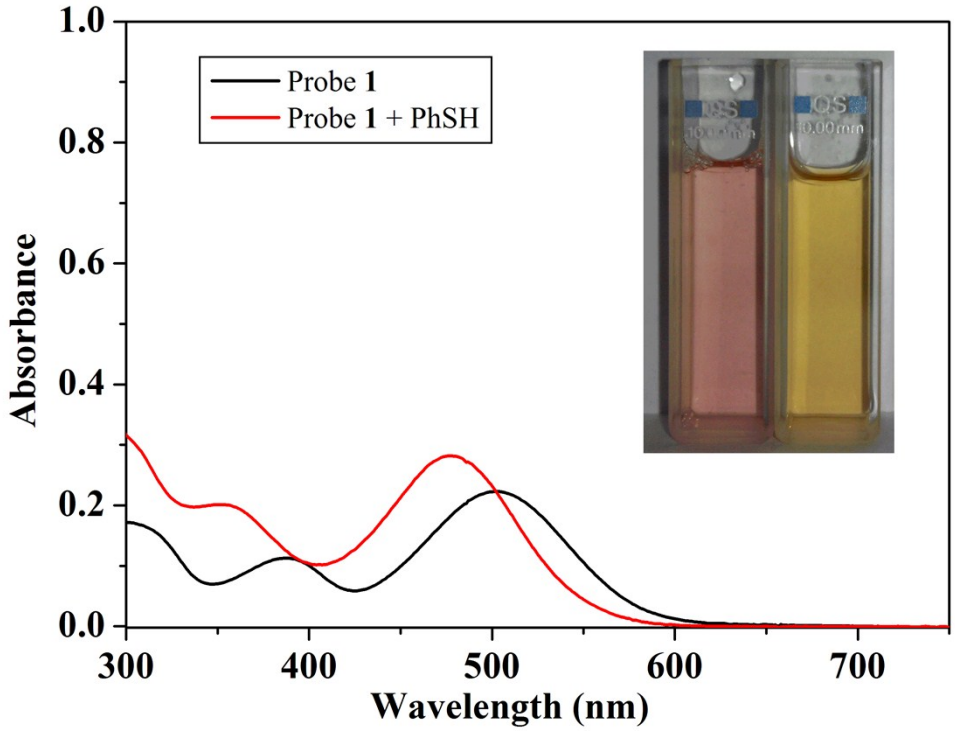


Fig. S1 Absorption spectra of Probe **1** (10.0 μM) in the absence/presence of thiophenol (5.0 equiv.) in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB). Inset: photographs of Probe **1** before (left) and after (right) addition of thiophenol.

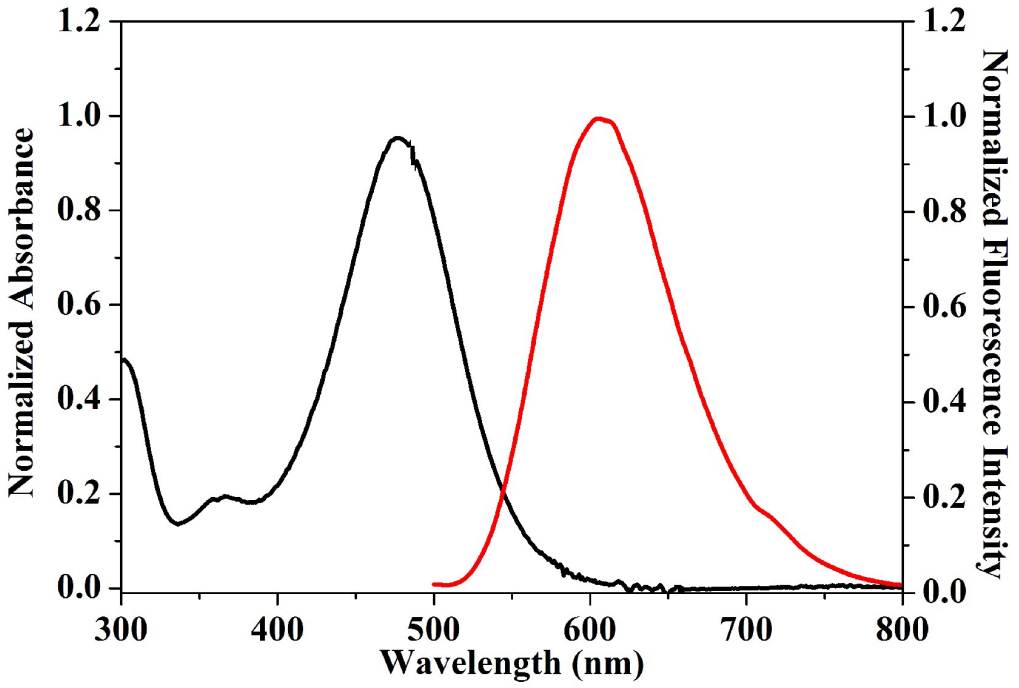


Fig. S2 Normalized absorption (black line) and fluorescence (red line) spectra of dye **4** in HEPES buffer (10.0 mM, pH = 7.4, 1.0 mM CTAB).

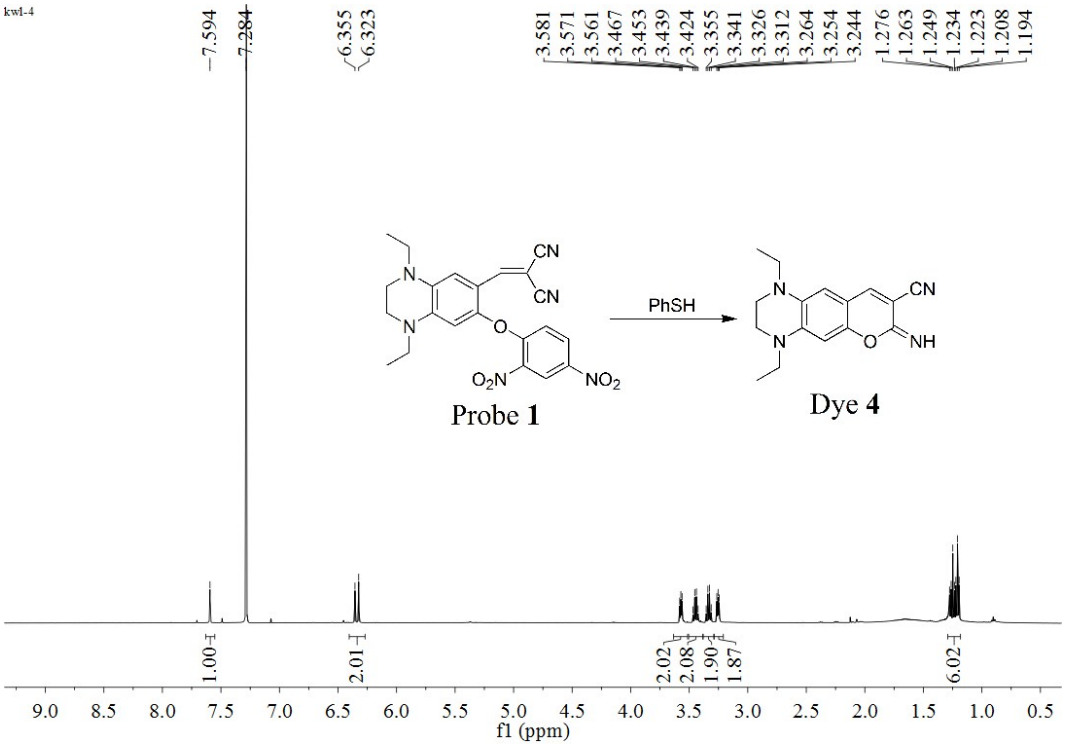


Fig. S3 ^{1}H NMR spectrum of the reaction product of Probe **1** with thiophenol in $CDCl_3$.

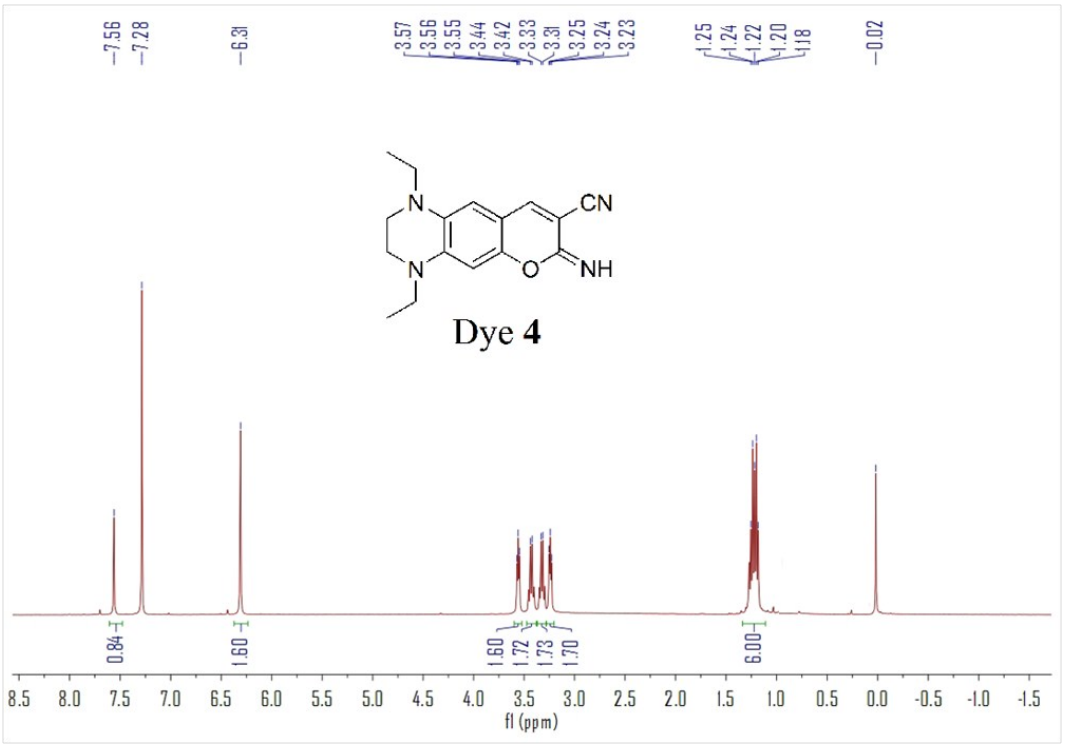


Fig. S4 ^{1}H NMR spectrum of dye **4** in $CDCl_3$.

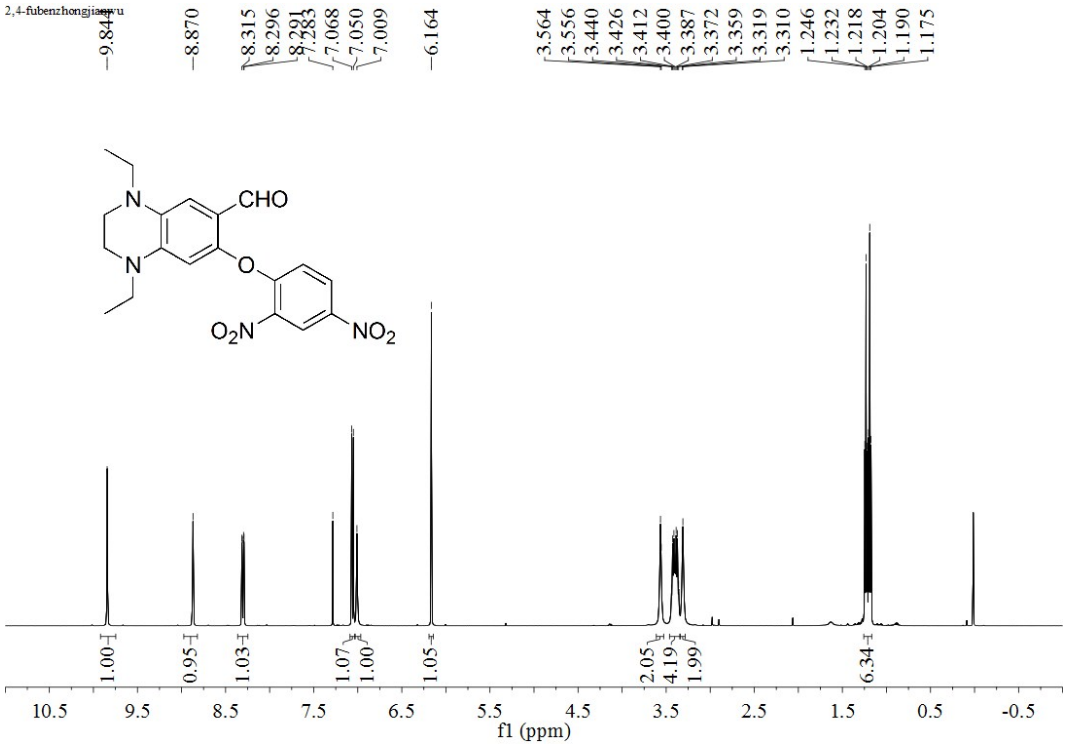


Fig. S5 ^{1}H NMR spectrum of compound **3** in $CDCl_3$.

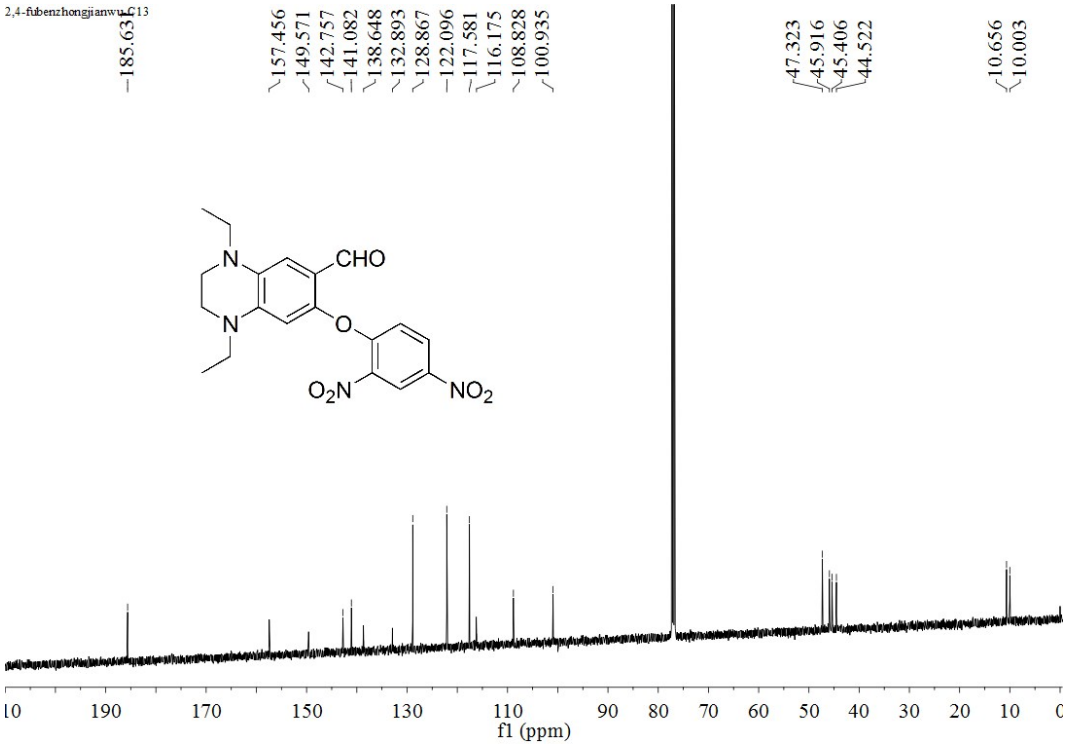


Fig. S6 ^{13}C NMR spectrum of compound **3** in $CDCl_3$.

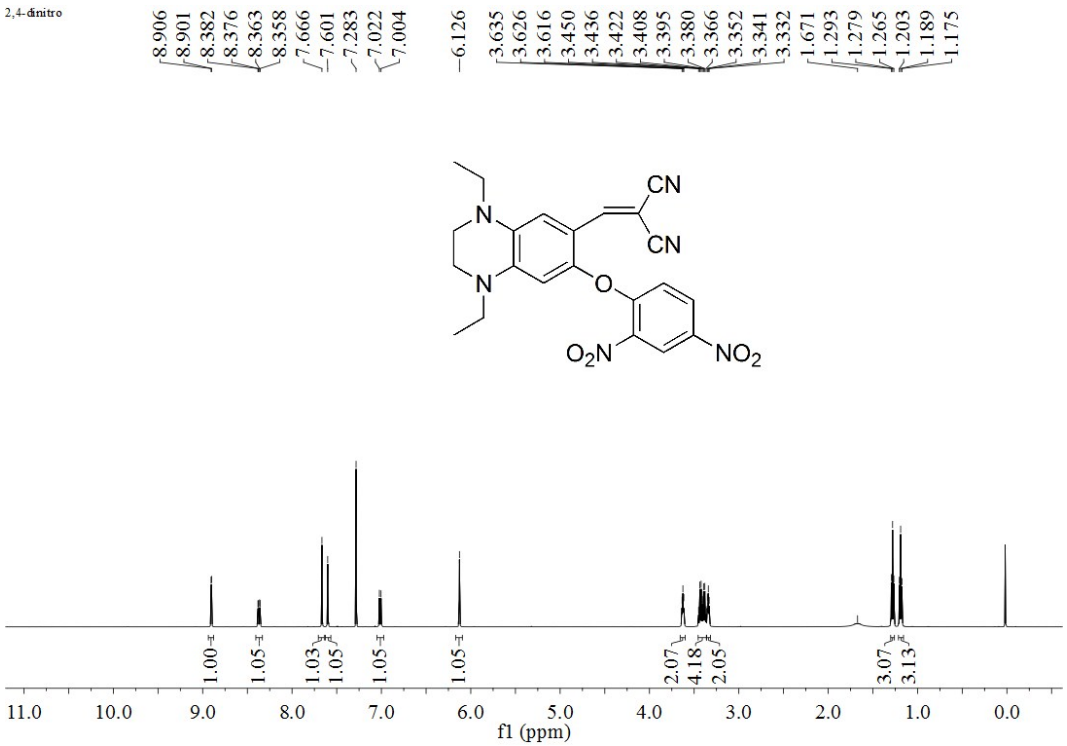


Fig. S7 ^{1}H NMR spectrum of Probe **1** in $CDCl_3$.

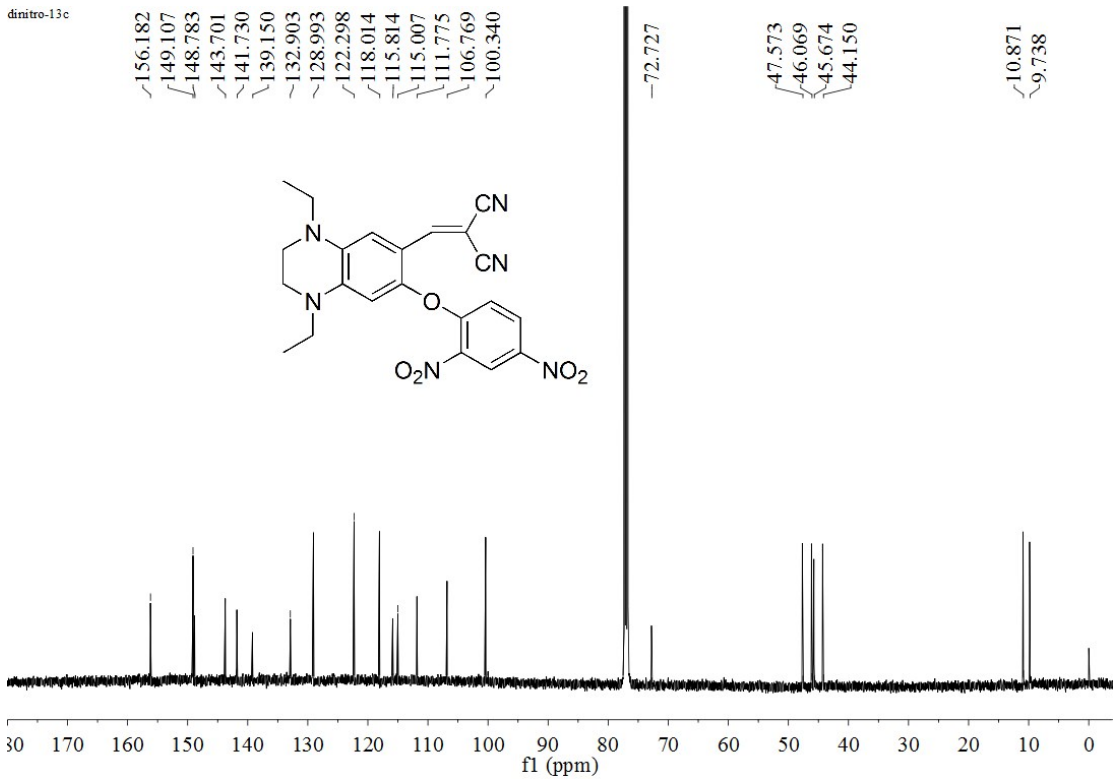


Fig. S8 ^{13}C NMR spectrum of Probe **1** in $CDCl_3$.

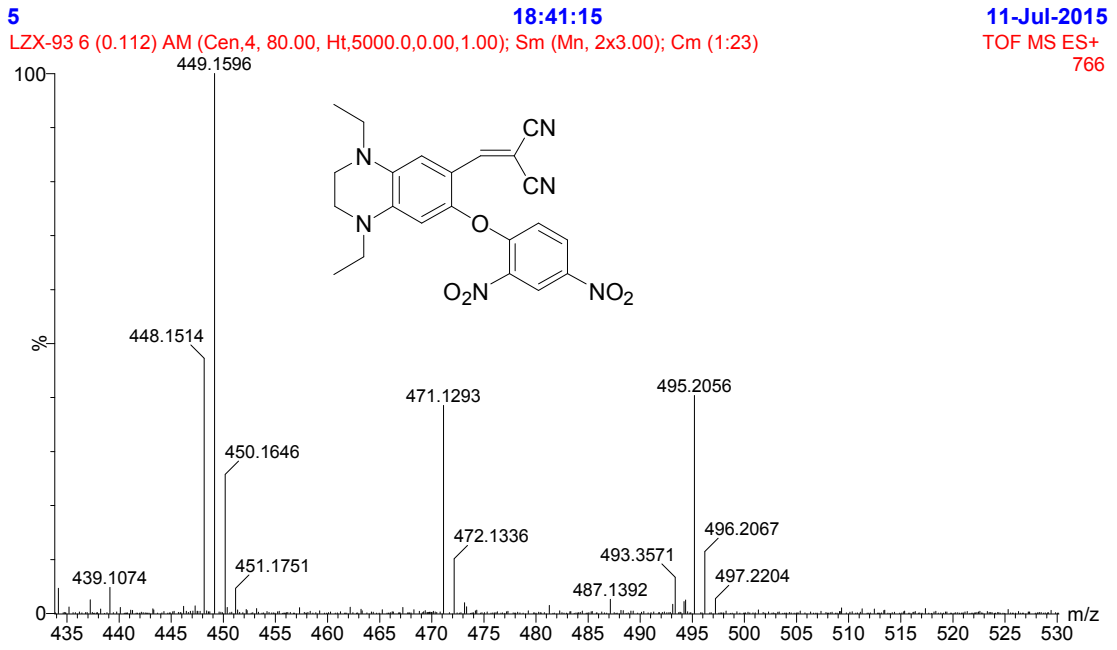


Fig. S9 HRMS spectrum of Probe **1**.

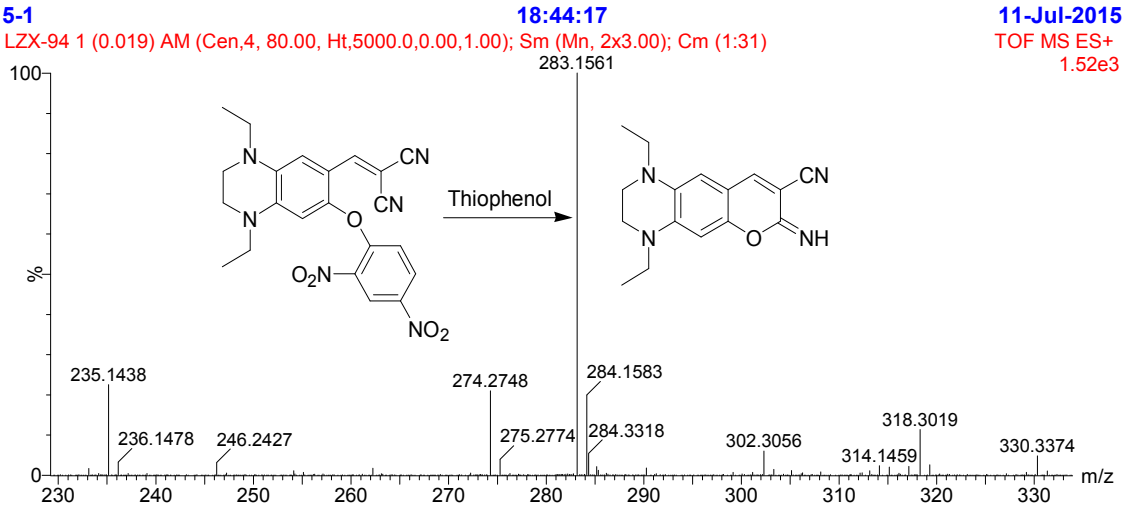


Fig. S10 HRMS spectrum of the reaction product of Probe **1** with thiophenol.

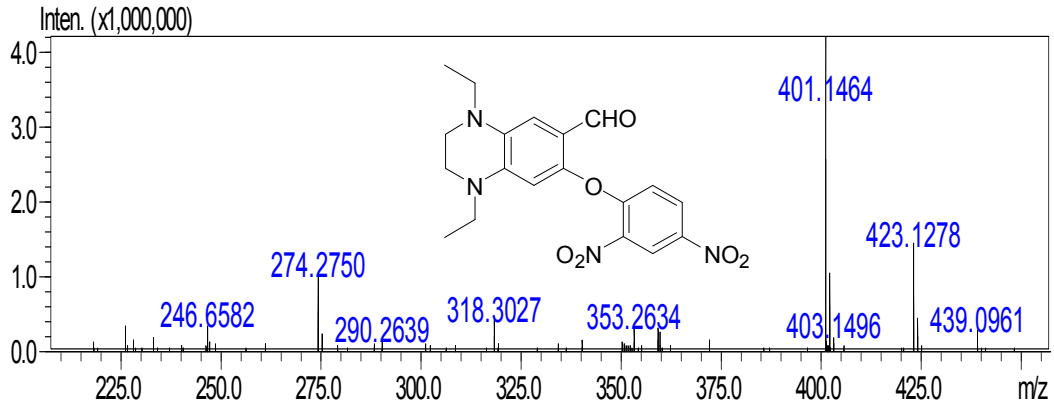


Fig. S11 HR MS spectrum of compound **3** in $CDCl_3$.

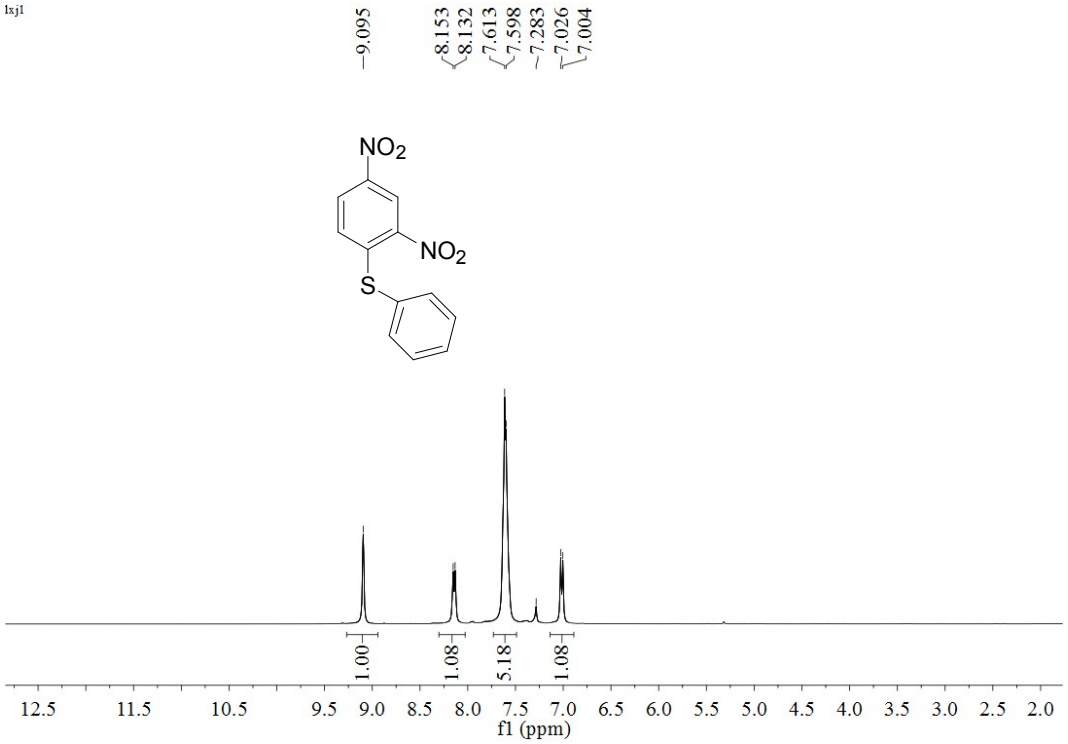


Fig. S12 ^{1}H NMR specrum of the product from the reaction of Probe **1** with thiophenol.

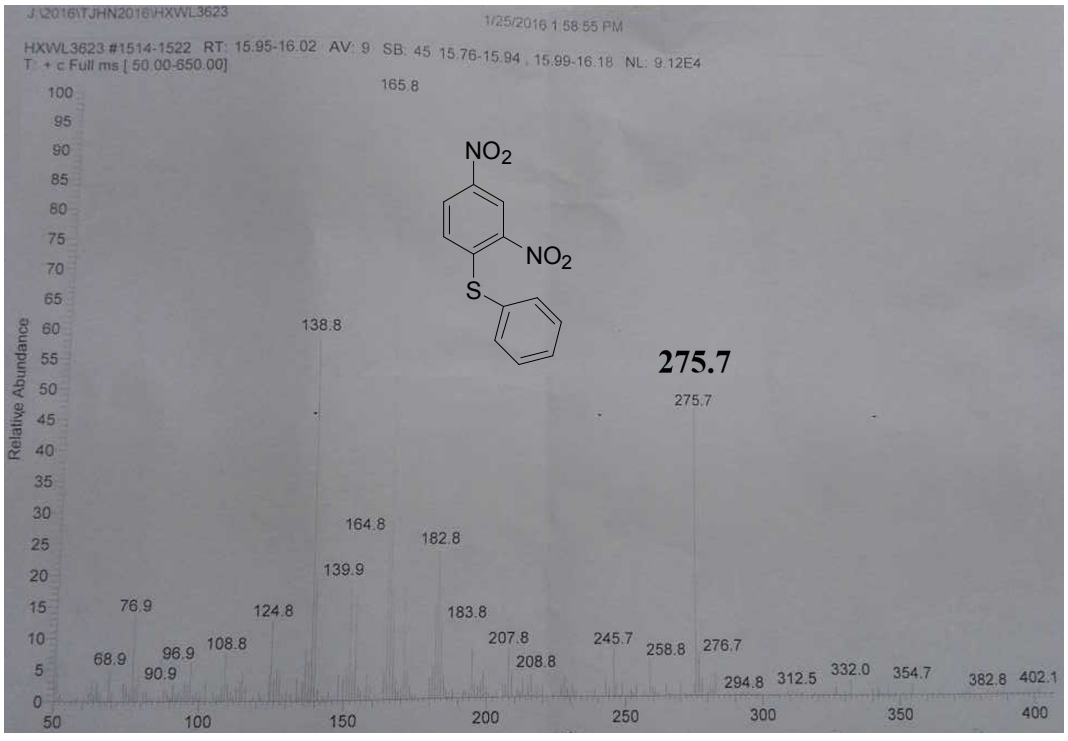


Fig. S13 The low-resolution mass spectrum of the reaction product of Probe **1** with thiophenol.

Photo- and thermo- stability studies of Probe 1

We investigated the photo- and thermo- stability of Probe **1** and found that this probe showed excellent photo- and thermo- stability. As shown in Fig. S14, only 8.7% of the probe was degraded in CH_3CN after 3 hours irradiation. The thermal gravimetric analysis (TGA) curve of Probe **1** demonstrated that the decomposition temperature was from 217 to 242 °C (Fig. S15). Furthermore, we studied the stabilities of Probe **1** under the physiological condition. We conducted the experiments in HEPES buffer (20.0 mM, pH = 7.4) containing 20% DMSO (DMSO was used to improve the solubility of Probe **1** in aqueous media) instead of the micell system (PBS buffer (20.0 mM, pH = 7.4) with 1.0 mM CTAB)，which may be vulnerable during heating or irradiation. The photostability studies showed that 8.6% and 34.4% of the probe was degraded after 1 hour and 2 hour irradiation under a 300 mW, 532 nm continuous wave laser, respectively (Fig. S18). We also investigated the fluorescence of HeLa cells incubated with Probe **1** (10.0 μM) under the continuous irradiation with 488 nm wave laser (Fig. S19). After 30 min irradiation, the fluorescence change of the cells was negligible. In addition, the thermo-stability of Probe **1** was studied under physiological condition at 25 °C, 37 °C, and 45 °C, respectively, by observing its absorption spectral change. As shown in Figure S20, the absorption spectra of this probe stayed unchanged after incubation for 1 hour at 25 °C and 37 °C, and negligible change was observed at 45 °C. Therefore, it can be concluded that Probe **1** exhibited good photo- and thermo- stability under physiological condition and can serve as a good candidate for potential biological use.

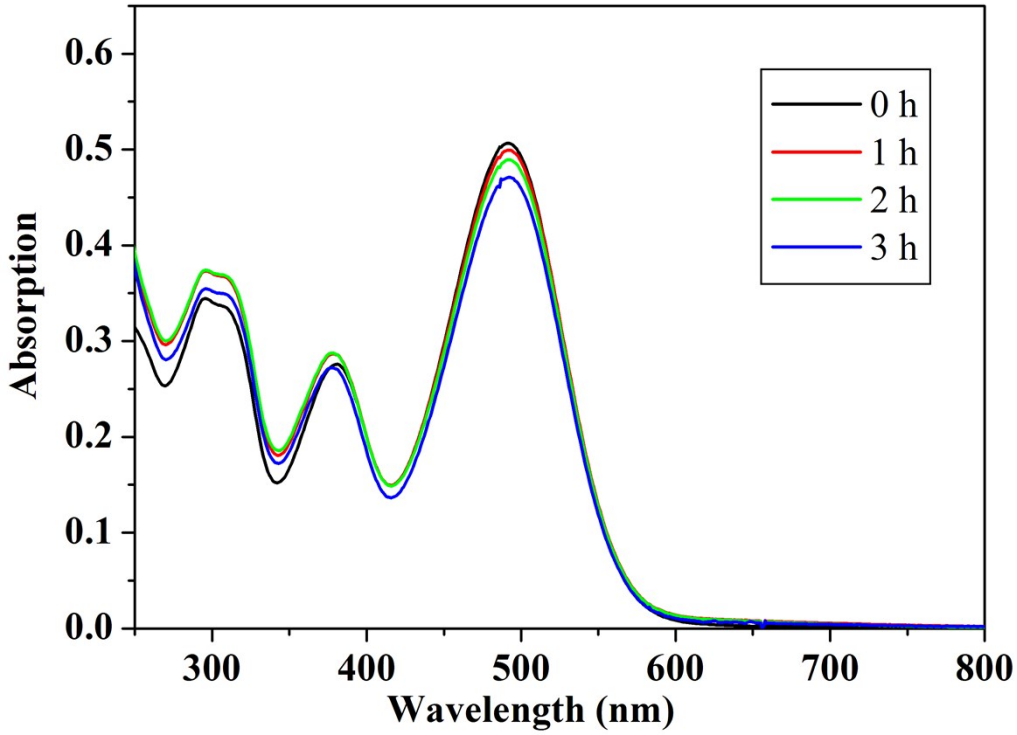


Fig. S14 Photodegradation of Probe **1** in CH_3CN under the continuous irradiation with a 300 mW, 532 nm continuous wave laser. The distance between the light source and the sample is 10 cm.

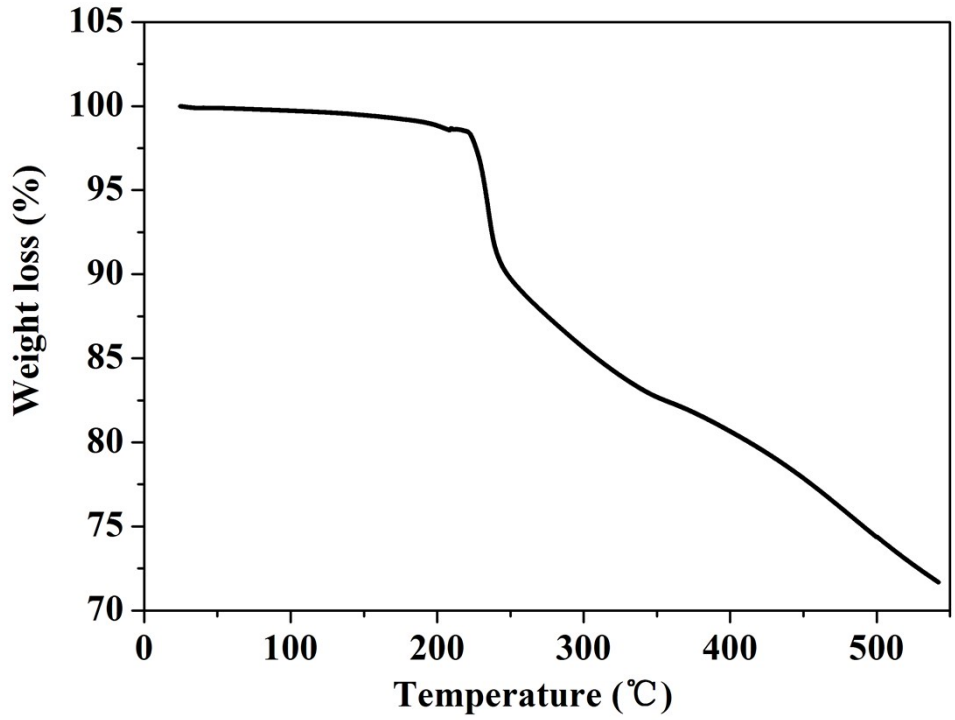


Fig. S15 Thermal gravimetric analysis (TGA) curve of Probe **1**. Heating rate: 10 °C min^{-1}.

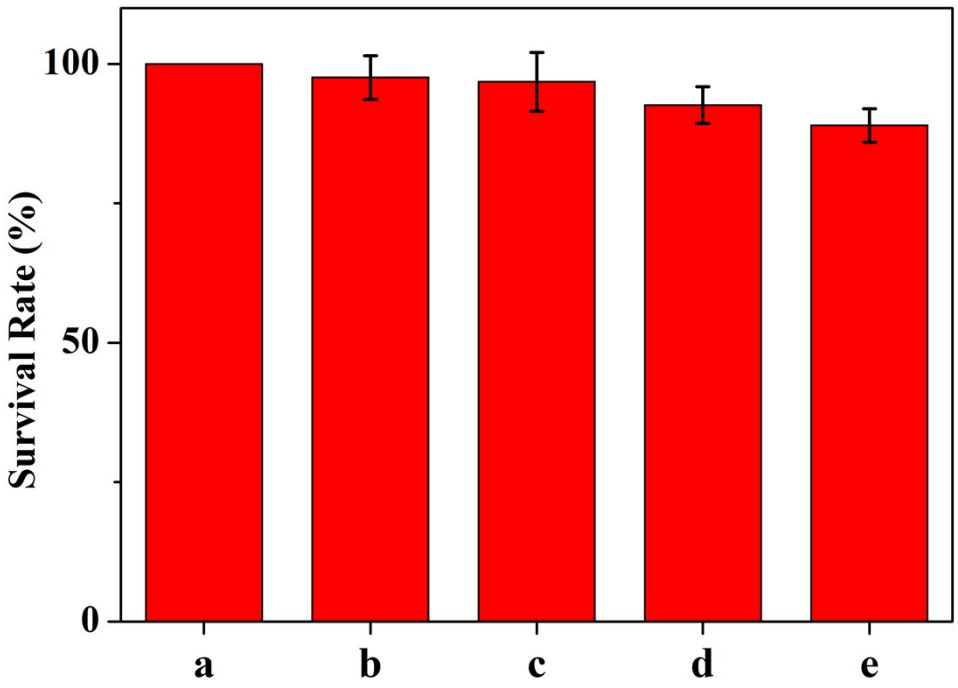


Fig. S16 Cytotoxicity assay of Probe **1** at different concentrations (a: 0.0 μM; b: 2.0 μM; c: 5.0 μM; d: 7.0 μM; e: 10.0 μM) for HNE-2 cells.

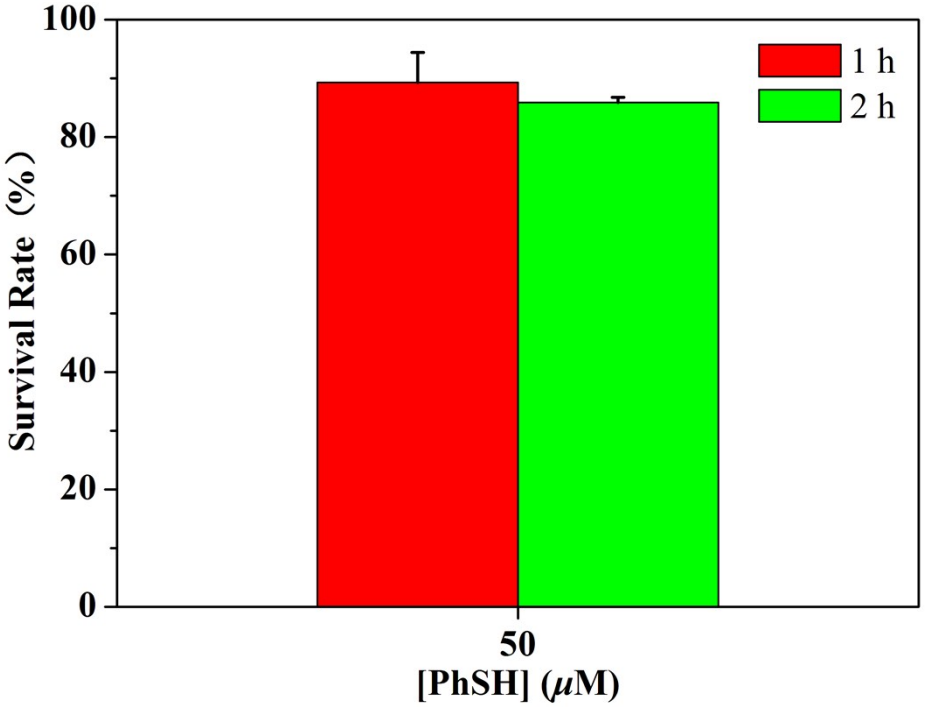


Fig. S17 Cytotoxicity assay of thiophenol for HNE-2 cells.

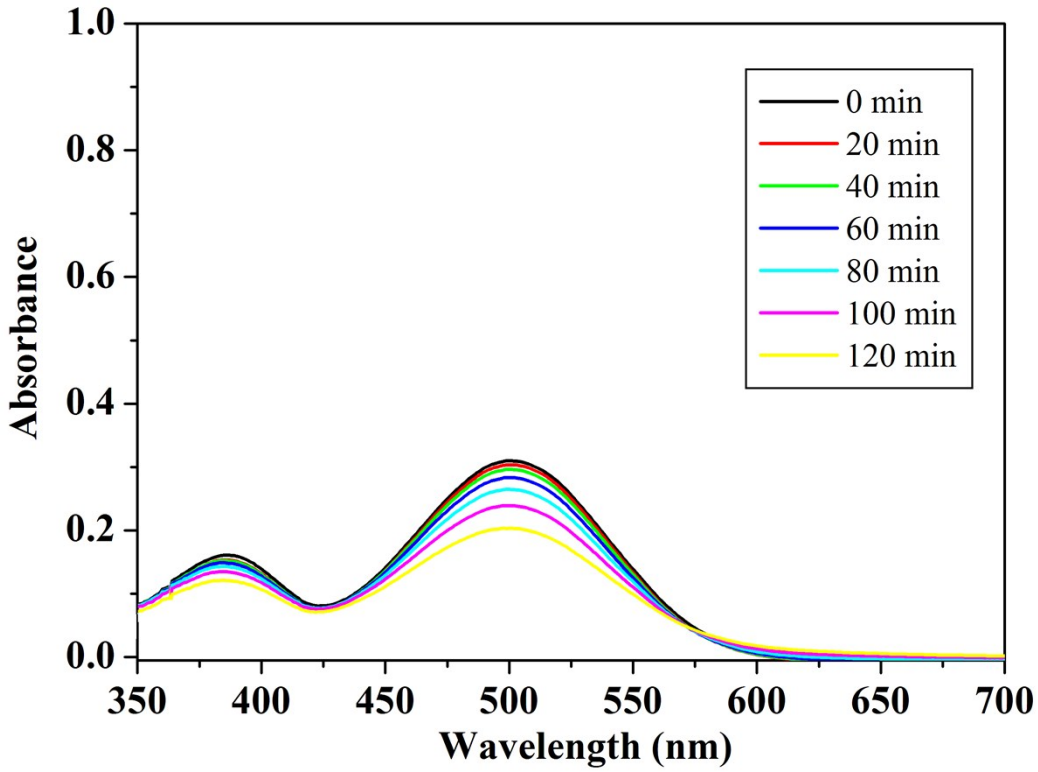


Fig. S18 Photodegradation of Probe **1** in HEPES buffer (10.0 mM, pH 7.4) containing 20% DMSO under the irradiation with a 300 mW, 532 nm continuous wave laser by observing its absorbance. The distance between the light source and the sample is 10 cm.

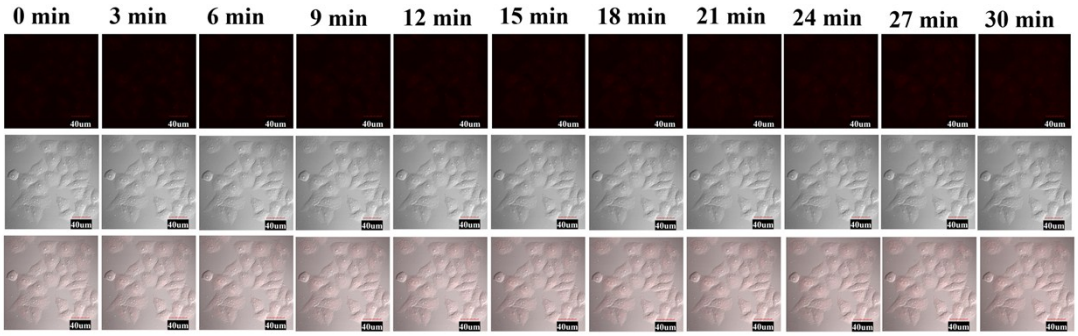


Fig. S19 Fluorescence (top row), bright field (middle row) and merged (bottom row) images of Hela cells after incubation with Probe **1** (10.0 μM) under the continuous irradiation with 488 nm wave laser at different time. Images were acquired from 560-630 nm for red fluorescence.

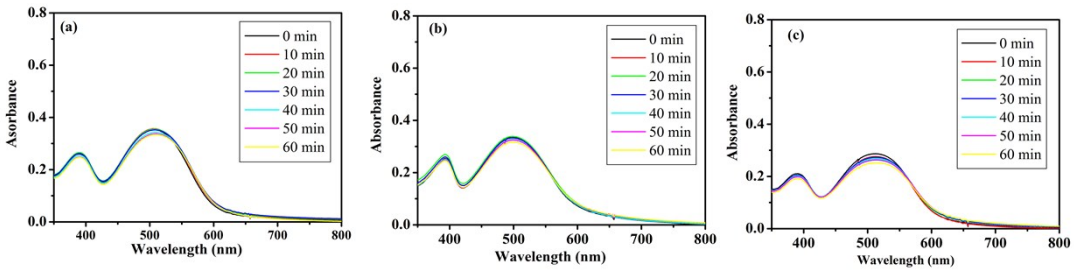


Fig. S20 Thermo-stability of Probe **1** at 25 °C (a), 37 °C (b) and 45 °C (c) in HEPES buffer (20.0 mM, pH = 7.4) containing 20% DMSO.

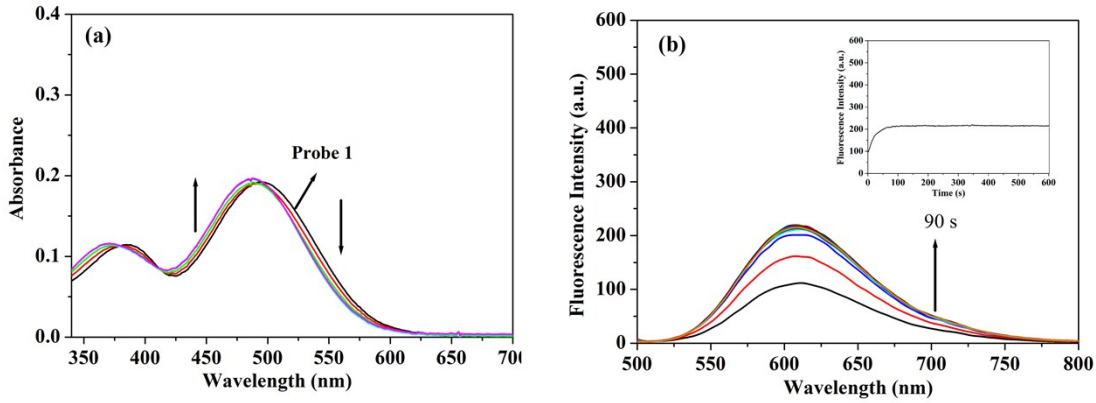


Fig. S21 Time-dependent absorption (a) and fluorescence (b) spectra of Probe **1** (10.0 μM) with 1 equiv. of PhSH in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB). Inset in Fig. R4(b): Kinetic of fluorescence enhancement rate at 606 nm for Probe **1** (10.0 μM) in the presence of PhSH (1.0 equiv.) in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB).

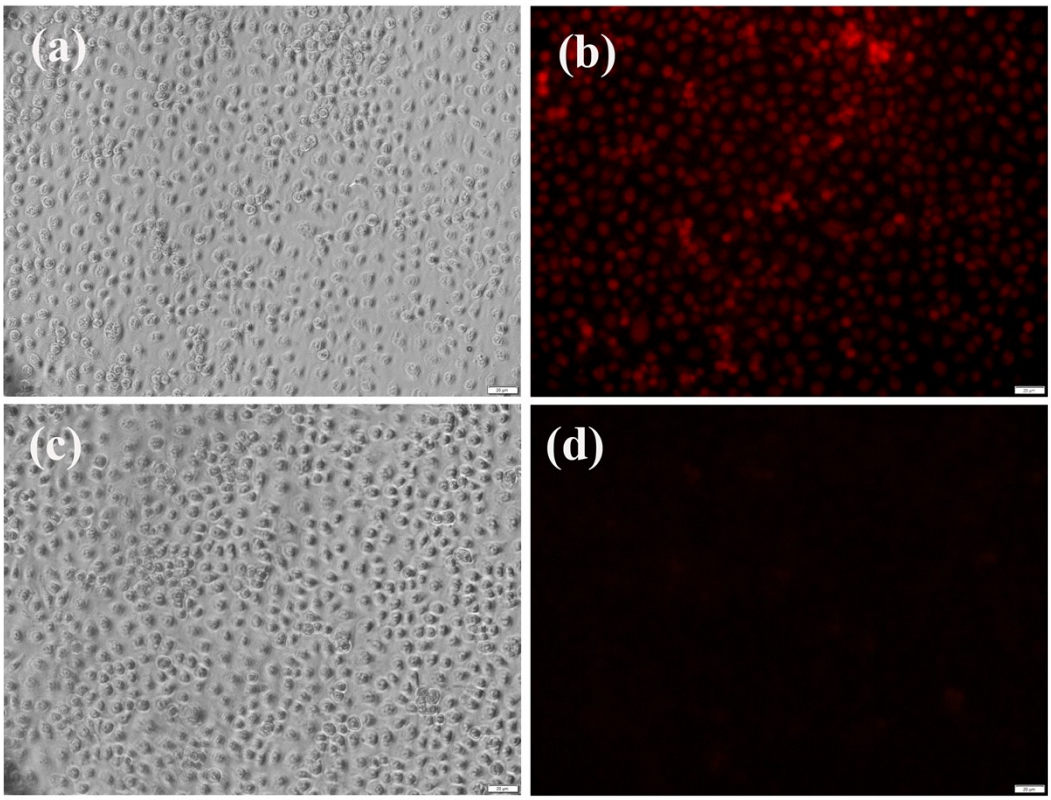


Fig. S22 Fluorescence images of thiophenol in living HNE-2 cells. (a) Bright field and (b) fluorescence images of cells pretreated with thiophenol (50.0 μM) and then incubated with Probe **1** (10.0 μM) for 30 min at 37 °C. (c) Bright field and (d) fluorescence images of cells incubated with Probe 1 (10.0 μM) for 30 min at 37 °C. The cells were magnified 20 times.