Supporting Information for

A Highly Sensitive Fluorescent Probe for Detection of Benzenethiols in the Environmental Samples and Living Cells

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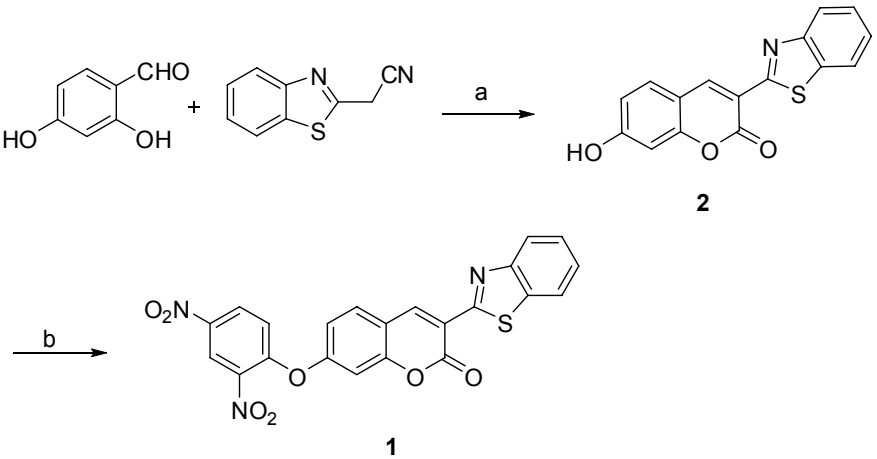
Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Melting points of compounds were measured on a Beijing Taike XT-4 microscopy melting point apparatus, and all melting points were uncorrected. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer with the excitation and emission slit widths at 1.0 and 2.5 nm respectively. Cell imaging was performed with a Nikon Eclipse TE300 inverted microscope using FITC filters. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.



Scheme S1. Synthesis of probe **1**. Reagents and conditions: (a) piperidine, ethanol, room temperature, overnight, then 10% hydrochloric acid, 130°C, overnight; (b) 2,4-dinitrofluorobenzene, K_2CO_3, anhydrous DMF, 75°C, 8 h..

Synthesis of 3-benzothiazolyl-7-hydroxycoumarin (2). 2, 4-dihydroxybenzaldehyde (345.3 mg, 2.5 mmol) and 2-(1, 3-benzothiazol-2-yl)acetonitrile (435.55 mg, 2.55 mmol) were dissolved in 5 mL ethanol, and five drops of piperidine were then added. The mixture was stirred at room temperature overnight. After filtration, the yellow solid was treated with 10% hydrochloric acid. The suspended solution was stirred at 130°C overnight, and the resulting yellow residue was collected by filtration, washed with water, dried under reduced vacuum, and then purified by silica gel column chromatography (dichloromethane/ethanol = 5:1, V/V) to afford compound **2** (530 mg, 71.8%). mp >300^{o}C. ^{1}H NMR (400 MHz, d_6-DMSO):δ 9.15 (s, 1H), 8.15 (d, J = 7.6Hz, 1H), 8.05 (d, J = 8Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.57 (t, J = 8.4 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 6.93 (dd, J = 8.4 Hz, J = 2 Hz, 1H), 6.87 (d, J = 2Hz,1H). MS (ESI): m/z = 296.1$[M+H]^{+}$.

Synthesis of 3-benzothiazolyl-7-(2,4-dinitrophenoxy) coumarin (1). 3-benzothiazolyl-7-hydroxycoumarin (59 mg, 0.2 mmol), 2,4-dinitrofluorobenzene (111.6 mg, 0.6 mmol), and anhydrous potassium carbonate (276 mg, 2 mmol) were added into a three-neck bottom flask under N_2 atmosphere, and then anhydrous DMF (1 mL) was injected into the reaction flask with a syringe. The mixture was stirred at 75 ^{o}C for 8 h. Subsequently, the solvent was removed under reduced pressure, and the resulting residue was purified by chromatography on silica gel (petroleum ether / dichloromethane = 1 : 2, V/V) to give compound **1** as a yellow solid (64.5 mg, yield 70.1%). mp 248-250^{o}C. ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.31 (s,1H), 8.96 (d, J = 2.8Hz, 1H), 8.56 (dd, J = 9.2 Hz, J = 3.2 Hz,1H), 8.22 (t, J = 8.4Hz, 2H), 8.10 (d, J = 8.0 Hz, 1H), 7.60 (t, J = 8.4 Hz, 1H), 7.52 (m, 3H), 7.37 (dd, J = 8.4 Hz, J = 2.0 Hz,1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ 160.1, 159.6, 158.9, 155.2, 153.4, 152.3, 143.4, 141.9, 140.8, 136.3, 132.8, 130.4, 127.2, 125.9, 122.9, 122.7, 122.5, 118.9, 116.9, 116.7, 107.3. MS (ESI): m/z = 462.0 $[M+H]^{+}$.

Preparation of the test solution.

A stock solution of probe **1** (1.0×10^{-3} M) was prepared in DMF. The test solution of probe **1** (1 or 5 μM) in 2 mL neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 45% DMF as a co-solvent) was prepared by placing 0.002 or 0.01 mL of the probe stock solution, 0.89 mL DMF, and 1.1 mL of 45.5 mM sodium phosphate buffer (pH = 7.0). The solutions of various testing species were prepared from Gly, Lys, Arg, Leu, glucose, vitamin C, phenol, aniline, homocysteine, cysteine, glutathione, 2-mercaptoethanol, 4-chlorobenzenethiol, 4-aminobenzenethiol, or benzenethiol, respectively.

Benzenethiol vapor detection by probe 1 in the paper strips.

The test papers of probe **1** were prepared by immersing the filter papers into the probe **1** solutions with different concentrations (10, 20, 60, and 100 μM) in DMF. The test papers were placed in a chemical storage cabinet where an uncapped bottle of volatile benzenethiol was stored. For comparison, a test paper prepared from the probe **1** solution (100 μM) was put into another chemical storage cabinet where an uncapped bottle of volatile 2-mercaptoethanol was stored. After 30 min, the test papers were removed and the photographs were taken under a handheld 365 nm UV lamp.

Detection of benzenethiol in water sample.

The crude water samples from YueLu spring and Xiang River were filtered through microfiltration membrane before use. 50 mL of the water samples were adjusted to 45.5 mM sodium phosphate buffer (pH 7.0), and aliquots (1.1 mL) of the phosphate buffer were then spiked with different concentrations of benzenethiol (0.5, 1, 10, or 100 μM) in ethanol (20 μL). The resulting samples (1.1 mL) were further treated with probe **1** (2.2 μM) in DMF (0.9 mL) to give DMF/pH 7.0 buffer solutions (2 ml, 25 mM sodium phosphate buffer, pH 7.0, 45% DMF as a co-solvent) containing probe **1** (final concentration = 1μM) and benzenthiol (final concentration = 0.005, 0.01, 0.1, or 1 μM). The solutions were incubated for 30 min at room temperature before fluorescence measurement.

Detection of benzenethiol in soil.

To DMF/pH 7.0 buffer (3 ml) containing probe **1** (final concentration = 1 μM) were added soil samples (90 mg) pre-spiked with benzenethiol (37.5 μL of 400 μM stock, final concentration = 5 μM) or 2-mercaptoethanol (37.5 μL of 400 μM stock, final concentration = 5 μM) in ethanol. After 30 min incubation at room temperature, the photographs were taken under a handheld 365 nm UV lamp and the solutions were filtered through microfiltration membrane for fluorescence measurement.

Cell culture and fluorescence imaging.

Hela cells were seeded in a 6-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum for 24 h. Hela cells were then incubated with benzenethiol (1 μM) in the culture medium for 30 min at 37^{o}C. After washing with PBS three times to remove the remaining benzenethiol, the cells were further incubated with probe **1** (1 μM) for 30 min at 37^{o}C. After washing the cells with PBS three times, the fluorescence images were acquired with a Nikon Eclipse TE300 equipped with FITC filters and a CCD camera. For the N-ethylmaleimide control experiment, Hela cells were incubated with benzenethiol (1 μM) in the culture medium for 30 min at 37^{o}C. After washing with PBS three times to remove the remaining benzenethiol, the cell were treated with N-ethylmaleimide (1 mM) for 30 min at 37^{o}C followed by washing with PBS three times, and then incubated with probe **1** (1 μM) for 30 min at 37^{o}C. Fluorescence imaging was then carried out after washing the cells with PBS buffer three times.

Determination of the fluorescence quantum yield: [1-3]

Fluorescence quantum yield was determined using optically matching solutions of quinine sulfate (Φ_r = 0.546 in 1N H_2SO_4)[4] as standard and the quantum yield was calculated using the following equation:

$$\Phi_s = \Phi_r (A_r F_s / A_s F_r) (n_s / n_r)^2 \qquad (1)$$

where, A_s and A_r are the absorbance of the sample and the reference, respectively, at the excitation wavelength, F_s and F_r are the corresponding relative integrated fluorescence intensities, and n is the refractive index of the solvent.

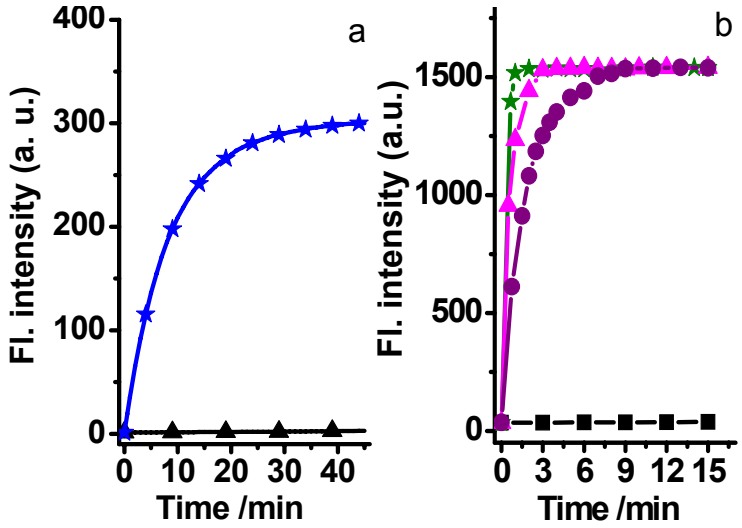


Figure S1. (a) Time course for the change in the fluorescence intensities of probe **1** (1 μM) in the absence (▲) and presence (★) of 5 equiv. of benzenethiol in the neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 45% DMF as a co-solvent). (b) Time course for the change in the fluorescence intensities of probe **1** (5 μM) in the absence (■) and presence of 5 equiv. (●), 20 equiv. (▲), and 50 equiv. (★) of benzenethiol in the neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 45% DMF as a co-solvent). Kinetic studies were performed at room temperature. The fluorescence intensities at 494 nm were monitored at time intervals. For clarity, the data were separately presented as Figures S 1a and b.

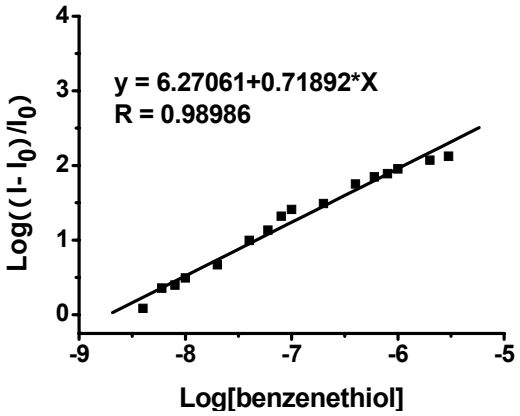


Figure S2. Plot of log[(I - I_0)/I_0] as a function of log[benzenethiol], I is the fluorescence intensity at 494 nm.

Detection limit:

The detection limit was determined from the fluorescence titration data based on a reported method.[5] According to the result of titration experiment, the fluorescent intensity data at 494 nm were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to these normalized fluorescent intensity data (Figure S3), and the point at which this line crossed the axis was considered as the detection limit (1.8×10^{-9} M).

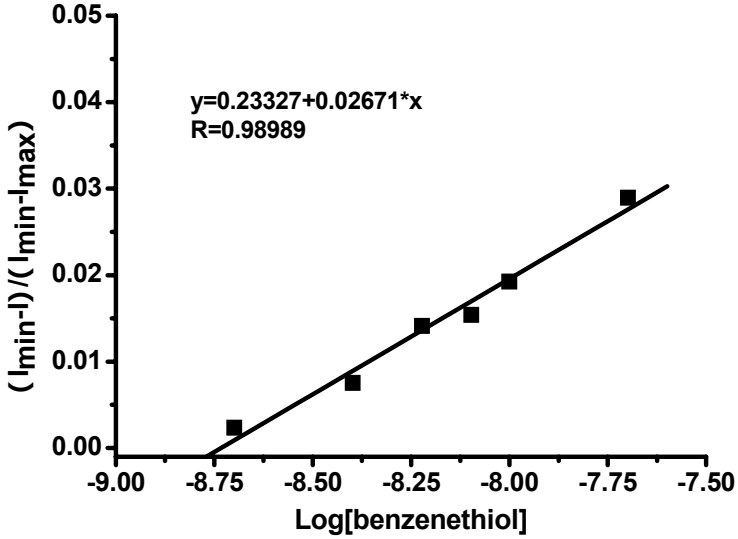


Figure S3. Normalized response of the fluorescence signal to changing benzenethiol concentrations.

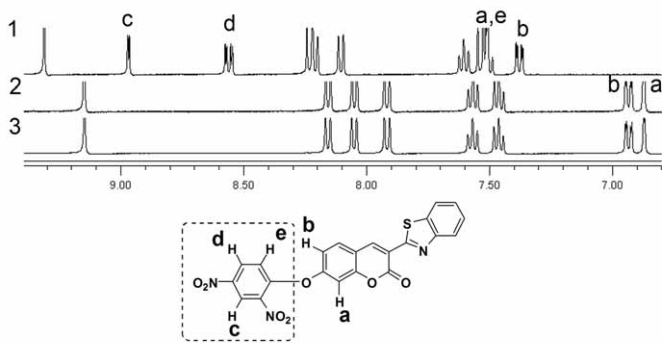


Figure S4. Partial ^{1}H NMR (400 MHz) spectra of: 1) probe **1**, 2) the isolated product of probe **1** + benzenethiol, and 3) the standard compound **2**

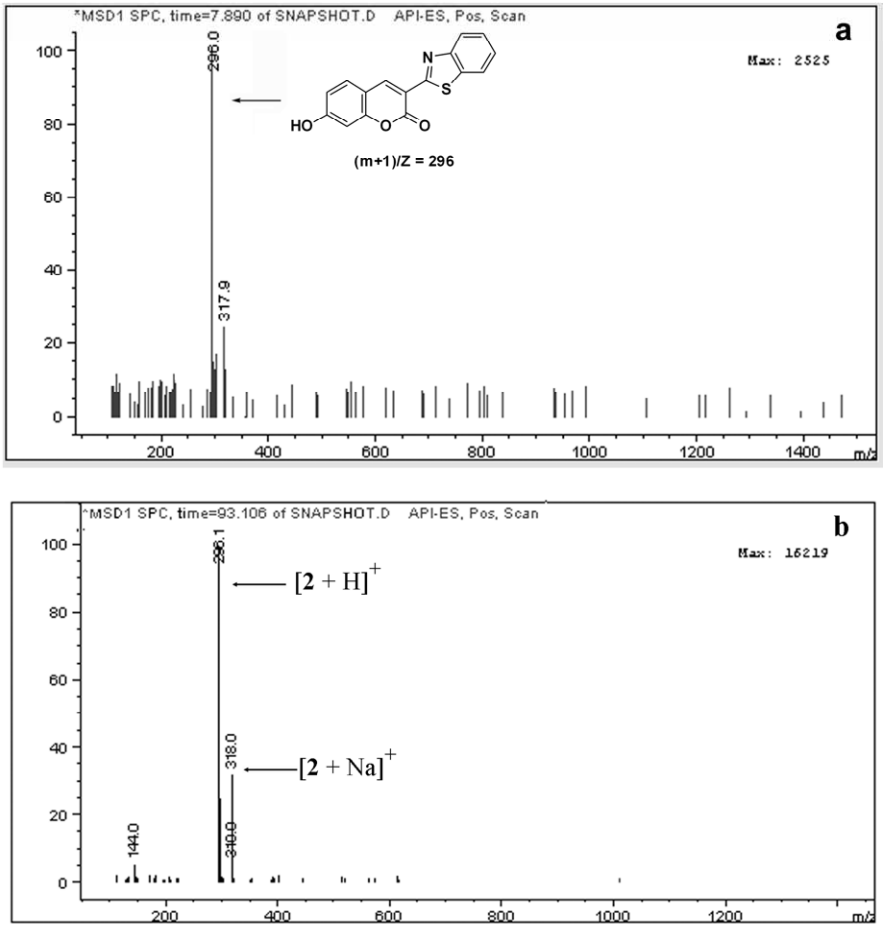


Figure S5. (a) The mass spectrum of the isolated product of probe **1** + benzenethiol.
(b) The mass spectrum of the standard compound **2**.

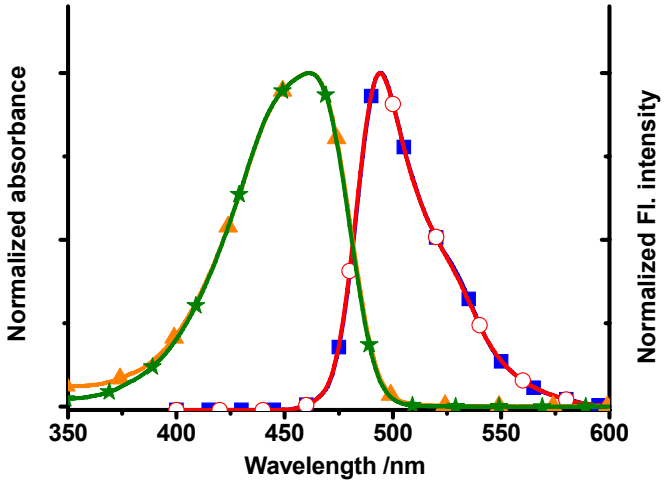


Figure S6. The normalized absorption spectra of the standard compound **2** (▲) and the isolated product of probe **1** + benzenethiol (★) and the normalized emission spectra of the standard compound **2** (■) and the isolated product of probe **1** + benzenethiol (○).

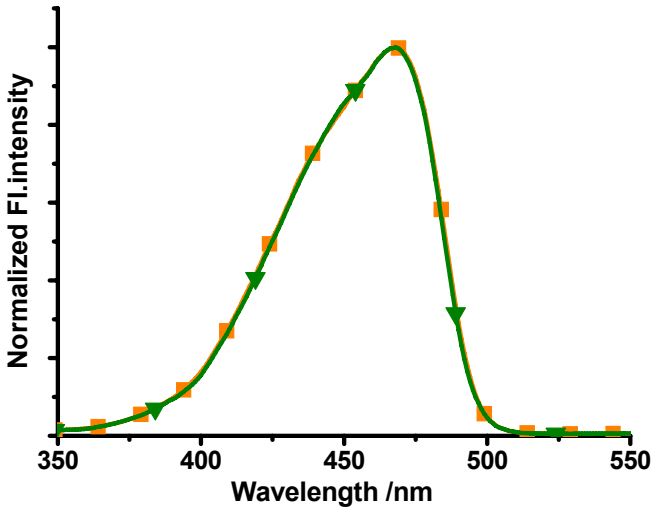


Figure S7. The normalized excitation spectra of the standard compound **2** (■) and the isolated product of probe **1** + benzenethiol (▼). The excitation spectra were recorded at 494 nm.

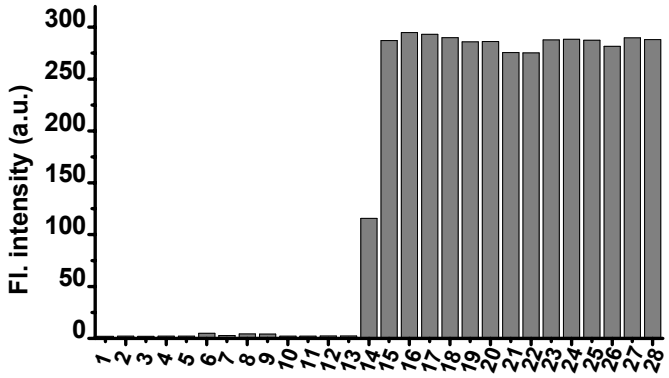


Figure S8. Fluorescence response of probe **1** (1 μM) to the various species (5 equiv.) in the neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 45% DMF as a co-solvent) for 30 min. 1: blank; 2: Gly; 3: Lys; 4: Arg; 5: Leu; 6: GSH; 7: Hcy; 8: Cys; 9: 2-mercaptoethanol; 10: glucose; 11: vitamin C; 12: phenol; 13: aniline;14: 4-mercaptobenzoic acid; 15: 4-bromobenzenethiol; 16: 4-chlorobenzenethiol; 17: 4-aminobenzenethiol; 18: benzenethiol; 19: Gly + benzenethiol; 20: Lys + benzenethiol; 21: GSH + benzenethiol; 22: Hcy + benzenethiol; 23: 2-mercaptoethanol + benzenethiol; 24: Cys + benzenethiol; 25: glucose + benzenethiol; 26: vitamin C + benzenethiol; 27: phenol + benzenethiol; 28: aniline + benzenethiol.

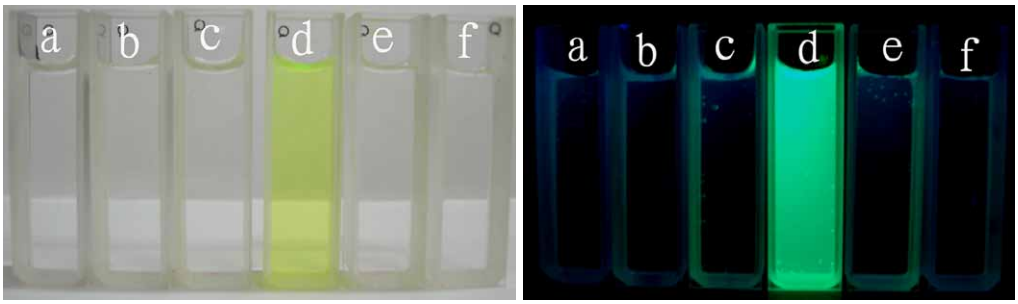


Figure S9. Color changes of probe **1** (5 μM) in the presence of 5 equiv. of the various species in the neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 45% DMF as a co-solvent) for 10 min. (a) blank; (b) Gly; (c) GSH; (d) benzenethiol; (e) aniline; and (f) phenol. Left: visible color; Right: visual fluorescence color on excitation at 365 nm using a handheld UV lamp.

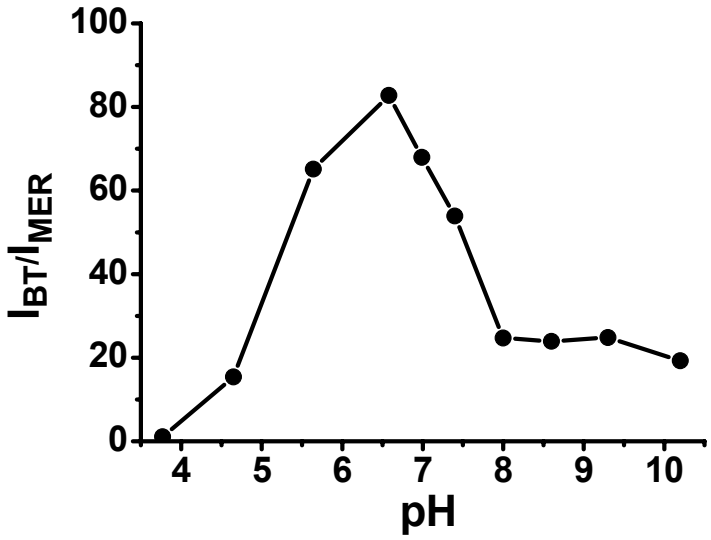


Figure S10. Fluorescence emission ratios (I_{BT}/I_{MER}) as a function of pH values. Probe **1** (1 μM) was treated with benzenethiol (5 equiv.) or 2-mercaptoethanol (5 equiv.) at various pH values, and the fluorescence intensity ratios (I_{BT}/I_{MER}) at 494 nm were determined. Excitation wavelength was 461 nm. BT= benzenethiol; MER = 2-mercaptoethanol.

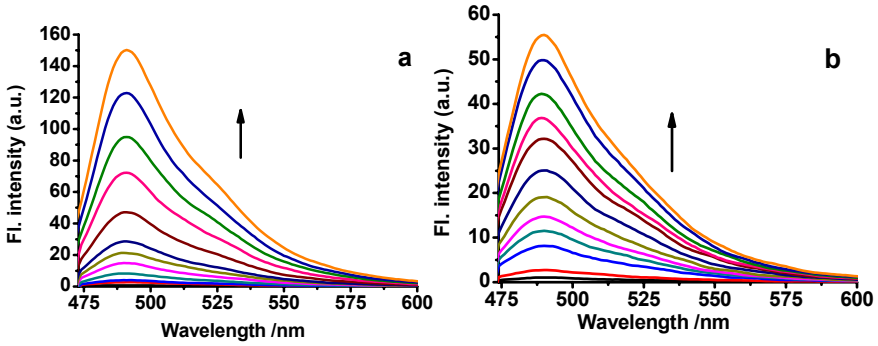


Figure S11. (a) Fluorescence spectral changes of probe **1** (1 μM) upon addition of increasing concentrations (0 - 50 equiv.) of benzenethiol (λ_{ex} = 461 nm) for 45 min in the neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 20% DMF as a co-solvent); (b) Fluorescence spectral changes of probe **1** (1 μM) upon addition of increasing concentrations (0 - 125 equiv.) of benzenethiol (λ_{ex} = 461 nm) for 60 min in the neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 10% DMF as a co-solvent).

Table S1. Determination of benzenethiol concentrations in water samples.

Sample	Benzenethiol spiked (mol L^{-1})	Benzenethiol recovered (mol L^{-1}) [a]	Recovery (%)
YueLu spring 1	0	Not detected	—
YueLu spring 2	5.00×10^{-9}	$(5.27\pm0.120)\times 10^{-9}$	105.4
YueLu spring 3	1.00×10^{-8}	$(1.01\pm0.024)\times 10^{-8}$	101.0
YueLu spring 4	1.00×10^{-7}	$(1.03\pm0.004) \times 10^{-7}$	103.0
YueLu spring 5	1.00×10^{-6}	$(1.00\pm0.013) \times 10^{-6}$	100.0
Xiang river 1	0	Not detected	—
Xiang river 2	5.00×10^{-9}	$(4.87\pm0.043)\times 10^{-9}$	97.4
Xiang river 3	1.00×10^{-8}	$(0.97\pm0.013)\times 10^{-8}$	97.0
Xiang river 4	1.00×10^{-7}	$(1.00\pm0.020) \times 10^{-7}$	100.0
Xiang river 5	1.00×10^{-6}	$(1.00\pm0.032) \times 10^{-6}$	100.0

[a] Relative standard deviations were calculated on the basis of three measurements.

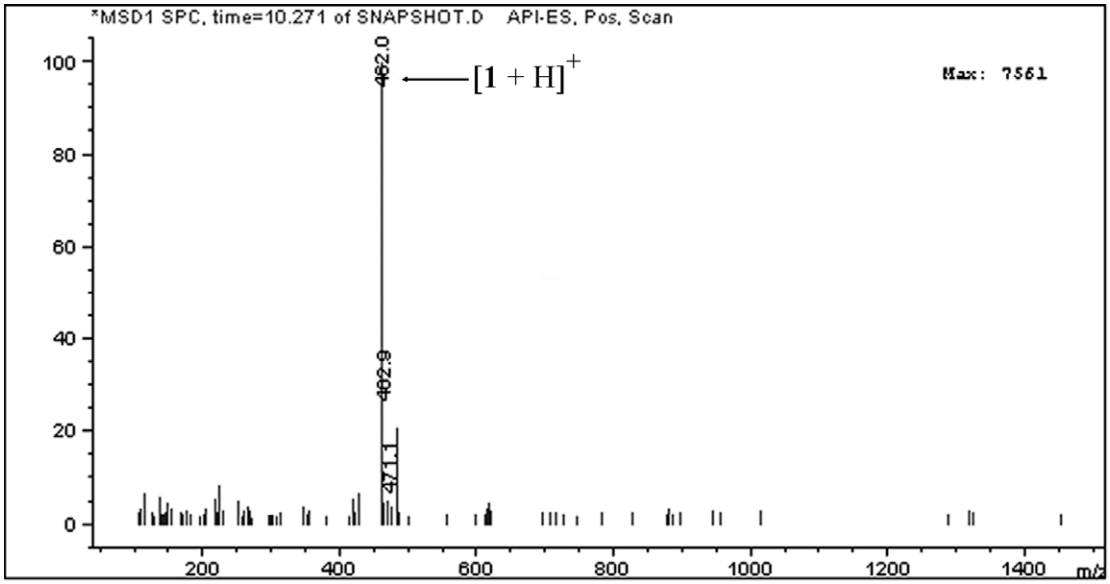


Figure S12. The mass spectrum of probe **1**.

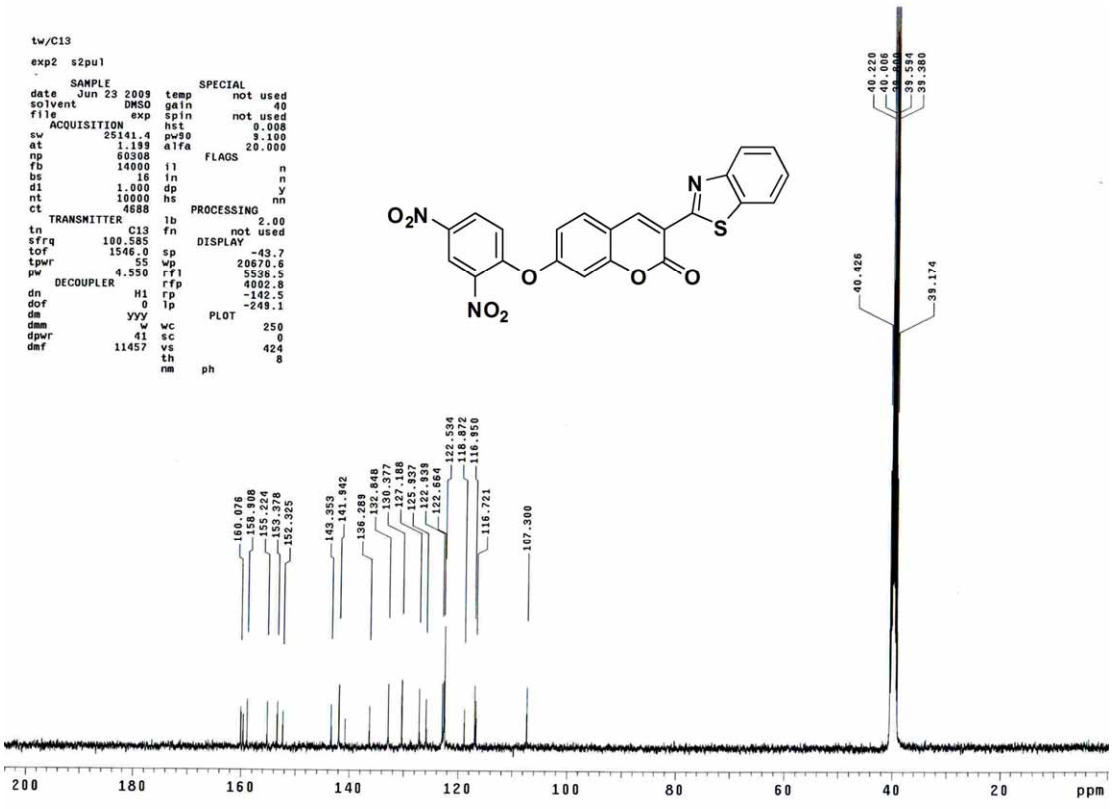


Figure S13. The ^{13}C NMR spectrum of probe **1.**

References:

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2 (a) I. B. Berlman, *Handbook of fluorescence spectra of aromatic molecules*, Academic Press, New York, 1971. (b) A. Ajayaghosh, P. Carol, S. Sreejith, *J. Am. Chem. Soc.* 2005, **127**, 14962.

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5 (a) M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland, *Anal. Chem.* 1996, **68**, 1414; (b) A. Caballero, R. Martınez, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tarraga, P. Molina, J. Veciana, *J. Am. Chem. Soc.* 2005, **127**, 15666. (c) W. Lin, L. Yuan, Z. Cao, Y. Feng, L. Long, *Chem. Eur. J.* 2009, **15**, 5096.