Supporting Information

A new fluorescent probe for gasotransmitter H₂S: high sensitivity, excellent selectivity, and significant fluorescence *off-on* response

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1. General information and methods. All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Deionized water was used throughout all experiments. All reactions were magnetically stirred and monitored by thin layer chromatography (TLC). Column chromatography was conducted over silica gel (mesh 200–300). Fluorescence spectra were taken on at room temperature on a Hitachi Fluorescence Spectrophotometer F-7000 with the excitation and emission slit widths at 10.0 and 10.0 nm respectively. Absorption spectra were recorded on Cary 4000 UV-vis Spectrophotometer. The ¹H NMR and ¹³C NMR spectra were recorded at 600 and 150 MHz, respectively. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. Fluorescence imaging was performed with a DeltaVision Microscope.

2. Synthesis and Characterization of Compounds

2.1 Synthesis of ABT



A mixture of 2-aminobenzenethiol (1.88 g, 15 mmol) and 2-aminobenzoic acid (2.06 g, 15 mmol) in PPA (30 mL) was heated slowly to 240 °C for 6 h. After completion of the reaction, the mixture was cooled to 100 °C and poured into a mixture of ice and water (300 mL) to afford abundant of precipitate, then adjusted to alkaline (pH 9-10) with 50 % aqueous NaOH. The precipitate was filtered off, washed with water to give the crude product, which was purified by silica gel column chromatography (CH₂Cl₂ : PE = 1 : 3) to give a white solid (68%). ¹H NMR (CDCl₃, 600 MHz) δ (ppm) : 7.96 (d, 1H, *J* = 8.10 Hz), 7.85 (d, 1H, *J* = 7.92 Hz), 7.69 (m, 1H), 7.43 (t, 1H, *J* = 7.68 Hz), 7.33 (t, 1H, *J* = 7.56 Hz), 7.21 (m, 1H), 6.77 (d, 1H, *J* = 8.16 Hz), 6.73 (t, 1H, *J* = 7.50 Hz), 6.39 (s, 2H). ¹³C NMR (CDCl₃, 150 MHz) δ (ppm) : 172.1, 156.6, 149.6, 136.1, 134.4, 133.2, 128.9, 127.7, 125.3, 124.1, 119.8, 119.7, 118.2. HRMS: calcd for [M+H]⁺ 227.0637, found 227.0641.

2.2 Synthesis of Compound 1



To a mixture of concentrated hydrochloric acid (4 mL), concentrated sulphuric acid (5 mL) and water (10 mL) cooled at 0 °C was added ABT (1.1 g, 5 mmol), then sodium nitrite (0.36 g, 5.25 mmol) in water (10 mL) was added dropwise, stirred for 15 min to afford a clear solution. Sodium azide (0.5 g, 7.5 mmol) in saturated aqueous sodium acetate (25 mL) was added dropwise to the above solution. The precipitate was filtered off, washed with water to give the crude product, which was purified by silica gel column chromatography (CH₂Cl₂ : PE = 1 : 3) to give a white solid (89 %). ¹H NMR (CDCl₃, 600 MHz) δ (ppm) : 8.46 (m, 1H), 8.11 (d, 1H, *J* = 8.10 Hz), 7.94 (d, 1H, *J* = 8.04 Hz), 7.51 (m, 2H), 7.40 (t, 1H, *J* = 7.11 Hz), 7.30 (m, 2H). ¹³C NMR (CDCl₃, 150 MHz) δ (ppm) : 165.5, 155.2, 140.6, 139.2, 134.4, 133.4, 129.0, 128.0,

128.0, 127.9, 126.0, 124.2, 121.9. HRMS: calcd for [M+H]⁺ 253.0542, found 253.0542.

3. Preparation of the test solution

All solutions of the anions were prepared from their sodium salts in deionized water. All solutions of the cations were prepared from their chloride salts in deionized water. Superoxide solution was prepared by adding KO₂ (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min. Hydroxyl radical ('OH) was generated through the Fenton reaction of Fe(ClO₄)₂ and H₂O₂. Singlet oxygen (¹O₂) was generated by the reaction of NaClO with H₂O₂. NO was generated from DEA/NO (NO donor): DEA/NO was dissolved in 0.01 M NaOH as a stock solution (500 mM), stored at 0 °C, and prepared daily. To initiate the release of NO, 0.4 µL of the stock alkaline solution of DEA/NO was dissolved in 2 mL of 10 mM PBS buffer (pH 7.4) to give a 0.1 mM final concentration. The stock solution of probe **1** (2 mM) was prepared in CH₃CN, then diluted to 10 µM for testing with the solution of PBS (10 mM, pH = 7.4, containing 1 mM CTAB). The stock solution of NaHS (20 mM) was prepared in deionized water, which was freshly prepared each time before use.

4. Quantum Yields.

Fluorescence quantum yields of **1** and **ABT** were determined in PBS buffer (10 mM, pH 7.4, containing 1 mM CTAB) with quinine sulfate ($\Phi = 0.58$, in 0.1 M H₂SO₄) as a reference. **ABT** was obtained in the experiment by addition of 10 equiv. of NaSH to the solution of probe **1**. The quantum yields were calculated using Eq.1:

$$\Phi_{\rm u} = \left[(A_{\rm s}FA_{\rm u}\eta^2) / (A_{\rm u}FA_{\rm s}\eta_0^2) \right] \Phi s. \qquad \text{Eq.1}$$

Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, FA_s and FA_u are the corresponding integrated fluorescence intensity, and η and η_0 are the solvent refractive indexes of sample and reference, respectively. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05.

Quantum yield of **1**: $\Phi = 0.0064$ Quantum yield of **ABT**: $\Phi = 0.4138$

5. Cell culture and fluorescence imaging: The B16 cell line was provided by Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education (China). Cells were grown in RPMI 1640 medium supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37 °C in humidified environment of 5% CO2. Cells were plated on 6-well plate at 5×10^6 cells per well and allowed to adhere for 12 hours. Fluorescence imaging was performed with a Olympus FluoView FV1000 confocal microscope. Before the experiments, cells were washed with PBS 3 times. Then, the cells were incubated with 1 (10 µM) in the presence of CTAB (1 mM), or pretreated with NaSH (100 µM, 30 min), or Cys (100 µM, 30 min), or Cys (100 µM, 30 min) and then 2 µL (1 µg/mL) PMA (30 min), in RPMI 1640 medium for 30 min at 37 °C. After each treatment, the cells were washed with PBS 3 times. Emission was collected at 420–520 nm for blue channel (excited at 405 nm).

6. Supplemental spectra



Fig. S1 Absorption intensities at 308 nm *vs* the varied concentrations of **1** in pure PBS buffer (10 mM, pH 7.4) at 25 °C.



Fig. S2 HRMS chart of 1+NaHS.



Fig. S3 Changes in fluorescence intensity of 1 (10 μ M) in PBS buffer (10 mM, containing 1 mM CTAB) measured with and without NaHS (100 μ M) as a function of pH. The fluorescence intensities of 1 upon treated with NaSH were obtained after 10 min at 25 °C. $\lambda_{ex} = 375$ nm, $\lambda_{em} = 450$ nm. Slits: 10/10 nm.



7. ¹H NMR, ¹³C NMR and HRMS chart of compounds 1 and ABT

Fig. S4 ¹H NMR chart of **1** (CDCl₃, 600 MHz).



Fig. S5 ¹³C NMR chart of 1 (CDCl₃, 150 MHz).



Fig. S6 HRMS chart of 1.



Fig. S7 ¹H NMR chart of ABT (CDCl₃, 600 MHz).



Fig. S8 ¹³C NMR chart of ABT (CDCl₃, 150 MHz).