Supporting Information

A new phenylsulfonamide-based Golgi-targeting fluorescent probe for H_2S and its bioimaging applications in living cells and zebrafish

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1. Materials and instruments

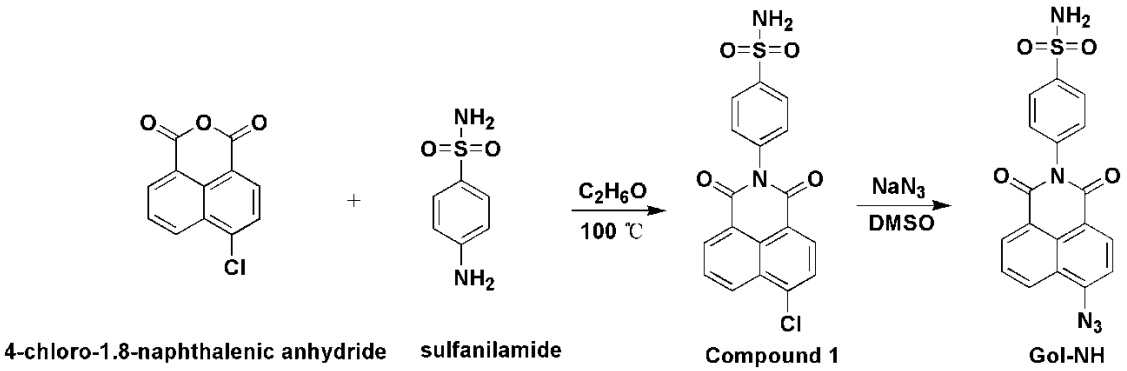
Except for special labels, chemical reagents were obtained from commercial vendor and employed without further purification. High resolution mass spectra (HRMS) were obtained by LC-MS2010A instrument. ^{1}H and ^{13}C NMR data were obtained by Bruker AV-400 NMR spectrometer. Absorption spectra were obtained by UV-3101PC spectrophotometer. Fluorescence spectra were obtained by Horiba FluoroMax-4 spectrophotometer (λ_{ex}: 440 nm, Slit widths: W_{ex} = W_{em} = 4 nm). Fluorescence imaging of H_2S in live cells and zebrafish were carried out on an Olympus FV1000-IX81 confocal fluorescence microscope. Except for special instructions, all optical measurements in this work were carried out 30 minutes after the substance was added in PBS solution (10 mM, pH = 7.4).

2. Synthesis of compound 1

4-chloro-1.8-naphthalenic anhydride (465 mg, 2 mmol) and sulfanilamide (344 mg, 2 mmol) were dissolved in ethanol (15 mL). The mixture was heated and stirred at 100 ℃ for 12 h, and then subjected to rotary distillation to obtain pale yellow crude product which was purified by chromatography to give a pure compound **1** (577 mg, 75%).

Compound **1** (0.35 g, 1 mmol), NaN_3 (0.19 g, 3 mmol) were dissolved in DMSO (7 mL) and stirred at 100 ℃ for 2 h. The product was extracted by water and dichloromethane, and then passed through the chromatographic column to obtain the light-yellow pure product probe **Gol-NH** (0.25 g, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) (ppm): 7.528(s, 2H), 7.621(d, J = 8.8 Hz, 2H), 7.810(d, J = 8.0 Hz, 1H), 7.917(t, J = 8.0 Hz, 1H), 7.974(d, J = 8.4 Hz, 2H), 8.508(d, J = 8.0 Hz, 2H), 8.559(d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) (ppm): 116.42, 118.91, 122.98, 124.10, 126.86, 127.79, 129.12, 129.24, 130.37, 132.07, 132.17, 139.46, 143.60,

144.38, 163.38, 163.85. HRMS (ESI): Calcd for $C_{18}H_{12}N_5O_4S$ $[M+H]^+$ 394.0605; Found, 394.0605.



Scheme S1. The synthesis of compound **1** and probe **GoI-NH**.

3. HRMS data for probe GoI-NH and its reaction products with H_2S

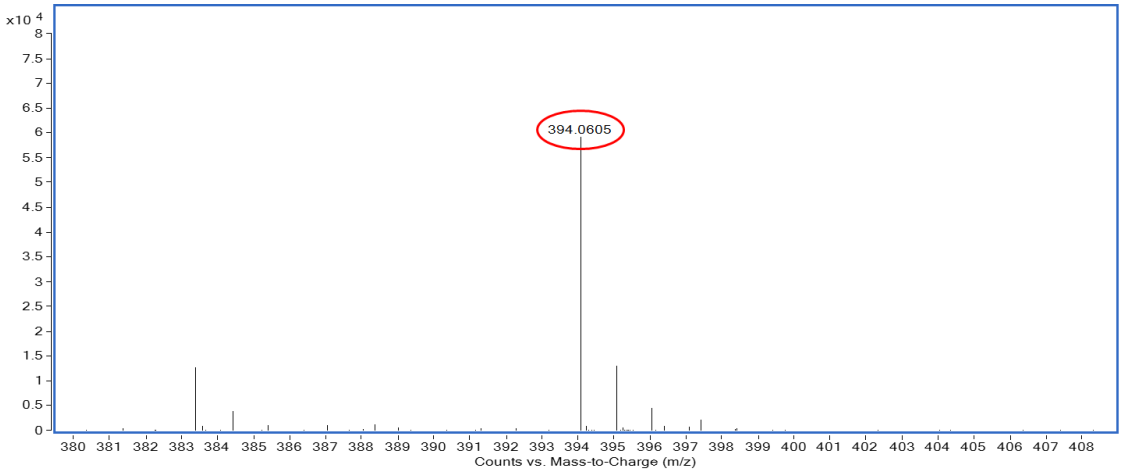


Figure S1. HRMS of probe **GoI-NH**.

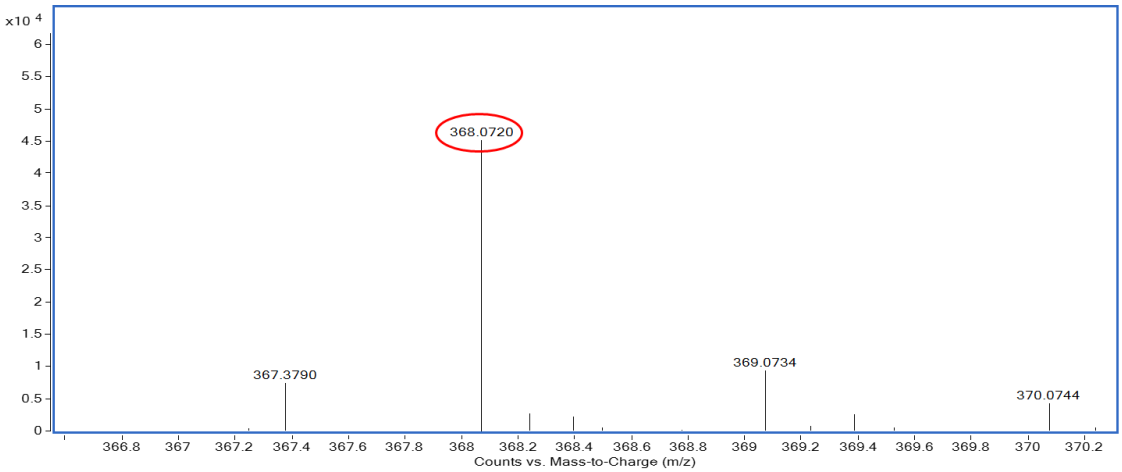


Figure S2. HRMS of the reaction products of probe **GoI-NH** and H_2S.

4. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence spectra of free probe **Gol-NH** were measured by five times and its standard deviation was obtained. To gain the slope, the fluorescence intensities at 550 nm were plotted as the increasing concentrations of the corresponding H_2S. So, the detection limit was calculated with the following equation (1):

$$\text{Detection limit} = 3\sigma/k \qquad (1)$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of H_2S.

5. Selectivity study of Gol-NH

We explored the specific detection of probe **Gol-NH** for H_2S. Compared with other analytes, probe **Gol-NH** has higher selectivity for H_2S. In the probe solution, only the addition of H_2S produced significant fluorescence enhancement at 550 nm. The addition of other substances had little effect on the probe solution (Fig. S3). These data demonstrated that probe **Gol-NH** could selectively detect H_2S.

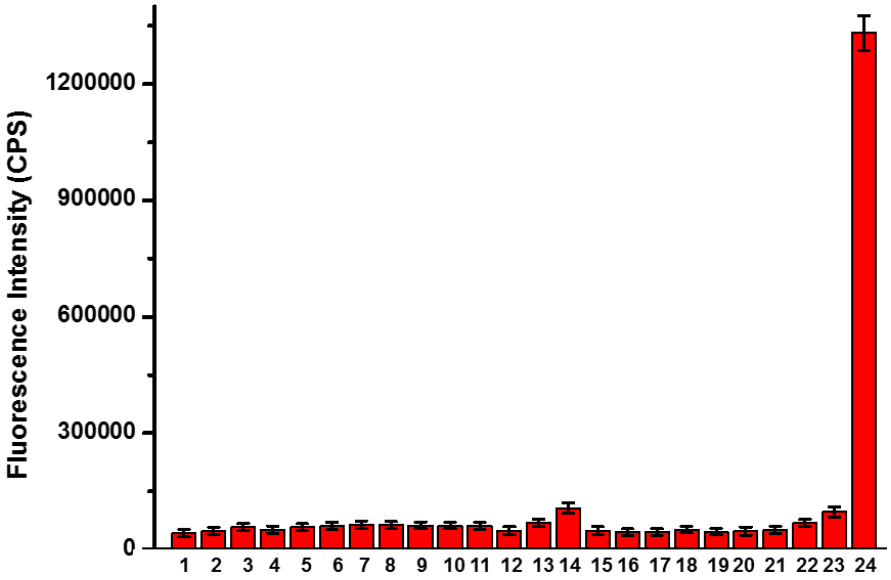


Figure S3. The spectral changes of probe **Gol-NH** (5 μM) were observed in the

presence of (1) Blank (2) K^+, (3) Ca^{2+}, (4) Na^+, (5) F^-, (6) Cl^-, (7) Br^-, (8) ClO^-, (9) CO_3^{2-}, (10) HCO_3^-, (11) NO_3^-, (12) SO_3^{2-}, (13) $S_2O_4^{2-}$, (14) HSO_3^-, (15) H_2O_2, (16) GSSG, (17) Ala, (18) Lys, (19) His, (20) Glu, (21) Cys, (22) Hcy, (23) GSH, (24) H_2S. Concentration: 1 mM for (2-23), 100 μM for H_2S. Spectra were collected at 550 nm 30 minutes after adding the substance. Data represent the mean ± S.D. of three independent experiments.

6. Kinetic studies of probe Gol-NH

Then, we tested the response time of probe **Gol-NH** to H_2S (Fig. S4). When H_2S were added into the system of probe **Gol-NH**, the fluorescent signal at 550 nm increased steadily, it takes about 25 minutes to reach the peak, indicating that the probe can quickly detect H_2S and has potential for biological imaging applications.

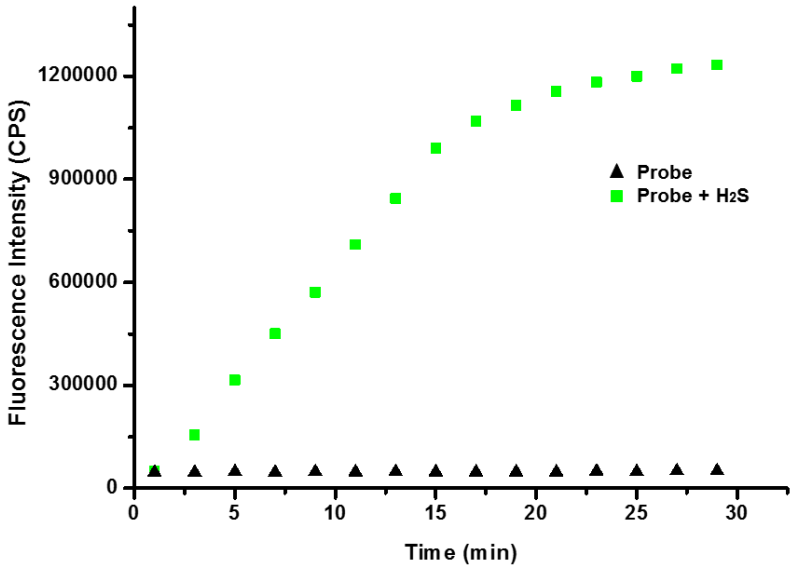


Figure S4. Fluorescence intensity changes with time at 550 nm after H_2S (0 μM / 100 μM) was added into the probe **Gol-NH** solution.

7. Live subject statement

All experiments were performed in strict compliance with the National Institute of Health (NIH) guidelines for the Care and Use of Laboratory Animals and the regulations of Qilu University of Technology on the ethical use of animals. All experiments were approved by the faculty Ethical Committee of the Biology Institute of the Shandong Academy of Sciences. All efforts were made to minimize the number of animals used and their suffering. The sources of biological samples in our experiments were all from Shanghai Institute of Biochemistry and Cell Biology, China Academy of Sciences (Shanghai, China).

8. Cytotoxicity assays

The cell viability of Hela cells, treated with probe **Gol-NH**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, Hela cells, seeded at a density of 1×10^6 cells mL^{-1} on a 96-well plate, were maintained at 37 ℃ in a 5% CO_2 / 95% air incubator for 12 h. Then the live Hela cells were incubated with various concentrations (0, 2, 5, 10, and 20 μM) of probe **Gol-NH** suspended in culture medium for 24 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 450 nm was measured.

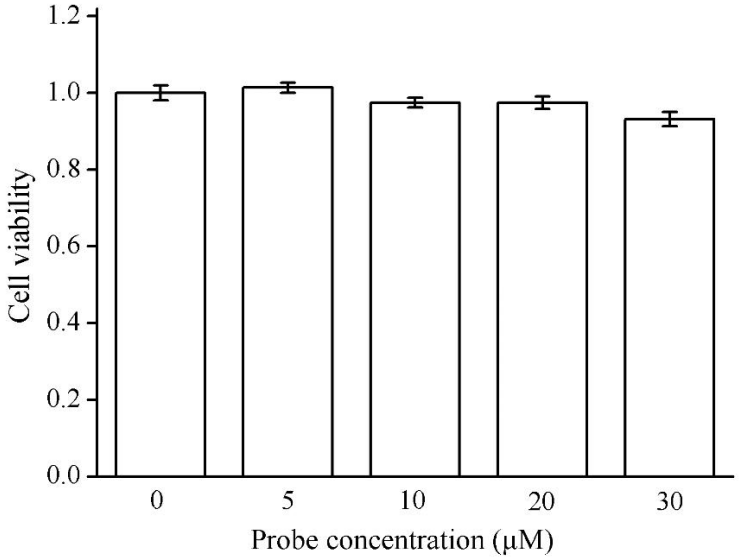


Figure S5. Cytotoxicity assays of probe **Gol-NH** at different concentrations for HeLa cells.

9. Imaging studies of live cells

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and incubated under an atmosphere containing 5% CO_2 at 37 ℃ humidified air for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin-streptomycin. When they grew and covered 80-90% of coverslips, they were treated by the specific probe **Gol-NH** (5 μM) (or H_2S, 200 μM; Cys, 0.5 mM; $ZnCl_2$, 1 mM; BODIPY TR Ceramide, 2 μg/mL; λ_{ex}: 633 nm; λ_{em}: 640-740 nm. MitoTracker Red CMXRos, (0.5 μM); λ_{ex}: 578 nm; λ_{em}: 590-640 nm. LysoTracker Red DND-99, (0.5 μM); λ_{ex}: 559 nm; λ_{em}: 585-620 nm. ER-Tracker Red, (0.5 μM); monensin, 10 μM); λ_{ex}: 594 nm; λ_{em}: 600-670 nm. AOAA (0.5 mM), PPG (0.5 mM) After 30 min incubation (monensin nigericin, brefeldin A, incubated HeLa cells for 4 h), probe **Gol-NH** (5 μM) was used as a bioimaging reagent to incubate live cells for 30 min, the culture medium was then removed and rinsed with phosphate buffered saline for three times before

fluorescence imaging was performed. In the fluorescence colocalization test, fluorescence images were acquired using a Fluoview FV1000 confocal microscopes (Olympus Corporation, Tokyo, Japan) under a 100× oil-immersion objective lens (N.A.1.40).

10. Imaging studies of zebrafish

Healthy male and female zebrafish (AB stain) were maintained in different tanks with a 14 h light / 10 h dark cycle at 28 ℃. Then, sexually mature zebrafish were selected to induce spawning in tanks and the zebrafish eggs were obtained by giving light stimulation in the morning. After sterilizing and cleaning, the fertilized eggs were added to zebrafish embryo culture water (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) and cultured in illumination incubator at 28 ℃. The 3-day-old zebrafish (or 5-day-old zebrafish) were treated by the specific probe **Gol-NH** (5 μM) (or H_2S, 200 μM; Cys, 1 mM; $ZnCl_2$, 1 mM; monensin, 10 μM). After 30 min incubation (monensin incubated 5-day-old zebrafish for 18 h), probe **Gol-NH** (5 μM) was used as a bioimaging reagent to incubate zebrafish for 30 min, the culture medium was used to clean and remove the residual probe, and then confocal fluorescence microscopy was used to observe.