Electronic Supplementary Information for

A colorimetric and ratiometric fluorescent probe for imaging of endogenous hydrogen sulphide in living cells and sulphide determination in mouse hippocampus

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General Information

Thin layer chromatography was performed on silica gel 60 F254 plates (250 μm) and column chromatography was conducted over silica gel (300-400 mesh). Visualisation of the developed chromatogram was accomplished by a UV lamp. Nuclear magnetic resonance (NMR) spectra were acquired on Bruker DRX-500/400 operated at 125/100 MHz for 1H NMR and 13C NMR, respectively, residual protio solvent signals serving as internal criteria for calibration purposes. Data for 1H NMR are reported as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), integration, coupling constant (Hz). High-Resolution Mass was performed by Mass Spectrometry. UV-Vis absorption spectra were recorded on a Shimadzu PharmaSpec UV-2401PC UV-Visible spectrophotometer. All fluorescence measurements were recorded on a Hitachi F4600 Fluorescence Spectrophotometer. The pH measurements were performed on a Mettler-Toledo Delta 320 pH meter. All fluorescence imaging experiments were conducted on a FV1000 confocal laser scanning microscope (Olympus, Japan).

Synthesis and Characterisation of compounds

Synthesis of 1 (4-bromo-N-butyl-1,8-naphthalimide). Compound 1 was synthesized according to the method reported by Zhu et al.1 A solution of 4-bromo-1,8-naphthalic anhydride (1.50 g, 5.4 mmol) and n-butylamine (3.55 g, 48.6 mmol) in acetic acid (30 mL) was heated to reflux under N2 for 6 h. The solution was poured into a beaker containing distilled water, forming a precipitate, which was filtered and rinsed with water. The crude product (yellow powder) was collected and recrystallized with ethanol to afford compound 1 (white crystals).

Yield: 1.4 g, 78.2 %. m.p. 101-102 °C. 1H NMR (500 MHz, CDCl3): δ 8.61 (d, J = 6.5 Hz, 1 H, ArH), 8.50 (d, J = 8.5 Hz, 1 H, ArH), 8.36 (d, J = 8.0 Hz, 1 H, ArH), 7.99 (d, J = 7.5 Hz, 1 H, ArH), 7.80 (t, J = 7.0 Hz, J = 8.5 Hz, 1 H, ArH), 4.16 (t, J = 7.5 Hz, 2 H, CH2N), 1.69-1.74 (m, 2 H, NCH2CH2-), 1.41-1.48 (m, 2 H, -CH2CH3), 0.98 (t, J = 7.5 Hz, J = 7.0 Hz, 3 H, -CH3).

13C NMR (125 MHz, CDCl3): δ 163.5, 133.1, 131.9, 131.1, 131.0, 130.5, 130.1, 128.9, 127.9, 123.2, 122.3, 104.0, 30.2, 20.4, 13.8; HRMS (ESI+): (M+H)+ calcd. for C16H15BrNO2, 332.0286; found, 332.0239.

Synthesis of 2 (4-azido-N-butyl-1,8-naphthalimide). Compound 2 was synthesized according to the previous method.2 A solution of 1 (0.5 g, 1.5 mmol) and sodium azide (0.97 g, 15.0 mmol) in DMF (10 mL) were stirred for 4 h at 40 °C. The solution was poured into a beaker containing water, forming a precipitate, which was filtered and rinsed with water. The crude product (yellow powder) was collected and recrystallized with ethanol to afford compound 2 (white crystals).

Yield: 0.3 g, 68.2 %. m.p. 126.2-127.6 °C. TLC (silica, hexane: EtOAc, 10:1 v/v): Rf 0.5; 1H NMR (500 MHz, CDCl3): δ 8.64 (d, J = 7.5 Hz, 1 H, ArH), 8.58 (d, J = 8.0 Hz, 1 H, ArH), 8.44 (d, J = 8.5 Hz, 1 H, ArH), 7.74 (t, J = 7.5 Hz, J = 7.5 Hz, 1 H, ArH), 7.47 (d, J = 7.5 Hz, 1 H, ArH), 4.18 (t, J = 7.0 Hz, J = 8.0 Hz, 2 H, CH2N), 1.69-1.75 (m, 2 H, NCH2CH2-), 0.98 (t, J = 7.5 Hz, J = 7.0 Hz, 3 H, -CH3).

13C NMR (125 MHz, CDCl3): δ 163.9, 163.5, 143.3, 131.6, 129.2, 128.6, 126.8, 124.4, 122.8, 119.1, 114.6, 40.3, 30.2, 20.4, 13.8; HRMS (ESI+): (M+H)+ calcd. for C16H15N4O2, 295.1195; found, 295.1193.

Synthesis of 3 (4-amino-N-butyl-1,8-naphthalimide). Compound 2 (0.15 g, 0.51 mmol) was dissolved in acetonitrile (25 mL). With the bubbling of H2S, the mixture was stirred for 3 h at 30 °C. The mixture for reaction was dried by evaporation under reduced pressure and an orange powder was obtained. Yield: 0.9 g, 66.2 %. m.p. 262.2-276.6 °C. TLC (silica, hexane:EtOAc, 10:1 v/v): Rf 0.5; 1H NMR (500 MHz, CDCl3): δ 8.59 (d, J = 8.4 Hz, 1 H, ArH), 8.40 (d, J = 7.2 Hz, 1 H, ArH), 8.17 (d, J = 8.4 Hz, 1 H, ArH), 7.62 (t, J = 8.0 Hz, J = 7.6 Hz, 1 H, ArH), 7.40 (s, 2 H, ArH), 6.83 (d, J = 8.4 Hz, 1 H, ArH), 3.99 (t, J = 7.2 Hz, 2 H, CH2N), 1.52-1.60 (m, 2 H, CH2CH2N), 1.28-1.34 (m, 2 H, CH3, -CH3). 13C NMR (125 MHz, CDCl3): δ 164.1, 163.5, 152.9, 154.2, 137.1, 129.9, 129.6, 124.3, 122.1, 119.7, 108.5, 107.9, 39.3, 30.2, 20.2, 14.1; HRMS (ESI+): (M+H)+ calcd. for C16H17N2O2, 295.1190; found, 295.1193.
Evidence of mechanism detection

NAP-1 (44 mg, 0.1 mmol) was dissolved in CH$_3$CN (15 mL), followed by the addition of the solution of Na$_2$S-9H$_2$O (80 mg, 0.33 mmol) in PBS buffer (16.5 mL, 20 mM, pH = 7.4). The resultant mixture was stirred for 3 h at room temperature. Subsequently, EtOAc (3 x 10 mL) was added into the solution for extraction. The fluorescent product was thereafter purified by column chromatography, and the spectra obtained by $^1$H NMR and $^{13}$C NMR were consistent with those of compound 3, hence the confirmation of the fluorescent product as compound 3.

Quantum Yields

Quantum yields were determined using fluorescein as a standard according to a published method.$^3$ For NAP-1 and fluorescein, the absorbance spectra were measured within an absorbance range of 0.01 to 0.1. The quantum yield was calculated according to the equation:

$$\Phi_{\text{sample}} = \Phi_{\text{standard}} \left( \frac{\text{Grad}_{\text{sample}}}{\text{Grad}_{\text{standard}}} \right) \left( \frac{\eta^2_{\text{sample}}}{\eta^2_{\text{standard}}} \right)$$

where $\Phi$ is the quantum yield, $\Phi_{\text{fluorescein}} = 0.79$ in 0.1 M NaOH, Grad is the slope of the plot of absorbance versus integrated emission intensity, and $\eta$ is the refractive index of the solvent.

Preparation of the test solution

NAP-1 stock solution preparation: NAP-1 (4.4 mg, 0.01 mmol) was dissolved into CH$_3$CN (5 mL) to get 2.0 mM stock solution.

Cys (L-Cysteine) stock solution preparation: Cys (24.2 mg, 0.2 mmol) was dissolved into DI H$_2$O (10 mL) to get 20.0 mM stock solution, which was then diluted to 1.0 mM and 100 µM solution for general use.

Hcy (Homocysteine) stock solution preparation: Hcy (27.0 mg, 0.2 mmol) was dissolved into DI H$_2$O (10 mL) to get 20.0 mM stock solution, which was then diluted to 1.0 mM and 100 µM solution for general use.

GSH (Glutathione) stock solution preparation: GSH (61.5 mg, 0.2 mmol) was dissolved into DI H$_2$O (10 mL) to get 20.0 mM stock solution, which was then diluted to 1.0 mM and 100 µM solution for general use.

Na$_2$S stock solution preparation: 5 mg EDTA was dissolved in 10 mL DI H$_2$O in a 25 mL Schlenk tube. The solution was purged vigorously with nitrogen for 15 min. Then 48 mg sodium sulfide (Na$_2$S-9H$_2$O) was dissolved in the solution under nitrogen. The resulting solution was 20 mM Na$_2$S, which was then diluted to 1.0 mM-100 µM stock solution for general use.

Stock solutions of other biological analytes, including Ala, Glu, Trp, Met, Tyr, Leu, Val, Ser, Pro, Arg, Gly, Phe, His, Gln, Asn, Ile, Thr, KCl, CaCl$_2$, NaCl, MgCl$_2$, ZnSO$_4$, FeCl$_3$, NaH$_2$PO$_4$, H$_2$O$_2$, ·OCl', O$_2'$, ·OH, 'BuOOH, NO', NO, Na$_2$S$_2$O$_3$, Na$_2$S$_2$O$_5$, Na$_2$SO$_4$, Na$_2$S$_2$O$_4$, Na$_2$SO$_3$, KSCN', NADH, and Glucose, were prepared in DI H$_2$O. Superoxide radicals (O$_2^-$) were generated according to the previous reported method.$^5$ ·OH was generated by Fenton reaction between Fe$^{II}$(EDTA) and H$_2$O$_2$ quantitively.$^6$ NO is generated in form of 3-(Aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5, 50 µmol/ml).
**Fig. S1** (a) Fluorescence spectra of compound 3 in PBS buffer (20 mM, pH = 7.4, 5 % CH₃CN). Excitation: 415 nm, emission: 430 nm to 650 nm. (b) Absorption spectra of compound 3 in PBS buffer (20 mM, pH = 7.4, 5 % CH₃CN).

**Determination of the detection limits**

The detection limit was calculated based on the method reported in the previous literature. The fluorescence emission spectrum of NAP-1 without Na₂S was measured by 10 times and the standard deviation of blank measurement was obtained. Then the solution was treated with Na₂S of concentration from 0 to 100 μM. A linear regression curve was then achieved according to the intensity ratio $F_{541}/F_{474}$ in the range of Na₂S from 0 to 4 μM. The detection limit was calculated with the following equation: Detection limit = 3σ/k. Where σ is the standard deviation of blank measurements, k is the slope between the fluorescence intensity ratios versus Na₂S concentrations. The detection limit was 110 nM and 120 nM in PBS buffer and bovine serum, respectively.

**Fig. S2** The emission intensity ratios ($F_{541}/F_{474}$) of NAP-1 (10 μM) to various concentrations of Na₂S (0-4 μM) in PBS buffer (right) (20 mM, pH 7.4, 5 % CH₃CN) and bovine serum (left) (5 % CH₃CN) at 37 °C for 20 min.

**Fig. S3** Absorption intensity ratios changes of NAP-1 (10 μM) after incubation with different concentrations of Na₂S (0-300 μM) for 20 min in PBS buffer (20 mM, pH = 7.4, 5 % CH₃CN). Insert: The linear relationship between the absorption intensity ratios ($A_{430}/A_{368}$) and the concentrations of Na₂S (0 to 80 μM) in PBS buffer.
Fig. S4 (a) Fluorescence spectra of NAP-1 (10 μM) with Na₂S 100 μM in PBS buffer (20 mM, pH 7.4, 5 % CH₃CN) at 37°C for 0, 2, 5, 10, 12, 14, 16, 18, 20, 25 and 30 min. (b) Time profile of NAP-1 (10 μM) toward Na₂S (100 μM) in PBS buffer (20 mM, pH 7.4, 5 % CH₃CN) at 37°C for 0, 2, 5, 10, 12, 14, 16, 18, 20, 25 and 30 min.

Fig. S5 (a) *Pseudo* first-order kinetic plot of the reaction of NAP-1 (10 μM) with Na₂S (10 equiv.) in PBS buffer (20 mM, pH 7.4, 5 % CH₃CN). $k_{oobs} = 3.0 \times 10^{-3}$ s⁻¹. (b) Plots of $k_{oobs}$ vs Na₂S concentrations. Second-order rate constant, $k_2 = 5.0$ M⁻¹ s⁻¹.
Fig. S6 (a) Fluorescence spectra of NAP-1 (10 μM) with Na₂S (100 μM) in different pH buffer (20 mM, pH 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, 6.6, 7.0, 7.4, 7.8, 8.2, 8.6, and 9.0, 5 % CH₃CN) at 37 °C for 20 min. (b) The emission intensity ratios (F₅₄₁/F₄₇₄) of NAP-1 (10 μM) to Na₂S (100 μM) in different pH buffer at 37 °C for 20 min.

![Fluorescence spectra of NAP-1 with Na₂S in different pH buffers](image)

Fig. S7 Photosatbility of NAP-1 under visible light and UV light. The last column is the same solution with the addition of 100 μM Na₂S.

![Photosatbility of NAP-1](image)

Fig. S8 Fluorescence spectra of NAP-1 (10 μM) with Na₂S and various thiols in 20 mM PBS (pH 7.4, 5 % CH₃CN) at 37 °C for 20 min. 1) Na₂S (0 μM); 2) Cys (100 μM); 3) Cys (1 mM); 4) Hcy (100 μM); 5) Hcy (1 mM); 6) GSH (1 mM); 7) GSH (10 mM); 8) Na₂S (100 μM); 9) Na₂S (100 μM) + Cys (100 μM); 10) Na₂S (100 μM) + Cys (1 mM); 11) Na₂S (100 μM) + Hcy (100 μM); 12) Na₂S (100 μM) + Hcy (1 mM); 13) Na₂S (100 μM) + GSH (1 mM); 14) Na₂S (100 μM) + GSH (10 mM).

![Fluorescence spectra of NAP-1 with Na₂S and various thiols](image)
**Fig. S9** (a) Fluorescence responses of NAP-1 (10 μM) towards Na₂S (100 μM) and other amino acids (1 mM). (1) Blank, (2) Na₂S, (3) Ala, (4) Glu, (5) Trp, (6) Met, (7) Tyr, (8) Val, (9) Ser, (10) Pro, (11) Arg, (12) Gly, (13) Phe, (15) His, (16) Gln, (17) Asn, (18) Ile, (19) Thr. (b) Fluorescence spectra of NAP-1 (10 μM) with Na₂S (100 μM) and various amino acids (1 mM) in 20 mM PBS (pH 7.4, 5 % CH₃CN) at 37 °C for 20 min.

**Fig. S10** Fluorescence spectra of NAP-1 (10 μM) with Na₂S (100 μM), reactive oxygen species (1 mM), reactive nitrogen species (1 mM), sulfur-containing inorganic ions (1 mM), reducing agents and inorganic salts (1 mM) in 20 mM PBS (pH 7.4, 5 % CH₃CN) at 37 °C for 20 min. (1) Blank, (2) Na₂S, (3) H₂O₂, (4) OCl⁻, (5)O₂⁻, (6)·OH, (7) tBuOOH, (8) NO₂⁻, (9) NO, (10) S₂O₃²⁻, (11) S₂O₅²⁻, (12) SO₄²⁻, (13) S₂O₄²⁻, (14) SO₃²⁻, (15) SCN⁻, (16) S-nitroso glutathione, (17) NADH, (18) Glucose, (19) KCl, (20) CaCl₂, (21) NaCl, (22) MgCl₂, (23) ZnSO₄, (24) FeCl₃, (25) NaH₂PO₄.

**MTT assay**

Cell growth inhibitory effect of NAP-1 and compound 3 were measured using a colorimetric MTT assay kit (Sigma-Aldrich). MCF-7 cells were seeded in 96-well plates at a density of 50,000 cells/well and then maintained at 37 °C in a 5 % CO₂ incubator. The cells were incubated with different concentrations of NAP-1 and compound 3 for 24 h, respectively. Cells in culture medium without NAP-1 were used as control. After the incubation time, 20 μL of MTT dye (3-[4, 5-dimethylthiazol-2-yl]- 2, 5-diphenyl tetrazolium bromide, 5 mg/ml in phosphate buffered saline), was added to each well, and the plates were incubated for 4 h at 37 °C. Then, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan.
crystals. The plate was shaken for 10 min and the absorbance was measured at 570 nm on a microplate reader (ELX808IU, Bio-tek Instruments Inc, USA). Each sample was performed in triplicate, and the entire experiment was repeated three times. Calculation of IC_{50} values was done according to Huber and Koella. IC_{50} of NAP-1 and compound 3 is calculated to be of 101.2 ± 1.3 μM and 81.7 ± 1.1 μM, respectively. The cell viability of NAP-1 and compound 3 (10 μM) at 0, 6, 12, 18 and 24 h further demonstrated that the NAP-1 and 3 are of low toxicity to cultured MCF-7 cells.

**Fig. S11** (a) The inhibitory effect of NAP-1 on cell growth in MCF-7 cells treated for 24 h. (b) Cell viability of NAP-1 (10.0 μM) at different times in MCF-7 cell.

**Fig. S12** (a) The inhibitory effect of compound 3 on cell growth in MCF-7 cells treated for 24 h. (b) Cell viability of compound 3 (10.0 μM) at different times in MCF-7 cell.
Fig. S13 The corresponding bright images of Fig. 6.

Fig. S14 The corresponding bright images of Fig. 7.
Fig. S15 Determination of sulphide concentration in the fresh human plasma with NAP-1 probe (see the H₂S detection protocols). Sulphide concentrations in the test solution (dilution to 7%) were 0.31 μM, 0.37 μM, 0.33 μM, 0.38 μM, 0.30 μM and 0.30 μM, respectively. Accordingly, H₂S concentrations in human plasma were 0.45 μM, 0.53 μM, 0.47 μM, 0.55 μM, 0.43 μM and 0.43 μM, respectively. The average H₂S concentration was 0.48 ± 0.05 μM.

Determination of sulphide in fresh human plasma with SFP-3 probe

The experiment protocol was approved by the Ethics Committee of The Affiliated Hospital of Xuzhou Medical College, and all individuals were fully informed of the purpose of the study. Human plasma from healthy individuals was provided by The Affiliated Hospital of Xuzhou Medical College. To measure sulphide, human plasma was immediately deproteined by the addition of acetonitrile (7:1 v/v plasma/acetonitrile) and then centrifuged at 10,000 r/min for 20 min at 4 °C. The resultant supernatant was immediately transferred for analyses. The analytical procedures were performed in ice bath. All procedures in fluorescence measurements were conducted on a Hitachi F4600 Fluorescence Spectrophotometer.

Determination of sulphide concentration in spiked human plasma with Na₂S as the internal criterion (X, X+0.2, X+0.4, X+0.6, X+0.8 μM). Deproteined human plasma (80 μL) was added into Eppendorf tubes containing 10 μL PBS buffer (100 mM, pH 7.4). With Na₂S stock solution (10 μM) of 0, 2, 4, 6 and 8 μL spiked into the samples as the internal criteria, DI H₂O (10, 8, 6, 4 and 2 μL, respectively) was correspondingly introduced into the samples, followed by the addition of SFP-3 probe (1 μL, 1.0 mM), rendering the final concentration at 10 μM. Emission spectra (λ<sub>ex</sub> = 400 nm, emission at 520 nm) were determined at the end of the 30-minute incubation of mixture at 37 °C. Zero point was obtained by adding 1 μL, 100 mM (final concentration 1 mM) ZnCl₂ to scavenge H₂S in the sample. The data represent the average of three independent experiments.
Determination of sulphide concentration in the fresh human plasma with SFP-3 probe (see the H₂S detection protocols). Sulphide concentrations in the test solution (dilution to 7%) were 0.40 \( \mu \text{M} \), 0.42 \( \mu \text{M} \), 0.41 \( \mu \text{M} \), 0.39 \( \mu \text{M} \), 0.40 \( \mu \text{M} \) and 0.41 \( \mu \text{M} \), respectively. Accordingly, H₂S concentrations in human plasma 1, 2, 3, 4, 5 and 6 were 0.57 \( \mu \text{M} \), 0.60 \( \mu \text{M} \), 0.58 \( \mu \text{M} \), 0.55 \( \mu \text{M} \), 0.57 \( \mu \text{M} \) and 0.58 \( \mu \text{M} \), respectively. The average sulphide concentration was 0.58 ± 0.02 \( \mu \text{M} \).

**Determination of sulphide in mouse hippocampus with SFP-2 probe**

For the measurement of H₂S, mice were sacrificed, followed by immediate removal of hippocampus, homogenization with a 9 volumes (w/v) of ice-cold 100 mM PBS buffer (pH 7.4) and centrifugation at 10,000 g for 10 min at 4 °C. All the procedures were performed in ice bath, and the homogenate supernatants were immediately transferred to sulphide determination. All fluorescence measurements were recorded on a Hitachi F4600 Fluorescence Spectrophotometer (Tokyo, Japan). Protein concentrations of mouse hippocampus were determined using a Pierce BCA Protein Assay Kit.

Determination of sulphide concentration in spiked hippocampus homogenates using Na₂S as internal standard (X, X+0.4, X+0.8, X+1.2 and X+1.6 \( \mu \text{M} \)). 20 \( \mu \text{L} \) of 10 % homogenate supernatant (final concentration 2 %, v/v) was added into Eppendorf tubes containing 69 \( \mu \text{L} \) PBS buffer (100 mM, pH 7.4) and DI H₂O (10, 9.6, 9.2, 8.8 and 8.4 \( \mu \text{L} \), respectively). Then 0, 0.4, 0.8, 1.2, 1.6 \( \mu \text{L} \) Na₂S stock solution (100 \( \mu \text{M} \)) were spiked into the sample as internal standard, followed by the addition of 1 \( \mu \text{L} \) 1.0 mM SFP-2 probe (final concentration 10 \( \mu \text{M} \)). Emission spectra (\( \lambda_{\text{ex}} = 450 \text{ nm} \), emission at 512 nm) were collected after the mixture was incubated at 37 °C for 30 min. Zero point was obtained by the addition of 1 \( \mu \text{L} \), 100 mM (final concentration 1 mM) ZnCl₂ to trap H₂S...
in the sample. The $\text{H}_2\text{S}$ concentration in each sample was calculated by a calibration curve of $\text{Na}_2\text{S}$ and results were expressed as $\mu\text{mol g}^{-1}\text{ protein}$. The data represent the average of three independent experiments.

**Fig. S17** Determination of sulphide concentrations in mouse hippocampus with NAP-1 probe (see the $\text{H}_2\text{S}$ detection protocols). Sulphide concentrations in hippocampus homogenates (the test solution, 2 %, v/v) were 1.0 $\mu\text{M}$, 0.93 $\mu\text{M}$, 0.80 $\mu\text{M}$, 0.86 $\mu\text{M}$, 0.85 $\mu\text{M}$ and 1.2 $\mu\text{M}$, respectively. Total protein concentrations in hippocampus homogenates (1 %, w/v) were 0.3 g/L, 0.27 g/L, 0.25 g/L, 0.23 g/L, 0.29 g/L and 0.32 g/L, respectively. The sulphide concentrations in mouse
Hippocampus were expressed as $\mu$mol g$^{-1}$ protein. The sulphide concentrations in mouse hippocampus were 1.67$\mu$mol g$^{-1}$ protein, 1.72$\mu$mol g$^{-1}$ protein, 1.60$\mu$mol g$^{-1}$ protein, 1.87$\mu$mol g$^{-1}$ protein, 1.47$\mu$mol g$^{-1}$ protein and 1.88$\mu$mol g$^{-1}$ protein, respectively. The average sulphide concentration was 1.70 ± 0.16 $\mu$mol g$^{-1}$ protein.

Fig. S18 Determination of sulphide concentrations in mouse hippocampus with SFP-2 probe (see the H$_2$S detection protocols). Sulphide concentrations in hippocampus homogenates (the test solution, 2 %, v/v) were 1.10 $\mu$M, 0.85 $\mu$M, 1.10 $\mu$M, 0.90 $\mu$M, 1.20 $\mu$M and 0.96 $\mu$M, respectively. Total protein concentrations in hippocampus homogenates (1 %, w/v) were 0.27 g/L, 0.23 g/L, 0.29 g/L, 0.23 g/L, 0.28 g/L and 0.28 g/L, respectively. The sulphide concentrations in mouse
hippocampus were expressed as μmol g⁻¹ protein. The H₂S concentrations in mouse hippocampus were 2.04 μmol g⁻¹ protein, 1.85 μmol g⁻¹ protein, 1.90 μmol g⁻¹ protein, 1.92 μmol g⁻¹ protein, 2.14 μmol g⁻¹ protein and 1.71 μmol g⁻¹ protein, respectively. The average sulphide concentration was \(1.93 ± 0.15 \text{ μmol g}^{-1}\) protein.

**Table S1** Measurement of sulphide concentrations in mouse hippocampus (n = 6)

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Mean ± SEM

1.70 ± 0.06 0.93 ± 0.04 1.54 ± 0.05

a H₂S concentrations in hippocampus homogenates (the test solution, 2 %).

b Total protein concentrations in hippocampus homogenate (dilution to 1 % homogenate).

c H₂S concentrations in mouse hippocampus.

![Fig. S19 IL-1β mRNA levels in mouse hippocampus. Data presented as mean ± SEM, n = 3. \(^*P< 0.001\) vs control, \(^{**}P< 0.001\) vs model.](image)
Fig. S20  TNF-α mRNA levels in mouse hippocampus. Data presented as mean ± SEM, n = 3. #P< 0.001 vs control, ##P< 0.001 vs model.

References

Fig. S21 HR-MS identification of compound 1 (calculated for C\textsubscript{16}H\textsubscript{15}BrNO\textsubscript{2} (M+H)+, 332.0286; found, 332.0239).
Fig. S22 HR-MS identification of compound 2 (calculated for $\text{C}_{16}\text{H}_{15}\text{N}_{4}\text{O}_{2} \ (M+\text{H})^{+}$ 295.1195; found 295.1193).
Fig. S23 HR-MS identification of compound 3 (calculated for C_{16}H_{17}N_{2}O_{2} (M+H)^{+} 269.1290; found 269.1282).
**Fig. S24** IR identification of NAP-1.

**Fig. S25** HR-MS identification of NAP-1 probe (calculated for C_{24}H_{20}N_{5}O_{4} (M-H)^+ 442.1516; found 442.1523).
Fig. S26 $^1$H NMR spectra of the isolated fluorescent product of NAP-1 + Na$_2$S (b) in DMSO-$d_6$.

Fig. S27 $^{13}$C NMR spectra of the isolated fluorescent product of NAP-1 + Na$_2$S (b) in DMSO-$d_6$. 