Electronic Supplementary Information

A small-molecule probe for ratiometric photoacoustic imaging of hydrogen sulfide in living mice

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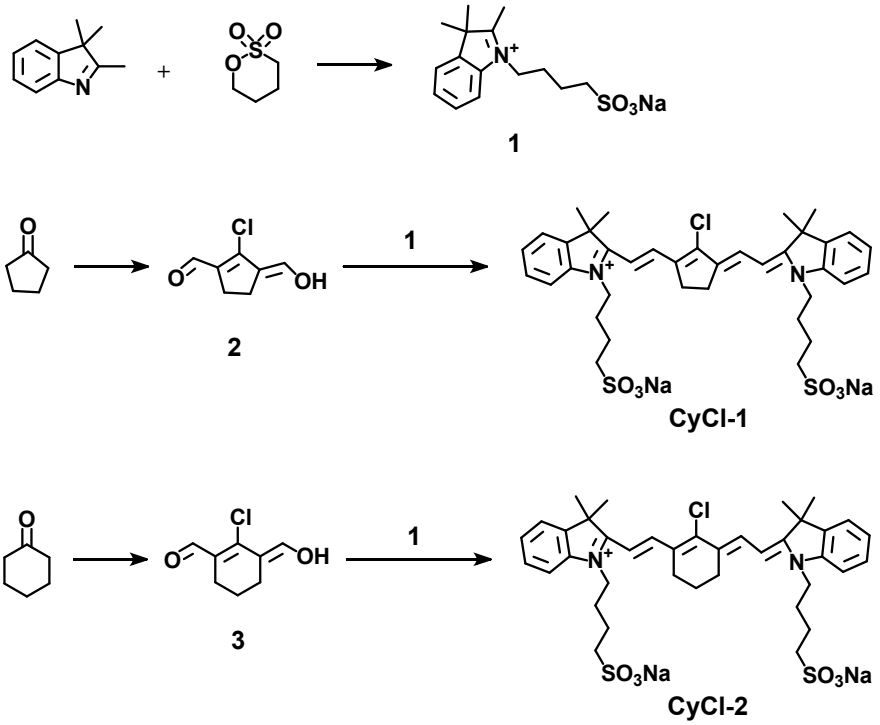
1. Experimental details

Materials: All chemical reagents and solvents were purchased from Sigma-Aldrich and used without further purification.

Characterization: NMR spectra were recorded on a Bruker Ultra Shield Plus 400 MHz spectrometer using tetramethylsilane (TMS) as the internal reference. Mass analysis was performed on a Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MASS, Bruker Autoflex) under the reflector mode

for data acquisition. The UV-visible absorption spectra were obtained on a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. In vivo fluorescence imaging for pharmacokinetics studies were performed with IVIS Lumina K (Perkin Elimer). All photoacoustic imaging results were obtained in the PAI tomography system (Nexus-128; Endra Inc., Ann Arbor, MI), accompanied with an adjustable nanosecond pulsed laser (20 Hz pulse repetition frequency, 5 ns pulses, 680−950 nm).

Synthetic route for target molecule



Scheme S1. Synthetic routes of CyCl-1 and CyCl-2.

Synthesize of N-(4-butanesulfonate)-2,3,3-trimethylindolinium (1).

2,3,3-trimethylindolenine (10 g, 63 mmol) was mixed with 1,4-Butanesultone (13 g, 95 mmol) in 30 mL 1,2-dichlorobenzene and heated for 12 h at 120 °C. The mixture

was cooled down to room temperature and precipitated in acetone. The obtained solid was further washed with acetone for several times to provide a purple solid of 1. The products were used for the next step without characterization.

Synthesize of 2-chloro-1-formyl-3-hydroxymethylenecyclopentene (2).

Forty milliliters of dimethylformamide previously mixed with 40 mL of methylene chloride was chilled in an ice bath, and then 37 mL of phosphorus oxychloride dissolved in 35 mL of methylene chloride was added dropwise with stirring, followed by 8.5 g of cyclopentene. The solution was refluxed for 3 h, cooled, poured into 200 g of ice. The mixture was allowed to stand over night, then filtered. The solide was further crystallized from acetone to obtained 7.8 g of orange crystal (yeild 49%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.59 (s, 1H, -CHO), 2.72 (s, 4H, -CH_2-).

Synthesize of CyCl-1.

A mixture of Compound 1 (0.688 g, 2 mmol), compound 2 (0.158 g, 1 mmol) and sodium acetate (0.16 g) were dissolved in acetic anhydride (10 mL). The mixture was stirred at 40 °C for 4 h. After cooled, the reaction mixture was added into 200 mL of diethyl ether. The precipitated was further purified by column chromatography (SiO_2, methanol/ dichloromethane 1:10) to give 0.61 g of glossy green solid products (yield 76%). ^{1}H NMR (400 MHz, methanol-D_4, δ): 7.96 (d, J = 14.1 Hz, 2H, Ar-H), 7.52 (d, J = 7.5 Hz, 2H, Ar-H), 7.48 – 7.34 (m, 4H, Ar-H), 7.28 (t, J = 7.5 Hz, 2H, Ar-H), 6.19 (d, J = 13.9 Hz, 2H, Ar-H), 4.22 (t, J = 7.1 Hz, 4H, -CH_2-), 3.03 (s, 4H, -CH_2-), 2.92 (t, J = 7.1 Hz, 2H, -CH_2-), 2.09 – 1.87 (m, 8H, -CH_2-), 1.74 (s, 12H, -CH_3). MALDI-TOF-MS m/z: 756.892 $[M]^+$.

Synthesize of 2-chloro-1-formyl-3-hydroxymethylenecyclopentene (3).

Compound 3 was synthesized similarly to compound 2, using cyclohexene as raw material (5.6 g, yield 67%). ^{1}H NMR (400 MHz, CDCl3, δ): 8. 90 (s, 1H, -CHO), 2.49 (t, J = 6.2 Hz, 4H, -CH2-), 1.78 – 1.68 (m, 2H, -CH2-) .

Synthesize of CyCl-2.

The synthesis of CyCl-2 was analogous to CyCl-1 (0.62 g, yield 81%). ^{1}H NMR (400 MHz, methanol-D4, δ): δ 8.44 (d, J = 14.2 Hz, 2H, Ar-H), 7.51 (d, J = 7.6 Hz, 2H, Ar-H), 7.45 – 7.34 (m, 4H, Ar-H), 7.28 (t, J = 7.3 Hz, 2H, Ar-H), 6.34 (d, J = 14.2 Hz, 2H, Ar-H), 4.22 (t, J = 7.2 Hz, 4H, -CH2-), 2.89 (t, J = 7.1 Hz, 4H, -CH2-), 2.76 (t, J = 6.1 Hz, 4H, -CH2-), 2.08 – 1.88 (m, 10H, -CH2-), 1.74 (s, 12H, -CH3). MALDI-TOF-MS m/z: 770.936 $[M]^{+}$.

Cell viability.

The cytotoxicity of CyCl-1 was assessed in Hella cells by MTT proliferation assay. Hella cells were seeded on 96-well plate (5 × 10^{4}/well) and incubated in DMEM containing 10% FBS and 1% penicillin/streptomycin at 37 ^{o}C in 5% CO_2 humidified atmosphere for 24 h. Different dose of CyCl-1 (0, 10, 20, 30, 40, 50 μg/mL) were added to the medium of cells and incubated at 37 °C for 24 h. The cells were treated with 10 μL MTT solution (0.5 mg/mL) at 37 °C for 4 h. Then the supernatant was removed and 200 μL of DMSO was added to dissolve the formazan. The absorbance of each well were recorded at 490 nm by a microplate reader. The cellular viability of each group was determined by defining the absorbance of the blank group to be 100% viability.

In vivo PA imaging.

All animal experiments were carried out in accordance with the guidelines of the Laboratory Animal Center of Jiangsu KeyGEN Biotech Corp., Ltd. for the care and use of laboratory mice and the experiments were approved by the Animal Ethics Committee of Simcere Bio Tech Corp., Ltd.

The mice for PA imaging eperiments were purchased from Jiangsu KeyGEN Biotech Corp., Ltd. and all animal experiments were carried out in accordance with the guidelines of the Laboratory Animal Center of Jiangsu KeyGEN Biotech Corp., Ltd.. For imaging of exogenous H_2S, NaHS (500 μM, 100 μL) was subcutaneously injected into the thigh of living mice, followed by the injection of CyCl-1 (50 μM, 10 μL) at the same area. The control group was conducted by the injection of PBS (100 μL) and then CyCl-1 (50 μM, 10 μL) in the thigh of living mice. The mice were anesthetized by isoflurane in a flow of oxygen and placed in a receptacle filled with 38 °C water during the imaging experiment. The PA signals of the injection areas were recorded at different post-injection time on the Nexus-128 PA tomography system (Endra Inc.) with alternating acquisition at 720 nm and 800 nm. For imaging of endogenous H_2S, a mouse model of drug-induced local H_2S production was employed as previously reported.[1] The mice were subcutaneously injected with Cys (2.0 mM, 100 μL) in the thigh. Following an incubation period of 30 min, CyCl-1 (50 μM, 10 μL) was injected at the same location in the thigh. The PA signals were recorded at different time after injection with alternating acquisition at 720 nm and 800 nm.

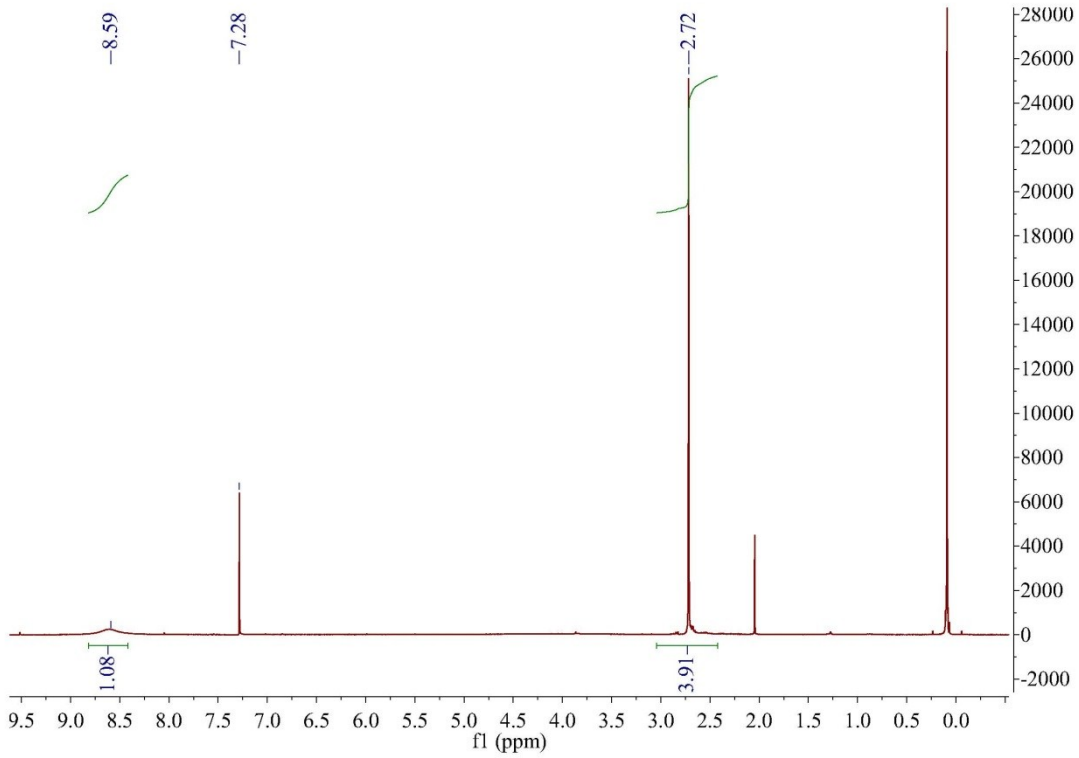


Figure S1. ^{1}H spectrum of compound 2 in $CDCl_3$.

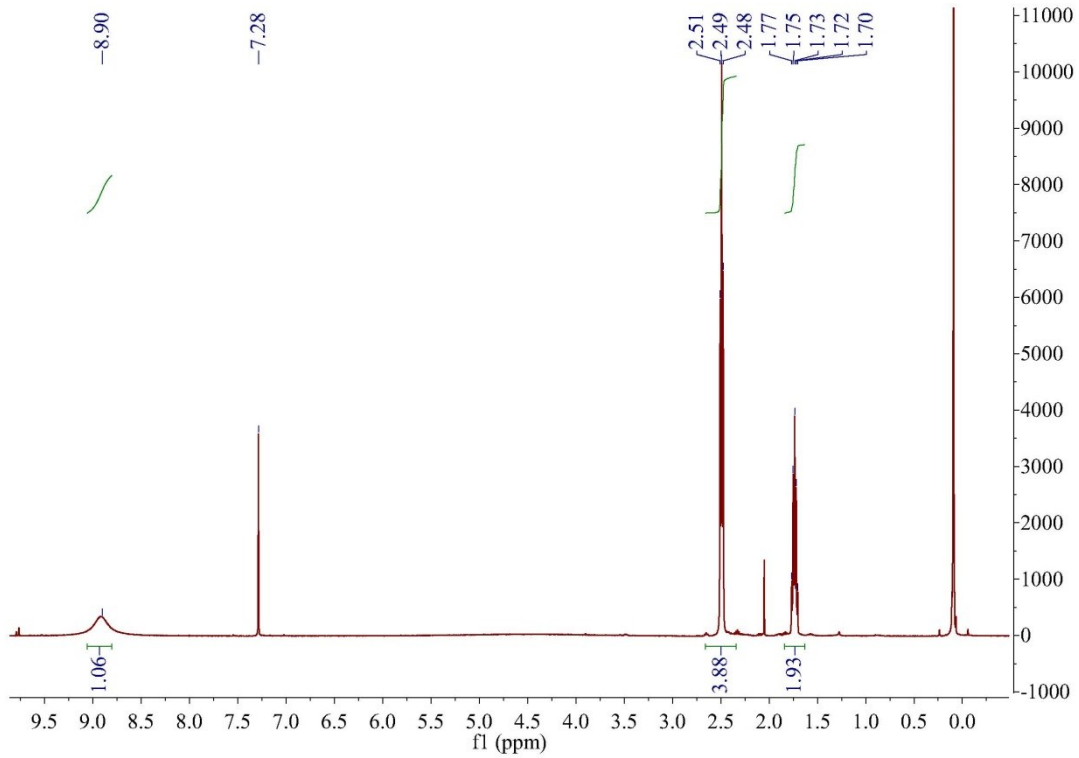


Figure S2. ^{1}H spectrum of compound 3 in $CDCl_3$.

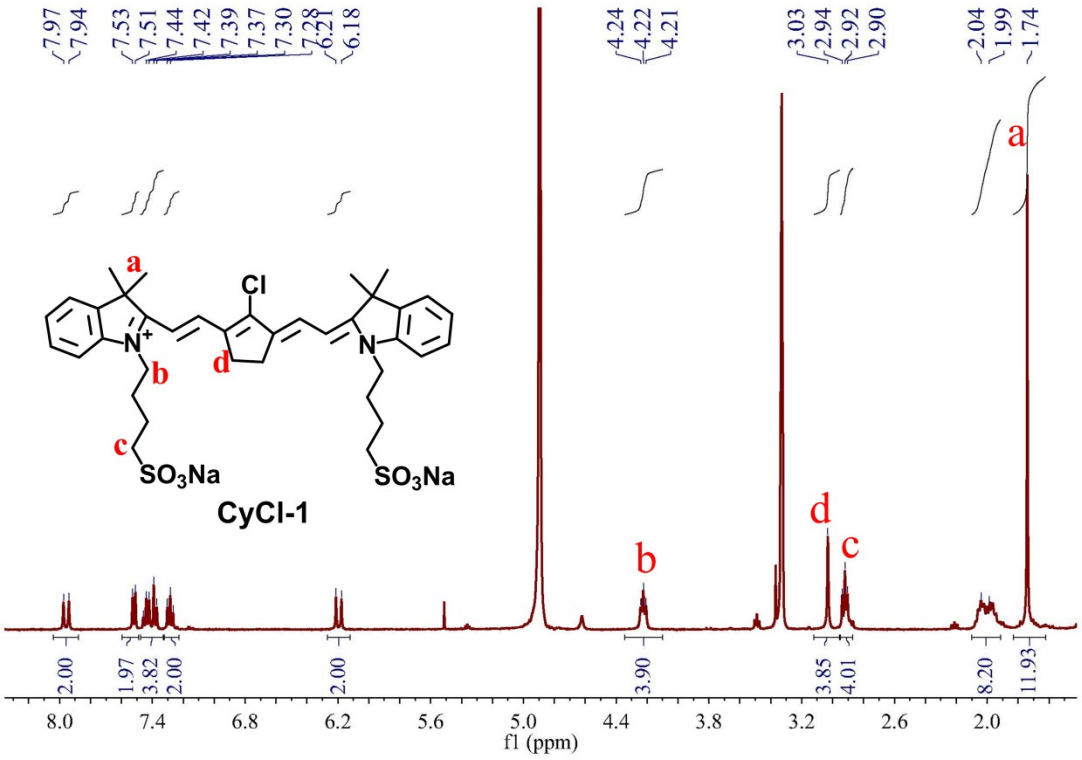


Figure S3. ^{1}H spectrum of CyCl-1 in methanol-D_4.

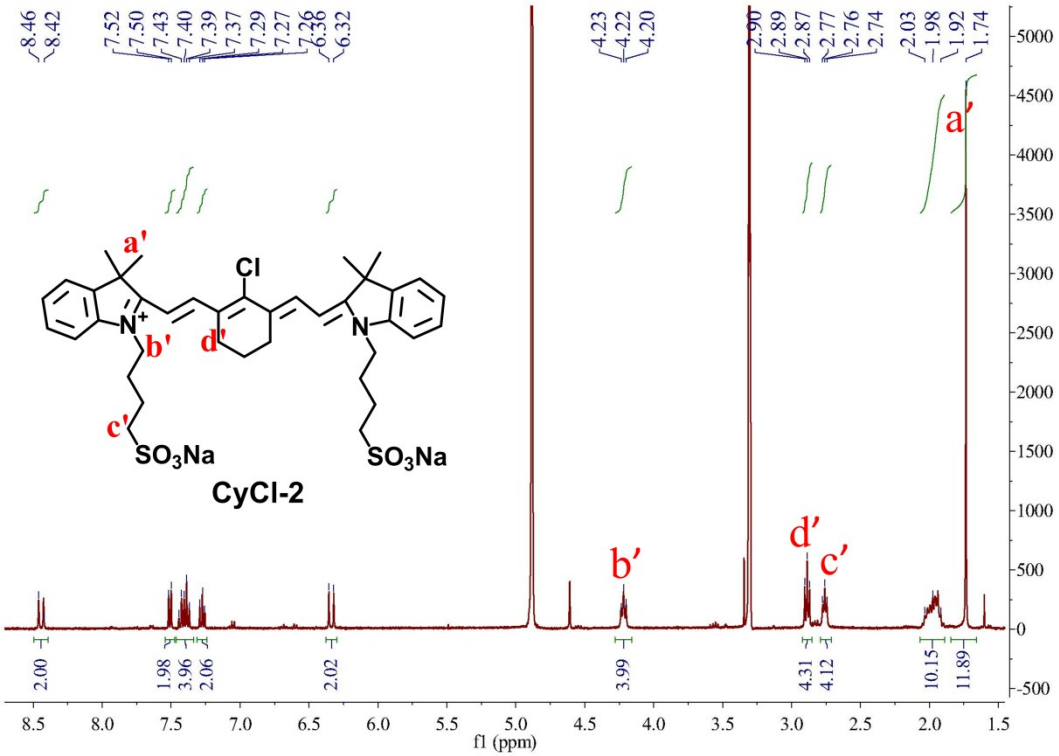


Figure S4. ^{1}H spectrum of CyCl-2 in methanol-D_4.

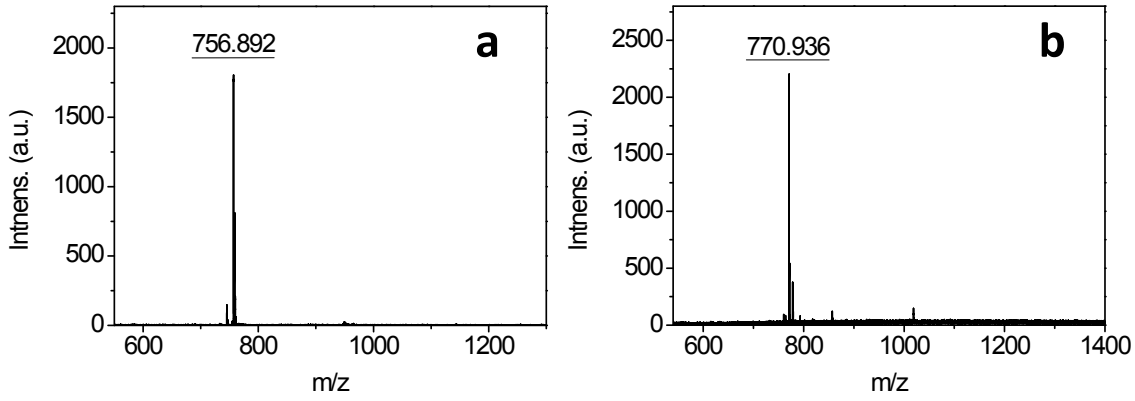


Figure S5. MALDI-TOF mass spectra of CyCl-1 (a) and CyCl-2 (b).

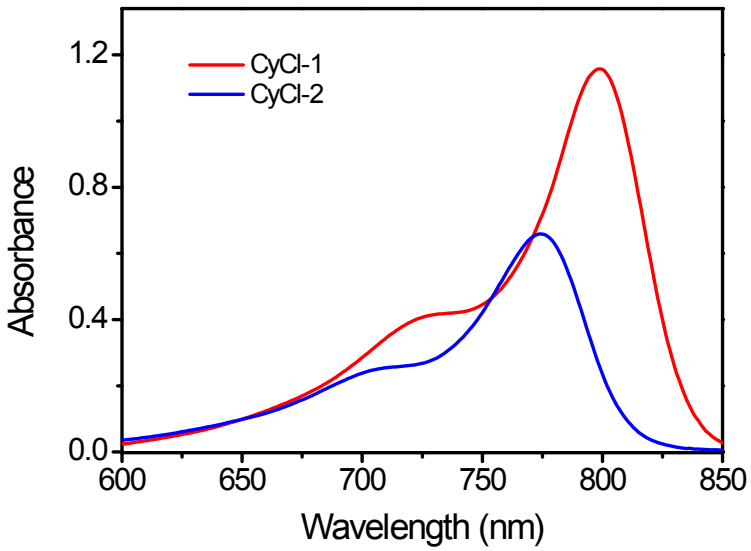


Figure S6. Absorption spectra of CyCl-1 (red line) and CyCl-2 (blue line) (10 μM) in PBS (pH 7.4).

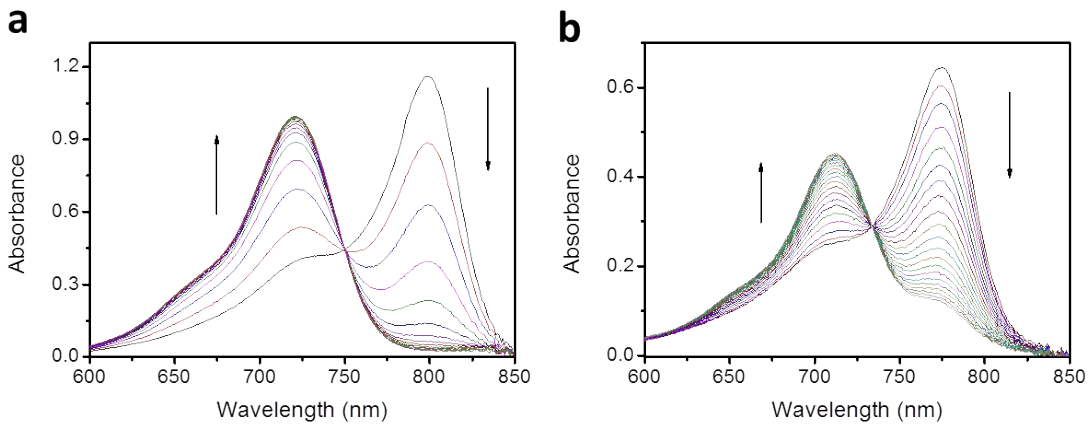


Figure S7. Absorption spectra evolution of (a) CyCl-1 and (b) CyCl-2 (10 μM) upon

addition of NaHS (80 μM) in PBS (pH 7.4). Spectra were acquired from 0 to 10 min with an interval of 30 s.

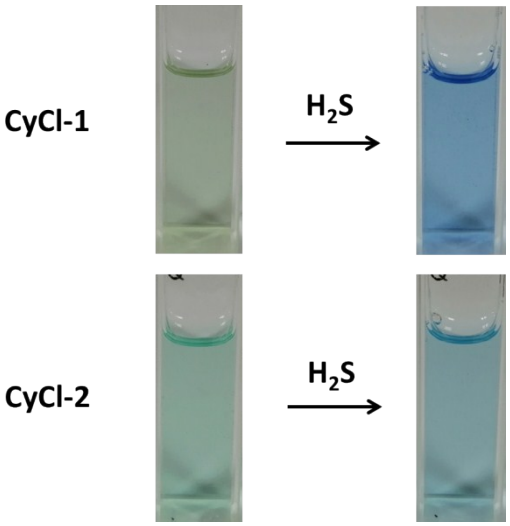


Figure S8. The color response of CyCl-1 and CyCl-2 to H_2S in PBS (pH 7.4).

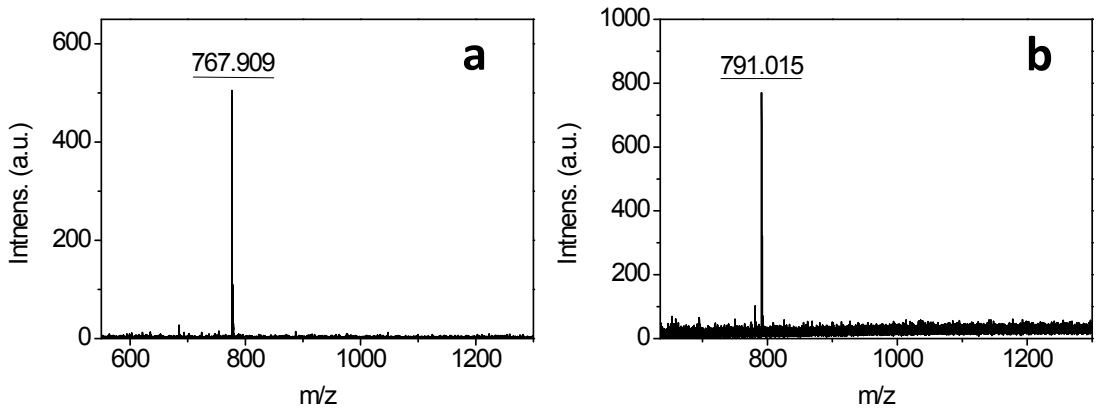


Figure S9. MALDI-TOF mass spectra of the reaction products of CyCl-1 (a) or CyCl-2 (b) with excess NaHS.

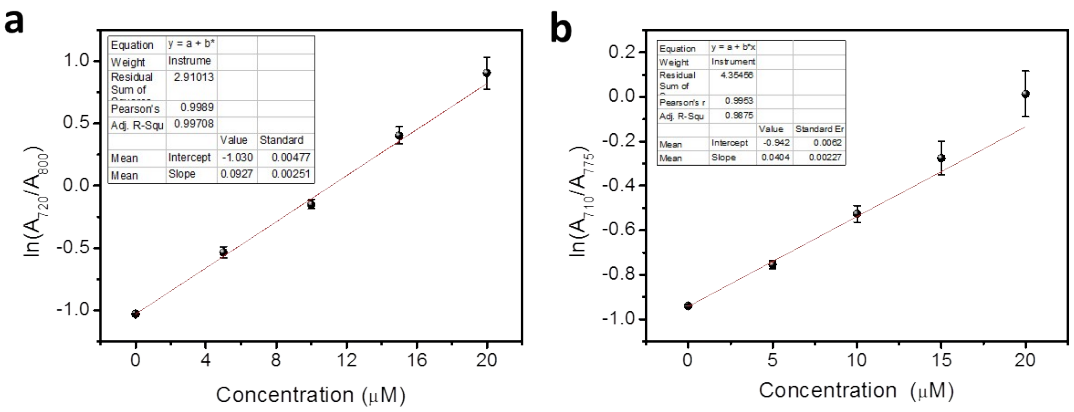


Figure S10. Detection limit analysis of CyCl-1 (a) and CyCl-2 (b) to H_2S using the logarithmic value of absorption intensity ratio as the quantized signal ($\ln(A_{720}/A_{800})$ for CyCl-1 and $\ln(A_{710}/A_{775})$ for CyCl-2).

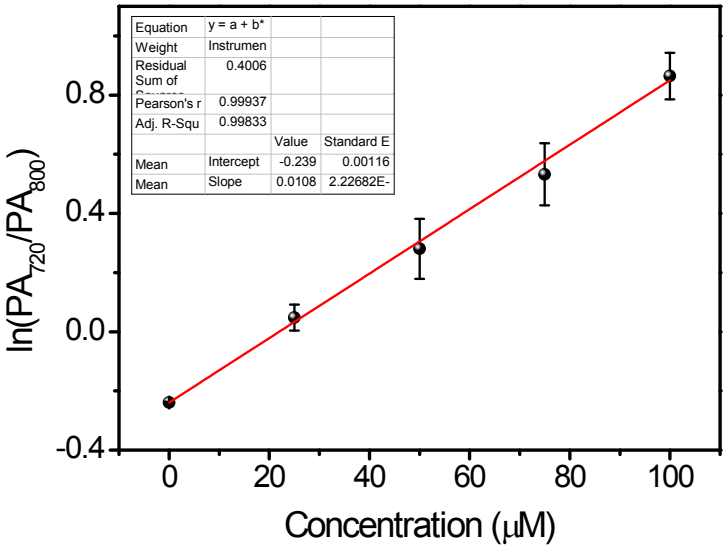


Figure S11. Detection limit analysis of CyCl-1 to H_2S using the logarithmic value of PA intensity ratio ($\ln(PA_{720}/PA_{800})$) as the quantized signal.

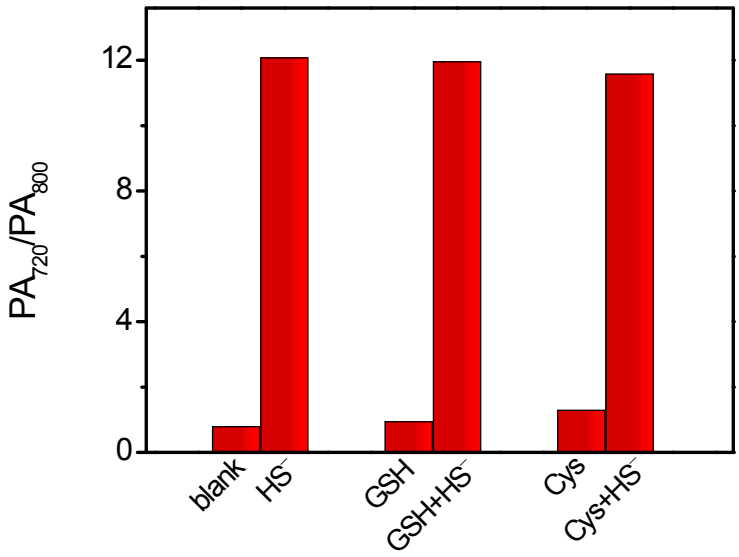


Figure S12. Competition assay of the ratiometric PA response of CyCl-1 to H_2S with excess GSH and Cys.

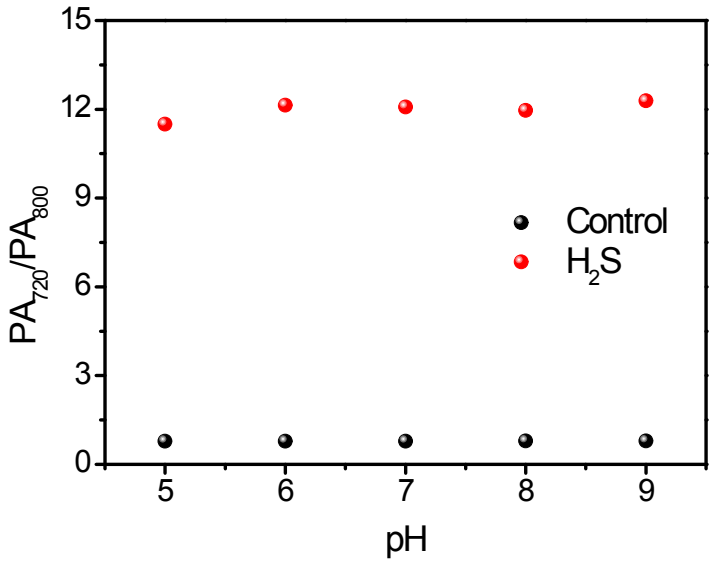


Figure S13. The effect of pH on the ratiometric PA signal (PA_{720}/PA_{800}) of CyCl-1 in the absence (black) or presence (red) of NaHS.

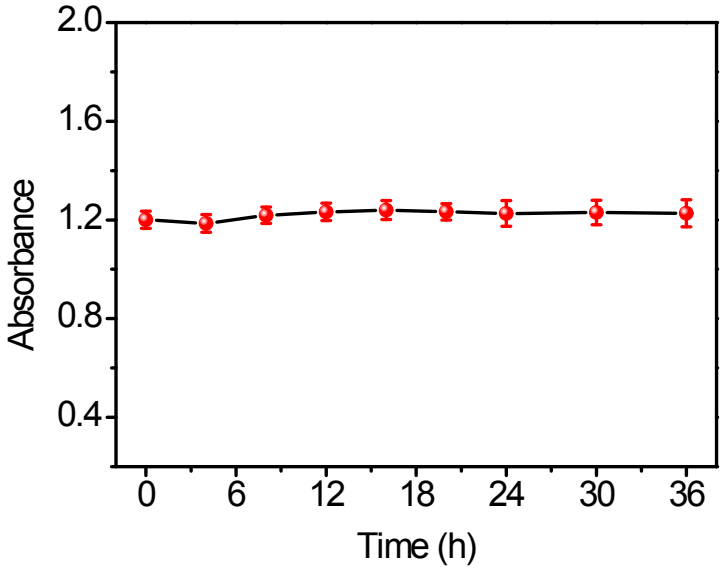


Figure S14. The absorbnce of the probe solution (10 μM) at 800 nm after incubation in PBS containing 10% fetal bovine serum for different time (0 to 36 h).

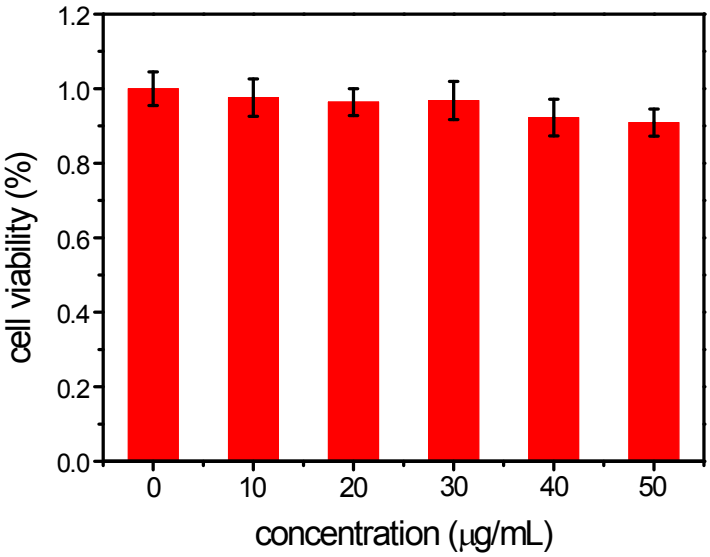


Figure S15. In vitro cell viability of HeLa cells incabuted with CyCl-1 in different concentrations from 0 to 50 μg/mL for 24 h.

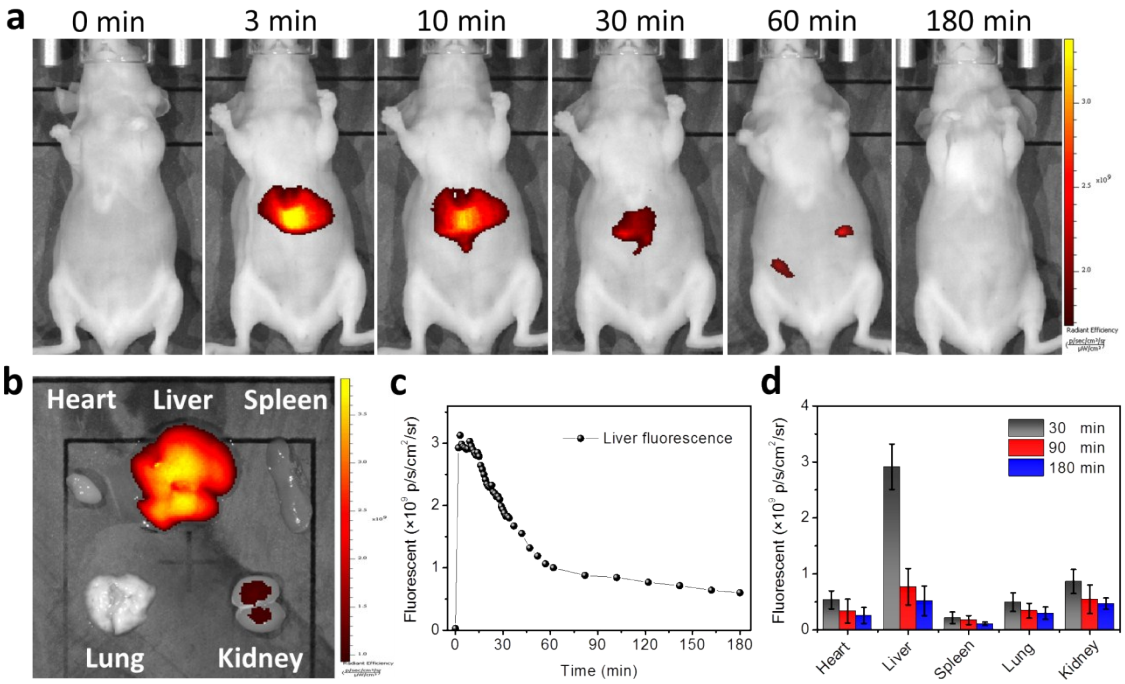


Figure S16. (a) Time-course fluorescence images of living mice after intravenous injection of CyCl-1 (50 μg mL^{-1}, 200 μL). Excitation: 720 nm, emission: 790 nm. (b) Fluorescence images of main organs at 30 min after intravenous injection of CyCl-1. (c) Quantification of fluorescence intensities of liver region in living mice as a function of postinjection time. (d) Bio-distribution of CyCl-1 in mice at 30, 90 and 180 min after intravenous injection determined by the fluorescence intensity in each organ.

References

S1. Z. Du, B. Song, W. Zhang, C. Duan, Y. Wang, C. Liu, R. Zhang, and J. Yuan, *Angew. Chem., Int. Ed.,* 2018, **57**, 3999-4004.