Electronic Supplementary Material

N-Positive ion activated rapid addition and mitochondrial targeting ratiometric fluorescent probes for in vivo cell H_2S imaging

Yan Shi, [1,2] Fangjun Huo, [3] Yongkang Yue,[1] Caixia Yin (✉)[1]

1 Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, Institute of Molecular Science, Shanxi University, Taiyuan 030006, China

2 College of Food Sciences, Shanxi Normal University, Linfen 041004，China

3 Research Institute of Applied Chemistry, Shanxi University, Taiyuan 030006, China

E-mail: yincx@sxu.edu.cn

Contents:

1. Materials and instruments

The materials and reagents used are commercially available and have not been further purified. The solution of compounds was prepard of deionized water. UV-vis spectroscopy was performed using HITACHI U-3900 spectrophotometer, and

fluorescence spectroscopy was performed using HITACHI F-7000 spectrophotometer. All related compounds were characterized by ^{1}H NMR and ^{13}C NMR using a Bruker AVANCE-600 MHz spectrometer, followed by mass spectrometry using an AB Triple TOF 5600plus System (AB SCIEX, Framingham, USA). The final bioimaging application were measured the Zeiss LSM880 Airyscan confocal laser scanning microscope.

2. Bio-imaging

All the animal experiments were performed by following the protocols approved by Radiation Protection Institute of Drug Safety Evaluation Center in China (Production license: SYXK (Jin) 2018-0005). Balb/c type mouse (4-6 weeks, male) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Experiments were conducted according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Imaging procedures were conducted with adult nude mice under general anesthesia by injection of sodium pentobarbital (0.5 mL/0.03 %). Then, **probe 1** and analytes (NaHS) was carefully injected into Balb/c type mouse according to designed experiment. Images were taken using an excitation laser of 405 nm and emission 590±20 nm, respectively.

3. Experimental Section

Precursors were synthesized using literature method. Synthesis of **probe 1** (Scheme 1). In a 25.0 mL round bottom flask, 6-methoxyquinoline-2-carbaldehyde (0.245 g, 1.00 mmol) and 1,2,3,3-tetramethyl-3H-indol-1-ium (0.186 g, 1.00 mmol) were dissolved in ethanol (10.0 mL). Piperidine (0.150 mL) was added and the mixture was refluxed for 8 h. The reaction was cooled to room temperature, the crude product was purified by silica gel column chromatography using ethyl acetate and petroleum ether (1:8) as eluent to afford pure compound **probe 1** as an yellow solid (0.240 g, yield 58.5%).^{1}H NMR (600 MHz, DMSO) δ 8.52, 8.48, 8.29, 8.09, 8.01, 7.93, 7.68, 7.54, 7.49, 4.22, 3.96, 1.84. (Fig. S1); ^{13}C NMR (151 MHz, DMSO) δ 191.77, 170.77, 164.66, 161.05, 159.95, 157.27, 156.90, 138.16, 132.27, 130.93, 130.09, 129.10, 127.68, 123.17, 122.27, 114.96, 114.51, 114.27, 113.70, 56.15, 40.09, 32.13, 27.90, 27.56. (Fig. S2); HR-MS m/z: calcd for 343.18049; found: m/z: 343.18040(Fig. S3).

4. Spectral test preparation

Probe 1 was dissolved in DMSO to make a 2.0 mM stock solution. Through testing, we finally chose DMSO / PBS (2/8, v/v, pH = 7.4) as the test system, the probe concentration was 10.0 μM. The concentration of interfering ions is 0.1 M. Therein $NaNO_2$ and $NaNO_3$ are the donors of NO_2^- and NO_3^-.

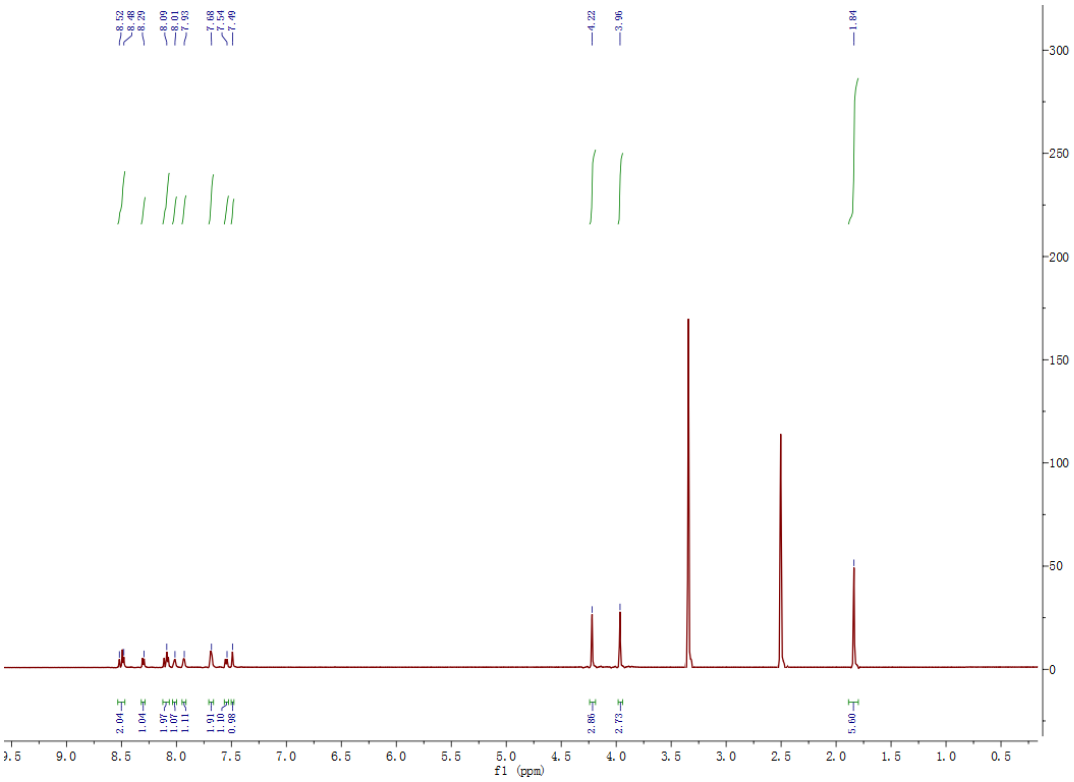

Figure S1: ^{1}H NMR(600 MHz) of **probe 1** in DMSO -d_6.

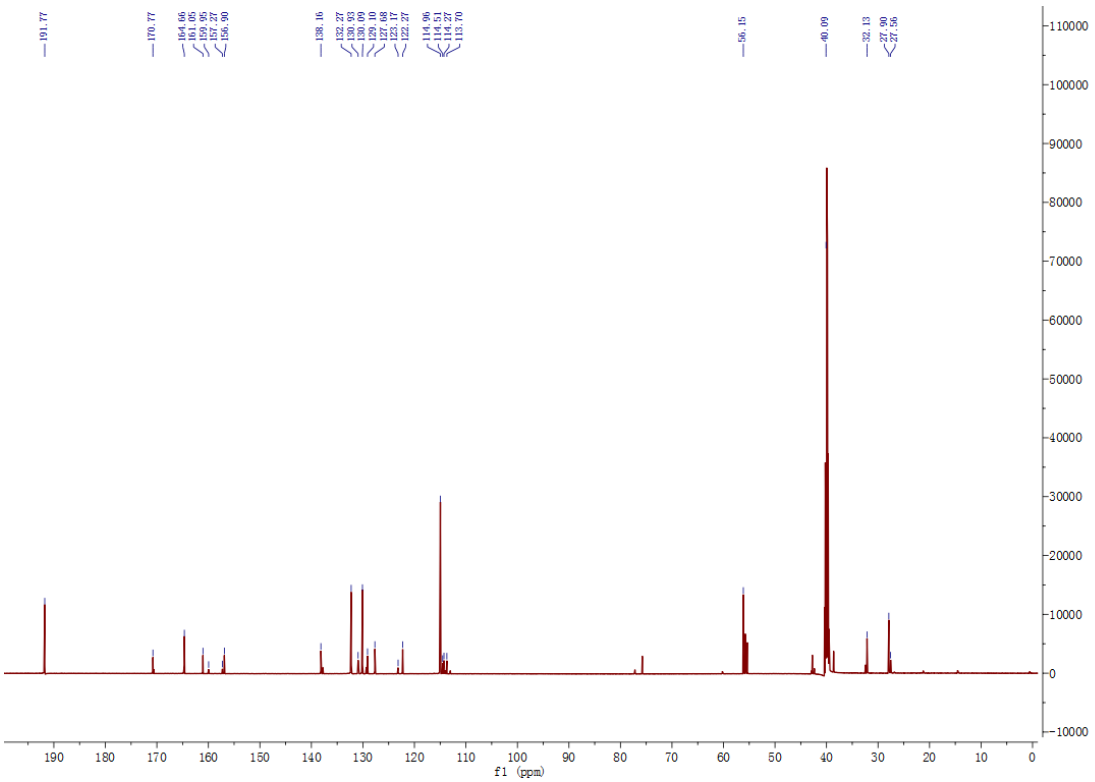

Figure S2: ^{13}C NMR(150 MHz) **probe 1** in DMSO -d_6.

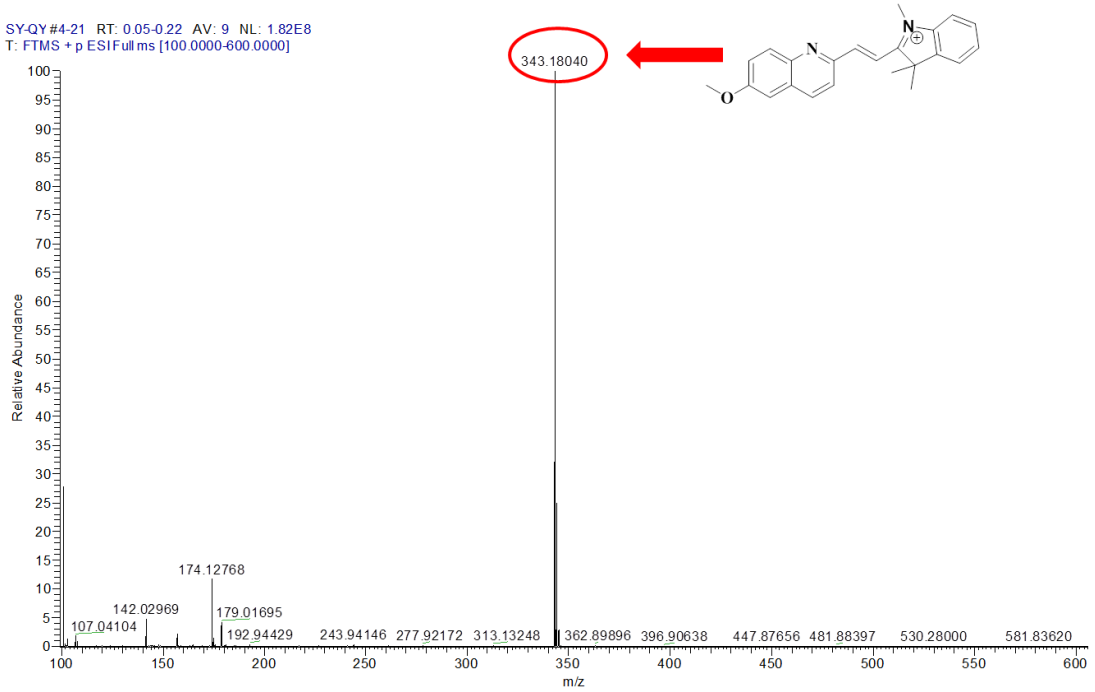

Figure S3: HR-MS spectrum of **probe 1**.

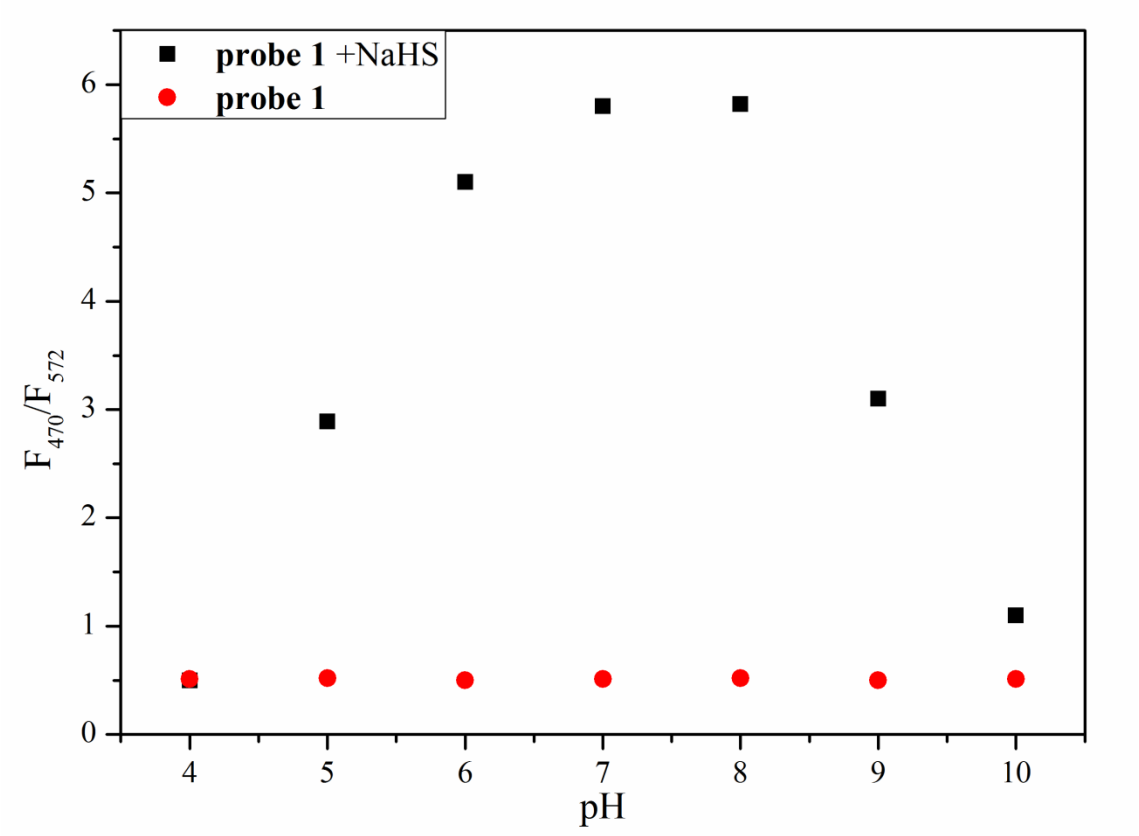

Figure S4: pH effect of **probe 1** (10 μM) towards H_2S (2 mM) detection process, each
spectrum was obtained 10 min after addition, λ_{ex} = 400 nm, slit: 5 nm/5 nm.

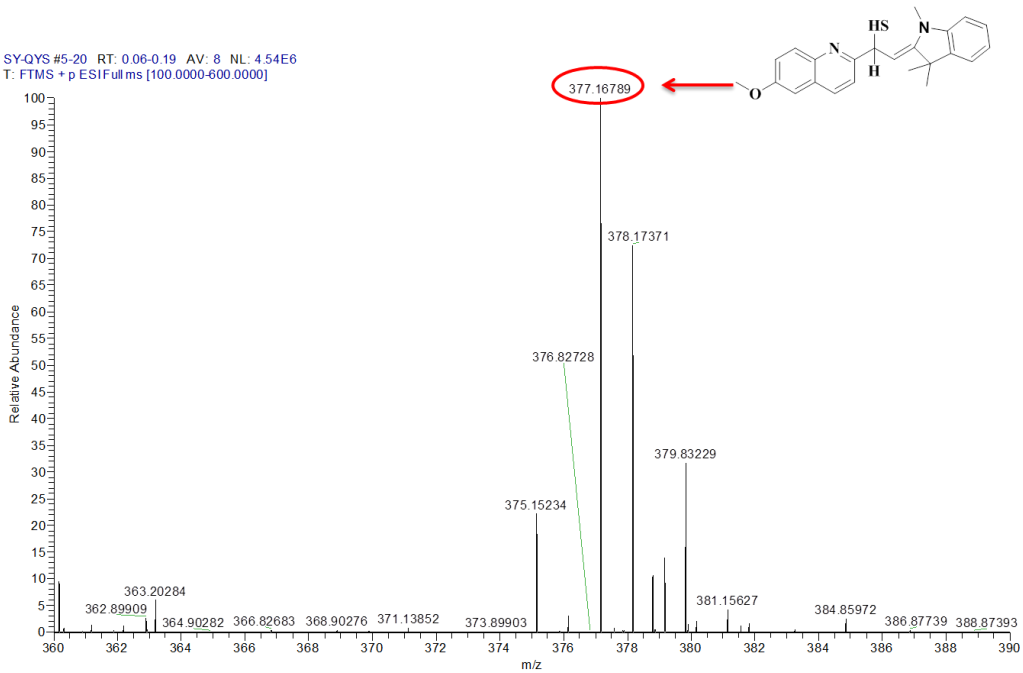

Figure S5: HR-MS spectrum of **probe 1** +NaHS.

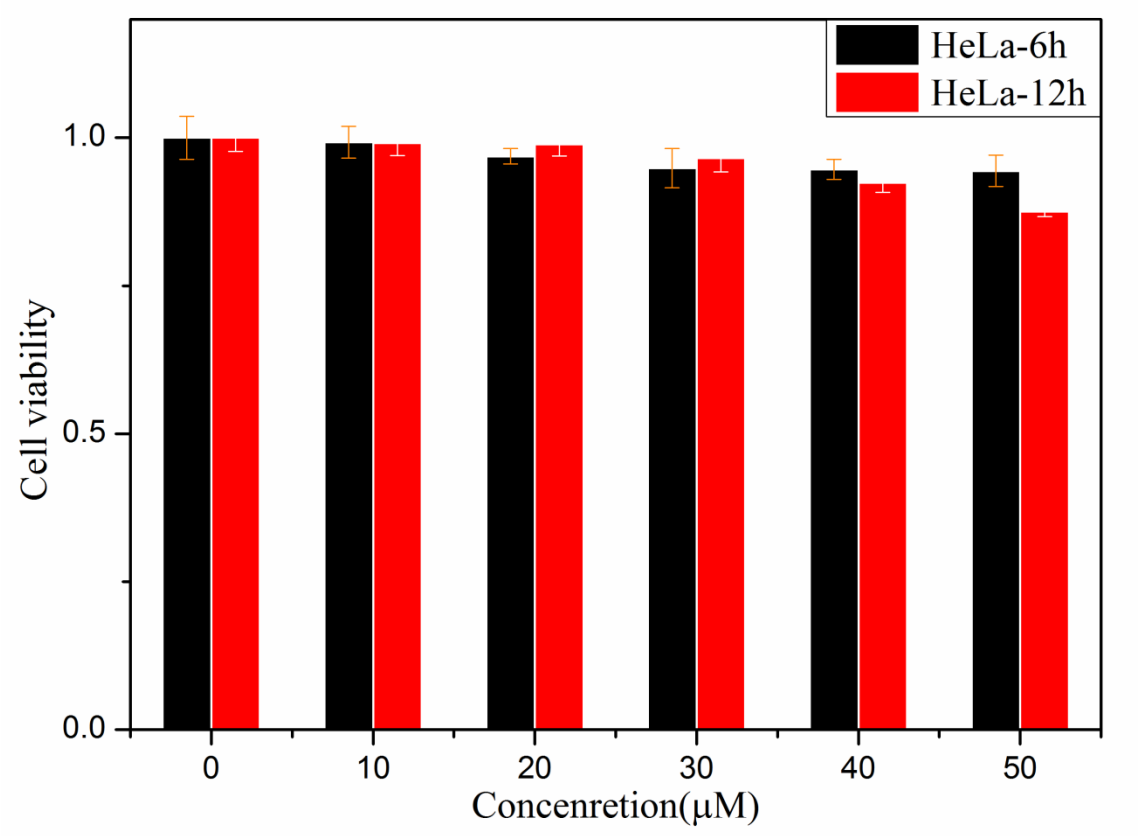

Figure S6: Cell viability values estimated by CCK-8 assay with HeLa cells.