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Electronic Supplementary Material (ESI)

Imaging and evaluation of sulfane sulfur in acute brain ischemia using a mitochondria-targeted near-infrared fluorescent probe

Min Gao,[ac] Rui Wang,[a] Fabiao Yu,[ab]* Jinmao You,[b] Lingxin Chen[ab]*

[a] Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Research Centre for Coastal Environmental Engineering and Technology, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China.

[b] Key Laboratory of Life-Organic Analysis, Key Laboratory of Pharmaceutical Intermediates and Analysis of Natural Medicine, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu 273165, China.

[c] University of Chinese Academy of Sciences, Beijing 100049, China.

E-mail: fbyu@yic.ac.cn; lxchen@yic.ac.cn

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1. General experimental section

Materials: aza-BODIPY was synthesized in our laboratory according to previous report with some modification.[1] Human neuroblastoma (SH-SY5Y) cells, mouse macrophage (RAW 264.7) cells, human lung carcinoma (A549) cells, human cervical carcinoma (Hela) cells , Human embryonic kidney 293 (HEK 293) cells, human hepatocellular liver carcinoma (HepG2) cells, human hepatocellular liver carcinoma (HL-7702) cells were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Mito-SH (1.0 mM, 10 mL) was prepared in DMSO and stored at 4 ^{o}C in darkness. All other reagents, fine chemicals and LC solvents with the highest grade commercially available were obtained from J&K Chemical Ltd (Shanghai, China) and Energy Chemical (Shanghai, China). Ultrapure water (Millipore, Bedford, MA, USA) was used throughout.

Instruments: Absorption spectra were obtained on EVOLUTION 220 UV-visible spectrophotometer (Thermo Fisher Scientific, USA). Fluorescence spectra were obtained by FluoroMax-4 Spectrofluorometer with a Xenon lamp and 1.0-cm quartz cells (HORIBA Scientific, France). The pH measurements were constructed with a pH-3c digital pH meter with a combined glass–calomel electrode (Shanghai Lei Ci Device Works, Shanghai, China). Mass spectra were taken on LCQ Fleet LC-MS System (Thermo Fisher Scientific, USA). ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker spectrometer. Fluorescent images were acquired on an Olympus Fluo View FV1000 confocal laser-scanning microscope (Japan) with an objective lens. In-vivo imaging was achieved by IVIS Lumina XRMS Series Ⅲ (Perkinelmer, USA).

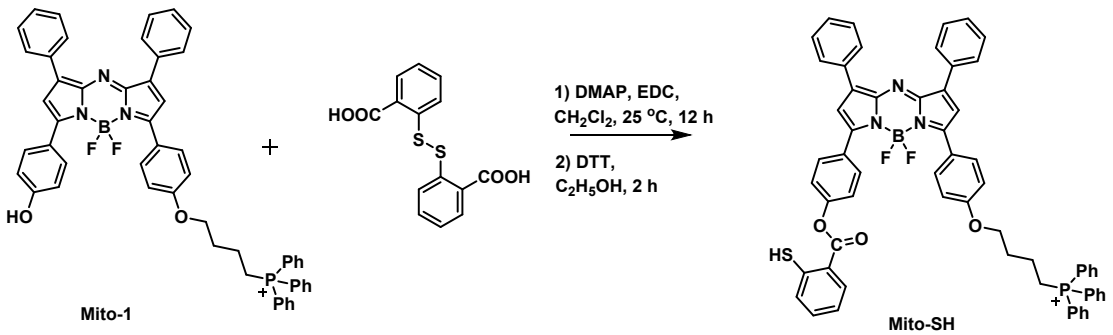
Absorption analysis: The probe (DMSO, 1.0 mM, 0.1 mL) was added to 10.0 mL color comparison tube and diluted to 10.0 μM with 10 mM HEPES (pH 7.4). Then Na_2S_4 (10 μM) was added to the color comparison tube. The mixture was equilibrated for 5 min before measurement.

Fluorescence analysis: The probe (DMSO, 1.0 mM, 0.1 mL) was added to 10.0-mL color comparison tube and diluted to 10.0 μM with 10 mM HEPES (pH 7.4). Then various concentration of Na_2S_4 (0-10 μM) was added to the color comparison tube. The mixture was equilibrated for 5 min before measurement.

Cytotoxicity assays: Cell viability was investigated by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide (MTT) assay. SH-SY5Y cells were cultured in RPMI 1640 Medium

supplemented with 10% FBS at 37 °C in a 5% CO_2 incubator. The cells were seeded in 96-well plate at a concentration of 1× 10^4 /mL in 200 μL of RPMI 1640 Medium supplemented with 10% FBS at 37 °C and maintained in a humidified atmosphere containing 5% CO_2 for 24 h. Then different concentrations of Mito-SH (0, 5.0, 10.0, 20.0, 30.0 and 40.0 μM, final concentration) were added to the wells and cultured for 24 h. The cells were washed with PBS and loaded with MTT (5 mg/mL, 20 μL). After incubation for 4 h, the remaining MTT was removed and DMSO was added to each well to dissolve the formazan crystals. The absorbance of treated cells was compared with the absorbance of the controls, which cells were exposed only to the vehicle and were considered as 100% viability value. The absorbance was determined using a microplate reader (Tecan, Austria).

2. Synthesis route of Mito-SH



Scheme S1 Synthesis route of Mito-SH.

3. Effect of pH values

To test feasibility of Mito-SH as a sulfane sulfur-selective probe, we evaluated the effects of pH values on the fluorescence intensity of Mito-SH (10 μM) and its reaction product Mito-1 in HEPES buffer (10 mM, 0.5% DMSO, 0.4% Tween 80), respectively. As shown in Fig. S1, Mito-SH showed a stable fluorescence at the range of pH 4.0 to 10.0 in a HEPES buffer solution with no addition of Na_2S_4 (10 μM). Mito-1 almost has no effect on the fluorescence by the pH of mediums within the range from 4.0 to 7.8. These results showed that Mito-SH could be applied for detecting sulfane sulfur without interference of pH in the biological environment.

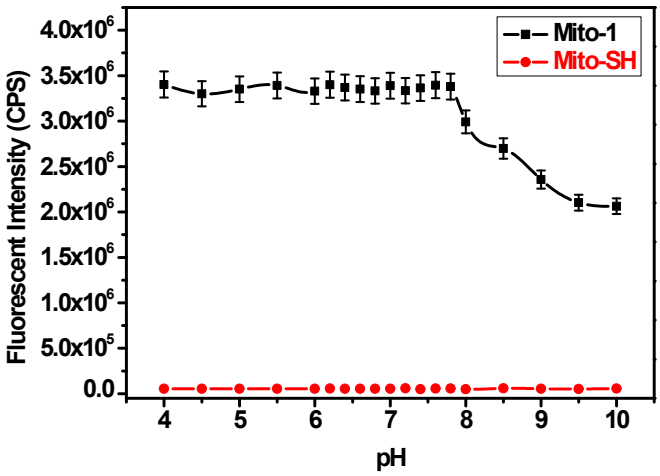


Fig. S1 The effects of pH values on the fluorescence intensity of Mito-SH (10 μM) and Mito-1 in HEPES buffer (10 mM, 0.5% DMSO, 0.4% Tween 80), respectively. pH values: 4.0, 4.5, 5.0, 5.5, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.5, 9.0, 9.5, 10.0. λ_{ex} = 635 nm, λ_{em} = 737 nm.

4. Selectivity

To fully investigate the selectivity of Mito-SH, the changes in fluorescent intensity of Mito-SH towards some reactive oxygen species (ROS) were evaluated. As illustrated in Fig. S2, the addition of ROS, including $O_2^{\bullet-}$, $^{\bullet}OH$, H_2O_2, $^1O_2$, methyl linoleate hydroperoxide (MeLOOH) and cumene hydroperoxide (CuOOH), did not interfere fluorescence change of Mito-SH.

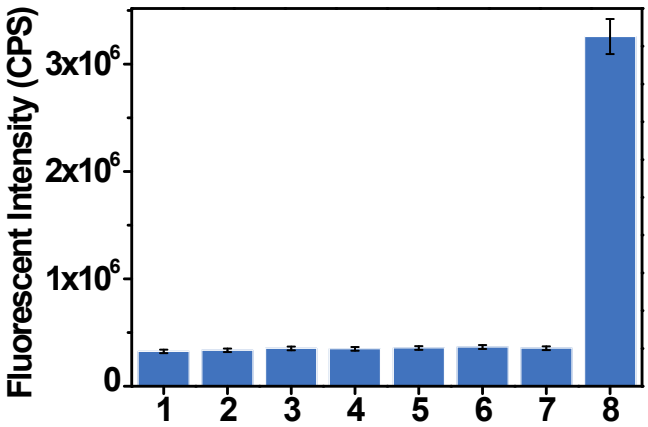


Fig. S2 Fluorescence responses of Mito-SH to ROS. (1) $O_2^{\bullet-}$ (100 μM), (2) $^{\bullet}OH$ (100 μM), (3) H_2O_2 (100 μM), (4) $^1O_2$ (100 μM), (5) methyl linoleate hydroperoxide (MeLOOH, 100 μM), (6) cumene hydroperoxide (CuOOH, 100 μM), (7) $ONOO^-$ (100 μM), (8) Na_2S_4 (10 μM). Data were acquired in 10 mM HEPES buffered at pH 7.4 (0.5% DMSO, 0.4% Tween 80). λ_{ex} = 688 nm, λ_{em} = 737 nm.

5. MTT assay

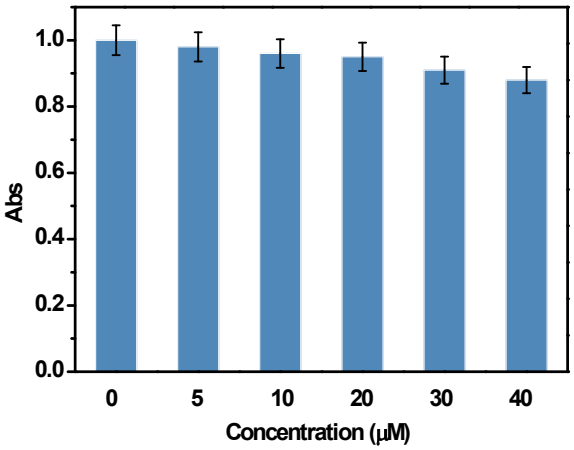


Fig. S3 MTT assay of SH-SY5Y cells with various concentrations of Mito-SH (0-40 μM). The experiment was repeated four times and the data are shown as mean (±S.D.).

6. Photostability of Mito-SH

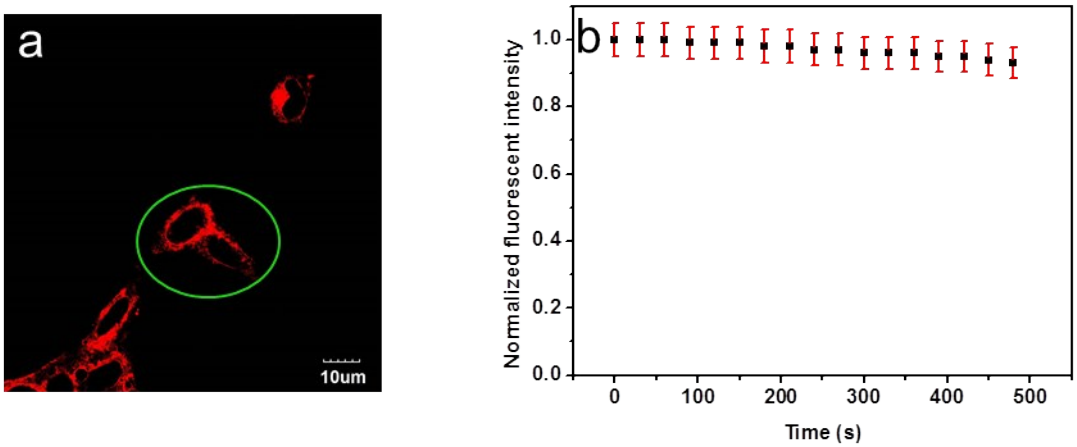


Fig. S4 Photostability test of Mito-SH for the detection of sulfane sulfur. (a) Firstly, SH-SY5Ycells were incubated with Na_2S_4 (1 μM) for 15 min, and then loaded with Mito-SH (1 μM) for 20 min. Representative fluorescence images (0-480 s) were acquired by time-sequential scanning using 635 nm excitation on Olympus FluoView FV1000 confocal laser-scanning microscope (Japan) with an objective lens (×60). (b) Normalized fluorescent intensity of the selected regions of (a) from 0 to 480 s. And the normalized data are versus with the first data point.

7. Imaging of sulfane sulfur *in vivo*

Fluorescent probe functions in the NIR region (650 - 900 nm) can penetrate deep tissues and reduce the interference of background autofluorescence. Therefore, our probe is a preferential candidate for fluorescent imaging of sulfane sulfur *in vivo*. BALB/c mice were divided into two

groups to investigate the utility of our probe *in vivo*. Group a was injected introperitoneal (i.p.) cavity with Mito-SH as the control experiment. Group b was then given an i.p. injection of Na_2S_4 after the same disposal of the control group. Obviously, a significant fluorescence increase was obtained in Group b, while the control experiment showed weak fluorescence (Fig. 7a). Then we quantified the mean fluorescent intensity of each group. Compared with Group a, Group b exhibited 12-fold fluorescence intensity. Taken together, these experiments established that our probe was capable of visualizing sulfane sulfur successfully *in vivo*.

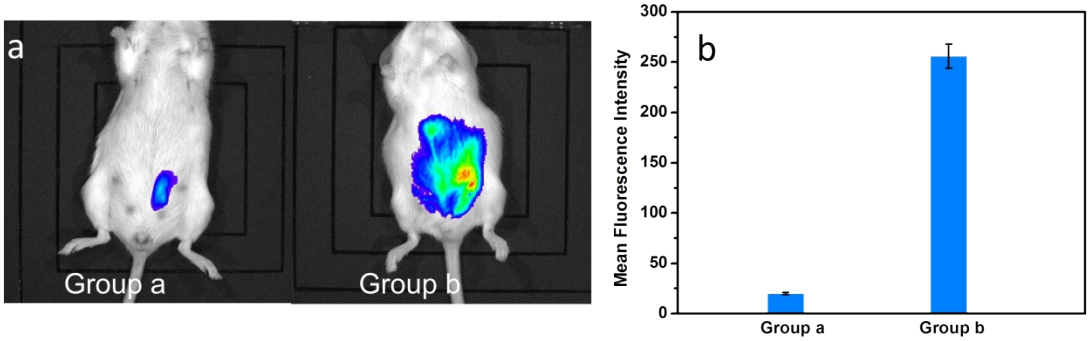


Fig. S5 (a) Representative fluorescence images of visualizing sulfane sulfur levels in mice using Mito-SH. Group a was given an i.p. injection with Mito-SH (10 μM, 50 μL in 1:9 DMSO/saline v/v). Group b was given an i.p. injection with Na_2S_4 (100 μM, 50 μL in saline) after the same treatment with Group a. (b) Quantification of total photon flux from each group. The total number of photons from the entire peritoneal cavity of the mice was integrated. Images displayed represent fluorescence emission collected windows: 720 nm (λ_{ex} = 670 nm). n = 5. Error bars are ± SEM.

8. Normalized fluorescent intensity of Fig. 6b, 6c, 6h and 6i.

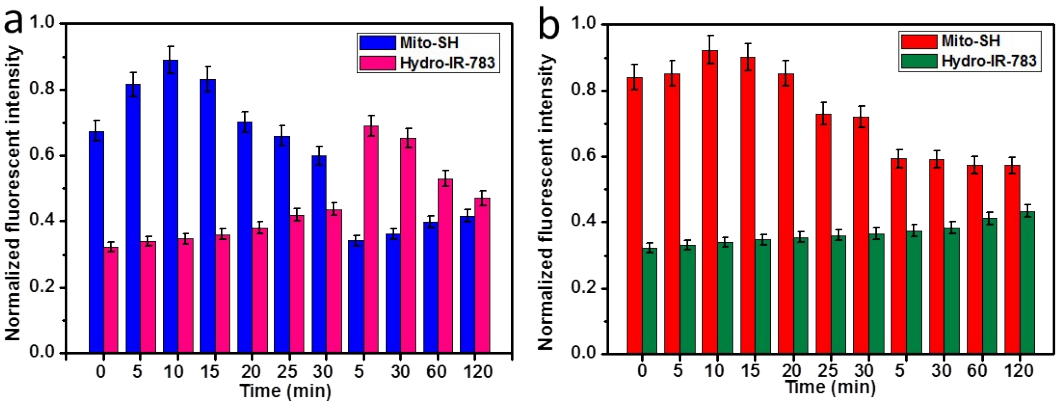


Fig. S6 (a) Normalized fluorescent intensity of Fig. 6b and 6c. (b) Normalized fluorescent intensity of Fig. 6h and 6i.

9. Bright-Field Images

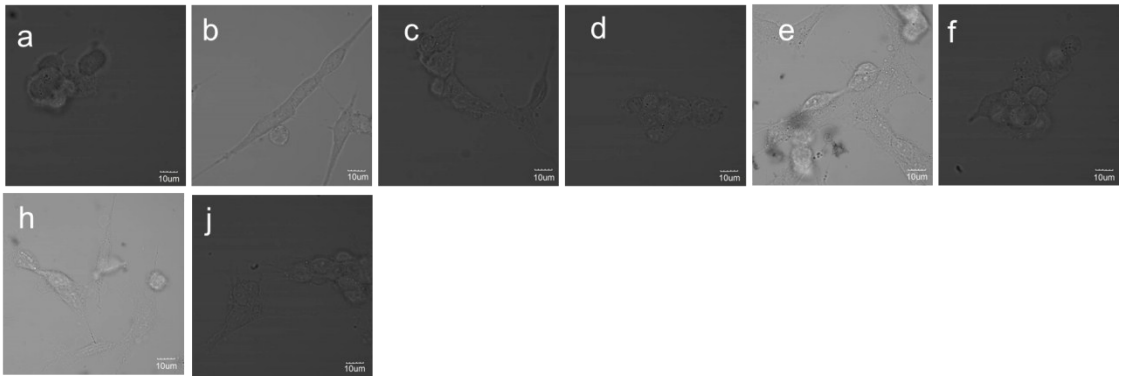


Fig. S6 Bright-field images of Fig. 2.

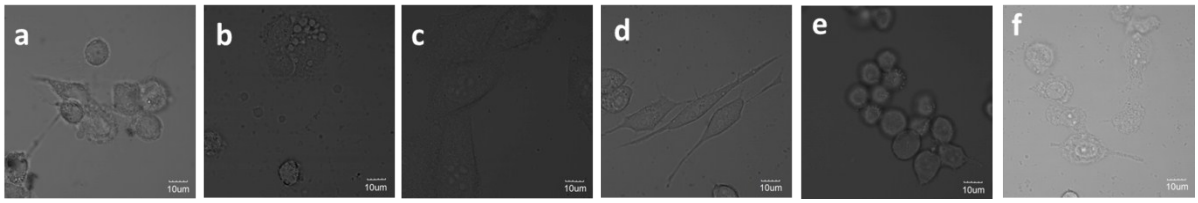


Fig. S7 Bright-field images of Fig. 3.

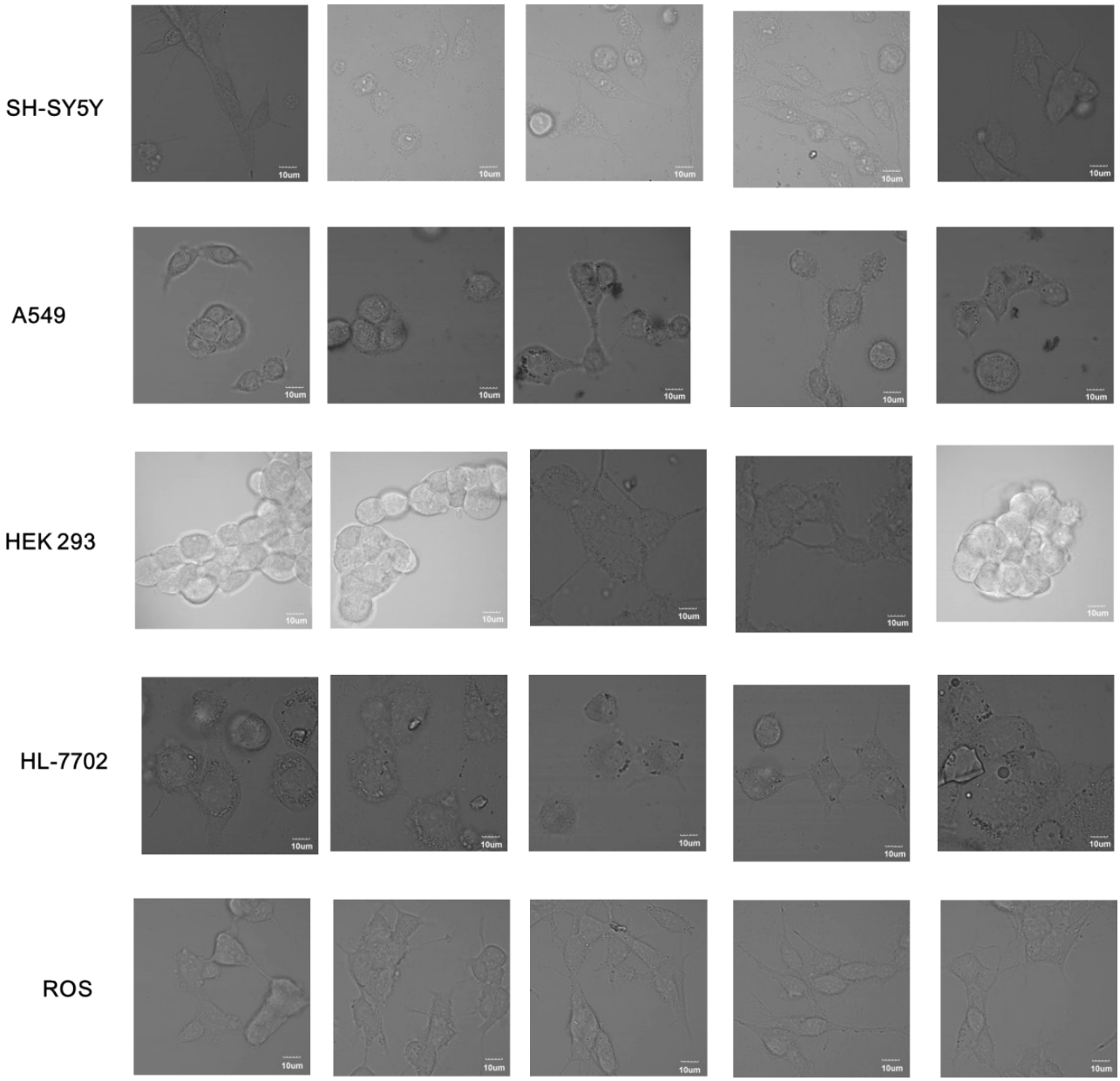


Fig. S8 Bright-field images of Fig. 5.

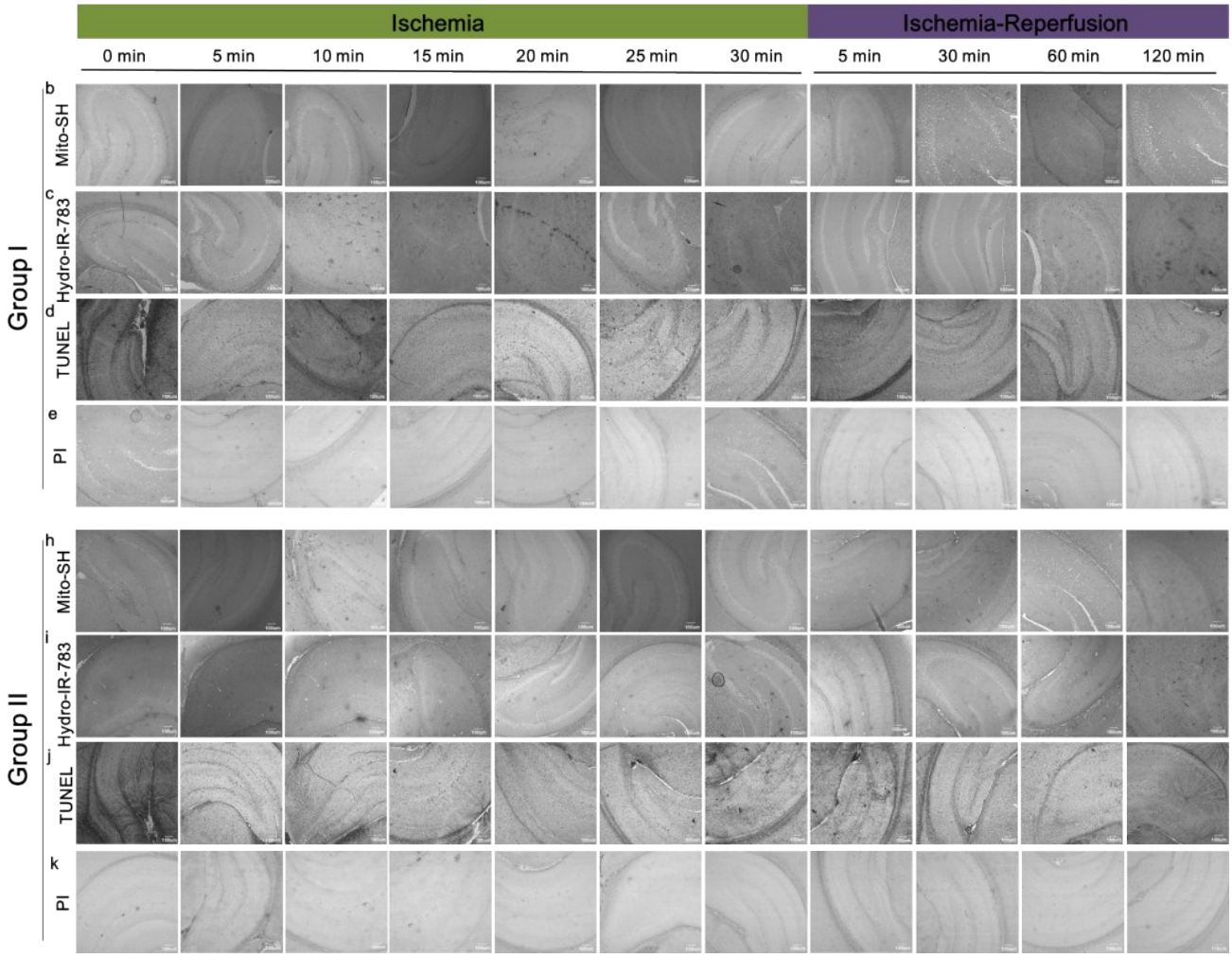
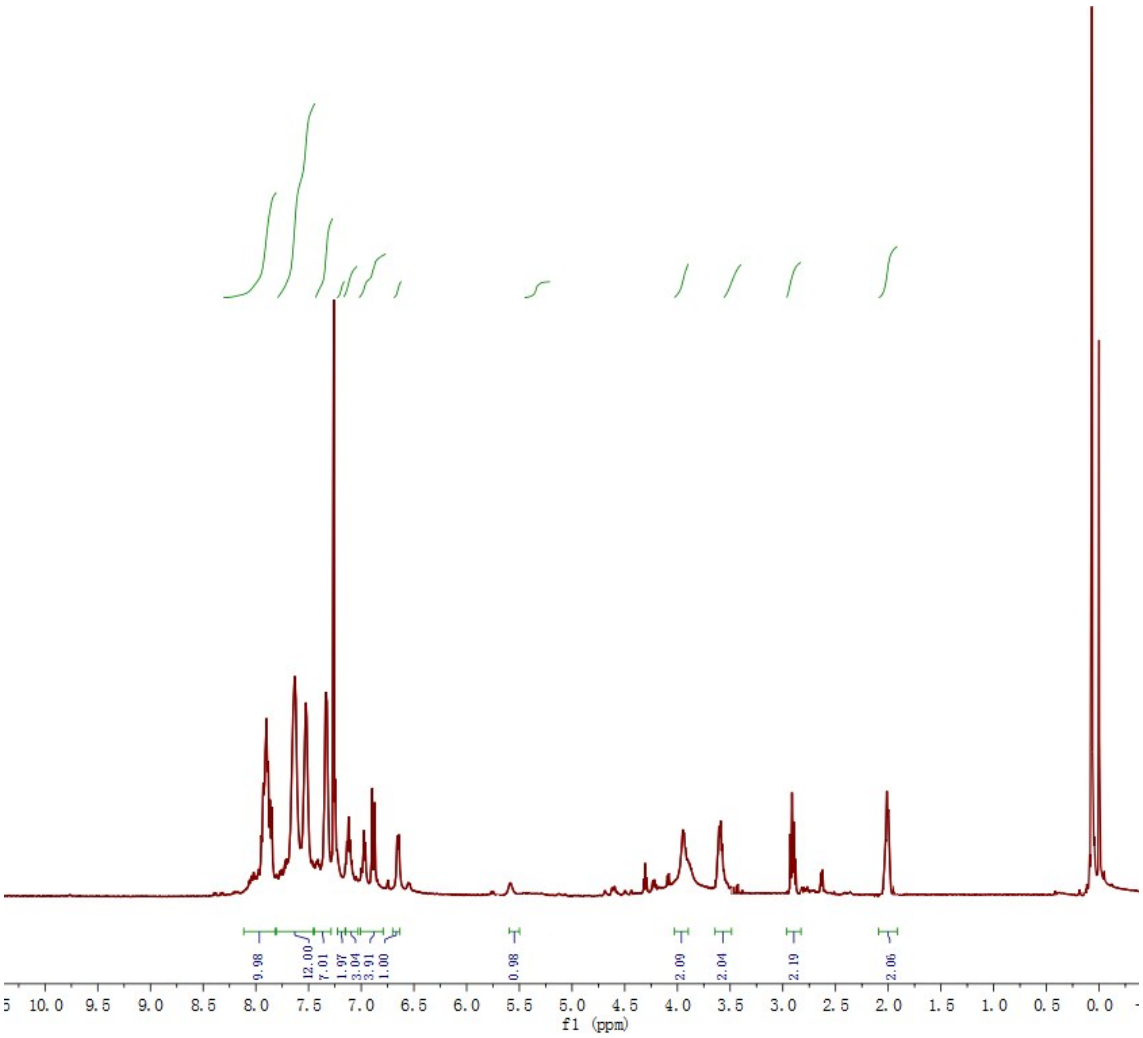
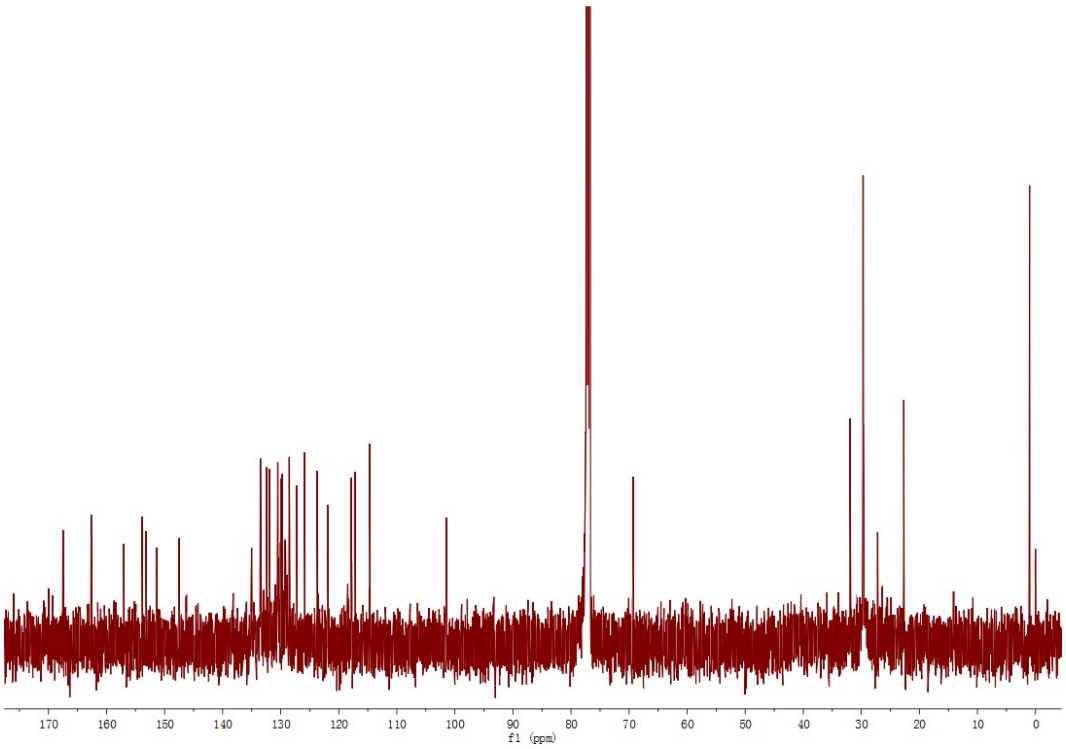
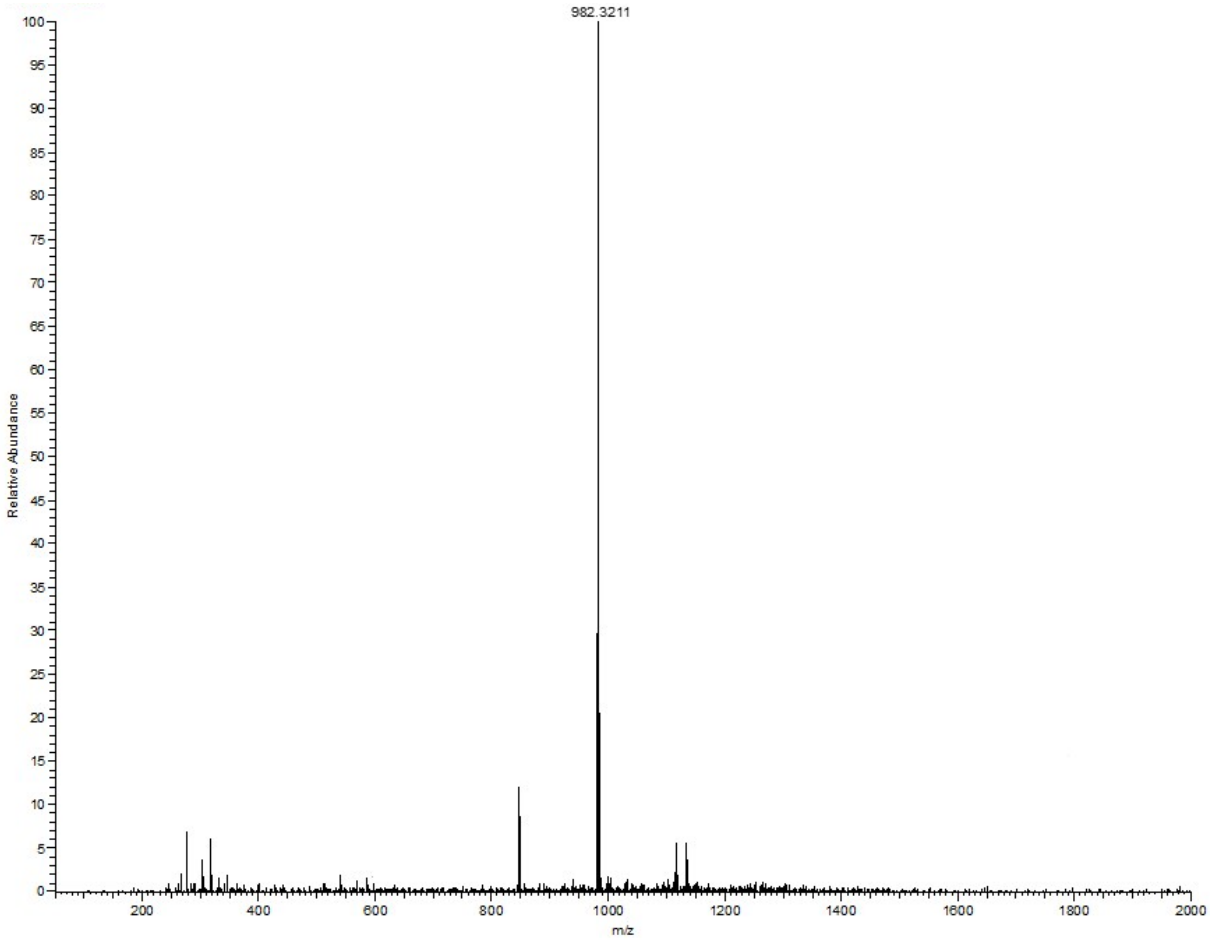


Fig. S9 Bright-field images of Fig. 6.

10. ^{1}H-NMR, ^{13}C-NMR and HR-MS spectra of Mito-SH







11. Reference

1. Gao, M.; Wang, R.; Yu, F.; You, J.; Chen, L., A near-infrared fluorescent probe for the detection of hydrogen polysulfides biosynthetic pathways in living cells and in vivo. *Analyst* **2015,** *140* (11), 3766-3772.