Supporting Information

Near-infrared frequency upconversion fluorescent probe for rapid and sensitive visual detection of sulfur dioxide

Hong Zeng, [a,‡] Xiao Ma,[a,‡] Shufen Pan,[a] Yuting Han,[a] Yanyan Tang,[a] Yulan Fan,[a] and Yongquan Wu [a,*]

[a] Jiangxi Provincial Key Laboratory of Synthetic Pharmaceutical Chemistry, School of Chemistry and Chemical Engineering, Gannan Normal University, Ganzhou, 341000, P R China. E-mail: wyq@gnnu.edu.cn

‡ These authors contributed equally to this work

Contents

1. Synthesis of compound A-C

2. Spectral measurements

3. Cell culture and MTT assay

4. Imaging of HSO_3^- in cells

5. Imaging of HSO_3^- in vitro

6. Imaging of HSO_3^- in mice

7. Supporting figures

8. NMR and HR-MS data

1. Synthesis of compound A-C

Synthesis of compound A

DMF (10 mL) and $CHCl_3$ (50 mL) were added to a round-bottomed flask, followed by the addition of phosphorus tribromide (6 mL) and stirring for 1 h at 0 °C in an ice bath, and then cyclopentanone (0.421 g, 5.0 mmol) was added and stirred at room temperature overnight. At the end of the reaction, the reaction was slowly added to 200 mL of ice water and neutralised with sodium bicarbonate. Finally, the aqueous layer was extracted several times with dichloromethane, and the extracted organic layer was dried with anhydrous Na_2SO_4, and the solvent was evaporated to obtain the crude product compound A. The obtained crude product were directly used for the next reaction.

Synthesis of compound B

Compound A (0.869 g, 5.0 mmol) was dissolved with 2,4-dihydroxybenzaldehyde (0.691 g, 5.0 mmol) in 10 mL of DMF, followed by the addition of $CsCO_3$ (4.5 g, 13.8 mmol). After 12 h at room temperature, the reaction solution was filtered and washed with dichloromethane. The obtained solution was spun and added to 300 mL of distilled water, the aqueous layer was extracted with dichloromethane, the water in the dichloromethane was removed by anhydrous Na_2SO_4, the solvent was filtered and spun, and the pure product was obtained as a yellow solid by column chromatography (PE:EA=5:1). HR-MS (ESI): calculated for $C_{17}H_{19}NO_2$ $[M]^+$, 270.1488; found, 270.1491.

Synthesis of compound C

2-Methyl-5-methoxybenzothiazole (0.538 g, 3.0 mmol) was dissolved in 15 mL of anhydrous acetonitrile and iodomethane (2.1 g, 15.0 mmol) was added. The reaction mixture was refluxed at 85 ℃ for 24 h. A white solid was precipitated during the reaction, and the pure product was obtained by filtration of the white solid after the reaction. ^{1}H NMR (400 MHz, D_2O) δ 7.90 (d, *J* = 9.1 Hz, 1H), 7.41 (d, *J* = 2.4 Hz, 1H), 7.27 (dd, *J* = 9.1, 2.4 Hz, 1H), 4.04 (s, 3H), 3.86 (s, 3H), 2.97 (s, 3H). HRMS (ESI): calculated for $C_{10}H_{12}NOS$ $[M]^+$, 194.0634; found, 194.0647.

2. Spectral measurements

The NIRX-1 was dissolved in DMSO to make a 10 mM stock solution. The analytes stock solution (10 mM) were prepared in deionized water, SO_2 was added to the probe system in the form of HSO_3^-.

All absorption and fluorescence spectra were tested in a mixed solution (DMSO:PBS, 1:1, v/v, pH 7.4, 37 °C). The probe NIRX-1 (2 μL, 10 mM) was dissolved and diluted in a cuvette containing 2 mL of buffer solution, and then different concentrations of $NaHSO_3$ were added, and the spectra of the mixed solution were tested after incubation for 3 min at 37 ℃. The absorption spectra were collected from 350 nm to 850 nm. The FUCL spectra were collected in the range of 700–850 nm, λ_{ex}= 808 nm.

Selective and competitive experiments of probe NIRX-1 (10 μM) in the presence of $NaHSO_3$ (150 μM) and with potential interference (150 μM) were conducted in mixed solution after 3 minutes.

3. Cell culture and MTT assay

MCF-7 cells were provided by the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Cells were cultured in cell culture flasks at 37 ℃ in 5% CO_2 humidified environment.

The cytotoxicity of the probe to MCF-7 cells were studied by standard microculture tetrazolium (MTT) assays. MCF-7 cells were seeded in a 96-well plate, and these cells were cultured at 37 °C with humidified 5% CO_2 for 12 h. Fresh RPMI 1640 containing various concentrations of NIRX-1 (0-15 μM) was then added and incubated for another 24 h. The medium was then removed and 20 μL (5 mg mL^{-1}) of MTT/RPMI 1640 and 100 μL of RPMI 1640 solution was added. After 5 h, the medium was aspirated and the purple crystals were dissolved with 150 μL of DMSO. The absorbance at 490 nm was read by the microplate reader. The cell viability (%) = $(OD_{sample}) / (OD_{blank}) \times 100$ %.

4. Imaging of HSO_3^- in cells

MCF-7 cells were divided into four groups: (I) 10 μM NIRX-1 was used for fluorescence imaging after incubating the cells for 5 min, 15 min, 30 min, and 60 min, respectively. (II) For exogenous experiments, cells were pre-incubated with different concentrations of HSO_3^- (25, 50, and 100 μM) for 30 min, and then incubated with the probe NIRX-1 for 30 min. (III) For endogenous experiments, cells were pre-incubated with NEM (100 μM) for 30 min, and then incubated with NIRX-1 for 30 min. (IV) cells were pre-incubated with Cys (100 and 200 μM), and then incubated with NIRX-1 for 30 min. Imaging was then performed after PBS washing in bright-field and fluorescence mode. λ_{ex}=635 nm, λ_{em}=700–800 nm.

5. Imaging of HSO_3^- in vitro

The specification of Eppendorf (EP) pipe is 500 μL, 20 μM NIRX-1 was added into EP tubes 1–5 in turn, and then different concentrations of HSO_3^- (1-5: 0, 20, 40, 60, and 80 μM) was added to incubate for 10 min, and then imaged under Clinx IVScpoe 7550 imaging system. FUCL imaging: λ_{ex}=808 nm, a shortpass filter was used to collect signals at ≤765 nm. Fluorescence imaging: λ_{ex}=635 nm, a longpass filter was used to collect signals at ≥ 650 nm. The results were analyzed by Clinx IVScope EQ software.

6. Imaging of HSO_3^- in mice

Mice were anaesthetised and carefully shaved before imaging of the inflammation model. Mice were injected with 100 μL of lipopolysaccharide (LPS) solution (0, 1.0, and 2.0 mg/mL) at different concentrations in the abdomen to cause local inflammation 12 h later. Then FUCL imaging was performed after injecting probe NIRX-1 (20 μM) at the inflammation site for 30 min. All experiments were performed Clinx IVScpoe 7550 imaging system. FUCL imaging was performed using a 808 nm laser excitation, λ_{ex} = 808 nm, shortpass filter at < 765 nm (filter was purchased from Guangzhou Hengyang Electronic Technology, China). FL imaging was performed using a 635 nm laser excitation, λ_{ex} = 635 nm, longpass filter at ≥ 650 nm. The results were analyzed by Clinx IVScope EQ software.

7. Supporting Figures

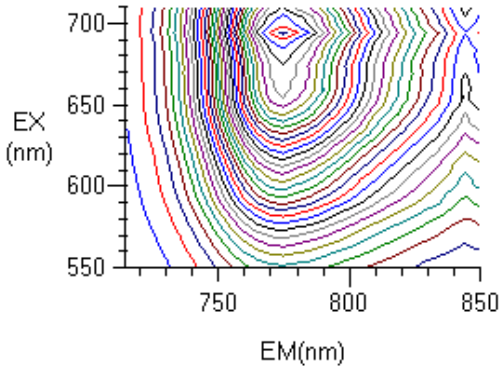

Figure S1 The 3D fluorescence of NIRX-1. λ_{ex} = 550-710 nm, λ_{em}= 715-850 nm. Intervals = 5 nm.

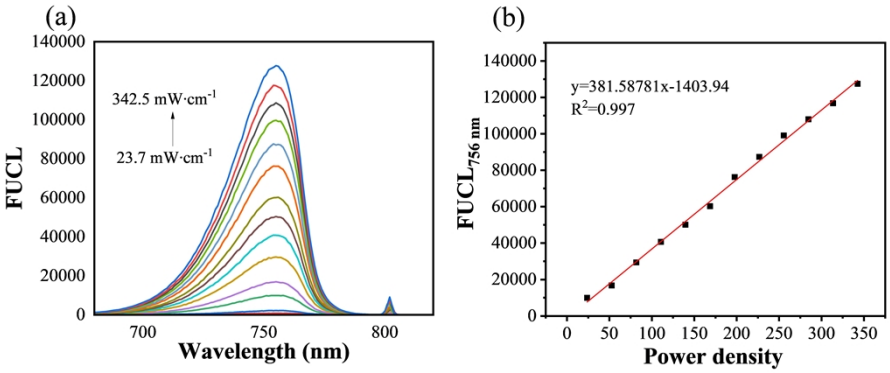

Figure S2 FUCL spectra (a) and FUCL intensity fitting plot (b) versus laser power density for NIRX-1

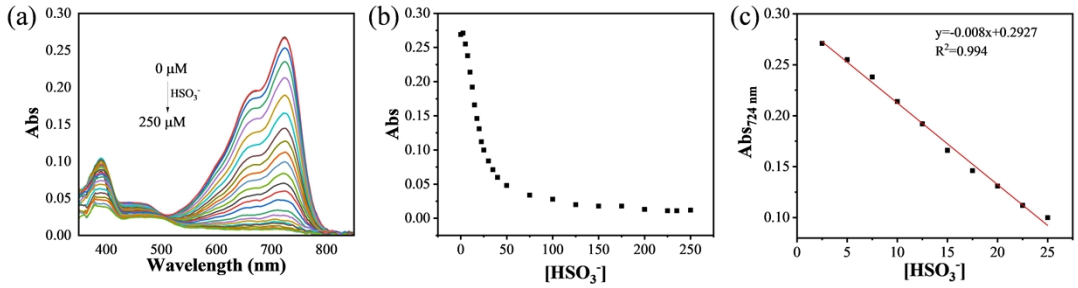

Figure S3 Absorption spectra (a) of NIRX-1 (10 μM) on addition of different amounts of HSO_3^- (0–250 μM). (b) The absorbance of the NIRX-1 at 724 nm under different concentrations of HSO_3^-, and (c) linear relationship between the absorbance at 724 nm and HSO_3^- concentration

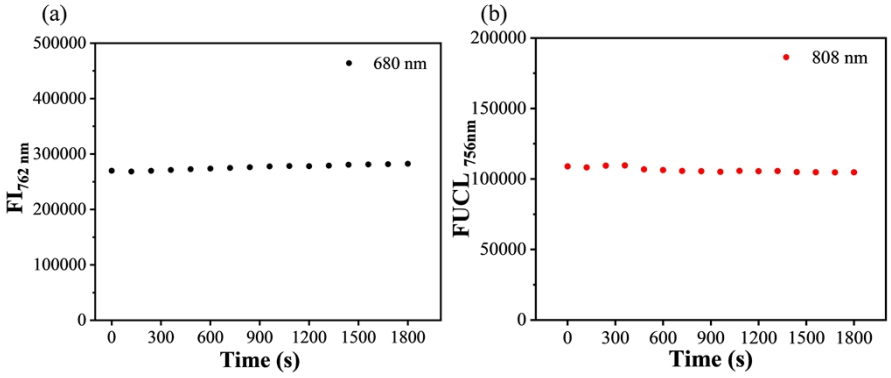

Figure S4 (a) Fluorescence intensity (λ_{ex}= 680 nm, 205.6 mW cm^{-1}) and (b) FUCL intensity (λ_{ex}= 808 nm, 208.7 mW cm^{-1}) for NIRX-1 versus time

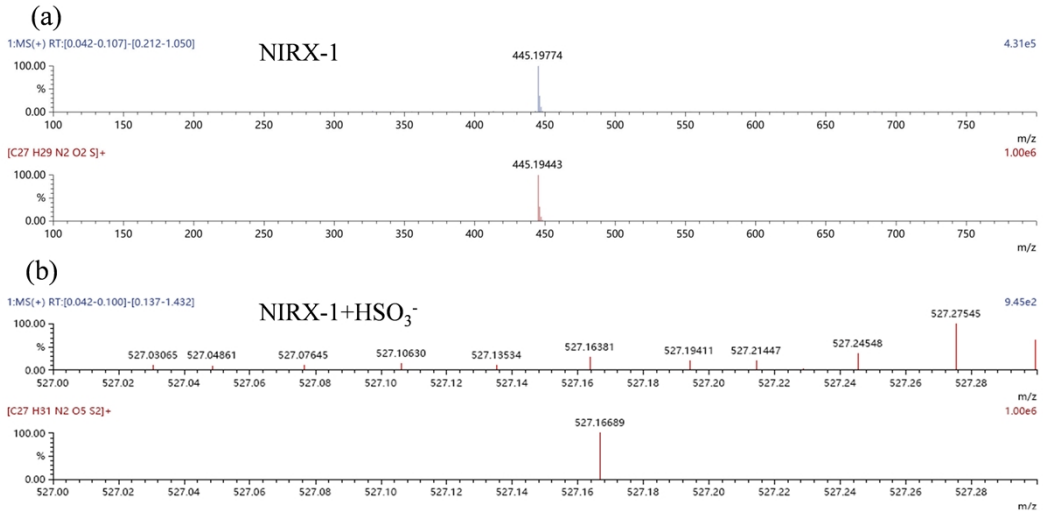

Figure S5 HR-MS for products of NIRX-1 upon addition of HSO_3^-

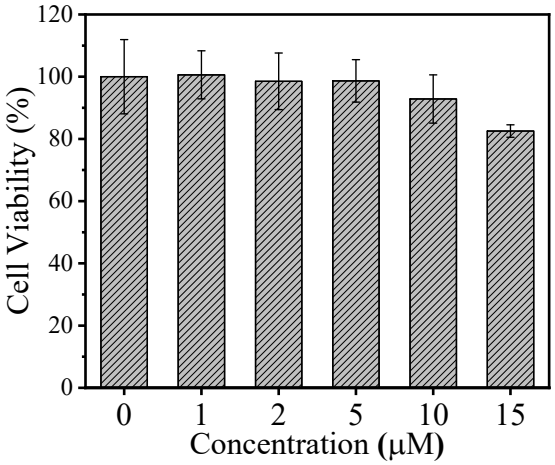

Figure S6 Cell viability of MCF-7 cells treated with the probe NIRX-1 (0–15 μM) for 24 h

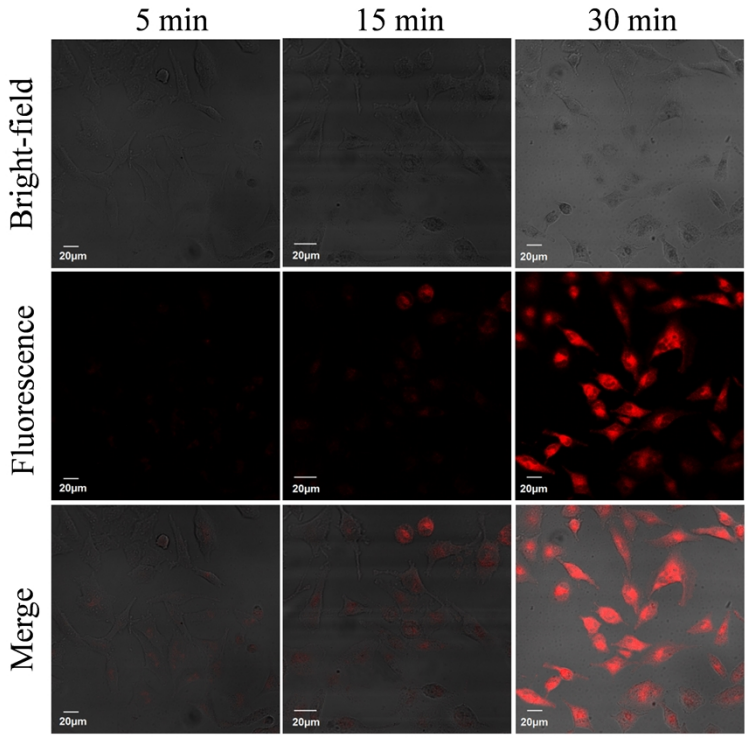

Figure S7 Fluorescence imaging of MCF-7 cells incubated with 10 μM NIRX-1 at different times, λ_{ex}= 635 nm, $\lambda_{collect}$= 700-800 nm

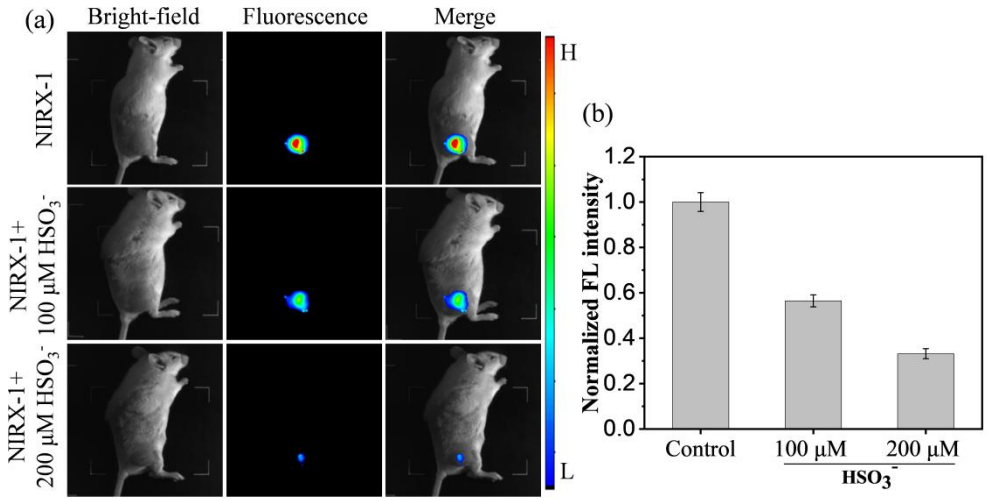

Figure S8 Fluorescence imaging of HSO_3^- in mice. (a) Control group: mice were injected subcutaneously with NIRX-1 (20 μM) and imaged after 30 min. Probe +HSO_3^- group: mice were pre-injected with HSO_3^- (100 and 200 μM), then injected with NIRX-1 (20 μM), and imaged 30 min later. (b) Relative fluorescence intensity found in Figrue A. λ_{ex} = 635 nm, $\lambda_{collect} \geq$ 650 nm

8. NMR and HR-MS data

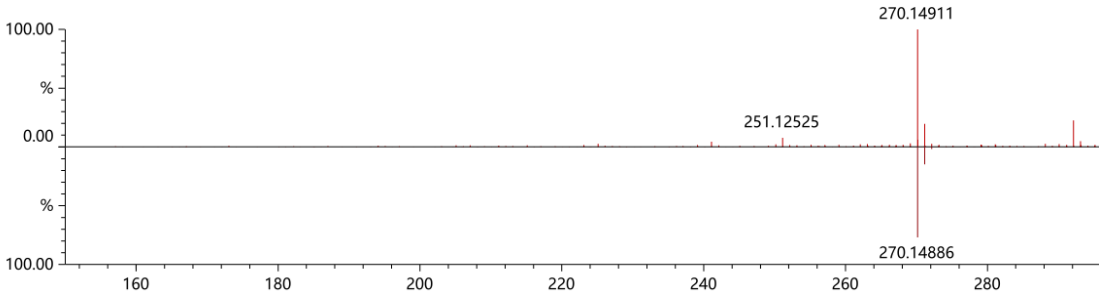

Figure S9 HR-MS of compound B

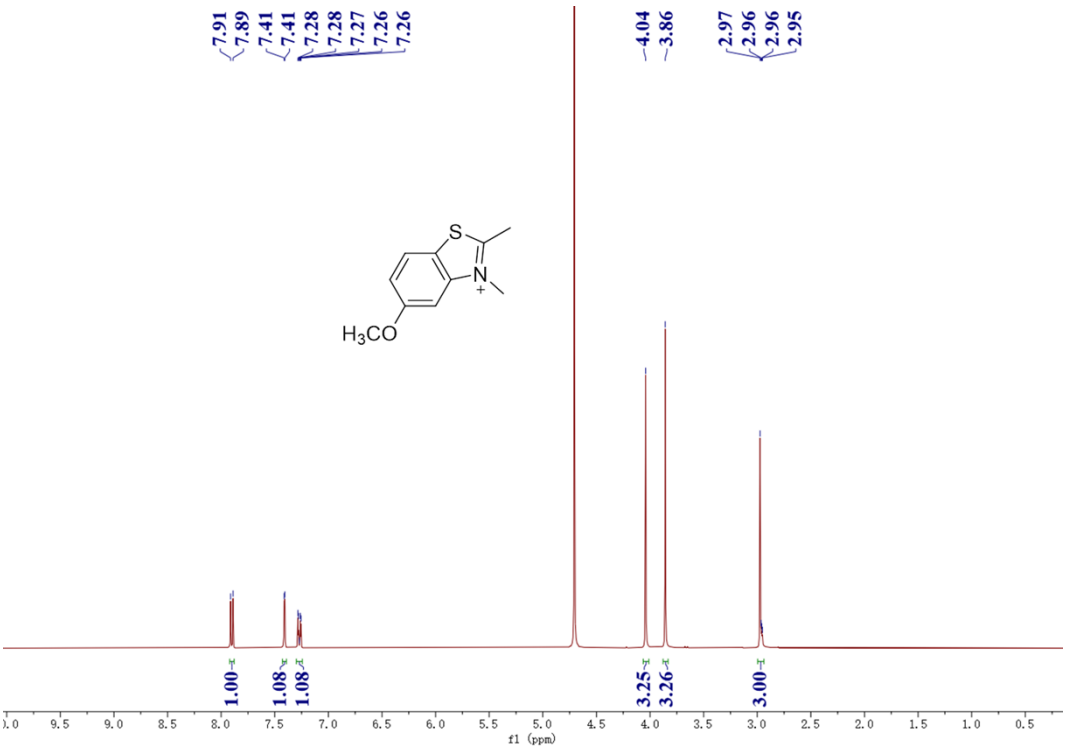

Figure S10 ^{1}H NMR of compound C

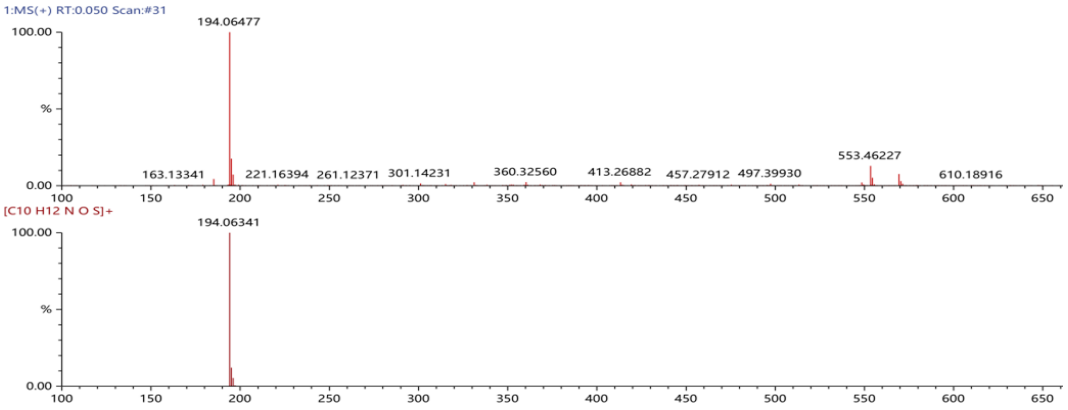

Figure S11 HR-MS of compound C

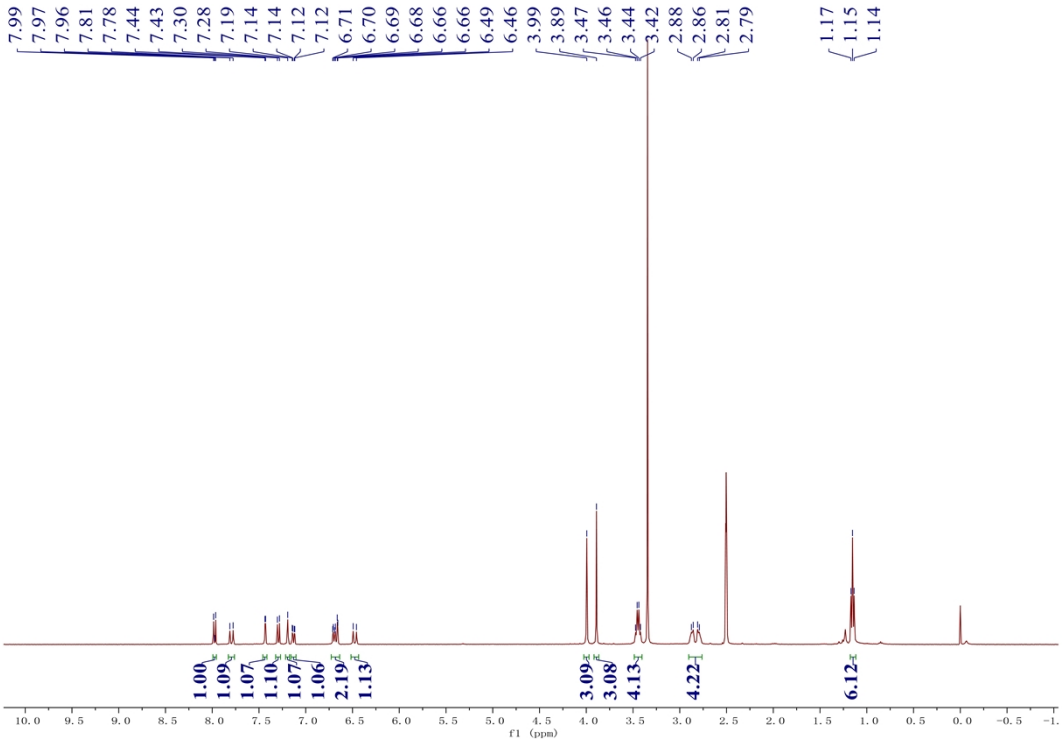

Figure S12 ^{1}H NMR of NIRX-1

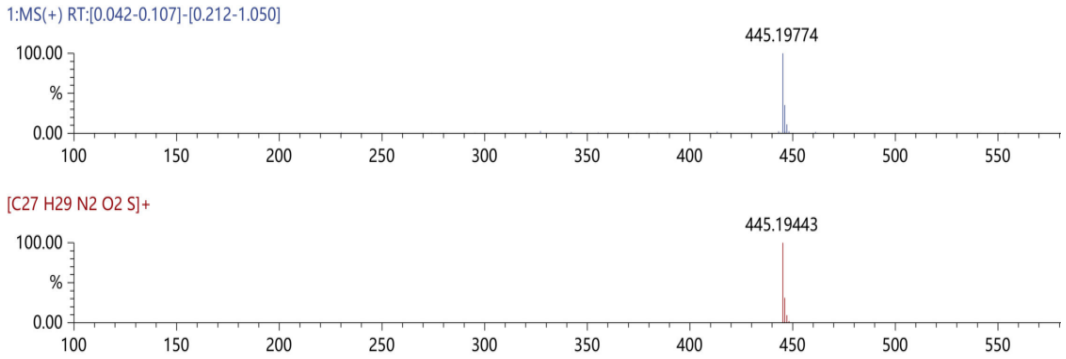

Figure S13 HR-MS of NIRX-1