Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Near-infrared fluorescent probe for in-*situ* imaging SO_2 Flux in drug-induced liver injury

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1. Reagents and Instruments

Materials

All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. PBS buffer solution was obtained by mixing of 0.05 mol/L Na_2HPO_4 water solution and 0.05 mol/L KH_2PO_4 water solution with the volume ratio 4:1. Distilled water was used after passing through a water ultra-purification system. All chemicals and solvents used were of analytical grade. All solution samples were made by dissolving their each solid in water or DMSO.

Instruments

TLC analysis was performed using precoated silica plates. Ultraviolet–visible (UV–*vis*) spectra were recorded on U-3900 UV-Visible spectrophotometer. Edinburgh Integrated fluorescence spectrometer FS5 was used to measure the fluorescence spectrum. Shanghai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ^{1}H NMR and ^{13}C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI-MS was measured with a Thermo Scientific Q Exactive. The cells imaging experiments were measured by a Zeiss LSM880 Airyscan confocal laser scanning microscope. The imaging assays of living body were performed in Bruker In-Vivo FX Pro small animal optical imaging system.

2. Synthesis and characterization

Synthesis

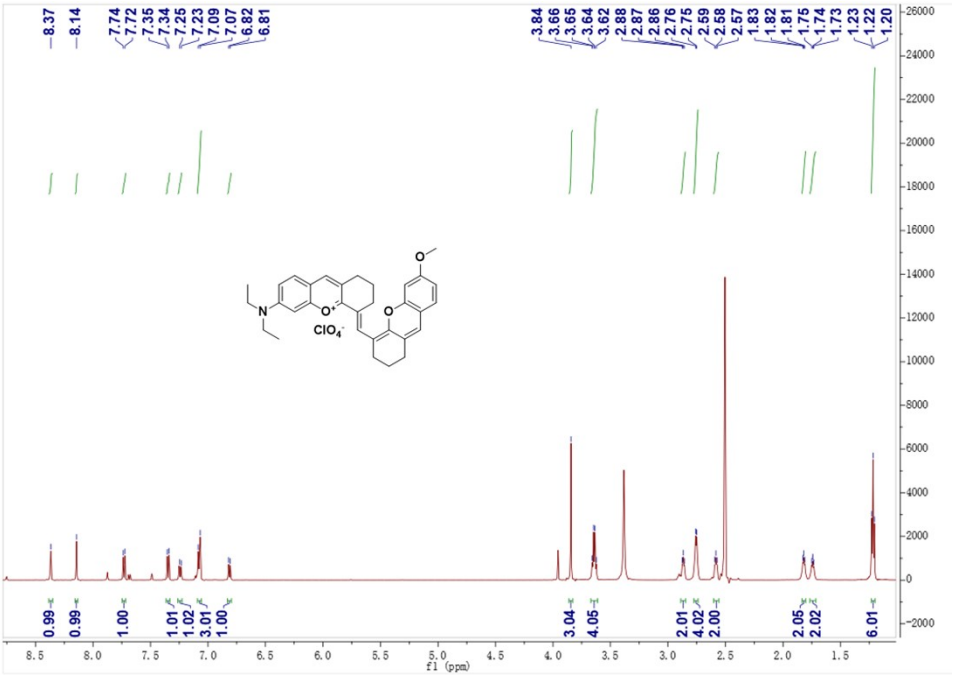
Compounds A and B were synthesized according to literature reports.[1-2]

Synthesis of **NTO**. Compound A (0.71 g, 2 mmol,) and compound B (0.97 g, 4 mmol,) were added to 25 mL glacial acid. The reaction system was refluxed at 110 °C. After reaction for 2 h, cool to room temperature. Vacuum concentrate to removes solvent and obtains crude product. This was followed by purification using a silica gel column chromatography (CH_2Cl_2/CH_3OH=20/1) to obtain a brown-black solid probe **NTO** (0.682 g, 71%). ^{1}H NMR (600 MHz, DMSO-*d6*) δ 8.37 (s, 1H), 8.14 (s, 1H), 7.73 (d, *J* = 9.2 Hz, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.24 (d, *J* = 9.2 Hz, 1H), 7.08 (d, *J* = 9.9 Hz, 3H), 6.81 (d, *J* = 8.5 Hz, 1H), 3.84 (s, 3H), 3.64 (q, *J* = 7.0 Hz, 4H), 2.87 (t, *J* = 5.5 Hz, 2H), 2.75 (d, *J* = 5.7 Hz, 4H), 2.60 −

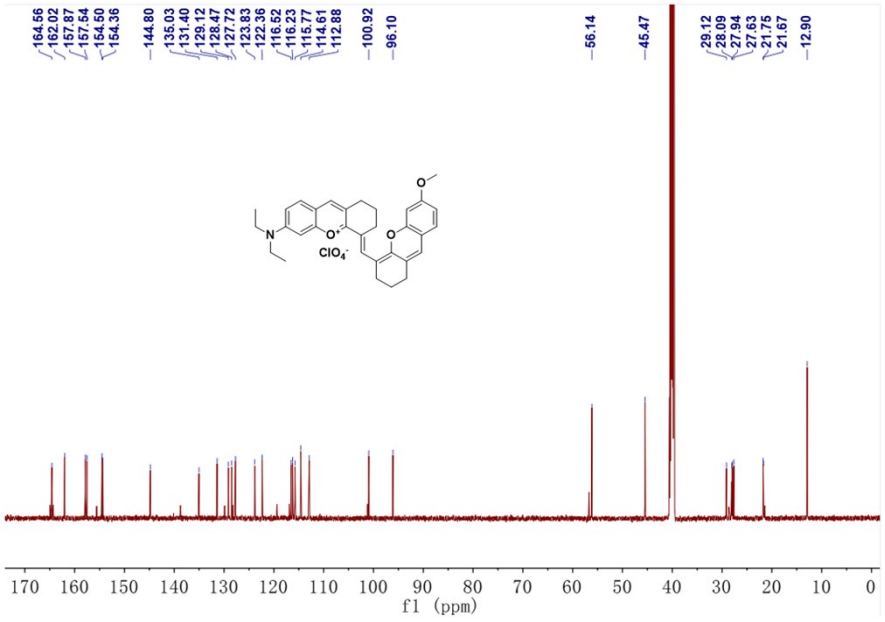
2.56 (m, 2H), 1.83 − 1.80 (m, 2H), 1.77 − 1.71 (m, 2H), 1.22 (t, J = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-*d6*) δ 162.02, 157.87, 157.54, 154.50, 144.80, 135.03, 131.40, 129.12, 128.47, 127.72, 123.83, 122.36, 116.52, 116.23, 115.77, 114.61, 112.88, 100.92, 96.10, 56.14, 45.47, 29.12, 28.09, 27.94, 27.63, 21.75, 21.67, 12.90. ESI-MS m/z: $[C^+H]^+$ calculated for $[C_{32}H_{34}NO_3]^+$, 480.25332, found, 480.25399.

Characterization

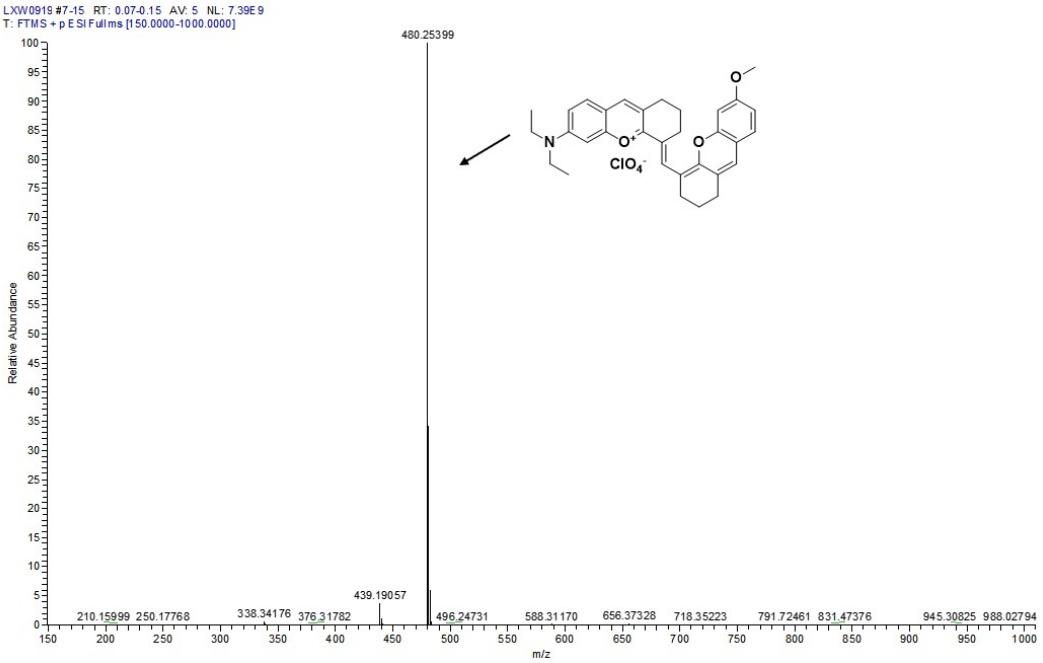
Figure S1: Structure characterization of probe **NTO.**



^{1}H-NMR spectrum of **NTO** in DMSO-d_6



^{13}C-NMR spectrum of **NTO** in DMSO-d_6



ESI-MS m/z: $[C+H]^+$ calculated for $[C_{32}H_{34}NO_3]^+$, 480.25332, found, 480.25399.

3. Spectral responses of probe NTO toward HSO_3^-

Figure S2: The linear relationship between fluorescence intensity and HSO_3^-.

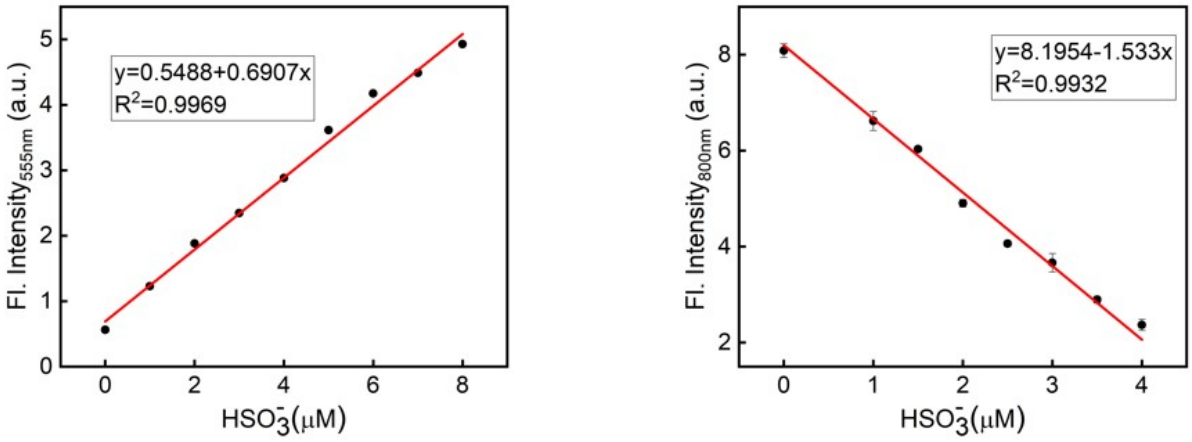
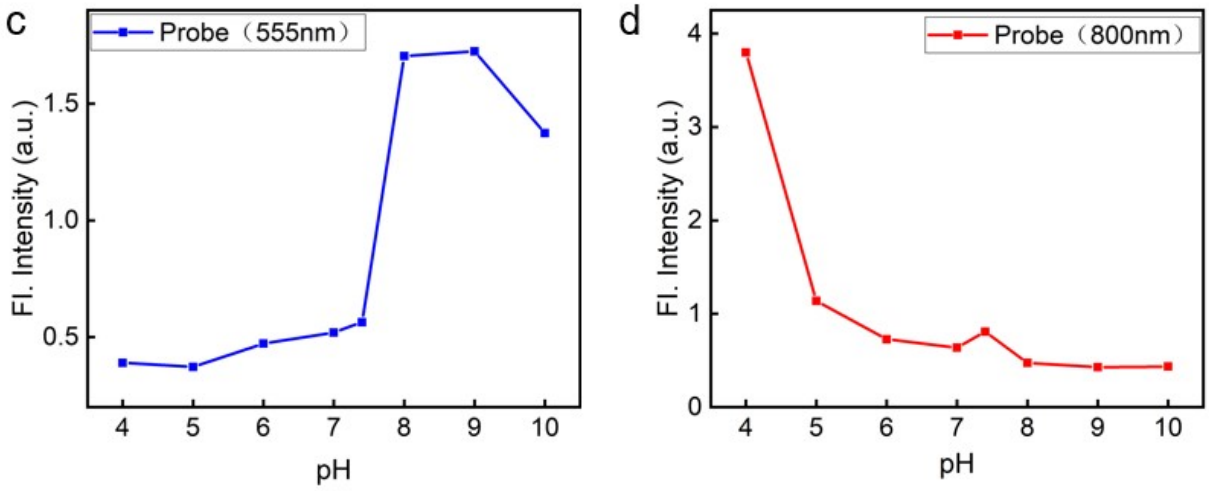


Figure S3: pH interference of the probe **NTO** at 800 nm and 555 nm.



4. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence spectra of free probe **NTO** were measured by ten times and its standard deviation was obtained. To gain the slope, the fluorescence intensities at 555 nm were plotted as the increasing concentrations of the corresponding HSO_3^-. So, the detection limit was calculated with the following equation:

$$LOD = 3\sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensities versus the concentrations of HSO_3^-.

5. Cytotoxicity assays

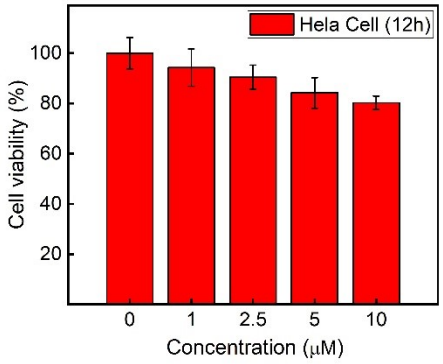


Figure S4. HeLa cells viability with different concentration **NTO**.

The cell viability of HeLa cells, treated with probe **NTO**, was assessed by a cell counting kit-8 (CCK-8). Briefly, HeLa cells, seeded at a density of 1×10^6 cells / mL on a 96-well plate, were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 16 h. Then, the cells were treated with increasing amounts of **NTO** (0, 1, 2.5, 5, 10 μM) for 12 h. Subsequently, CCK-8 solution was added into each well for 50 min, and absorbance at 450 nm was measured (Figure S4).

6. Bioimaging

Living mice 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 (14 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. Mouse model of acute liver injury was established by intraperitoneal injection of APAP. APAP was dissolved in warm saline (15 mg/mL). The study divided mice into two groups. The first group of mice was the control group, which was intraperitoneally injected with normal saline. Mice in the second group were intraperitoneally injected with 300 mg/kg APAP. All mice were intravenously injected with 100 μL PBS (pH=7.4, containing 1% DMSO and 200 μM probe **NTO**) 12 h after APAP at injection. In vivo imaging was performed 15 min after injection to assess changes in SO_2 during liver injury. (λ_{ex} = 720 nm, λ_{em} = 780-810 nm, power density on the surface of mice was set to 7 mw/cm^2, exposure time was set to 500 ms).

7. Determination of SO_2 in food.

Determination of HSO_3^- in food samples.

The liquid sample was diluted 10 times with distilled water. The solid sample was first crushed, then 0.25 g was weighed and dissolved in 2 mL distilled water. 20 μL of sample solution was taken into the test system to react with **NTO** (20 μM) for 30 s. The recovery is determined by adding HSO_3^- (5, 10, 20 μM). Then, the fluorescence change of the **NTO** at 555 nm was measured. Each experiment was conducted in parallel three times.

Sample	Content (g/kg)	Chinese Standards for Food Additives-GB2760-2015 (g/kg)	RSD (%)
Beer	0.0089	0.01	1.436
Seabuckthorn juice	0.0214	0.05	1.322
Crystal sugar	0.055	0.1	0.459
Starch	0.0281	0.03	0.136
Yuba	0.0925	0.2	1.841
Raisins	0.0133	0.1	2.102
Fungus	0.0272	0.05	1.586
Dried vegetable	0.1952	0.4	2.498

Table S1: Comparison of HSO_3^- content determination values of various foods with national food additive standards.

5. References

(1) Wu, Y.; Yin, C.; Zhang, W.; Zhang, Y.; Huo, F. *Anal. Chem.* 2022, **94**, 5069−5074.

(2) Yang, Y.; Zhou, T.; Jin, M.; Zhou, K.; Liu, D.; Li, X.; Huo, F.; Li, W.; Yin, C. *J. Am. Chem. Soc.* 2020, **142**, 1614−1620.