Electronic Supplementary Information

An Activatable Probe for Detecting Alcoholic Liver Injury via Multispectral Optoacoustic Tomography and Fluorescence Imaging

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General experimental procedures

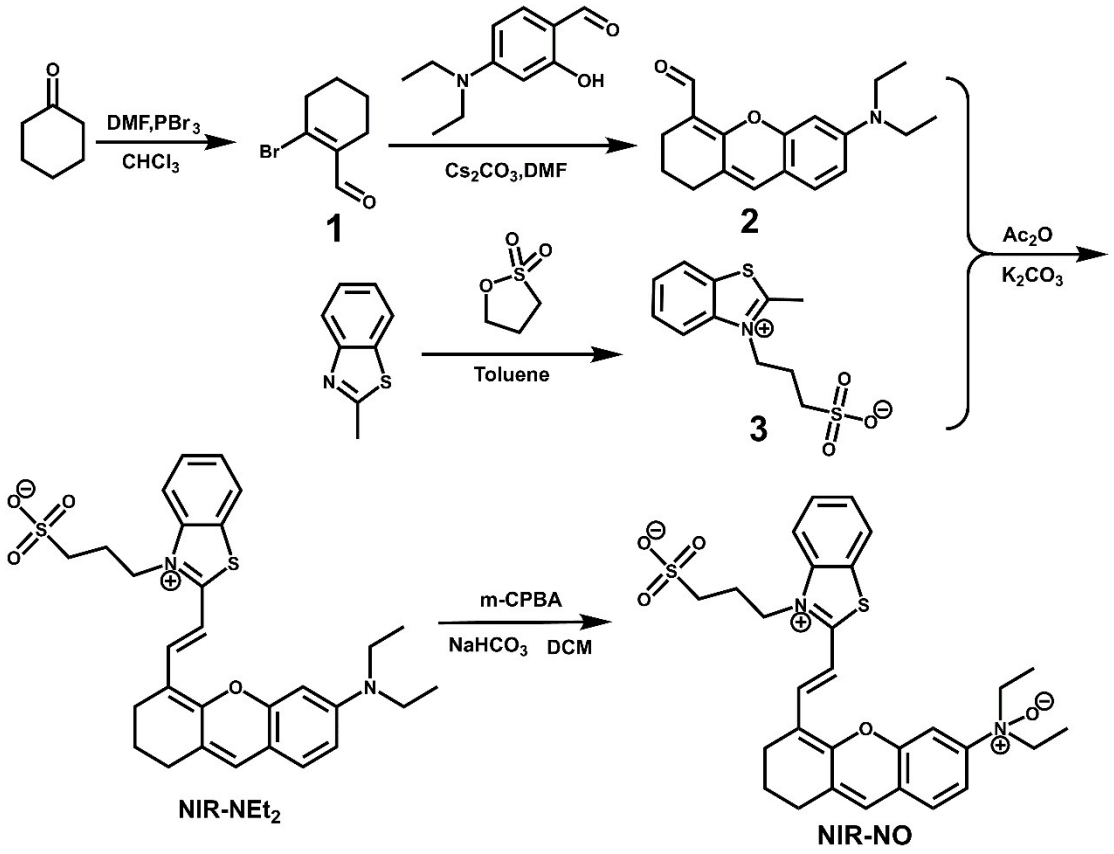
1. Reagents and Materials：

Phosphorus tribromide (PBr_3), cyclohexanone, 4-(diethylamino) salicylaldehyde, cesium carbonate (Cs_2CO_3), 2-methyl-1,3-benzothiazole, 1,3-propanesultone, potassium carbonate (K_2CO_3), sodium bicarbonate ($NaHCO_3$) and 3-chloroperbenzoic acid (m-CPBA) were purchased from Aladdin Reagents and used as received. Mouse liver microsomes was purchased from Wuxi Xinrun Biological Technology Co., Ltd. Cytochrome P450 reductase was purchased from Sigma. All solvents were of spectroscopic grade and used as received.

2. Apparatus

^1H NMR spectra were obtained with a Bruker Avance 600 MHz NMR Spectrometer. High resolution mass spectrometer (MS) was recorded on a Bruker MAXIS IMPACT mass spectrometer. HPLC measurements were carried out on Agilent 1260 Infinity liquid chromatograph (with DAD). UV-vis spectra were collected on a Hitachi U-3010 UV-vis spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4700 fluorescence spectrophotometer. Fluorescence imaging in vivo and ex vivo was performed on AMI small animal fluorescence imaging system (Spectral Instruments Imaging Co.). Optoacoustic imaging was conducted with inVision128 multispectral optoacoustic tomographic (MSOT) imaging system (iThera Medical GmbH).

3. Synthetic Procedures



Scheme S1. Synthetic route of NIR-NO

Synthesis of compound 1 and compound 2:

A solution of DMF (12 mL, 155 mmol) and $CHCl_3$ (50 mL) were placed in a 250mL round-bottomed flask at 0 °C. Subsequently PBr_3 (9 mL, 94.7 mmol) was added slowly to the flask and stirred for 45 min at 0 ℃. After that, cyclohexanone (4 mL, 39 mmol) was added and the mixture was stirred for 16 h at room temperature. After completion of the reaction, the resultant mixture was poured into ice water and then $NaHCO_3$ was added slowly until pH ≈ 7. The mixture was poured into dichloromethane and washed three times with brine. The organic layer was then separated and dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, and compound 1 was obtained.

4-(Diethylamino) salicylaldehyde (487 mg, 2.52 mmol) and Cs_2CO_3 (2.4 g, 7.36 mmol) were dissolved in 12 mL anhydrous DMF in a 50 mL round-bottomed flask. Compound 1 (980 mg, 5.12 mmol) was dissolved in 2 mL anhydrous DMF and subsequently injected into the flask. The mixture was stirred at room temperature for 48 hours. Afterward, the resulting mixture was filtered, and the filtrate was concentrated under reduced pressure. Then the mixture was dissolved into dichloromethane and washed with brine for three times. The combined organic layer was dried over anhydrous Na_2SO_4 and the crude product was purified by silica gel column chromatography CH_2Cl_2 / AcOEt (100:1 to 20:1, v/v) as an eluent to obtain compound 2 as an orange solid. Yield: 506 mg (71 %) ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.28 (s, 1H), 7.02-7.01 (d, J = 8.6 Hz, 1H), 6.64 (s, 1H), 6.47 (s, 1H), 6.41 (s, 1H) , 3.38-3.41 (t, J = 7.1 Hz, 4H), 2.56 – 2.54 (m, 2H), 2.46-2.44 (t, J = 6.1 Hz, 2H), 1.72 – 1.68(m, 2H), 1.22-1.19 (t, J = 7.1 Hz, 6H). ESI-MS (m/z) $[M+H]^+$ calcd. 284.1650, found 284.1651.

Synthesis of compound 3:

2-Methyl-1,3-benzothiazole (1 g, 6.70 mmol) was dissolved in toluene (5 mL), and then 1,3-propanesultone (1.23 g, 10.05 mmol) was added. The mixture was heated at 120 °C for 4 h in a sealed tube. The reaction mixture was dissolved in minimum quantity of methanol and recrystallized by excess amount of diethyl ether for three times, providing the white powder (1.5 g, 83 %). ^{1}H NMR (600 MHz, CD_3OD) δ 8.39-8.38 (d, J = 8.6 Hz, 1H), 8.29-8.27 (d, J = 8.2 Hz, 1H), 7.92 – 7.90 (m, 1H), 7.82-7.79 (t, J = 7.8 Hz,

1H), 5.01 – 4.99(m, 2H), 3.24 (s, 3H), 3.04 – 3.02 (m, 2H), 2.38 (m, 2H). ESI-MS (m/z) $[M+Na]^+$ calcd. 294.0235, found 294.0234.

Synthesis of NIR-NEt_2:

In a dry nitrogen atmosphere, compound 2 (177 mg, 0.625 mmol) and compound 3 (203 mg, 0.75 mmol) were dissolved with 5 mL anhydrous Ac_2O in a 50 mL one-neck round-bottom flask. Then K_2CO_3 (173 mg, 1.25 mmol) was added subsequently and stirred for overnight at room temperature. At the end of reaction, the mixture was concentrated and the resulting residue was dissolved in dichloromethane and washed with brine for three times. The combine organic layer was dried with anhydrous Na_2SO_4 and purified using silica chromatography using CH_2Cl_2/CH_3OH (10:1, v/v) as an eluent to give a blue-green solid. (300 mg, 90 %). ^1H NMR (600 MHz, DMSO-d_6) δ 8.25-8.23 (d, *J* = 14.1 Hz, 1H), 8.14-8.13 (d, *J* = 7.9 Hz, 1H), 8.04-8.03 (d, *J* = 8.4 Hz, 1H), 7.67-7.65 (t, *J* = 7.8 Hz, 1H), 7.55-7.53 (t, *J* = 7.6 Hz, 1H), 7.35-7.34 (d, *J* = 8.6 Hz, 1H), 7.31 (s, 1H), 6.91-6.88 (d, *J* = 14.1 Hz, 1H), 6.77 (s, 2H), 4.79-4.76(t, *J* = 7.6 Hz, 2H), 3.51-3.47 (d, *J* = 7.1 Hz, 4H), 2.66 (s, 4H), 2.63-2.61 (d, *J* = 6.6 Hz, 2H), 2.10 (s, 2H), 1.79 (s, 2H), 1.19-1.16 (t, *J* = 7.0 Hz, 6H). ESI-MS (m/z) $[M+H]^+$ calcd. 537.1881, found 537.1879.

Synthesis of NIR-NO:

To a solution of NIR-NEt_2 (100 mg, 0.1865 mmol) in dry CH_2Cl_2 (5 mL) was added $NaHCO_3$ (38 mg, 0.4476 mmol) and stirred for 15min at 0 ℃. Subsequently, m-CPBA (80 % w/w，96 mg, 0.4476 mmol) was dissolved with 2 mL CH_2Cl_2 and added slowly into the flask in four portions every 30 minutes. At the end of last addition, the mixture was quenched with saturated $NaHCO_3$ and concentrated by vacuum-rotary evaporation procedure. Then the crude product was purified with neutral alumina chromatography using CH_2Cl_2/CH_3OH (10:1, v/v) as an eluent to afford a purple solid. (20 mg, 20 %). ^1H NMR (600 MHz, CD_3OD) δ 8.55-8.53 (d, *J* = 14.7 Hz, 1H), 8.33-8.31 (d, *J* = 13.7 Hz, 1H), 7.90-7.89 (d, *J* = 7.3 Hz, 1H), 7.68-7.66 (t, *J* = 7.7 Hz, 1H), 7.59-7.56 (d, *J* = 8.3 Hz, 1H), 7.51-7.49 (d, *J* = 8.5 Hz, 1H), 7.27-7.26 (d, *J* = 8.8 Hz, 1H), 7.20-7.18 (d, J = 13.9 Hz, 1H), 7.04 (s, 1H), 6.66-6.64 (d, *J* = 14.2 Hz, 1H), 4.98-4.93 (d, *J* = 15.8 Hz, 4H), 4.67– 4.65 (m, 2H), 3.00 (dt, *J* = 14.1, 6.2 Hz, 4H), 2.65 (s, 2H), 2.35 (s, 2H), 1.90 (s, 2H), 1.17-1.15 (t, *J* = 7.0

Hz, 6H). ESI-MS (m/z) $[M+H]^+$ calcd. 553.1831, found 553.1833.

4. Measurement of absorption and fluorescence spectra

The cytochrome P450 reductase activity assays were carried out according to the following procedures. The probe NIR-NO was prepared in a stock solution (1 mM) in PBS (10 mM, pH = 7.4). PBS (10 mM, pH 7.4) and NADPH (500 μM) were degassed with N_2 for 30 min before use.

For concentration-dependent experiments, CYP450 reductase with different levels from 0 - 250 μg/mL was added into PBS and followed by the probe NIR-NO (final concentration 10 μM). The solutions were kept at 37 ℃ for 15 min under N_2 atmosphere. Then the absorption and fluorescence spectra of each of the solutions were measured.

For time-dependent experiments, the reactions were kept at 37 °C for different time period before spectral measurements.

For experiments with mouse liver microsomes (MLM), the mouse liver microsomes with 400 μg/mL added into PBS (10 mM, pH 7.4) containing NADPH (500 μM) under nitrogen atmosphere. Then the absorption and fluorescence spectra of the solutions were measured.

For selectivity experiments, the stock solutions of hyaluronidase, γ-glutamyl transferase, β-D-galactosidase, alkaline phosphatase, acetylcholinesterase, nitroreductase and heparinase were prepared in distilled water. The solution of NIR-NO was incubated with a certain amount of analyte for 10 min in PBS (10 mM, pH 7.4) containing NADPH (500 μM) at 37 ℃. Then the fluorescence spectra and the relative optoacoustic intensity of the solutions were measured. The fluorescence emission spectra were measured with the excitation at 700 nm. The excitation slit and emission slit were both set at 10.0 nm.

5. Cell culture

HepG2 cells and L929 cells were obtained from KeyGen Biology Co. Ltd (Nanjing, China). HepG2 cells were incubated in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS, 1% penicillin and streptomycin at 37 °C in an incubator (containing 5% CO_2 gas). When the cell density reached 70-80% of confluence, sub-culturing was regarded

complete. The medium was changed approximately every 1-2 days. Hypoxic incubator was performed by incubating the cells in an Anaero Pack® (Mitsubishi Gas Chemical Company, Inc.) and a 2.5 L hypoxic incubator chamber (Mitsubishi Gas Chemical Company, Inc.).

6. Cell viability assays

The in vitro cytotoxicity of probe was evaluated by MTT assay in L929 and HepG2 cells. Briefly, the cells were seeded in 96-well plates at 5000 cells per well and cultured for 24 h. The medium was washed three times with PBS and incubated with various concentrations (0, 5, 10, 20, 30, 50 μM) of the probe for an additional 24 h. After that the medium was replaced with new medium containing 0.5 mg/mL MTT solution for another 4 h. Finally, the medium was washed three times with PBS solution and replaced with 150 μL DMSO in order to dissolve the precipitates. The thermo MK3 ELISA reader was used to measure the absorbance at 570 nm and estimate the viability of cells.

7. Cell imaging

The HepG2 cells ($1x10^6$) were seeded in the 6-well plates and incubated for 2 h. After cells adhering to the plates, the cells were washed with PBS and then incubated in medium supplemented with 10% FBS containing the various concentrations (10, 30, 50 μM) of the probe at 37 °C under 5% CO_2 for 4 h under normoxia or hypoxia. As for the inhibition experiment, the cells were pretreated with 500 μM diphenyliodonium chloride for 2 h. As for cell culture under hypoxia, an O_2 concentrations of 0.1% was generated with an Anaero Pack® (Mitsubishi Gas Chemical Company, Inc.) and a 2.5 L hypoxic incubator chamber (Mitsubishi Gas Chemical Company, Inc.) Afterwards, the treated cells were washed, tryosinized, centrifuged, suspended in PBS and then subjected to fluorescence imaging using Ami small animal imaging system (Spectral Instruments Imaging Co.) (Excitation filter: 710 nm, emission filter: 750 nm) and optoacoustic imaging (Excitation: 718 nm. In the MSOT system, the wavelength of excitation laser light is in the range of 680 – 980 nm). For cell experiments in MSOT imaging, the control (PBS) or the treated cells suspended in PBS were fully filled in commercial Wilmad NMR

tubes respectively and fixed on the holder of the imaging instrument.

8. Phantom optoacoustic imaging

Phantom optoacoustic imaging was conducted with MOST system(in Vision 128, iThera Medical GmbH).The test solutions containing 10 μM NIR-NO in PBS (10 mM, pH = 7.4), 500 μM NADPH and different concentrations of CYP450 reductases (0, 50, 100, 150, 200, 250 $\mu g \cdot mL^{-1}$) were stirred for 15 min at 37 °C under hypoxia condition. Then the solutions were added into commercial Wilmad NMR tubes for phantom optoacoustic imaging at room temperature.

9. Animal experiments

The BALB/c mice (male, 6-7 weeks old) were purchased from Guangdong Medical Laboratory Animal Center (GDMLAC) and kept in the Laboratory Animal Center of South China Agricultural University. All animal operation was carried out in accordance with the guidelines for the care and use of laboratory animals, as well as in accordance with the regulations on the management of laboratory animals of China and the Regulations on the Administration of Laboratory Animals of Guangdong Province.

10. Mice model of alcoholic liver injury

The BALB/c mice (male, 6-7 weeks old) were randomly divided into four groups (n = 5 per group).

For alcohol-induced liver injury model, the animals were treated with varied dosage of ethanol (4 g/kg or 8 g/kg) via oral gavage once a day for three consecutive days.

For liver rehabilitation model, animals were administered with 200 mg/kg of metadoxine via oral gavage once a day after 2 hours of 8 g/kg ethanol gavage for three consecutive days.

For the control group, the animals were treated with isovolumic PBS solution by oral gavage.

11. Fluorescence imaging

In the study, six hours after the mice were last treated with ethanol gavage (on the third day of ethanol gavage), the mice were anesthetized with 2% isoflurane in oxygen and then were given an intravenous injection of 2.7 mg·kg^{-1} NIR-NO for vivo fluorescence imaging.

For fluorescence imaging of organs, six hours after the mice were last treated with ethanol or PBS gavage (on the third day of gavage), 10 min after the probe injection, mice were sacrificed by CO_2 asphyxiation, then the major organs, such as heart, liver, spleen, lung and kidney were collected for fluorescence imaging. For fluorescence imaging, the experiments were conducted on the excitation filter of 710 nm and emission filter of 750 nm.

12. Optoacoustic imaging

All in vitro / ex vivo phantom and in vivo mice optoacoustic imaging experiments were performed on a multispectral optoacoustic tomographic imaging system (in Vision 128, iThera Medical GmbH). For in vivo optoacoustic experiment, the mice were anesthetized by 2% isoflurane delivered via a nose cone for the duration of the experiments. A catheter was then inserted into the tail vein and the mice were placed in the prone position in a water bath maintained at 34 ℃, and anesthesia and oxygen are supplied through a breathing mask. Then 2.7 mg·kg^{-1} of NIR-NO in PBS was injected into the mice via tail vein. The following imaging wavelengths were selected for correspondence with the major turning points in the absorption spectra of NIR-NEt_2 and endogenous photoabsorbers including hemoglobin: 680, 690, 700, 710, 718, 720, 750, 800 (background), 850, 900 nm. For each wavelength, we recorded 10 individual frames. In vivo MSOT images were acquired before probe injection (0 min) and at different time points post probe injection (e.g. 5, 10, 15, 30 min) using the MOST system. Cross-sectional images were acquired with a step size of 0.3 mm spanning through the liver region. For ex vivo optoacoustic experiment, 10 min after the probe injection, mice were sacrificed by CO_2 asphyxiation, then the major organs were collected for MSOT imaging. In this study, the MSOT images were reconstructed using

a standard backprojection algorithm. After the images are generated, the z-stack was rendered as orthogonal MIP images. Afterwards, guided ICA spectral unmixing was utilized to separate signals from the activated probe and those from the photo-absorbing tissue elements in the body (e.g. hemoglobin). In in-vivo mouse optoacoustic imaging experiments, for each group, five mice were tested.

13. Tissue histological evaluation

For the study of biosafety, mice were i.v. injected with the probe or PBS for 24 h. Then the major organs such as heart, liver, spleen, lung, and kidney were excised and tissue-fixed for hematoxylin and eosin (H&E) staining.

For the evaluation of alcohol-induced liver injury, 6 h after the last treatment (ethanol gavage) on the third day, the mice in four different groups were euthanized and the liver was excised and then embedded in paraffin and sectioned to 4 μm for H&E staining.

14. Serum biochemistry assessment

Serum biochemistry index alanine aminotransferase (ALT) was measured by Elisa kits.

15. Relative OA intensity

Relative OA intensity was calculated with the equation:

For measurements involving CYP450 reductase in PBS, relative OA intensity = $[(OA_{718})_{\text{CYP450 reductase}} - (OA_{718})_{\text{control}}]/ (OA_{718})_{\text{control}}$.

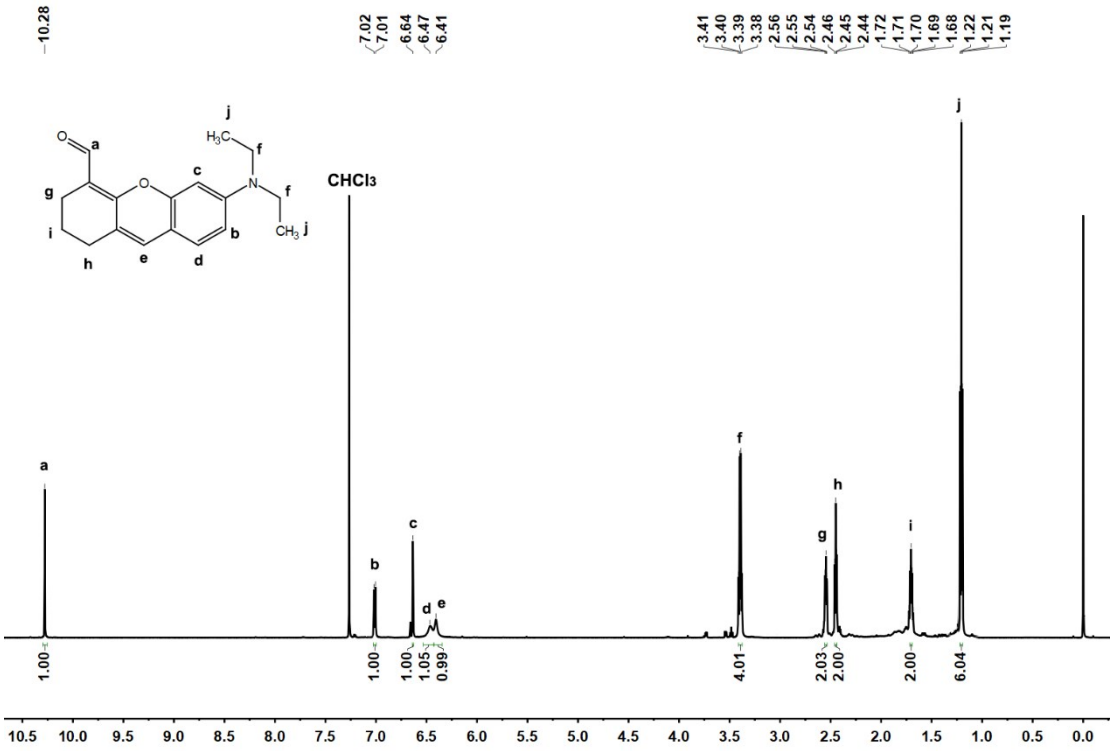


Fig. S1 ^{1}H NMR Spectrum of Compound 2 (600 Hz, $CDCl_3$).

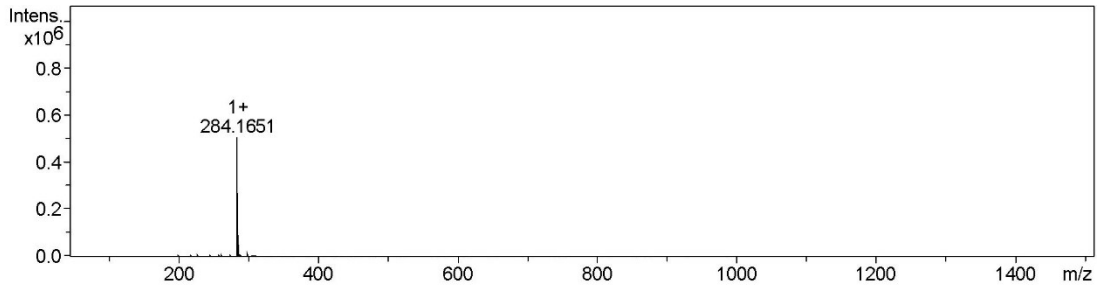


Fig. S2 HR Mass spectrum of Compound 2.
m/z $[M+H]^+$ 284.1651

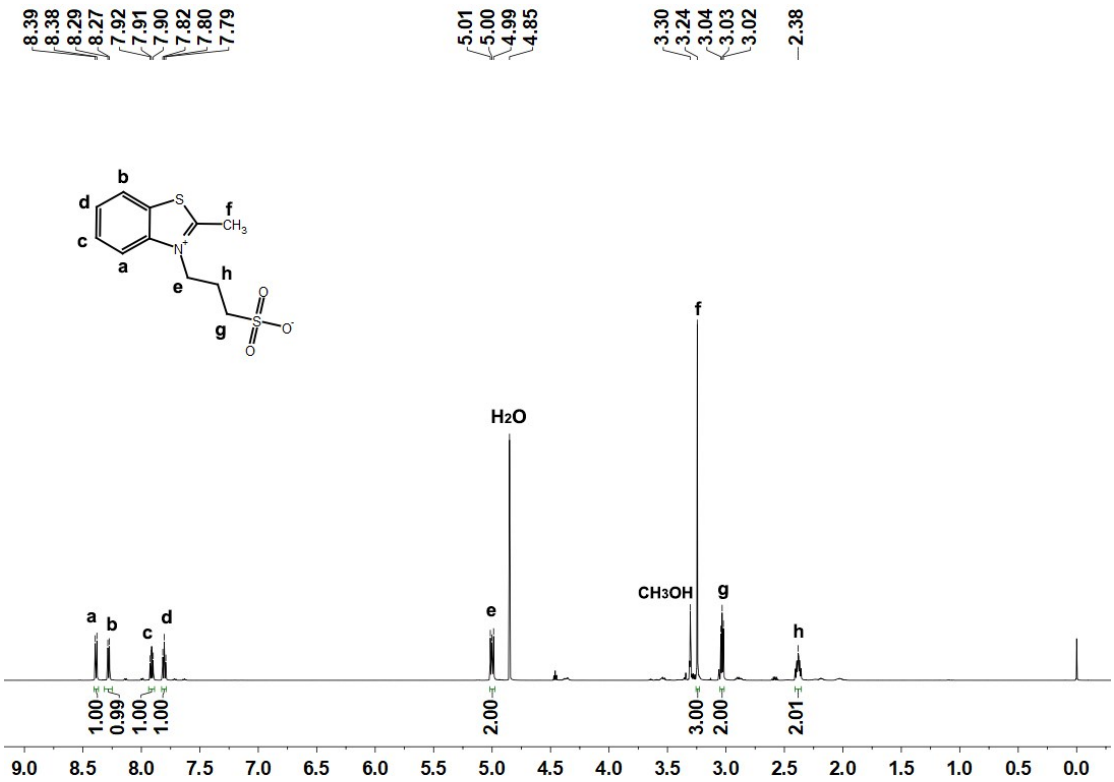


Fig.S3 ^{1}H NMR Spectra of Compound 3 (600 Hz, CD_3OD).

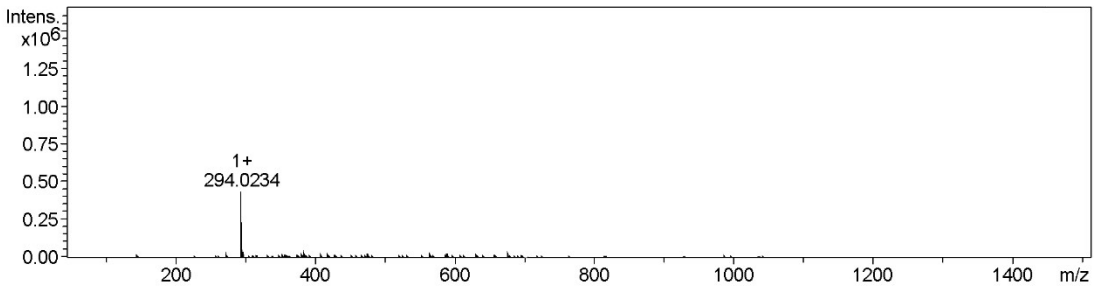


Fig. S4 HR Mass spectrum of Compound 3.
m/z $[M+Na]^+$ 294.0234

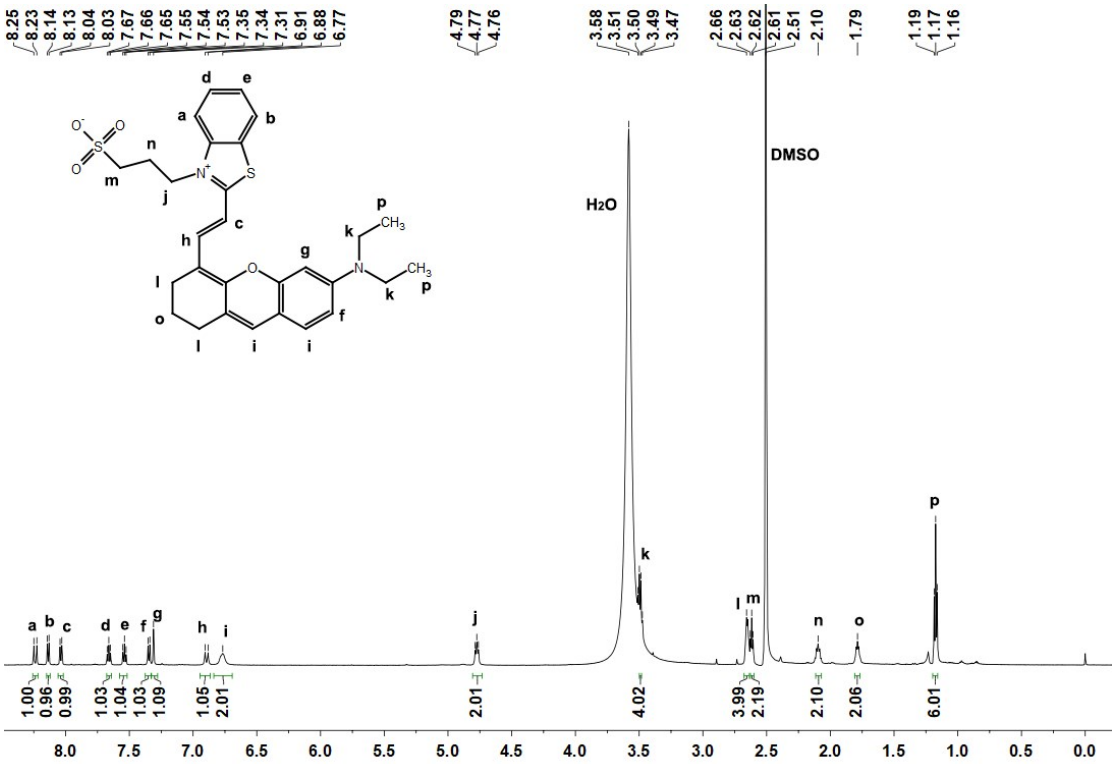


Fig S5 ^{1}H NMR Spectra of NIR-NEt_2 (600 Hz, DMSO-d_6).

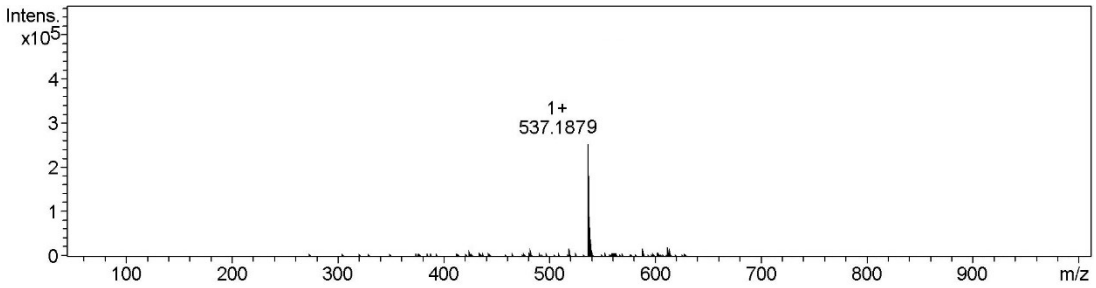


Fig. S6 HR Mass spectrum of NIR-NEt_2.

m/z $[M+H]^+$ 537.1879

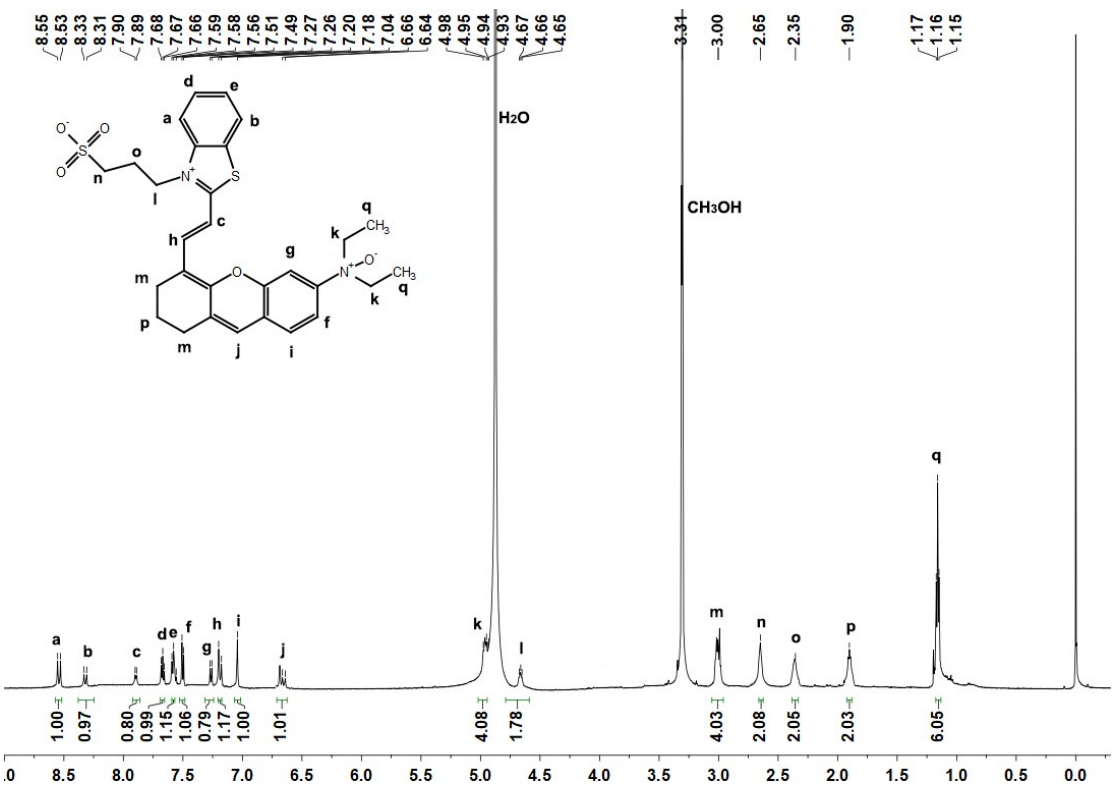


Fig. S7 ^{1}H NMR Spectra of NIR-NO (600 Hz, CD_3OD).

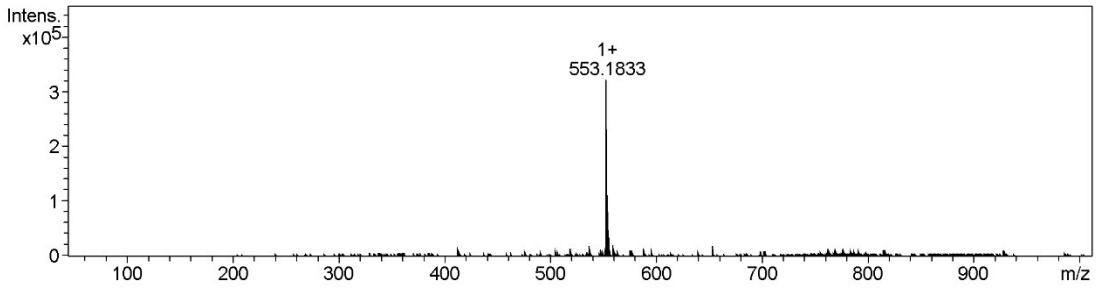
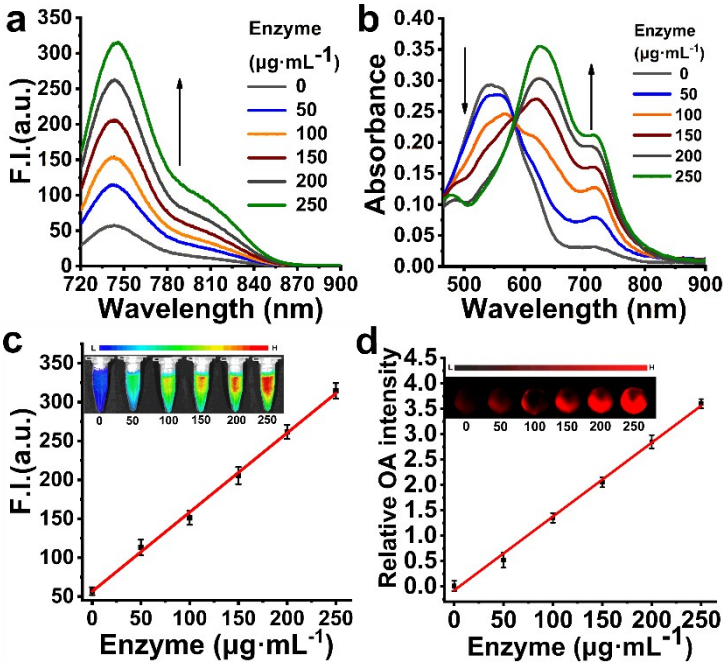


Fig. S8 HR Mass spectrum of NIR-NO.

m/z $[M+H]^+$ 553.1833

I



II

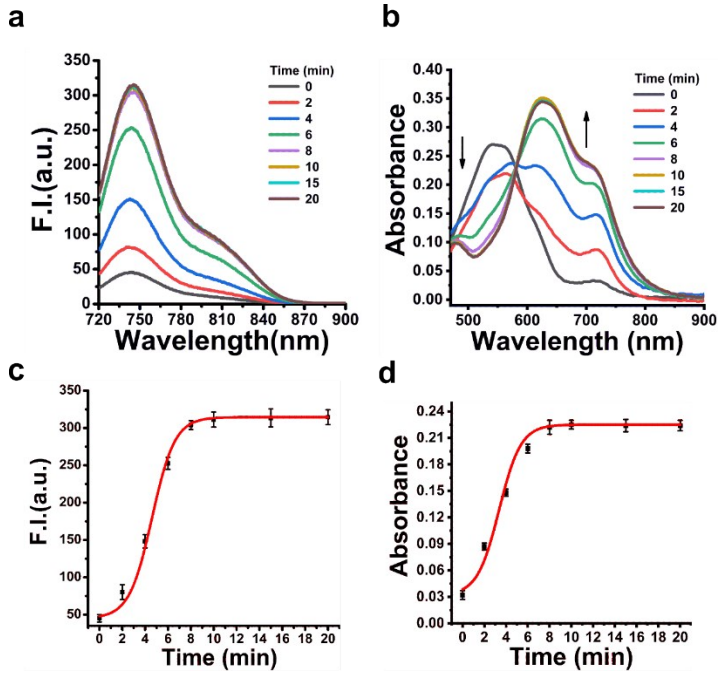


Fig. S9 I: Fluorescence spectra (a) (excitation: 700 nm) and Absorption spectra (b) of NIR-NO (10 μM) before and after incubation with different concentrations of CYP450 reductase with nitrogen bubbling. (c) Fluorescence intensity at 745 nm for NIR-NO (10 μM) as a function of CYP450 reductase level (n=3). Inset: Representative fluorescent images of the probe towards varied concentrations of CYP450 reductase.

(d) Relative optoacoustic intensity [$(I-I_0)/I_0$] for the probe upon incubation with varied concentrations of CYP450 reductase (n=3). Inset: Representative optoacoustic images of the NIR-NO in phantom after being incubated with varied concentrations of CYP450 reductase with nitrogen bubbling.

II: (a) Time-dependent fluorescence spectra of NIR-NO (10 μM) in PBS containing 250 μg/mL CYP450 reductase under hypoxia with an excitation wavelength at 700 nm. (b) Time-dependent absorption spectra of NIR-NO (10 μM) in PBS containing 250 μg/mL CYP450 reductase under hypoxia. (c) The plot of fluorescent intensity at 745 nm after incubation with 250 μg/mL CYP450 reductase under hypoxia for varied time. (d) The plot of absorbance at 718 nm after incubation with 250 μg/mL CYP450 reductase under hypoxia for varied time.

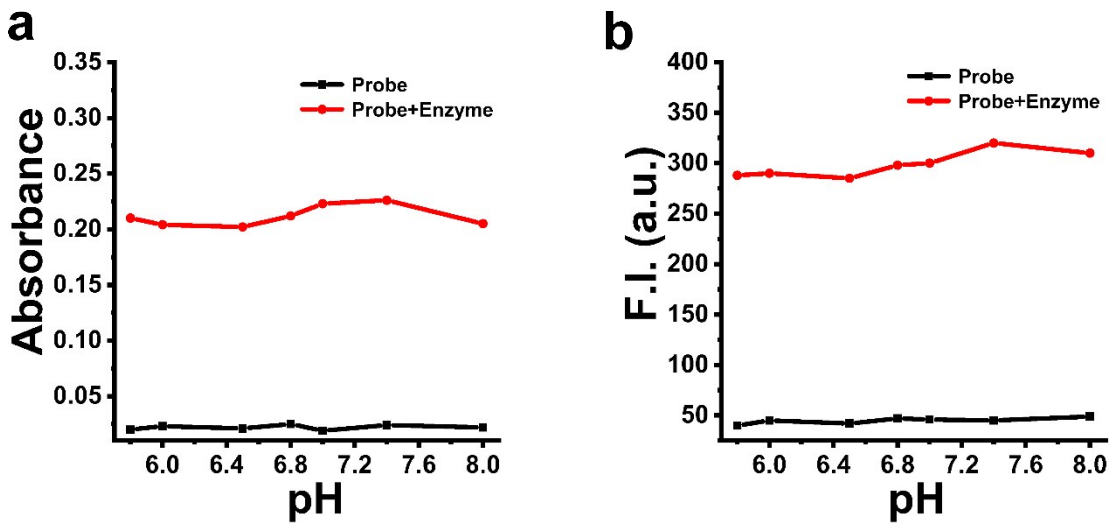


Fig. S10 (a) Effect of pH on the absorbance at 718 nm for NIR-NO (10 μM) in the presence (red) and absence (black) of CYP450 reductase under hypoxia condition. (b) Effect of pH on the fluorescence intensity at 745 nm for NIR-NO (10 μM) in the presence (red) and absence (black) of CYP450 reductase under hypoxia condition.

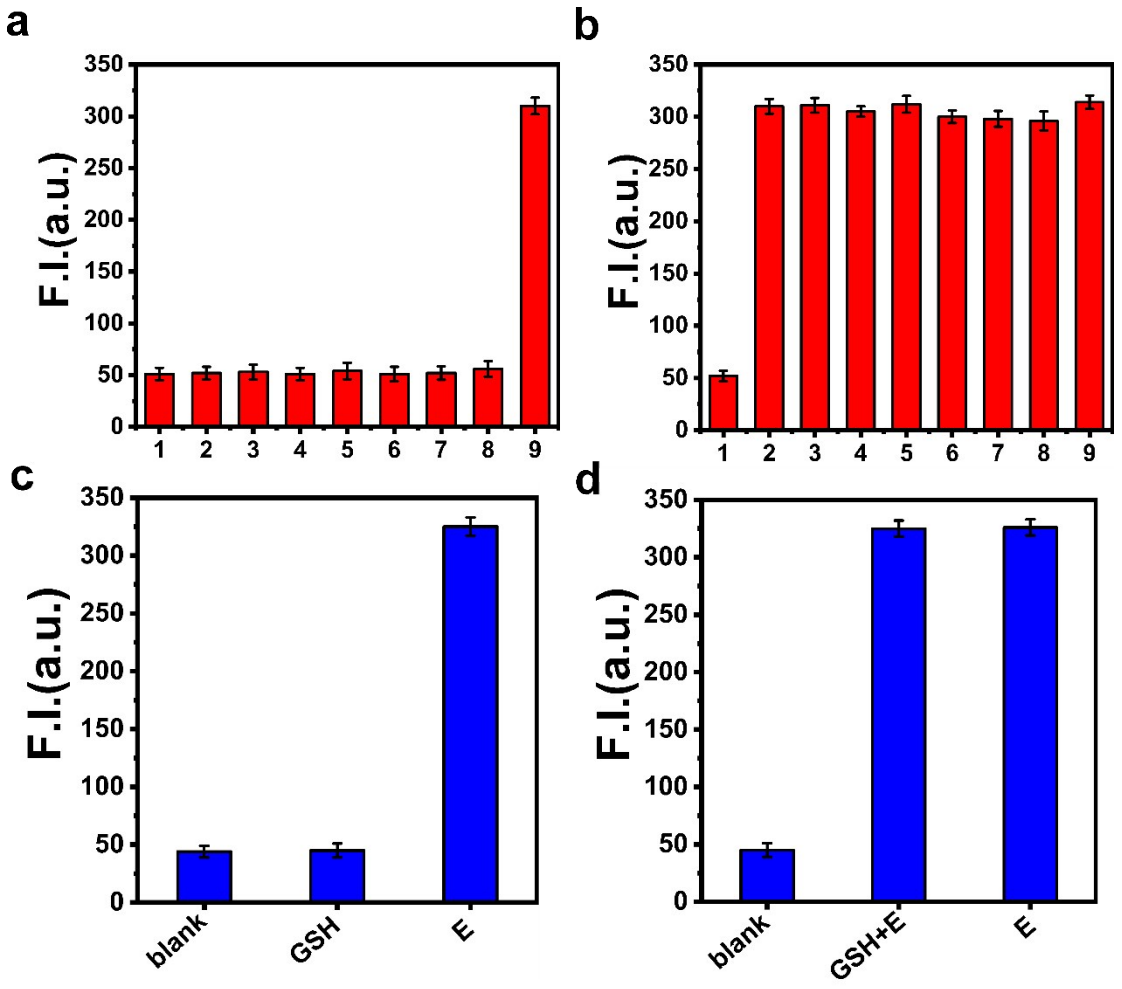


Fig. S11 (a) Fluorescent intensity at 745 nm of NIR-NO (10 μM) in the presence of different species respectively for 10 min under hypoxia condition. (b) The fluorescence response of NIR-NO upon treatment with 250 μg·mL^{-1} CYP450 reductase and simultaneously in the presence of respective potential interferents for 10 min under hypoxia condition. (1.blank; 2. Hyaluronidase : 300 μg·mL^{-1}; 3.γ-glutamyl transferase: 300 μg·mL^{-1}; 4. β-D-galactosidase: 300 μg·mL^{-1}; 5. Alkaline phosphatase: 300 μg·mL^{-1}; 6. Acetylcholinesterase: 300 μg·mL^{-1}; 7. Nitroreductase: 300 μg·mL^{-1}; 8. Heparinase: 300 μg·mL^{-1}; 9. CYP450 reductase: 250 μg·mL^{-1}). (c) Fluorescent intensity at 745 nm of NIR-NO (10 μM) in the presence of different species respectively for 10 min under hypoxia condition. (d) The fluorescence response of NIR-NO upon treatment with 250 μg·mL^{-1} CYP450 reductase and simultaneously in the presence of respective potential interferents for 10 min under hypoxia condition. E: CYP450 reductase: 250 μg·mL^{-1}.
GSH: 10 mM.

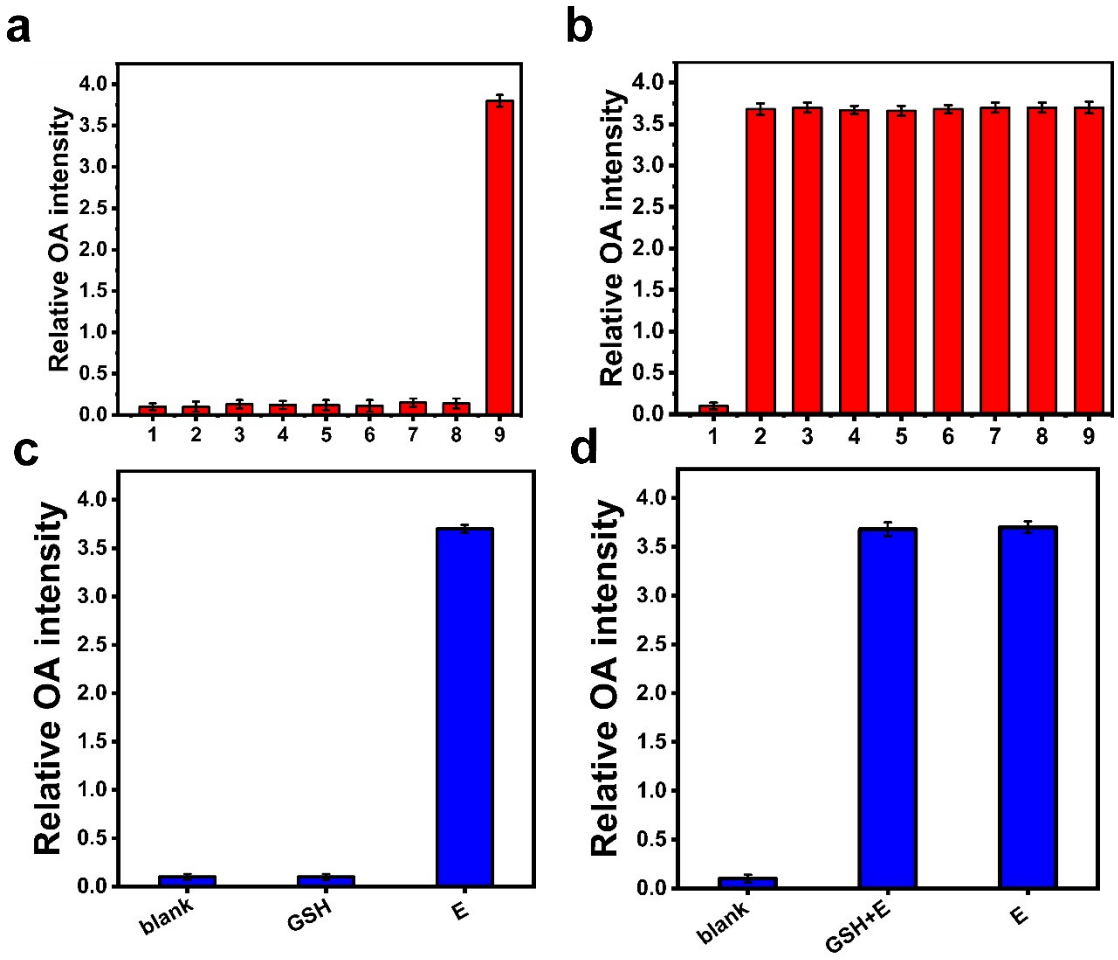


Fig. S12 (a) Relative optoacoustic intensity of NIR-NO (10 μM) in the presence of different species respectively for 10 min under hypoxia condition. (b) Relative optoacoustic intensity of NIR-NO (10 μM) in the presence of CYP450 reductase and simultaneously in the presence of respective potential interferents for 10 min under hypoxia condition. (1.blank; 2. Hyaluronidase : 300 μg·mL^{-1}; 3.γ-glutamyl transferase: 300 μg·mL^{-1}; 4. β-D-galactosidase: 300 μg·mL^{-1}; 5. Alkaline phosphatase: 300 μg·mL^{-1}; 6. Acetylcholinesterase: 300 μg·mL^{-1}; 7. Nitroreductase: 300 μg·mL^{-1}; 8. Heparinase: 300 μg·mL^{-1}; 9. CYP450 reductase: 250 μg·mL^{-1}). (c) Relative optoacoustic intensity of NIR-NO (10 μM) in the presence of different species respectively for 10 min under hypoxia condition. (d) Relative optoacoustic intensity of NIR-NO (10 μM) in the presence of CYP450 reductase and simultaneously in the presence of respective potential interferents for 10 min under hypoxia condition. E: CYP450 reductase: 250 μg·mL^{-1}. GSH: 10 mM.

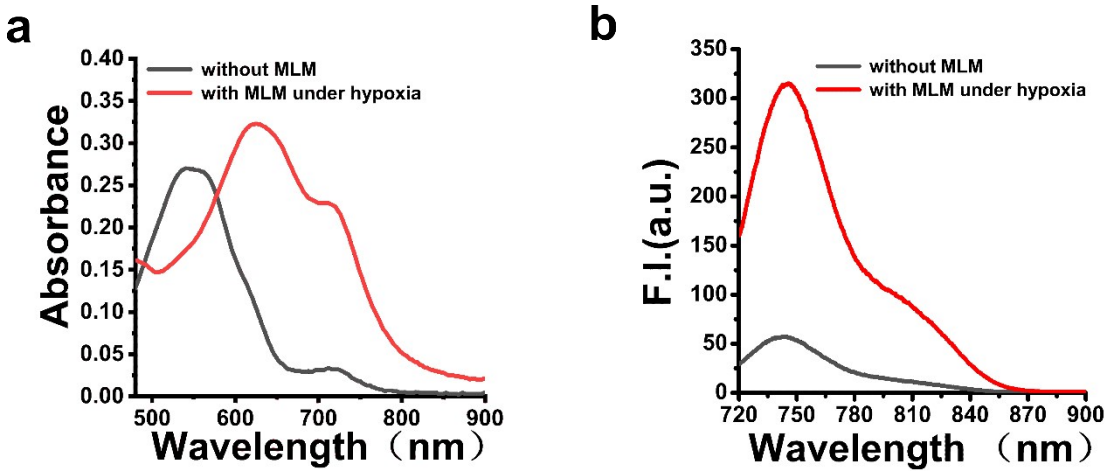


Fig. S13 (a) Fluorescence spectra of NIR-NO before and after treatment with 400 μg·mL^{-1} MLM with nitrogen bubbling (excitation: 700 nm). (b) Absorption spectra of NIR-NO before and after treatment with 400 μg·mL^{-1} MLM under hypoxia.

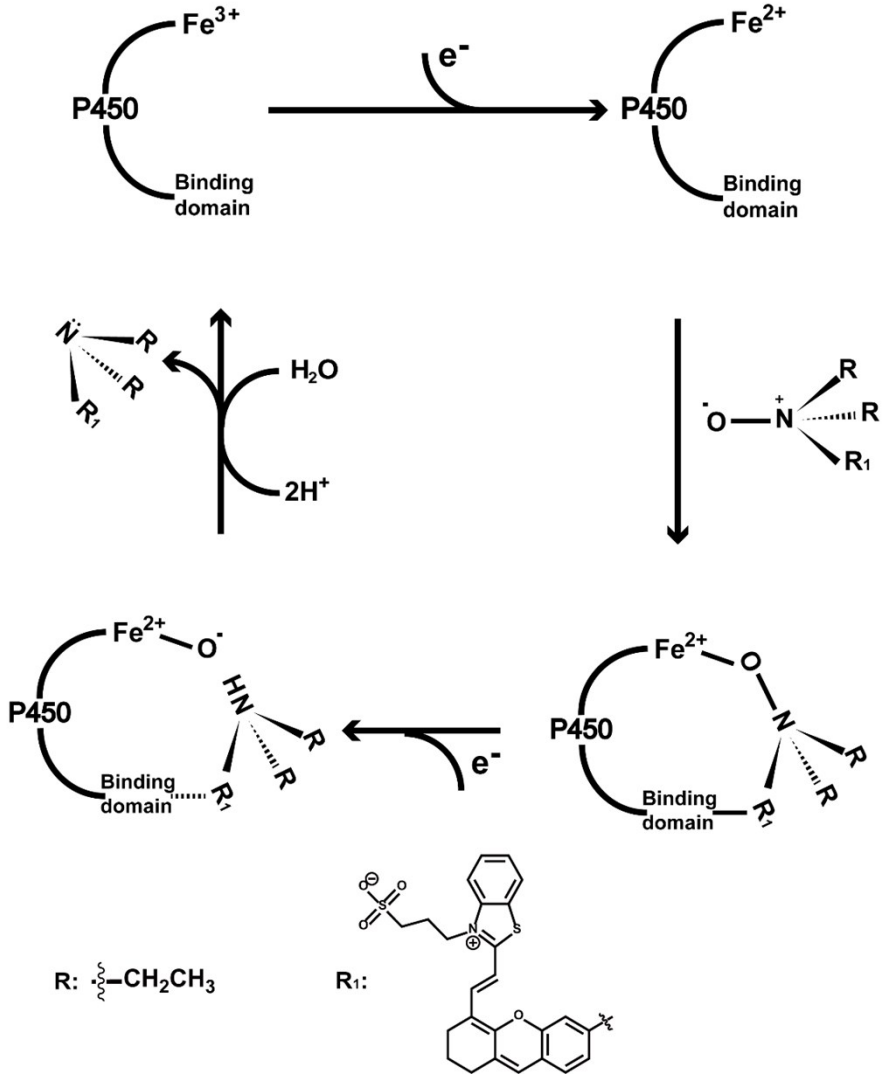


Fig. S14 Scheme for the proposed mechanism of cytochrome P450 mediated N-oxide reduction of the probe.

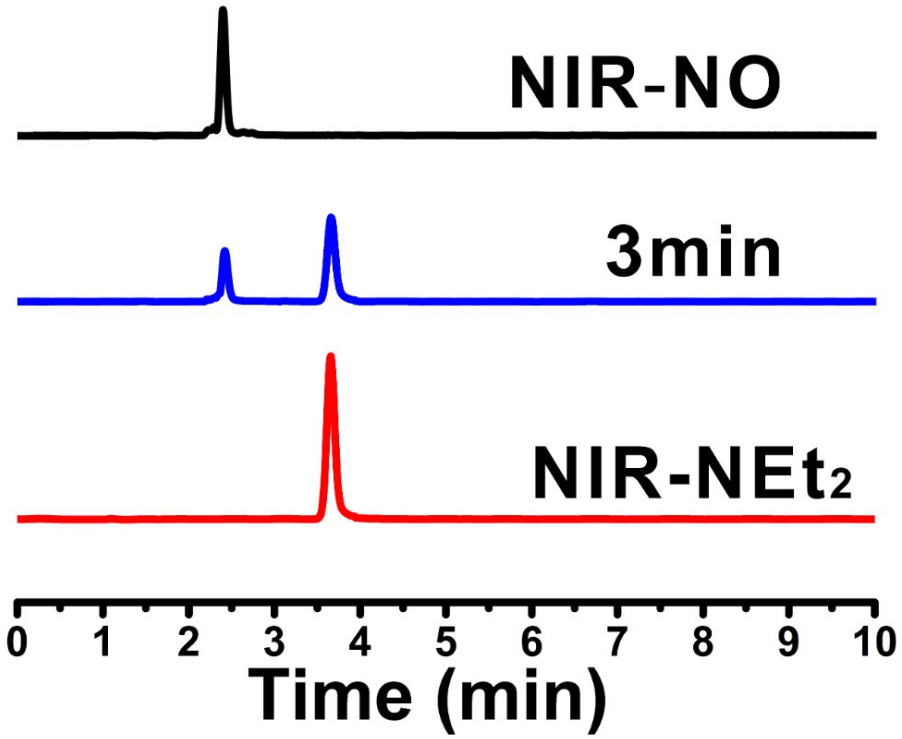


Fig. S15 HPLC profiles for pure NIR-NO, mixture of NIR-NO and CYP450 reductase upon reaction for 3 min with nitrogen bubbling, and pure NIR-NEt_2. Mobile phase: methanol/water = 90/10.

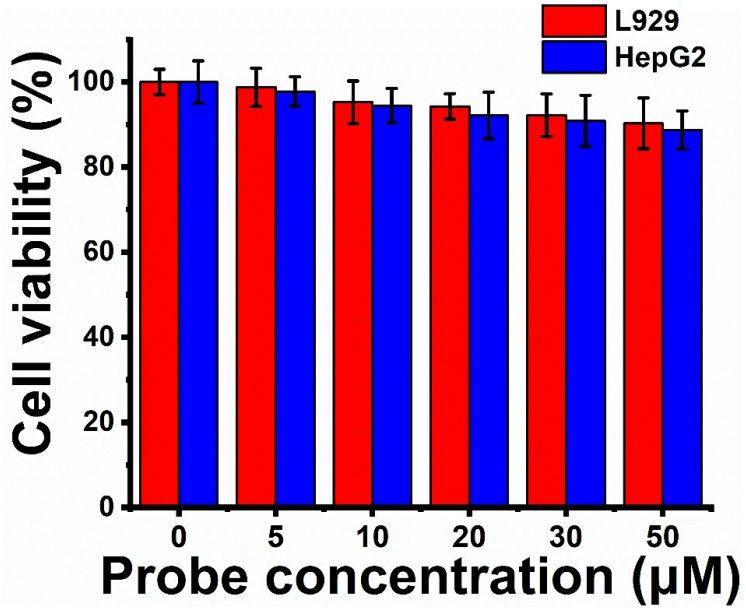


Fig. S16 Cell viability for L929 and HepG2 cells in the presence of NIR-NO at varied concentrations.

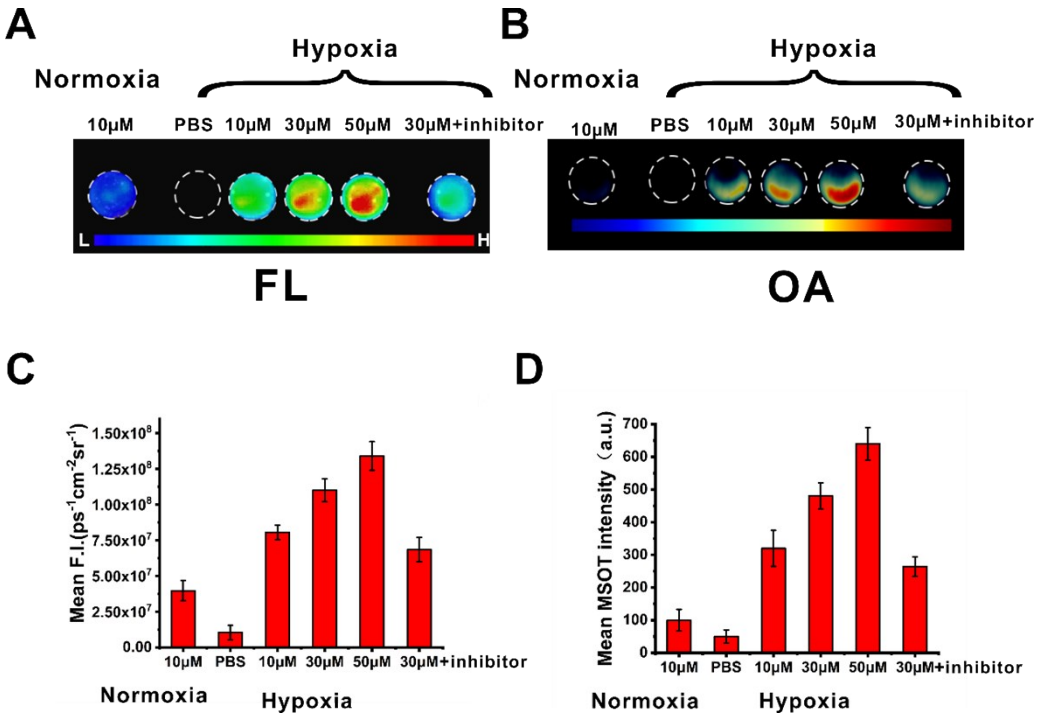


Fig. S17 (A) Fluorescent (FL) images and optoacoustic (OA) images for HepG2 cells incubated with the probe NIR-NO of varied concentrations for 4 h under hypoxia or in normoxia, respectively. For fluorescence imaging: excitation filter: 710 nm, emission filter: 750 nm. For optoacoustic imaging: excitation at 718 nm. (C) Mean fluorescence intensities corresponding to the fluorescent images in (A) for HepG2 cells. (D) Mean MSOT intensities corresponding to the optoacoustic images in (B) for HepG2 cells.

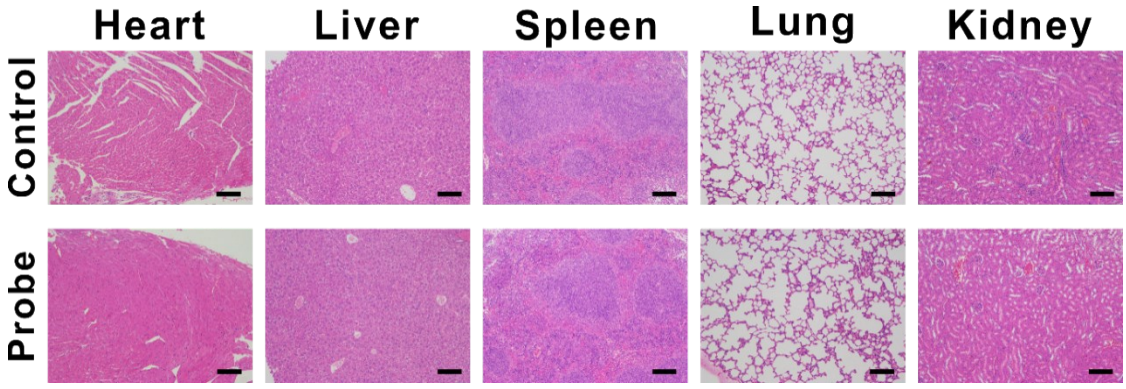


Fig. S18 Representative histological section (H&E staining) of heart, liver, spleen, lung and kidney for the healthy mice upon intravenously injected with PBS (the control) or the probe NIR-NO for 24 h. Scale bar: 100 μm.

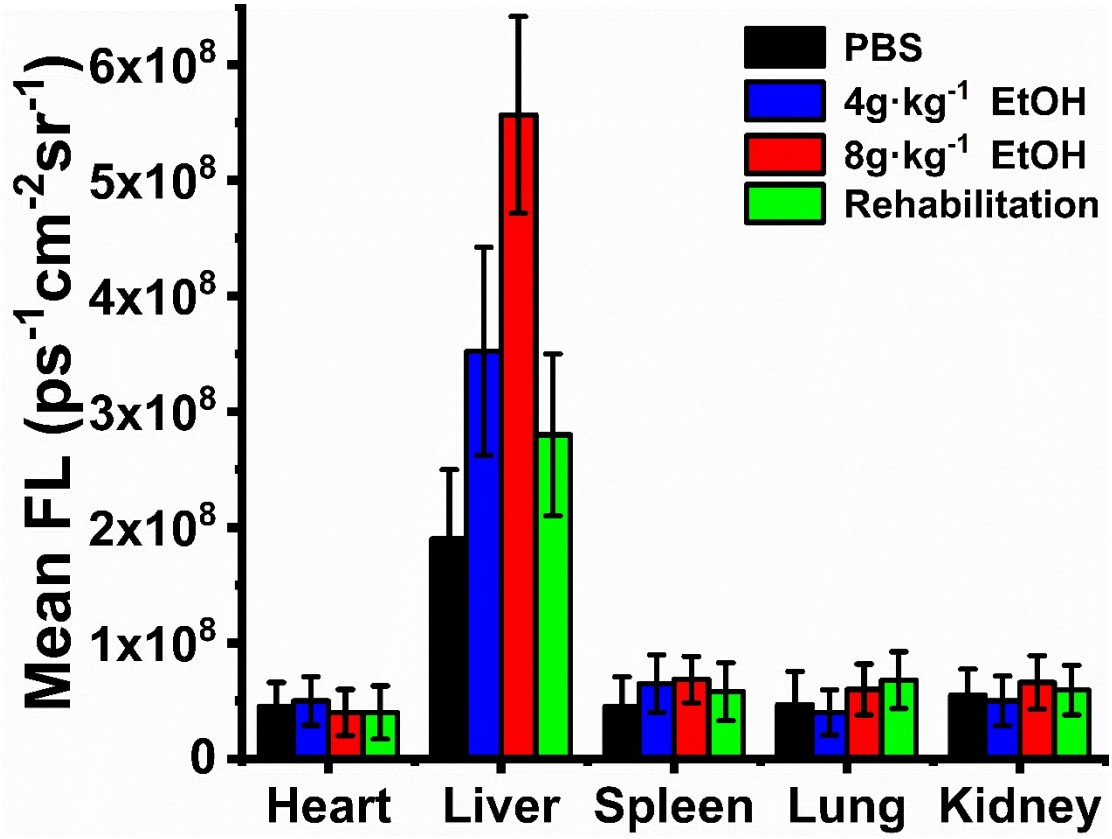


Fig. S19 Mean fluorescence intensity for major organs. (corresponding to Fig. 2C)

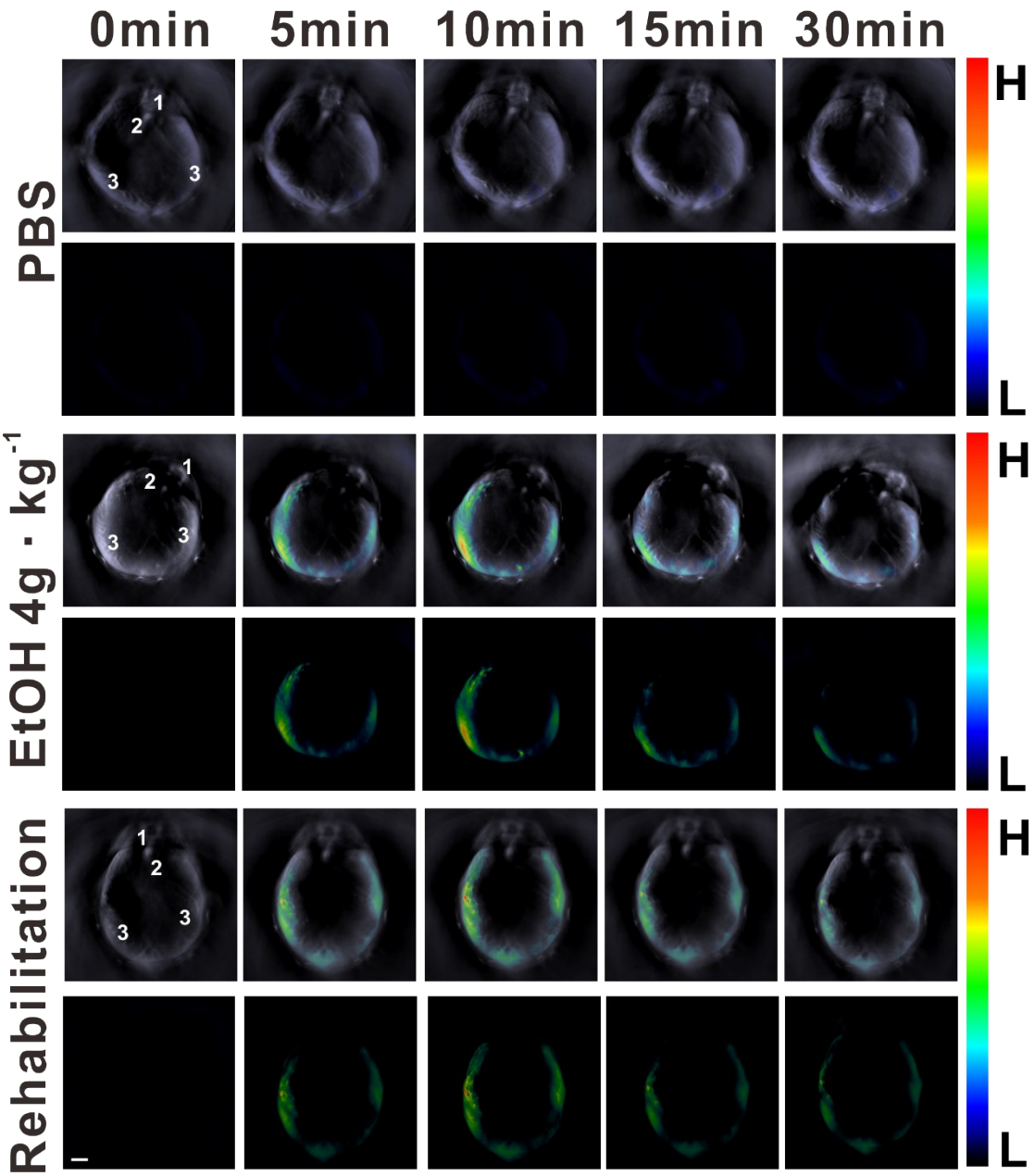


Fig. S20 Representative cross-sectional MSOT images of the mice at varied time points upon injection of NIR-NO. The mice groups treated with PBS and 4 g·kg^{-1} EtOH and the rehabilitation group. Organ labelling: 1. Spinal cord; 2. Aorta; 3. Liver. Scale bar: 3 mm.

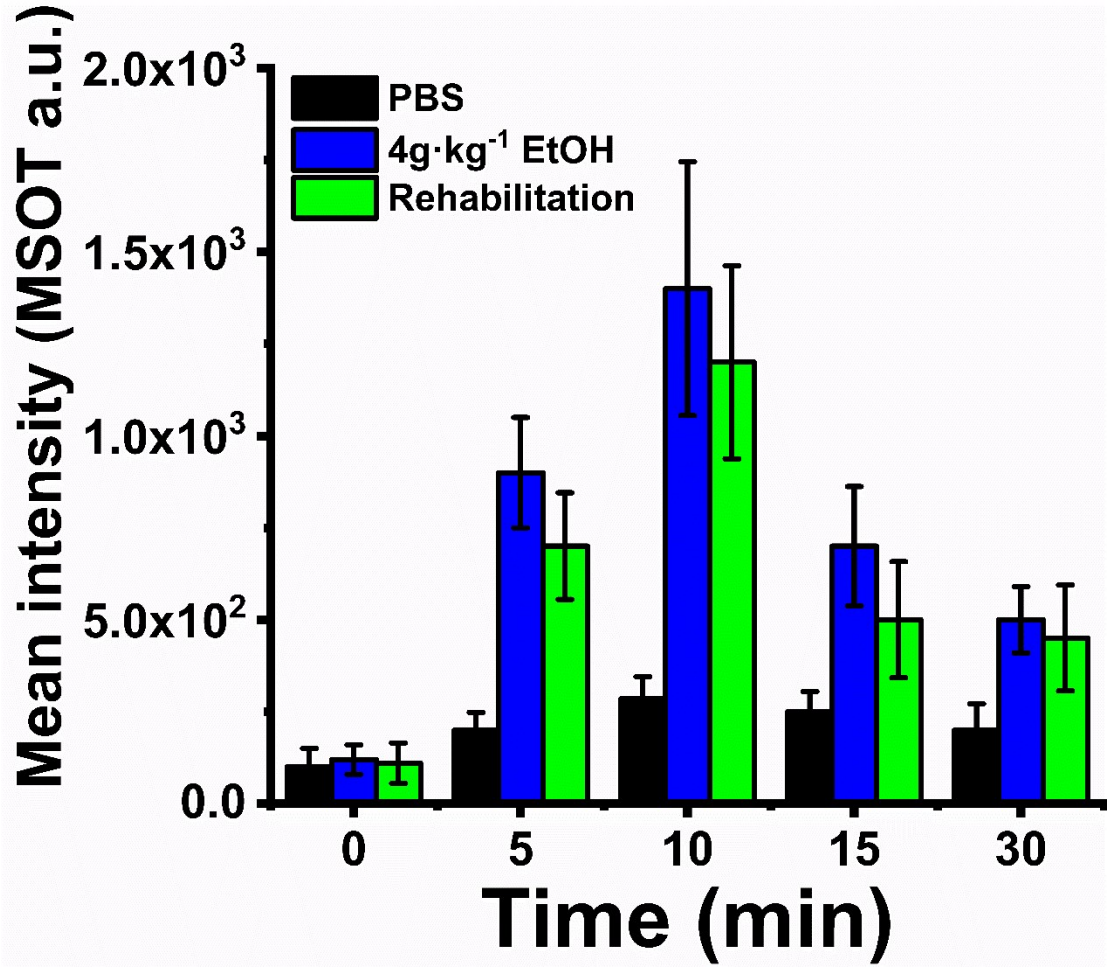


Fig. S21 Mean MSOT intensities in different mice groups for varied time upon probe injection (corresponding to Fig. S20).

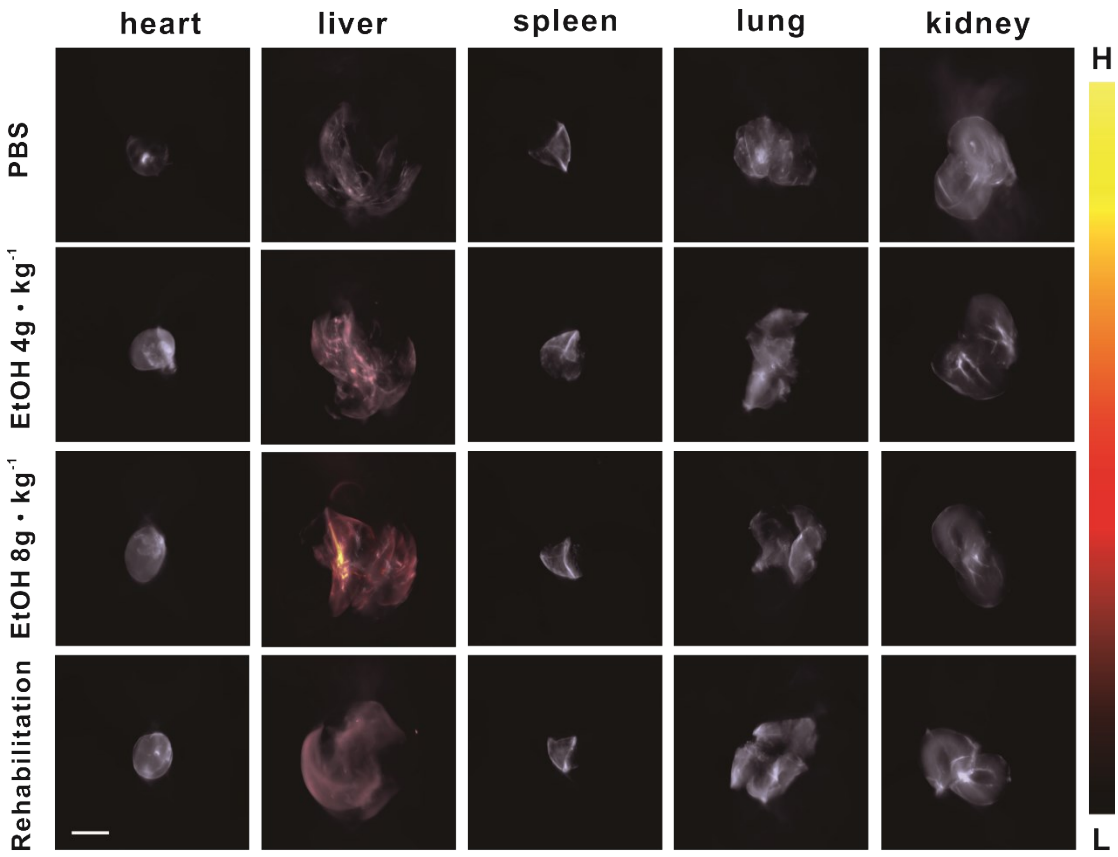


Fig. S22 Representative cross-sectional MSOT images for excised organs (heart, liver, spleen, lung, kidney) of the mice after pretreatment with PBS, 4 g·kg^{-1} EtOH, 8 g·kg^{-1} EtOH and then injection of the probe for 10 min, as well as the rehabilitation group 10 min upon the probe injection, the excised organs underwent immediate embedment in phantom. scale bar: 5 mm.

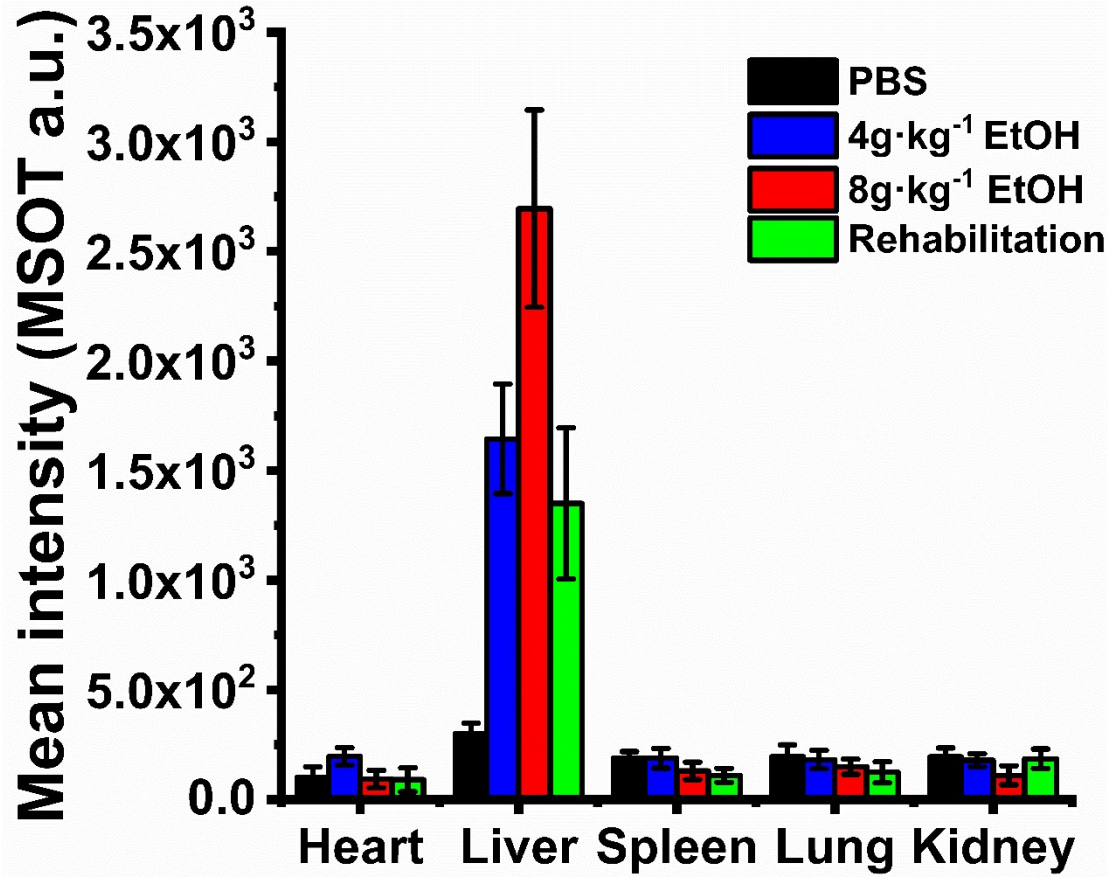


Fig. S23 Mean MSOT intensities in major organs corresponding to Fig. S22.