Supporting Information

Visualization of Oxidative Injury in the Mouse Kidney by Selective Superoxide Anion Fluorescent Probes

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1. Materials and General Experimental Methods

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Mass spectra were performed using LCQ advantage trap mass spectrometer from Thermo Finnigan. NMR spectra were recorded on a Bruker-400 spectrometer, using TMS as an internal standard. High-resolution electronspray (ESI-HRMS) spectra were obtained from The Thermo Fisher Scientific LTQ FT Ultra (Shanghai Institute of Organic Chemistry Chinese Academic of Sciences). High-performance liquid chromatography (HPLC) analyses were performed on an Agilent 1260 system equipped with a G1311x pump and inertsil ODS-3 5020-01732 column. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer (1 cm standard quartz cell). The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; the fluorescence images were acquired with a two photon confocal laser scanning microscope (Nikon, Japan) or an Olympus FV1000 equipped with a CCD camera; The *in vivo* (living mice) imaging was carried out using an IVIS Lumina XR (IS1241N6071) *in vivo* imaging system; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals.

2. Determination of the fluorescence quantum yield:

Fluorescence quantum yield for **Naph-$O_2^{\cdot-}$** and **NIR-$O_2^{\cdot-}$** was determined by using rhodamine B (Φ_f = 0.65 in EtOH) or ICG (Φ_f = 0.13 in DMSO) as a fluorescence standard.[1, 2] The quantum yield was calculated using the following equation[3]:

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

3. Spectrometric Studies

Measurement of photophysical properties. For photophysical characterization, the compound **1, 2, 3, 4**, probe **Naph-$O_2^{\cdot-}$** and **NIR-$O_2^{\cdot-}$** were dissolved in DMSO and CH_3CN to make the stock solutions (500 μM), which were diluted to 5 μM or 10 μM as the testing solutions with PBS buffer solution. Absorption and fluorescence spectroscopic studies were performed on a UV 1800 ultraviolet and visible spectrophotometer; a Hitachi F-4600 fluorescence spectrophotometer. Sources for different ROS/RNS are described as follows. Specifically, H_2O_2 solution was purchased from Sigma-Aldrich and added into the probe solution directly. Superoxide ($O_2^{\bullet-}$) generated from KO_2 was dissolved in DMSO. The source of NaOCl was commercial bleach. Peroxynitrite ($ONOO^-$) solution was prepared from SIN-1.[4]

4. Fluorescence Microscopic Studies

Cell culture. HepG2 cells were cultured in high glucose Dulbecco's Modified Eagle Medium(DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, BI), and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) at 37 °C and 5% CO_2. Cells were carefully harvested and split when they reached 80% confluence to maintain exponential growth.

Fluorescence microscopic imaging. All the experiments were conducted in live cells. The microscopic imaging uses a confocal laser scanning microscope (Nikon, Japan) with an excitation filter of 488 and 640 nm and the collection wavelength range is from 500-550 nm and 663-738 nm, respectively.

For the cell specific experiment, the cell experiment can be divided into seven groups. The first group is intact HepG2 cells. The second group is that HepG2 cells were incubated with probe 5 μM **Naph-$O_2^{\cdot-}$** and **NIR-$O_2^{\cdot-}$** for 30 min. Then the cells were washed by PBS buffer before imaging. In the third and fourth group, HepG2 cells were incubated with probe 5 μM **Naph-$O_2^{\cdot-}$** and **NIR-$O_2^{\cdot-}$** for 30 min, then washed, and incubated with 100 μM H_2O_2 or Na_2SO_3, respectively, for 30 min prior to imaging. In the fifth group, HepG2 cells were pre-incubated with lipopolysaccharide (LPS, 1 μg/mL) and interferon-gamma (IFN-γ, 50 ng/mL) for 12 h, and then 5 μM **Naph-$O_2^{\cdot-}$** or **NIR-$O_2^{\cdot-}$** was further added for 30 min. Then the cells were washed by PBS prior to imaging. In the sixth and seventh group, HepG2 cells were incubated with LPS (1 μg/mL) and IFN-γ (50 ng/mL) in the presence of 2, 2, 6, 6-tetramethylpiperidine-N-oxyl (TEMPO, 300 μM) or Tiron (10 μM) for 12 h, respectively, and then incubated with probe 5 μM **Naph-$O_2^{\cdot-}$** or **NIR-$O_2^{\cdot-}$** for 30 min prior to imaging.

For the cell experiment in HK-2 cells, the cell experiment can be divided into nine groups. The first group is that the HK-2 cells were treated with 5 μM **NIR-$O_2^{\cdot-}$**. Then the cells were washed by PBS buffer before imaging. The second-fifth group is that HK-2 cells were pre-treated with different concentrations of cisplatin (100 μM, 300 μM, 600 μM and 1000 μM) for 12 h, respectively, and then treated with 5 μM probe **NIR-$O_2^{\cdot-}$** for 30 min. Then the cells were washed by PBS buffer before imaging. The sixth-ninth group, cells were pre-incubated with different concentrations of L-carnitine (LC) (100 μM, 400 μM, 800 μM, 1000 μM) in the presence of cisplatin (1000 μM) for 12 h, respectively, and then treated with 5 μM **NIR-$O_2^{\cdot-}$** for 30 min. Then the cells were washed by PBS buffer before imaging.

Cell cytotoxicity in MTT assay. Cells were plated in 96-well flat-bottomed plates at 1×10^5cells per well and allowed to grow overnight prior to exposure to **Naph-$O_2^{\cdot-}$** or **NIR-$O_2^{\cdot-}$**. Then the MTT (0.5 mg/mL) reagent was added for 4 h at 37 °C and DMSO (100 μL/well) was further incubated with cells to dissolve the precipitated formazan violet crystals at 37 °C for 15min. The absorbance was measured at 490 nm by a multidetection microplate reader. The following formula was used to calculate the viability of cell growth: Cell viability (%) = (mean of *A* value of treatment group / mean of *A* value of control) ×100.

Determining the subcellular location of probes. Live HepG2 cells cultured in 35-mm glass bottom culture dishes were incubated with LPS (1 μg/mL)/IFN-γ (50 ng/mL) for 12 h followed by treated with 5 μM **Naph-$O_2^{\cdot-}$** or **NIR-$O_2^{\cdot-}$** for 30 min. After 30 min treatment at 37 °C, Mito-Tracker Green (1.0 μM) and Lyso-Tracker Red (1.0 μM) were added for another 10 min. Then the cells were washed by PBS prior to imaging.

5. Two-photon Imaging Studies

Two-photon microscopic Imaging Studies in living cells. All the experiments were conducted in live cells. For the cell specific experiment, the cell experiment can be divided into seven groups. The first group is intact HepG2 cells. The second group is that HepG2 cells were incubated with 5 μM **Naph-$O_2^{\cdot-}$** for 30 min. Then the cells were washed by PBS buffer before imaging. In the third and fourth group, HepG2 cells were incubated with 5 μM **Naph-$O_2^{\cdot-}$** for 30 min, then washed, and incubated with 100 μM H_2O_2 or Na_2SO_3 respectively for 30 min prior to imaging. In the fifth group, HepG2 cells were pre-incubated with lipopolysaccharide (LPS, 1 μg/mL) and interferon-gamma (IFN-γ, 50 ng/mL) for 12 h, and 5 μM **Naph-$O_2^{\cdot-}$** was further added for 30 min. Then the cells were washed by PBS prior to imaging. In the sixth and seventh group, HepG2 cells were incubated with LPS (1 μg/mL) and IFN-γ (50 ng/mL) in the presence of 2, 2, 6, 6-tetramethylpiperidine-N-oxyl (TEMPO, 300 μM) or Tiron (10 μM) for 12 h, respectively, and then incubated with 5 μM **Naph-$O_2^{\cdot-}$** for 30 min prior to imaging. The microscopic imaging uses a confocal laser scanning microscope (Nikon, Japan) with an excitation filter of 800 nm and the collection wavelength range is 500-550 nm.

For the cell experiment in HK-2 cells, the cell experiment can be divided into fourth groups. The first group is that the HK-2 cells were treated with 5 μM **Naph-$O_2^{\bullet-}$**. Then the cells were washed by PBS buffer before imaging. The second and third group is that HK-2 cells were pre-treated with different concentrations of cispaltin (500 μM, 1000 μM) for 12 h, respectively, and then treated with 5 μM **Naph-$O_2^{\bullet-}$** for 30 min. Then the cells were washed by PBS buffer before imaging. The fourth group, cells were pre-incubated with L-carnitine (LC) (1000 μM) in the presence of cisplatin (1000 μM) for 12 h, and then treated with 5 μM **Naph-$O_2^{\bullet-}$** for 30 min. Then the cells were washed by PBS buffer before imaging. The microscopic images were conducted using OLYMPUS FV1000 (TY1318) confocal microscope with an excitation filter of 800 nm and the collection wavelength range is 520-580 nm.

Two-photon Tissues Imaging Studies. Slices were prepared from the kidney of BALB/c mice. Slices were cut by using a vibrating-blade microtome in 25 mM PBS (pH 7.4). For the control experiments, slices were incubated with 10 μM **Naph-$O_2^{\cdot-}$** in PBS buffer bubbled with 95% O_2 and 5% CO_2 for 30 min at 37 °C. Slices were then washed three times with PBS, transferred to glass-bottomed dishes, and observed under a two-photon confocal microscope (Nikon, Japan). To obtain the two-photon fluorescence images of the tissues incubated with both the probe and superoxide anion, the mice were injected intraperitoneally with cisplatin (20 mg/kg) for 48 h, then 10 μM **Naph-$O_2^{\cdot-}$** was added. Following this incubation for 30 min at 37 °C, the slices were washed three times and imaged. The two-photon fluorescence emission was collected at between 500-550 nm upon excitation at 800 nm.

Cisplatin-induced acute kidney injury. Cisplatin was dissolved in 0.9 % saline. BALB/c mice were intraperitoneally preinjected with cisplatin (20 mg/kg) for 24 h, then intravenously injected with the probe **NIR-$O_2^{\bullet-}$** (100 μL, 200 μM). After 45 min, all the mice were anaesthetized and performed surgical procedure to expose the kidney. Imaging was observed under an IVIS Lumina XR (IS1241N6071) *in vivo* imaging system.

For BALB/c mice, cisplatin with varied concentrations (0, 10 mg/kg, 20 mg/kg) for different time periods (12 h, 24 h, 48 h, 72 h) and then intravenous injected with the **NIR-$O_2^{\bullet-}$** (100 μL, 100 μM). After 45 min, the kidney were harvested and transferred to glass-bottomed dishes, and observed under an IVIS Lumina XR (IS1241N6071) *in vivo* imaging system.

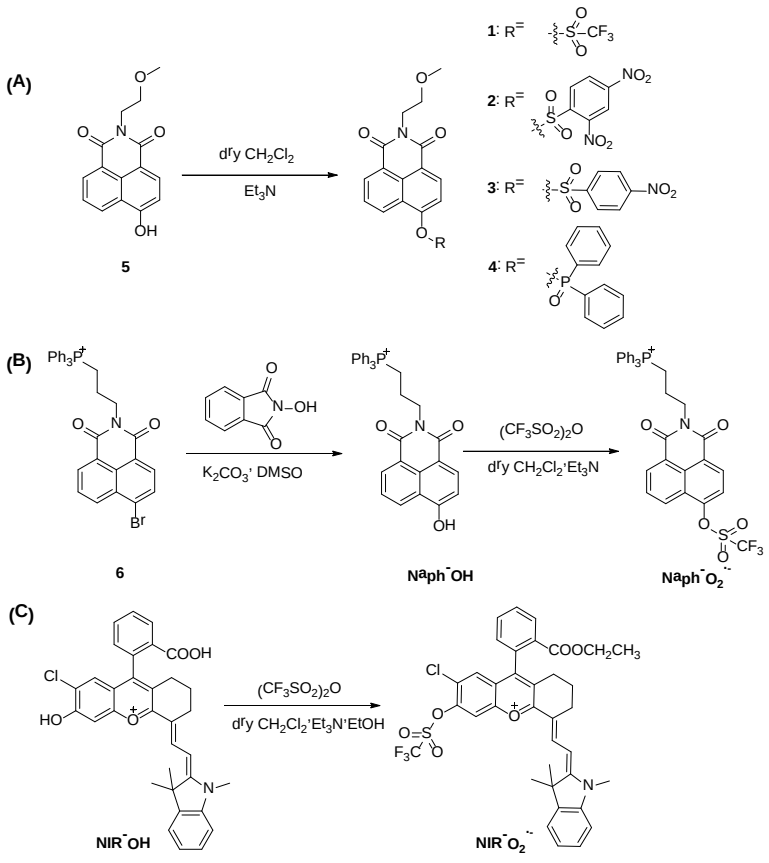
For the protective effects of L-carnitine (LC) against cisplatin induced acute kidney injury, the

BALB/c mice were divided into three groups. One group was given PBS (320 μL) in the peritoneal cavity, after 48 h, followed by intravenous injection with **NIR-$O_2^{\bullet-}$** (100 μL, 100 μM) as the negative control group; The second group was given an intraperitoneal injection of cisplatin (320 μL, 20 mg/kg) and followed by intravenous injection with the probe **NIR-$O_2^{\bullet-}$** (100 μL, 100 μM) after 48 h; and the third group were pre-injected intraperitoneally with LC (60 μL, 400 mg/kg) for 48 h before injected intraperitoneally with cisplatin (320 μL, 20 mg/kg), and followed by intravenous injection with the probe **NIR-$O_2^{\bullet-}$** (100 μL, 100 μM) after 48 h. The mice were anesthetized, and the kidney was dissected for imaging using an IVIS Lumina XR (IS1241N6071) in vivo imaging system.

6. Histopathological Studies

Renal tissues of BALB/c mice were fixed in 10% formaldehyde immediately following sacrifice, processed for histological examination according to a conventional method, and stained with hematoxylin and eosin (H&E). The morphology of any observed lesions was classified and recorded according to the classification criteria.[5]

7. Synthesis and Characterization



Scheme S1. Synthetic routes of compounds **1-4** (A), **Naph-$O_2^{\bullet-}$** (B) and **NIR-$O_2^{\bullet-}$** (C).

Synthesis of compound 1. Compound **5** (135.5 mg, 0.5 mmol) was dissolved in 4 mL anhydrous CH_2Cl_2 under nitrogen atmosphere. Then trifluoromethanesulfonic anhydride (265 μL) and triethylamine (200 μL) were added to the solution and stirred for 30 min at room temperature. Then the solvent was removed under vacuum and the residue was purified by silica gel

chromatography with CH_2Cl_2/Petroleum Ether (v/v, 3:1) as eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, *J* = 7.3 Hz, 1H), 8.65 (d, *J* = 8.1 Hz, 1H), 8.41 (d, *J* = 8.5 Hz, 1H), 7.92 (t, *J* = 7.9 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 4.44 (t, *J* = 5.7 Hz, 2H), 3.73 (t, *J* = 5.6 Hz, 2H), 3.37 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 162.9, 149.2, 132.6, 131.4, 129.6, 128.7, 127.0, 124.9, 123.1, 122.8, 119.0, 69.5, 58.8, 39.5. MS (ESI): calcd for $C_{16}H_{12}F_3NO_6S$ $(M+H)^+$ 404.0, found 404.1.

Synthesis of compound 2. Compound **5** (135.5 mg, 0.5 mmol) was dissolved in 4 mL anhydrous CH_2Cl_2 under nitrogen atmosphere. Then 2,4-dinitrobenzenesulfonyl chloride (133.1 mg, 0.5 mmol) and triethylamine (200 μL) were added to the solution and stirred for 30 min at room temperature. Then the solvent was removed under vacuum and the residue was purified by silica gel chromatography with CH_2Cl_2/Petroleum Ether (v/v, 3:1) as eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.70 (d, *J* = 7.3 Hz, 1H), 8.59 (d, *J* = 8.1 Hz, 1H), 8.46 (dd, *J* = 15.3, 9.8 Hz, 2H), 7.84 (t, *J* = 7.9 Hz, 1H), 7.25 – 7.20 (m, 2H), 4.45 (t, *J* = 5.7 Hz, 2H), 3.74 (t, *J* = 5.7 Hz, 2H), 3.38 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ162.3, 131.6, 131.1, 128.3, 126.9, 126.7, 121.4, 120.1, 113.4, 68.6, 57.8, 38.4. MS (ESI): calcd for $C_{21}H_{15}N_3NaO_{10}S^+$ $(M+Na)^+$ 524.0 , found 524.0.

Synthesis of compound 3. Compound **5** (135.5 mg, 0.5 mmol) was dissolved in 4 mL anhydrous CH_2Cl_2 under nitrogen atmosphere. Then 4-nitrobenzene sulfonyl chloride (111.2 mg, 0.5 mmol) and triethylamine (200 μL) were added to the solution and stirred for 1 h at room temperature. Then the solvent was removed under vacuum and the residue was purified by silica gel chromatography with CH_2Cl_2/Petroleum Ether (v/v, 1:1) as eluent. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (d, *J* = 7.3 Hz, 1H), 8.45 (t, *J* = 7.6 Hz, 3H), 8.27 (t, *J* = 8.6 Hz, 3H), 7.88 (t, *J* = 7.9 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 4.22 (t, *J* = 6.0 Hz, 2H), 3.57 (t, *J* = 6.0 Hz, 2H), 3.25 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ163.5, 162.9, 151.8, 149.1, 139.4, 132.2, 131.8, 130.8, 129.2, 129.0, 128.1, 125.7, 125.3, 122.9, 122.0, 120.4, 69.0, 58.4, 39.1. MS (ESI): calcd for $C_{21}H_{16}N_2O_8S$ $(M-H)^-$ 455.1, found 455.0.

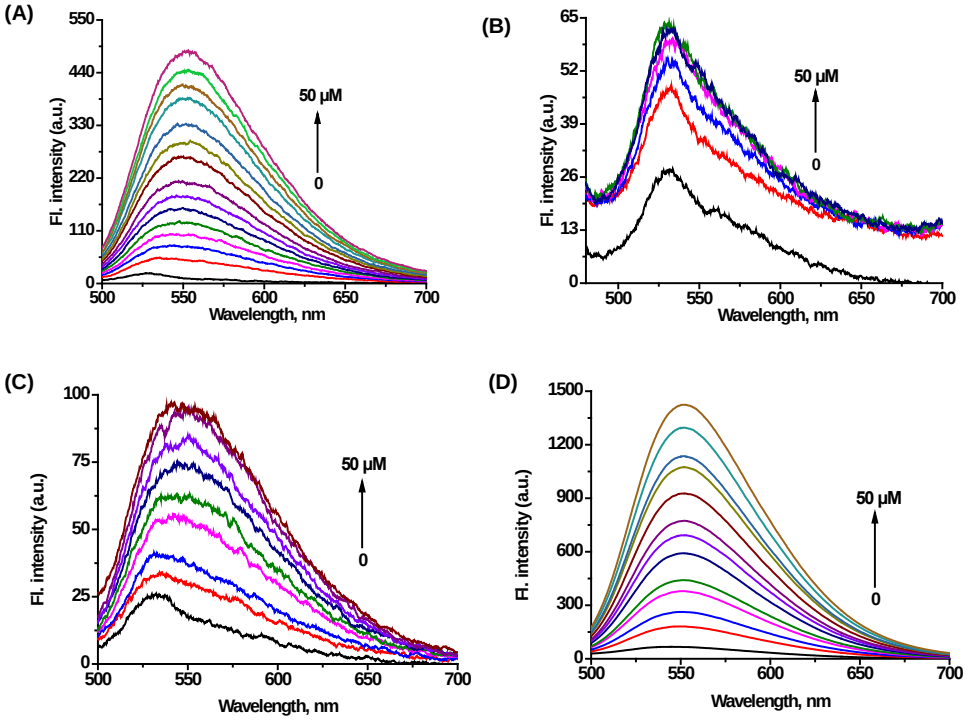
Synthesis of compound 4. Compound **2** (135.5 mg, 0.5 mmol) was dissolved in 4 mL anhydrous CH_2Cl_2 under nitrogen atmosphere. Then diphenylphosphinyl chloride (210 μL) and triethylamine (200 μL) were added to the solution and stirred for 30 min at room temperature. Then the solvent was removed under vacuum and the residue was purified by silica gel chromatography with CH_2Cl_2/Petroleum Ether (v/v, 3:1) as eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 7.3 Hz, 1H), 8.51 (d, *J* = 8.4 Hz, 1H), 8.41 (d, *J* = 8.2 Hz, 1H), 7.95 (m, 3H), 7.78 – 7.70 (m, 2H), 7.58 (t, *J* = 7.2 Hz, 2H), 7.49 (m, 5H), 4.39 (t, *J* = 5.8 Hz, 2H), 3.68 (t, *J* = 5.8 Hz, 2H), 3.34 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 162.5, 151.3, 132.1, 131.4, 130.9, 130.7, 130.6, 129.6, 128.6, 128.3, 128.0, 127.9, 127.5, 127.1, 126.1, 123.9, 121.7, 117.7, 115. 5, 68.6, 57.7, 38.2. MS (ESI): calcd for $C_{27}H_{22}NO_5P$ $(M+H)^+$ 472.1, found 472.6.

Synthesis of compound Naph-OH. Compound **6** was prepared by reported method.[6] Compound **6** (58 mg, 0.1 mmol), 2-hydroxyisoindoline-1, 3-dione (32.6 mg, 0.2 mmol) and potassii (41.4 mg, 0.3 mmol) was dissolved in 5 mL DMSO under nitrogen atmosphere. The reaction mixture was heated to 80 °C and stirred for 8 h. Then the solution was poured onto water and added hydrochloric acid to regulate pH to 2-3, the yellow precipitate was obtained by filtration, washed with water to offer compound **Naph-OH** as a yellow powder.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, *J* = 8.3 Hz, 1H), 8.44 (d, *J* = 7.3 Hz, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 7.88 (t, *J* = 6.8 Hz, 3H), 7.81 – 7.72 (m, 13H), 7.18 (d, *J* = 8.1 Hz, 1H), 4.20 (t, *J* = 6.6 Hz, 2H), 3.76 (d, *J* = 14.3 Hz, 2H), 1.97 (d, *J* = 6.5 Hz, 2H). MS (ESI): calcd for $C_{33}H_{27}NO_3P^+$ $(M+H)^+$ 517.2, found 517. 2.

Synthesis of probe Naph-$O_2^{\cdot-}$. Compound **Naph-OH** (103.2 mg, 0.2 mmol) was dissolved in 3mL anhydrous under nitrogen atmosphere. Then trifluoromethanesulfonic anhydride (150 μL) and triethylamine (100 μL) were added to the solution and stirred for 30 min at room temperature. After completion of reaction, the solvent was removed under vacuum and the residue was purified by silica gel chromatography with CH_2Cl_2/Petroleum Ether (v/v, 3:1) as eluent.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, *J* = 7.2 Hz, 1H), 8.55 (d, *J* = 8.1 Hz, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 7.89 (t, *J* = 7.8 Hz, 1H), 7.79 – 7.62 (m, 16H), 4.36 (t, *J* = 6.7 Hz, 2H), 3.60 (t, *J* = 14.1 Hz, 2H), 2.11 (d, *J* = 7.2 Hz, 2H).^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 161.9, 148.3, 134.2, 132.5, 132.4, 131.6, 130.5, 129.7, 129.5, 127.7, 126.2, 118.0, 117.2, 116.4, 39.4, 28.7, 20.3. HRMS (ESI): calcd for $C_{34}H_{26}F_3NO_5PS^+$ (M^+) 648.1216, found 648.1205.

Synthesis of probe NIR-$O_2^{\cdot-}$. Compound **NIR-OH** was prepared by reported method.[7] Compound **NIR-OH** (53.8 mg, 0.1 mmol) was dissolved in 0.2 mL EtOH and 2 mL anhydrous CH_2Cl_2 mixed solution under nitrogen atmosphere. Then trifluoromethanesulfonic anhydride (80 μL) and triethylamine (50 μL) were added to the solution and stirred for 30 min at room temperature. Then the solvent was removed under vacuum and the residue was purified by silica gel chromatography with CH_2Cl_2/CH_3CN (v/v, 10:1) as eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 15.3 Hz, 1H), 8.17 (d, *J* = 7.7 Hz, 1H), 7.68 (t, *J* = 7.2 Hz, 1H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 4H), 7.19 (d, *J* = 6.0 Hz, 1H), 7.14 (d, *J* = 7.3 Hz, 1H), 6.76 – 6.65 (m, 2H), 4.15 (dd, *J* = 13.7, 6.7 Hz, 2H), 3.99 (s, 3H), 2.18 (d, *J* = 25.6 Hz, 4H), 1.94 (s, 2H), 1.76 (s, 6H), 1.58 (d, *J* = 23.3 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ178.6, 164.3, 156.6, 145.4, 144.4, 141.2, 140.9, 133.6, 132.6, 130.5, 128.8, 128.7, 128.5, 128.1, 127.5, 126.3, 123.2, 121.7, 121.3, 116.0, 112.6, 109.8, 107.3, 60.5, 50.3, 32.8, 30.6, 28.5, 28.2, 26.7, 22.8, 21.6. HRMS (ESI): calcd for $C_{36}H_{32}ClF_3NO_6S^+$ (M^+) 698.1585, found 698.1578.

8. Supplemental Figures



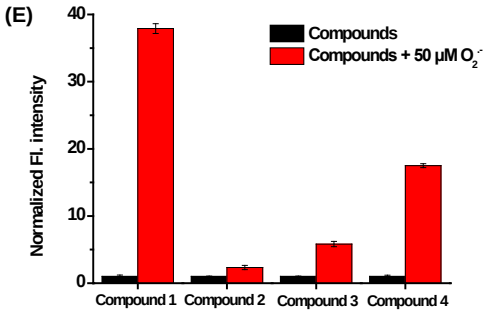


Fig. S1 Fluorescence spectra of (A) compound **1**, (B) compound **2**, (C) compound **3**, (D) compound **4** (10 μM) upon addition of $O_2^{\cdot-}$ (0-50 μM) in PBS buffer solution (25 mM, 30% DMSO, pH 7.4 (A-C) or 25 mM, 30% CH_3CN, pH 7.4 (D)). (E) The normalized fluorescence intensity of compounds **1**-**4** upon addition of $O_2^{\cdot-}$ (0 and 50 μM) in PBS buffer solution (25 mM, 30% DMSO, pH 7.4 (compounds **1**-**3**) or 25 mM, 30% CH_3CN, pH 7.4 (compound **4**)). Excitation wavelength was 450 nm.

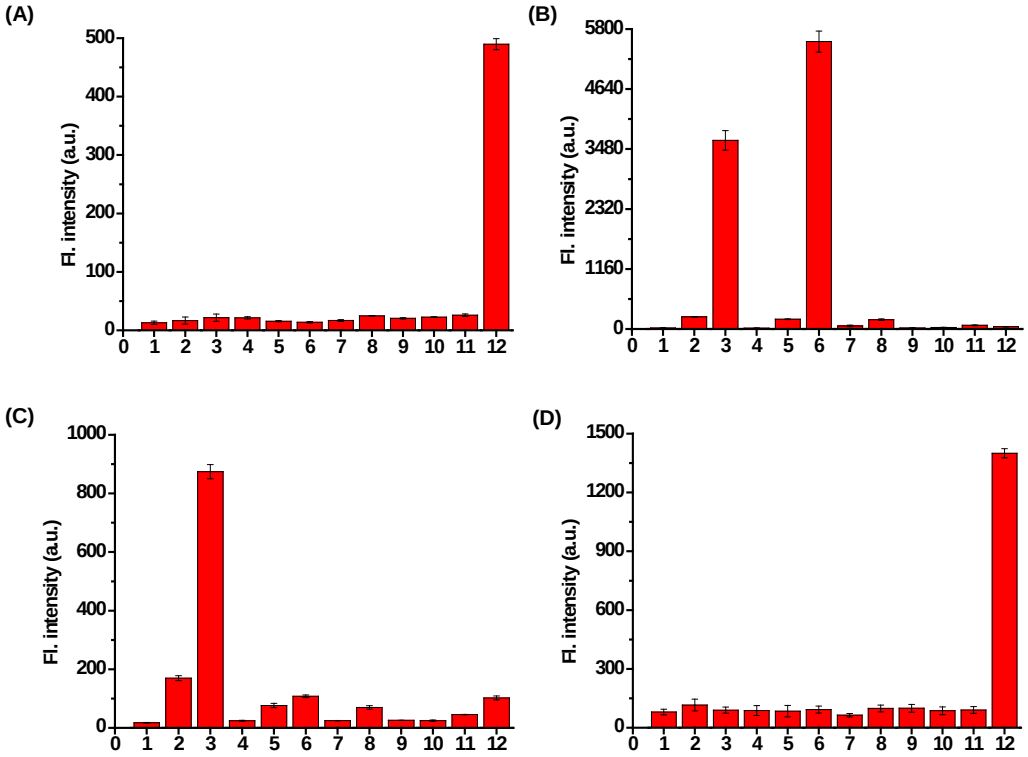


Fig. S2 Fluorescence spectra of (A) compound **1**, (B) compound **2**, (C) compound **3** (10 μM) in PBS buffer solution (25 mM, 30% DMSO, pH 7.4) and (D) compound **4** (10 μM) in PBS buffer solution (25 mM, 30% CH_3CN, pH 7.4) toward $O_2^{\cdot-}$ (50 μM) and other analytes: 1, blank; 2,Cys (100 μM); 3, GSH (1 mM); 4, H_2O_2 (100 μM); 5, H_2S (100 μM); 6, H_2S_2 (100 μM); 7, HOCl (100 μM); 8, HSO_3^- (100 μM); 9, NO (100 μM); 10, SIN-1 (100 μM); 11, SO_3^{2-} (100 μM); 12, $O_2^{\cdot-}$ (50 μM). Excitation wavelength was 450 nm.

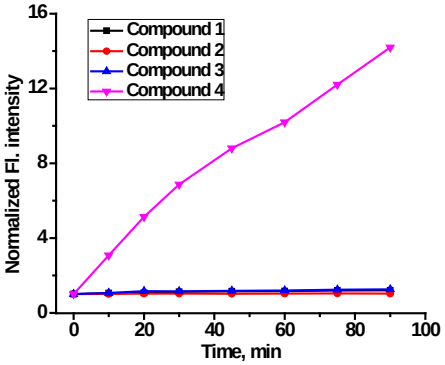


Fig. S3 Time profile of fluorescence for compound **1**, **2**, **3** (10 μM) in PBS solution (25 mM, 30% DMSO, pH 7.4) and compound **4** (10 μM) in PBS solution (25 mM, 30% CH_3CN, pH 7.4).

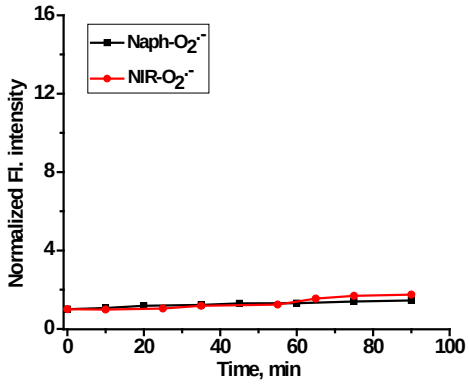


Fig. S4 Time profile of fluorescence for probe **Naph-$O_2^{\cdot-}$** (5 μM) in PBS solution (pH 7.4, 25 mM, 30% DMSO) and **NIR-$O_2^{\cdot-}$** (5 μM) in PBS solution (pH 7.4, 25 mM, 20% CH_3CN).

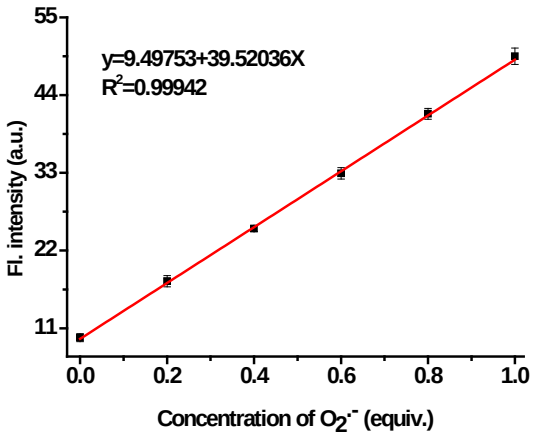


Fig. S5 Linear correlation between the fluorescence emission intensity of **NIR-$O_2^{\bullet-}$** (5 μM) in PBS buffer solution (25 mM, 20% CH_3CN, pH 7.4) and superoxide anion concentration.

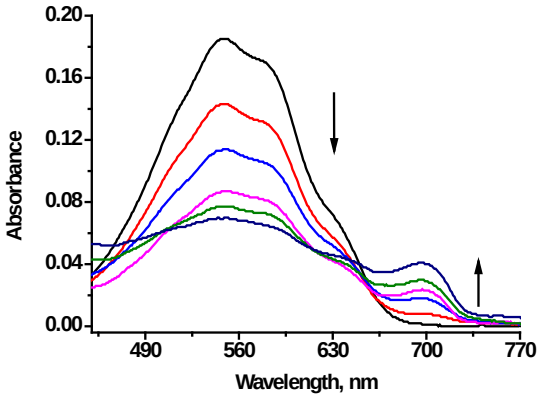


Fig. S6 Absorption spectra of **NIR-$O_2^{\bullet-}$** (5 μM) in the presence of $O_2^{\bullet-}$ (0-30 μM) in PBS buffer solution (25 mM, 20% CH_3CN, pH 7.4).

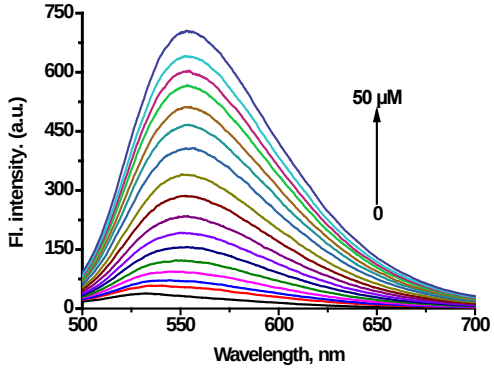


Fig. S7 Fluorescence spectra of **Naph-$O_2^{\bullet-}$** (5 μM) in PBS buffer solution (25 mM, 30% DMSO, pH 7.4) upon addition of $O_2^{\bullet-}$ (0-50 μM). Excitation wavelength was 450 nm.

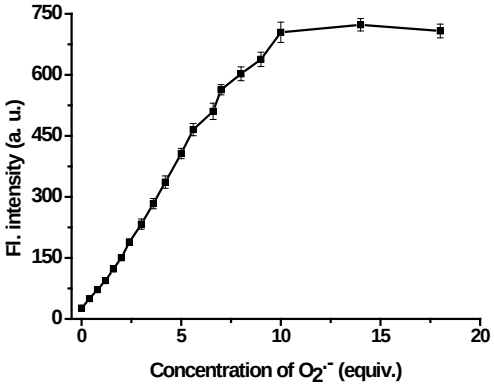


Fig. S8 Fluorescence intensity (554 nm) of probe **Naph-$O_2^{\bullet-}$** (5 μM) in PBS buffer solution (pH 7.4, 25 mM, 30% DMSO) reacts with $O_2^{\bullet-}$. Excitation wavelength was 450 nm.

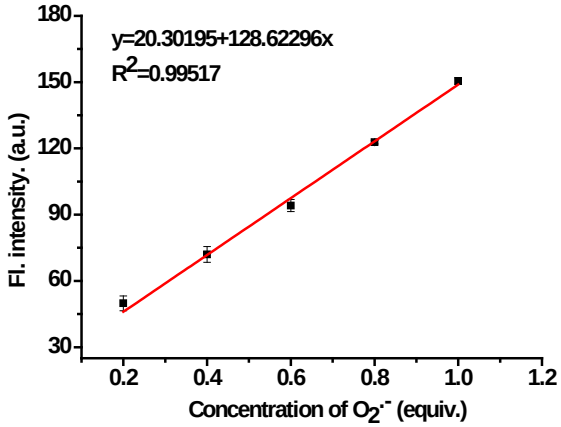


Fig. S9 Linear correlation between the fluorescence emission intensity of **Naph-$O_2^{\cdot-}$** (5 μM) in PBS buffer solution (25 mM, 30% DMSO, pH 7.4) and superoxide anion concentration.

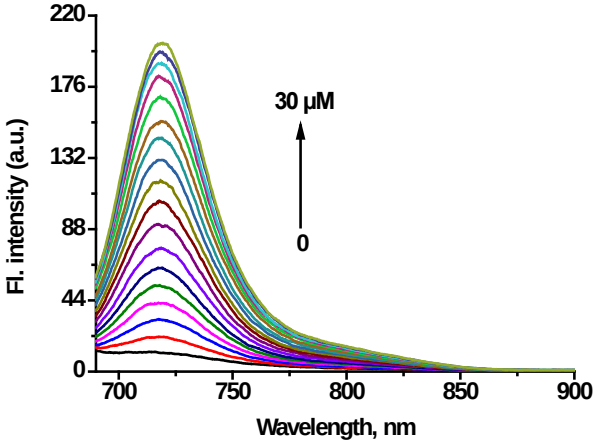


Fig. S10 Fluorescence spectra of **NIR-$O_2^{\cdot-}$** (5 μM) in PBS buffer solution (25 mM, 20% CH_3CN, pH 7.8) upon addition of $O_2^{\cdot-}$(0-30 μM). Excitation wavelength was 660 nm.

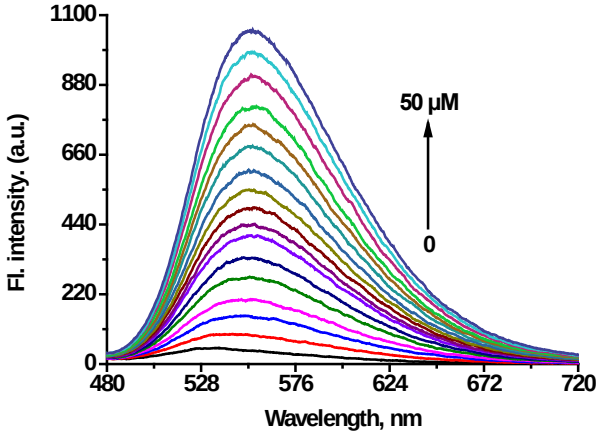


Fig. S11 Fluorescence spectra of **Naph-$O_2^{\cdot-}$** (5 μM) in PBS buffer solution (25 mM, 30% DMSO, pH 7.8) upon addition of $O_2^{\cdot-}$ (0-50 μM). Excitation wavelength was 450 nm.

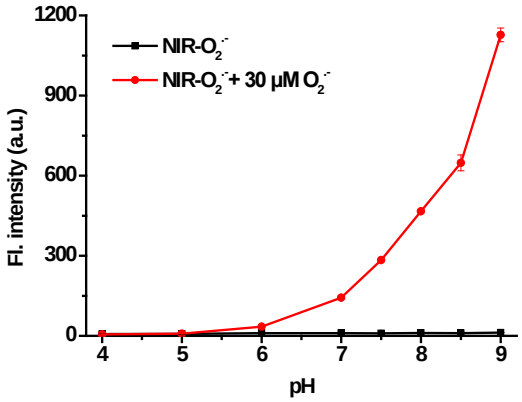


Fig. S12 pH-dependent fluorescence intensity changes of **NIR-$O_2^{\cdot-}$** (5 μM) toward $O_2^{\cdot-}$ (30 μM) in PBS buffer solution (25 mM, 20% CH_3CN, pH = 4.0-9.0), λ_{ex}: 660 nm.

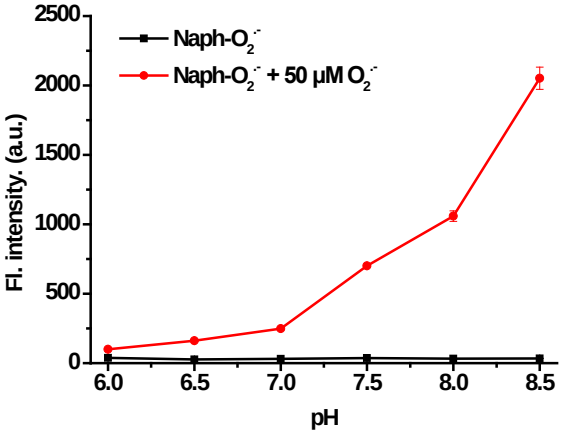


Fig. S13 pH-dependent fluorescence intensity changes of **Naph-$O_2^{\cdot-}$** (5 μM) toward $O_2^{\cdot-}$ (50 μM) in PBS buffer solution (25 mM, 30% DMSO, pH = 6.0-8.5), λ_{ex}: 450 nm.

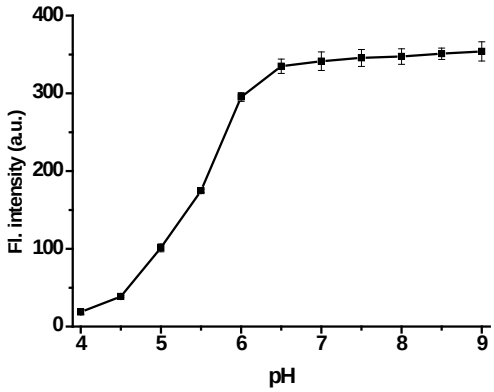


Fig. S14 pH-dependent fluorescence intensity changes of **NIR-OH** (5 μM) in PBS buffer solution (25 mM, 20% CH_3CN), λ_{ex}: 660 nm.

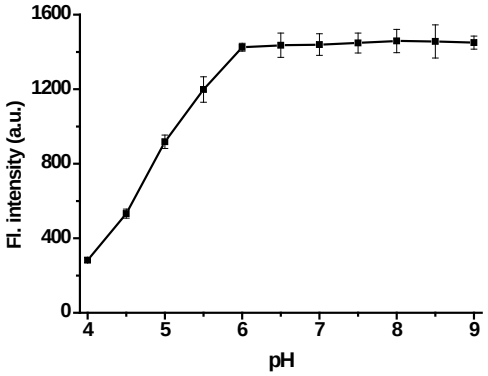


Fig. S15 pH-dependent fluorescence intensity changes of **Naph-OH** (5 μM) in PBS buffer solution (25 mM, 30% DMSO), λ_{ex}: 450 nm.

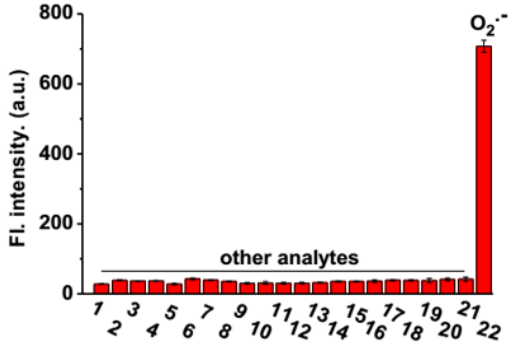
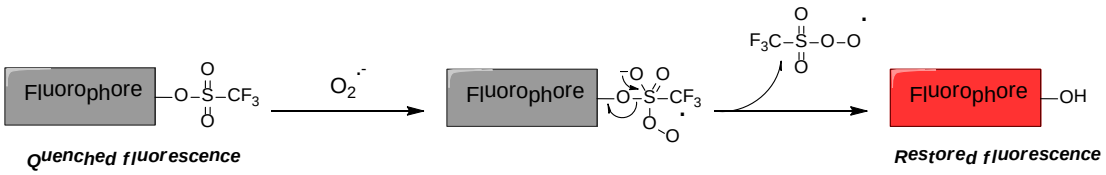


Fig. S16 Fluorescence intensity of **Naph-$O_2^{\bullet-}$** in PBS buffer solution (25 mM, 30% DMSO, pH 7.4) toward $O_2^{\bullet-}$ (50 μM) and other analytes: 1: blank; 2: Ca^{2+} (100 μM); 3: CH_3COO^- (100 μM); 4: Cu^{2+} (100 μM); 5: Cys (100 μM); 6: Fe^{3+} (100 μM); 7: GSH (1 mM); 8: H_2O_2 (100 μM); 9: H_2S (100 μM); 10: H_2S_2 (50 μM); 11: HOCl (100 μM); 12: HSO_3^- (100 μM); 13: K^+ (100 μM); 14: Mg^{2+} (100 μM); 15: Na^+ (100 μM); 16: NO (100 μM); 17: NO_2^- (100 μM); 18: SIN-1 (25 μM); 19: SO_3^{2-} (100 μM); 20: SO_4^{2-} (100 μM); 21: Zn^{2+} (100 μM); 22: $O_2^{\bullet-}$.



Scheme S2. Proposed mechanism for recognizing $O_2^{\bullet-}$ based on a fluorophore accommodating trifluoromethanesulfonate.

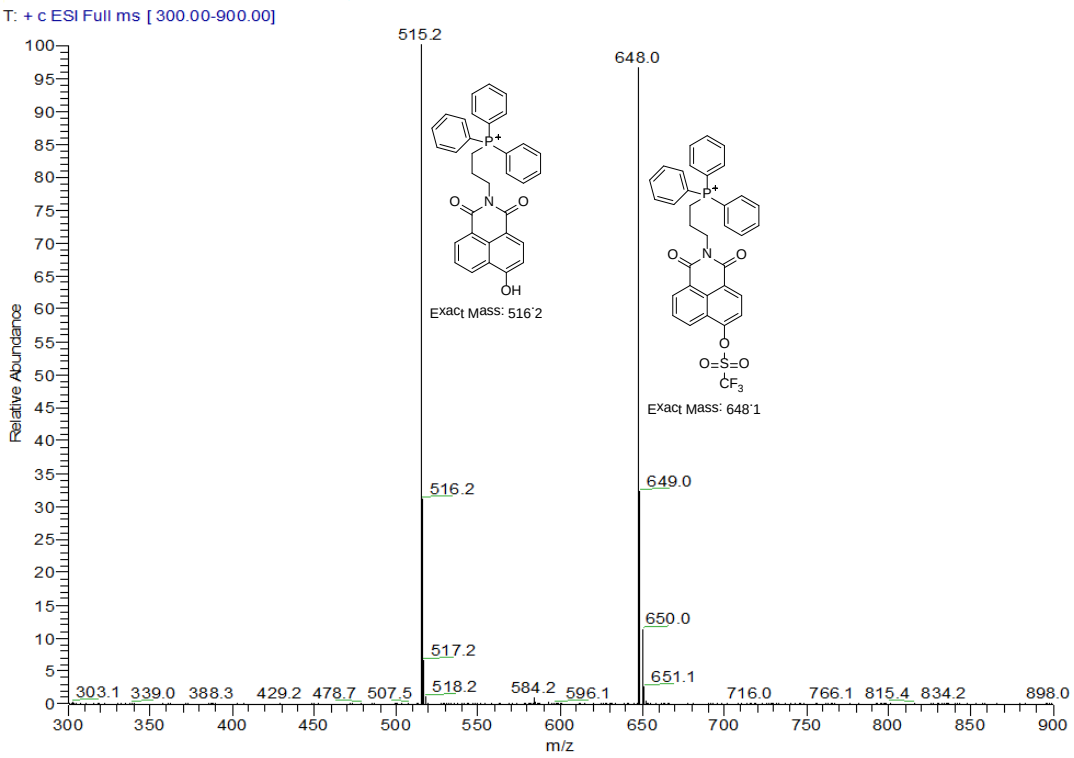


Fig. S17 The ESI mass spectrum of compound **Naph-$O_2^{\cdot-}$** in the presence of $O_2^{\cdot-}$.

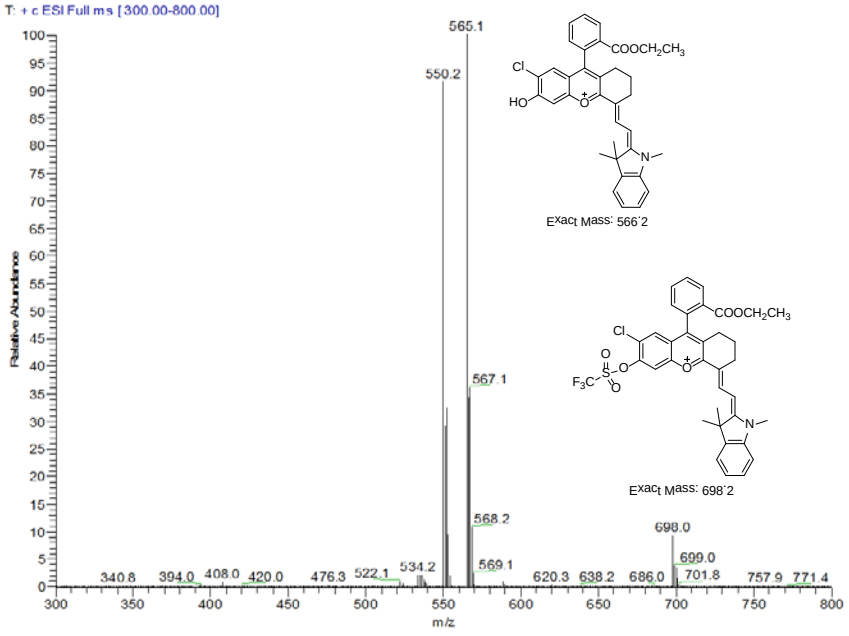


Fig. S18 The ESI mass spectrum of compound **NIR-$O_2^{\cdot-}$** in the presence of $O_2^{\cdot-}$.

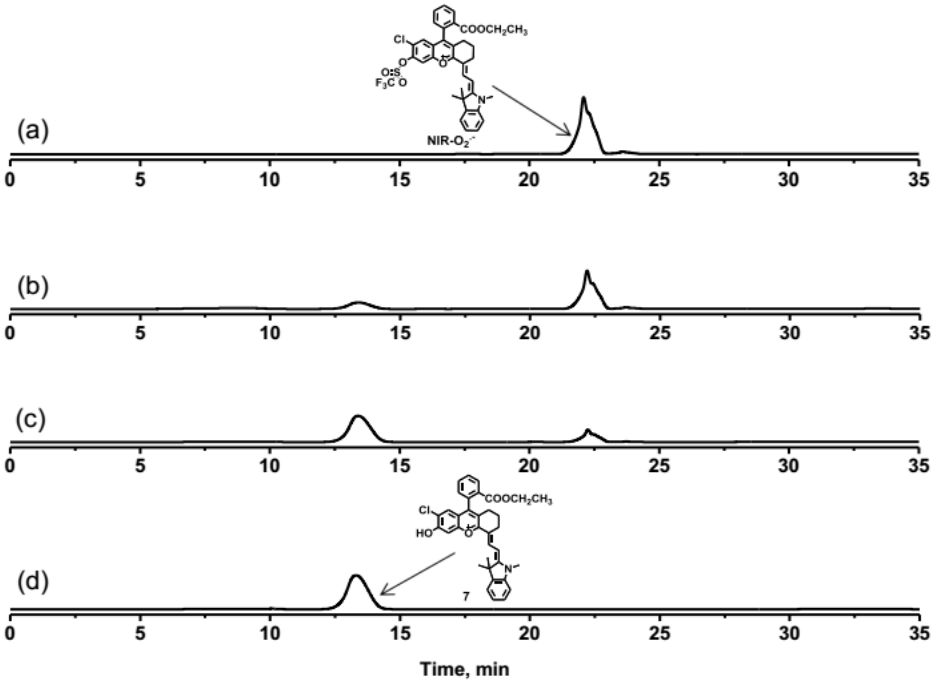


Fig. S**19** Reversed-phase HPLC with absorption (350 nm) detection. (a) Reversed-phase HPLC chromatograms of 100 μM **NIR-$O_2^{\cdot-}$**. (b) Reversed-phase HPLC chromatograms of 100 μM **NIR-$O_2^{\cdot-}$** in the presence of 100 μM $O_2^{\cdot-}$. (c) Reversed-phase HPLC chromatograms of 100 μM **NIR-$O_2^{\cdot-}$** in the presence of 400 μM $O_2^{\cdot-}$. (d) Reversed-phase HPLC chromatograms of 100 μM compound **7**.

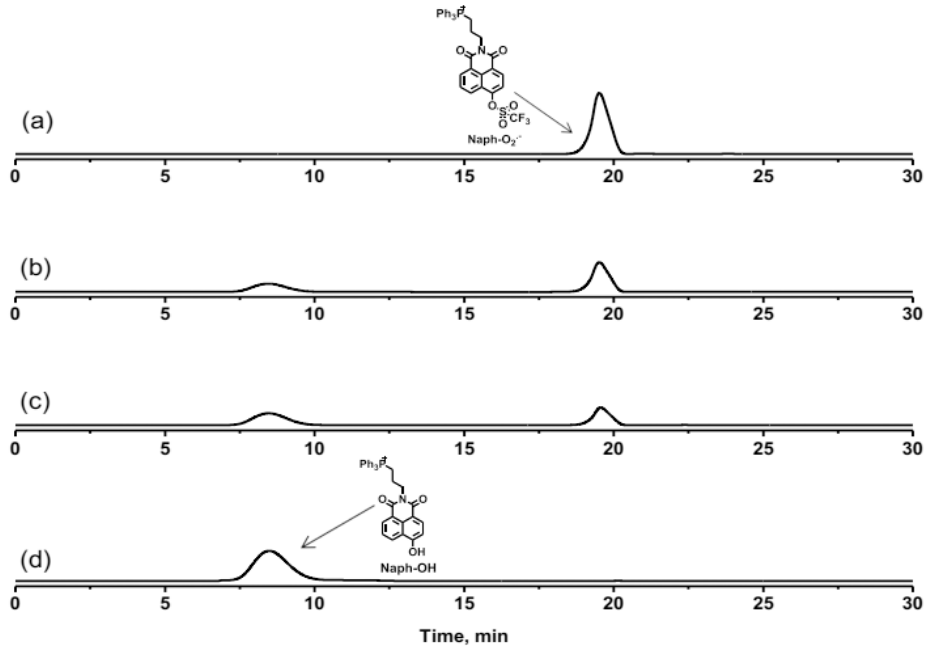


Fig. S**20** Reversed-phase HPLC with absorption (350 nm) detection. (a) Reversed-phase HPLC chromatograms of 100 μM **Naph-$O_2^{\cdot-}$**. (b) Reversed-phase HPLC chromatograms of 100 μM **Naph-$O_2^{\cdot-}$** in the presence of 100 μM $O_2^{\cdot-}$. (c) Reversed-phase HPLC chromatograms of 100 μM **Naph-$O_2^{\cdot-}$** in the presence of 200 μM $O_2^{\cdot-}$. (d) Reversed-phase HPLC chromatograms of 100 μM compound **Naph-OH**.

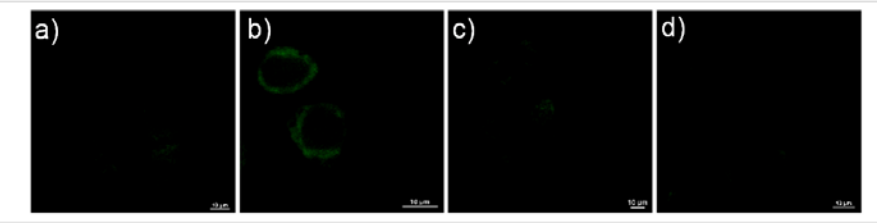


Fig. S21 Confocal fluorescence images of the HepG2 cells with probe **Naph-$O_2^{\cdot-}$**. (a) HepG2 cells treated with probe **Naph-$O_2^{\cdot-}$** (5 μM) for 30 min, then imaged; (b) Cells were pre-stimulated with LPS (1 μg/mL) and IFN-γ (50 ng/mL) for 12 h, subsequently incubated with probe **Naph-$O_2^{\cdot-}$** (5 μM, 30 min), then imaged; (c-d) Cells pretreated with $O_2^{\cdot-}$ scavenger TEMPO (300 μM) or Tiron (10 μM) during stimulation with LPS (1 μg/mL)/IFN-γ (50 ng/mL) for 12 h, subsequently incubated with probe **Naph-$O_2^{\cdot-}$** (5 μM, 30 min), then imaged. Scale bar: 10 μm. The fluorescence images were captured from the green channel of 500-550 nm with an excitation at 488 nm.

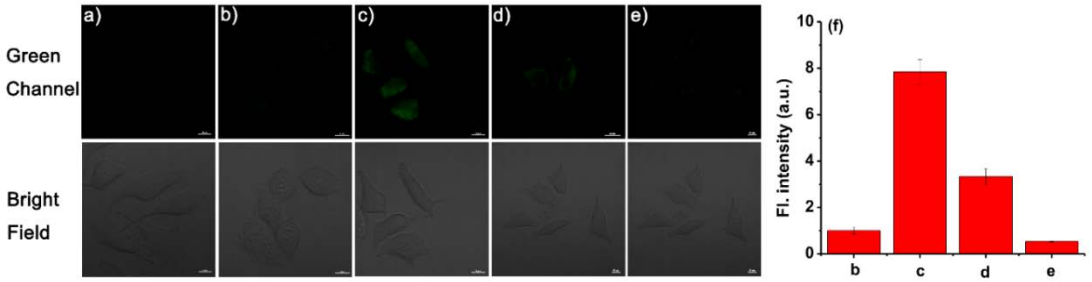


Fig. S22 Confocal fluorescence images of the HeLa cells with probe **Naph-$O_2^{\cdot-}$**. (a) Intact HeLa cells; (b) HeLa cells treated with probe **Naph-$O_2^{\cdot-}$** (5 μM) for 30 min; (c) Cells were pre-stimulated with LPS (1 μg/mL) and IFN-γ (50 ng/mL) for 12 h, subsequently incubated with **Naph-$O_2^{\cdot-}$** (5 μM, 30 min), then imaged; (d, e) Cells pretreated with $O_2^{\cdot-}$ scavenger TEMPO (300 μM) (d) or Tiron (10 μM) (e) during stimulation with LPS (1 μg/mL)/IFN-γ (50 ng/mL) for 12 h, subsequently incubated with **Naph-$O_2^{\cdot-}$** (5 μM, 30 min), then imaged. Scale bar: 10 μm. The fluorescence images were captured from the green channel of 500-550 nm with an excitation at 488 nm. (f) The relative intensity of the corresponding fluorescence images in Figure S18 (a-e).

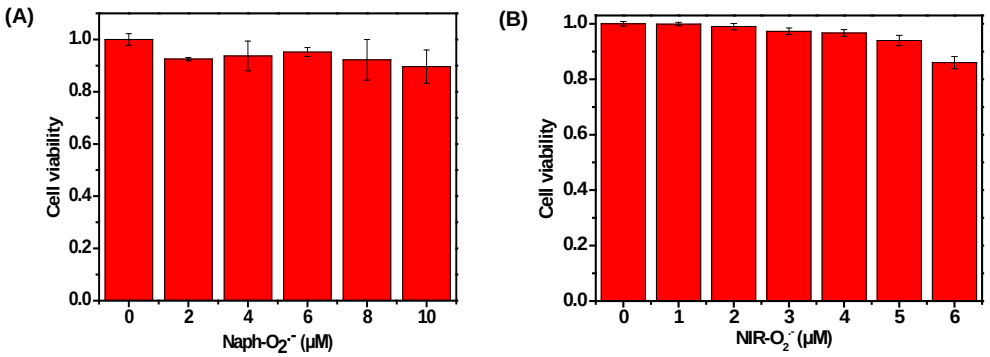


Fig. S23 Cytotoxicity of **Naph-$O_2^{\bullet-}$** (A) and **NIR-$O_2^{\bullet-}$** (B) in HepG2 cells. Cells were incubated with these probes at corresponding concentrations for 12 h. Cell viability was measured by MTT assay and the results are reported as percentage relative to untreated cells (mean ± SD).

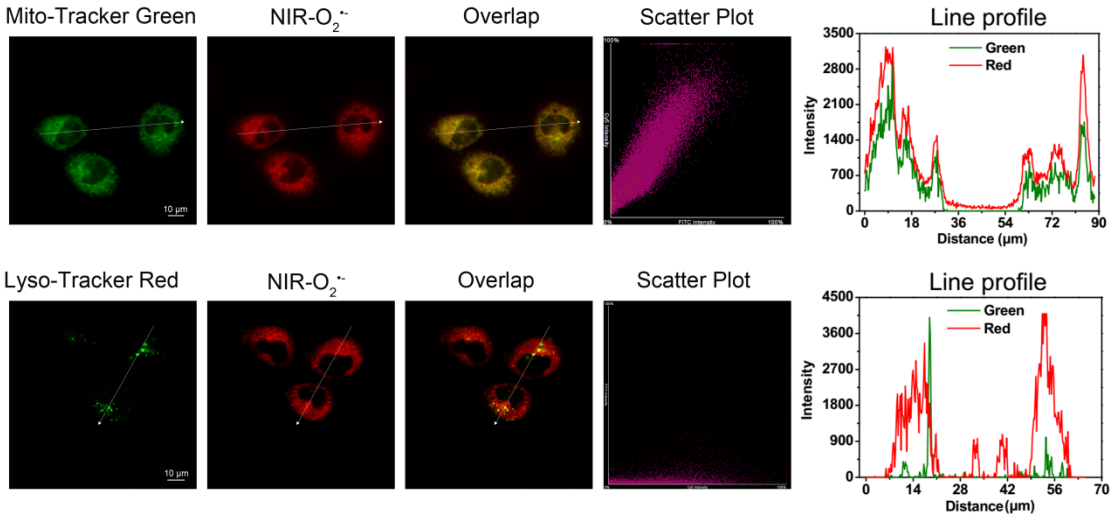


Fig. S24 Intracellular localization of **NIR-$O_2^{\bullet-}$** in HK-2 cells. Images of HK-2 cells pre-treated with LPS (1 μg/mL) and IFN-γ (50 ng/mL) for 12 h, then incubated with 5 μM **NIR-$O_2^{\bullet-}$** for 30 min and subsequently added 1 μM Mito-Tracker Green (or 1 μM Lyso-Tracker Red) for 10 min. First column: green channel of Mito-Tracker Green (λ_{ex}= 488 nm, λ_{em} = 500-550 nm) and Lyso-Tracker Red fluorescence (λ_{ex}= 561 nm, λ_{em} = 570-620 nm); Second column: red channel of **NIR-$O_2^{\bullet-}$** (λ_{ex} = 640 nm, λ_{em} =663-738 nm); Third column: merged signal. Scatter plot: the overlap of green and red channel images. Line profile: Intensity profile of the white line in image overlap. Scale bar: 10 μm.

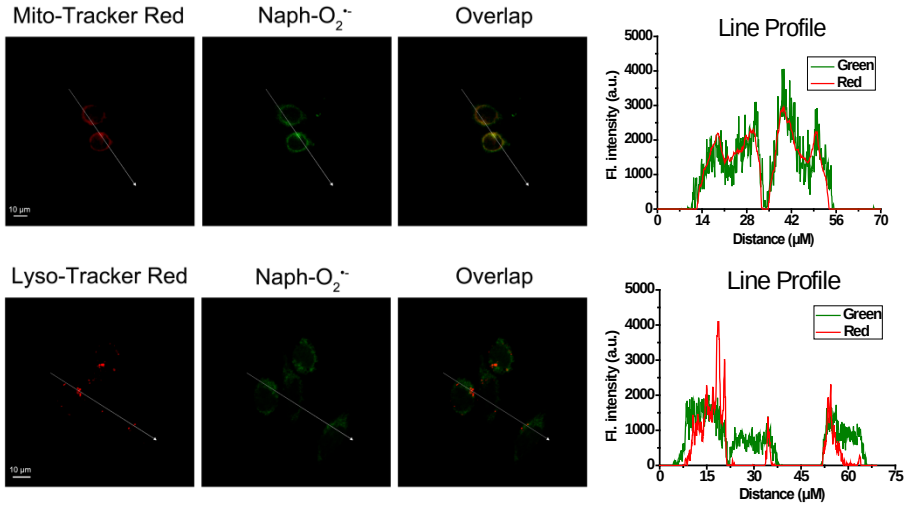


Fig. S25 Intracellular localization of **Naph-$O_2^{\bullet-}$** in HepG2 cells. Images of HepG2 cells pre-treated with LPS (1 μg/mL) and IFN-γ (50 ng/mL) for 12 h, then incubated with 5 μM **Naph-$O_2^{\bullet-}$** for 30 min and subsequently 1 μM Mito-Tracker Red (or 1 μM Lyso-Tracker Red) for 10 min. Red channel: Mito-Tracker Red (λ_{ex}= 640 nm, λ_{em}=663-738 nm) and Lyso-Tracker Red fluorescence (λ_{ex} = 561 nm, λ_{em} = 570-620 nm); green channel: **Naph-$O_2^{\bullet-}$** fluorescence (λ_{ex} = 488 nm, λ_{em} = 500-550 nm); yellow: merged signal. Line profile: Intensity profile of the white line in image overlap. Scale bar: 10 μm.

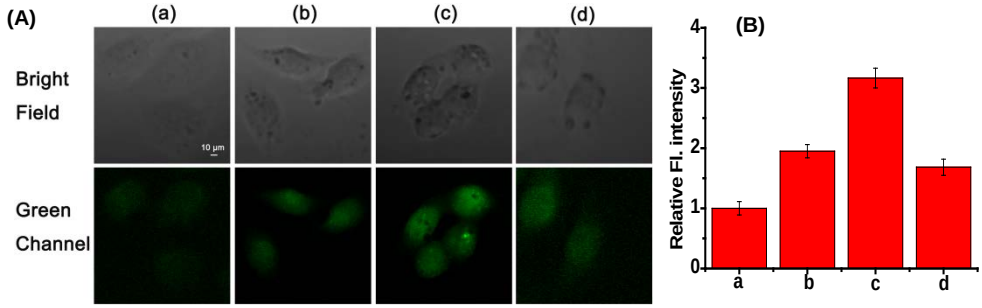


Fig. S26 Two photon fluorescence images of $O_2^{\bullet-}$ in HK-2 cells. Images were constructed with the addition of 5 μM **Naph-$O_2^{\bullet-}$** in the same confocal dish (a–d). (a) The HK-2 cells were treated with 5 μM **Naph-$O_2^{\bullet-}$**; (b, c) Cells were pre-treated with different concentrations of cisplatin (500 μM, 1000 μM) for 12 h, and then treated with 5 μM probe **Naph-$O_2^{\bullet-}$** for 30 min; (d) Cells were pre-incubated with LC (1000 μM) in the presence of cisplatin (1000 μM) for 12 h, and then treated with 5 μM probe **Naph-$O_2^{\bullet-}$** for 30 min. (B) Average intensity in Figure S22 (a-d), respectively. Data are expressed as mean ± SD of three experiments. Excitation wavelength at 800 nm. The emission band at 520-580 nm. Scale bar = 10 μm.

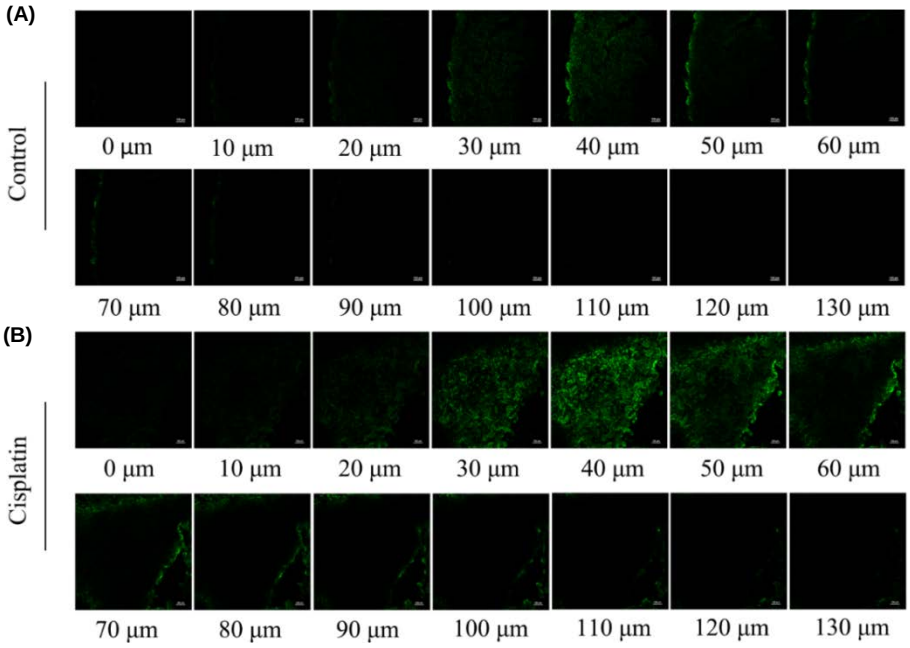


Fig. S27 Two-photon fluorescence images of a fresh rat kidney slice. PBS (320 μL) or cisplatin (320 μL, 20 mg/kg) was subcutaneously injected into abdominal cavity of mice to be as the control group (A) and to cause acute kidney injury (B). After 48 h, the mice were anesthetized and the kidney was dissected and then incubated with **Naph-$O_2^{\bullet-}$** (10 μM) for 30 min for imaging at the depths of approximately 0~130 μm. Excitation at 800 nm. Scale bar: 100 μm.

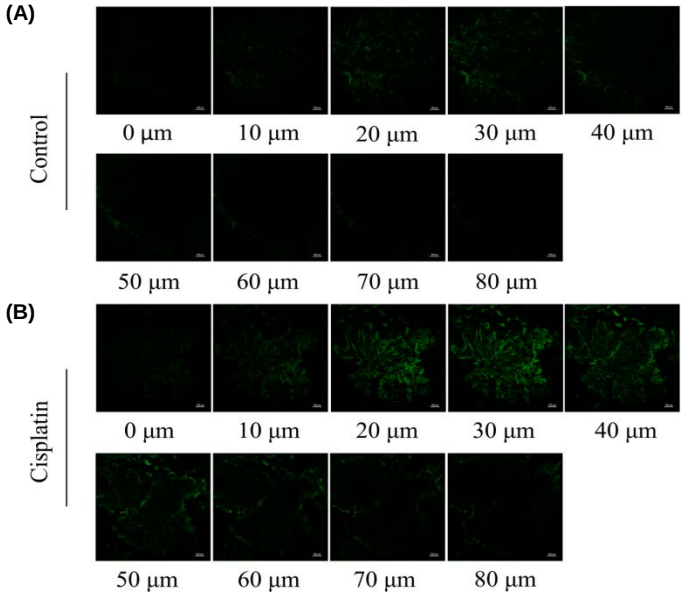


Fig. S28 Confocal fluorescence images of a fresh rat kidney slice. PBS (320 μL) or cisplatin (320 μL, 20 mg/kg) was subcutaneously injected into abdominal cavity of mice to be as the control group (A) and to cause acute kidney injury (B). After 48 h, the mice were anesthetized and the kidney was dissected and then incubated with **Naph-$O_2^{\bullet-}$** (10 μM) for 30 min for imaging at the depths of approximately 0~80 μm. Excitation at 488 nm. Scale bar: 100 μm.

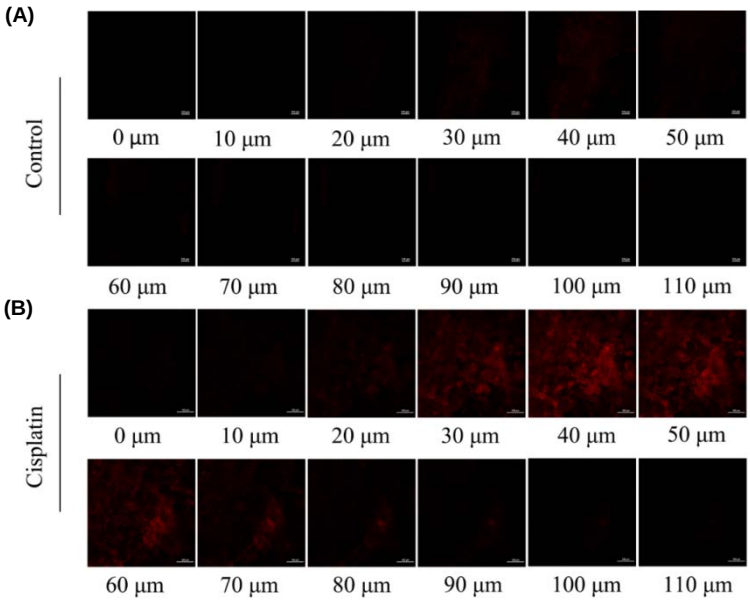


Fig. S29 Confocal fluorescence images of a fresh rat kidney slice. PBS (320 μL) or cisplatin (320 μL, 20 mg/kg) was subcutaneously injected into abdominal cavity of mice to be as the control group (A) and to cause acute kidney injury (B). After 48 h, the mice were anesthetized and the kidney was dissected and then incubated with **NIR-$O_2^{\bullet-}$** (5 μM) for 30 min for imaging at the depths of approximately 0~110 μm. Excitation at 640 nm. Scale bar: 100 μm.

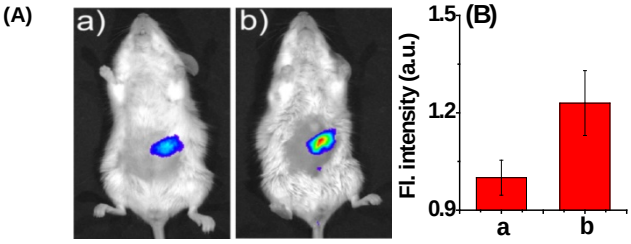


Fig. S30 (A) *In vivo* fluorescence imaging of mice that were intraperitoneally preinjected with (a) PBS and (b) 20 mg/kg cisplatin before intravenous injection of **NIR-$O_2^{\bullet-}$** (100 μL× 200 μM) in PBS. (B) Average fluorescence intensity in A. Values are the mean ± s.d. for n=4.

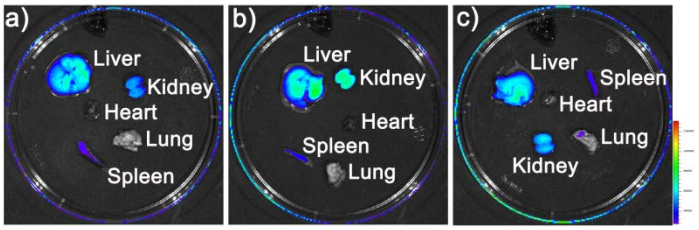


Fig. S31 Confocal fluorescence images of the various organs by using **NIR-$O_2^{\bullet-}$**. (a) The mice were injected intraperitoneally with PBS (320 μL), then intravenous injected with the **NIR-$O_2^{\bullet-}$** (100 μL, 100 μM) after 48 h. Then the various organs were dissected for imaging. (b) The mice were injected intraperitoneally with cisplatin (320 μL, 20 mg/kg), then intravenous injected with the **NIR-$O_2^{\bullet-}$** (100 μL, 100 μM) after 48 h. Then the various organs were dissected for imaging. (c) The mice were preinjected intraperitoneally with LC (60 μL, 400 mg/kg) for 48 h before injected intraperitoneally with cisplatin (320 μL, 20 mg/kg), after 48 h, then intravenous injected with the **NIR-$O_2^{\bullet-}$** (100 μL, 100 μM). Then the various organs were dissected for imaging. Excitation at 640 nm. Emission channel at 695-770 nm.

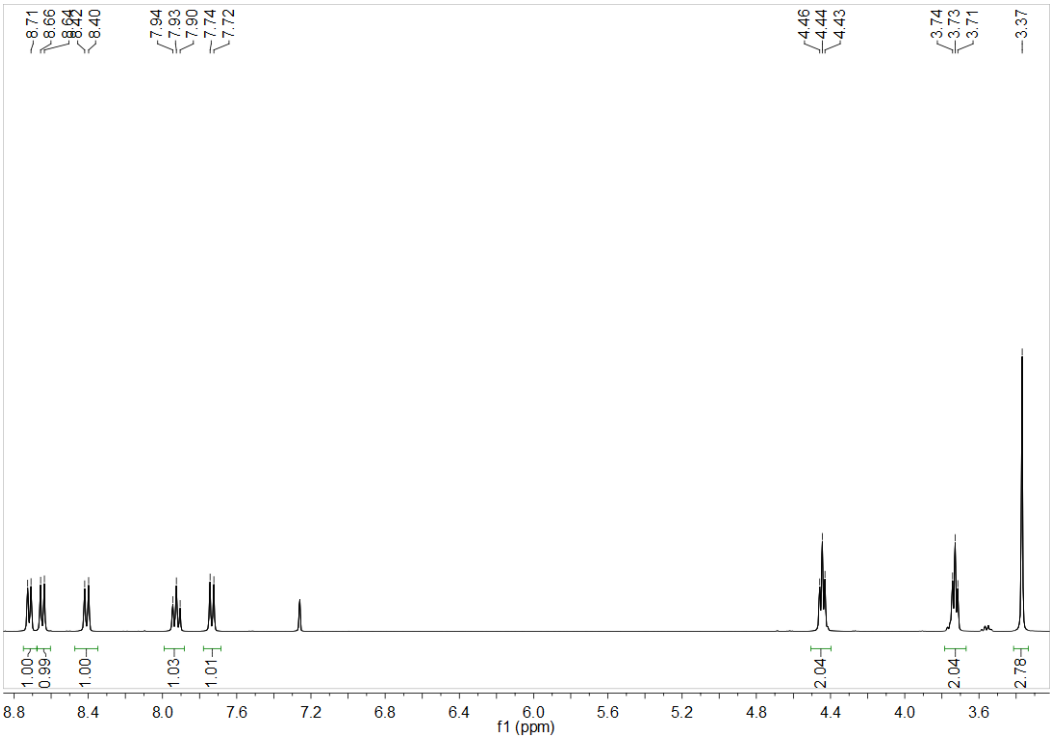


Fig. S32 The ^{1}HNMR spectrum of compound **1** ($CDCl_3$)

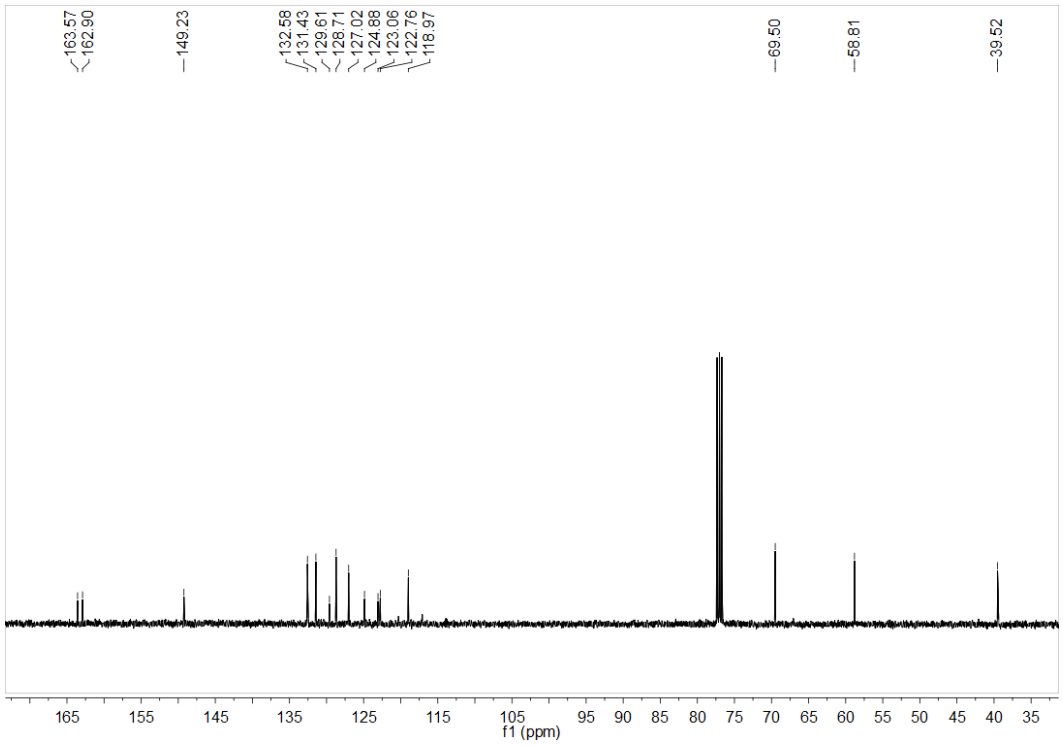


Fig. S33 The ^{13}CNMR spectrum of compound **1** ($CDCl_3$)

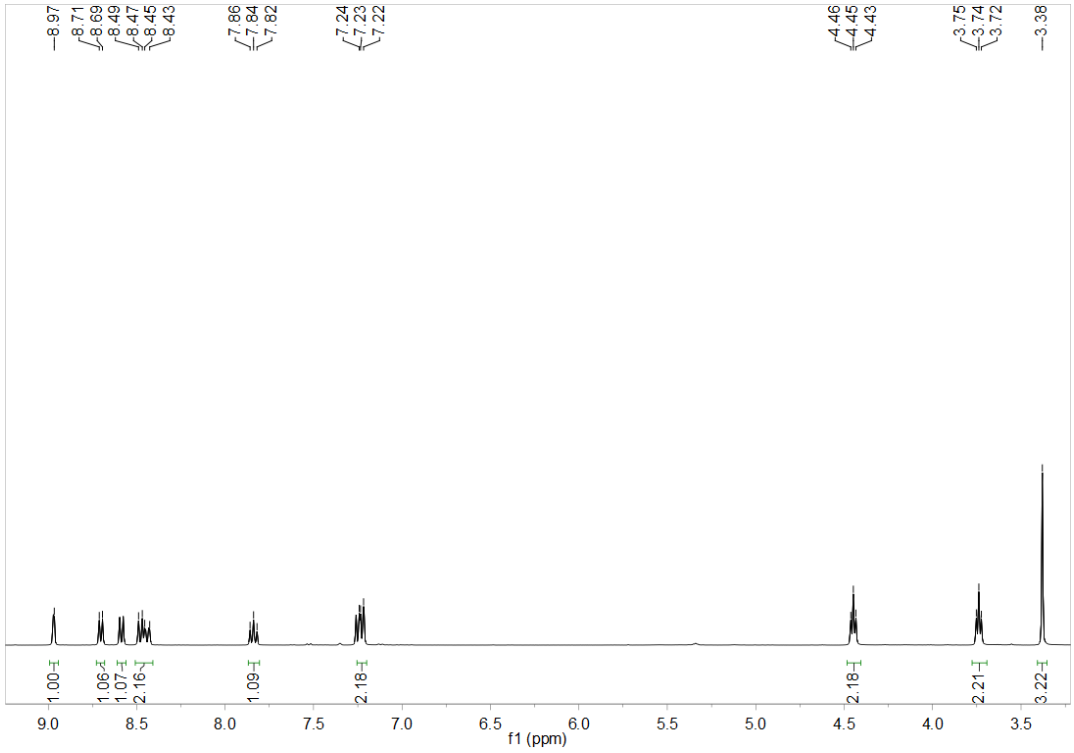


Fig. S34 The ^{1}HNMR spectrum of compound **2** ($CDCl_3$)

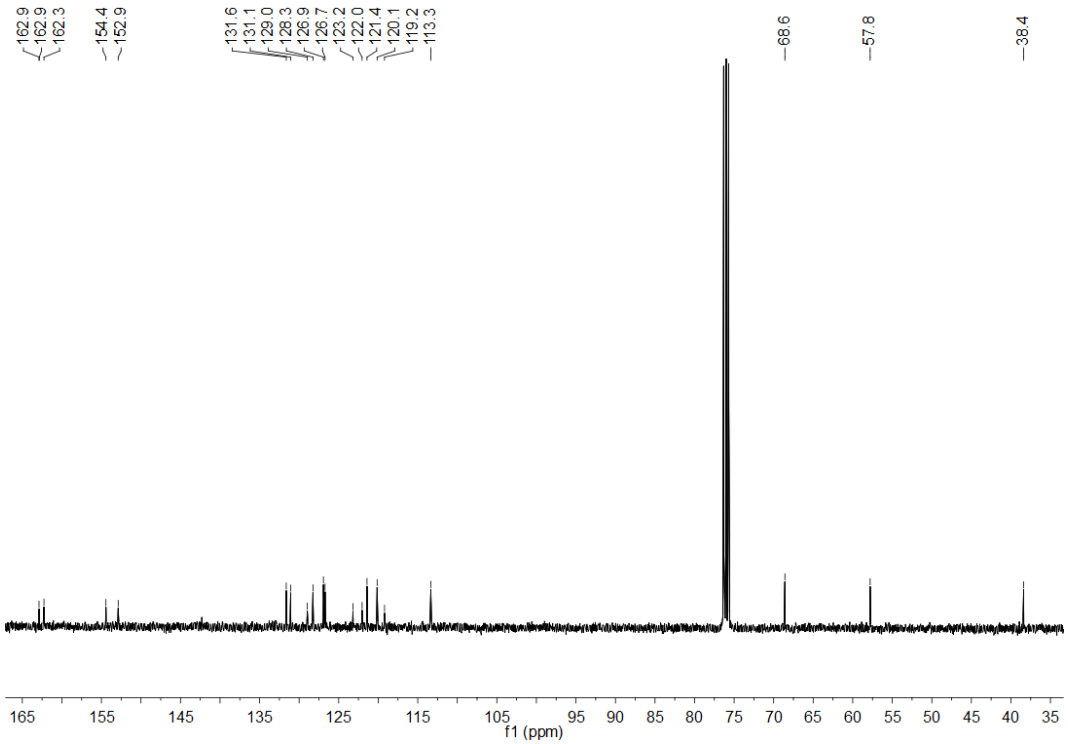


Fig. S35 The ^{13}CNMR spectrum of compound **2** ($CDCl_3$)

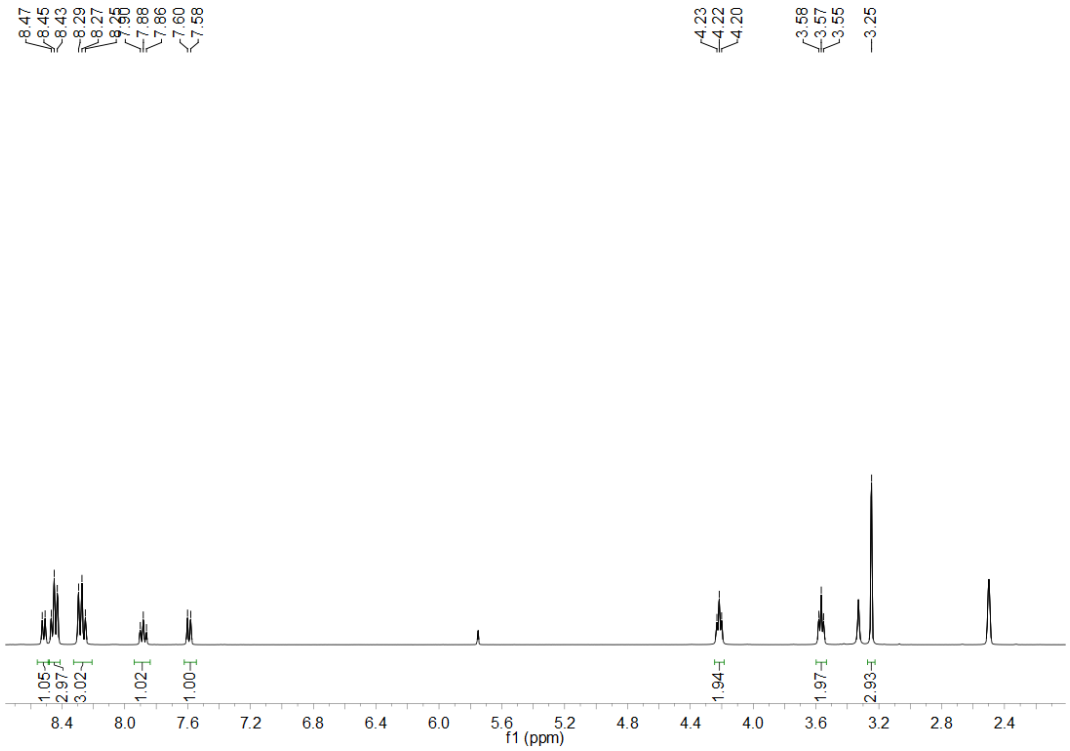


Fig. S36 The ^{1}HNMR spectrum of compound **3** (DMSO-d_6)

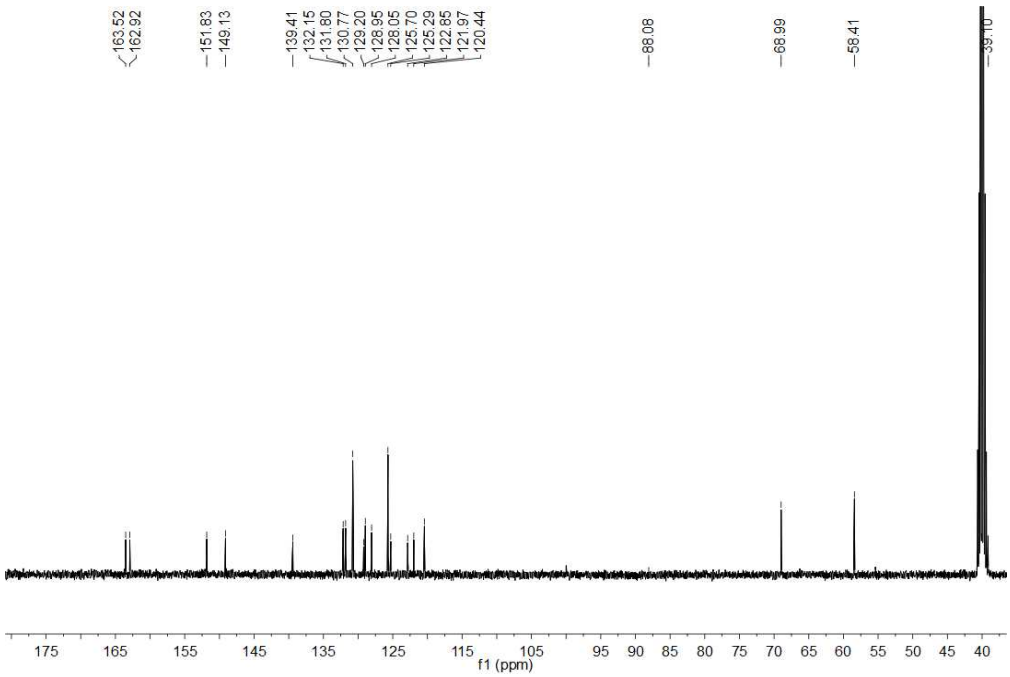


Fig. S37 The ^{13}CNMR spectrum of compound **3** (DMSO-d_6)

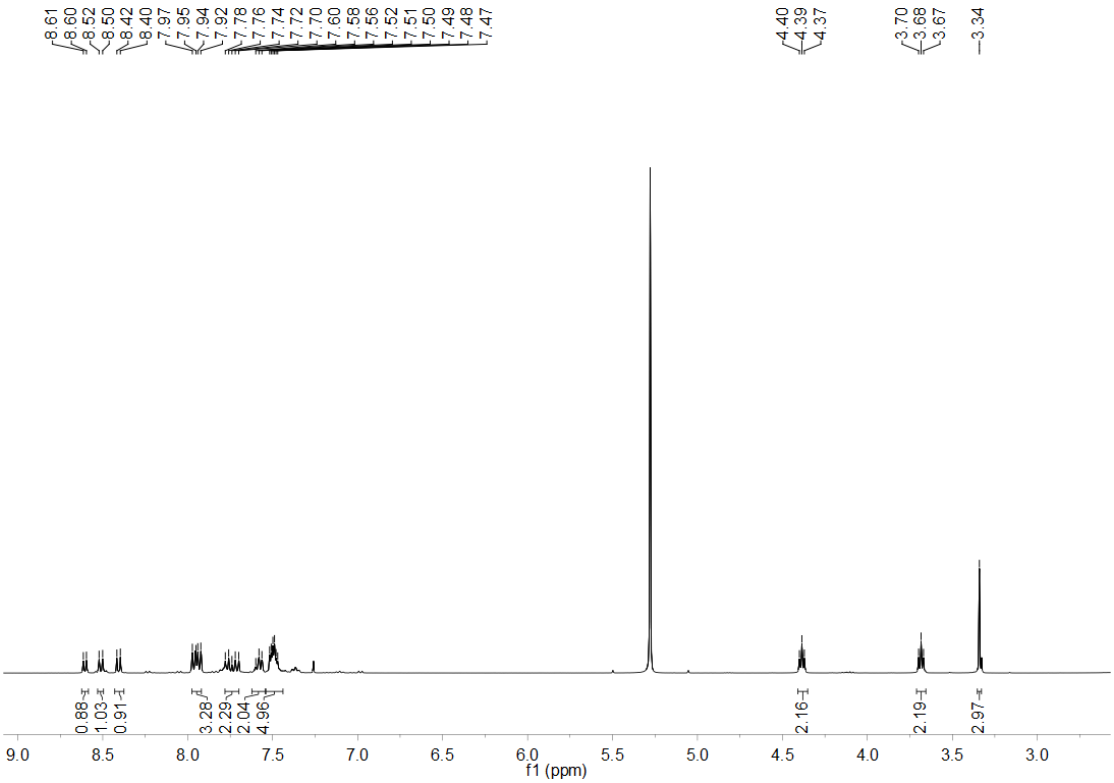


Fig. S38 The ^{1}HNMR spectrum of compound **4** ($CDCl_3$)

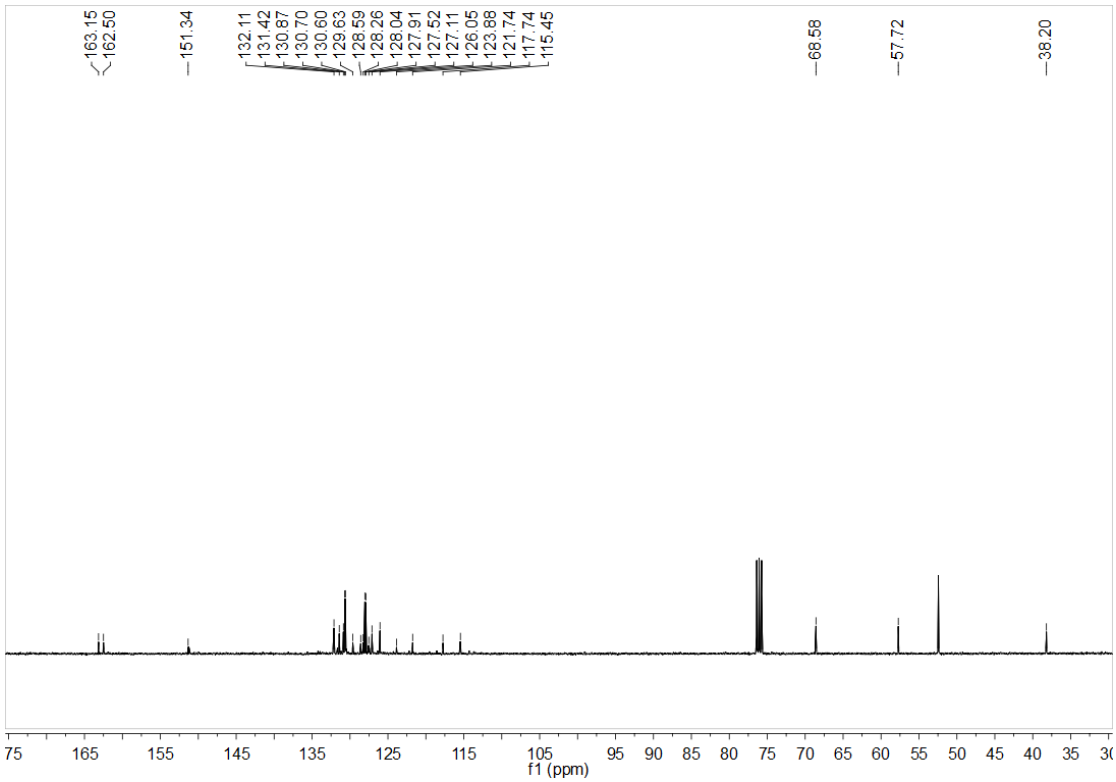


Fig. S39 The ^{13}CNMR spectrum of compound **4** ($CDCl_3$)

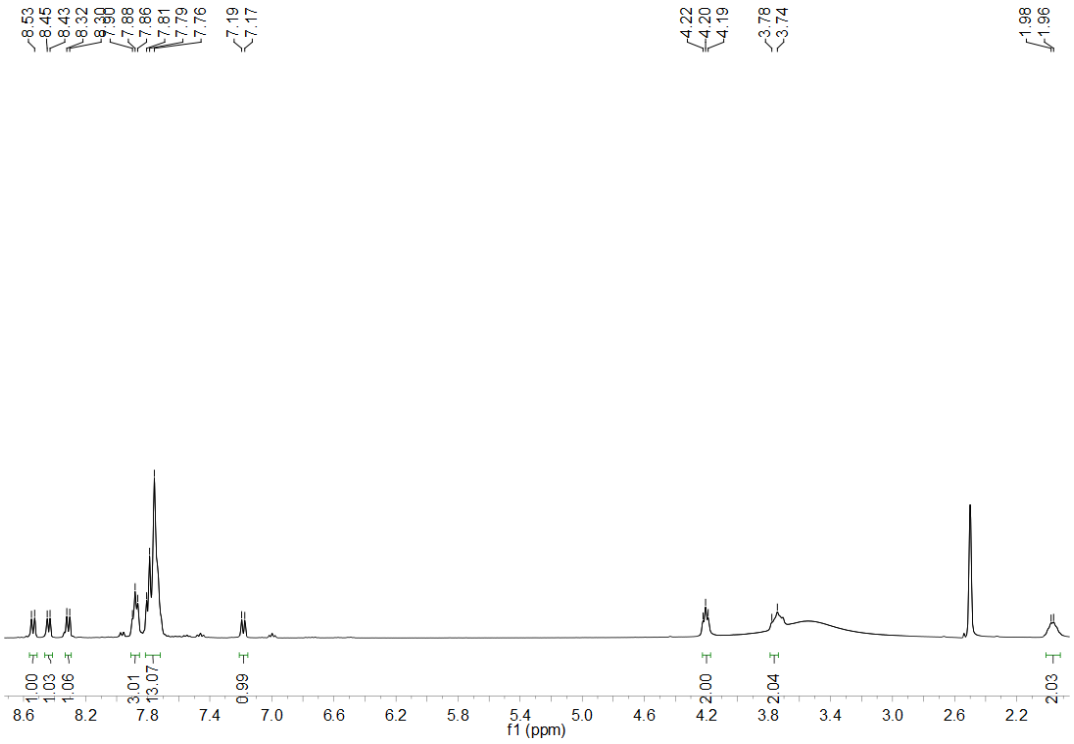


Fig. S40 The ^{1}HNMR spectrum of compound **Naph-OH** (DMSO-d_6)

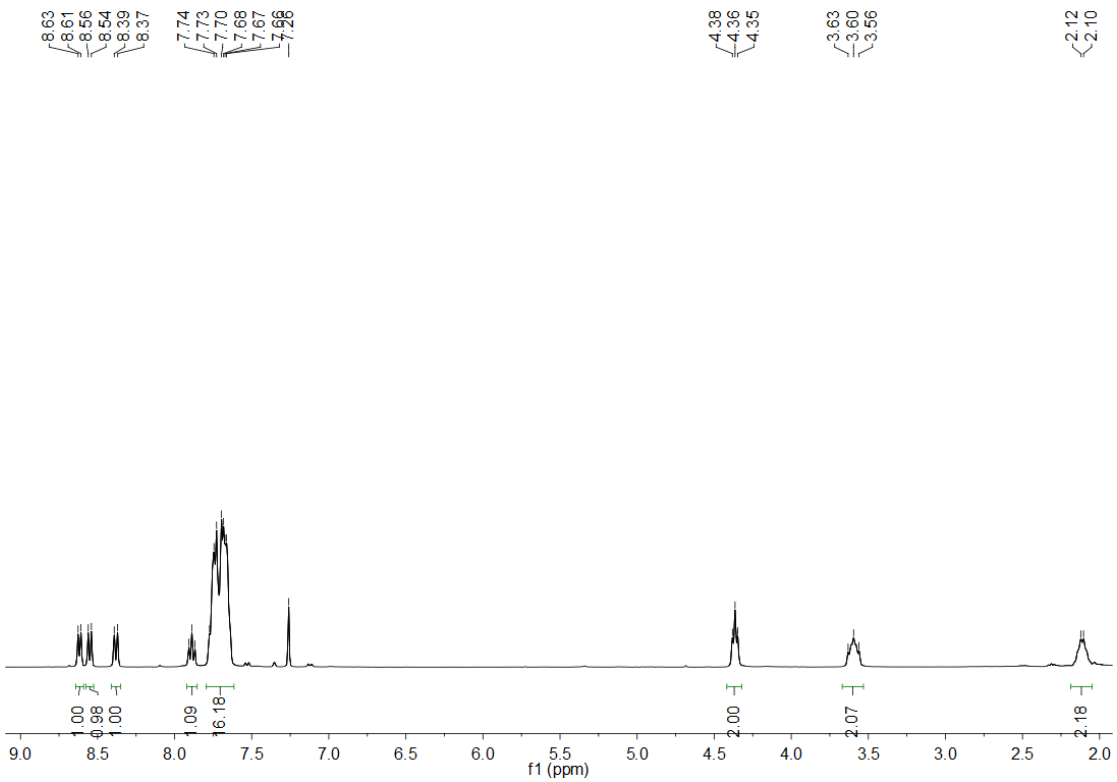


Fig. S41 The[1]HNMR spectrum of probe **Naph-$O_2^{\cdot-}$** ($CDCl_3$)

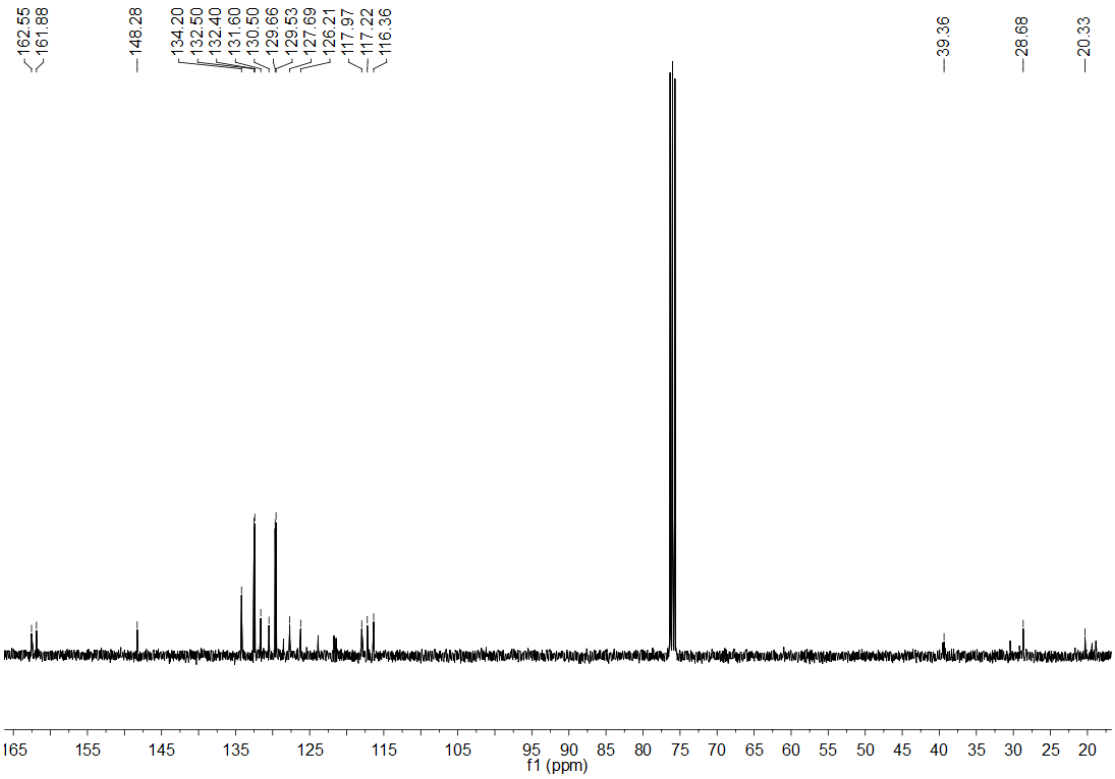


Fig. S42 The[13]CNMR spectrum of probe **Naph-$O_2^{\cdot-}$** ($CDCl_3$)

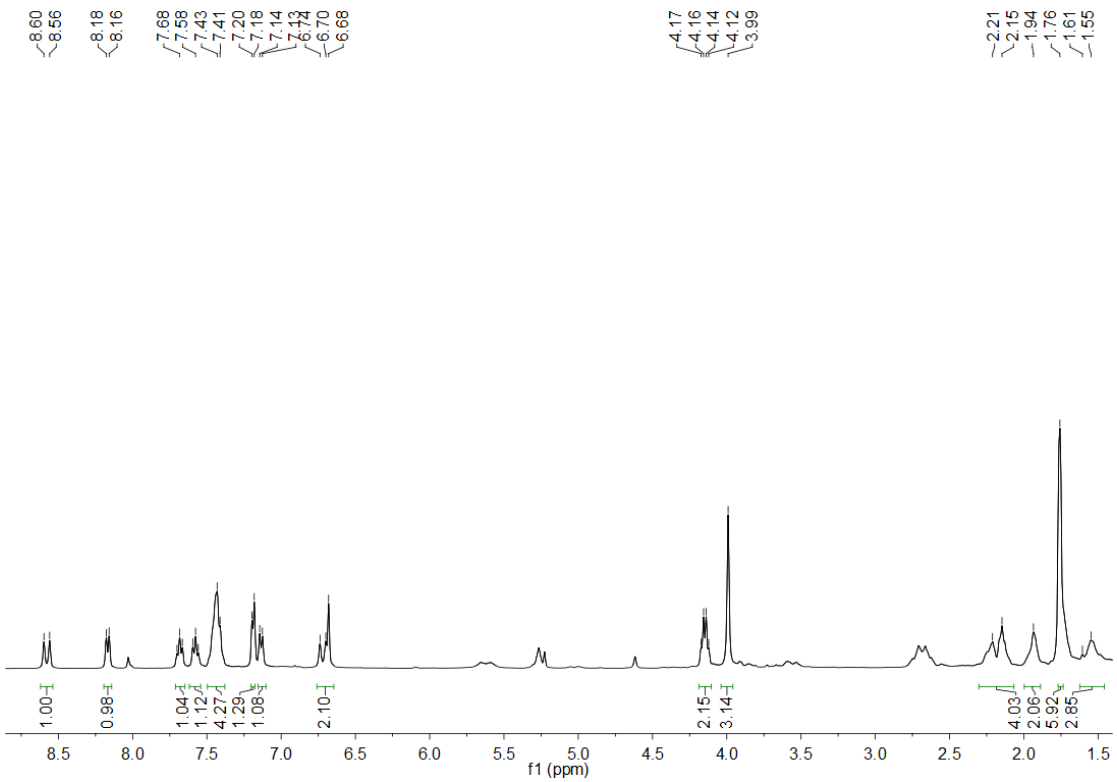


Fig. S43 The ^{1}HNMR spectrum of probe **NIR-$O_2^{\cdot-}$** ($CDCl_3$)

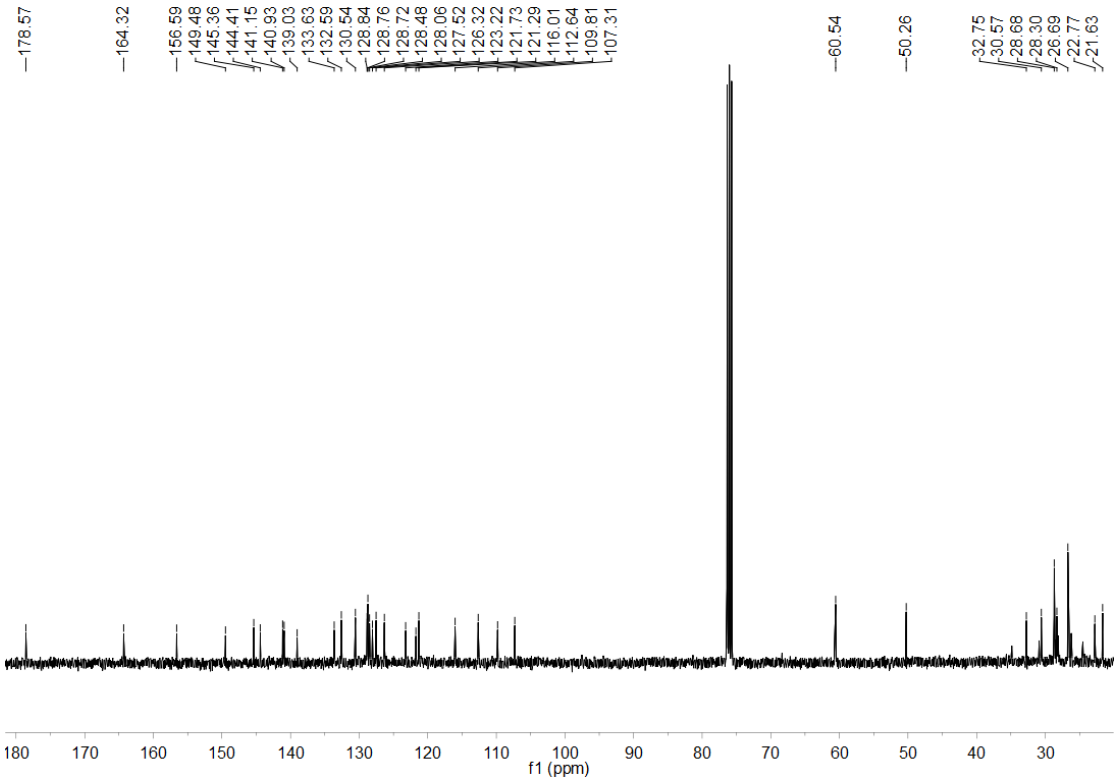


Fig. S44 The^{13}CNMR spectrum of probe **NIR-$O_2^{\cdot-}$** ($CDCl_3$)

9. References

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