Supporting Information

Chlorination-induced *pKa* decrease: an improved strategy to design ratiometric hypochlorite fluorescent probes with ideally high selectivity

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1. Materials and Instruments.

All commercial chemicals were purchased from suppliers and used as received. 1H NMR and 13C NMR spectra were obtained using a Bruker 400 spectrometer. The mass spectra were obtained using a Bruker Daltonics micr-OTOF-Q Ⅱ spectrometer. Emission spectra were recorded on a Hitachi F-7000 fluorometer, and UV-vis absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer. A Leici PHS-3C pH meter was used for pH measurements. Fluorescence imaging experiments were conducted using an Opera Phenix/Operetta CLS system from PerkinElmer, a Nikon confocal microscope and an Olympus IX83 inverted microscope. HeLa cells were obtained from Xiangya Hospital at Central South University, China. Zebrafish were purchased from Nanjing Eze-Rinka Biotechnology Co., Ltd., China. For the study in the LPS-induced acute lung injury, lung histology slides of mouse were purchased from Wuhan Servicebio Technology Co., Ltd., China.

One-photon and Two-photon Fluorescence Imaging of HeLa cells and Zebrafish. HeLa cells and zebrafish were cultured according to the traditional protocols and the detailed procedures were described in the Supporting Information. For one photon fluorescence imaging experiments, signals were collected from green channel (490-520 nm) and red channel (620-650 nm) with an excitation at 430 nm. For two photon fluorescence imaging experiments, signals were collected from green channel (500-550 nm) and red channel (560-750 nm) with an excitation at 900 nm.

Fluorescence Imaging of Acute Lung Injury in A Mice Model. All animal experiments were performed in accordance with the regulations established by the Committee on the Ethics of Animal Experiments of Central South University. Lung histology slides of mouse were incubated with **Naph-DFOB** for 30 min and imaged under an inverted fluorescence microscope after being sealed in coverslip.

2. Synthesis

Synthesis of Compound 1. Compound **1** was synthesized according to the literature method (Cao, S. X. B., Pierre-Yves; Chung, Hyun-Ho, et al., Preparation of Phenol and

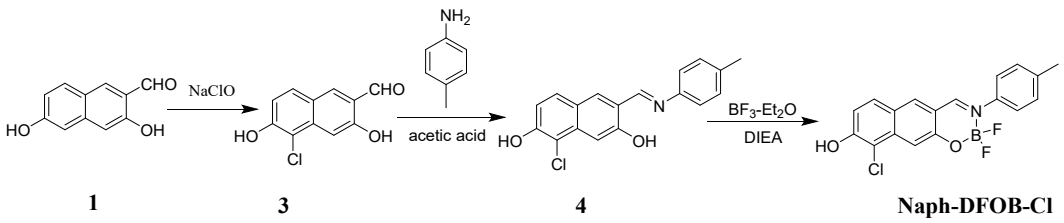
Hydroxynaphthalene Based Inhibitors of Protein Kinase for the Treatment of Disease. PCT Int. Appl. 2002, WO 2002096867).

Synthesis of Compound 2. The solution of compound **1** (0.5 mmol, 94 mg), *p*-methylaniline (0.6 mmol, 64 mg) and 10 μL acetic acid in 5 mL of anhydrous ethanol was stirred for 2 hours at room temperature and a yellow solid was precipitated out. The solid was collected by filtration and washed with ethanol to give pure compound **2**. 109 mg, yield 79%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 7.76 (d, J = 9.1 Hz, 1H), 7.69 (s, 1H), 7.61 (d, J = 8.6 Hz, 1H), 7.50 (d, J = 8.2 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 6.89 (d, J = 10.4 Hz, 1H), 6.74 (d, J = 9.1 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.1, 158.3, 155.3, 142.2, 137.1, 136.3, 135.78, 131.2, 130.6, 121.3, 120.7, 118.8, 114.8, 108.1, 104.1, 21.1.

Synthesis of Naph-DFOB. The solution of compound **2** (0.2 mmol, 55 mg) and DIEA (0.5 mmol, 64 mg) in 10 mL anhydrous dichloroethane was stirred for 10 minutes at 80 °C. Then, $BF_3{\cdot}OEt_2$ (0.5 mmol, 71 mg) was added to the reaction mixture and the obtained solution was stirred at 80 °C overnight. The reaction mixture was cooled, and 20 mL DCM was added. The obtained solution was washed with 30 mL saturated aqueous $NaHCO_3$ solution. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to give a residue, which was subjected to further purification by column chromatography (PE/DCM = 1/1) to obtain **Naph-DFOB**. 39 mg, yield 61%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 9.39 (s, 1H), 8.17 (d, J = 8.9 Hz, 1H), 7.82 (d, J = 8.7 Hz, 1H), 7.71 (s, 1H), 7.60 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 7.04 (dd, J = 19.8, 8.1 Hz, 2H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 159.3, 141.3, 138.6, 134.6, 131.6, 130.2, 124.1, 122.5, 116.8, 116.1, 104.9, 100.0, 21.1. HRMS (ESI) m/z: calcd for $C_{18}H_{14}BF_2NO_2Na$ $[M+Na]^+$, 348.0978; found, 348.0946.

Synthesis of NaphOH-CN-Cl. To a solution of **NaphOH-CN** (0.59 mmol, 100 mg) in 3 mL methanol was added 0.5 mL of NaClO (10% available chlorine) at room temperature. After the reaction solution was stirred for 10 min, 20 mL water was added. The mixture was extracted with DCM (3×20 mL). And then the organic layers were combined, dried with Na_2SO4, and removed under a reduced pressure, the crude

product was purified by silica gel column chromatography (DCM/PE = 1:4, v/v) to obtain compound **NaphOH-CN-Cl** (74 mg, 61.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.09 (d, J = 8.8 Hz, 1H), 7.90 (d, J = 9.0 Hz, 1H), 7.80 (dd, J = 8.8, 1.6 Hz, 1H), 7.51 (d, J = 8.9 Hz, 1H).



1 **3** **4** **Naph-DFOB-Cl**

Scheme S1. Synthetic Route of **Naph-DFOB-Cl**.

To a solution of compound **1** (0.5 mmol, 94 mg) in 5 mL methanol was added 0.2 mL 10% aqueous sodium hypochlorite at room temperature. After stirring for 10 min, 20 mL water was added to the reaction mixture. The resultant mixture was extracted with 20 mL ethyl acetate. The organic layer was separated, washed with water and dried over anhydrous Na_2SO_4. After removing the solvent, the obtained solid was purified by column chromatography (silica gel, DCM as an eluent) to offer compound **3** as a yellow powder. 42 mg, 38% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 11.09 (s, 1H), 10.94 (s, 1H), 8.04 (d, J = 9.0 Hz, 1H), 7.79 (d, J = 8.7 Hz, 1H), 7.21 (d, J = 8.7 Hz, 1H), 7.01 (d, J = 8.9 Hz, 1H).

The solution of compound **3** (0.18 mmol, 40 mg), *p*-methylaniline (0.6 mmol, 68 mg) and 20 μL acetic acid in 2 mL anhydrous ethanol was stirred for 2 hours at room temperature and a yellow solid was precipitated out. The solid was collected by filtration and washed with ethanol to give pure compound **4**.

Compound **4** obtained from the above step and DIEA (0.45 mmol, 58 mg) were dissolved in 10 mL freshly distilled dichloroethane. The mixture was stirred at 80 °C for 10 minutes. Then, $BF_3{\cdot}OEt_2$ (0.45 mmol, 64 mg) was added and the reaction mixture was stirred at 80 °C overnight. Finally, the reaction mixture was cooled to room temperature and 20 mL CH_2Cl_2 was added. The obtained solution was washed with 30 mL saturated aqueous $NaHCO_3$ solution. The organic layer was separated and dried

over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to give a residue, which was subjected to further purification by column chromatography (PE/DCM = 3/2) to yield **Naph-DFOB-Cl**. 26 mg, yield 58%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 10.10 (s, 1H), 8.24 (d, J = 8.9 Hz, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.54 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 8.7 Hz, 1H), 7.15 (d, J = 8.9 Hz, 1H), 2.38 (s, 3H). HRMS (ESI) m/z: calcd for $C_{18}H_{13}BClF_2NNaO_2$ $[M+Na]^+$, 382.0588; found, 382.0563.

3. TLC Analysis

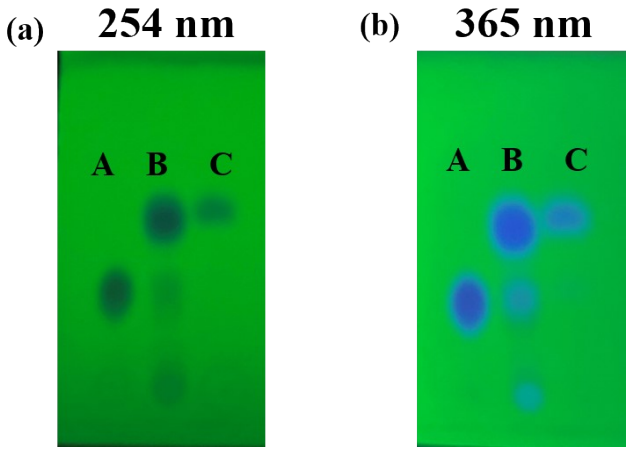


Fig. S1. TLC analysis of **NaphOH-CN** (A), the mixture of **NaphOH-CN** and ClO^- (B), and **NaphOH-CN-Cl** (C) under a UV lamp ((a):254 nm; (b): 365 nm). Stationary phase: silica gel. Solvent: petroleum ether: ethyl acetate = 3:1 (V/V).

4. Optical, NMR and HRMS Spectra

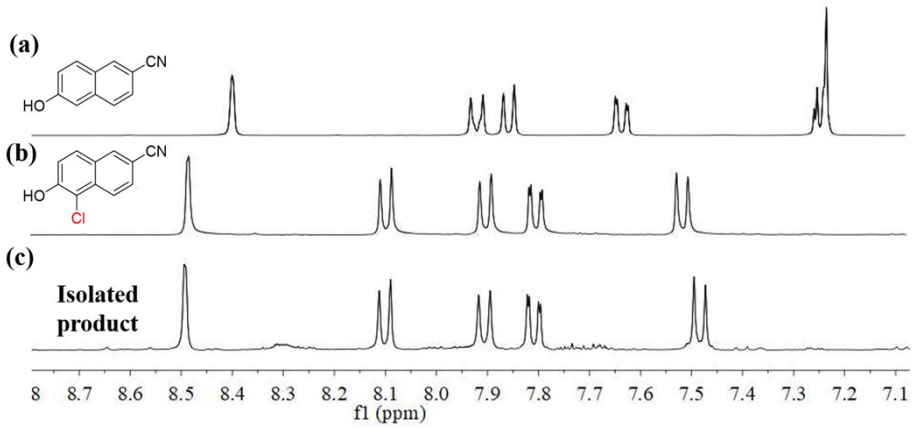


Fig. S2. ^{1}H NMR spectrum (DMSO-d_6) of **NaphOH-CN** (a), **NaphOH-CN-Cl** (b), and the isolated product from the reaction of **NaphOH-CN** with ClO^- (c).

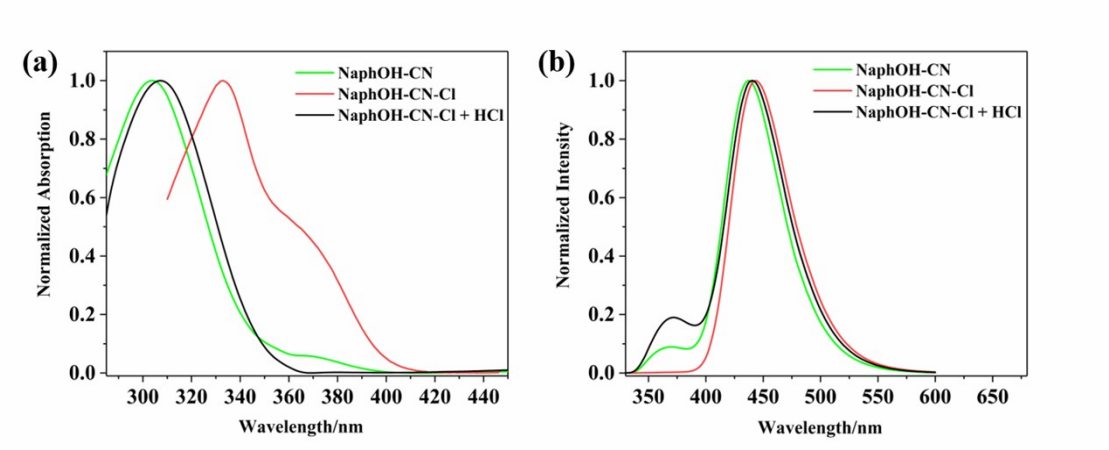


Fig. S3. Normalized absorption (left) and emission (right) spectra of **NaphOH-CN**, **NaphOH-CN-Cl**, and the mixture of **NaphOH-CN-Cl** with HCl (500.0 equiv.). Test medium: PBS buffer (10.0 mM, pH = 7.4, containing 50% EtOH). Excitation wavelength: 310 nm.

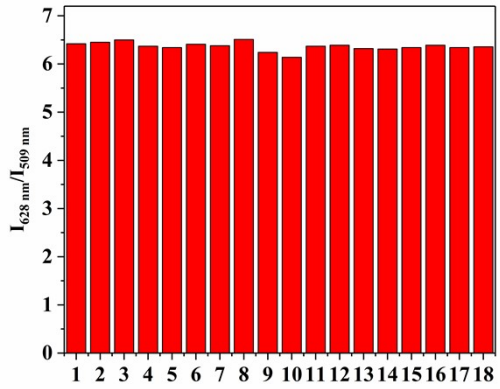


Fig. S4. Fluorescence intensity ratio ($I_{628\ nm}/I_{509\ nm}$) of **Naph-DFOB** (10.0 μM) in response to ClO^- in the presence of other interfering analytes in PBS buffer (10.0 mM, pH = 7.4, containing 50% EtOH. 1, Blank; 2, $HO\cdot$; 3, $^1O_2$; 4, H_2O_2; 5, KO_2; 6, $NO\cdot$; 7, $ONOO^-$; 8, $ROO\cdot$; 9, TBHP; 10, *t*-$BuO\cdot$; 11, S^{2-}; 12, HSO_3^-; 13, Cl^-; 14, NO_3^-; 15, Ca^{2+}; 16, Mg^{2+}; 17, Zn^{2+}; 18, Na^+. Excitation wavelength: 430 nm.

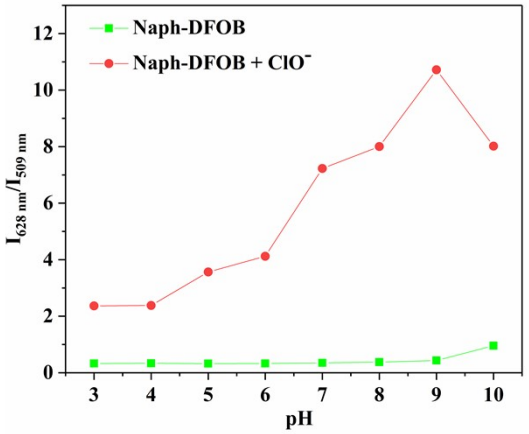


Figure S5. Fluorescence intensity ratio ($I_{628\ nm}/I_{509\ nm}$) of **Naph-DFOB** (10.0 μM) in response to ClO^- (500.0 μM) at different pH value. Excitation wavelength: 430 nm. Test media: PBS buffer (10.0 mM, pH = 7.4, containing 50% EtOH).

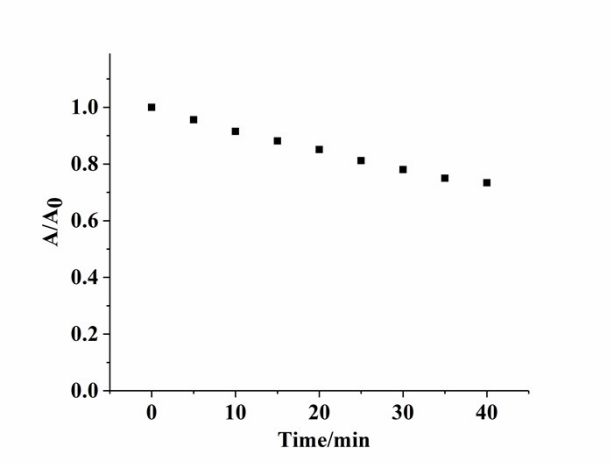


Figure S6. Time-dependent absorbance changes of probe **Naph-DFOB** (10.0 μM) under a Xe lamp irradiation (light intensity: 200 mW/cm^2) in PBS buffer (10.0 mM, pH = 7.4, containing 50% EtOH).

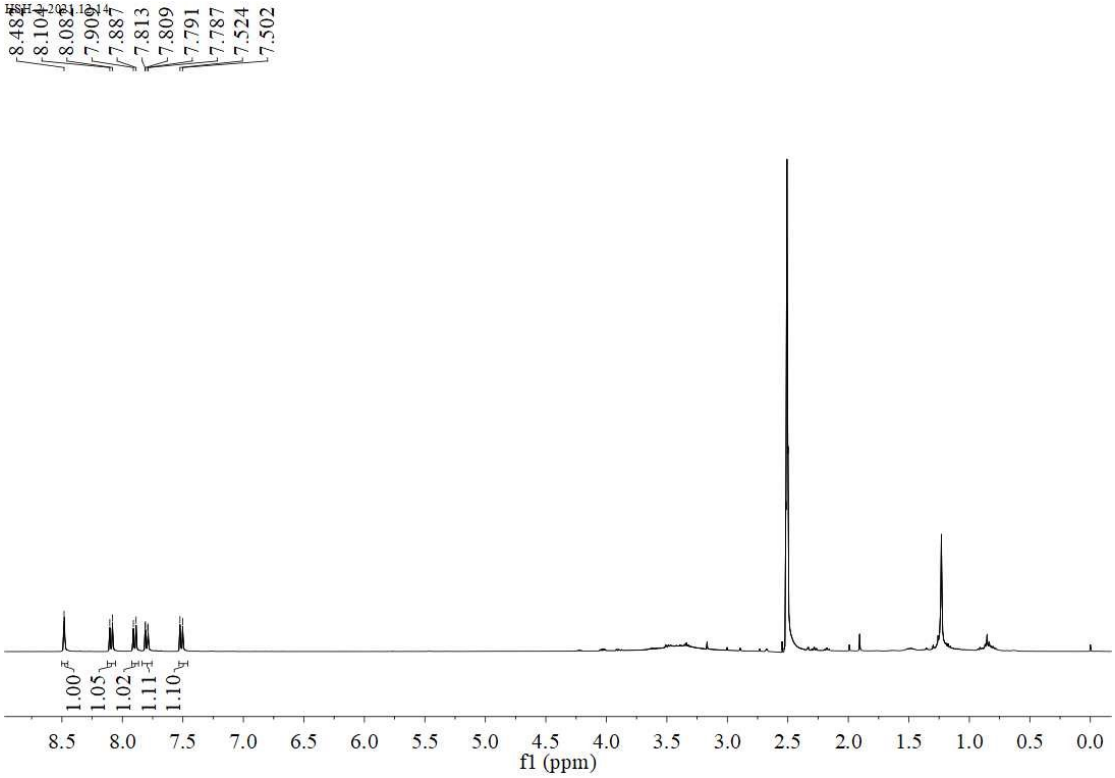


Fig. S7. ^{1}H NMR spectrum of **NaphOH-CN-Cl** in DMSO-d_6.

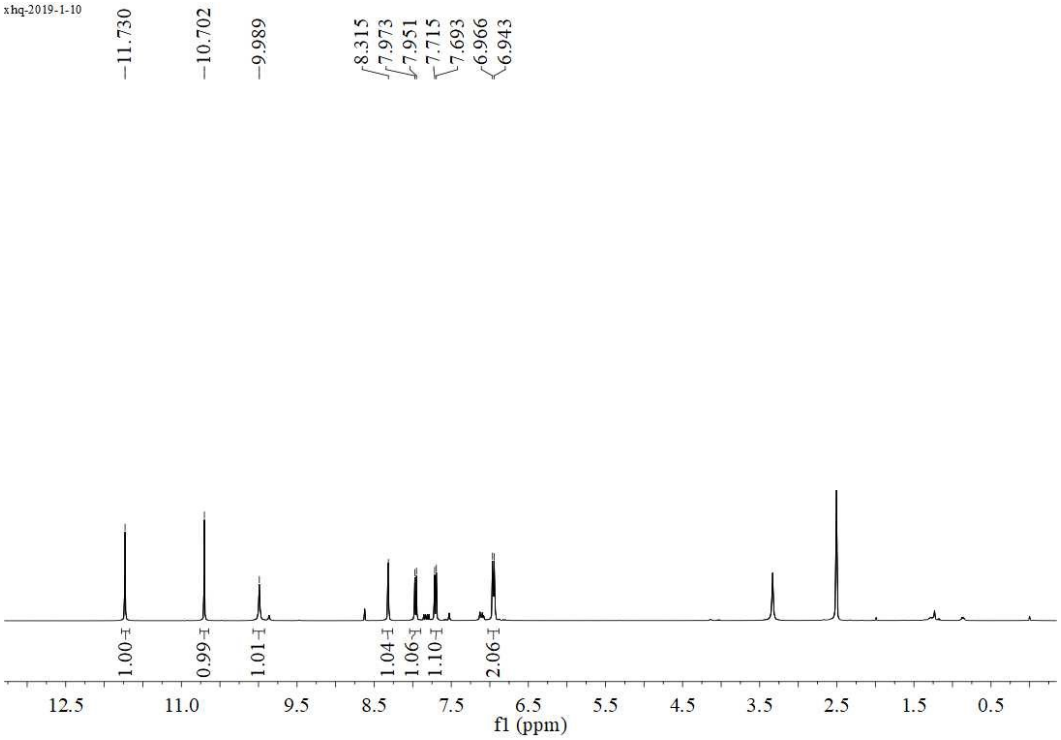


Fig. S8. ^{1}H NMR spectrum of compound **1** in DMSO-d_6.

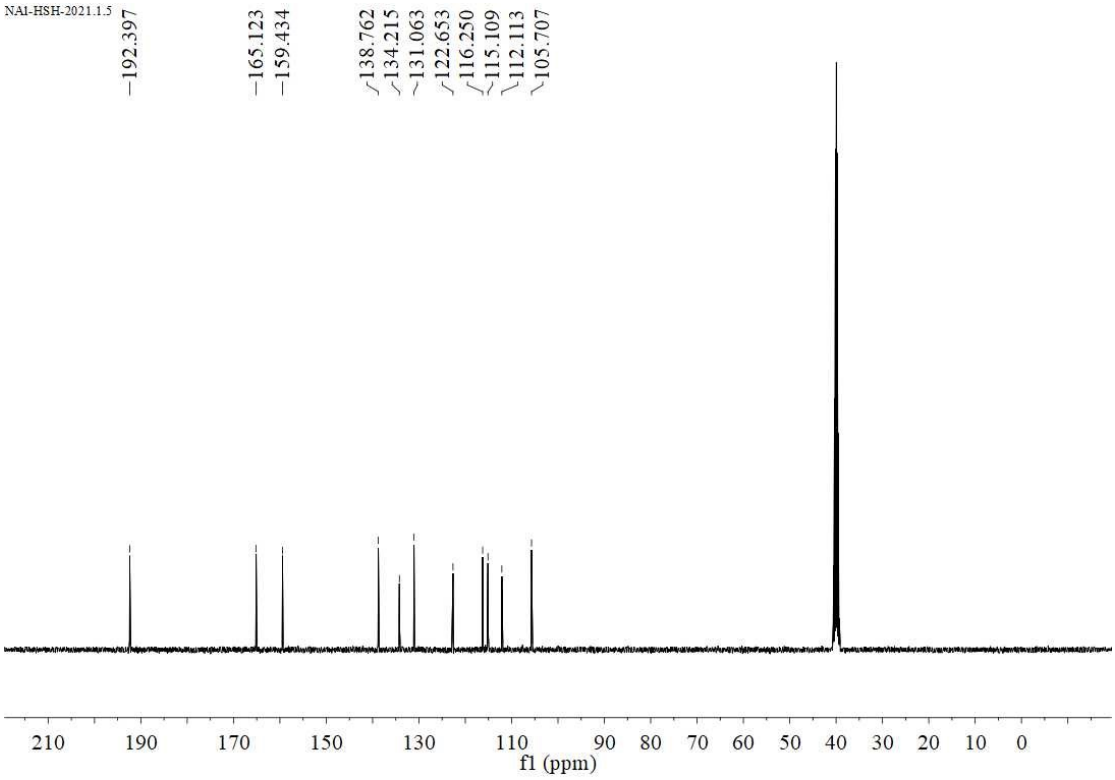


Fig. S9. ^{13}C NMR spectrum of compound **1** in DMSO-d_6.

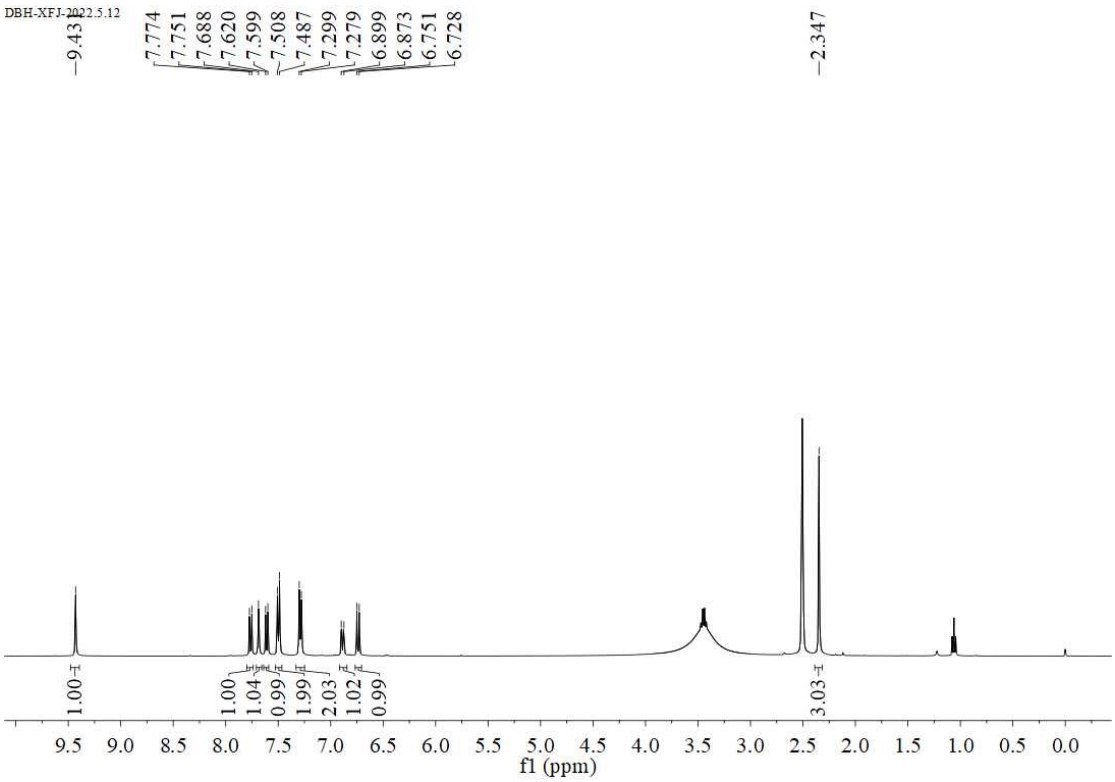


Fig. S10. ^{1}H NMR spectrum of compound **2** in DMSO-d_6.

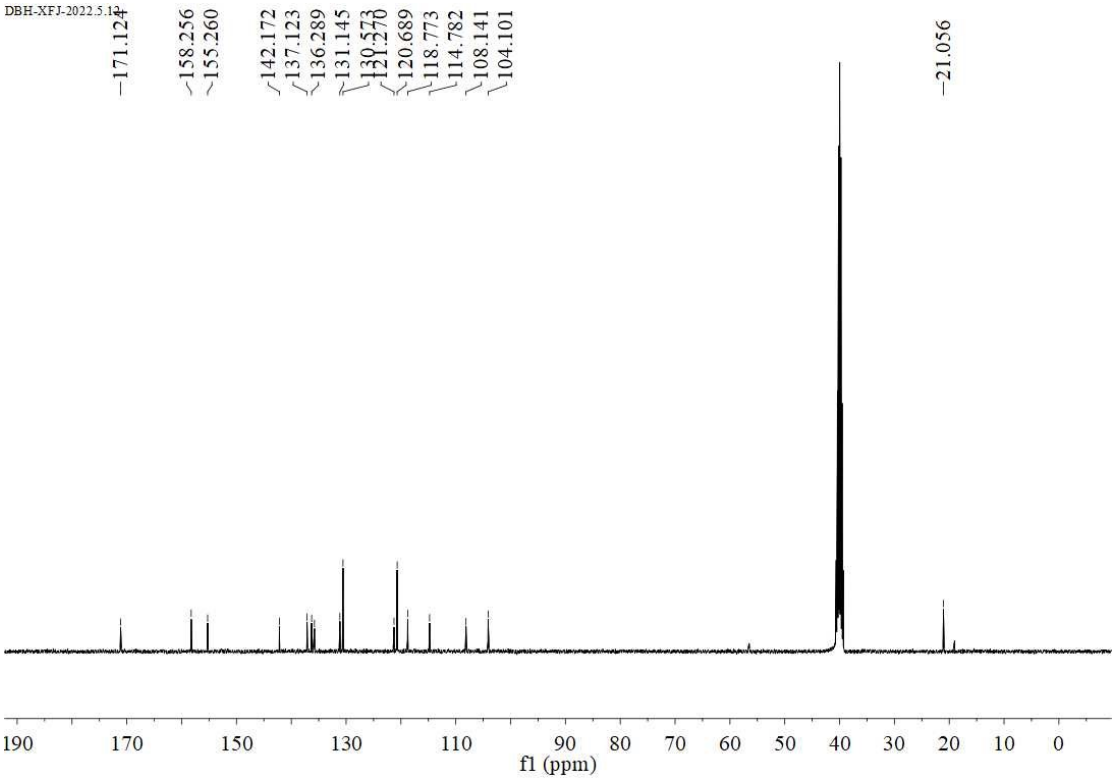


Fig. S11. ^{13}C NMR spectrum of compound **2** in DMSO-d_6.

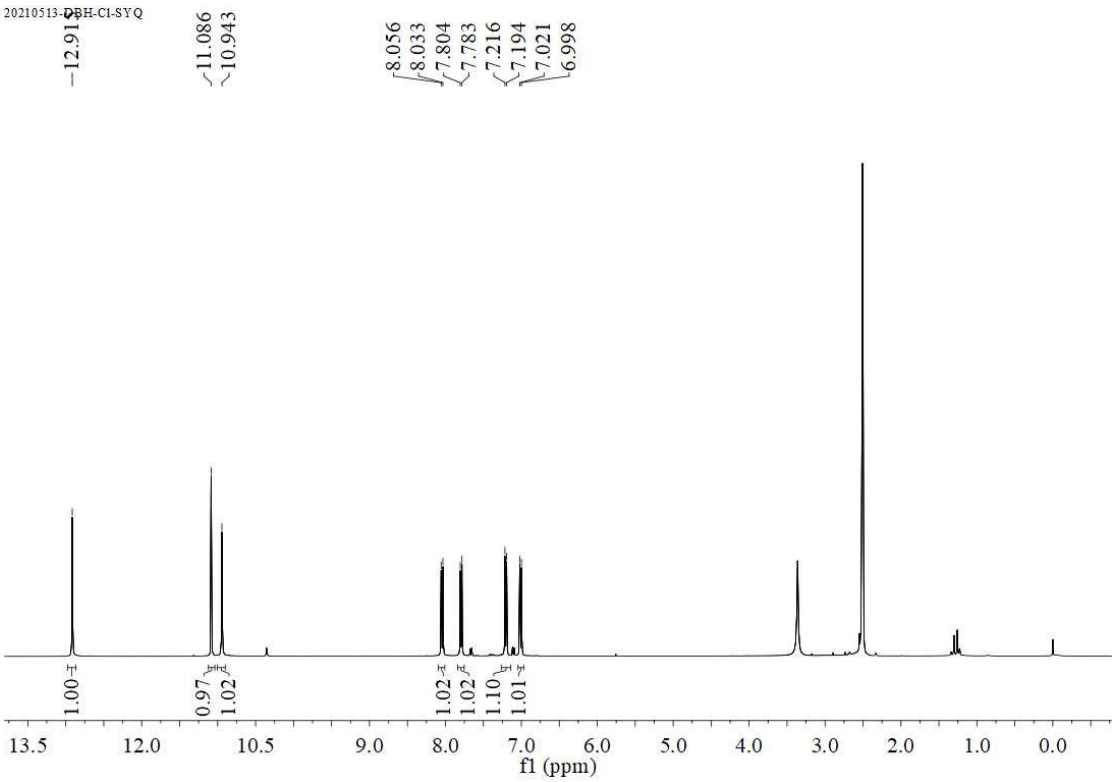


Fig. S12. [1]H NMR spectrum of compound **3** in DMSO-d_6.

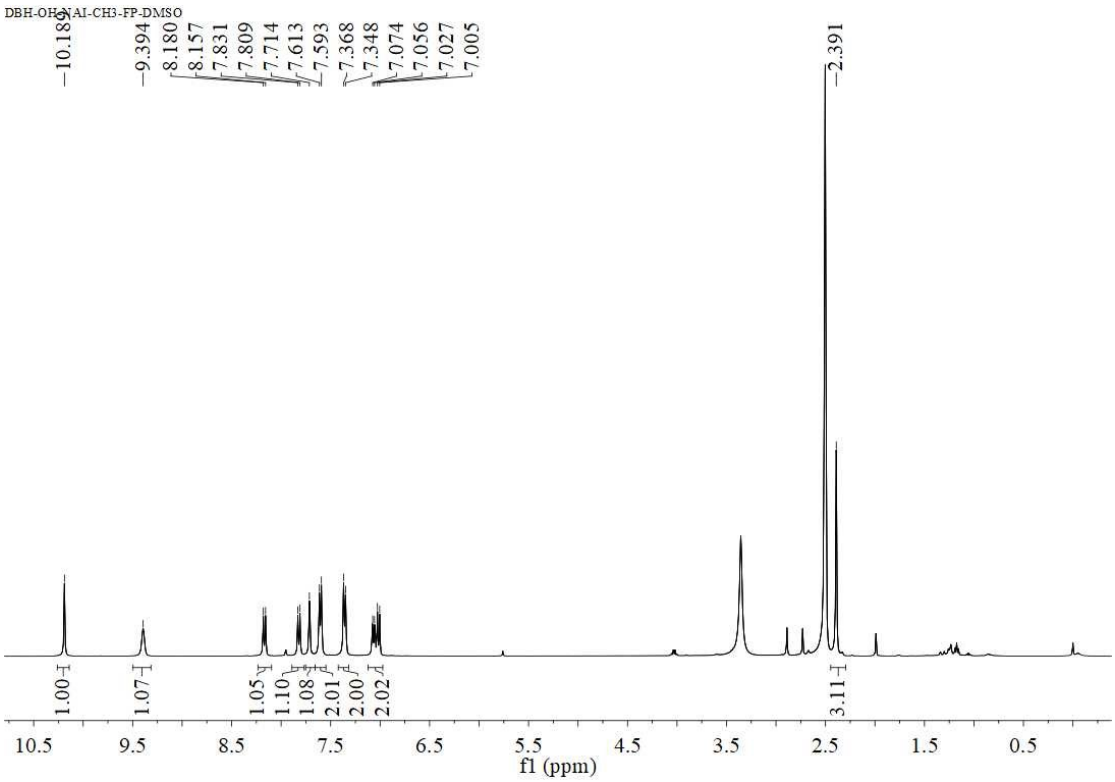


Fig. S13. [1]H NMR spectrum of **Naph-DFOB** in DMSO-d_6.

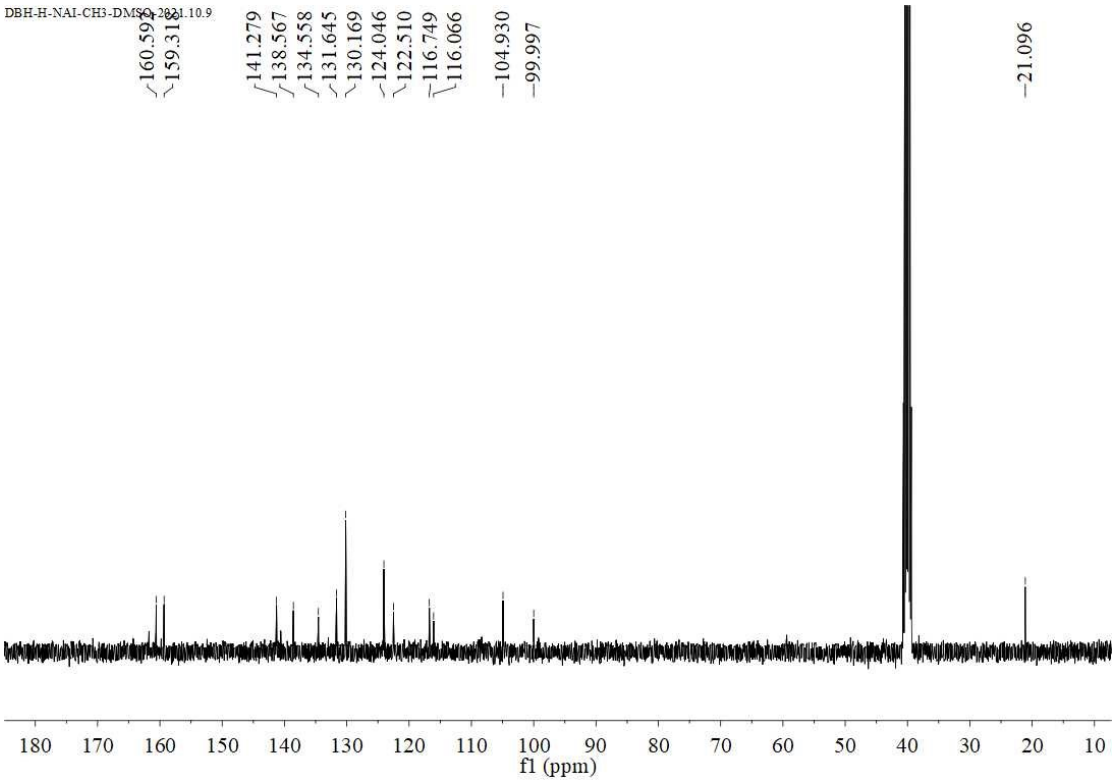


Fig. S14. ^{13}C NMR spectrum of **Naph-DFOB** in DMSO-d_6.

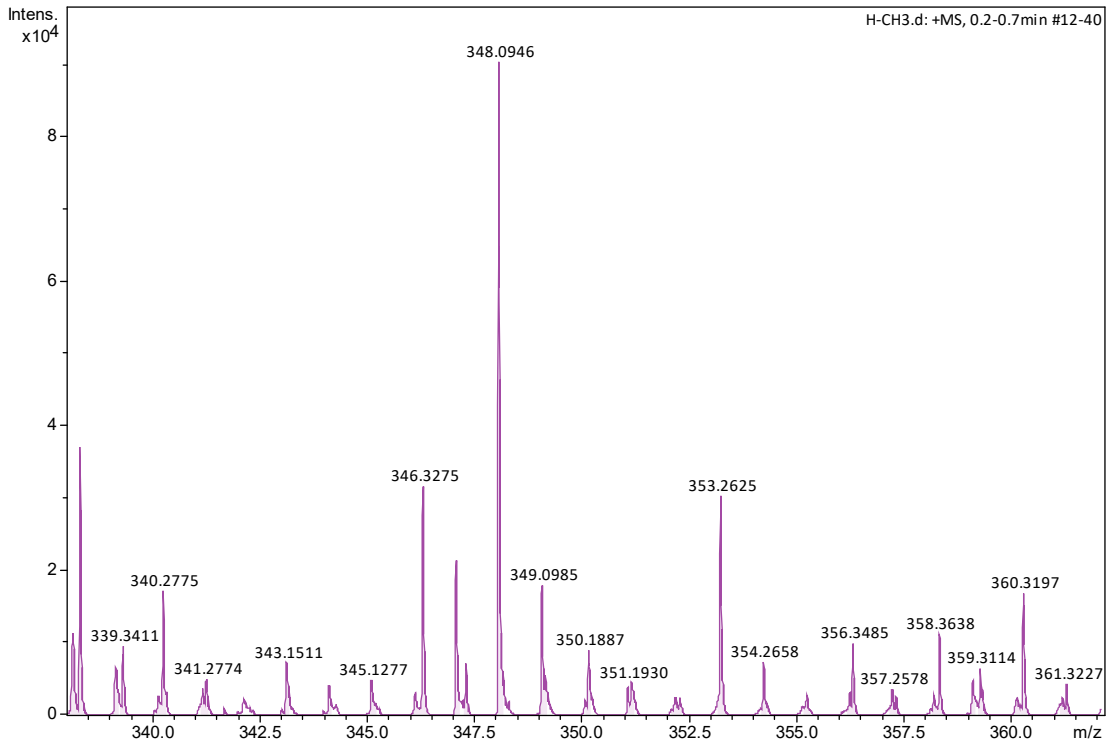


Fig. S15. HRMS spectrum of **Naph-DFOB**.

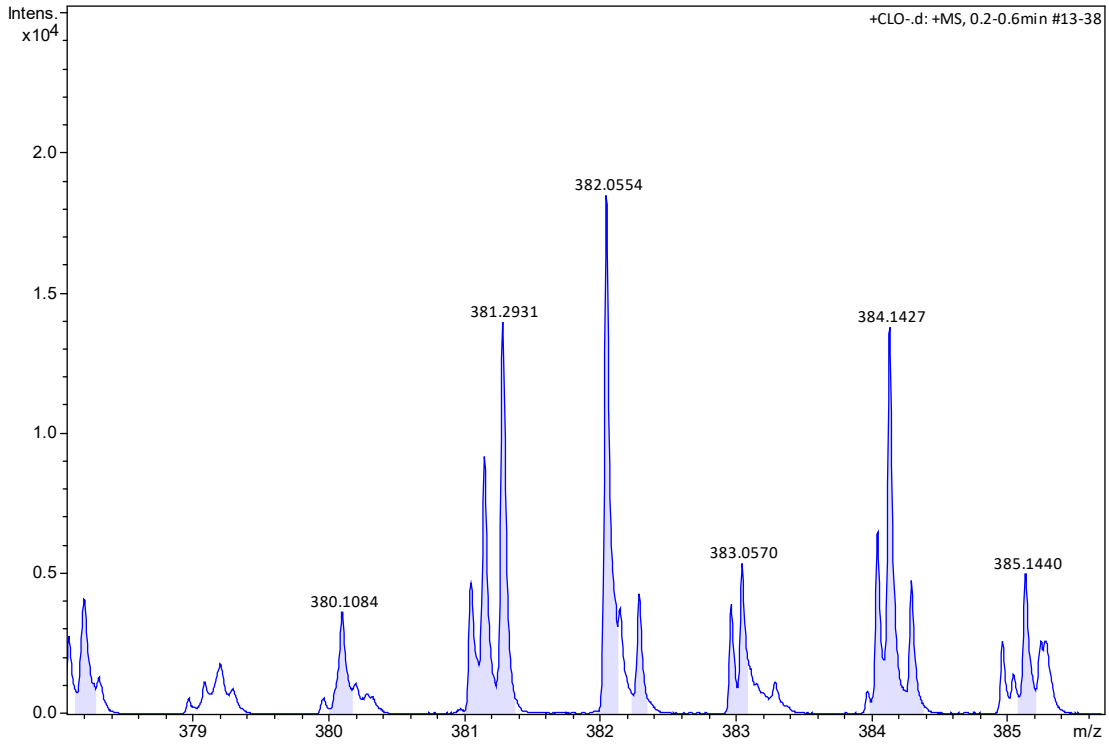


Fig. S16. HRMS spectrum of **Naph-DFOB** with ClO^-.

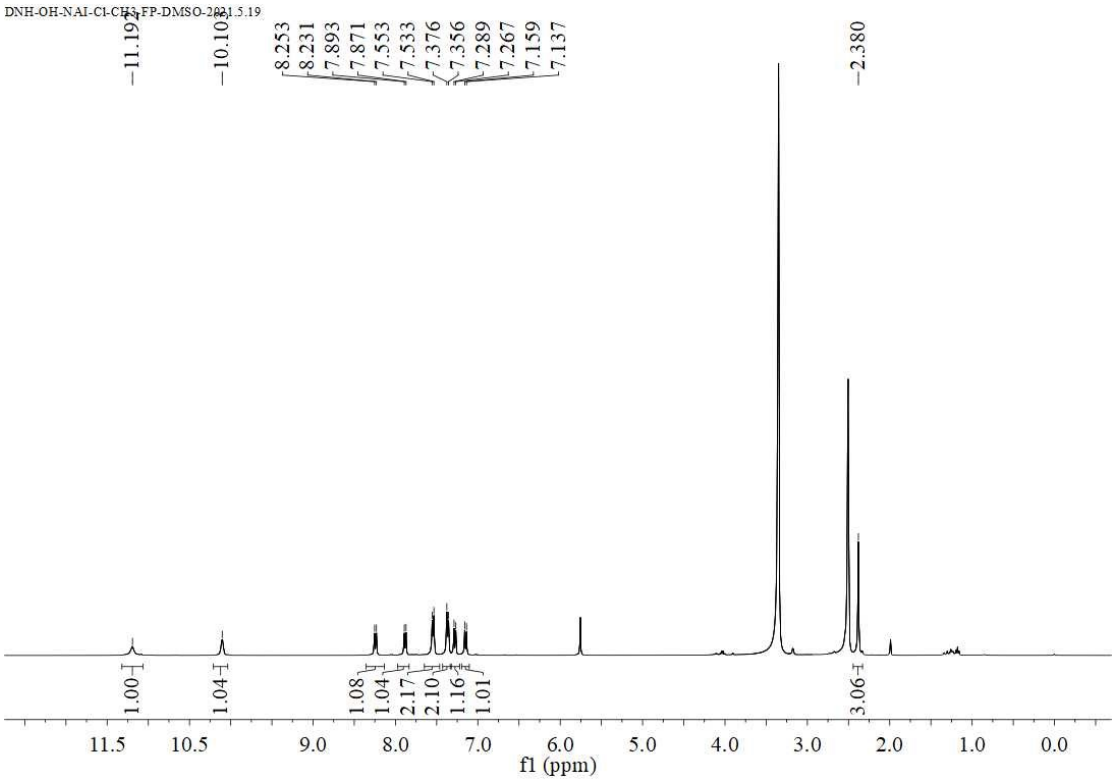


Fig. S17. ^1H NMR spectrum of **Naph-DFOB-Cl** in DMSO-d_6.

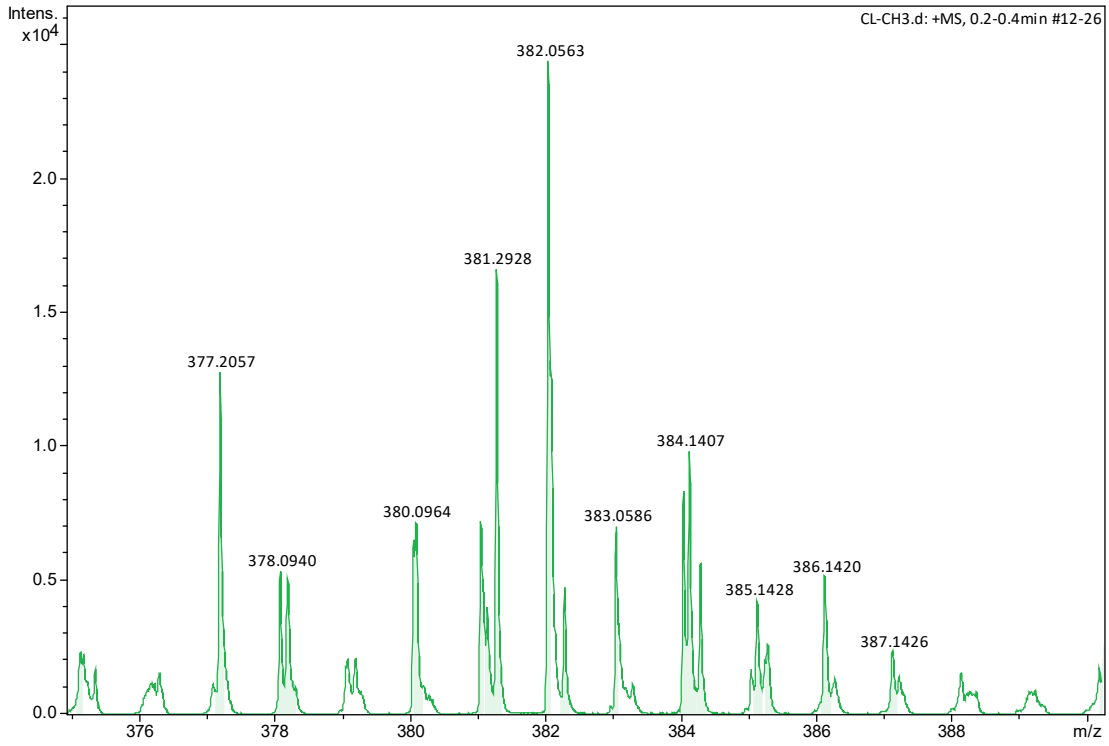


Fig. S18. HRMS spectrum of **Naph-DFOB-Cl**.

5. Imaging Experiments

Cell and zebrafish Culture

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin in humidified environment of 5% CO_2 and 95% air. Before imaging experiments, the growth medium was removed and cells were washed with PBS buffer three times. Cells were divided into two groups for different purposes. The first group were treated with **Naph-DFOB** (10.0 μM) for 30 min. The second group were pretreated with 500.0 μM ClO^- for 30 min, and then incubated with **Naph-DFOB** (10.0 μM) for another 30 min. After washing with PBS buffer three times, the imaging experiments were carried out.

All the experiments on living zebrafish were performed in compliance with the relevant local laws and institute guidelines, and the institution committee of Central South University has approved the experiments. Three-day old zebrafish were incubated in E3 media (15 mM NaCl, 0.5 mM KCl, 1.0 mM $MgSO_4$, 1.0 mM $CaCl_2$,

0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10^{-5}% methylene blue; pH 7.5). Before imaging experiments, zebrafish were washed with E3 media three times.

H&E Staining

After sacrificing the mice, lungs were obtained, fixed in 10% paraformaldehyde solution, and embedded in paraffin. The lung sections were observed under an optical microscope after being stained with H&E.

Imaging of ClO^- in Lung Sections

Lung sections of each group were cultured with **Naph-DFOB** (10.0 μM) for 30 min, washed three times with PBS and imaged with inverted fluorescence microscope.

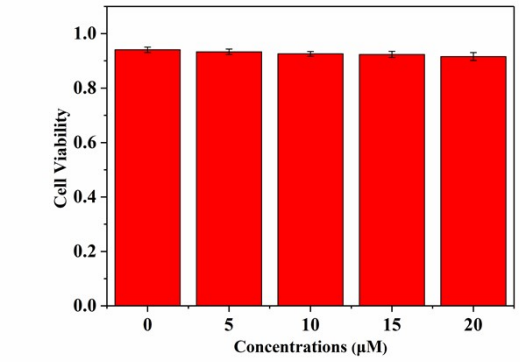


Fig. S19. MTT assay of HeLa cells incubated with different concentrations of **Naph-DFOB** for 24 h.