Transforming the recognition site of 4-hydroxyaniline into 4-methoxyaniline grafted onto a BODIPY core switches the selective detection of peroxynitrite to hypochlorous acid

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Table of contents

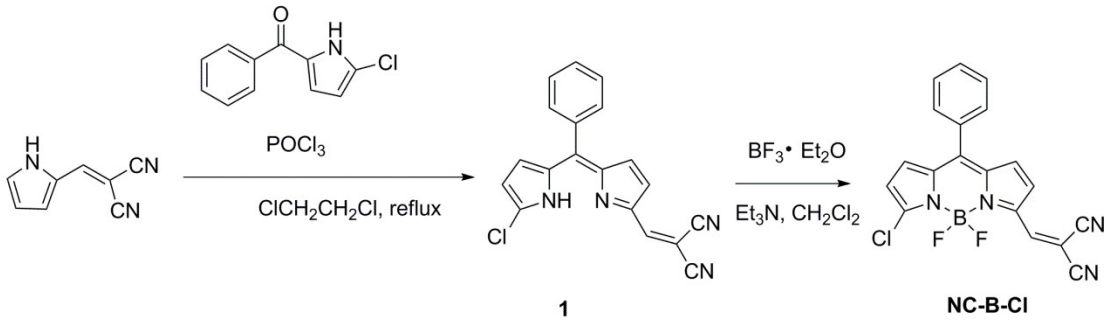
1. **Materials and instruments**

Chemicals for synthesis were obtained from commercial suppliers and used without further purification unless otherwise stated. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at ambient temperature. Mass spectra were measured on a HP 1100 LC-MS spectrometer. In the experiments of absorption or fluorescence measurements, compounds were dissolved in CH_3CN to obtain stock solutions (1.0 mM). These stock solutions were then diluted to the desired concentration. UV-vis absorption spectra were obtained on a Varian Cary 100 spectrophotometer. Fluorescence spectra were measured with a Varian Cary Eclipse Fluorescence spectrophotometer.

Cell Cultures. In a humidified atmosphere of 5/95 CO_2/air incubator, raw264.7 macrophages and hepatocytes were cultured in Roswell Park Memorial Institute 1640 medium (RPMI-1640) supplemented with 10% fetal bovine serum (FBS) at 37 °C.

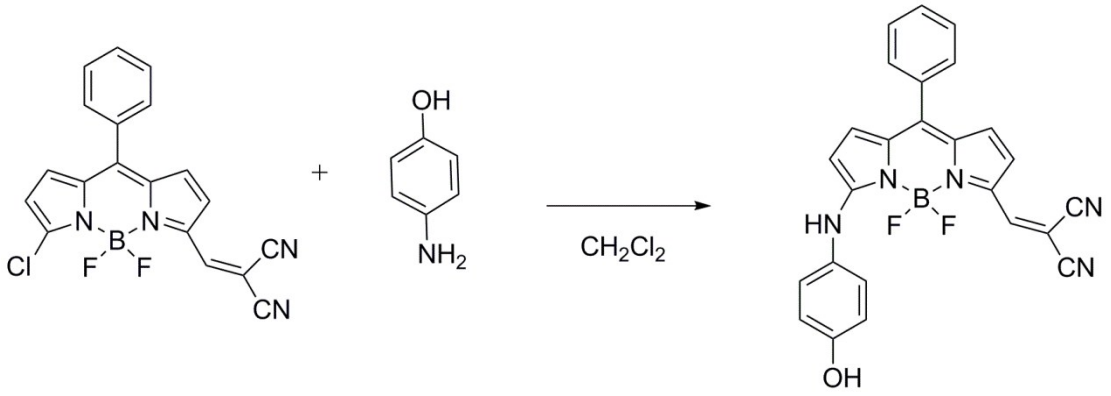
Confocal image of cells. Cells were grown in a glass bottom dish for 24 h. For image of $ONOO^-$, cells were stained with **BHAni** (10 μM) in culture medium for 15 min at 37 °C, washed with D-Hanks. Then peroxynitrite donor 3-morpholinosydnonimine (SIN-1) (50 μM) was introduced for 30 min. For HOCl, cells were stimulated with lipopolysaccharides (LPS, 1 μg/mL) for 12 h, followed by phorbol myristate acetate (PMA, 1 μg/mL) for 1h, then loaded with **BMAni** (10 μM) for 30 min. The excitation wavelength was 488 nm, and images were collected at 500-600 nm.

Synthesis of NC-B-Cl.



The mixture of 2-benzoyl-5-chloropyrrole (206 mg, 1 mmol) and $POCl_3$ (0.5 mL) in $ClCH_2CH_2Cl$ (5 mL) was stirred for 12 h at room temperature. 2-(1H-2-pyrrolylmethylene) malononitrile (172 mg, 1.2 mmol) in $ClCH_2CH_2Cl$ (15 mL) was then added to the reaction mixture, and further stirred at 85 ℃ for 12 h under N_2. After cooling to room temperature, the mixture was neutralized with saturated aqueous $NaHCO_3$, washed with water, and then dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and intermediate **1** was obtained, which was used for the next step without purification. Intermediate **1** was re-dissolved in CH_2Cl_2 (20 mL). Et_3N (0.8 mL) was then added to the mixture and further stirred for another 30 min, then $BF_3{\cdot}Et_2O$ (0.8 mL) was added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was washed with water, and dried over anhydrous Na_2SO_4. After evaporating the solvent in vacuo, the residue was purified by column chromatography to afford compound **NC-B-Cl** (176 mg, 47%). ^1H NMR ($CDCl_3$, 400 MHz): δ 8.29 (s, 1H), 7.69-7.65 (m, 2H), 7.61-7.57 (t, 2H), 7.53-7.52 (m, 2H), 7.16-7.15 (d, 1H), 6.90-6.89 (d, 1H), 6.67-6.66 (d, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 153.1, 146.7, 145.5, 142.5, 137.6, 136.8, 135.9, 132.1, 131.7, 130.5, 128.9, 128.7, 123.0, 121.2, 113.7, 113.1, 82.8; HRMS (ESI): calcd for $C_{19}H_{10}BClF_2N_4Na$: 401.0553, Found: 401.0543 $[M + Na]^+$.

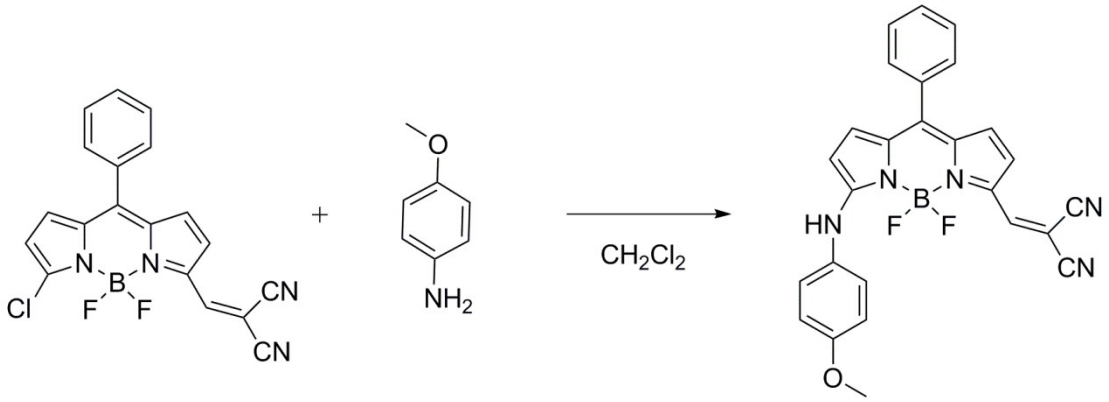
Synthesis of BHAni.



NC-B-Cl **BHAni**

Compound **NC-B-Cl** (37.8 mg, 0.1 mmol) and 4-hydroxyaniline (13.1 mg, 0.12 mmol) were mixed in CH_2Cl_2, and the mixture was stirred at room temperature for 30 min. The reaction mixture was washed with water, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by column chromatography to afford compound **BHAni** (36.2 mg, 80%). ^{1}H NMR (d_6-DMSO, 400 MHz): δ 10.94 (s, 1H), 9.83 (s, 1H), 8.07 (s, 1H), 7.57-7.54 (m, 4H), 7.53-7.51 (m, 2H), 7.23-7.21 (d, 2H), 7.12-7.11 (d, 1H), 6.86-6.84 (d, 2H) , 6.56-6.55 (d, 1H) , 6.53-6.52 (d, 1H); ^{13}C NMR (d_6-DMSO, 100 MHz): δ161.8, 156.8, 145.8, 139.4, 138.0, 136.9, 132.9, 132.7, 130.1, 129.4, 128.6, 128.1, 127.0, 125.2, 120.8, 119.4, 118.6, 115.7. 115.5, 114.5, 71.3; HRMS (ESI): calcd for $C_{25}H_{16}BF_2N_5ONa$: 474.1314, Found: 474.1313 [M + Na]$^+$.

Synthesis of BMAni.



NC-B-Cl **BMAni**

The mixture of compound **NC-B-Cl** (37.8 mg, 0.1 mmol) and 4-methoxyaniline (14.8 mg, 0.12 mmol) was stirred at room temperature for 30 min, then washed with water, dried over anhydrous Na_2SO_4, evaporated. The residue was purified by column chromatography to afford **BMAni** (38.0 mg, 82%). ^{1}H NMR (d_6-DMSO, 400 MHz): δ 10.83 (broad, 1H), 8.08 (s, 1H), 7.58-7.55 (m, 4H), 7.53-7.51 (m, 2H), 7.37-7.34 (d, 2H), 7.14-7.13 (d, 1H), 7.06-6.03 (d, 2H) , 6.58-6.56 (d, 1H) , 6.54-6.53 (d, 1H), 3.80 (s, 3H); ^{13}C NMR (d_6-DMSO, 100MHz): δ 161.9, 158.4, 145.9, 139.5, 138.0, 137.0, 133.0, 132.8, 130.2, 129.8, 129.5, 128.7, 127.0, 125.6, 120.9, 119.4, 118.8, 115.5, 114.6, 114.5, 71.5, 55.4; HRMS (ESI): calcd for $C_{26}H_{17}BF_2N_5O$: 464.1494, Found: 464.1502 $[M - H]^-$.

2. Time-dependent fluorescence intensity changes of BHAni.

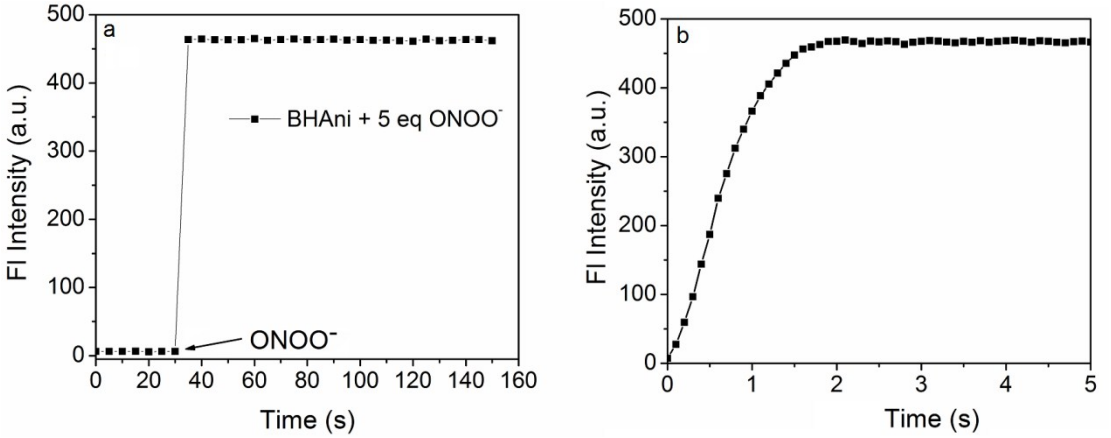


Figure S1. (a) and (b) Time-dependent fluorescence intensity changes of **BHAni** (1 μM) at 530 nm in the presence of $ONOO^-$; Kinetic studies in b were explored using a stopped-flow spectrophotometer at 25 ^{0}C.

3. Fluorescence responses of BHAni toward ROS.

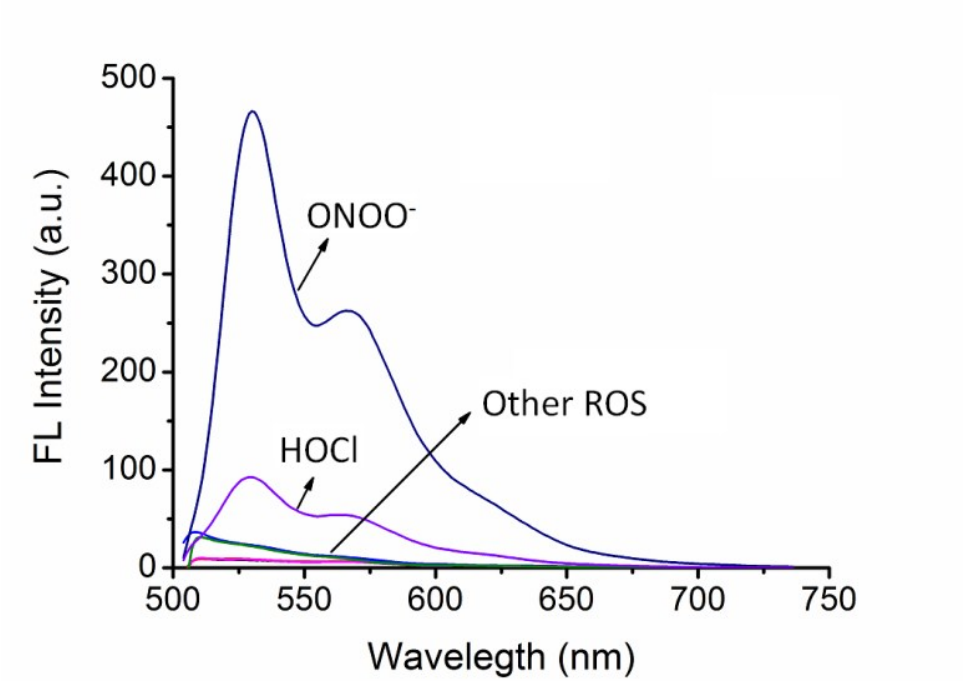


Figure S2. Fluorescence responses of **BHAni** (1 μM) toward ROS; $ONOO^-$ (5 μM) and other ROS (10 μM).

4. Fluorescence responses of BMAni toward ROS.

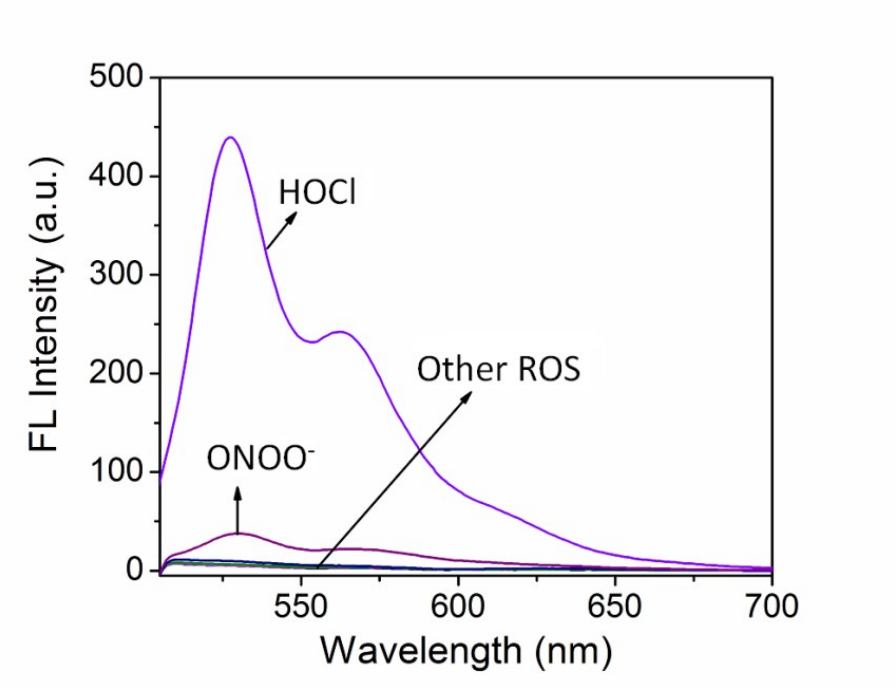


Figure S3. Fluorescence responses of **BMAni** (1 μM) toward ROS. HOCl (5 μM), other ROS (10 μM).

5. Time-dependent fluorescence changes of BMAni.

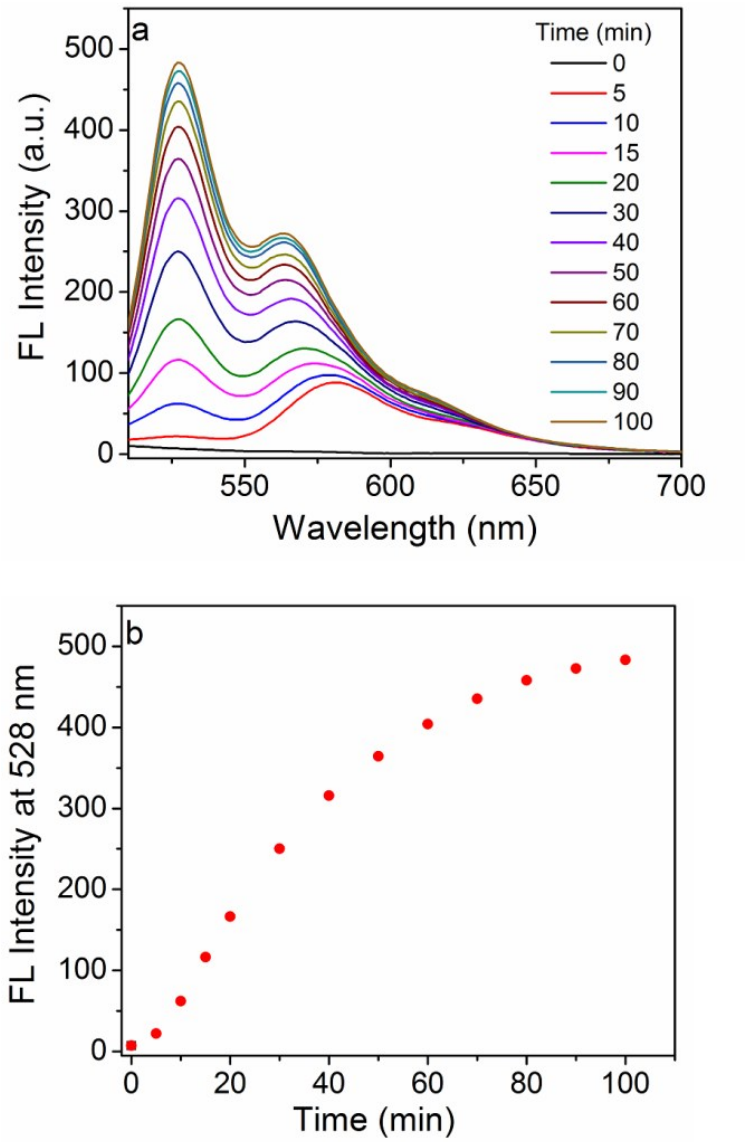


Figure S4. (a) Time-dependent fluorescence changes of **BMAni** (1 μM) in the presence of 10 μM HOCl. (b) Time course of fluorescence intensity of **BMAni** at 528 nm in the presence of HOCl.

6. pH dependent fluorescence changes.

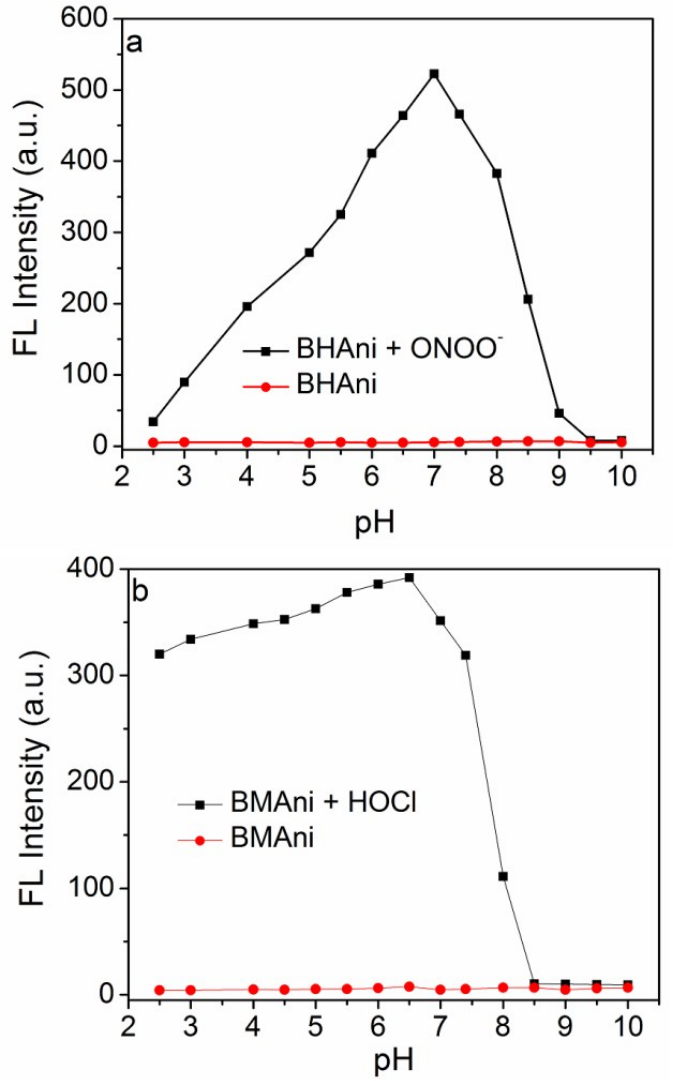
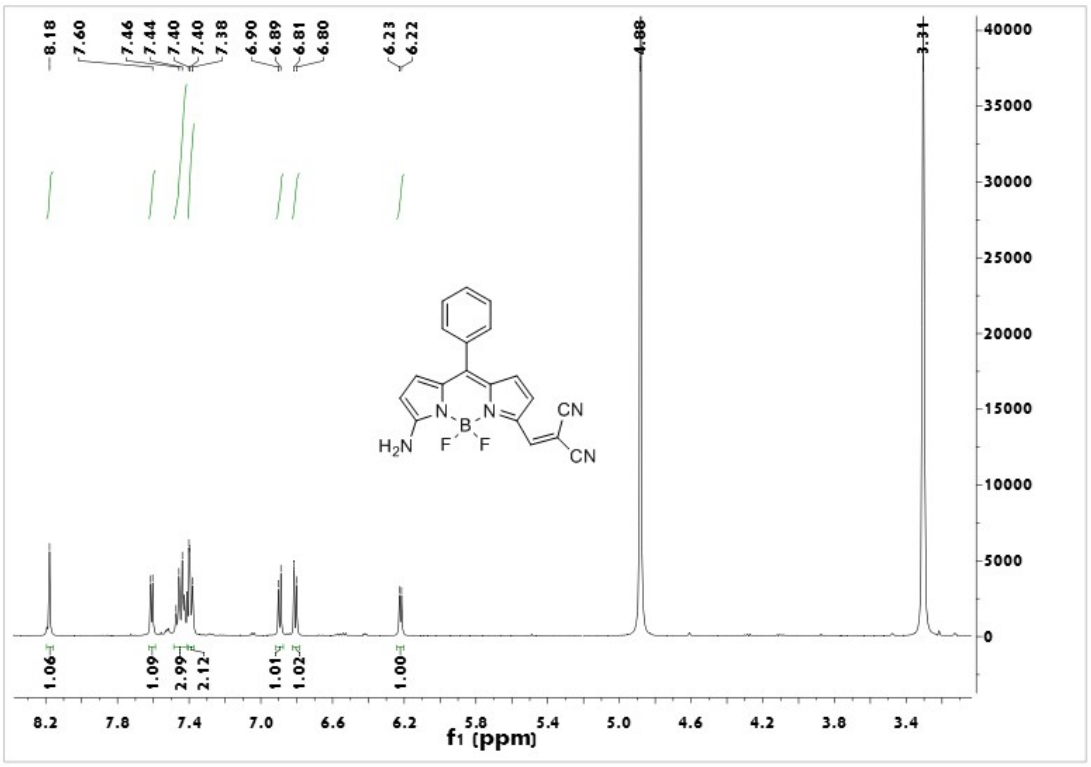


Figure S5. pH dependent fluorescence changes of (a) **BHAni** at 530 nm in the absence and presence of $ONOO^-$ and (b) **BMAni** at 528 nm in the absence and presence of HOCl.

7. Characterization of oxidation product.



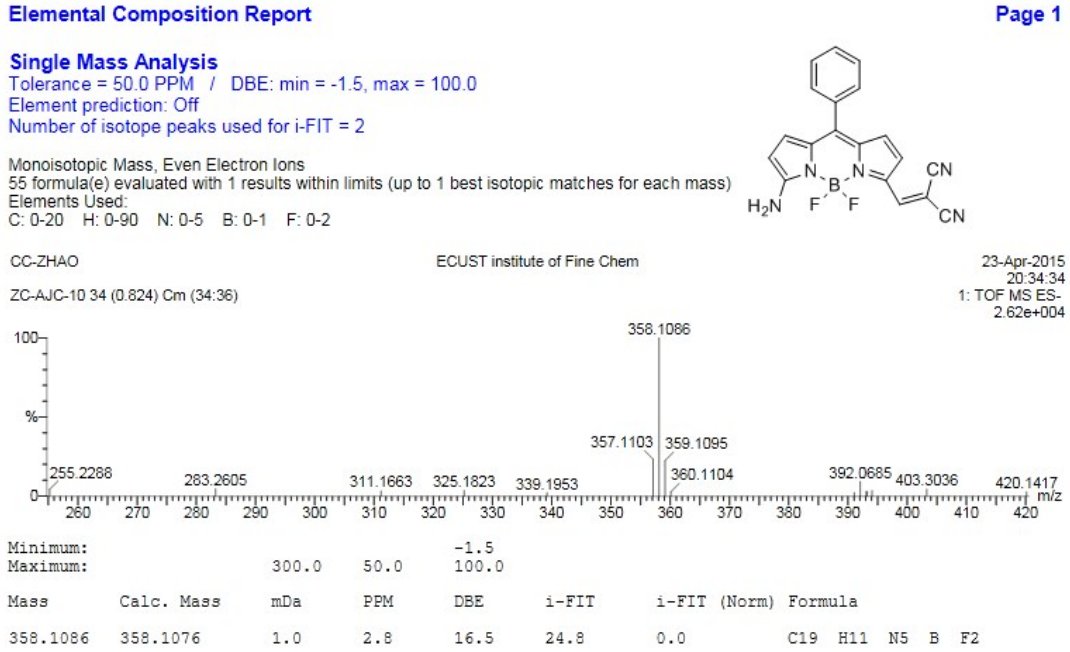


Figure S6. ^{1}NMR (CD_3OD) and HRMS characterization of oxidation product from the reactions of **BHAni** + $ONOO^-$ or **BMAni** + HOCl.

8. Confocal fluorescence microscopy images of $ONOO^-$.

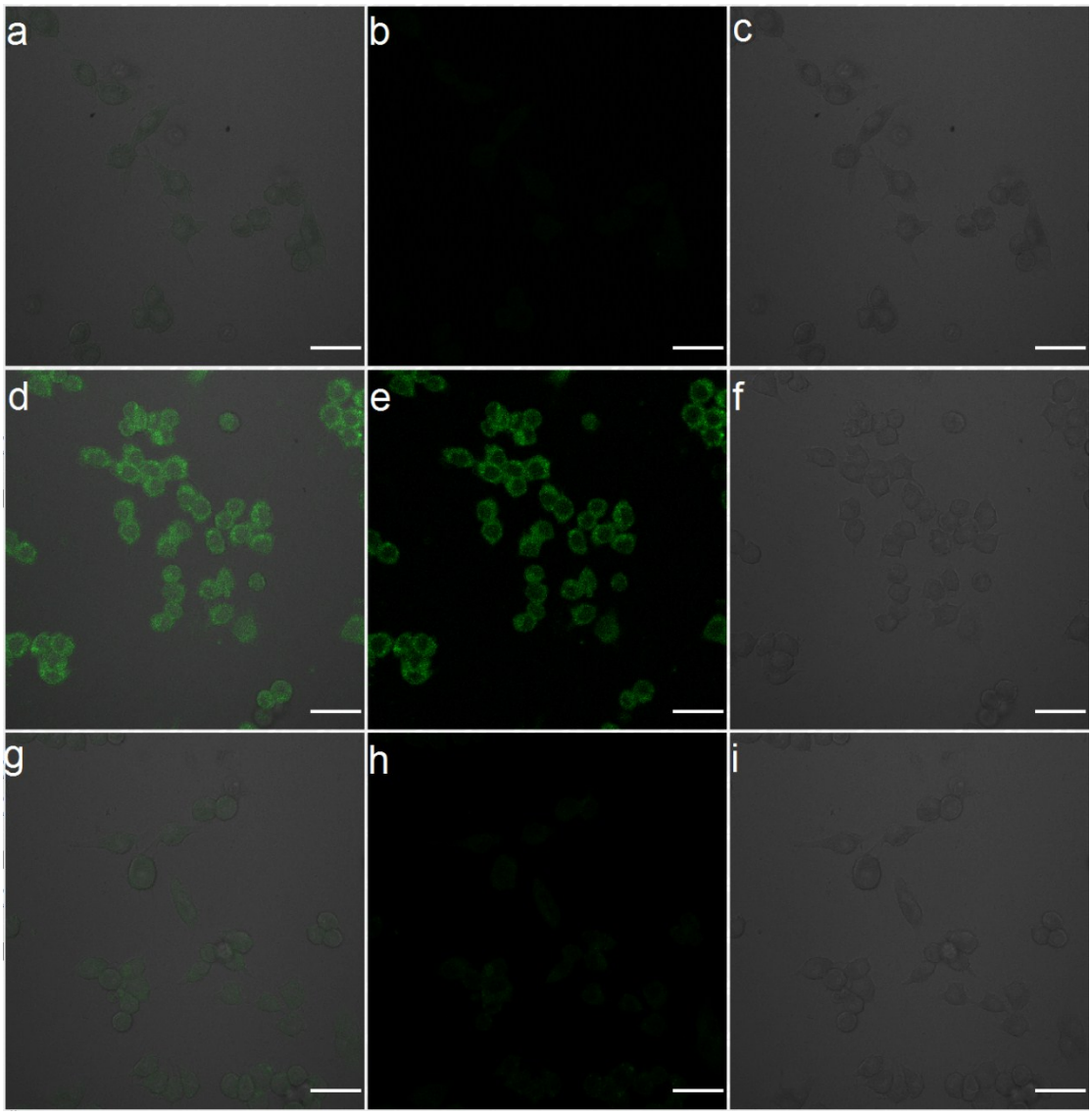


Figure S7. Confocal fluorescence microscopy images of $ONOO^-$ in raw264.7 macrophages cells, the excitation wavelength was 488 nm and the emission was collected at 500-600 nm. a-c) cells stained with **BHAni** (10 μM) for 30 min; d-f) cells loaded with **BHAni** for 15 min, followed by treated with SIN-1 (50 μM) for 30 min; g-i) cells pretreated with urate (100 μM) for 30 min were incubated with **BHAni** for 15 min, subsequently loaded with SIN-1 for 30 min. The scale bars are 20 μm.

9. Confocal fluorescence microscopy images of HOCl.

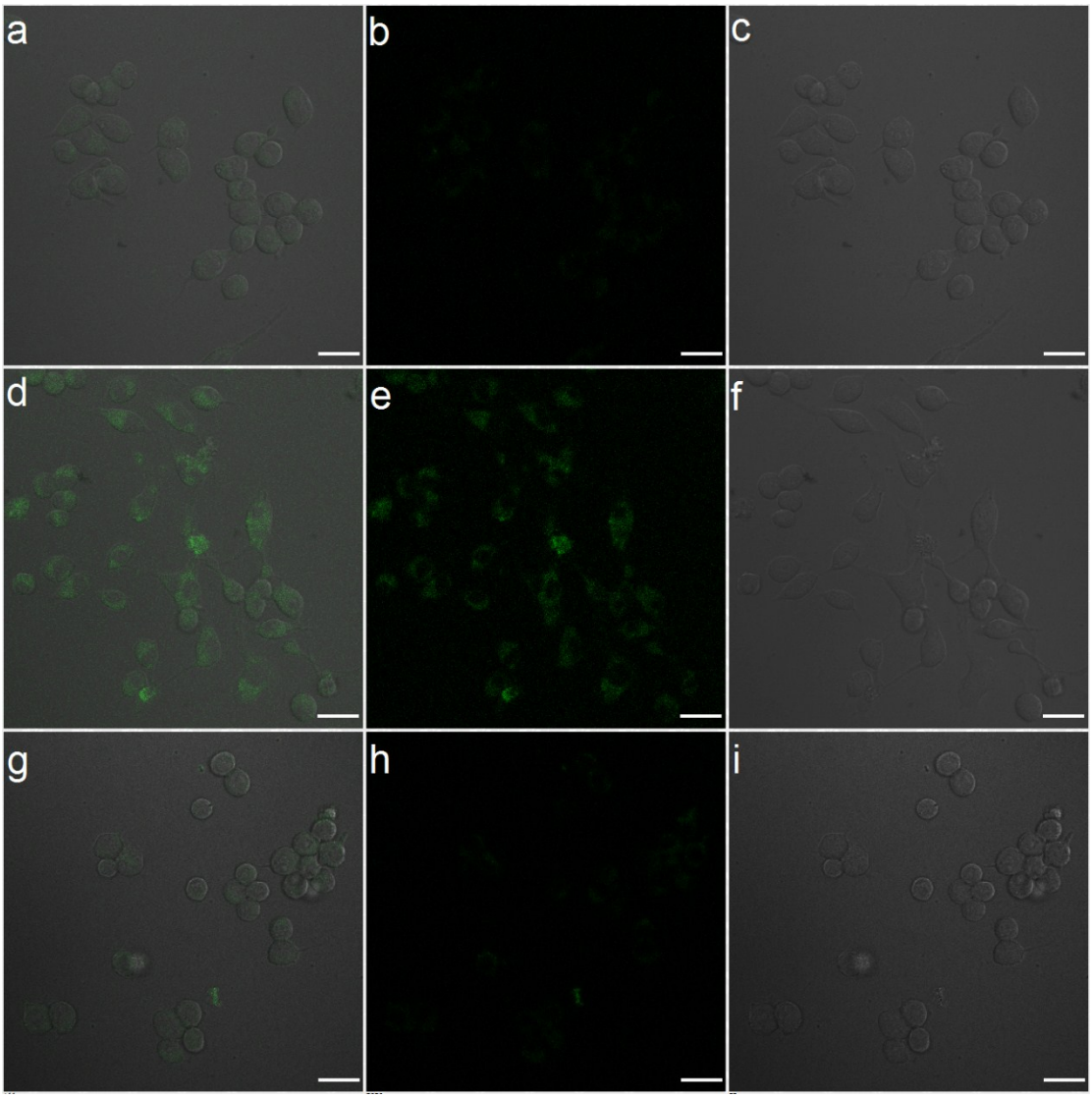
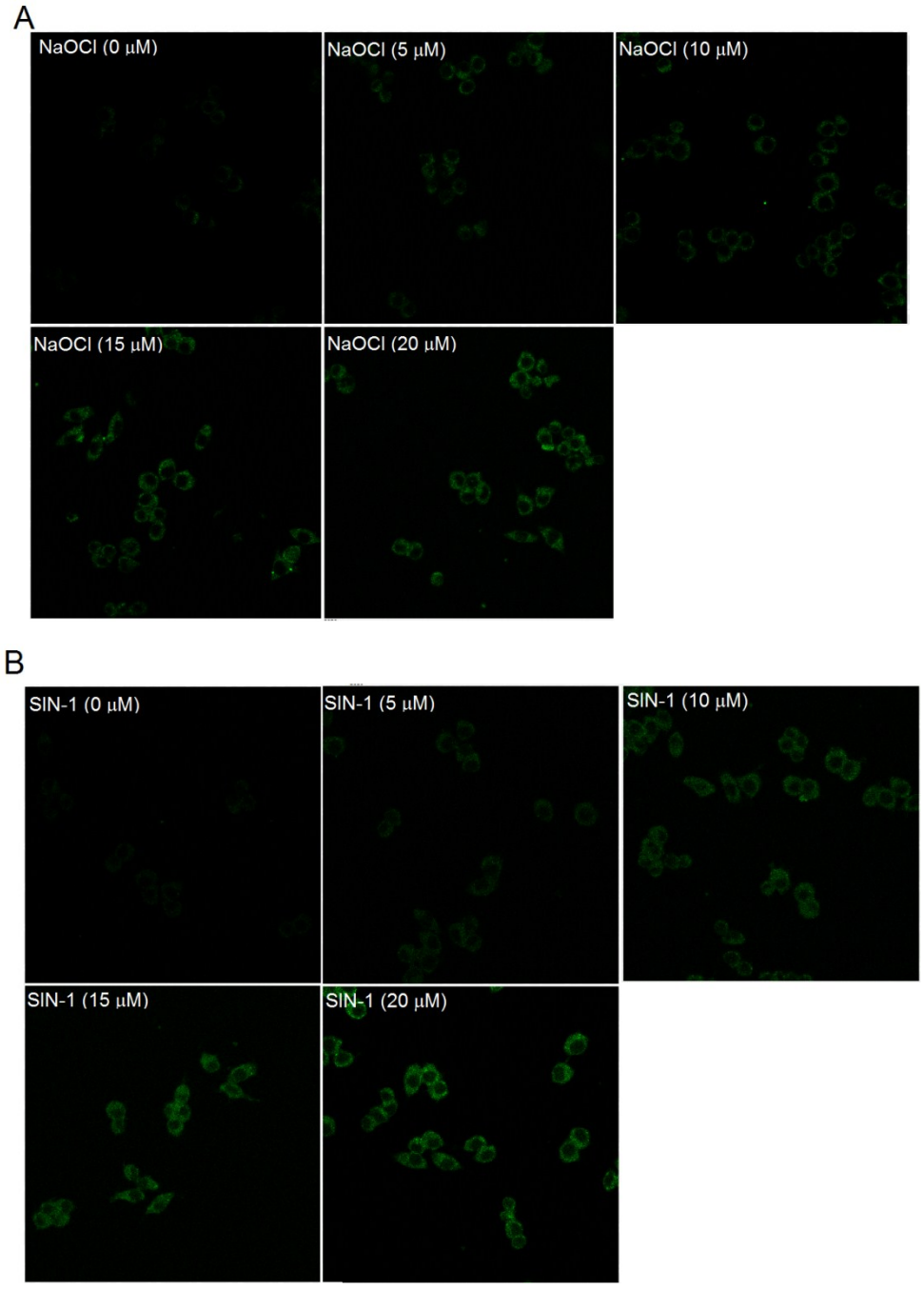


Figure S8. Confocal fluorescence microscopy images of HOCl in raw264.7 macrophages cells, the excitation wavelength was 488 nm and the emission was collected at 500-600 nm. a-c) cells stained with **BMAni** (10 μM) for 30 min; d-f) cells were stimulated with LPS (1.0 μg/mL) for 12 h and followed by PMA (1.0 μg/mL) for 1 h, then treated with **BMAni** for 30 min; g-i) cells pretreated with ABH (200 μM) for 15 min were stimulated with LPS (1.0 μg/mL) for 12 h and followed by PMA (1.0 μg/mL) for 1 h, then stained with **BMAni** for 30 min. The scale bars are 20 μm.

10. A concentration-dependent fluorescence relationship



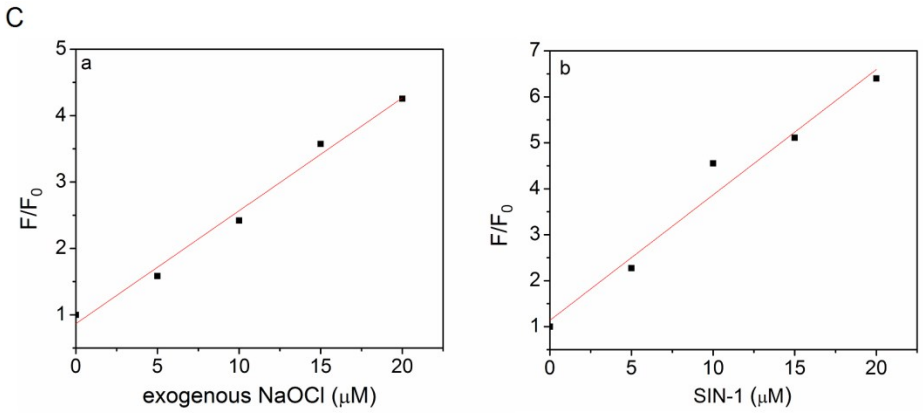
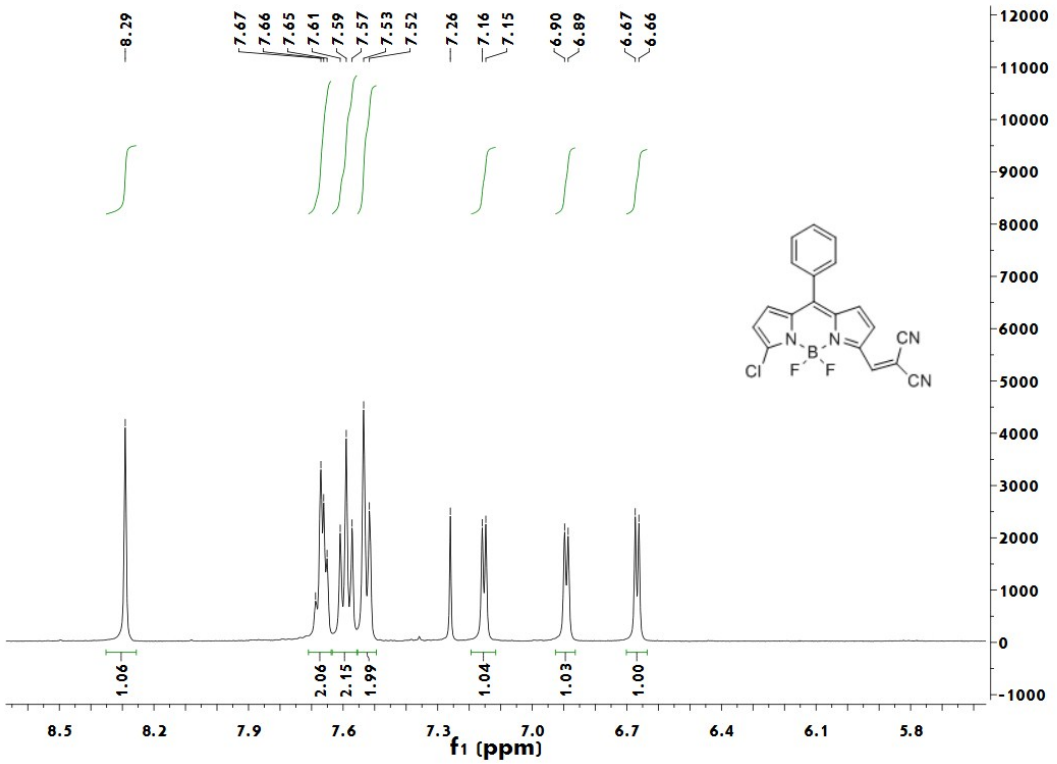
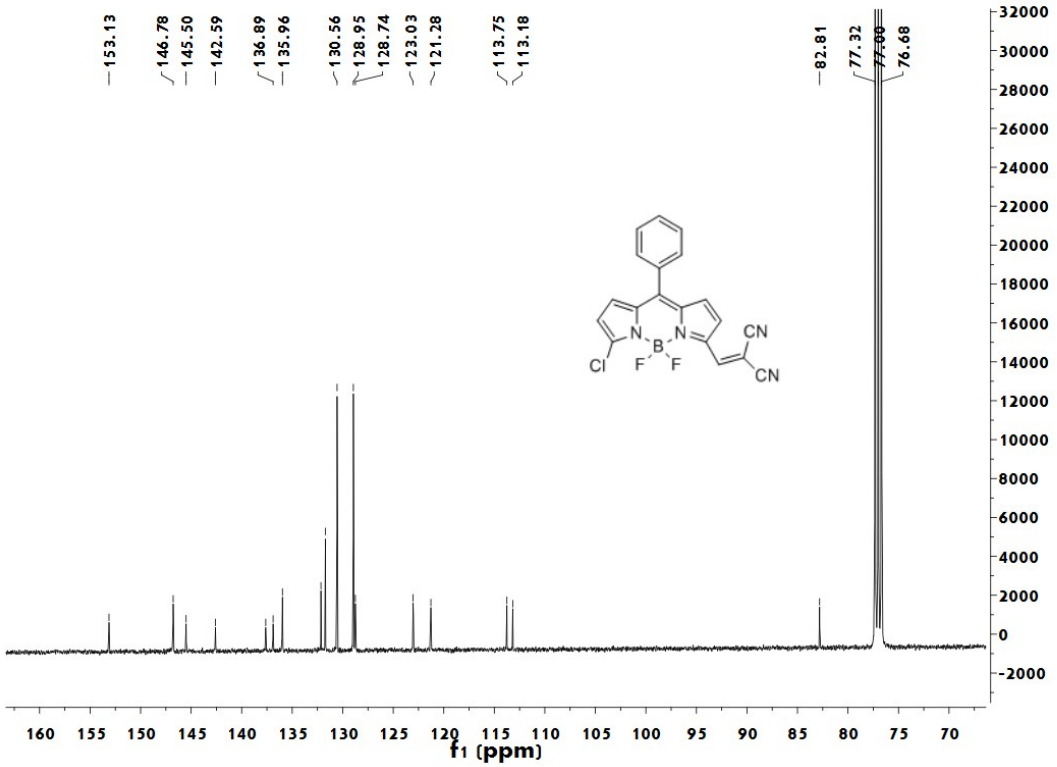


Figure S9 A) Image intensity of **BMAni** (10 μM) in the presence of various exogenous NaOCl; B) Image intensity of **BHAni** (10 μM) in the presence of various 3-morpholinosydnonimine hydrochloride (SIN-1); C) Image intensity changes of (a) **BMAni** (10 μM) (estimated from A) as a function of exogenous NaOCl increasing concentrations and (b) **BHAni** (10 μM) (estimated from B) as a function of SIN-1 increasing concentrations.

11. NMR and HRMS spectra.





Elemental Composition Report **Page 1**

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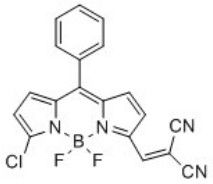

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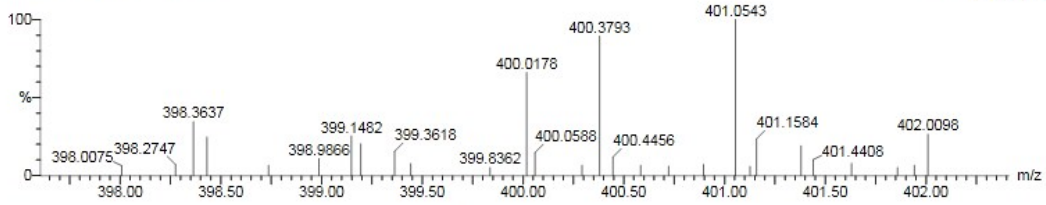

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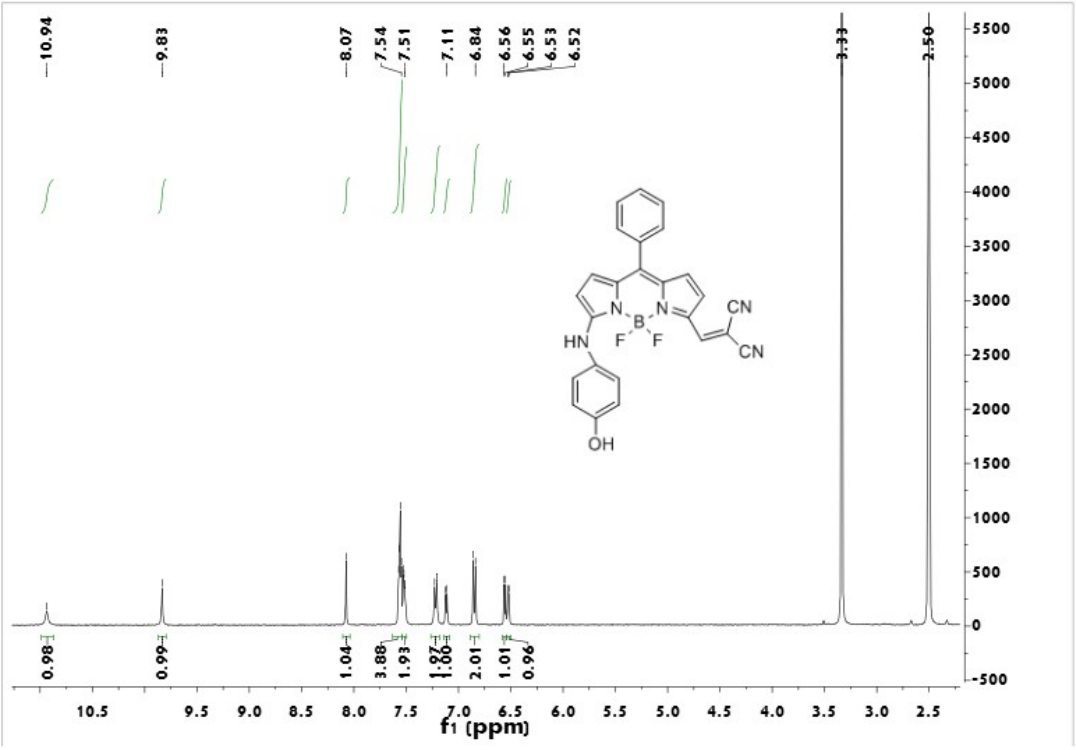
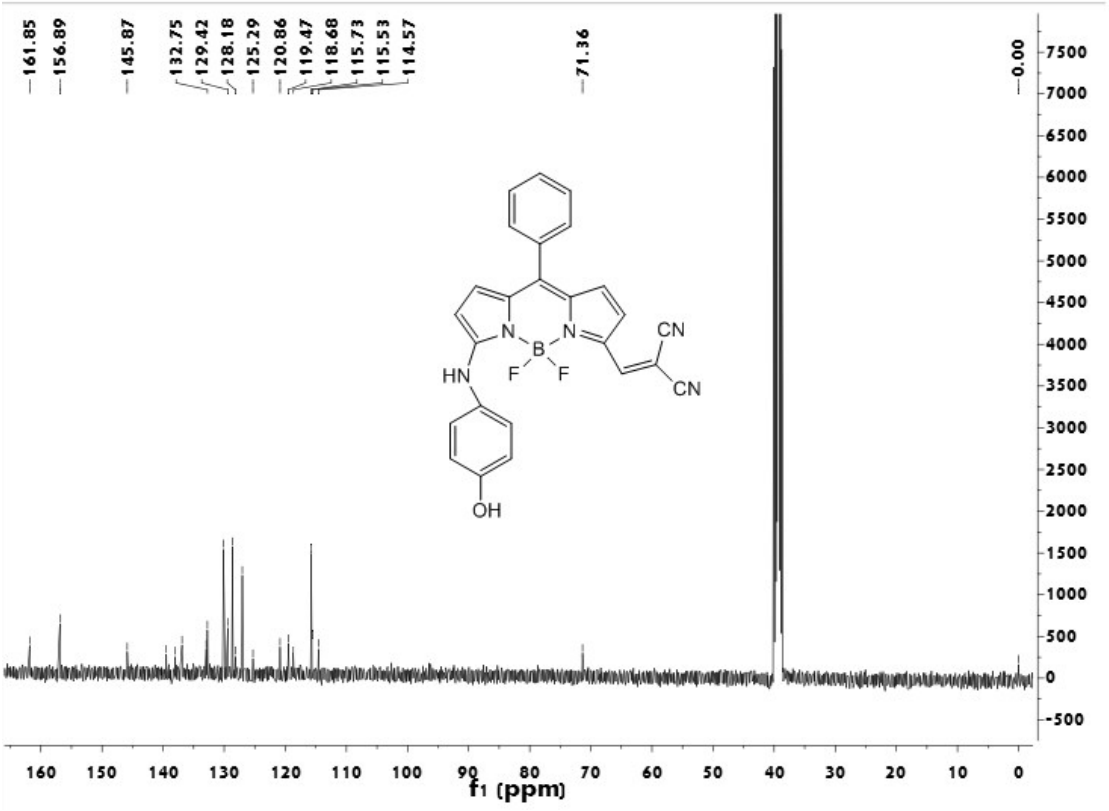
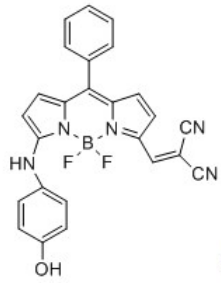




Elemental Composition Report **Page 1**



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Element prediction: Off
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Monoisotopic Mass, Even Electron Ions
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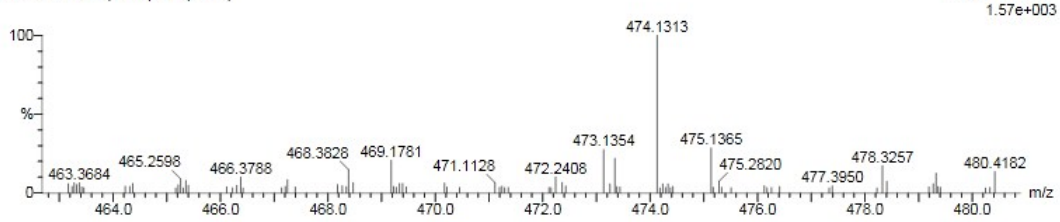

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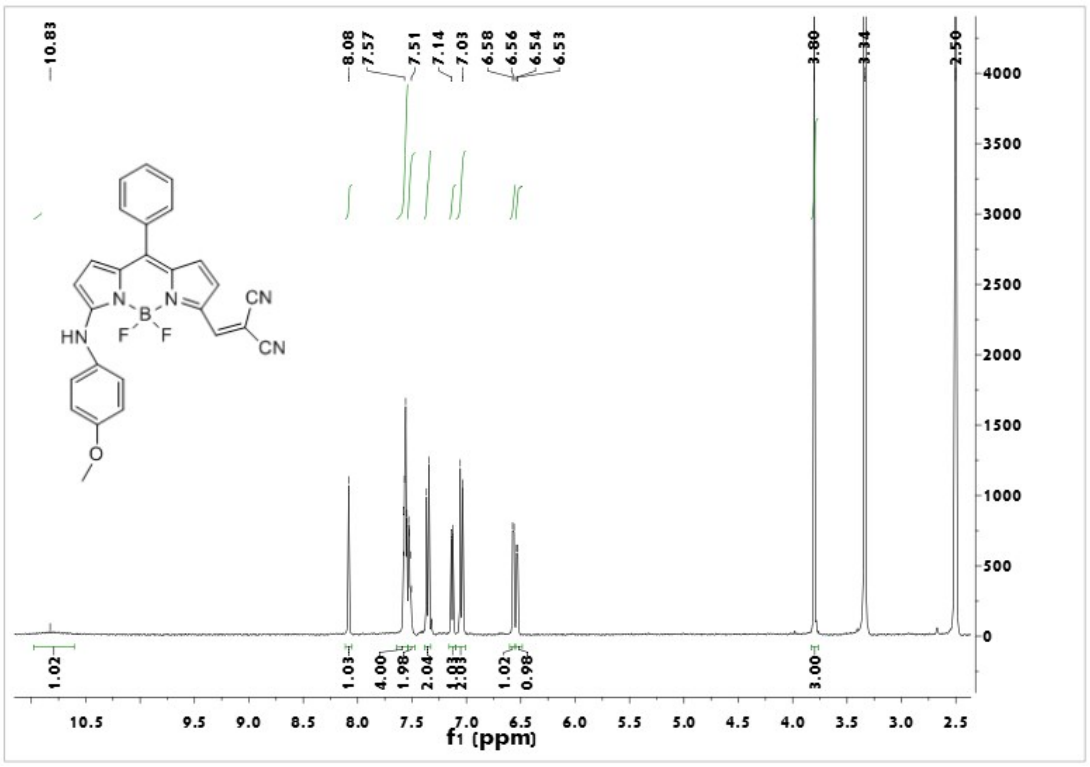
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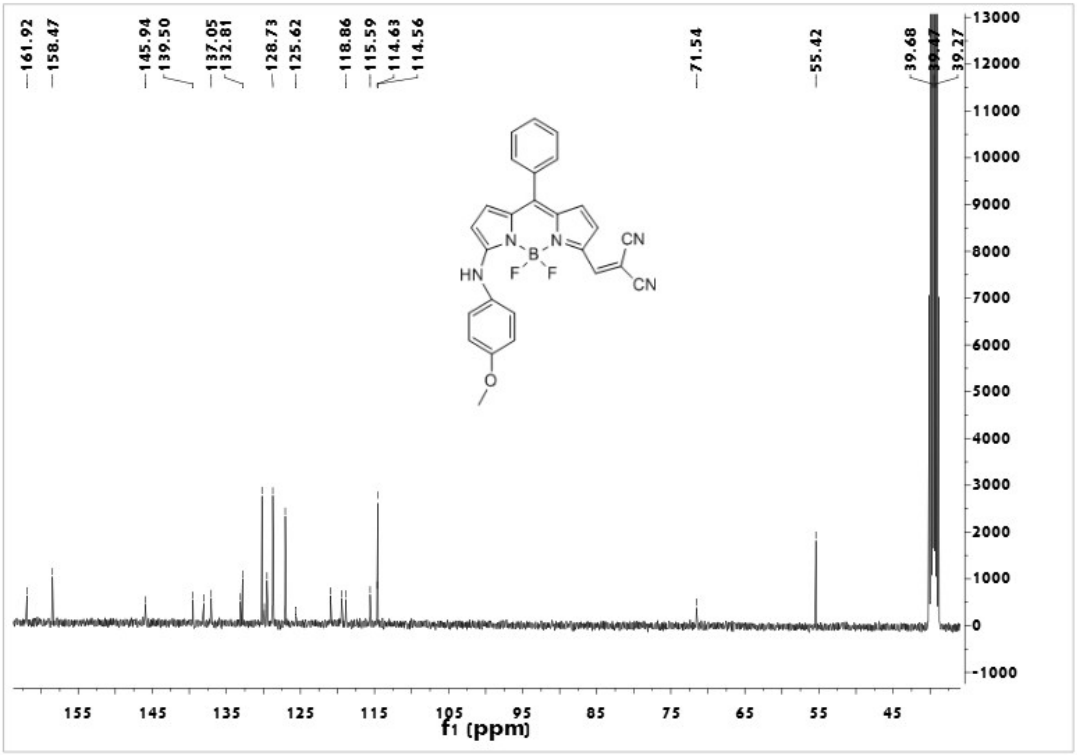
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HN
B
F
F
N
N
CN
CN
O
f1 (ppm)

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
50 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass
Elements Used:
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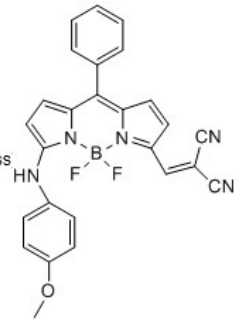

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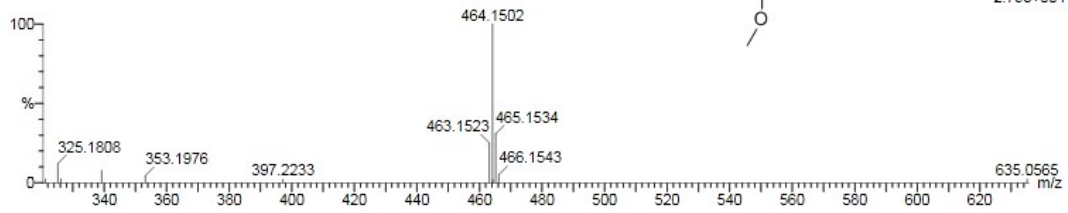

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