Electronic Supplementary Information (ESI) for

BODIPY-based hydrazine as fluorescent probe for sensitive and selective detection of nitric oxide: a new strategy

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Contents:

1. Materials and Methods

All reagents for synthesis were purchased from commercial suppliers and used without further purification. Solvents of technical quality were distilled prior to use. Water for preparation of solutions was purified with a Millipore water system. UV−vis absorption spectra and fluorescence spectra were measured on a Shimadzu 2450 UV/vis spectrometer and a Shimadzu RF-5301PC luminescence spectrometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV spectrometer performing at 400 and 100 MHz, respectively, and chemical shifts were reported in ppm using tetramethylsilane (TMS) as the internal standard. HRMS data were obtained with a Thermo LTQ Orbitrap mass spectrometer.

Preparation of the test solutions. A bulk solution of 8-HB was prepared in DMSO and then diluted to a given concentration with a 0.1 M PBS buffer (pH 7.4) in the ratio of DMSO to the buffer (v/v 1:100). The NO saturated solution in deionized water was prepared by bubbling NO gas into a NaOH solution to eliminate NO_2 generated from the reaction of NO and O_2, then into deoxygenated deionized water for 30 min. The concentration of the NO saturated solution is 1.9 mM.[S1]

Various analytes were prepared in the terms of methods as follows. Peroxynitrite ($ONOO^-$) solution was prepared according to literature,[S2] and its concentration was estimated in terms of the extinction coefficient of 1670 M^{-1} cm^{-1} at 302 nm. ClO^- solutions were from aqueous solutions of NaClO freshly prepared. Hydroxyl radical ($HO^{\bullet}$) was generated in situ by the Fenton reaction. H_2O_2 solution was obtained by dilution of commercial H_2O_2 solution with deionized water. Superoxide ($O_2^{\bullet-}$) solution was prepared through adding KO_2 (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min. For spectral measurements, various analytes including NO, $ONOO^-$, ClO^-, $HO^{\bullet}$, H_2O_2, $O_2^{\bullet-}$, DHA and AA were added to the solution of 8-HB (10 μM) in PBS (0.1 M, pH 7.4, containing 1% DMSO).

The mixture solutions cover a pH range from 2 to 11, involving 0.1 M citric acid-0.1 M disodium hydrogen phosphate buffer for pH 2-5, 0.1 M Na_2HPO_4-0.1 M NaH_2PO_4 for pH 6-8, and a 0.1 M

K_2CO_3-0.1 M $NaHCO_3$ buffer for pH > 8. All pH values of solutions were further measured with a MQK-PHS-3C pH meter.

Cell culture, MTT assay and Cell imaging experiments. HepG2 and Raw 264.7cells were seeded in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37ºC. The cells were placed in a 96-well plate, and the added various concentrations of 8-HB (0 - 60 μM), respectively, and then incubated at 37ºC in an atmosphere of 5% CO_2 and 95% air for 24 h for MTT assay. The culture medium was removed and 100 μL of 5 mg mL^{-1} MTT reagent in PBS was added to each well. Thereafter, it was incubated for 4 h; during this period active mitochondria of viable cells reduced MTT to purple formazan. Unreduced MTT was then discarded and DMSO (100 μL) was added into each well to dissolve the formazan precipitate, which was determined by measuring the absorbance at 570 nm with a microplate reader. The cytotoxic effect of each treatment was expressed as a percentage of cell viability relative to the untreated control cells. Cell imaging experiments were performed on the IX71 Olympus Reflected Fluorescence System with an excitation filter (420 - 445 nm).

Synthesis and Characterization of the probe 8-HB and compounds 2 and 3

5,5-difluoro-10-hydrazinyl-3,7-dimethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (8-HB). Compound **1**[S3] (500 mg, 2.0 mmol) was dissolved in 10 mL absolute alcohol, then 80% hydrazine hydrate (5 drops) was slowly added to the solution, and stirred for 5 min at room temperature. The solvent in the reaction mixture was removed in vacuo, and the crude was purified by flash chromatography on silica gel to afford a light yellow solid (300 mg, 60%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.76 (s, 2H, NH), 7.89 (d, J = 4.0 Hz, 1H, pyrrole-H), 7.15 (d, J = 4.0 Hz, 1H, pyrrole-H), 6.22 (d, J = 4.0 Hz, 1H, pyrrole-H), 6.05 (d, J = 3.6 Hz, 1H, pyrrole-H), 5.55 (s, 2H, NH_2), 2.43 (s, 3H, CH_3), 2.36 (s, 3H, CH_3) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ= 144.81, 141.28, 125.60, 122.54, 121.54, 114.54, 114.19, 112.85, 14.35, 14.03 ppm. TOFMS (ESI) calcd for $[M+H]^+$: 251.1201, found 251.1268.

5,5-difluoro-3,7-dimethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (**2**)[S4] and 10-azido-5,5-difluoro-3,7-dimethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (**3**).

8-HB (1.0 g, 4.0 mmol) was dissolved in 5 mL acetonitrile and diverted into 10 mL water. Then, NO gas was bubbled into the solution for 5 min. The mixture extracted with dichloromethane (50 mL × 3). The organic phase was collected, washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtrated and evaporated to give a crude product. The crude product was purified by column chromatography to give compounds **2** (528 mg, red solid, 60 % yield) and **3** (157 mg, red solid, 15 % yield). Compound **2**: R_f = 0.13 (PE/EA 10:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.06 (s, 1H, pyrrole-H), 6.94 (d, J = 4.0 Hz, 2H, pyrrole-H), 6.27 (d, J = 4.0 Hz, 2H, pyrrole-H), 2.62 (s, 6H, pyrrole-H) ppm. TOFMS (ESI) calcd for $[M+H]^+$: 221.0983, found 221.1051. Compound **3**: R_f = 0.25 (PE/EA 10:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.23 (d, J = 4.4 Hz, 2H, pyrrole-H), 6.27 (d, J = 4.0 Hz, 2H, pyrrole-H), 2.60 (s, 6H, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.6, 139.5, 127.9, 123.8, 118.6, 14.7 ppm. TOFMS (ESI) calcd for $[M+H]^+$: 262.0997, found 262.1067.

References

S1 J. Hartung, *Chem. Rev*. 2009, **109**, 4500-4517.

S2 R. M. Uppu and W. A. Pryor, *Anal. Biochem.* 1996, **236**, 242-249.

S3 L. Esnal, L. Valois-Escamilla, C. F. A. Gómez-Durán, A. Urías-Benavides, M. L. Betancourt-Mendiola, I. López-Arbeloa, J. Bañelos, I. García-Moreno, A. Costela and E. Peña-Cabrera, *Chem. Phys. Chem.* 2013, **14**, 4134-4142.

S4 S. Kim, J. Bouffard and Y. Kim, *Chem. Eur. J*. 2014, **20**, 5064-5074.

2. Spectral response of 8-HB toward NO

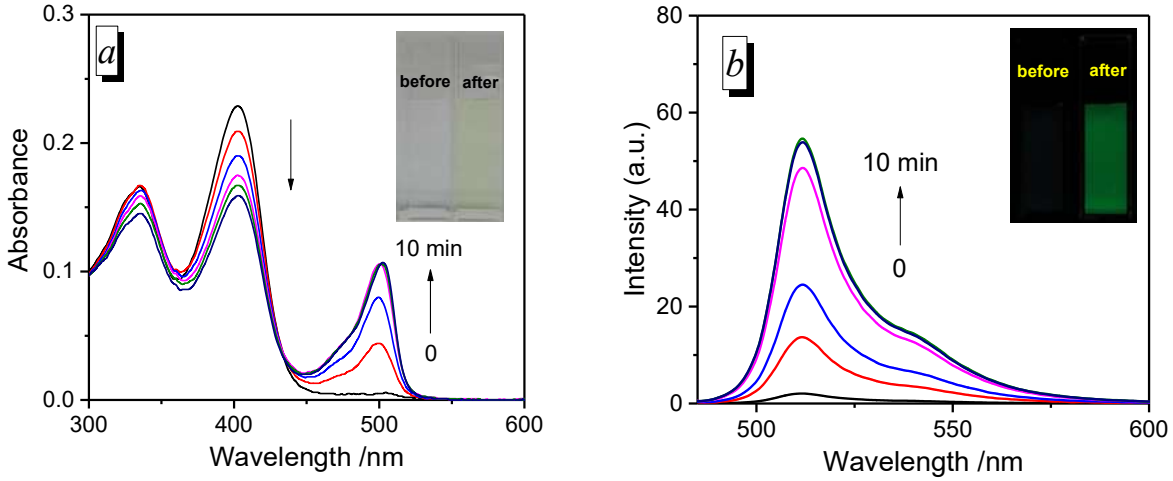


Figure S1. Time-dependent absorption (a) and fluorescence spectra (b) of 10 μM 8-HB (0.1 M PBS, pH 7.4, 1% DMSO) before and after the addition of NO (20 μM), λ_{ex} = 480 nm, the time intervals 2 min. Insets: photos of solutions under room light (a) or 365 nm light (b).

3. HRMS analysis of the reaction mixture of 8-HB with NO

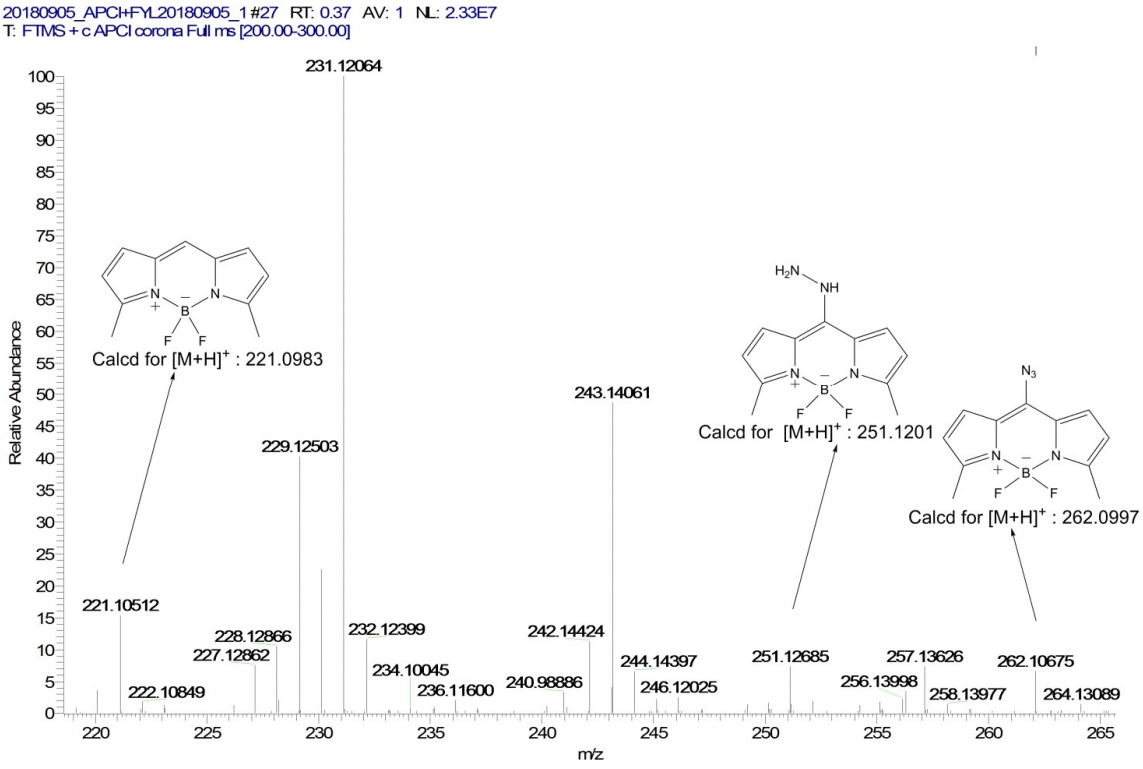


Figure S2. High-resolution mass spectrum (HRMS) for the mixture of 8-HB with NO in an aerobic aqueous solution (pH 7.4).

4. pH Effects and cytotoxicity

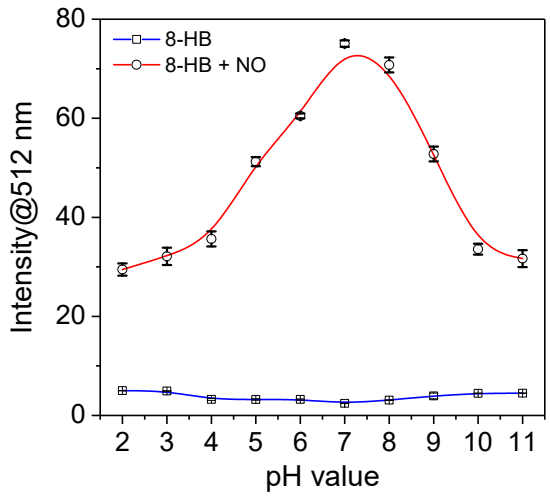


Figure S3. Fluorescence intensities at 512 nm of 10 μM 8-HB and its reaction mixture with 20 μM NO in PBS buffer solutions (pH 2−11).

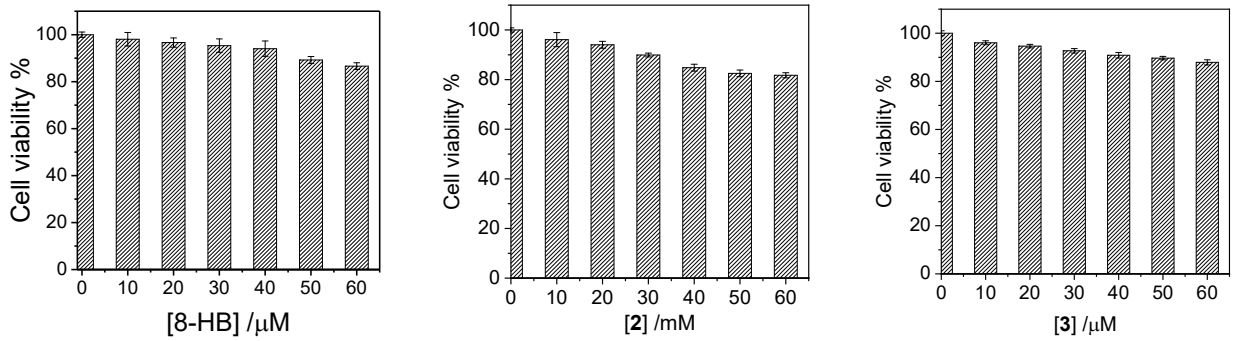


Figure S4. MTT assay of HepG2 cells in the presence of different concentrations of 8-HB, sensing products **2** and **3** incubated for 24 h.

5. Cell imaging

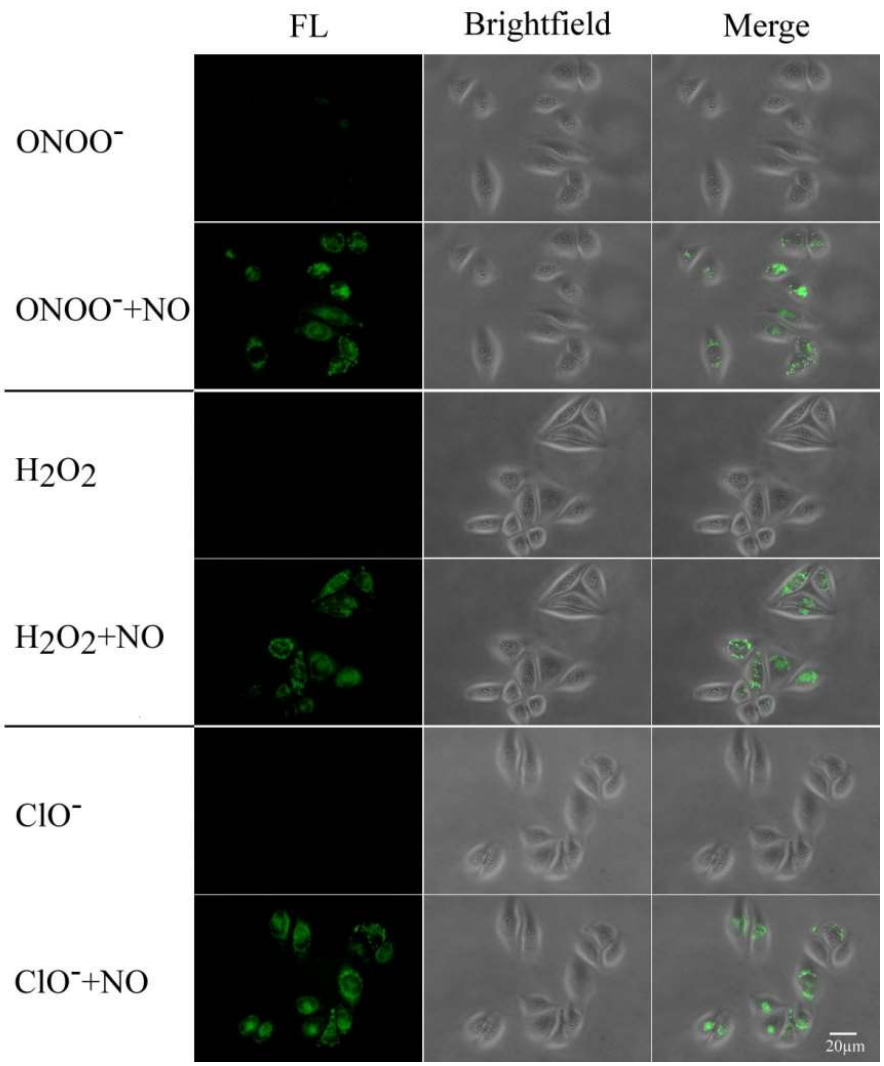
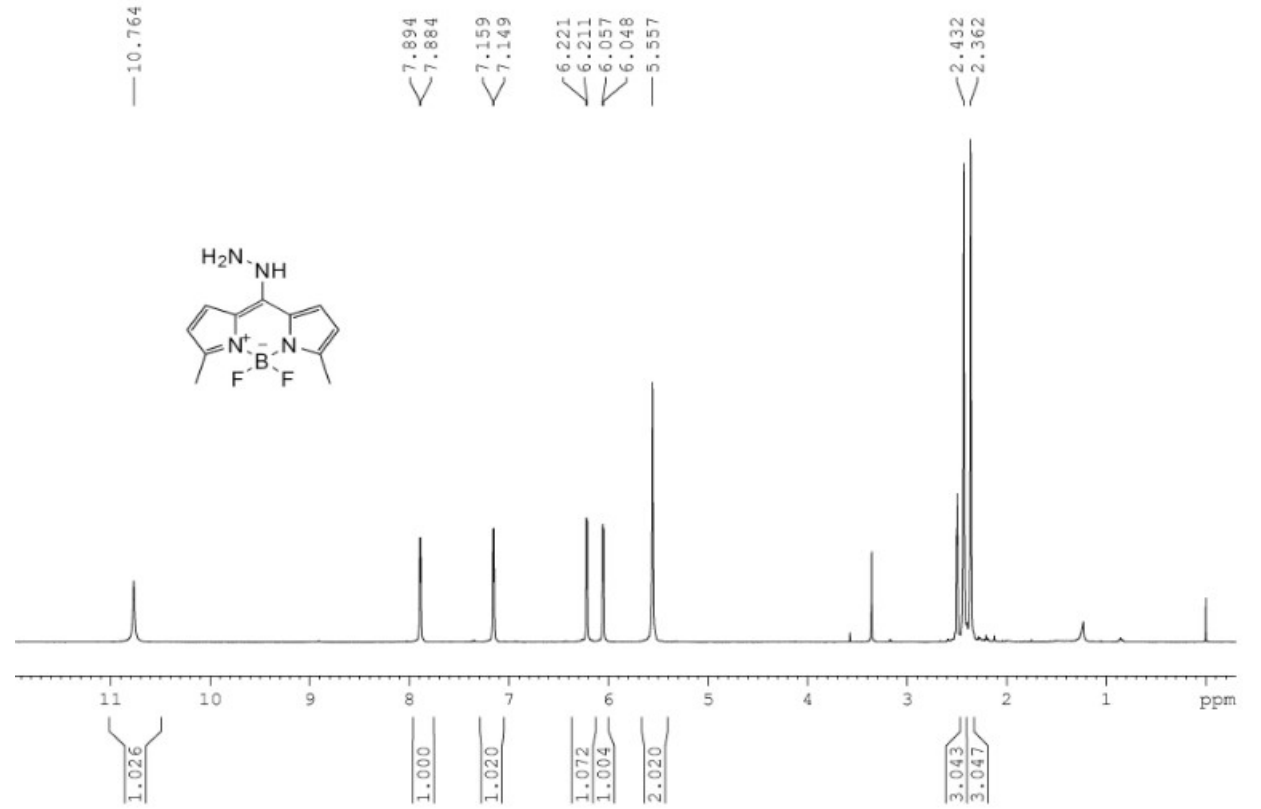
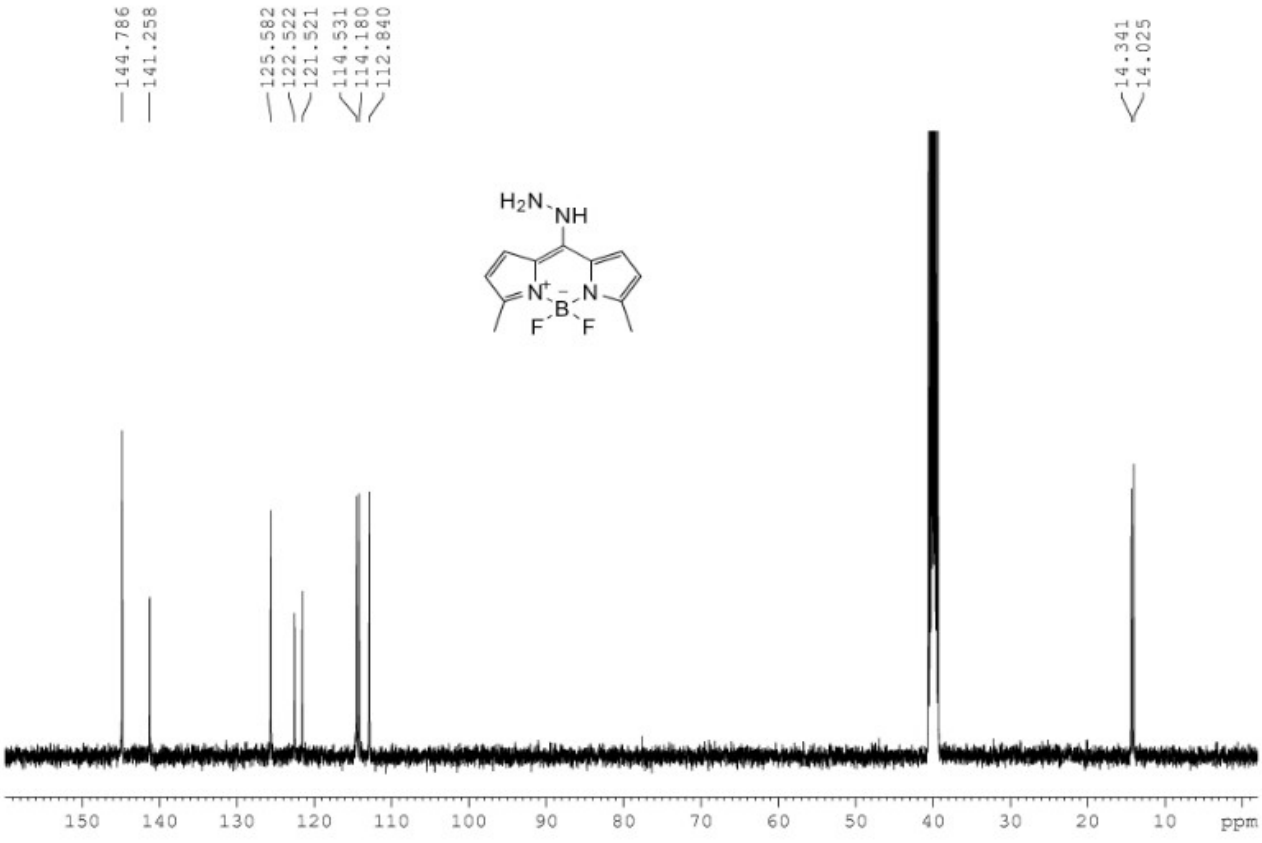
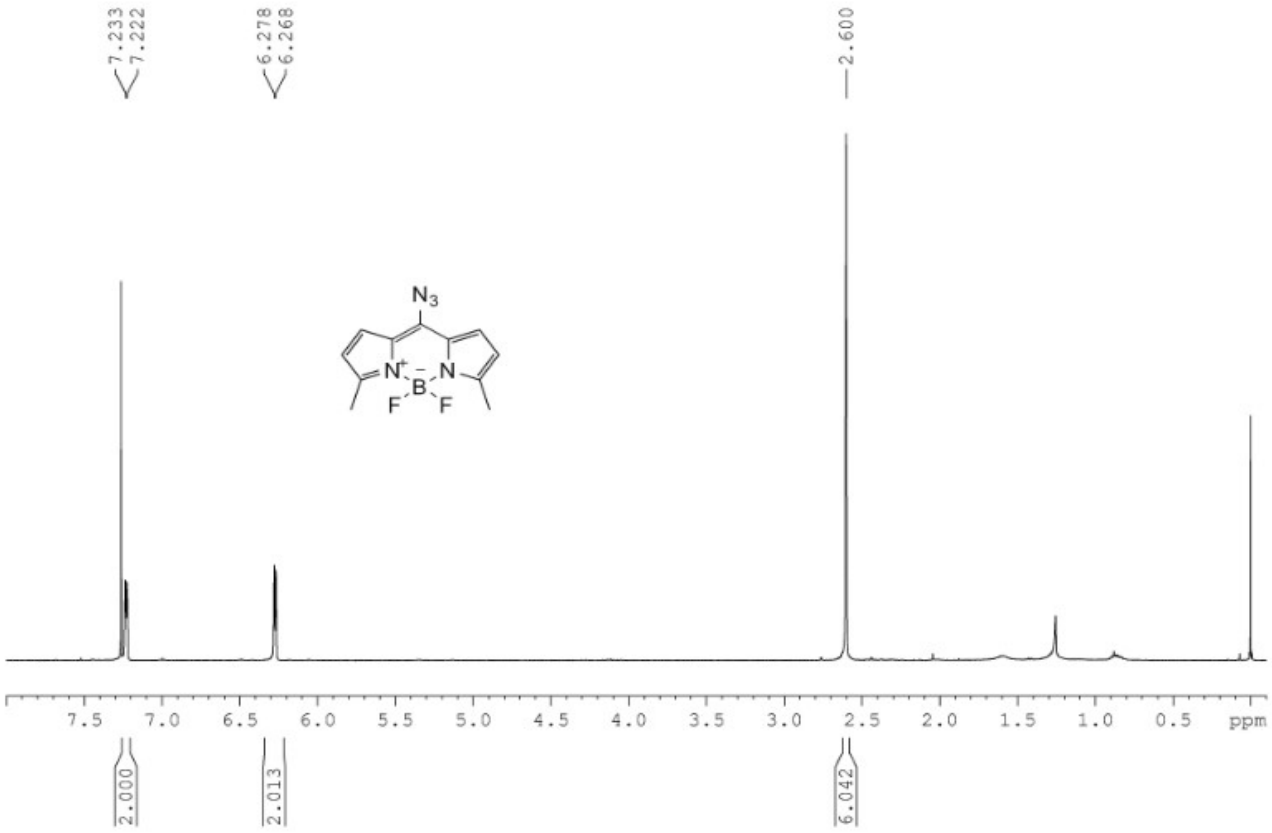


Figure S5. Fluorescence images of HepG2 cells stained with 8-HB (10 μM, 30 min) and then treated with $ONOO^-$ (50 μM, 30 min), H_2O_2 (50 μM, 30 min) or ClO^- (50 μM, 30 min), and finally incubated with NO solution (50 μM, 10 min), respectively.

6. Copies of NMR spectra for compounds 8-HB, **3** and **2**



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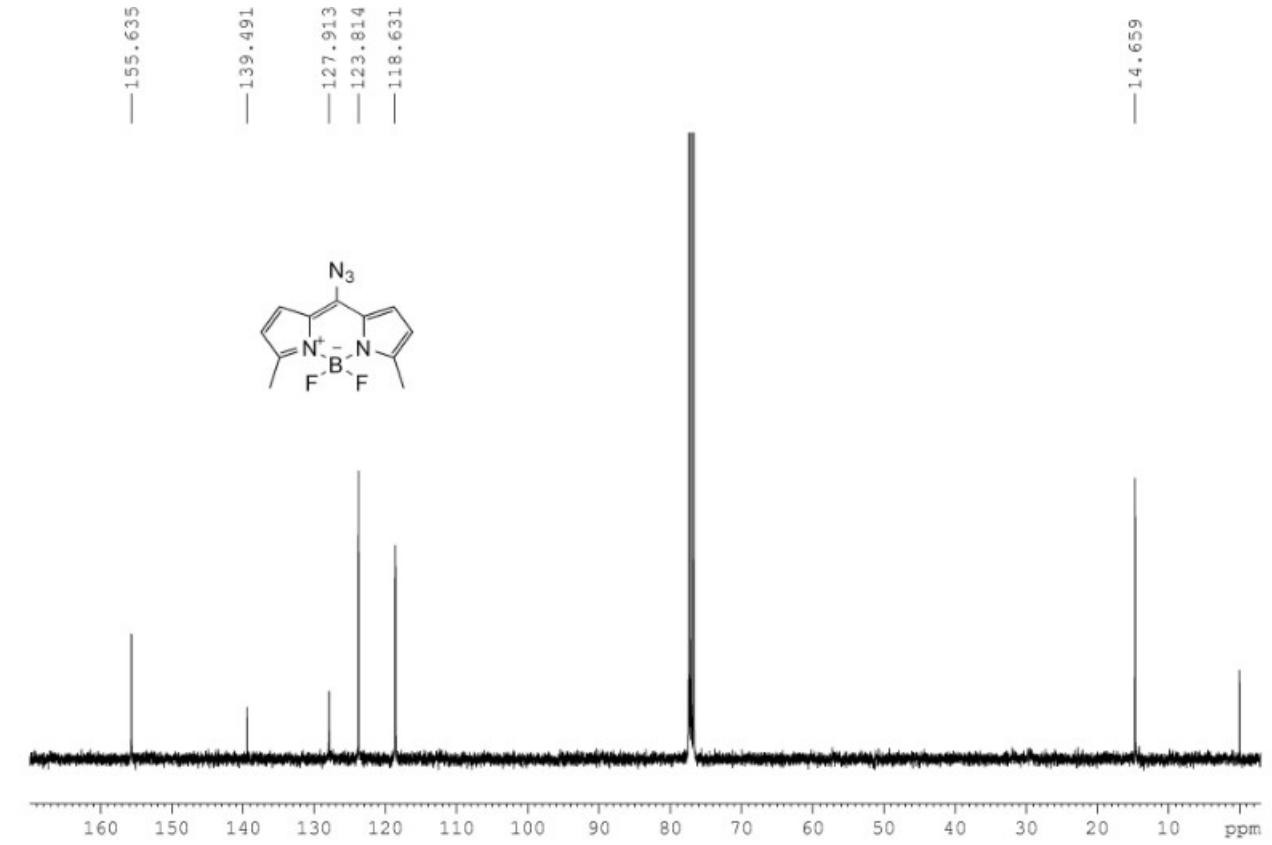
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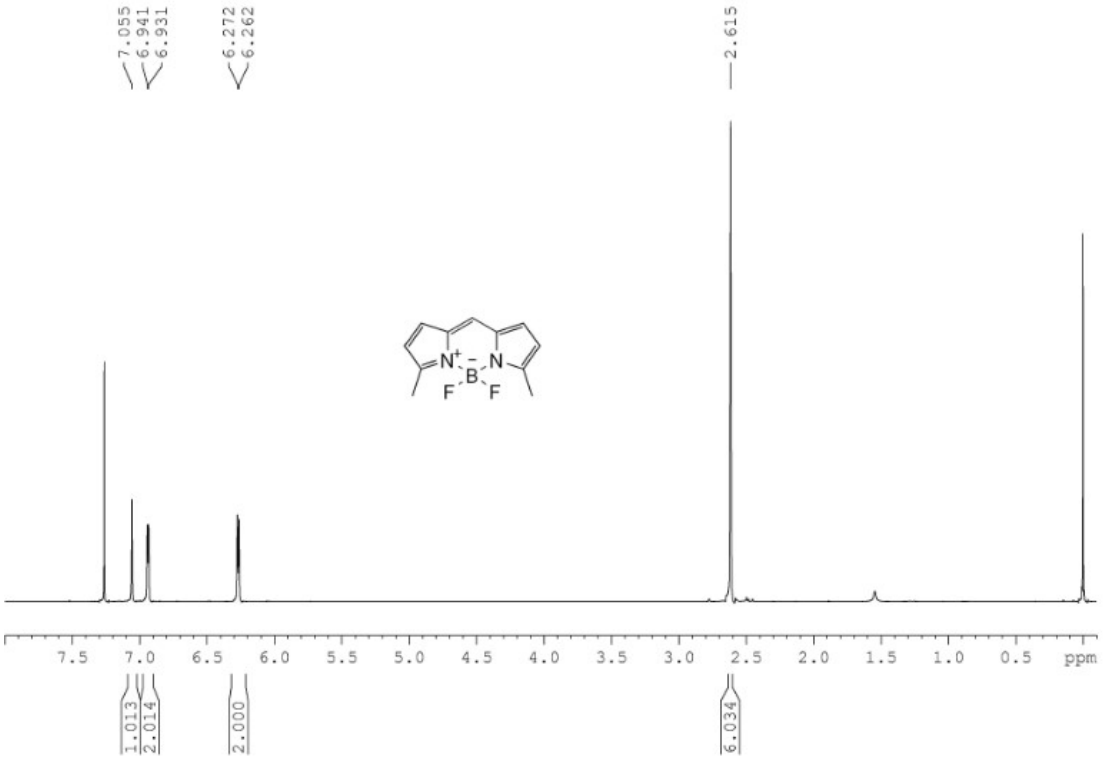
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