Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

An arylboronate-based fluorescent probe for peroxynitrite with fast response and high Selectivity

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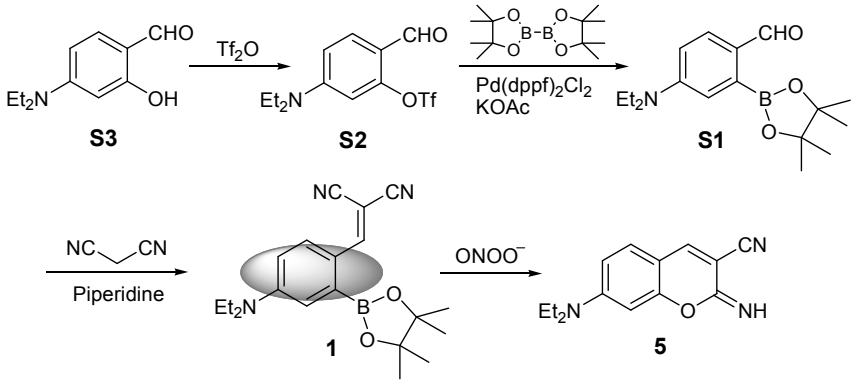
1. General methods

Reagents and solvents were purchased from commercial sources and were of the highest grade. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography was performed using silica gel 60 (200–300 mesh). Absorption spectra were taken on a Varian Carry 4000 spectrophotometer. Fluorescence spectra were taken on Hitachi F-7000 fluorescence spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer.

2. Synthesis



Compound S2: 4-Diethylaminosalicylaldehyde **S3** (0.579 g, 3 mmol) and trifluoromethanesulfonic anhydride (1.692 g, 6 mmol) were dissolved in 24 mL of anhydrous THF. N,N-Diisopropylethylamine (DIEA, 7.5 mmol) was added via syringe, and the resulting solution was stirred at room temperature overnight. The reaction was taken to dryness by rotary evaporation, and purification by flash column chromatography (silica gel, EA/PE 1:10) gave **S2** as a green solid (0.417 g, 43% yield). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.95 (s, 1H), 7.79 (d, J = 9 Hz, 1H), 6.65 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 6.43 (d, J = 2.4 Hz, 1H), 3.44 (q, J = 7.2 Hz, 4H), 1.23 (t,

J = 7.2 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.0, 159.7, 154.8, 150.8, 145.6, 133.3, 112.9, 99.8, 47.9, 15.3.

Compound S1: Compound **S2** (113 mg, 0.35 mmol), bis(pinacolato)diboron (178 mg, 0.7 mmol), Pd(dppf)$Cl_2CH_2Cl_2$, (12 mg, 5%), potassium acetate (137 mg, 1.4 mmol), and anhydrous 1,4-dioxane (10 mL) were combined in a 50-mL Schlenk flask. The vessel was stirred at 100 °C for 12 h under nitrogen. The reaction was then cooled to room temperature, diluted with dichloromethane, and washed three times with brine. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to give a yellow residue. The residue was purified by flash column chromatography (silica gel, EA/PE 1:10) gave **S2** as a yellow oil (57 mg, 54% yield). ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.15 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 2.4 Hz, 1H), 6.71 (dd, J_1 = 9 Hz, J_2 = 2.4 Hz, 1H), 3.45 (q, J = 6.6 Hz, 4H), 1.39 (s, 12H), 1.20 (t, J = 6.6 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 194.8, 153.9, 133.9, 132.0, 119.4, 114.9, 86.9, 47.3, 27.7, 15.4.

Probe 1: To a stirred solution of **S1** (20 mg, 0.07 mmol) and malononitrile (4.79 mg, 0.072 mmol) in ethanol (5 mL) was added piperidine (0.04 mL) under nitrogen. The reaction mixture was allowed to stir at room temperature for 1 h. The reaction process was monitored by TLC. After the reaction was completed, The solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography (DCM/PE 1:1) to afford probe **1** as a orange solid (21.7 mg, 93%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.61 (s, 1H), 8.42 (d, J = 9 Hz, 1H), 7.17 (d, J = 2.4 Hz, 1H), 6.77 (dd, J_1 = 9.6 Hz, J_2 = 3 Hz, 1H), 3.49 (q, J = 7.2 Hz, 4H), 1.36 (s, 12H), 1.24 (t, J = 7.2 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 161.8, 153.7, 133.8, 126.2, 121.9, 119.4, 118.3, 116.1, 87.4, 75.0, 47.5, 27.7, 15.4. ESI-MS: Calcd 351.2227, Found 351.2230.

Product 5: Probe **1** (100 mg, 0.28 mmol) were dissolved in the mixture of methanol (15 mL) and H_2O (5 mL), the solution of $ONOO^-$ (60 mM, 5mL) was added. The reaction mixture was allowed to stir at room temperature under nitrogen for 30min. The reaction was then diluted with dichloromethane, and washed three times with

brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography (silica gel, DCM/EA 4:1) to afford compound **5** as a yellow solid (46 mg, 68%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.54 (s, 1H), 7.12 (d, J = 9 Hz, 1H), 6.45 (dd, J_1 = 9 Hz, J_2 = 2.4 Hz, 1H), 6.29 (d, J = 2.4 Hz, 1H), 3.41 (q, J = 7.2 Hz, 4H), 1.21 (t, J = 7.2 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 159.5, 155.5, 148.0, 133.0, 119.0, 109.3, 100.0, 98.1, 47.9, 15.3. ESI-MS: Calcd 242.1287, Found 242.1287.

3. Preparation of the test solution

Deionized water and spectroscopic grade MeOH were used for spectroscopic studies. Peroxynitrite ($ONOO^-$) solution was synthesized as reported,[1] and its concentration was estimated by using an extinction coefficient of 1670 $M^{-1}cm^{-1}$ at 302 nm [C_{ONOO^-} = $Abs_{302\ nm}$/1.67 (mM)]. The NO solution in de-ionized water was prepared by bubbling NO gas into a saturated NaOH solution, and then into deoxygenated deionized water for 30 min. The concentration of the resulting NO stock solution was determined to be 1.8 mM by Griess method.[2] Superoxide solution (O_2^-) was prepared by adding KO_2 (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min. Hydroxyl radical (OH•) was generated *in situ* by the Fenton reaction. Singlet oxygen ($^1O_2$) was generated from HClO and H_2O_2. H_2O_2 solution was prepared by dilution of commercial H_2O_2 solution in deionized water. The aqueous solutions of $NaNO_2$ and NaClO were freshly prepared and used as NO_2^- and ClO^- sources, respectively. For spectra studies, various analytes, represented by NO, H_2O_2, NO_2^-, ClO^-, O_2^-, $^1O_2$, OH•, $ONOO^-$, were added to the solution of probe **1** (5 μM) in Tris-HCl buffer (100 mM, pH 7.4, containing 20% methanol). The fluorescence spectra were recorded in an indicated reaction time.

4. Cell culture and fluorescence imaging

Raw 264.7 macrophage cell line was provided by Institute of Biotechnology, Shanxi University, China. Cells were grown in Dulbecco's modification of Eagle's medium

(DMEM) supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in humidified environment of 5% CO_2. Grow Raw 264.7 macrophage cells in the exponential phase of growth on glass-bottom cell culture dishes (Φ 15 mm) for 1 day. The fluorescence images were acquired through Olympus FluoView™ FV1000 confocal microscope.

Imaging exogenously added $ONOO^-$ in Raw 264.7 macrophage cells. Before the experiments, Raw 264.7 macrophage cells were washed with PBS 3 times. Then, the cells were incubated with **1** (10 μM) for 20 min in DMEM medium at 37 °C. After washed with PBS 3 times, the cells were treated with the $ONOO^-$ donor SIN-1 (1 mM) for 30 min in DMEM medium at 37 °C.

Imaging endogenously generated $ONOO^-$ in Raw 264.7 macrophage cells. Before the experiments, the cells were washed with PBS 3 times. The cells were pretreated with 1 μg/mL LPS and 50 ng/mL INF-γ for 12 h in DMEM medium at 37 °C, and after washed with PBS 3 times, the cells were incubated with probe **1** (10 μM) for 20 min at 37 °C in the same medium. For inhibition assays, the cells were activated with LPS (1 μg/mL) and INF-γ (50 ng/mL) in the presence of AG (5 mM) or TEMPO (300 μM) for 12 h, and then loaded with 10 μM probe **1** for 20 min. Emission was collected at 467–600 nm for green channel (excited at 458 nm).

5. Supplementary Data

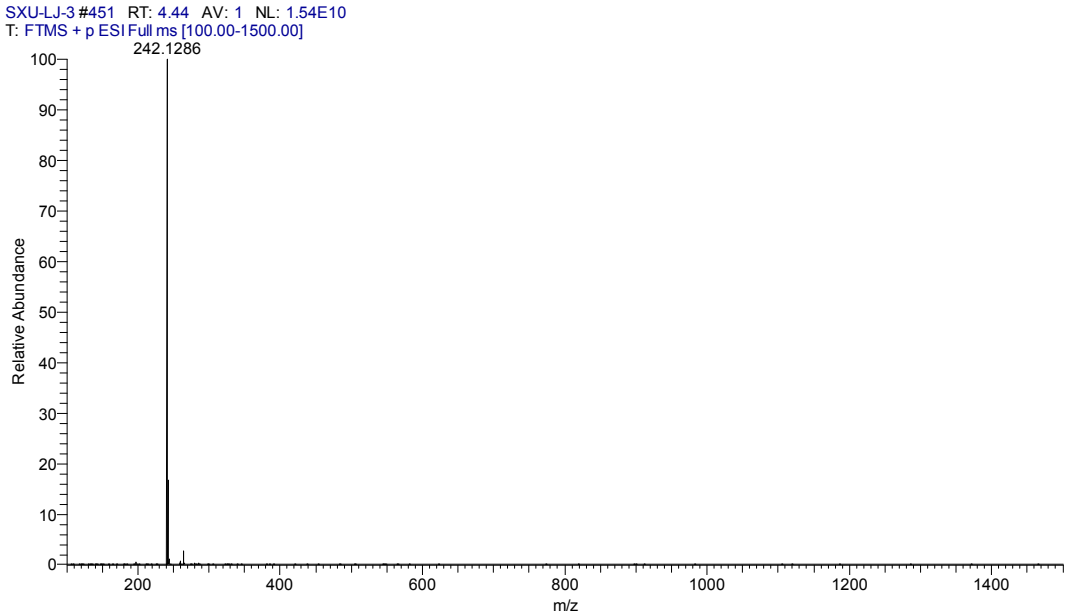


Figure S1. HRMS chart of probe **1** treated with $ONOO^-$.

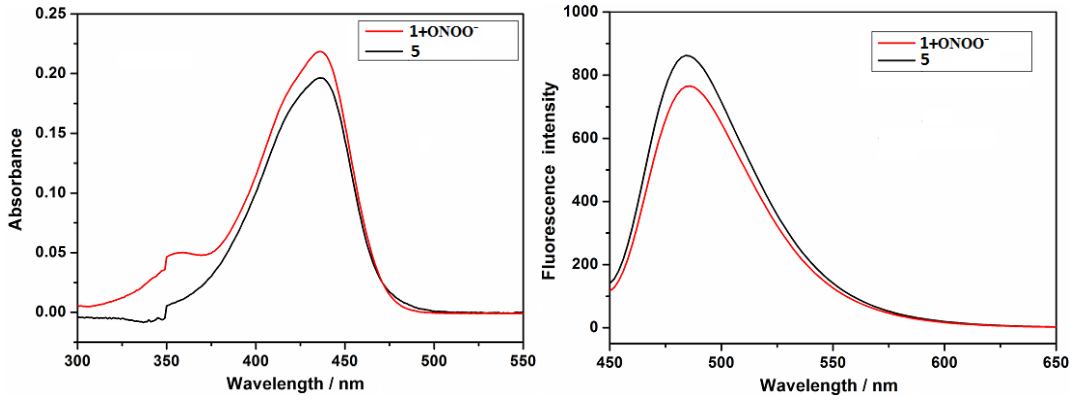


Figure S2. Absorption (Left) and fluorescence (Right) spectra of **1** (5 μM) treated with 10 equiv of $ONOO^-$ *vs* those of product **5**. Conditions: Tris-HCl buffer (100 mM, pH 7.4, containing 20% methanol); λ_{ex} = 436 nm; λ_{em} = 486 nm; Slits: 5/10 nm; voltage: 500 V; T: 25 °C.

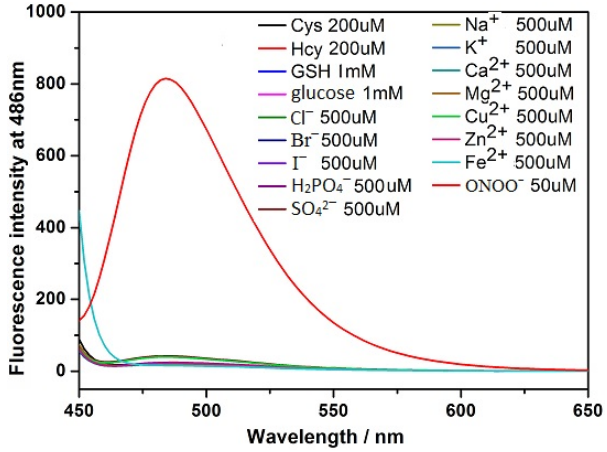


Figure S3. The fluorescence spectra changes of **1** (5 μM) treated with various species, including anions, cations, biothiols, glucose, and $ONOO^-$. Conditions: Tris-HCl buffer (100 mM, pH 7.4, containing 20% methanol); λ_{ex} = 436 nm; λ_{em} = 486 nm; Slits: 5/10 nm; voltage: 500 V; T: 25 °C.

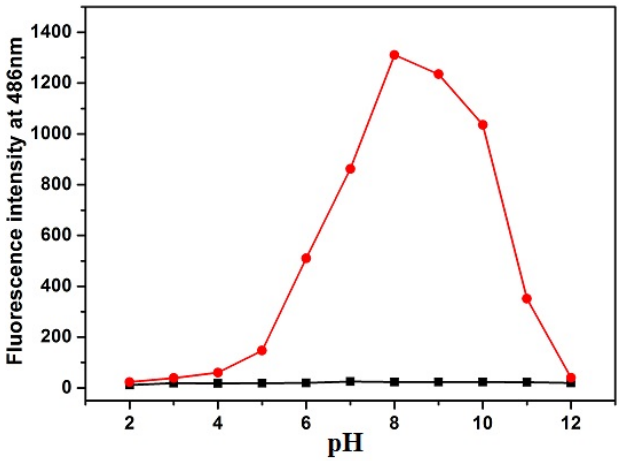


Figure S4. The fluorescence intensities at 486 nm of **1** (5 μM) in the absence and presence of $ONOO^-$ (10 equiv) at varied pH values.

6. ^{1}H NMR, ^{13}C NMR, and HRMS charts

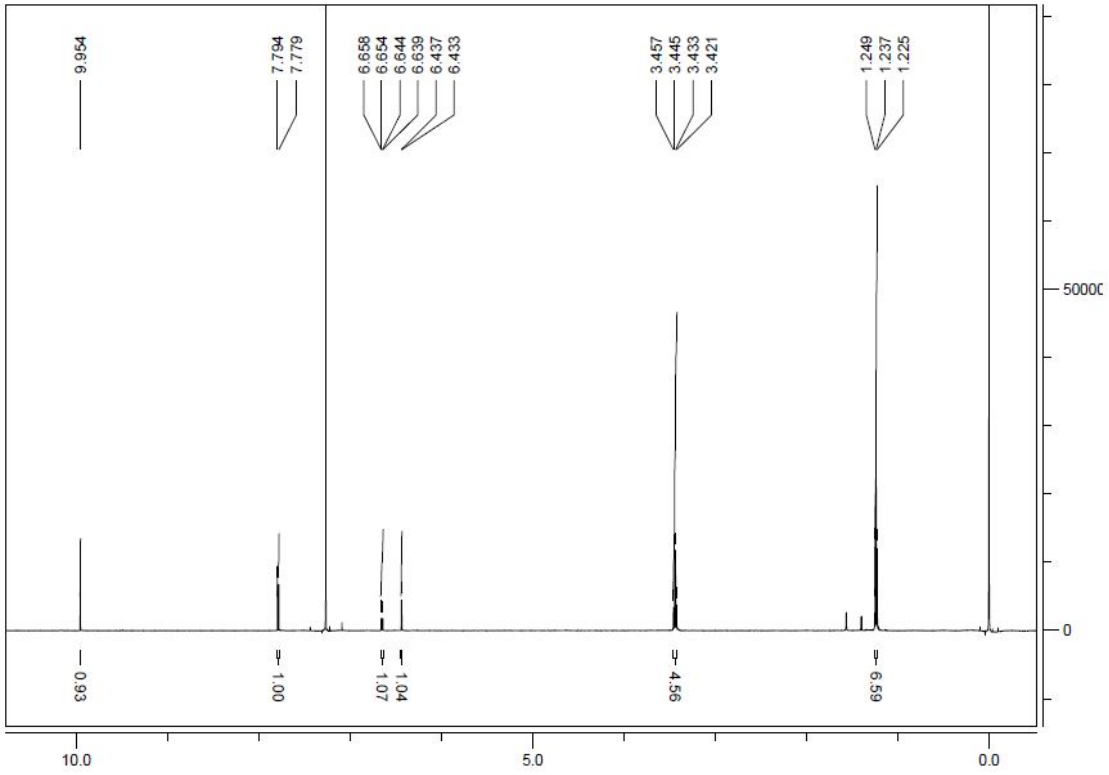


Figure S5 ^{1}H NMR chart of compound **S2** (600 MHz, $CDCl_3$).

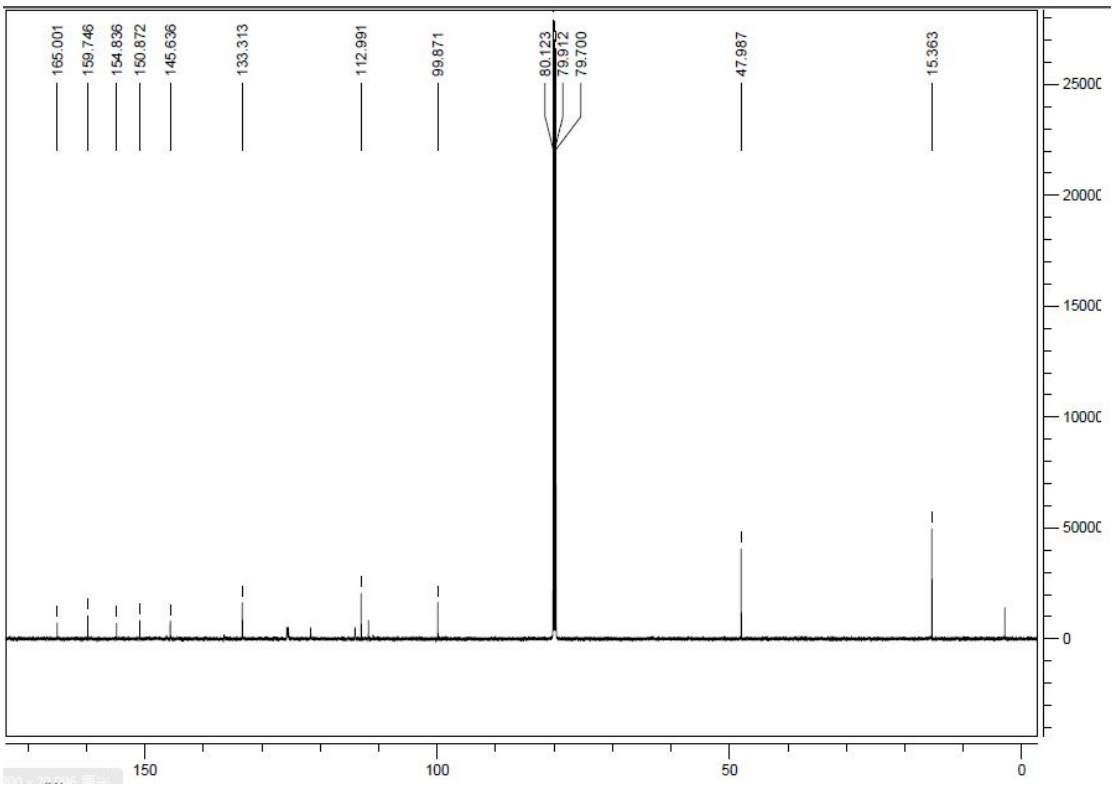


Figure S6 ^{13}C NMR chart of compound **S2** (150MHz, $CDCl_3$).

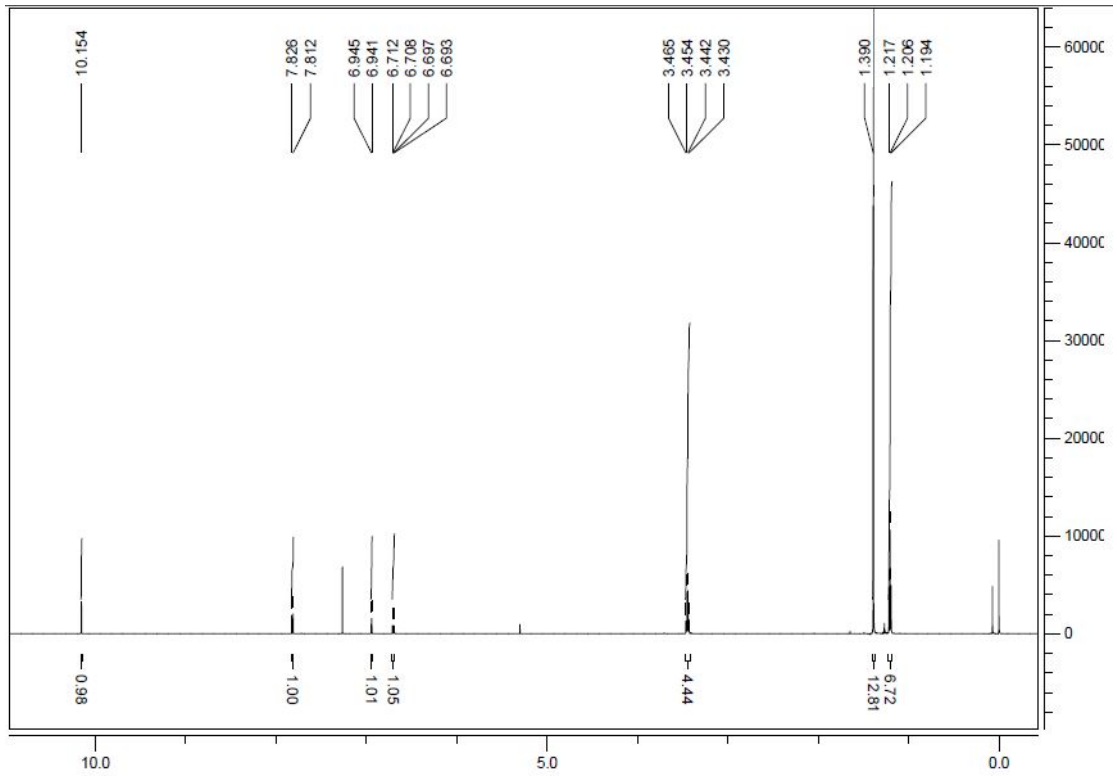


Figure S7 ^{1}H NMR chart of compound **S1** (600 MHz, $CDCl_3$).

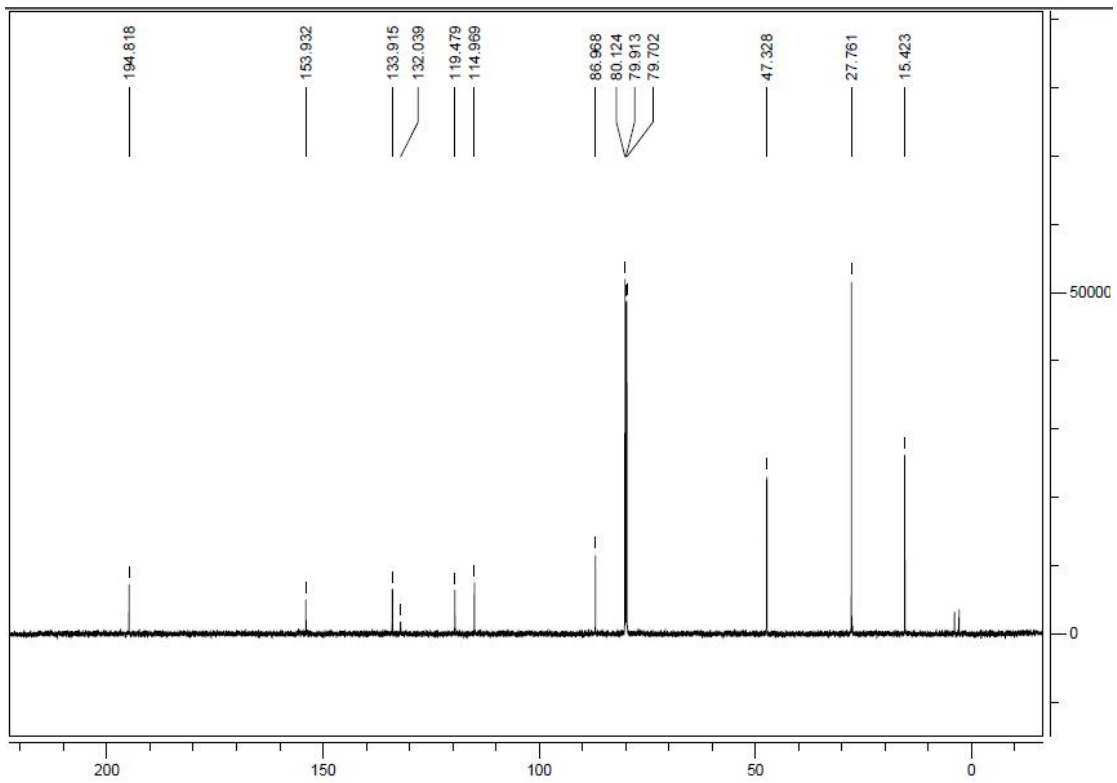


Figure S8 ^{13}C NMR chart of compound **S1** (150MHz, $CDCl_3$).

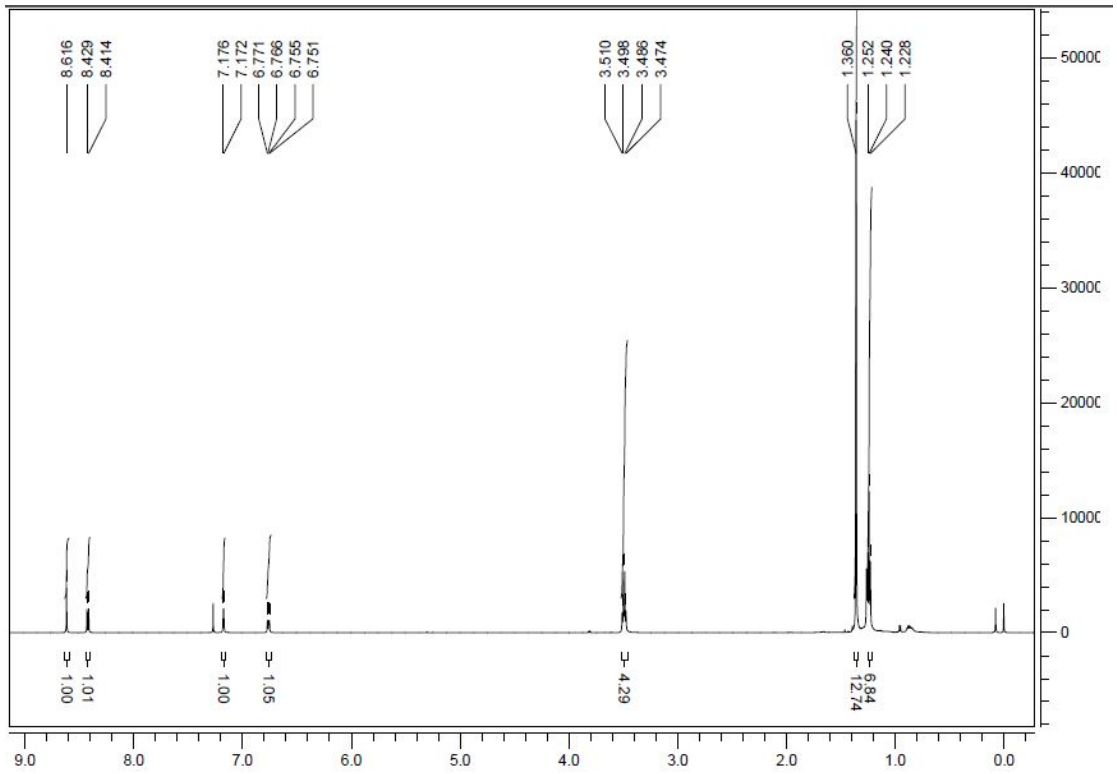


Figure S9 ^{1}H NMR chart of probe **1** (600 MHz, $CDCl_3$).

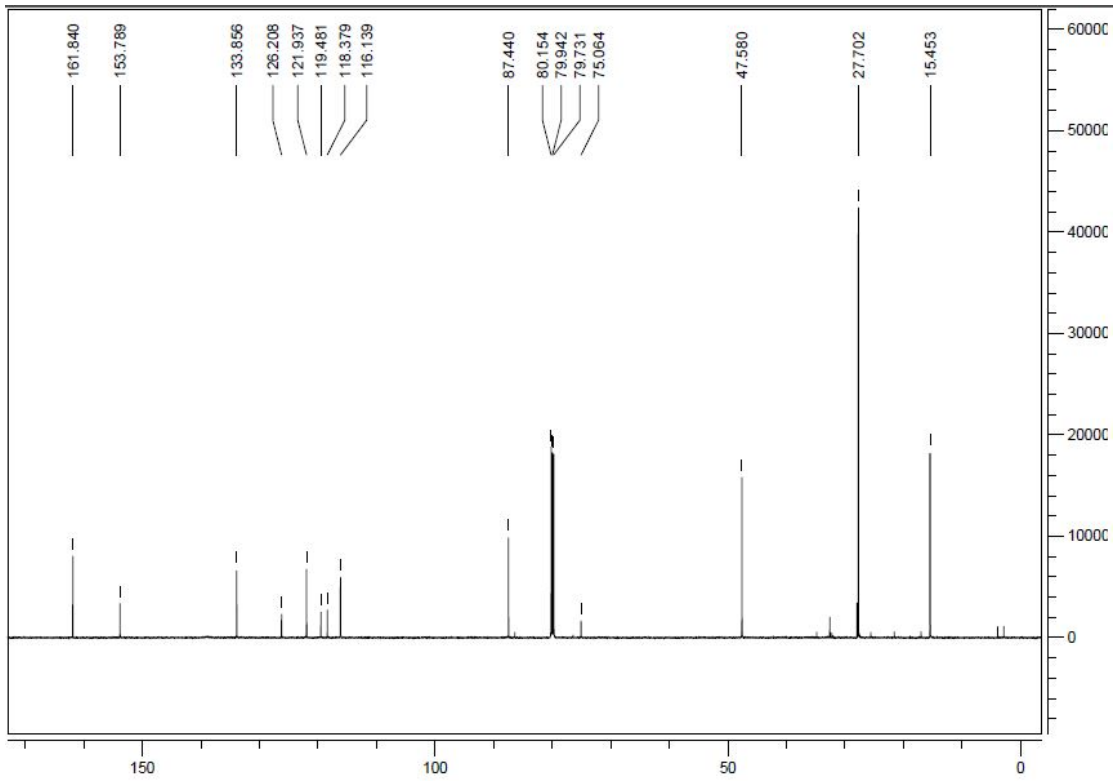


Figure S10 ^{13}C NMR chart of probe **1** (150MHz, $CDCl_3$).

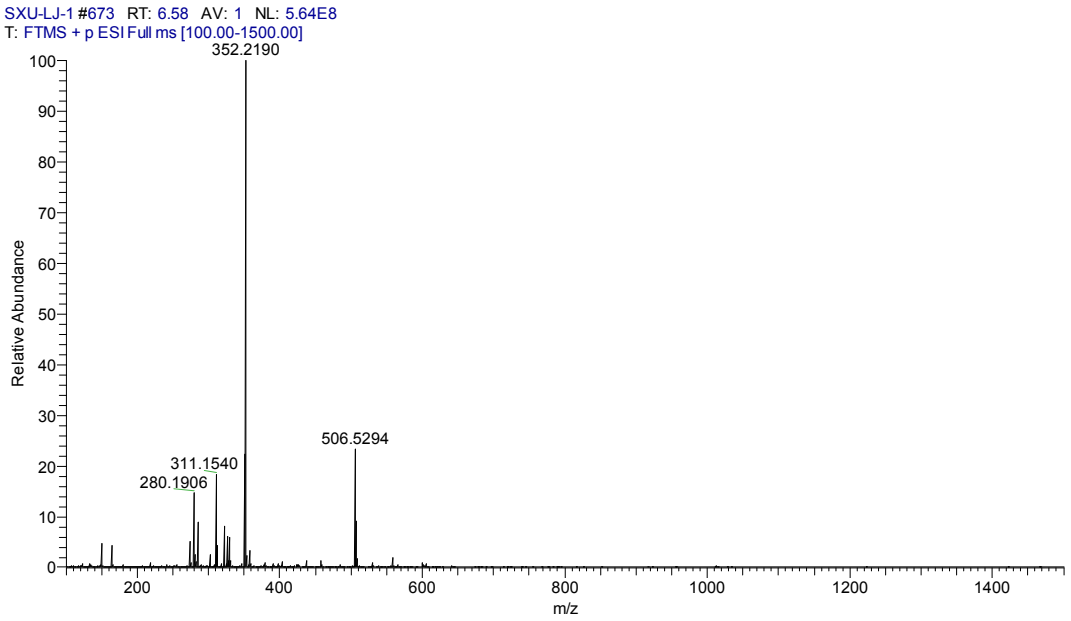


Figure S11 HRMS chart of probe **1**.

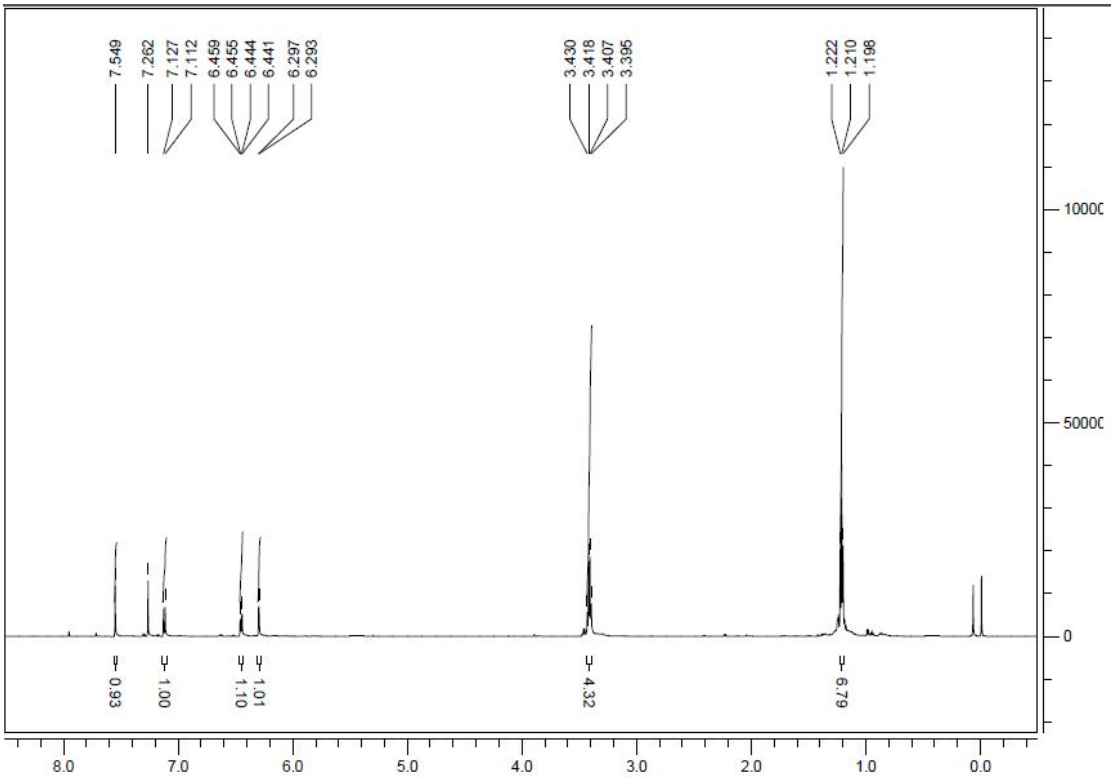


Figure S12 ^{1}H NMR chart of aminocoumarin **5** (600 MHz, $CDCl_3$).

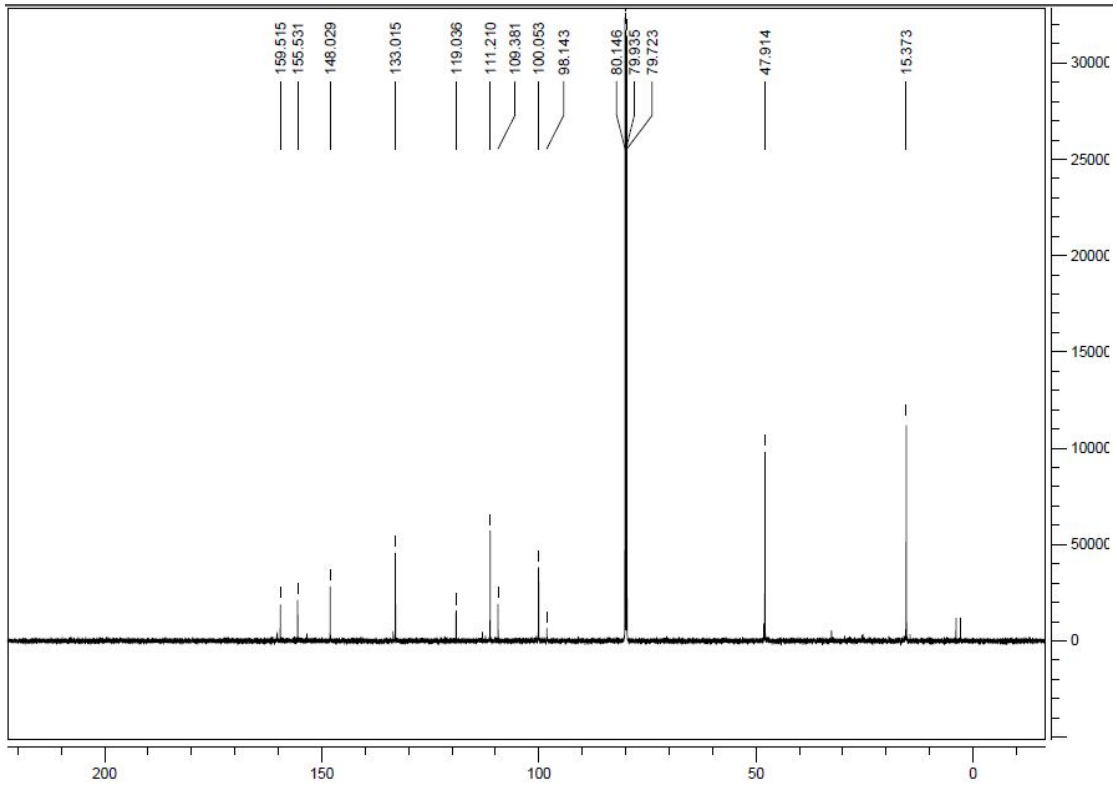


Figure S13 ^{13}C NMR chart of aminocoumarin **5** (150MHz, $CDCl_3$).

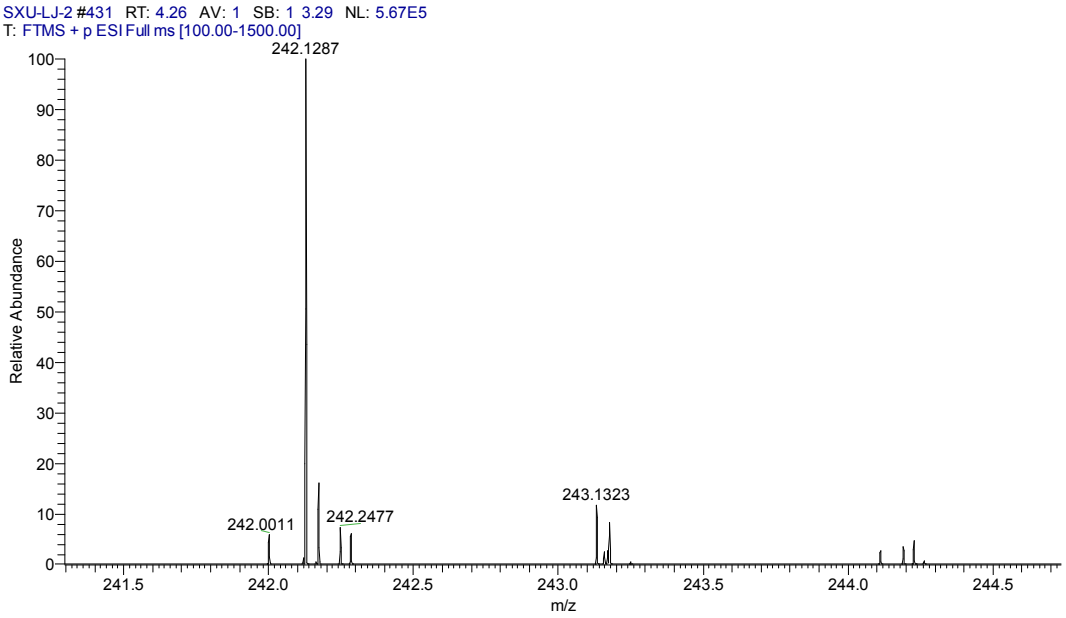


Figure S14 HRMS chart of aminocoumarin **5**.

7. References

(1) Uppu, R. M.; Pryor, W. A. *Anal. Biochem.* **1996**, *236*, 242.

(2) Yu, H.; Zhang, X.; Xiao, Y.; Zou, W.; Wang, L.; Jin, L. *Anal. Chem.* **2013**, *85*, 7076.