Aromatization of 9, 10-Dihydroacridine Derivatives: Discovering a Highly Selective and Rapid-Responding Fluorescent Probe for Peroxynitrite

Zhiheng Li,[†] Rui Liu,[†] Zhengli Tan,[†] Lan He,[§] Zhonglin Lu,[†*] and Bing Gong[†¶*]

[†] College of Chemistry, Beijing Normal University, Beijing 100875, China

[¶] Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York 14260, United States

[§] National Institute for Food and Drug Control, Beijing 100050, China

Supporting Information

Contents

General Experimental Section

Materials

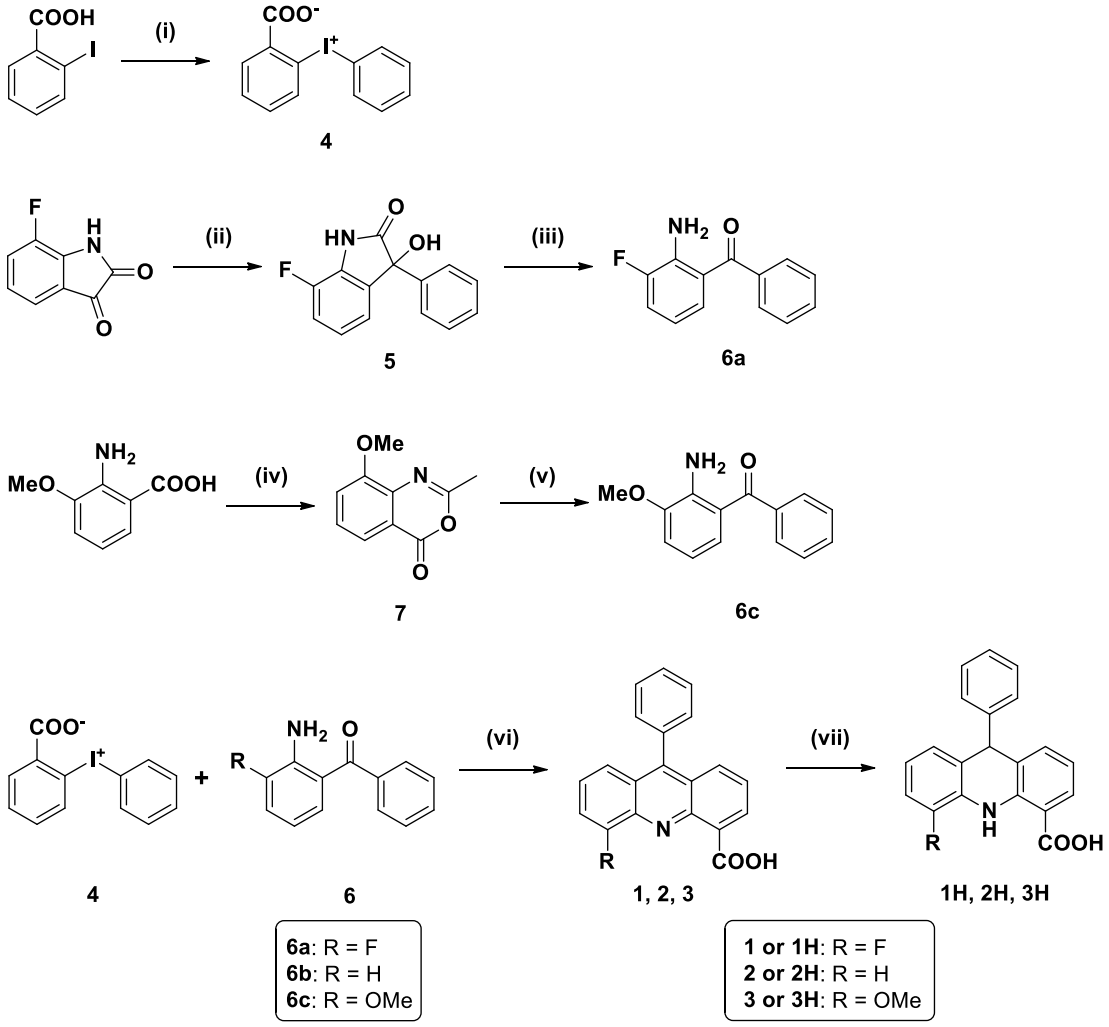
Probe **2H** was maintained in a refrigerator at 4 °C, and a 2 mM solution of probe **2H** (6.04 mg, 0.02 mmol) was prepared in 10 mL of DMSO and stored in a refrigerator at 4 °C as the stock solution. Peroxynitrite ($ONOO^-$) was prepared following the reported method[1] and assayed with UV spectral using ε_{302} = 1670 $cm^{-1}M$. The nitric oxide (NO) stock solution in de-ionized water was prepared according to the method mentioned in the literature.[2] Superoxide ($\bullet O_2^-$) was prepared by stirring KO_2 (1 mg) in dry DMSO (1 mL) for 10 min. Hydroxyl radicals (•OH) was generated *in situ* by addition of Fe^{2+} into a solution containing 10 equiv. of H_2O_2 through Fenton reaction. Singlet oxygen ($^1O_2$) was generated *in situ* by addition of NaClO into a solution containing 10 equiv. of H_2O_2. Other reactive oxidative species (ROS), such as $NaNO_2$ and $NaNO_3$, were prepared by dilution of commercial suppliers in deionized water. All other chemicals were purchased from commercial suppliers and used without further purification, unless indicated otherwise. Deionized water was used throughout all experiments.

Instruments

^1H NMR and ^{13}C NMR spectra were recorded on a 400 MHz Bruker Arance III spectrometer. Mass spectra were obtained from a Water LCT Premier XE spectrometer. Fluorescence spectra were recorded on a Cary Eclipse Fluorescence spectrophotometer. Absorption spectra were recorded on a Cary-50 UV-Vis spectrophotometer. Fluorescence quantum yield were obtained from a Fluormax-4 spectrophotometer with an integrating sphere system. Melting points were obtained from an X-5B precise micro melting point apparatus.

Synthesis and Characterization

Synthetic Routes



Scheme S1. Strategy and Synthesis the target compounds**.** Notes: (i) $K_2S_2O_8$, 98% H_2SO_4, benzene, NaOH, (80%); (ii) C_6H_5BrMg, THF, (85%); (iii) $K_4Fe(CN)_6$, NaOH, DMF, (66%); (iv) $(Ac)_2O$, (100%); (v) 1) C_6H_5BrMg, THF; 2) HCl, AcOH, $NaHCO_3$, (40%); (vi) $Cu(OAc)_2$, DMF, AcOH, 98% H_2SO_4, (**1**: 18%, **2**: 19%, **3**: 30%); (vii) $NaBH_4$, EtOH, (**1H**: 90%, **2H**: 95%, **3H**: 95%).

Synthetic Procedures

2-(phenyliodonio)benzoate (4). Potassium persulfate (27.1 g, 100 mmol) was added into a solution of 2-iodobenzoic acid (12.4 g, 50 mmol) in 50 mL of 98% H_2SO_4 and

the reaction mixture was stirred for 30 min at 0 °C. Then benzene (17.8 mL, 200 mmol) was added and stirred acutely for 5 h at room temperature. The reaction mixture was poured onto 200 g of ice, adjusted to pH>10 with 5 M NaOH and extracted with 300 mL of DCM. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuum to obtain product **4** as a white solid (13.0 g, 80% yield). M.p. 229~230 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ8.42 (d, *J* = 6.4 Hz, 1H), 8.00 (d, *J* = 7.0 Hz, 2H), 7.76 (t, *J* = 7.5 Hz, 1H), 7.57 (dt, *J* = 14.8, 7.5 Hz, 3H), 7.37 (d, *J* = 7.1 Hz, 1H), 6.74 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-*d*6) δ165.84, 137.26, 134.49, 133.52, 132.19, 131.57, 130.31, 127.38, 116.86, 115.97; ESI-MS: m/z $C_{13}H_{10}IO_2$ $[M+H]^+$, calcd. 324.98, found 324.98.

7-fluoro-3-hydroxy-3-phenylindolin-2-one (5). Compound **5** was synthesized according to the reported procedure[3]. Yellow solid, 85% yield. ^{1}H NMR (400 MHz, Acetone-d_6) δ9.84 (s, 1H), 7.49 – 7.43 (m, 2H), 7.41 – 7.11 (m, 4H), 7.06 (q, *J* = 3.2 Hz, 2H), 5.74 (s, 1H).

(2-amino-3-fluorophenyl)(phenyl)methanone (6a). Compound **6a** was synthesized according to the reported procedure[3]. Yellow solid, 66% yield. ^{1}H NMR (400 MHz, CD_2Cl_2) δ7.67 (d, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 7.4 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.20 (dd, *J* = 11.5, 7.8 Hz, 1H), 6.59 (td, *J* = 7.9, 5.1 Hz, 1H).

8-methoxy-2-methyl-4H-benzo[d][1,3]oxazin-4-one (7). Compound **7** was synthesized according to the reported procedure[3]. White solid, 100% yield. ^{1}H NMR (400 MHz, Acetone-d_6) δ7.65 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.54 – 7.40 (m, 2H), 3.97 (s, 3H), 2.43 (s, 3H).

(2-amino-3-methoxyphenyl)(phenyl)methanone (6c). Compound **6c** was synthesized according to the reported procedure[3]. White solid, 40% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ7.68 – 7.61 (m, 2H), 7.55 – 7.48 (m, 1H), 7.48 – 7.41 (m, 2H), 7.08 (dd, *J* = 8.2, 1.3 Hz, 1H), 6.89 (dd, *J* = 7.9, 1.3 Hz, 1H), 6.55 (t, *J* = 8.0 Hz, 1H), 3.91 (s, 3H).

5-fluoro-9-phenylacridine-4-carboxylic acid (1). A mixture of compound **6a** (6.5 g, 30 mmol), compound **4** (10.7 g, 33 mmol), and cupric acetate (0.6 g, 3 mmol) in 30

mL of DMF was heated to 120 °C under nitrogen atmosphere and stirred for 5 h. The reaction mixture was concentrated in vacuum, and the residue was dissolved in 30 mL of AcOH with 10 mL of 98% H_2SO_4. The reaction mixture was heated to 100 °C and stirred for 1 h, cooled, and poured into 200 mL of ice-cold water, filtered and 30 mL of pyridine was added into the filtrate. The precipitate was further purified by column chromatography with PE/ EtOAc (3:1, v/ v) as the eluent to obtain product **1** as a yellow solid (1.7 g, 18% yield). M.p. 250~252 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ8.99 (dd, *J* = 7.0, 1.3 Hz, 1H), 8.01 (dd, *J* = 8.8, 1.4 Hz, 1H), 7.76 – 7.40 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ166.93, 157.29, 154.71, 150.82, 145.15, 137.08, 136.69, 136.56, 134.02, 132.08, 129.79, 129.08, 128.51, 126.17, 126.02, 125.75, 125.68, 125.22, 124.06, 122.72, 122.67, 114.61, 114.44. ESI-HRMS: m/z $C_{20}H_{13}FNO_2$ $[M+H]^+$, calcd. 318.0924, found 318.0919.

9-phenylacridine-4-carboxylic acid (2). Compound **2** was synthesized according to the above procedure, from compound **6b**. Yellow solid, 1.6 g, 19% yield. M.p. 184~186 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ8.77 (dd, *J* = 7.0, 1.5 Hz, 1H), 8.42 – 8.35 (m, 1H), 8.11 – 8.02 (m, 1H), 7.95 (dd, *J* = 8.7, 1.5 Hz, 1H), 7.82 – 7.67 (m, 6H), 7.60 – 7.51 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ166.57, 151.15, 145.40, 145.24, 136.50, 134.24, 133.27, 132.36, 130.23, 129.35, 128.89, 127.58, 127.53, 127.00, 126.18, 124.76, 124.74, 123.83; ESI-HRMS: m/z $C_{20}H_{14}NO_2$ $[M+H]^+$, calcd. 300.1019, found 300.1021.

5-methoxy-9-phenylacridine-4-carboxylic acid (3). Compound **3** was synthesized according to the above procedure, from compound **6c**. M.p. 292~294 °C; Yellow solid, 2.9 g, 30% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ8.82 (dd, *J* = 7.0, 1.4 Hz, 1H), 7.87 (dd, *J* = 8.8, 1.5 Hz, 1H), 7.62 – 7.49 (m, 4H), 7.43 – 7.32 (m, 3H), 7.23 (d, *J* = 8.8 Hz, 1H), 7.09 (d, *J* = 7.5 Hz, 1H), 4.10 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ167.63, 153.36, 150.37, 143.88, 138.05, 136.16, 134.51, 131.73, 131.62, 129.80, 128.81, 128.37, 126.62, 125.80, 125.77, 125.02, 123.90, 118.12, 108.17, 56.06.

5-fluoro-9-phenyl-9,10-dihydroacridine-4-carboxylic acid (1H). Compound **1** (635 mg, 2 mmol) was dissolved in 10 mL of ethanol, and sodium borohydride (152 mg, 4 mmol) was added. The reaction mixture was kept at room temperature and stirred for

1 h under nitrogen atmosphere. 0.5 mL of 2 M HCl was added, then the reaction mixture was poured into 50 mL of water and followed by adding 0.5 mL of pyridine to neutralize the residual HCl. Filtered and the precipitate was further purified by recrystallized from acetone/ n-Hexane to obtain product **1H** as a light yellow solid (575 mg, 90% yield). M.p. 259~261 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ10.48 (s, 1H), 7.96 (dd, J = 7.9, 1.5 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.27 (d, J = 4.3 Hz, 4H), 7.18 (h, J = 4.3 Hz, 1H), 7.12 – 6.83 (m, 4H), 5.53 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ170.09, 151.17, 148.79, 147.28, 140.64, 135.00, 130.11, 128.83, 127.14, 126.57, 126.27, 126.15, 125.78, 124.80, 124.76, 121.14, 121.07, 119.95, 113.36, 113.18, 112.04, 45.79; ESI-HRMS: m/z $C_{20}H_{13}FNO_2$ $[M-H]^-$, calcd. 318.0935, found 318.0930.

9-phenyl-9,10-dihydroacridine-4-carboxylic acid (2H). Compound **2H** was synthesized according to the above procedure, from compound **2**. Light yellow solid, 572 mg, 95% yield. M.p. 232~234 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ10.17 (s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 7.4 Hz, 1H), 7.28 – 7.06 (m, 7H), 7.01 (d, J = 8.1 Hz, 1H), 6.85 (dt, J = 12.5, 7.5 Hz, 2H), 5.41 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ169.99, 147.64, 141.69, 137.59, 134.69, 129.92, 129.16, 128.72, 127.65, 127.10, 126.37, 125.02, 123.24, 121.61, 119.16, 115.18, 111.49, 46.25; ESI-HRMS: m/z $C_{20}H_{16}NO_2$ $[M+H]^+$, calcd. 302.1175, found 302.1179.

5-methoxy-9-phenyl-9,10-dihydroacridine-4-carboxylic acid (3H). Compound **3H** was synthesized according to the above procedure, from compound **3**. Light yellow solid, 630 mg, 95% yield. M.p. 248~250 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ10.36 (s, 1H), 7.82 (dd, J = 7.9, 1.6 Hz, 1H), 7.31 (dt, J = 7.5, 1.0 Hz, 1H), 7.22 – 7.07 (m, 4H), 7.03 (ddd, J = 8.6, 5.2, 3.2 Hz, 1H), 6.82 – 6.70 (m, 3H), 6.67 (dd, J = 7.1, 1.9 Hz, 1H), 5.35 (s, 1H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, Acetone-d_6) δ168.83, 168.78, 147.67, 146.16, 141.55, 134.68, 129.75, 128.16, 127.23, 126.93, 125.92, 124.85, 123.28, 120.64, 118.43, 110.73, 108.19, 55.05, 46.69; ESI-HRMS: m/z $C_{21}H_{16}NO_3$ $[M-H]^-$, calcd. 330.1135, found 330.1133.

Fluorescence Analysis

The solutions of probe **2H** were prepared by diluting the stock solution to certain volumes using PBS buffer (50 mM, pH 7.4). All the fluorescence spectral properties of the probe were obtained with 1.0-cm quartz cuvettes. The slit width was 5 nm for both excitation and emission. The photon multiplier voltage was 600 V. Test temperature was 25 °C. Every fluorescent titration experiment was performed at least three times for calculating error bars.

$ONOO^-$ solutions were quickly added to a 2 μM **2H** solution with vigorously stirring with various final concentrations. The volume change must be less than one percent.

The fluorescence spectra the 2 μM **2H** solutions with $ONOO^-$ (2 μM, 1 equiv.) or other ROS (20 μM, 10 equiv.), including singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hypochlorite (ClO^-), nitrite (NO_2^-), nitrate (NO_3^-), nitric oxide (NO), hydroxyl radicals (•OH) and superoxide ($•O_2^-$) were measured using the same conditions as described above.

Calculation of detection limit

The detection limit was calculated according to the method mentioned in the literature.[4] The fluorescence emission spectrum of probe **2H** was determined by three times. The fluorescence intensity was plotted as a function of the concentration of $ONOO^-$, and the fluorescence intensities at 496 nm was linearly proportional to the $ONOO^-$ concentration in the range of 0 - 2 μM ($R^2 = 0.999$). The detection limit was calculated according to the following formula:

$$\text{Detection limit} = 3s/k$$

Where s is the standard deviation of blank measurement, k is the slope between the fluorescence intensity at 496 nm versus $ONOO^-$ concentration.

Cell Culture

RAW264.7 cells were obtained from Cell Resource Center (IBMS, CAMS/PUMC), and incubated in DMEM medium with 10% FBS. For imaging studies, cells were seeded in glass bottom cell culture dishes (Nest) with 1 mL of DMEM medium and incubated at 37 °C under a simulated humidified atmosphere containing 5% CO_2 for 24 h.

Cell Cytotoxicity

Cell viability was examined based on the known method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, the MTT reagent. The mouse macrophage cell RAW264.7 cells were grown in 96-well plates (10^4 cell per well). Plates were maintained at 37 °C in a 5% CO_2/ 95% air for 4 h and then RAW264.7 cells were incubated for 24 h. Subsequently, the cells were treated with 2 μM, 10 μM, 20 μM, 40 μM of probe **2H** and compound **2** and were incubated for 12 h respectively. MTT solution (20 μL, 5 mg/ mL, PBS) was then added to each well and kept for another 4 h. The remaining MTT solution was removed and 100 μL of DMSO was added to each well. Absorbance at 490 nm was measured on a plate reader. The cell viabilities showed that neither probe **2H** nor compound **2** were toxic to the cells in the experimental concentrations, meaning the probe **2H** can be used in biomedical applications.

Cell Imaging Experiments

The stock solution (2 mM) of probe **2H** or compound **2** were mixed with dioleoylphosphatidyl ethanolamine (DOPE, 2 mM) to enhance the permeability of probe **2H** and compound **2** to cell membrane. The mouse macrophage cell RAW264.7 cells were divided into nine groups and cultured for 24 h before the tests. Each group was treated with the following conditions:

(A) **2** (5 μM, mixed with 5 μM of DOPE), cultured for 15 min;

(B) **2H** (5 μM, mixed with 5 μM of DOPE), cultured for 15 min;

Group C~I were first treated with different induces, then loaded with probe **2H** (5 μM, mixed with 5 μM of DOPE) and cultured for 15 min.

(C) SIN-1 (1 mM), 0.5 h;

(D) LPS (1 μg/ mL), and IFN-γ (50 ng/ mL), 4 h;

(E) LPS (1 μg/ mL), IFN-γ (50 ng/ mL) and AG (1 mM), 4 h;

(F) H_2O_2 (50 μM) for 0.5 h;

(G) NaClO (50 μM) for 0.5 h;

(H) NO (50 μM) for 0.5 h;

(I) PMA (10 nM) for 0.5 h;

Cells were washed with 1 mL of PBS for 5 times before imaging. Bright field and fluorescence images were taken with a 40× objective lens.

^{1}H, ^{13}C-NMR Spectra and HR-MS of compounds

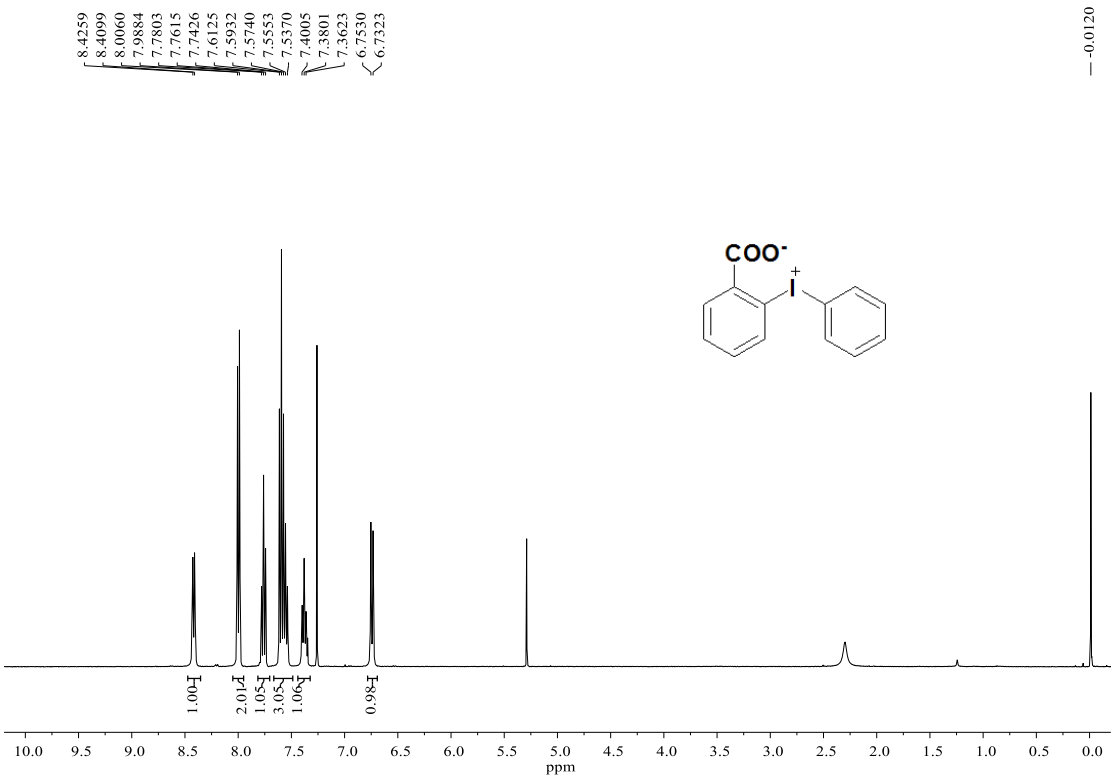


Figure S1 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **4**.

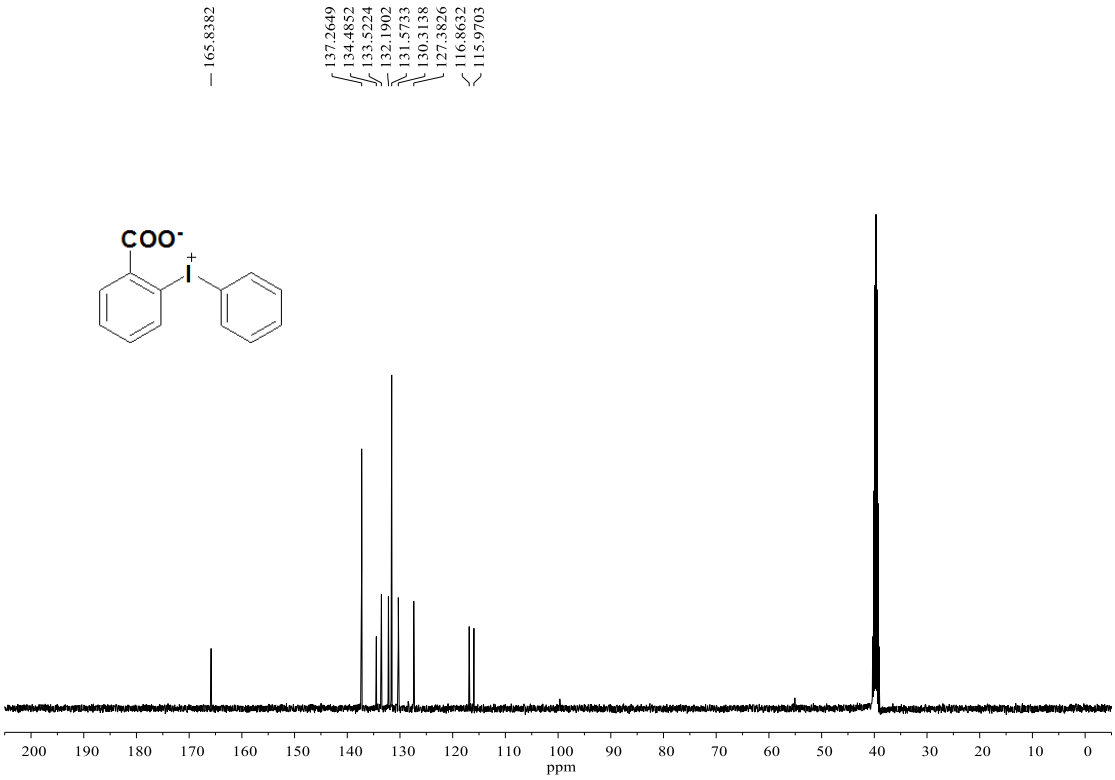


Figure S2 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of compound **4**.

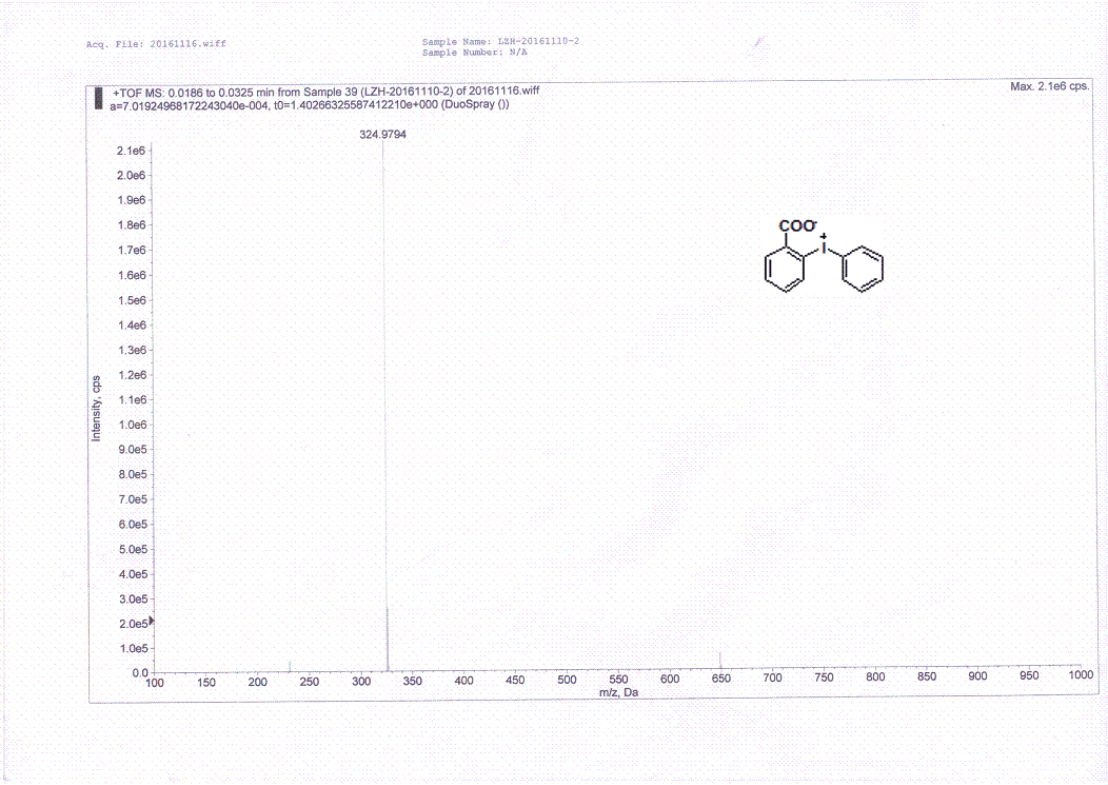


Figure S3 MS spectrum of compound **4**.

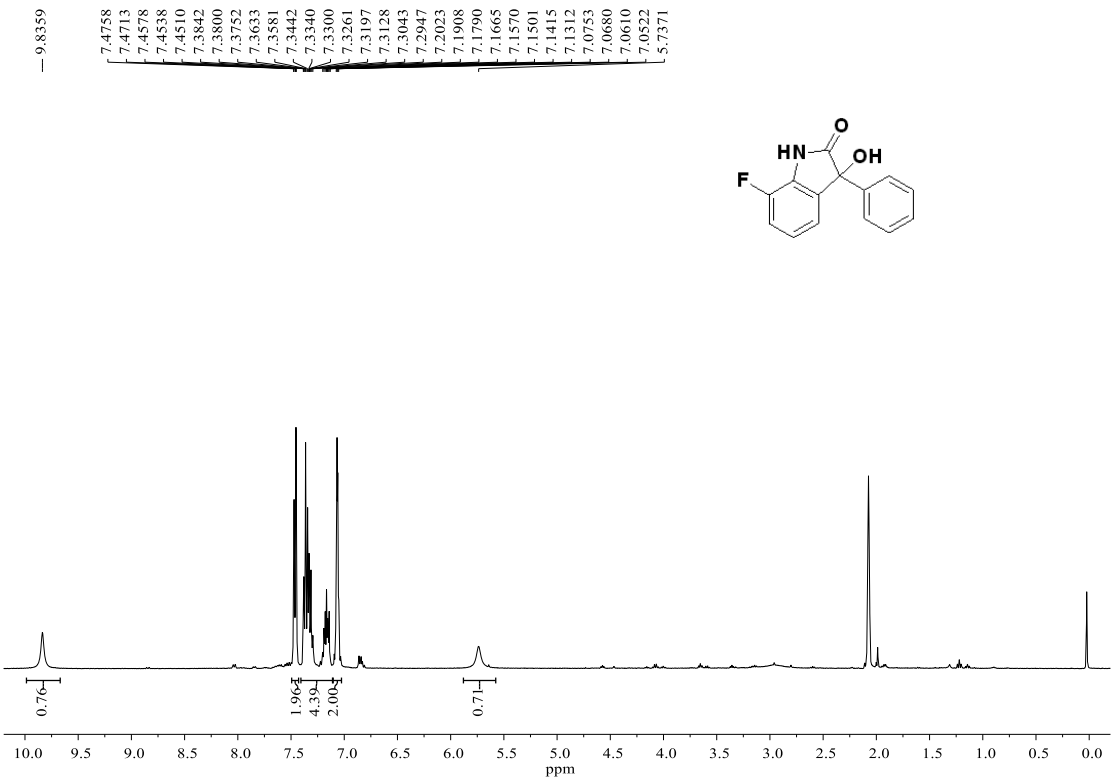


Figure S4 ^{1}H NMR spectrum (400 MHz, Acetone-d_6, 298 K) of compound **5**.

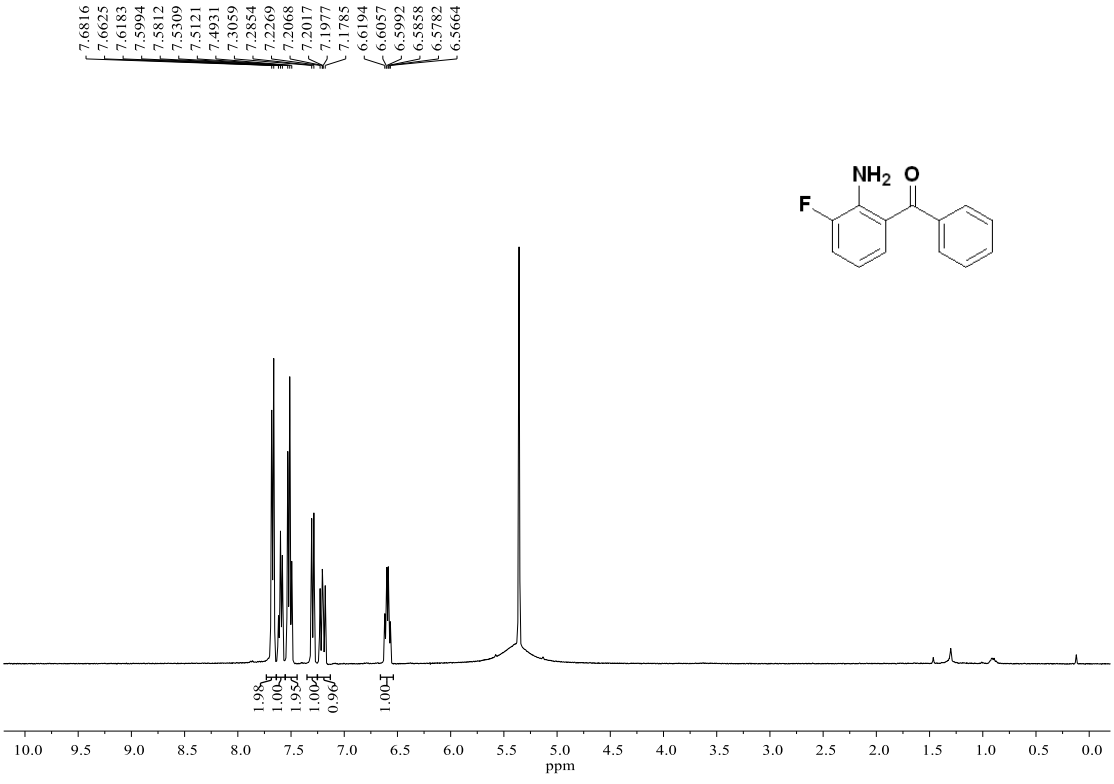


Figure S5 ^{1}H NMR spectrum (400 MHz, CD_2Cl_2, 298 K) of compound **6a**.

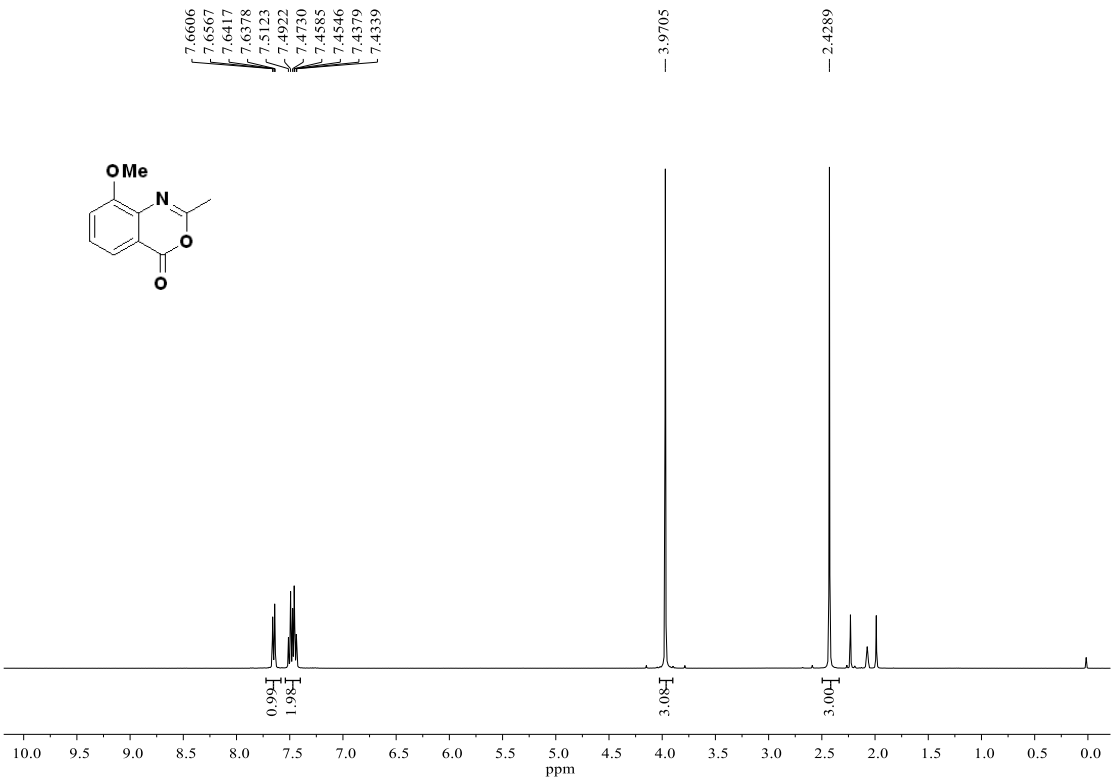


Figure S6 ^{1}H NMR spectrum (400 MHz, Acetone-d_6, 298 K) of compound **7**.

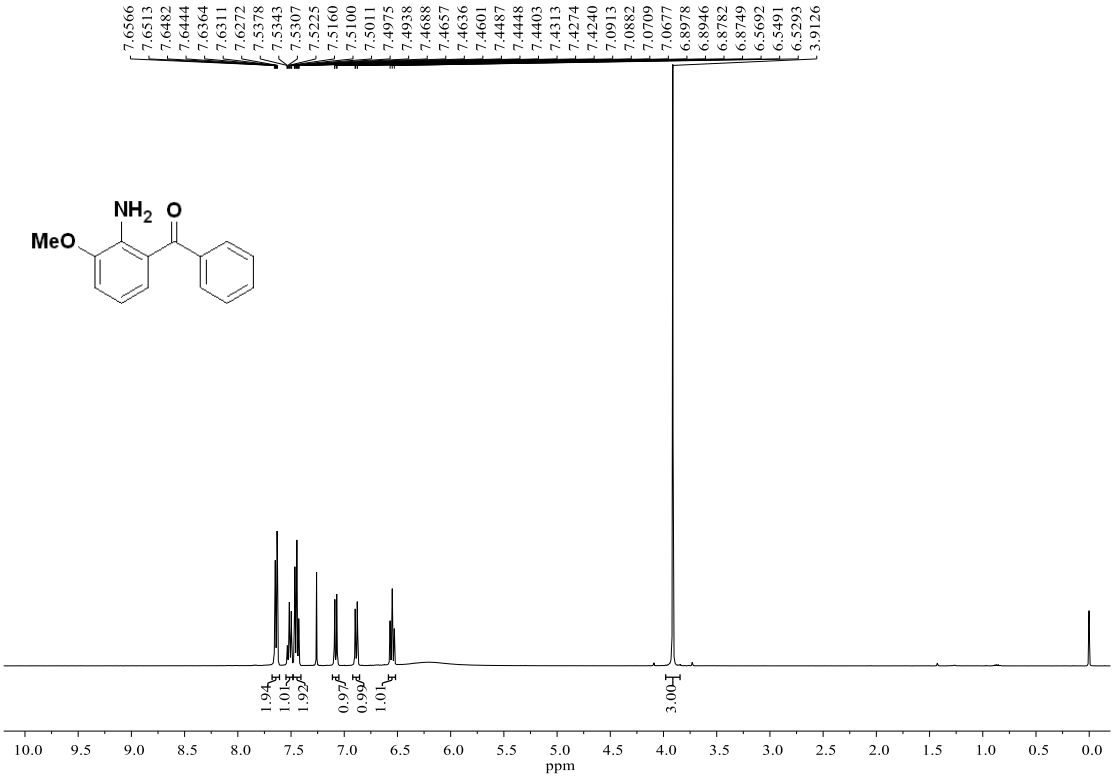


Figure S7 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **6c**.

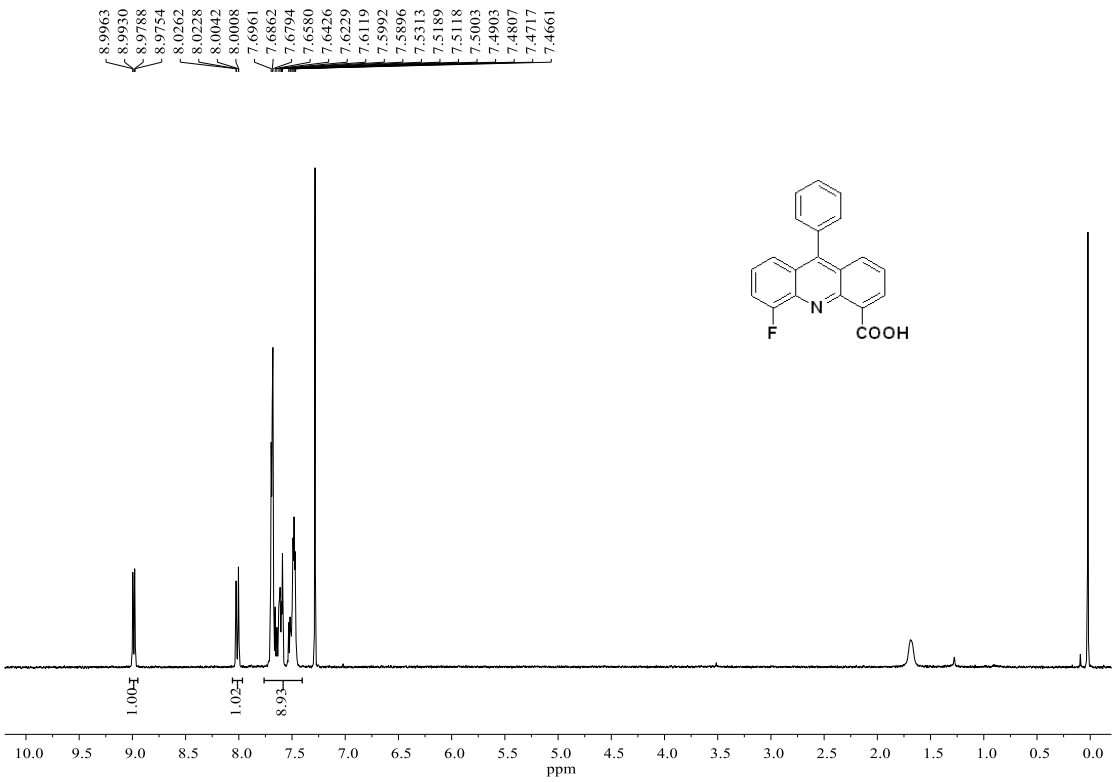


Figure S8 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **1**.

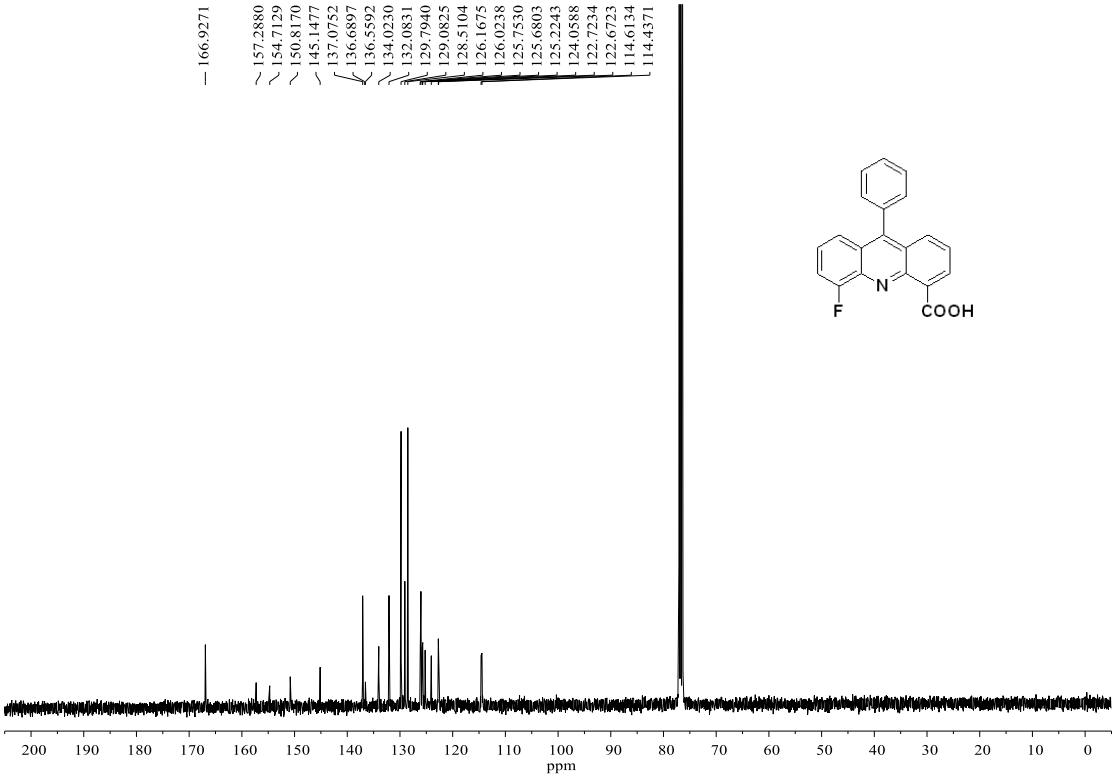
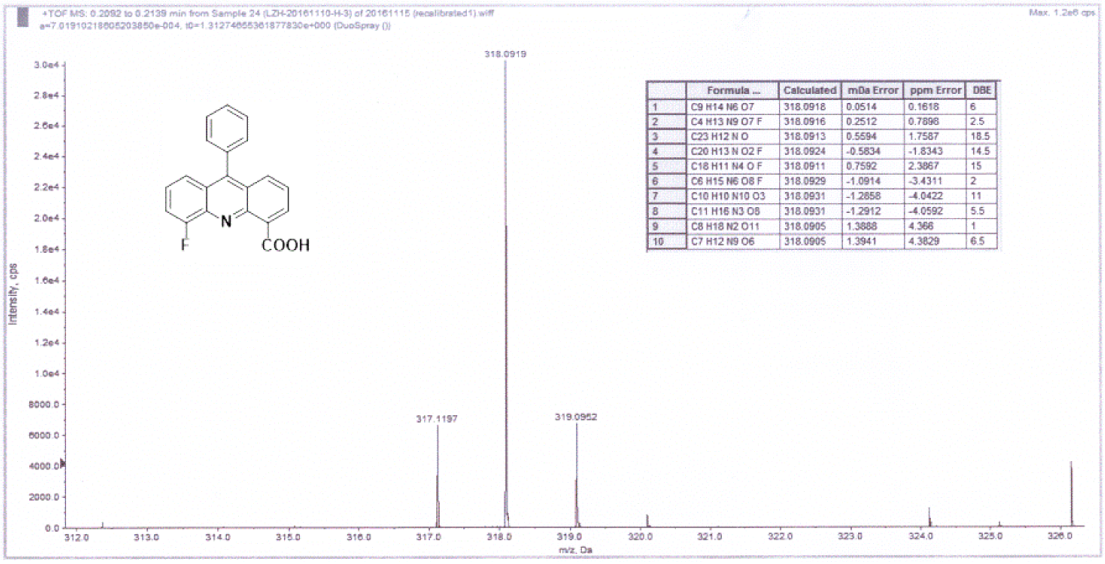


Figure S9 ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of compound **1**.



	Formula ...	Calculated	mDa Error	ppm Error	DBE
1	C9 H14 N6 O7	318.0918	0.0514	0.1618	6
2	C4 H13 N9 O7 F	318.0916	0.2512	0.7898	2.5
3	C23 H12 N O	318.0913	0.5594	1.7587	18.5
4	C20 H13 N O2 F	318.0924	-0.5834	-1.8343	14.5
5	C18 H11 N4 O F	318.0911	0.7592	2.3867	15
6	C6 H15 N6 O8 F	318.0929	-1.0914	-3.4311	2
7	C10 H10 N10 O3	318.0931	-1.2858	-4.0422	11
8	C11 H16 N3 O8	318.0931	-1.2912	-4.0592	5.5
9	C8 H18 N2 O11	318.0905	1.3888	4.366	1
10	C7 H12 N9 O6	318.0905	1.3941	4.3829	6.5

Figure S10 HRMS spectrum of compound **1**.

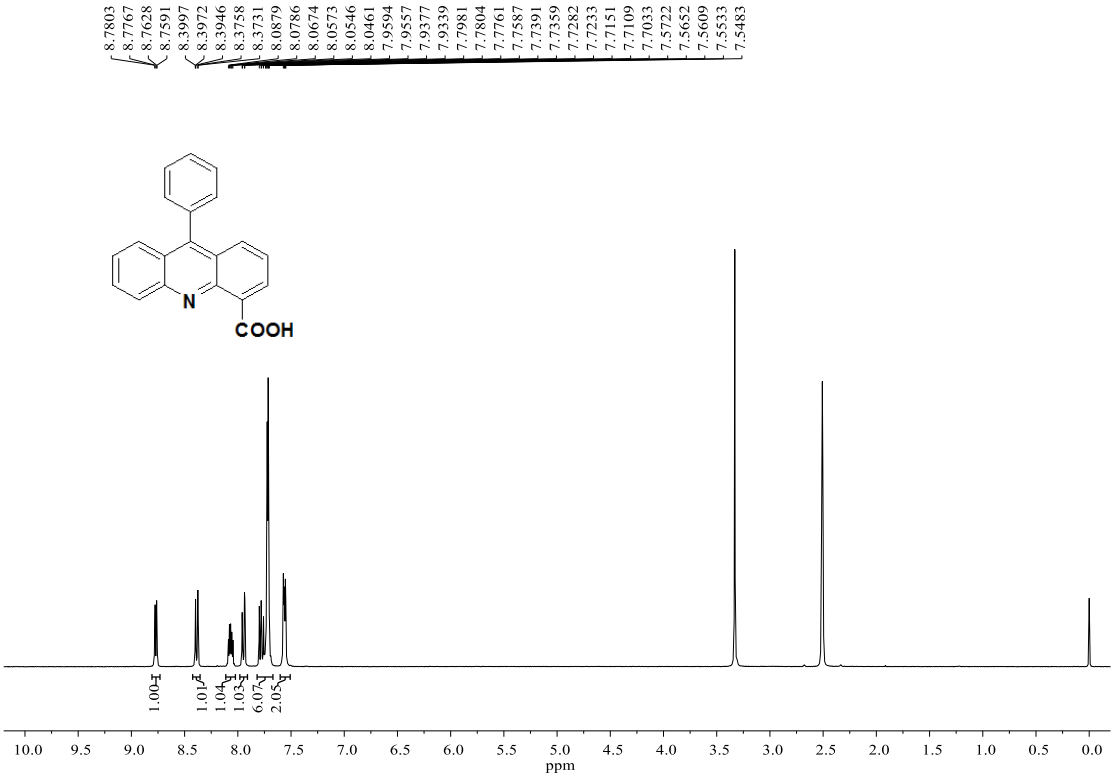


Figure S11 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of compound **2**.

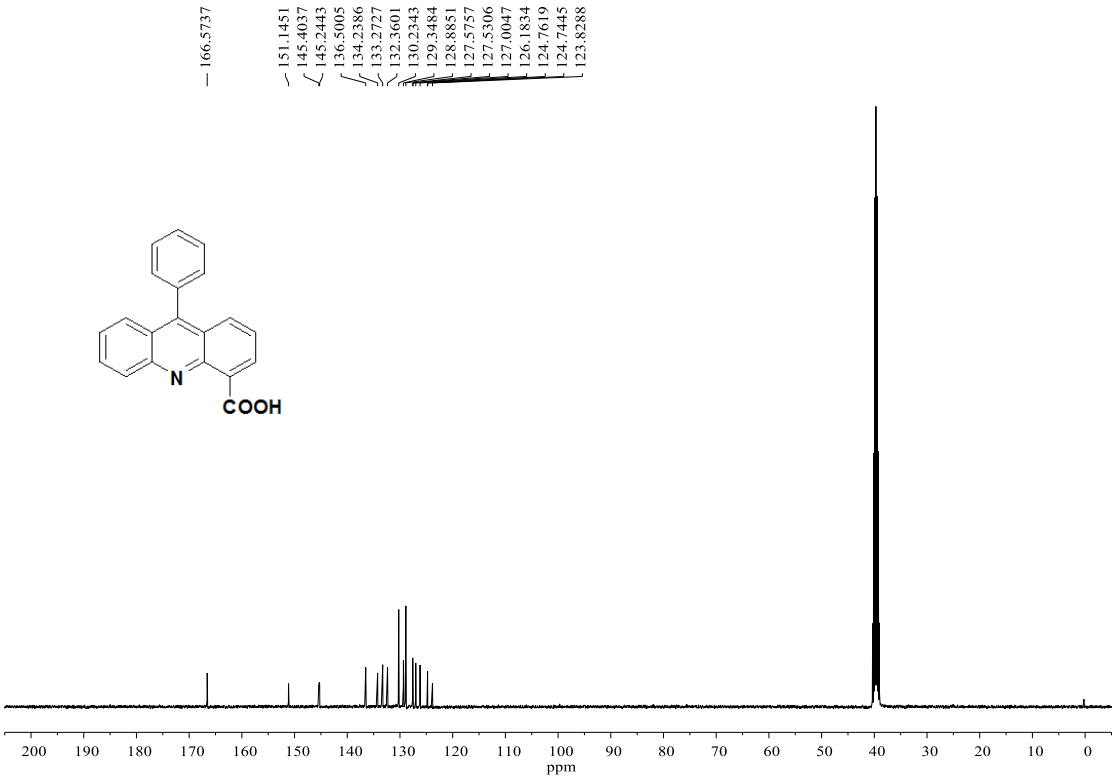


Figure S12 ^{13}C NMR spectrum (100 MHz, DMSO-d6, 298 K) of compound **2**.

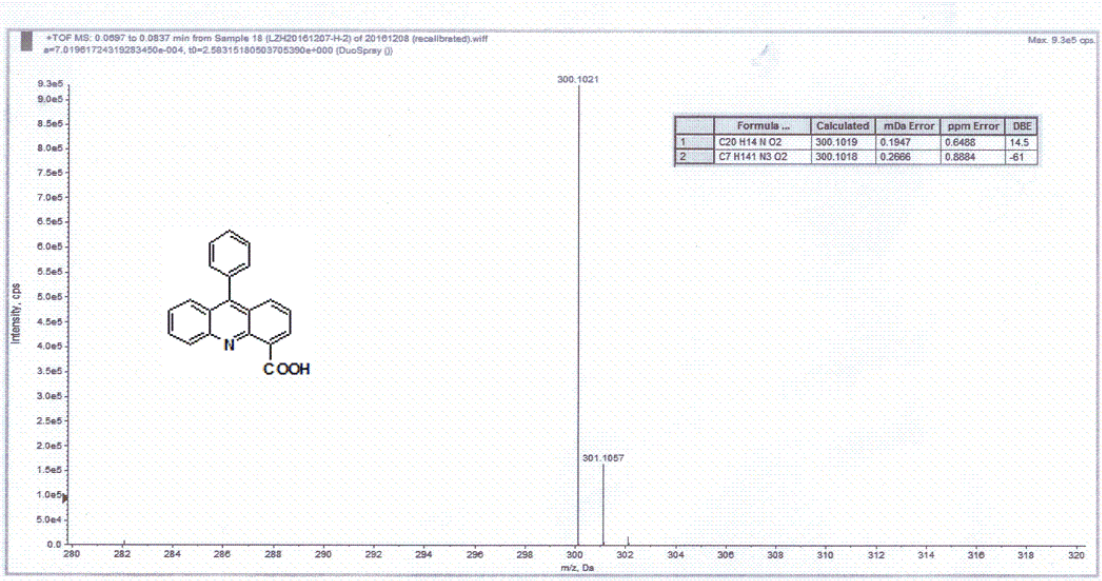


Figure S13 HRMS spectrum of compound **2**.

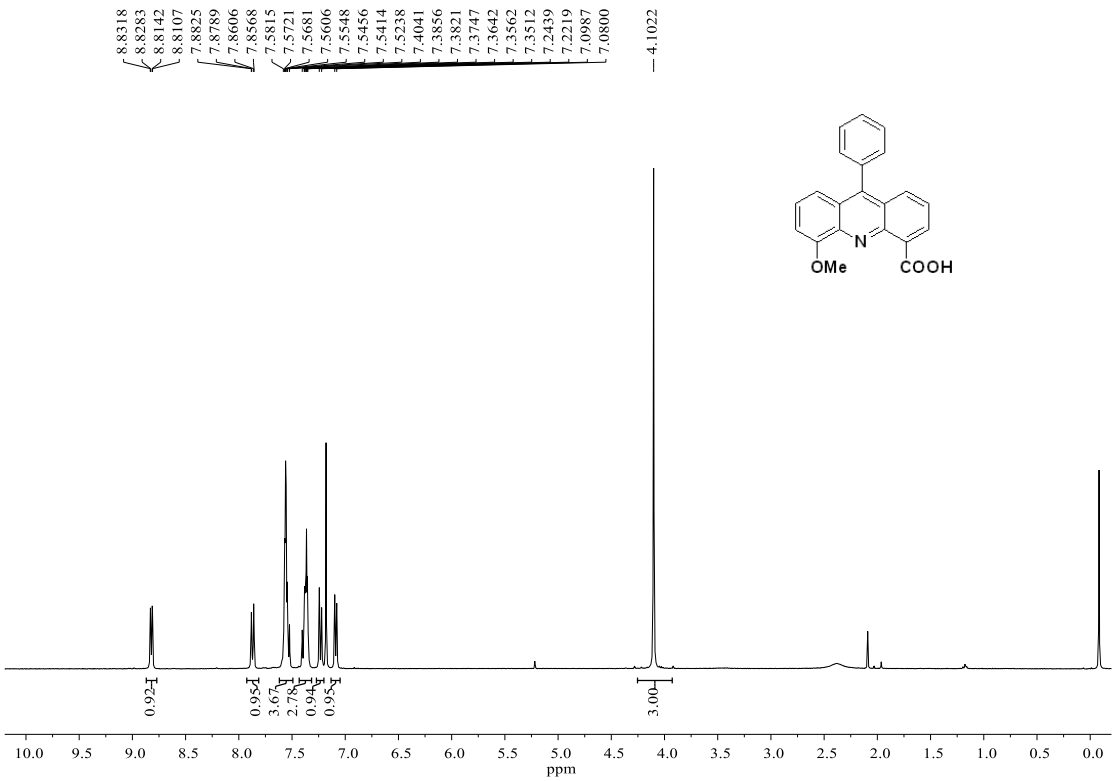


Figure S14 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of compound **3**.

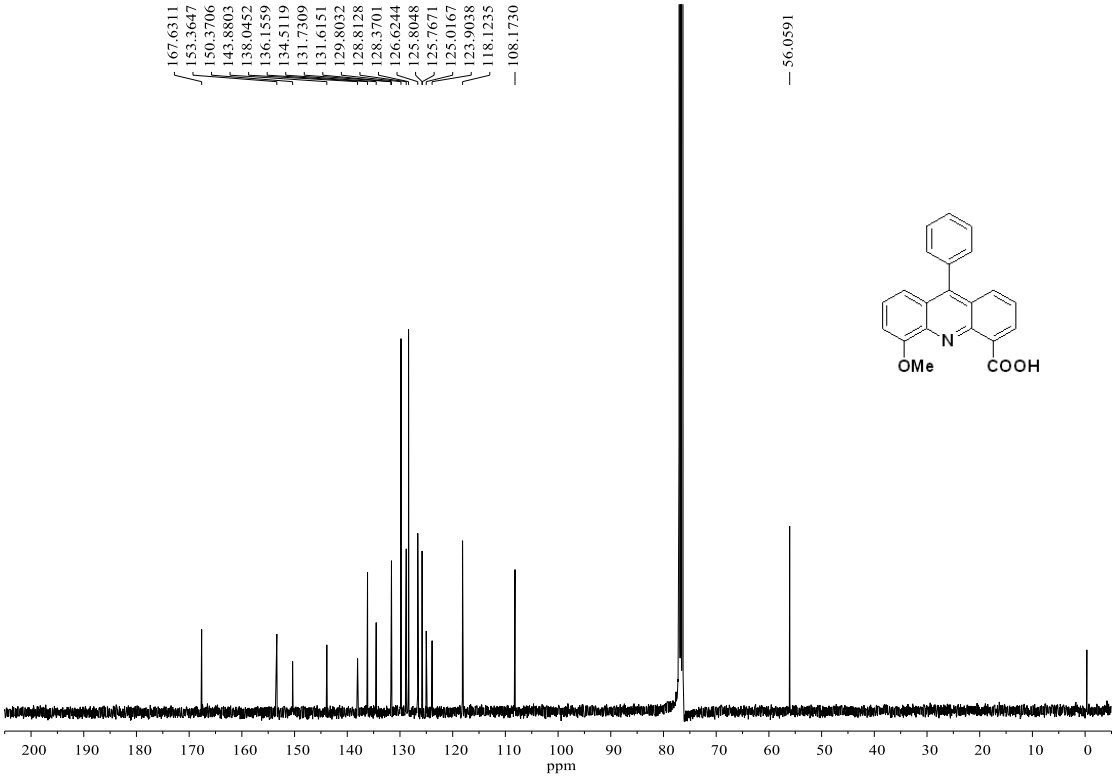


Figure S15 ^{13}C NMR spectrum (100 MHz, $CDCl_3$, 298 K) of compound **3**.

Figure S16 HRMS spectrum of compound **3**.

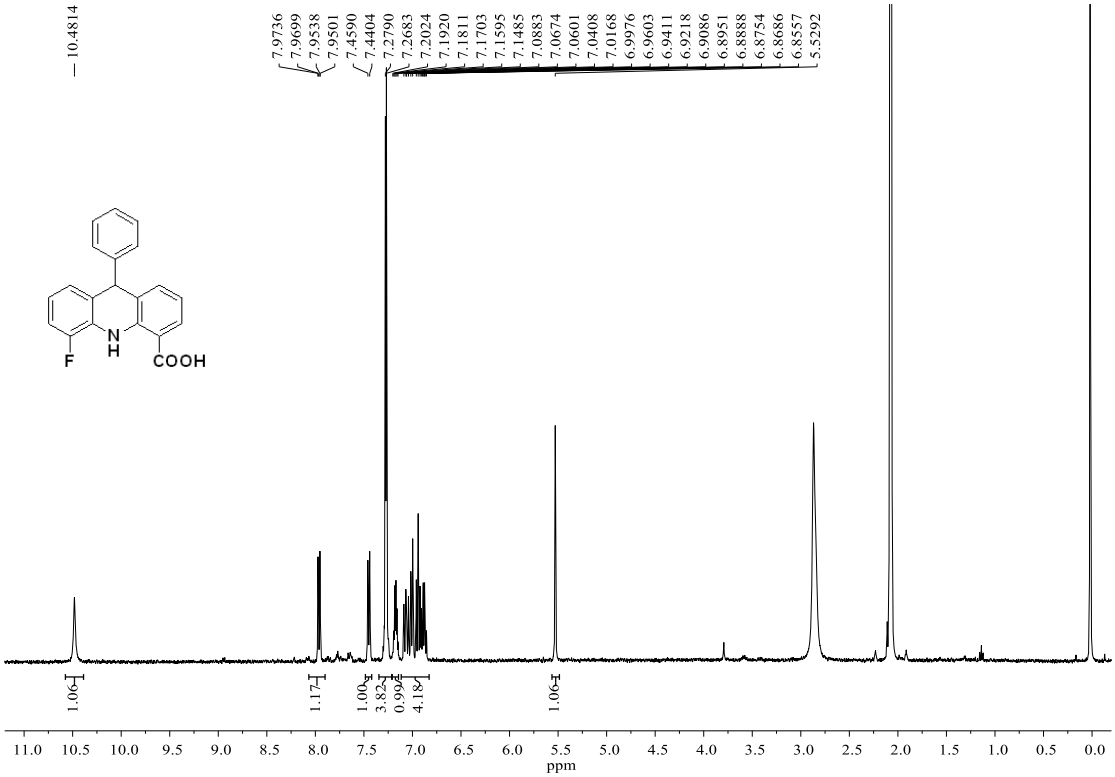


Figure S17 ^{1}H NMR spectrum (400 MHz, Acetone-d_6, 298 K) of **1H**.

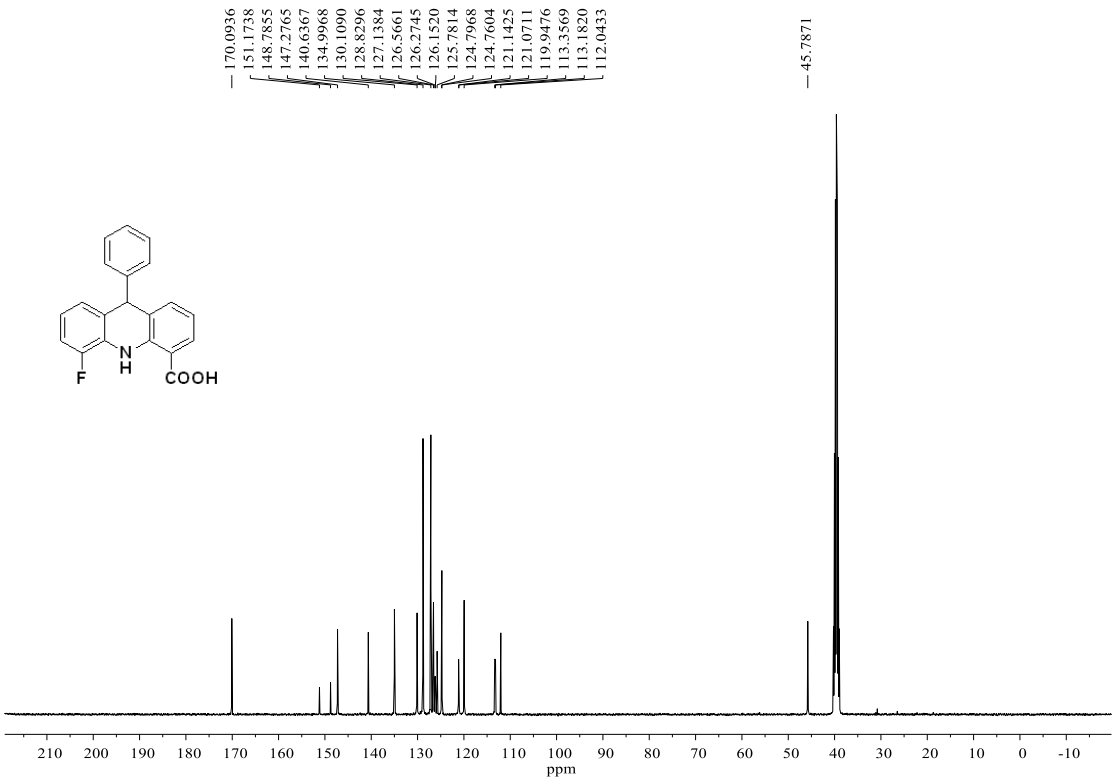


Figure S18 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of **1H**.

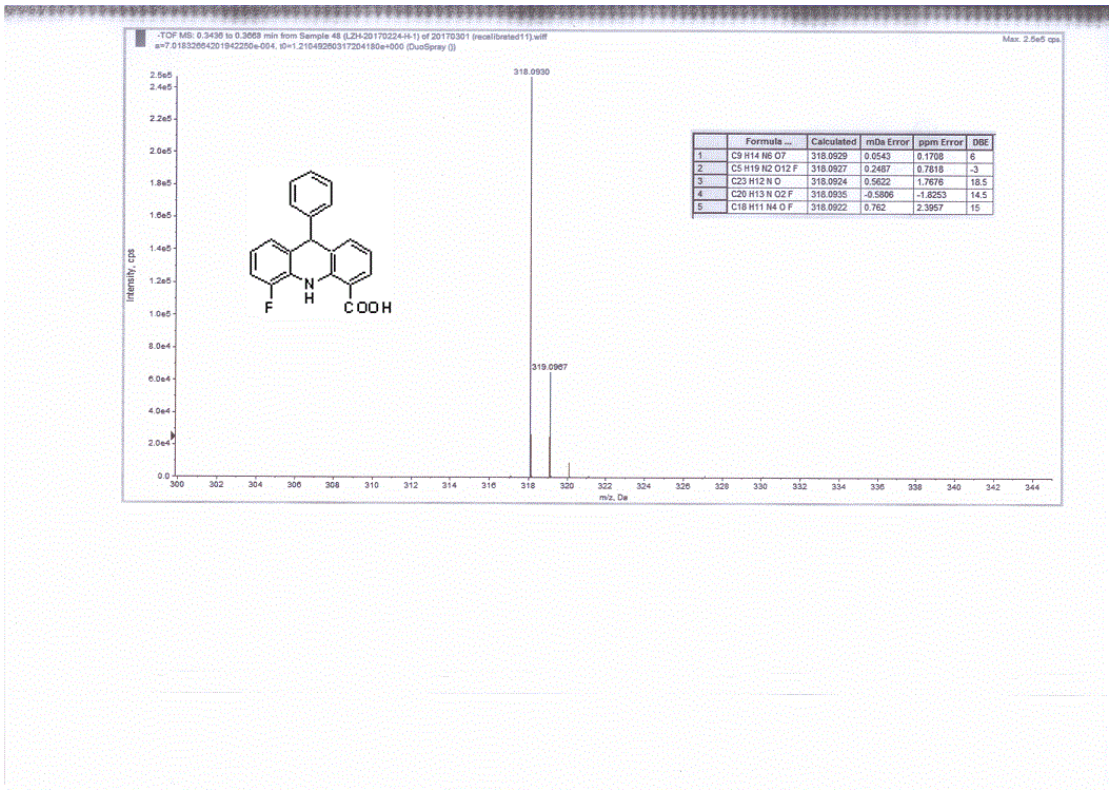


Figure S19 HRMS spectrum of compound **1H**.

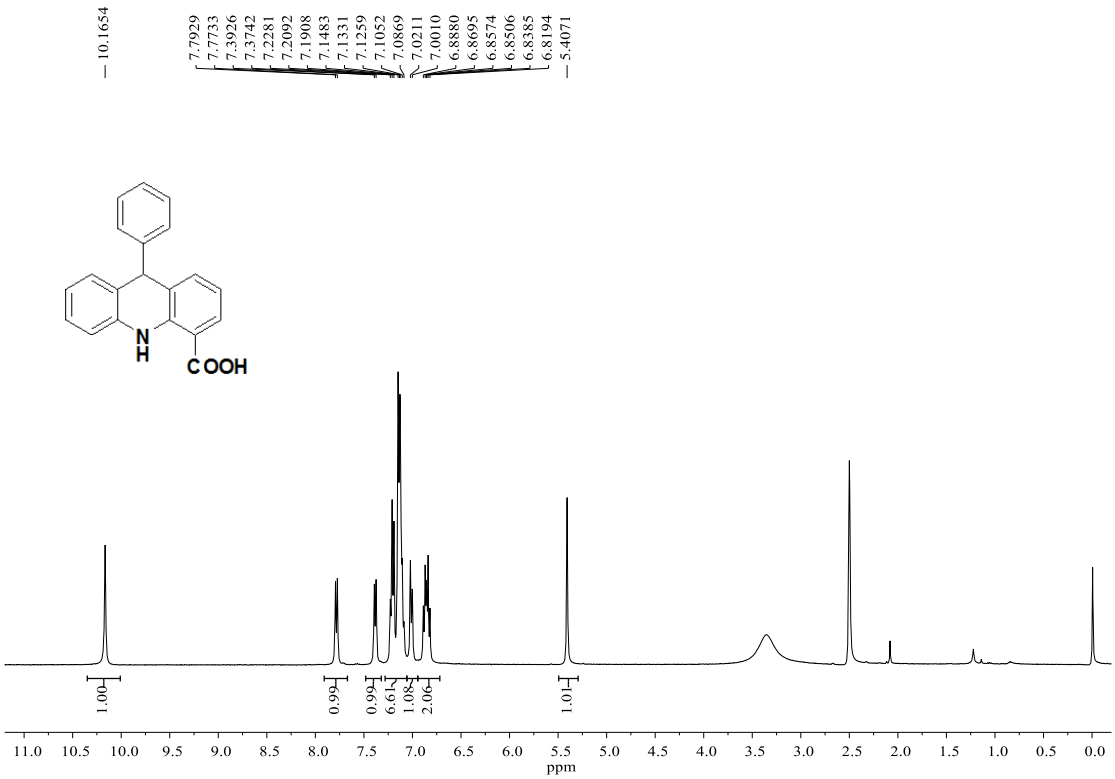


Figure S20 ^{1}H NMR spectrum (400 MHz, DMSO-d_6, 298 K) of **2H**.

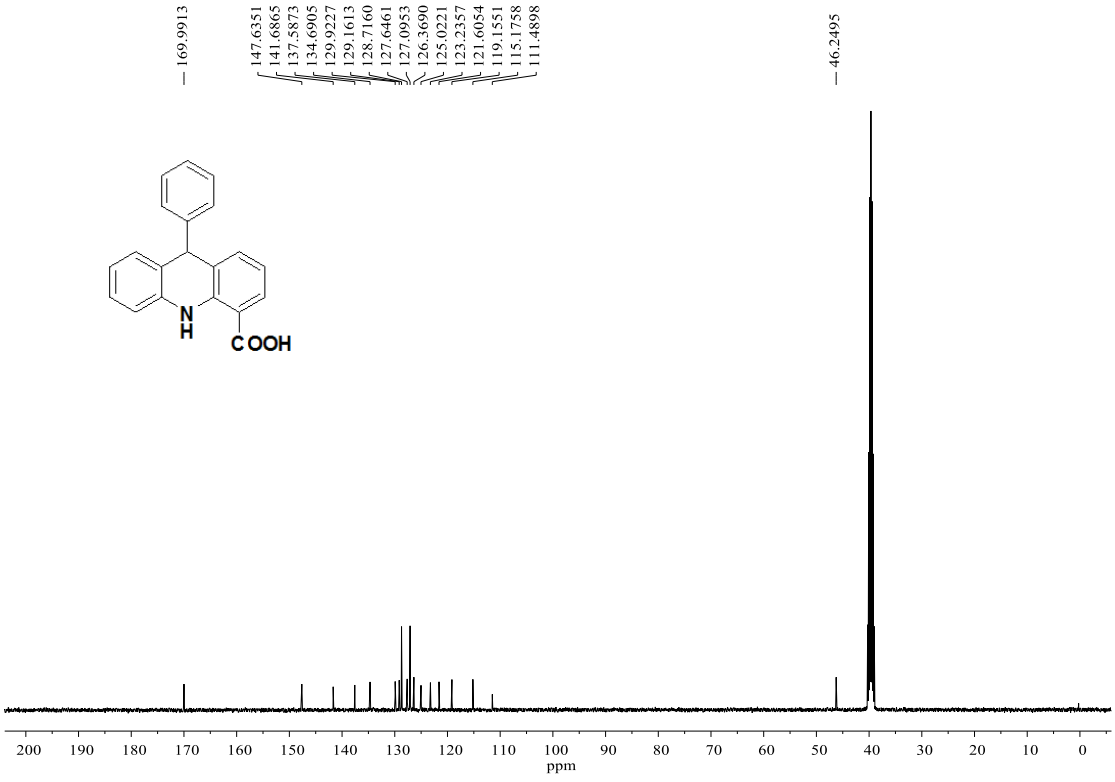


Figure S21 ^{13}C NMR spectrum (100 MHz, DMSO-d_6, 298 K) of **2H**.

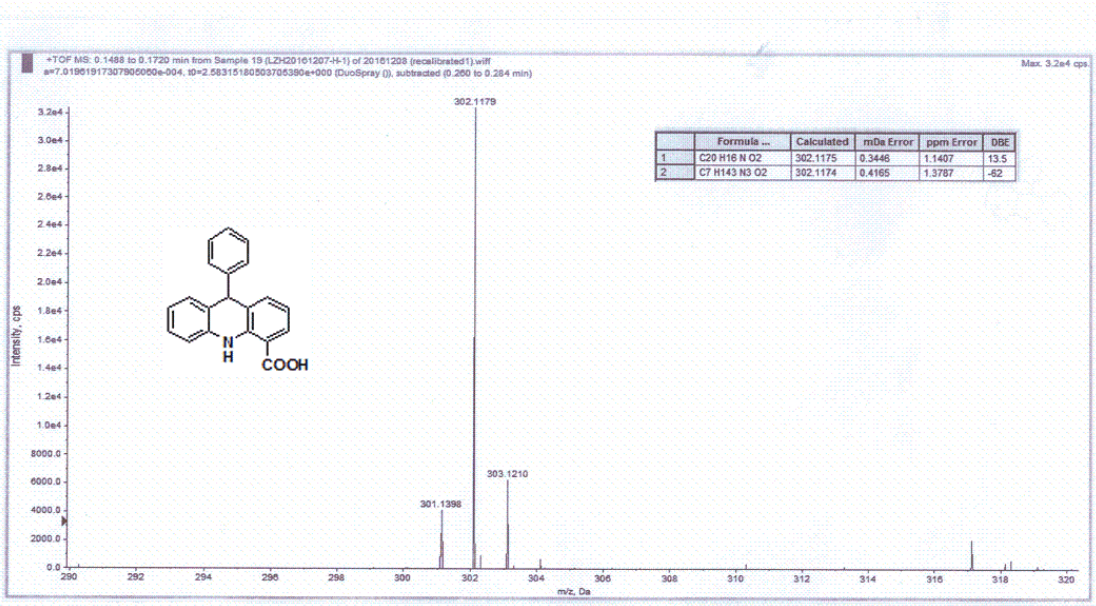


Figure S22 HRMS spectrum of **2H**.

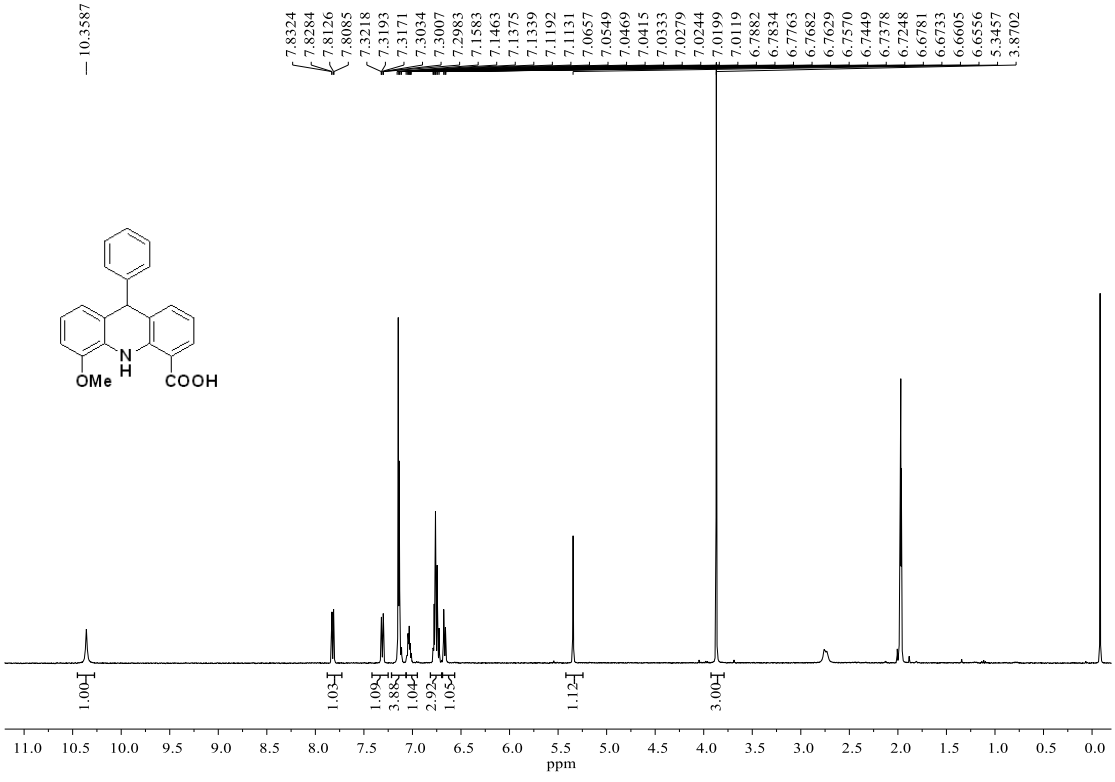


Figure S23 ^{1}H NMR spectrum (400 MHz, Acetone-d_6, 298 K) of **3H**.

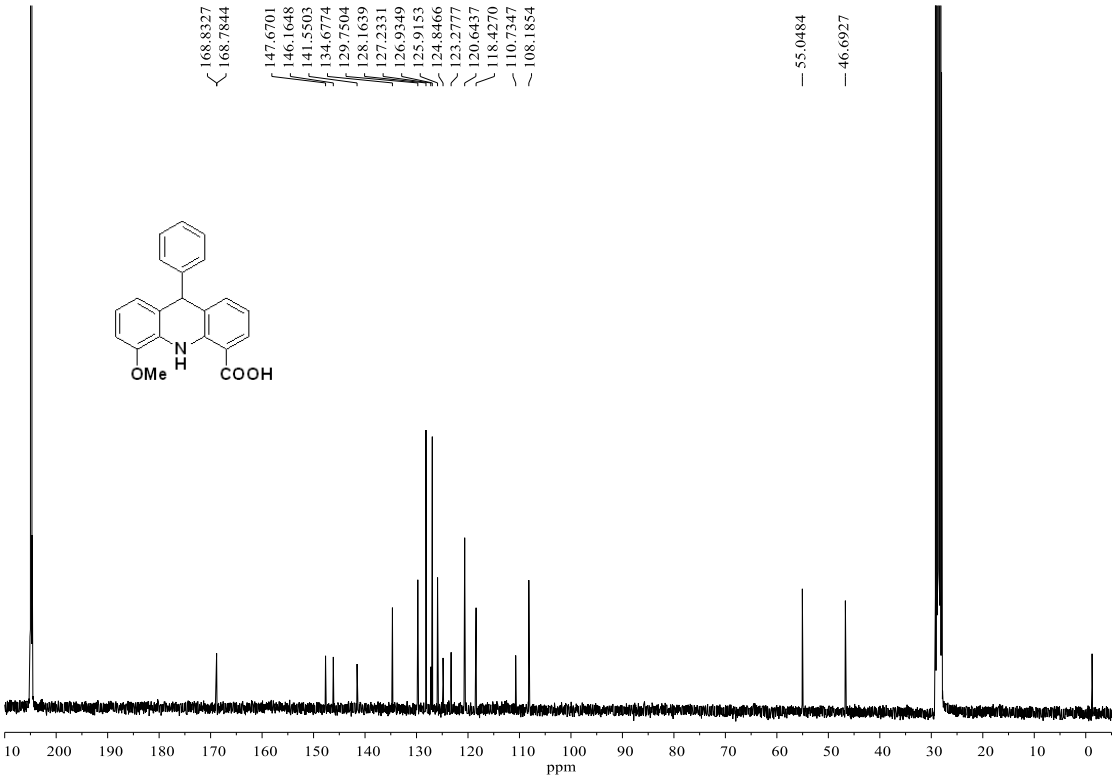
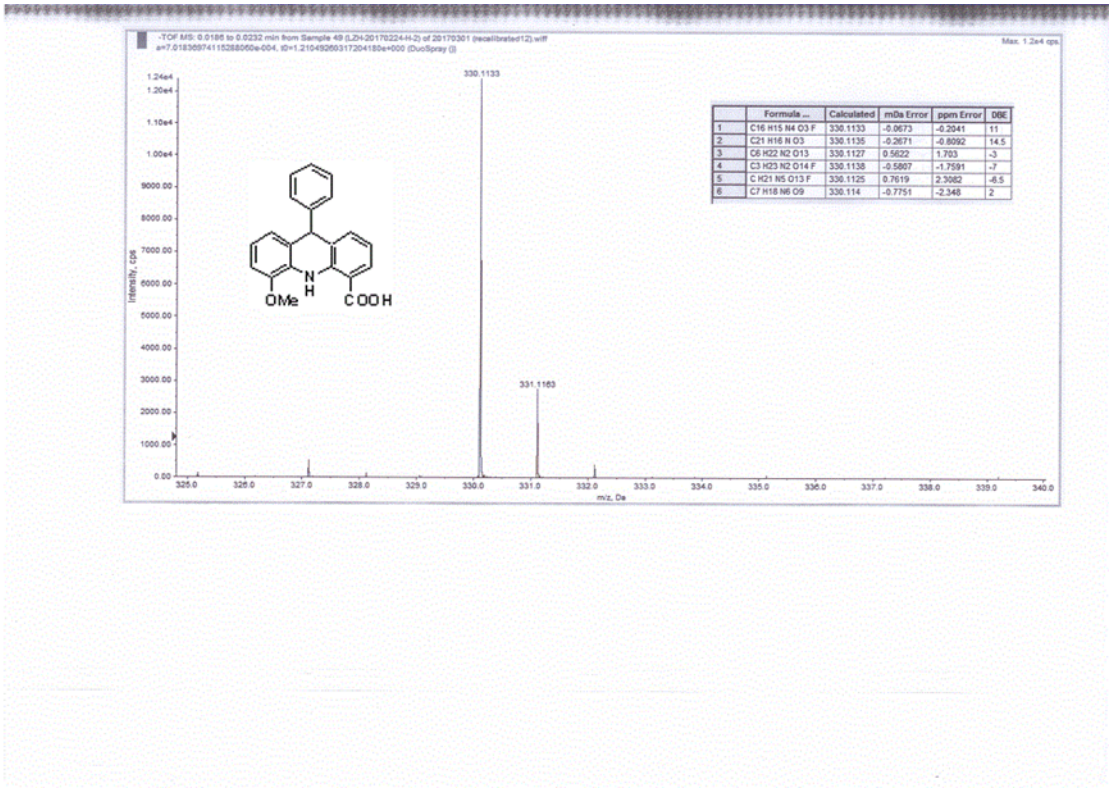


Figure S24 ^{13}C NMR spectrum (100 MHz, Acetone-d_6, 298 K) of **3H**.



	Formula ...	Calculated	mDa Error	ppm Error	DBE
1	C16 H15 N4 O3 F	330.1133	-0.0673	-0.2041	11
2	C21 H16 N O3	330.1135	-0.2671	-0.8092	14.5
3	C6 H22 N2 O13	330.1127	0.5822	1.763	-3
4	C3 H23 N2 O14 F	330.1138	-0.5807	-1.7591	-7
5	C H21 N5 O13 F	330.1125	0.7619	2.3082	-6.5
6	C7 H18 N6 O9	330.114	-0.7751	-2.348	2

Figure S25 HRMS spectrum of **3H**.

Proposed Reaction Mechanism

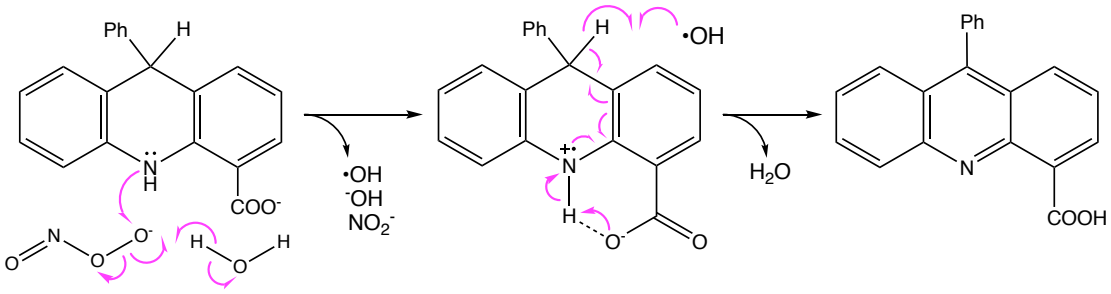


Figure S26 Proposed reaction mechanism of **2H** with $ONOO^-$.

ESI-HRMS Spectrum of the Reaction Mixture of $ONOO^-$ with 2H

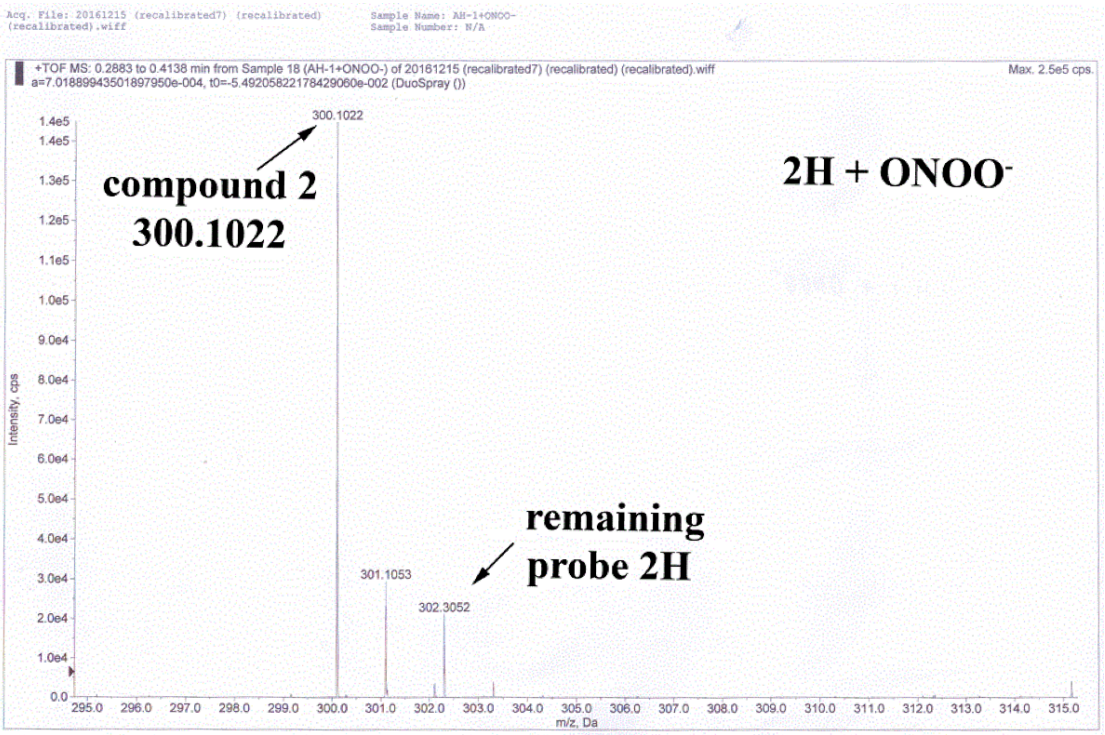


Figure S27 ESI-HRMS spectrum of the reaction mixture of $ONOO^-$ (1 equiv.) with **2H**.

pH-Dependent Change in the Fluorescent Intensities of 2, 2H, and the 1:1 mixture of 2H and ONOO-

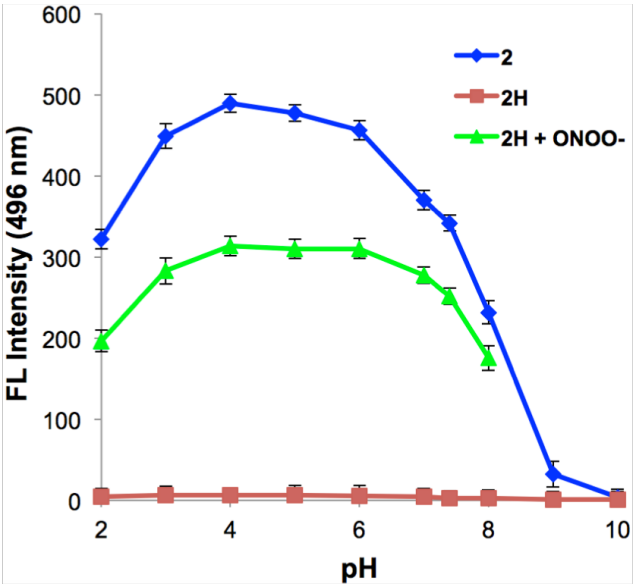


Figure S28 Fluorescence intensity (at 496 nm) of **2H** (2 μM, red), **2** (2 μM, blue) and **2H** (2 μM) in the presence of one equivalent (2 μM) of $ONOO^-$ (green) at different pH values. λ_{ex} = 356 nm, λ_{em} = 496 nm.

Results from Cell Viability Assays

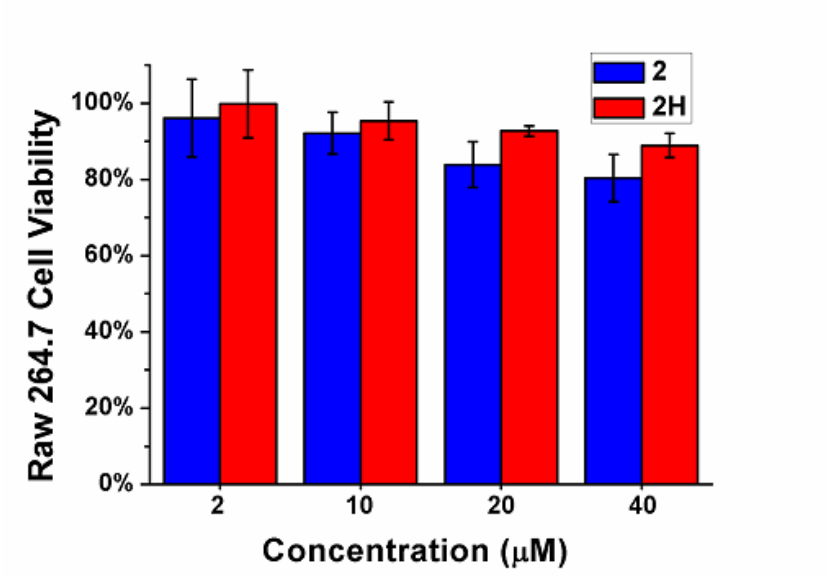


Figure S29 MTT assay of RAW264.7 cells treated with different concentrations of probe **2H** and compound **2**.

Additional fluorescence microscopic images of RAW264.7 macrophages loaded with 2H and oxidants

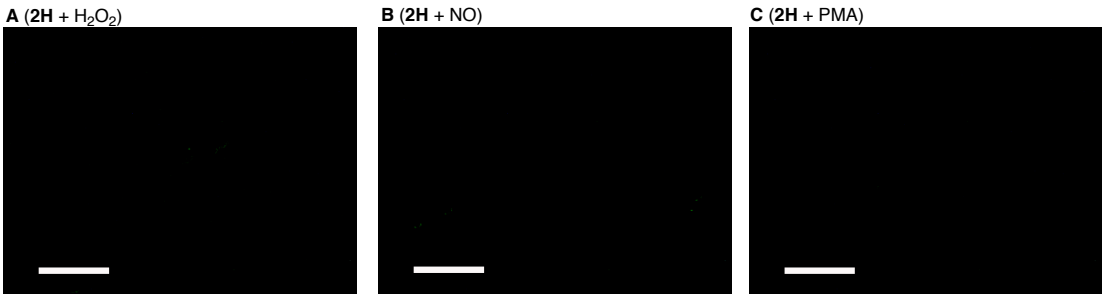


Figure S30 Fluorescence microscopic images of RAW264.7 macrophages loaded with **2H**. Cells were first with different induces and then loaded with probe **2H** (5 μM, mixed with 5 μM of dioleoylphosphatidyl ethanolamine, DOPE) for 15 min. (A) **2H** and H_2O_2 (50 μM) for 0.5 h. (B) **2H** and NO (50 μM) for 0.5 h. (C) **2H** and PMA (10 nM) for 0.5 h. Scale bars: 50 μm.

References

1. Uppu, R. M.; Pryor, W. A., Synthesis of Peroxynitrite in a Two-Phase System Using Isoamyl Nitrite and Hydrogen Peroxide. *Analytical Biochemistry* **1996,** *236*, 242-249.

2. Huang, K.-J.; Wang, H.; Ma, M.; Zhang, X.; Zhang, H.-S., Real-time imaging of nitric oxide production in living cells with 1,3,5,7-tetramethyl-2,6-dicarbethoxy-8-(3',4'-diaminophenyl)-difluoroboradiaza-s-indacence by invert fluorescence microscope. *Nitric Oxide* **2007,** *16*, 36-43.

3. Gavai, A. V.; Zhao, Y.; O'Malley, D.; Quesnelle, C. A.; Fink, B. E.; Norris, D. J.; Han, W. C.; Delucca, G. V., Alkyl, fluoroalkyl-1,4-benzodiazepinone compounds. *WO2014047374A1*.

4. Zhu, B.; Gao, C.; Zhao, Y.; Liu, C.; Li, Y.; Wei, Q.; Ma, Z.; Du, B.; Zhang, X., A 4-hydroxynaphthalimide-derived ratiometric fluorescent chemodosimeter for imaging palladium in living cells. *Chem. Commun. (Cambridge, U. K.)* **2011,** *47*, 8656-8658.