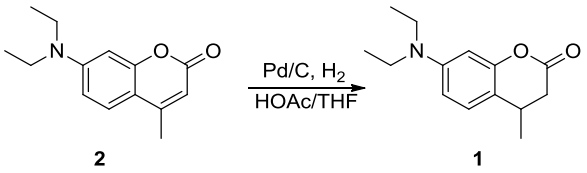
Electronic Supplementary Material (ESI) for ChemComm.

Taking advantage of the aromatisation of 7-diethylamino-4-methyl-3, 4-dihydrocoumarin to fluorescently sense superoxide anion

1. Chemistry

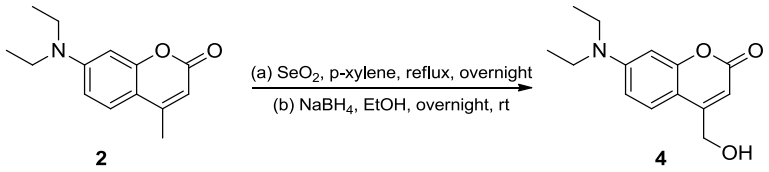
1.1. Synthesis of probe 1 and 3

General: All the solvents and chemicals were purchased from commercial sources: Sigma-Aldrich Chemical Co., Beijing Ou-he Reagents Co., Beijing Shiji-Aoke Biotechnology Co. and Shanghai Jingke Chemistry Technology Co. with the purity of more than 95%. Flash column chromatography was performed on Biotage Isolera one. ^{1}H NMR and ^{13}C NMR were recorded on Mercury300, Mercury400, Bruker AVANCEIII 400 spectrometer. Chemical shifts are referenced to the residual solvent peak and reported in ppm (δ scale) and all coupling constant (J) values are given in Hertz (Hz). The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. ESI-HRMS data were measured on Thermo Exactive Orbitrap plus spectrometer. Purity was determined using both LCMS and NMR spectroscopy. All of the synthesized compounds have a purity of > 95%.



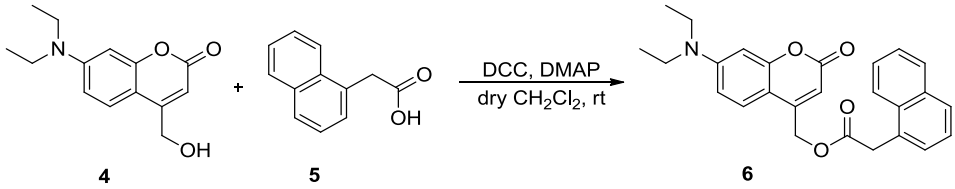
2 1

General procedure of 7-diethylamino-4-methyl-3, 4-dihydrocoumarin[1]: A solution of 7-diethylamino-4-methyl coumarin **2** (462 mg, 2 mmol) and 10% Pd/C (46 mg) in acetic acid/THF = 1:3 (20 mL) was stirred overnight at rt under H_2. Then the solvent was evaporated, and the crude product was further purified by flash chromatography (silica gel, ethyl acetate/petroleum ether = 20:80) to give pure compound **1** (329 mg). Colorless oil, yield: 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.06 (d, J = 8.6 Hz, 1H), 6.44 (dd, J = 8.5, 2.6 Hz, 1H), 6.29 (d, J = 2.6 Hz, 1H), 3.30 (q, J = 7.0 Hz, 4H), 3.04 (h, J = 6.8 Hz, 1H), 2.82 (dd, J = 15.7, 5.5 Hz, 1H), 2.50 (dd, J = 15.7, 7.5 Hz, 1H) , 1.17 (d, J = 6.9 Hz, 3H), 1.06 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.03 , 152.46 , 147.94 , 127.60 , 114.45 , 108.14 , 99.45 , 44.20 , 37.34 , 28.19 , 20.54 , 12.78 . HRMS (ESI): m/z calcd for $C_{14}H_{20}O_2N$ $[M+H]^+$, 234.14886; found, 234.14853.



2 4

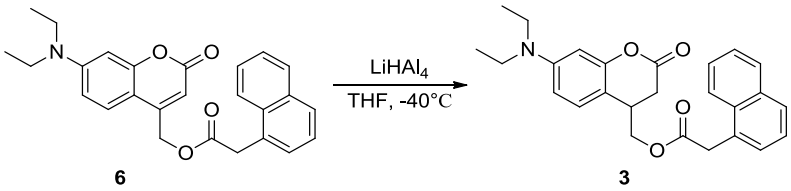
General procedure of 7-diethylamino-4-hydroxymethyl coumarin[2]: To a solution of 7-diethylamino-4-methyl coumarin **2** (4.63 g, 20 mmol, 1 eq) in hot p-xylene (120 mL) was added SeO_2 (3.33 g, 30 mmol, 1.5 eq). The mixture was heated under reflux with vigorous stirring overnight. The mixture was filtered and concentrated under reduced pressure. The dark brown residual oil was dissolved in ethanol (130 mL), and sodium borohydride (380 mg, 10 mmol, 0.5 eq) was added to the solution followed by stirring overnight at room temperature. The suspension was carefully hydrolyzed with 1 M HCl (20 mL), diluted with water (30 mL) and extracted with DCM (3 × 50 mL). The organic phase was washed with water (2 × 100 mL), brine (100 mL) and dried over Na_2SO_4 and concentrated in vacuo. The crude material was purified by flash chromatography (silica gel, ethyl acetate/dichloromethane = 20:80) to yield **4** as a yellow solid (2.16 g, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (d, J = 9.0 Hz, 1H), 6.65 (dd, J = 9.0, 2.6 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 6.06 (t, J = 1.2 Hz, 1H), 5.49 (s, 1H), 4.66 (s, 2H), 3.41 (q, J = 7.0 Hz, 4H), 1.11 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.59 , 157.29 , 156.07 , 150.63 , 125.51 , 108.98 , 106.15 , 104.36 , 97.23 , 59.50 , 44.41 , 12.78 . HRMS (ESI): m/z calcd for $C_{14}H_{18}O_3N$ $[M+H]^+$, 248.12812; found, 248.12881.



4 5 6

General procedure of 7-diethylamino-4-hydroxymethyl coumarin 4-naphthylacetate[3, 4] : A solution of compound **4** (2.47 g, 10 mmol, 1 eq), 1-Naphthylacetic acid **5** (2.23 g, 12 mmol, 1.2 eq), N,N'-dicyclohexylcarbodiimide (DCC, 2.47 g, 12 mmol, 1.2 eq), and 4-dimethylaminopyridine (DMAP, 25 mg, 0.2 mmol, 0.2 eq) in dry CH_2Cl_2 (60 mL) was stirred at room temperature. When the reaction was complete as checked by TLC analysis, the solution of the mixture was diluted with CH_2Cl_2 (40mL). Subsequently, the solution was washed by water (40 mL), 0.1 M aqueous HCl (40 mL), saturated aqueous $NaHCO_3$ (40 mL), and brine (40 mL), dried over anhydrous Na_2SO_4, concentrated

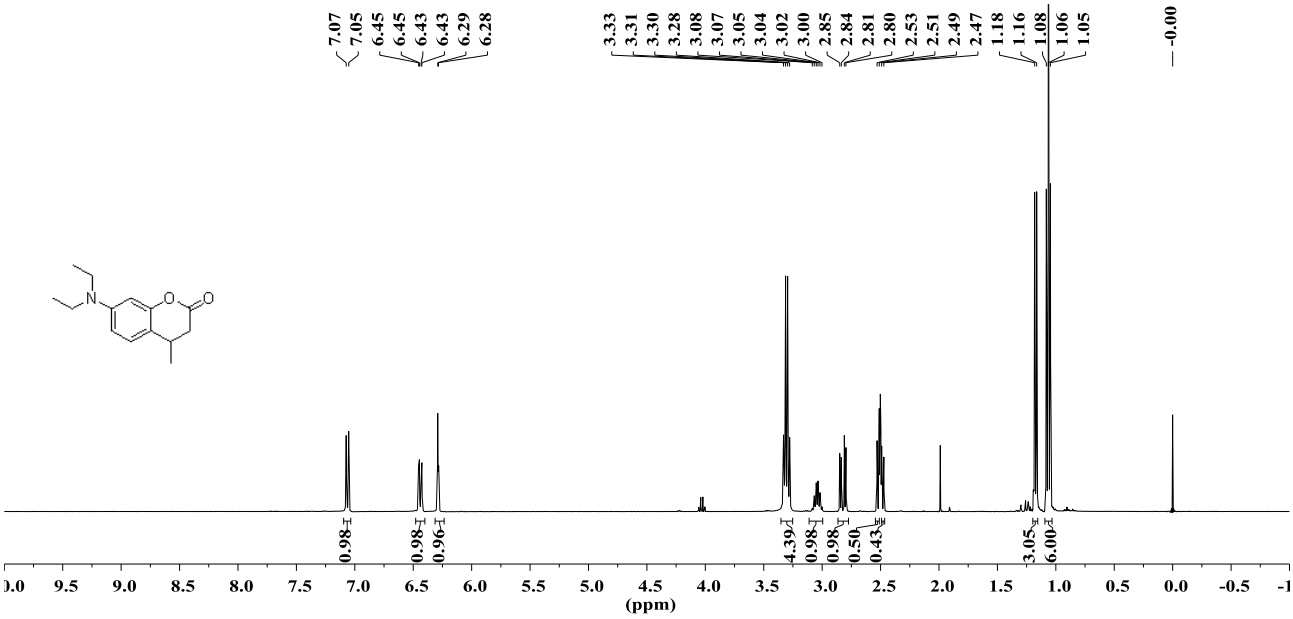
in vacuo, and purified by flash chromatography (silica gel, ethyl acetate/petroleum ether = 20:80) to give the target product **6** (3.07g, 74%). ^{1}H NMR (400 MHz,) δ 7.88 (ddd, *J* = 17.2, 10.8, 5.8 Hz, 4H), 7.58 – 7.43 (m, 3H), 7.38 (d, *J* = 9.0 Hz, 1H), 6.57 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.52 (d, *J* = 2.5 Hz, 1H), 5.98 (s, 1H), 5.32 (s, 2H), 4.03 (s, 2H), 3.40 (q, *J* = 7.0 Hz, 4H), 1.10 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.18 , 161.02 , 156.23 , 150.88 , 133.41 , 132.44 , 132.19 , 128.36 , 128.34 , 128.25 , 127.98 , 127.92 , 126.69 , 126.32 , 109.09 , 105.70 , 97.27 , 62.19 , 44.43 , 40.71 , 12.76 . HRMS (ESI): m/z calcd for $C_{26}H_{26}O_4N$ $[M+H]^+$, 416.18563; found, 416.18576.

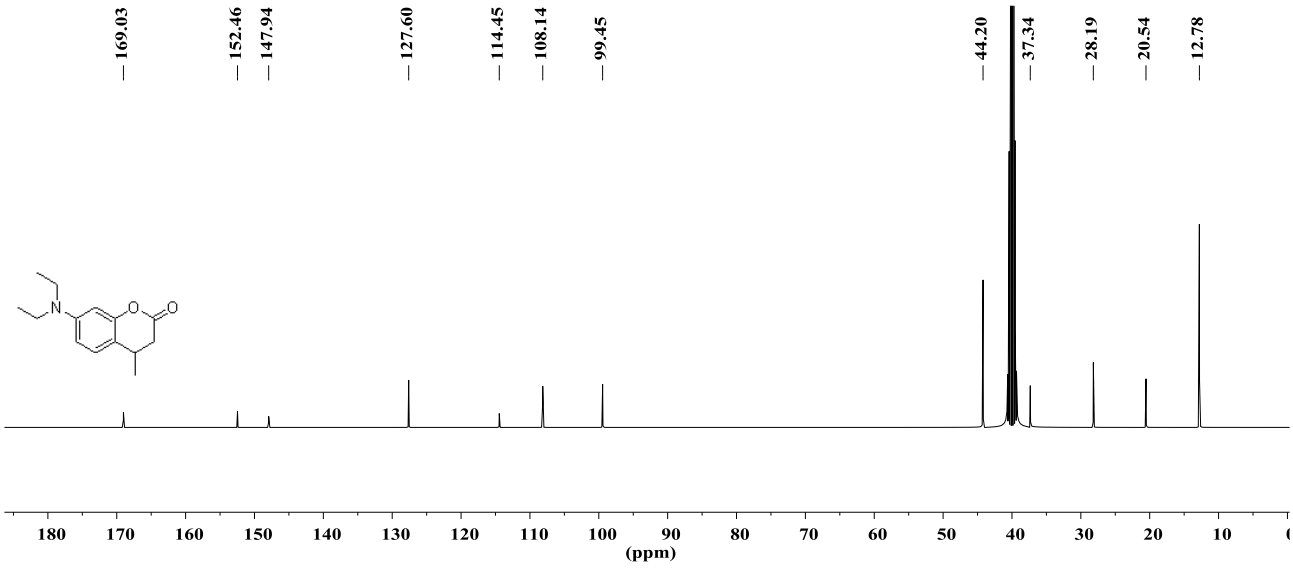


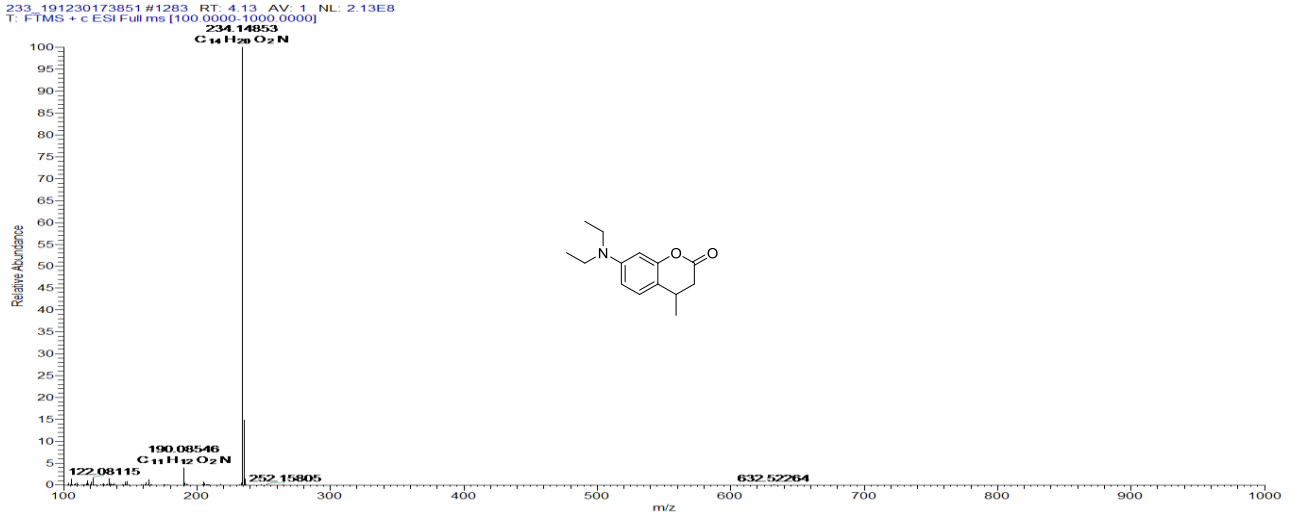
6 **3**

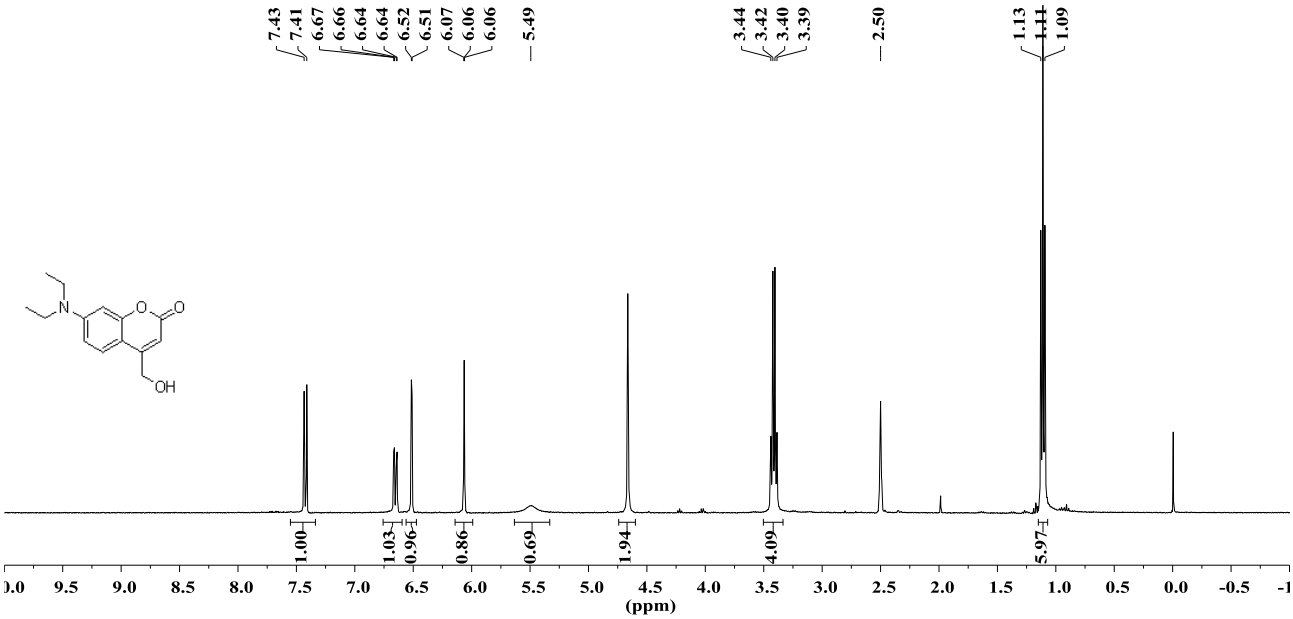
General procedure of 7-diethylamino-4-hydroxymethyl-3, 4-dihydrocoumarin 4-naphthylacetate[5-7] : To a round bottomed flask equipped with a drying tube ($CaCl_2$) containing a solution of **6** (2.07 g, 5 mmol, 1 eq) in THF (60 mL) at -40℃ was added 0.1 M $LiAlH_4$ solution in THF (55 mL， 5.5 mmol, 1.1 eq) slowly. After stirring the solution at the same temperature for 2 hours the reaction was warmed to room temperature and stirred for 20 min before be quenched with a saturated solution of NH_4Cl (40 mL). The solution was extracted with ethyl acetate (60 mL×3). The organic phase was washed with brine, dried and concentrated in vacuo, which was purified by flash chromatography (silica gel, ethyl acetate/petroleum ether = 20:80) to give the target product **3** (130 mg, 6.2%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (d, *J* = 8.1 Hz, 1H), 7.97 – 7.90 (m, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.61 – 7.46 (m, 4H), 6.90 (d, *J* = 8.7 Hz, 1H), 6.45 (dd, *J* = 8.7, 2.6 Hz, 1H), 6.26 (d, *J* = 2.6 Hz, 1H), 4.33 (s, 2H), 3.86 – 3.69 (m, 2H), 3.27 (q, *J* = 7.0 Hz, 4H), 2.86 (p, *J* = 8.0 Hz, 1H), 2.28 – 2.05 (m, 2H), 1.11 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 176.55 , 170.01 , 149.72 , 147.97 , 133.91 , 131.90 , 130.09 , 129.14 , 128.65 , 128.50 , 127.11 , 126.68 , 126.18 , 125.69 , 123.56 , 116.90 , 109.84 , 105.31 , 73.22 , 44.42 , 39.98 , 34.56 , 33.85 , 12.48 . HRMS (ESI): m/z calcd for $C_{26}H_{28}O_4N$ $[M+H]^+$, 418.20128; found, 418.20294.

1.2. ^{1}H, ^{13}C NMR spectra





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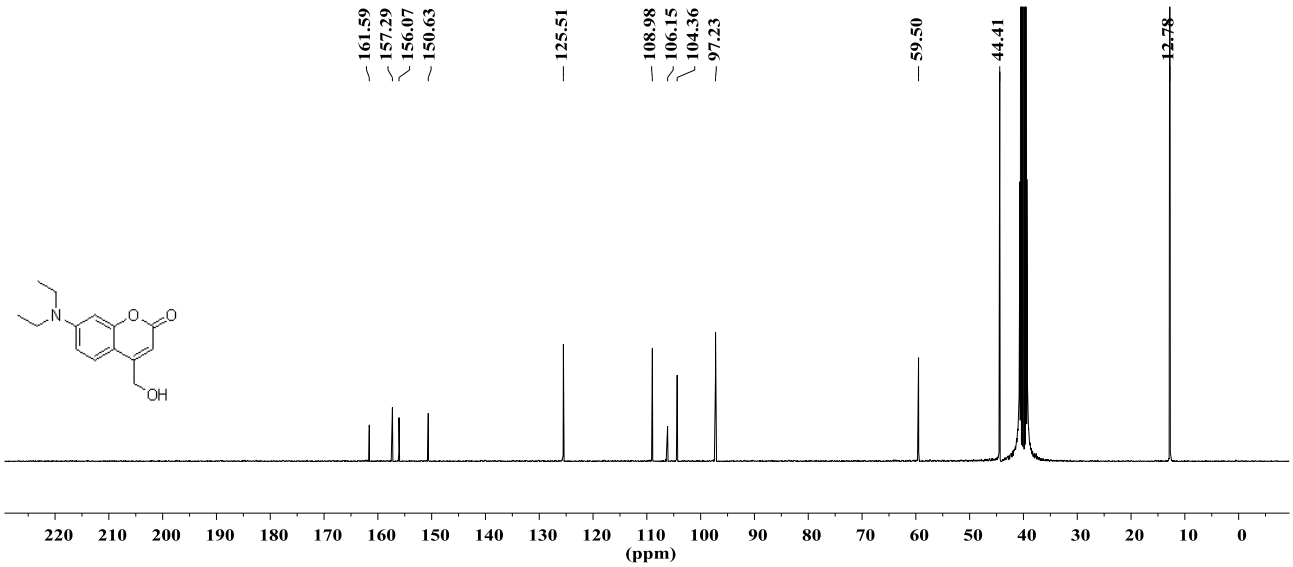
6.66
6.64
6.64
6.52
6.51
6.07
6.06
6.06
5.49
3.44
3.42
3.40
3.39
2.50
1.13
1.11
1.09
1.00
1.03
0.96
0.86
0.69
1.94
4.09
5.97
(ppm)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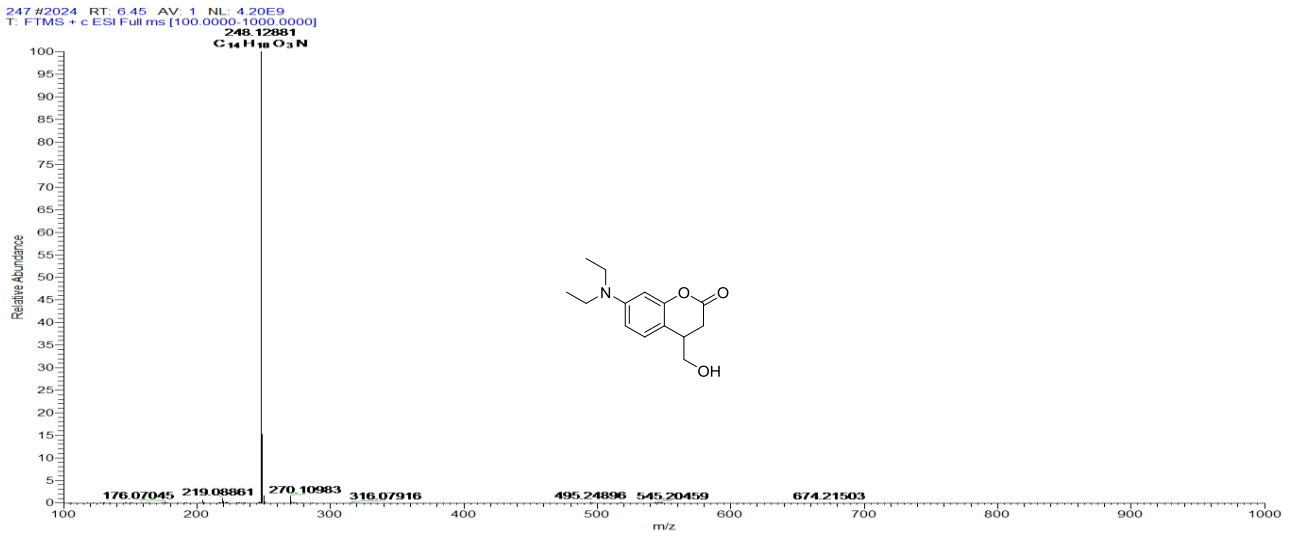
97.23
59.50
44.41
12.78
(ppm)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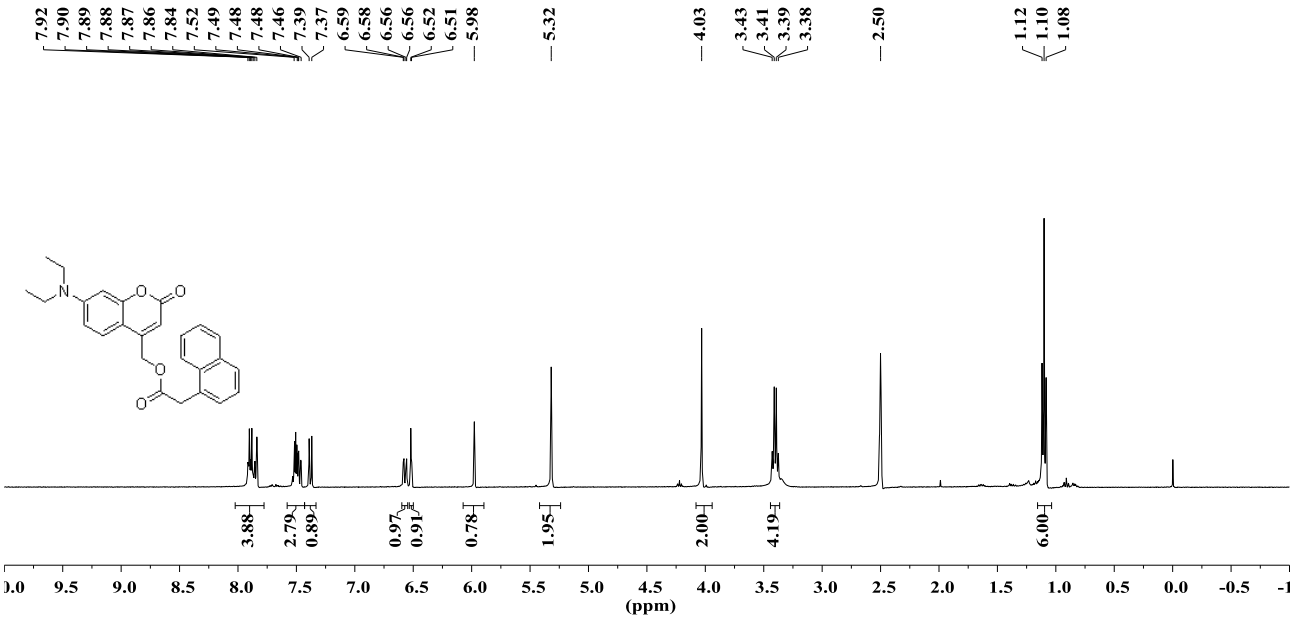
495.24896
545.20459
674.21503
m/z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7.86
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7.52
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6.58
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3.43
3.41
3.39
3.38
2.50
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1.10
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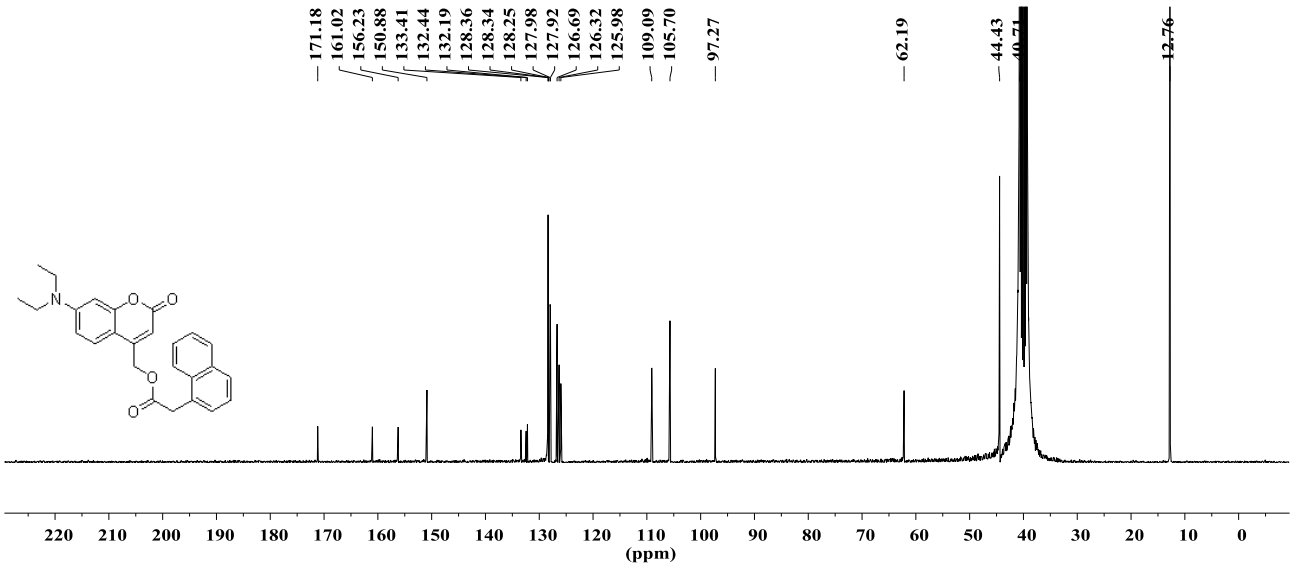
(ppm)


171.18
161.02
156.23
150.88
133.41
132.44
132.19
128.36
128.34
128.25
127.98
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97.27
62.19
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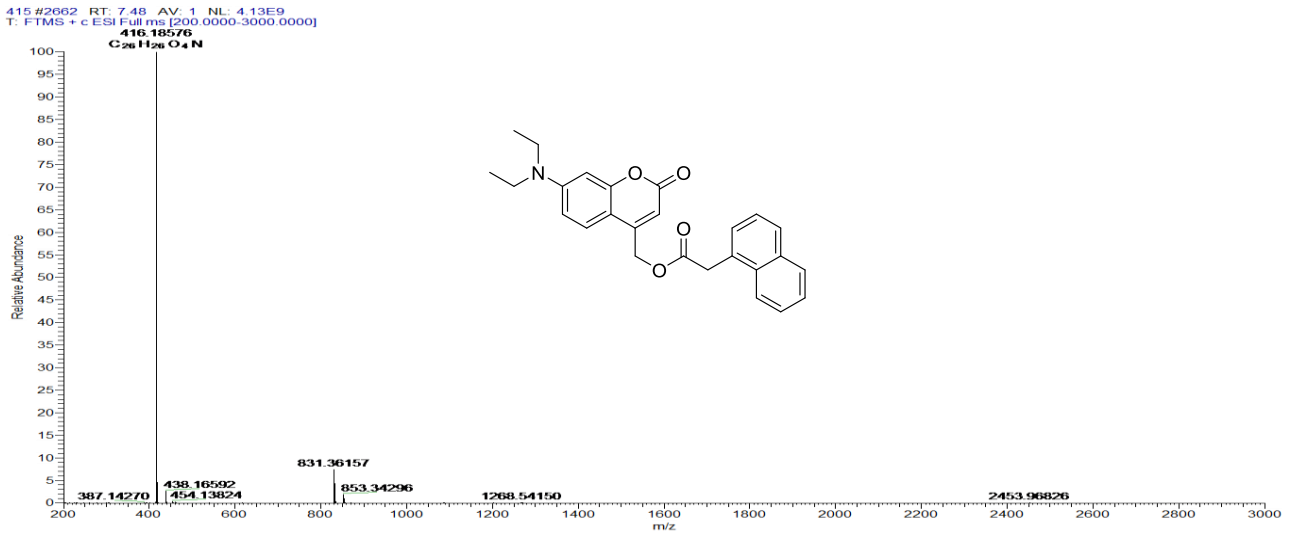
(ppm)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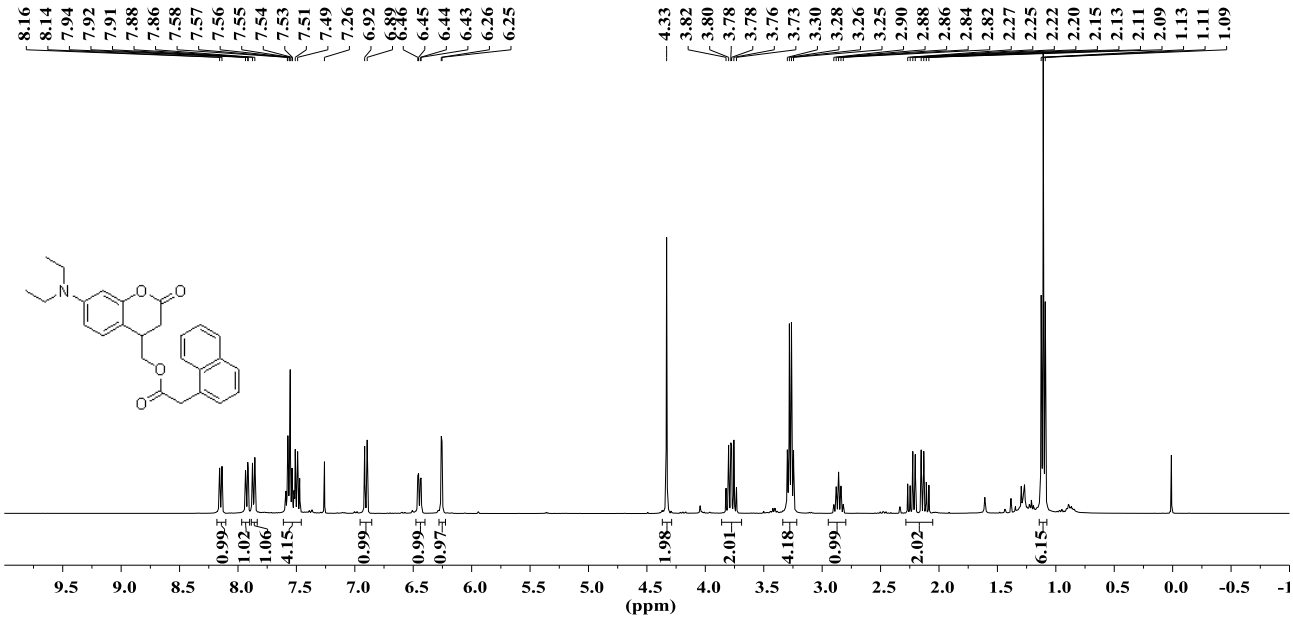
m/z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7.51
7.49
7.26
6.92
6.89
6.46
6.45
6.44
6.43
6.26
6.25
4.33
3.82
3.80
3.78
3.78
3.76
3.73
3.30
3.28
3.26
3.25
2.90
2.88
2.86
2.84
2.82
2.27
2.25
2.22
2.20
2.15
2.13
2.11
2.09
1.13
1.11
1.09
0.99
1.02
1.06
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0.99
0.99
0.97
1.98
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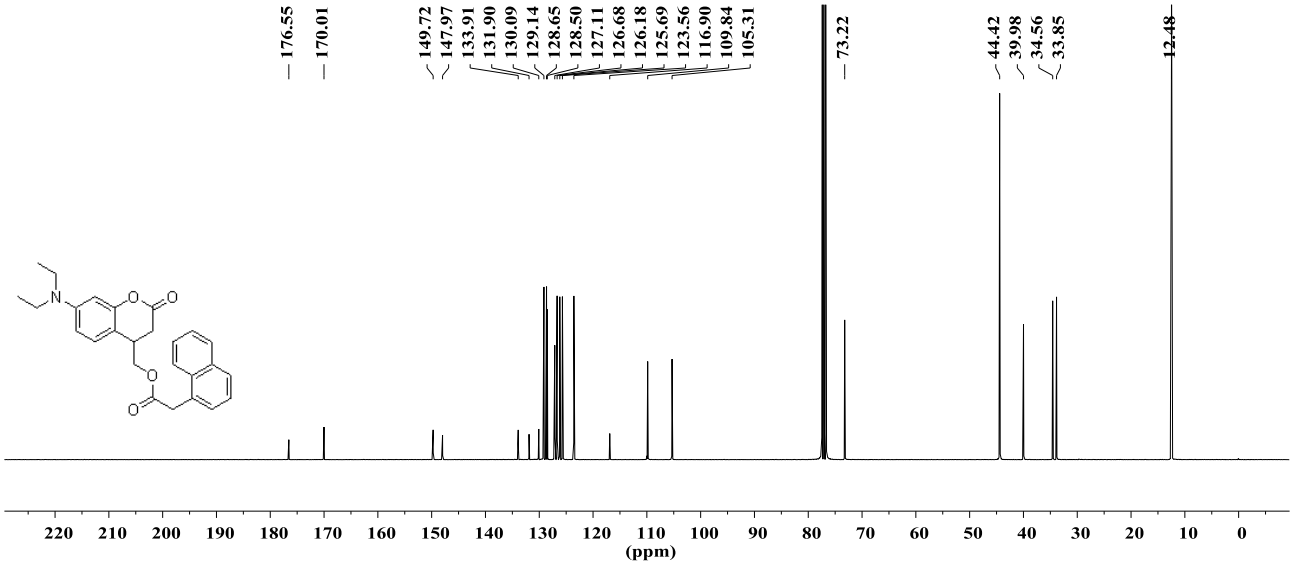
4.18
0.99
2.02
6.15
(ppm)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130.09
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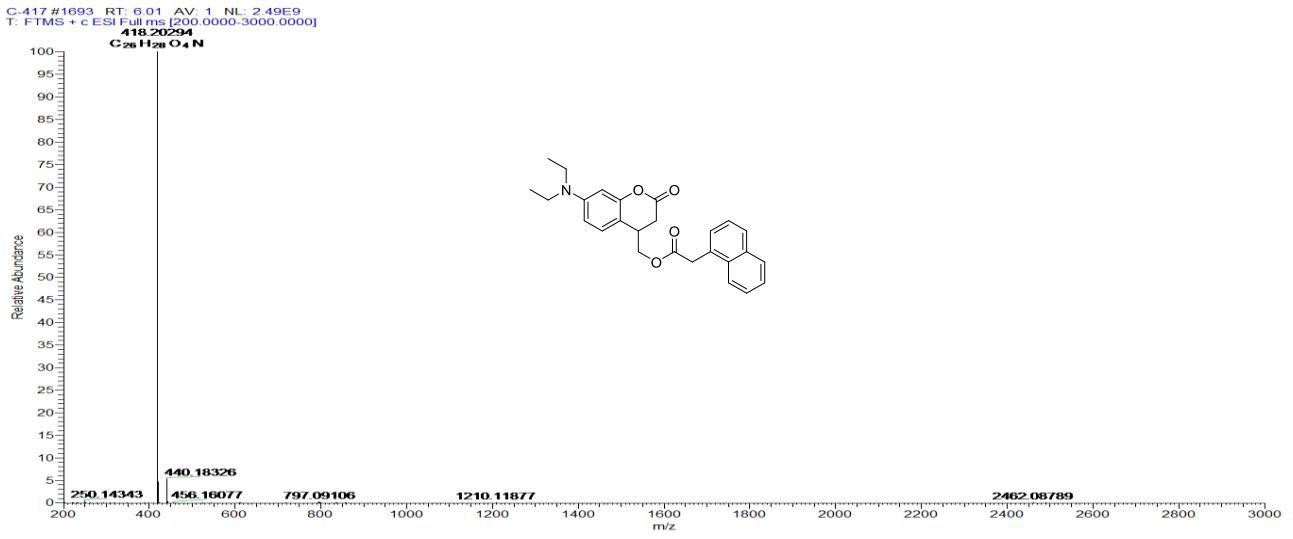
33.85
12.48
(ppm)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C26 H28 O4 N
Relative Abundance
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250.14343
456.16077
797.09106
1210.11877
2462.08789
m/z

2. Biological Analysis

2.1 The solubility of probe 1 in various solution.

Probe **1** (10mM stock solution in DMSO) was dissolved in 0.1M Tris-HCl buffer, pH8.0, 0.01M PBS buffer, pH7.4, 0.1M HEPES buffer, pH7.4 at the concentration of 500 μM. The final solution is clear and no precipitation was observed.

Figure S1, The solubility of Probe 1 (500μM) in 0.1M Tris-HCl buffer, pH8.0, 0.01M PBS buffer, pH7.4, 0.1M HEPES buffer, pH7.4.

Probe 1 was also dissolved in various solvents (DMF, DMSO, Ethanol, Acetonitrile, Isopropanol, Acetone, Tetrahydrofuran, Dioxane) as the 10mM stock solution. All of the stock solutions were well dissolved, and the solution is clear.

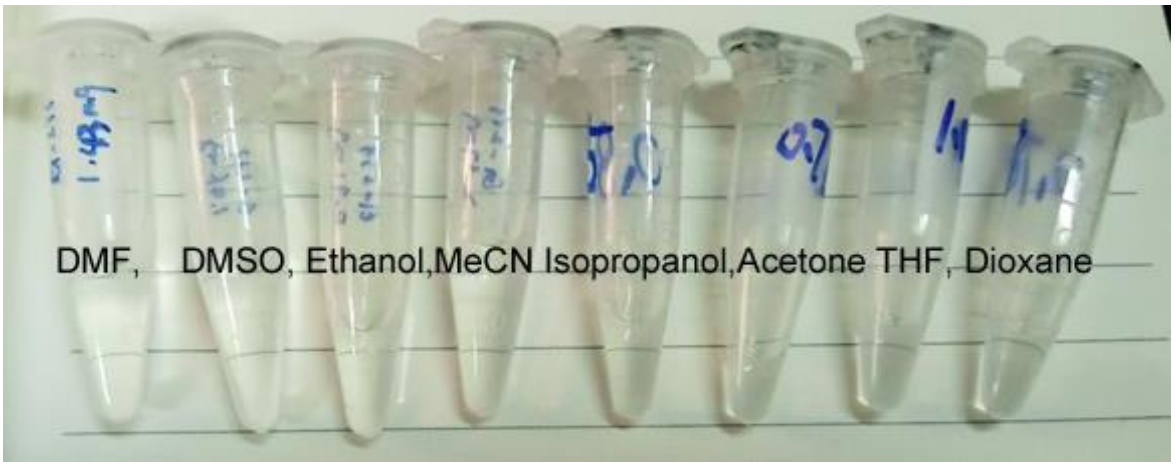


Figure S2, The 10mM stock solution of Probe **1** in various solvents (DMF, DMSO, Ethanol, Acetonitrile, Isopropanol, Acetone, THF, Dioxane).

Various stock solutions of probe **1** were stored at room temperature for over than a month, and the contents in the solutions were simply examined by TLC (EA/PE=1:9). TLC results show that there is only one UV active compound in the solution, which is probe **1**. Probe **1** remains stable in all of the solvent for a month.

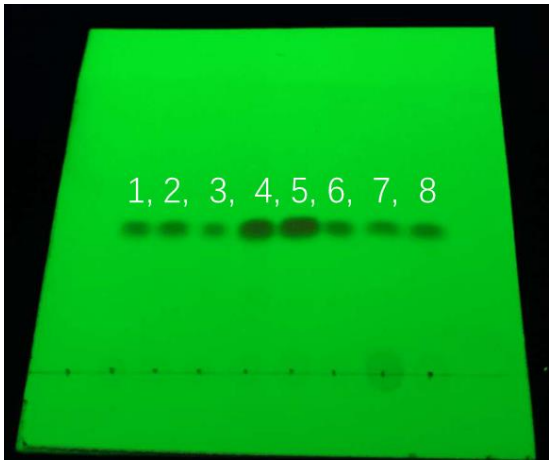


Figure S3, The 10mM stock solution of Probe **1** in various solvents (1-Ethanol, 2-Acetonitrile, 3-Isopropanol, 4-Acetone, 5-THF, 6-Dioxane, 7-DMSO, 8-DMF).

2.2 The stability of probe 1 and compound 2 in various pH in 200mM Phosphate buffer.

Probe 1 and compound 2 were dissolved with the concentration of 10 μM in 200mM phosphate buffer (pH3, 7, and 11). The fluorescence was measured in 20 minutes (E_x = 371 nm/E_m = 468 nm).

Both probe **1** and cmpd **2** remain stable in various pH (3, 7, 11) conditions. The above right picture show the plate layout of the six samples, and general graphs of these six samples.

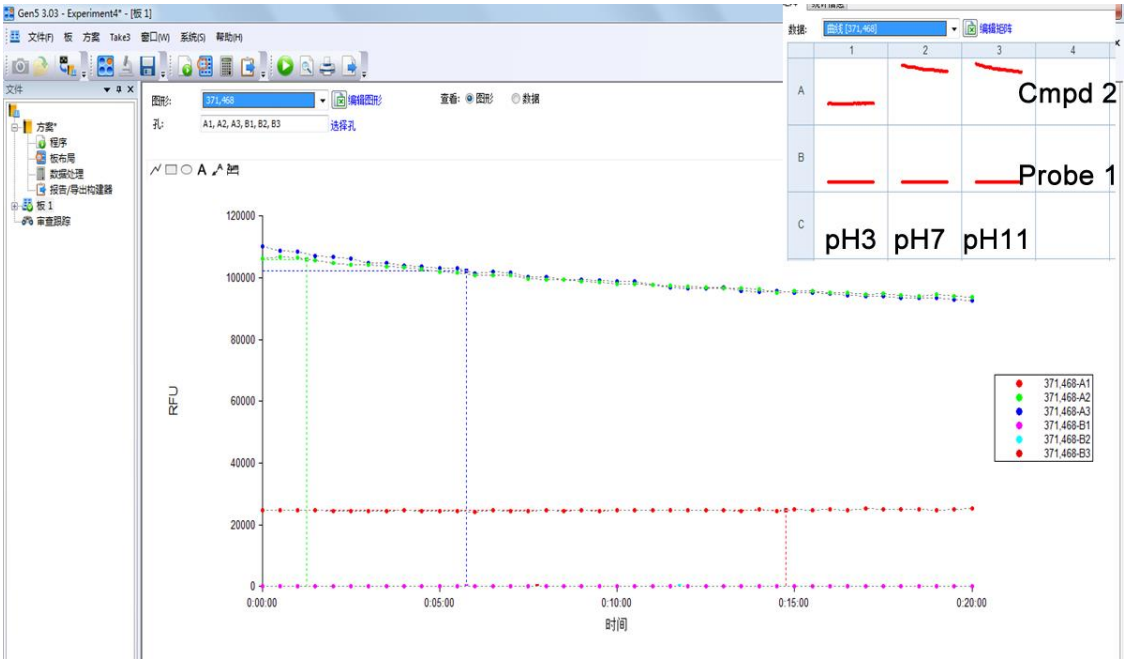


Figure S4, The stability of Cmpd **2** (A1, A2, A3) and Probe **1** (B1, B2, B3) in various pH conditions (pH 3, pH 7 and pH 11).

2.3. Fluorescence responses of probe 1 to various ROS

The fluorescence was measured with the Biotek Synergy™ H1, E_x = 371 nm/E_m = 468 nm. Probe **1** was dissolved in Acetonitrile as the 10mM stock solution, and used in this section. The buffer used in this section was 0.01 M PBS buffer, pH 7.4.

H_2O_2: Purchased commercial available H_2O_2 (~8.8 M) was used and dissolved in 0.01 M PBS buffer, pH 7.4 as the final concentration of 500 μM. Probe 1 was added into the solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37^{o}C for measurement.

TBHP: Purchased commercial available TBHP (~6.0 M) was used and dissolved in 0.01 M PBS buffer, pH 7.4 as the final concentration of 500 μM. Probe 1 was added into the solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37^{o}C for measurement.

OCl^-: Purchased commercial available NaOCl (~1.4 M) was used and dissolved in 0.01 M PBS buffer, pH 7.4 as the final concentration of 500 μM. Probe 1 was added into the solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37^{o}C for measurement.

$ONOO^-$: Peroxynitrite (ONOO-) solution was prepared following the protocols in the literature. The concentration of peroxynitrite was determined as 60 mM and was stored at -20 ^{o}C.

A, The chemistry of pernitrites. Part I. Kinetics of decomposition of pernitrous acid, 1968, J. Chem. SOC. (A), 450.

B, Molecular Imaging of Peroxynitrite with HKGreen-4 in Live Cells and Tissues.2014, J. Am. Chem. Soc. 136, 11728.

$ONOO^-$ (~0.06 M) was used and dissolved in 0.01 M PBS buffer, pH 7.4 as the final concentration of 500 μM. Probe 1 was added into the solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37^{o}C for measurement.

$^1O_2$: Purchased commercial available NaOCl (~1.4 M) was used and dissolved in 0.01 M PBS buffer, pH 7.4 as the final concentration of 500 μM. Twice dose of H_2O_2 was also added to the solution to make the final concentration of 1mM. The solution was mixed, and probe 1 was added into the solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37°C for measurement.

•OH: Purchased commercial available $FeSO_4$ was dissolved in 0.01 M PBS buffer, pH 7.4 as the final concentration of 500 μM. Twice dose of H_2O_2 was also added to the solution to make the final concentration of 1mM. The solution was mixed, and probe 1 was added into the solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37°C for measurement.

2.4. Fluorescence responses of probe 1 to XO/HPX system

100 mM HEPES buffer (pH 7.4) was prepared and used for this enzymatic assay. 50 μL of xanthine oxidase (XO, 5 U/890 μL) was added to 150 μL of 100 mM HEPES buffer (pH 7.4) to prepare the enzymatic solution. HPX was added into the enzymatic assay to make the final concentration of 1mM, probe **1** was added into the solution to make the final concentration of 10 μM or 50 μM. Fluorescence intensity was measured every 5 minutes for 70 minutes with Biotek Synergy™ H1, E_x = 371 nm/E_m = 468 nm.

In order to compare the reactivity with other ROS, we also repeated 10 μM probe **1** to incubate with the XO/HPX system (1.4U/ml, 1mM) for 5 minutes at 37 °C. The fluorescence was measured with Biotek Synergy™ H1, E_x = 371 nm/E_m = 468 nm.

2.5. Fluorescence responses of probe 1 to $O_2^{\bullet-}$ in anhydrous DMSO

750 μL 10 mM KO_2 in DMSO was incubated with 250 μL 10 mM probe **1** in DMSO at 37°C. Fluorescence intensity was measured every 20 s for 10 minutes with Biotek Synergy™ H1, E_x = 371 nm/E_m = 468 nm.

2.6. Fluorescence responses of probe 1 to various concentration of $O_2^{\bullet-}$ in anhydrous DMSO

Various volume of 10 mM KO_2 was added into various 1ml DMSO to make the final concentration of 0, 25, 50, 75, and 100 μM. Probe **1** was added into these solution to make the final concentration of 1 mM. The reaction was vortexed for 10 s, and incubated for 5 min at 37ºC for measurement. E_x = 371 nm/E_m = 468 nm. LOD was calculated.

2.7. Fluorescence excitation and emission spectra of reaction mixture or 2 in DMSO

Compound **2** was dissolved in DMSO to a final concentration of 1 μM. The reaction mixture of section 2.5 was also diluted in DMSO. Both solution was scanned the fluorescence excitation spectra (E_x = 300-420 nm/E_m = 468 nm) and emission spectra (E_x = 371 nm/E_m = 400-600 nm). When prepare the graph of the fluorescence spectra, the normalized fluorescence was used.

2.8. The oxidization product analysis of 2.5.

The reaction mixture of probe **1** and $O_2^{\bullet-}$ was extracted with ethyl acetate (2 mL×3). The organic phase concentrated in vacuo, dissolved with 1 mL MeOH and analyzed by LC-MS. Probe **1** and cmpd **2** were found in the reaction mixture with the retention time of 2.82 min and 4.52 min.

LC-MS system was performed on a ThermoFisher Exactive Plus mass spectrometer (ThermoFisher Scientific, Bremen, Germany) equipped with a ThermoFisher Accela HPLC system (ThermoFisher Scientific, Bremen, Germany). The mobile phase gradient for LC-MS monitoring was: A/H_2O+0.1% formic acid; B/Acetonitrile; t = 0 min 5% B; t =4 min 95% B; t =5.5 min 95% B; t = 5.6-8 min 5% B; flow rate: 0.3 $mLmin^{-1}$. Detector Wavelength： 254nm, Column: Agilent Zorbax SB-C18, 5 μm, 2.1 × 50 mm.

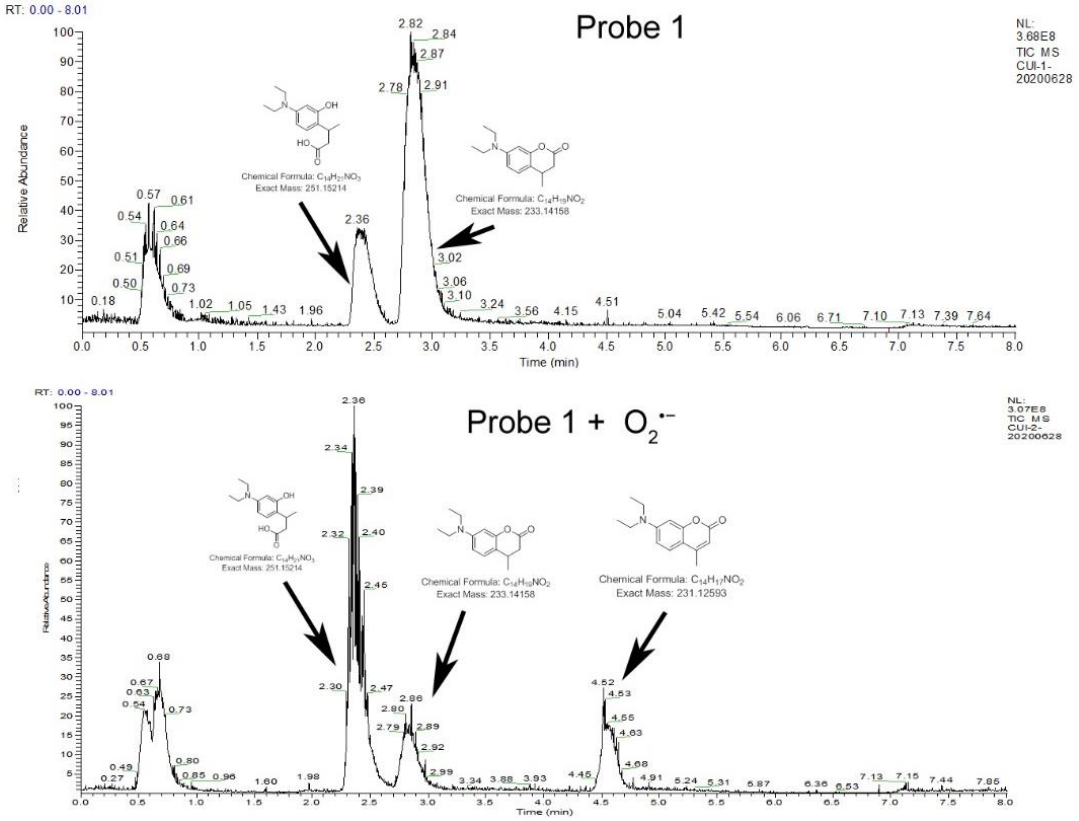
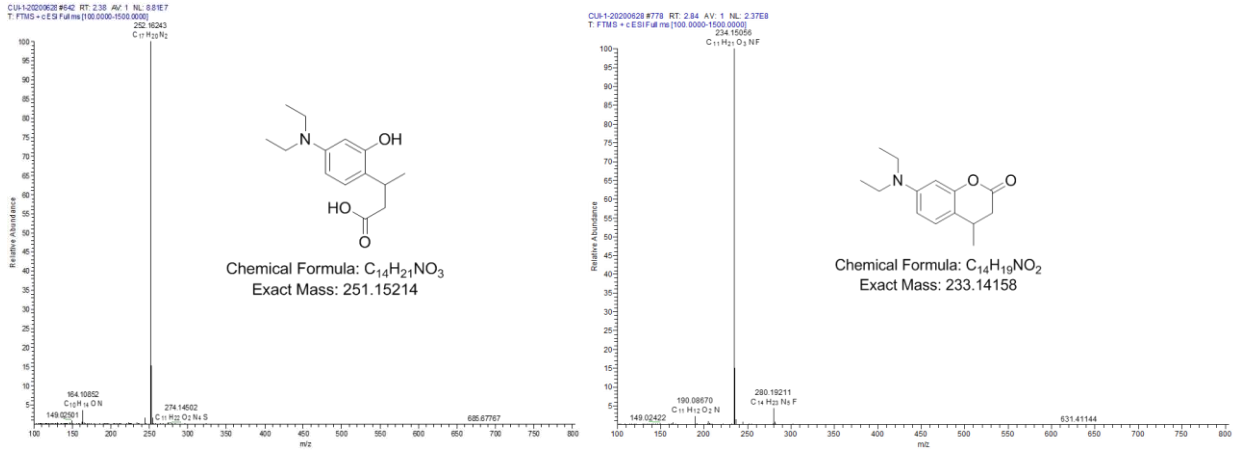
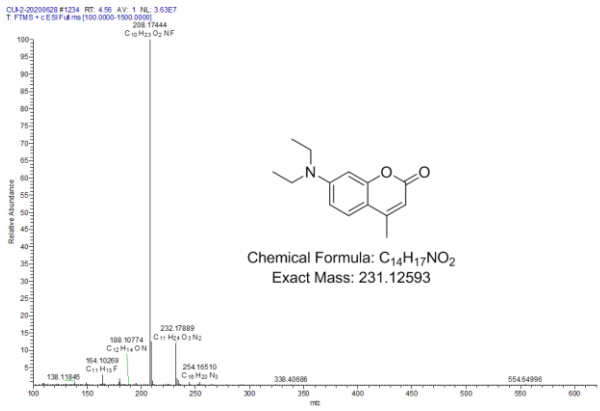


Figure S5, The analysis of the reaction mixture of Probe **1** and KO_2 in DMSO. Compound **2** was found as the major product in the reaction mixture, which was separated by HPLC and identified by MS. The MS analysis and the structures of three main peaks were also shown as follow.





2.9. The reaction between probe 1 and $O_2^{\bullet-}$ in aqueous buffer.

Probe **1** was dissolved in 10 mM PBS buffer (pH 7.4) to make a final concentration of 1mM. Solid KO_2 was added to this solution to make a final concentration of 10 mM. The reaction was vortexed for 10 s, and incubated for 5 min at 37°C for measurement. E_x = 371 nm/E_m = 468 nm.

The blank solution was made as follow. Probe **1** was dissolved in 10 mM PBS buffer (pH 7.4) to make a final concentration of 1mM. There was no KO_2 added.

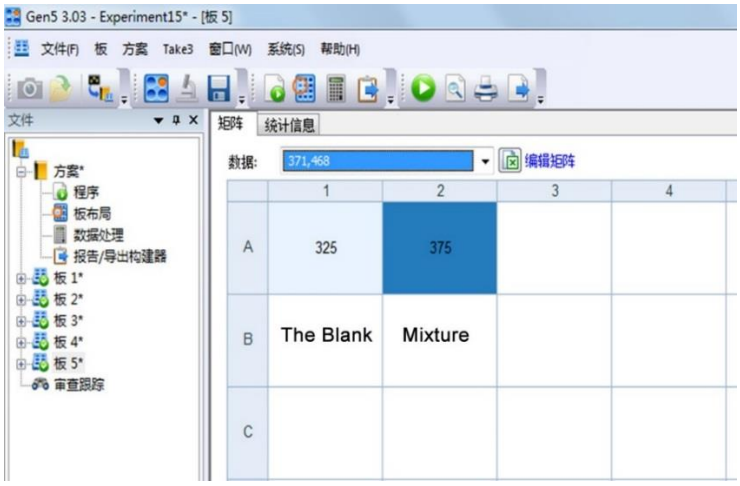


Figure S6, The fluorescence intensity of Probe **1** with solid KO_2 in 10 mM PBS buffer (pH 7.4).

2.9. Fluorescence responses of probe 1 to ClO^- in phosphate buffer (pH 7.4)

100 mM phosphate buffer, 150mM NaCl (pH 7.4) was firstly prepared. Probe **1** was dissolved in various solvents (Ethanol, Acetonitrile, Isopropanol, Acetone, Tetrahydrofuran, Dioxane) to make the 10mM stock solution.

For the detection of OCl^-: Purchased commercial available NaOCl (~1.4 M) was used and dissolved in 0.01 M PBS buffer, pH 7.4 as the final concentration of 500 μM. Probe **1** was added into the

solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37ºC for measurement.

The blank solution is absence of probe **1**. Thus, there is no reaction between probe **1** and the ClO^-.

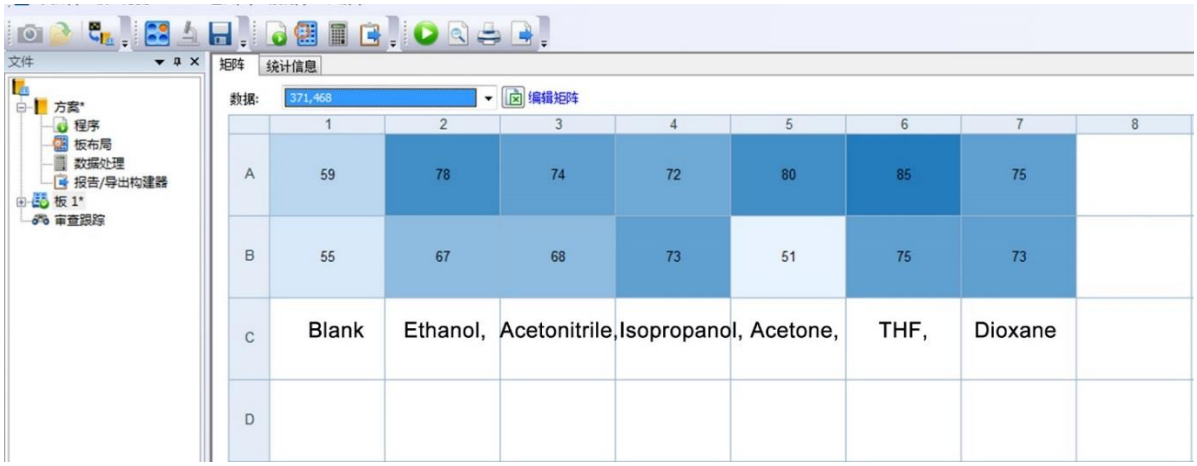


Figure S7, The fluorescence intensity of Probe **1** from various stock solution react with ClO^- in 100 mM PBS buffer (pH 7.4).

2.10. Fluorescence responses of probe 1 in various solvents to various ROS in phosphate buffer (pH 7.4)

100 mM phosphate buffer, 150mM NaCl (pH 7.4) was firstly prepared. Probe **1** was dissolved in various solvents (Ethanol, Acetonitrile, Isopropanol, Acetone, Tetrahydrofuran, Dioxane) to make the 10mM stock solution.

For the detection of various ROS, the surrogate of various ROS (500μM) was prepared separately as **2.3.** Probe **1** from various stock solution was added into the each solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37ºC for measurement. The blank solution is absence of probe **1**.

In general, all six stock solvent (final concentration 0.1%) does not affect the reactions between probe **1** and ROS.

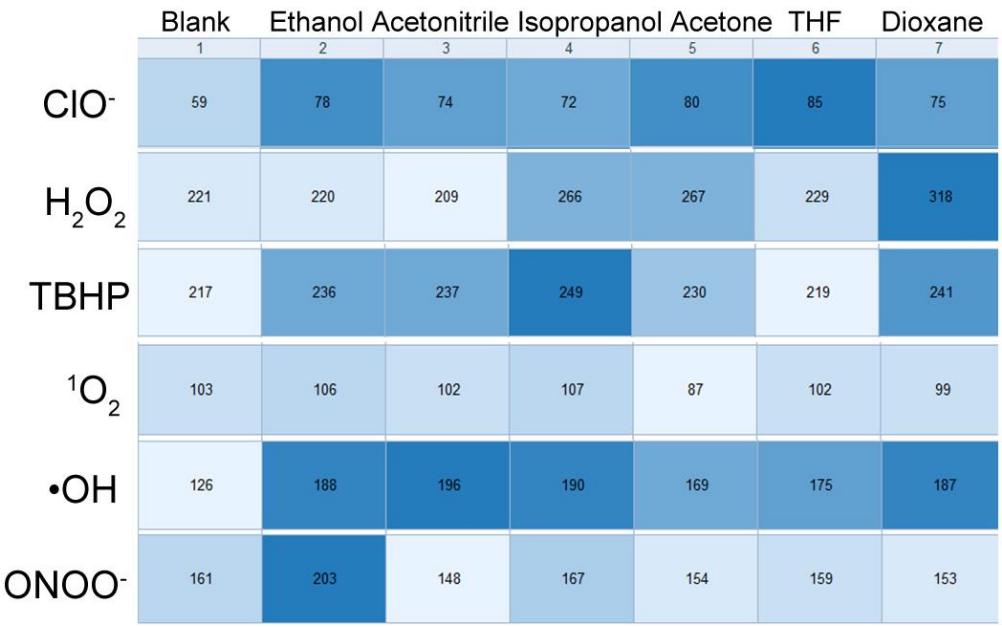


Figure S8, The fluorescence intensity of Probe **1** from various stock solution with 500 μM various ROS (ClO^-, H_2O_2, *tert*-butyl hydroperoxide, $^1O_2$, •OH, $ONOO^-$) in 100 mM PBS buffer (pH 7.4).

2.11. Fluorescence responses of probe 1 in various solvents to $O_2^{\bullet-}$ in anhydrous DMSO.

Probe **1** was dissolved in various solvents (Ethanol, Acetonitrile, Isopropanol, Acetone, Tetrahydrofuran, Dioxane) to make the 10mM stock solution.

For the detection of $O_2^{\bullet-}$, KO_2 was dissolved in DMSO to make the final concentration of 500μM**.** Probe **1** from various stock solution was added into the each solution as the final concentration of 10 μM. The reaction was vortexed for 10 s, and incubated for 5 min at 37°C for measurement. The blank solution is absence of probe **1**.

In general, the stock solvent of Ethanol, Acetonitrile, Isopropanol, Dioxane, Tetrahydrofuran, Acetone, DMF, DMSO do not affect the reaction between probe **1** and $O_2^{\bullet-}$. In all of these groups, probe **1** were oxidized by $O_2^{\bullet-}$ and fluorescence were turned on.

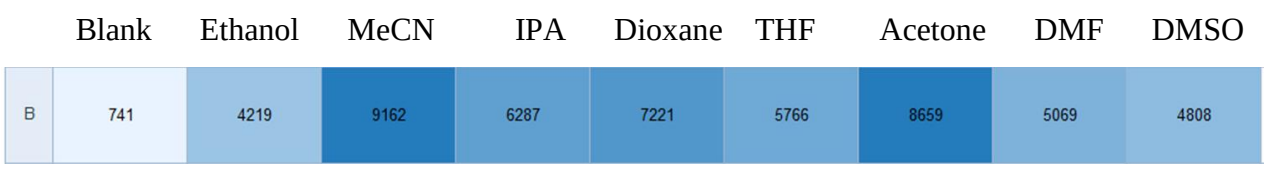


Figure S9, The fluorescence intensity of Probe **1** (10μM) from various stock solution with 500 μM $O_2^{\bullet-}$ in anhydrous DMSO.

The reaction mixture of probe **1** and $O_2^{\bullet-}$ were diluted in various pHs phosphate buffers, and the fluorescence was pH dependent, and at pH9 the fluorescence intensity is the most strong.

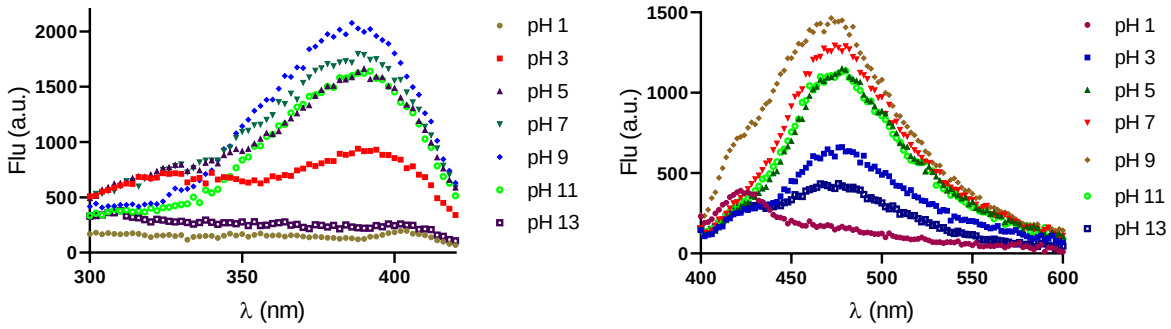


Figure S10, The fluorescence excitation and emission spectra of reaction mixture or compound **2** in various pHs of 200mM phosphate buffer.

The reaction mixture of probe **1** and $O_2^{\bullet-}$ were diluted in various buffers, 0.01M PBS buffer, pH7.4, 0.1M Tris-HCl buffer, pH8.0, 0.1M HEPES buffer, pH7.4. All three groups show good fluorescence spectra.

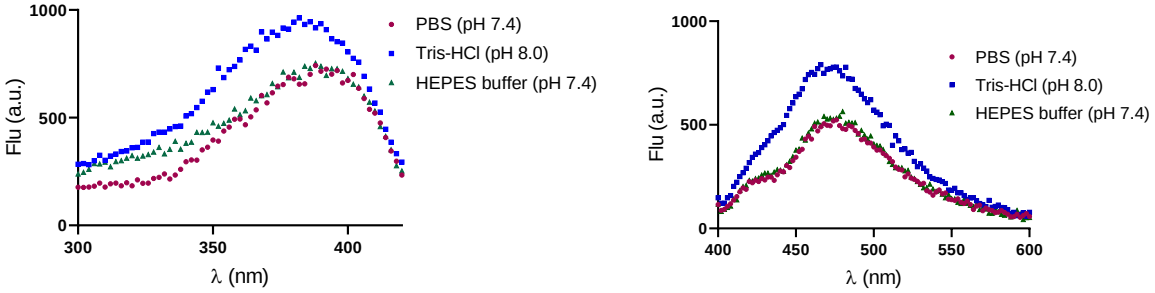


Figure S11, The fluorescence excitation and emission spectra of reaction mixture in various buffers, 0.01M PBS buffer, pH7.4, 0.1M Tris-HCl buffer, pH8.0, 0.1M HEPES buffer, pH7.4.

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