Electronic Supplementary Information (ESI)

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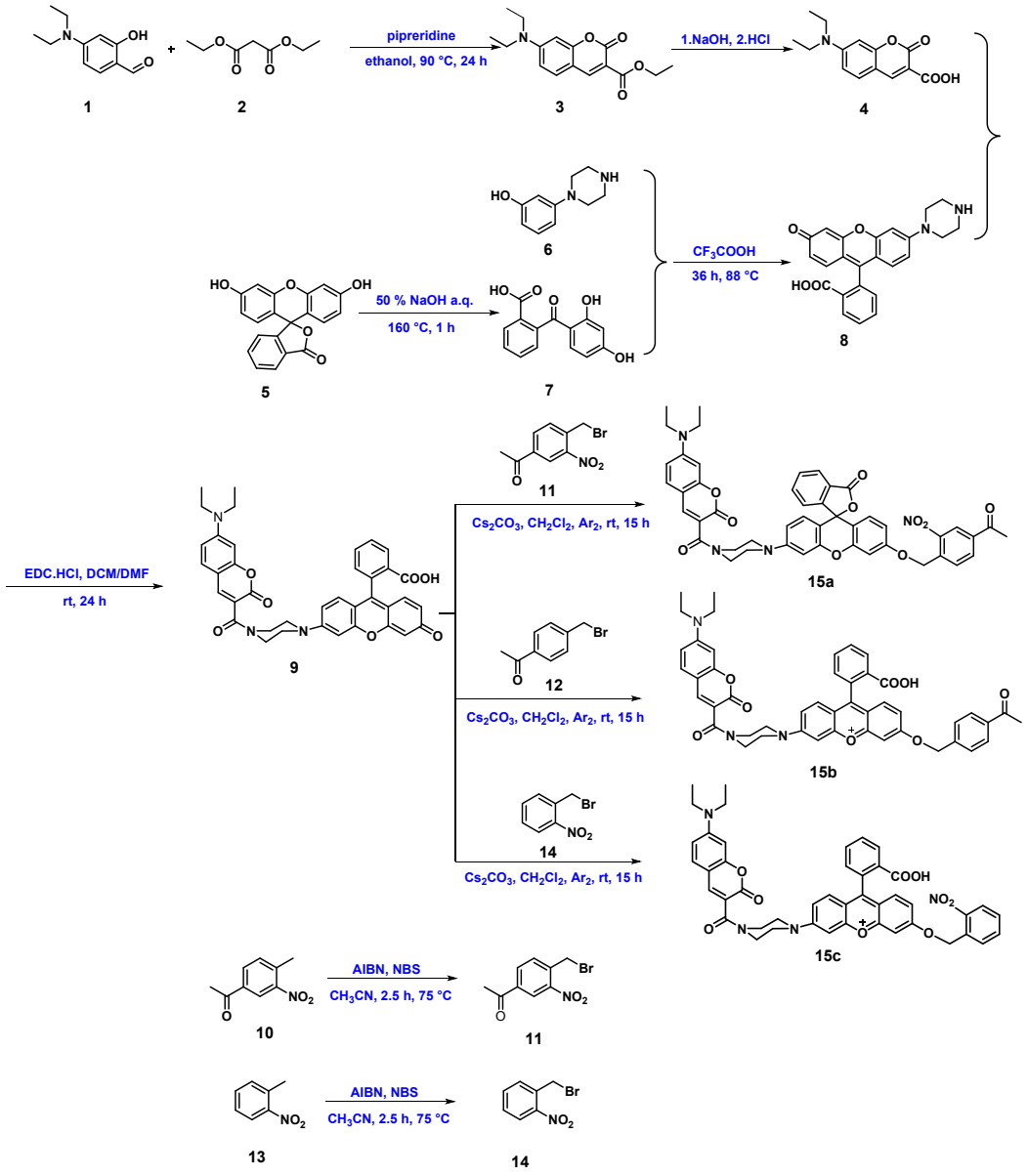
1. Synthesis and characterization of compounds

All chemical reagents and dry solvents for synthesis were purchased from commercial suppliers TCI (Shanghai) Development Co., Ltd., Adamas-beta Co., Ltd., and Merck KGaA, which were used without further purification. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on an AVANCE III 400 Nanobay (Bruker, 400 MHz and 600 MHz for ^{1}H NMR, 101 MHz for ^{13}C NMR).



Scheme S1. The synthetic procedure of compounds.

ethyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate (3)

Compound 4-diethylaminosalicyl aldehyde (2.0 g, 10.47 mmol) was dissolved in 120 mL of ethanol, and then added diethyl malonate (3.0 mL) and piperidine (1 mL). The reaction was then heated to reflux and stopped 24 hours later. The organic solvent was removed, The crude product was purified by silica gel chromatography to provide the final product as yellow liquid(1.70 g, 56.1 %) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.24 (s, 1H), 7.22 (d, *J* = 8.9 Hz, 1H), 6.47 (d, *J* = 8.7 Hz, 1H), 6.22 (s, 1H), 4.22 (dd, *J* = 14.0, 6.9 Hz, 2H), 3.31 (dd, *J* = 13.7, 6.7 Hz, 5H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.08 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.26, 158.47, 158.23, 152.85, 149.13,

130.98, 109.48, 109.12, 107.70, 96.77, 61.12, 45.07, 12.41.

7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (4)

Then compound **2** (1.700 g, 5.87 mmol) was added to 25 mL of 0.5 M aqueous NaOH solution and 25 mL of ethanol, After the solution was completely dissolved, then pH was adjusted to acidic by 1.0 M aqueous HCl solution. The precipitate was filtered off to afford the final product as orange red solid (0.590 g, 34.9 %).^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.29 (s, 1H), 8.64 (s, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 6.71 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.53 (d, *J* = 2.3 Hz, 1H), 3.50 (q, *J* = 7.1 Hz, 4H), 1.27 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 165.49, 164.35, 158.06, 153.78, 150.22, 131.90, 110.90, 108.58, 105.72, 96.88, 45.31, 12.37.

2-(2,4-dihydroxybenzoyl) benzoic acid (7)

A mixture of Fluorescein (1.700 g, 5.12 mmol) and 12 mL of 2.5 M aqueous NaOH solution was heated at 160 °C for 1 h. After the solution was cooled to room temperature, the mixture was dissolved in 40 mL of H_2O, and the pH was adjusted to 2 by concentrated hydrochloric acid. The mixture was kept still for 2 h. The precipitate was filtered off to afford the final product as beige solid (1.220 g, 83.8 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ12.24 (s, 1H), 10.77 (s, 1H), 8.00 (dd, *J* = 7.7, 0.9 Hz, 1H), 7.72 (td, *J* = 7.5, 1.3 Hz, 1H), 7.64 (td, *J* = 7.6, 1.3 Hz, 1H), 7.42 (dd, *J* = 7.5, 0.9 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 6.34 (d, *J* = 2.3 Hz, 1H), 6.29 (dd, *J* = 8.8, 2.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.90, 167.15, 165.46, 164.80, 140.41, 135.12, 132.73, 130.39, 130.16, 129.89, 127.86, 113.68, 108.76, 102.96.

2-(3-oxo-6-(piperazin-1-yl)-3H-xanthen-9-yl) benzoic acid (8)

Compound **7** (0.590 g，4.0 mmol)、**8** (1.020 g，3.3 mmol) were dissolved in 11.8 mL of TFA and heated to reflux and stirred for 36 h. the solvent was distilled off under reduced pressure. The mixture was extracted by ethyl acetate. The filtrate was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The mixture was dissolved in 40 mL of H_2O, and the pH was adjusted to 8 by 0.5 M aqueous NaOH solution. The mixture was kept still for 24 h. The precipitate was filtered off to afford the final product as red solid (0.620 g, 58.8 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (d, *J* = 7.7 Hz, 1H), 7.83-7.72 (m, 2H), 7.28 (d, *J* = 7.4 Hz, 1H), 6.81-6.69 (m, 3H), 6.56 (m, 3H), 3.18 (s, 4H), 2.87 (s, 4H).

2-(6-(4-(7-(diethylamino)-2-oxo-2H-chromene-3-carbonyl)piperazin-1-yl)-3-oxo-3H-xanthen-9-yl) benzoic acid (9)

To a solution of compound **4** (0.629 g, 2.46 mmol) in 30 mL of DCM/DMF (v/v, 5:1) was added compound **8** (0.978 g, 2.46 mmol), EDC•HCl (0.561 g, 2.94 mmol) then stirred at room temperature for 24 h. The solvent was removed by rotavapor, and then the product was purified through column chromatograph and obtains as a red solid (0.214 g, 13.54 %).^{1}H NMR (600 MHz, TFA/DMSO) δ 10.33 (s, 1H), 8.06 – 8.02 (m, 2H), 7.84 – 7.80 (m, 1H), 7.75 (t, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 8.9 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 2.5 Hz, 1H), 6.79 (ddd, *J* = 9.1, 4.5, 2.4 Hz, 2H), 6.72 (d, *J* = 2.1 Hz, 1H), 6.60 (dt, *J* = 8.6, 2.4 Hz, 4H), 3.75 (s, 2H), 3.50 (dd, *J* = 13.2, 6.2 Hz, 4H), 3.34 (d, *J* = 32.1 Hz, 4H), 2.58 – 2.53 (m, 2H), 1.17 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, TFA/DMSO) δ 166.72, 162.13, 156.45, 154.66, 150.24, 150.20, 149.91, 149.29, 141.99, 133.31, 128.14, 127.98, 127.05, 126.48, 124.67, 122.73, 122.14, 113.77, 111.00, 109.92, 107.69, 107.40, 106.76, 105.14, 100.26, 99.39, 94.31, 45.69, 45.12, 44.03, 42.16, 39.09, 10.28.

1-(4-(bromomethyl)-3-nitrophenyl) ethan-1-one (11)

N-bromosuccinimide (NBS, 3.600 g, 18.6 mmol) and subsequently azobisisobutyronitrile (AIBN, 0.276 g, 1.62 mmol) were added to a solution of compound **10** (3.000 g, 16.8 mmol) in acetonitrile (40 mL). The mixture was refluxed for 2.5 h and then cooled to 20 °C. The solvent was then removed under reduced pressure, toluene (90 mL) was added, and the resulting precipitate (unreacted reagents) was filtered off. The filtrate was dried over $MgSO_4$ and the solvent was removed under reduced pressure. the product was purified through column chromatograph and obtains as yellow oil(1.591 g, 36.7 %).^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.50 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 4.85 (s, 2H), 2.67 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 195.03, 148.14, 138.00, 136.97, 133.11, 132.65, 125.03, 27.93, 26.57.

1-(bromomethyl)-2-nitrobenzene (14)

N-bromosuccinimide (NBS, 3.600 g, 18.6 mmol) and subsequently azobisisobutyronitrile (AIBN, 0.276 g, 1.62 mmol) were added to a solution of

compound **13** (2.303 g, 16.8 mmol) in acetonitrile (40 mL). The mixture was refluxed for 2.5 h and then cooled to 20 °C. The solvent was then removed under reduced pressure, toluene (90 mL) was added, and the resulting precipitate (unreacted reagents) was filtered off. The filtrate was dried over $MgSO_4$ and the solvent was removed under reduced pressure. the product was purified through column chromatograph and obtains as yellow oil (1.351 g, 37.2 %). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.06 (d, *J* = 9.7 Hz, 1H), 7.66 – 7.62 (m, 1H), 7.62 – 7.59 (m, 1H), 7.54 – 7.49 (m, 1H), 4.86 (s, 2H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 148.02, 133.77, 132.84, 132.58, 129.68, 125.51, 28.92.

3'-((4-acetyl-2-nitrobenzyl)oxy)-6'-(4-(7-(diethylamino)-2-oxo-2H-chromene-3-carbonyl)piperazin-1-yl)-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (15a)

In an atmosphere of dry Ar_2, a mixture of compound **9** (0.107 g, 0.167 mmol), compound **11** (0.043 g, 0.167 mmol), Cs_2CO_3 (0.059 g, 0.183 mmol) and DCM (20 mL) was stirred at room temperature for 15 h. The solvent was removed by rotavapor, and then the product was purified through column chromatograph and obtains as a red solid (0.117 g, 85.40 %).^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.46 (s, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.88 (s, 1H), 7.69 (d, *J* = 7.4 Hz, 1H), 7.67 – 7.62 (m, 1H), 7.26 (d, *J* = 8.8 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 1H), 7.12 (d, *J* = 7.9 Hz, 1H), 6.73 (dd, *J* = 9.3, 2.8 Hz, 2H), 6.59 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.58 – 6.54 (m, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.44 – 6.40 (m, 2H), 6.12 (d, *J* = 1.9 Hz, 1H), 5.37 – 5.26 (m, 2H), 3.87 (s, 2H), 3.58 (s, 2H), 3.50 (d, *J* = 14.3 Hz, 4H), 3.38 (q, *J* = 7.0 Hz, 4H), 2.63 (s, 3H), 1.17 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 195.60, 185.30, 165.49, 165.17, 159.38, 158.70, 157.60, 154.54, 154.18, 152.10, 150.05, 147.50, 146.16, 137.99, 134.65, 134.49, 133.14, 133.09, 131.66, 130.71, 130.67, 130.19, 130.16, 129.91, 129.87, 129.18, 129.15, 124.91, 116.23, 115.57, 112.51, 111.81, 109.66, 107.93, 105.56, 99.61, 97.07, 63.87, 45.13, 29.81, 26.93, 12.54.

3'-((4-acetylbenzyl)oxy)-6'-(4-(7-(diethylamino)-2-oxo-2H-chromene-3-carbonyl)piperazin-1-yl)-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (15b)

In an atmosphere of dry Ar_2, a mixture of compound **9** (0.107 g, 0.167 mmol), compound **12** (0.036 g, 0.167 mmol), Cs_2CO_3 (0.059 g, 0.183 mmol) and DCM (20 mL)

was stirred at room temperature for 15 h. The solventwas removed by rotavapor, and then the product was purified through column chromatograph and obtains as a red solid (0.103 g, 79.84 %). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.97 (s, 1H), 7.77 (d, *J* = 1.8 Hz, 1H), 7.75 (d, *J* = 1.9 Hz, 1H), 7.73 – 7.65 (m, 2H), 7.34 (d, *J* = 8.9 Hz, 1H), 7.27 (d, *J* = 7.7 Hz, 2H), 6.98 (d, *J* = 8.1 Hz, 2H), 6.81 (d, *J* = 4.1 Hz, 1H), 6.78 (d, *J* = 3.5 Hz, 1H), 6.63 (ddd, *J* = 8.9, 4.6, 2.4 Hz, 2H), 6.58 (d, *J* = 2.4 Hz, 1H), 6.50 (dt, *J* = 5.1, 2.4 Hz, 2H), 6.27 (d, *J* = 1.9 Hz, 1H), 4.99 (d, *J* = 2.2 Hz, 2H), 3.72 (q, *J* = 7.0 Hz, 2H), 3.65 (s, 2H), 3.55 (t, *J* = 6.6 Hz, 4H), 3.46 (t, *J* = 7.1 Hz, 4H), 2.64 (s, 3H), 1.23 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 196.83, 184.16, 164.42, 164.35, 158.25, 157.70, 156.46, 153.47, 152.98, 150.97, 149.14, 145.03, 138.27, 135.93, 133.27, 131.74, 130.33, 129.49, 129.20, 129.10, 129.06, 128.61, 127.97, 127.91, 127.58, 127.16, 115.32, 114.41, 111.67, 110.57, 108.52, 106.79, 104.40, 98.79, 95.93, 65.67, 46.20, 45.63, 45.41, 43.99, 40.91, 25.80, 11.41.ESI-MS cald for compound **15b** ($C_{47}H_{42}N_3O_8^+$, positive): 776.29, found: 776.41.

3'-(4-(7-(diethylamino)-2-oxo-2H-chromene-3-carbonyl)piperazin-1-yl)-6'-((2-nitrobenzyl)oxy)-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (15c)

In an atmosphere of dry Ar_2, a mixture of compound **9** (0.107 g, 0.167 mmol), compound **14** (0.036 g, 0.167 mmol), Cs_2CO_3 (0.059 g, 0.183 mmol) and DCM (20 mL) was stirred at room temperature for 15 h. The solvent was removed by rotavapor, and then the product was purified through column chromatograph and obtains as a red solid (0.103 g, 79.84 %). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.23 (d, *J* = 7.8 Hz, 1H), 7.94 (d, *J* = 7.2 Hz, 1H), 7.88 (s, 1H), 7.71 – 7.65 (m, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.43 (s, 2H), 7.26 (d, *J* = 8.9 Hz, 1H), 7.22 (d, *J* = 7.4 Hz, 1H), 7.05 – 7.00 (m, 1H), 6.76 (dd, *J* = 9.4, 3.6 Hz, 2H), 6.61 (d, *J* = 9.0 Hz, 1H), 6.56 (dd, *J* = 7.7, 4.6 Hz, 2H), 6.46 (d, *J* = 10.5 Hz, 1H), 6.43 (s, 1H), 6.24 (s, 1H), 5.26 (s, 2H), 3.86 (s, 2H), 3.56 (s, 2H), 3.51 (s, 4H), 3.38 (q, *J* = 7.1 Hz, 4H), 1.17 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 164.36, 164.10, 158.25, 157.62, 156.48, 153.60, 150.98, 146.24, 145.11, 133.31, 133.03, 131.85, 130.53, 129.48, 129.19, 129.07, 128.99, 128.72, 128.53, 128.14, 124.19, 115.17, 114.38, 111.71, 110.91, 108.53, 106.79, 104.54, 98.54, 95.92, 63.18,

46.28, 45.41, 44.00, 11.40. ESI-MS cald for compound **15c** ($C_{45}H_{39}N_4O_9^+$, positive): 779.27, found: 779.27.

2. Preparation of the test solution

UV/vis spectra were recorded on an Agilent Cary 60 Ultraviolet-visible spectrophotometer with 2 nm resolution at room temperature. The fluorescence spectra were taken on a Thermo Scientific Lumina fluorescence spectrophotometer. Fluorescence properties of compounds were evaluated in EtOH: HEPES (1:1, v/v) at different pH values. Stock solution of compounds were prepared at the concentration of 10^{-3} mol/L in 25 mL of EtOH and then diluted to the desired concentration. The pH value of solution was adjusted by adding small aliquots of aqueous solutions of HCl or NaOH. For the fluorescence measurements, excitation wavelength was set to 400 nm (slit width = 5/5 nm) and the emission was acquired from 410 nm to 700 nm.

3. Cell culture and fluorescence imaging

Hela cells were seeded in a 24-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5 % CO_2 and 95 % air at 37 °C for 36 h. Compound **15a** to be cultured was dissolved in DMSO, Hela cells were then incubated with the compound **15a** (10 μmol/L) for 30 min at 37 °C. After washing with physiological saline three times to remove the remaining compound **15a**, put the petri dish into Olympus FV1000 laser confocal microscope for fluorescence imaging. The excitation wavelength was set as λex=405 nm, and the images of corresponding wavelength channels were collected respectively.

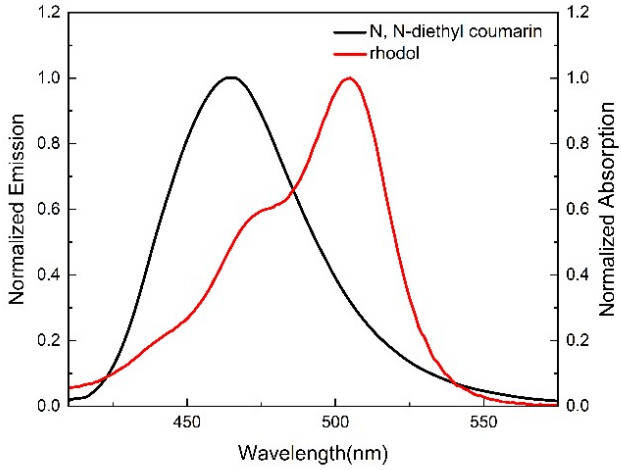


Fig. S1 The absorption spectrum of N, N-diethyl coumarin (**4**) overlaps well with the emission spectrum of rhodol (**8**).

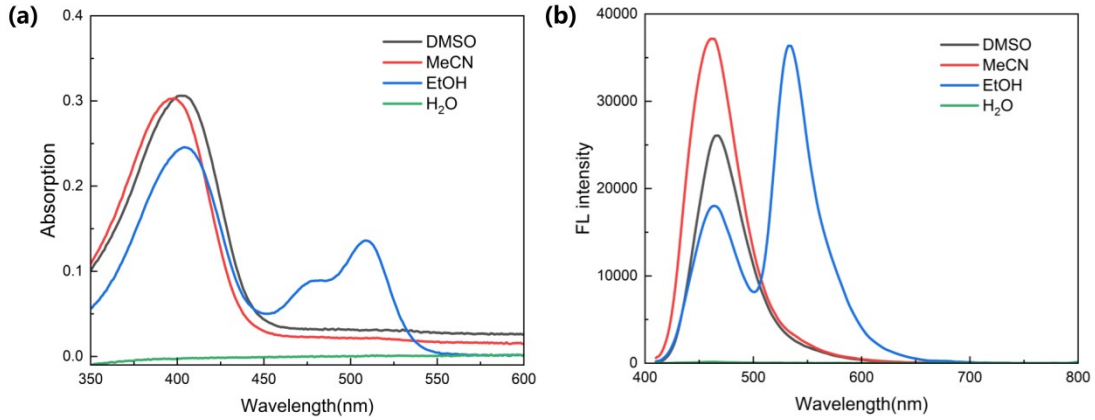


Fig. S2 The UV spectrum and fluorescence spectrum of compound **9** in different solution.

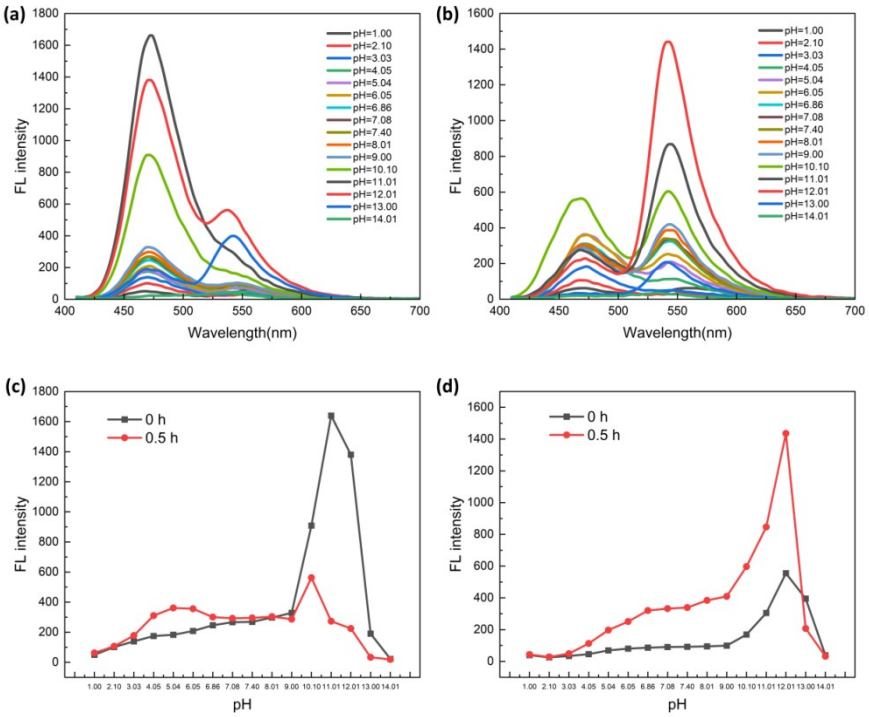


Fig. S3 The fluorescence property of compound **15a** at various pH before and after photo illumination with UV light in HEPES, containing 50% EtOH as a cosolvent (Ex = 400 nm). (a) The emission spectra of **15a** at various pH; (b) The emission spectra of **15a** at various pH after photo illumination for 30 min with UV light; (c) The fluorescence intensity of **15a** at 469 nm at various pH before and after photo illumination for 30 min with UV light; (d) The fluorescence intensity of **15a** at 540 nm at various pH before and after photo illumination for 30 min with UV light.

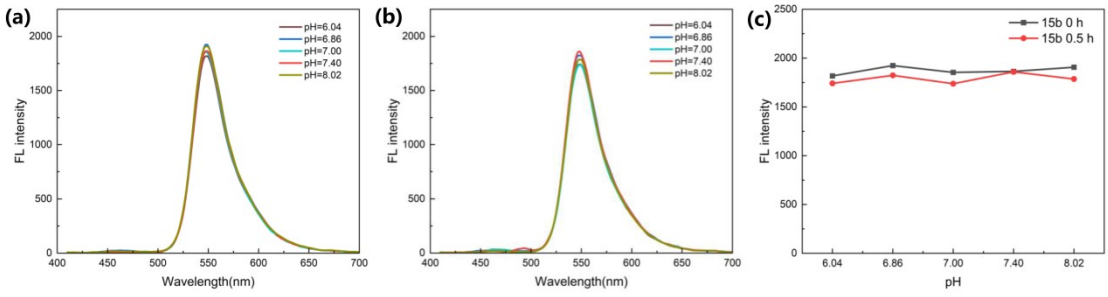


Fig. S4 The fluorescent properties of compound **15b** at pH = 6-8 before and after photo illumination with UV light in HEPES, containing 50% EtOH as a cosolvent (Ex = 400 nm). (a) The emission spectra of **15b** before the illumination; (b) The emission spectra of **15b** at pH=6-8 after photo illumination for 30 min with UV light; (c) The fluorescence intensity of **15b** at 540 nm at pH=6-8 before and after photo illumination for 30 min with UV light.

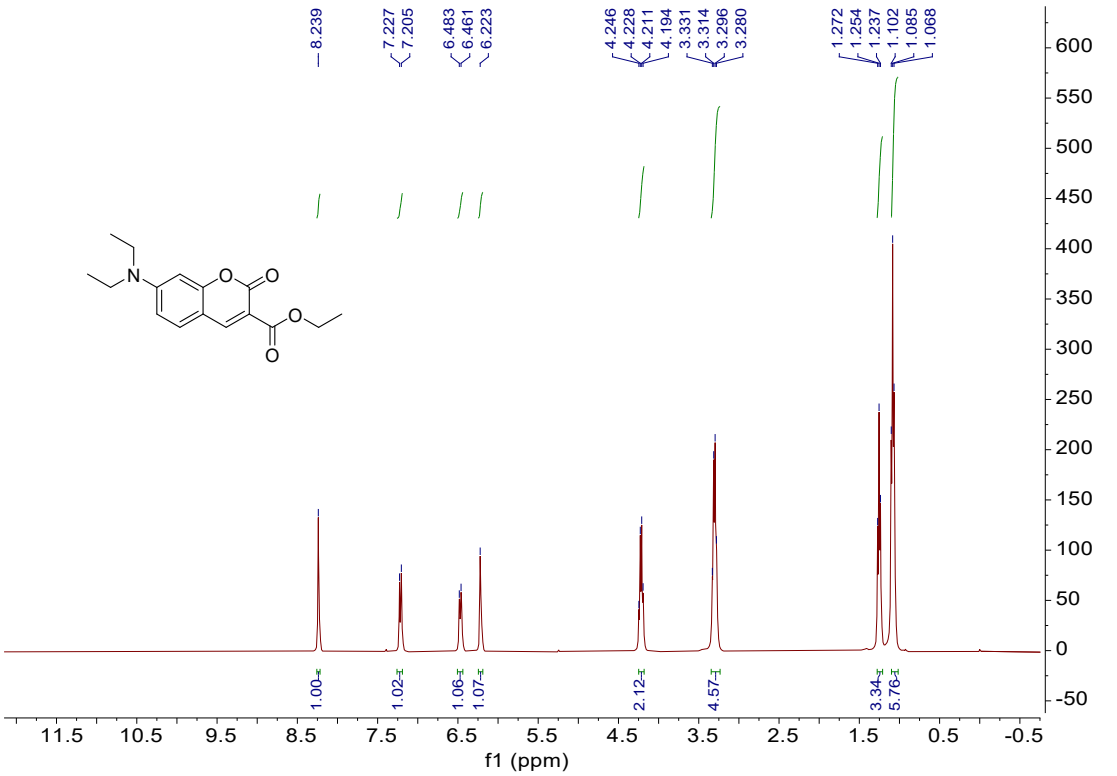


Fig. S5 ^{1}H NMR of compound **3**.

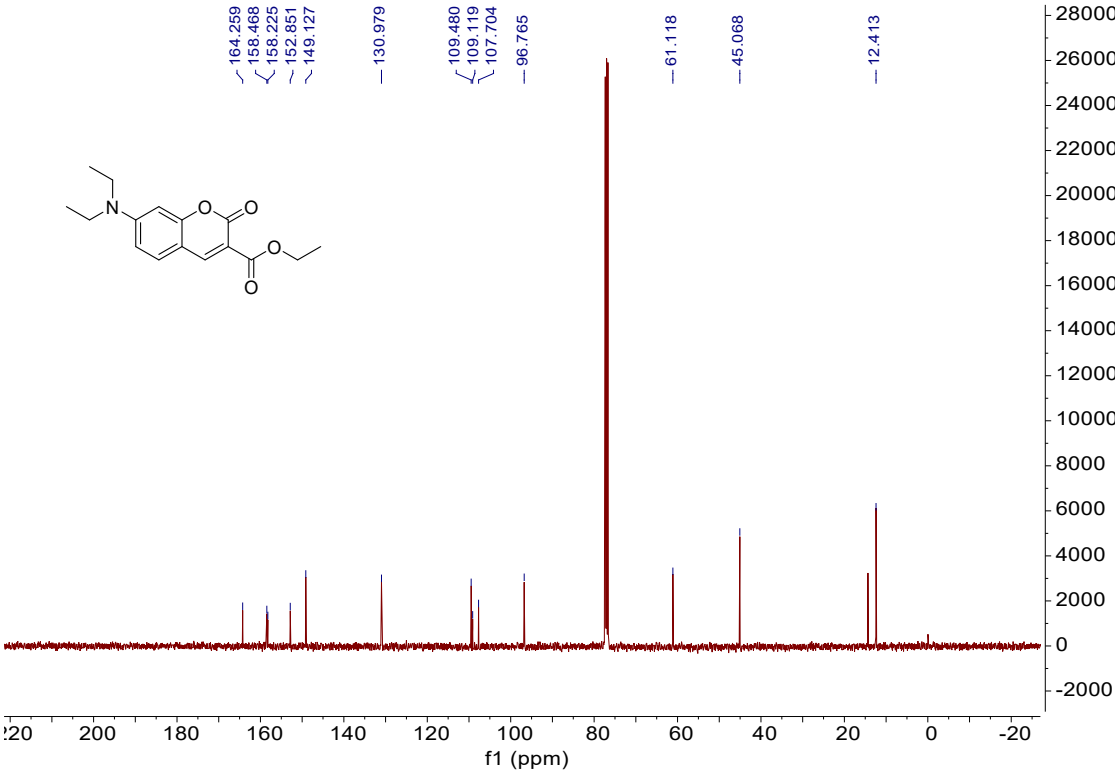


Fig. S6 ^{13}C NMR of compound **3**.

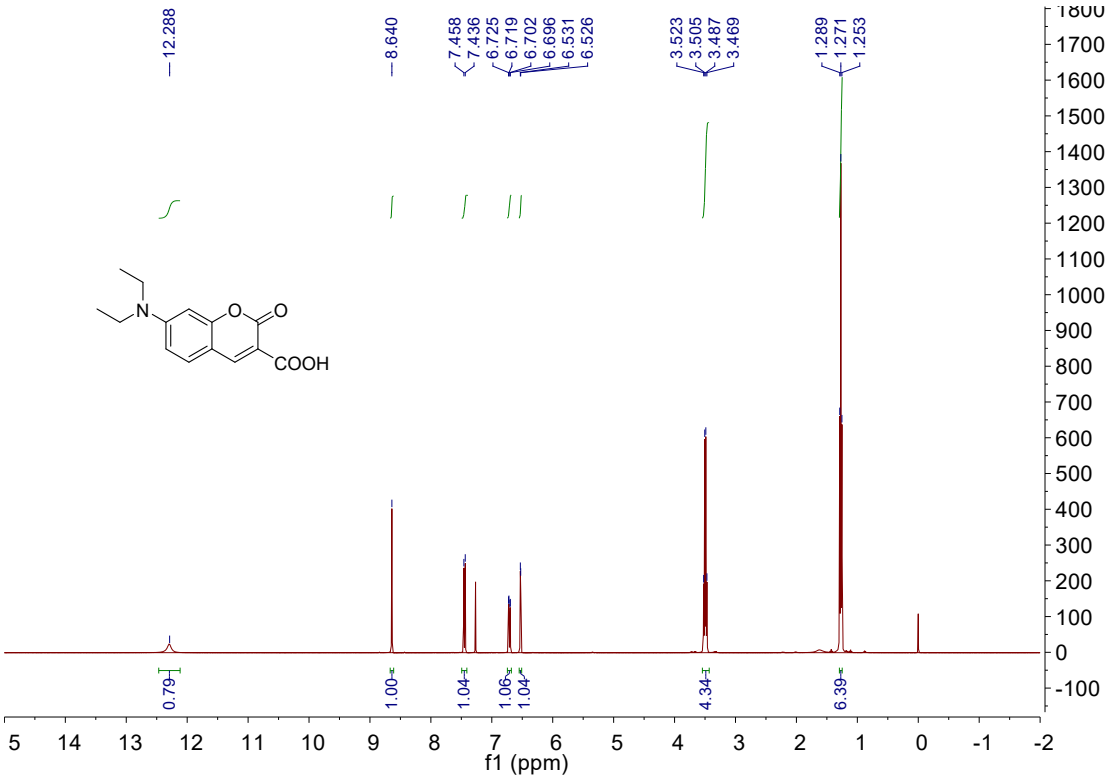


Fig. S7 ^{1}H NMR of compound **4**.

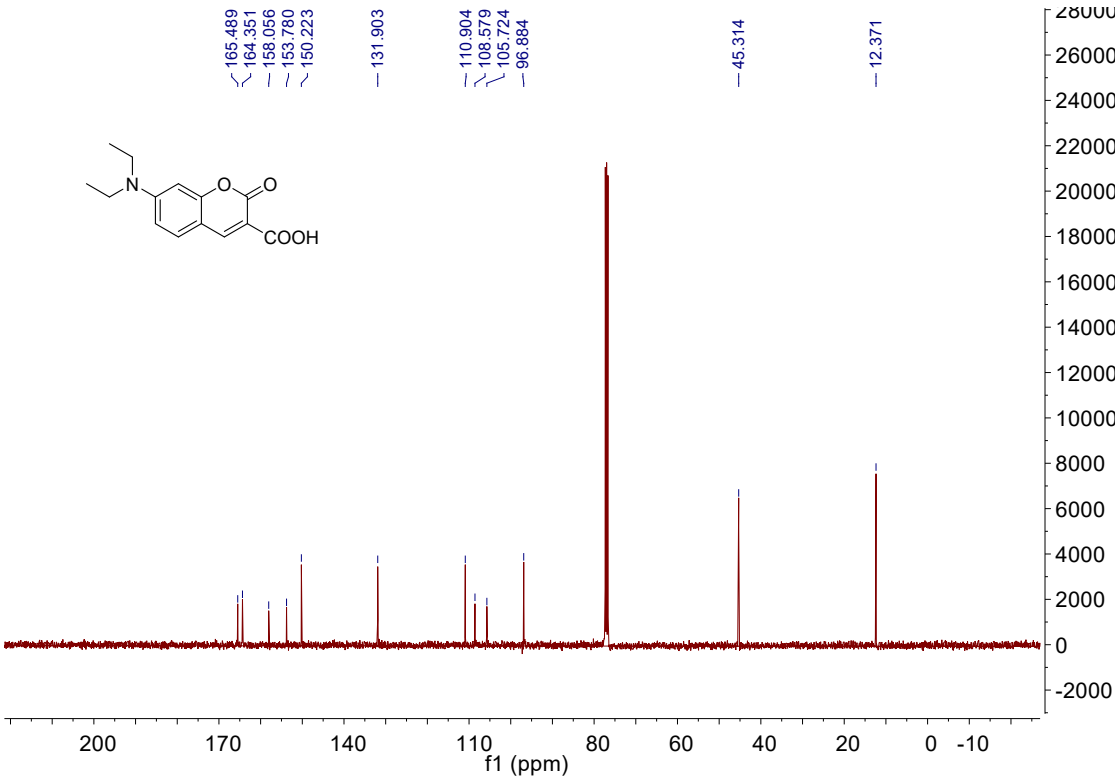


Fig. S8 ^{13}C NMR of compound **4**.

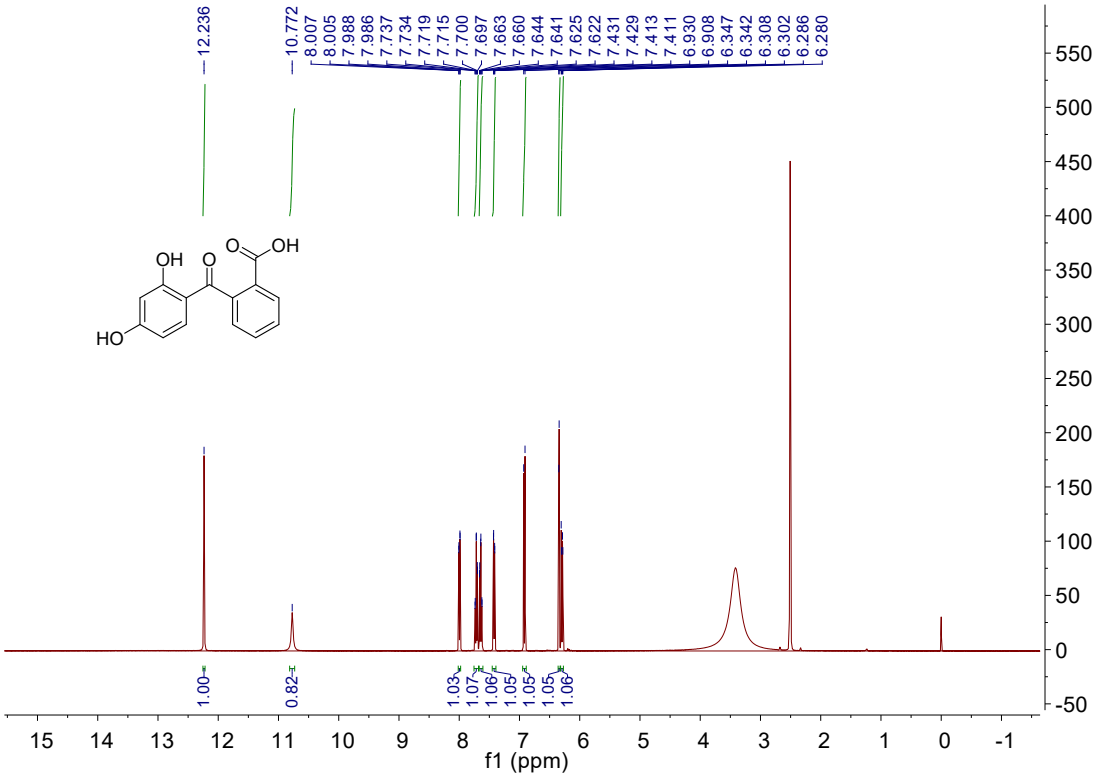


Fig. S9 ^{1}H NMR of compound **7**.

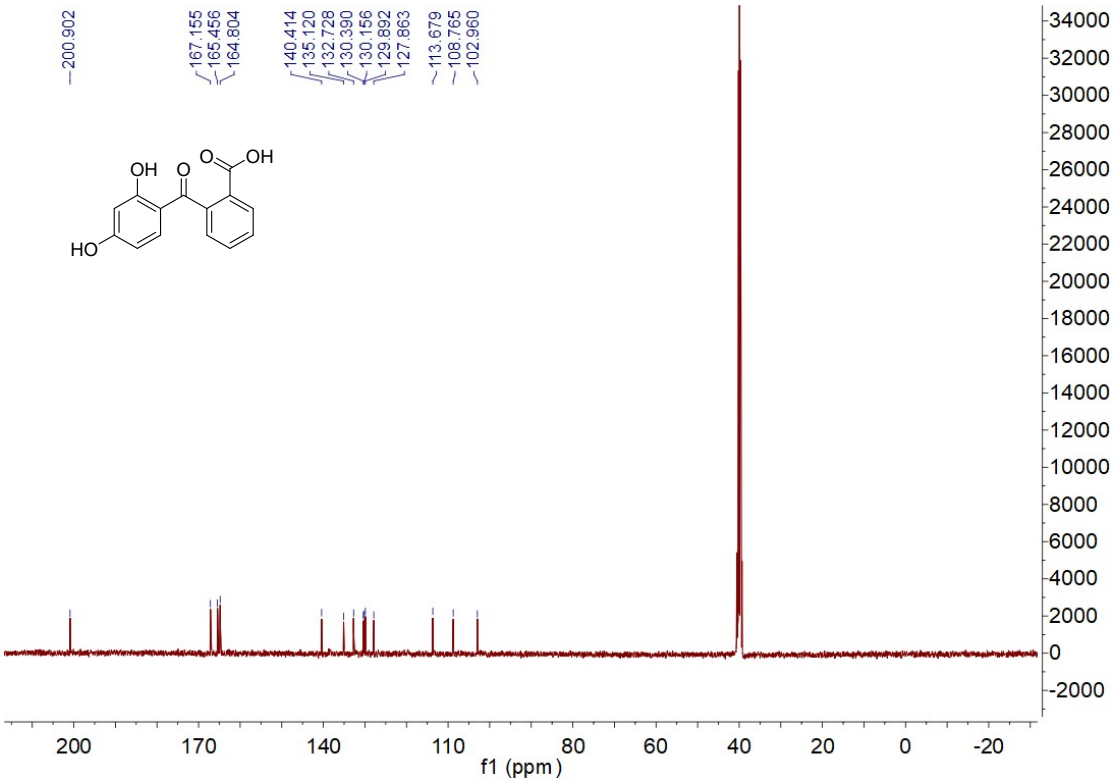


Fig. S10 ^{13}C NMR of compound **7**.

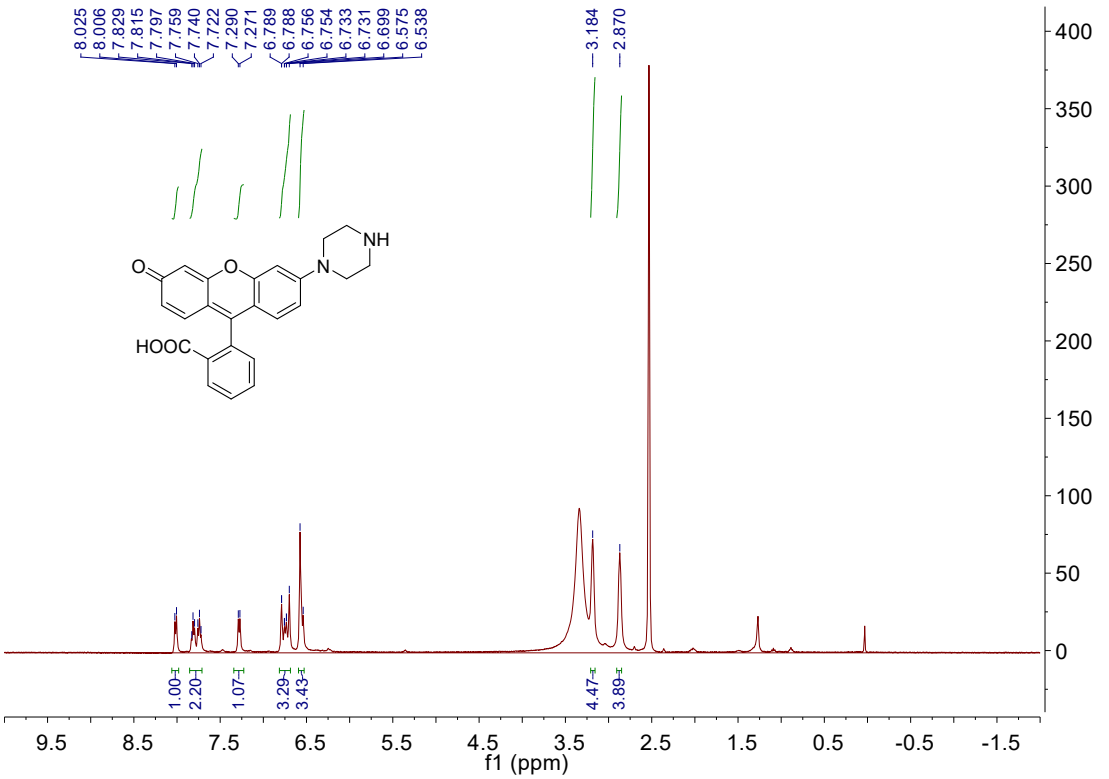


Fig. S11 ^{1}H NMR of compound **8**.

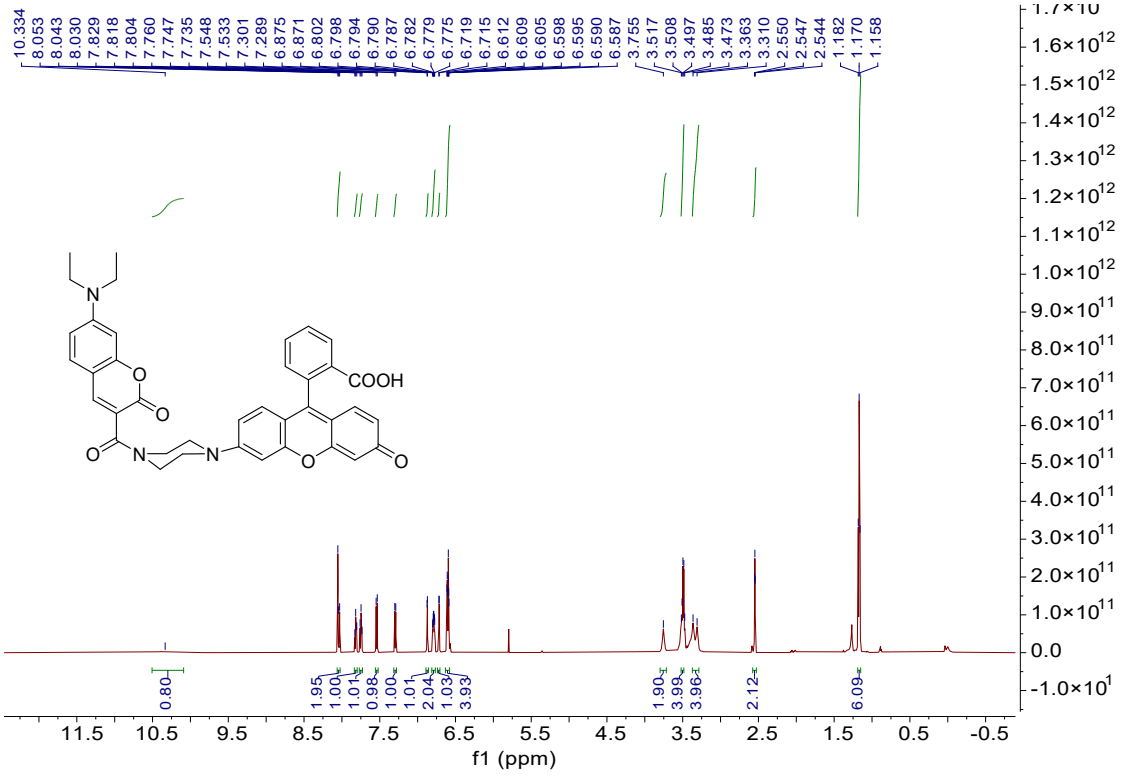


Fig. S12 ^{1}H NMR of compound **9**.

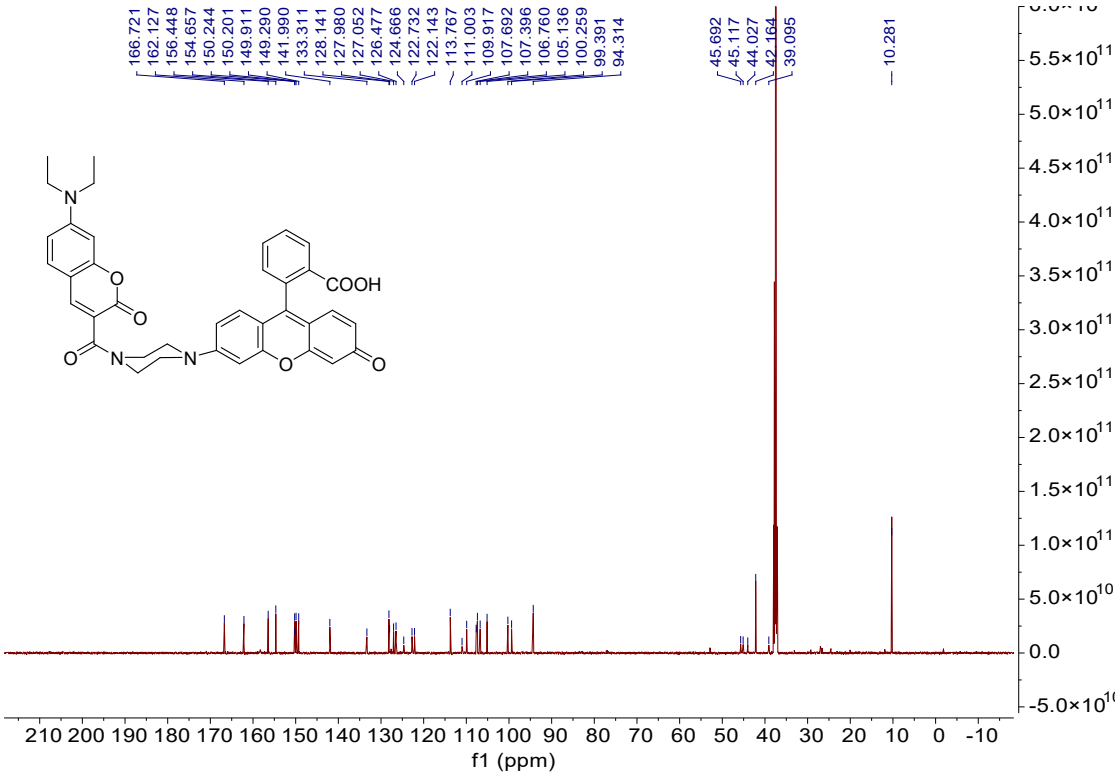


Fig. S13 ^{13}C NMR of compound **9**.

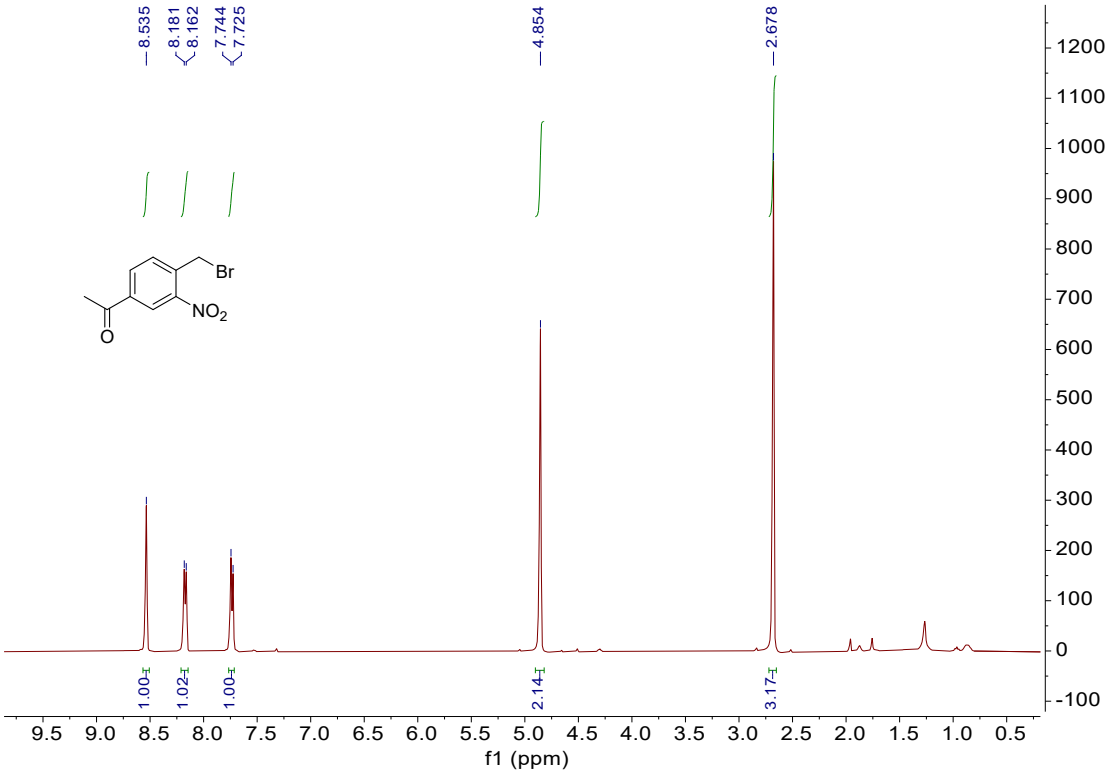


Fig. S14 ^{1}H NMR of compound **11**.

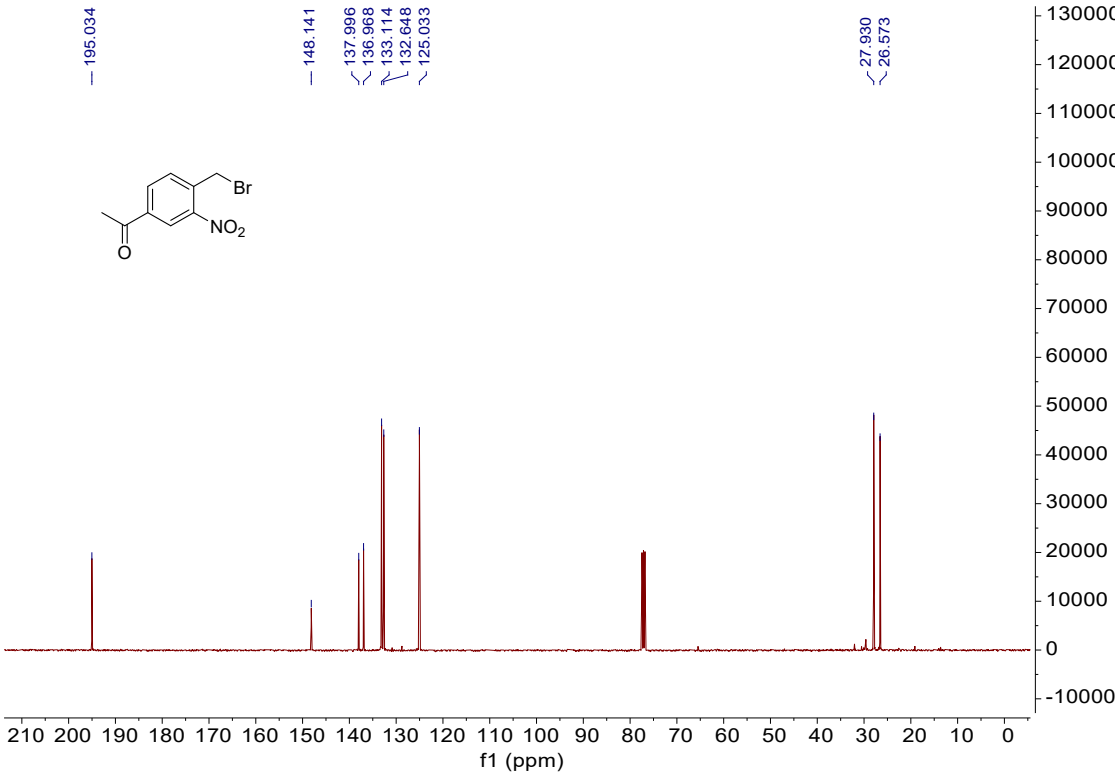


Fig. S15 ^{13}C NMR of compound **11**.

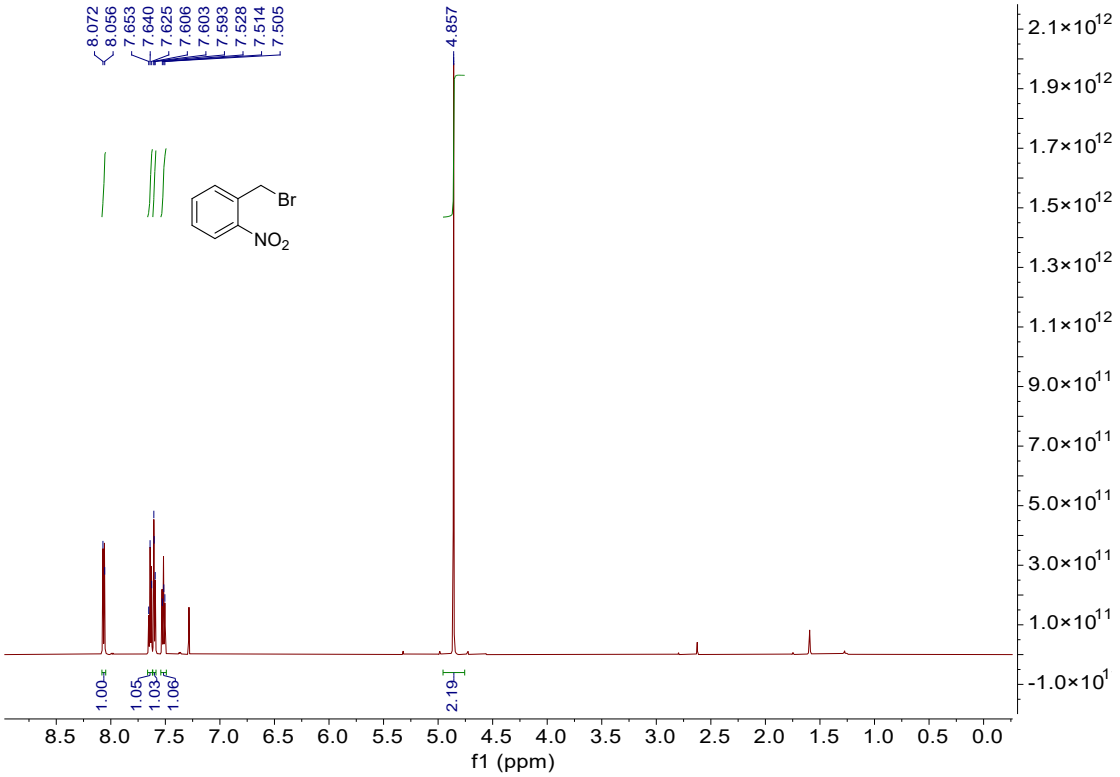


Fig. S16 ^{1}H NMR of compound **14**.

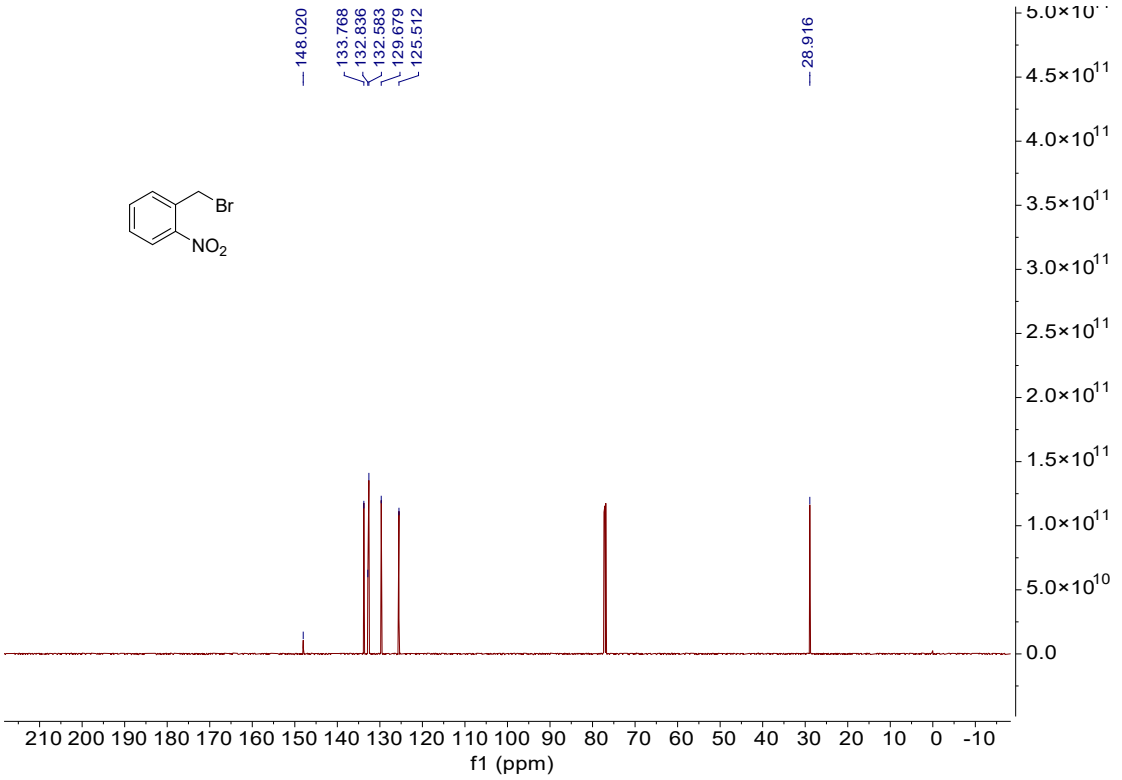


Fig. S17 ^{13}C NMR of compound **14**.

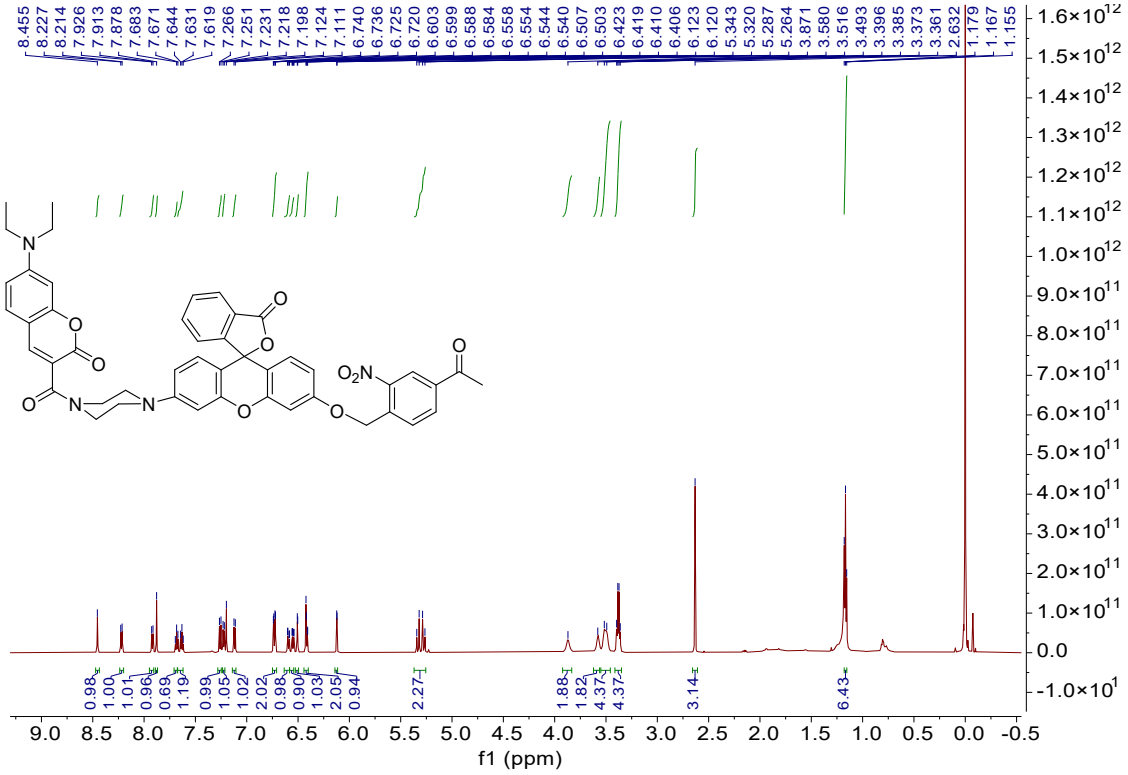


Fig. S18 ^{1}H NMR of compound **15a**.

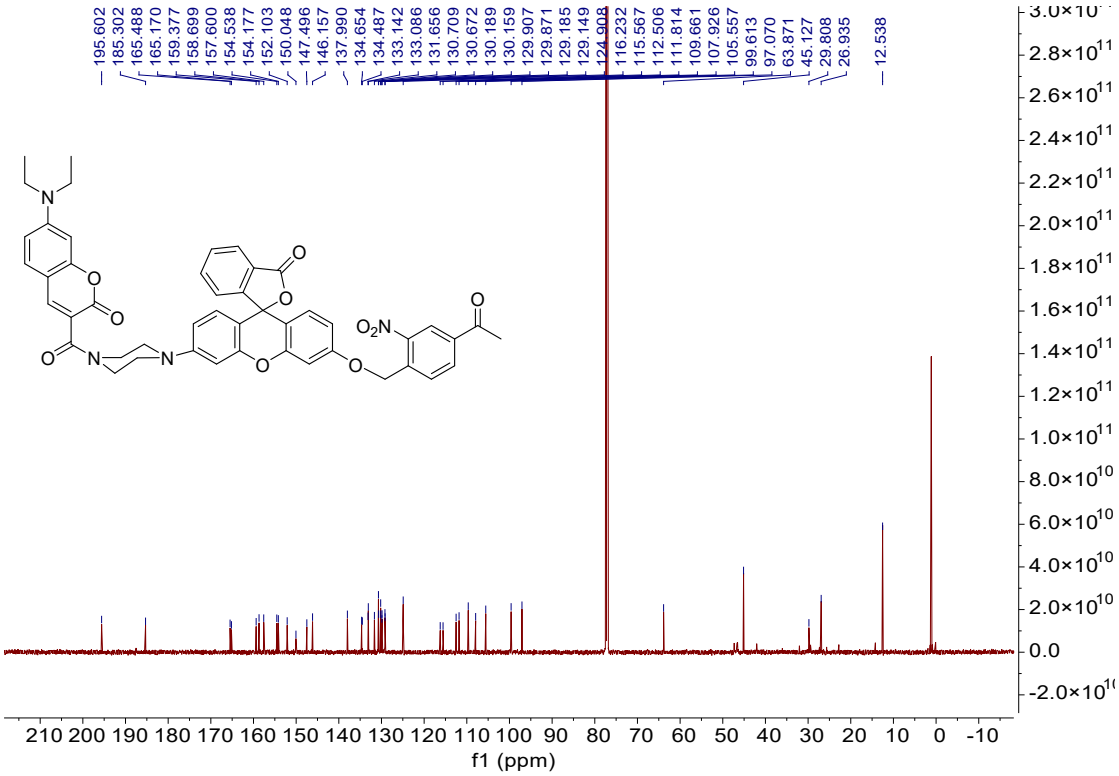


Fig. S19 ^{13}C NMR of compound **15a**.

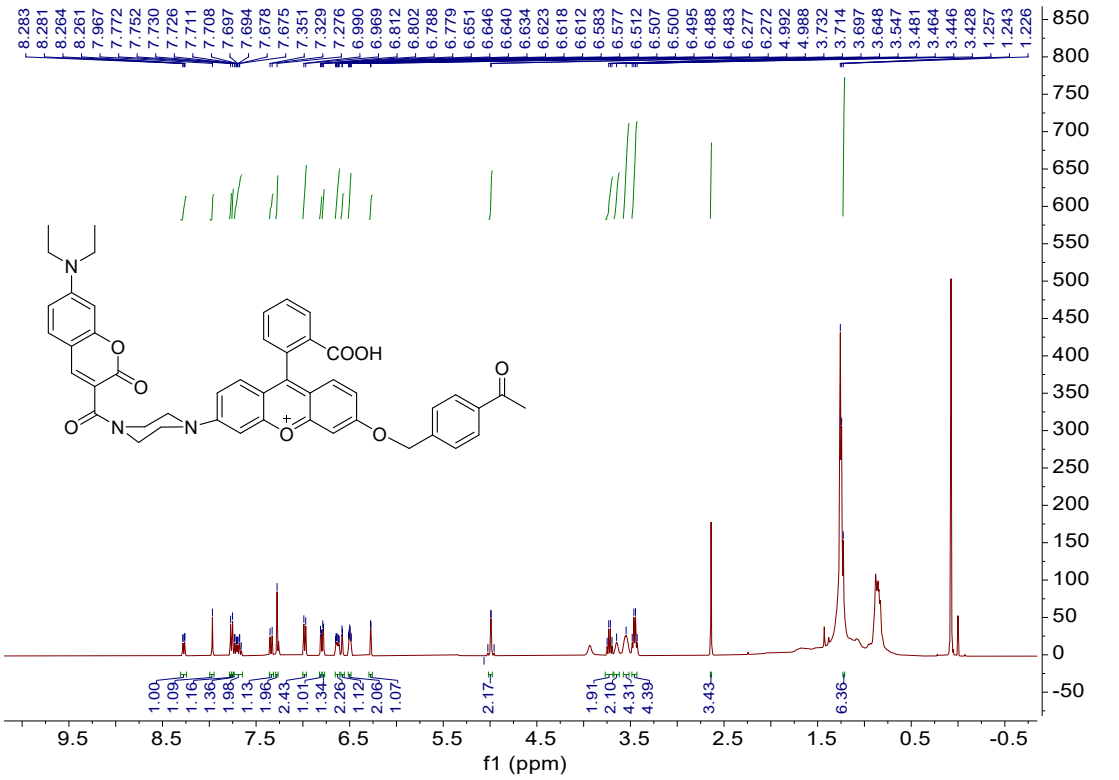


Fig. S20 ^{1}H NMR of compound **15b**.

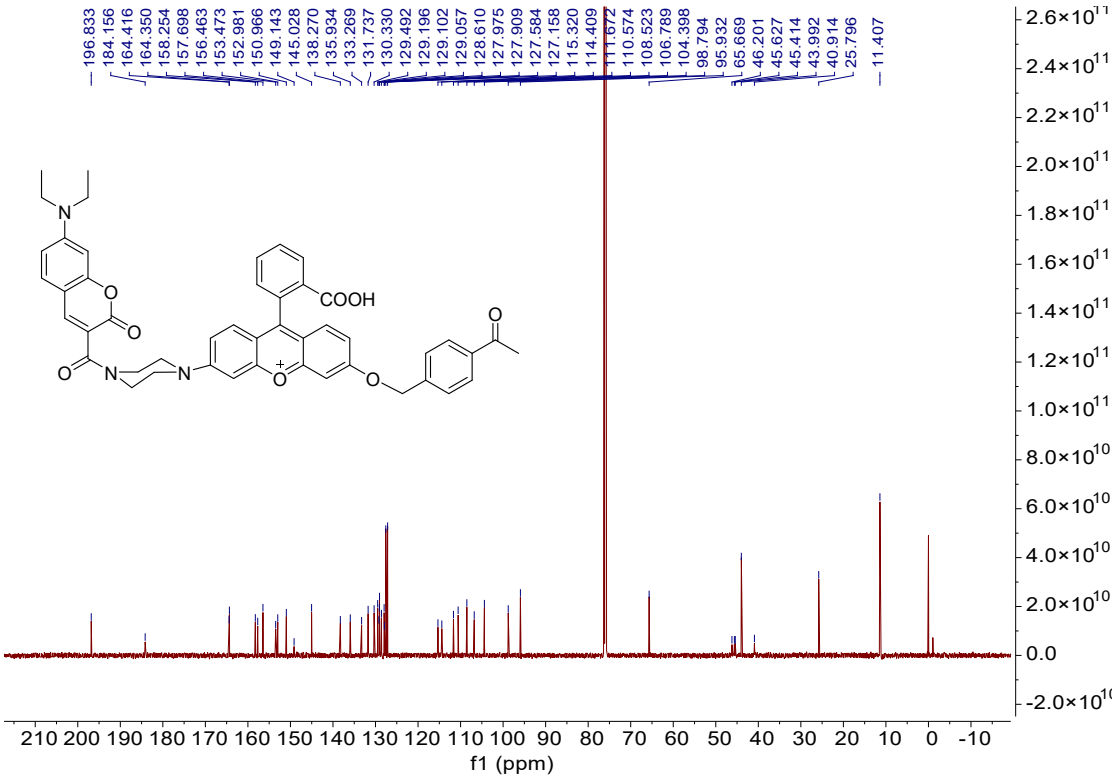
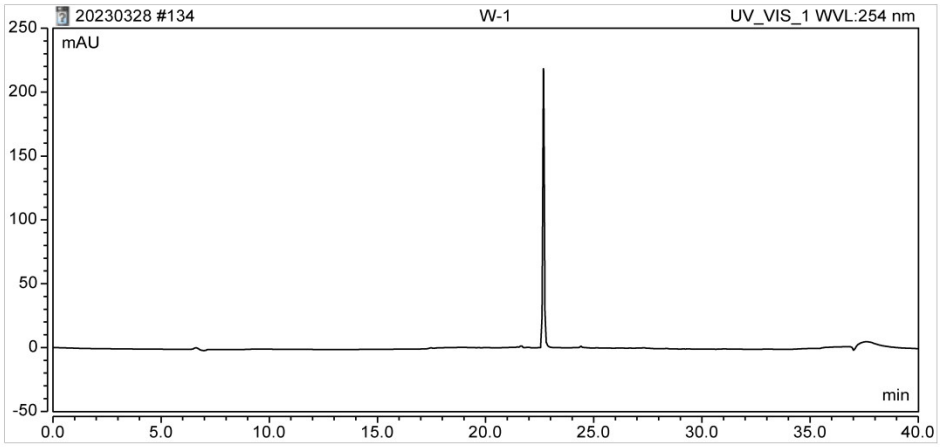


Fig. S21 ^{13}C NMR of compound **15b**.



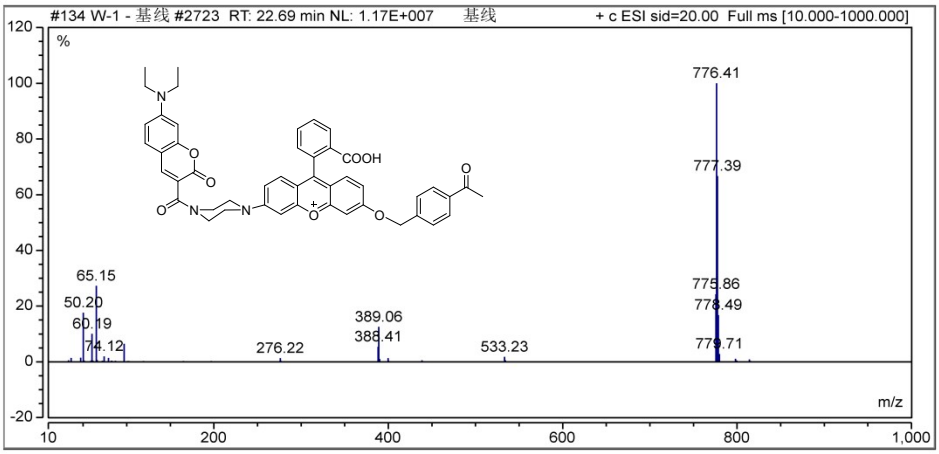


Fig. S22 HPLC-MS of compound **15b**.

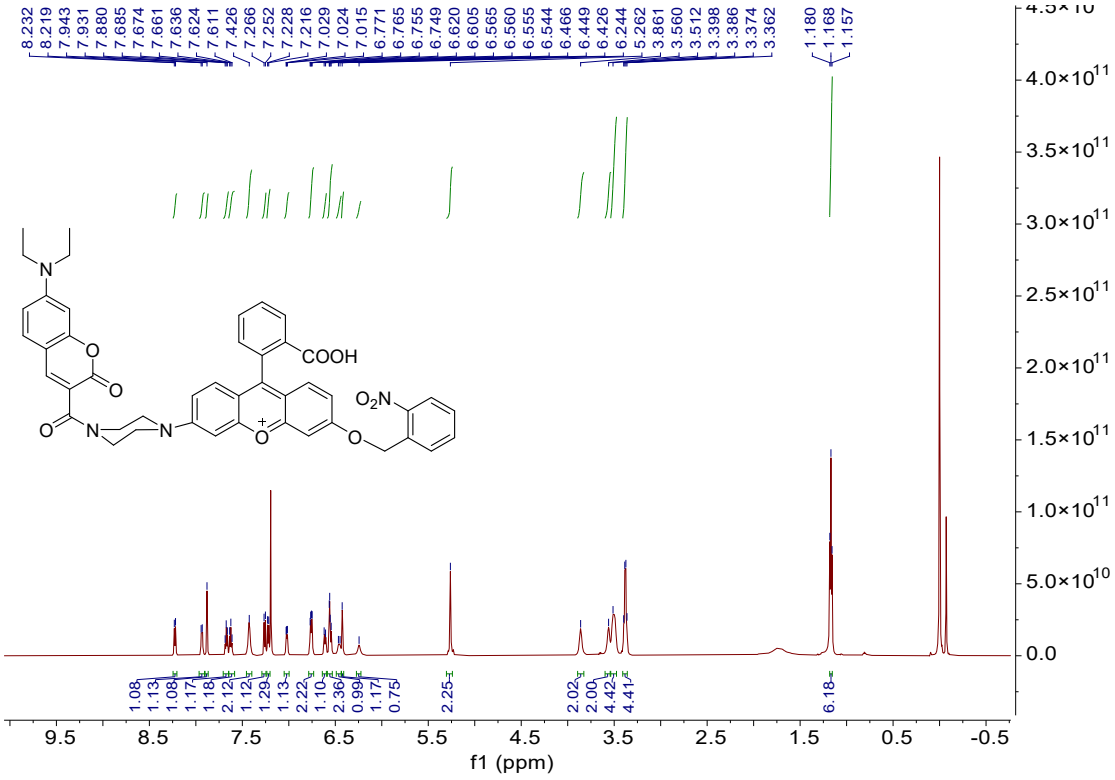


Fig. S23 ^{1}H NMR of compound **15c.**

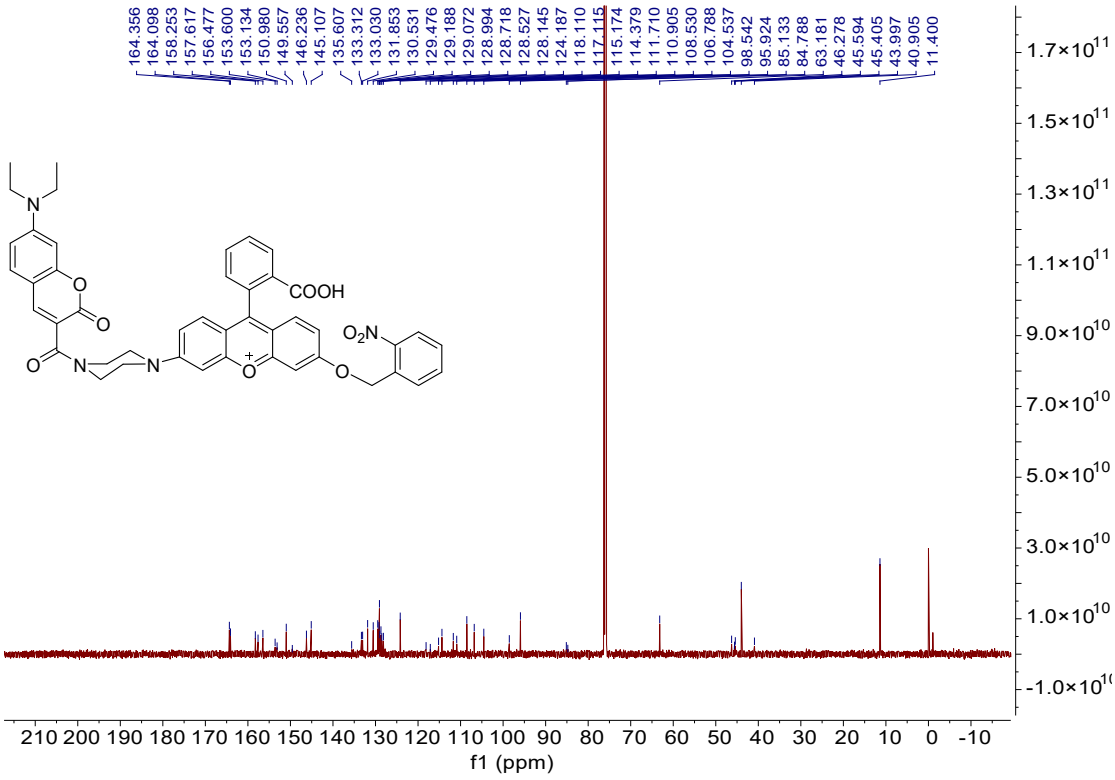


Fig. S24 ^{13}C NMR of compound **15c.**

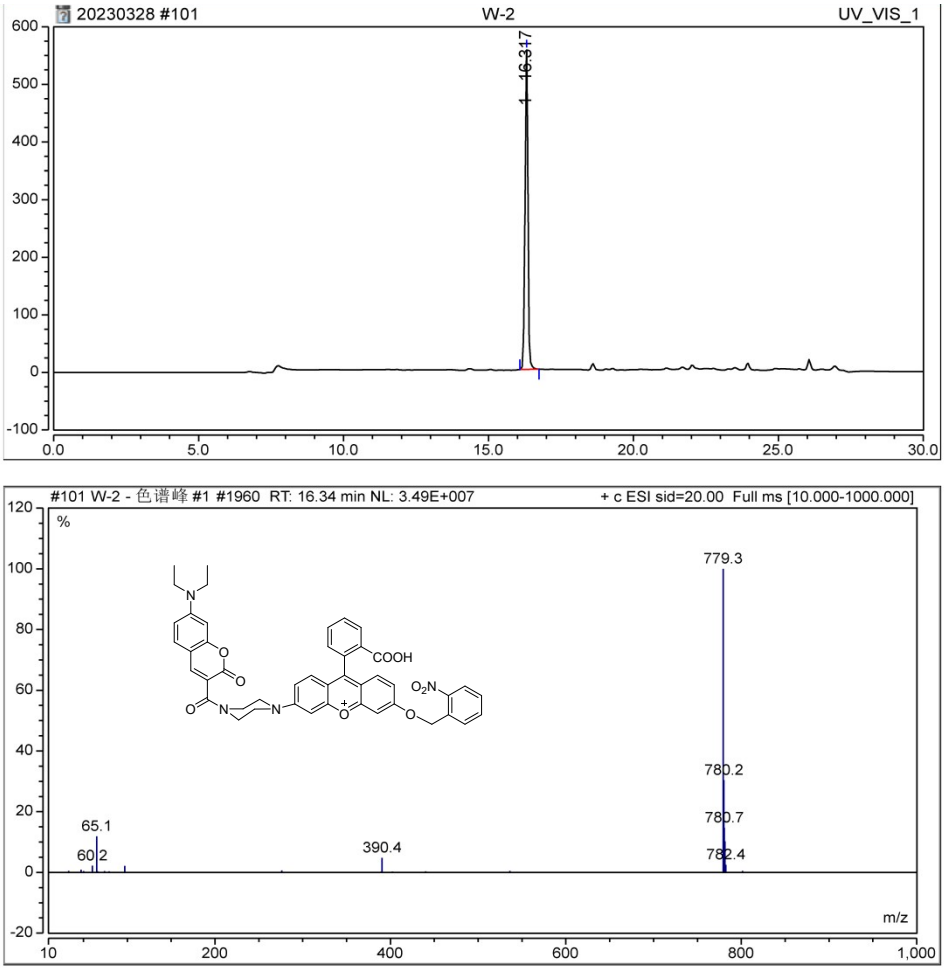


Fig. S25 HPLC-MS of compound **15c**.