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Supporting Information

An 'AND'-based ratiometric fluorescence probe for the sequential detection of biothiols and hypochlorous acid

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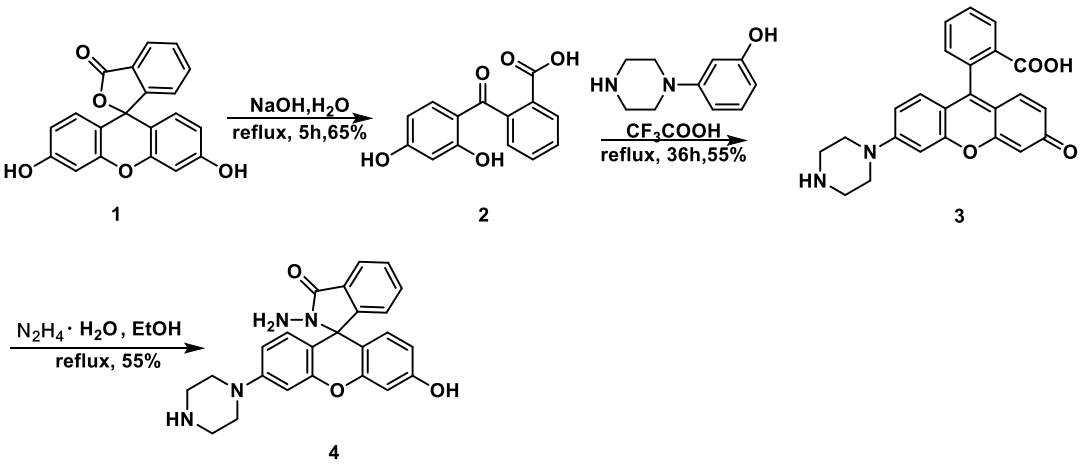
1. Synthesis and Characterization



Synthesis and Characterization of 2

NaOH (50g, 1.25 mol) was dissolved in 100 mL ultrapure water under ice bath. Then the fluorescein (compound 2-5, 10 g, 30 mmol) was added to the NaOH solution and refluxed for 5 h. After cooling down, adjusted the pH to 7 with concentrated hydrochloric acid and extracted with ether (3 × 100 mL). Discard ed the ether solution and the aqueous solution was further adjusted to pH = 2 with concentrated hydrochloric acid. The acidified aqueous was then extracted with ether (3 × 100 mL) and dried over anhydrous sodium sulfate. The organic solvent was removed with a rotary evaporator to obtain the crude product as a brown solid. The brown solid was recrystallized from hot water to obtain 5 g of crystals with a yield of 65%. ^{1}H NMR (400 MHz, DMSO–d_6）: δ 13.14 (s, 1H), 12.20 (s, 1H), 10.69 (s, 1H), 7.96 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 30.3, 7.2 Hz, 2H), 7.39 (d, J = 7.3 Hz, 1H), 6.88 (d, J = 8.7 Hz, 1H), 6.36 – 6.16 (m, 2H). ^{13}C NMR (100 MHz, DMSO–d_6): δ 200.9, 167.1, 165.4, 164.8,140.4, 135.1, 132.7, 130.4, 130.1, 129.8, 127.8, 113.7, 108.7, 102.9. HRMS（ESI）calcd. for $C_{14}H_9O_5$, [M – H]$^-$:257.0455, found: 257.0454.

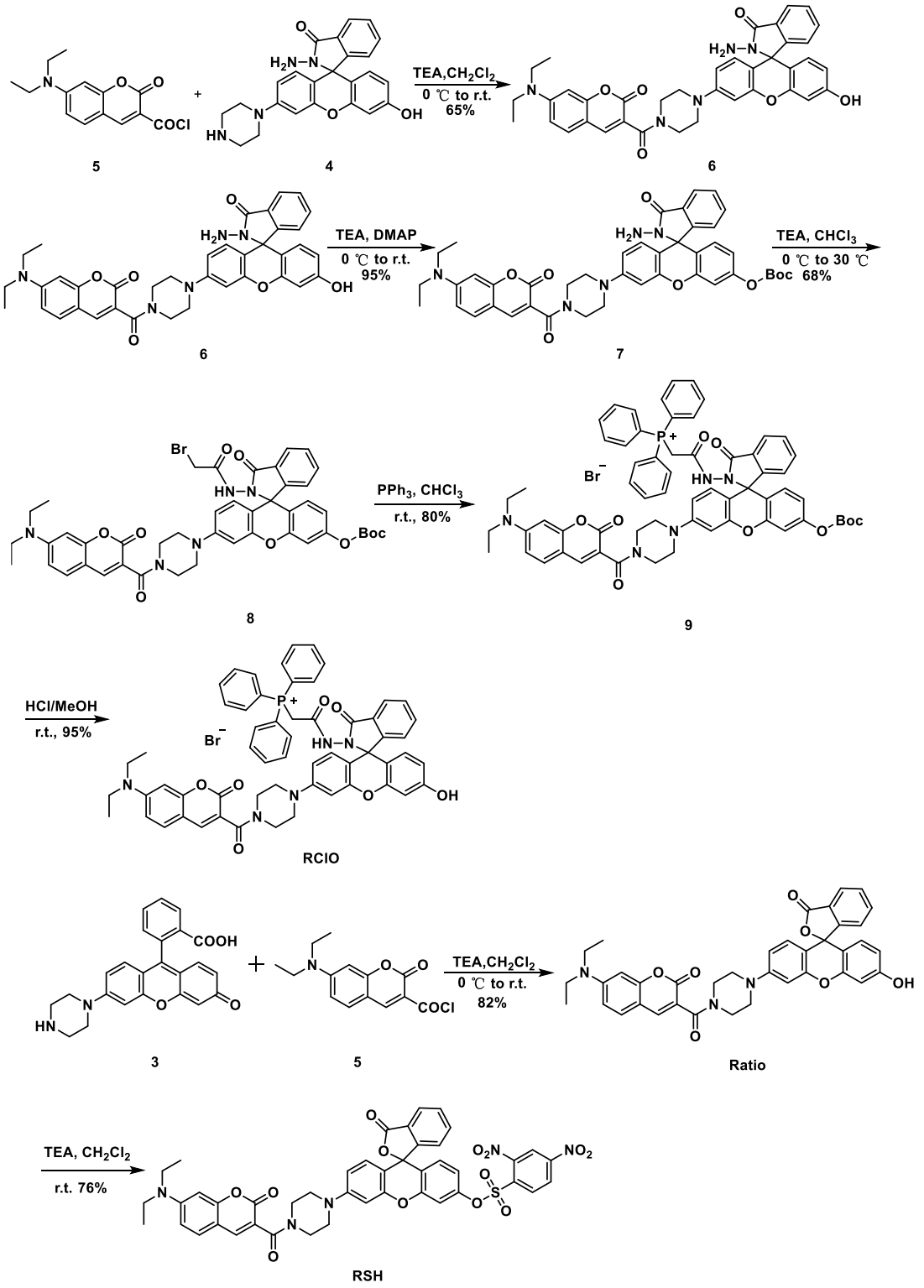
Synthesis and Characterization of 3

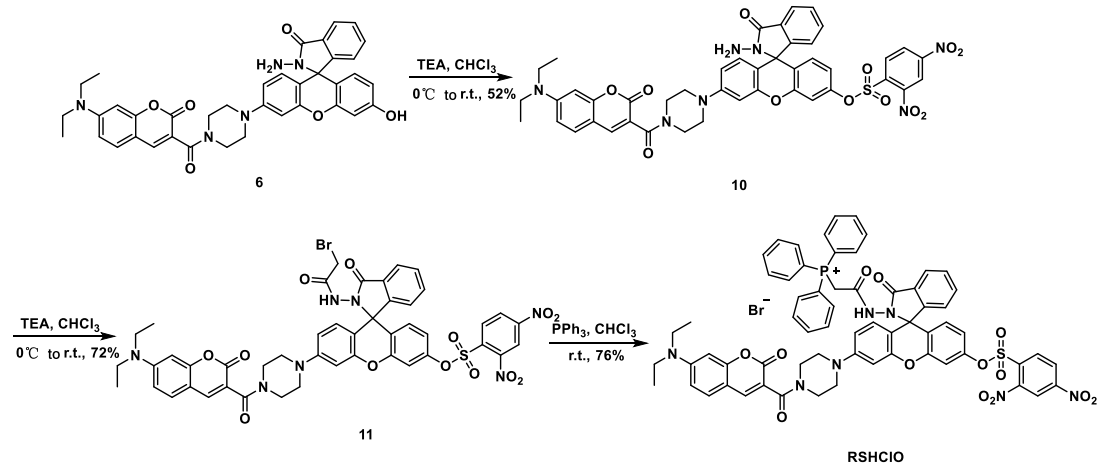
Compound 2 (4 g, 15.4 mmol) and m-hydroxyphenylpiperazine (25.7 mL, 12.84 mmol) were dissolved in 50 mL of trifluoroacetic acid and heated to reflux. The reaction was complete about 36 hours later. The solvent was distilled off under reduced pressure to obtain a crude product. The crude product was separated through 300-400 mesh silica

gel column chromatography, and dichloromethane / methanol = 30: 1 (v / v) was used as eluent to obtain compound 2-7 (3.762 g , 61%). ^{1}H NMR (400 MHz, DMSO–d_6) δ 7.77 – 7.71 (m, 1H), 7.49 – 7.40 (m, 2H), 6.96 – 6.91 (m, 1H), 6.68 – 6.52 (m, 3H), 6.39 (ddd, *J* = 19.0, 8.7, 2.1 Hz, 3H), 4.33 (s, 2H), 3.13 (s, 1H), 3.08 – 2.96 (m, 4H), 2.82 – 2.69 (m, 4H), 2.47 (dt, *J* = 3.5, 1.7 Hz, 2H). HRMS (ESI) calcd. for $C_{24}H_{21}N_2O_4$, $[M+H]^+$: 401.1501, found: 401.1494.

Synthesis of compound **4**:

Compound 3 (0.8 g, 2.0 mmol) was dissolved in 30 mL of ethanol, and then excess hydrazine hydrate (40 mL) was added dropwise. The reaction was then heated to reflux and stopped 6 hours later. The solvent was distilled off under reduced pressure, and the crude product was separated through 300-400 mesh silica gel column chromatography using dichloromethane / methanol = 15: 1 (v / v) as the eluent to obtain 2-8 (513.9 mg, 62%). ^{1}H NMR（400 MHz, DMSO–d_6）δ 7.77 – 7.71 (m, 1H), 7.49 – 7.40 (m, 2H), 6.96 – 6.91 (m, 1H), 6.68 – 6.52 (m, 3H), 6.45 – 6.31 (m, 3H), 4.33 (s, 2H), 3.13vs, 1H), 3.03 (dd, *J* = 6.4, 3.8 Hz, 4H), 2.76 (dd, *J* = 6.4, 3.7 Hz, 4H), 2.47 (s, 2H). HRMS（ESI）calcd. for $C_{24}H_{23}N_4O_3$, $[M+H]^+$: 415.1770, found:415.1777.


TEA,CH2Cl2
0 ℃ to r.t.
65%
5
4
6
TEA, DMAP
0 ℃ to r.t.
95%
TEA, CHCl3
0 ℃ to 30 ℃
Boc 68%
7
PPh3, CHCl3
r.t., 80%
8
9
HCl/MeOH
r.t., 95%
RClO
TEA,CH2Cl2
0 ℃ to r.t.
82%
3
Ratio
TEA, CH2Cl2
r.t. 76%
RSH



Scheme S1. Synthetic route of **RSH, RClO** and **RSHClO**.

Synthesis of compound 6:

Compounds 4 (2.1 g, 5 mmol) and triethylamine (TEA, 1.6 mL) were dissolved in 40 mL of anhydrous dichloromethane, and then the intermediates 5 (1.21 g, 4.4 mmol) of anhydrous dichloromethane (5 mL) was added to the solution in portions in an ice bath. After the addition was completed, the ice bath was removed and the reaction was returned to room temperature with continued stirring overnight. Then, the solvent was distilled off under reduced pressure and the crude product was directly subjected to silica gel column chromatography. Dichloromethane / ethyl acetate = 5: 1 (v / v) was used as an eluent to obtain a yellow solid 6 (2.513 g). Yield: 76.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 2H), 7.39 (s, 2H), 7.26 (d, *J* = 8.8 Hz, 1H), 6.95 (s, 1H), 6.73 (s, 1H), 6.64 (s, 1H), 6.48 (ddd, *J* = 32.7, 25.5, 10.5 Hz, 6H), 3.83 (s, 2H), 3.51 (s, 2H), 3.38 (d, *J* = 6.2 Hz, 4H), 3.22 (s, 4H), 1.17 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR（100 MHz, $CDCl_3$）δ 166.6, 165.3, 159.3, 158.4, 157.3, 153.12, 151.9, 151.1, 145.8, 133.0, 130.1, 129.3, 128.6, 128.0, 123.8, 123.2, 115.1, 112.6, 112.5, 109.6, 108.9, 107.7, 103.5, 103.3, 96.8, 65.8, 48.8, 48.4, 46.9, 45.0, 42.0, 12.4; HRMS（ESI）calcd. for $C_{38}H_{35}N_5NaO_6$, $[M+Na]^+$:680.2485, found: 680.2473.

Synthesis of **Ratio**:

Compound 3 (400 mg, 1.0 mmol) and triethylamine (TEA) (111 mg, 1.1 mmol) were dissolved in 20 mL dichloromethane. Compound 3 (279 mg, 1.0 mmol) was also dissolved in another 5 mL of dry dichloromethane. Then, the solution of Compound 5 was dropped into the solution of Compound 3 and TEA in an ice bath. After the dropwise addition was completed, the temperature was raised to room temperature and stirred overnight. The reaction solution was subjected to rotary evaporation to remove the solvent, and the obtained crude product was passed through a column. The mobile phase was dichloromethane / ethyl acetate = 1: 1 (v / v). The pure product obtained was an orange solid (528 mg, 82%). ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.98 (d, *J* = 7.4 Hz, 1H), 7.88 (s, 1H), 7.59 (m, *J* = 20.7, 7.3 Hz, 2H), 7.27 (d, *J* = 9.0 Hz, 1H), 7.11 (d, *J* = 7.4 Hz, 1H), 6.73 (s, 1H), 6.58 (m, *J* = 24.0, 18.5, 9.8 Hz, 6H), 6.45 (s, 1H), 3.88 (s, 2H), 3.55 (s, 2H), 3.41 (q, *J* = 7.1 Hz, 4H), 3.30 (d, *J* = 9.0 Hz, 4H), 1.20 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO–*d*$_6$) δ 169.2, 164.5, 159.9, 158.9, 157.1, 152.9, 152.6, 152.4, 152.2, 151.7, 144.5, 136.0, 130.6, 130.5, 129.5, 128.9, 126.6, 125.0, 124.4, 116.1, 113.0, 112.4, 110.1, 109.8, 109.0, 107.6, 102.6, 101.9, 96.7, 83.6, 44.6, 12.7. HRMS （ESI）calcd. for $C_{38}H_{34}N_3O_7$, $[M+H]^+$: 644.2397, found: 644.2396.

Synthesis of compound **RSH:**

The compound Ratio (103 mg, 0.16 mmol) and triethylamine (TEA, 32 mg) were dissolved in 10 mL of anhydrous dichloromethane, and then 2,4-dinitrobenzenesulfonyl chloride (52 mg, 0.19 mmol) dissolved in dichloromethane (30 mL)was added dropwise. The solvent was distilled off under reduced pressure and the crude product was directly subjected to silica gel column chromatography. Dichloromethane / ethyl acetate = 5: 1 (v / v) was used as the eluent to obtain the compound RSH (106.3 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 2.1 Hz, 1H), 8.50 (dd, *J* = 8.6, 2.2 Hz, 1H), 8.22 (d, *J*= 8.6 Hz, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.88 (s, 1H), 7.64 (dt, *J* = 22.4, 7.4 Hz, 2H), 7.30 (d, *J* = 8.9 Hz, 1H), 7.16 – 7.10 (m, 2H), 6.85 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.76 (d, *J*= 8.7 Hz, 1H), 6.60 (dd, *J* = 20.1, 9.9 Hz, 4H), 6.46 (d, *J* = 2.1 Hz, 1H), 3.86 (s, 2H), 3.55 (s, 2H), 3.42 (q, *J* = 7.0 Hz, 4H), 3.31 (d, *J* = 9.0 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 165.1, 159.2, 157.3, 152.7, 152.5, 152.2, 151.8, 151.8, 151.0, 149.4, 148.9, 145.6, 135.3, 134.1, 133.1, 130.1, 130.0, 130.0, 128.7, 126.8, 126.4, 125.2, 123.9, 120.5, 119.5, 117.0, 115.6, 112.6, 110.9, 109.4, 108.6, 107.7, 102.1, 96.8, 82.1, 48.3, 47.8, 46.8, 45.0, 41.9, 33.9, 12.4; HRMS (ESI) calcd. For $C_{44}H_{35}N_5O_{13}S$, $[M+H]^+$: 874.2030, found: 874.2027.

Synthesis of compound **RClO**:

Compound 9 (50 mg, 0.05 mmol) was dissolved in 30 mL of a saturated solution of hydrogen chloride in methanol and stirred at room temperature overnight. The solvent was distilled off under reduced pressure, and the crude product was directly subjected to silica gel column chromatography using dichloromethane / methanol as 30: 1 (v / v) eluent to obtain compound RClO (49.4 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.31 (s, 1H), 7.85 (s, 1H), 7.71 (dd, *J* = 7.8, 4.8 Hz, 5H), 7.52 (d, *J* = 7.9 Hz, 10H), 7.40 – 7.34 (m, 2H), 7.31 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 5.8 Hz, 1H), 6.76 – 6.63 (m, 2H), 6.58 (d, *J* = 9.0 Hz, 1H), 6.50 (d, *J* = 7.5 Hz, 1H), 6.47 – 6.28 (m, 4H), 4.82 (d, *J*= 45.0 Hz, 2H), 3.76 (s, 2H), 3.48 (s, 2H), 3.40 (dd, *J* = 12.9, 6.1 Hz, 4H), 3.15 (s, 4H), 1.19 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 164.6, 161.4, 161.4, 159.3, 158.7, 157.2, 152.7, 152.5, 152.0, 151.8, 151.6, 145.2, 135.12, 133.8, 133.7, 133.4, 132.1, 132.0, 131.2, 131.1, 130.2, 130.1, 129.3, 128.6, 128.5, 128.4, 127.6, 123.8, 123.3, 118.1, 117.2, 115.6, 112.4, 112.0, 109.5, 108.1, 107.7, 107.6, 103.3, 102.7, 96.8, 65.8, 48.7, 48.1, 46.9, 45.0, 41.9, 30.5, 29.9, 12.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.63 (s) ; HRMS (ESI) calcd. for $C_{58}H_{51}N_5O_7P$, $[M]^+$: 960.3521, found: 960.3521.

Synthesis of compound 10:

Compound 6 (1 g, 1.52 mmol) and triethylamine (316 μL, 2.28 mmol) were dissolved in 60 mL of anhydrous chloroform, and added dropwise to the solution of 2,4-dinitrobenzenesulfonyl chloride (608 mg, 2.28 mmol) in chloroform in an icebath. Then stirred at room temperature overnight. The solvent was distilled off under reduced pressure, and the crude product was directly subjected to silica gel column chromatography. Dichloromethane / ethyl acetate = 1: 1 (v / v) was used as the eluent to obtain compound 10 (701.8 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (d, *J* = 2.7 Hz, 1H), 8.36 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.98 – 7.93 (m, 1H), 7.89 (s, 1H), 7.56 – 7.50 (m, 2H), 7.30 (d, *J* = 8.9 Hz, 1H), 7.18 (d, *J* = 9.2 Hz, 1H), 7.13 – 7.08 (m, 1H), 7.00 (d, *J* = 2.3 Hz, 1H), 6.78 – 6.74 (m, 1H), 6.69 (d, *J* = 2.4 Hz,1H), 6.60 (ddd, *J* = 8.9, 4.4, 2.4 Hz, 2H), 6.56 (d, *J* = 8.8 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 3.89 (s, 2H), 3.80 (s, 2H), 3.56 (s, 2H), 3.43 (q, *J* = 7.1 Hz, 4H), 3.31 (d, *J* = 14.1 Hz, 4H), 1.22 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 165.1, 159.2, 157.3, 153.1, 152.5, 152.1, 151.8,

151.0, 150.3, 148.9, 148.7, 145.6, 133.8, 133.4, 133.2, 120.0, 129.4, 129.3, 129.1, 127.9, 126.6, 123.8, 123.4, 120.5, 119.5, 117.0, 115.7, 112.9, 111.0, 109.4, 108.4, 107.7, 102.8, 96.9, 83.7, 64.9, 48.5, 48.1, 46.9, 45.0, 42.0, 12.4; HRMS (ESI) calcd. for $C_{44}H_{37}N_7NaO_{12}S$, $[M+Na]^+$: 910.2119, found: 910.2119.

Synthesis of compound 11:

Compound 10 (914.6 mg, 1.02 mmol) and triethylamine (286 μL, 2.06 mmol) were dissolved in 40 mL of anhydrous chloroform, and then bromoacetyl bromide (190 μL) was dissolved in 14.5 mL of chloroform. It was added dropwise to the system dropwise, then the temperature was raised to 25 °C and stirred overnight. The solvent was distilled off under reduced pressure, and the crude product was directly subjected to silica gel column chromatography. Petroleum ether / ethyl acetate = 3: 4 (v / v) was used as the eluent to obtain compound 11 (740.9 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 2.2 Hz, 1H), 8.51(dd, *J* = 8.6, 2.2 Hz, 2H), 8.20 – 8.13 (m, 3H), 7.92 (dd, *J* = 6.7, 1.7 Hz, 2H), 7.87 (d, *J* = 9.5 Hz, 2H), 7.57 (tt, *J* = 8.7, 6.6 Hz, 4H), 7.33 (d, *J* = 8.9 Hz, 2H), 7.14 – 7.08 (m, 3H), 6.83 (d, *J* = 8.8 Hz, 2H), 6.68 (dd, *J* = 8.8, 2.5 Hz, 2H), 6.61 (h, *J* = 2.2 Hz, 4H), 6.54 (d, *J* = 2.4 Hz, 3H), 6.47 (d, *J* = 2.4 Hz, 2H), 5.29 (s, 1H), 3.81 (s, 4H), 3.71 – 3.57 (m, 4H), 3.52 (s, 3H), 3.44 (q, *J* = 7.2 Hz, 8H), 3.27 (s, 8H), 1.23 (t, *J* = 7.1 Hz, 11H). ^{13}C NMR (100 MHz, $CDCl_3$) δ161.7, 160.3, 154.4, 152.6, 150.3, 149.6, 148.9, 147.9, 147.40, 147.0, 145.7, 140.8, 137.2, 128.3, 125.1, 124.9, 124.7, 124.2, 124.1, 123.1, 119.0, 117.4, 114.7, 112.6, 111.0, 110.60, 108.1, 104.6, 103.9, 103.8, 103.0, 98.1, 92.1,72.5, 60.2, 40.2. HRMS (ESI) calcd. for $C_{46}H_{38}BrN_7NaO_{13}S$, $[M+Na]^+$:1030.1329, found: 1030.1328.

Synthesis of compound **RSHClO**:

Compound 11 (156.6 mg, 0.15 mmol) and triphenylphosphine (39 mg, 0.15 mmol) were dissolved in 20 mL of anhydrous chloroform and stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the crude product was directly subjected to silica gel column chromatography. Dichloromethane / methanol = 30: 1 (v / v) was used as an eluent to obtain the compound RSHClO (144.9 mg, 76%). ^{1}H NMR （400 MHz, $CDCl_3$）δ 10.14 (s, 1H), 8.71 (t, *J* = 11.7 Hz, 2H), 8.58 (s, 1H), 7.89 (s, 1H), 7.85 (d, *J* = 6.7 Hz, 1H), 7.77 (t, *J* = 6.6 Hz, 3H), 7.65 (dd, *J* = 21.3, 7.6 Hz, 11H), 7.51 – 7.34 (m, 3H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.06 (s, 1H), 6.93 (d, *J* = 6.6 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 6.70 (d, *J* = 7.9 Hz, 1H), 6.60 (d, *J* = 9.0 Hz, 1H), 6.53 (s, 1H), 6.47 (s, 2H), 5.17 (s, 2H), 3.87 (s, 2H), 3.55 (s, 2H), 3.43 (dd, *J* = 13.5, 6.6 Hz, 4H), 3.27 (s, 4H), 1.22 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 163.8, 161.1, 161.0, 159.3, 157.3, 152.6, 152.3, 151.9, 151.8, 150.9, 150.6, 149.4, 148.6, 145.5, 135.2, 135.2, 134.0, 133.9, 133.6, 132.7, 132.1, 132.0, 130.7, 130.2, 130.0, 128.9, 128.6, 128.5, 128.4, 127.7, 123.8, 119.9, 118.2, 118.0, 117.1, 115.7, 111.1, 109.5, 107.7, 107.3, 102.4, 96.9, 64.9, 48.5, 48.2, 46.9, 45.0, 41.9, 31.7, 31.1, 12.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.91 (s). HRMS (ESI) calcd. for $C_{64}H_{53}N_7O_{13}PS$, $[M]^+$: 1190.3154, found: 1190.3174.

Compound 5 were prepared referring to the literature reports. (*Chemical Communications*, **2015**, 51, 6781-6784)

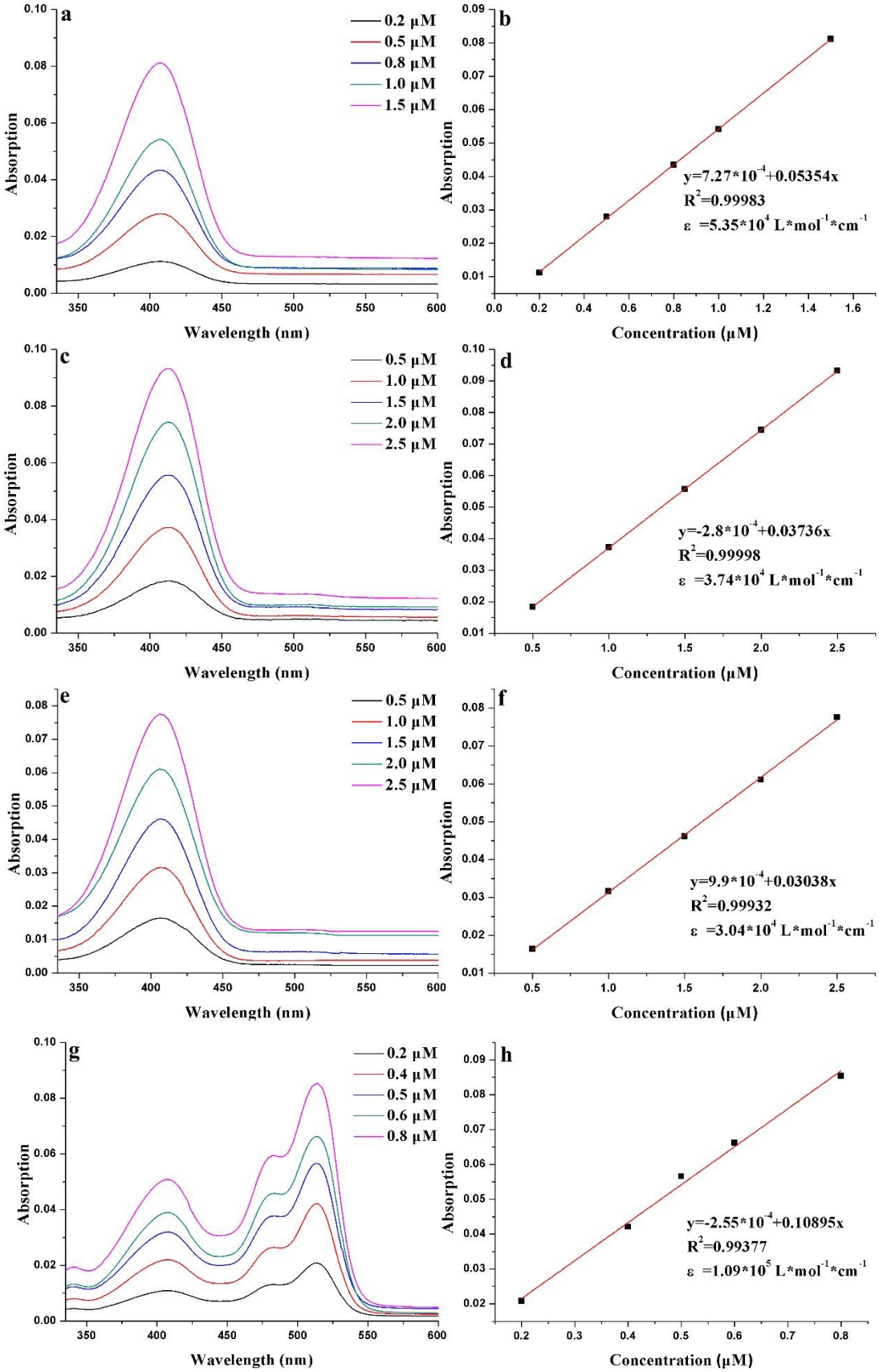


Figure S1. UV spectra of （a）RSHClO,（c）RSH,（e）RClO,（g）Ratio at different concentrations. Absorption-concentration curve of（b）RSHClO,（d）RSH,（f）RClO,（h）Ratio.

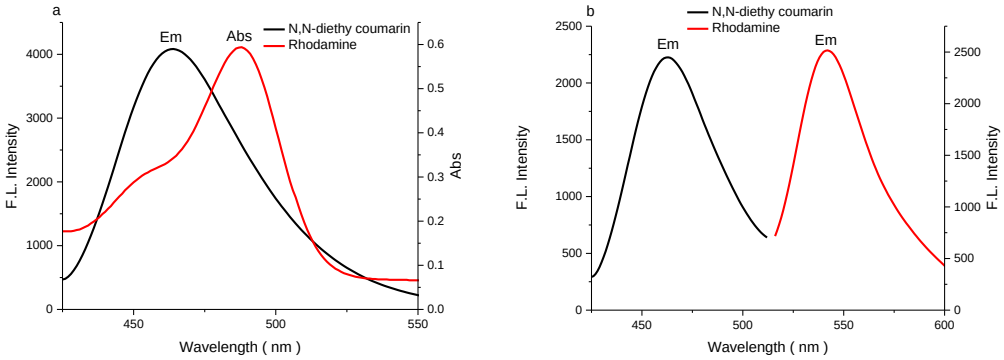


Figure S2. (a) UV spectra of Rhodamine and fluorescence spectra of N,N-diethy coumarin. (b) Fluorescence spectra of Rhodamine and N,N-diethy coumarin.

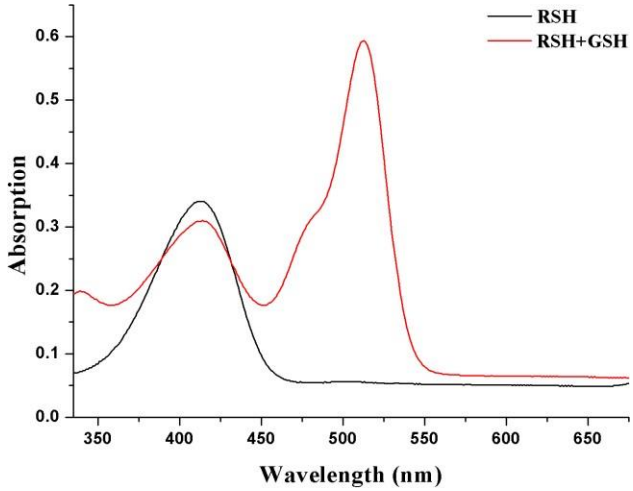


Figure S3. UV spectra of compound **RSH** (5 μM) before the reaction with GSH and after the reation with GSH (100 μM).

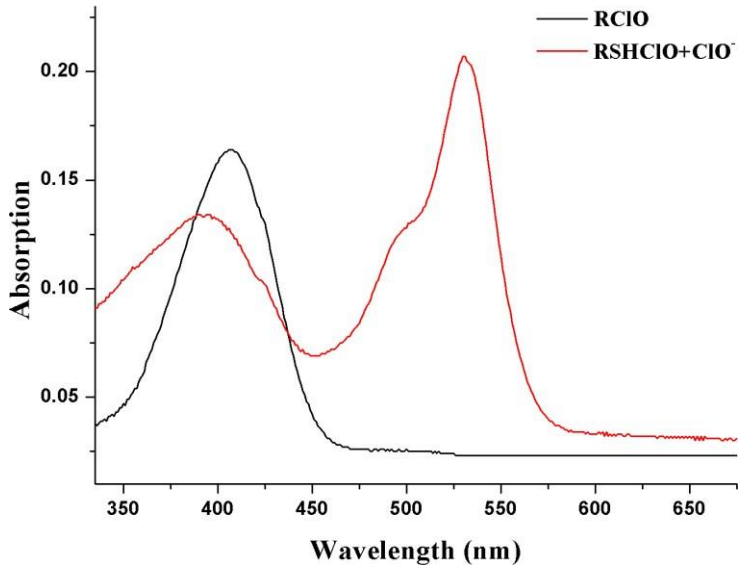


Figure S4. UV spectra of compound **RClO** (5 μM) before the reaction with ClO^- and after the reation with ClO^- (100 μM).

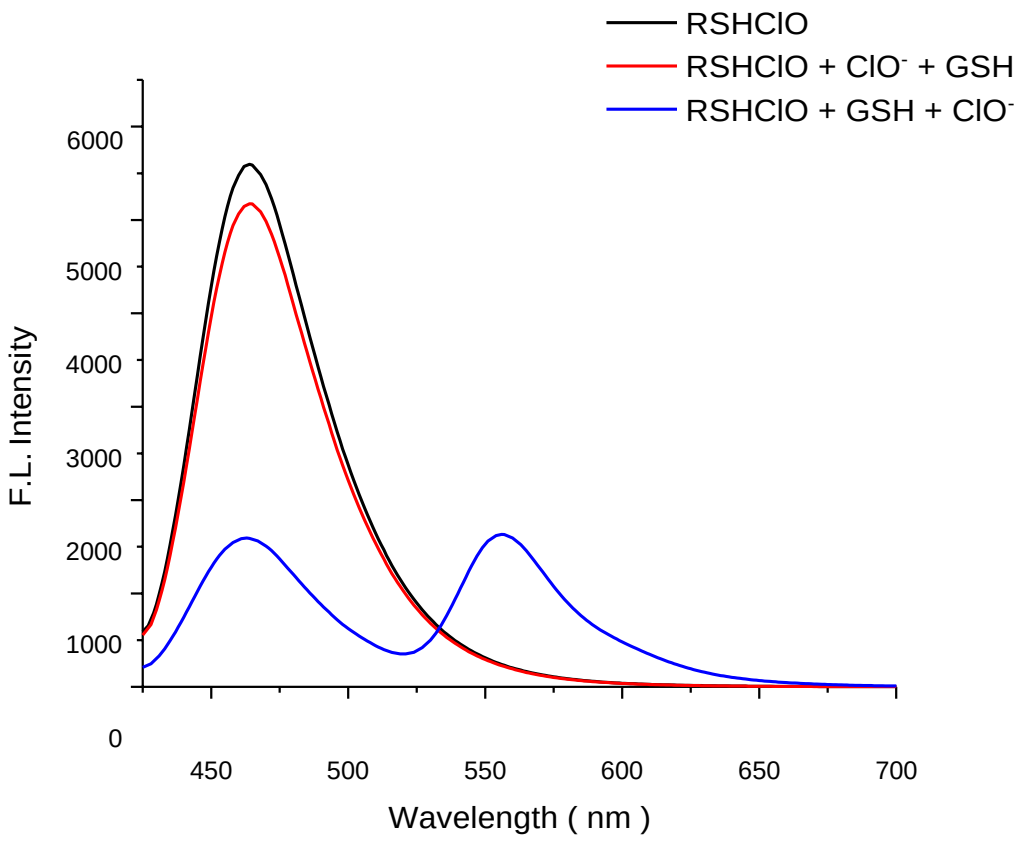
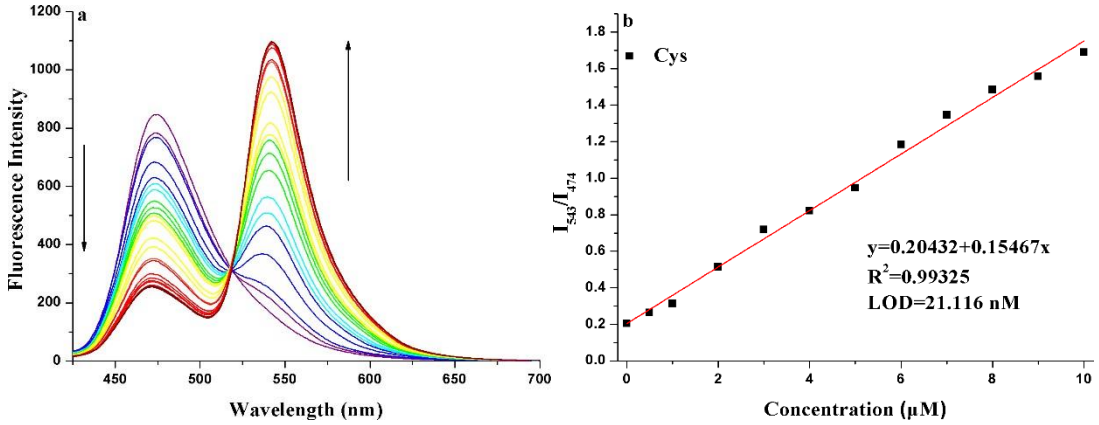


Figure S5. Fluorescence spectra of compound **RSHClO** (5 μM) before the reaction with GSH (10 μM) / ClO^- (200 μM) and after the reation with GSH (10 μM) /ClO^- (200 μM) in PBS (10 mM, pH = 7.4, containing 1 mM CTAB).



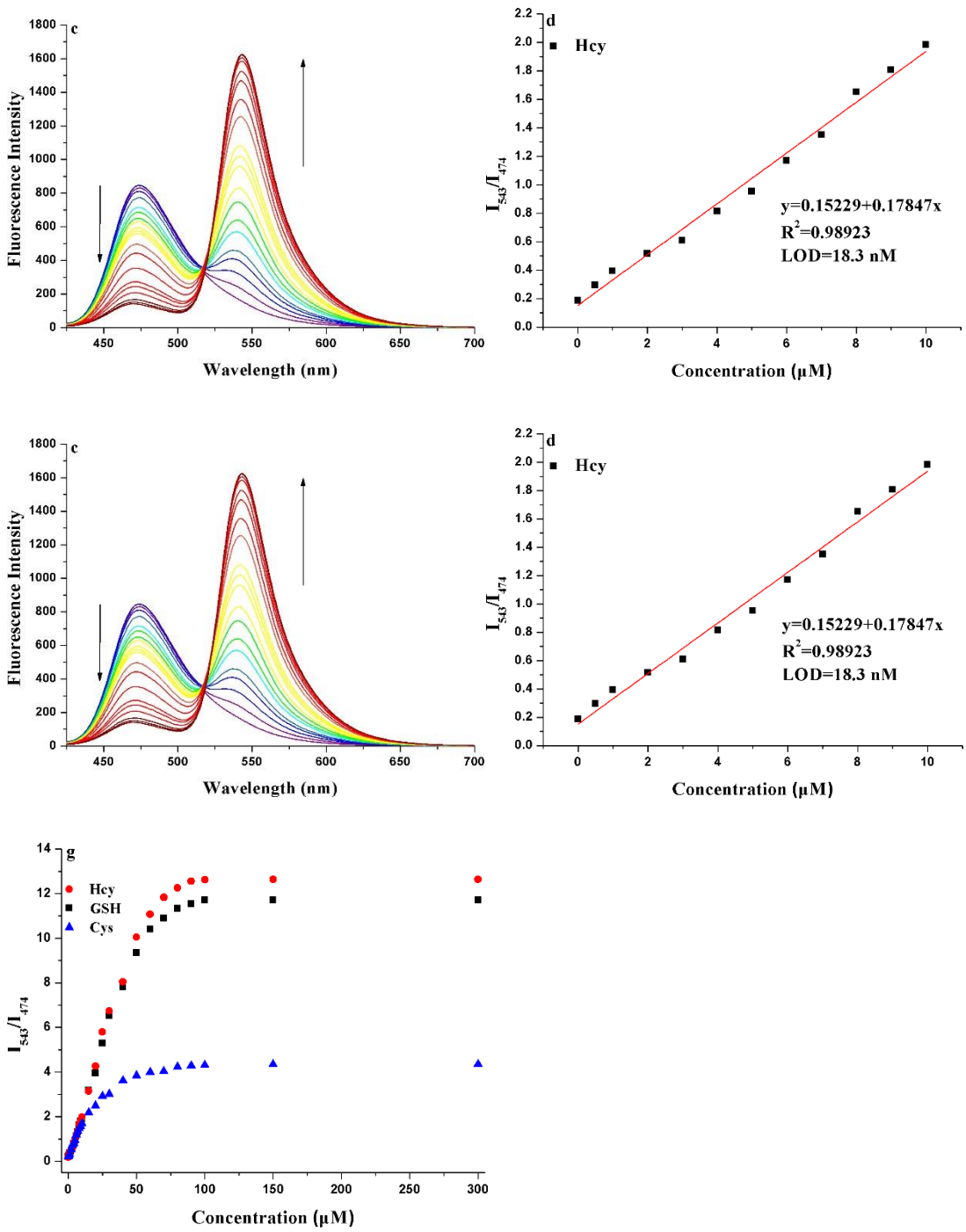


Figure S6. (a), (c), (e) Fluorescence titration spectra of **RSH** (5 μM) with Cys, Hcy and GSH respectively in PBS (10 mM, pH = 7.4, containing 1 mM CTAB) ; (b), (d), (f) Linear diagram between I_{558}/I_{464} and Cys, Hcy, GSH respectively ; (g) Ratio of fluorescence intensity at 558 nm and 464 nm (I_{558}/I_{464}) as a function of Cys, Hcy and GSH respectively.

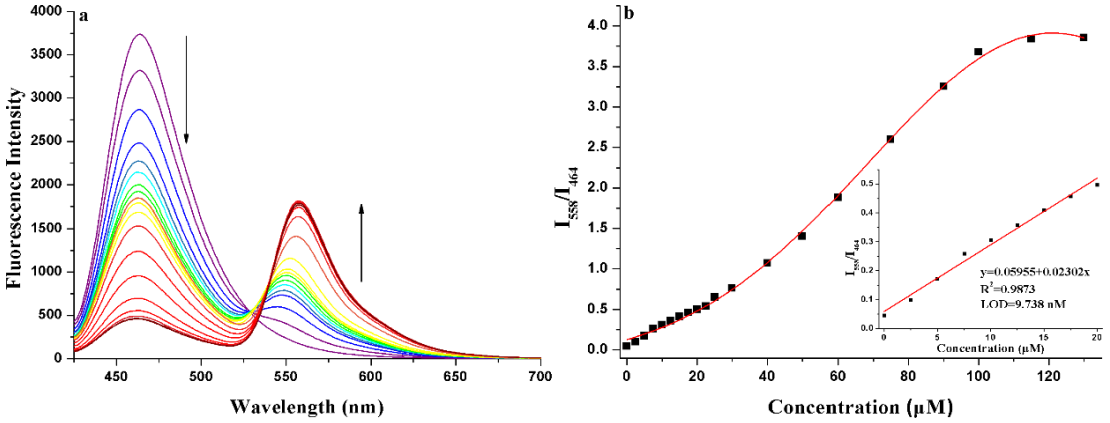


Figure S7. (a) Fluorescence titration spectra of **RClO** (5 μM) with ClO^- in PBS (10 mM, pH = 7.4, containing 1 mM CTAB) ; (b) Ratio of fluorescence intensity at 558 nm and 464 nm (I_{558}/I_{464}) as a function of ClO^-, inserted plot: linear diagram between I_{558}/I_{464} and [NaClO].

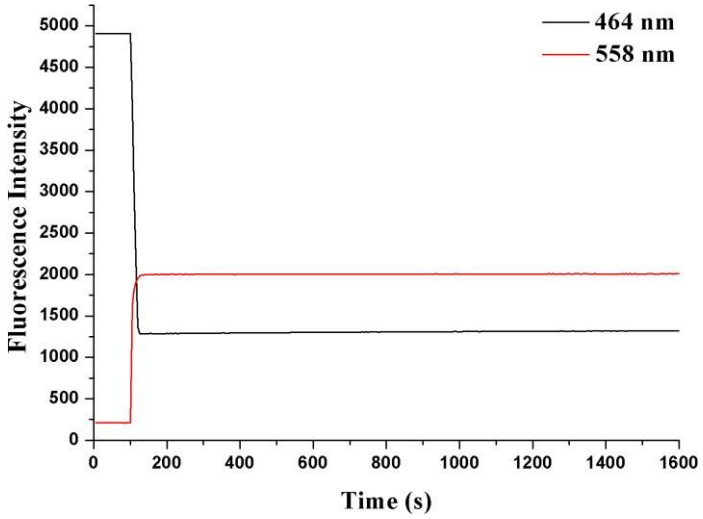


Figure S8. Time-dependent fluorescence curve of **RSHClO** (5 μM) with 20 equiv. of GSH and than incubated with 20 equiv. of ClO^- in PBS (10 mM, pH = 7.4, containing 1 mM CTAB).

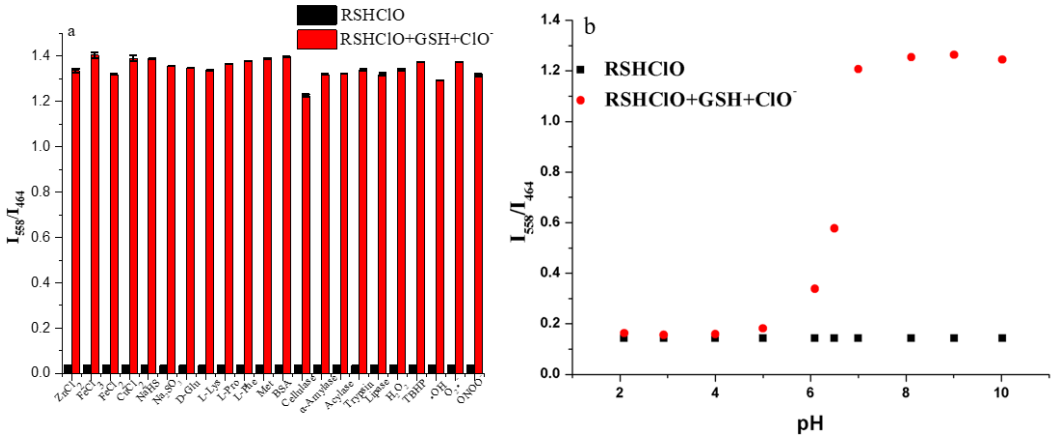


Figure S9. (a) Selectivity of RSHClO (5 μM) for biothiols and ClO– over other biologically relevant

species in PBS (10 mM, pH = 7.4, containing 1 mM CTAB), the black bars represent the addition of interferents (10 μg/mL for all the proteins and 100 μM for other interferents), the red bars represent the subsequent addition of 10 μM GSH followed by 100 μM NaClO (reactive oxygen species including H2O2, TBHP, •OH, O2•− and ONOO– were added after the addition of GSH); (b) Ratio of fluorescence intensity at 558 nm and 464 nm (I558/I464) as a function of pH. λex = 405 nm.

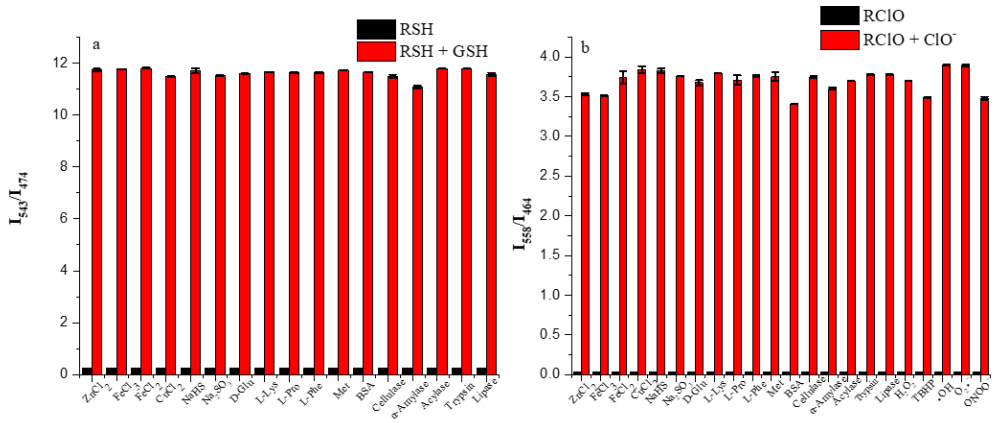


Figure S10. (a) Selectivity of **RSH** (5 μM) for biothiols and other biologically relevant species in PBS (10 mM, pH = 7.4, containing 1 mM CTAB), the black bars represent the addition of interferents (10 μg/mL for all the proteins and 100 μM for other interferents), the red bars represent the subsequent addition of 10 μM GSH followed by 100 μM interferents; (b) Selectivity of **RClO**$^-$ (5 μM) for ROS and other biologically relevant species in PBS (10 mM, pH = 7.4, containing 1 mM CTAB), the black bars represent the addition of interferents (10 μg/mL for all the proteins and 100 μM for other interferents), the red bars represent the subsequent addition of 10 μM ClO^- followed by 100 μM interferents

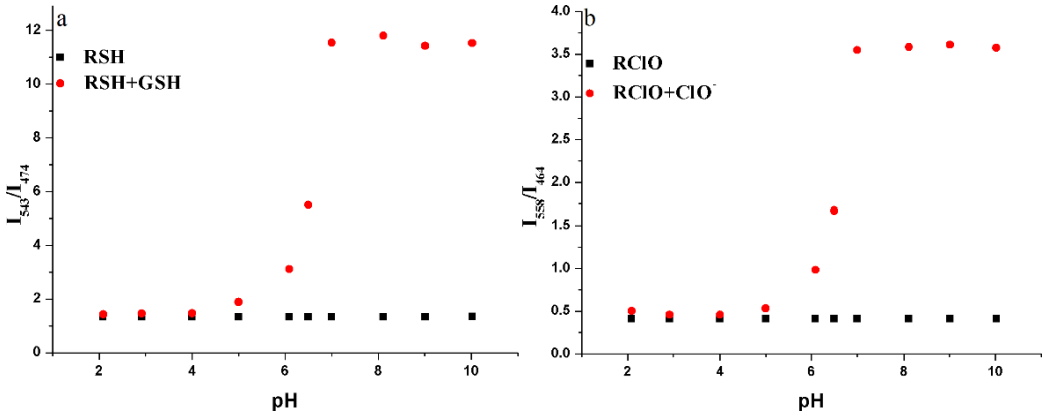


Figure S11. (a) Ratio of fluorescence intensity at 558 nm and 464 nm (I_{558}/I_{464}) as a function of pH of **RSH;** (b) Ratio of fluorescence intensity at 558 nm and 464 nm (I_{558}/I_{464}) as a function of pH of **RClO**. λex = 405 nm.

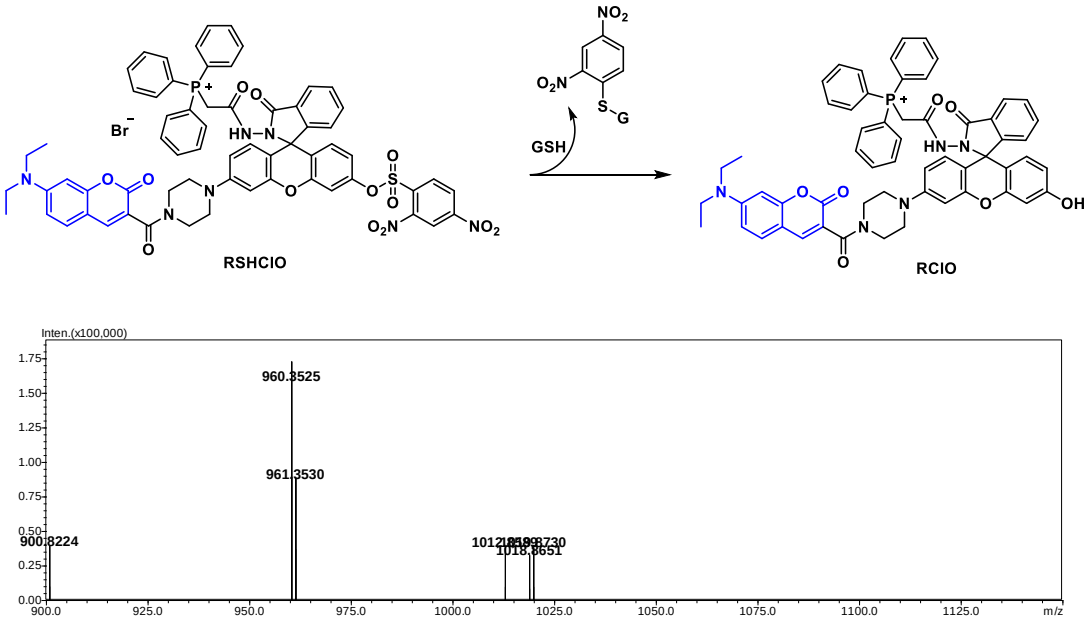


Figure S12. The reaction mechanism of **RSHClO** with GSH and the ESI–MS spectra of this reaction, **RClO**$^{+}$ = 960.3521, Found for 960.3525.

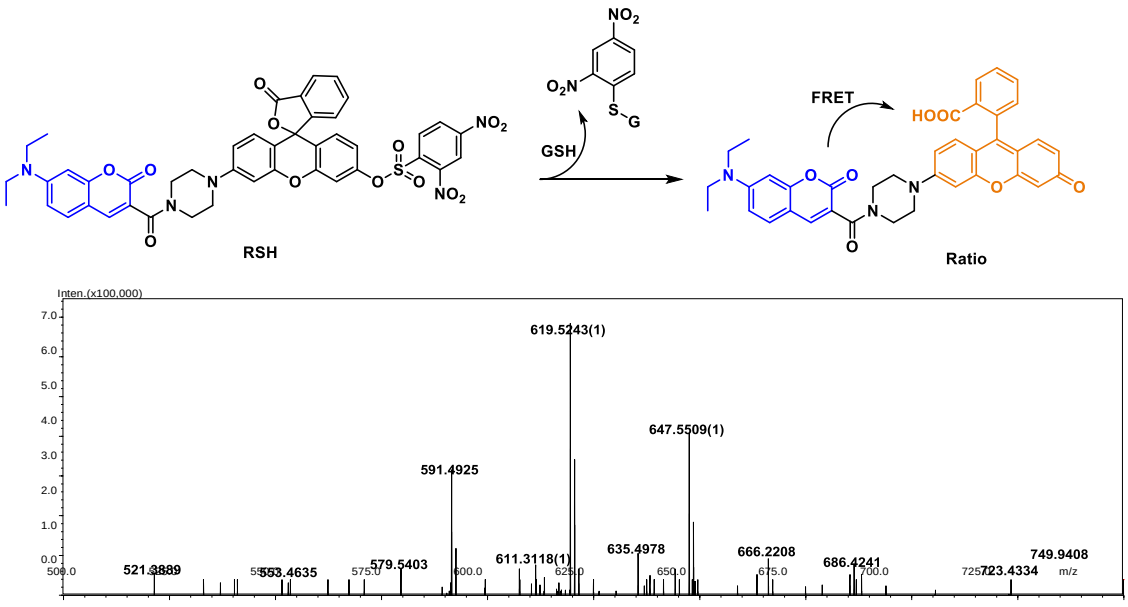


Figure S13. The reaction mechanism of **RSH** with GSH and the ESI–MS spectra of this reaction, [**Ratio** + Na]$^{+}$ = 666.2216, Found for 666.2208

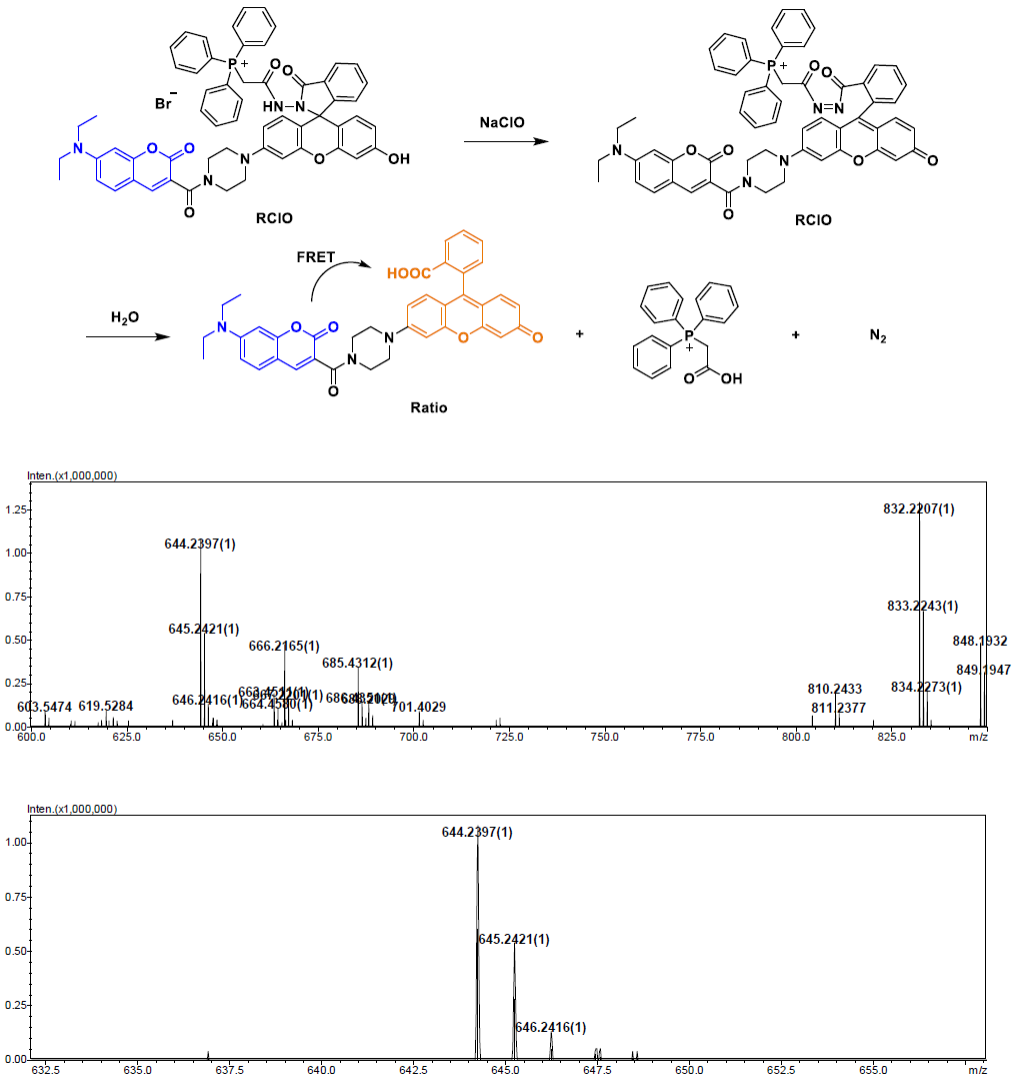


Figure S14. The reaction mechanism of **RClO** with ClO^- and the ESI–MS spectra of this reaction, **[Ratio+H]**$^+$= 644.2397, Found for 644.2397.

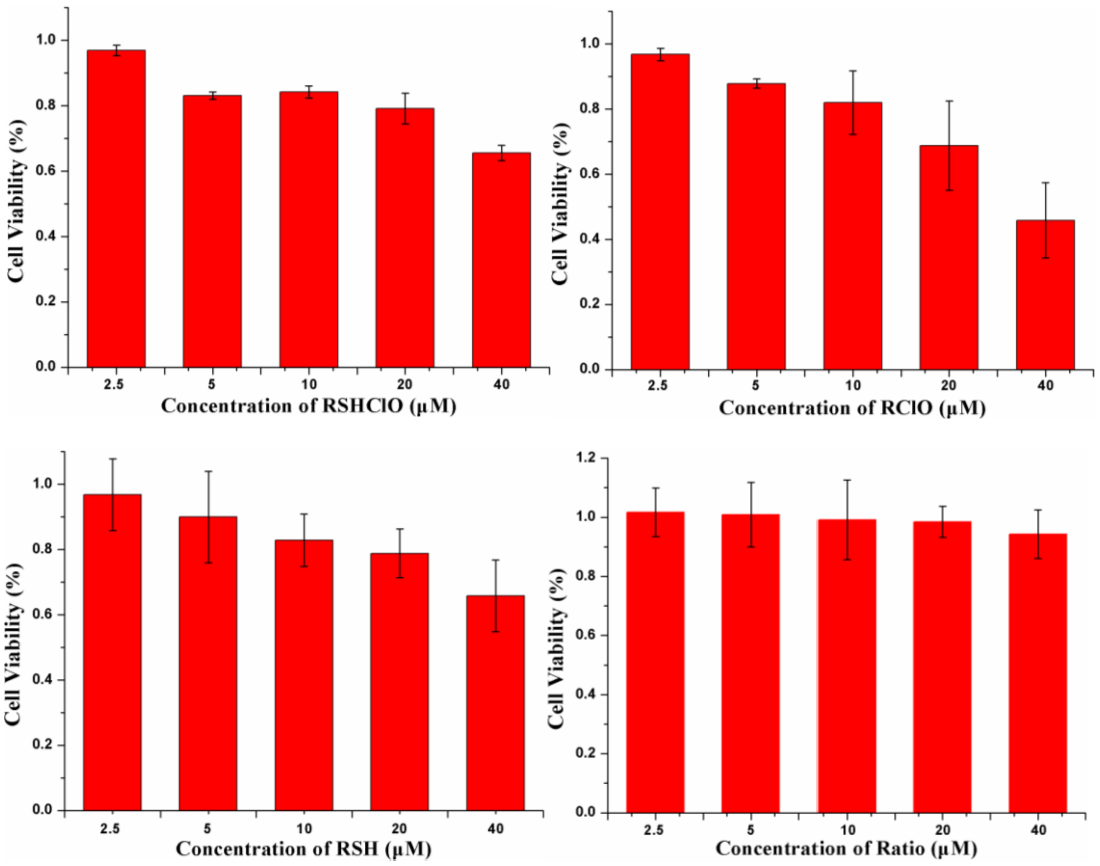


Figure S15. Cytotoxicity of different concentrations of RSHClO, RClO, RSH and Ratio to B16 cells by a standard MTS assay, the experiment was repeated three times and the data are shown as mean (±S.D.).

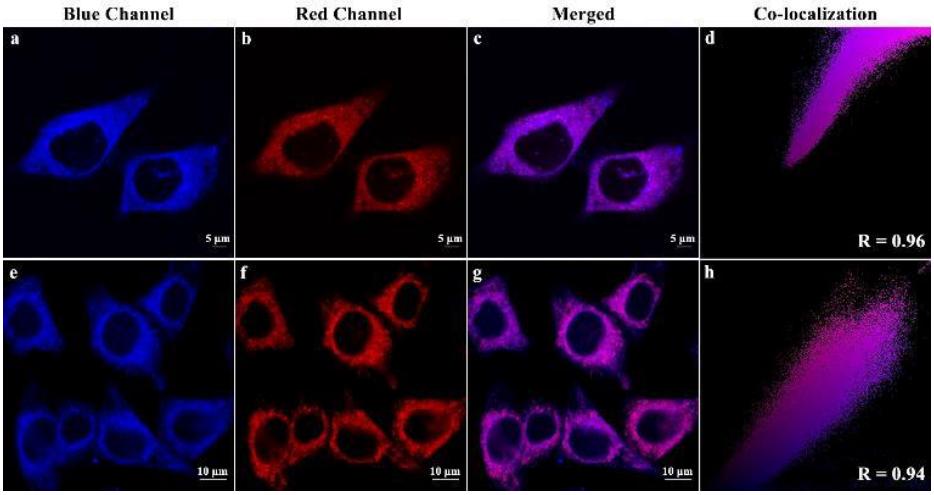


Figure S16. Confocal fluorescence images of B16 cells stained with 1 μM Mitotracker Deep Red and (a-c) 1 μM RSHClO ; (e-g) 1 μM RClO; (d, h) Co-localization images; Blue channel: λex = 405 nm, λem = 425−520 nm, red channel: λex = 632 nm, λem = 650–720 nm.

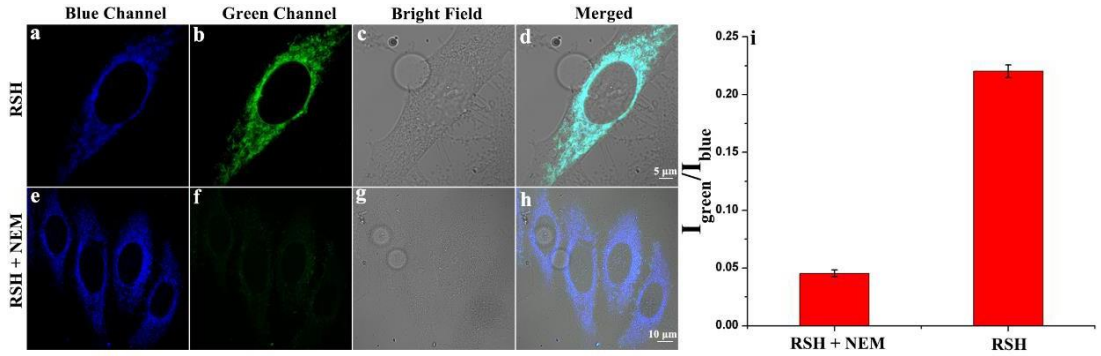


Figure S17. (a)-(d) The endogenous biothiol fluorescence imaging of **RSH** (1 μM) incubated in B16 cells for 60 min at 37 ℃; (e)-(h) The fluorescence imaging of **RSH** (1 μM) in B16 cells that pre-treated with NEM (200 μM) for 60 min at 37 ℃ ; (i) The ratio of fluorescence intensity from blue channel and green channel.

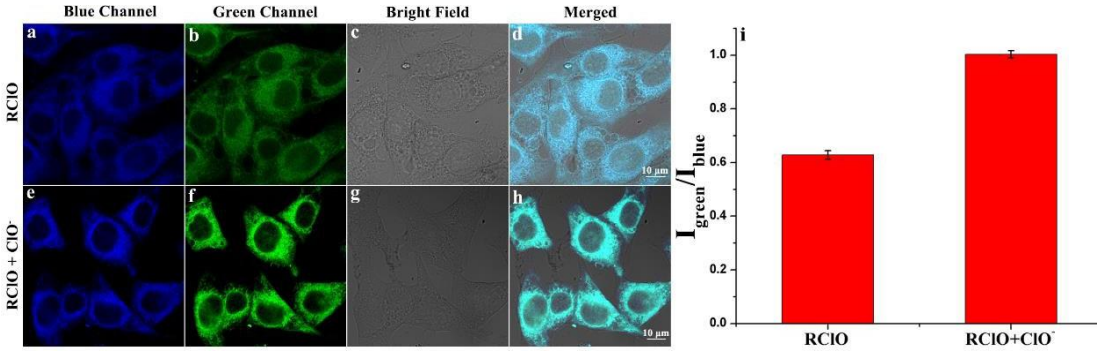


Figure S18. (a)-(d) The fluorescence imaging of **RClO** (1 μM) incubated in B16 cells for 15 min at 37 ℃; (e)-(h) The fluorescence imaging of **RClO** (1 μM) incubated in B16 cells for 15 min and then treated with ClO^- (75μM) for 30 min; (i) The ratio of fluorescence intensity from blue channel and green channel.

1. NMR, HRMS and Modi-tof spectra of synthetic products

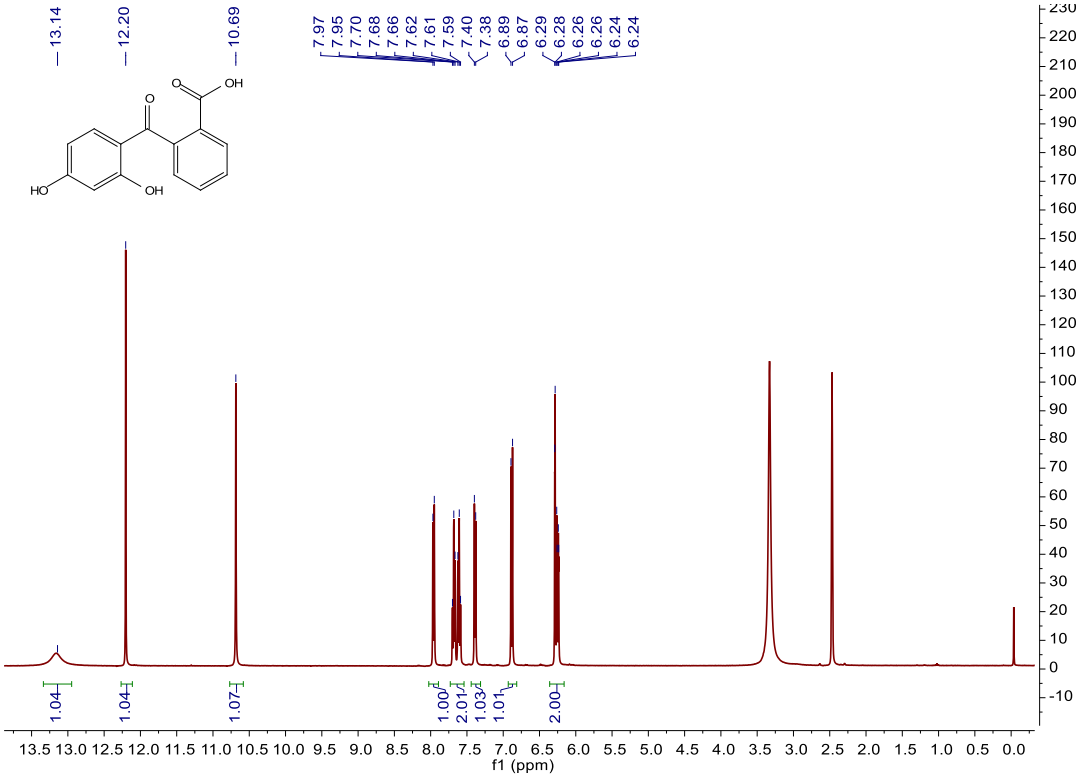


Figure S19. ^{1}H NMR spectrum of compound **2** in $CDCl_3$.

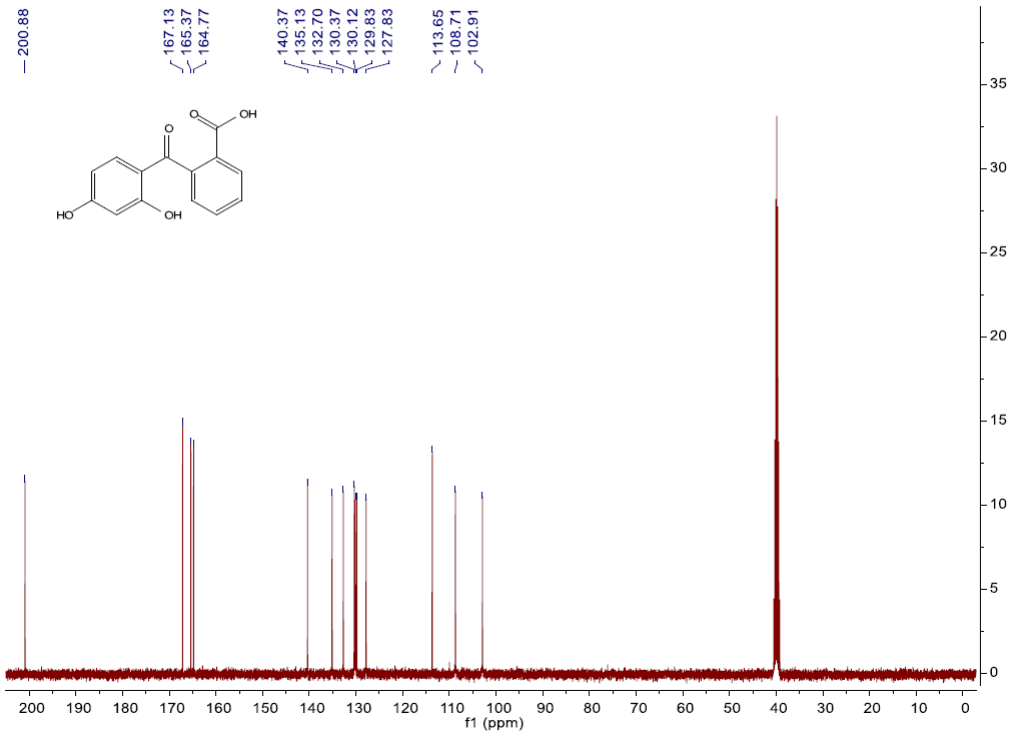


Figure S20. ^{13}CNMR spectrum of compound **2** in $CDCl_3$.

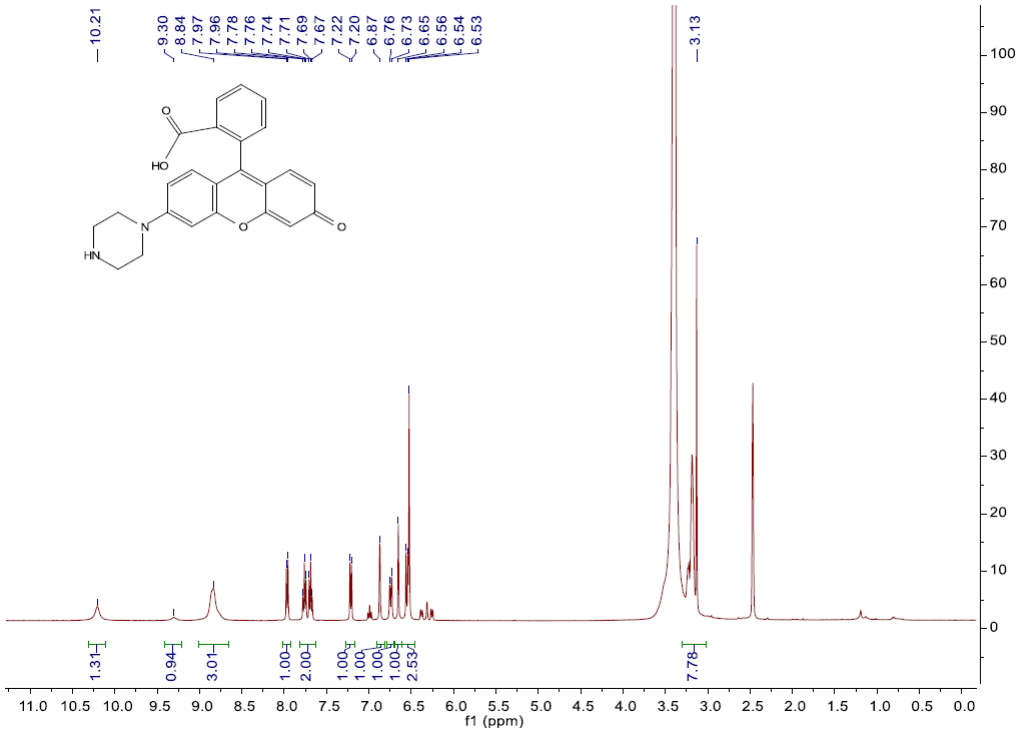


Figure S21. ^{1}H NMR spectrum of compound **3** in $CDCl_3$.

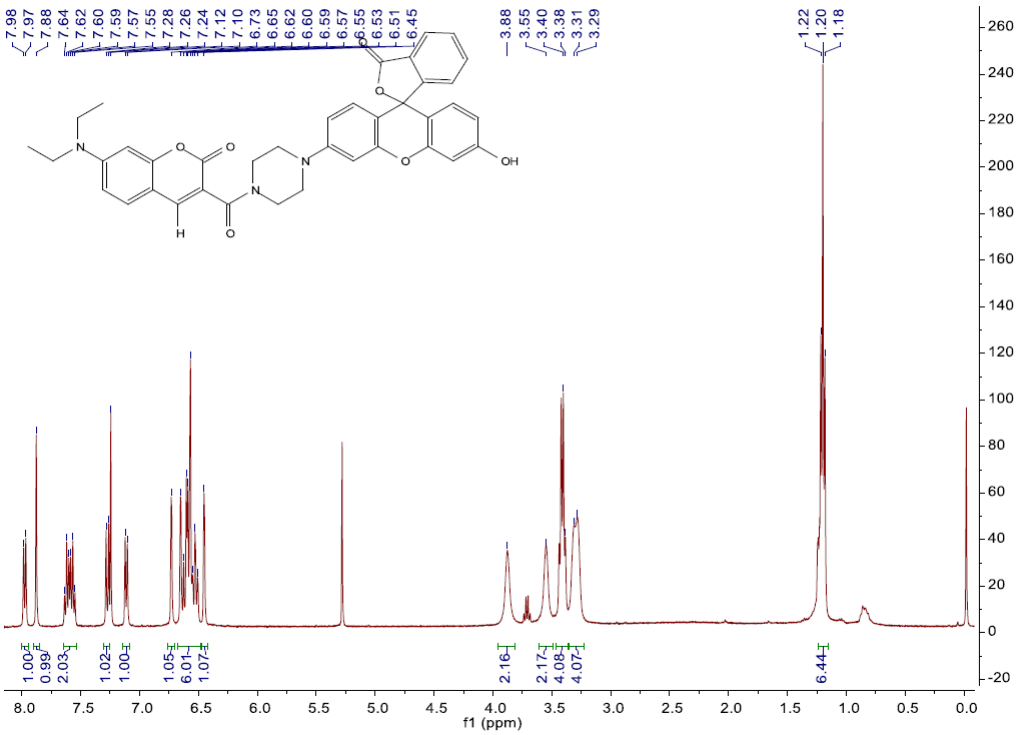


Figure S22. ^{1}H NMR spectrum of compound **Ratio** in $CDCl_3$.

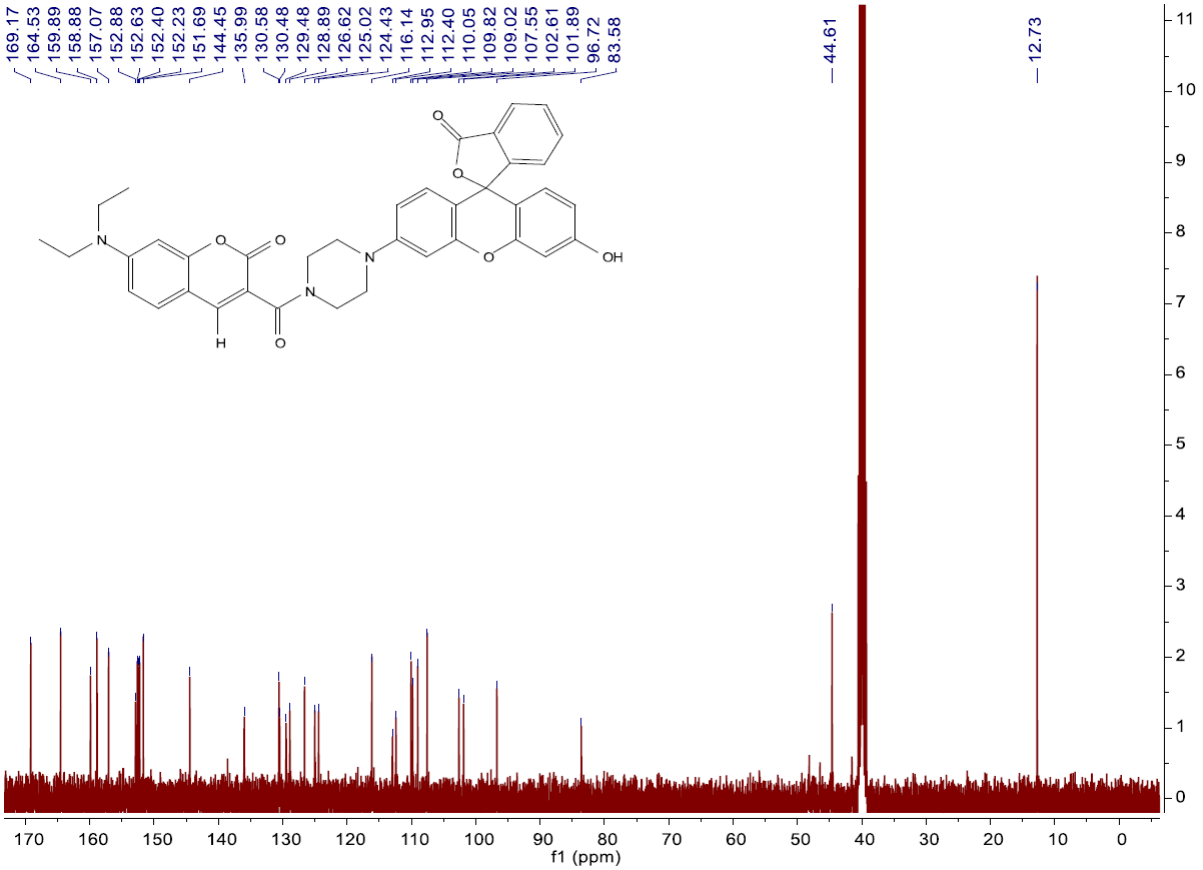


Figure S23. ^{13}C NMR spectrum of compound **Ratio** in $CDCl_3$.

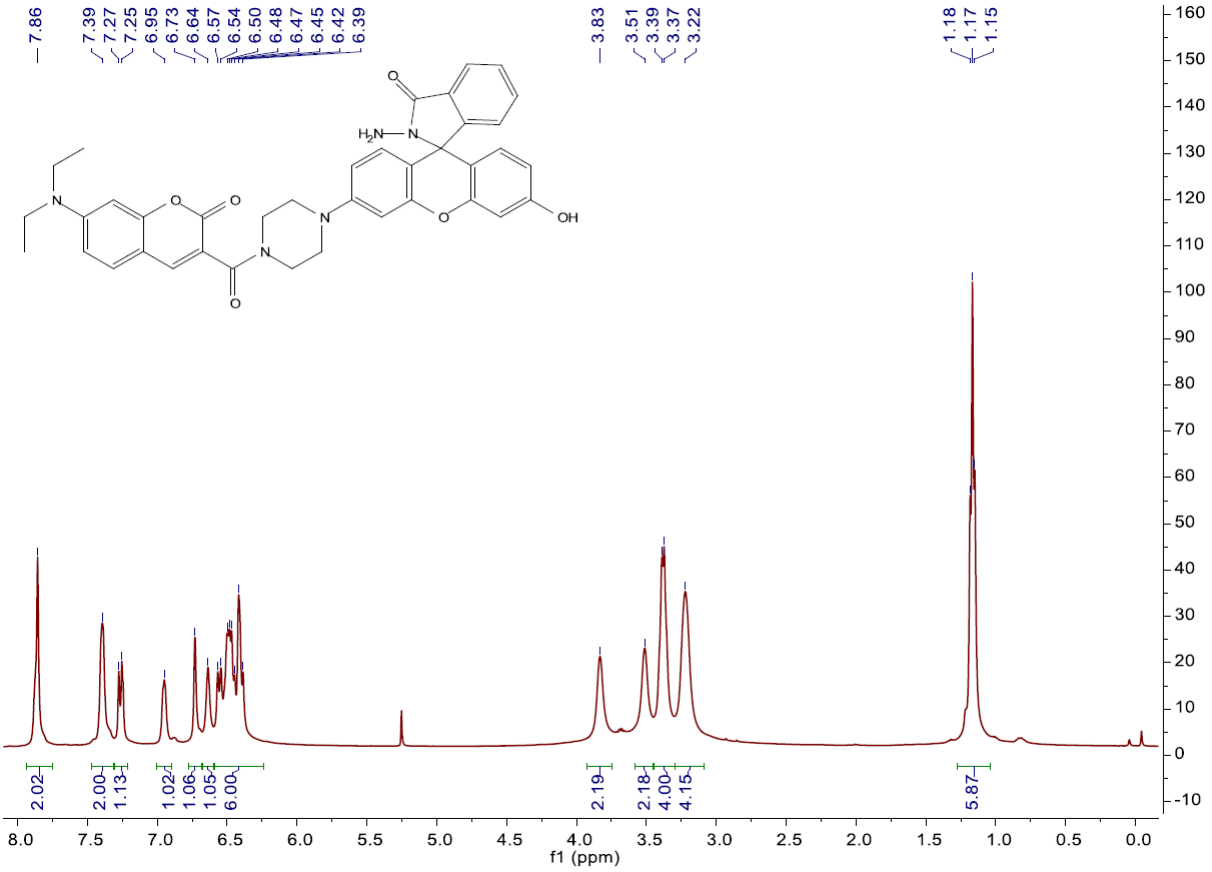


Figure S24. ^{1}H NMR spectrum of compound **6** in $CDCl_3$.

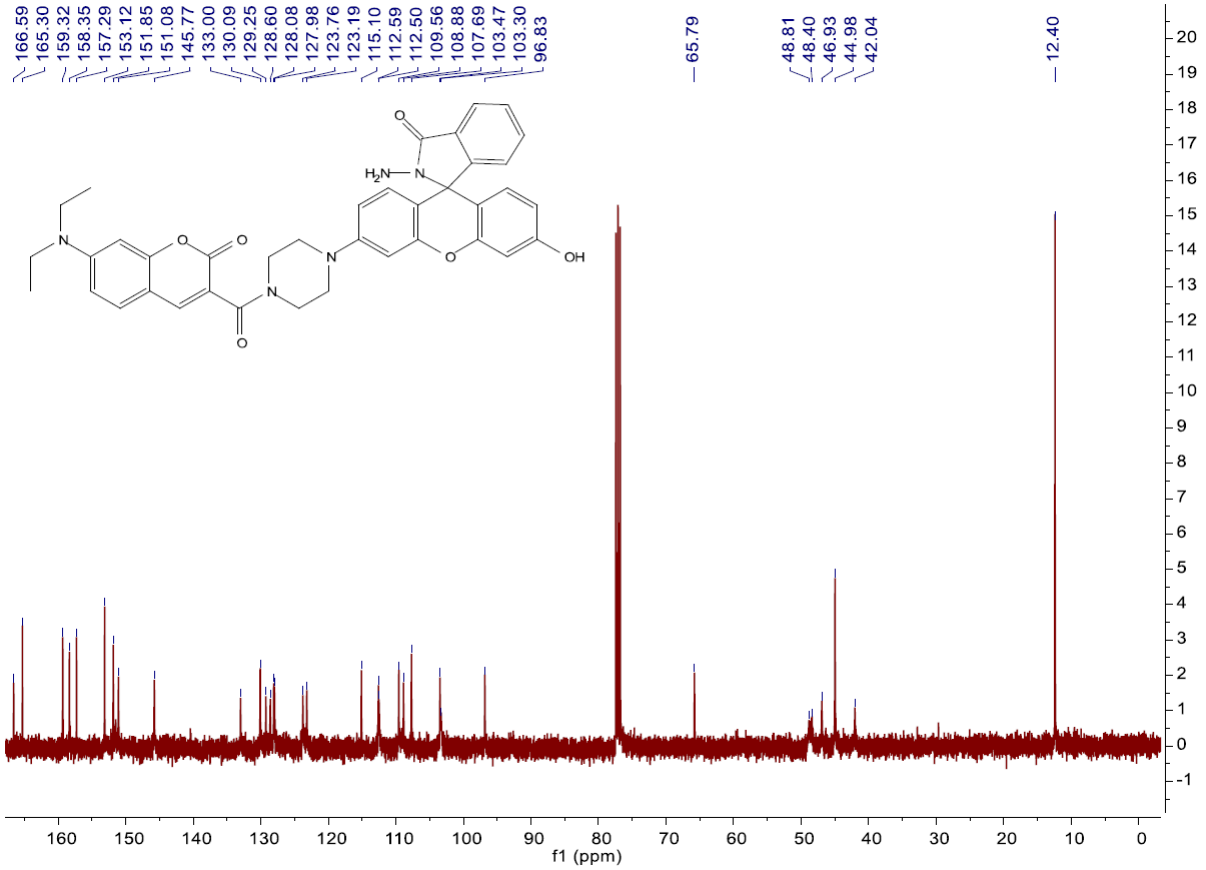


Figure S25. ^{13}C NMR spectrum of compound **6** in $CDCl_3$.

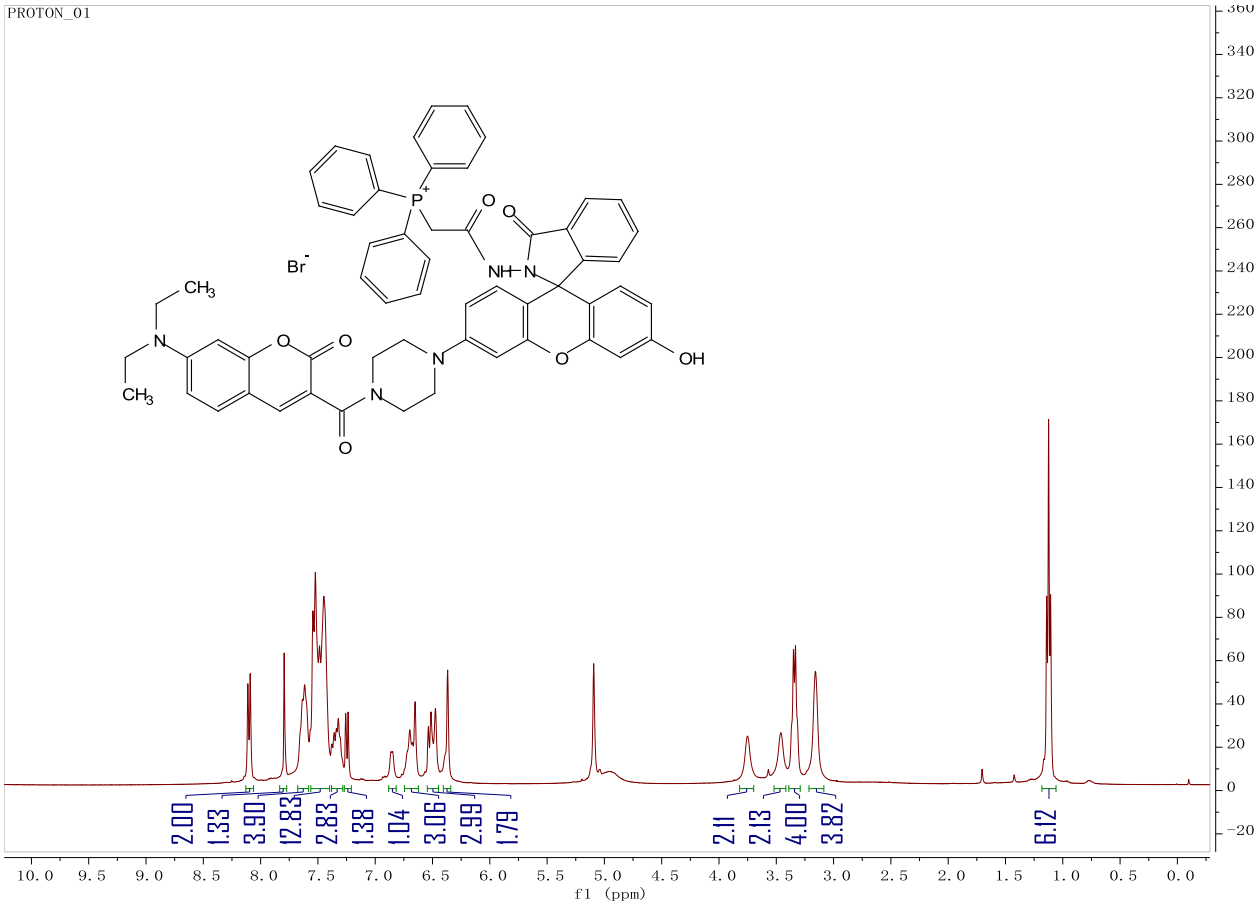


Figure S26. ^{1}H NMR spectrum of compound **RClO** in $CDCl_3$.

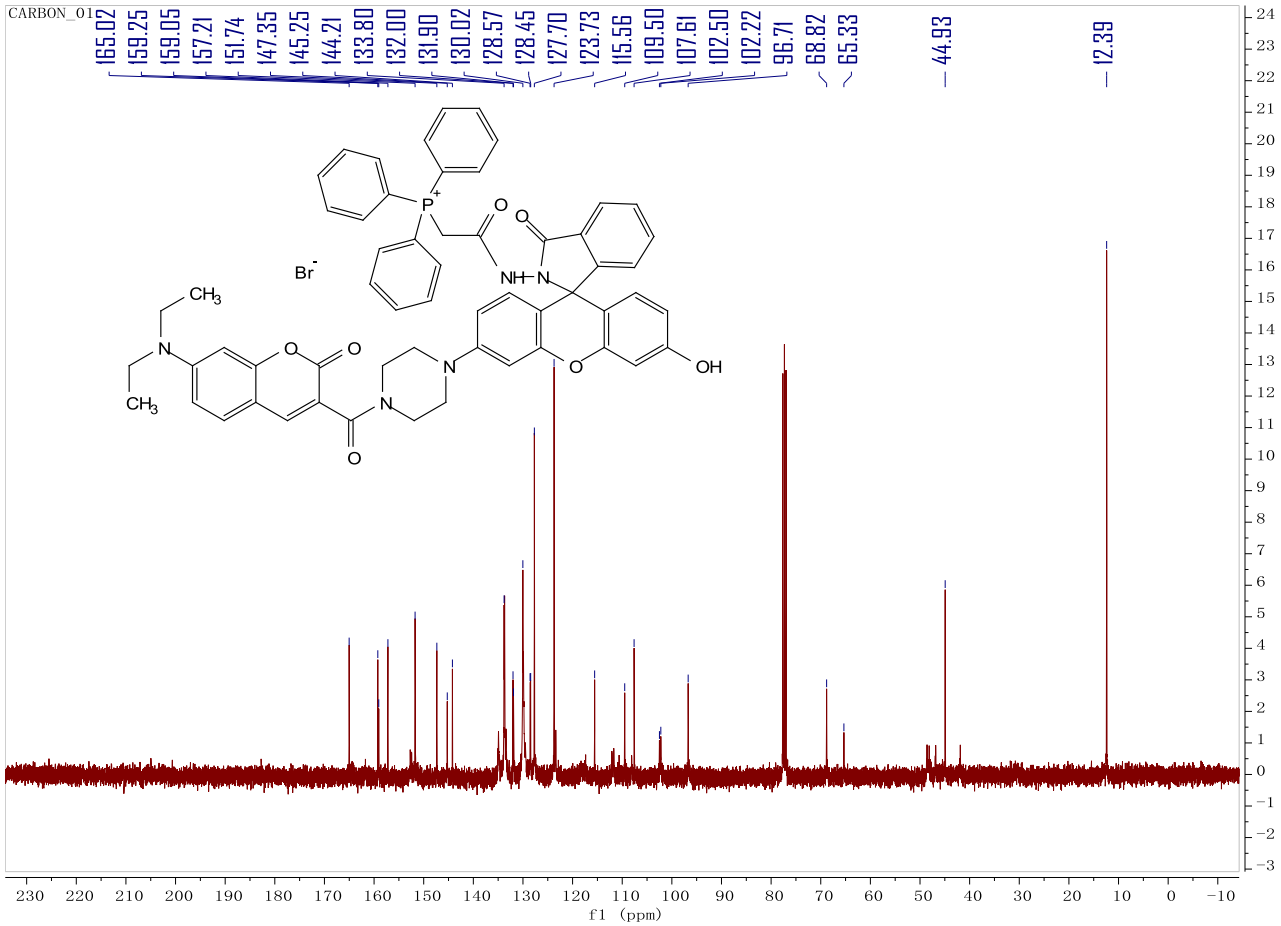


Figure S27. ^{13}C NMR spectrum of compound **RClO** in $CDCl_3$.

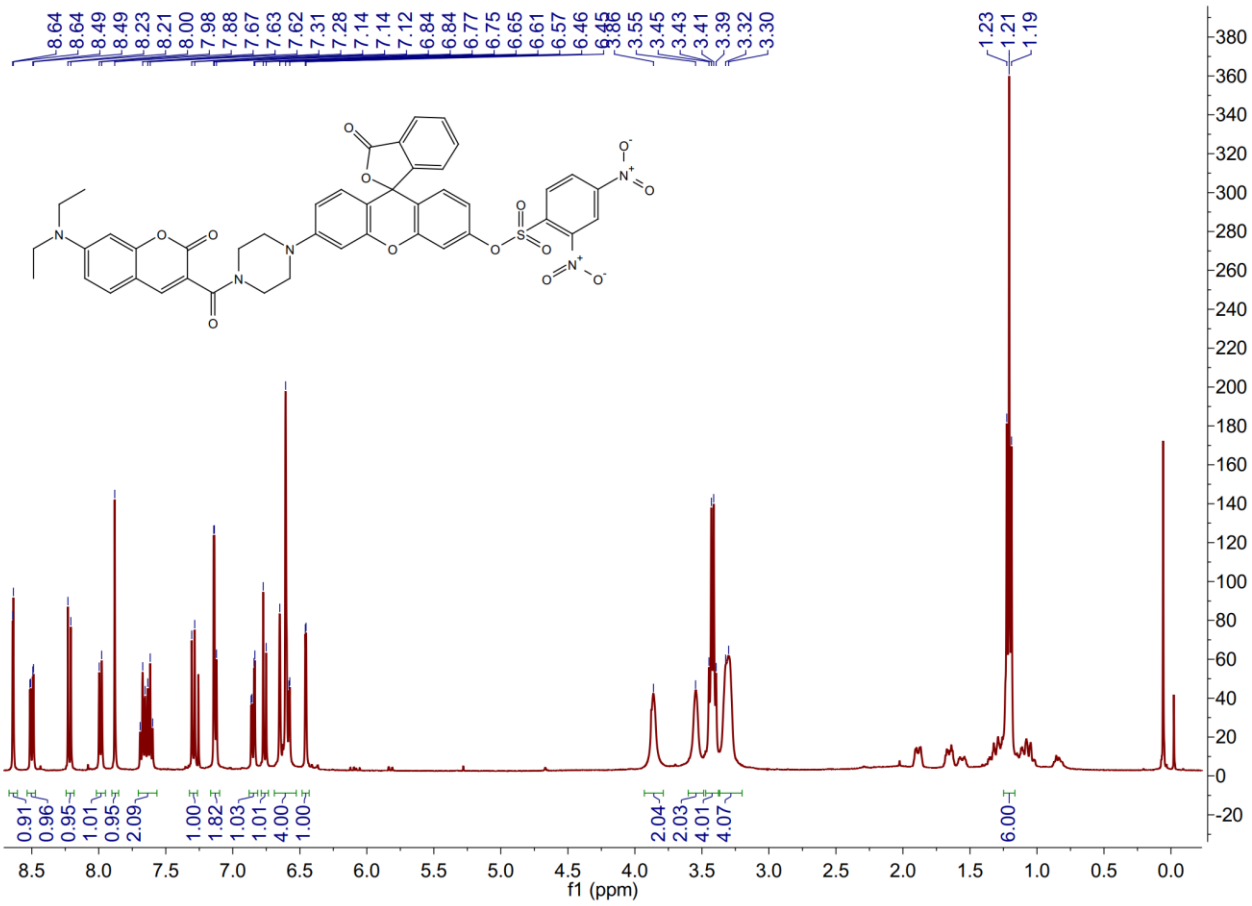


Figure S28. ^{1}H NMR spectrum of compound **RSH** in $CDCl_3$.

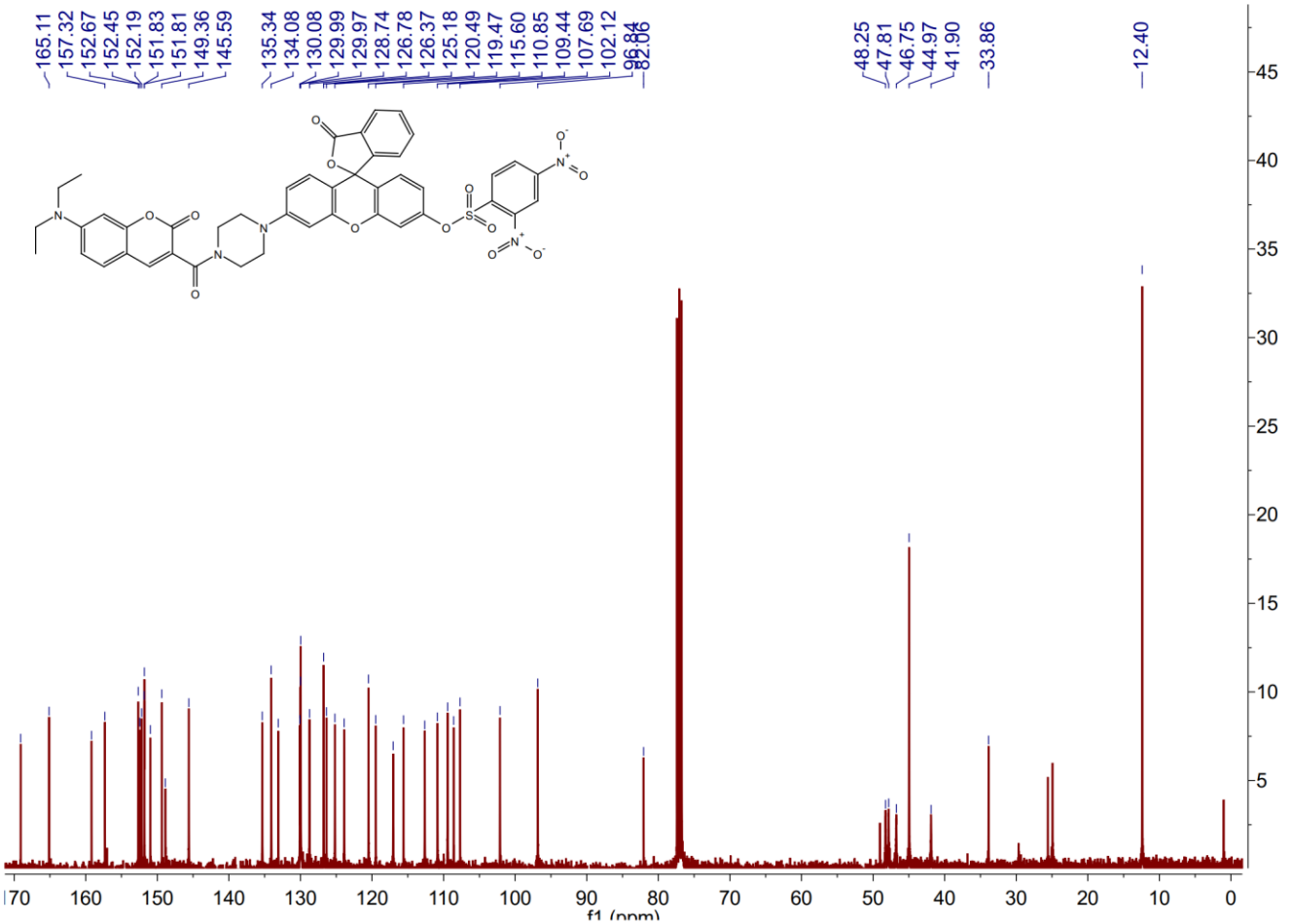


Figure S29. ^{13}C NMR spectrum of compound **RSH** in $CDCl_3$.

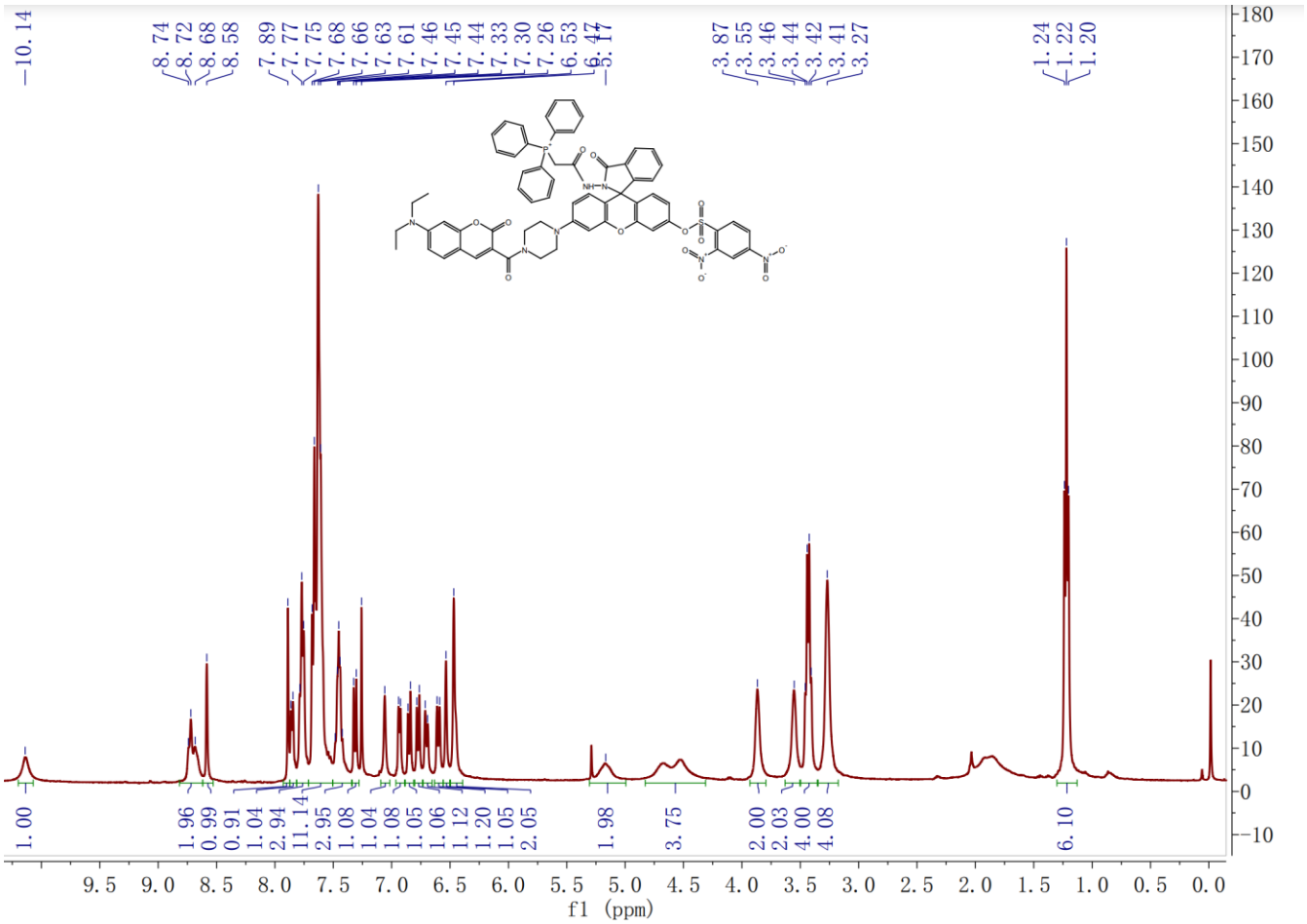


Figure S30. ^{1}H NMR spectrum of compound **RSHClO** in $CDCl_3$.

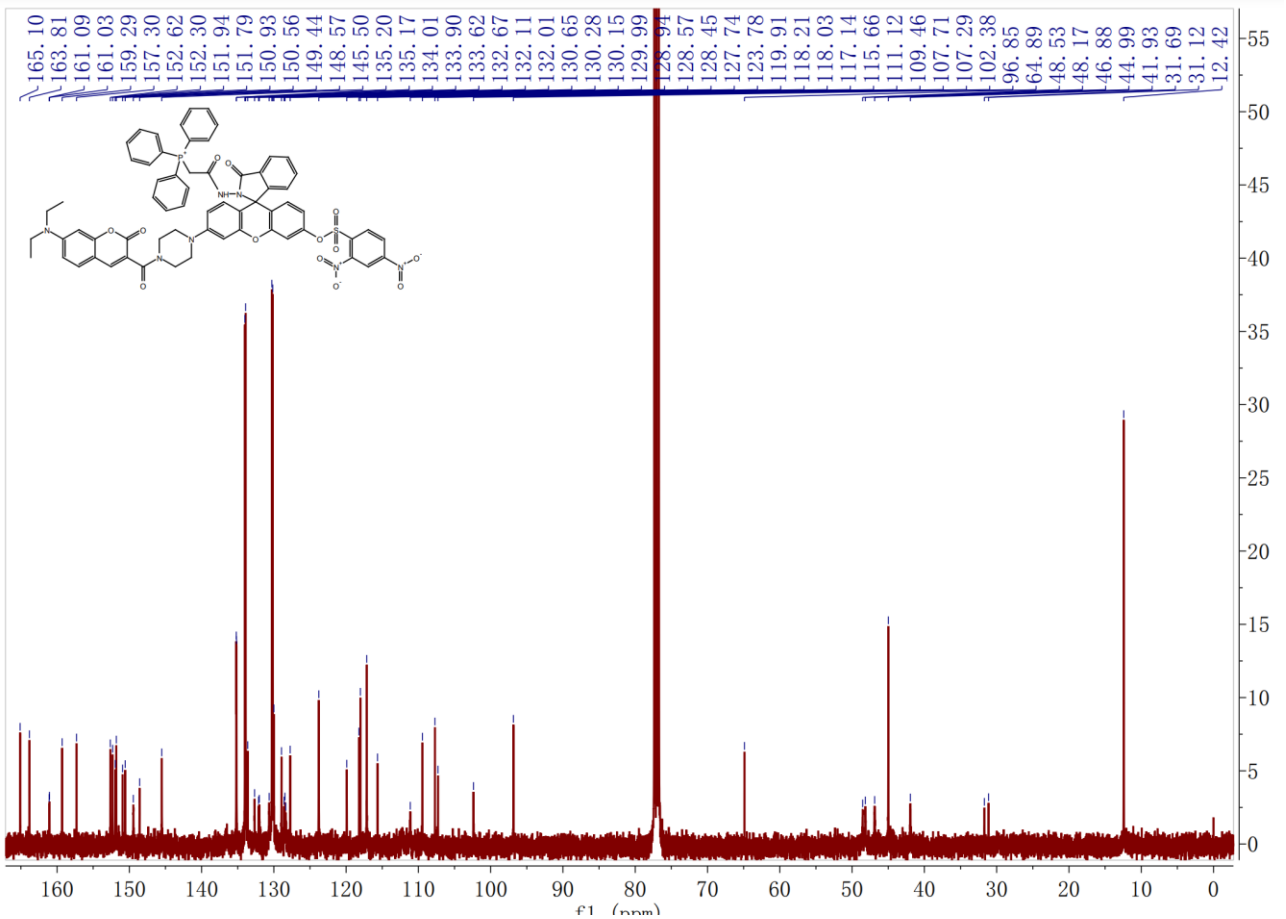


Figure S31. ^{13}C NMR spectrum of compound **RSHClO** in $CDCl_3$.