Supporting information

Simultaneous Detection of Glutathione and Hydrogen Polysulfides from Different Emission Channels

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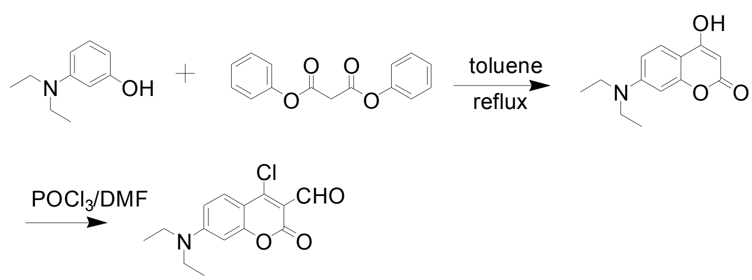
Table of contents

Cell viability assay

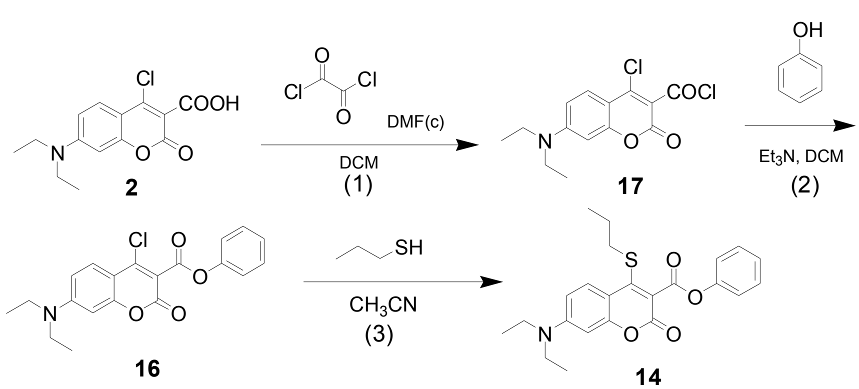
RAW264.7 Cells were grown in CMEM medium supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37ºC under an atmosphere of 5% CO_2. Immediately before the experiment, the cells well placed in a 96 well plate, followed by addition of increasing concentrations of ACC-Cl. The final concentrations of the probe were kept from 0 to 12 μM. The cells were then incubated at 37ºC in an atmosphere of 5% CO_2 and 95% air for 24 h, followed by MTT assays (n= 5). Untreated assay with CMEM (n = 5) was also conducted under the same conditions.

Synthesis of compound 1



Compound **1** was synthesized according the literature method.[2] ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.32 (s, 1H), 7.87 (d, J = 9.3 Hz, 1H), 6.74 (dd, J = 9.3, 2.5 Hz, 1H), 6.48 (d, J = 2.5 Hz, 1H), 3.51 (q, J = 7.2 Hz, 4H), 1.29 (t, J = 7.1 Hz, 6H).

Synthesis of control compound 14

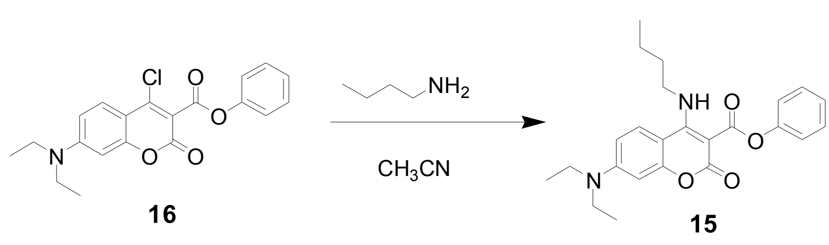


(1) Oxalyl chloride (90 μL, 1 mmol) was added to a solution of **2** (30 mg, 0.1 mmol) in dry CH_2Cl_2 (5 mL). DMF (2 μL) was then added as a catalyst. The reaction mixture was stirred at room temperature for 2 hours under an argon atmosphere. After removal of the solvent, the residue (compound **17**) was used directly for next step without further purification.

(2) To a mixture of the corresponding phenol (0.2 mmol), Et_3N (27 μL, 0.2 mmol) and 17 (0.1 mmol) was added dry CH_2Cl_2 (3 mL) at room temperature. The mixture was stirred overnight. The solvent was removed under reduced pressure and the resultant residue was further purified by silica gel chromatography to afford the compound **16** in 82% yield. Mp: 160-162°C. ^{1}H NMR (300 MHz, CDCl3) δ 7.73 (d, J = 9.2 Hz, 1H), 7.45 (dd, J = 8.7, 6.9 Hz, 2H), 7.31 (dd, J = 7.5, 1.3 Hz, 3H), 6.69 (dd, J = 9.2, 2.5 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 3.48 (q, J = 7.1 Hz, 4H), 1.26 (t, J = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, CDCl3) δ 162.05, 157.70, 155.41, 152.59, 150.59, 149.06, 129.53, 129.53,127.82, 126.26, 121.63, 121.63,112.38, 109.85, 106.23, 96.80, 45.09, 45.09,12.41, 12.41; HRMS (ESI) m/z: calcd for $C_{20}H_{18}ClNO_4Na$ $[M+Na]^+$ 394.0822, found 394.0824.

(3) To a solution of compound **16** (43 mg, 0.1 mmol) and 1-propanethiol (14 μL, 0.15 mmol) in anhydrous CH_3CN (2 mL) was added Et_3N (21 μL, 0.15 mmol). The reaction mixture was stirred at room temperature for 20 min. After removal of the solvent under reduced pressure, the resultant residue was further purified by silica gel chromatography to afford compound **14** (31 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J = 9.1 Hz, 1H), 7.51 – 7.39 (m, 2H), 7.38 – 7.27 (m, 3H), 6.67 (dd, J = 9.2, 2.5 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 3.46 (q, J = 7.1 Hz, 4H), 3.02 (t, J = 7.3 Hz, 2H), 1.72 (dd, J = 14.7, 7.3 Hz, 2H), 1.25 (t, J = 7.1 Hz, 7H), 1.02 (t, J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.76, 158.07, 155.32, 152.30, 151.73, 150.83, 129.51, 128.45, 126.13, 121.64, 117.89, 109.31, 108.35, 97.32, 44.95, 38.07, 23.42, 13.34, 12.46. HRMS (ESI) m/z: calcd for $C_{23}H_{25}NO_4SNa$ $[M+Na]^+$ 434.1402, found 434.1406.

Synthesis of control compound 15



To a solution of compound **16** (43 mg, 0.1 mmol) and 1-propanethiol (14 μL, 0.15 mmol) in anhydrous CH_3CN (2 mL) was added Et_3N (21 μL, 0.15 mmol). The reaction mixture was stirred at room temperature for 20 min. After removal of the solvent under reduced pressure, the resultant residue was further purified by silica gel chromatography to afford compound **15** (31 mg, 75%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.58 (s, 1H), 7.79 (d, J = 9.1 Hz, 1H), 7.37 (t, J = 7.7 Hz, 2H), 7.20 (dd, J = 16.7, 7.8 Hz, 3H), 6.50 (d, J = 8.6 Hz, 1H), 6.44 (s, 1H), 3.75 (d, J = 5.0 Hz, 2H), 3.40 (dd, J = 13.5, 6.6 Hz, 4H), 1.75 – 1.66 (m, 2H), 1.45-1.41 (m, 2H), 1.20 (t, J = 6.8 Hz, 6H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.94, 161.65, 159.65, 156.87, 151.63, 150.96, 129.19, 128.62, 125.46, 122.42, 107.42, 101.71, 97.64, 83.83, 48.26, 44.75, 32.51, 19.98, 13.65, 12.48.

Reference

[1] Takata, T.; Saeki, K.; Makita, Y.; Yamada, N.; Kihara, N. *Inorg. Chem.* **2003**, 42, 3712.
[2] Liu, J.; Sun, Y.-Q.; Huo, Y.; Zhang, H.; Wang, L.; Zhang, P.; Song, D.; Shi, Y.; Guo, W. *J. Am. Chem. Soc.* **2014**, 136, 574.

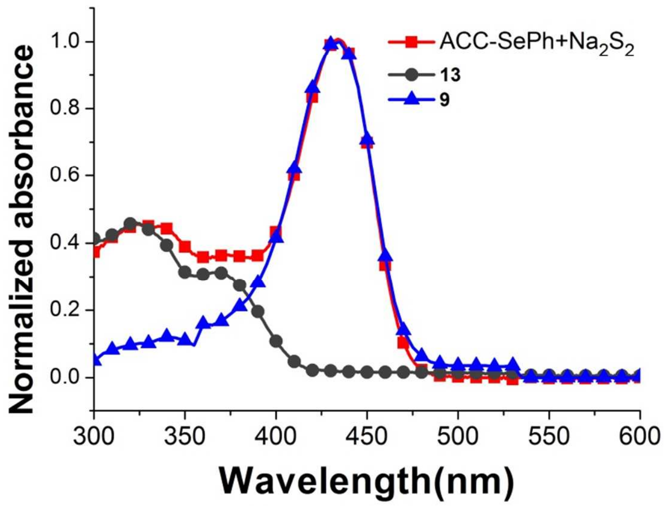


Figure S1. Normalized absorption spectra of ACC-SePh +10.0 equiv of Na_2S_2, compound **13** and compound **9** in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

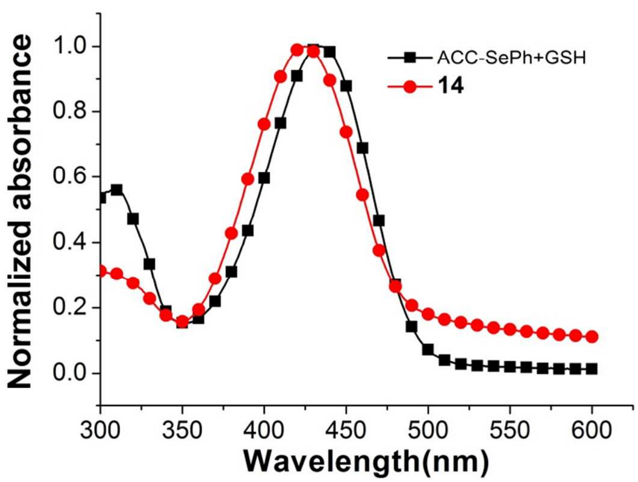


Figure S2. Normalized absorption spectra of ACC-SePh +10.0 equiv of GSH, and compound **14** in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

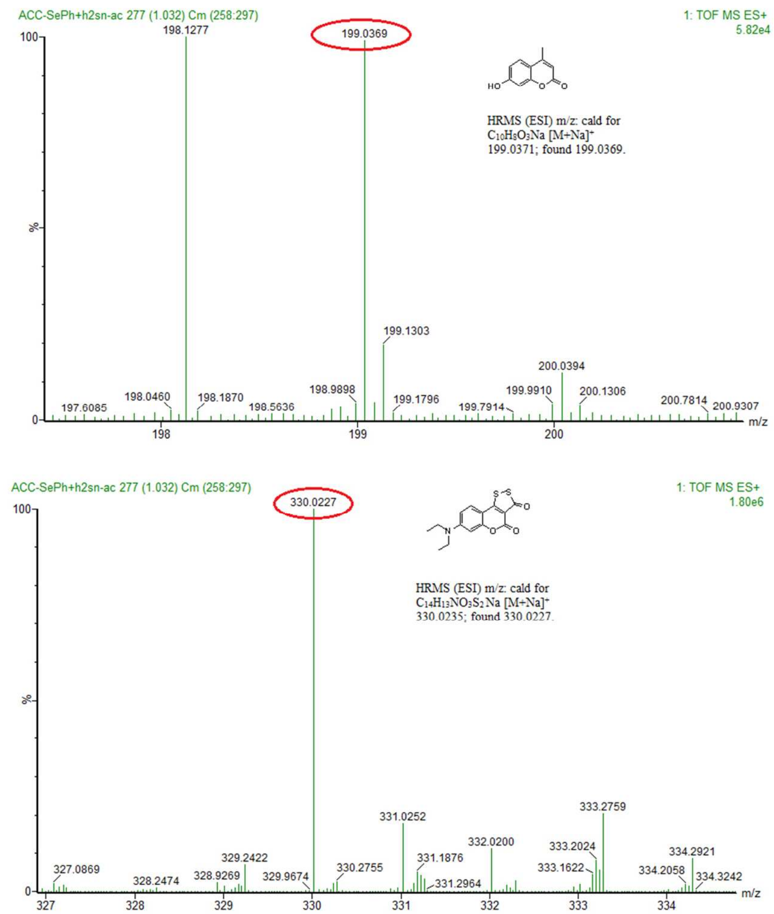


Figure S3. HRMS chart of ACC-SePh upon addition of 6.0 equiv of Na_2S_2 in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

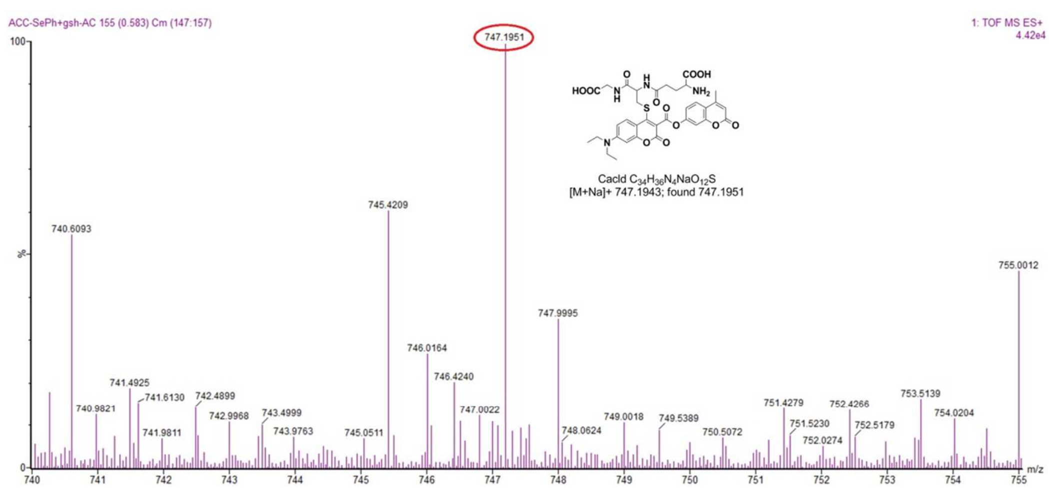


Figure S4. HRMS chart of ACC-SePh upon addition of 6.0 equiv of GSH in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

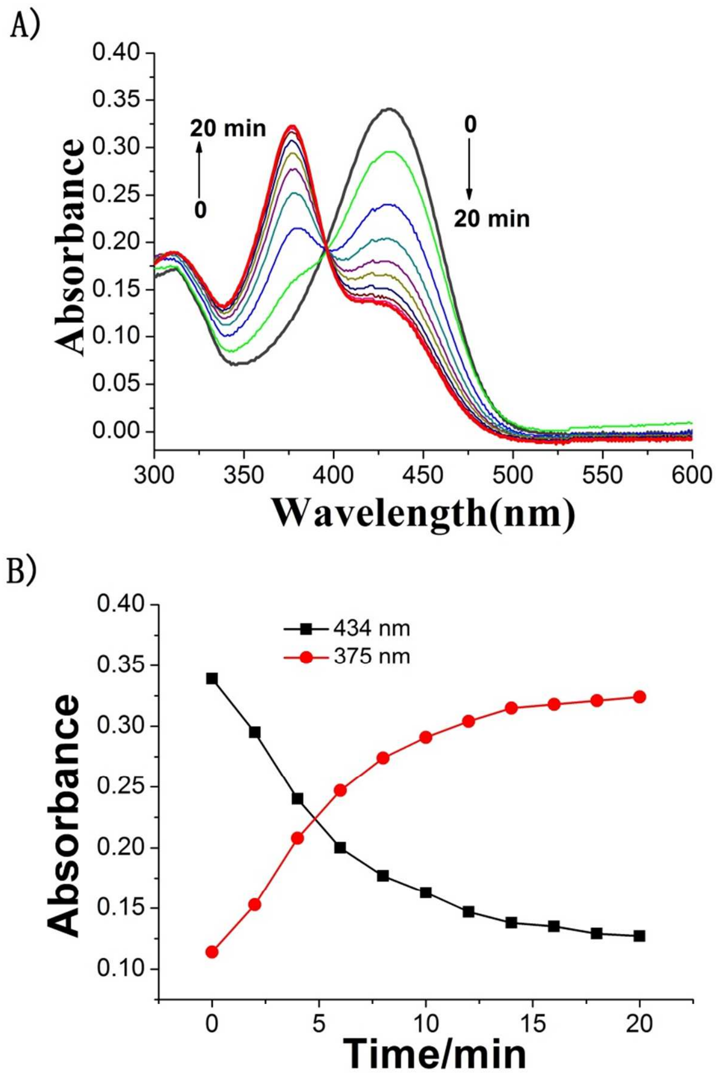


Figure S5 Time-dependent Uv-vis absorption spectra of ACC-SePh (10 μM) in the presence of 10.0 equiv of Cys (A) and the corresponding time-dependent absorption intensity changes (B) in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

A)

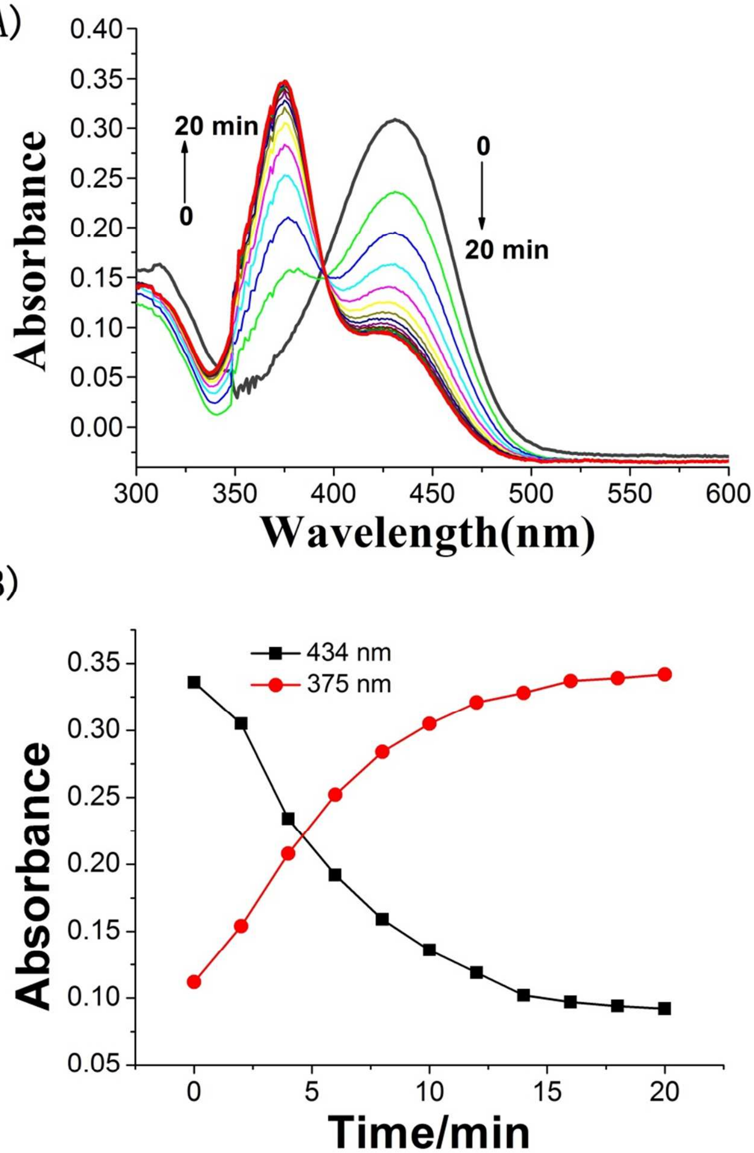


Figure S6. Time-dependent Uv-vis absorption spectra of ACC-SePh (10 μM) in the presence of 10.0 equiv of Hcy (A) and the corresponding time-dependent absorption intensity changes (B) in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

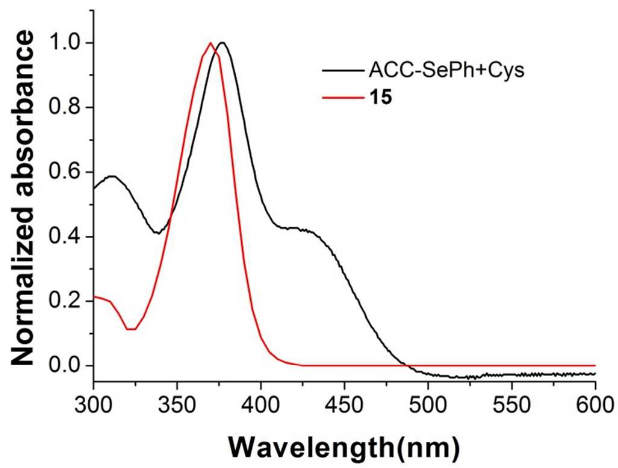


Figure S7. Normalized absorption spectra of ACC-SePh +10.0 equiv of Cys and compound **15** in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

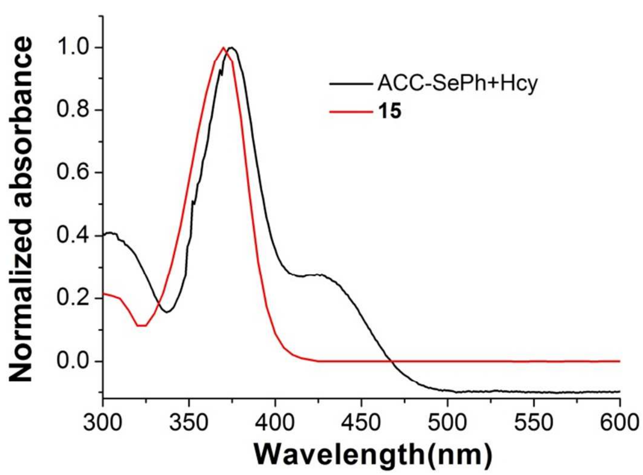


Figure S8. Normalized absorption spectra of ACC-SePh +10.0 equiv of Hcy and compound **15** in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

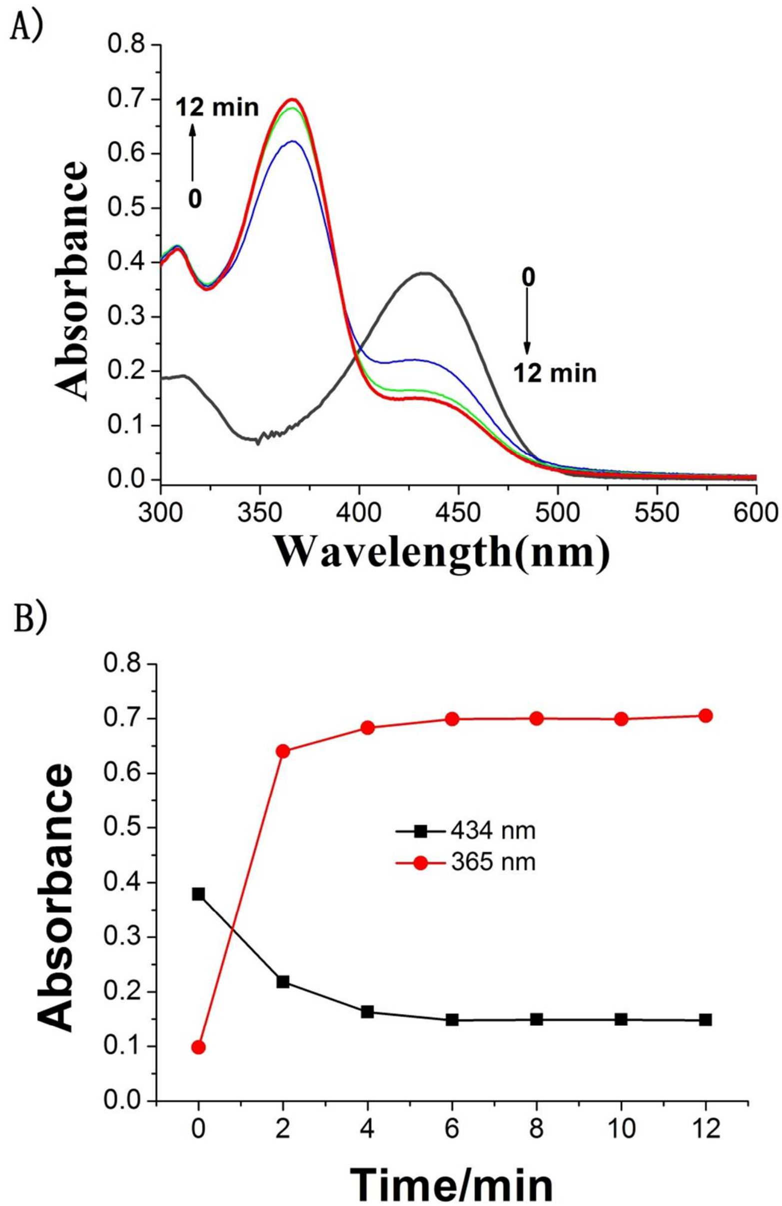


Figure S9. Time-dependent Uv-vis absorption spectra of ACC-SePh (10 μM) in the presence of 10.0 equiv of Na_2S (A) and the corresponding time-dependent absorption intensity changes (B) in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

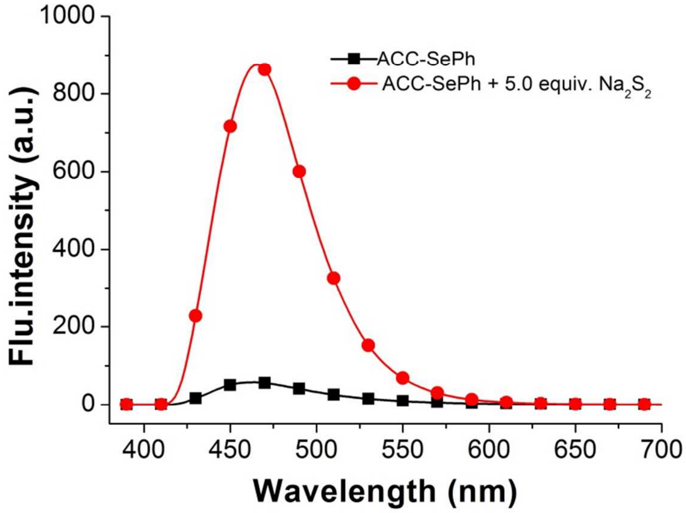


Figure S10. Fluorescence spectra of ACC-SePh (10 μM) in the absence/presence of 5.0 equiv. of Na_2S_2 in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

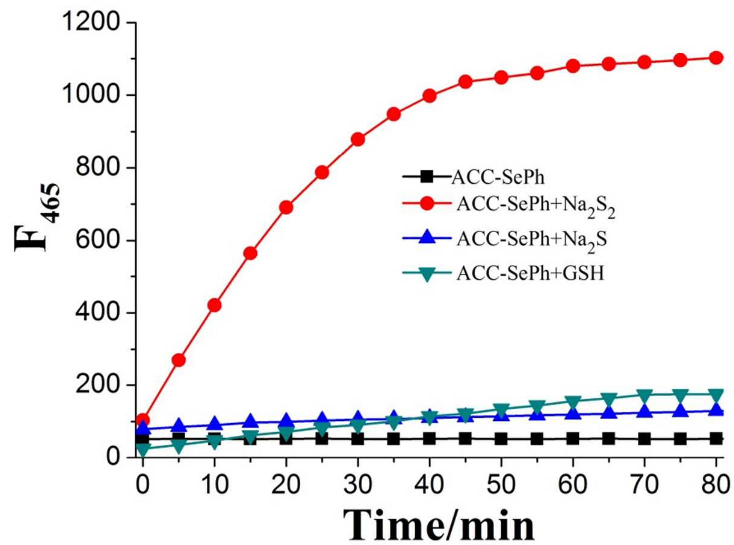


Figure S11. Time-dependent fluorescence intensity changes of ACC-SePh (10 μM) toward 10 equiv. of Na_2S_2, Na_2S and GSH excited at 366 nm in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

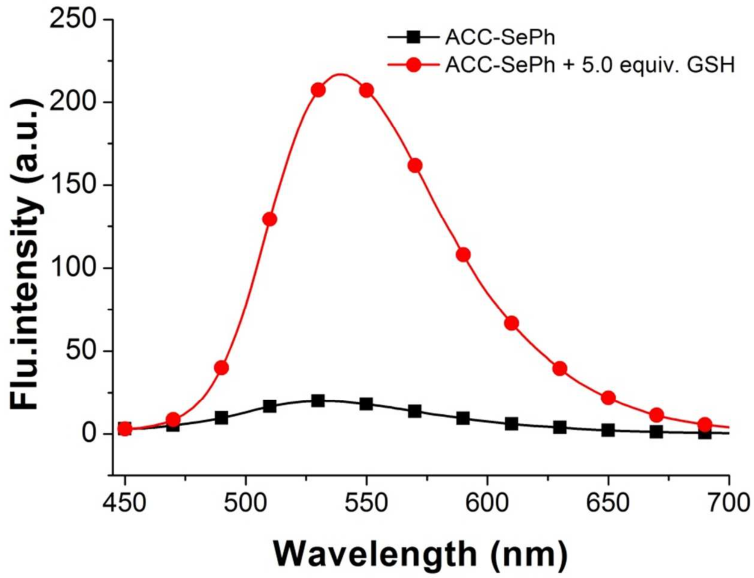


Figure S12. Fluorescence spectra of ACC-SePh (10 μM) in the absence/presence of 5.0 equiv. of GSH in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

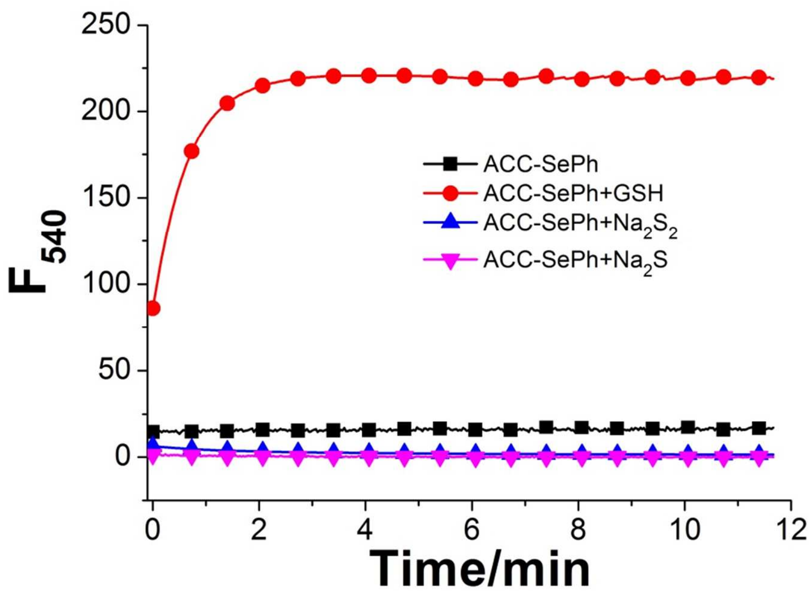


Figure S13. Time-dependent fluorescence intensity changes of ACC-SePh (10 μM) toward 10.0 equiv. of Na_2S_2, Na_2S and GSH excited at 430 nm in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

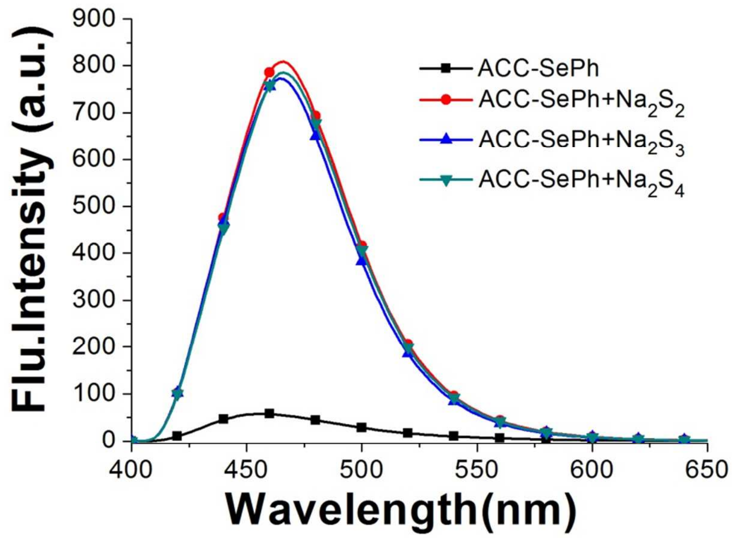


Figure S14. Fluorescence spectra of ACC-SePh in the presence of 6.0 equiv of Na_2S_2, Na_2S_3 or Na_2S_4 in PBS buffer (10 mM, pH 7.4, containing 1mM CTAB).

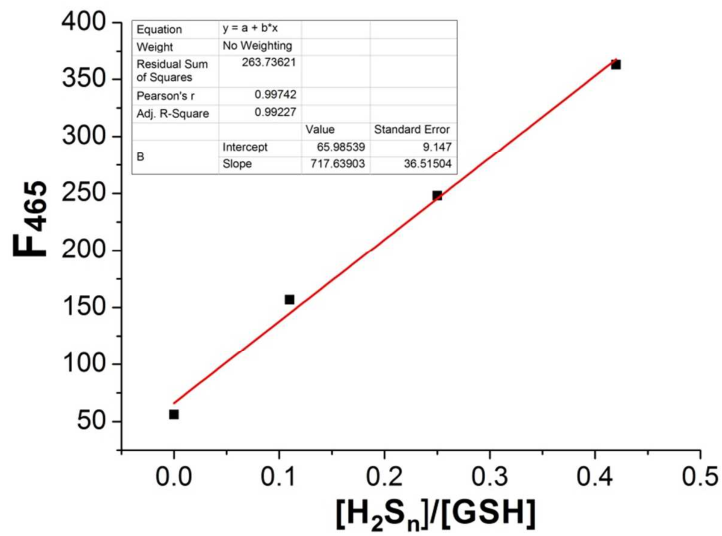


Figure S15. The linear fitting curve between the fluorescence intensity at 465 nm (F_{465}) and the concentration ratios ($[H_2S_n]/[GSH]$). λ_{ex} = 366 nm.

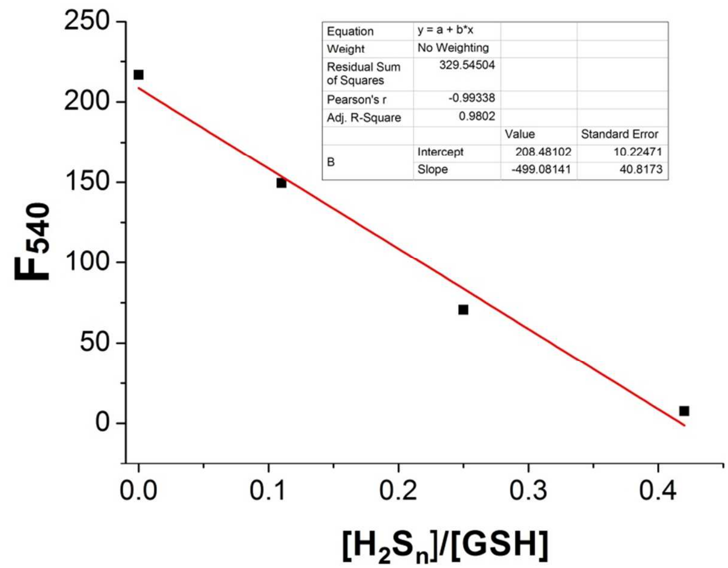


Figure S16. The linear fitting curve between the fluorescence intensity at 540 nm (F_{540}) and the concentration ratios ($[H_2S_n]/[GSH]$). λ_{ex} = 430 nm.

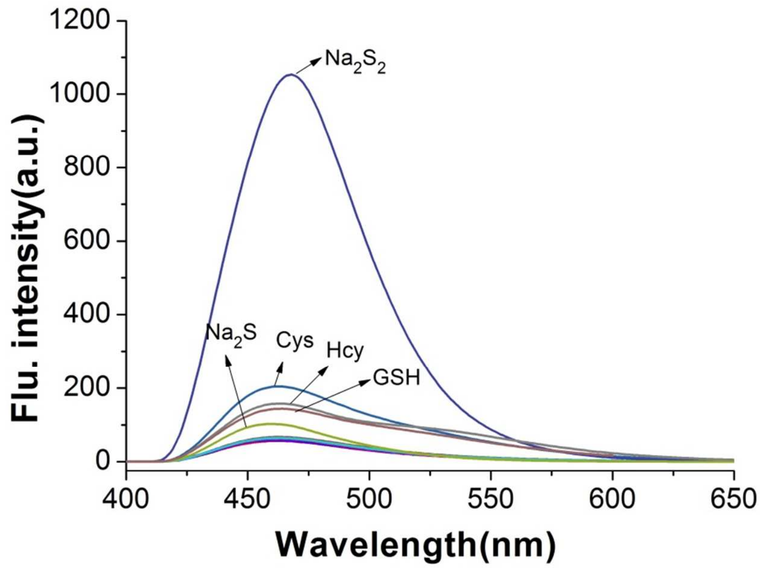


Figure S17. Fluorescence spectra of ACC-SePh (10 μM) in the presence of 10.0 equiv of various species (KCl, $CaCl_2$, $ZnCl_2$, $MgCl_2$, NaClO, L-Glu, L-Ser, DL-Tyr, D-Pro, Cys, DL-Met, $NaHSO_3$, $Na_2S_2O_3$, Na_2SO_4, H_2O_2, Na_2S, Hcy, GSH, Na_2S_2) in PBS buffer (10 mM, pH 7.4, containing 1 mM CTAB). λ_{ex} = 366 nm.

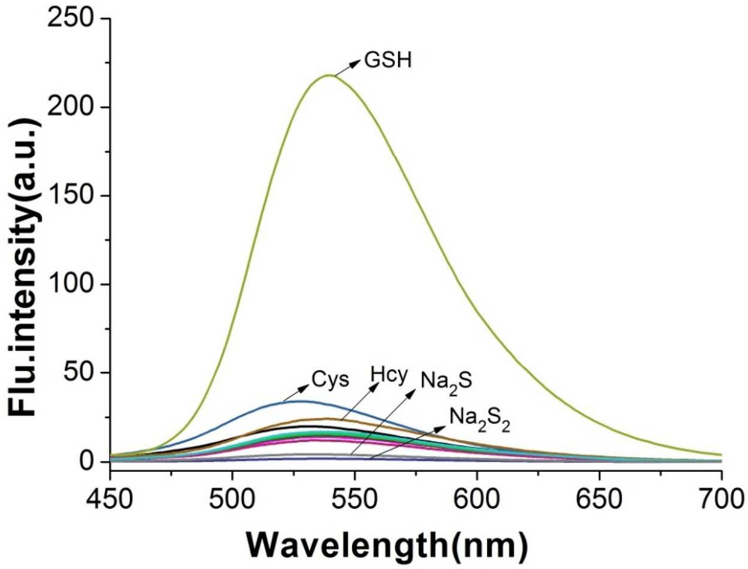


Figure S18. Fluorescence spectra of ACC-SePh (10 μM) in the presence of 10.0 equiv of various species (KCl, $CaCl_2$, $ZnCl_2$, $MgCl_2$, NaClO, L-Glu, L-Ser, DL-Tyr, D-Pro, Cys, DL-Met, $NaHSO_3$, $Na_2S_2O_3$, Na_2SO_4, H_2O_2, Na_2S, Hcy, GSH, Na_2S_2) in PBS buffer (10 mM, pH 7.4, containing 1 mM CTAB). λ_{ex} = 430 nm.

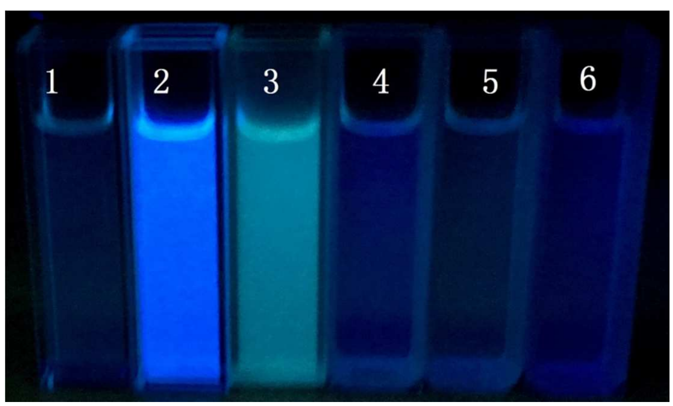


Figure S19. Fluorescence images of ACC-SePh alone (**1**) and in the presence of Na_2S_2 (**2**), GSH (**3**), Cys (**4**), Hcy (**5**), and Na_2S (**6**) under illumination by a 365 nm UV lamp.

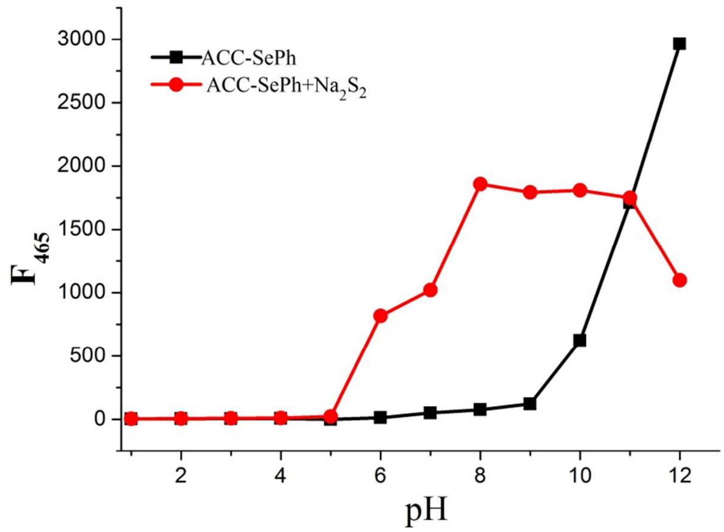


Figure S20. Fluorescence intensity at 465 nm of ACC-SePh (10 μM) at different pH values in the absence/presence of 5.0 equiv. of Na_2S_2 upon excited at 366 nm.

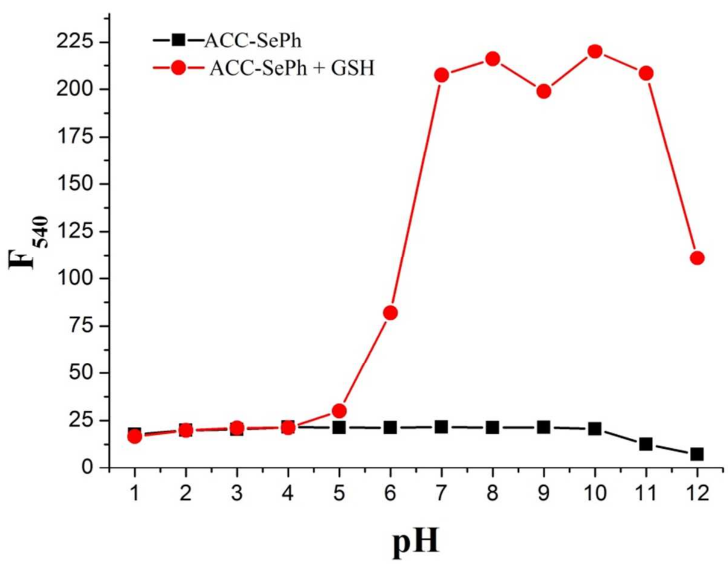


Figure S21. Fluorescence intensity at 540 nm of ACC-SePh (10 μM) at different pH values in the absence/presence of 5.0 equiv. of GSH upon excited at 430 nm.

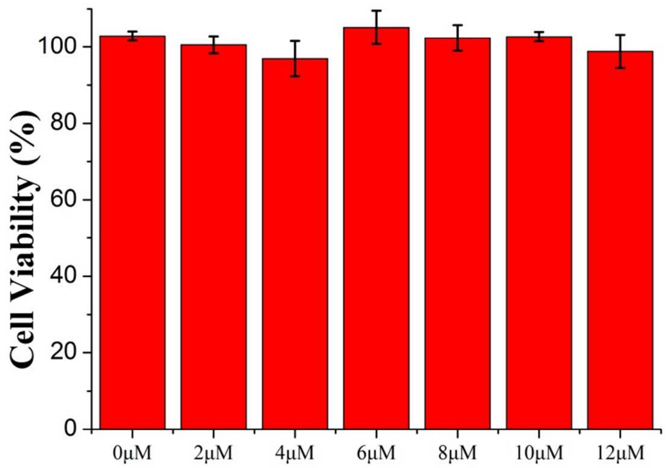


Figure S22. Percentage of viable RAW264.7 cells after treatment with indicated concentrations of ACC-SePh after 24 hours.

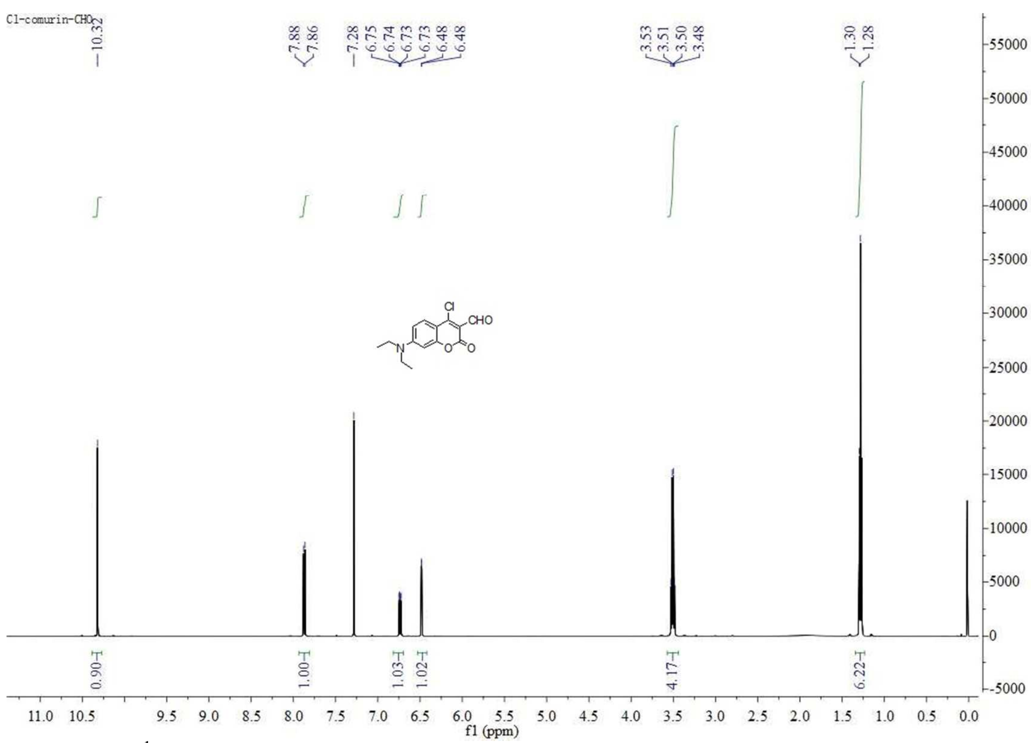


Figure S23. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

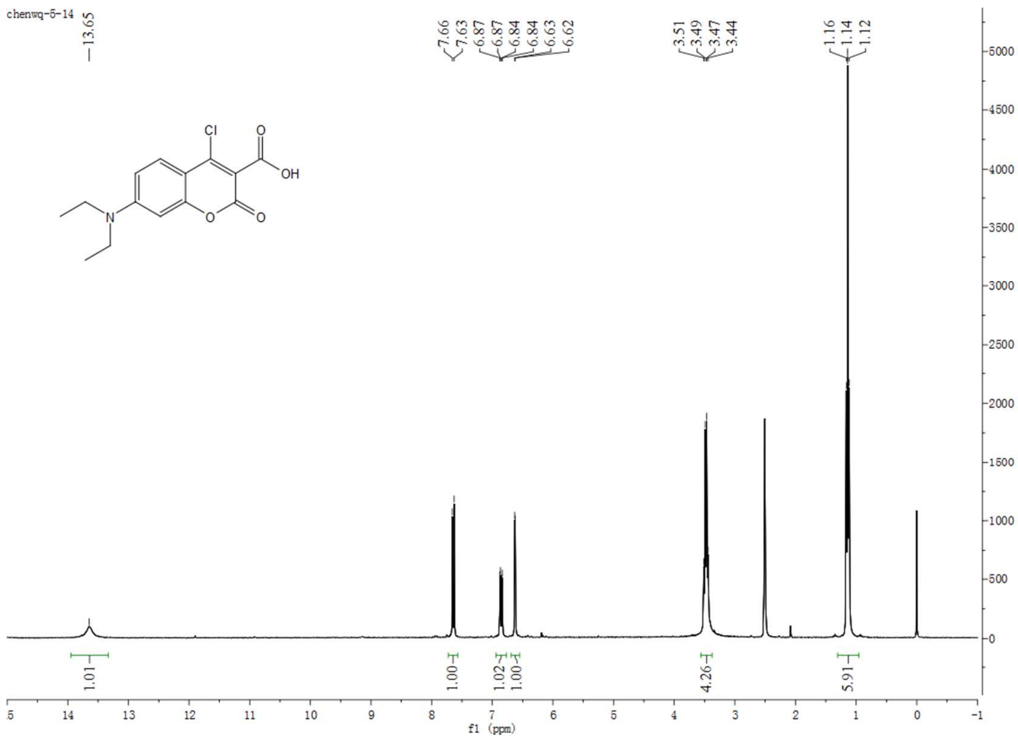


Figure S24. ^{1}H NMR spectrum of compound **2** in d_6-DMSO.

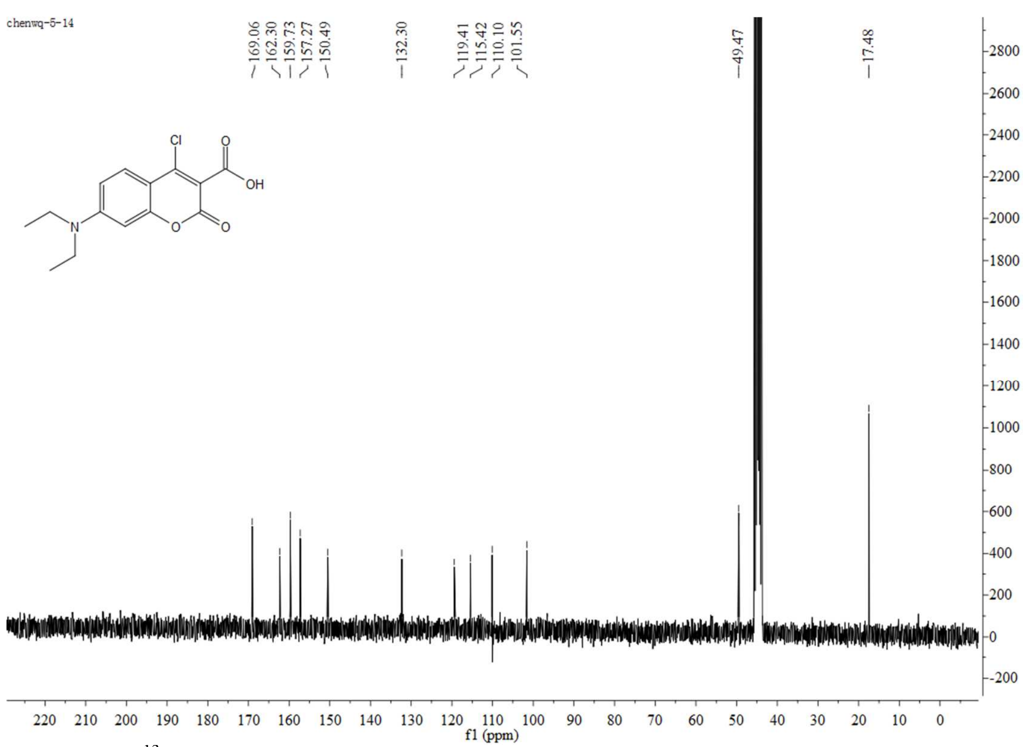


Figure S25. ^{13}C NMR spectrum of compound **2** in d_6-DMSO.

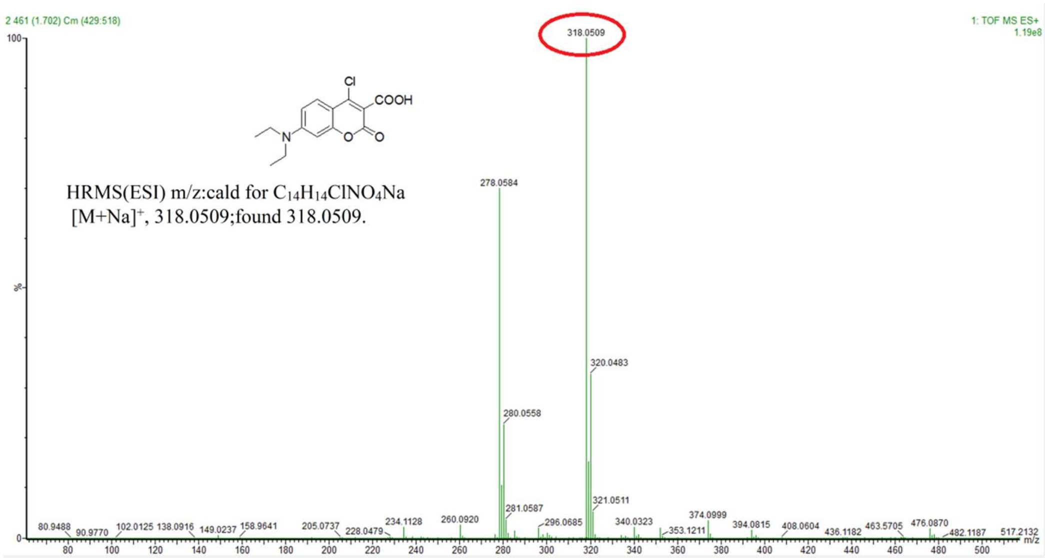


Figure S26. HRMS spectrum of compound **2**.

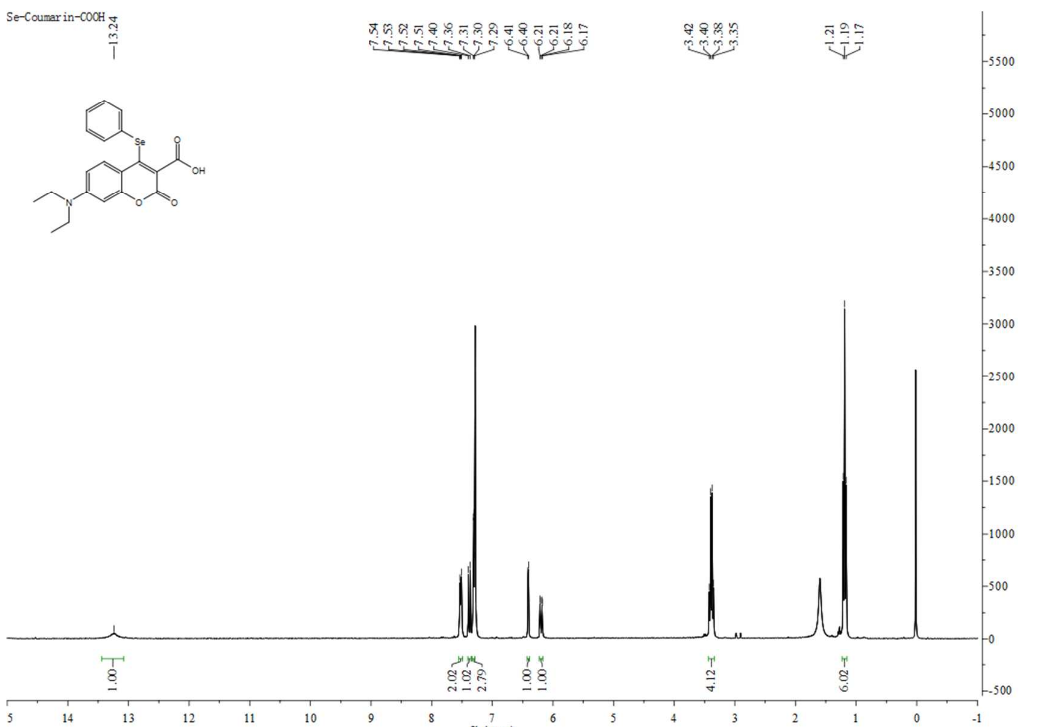


Figure S27. ^{1}H NMR spectrum of compound **5** in $CDCl_3$.

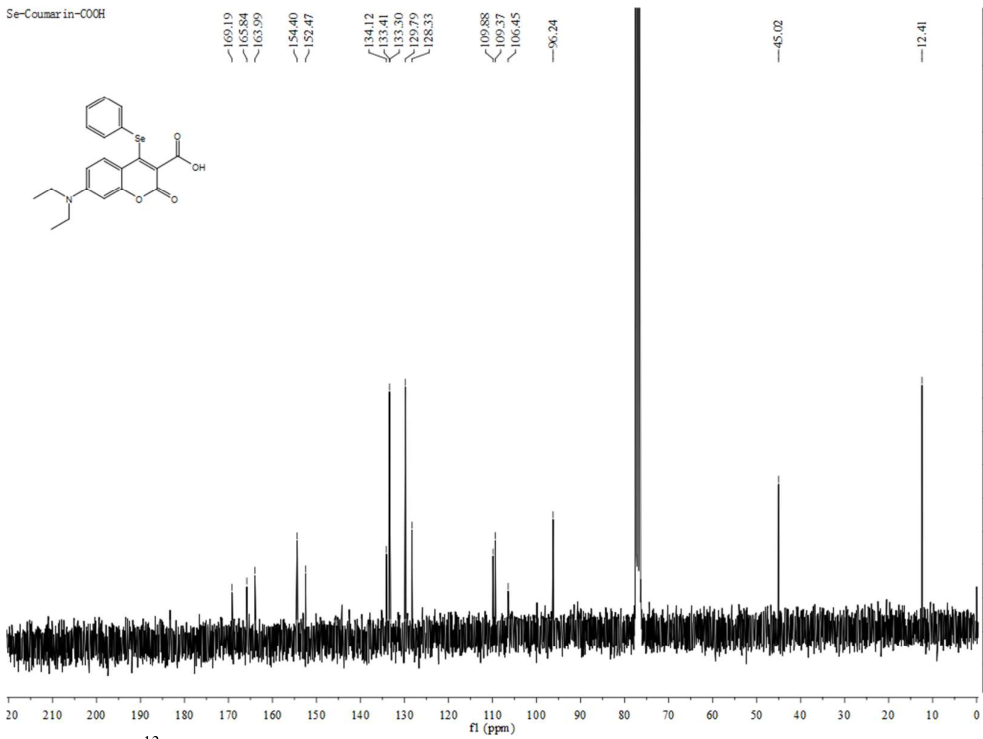


Figure S28. ^{13}C NMR spectrum of compound **5** in $CDCl_3$.

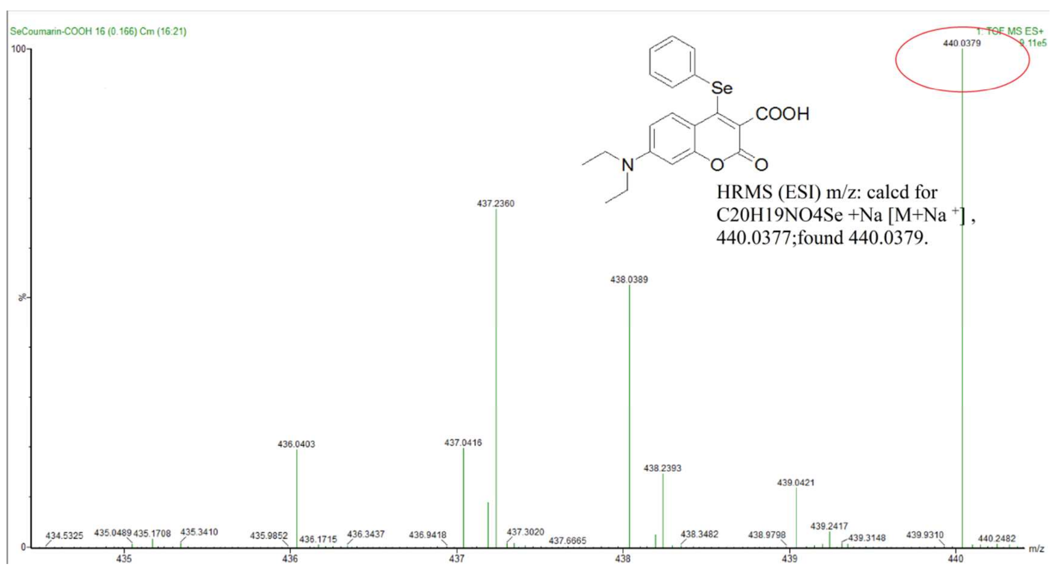


Figure S29. HRMS spectrum of compound **5**.

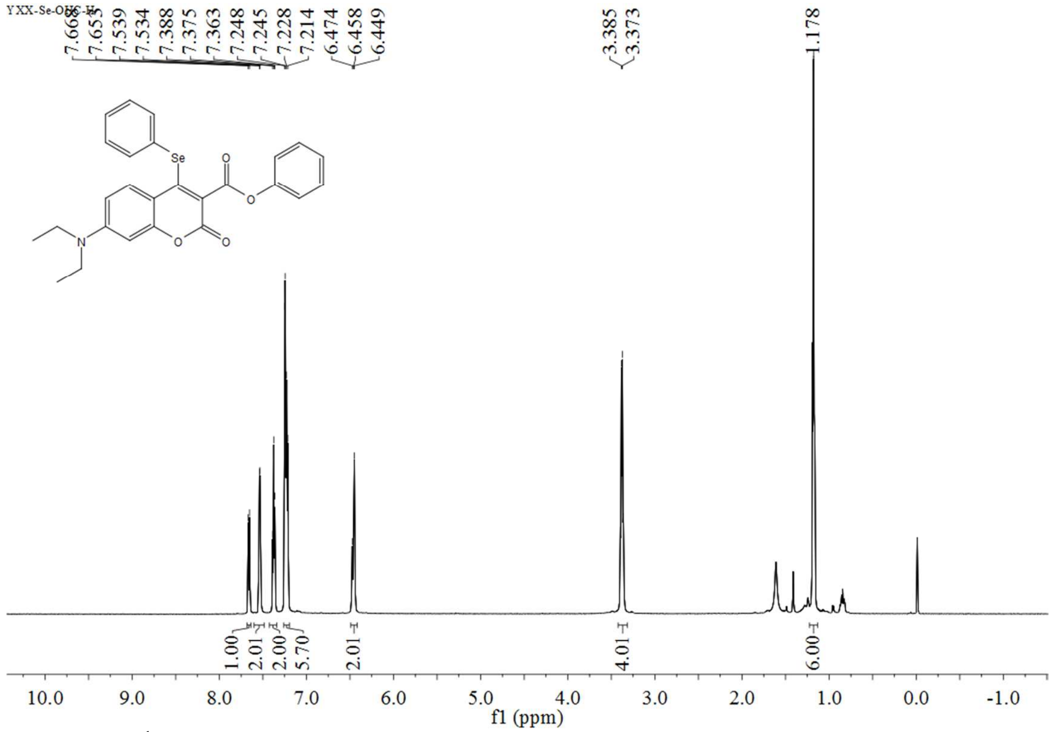


Figure S30. ^{1}H NMR spectrum of compound **6** in $CDCl_3$.

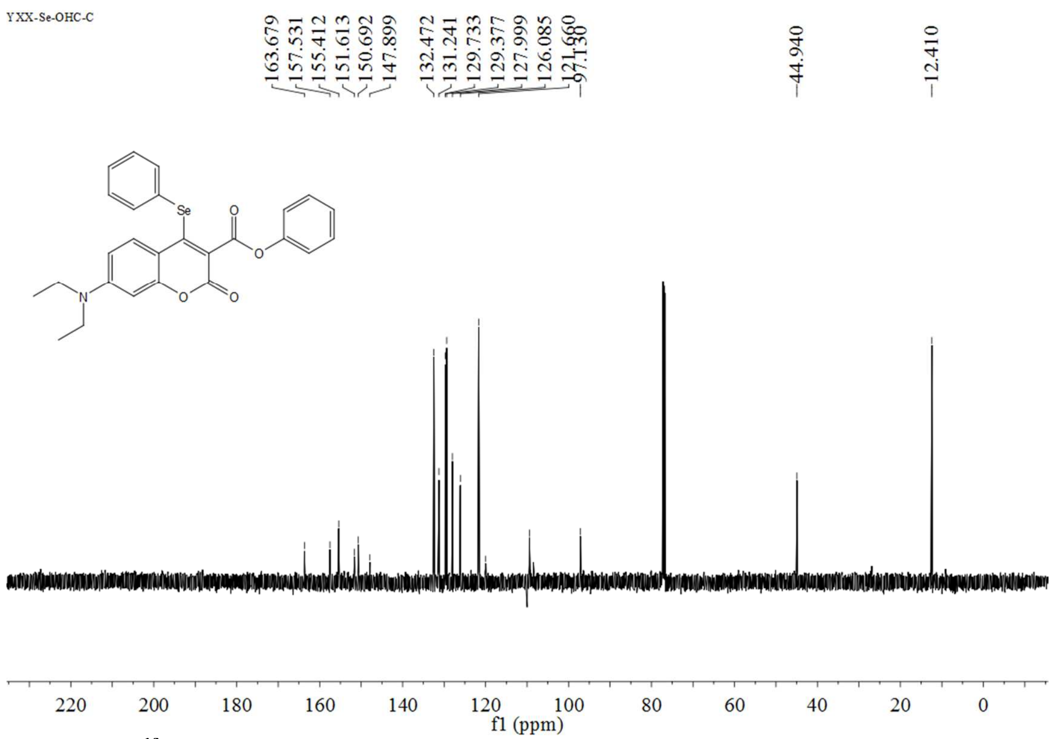


Figure S31. ^{13}C NMR spectrum of compound **6** in $CDCl_3$.

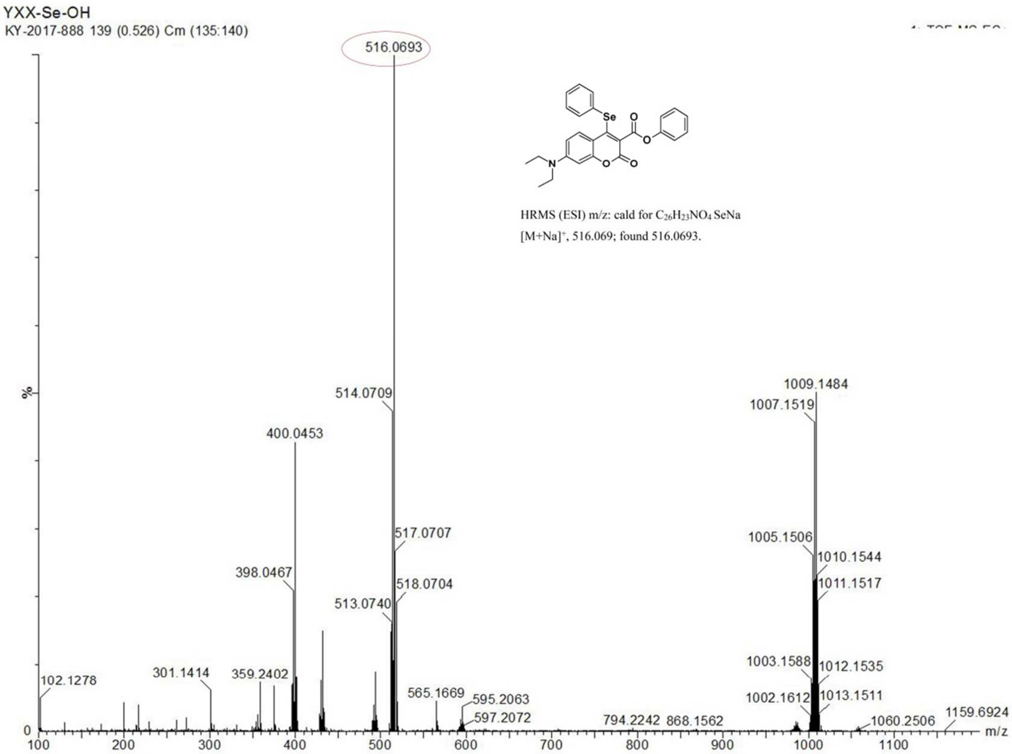


Figure S32. HRMS spectrum of compound **6**.

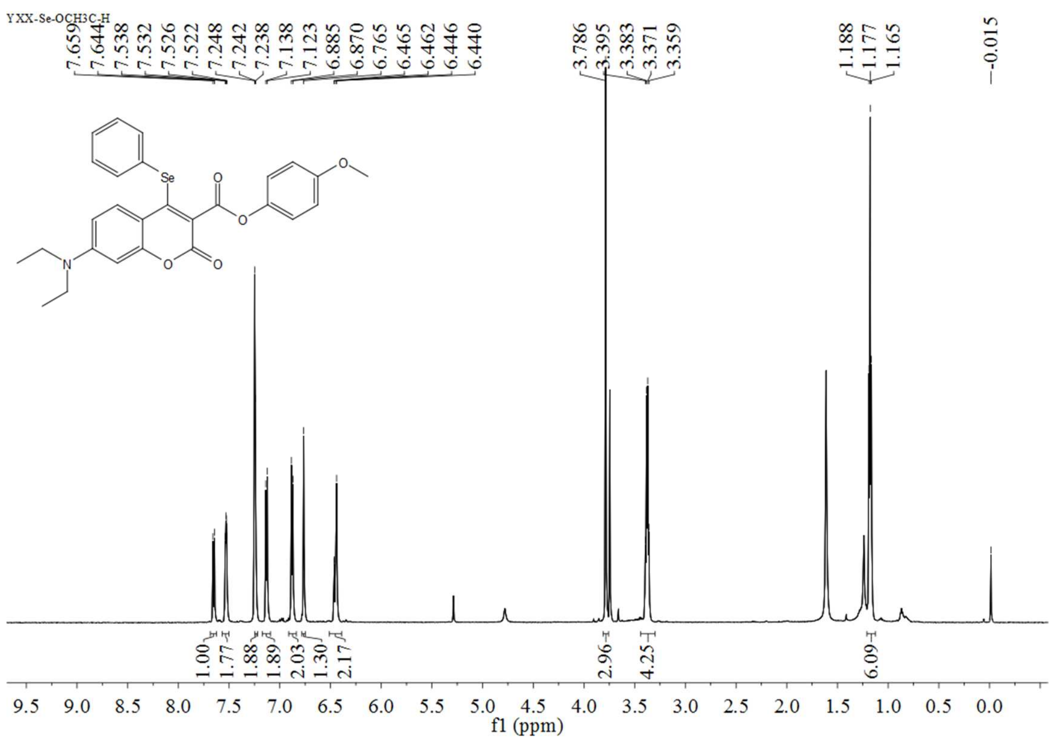


Figure S33. ^{1}H NMR spectrum of compound **7** in $CDCl_3$.

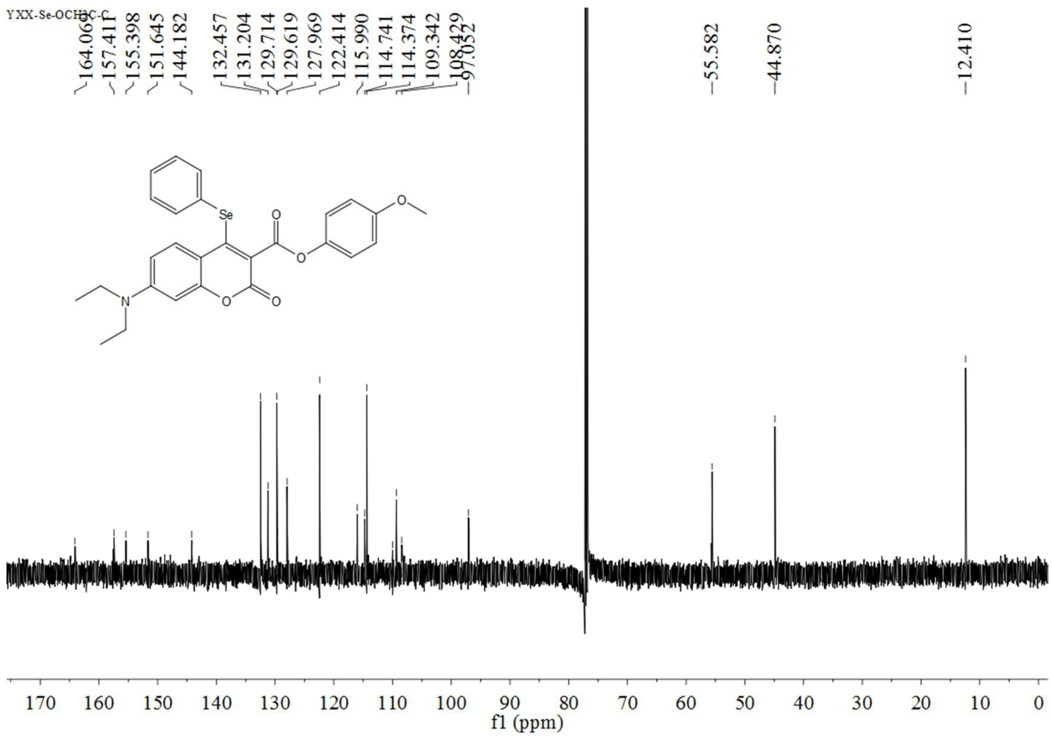


Figure S34. ^{13}C NMR spectrum of compound **7** in $CDCl_3$.

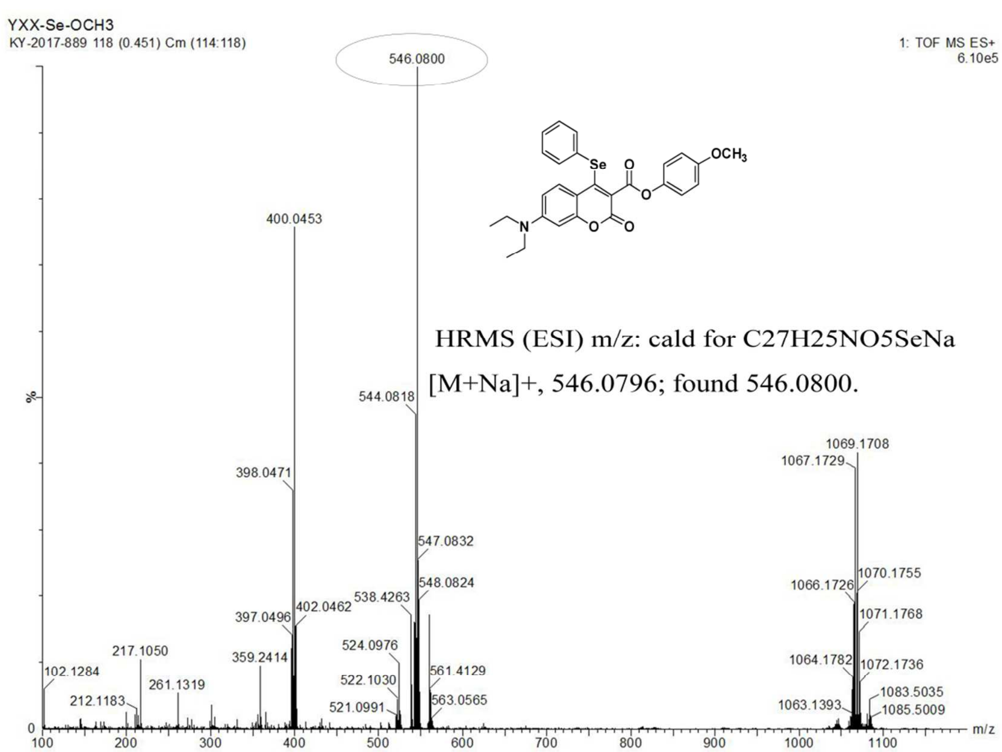


Figure S35. HRMS spectrum of compound **7**.

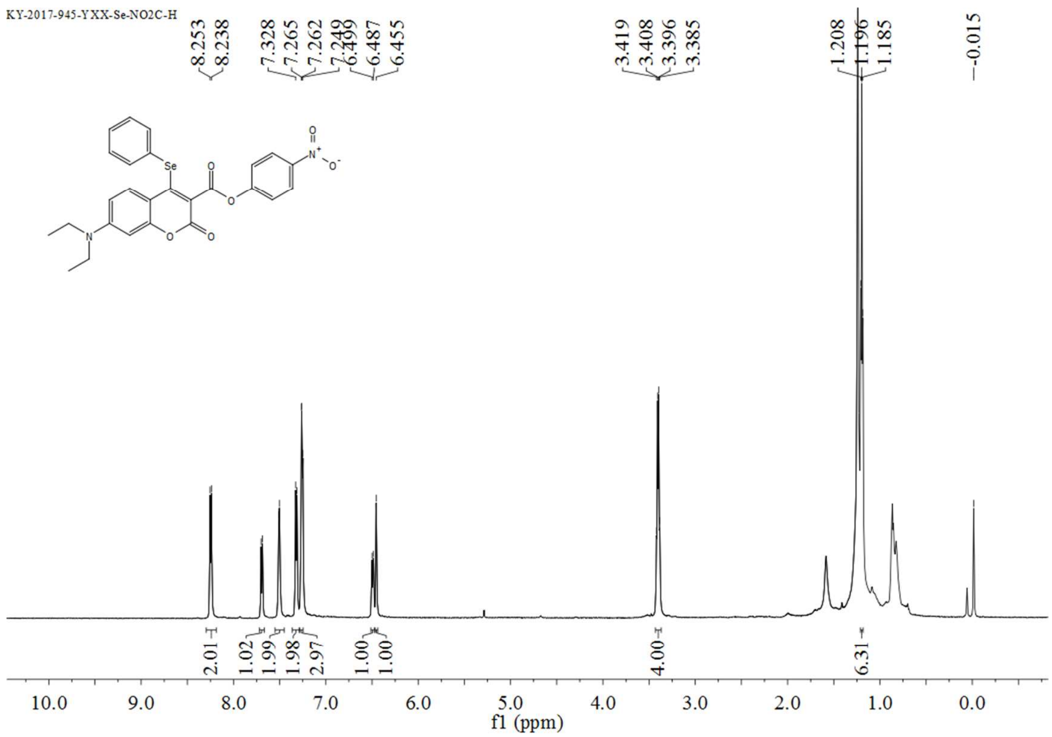


Figure S36. ^{1}H NMR spectrum of compound **8** in $CDCl_3$.

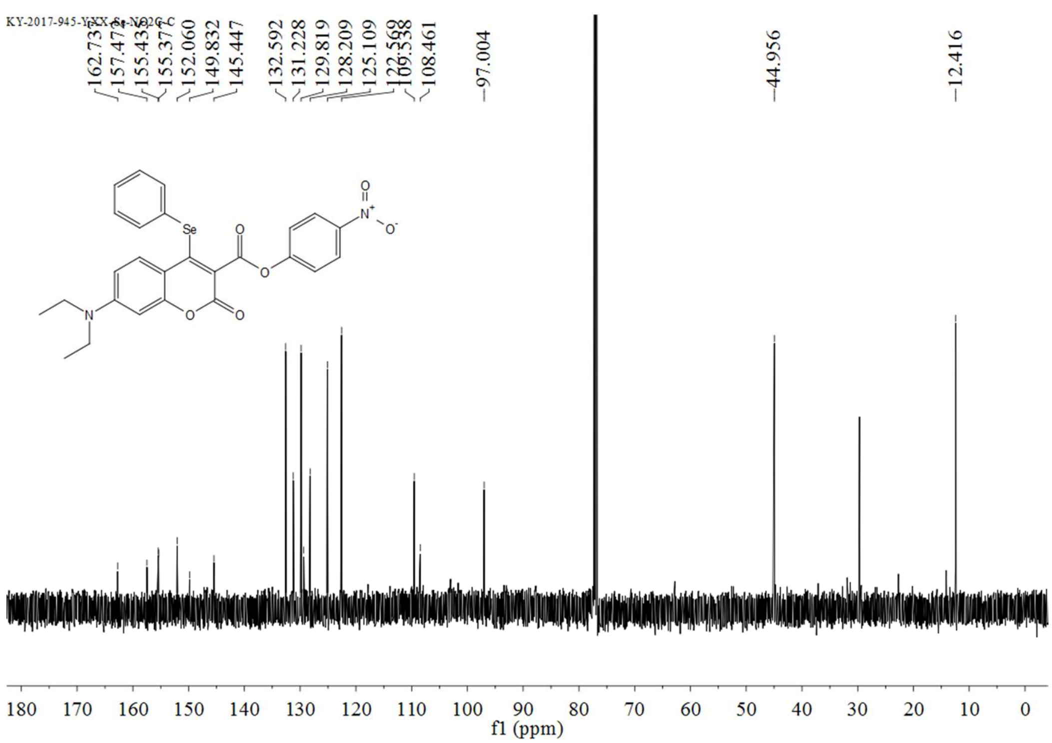


Figure S37. ^{13}C NMR spectrum of compound **8** in $CDCl_3$.

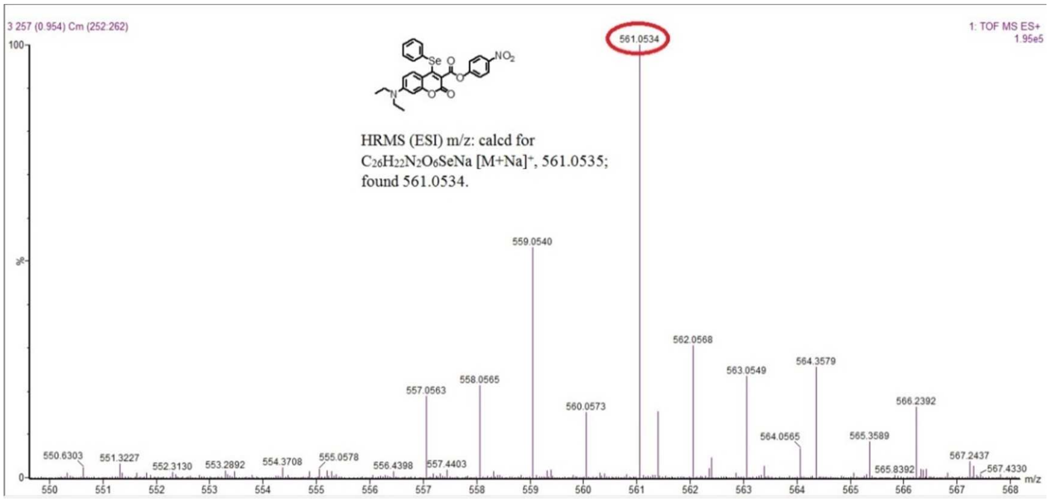


Figure S38. ^{13}C NMR spectrum of compound **8** in $CDCl_3$.

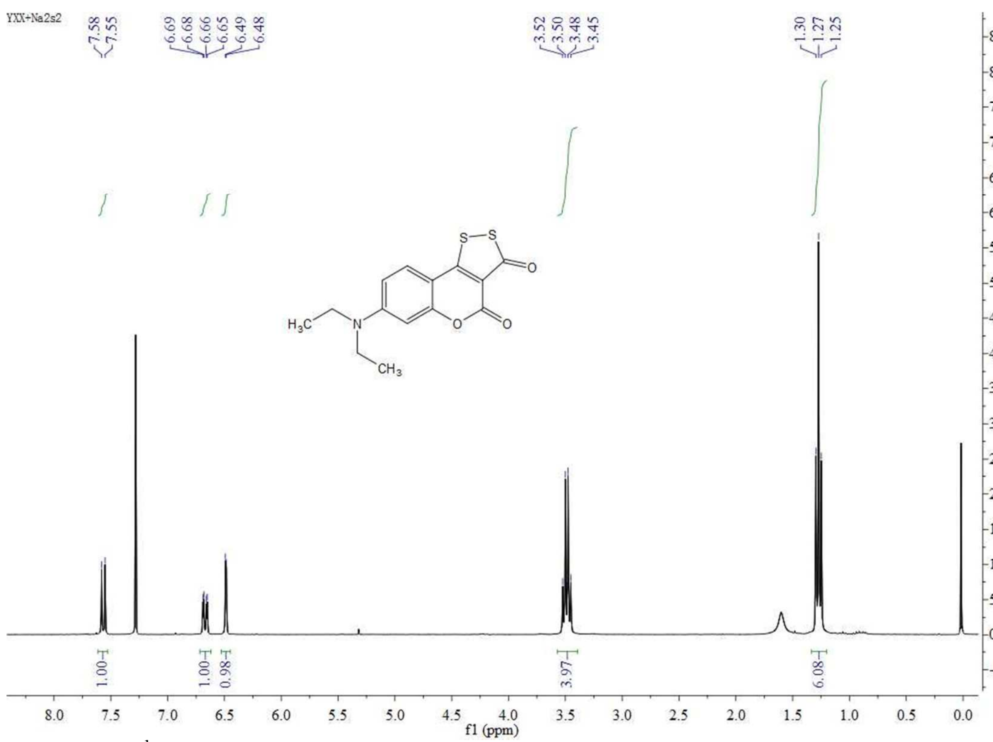


Figure S39. ^{1}H NMR spectrum of compound **9** in $CDCl_3$.

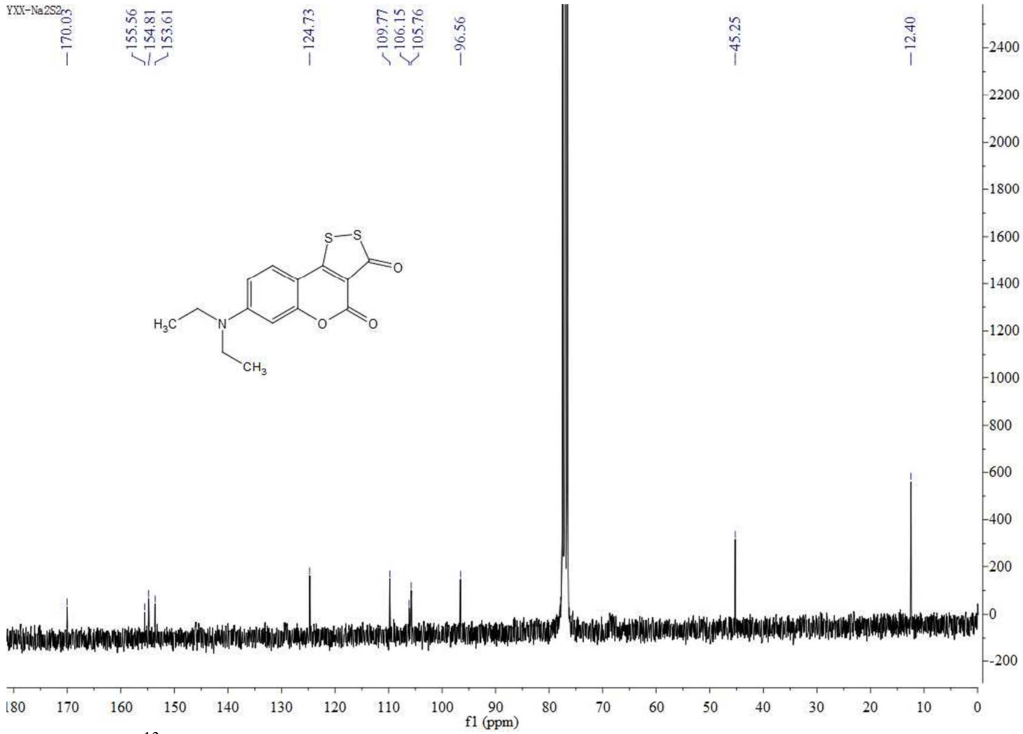


Figure S40. ^{13}C NMR spectrum of compound **9** in $CDCl_3$.

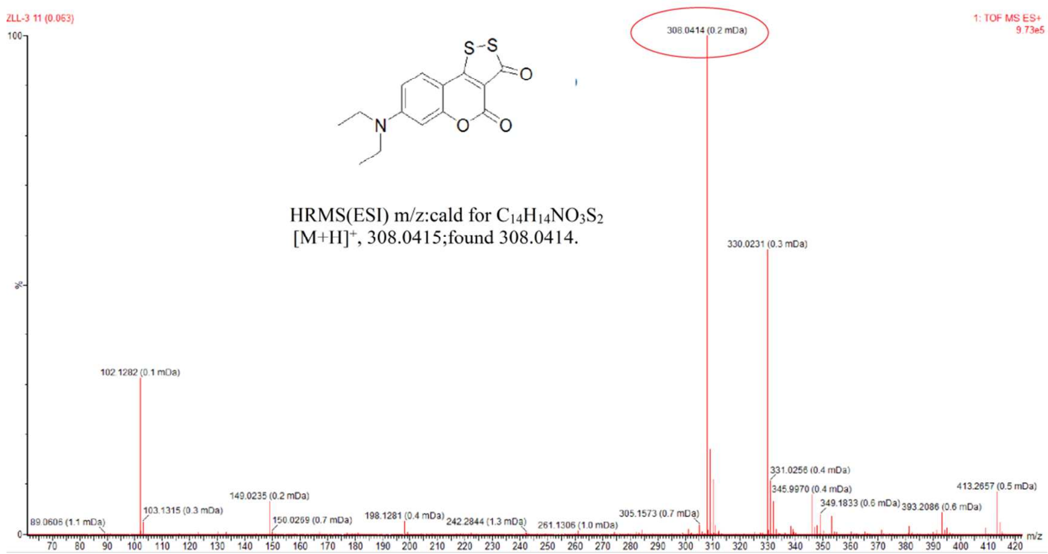


Figure S41. HRMS spectrum of compound **9**.

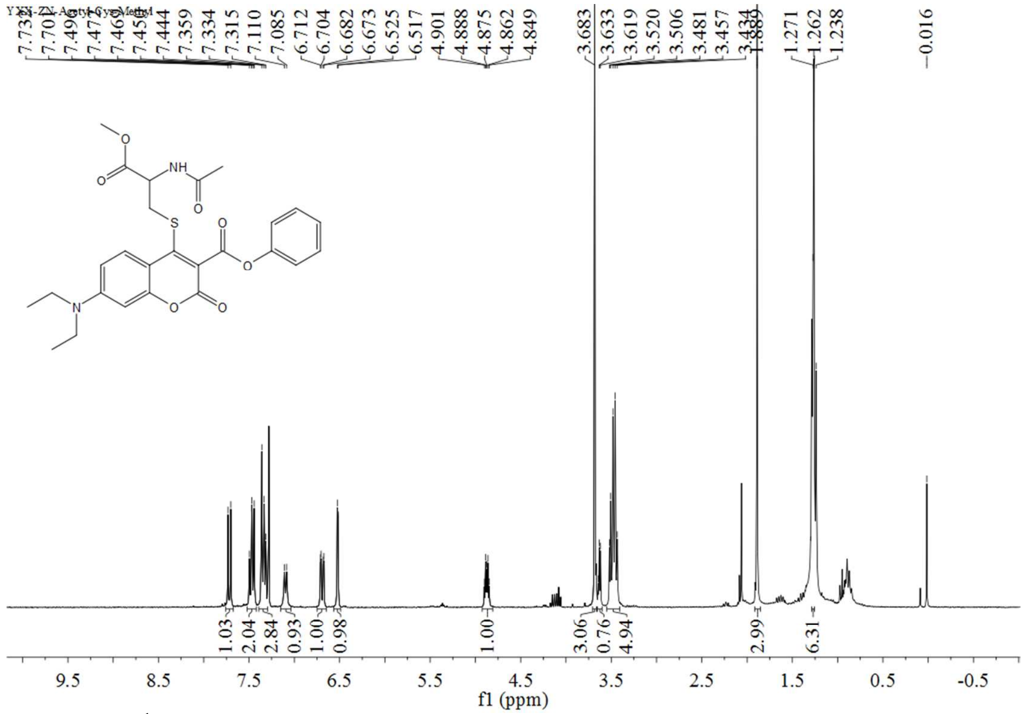


Figure S42. ^{1}H NMR spectrum of compound **10** in $CDCl_3$.

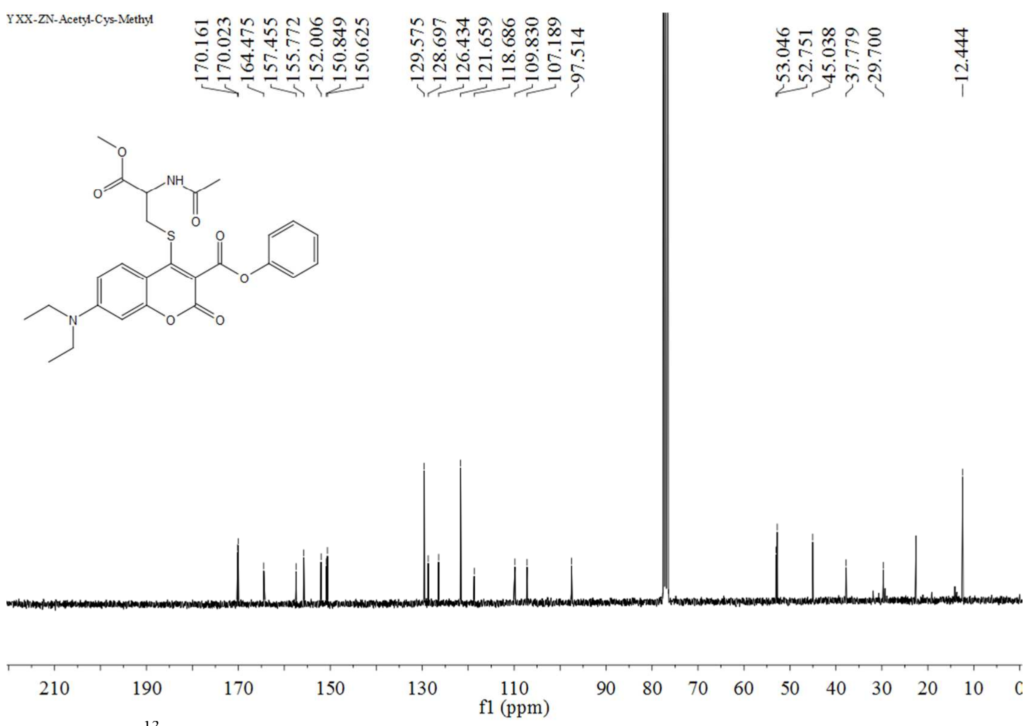


Figure S43. ^{13}C NMR spectrum of compound **10** in $CDCl_3$.

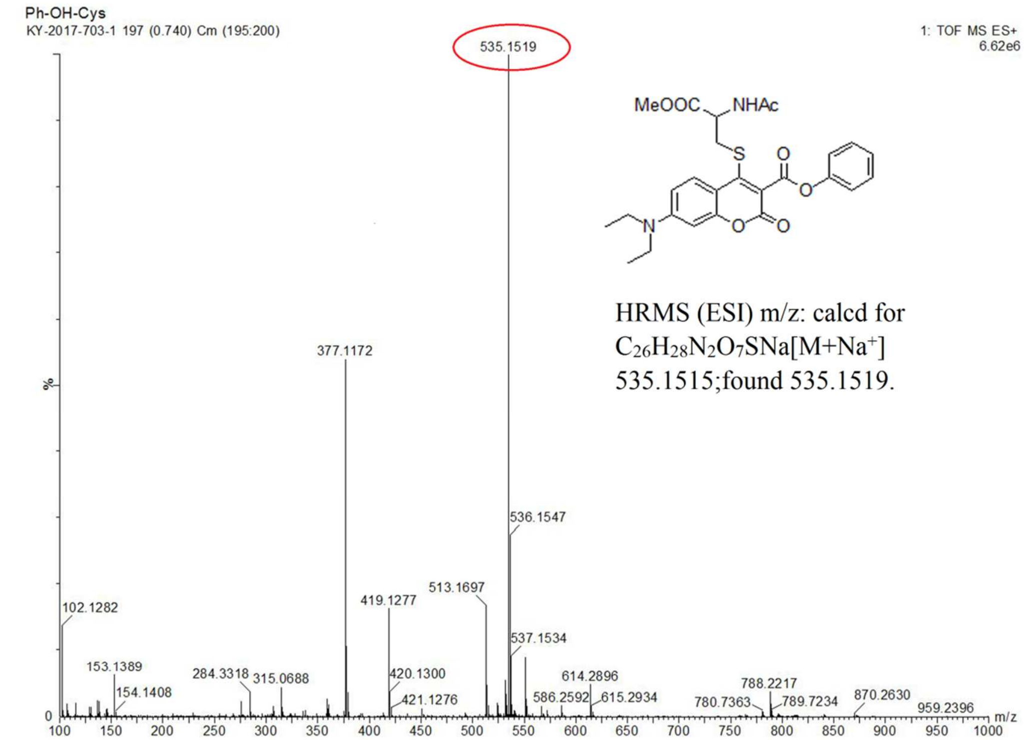


Figure S44. HRMS spectrum of compound **10**.

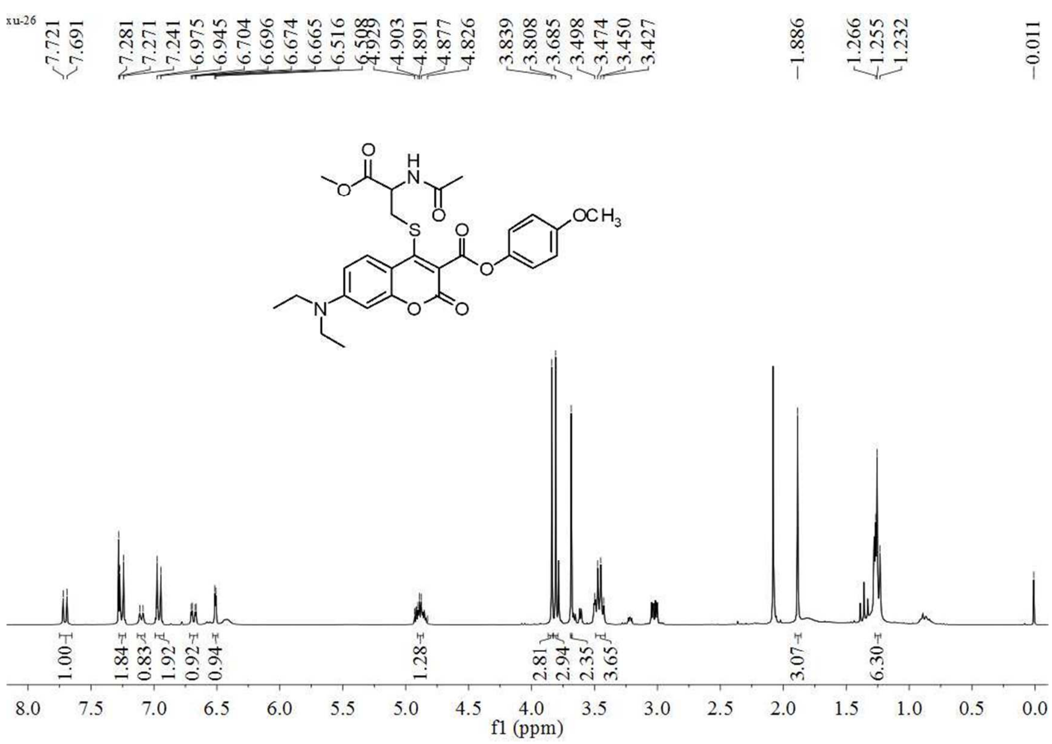


Figure S45. ^{1}H NMR spectrum of compound **11** in $CDCl_3$.

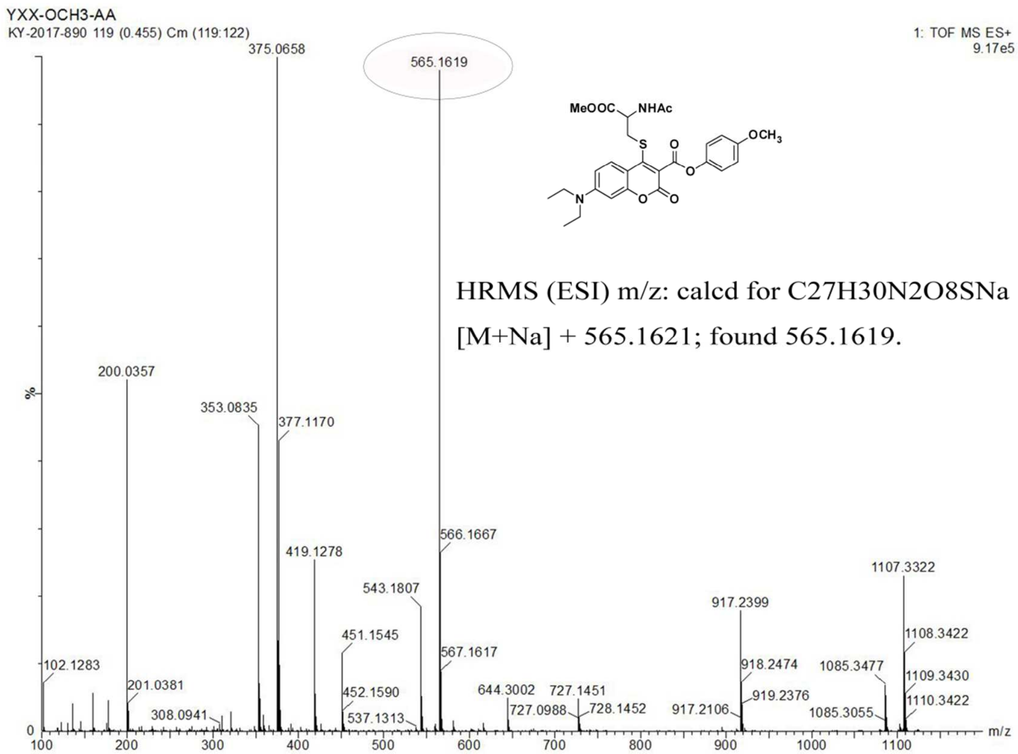


Figure S46. HRMS spectrum of compound **11**.

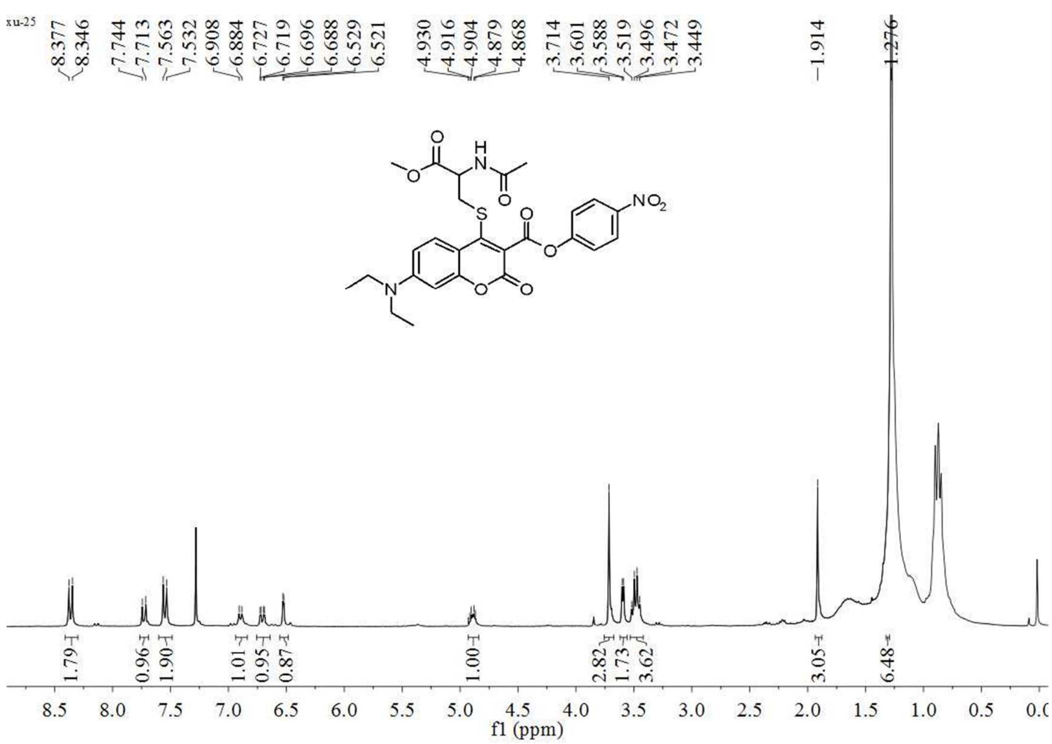


Figure S47. ^{1}H NMR spectrum of compound **12** in $CDCl_3$.

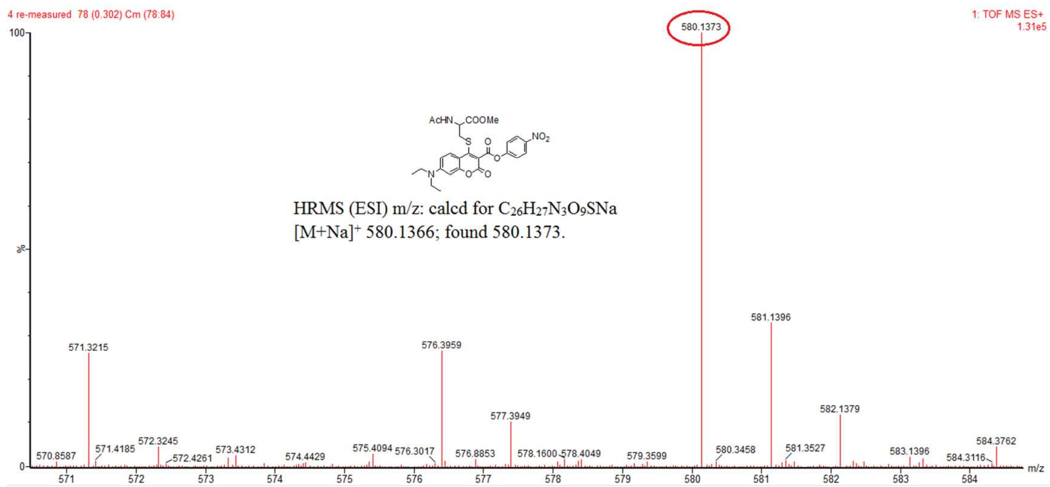


Figure S48. HRMS spectrum of **12**.

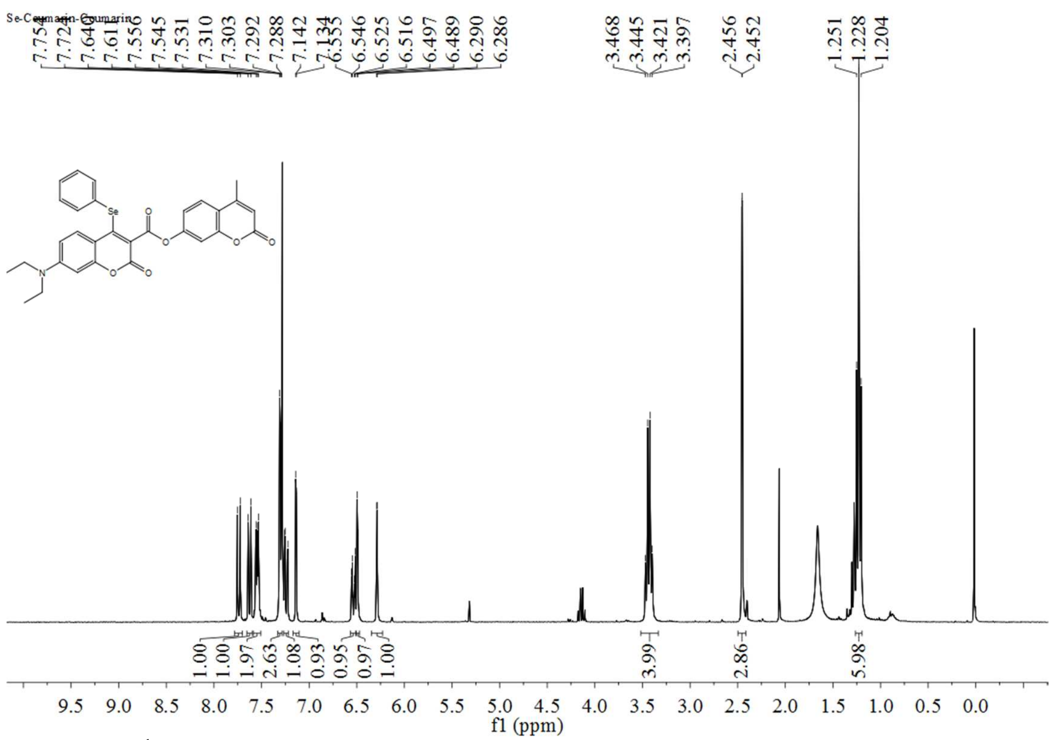


Figure S49. ^{1}H NMR spectrum of ACC-SePh in $CDCl_3$.

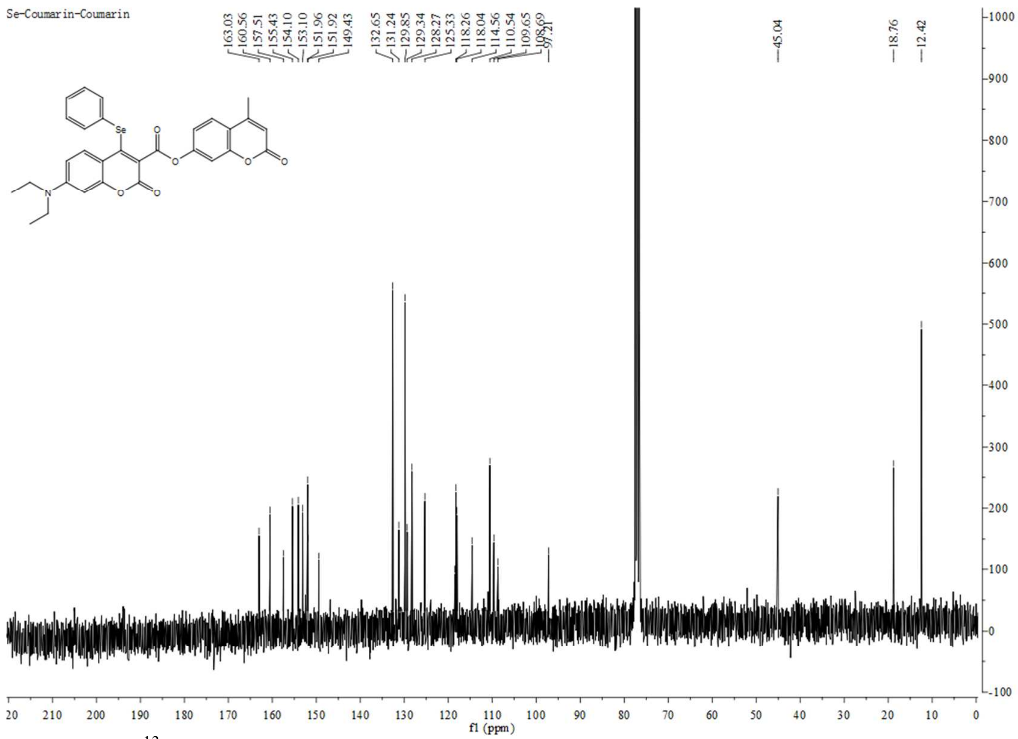


Figure S50. ^{13}C NMR spectrum of ACC-SePh in $CDCl_3$.

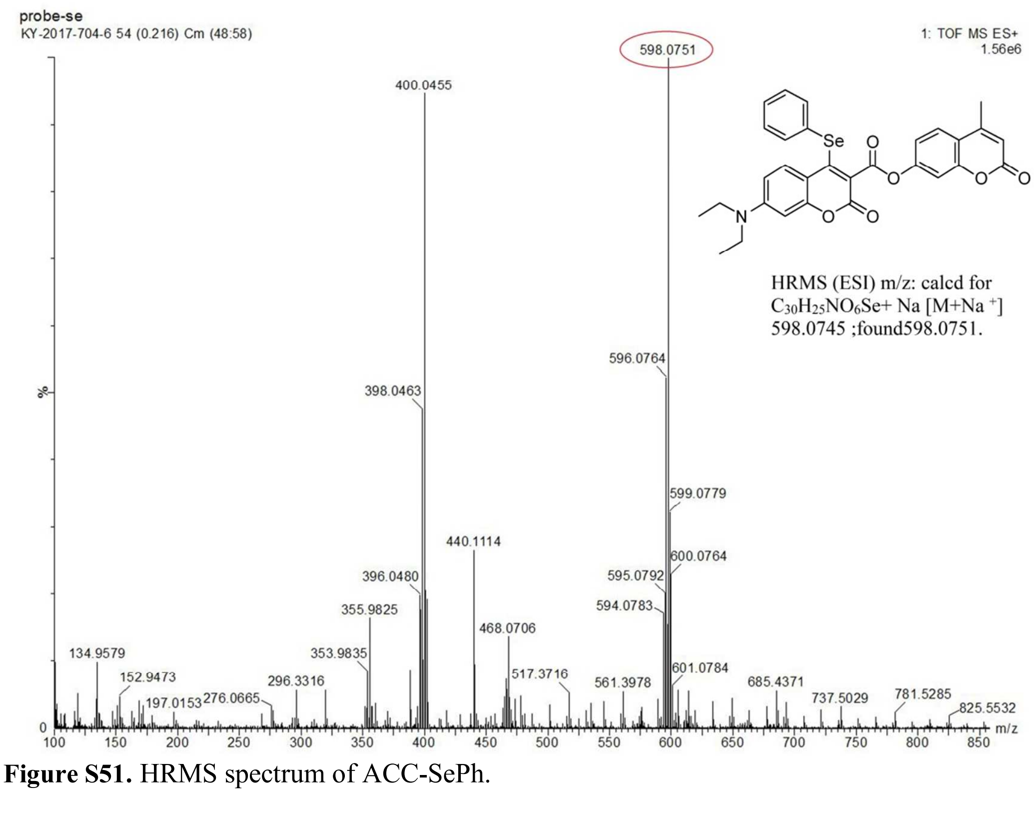


Figure S51. HRMS spectrum of ACC-SePh.

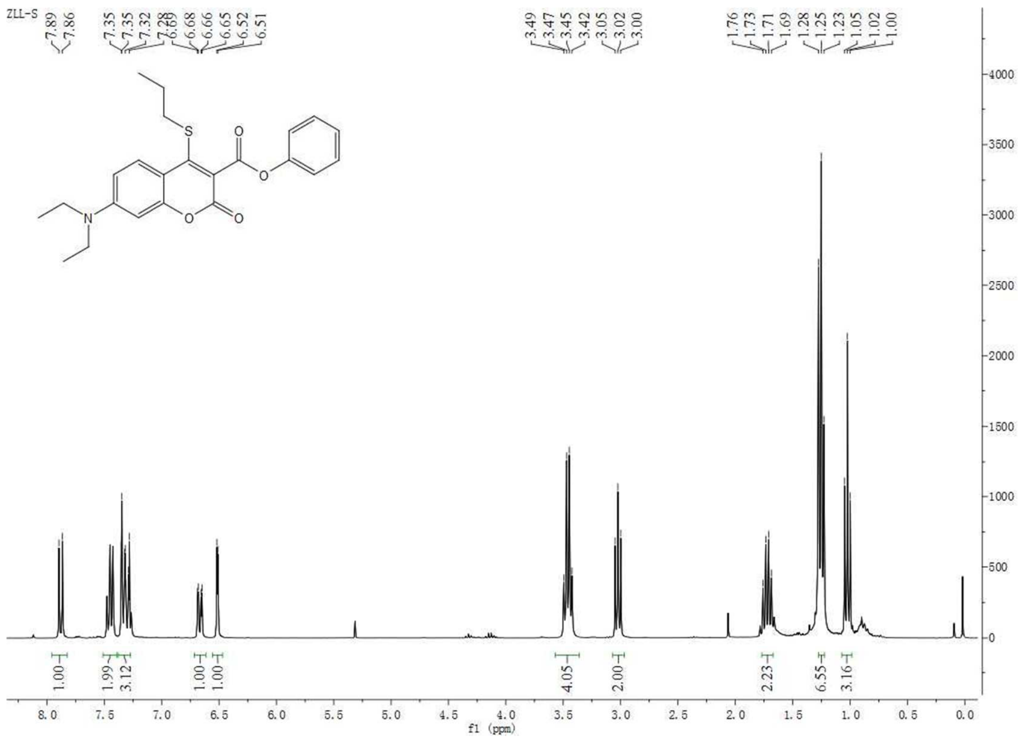


Figure S52. ^{1}H NMR spectrum of **14** in $CDCl_3$.

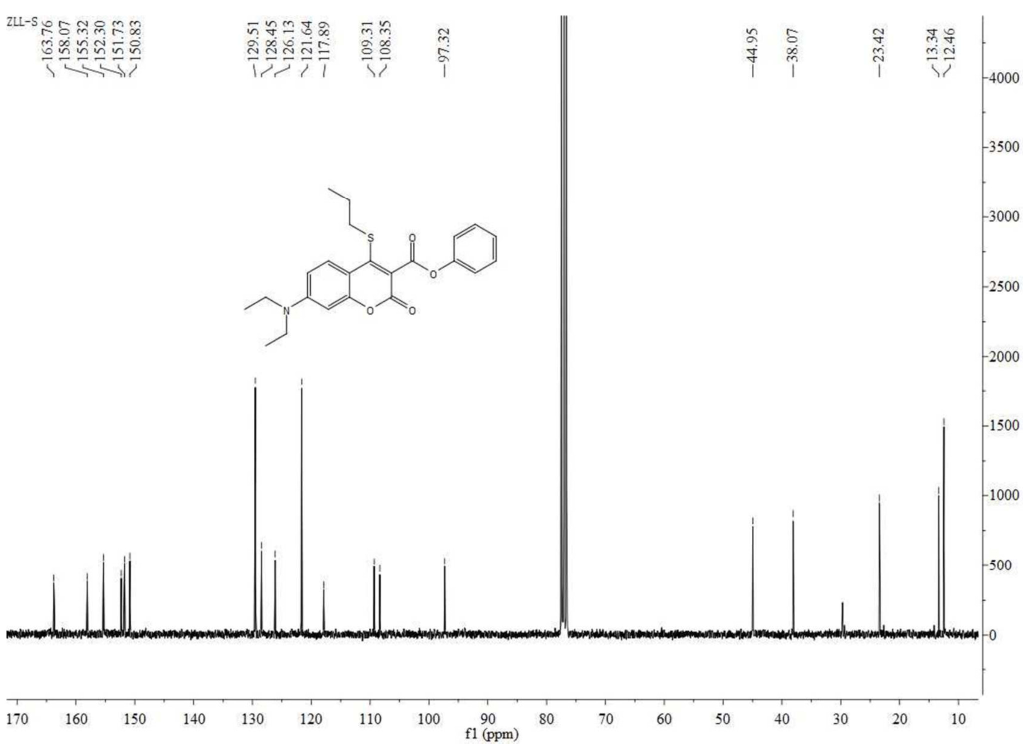


Figure S53. ^{13}C NMR spectrum of **14** in $CDCl_3$

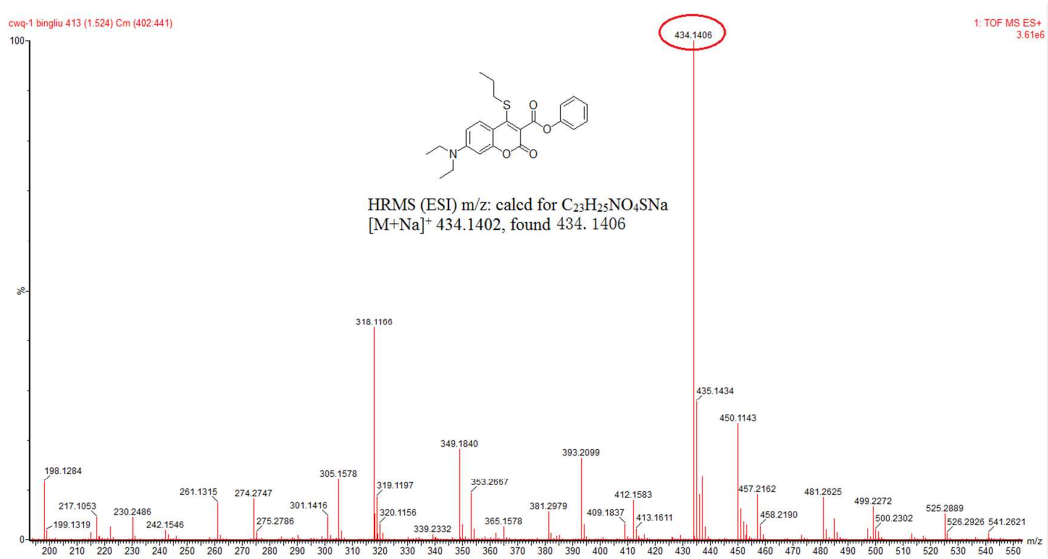


Figure S54. HRMS spectrum of **14**.

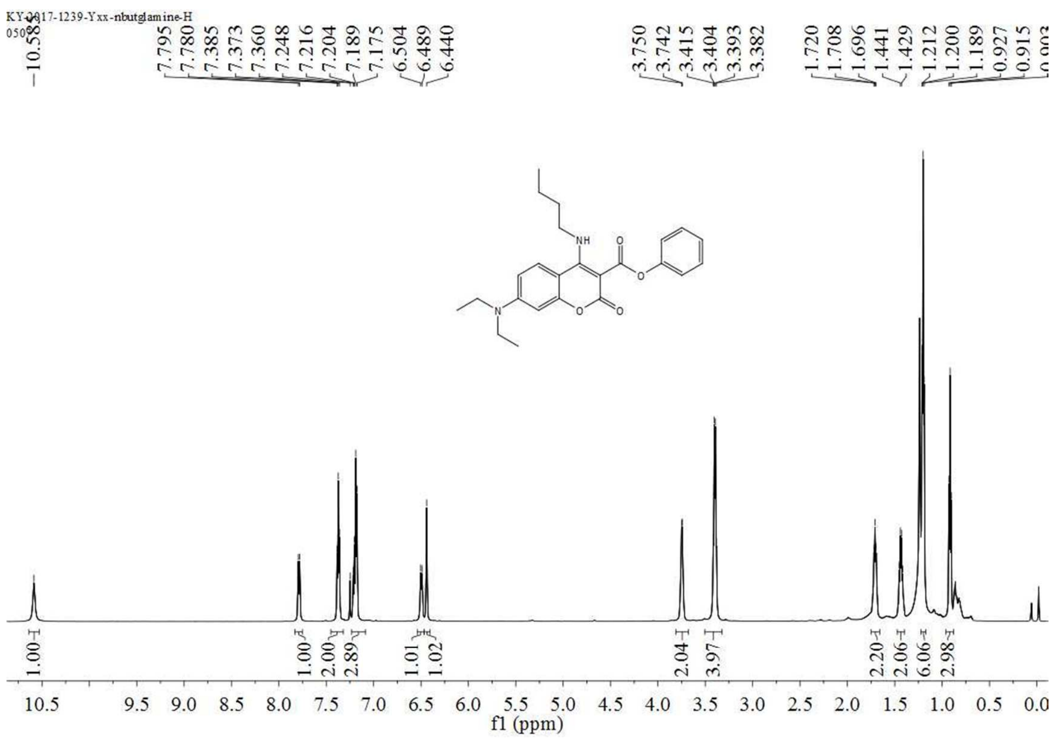


Figure S55. ^{1}H NMR spectrum of **15** in $CDCl_3$.

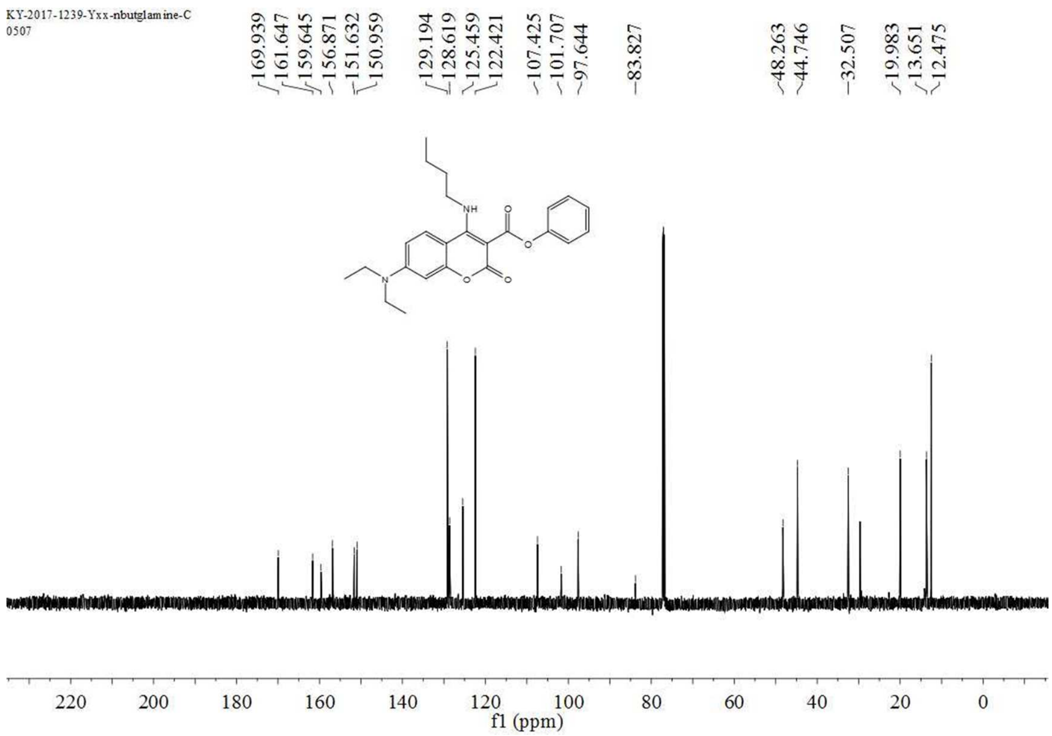


Figure S56. ^{13}C NMR spectrum of **15** in $CDCl_3$.

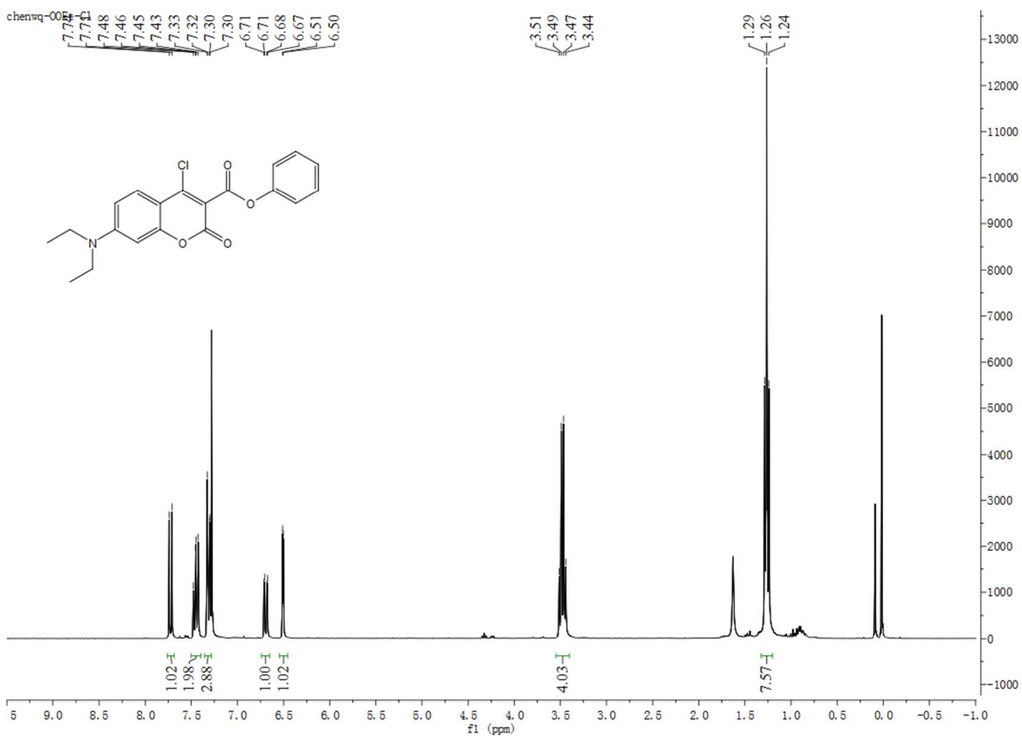


Figure S57. ^{1}H NMR spectrum of compound **16** in $CDCl_3$.

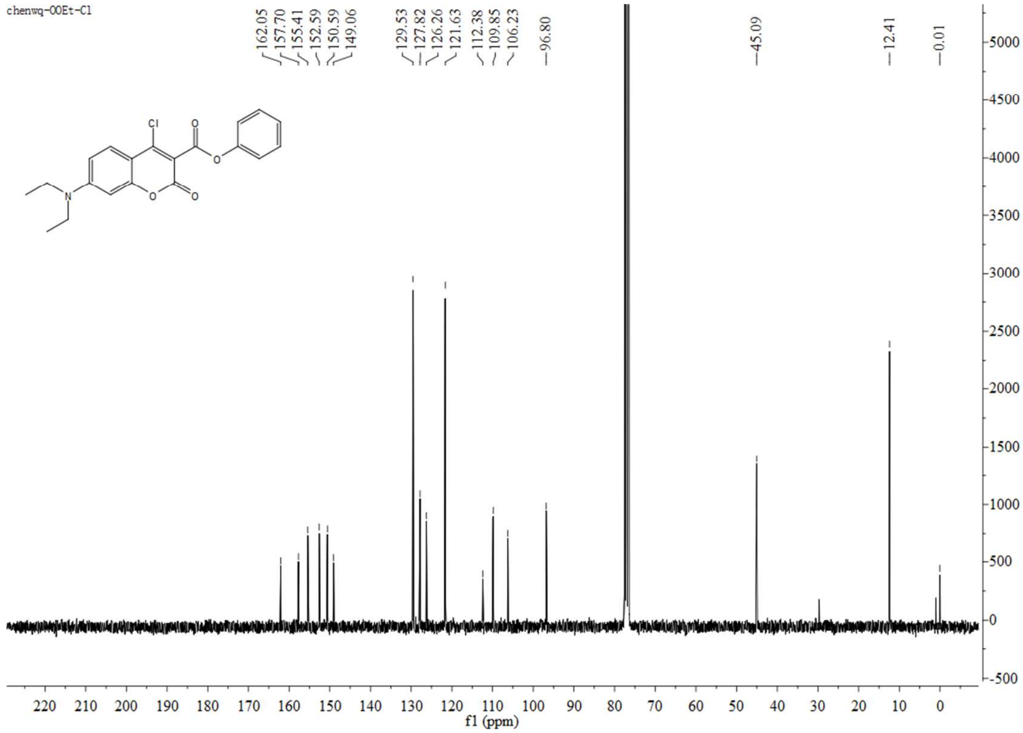


Figure S58. ^{13}C NMR spectrum of compound **16** in $CDCl_3$.

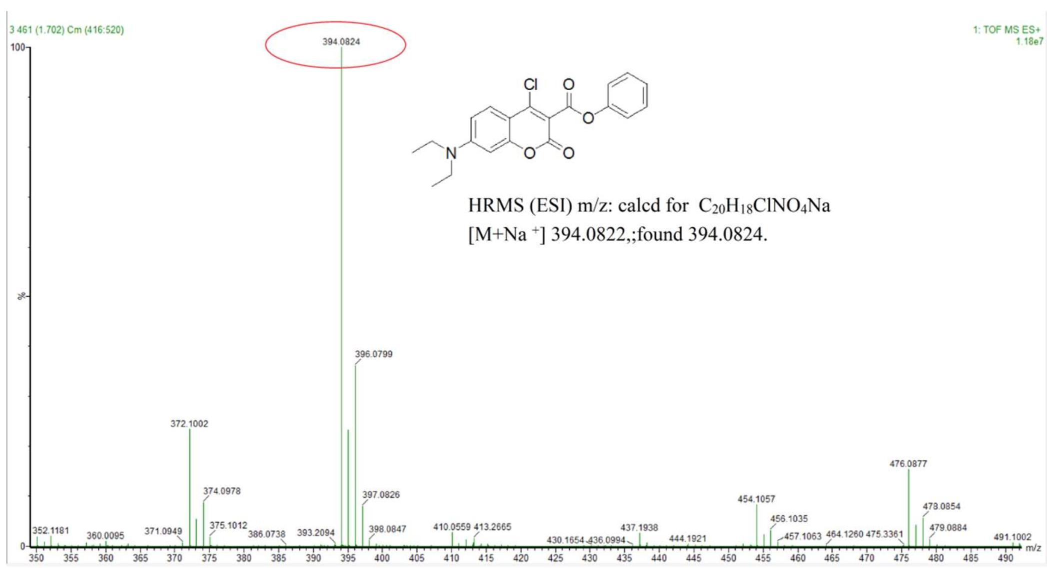


Figure S59. HRMS spectrum of compound **16**.