Electronic Supplementary Material (ESI) for Chemical Science.

SUPPORTING INFORMATION

Exceptional Time Response, Stability and Selectivity in Doubly-Activated Phenyl Selenium-Based Glutathione-Selective Platform

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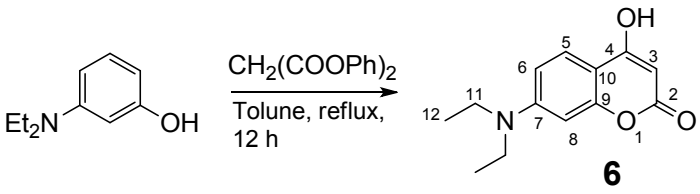
EXPERIMENTAL SECTION

General considerations. All chemicals used herein were used as received from commercial suppliers (Aldrich, Tokyo Chemical Industry). ^{1}H, ^{13}C, ^{77}Se NMR spectra were acquired using a Bruker Avance 400 and Agilent-NMR-vnmrs 600 MHz spectrometer. TMS and dimethyl selenide was used as external standards. ESI-mass spectrometry was performed on a BRUKER micrOTOF-QⅡ by the research support staff at KAIST. A Time-of-Flight mass spectrometer was operated at a resolution of 20,000. Absorption spectra and stopped-flow absorption spectra were measured using a JASCO V–530 and JASCO-815 Uv/Vis spectrophotometer, respectively. Fluorescence measurements were carried out with a Shimadzu RF–5301pc spectrofluorophotometer.

Cell culture and probe treatment. Hep3B cells from the Korean Cell Line Bank were maintained as a monolayer in a humidified incubator (5% CO_2) at 37 ℃ in Dulbecco's Modified Eagle's Medium (DMEM, WELGENE) supplemented with 10% (v/v) fetal bovine serum (FBS, WELGENE), 100 IU/mL penicillin-streptomycin solution (WELGENE). Before treatment of the probe, the cells were incubated with media containing 1 mM of *N*-ethylmaleimide (NEM) for 30 min. Finally, the probe (10 μM) in PBS was used to treat the cells; and after 30 min incubation, confocal fluorescence images were recorded using a 405 nm Argon laser and a 410-585 nm band pass emission filter for blue fluorescence signal, a 488 nm Argon laser and a 490-562 nm band pass emission filter for green fluorescence, and a 633 nm Argon laser and a 661-759 nm band pass emission filter for DRAQ5 nucleus stain signal.

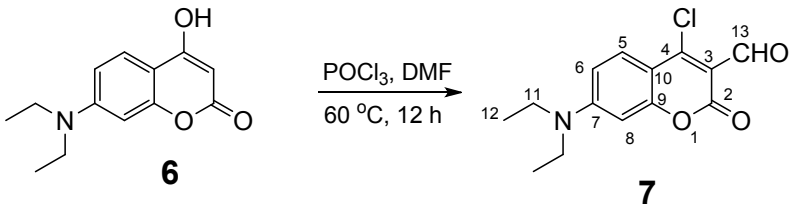
Cell Viability Assay. Proliferation of Hep3B cells was determined by WST-1 assay kit (Roche) with the manufacturer's protocols. Hep3B cells pre-treated with the various probe concentrations and control cells were seeded with 5 ×10^3 cells / well in 96-well plate (n = 6). The cell Proliferation Reagent WST-1 was added (10 μL) to each well and the reagent-applied cells were incubated for 4 h at 37°C, 5% CO_2. Absorbances were measured using a microplate ELISA reader at 450 nm.

7-(Diethylamino)-4-hydroxy-2H-chromen-2-one (6)



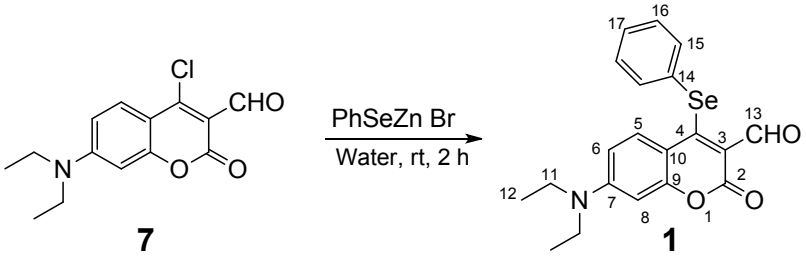
To a solution of 3-(*N,N*-diethylamino)phenol (5.0 g, 30.3 mmol, 1 equiv) in dry toluene (60 mL) was added diphenyl malonate (7.75 g, 30.3 mmol); this reaction mixture was refluxed for 12 h. The reaction mixture was then cooled to room temperature and crystals formed and were filtered off and washed with hexane (3 × 30 mL), dried under vacuum to give **6** as a grey amorphous powder (6.42 g, 91%). ^{1}H-NMR (400 MHz, DMSO-d6/TMS): δ 1.09 (t, $^{3}J_{H\text{-}H}$ = 7.0, Hz, 6H, H_{12}), 3.38 (q, $^{3}J_{H\text{-}H}$ = 7.0 Hz, 4H, H_{11}), 5.26 (s, 1H, H_3), 6.44 (d, $^{4}J_{H\text{-}H}$ = 2.4 Hz, 1H, H_8), 6.63 (dd, $^{3}J_{H\text{-}H}$ = 9.0 Hz, $^{4}J_{H\text{-}H}$ = 2.4 Hz, 1H, H_6), 7.54 (d, $^{3}J_{H\text{-}H}$ = 9.0 Hz, 1H, H_5), 11.91 (bs, 1H, *OH*); ^{13}C-NMR (100 MHz, DMSO): δ = 12.3 (C_{12}), 44.0 (C_{11}), 86.2 (C_3), 96.4 (C_8), 103.5 (C_{10}), 108.2 (C_6), 124.2 (C_5), 150.9 (C_7), 156.2 (C_9), 162.8 (C_2), 166.5 (C_4).

4-Chloro-7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (7)



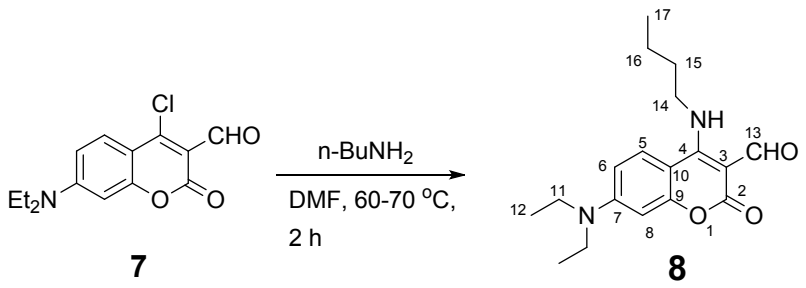
To a solution of 7-diethylamino-4-hydroxycoumarin **6** (3.0 g, 12.9 mmol, 1 equiv) in dry DMF (15 mL) was added phosphorus oxychloride (1.56 mL, 16.7 mmol, 1.3 equiv) and stirred at 60 °C 12 h. Then, the reaction mixture was cooled to room temperature, poured into aqueous solution of sodium acetate (10 g in 200 mL) and stirred for 1 h. The orange precipitate formed was recovered by filtration and washed with water (2 × 50 mL) to give crude a product which was recrystallized from absolute ethanol afforded **7** as orange crystals (2.84 g, 79 %). ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 1.25 (t, $^{3}J_{H\text{-}H}$ = 7.1 Hz, 6H, H_{12}), 3.48 (q, $^{3}J_{H\text{-}H}$ = 7.1 Hz, 4H, H_{11}), 6.42 (d, $^{4}J_{H\text{-}H}$ = 2.5 Hz, 1H, H_8), 6.68 (dd, $^{3}J_{H\text{-}H}$ = 9.3 Hz, $^{4}J_{H\text{-}H}$ = 2.5 Hz, 1H, H_6), 7.83 (d, $^{3}J_{H\text{-}H}$ = 9.3, Hz, 1H, H_5), 10.28 (s, 1H, H_{13}); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 12.6 (C_{12}), 45.5 (C_{11}), 96.7 (C_8), 107.8 (C_{10}), 110.7 (C_6), 111.0 (C_3), 129.4 (C_5), 153.8 (C_7), 154.2 (C_4), 156.5 (C_9), 160.0 (C_2), 187.1 (C_{13}).

7-(Diethylamino)-2-oxo-4-(phenylselanyl)-2H-chromene-3-carbaldehyde (1)



To the mixture of coumarin **7** (280 mg, 1.0 mmol) and (phenylselenenyl)zinc bromide (301 mg, 1.0 mmol) was added distilled water (6 mL). The reaction mixture was vigorously stirred at room temperature for 2 h. CH_2Cl_2 (15 mL) was added to the reaction mixture and separated aqueous layer extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under vacuum. The crude product was purified by silica gel column chromatography using dichloromethane and ethyl acetate (19:1) to give **1** as an orange solid (324 mg, 81%). M.P. = 171–173 °C; ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 1.09 (t, $^3J_{H\text{-}H}$ = 7.1 Hz, 6H, H_{12}), 3.29 (q, $^3J_{H\text{-}H}$ = 7.1 Hz, 2H, H_{11}), 6.12 (dd, $^3J_{H\text{-}H}$ = 9.4, $^4J_{H\text{-}H}$ = 2.6 Hz, 1H, H_6), 6.26 (d, $^4J_{H\text{-}H}$ = 2.6 Hz, 1H, H_8), 7.15–7.20 (m, 3H, $H_{16,17}$), 7.30 (d, $^3J_{H\text{-}H}$ = 9.4 Hz, 1H, H_5), 7.37–7.41 (m, 2H, H_{15}), 10.24 (s, 1H, H_{13}); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 12.3 (C_{12}), 44.9 (C_{11}), 96.5 (C_8), 109.0 (C_6), 109.2 (C_{10}), 115.3 (C_3), 128.2 (C_5), 129.6 (C_{16}), 132.0 (C_{14}), 132.8 (C_{17}), 133.0 (C_{15}), 152.2 (C_7), 155.6 (C_9), 160.0 (C_2), 162.2 (C_4), 189.0 (C_{13}); ^{77}Se-NMR (76.3 MHz, CDCl3): δ 454.9; HRMS (ESI): calcd for $C_{20}H_{19}NO_3Se$ + Na: 424.0428, found: m / z 424.0427 $(M + Na)^+$.

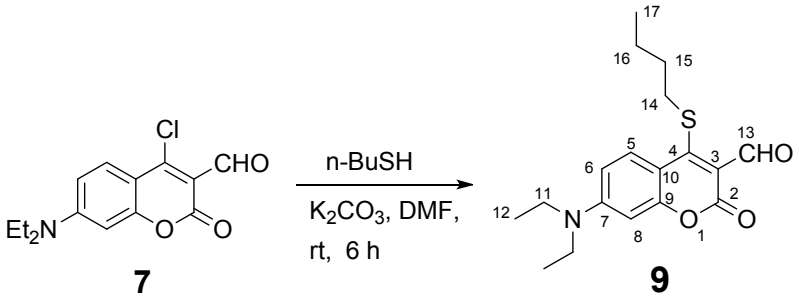
4-(Butylamino)-7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (8)



To a stirred solution of compound **7** (140 mg, 0.5 mmol) in anhydrous DMF (10 mL) was added *n*-$BuNH_2$ (49 µL, 0.5 mmol). The mixture was stirred at 60-70ºC under N_2 for 2 h. Then, the

reaction mixture was cooled to room temperature and diluted with water (20 mL). The aqueous layer extracted with EtOAc (4 × 20 mL) and combined organic layers were washed with water, brine, dried (Na_2SO_4) and concentrated. The crude residue was purified by silica gel column chromatography using hexane:EtOAc (5:1) to give **8** as a yellow solid (106 mg, 67 %). ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 0.98 (t, $^3J_{H\text{-}H}$ = 7.3, Hz, 3H, H_{17}), 1.20 (t, $^3J_{H\text{-}H}$ = 7.1, Hz, 6H, H_{12}), 1.49-1.54 (m, 2H, H_{16}), 1.74-1.80 (m, 2H, H_{15}), 3.40 (q, $^3J_{H\text{-}H}$ = 7.1 Hz, 4H, H_{11}), 3.71-3.76 (m, 2H, H_{14}), 6.40 (d, $^4J_{H\text{-}H}$ = 2.6 Hz, 1H, H_8), 6.51 (dd, $^3J_{H\text{-}H}$ = 9.4 Hz, $^4J_{H\text{-}H}$ = 2.7 Hz, 1H, H_6), 7.80 (d, $^3J_{H\text{-}H}$ = 9.4 Hz, 1H, H_5), 10.00 (s, 1H, H_{13}), 11.67 (s, 1H, *NH*); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 12.5 (C_{12}), 13.8 (C_{17}), 20.1 (C_{16}), 32.1 (C_{15}), 44.9 (C_{11}), 47.3 (C_{14}), 94.7 (C_3), 98.4 (C_8), 101.6 (C_{10}), 108.2 (C_6), 129.3 (C_5), 151.9 (C_7), 157.8 (C_9), 159.1 (C_2), 164.1 (C_4), 190.7 (C_{13}).

4-(Butylthio)-7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (9)



To a stirred solution of compound **7** (140 mg, 0.5 mmol) in anhydrous DMF (10 mL) were added K_2CO_3 (69 mg, 0.5 mmol) and *n*-BuSH (54 µL, 0.5 mmol). The mixture was stirred at room temperature for 6 h. Then, the reaction mixture was diluted with water (20 mL). The aqueous layer was extracted with EtOAc (4 × 20 mL) and then combined organic layers which were washed with water, brine, dried (Na_2SO_4) and then concentrated. The crude residue was purified by silica gel column chromatography using hexane:EtOAc (5:1) to give **9** as a yellow solid (132 mg, 79 %). ^{1}H-NMR (400 MHz, $CDCl_3$/TMS): δ 0.85 (t, $^3J_{H\text{-}H}$ = 7.3, Hz, 3H, H_{17}), 1.22 (t, $^3J_{H\text{-}H}$ = 7.1, Hz, 6H, H_{12}), 1.36-1.41 (m, 2H, H_{16}), 1.53-1.60 (m, 2H, H_{15}), 3.02 (t, $^3J_{H\text{-}H}$ = 7.4 Hz, 2H, H_{14}), 3.44 (q, $^3J_{H\text{-}H}$ = 7.1 Hz, 4H, H_{11}), 6.38 (d, $^4J_{H\text{-}H}$ = 2.6 Hz, 1H, H_8), 6.61 (dd, $^3J_{H\text{-}H}$ = 9.3 Hz, $^4J_{H\text{-}H}$ = 2.6 Hz, 1H, H_6), 7.03 (d, $^3J_{H\text{-}H}$ = 9.3 Hz, 1H, H_5), 10.32 (s, 1H, H_{13}); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 12.6 (C_{12}), 13.6 (C_{17}), 21.9 (C_{16}), 32.1 (C_{15}), 38.3 (C_{14}), 45.2 (C_{11}), 96.9 (C_8), 109.8 (C_6), 110.3 (C_{10}), 113.8 (C_3), 129.8 (C_5), 153.1 (C_7), 155.9 (C_9), 160.6 (C_2), 162.4 (C_4), 188.2 (C_{13}).

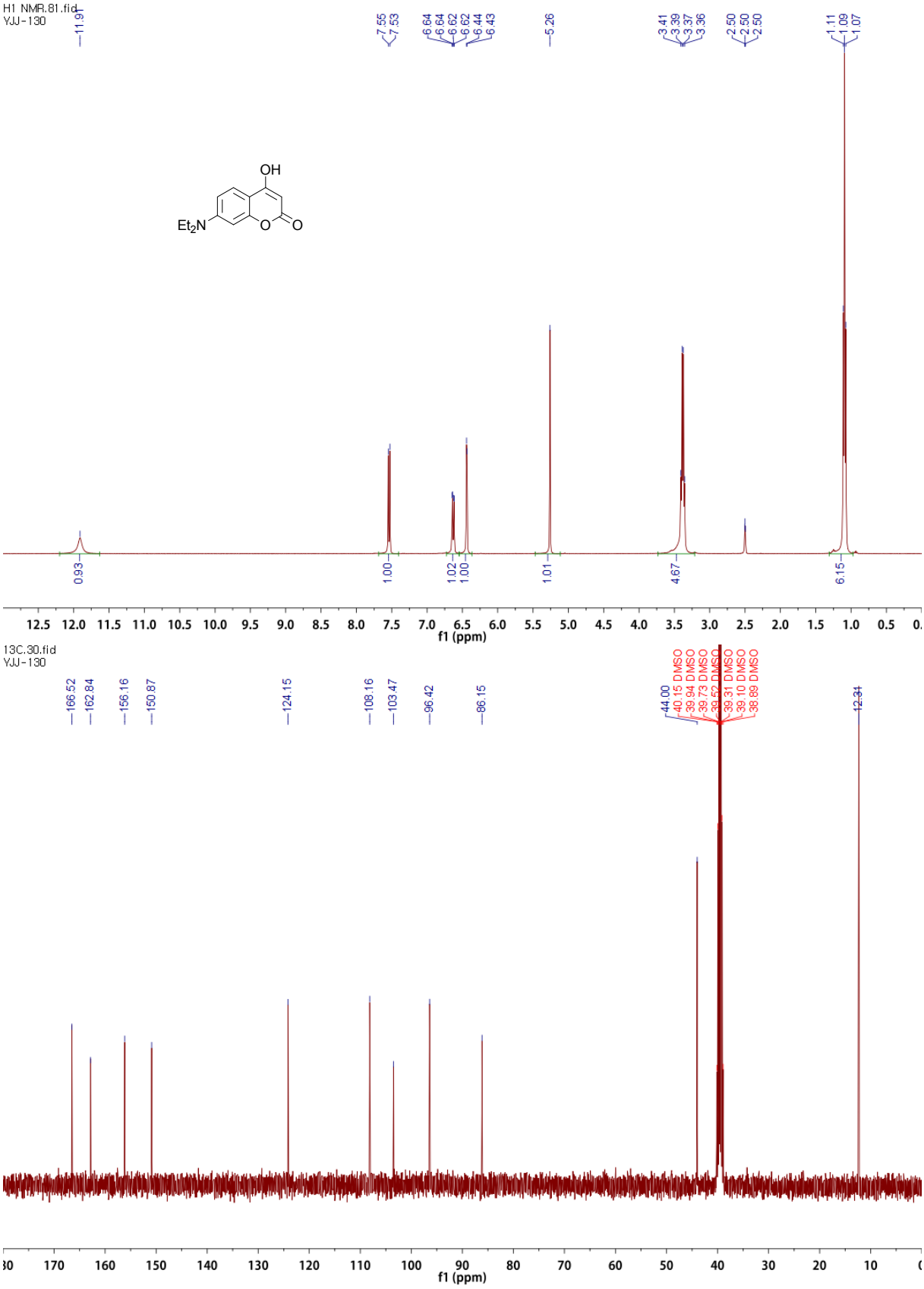


Fig. S1. (top) ^{1}H and (bottom) ^{13}C NMR spectrum of **6**[1].

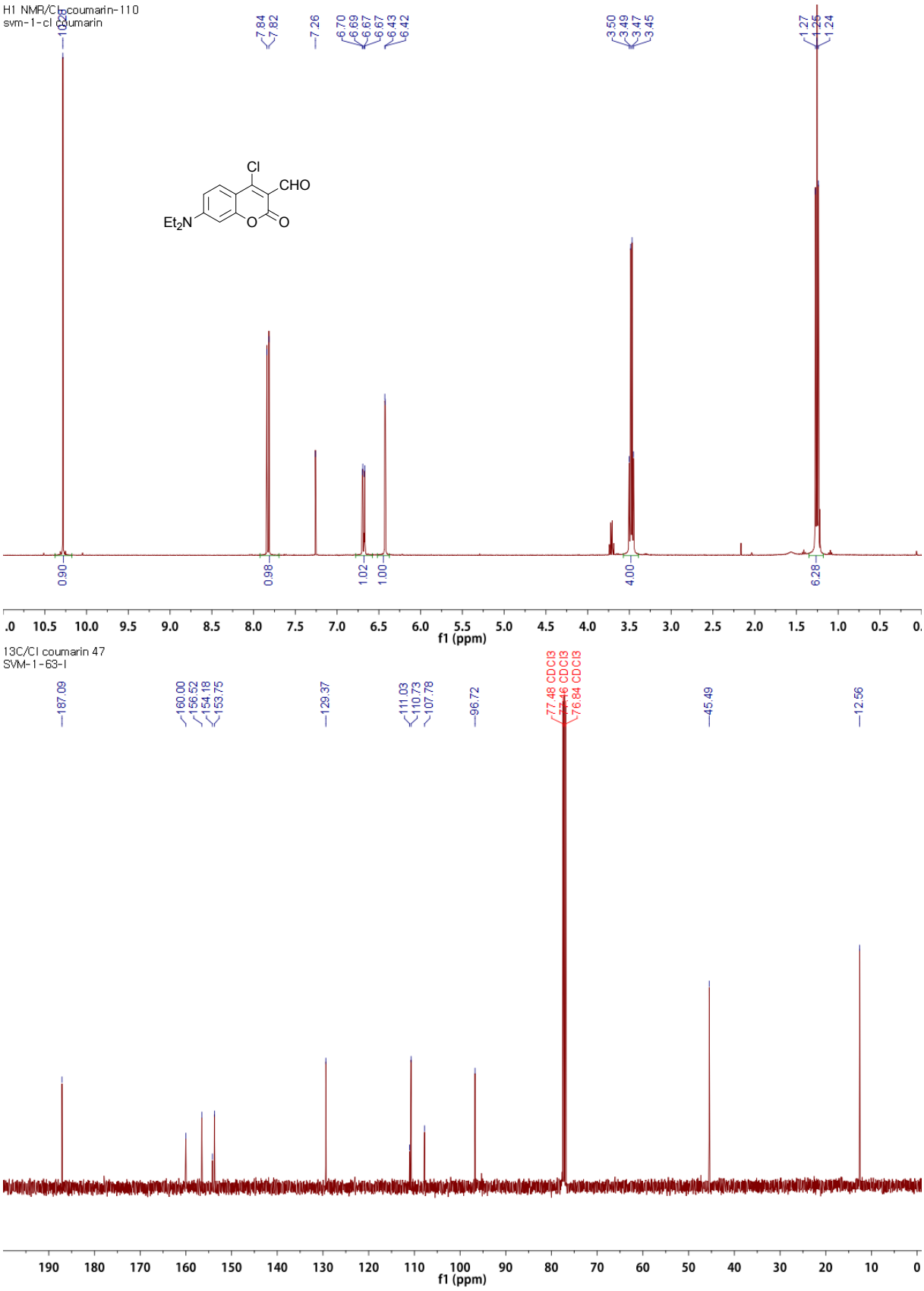


Fig. S2. (top) ^{1}H and (bottom) ^{13}C NMR spectrum of **7**[1].

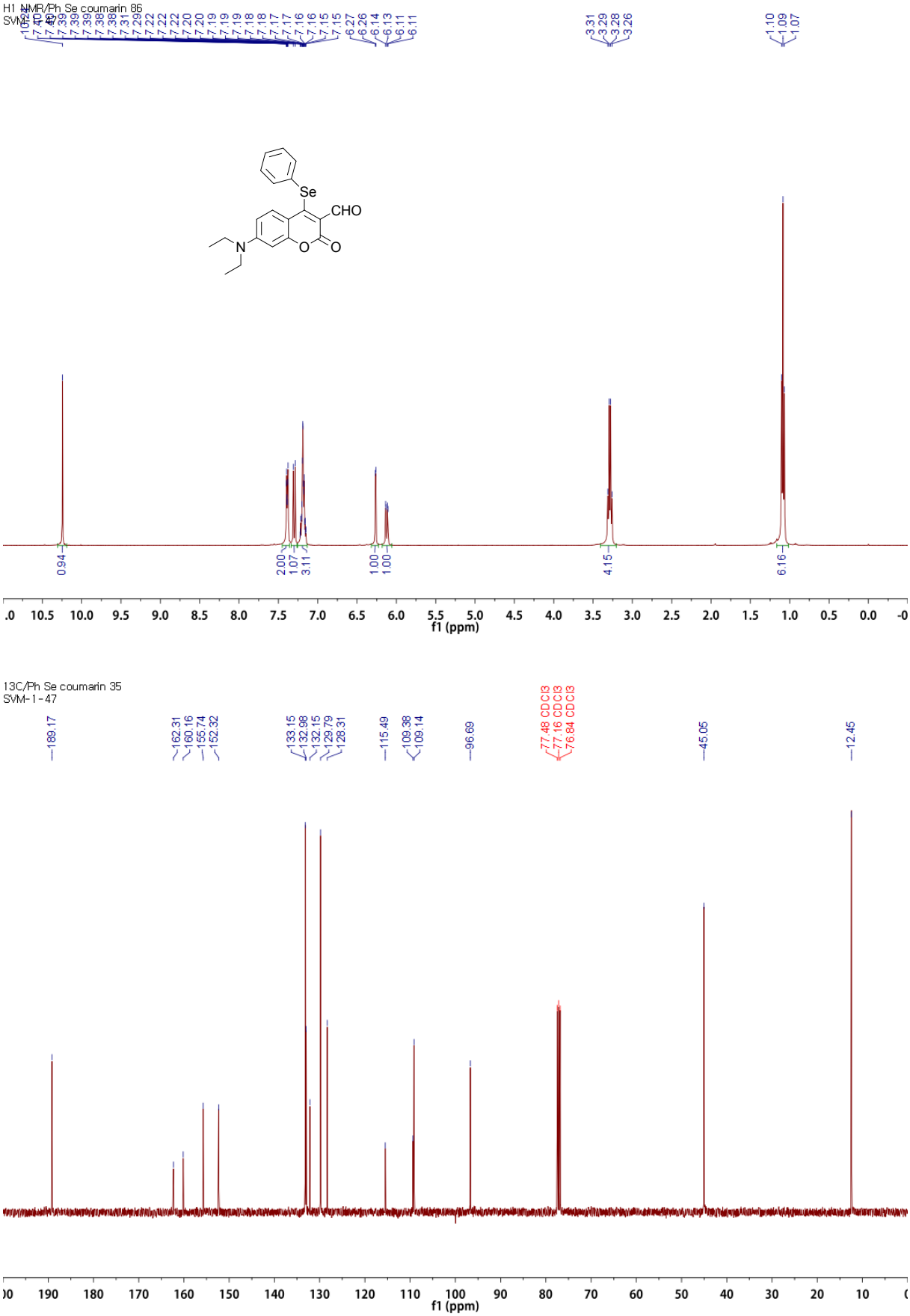


Fig. S3. (top) ^{1}H and (bottom) ^{13}C NMR spectrum of **1**.

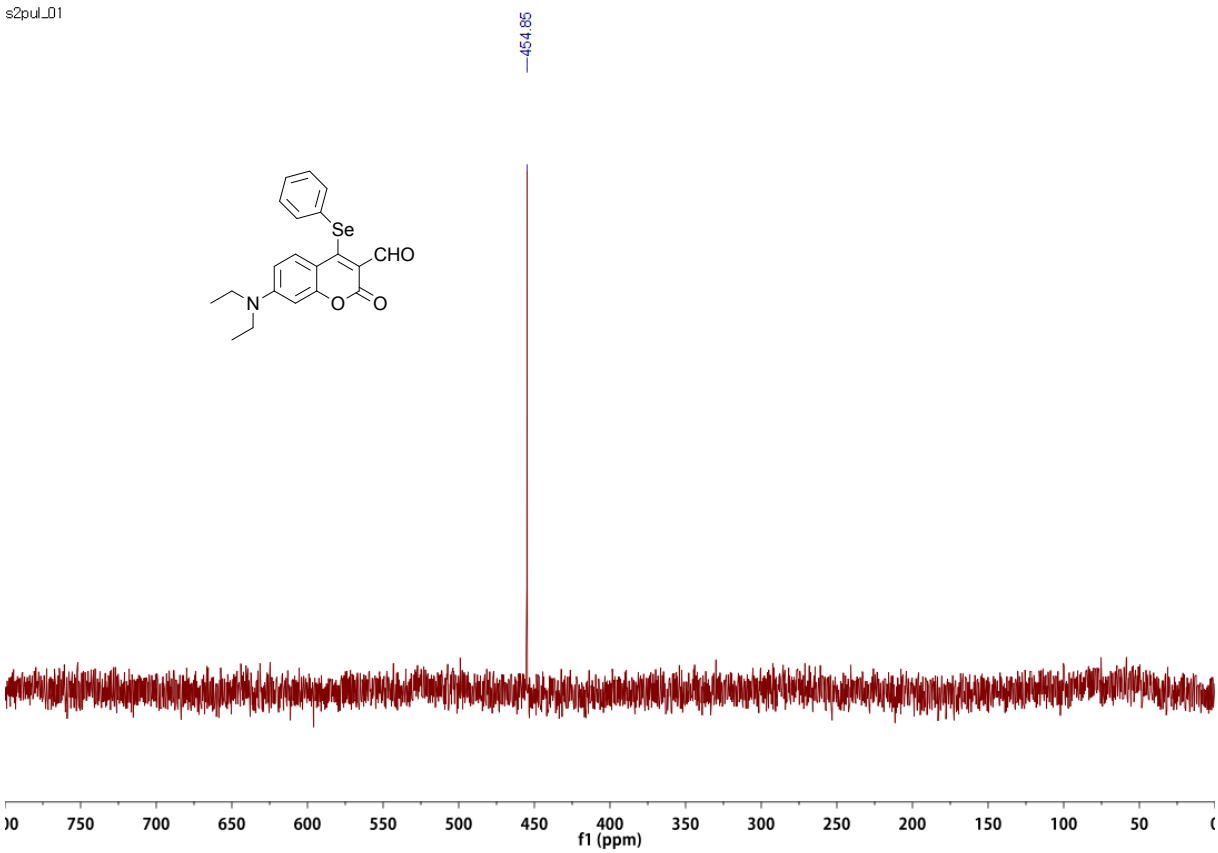


Fig. S4. ^{77}Se NMR spectrum of **1**.

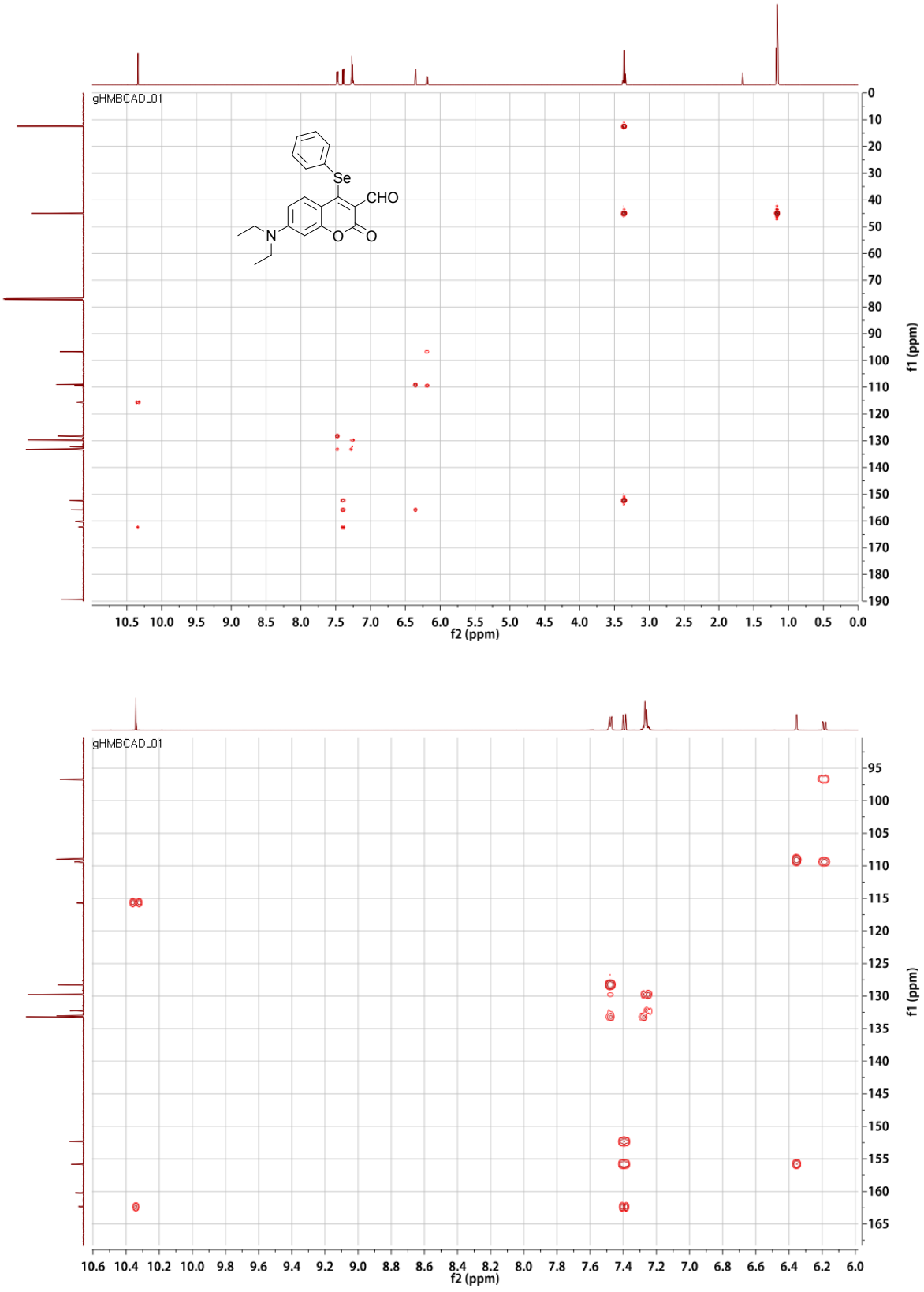


Fig. S5. (top) ^{1}H – ^{13}C HMBC NMR spectrum of **1** and (bottom) expanded aromatic region.

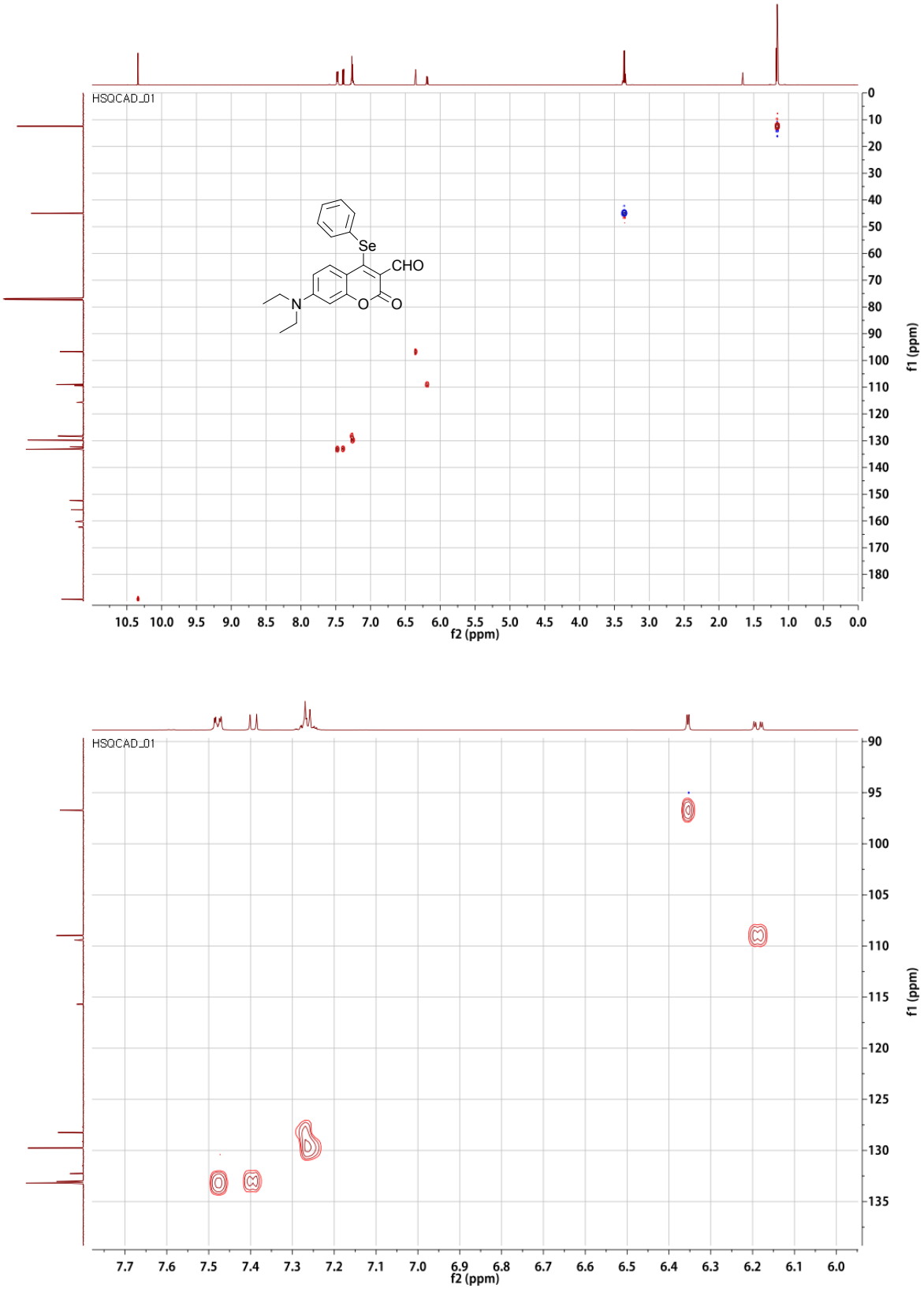
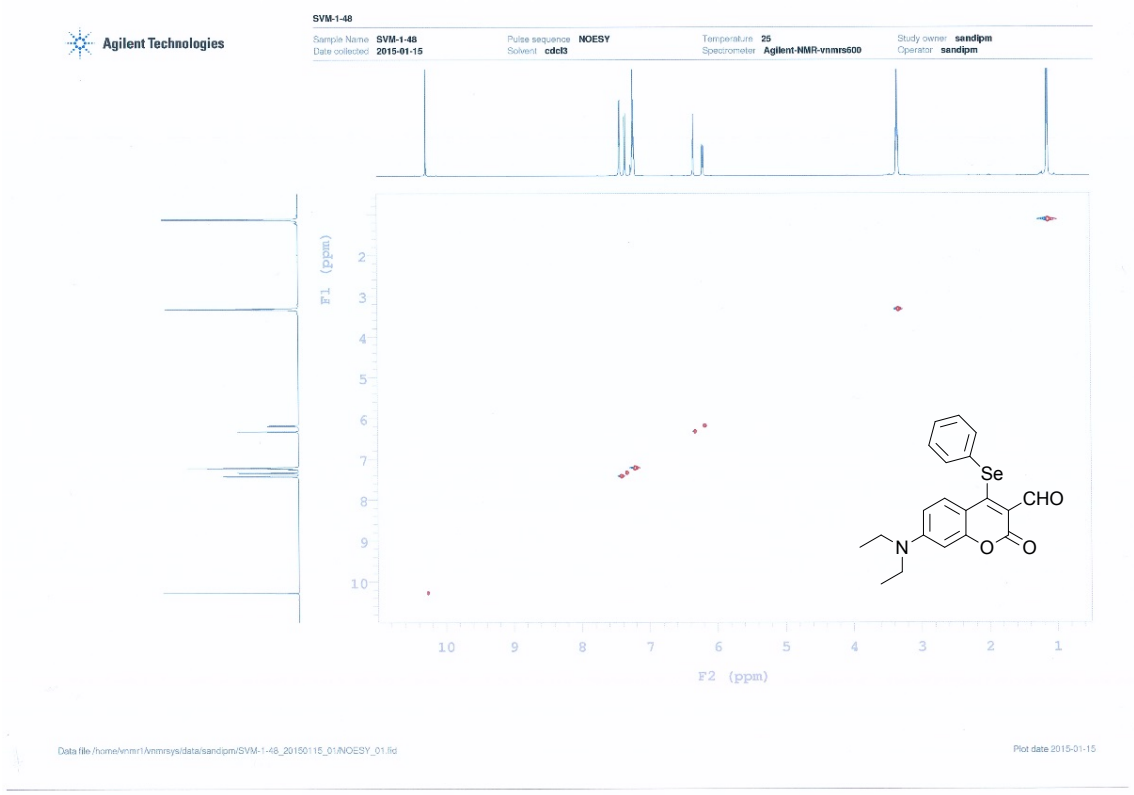


Fig. S6. (top) ^{1}H – ^{13}C HSQC NMR spectrum of **1** and (bottom) expanded aromatic region.



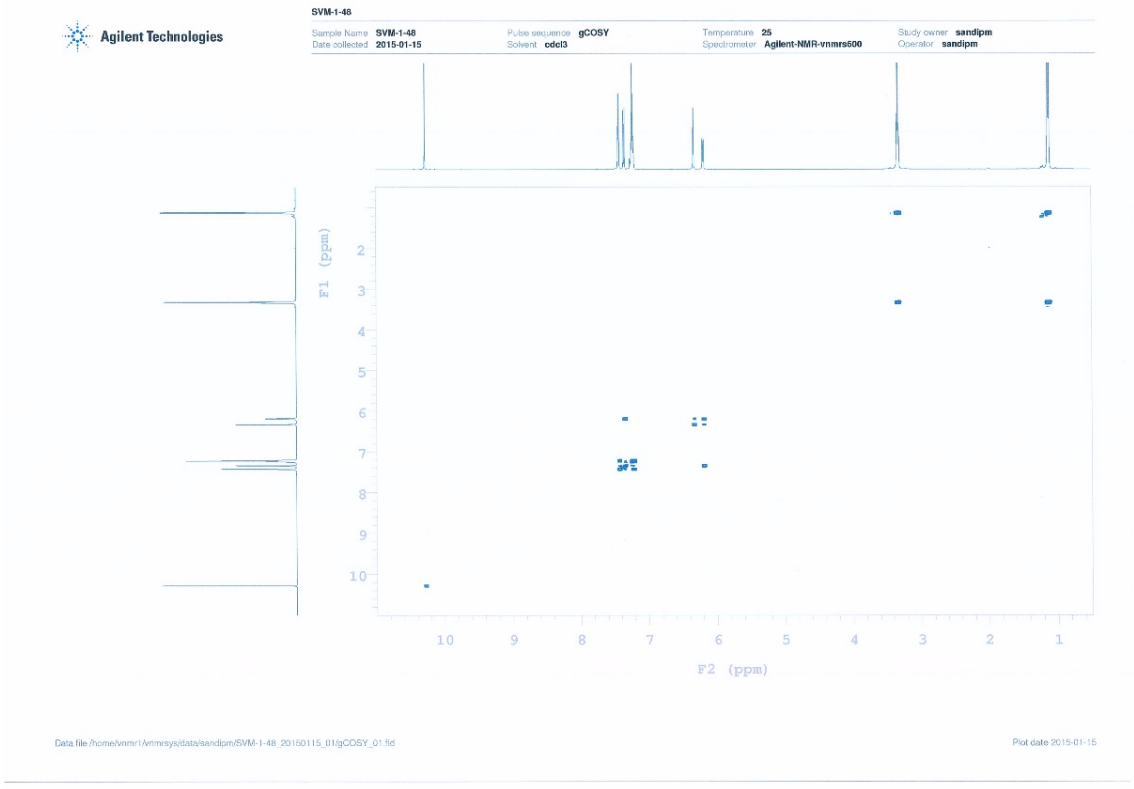


Fig. S7. (top) COSY and (bottom) NOESY NMR spectrum of **1**.

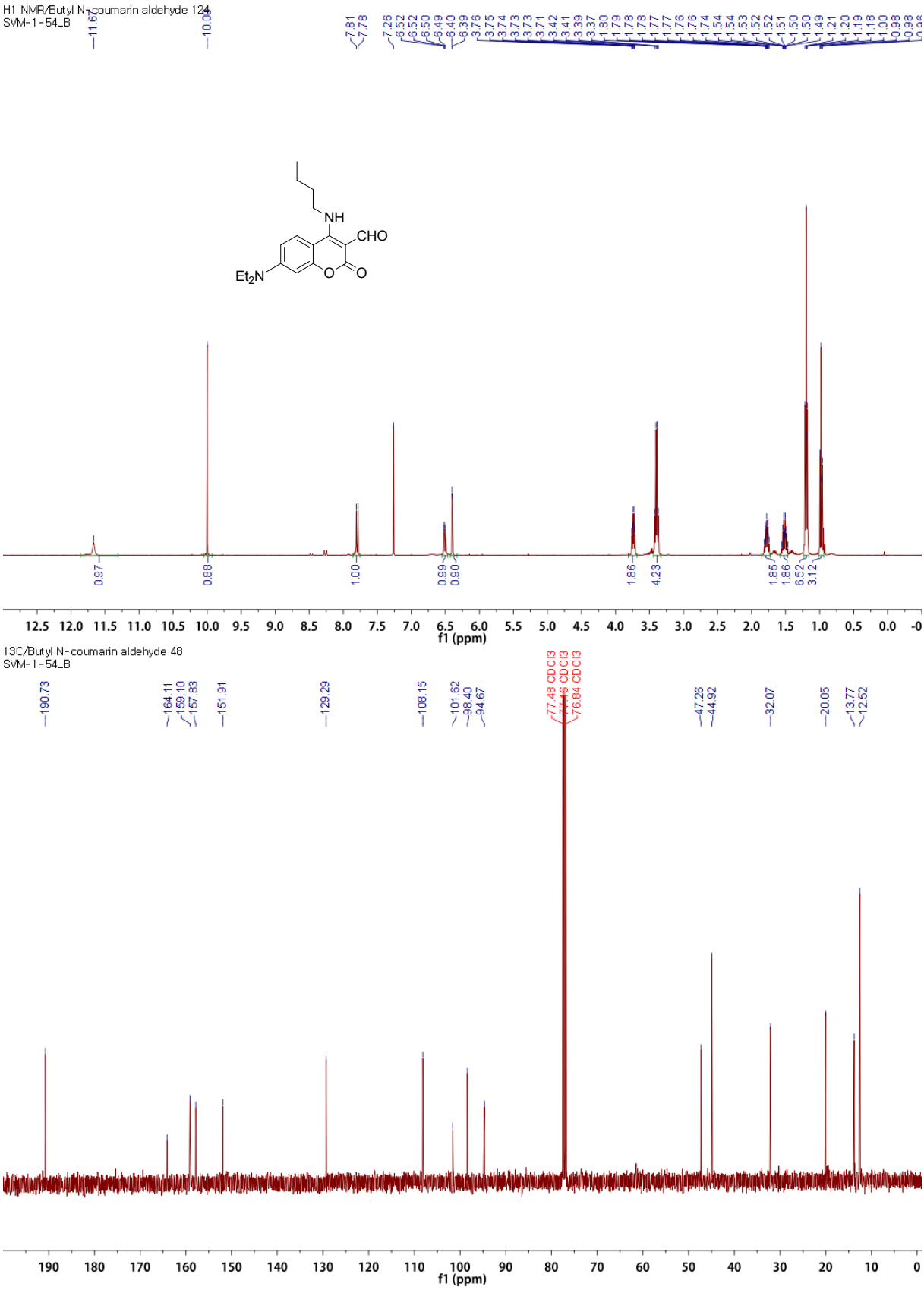


Fig. S8. (top) ^{1}H and (bottom) ^{13}C NMR spectrum of **8**.

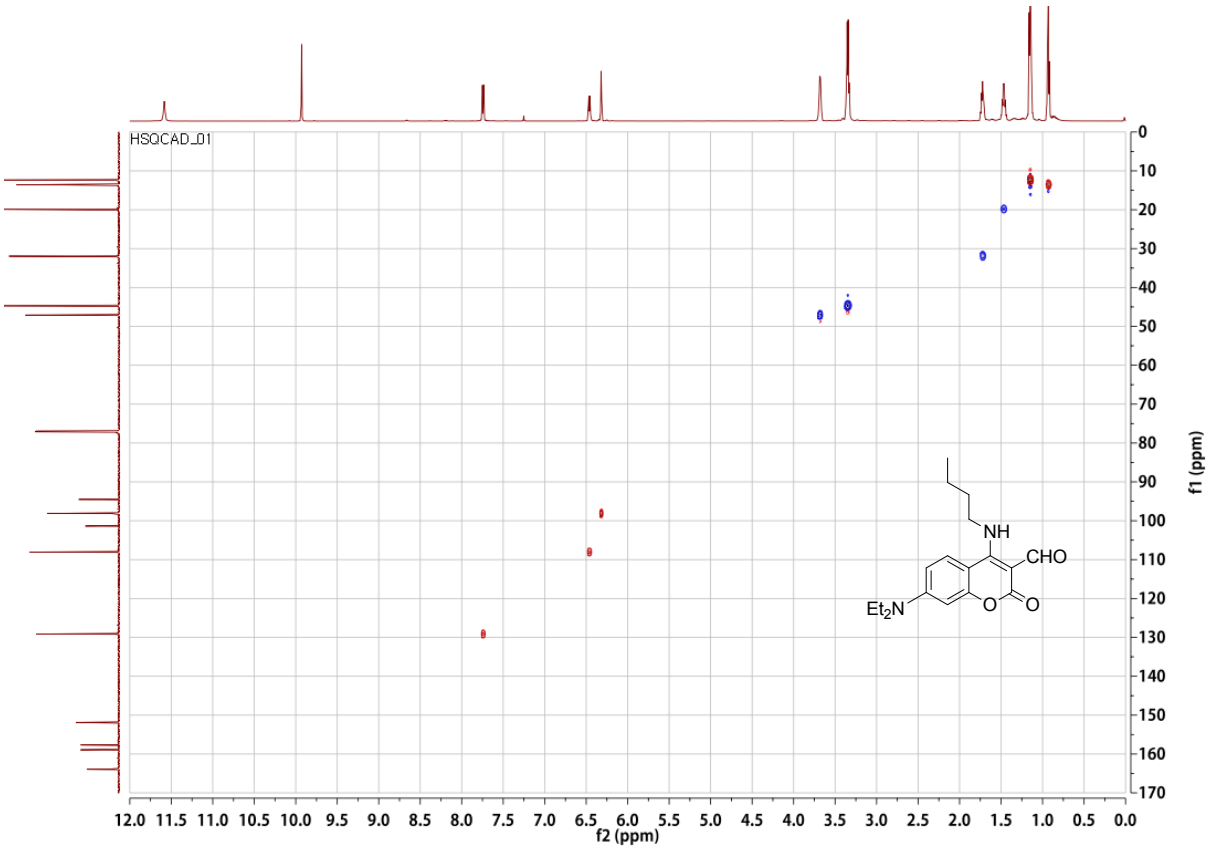


Fig. S9. ^{1}H – ^{13}C HSQC NMR spectrum of **8**.

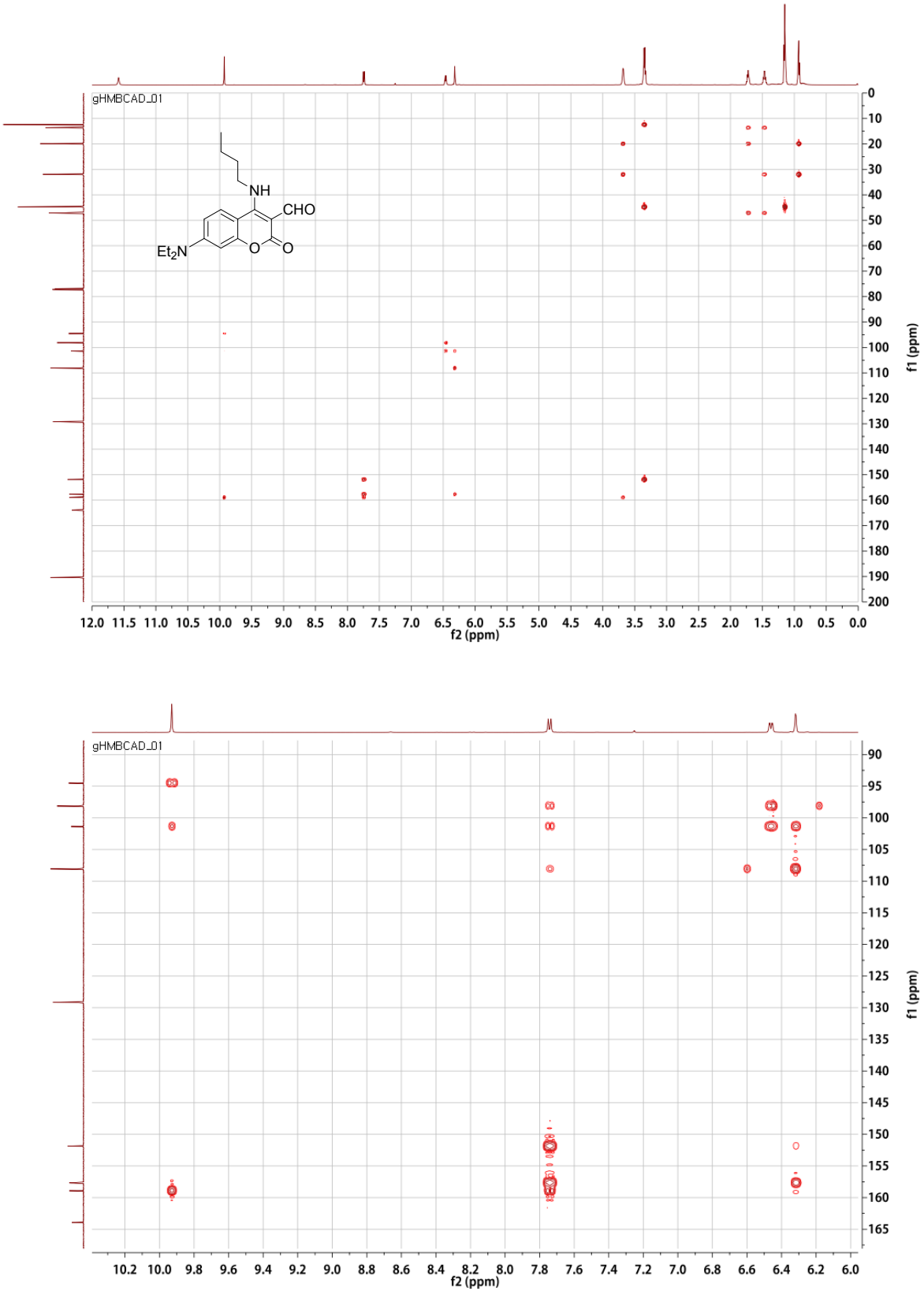


Fig. S10. (top) ^{1}H – ^{13}C HMBC NMR spectrum of **8** and (bottom) expanded aromatic region.

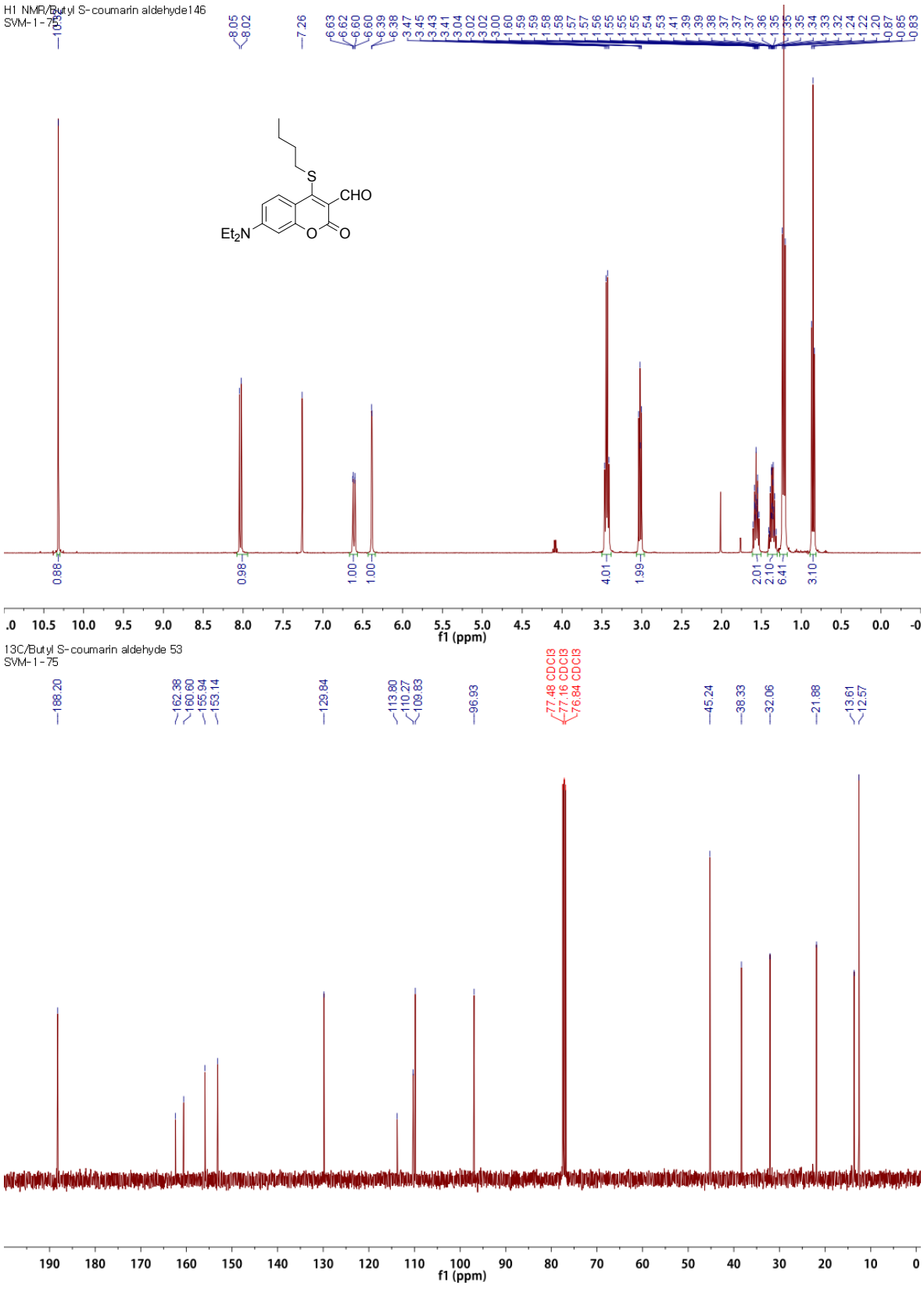


Fig. S11. (top) ^{1}H and (bottom) ^{13}C NMR spectrum of **9**.

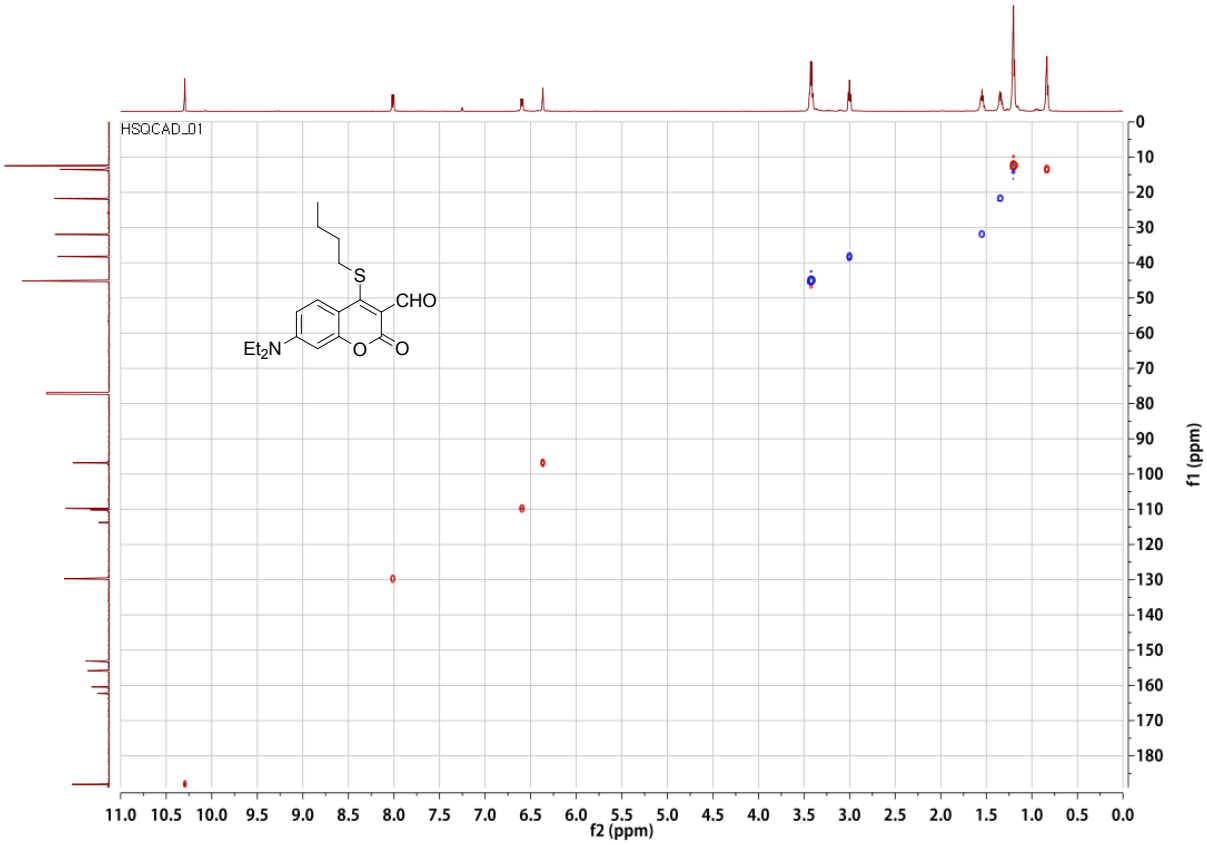


Fig. S12. ^{1}H – ^{13}C HSQC NMR spectrum of **9**.

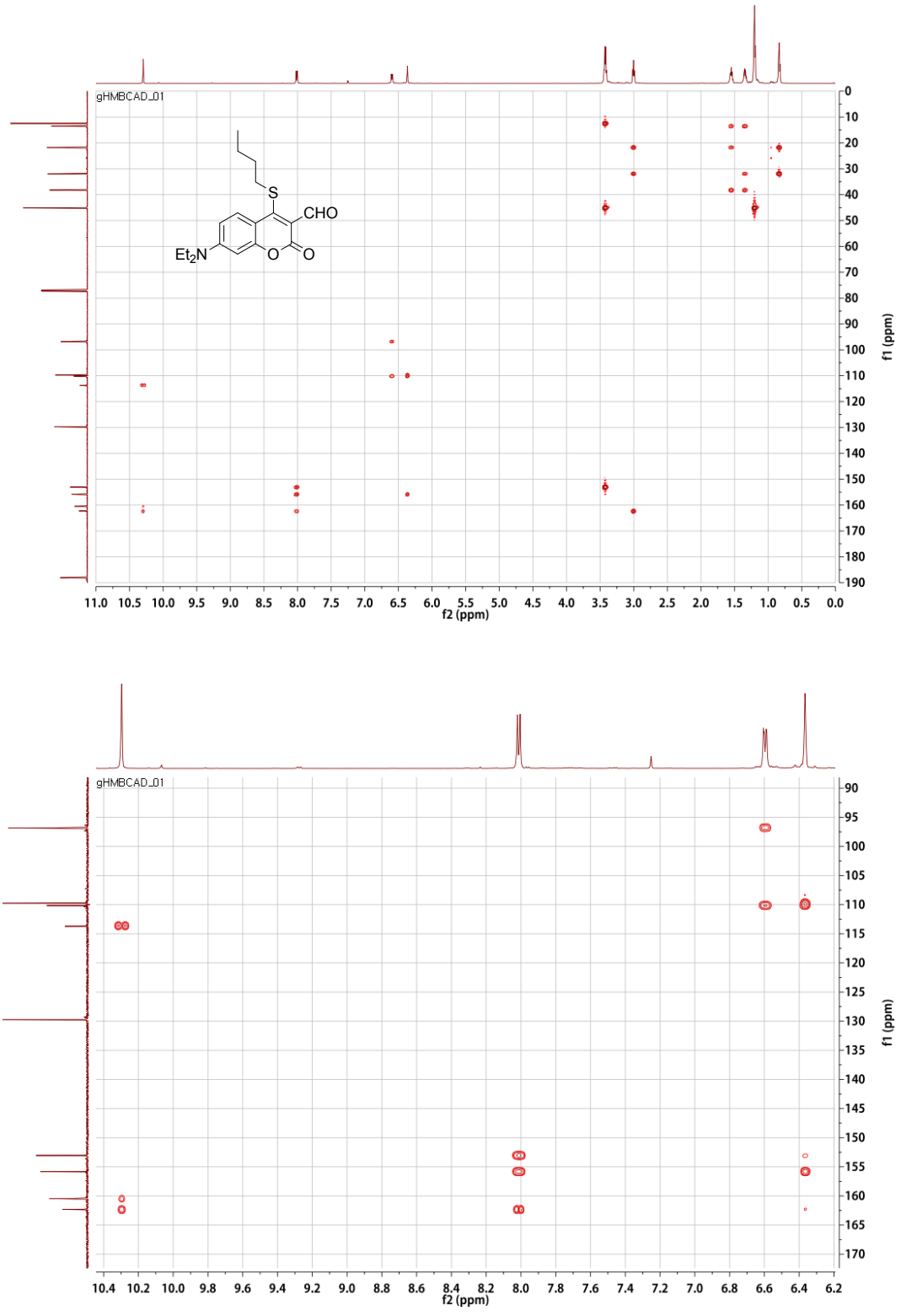


Fig. S13. (top) ^{1}H – ^{13}C HMBC NMR spectrum of **9** and (bottom) expanded aromatic region.

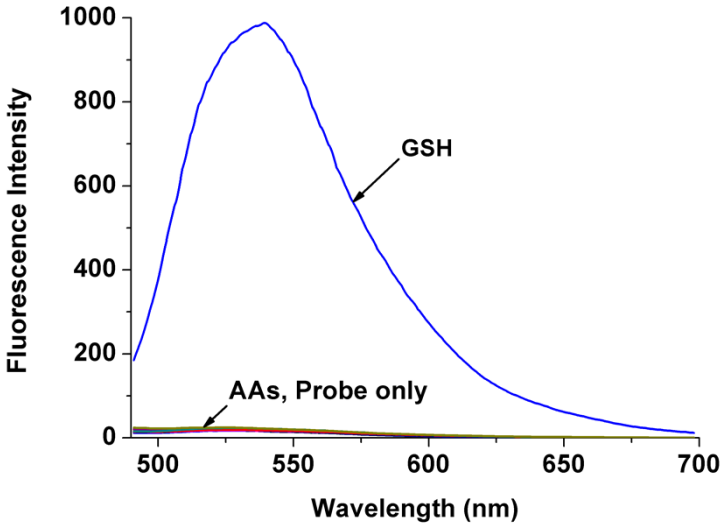


Fig. S14. Emission spectra of the **1** (20 μM) with 10 equiv of amino acids (Pro, Thr, Val, Leu, Asn, Trp, Ile, Ala, His, Glu, Tyr, Lys, Met, Asp, Phe, Arg, Gly, Gln, Ser), biothiols (Cys, Hcy, GSH) in the solution (DMSO/ 10 mM PBS pH 7.4, 1 : 3 v/v) incubated for 5 min at r.t. λ_{ex}: 471 nm.

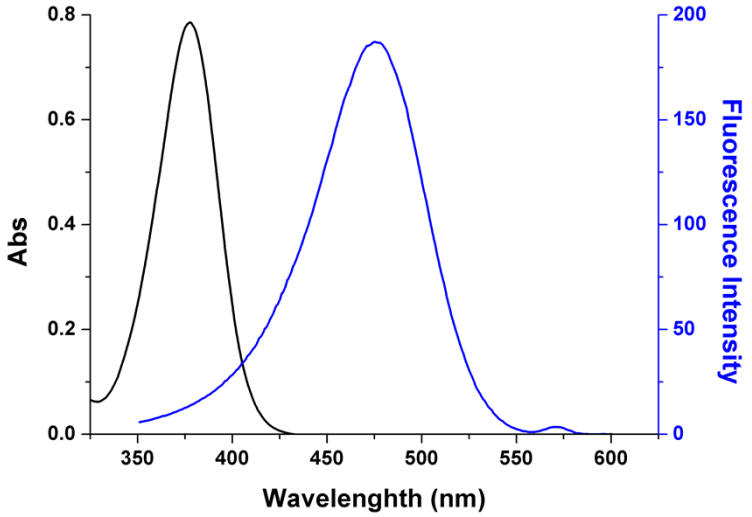


Fig. S15. Absorption and emission spectra of **8** in the solution (DMSO: PBS, v/v, 3: 1). λ_{ex}: 380 nm

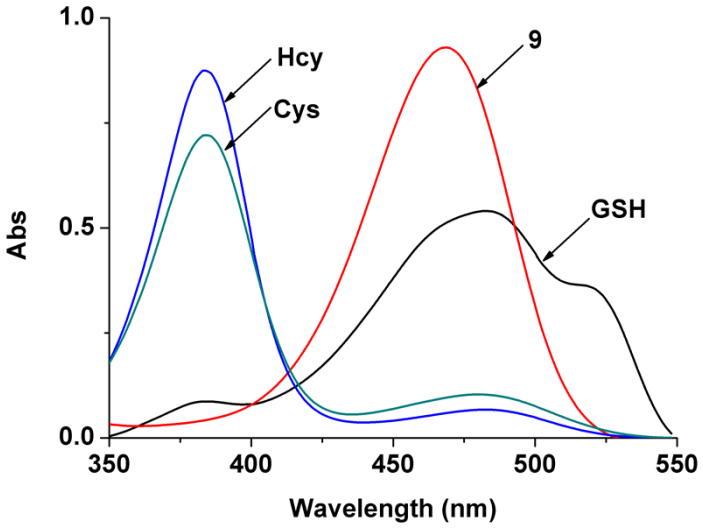


Fig. S16. Absorption spectra of the **9** (20 μM) with 10 equiv biothiols (Cys, Hcy, GSH) in the solution (DMSO/ 10 mM PBS pH 7.4, 1 : 3 v/v) incubated for 5 min at r.t.

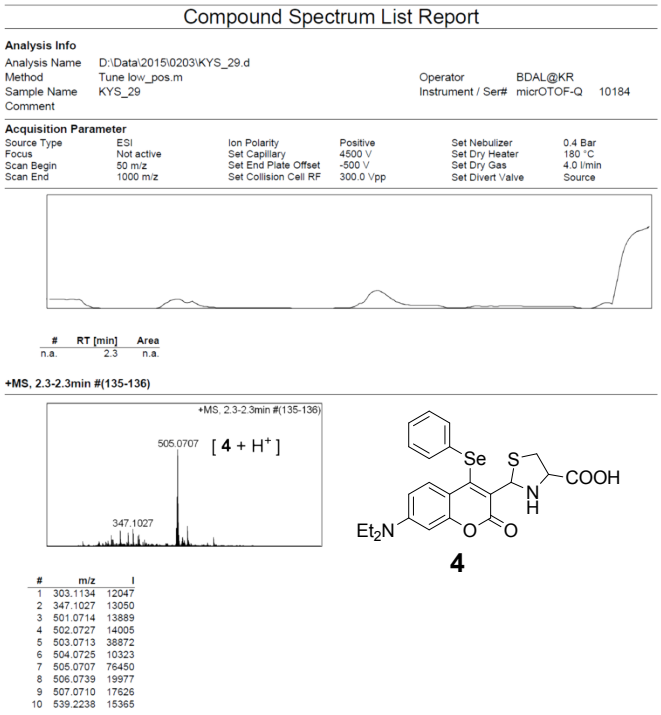


Fig. S17. Mass spectrum of **1** with 1 equiv of Cys. ESI-MS (*calc. 505.0700 obtained 505.0707 [**4** + H]*$^{+}$).

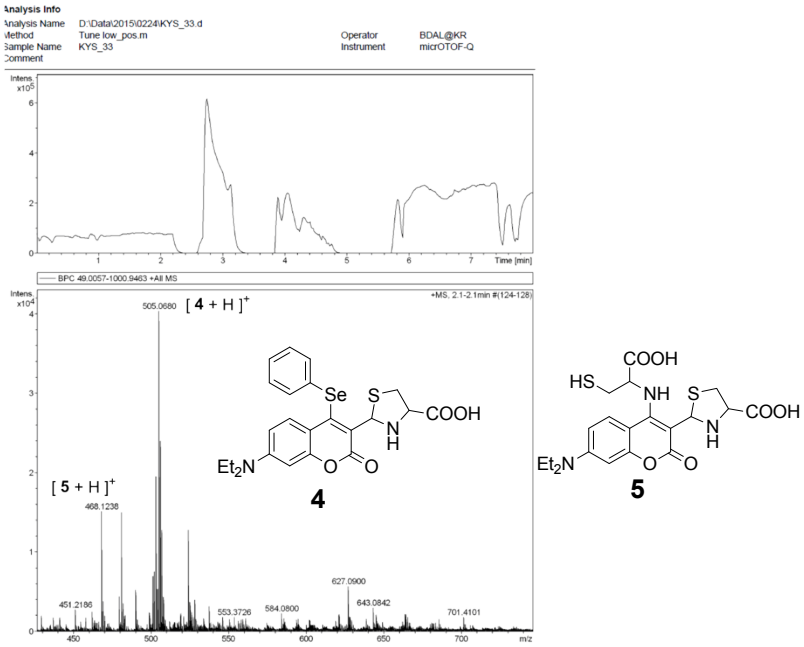


Fig. S18. Mass spectrum of **1** with 10 equiv of Cys. ESI-MS (*calc. 505.0700 obtained 505.0680 [**4** + H]$^+$, calc. 468.1238 obtained 468.1263 [**5** + H]$^+$*).

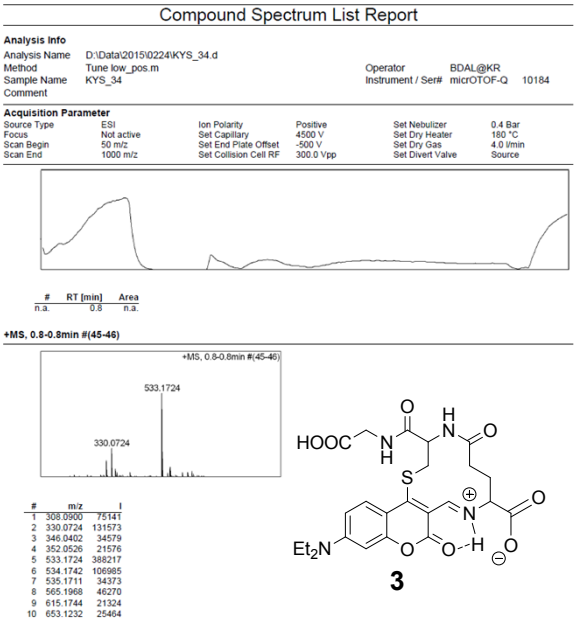


Fig. S19. Mass spectrum of **1** with 10 equiv of GSH. ESI-MS (*calculated 533.1706 obtain 533.1724 [3 + H]$^+$*).

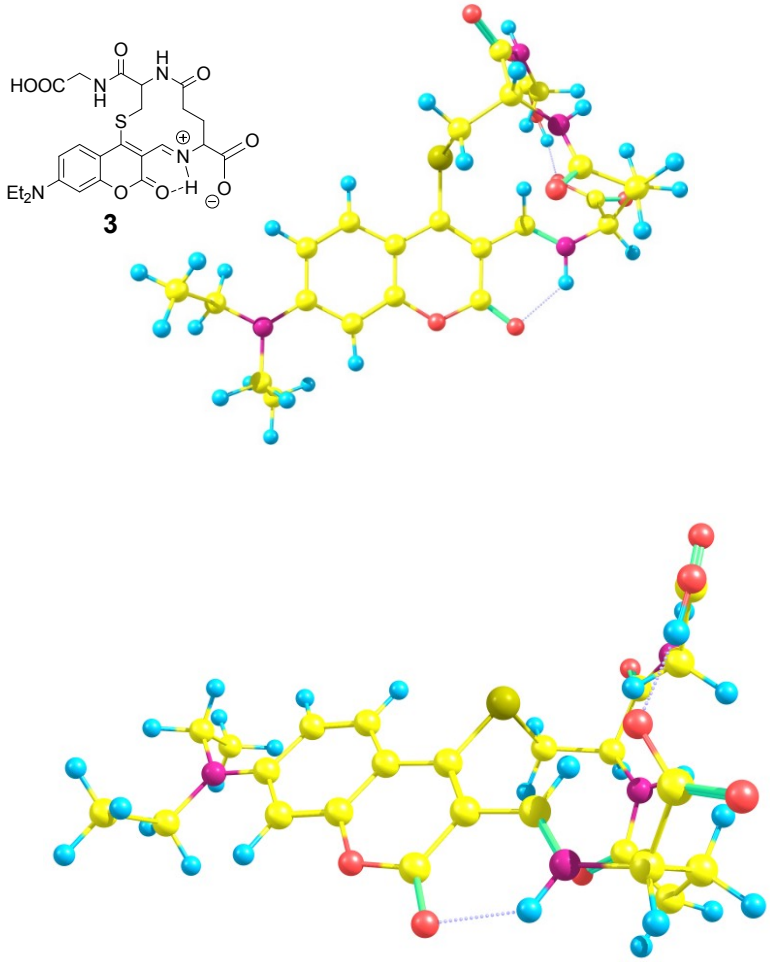


Fig. S20. Different views of optimized structure of **3** by DFT calculation (B3LYP/6-31g*, G09).

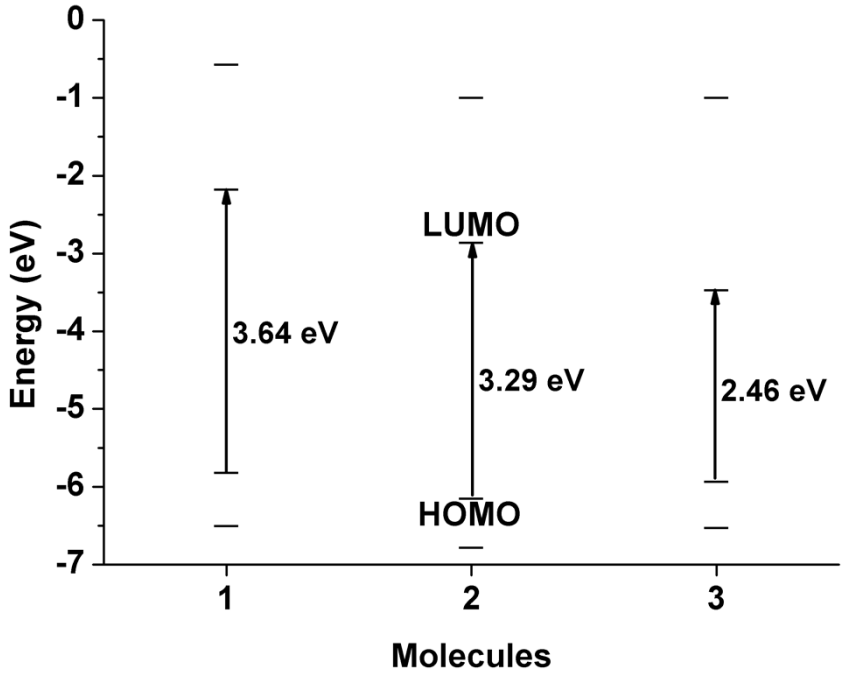


Fig. S21. The calculated HOMO-LUMO levels of compounds **1**, **2** and **3**.

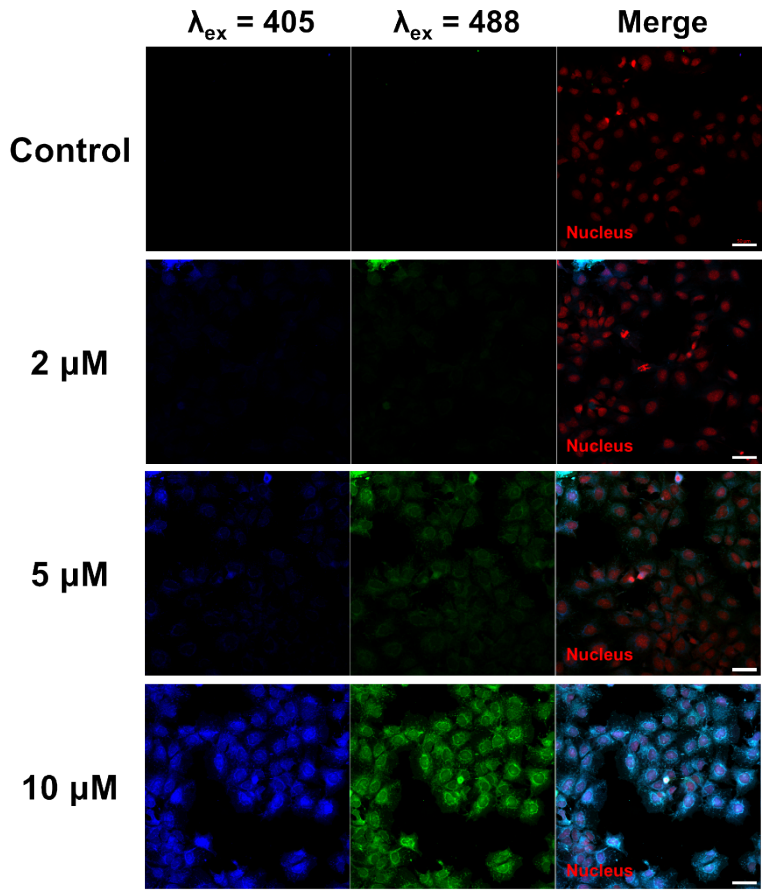


Fig. S22. Hep3B cells were treated with various concentrations of **1** (0 μM, 2 μM, 5 μM, and 10 μM) incubated for 30 min.

References

1. J. Liu, Y. Q. Sun, Y. Huo, H. Zhang, L. Wang, P. Zhang, D. Song, Y. Shi and W. Guo, *J. Am. Chem. Soc.*, 2014, **136**, 574.

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