Supporting Information

A lysosome-targetable fluorescent probe for the simultaneous sensing of Cys/Hcy and GSH from different emission channels

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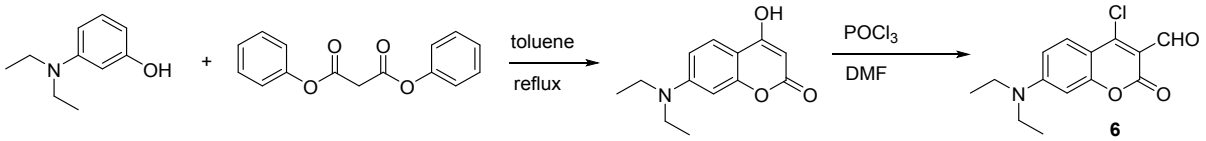
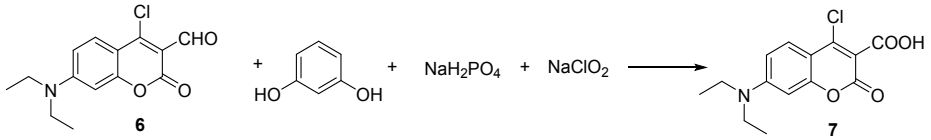
Synthesis of compound 6



Compound 6 was synthesized according the literature method. [1] ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.32 (s, 1H), 7.87 (d, J = 9.3 Hz, 1H), 6.74 (d, 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 compound 7



Compound 7 was synthesized according the literature method.[2] ^{1}H NMR (300 MHz, DMSO-d_6) δ13.65 (s, 1H), 7.64 (d, J = 9.2 Hz, 1H), 6.86 (d, J = 9.2, 2.4Hz, 1H), 6.63 (d, J = 2.4 Hz, 1H), 3.48 (q, J = 7.0 Hz, 4H), 1.14 (t, J = 7.0 Hz, 6H).^{13}C NMR (75 MHz, DMSO-d_6) δ169.06, 162.30, 159.73, 157.27, 150.49, 132.30, 119.41, 115.42, 110.10, 101.55, 49.47, 49.47, 17.48, 17.48.

Synthesis of compound A1

Compound **A1** was synthesized according the literature method.[2] Compound **A1,** ^{1}H NMR (600 MHz, $CDCl_3$) δ10.58 (s, 1H), 7.79 (d, J = 9.1 Hz, 1H), 7.37 (t, J = 7.7 Hz, 2H), 7.20 (dd, J = 16.7, 7.8Hz, 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.

Synthesis of compound A2

Compound **A1, A2** was synthesized according the literature method.[2] ^{1}H NMR (300 MHz, $CDCl_3$) 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 = 7.1Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) 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.

Reference

[1] 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.

[2] Chen, W.-Q.; Yue, X.-X.; Zhang, H.; Li, W.-X.; Zhang, L.-L; Xiao, Q.;Huang, C.-S.; Sheng, J.-R.; Song, X.-Z. Anal. Chem. 2017, 189, 12984.

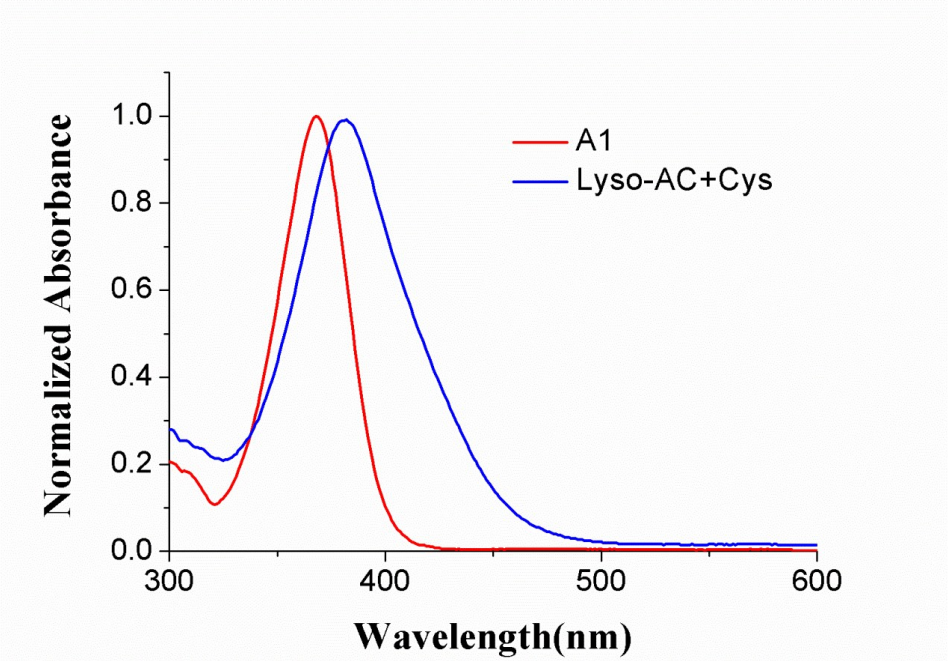


Figure S1. Normalized absorption spectra of Lyso-AC +10.0 equiv of Cys and compound A1 in PBS buffer (10mM, pH 7.4, containing 20% DMSO)

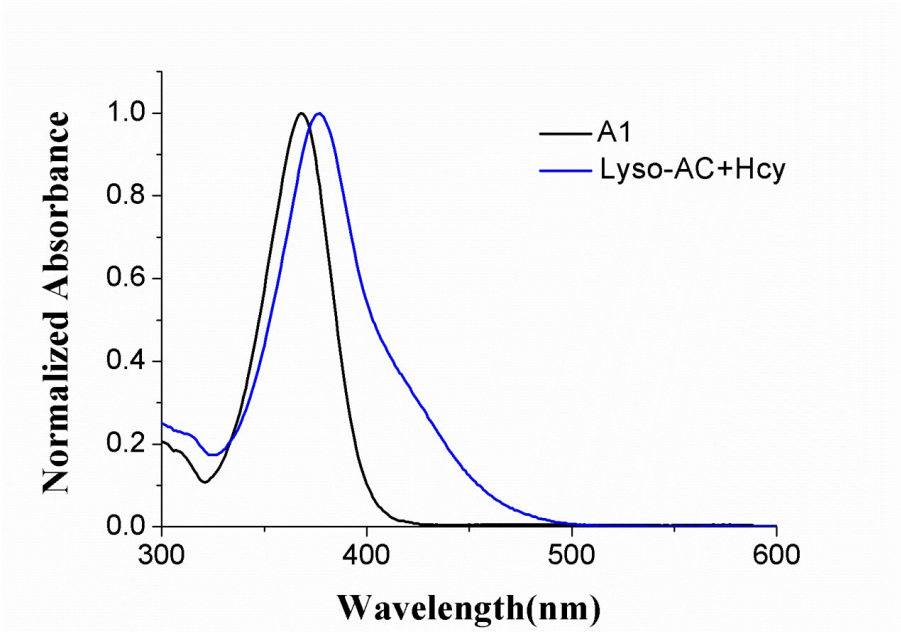


Figure S2. Normalized absorption spectra of Lyso-AC +10.0 equiv of Hcy and compound A1 in PBS buffer (10mM, pH 7.4, containing 20% DMSO)

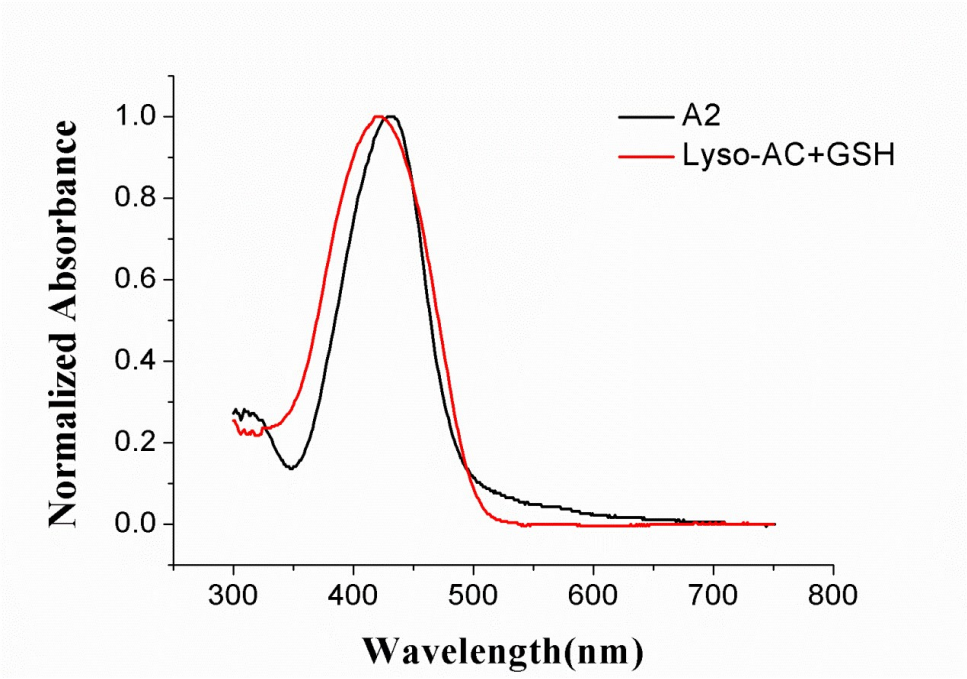


Figure S3. Normalized absorption spectra of Lyso-AC +10.0 equiv of GSH and compound A2 in PBS buffer (10mM, pH 7.4, containing 20% DMSO)

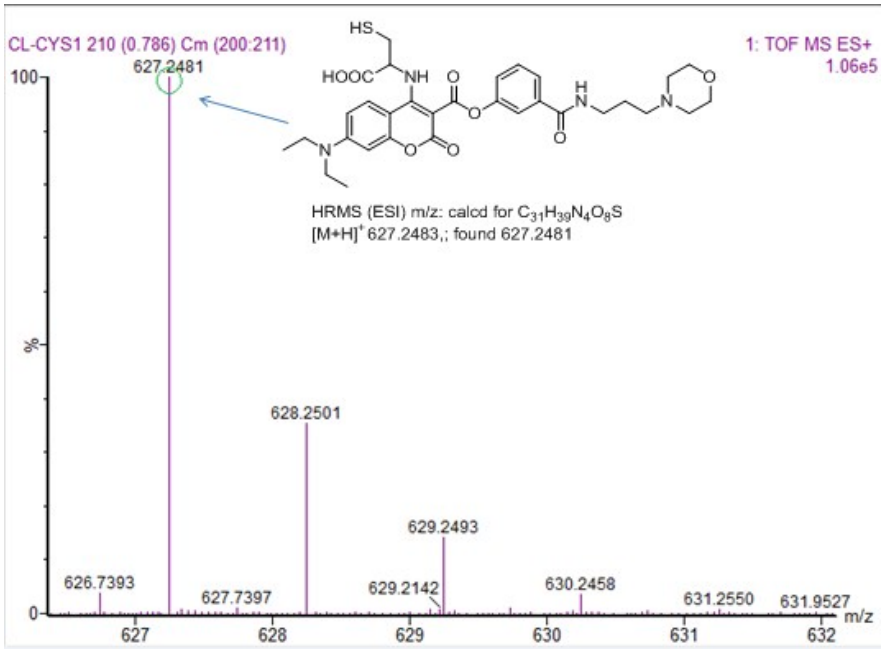


Figure S4. HRMS sprectrum of Lyso-AC in the presence of Cys.

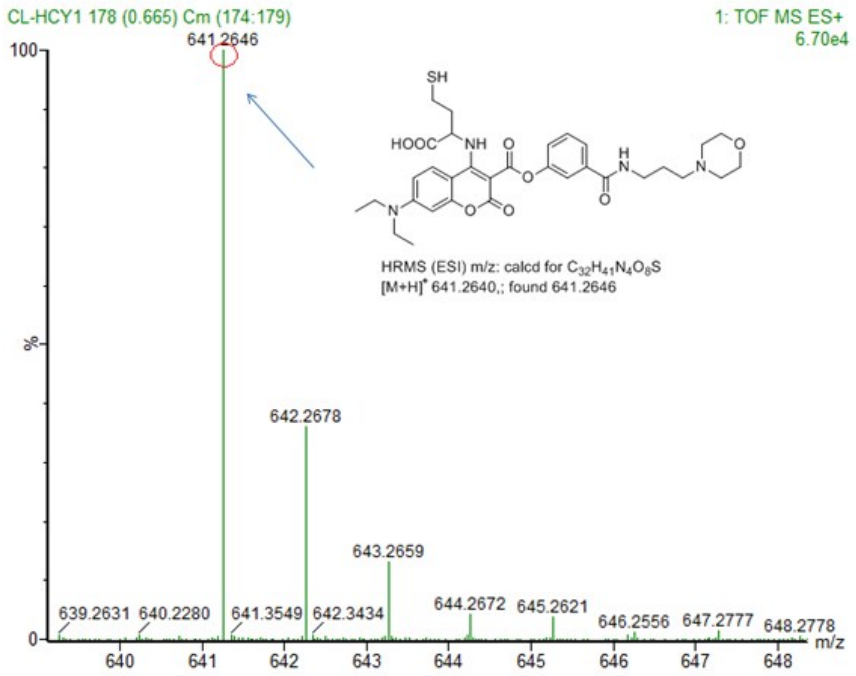


Figure S5. HRMS sprectrum of Lyso-AC in the presence of Hcy.

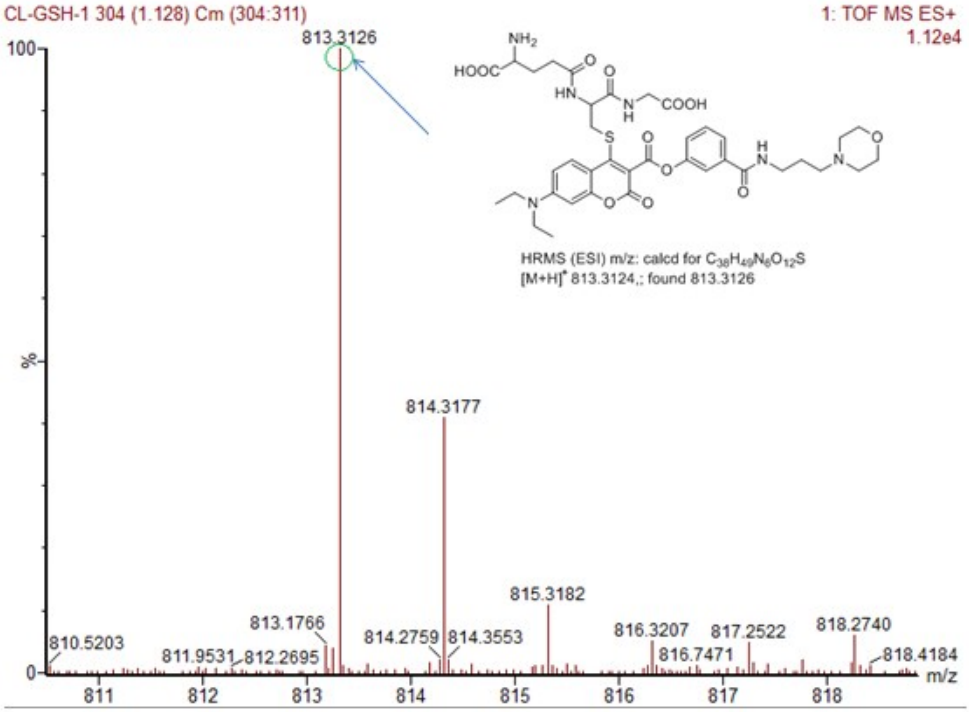


Figure S6. HRMS sprectrum of Lyso-AC in the presence of GSH.

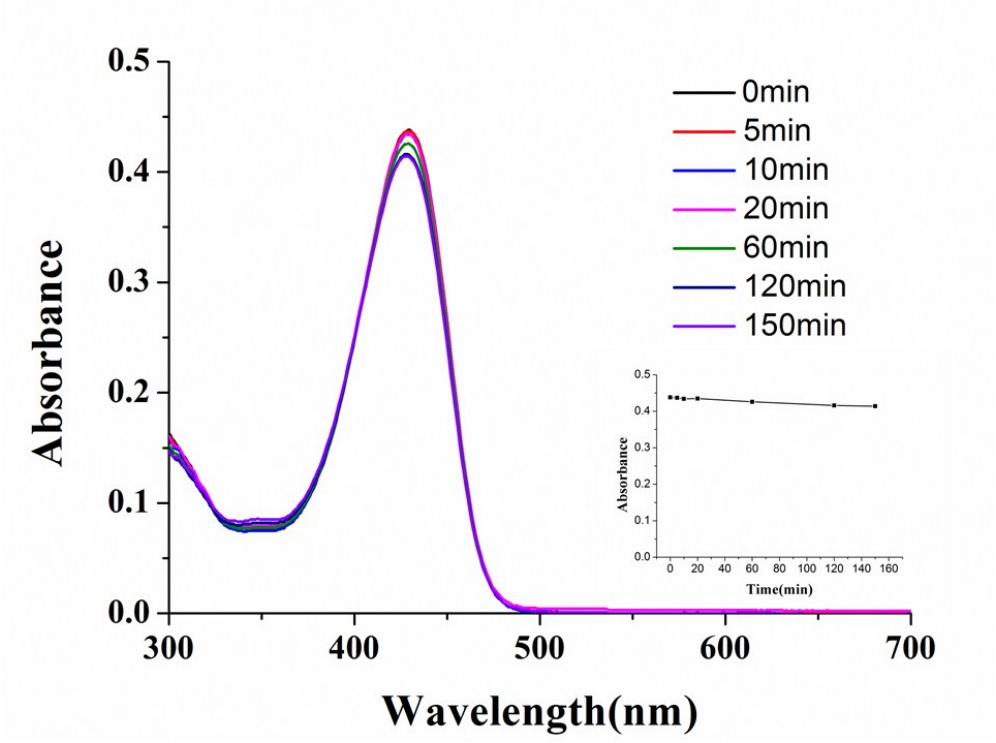


Figure S7. The photostability of Lyso-AC.

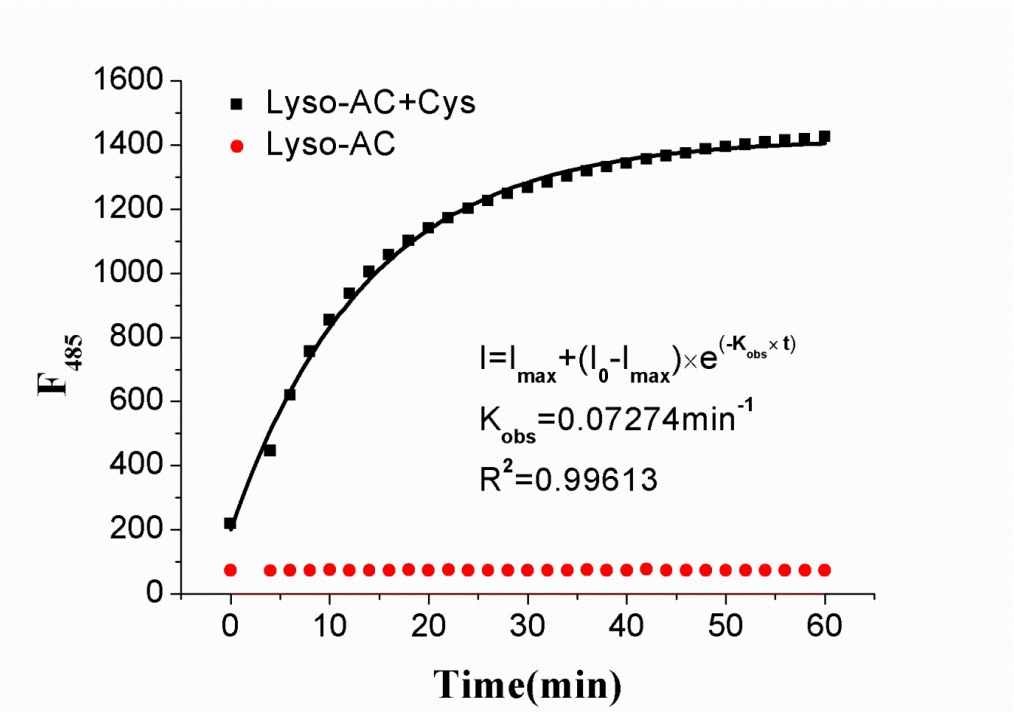


Figure S8. Time-dependent fluorescence intensity changes of Lyso-AC (10 μM) in the absence/presence of 10 equiv. of Cys in PBS buffer (10mM, pH 7.4, containing 20% DMSO). Excition at 383 nm. Slits: 5/5 nm, voltage: 700V.

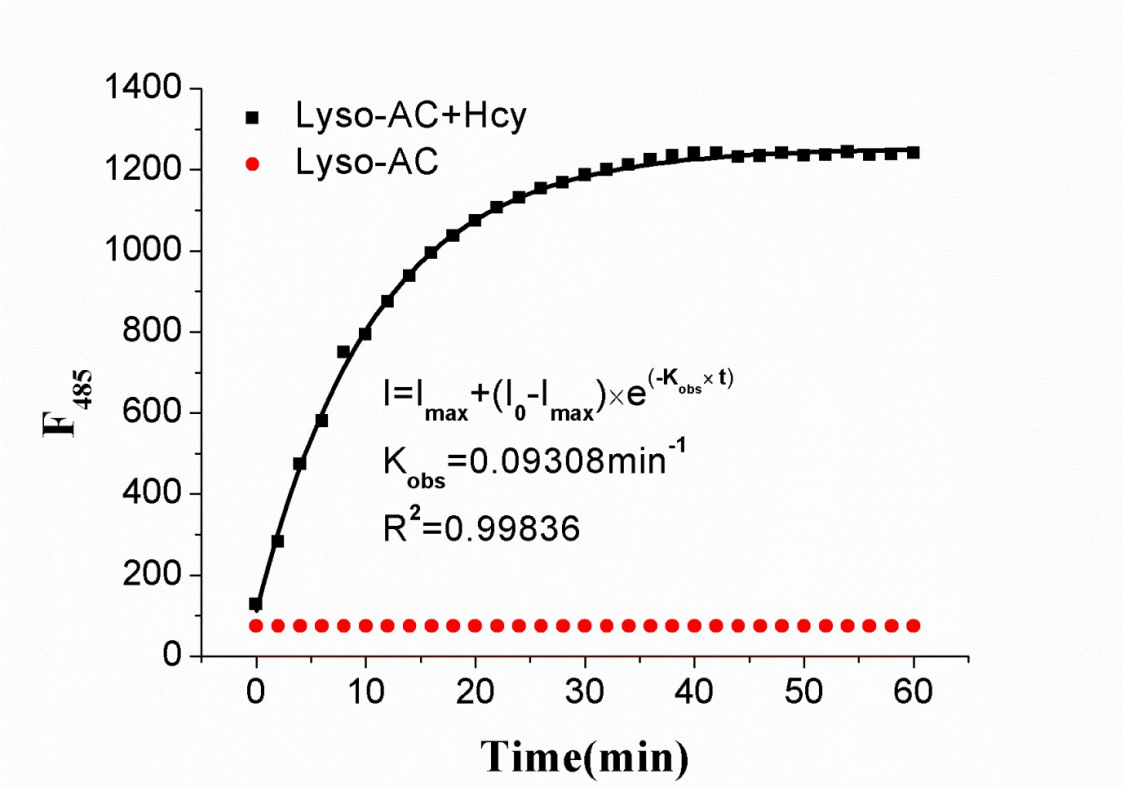


Figure S9. Time-dependent fluorescence intensity changes of Lyso-AC (10 μM) in the absence/presence of 10 equiv. of Hcy in PBS buffer (10mM, pH 7.4, containing 20% DMSO). Excition at 383 nm. Slits: 5/5 nm, voltage: 700V.

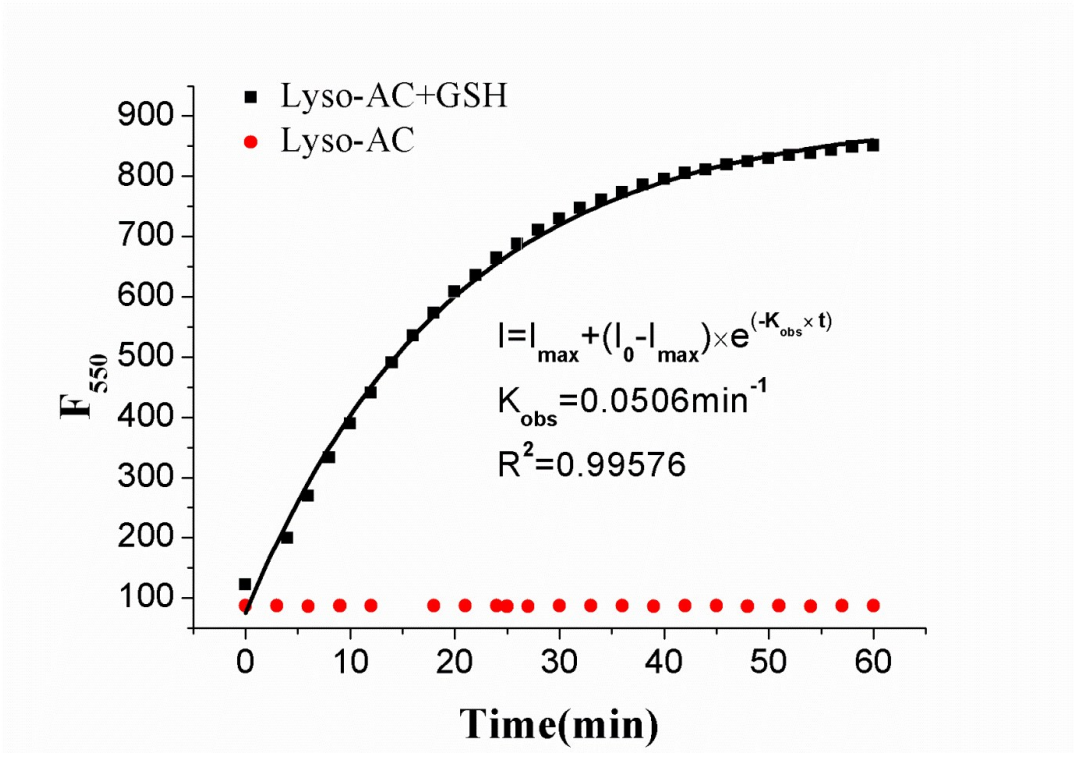


Figure S10. Time-dependent fluorescence intensity changes of Lyso-AC (10 μM) in the absence/presence of 10 equiv. of GSH in PBS buffer (10mM, pH 7.4, containing 20% DMSO). Excition at 425 nm. Slits: 5/5 nm, voltage: 700V.

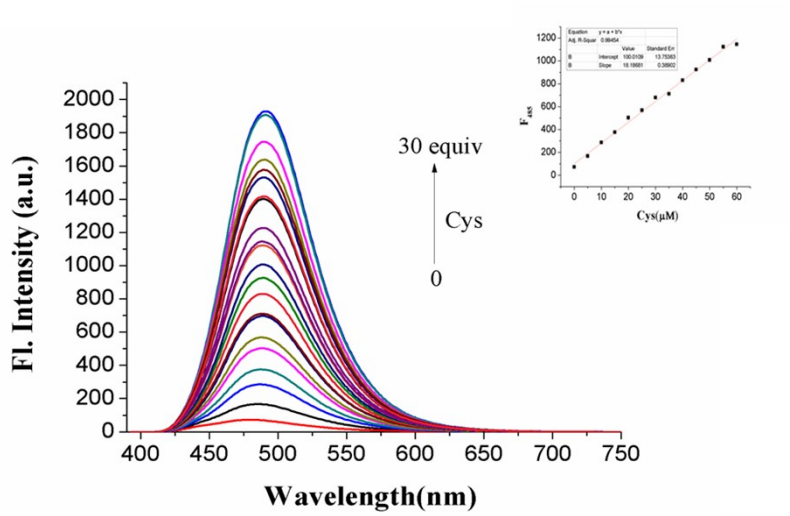


Figure S11. Fluorescence spectra changes of Lyso-AC (10 μM) upon addition 0 to 30 equiv of Cys in PBS buffer (10 mM, pH 7.4, containing 20% DMSO) for 60min at 25°C. λ_{ex} = 383 nm. Slits: 5/5 nm, voltage: 700V.

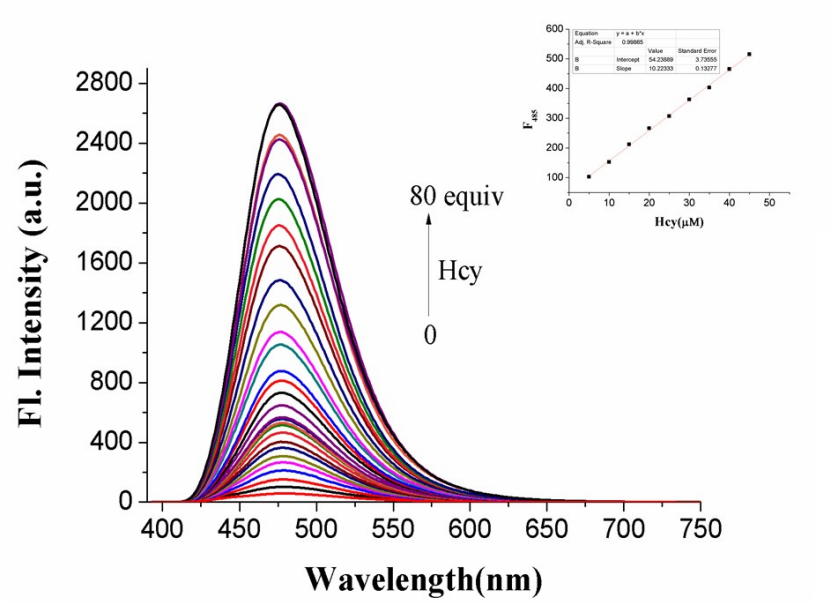


Figure S12. Fluorescence spectra changes of Lyso-AC (10 μM) upon addition 0 to 80 equiv of Hcy in PBS buffer (10 mM, pH 7.4, containing 20% DMSO) for 60min at 25°C. λ_{ex} = 383 nm. Slits: 5/5 nm, voltage: 700V.

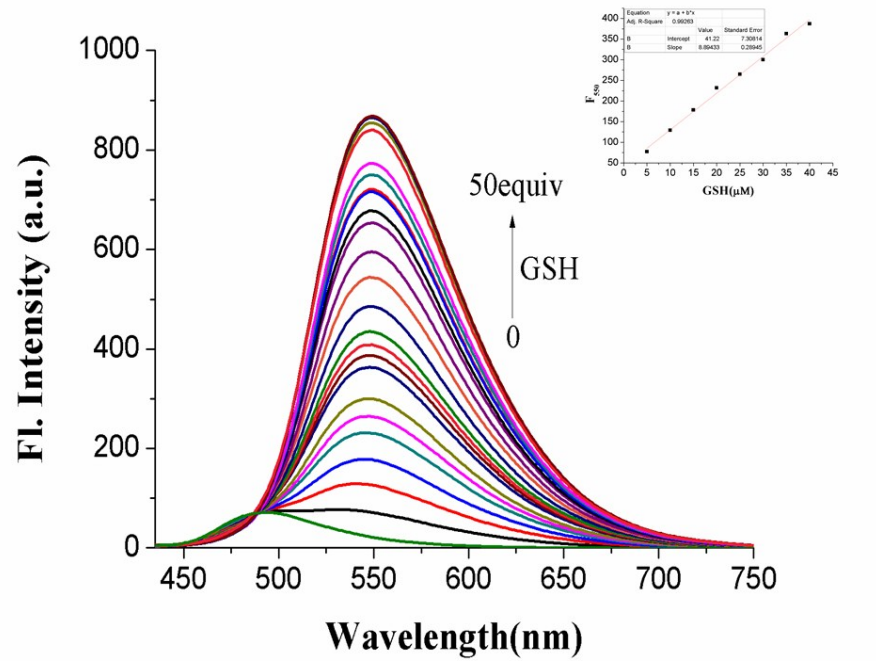


Figure S13. Fluorescence spectra changes of Lyso_AC (10 μM) upon addition 0 to 50 equiv of GSH in PBS buffer (10 mM, pH 7.4, containing 20% DMSO) for 60min at 25°C. λ_{ex} = 425 nm. Slits: 5/5 nm, voltage: 700V.

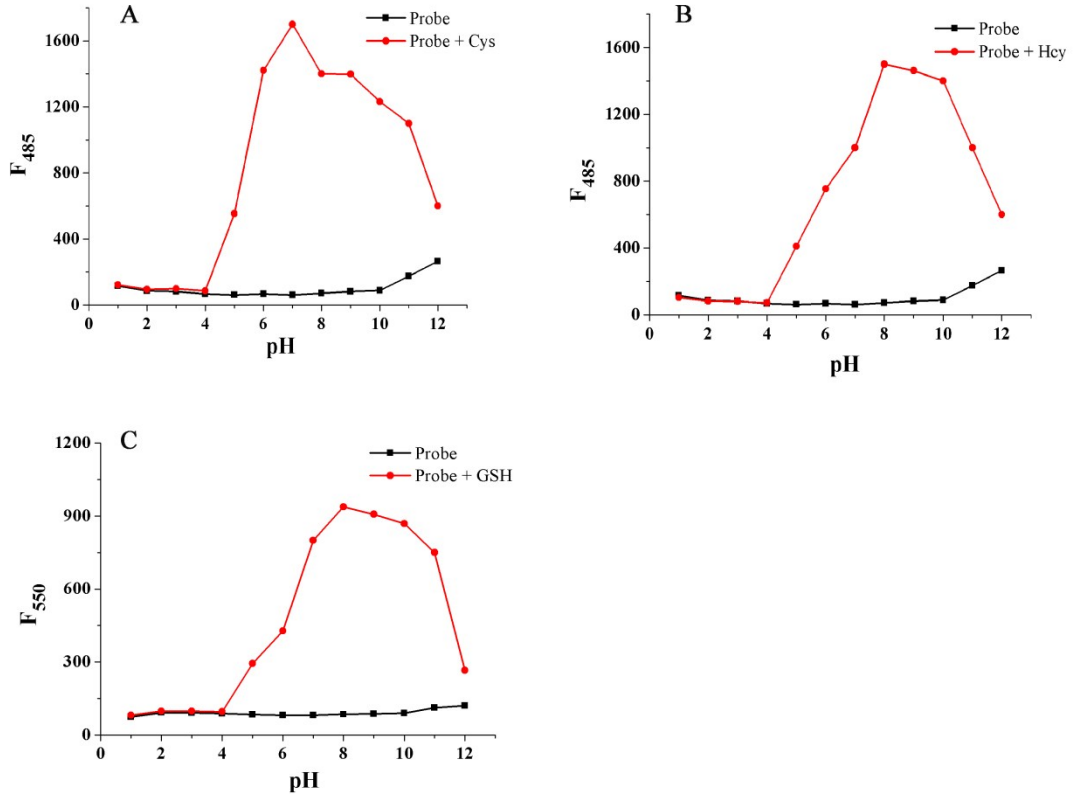


Figure 14. The pH effect on the fluorescence intensity of the probe Lyso-AC (10.0 μM) in the absence and presence of 30.0 equivalents of Cys and Hcy emission at 485 nm (A, B) and emission at 550 nm GSH (C). Spectrum recorded at 38 min after addition of the corresponding species.

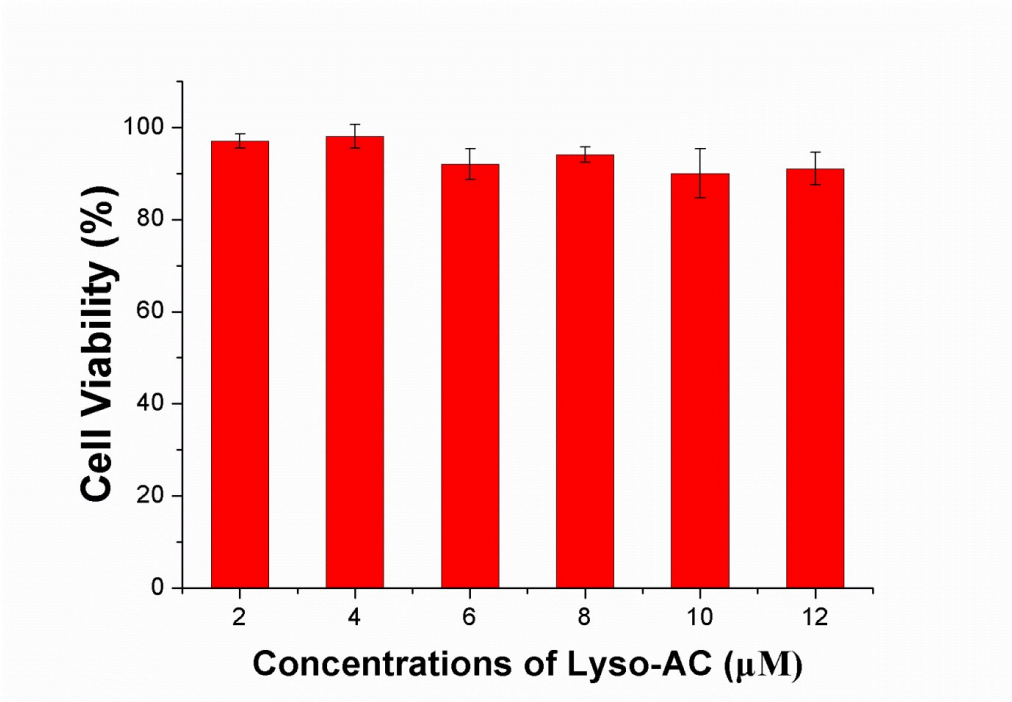


Figure S15. Percentage of living HeLa cells after treatment with indicated concentrations of Lyso-AC after 24 hours.

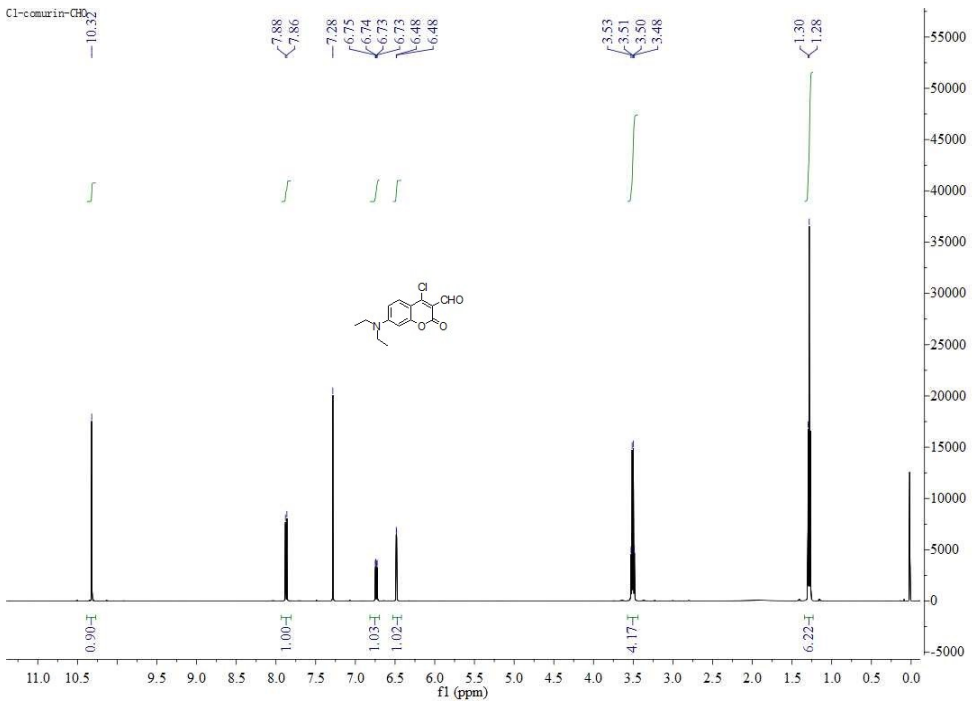


Figure S16. [1]HNMR sprectrum of compound **6**.

Figure S17. [1]HNMR sprectrum of compound **7**.

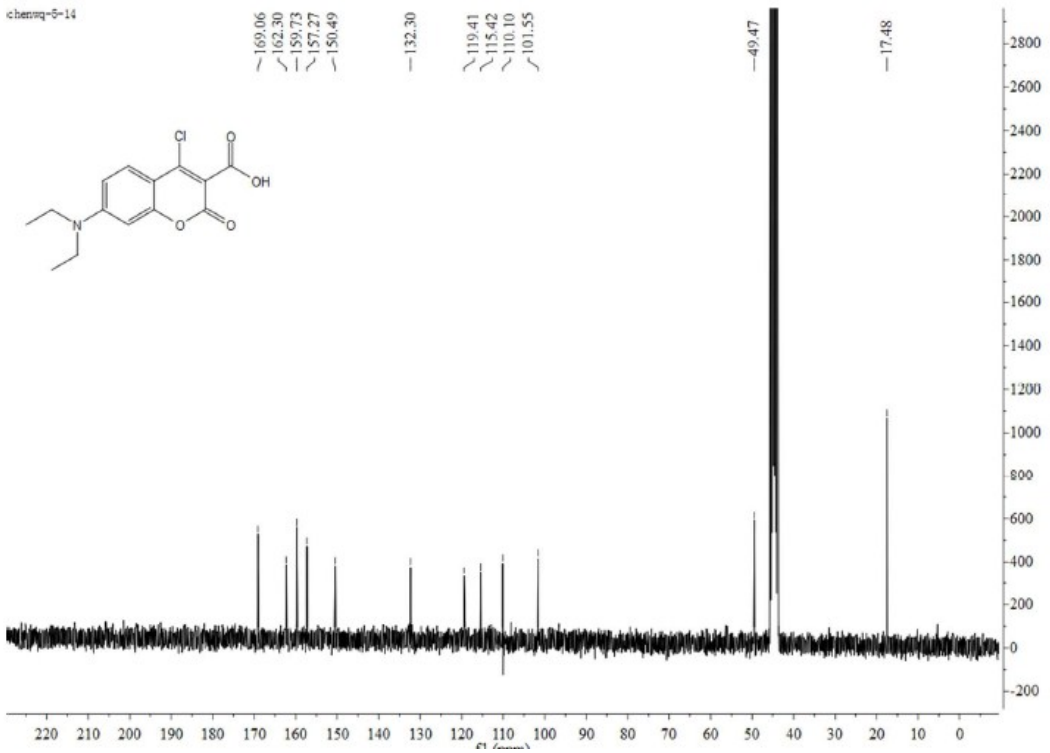


Figure S18. [13]CNMR sprectrum of compound **7**.

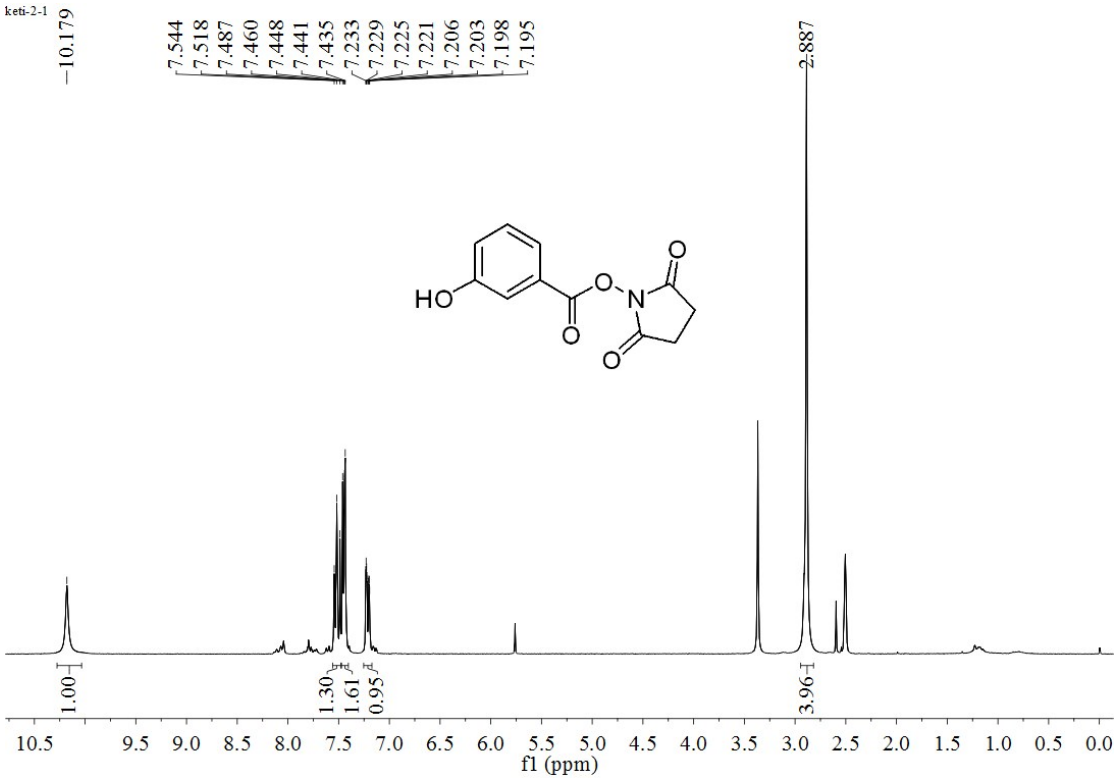


Figure S19. [1]HNMR sprectrum of **2**.

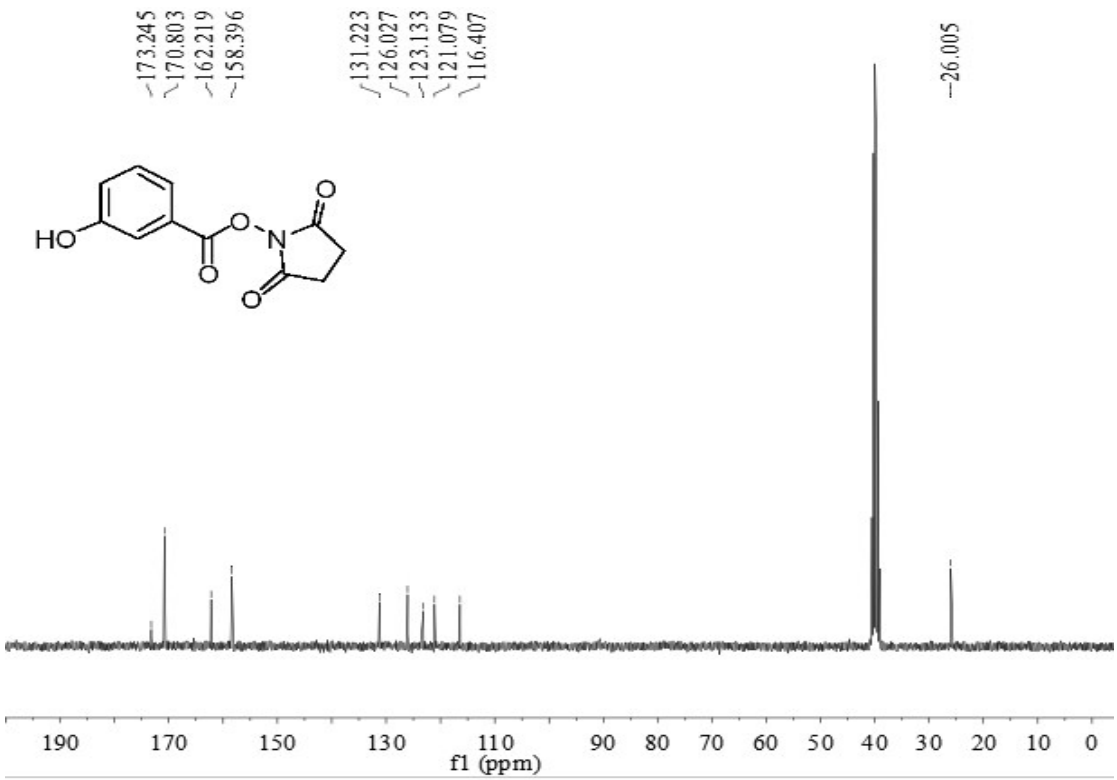


Figure S20. [13]CNMR sprectrum of compound 2

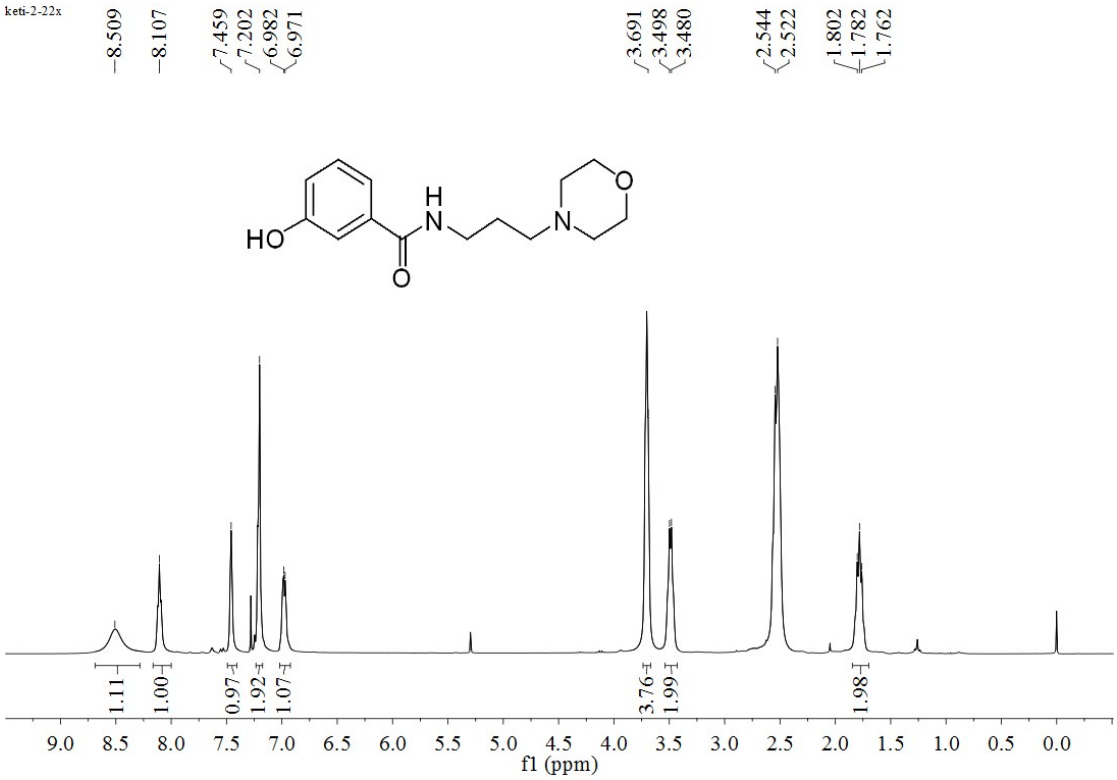


Figure S21. [1]HNMR sprectrum of compound **3**.

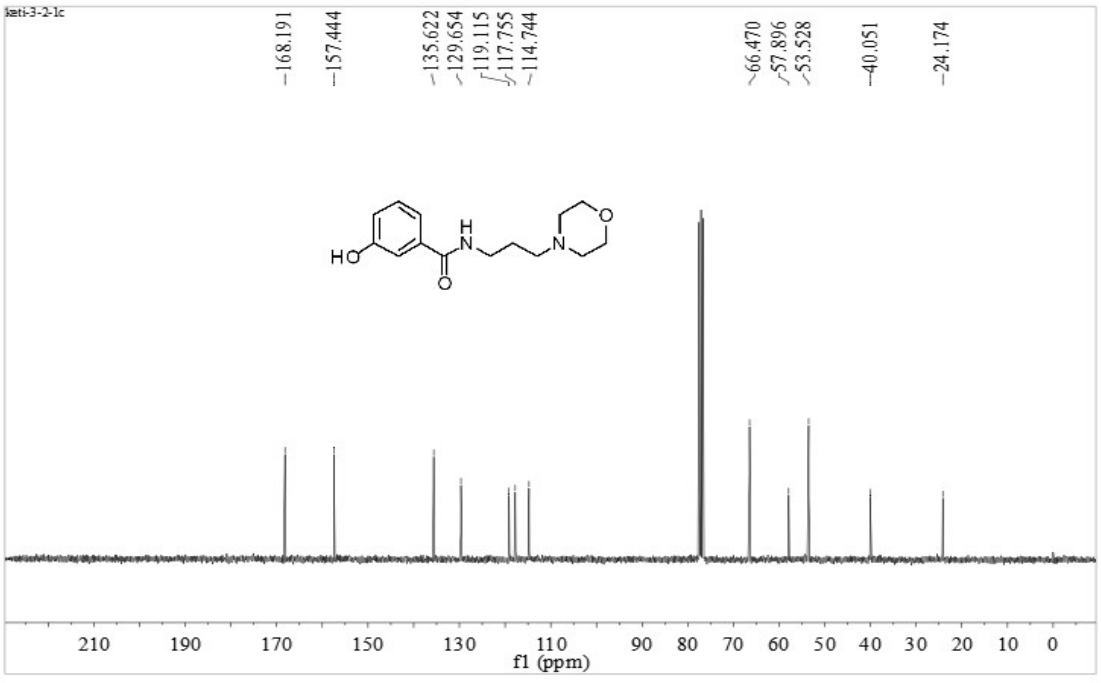


Figure S22. [13]CNMR sprectrum of compound **3**.

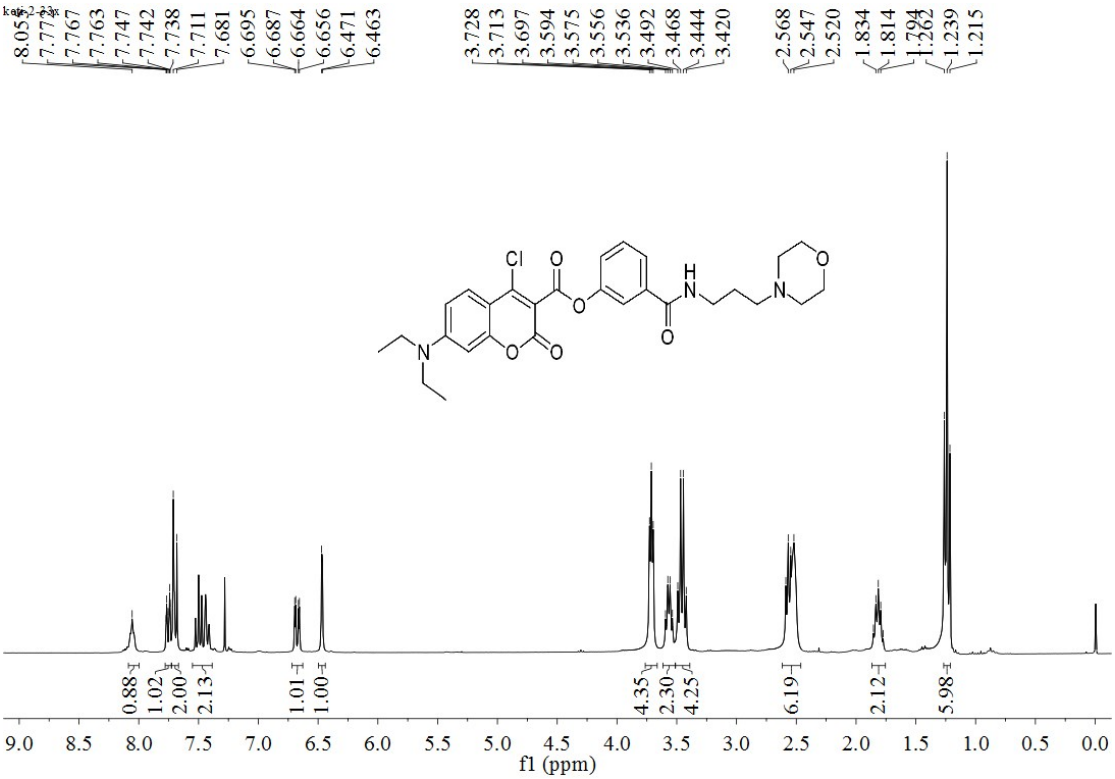


Figure S23. [1]HNMR sprectrum of compound 5.

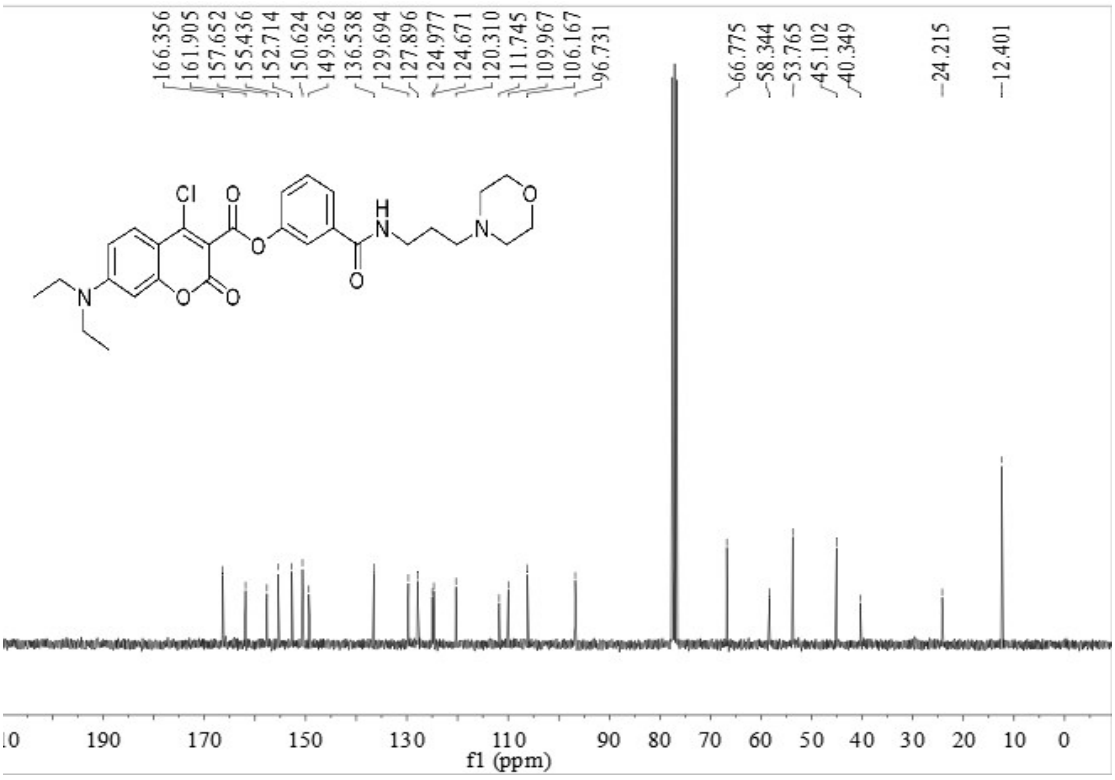


Figure S24. [13]CNMR sprectrum of compound **5**.

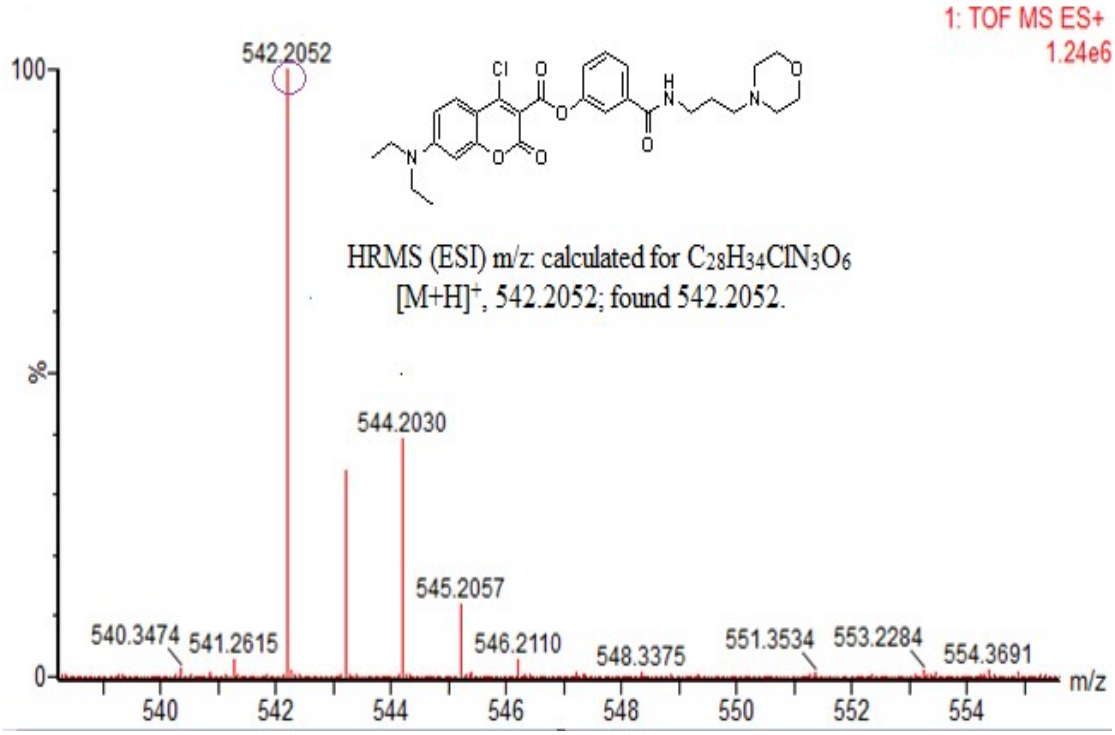


Figure S25. HRMS sprectrum of compound **5**.

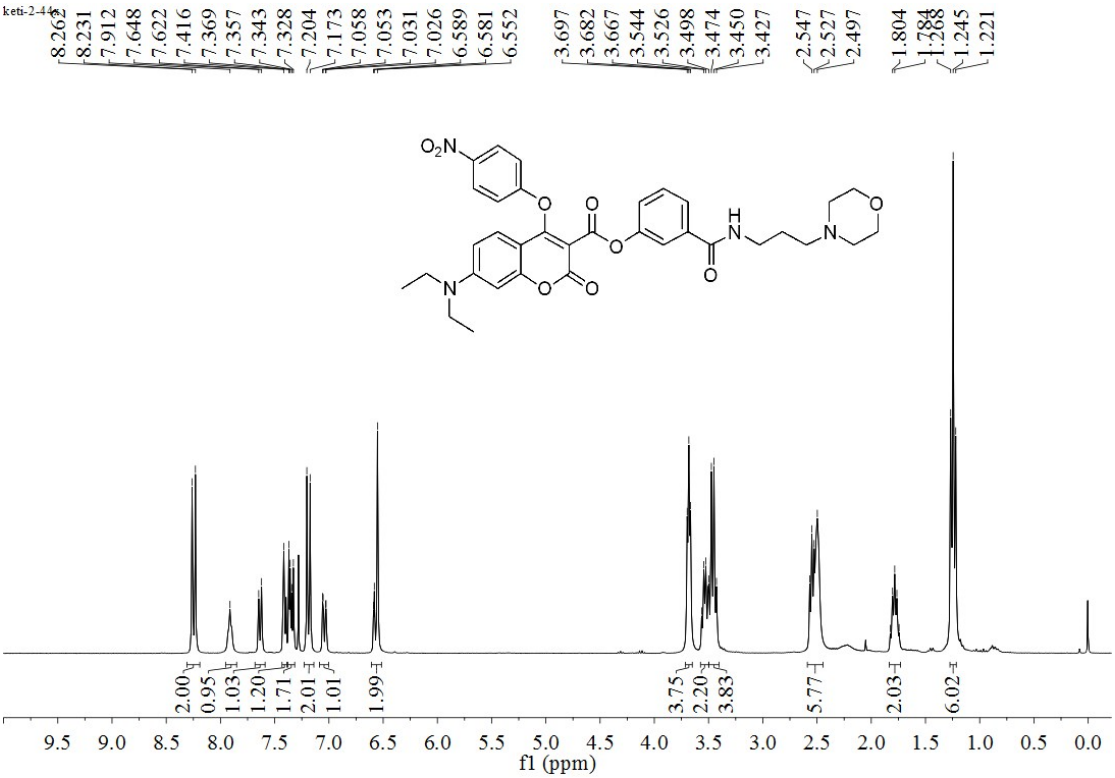


Figure S26. [1]HNMR sprectrum of Lyso-AC.

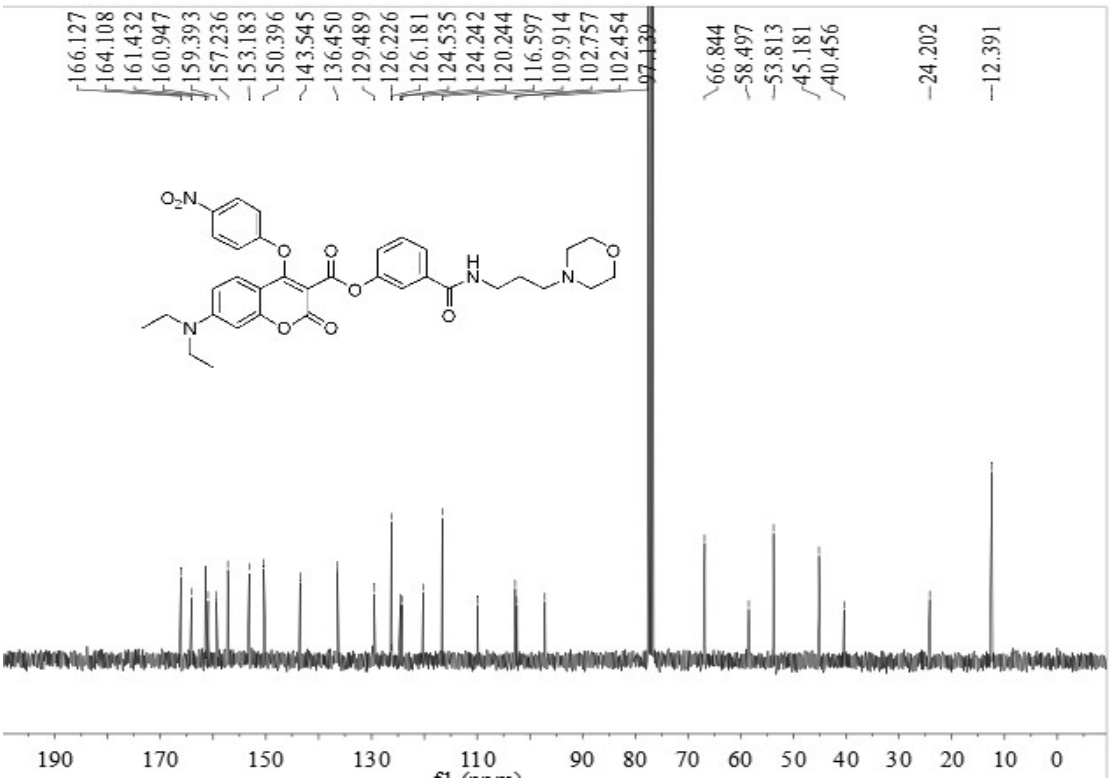


Figure S27. ^{13}CNMR sprectrum of Lyso-AC.

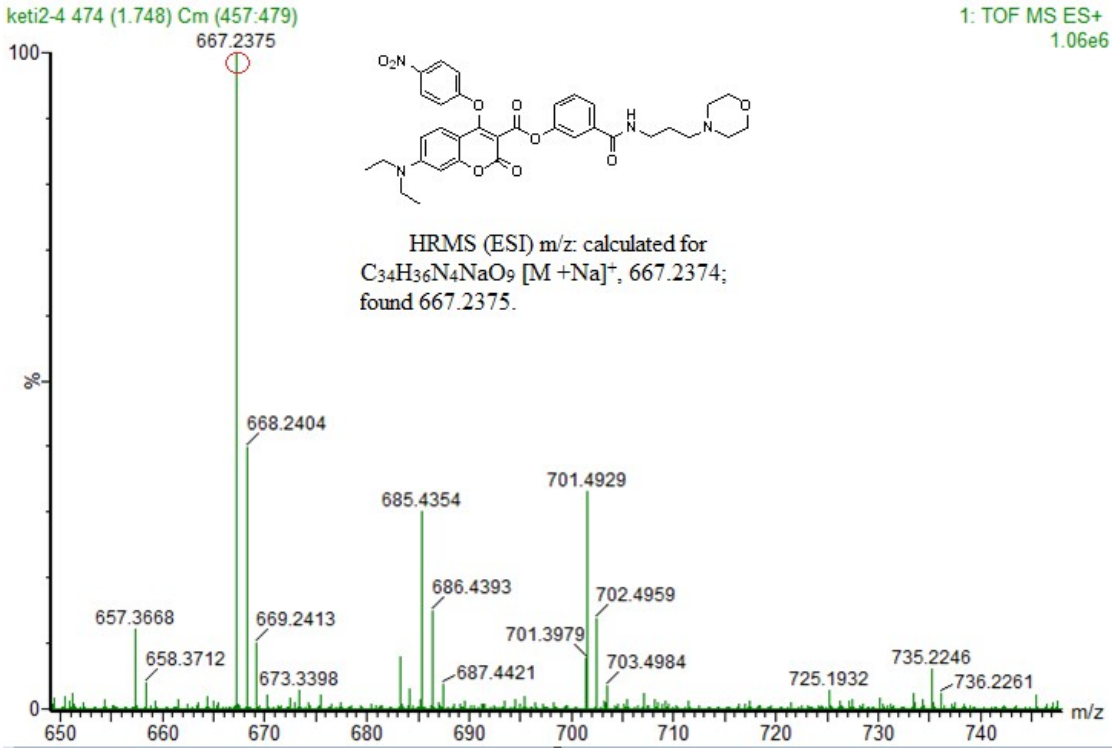


Figure S28. HRMS sprectrum of Lyso-AC.

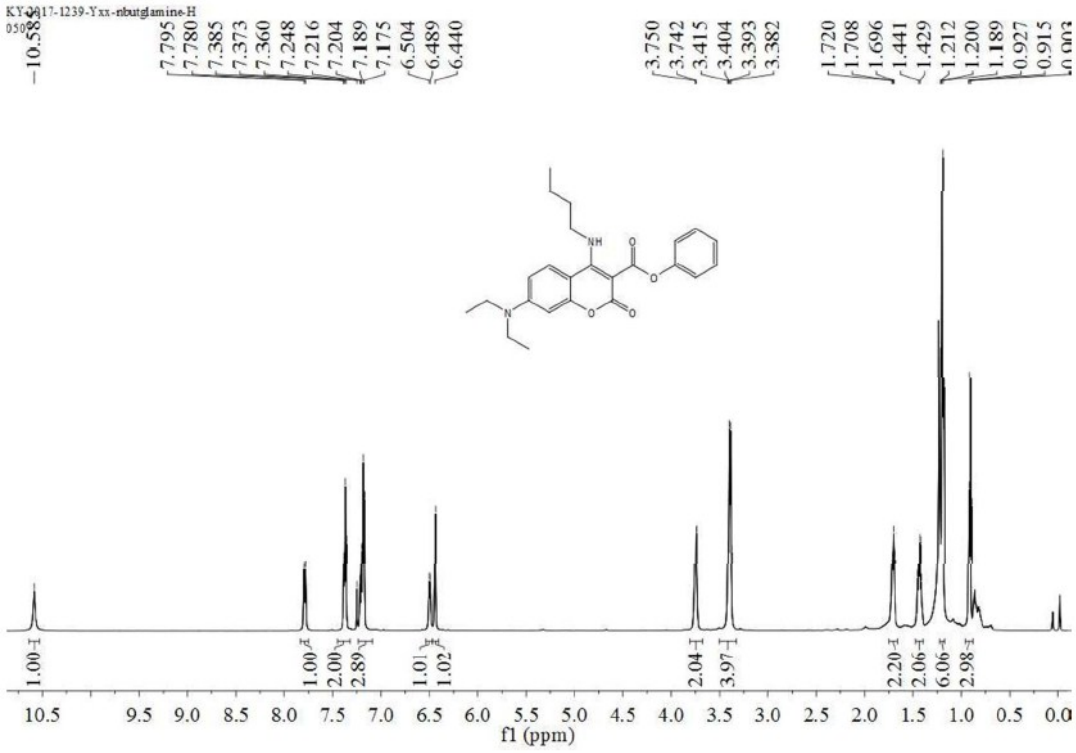


Figure S29. HNMR sprectrum of A1.

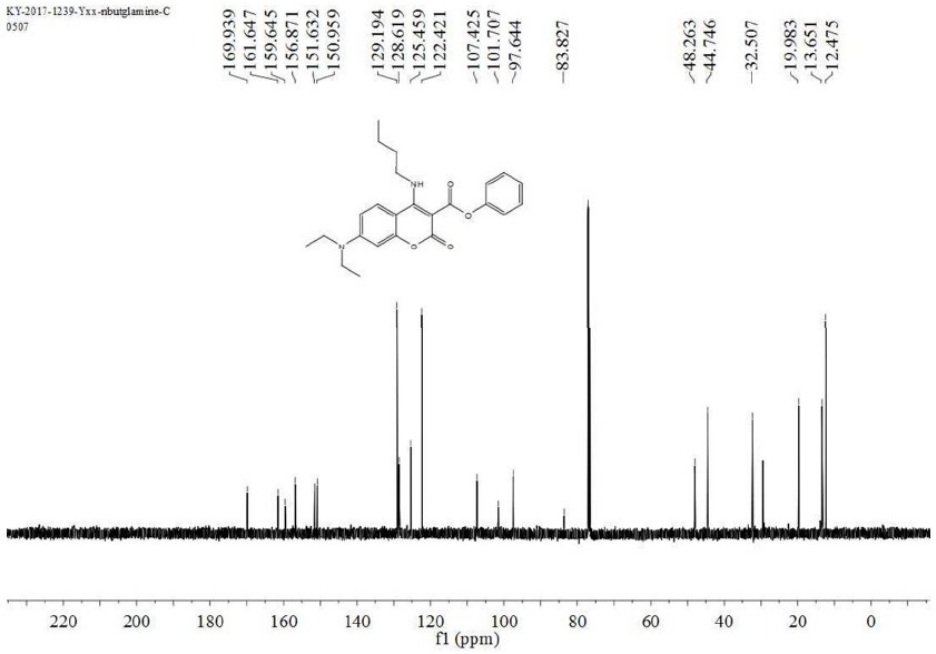


Figure S30. ^{13}CNMR sprectrum of A1.

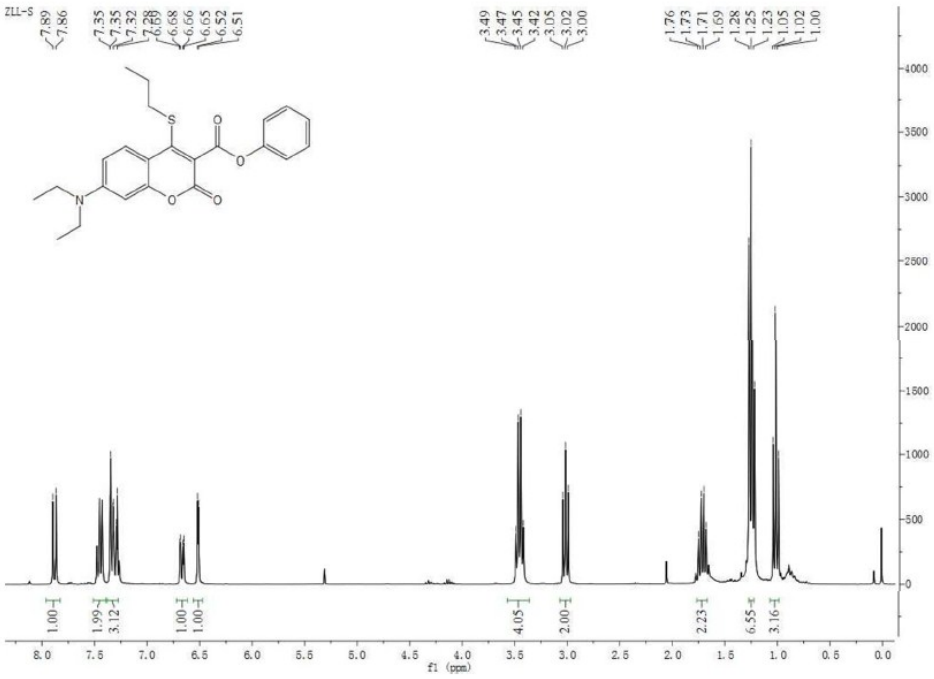


Figure S31. HNMR sprectrum of A2.

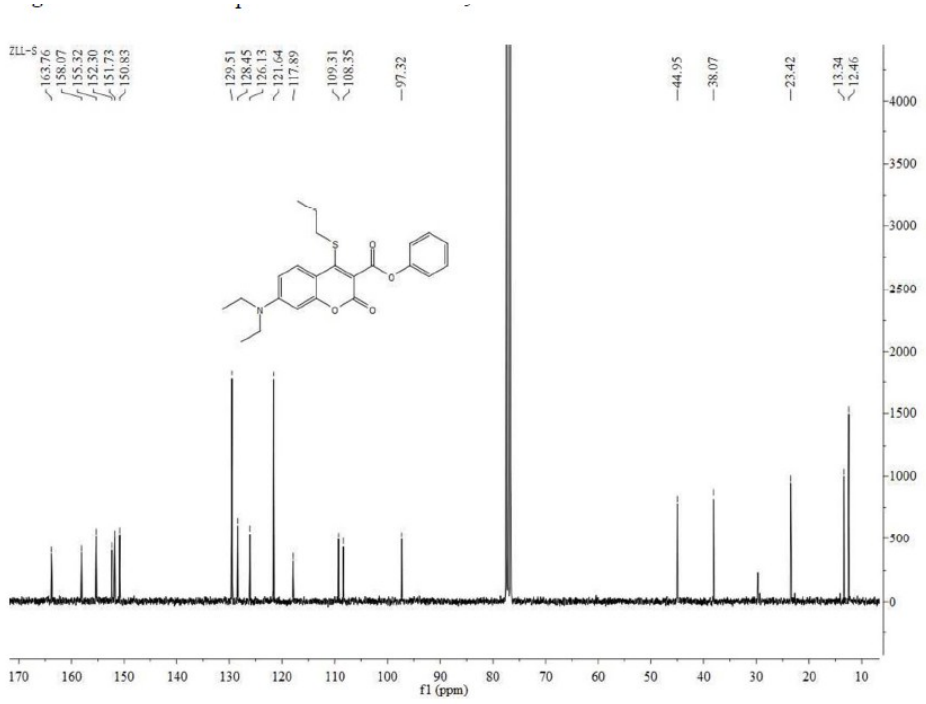


Figure S32. ^{13}CNMR sprectrum of A2.