

Electronic Supplementary Information (ESI)

for

Highly sensitive and selective detection of biothiols by a new low dose colorimetric and fluorescent probe

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1. The optical spectra of probe 1 and compound 2

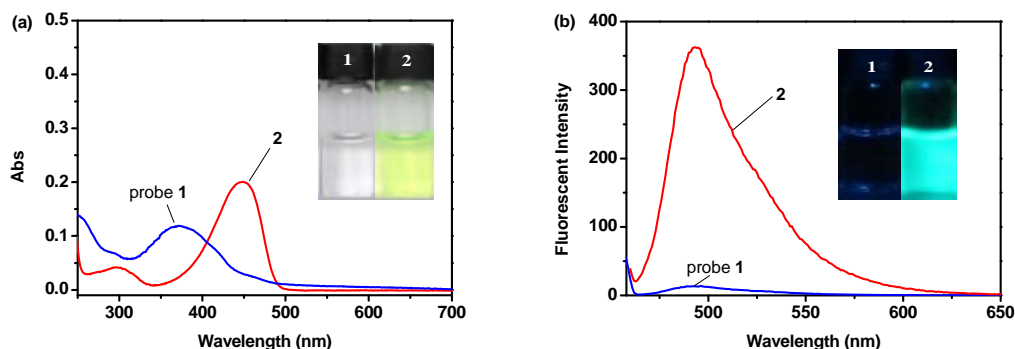


Fig. S1 (a) The UV-Vis spectra of probe **1** (5 μM) and compound **2** (5 μM) in PBS buffer (10 mM, pH 7.4, with 20% DMSO, v:v) at 37°C. (b) The fluorescence spectra of probe **1** (1 μM) and compound **2** (1 μM) in PBS buffer (10 mM, pH 7.4, with 20% DMSO, v:v) at 37°C. $\lambda_{\text{ex}} = 453 \text{ nm}$, slit width: $d_{\text{ex}} 2.5 \text{ nm}/d_{\text{em}} 5 \text{ nm}$. Color and emission color under a 365 nm light of **1** and **2** are inserted, respectively. The fluorescence quantum yield (Φ) for **1** and **2** under this experimental condition was determined to be 0.07 and 0.65, respectively, using rhodamine B as standard.

2. Data for investigation of the sensing mechanism

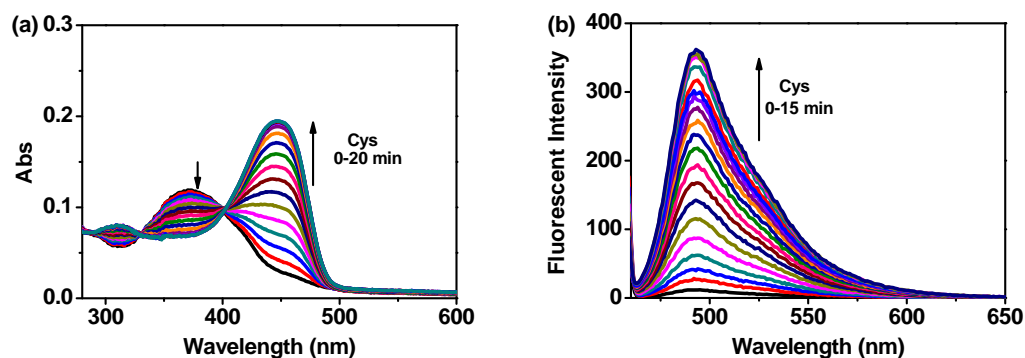


Fig. S2 (a) UV-vis absorption spectra changes of probe **1** (5 μM) against time (0-20 min) upon addition of 5 equiv of Cys. (b) Fluorescence spectra changes of probe **1** (1 μM) against time (0-15 min) upon addition of 5 equiv of Cys. Data were collected in PBS buffer (10 mM, pH 7.4, 20% DMSO, v/v) at 37°C. For fluorescence measurement, $\lambda_{\text{ex}} = 453 \text{ nm}$, slit: $d_{\text{ex}} 2.5 \text{ nm}/d_{\text{em}} 5 \text{ nm}$. These data showed the conversion process of probe **1** to compound **2** in the presence of Cys.

4. Interference experiments of probe 1 for detection of biothiols

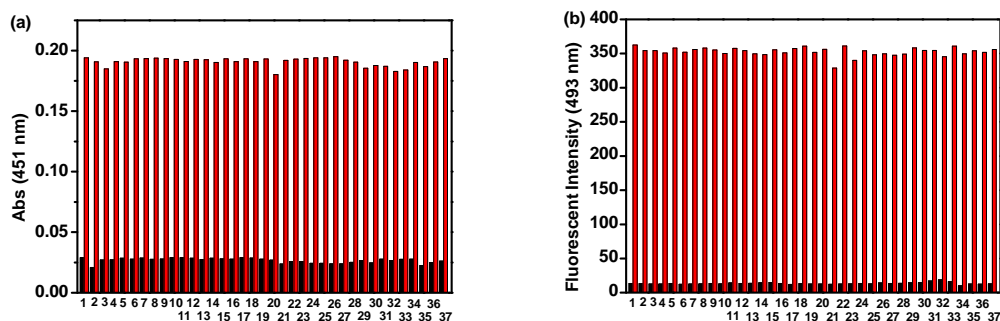


Fig. S5 (a) Absorbance responses of probe 1 (5 μ M) at 451 nm and (b) fluorescence responses of probe 1 (1 μ M) at 493 nm to GSH (50 μ M) in the presence of 100 μ M of various analytes (1. blank, 2. F^- , 3. Cl^- , 4. Br^- , 5. I^- , 6. ACO^- , 7. $C_2O_4^{2-}$, 8. NO_3^- , 9. NO_2^- , 10. PO_4^{3-} , 11. HPO_4^{2-} , 12. $H_2PO_4^-$, 13. S^{2-} , 14. HS^- , 15. $S_2O_4^{2-}$, 16. $S_2O_7^{2-}$, 17. IO_4^- , 18. ClO_4^- , 19. SCN^- , 20. Ala, 21. Glu, 22. Thr, 23. Trp, 24. Phe, 25. Gln, 26. Gly, 27. Lys, 28. Arg, 29. Ile, 30. Asp, 31. Leu, 32. Ser, 33. Met, 34. His, 35. $H_2NCH_2CH_2NH_2$, 36. $HOCH_2CH_2NH_2$, 37. $C_6H_5CH_2NH_2$). Black bars represent the blank and the addition of a single analyte (2-37). Red bars represent the subsequent addition of GSH (50 μ M) to the mixture. Each spectrum was collected after 15 min of addition of analyte in PBS buffer (10 mM, pH 7.4, 20% DMSO) at 37°C. For fluorescence measurement, λ_{ex} = 453 nm, slit: 2.5 nm/5 nm.

5. Kinetics of probe 1 with Cys, Hcy and GSH

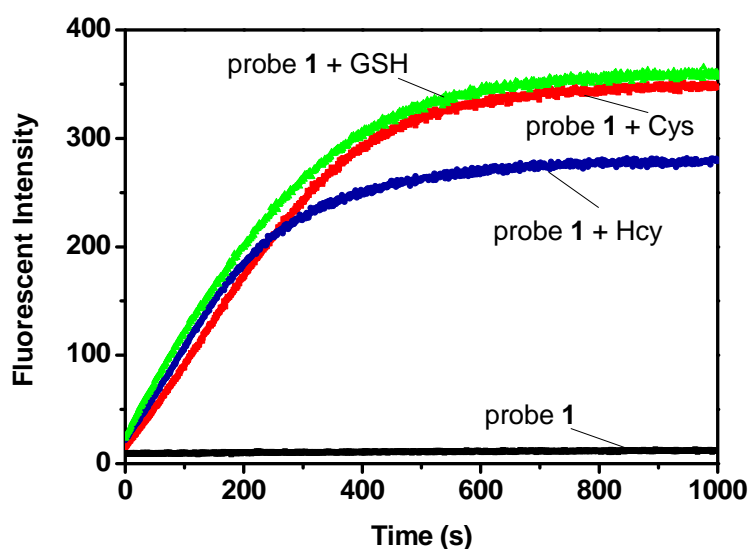


Fig. S6 Fluorescent kinetics of probe 1 (1 μ M) in the absence and presence of 5 μ M Cys, Hcy and GSH in DMSO-PBS buffer (10 mM, pH 7.4, 20% DMSO) at 37°C. The reactions are monitored at 493 nm with λ_{ex} = 453 nm, slit: 2.5 nm/5 nm.

6. Additional data

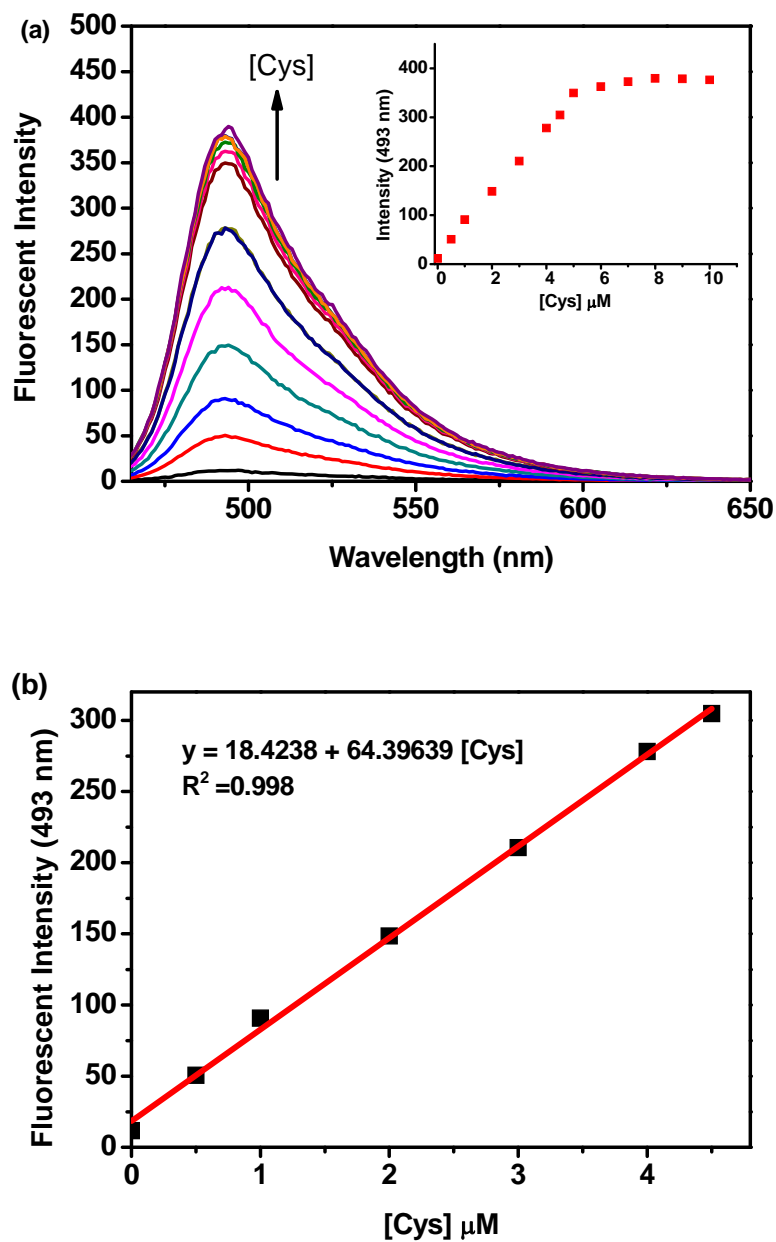


Fig. S7 (a) Fluorescence spectra changes of probe 1 (1 μM) in DMSO-PBS buffer (10 mM, pH 7.4, 20% DMSO) at 37°C upon addition of different concentrations of Cys (0–10 μM). Each spectrum was obtained 15 min after Cys addition. Insert: Fluorescence intensity changes at 493 nm as a function of [Cys]. $\lambda_{\text{ex}} = 453 \text{ nm}$, slit: 2.5 nm/5 nm. (b): Linear relationship of fluorescence intensity at 493 nm as a function of Cys concentration (0–4.5 μM).

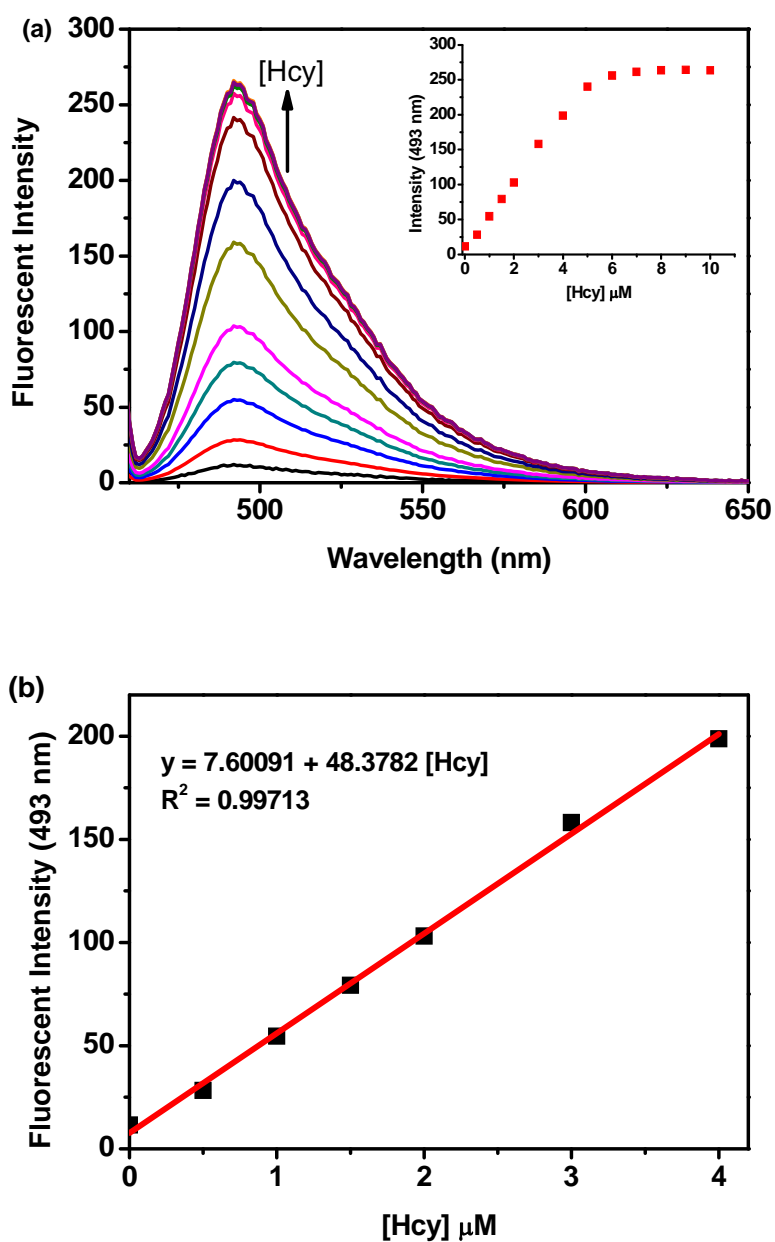


Fig. S8 (a) Fluorescence spectra changes of probe 1 (1 μM) in DMSO-PBS buffer (10 mM, pH 7.4, 20% DMSO) at 37°C upon addition of different concentrations of Hcy (0–10 μM). Each spectrum was obtained 15 min after Hcy addition. Insert: Fluorescence intensity changes at 493 nm as a function of [Hcy]. $\lambda_{\text{ex}} = 453$ nm, slit: 2.5 nm/5 nm. (b): Linear relationship of fluorescence intensity at 493 nm as a function of Hcy concentration (0–4 μM).

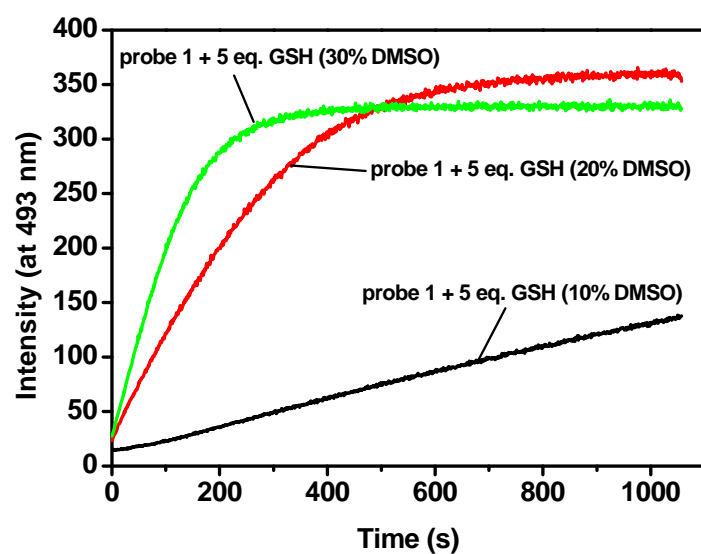


Fig. S9 The fluorescence kinetics of probe **1** (1 μM) upon addition 5 equiv of GSH in PBS buffer (10 mM, pH 7.4, with 10-30% DMSO, v:v) at 37°C. $\lambda_{\text{ex}} = 453 \text{ nm}$, slit width: $d_{\text{ex}} 2.5 \text{ nm}/d_{\text{em}} 5 \text{ nm}$.

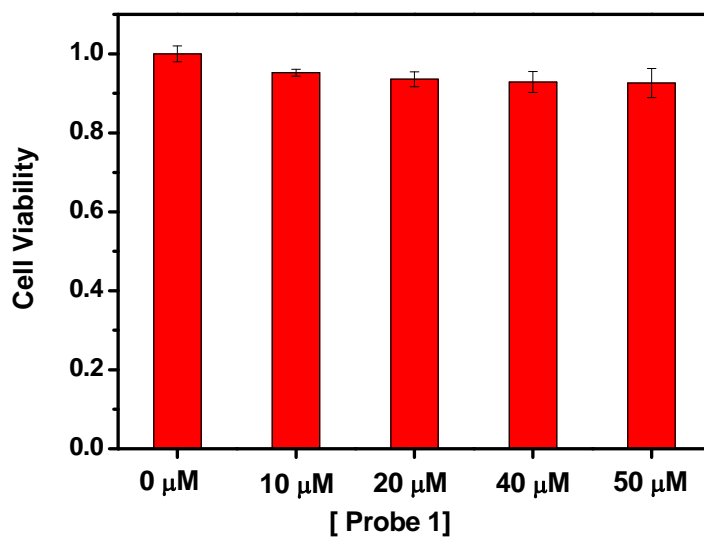
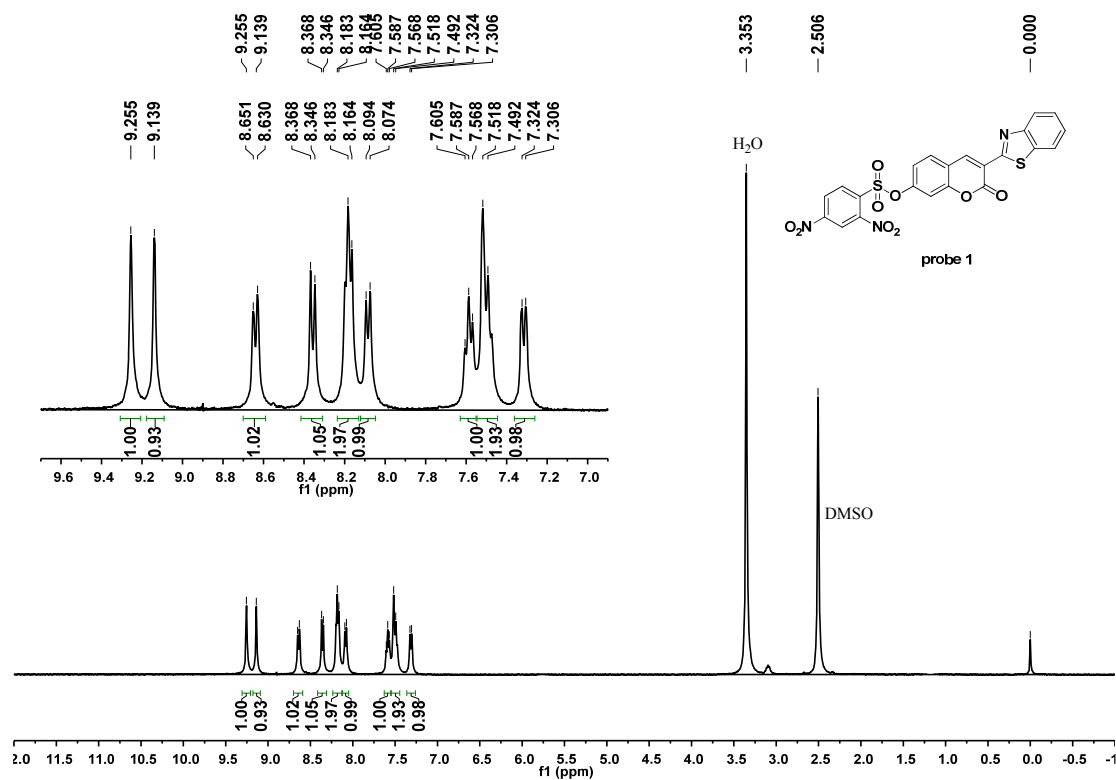
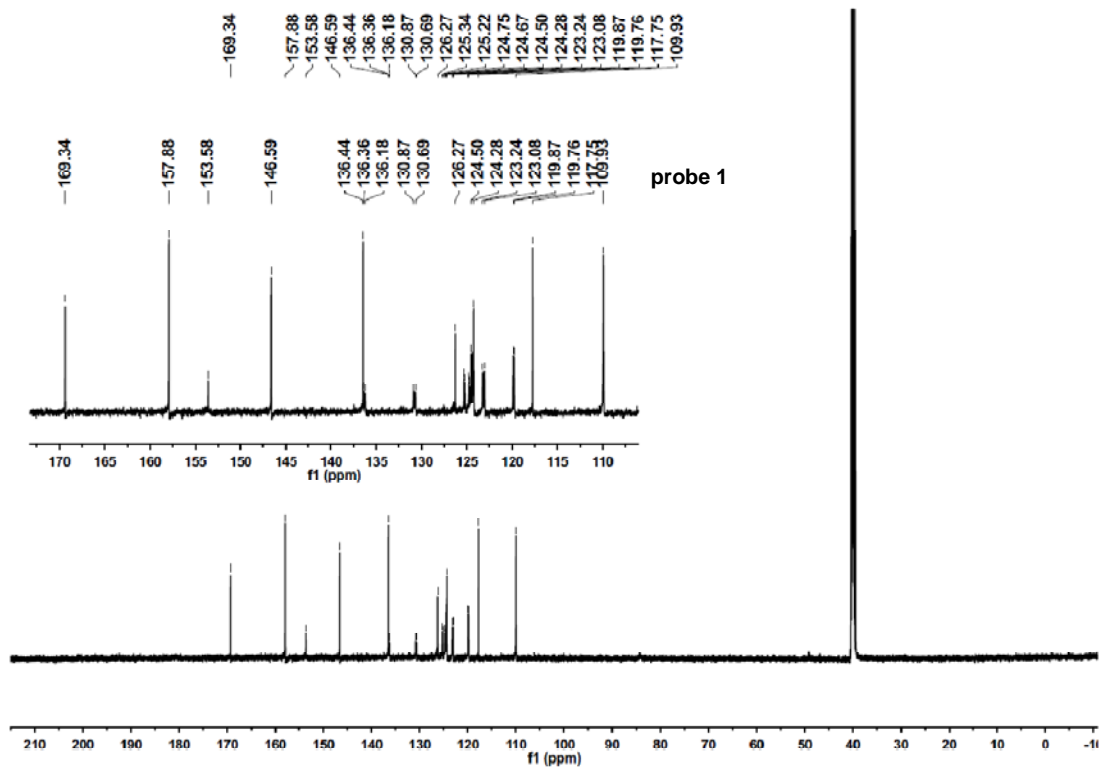


Fig. S10 Percentage of viable HeLa cells after treatment with indicated concentrations of probe **1** after 12 hours. The cell viability was observed via MTT assay.

7. Structure characterizations of probe 1

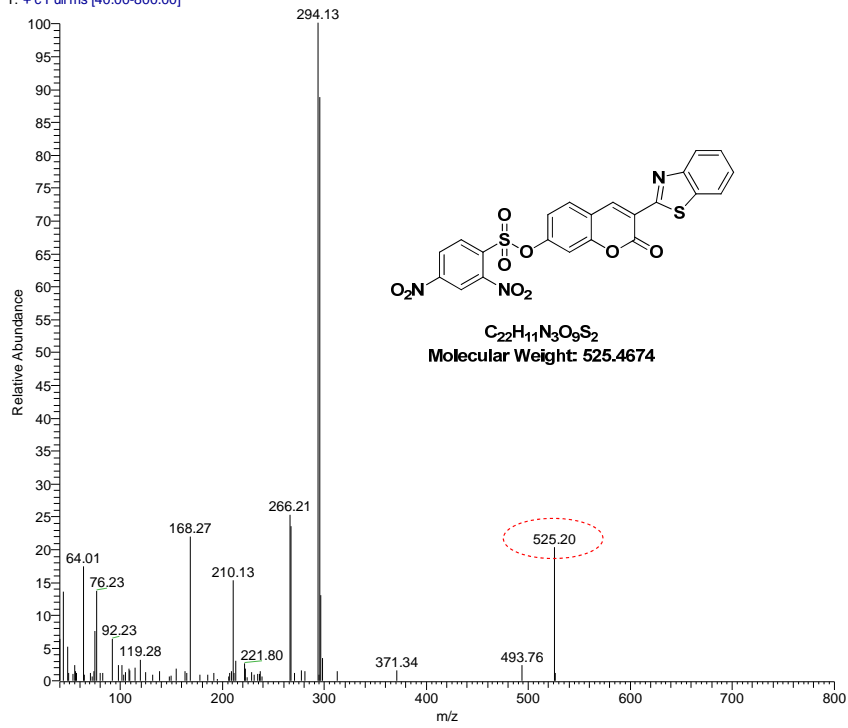


¹H-NMR spectrum of probe 1 in *d*₆-DMSO

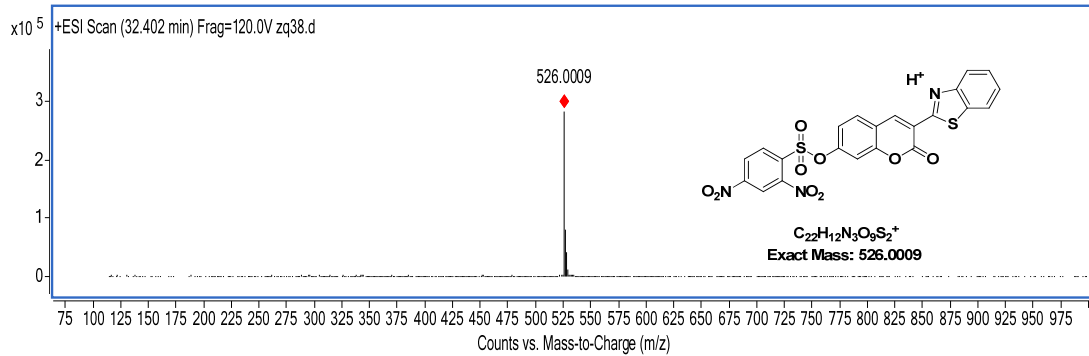


¹³C-NMR spectrum of probe 1 in *d*₆-DMSO

ZQ-38 #481 RT: 3.58 AV: 1 SB: 538 0.04-3.35, 3.62-4.26 NL: 2.13E4
T: + c Fullms [40.00-800.00]



MS (EI) spectrum of probe 1



HR-MS spectrum of probe 1