Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supporting Information

Rapid Detection of Thioredoxin Reductase with a fluorescence probe via Tag-Sec Method

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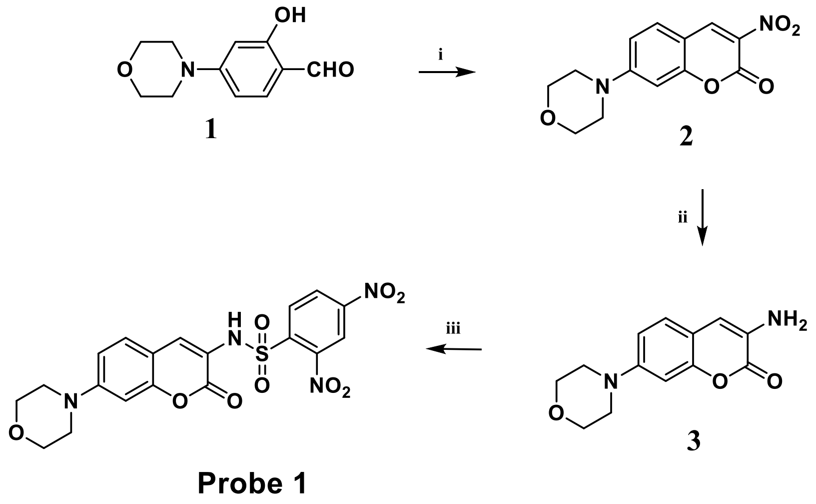
General information.

Unless otherwise noted, chemicals and solvents were purchased and used without further purification. Purification of products was conducted by column chromatography on silica gel (200-300 mesh). NMR spectra were measured on a Bruker ARX400 (^{1}H at 400 MHz, ^{13}C at 101 MHz) magnetic resonance spectrometer. Chemical shifts (δ) are reported in ppm using tetramethylsilane as internal standard (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, m = multiplet), and coupling constants (*J*) were reported in Hertz (Hz). HRMS data were obtained on a VG ZAB-HS mass spectrometer, Brucker Apex IV FTMS spectrometer. UV-visible spectra were measured on a Quawell UV/Vis spectrophotometer (Q5000) by using droplet measurement with a sample volume of 10 μL. Fluorescence spectra were acquired on a HITACHI F-4600 fluorescence spectrophotometer using fluorescence cuvettes (Fisher Scientific) with a sample volume of 1.5 mL. The confocal fluorescence images were taken with a Leica TCS SP8 fluorescence microscope. Proteins and enzymes were purchased from Gene Company (Shanghai) Co., Ltd and stored at 4 °C beforehand. The human cells HEK293 were obtained from ATCC.

Sequence of TrxR:

MNDSKDAPKSYDFDLIIIGGGSGGLAAAKEAAKFDKKVMVLDFVTPTPLGTR
WGLGGTCVNVGCIPKKLMHQAALLGQALKDSRNYGWKLEDTVKHDWEKM
TESVQNHIGSLNWGYRVALREKKVVYENAYGKFIGPHKIMATNNKGKEKVYS
AERFLIATGERPRYLGIPGDKEYCISSDDLFSLPYCPGKTLVVGASYVALECAG
FLAGIGLDVTVMVRSILLRGFDQDMANKIGEHMEEHGIKFIRQFVPTKIEQIEA
GTPGRLKVTAKSTNSEETIEDEFNTVLLAVGRDSCTRTIGLETVGVKINEKTGK
IPVTDEEQTNVPYIYAIGDILEGKLELTPVAIQAGRLLAQRLYGGSTVKCDYDN
VPTTVFTPLEYGCCGLSEEKAVEKFGEENIEVYHSFFWPLEWTVPSRDNNKCY
AKVICNLKDNERVVGFHVLGPNAGEVTQGFAAALKCGLTKQQLDSTIGIHPV
CAEIFTTLSVTKRSGGDILQSGCUG

Experimental section



Scheme 1. Synthesis route to **probe 1**. (i) ethyl nitroacetate, n-BuOH, reflux; (ii) SnCl$_2$, 30 % HCl, r.t.; (iii) 2,4-dinitrobenzene-1-sulfonyl chloride, Et$_3$N, CH$_2$Cl$_2$, r.t.

Synthesis route was following previously reported literatures [1-4].

7-morpholino-3-nitro-2H-chromen-2-one (**2**)

2-hydroxy-4-morpholinobenzaldehyde (**1**) (2.34 g, 10.0 mmol), ethyl nitroacetate (1.33 g, 10.0 mmol), 0.1 mL piperidine and 0.2 mL acetate acid were dissolved in 20 mL absolute ethanol. Then, the mixture was refluxed at 78 °C under nitrogen overnight. After cooling to room temperature, the bright orange precipitate was extract with DCM (100 mL X 3) and further purified by silica gel column chromatography to afford compound **3.** (0.99 g, 3.29 mmol). Yield: 32.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (s, 1H), 7.79 (d, J = 9.1 Hz, 1H), 7.13 (dd, J = 9.1, 2.4 Hz, 1H), 6.91 (d, J = 2.3 Hz, 1H), 3.71 (t, J = 4.8 Hz, 4H), 3.54 (t, J = 4.9 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.51, 156.87, 153.28, 144.65, 133.52, 128.19, 112.55, 107.75, 98.45, 66.16, 47.01. HRMS (ESI): calcd for $C_{13}H_{13}N_2O_5^+$ [M+H]$^+$: 277.0819; found: 277.0818.

3-amino-7-morpholino-2H-chromen-2-one (**3**)

A suspension containing 30 % HCl (15 mL) and stannous chloride dihydrate (0.76 g, 4.0 mmol) was stirred at room temperature under nitrogen. Then, 3 (0.303 g, 1.0 mmol) was added in small portion carefully in an ice bath. Stirring was continued for 12 h under nitrogen to avoid oxidization before the solution was poured onto ice and made alkaline using sodium hydroxide solution (5 M) in an ice bath. The resulting suspension was then extracted with DCM. The organic layer was dried over anhydrous sodium sulfate and concentrated. Gel column chromatography to afford a pale-yellow solid of 0.222 g as 3-amino-7-(4-ethylpiperazin-1-yl)-2H-chromen-2-one (**3**) was obtained (yield 81.2 %).^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 (d, *J* = 8.6 Hz, 1H), 6.80 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.74 (d, *J* = 2.4 Hz, 1H), 6.67 (s, 1H), 4.02 (s, 2H), 3.88 – 3.84 (m, 4H), 3.21 – 3.17 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 159.89, 150.61, 150.53, 129.42, 125.67, 113.35, 112.68, 112.53, 102.06, 66.66, 48.76. HRMS (ESI): calcd for $C_{13}H_{15}N_2O_3^+$ $[M+H]^+$: 247.1077; found: 247.1073.

Synthesis of **Probe 1**.

Compound **3** (50 mg, 0.156 mmol) and Et_3N (16 mg, 0.156 mmol) were dissolved in 5 mL of anhydrous DCM. Then, 2,4-Dinitrobenzenesulfenyl chloride (50 mg, 0.19 mmol) in 6 mL of anhydrous DCM was slowly added to the solution of compound **3**. The mixture was stirred overnight under argon at room temperature. After the completion of reaction, the mixture was wash in turn with 30 mL distilled water and brine. Then, the organic phase was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and further purified by column chromatography to give a dark red solid (37 mg, 50.2 %).^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1H), 8.85 (d, *J* = 2.3 Hz, 1H), 8.63 (dd, *J* = 8.8, 2.3 Hz, 1H), 8.33 (d, *J* = 8.7 Hz, 1H), 7.86 (s, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 7.00 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.81 (s, 1H), 3.76 – 3.67 (m, 4H), 3.30 (t, *J* = 4.9 Hz, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.45, 154.76, 154.13, 150.31, 147.86, 139.65, 138.27, 132.59, 129.93, 127.47, 120.21, 116.90, 112.07, 110.12, 100.05, 66.23, 47.39. HRMS (ESI): calcd for $C_{19}H_{17}N_4O_9S^+$ $[M+H]^+$: 477.0711; found: 477.0700.

References:

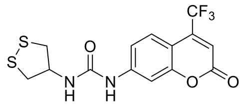
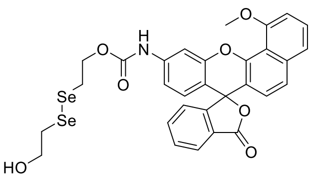
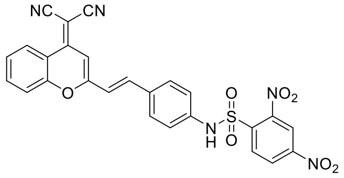
(1) Yi, L.; Li, H.; Sun, L.; Liu, L.; Zhang, C.; Xi, Z. A highly sensitive fluorescence probe for fast thiol-quantification assay of glutathione reductase. *Angewandte Chemie International Edition* **2009**, *48*, 4034-4037.

(2) Chaurasia, C. S.; Kauffman, J. M. Synthesis and fluorescent properties of a new photostable thiol reagent “BACM”. *Journal of heterocyclic chemistry* **1990**, *27*, 727-733.

(3) Li, W.; Sun, W.; Yu, X.; Du, L.; Li, M. Coumarin-based fluorescent probes for H 2 S detection. *Journal of fluorescence* **2013**, *23*, 181-186.

(4) Chen, Z.; Sun, Q.; Yao, Y.; Fan, X.; Zhang, W.; Qian, J. Highly sensitive detection of cysteine over glutathione and homo-cysteine: new insight into the Michael addition of mercapto group to maleimide. *Biosensors and Bioelectronics* **2017**, *91*, 553-559.

Table S1. Comparison with several related probes

Probe structure	Detecting target	Quntumn yield
	TrxR	0.40
	TrxR	NA
	Sec	0.66
This work	TrxR & Sec	0.32

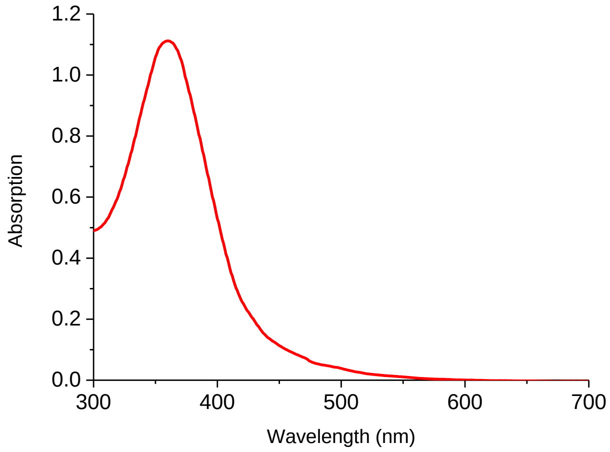


Figure S1. Normalized absorption spectrum of **probe 1**.

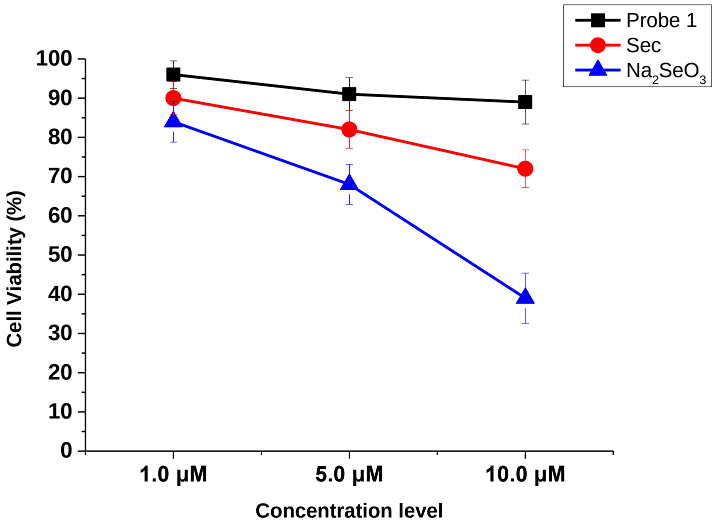


Figure S2. Cell viability study of **probe 1** by applying MTT assay.

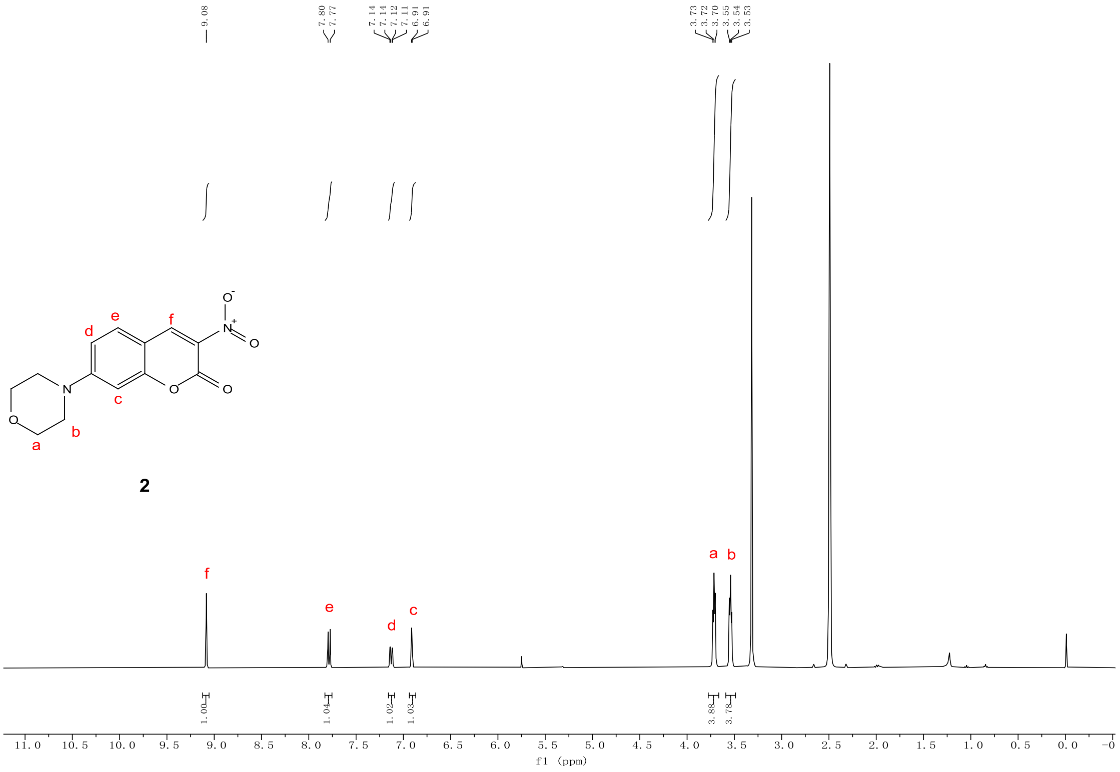


Figure S3. Proton NMR of compound **2**

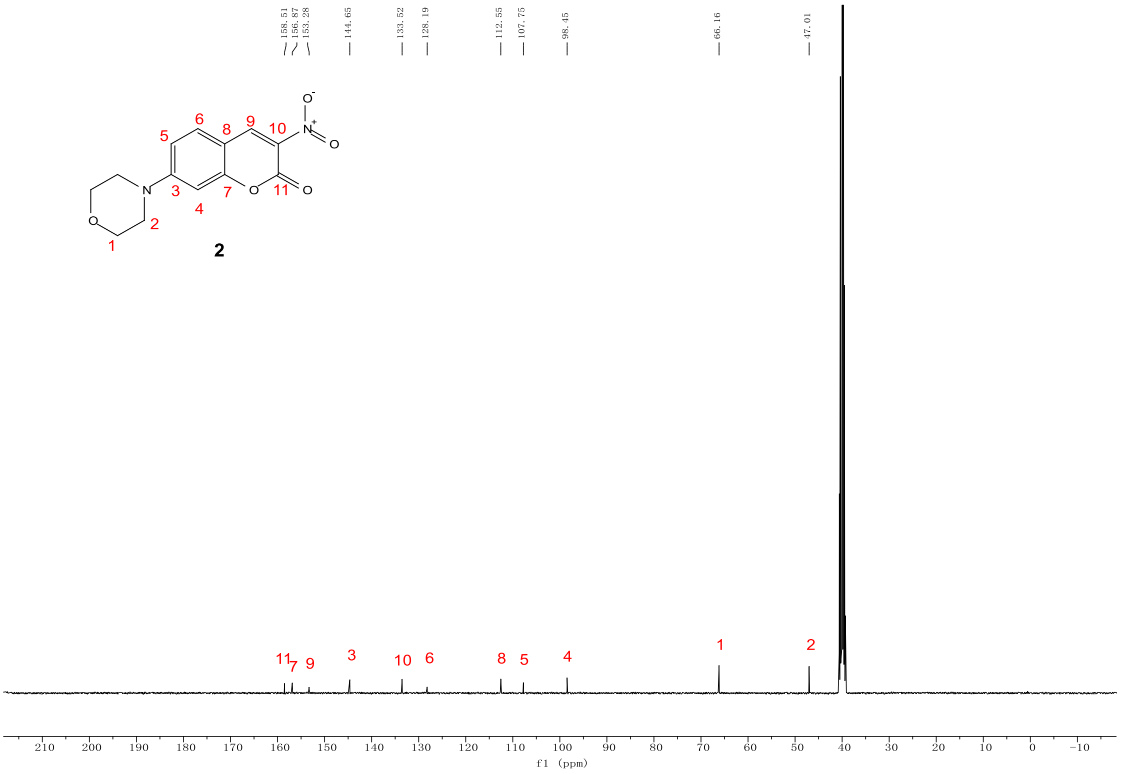


Figure S4. Carbon NMR of compound **2**

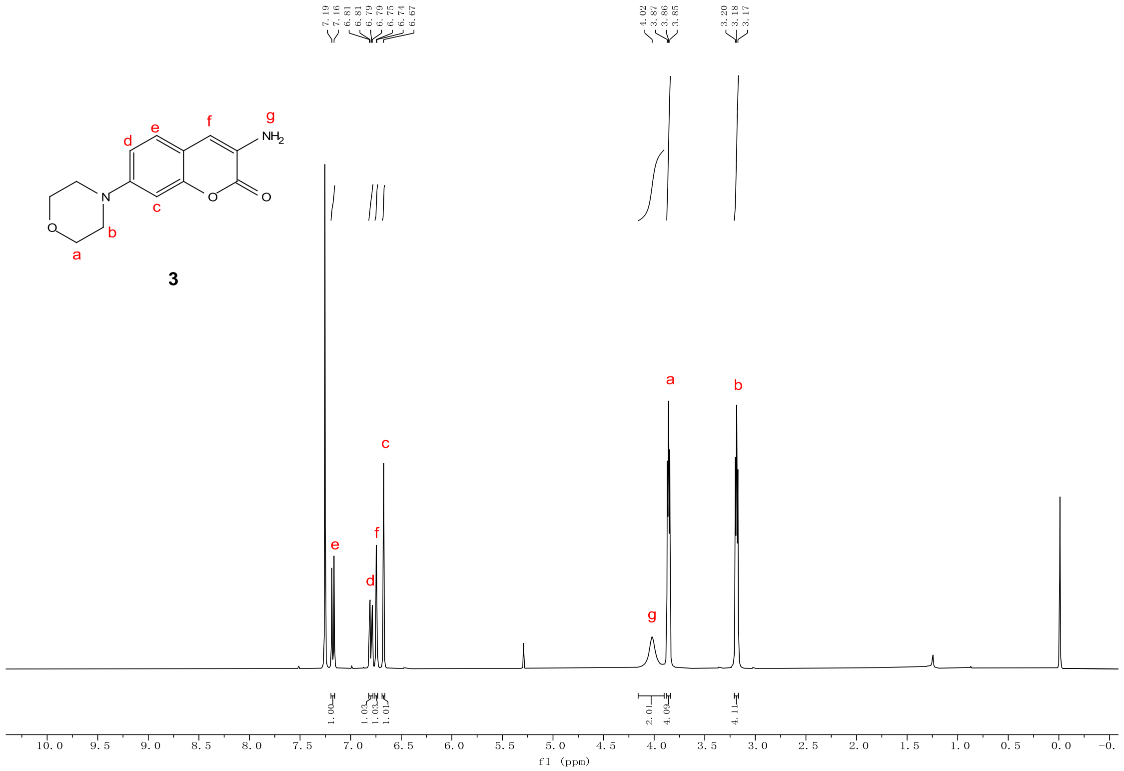


Figure S5. Proton NMR of compound **3**

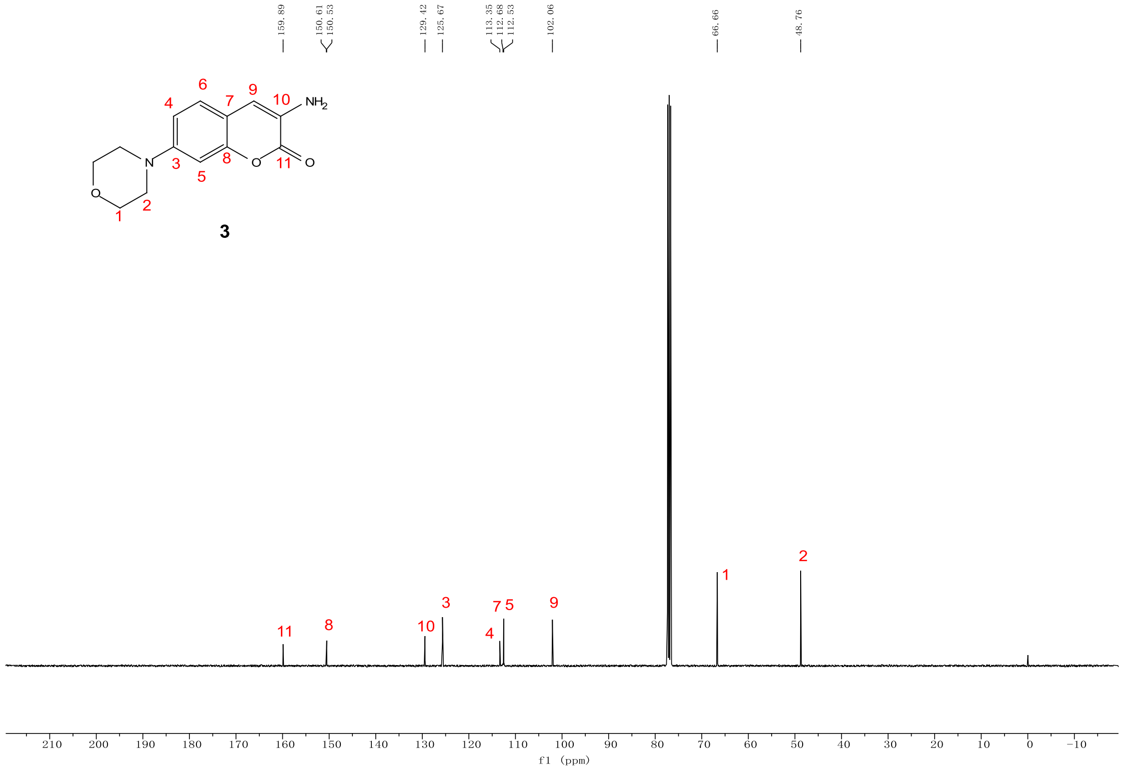


Figure S6. Carbon NMR of compound **3**

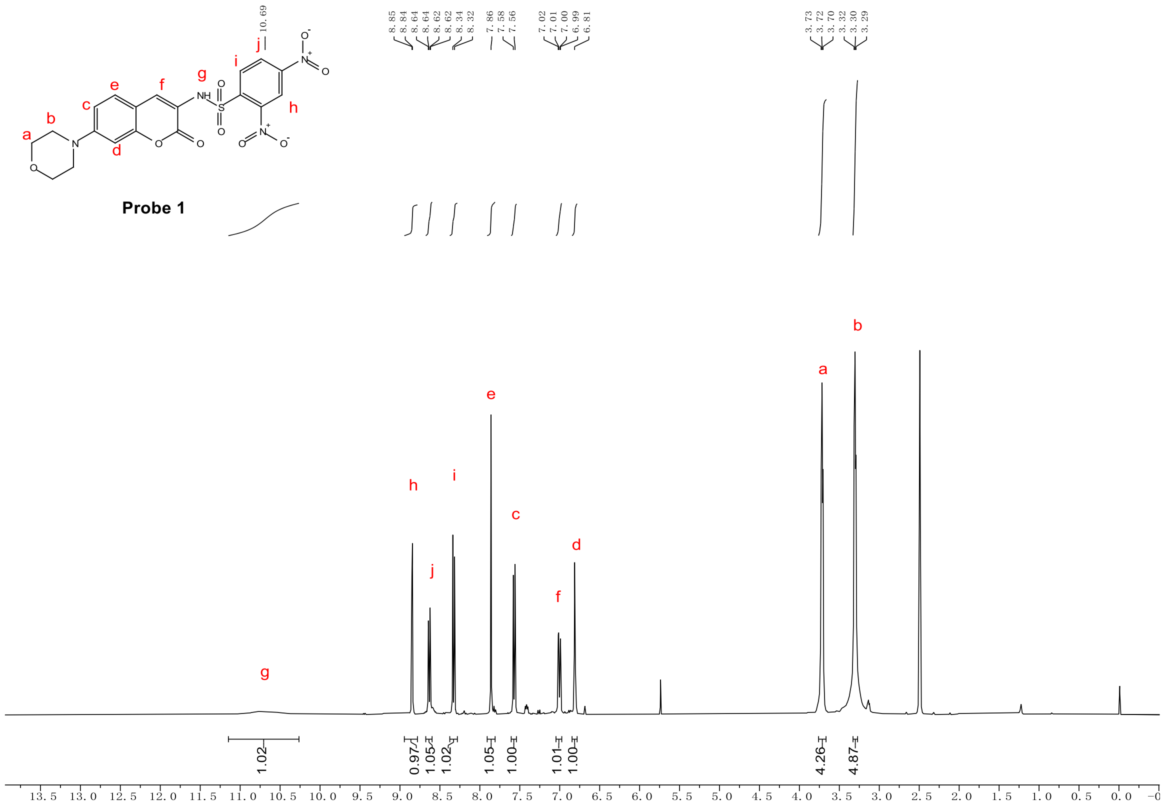


Figure S7. Proton NMR of compound **Probe 1.**

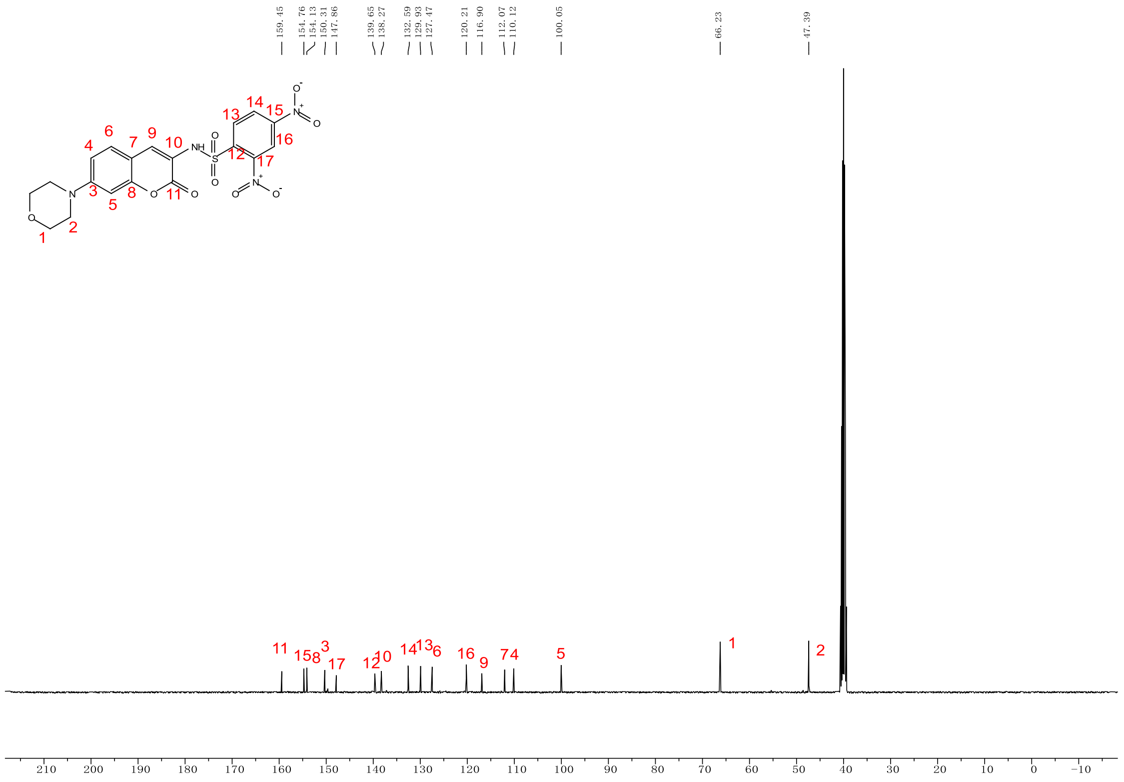


Figure S8. Carbon NMR of compound **Probe 1**.

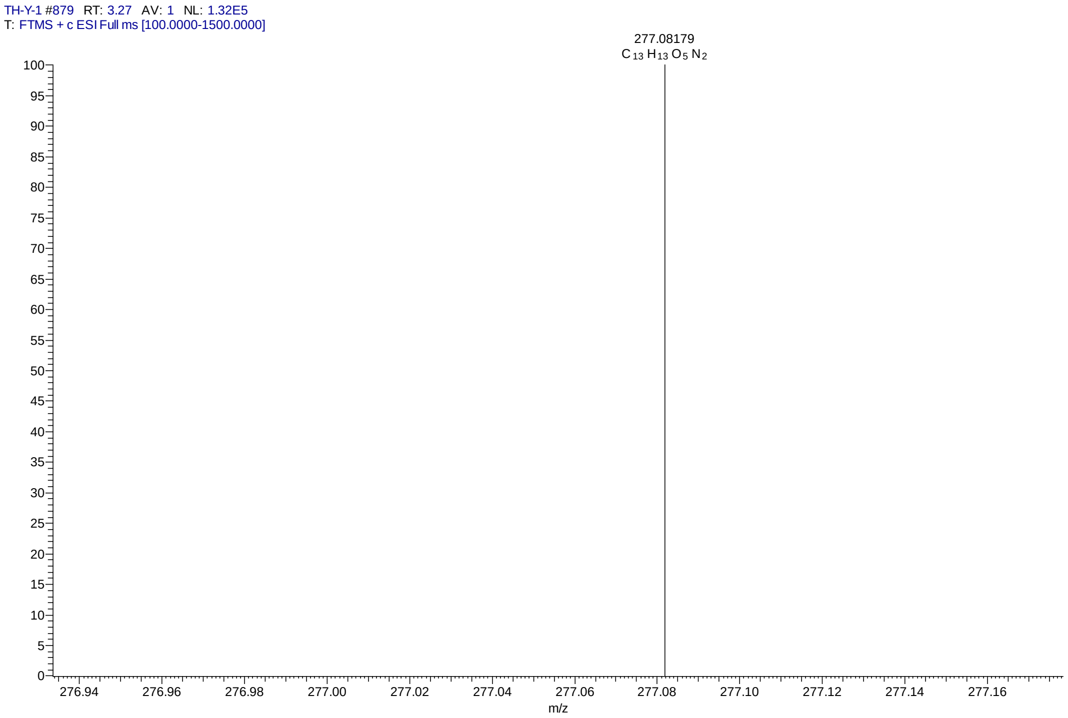


Figure S9. HRMS spectra of compound **2**.

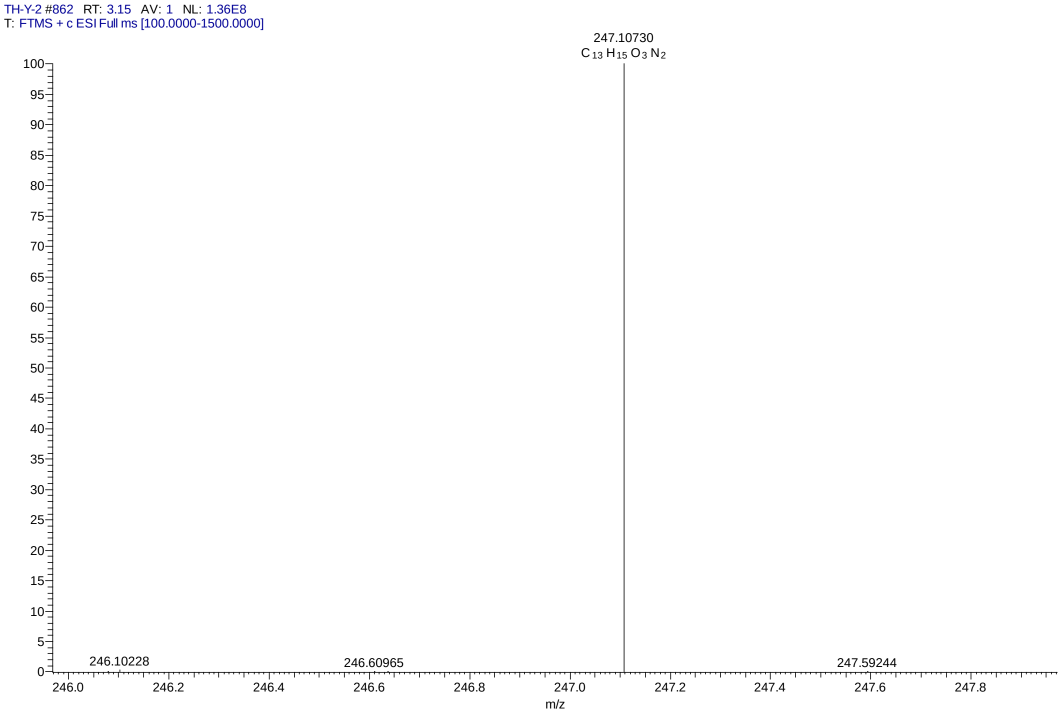


Figure S10. HRMS spectra of compound **3**.

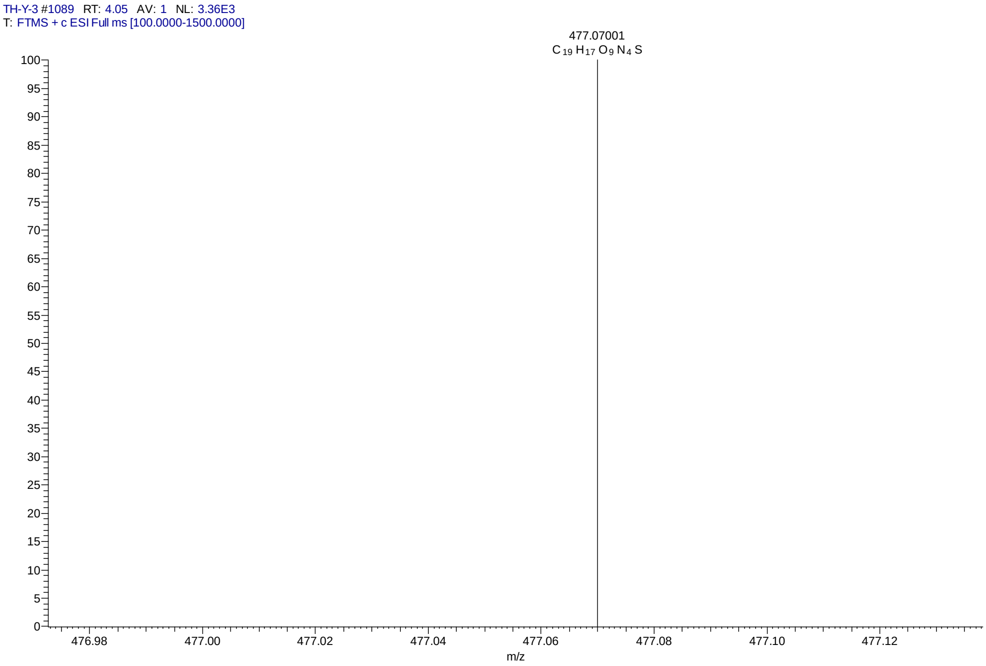


Figure S11. HRMS spectra of compound **Probe 1**.