Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Mutual correlation evaluation of Cys and Hcy in serum through reaction activity regulated fluorescence quantification

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1. Experimental section

Materials

Immobilized TCEP Disulfide Reducing Gel was purchased from ThermoFisher. All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. The human serum samples were obtained from Second Hospital of Shanxi Medical University. TLC analysis was performed using precoated silica plates. Hitachi F−7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Hitachi U-3900 UV-vis spectrophotometer was employed to measure UV-vis spectra. Shanhai Huamei Experiment Instrument Plants provided a PO-120 quartz cuvette (10 mm). ^{1}H NMR and ^{13}C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. HR-MS determinations were carried out on a Thermo Scientific Q Exactive Instrument.

Synthesis

Synthesis of **2-IC**. 3-acetyl-7-diethylaminocoumarin (1 mmol, 0.259 g) and 1H-imidazole-2-carbaldehyde (1 mmol, 0.096 g) were dissolved in 20 mL EtOH, 3 drops of piperidine was added. The mixture was heated under reflux for 6h and the solvent was removed under

reduced pressure. The crude product was purified by column chromatography using EA:PE (1/3) as eluent. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.56 (s, 1H), 8.54 (s, 1H), 7.83 (d, J = 14.9 Hz, 1H), 7.79 (s, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.61 (d, J = 15.4 Hz, 1H), 7.59 (s, 1H), 6.79 (dd, J = 9.0, 2.2 Hz, 1H), 6.59 (d, J = 1.9 Hz, 1H), 3.49 (q, J = 7.0 Hz, 4H), 1.14 (t, J = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 185.57, 159.86, 158.11, 152.80, 148.08, 132.21, 121.13, 115.96, 110.10, 107.90, 95.91, 44.46, 12.40. HR-MS [probe]+: m/z Calcd 338.1499, Found 338.1501.

Synthesis of **4-IC**. **4-IC** was synthesized through similar procedure with **2-IC**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.79 (s, 1H), 8.59 (s, 1H), 8.01 (d, J = 15.7 Hz, 1H), 7.70 (d, J = 9.0 Hz, 1H), 7.46 (d, J = 15.7 Hz, 1H), 7.26 (d, J = 126.6 Hz, 2H), 6.82 (d, J = 9.1 Hz, 1H), 6.62 (s, 1H), 3.51 (q, J = 7.0 Hz, 4H), 1.15 (t, J = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 185.78, 160.34, 158.73, 153.51, 148.94, 144.05, 132.89, 130.52, 124.89, 115.78, 110.72, 108.41, 96.39, 44.95, 12.84. HR-MS [probe]+: m/z Calcd 338.1499, Found 338.1500.

Preparation of solutions of probes and analytes

Stock solution of **2-IC** (2 mM) were prepared in DMSO. Stock solutions of 20 mM Cys, Hcy, 200 mM GSH and other amino acids were prepared by direct dissolution in deionized water. All chemicals used were of analytical grade.

General fluorescence spectra measurements

The detection experiments were measured in PBS (pH 7.4, 10 mM) and PB (pH 8.0, 200 mM). The procedure was as follows: into a PBS solution, containing 10 μM **2-IC**, an analyte sample was added. The process was monitored by fluorescence spectrometer.

Quantificational study

The fluorescent spectrum was analyzed by the FL solutions 4.0 software to obtain the peak area from 470 nm to 550 nm. The working curve was fitted based on the peak area data.

Detection of thiols in human serum by our proposed strategy

Human serum samples were obtained and stored at −20 °C until further analysis. After being thawed at room temperature, 200 μL of human serum was transferred to a 2 mL centrifuge tube and 100 μL immobilized TCEP slurry was added. The mixture was vortexed and incubated for 30 min at 25 °C. Then, 1 mL MeOH was added and vortexed for 2 min. After

that, the tube was centrifuged at 8000 r/min for 3 min. The supernatant (1 mL) was collected and dried under reduced pressure.

The obtained powder was further dissolved in 50 μL deionized water, 50 μL immobilized TCEP slurry was added. The mixture was vortexed for 10 min at 25 °C and further centrifuged at 8000 r/min for 2 min. 20 μL supernatant was added in the detection system (380 μL PBS or PB with 2 μL **2-IC**) and incubated for 4 min (in PBS) or 10 min (in PB) at 25 °C before testing on the fluorescence spectrometer.

The actual concentrations of thiols in serum (c_a) can be calculated according to the following equation:

$$c_a = 13\,c$$

c represents the final concentrations in the detection system calculated the equations a and b.

Software

FL solutions 4.0; Origin Pro 9.0.

2. Additional figures and tables

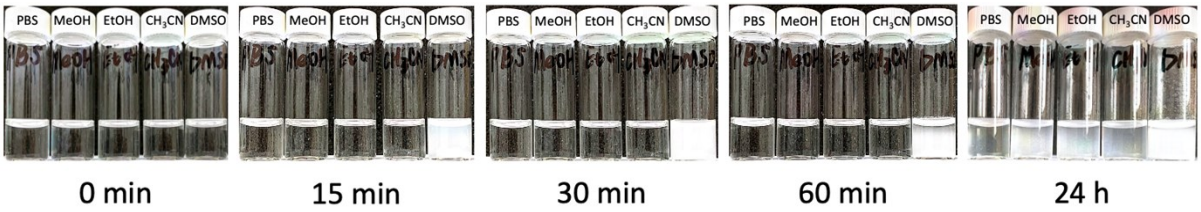


Fig. S1. Time dependent conversion of cysteine (100 mM) to cystine in various solutions (20% organic solvents in PBS) at 25 °C.

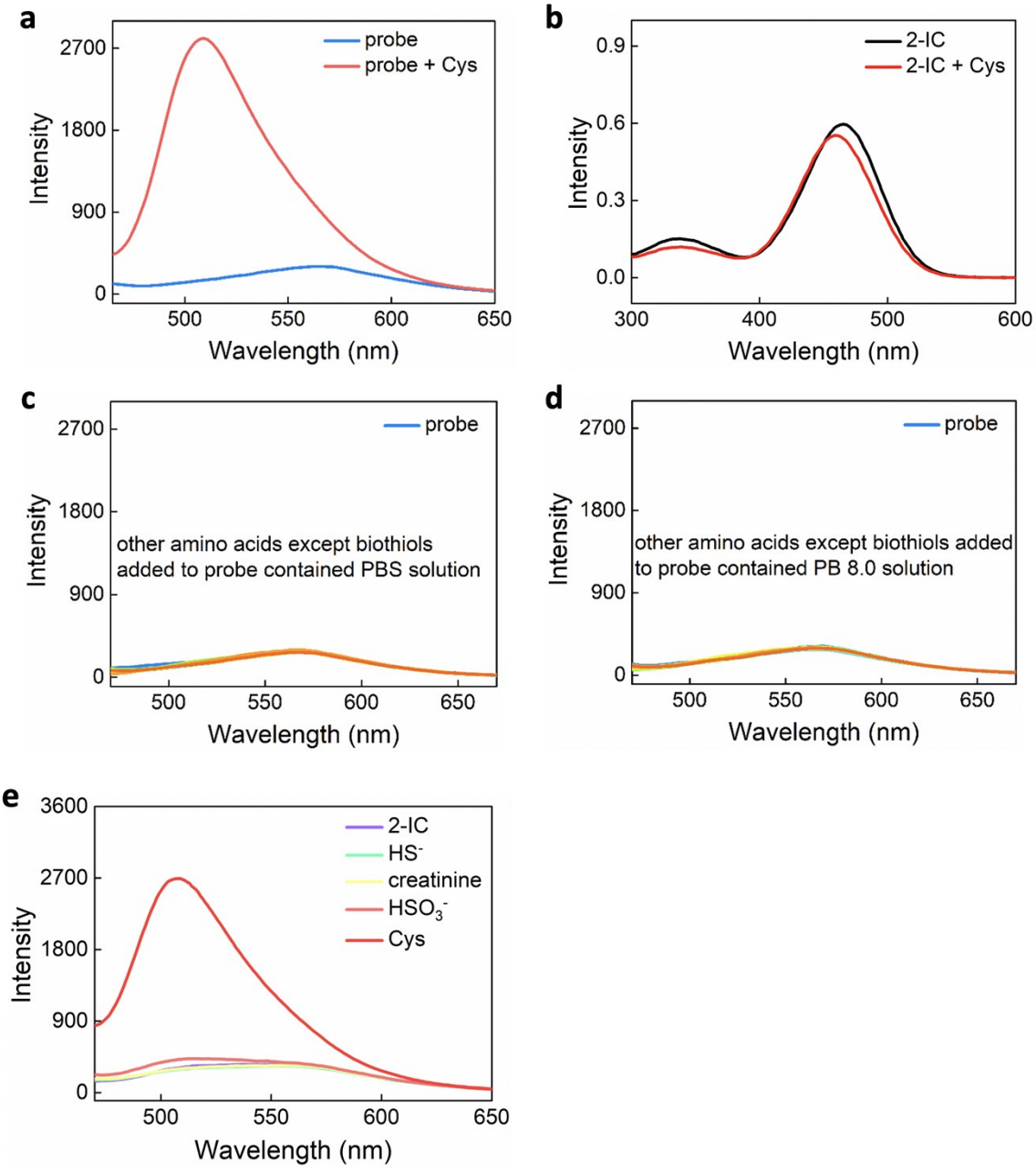


Fig. S2. a) Fluorescent response of 10 μM **2-IC** toward 200 μM Cys in PBS and the corresponding UV-vis spectra (b). Fluorescent responses of 10 μM **2-IC** toward 2 mM various

amino acids including Ala, Arg, Gln, Ile, Thr, Leu, Gly, Glu, Met, Ser, Asp, Tyr, His, Lys, Trp, Asn and Val in PBS (c) and PB (d) solutions. Fluorescent responses of 10 μM **2-IC** toward 100 μM HS^-, HSO_3^-, creatinine and 200 μM Cys in PBS (e). λ_{ex} = 452 nm; slit, 5 nm/5 nm.

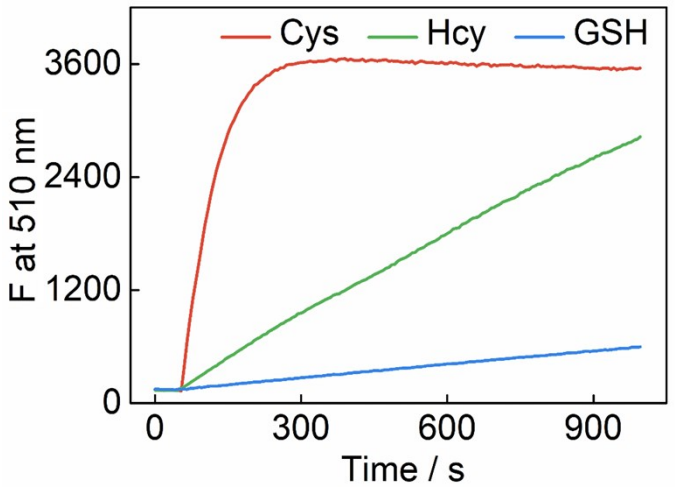


Fig. S3. Time dependent fluorescent responses of 10 μM **4-IC** toward 200 μM Cys, 200 μM Hcy and 2 mM GSH in PBS. λ_{ex} = 453 nm; slit, 5 nm/5 nm.

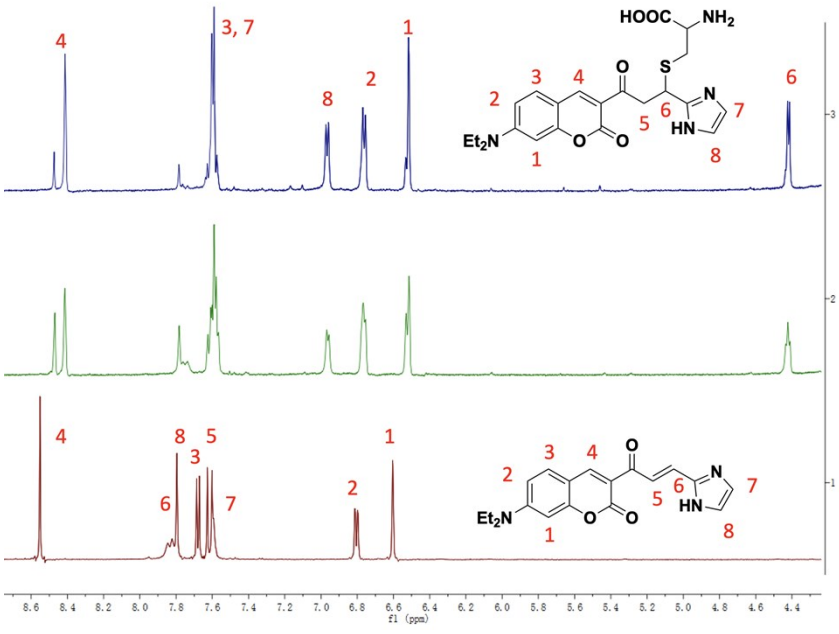


Fig. S4. ^{1}H NMR titration experiment upon addition of Cys (D_2O solution) to the **2-IC** containing DMSO-d_6 solution. Cys addition induced decrease of the original signal at 7.82 (proton 6 in **2-IC**) and appearance of a new signal at 4.41 (proton 6 in **2-IC-Cys**). The results demonstrated

that the nucleophilic addition reaction process between the thiols and the α, β-unsaturated ketone moiety of **2-IC** supported thiols detection.

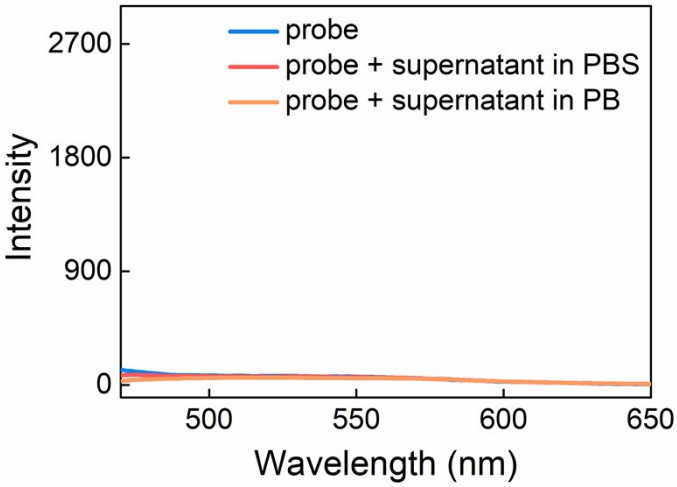


Fig. S5. Fluorescent response of 10 μM **2-IC** toward the supernatant of immobilized TCEP slurry after centrifuged at 8000 r/min for 2 min.

Table S1. The corresponding peak area data of the fluorescent spectra upon addition of spiked thiols in PBS (pH 7.4) and PB (pH 8.0), respectively, obtained by the FL solution 4.0 software.

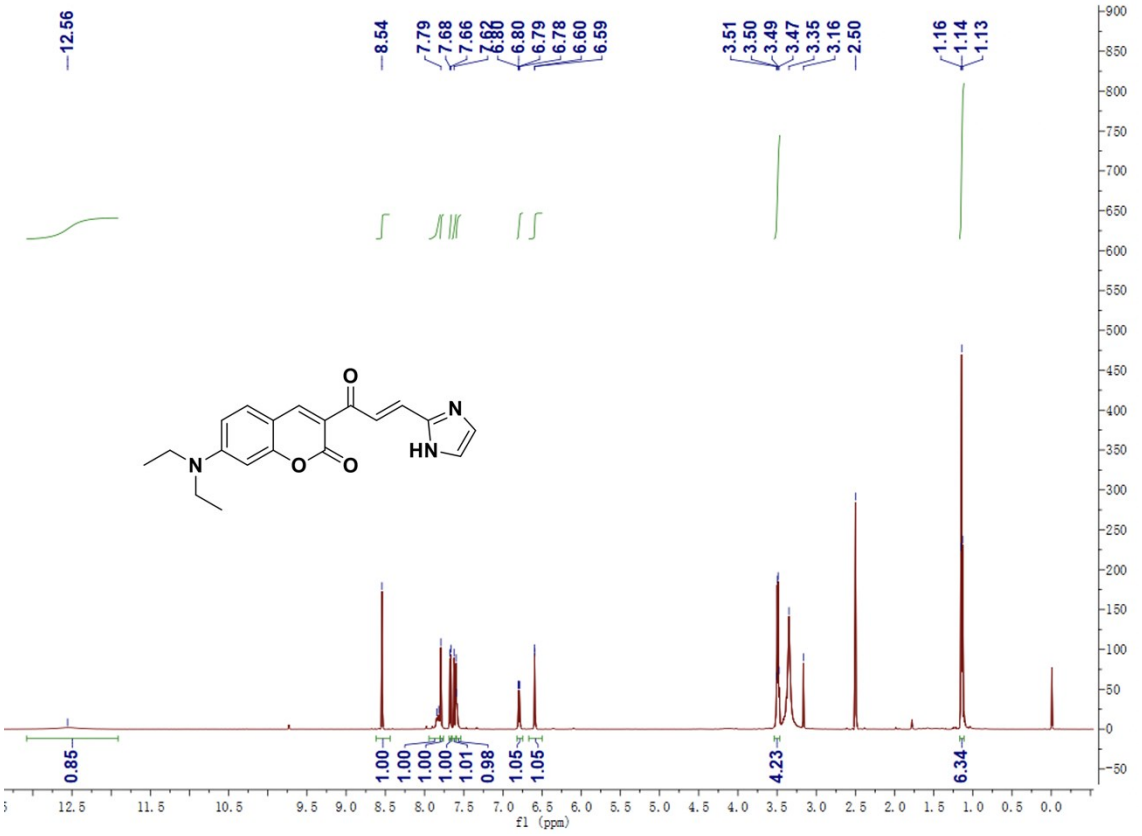
No.	A_{PBS}	A_{PB}	No.	A_{PBS}	A_{PB}
1	412.532	730.395	4	652.429	1138.12
2	623.529	1120.078	5	681.494	1239.95
3	617.043	1014.87	6	434.334	776.192

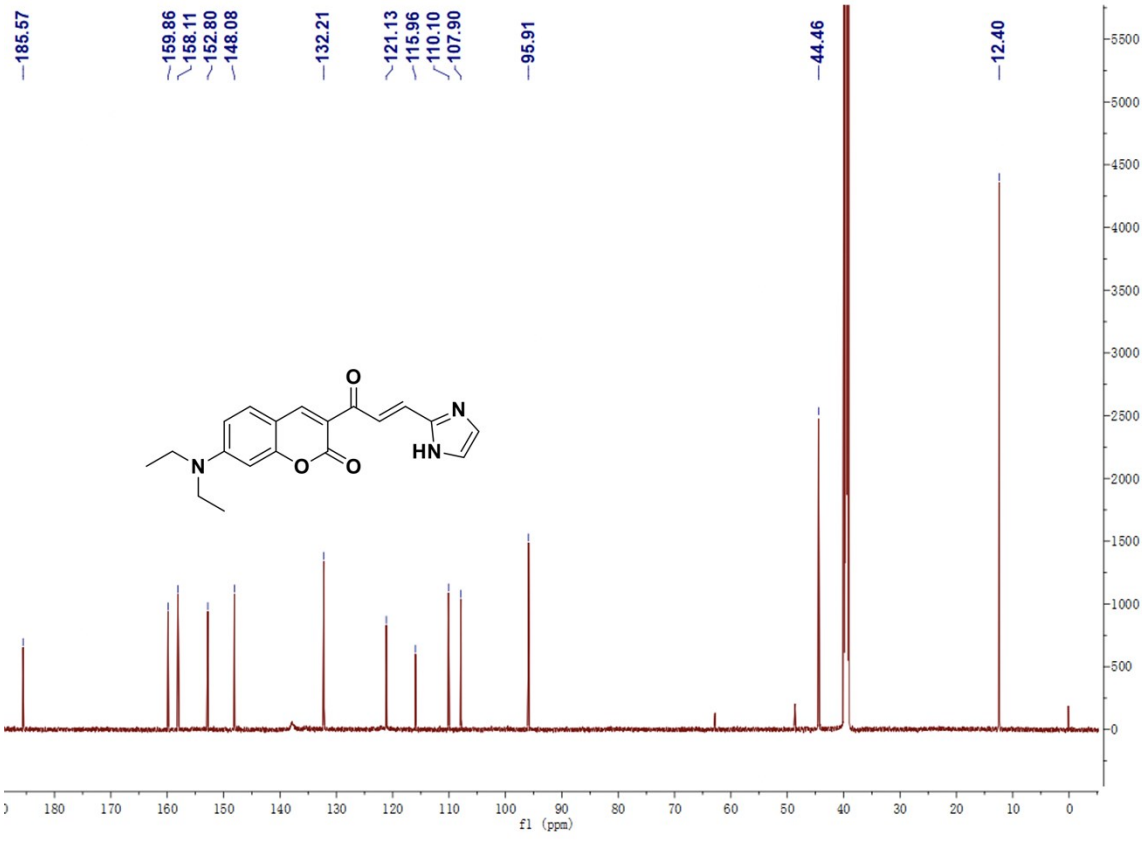
Table S2. The peak area data of the fluorescent spectra for serum detection, obtained by the FL solution 4.0 software. A_{PBS} and A_{PB} are the data obtained in PBS (pH 7.4) and PB (pH 8.0), respectively. All the serum samples were tested twice independently.

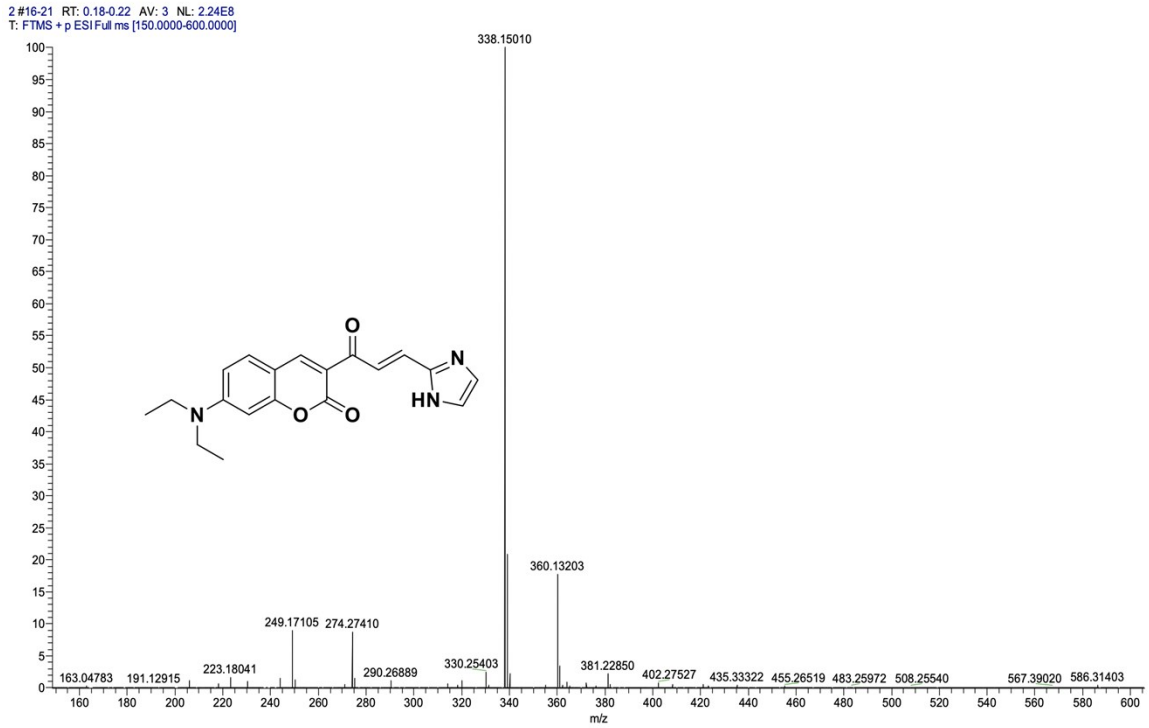
No.	A_{PBS1}	A_{PB1}	A_{PBS2}	A_{PB2}	c_{Cys} (μM)	c_{Hcy} (μM)	$c_{a\text{-}Cys}$ (μM)	$c_{a\text{-}Hcy}$ (μM)
1	1065.545	1046.361	1600.988	1519.522	154.265 ± 2.352	N. D.	2005.4 ± 30.6	47.1

2	946.203	902.972	1295.371	1335.576	131.490 ± 5.300	N. D.	1709.4 ± 68.9	9.5
3	866.355	880.954	1169.329	1195.864	122.660 ± 1.790	N. D.	1594.6 ± 23.3	10.5
4	861.624	863.804	1260.582	1312.72	120.763 ± 0.267	N. D.	1569.9 ± 3.5	12
5	812.7	813.407	1121.014	1076.735	112.153 ± 0.087	N. D.	1458.0 ± 1.1	7.2
6	782.681	774.62	1053.616	1034.259	106.189 ± 0.988	N. D.	1380.4 ± 12.8	19.6
7	757.27	701.914	950.419	963.853	97.683 ± 6.786	N. D.	1269.9 ± 88.2	11.7
8	747.118	708.15	937.728	895.269	97.344 ± 4.777	N. D.	1265.5 ± 62.1	7.7
9	723.876	724.686	1099.088	1054.784	96.763 ± 0.099	N. D.	1257.9 ± 1.3	23.8
10	708.125	750.163	947.433	918.433	97.606 ± 5.153	N. D.	1268.9 ± 67.0	10.9
11	626.127	641.281	845.695	888.528	81.059 ± 1.858	N. D.	1038.8 ± 6.6	11.2
12	629.144	625.025	835.801	781.081	79.912 ± 0.505	N. D.	1053.8 ± 24.2	14.1
13	612.634	618.238	751.811	801.348	77.892 ± 0.687	N. D.	1012.6 ± 8.	9.2
14	596.5	631.625	654.317	663.158	77.654 ± 4.306	N. D.	1009.5 ± 56.0	13.2
15	591.915	587.375	813.768	819.743	73.421 ± 0.556	N. D.	954.5 ± 7.2	10.6
16	590.018	581.828	785.38	745.042	72.775 ± 1.004	N. D.	946.1 ± 13.0	5.8
17	588.324	574.296	767.593	769.704	71.976 ± 1.720	N. D.	935.7 ± 22.4	14.4
18	549.023	576.907	671.336	676.374	68.795 ± 3.418	N. D.	874.4 ± 14.1	6.6
19	549.65	558.553	745.099	736.24	67.258 ± 1.091	N. D.	894.3 ± 44.4	15.1
20	510.124	533.955	586.494	579.751	61.700 ± 2.921	N. D.	802.1 ± 38.0	4.9
21	505.669	538.168	659.606	706.165	61.679 ± 3.984	N. D.	801.8 ± 51.8	8.2
22	505.34	503.319	716.315	694.665	58.630 ± 0.248	N. D.	762.2 ± 3.2	11.9
23	495.499	539.348	564.816	568.875	60.900 ± 5.376	N. D.	791.7 ± 69.9	13
24	339.683	380.132	430.904	454.469	33.591 ± 4.959	N. D.	436.7 ± 64.5	13.4

3. Characterization data



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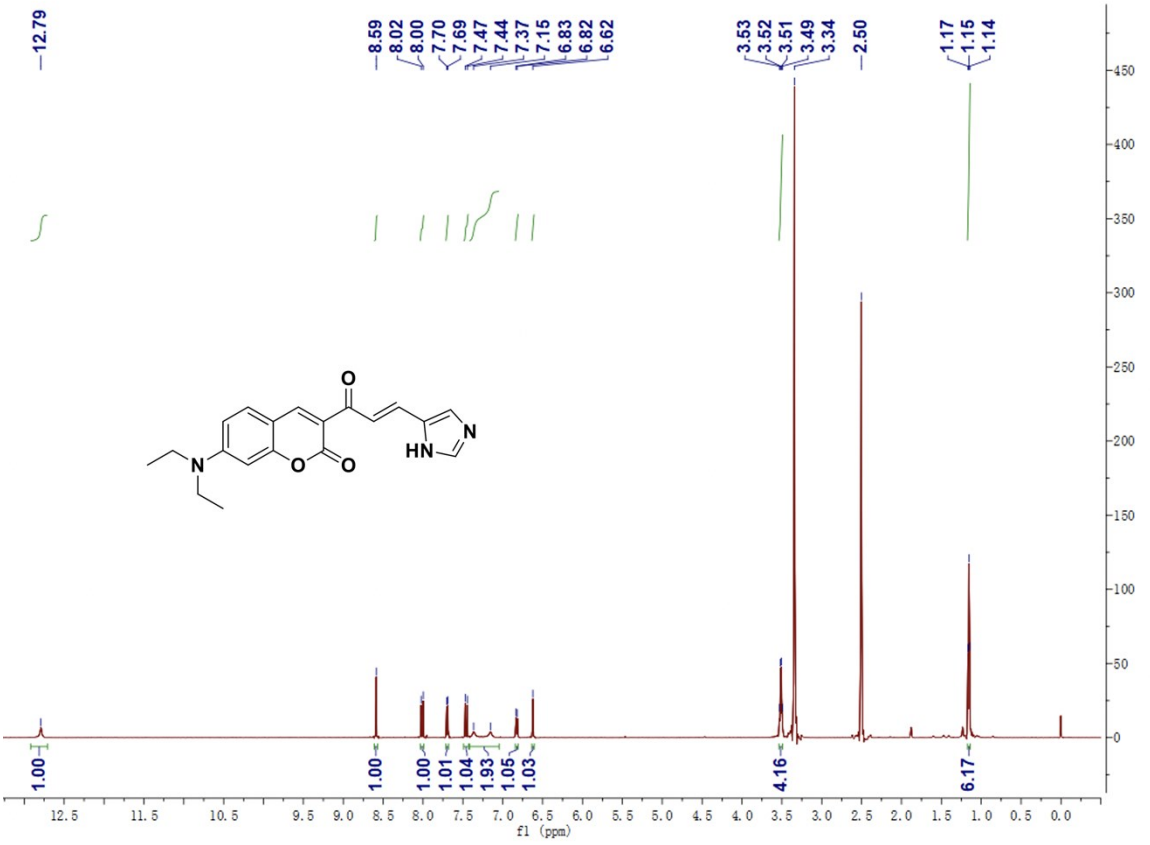
m/z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7.15
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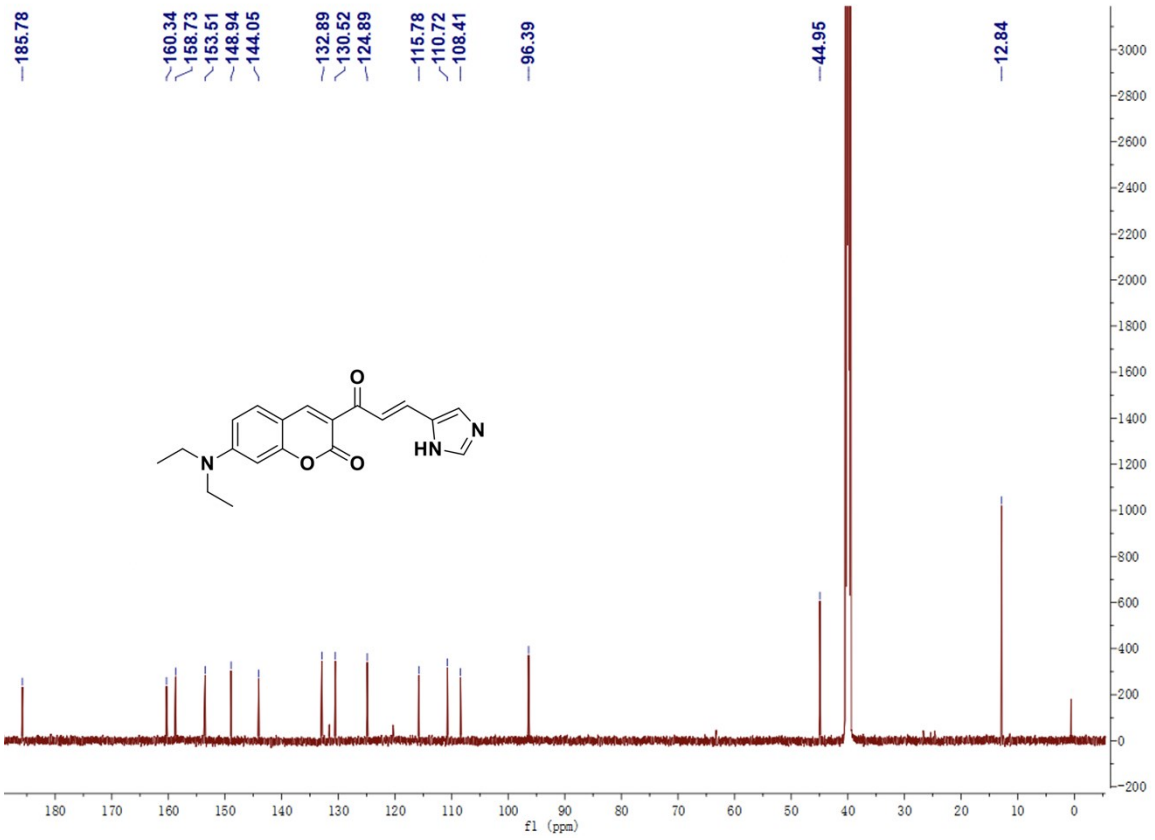
1.03
4.16
6.17
f1 (ppm)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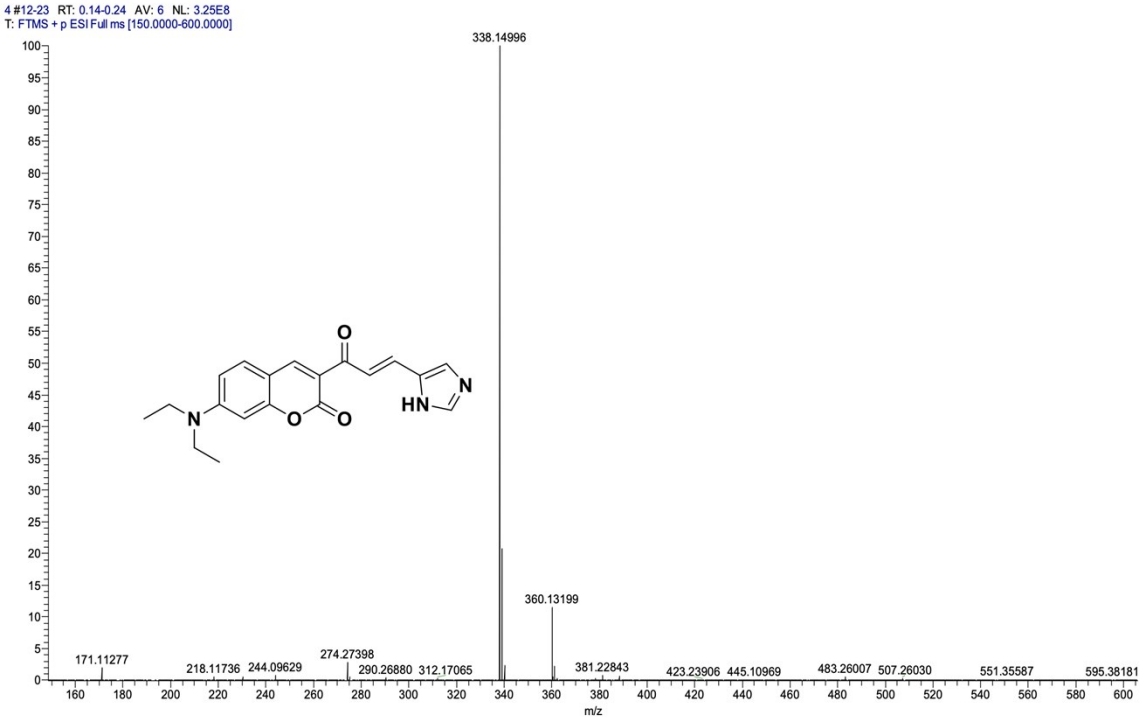
N
f1 (ppm)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m/z