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Supporting Information

A chemical covalent tactic for bio-thiol sensing and protein labeling agent designing

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1. Materials and instruments

All reagents were purchased from commercial supplies without further purification. NMR spectra were recorded on Bruker 400 and 500 MHz instruments. MS spectrum was performed on Trace DSQ GC-MS spectrometer (Thermo). Live cell imaging was carried out on inverted fluorescent microscope (Leica DMI 4000B). Fluorescence studies were recorded on an Agilent Cary Eclipse Fluorescence Spectrophotometer. The gel fluorescence imaging was performed by an image Quant 400.

2 HPLC analysis

HPLC assays were performed on an Agilent 1100 series HPLC system and Agilent Technologies 1260 Infinity HPLC system. C-SOMe (20 μM) was incubated with 2- mercaptoethanol (100 μM) at 37 ^{o}C in PBS buffer. C-SOMe and the Coum-S were both prepared as a 20 μM solution and were used as standard samples. Coum-S was obtained as the followed procedure. Coum-S (28 mg) was dissolved in 20 mL acetonitrile, and then 20 mL PBS buffer was added to the mixture. 2- mercaptoethanol was added to the solution and stirred for 30 min at 37 ^{o}C. The acetonitrile was removed under reduced pressure, and the residue was extracted by ethyl acetate. The crude product was purified by silica gel column chromatography with a yield of 80%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, *J* = 9.0 Hz, 1H), 6.57 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.46 (d, *J* = 2.6 Hz, 1H), 5.93 (s, 1H), 3.97 (q, *J* = 6.0 Hz, 2H), 3.41 (q, *J* = 7.1 Hz, 4H), 3.24 (t, *J* = 6.2 Hz, 2H), 2.26 (t, *J* = 5.9 Hz, 1H), 1.21 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.73, 155.71, 154.65, 150.89, 124.86, 108.46, 107.16, 100.99, 97.34, 60.00, 44.74, 33.19, 12.41. ESI-MS: $[M+H]^+$, 294.12. All samples were passed through a 0.22 µm filter, and 20 µL of each sample was loaded onto Agilent ZORBAX SB-C18, reversed-phase column (5 µm, 4.6×150 mm). The column was eluted with methanol/water (75:25). The flow rate was set at 0.6 mL min^{-1}. A UV/vis detector was used to monitor the desiring product at the wavelength of 400 nm.

3. Live cell imaging.

Hep G2 cells (4x10^{5}) were cultured in Dulbecco's Modified Eagle Medium (DMEM) in 6-well plates and allowed to grow overnight. Then C-SOMe (10 µM) was added to the plate and continued culture for certain time. With difference, the intracellular thiols is blocked by addition of NEM 30 minutes ahead the addition of the probe. The cells were visualized and photographed on inverted fluorescent microscope (Leica DMI 4000B).

4. BSA labeling

BSA (4 mg/mL) was dissolved in Tris-HCl (50 mM, pH = 7.4) and further dealt with 10% SDS at 37 °C for 30 min. the precipitate was collected by addition of cold acetone (v/v, 1:2) kept at −20 °C for 1 h and centrifuging at 4 °C for 10 min. The precipitate was further dealt with 0.1% SDS and reduced by 5 mM Tris(2-chloroethyl) phosphate (TCEP) at 37 °C for 15 min. Excess TCEP was removed by acetone precipitation. The precipitate was dissolved in 0.1% SDS

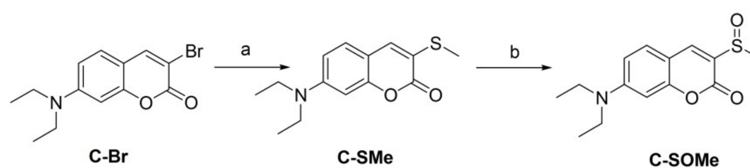
and labeled with C-SOMe (10 μM) at 37 °C, and then the protein samples were separated on a SDS-Page gel. The same gel was also stained by Coomassie brilliant blue (CBB) after the fluorescent image was obtained.

Table S1 Screen of the probes.[a] (From Chem.Sci., 2017, 8, 2966–2972)

Probes	λ_{ex} / λ_{em} (nm)	DTT (F/F_0)	Probes	λ_{ex} / λ_{em} (nm)	DTT (F/F_0)
1	395/505	11.2	**13**	370/497	1.32
2	390/500	14.5	**14**	375/550	10.7
3	385/505	216	**15**	335/438	1.08
4	425/570	1.7	**16**	420/490	2.0
5	508/528	1.2	**17**	390/505	24.9
6	508/520	1.7	**18**	375/480	1.0
7	440/570	0.97	**19**	380/580	1.0
8	430/640	1.0	**20**	400/510	0.37
9	450/625	0.22	**21**	340/490	0.57
10	—[b]	—[b]	**22**	—[b]	—[b]
11	390/550	1.92	**23**	330/450	2.1
12	410/550	0.81			

[a] The assays were performed by incubating probes (10 μM) with DTT (5 mM) at 37 °C in TE buffer (50 mM Tris-HCl, 1 mM EDTA, pH 7.4). [b] There is only very weak fluorescence in the aqueous solution. All compounds were dissolved in DMF to prepare stock solutions, and the organic solvent in final assay mixture is no more than 0.1 % (v/v). The fold of fluorescence changes was expressed as F/F_0.

Notes for activity analysis of Compound for thiol: *There are two main reasons to exclude this kind of naphthalimide derivatives (1-4) baring sulfoxide group. One is that the fluorescence in whole cell was quickly quenched in several seconds as shown in Fig.S1. The other is that two products were produced during naphthalimide derivatives react with thiol and not suitable for quantitative studies, which has been proved already in previous studies (Ref. 37: Bioorg. Med. Chem. Lett., 2015, 25, 59-61) and greatly limited their application. From experimental results, we find the compounds (7-12 and 18-21) had not exhibited significant fluorescence response. Therefore, the compounds were first excluded. Although bearing the electron with-drawing group, they were linked by carbon-carbon double bond, and then the distance may erode its site activity. The three compounds (15-17) have same skeleton and their recognition group at different substituted sites. The compound 15 and 16 with recognition group at same substituted sites (7-position) and showed slight fluorescence enhancement. The compound 17 (C-SOMe) with recognition group at 4-substituted sites, and its adjacent site, C=O, is a strong electron-withdrawing group, eventually resulting in significant fluorescence response. In short, the activity of recognition group may ascribe to the comprehensive results of push-pull electron system.*



Scheme S1 Synthesis of C-SOMe. (a) DMF/CH_3SNa, rt, 62%; (b) *m*-CPBA/DCM, rt, 81%;

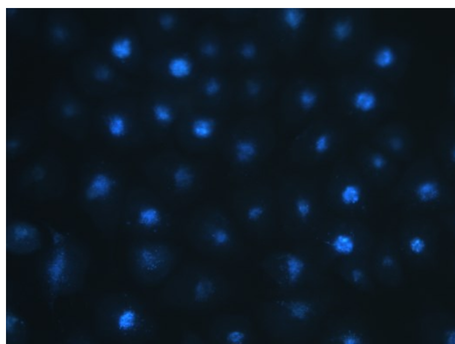


Figure S1 Fluorescence imaging of Nap derivatives (The fluorescence was quickly quenched in several seconds).

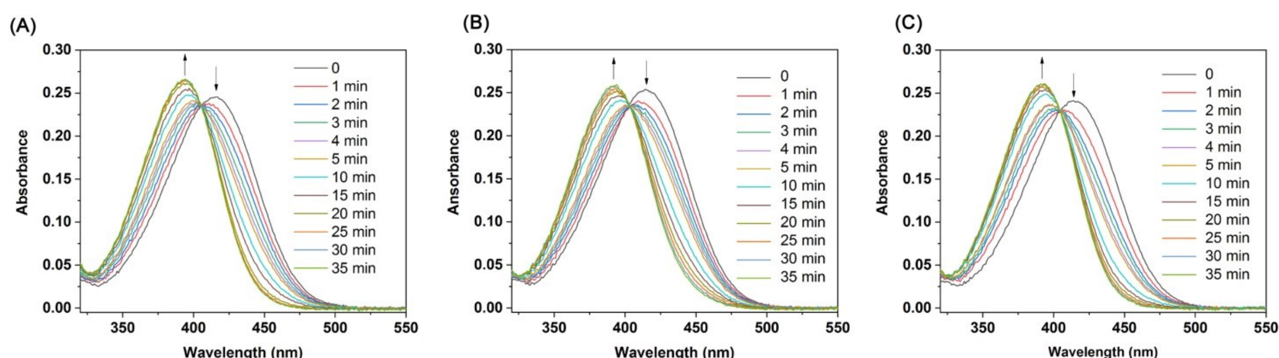


Figure S2 Time-dependent UV-vis absorption response of C-SOMe (10 μM) with 5 equiv. of Cys (A), GSH (B), and Hcy (C).

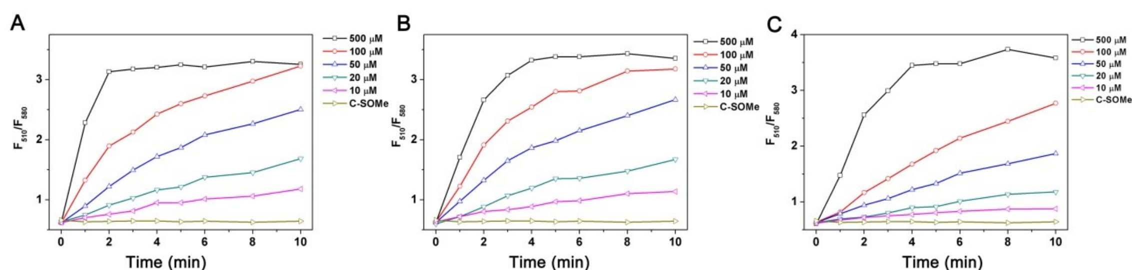


Figure S3 Time-dependent fluorescence response of C-SOMe (10 μM) with various concentrations of Cys (A), GSH (B), and Hcy (C).

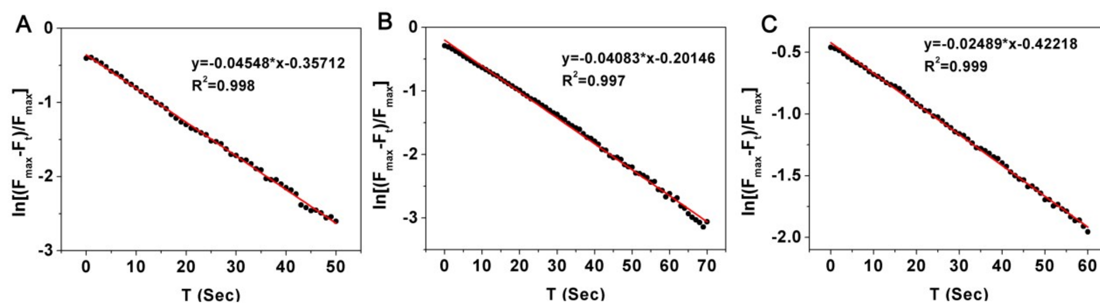


Fig.S4 Pseudo-first-order kinetic plot of the reaction of C-SOMe incubated with Cys (A), GSH (B), and Hcy (C) in PBS buffer at room temperature.

Table S2 The pseudo-first-order rate constants for the reaction of C-SOMe with Cys, Hcy and GSH

Thiol	Cys	GSH	Hcy
K'	0.0455[a] (1.83)[b]	0.0408[a] (1.64)[b]	0.0249[a] (1.00)[b]

[a] The unit of the rate constants is S^{-1}

[b]The data in parentheses represent the ratio of the reaction rate according to Hcy

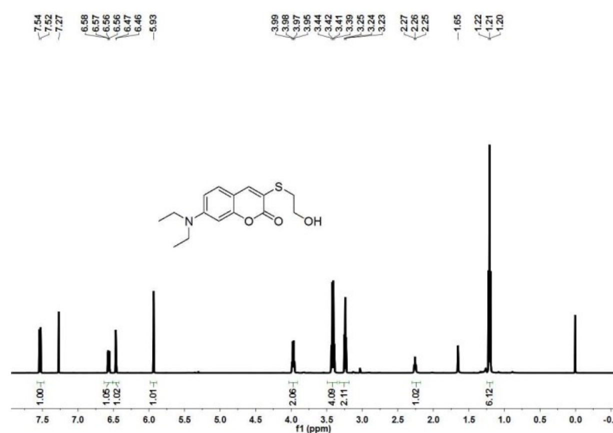


Fig. S5 ^{1}H NMR spectrum of Coum-S

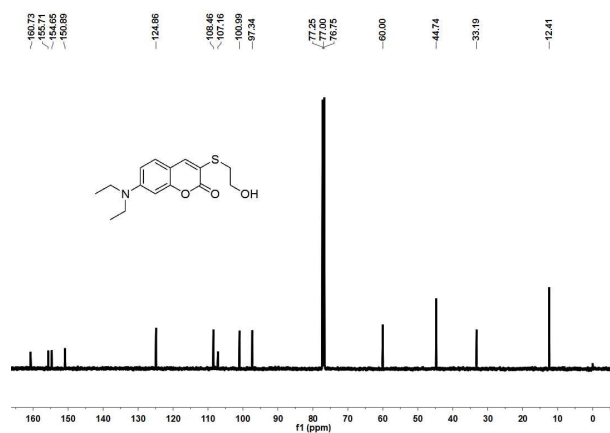


Fig. S6 ^{13}C NMR spectrum of Coum-S

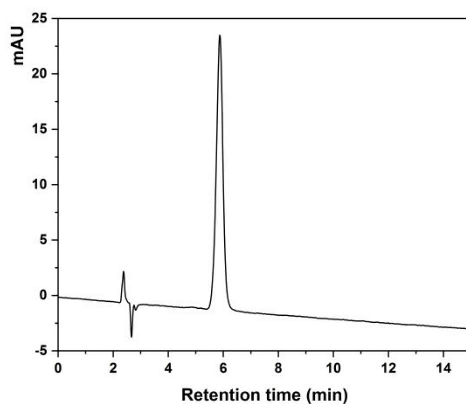


Fig. S7 Analyses of the purity of Coum-S by HPLC (>99%). (This carried out on Agilent Technologies 1260 Infinity HPLC system. That is why the retention time is different from Fig.4D).

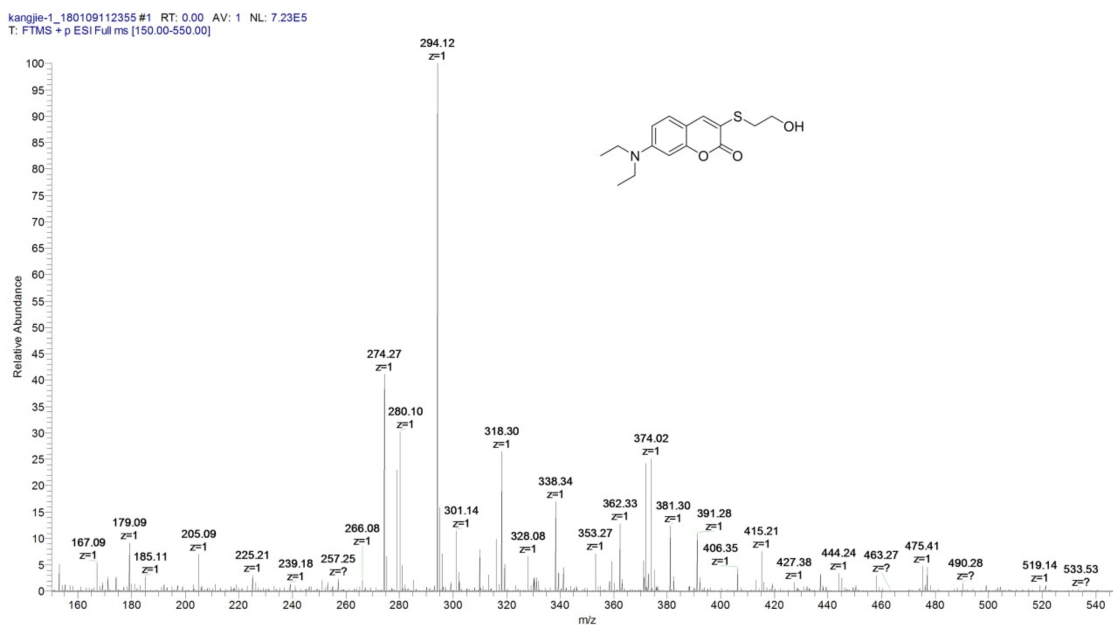


Fig.S8 MS spectrum of Coum-S

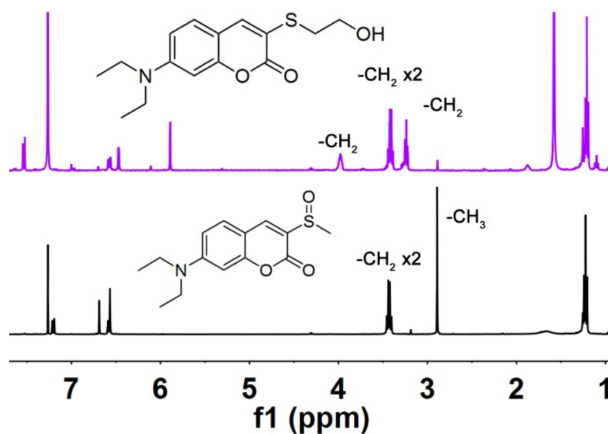


Figure S9 Enlarged NMR spectra of Fig.4C.

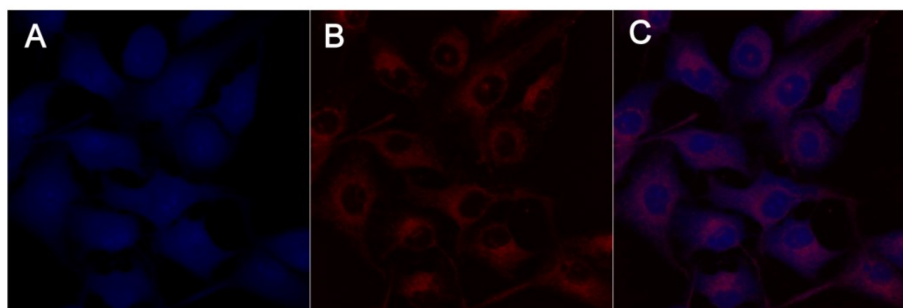


Figure S10 Colocalization experiments of C-SOMe and Bcy-Keto (Our reported red-emission fluorophore which was confirmed without staining nucleic acid).[1] The Hep G2 cells were incubated with C-SOMe (A) and Bcy-Keto (B) for 5min. (C) Overlay of blue and red channel. Blue channel: λem= 480−580 nm, λex= 405 nm; Red channel: λem= 700−800 nm, λex= 635 nm.

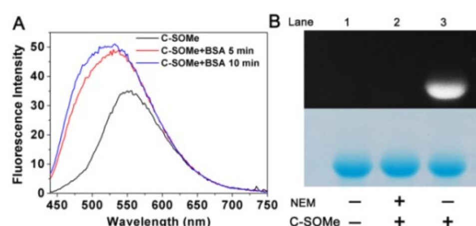


Fig. S11 (A) Fluorescence response of C-SOMe (10 μM) in presence of BSA (2 mg/mL). (B) Fluorescence and white-light image of BSA (4 mg/mL) labeled with C-SOMe (10 μM). Lane 1, BSA; lane 2, BSA incubated with NEM (100 μM) for 30 min followed by further treated with C-SOMe (10 μM); lane 3, BSA treated with C-SOMe (10 μM).

5. Comparsion of C-SOMe with previous agents

Previous thiols probes were rarely considered for thiols labeling, notably, C-SOMe showed excellent performance on thiols sensing and BSA labeling, and we mainly focused on its application in thiol labeling. Compared with previous agents with thiol, illustrated in Fig. 1, C-SOMe shows high selectivity, photostability and fast response time toward thiols (within 80 seconds, Fig. 3E), leading to its exclusive nucleophilic substituted compound, which greatly elevated the performances of thiol labeling. The early example 4NBN reported by Triboni and co-workers, although it can react with thiols, suffered from long response time.[2] Kong and coworkers have designed a series of 1, 8-naphthalimide derivatives containing sulfoxide or sulfone group and their response time was greatly improved. The sulfoxide substituent underwent a different mechanism and gave two types of thioether, nucleophilic substituent and reductive compounds, which tremendously hinder its utility in protein thiols labeling.[3] Fang applied the sulfone substituents for protein thiols labeling.[4] In addition, the response time of probes needed at least half an hour in presence of large amounts of thiols (millimole level).

6. References

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