Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Highly Selective Fluorescence off-on Probes for Biothiols and Imaging in Live Cells

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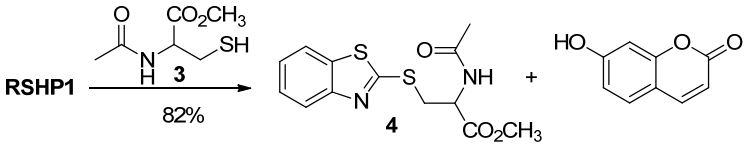
Model reaction between RSHP1 and compound 3



To the solution of **RSHP1** (55 mg, 0.15 mmol) in THF (2 mL) and PBS buffer (2 mL, 50 mM, pH = 7.4) was added compound **3** (80 mg, 0.45 mmol). The mixture was stirred for 30 min at 37 ^{o}C. No starting material was observed on TLC. Then DCM (50 mL) was added into the solution to extract the reaction mixture. The organic layer was separated, dried by $MgSO_4$, and concentrated. Compound **4** and 7-OH-coumarin were isolated in 82 % yields by column chromatography. The structure of compound **4** was confirmed by NMR.[1]

Preparation of the solutions and fluorescence measurements

The stock solutions of **RSHP1, RSHP2, RSHP3** (2 mM) were prepared in DMSO. The solutions of various testing species were prepared from cysteine (Cys), GSH, homocysteine (Hcy), glutathione disulfide (GSSG), $Na_2S_2O_3$, Na_2SO_3, H_2O_2, NaClO, $NaNO_2$ in 50 mM PBS buffer (pH 7.4), and KO_2 solution was prepared in DMSO. All the test solutions needed to be freshly prepared.

Unless otherwise noted, all the measurements were carried out for 15 min at 37 ^{o}C in 50 mM PBS buffer (pH 7.4) with 10% DMSO according to the following procedure. For **RSHP3**, in a test tube, 3.5 mL of 50 mM PBS buffer (pH 7.4) was added, and then added a requisite volume of testing species sample solution. The resulting solution was mixed well, followed by addition of 20 μL of the stock solution of **RSHP3**. The final volume of the reaction

solution was adjusted to 4 mL with 50 mM PBS buffer (pH 7.4) with 10% DMSO. After mixing and then standing for 15 min at 37 ^{o}C, a 4-mL portion of the reaction solution was transferred into a 1-cm quartz cell to measure fluorescence with λ_{ex} = 490 nm. PMT detector voltage = 450 V. In the meantime, a blank solution containing no testing species was prepared and measured under the same conditions for comparison. Error bars represent the standard deviation from triplicate experiments.

1. D. Zhang, N. O. Devarie-Baez, Q. Li, J. R. Lancaster, Jr., M. Xian, *Org. Lett.*, 2012, **14**, 3396.

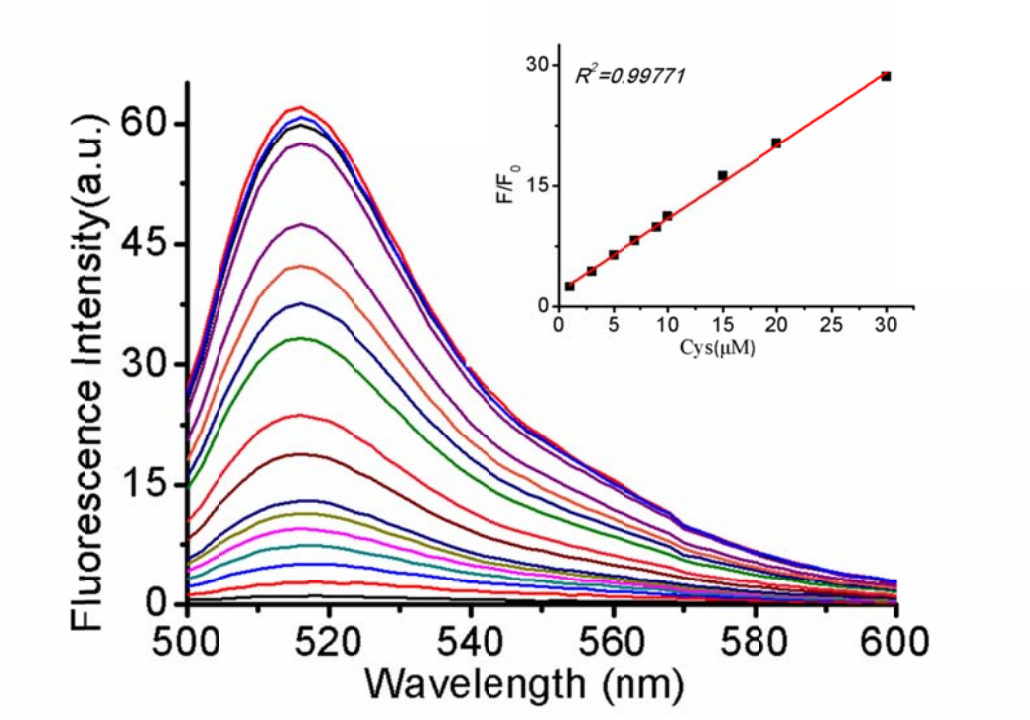


Figure S1. Fluorescence emission spectra of **RSHP3** (10 μM) with varied concentrations of Cys (0, 1, 3, 5, 7, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100 μM for curves 1-17, respectively). The reactions were carried out for 15 min at 37 ^{o}C in PBS buffer (50 mM, pH = 7.4) with 10% DMSO. Inset: linear plot of **RSHP3** (10 μM) upon the addition of different amounts (0, 1, 3, 5, 7, 9, 10, 15, 20, 30 μM) of Cys in PBS buffer (50 mM, pH = 7.4) with 10% DMSO. ($\lambda_{ex/em}$ = 490/518 nm).

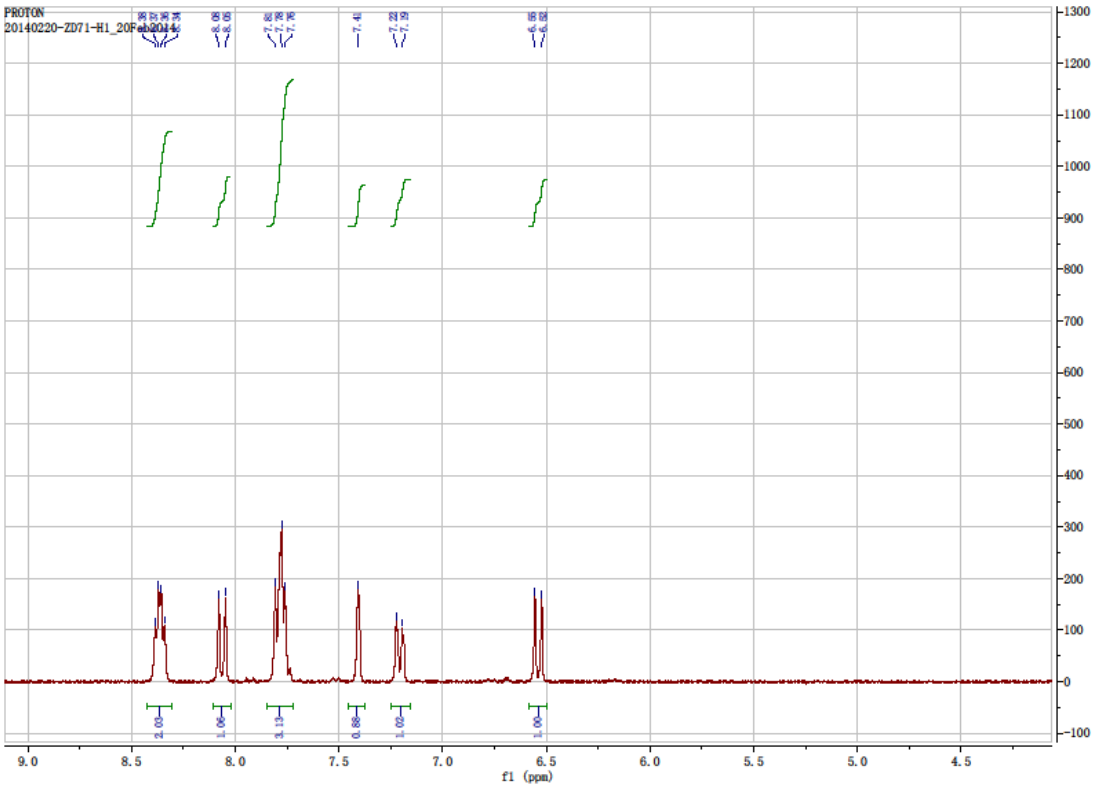


Figure S2. ^{1}H NMR (300 MHz, DMSO) of **RSHP1**

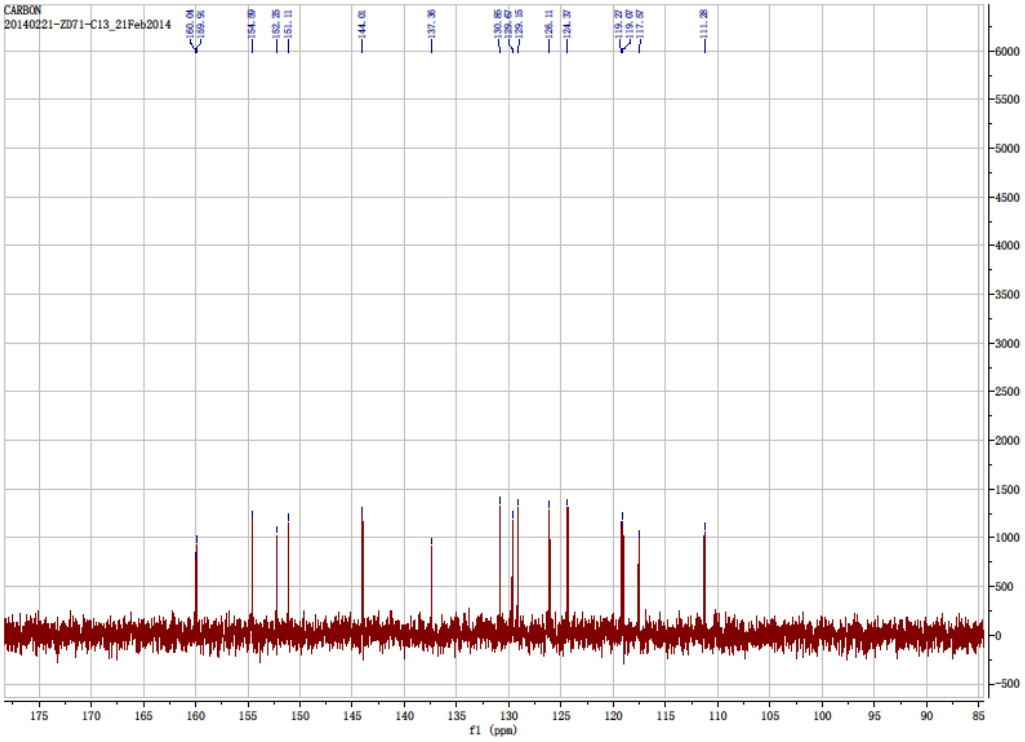


Figure S3. ^{13}C NMR (75 MHz, DMSO) of **RSHP1**

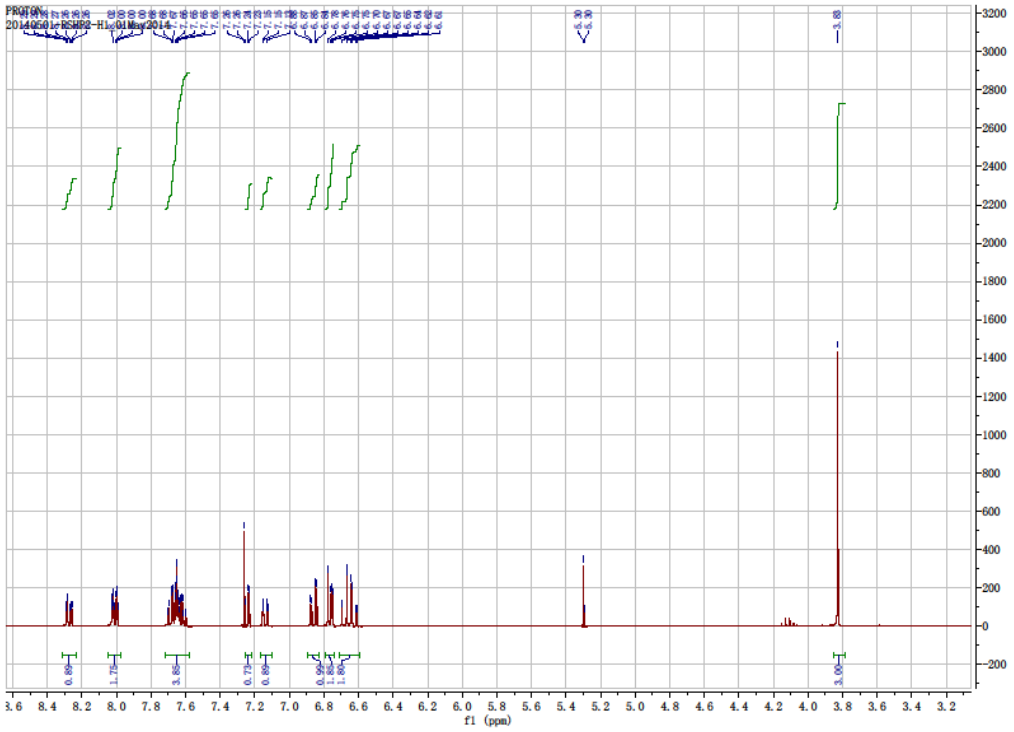


Figure S4. ^{1}H NMR (300 MHz, $CDCl_3$) of **RSHP2**

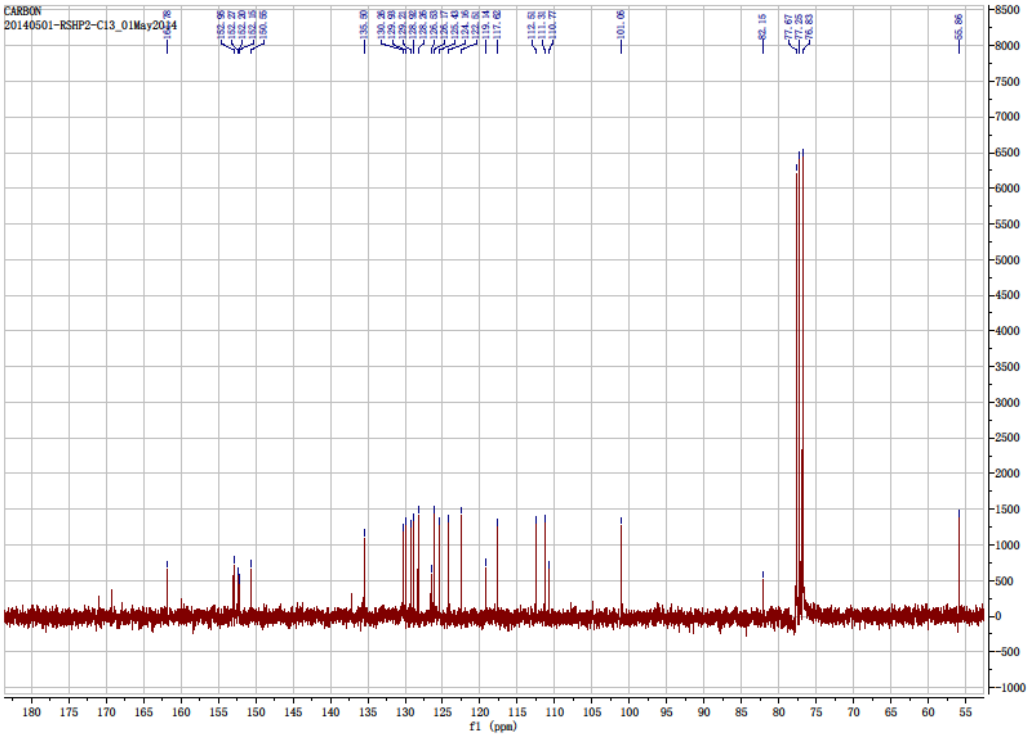


Figure S5. ^{13}C NMR (75 MHz, $CDCl_3$) of **RSHP2**

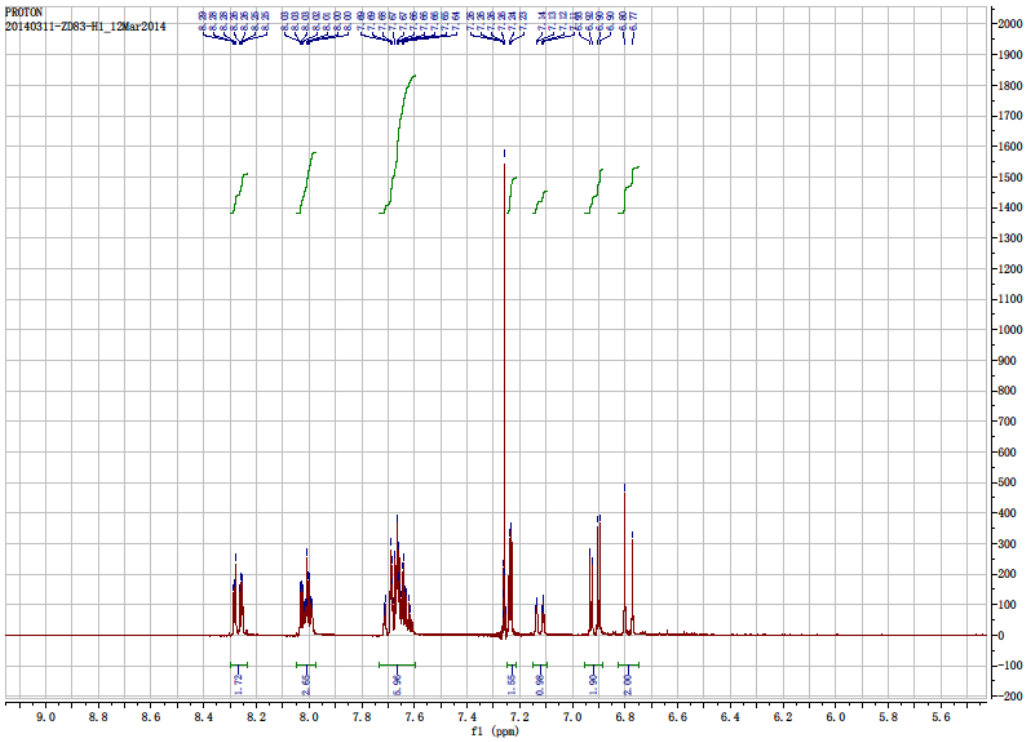


Figure S6. ^{1}H NMR (300 MHz, $CDCl_3$) of **RSHP3**

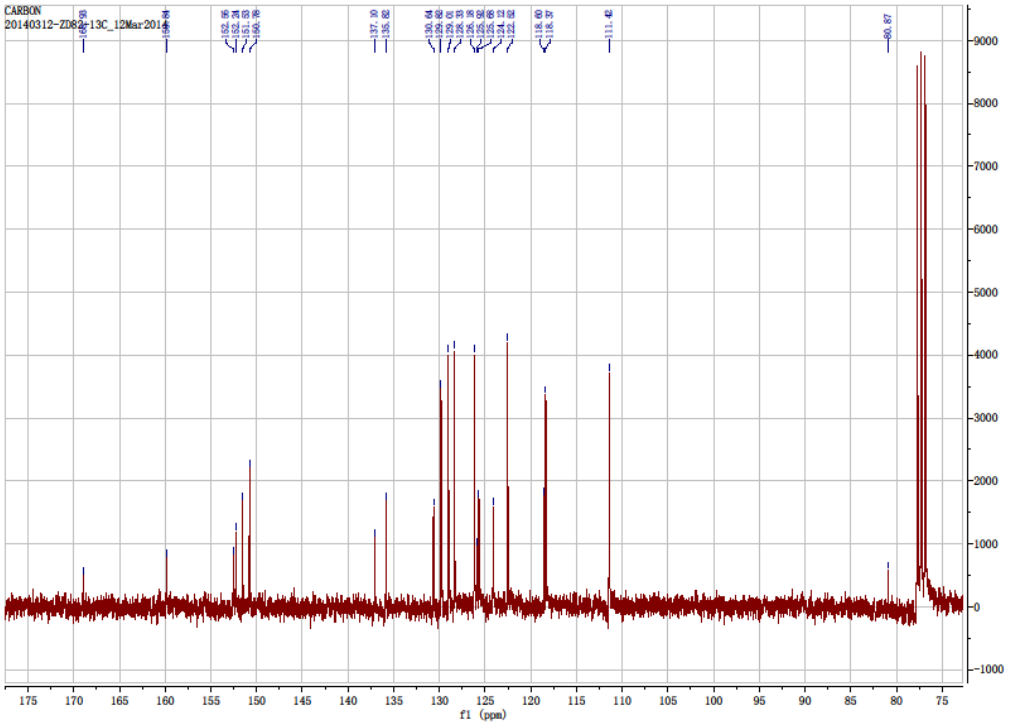


Figure S7. ^{13}C NMR (75 MHz, $CDCl_3$) of **RSHP3**