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Supplementary Information

A selective fluorescent sensor for Cysteine detection with potential of white light emitting fluorophor in living cell imaging

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Experimental section

1 The experimental materials and equipment

All chemicals purchased were used as received without further purification. Chromatographic purification was performed using 300-400 mesh chromatography silica gel. Melting point was determined on a WRS-1C digital melting point apparatus. The NMR data were collected in DMSO-d_6 using Bruker (Rhenistetten-Forchheim, Germany) AM 600 MHz and dealt with MestReNova software. Mass spectra were acquired from an Agilent 6540 UHD Accurate Mass Q-TOF LC/MS. All pH measurements were conducted on pH meter of PHS-25. UV-vis spectra were accomplished on Shimadzu UV-2550 spectrometer. All the fluorescence measurements were recorded on Hitachi Fluorescence Spectrophotometer F-7000. Cell viability experiments were carried out using MTT method. The imaging experiments were performed using single-photon confocal fluorescent microscope (Carl Zeiss LSM 880).

2 Synthesis experiments

For the synthesis of Intermediate **3**, 4-(diethylamino)-2-hydroxybenzaldehyde (800 mg, 4.14 mmol) was added slowly to DMF (6 mL) at 60 ℃ and the solution was stirred for 30 min. Then 2-aminobenzenethiol (0.45 mL, 4.2 mmol) in DMF (1 mL) and potassium iodide (120 mg, 0.72 mmol) was added and the mixture was stirred at 110 ℃ for 18 h. The reaction solution was poured into ice water and washed with brine. The aqueous layer was extracted with DCM (50 mL) for twcie. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The yellow crude product was purified by column chromatography (PE:EA = 20:1, v/v) to afford intermediate **3** as pale yellow (500 mg, 40%).

Intermediate **3** (600 mg, 2.0 mmol) and 4-chloro-7-nitro-1,2,3-benzoxadiazole (400 mg, 2.0 mmol) were dissolved in DMF (5 mL). A few drops of triethylamine was added slowly into the solution and the mixture was stirred at 110 ℃ for 12 h. After the completion of the reaction confirmed by TLC, the solvent was removed by evaporation and the residue was then purified by column chromatography (PE:EA = 10:1, v/v) to afford **CysW-1** as dark solid (100 mg). m. p. : 236-238 ℃. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.61 (d, J = 8.4 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 7.7 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.42 (t, J =8.1 Hz, 1H) 7. 29(t, J = 8.5 Hz, 1H), 6.92 (dd, J = 9.2, 2.6 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 3.45 (q, J = 7.0 Hz, 4H), 1.14 (t, J = 7.0 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.91, 153.51, 152.65, 152.43, 151.45, 145.97, 136.08, 134.55, 131.70, 126.80, 125.02, 122.30, 122.24, 111.55, 111.03, 109.77, 103.94, 44.50, 12.86. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd. for $C_{23}H_{20}N_5O_4S$ 285.0275, Found 285.0290.

After the cleavage reaction, the corresponding products **Prod-B** and **Prod-Y** were purified and obtained through prep-HPLC (preparative- High Performance Liquid Chromatography). Then their HR-MS spectra were acquired.

3 Determination of the fluorescence quantum yield

The participation ratio method was used in estimating the fluorescence quantum yield Φ_u with the ethanol solution of rhodamine B as control. The fluorescence quantum yield of **CysW-1** was calculated as 0.03 for **CysW-1**, 0.52 for **Prod-B** and 0.44 for **Prod-Y**.

4 Ethic statement

All cell lines were cultured and passaged in accordance with the guidelines established by the National Science Council of Republic China. The primary cell lines were purchased from ATCC. All experimental protocols were approved by Academic Committee of Nanjing University.

5 Confocal microscopy experiments

HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum), 100 U/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, water saturated incubator at 37 ℃. For the group of initial status, cells were incubated with 10 μM of **CysW-1** (containing 10% DMSO, v/v) for 30 min at 37 ℃, washed three times with PBS, incubated with the same buffer as other groups for another 30 min, and imaged. For experimental groups, cells were treated with NEM (*N*-Ethylmaleimide, a Cys scavenger) for 30 min, washed three times with PBS, **CysW-1** for 30 min at 37 ℃, washed three times with PBS, and then incubated with different concentrations of Cys (0, 20, 200 μM) for 30 min before finally imaged. The usual signal collection range of the dyes DAPI (4',6-diamidino-2-phenylindole) and HEX (5-hexachloro-fluorescein) were employed as blue and yellow channels, respectively.

6 Cell viability assay

HeLa (human cervical cancer cell line) and L02 (human embryonic liver cell line) were cultured in 96-well plates at a density of 5×10^5 cells and different concentrations of **CysW-1** were added. Cytotoxicity evaluation was performed after culturing for 48 h at 37 ℃ in a 5% CO_2 atmosphere. 4 h before the end of the incubation, 20 μL MTT reagent (5 mg/mL) was added to each well. Then the plate was centrifuged at 1200 rpm for 5 min. The supernatant were removed and 150 μL DMSO was added. The absorbance was measured at a wavelength of 490 nm on an ELISA microplate reader. The experiment was conducted in triplicate.

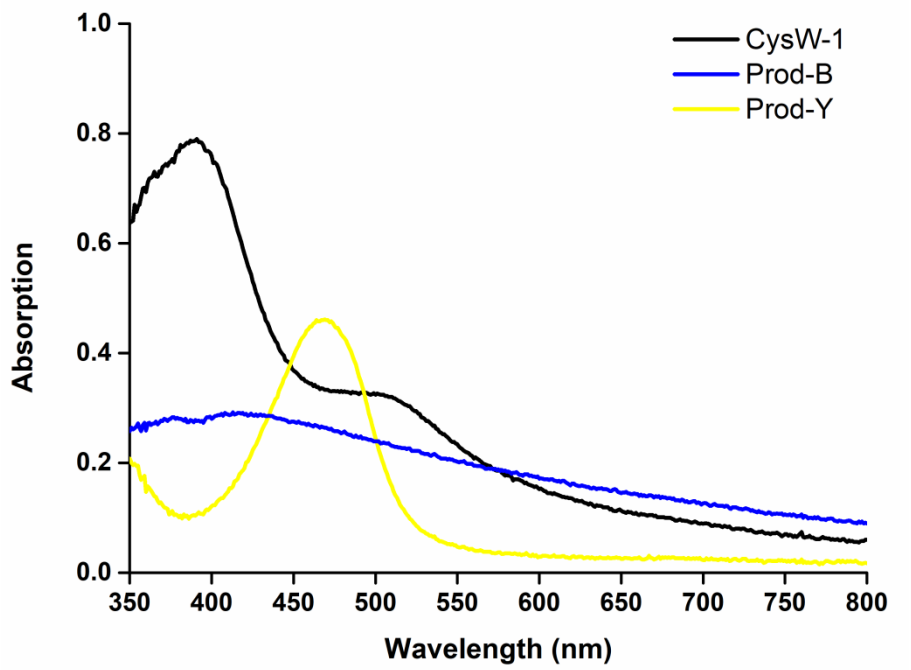

Figure S1. The absorption spectrum of **CysW-1** (50 μM), **Prod-B** (50 μM) and **Prod-Y** (50 μM) in PBS buffer (pH 7.4, 10 mM, 10% DMSO, v/v) at 37 ℃.

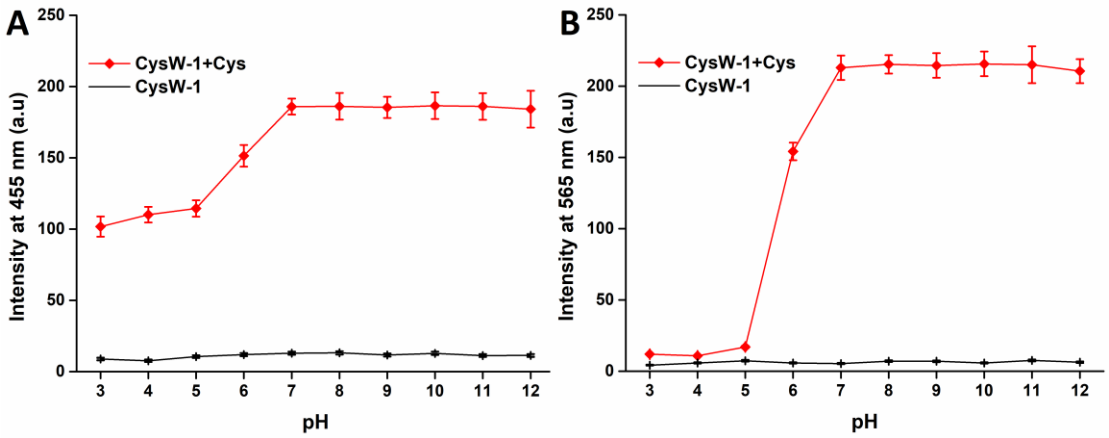

Figure S2. The fluorescence intensity changes in various pH environments (Black: **CysW-1**; Red: **CysW-1**+Cys.)

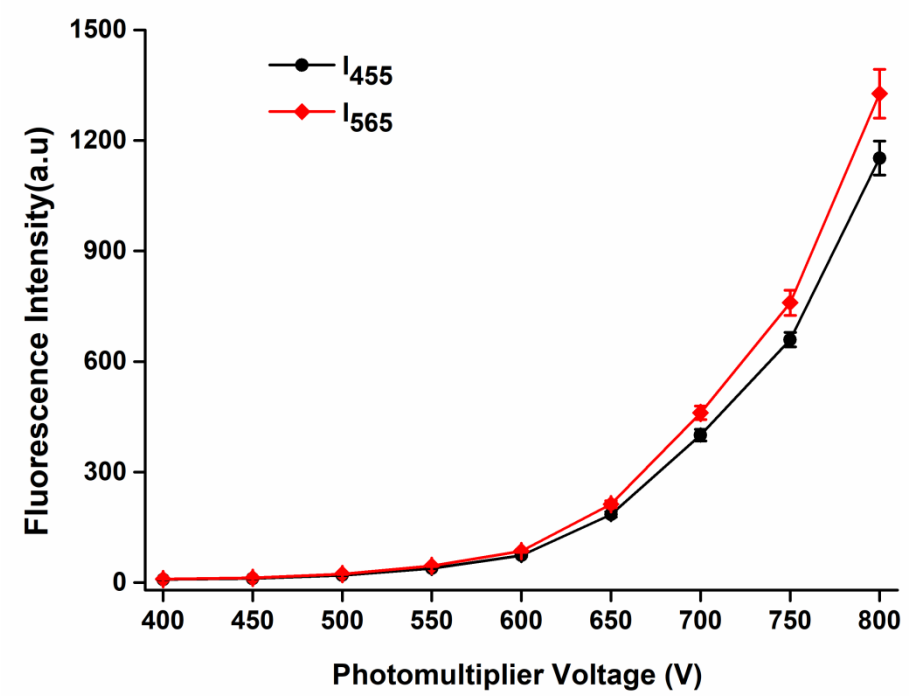

Figure S3. The fluorescence intensity changes with increasing photomultiplier voltage from 400 V to 800 V.

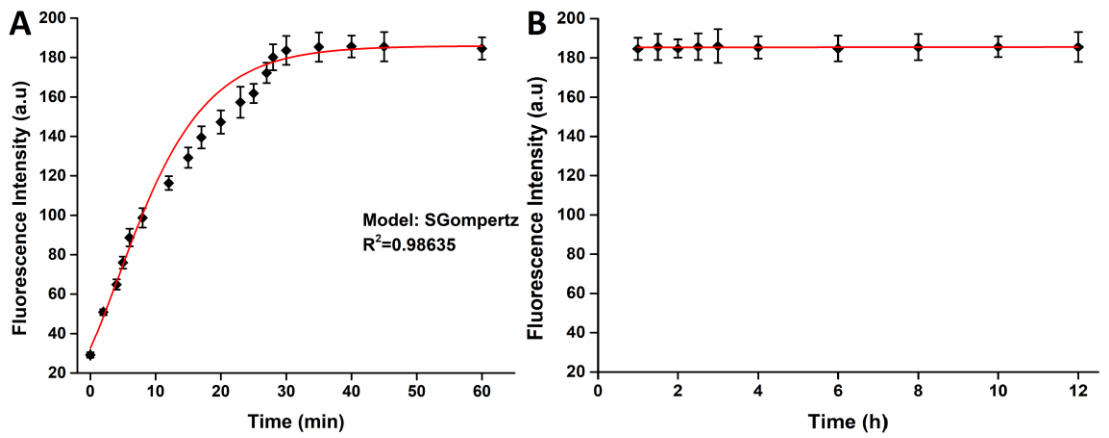

Figure S4. The fluorescence intensity changes with different incubation time: (A) 0-60 min; (B) 1-12 h.

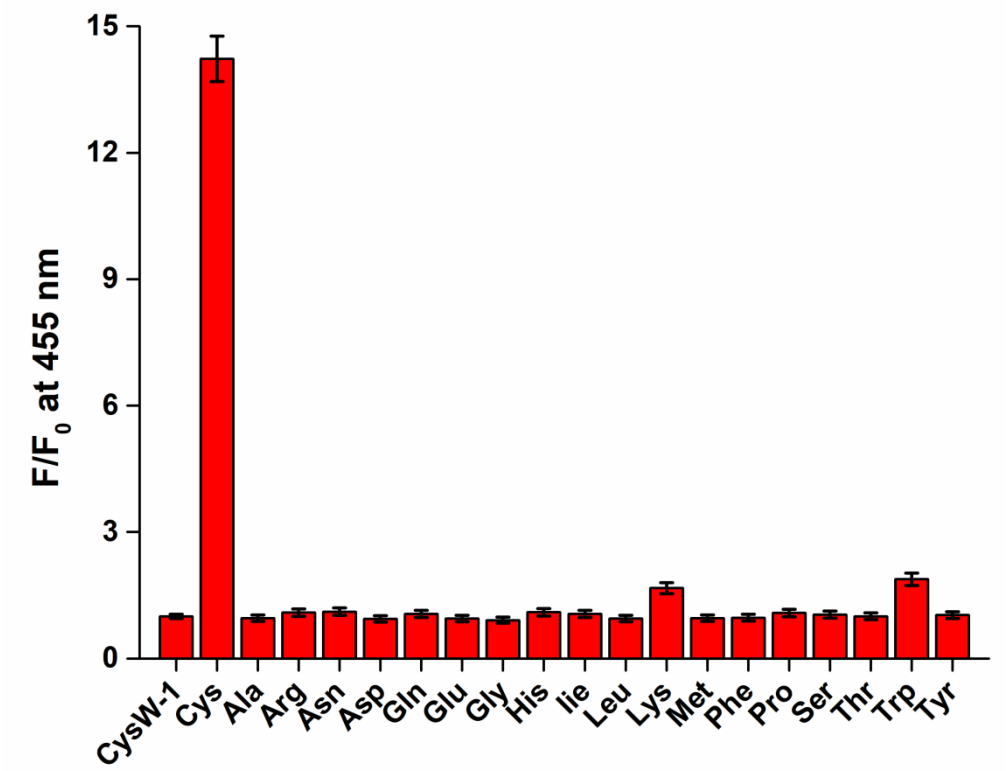

Figure S5. The selectivity of **CysW-1** (10 μM) towards Cys over various amino acids. The concentration of Cys was 100 μM and that of all the other analytes were 1 mM. The data were obtained after incubation in PBS buffer (pH 7.4, 10 mM, 10% DMSO v/v) at 37 ℃.

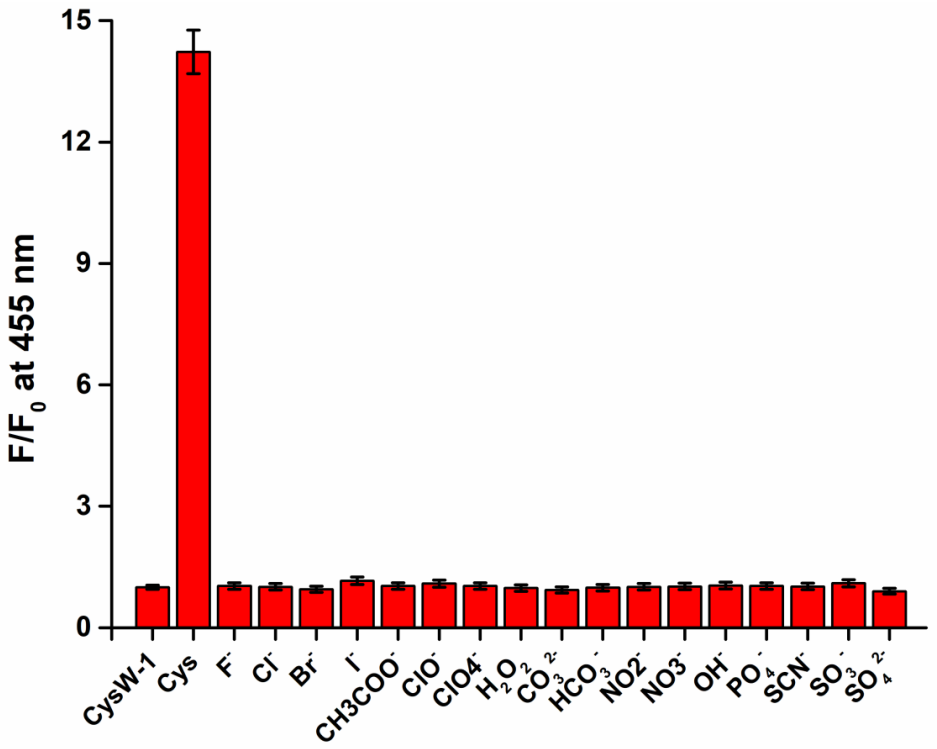

Figure S6. The selectivity of **CysW-1** (10 μM) towards Cys over various anions. The concentration of Cys was 100 μM and that of all the other analytes were 1 mM. The data were obtained after incubation in PBS buffer (pH 7.4, 10 mM, 10% DMSO v/v) at 37 °C.

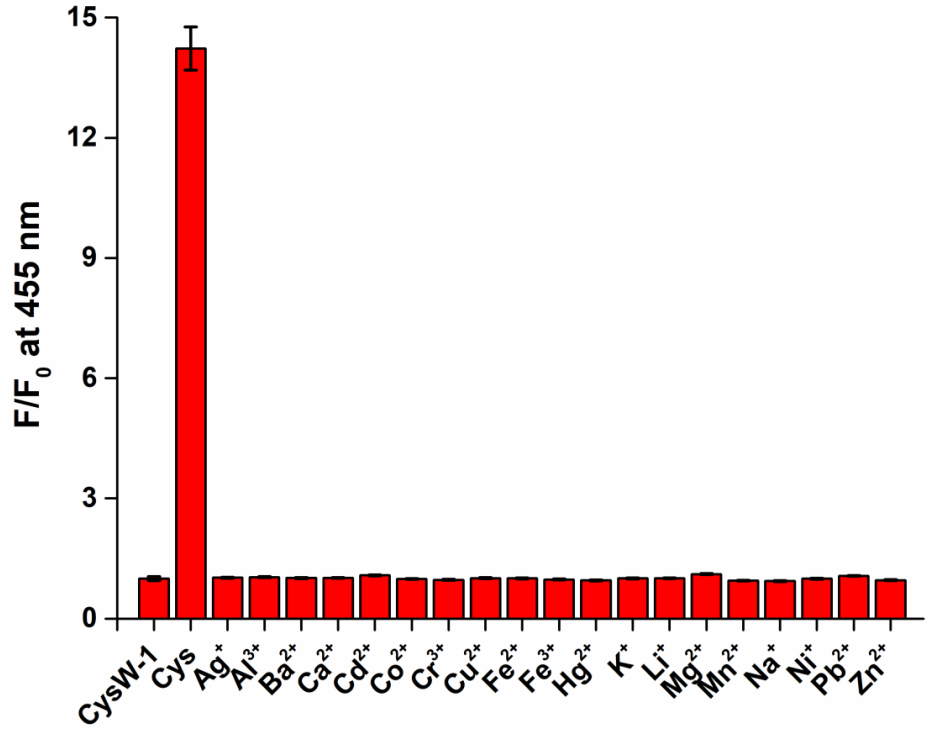

Figure S7. The selectivity of **CysW-1** (10 μM towards Cys over various metallic ions. The concentration of Cys was 100 μM and that of all the other analytes were 1 mM. The data were obtained after incubation in PBS buffer (pH 7.4, 10 mM, 10% DMSO v/v) at 37 °C.

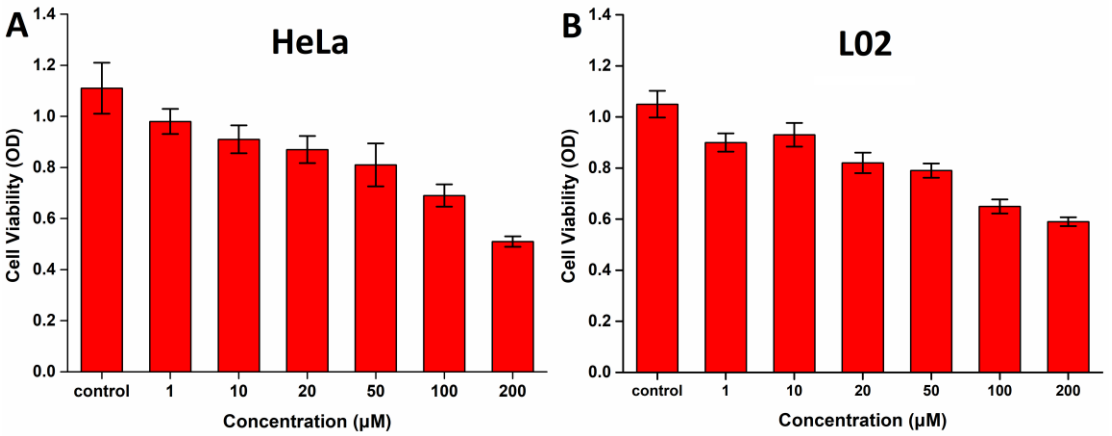

Figure S8. Cell viability of HeLa (human cancer cell line) and L02 (human embryonic liver cell line) with different concentrations of **CysW-1**.

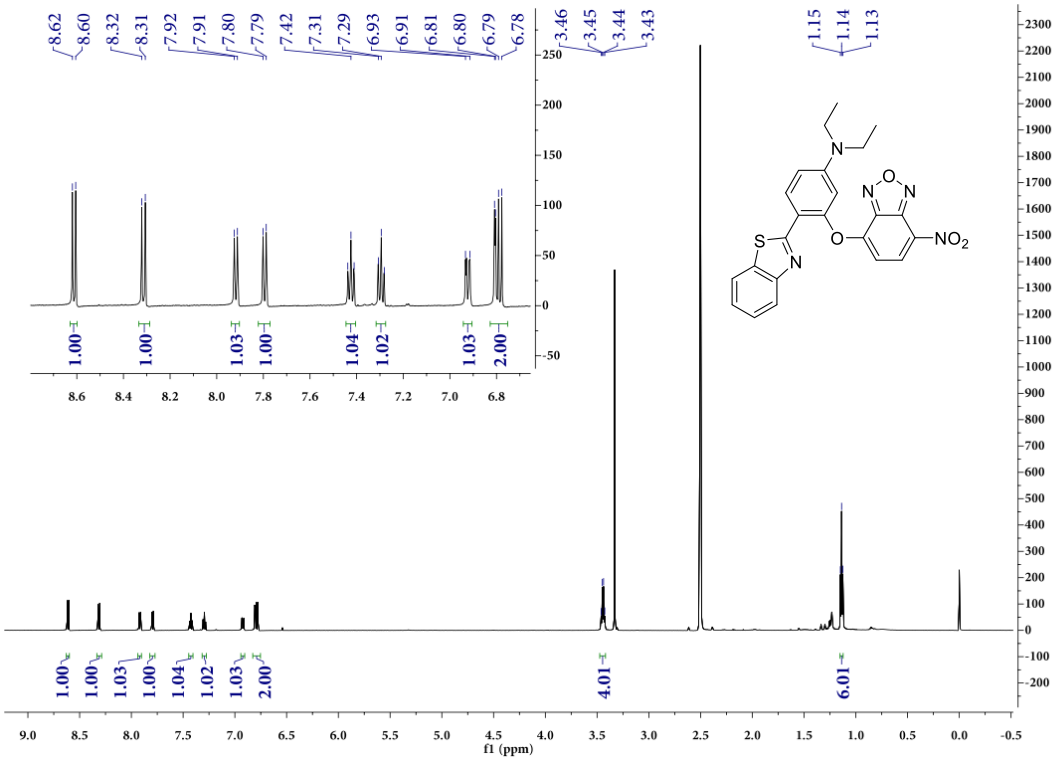

Figure S9. ^{1}H NMR of compound **CysW-1** (600 MHz, in DMSO-d_6).

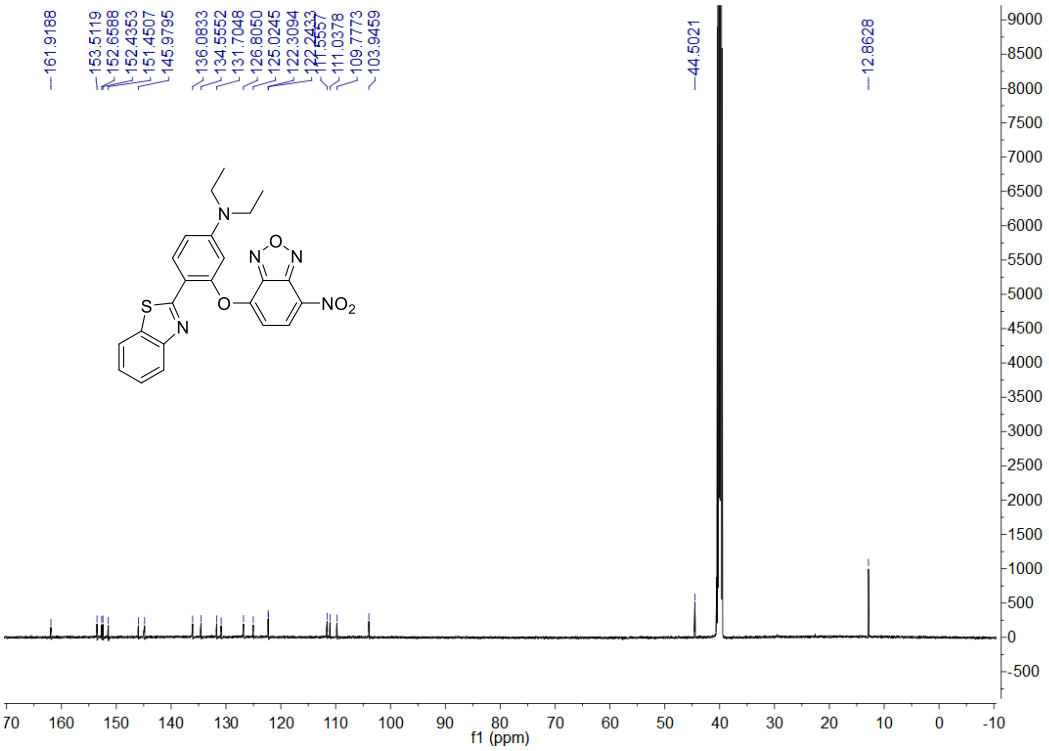

Figure S10. ^{13}C NMR of compound **CysW-1** (600 MHz, in DMSO-d_6).

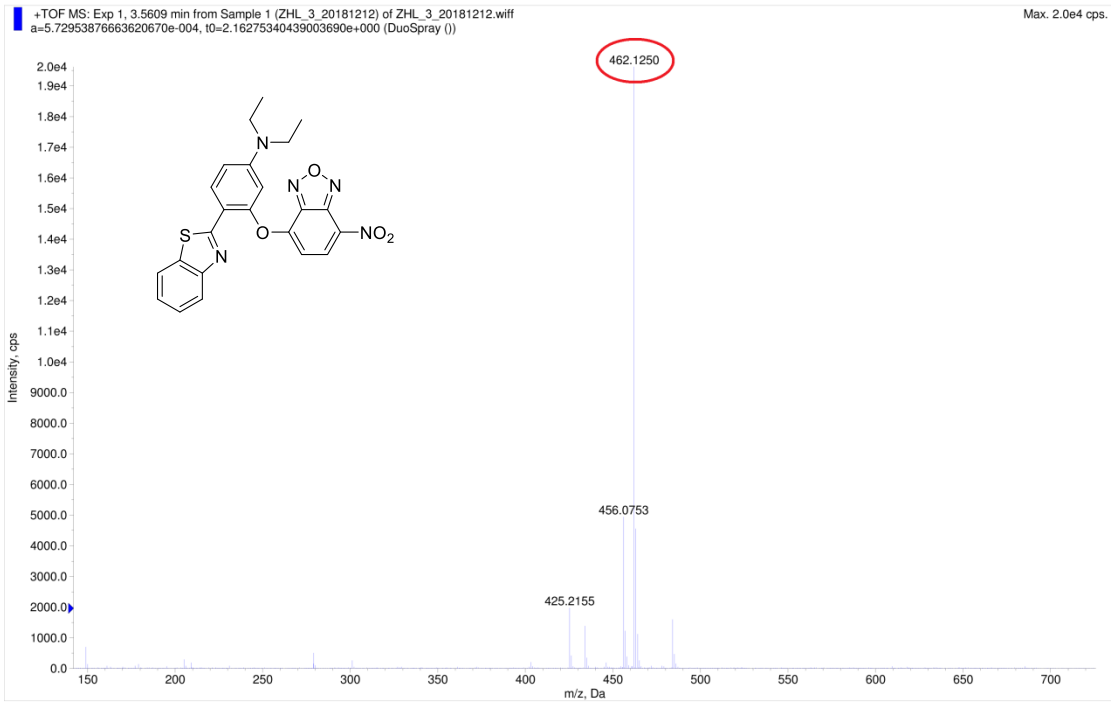

Figure S11. HR-MS spectrum of **CysW-1** in acetonitrile.

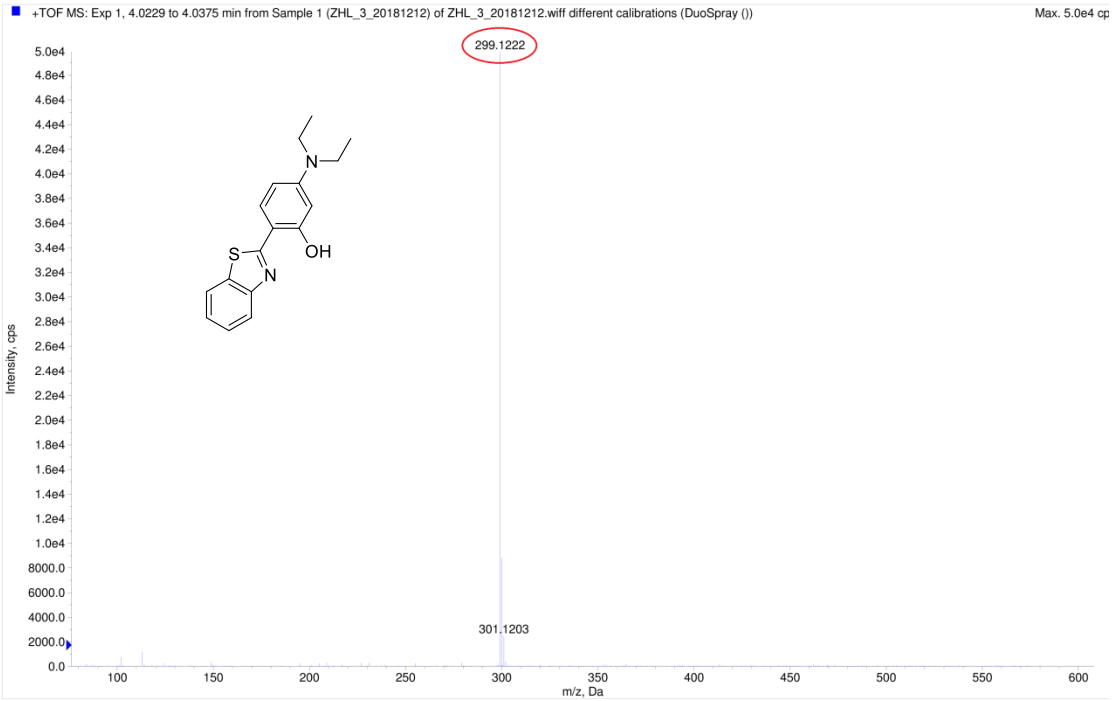

Figure S12. HR-MS spectrum of **Prod-B** in acetonitrile.

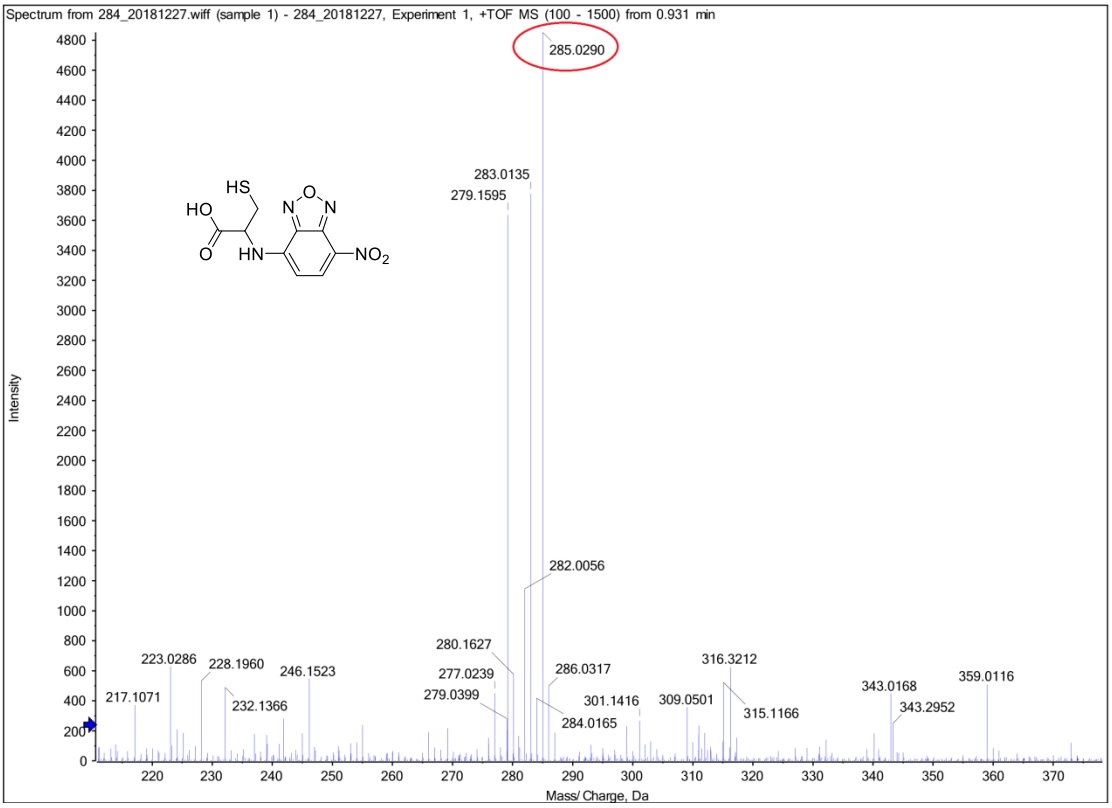

Figure S13. HR-MS spectrum of **Prod-Y** in acetonitrile.