Supporting Information

Rational design of large Stokes shift xanthene-benzothiozolium dyad for probing cysteine in mitochondria

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Contents

Materials and instruments

All reagents and organic solvents used in this work were analytical grade and were purchased from Aladdin Ltd, and were used directly unless otherwise stated. The silica gel (200-400 mesh) used in the column chromatography was purchased from Qingdao Ocean Chemicals. Spectra were measured by using UV-2550 UV/Vis spectrophotometer (Hitachi Japan) and F-4600 fluorescence spectrophotometer (Hitachi Japan). The chemical structures were characterized by nuclear magnetic resonance (NMR) spectra (Bruker AVANCE III 400 M/300 M) and high resolution mass spectra (Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA)), respectively. The pH was examined by FE 20/EL 20PH meter (Mettler-Toledo Instruments (Shanghai) CO., Ltd.). Cell imaging was carried out by the Olympus FV 1000-IX81 laser scanning confocal microscope.

Synthesis of PhCy:

The potassium acetate (1.0 g, 10 mmol), 2-chloro-1-formyl-3-(hydroxymethylene)cyclohex-1-ene 4 (0.85 g, 5 mmol) and 1-ethyl-2,3,3-trimethylbenzoindoleninium tetrafluoroborate (3.25 g, 10 mmol) was added to acetic anhydride (30 mL), then the solution was heated to 70 °C for 1 h. After cooling to room temperature, the solvent was evaporated, and the residue was purified by silica gel flash column to obtain brick red solid **PhCy** (2.5 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.47 (d, J = 14.1 Hz, 2H), 8.14 (d, J = 8.5 Hz, 2H), 8.03 – 7.92 (m, 4H), 7.63 (t, J = 7.6 Hz, 2H), 7.49 (t, J = 8.2 Hz, 4H), 6.27 (d, J = 14.2 Hz, 2H), 4.40 (q, J = 6.8 Hz, 4H), 2.79 (t, J = 5.6 Hz, 4H), 2.03 (s, 12H), 1.53 (t, J = 7.0 Hz, 6H).

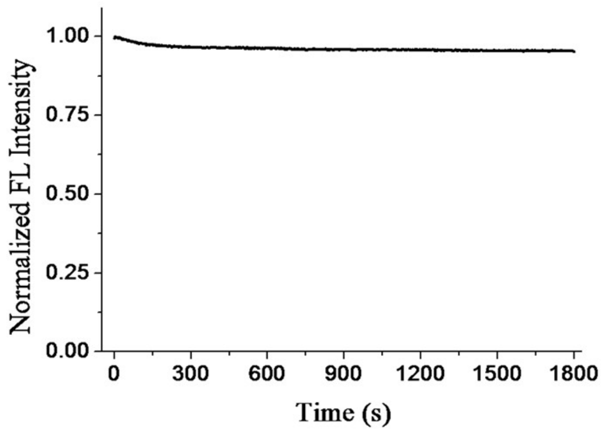


Fig. S1 Time-dependent fluorescence intensity of **PhCy-OH** in PBS buffer (10 mM, pH 7.4, containing 20% EtOH). λ_{ex} = 730 nm, slit = 10/10 nm.

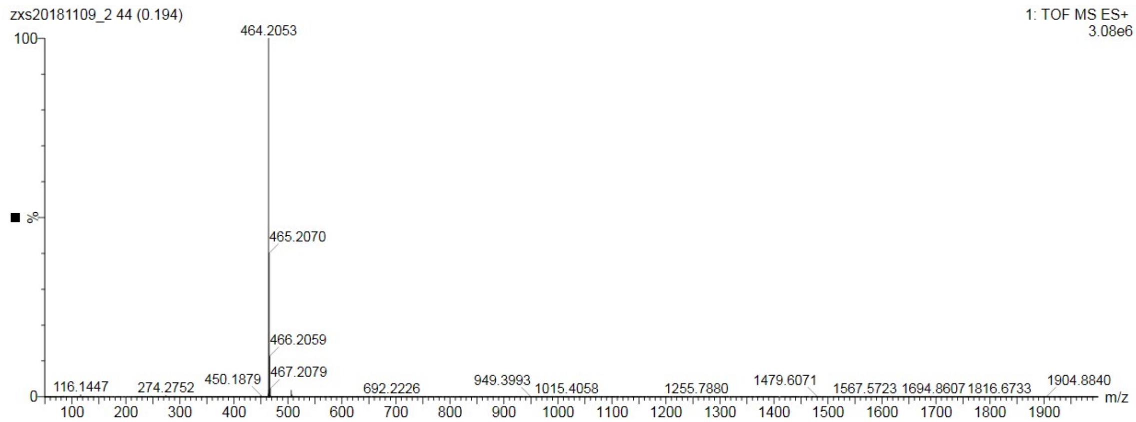
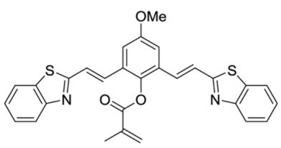
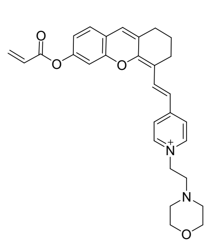
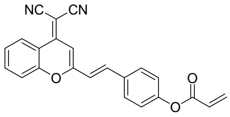
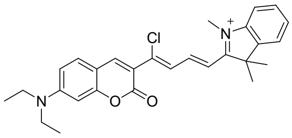
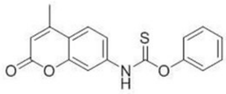
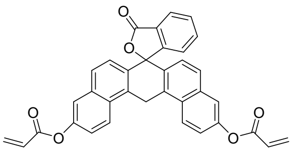
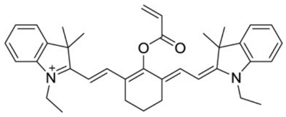
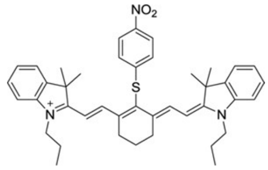
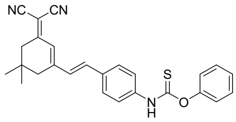
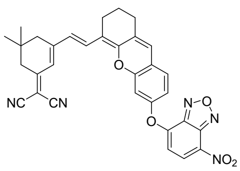
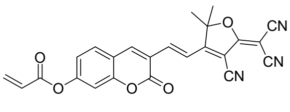
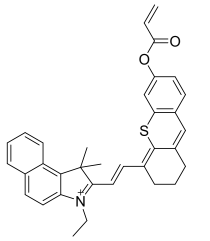


Fig. S2 HRMS spectrum of **PhCy-Cys** recorded after reaction with Cys.

Table S1. Comparisons of **PhCy-Cys** with the reported Cys probes.

No.	Structures	$\lambda_{em}/\lambda_{abs}$ (*nm*)	LOD (*nM*)	Time (*min*)	Ref.
1		710/396	500	120	*Front. Chem. 2019, 7, 32*
2		674/530	960	--	*Talanta 2019, 204, 747–752*

3		700/557	200	70	*ACS Sens. 2016, 1, 882−887*
4		725/578	2965	60	*Anal. Chem. 2019, 91, 1472−1478*
5		443/340	160	14	*Anal. Chem. 2019, 91, 8591–8594.*
6		690/612	180	5	*Biosensor. Bioelector. 2015, 68, 316–321*
7		794/750	90	30	*Sens. Actuators, B Chem. 2019, 282, 69–77*
8		750/650	126	60	*RSC Adv. 2014, 4, 8360–8364*
9		660/450	79	60	*Talanta 2021, 223, 121758*
10		756/588	1010	8	*Dye. Pigment. 2021, 186, 109015*
11		675/584	200	10	*Dye. Pigment. 2017, 146, 103-111*
12		**803/735**	**166**	**10**	***This work***

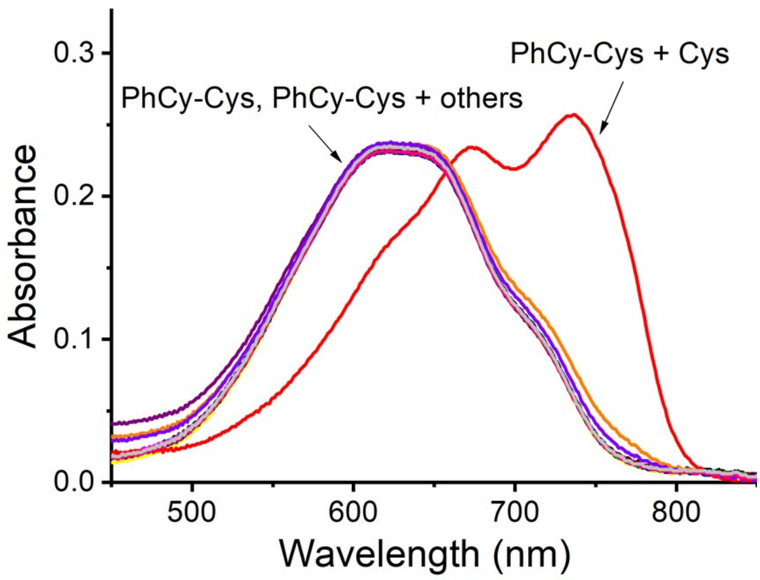


Fig. S3 Absorption spectra of **PhCy-Cys** upon addition of analytes (100 μM): Cys, Hcy, GSH (5.4 mM), H_2S, Gly, Ser, Met, Val, Leu, Tyr, His, Trp, Arg, Ala, Glu, Pro, Thr, and Phe.

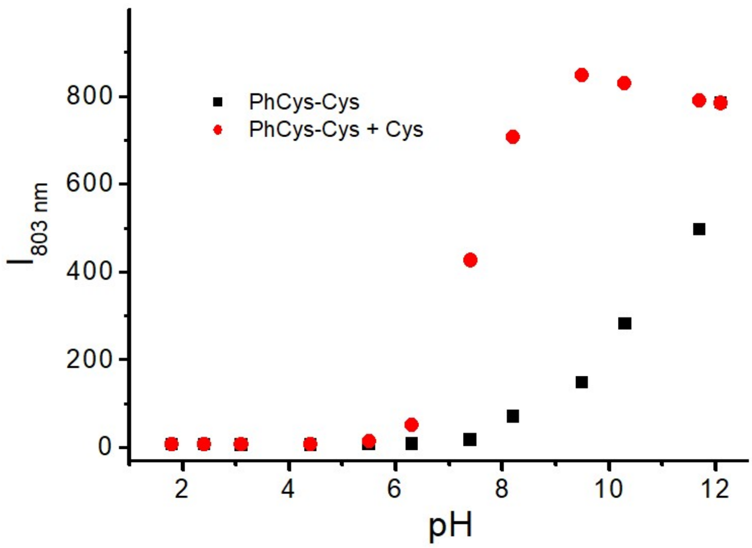


Fig. S4 pH-dependent fluorescence changes of **PhCy-Cys** in the absence/presence of Cys (100 μM). The conditions: PBS buffer (10 mM, pH 7.4, containing 50% EtOH), λ_{ex} = 730 nm, slit = 10/10 nm.

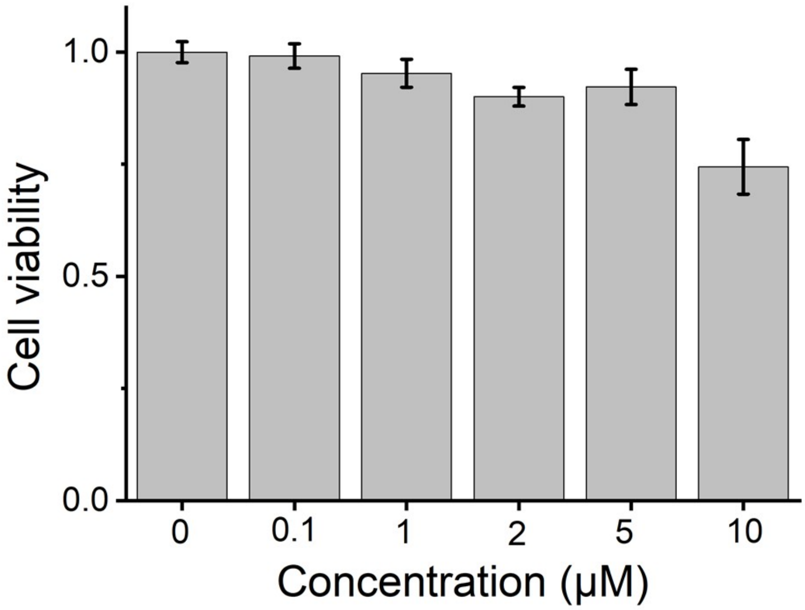


Fig. S5 MTT assay for the survival rate of HeLa cells treated with **PhCy-Cys** for 24 h. Error bars represent the standard deviations of 5 trials.

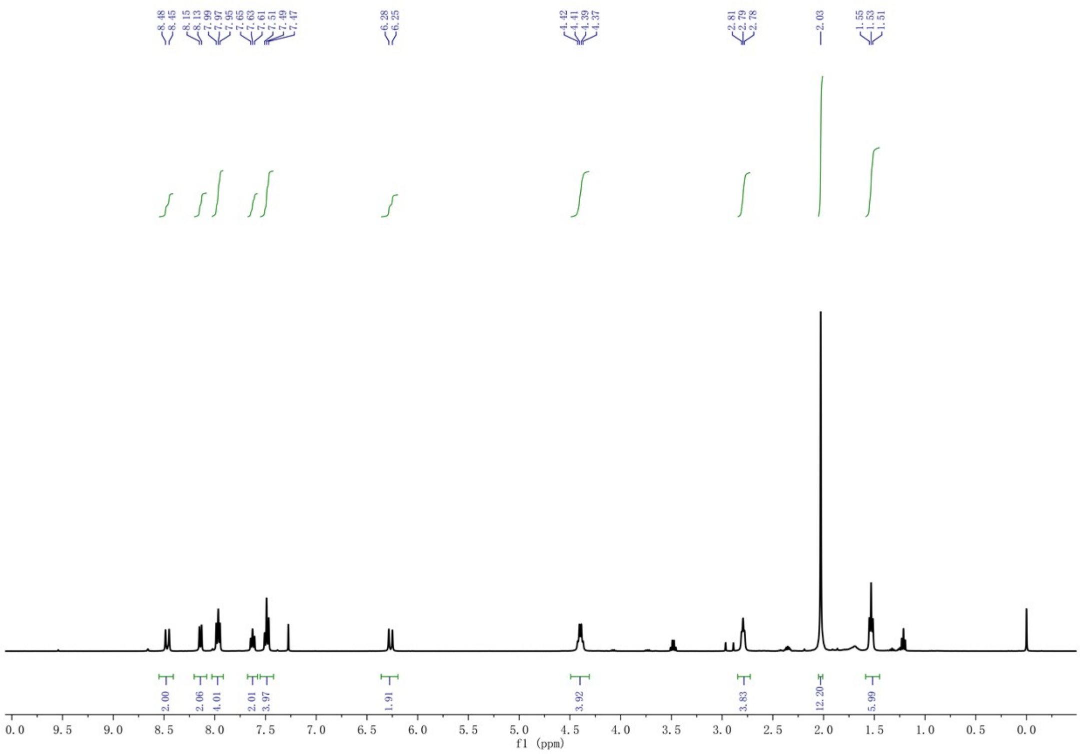


Fig. S6 ^{1}H NMR spectra of compound **PhCy** in $CDCl_3$.

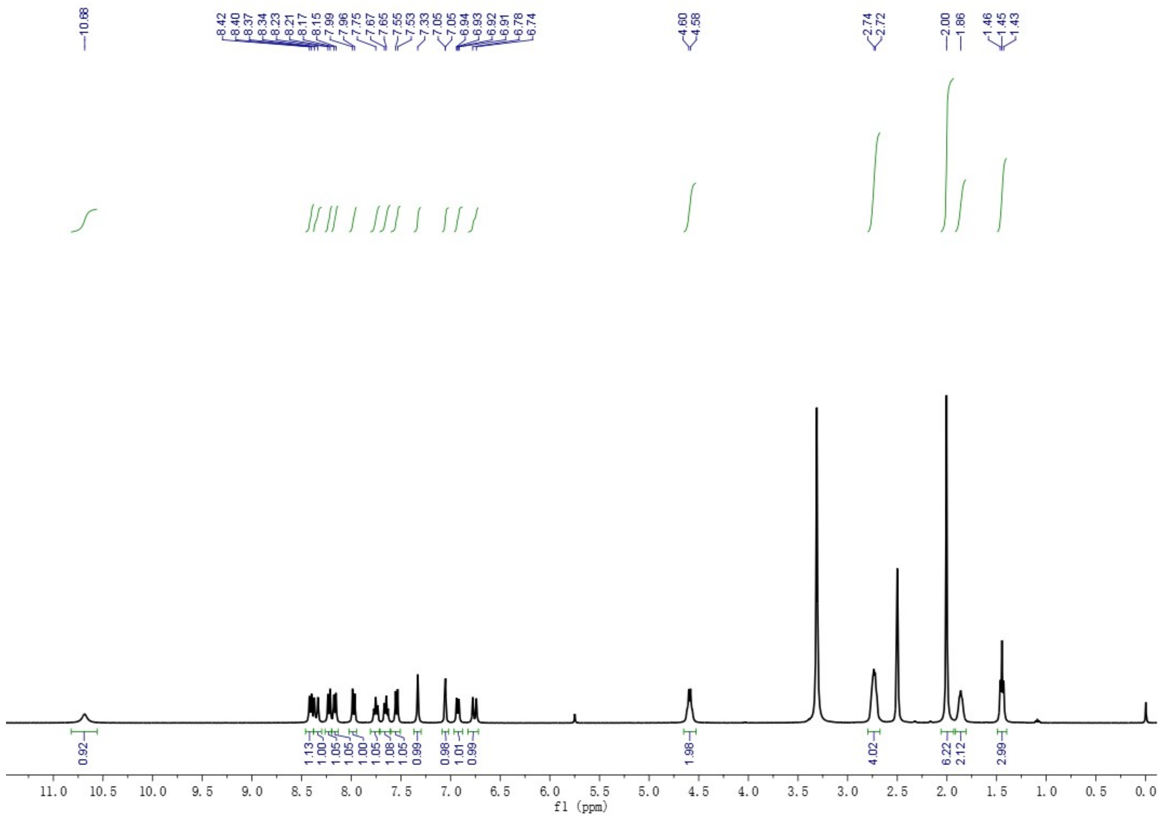


Fig. S7 ^{1}H NMR spectra of compound **PhCy-OH** in DMSO-d_6.

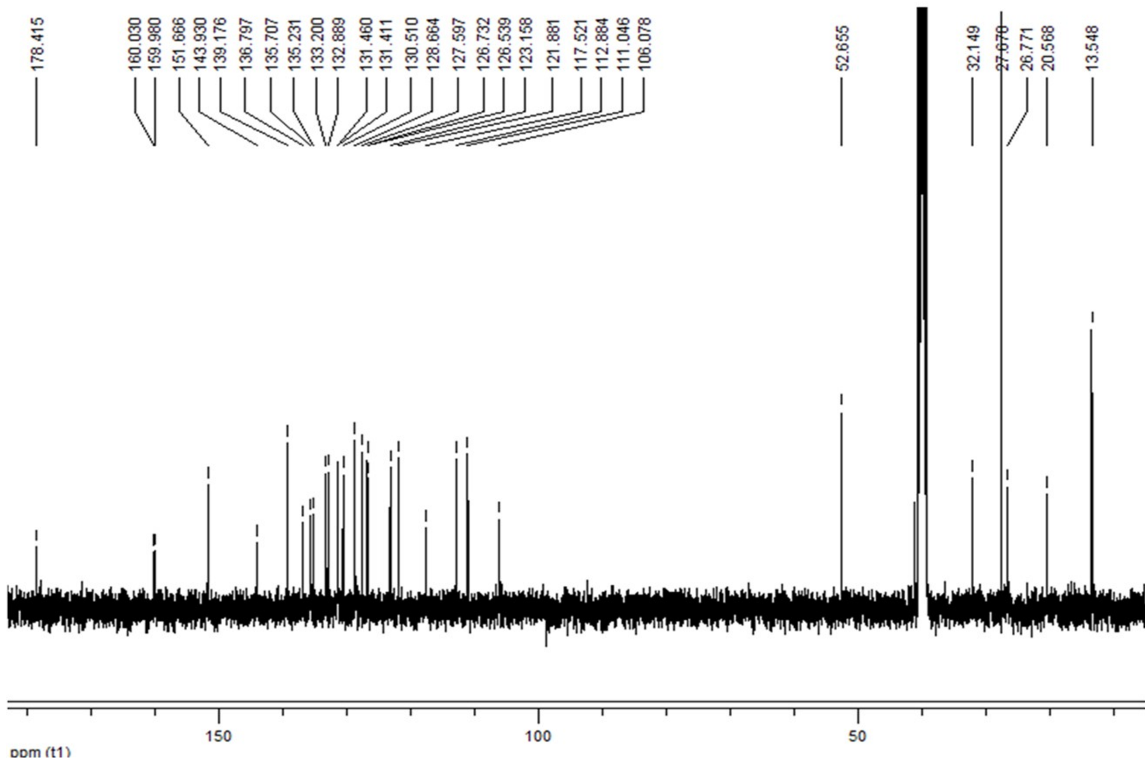


Fig. S8 ^{13}C NMR spectra of compound **PhCy-OH** in DMSO-d_6.

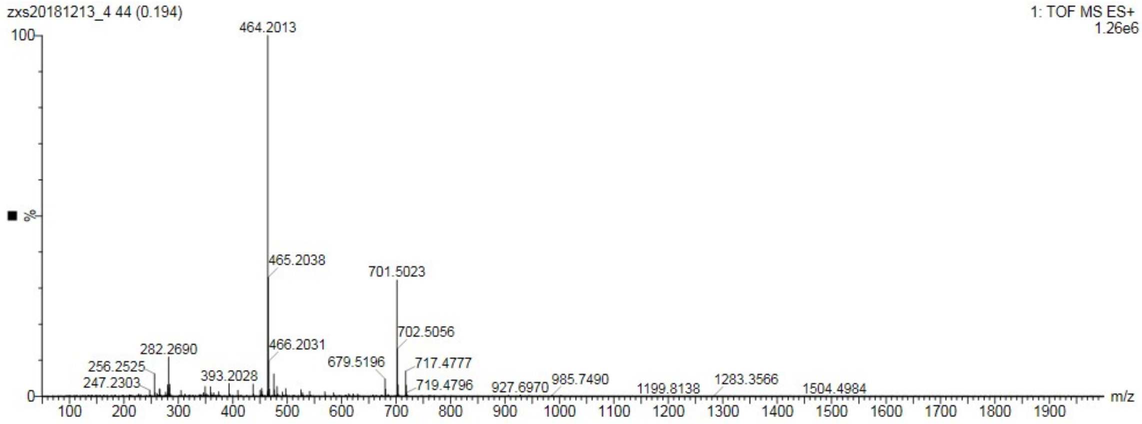


Fig. S9. HRMS spectrum of compound **PhCy-OH**.

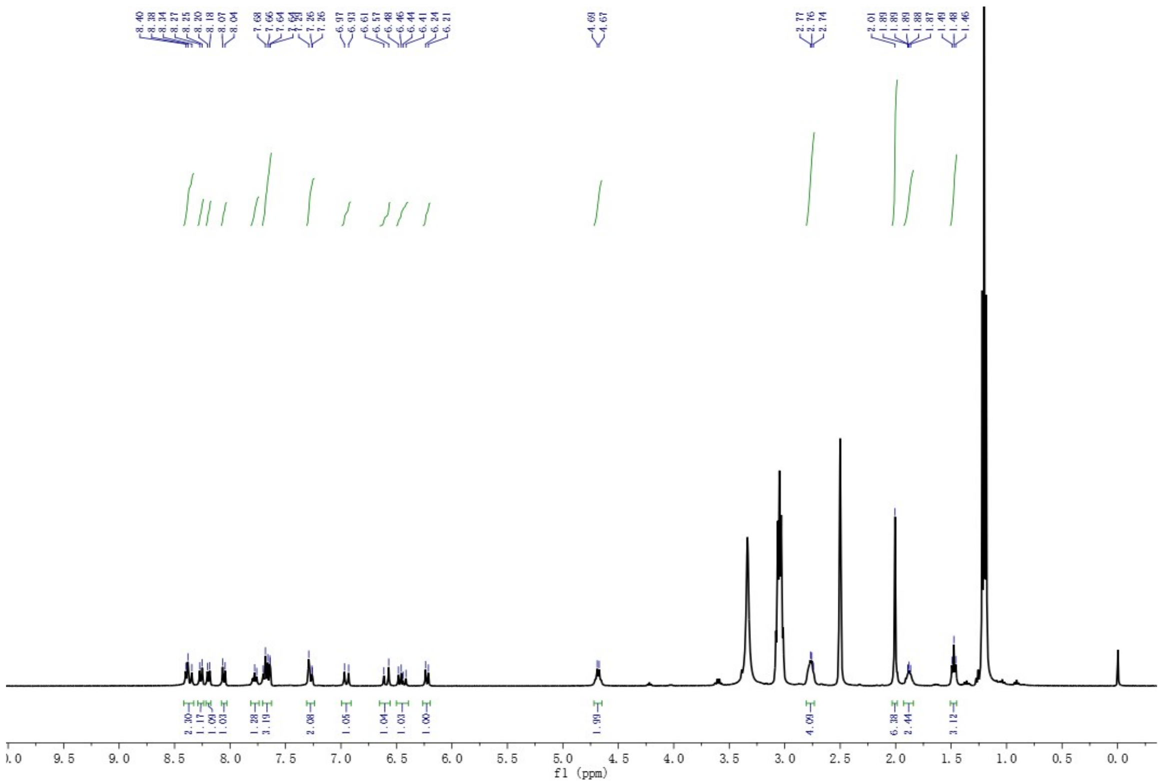


Fig. S10 ^{1}H NMR spectra of compound **PhCy-Cys** in DMSO-d_6.

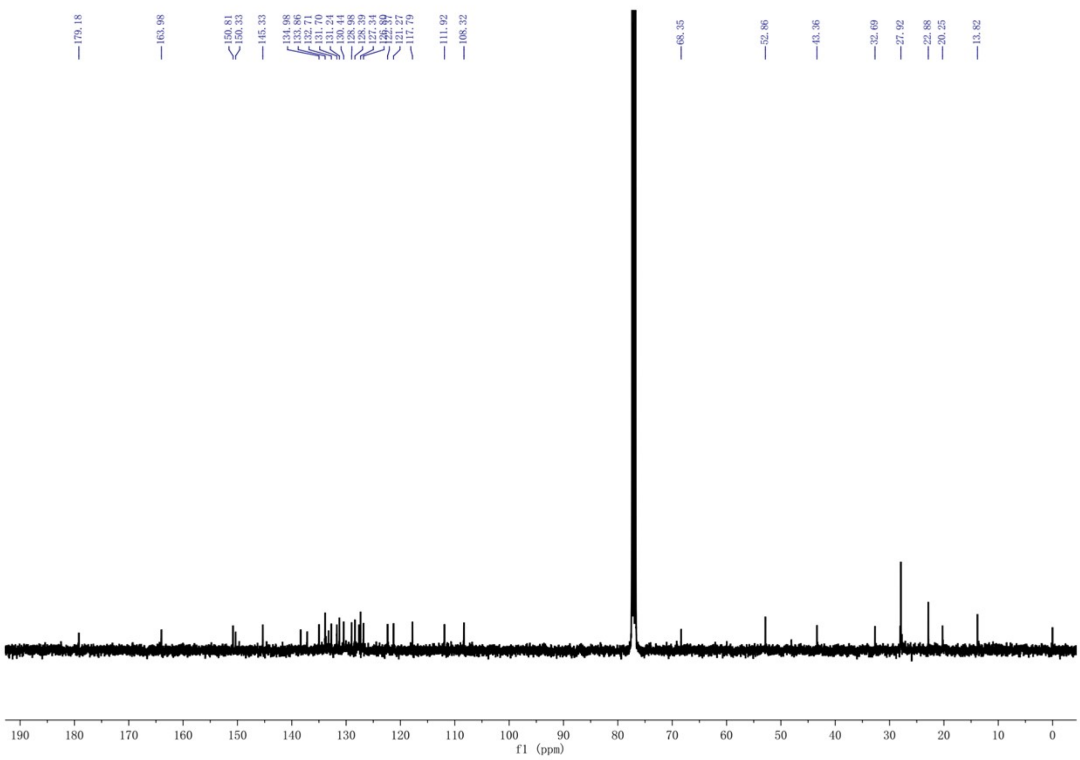


Fig. S11 ^{13}C NMR spectra of compound **PhCy-Cys** in DMSO-d_6.

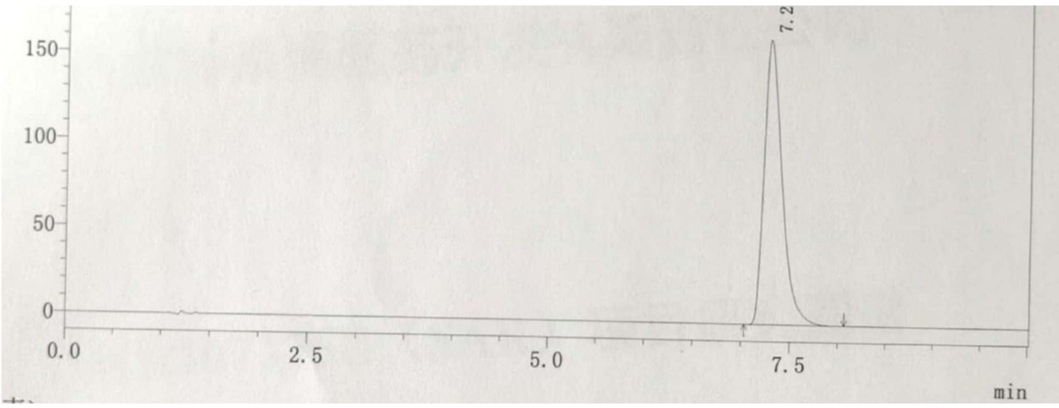


Fig. S12. HPLC of compound **PhCy-Cys.**

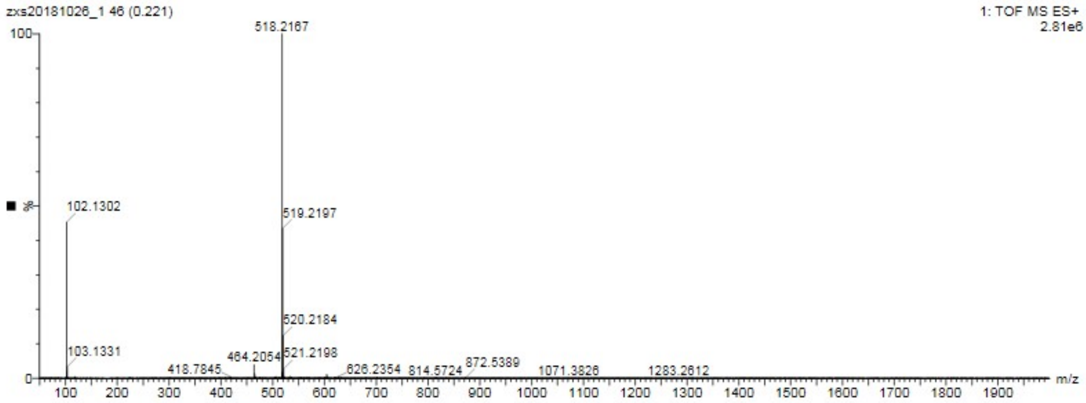


Fig. S13 HRMS spectra of compound **PhCy-Cys**.