Supporting information

A novel NIR fluorescent probe with ratiometric imaging of cysteine in Endoplasmic Reticulum

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Contents

Experimental part

1. Apparatus

Absorption spectra were accurately measured on a HP-8453 UV/Vis (Agilent) spectrometer. Fluorescence spectra were measured on the F-4500 Spectrophotometer and the EX Slit and EM Slit were both set at 10.0 nm. The pH was measured by a Model PHs-3C meter (Shanghai, China). ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker DTX-400 spectrometer using TMS as internal reference. HR-MS (high-resolution mass spectrometry) spectra were collected using the Q-T of HR-MS spectrometer (Waters Micromass). Cells were imaged using LEICA TCS SP8 laser scanning confocalmicroscope.

2. Materials

All the reagents were purchased from reagent companies without further purification and directly used in the experiment. The deionized water was purified by Milli-Q. The probe was put into 10 mL DMSO to get a 1mM stock solution. Then it was diluted to 10 μM in 10 mM HEPES solution with 1 mM CTAB for spectroscopic determination. The interfering ions include: Na^{+}, K^{+}, Fe^{2+} , F^{-}, Br^{-}, I^{-}, CH_3COO^{-}, CO_3^{2-}, HCO_3^{-}, HPO_4^{2-}, $H_2PO_4^{-}$, SO_4^{2-}, NO_3^{-}, NO_2^{-}, HS^{-}, SO_3^{2-}, HSO_3^{-}, GSH, Hcy, Cys, NO, HNO, $^{1}O_2$, H_2O_2, HClO, ·OH,$ONOO^{-}$.

3. Cell culture

HeLa cells were inoculated in 96-well plates, cultured in 5% CO_2, 37℃for 24 h, removed the old medium, washed with PBS for 3 times. Then, the preprepared medium containing different concentrations of probe **HL-Cys** (0, 5 μM, 10 μM, 15 μM and 20 μM) was added and placed in an incubator for further culture for 24 h. Cell Counting Kit (CCK-8) staining was used to evaluate the cytotoxicity of probe **HL-Cys**.Cell survival rate = experimental group/control group × 100%.

4. Synthesis

Compound **1** and Compound **4** was synthesized according to the previous literatures[a,b]

Synthesis of Compound **2**:Compound **1** (242 mg, 1.3 mmol), 5-Formylsalicylic Acid (166 mg, 1 mmol) and piperidine (0.1mL) were dissolved in 7 mL EtOH under argon atmosphere, the mixture was refluxed 10 h. At the end of the reaction, the solvent was removed under reduced pressure and the residue was purified by column chromatography with dichloromethane: methanol (30:1) as the eluent to afford the desire product as a red solid (230 mg, yield 69%). ^{1}H NMR (400 MHz, DMSO-d_6)δ(ppm):8.06(d,*J*=2.12,1H),7.73(dd,*J*=8.6,2.16Hz,1H),7.24(q,*J*=16.08Hz, 2H),6.83(t,*J*=4.64Hz,2H),2.59(s,2H),2.53(s,2H),1.01(s,6H).^{13}CNMR(100MHz,DMSO-d_6)δ(ppm):171.9,170.7,164.8,157.2,138.7,133.3,131.4,126.7,126.0,121.8,118.1,117.6, 114.6, 113.8, 75.2, 42.8, 38.6, 32.1, 27.9.HR-MS: *m/z* calcd for $C_{20}H_{18}N_2O_3$[M - H]$^-$: =333.1244, found: 333.1250.

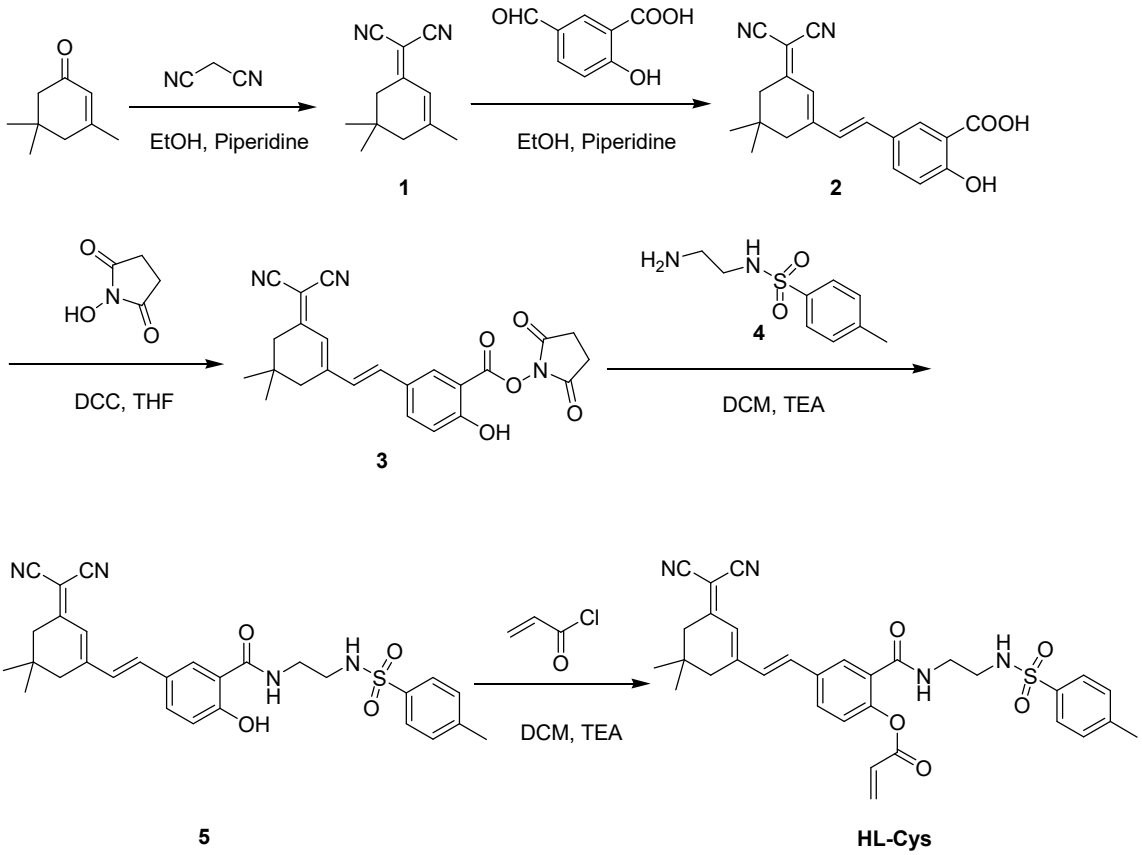
Synthesis of Compound **3**: Compound **2** (100 mg, 0.3 mmol) and N-hydroxysuccinimide (41.4mg,0.36 mmol) were dissolved in THF (4 mL) was added DCC (74.2 mg, 0.36 mmol). The mixture was stirred at room temperature for 30 min. At the end of the reaction, the solvent was removed under reduced pressure and the residue was purified by column chromatography with Petroleum ether: dichloromethane (1:1) as the eluent to afford the desire product as a yellow solid (40 mg, yield 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.21 (s, 1H), 8.12 (d, *J* = 1.88 Hz, 1H), 8.00 (dd, *J* = 1.88, 1.92 Hz, 1H), 7.38 - 7.29 (m, 2H), 7.11 (d, *J* = 8.72 Hz, 1H), 6.87 (s, 1H), 2.90 (s, 4H).2.61 (s, 2H), 2.54 (s, 2H), 1.01 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 170.9, 170.8, 161.1, 161.0, 156.5, 136.8, 135.0, 132.4, 128.6, 128.0, 122.8, 119.0, 114.4, 113.6, 112.0, 76.4, 42.7, 38.6, 32.1, 27.9, 26.0.HR-MS: *m/z* calcd for $C_{24}H_{21}N_3O_5$[M - H]$^-$: = 430.1408, found: 430.1413.

Synthesis of Compound **5**: Compound **3** (86.2 mg, 0.2 mmol) and Compound **4** (55.6mg, 0.3 mmol) were dissolved in DCM (6 mL) was added triethylamine(0.06 mL). The mixture was stirred at room temperature for 2 h. At the end of the reaction, the solvent was removed under reduced pressure and the residue was purified by column chromatography with dichloromethane: methanol (100 :1) as the eluent to afford the desire product as an orange solid (91 mg, yield 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.77 (s, 1H), 8.87 (t, *J* = 5.56 Hz, 1H), 8.15 (d, *J* = 1.88 Hz,1H) 7.76 – 7.72 (m, 2H), 7.69 (s, 1H), 7.67 (s, 1H), 7.36 (s, 1H).7.34 (s, 1H), 7.23 (s, 2H),

6.95 (d, *J* =8.60 Hz, 1H), 6.81 (s, 1H), 3.37 (q, *J* = 6.28 Hz, 2H), 2.94 (q, *J* = 6.28 Hz, 2H), 2.62 (s, 2H),2.54 (s,2H),2.34(s,3H),1.02(s,6H).^{13}CNMR(100MHz,DMSO-d_6)δ(ppm):170.6,169.1,161.7,156.4,143.1,137.9,137.6,134.0,130.7,127.9,127.8,127.3,127.0,122.4,118.5,116.1,114.4,76.2,42.7,42.0,38.6,32.1,27.9,21.4.HR-MS:*m/z*calcdfor$C_{29}H_{30}N_4O_4S$[M-H]$^-$:=529.1915,found: 529.1919.

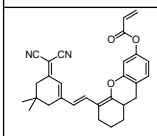
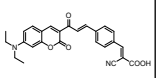
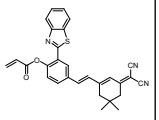
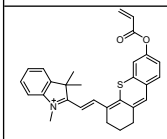
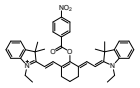
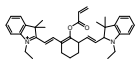
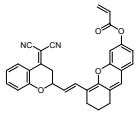
5. References

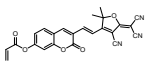
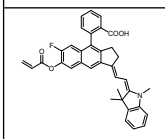
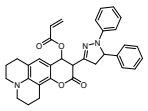
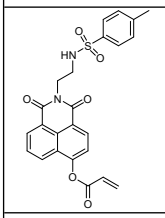
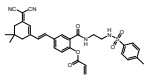
a) Ma Y, Gao W and Ma S, *Anal. Chem.*, 2020, **92**, 13405-13410.
b) Song W, Dong B and Lu Y, *New J. Chem.*, 2019, **43**, 12103-12108.



Scheme S1. Synthetic route of **HL-Cys**

Table S1 Comparison of reported probes for recognition of Cys

Structure	$\lambda_{ex}/\lambda_{em}$ (nm)	Stock's Shifts(nm)	Detection Limit	Response time	Targeting	Rational	References
	590/770	180	0.4μM	10min	-	-	Anal Chimica Acta 2021, 1171, 338655
	445/500	55	0.122μM	200s	-	-	Sens. Actuators B: Chem. 2018, 267, 76-82
	540/714	174	28.6 nM	5min	-	-	Sens. Actuators B: Chem. 2022, 357, 131430
	700/770	70	16 nM	10min	Lyso-	-	J. Mater. Chem. B, 2020, 8, 2269
	560/640	80	0.2 μM	5min	Mito-	F_{640}/F_{785}	Biosensors and Bioelectronics 2015, 74 156-164
	535/635	100	0.09 μM	30min	Mito-	F_{635}/F_{794}	Sens. Actuators B: Chem. 2019, 282, 69-77
	600/760	160	48 nM	5min	-	-	Anal. Chem. 2018, 90, 1014–1020

	481/675	194	0.2 μM	5min	-	F_{675}/F_{530}	Dyes. Pigments, 2017, 146 103-111
	610/716	116	0.083 μM	-	Mito-	F_{716}/F_{660}	Sens. Actuators B: Chem. 2018, 259, 219-225
	441/514	73	8.95 nM	30min	ER	-	Chem Commun 2019, 55, 9629.
	390/550	160	1.8 μM	20min	ER	F_{550}/F_{440}	Anal. Chem. 2019 , 91, 5513–5516
	**410/650**	**240**	**490 nM**	**6min**	**ER**	**F_{650}/F_{545}**	**This work**

Characterization of compounds

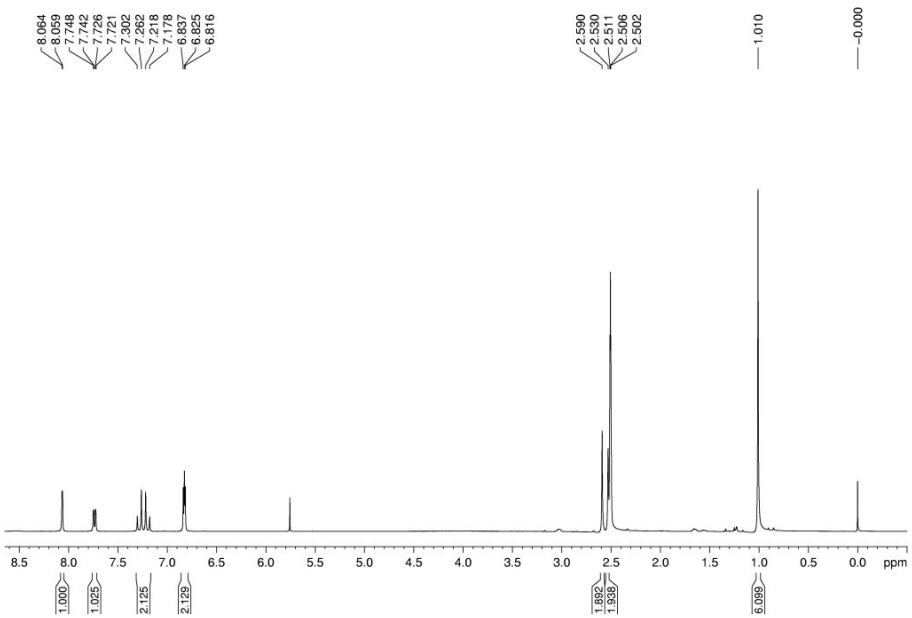


Figure S1. ^{1}H NMR spectra of **2** in DMSO-d_6.

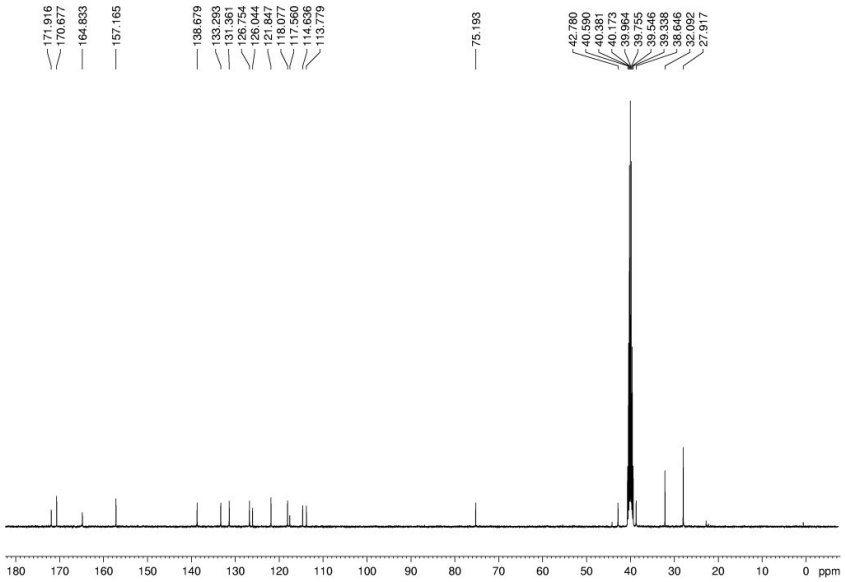


Figure S2. ^{13}C NMR spectra of **2** in DMSO-d_6.

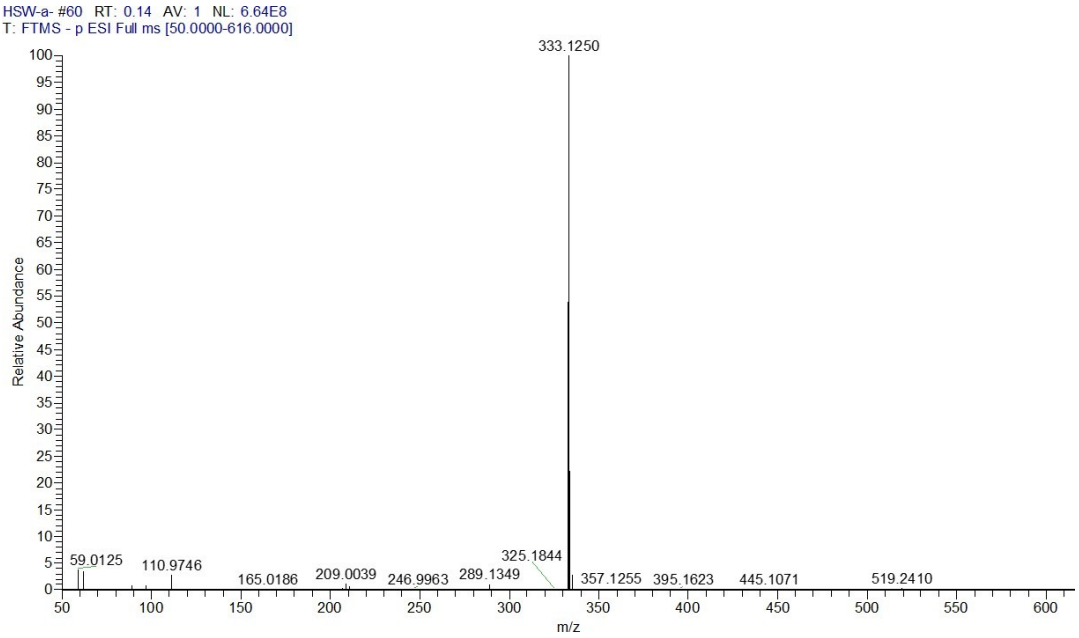


Figure S3. ESI-HRMS spectra of **2**.

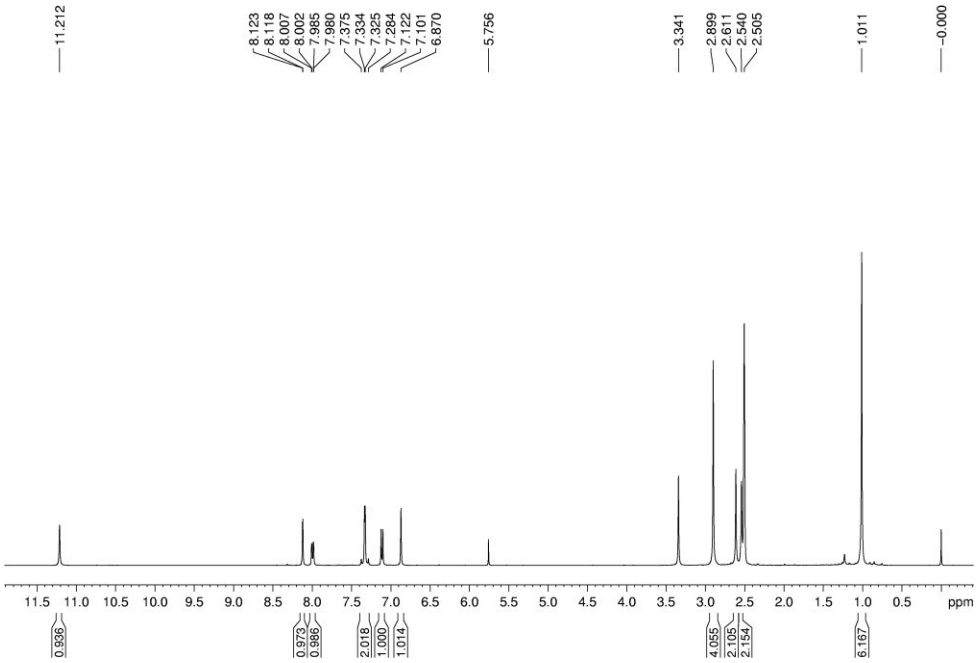


Figure S4. ^{1}H NMR spectra of **3** in DMSO-d_6.

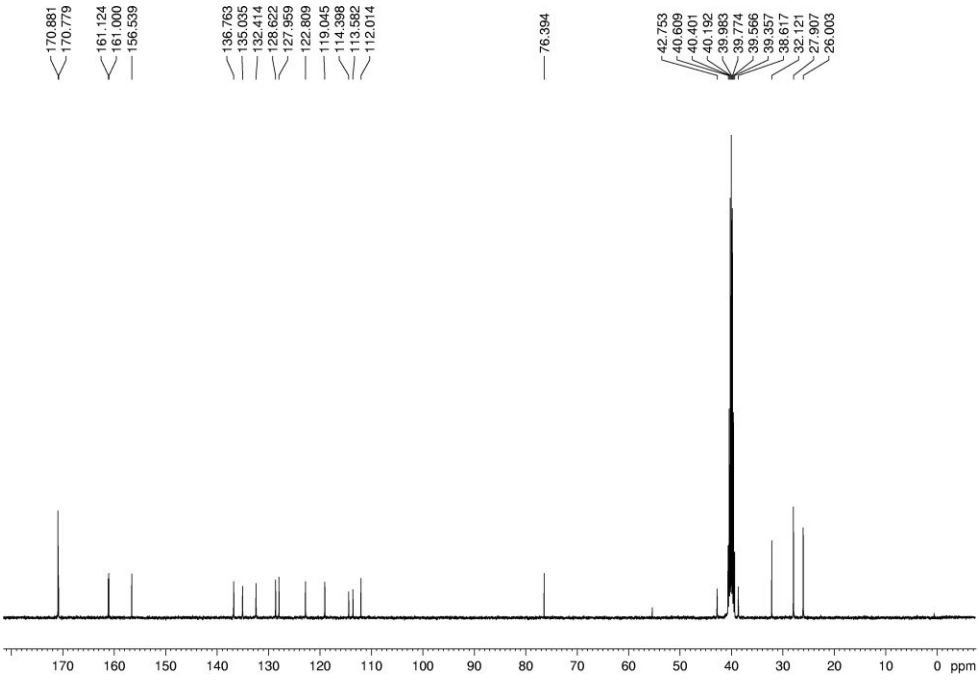


Figure S5. ^{13}C NMR spectra of **3** in DMSO-d_6.

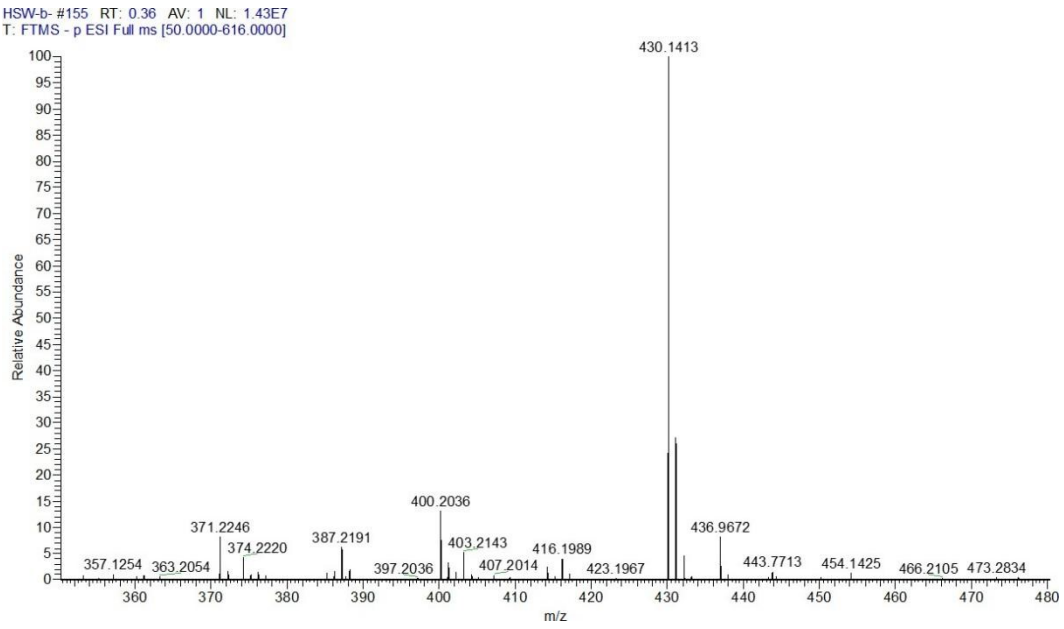


Figure S6. ESI-HRMS spectra of **3**.

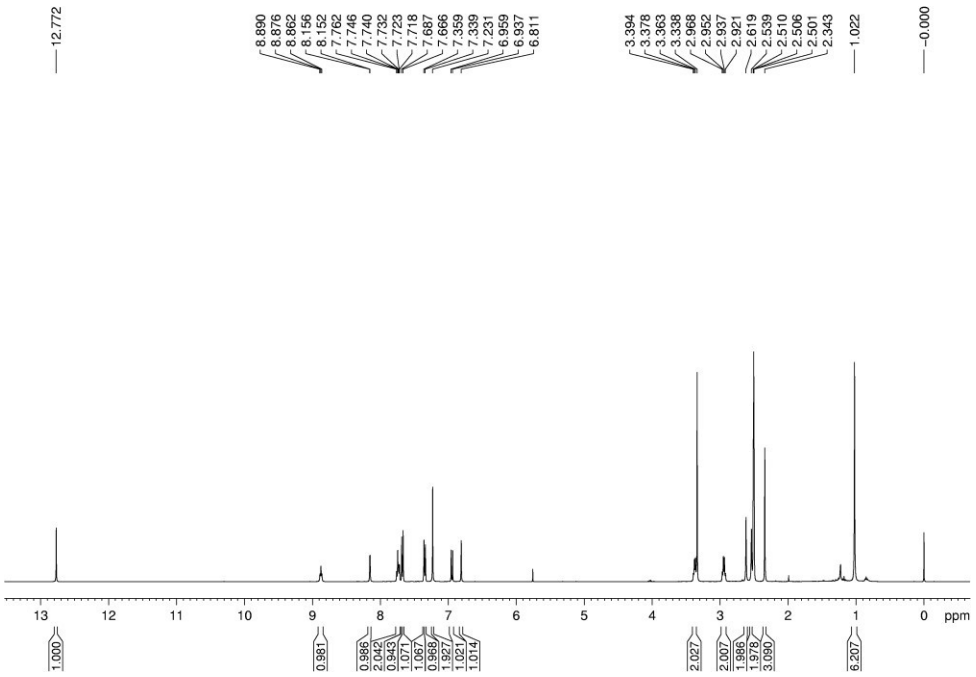


Figure S7. ^{1}H NMR spectra of **5** in DMSO-d_6.

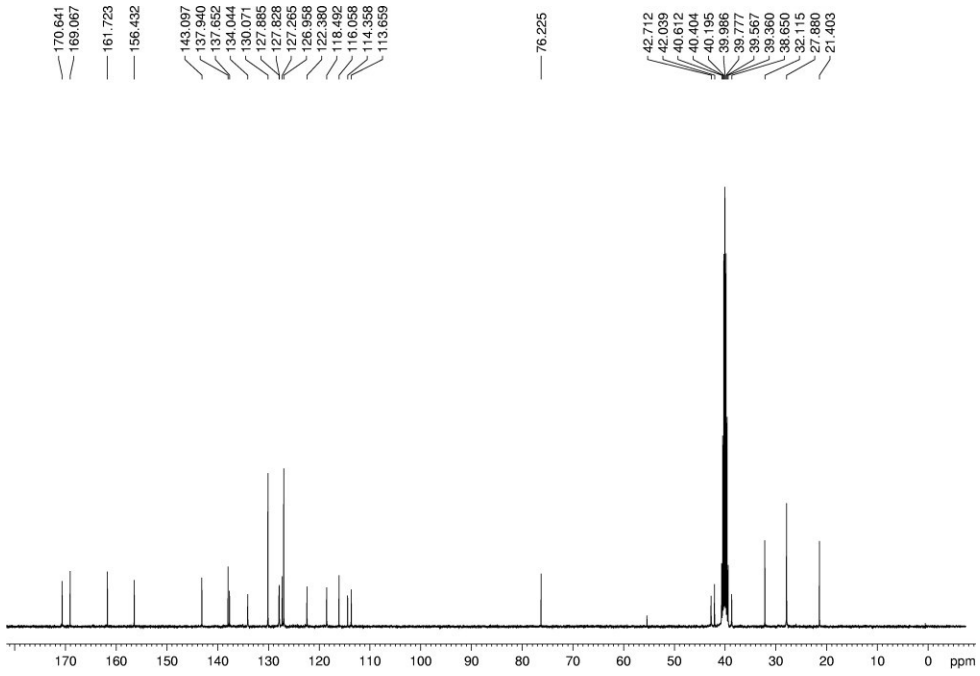


Figure S8. ^{13}C NMR spectra of **5** in DMSO-d_6.

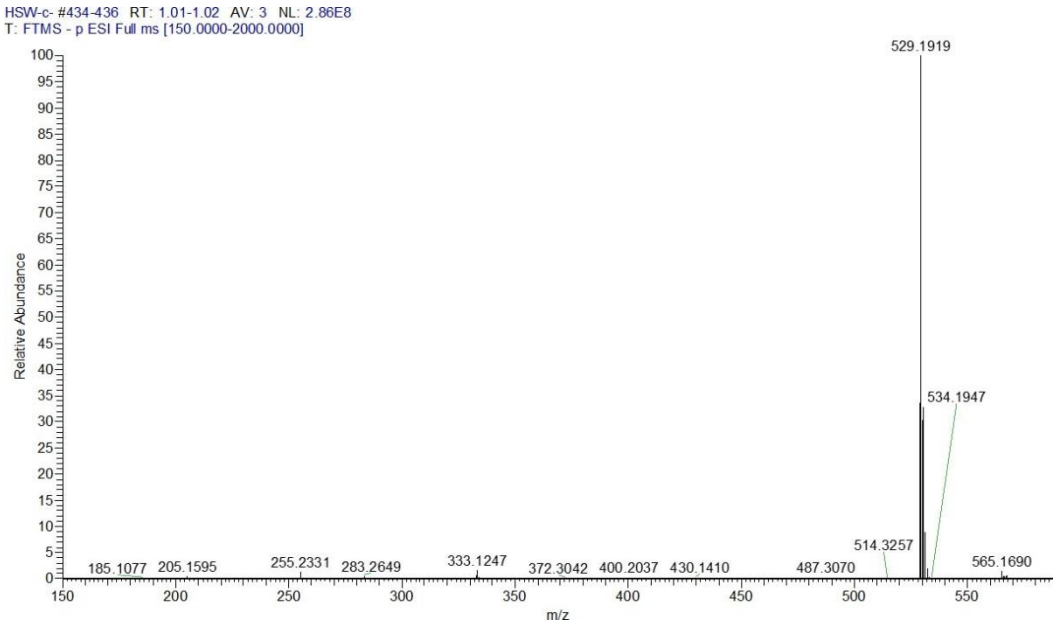


Figure S9. ESI-HRMS spectra of **5**.

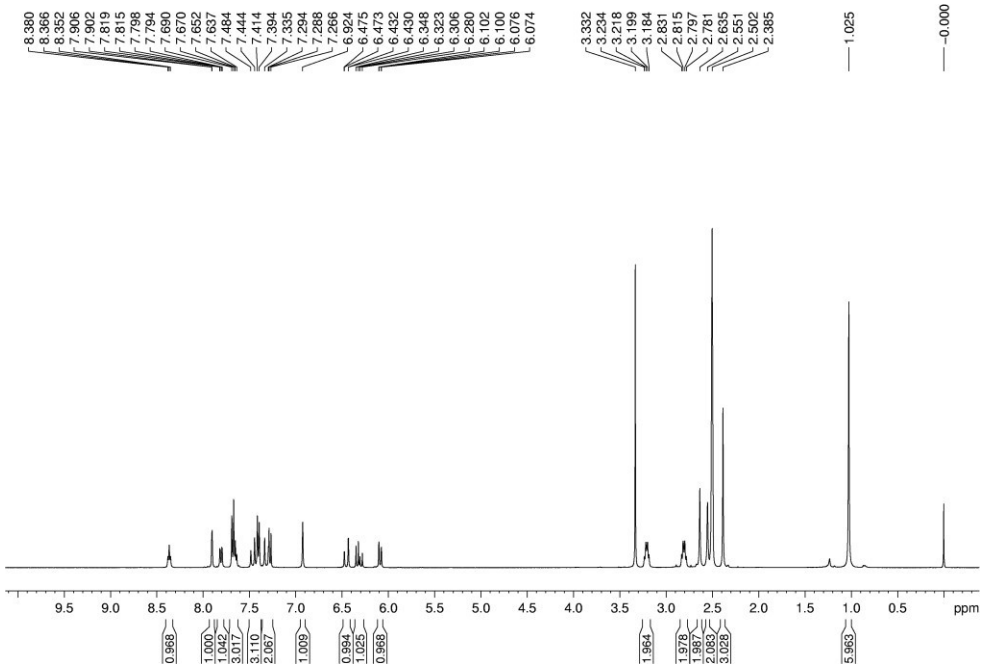


Figure S10. ^{1}H NMR spectra of **HL-Cys** in DMSO-d_6.

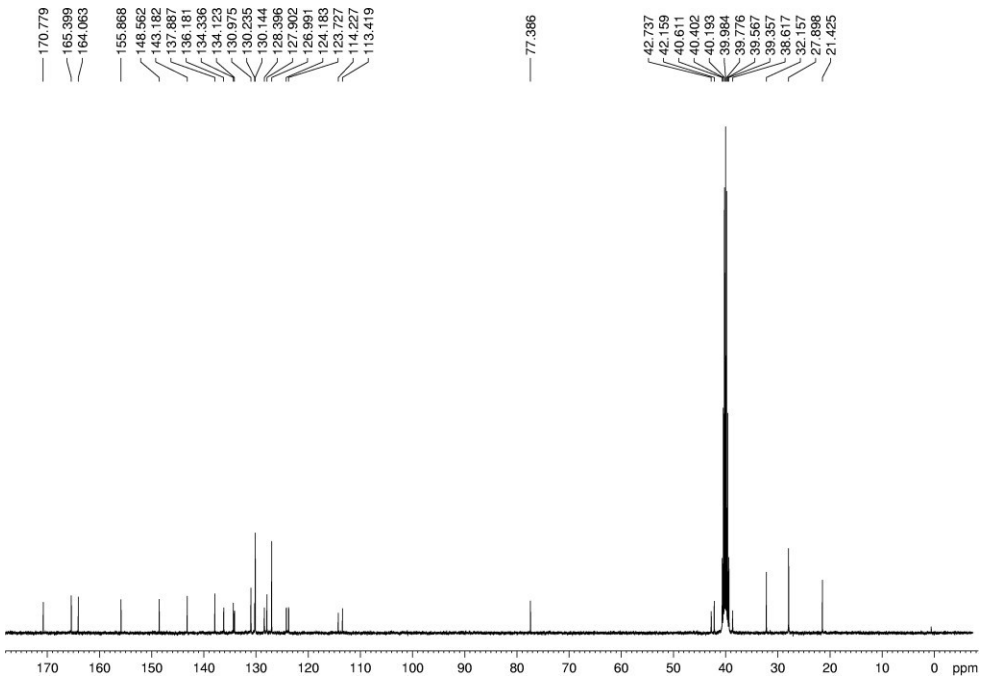


Figure S11. ^{13}C NMR spectra of **HL-Cys** in DMSO-d_6.

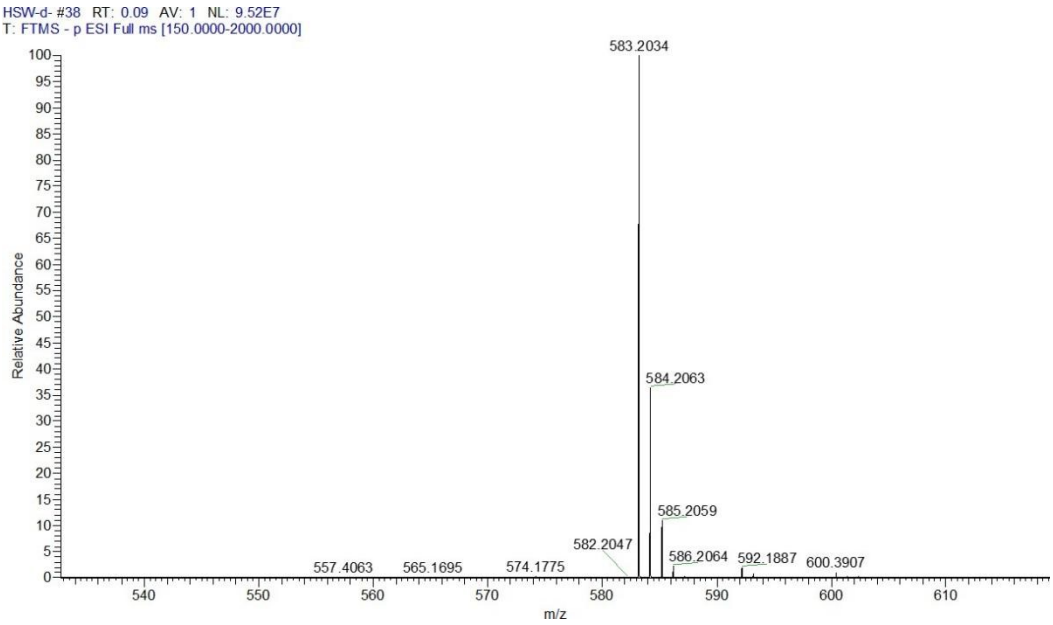


Figure S12. ESI-HRMS spectra of **HL-Cys**.

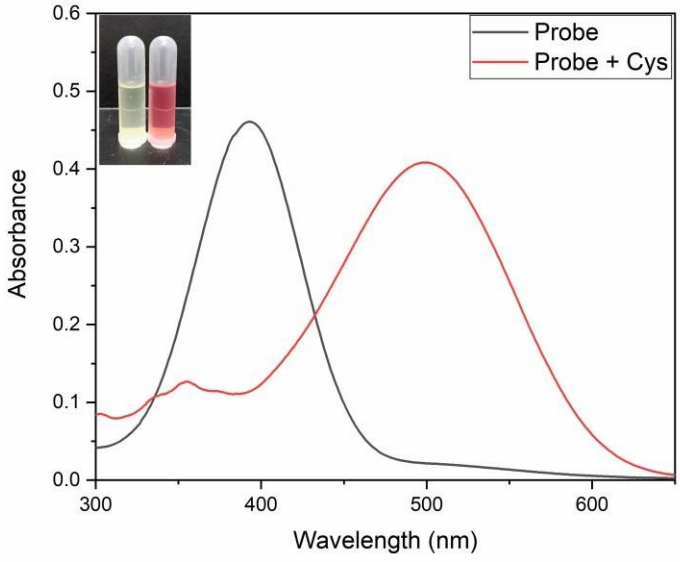


Figure S13. UV−vis absorption spectra of **HL-Cys** (10 μM) before and after reaction with Cys (100 μM)in PBS buffer solution (containing 30% DMSO, 10mM, pH=7.4). Inset: left,**HL-Cys**(10 μM); right, **HL-Cys** (10 μM) + Cys (100 μM).

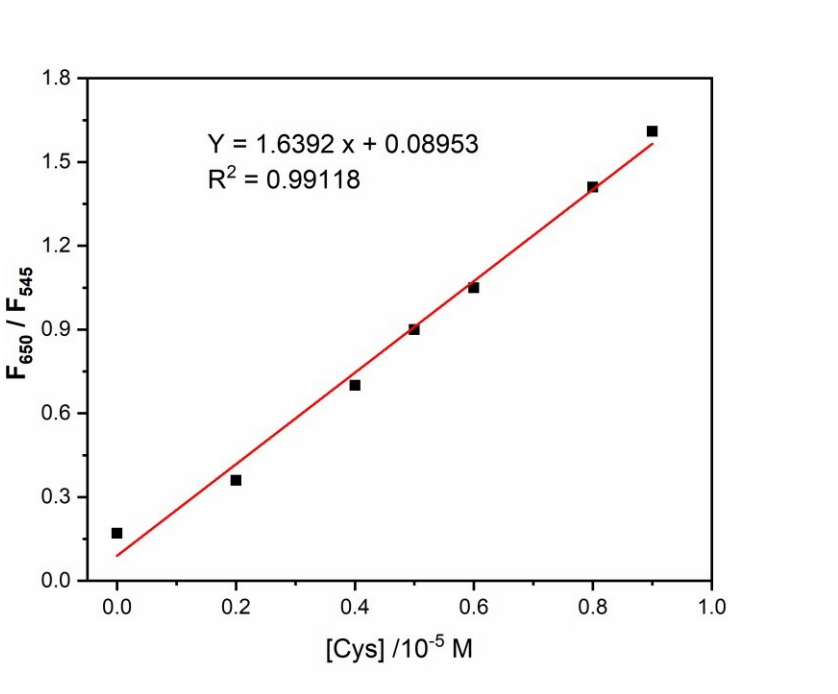


Figure S14. The linear fitting of fluorescence intensity ratio (F_{650}/F_{545}) against concentrations of Cys (0-9 μM) in PBS buffer solution (containing 30% DMSO, 10mM, pH = 7.4).

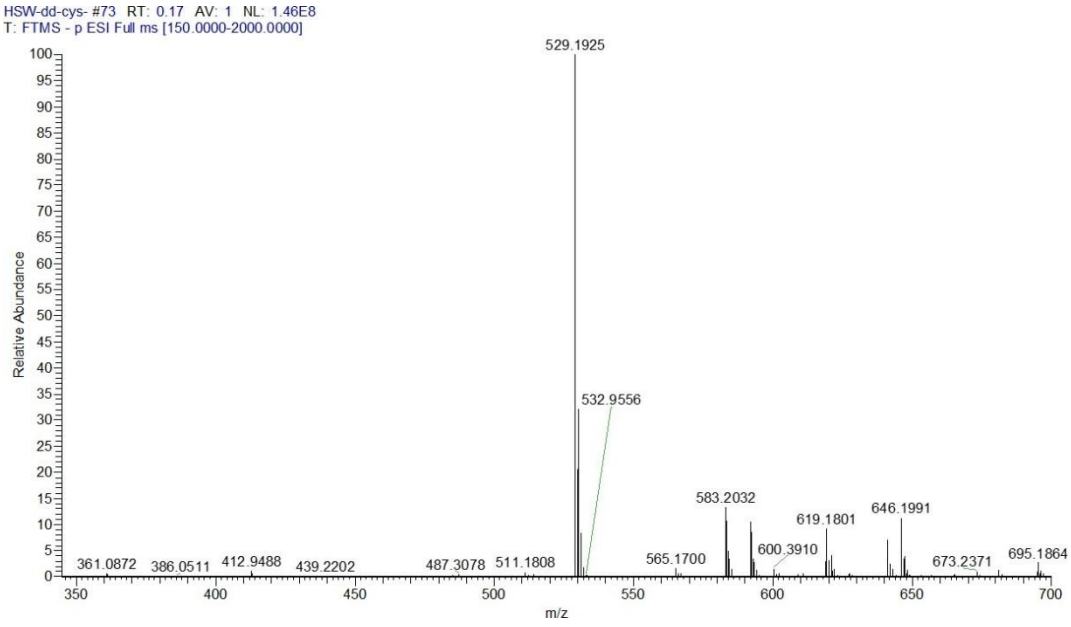


Figure S15. HR-MS spectra of **HL-Cys** in response to Cys.

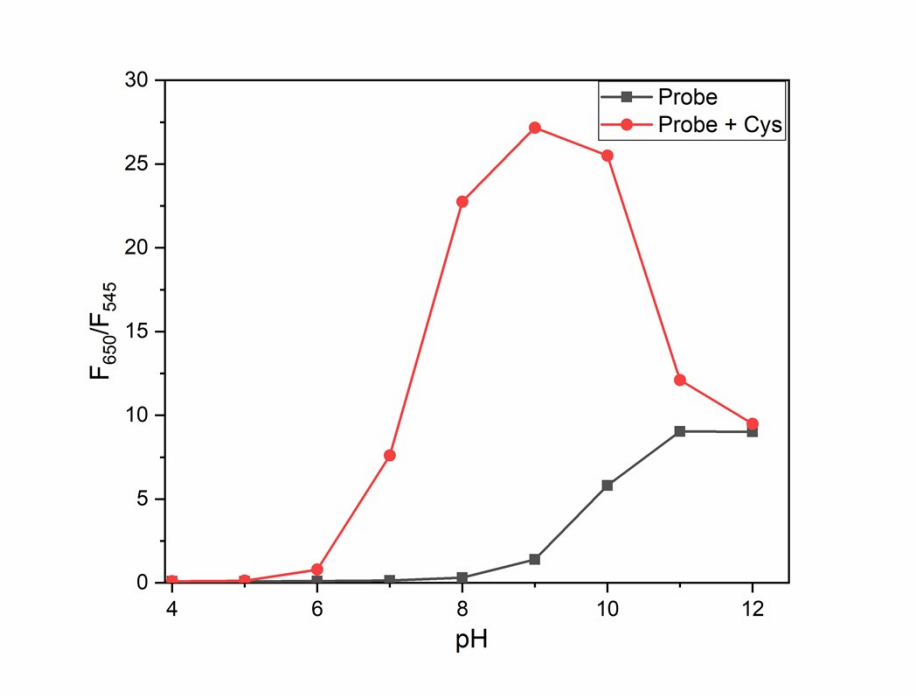


Figure S16. The fluorescence emission intensity of **HL-Cys** (10 μM) in solution of different pH value (4-12).

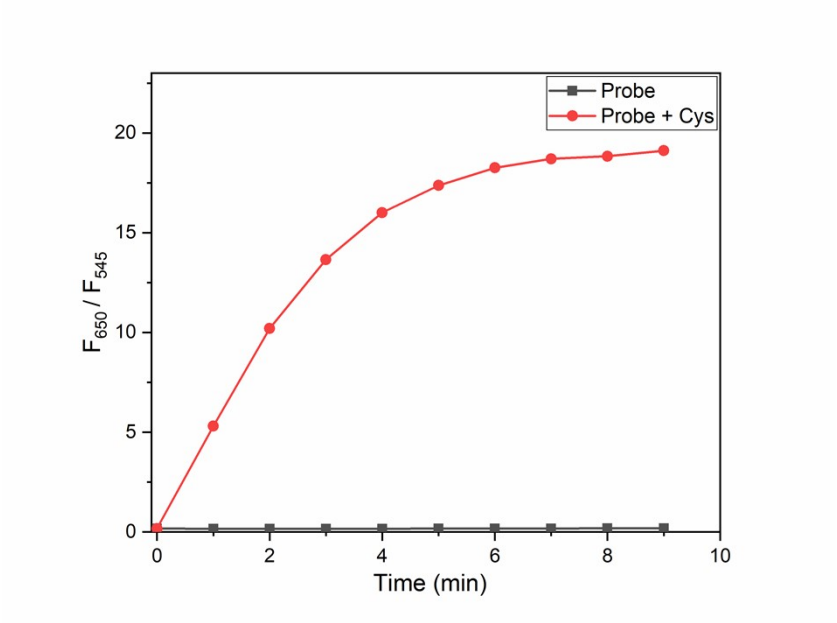


Figure S17. Time-dependent fluorescence intensity ratio (F_{650}/F_{545}) of the **HL-Cys** (10 μM) without (black) and with (red) 100 μM Cys in PBS buffer solution (containing 30% DMSO, 10 mM, pH = 7.4).

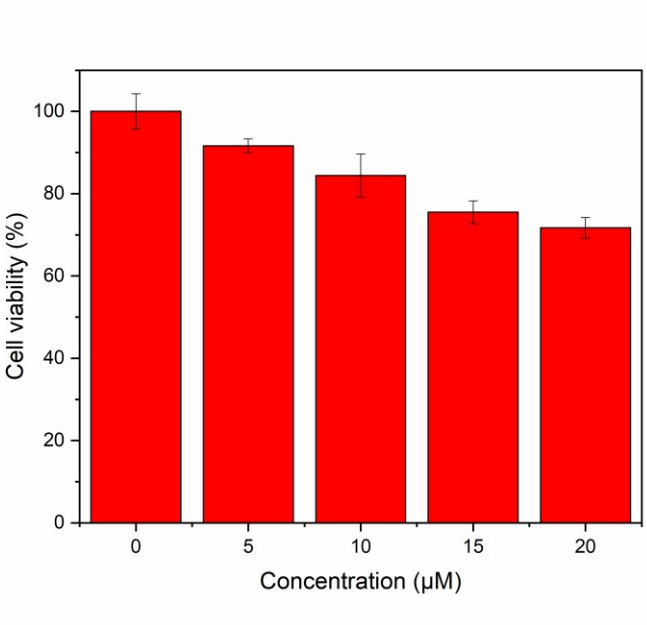


Figure S18. The cytotoxicity test of different concentrations **HL-Cys** in living HeLa cells for 24 h.