Electronic Supplementary Information

HBT-based turn-on fluorescent probe for discrimination of Homocysteine from Glutathione/Cysteine and its bioimaging applications

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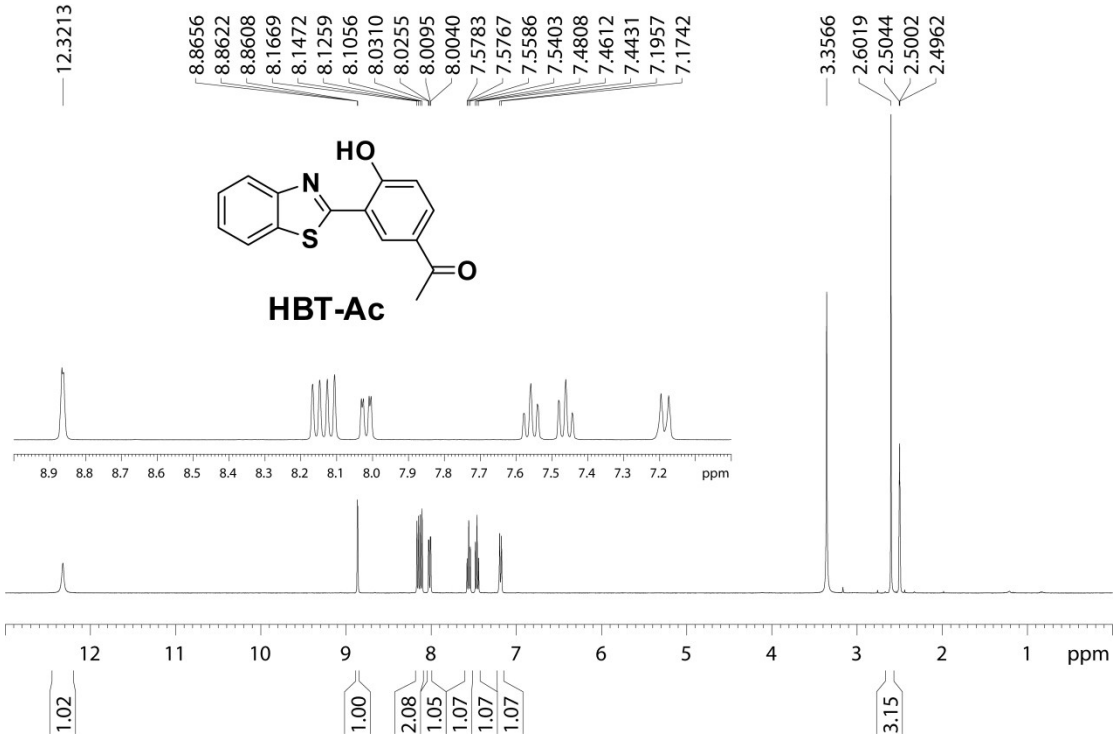
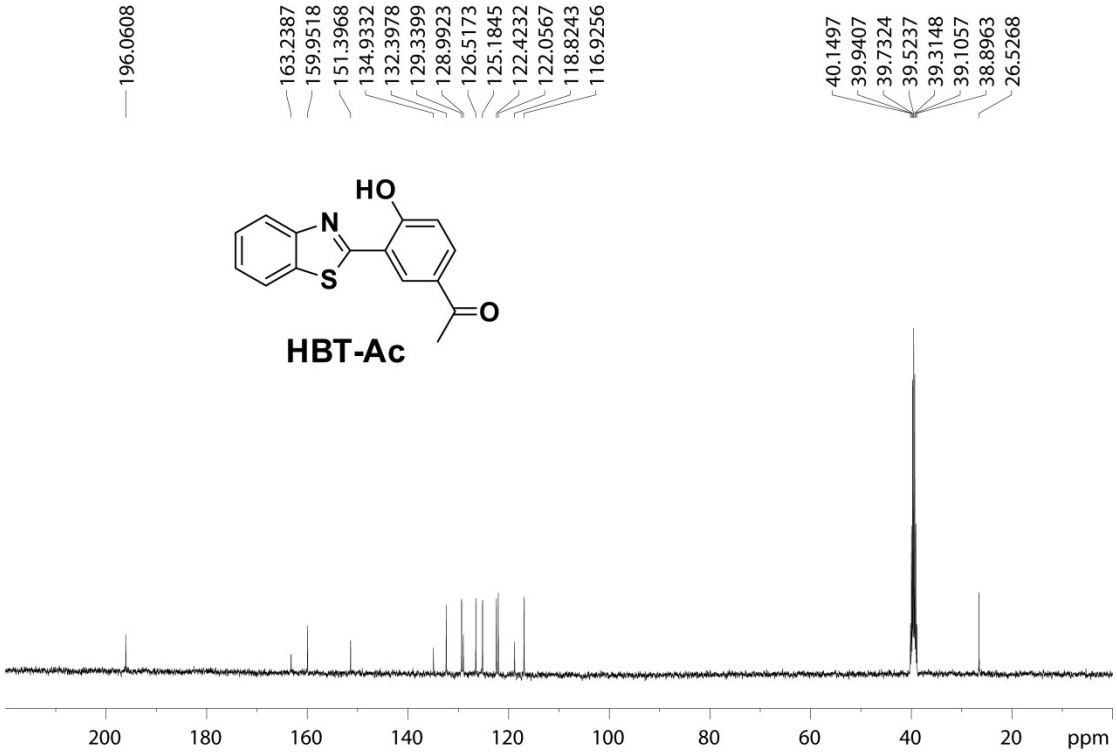
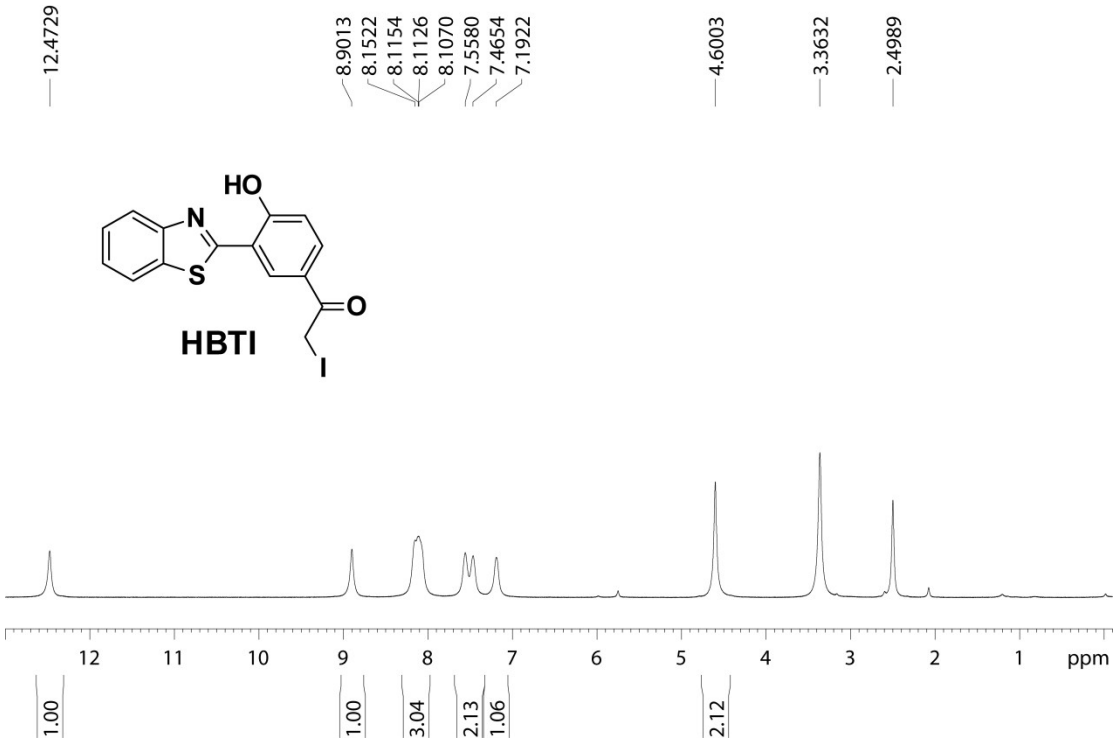
1. Structure characterizations for HBT-Ac and HBTI



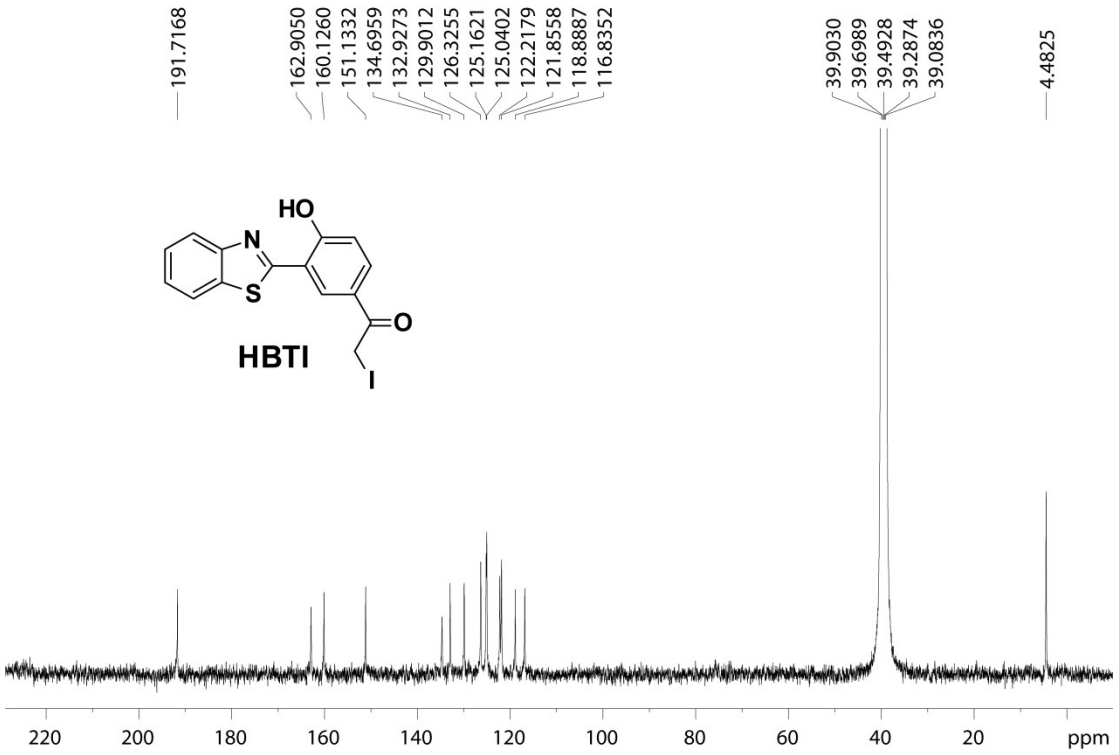
^{1}H NMR (400MHz) spectra of HBT-Ac in d_6-DMSO



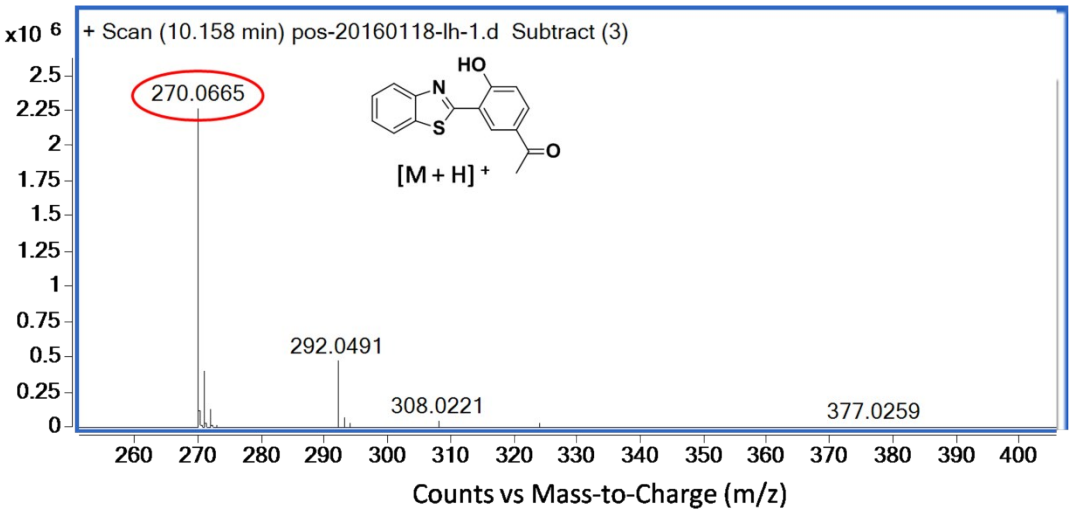
^{13}C NMR (400MHz) spectra of HBT-Ac in d_6-DMSO



^{1}H NMR (400MHz) spectra of HBTI in d_6-DMSO



Mass spectrum of HBTI



ESI-MS of HBT-Ac

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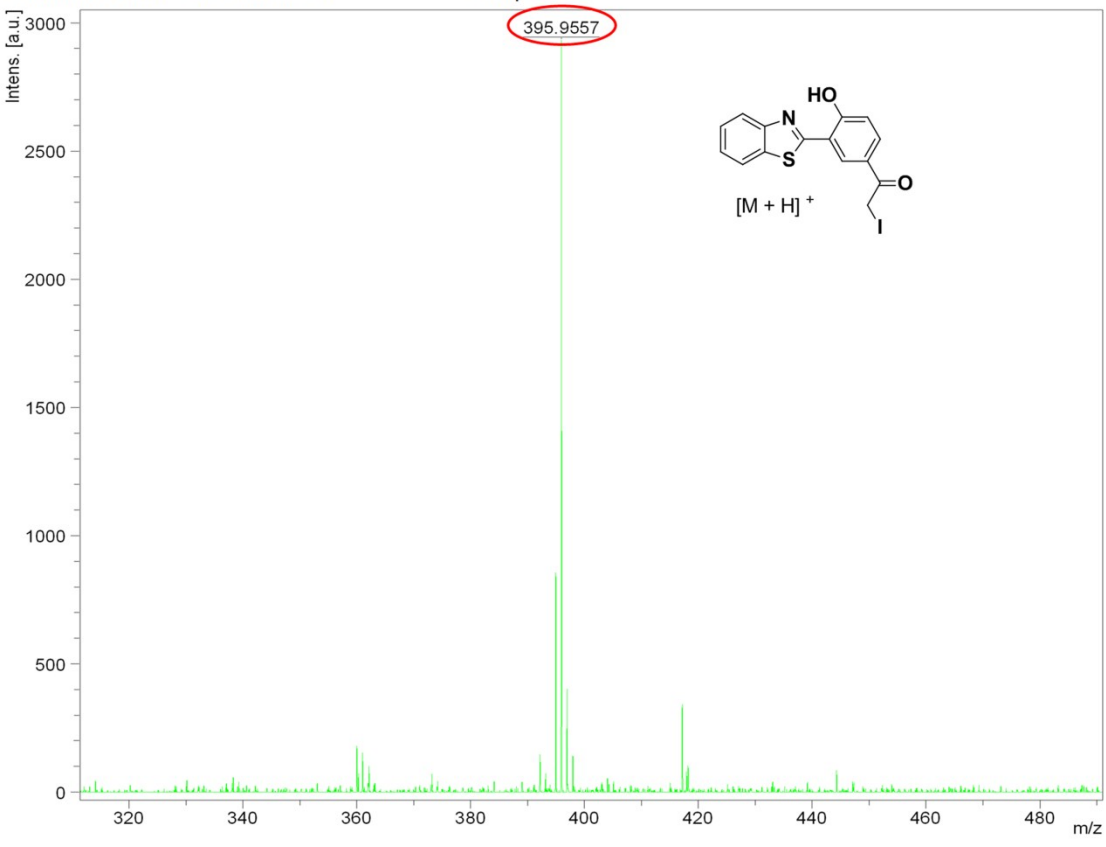
Aquisition operation mode	Reflector
Voltage polarity	POS
Number of shots	500
Name of spectrum used for calibration	
Calibration reference list used	sample



HRMS (ESI^{+}) of HBTI

2. Additional experiment data

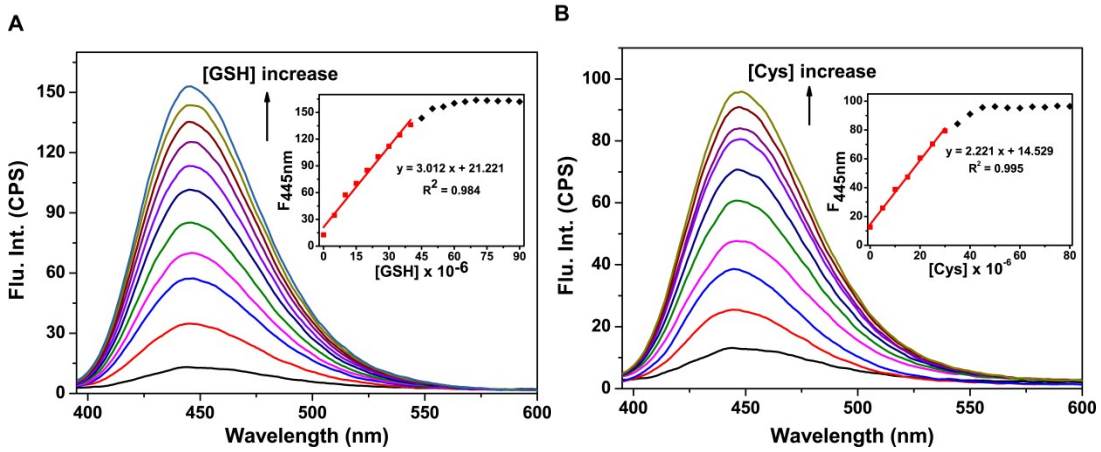


Fig. S1. Titration graph of the fluorescence response of probe HBTI (10 μM) toward GSH (A) and Cys (B) in PBS buffer (10 mM, pH 7.4, containing 1% CH_3CN, v/v) at 25℃. Inset: plot of the fluorescence intensity at 445 nm of probe HBTI versus GSH and Cys concentrations. Each spectrum was performed after 15 min. E_x = 385 nm, d_{ex} = d_{em} = 3 nm.

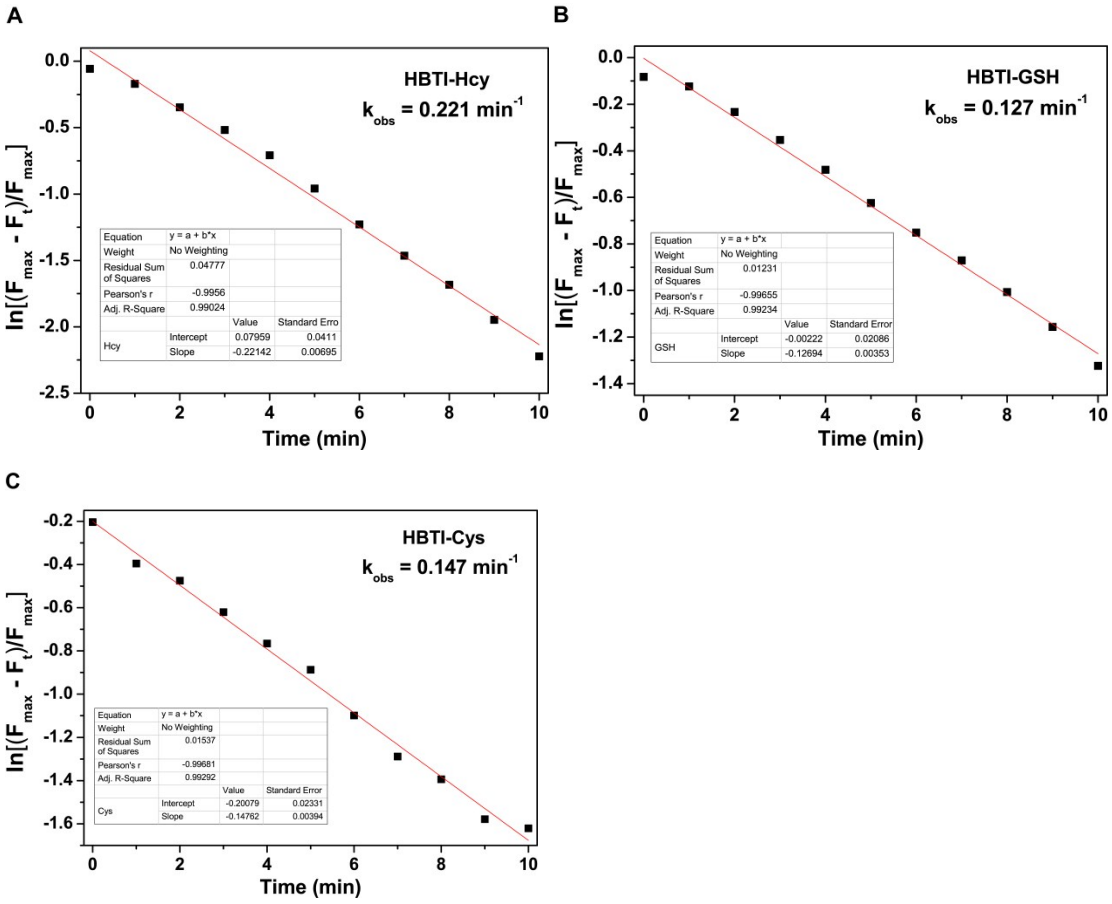


Fig. S2. The pseudo-first-order kinetic analysis of probe HBTI (10 μM) to Hcy, GSH and Cys (50 μM each) in PBS buffer (10 mM, pH 7.4, containing 1% CH_3CN, v/v) at 25℃. E_x = 385 nm, d_{ex} = d_{em} = 3 nm.

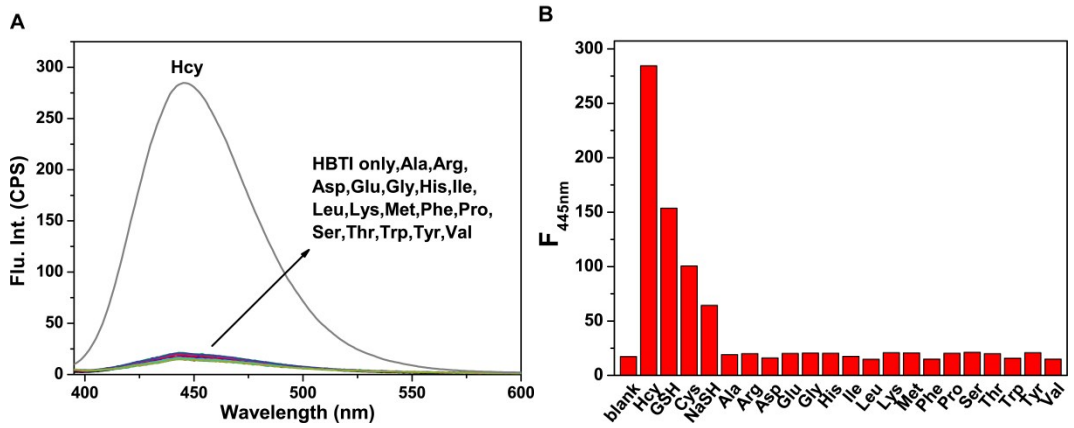


Fig. S3. Fluorescence spetrum (A) and bar graph (B) of probe HBTI (10 μM) upon addition of Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val and NaSH (various analytes: 100 μM; Hcy: 50 μM; GSH: 1 mM; Cys: 200 μM) in PBS buffer (10 mM, pH 7.4, containing 1% CH_3CN, v/v) at 25℃. Ex = 385 nm, d_{ex} = d_{em} = 3 nm.

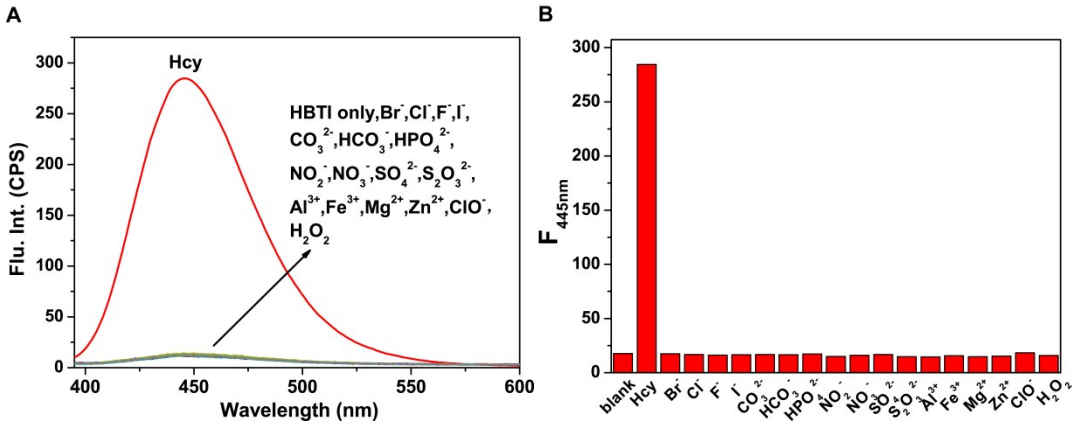


Fig. S4. Fluorescence spetrum (A) and bar graph (B) of probe HBTI (10 μM) upon addition of various analytes (100 μM) in PBS buffer (10 mM, pH 7.4, containing 1% CH_3CN, v/v) at 25℃. Ex = 385 nm, d_{ex} = d_{em} = 3 nm.

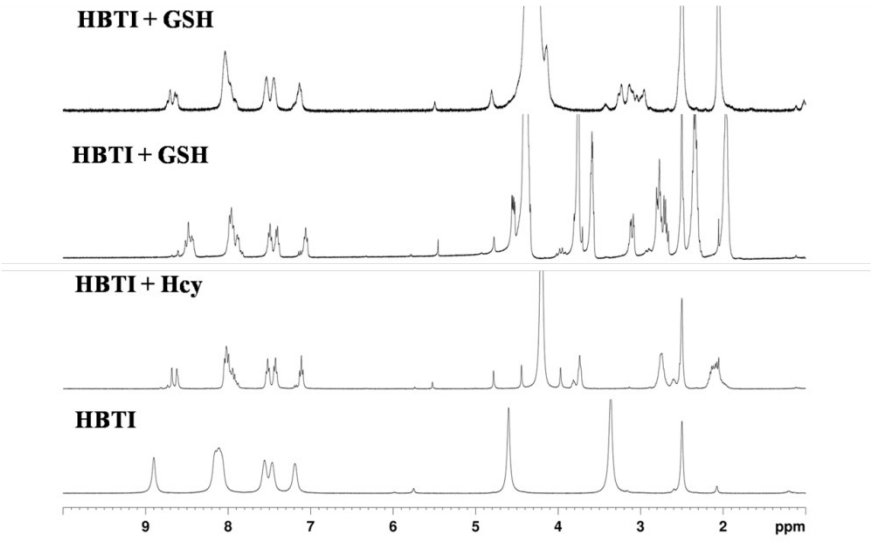


Fig. S5.[1]H NMR spectra changes of probe HBTI in the presence of excessive Hcy, GSH and Cys in d_6-DMSO/D_2O (9:1, v/v), respectively.

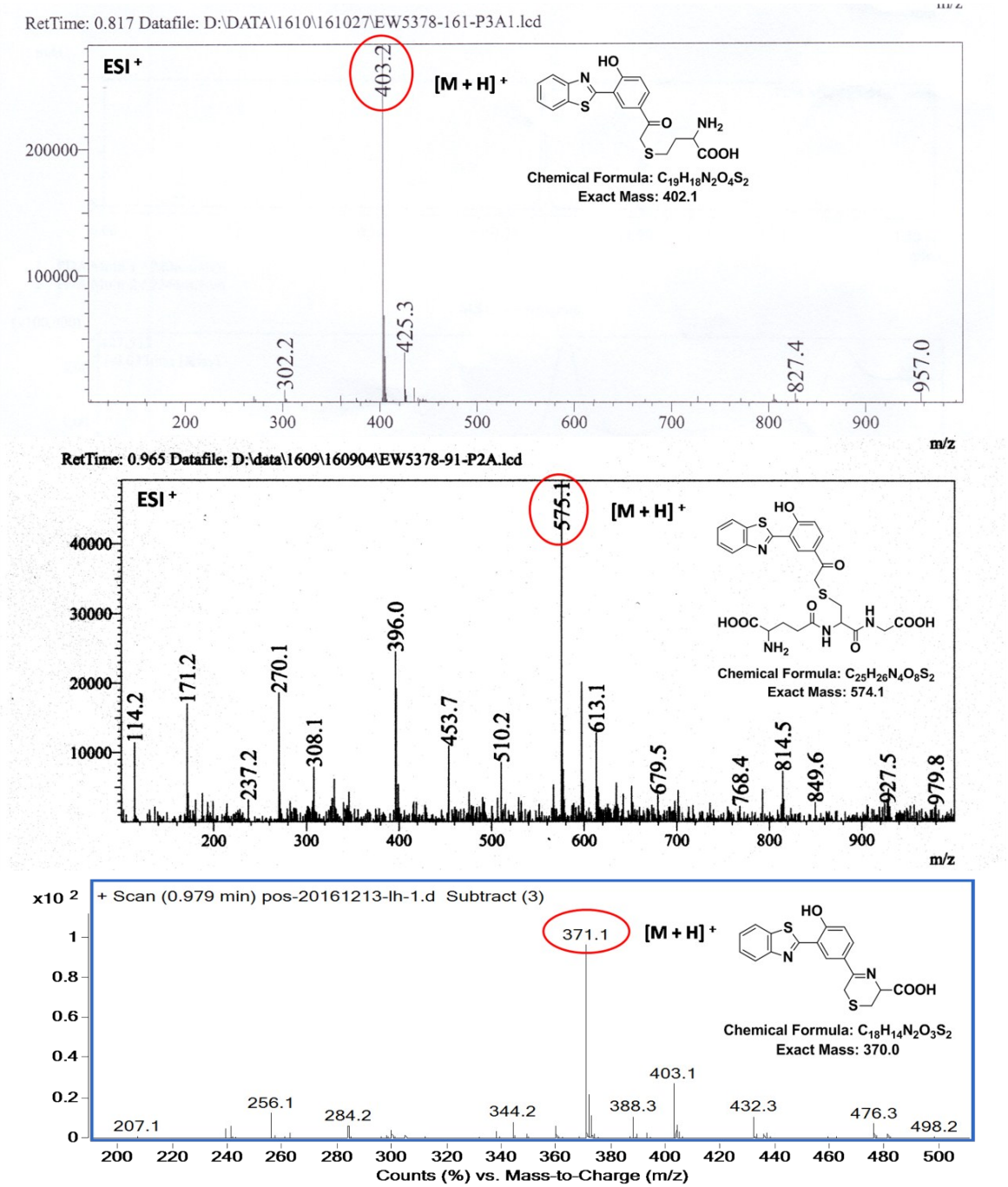


Fig. S6. MS (ESI$^+$) of probe HBTI in the presence of Hcy, GSH and Cys, respectively.

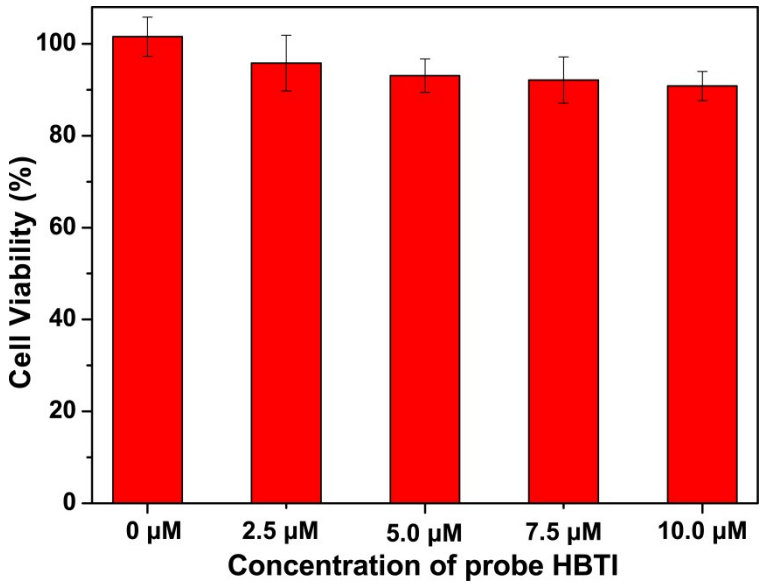
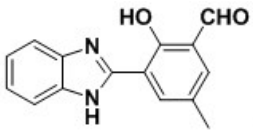
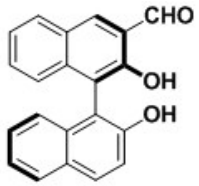
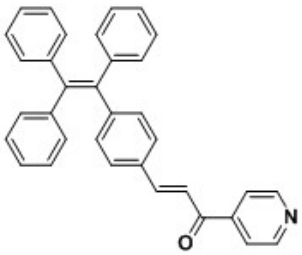
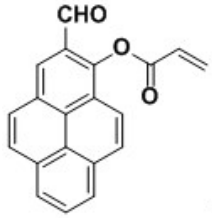
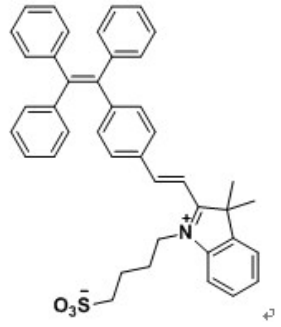
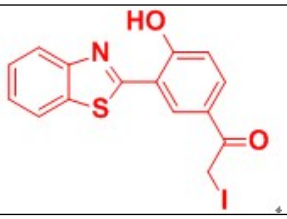


Fig. S7. Cell viability of HeLa cells treated with different concentrations of probe HBTI (0, 2.5, 5.0, 7.5 and 10.0 μM) for 12 h. The cell viability was observed via CCK-8 assay.

3. Table S1. Comparison of fluorescent probe for Hcy

Probes	Detection system	Response time	Detection limit	Reference
	HEPES buffer (pH 7.4) containing 10% EtOH	80 min	9.02×10^{-6} M	Tetrahedron Lett. 2016, 57, 5227.
	HEPES buffer (pH 7.0) containing 98% EtOH	100 min	5.4×10^{-5} M	Chem Commun., 2016,52, 827.
	PB buffer (pH 8.0) containing 20% CH_3CN	3 min	3.46×10^{-7} M	J. Mater. Chem. C, 2015, 3,8397.
	HEPES buffer (pH 7.4) containing 10% DMSO	10 min	1.94×10^{-6} M	Chem. Commun., 2014, 50, 6967.

	Buffer (pH 8.0) containing 1% DMSO	5 min	1.2×10^{-5} M	J. Mater. Chem. B, 2014, 2, 3919.
	PBS buffer (pH 7.4) containing 1% CH_3CN	15 min	1.6×10^{-7} M	This work