

Supporting information for

Lysosome-targeted two-photon fluorescent probes for rapid detection of H₂S in live cells

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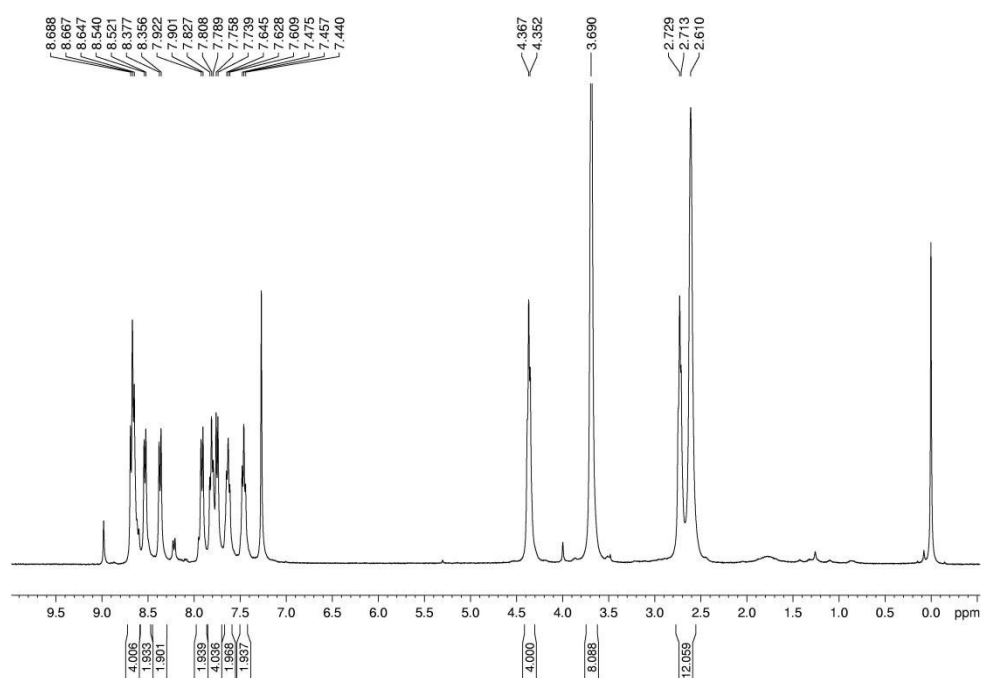


Figure S1. ¹H-NMR spectra of probe BHNP-DA in CDCl₃

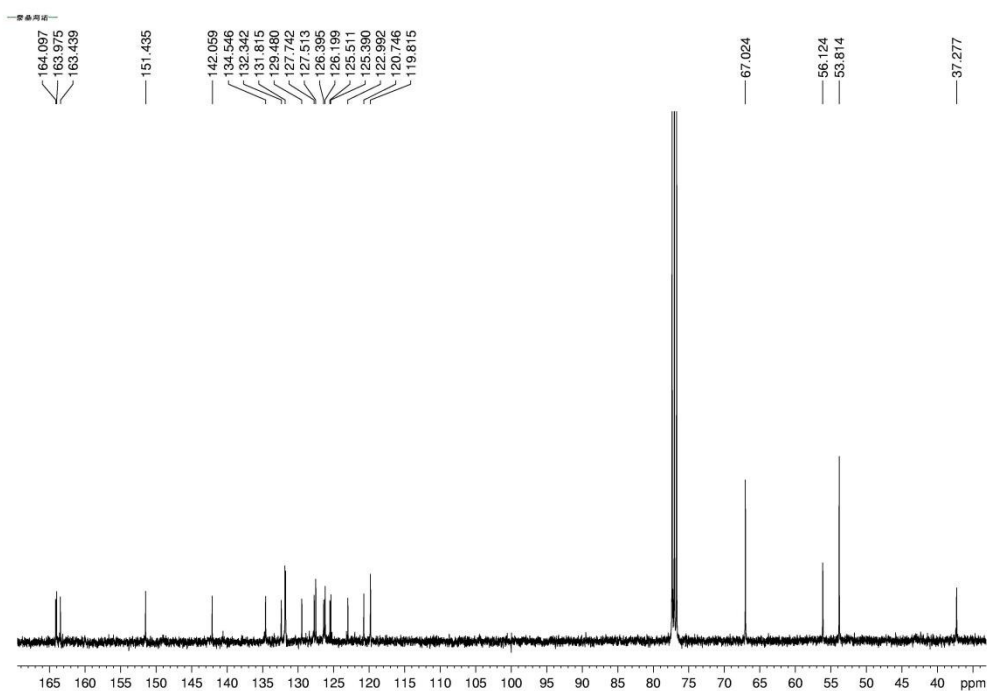


Figure S2. ^{13}C -NMR spectra of probe **BHNP-DA** in CDCl_3

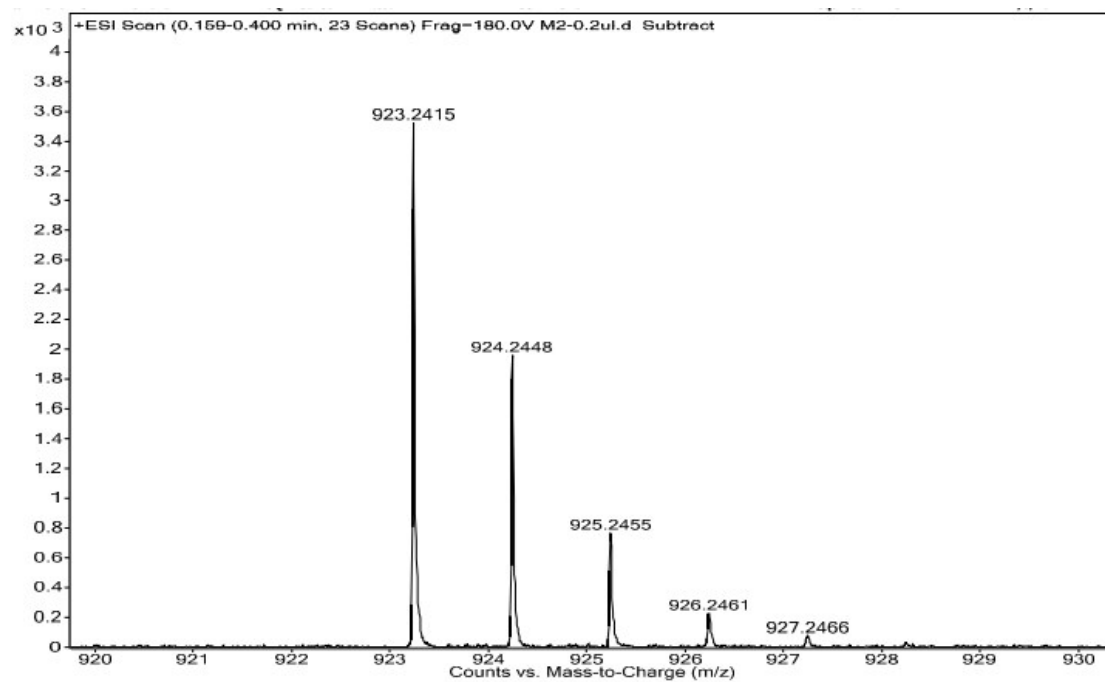


Figure S3. ESI-MS spectrum of probe **BHNP-DA** in MeOH

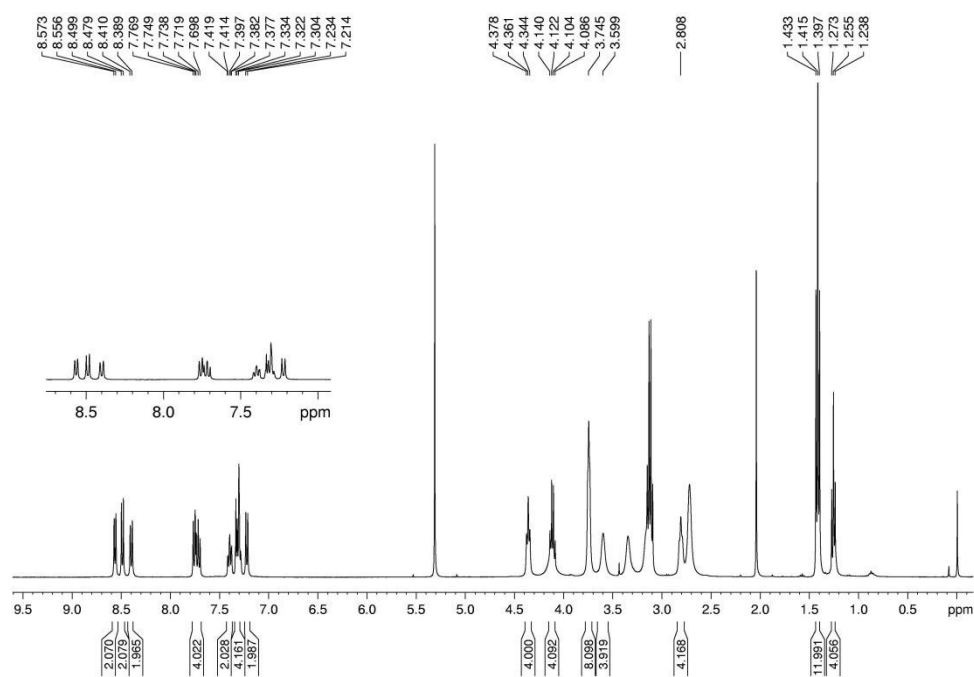


Figure S4. ^1H -NMR spectra of probe **M2** in CDCl_3

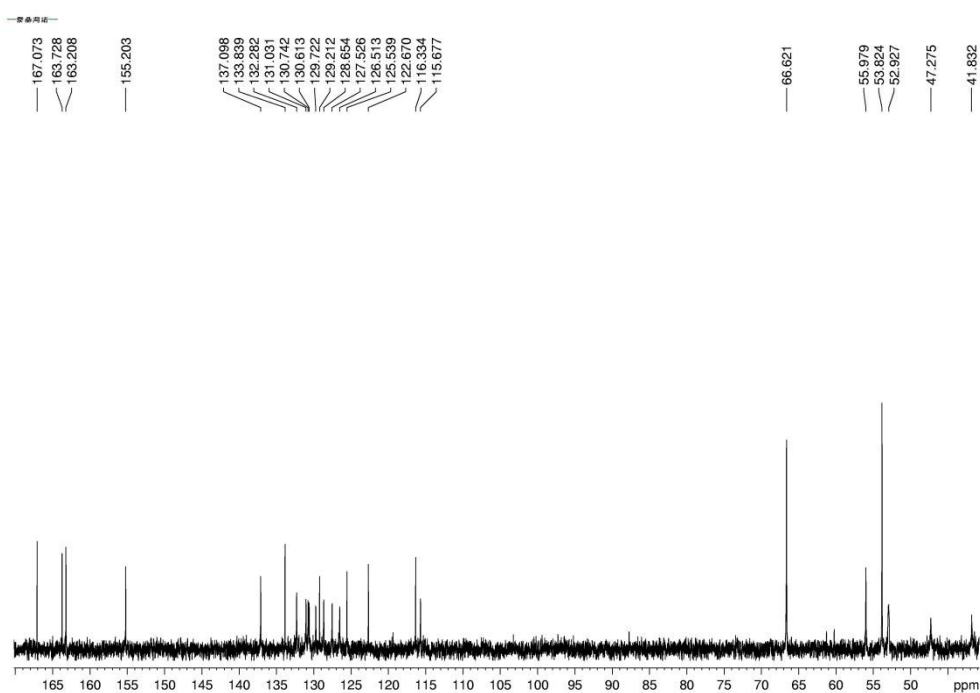


Figure S5. ^{13}C -NMR spectra of probe **M2** in DMSO

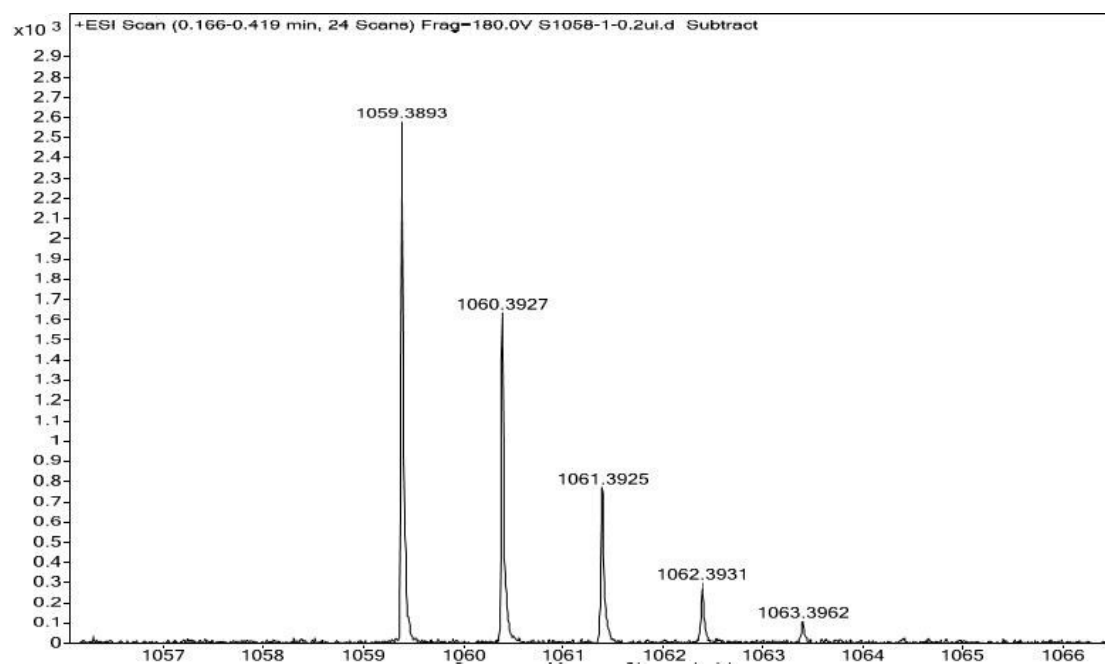


Figure S6. ESI-MS spectrum of probe **M2** in MeOH

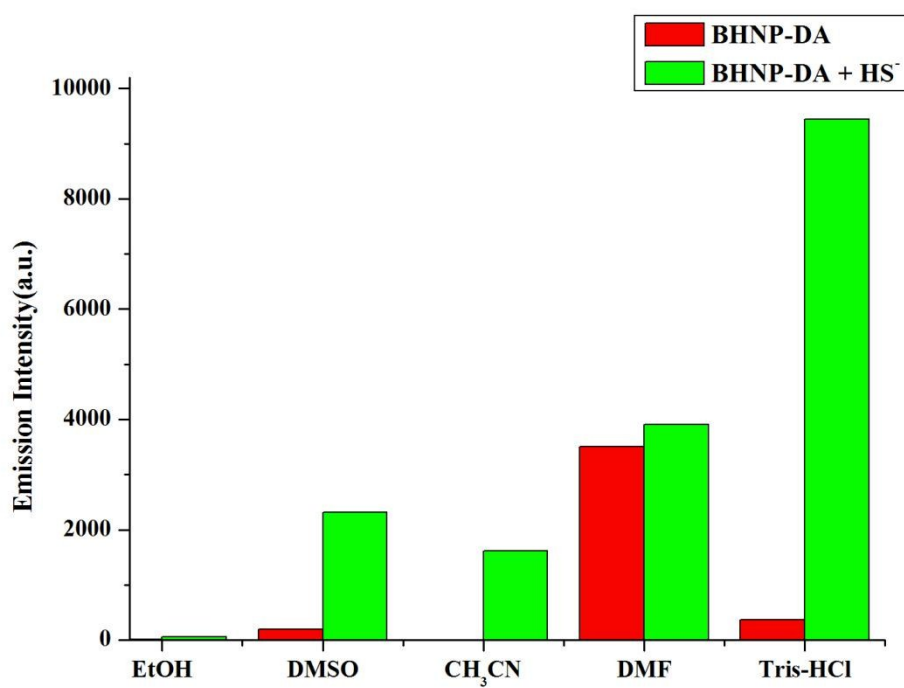


Figure S7. The fluorescence intensity (at 550 nm) of probe **BHNP-DA** (10 μM) in presence of NaHS (10 eq.) in different media.

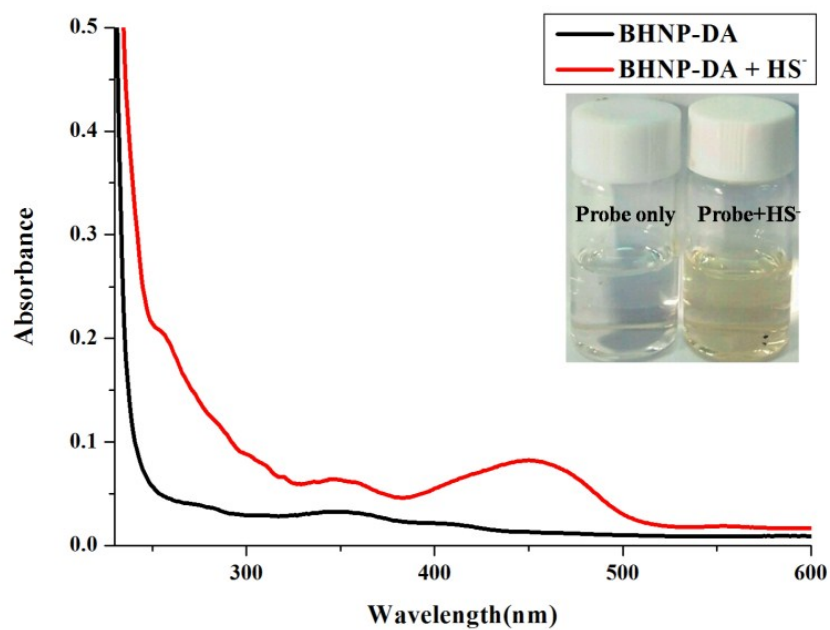


Figure S8. Absorption spectra of **BHNP-DA** (10 μM) in Tris-HCl buffer (10 mM, pH=7.4) with 1 mM CTAB with the presence of 10 eq. of NaHS. Insert shows the photo of probe **BHNP-DA** with 10 eq. of NaHS.

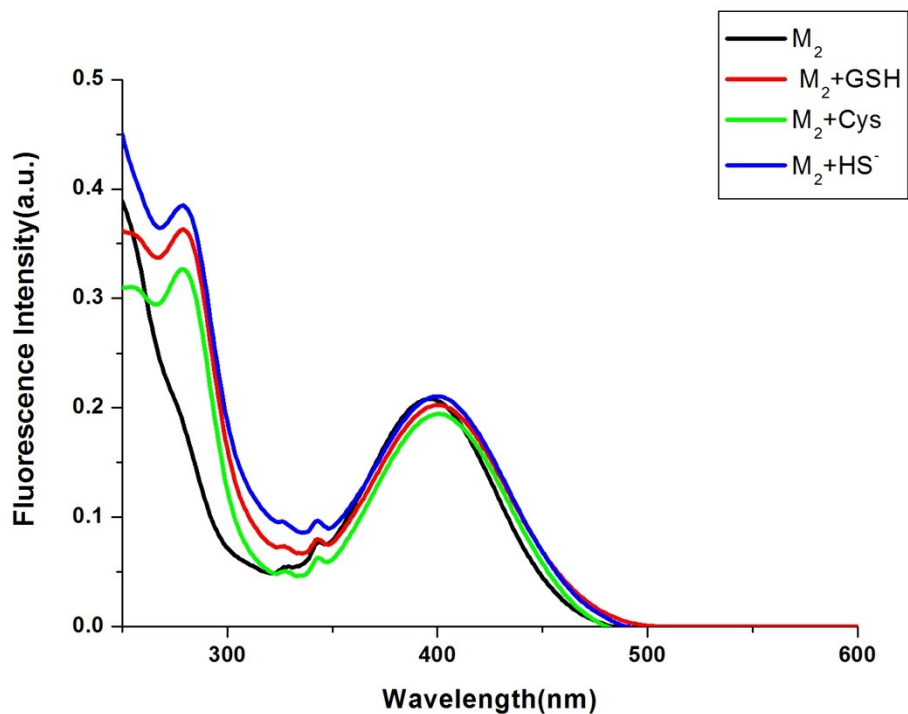
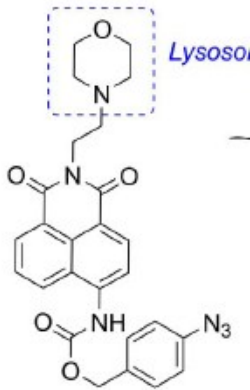
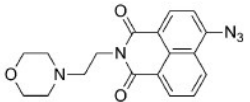
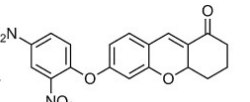
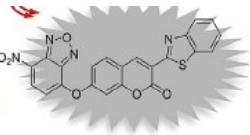
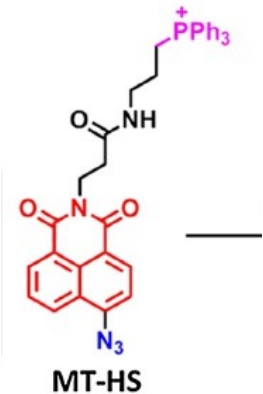
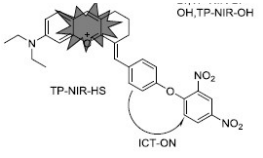
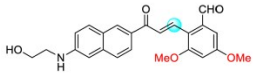
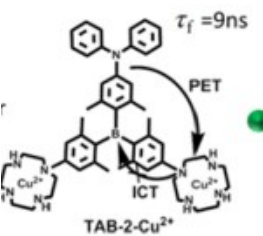
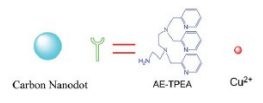
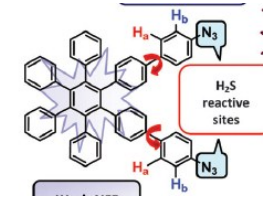
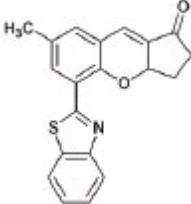
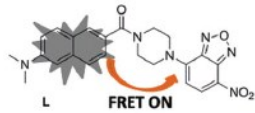
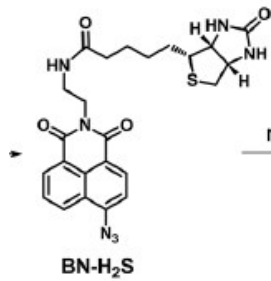
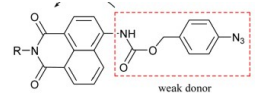


Figure S9. Absorption spectra of **M2** (10 μM) in Tris-HCl buffer (10 mM, pH=7.4) with 1 mM CTAB with the presence of 10 eq. of HS^- , GSH and Cys.

Table S1. Comparison of Some Reported probes for H₂S

Probe	solvent	Detecti on limit	Response time	applicati ons	Ref.
	PBS/CH ₃ CN =4:1 (v/v)	0.70 μM	/	SGC- 7901 cells	<i>Talanta</i> , 2017, 167 134
	pH 4.4 PBS buffer solution (containing 5% DMSO)	3.2 uM	5 min	A549 cells	<i>Chem. Commun</i> . 2016, 52, 7016
	CH ₃ CN– HEPES 5 : 5, pH 7.4	/	30 min	Hela cells.	RSC Adv., 2014, 4, 25790
	PBS buffer– ethanol (8:2, v/v)	3.02 uM	40 min	HeLa cells.	Analytica Chimica Acta 2015, 853 548
	HEPES buffer solution (20 mM, pH 7.4) containing 10% CH ₃ CN	0.12 uM	about 2 min	HeLa cells	Analytica Chimica Acta 2015, 896 128

 MT-HS	pH 7.4 PBS buffer (30% DMSO)	be1.65 uM.	5–6 min	HeLa cells	Sensors and Actuators B 2017, 248. 50– 56
	PBS with 0.5% EtOH	83 nM,	/	HeLa cells by	<i>Biosensor s and Bioelectr onics,</i> 2017, 91 699–705
	pH HEPES buffer containing 1% CH3CN	7.4 50 nM	5 min	HeLa cells	Anal. Chem. 2015, 87, 1188–11 95
 TAB-2-Cu²⁺	10 mM PBS physiological buffer DMSO (1%) as a cosolvent	47 nM	5min	NIH/3T3 cells.	Anal. Chem. 2016, 88, 1052–10 57
 C-Dot-TPEA–Cu²⁺	phosphate buffer solution (PBS, pH 7.4).	0.7 uM.	200 s	A549 cells. HeLa cells.	Analyst, 2014, 139, 1945
	H2O/DMSO (7: 3, v/v) buffered with HEPES, pH = 7.05,	0.33 uM	16min	HeLa cells.	Chem. Commun . 2015, 51, 15570
	10 vol% DMF–90 vol% PBS solution	41 nM	20 min.	HeLa cells	Chem. Commun . 2017, 53, 4791

					
	PBS, 25 mM, pH 7.4, containing 20% EtOH as co- solvent	24 nM.	10 min	HeLa	<i>New J.Chem.</i> , 2017, 41, 6769
	PBS (20 mM, pH 7.4, 5% MeOH)	71 nM	25 min	HeLa and NIH 3T3 cells	RSC Adv., 2017, 7, 15817
	EtOH/PBS (v/v1:4)	50–85 nM	2h	MCF-7 cells	J. Org. Chem. 2014, 79, 9481–94 89
naphthalimide- based fluorescent probe	Tris-HCl buffer	0.06 μmol/L	<1 min	MCF-7 cells	This work

“/” not mentioned

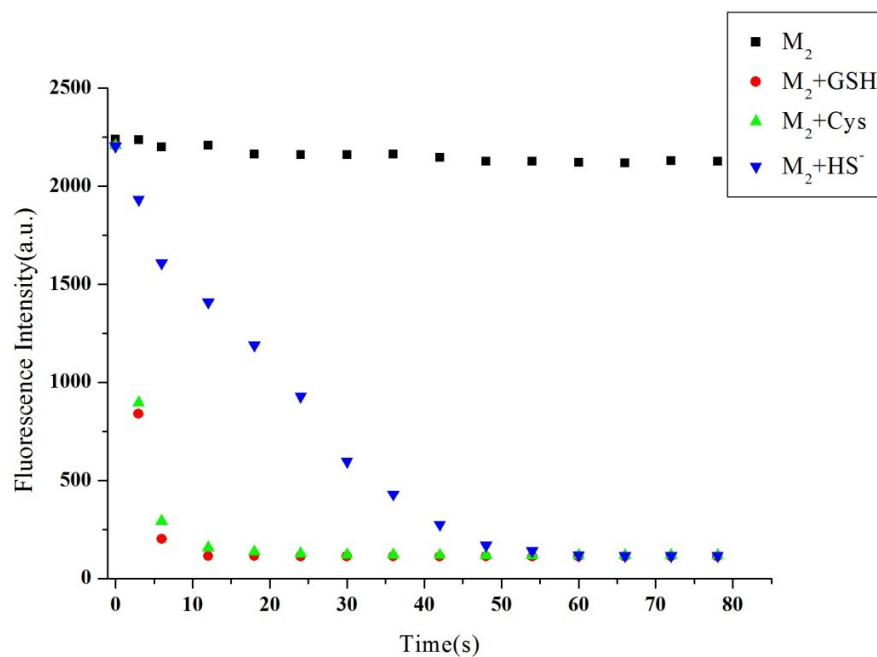


Figure S10 Effect of reaction time on the fluorescence intensity (at 550 nm) of **M2** (10 μ M) in Tris-HCl buffer (10 mM, pH=7.4) with 1 mM CTAB in the absence and presence of 10 eq. HS^- , GSH and Cys (λ_{ex} =400nm, slit=5 nm).

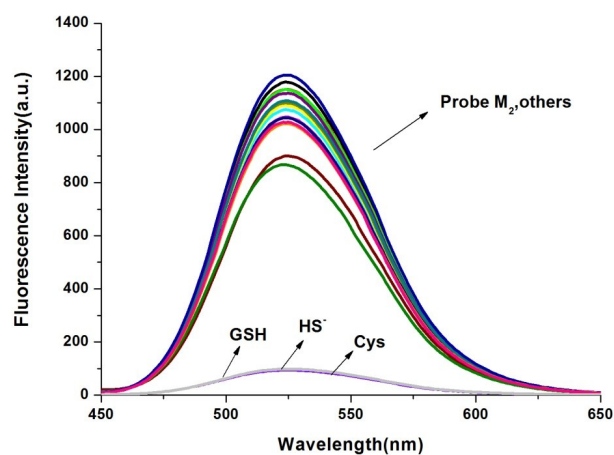


Figure S11 Fluorescence spectra of **M2** (10 μ M) in Tris-HCl buffer (10 mM, pH=7.4) with 1mM CTAB in the presence of 10 eq. of various species (λ_{ex} =410 nm, slit=5 nm). Others species are His, Glu, Gln, Asp, Val, Phe, Tyr, Ala, Ser, Leu, Arg, Pro, Thr, Lys, Gly

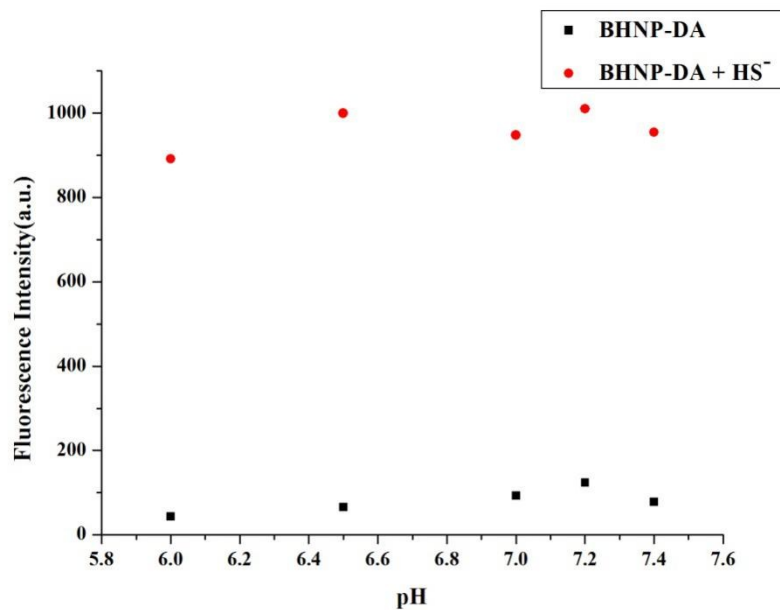


Figure S12. The fluorescence intensity (at 550 nm) of probe **BHNP-DA** (10 μ M) in presence of NaHS (10 eq.) in different pH of Tris-HCl buffer (10 mM) containing 1.0 mM CTAB.

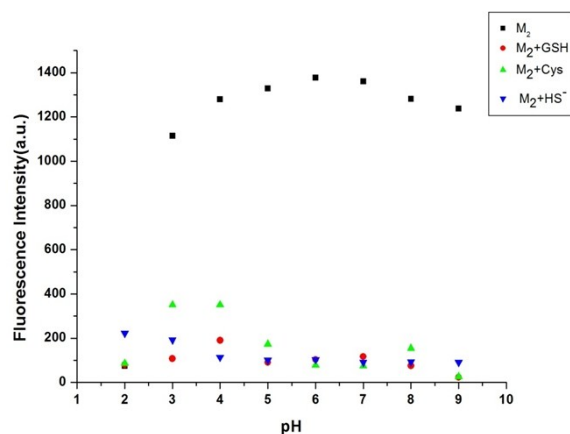


Figure S13 The fluorescence intensity (at 550 nm) of probe **M2** (10 μ M) in presence of NaHS, GSH and Cys (10 eq.) in different pH of Tris-HCl buffer (10 mM) containing 1.0 mM CTAB.

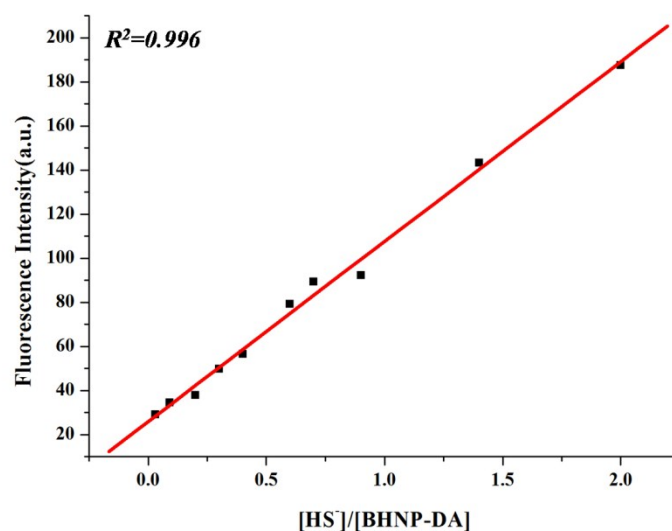


Figure S14. The fluorescence intensity (at 550 nm) of probe **BHNP-DA** (10 μ M) upon addition of increasing concentrations of NaHS (0-20 μ M) in Tris-HCl buffer (10 mM, pH 7.4) with 1 mM CTAB (λ_{ex} = 410 nm, slit = 5 nm).

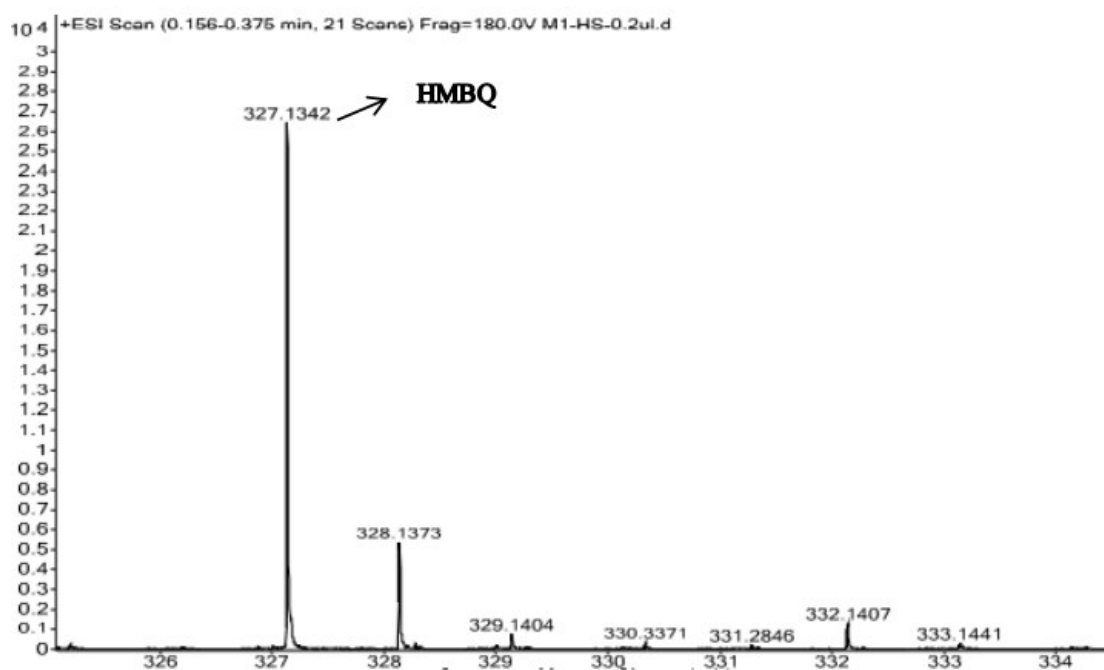


Figure S15. ESI-MS spectrum of HMBQ obtained and isolated from reaction mixture of probe **BHNP-DA** with NaHS in 1.0 mM CTAB media buffered at pH 7.4 (Tris-HCl buffer, 10 mM).

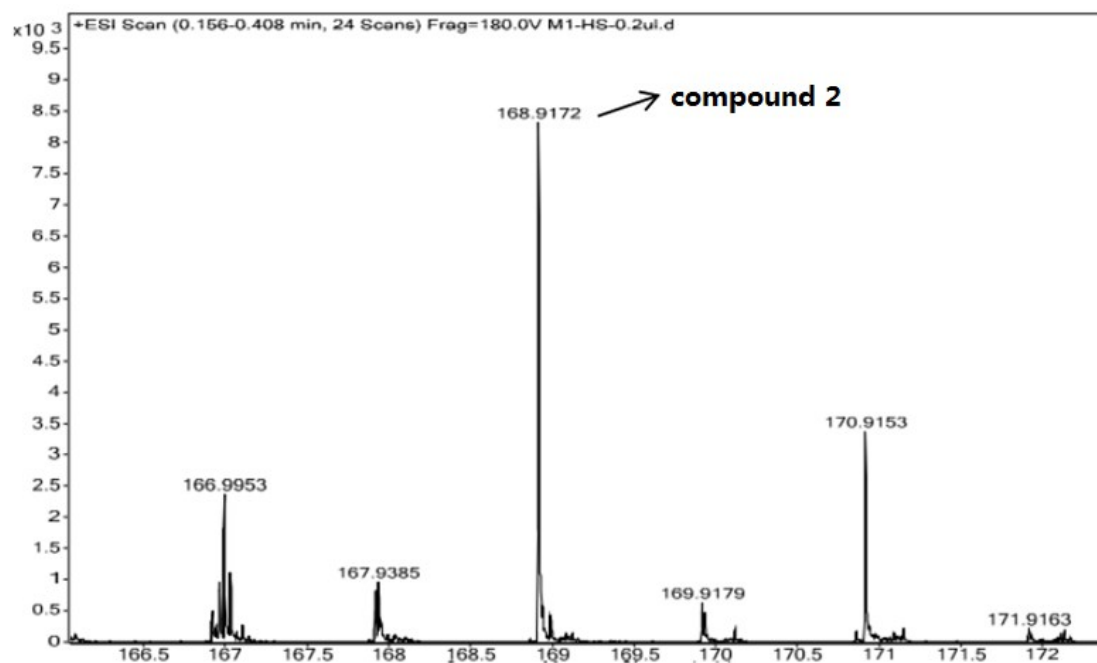
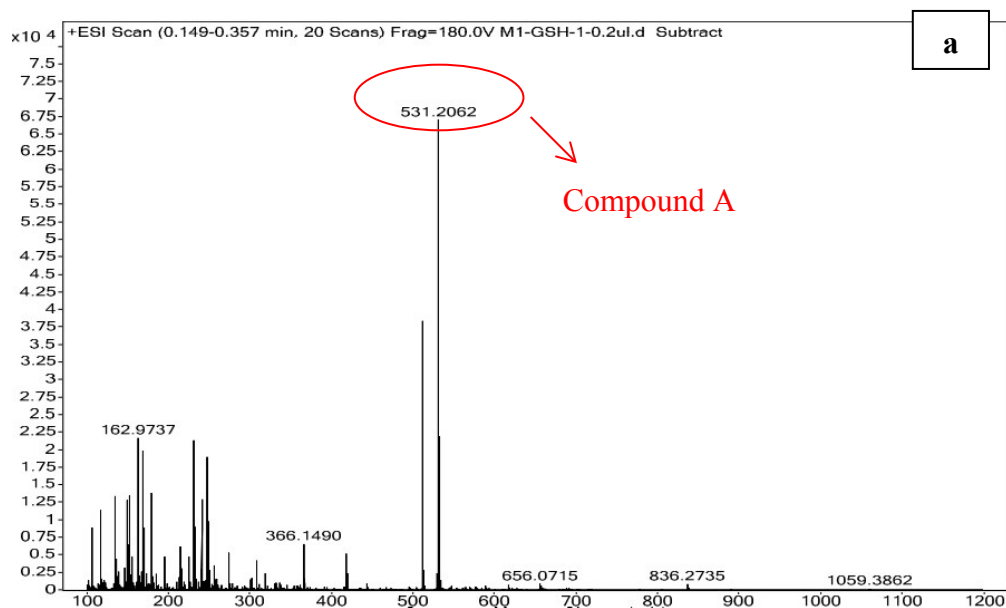


Figure S16. ESI-MS spectrum of **compound 2** obtained and isolated from reaction mixture of probe **BHNP-DA** with NaHS in 1.0 mM CTAB media buffered at pH 7.4 (Tris-HCl buffer, 10 mM).



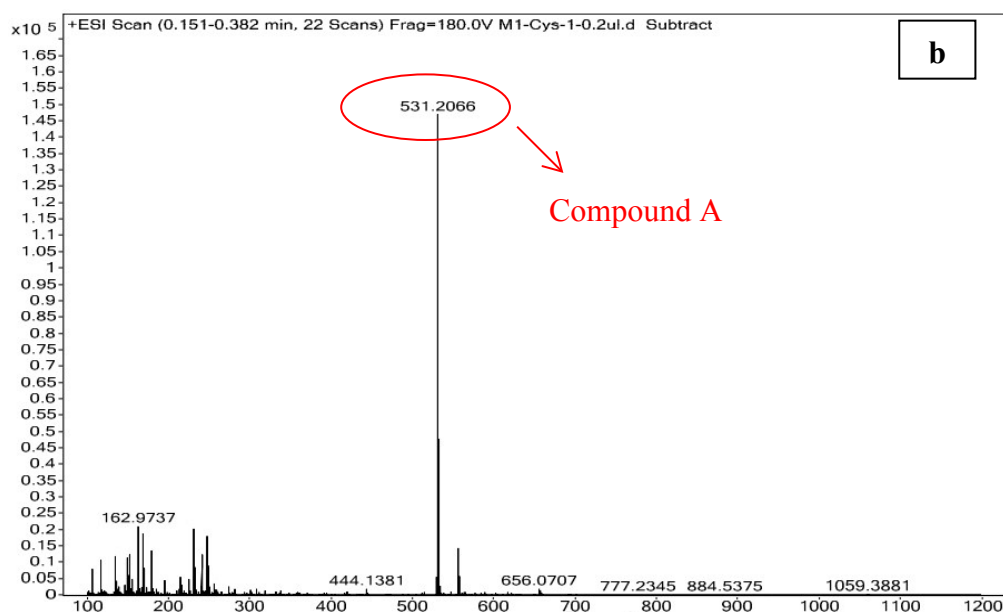


Figure S17. ESI-MS spectrum of reaction mixture of probe **M2** with GSH (a) and Cys (b) in 1.0

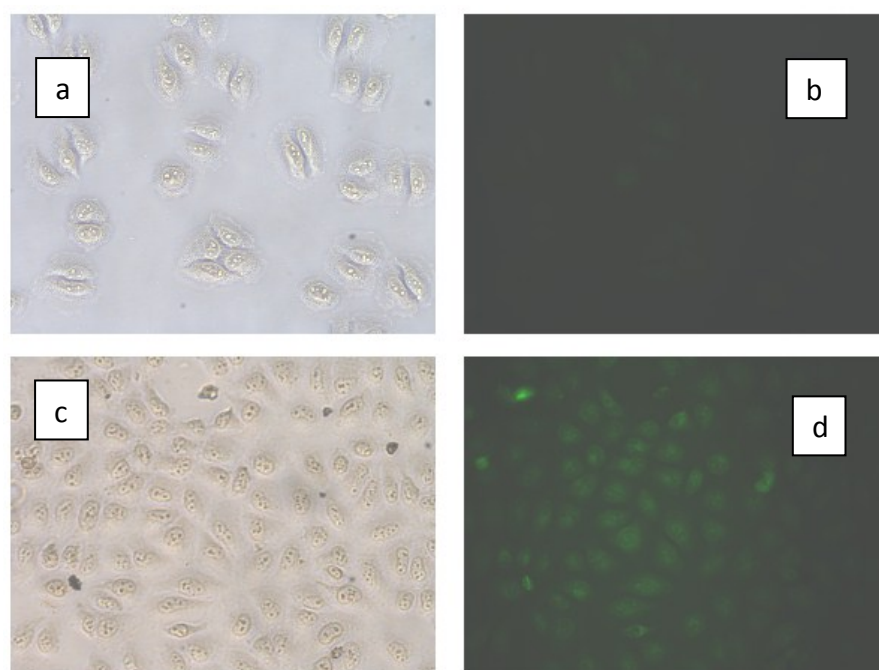


Figure S18. Fluorescent images of MCF-7 cells pre-treated with 5.0 μM probe BHNP-DA for 30 min at 37 $^{\circ}\text{C}$ (a, b) and followed by incubation with 100.0 μM NaHS for 30 min at 37 $^{\circ}\text{C}$ (c, d). Bright field images (a, c), Fluorescent images (b, d). (λ_{ex} = 410 nm, green channel)