Supporting Information

A NIR fluorescent sensor for biothiols based on dicyanoisophorone derivative with large Stokes shift and high quantum yield

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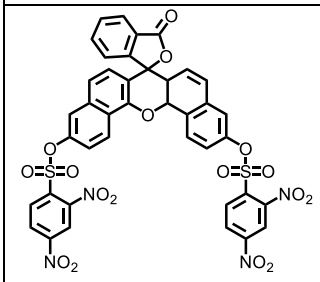
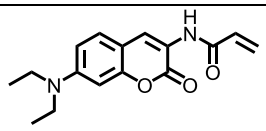
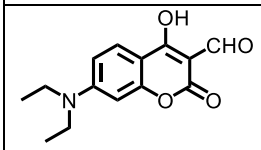
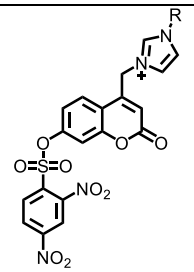
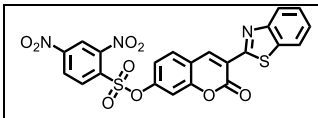
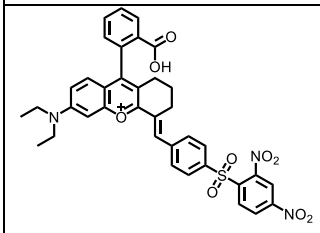
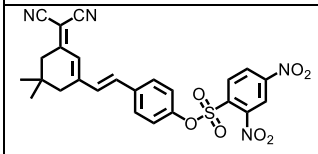
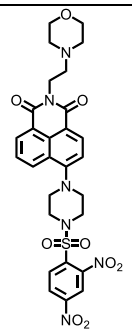
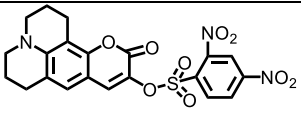
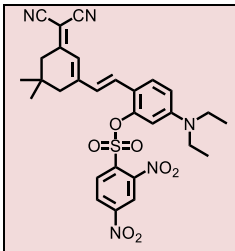
Table S1. The comparison of fluorescent probes for biothiols.

Probe	Properties	Stokes shift	Fluorescence enhancement	Detection limit	quantum yield
 Sensors and Actuators B 246 (2017) 988–993	Abs: 620 nm Em: 688 nm NIR emission	68 nm	Cys: 160-fold GSH: 120-fold Hcy: 70-fold	Cys: 2.93 μM GSH: 59 nM Hcy: 1.29 μM	Not mentioned
 Sensors and Actuators B 232 (2016) 732–737	Abs: 400 nm Em: 490 nm UV-Vis region	90 nm	Cys: 44-fold GSH: 31-fold Hcy:35-fold	Cys: 192 nM GSH: 155 nM Hcy: 158 nM	0.262 0.208 0.186
 Biosensors and Bioelectronics 85 (2016) 46–52	Abs: 384 nm Em: 480 nm UV-Vis region	96 nm	About 5-fold	Cys: 0.874 μM	Not mentioned
 Sensors and Actuators B 255 (2018) 193–202	Abs :380 nm Em: 480 nm Two-photon mode	100 nm	About 10-fold	GSH: 31.4 nM	0.60

 RSC Advance, 5 (2015), 62325-62330.	Abs: 451 nm Em: 493 nm UV-Vis region	42 nm	35-fold	Cys: 7 nM GSH: 6 nM Hcy: 9 nM	0.65
 Journal of Materials Chemistry B, 5 (2017), 3836-2841	Abs :550 nm Em: 660 nm NIR emission	110 nm	6-fold	Cys: 43 µM	0.0216
 Sensors and Actuators B 248 (2017) 338–345	Abs: 550 nm Em: 676 nm NIR emission	126 nm	About 25-fold	Cys: 12.1 nM GSH: 11.9 nM Hcy: 14.5 nM	Not mentioned
 Scientific Reports 6 (2016) 19562-19569.	Abs: 400 nm Em: 540 nm Two-photon mode	54 nm	Cys: 74-fold GSH: 63-fold Hcy: 60-fold	Cys: 0.26 µM GSH: 2.41 µM Hcy: 4.87 µM	Cys: 0.52 GSH: 0.45 Hcy: 0.43
 Sensors and Actuators B 241 (2017) 327–334	Abs: 387 nm Em: 517nm UV-Vis region	137 nm	60-fold	Cys:17.1 nM GSH:14.5 nM Hcy:40 nM	0.255

 **This work**	Abs: 560 nm Em: 680 nm NIR emission	120 nm	Cys: 208-fold GSH: 204-fold Hcy: 168-fold	Cys: 36.93 nM GSH: 32.56 nM Hcy: 65.03 nM	0.2971

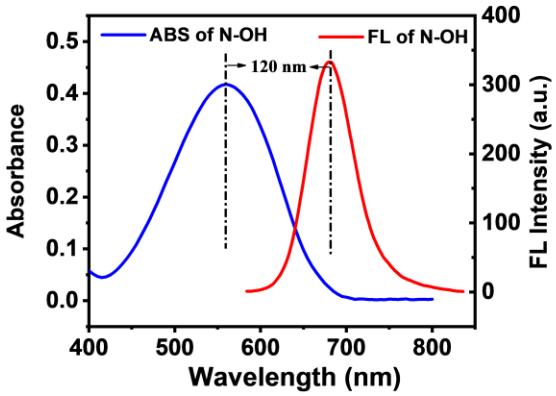


Figure S1. The absorption spectrum and fluorescence spectrum of **N-OH** in PBS-ethanol buffer (10 mM, 1:1, v/v).

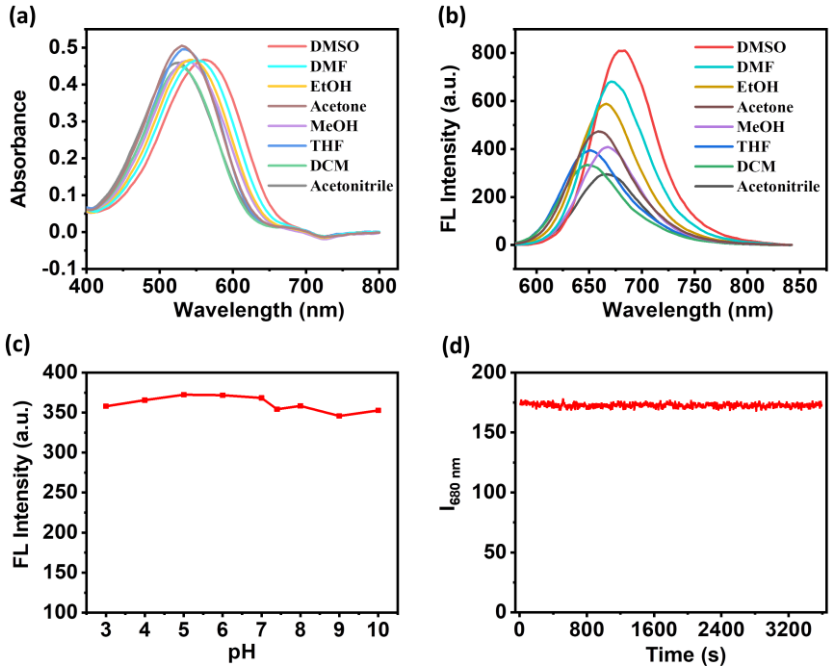


Figure S2. The optical properties of fluorophore **N-OH.** (a) The absorption spectra of **N-OH** in different solvents. (b) The fluorescence spectra of **N-OH** in different solvents. (c) The pH effect on the **N-OH** in PBS-ethanol buffer (10 mM, 1:1, v/v). (d) The photostability of **N-OH** under the exposure to UV light.

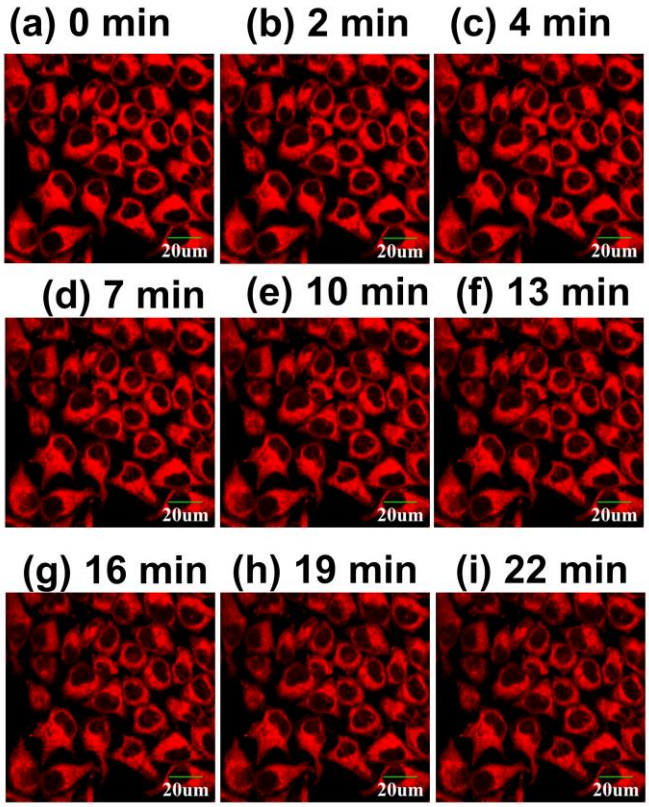


Figure S3. The intracellular photostability of **N-OH** with ceaseless laser exposure for different time. Laser exposure time: (a) 0 min, (b) 2 min, (c) 4 min; (d) 7 min, (e) 10 min, (f) 13 min, (g) 16 min, (h) 19 min, (i) 22 min. Optical parameters: λ_{ex} = 543 nm; λ_{em} = 620-720 nm; Bar = 20 μm.

A

Fluorophore	DCM-OH	NDCM-OH	TCF-OH	N-OH
quantum yield	0.008	0.005	0.009	0.30

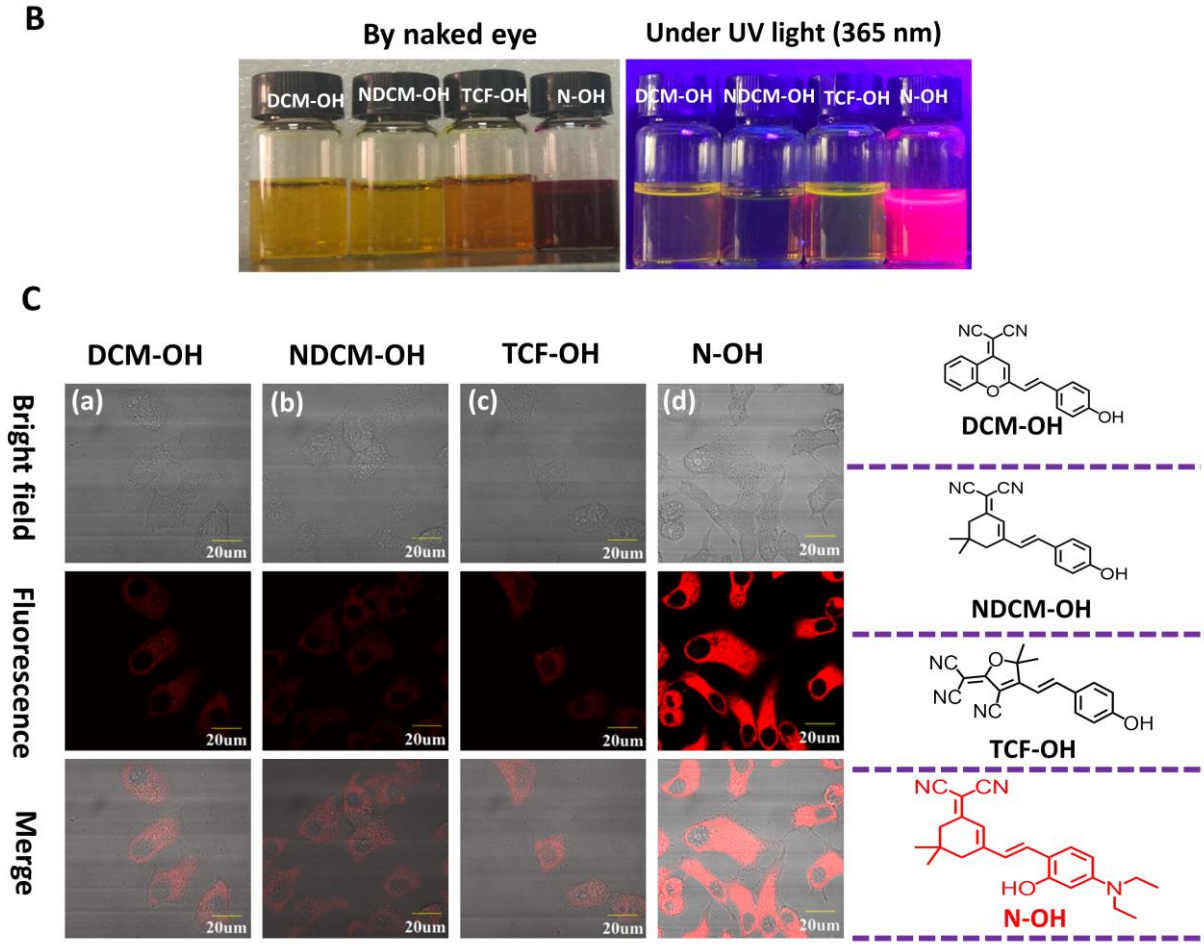


Figure S4. The comparsion of four dicyano-derivative based fluorophores. (A) the comparison of quantum yield of four fluorophores, the data were obtained in ethanol with Rodanmine B (0.66 in ethanol) as the reference compound. (B) observing the solution of four fluorophores by naked eye and under UV light (365 nm). (C) evaluating the brightness of four fluorophores by confocal fluorescence microscopy (a): **DCM-OH;** (b): **NDCM-OH;** (c): **TCF-OH;** (d): **N-OH.**

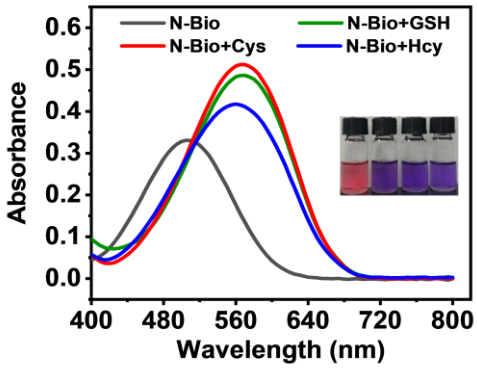


Figure S5. The absorption response of **N-Bio** toward biothiols in PBS-ethanol buffer (10 mM, 1:1, v/v).

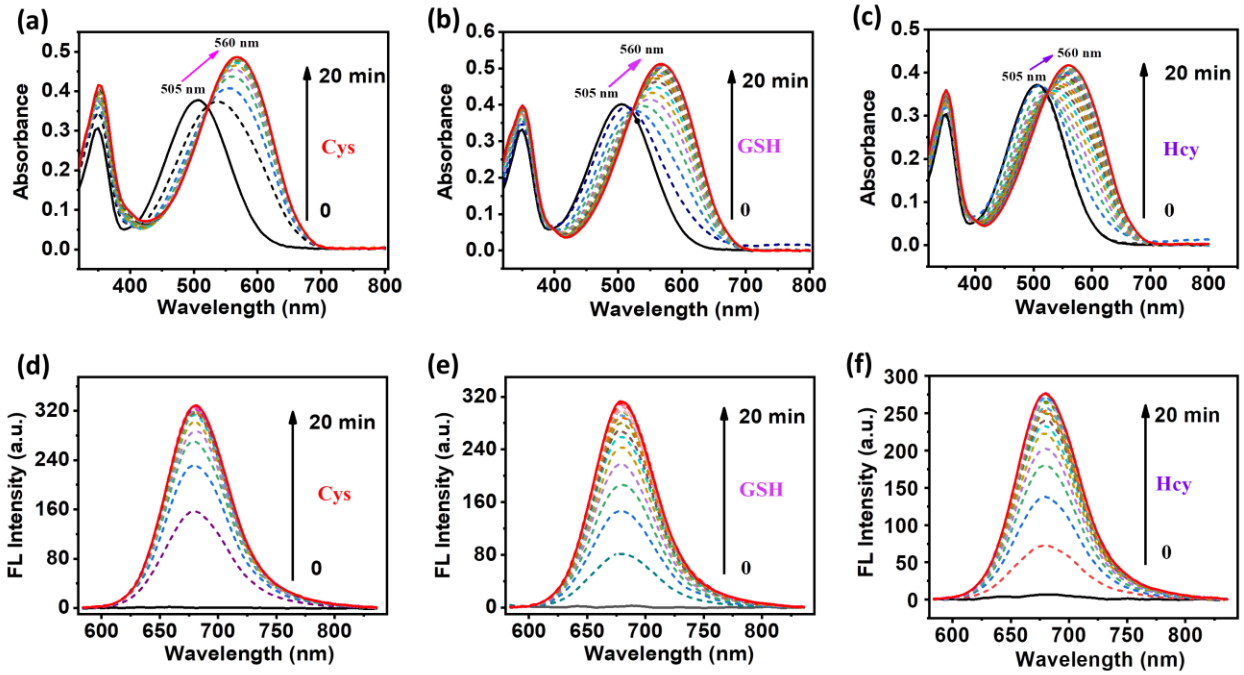


Figure S6. The time-dependent response of **N-Bio** toward 100 μM biothiols in PBS-ethanol buffer (10 mM, 1:1, v/v). Up: the time-dependent absorption spectra change of **N-Bio** toward Cys(a), GSH(b) and Hcy(c). Down: the time-dependent fluorescence spectra change of **N-Bio** toward Cys(d), GSH(e) and Hcy(f).

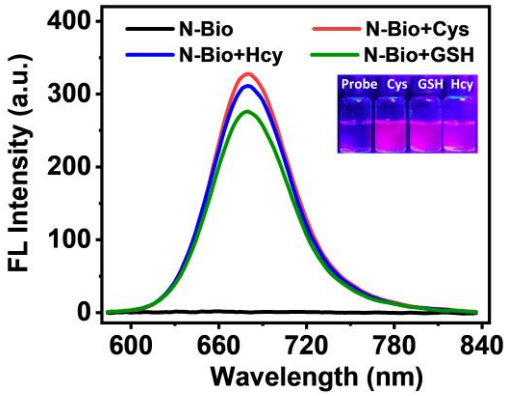


Figure S7. The fluorescence response of **N-Bio** toward biothiols in PBS-ethanol buffer (10 mM, 1:1, v/v).

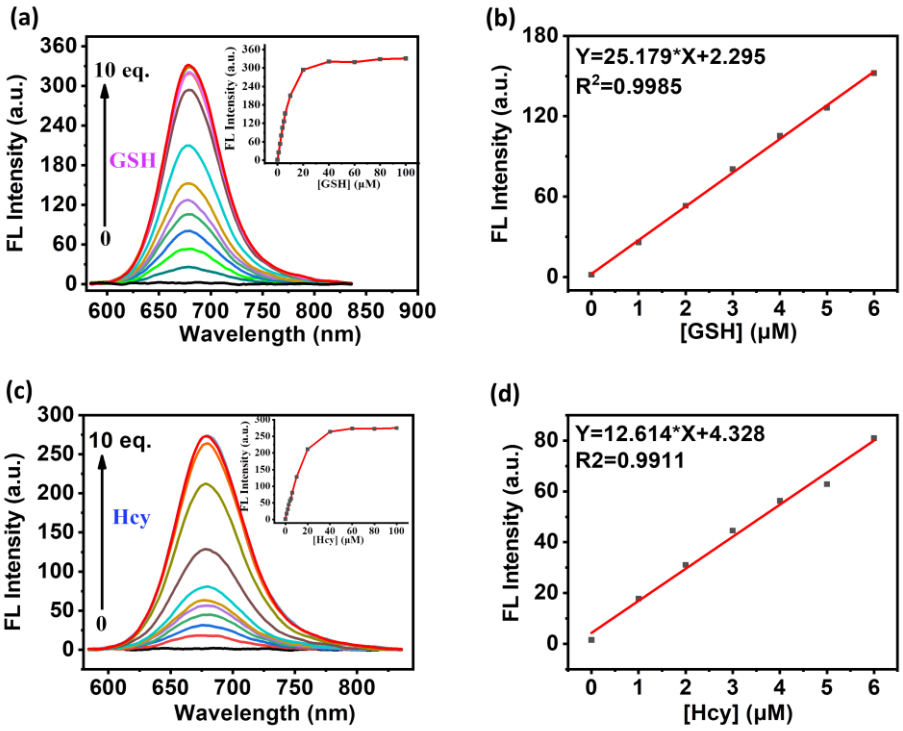


Figure S8. Fluorescence titration of **N-Bio** toward Hcy and GSH in PBS-ethanol buffer (10 mM, 1:1, v/v). (a) Fluorescence spectral change of **N-Bio** (10 μM) with the addition of different GSH concentrations (0, 1, 2, 3, 4, 5, 6, 10, 20, 40, 60, 80, and 100 μM) for 20 min; inset: the

fluorescence intensity at 680 nm changes toward different concentration of Cys. (b) Linear relationship between the fluorescence intensity at 680 nm and the low concentrations (0-6 μM) of Cys. (c) Fluorescence spectral change of **N-Bio** (10 μM) with the addition of different concentrations of Hcy (0, 1, 2, 3, 4, 5, 6, 10, 20, 40, 60, 80, and 100 μM) for 20 min; inset: the fluorescence intensity at 680 nm changes toward different concentrations of Hcy. (c) Linear relationship between the fluorescence intensity at 680 nm and the low concentrations (0-6 μM) of Hcy.

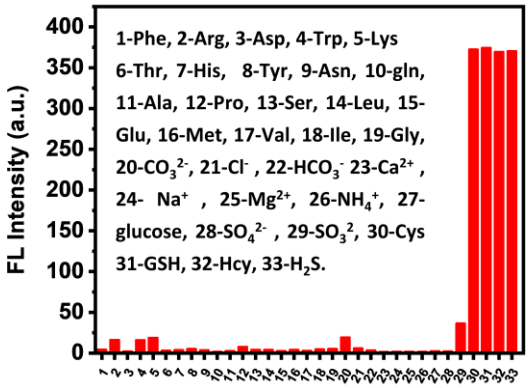


Figure S9. Fluorescence responses of **N-Bio** with the addition of various testing analytes (the concentration is set as: 100 μM for the Cys, GSH, H_2S, Hcy, 500 μM for SO_3^{2-}, 10 mM for the other amino acids and other ions).

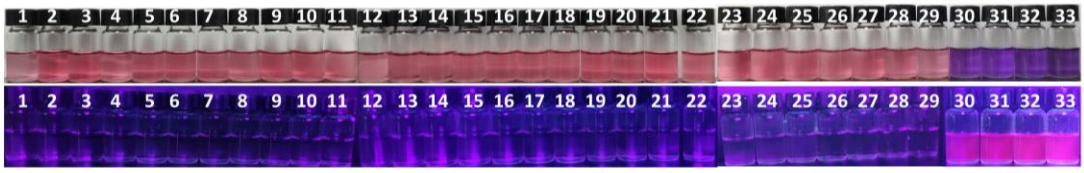


Figure S10. The color change of **N-Bio** solution incubated with various analytes, up: observed by naked eye, down: excited by a UV lamp (365 nm). All the selectivity measurements were conducted in PBS-ethanol buffer (10 mM, 1:1) for 15 min. The concentration is set as: 100 μM for the Cys, GSH, H_2S, Hcy, SO_3^{2-}, 2 mM for the other amino acids and other ions. 1: Phe. 2: Arg, 3: Asp, 4: Trp, 5: Lys, 6: Thr, 7: His, 8: Tyr, 9: Asn, 10: gln, 11: Ala, 12: Pro, 13: Ser, 14: Leu, 15: Glu,

16: Met, 17: Val, 18: Ile, 19: Gly, 20: CO_3^{2-}, 21:Cl^-, 22: HCO_3^-, 23: Ca^{2+}, 24: Na^+, 25: Mg^{2+}, 26: NH_4^+, 27: glucose, 28: SO_4^{2-}, 29: SO_3^{2-}, 30: Cys, 31: GSH, 32: Hcy, 33: H_2S.

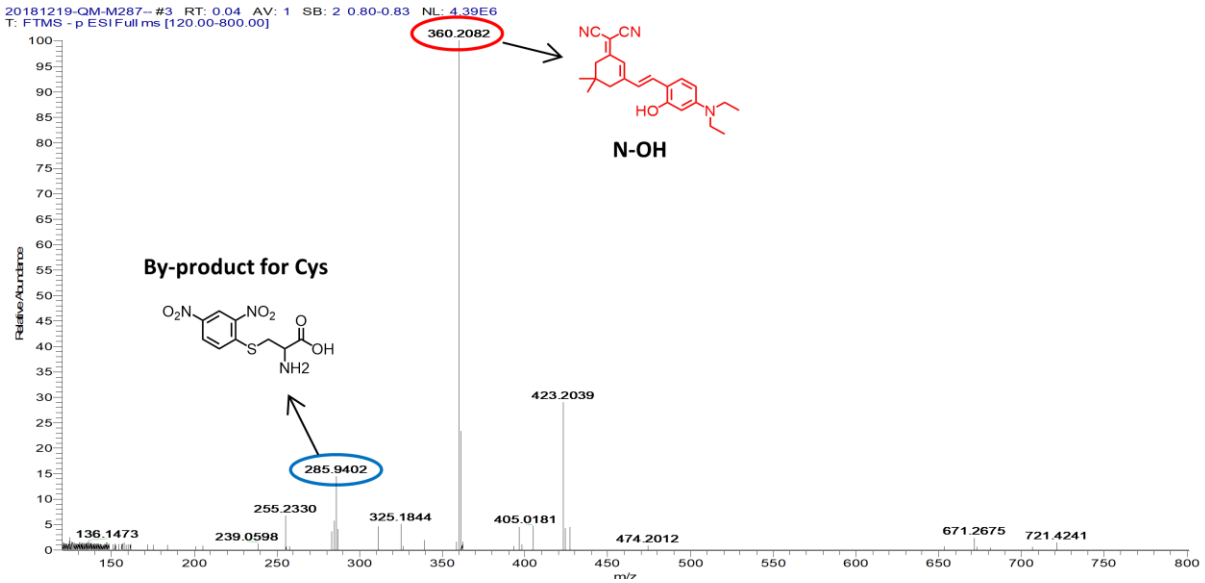


Figure S11. Verifying the sensing mechanism of **N-Bio** toward Cys using HRMS analysis method (mass negative mode)

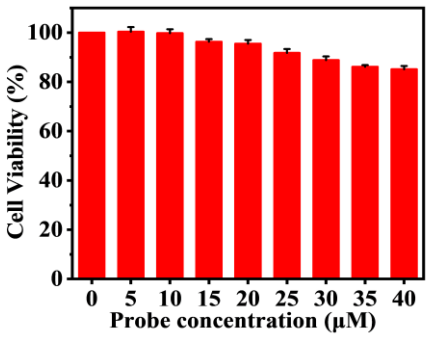


Figure S12. Cytotoxicity of the probe **N-Bio** against HeLa cells evaluated by a standard MTT assay, the data are presented as mean ± S.D.

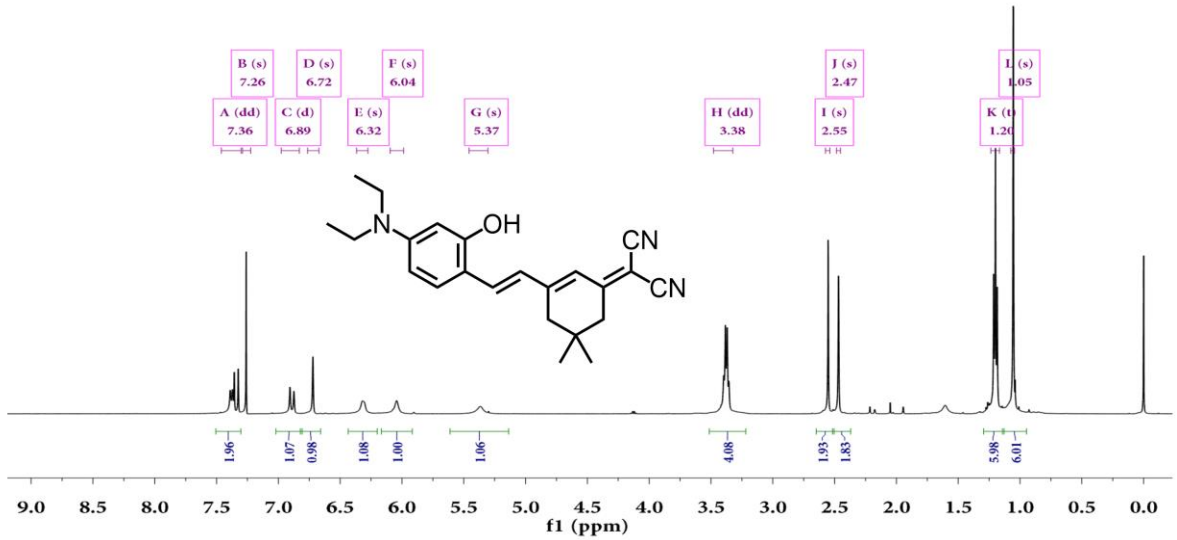


Figure S13. ^{1}H NMR spectrum of **N-OH** in $CDCl_3$

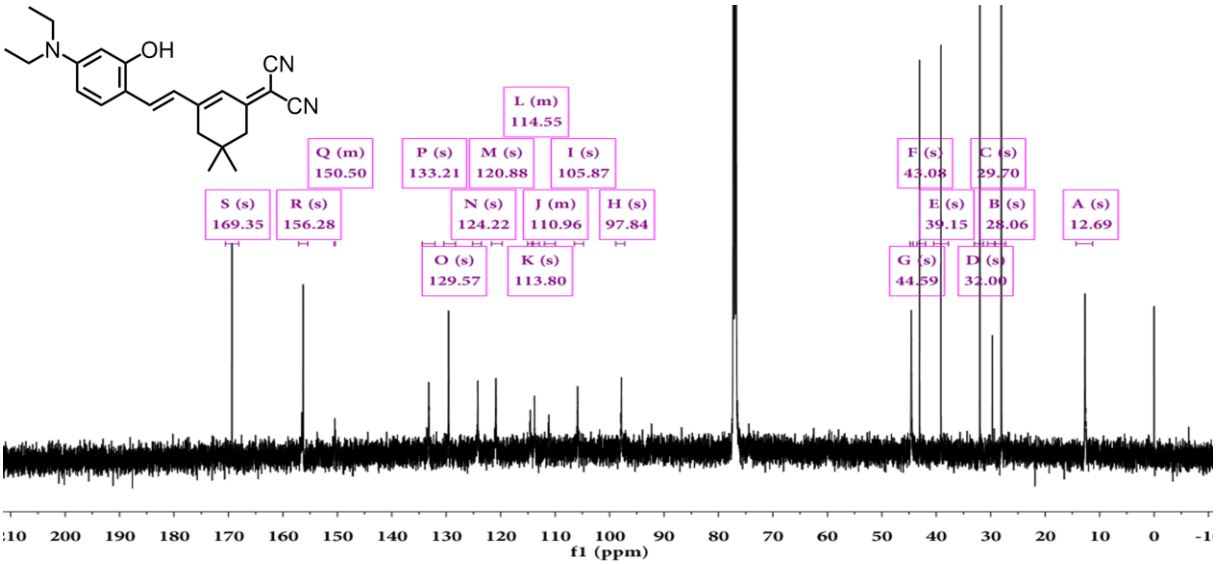


Figure S14. ^{13}C NMR spectrum of **N-OH** in $CDCl_3$

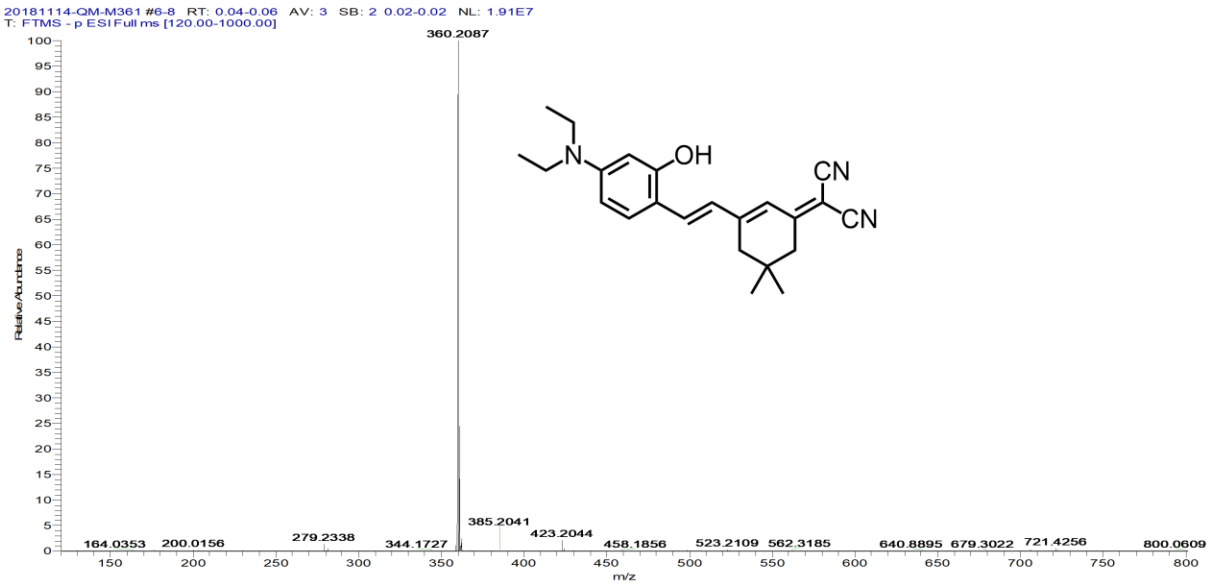


Figure S15. HRMS spectrum of **N-OH**

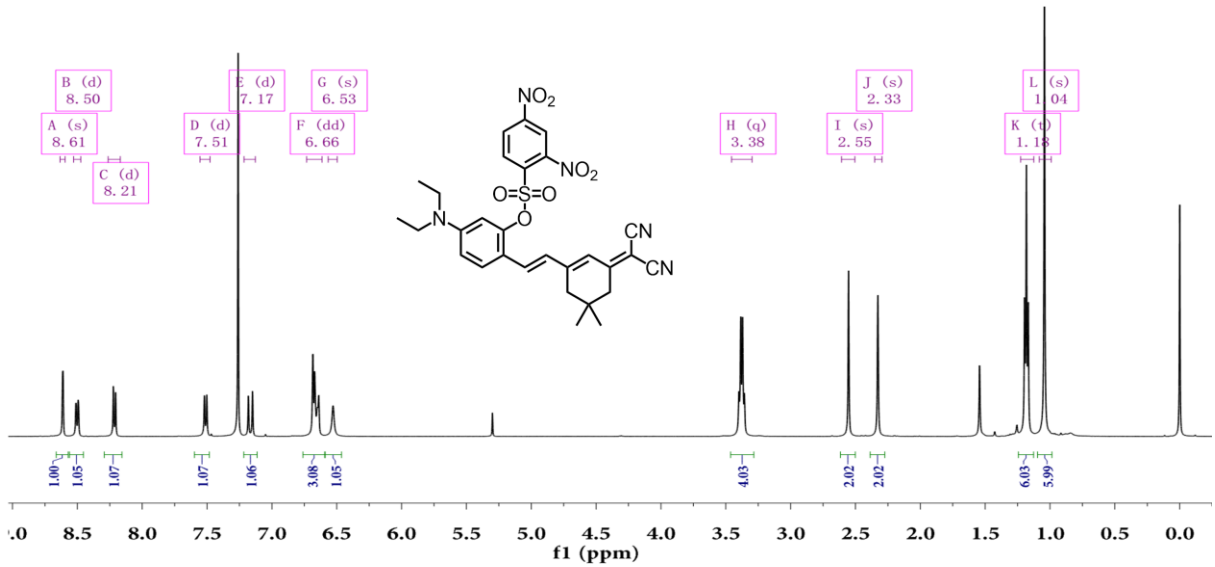


Figure S16. ^{1}H NMR spectrum of **N-Bio** in $CDCl_3$

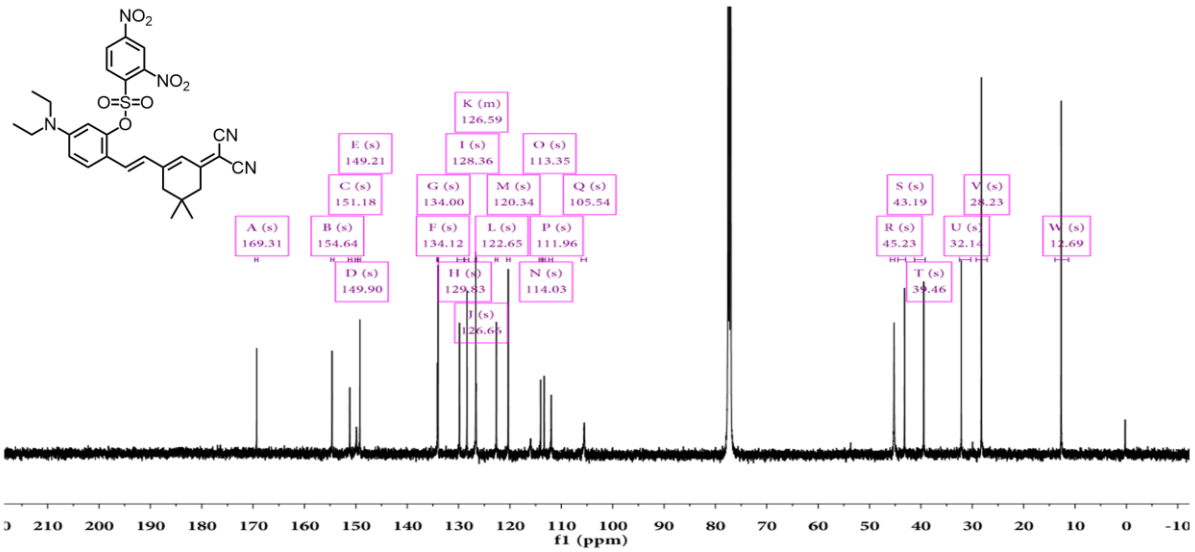


Figure S17. ^{13}C NMR spectrum of **N-Bio** in $CDCl_3$

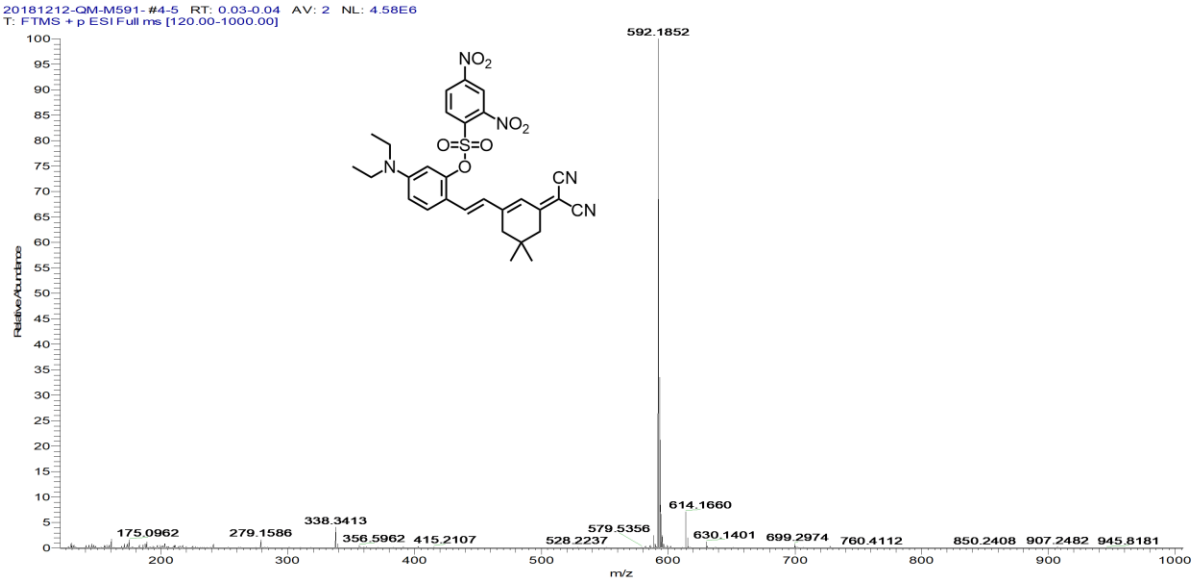


Figure S18. HRMS spectrum of **N-Bio**