Supporting Information for

A Dual-Color ESIPT-based Probe for Simultaneous Detection of Hydrogen Sulfide and Hydrazine

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1. Materials and instrument

Common reagents or materials were obtained from commercial suppliers without further purification except as otherwise noted. All experiments used ultra-pure water. The pH measurements were performed with a PHS-3E pH meter. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer, and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer. The fluorescence imaging of cells was performed with a Leica TCS SP8 CARS confocal microscope. ^{1}H and ^{13}C NMR spectra were measured on an AVANCE III HD600 digital NMR spectrometer, using tetramethylsilane (TMS) as the internal reference. High-resolution mass spectrometric (HRMS) analyses were measured on Brooke solanX 70 FT-MS, Agilent 6540T.

2. Test solution configuration of the BDM-DNP

Except for special circumstances, all probe solutions, ion solutions, and related solutions required in this article were prepared and used by the following methods. (a) Preparation of **BDM-DNP** reserve solution: 5.9 mg of **BDM-DNP** solid dissolved in 1 mL DMSO solution to prepare reserve solution; (b) Manufacture of **BDM-DNP** test solution: 1.2 μL of the stock solution diluted with DMSO to 1.2 mL, then the diluted solution diluted with PBS buffer to 3 mL to prepare the final test solution required for testing. The final probe was tested at the concentration of 10 μM, and the solution was 60% DMSO and 40% PBS buffer. The spectral properties of the solutions were measured using a Shimadzu UV-2700 spectrophotometer and a HITACHI F4700 fluorescence spectrophotometer.

3. Cytotoxicity assay

In vitro, cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on Hela cells. Cells were seeded into the 96-well tissue

culture plate in the presence of 100 μL Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2 atmosphere for 24h. The cell DMEM medium from each well was replaced with fresh medium containing increasing concentrations of **BDM-DNP**, i.e., 0, 1, 2, 5, 10, 20, 30, and 40 μM. Then the cell culture medium was removed and rinsed twice with PBS. Subsequently, 90 μL of fresh DMEM medium and 10 μL of MTT (5 mg/mL) were added to each well and incubated for 4 h. Absorbance of the solution was measured using a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **BDM-DNP**.

4. Cells fluorescence imaging

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin sulfate and penicillin, incubated at 37°C in a 5% CO_2 environment. For the fluorescence imaging study of exogenous H_2S and N_2H_4 in living Hela cells, we conducted a control experiment, in which the Hela cells were incubated with 10 μM **BDM-DNP** in the culture medium.

To investigate exogenous H_2S and N_2H_4 in live cells, three HeLa cell lines were treated with NaHS and N_2H_4 for imaging studies, respectively, then cultured with **BDM-DNP** (10 μM) for 15 minutes. Firstly, 10 mM stock solutions of **BDM-DNP** in DMSO and 100 mM hydrogen sulfide and hydrazine in water were prepared. The first HeLa cell lines were only cultured with **BDM-DNP** (10 μM) for 15 minutes. The second cell lines were pretreated with H_2S and N_2H_4 (100 μM) for 15 minutes and then incubated with **BDM-DNP** (10 μM) for 15 minutes. The third cell lines were pretreated with H_2S and N_2H_4 (100 μM) for 30 minutes before being incubated with **BDM-DNP** (10 μM) for 15 minutes.

5. Supporting figures and tables

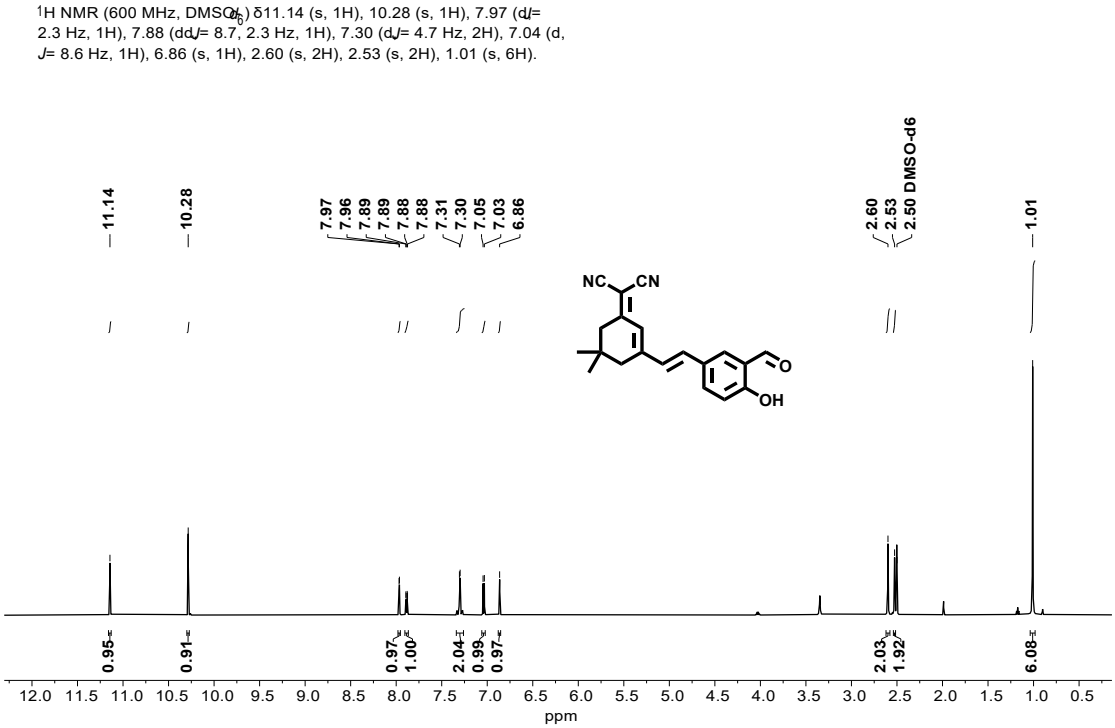

Fig. S1. [1]HNMR (600 MHz, DMSO-d_6) spectrum of compound 2.

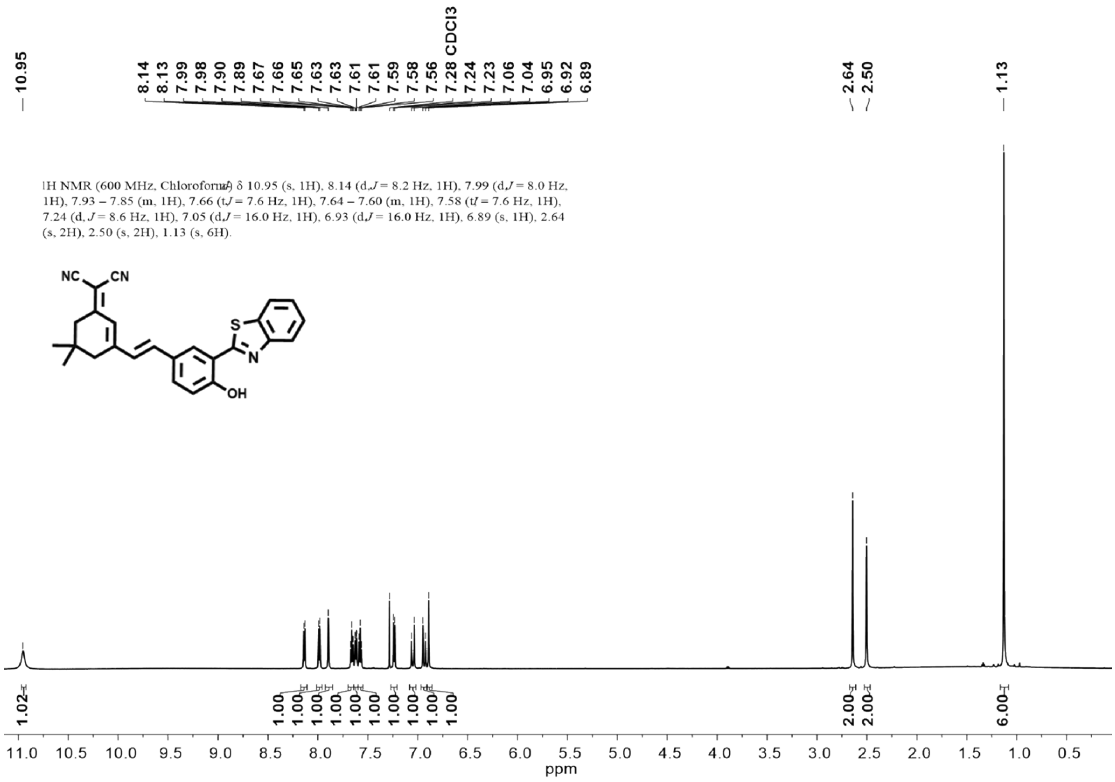

Fig. S2. [1]HNMR (600 MHz, Chloroform-*d*) spectrum of **BDM-OH**.

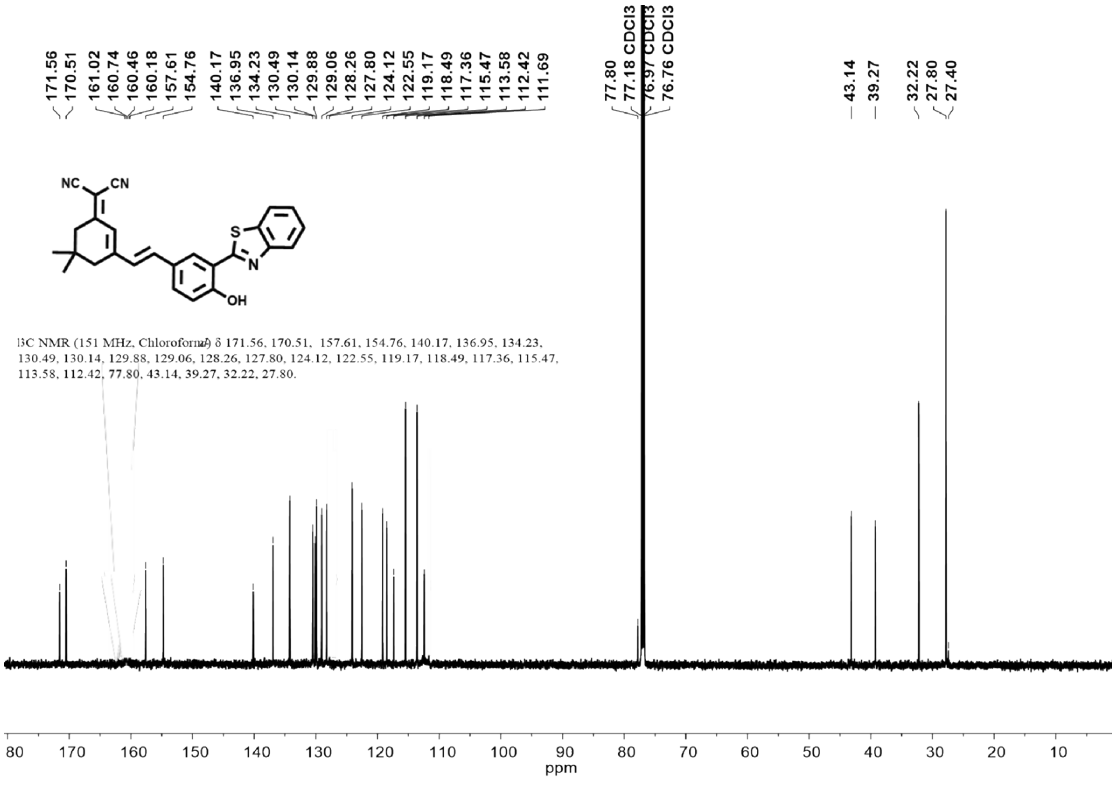

Fig. S3. [13]C NMR (151 MHz, Chloroform-*d*) spectrum of **BDM-OH**.

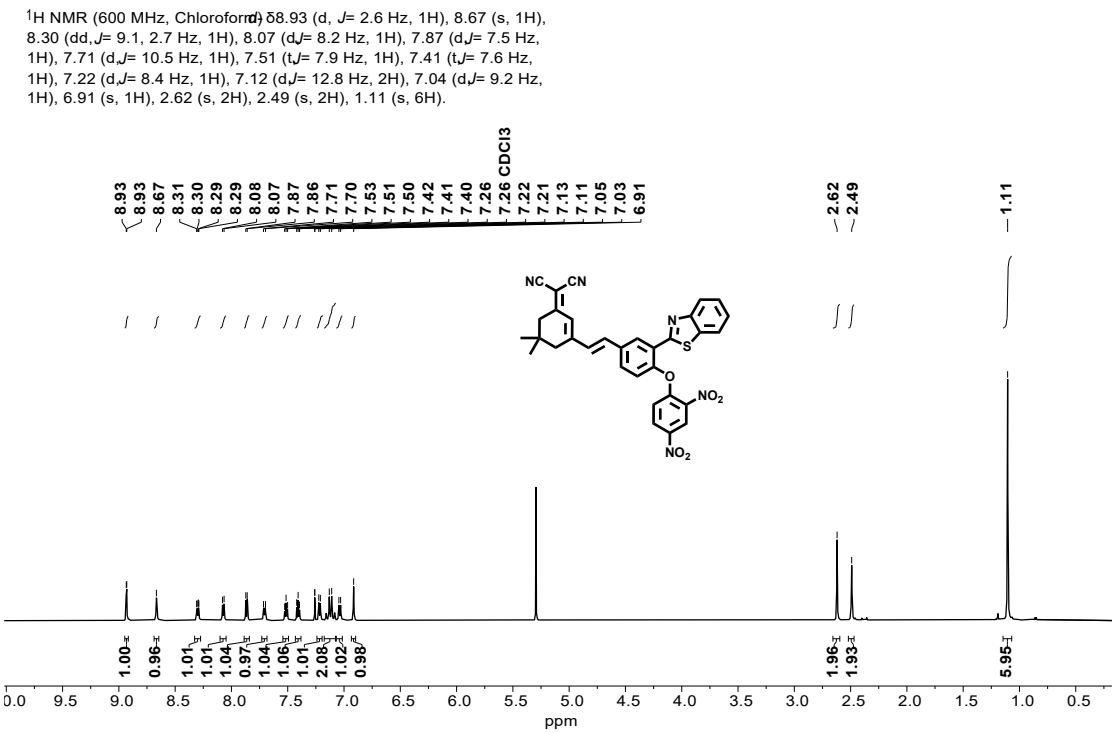

Fig. S4. [1]HNMR (600 MHz, Chloroform-*d*) spectrum of **BDM-DNP**.

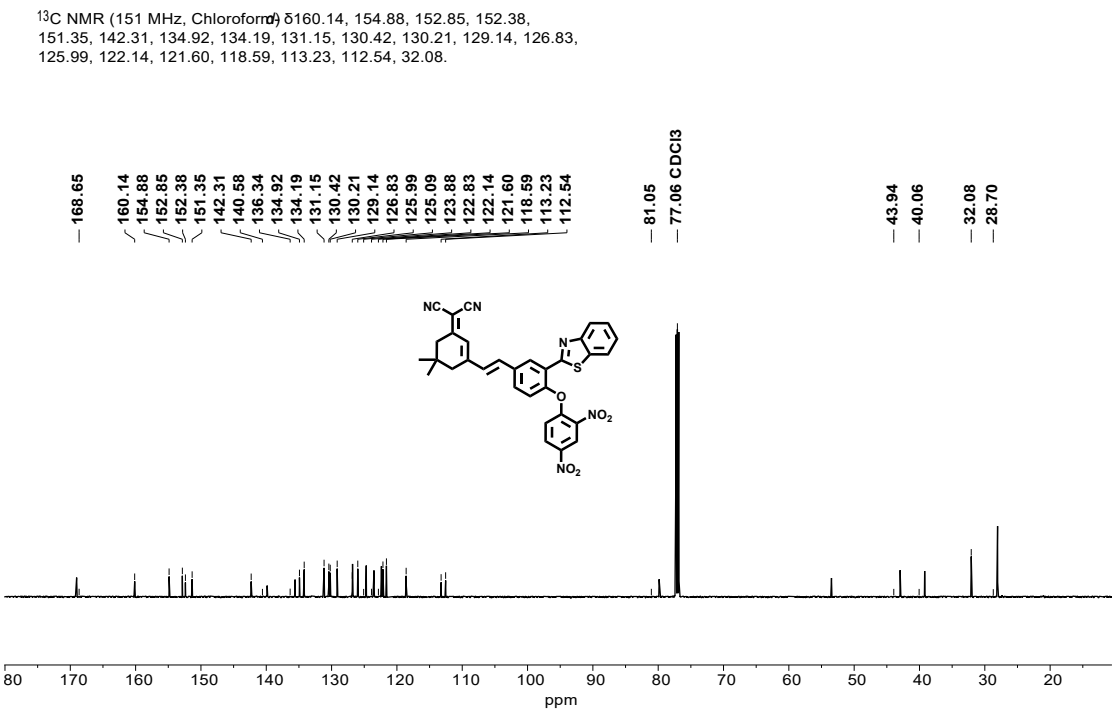

Fig. S5. [13]C NMR (151 MHz, Chloroform-*d*) spectrum of **BDM-DNP**.

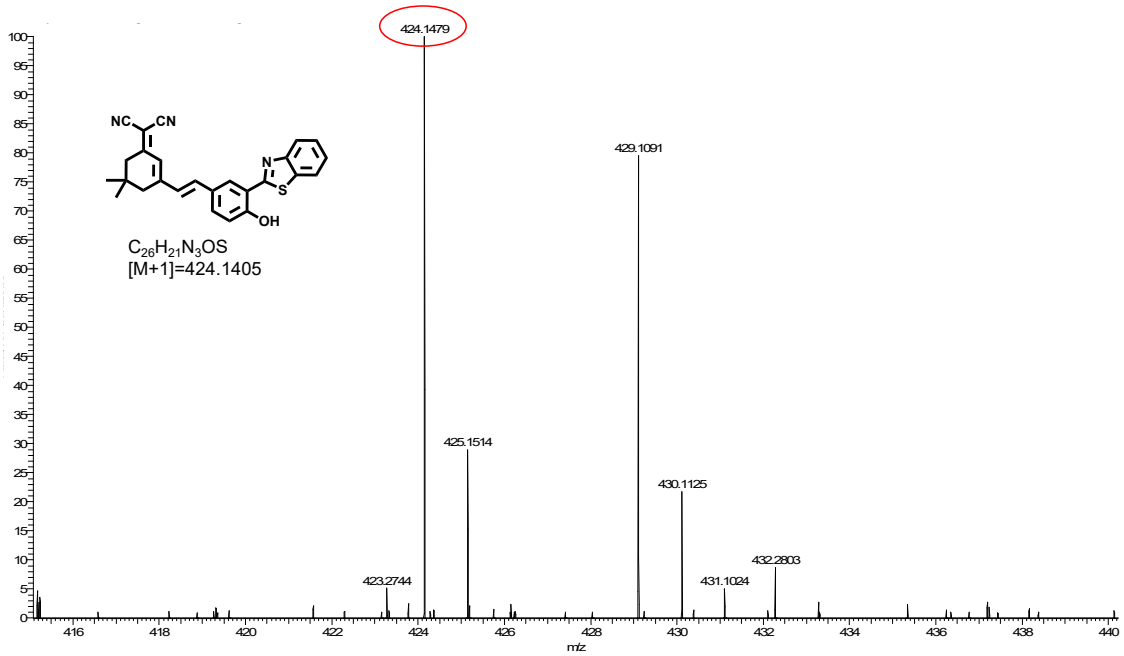

Fig. S6. HRMS (ESI) spectrum of **BDM-OH**

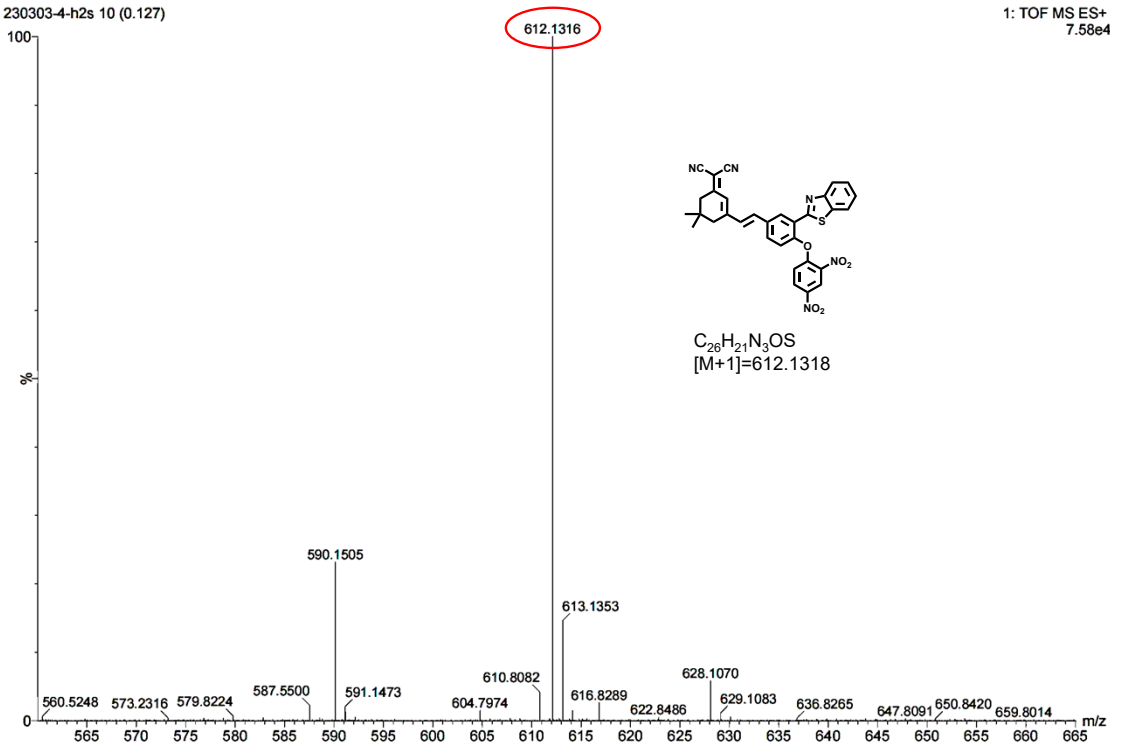

Fig. S7. HRMS (ESI) spectrum of **BDM-DNP**.

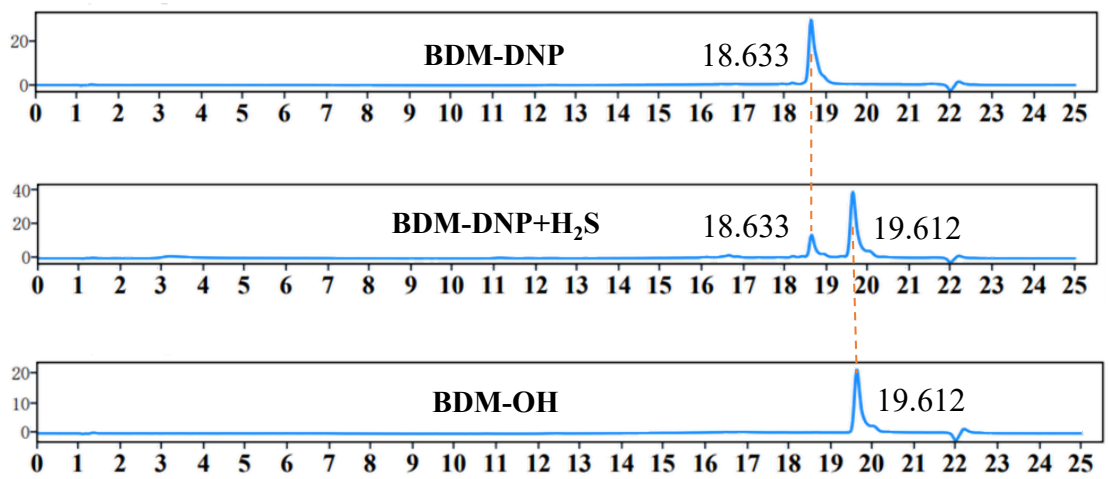

Fig. S8. The high-performance liquid chromatography (HPLC) of **BDM-DNP** and **BDM-OH** were tested in 10 mM PBS buffer solution (methyl alcohol: water = 1:9, pH = 7.4). (A) **BDM-DNP**; (B) **BDM-DNP** + H_2S; (C) **BDM-OH**.

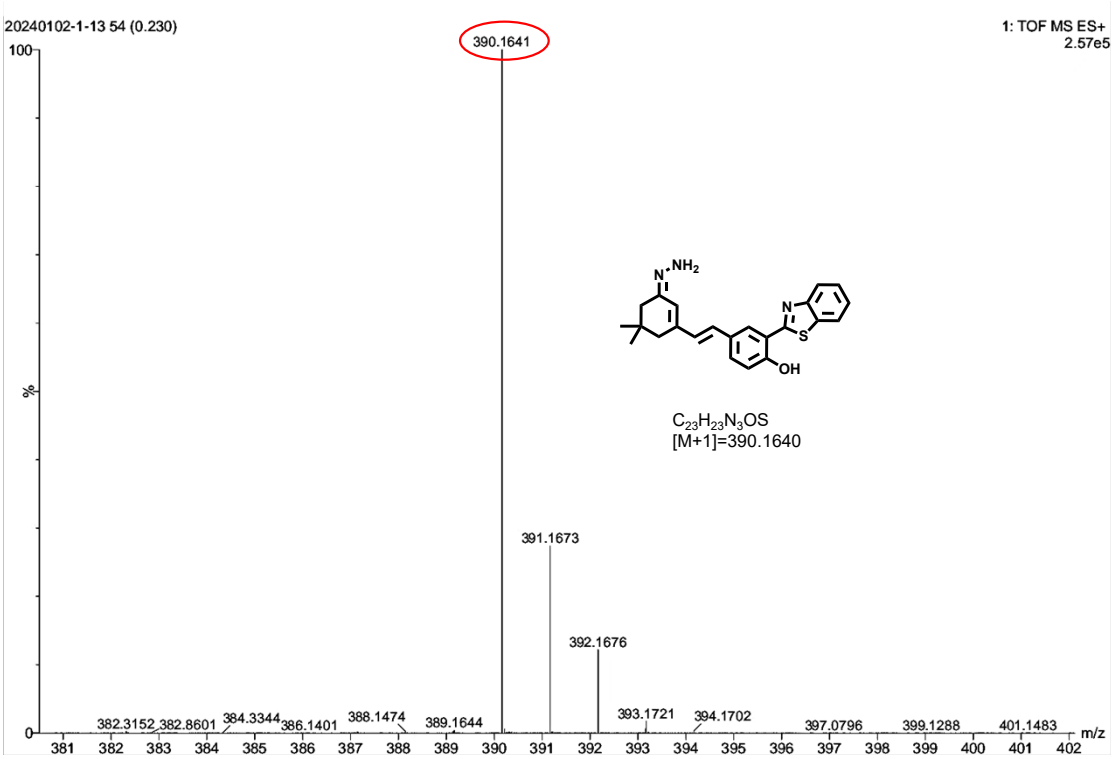

Fig. S9. HRMS (ESI) spectra of the reaction of **BDM-DNP** with N_2H_4.

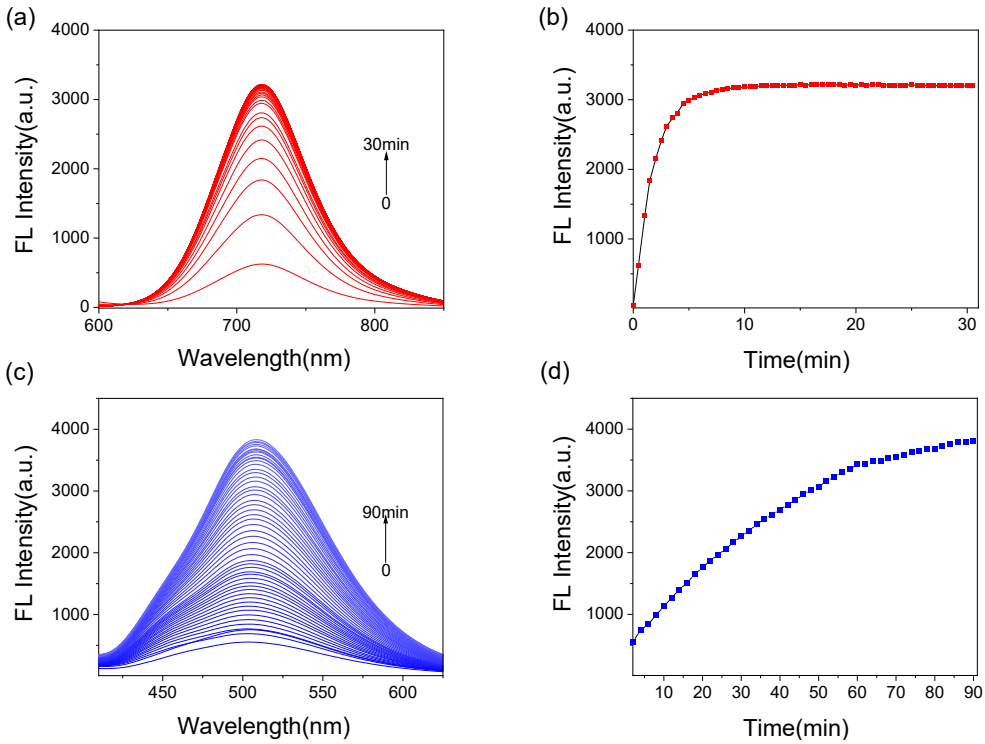

Fig. S10. (a) Fluorescence spectra of **BDM-DNP** (10 μM) with 1 mM H_2S at different times; (b) The response time of **BDM-DNP** with H_2S at 713 nm; (c) Fluorescence spectra of **BDM-DNP** (10 μM) with 1 mM N_2H_4 at different time; (d) The response time of **BDM-DNP** with N_2H_4 at 713 nm.

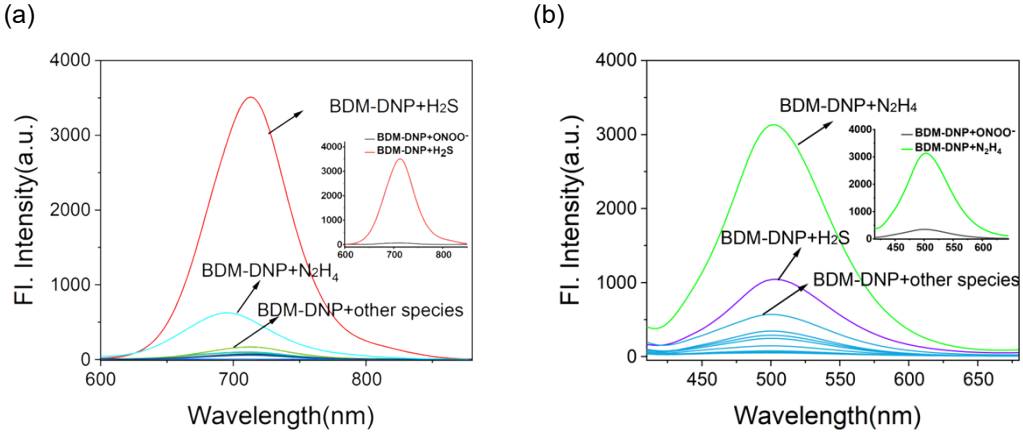

Fig. S11. The selectivity of **BDM-DNP** (10 μM) towards diverse relevant analytes (1 mM) against H_2S (1 mM) (a) and N_2H_4 (1 mM) (b) in the presence of coexisting analytes (1 mM). Insert: 1.H_2S; 2. N_2H_4 3. Cys; 4. Hcy; 5. GSH; 6. Na_2S; 7. NaS_2O_3; 8. NaSCN; 9. NaCl; 10. NaH_2PO4; 11. $NaHCO_3$; 12. $NaNO_3$; 13.CH_3COONa; 14. H_2O_2; 15. Gly; 16. Asp; 17$ONOO^-$.

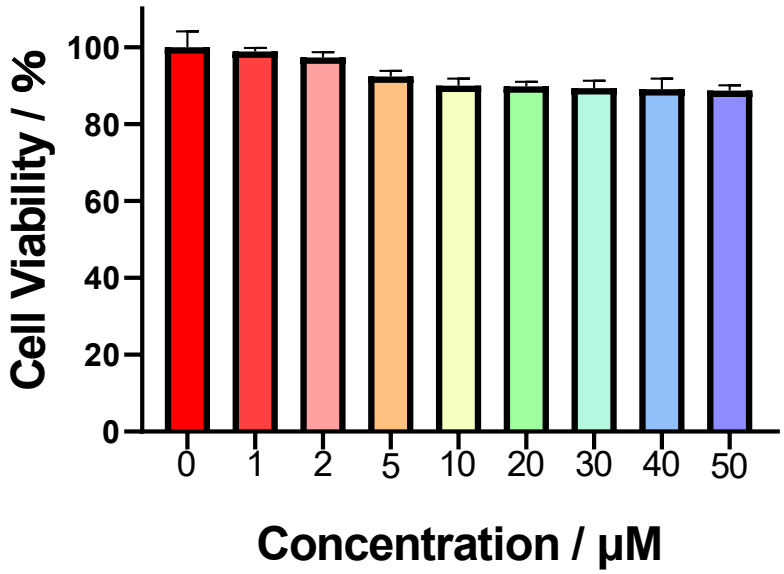

Fig. S12. The cytotoxicity of **BDM-DNP** in Hela cells.

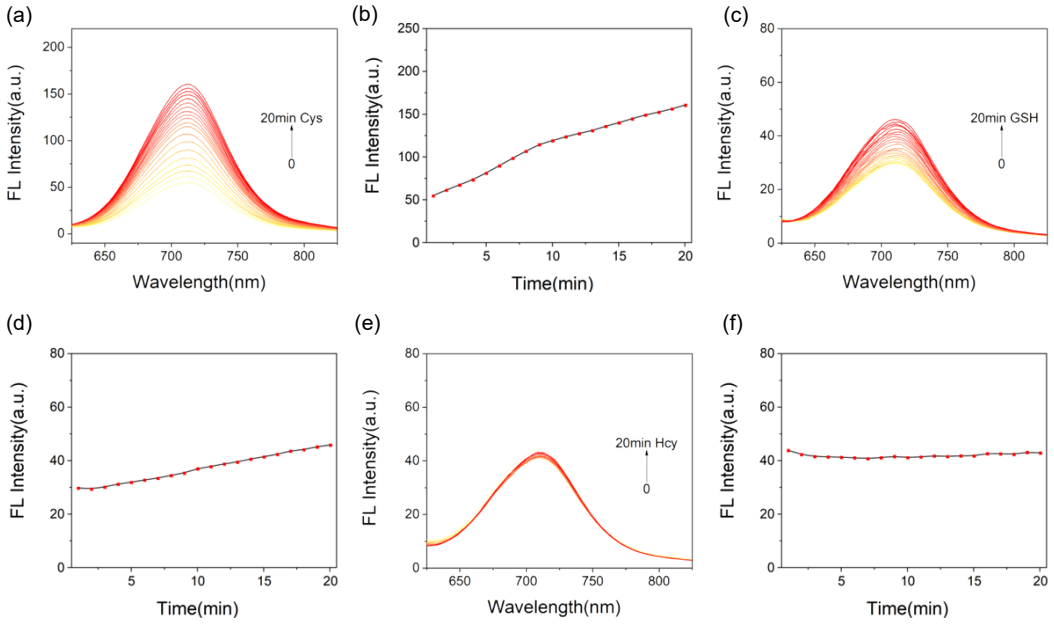

Fig. S13. Fluorescence spectra of **BDM-DNP** (10 μM) with 500 μM Cys (a), 500 μM GSH (c), and 500 μM Hcy (e) at different times; The response time of **BDM-DNP** with Cys (b), GSH (d) and Hcy (f) at 713 nm.

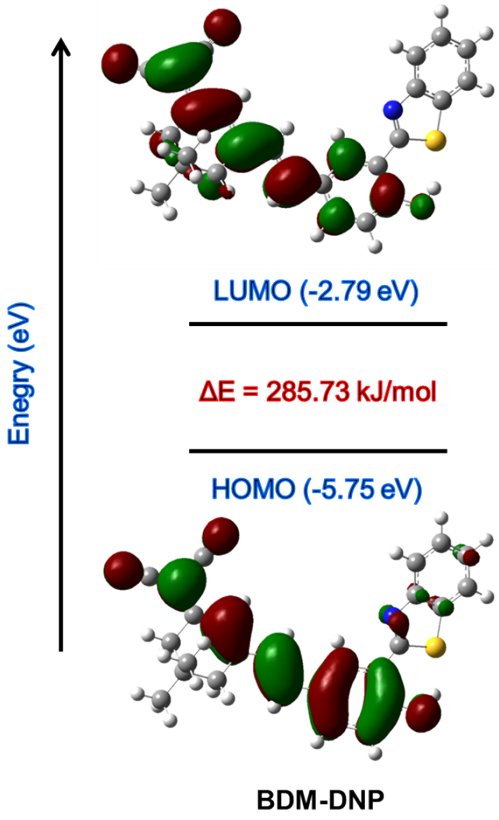

Fig. S14. Theoretical calculation of HOMO/LUMO energy for **BDM-DNP**.

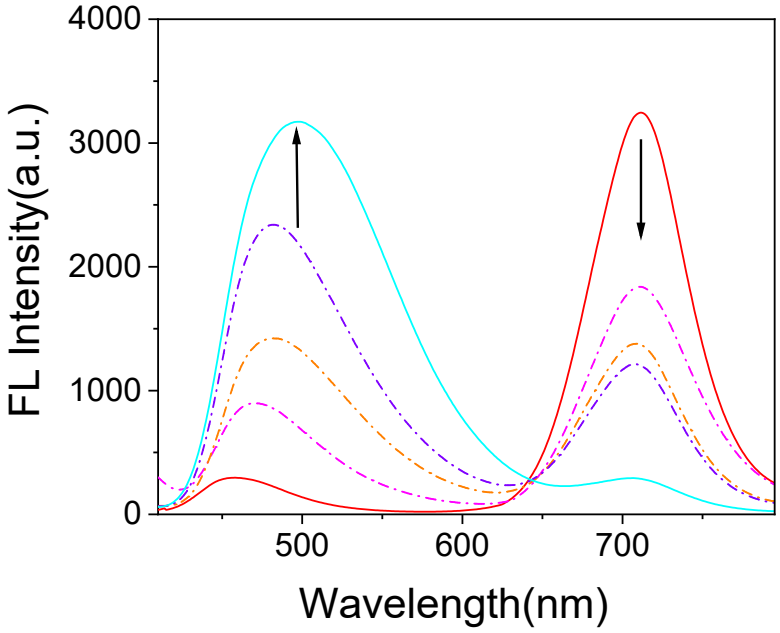

Fig. S15. Fluorescence spectra of **BDM-DNP** (10 μM) coexisting with 1 mM H_2S and 1 mM N_2H_4.