Supporting information

Endoplasmic reticulum targeted fluorescent probe for the detection of hydrogen sulfide based on twist-blockage strategy

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1. *Synthesis of compound 1*

A mixture of o-aminothiophenol (2.5g, 0.02 mol), glacial AcOH (1 mL) and malononitrile (1.32 g, 0.01 mol) was stirred in absolute ethanol (15 mL) at room temperature for overnight. The precipitate was filtered and the crude product was purified by column chromatography (silica gel, dichloromethane as eluent) to give 1（1.67g, 48%） for the next step.

2. *Synthesis of compound 2*

3-aminophenol (436.5 mg, 4 mmol), 1-bromo-3-chloropropane (1.32 g, 8.4 mmol) and sodium bicarbonate (705.2 mg, 8.4 mmol) were dissolved in 10 mL DMF. The mixture was stirred at 70 °C for overnight . Then, the mixture was cooled to room

temperature and extracted by EtOAc (30 mL), washed with water (3×30 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography using pure ethyl acetate as the eluent to afford the product as a white solid (514 mg, 68%) and used directly for the next step.

3. *Synthesis of compound 3*

To a round-bottom flask equipped with an ice-water bath were added phosphorous oxychloride (2.4 mL, 27.0 mmol) and DMF (3.0 mL, 38.0 mmol) at 0 °C. The resulting solution was stirred for 30 min at room temperature. To above reaction mixture a solution of compound **2** (3.3 g, 17.5 mmol) in DMF (2.0 mL) was added. And the resulting solution was stirred for 30 min at room temperature, then heated to 60 °C for 1h. After cooling to room temperature, the reaction mixture was poured into crash ice (100 g) and then the precipitate was formation. Next, the precipitate was filtered and dried for overnight. Then the product was obtained as a grass green solid（2.96 g, 78%）（and used directly for the next step）.

4. *Synthesis of compound 4*

A mixture of compound 3 (217 mg, 1.0 mmol), 1-fluoro-2,4-dinitrobenzene (205 mg, 1.1 mmol), and K_2CO_3 (274 mg, 2 mmol) in DMF (5 mL) was stirred at room temperature. After the reaction completed (about 1 h, monitored by TLC), the reaction mixture was poured into ice water. The precipitate was collected by filtration and washed with cold water to afford an yellow solid (394 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.96 (s, 1H), 8.87 (d, J=2.4 Hz, 1H), 8.25 (d, 1H), 7.37 (s, 1H), 6.86 (s, 1H), 3.36 (t, J=2.4 Hz, 2H), 3.34 (t, J=11.6 Hz, 2H), 2.81 (m, J=11.2 Hz, 3H), 2.28 (s, 1H), 2.02 (m, 2H), 1.94 (m, 2H).

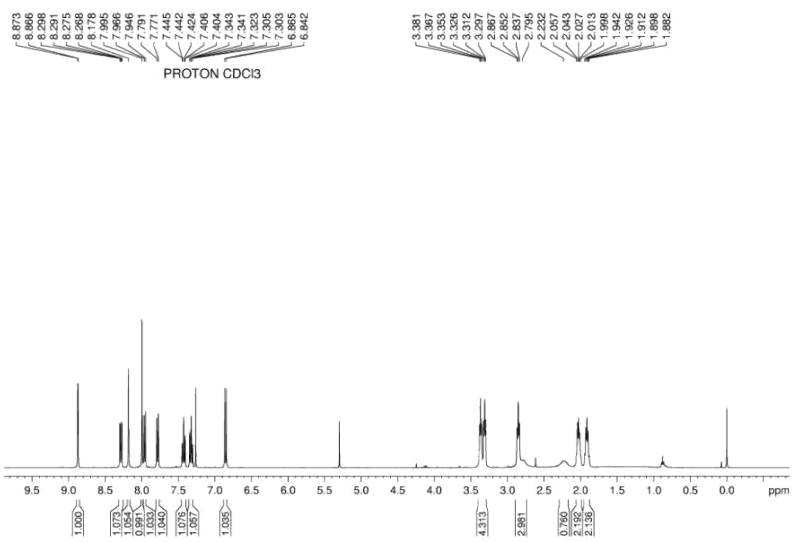

Figure S1: ^{1}H NMR spectra of Z1 (400 MHz, $CDCl_3$)

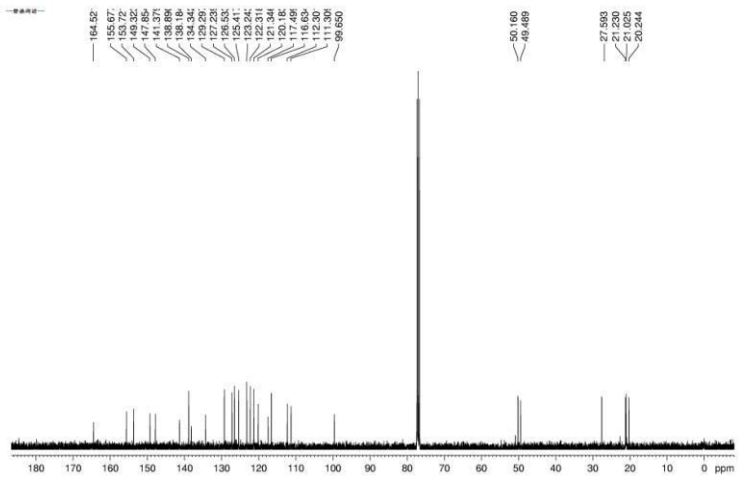

Figure S2: ^{13}C NMR spectra of Z1 (400 MHz, $CDCl_3$)

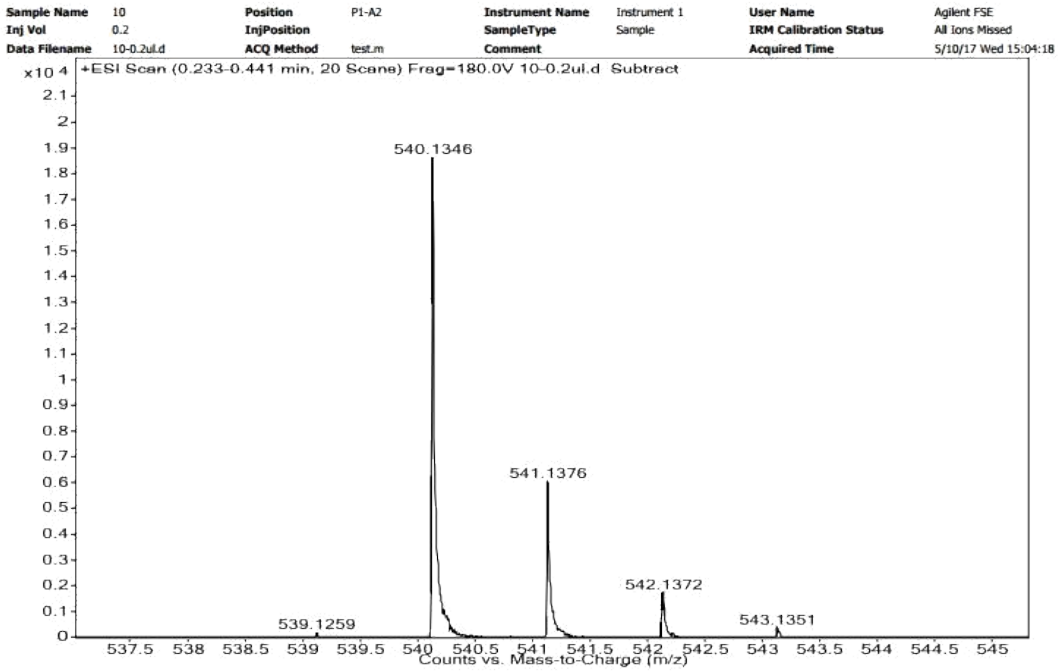

Figure S3. HRMS spectrum of **Z1**.

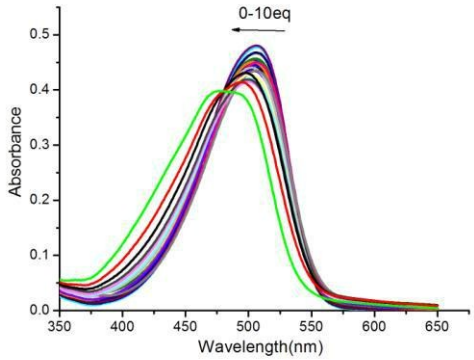

Figure S4 Absorption titration of **Z1** (10 μM) in HEPES (pH=7.4, 10 mM, containing 1mM CTAB) with addition of increasing concentrations of Na_2S (0, 1, 2, 3, 4, 5, 6, 7, 8, 9,10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μM).

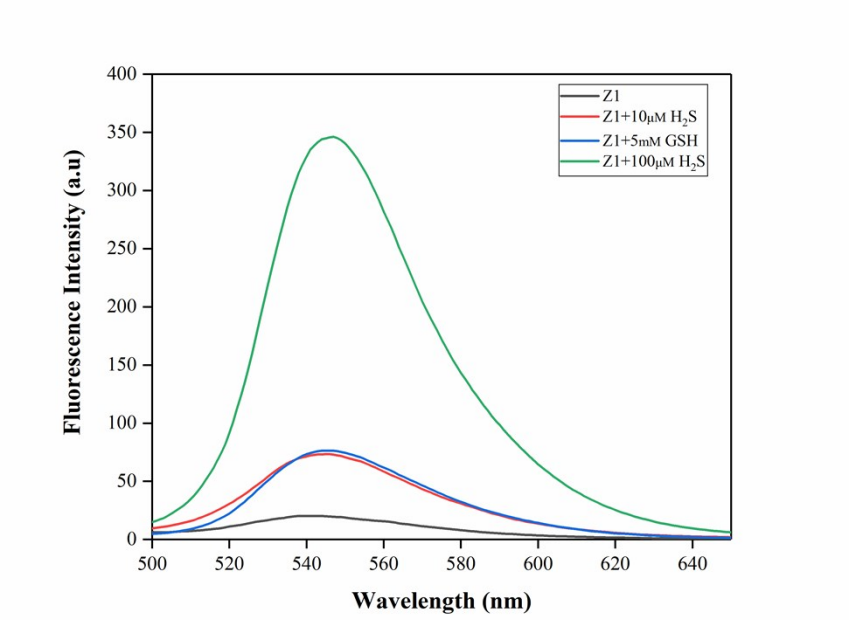

Figure S5. The fluorescent spectra of **Z1** with H_2S or GSH (recorded in 5 mins)

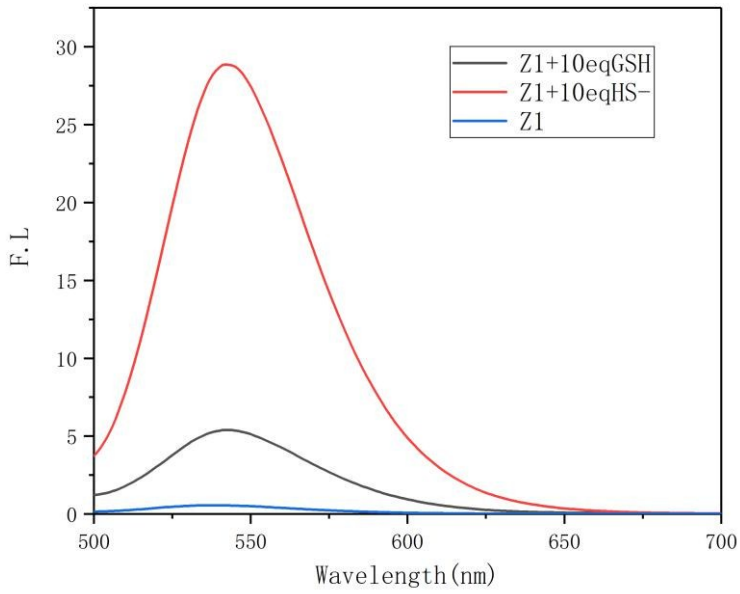

Figure S6. The fluorescent spectra of Z1 with H_2S or GSH (recorded in 5 mins)

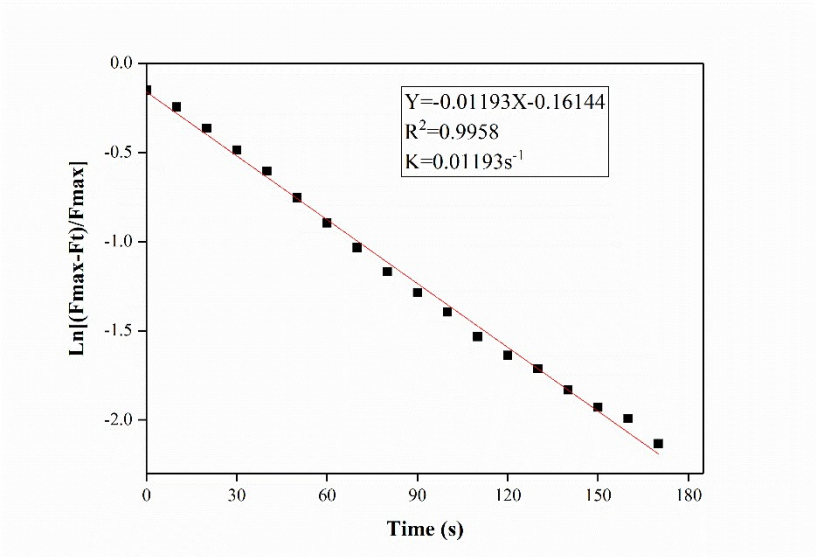

Figure S7.*Pseudo* first-order kinetic plot of the reaction of **Z1**(10 μM)with H_2S (10eq). $t_{1/2}$=45s

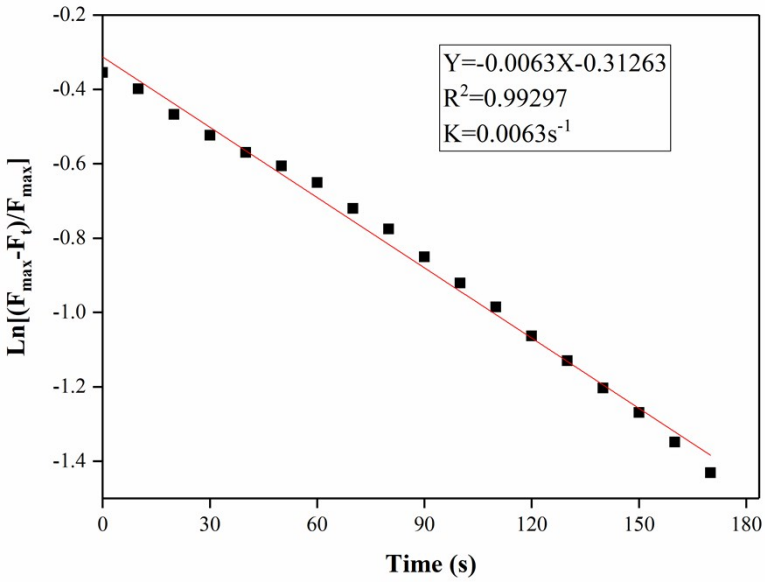

Figure S8.*Pseudo* first-order kinetic plot of the reaction of **Z1**(10 μM)with H_2S (50eq). $t_{1/2}$=60s

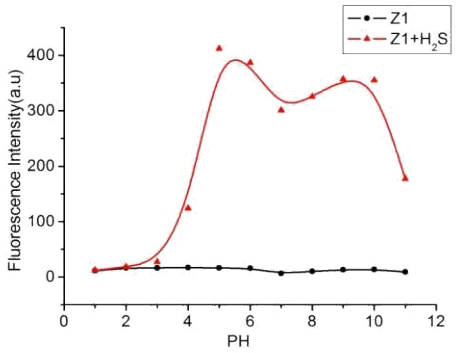

Figure S9. Fluorescence intensity at 537 nm of **Z1** (5.0 M) at different pH values in the absence/presence of Na_2S (10.0 equiv).

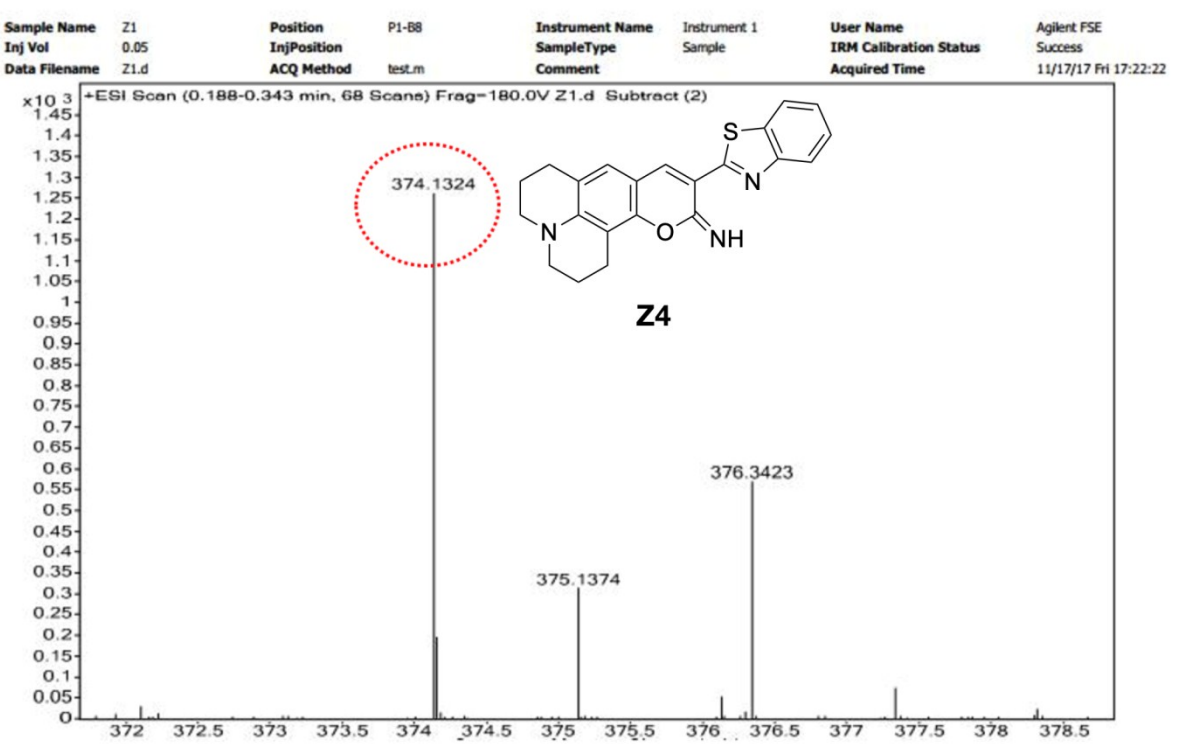

Figure S10 HRMS spectrum of the reaction product from **Z1** with Na_2S.

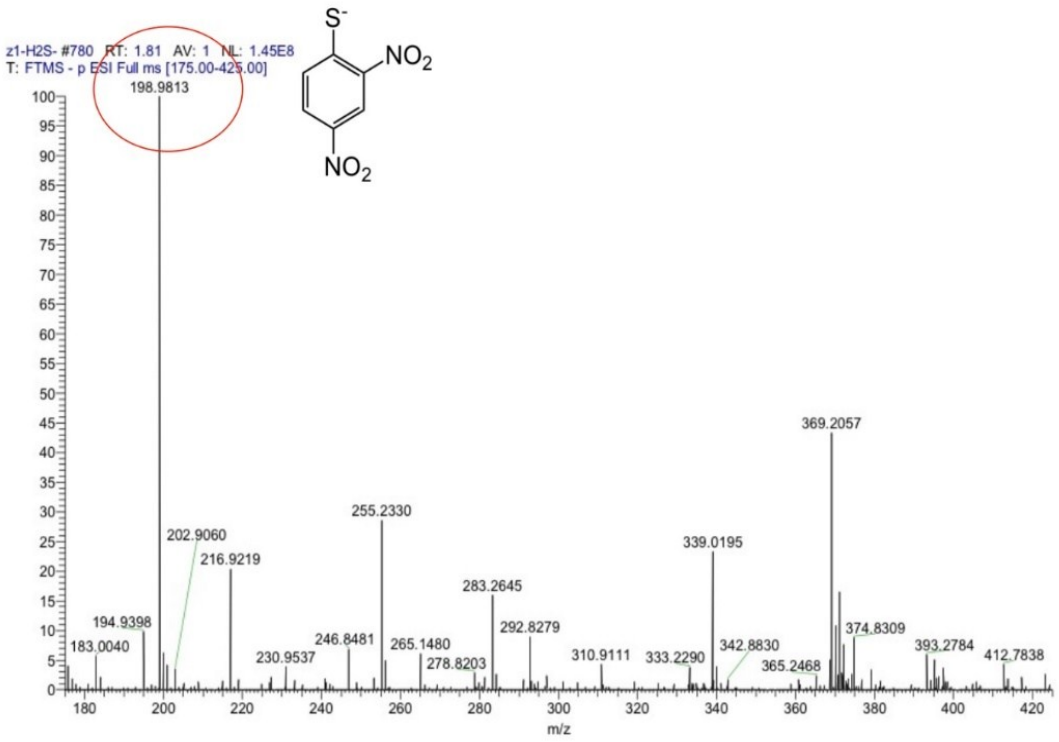

Figure S11 HRMS spectrum of the reaction product from **Z1** with Na_2S.