Supporting information

Benzophenoxazine-based NIR fluorescent probe for the detection of hydrogen sulfide and imaging in living cells

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Contents

Synthesis of 4-nitrosobenzene-1,3-diol

For synthesizing compound 4-nitrosobenzene-1,3-diol, 350 mL of ice water and 6.3 mL of concentrated sulfuric acid were added in a 1000 mL round bottom flask, and a mixture of resorcinol (14.5 g, 0.13 M) and sodium nitrite (10.8 g, 0.15 M) was added into the round bottom flask and thoroughly mixed with constant magnetic stirring. The whole process temperature was maintained at 0 - 5 ℃, keeping reaction for 20 min. Then, the temperature was raised to 8 - 15 °C, and water was added to make the volume up to 550 mL, filtered, washed with water and dried to obtain the product (17.93 g; yield, 96%).

Synthesis of NR

At room temperature, 4-nitrosobenzene-1,3-diol (0.153 g, 1.1 mM) and 1-naphthol (0.144 g, 1 mM) were added to 5 mL of DMF in the reaction bottle and stirred evenly. Then, 3 mL of hydrochloric acid was added dropwise to the reaction bottle. After the overnight reaction, the product was purified through silica gel chromatography using a mixture of methanol and dichloromethane (v/v = 1:30) as the mobile phase, which afforded NR as a red solid (130 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 8.61 (d, J = 7.7 Hz, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.81 (ddd, J = 28.5, 17.8, 7.7 Hz, 3H), 7.02 - 6.67 (m, 2H), 6.39 (s, 1H).

MS (ESI-MS, position mode, m/z) Calcd for $C_{16}H_{10}N_2O_2$ $[M + H]^+$: 264.07, found: 263.95.

Photostability

To investigate the photostability of the probe system, the UV-vis absorption spectra of NRDNP in PBS solution were monitored by a UV-vis spectrophotometer, upon continuous irradiation with a UV lamp (20 W). The photostability of NRDNP was demonstrated by plotting A versus t, where A is the absorbance of NRDNP and t is irradiation time.

Determination of Limit of Detection

The Limit of Detection (LOD) was calculated according to the formula LOD = 3σ/k. In this formula, σ was the standard deviation of the blank which was obtained through 11

times tests of the probe in buffer, and k was the slope of the calibration curve. The coefficient 3 was according to the Compendium of Analytical Nomenclature (IUPAC) standard.

Kinetic studies

The reaction of NRDNP (10 μM) with Na_2S 100 μM) in pH 7.4 PBS buffer (containing 10 % DMSO as co-solvent) was monitored using the absorbance (A) at 582 nm. The pseudo-first-order rate constant for the reaction was determined by fitting the A of the samples to the pseudo-first-order equation:

$$\mathrm{Ln}\,[(A_{max} - A_t)/A_{max}] = k_t$$

where A_t and A_{max} are the absorbance at 582 nm at time t and the maximum absorbance obtained after the reaction was complete, respectively. k is the pseudo-first-order rate constant. The pseudo-first-order plot for the reaction of NRDNP with 10 equivalents Na_2S is shown in Fig. S9B. The slope of the line provides the pseudo-first-order rate constant.

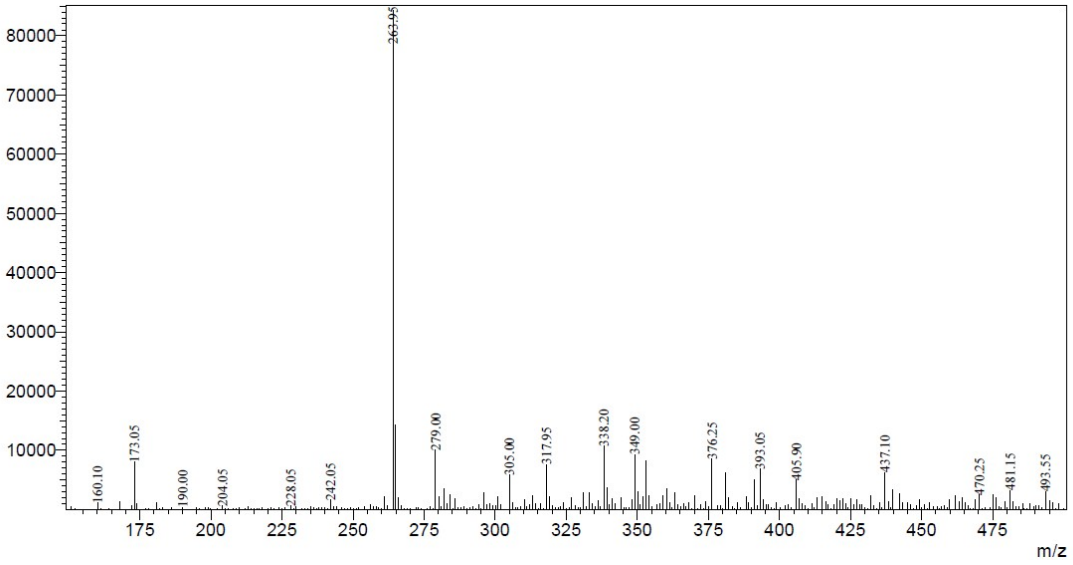

Figure S1. ESI-MS of intermediate NR (positive ion mode).

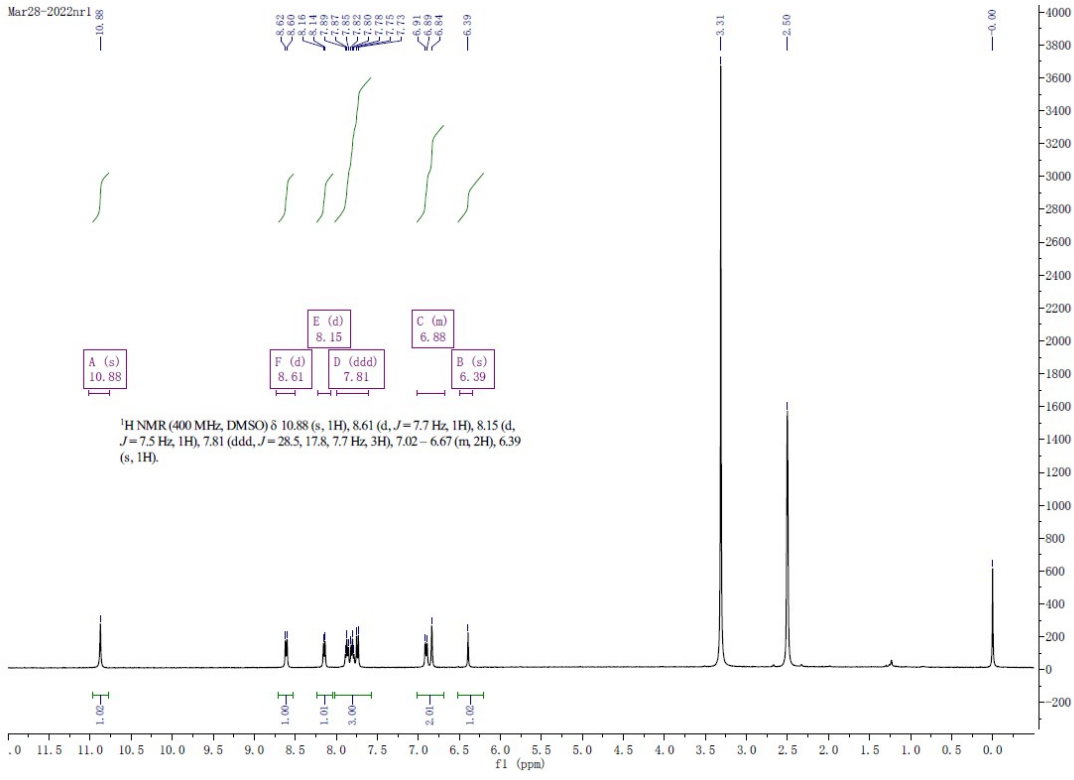

Figure S2. ^{1}H NMR of intermediate NR in DMSO-d_6.

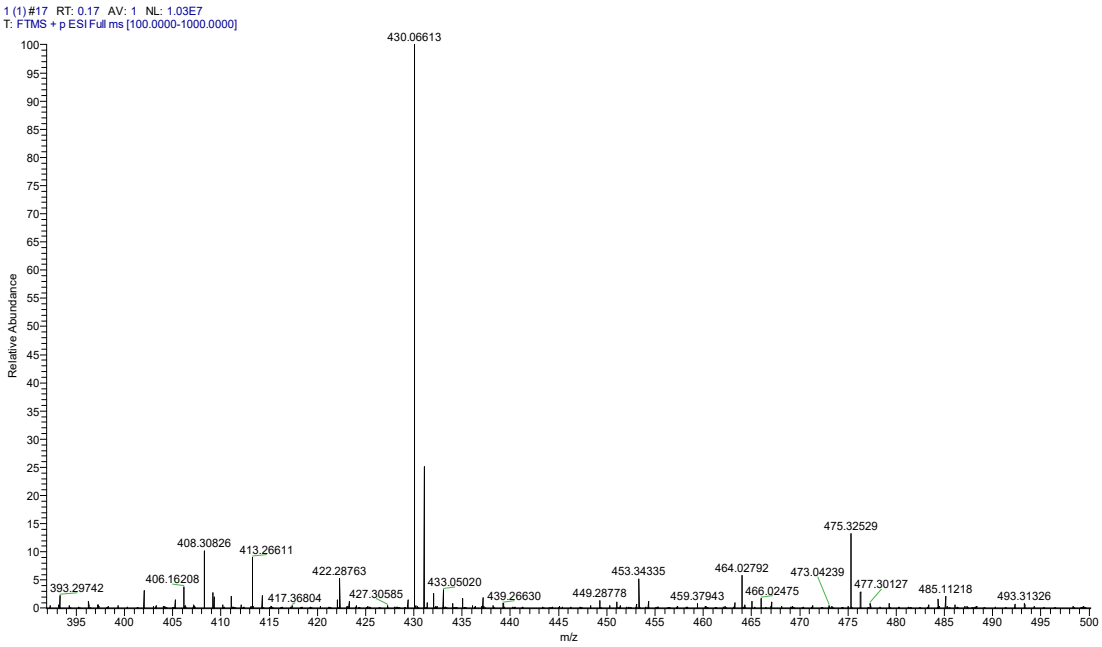

Figure S3. ESI-HRMS of NRDNP.

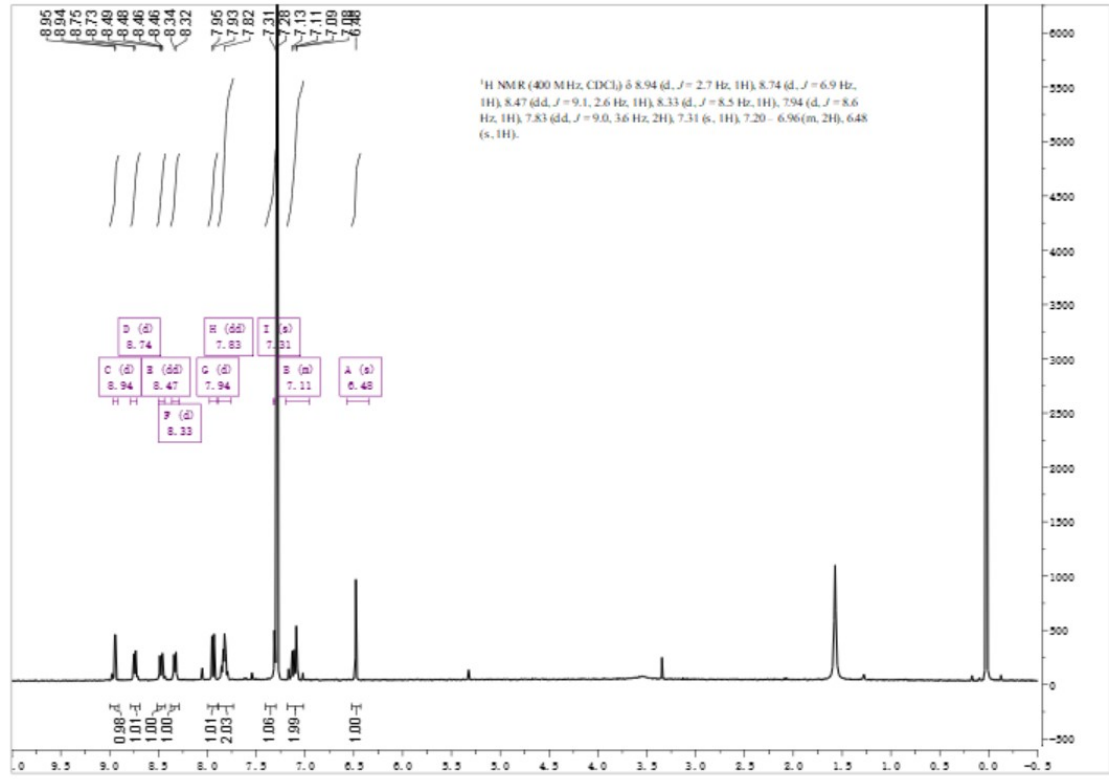

Figure S4. ^{1}H NMR of NRDNP in $CDCl_3$.

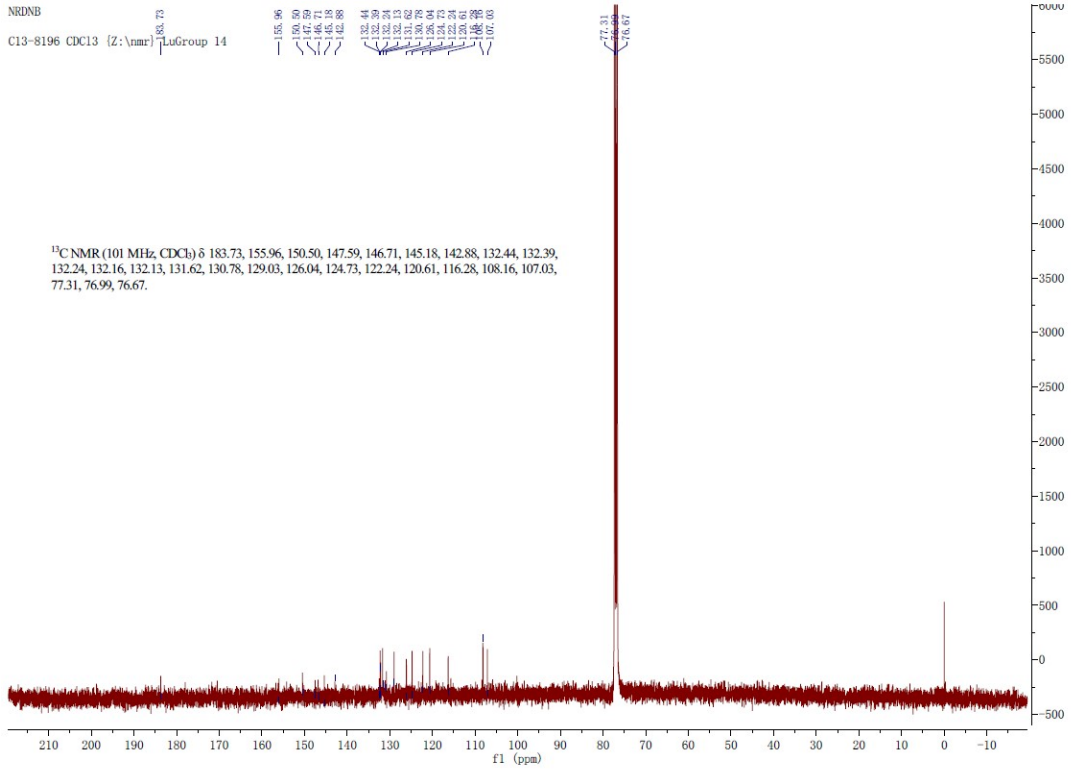

Figure S5. ^{13}C NMR of NRDNP in $CDCl_3$.

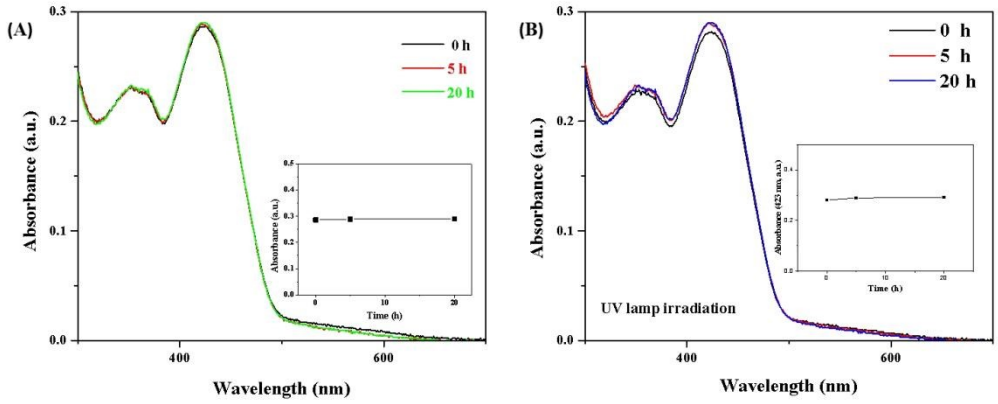

Figure S6. UV-vis spectra for stability analyses of NRDNP in different time. (A) with natural place; (B) under ultraviolet lamp irradiation.

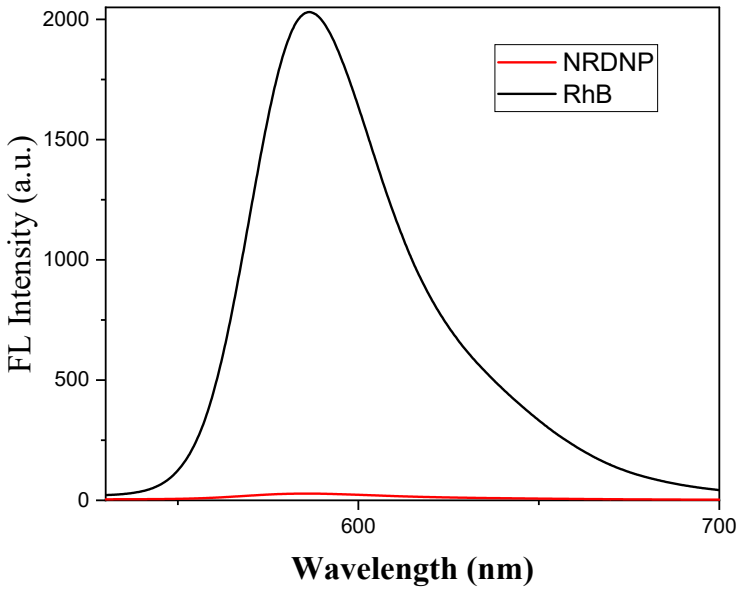

Figure S7. Relative quantum yield of NRDNP (Φ) measurement using Rhodamine B as reference.

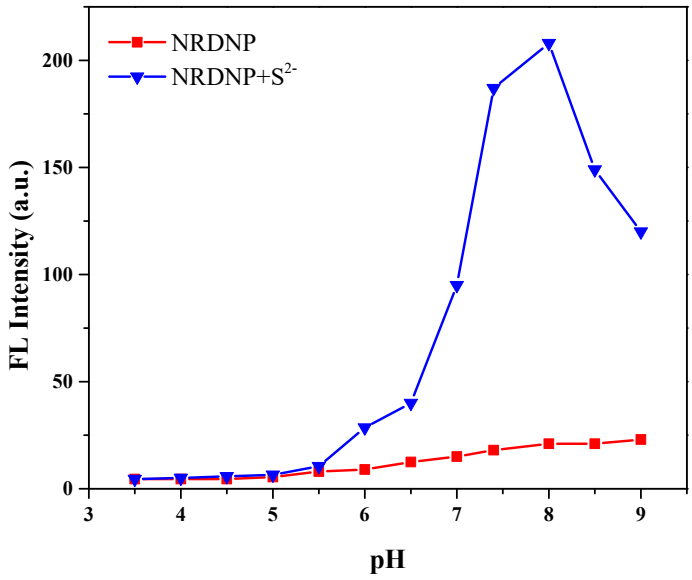

Figure S8. Fluorescence intensity of NRDNP (5 μM) with / without Na_2S (50 μM) at 612 nm in different pH conditions.

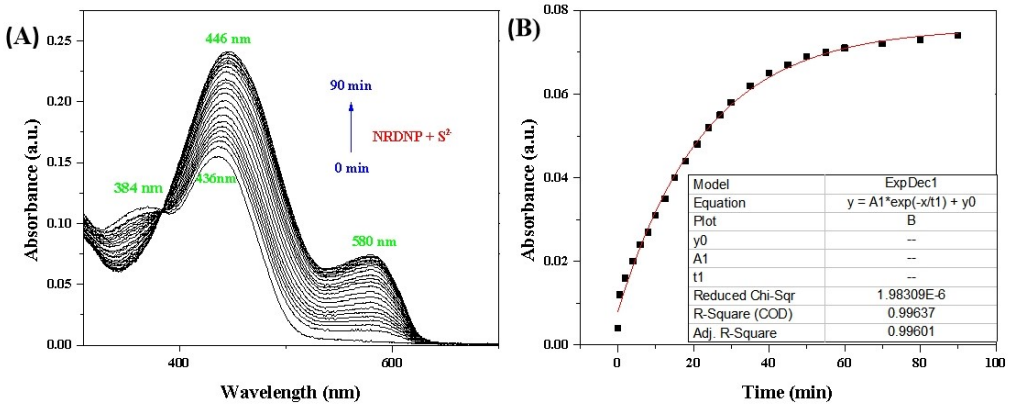

Figure S9. (A)Time dependent absorbance changes of probe NRDNP (10 μM) with 100 μM Na_2S in 10 mM PBS (10 % DMSO with 0.1% CTAB) at pH = 7.4; (B) Pictures of absorbance (A) at 582 nm versus time (t).

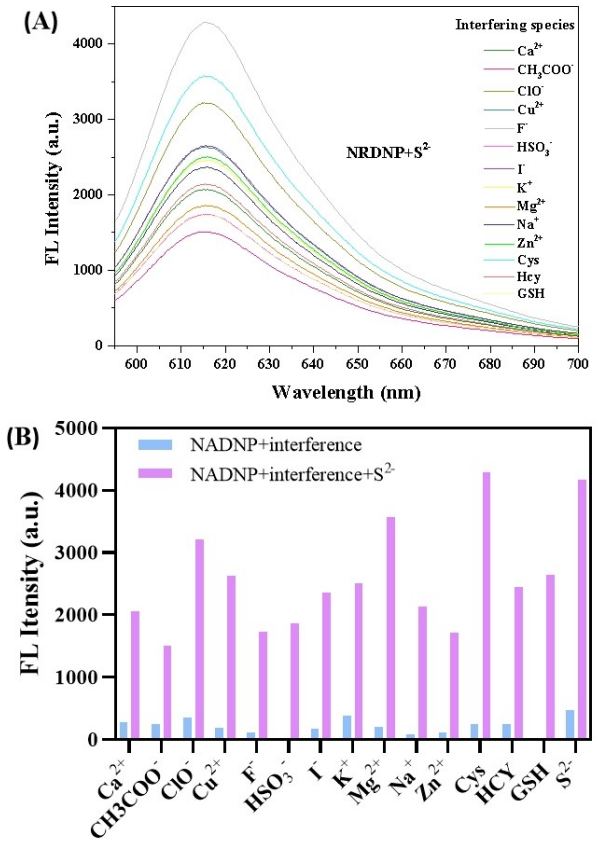

Figure S10 (A) and (B). Fluorescence spectra of NRDNP (2 μM) upon the addition Na_2S (20 μM) and 20 μM of various interference species (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, F^-, I^-, OAc^-, ClO^-, HSO_3^-, Cys, GSH, Hcy,), respectively, in DMSO : PBS = 1 : 1 (v/v) (λ_{ex} = 582 nm, slit width: 5 nm/5 nm).

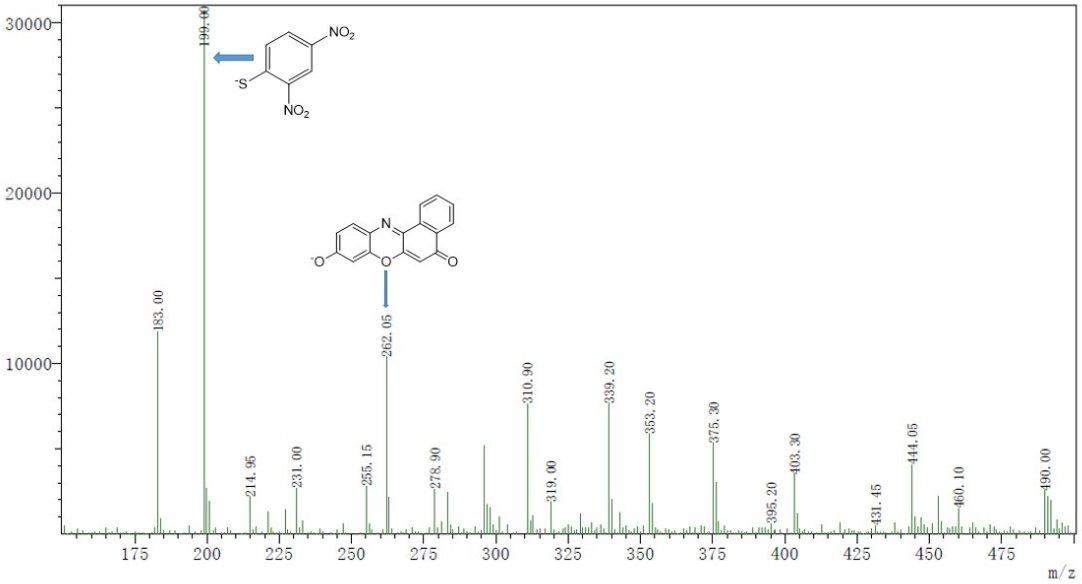

Figure S11. ESI-MS of NRDNP + S^{2-} (Negative ion mode).

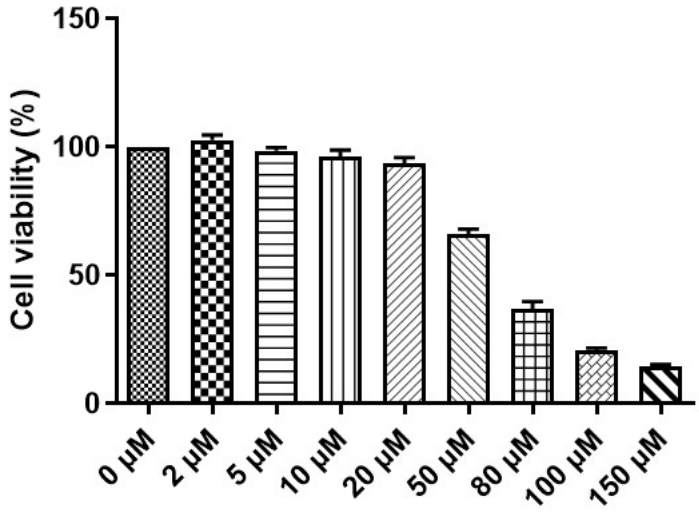

Figure S12. Cell viability was assessed by CCK-8 assay with RAW 264.7 cells after culturing in the presence of 0 -150 μM NRDNP for 24 h.

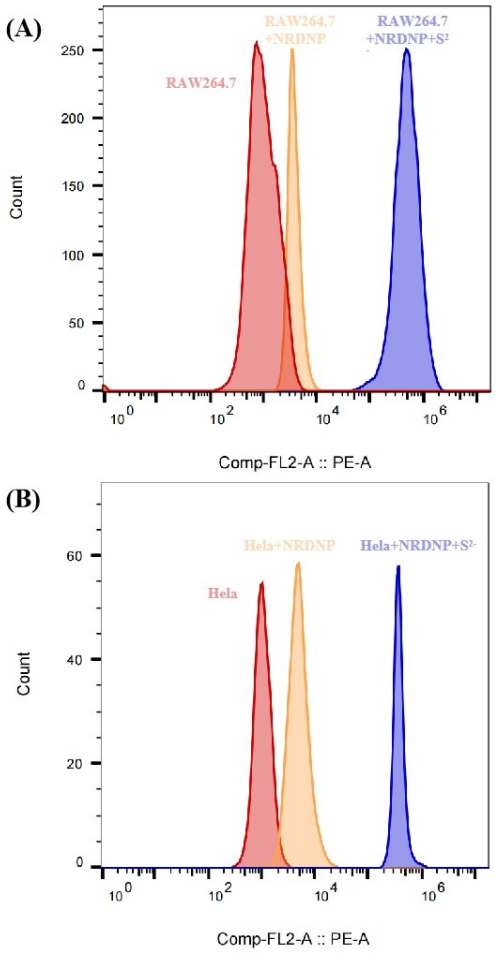

Figure S13. Flow cytometry study of RAW264.7 cells (A) and Hela cells cells (B). Mock (red), treated without (orange) and with (blue) S^{2-} in the presence of NRDNP.