# Supporting Information

## Dual-activated fluorescent probe for the study of the mechanism of $SO_2$ and NO in cisplatin resistance of nasopharyngeal carcinoma

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## 1. General methods

**Apparatus and Materials.** $^{1}$H NMR and $^{C}$NMR spectra were obtained on a Bruker spectrometer. High-resolution mass spectrometry analysis was performed using a Bruker microTOF-Q II mass spectrometer. Absorption and fluorescence spectra were tested using UV-2450 and F-7000, respectively. Cellular imaging was acquired on a ZEISS LSM 900.

**Preparation of aqueous NO solution.** Take 10 mL of deionized water and stir it continuously for 30 minutes to effectively remove dissolved oxygen. Then, purify the NO gas generated by the reaction between sulfuric acid and sodium nitrite by passing it through a saturated NaOH solution. Subsequently, continuously introduce the NO gas into deionized water for 30 minutes to achieve a NO aqueous solution concentration of 1.9 mM. The solution is then sterilized under high temperature and high pressure for biological experiments.

**Preparation of aqueous cisplatin solution.** Due to the instability of cisplatin, it is prepared and used immediately for each experiment. Cisplatin is dissolved in physiological saline to prepare a 1 mM stock solution. Before each experiment, the stock solution is diluted to the desired concentration using the culture medium. Since the solvent of the prepared stock solution is physiological saline and the diluent is complete culture medium, which do not cause any damage to the cells, the influence of the solvent on the cells can be ignored.

**CCK-8 assay for IC50.** The cells were inoculated into 96-well plates according to experimental requirements, with 100 μL of cell suspension per well. The cells were cultured overnight or for 24 h to ensure adhesion or stable growth. Drug solutions were prepared at different concentration gradients, with at least three replicate wells per concentration. The old medium was removed from the 96-well plates, and fresh medium containing different drug concentrations was added, 100 μL per well. The drug-treated cells were incubated for 24-72 h in a 37 °C, 5% $CO_2$ incubator. After incubation, 10 μL of CCK-8 reagent was added per well. The plates were gently shaken to mix, then incubated at 37 °C for 2 h. After incubation, absorbance (OD value) was measured per well using a microplate reader at 450 nm wavelength. The drug concentrations and corresponding cell survival rates were imported into GraphPad Prism. The "XY" data format was selected, with the X-axis representing log (drug concentration) and the Y-axis showing cell survival rate (%). The "Non-linear regression" method was chosen with the "log (inhibitor) vs. response–Variable slope" mode to plot the dose-response curve, automatically marking the $IC_{50}$ value.

**2. Synthesis and characterization of compounds**

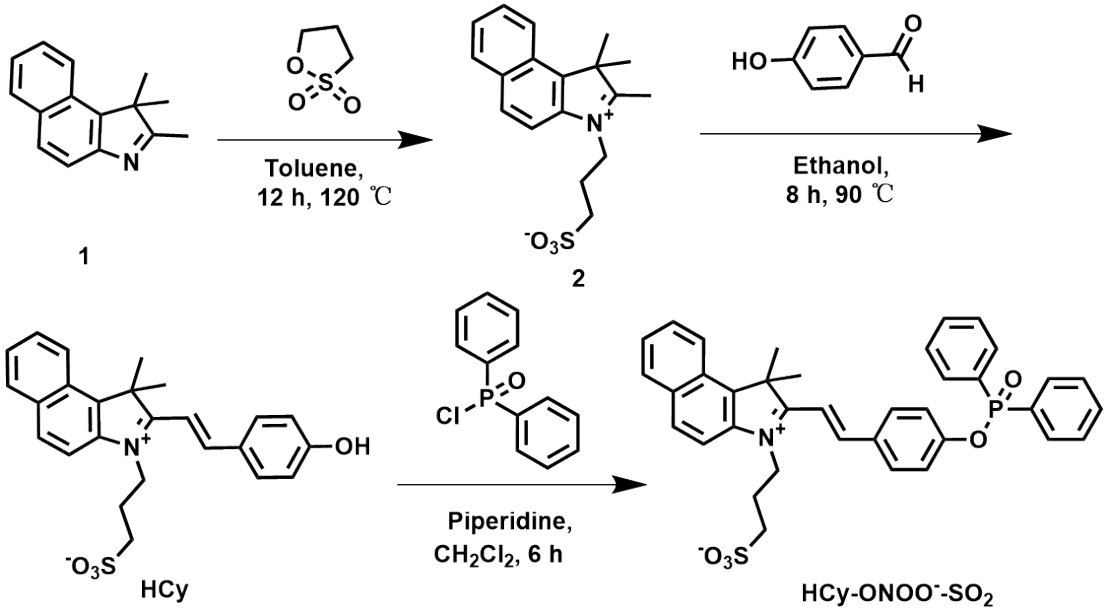


Figure S1 The Synthesis steps of Hcy-ONOO$^-$-$SO_2$.

**Synthesis of Compound 2.**

A mixture of 2.1 g of compound 1 (2,3, 3-trimethyl-3H-benz[g]indole, 10 mmol) and 1.9 mL of 2-oxo-sulfido-cyclohexane 2, 2-disulfide (22 mmol) was introduced into a 50 mL round-bottomed flask. Subsequently, 30 mL of toluene was added, and the reaction mixture was stirred under reflux at 120 ℃ for 24 h. Upon completion, a substantial amount of solid precipitated and was collected by filtration. Compound 2 (2.38 g, yield 72%) was obtained in crude form without the need for further purification and was used directly in the subsequent step. Compound 2: $^1$H NMR (400 MHz, DMSO-D6) δ 8.39 – 8.16 (m, 4H), 7.83 – 7.67 (m, 2H), 4.77 (t, J = 8.0 Hz, 2H), 2.93 (s, 3H), 2.67 (t, J = 6.5 Hz, 2H), 2.21 (t, J = 7.8 Hz, 2H), 1.75 (s, 6H).

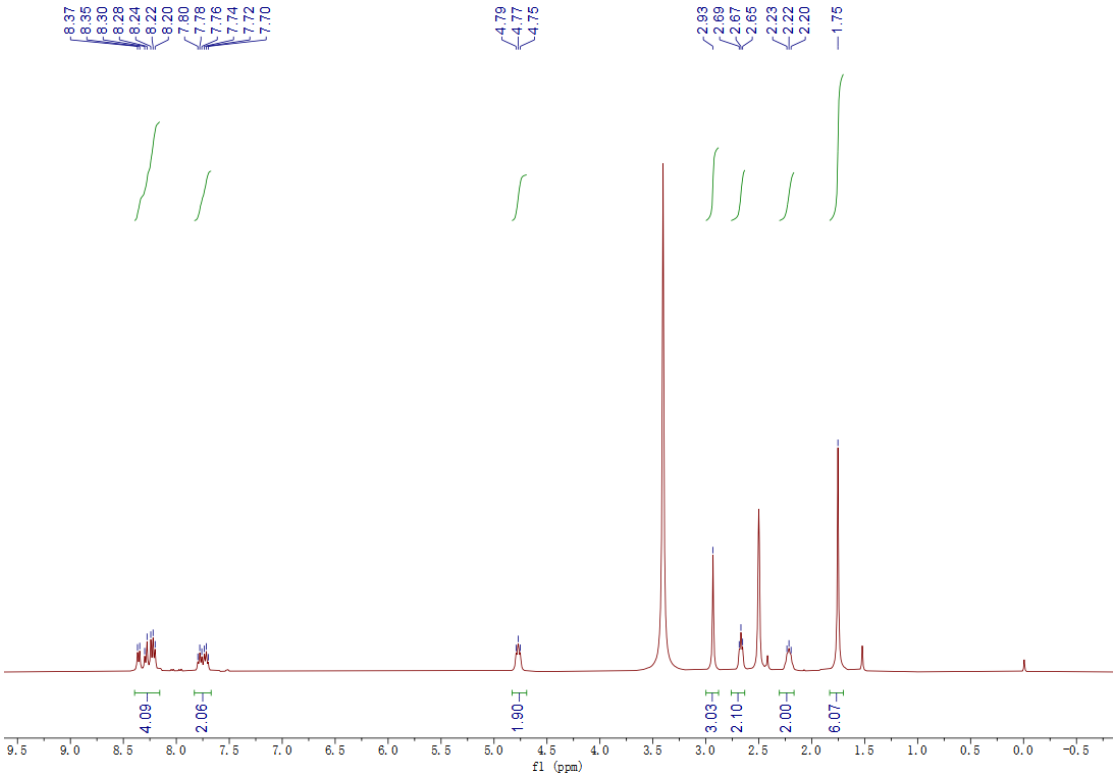


Figure S2 $^{1}$H NMR spectrum of Compound 2.

**Synthesis of Compound HCy.** Add compound 2 (0.16 g, 0.5 mmol) and 4-hydroxybenzaldehyde (0.07 g, 0.6 mmol) to a round bottom flask containing anhydrous acetonitrile (10 mL).The mixture was refluxed at 90 °C overnight. After completion of the reaction, the solvent was removed under reduced pressure. The crude product was then purified by column chromatography ($CH_2Cl_2$/$CH_3OH$ = 15:1, v/v) to afford the final compound HCy (217 mg, yield 41%).

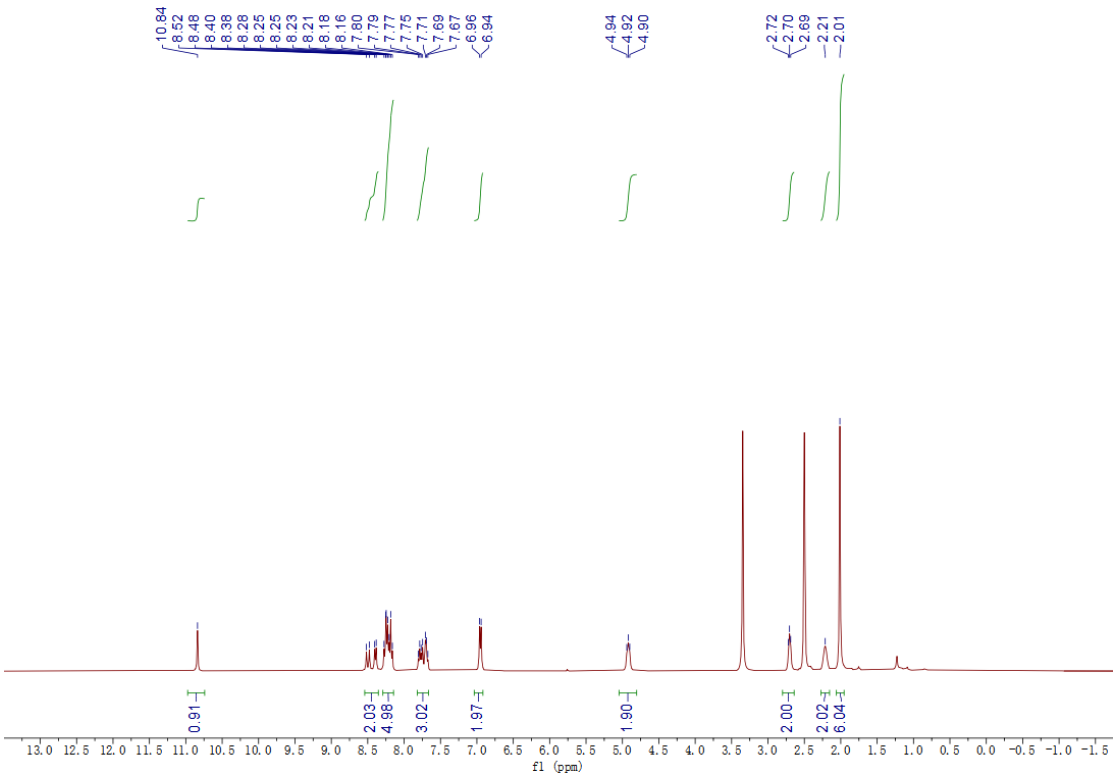

Figure S3 $^{1}$H NMR spectrum of Hcy.

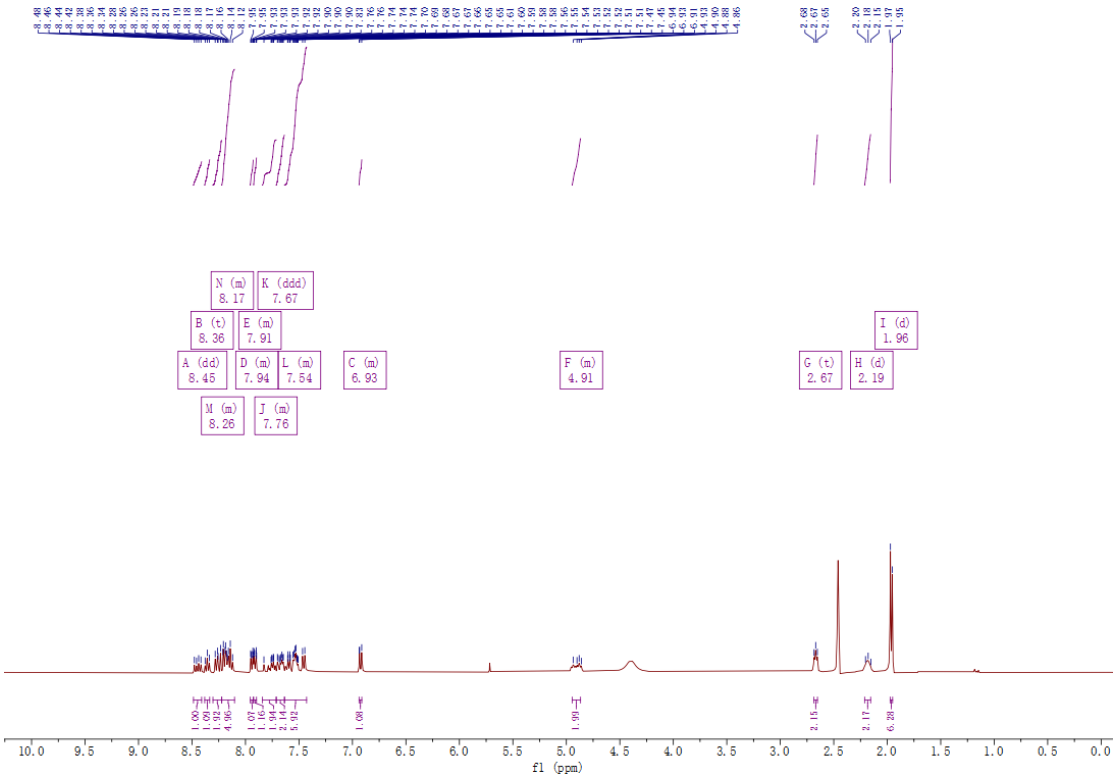

Figure S4 $^{1}$H NMR spectrum of HCy-ONOO$^{-}$-$SO_2$.

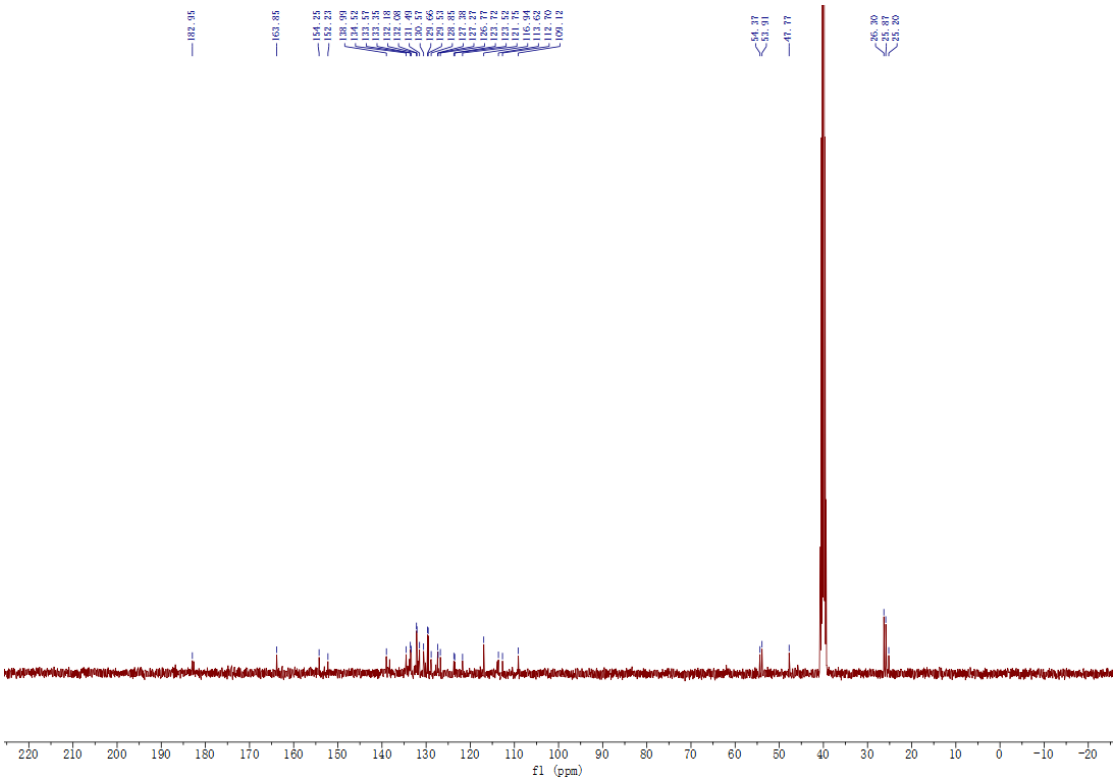


Figure S5 $^{13}C$ NMR spectrum of HCy-ONOO$^-$-$SO_2$.

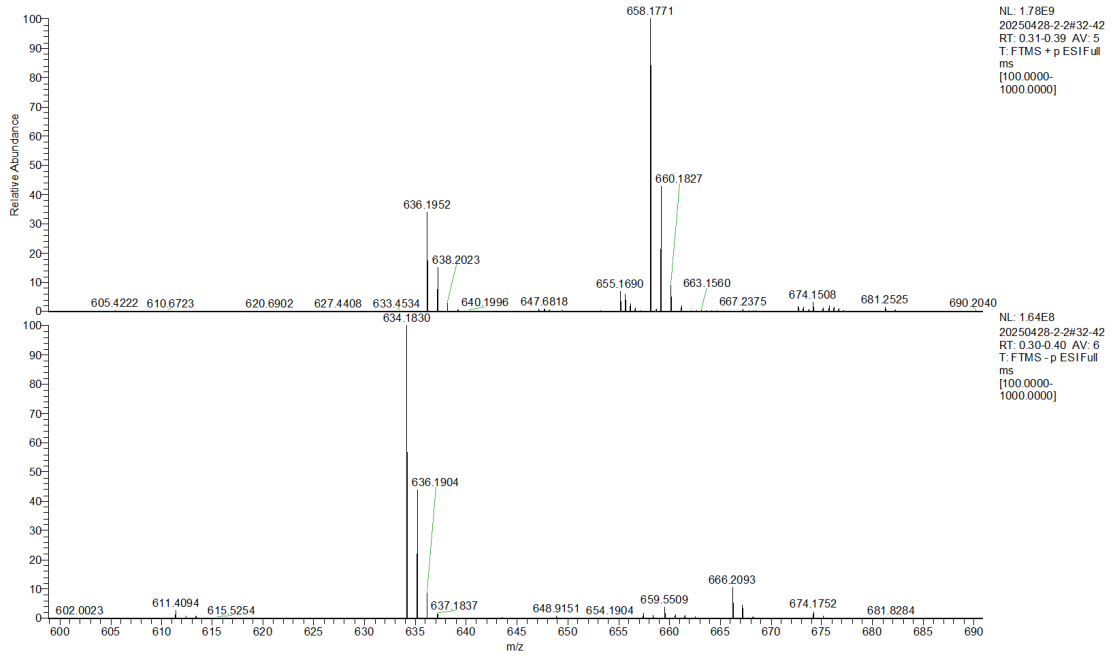


Figure S6 The HRMS spectrum of HCy-ONOO$^-$-$SO_2$.

FT-IR (KBr, $cm^{-1}$): 3441 (br, O–H stretching, sulfonate group and hydrogen bonding), 3065 (aromatic C–H stretching), 1590, 1572, 1522, 1464 (aromatic C=C skeletal vibrations), 1358 (asymmetric S=O stretching of $–SO_3^-$), 1297, 1229 (P=O and P–O–C stretching vibrations of aryl phosphate ester), 1171, 1134, 1111 (symmetric S=O stretching of $–SO_3^-$), 1041 (C–O–C stretching vibration of aromatic ether), 995, 948,

910 (=C–H out-of-plane bending), 848, 817, 786, 756, 733, 694, 664 (out-of-plane bending vibrations of aromatic C–H).

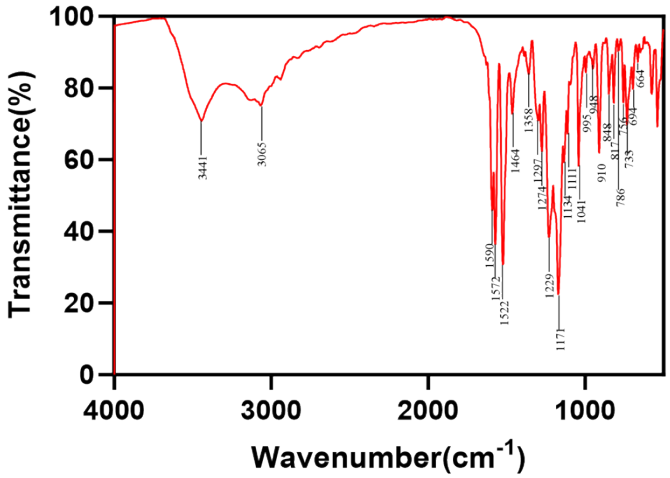


Figure S7 The infrared spectrum of HCy-ONOO⁻-$SO_2$.

**3. Absorption spectroscopic studies of the probe HCy-$ONOO^-$-$SO_2$.**

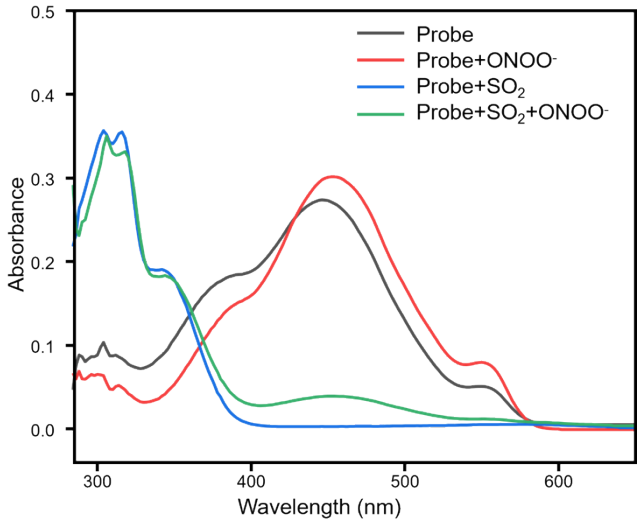


Figure S8 The UV-Vis absorption spectrum of HCy-$ONOO^-$-$SO_2$ to various substances, probe, probe + $ONOO^-$, probe + $SO_2$, and probe + $ONOO^-$ + $SO_2$.

**4.Reaction kinetics and pH studies of the probe HCy-$ONOO^-$-$SO_2$.**

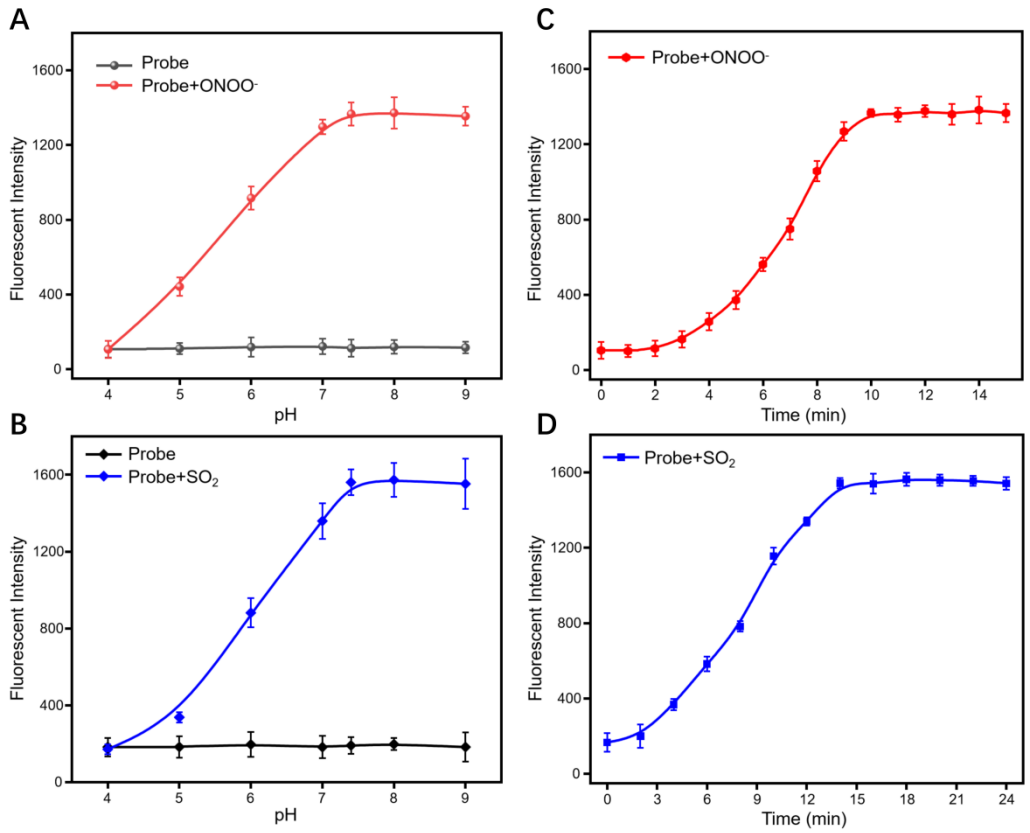


Figure S9. (A) pH influence on fluorescence intensity of HCy-$ONOO^-$-$SO_2$ before and after the addition of 20 μM $ONOO^-$. $\lambda_{ex/em}$ =480/575 nm. (B) pH influence on fluorescence intensity of HCy-$ONOO^-$-$SO_2$ before and after the addition of 50 μM $SO_2$. $\lambda_{ex/em}$ =320/450 nm. (C) Time-dependent fluorescence intensity of HCy-$ONOO^-$-$SO_2$ in the presence of $ONOO^-$. $\lambda_{ex/em}$ =480/585 nm. (D) Time-dependent fluorescence intensity of HCy-$ONOO^-$-$SO_2$ in the presence of $SO_2$. $\lambda_{ex/em}$ =320/465 nm.

**5. The cytotoxicity of HCy-$ONOO^-$-$SO_2$.**

Figure S10 The CCK-8 method was employed to evaluate the cytotoxic effects of HCy-$ONOO^-$-$SO_2$ at various concentrations (0-60 μM) on NP69 cells, C666-1 cells, and C666-1/DDP cells.The experiments were repeated three times and the data were shown as mean (± S.D.).

**6. Bright-field images of different fluorescence imaging in the article.**

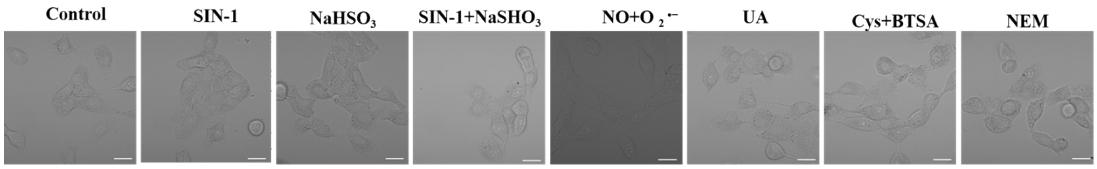


Figure S11 Bright-field images of Figure 2B. Scale bar: 40 μm.

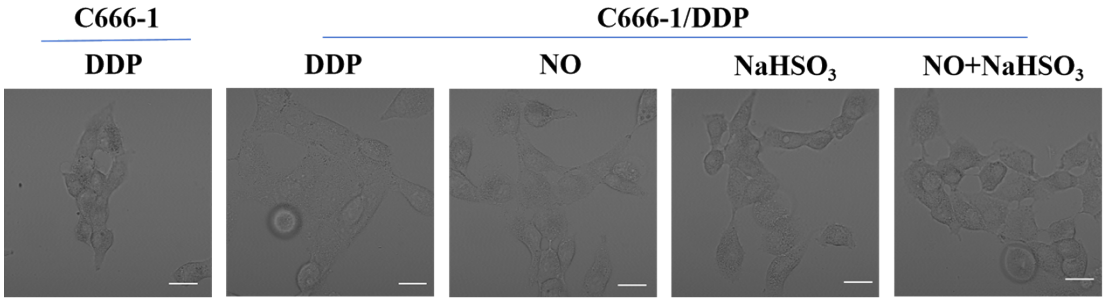


Figure S12 Bright-field images of Figure 3B. Scale bar: 40 μm.

7. **The $IC_{50}$ of NO and $SO_2$ for C666-1/DDP.**

Figure S13 (A) The cytotoxicity of NO to C666-1/DDP cells. (B) The cytotoxicity of $SO_2$ to C666-1/DDP cells.