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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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Cisplatin is widely used in the treatment of advanced nasopharyngeal carcinoma; however, its therapeutic application is often limited by a high incidence of drug resistance. Recent studies have demonstrated that nitric oxide (NO) and sulfur dioxide (SO_2), acting as gaseous signaling molecules, exhibit anti-cisplatin resistance properties in tumor cells. Nevertheless, developing appropriate chemical tools to investigate the mechanisms underlying NO and SO_2 mediated cisplatin resistance to cisplatin remains challenging. This study designed and synthesized a dual-responsive fluorescent probe to detect peroxynitrite (ONOO^-) and SO_2 , enabling them to be visualized within cells. Using this probe to detect and image ONOO^- and SO_2 in cisplatin-resistant cell lines revealed that NO and SO_2 combat cisplatin resistance by generating highly reactive ONOO^- and depleting intracellular glutathione. The IC_{50} values of cisplatin-resistant cells treated with NO and SO_2 were significantly lower than those of the control group. These results indicate that $\text{HCy-ONOO}^--\text{SO}_2$ can serve as a powerful chemical tool for investigating the mechanisms of cisplatin resistance in nasopharyngeal carcinoma.

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1. Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor that originates in the epithelium of the nasopharynx, often appearing in the pharyngeal recess.¹ Due to its unique anatomical location, the early symptoms of NPC are not obvious. Among patients presenting for the first time with NPC, more than 70% have locally advanced nasopharyngeal carcinoma.²⁻⁴ Therefore, chemotherapy plays a crucial role in NPC treatment, particularly in R/M NPC cases.⁵⁻⁷

Cisplatin (also known as DDP) is a widely used platinum-based chemotherapeutic agent in clinical practice. It has become a cornerstone in the treatment of various solid tumors, including NPC, ovarian cancer, bladder cancer, lung cancer, and colorectal cancer.^{8,9} However, many tumors that initially respond to cisplatin gradually develop resistance during treatment, significantly limiting its clinical efficacy.^{10,11} Due to the non-specific interactions between cisplatin and intracellular components, as well as the various mechanisms through which cisplatin induces DNA damage and apoptosis, tumor cells can acquire resistance *via* multiple molecular pathways.^{12,13} Numerous studies have demonstrated that inhibiting ATPase activity and reducing intracellular cisplatin efflux are effective strategies to enhance cisplatin sensitivity. Following its

hydrolysis in the cytoplasm, cisplatin readily binds to sulphur-containing intracellular molecules, such as glutathione (GSH), sequestering the drug in the cytoplasm and preventing its translocation to the nucleus for DNA binding. Consequently, depleting intracellular GSH is another viable approach to increasing cisplatin chemosensitivity.

Gaseous messenger small molecules are a type of gas molecules produced by metabolism within the body and play an important role in signal transduction under normal physiological and pathological conditions. Studies have shown that nitric oxide (NO), an important gaseous messenger, can overcome the multidrug resistance in tumor cells by reducing *P*-glycoprotein expression and increasing sensitivity to chemotherapy. NO interacts with superoxide anion free radicals to generate highly reactive peroxynitrite (ONOO^-), which can inhibit the mitochondrial respiratory chain and lead to mitochondrial dysfunction and the oxidation of ATPase.¹⁴ Therefore, the ability of NO to reduce intracellular cisplatin efflux and enhance the killing effect of cisplatin on mitochondria in order to overcome cisplatin resistance by generating ONOO^- requires further exploration. On the other hand, once hydrolysed in the cytoplasm, cisplatin is prone to binding with intracellular sulphur-containing molecules such as GSH, thereby preventing it from entering the nucleus and binding to its target DNA. Therefore, depleting intracellular GSH is also a way to increase sensitivity to cisplatin.

In conclusion, monitoring intracellular levels of ONOO^- and sulfur dioxide (SO_2) is crucial for studying the role and mechanism of SO_2/NO in anti-cisplatin resistance. The high

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sensitivity, selectivity, and spectral characteristics of small-molecule fluorescent probes make them indispensable tools in modern analytical chemistry and biomedical research.¹⁵⁻¹⁷ Furthermore, the use of these probes in biological imaging and *in vivo* monitoring enables researchers to observe the dynamic molecular changes in the body in real time. However, most probes cannot conduct separate and simultaneous detection without interference. Based on previous studies, this research has innovatively developed a small molecule fluorescent probe for the first time that can simultaneously detect and image ONOO^- and SO_2 . Using the developed ONOO^- and SO_2 small molecule fluorescent probes, the anti-cisplatin resistance effect and mechanism of NO/SO_2 in NPC were explored.

2. Materials and methods

2.1. Materials

All organic synthetic materials are of analytical grade, while all cell reagents are of biological grade. The organic synthetic reagents were purchased from Energy Chemical in Anhui, China. Normal nasopharyngeal cells (NP69) were purchased from the Xiang Ya Central Experiment Laboratory in Changsha, China. NPC cells (C666-1) were purchased from Fuheng Biology in Shanghai, China. DMSO (BL165B), CCK-8 (BS350B), RPMI-1640 (PM150110) and Trypsin-EDTA Solution (BL501A) were purchased from Biosharp in Anhui, China. Fetal bovine serum (S660JY) was purchased from Basalmedia (Shanghai, China). The Reduced Glutathione Content Assay Kit (BC1175) was purchased from Solarbio, Beijing, China.

2.2. Construction of cisplatin-resistant cells

First, a stable *in vitro* model was obtained through the traditional continuous culture method. By continuously culturing the NPC cell line C666-1 for 6 months and gradually increasing the cisplatin dose to 4 $\mu\text{mol mL}^{-1}$, we established a cisplatin-resistant NPC cell line (C666-1/DDP). The drug concentration escalation method¹⁸ was employed. C666-1 cells in logarithmic growth phase were treated with low-concentration DDP for 24 h, followed by medium replacement. After cell recovery, the cells were digested and subcultured, then re-exposed to low-concentration cisplatin for 24 hours. When cells reached normal morphology, the drug shock protocol was repeated 6-8 times at each concentration (0 $\mu\text{mol mL}^{-1}$, 1 $\mu\text{mol mL}^{-1}$, 2 $\mu\text{mol mL}^{-1}$, 3 $\mu\text{mol mL}^{-1}$ and 4 $\mu\text{mol mL}^{-1}$). Once stable growth was achieved, the drug concentration was gradually increased during subsequent cultures. The drug induction period lasted 6 months until cells could grow stably in 4 $\mu\text{mol L}^{-1}$ DDP. CCK-8 assay was used to evaluate the resistance index (RI) of drug-resistant and parental strains, with RI > 5 indicating drug resistance.

3. Results and discussion

3.1. Design and synthesis of HCy- ONOO^- - SO_2

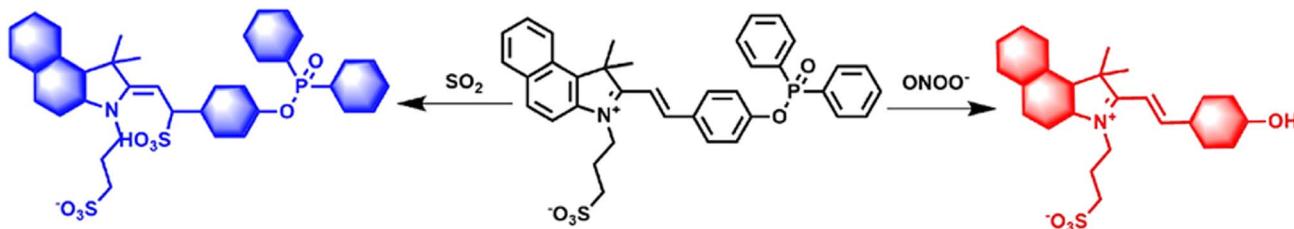
Fig. S1 shows the preparation method of the probe. Under ice bath conditions and in an argon atmosphere, HCy (217 mg, 0.5 mmol) was dissolved in 10 mL of anhydrous CH_2Cl_2 , followed

by the addition of 50 μL of piperidine. A dropwise addition was then performed of a solution of 236 mg (2 mmol) solution of diphenylphosphine oxychloride in 5 mL of dichloromethane was performed. The reaction was maintained at room temperature for 6 hours after an initial one-hour incubation period. Fig. S1 shows the preparation method of the probe.

Dissolve HCy (217 mg, 0.5 mmol) in 10 mL anhydrous CH_2Cl_2 in an ice bath and argon atmosphere, and then add 50 μL of piperidine. Subsequently, add dropwise a solution of diphenylphosphine oxychloride (472 mg, 2 mmol) dissolved in 5 mL of dichloromethane. After stirring for 1 h in an ice bath, return to room temperature and continue the reaction for another 6 h. The solvent was then removed and the product purified by column chromatography ($\text{CH}_2\text{Cl}_2 : \text{CH}_3\text{OH} = 30 : 1$, v/v), yielding the final compound HCy- ONOO^- - SO_2 (117 mg, yield 37%). As shown in Fig. S3-S5, the structure of the probe was verified. ^1H NMR (400 MHz, DMSO- D_6) δ 8.45 (dd, $J = 16.2$, 8.4 Hz, 1H), 8.36 (t, $J = 7.9$ Hz, 1H), 8.30-8.22 (m, 2H), 8.22-8.10 (m, 5H), 7.95-7.93 (m, 1H), 7.92-7.90 (m, 1H), 7.84-7.71 (m, 2H), 7.67 (ddd, $J = 11.1$, 7.4, 2.1 Hz, 2H), 7.63-7.43 (m, 6H), 6.94-6.91 (m, 1H), 4.95-4.87 (m, 2H), 2.67 (t, $J = 6.1$ Hz, 2H), 2.19 (d, $J = 9.9$ Hz, 2H), 1.96 (d, $J = 6.9$ Hz, 6H). ^{13}C NMR (400 MHz, DMSO- D_6) 182.95, 163.85, 154.25, 152.23, 138.99, 133.57, 133.35, 132.18, 132.08, 131.49, 130.57, 129.66, 129.53, 128.85, 127.27, 126.77, 123.72, 123.52, 121.75, 116.94, 113.62, 112.70, 109.12, 54.37, 53.91, 47.77, 26.30, 25.87, 25.20. HRMS m/z : calculated for $[\text{C}_{37}\text{H}_{34}\text{NO}_5\text{PS}]$: 635.1895, found $[\text{C}_{37}\text{H}_{34}\text{NO}_5\text{PS} + \text{H}]^+$: 636.1952. Furthermore, we measured the infrared spectrum of the compound HCy- ONOO^- - SO_2 (Fig. S7). These characteristic absorption bands are consistent with the presence of an extended aromatic conjugated system, phosphate ester moieties, and sulfonate groups, confirming the successful formation of the target compound HCy- ONOO^- - SO_2 .

Scheme 1 shows the structure of the probe HCy- ONOO^- - SO_2 and how it reacts with SO_2 and ONOO^- . This semi-carbocyanine compound has excellent spectral properties and is widely used in the fields of biomedical science, dye-sensitized science and environmental science, making it a recommended luminescent group.^{19,20} Due to the presence of the indole cation, HCy- ONOO^- - SO_2 can accumulate effectively at mitochondrial sites. To further improve the probe's water solubility, we introduced a sulfonic acid group alongside the indole cation, which significantly enhances its biocompatibility. Recent studies have found that the diphenylphosphinoyl ester group exhibits a rapid response, excellent sensitivity, and remarkable selectivity towards ONOO^- .^{21,22} Based on this, we selected the diphenylphosphinoyl ester group as the specific ONOO^- trigger in this design. Its introduction inhibits the probe's intramolecular charge transfer process, resulting in inert fluorescence. In the presence of ONOO^- , HCy- ONOO^- - SO_2 generates a strong fluorescence signal at 575 nm within 10 minutes. Furthermore, under SO_2 conditions, a Michael addition reaction occurs, disrupting the large conjugated system and leading to enhanced fluorescence at 450 nm within 15 minutes. Therefore, this probe can be used to detect of SO_2 and ONOO^- separately through distinct signals, providing a solid basis for studying their interrelationships.





Scheme 1 The molecular structure of HCy-ONOO⁻-SO₂ and its reaction mechanisms with ONOO⁻ and SO₂.

3.2. Spectral characterization of the probe for ONOO⁻ and SO₂

First, the spectral properties of HCy-ONOO⁻-SO₂ were investigated in phosphate buffer solution. A detailed analysis of the ultraviolet-visible (UV-vis) absorption spectral changes of the HCy-ONOO⁻-SO₂ probe was conducted. As can be seen in Fig. S7, the probe exhibits a distinct absorption band around 450 nm. The addition of ONOO⁻ results in the emergence of a stronger absorption peak at 460 nm, indicating the formation of a new product from the reaction between the probe and ONOO⁻. Conversely, in the presence of SO₂, HCy-ONOO⁻-SO₂ exhibits a new absorption band at 315 nm while the original absorption peak at 450 nm disappears completely. This distinct spectral response demonstrates that HCy-ONOO⁻-SO₂ can effectively detect SO₂. Furthermore, in the presence of both SO₂ and ONOO⁻, the probe exhibits a strong absorption band at approximately 315 nm and weak absorption features around 460 nm, while the original absorption peak at 450 nm disappears completely. These results demonstrate the potential of HCy-ONOO⁻-SO₂ for use in the detecting of ONOO⁻ and SO₂ under co-existing conditions.

In addition, the fluorescence spectral changes of ONOO⁻ and SO₂ in the HCy-ONOO⁻-SO₂ system were investigated systematically. As illustrated in Fig. 1A, the addition of ONOO⁻ resulted in a progressive increase in the fluorescence signal of HCy-ONOO⁻-SO₂ at 585 nm with increasing concentration. This indicates that the probe can effectively monitor variations in ONOO⁻ levels. In the presence of SO₂, a gradual enhancement of the fluorescence signal at 465 nm was observed (Fig. 1B), which further demonstrates the potential of this probe for SO₂ detection. Furthermore, a strong linear correlation was established between fluorescence intensity and the concentrations of ONOO⁻ and SO₂. The calibration curves were described by the following equations: $Y_1 = 64.1584X + 87.9061$ ($R^2 = 0.9994$) for ONOO⁻ and $Y_2 = 28.4919X + 151.7518$ ($R^2 = 0.9996$) for SO₂ (Fig. 1C and D).

To evaluate the probe's ability to detect ONOO⁻ and SO₂ *in vivo*, we examined how pH affects the fluorescence response of HCy-ONOO⁻-SO₂. As illustrated in Fig. S9A and B, HCy-ONOO⁻-SO₂ exhibits a stable and minimal fluorescence signal across the pH range of 4 to 9, indicating that the probe's performance remains unaffected by physiological pH variations. In contrast, the presence of ONOO⁻ and SO₂ causes HCy-ONOO⁻-SO₂ to generate distinct fluorescence emissions at 575 nm and 450 nm, respectively, within the physiologically

relevant pH range of 4–7. Next, we investigated the response kinetics of HCy-ONOO⁻-SO₂ towards ONOO⁻ and SO₂. As illustrated in Fig. S9C, the fluorescence signal of HCy-ONOO⁻-SO₂ reached its maximum within approximately 10 min upon addition of ONOO⁻, indicating a rapid response to ONOO⁻. As shown in Fig. S9D, when exposed to different SO₂ concentrations, the fluorescence intensity reached saturation within approximately 15 min, demonstrating that the probe is also effective for SO₂ detection. Notably, the distinct reaction kinetics of the probe towards ONOO⁻ and SO₂ enable discrimination between the two, offering potential for selective and sequential detection. These results suggest that HCy-ONOO⁻-SO₂ can be used for the *in vivo* monitoring of both ONOO⁻ and SO₂. Additionally, we examined the fluorescence responses of this probe to various reactive species, amino acids, and common anions. As shown in Fig. 1E and F, ONOO⁻ and SO₂ produced distinct fluorescence enhancements at 575 nm and 450 nm, respectively, no significant changes in signal were observed in the presence of the other tested substances. These findings demonstrate the high selectivity of HCy-ONOO⁻-SO₂ towards ONOO⁻ and SO₂ in complex biological environments.

3.3. Visualization of ONOO⁻ and SO₂ in living cells

Subsequently, the cytotoxicity and biocompatibility of the probe HCy-ONOO⁻-SO₂ were evaluated using the CCK-8 assay. As shown in Fig. S10, the cells exhibited high viability even at elevated probe concentrations, indicating that the designed probe exhibits low cytotoxicity and favorable biocompatibility. To evaluate the probe's ability to detect ONOO⁻ and SO₂ in living cells, we used it to visualise and image these species in the normal nasopharyngeal cells (NP69). As shown in Fig. 2A and B, no fluorescence was observed in the absence of ONOO⁻ and SO₂. However, upon exposure to ONOO⁻ and SO₂, a strong red fluorescence signal was detected in NP69 cells after 20 min, indicating that HCy-ONOO⁻-SO₂ can detect ONOO⁻ in cellular environments. However, as the incubation time increased, the blue fluorescence intensity increased while the red fluorescence gradually diminished within 10 min. We hypothesise that this phenomenon may be attributed to SO₂ disrupting the extended conjugated system, which reduces the red fluorescence signal while enhancing the blue fluorescence signal. This study demonstrates that dual-target detection of ONOO⁻ and SO₂ can be achieved by monitoring the temporal evolution of dual fluorescence signals.



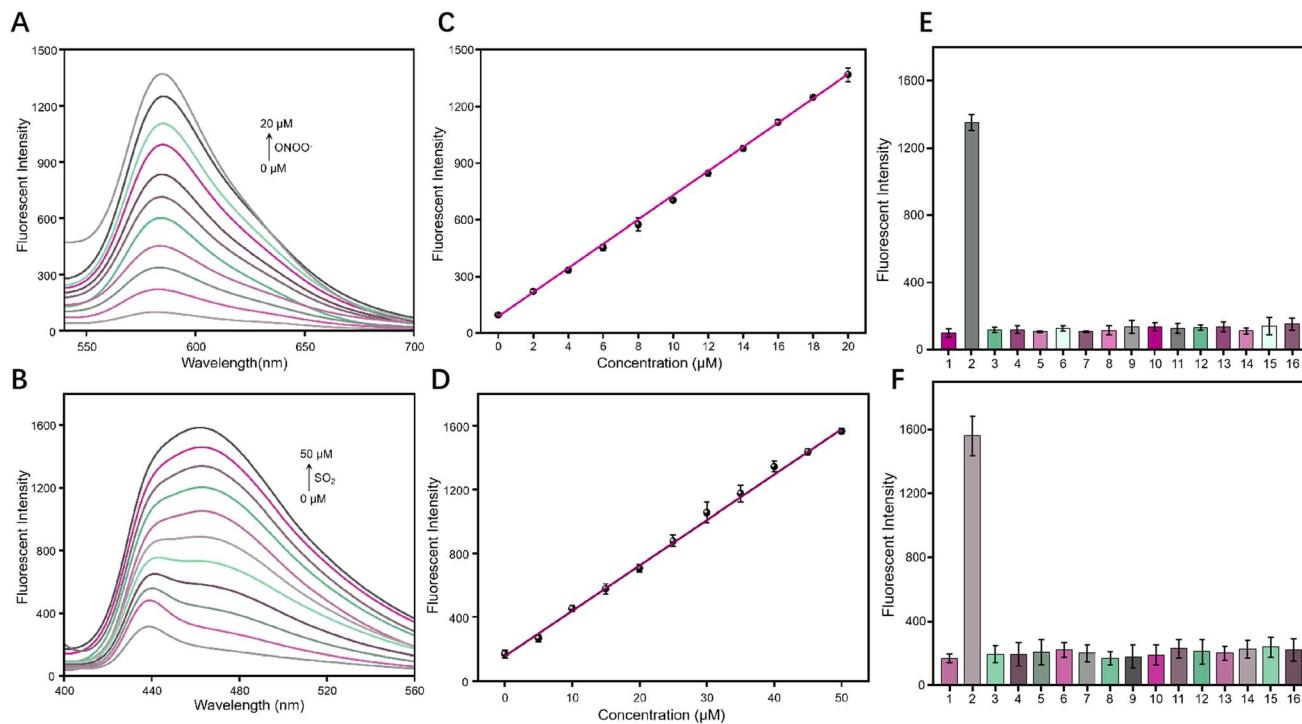


Fig. 1 Spectral properties of HCy-ONOO[−]-SO₂ in the presence of ONOO[−] and SO₂. Fluorescence emission spectra were recorded in PBS buffer (10 mM, pH 7.4, PBS : DMSO = 19 : 1 v/v). (A) Fluorescence response of HCy-ONOO[−]-SO₂ to ONOO[−] (0–20 μM) was measured at an excitation wavelength of 575 nm, and data were collected after a 10 min incubation. (B) Response to SO₂ (0–50 μM) was monitored at an excitation wavelength of 450 nm under the same instrumental conditions, with a 20 min incubation period. (C) and (D) Linear calibration curves for ONOO[−] (0–20 μM) and SO₂ (0–50 μM), respectively, showing the quantitative relationship between fluorescence intensity and analyte concentration. (E) and (F) The probe exhibited distinct emission signals for different species, which were recorded at (E) 575 nm and (F) 450 nm. (E) (1) blank; (2) ONOO[−] (20 μM); (3) HPO₄^{2−} (500 μM); (4) PO₄^{3−} (500 μM); (5) CO₃^{2−} (500 μM); (6) SO₄^{2−} (500 μM); (7) NO₃[−] (500 μM); (8) Na⁺ (500 μM); (9) K⁺ (500 μM); (10) Mg²⁺ (200 μM); (11) Ca²⁺ (200 μM); (12) Zn²⁺ (200 μM); (13) GSH (1 mM); (14) H₂O₂ (50 μM); (15) ClO[−] (50 μM); (16) ·OH (50 μM). (F) (1) blank; (2) HSO₃[−] (50 μM); (3) HPO₄^{2−} (500 μM); (4) PO₄^{3−} (500 μM); (5) CO₃^{2−} (500 μM); (6) SO₄^{2−} (500 μM); (7) NO₃[−] (500 μM); (8) Na⁺ (500 μM); (9) K⁺ (500 μM); (10) Mg²⁺ (200 μM); (11) Ca²⁺ (200 μM); (12) Zn²⁺ (200 μM); (13) GSH (1 mM); (14) H₂O₂ (50 μM); (15) ClO[−] (50 μM); (16) ·OH (50 μM).

We subsequently investigated the capability of selectively monitoring ONOO[−] and SO₂ in living cells. As illustrated in Fig. 3, different substances were introduced into NP69 cells to alter the concentrations of ONOO[−] and SO₂. When 3-

morpholino-syndnonimine (SIN-1, 50 μM), a ONOO[−] generator, was added to the cells and pre-treated for 1 h, the red (ONOO[−]) channel fluorescence signal was significantly higher than in the control group, while the blue (SO₂) fluorescence remained

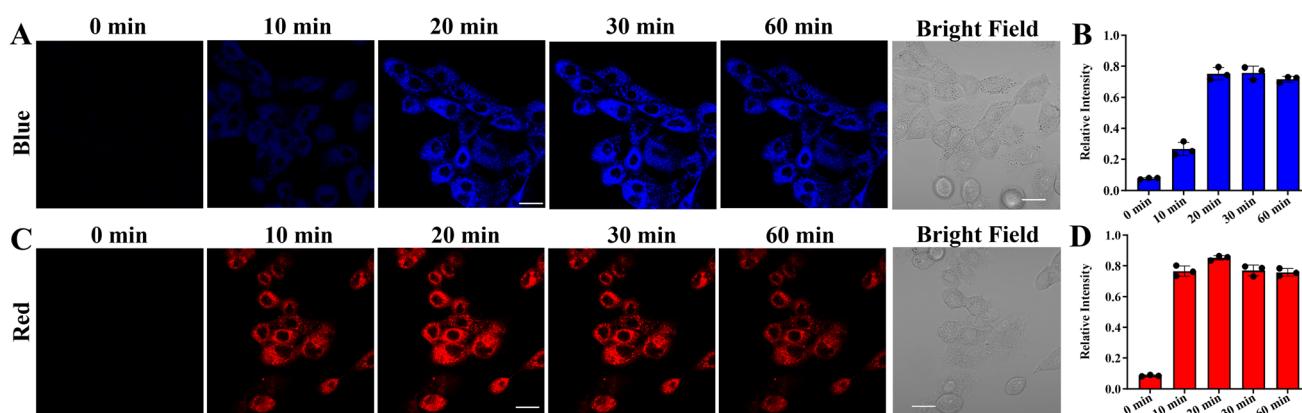


Fig. 2 Visualization of ONOO[−] and SO₂ in NP69 Cells. (A) Fluorescence imaging using probes after adding exogenous SO₂ to NP69 cells. (B) Statistical chart of average fluorescence intensity in figure (A). (C) Fluorescence imaging using probes after adding exogenous ONOO[−] to NP69 cells. (D) Statistical chart of average fluorescence intensity in figure (C). $\lambda_{\text{ex/em}} = 405/430-470$ nm (blue channel), $\lambda_{\text{ex/em}} = 488/560-620$ nm (ONOO[−] channel). Data are shown as mean \pm S.D. Scale bar: 40 μm.

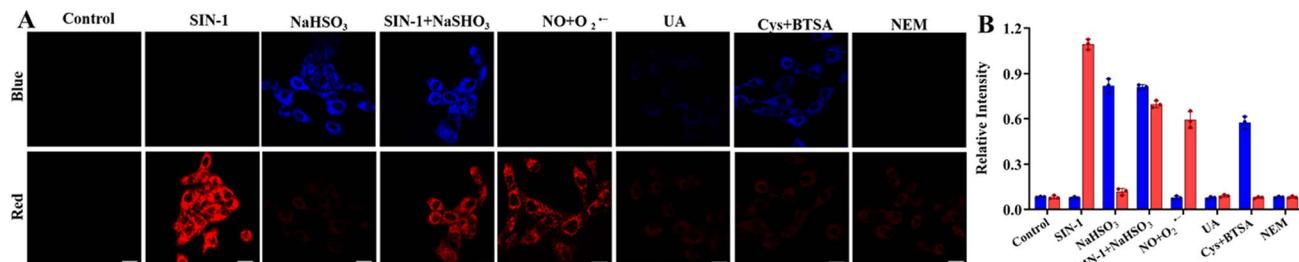


Fig. 3 The probe monitors intracellular ONOO^- and SO_2 fluctuations. (A) Fluorescence imaging using probes after different substance pretreatments of NP69 cells: Group SIN-1, NaHSO_3 , SIN-1+ NaHSO_3 , $\text{NO} + \text{O}_2^-$, and Cys + BTSA (pre-treated with NaHSO_3 (50 μM , 1 h), SIN-1 (50 μM , 1 h), NaHSO_3 (50 μM , 1 h), NO (50 μM , 1 h) + O_2^- (50 μM , 1 h), and Cys (50 μM , 1 h) + BTSA (40 μM , 1 h) respectively). Group UA: pretreated with NO (50 μM) and O_2^- (50 μM) for 1 hour, then treated with UA (500 μM) for 1 hour, followed by probe incubation for 10 min for imaging. Group NEM was pre-treated with Cys (50 μM) and BTSA (40 μM) for 1 hour, then treated with NEM (100 μM) for 1 h, followed by probe incubation for 10 minutes for imaging. (B) Statistical chart of average fluorescence intensity in figure (A). $\lambda_{\text{ex/em}} = 405/430-470$ nm (blue channel), $\lambda_{\text{ex/em}} = 488/560-620$ nm (ONOO^- channel). Data are shown as mean \pm S.D. Scale bar: 40 μm .

almost unchanged. This indicates that our probe can detect exogenous ONOO^- . When sodium hydrosulfite (NaHSO_3 , 50 μM), an SO_2 donor, was added to NP69 cells and pre-treated for 1 h, the blue (SO_2) channel fluorescence was significantly higher than in the control group, with faint red (ONOO^-) channel fluorescence. After the simultaneous addition of SIN-1 and NaHSO_3 for the pre-treatment of NP69 cells, significant fluorescence was exhibited by both channels. These results demonstrate that HCy- ONOO^- - SO_2 enables the visual monitoring of ONOO^- and SO_2 in cells. Nitric oxide and superoxide anion can generate ONOO^- within cells. When NP69 cells were pre-treated with NO and superoxide anion, the red fluorescence signal of the ONOO^- channel was significantly higher than in the control group. After using UA to neutralize the intracellular ONOO^- , the red fluorescence signal almost disappeared, further demonstrating that HCy- ONOO^- - SO_2 can detect the presence of endogenous ONOO^- in cells. Previous studies have shown that sulfanilamide (BTSA) reacts with cysteine (Cys) to

produce endogenous sulfur dioxide.²⁰ Therefore, we first pre-treated cells with BTSA and Cys, then added the probe for imaging, obtaining a bright blue fluorescence signal. To verify whether this fluorescence signal was generated by SO_2 , cells were treated with *N*-ethylmaleimide (NEM) for 0.5 h, followed by imaging with the probe (NEM group). We found that the blue fluorescence signal was almost imperceptible, indicating that HCy- ONOO^- - SO_2 can detect SO_2 in biological systems. These results demonstrate that HCy- ONOO^- - SO_2 can dynamically monitor SO_2 and ONOO^- levels.

3.4. NO and SO_2 may reverse cisplatin resistance in nasopharyngeal carcinoma cells

Cisplatin is a first-line chemotherapeutic agent for nasopharyngeal carcinoma and typically demonstrates favourable efficacy in the treatment of early-stage disease. However, drug resistance significantly hinders its clinical application.

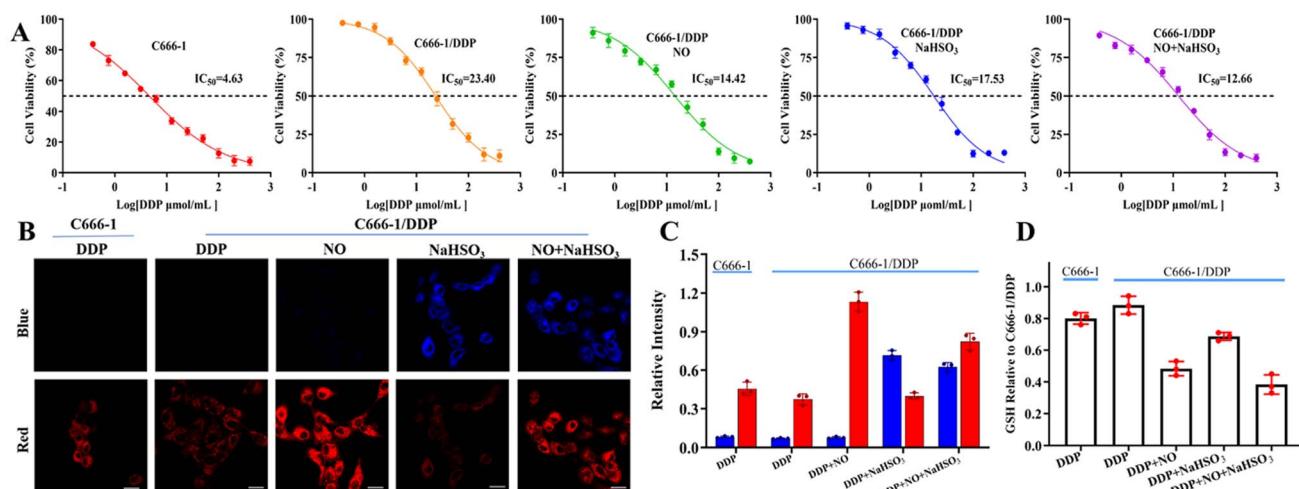


Fig. 4 NO and SO_2 reverse cisplatin resistance in nasopharyngeal carcinoma cells. (A) CCK-8 assay demonstrates NO and SO_2 resistance to C666-1/DDP cells. (B) Fluorescence probe analysis reveals the mechanism of NO and SO_2 resistance in C666-1/DDP cells. (C) Statistical chart of average fluorescence intensity in figure (B). (D) GSH assay detects intracellular GSH changes after NO and SO_2 treatment. $\lambda_{\text{ex/em}} = 405/430-490$ nm (blue channel), $\lambda_{\text{ex/em}} = 488/560-620$ nm (red channel). Data are presented as mean \pm S.D. Scale bar: 40 μm .



Numerous cisplatin-resistant tumor models exhibit decreased reactive oxygen species (ROS) and elevated glutathione (GSH) levels. These findings have been further validated in clinical studies.^{23–25} Recent research highlights the therapeutic potential of NO and SO₂. NO reacts with superoxide anion radicals to form highly reactive nitroxyl radicals (ONOO[−]), which disrupt mitochondrial respiratory chains and induce dysfunction. SO₂ depletes GSH and disrupts redox balance in tumor cells, leading to increased ROS levels. We hypothesize that NO and SO₂ may counteract drug resistance by generating reactive nitrogen species (RNS) and depleting GSH. As shown in Fig. S13, we first ruled out the effects of trace NO and SO₂ on resistant cells. Subsequently, we pretreated cisplatin-resistant nasopharyngeal carcinoma cells (C666-1/DDP) with NO (20 μM) and SO₂ (NaHSO₃, 20 μM), then measured DDP IC₅₀ using CCK8 assay. Fig. 4A demonstrates that the IC₅₀ for cisplatin-sensitive cells (Group C666-1) was approximately 4.63 μmol mL^{−1}, whereas the IC₅₀ for resistant cells (Group C666-1/DDP) was 23.40 μmol mL^{−1}. Pretreatment with NO reduced the IC₅₀ of cisplatin in drug-resistant nasopharyngeal carcinoma cells was 14.42 μmol mL^{−1}, while pretreatment with SO₂ resulted in an IC₅₀ of 17.53 μmol mL^{−1}. Concurrent pretreatment with both NO and SO₂ reduced the IC₅₀ to 12.66 μmol mL^{−1}, significantly decreasing the required cisplatin dosage compared to the untreated group and reversing cisplatin resistance in nasopharyngeal carcinoma. Subsequent probe analysis of ONOO[−] and SO₂ levels in these groups showed that the red channel fluorescence signal increased markedly after NO treatment (Fig. 4B and C), indicating elevated intracellular ONOO[−] levels. The blue channel fluorescence signal also significantly intensified after NaHSO₃ treatment, suggesting elevated intracellular SO₂ levels. Notably, cisplatin-resistant cells exhibited lower ONOO[−] content than cisplatin-sensitive cells, which may be related to intracellular GSH levels. As shown in Fig. 4D, both NO and NaHSO₃ treatments significantly reduced intracellular GSH levels compared to the untreated group, with cisplatin-sensitive cells demonstrating lower GSH content than resistant cells. These results validated our hypothesis that NO and SO₂ induce RNS production and deplete intracellular GSH to counteract cisplatin resistance.

4. Conclusion

In summary, we designed and synthesized a small-molecule fluorescent probe, HCy-ONOO[−]-SO₂, that responds to both ONOO[−] and SO₂. The probe had excellent stability and strong biocompatibility in complex biological systems, enabling the sensitive detection of changes in ONOO[−] and SO₂ within cells. Leveraging the probe's exceptional biochemical properties, we investigated the ability of NO and SO₂ to counteract cisplatin resistance in nasopharyngeal carcinoma, shedding light on their mechanisms. The results showed that NO and SO₂ can induce cisplatin resistance in nasopharyngeal carcinoma cells in a reversible manner by generating reactive nitrogen species and depleting glutathione. We believe that this probe has significant potential for evaluating the relationship between NO/SO₂ and cisplatin resistance and could provide a novel

chemical tool for studying cisplatin resistance in nasopharyngeal carcinoma.

Author contributions

Xiaofeng Wang: conceptualization, methodology, formal analysis, investigation, writing – original draft. Xinyu Li: data curation, validation, resources, software, visualization. Xiaoqiang Chen: supervision, project administration, writing – review & editing. Desheng Wang: supervision, conceptualization, writing – review & editing, funding acquisition.

Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

Data availability

Data is provided within the manuscript or supplementary information (SI). Supplementary information: the general methods, reaction kinetics, and cell bright-field images, *etc*. See DOI: <https://doi.org/10.1039/d5ra08282j>.

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