Electronic Supplementary Information

Water soluble ratiometric fluorescent probe to mitochondrial targeted SO_2 based on conjugated biquinolines

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Contents:

I: Material and Methods

1.1 Materials and Physical measurements

All chemical reagents and solvents were obtained from commercial suppliers, such as Aladdin Industries (Shanghai, China), Shanghai Experimental Reagent Co., Ltd. (Shanghai, China), Beyotime Co., Ltd. (Shanghai, China). Among them, 6-methoxy-2-methylquinoline and 1.4-dioxane were purchased from Aladdin Industries (Shanghai, China). Mitochondrial red, CCK-8 and cell culture reagents were purchased from Beyotime Co., Ltd (Shanghai, China). The fluorescence spectrum was measured using a Hitachi F-7000 fluorescence spectrophotometer. The ultraviolet-visible spectrum was monitored by Hitachi U-3900 ultraviolet-visible spectrophotometer. ^{1}H NMR and ^{13}C NMR data were performed with BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometers (Bruker, Billerica, MA). The HR-MS measurement was performed on the AB SCIEX Tripple TOF5600 instrument. Zeiss LSM880 confocal laser scanning microscope was used to evaluate the probe's ability to respond in living cells. PerkinElmer IVIS Lumina LT Ⅲ living imaging instrument was used for evaluating the imaging of **Probe** in living cells and mice.

1.3. Synthesis of Probe

Compound 1: 6-methoxy-2-methylquinoline (346 mg, 2 mmol) and SeO_2 (0.45 g, 4 mmol) were dissolved in 1.4-dioxane at 80 °C for 4 hours. TLC spot check. The solvent was removed under reduced pressure to obtain a light pink solid. (EA: PE = 1:3).

Probe: The Compound 1 (0.55 g, 2.6 mmol) was added dropwise to a solution of 6-

methoxy-1,2-dimethylquinolin-1-ium (0.64 g, 3.5 mmol) dissolved in ethanol, and 110 ul of piperidine was added. The mixture was heated to 90 °C, refluxed, and protected with N_2. TLC spot check. After the reaction, the solvent was removed under reduced pressure. The product was purified by column chromatography to obtain a light yellow solid product 0.25 g, 45%, (CH_2Cl_2 :CH_3OH = 20:1).

1.4. Imaging Experiments

Hela cells were cultured in humidified air containing 5% CO_2 at 37 °C in DMEM medium supplemented with 10% fetal bovine serum (additional 100 μg/mL streptomycin and 100 u/mL penicillin). The cells were washed 3 times with PBS (pH = 7.4) and then incubated with 10 μM **Probe** in pure PBS (pH = 7.4) for 20 min. After washing three times, the cells were CLSM imaged with a Zeiss LSM-880 microscope. The yellow channel was set to 570 ± 30 nm (λ_{ex} = 488 nm), and the blue channel was set to 445 ± 30 nm (λ_{ex} = 405 nm). In the mitochondrial localization experiment, we set the parameters: λ_{ex} = 488 nm, λ_{ex} = 490-550 nm. For the endogenous group, the loaded **Probe** cells were pre-incubated with Cys (150 μM) for 0.5 h.

*1.3. L*iving mice imaging.

All animal experiments were conducted in accordance with the procedures approved by the Radiation Protection Research Institute of China Drug Safety Evaluation Center (production license: SYXK (Jin) 2018-0005). Balb/c type mice (12-14 weeks, male) were purchased from Beijing Life River Experimental Animal Technology Co., Ltd. The experiment was conducted according to the guidelines for the care and use of laboratory animals of the National Institutes of Health. During the

experiment, the mice was under general anesthesia. The fluorescence image is recorded in the PerkinElmer IVIS Lumina LTIII real-time imager.

II:

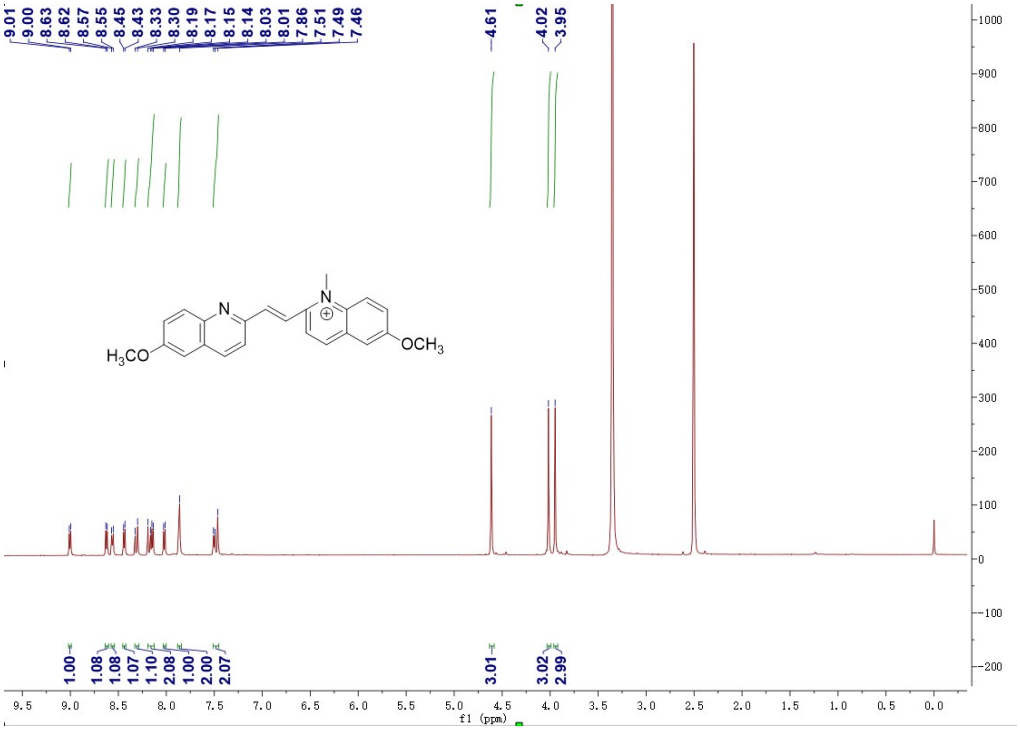


Figure S1. The ^{1}H NMR (600 MHz) Spectrum of **Probe** in DMSO-d_6

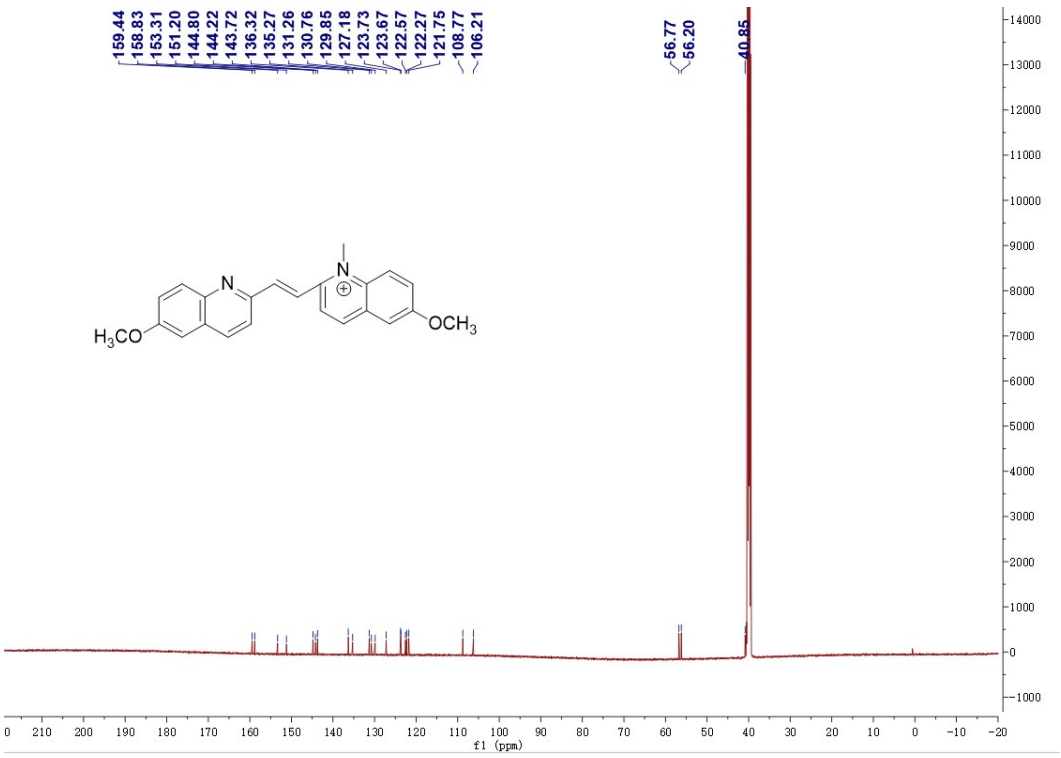


Figure S2. The ^{13}C NMR (151 MHz) Spectrum of **Probe** in DMSO-d_6

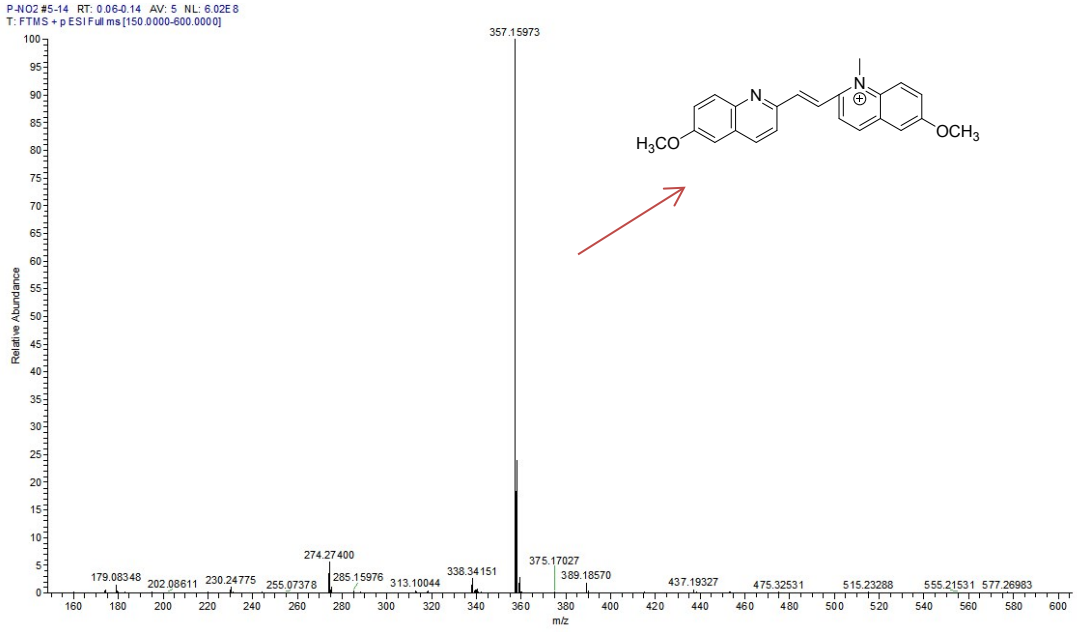


Figure S3: The HR-MS spectrum of the **Probe**.

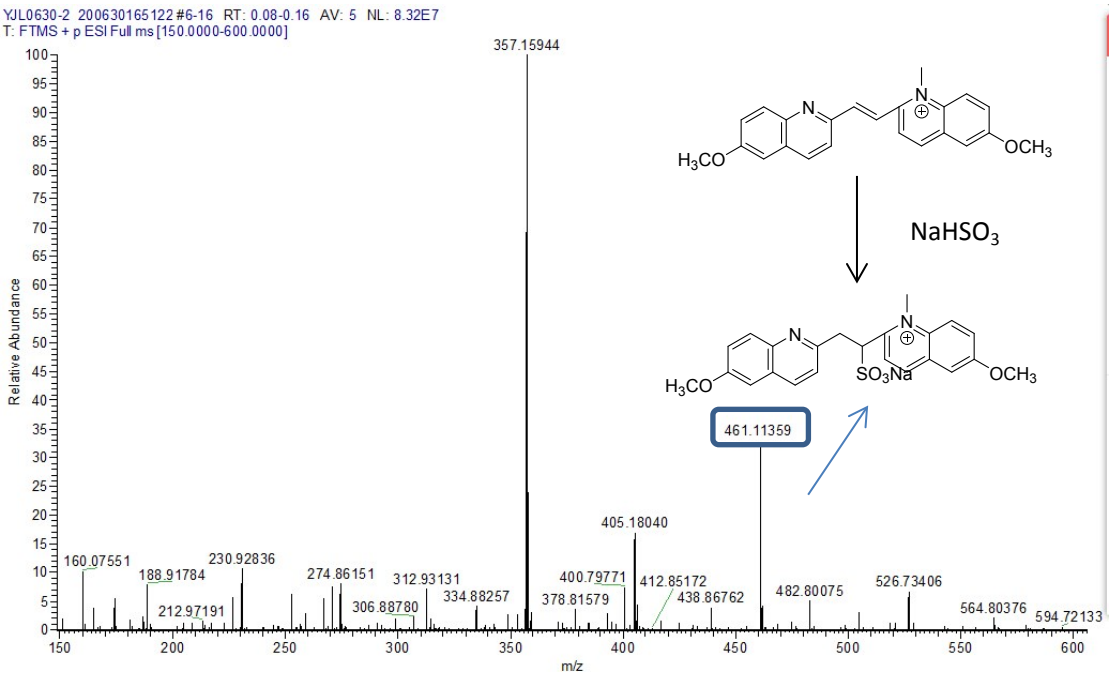


Figure S4:The HR-MS of the reaction mechanism.

Probe+$NaHSO_3$： HR-MS m/z: Calcd for $[C_{23}H_{22}N_2NaO_5S^+]$:461.11416;found: m/z: 461.11359.

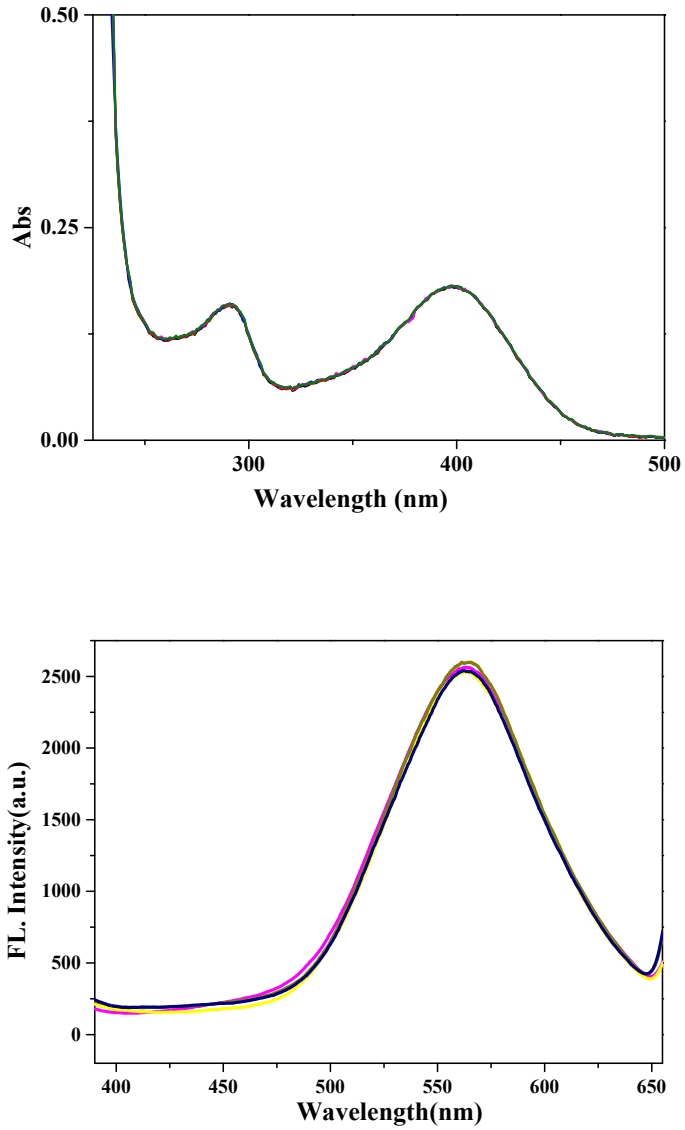
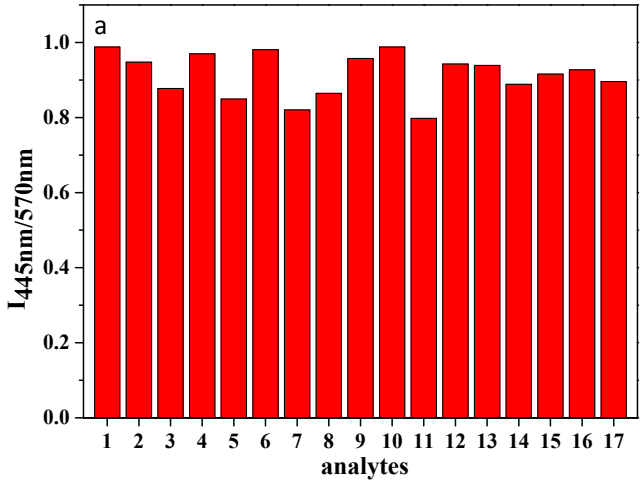


Figure S5: UV-Vis absorption spectrum and fluorescence emission spectra (10 μM) of **Probe** under LED in PBS within 40 min.



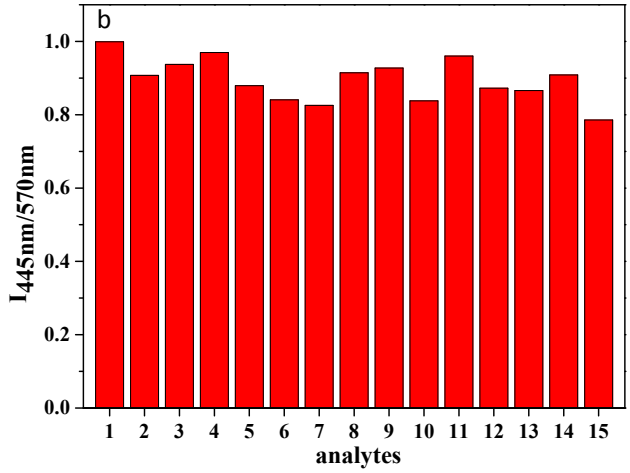


Figure S6: Fluorescence competition of **Probe** (10 μM) in response to various other biologically relevant species (5 mM) at $I_{445/570}$. (a) 1.$NaHSO_3$, 2.H_2S + $NaHSO_3$, 3.L-Threonine + $NaHSO_3$, 4.L-Methionine + $NaHSO_3$, 5.L-Proline + $NaHSO_3$, 6.L-Aspartic acid + $NaHSO_3$, 7.L-Valine + $NaHSO_3$, 8.trans-4-Hydroxy-L-proline + $NaHSO_3$, 9.L-Asparagine + $NaHSO_3$, 10.L-Glutamic acid + $NaHSO_3$, 11.L-phenylalanine + $NaHSO_3$, 12.L-Arginine + $NaHSO_3$, 13.L-Isoleucine + $NaHSO_3$, 14.Cys + $NaHSO_3$, 15.GSH + $NaHSO_3$, 16.Hcy + $NaHSO_3$, 17.L-Leucine + $NaHSO_3$.(b) Competition response of **Probe** (10 μM) in the presence of various other anions (5 mM) at $I_{445/570}$. 1.$NaHSO_3$, 2.$NaHSO_3$+CH_3COO^-, 3. $NaHSO_3$+Cl^-, 4. $NaHSO_3$+CO_3^{2-}, 5.$NaHSO_3$+F^-, 6.$NaHSO_3$+$H_2PO_4^-$, 7.$NaHSO_3$+ClO^-, 8. $NaHSO_3$+HCO_3^-, 9. $NaHSO_3$+HPO_4^{2-}, 10. $NaHSO_3$+SO_4^{2-} 11. $NaHSO_3$+Br^-, 12. $NaHSO_3$+NO_2^-, 13. $NaHSO_3$+SCN^-, 14. $NaHSO_3$+$S_2O_3^{2-}$, 15. $NaHSO_3$+H_2O_2, All data were obtained after 3 minutes.

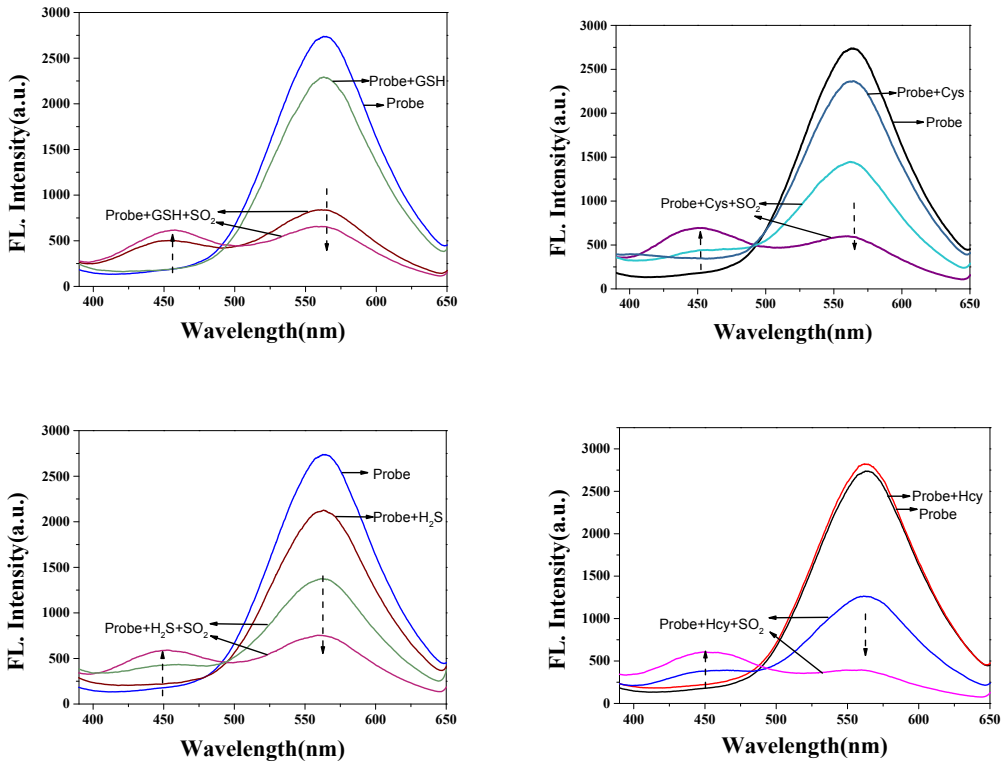


Figure S7: Fluorescence intensity curves of **Probe** (10 μM) in the presence or absence of $NaHSO_3$ (200 μM), GSH, Cys and Hcy, and H_2S (10 mM) in the presence or absence of $NaHSO_3$ (200 μM) in PBS buffer (pH 7.4).

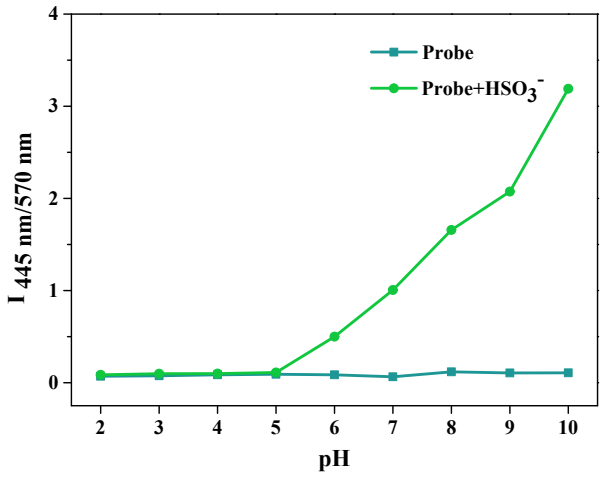


Figure S8. Fluorescence spectra of free **Probe** and **Probe** (10 μM) after addition of $NaHSO_3$ (120 μM) under different pH conditions.

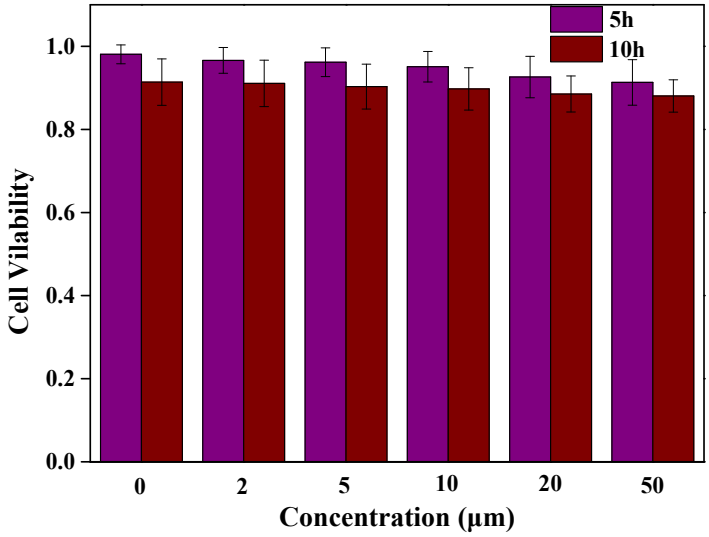
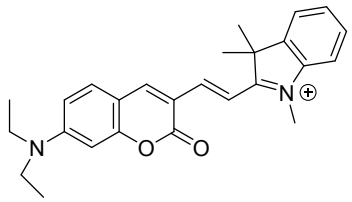
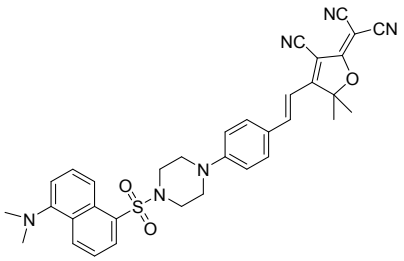
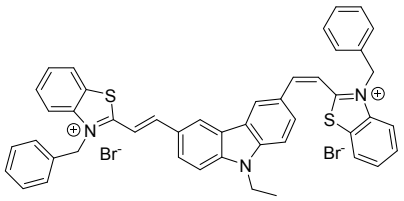
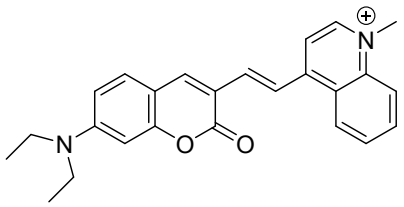
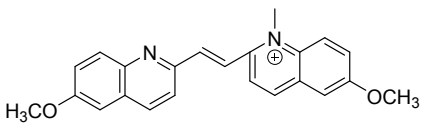


Figure S9: Cell viability estimated by CCK-8 assay with Hela cells, which were cultured in the presence of 0−50 μM **Probe** for 5 and 10 h.

Table 1: The water solubility comparison chart of the **Probe** and other probes.

Probe	Structure	Reaction system	Reference
Probe 1		PBS (contain 15% EtOH)	Ref (1)
Probe 2		PBS: EtOH = 5:5	Ref (2)
Probe 3		PBS: DMSO= 1:1	Ref (3)
Probe 4		PBS: DMSO= 1:1	Ref (4)
This **Probe**		PBS	This work

References

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