Supplementary Information (SI) for Analytical Methods.

Supporting Information for

Remarkable membrane permeability fluorescent probe for real-time imaging mitochondrial SO_2 with high-fidelity

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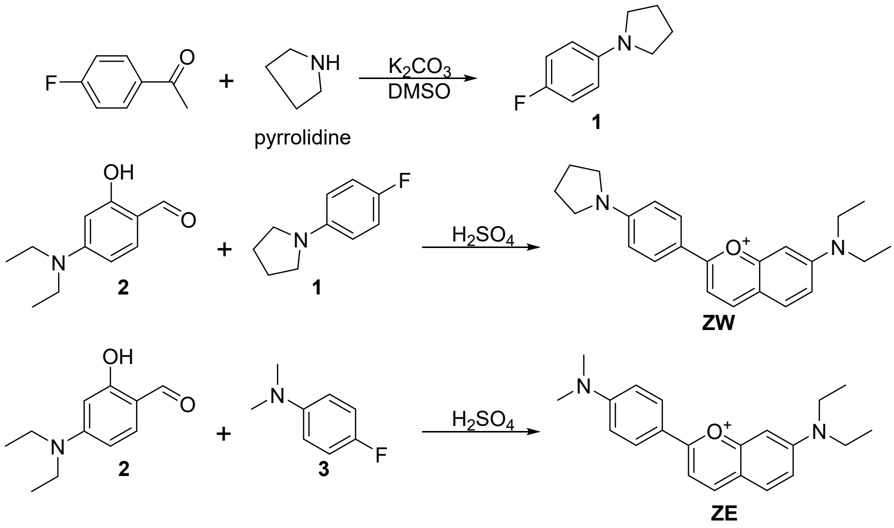
1. **Materials and apparatus**.

NMR data were obtained with an AVANCE III 600 MHz Digital NMR spectrometer with tetramethylsilane (TMS) as an internal standard. High Resolution Mass Spectrometric (HRMS) data were got at an Agilent 1100 HPLC/MSD spectrometer. The pH experiments were tested using a Mettler-Toledo Delta 320 pH meter. Absorption experiments were conducted on a Shimadzu UV-2700 spectrometer. Photoluminescent spectra were obtained using a HITACHI F4600 fluorescence spectrophotometer. Biological imaging experiments were accomplished with Nikon A1 fluorescence microscopy.

2. **Experimental section**



Scheme S1 Detail synthetic routes of probes **ZW** and **ZE**.

*2.1 The synthesis of compound **1***

4-Fluoroacetophenone (1.38 g, 10 mmol) and pyrrolidine (1.42 g, 20 mmol) was dissolved in 6 mL DMSO, then added K_2CO_3 (4.15 g, 30 mmol) to the solution. The mixture were stirred at 120℃ for 10 h. After the reaction was completed, then mixture was cooled to room temperature, 100 mL saturated salt water was poured into reaction solution, and 100 mL dichloromethane was add for extraction. Repeated this operation three times, and dried with anhydrous sodium sulfate to obtain crude product compound **1**. Finally, compound **1** further purified using silica gel column chromatography to obtain pure solid compound **1** (1.4 g, yield: 74%).

2.4 Cell culture and imaging

HeLa cells were employed to test the imaging feasibility of probes ZW and ZE. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Before imaging, cells were passaged onto 20 mm glass dishes and further cultured for around 24 h to obtain the confluence of 80%.

3. Figures

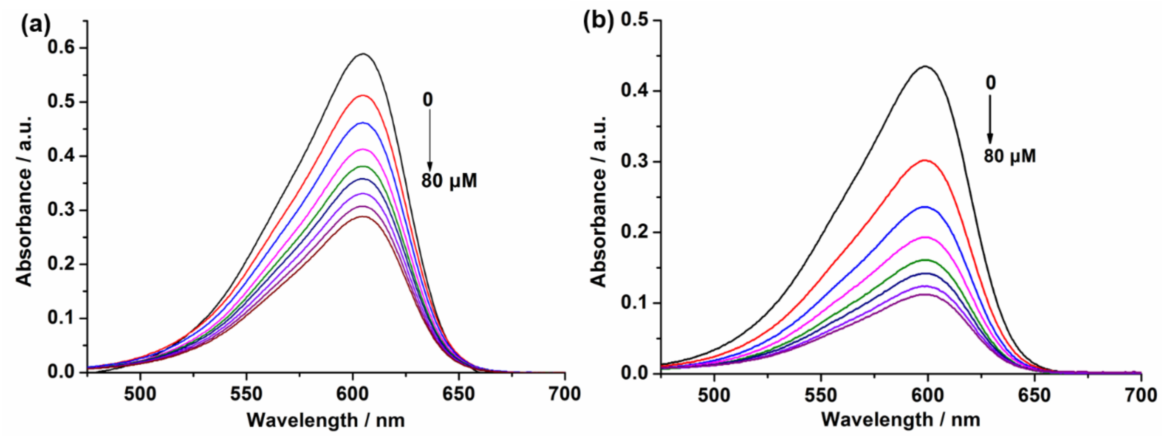
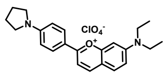
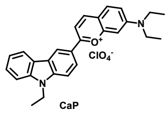
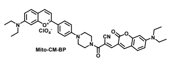
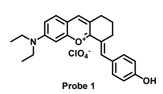
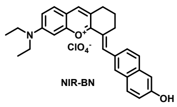
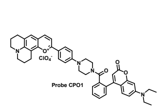
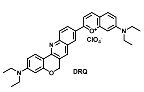
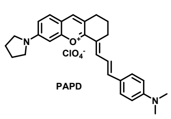


Fig.S1 Absorption spectra of **ZW** (a) and **ZE** (b) with $NaHSO_3$ (0-80 μM) in PBS (10 mM, pH=7.4) containing 10% DMSO.

Table S1. Optical and application properties of the representative known benzopyrylium-based SO_2 fluorescent probes and the new probe **ZW** designed herein.

Reference	Structure	Absorption	Excitation & emission	Quantum yield(Φ)	LOD[a]	Concentrations of imaging in living cells	Applications
This work	 ZW	590 nm	λ_{ex}: 590 nm; λ_{em}: 670 nm	0.237	190 nM	200 nM & 500 nM	Exogenous and endogenous SO_2 imaging & Ferroptosis imaging in cells
1. Anal. Chem. 2019,91,10723-10730	 CaP	570 nm	λ_{ex}: 270 nm; λ_{em}: 370 nm/630 nm	ND[b]	730 nM	10 µM	SO_2/formaldehyde reversible imaging in cells
2. J. Am. Chem. Soc. 2020, 142, 6324-6331	 Mito-CM-BP	570 nm	λ_{ex}: 488 nm; λ_{em}: 638 nm	ND	160 nM	10 µM	Exogenous SO_2 imaging & endogenous SO_2 metabolism in cells
3. Sensor. Actuat-B. Chem., 2019, 297, 126747	 Probe 1	570 nm	λ_{ex}: 570 nm; λ_{em}: 660 nm	0.026	121 nM	10 µM	SO_2/H_2O_2 reversible imaging in cells
4. Anal. Methods, 2021, 13, 3535-3542	 NIR-BN	320 nm & 580 nm	λ_{ex}: 580 nm; λ_{em}: 680 nm	ND	170 nM	10 µM	Exogenous and endogenous SO_2 imaging in cells & SO_2 detection of food
5. New J. Chem., 2022, 46, 18090-18099	 Probe CPO1	570 nm & 610 nm	λ_{ex}: 410 nm; λ_{em}: 645 nm	ND	60 nM	5 µM	Exogenous and endogenous SO_2 imaging & SO_2 detection of water
6. Talanta, 2020, 217, 121086	 DRQ	580 nm & 405 nm	λ_{ex}: 580 nm; λ_{em}: 613 nm	0.33	103 nM	10 µM	Two-photon imaging of exogenous and endogenous SO_2 in cells & zebrafish
7. Dyes Pigments,2023, 216,111308	 PAPD	373 nm & 688 nm	λ_{ex}: 400 nm & λ_{em}: 495 nm; λ_{ex}: 680 nm & λ_{em}: 835nm;	0.13	27.36 nM	2.5 µM	SO_2 & viscosity imaging in cells

[a]LOD: Limit of Detection; [b] ND: Not determinated.

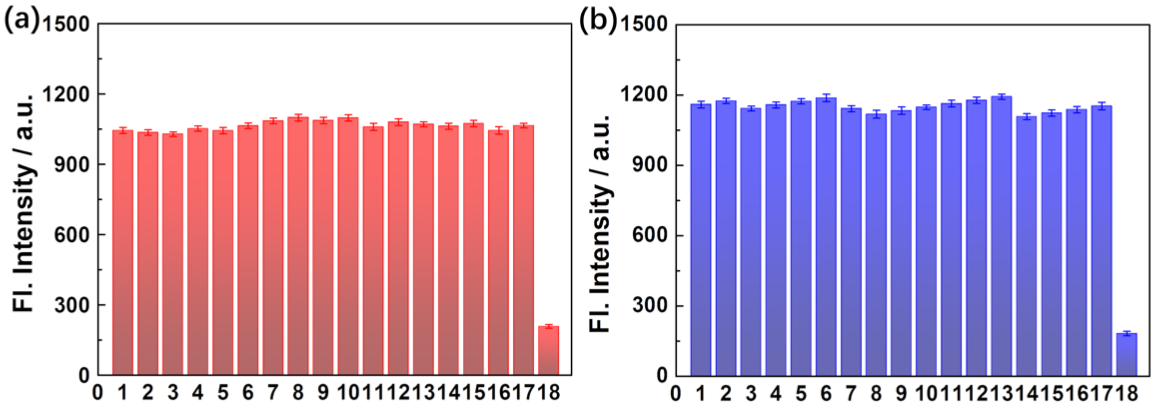


Fig. S2 Fluorescence changes of **ZW** (10 μM) (a) and **ZE** (10 μM) (b) in the presence of various analytes (200 μM) for 20 min. Number1-18 denotes: free probes (ZW or ZE); $FeCl_2$; H_2O_2; VC; H_2S; HClO; K_2CO_3; KCl; $MgCl_2$; $ZnCl_2$; NaBr; $CaCl_2$; $FeCl_3$; Na_2SO_4; $NaNO_2$; GSH; Cys; Na_2HCO_3. $\lambda_{ex} = 590$ nm.

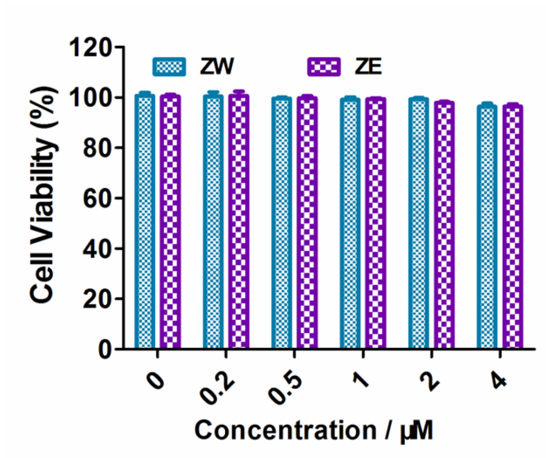


Fig. S3 HeLa cell viability after treatment with probes **ZW** and **ZE** (0-4 μM) for 24 h.

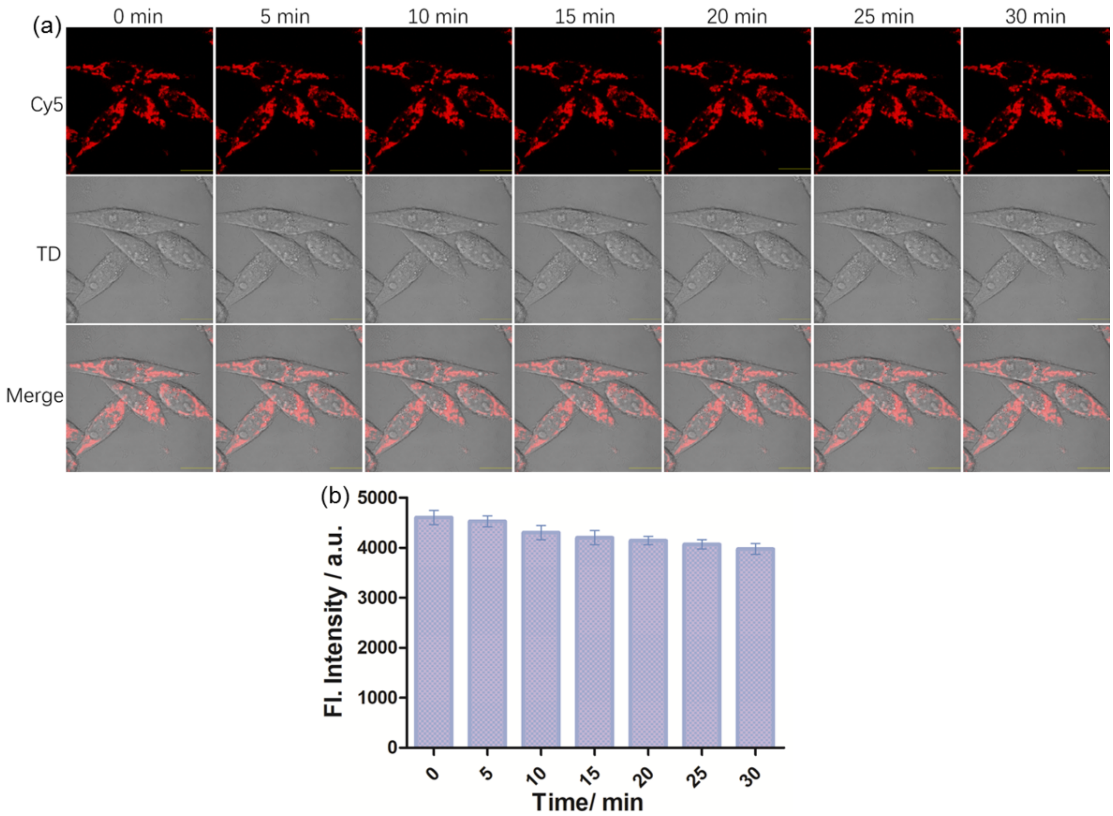


Fig.S4 (a) Fluorescence images of cells continuous irraditon for 30 min after the incubation with 200 nM **ZW** for 18 min. (b) Fluorescence intensities of cells at different time. Cy5 Channel: λ_{ex}=561 nm, λ_{em} = 663-738 nm. Scale bar: 20 μm.

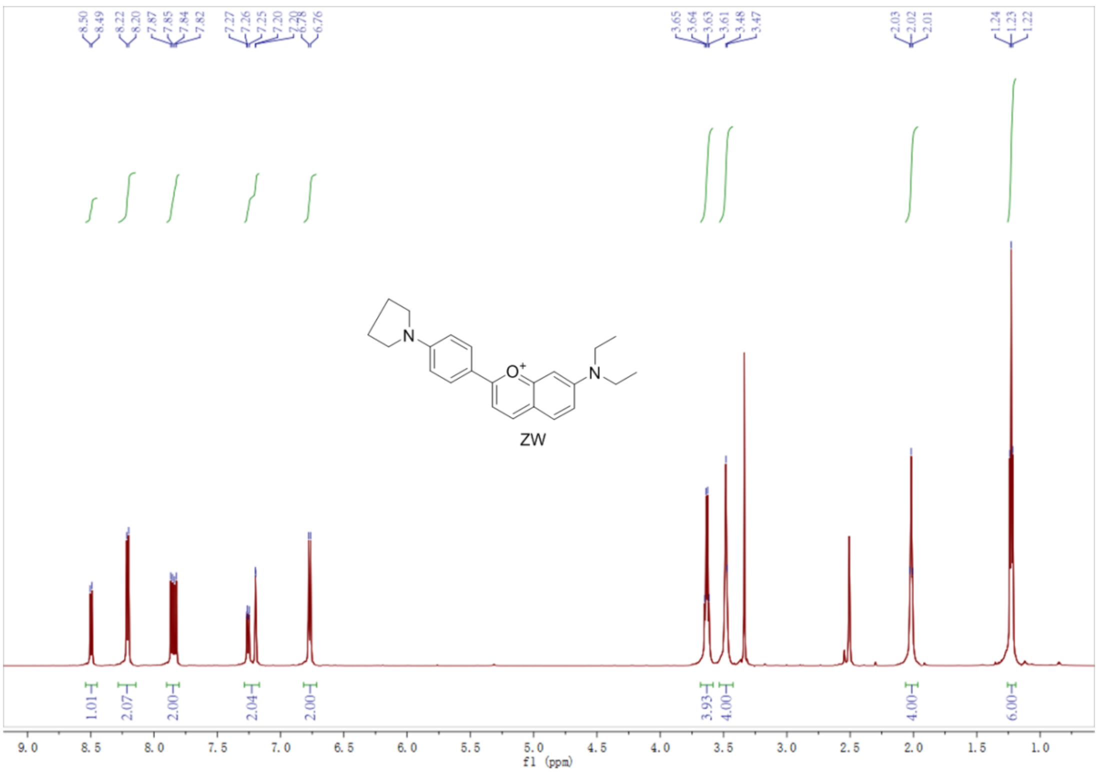


Fig. S5. ^{1}HNMR (600 MHz) spectrum of **ZW** in DMSO-d_6.

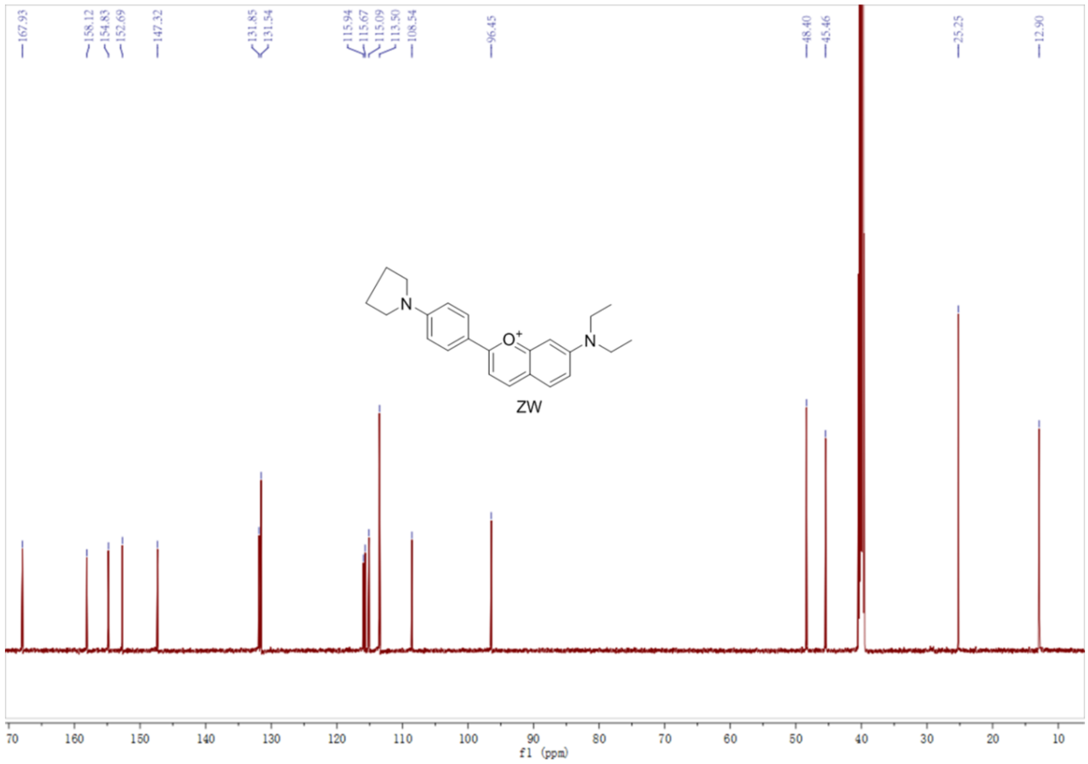


Fig. S6. [13]CNMR (150 MHz) spectrum of **ZW** in DMSO-d_6.

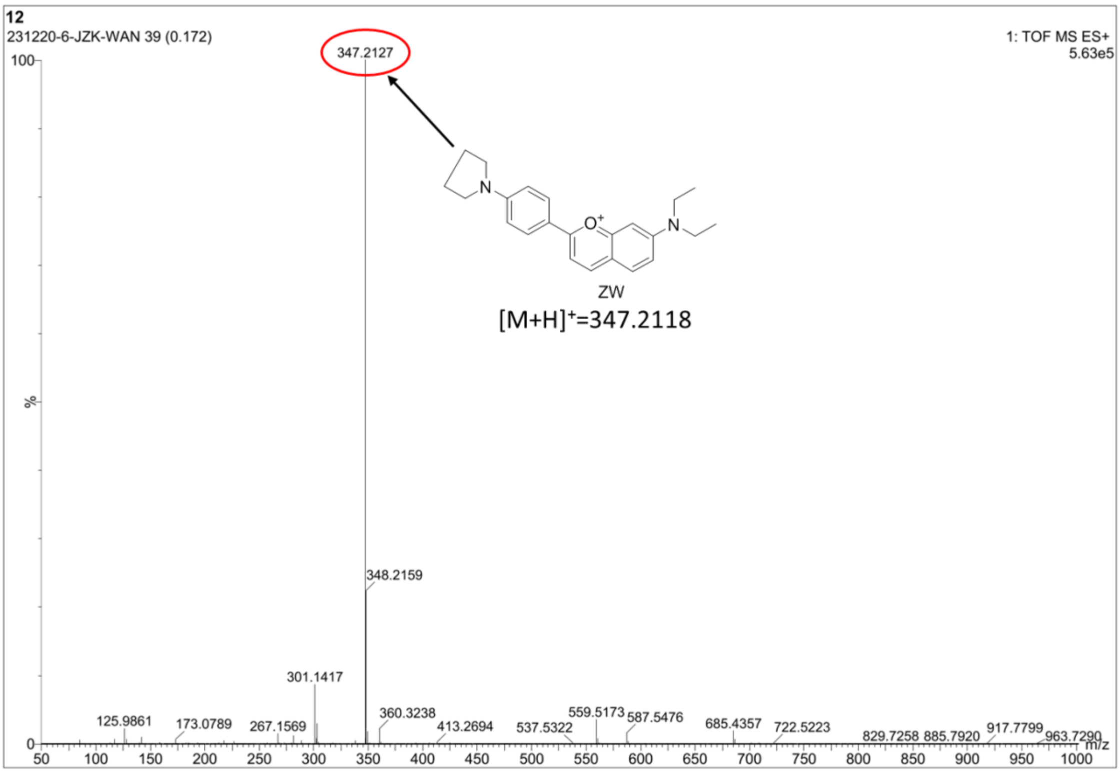


Fig. S7. HRMS spectrum of **ZW**.

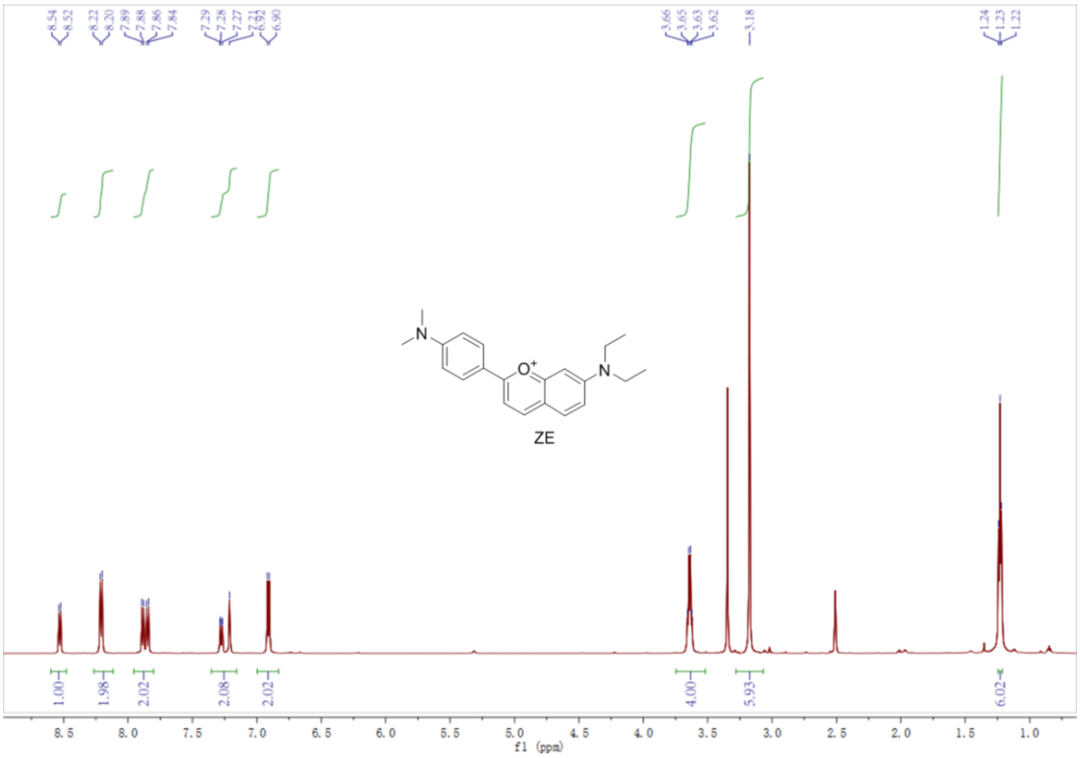


Fig. S8. [1]HNMR (600 MHz) spectrum of **ZE** in DMSO-d_6.

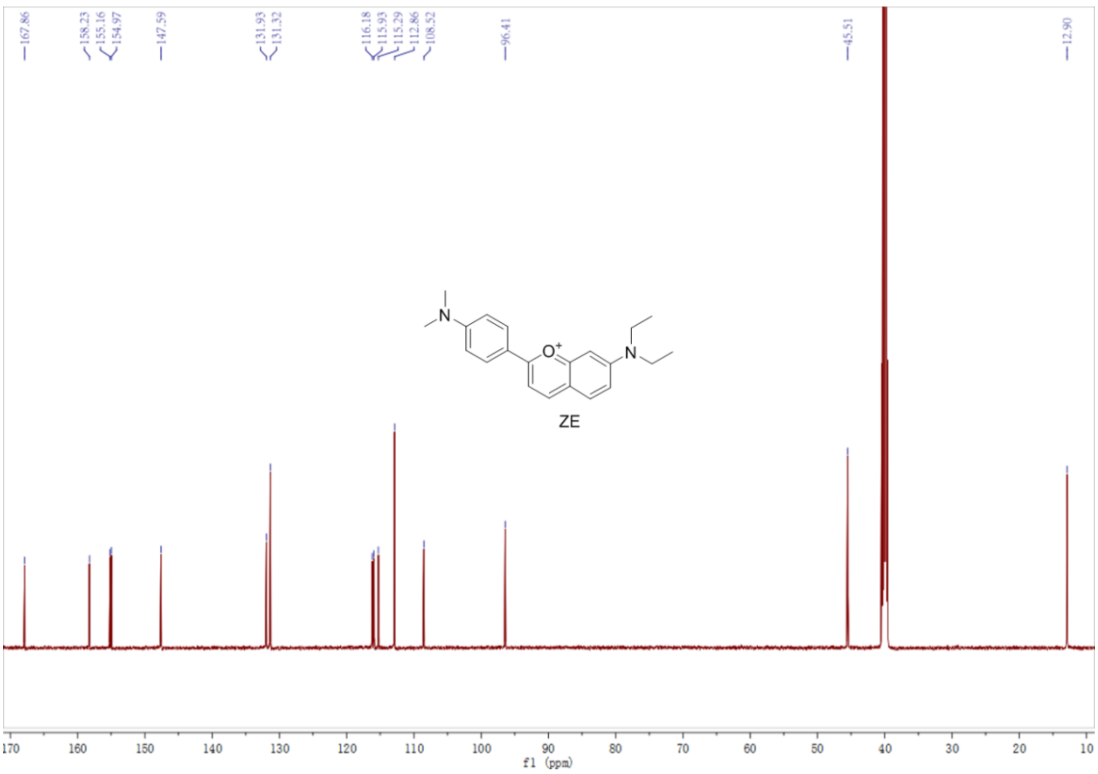


Fig. S9. [13]CNMR (150MHz) spectrum of **ZE** in DMSO-d_6.

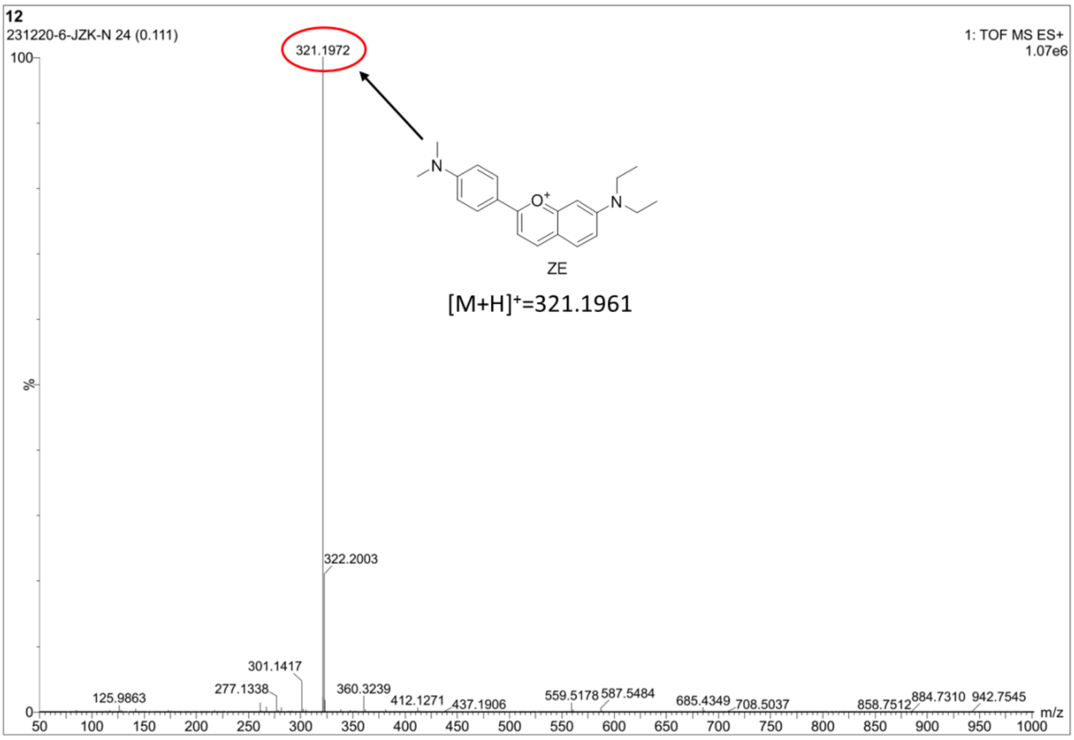


Fig. S10. HRMS spectrum of **ZE**.