Supporting Information for

A ratiometric fluorescent probe for reversible monitoring of endogenous SO_2/formaldehyde in cytoplasm and nucleoli regions and its applications in living mice

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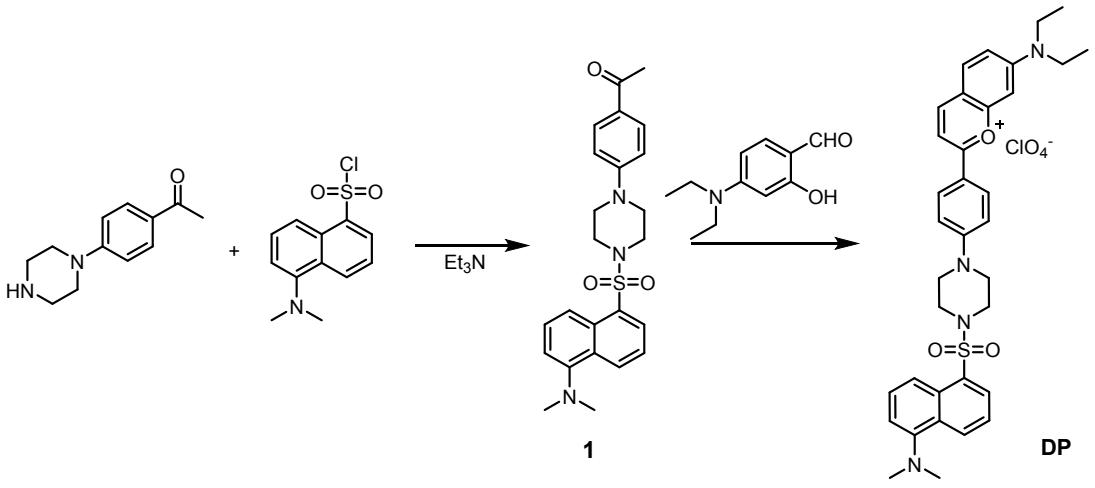
1. Materials and instruments

Unless specifically stated, All reagents are purchased from commercial sources and used directly without further purification. Solvents were purified by standard methods before used. TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200−300), both of them were obtained from the Qingdao Ocean Chemicals. ^{1}H and ^{13}C NMR spectra were recorded on an AVANCE III 400 Nanobay (Bruker, 400 MHz for ^{1}H, 100 MHz for ^{13}C), using etramethylsilane (TMS) as internal reference. High-resolution mass spectrometric (HRMS) analyses were performed on a Bruker apex-Ultra mass spectrometer (Bruker Daltonics Corp., USA) in electrospray ionization (ESI) mode. HRMS for response mechanism was collected using Agilent 6510 Q-TOF LC/MS. The pH measurements were obtained on a Mettler-Toledo Delta 320 pH meter. UV−vis absorption spectra were performed with a Shimadzu UV-2700 spectrophotometer and fluorescence spectra were recorded on a HITACHI F4600 fluorescence spectrophotometer. MTT was obtained from J&K Scien-tific Ltd. Fluorescence imaging experiments were measured on Nikon A1MP confocal microscopy. The 4-week old female balb/c mice were purchased from School of Pharmaceutical Sciences, Shandong University. The procedures for care and use of animals were approved by the Ethics Committee of the Institutional Animal Care and Use Committee (IACUC) and all applicable institutional and governmental regulations concerning the ethical use of animals were followed. In vivo imaging was carried out on a PerkinElmer IVIS Spectrum imaging system equipped with a -90 °C CCD camera.

2. Synthesis of compounds



Scheme S1 Synthesis route of the probe **DP**.

Synthesis of compound 1

4-piperazinoacetophenone (204 mg, 1 mmol) and 100 μL triethylamine were added to 10 ml CH_2Cl_2 solution at 0 °C, then dansyl chloride (323 mg, 1.2 mmol) dissolved in 5 mL CH_2Cl_2 and dripped into the above solution. The mixture was stirred for 6 h at room temperature and then evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2: MeOH=30:1) to afford compound **1** (yield 75%). ^{1}H NMR (400 MHz, DMSO) δ 8.54 (d, *J* = 8.0 Hz, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.18 (d, *J* =8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 2H), 7.74 – 7.59 (m, 2H), 7.28 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 12 Hz, 2H), 3.41 – 3.36 (m, 4H), 3.23 – 3.18 (m, 4H), 2.83 (s, 6H), 2.43 (s, 3H).

3. Measurements

Spectral measurements of selectivity

All of spectral experiments were conducted on phosphate buffered saline (PBS, pH 7.4, 10 mM) containing 20% CH_3CN. The stock solution of **DP** (1 mM) was dissolved in DMSO. The various testing analytes stock solutions (KCl, CH_3CHO, TBHP, KNO_3, $MgCl_2$, NaBr, KI, $NaNO_2$, benzaldehyde, KSCN, KF, NH_4OAc, H_2O_2, Hcy, GSH, $CaCl_2$, $AlCl_3$, Cys, $ZnCl_2$, $CuCl_2$, Na_2HPO_4, Na_2S, $BaCl_2$, NaClO, FA and $NaHSO_3$) were prepared at 100 mM in ultrapure water, and the di-*t*-butyl peroxide (DTBP) stock solution (100 mM) was prepared in the DMSO. The test solution contained **DP** (10 μM), PBS buffer (pH 7.4, 10 mM, 20% CH_3CN) and the anions and cations at 1 mM, ROS at 100 μM, representative amino acid at 500 μM, GSH at 2.5 mM, Na_2S at 50 μM and other analytes at 500 μM. Stock solution of $NaHSO_3$ was freshly prepared every time before use.

For the selectivity of FA, the probe were pre-treated with 50 μM $NaHSO_3$ and an appropriate aliquot of each testing species were added. After that, the fluorescence spectra were measured. Unless otherwise stated, the excitation wavelength was 365 nm, the excitation slit widths were 5 nm, and emission slit widths were 5 nm for all the measurements.

Detection limit of DP for SO_2

The detection limit was based on a reported method[1]. According to the result of fluorescence spectral titrating experiment, the fluorescence ratio intensities (I_{550}/I_{635}) of **DP** treated with various concentration of $NaHSO_3$ were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to the normalized fluorescent intensity data and the point at

which this line crossed the axis was considered as the detection limit.

The reversibility cycle of DP

DP (10 μM) was added to 10 mM PBS buffer containing 20% CH_3CN as a cosolvent. $NaHSO_3$ and FA with mole ratio of 1:4 were added alternately for reversibility.

Cytotoxicity assays and cell culture

HeLa cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Gibico) supplemented with 10 % fetal bovine serum (FBS, Sijiqing), penicillin (100 U/ml, Hyclone) and streptomycin sulfate (100 U/ml, Hyclone) under an atmosphere of 5% CO_2 and 95% air at 37 °C.

The cytotoxicity of **DP** to HeLa cells were performed by MTT assays. 2×10^4 cells/mL HeLa cells were seeded in 96-well plates and then incubated with various concentrations of **DP** (0 - 20 μM) (n=4) for 24 h. After that 10 μL MTT (5 mg/mL) was added into per well and incubated for 4 h, and then the 100 μL DMSO were added to substitute the previous media and solve the formazan crystals. The absorbance of the solution at 570 nm was recorded by microplate reader. The cell viability (%) = $(OD_{sample}-OD_{blank}) / (OD_{control}-OD_{blank}) \times 100$ %.

OD_{sample} denotes the cells cultured with different concentrations of **DP**, $OD_{control}$ denotes the cells incubated with culture medium, OD_{blank} denotes only the culture medium.

Co-localization experiment

HeLa cells were pre-incubated with 10 μM **DP** for 30 min and then 0.5 μM SYTO RNA-Select was added to the cells and incubated for 10 min. Then, the HeLa cells were washed with PBS for three times. Finally, confocal fluorescence imaging was carried out. The **DP** probe was excited at 405 nm and fluorescence was collected at 663-738 nm. SYTO RNA-Select was excited at 488 nm and fluorescence was collected at 500-550nm.

RNase digest test of fixed cells

The HeLa cells were first fixed by 4% paraformaldehyde for 1 h and then permeabilized by 0.5% Trition X-100 for 5 min at ambient temperature. After rinsing with PBS twice, DNase-Free RNase (GE) was added into the other set of cells and incubated for 2 h. Then two sets of cells were incubated with **DP** 20 μg/mL for 30 min. Finally, confocal fluorescence imaging was carried out. Emission was

collected between 500 -550 nm for green channel and 663-738 nm for red channel with a 405 nm laser excitation.

Reversible imaging SO_2 and FA in living mice

The 4-week old female balb/c mice were purchased from School of Pharmaceutical Sciences, Shandong University. The procedures for care and use of animals were approved by the Ethics Committee of the Institutional Animal Care and Use Committee (IACUC) and all applicable institutional and governmental regulations concerning the ethical use of animals were followed.

For the imaging of exogenous SO_2 and FA in living mice, the abdominal fur of mice was removed by an electric shaver, then they were anesthetized by 4% chloral hydrate aqueous solution. The mice was the injected 100 μL **DP** (50 μM) by intraperitoneal injection manner and imaged. The *in vivo* images were collected with an IVIS Lumina XR in vivo imaging system under an excitation filter of 580 nm and an emission filter of 660 nm. Subsequently, 100 μL $NaHSO_3$ (500 μM in PBS) was injected into the same location and the fluorescence was collected under the same instrumental condition. Finally, 100 μL FA (2 mM in PBS) was injected into the same location and imaged under the same instrumental condition.

For the imaging of endogenous SO_2 in living mice, the mice were divided into two groups and both of them were depilated by an electric shaver. The mice of one group was firstly intraperitoneal injection 100 μL FA (2 mM in PBS), after 20 min, 100 μL **DP** (50 μM) was injected into the same location and imaged. The other group was only injected with 100 μL **DP** (50 μM) and then imaged by IVIS Lumina XR in vivo imaging system.

For the imaging of endogenous SO_2 and FA in living mice, the mice was injected 100 μL **DP** (50 μM) by intraperitoneal injection manner after depilation and anesthesia. The fluorescence image was collected with an IVIS Lumina XR in vivo imaging system. After that 100 μL Tet (4 mM in PBS) was injected into the same location and the fluorescence was collected under the same instrumental condition. The statistical analysis was performed from three separate biological replicates. A region of interest (ROI) was created around in each image and the mean fluorescence intensity of each sample was measured (via “Measure” function) and averaged across the three fields imaged.

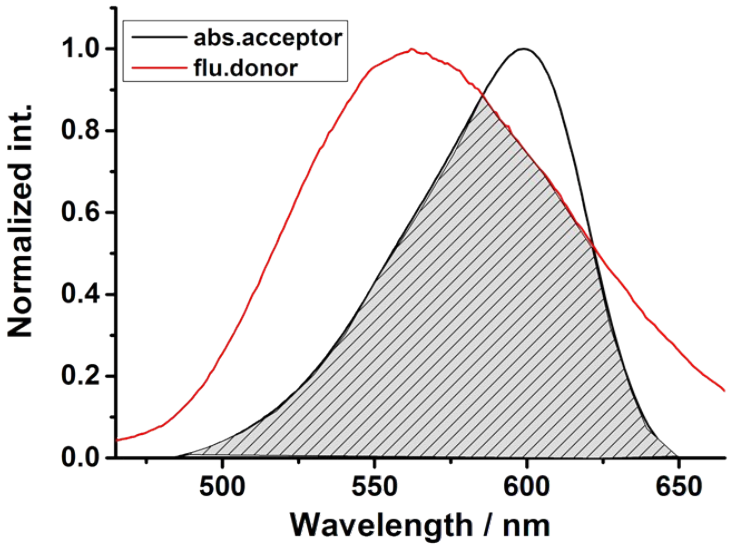


Fig. S1. Normalized absorption spectra (abs) and fluorescence spectra (flu) of acceptor benzopyrylium unit and donor dansyl at pH 7.4 in PBS buffers (20% CH_3CN). The gray area represents the overlap between the fluorescence spectrum of the donor and the absorption spectrum of the acceptor. Fluorescence spectra for dansyl, λ_{ex} = 365 nm.

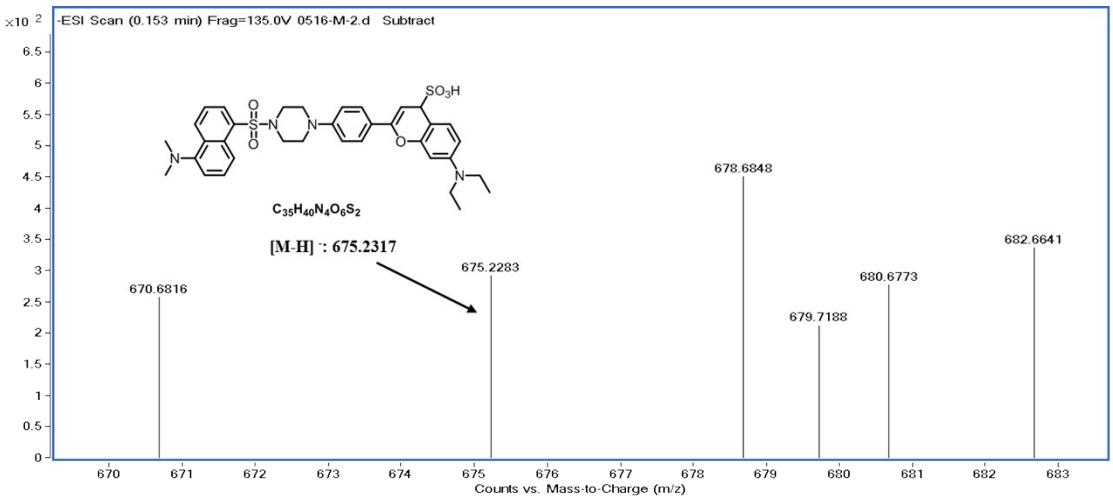


Fig. S2. HR-MS spectrum of 20 μM **DP** with 100 μM $NaHSO_3$.

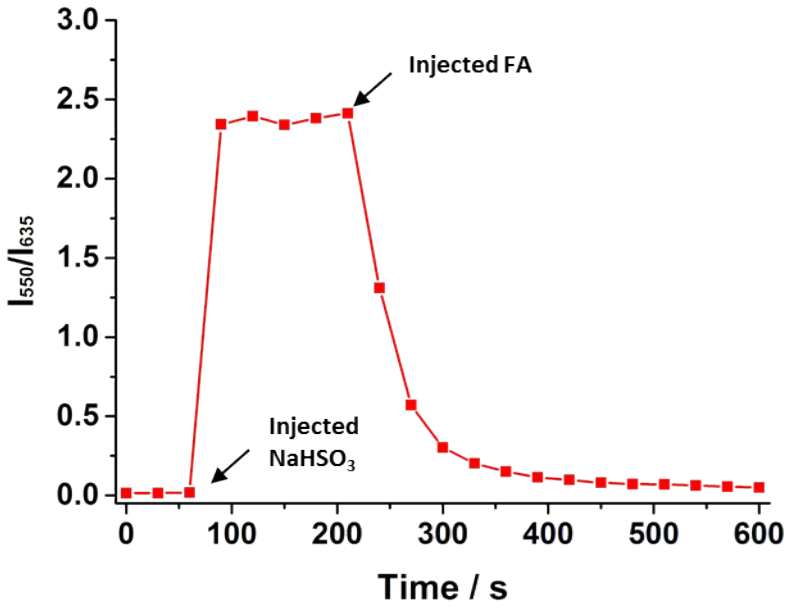


Fig. S3. Time-dependent fluorescence intensity ratios (I_{550}/I_{635}) of **DP** upon addition of $NaHSO_3$ (50 μM) and FA (200 μM).

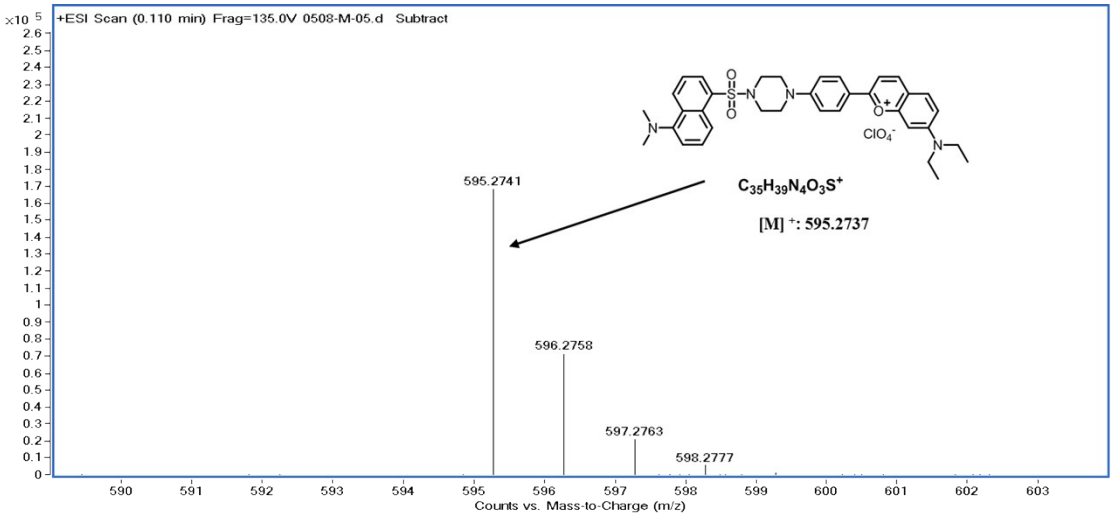


Fig. S4. HR-MS spectrum of the mixture of 20 μM **DP** with 100 μM $NaHSO_3$ upon addition of 400 μM FA.

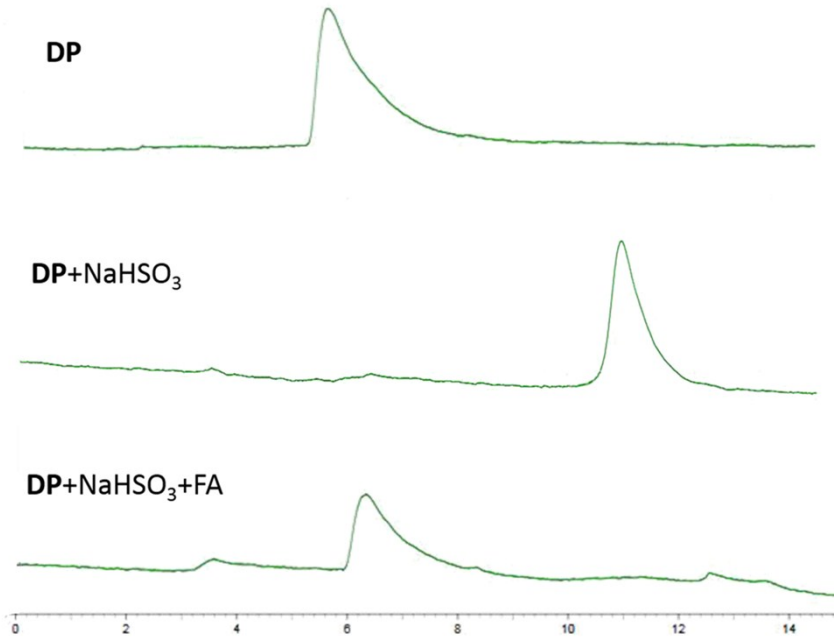


Fig. S5. The HPLC chromatograms for the mixture of **DP**, $NaHSO_3$ and FA in PBS buffer (10 mM, pH 7.4, containing 20% CH_3CN).

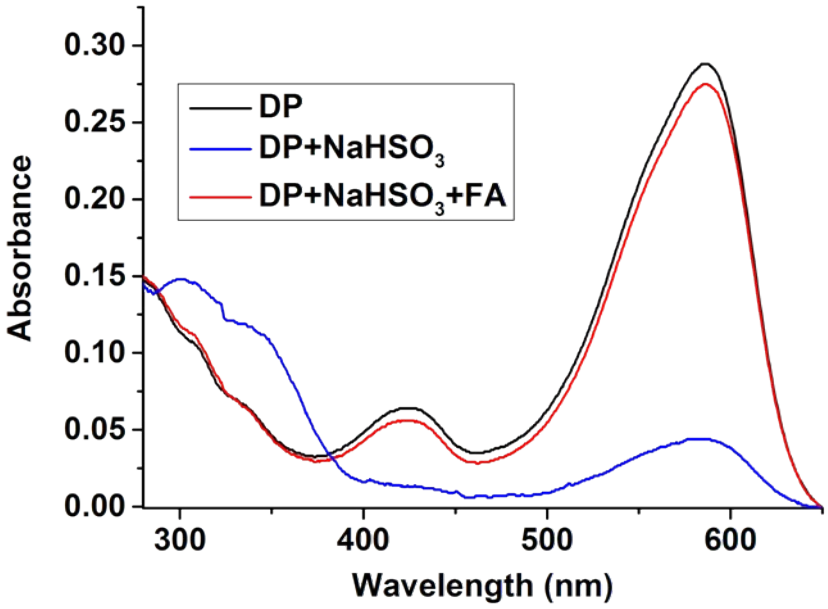


Fig. S6. The absorption spectra of **DP** in the absence or presence of $NaHSO_3$ (50 μM) and FA (200 μM).

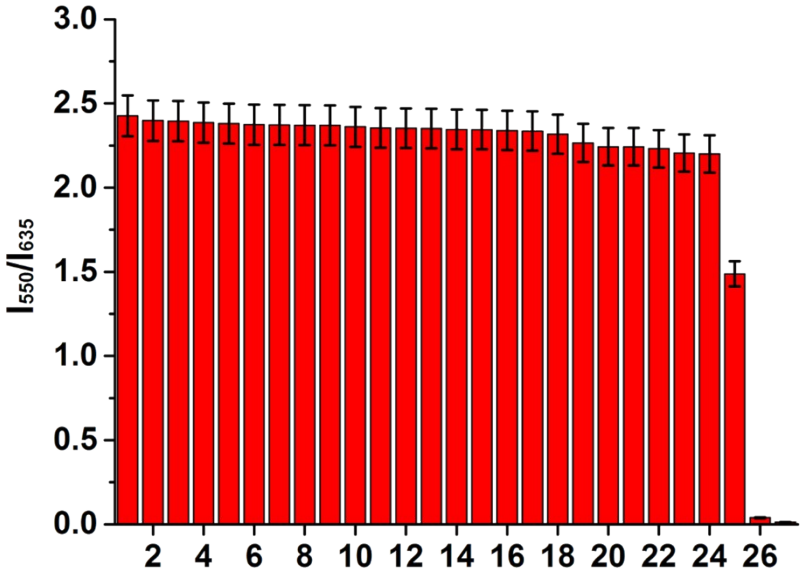


Fig. S7. Fluorescent ratio (I_{550}/I_{635}) of the probe **DP** (10 μM) in the presence of 50 μM $NaHSO_3$ upon addition of various relevant analytes: 1, Na_2HPO_4 (1 mM); 2, Na_2S (50 μM); 3, KI (1 mM); 4, NaBr (1 mM); 5, KNO_3 (1 mM); 6, $MgCl_2$ (1 mM); 7, Benzaldehyde (500 μM); 8, KSCN (1 mM); 9, Hcy (500 μM); 10, KF (1 mM);11, Cys (500 μM); 12, $CaCl_2$ (1 mM);13, NH_4OAc (1 mM); 14, $NaNO_2$ (1 mM); 15, KCl (1 mM); 16, GSH(2.5 mM); 17, $BaCl_2$ (1 mM); 18, $NaHSO_3$ (50 μM); 19, *ditert*-butyl peroxide (DTBP) (100 μM); 20, $AlCl_3$ (1 mM); 21, NaClO (100 μM); 22, $ZnCl_2$ (1 mM); 23, H_2O_2 (100 μM); 24, TBHP (100 μM); 25, CH_3CHO (500 μM); 26, FA (200 μM). 27, only probe **DP** (10 μM). Error bars represent mean values ± SD. (n = 3)

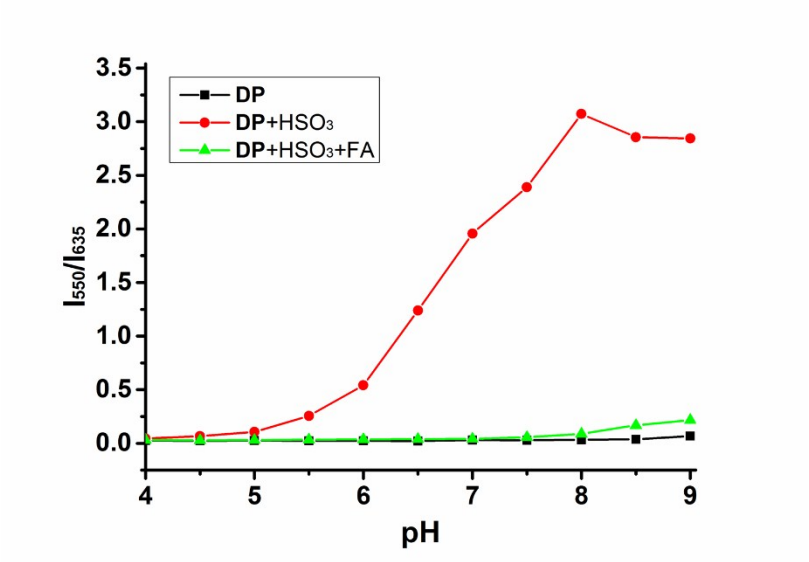


Fig. S8. Fluorescent ratios (I_{550}/I_{635}) of **DP** (10 μM) toward $NaHSO_3$ (50 μM) and FA (200 μM) in PBS (20 % CH_3CN) at various pH values.

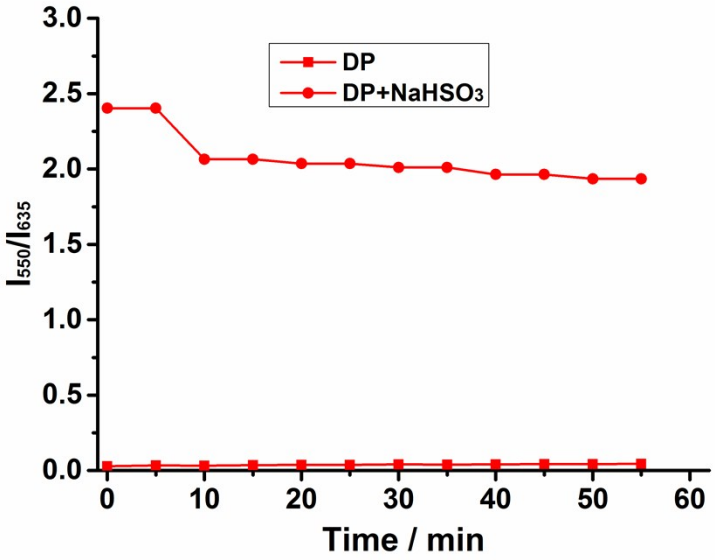


Fig. S9. Photostability profiles of the probe **DP** in the absence or presence of $NaHSO_3$.

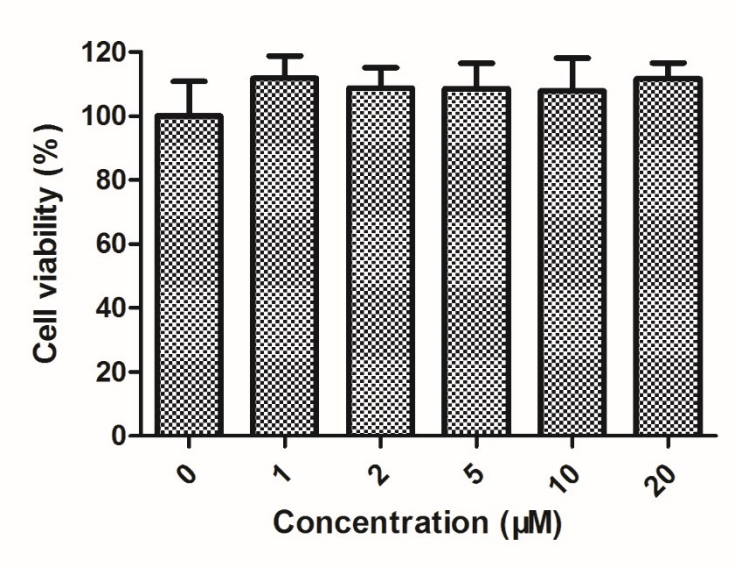


Fig. S10. Viability of HeLa cells incubated with different concentrations (0 - 20 μM) of **DP** for 24 h. Error bars represent mean values ± SD. (n = 3).

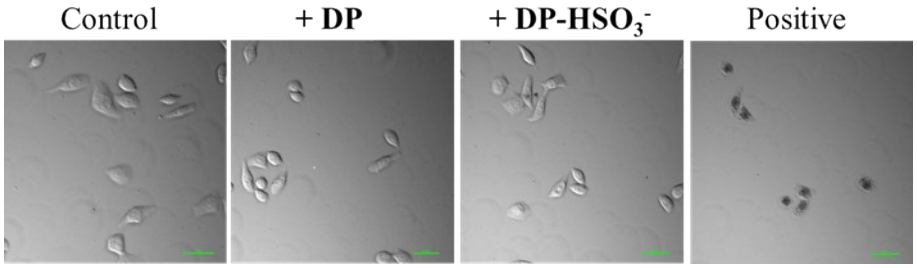


Fig. S11. Images of HeLa cells stained with **DP** in the absence or presence of $NaHSO_3$ and then treated with 0.4% trypan blue. Control group: HeLa cells were treated with only 0.4% trypan blue. Positive group: The HeLa cells were first fixed by 4% paraformaldehyde for 1 h and then permeabilized by 0.5% Trition X-100 for 5 min at ambient temperature. Finally, 0.4% trypan blue were added.

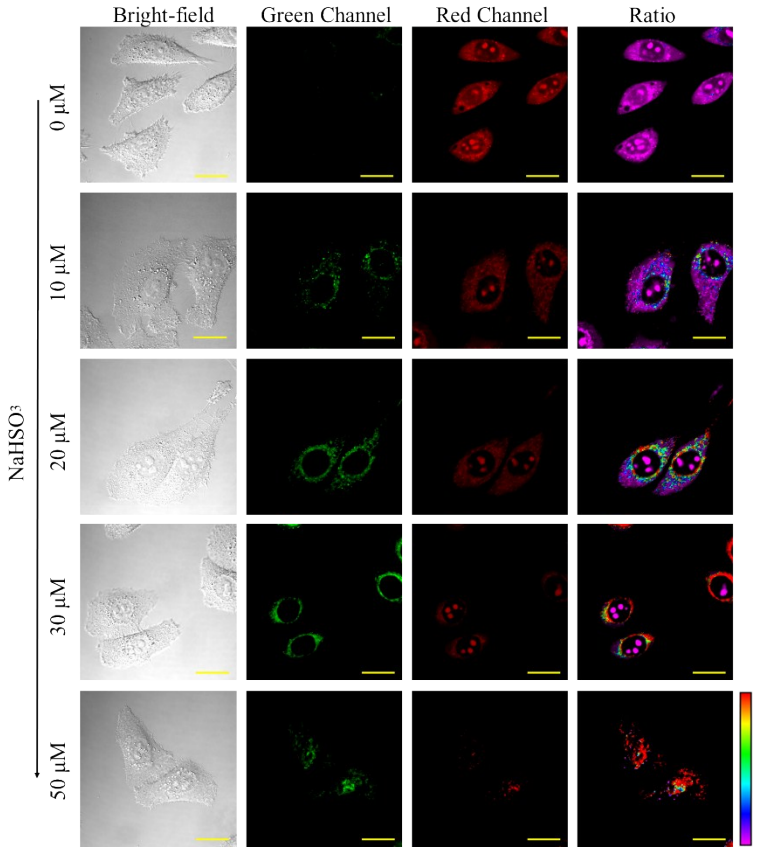


Fig. S12. Confocal images of HeLa cells stained with 10 μM **DP** and the increasing concertation of $NaHSO_3$ (10 μM, 20 μM, 30 μM and 50 μM). Green channel, λ_{ex} = 405 nm, λ_{em} = 500−550 nm. Red channel, λ_{ex} = 405 nm, λ_{em} = 663−738 nm. Fluorescence ratio in green and red channels. Scale bar: 20 μm.

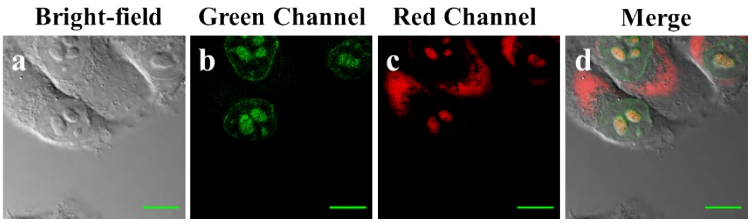


Fig. S13. Confocal images of HeLa cells (a) co-stained with (b) 10 μM **DP** and (c) 0.5 μM SYTO RNAselect; (d) Merged image of (a), (b) and (c);

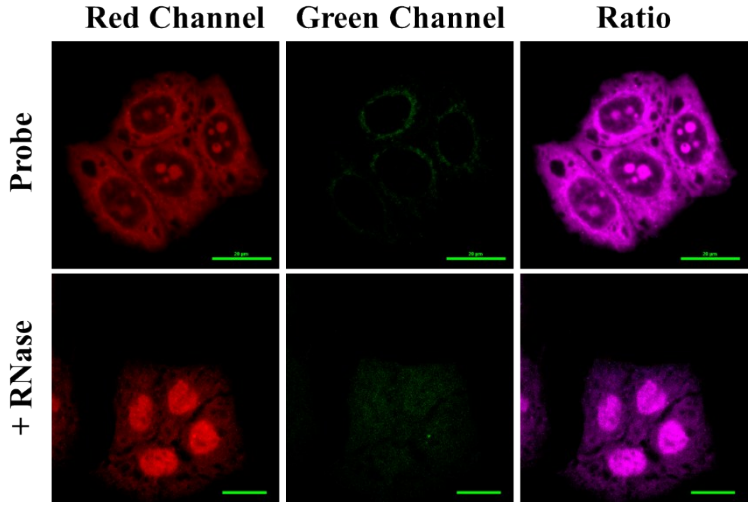


Fig. S14. Fluorescence images of fixed HeLa cells treated with **DP** and RNase. The fixed cells were incubated with RNase for 2 h and stained with 10 μM **DP** in culture media for 30 min. Scale bar: 20 μm.

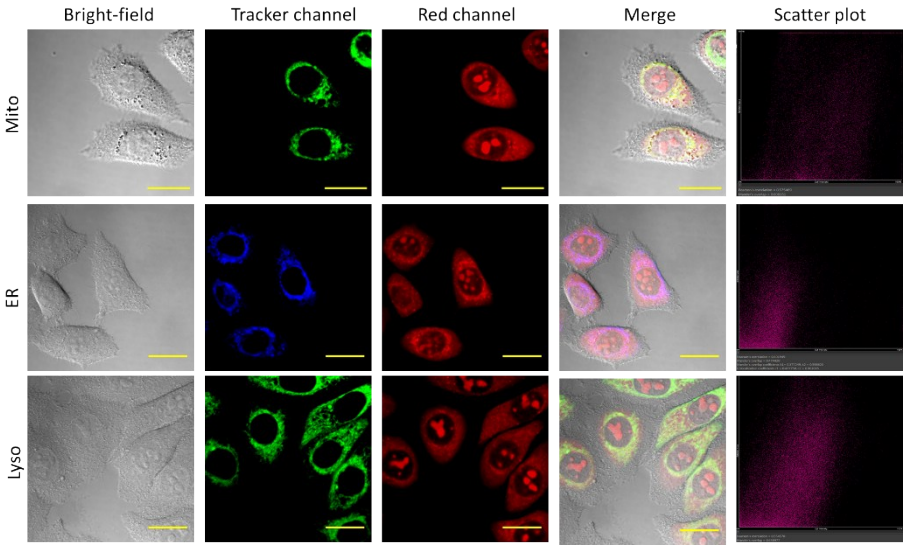


Fig. S15. Confocal microscopy images of HeLa cells stained with **DP** and the commercial organelle tracker (Mito-Tracker Green, Lyso-Tracker Green and ER-Tracker Blue). Scale bar = 20 μm.

Table S1 Quantification of the co-localization experiment. The Pearson's Coefficients were calculated using the software NIS-Elements AR.

	Pearson's Coefficient
Mito-Tracker™ Green	0.50
Lyso-Tracker Green	0.55
ER-Tracker Blue	0.40

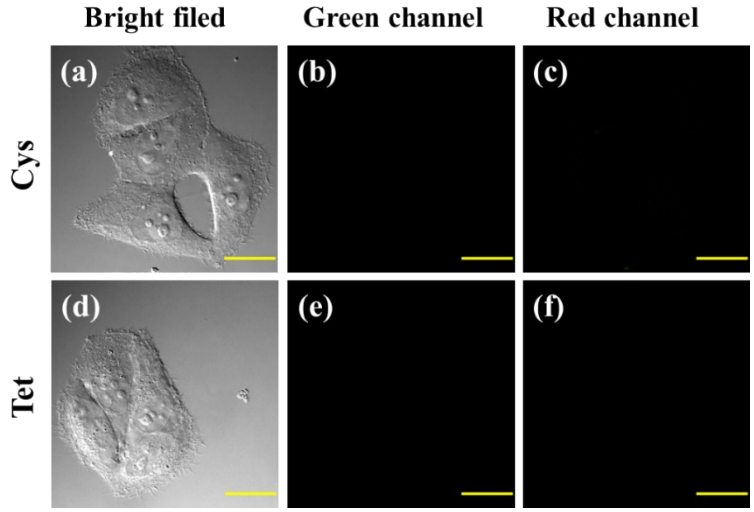


Fig. S16. Fluorescence imaging of HeLa cells treated with 200 μM Cys (a)-(c) and 400 μM Tet (d)-(f). (a) and (d) Bright-field images of HeLa cells. (b) and (e) are the fluorescence images in green channel. (c) and (f) are the fluorescence images in red channel red channel.

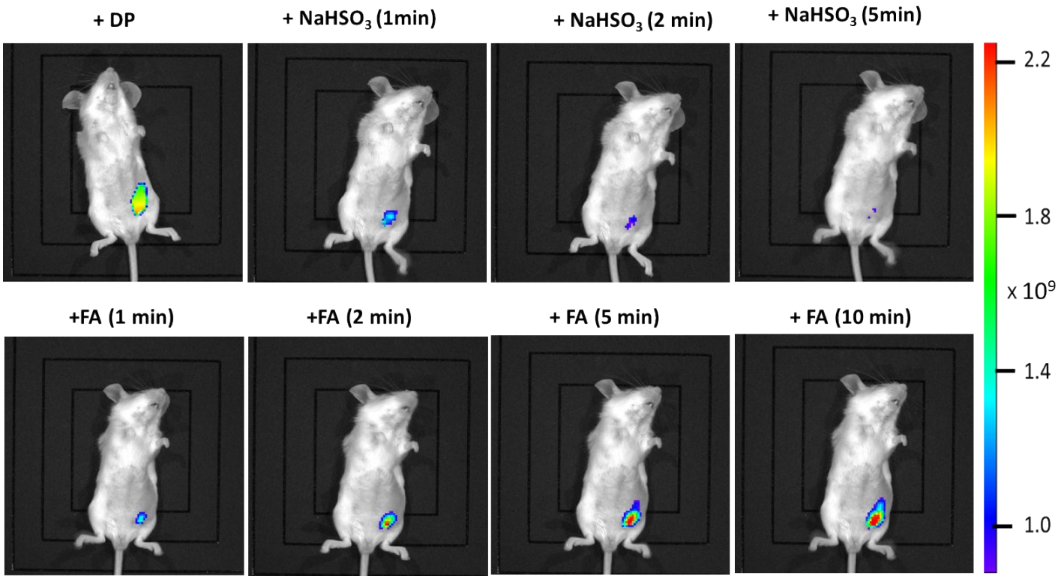


Fig. S17. Time-dependent *in vivo* fluorescence images of exogenous SO_2 and FA in living mice by using **DP**. First row: the mice was injected with 100 μL of 50 μM **DP** and then injected 100 μL of 500 μM

$NaHSO_3$. Second row: 100 μL of 50 μM **DP** and 100 μL of 500 μM $NaHSO_3$ were injected, then 100 μL of 2 mM FA was injected and imaged for different time. λ_{ex} = 580 nm, λ_{em} = 660 nm.

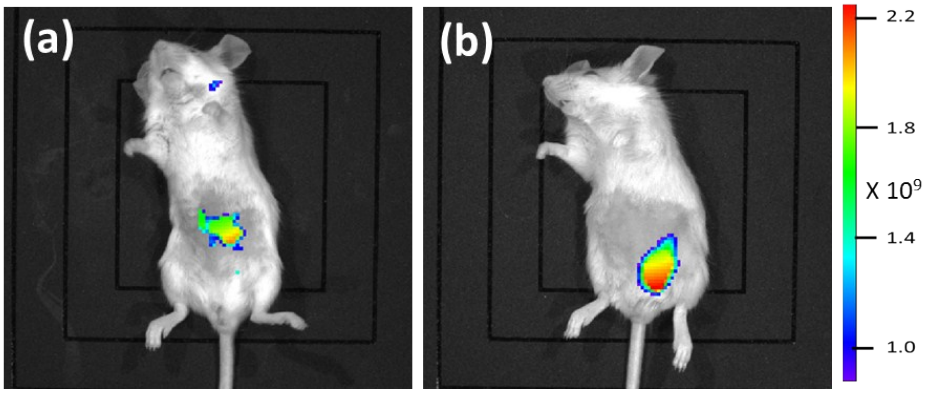


Fig. S18. *In vivo* fluorescence images of reversible detection endogenous SO_2 in living mice by **DP**. (a) The living mouse injected with 100 μL of 50 μM **DP** and imaged. (b) The mice was pre-injected with 100 μL of 2 mM FA and then injected with 100 μL of 50 μM **DP**. λ_{ex} = 580 nm, λ_{em} = 660 nm.

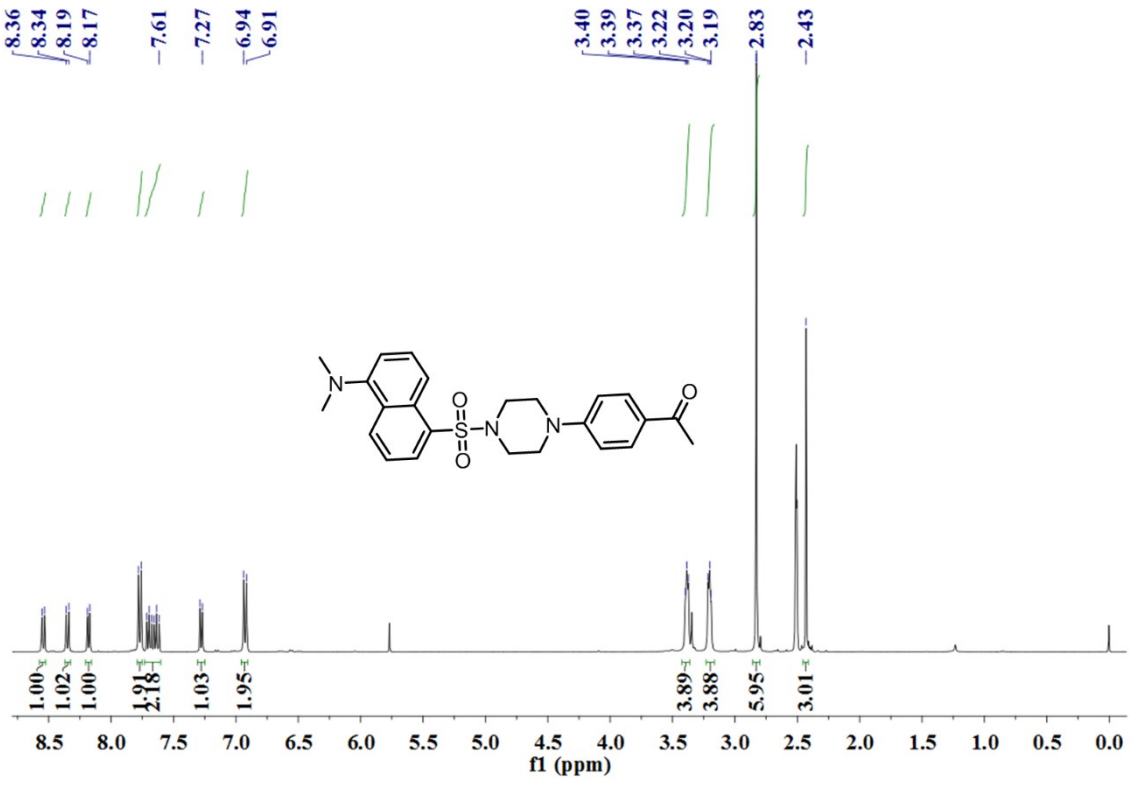


Fig. S19. ^{1}H NMR spectrum of Compound **1** in DMSO-d_6.

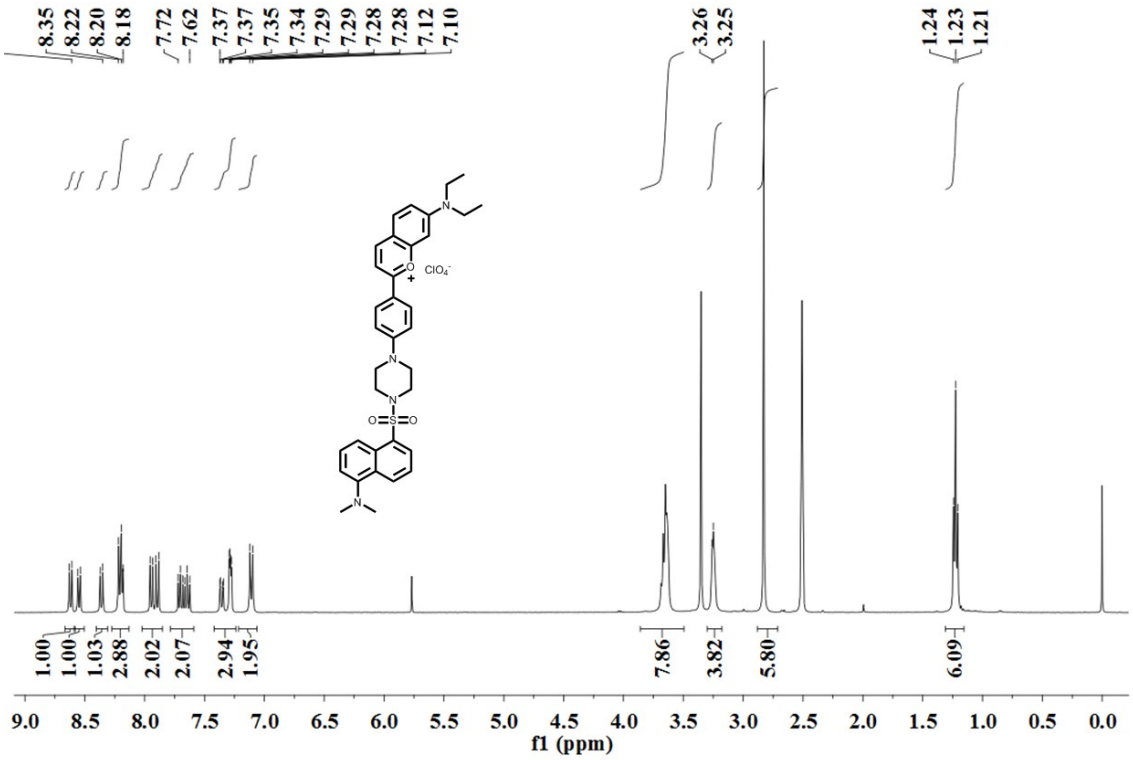


Fig. S20. ^{1}H NMR spectrum of the probe **DP** in DMSO-d_6.

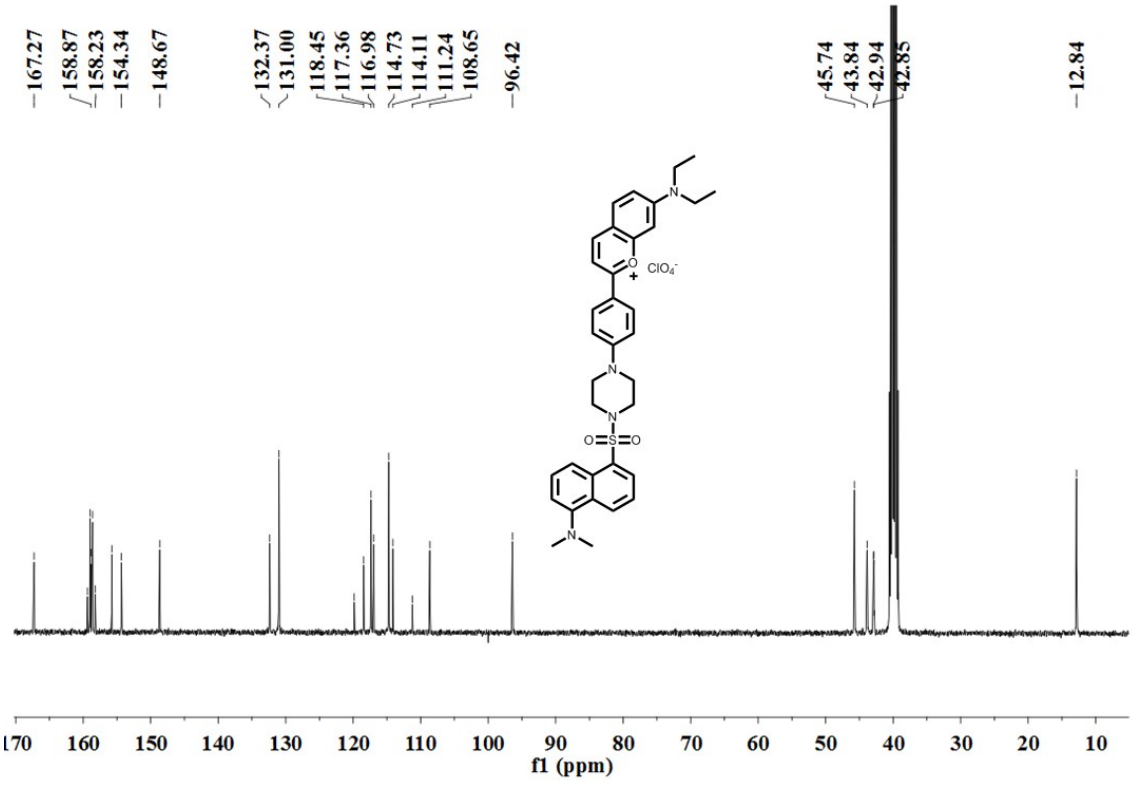


Fig. S21. ^{13}C NMR spectrum of **DP** in DMSO-d_6.

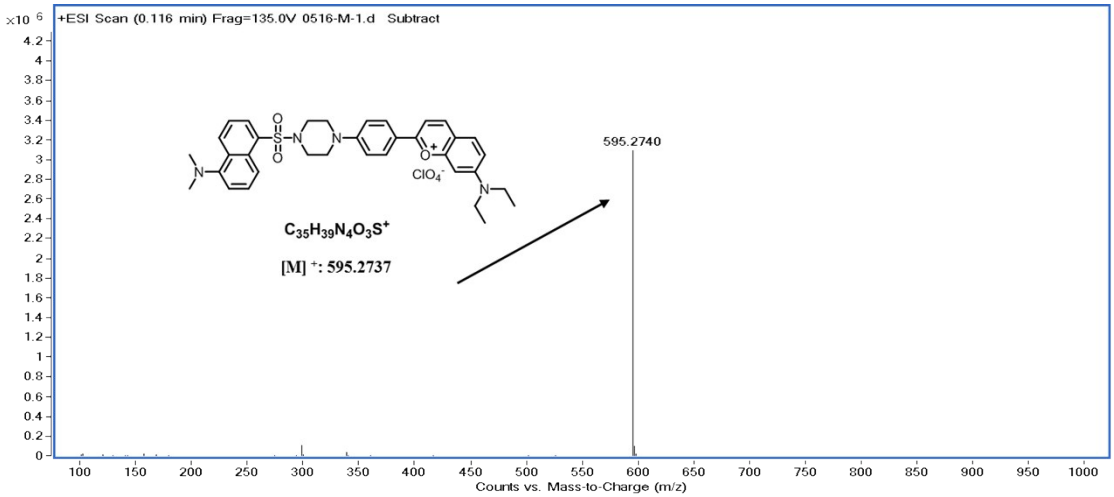


Fig. S22. HR-MS spectrum of **DP**.

Supplementary References

(1) Shortreed, M., Kopelman, R., Kuhn, M. and Hoyland, B. *Anal. Chem.,* 1996, **68**, 1414-1418.