# Supporting Information

# A novel fluorescent probe for the detection of sulfur dioxide derivatives and its application in biological imaging

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# Contents

# 1. Experimental

## 1.1. Chemicals and materials

All chemicals and solvents were purchased from Energy Chemical Reagent Co. (Shanghai, China). All ions (nitrate salts), anions (sodium salts) and biothiol for testing were purchased from Sigma-Aldrich. All solvents and chemicals, unless special stated, were purchased commercially in analytical grade and used without further purification. Deionized water was used to prepare all aqueous solutions. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker AV-400NMR and AV-600NMR spectrometer, and tetramethylsilane was used as the internal standard to measure the chemical shifts. High resolution mass spectra (HRMS) were recorded on an Agilent 6530 QTOF spectrometer. Absorption spectra were measured with Shimadzu UV-2700. Fluorescence spectra were measured with Zolix OmniFluo 900. Confocal fluorescence images were performed on confocal laser scanning microscope (CLSM, Nikon A1R).

## 1.2. Fluorescence Assay

The stock solution of probe **CA-$SO_2$** (1 mM) was prepared in DMSO. Stock solutions (10 mM) of amino acids (Cys, Hcy, GSH), reactive oxygen species ($ClO^-$, $H_2O_2$) and sodium salts ($F^-$, $Br^-$, $Cl^-$, $I^-$, $CH_3COO^-$, $NO_3^-$, $NO_2^-$, $SO_4^{2-}$, $S_2O_3^{2-}$, $S_2O_8^{2-}$, $H_2PO_4^-$, $S^{2-}$, $HSO_3^-$) were prepared in deionized water. Dilute the stock solution to required concentration when in use. When measuring, the concentration of the probe is 10 μM (containing 0.1% DMSO), and the solvent system is DMSO/PBS (v/v: 2/8, 20 mM, pH=7.4).

## 1.3. Cell culture and cell imaging

Human lung adenocarcinoma A549 cells were cultured at 37 ℃ under a humidified 5% $CO_2$ atmosphere in DMEM (GIBCO/Invitrogen, Camarillo, CA, USA), which were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. In *vivo* cell imaging experiment, the cells were cultured in confocal culture plate for 24 h. Then cells were incubated with **CA-$SO_2$** (10 μM) and followed by incubation with $HSO_3^-$ or cysteine, and imaging in confocal laser scanning microscope.

1.4. Fluorescence imaging in zebrafish

Zebrafish eggs are provided by State Key Laboratory of Military Stomatology, Department of Oral Biology, School of Stomatology, The Fourth Military Medical University, and cultured in embryonic medium at 28 ℃ for 96 h. In fluorescence imaging experiment, the zebrafish were incubated with **CA-$SO_2$** (10 μM) and followed by incubation with $HSO_3^-$ or cysteine, and imaging in confocal laser scanning microscope.

## 2. Theoretical computations

The ground state structures of all compounds were optimized using the DFT with the Beck's three parameter hybrid functional with the Lee-Yang-Parr correlation functional (B3LYP). All atoms were treated with 6-311+G (d, p) basis set. The polarized continuum model (PCM) method was employed to account for the solvent effects in optimization and emission calculations. All quantum chemistry calculations were performed using the Gaussian 16 package.

## 3. Spectral data of probe CA-$SO_2$

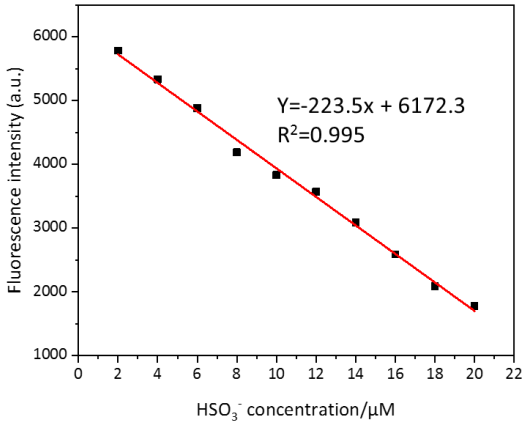


**Fig. S1.** Linear relationship between fluorescence intensity at 589 nm and concentrations of $HSO_3^-$ (0-20 μM)

## 4. *In vitro* cytotoxicity of CA-$SO_2$

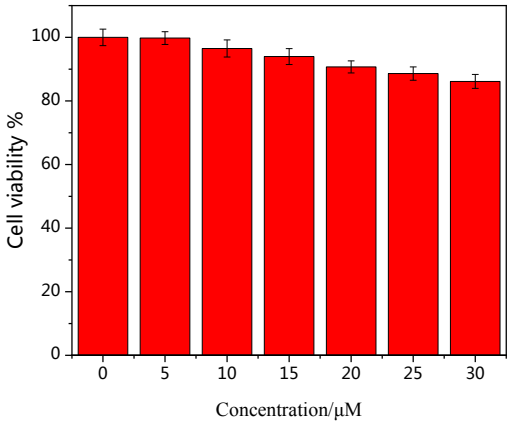


**Fig. S2.** Cell viability values (%) estimated by MTT proliferation test versus concentrations of CA-$SO_2$. A549 cells were cultured in the presence of 0-30 μM CA-$SO_2$.

## 5. Characterization of compounds

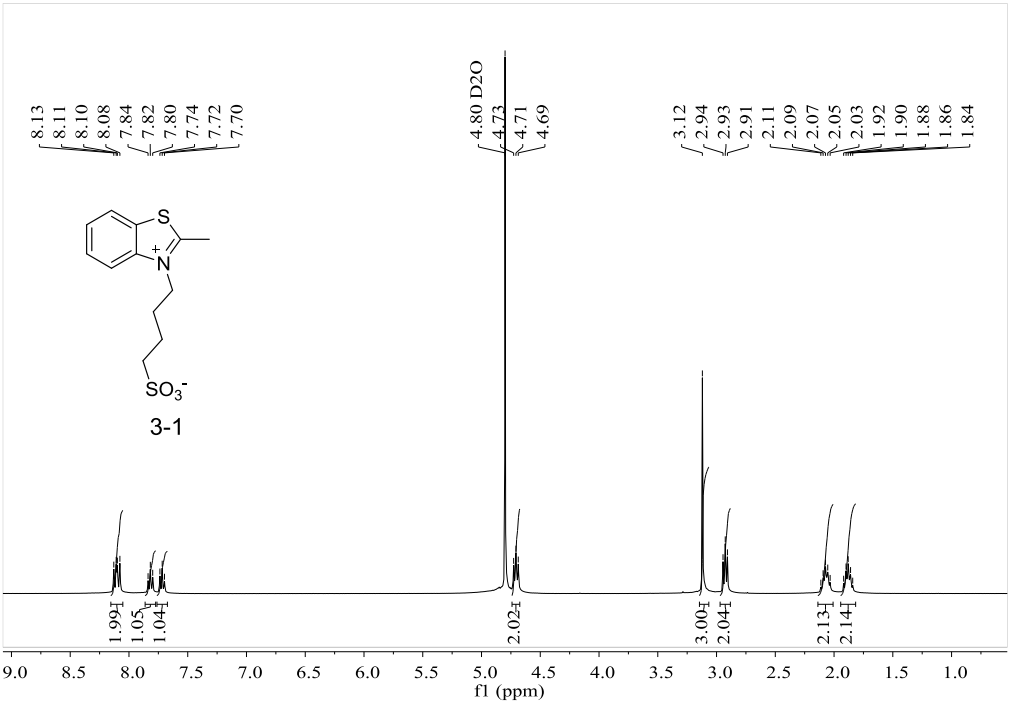


**Fig. S3** $^1$H NMR spectrum of compound 3-1 in $D_2O$

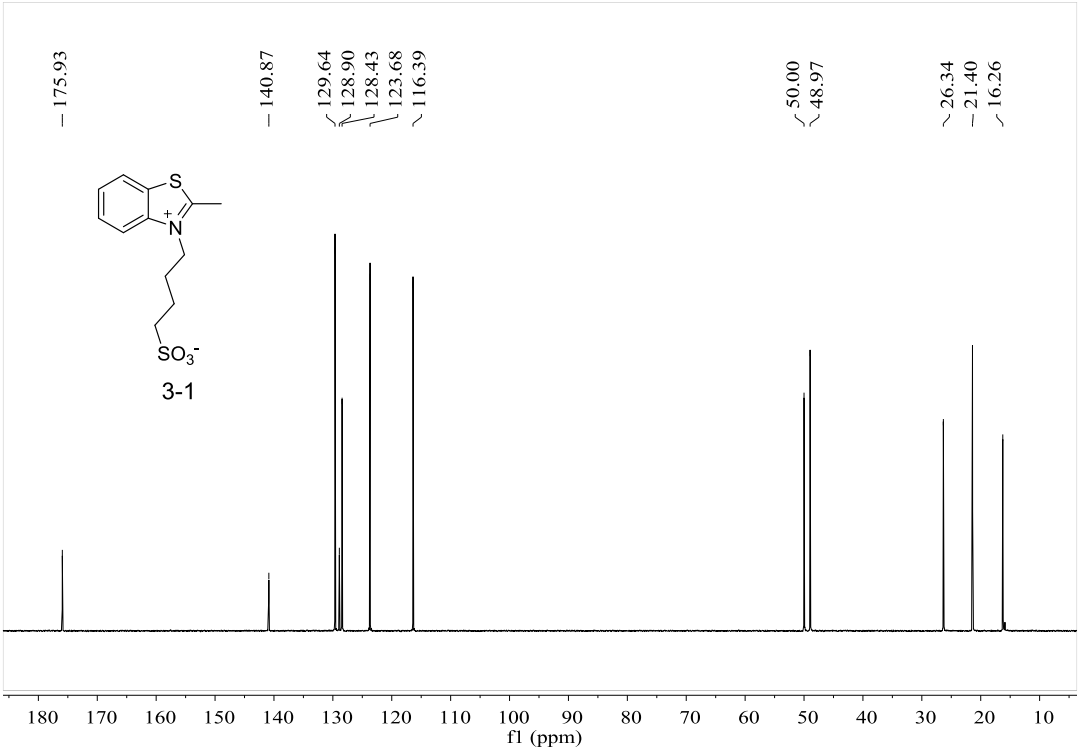


**Fig. S4** $^{13}$C NMR spectrum of compound 3-1 in $D_2O$

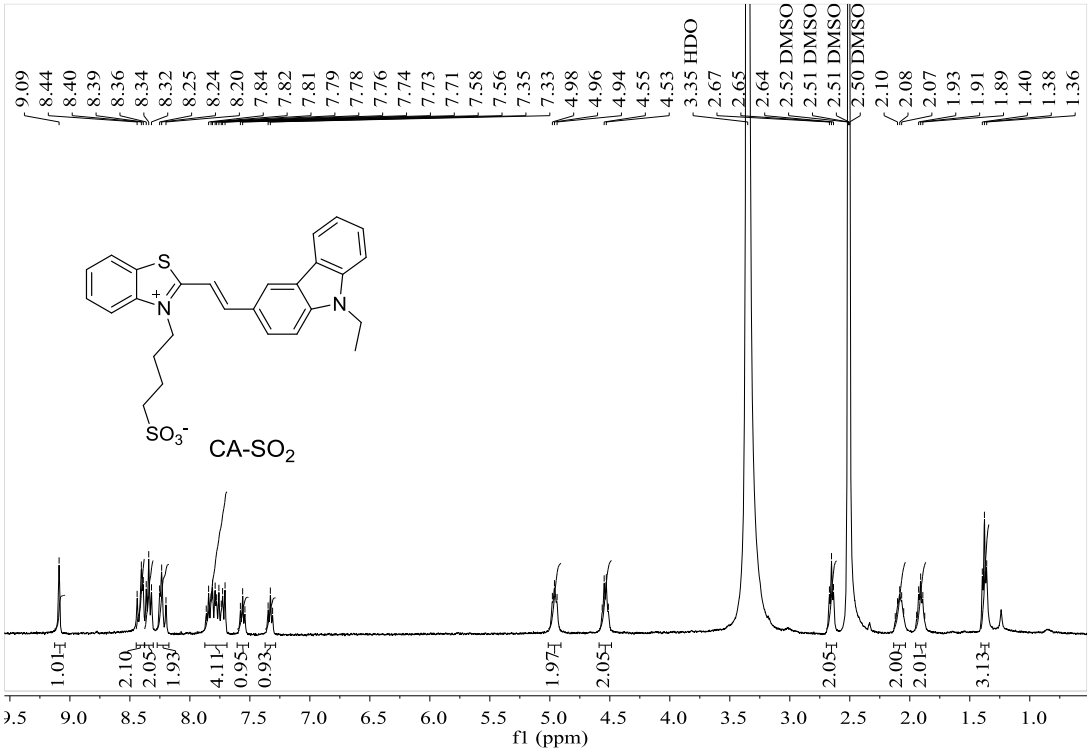


**Fig. S5** $^{1}$H NMR spectrum of CA-$SO_2$ in DMSO-$d_6$

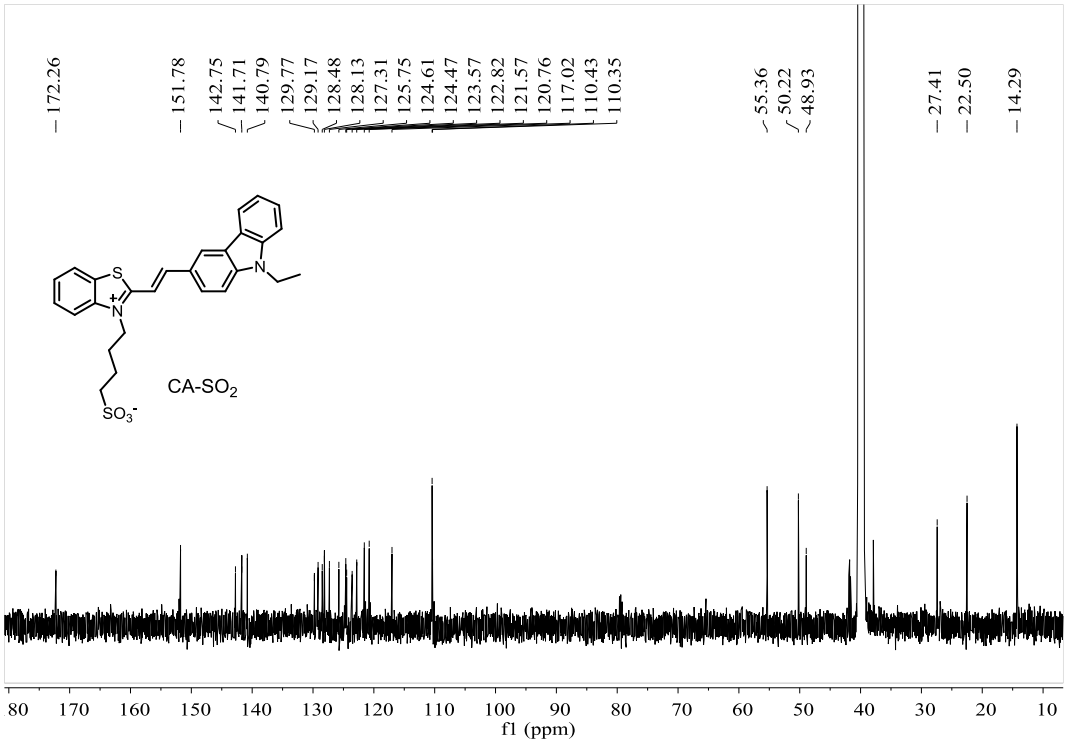


**Fig. S6** $^{13}$C NMR spectrum of CA-$SO_2$ in DMSO-$d_6$

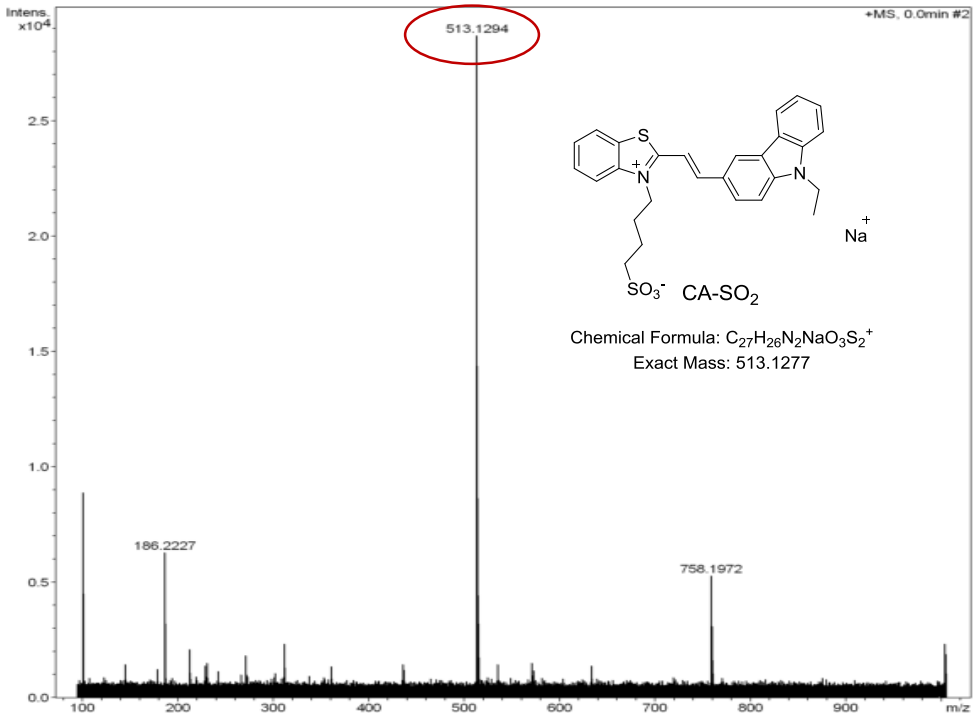


**Fig. S7** HRMS spectrum of CA-$SO_2$