Supporting information for

Chromenoquinoline-based two-photon fluorescent probe for highly specific and ultrafast visualizing sulfur dioxide derivatives in living cells and zebrafish

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Materials and instruments. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. The fluorescence imaging of cells was performed with OLYMPUS FV1000 (TY1318) confocal microscopy. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the fluorescence quantum yield[1-3]: Fluorescence quantum yields for chromenoquinoline **1** and **CQ-SO2** were determined by using rhodamine 6G (Φ_f = 0.95 in H_2O) as a fluorescence standard. The quantum yield was calculated using the following equation:

$$\Phi_{F(X)} = \Phi_{F(S)} \, (A_S F_X / A_X F_S) \, (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts $_S$ and $_X$ refer to the standard and to the unknown, respectively.

References

1. B. Valeur, Molecular Fluorescence: Principles and Applications, Wiley-VCH, 2001.
2. D. Magde, G. E. Rojas and P. Seybold, *Photochem. Photobiol.*, 1999, **70**, 737.
3. D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano and T. Nagano, *J. Am. Chem. Soc*., 2010, **132**, 2795.

Measurement of two-photon cross-sections. The two-photon cross-section (σ) was determined by using a femtosecond (fs) fluorescence measurement technique. Chromenoquinoline **1** was dissolved in EtOH and PBS, at a concentration of 5.0× 10^{-5} M, and then the two-photon fluorescence was excited at 700-900 nm by using fluorescein in pH = 11 aqueous solution (σ = 32 GM in 810 nm) as the standard, whose two-photon property has been well characterized in the literature. The two-photon cross-section was calculated by using $\sigma = \sigma_r(F_t n_t^2 \Phi_r C_r)/(F_r n_r^2 \Phi_t C_s)$, where the subscripts t and r stand for the sample and reference molecules. *F* is the average fluorescence intensity integrated from two-photon emission spectrum, n is the refractive index of the solvent, *C* is the concentration, Φ is the quantum yield, and σ_r is the two-photon cross-section of the reference molecule.

HeLa cell culture and imaging using CQ-SO_2. HeLa cells were seeded in Dulbecco's modified Eagle'smedium (DMEM) supplemented with 10% fetal bovine serum for 24 h. Hella cells were incubated with 5.0 μM **CQ-SO_2** for 30 min, and then treated with exogenous HSO_3^- at 20 mM in the culture medium for 30 min at 37 °C. After washing with PBS three times to remove the remaining HSO_3^-. the cells were imaged using OLYMPUS FV1000 (TY1318) confocal microscopy with an excitation filter of 405 nm or 800 nm.

Cytotoxicity assays. The toxicity of **CQ-SO_2** towards living HeLa cells was determined by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assays. HeLa cells were grown in the modified Eagle's medium (MEM) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 ºC. Immediately before the experiments, the cells were placed in a 96-well plate, followed by addition of increasing concentrations of probe **CQ-SO_2** (99% MEM and 1% DMSO). The final concentrations of **CQ-SO_2** was 0, 5, 10, 20, 50, 100 μM (n = 5), respectively. The cells were then incubated at 37 ºC in an atmosphere of 5% CO_2 and 95% air at 37 ºC for 24 h, followed by MTT assays. Untreated assay with MEM (n = 5) was also conducted under the same conditions.

Fluorescence imaging in living zebrafish.

Wild type zebrafish were purchased from the Nanjing EzeRinka Biotechnology Co. Ltd. Animal handling procedures were approved by the Animal Ethics Committee of Guangxi Normal University (No. 20150325-XC). The zebrafish were kept at 28 °C and optimal breeding conditions. The zebrafish then incubated with $NaHSO_3$ (30 μM) in the culture medium for 30 min at 28 °C. After washing with PBS three times to remove the remaining $NaHSO_3$. Then, the zebrafish were further incubated with probe **CQ-SO₂** (10 μM) for 30 min at 28°C. The imaging experiments were recorded with a 10x objective lens using OLYMPUS FV1000 (TY1318) confocal microscopy. The fluorescence emission was collected at green channel when excitation at 800 nm.

Synthesis of intermediate 1

In a 50 ml round bottomed flask were added 3-bromoprop-1-yne (2.60 g, 22.0 mmol), 4-(diethylamino)-2-hydroxybenzaldehyde (3.86 g, 20.0 mmol), K_2CO_3 (3.04 g, 22.0 mmol) and then was dissolved in DMF (15.0 ml), the reaction mixture was stirred at room temperature for 10 h. After completion of the reaction, the reaction mixture was poured on cold water (50 ml) and extracted with DCM (15 ml x 3). The combined organic layer was washed with water (50 ml x 3). The solvent was removed under reduced pressure to obtain a brown residue which was purified by column chromatography over silica gel to obtain the pure **intermediate 1** (3.97 g, 85.8%) as a sandy beige solid. ^{1}H NMR (400 MHz, $CDCl_3$) 10.13 (s, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 6.33 (dd, *J* = 8.8Hz, 1H), 6.23 (d, *J* = 2.3 Hz, 1H), 4.80 (d, *J* = 2.4 Hz, 2H), 3.43 (q, *J* = 7.1 Hz, 4H), 2.58 (t, *J* = 2.4 Hz, 1H), 1.23 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) 185.92, 160.99, 152.53, 129.64, 113.62, 104.08, 93.37, 77.26，75.15, 55.09, 43.94, 11.60. MS (ESI) m/z =232.1 $[M+H]^+$; HRMS (ESI) Calcd for $C_{14}H_{18}NO_2^+$ ($[M+H)^+$: 232.1338, Found 232.1326.

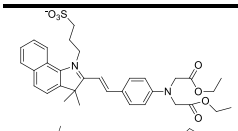
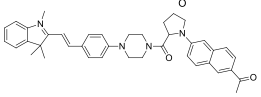
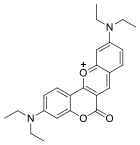
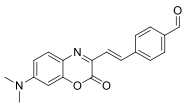
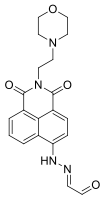
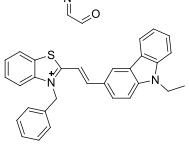
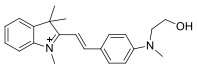
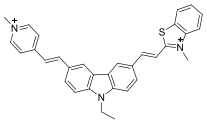
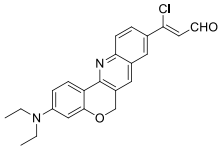
Synthesis of chromenoquinoline 1

In a 25 ml round bottomed flask placed under nitrogen atmosphere were added **intermediate 1** (231 mg, 1.0 mmol), 4-aminoacetophenone (135 mg, 1.0 mmol), and CuCl (29 mg, 0.3 mmol) was dissolved in dry DMF (5.0 ml), the reaction mixture was stirred at 110 °C for 5 h. After completion of the reaction, the reaction mixture was poured on cold water (20 ml) and extracted with DCM (10 ml x 3). The combined organic layer was washed with water (20 ml x 3). The solvent was removed under reduced pressure to obtain a black residue which was purified by column chromatography over silica gel to obtain the **chromenoquinoline 1** (129.8 mg, 37.5 %) as a straw yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37-8.26 (m, 2H), 8.17 (dd, *J* = 8.8, 1.9 Hz, 1H), 8.12-8.04 (m, 1H), 7.82 (s, 1H), 6.51 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.22 (d, *J* = 2.5 Hz, 1H), 5.28 (s, 2H), 3.49-3.36 (q, *J* = 7.1 Hz, 4H), 2.70 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) 197.40, 159.65, 151.82, 133.28, 131.62, 129.36, 128.74, 127.90, 127.35, 125.40, 107.20, 98.01, 68.41, 44.67, 26.70, 12.71. MS (ESI) m/z =347.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{22}H_{23}N_2O_2^+$ ($[M+H]^+$): 347.1760, Found, 347.1746.

Synthesis of probe CQ-SO_2

Phosphorous oxychloride ($POCl_3$) (5 mL) was slowely added to dimethylformamide (DMF) (5 mL) at 0-5 °C under the stirring about half an hour. Then the **chromenoquinoline 1** (35 mg)by dissolving it into DMF (3 mL) was added to this cooled system under the stirring for 3 h, and the reaction mixture was subsequently heated at 75 °C for 6 h. The reaction mixture was cooled to room temperature and then poured into ice cold water (30 mL). Then the reaction system was neutralised with sodium carbonate and subsequently was filtered and washed with cold water, dried and crystallised from ethanol to give brick-red solid probe **CQ-SO_2** (21 mg, 53 %). ^{1}H NMR (400 MHz, $CDCl_3$) 10.20 (d, *J* = 6.8 Hz, 1H), 8.22 (d, *J* = 8.9 Hz, 1H), 8.11 (d, *J* = 2.1 Hz, 1H), 8.00 (d, *J* = 8.7 Hz, 1H), 7.84 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.73 (s, 1H), 6.74 (d, *J* = 6.8 Hz, 1H), 6.45 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.16 (d, *J* = 2.4 Hz, 1H), 5.22 (s, 2H), 3.35 (t, *J* = 7.1 Hz, 4H), 1.16 (q, *J* = 7.1, 5.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) 190.38, 158.84, 150.58, 130.24, 126.71, 125.66, 125.10, 124.74, 123.14, 106.19, 96.99, 67.38, 43.65, 11.68. MS (ESI) m/z =393.1 $[M+H]^+$. HRMS (ESI) Calcd for $C_{23}H_{22}ClN_2O_2^+$ ($[M+H]^+$): 393.1370, Found, 393.1353.

Table S1. Comparison of some two-photon fluorescent probes for selective detection of SO_2 derivatives.

Probes	Detection limit	Living cell Imaging	Living Animals Imaging	Response time	Reference
	0.11 μM	endogenous	no	5 min	Sensors and Actuators B 2018, 255, 1228-1237
	50 nM	endogenous	no	3 min	Chem. Commun., 2016, 52, 10289-10292; Anal. Chim. Acta, 2016, 937, 136-142
	0.3 μM	exogenous	yes	5 seconds	Anal.Chem., 2017, 89, 9388-9393
	1.86 μM	exogenous	no	4.5 min	J. Mater. Chem. B, 2016, 4, 7888-7894
	3.75 μM	exogenous	yes	15 seconds	Sensors and Actuators B 2018, 268, 157-163
	0.16 μM	endogenous	yes	35 seconds	Sensors and Actuators B 2018, 254,709-718
	0.16 μM	exogenous	no	No data	Sensors and Actuators B 2016, 233, 1-6
	2.21 nM	endogenous	no	10min	Dyes and Pigments 2016, 134, 297-305
	16 nM	endogenous	yes	5 seconds	This work

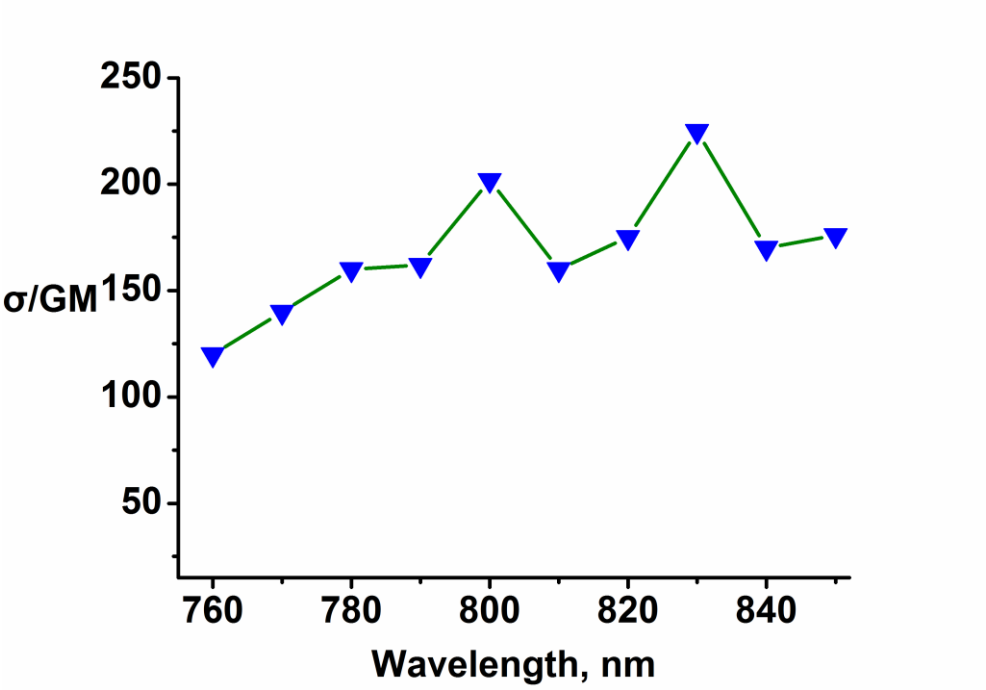


Figure S1. Two-photon action cross-sections of chromenoquinoline **1** in EtOH.

Table S2. Photophysical data of chromenoquinoline **1** in diverse solvents.

	DMSO	DMF	CH_3CN	EtOH	PBS
$\lambda_{max\ (nm)}$	423	418	414	418	422
$\lambda_{em\ (nm)}$	536	526	525	541	556
Stokes Shifts (nm)	113	108	111	123	134
Φ	0.81	0.65	0.65	0.46	0.16
ε_{max}	1.68×10^4	2.03×10^4	2.23×10^4	2.00×10^4	1.12×10^4

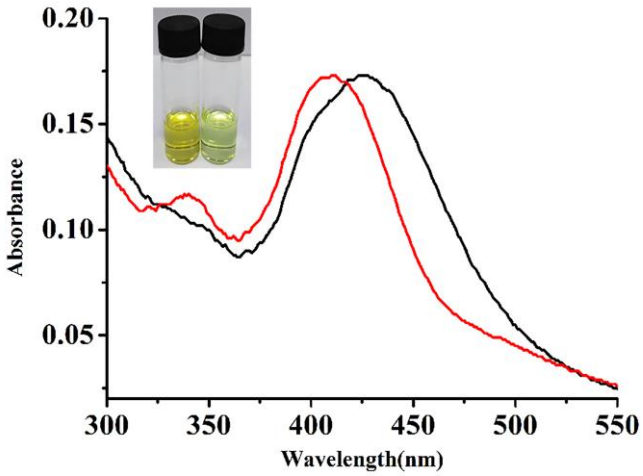


Figure S2. Absorption spectra of the probe **CQ-SO$_2$** (5 μM) in the absence (■) and presence (●) of SO_2 derivatives (25 μM) in PBS buffer (pH 7.4, 25 mM, containing 20% DMSO as a cosolvent). Insets: images of aqueous probe **CQ-SO$_2$** before (left) and after (right) treatment with $NaHSO_3$.

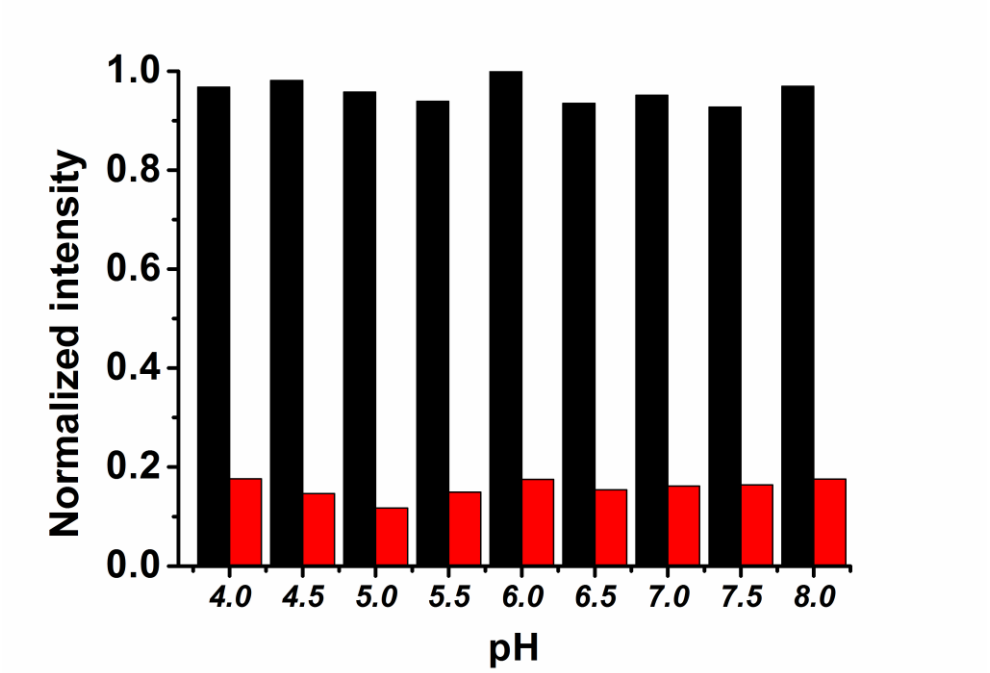


Figure S3. The pH effects of probe **CQ-SO$_2$** in the absence (●) or presence (■) of SO_2 derivatives, excitation at 405 nm and emission at 520 nm.

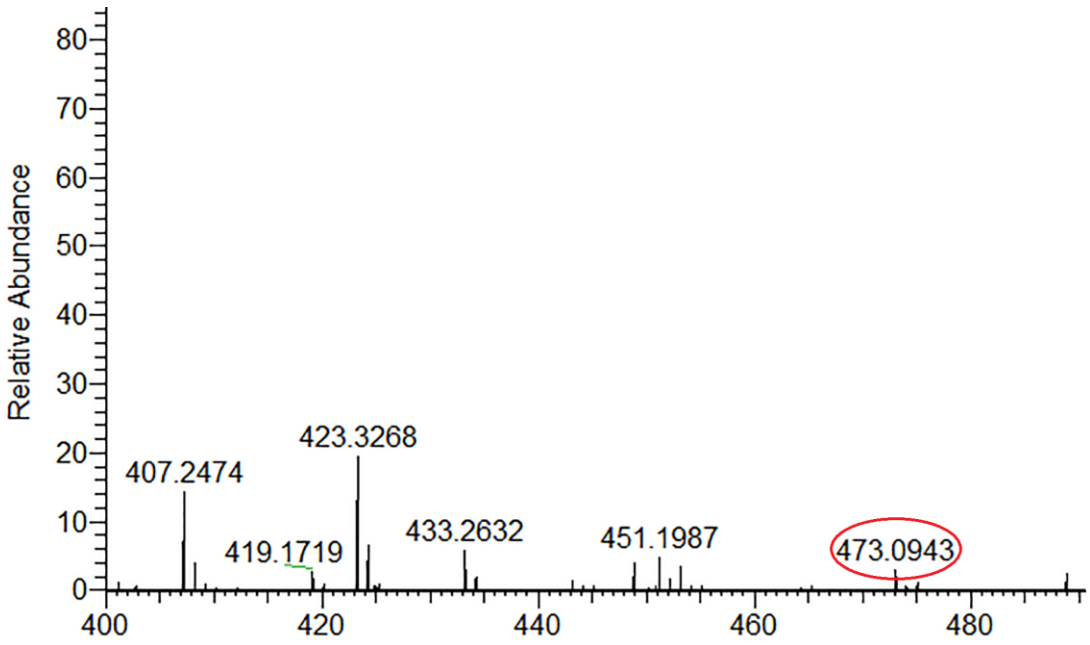
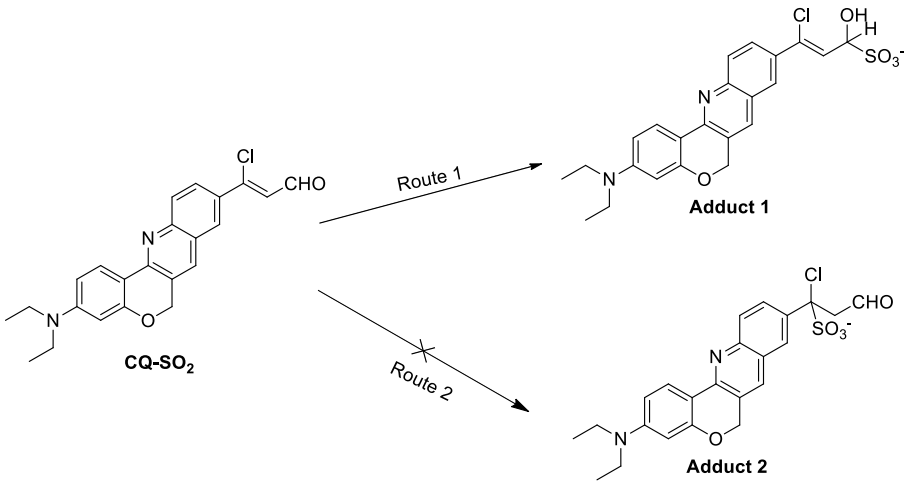


Figure S4. HRMS (ESI) of the reaction mixture of **CQ-SO$_2$** towards SO_2 derivatives.



Scheme S1. The two proposed response mechanism of probe **CQ-SO$_2$** towards SO_2 derivatives.

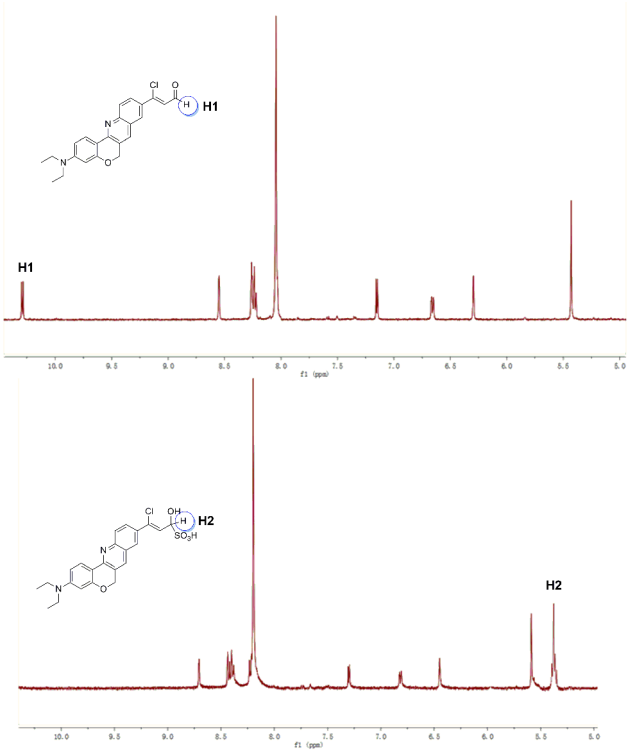


Figure S5. ^{1}H-NMR comparison of the probe **CQ-SO$_2$** with the product upon addition of HSO_3^- in DMSO-*d*6 and D_2O (V/V=7:3).

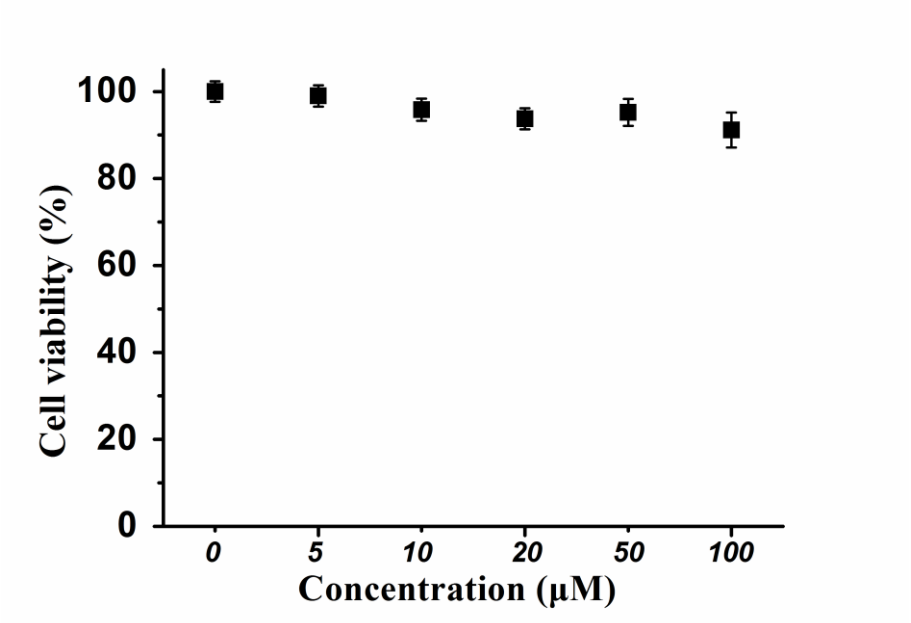


Figure S6. Cytotoxicity of the probe **CQ-SO$_2$** on Hella cells determined by MTT.

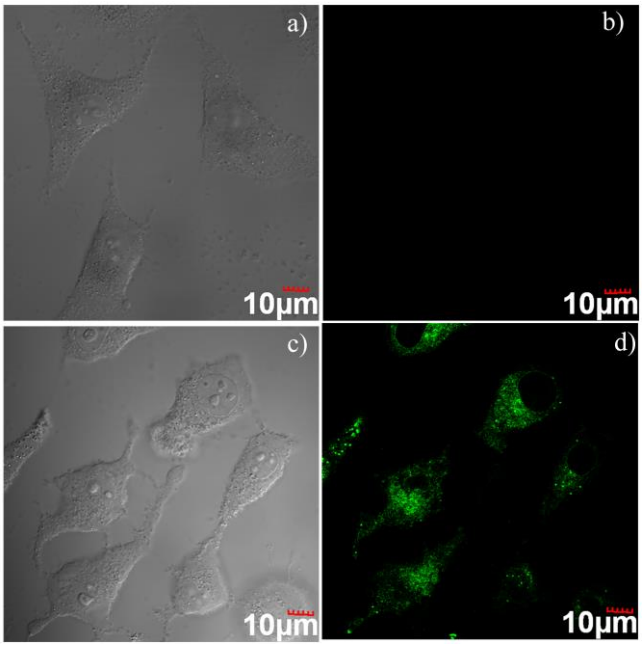


Figure S7. One-photon fluorescence images of Hella cells. (a-b) The cells incubated with the probe **CQ-SO₂** (5 μM) for 30 min: (a) Bright field image and (b) fluorescent image generated from green channel; (c-d) The cells incubated with the probe **CQ-SO₂** (5 μM) for 30 min after pre-incubation with 20 μM $NaHSO_3$ for 30 min: (c) Bright field image and (d) fluorescence image generated from green channel. Excitation at 405 nm. Scale bar =10 μm.

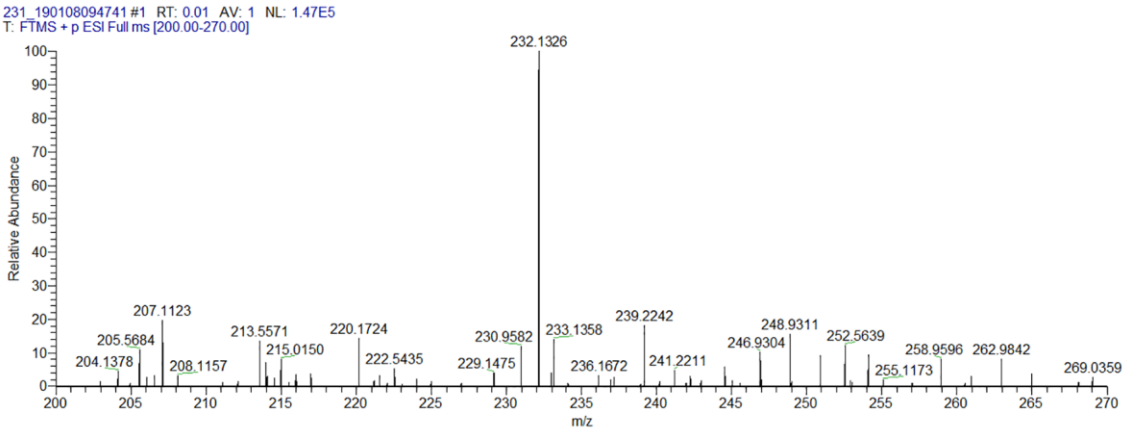


Figure S8. The HRMS of **intermediate 1.**

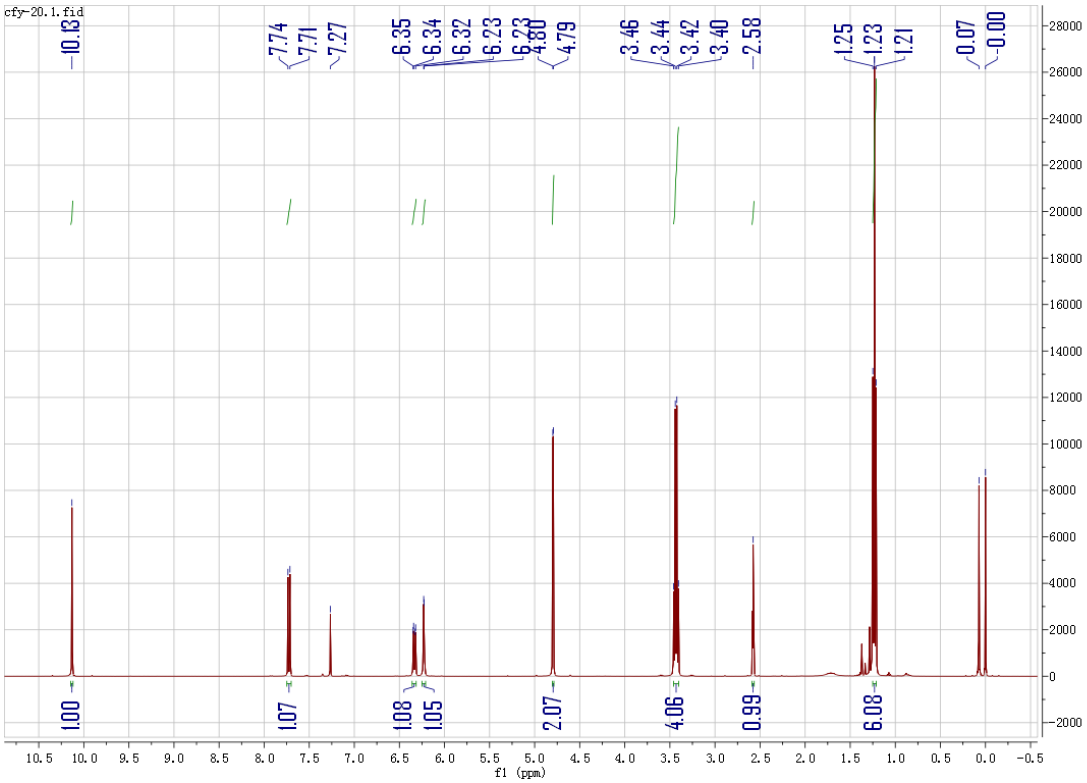


Figure S9. The ^{1}H NMR of **intermediate 1.**

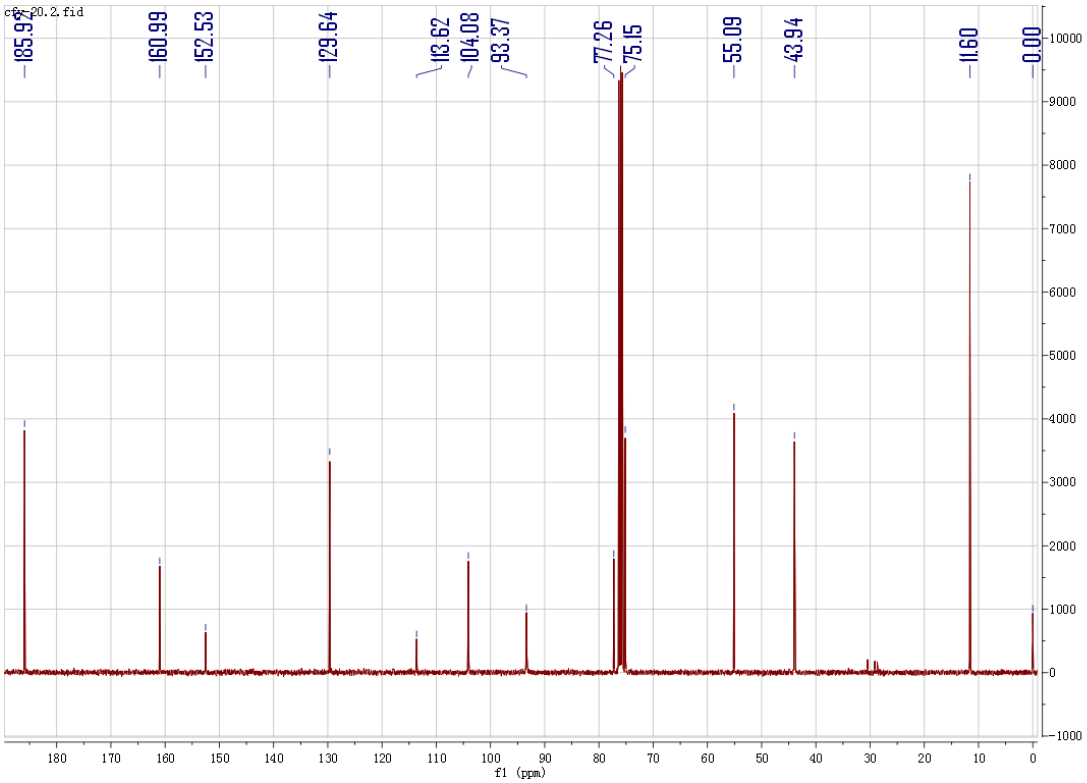


Figure S10. The ^{13}C NMR of **intermediate 1.**

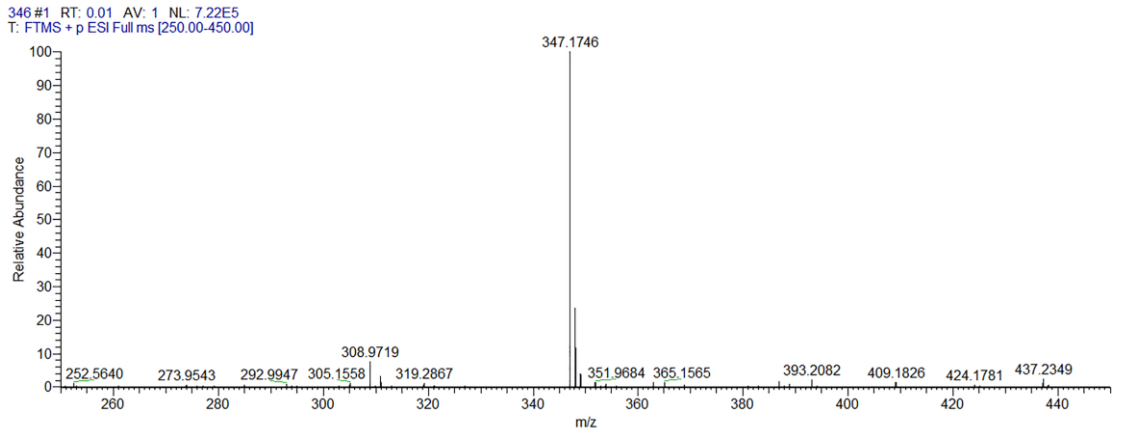


Figure S11. The HRMS of chromenoquinoline **1**.

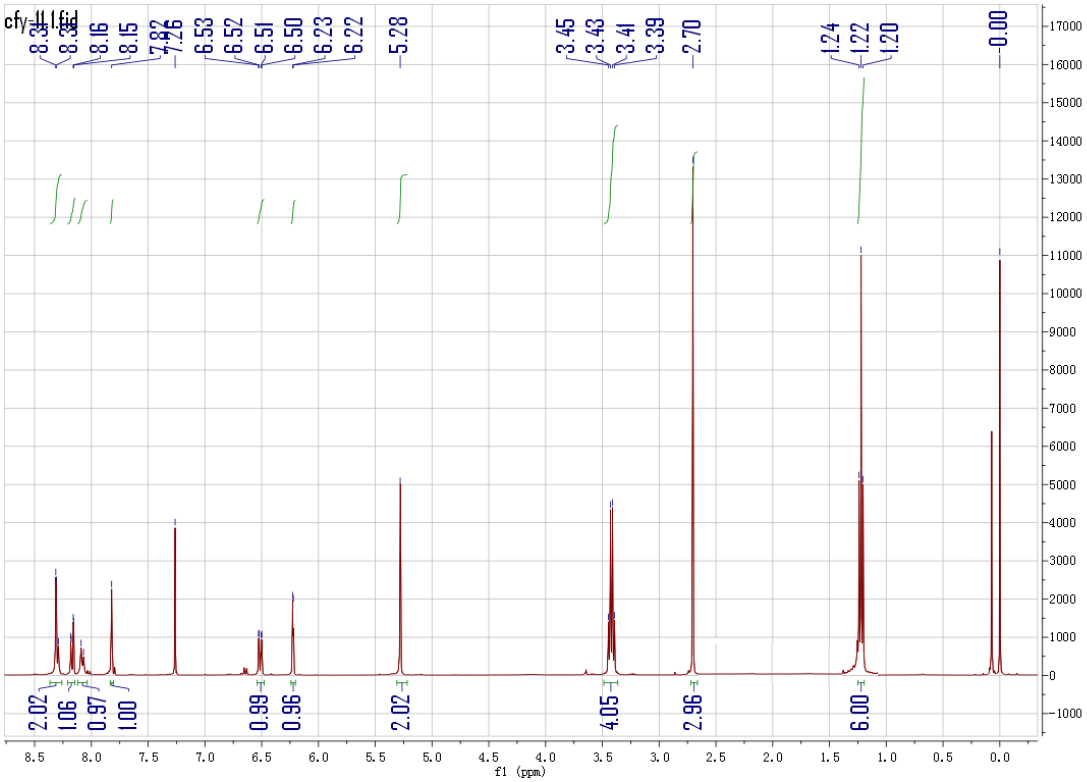


Figure S12. The ^{1}H NMR of chromenoquinoline **1.**

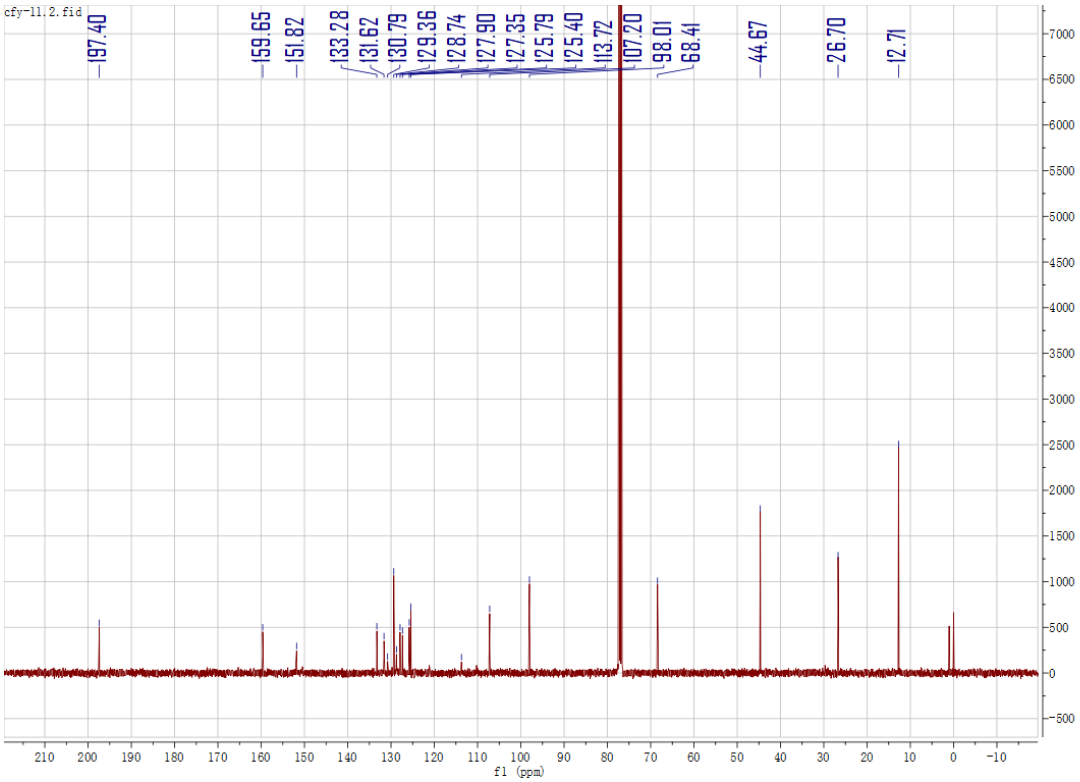


Figure S13. The ^{13}C NMR of chromenoquinoline **1.**

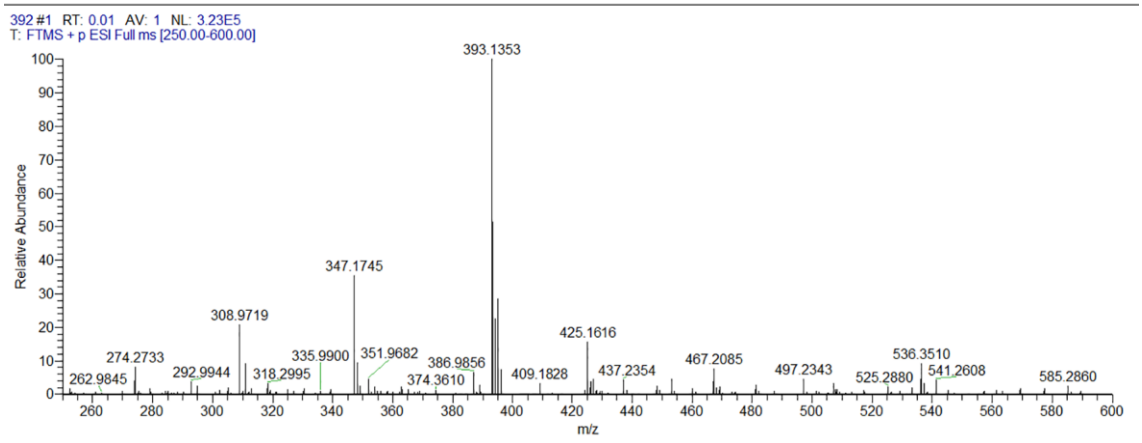


Figure S14. The HRMS of probe **CQ-SO$_2$**.

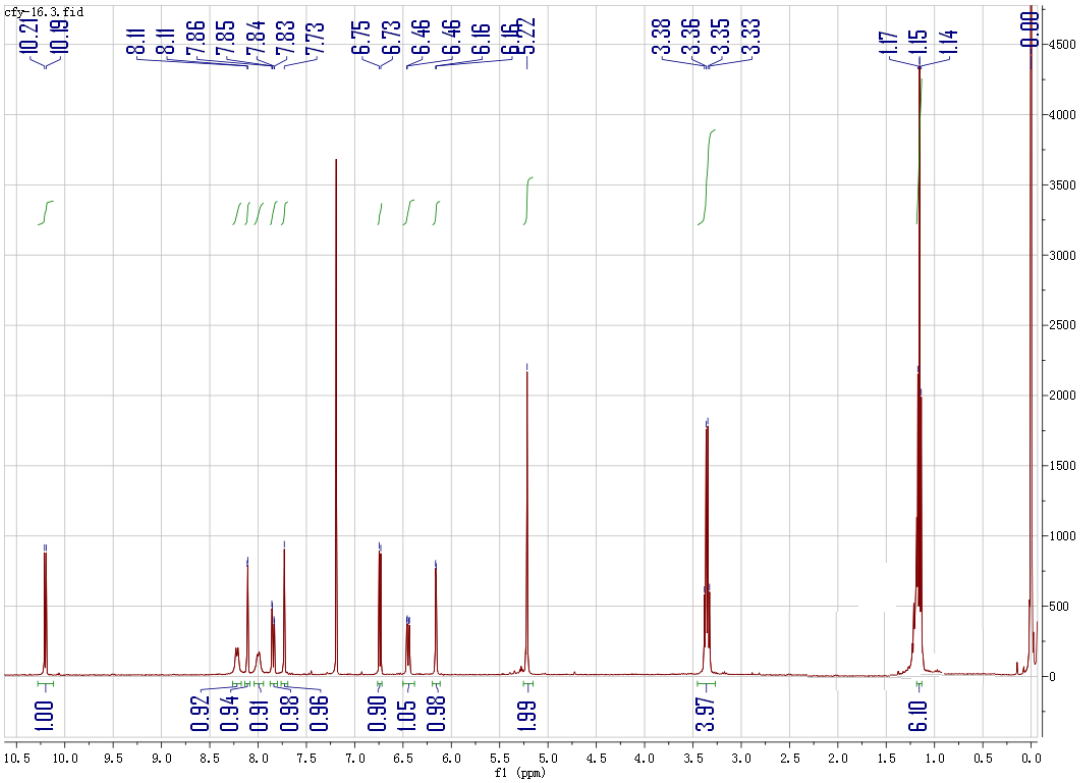


Figure S15. The ^{1}H NMR of probe **CQ-SO$_2$.**

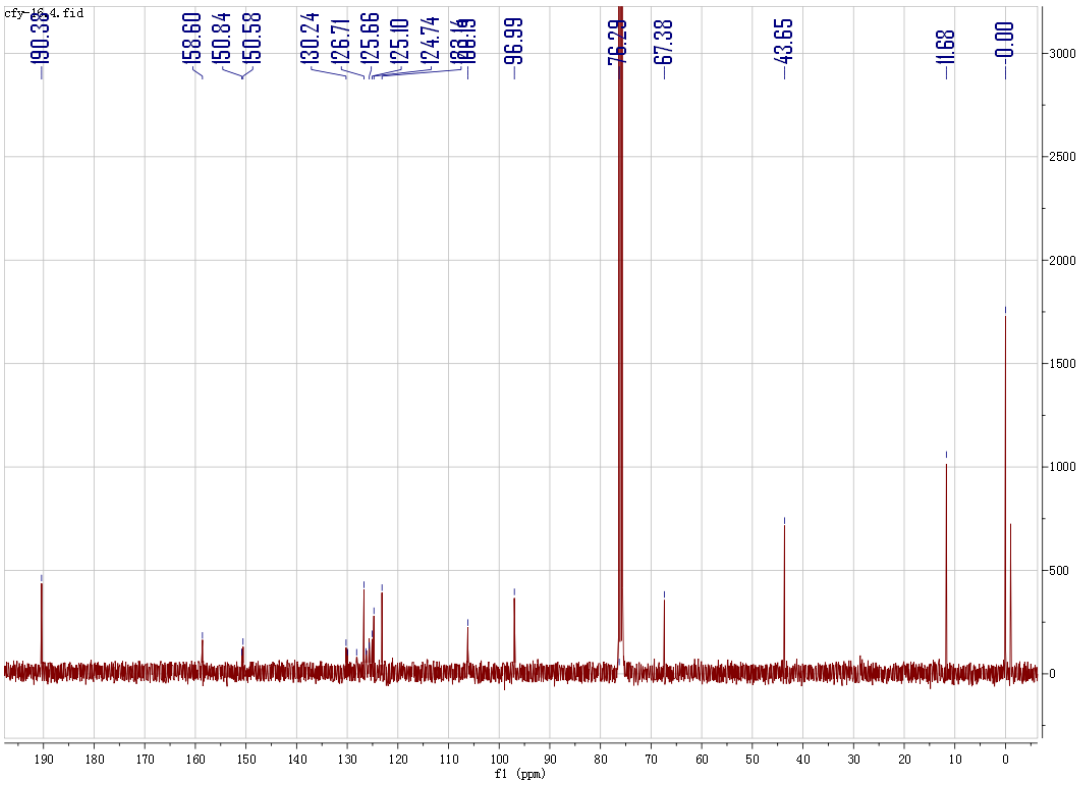


Figure S16. The ^{13}C NMR of probe **CQ-SO$_2$.**