Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information for

A Novel Dual-functional Fluorescent Probe for Imaging Viscosity and Cysteine in Living System

Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering,

Zhejiang University of Technology, Hangzhou 310014, P. R. China

Email: zhuq@zjut.edu.cn.

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Experimental section

Materials and apparatus

All chemicals were commercially available from Energy Chemical and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Avance 500 MHz spectrometers. The spectra were reported in ppm (δ) and referenced to a tetramethylsilane (TMS) standard in $CDCl_3$, DMSO-d_6. Thin layer chromatography (TLC) for reaction monitoring was performed on pre-coated silica gel plates (Merck 60 F254 nm) with a UV254 fluorescent indicator and column chromatography was conducted over silica gel (mesh 300-400). The fluorescence and UV–vis spectra were acquired on a SpectraMax M5 (Molecular Devices).

General procedure for viscosity analysis

The parent stock solution was prepared by dissolving probe **FCV** (5 mM) in DMSO. The test solution was prepared by diluting **FCV** to PBS/ Glycerol solutions (5 μM, 1.0 mL). The viscosity solution was prepared by mixing PBS and Glycerol in different proportion. These solutions were ultrasonically treated for 30 minutes. Then, the data of fluorescence spectrum was recorded under the excitation of 500 nm, and the slit width were set at 5 nm. The relationship between the fluorescence emission intensity of the probe and the viscosity of the solvent is well expressed by the Forster Hoffmann equation as follows:

$$\text{Log (If)} = c + x \log \eta$$

Where if is the fluorescence intensity, η is the viscosity of the solution, and X and C are constant.

Determination of the lowest detection limit LOD

The detection limit (LOD) of probe **FCV** for hydrogen sulfide was determined by fluorescence titration. The equation is as follows:

$$LOD = 3\delta/m$$

Where LOD is the lower detection limit, δ is the standard deviation of the background (obtained by measuring the fluorescence emission intensity of **FCV** solution of 20 probes and calculating the standard deviation), and M is the sensitivity (slope of the linear curve between the fluorescence intensity and the concentration of hydrogen sulfide).

Cellular imaging

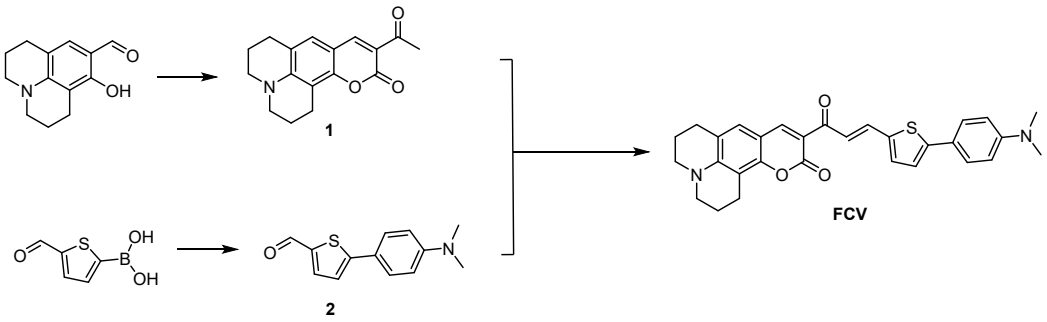
To prepare the **FCV** solution, stock solutions of Lyso-BTC (DMSO, 10 mM) were prepared. 100 μL of stock solution of **FCV** (DMSO, 10 mM) was added dropwise into 9.9 mL PBS under ultrasonic conditions to obtain the sample solution for cellular imaging.

Cells were cultured in DMEM containing 10% fetal bovine serum, 1% penicillin, and 1% streptomycin at 37 °C (w/v) in a 5% CO_2 and 95% air incubator MCO-15AC (Sanyo, Tokyo, Japan). The concentrations of counted cells were adjusted to 1×10^6 cells mL^{-1} for confocal imaging in high-glucose DMEM (4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2.0 ng/L), and 1% antibiotics (penicillin/streptomycin, 100.0 U/mL). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2.

Imaging in zebrafish

We incubated 3-day-old zebrafish with **FCV** (10 μM) for 30 min, then washed with PBS buffer and imaged as control group. Another group treated with LPS (2 μg/L) for 4 h, and then incubated with **FCV** (10 μM) for 30 min. Thereafter, the treated zebrafish

was washed with PBS buffer three times and imaged using a confocal microscope. Fluorescence images were acquired with Nikon A1R confocal microscope.



Scheme S1. Synthesis route of **FCV**.

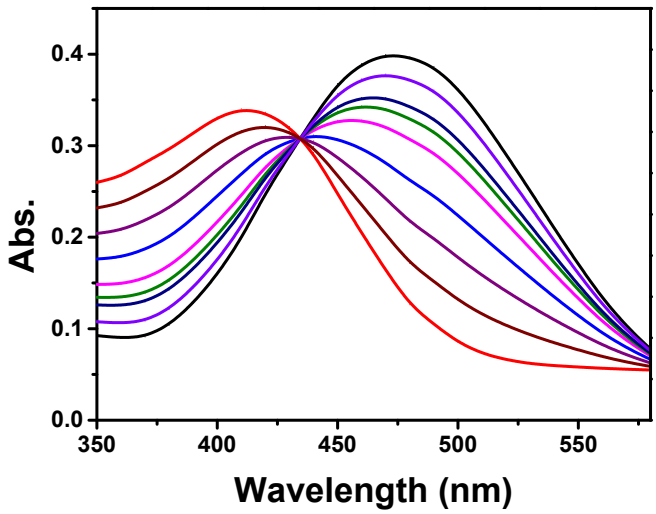


Fig. S1. Absorbance spectra of the probe **FCV** (5 μM) in DMSO/PBS = 1:99 (v/v = 1:1, pH = 7.4) buffer with different concentration of Cys.

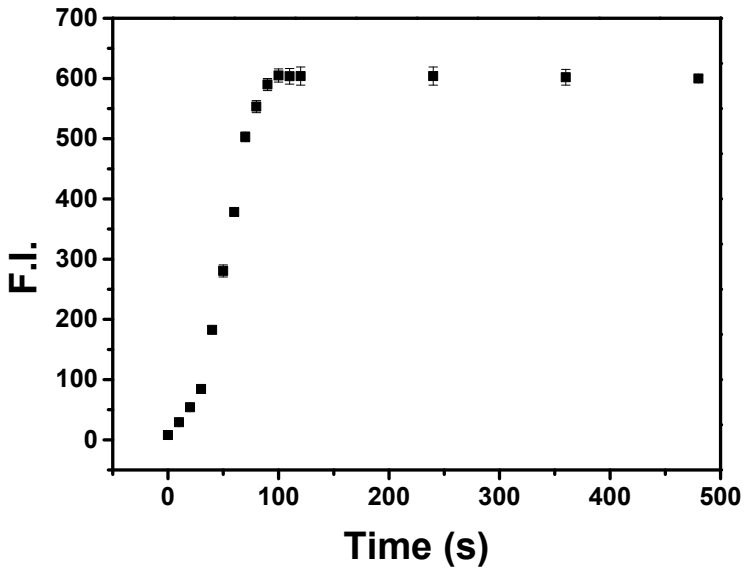


Fig. S2. Time course of the probe **FCV** fluorescence upon the addition of Cys (20 μM).

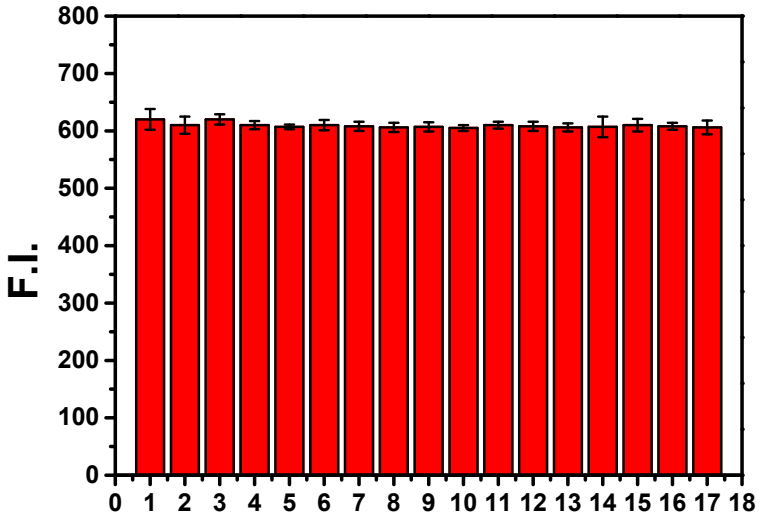


Fig. S3. The fluorescent responses of probe **FCV** (5.0 μM) to Cys (20.0 μM) in the presence of various relevant species (1, Serine; 2, Asparticacid; 3, Arginine; 4, Alanine; 5, Lysine; 6, Glutamicacid; 7, TBHP; 8, H_2O_2; 9, NO; 10, Glutamicacid; 11, Tyrosine; 12, Histidine; 13, Tryptophan; 14, Valine, 15, GSH; 16, Homocysteine; 17, Cysteine, 100 μM) in DMSO/PBS buffer (1:99, v/v, pH 7.4) at 37 °C.

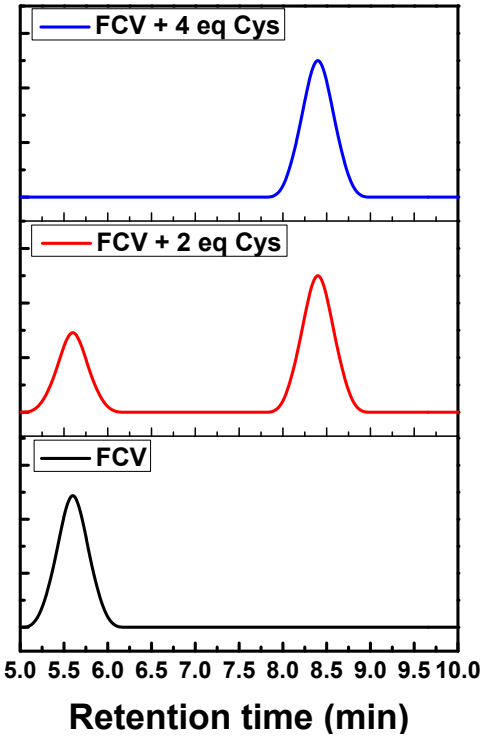


Fig. S4. HPLC results for probe **FCV** (5.0 μM, black line), probe **FCV** (5.0 μM, red line) incubated with cysteine (2 eq., 10.0 μM) for 30 min, probe **FCV** (5.0 μM, blue line) incubated with cysteine (4 eq., 20.0 μM) for 30 min. The HPLC method for the determination of the above-mentioned analytes with isocratic elution was established, and the mobile phase is 80% methanol and 20% water.

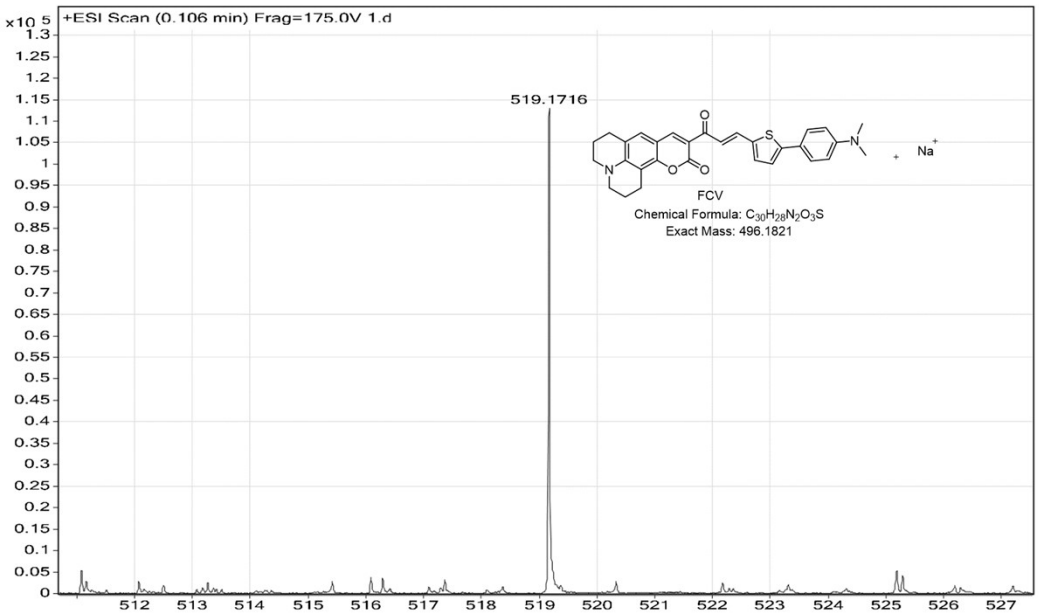


Fig. S5 HRMS spectrum of **FCV**.

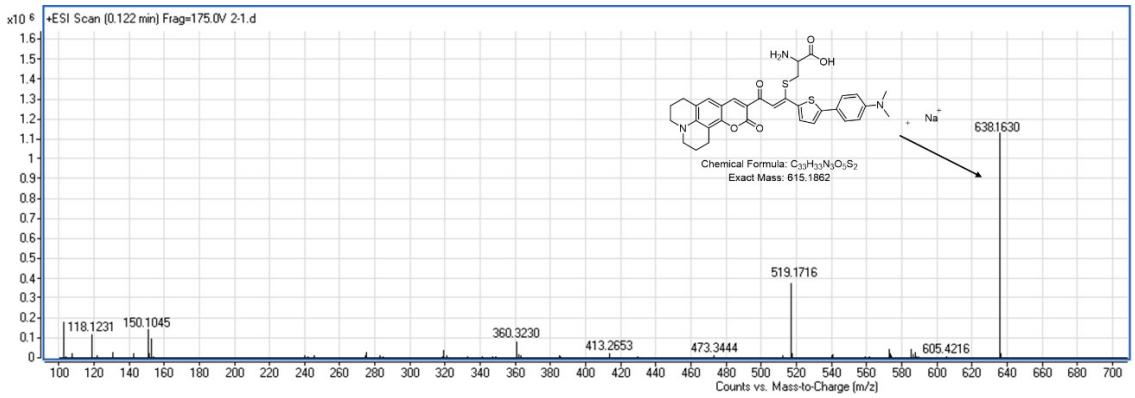


Fig. S6 In situ HRMS spectrum of **FCV** after adding Cys.

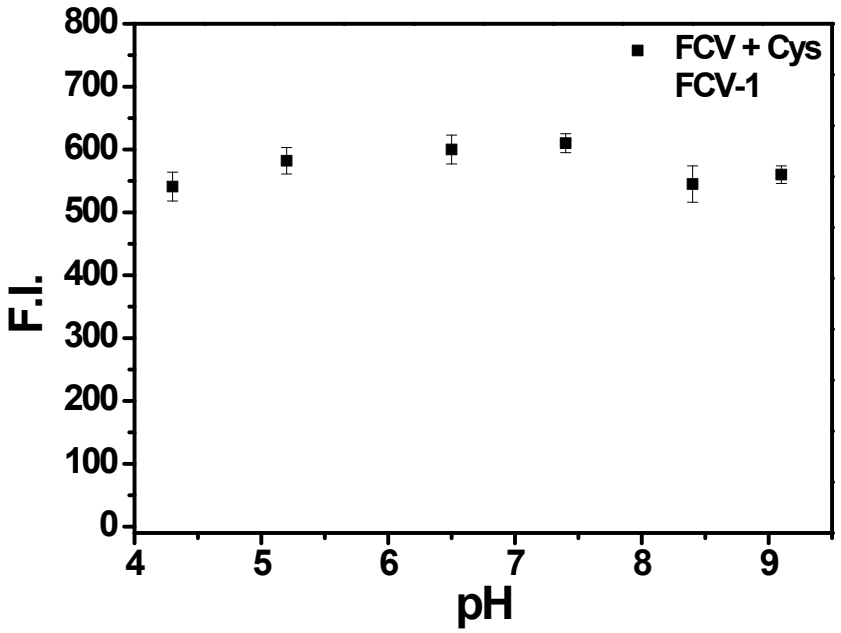


Fig. S7. The effect of pH on the fluorescence intensity changes of **FCV-1** (5 μM).

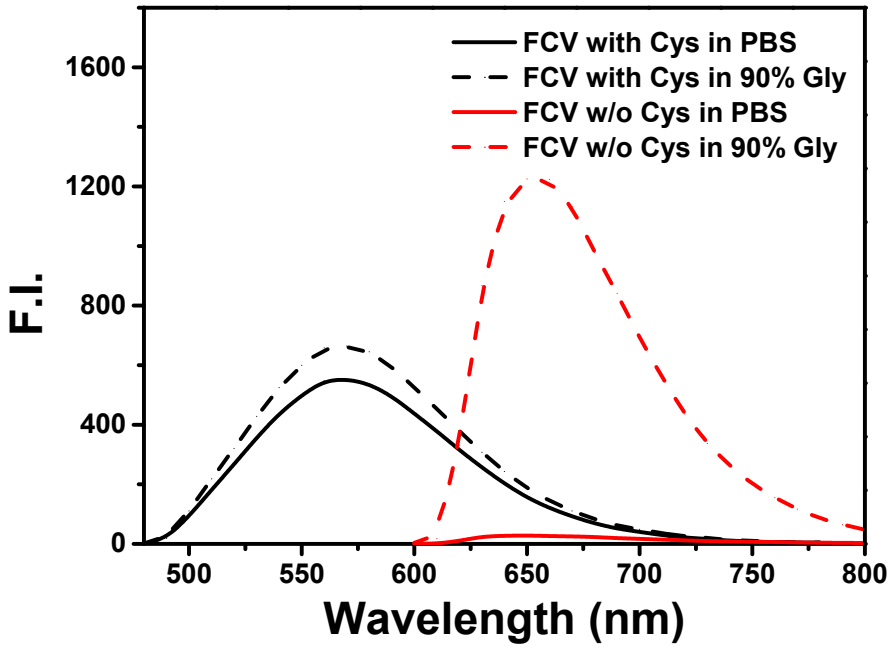


Fig. S8. The fluorescent spectra of **FCV** (5.0 μM) in the absence and presence of Cys in PBS solution (5 mM, ~0 CP, pH=7.4) and 90% Gly solution (~900 CP).

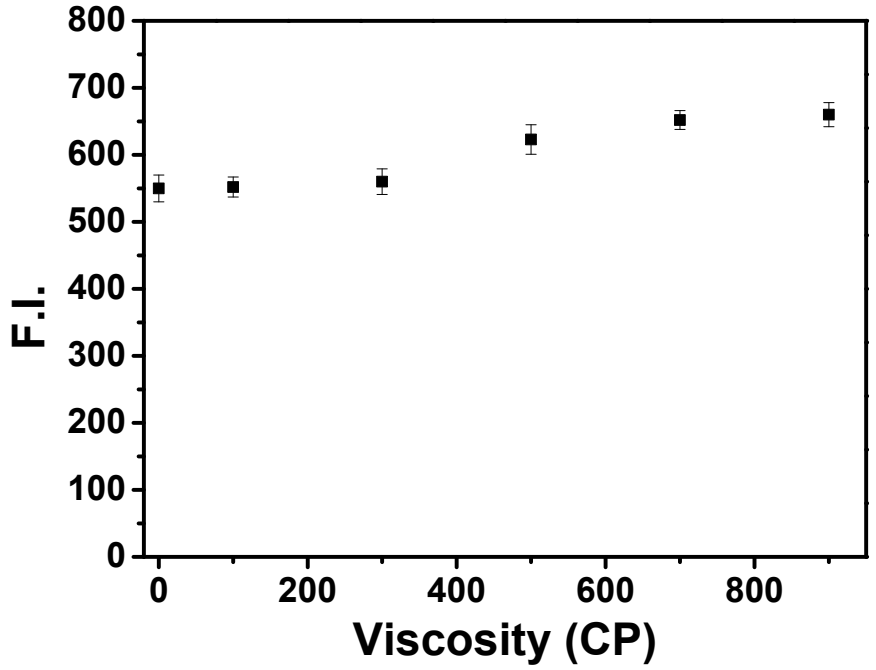


Fig. S9. The fluorescent intensity of **FCV-1** (5.0 μM) in varies viscosity environments provided by PBS–glycerol (0%, 10%, 30%, 50%, 70%, 90% glycerol).

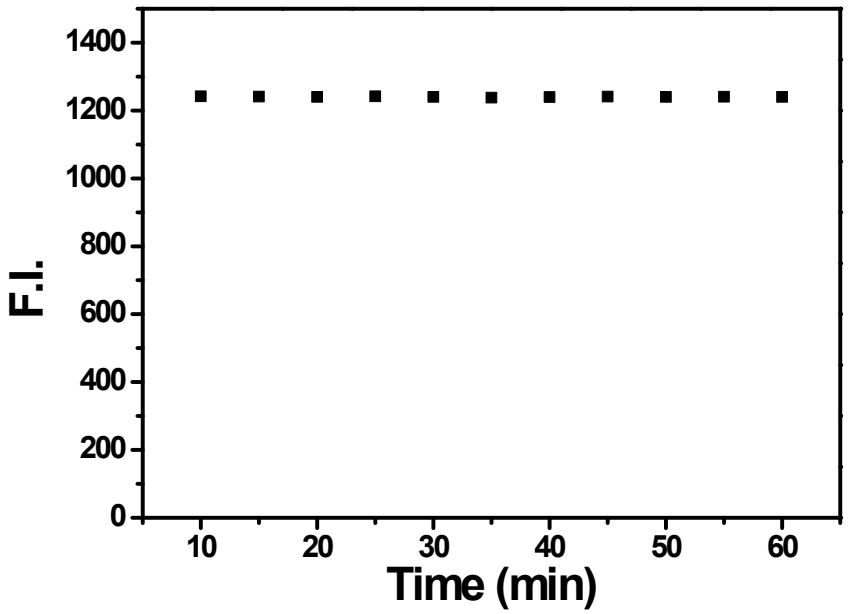


Fig. S10. Light stability of fluorescence intensity of probe **FCV**.

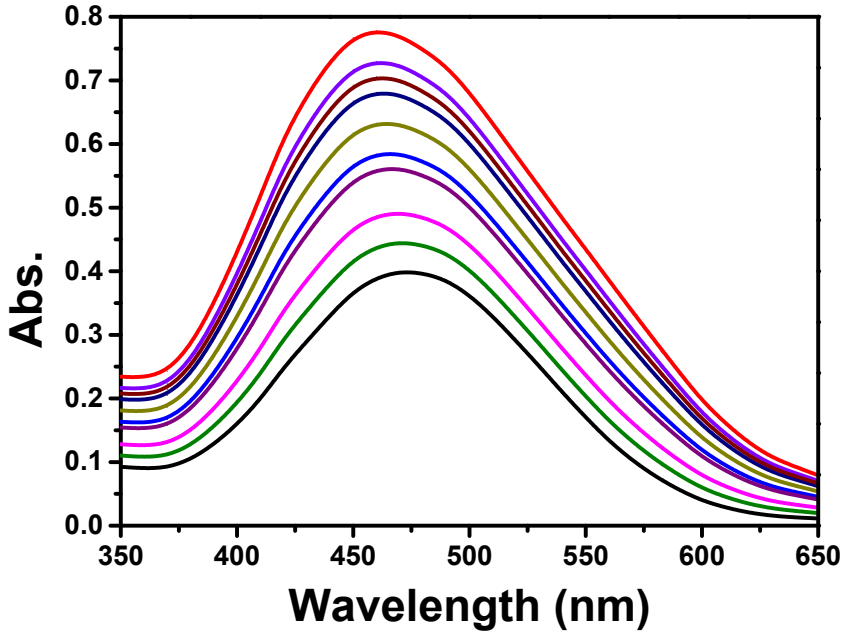


Fig. S11. Absorbance spectra of the probe **FCV** (5 μM) in DMSO/PBS = 1:99 (v/v = 1:1, pH = 7.4) buffer to 90% glycerol.

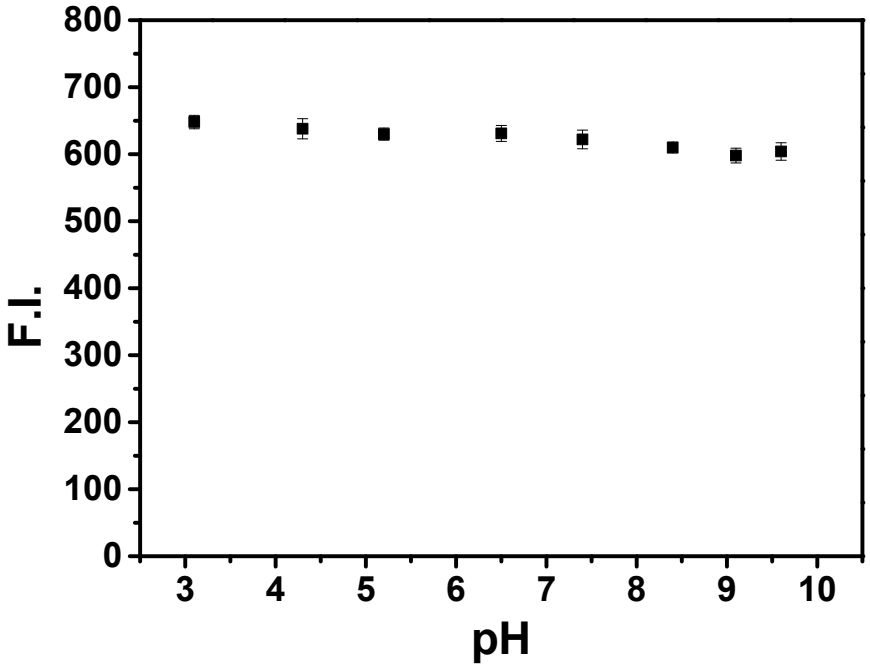


Fig. S12. The effect of pH on the fluorescence intensity changes of **FCV** (5 μM) in 50% glycerol/PBS solution.

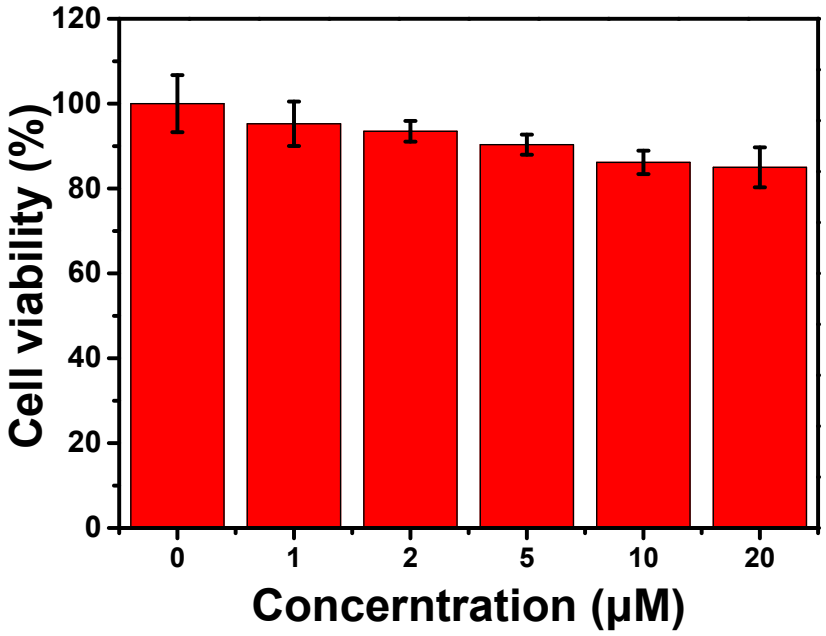


Fig. S13. MTT results of Hela cells viabilities after incubation with **FCV** for 24 h. Data are expressed as mean ± SD (experiment times n = 3).

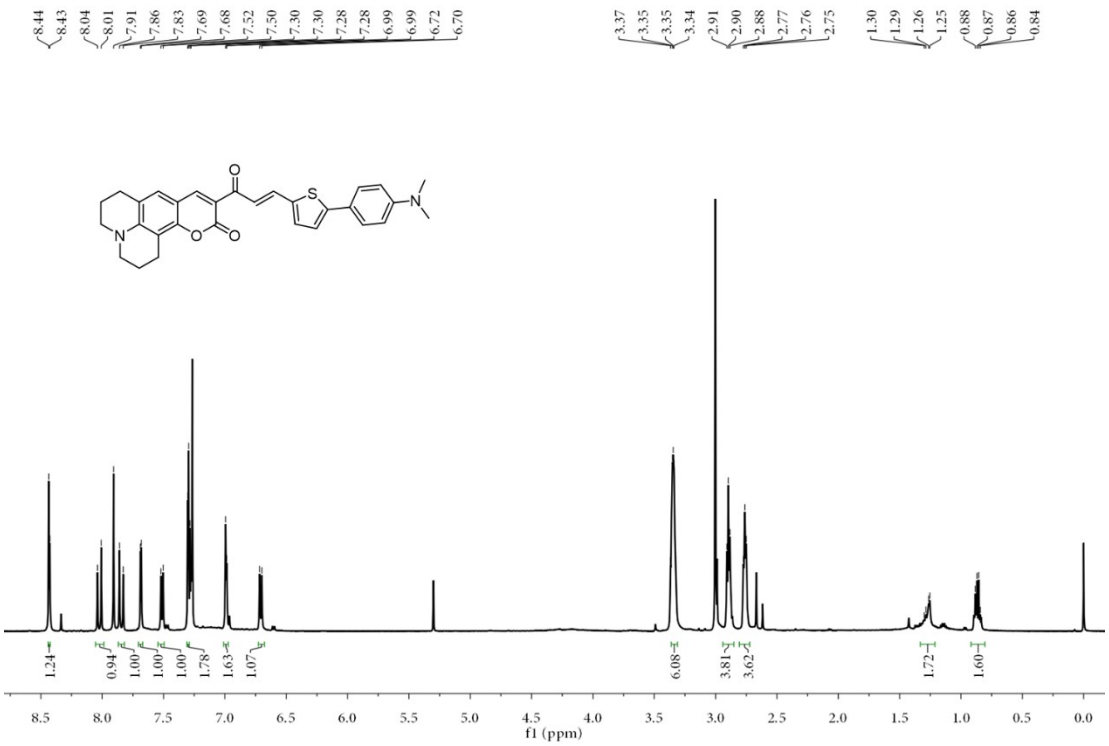


Fig. S14 ^{1}H NMR spectrum of **FCV** in $CDCl_3$

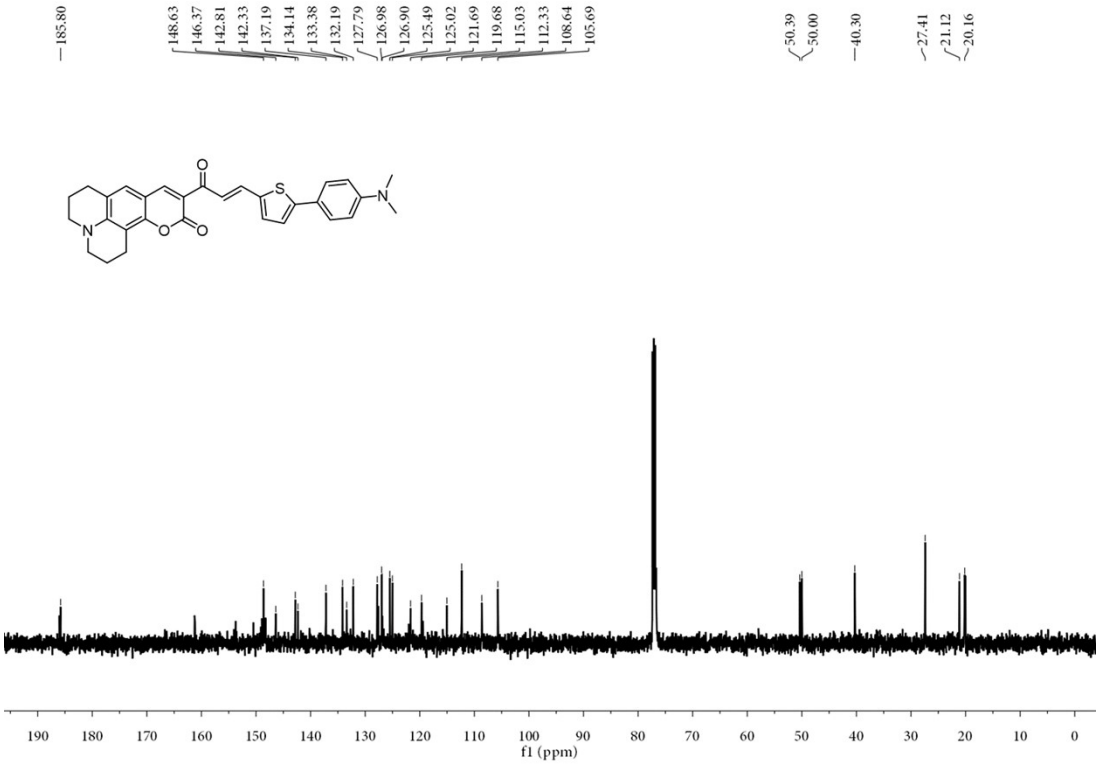


Fig. S15 ^{13}C NMR spectrum of **FCV** in $CDCl_3$