Supporting Information

for

Design, Synthesis and Performance Evaluation of a Fluorescent Probe for the Detection of Sulfur Dioxide In Vitro and Intracellularly

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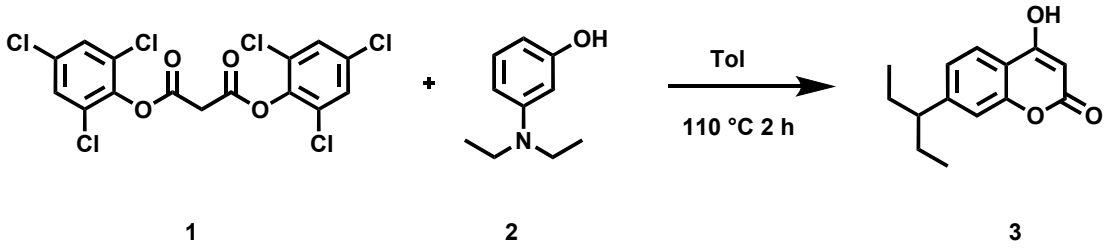
Materials and instruments

The reagents used in this experiment were purchased from commercial suppliers without further purification. All reagents were purchased from commercial suppliers and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded by avance III 400 MHz, using $CDCl_3$ or DMSO as solvent and tetramethylsilane (TMS) as internal standard. UV-vis absorption spectra were recorded using a UV-2700PC spectrophotometer. The fluorescence spectra were measured using HITACHI F-4500 spectroflu orometer. The instruments used for cytotoxicity experiments and cellular kinetic processes are a multifunctional enzyme labeller (F200 pro) and a confocal microscope (SP8). Both TLC and silica gel were purchased from the Qingdao Ocean Chemicals.

Preparation of Substrates (3)

Compound **3** was reacted with 3-diethylaminophenol in toluene under reflux for 2 h. The precipitate was cooled to room temperature and filtered to yield a gray product. The gray crude product was washed with toluene to afford pale yellow solid target compound **3** in 91% yield[1].



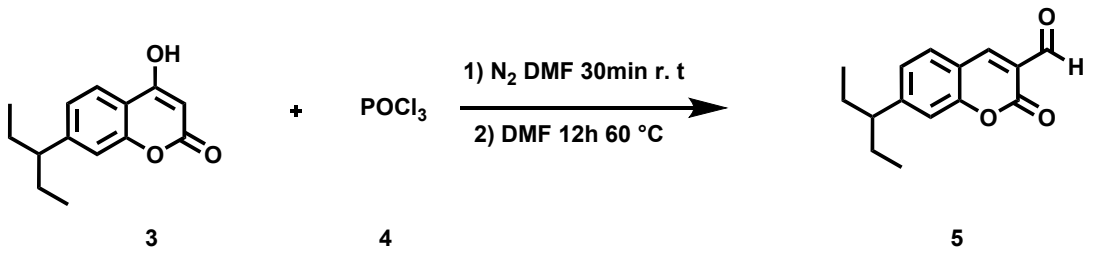
1 2 3

Scheme**. S1.** Synthetic pathway of compound **3**.

Preparation of Substrates (5)

Freshly distilled DMF was added dropwise to $POCl_3$ at room temperature, and the mixture

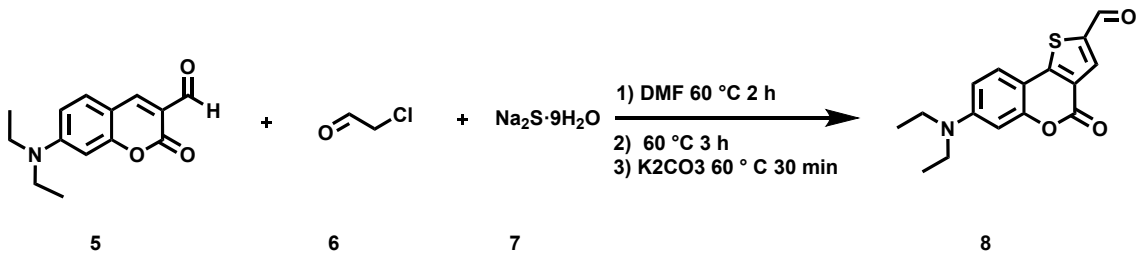
was stirred under a nitrogen atmosphere for 30 min to obtain a red liquid. Compound **3** was dissolved in DMF, and this DMF solution of compound **3** was then added dropwise to the red liquid, resulting in a scarlet suspension. The scarlet suspension was reacted at 60 °C for 12 h. Upon completion of the reaction, the mixture was poured into ice water, and the pH was adjusted with 20% NaOH aqueous solution until a sufficient amount of orange precipitate was obtained. The precipitate was identified as compound **5** in a yield of about 96%[2].



Scheme**. S2.** Synthetic pathway of compound **5.**

Preparation of Substrates (8)

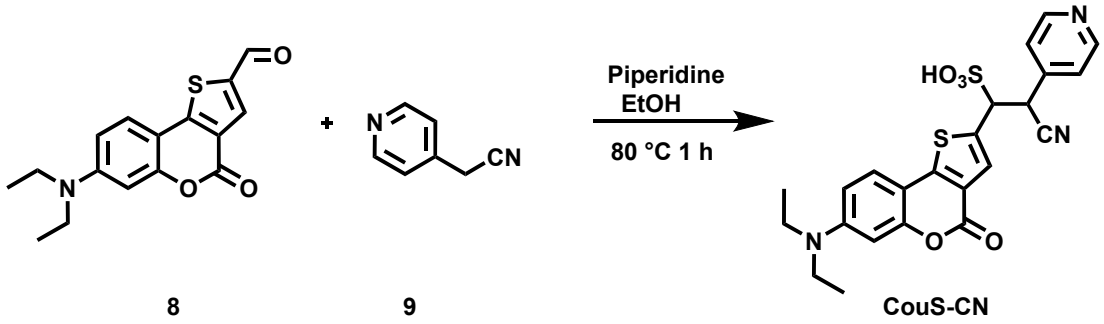
A solution of $Na_2S·9H_2O$ was added to DMF. The mixture was stirred at 60 °C for 2 h. Subsequently, chloroacetaldehyde was added rapidly, and the reaction was stirred at 60 °C for another 3 h. K_2CO_3 was dissolved in water, and this aqueous solution was added to the reaction mixture. The resulting mixture was stirred at 60 °C for 10 minutes, cooled to room temperature, and quenched in water. Upon adding a large amount of distilled water to the reaction solution, a substantial precipitate formed. The precipitate was filtered to give a crude yellow solid, which was dissolved in dichloromethane. The sample was prepared by adding silica gel powder, and column chromatography was carried out with PE/EtOAc (1:5, v/v) as the eluent to obtain solid product **8** in 88% yield[3].



Scheme**. S3.** Synthetic pathway of compound **8**.

Preparation of CouS-CN

Compound **8** was further reacted with 2-(pyridin-4-yl)acetonitrile and piperidine in anhydrous ethanol at 80 °C for 1 h. After the reaction mixture was cooled, the excess solvent was removed by reduced-pressure distillation. The residue was dissolved and mixed with dichloromethane for sample preparation, then subjected to column chromatography. The target product **CouS-CN** was afforded as a red solid by elution with PE/EtOAc (1:5, v/v), in 86% yield. Product CouS-CN m.p. 271.1–272.0 °C. ^{11}H NMR (600 MHz, $CDCl_3$) δ 8.70-8.68 (m, 2H), 7.92 (s, 1H), 7.87 (d, J = 8.8 Hz, 1H), 7.56-7.54 (m, 3H), 6.66-6.55 (m, 2H), 3.47-3.42 (q, J = 7.2 Hz, 4H), 1.26-1.22 (t, J = 7.2 Hz, 6H).^{13}C NMR (151 MHz, $CDCl_3$) δ 154.77, 151.13, 150.60, 136.73, 135.69, 133.52, 125.54, 120.75, 119.67, 109.61, 105.60, 97.94, 77.00 ($CDCl_3$), 45.05, 12.56.



Scheme**. S4.** Synthetic pathway of compound **CouS-CN**.

Cell cytotoxicity assays

HeLa cells were provided by Jiangsu Kaiji Biotechnology Co., Ltd. The living HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10% FBS) under the atmosphere containing 5% CO2 and 95% air at 37 °C. The cytotoxic effects of the probe **CouS-CN** were tested by the MTT assay.

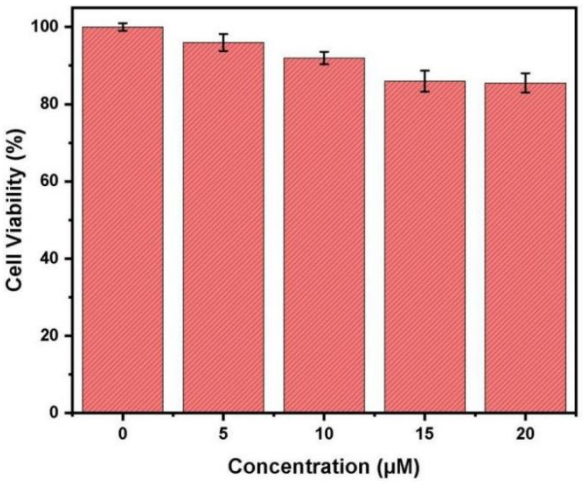


Fig. S1 Cytotoxicity assays of probe **CouS-CN** at different concentrations (0, 5, 10, 15 and 20 μM) and at 24h for HeLa cells.

Cell imaging

The concentration of the probe in the cell imaging experiments was 10 μM, and the cells were incubated at a temperature of 37°C with 5% CO2 for 30 min. To remove the residual probe, the cells were rinsed three times with PBS buffer solution before imaging. Finally, the cells were imaged with a Leica SP8 inverted fluorescence confocal microscope with an excitation wavelength of 620 nm.

Results and Discussion

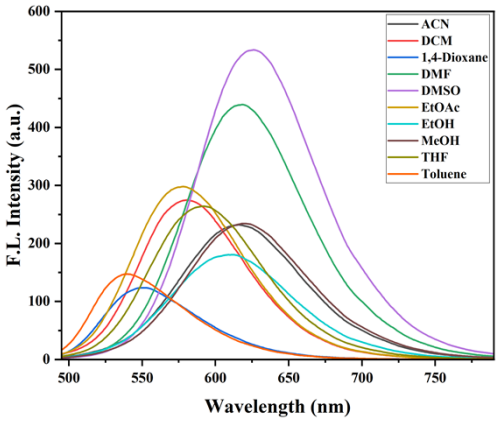


Fig. S2. Fluorescence emission spectra of **CouS-CN**.

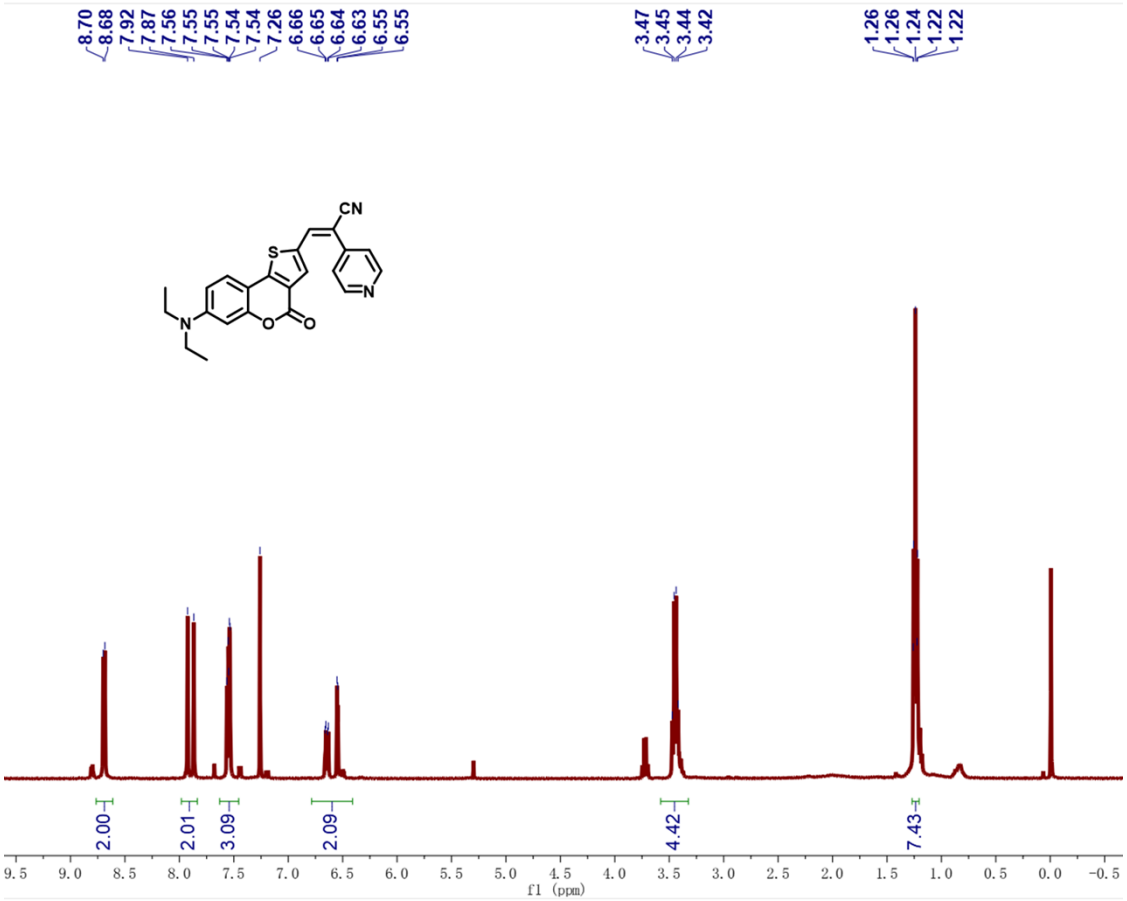


Fig. S3 ^{1}H NMR spectra of **CouS-CN**.

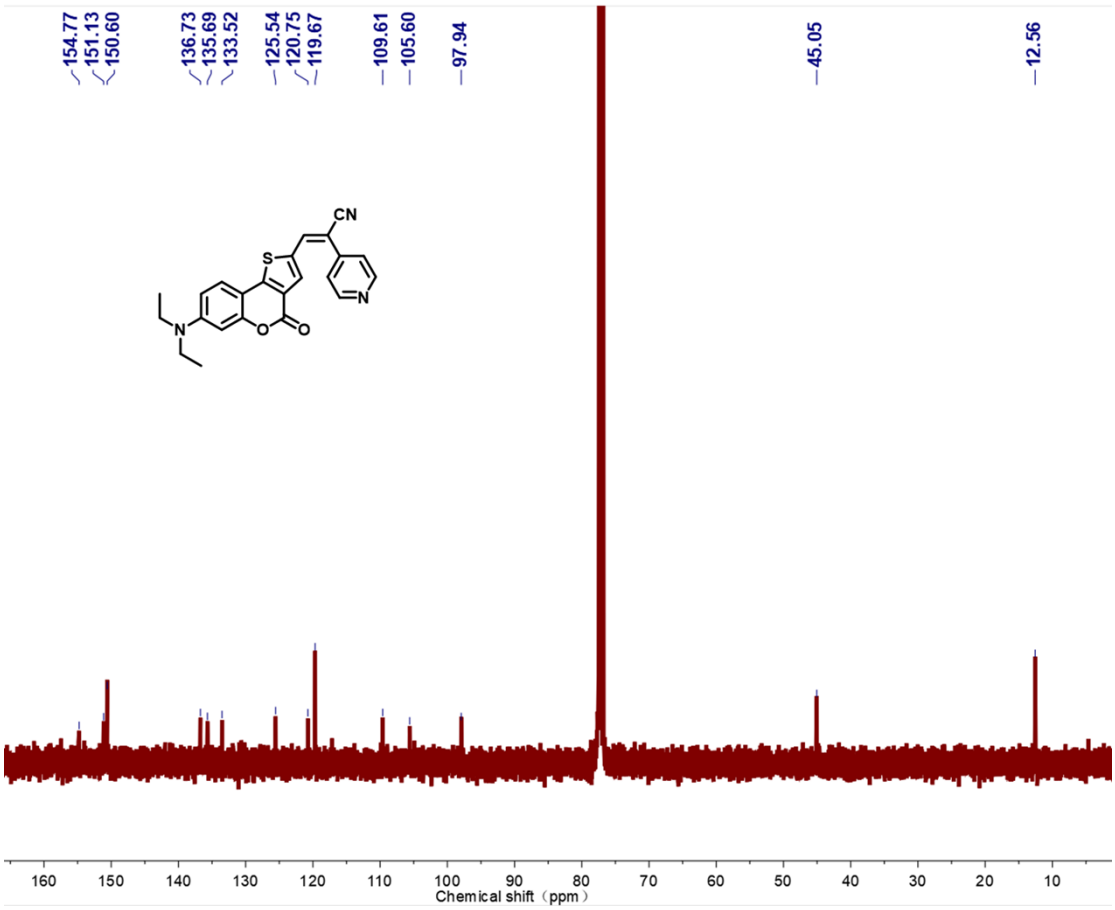


Fig. S4 ^{13}C NMR spectra of **CouS-CN**.

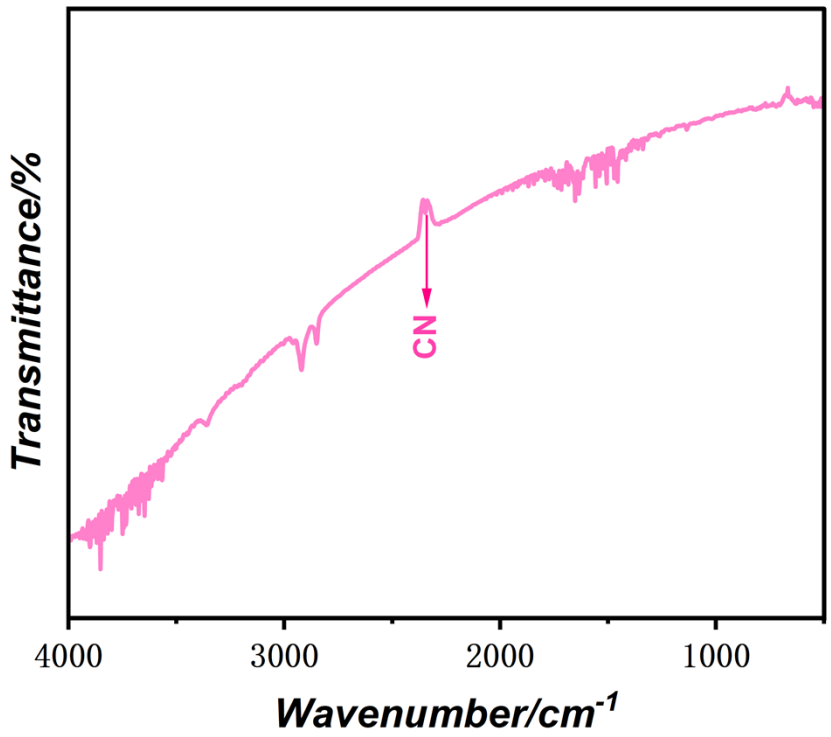


Fig. S5 FT-IR spectrum of **CouS-CN**.

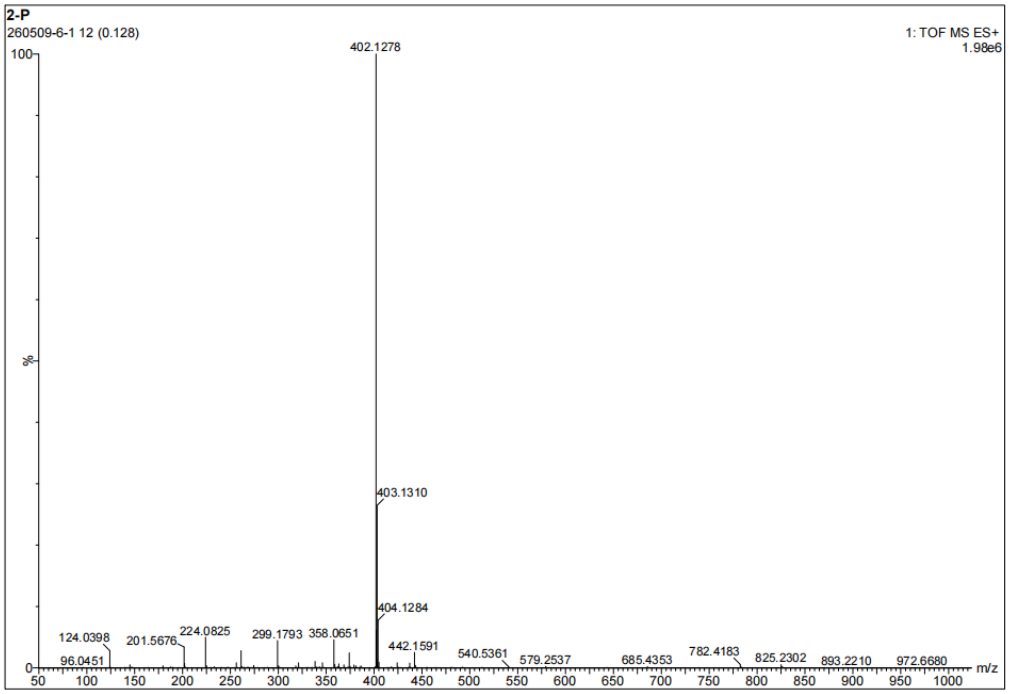


Fig. S6 HR-MS spectra of **CouS-CN**.

Notes and references

1. Chevalier, P.-Y. Renard and A. Romieu, Chem. Eur. J., 2014, 20(27), 8330–8337. DOI: 10.1002/chem.201402306.
2. K. Renault, P.-Y. Renard and C. Sabot, Eur. J. Org. Chem., 2018, 2018(46), 6494–6498. DOI: 10.1002/ejoc.201801157.
3. L. Yang, M. Liu, K. Sheng, X. Li, J. Du, Y. Ning, X. Wang, J. Li, Y. Zhang and S. Wu, New J. Chem., 2019, 43, 4188–4195. DOI: 10.1039/C8NJ06326E