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A dual responsive fluorescent probe for selective detection of cysteine and bisulfite and its application in bioimaging

Xiaofeng Wang[1§], Mingshun Li[2§], Tingting Duan[1,*], Yuxia Zou[1,*], Xuejun Zhou[1,*]

[1]Department of Otolaryngology, Head and Neck Surgery, The First Affiliated Hospital of Hainan Medical University, Haikou 570102, China

[2]School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, 510515, China.

[*]Corresponding author.

E-mail: xuejunzhouent@hainmc.edu.cn; denieceduan@163.com; zouyuxiazzz@163.com

[§] These two authors contributed equally to this work.

Contents:

1. Materials and Instruments

8-hydroxy nonalonidine-9-formaldehyde, cysteine, phosphorus oxychloride, and diethyl malonate were purchased from Aladdin and used as received. Fluorescent spectra were obtained on a F-4600 fluorescence spectrophotometer. NMR spectra were performed on a Bruker-500 NMR spectrometer. HRMS were obtained on Agilent 1290. Confocal fluorescence imaging experiments were performed with an Olympus FV-1000 laser scanning microscopy system, based on an IX81 (Olympus, Japan) inverted microscope. Images were collected and processed with Olympus FV10-ASW Ver.2.1b software. The Dulbecco's Modified Eagle Medium were purchased from Gibco, American. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo, Shanghai, China. Human macrophage cell line (RAW264 .7, blood) purchased from the Cell Bank of the Chinese Academy of Sciences.

2. Experimental methods

2.1. Spectral experiments

The probe was dissolved in dimethyl sulfoxide to prepare 1 mM stock solutions. All spectral experiments were performed at 37 ℃. All aqueous solutions were prepared with ultrapure water. For Cou-F, the fluorescence intensity was measured at $\lambda_{ex}/_{em}$ = 432/500 nm and $\lambda_{ex}/_{em}$ = 452/521 nm, respectively. The spectral test procedure was as follows: add Cou-F (0.10 ml, 0.10 mM) to a 1 ml centrifuge tube, dilute to 10 μM with 10 mM HEPES buffer and DMSO and add various analytes to different centrifuge tubes. The system was incubated at 37°C, and the spectral test was performed.

2.2. Cell Counting Kit-8 assay for Cou-F

The mouse macrophage cell line (RAW246.7) was purchased from the Cell Bank of the Chinese Academy of Sciences. Cells were cultured in an atmosphere containing 5% CO_2 and 95% air at 37 ^{o}C, and a high-sugar DMEM medium containing 10% fetal bovine serum (FBS) was provided. The cells (8000 cells/well) were seeded in a 96-well plate and allowed to adhere and culture for 24 hours. Subsequently, the cells were incubated with 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μM probe Cou-F under the above conditions for 24 h. Under the same conditions, the untreated blank group high-sugar DMEM medium was also analyzed. Add 10 μM CCK-8 solution to each well. After incubating for 4 h, the absorbance at 450 nm with a microplate reader was measured.

2.3. Cell culture and imaging experiments

The RAW246.7 cell line was used in this experiment. The complete medium contains 89% high-sugar DMEM medium, 10% FBS and 1% penicillin-streptomycin double antibody solution added to the T25 culture flask, and the cells are cultured at 37 ℃ with 5% CO_2 and 95% air 24 h. Before cell imaging, the cells were inoculated into a confocal culture dish under the same conditions with 1 ml of fresh complete medium and incubated for 24 h, and then incubated with Cou-F.

3. Synthesis of probe Cou-F

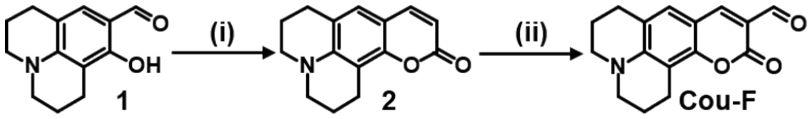

Figure S1. The synthesis of probe Cou-F.

Synthesis of compound 2

8-Hydroxyjulonidine-9-formaldehyde (0.504 g, 2.3 mmol) and diethyl malonate (0.736 mL, 4.6 mmol) were dissolved in 20 mL of absolute ethanol, and then 0.23 mL of piperidine was added to the solution. The mixed solution was heated to reflux for 24 h, and the progress of the reaction was monitored by TLC during the reaction. After the reaction was completed, the solution was subjected to rotary evaporation to remove excess solvent to obtain a solid. Adding 8 mL of concentrated hydrochloric acid and glacial acetic acid to the newly obtained solid, the mixture solution was stirred and heated to 80 ℃. After the reaction was completed, the reaction solution was poured into 400 mL of ice water, and 20% sodium hydroxide solution was added dropwise to adjust the pH to 7.0. The mixed solution was stirred for 30 min, and a yellow precipitate slowly precipitated out of the solution. Finally, the mixed solution was filtered, washed, vacuum dried, and recrystallized using toluene to obtain yellow powdery solid compound 2 (yield 79.1%). ^{1}H NMR ($CDCl_3$-d_1, 400 MH_Z) δ (ppm): 7.72-7.70 (d, 1H), 7.00 (s, 1H), 5.93-5.91 (d, 1 H), 3.26-3.23 (t, 4H), 2.73-2.68 (t, 4H), 1.90-1.85 (m, 4H). ^{13}C NMR ($CDCl_3$-d_1, 125 MH_Z) δ (ppm): 161.61, 151.65, 145.13, 125.68, 118.43, 107.98, 105.98, 56.49, 49.24, 27.28, 20.25, 19.03. HRMS (ESI^+): m/z $C_{15}H_{15}NO_2$ calcd. 241.1103, found $[M+H]^+$ 242.1175.

Synthesis of probe Cou-F

Under the protection of argon atmosphere, phosphorus oxychloride ($POCl_3$) was added dropwise to 2 mL of anhydrous DMF. After the mixture was stirred at room temperature for 30 min, the color of the reaction solution gradually changed to light yellow. The compound 2 obtained in the previous step was dissolved in 3 mL of anhydrous DMF, and all this solution was added to the light-yellow solution. Then, the solution was heated to 60 ℃ and reacted for 12 h. The reacted solution was slowly poured into 100 mL of ice water. The pH was adjusted to 7.0 with 20% sodium hydroxide solution. The precipitate gradually precipitated in the solution. The mixture was filtered to obtain a

solid. The obtained solid was recrystallized in ethanol to obtain probe Cou-F (yield 62.3%). ^{1}H NMR ($CDCl_3$-d_1, 400 MH_Z) δ (ppm): 9.87 (s, 1H), 8.23 (s, 1H), 7.25 (s, 1H), 3.39-3.36 (t, 4H), 2.74-2.68 (t, 4H), 1.91-1.84 (m, 4H). ^{13}C NMR ($CDCl_3$-d_1, 125 MH_Z) δ (ppm): 186.94, 161.05, 149.35, 145.43, 145.21, 128.61, 119.82, 111.62, 107.45, 105.14, 49.29, 26.69, 20.41, 19.45. HRMS (ESI^+): m/z $C_{16}H_{15}NO_3$ calcd. 269.1052, found $[M+H]^+$ 270.1126.

4. ^{1}H NMR ,^{13}C NMR and HRMS of compound 2 and Cou-F

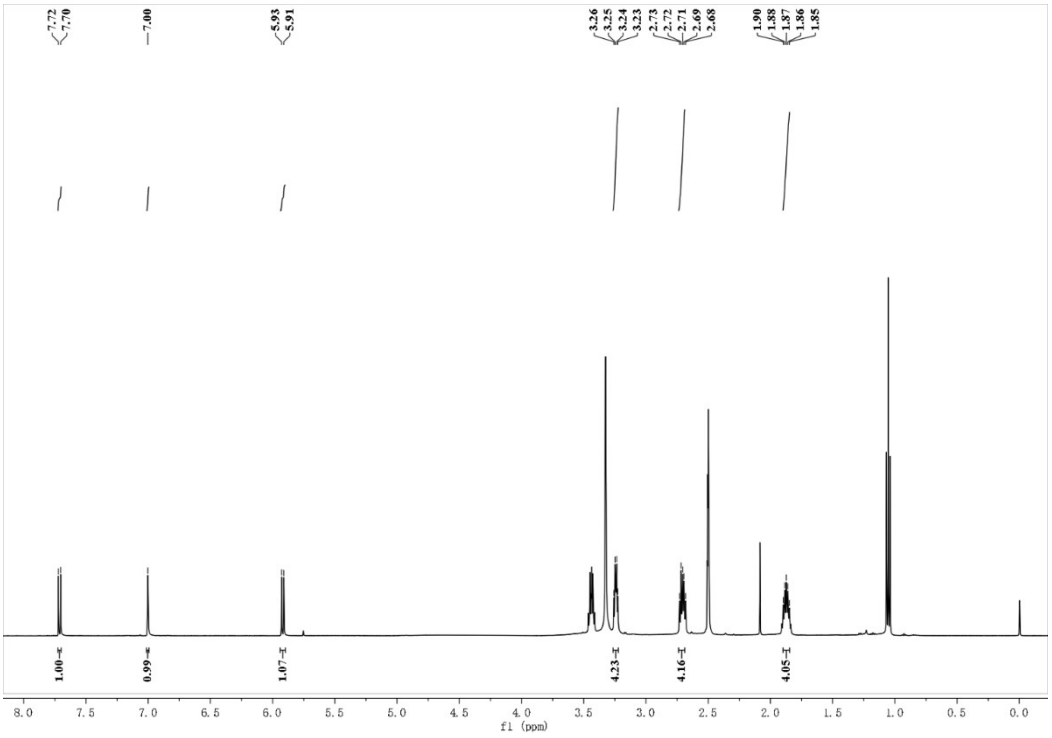

Figure S2. ^{1}H NMR of compound 2

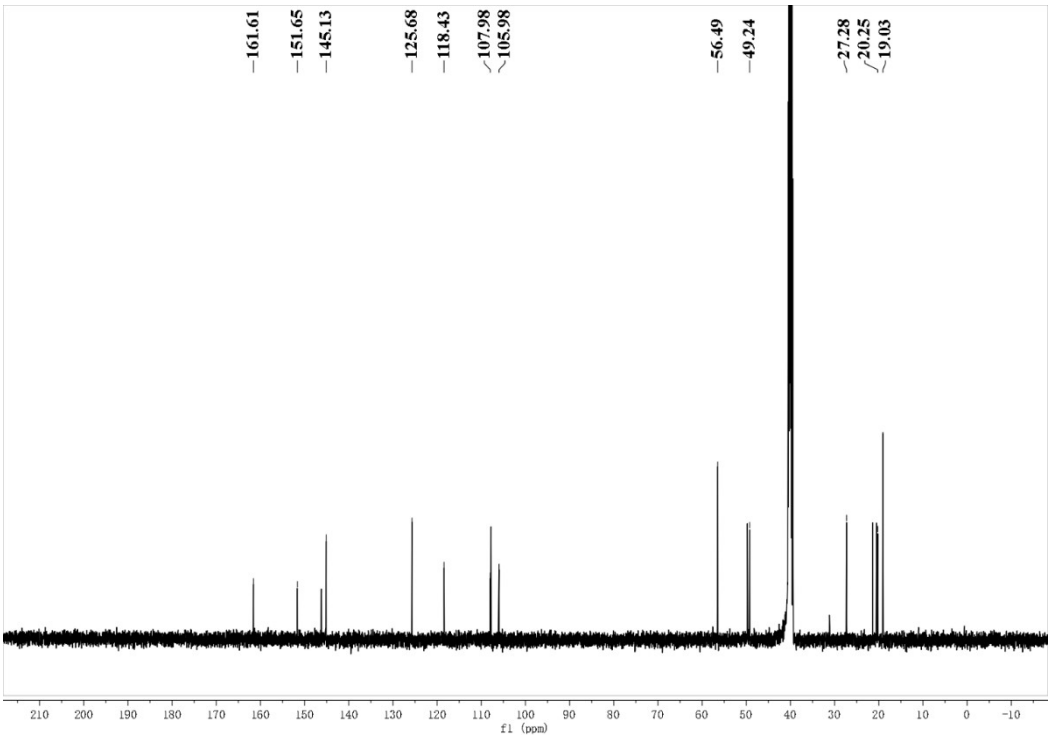

Figure S3. ^{13}C NMR of compound 2

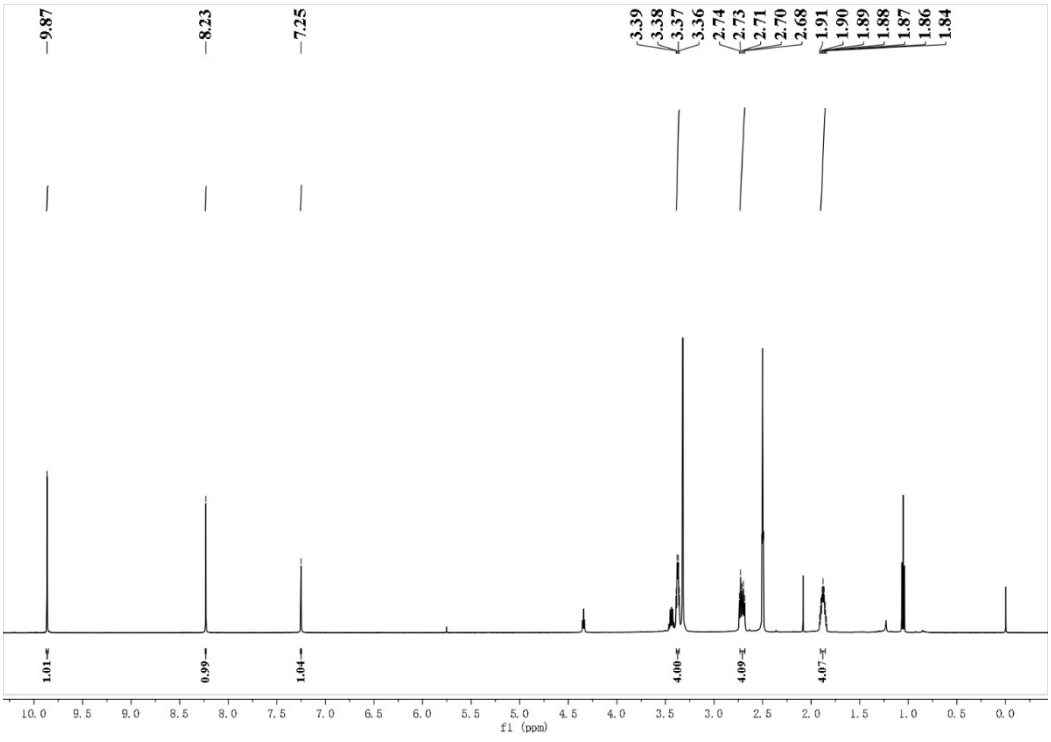

Figure S4. ^{1}H NMR of Cou-F

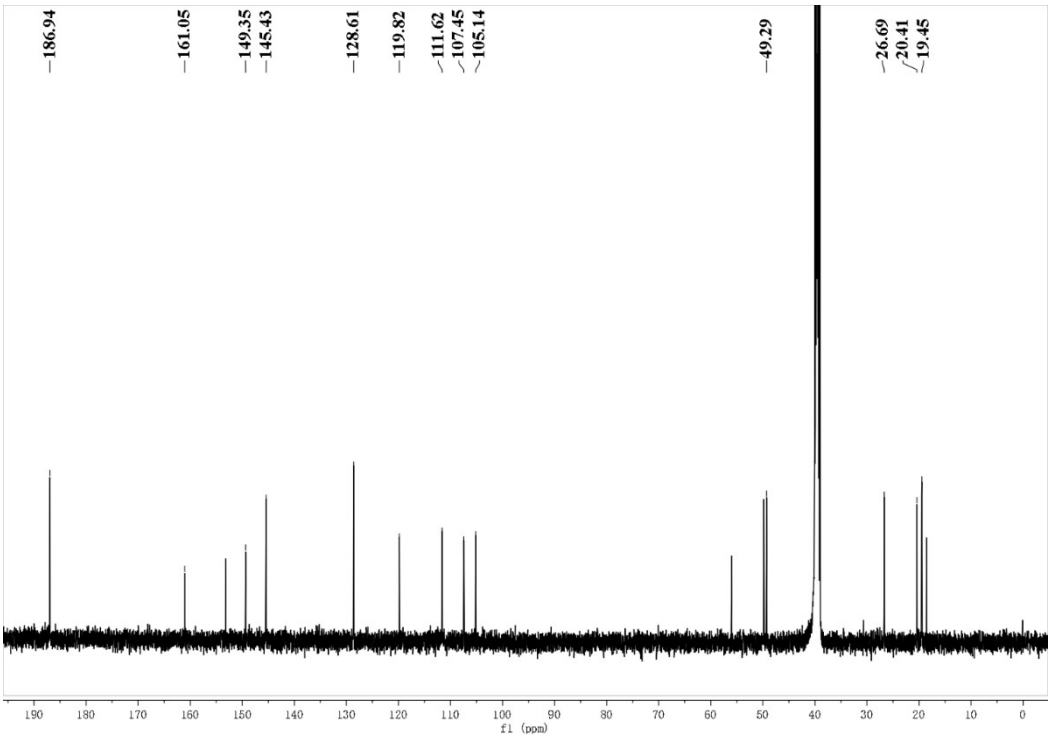

Figure S5. ^{13}C NMR of Cou-F

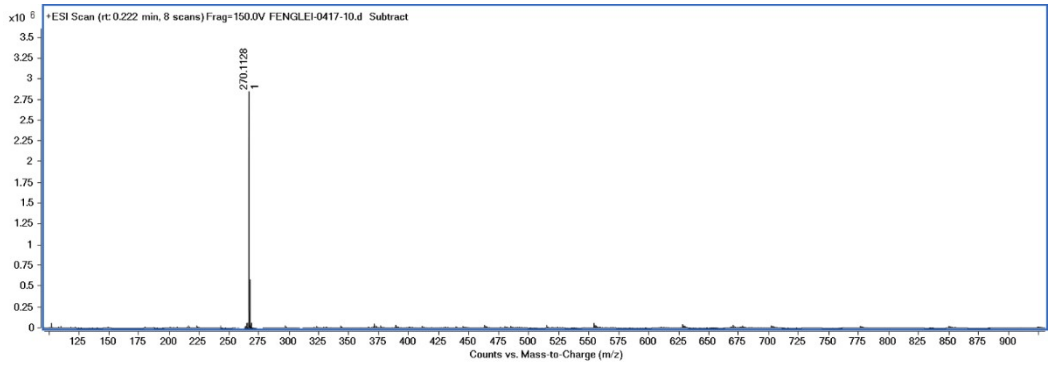

Figure S6. HRMS of Cou-F

5. The reaction of Cou-F with Cys and HSO_3^- monitored by HRMS

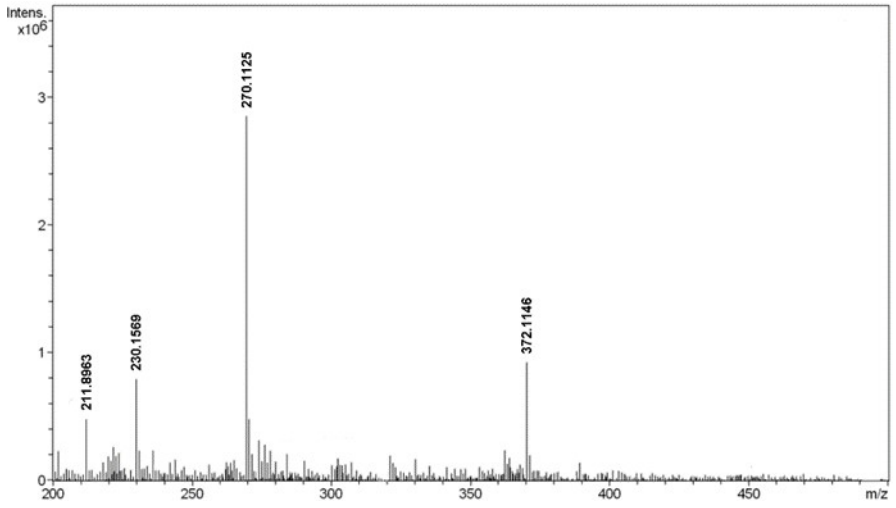

Figure S7. HRMS of the reaction product of Cou-F and Cys in DMSO/H_2O (v/v = 1/1)

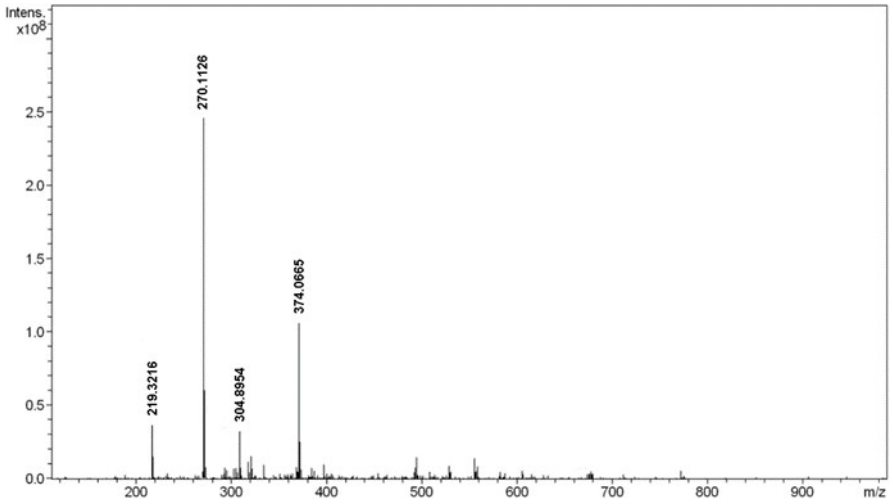

Figure S8. HRMS of the reaction product of Cou-F and HSO_3^- in DMSO/H_2O (v/v = 1/1)

6. The selectivity of probe Cou-F

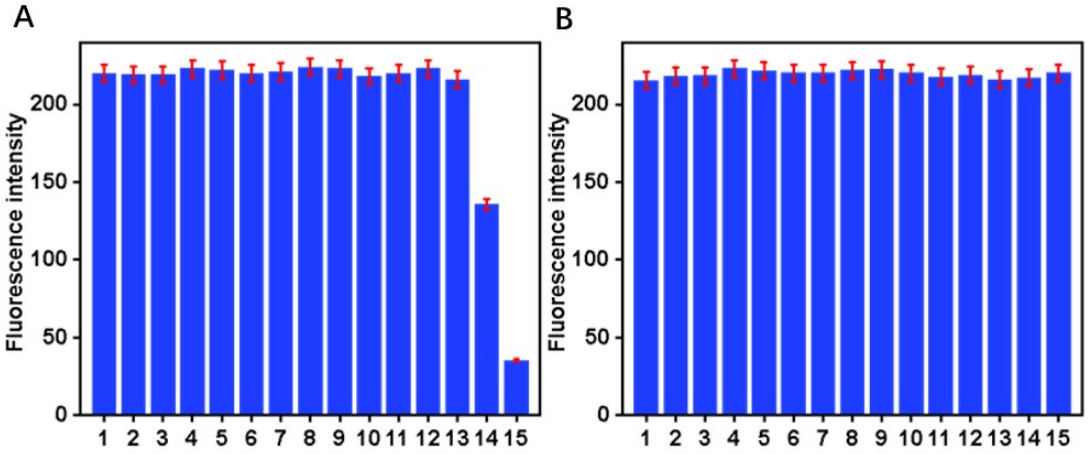

Figure S9. The selectivity of Cou-F (10 μM) toward Cys in HEPES (pH 7.4, 10 mM) at 37 ºC. (A) 1. 100 μM Ala, 2. 100 μM Asn, 3. 100 μM Arg, 4. 100 μM Asp, 5. 100 μM Gln, 6. 100 μM Glu, 7. 100 μM Gly, 8. 100 μM His, 9. 100 μM Ile, 10. 100 μM Leu, 11. 100 μM Lys, 12. 100 μM Met, 13. 1 mM GSH, 14. 100 μM Hcy, 15. 100 μM Cys. (B) 1. 100 μM Pro, 2. 100 μM Ser, 3. 100 μM Thr, 4. 100 μM Trp, 5. 100 μM Tyr, 6. 100 μM Val, 7. 100 μM HClO, 8. 100 μM H_2O_2, 9. 15 μM NO, 10. 100 μM HS^-, 11. 100 μM SO_4^{2-}, 12. 100 μM Cl^-, 13. 100 μM $H_2PO_4^{2-}$, 14. 100 μM SCN^-, 15. 100 μM $S_2O_3^{2-}$. The experiments were repeated three times and the data were shown as mean (± S.D.)

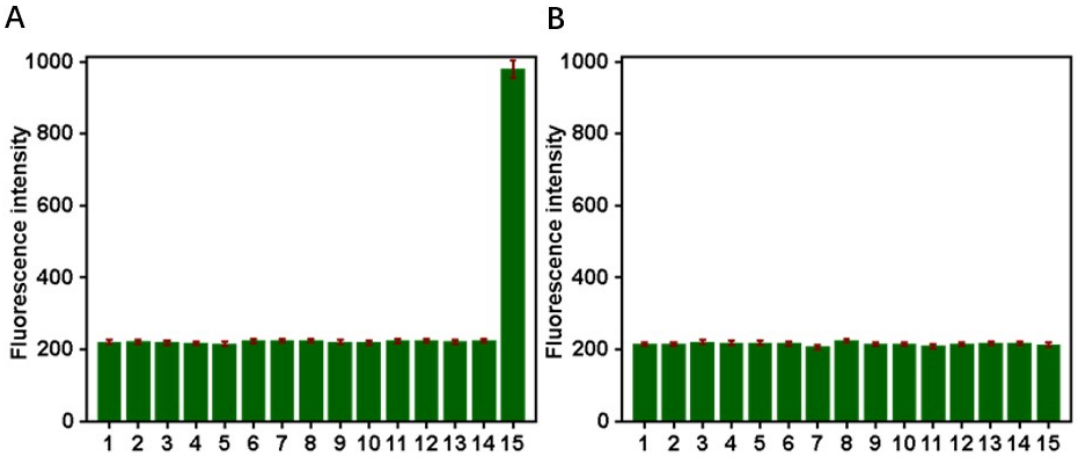

Figure S10. The selectivity of Cou-F (10 μM) toward HSO_3^- in HEPES (pH 7.4, 10 mM) at 37 °C. (A) 1. 100 μM Ala, 2. 100 μM Asn, 3. 100 μM Arg, 4. 100 μM Asp, 5. 100 μM Gln, 6. 100 μM Glu, 7. 100 μM Gly, 8. 100 μM His, 9. 100 μM Ile, 10. 100 μM Leu, 11. 100 μM Lys, 12. 100 μM Met, 13. 100 μM Trp, 14. 100 μM Tyr, 15. 100 μM HSO_3^-. (B) 1. 100 μM Pro, 2. 100 μM Ser, 3. 100 μM Thr, 4. 100 μM Val, 5. 100 μM HS^-, 6. 1 mM GSH, 7. 15 μM NO, 8. 100 μM HPO_4^{2-}, 9. 100 μM $H_2PO_4^-$, 10. 100 μM SO_4^{2-}, 11. 100 μM F^-, 12. 100 μM Cl^-, 13. 100 μM SCN^-, 14. 100 μM H_2O_2 and 15. 100 μM $S_2O_3^{2-}$

7. The cytotoxicity assay of Cou-F by using CCK-8

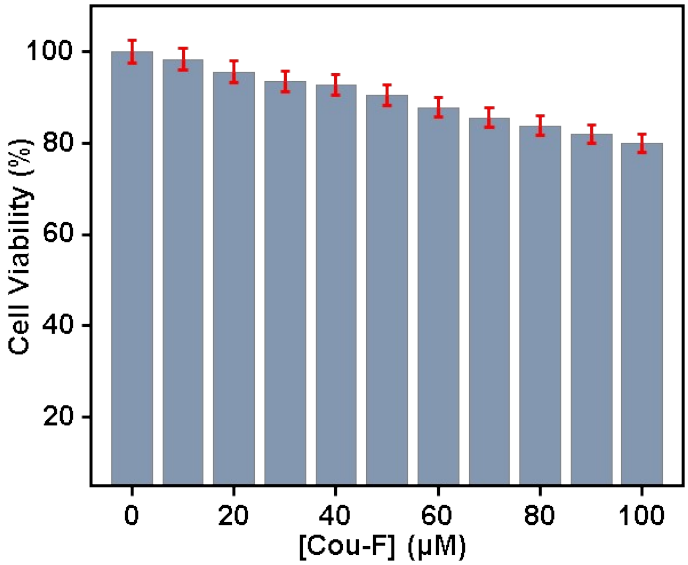

Figure S11. The cytotoxicity assay of Cou-F