Development of an ultrafast fluorescent probe for specific recognition of hypochlorous acid and its application in live cells

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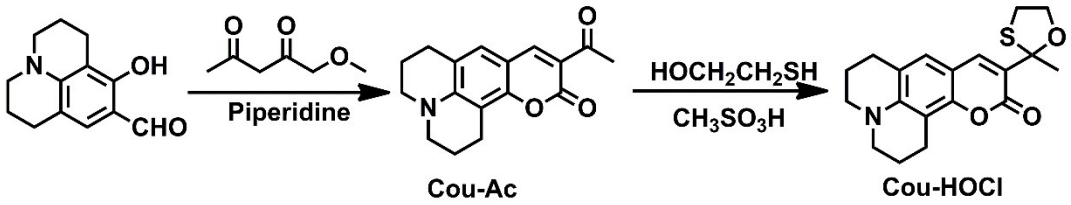
1. Materials and Instruments

Piperidine, methanesulfonic acid, 2-mercaptoethanol, 8-hydroxy nonalonidine-9-formaldehyde, dimethyl sulfoxide and sodium hypochlorite were purchased from Aladdin and used as received. UV/vis spectra were recorded on a GBC Cintra 2020 UV-vis spectrometer. Fluorescent spectra were obtained on a HITACHI F-4500 fluorescent spectrophotometer. NMR spectra were performed on a Bruker DPX-400 NMR spectrometer. HPLC-MS were obtained on Agilent 1100 series and LC/MSD Trap XCT. High resolution mass spectra were ensured on a MALDI-FTMS. 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 fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium and phosphate buffer saline were purchased from Gibco, American. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo, Shanghai, China. Different cell lines, including human cervical cancer cell line (HeLa, pelvic), human non-small cell lung cancer cell line (A549, chest), human hepatocellular carcinoma cell line (HepG2, abdomen), and human macrophage cell line (RAW264 .7, blood) purchased from the Typical Culture Collection Committee of the Chinese Academy of Sciences (Shanghai, China)

2.Synthesis and Characterization of Cou-HOCl



Scheme S1. Synthesis procedures of Cou-HOCl

Synthesis of Cou-Ac

8-Hydroxy nonulonidine-9-formaldehyde (651.8 mg, 3 mmol), ethyl acetoacetate (774.8 mg, 3 mmol) and piperidine (0.5 ml) were dissolved in 24 ml of absolute ethanol. The mixture was heated and refluxed for 6 hours and then cooled to room temperature. The suspension was then collected by filtration and washed with cold absolute ethanol. Finally, the product was dried under vacuum to obtain Cou-Ac as orange crystals (671.5 mg,79 %).

Synthesis of Cou-HOCl

Cou-Ac (283.3 mg, 1 mmol), methanesulfonic acid (80 μl, 1.1 mmol) and 2-mercaptoethanol (250 μl, 1 mmol) were dissolved in 100 ml of dichloromethane. Heat to reflux for 3 hours under the protection of argon, and then cool to room temperature. The obtained precipitate was filtered and purified by silica gel column chromatography (eluent: dichloromethane: methanol = 10:1) to obtain yellow solid (133.6 mg, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.56 (s, 1H), 6.90 (s, 1H), 4.41-4.36 (m, 1H), 4.15-4.09 (m, 1H), 3.26 (t, J = 5.9 Hz, 4H), 3.16-3.10 (m, 1H), 2.98-2.93 (m, 1H), 2.89 (t, J = 6.5 Hz, 2H), 2.78 (t, J = 6.4 Hz, 2H), 2.01 (t, J = 5.9 Hz, 4H), 1.95 (s, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$), δ (ppm): 161.14, 150.86, 145.39, 134.93, 125.20, 124.92, 118.47, 107.97, 106.40, 92.00, 71.11, 49.93, 49.57, 33.26, 29.52, 27.43, 21.46, 20.55, 20.26. HRMS (ESI): calcd for $C_{19}H_{22}NO_3S$ ($[M + H]^+$): 344.1320, found: 344.1346.

3. Cell Culture and confocal imaging

For imaging experiments, Cou-HOCl has a purity of above 95% was used. HeLa, A549, HepG2 and RAW264.7 cells were used and incubated in DMEM supplemented with 10% fetal bovine serum. The culture was maintained at 37°C, 95% atmosphere and 5% CO_2 conditions. First inoculate live cells on a confocal plate, then add 1 ml of fresh complete medium, and then culture for 24 h. Before performing the imaging experiment, the cells were incubated with 10 μM Cou-HOCl. Confocal images of cells were obtained using Olympus FV-1000 laser scanning microscopy and 60× oil objective lens. Green channel: the excitation wavelength was selected as 480nm, and the collection wavelength was selected as 500-540nm.

4. Detection limit of Cou-HOCl to HOCl

According to the following formula, the detection limit (D) of Cou-HOCl for HOCl is calculated: D = 3σ/k, σ stands for the standard deviation of the blank measurement of Cou-HOCl's fluorescence intensity at 510 nm within 10 times; And k represents the slope between the fluorescence intensity and the concentration of HOCl in the fluorescence titration curve of Cou-HOCl in the presence of HOCl (0-50 μM). The experiments were repeated three times and the data were shown as mean (± s.d.)

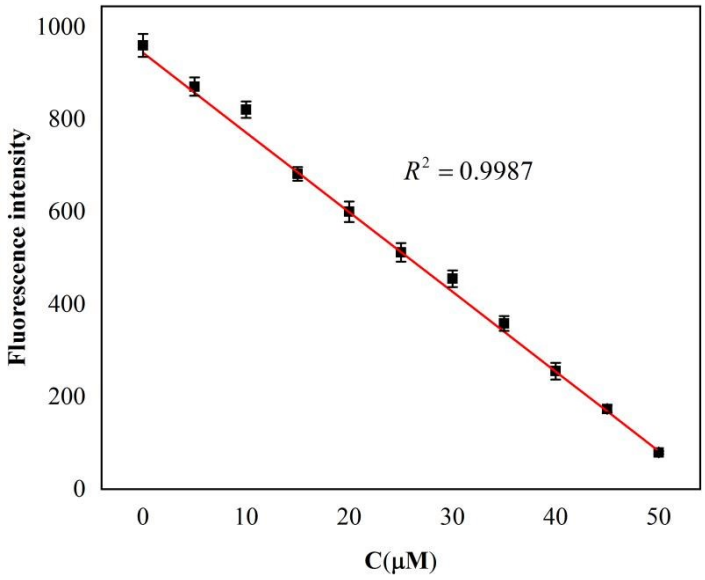


Figure S1. The linear relationship between the fluorescence intensity of Cou-HOCl and the concentration of HOCl.

5. The selectivity of Cou-HOCl

(a) Fluorescence response of 10 μM Cou-HOCl to amino acids (200 μM) and metal ions (50 μM). 1: GSH; 2: Cys; 3: Hcy; 4: Fe^{2+}; 5: Fe^{3+}; 6: Zn^{2+}; 7: Cu^{2+}. (b) Fluorescence response of 10 μM Cou-HOCl to different ROS (100 μM. 1:NO; 2: KO_2; 3: H_2O_2; 4:

$^1O_2$; 5: HO^-; 6: *t*-BuOOH; 7: HOCl). The experiments were repeated three times and the data were shown as mean (± S.D.)

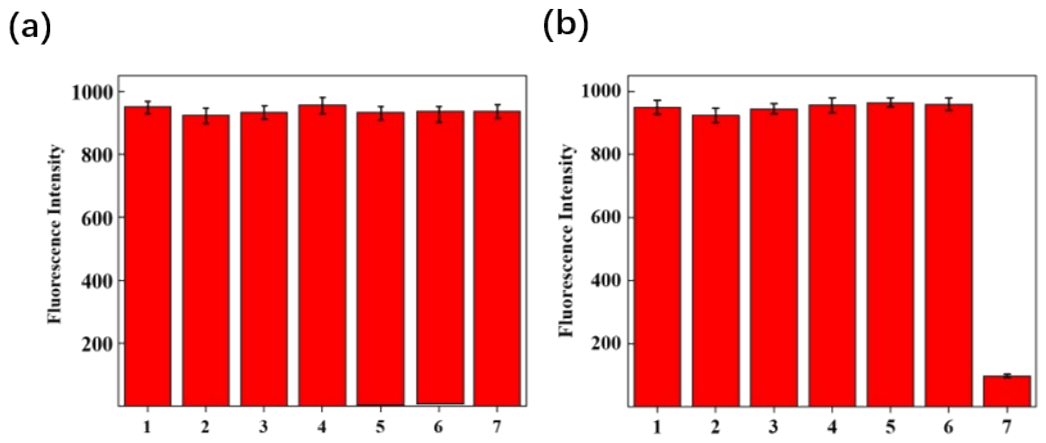


Figure S2. The selectivity of Cou-HOCl toward various ROS, RNS and metal ions

6. The reaction of Cou-HOCl with HOCl monitored by ^{1}H NMR spectra

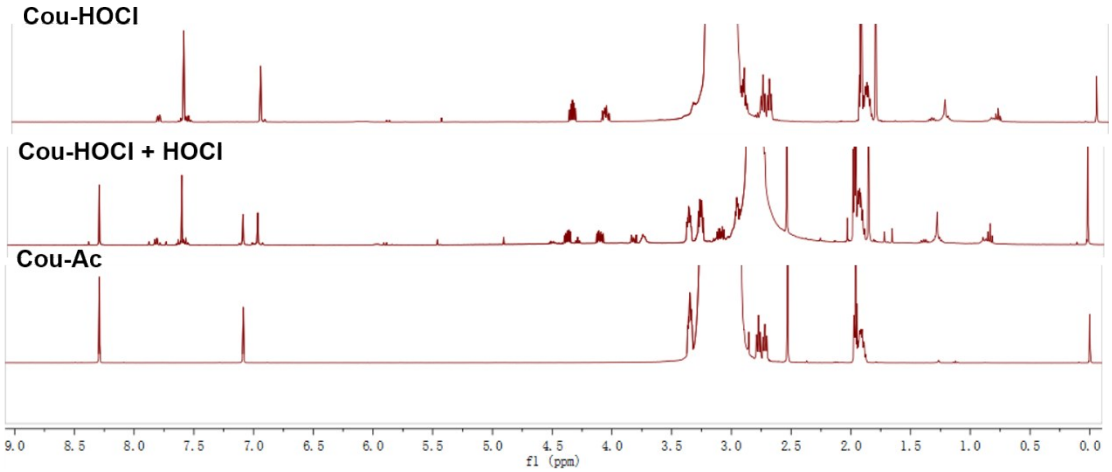


Figure S3. ^{1}H NMR spectra of Cou-HOCl upon addition of 2 equiv. HOCl in CD_3CN/H_2O (v/v = 9/1)

7. Cell toxicity assay of Cou-HOCl

A549, HepG2, HeLa and RAW264.7 cells were cultured in DMEM supplemented with 10% FBS in 5% carbon dioxide (CO_2) and 95% air at 37°C. The cells (8000 cells/well) were seeded in a 96-well plate and allowed to adhere for 24 hours. Subsequently, the cells were combined with 0, 10, 20, 30, 40, 50, 60, 70, 80 and 100 μM (final concentration) of Cou-HOCl (dissolved in DMSO) in 5% CO_2 and 95% air at 37°C After incubating for 24 hours, under the same conditions as the control, untreated DMEM was also tested. Add CCK-8 solution (5.0 mg/mL, 10 μL) to each well. After the plate was incubated for 1 hour in 5% CO_2 and 95% air, the absorbance was measured at 450 nm using a microplate reader (TECAN infinite M200pro). The experiments were repeated three times and the data were shown as mean (± S.D.)

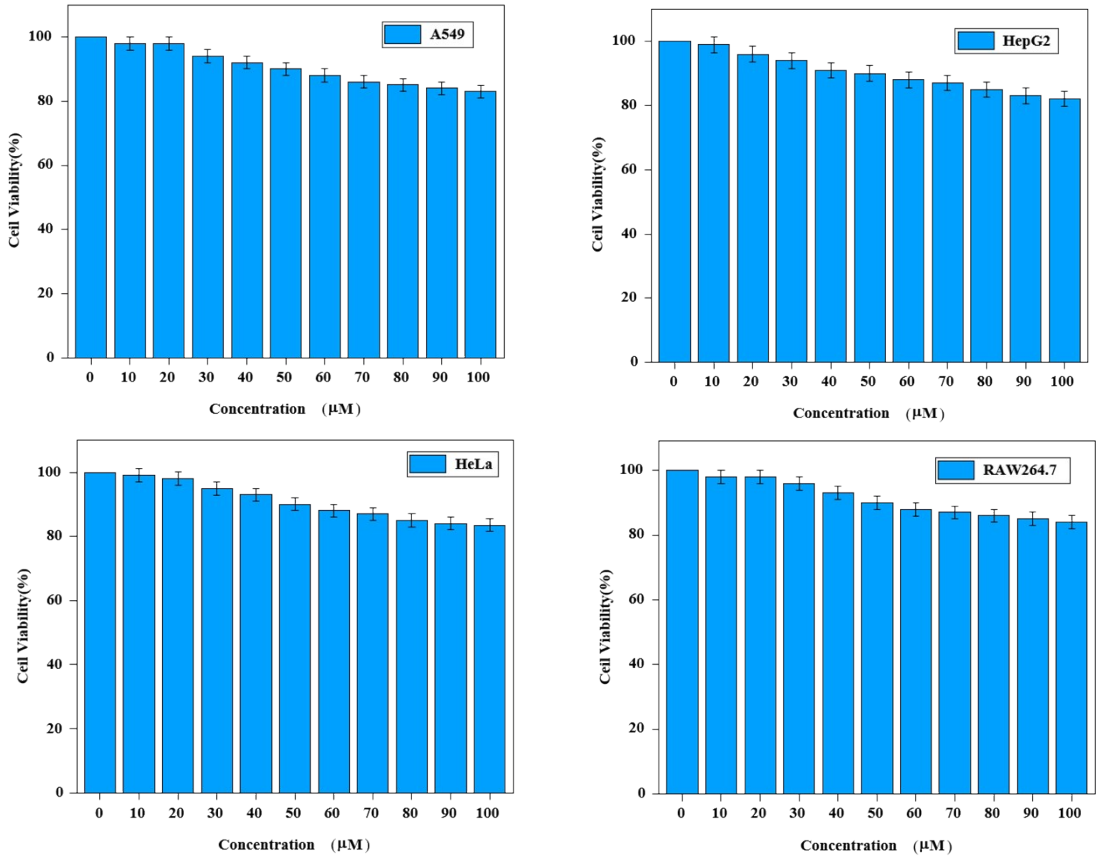


Figure S4. Cell toxicity assay of Cou-HOCl

8. Bright-field imaging of Figure 3

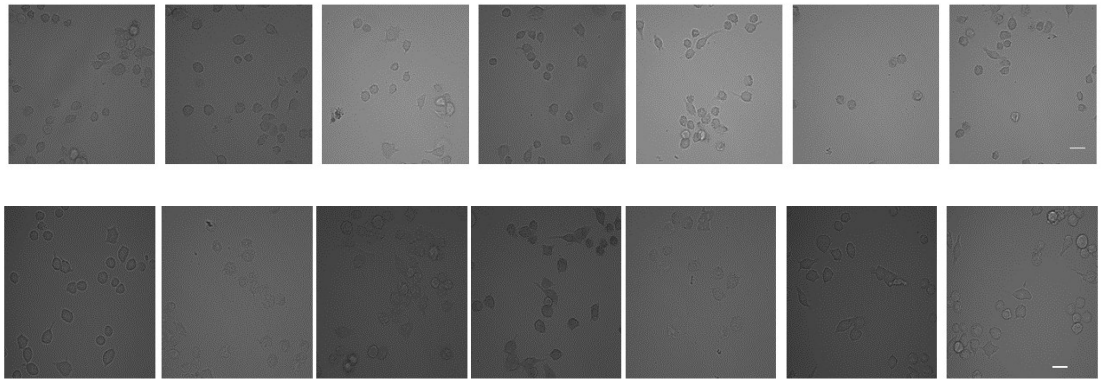


Figure S4. Bright-field imaging of Figure 3. Scale bar = 30 μm.

9.^{1}H NMR ,^{13}C NMR and HR-MS of Cou-HOCl

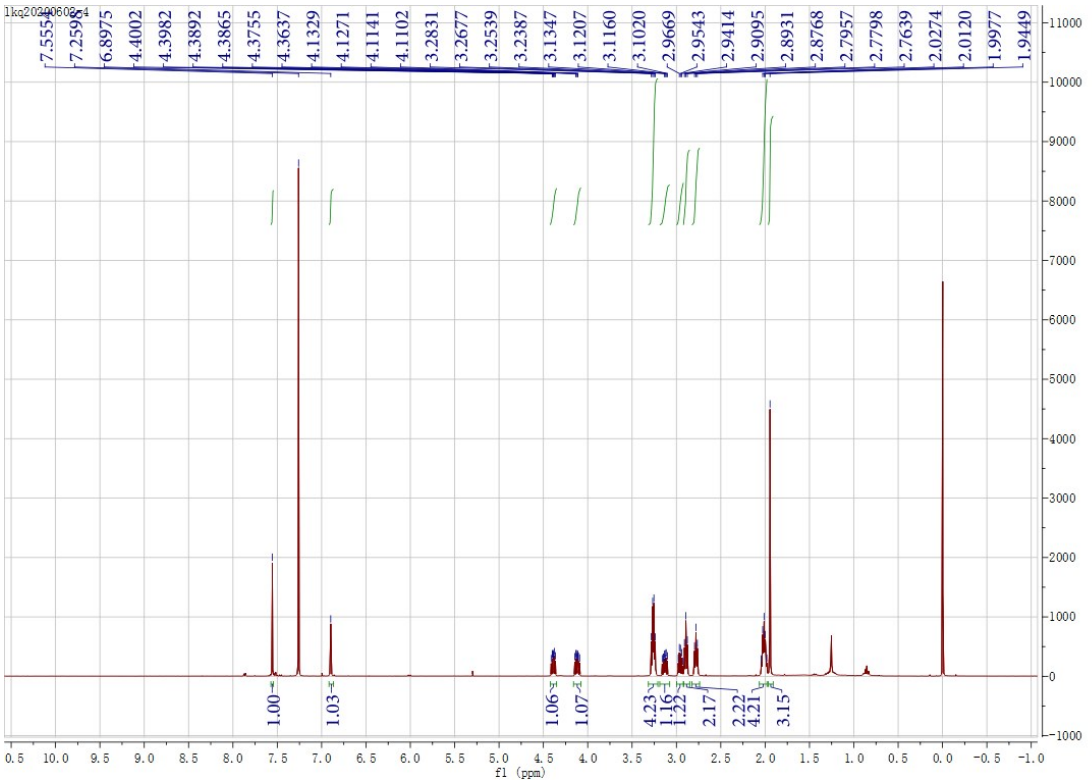


Figure S5. ^{1}H NMR of Cou-HOCl

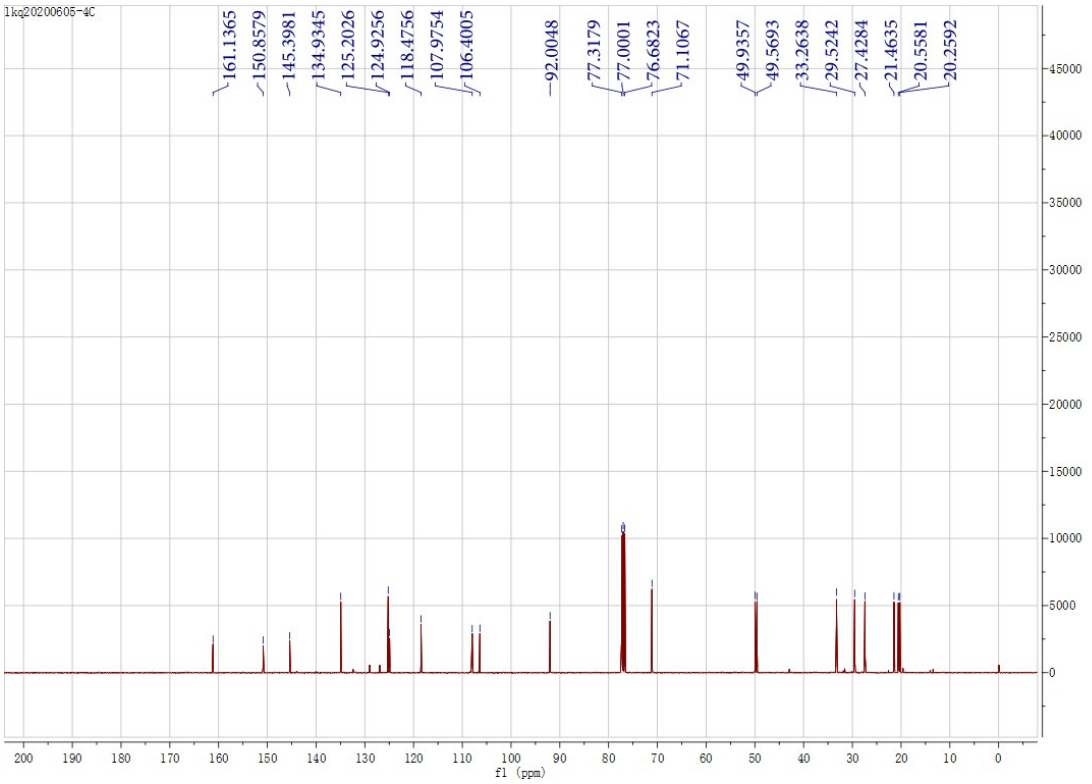


Figure S6. ^{13}C NMR of Cou-HOCl

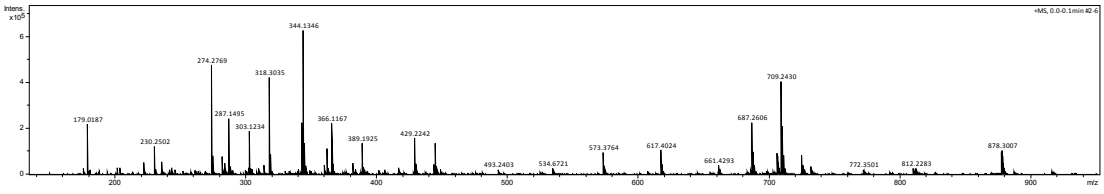


Figure S7. HR-MS of Cou-HOCl