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SUPPLEMENTAL DATA

Hypochlorite sensing and real-time imaging with XY-01: A red-emitting fluorescent turn-on probe for living cells and colorectal cancer organoids

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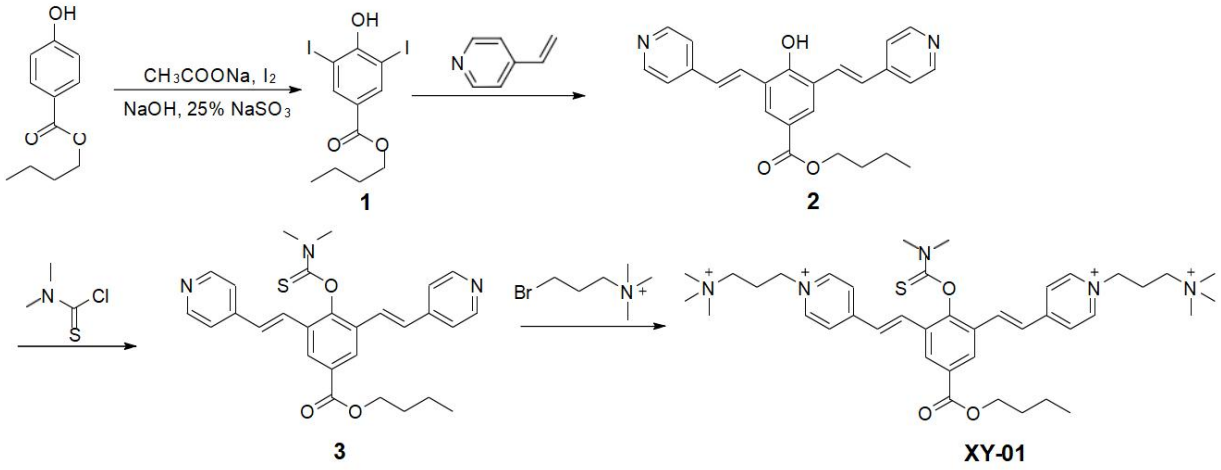
Full article is available at the following link: Hypochlorite sensing and real-time imaging with XY-01: A red-emitting fluorescent turn-on probe for living cells and colorectal cancer organoids

Experimental

Materials and measurements

All solvents and reagents utilized in this study were of analytical grade. Proton nuclear magnetic resonance (1H NMR) and carbon-13 nuclear magnetic resonance (13C NMR) spectra were acquired using a Bruker AV 400 spectrometer at room temperature. Ultraviolet-visible (UV-Vis) absorption spectra were measured with a Techcomp UV2310II spectrophotometer. Photoluminescence (PL) spectra were recorded using a Hitachi F-4500. Fluorescence microscopy images were obtained with a Leica Stellaris 5 confocal microscope.

Synthesis of the compounds



Scheme S1. Synthesis route of probe XY-01. The synthesis methods of compounds 1, 2 and 3 can be referred to the following literatures [31-32].

Compound 2

Butyl 4-hydroxy-3,5-bis((E)-2-(pyridin-4-yl)vinyl)benzoate（2）

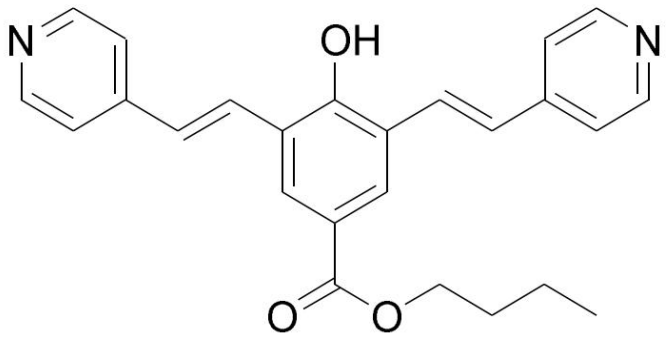


Figure S1. The structure of compound 2. Brownish-yellow solid (3.65 g, 73.13% yield); 1H NMR (500 MHz, DMSO-d6) δ 8.55 (d, J = 6.0 Hz, 4H), 8.18 (s, 2H), 7.8 6 (d, J = 16.3 Hz, 2H), 7.59 (d, J = 6.2 Hz, 4H), 7.29 (d, J = 16.3 Hz, 2H), 4.27 (s, 3H), 1.76 (m, 2H), 1.48 (m, 2H), 1.07(t, 3H); 13C NMR (125 MHz, DMSO-d6) δ 166.3, 157.9, 150.4, 144.9, 128.3, 127.9, 127.6, 125.6, 121.7, 121.5, 64.4, 31.1, 18.9, 13.8; MS [M+H]+ m/z calcd for C25H24N2O3 401.18; found 401.16.

Compound 3

Butyl 4-((dimethylcarbamothioyl)oxy)-3,5-bis((E)-2-(pyridin-4yl)vinyl) benzoate (3)

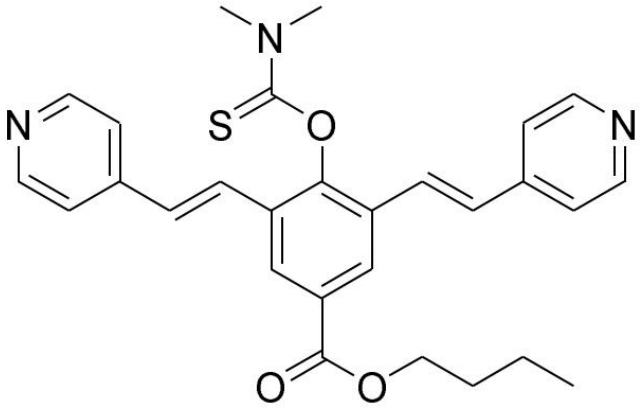


Figure S2. Structure of compound 3. A solution of compound 3 (3.60 mmol, 1.44 g) was prepared in 20 mL of DMF and cooled to 0 °C. Potassium carbonate (5.40 mmol, 745.20 mg) was then added, and the mixture was stirred at this temperature for 10 minutes. Following this, dimethylthiocarbamoyl chloride (7.20 mmol, 889.85 mg) was introduced, and the reaction was allowed to proceed at room temperature for 12 hours. The progress of the reaction was monitored using thin-layer chromatography (TLC). Upon completion, extraction was carried out three times with ethyl acetate and water. The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Purification was carried out by column chromatography (eluent: PE/EA = 1~1 to DCM/MeOH = 100:1~20:1), yielding the target compound 3 (yellow solid, yield 35.03%).1H NMR (500 MHz, DMSO-d6) δ 8.66-8.61 (m, 4H), 8.38 (s, 2H), 7.41-7.36 (m, 4H), 7.27-7.03 (m, 4H), 4.01 (s, 3H), 3.56 (d, J = 1.8 Hz, 6H), 4.27 (s, 3H), 1.71 (m, 2H), 1.39 (m, 2H), 1.04(t, 3H); 13C

NMR (125 MHz, DMSO-d6) δ 181.1, 161.2, 147.8, 145.1, 139.9, 126.6, 124.9, 123.8, 123.2, 121.4, 116.5, 47.8, 64.5, 31.2, 18.9, 14.3.

Probe XY-01

4,4'-((1E,1'E)-(2-((dimethylcarbamothioyl)oxy)-5-(methoxycarbonyl)-1,3-phenylene)bis(ethene-2,1-diyl))bis(1-(3-(trimethylammonio)propyl)pyridin-1-ium（) XY-01）

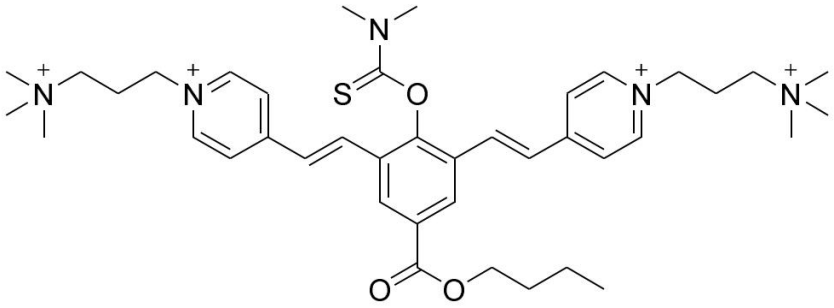


Figure S3. Structure of probe XY-01. Under nitrogen atmosphere, compound 3 (0.2 mmol, 97.53 mg) was dissolved in 3 mL of acetonitrile, followed by the addition of (3-bromopropyl)trimethylammonium bromide (0.4 mmol, 104.40 mg). The reaction mixture was heated to 90 °C and refluxed for 24 hours. Upon completion of the reaction, the mixture was cooled to room temperature, and ethyl acetate was added. The mixture was then allowed to stand for 1 hour, resulting in the formation of a significant amount of yellow precipitate. The solid product was collected by filtration, washed with ethyl acetate more than three times, and dried to obtain the target compound XY-01(yellow solid 63.30 mg, 31.21% yield); 1H NMR (500 MHz, DMSO-d6) δ 9.11 (d, J = 6.4 Hz, 4H), 8.55 (s, 2H), 8.42 (d, J = 6.4 Hz, 4H), 7.82-7.70 (m, 4H), 4.68 (t, J = 7.51 Hz, 4H), 4.00 (s, 3H), 3.67 (s, 3H), 3.52-3.45 (m, 3H), 3.43 (s, 1H), 3.12 (s, 18H), 2.47 (m ,4H); 13C NMR (125 MHz, DMSO-d6) δ 184.6, 165.6, 153.8, 152.6, 145.4, 132.8, 131.4, 129.6, 128.8, 127.8, 125.2, 62.2, 57.3, 53.2, 52.9, 43.9, 24.7; HRMS (ESI) m/z calcd for C40H59N5O3S+4 172.3579; found:172.350201 [M]4+.

The compound of XY-01-OH

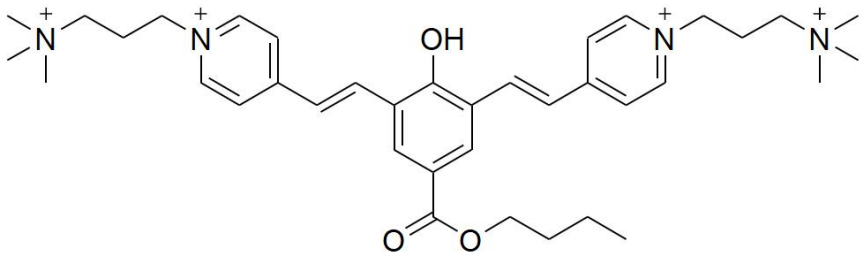


Figure S4. The structure of XY-01-OH. 4,4'-((1E,1'E)-(2-hydroxy-5-(methoxycarbonyl)-1,3-phenylene)bis(ethene-2,1-diyl))bi s(1-(3-(trimethylammonio)propyl)pyridin-1-ium) ; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.81 (d, J = 6.8 Hz, 4H), 8.10 (d, J = 6.8 Hz, 2H), 8.05 (d, J = 15.6 Hz, 4H), 7.97 (s, 2H), 3.77 (s, 4H), 3.50-3.41 (m, 3H), 2.44 (p, J = 7.7 Hz, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 178.2, 166.9, 156.3, 143.8, 143.4, 137.1, 125.7, 122.6, 119.3, 109.6, 62.3, 56.2, 52.9, 51.5, 24.7; HRMS(ESI) m/z calcd for C H N O $^{4+}$ 150.6044; found 150.6099 $[M]^{4+}$.

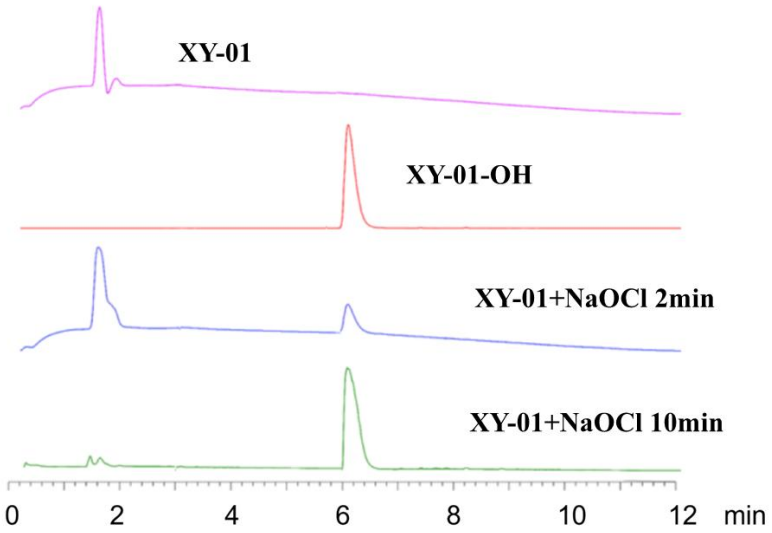


Figure S5. HPLC analysis of ClO^--mediated oxidation of XY-01. HPLC chromatograms of XY-01 (purple) and the oxidized product XY-01-OH (red),

together with reaction mixtures collected 2 min (blue) and 10 min (green) after addition of NaOCl/HOCl. The XY-01 peak appears at 1.7 min, while the XY-01-OH peak appears at 6.2 min and increases over time, supporting conversion of XY-01 to XY-01-OH upon ClO^- treatment. Abbreviations: HPLC: High-performance liquid chromatography; NaOCl: Sodium hypochlorite; HOCl: Hypochlorous acid; ClO^-: Hypochlorite.

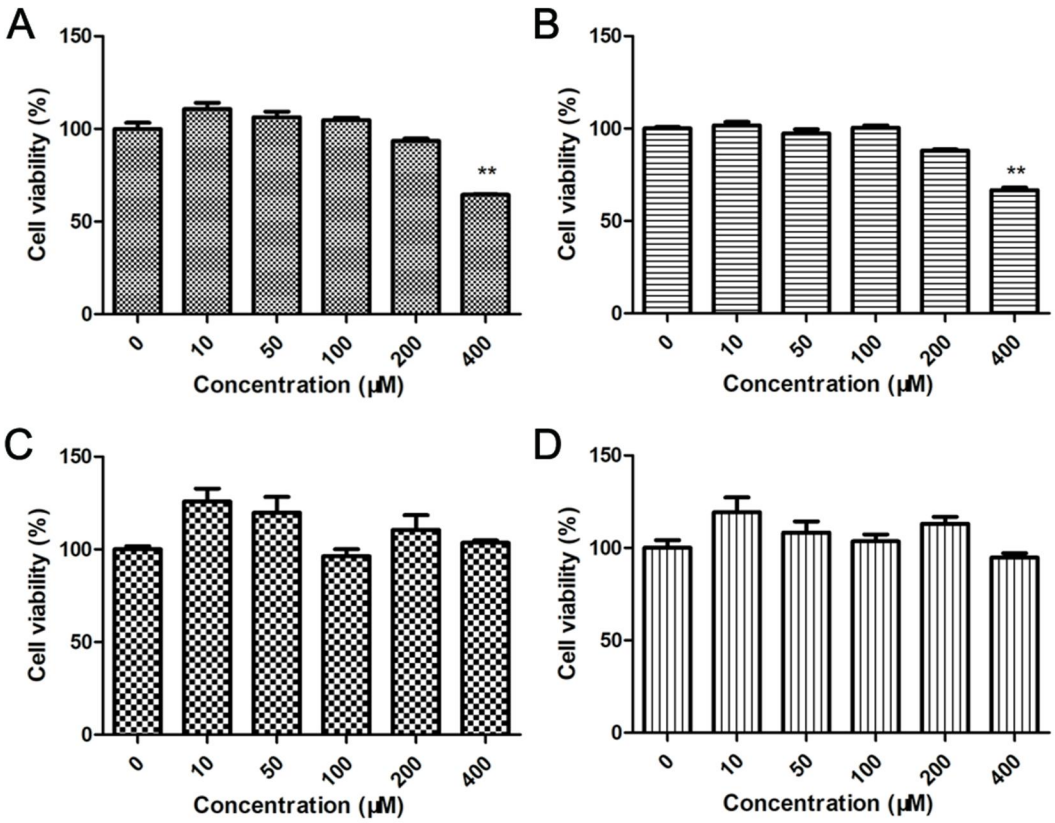


Figure S6. (A) Survival of HCT-116 cells after a 12-hour treatment with varying dosages of probe XY-01. (B) Survival of HCT-116 cells following a 24-hour treatment with varying dosages of probe XY-01. (C) Survival of colorectal cancer organoids after a 12-hour treatment with varying dosages of probe XY-01. (D) Survival of colorectal cancer organoids following a 24-hour treatment with varying dosages of probe XY-01.