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Electronic Supplementary Information

ICT-Based Fluorescent and Colorimetric Dual Sensing of Endogenous Hypochlorite in Living Cells, Bacteria and Zebrafish

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2. Experimental Section

2.1. Materials and Apparatus

Unless otherwise stated, all chemicals and solvents were obtained from commercial suppliers with analytical reagents grade. Sodium hypochlorite was purchased from Aladdin stored at 4 ºC, and all of other chemicals were purchased from Aladdin and Sigma-Aldrich. MRC-5 cells were provided by ATCC (Manassas, VA) and zebrafish were got from the Animal Ethics Committee of Wenzhou Medical University. Absorption spectrum and emission spectrum were recorded on UV-2600 form SHIMADZU and RF-45301 from SHIMADZU, respectively. NMR spectra were obtained on a Bruker AVANCE-600 spectrometer in DMSO-d_6, and chemical shift values are reported in δ (ppm) and coupling constants in Hertz. HRMS spectra were recorded on the AB Sciex 5600 Triple TOF mass spectrometer (Foster, CA, USA). Cells and zebrafish fluorescent images were acquired on confocal microscopy with Nikon-A1.

*2.2. Synthesis of **SPTPA***

To a solution of 6-(tert-butyl)-1',3',3'-trimethylspiro[chromene-2,2'-indoline]-8-carbaldehyde (300 mg, 0.83 mmol) in absolute ethanol (EtOH, 30 mL) was added (E)-4-(hydrazineylidenemethyl)-N, N-diphenylaniline (238 mg, 0.83 mmol).[1] The reaction was thoroughly mixed and stirred at room temperature for 16 hours, then the reaction mixture was filtered using a Buchner funnel and then washed with ethanol to afford 183 mg of **SPTPA** as a yellow solid. Yield: 35%; ^{1}H NMR (600 MHz, DMSO-d_6) δ in ppm: 8.80 (s, 1H), 8.49 (s, 1H), 7.75 (s, 1H), 7.62 – 7.47 (m, 2H), 7.20 (br s, 6H), 7.11 – 6.87 (m, 10H), 6.76 (br s, 3H), 2.33 (s, 3H), 1.12 (s, 9H), 1.10 (s, 3H), 1.04 (s, 3H); HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{43}H_{43}N_4O$, 630.34, found, 631.3425. ^{13}C NMR (101 MHz, DMSO) δ 162.38, 160.05, 150.81, 146.80, 146.71, 146.66, 130.42, 130.32, 130.28, 130.05, 126.57, 125.98, 125.83, 125.78, 125.69, 125.02, 124.87, 124.82, 121.14, 120.78, 119.82, 31.55. The SPTPA probe after reaction to ClO^-: HRMS (ESI) m/z $[M+1]^+$: Calcd for $C_{43}H_{43}N_4O$, 631.34, found, 632.35. ^{1}H NMR (400 MHz, DMSO) δ 8.83 (s, 1H), 8.40 (s, 1H), 7.66 (s, 1H), 7.63 – 7.40 (m, 3H), 7.19 (br s, 6H), 6.93 (br s, 10H), 6.77 (s, 3H), 2.35 (s, 3H), 1.08 (s, 9H), 1.04 (s, 3H), 0.93 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 149.86, 146.88, 146.71, 146.63, 130.31, 130.27, 130.06, 129.68, 128.02, 125.84, 125.78, 125.54, 124.90, 124.65, 121.51, 121.00, 31.92, 31.54.

2.3. Spectrum Measurements

The stock solution of **SPTPA** was prepared in DMSO with the concentration of 1 mM, which prepared working solution with DMSO/H_2O (v/v, 5:1) system in 10 μM and 2.0 μM for absorption spectrum and emission spectrum tests, respectively. The detecting ions (ClO^-) and other various interferential analytes stock solutions [F^-, Cl^-, NO_2^-, ClO_4^-, HCO_3^-, $H_2PO_4^-$, SO_4^{2-}, $S_2O_3^{2-}$, CO_3^-, H_2O_2] were prepared at 100 μM in deionized water. The work system solution contained **SPTPA**, DMSO/H_2O (5:1) and 3.0 equiv. of each analytes.

2.4. Cytotoxicity Assays

The toxicological evaluation of **SPTPA** to MRC-5 cells were employed by traditional methods MTT assays.[2, 3] MRC-5 cells were cultured and seeded in 96-well plates with 2×10^4 cells/mL, and after treatment by various concentrations of **SPTPA** from 0 μM to 80 μM at 37 °C for 24h. After that 10 μL MTT (5 mg/mL) was added to each well, and incubated with 4 h under the same condition, and was added 150 μL DMSO to dissolve precipitation after the supernatants were aspirated. To record the absorbance at 570 nm by microplate reader. The cell viability (%) = (ODs-ODb) / (ODc-ODb) × 100 %, there s-sample, b-blank and c-control.

2.5. Cell Culture and Imaging

Dulbecco's Modified Eagle Medium (DMEM, Hyclone) used for MRC-5 cells cultured, and 10% fetal calf serum (FBS, Sijiqing), penicillin (100 U / ml, Hyclone) and streptomycin sulfate (100 U / ml, Hyclone) were added in above medium, and cultured at 37 °C of 5% CO_2 and 95% air atmosphere condition.[4, 5] MRC-5 cells were seeded in 6-well plates processing for the night prior to imaging experiments. After that the cells were pretreated with 2.0 μM free **SPTPA** probe for 20 min, and then incubated with 6.0 μM of ClO^- for 20 min. Cellular imaging was performed after washing the cells thrice with PBS, and the negative control was treated with N-acetylcysteine (100 μM), which was inhibited produce of endogenous ClO^-. Fluorescent imaging was taken in green/blue channels on confocal microscopy with Nikon-A1.

2.6. Fluorescence Imaging in Zebrafish

The 5-day old zebrafish were cultured and transferred to 24-well plate, and 2.0 μM of free **SPTPA** probe was added for 20 minutes and washed with PBS, and the negative control was treated with N-acetylcysteine (100 μM), which was inhibited produce of endogenous ClO^-, and then 6.0 μM of ClO^- was added respectively, which were incubated with 20 minutes further. The zebrafish were washed and transferred to a new confocal dish for narcosis (3-Aminobenzoic acid ethyl ester methanesulfonate, MS222) and imaging.

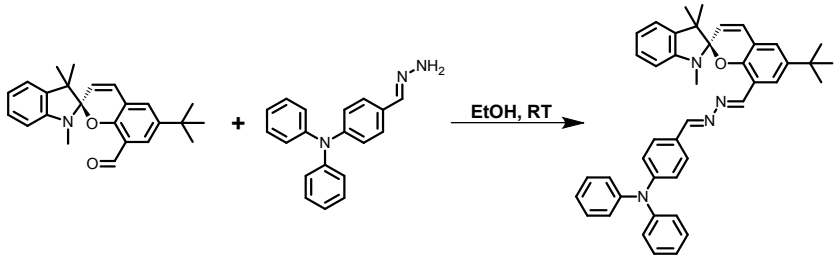
2.7. Quantum calculations

The geometry of the free **SPTPA** probe and the **SPTPA** complexes in the ground state was optimized by density functional theory (DFT) based on the RB3LYP / 6-31G basis set.[6, 7] The electronic cloud distribution and related optical properties of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) states are calculated by DFT at the same theoretical level. Vibration analysis calculations were employed to confirm that the local minimum of the resulting optimized geometry over the entire molecular potential energy surface.

2.8. Live subject statement

All animal operations (fluorescence imaging in zebrafish) were performed in accordance with the guidelines for laboratory animal care and use at Wenzhou Medical University and approved by the Animal Ethics Committee of Wenzhou Medical University.

Scheme/Figure Captions:



Scheme S1 Synthesis procedure of **SPTPA**.

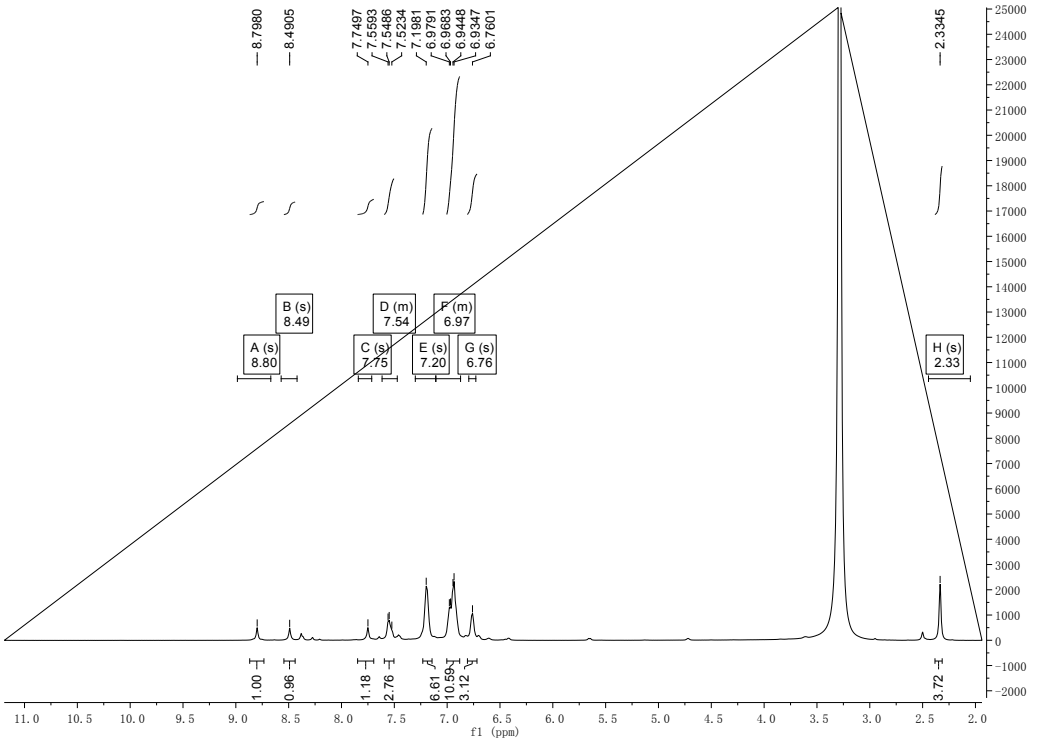


Fig.S1 ^{1}H NMR spectrum of **SPTPA** in DMSO-d6.

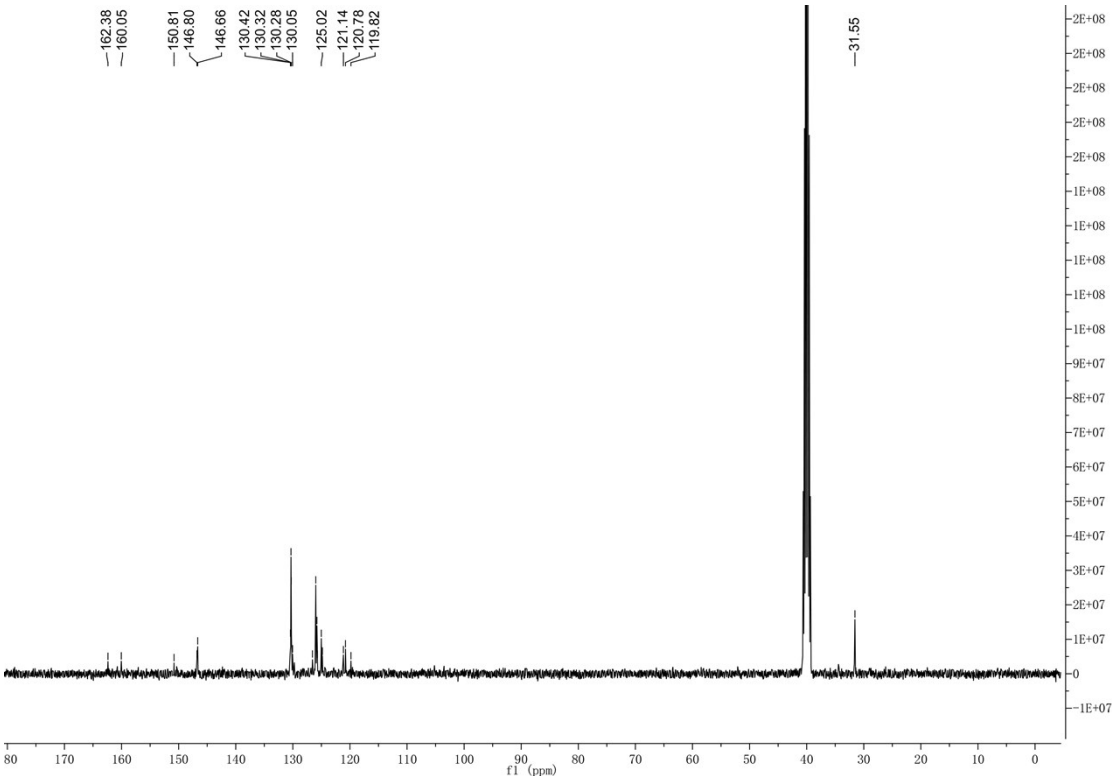


Fig.S2 ^{13}C NMR spectrum of **SPTPA** in DMSO-d6.

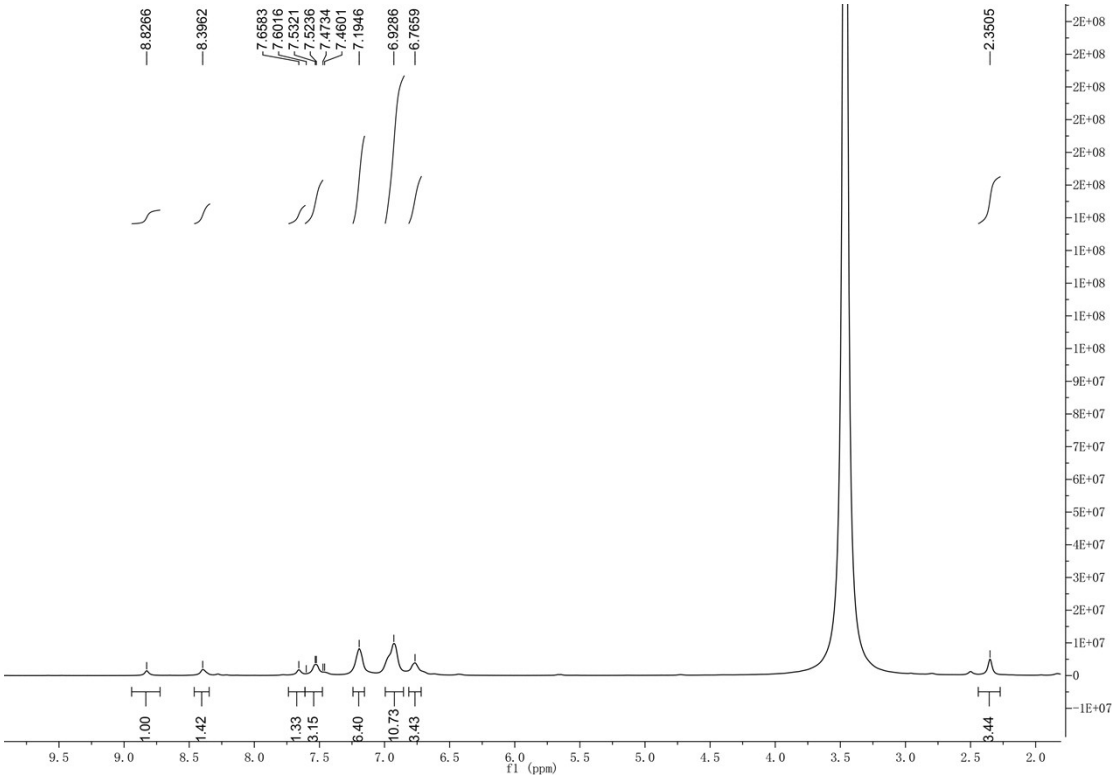


Fig.S3 ^{1}H NMR spectrum of **SPTPA** after reaction with ClO^- in DMSO-d6.

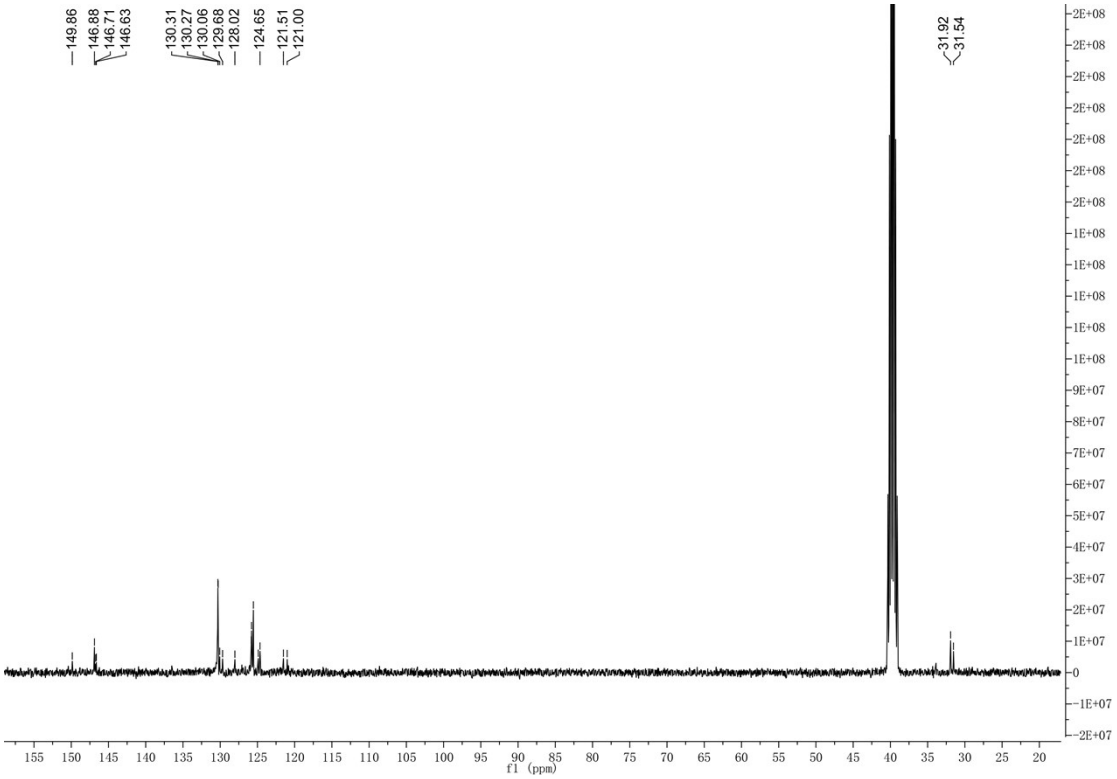


Fig.S4 ^{1}H NMR spectrum of **SPTPA** after reaction with ClO^- in DMSO-d6.

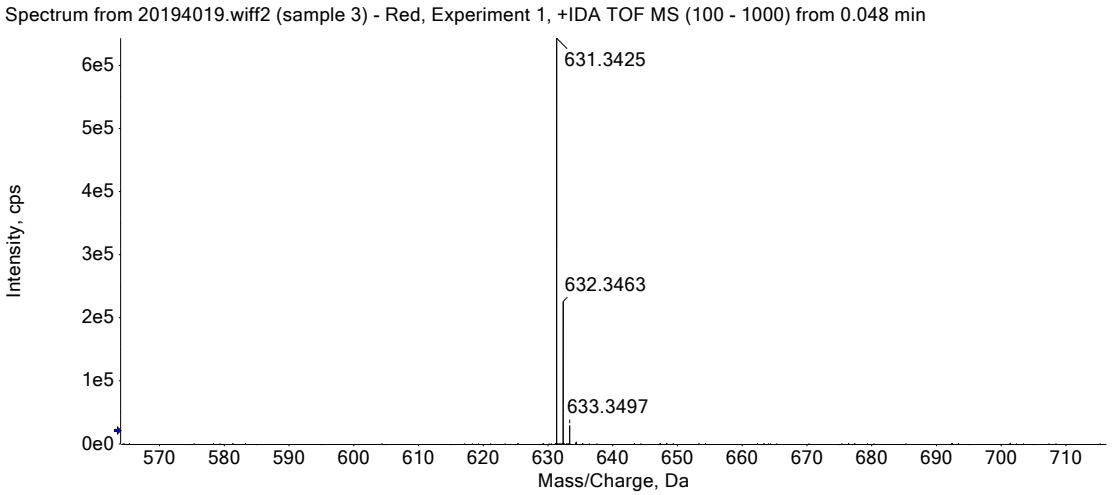


Fig.S5 HR-MS spectrum of free **SPTPA**.

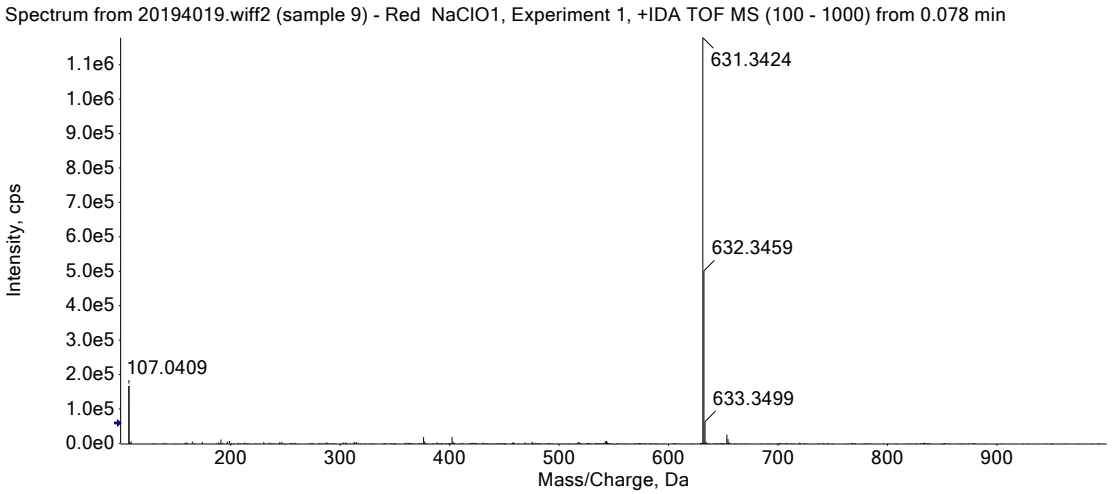


Fig.S6 HR-MS spectrum of **SPTPA** response to hypochlorite.

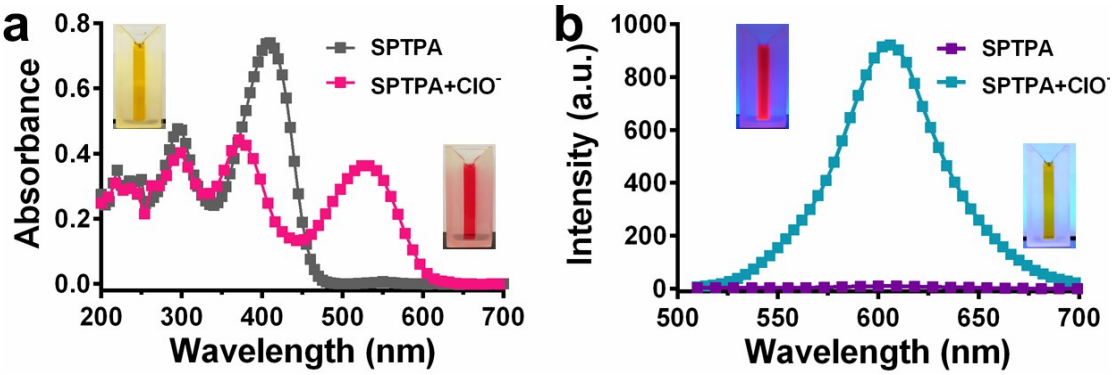


Fig.S7 (a) Absorption spectra (10 μM) and (b) fluorescence emission spectra (2.0 μM) as well as the color change (insets) of **SPTPA** before and after reaction with ClO^- (3.0 eq.) at in DMSO/H_2O solution system.

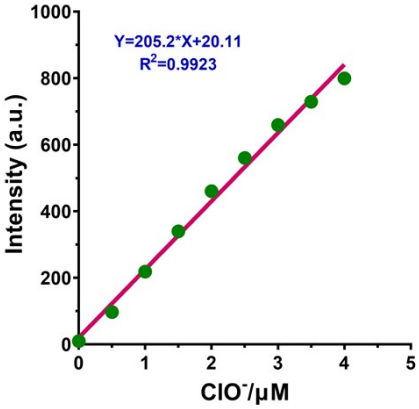


Fig.S8 Linear dynamic fluorescence response for the titration of **SPTPA** probe with ClO^- to determine the limit of detection (LOD).

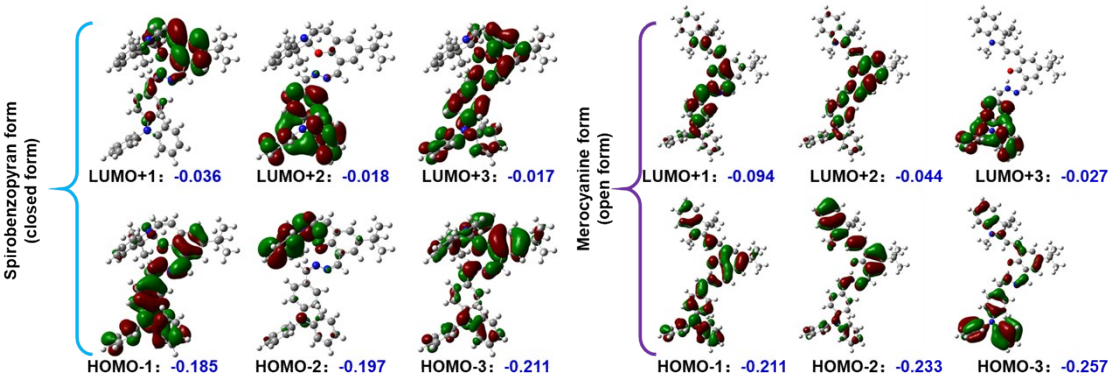


Fig.S9 Frontier molecular orbitals of **SPTPA** and after sensing ClO^- with spirobenzopyran form (closed form) and merocyanine form (open form).

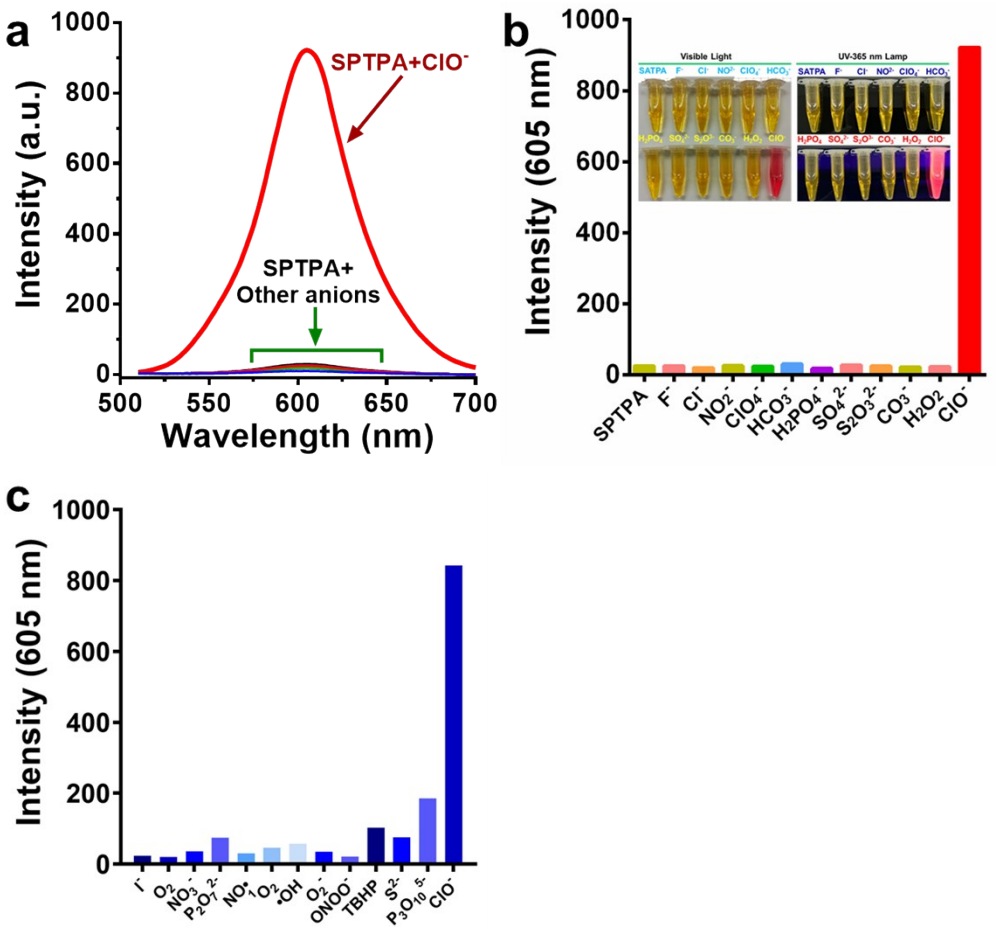


Fig.S10 (a) Emission spectra of **SPTPA** (2.0 μM) for ClO^- over various species (3.0 eq.) in DMSO/H_2O solution system. (b) and (c) Fluorescence intensity changes at 605 nm of **SPTPA** addition of analyte, including F^-, Cl^-, NO_2^-, ClO_4^-, HCO_3^-, $H_2PO_4^-$, SO_4^{2-}, $S_2O_3^{2-}$, CO_3^-, H_2O_2, I^-, O_2, NO_3^-, S^{2-}, $P_2O_7^{2-}$, $P_3O_{10}^{5-}$, NO•, $^1O_2$, •OH, O_2^-, $ONOO^-$, TBHP and ClO^-. λex = 408 nm.

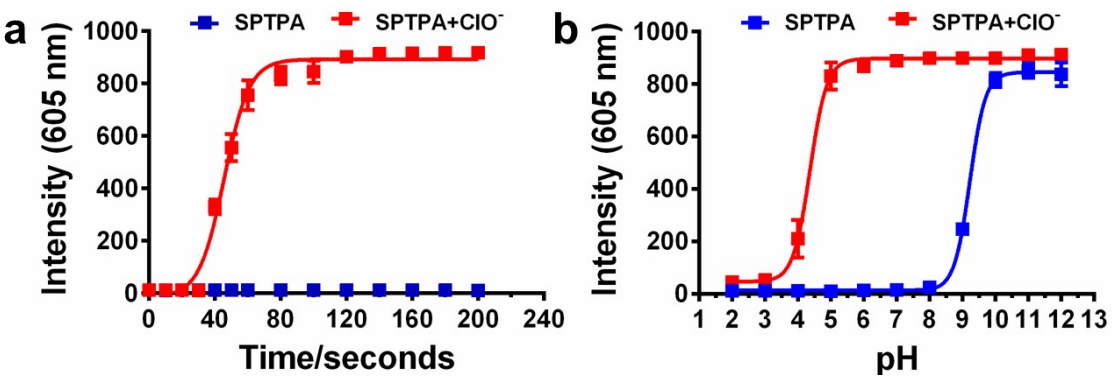


Fig.S11 (a) Time-dependent fluorescence of **SPTPA** before and after treated with 3.0 equiv. of ClO^-. (b) Effects of pH on **SPTPA** (2.0 μM) and its recognition ability for ClO^-. The data represent the fluorescence intensities at 605 nm and each spectrum was acquired 3 min after ClO^- addition at 25 °C. Conditions: λex = 400 nm, λem = 605 nm.

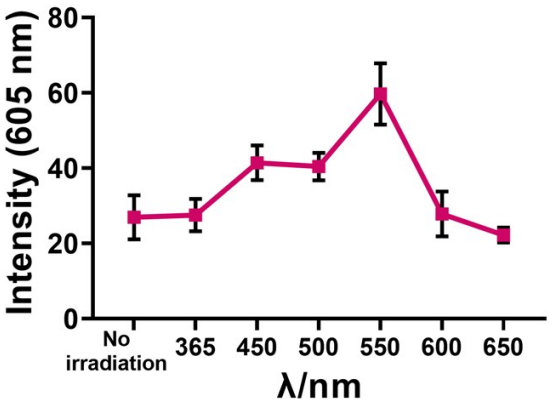


Fig.S12 Fluorescence intensity change of **SPTPA** at 605 nm after irradiation by various excitation wavelength for 1 min.

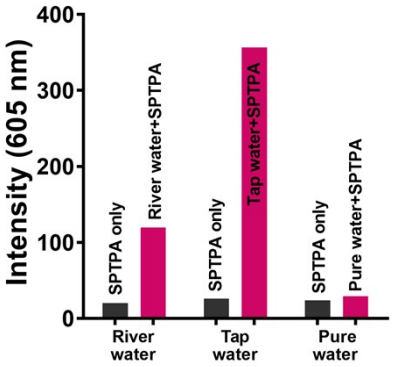


Fig.S13 Fluorescence intensity change of **SPTPA** (2.0 μM) at 605 nm after addition of river, tap and pure water.

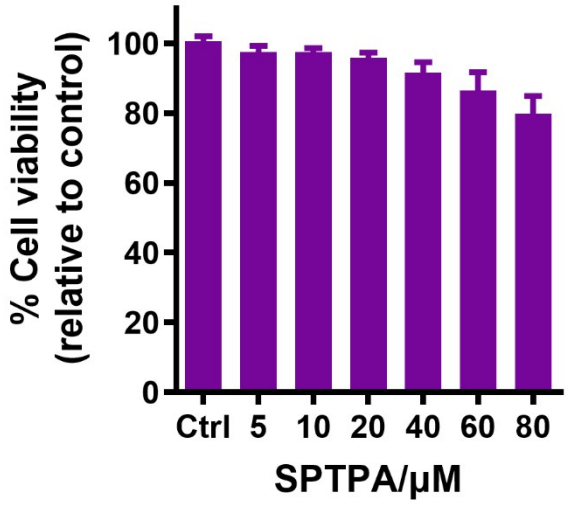


Fig.S14 Different concentrations of **SPTPA** (0 μM, 5 μM, 10 μM, 20 μM, 40 μM, 60 μM and 80 μM) were tested in MRC-5 cells for toxicity with MTT assay.

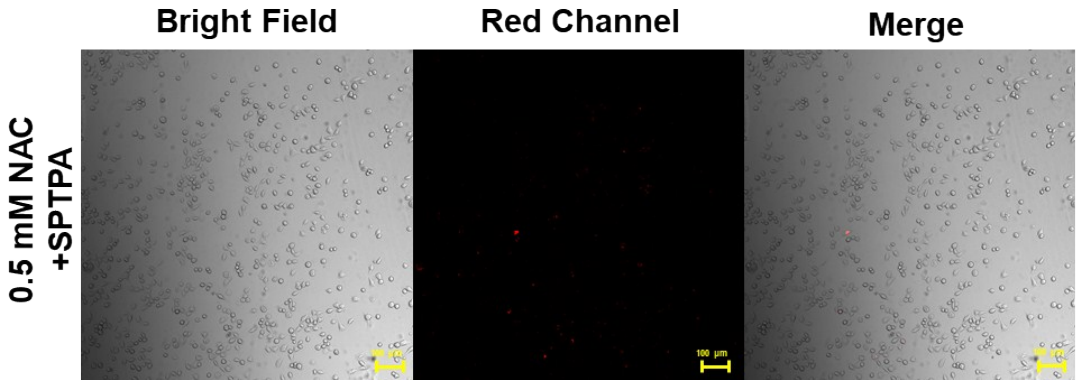


Fig.S15 Confocal fluorescence images of MRC-5 cells. MRC-5 cells were incubated with N-acetylcysteine (100 μM) for 20 min, further incubated with **SPTPA** (2.0 μM) for 20 min.

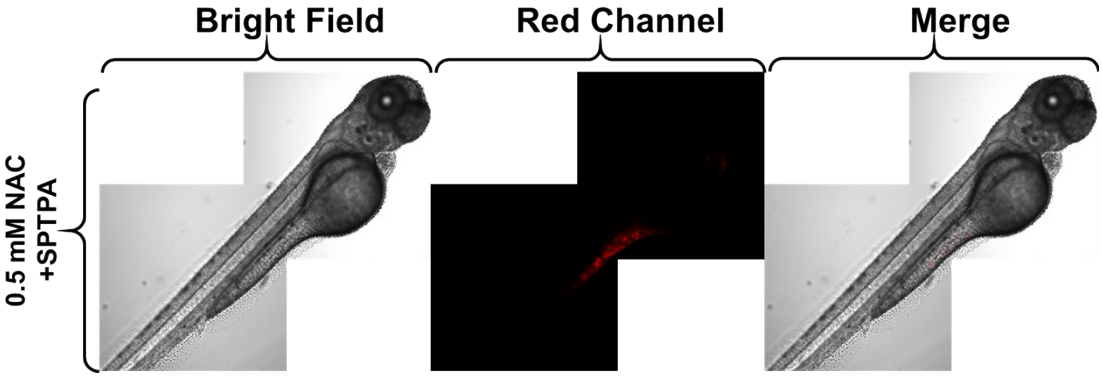


Fig.S16 Confocal fluorescence images of zebrafish. Zebrafish were incubated with N-acetylcysteine (100 μM) for 20 min, further incubated with **SPTPA** (2.0 μM) for 20 min.

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