Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A highly sensitive and selective fluorescent probe for fast sensing of endogenous HClO in living cells

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Table of contents

1. Reagents and instruments

All chemicals and solvents used for synthesis were purchased from commercial suppliers and applied directly in the experiments without further purification. The progress of the reaction was monitored by TLC on pre-coated silica plates (Merck 60F-254, 250 μm in thickness), and spots were visualized by UV light. Merck silica gel 60 (70-200 mesh) was used for general column chromatography purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 (400 MHz ^{1}H and 101 MHz ^{13}C) spectrometer at room temperature. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ($Si(CH_3)_4$ = 0.00 ppm) or residual solvent peaks ($CDCl_3$ = 7.26 ppm; DMSO-d_6 = 2.50 ppm). ^{1}H NMR coupling constants (*J*) are reported in Hertz (Hz), and multiplicity is indicated as the following: *s* (singlet), *d* (doublet), *dd* (doublet doublet), *m* (multiple). High-resolution mass spectra (HRMS) were obtained on an Agilent 6540 UHD Accurate-Mass Q-TOFLC/MS. The UV-visible spectra were recorded on a UV-3600 UV-VIS-NIR spectrophotometer (SHIMADZU, Japan). Fluorescence studies were carried out using F-280 spectrophotometer (Tianjin Gangdong Sci & Tech., Development. Co., Ltd). The cellular bioimaging was performed on a confocal microscope (Leica TCS SP8).

2. Synthesis

Synthesis of 2. Synthesis of **2** was according the literature.[1] To a solution of fluorescein (996 mg, 3 mmol) in anhydrous DMF (10 ml), K_2CO_3 (830 mg, 6mmol) and CH_3I (375 μl, 6 mmol) were added. After stirred for 24 h, the reaction mixture was diluted with H_2O (100 ml). The resulted precipitate was filtered and redissolved with ethyl acetate, the organic phase was washed with brine, dried by anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was subjected to silica gel chromatography with CH_2Cl_2/CH_3OH (100/1) to give a yellow solid 2 (930 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 8.00 (d, *J* = 7.5 Hz, 1H), 7.78 (t, *J* = 7.4 Hz, 1H), 7.71 (t, *J* = 7.4 Hz, 1H), 7.26 (d, *J* = 7.6 Hz, 1H), 6.93 (d, *J* = 2.1 Hz, 1H), 6.74-6.63 (m, 3H), 6.59 (s, 2H), 3.80 (s, 3H); HRMS (ESI): m/z 347.0916 $[M+H]^+$ (calcd for $C_{14}H_{13}O_5^+$, 347.0914).

Synthesis of 3. To a solution of **2** (170 mg, 0.5 mmol) in anhydrous DMF (5 ml), Cs_2CO_3 (325 mg, 1 mmol) and NaH (100 mg, 2.5 mmol) were added one by one, the mixture was stirred for 20 min at room temperature and then *p*-fluoronitrobenzene (350 mg, 2.5 mmol) was added. The above solution was heated to 120 °C for 8 h. After cooled to room temperature, the mixture was diluted with H_2O (100 mL), which was extracted with ethyl acetate, and the organic phase was washed with brine, dried by anhydrous Na_2SO_4. After removed the solvent under reduced pressure, the resulted residue was purified by silica gel column chromatography with CH_2Cl_2 to get a light yellow solid **3** (201 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 9.0 Hz, 2H), 8.03 (d, J = 7.6 Hz, 1H), 7.71 (t, J = 7.4 Hz, 1H), 7.65 (t, J = 7.4 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.10 (d, J = 9.1 Hz, 2H), 6.98 (d, J = 2.0 Hz, 1H), 6.84 (d, J = 8.7 Hz, 1H), 6.78 – 6.68 (m, 3H), 6.63 (dd, J = 8.8, 2.1 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4, 162.1, 161.6, 156.7, 152.8, 152.7, 152.3, 143.4, 135.4, 130.1, 129.1, 126.7, 126.1, 125.3, 124.0, 118.2, 116.1, 115.8, 112.1, 110.9, 108.2, 101.0, 82.7, 55.7. HRMS (ESI): m/z 468.1095 $[M+H]^+$ (calcd for $C_{14}H_{13}O_5^+$, 468.1078).

Synthesis of probe 1. To a mixed solution (8 ml, CH_3OH/concentrated HCl/H_2O = 3/2/3), **3** (67 mg, 0.14 mmol) and $SnCl_2$•$2H_2O$ (472 mg, 2.1 mmol) were added. The reaction mixture was stirred for 12 h at room temperature. Under an ice base, the reaction solution was adjusted to alkalescence with saturated sodium carbonate solution, which was extracted with ethyl acetate, and the organic phase was washed with brine, dried by anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was subjected to silica gel chromatography with CH_2Cl_2/ ethyl acetate (15/1) to give a white solid **1** (30 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 7.5 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.61 (t, J = 7.1 Hz, 1H), 7.17 (d, J = 7.5 Hz, 1H), 6.90 (d, J = 8.7 Hz, 2H), 6.75 – 6.58 (m, 8H), 3.81 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.5, 161.5, 161.1, 153.2, 152.6, 152.5, 147.6, 143.0, 135.1, 129.8, 129.4, 129.2, 126.9, 125.1, 124.1, 121.8, 116.7, 113.4, 112.6, 111.8, 111.3, 104.3, 100.9, 83.2, 55.7. HRMS (ESI): m/z 438.1343 $[M+H]^+$ (calcd for $C_{14}H_{13}O_5^+$, 438.1336).

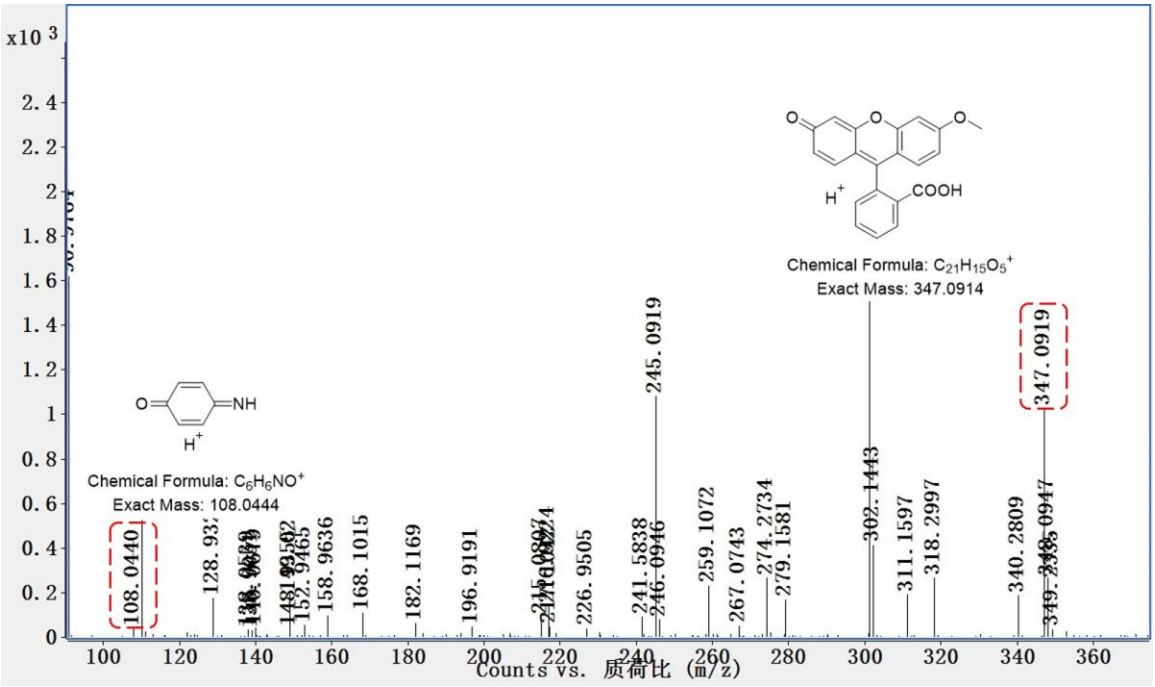


Fig. S1. HRMS spectrum of the products from the reaction between probe **1** and HClO. Probe **1** (500 μM) was treated with HClO (500 μM) in PBS buffer (50 mM, pH 7.4 containing 50% EtOH) for 1 h at room temperature.

3. Spectroscopic studies

Spectroscopic experiments were normally performed in phosphate buffer saline (PBS, 50 mM, pH 7.4 containing 5% EtOH) at room temperature. Probe **1** was dissolved into EtOH to prepare the stock solution with a concentration of 2.0 mM. Probe **1** was diluted in PBS buffer to afford the final concentration of 5 or 20 μM. All measurements were performed in a 3 ml corvette with 2 ml solution.

For the study on selectivity, HOCl was obtained from 14.5% NaOCl solution, and the concentration was determined from absorption at λ = 292 nm (ε = 350 $M^{-1}\cdot cm^{-1}$);[2] H_2O_2 was diluted from 30% solution, and the concentration was determined from absorption at λ = 240 nm (ε = 43.6 $M^{-1}\cdot cm^{-1}$); $^{\bullet}OH$ was generated by Fenton reaction between $FeSO_4$ and H_2O_2; $^{t}BuOO^{\bullet}$ was prepared from the reaction between $^{t}BuOOH$ and H_2O_2; $ONOO^-$ was obtained by the reaction of H_2O_2 and $NaNO_2$[3] and the concentration was determined from absorption at λ = 302 nm (ε = 1670 $M^{-1}\cdot cm^{-1}$) in 0.1 M NaOH solution;[2] Na_2S was used as the H_2S donor; KO_2 was dissolved into DMSO to provide O_2^-. All the analytes were prepared as stock solutions in PBS buffer except KO_2. Appropriate amount of bio-

related species were added to separate portions of the probe solution and mixed thoroughly. All species were incubated with **1** (5 μM) for 10 min at room temperature.

For evaluating the effects of pH, **1** (5 μM) was treated with HClO (1 eq.) in PBS buffer at diverse pH values (from 4.5 to 9.5) at room temperature and data were recorded at different time points.

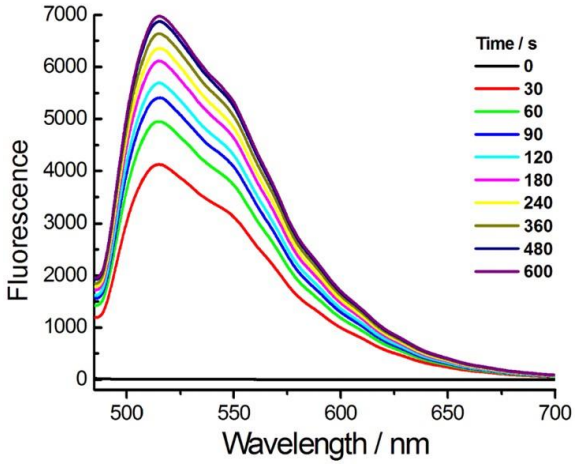


Fig. S2. Time-dependent fluorescence spectra of **1** (5 μM) toward HClO (5 μM) in PBS buffer (50 mM, pH 7.4, containing 5% EtOH).

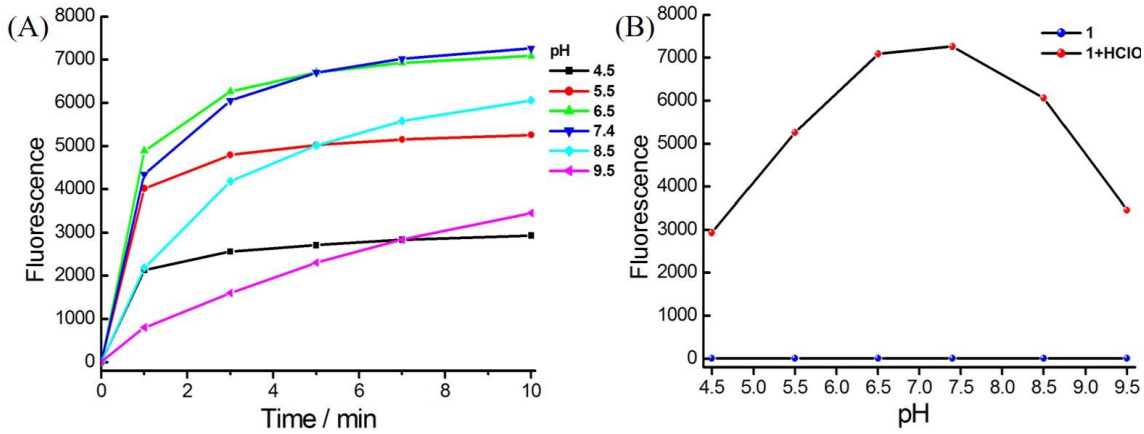


Fig. S3. (A) Time-dependent emission of **1** (5 μM) at 515 nm with HClO (5 μM) in PBS buffer (50 mM containing 5% EtOH) at different pH values. (B) Emission of **1** (5 μM) at 515 nm incubated with HClO (5 μM) or not for 10 min in PBS buffer at different pH values.

4. MTT assay

The *in-vitro* cytotoxicity was measured using standard methyl thiazolyl tetrazolium (MTT, Sigma-Aldrich) assay in RAW 264.7 cell lines. Briefly, cells growing in log phase

were seeded into 96 well cell-culture plate at 1×10^4/well. The probe (100 μl/well) at concentrations of 0-10 μM was added to the wells of the treatment group, and 100 μl/well EtOH diluted in DMEM at final concentration of 1.65% to the negative control group, respectively. The cells were incubated for 24 h at 37 °C under 5% CO_2, respectively. The combined MTT/PBS solution was added to each well of the 96-well assay plate and incubated for another 4 h. An enzyme-linked immunesorbent assay (ELISA) reader (Bio-Rad Laboratories, Hercules, CA, USA) was used to measure the OD490 (absorbance value) of each well referenced at 490 nm. The following formula was used to calculate the viability of cell growth: viability (%T) = $A_1/A_2 \times 100\%$, where A_1 denotes absorbance value of treatment group, and A_2 denotes absorbance value of control.

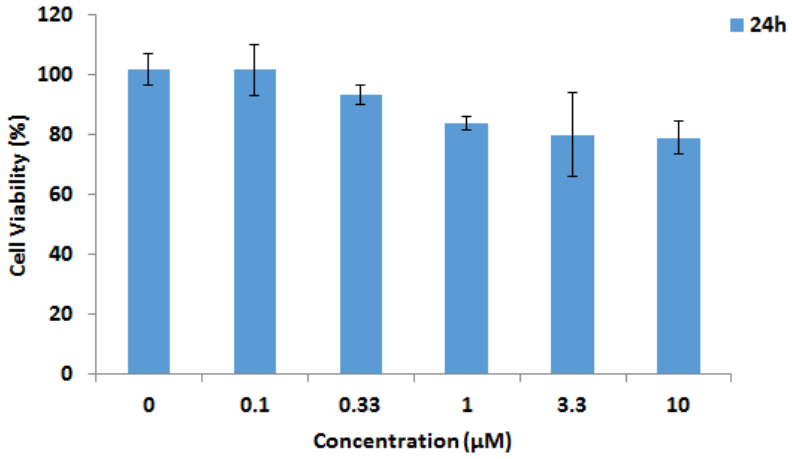


Fig. S4. The cytotoxicity of the probe **1** evaluated by the MTT assay.

5. Cell culture and bioimaging

RAW 264.7 cells (mouse macrophage cell line) were grown in RPMI 1640 supplemented with FBS (10%), penicillin (100 U/ml) and streptomycin (100 U/ml). All cells were maintained in an incubator at 37 ℃ with 5% CO_2/air environment. Cells were passaged every 2-3 days and used between passages 3 and 10.

For the exogenous HClO detection, cells were incubated with **1** (5 μM) for 30 min, washed with PBS and then treated with NaClO (5 or 20 μM) for 30 min before imaging. For the LPS/PMA-stimulated endogenous HClO detection, cells were treated with LPS (1 μg/mL) for 12 h, then PMA (1 μg/mL) for 1 h, washed by PBS and then incubated with **1** (5 μM) for 30 min before imaging. Scavenger-treated cells were pretreated with ebselen

(100 μM) for 1 h before incubation with **1**. For the elesclomol-induced endogenous HClO detection, cells were treated with elesclomol (2 μM) for 1 or 2 h, washed with PBS and then incubated with **1** (5 μM) for 30 min before imaging. For the H_2S-induced endogenous HClO detection, cells were treated with H_2S (50, 100 and 150 μM) for 1 h, washed with PBS and then incubated with **1** (5 μM) for 30 min before imaging. Scavenger-treated cells were pretreated with ebselen (100 μM) for 30 min. For the study on the order of **1** and H_2S incubated with cells, their orders were exchanged but the incubation time remained unchanged. The cells incubated with probe **1** (5 μM) alone were treated for 30 min and then imaged. Cetyltrimethyl ammonium bromide (CTAB, 1 mM) was introduced into the bioimaging experiments. Emissions were collected at green channel (500-600 nm) with 458 nm excitation.

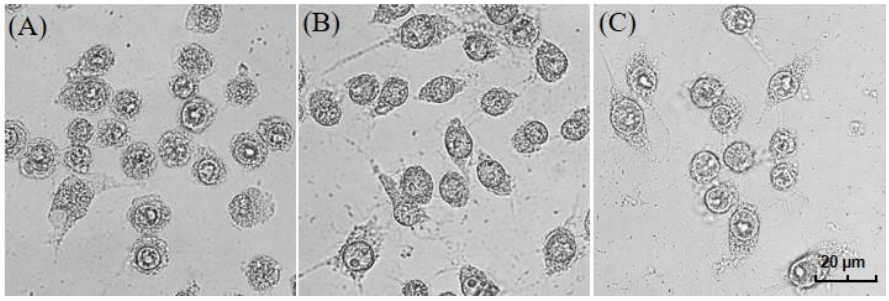


Fig. S5. Bright-field microscopy images of exogenous HClO detection in living RAW 264.7 cells using **1**. Cells were incubated with (A) **1** (5 μM) alone for 30 min; (B) **1** (5 μM) for 30 min and then NaClO (5 μM) for another 30 min; (C) **1** (5 μM) for 30 min and then NaClO (20 μM) for another 30 min. Scale bar, 20 μm.

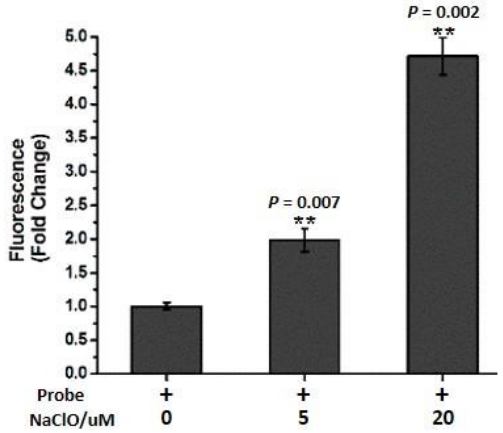


Fig. S6. The relative fluorescence ratio of confocal microscopy images for detection of exogenous HClO in living RAW 264.7 cells using **1**. N = 3 fields of cells, error bars are ± sd.

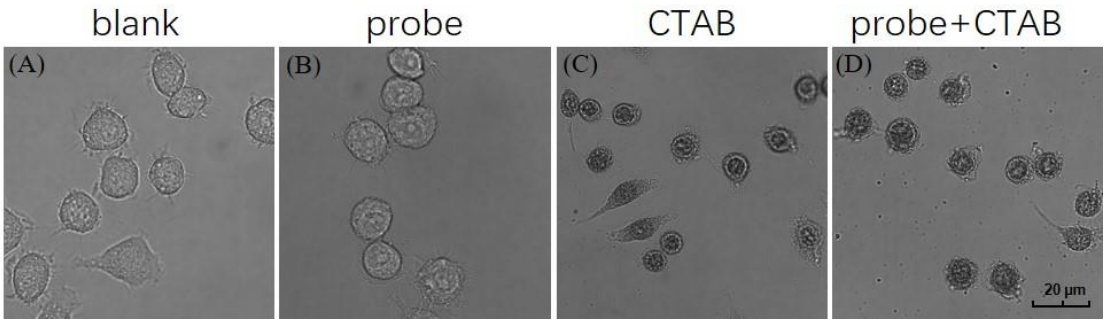


Fig. S7. Investigation about the influence of probe **1** or CTAB on the cell morphology. Bright-field microscopy images of (A) untreated cells; (B) cells incubated with **1** (5 μM) alone for 30 min; (c) cells treated by CTAB (1 mM) alone for 30 min; (D) cells coincubated with **1** (5 μM) and CTAB (1 mM) for 30 min. The probe itself had no unfavorable influence on the cell health.

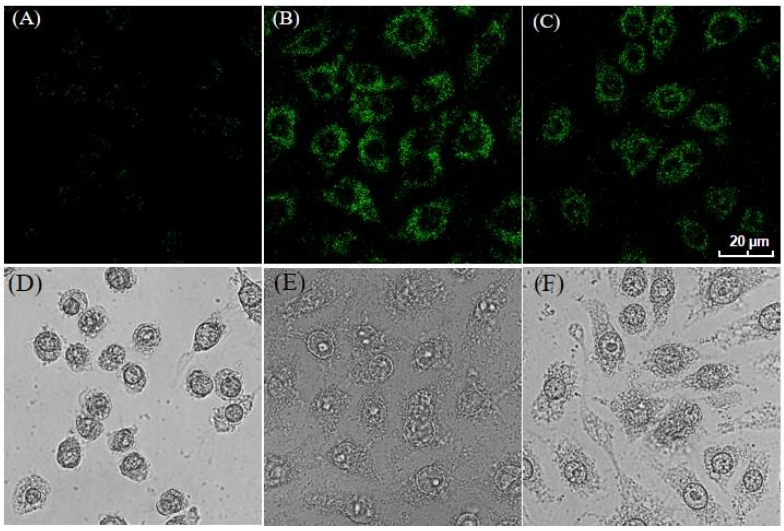


Fig. S8. Confocal microscopy images of LPS/PMA-stimulated endogenous HClO detection in living RAW 264.7 cells using **1**. Cells were incubated with (A) **1** (5 μM) alone for 30 min; (B) LPS (1 μg/ml) for 12 h, then PMA (1 μg/ml) for 1 h, and then **1** (5 μM) for 30 min. (C) Cells were treated with LPS (1 μg/mL) for 12 h, PMA (1 μg/mL) for 1 h, and then ebselen (100 μM) for 1h before incubation with **1** (5 μM) for another 30 min. (D-F) Bright-field microscopy images of A-C. Emissions were collected at green channel (500-600 nm) with 458 nm excitation. Scale bar, 20 μm.

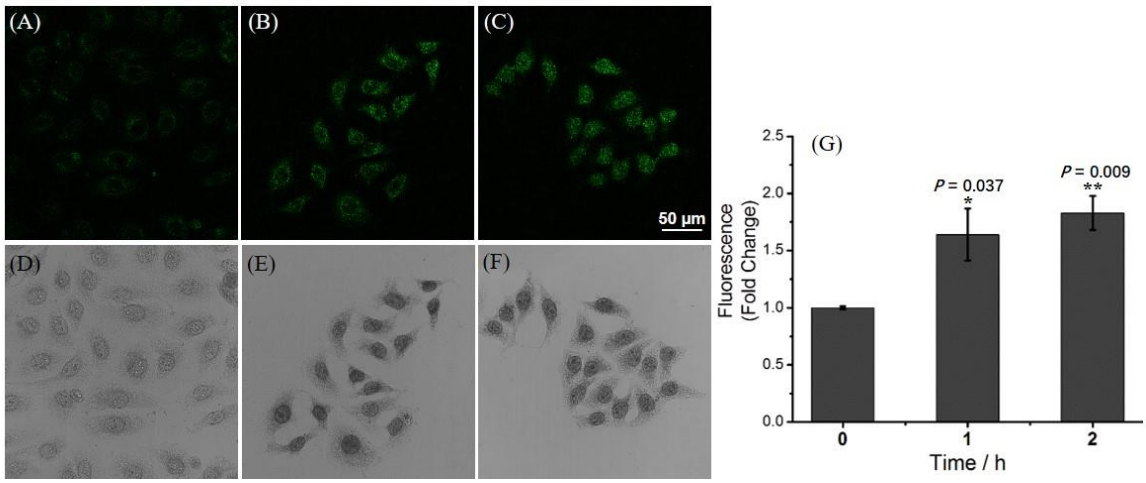


Fig. S9. Confocal microscopy images of elesclomol-induced endogenous HClO detection in living MCF-7 cells using **1**. Cells were incubated with (A) **1** (5 μM) alone for 30 min; (B) elesclomol (2 μM) for 1 h, then **1** (5 μM) for 30 min; (C) elesclomol (2 μM) for 2 h, then **1** (5 μM) for 30 min. (D-F) Bright-field microscopy images of A-C. (G) The relative fluorescence ratio for images A-C. Emissions were collected at green channel (500-600 nm) with 458 nm excitation. Scale bar, 50 μm. *N* = 3 fields of cells, error bars are ± sd.

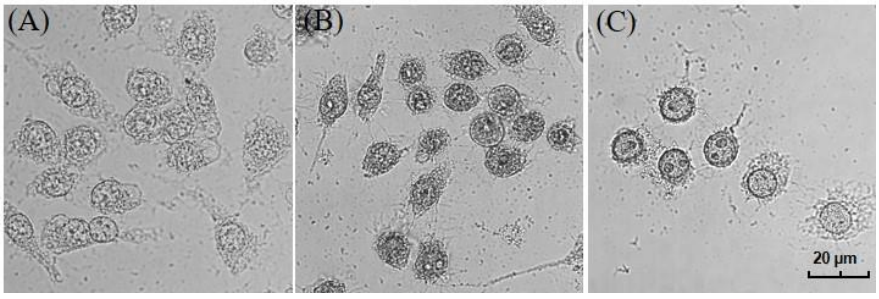


Fig. S10. Bright-field microscopy images of H_2S-induced endogenous HClO detection in living RAW 264.7 cells using **1**. Cells were incubated with (A) **1** (5 μM) alone for 30 min; (B) Na_2S (150 μM) for 1 h, then **1** (5 μM) for 30 min. (C) Cells were pretreated with ebselen (100 μM) for 30 min. Scale bar, 20 μm.

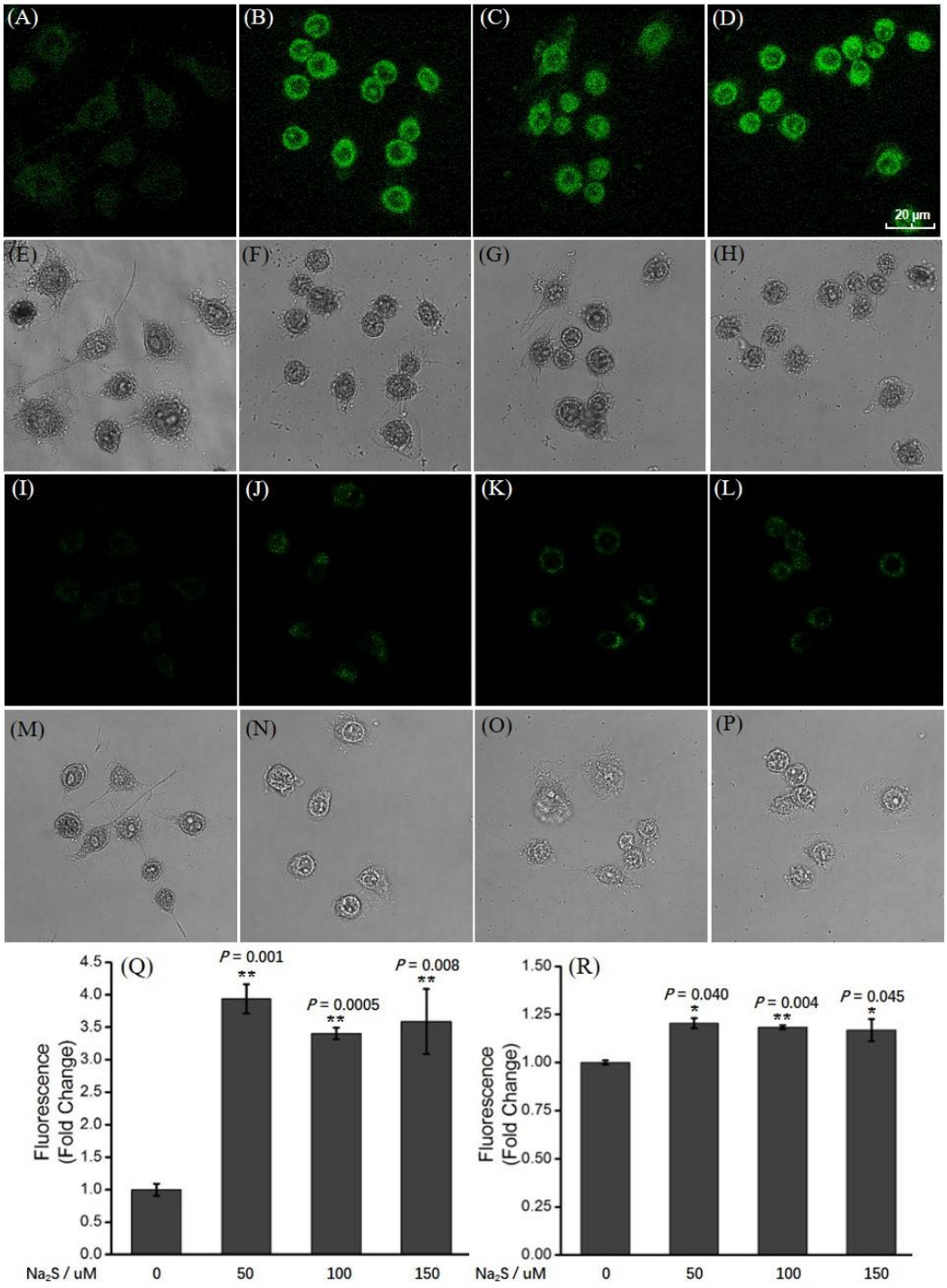
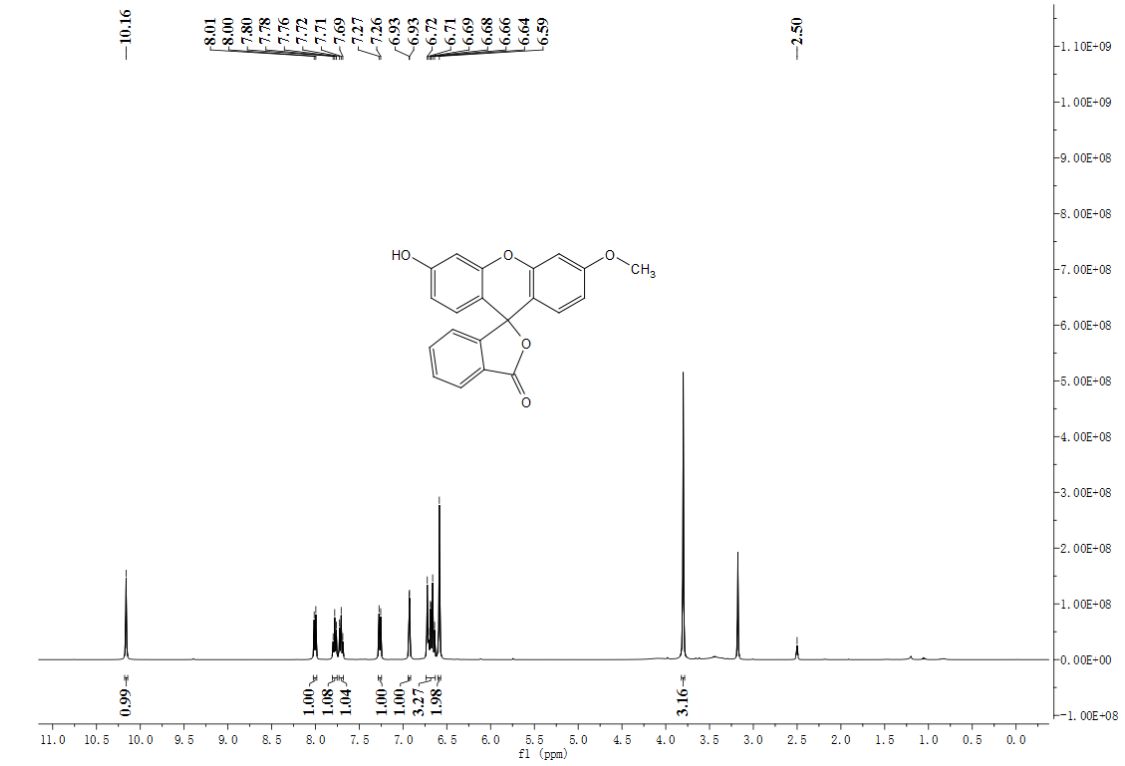


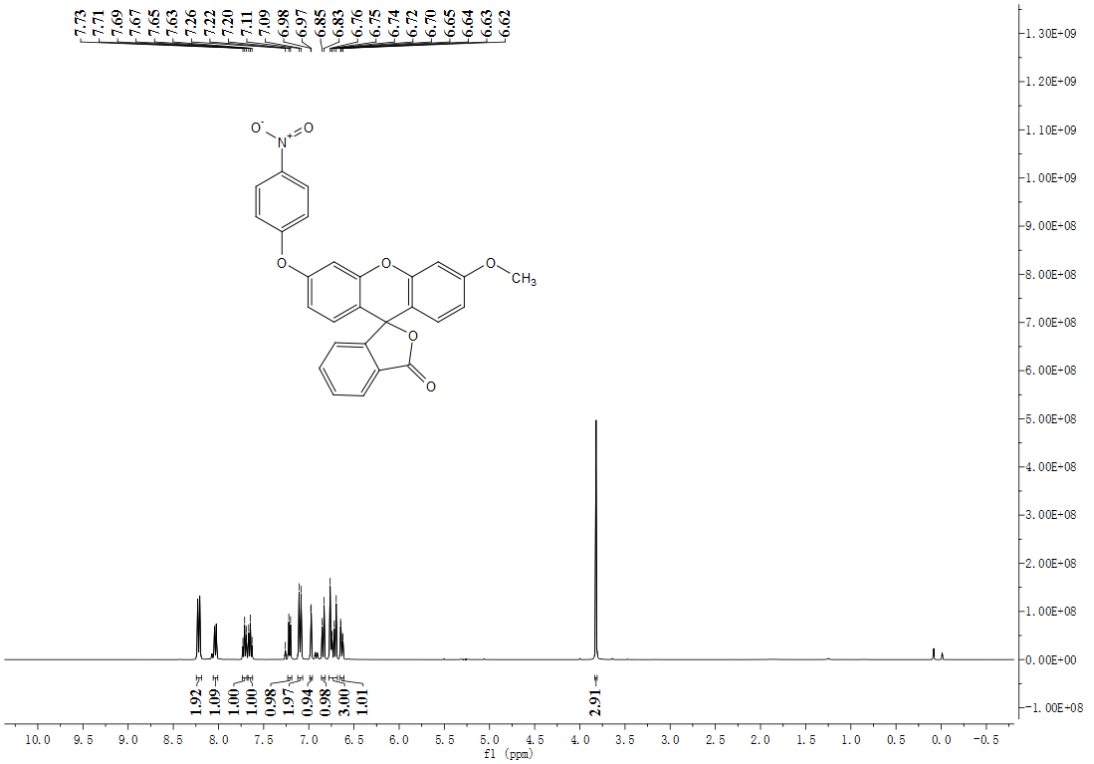
Fig. S11. Confocal microscopy images of H_2S-induced endogenous HClO detection in living RAW 264.7 cells using **1**. (A and I) Cells were incubated with **1** (5 μM) alone for 30 min. Cells were incubated with Na_2S (B, 50 μM; C, 100 μM; D, 150 μM) for 1 h, then **1** (5 μM) for 30 min. Cells were incubated with **1** (5 μM) for 30 min, washed by PBS, then treated by Na_2S (J, 50 μM; K, 100 μM; L, 150 μM) for 1 h. (E-H, M-P) Bright-field microscopy images. (Q) The relative fluorescence ratio for images A-D. (R) The relative fluorescence ratio for images I-L. Emissions were collected at green channel (500-600 nm) with 458 nm excitation. Scale bar, 20 μm. N = 3 fields of cells, error bars are ± sd.

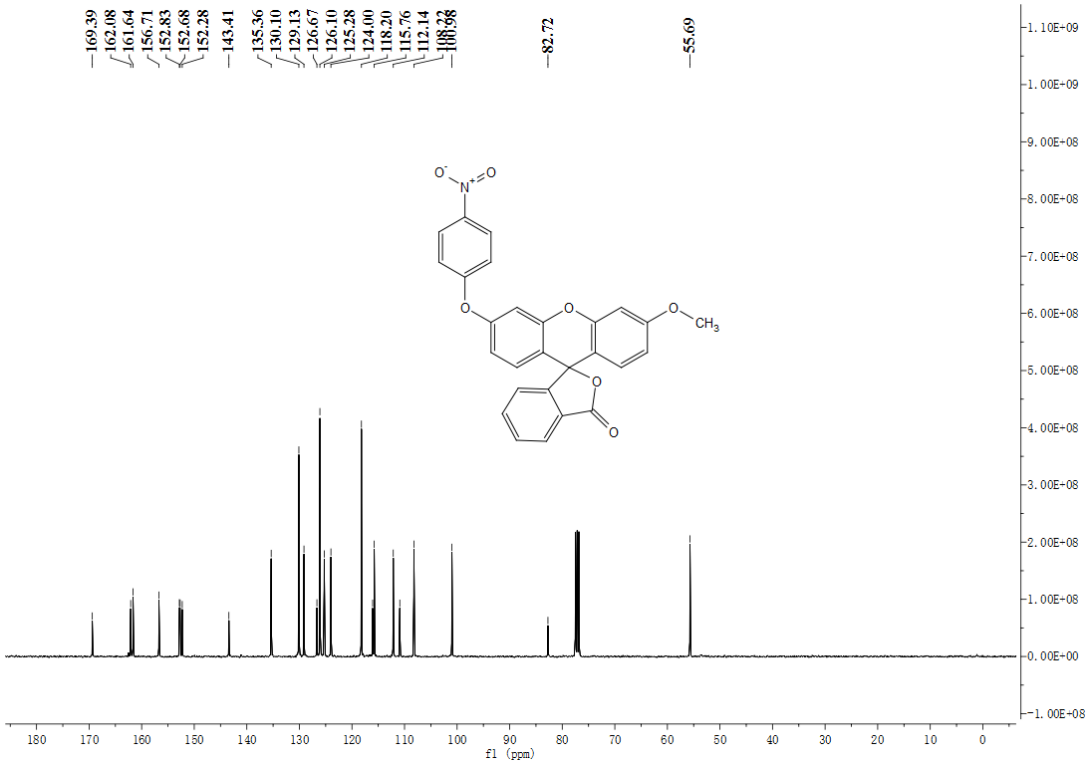
6. References

1. J. Zhang, Y.-Q. Sun, J. Liu, Y. Shi and W. Guo, *Chem. Commun.*, 2013, ***49***, 11305-11307.
2. M. Abo, Y. Urano, K.Hanaoka, T. Terai, T. Komatsu, T. Nagano, *J. Am. Chem. Soc.*, 2011, ***133***, 10629-10637.
3. J. S. Beckman, J. Chen, H. Ischirpoulos, J. P. Crow, *Methods enzymol*., 1994, ***233***, 229-240.

7. Supplementary figures



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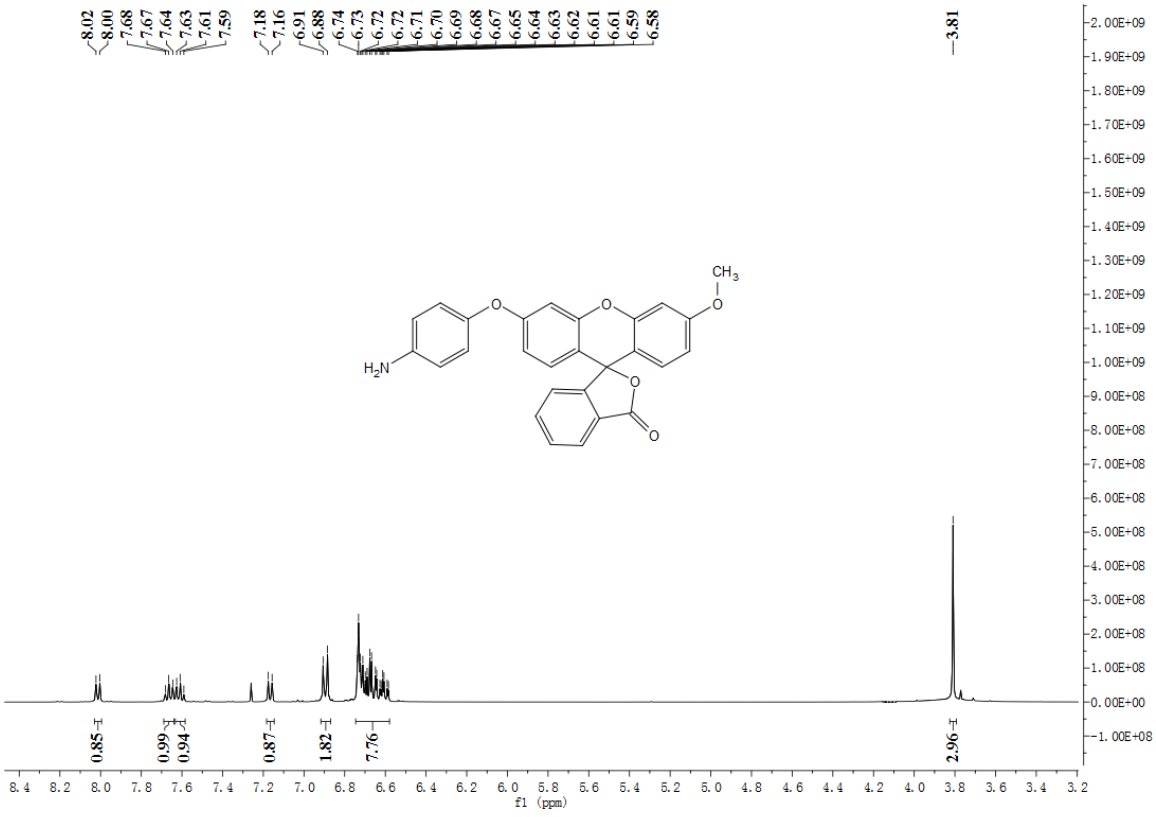
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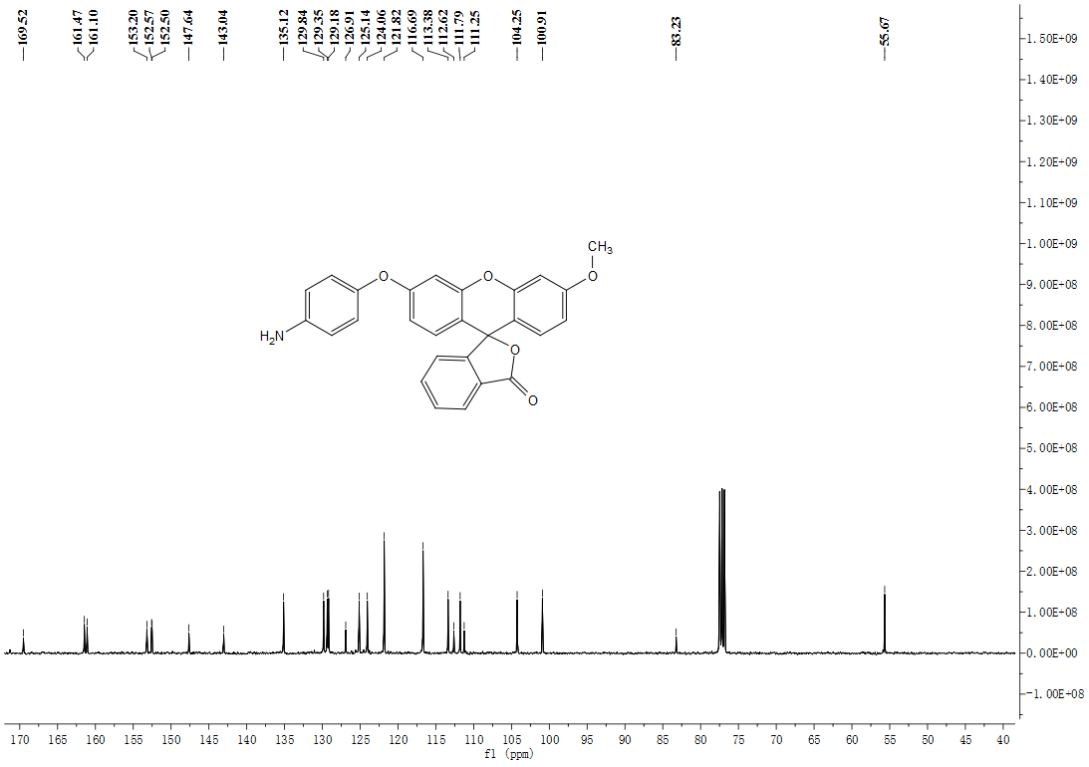
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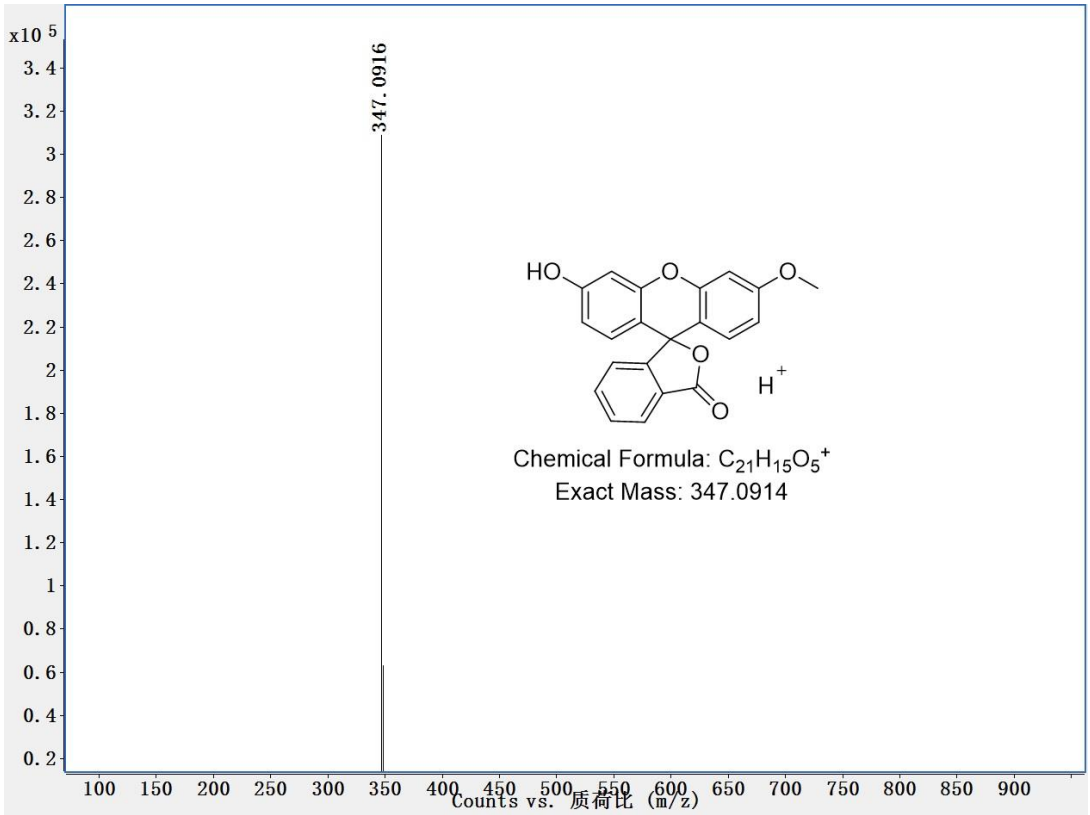
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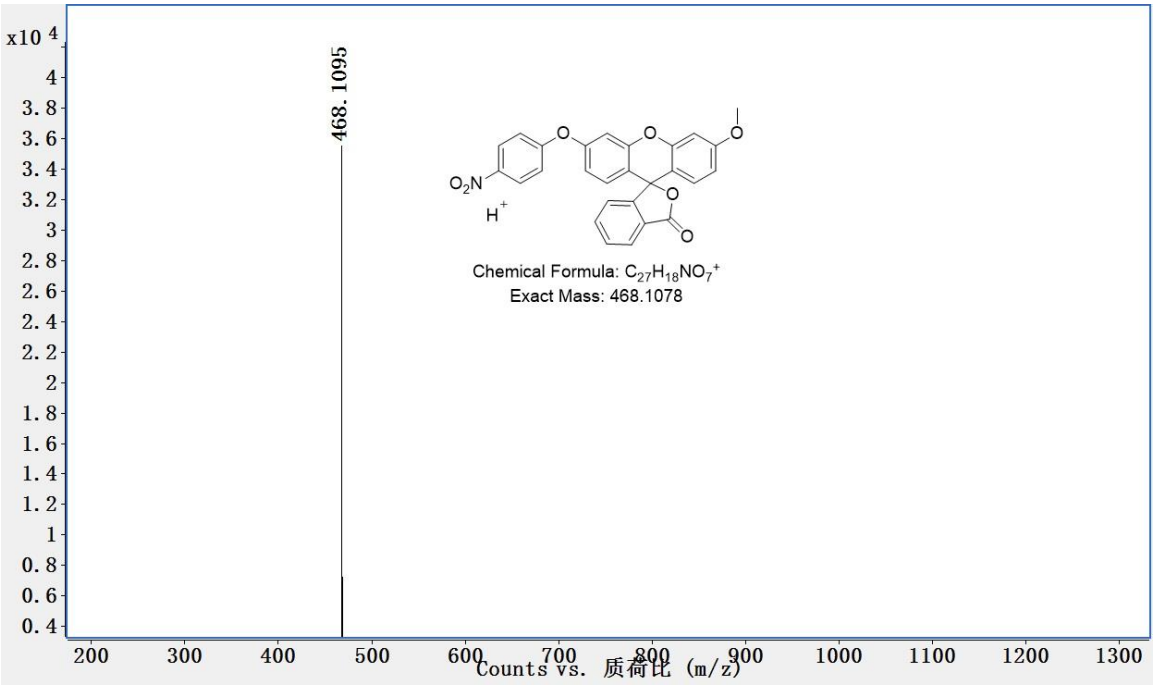
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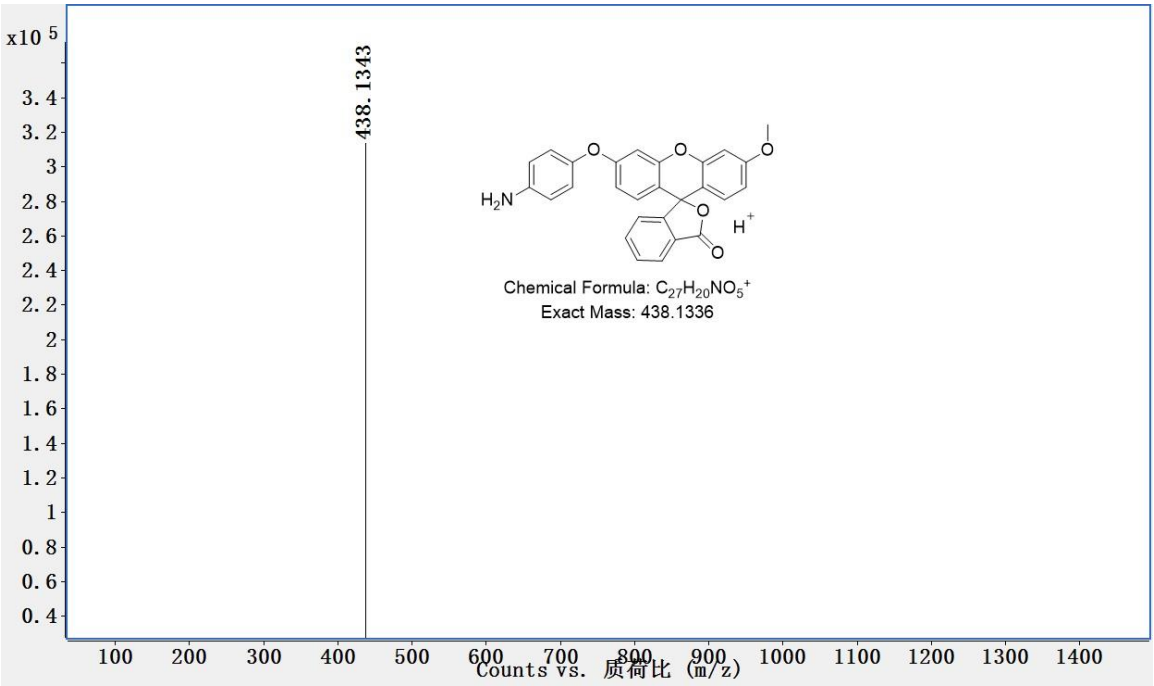
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