Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A S-Alkyl Thiocarbamate-Based Biosensor for Highly Sensitive and Selective Detection of Hypochlorous Acid

Jian Sun and Fude Feng*

Key Laboratory of High Performance Polymer Material and Technology of Ministry of Education, Department of Polymer Science & Engineering, School of Chemistry & Chemical Engineering, Nanjing University, Nanjing, 210023, China

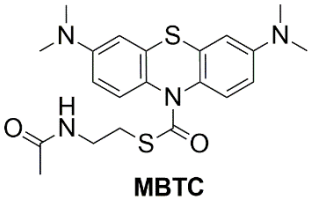
*E-Mail: fengfd@nju.edu.cn

Table of Contents

1. General methods and materials

All the commercially reagents were used as received without further purification unless otherwise stated. 3,7-bis(dimethylamino)-10H-phenothiazine-10-carbonyl chloride (MB-Cl), *N*-acetylcysteamine, and 4-bromo-*N*-(2-mercaptoethyl)benzamide were synthesized according to the reported procedure.[1-3] NMR spectra were recorded on a Bruker AMX 400 spectrophotometer using TMS as the internal reference. High resolution mass spectra were aquired on an electrospray Agilent Q-TOF mass spectrometer. Reaction process was monitored by a Waters ACQUITY H-Class Ultra performance liquid chromatography (UPLC) equipped with ultrasensitive PDA and fluorescence detectors. Low-resolution mass spectra were obtained with a Waters SQ detector-2 mass spectrometer. Ultraviolet-visible (UV-vis) spectra were taken on a Shimadzu UV-2600 spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-7000 fluorimeter (PMT detector: 600 V, excitation and emission slit widths: 5 nm). Water purified by a Mill-Q system was used to prepare all aqueous solutions.

2. Synthesis and characterization

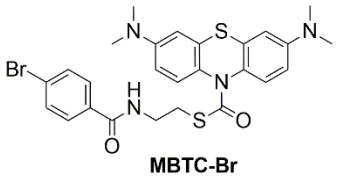


MBTC

Synthesis of S-(2-acetamidoethyl) 3,7-bis(dimethylamino)-10H-phenothiazine-10-carbothioate (MBTC)

To a solution of MB-Cl (35 mg, 0.1 mmol) and *N*-acetylcysteamine (18 mg, 0.15 mmol) in dry CH_2Cl_2 (10 mL) were added 4-dimethylaminopyridine (DMAP, 2.5 mg, 0.02 mmol) and triethylamine (28 μL, 0.2 mmol). The reaction mixture was stirred at 25 °C for 1 h under argon atmosphere and concentrated in vacuo. The residue was dissolved in ethyl acetate (50 mL), and successively washed with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness in vacuo. Silica-gel column chromatography using petroleum

ether/ethyl acetate (4:1) as eluent afforded MBTC (36 mg, 84%) as a white solid; ^{1}H NMR (400 MHz, CD_3CN): δ 7.34 (d, J = 8.9 Hz, 2H), 6.77 (d, J = 2.8 Hz, 2H), 6.6 (dd, J_1 = 2.8 Hz, J_2 = 8.9 Hz, 2H), 6.48 (br, 1H), 3.26 (m, 2H), 2.93 (s, 12H), 2.90 (m, 2H), 2.15 (s, 3H); ^{13}C NMR (100 MHz, CD_3CN): δ 169.3, 149.4, 127.6, 110.1, 109.8, 39.4, 38.8, 30.0, 21.7 ppm. HRMS (ESI+): *m/z* 431.1581 $[M+H]^+$, Calculated for $C_{21}H_{27}N_4O_2S_2$:431.1575; found 431.1581.



MBTC-Br

Synthesis of S-(2-(4-bromobenzamido)ethyl) 3,7-bis(dimethylamino)-10H-phenothiazine-10-carbothioate (MBTC-Br)

4-bromo-*N*-(2-mercaptoethyl)benzamide (39 mg, 0.15 mmol) and MB-Cl (35 mg, 0.1 mmol) was used to synthesize MBTC-Br using the procedure described for preparation of MBTC. Silica-gel column chromatography using petroleum ether/ethyl acetate (1:1) as eluent afforded MBTC (49 mg, 86%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, 2H), 7.41 (d, 2H), 7.34 (d, J = 8.8 Hz, 2H), 7.11 (br, 1H), 6.69 (d, J = 2.8 Hz, 2H), 6.61 (dd, J_1 = 2.8 Hz, J_2 = 8.8 Hz, 2H), 3.63 (m, 2H), 3.16 (m, 2H), 2.98 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 166.5, 149.6, 133.2, 131.5, 128.8, 127.6, 125.7, 110.4, 41.8, 40.6, 29.8 ppm.

3. UPLC analysis

Analytical LC was run on a Waters UPLC BEH C18 column (1.7 μm, 2.1×50 mm) eluted with a gradient of 30%–100% CH_3CN in 0.2% acetic acid for 4 min at 40 °C. A flow rate of 0.4 mL/min and an injection volume of 2 μL were applied. UPLC spectra were obtained in PDA channel (620 nm) or in fluorescence channel (λ_{ex} 620 nm, λ_{em} 690 nm).

4. Detection selectivity assay

Selectivity assay was performed by recording absorption spectra of reaction

mixture containing MBTC (5 μM) and various analytes at 10 min post reaction. The analytes include common ROS, RNS, thiols, amino acids, anions and transition metal cations. ROS and RNS solutions were freshly prepared in PBS according to the procedure as reported previously.[4] Hg^{2+}, Pb^{2+}, Ag^{+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, and Al^{3+} stock solutions were prepared and tested in HEPES (20 mM, pH 7.4). The reaction mixture was prepared by mixing 20 μL of analyte solution and 1.98 mL of MBTC (5 μM) solution in PBS or HEPES buffer (20 mM, pH 7.4).

5. Cell culture

Macrophage RAW 264.7 cells (American Type Culture Collection) were cultured in DMEM medium containing 5% fetal bovine serum (Biological Industries) at 37 °C in a humid 5% CO_2-containing atmosphere.

6. MTT assay

RAW 264.7 cells were seeded at a density of 1.5×10^4/mL cell in a 96 well plate and allowed to grow in 5% CO_2 at 37 ^oC for 24 h. The medium was replaced by the fresh growth medium containing MBTC at varying concentrations (0, 1, 2.5, 5 μM). After incubation for 24 h, the cells were washed three times with PBS before incubation with 20 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) at 37 ^oC for 4 h. The absorbance of formazan product was measured at 562 nm by a microplate reader.

7. CLSM imaging

RAW 264.7 cells were seeded in 4-well glass-bottomed plates (Φ = 24 mm) at a density of 5×10^4 cells per well. CLSM imaging of RAW 264.7 cells were performed with a Zeiss laser scanning microscope 710 with a 63× oil objective lens, using Zen 2008 software (Carl Zeiss). MB product was excited by a 633 nm diode laser, with emission collected between 670 and 750 nm.

8. Supplementary Figures

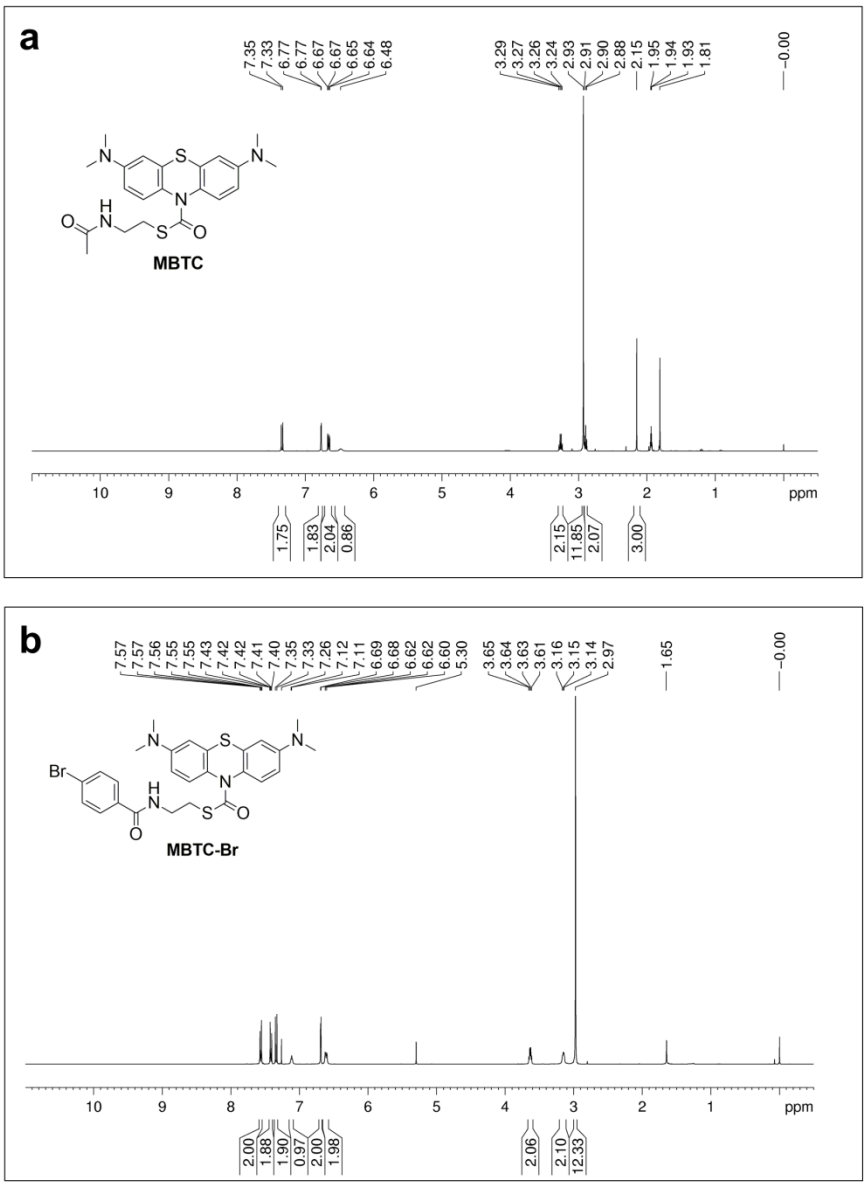


Fig. S1 ^{1}H NMR spectra of (**a**) MBTC in CD_3CN and (**b**) MBTC-Br in $CDCl_3$.

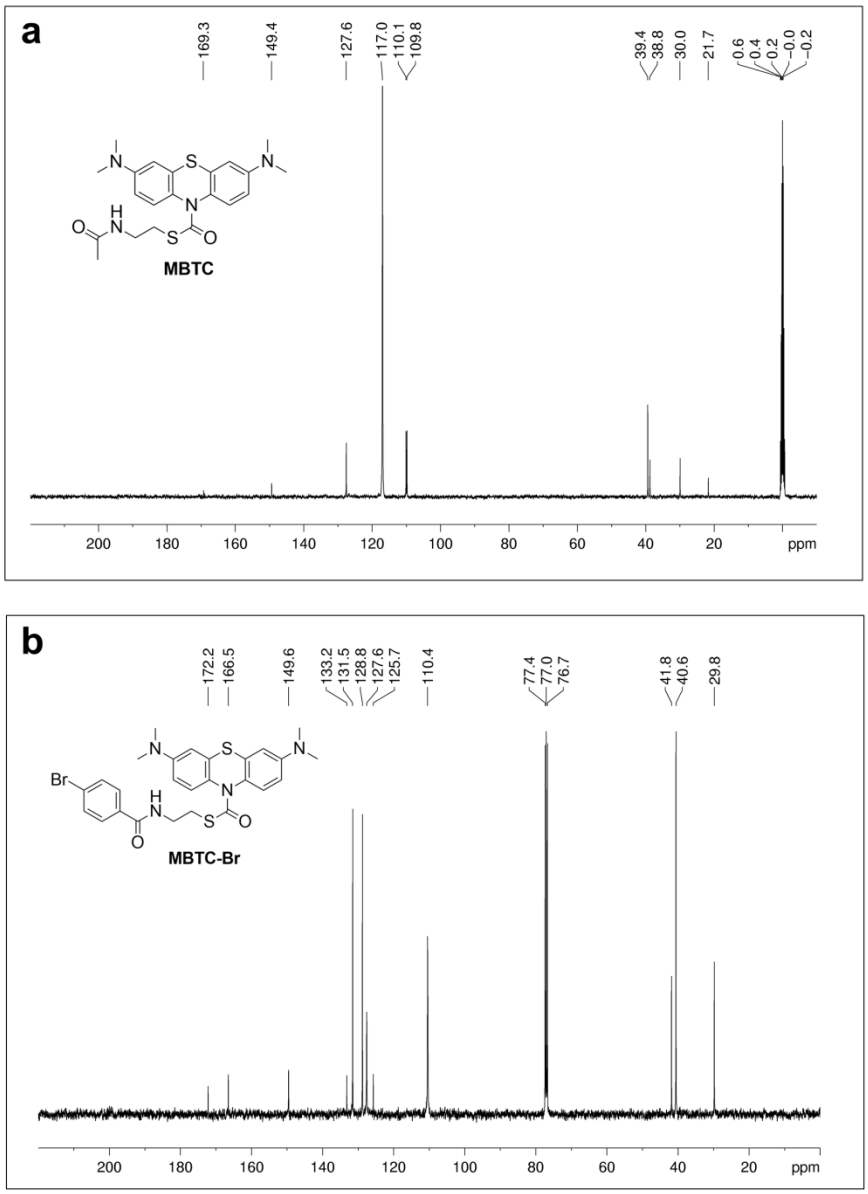


Fig. S2 ^{13}C NMR spectra of (**a**) MBTC in CD_3CN and (**b**) MBTC-Br in $CDCl_3$.

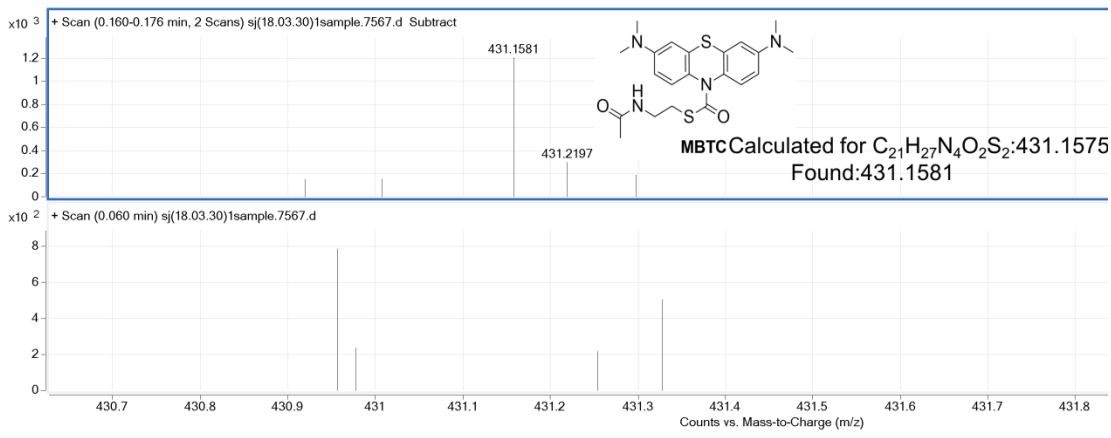


Fig. S3 HRMS spectra of MBTC.

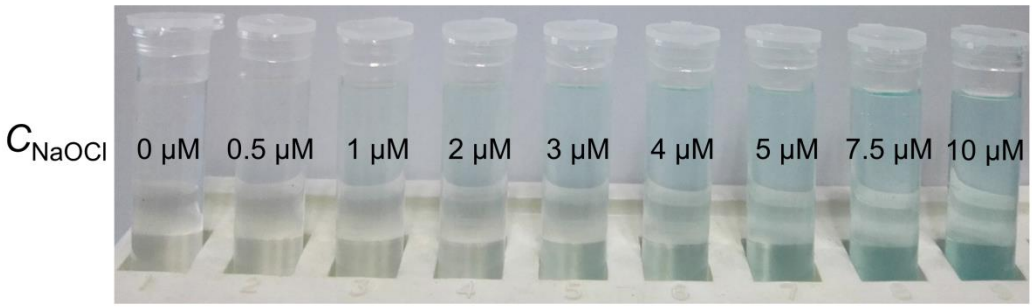


Fig. S4 Photograph of the solutions containing MBTC (5 μM) and varied concentrations of NaOCl (0-10 μM) in PBS (20 mM, pH 7.4).

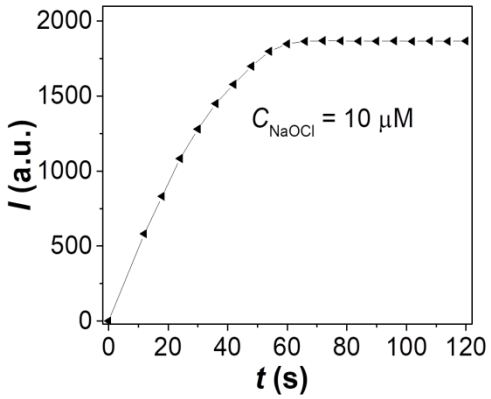


Fig. S5 Plot of fluorescence intensity at 690 nm of reaction mixture containing MBTC (5 μM) and NaOCl (10 μM) as a function of reaction time. The excitation was at 620 nm.

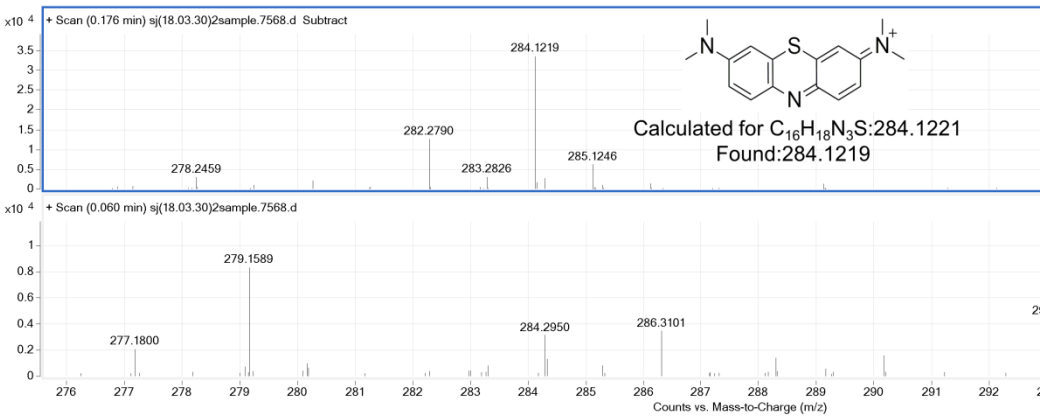


Fig. S6 HRMS spectra of the product from the reaction between MBTC (5 μM) and NaOCl (10 μM).

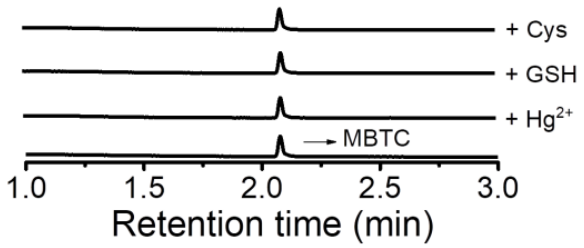


Fig. S7 UPLC analysis of reaction mixture containing MBTC (5 μM) and different analytes including Cys (500 μM), GSH (5 mM), and Hg^{2+} (500 μM), in PDA channel (λ_{abs} 620 nm).

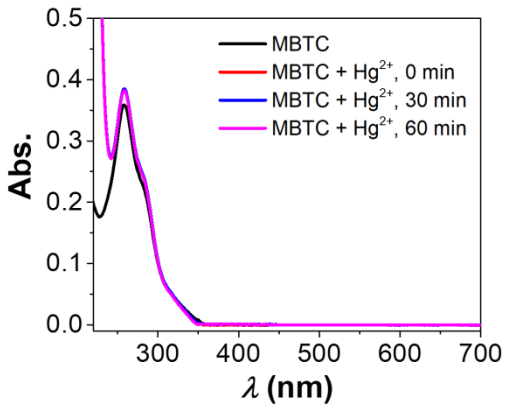


Fig. S8 Absorption spectra of the reaction mixture containing MBTC (5 μM) and Hg^{2+} (500 μM) in HEPES (20 mM, pH 7.4) at 60 ºC for 0 ~ 60 min.

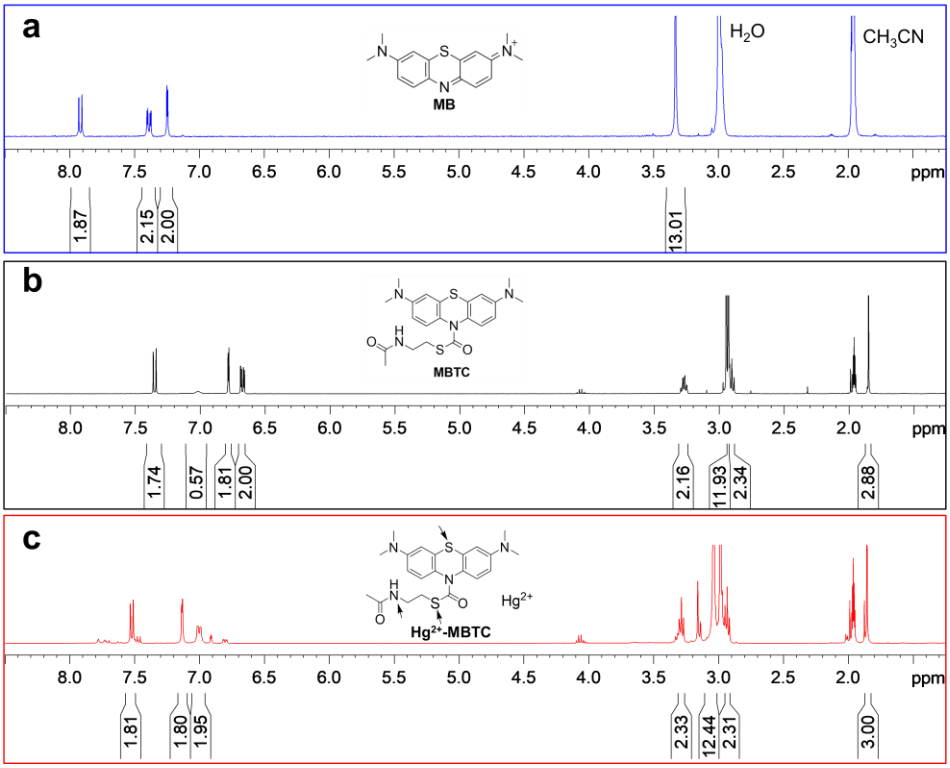


Fig. S9 ^{1}H NMR spectra of (**a**) MB, (**b**) MBTC, and (**c**) Hg^{2+}-MBTC in CD_3CN/D_2O (9:1, *v*/*v*). The arrows in (**c**) indicate the possible Hg^{2+} binding sites.

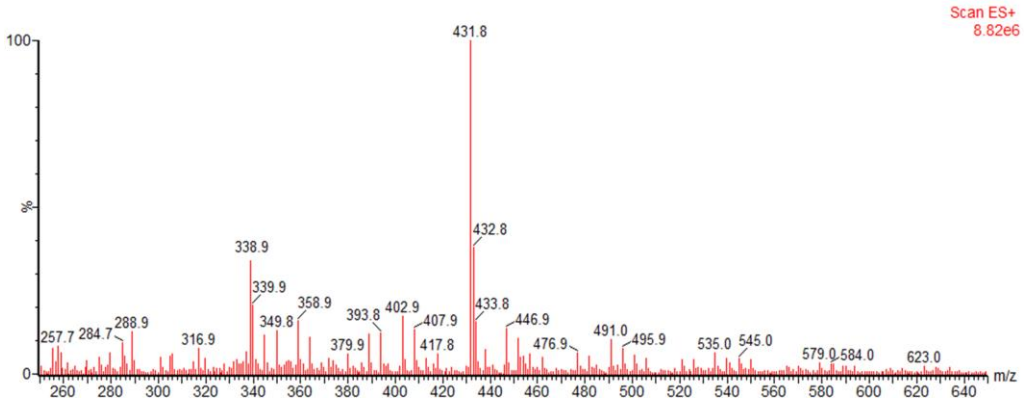


Fig. S10 MS spectrum of Hg^{2+}-MBTC in CD_3CN/D_2O (9:1, *v*/*v*).

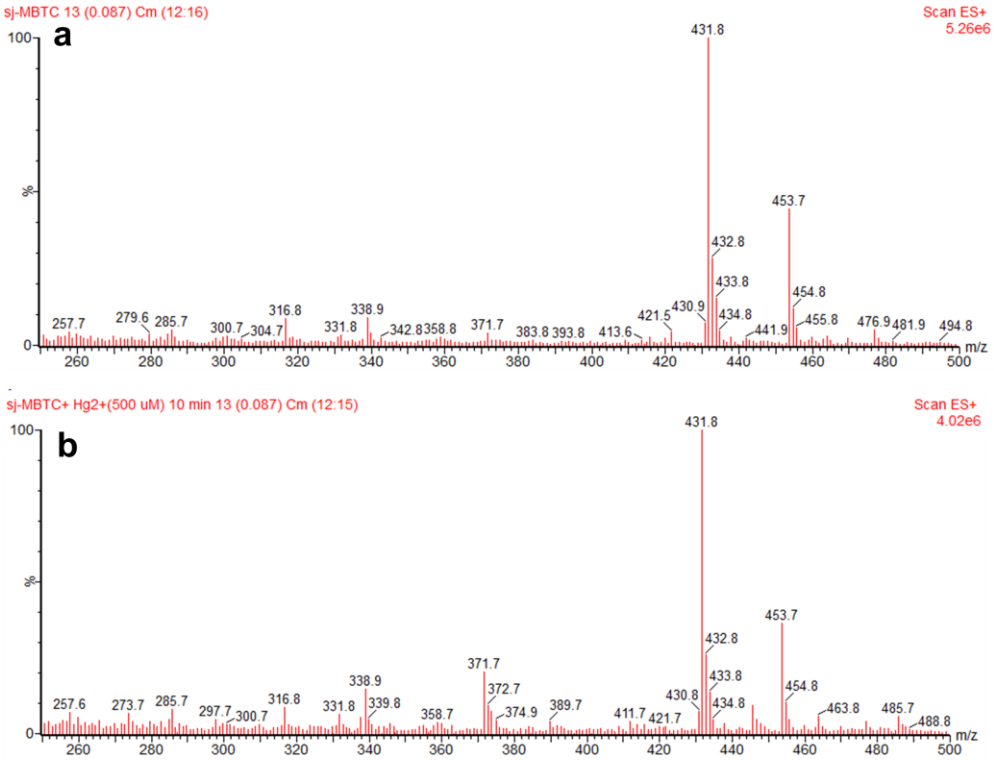


Fig. **S11** MS spectra of (a) MBTC and (b) Hg^{2+}/MBTC in PBS.

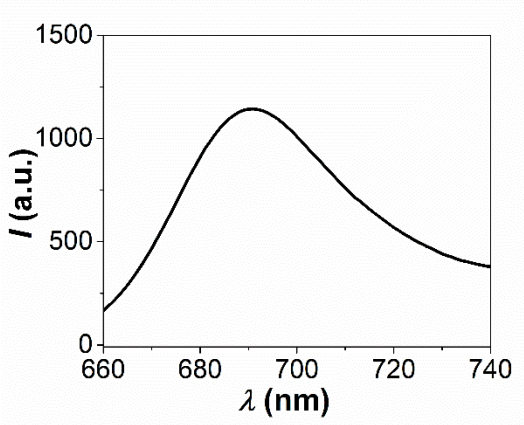


Fig. S12 The fluorescence spectra of the solution containing MBTC-Br (5 μM) and NaOCl (10 μM) in PBS.

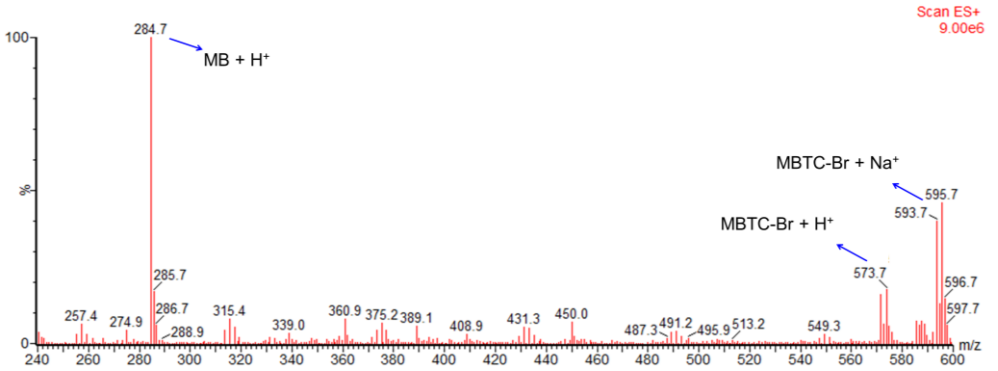


Fig. S13 MS analysis of the reaction mixture containing MBTC-Br (5 μM) and NaOCl (10 μM).

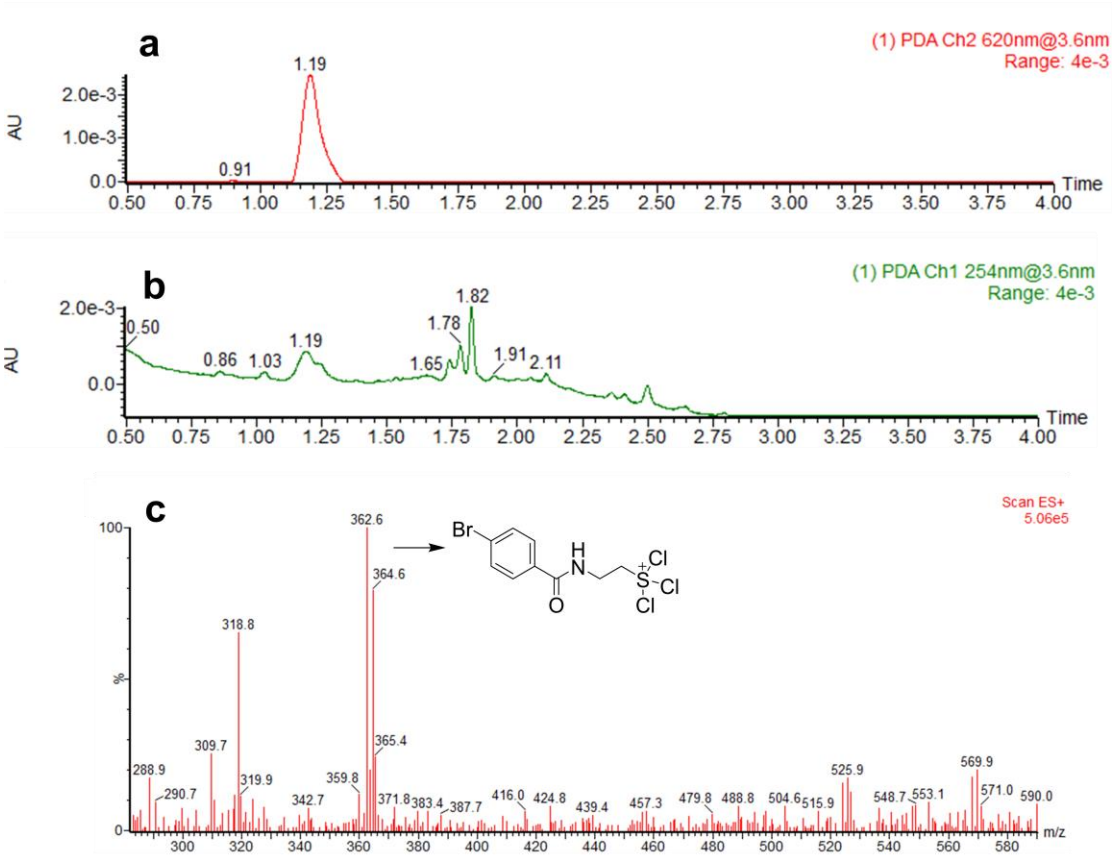


Fig. S14 UPLC-MS analysis of the reaction mixture containing MBTC-Br (5 μM) and NaOCl (10 μM) in (**a**) 620 nm and (**b**) 254 nm PDA channels, and (**c**) by SQ detector-2 for the intermediate with a retention time of 1.82 min. The chemical structure in the inset of (c) indicates the proposed structure of polychlorinated intermediate.

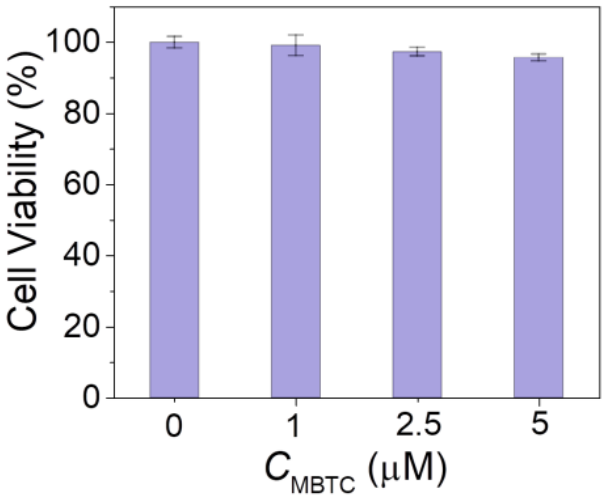


Fig. S15 MTT assay results of MBTC-treated RAW 264.7 cells.

9. References

1. P. Wei, W. Yuan, F. Xue, W. Zhou, R. Li, D. Zhang, T. Yi, *Chem. Sci.,* **2018,** *9*, 495.

2. A. J. Hughes, A. Keatinge-Clay, *Chem. Biol.,* **2011,** *18*, 165.

3. N. Mano,.; S. Aoki, T. Yamazaki, Y. Nagaya, M. Mori, K. Abe, M. Shimada, H. Yamaguchi, T. Goto, J. Goto, *Anal. Chem.,* **2009**, *81*, 9395.

4. X. Chen, K. Lee, X. Ren, J. Ryu, G. Kim, J. Ryu, W. Lee, J. Yoon, *Nat. Protoc.,* **2016,** *11*, 1219.