Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A benzocoumarin-based fluorescent probe for ultra-sensitive fast detecting endogenous/exogenous hypochlorous acid and its applications

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[a]*College of Chemistry and Chemical Engineering, Lanzhou University, Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province and State Key Laboratory of Applied Organic Chemistry, Lanzhou 730000, P. R. China*

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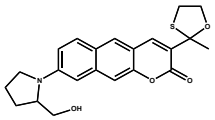
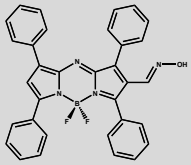
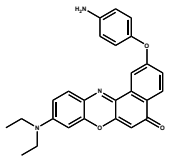
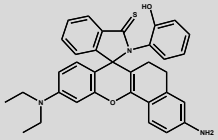
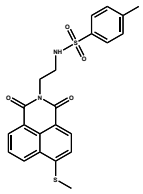
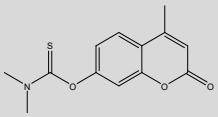
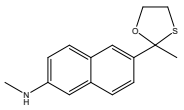
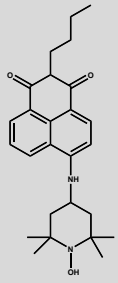
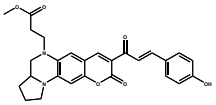
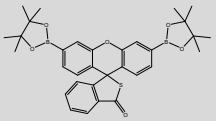
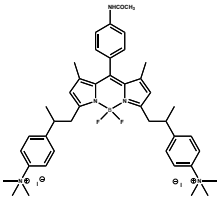
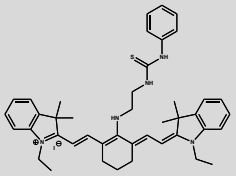
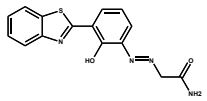
Contents

1. Comparison of the this work with reported HOCl probes

Table S1. Comparison of analytical parameters of representative fluorescent probes for HOCl.

Probe structures	LOD	Solvent	Reaction time	Endogenous detection	Ref.
	34.8 nM	1% DMSO	<60s	Yes	[1]
	2.33μM	HEPES/CH_3CN (1/1, pH=7.2)	-	Yes	[2]
	4.370μM	DMSO/PBS (5/95, pH=7.4)	60s	-	[3]
	40nM	DMSO/PBS (5/95, pH=7.4)	-	Yes	[4]
	120nM	DMSO/PBS (1/99, pH=7.4)	150s	-	[5]
	34.75nM	H_2O/C_2H_5OH (1/1, pH=7.4)	60s	Yes	[6]
	16.6 nM	H_2O/C_2H_5OH (1/1)	<60s	Yes	[7]
	10.3nM	CH_3CN/PBS (1/9, pH=7.4)	8s	Yes	[8]

	55 nM	PBS, 10 mM, pH = 7.4, containing 1% DMSO	-	Yes	[9]
	220 nM	aqueous soloution	<60s	Yes	[10]
	31.6nM	PBS(PH=7.4)	215s	Yes	[11]
	44nM	PBS/ DMF (2/8, pH=7.4)	15min	Yes	[12]
	8.2nM	100% water	20min	Yes	[13]
This work	**2.45 nM**	**H_2O (PBS buffer, pH=7.4)**	**3s**	**Yes**	-

2. Preparation of Testing Solutions and Measurements of Spectra

A stock solution of Probe BMH (1.0 mM) was dissolved in analytical grade DMF. The stock solutions of analytes (Gln, Cys, Glu, Gly, Trp, Val, Lys, Li^+, Na^+, K^+, Ag^+, Mg^{2+}, Pb^{2+}, Cu^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}, F^-, Cl^-, Br^-, I^-, OH^-, NO_3^-, NO_2^-, HCO_3^-, CO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, PO_4^{3-}, Ac^-, SCN^-) were prepared with ultrapure water at a concentration of 1×10^{-3} M. All optical studies were tested in the phosphate-buffered solution (PBS 10 mM, pH 7.4) with slit (3 nm/ 3 nm). Test solutions were prepared as follows: 10 μL of probe BMH solution (1.0 mM) and proper analyte's solution were added into a test tube, and the solution was diluted to 2 mL using PBS buffer. Absorption and fluorescence spectra were recorded at an indicated time and indicated temperature.

The solubility experiment tested the solubility of the probe BMH and the compound BM. The experimental results found that the solubility of BMH and BM in water were both at the insoluble level (less than 0.1 g/lit water). BMH and BM have excellent solubility in N, N-dimethylformamide (DMF) and are soluble (more than ten g/lit solvents). Therefore, we used N, N-dimethylformamide (DMF) solvent to configure the accurate test solution system for probe BMH and compound BM.

Phosphate buffers were prepared using distilled water. Hypochlorous acid (HOCl) was obtained by diluting a commercial aqueous NaClO solution. Hydrogen peroxide (H_2O_2) and *tert*-butyl hydroperoxide (*t*-BuOOH) were delivered from commercial aqueous solutions respectively. Singlet oxygen ($^1O_2$) was generated *in situ* by the addition of the H_2O_2 a stock solution into asolution containing 10 equiv. of HOCl. Hydroxyl radicals (•OH) were generated *in situ* by the reaction of Fe^{2+} with H_2O_2. Superoxide solution ($O_2•^-$) was prepared by adding KO_2 to dry dimethylsulfoxide and stirring vigorously for 10 min. Peroxynitrite ($ONOO^-$) solution was prepared as the previous method. Briefly, a mixture of sodium nitrite (0.6 M) and hydrogen peroxide (0.7 M) was acidified with hydrochloric acid (0.6 M), and sodium hydroxide (1.5 M) was added within 1~2 s to make the solution alkaline. The concentration of H_2O_2 was determined from the absorbance at 240 nm (ε = 43.6 $M^{-1}• cm^{-1}$). The concentration of HOCl was determined from the absorbance at 292 nm (ε = 350 $M^{-1}• cm^{-1}$). The concentration of $ONOO^-$ was determined from the absorbance at 302 nm (1670 $M^{-1}• cm^{-1}$).

3. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The detection limit for BMH and HOCl based on the IUPAC definition (signal-to-noise

ratio S/N=3) is calculated by the linear function and the following equation:

$$\text{Detection limit} = 3\sigma/k$$

$$\boldsymbol{\sigma} = \sqrt{\frac{\sum(\bar{x} - \boldsymbol{x_i})^2}{\boldsymbol{n-1}}}$$

Where σ is the standard derivation of fluorescence spectra experiments of 21 blank solutions, $\bar{x}$ is the mean of the blank measures; x_i is the values of blank measures, k is the slope of the linear calibration curve; the concentration of probe **BMH** is 1×10^{-6} mol·L^{-1}.

4. Cytotoxicity Tests

The MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) assays were carried out to evaluate the cytotoxicity of probe BMH. The live RAW 264.7 macrophage cells (1×10^6 cells mL^{-1}) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL well^{-1}. Plates were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 24 h. Then the live RAW 264.7 macrophage cells were incubated for 24 h upon different probe concentrations(0, 0.5, 1, 5, 10, 15, 20, 30 μM). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL^{-1}, HEPES) was then added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 470 nm in a Triturus microplate reader.

5. Excitation emission spectroscopy of BMH and compound BM

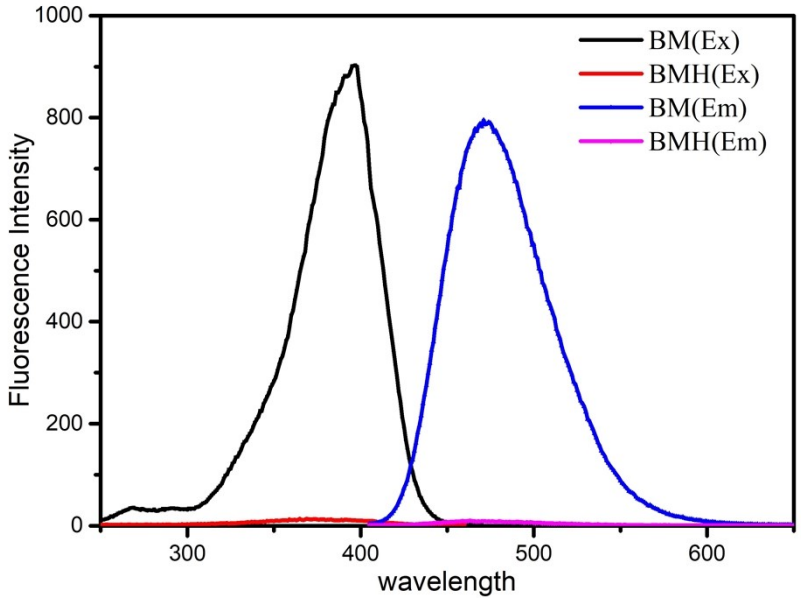


Fig. S1. Fluorescence spectrum of BMH (10.0 μM), BM (10.0 μM) in PBS (10 mM, pH 7.4) (λ_{Ex}= 395 nm, λ_{Em}= 470 nm, slit widths: 3 nm/3 nm).

6. Fluorescence spectrum of probe BMH, BMH+HOCl and BM

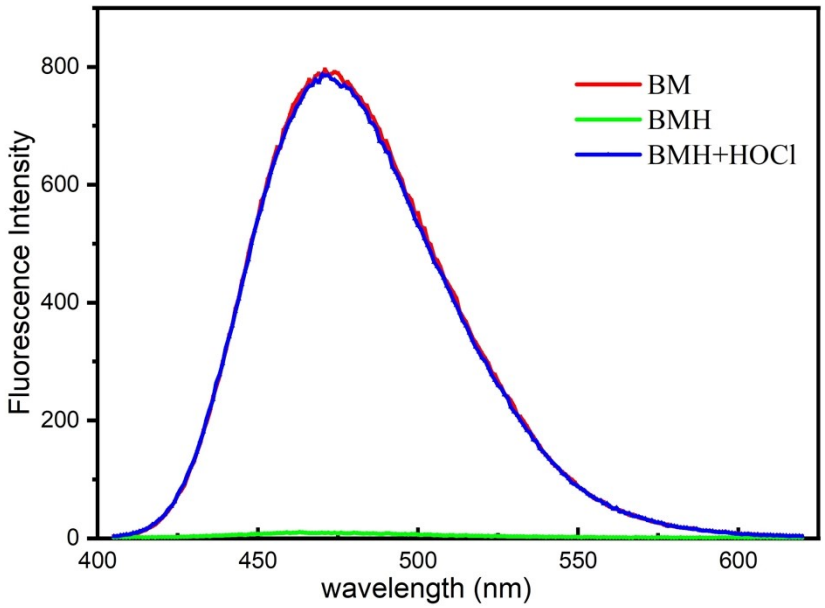


Fig. S2. Fluorescence spectrum of BMH (10.0 μM) , BMH +HOCl(10.0+10.0 μM), BM (10.0 μM) in PBS (10 mM, pH 7.4). (λ_{Ex}= 395 nm, slit widths: 3 nm/3 nm).

7. UV-vis absorption of BMH, BMH+HOCl and BM

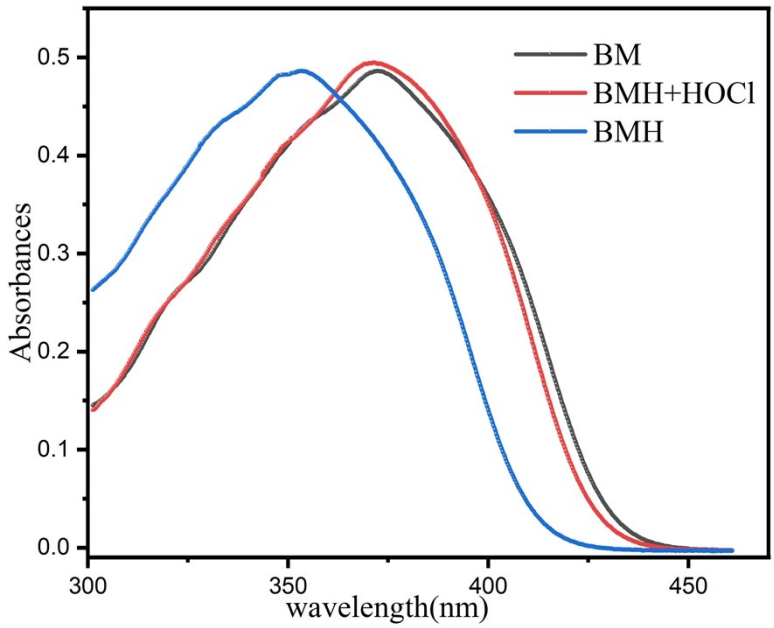


Fig. S3. UV spectrum of BMH +HOCl (50.0+50.0 μM), BM (50.0 μM).

8. The time-resolved curve of probe BMH

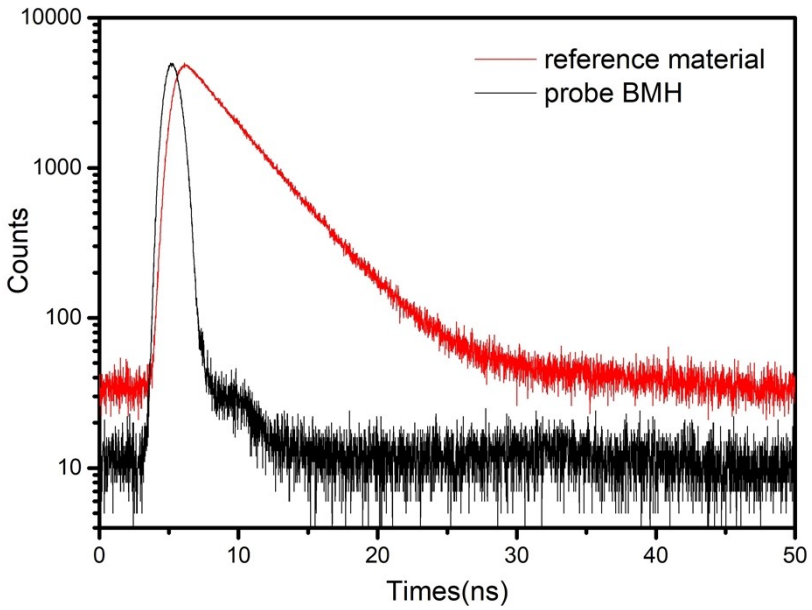


Fig. S4 Time-resolved curve of probe **BMH** in PBS (10 mM, pH 7.4), λ_{Ex}=405 nm.

9. Proposed reaction mechanism of BMH

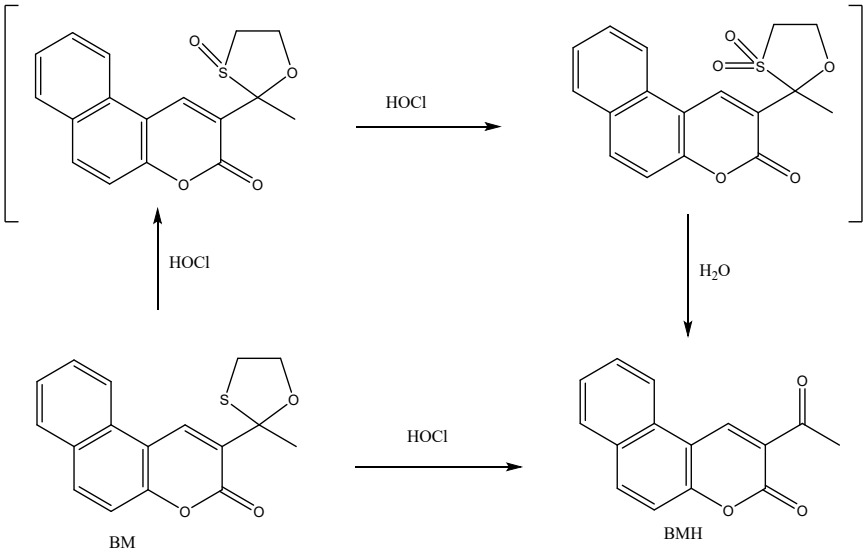


Fig. S5. The previously reported HOCl sensing mechanism of the probe BMH.

10. Spectra of ^{1}H-NMR, ^{13}C-NMR and ESI-MS of probe BMH (Fig. S6-S8)

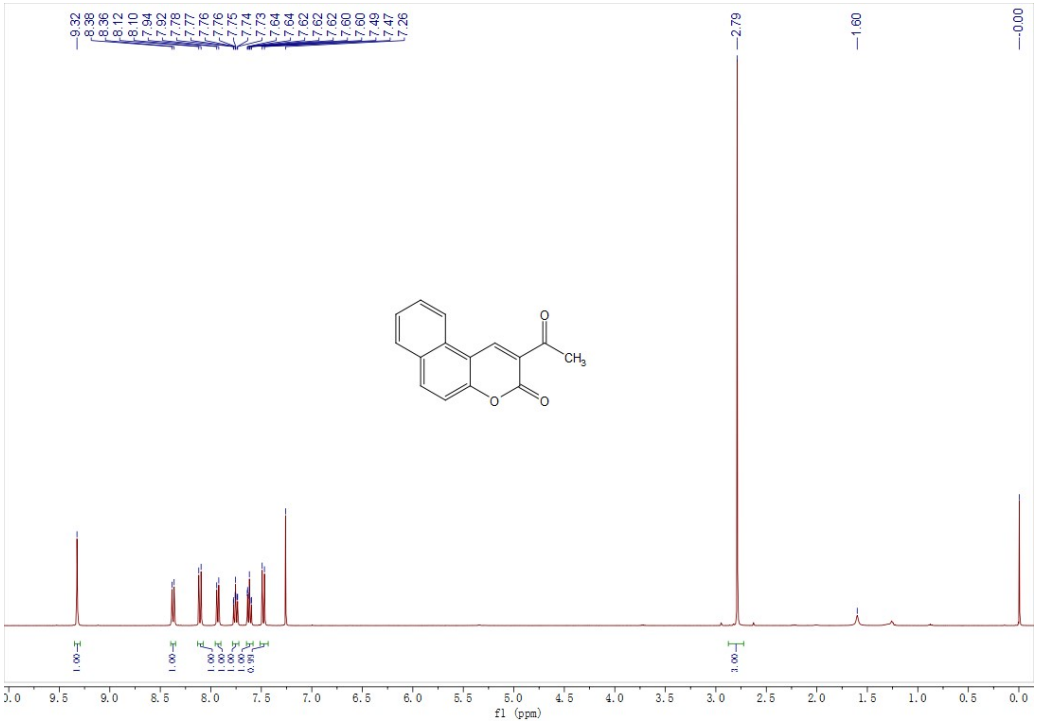


Fig. S6: ^{1}H MNR (400 MHz) spectrum of Compound **BM** in chloroform-d_6.

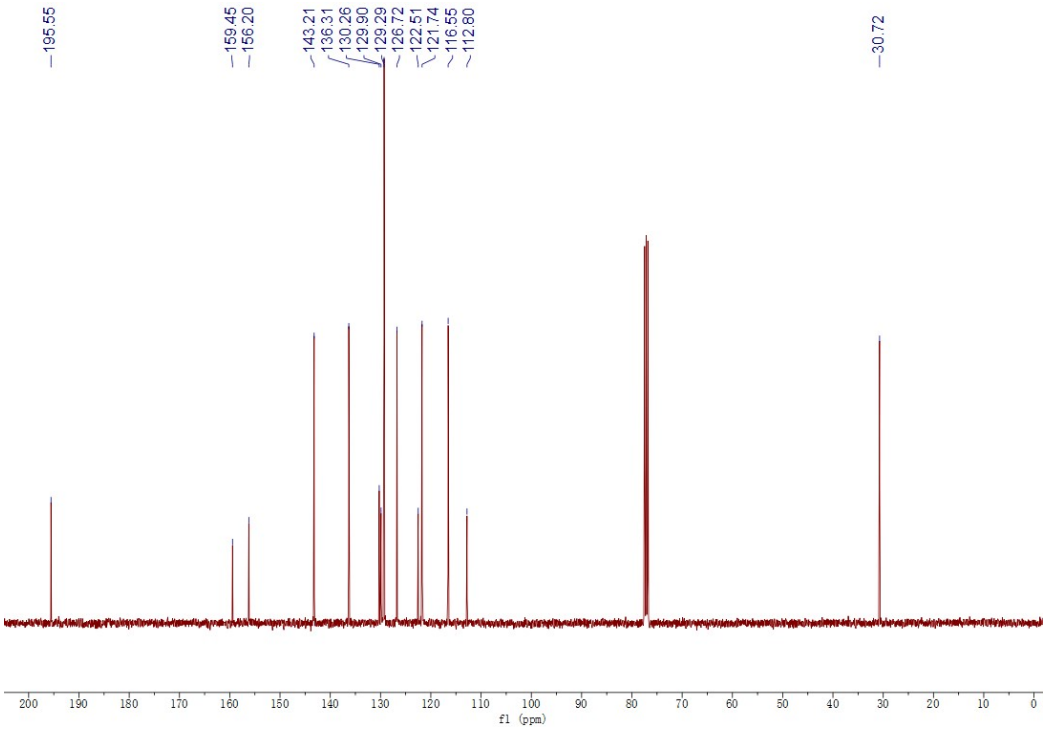


Fig. S7: ^{13}C MNR (400 MHz) spectrum of Compound **BM** in chloroform-d_6.

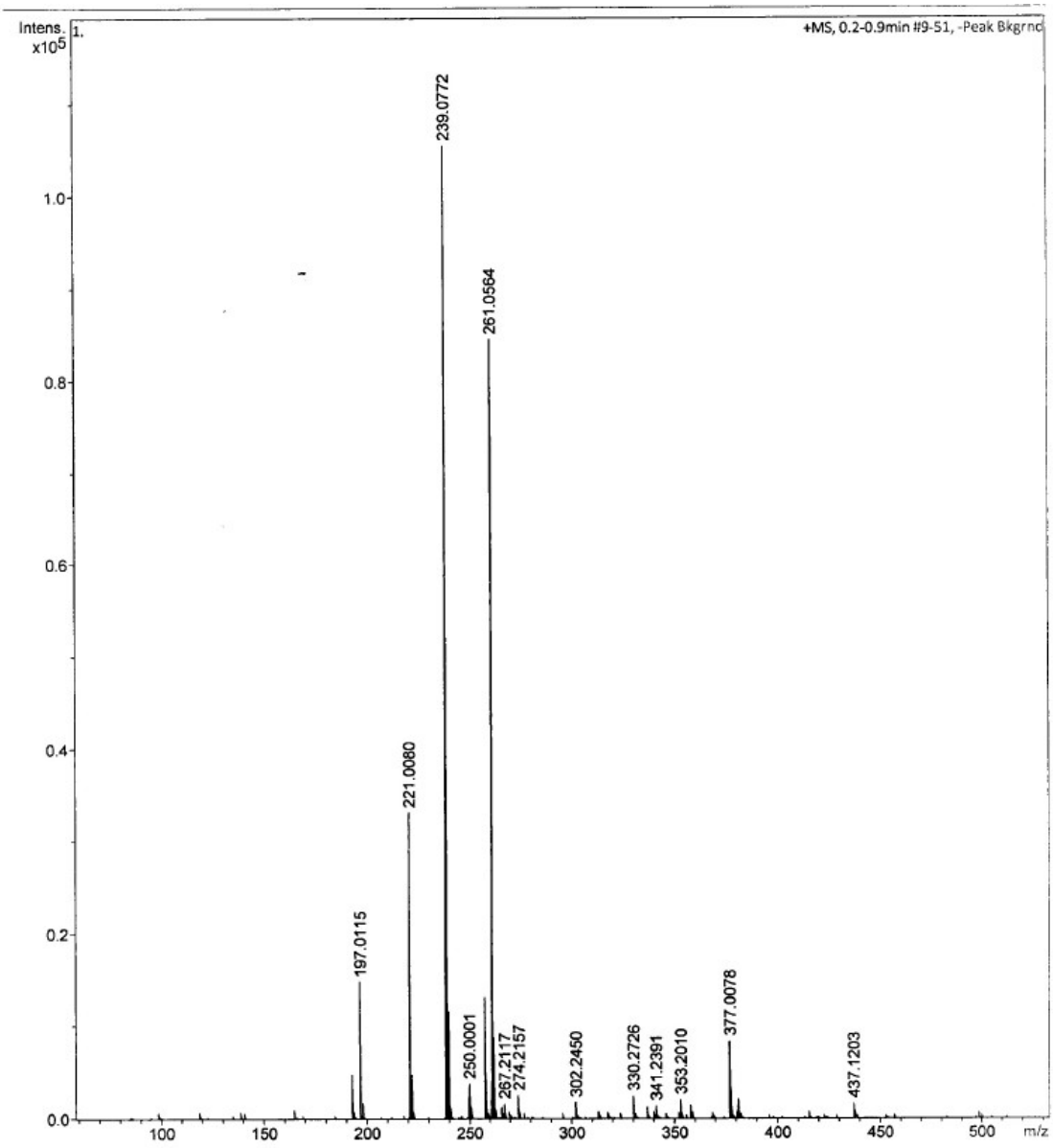


Fig. S8. ESI-MS spectrum of **BM**.

11. Spectra of ^{1}H-NMR, ^{13}C-NMR and ESI-MS of BM (Fig. S9-S11)

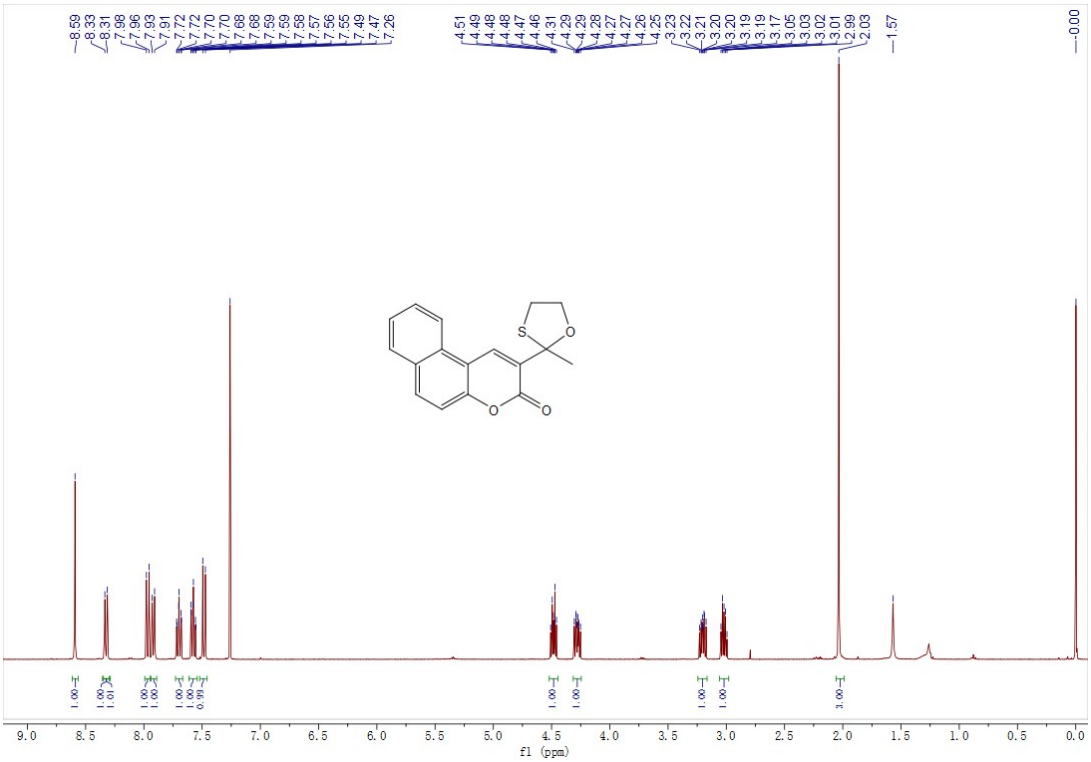


Fig. S9: ^{1}H MNR (400 MHz) spectrum of probe BMH in chloroform-d_6.

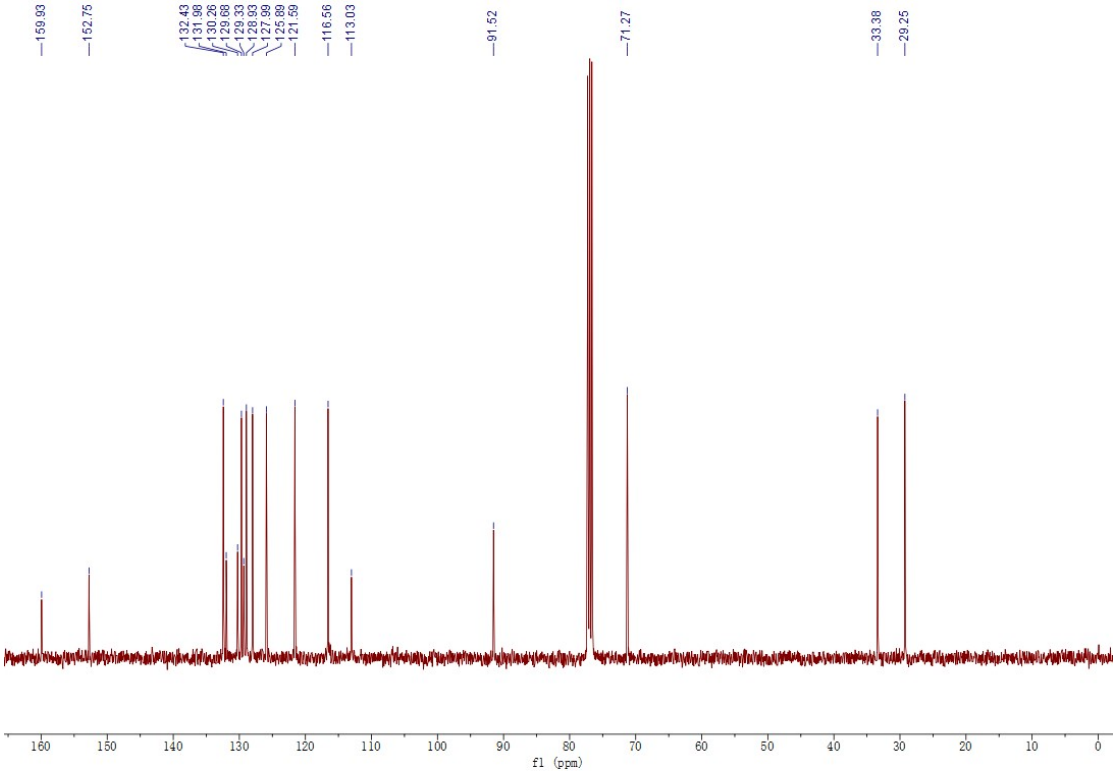


Fig. S10: ^{13}C MNR (400 MHz) spectrum of probe BMH in chloroform-d_6.

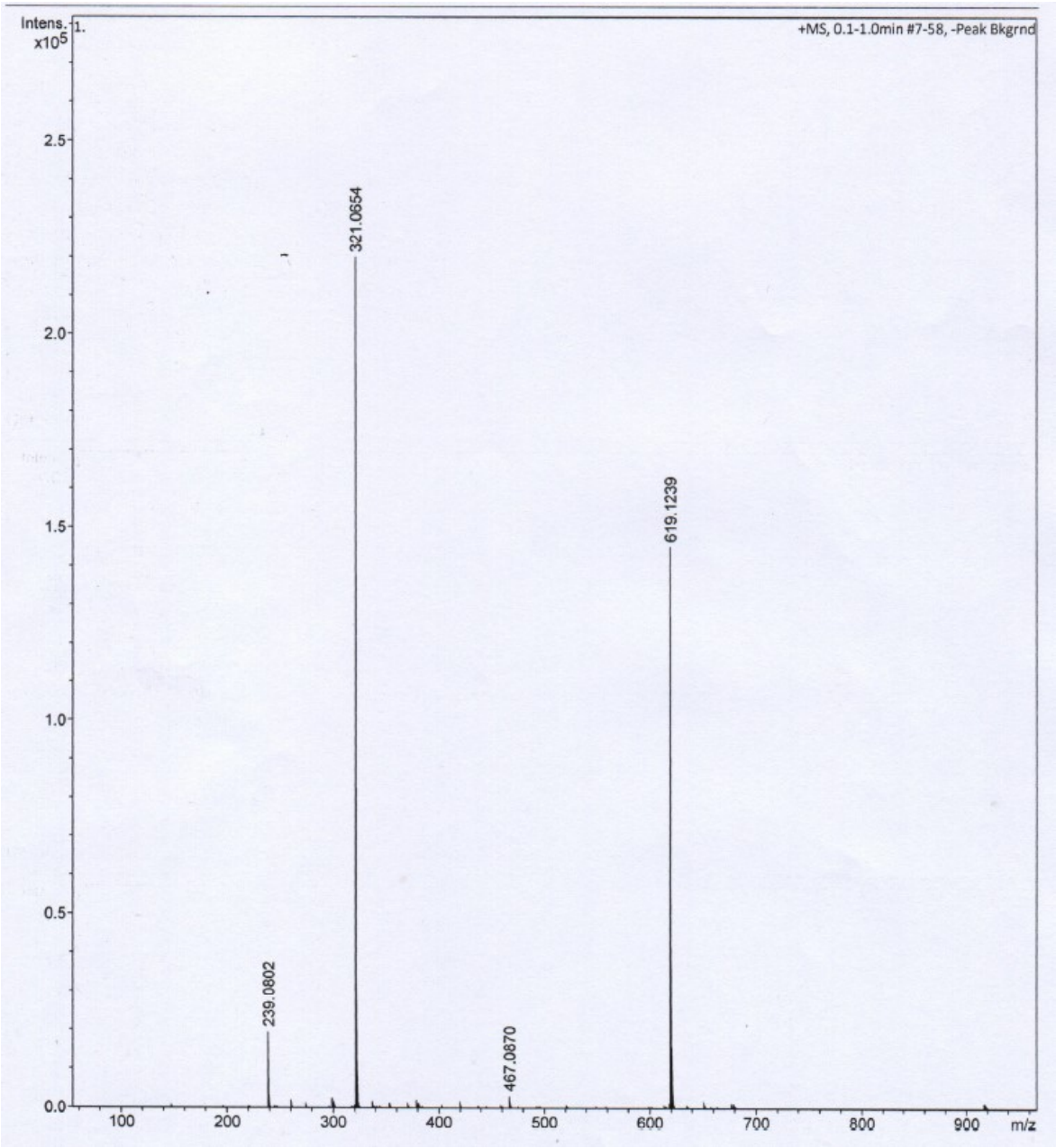


Fig. S11. ESI-MS spectrum of probe BMH.

12. Spectra of HRMS of the reaction solution of BMH and HOCl

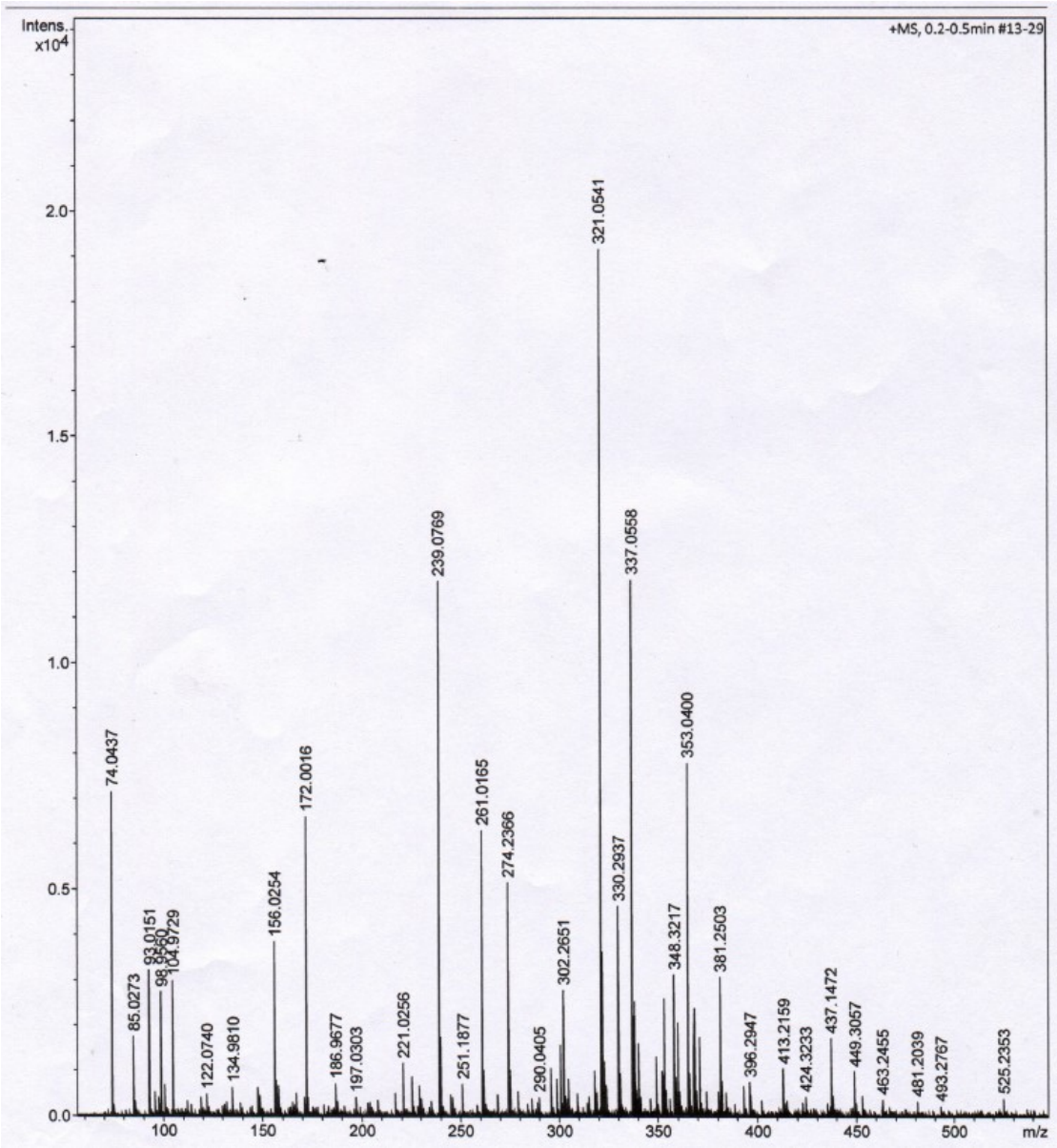


Fig. S12: Spectra of HRMS of the reaction solution of probe BMH and HOCl.

13. References

[1.] Y. W. Jun; S. Sarkar; S. Singha, et al., A two-photon fluorescent probe for ratiometric imaging of endogenous hypochlorous acid in live cells and tissues. *Chem. Commun.* **2017,** *53* (78), 10800-10803.

[2.] Y. Gao; Y. Pan; Y. Chi, et al., A "reactive" turn-on fluorescence probe for hypochlorous acid and its bioimaging application. *Spectrochim. Acta. A Mol. Biomol. Spectrosc.* **2019,** *206*, 190-196.

[3.] X. Lin; Y. Chen; L. Bao, et al., A two-photon near-infrared fluorescent probe for imaging endogenous hypochlorite in cells, tissue and living mouse. *Dyes and Pigments* **2020,** *174*, 108113.
[4.] Y.-J. Gong; M.-K. Lv; M.-L. Zhang, et al., A novel two-photon fluorescent probe with long-wavelength emission for monitoring HOCl in living cells and tissues. *Talanta* **2019,** *192*, 128-134.
[5.] Q. Xia; X. Wang; Y. Liu, et al., An endoplasmic reticulum-targeted two-photon fluorescent probe for bioimaging of HOCl generated during sleep deprivation. *Spectrochim. Acta. A Mol. Biomol. Spectrosc.* **2020,** *229*, 117992.
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[7.] L. Yuan; L. Wang; B. K. Agrawalla, et al., Development of Targetable Two-Photon Fluorescent Probes to Image Hypochlorous Acid in Mitochondria and Lysosome in Live Cell and Inflamed Mouse Model. *J. Am. Chem. Soc.* **2015,** *137* (18), 5930-5938.
[8.] M. He; H. Sun; J. Wei, et al., A highly sensitive, fast responsive and reversible naphthalimide-based fluorescent probe for hypochlorous acid and ascorbic acid in aqueous solution and living cells. *Spectrochim. Acta. A Mol. Biomol. Spectrosc.* **2021,** *247*.
[9.] Y. Gan; G. Yin; X. Zhang, et al., Turn-on fluorescent probe for sensing exogenous and endogenous hypochlorous acid in living cells, zebrafishes and mice. *Talanta* **2021,** *225*, 122030.
[10.] Q. Xu; K.-A. Lee; S. Lee, et al., A Highly Specific Fluorescent Probe for Hypochlorous Acid and Its Application in Imaging Microbe-Induced HOCl Production. *J. Am. Chem. Soc.* **2013,** *135* (26), 9944-9949.
[11.] S. Liu; D. Yang; Y. Liu, et al., A dual-channel and fast-response fluorescent probe for selective detection of HOCl and its applications in live cells. *Sens. Actuators B Chem.* **2019,** *299*, 126937.
[12.] Q.-S. Lin; Y.-L. Huang; X.-X. Fan, et al., A ratiometric fluorescent probe for hypochlorous acid determination: Excitation and the dual-emission wavelengths at NIR region. *Talanta* **2017,** *170*, 496-501.
[13.] Y. Yang; F. Qiu; Y. Wang, et al., A sensitive and selective off-on fluorescent probe for HOCl in 100% aqueous solution and its applications in bioimaging. *Sens. Actuators B Chem.* **2018,** *260*, 832-840.