Ratiometric fluorescent probe with aggregation-induced emission features for monitoring to HClO in living cell and zebra fish

Yanfei Li [a], Hui Li [a], Guilan Di [a,*]

[a] *College of Fisheries, Engineering Lab of Henan Province for Aquatic Animal Disease Control, Engineering Technology Research Center of Henan Province for Aquatic Animal Cultivation, Henan Normal University, Xinxiang, 453007, China*

[*] *Corresponding author: Guilan Di, College of Fisheries, Henan Normal University, Xinxiang, 453007, China, Tel: +86 3733326563, E-mail: gldi123@163.com;*

Contents

1. Experiment.....1

2. Spectroscopic Property.....2

3. ^{1}H NMR, ^{13}C NMR and HRMS spectra 5

1. Experiment

Cell culture. HeLa cells were seeded in culture dishes in Dulbecco's modified Eagle's medium (DMEM) which was supplemented with 10% fetal bovine serum and 1% penicillin and cultured in a humidified incubator containing 5% CO_2 and 95% air at 37 °C for 24 h. Before imaging, the cells were cultured in an 18 mm glass dish, during which dead cells and cell metabolites were washed away with Phosphate buffer saline (PBS) buffer.

Cytotoxicity assay. MTT assay was performed using HeLa cells, which were inoculated into 96-well plate and cultured in a cell culture tank. After the cell attachment was completed, different concentrations (0.0, 5.0, 10.0, 15.0, 20.0 μM) of probe **PTZ-HClO** were added into the 96-well plate and incubated in 5% CO_2 humidified incubator for 24 h. The MTT solution (1.0 mg/mL in PBS) was then added to each well and the cells were incubated in a cell culture tank for another 4 h. Finally, the MTT solution was dumped and DMSO (100.0 μL) was added to each well. The absorbance was determined at 490 nm and the cell viability was calculated using the following formula: cell viability = (mean absorbance of test wells - mean absorbance of medium control wells) / (mean absorbance of untreated wells - mean absorbance of medium control wells) × 100%.

Endogenous HClO imaging in living HeLa cells. Endogenous HClO imaging in living HeLa cells using **PTZ-HClO**. The first group: Cells were treated with LPS (1 μg/mL) and PMA (1 μg/mL) for 1 h, then 5 μM PTZ-HClO for additionally 15 min. The second group: Cells were treated with LPS (1 μg/mL), PMA (1 μg/mL) and ABH (200 μM) for 1 h, then 5 μM PTZ-HClO for additionally 15 min. The third group: Cells were treated with LPS (1 μg/mL), PMA (1 μg/mL) and NAC (1 mM) for 1 h, then 5 μM PTZ-HClO for additionally 15 min. Green channel: 515-580 nm (excited at 400 nm). Red channel: 615-750 nm (excited at 435-460 nm).

Fluorescence images of PTZ-HClO in HeLa cells incubated with different concentrations of HClO HeLa cells were incubated with **PTZ-HClO** (5 μM) at 37 °C for 30 min and then further treated with different concentrations of HClO for 15 min. Green channel: 515-580 nm (excited at 400 nm). Red channel: 615-750 nm (excited at 435-460 nm).

The kinetics of oxidation of the probe PTZ-HClO. Fluorescence spectra of probe **PTZ-HClO** (5.0 μM) in the presence of testing species (200 μM). Then test the fluorescence intensity ratio (I_{535}/I_{670}) of probe PTZ-HClO (5.0 μM) in the presence of other strongly oxidizing species. (1) HClO, (2) NO, (3) $ONOO^-$, (4) OH, (5) $^1O_2$, (6) O^{2-}, (7) ROO ·

2. Spectroscopic Property

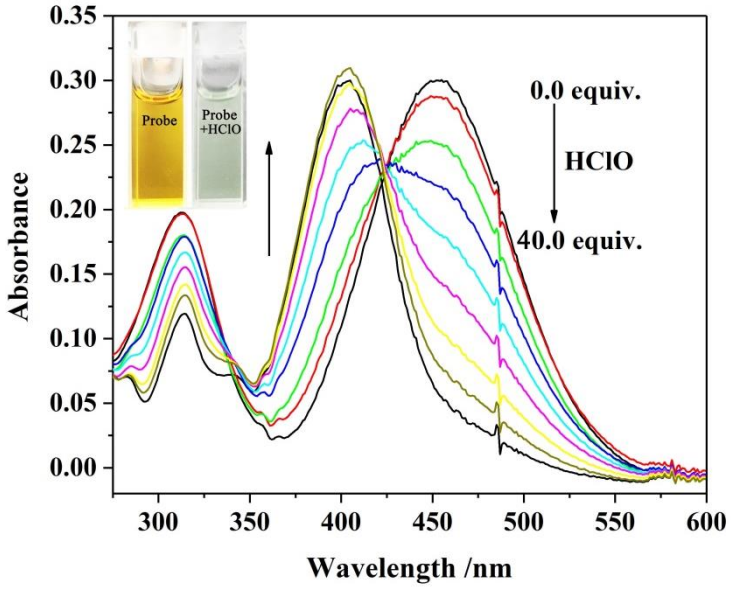


Fig. S1. Absorption spectra of probe **PTZ-HClO** (10.0 μM) in the presence and absence of (A) HClO in PBS buffer (pH 7.4, 10.0 mM, containing 20 % CH_3CN).

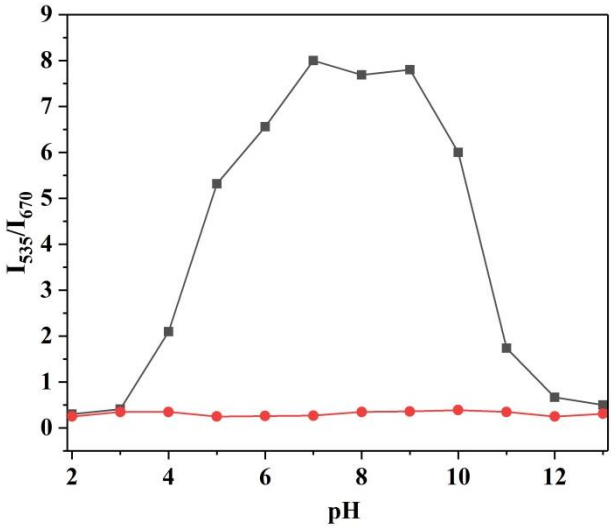


Fig. S2. The changes of fluorescence intensity ratio (I_{535}/I_{670}) of probe **PTZ-HClO** (5.0 μM) in the presence and absence of HClO (200.0 μM.) at different pH values.

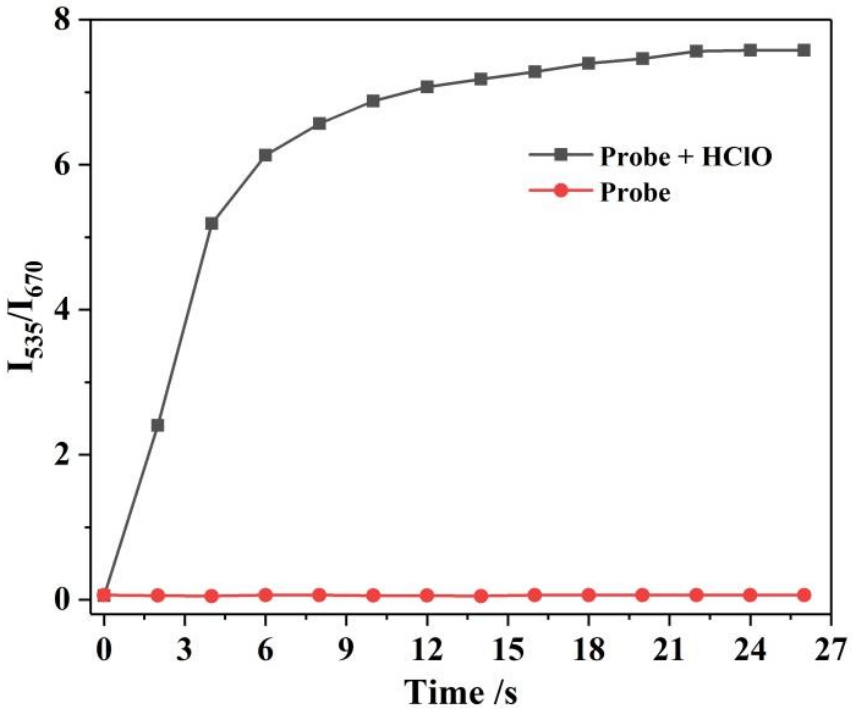


Fig. S3. Time-dependent fluorescence intensity ratio of I_{535}/I_{670} changes of probe **PTZ-HClO** (5.0 μM) with HClO (200.0 μM).

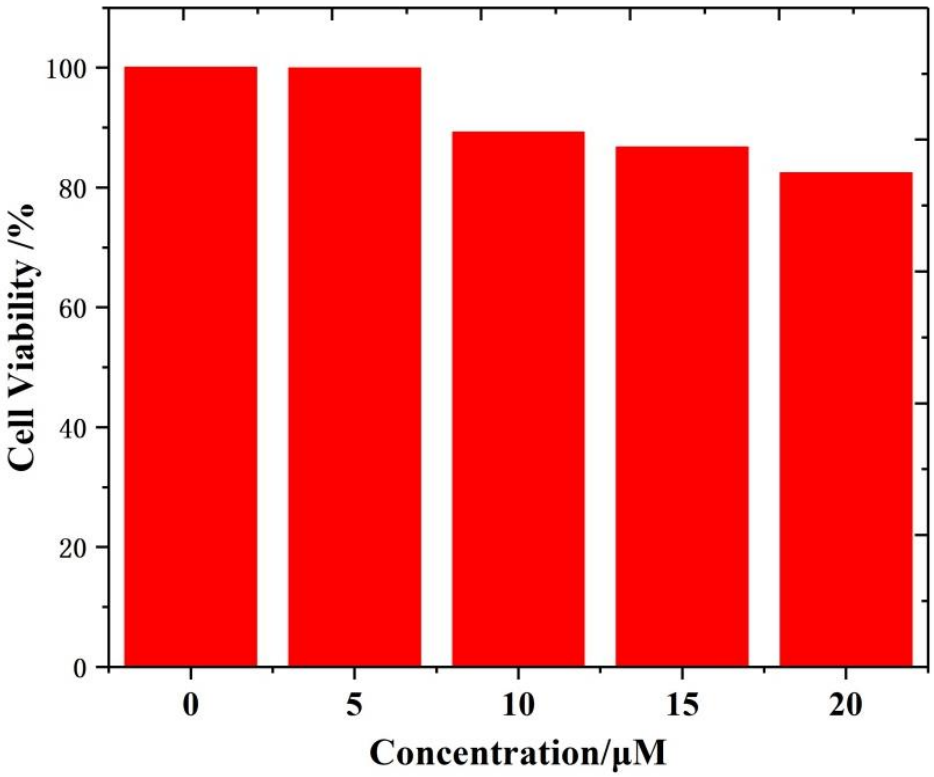


Figure S4. The cytotoxicity assay of HeLa cells with different concentrations of **PTZ-HClO**.

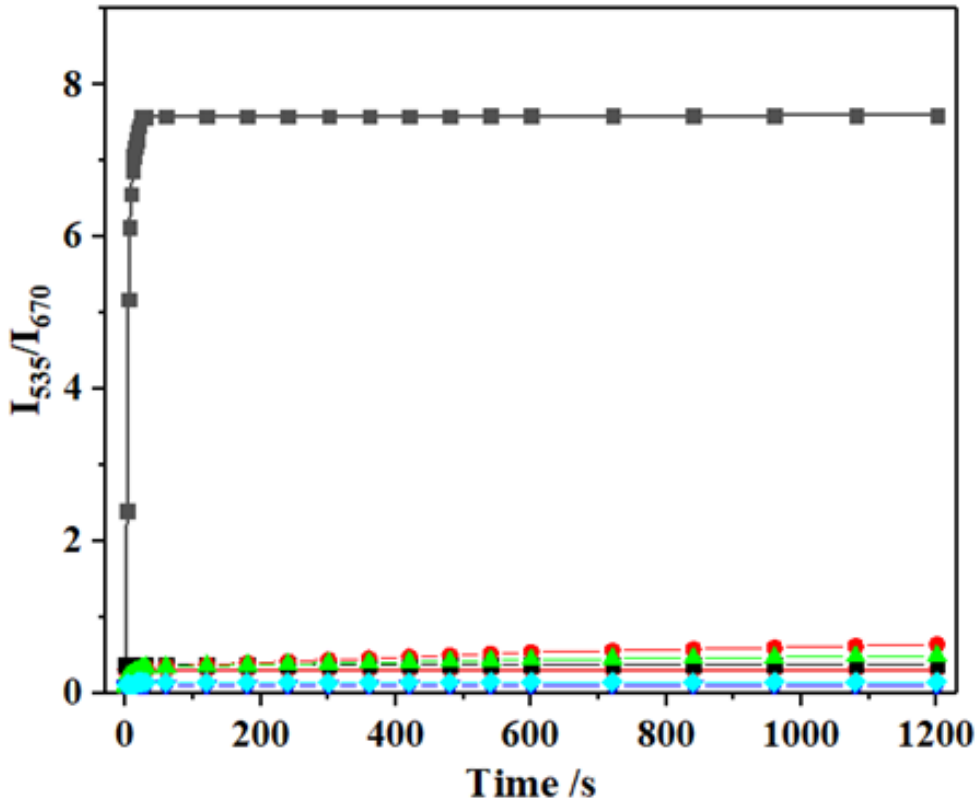
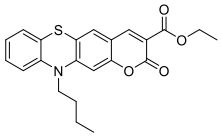
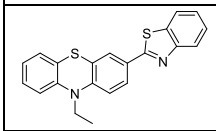
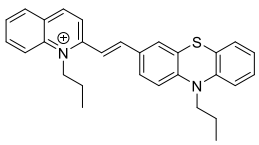
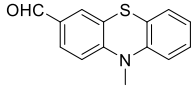
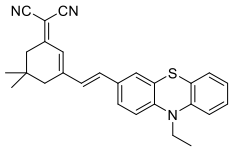
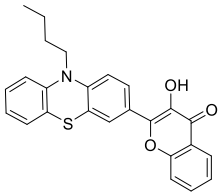
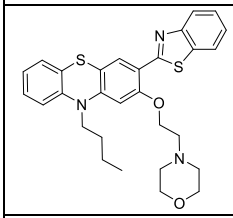
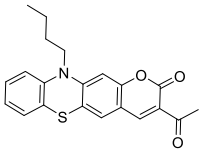


Figure S5. The kinetics of oxidation of the probe **PTZ-HClO**.

Table. S1 Some fluorescent probes for HClO.

Probes	Types	Emission wavelength /nm	Detection limit	Ref.
	Ratiometric	534, 626	15 nM	1
	OFF-ON	450	0.76 μM	2
	OFF-ON	588	15.6 nM	3
	OFF-ON	449.76 nM	513	4
	OFF-ON	39 nM	613	5

	Ratiometric	6.6 nM	524, 586	6
	Ratiometric	23 nM	418, 520	7
	Ratiometric	25 nM	535, 670	This work

1. W. Chen, G. Li, C. Chen, J. Sheng and L. Yang, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2020, **228**, 117724.

2. J.-Y. Wang, J. Qu, H. Zhang, K. Wei and S.-X. Ni, *RSC Advances*, 2019, **9**, 16467-16471.

3. H. Feng, Q. Meng, Y. Wang, C. Duan, W. Cuiping, H. Jia, Z.-Q. Zhang and R. Zhang, *Chemistry - An Asian Journal*, 2018, **13**.

4. H. Song, Y. Zhou, C. Xu, X. Wang, J. Zhang, Y. Wang, X. Liu, M. Guo and X. Peng, *Dyes and Pigments*, 2019, **162**, 160-167.

5. D. Zheng, X. Qiu, C. Liu, X. Jiao, S. He, L. Zhao and X. Zeng, *New Journal of Chemistry*, 2018, **42**, 5135-5141.

6. L. He, Y. Zhang, H. Xiong, J. Wang, Y. Geng, B. Wang, Y. Wang, Z. Yang and X. Song, *Dyes and Pigments*, 2019, **166**, 390-394.

7. P. Hou, S. Chen, G. Liang, H. Li and H. Zhang, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2020, **229**, 117866.

3. ^{1}H NMR, ^{13}C NMR and HRMS spectra

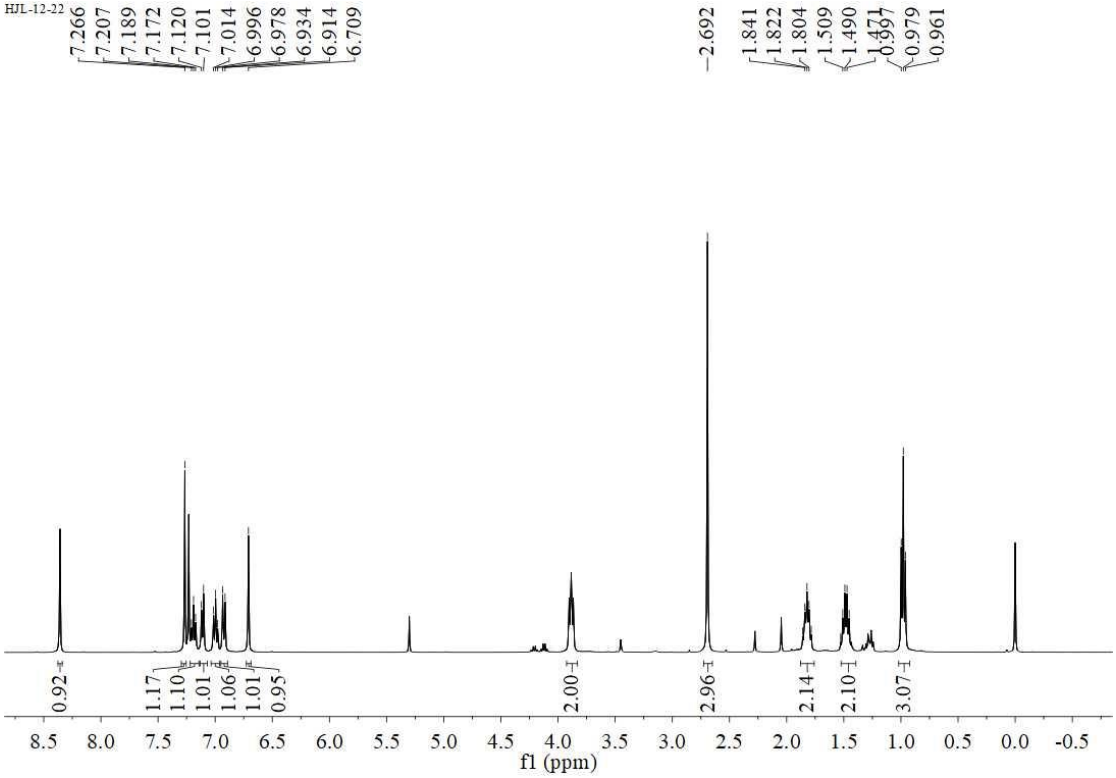


Figure S6. ^{1}H NMR spectrum of **PTZ-HClO** in $CDCl_3$.

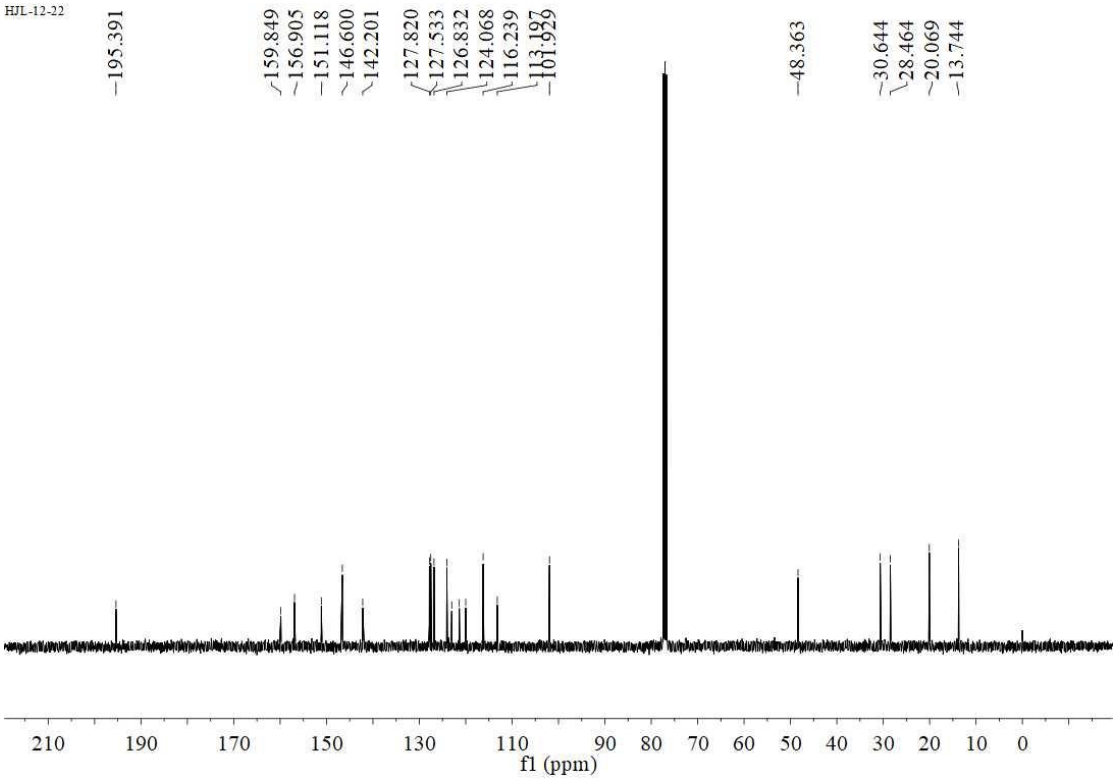


Figure S7. ^{13}C NMR spectrum of **PTZ-HClO** in $CDCl_3$.

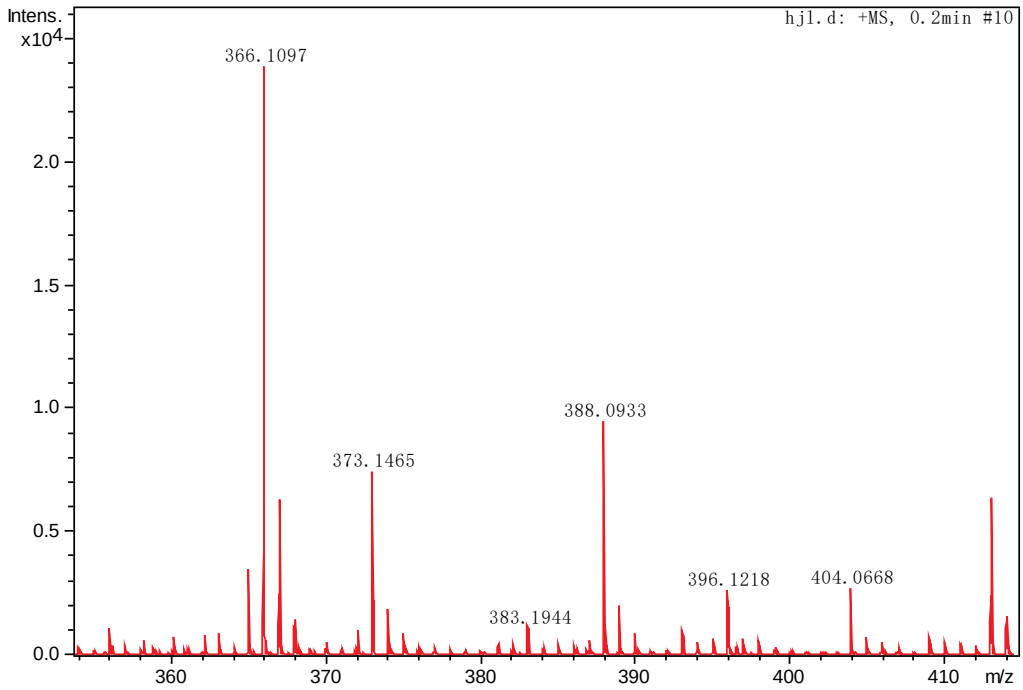


Figure S8. HR-MS spectrum of **PTZ-HClO**.

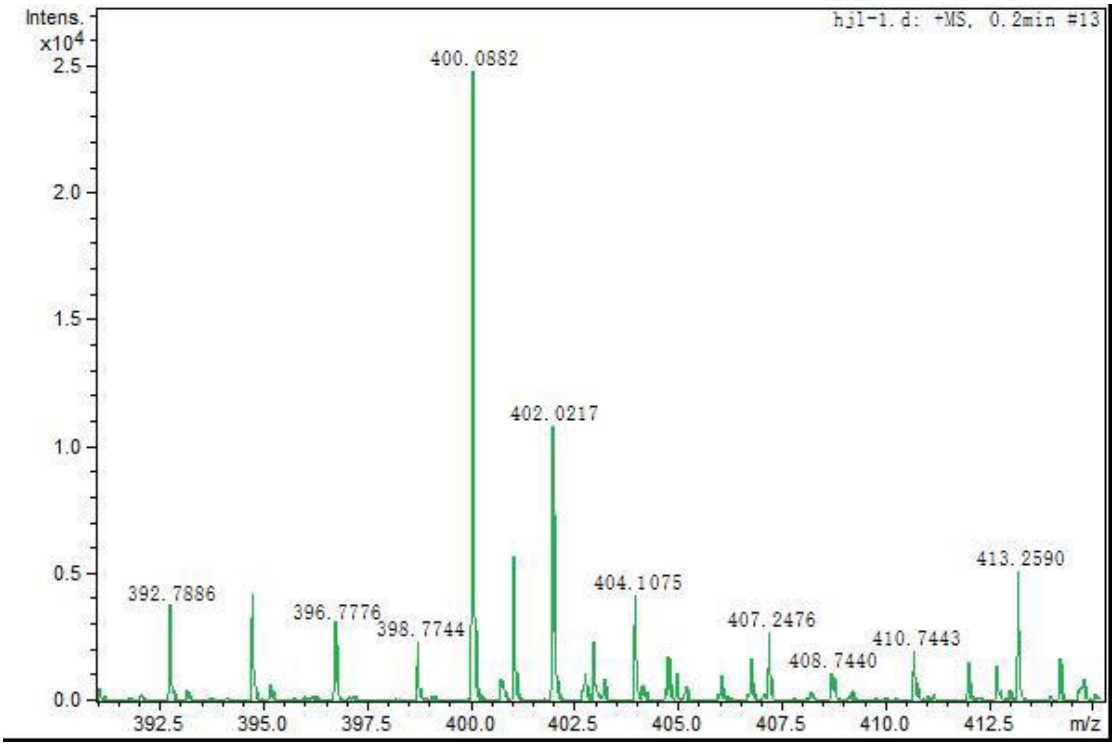


Figure S9. HR-MS spectrum of the reaction product of probe **PTZ-HClO** with HClO.