Supporting Information

Visualization of HOCl in the brains of Alzheimer's Disease models by an easily available two-photon fluorogenic probe

Jia Ke, Peixu Zhao, Jianfeng Li, Qiang Fu[*]

Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang 110016, China.

[*]E-mail addresses: fuqiang@syphu.edu.cn

Contents

1. Materials and instruments

Unless otherwise stated, all reagents in this work were obtained from commercial suppliers and used without further purification. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III for ^{1}H-NMR or ^{13}C-NMR with chemical shifts reported as ppm (TMS as internal standard and DMSO-d_6 as solvent reagent). High resolution mass spectra (HRMS) were measured using Bruker Apex IV Fourier transform mass spectrometry (FTMS) under electrospray ionization (ESI) model. For spectral properties, absorption spectra were recorded on a Purkinje TU-1901 spectrophotometer and fluorescent emission were conducted using a Hitachi F-7000 fluorescence spectrometer. pH measurements were carried out using a Mettler Toledo FE-30 pH meter. MTT assay was conducted utilizing Multiskan FC microplate reader, Thermo Fisher. Fluorescence imaging in living cells were acquired by confocal laser scanning microscope (CLSM, Olympus IX81).
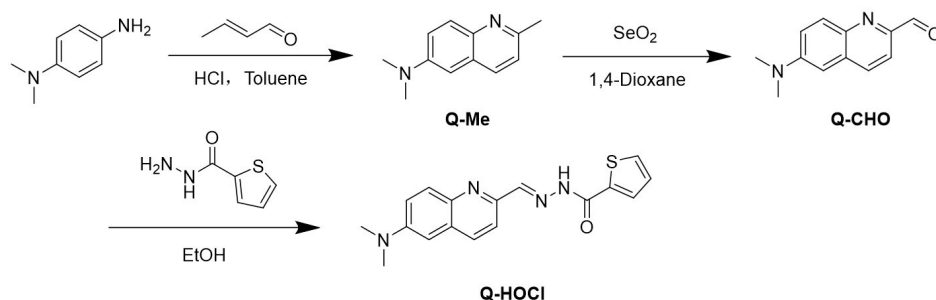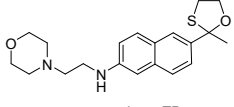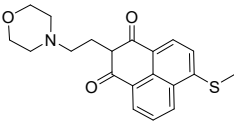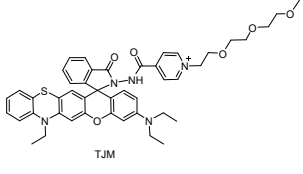
2. Synthesis route
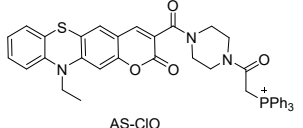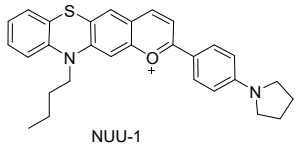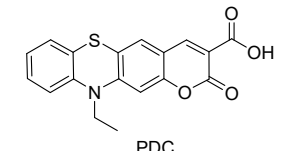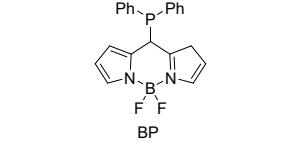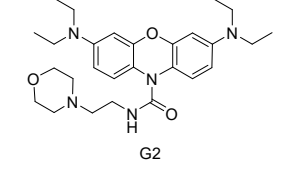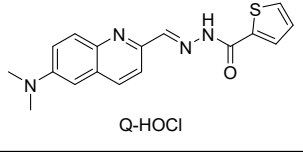
Figure S1. The synthesis route of Q-HOCl.

3. Comparison of fluorogenic probes for HclO

Table S1. Comparison of fluorogenic probes for HClO

Probes	$\lambda_{ex}/\lambda_{em}$ (nm)	Limit of detection	Response time	Quantum yield	Application	Ref.
Lyso-TP	356/500	16.6 nM	seconds	0.380	Cell & tissue imaging	[1]
L1	405/505	0.67 μM	2.5 min	not mentioned	Cell imaging	[2]

Structure						
TJM	585/730	0.11 μM	7 s	not mentioned	Cells & Zebrafish & Diabetic & tumor imaging	[3]
AS-ClO	383/520	12.53 nM	5 s	0.057	Cells & abdominal aorta sections imaging	[4]
NUU-1	365/503	25.8 nM	15 s	not mentioned	Cells & PD brain models imaging	[5]
PDC	400/503	16.1 nM	100 s	0.078	Cells & osteoarthritis model imaging	[6]
BP	546/564	1.9 nM	15 s	not mentioned	Cells & rheumatoid arthritis model imaging	[7]
G2	610/669	91 nM	10 s	not mentioned	Cells & psoriasis model imaging	[8]
Q-HOCl	405/520	12.5 nM	20 s	0.143	Cells & AD model mice & brain slices imaging	This work

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[2] B. Zhang, X. Yang, R. Zhang, Y. Liu, X. Ren, M. Xian, Y. Ye and Y. Zhao, Anal. Chem., 2017, 89, 10384-10390.

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[4] B. Wang, F. Yuan, S. Wang, R. Duan, W.X. Ren, J.-T. Hou, Detection of atherosclerosis-associated HOCl using a mitochondria-targeted fluorescent probe, Sens. Actuators B Chem., 348 (2021) 130695

[5] J. Chen, Y. Lu, Y. Wu, Z. Chen, X. Liu, C. Zhang, J. Sheng, L. Li, W. Chen, X. Song, ACS Chem. Neurosci, 2021, 12, 4058–4064.

[6] J. Hou, B. Wang, Y. Zou, P. Fan, X. Chang, X. Cao, S. Wang, F. Yu, ACS Sens. 2020, 5, 1949–1958.

[7] C. Ma, S. Hou, X. Zhou, Z. Wang, J. Yoon, Anal. Chem. 2021, 93, 9640–9646.

[8] P. Wei, Y. Guo, L. Liu, X. Zhou, T. Yi, J. Mater. Chem. B, 2022,10, 5211-5217.
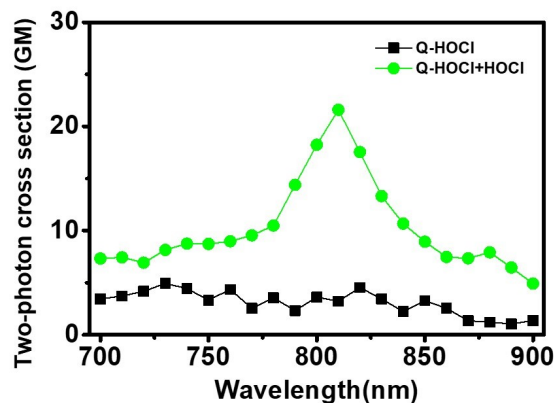
4. Two photon properties of probe Q-HOCl

Figure S2. Two-photon spectra of 10 μM Q-HOCl in the absence and presence of 20 μM HOCl in 10 mM PBS buffer (pH=7.4, containing 5% EtOH).
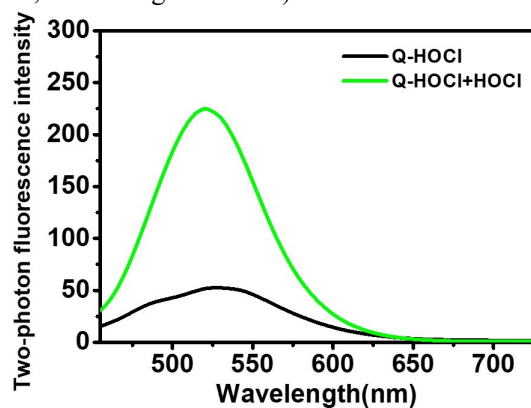

Figure S3. Two-photon fluorescence spectra of 10 μM Q-HOCl before and after reaction with 20 μM HOCl. (λ_{ex}= 810 nm).

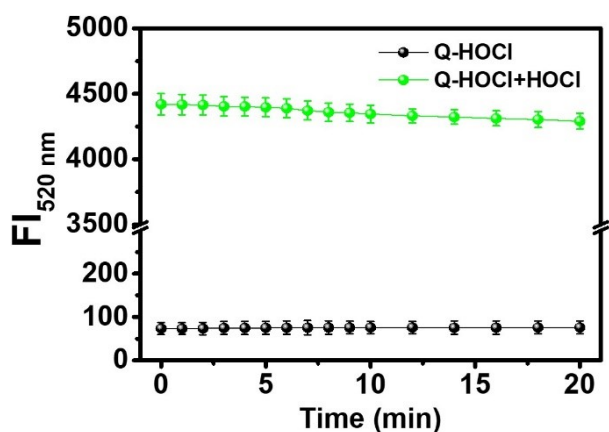# 5. Photostability of probe Q-HOCl

Figure S4. The photostability of probe Q-HOCl (Black dotted line) and the mixture of probe Q-

HOCl with the addition of 20 μM HOCl (Green dotted line) in 10 mM PBS buffer (pH=7.4, within 5% EtOH) at room temperature. λ_{ex} = 400 nm and slit width d_{ex} = d_{em} = 10 nm.

The detection limit was calculated on the basis of the fluorescence titration test in 10 mM PBS buffer (pH=7.4, within 5% EtOH) at room temperature, and using the following equation:

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

where σ is the standard deviation of blank measurements and k is the slope of the plot of fluorescent intensity at 520 nm vs HOCl concentration.

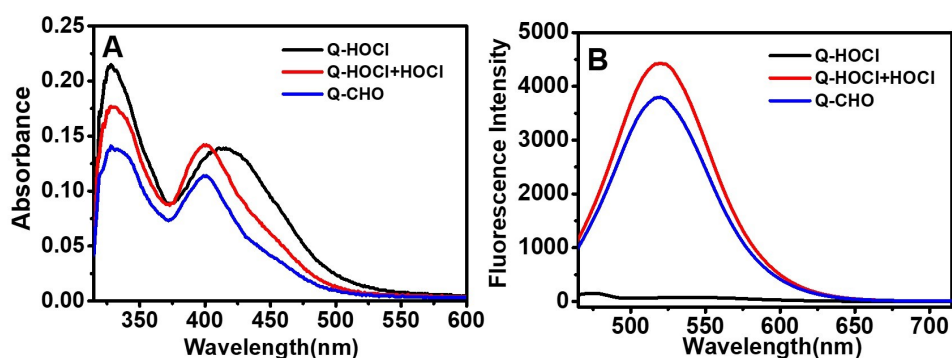# 6. The proposed reaction mechanism of probe with HOCl

Figure S5. Absorption spectra (A) and fluorescence spectra (B) of 10 μM Q-HOCl (Black line), the mixture of 10 μM Q-HOCl with 20 μM HOCl (Red line) and 5 μM Q-CHO (Blue line). The sample solutions were recorded by UV-vis or fluorescence spectrometer in PBS (10 mM, pH=7.4) with 5% EtOH. λ_{ex} = 400 nm and slit width d_{ex} = d_{em} = 10 nm.
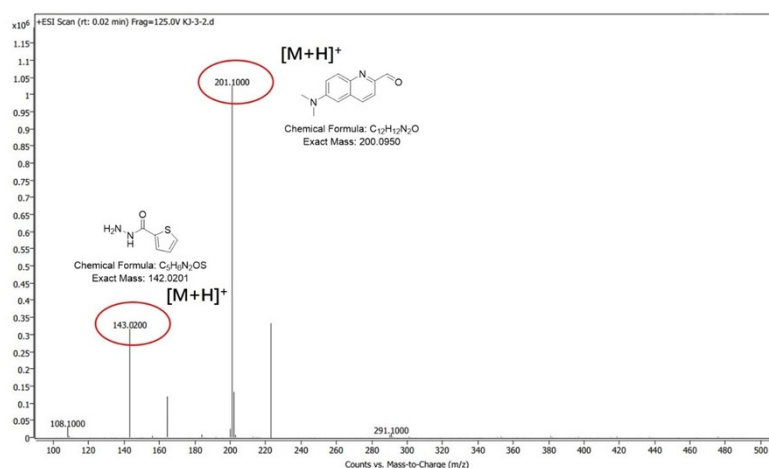

Figure S6. The mixture of probe Q-HOCl with HOCl (1 eq.) was purified by extraction followed by concentration, and then analyzed by ESI-MS spectral.
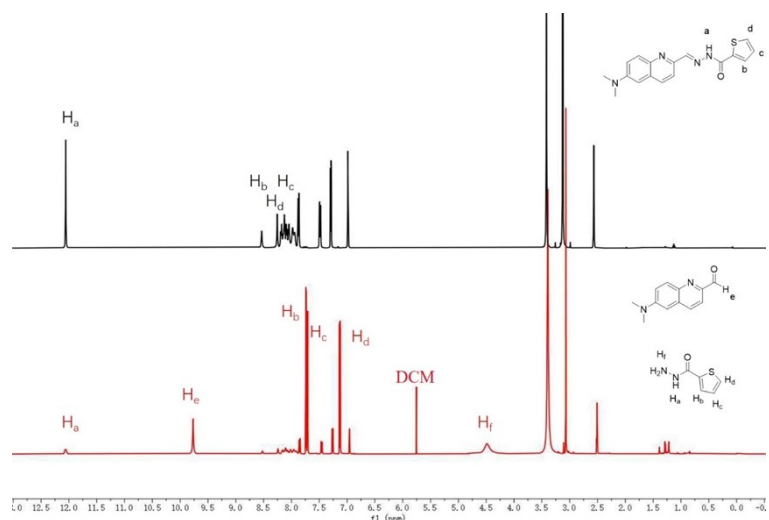
Figure S7. The mixture of probe Q-HOCl with HOCl (1 eq.) was purified by extraction followed by concentration, and then analyzed by the ^{1}HNMR spectra. Black ^{1}H-NMR spectrogram: probe Q-HOCl, Red ^{1}H-NMR spectrogram: the product of probe Q-HOCl with HOCl.
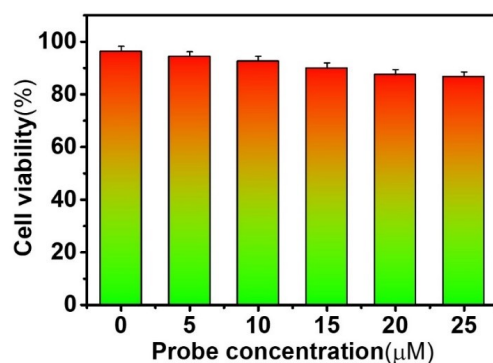
7. MTT assay

Figure S8. MTT assay for estimating cell viability (%) of PC12 cells were seeded into 96-well plates at a density of 5×10^3 cells per well in culture media after treatment with a series concentration of the probe system at 37 °C in an atmosphere of 5% CO_2 and 95% air for 24 h. The concentrations of the probe Q-HOCl were used: 1. blank, 2. 5 μM, 3. 10 μM, 4. 15 μM, 5. 20 μM, 6. 25 μM, respectively.
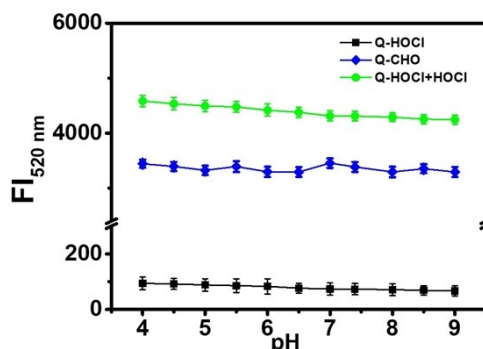
8. The influence of pH values

Figure S9. Fluorescence responses of 10 μM probe Q-HOCl in the absence (Black dotted line) and presence (Green dotted line) of 20 μM HOCl at different pH values and 5 μM compound Q-CHO (Blue dotted line). All dates were performed in 10 mM PBS buffer (pH=7.4, within 5% EtOH) at room temperature. λ_{ex} = 400 nm and slit width d_{ex} = d_{em} = 10 nm.

9. The determination of lipophilicity (Log *P*)

Lipophilicity (log *P*) was measured by typical flask-shaking method. Firstly, probe Q-HOCl was dissolved in a mixture solution of water and 1-octanol (1:1, V/V), and then shaken evenly for 72 h, and centri-fuged for 5.0 min. The contents of the probe-Q-HOCl in 1-octanol and water were measured by fluorescence spectrophotometry.

log *P* was calculated by the following equation:

$$\log p = \frac{[1 - octanol]}{[water]}$$

[1-octanol] represented the probe content in organic phase and [water] represented the probe content in aqueous phase.

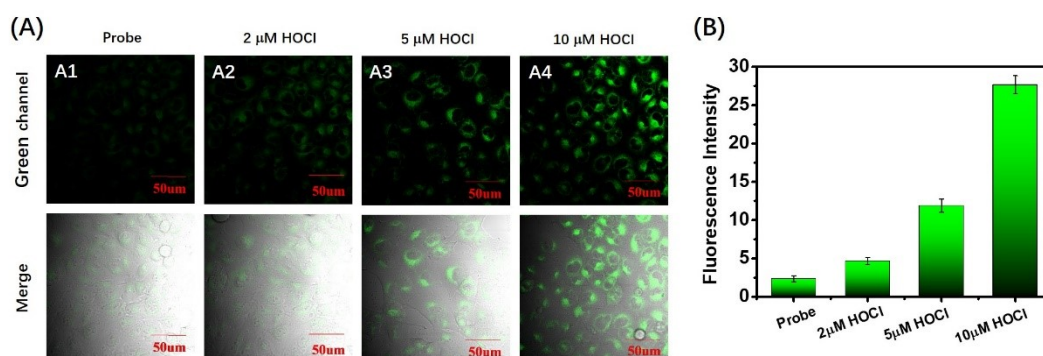# 10. Imaging of exogenous HOCl in PC12 cells

Figure S10. Two-photon cell imaging of exogenous HOCl in PC12 cells. (A) PC12 cells were incubated with 0 μM HOCl (A1), 2 μM HOCl (A2), 5 μM HOCl (A3), 10 μM HOCl (A4) for 10 min and then treated with 5 μM Q-HOCl for 10 min. (B) Relative fluorescence intensity of fluorescence imaging. Green channel (460–560 nm), λ_{ex} = 810 nm. Scale bar is 50 μm. Error bars

represent standard deviation (±SD).
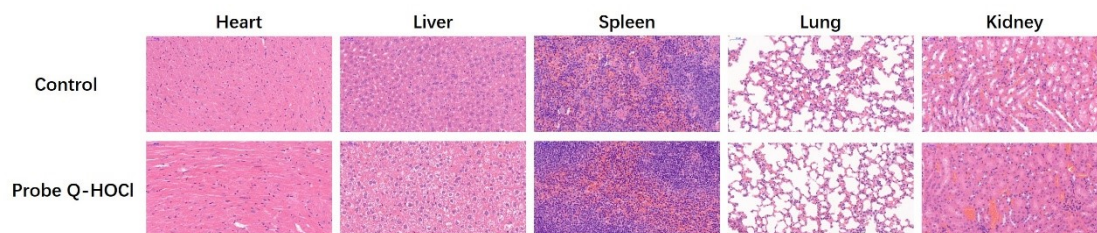
11. Hematoxylin and eosin (H&E) staining

Figure S11. H&E staining results of various organ sections (heart, liver, spleen, lung and kidney) collected from the control group mice and probe Q-HOCl (2 mg/kg) treated group.
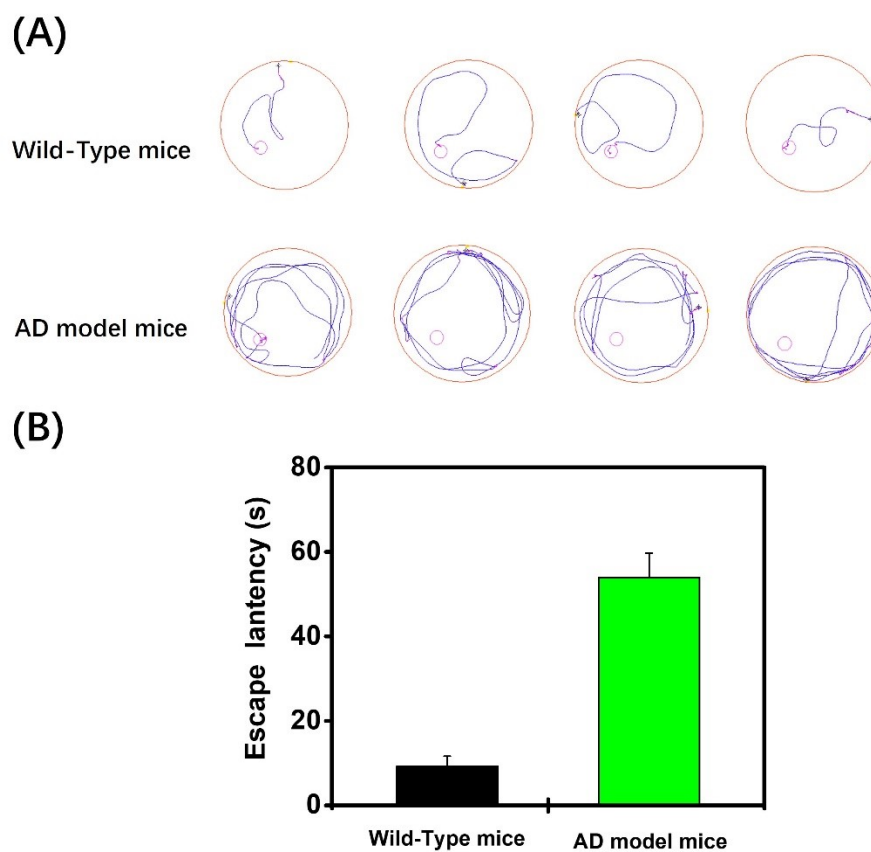
12. Water maze experiment

Figure S12. (A) Trajectories of wild-type mice group and AD model mice group. (B) The escape time collected from the wild-type mice group and AD model mice group in (A). Error bars represent standard deviation (±SD).
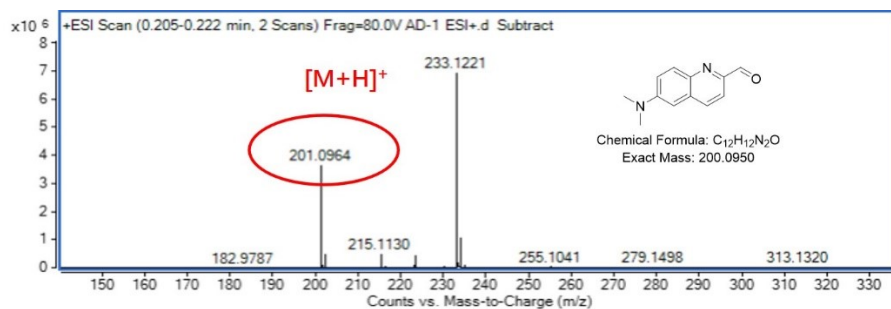
13. Mass and NMR characterization of compounds
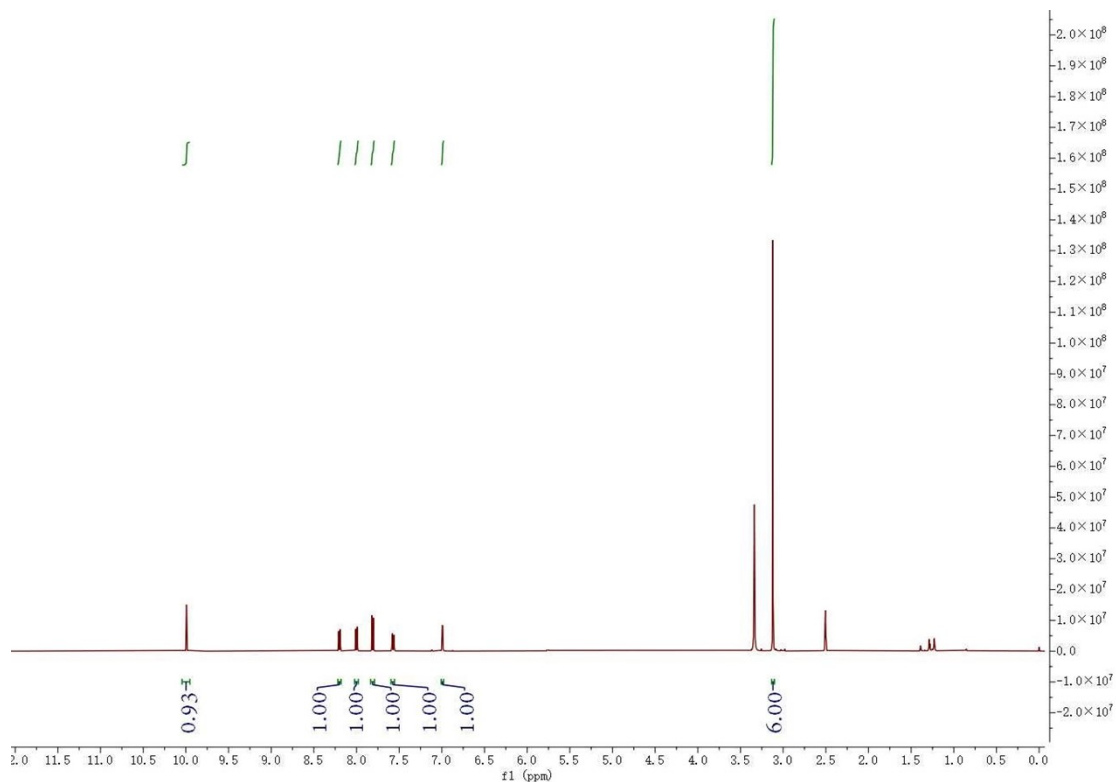

Figure S13. Mass spectrum of compound Q-CHO.
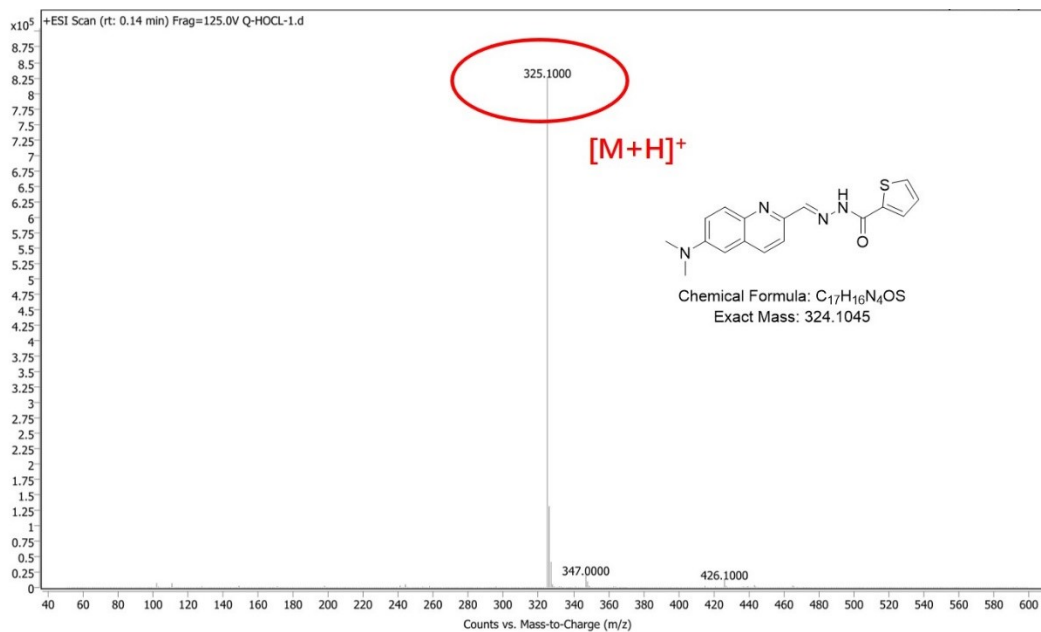

Figure S14. ^{1}H-NMR spectrum of compound Q-CHO.

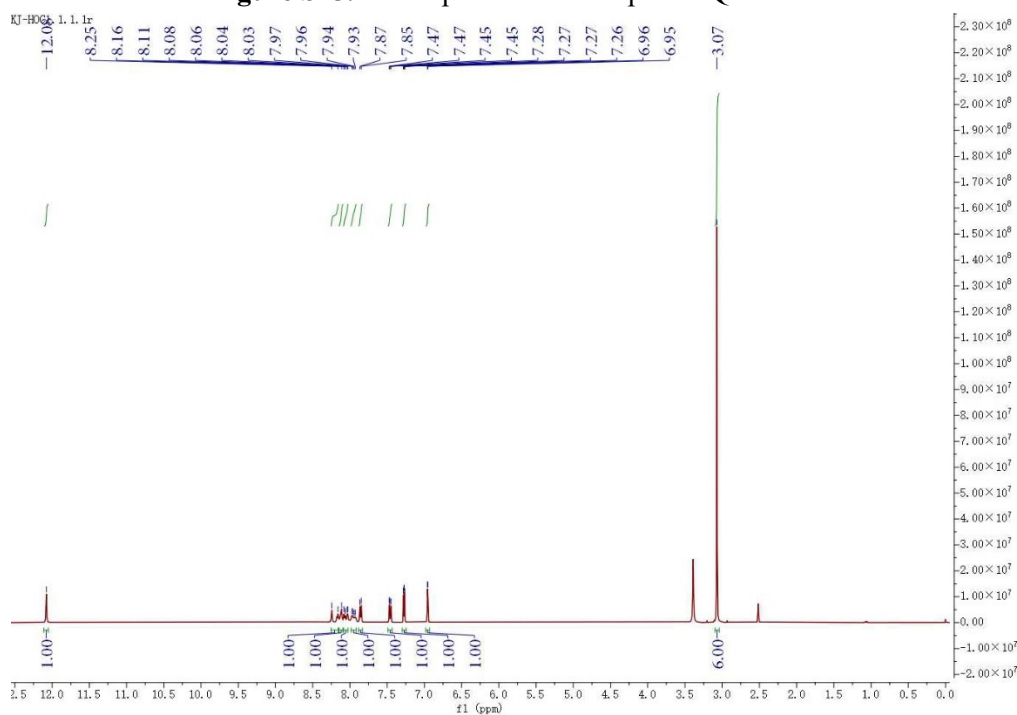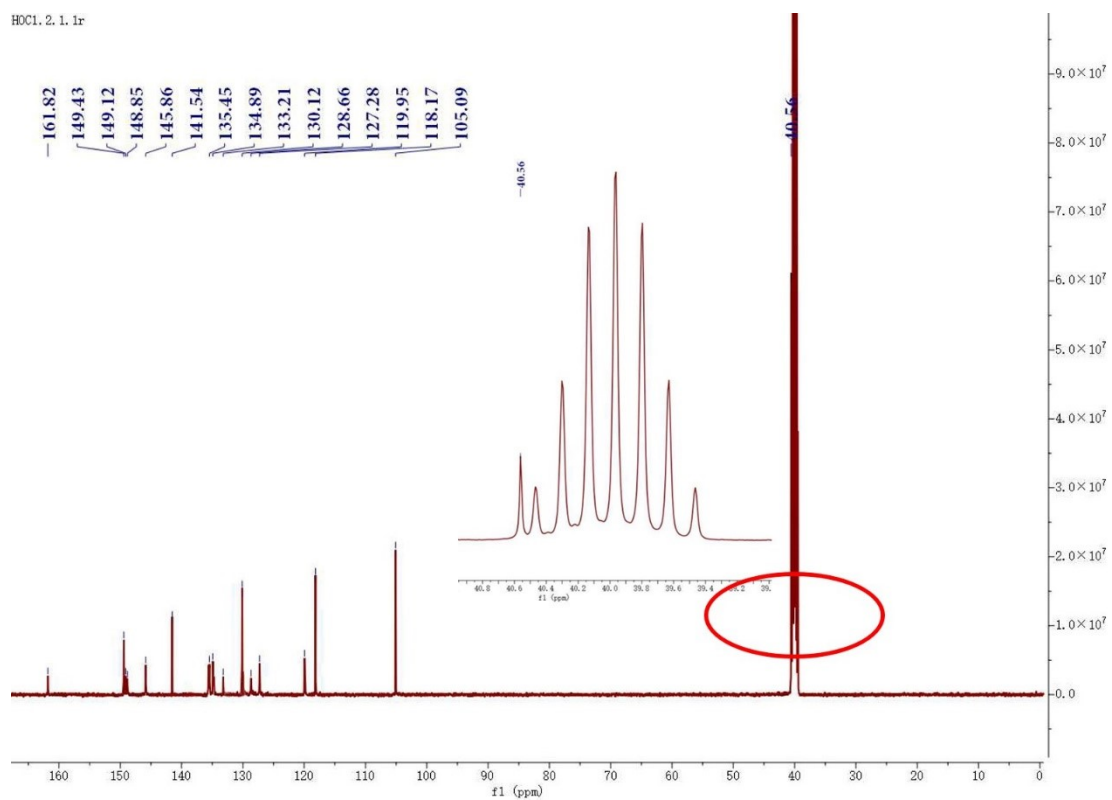**Figure S15.** Mass spectrum of compound Q-HOCl.

Figure S16. ¹H-NMR spectrum of compound Q-HOCl.

Figure S17. ^{13}C-NMR spectrum of compound Q-HOCl.