Electronic Supplementary Material (ESI) for Analyst.

Rational design of a facile camphor-based fluorescence turn-on probe for real-time tracking of hypochlorous acid in vivo and vitro

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1. Experiment

1.1 Materials and instruments

All chemicals were purchased from commercial sources and applied without further purification. Deionized water was adopted for preparing aqueous solution. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were determined on a Bruker AV 500 spectrometer in $CDCl_3$ or DMSO-$_{d6}$ solution. UV-vis absorption spectra were obtained on a Shimadzu UV-2450 spectrophotometer. Fluorescence emission spectra were measured on a PerkinElmer LS55 spectrophotometer. HRMS spectra was recorded on an America Agilent 5975c mass spectrometer. Confocal fluorescence images were captured on a Carl Zeiss LSM-710 confocal laser scanning fluorescence microscope.

1.2 Synthesis

1.2.1 Synthesis of compound **ACO**

9-anthraldehyde (0.206 g, 1.0 mmol), hydroxylamine hydrochloride (0.278 g, 2.0 mmol), and sodium hydroxide (0.2 g, 5.0 mmol) were successively dissovled in 15 mL of anhydrous ethanol and the reaction solution was then heated to to 80 ℃ for 6 h. After removing the superfluous solvent, the resulting residue was washed with distilled water serveral times and then recrystallized in ethanol to obtain compound **ACO** with a yield of 85.3%. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.49-7.56 (m, 5H), 8.01 (J = 6 Hz, 2H), 8.40 (J = 6 Hz, 2H), 8.48 (s, 1H), 9.21 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ:123.72, 125.12, 125.61, 127.03, 129.08, 129.61, 130.40, 131.46, 149.10; ESI-MS (m/z): 220.0 $[M+H]^+$.

1.2.2 Synthesis of compound **ATH**

A mixture of camphor (0.076 g, 0.5 mmol), 9-anthraldehyde (0.124 g, 0.6 mmol), and potassium *tert*-butoxide (0.168 g, 1.5 mmol) was dissolved in 25 mL *tert*-butanol and heated to 80 ℃ for 48 h. After the reaction finished, the solvent was removed and ethyl acetate was employed for extracting the resultant residue for several times. The retained organic layer after drying was evaporated to give a crude product. The crude product was purified by column chromatography (1:5 v/v, ethyl acetate/petroleum ether) to obtain compound **ATH** with a yield of 79.7%. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.84 (s, 3H), 1.03 (s, 3H), 1.11 (s, 3H), 1.26-1.30 (m, 1H), 1.35-1.39 (m, 1H), 1.62-1.67 (m, 1H), 1.71-1.77 (m, 2H), 2.19 (d, J = 6 Hz, 1H), 7.45-7.50 (m, 4H), 8.01-8.03 (m, 5H), 8.43 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 9.56, 18.26, 20.99, 26.08, 30.79, 46.30, 48.81, 58.34, 125.42, 125.52, 125.85, 126.04, 127.16, 127.39, 128.96, 129.45, 130.45, 131.47, 133.68, 134.27, 148.06, 207.38; ESI-MS (*m*/*z*): 341.1 $[M+H]^+$.

1.3 Measurement of HClO in real water samples

In this experiment, a series of environmental water samples including mineral water from our local supermarket, lake water from Xuanwu Lake, and river water from Yangtze River were adopted and mixed with pure DMF to prepare the test solutions of DMF/PBS buffer (4/6, v/v, 10 mM, pH = 7.4). Subsequently, different concentrations of HClO (5, 10, 20, 40 and 80 μM) were spiked into each water samples. The fluorescence intensity of each water sample at 453 nm was measured using the probe **ATHO** (2 μM). Each fluorescence measurement was performed in

triplicate.

1.4 Cytotoxicity testing

Before confocal fluorescence imaging, the cytotoxicity of **ATHO** to HeLa cells was estimated using a standard MTT method. HeLa cells were seeded into a 96-well plate with a density of 1×10^4 cell/well and cultivated in DMEM containing 10% FBS. After that, various concentrations of **ATHO** (0.5, 1.0, 2.0, 3.0, and 5.0 μM) were spiked and further incubated for 48 h. MTT solutions (5 mg/mL, 10 μL) were injected into each well, and these cells were allowed to be maintained for 4 h. After removing the cell supernatant, 200 μL of DMSO solution was supplemented. The absorbance of each plate was measured at 490 nm wavelength. Each cytotoxic test was conducted in triplicate.

1.5 Imaging of living cells

HeLa cells and RAW 264.7 cells were cultured at 37 ℃ in DMEM medium in a humid atmosphere of 5% CO_2. For imaging exogenous HClO in HeLa cells, the cells were stained with the probe **ATHO** (2 μM) and then further treated with HClO (50 μM) for 15 min. For imaging exogenous HClO in RAW 264.7 cells, one group of the cells were treated with PMA (2 μg/mL) for 30 min and further stained with probe **ATHO** (2 μM), and another group of the cells were treated with PMA (2 μg/mL) and then incubated with NAC (2 μg/mL), followed by staining with probe **ATHO** (2 μM) for 30 min. The fluorescent photographs of HeLa cells and RAW 264.7 cells were captured by using a Carl Zeiss LSM-710 confocal fluorescence microscope.

1.6 Imaging of living zebrafish

Zebrafish were grown in embryo media containing 1-phenyl-2-thiourea (PTU) at 30 ℃ for five days. The zebrafish were firstly incubated with probe **ATHO** (2 μM) for 15 min. After that, the zebrafish were then treated with HClO (50 μM) for 15 min. The fluorescence images of zebrafish were captured by using using a Carl Zeiss LSM-710 confocal fluorescence microscope.

2. Figures

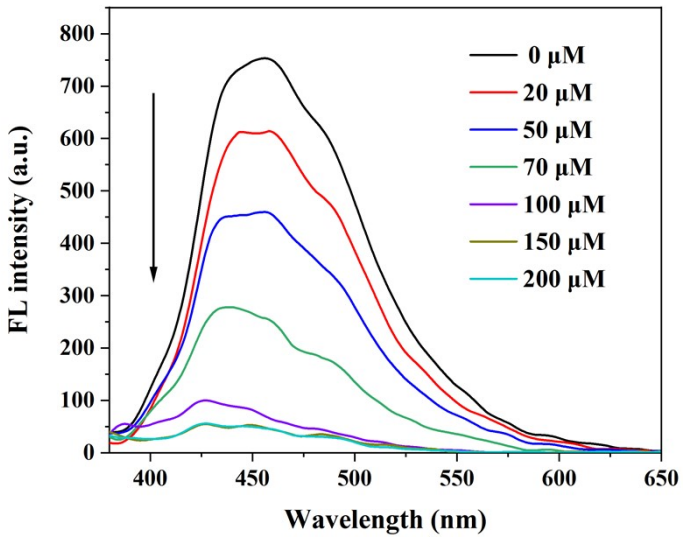


Fig. S1. Fluorescence spectra of compound **ACO** (2 μM) with the increase of HClO concentrations (0-200 μM). (λ_{ex} = 365 nm, slit width: d_{ex}/d_{em} = 10/10 nm).

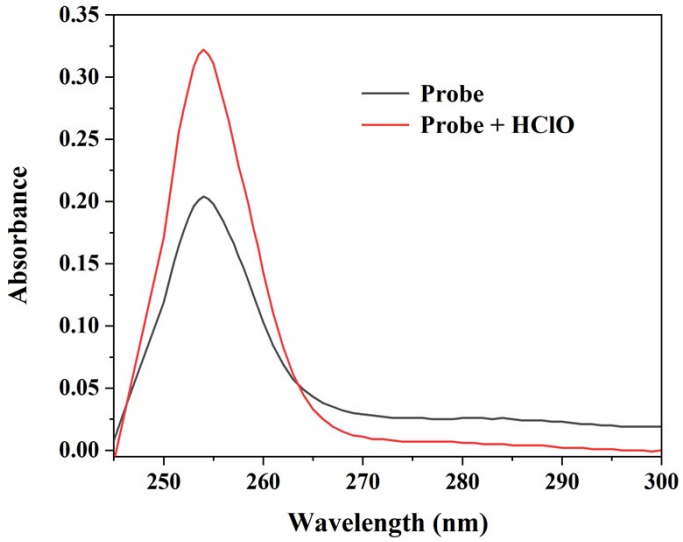


Fig. S2. Absorption spectra of probe **ATHO** (2 μM) with and without HClO (180 μM).

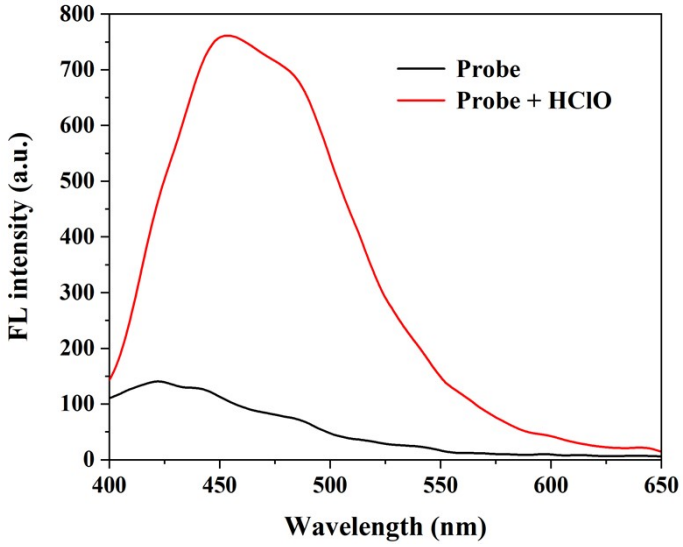


Fig. S3. Fluorescence spectra of probe **ATHO** (2 μM) with and without HClO (180 μM). (λ_{ex} = 365 nm, slit width: d_{ex}/d_{em} = 10/10 nm).

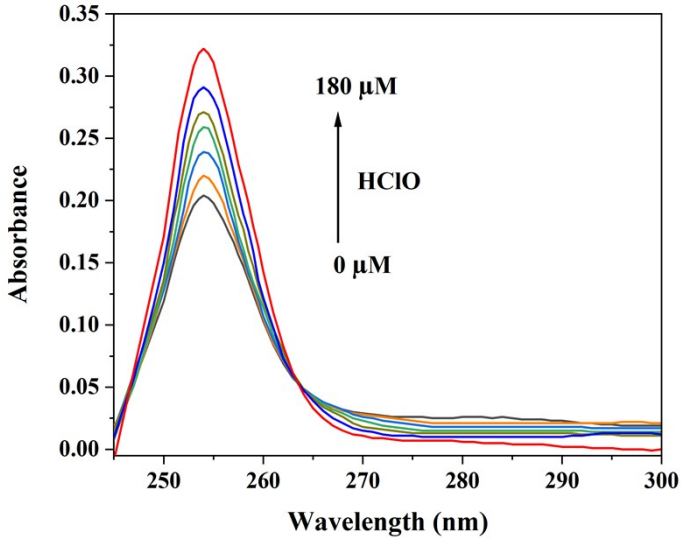


Fig. S4. Absorption spectra of probe **ATHO** (2 μM) after the addition of HClO (0-180 μM).

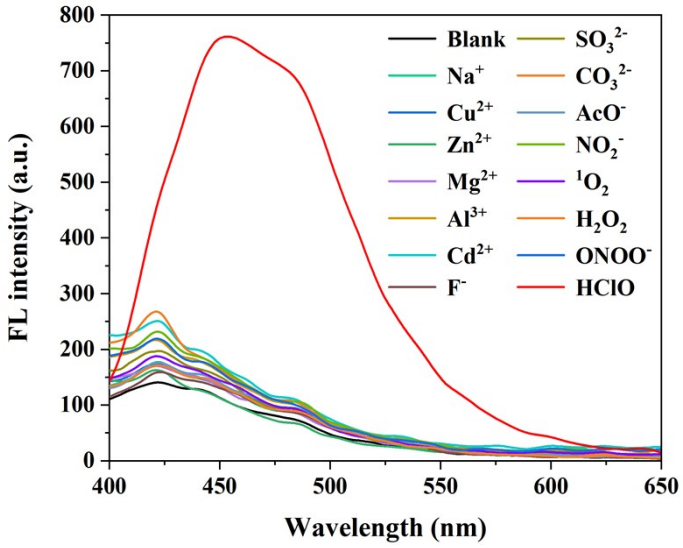
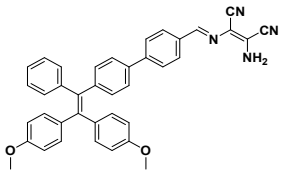
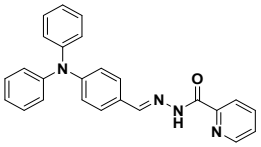
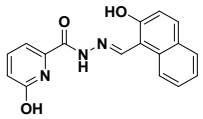
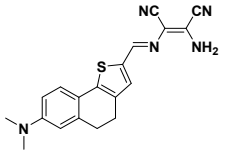
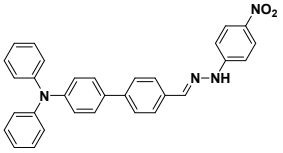
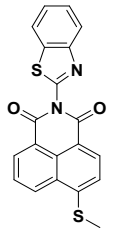
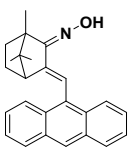


Fig. S5. Fluorescence spectra of probe **ATHO** (2 μM) after addition of various analytes (180 μM). (λ_{ex} = 365 nm, slit width: d_{ex}/d_{em} = 10/10 nm).

Table S1. Comparison of other reported HClO-specific fluorescent probes.

Probes	Response time	Probe concentration	Detection limit	Linear range	Biological imaging
 **Ref [1]**	14 min	10 μM	2.362 μM	0-110 μM	Living cells
 **Ref [2]**	30 s	10 mM	0.8 μM	0-50 μM	Living cells
 **Ref [3]**	–	20 μM	3.99 μM	0-100 μM	Living cells
 **Ref [4]**	1 min	10 μM	0.15 μM	0-60 μM	Living cells
 **Ref [5]**	90 s	10 μM	0.36 μM	0−140 μM	Living cells Living zebrafish
 **Ref [6]**	2 min	10 μM	0.237 μM	0−150 μM	Living cells
 **This work**	Within seconds	2 μM	0.118 μM	0−180 μM	Living cells Living zebrafish

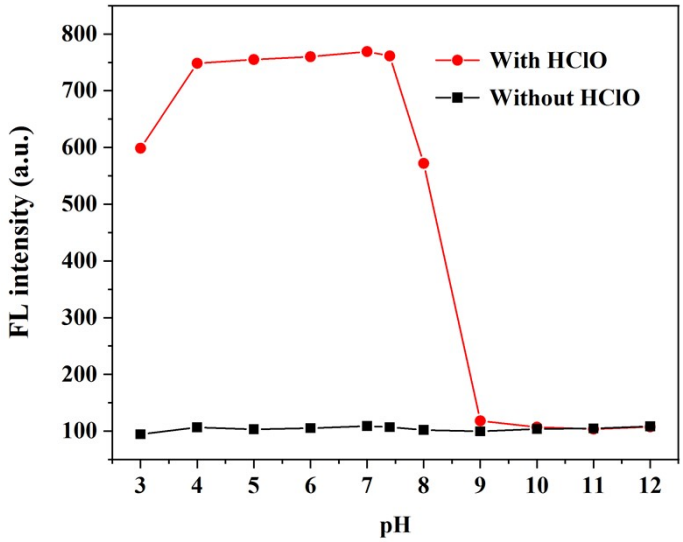


Fig. S6. Fluorescence intensity of probe **ATHO** (2 μM) with and without HClO (180 μM) at different pH values. (λ_{ex} = 365 nm, slit width: d_{ex}/d_{em} = 10/10 nm).

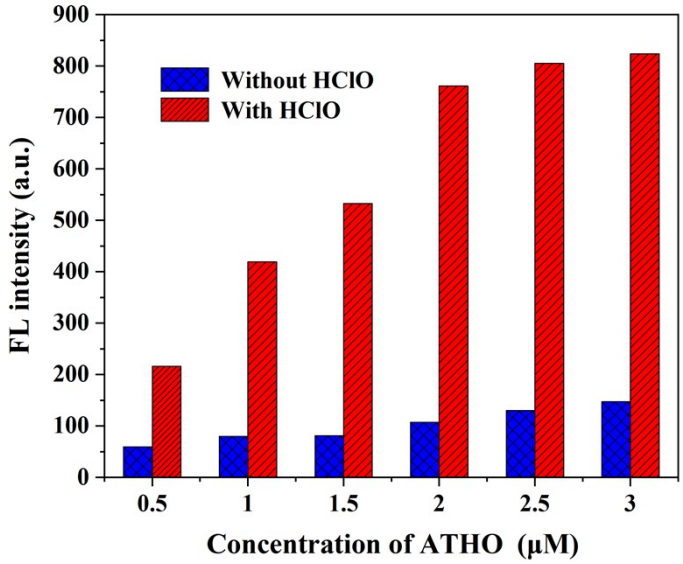


Fig. S7. Fluorescence intensity of probe **ATHO** at different concentrations (0.5-3.0 μM) with and without HClO (180 μM). (λ_{ex} = 365 nm, slit width: d_{ex}/d_{em} = 10/10 nm).

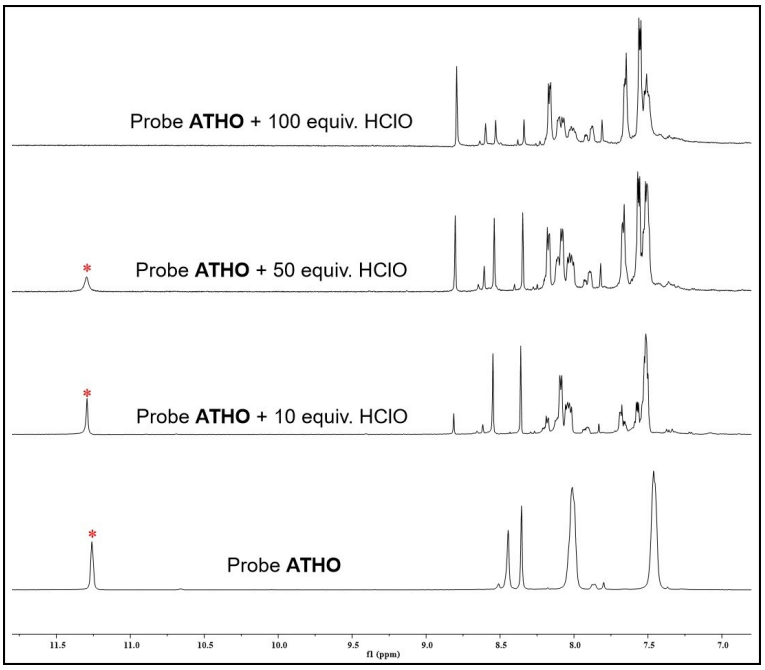
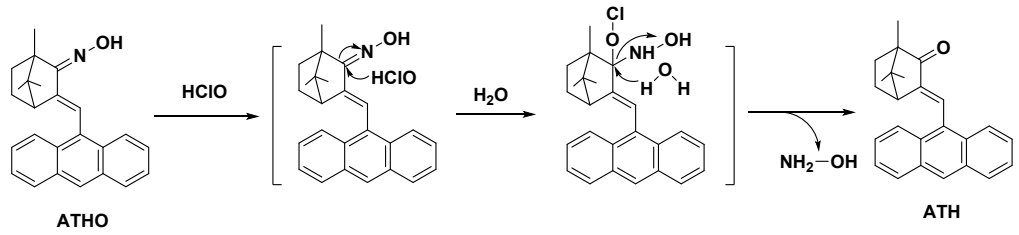


Fig. S8. ^{1}H NMR spectra of probe **ATHO** after the addition of 0-100 equiv. HClO in DMSO-$_{d6}$.



Scheme S1. Schematic diagram of the reaction process of probe **ATHO** with HClO.

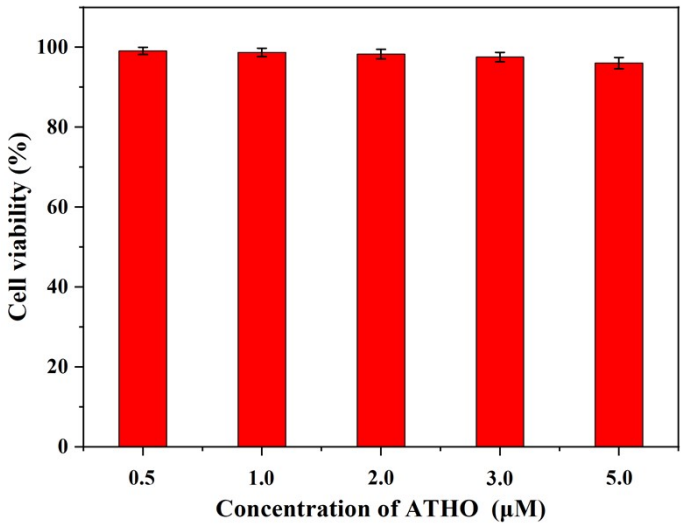


Fig. S9. Cell viability of Hela cells after incubation with different concentrations of **ATHO** (0.5−5.0 μM).

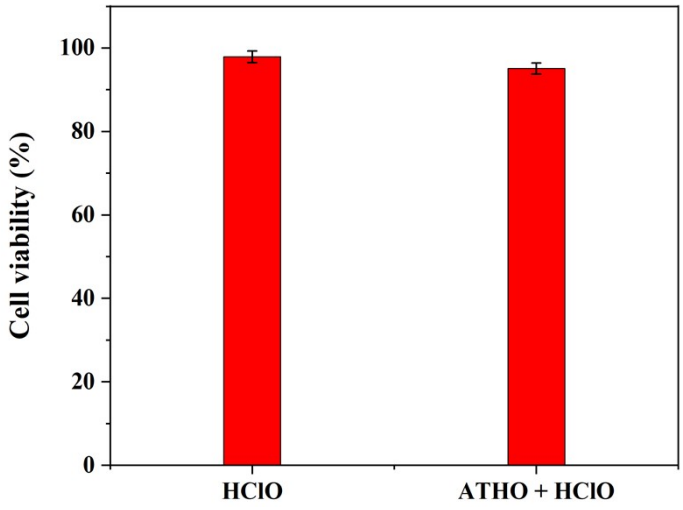


Fig. S10. Cell viability of Hela cells after incubation with HClO (50 μM) in the absence and presence of probe **ATHO** (2.0 μM).

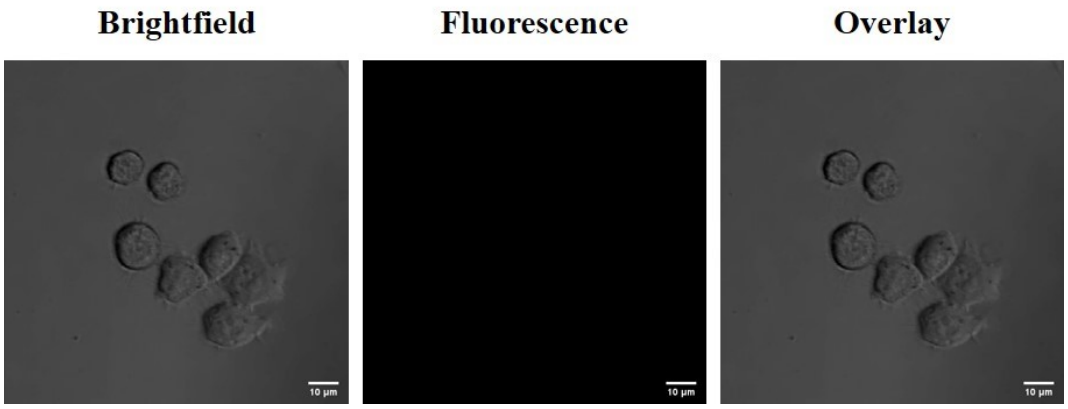


Fig. S11. Confocal fluorescence images of the RAW 264.7 cells only treated with PMA (2 μg/mL).

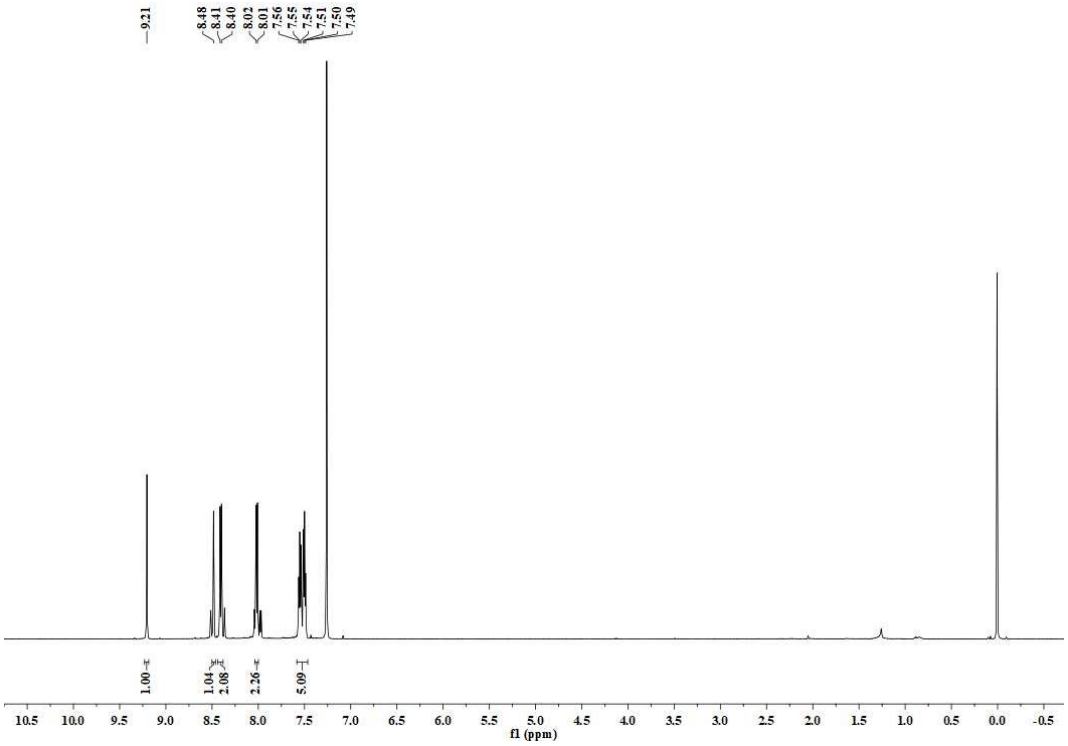


Fig. S12. ^{1}H NMR spectra of compound **ACO** in $CDCl_3$.

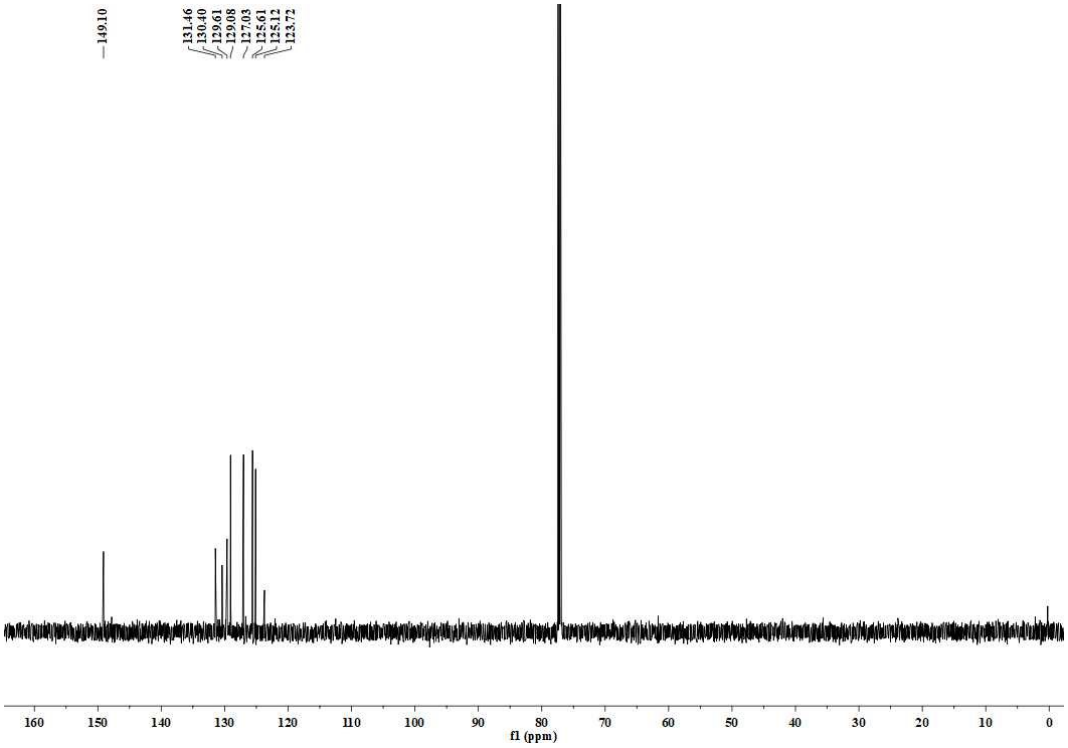


Fig. S13. ^{13}C NMR spectra of compound **ACO** in $CDCl_3$.

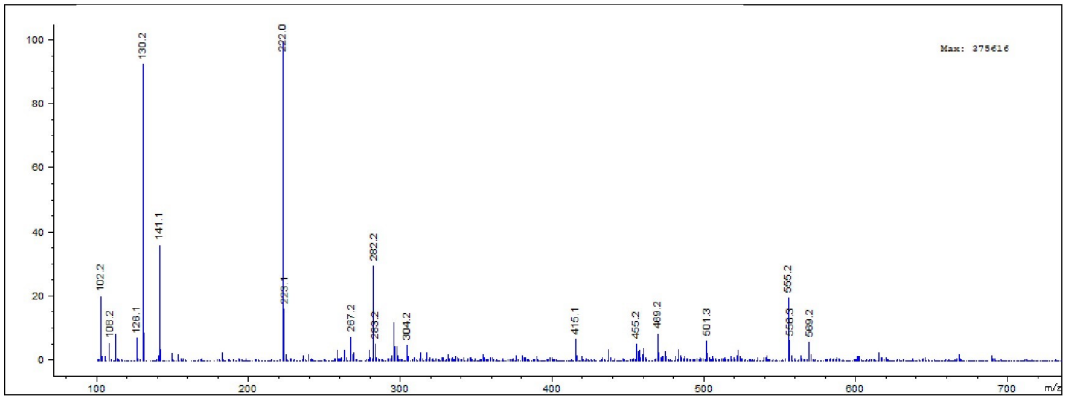


Fig. S14. ESI-MS spectra of compound **ACO**.

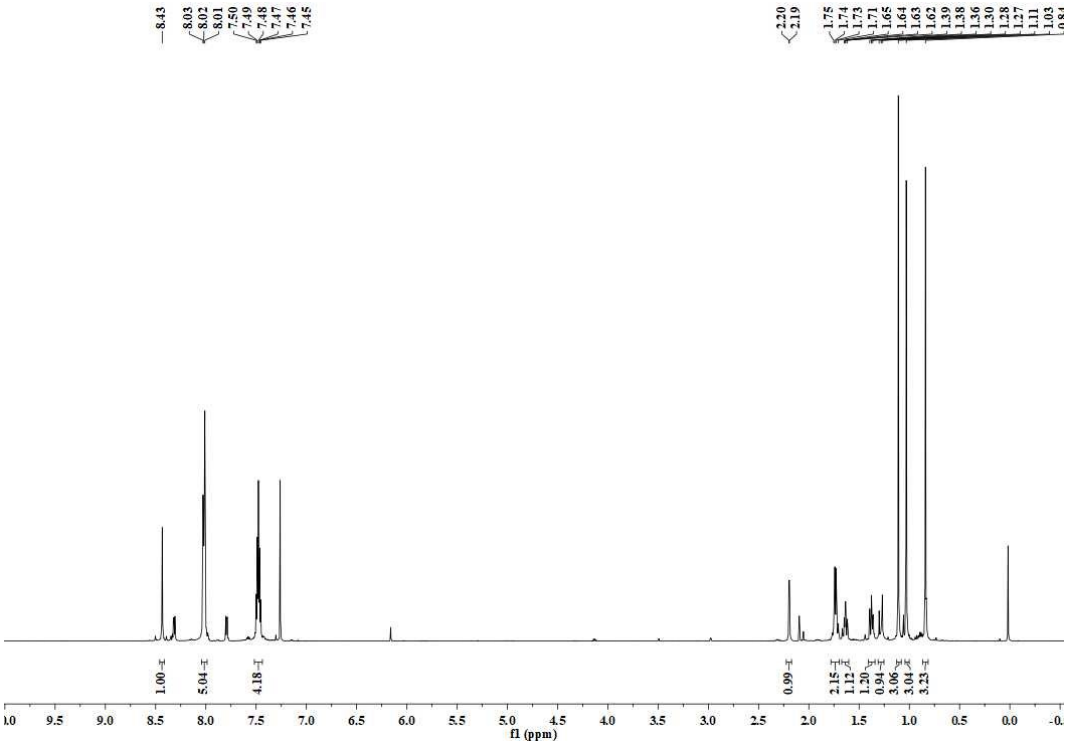


Fig. S15. ^{1}H NMR spectra of intermediate **ATH** in $CDCl_3$.

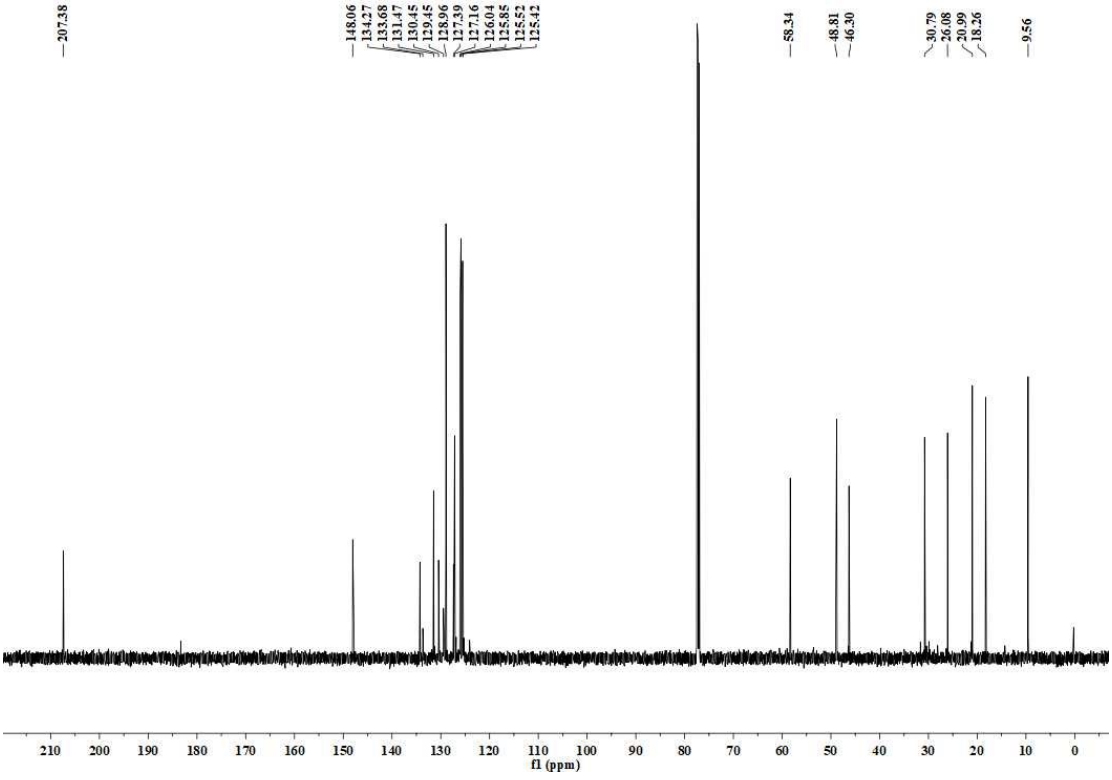


Fig. S16. ^{13}C NMR spectra of intermediate **ATH** in $CDCl_3$.

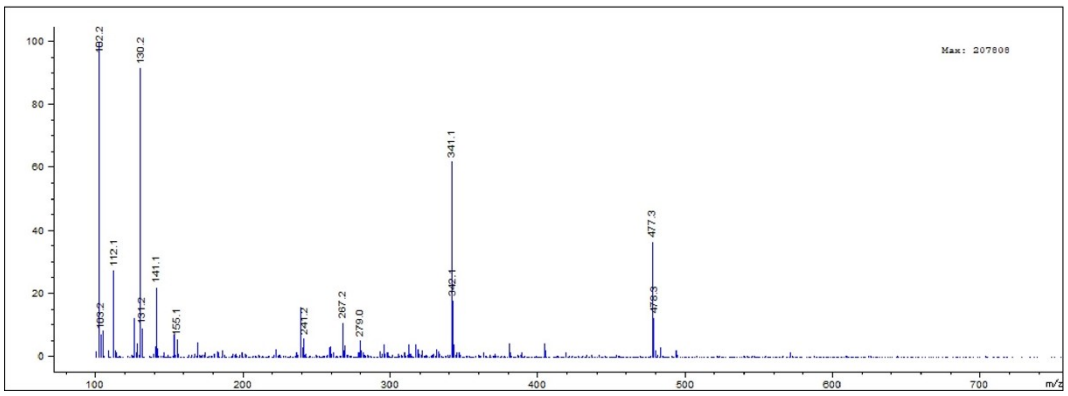


Fig. S17. ESI-MS spectra of compound **ATH**.

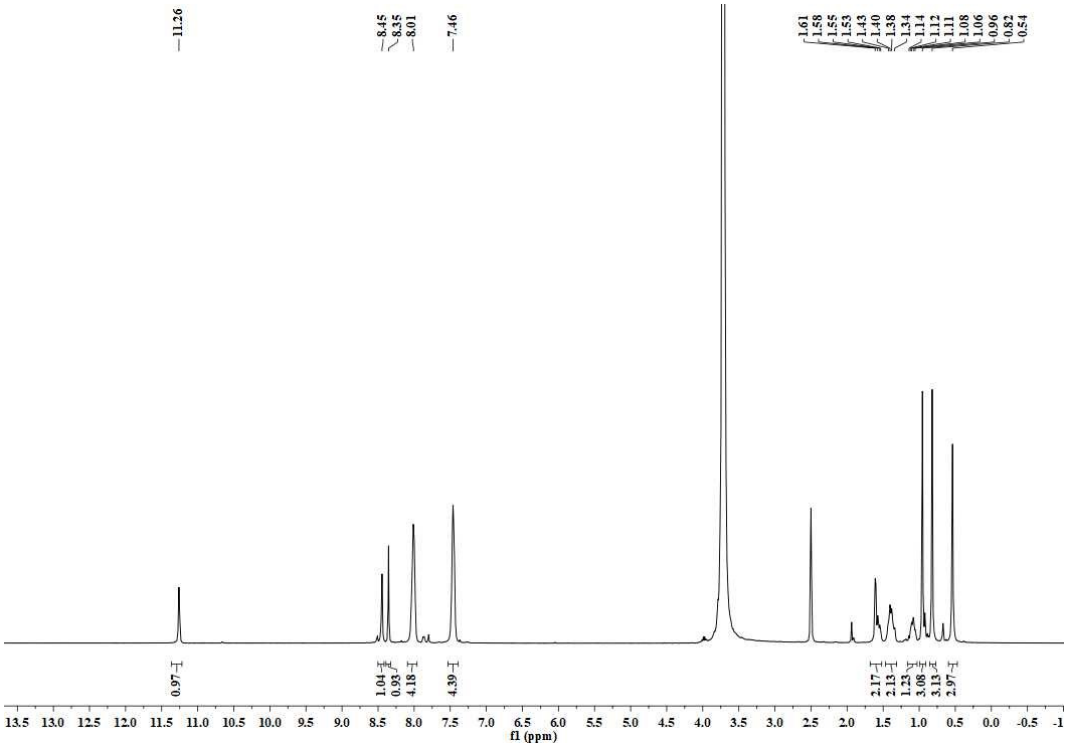


Fig. S18. ^{1}H NMR spectra of probe **ATHO** in DMSO-*d6*.

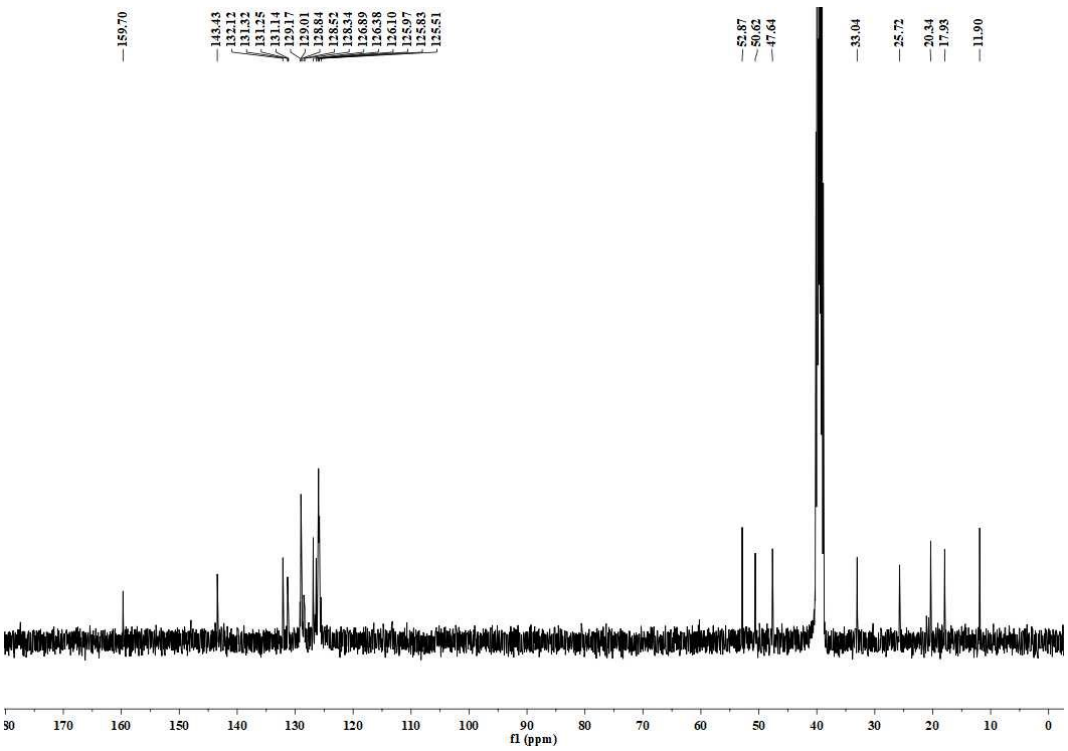


Fig. S19. ^{13}C NMR spectra of probe **ATHO** in DMSO-*d6*.

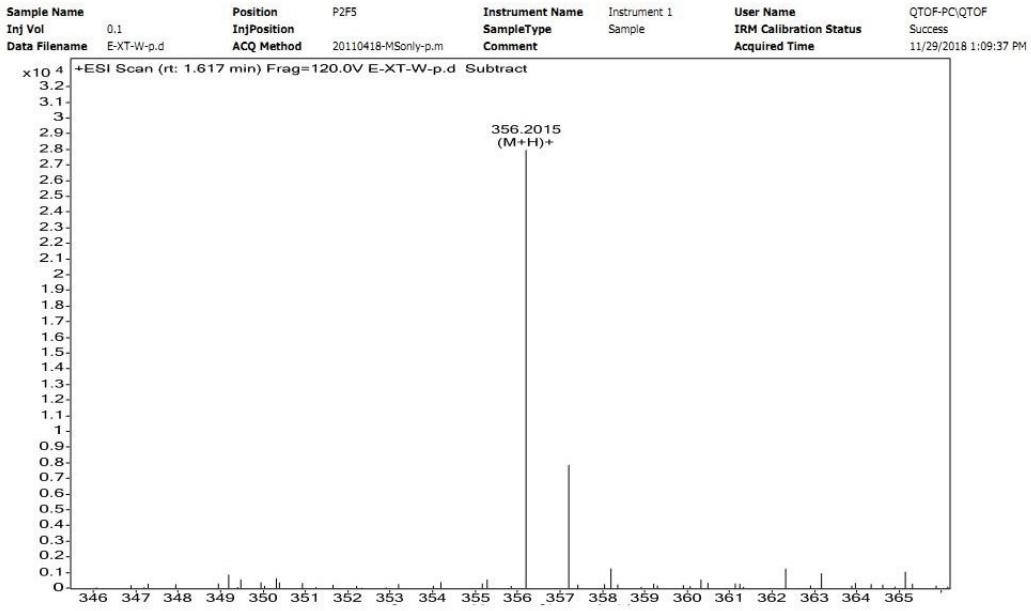


Fig. S20. HRMS spectra of probe **ATHO**.

References

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