Supporting information

An ESIPT-based AIE fluorescent probe to visualize mitochondrial hydrogen peroxide and its application in living cells and rheumatoid arthritis

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Experimental section

2.1 Materials and apparatus

5-Bromosalicylaldehyde, 2-aminobenzenethiol and 4-bromomethylphenylboronic acid pinacol ester were purchased from Aladdin, Bidepharm or Energy Chemical Co., Ltd (Shanghai, China). All chemical reagents and solvents are analytically pure and do not require further purification. LysoTracker-Green and MitoTracker-Green were purchased from Beyotime. The general biochemical agents such as Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), lipopolysaccharide (LPS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCEIII 500M spectrometer (Rheinstetten, Germany) and Bruker AVANCEIII 400 MHz spectrometer (Rheinstetten, Germany). High-resolution mass spectroscopy (HRMS) was obtained on an Orbitrap Velos Pro LC-MS spectrometer (Thermo Scientific). UV–vis absorption spectra were measured on a Shimadzu UV-2450 UV-VIS spectrophotometer. Fluorescence spectra were measured with a HITACHI F-2700 fluorescence spectrophotometer (HITACHI, Japan). The absorbance was recorded with microplate reader SpectraMax M2 in the cytotoxicity experiment. Fluorescence images of cells were acquired on a Leica TCS SP8 laser scanning confocal microscope (Germany).

2.2 Synthesis of probe HTQ-R

Synthesis of HTO-CHO

Compound **2** (1000 mg, 3 mmol) and 4-(diphenylamino) phenylboronic acid (900 mg, 3 mmol) were dissolved in 10 ml anhydrous methanol and 5 ml anhydrous THF, then potassium carbonate (621 mg, 4.5 mmol) and $Pd(dppf)_2Cl_2$ (30 mg) were added into the solution. After stirring for 6 h at 90 ℃ reflux under N_2 atmosphere, reaction mixture was rotary evaporated to remove the solvent and then purified by column chromatography on silica gel (PE: EA=30:1). 1048 mg of **HTO** as orange solid was obtained in 75% yield. **HTO** (1048 mg, yield 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.56 (s, 1H), 8.38 (s, 1H), 8.12 (d, *J* = 2.3 Hz, 2H), 7.97 (d, *J* = 7.9 Hz, 1H), 7.57 (dd, *J* = 11.4, 4.1 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.48 (dd, *J* = 11.2, 4.0 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 4H), 7.18 (d, *J* = 8.6 Hz, 2H), 7.15 (d, *J* = 7.6 Hz, 4H), 7.06 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.48, 173.42, 159.50, 152.16, 151.47, 147.62, 147.54, 135.89, 132.52, 132.49, 132.32, 131.18, 129.39, 127.42, 126.99, 126.01, 124.81, 124.58, 123.82, 123.20, 122.46, 121.66. HRMS: calcd for $[M+H]^+$: 499.1479; found: 499.1454.

Synthesis of HTQ

HTO-CHO (500 mg, 1 mmol) and p-toluenesulfonic acid (475 mg, 2.5 mmol) were dissolved in 15 ml DMF, then 4-methylquinoline (214 mg, 1.5 mmol) was added into the solution. After stirring for 10 h at 120 ℃ reflux under N_2 atmosphere, the reaction

was quenched by water and then extracted thrice with dichloromethane. The organic layers were combined, washed with water, dried over anhydrous potassium carbonate and rotary evaporated. The residue was purified by column chromatography on silica gel (PE: EA=15:1) to afford **HTQ** (498 mg) as red oil in 80% yield. **HTQ** (498 mg, yield 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.37 (s, 1H), 8.89 (d, J = 4.6 Hz, 1H), 8.27 (d, J = 8.2 Hz, 1H), 8.14 (d, J = 8.3 Hz, 1H), 8.10 (d, J = 16.2 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.91 – 7.88 (m, 2H), 7.80 (dd, J = 11.5, 9.2 Hz, 2H), 7.75 – 7.70 (m, 1H), 7.68 (d, J = 4.6 Hz, 1H), 7.61 – 7.56 (m, 1H), 7.54 – 7.49 (m, 3H), 7.44 – 7.40 (m, 1H), 7.29 (t, J = 7.9 Hz, 4H), 7.20 (d, J = 8.5 Hz, 2H), 7.16 (d, J = 7.6 Hz, 4H), 7.06 (t, J = 7.3 Hz, 2H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 169.27, 155.52, 151.58, 150.07, 148.51, 147.64, 147.33, 143.62, 133.76, 132.63, 132.43, 130.01, 129.90, 129.38, 129.14, 127.52, 126.90, 126.62, 126.53, 126.48, 125.99, 125.84, 124.48, 124.37, 124.04, 123.66, 123.11, 122.24, 121.60, 117.42, 117.01. HRMS: calcd for $[M+H]^+$: 624.2031; found: 624.20795.

Synthesis of HTQ-R

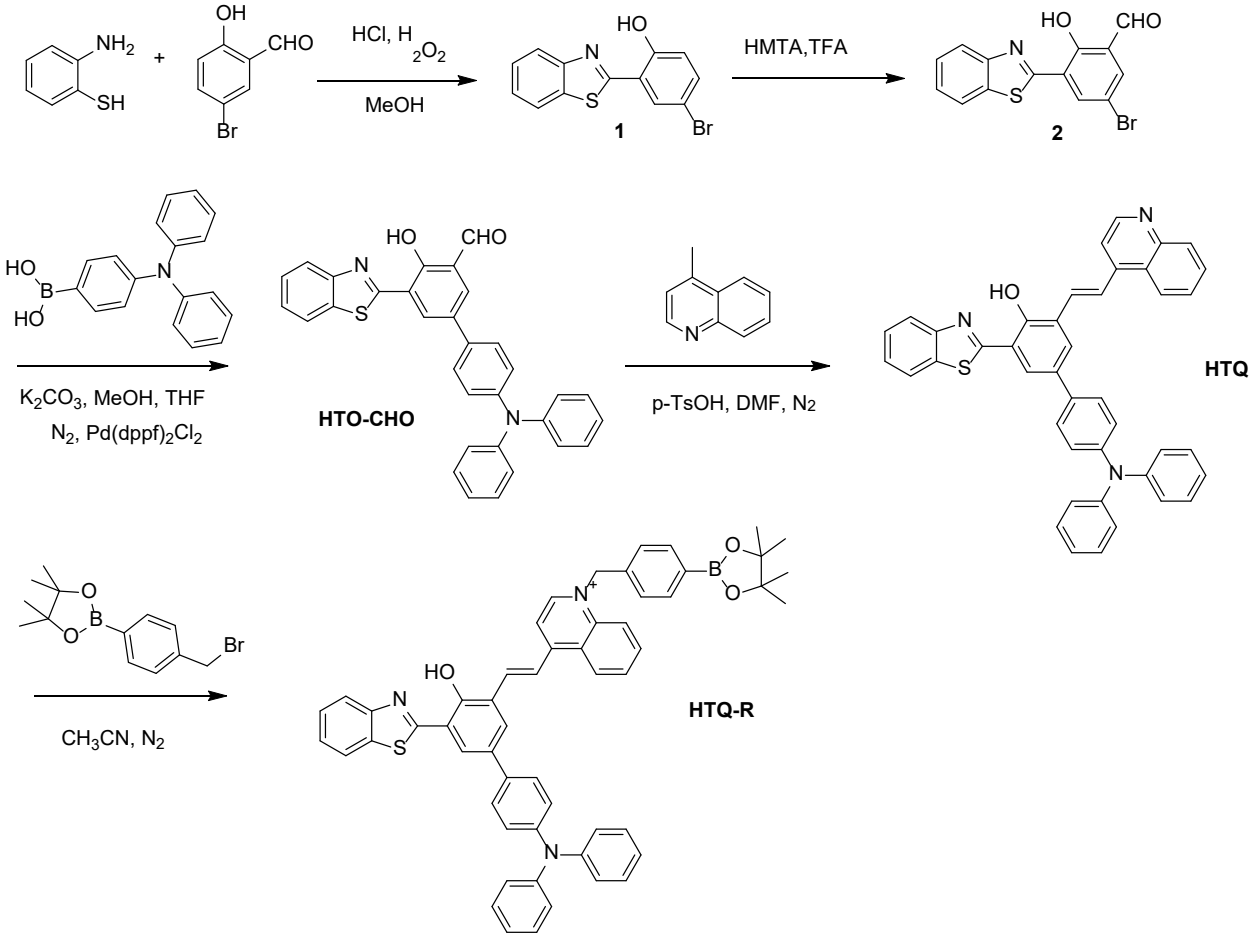
4-bromomethylphenylboronic acid pinacol ester (297 mg, 1mmol) was added to **HTQ** (300 mg, 0.5 mmol) in 13 ml acetonitrile, reaction was carried on at 90 ℃ reflux under N_2 atmosphere. After stirring for 4 h, the reaction was quenched and mixture was rotary evaporated to remove the solvent. The residue was purified by column chromatography on silica gel (DCM: MeOH=10:1). 417 mg of **HTQ-R** as black solid was obtained in 50% in yield. ^{1}H NMR (500 MHz, CDCl3) δ 10.18 (d, J = 6.3 Hz, 1H), 8.57 (d, J = 8.4 Hz, 1H), 8.40 (dd, J = 11.2, 4.5 Hz, 2H), 8.13 (dd, J = 22.6, 12.4 Hz, 3H), 7.97 (d, J = 8.1 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.75 (d, J = 8.1 Hz, 2H), 7.49 (dd, J = 15.6, 8.0 Hz, 4H), 7.42 – 7.35 (m, 2H), 7.31 – 7.27 (m, 5H), 7.25 (s, 1H), 7.18 (d, J = 8.6 Hz, 2H), 7.15 (d, J = 7.7 Hz, 4H), 7.06 (t, J = 7.3 Hz, 2H), 6.75 (d, J = 8.2 Hz, 1H), 5.29 (s, 2H), 1.30 (s, 12H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 168.76, 156.87, 154.50, 152.31, 151.25, 149.23, 147.56, 147.51, 142.38, 139.94, 138.18, 136.17, 135.76, 135.06, 132.67, 132.51, 132.46, 132.05, 129.41, 127.44, 127.28, 127.06, 126.21, 126.06, 124.63, 124.24, 123.86, 123.74, 123.47, 123.24, 122.26, 121.65, 119.43, 117.73, 116.86, 116.05, 84.04, 53.47, 24.84. HRMS: calcd for $[M+H]^+$: 840.3426; found: 840.04106.

2.5 Cell culture and imaging

HeLa cells were cultured at 37 °C in a DMEM high-glycemic medium containing 10% (V/V) fetal bovine serum. The HeLa cells were divided into four groups. The first group of cells were only incubated with **HTQ-R** (10 μM). The exogenous H_2O_2 imaging group of cells were treated with H_2O_2 (20, 40, 60 μM) for 30 min, then incubated with **HTQ-R** (10 μM) for another 30 min. The endogenous H_2O_2 imaging group of cells were treated with LPS (10 μg/mL in PBS) and **HTQ-R** (10 μM) for 20, 40, 60 min. The negative control group of cells were incubated with NAC (1 mM) for 30 min followed by LPS (10 μg/mL in PBS) stimulation, after that cells were incubated with **HTQ-R** (10 μM) for another 30 min. The cells were washed for three times with PBS before imaging, Leica TCS SP8 (MP+X) confocal laser scanning microscope (Leica, Germany), objective lens 40 times (excitation wavelength 405 nm) is used to collect

fluorescence signals. Red channels were collected at 600-650 nm (λ_{ex} = 405 nm) and green channels were at 510-550 nm (λ_{ex} = 488 nm).

Synthesis



Scheme S1. Synthesis route of probe HTQ-R.

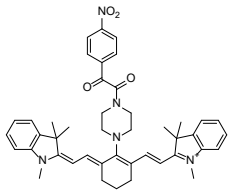
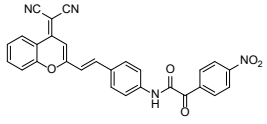
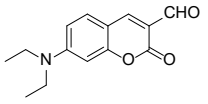
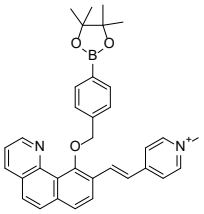
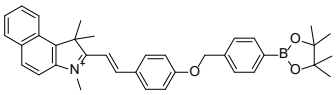
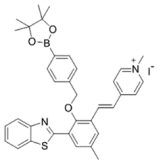
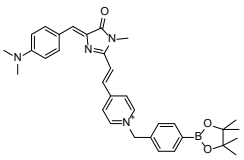
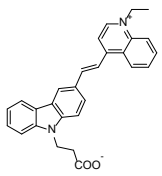
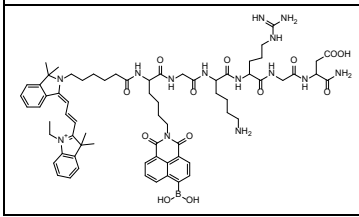
Structure	Mechanism	Response mode	$\lambda_{ex}/\lambda_{em}$	LOD
	PET	Turn on	λ_{ex}=730 nm λ_{em}=790 nm	65 nM
	ICT	Turn on	λ_{ex}=487 nm λ_{em}=653 nm	5300 μM
	Payne Dakin tandem reaction	Turn on	λ_{ex}=450 nm λ_{em}=502 nm	31nM
	ESIPT	Ratiometric	λ_{ex}=422 nm λ_{em}=656 nm	40.2 nM
	ICT	Turn on	λ_{ex}=524 nm λ_{em}=572 nm	87 nM
	ESIPT	Ratiometric	λ_{ex}=373 nm λ_{em}=669 nm	590 nM
	TICT	Turn on	λ_{ex}=514 nm λ_{em}=660 nm	2010 nM
	FRET	Ratiometric	λ_{ex}=526 nm λ_{em}=630 nm	400 nM
	FRET	Ratiometric	λ_{ex}=445 nm λ_{em}=545 nm	260 nM

Table S1 An overview for reported optical probes for sensing H_2O_2.

Figures

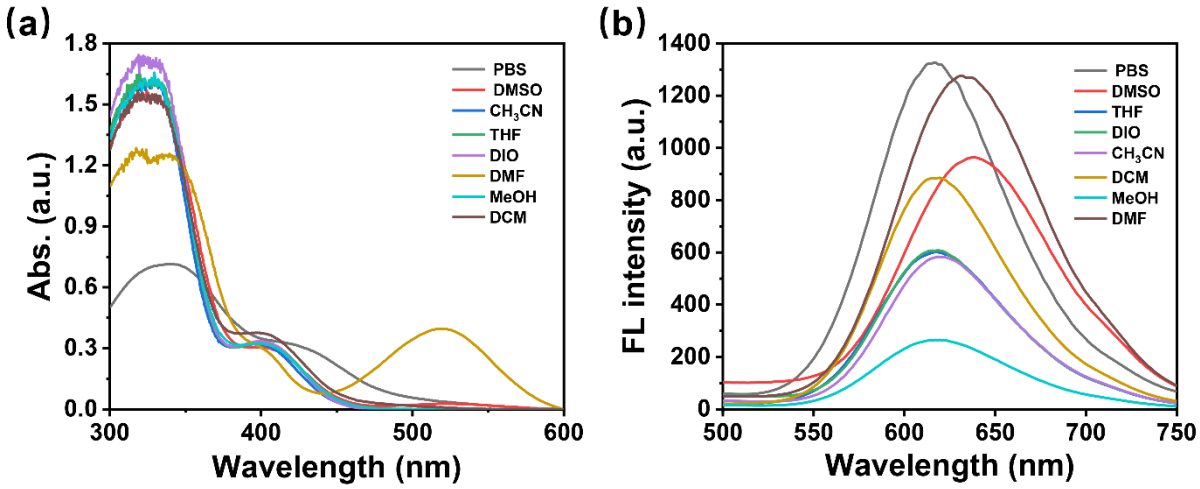


Fig.S1. (a) UV spectra of **HTQ** (10 μM) and (b) fluorescence spectra of **HTQ** (10 μM) in eight different solvents at room temperature. λex = 400 nm.

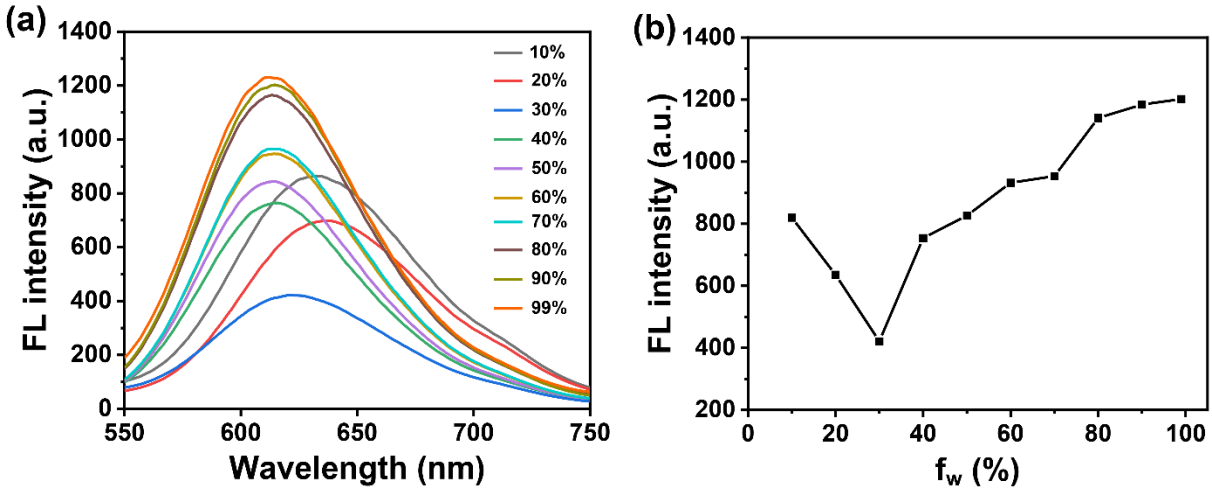


Fig.S2. Fluorescence spectra of **HTQ** (10 μM) in DMSO/H_2O system at room temperature. λex = 400 nm.

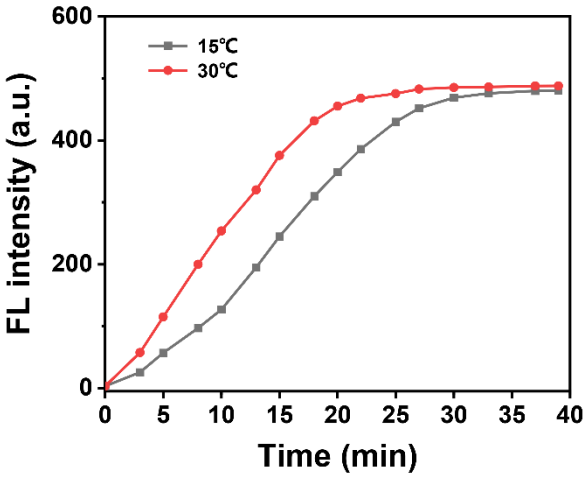


Fig.S3. Time-dependent fluorescence intensities of **HTQ-R** (10 μM) at 620 nm toward H_2O_2 (50 μM) at 15 ℃ and 30 ℃ respectively.

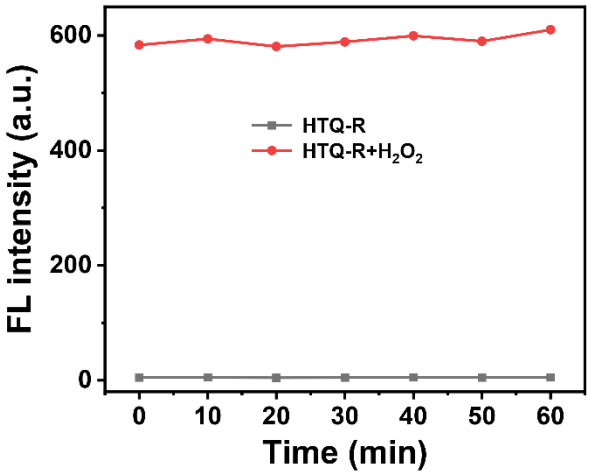


Fig.S4. Photostability of the **HTQ-R** (10 μM) in the absence and presence of H_2O_2 (50 μM) under ambient light illumination over a period of 60 min at ambient temperature. λ_{ex} / λ_{em} = 400 /620 nm.

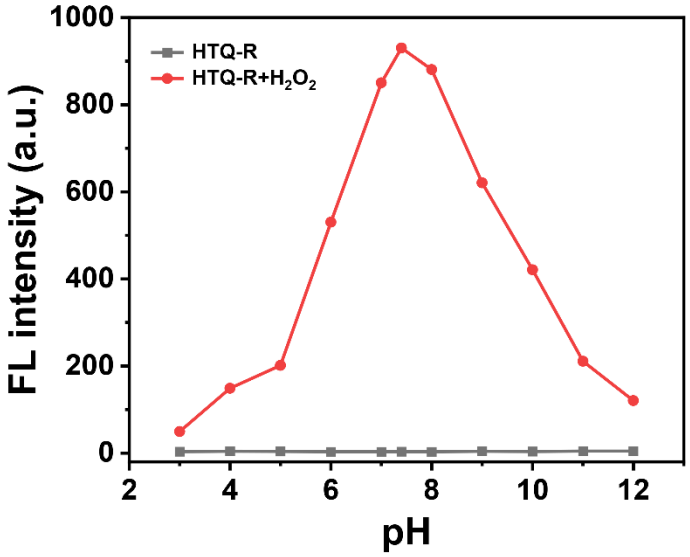


Fig.S5. Fluorescence intensity of **HTQ-R** (10 μM) in the absence and presence of H_2O_2 (100 μM) at different pH.

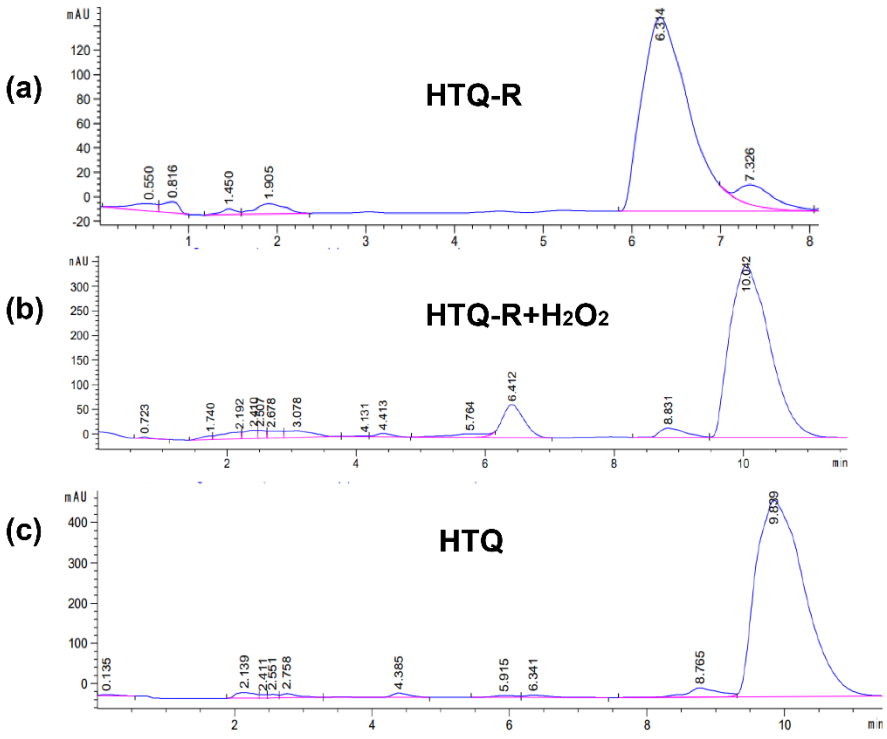


Fig.S6. HPLC traces of **HTQ-R**, **HTQ-R** incubated with H_2O_2, **HTQ**. The mixture of **HTQ-R** (10 μM) and H_2O_2 (50 μM) was incubated for 20 min at 37 ℃.

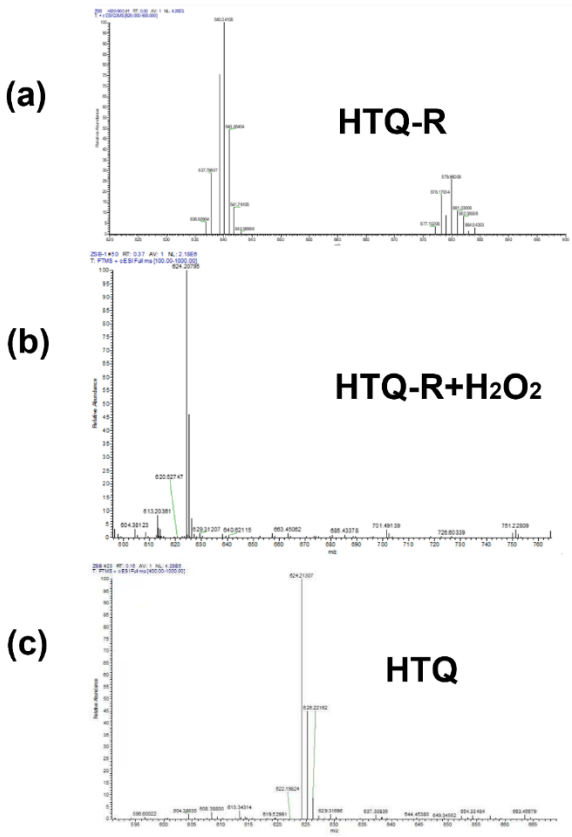


Fig.S7. HRMS analyses of (a) **HTQ-R**, (b) **HTQ-R** incubated with H_2O_2, and (c) **HTQ**. The mixture of **HTQ-R** (10 μM) and H_2O_2 (50 μM) was incubated for 20 min at 37 ℃. The peak at m/z = 840.04108 can be characterized by **HTQ-R**. The peak at m/z = 624.20795 and 624.21307 can be characterized by **HTQ**.

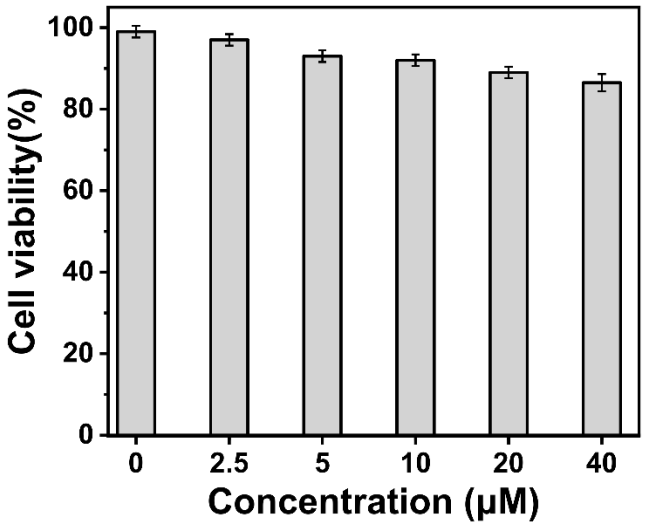


Fig.S8. Cell viability values (%) estimated by MTT proliferation test versus concentrations of **HTQ-R**. HeLa cells were cultured in the presence of 0-40 μM **HTQ-R**.

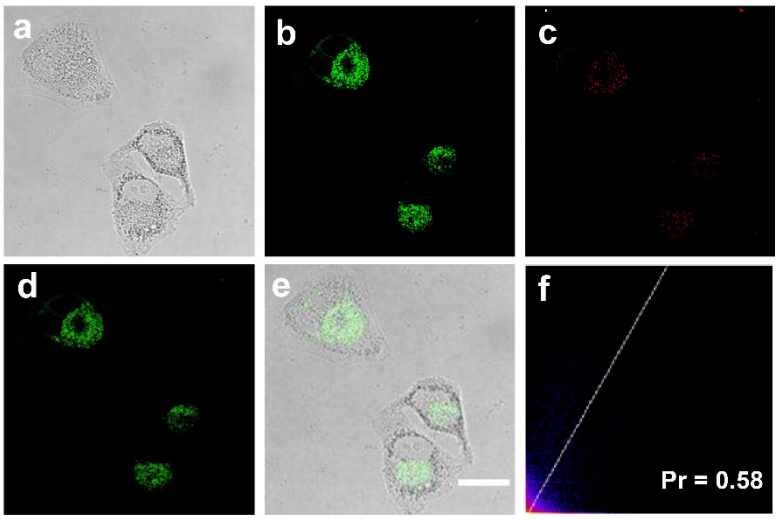


Fig.S9. Colocalization images of HeLa cells co-stained with **HTQ-R** (10 μM) (pretreated with 50 μM H_2O_2 for 30 min) and LysoTracker-Green (1 μM). (a) Bright field. (b) Green fluorescence of LysoTracker-Green. (c) Red fluorescence of **HTQ-R.** (d) Merge of (b) and (c). (e) Merge of (a) and (d). (f) Intensity scatter plot. The red fluorescence signal was collected at 600-650 nm (λ_{ex} = 405 nm) and the green

fluorescence was collected at 510-550 nm (λ_{ex} = 488 nm). Scale bar = 10 μm.

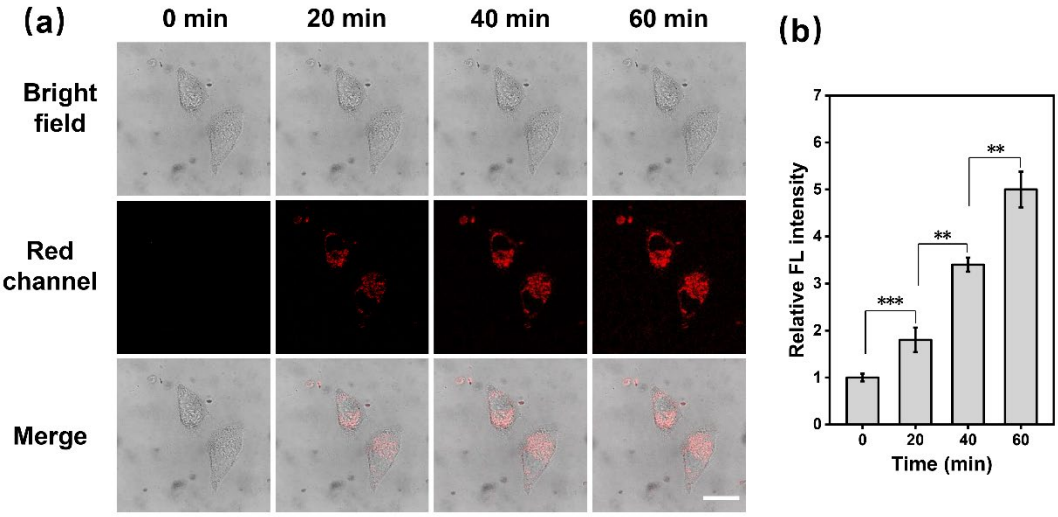


Fig.S10. Fluorescence imaging of endogenous H_2O_2 in HeLa cells. (a) HeLa cells were incubated with 0 min, 20 min, 40 min, 60 min LPS (10 μg / mL) and **HTQ-R** (10 μM). (b) Relative fluorescence intensity of (a). λex = 405 nm, λem = 600-650 nm. Statistical significance was defined as $p < 0.01$ (**), $p < 0.001$ (***). Scale bar = 10 μm.

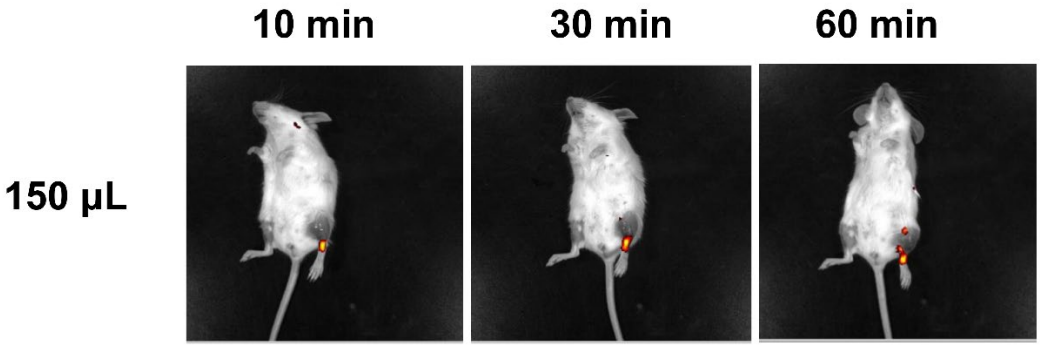


Fig.S11. Balb/C mice were subcutaneously injected with 150 μL λ-carrageenan (5 mg/mL) into the left joints, and an equal amount of PBS into the right joints. After 4 h, both legs were injected with **HTQ-R** (100 μM, 100 μL) for different periods (10 min, 30 min, 60 min).

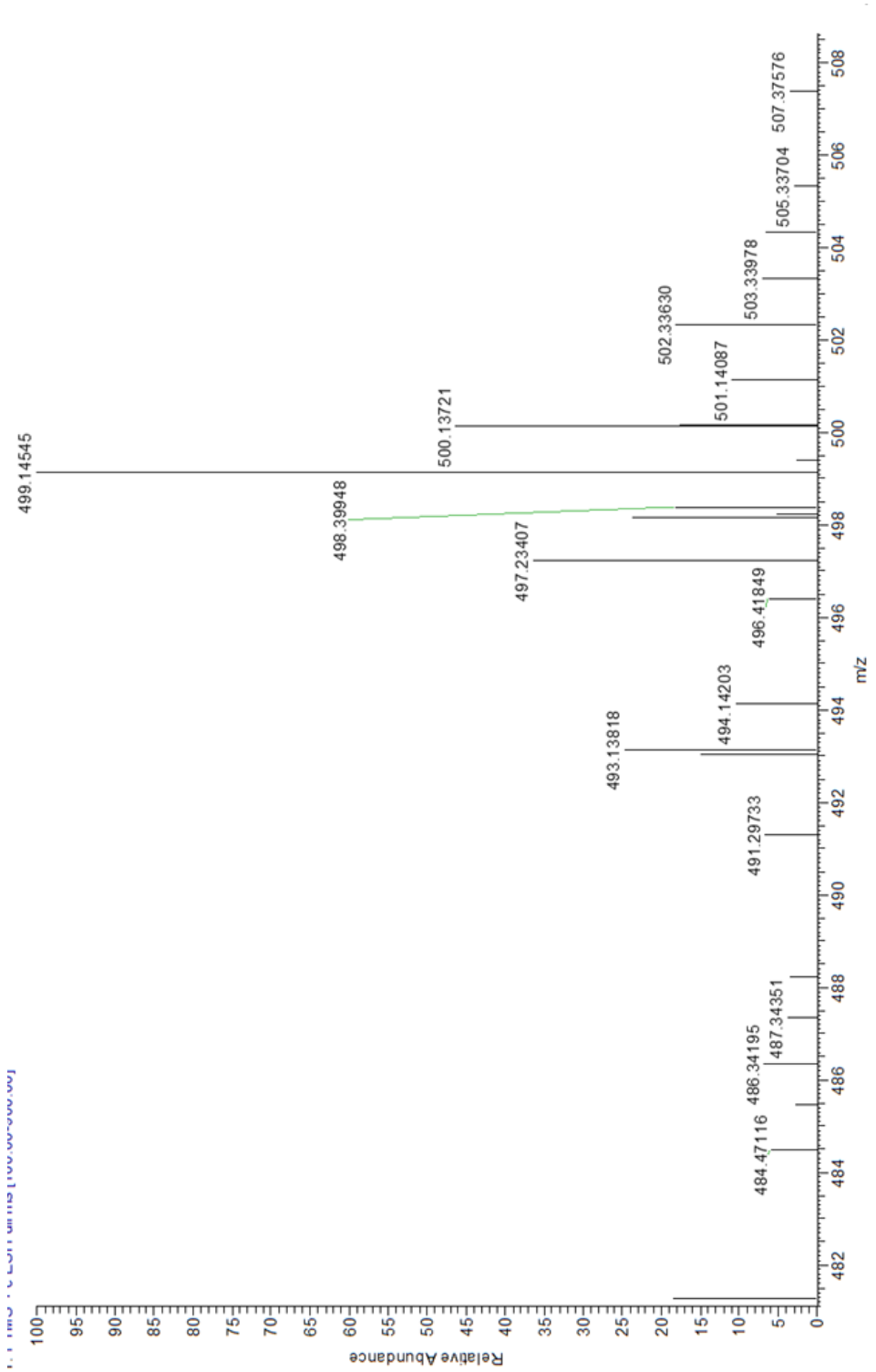


Fig.S12. HRMS spectrum of **HTO-CHO**.

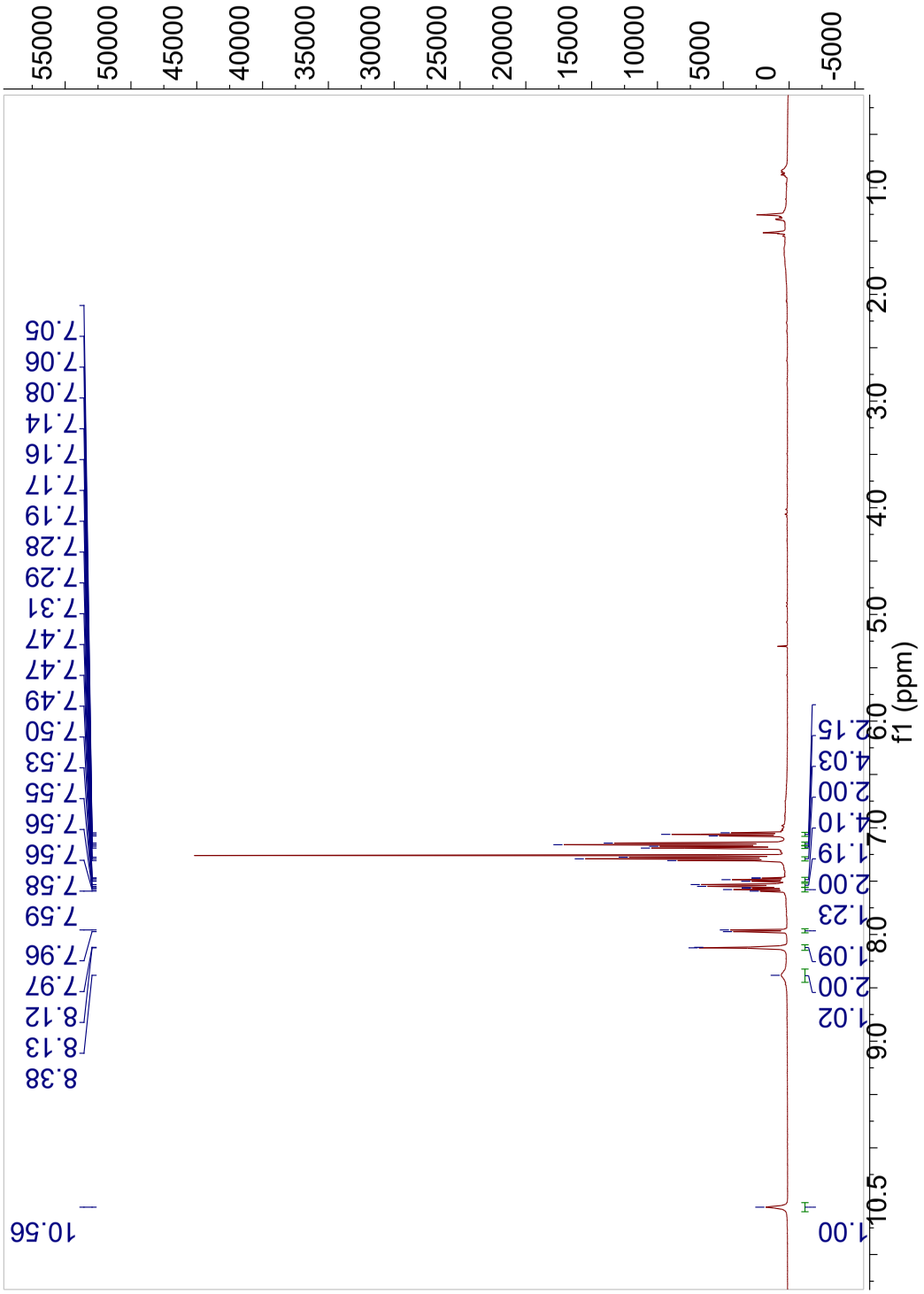


Fig.S13. ^{1}H-NMR spectrum of **HTO-CHO** in $CDCl_3$.

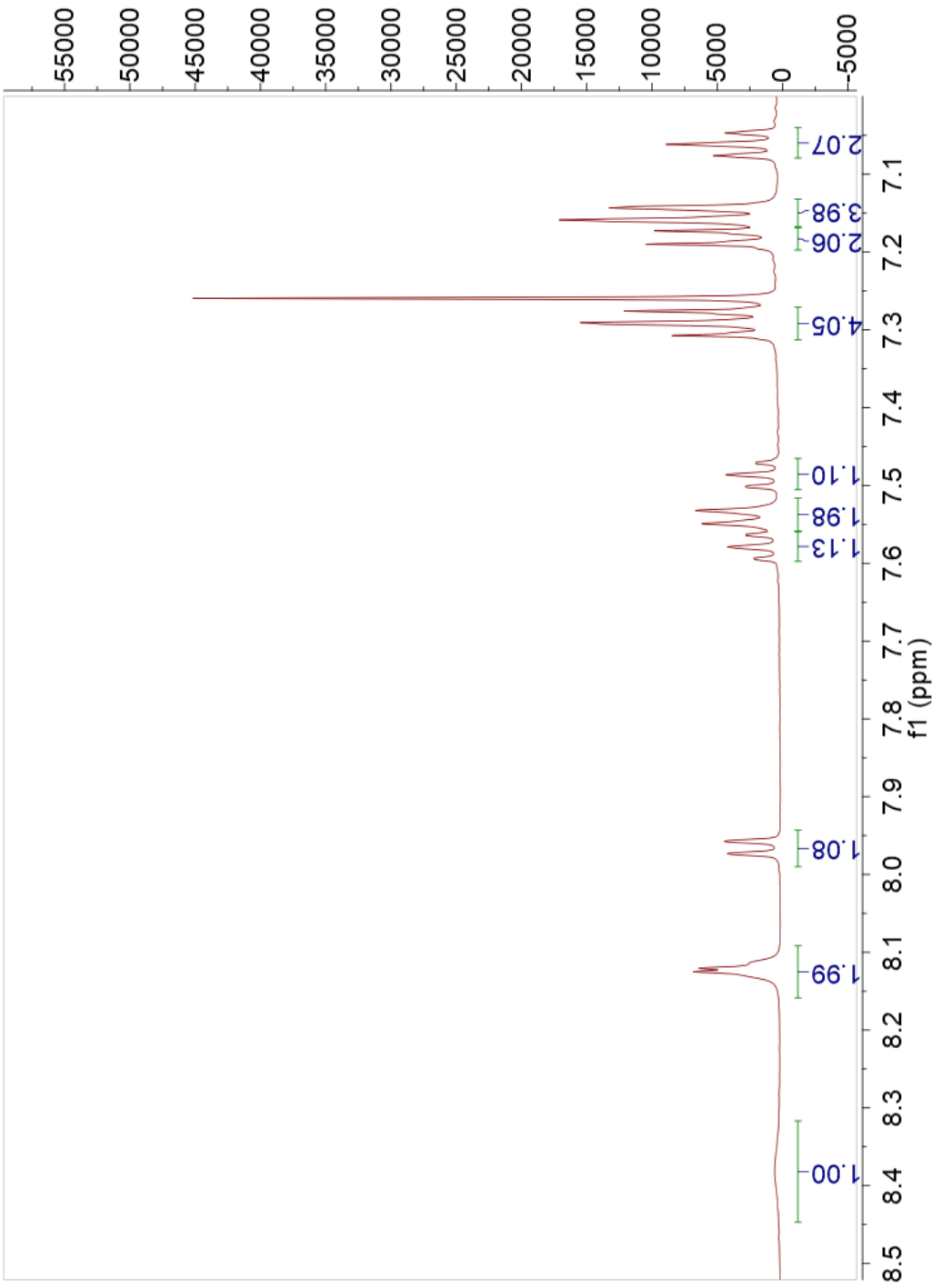


Fig.S14. The expanded ^{1}H-NMR spectrum of **HTO-CHO** in $CDCl_3$.

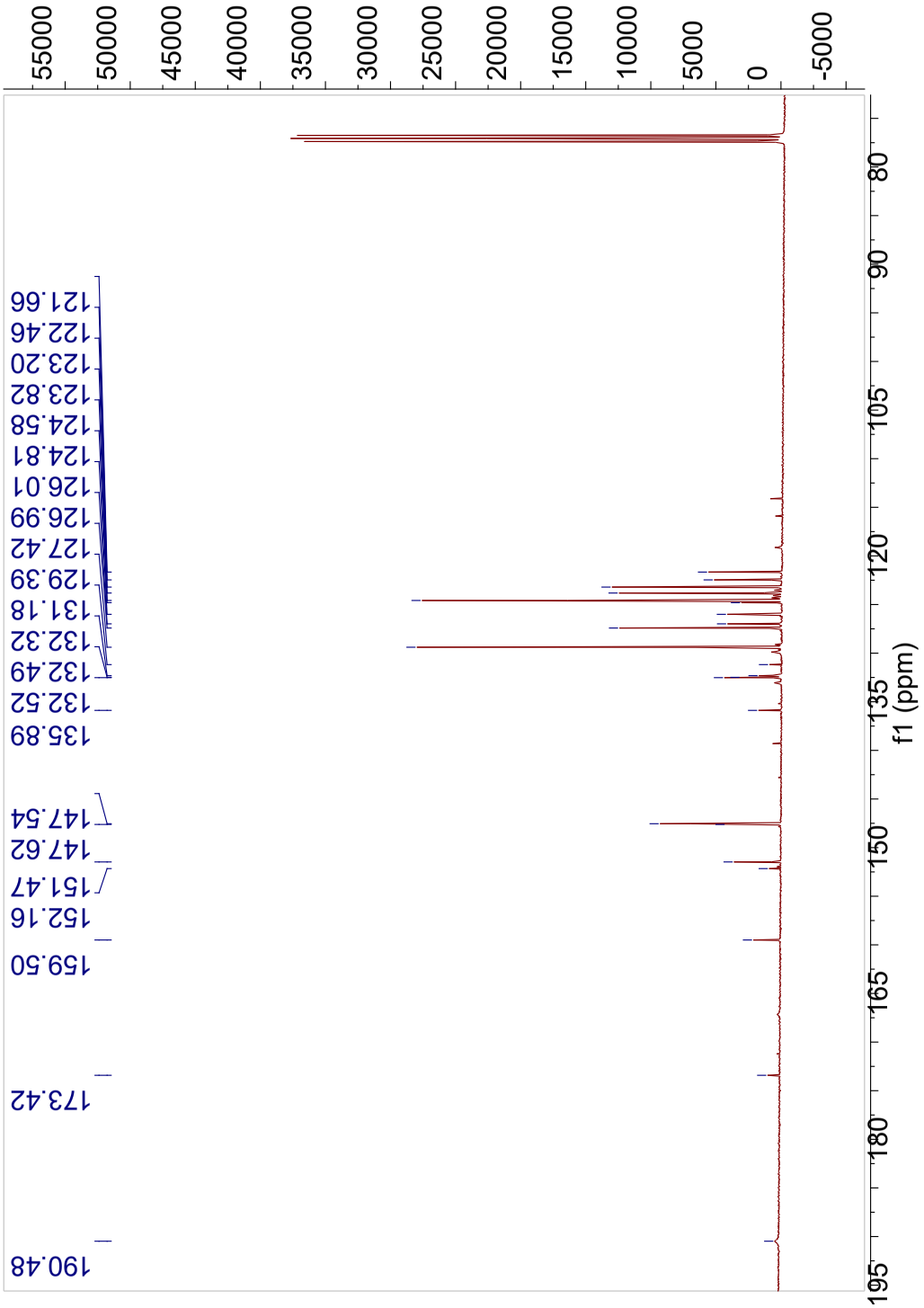


Fig.S15. ^{13}C-NMR spectrum of **HTO-CHO** in $CDCl_3$.

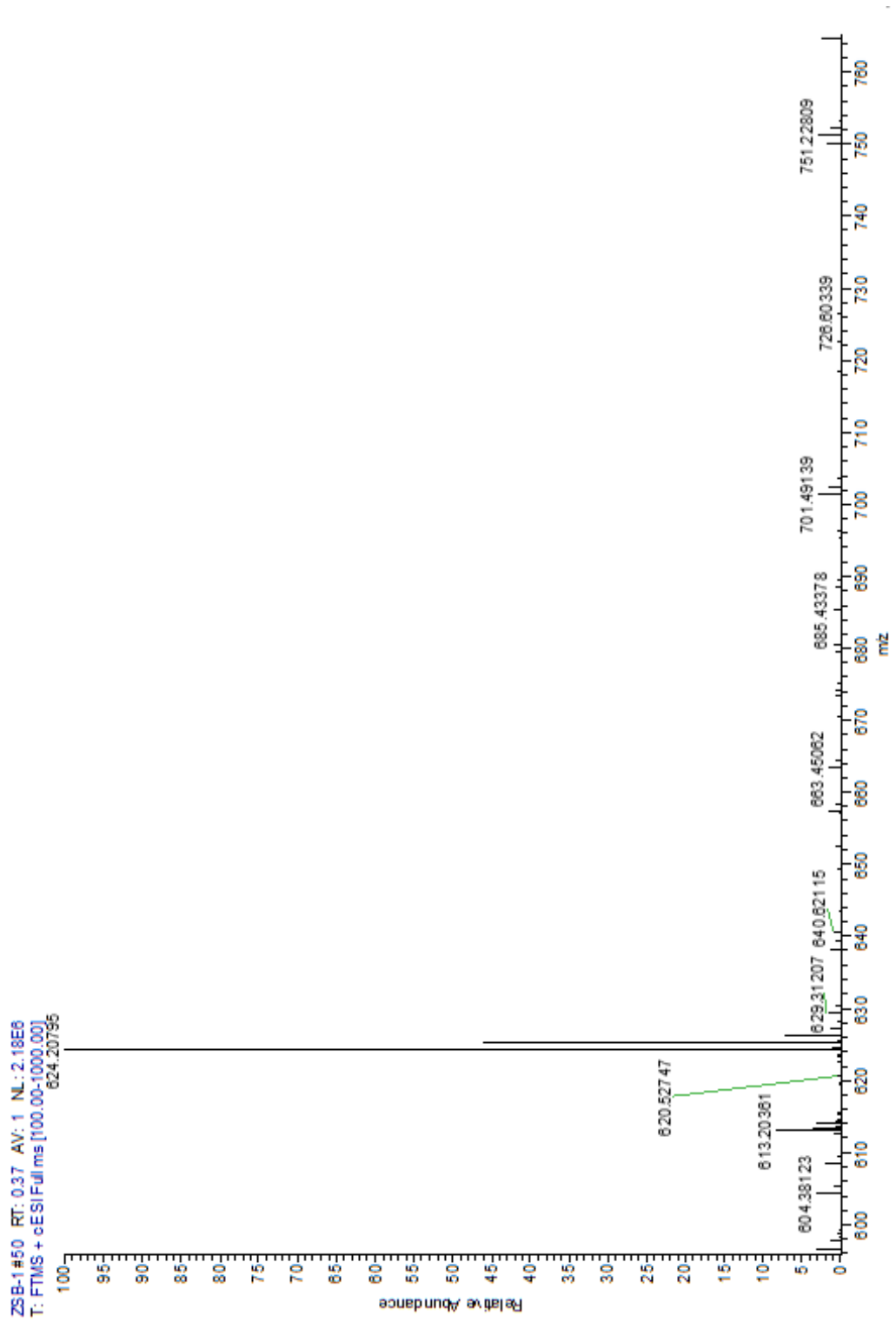


Fig.S16. HRMS spectrum of **HTQ**.

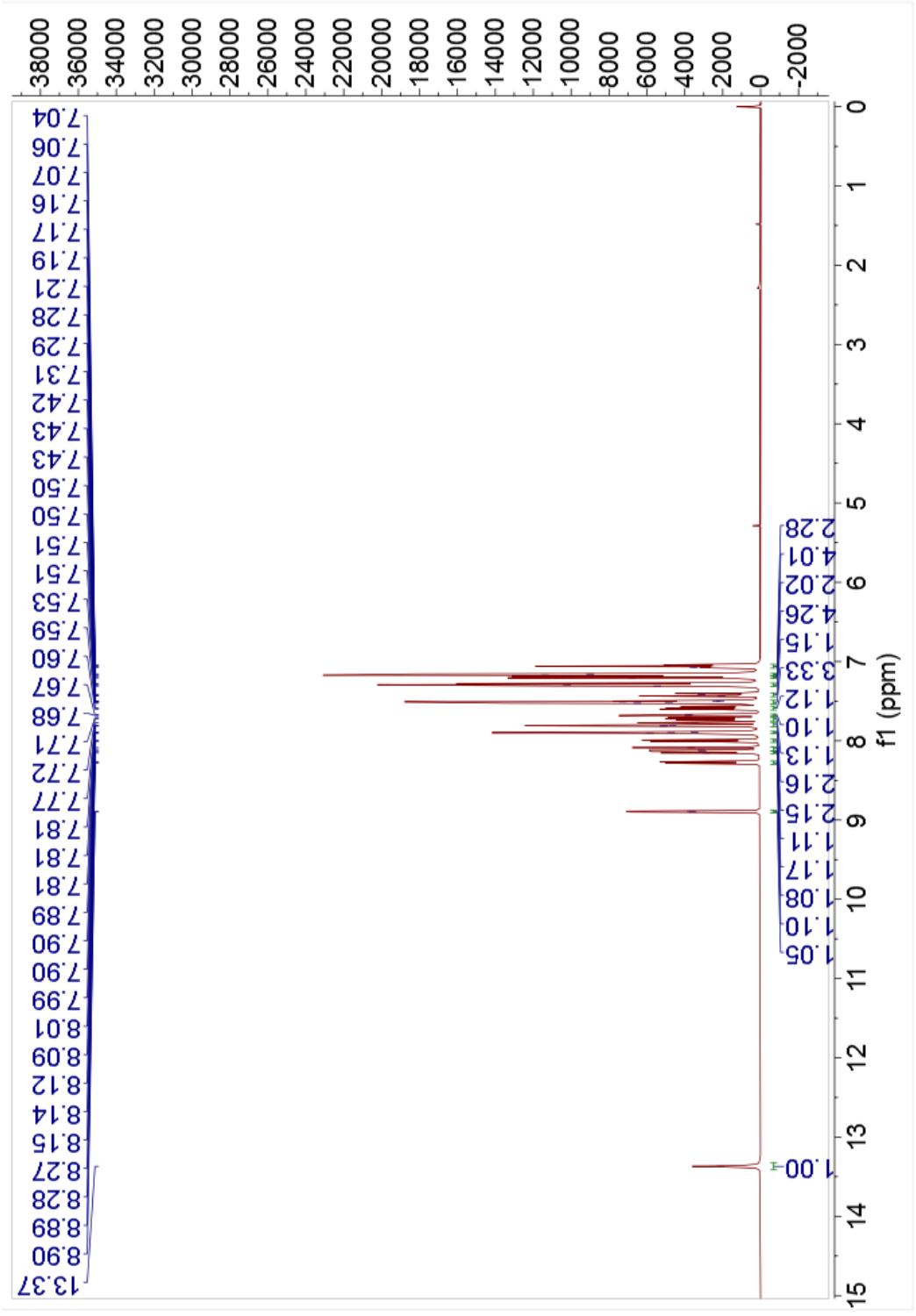


Fig.S17. ^{1}H-NMR spectrum of **HTQ** in $CDCl_3$.

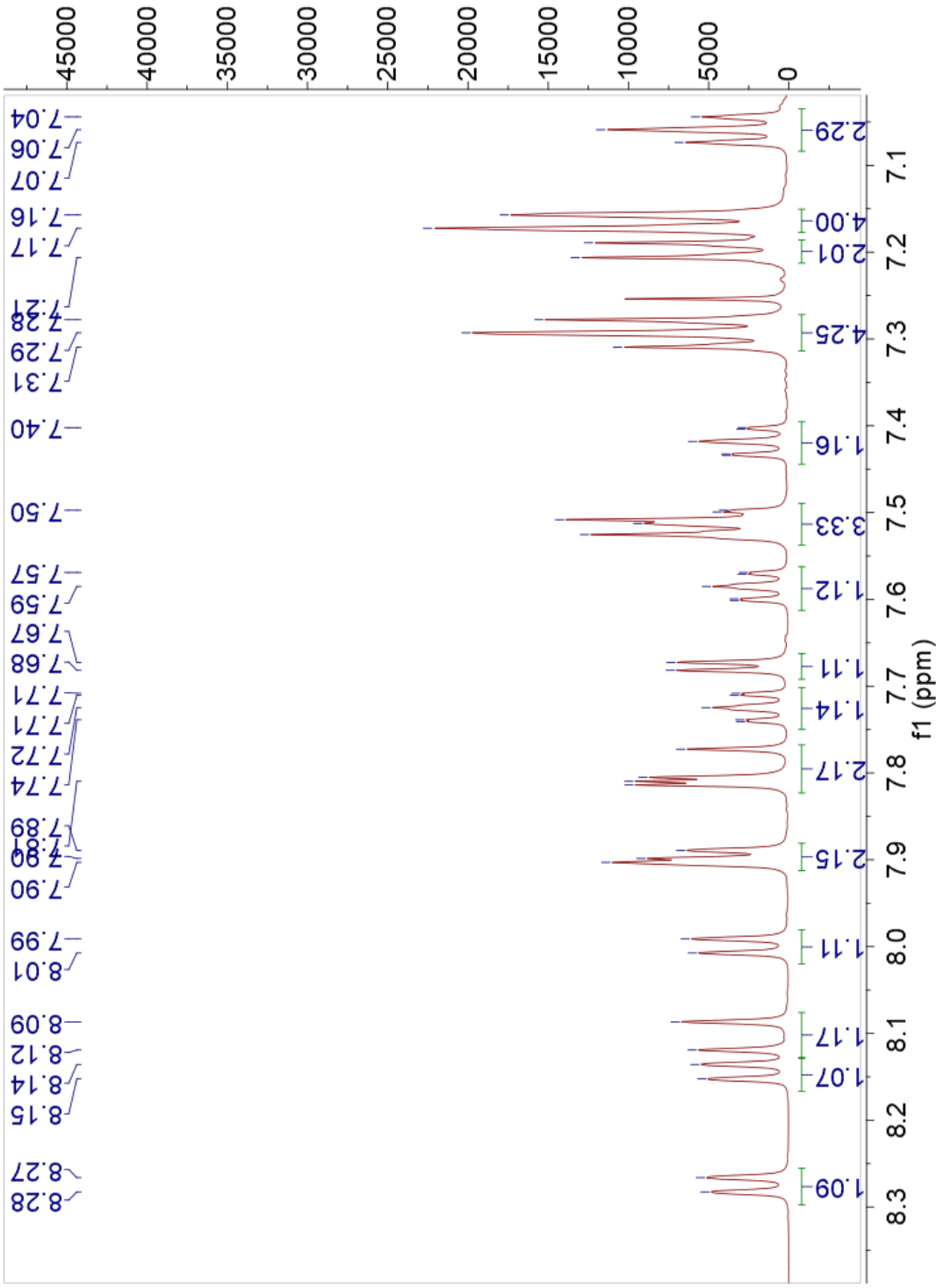


Fig.S18. The expanding ^{1}H-NMR spectrum of **HTQ** in $CDCl_3$.

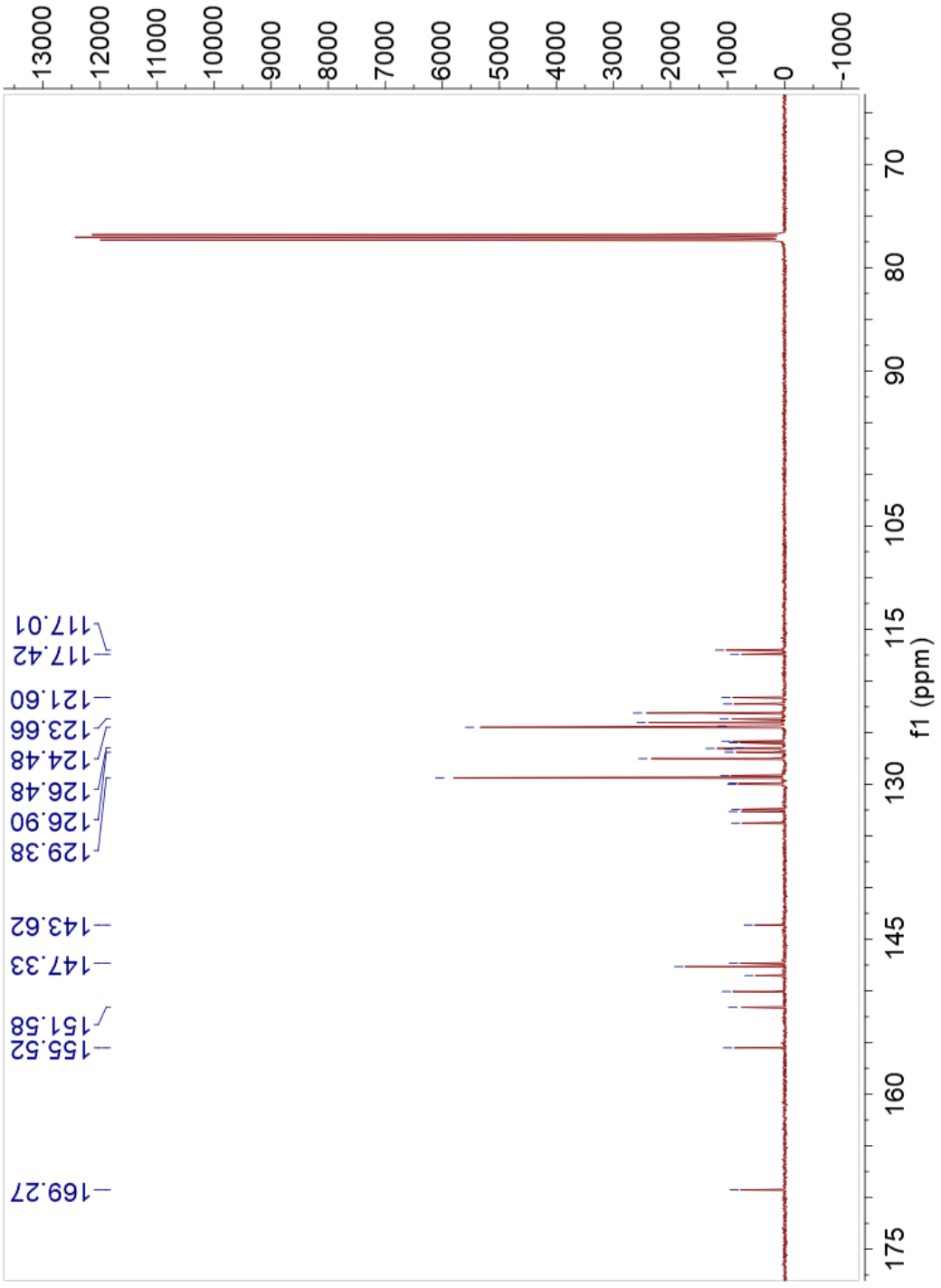


Fig.S19. ^{13}C-NMR of **HTQ** in $CDCl_3$.

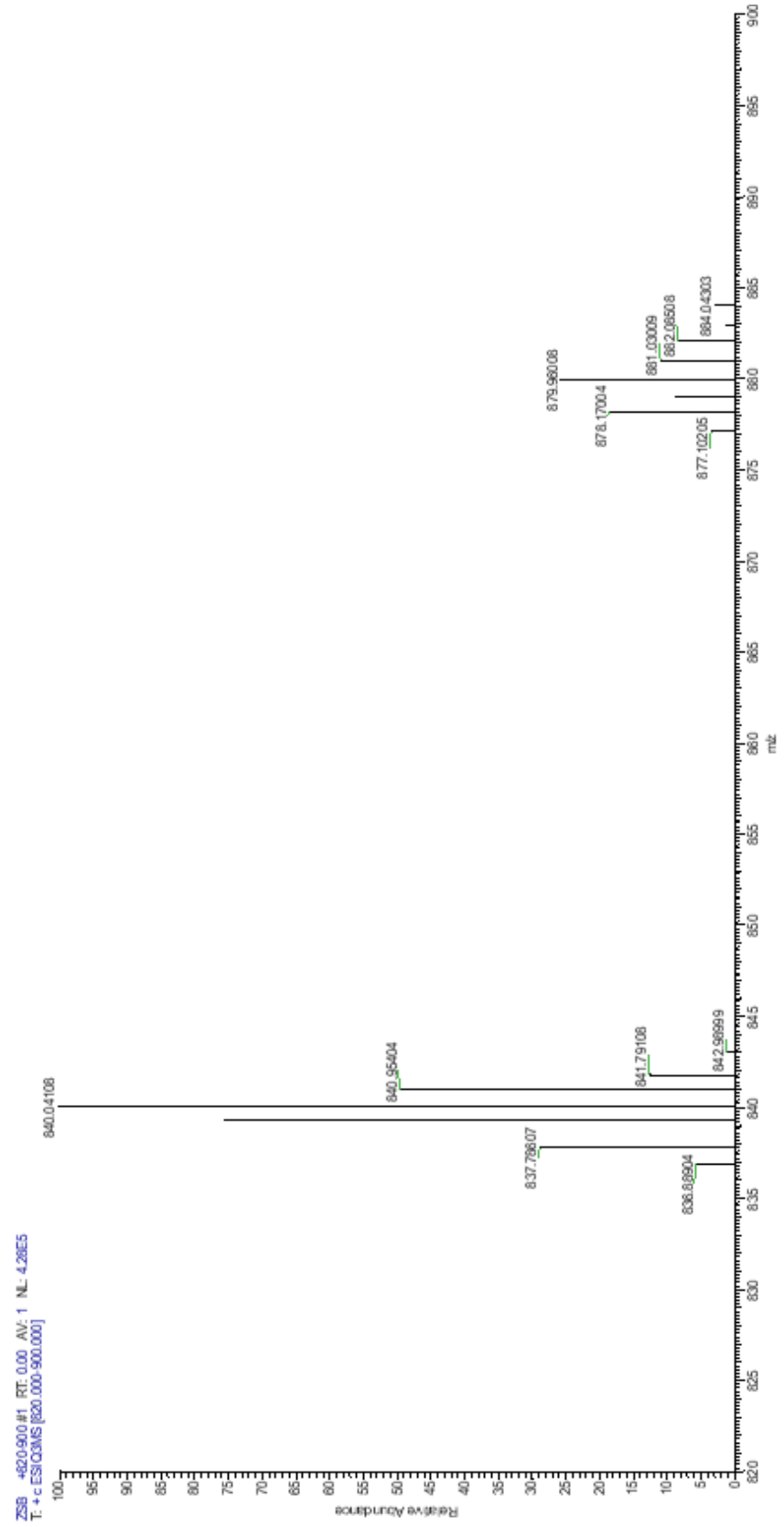


Fig.S20. HRMS spectrum of **HTQ-R**.

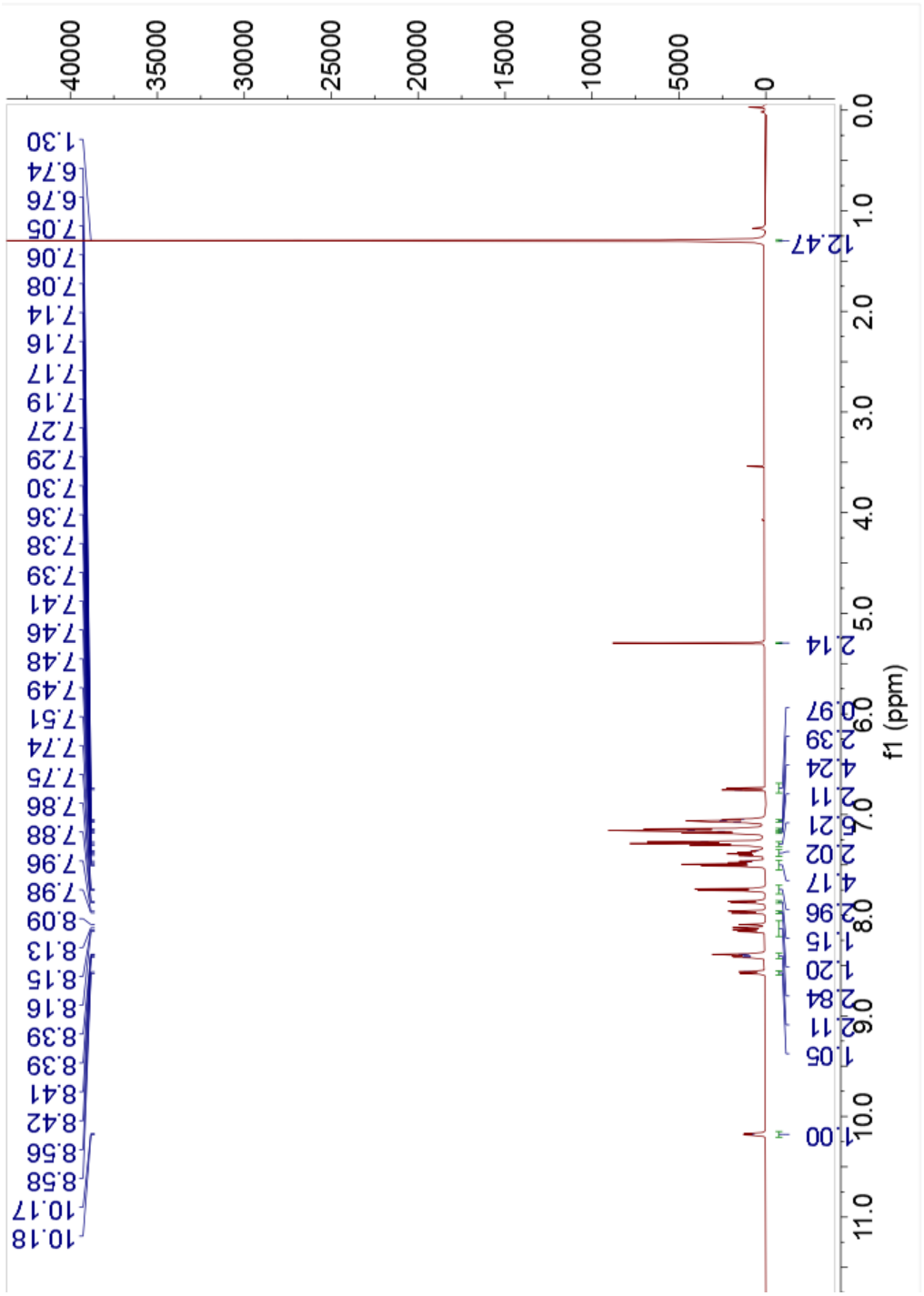


Fig.S21. ^{1}H-NMR spectrum of **HTQ-R**.

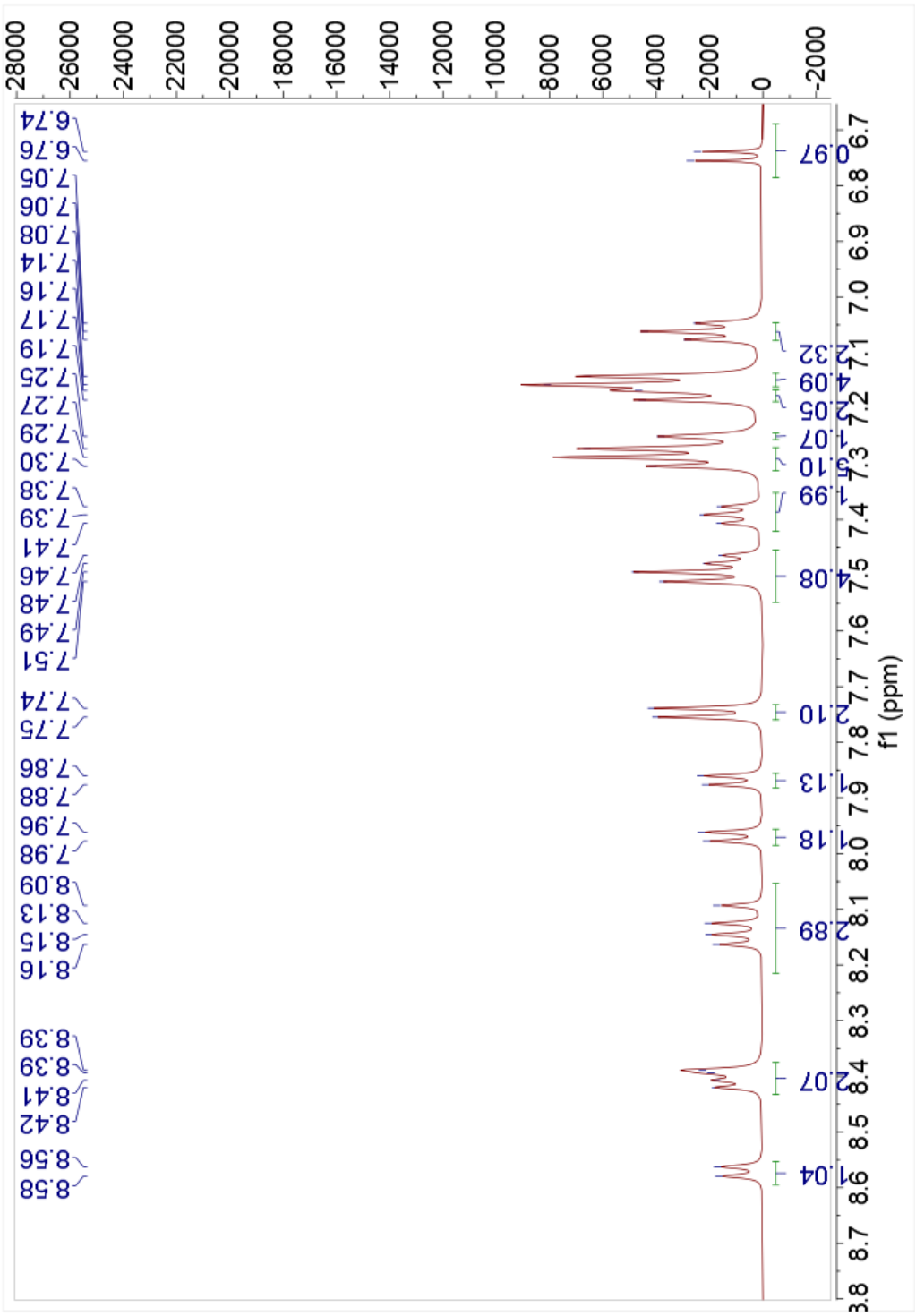


Fig.S22. The expanding ^{1}H-NMR spectrum of **HTQ-R**.

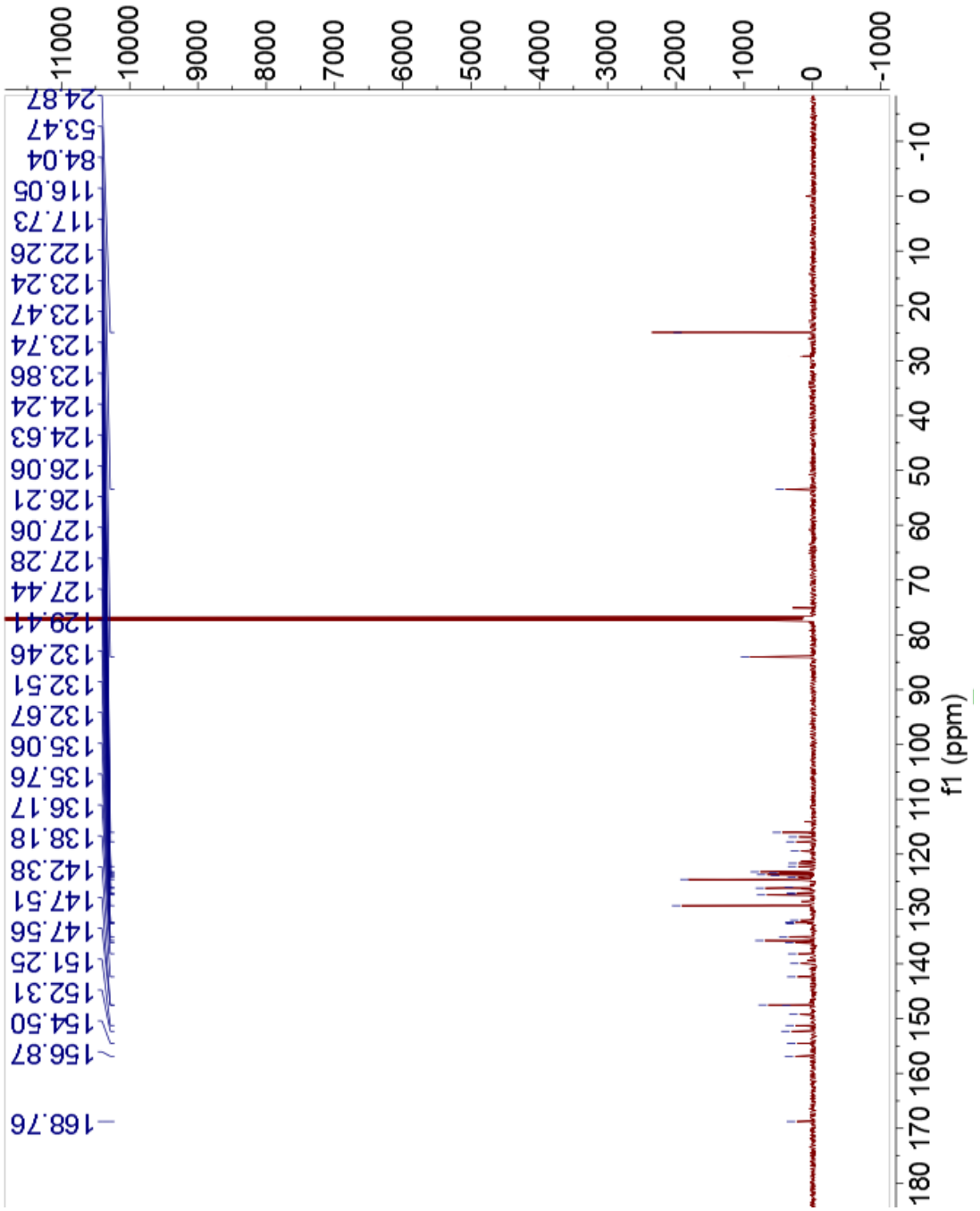


Fig.S23. ^{13}C-NMR spectrum of **HTQ-R**.