Supporting Information

Discovery of mitochondria-targeted fluorescent probe as TrxR inhibitors for cancer therapy

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Wenda Zhang and Wenying Yu have the equal contribution.

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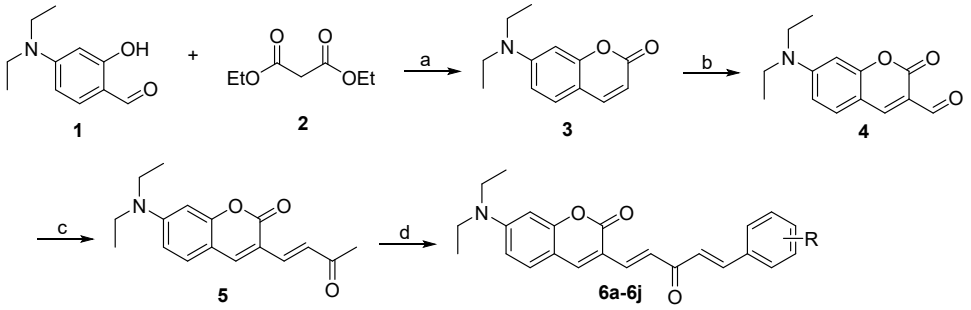
Synthesis of the designed TrxR inhibitors



Scheme S1. Synthesis of coumarin-based derivatives **6a-6l**. (a) i. Piperidine, EtOH, reflux, 12 h; ii. HOAc, HCl, reflux, 18 h; (b) $POCl_3$, DMF, 50 °C, 45 min, 60 °C, 2h; (c) (Acetylmethylene)triphenylphosphorane, THF; (d) Piperidine, HOAc, n-butyl alcohol. Chemical structures of R groups are shown in Table 1.

Synthesisof 7-(diethylamino)-2*H*-chromen-2-one (3). Piperidine (1 mL) was added to a solution of 4-(diethylamino)-2-hydroxybenzaldehyde **1** (4.0 g) and diethyl malonate **2** (9.9 mL) in ethanol (40 mL). The mixture was refluxed for 12 h. After the reaction was complete, the solvent was evaporated to obtain the crude ethyl 7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylate. The crude compound was dissolved in hydrochloric acid (20 mL) and glacial acetic acid (20 mL), and refluxed for 18 h. The mixture was cooled to room temperature, poured into ice water (150 mL), and adjusted to pH 5.0 by 1.0 M sodium hydroxide solution. The precipitate was filtered to obtain pure 7-(diethylamino)-2*H*-chromen-2-one (**3**) with 89% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, *J* = 9.3 Hz, 1H), 7.23 (d, *J* = 8.8 Hz, 1H), 6.60-6.53 (m, 1H), 6.49 (d, *J* = 1.8 Hz, 1H), 6.02 (d, *J* = 9.3 Hz, 1H), 3.40 (q, *J* = 7.1 Hz, 4H), 1.20 (t, *J* = 7.1 Hz, 6H).

Synthesis of 7-(diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde (4). $POCl_3$ (0.4 mL) was added dropwise to 4 mL DMF at 20-50°C. The mixture was stirred for 45 min at 50°C under a N_2 atmosphere. A suspension of 0.65 g of 7-(diethylamino)-2*H*-chromen-2-one **3** in 3 mL of dry DMF was added, and the mixture was warmed to 60 °C for 2 h, poured onto ice water, and stirred for 2 h. The crystalline precipitate was filtered off, thoroughly washed with water, and dried in vacuo at 50 °C to afford orange solid compound **4** with a yield of 72%.^{1}H NMR (500 MHz, $CDCl_3$) *δ* 10.09 (s, 1H), 8.22 (s, 1H), 7.39 (d, *J* = 9.0 Hz, 1H), 6.63 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.47 (d, *J* = 2.0 Hz, 1H), 3.46 (q, *J* = 7.1 Hz, 4H), 1.24 (t, *J* = 7.1 Hz, 6H).

Synthesis of (*E*)-7-(diethylamino)-3-(3-oxobut-1-en-1-yl)-2*H*-chromen-2-one (5). A solution of 7-(diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde **4** (1.0 g) in THF (15 mL) was added to (acetylmethylene)Triphenylphosphorane (1.37 g). The mixture was refluxed for 8 h. After completion of the reaction, the solvent was evaporated. The residue was purified by column chromatography and eluted with petroleum ether-ethyl acetate to afford product **5** (yield 92%). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.76 (s, 1H), 7.71 (d, *J* = 16.0 Hz, 1H),7.31 (d, *J* = 8.7 Hz, 1H), 7.13 (d, *J* = 16.0 Hz, 1H), 6.62 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.50 (d, *J* = 2.1 Hz, 1H), 3.44 (q, *J* = 7.0 Hz, 4H), 2.35 (s, 3H), 1.23 (t, *J* = 7.0 Hz, 6H).

General procedure for the preparation of compounds 6a-6l. A solution of compound **5** (0.1 g) in n-butyl alcohol (5 mL) was

added to piperidine (0.1 mL), glacial acetic acid (0.1 mL), and different substituted benzaldehyde (0.43 mmol) and refluxed for 10 h. After the reaction was complete, as determined by TLC, the mixture was concentrated under vacuum. The residue was dissolved in dichloromethane, purified by column chromatography, and eluted with petroleum ether-dichloromethane to afford products **6a-6l**.

7-(diethylamino)-3-((1*E*,4*E*)-5-(4-methoxyphenyl)-3-oxopenta-1,-4-dien-1-yl)-2*H*-chromen-2-one. (6a) Yield 55%, red solid; m.p. 160-161 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.71 (d, J = 15.9 Hz, 1H), 7.67 (d, J = 15.9 Hz, 1H), 7.60-7.55 (m, 3H), 7.32 (d, J = 8.9 Hz, 1H), 6.96-6.91 (m, 3H), 6.61 (dd, J = 8.9, 2.3 Hz, 1H), 6.50 (d, J = 2.1 Hz, 1H), 3.85 (s, 3H), 3.45 (q, J = 7.1 Hz, 4H), 1.23 (t, J = 7.1 Hz, 6H).^{13}C NMR (125 MHz, $CDCl_3$) δ 189.50, 161.55, 160.38, 156.46, 145.41, 142.86, 137.86, 134.40, 130.18 (two), 129.99, 128.58, 127.71, 126.41, 124.59, 124.28, 114.41 (two), 109.85, 97.36, 55.43, 45.33 (two), 12.44 (two). MS (ESI) *m/z* 404.2 $[M+H]^+$; HRMS (ESI) *m/z* 404.1858 $[M+H]^+$ (calcd for 404.1856 $C_{25}H_{26}NO_4$).

7-(diethylamino)-3-((1*E*,4*E*)-5-(3,4-dimethoxyphenyl)-3-oxopenta-1,4-dien-1-yl)-2*H*-chromen-2-one. (6b) Yield 49%, red solid; m.p. 156-158 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.79-7.72 (m, 2H), 7.62 (d, J = 15.4 Hz, 1H), 7.39 (d, J = 9.0 Hz, 1H), 7.25 (dd, J = 8.3, 2.0 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 6.98 (d, J = 15.9 Hz, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.70 (dd, J = 8.9, 2.4 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 3.50 (d, J = 7.1 Hz, 4H), 1.29 (t, J = 7.1 Hz, 6H).^{13}C NMR (125 MHz, $CDCl_3$) δ 189.40, 160.34, 156.41, 151.29, 149.24, 145.59, 143.13, 137.91, 132.09, 130.01, 128.50, 127.96, 126.36, 124.93, 123.30, 119.10, 115.45, 111.06, 109.68, 97.59, 56.01, 55.96, 45.49 (two), 12.40 (two). MS (ESI) *m/z* 434.2 $[M+H]^+$; HRMS (ESI) *m/z* 434.1958 $[M+H]^+$ (calcd for 434.1962 $C_{26}H_{28}NO_5$).

7-(diethylamino)-3-((1*E*,4*E*)-5-(3,5-dimethoxyphenyl)-3-oxopenta-1,4-dien-1-yl)-2*H*-chromen-2-one. (6c) Yield 61%, red solid; m.p. 158-160 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.75 (d, J = 15.4 Hz, 1H), 7.71 (d, J = 15.9 Hz, 1H), 7.63 (d, J = 15.5 Hz, 1H), 7.38 (d, J = 8.9 Hz, 1H), 7.06 (d, J = 15.9 Hz, 1H), 6.81 (d, J = 2.3 Hz, 2H), 6.67 (dd, J = 8.9, 2.5 Hz, 1H), 6.56 (t, J = 1.9 Hz, 2H), 3.89 (s, 6H), 3.51 (q, J = 7.1 Hz, 4H), 1.30 (t, J = 7.2 Hz, 31H)..^{13}C NMR (125 MHz, $CDCl_3$) δ 189.51, 161.05 (two), 160.42, 156.62, 151.94, 145.85, 142.94, 138.52, 136.95, 130.06, 127.30, 125.93, 114.93, 109.61, 109.02, 106.15 (two), 103.06, 97.00, 55.52 (two), 45.11 (two), 12.51 (two). MS (ESI) *m/z* 434.2 $[M+H]^+$; HRMS (ESI) *m/z* 434.1961 $[M+H]^+$ (calcd for 434.1962 $C_{26}H_{28}NO_5$).

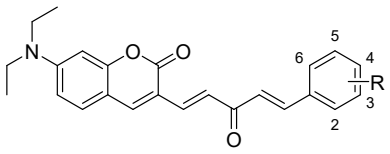
7-(diethylamino)-3-((1*E*,4*E*)-3-oxo-5-(3,4,5 trimethoxyphenyl)pe-nta-1,4-dien-1-yl)-2*H*-chromen-2-one. (6d) Yield 64%, red solid; m.p. 181-182°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.74 (d, J = 15.4 Hz, 1H), 7.66 (d, J = 15.9 Hz, 1H), 7.57 (d, J = 15.4 Hz, 1H), 7.33 (d, J = 9.0 Hz, 1H), 6.95 (d, J = 15.9 Hz, 1H), 6.85 (s, 2H), 6.62 (dd, J = 8.9, 2.5 Hz, 1H), 6.51 (d, J = 2.4 Hz, 1H), 3.92 (s, 6H), 3.89 (s, 3H), 3.45 (q, J = 7.1 Hz, 4H), 1.24 (t, J = 7.1 Hz, 6H).^{13}C NMR (125 MHz, $CDCl_3$) δ 189.36, 160.45, 156.56, 153.44 (two), 151.93, 146.00, 142.96, 140.14, 138.40, 130.50, 130.05, 126.38, 125.78, 114.82, 109.59, 108.96, 105.46 (two), 96.90, 61.02, 56.20 (two), 44.08 (two), 12.50 (two). MS (ESI) *m/z* 464.2 $[M+H]^+$; HRMS (ESI) *m/z* 464.2071 $[M+H]^+$ (calcd for 464.2068 $C_{27}H_{30}NO_6$).

7-(diethylamino)-3-((1*E*,4*E*)-5-(3-hydroxy-4-methoxyphenyl)-3-oxopenta-1,4-dien-1-yl)-2*H*-chromen-2-one. (6e) Yield 38%, red solid; m.p. 120-121°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 7.7 Hz, 1H), 7.67 (dd, J = 15.7, 6.1 Hz, 2H), 7.58 (t, J = 11.8 Hz, 1H), 7.34 (d, J = 8.9 Hz, 1H), 7.22 (d, J = 1.8 Hz, 1H), 7.13 (dd, J = 8.3, 1.8 Hz, 1H), 6.92 (d, J = 15.9 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 6.68 (d, J = 8.7 Hz, 1H), 6.56 (s, 1H), 5.65 (s, 1H), 3.94 (s, 3H), 3.45 (q, J = 7.1 Hz, 5H), 1.24 (t, J = 7.1 Hz, 6H).^{13}C NMR (125 MHz, $CDCl_3$) δ 189.45, 160.34, 156.45, 151.46, 148.64, 145.87, 145.35, 142.96, 137.91, 129.98, 128.64, 126.41, 125.05, 122.30, 115.35, 113.32, 110.58, 109.87, 109.34, 97.39, 56.03, 45.34 (two), 12.42 (two). MS (ESI) *m/z* 420.2 $[M+H]^+$; HRMS (ESI) *m/z* 420.1807 $[M+H]^+$ (calcd for 420.1805 $C_{25}H_{26}NO_5$).

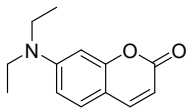
7-(diethylamino)-3-((1*E*,4*E*)-5-(4-(dimethylamino)phenyl)-3-oxo-penta-1,4-dien-1-yl)-2*H*-chromen-2-one. (6f) Yield 32%, red solid; m.p. 184-185°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.77 (d, J = 15.8 Hz, 1H), 7.73 (d, J = 15.5 Hz, 1H), 7.63-7.57 (m, 2H), 7.56 (s, 1H), 6.92 (d, J = 15.8 Hz, 1H), 6.74 (d, J = 8.5 Hz, 2H), 6.65 (dd, J = 9.0, 2.4 Hz, 1H), 6.55 (d, J = 2.4 Hz, 1H), 3.49 (q, J = 7.2 Hz, 4H), 3.08 (s, 6H), 1.28 (t, J = 7.2 Hz, 6H).^{13}C NMR (125 MHz, $CDCl_3$) δ 189.44, 160.51, 156.44, 151.93, 151.67, 145.15, 143.98, 137.09, 130.34 (two), 129.87, 126.78, 122.75, 122.01, 115.32, 111.88 (two), 109.44, 109.00, 96.94, 45.04, 40.17 (two), 12.50 (two). MS (ESI) *m/z* 417.2 $[M+H]^+$; HRMS (ESI) *m/z* 417.2170 $[M+H]^+$ (calcd for 417.2173 $C_{26}H_{29}N_2O_3$).

7-(diethylamino)-3-((1*E*,4*E*)-3-oxo-5-(3-(trifluoromethoxy)phenyl)-penta-1,4-dien-1-yl)-2*H*-chromen-2-one. (6g) Yield 39%, red solid; m.p. 161-163°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.74 (d, J = 15.7 Hz, 2H), 7.65 (d, J = 15.5 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.50 (s, 1H), 7.48 (d, J = 7.9 Hz, 1H), 7.39 (d, J = 8.9 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.10 (d, J = 16.0 Hz, 1H), 6.68 (dd, J = 8.9, 2.5 Hz, 1H), 6.57 (d, J = 2.4 Hz, 1H), 3.51 (q, J = 7.2 Hz, 4H), 1.29 (t, J = 7.1 Hz, 6H).^{13}C NMR (125 MHz, $CDCl_3$) δ 189.13, 160.35, 156.65, 151.91, 149.73, 145.92, 140.97, 138.96, 137.19, 130.33, 130.13, 128.16, 126.72, 125.82, 122.42, 120.38, 114.87, 109.77, 109.13, 97.15, 45.23 (two), 12.49 (two). MS (ESI) *m/z* 458.1 $[M+H]^+$; HRMS (ESI) *m/z* 458.1576 $[M+H]^+$ (calcd for 458.1574 $C_{25}H_{23}F_3NO_4$).

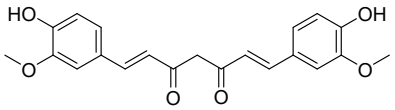
Table 1. Anti-proliferative activity of the designed compounds and reference Curcumin.



6a-6g



7-diethylamino-coumarin (3)



Curcumin

Compd	R	HCT116
		IC_{50} ± SD (μM)[a]
6a	4-OCH_3	3.57± 0.22
6b	3,4-$(OCH_3)_2$	1.01 ± 0.04
6c	3,5-$(OCH_3)_2$	4.40 ± 0.26
6d	3,4,5-$(OCH_3)_3$	1.80 ± 0.40
6e	3-OH-4-OCH_3	0.70 ± 0.09
6f	4-N,N-$(CH_3)_2$	5.26 ± 0.25
6g	3-OCF_3	3.05 ± 0.34
3	--	>100
Curcumin	--	37.20 ± 1.50
3 + Curcumin	--	23.76 ± 2.30

The inhibitory effects of the compounds on the proliferation of HCT116 cell line were determined by the MTT assay for 72 h. SD: standard deviation, all experiments were independently performed at least three times.

Table 2. Anti-proliferative activity of the designed compounds on L02 cells

Compd	R	L02
		IC_{50} ± SD (μM)[a]
6b	3,4-$(OCH_3)_2$	18.2 ± 1.65
6e	3-OH-4-OCH_3	14.3 ± 2.50

The inhibitory effects of the compounds on the proliferation of L02 cell line were determined by the MTT assay for 72 h. SD: standard deviation, all experiments were independently performed at least three times.

Maintenance of Cell Line Culture and Cell Viability Assays. All cell lines were purchased from the Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). The human colon carcinoma cell line HCT116 and the human normal liver cell line L02 were maintained in DMEM. All cells were supplemented with 12% fetal bovine serum containing 50 μg/mL penicillin and 50 μg/mL streptomycin. The cells were grown to 80% confluency in a tissue culture flask at 37 °C in a humidified atmosphere containing 5% CO_2 and then trypsinized with 1× Trypsin-Versene and split.

Cells (HCT116 and L02) were seeded in 96-well plates at a density of 4000-6000 cells per well. The cells were incubated at 37 °C overnight (16 h) in a humidified 5% CO_2 incubator. After medium removal, different concentrations of test compounds were added in triplicate to the plates in 200 μL of fresh media, and the plates were incubated at 37 °C for 72 h. The percentage of DMSO in the medium did not exceed 0.1%. 3-(4,5-Dimethylthiazolyl)-2,5-diphenyltetrazolium broth (MTT) was added to evaluate cell viability.

The absorbance was read by an ELISA reader (SpectraMax Plus384, Molecular Devices, Sunnyvale, CA) at a wavelength of 570 nm and a reference wavelength of 630 nm. Cell viability was calculated by the following formula:

% Cell viability = (At/As) × 100%

At and As denote the absorbance of the test substances and solvent control, respectively.

Detection of TrxR enzyme activity. HCT116 cells were seeded at a density of 3×10^5 per well in 6-well plates. The cells were treated with various concentrations of **6e** for 24 h. After trypsinization, the cells were treated with 1× RIPA lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.25% deoxycholic acid, 1% NP-40, 1 mM EDTA and protease inhibitors) (Amresco, Solon, USA) to extract the total proteins and quantified with a BCA Protein Assay Kit. The cell protein samples (50 μg per sample) were reacted in triplicate with NADPH (40 mg/mL) and DTNB (10 mM) in TE buffer in 96-well plates at 37 °C for 1 h. The contents of free TNB derived from free thiols were determined by a microplate spectrophotometer (412 nm). TrxR activity was calculated based on the standard curve established using the purified TrxR provided.

Detection of ROS. HCT116 cells were seeded at a density of 1.5×10^4 per well in 96-well plates or 3×10^5 per well in 6-well plates. The cells were treated with various concentrations of **6e** for 24 h. DCFH-DA was dissolved in serum-free medium and diluted to a final concentration of 10 μM. After treatment with **6e**, the growth media was replaced with serum-free media containing the probe. After incubation for 30 min at 37 °C, the cells were washed with serum-free medium twice, digested with trypsin and resuspended in prewarmed PBS buffer. The samples were then subjected to a flow cytometry assay using a BD FACS Calibur flow cytometer (Becton & Dickinson Company, Franklin Lakes, NJ) and an ImageXpress Micro Confocal analysis system.

Detection of apoptosis. HCT116 cells were seeded at a density of 1.5×10^4 per well in 96-well plates. The cells were treated with various concentrations of **6e** for 24 h. Hoeschst 33342 was dissolved in serum-free medium and diluted to a final concentration of 5 μg/mL. After incubation for 30 min at 37 °C, the cells were washed with serum-free medium twice, digested with trypsin and resuspended in prewarmed PBS buffer. The samples were then subjected to an ImageXpress Micro Confocal analysis.

Wound Healing Assays HCT116 cells were seeded at 6 x 10^5 cells per well into 6-well plates and allowed to grow overnight. Wounds were made by scratching the cells with pipette tips (1-10 μL). The cells were treated with **6e** and allowed to migrate into the scratched area for 24 h. The migration of the cells was visualized at 10X magnification using a Leica microscope at time 0 (right before the drug was added) and 24 h after vehicle or **6e** treatment.

***In vivo* Studies** The working solution was prepared in a mixture of cremophore and DMSO (cremophore 10% and DMSO 10% and corn oil 80%) to give a 1 mg/mL solution. Athymic BALB/c nude mice (16-18 g) were injected with human colon tumor HCT116 cells (3×10^6 cells in a volume of 0.2 mL) into the subcutaneous tissue of the right auxiliary region of the mice. When the tumor volume reached approximately 80 mm^3, the mice were randomly sorted into two groups (5 mice in each group): the vehicle group and 30 mg/kg compound **6e** group. The compounds were administered daily *via* intraperitoneal injection. The tumor size was measured by callipers three times a week to document tumor growth and calculated by the formula length ×$width^2/2$, and the body weight was measured and recorded. The therapeutic efficacy was evaluated based on body weight loss and tumor growth inhibition [determined by using callipers and calculated with the following formula: tumor inhibition rate (%) = (tumor vol_{Con} − tumor vol_{Tre})/tumor vol_{Con}]. On the 22^{th} day, all the mice were killed, and the tumors were harvested, weighed, and stored at −80 °C for later use.

intraperitoneal injection

When the tumor volume reached 100-200 mm3, the mice were randomly divided into 4 groups (6 mice in each group): the blank saline group, 5 mg/kg CC-PTX group, 10 mg/kg CC-PTX group, and 10 mg/kg positive paclitaxel group.

***In vivo* Imaging of Tumor-bearing Nude Mice.** For *in vivo* imaging, 5-week-old BALB/c nude mice were selected and inoculated with HCT116 cells in the auxiliary region via subcutaneous injection of 3×10^6 cells to establish a tumor model. Tumors were allowed to grow to 8-12 mm in diameter and then used for experiments. To investigate the *in vivo* bioimaging application, the tumor region was subcutaneously injected with probe **6e** (50 μM, 50 μL). *In vivo* imaging was performed by using a CRI Maestro small animal *in vivo* imaging system with excitation of a light filter (460 nm~560 nm).

The experimental mice were cared for and handled strictly according to the obligations of the Animal Ethics Committee of China Pharmaceutical University and the National Institutes of Health (NIH) standard guidelines for the Care and Use of Laboratory Animals. All experimental protocols were approved by the Animal Ethics Committee of China Pharmaceutical University.

^{1}H NMR and ^{13}C NMR spectra

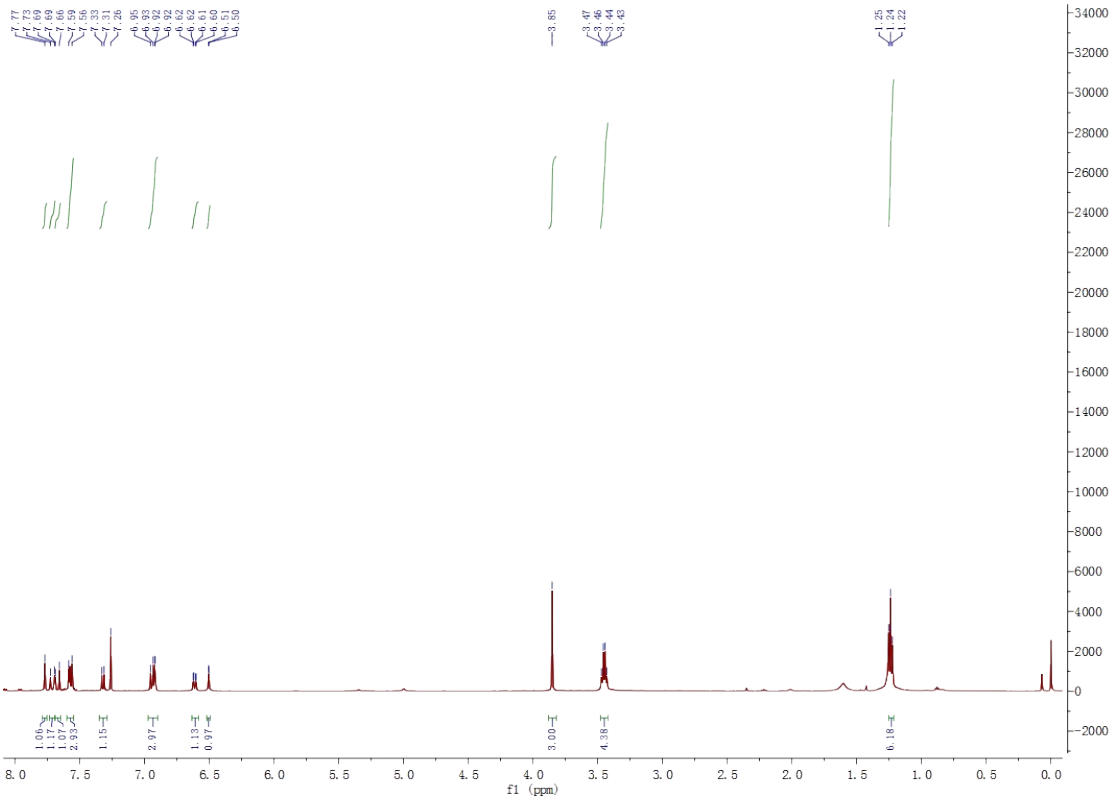


Fig. S1 ^{1}H NMR spectra (500 MHz) of compound **6a** in $CDCl_3$

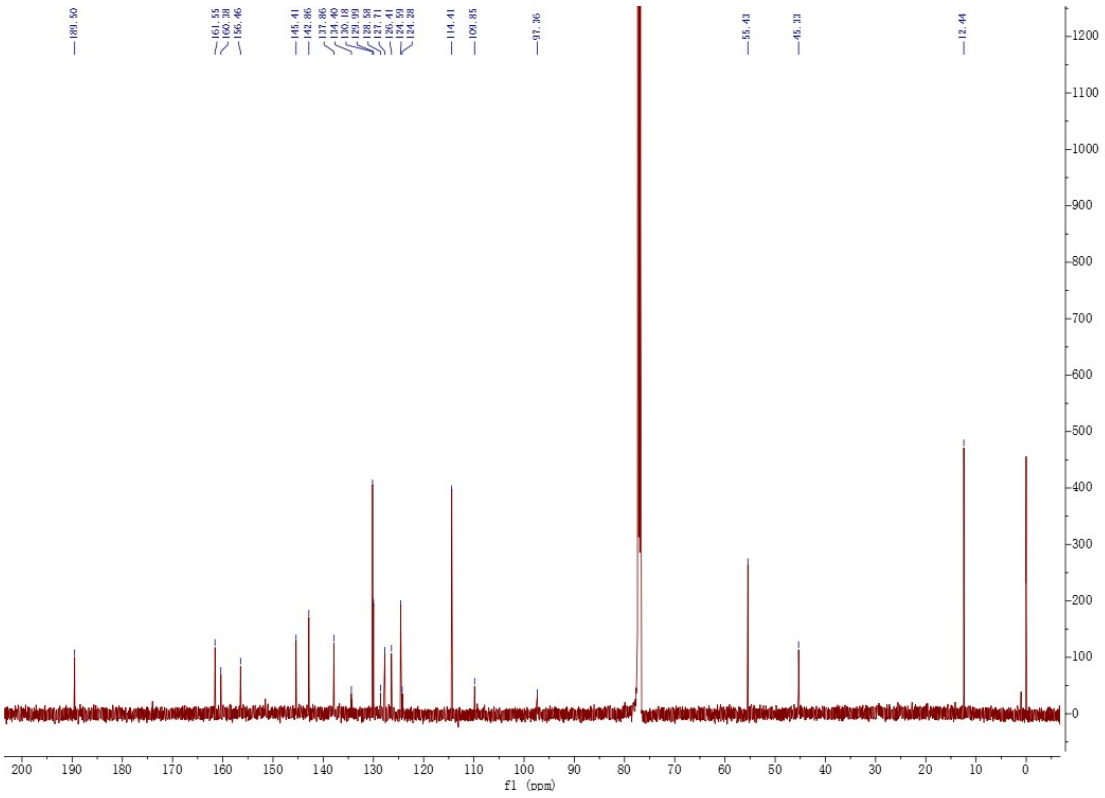


Fig. S2 ^{13}C NMR spectra (125 MHz) of compound **6a** in $CDCl_3$

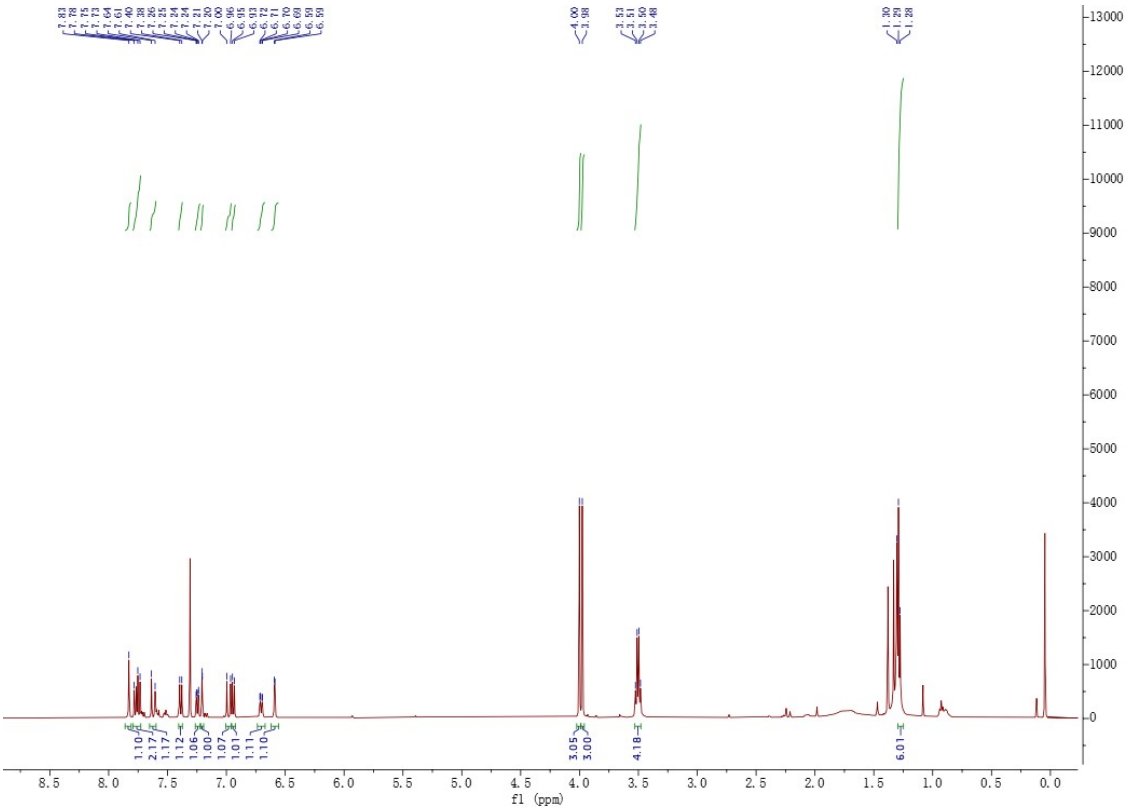


Fig. S3 ^{1}H NMR spectra (500 MHz) of compound **6b** in $CDCl_3$

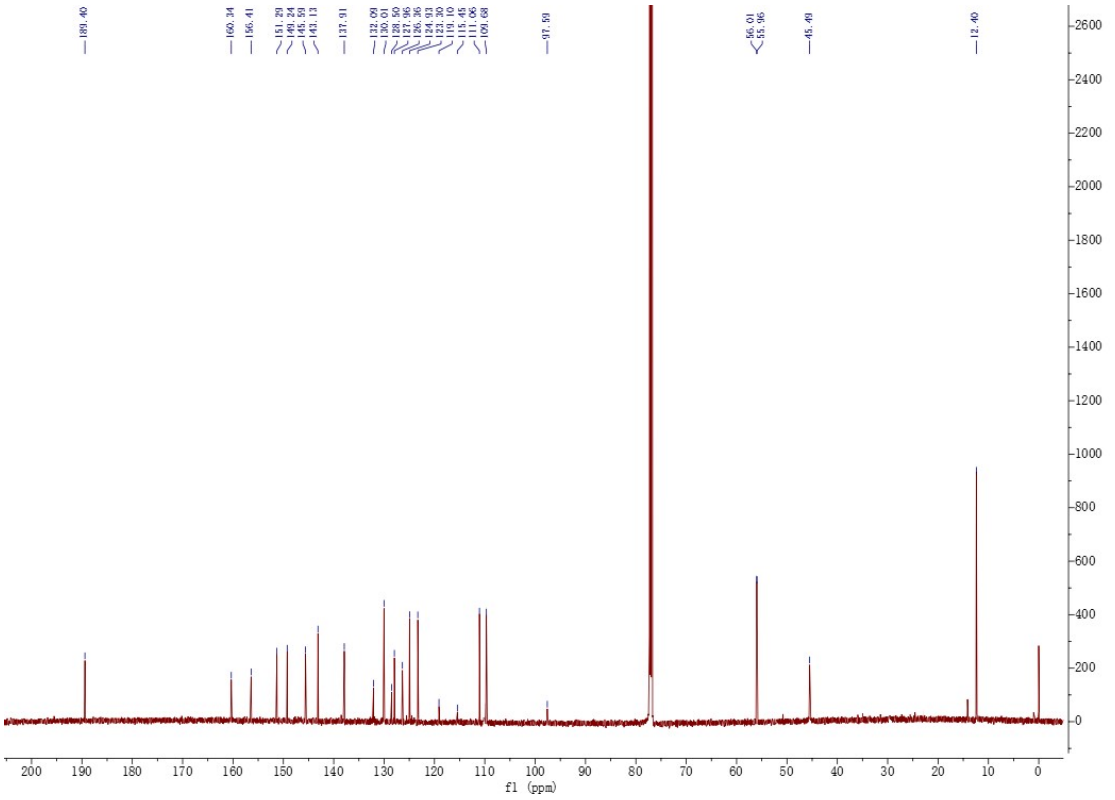


Fig. S4 ^{13}C NMR spectra (125 MHz) of compound **6b** in $CDCl_3$

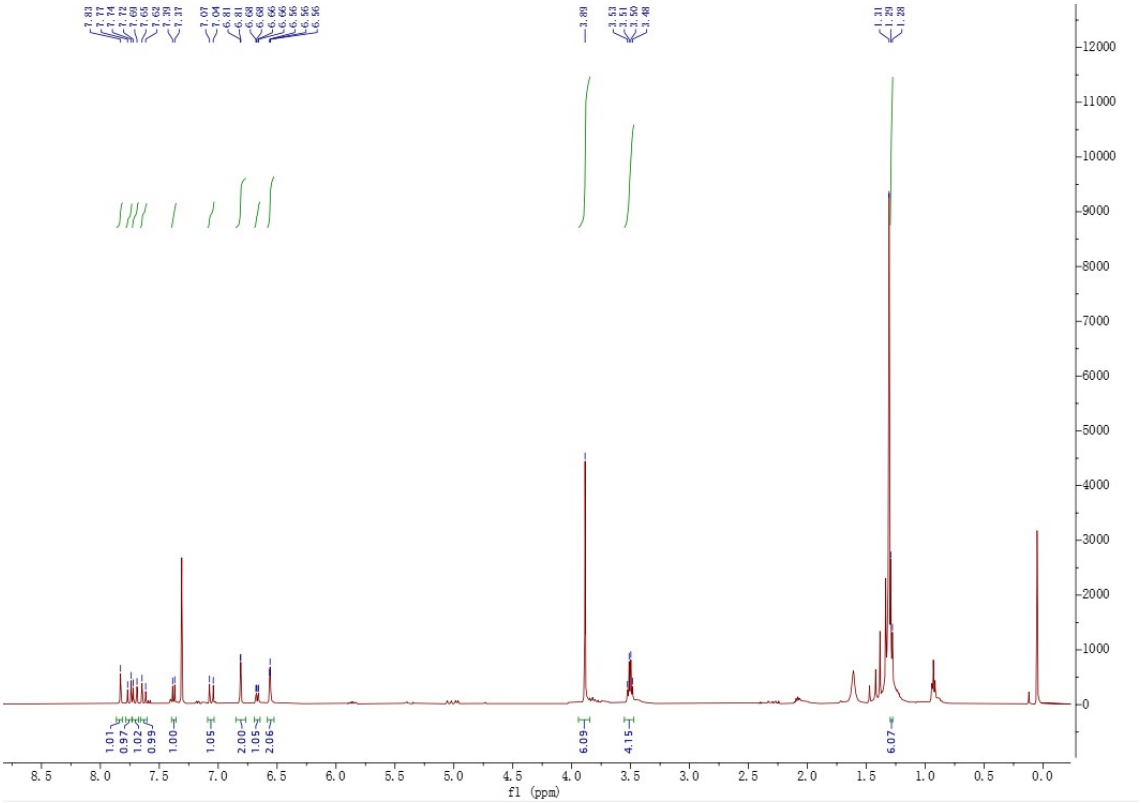


Fig. S5 ^{1}H NMR spectra (500 MHz) of compound **6c** in $CDCl_3$

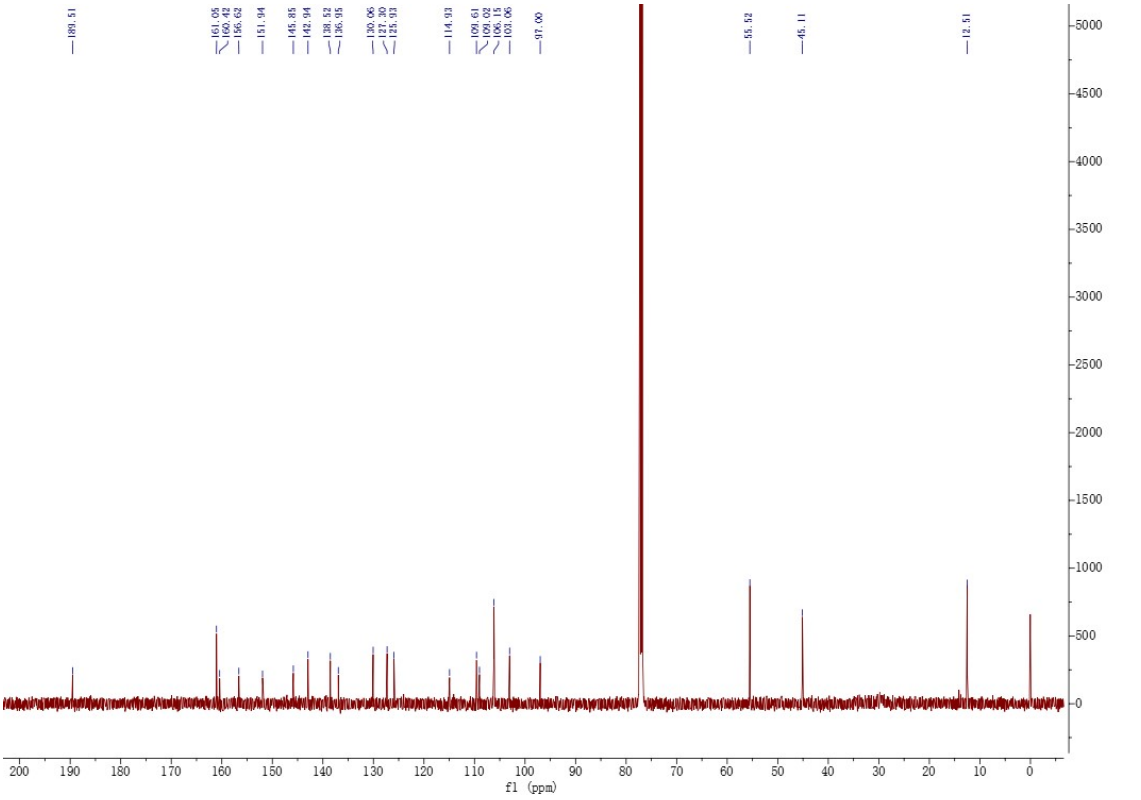


Fig. S6 ^{13}C NMR spectra (125 MHz) of compound **6c** in $CDCl_3$

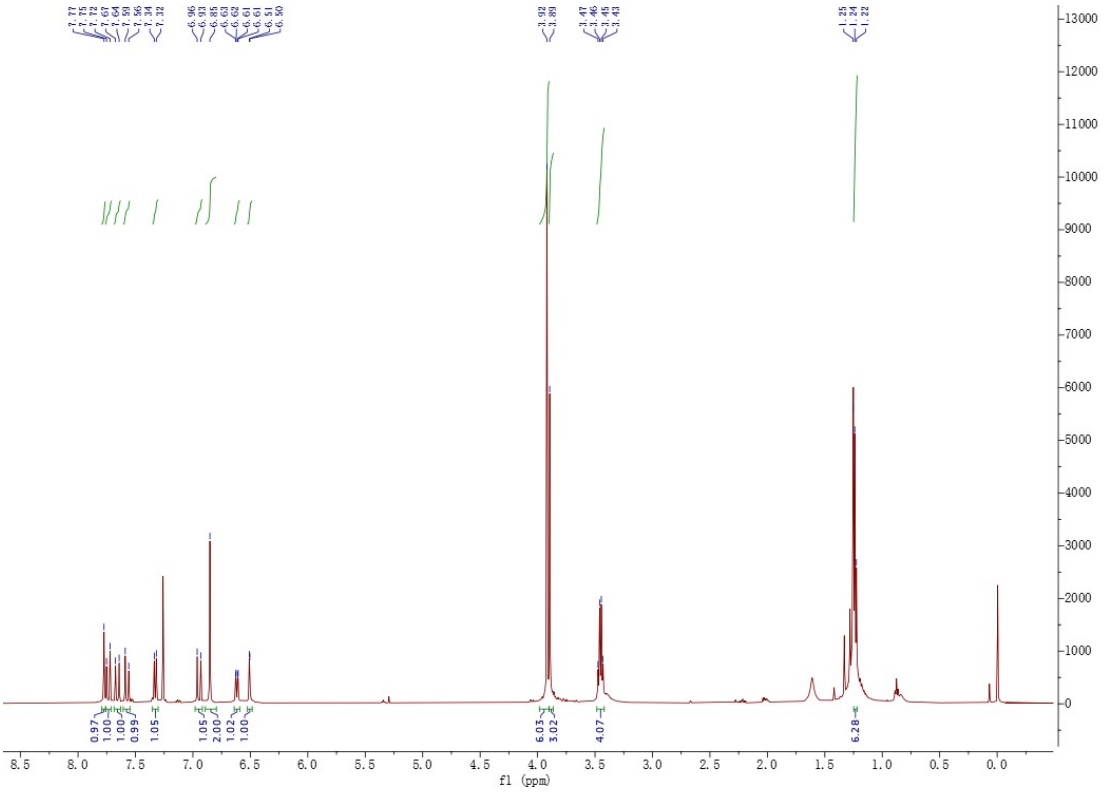


Fig. S7 ^{1}H NMR spectra (500 MHz) of compound **6d** in $CDCl_3$

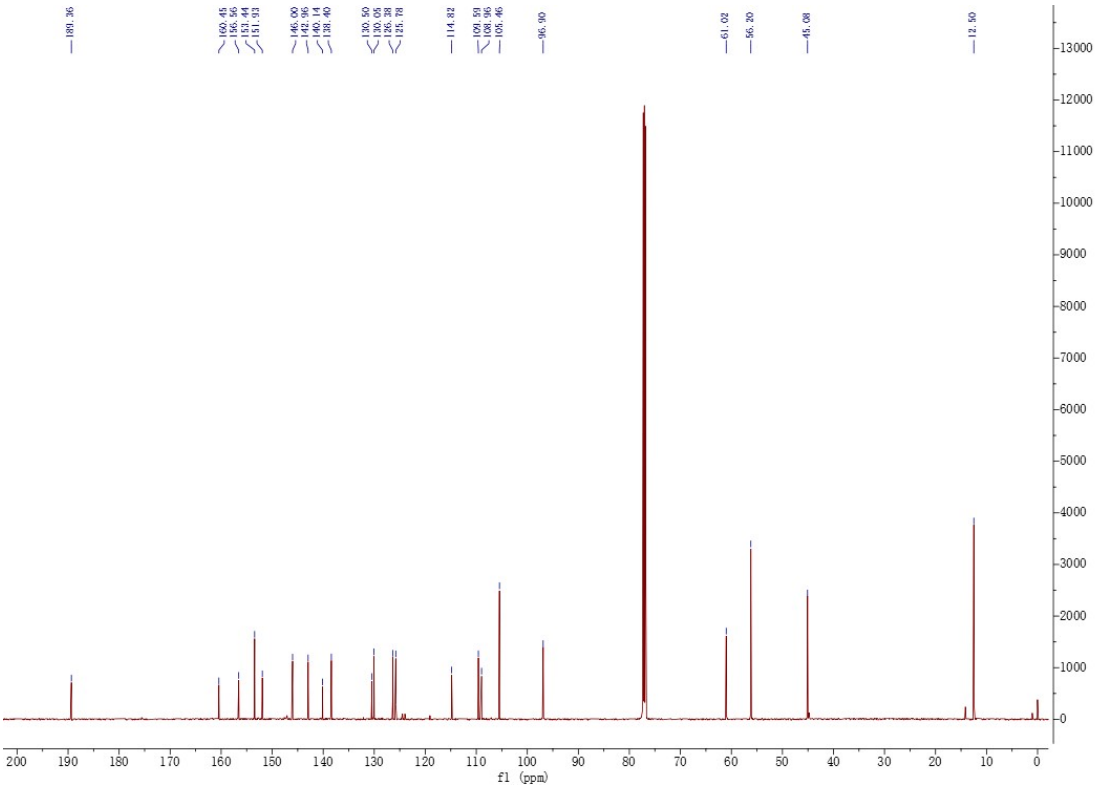


Fig. S8 ^{13}C NMR spectra (125 MHz) of compound **6d** in $CDCl_3$

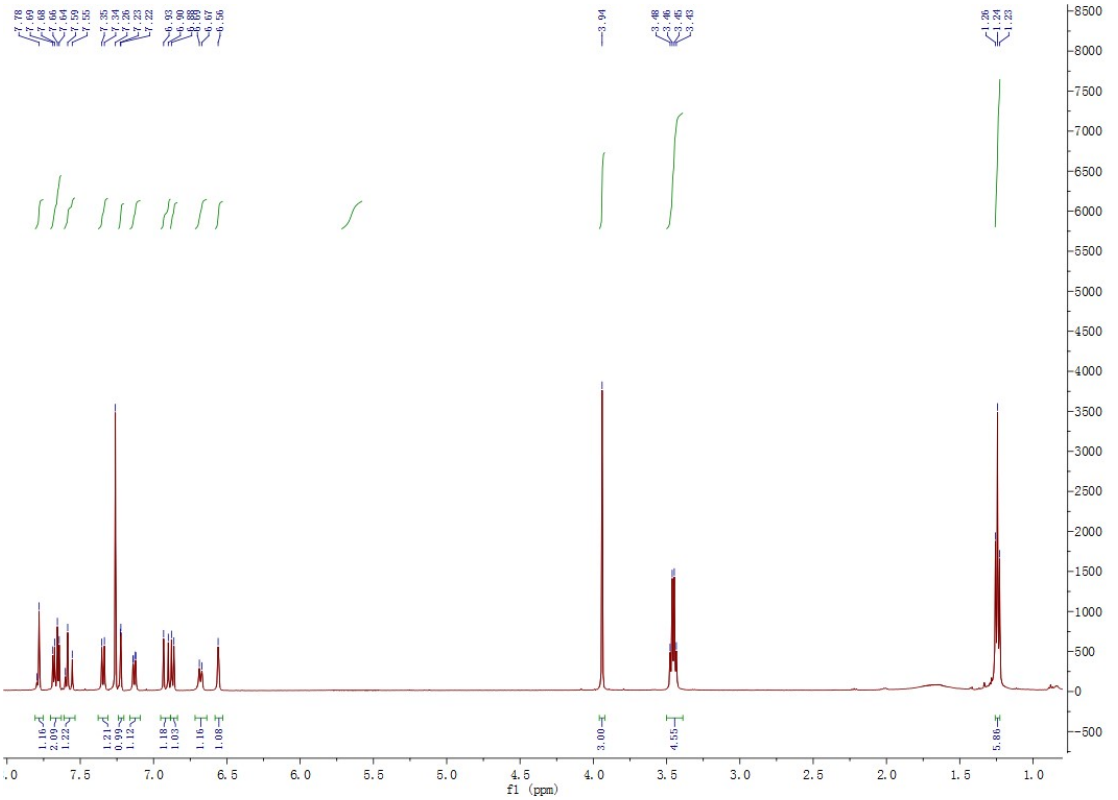


Fig. S9 ^{1}H NMR spectra (500 MHz) of compound **6e** in $CDCl_3$

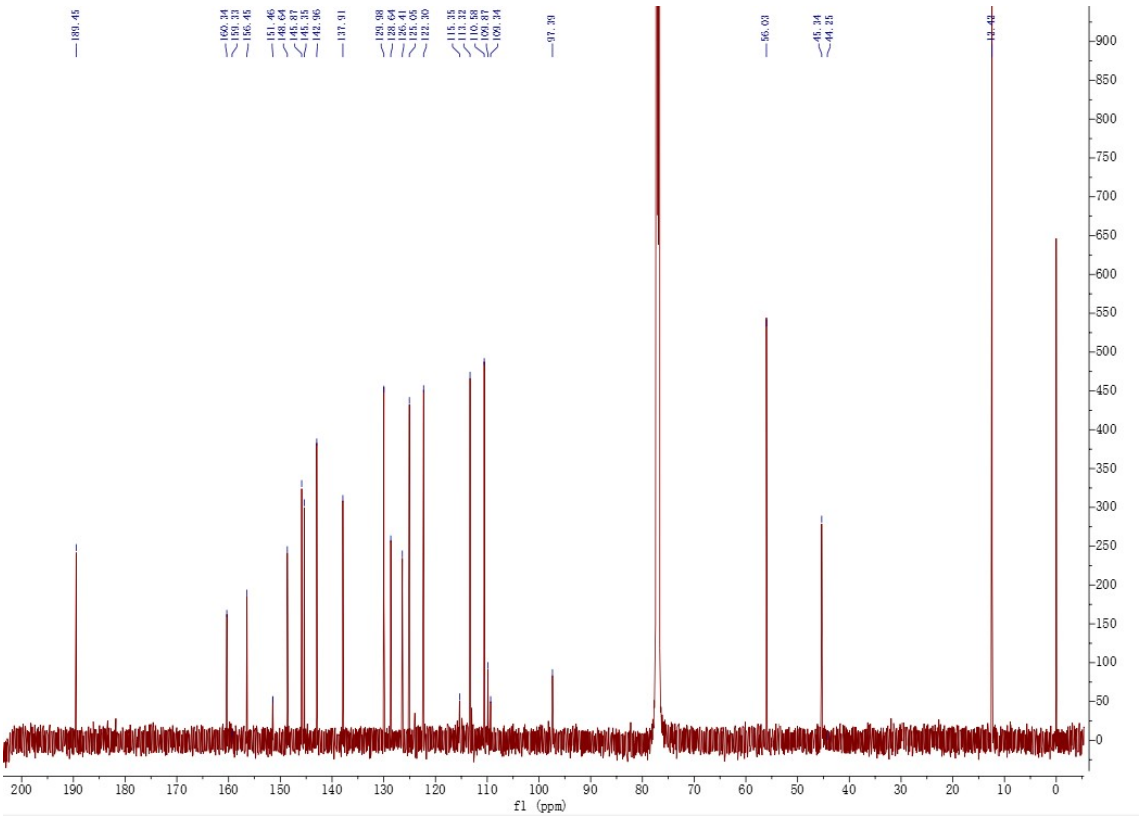


Fig. S10 ^{13}C NMR spectra (125 MHz) of compound **6e** in $CDCl_3$

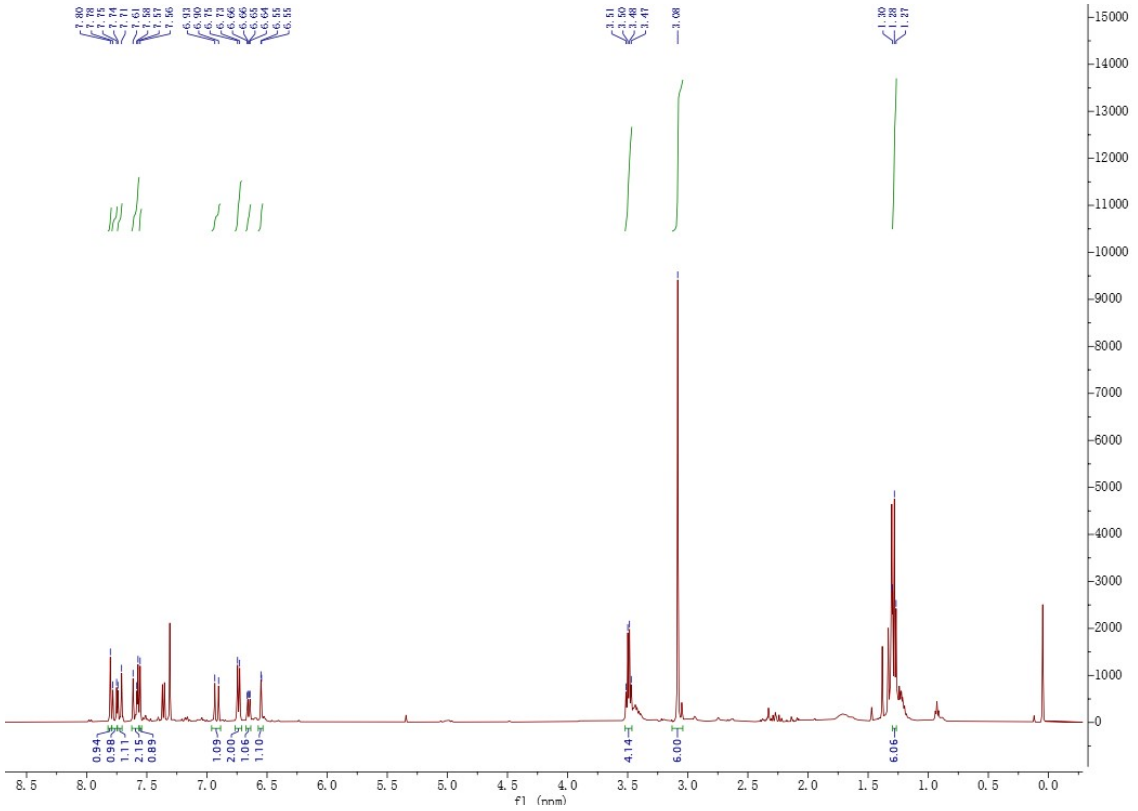


Fig. S11 ^{1}H NMR spectra (500 MHz) of compound **6f** in $CDCl_3$

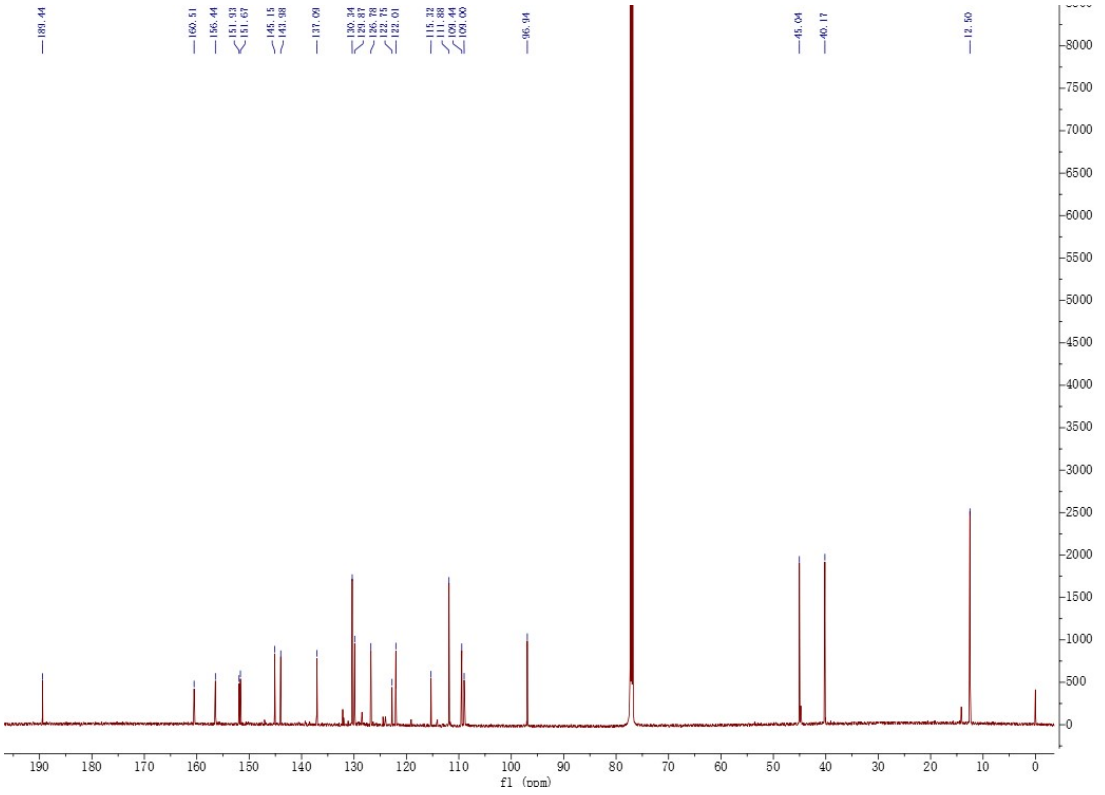


Fig. S12 ^{13}C NMR spectra (125 MHz) of compound **6f** in $CDCl_3$

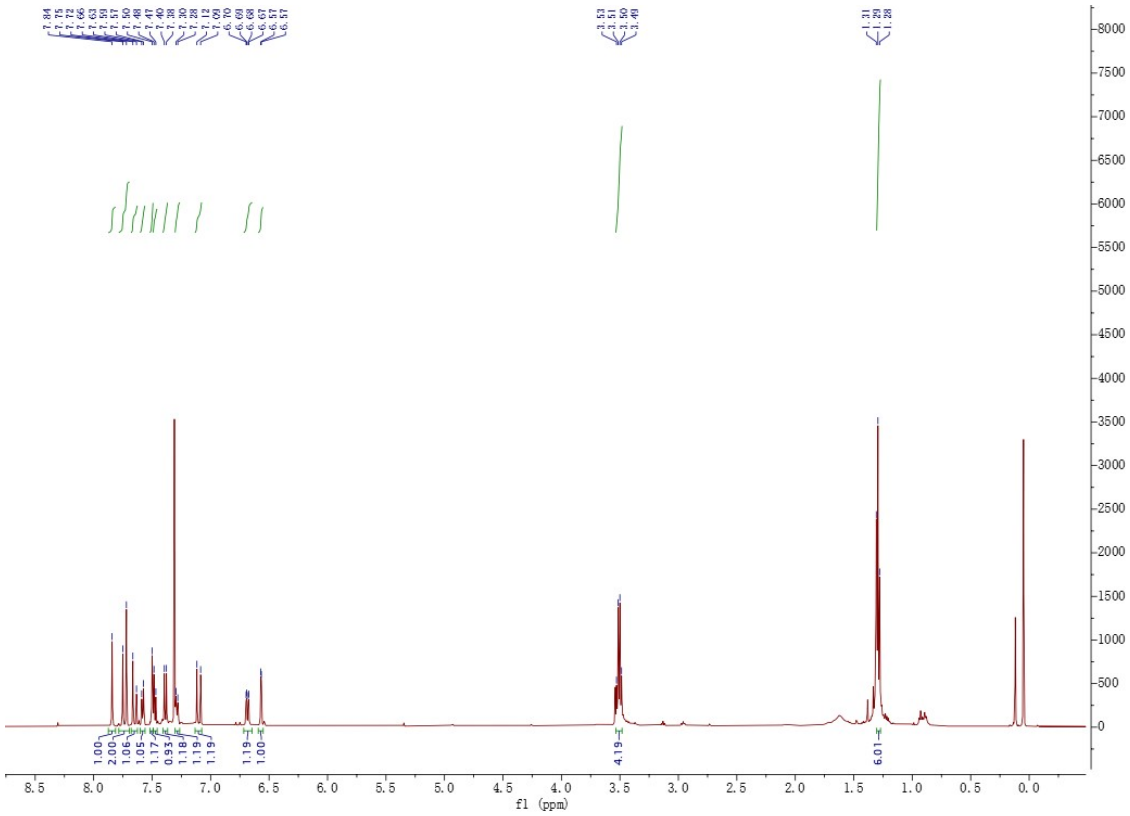


Fig. S13 ^{1}H NMR spectra (500 MHz) of compound **6g** in $CDCl_3$

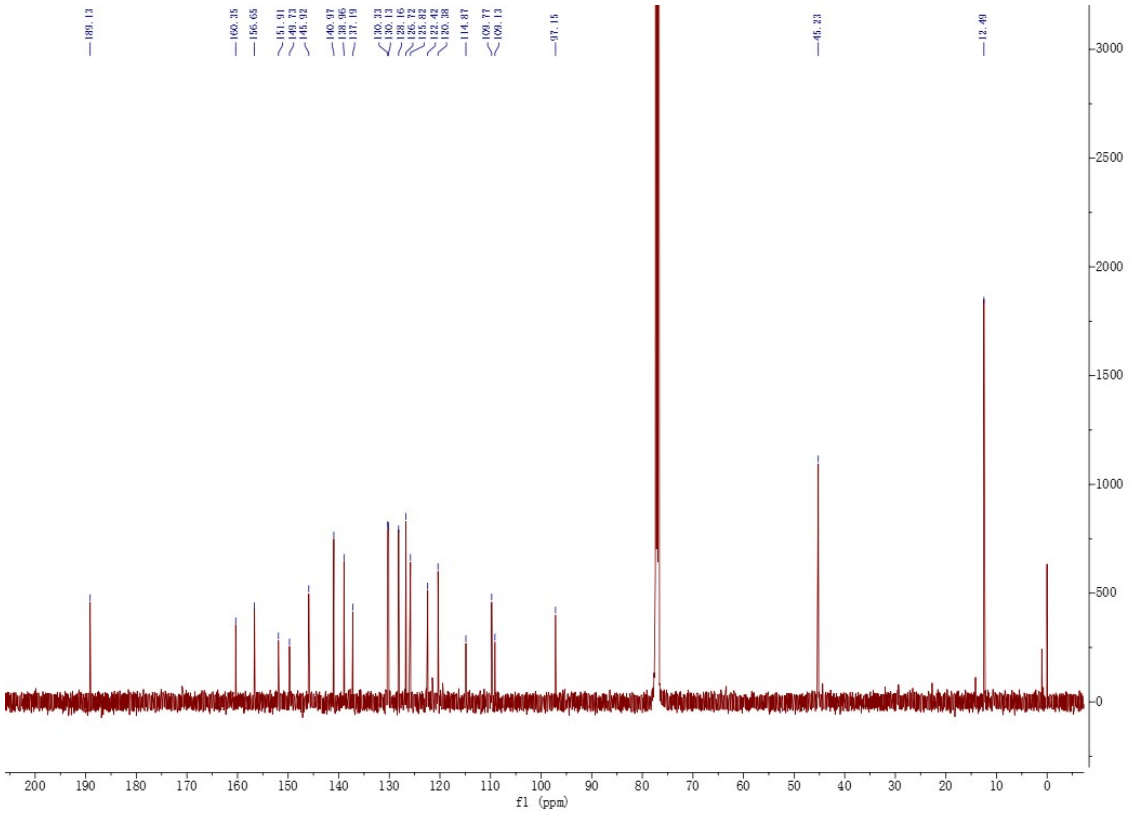


Fig. S14 ^{13}C NMR spectra (125 MHz) of compound **6g** in $CDCl_3$

HRMS (ESI) spectra

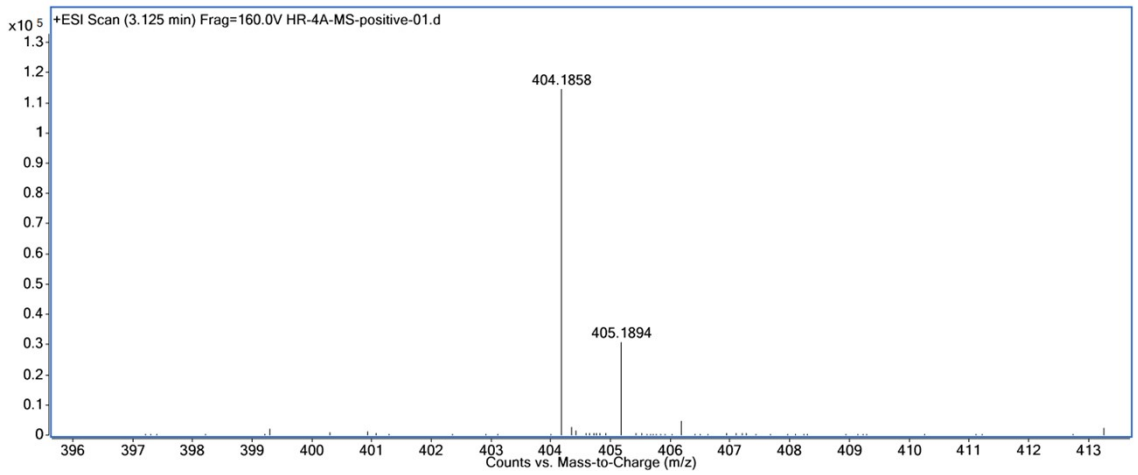


Fig. S15 HRMS (ESI) spectra of **6a**

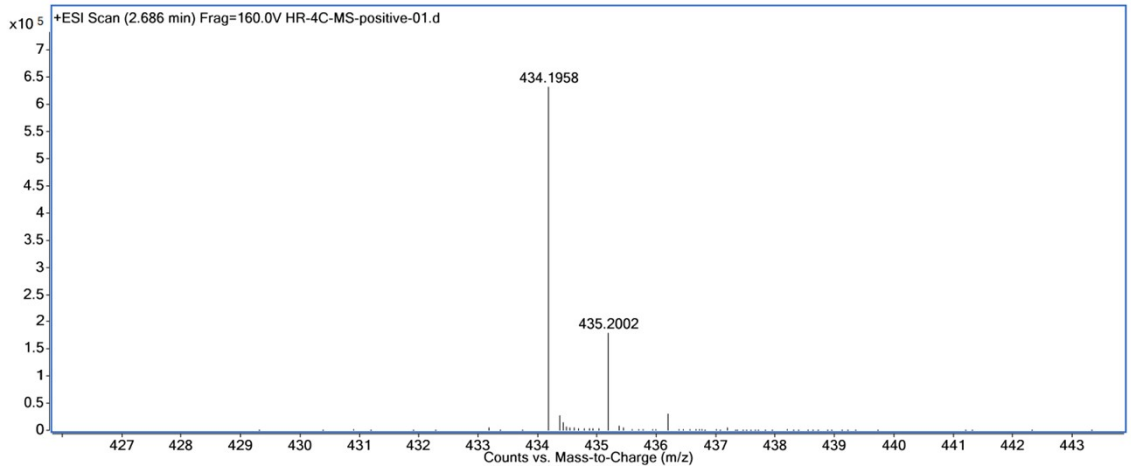


Fig. S16 HRMS (ESI) spectra of **6b**

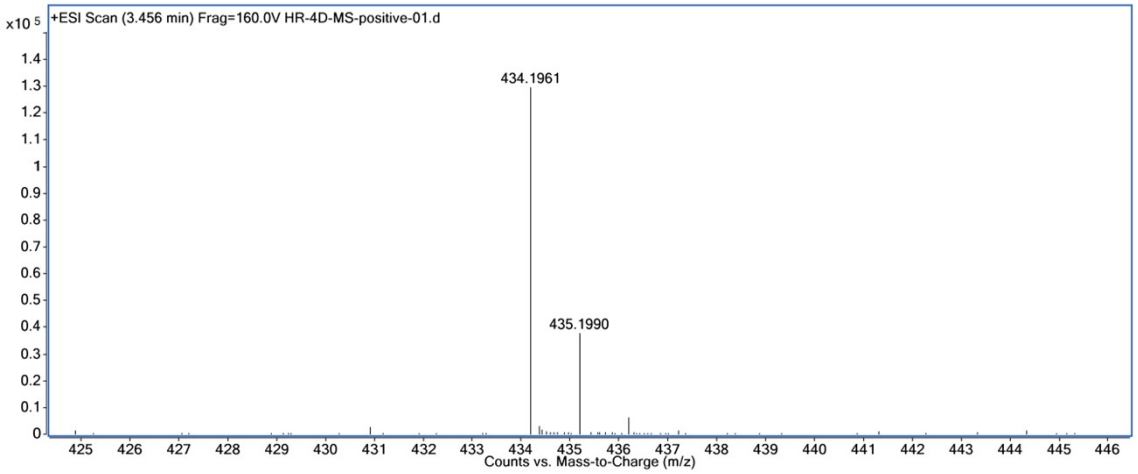


Fig. S17 HRMS (ESI) spectra of **6c**

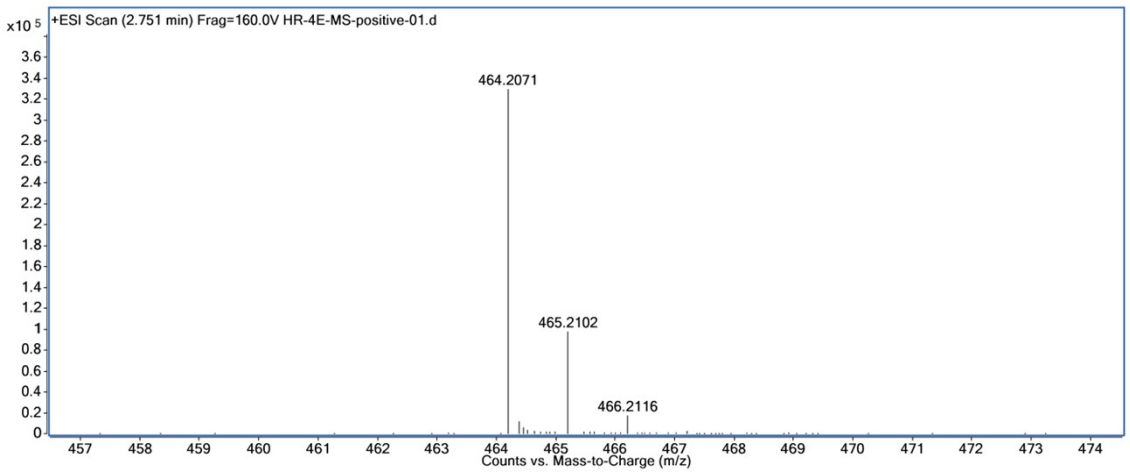


Fig. S18 HRMS (ESI) spectra of **6d**

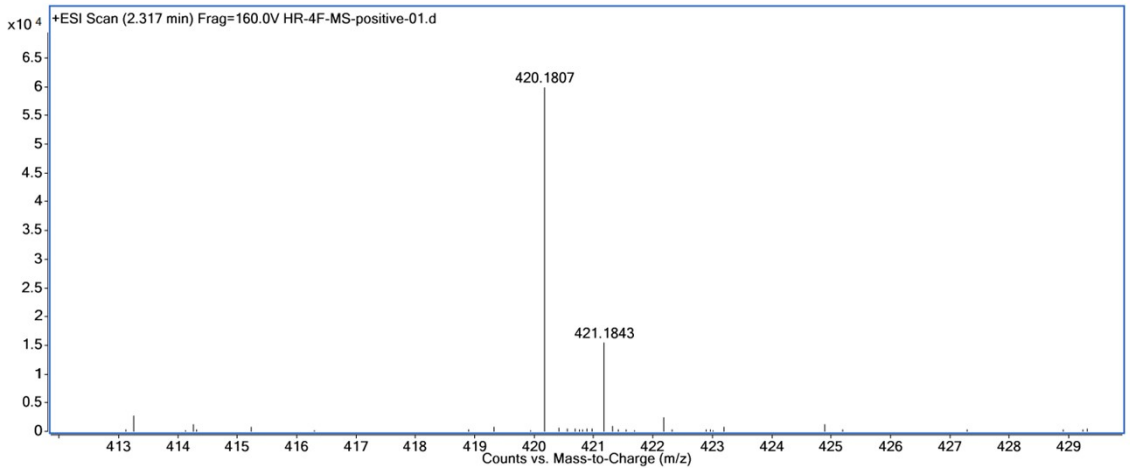


Fig. S19 HRMS (ESI) spectra of **6e**

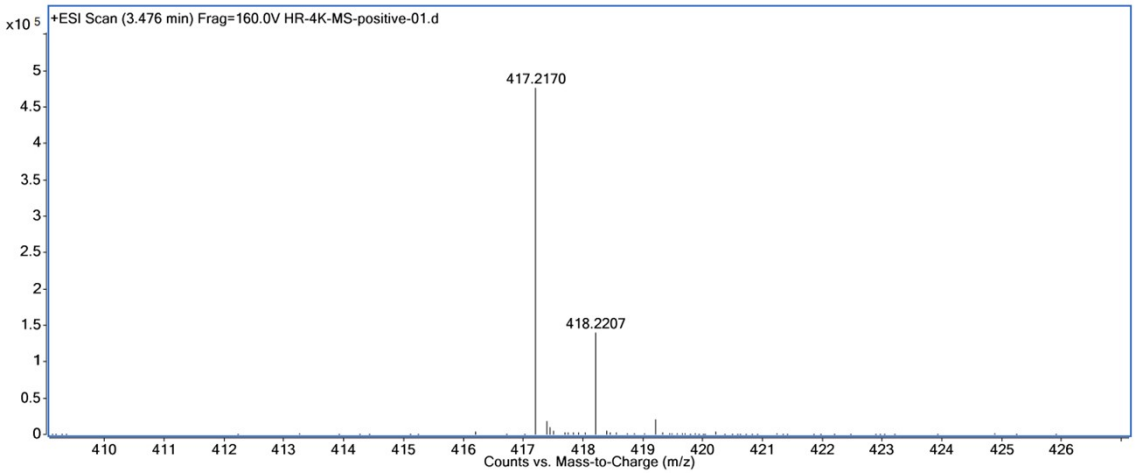


Fig. S20 HRMS (ESI) spectra of **6f**

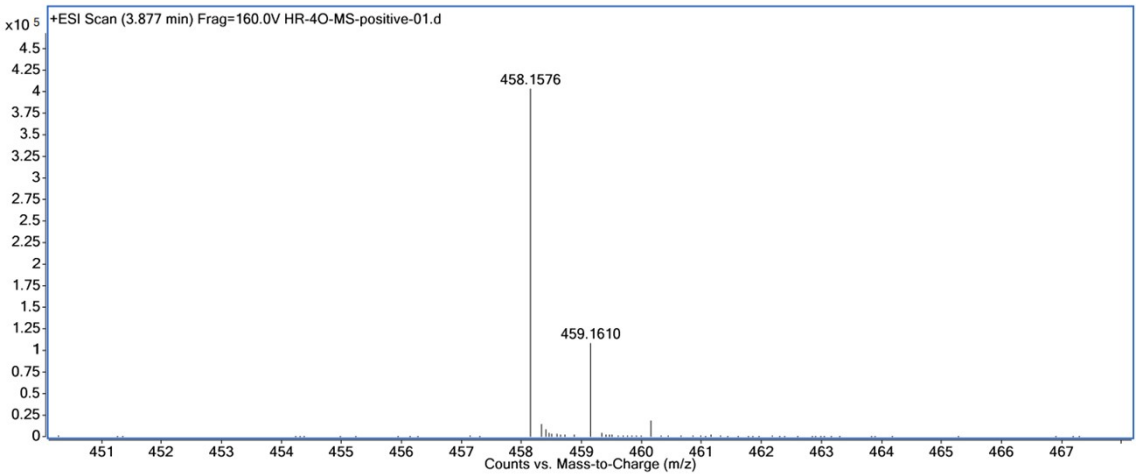


Fig. S21 HRMS (ESI) spectra of **6g**