Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information

Discovery of SKLB-0335 as a Paralog-Selective EZH2 Covalent Inhibitor

Qiangsheng Zhang, ‡[a] Xi Hu, ‡[a] Lu Li, ‡[b] Lidan Zhang, [a] Guoquan Wan, [a] Qiang Feng, [c] Yongxia Zhu, [d] Ningyu Wang, *[e] Zhihao Liu *[a] and Luoting Yu*[a]

a. State Key Laboratory of Biotherapy/Collaborative Innovation Center for Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu, Sichuan 610041, P. R. China.
b. Institute of Clinical Trials, West China Hospital, Sichuan University, Chengdu, Sichuan, 610041, P.R. China
c. College of Chemistry and Life Science, Chengdu Normal University, Chengdu 611130, P. R. China.
d. Department of Clinical Pharmacy, Sichuan Cancer Hospital & Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China, Chengdu, 610041, P. R. China.
e. School of Life Science and Engineering, Southwest JiaoTong University, Chengdu, Sichuan, 611756, P. R. China.
‡These authors contributed equally to this work.

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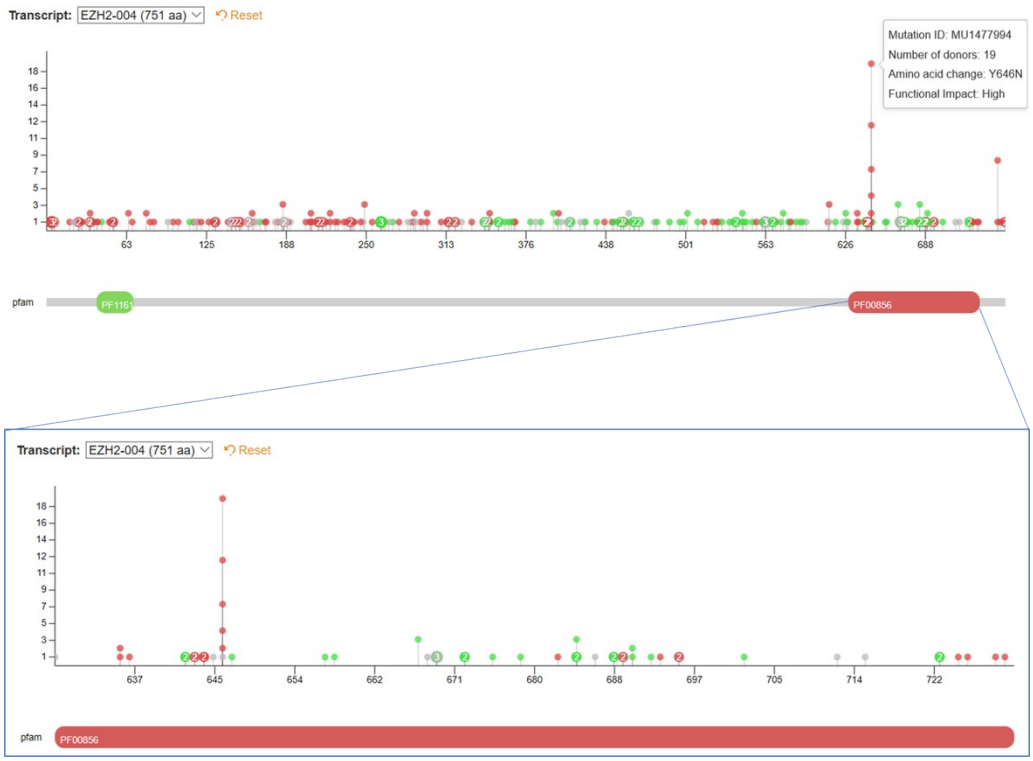


Fig. S1 There is no Cys663 in EZH2 mutation reported. Date from ICGC (https://dcc.icgc.org/genes/ENSG00000106462/protein).

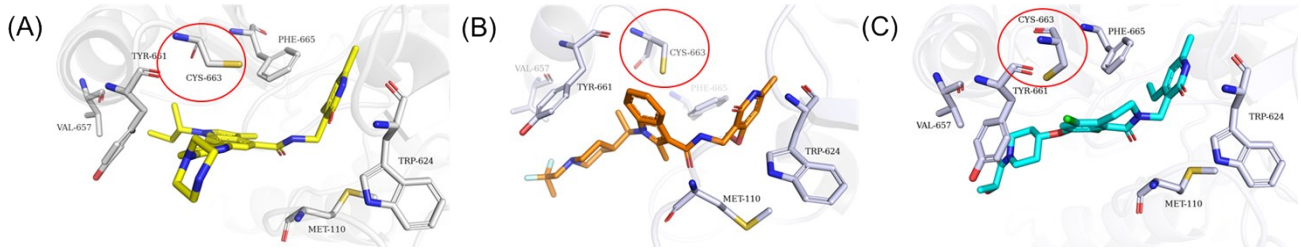


Fig. S2 Indole derivatives (such as CPI-1205) are more suitable for introducing electrophilic warheads. The co-crystal structure of A) GSK126 (yellow spheres, PDB code: 5WG6), B) CPI-1205 analog (orange spheres, PDB code: 5LS6) and C) PF-06821497 analog (bule spheres, PDB code:5IJ7) bound to the PRC2 complex.

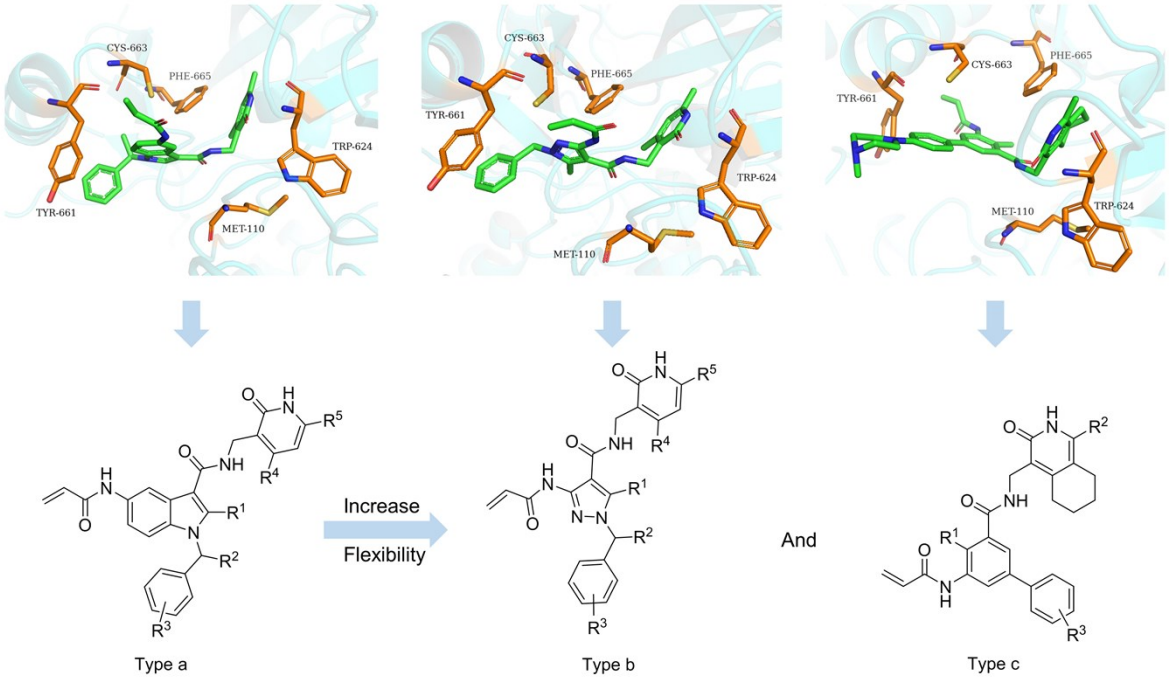


Fig. S3 Rational design of indole derivatives, pyrazole derivatives and benzene derivatives.

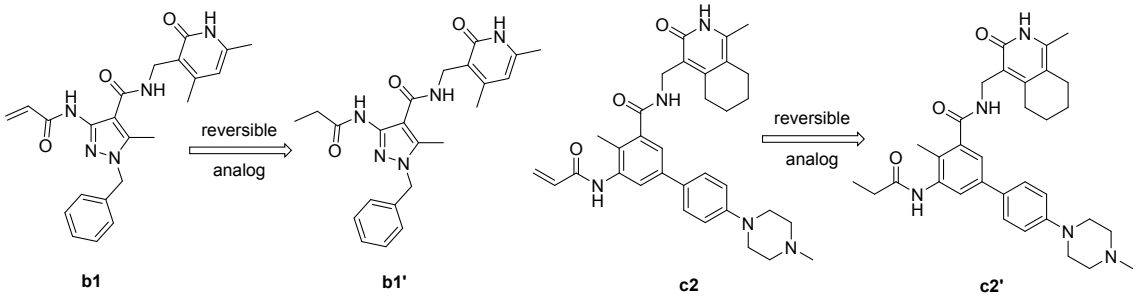


Fig. S4 Chemical structures of compounds **b1**, **c2** and their reversible analogs **b1′**, **c2′**.

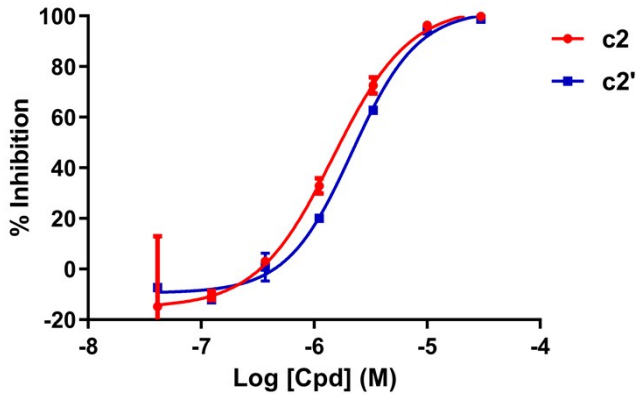
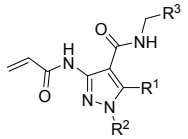


Fig. S5 Inhibition curves of compound **c2** and its reversible analog **c2′** on $EZH2^{WT}$. Data are expressed as the mean ± SD (n = 2).

Tab. S1 Inhibitory activity of compounds **b3**-**b15** on $EZH2^{WT}$.



Cpd.	R^1	R^2	R^3	IC_{50} (μM) [a]	Cpd.	R^1	R^2	R^3	IC_{50} (μM) [a]
b3	CH_3			5.243 ± 1.037	**b11**	H			>10
b4 (SKLB -0335)	CH_3			0.064 ± 0.002	**b12**	CH_3			0.109 ± 0.027
b5	CH_3			>10	**b13**	CH_3			>10
b6	CH_3			2.663 ± 0.161	**b14**	CH_3			2.545 ± 0.006
b7	CH_3			1.368 ± 0.110	**b15**	CH_3			0.710 ± 0.144
b8	H			7.661 ± 0.724	**(S)-b4**	CH_3			0.108 ± 0.016
b9	H			>10	**(R)-b4**	CH_3			0.042 ± 0.016
b10	H			>10					

[a] Data are expressed as the mean ± SEM for at least 2 independent experiments.

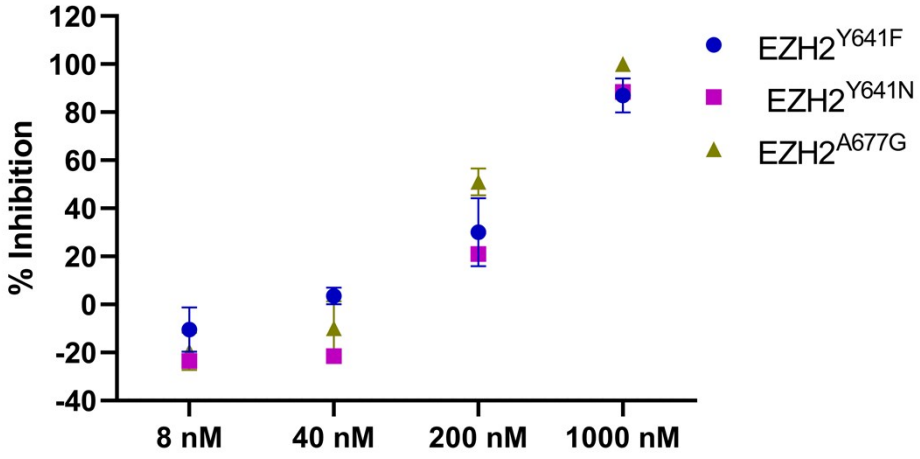


Fig. S6 Potency of SKLB-0335 against mutant EZH2. Data are expressed as the mean ± SD (n = 2).

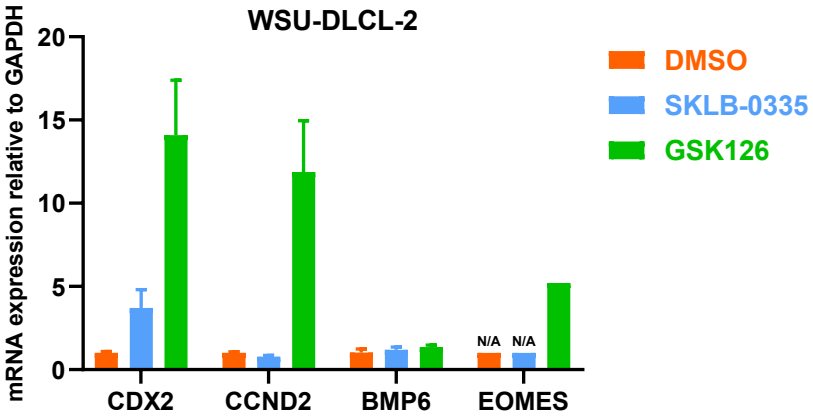
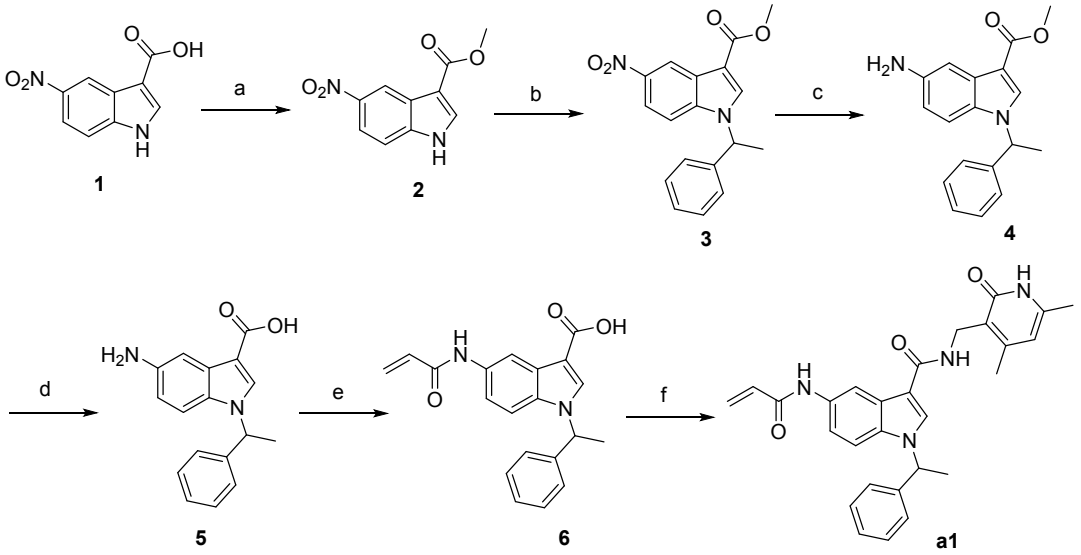


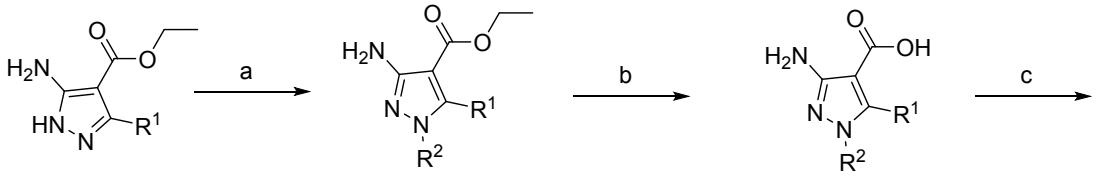
Fig. S7 The mRNA levels of genes silenced by EZH1 in WSU-DLCL-2 cells after treatment with compounds for 5 days. Data are expressed as the mean ± SD (n = 3).

Scheme S1[a]



[a]Reagents and conditions: (a) H_2SO_4, CH_3OH, 80 °C, 6 h; (b) NaH, DMF, r.t., 4 h; (c) Fe, NH_4Cl, CH_3OH/H_2O, reflux, 1 h; (d) NaOH, EtOH, 60 °C, 1 h; (e) Acryloyl chloride, K_2CO_3, DCM, r.t., 4 h; (f) 3-(aminomethyl)-4,6-dimethylpyridin-2(1*H*)-one, EDCI, HOAT, NMM, DMSO, r.t., overnight.

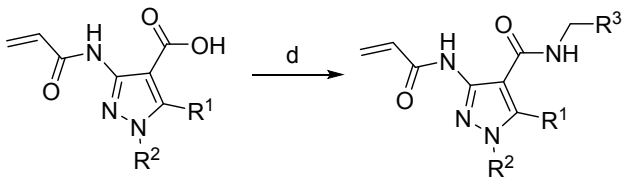
Scheme S2[b]



7a,R^1= Me
7b,R^1= H

8a,R^1= Me; R^2= benzyl
8b,R^1= Me, R^2= 1-phenylethyl
8c,R^1= Me, R^2= 1,1'-biphenyl
8d,R^1= Me, R^2= (S)-1-phenylethyl
8e,R^1= Me, R^2= (R)-1-phenylethyl
8f,R^1= H, R^2= 1-phenylethyl

9a,R^1= Me; R^2= benzyl
9b,R^1= Me, R^2= 1-phenylethyl
9c,R^1= Me, R^2= 1,1'-biphenyl
9d,R^1= Me, R^2= (S)-1-phenylethyl
9e,R^1= Me, R^2= (R)-1-phenylethyl
9f,R^1= H, R^2= 1-phenylethyl



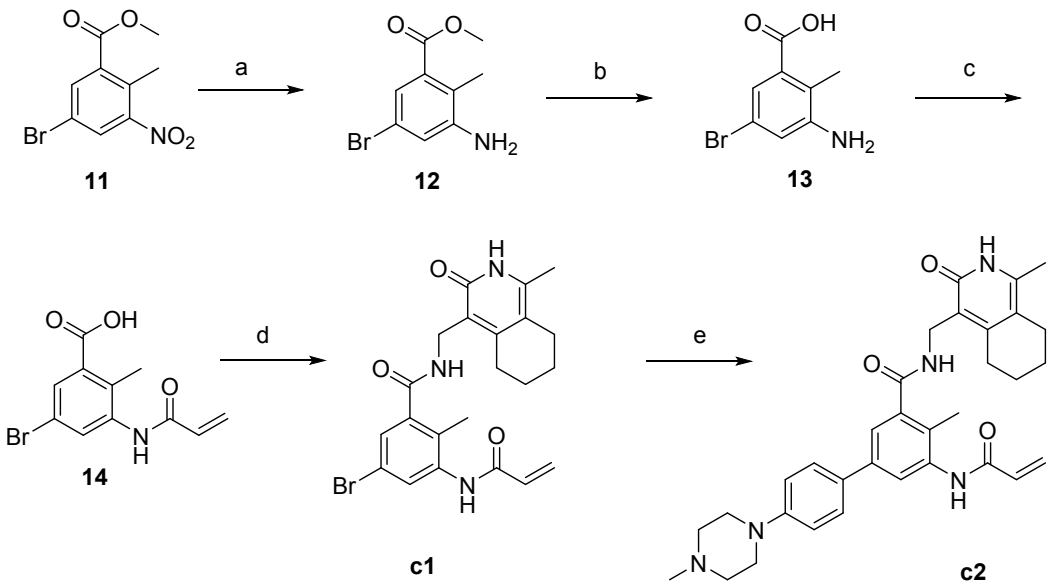
10a,R^1= Me; R^2= benzyl
10b,R^1= Me, R^2= 1-phenylethyl
10c,R^1= Me, R^2= 1,1'-biphenyl
10d,R^1= Me, R^2= (S)-1-phenylethyl
10e,R^1= Me, R^2= (R)-1-phenylethyl
10f,R^1= H, R^2= 1-phenylethyl

b1-b15,
(S)-b4,
(R)-b4;

b1,R^1= Me; R^2= benzyl; R^3= 4,6-dimethylpyridin-2(1*H*)-one
b2,R^1= Me; R^2= benzyl; R^3= 1-methyl-5,6,7,8-tetrahydroisoquinolin-3(2*H*)-one
b3,R^1= Me; R^2= benzyl; R^3= 4,6-diethylpyridin-2(1*H*)-one
b4,R^1= Me; R^2= 1-phenylethyl; R^3= 4,6-dimethylpyridin-2(1*H*)-one
b5,R^1= Me; R^2= 1-phenylethyl; R^3= 1-methyl-5,6,7,8-tetrahydroisoquinolin-3(2*H*)-one
b6,R^1= Me; R^2= 1-phenylethyl; R^3= 4,6-diethylpyridin-2(1*H*)-one
b7,R^1= Me; R^2= 1-phenylethyl; R^3= 4-ethyl-6-methylpyridin-2(1*H*)-one
b8,R^1= H; R^2= 1-phenylethyl; R^3= 4,6-dimethylpyridin-2(1*H*)-one
b9,R^1= H; R^2= 1-phenylethyl; R^3= 1-methyl-5,6,7,8-tetrahydroisoquinolin-3(2*H*)-one
b10,R^1= H; R^2= 1-phenylethyl; R^3= 4,6-diethylpyridin-2(1*H*)-one
b11,R^1= H; R^2= 1-phenylethyl; R^3= 4-ethyl-6-methylpyridin-2(1*H*)-one
b12,R^1= Me; R^2= 1,1'-biphenyl; R^3= 4,6-dimethylpyridin-2(1*H*)-one
b13,R^1= Me; R^2= 1,1'-biphenyl; R^3= 1-methyl-5,6,7,8-tetrahydroisoquinolin-3(2*H*)-one
b14,R^1= Me; R^2= 1,1'-biphenyl; R^3= 4,6-diethylpyridin-2(1*H*)-one
b15,R^1= Me; R^2= 1,1'-biphenyl; R^3= 4-ethyl-6-methylpyridin-2(1*H*)-one
(S)-b4,R^1= Me; R^2= (S)-1-phenylethyl; R^3= 4,6-dimethylpyridin-2(1*H*)-one
(R)-b4,R^1= Me; R^2= (R)-1-phenylethyl; R^3= 4,6-dimethylpyridin-2(1*H*)-one

[b]Reagents and conditions: (a) $CsCO_3$, DMF, r.t., 4 h; (b) NaOH, CH_3OH/H_2O,80 °C,4 h; (c) Acryloyl chloride, K_2CO_3, DCM, r.t., 4 h; (d) 3-(aminomethyl)- pyridin-2(1*H*)-one derivatives, EDCI, HOAT, NMM, DMSO, r.t., overnight.

Scheme S3[c]



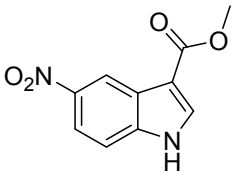
[c]Reagents and conditions: (a) Fe, NH_4Cl, CH_3OH/H_2O, 85 °C, 0.5 h; (b) NaOH, CH_3OH/H_2O,80 °C,4 h; (c) Acryloyl chloride, K_2CO_3, DCM, r.t., 4 h; (d) 4-(aminomethyl)-1-methyl-5,6,7,8-tetrahydroisoquinolin-3(2*H*)-one, EDCI, HOAT, NMM, DMSO, r.t., overnight; (e) 4-(4-methyl-1-piperazinyl)benzeneboronic acid pinacol ester, $PdCl_2(dppf)\cdot CH_2Cl_2$, Na_2CO_3, dioxane/water, 100 °C, 4 h.

General chemistry experiment and information.

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. The reference compound GSK126 was purchased from MedChemExpress (China). The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 25 °C using DMSO-d_6, CD_3OD or $CDCl_3$ as the solvent. Chemical shifts (δ) are reported in ppm relative to Me_4Si (internal standard), coupling constants (*J*) are reported in hertz, and peak multiplicity are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). High resolution mass analysis was performed on a Waters Q-TOF Premier mass spectrometer with electron spray ionization (ESI). Thin layer chromatography (TLC) was performed on 0.20 mm silica gel F-254 plates (Qingdao Haiyang Chemical, China). Visualization of TLC was accomplished with UV light and/or aqueous potassium permanganate or I_2 in a silica gel. Column chromatography was performed using silica gel 60 of 300-400 mesh (Qingdao Haiyang Chemical, China). Chemical purities were analyzed by HPLC using Acetonitrile / Water as the mobile phase with a flow rate of 1.0 mL/min on an Phenomenex Gemini C18 column (NO.00F-4435-EO).

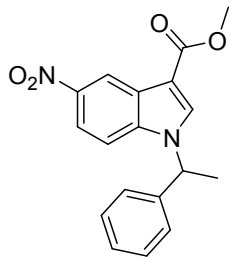
Chemistry experimental procedures.

The representative procedure for the preparation of indole derivatives.



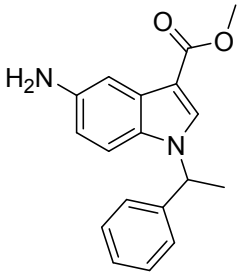
2

methyl 5-nitro-1*H*-indole-3-carboxylate (2). Sulfuric acid (0.5 ml) was added to the methanol solution (20 ml) of 5-nitro-1*H*-indole-3-carboxylic acid (206.15 mg, 1 mmol) and reacted at 80 °C for 6 h. After the reaction was completed (monitored by TLC), the reaction solution was concentrated in vacuo. Saturated sodium bicarbonate solution was added to adjust the pH to 8 ~ 9, and ethyl acetate was added for extraction. The combined organic phase was dried over sodium sulfate, filtered and concentrated in vacuo to provide compound **2**. Tan solid 216 mg, yield: 98.1%.^1H NMR (400 MHz, DMSO-d_6) δ 8.84 (d, *J* = 2.3 Hz, 1H), 8.34 (s, 1H), 8.05 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.64 (d, *J* = 8.9 Hz, 1H), 3.85 (s, 3H).



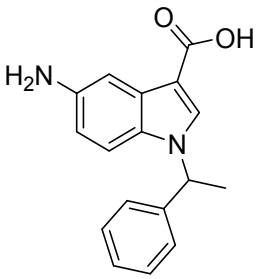
3

methyl 5-nitro-1-(1-phenylethyl)-1*H*-indole-3-carboxylate (3). Sodium hydride (76 mg, 60%, 1.90 mmol) was dissolved in 5 ml of N, N-dimethylformamide and added to the flask, methyl 5-nitro-1*H*-indole-3-carboxylate (216 mg, 0.95 mmol) dissolved in 10 ml N, N-dimethylformamide was added to the flask, and the mixed solution was stirred at 40 °C for 0.5 h. Then, 1-bromoethylbenzene (156 µl, 1.14 mmol) was added and stirred at room temperature for 4 h. After the reaction was detected by TLC, 30 ml of cold water was added to quench the reaction, and extracted with ethyl acetate. Dry over anhydrous sodium sulfate, filtered, and column chromatography to obtain the target compound. Light yellow solid 227 mg, yield: 73.7%.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (d, *J* = 2.4 Hz, 1H), 8.68 (s, 1H), 8.09 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.82 (d, *J* = 9.2 Hz, 1H), 7.38 -7.32 (m, 4H), 7.30 – 7.24 (m, 1H), 6.07 (q, *J* = 7.0 Hz, 1H), 3.89 (s, 3H), 1.96 (d, *J* = 7.0 Hz, 3H).



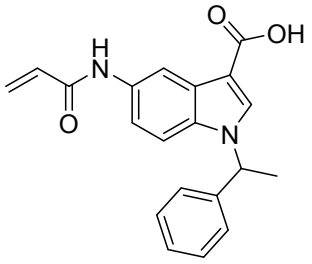
4

methyl 5-amino-1-(1-phenylethyl)-1*H*-indole-3-carboxylate (4). Compound **3** (227 mg, 0.7 mmol), ammonium chloride (187.25 mg, 3.5 mmol) was added to a solution of methanol / water (18 ml / 6 ml). While the reaction solution was refluxing, iron powder (196 mg, 3.5 mmol) was added, and the reaction was continued for 1 h. After the reaction was completed (monitored by TLC), added a layer of diatomaceous earth to filter the hot reaction liquid. The diatomaceous earth was washed twice with acetone and the filtrate was collected. The combined organic phase was dried over sodium sulfate, filtered and concentrated in vacuo to provide compound **4.** The product was used for the next step directly. Brown viscous liquid 202 mg, yield: 98.0%.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 7.33 – 7.21 (m, 5H), 7.19 – 7.17 (m, 1H), 7.15 (d, *J* = 8.7 Hz, 1H), 6.51 (dd, *J* = 8.7, 2.2 Hz, 1H), 5.73 (q, *J* = 7.0 Hz, 1H), 4.80 (s, 2H), 3.77 (s, 3H), 1.86 (d, *J* = 7.0 Hz, 3H).



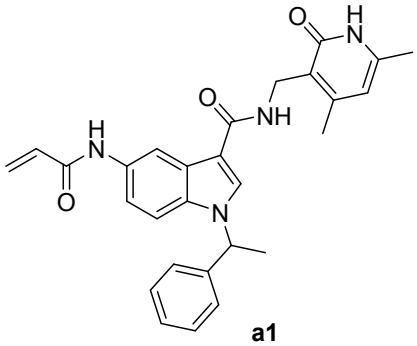
5

5-amino-1-(1-phenylethyl)-1*H*-indole-3-carboxylic acid (5). A mixture of compound **4** (160 mg, 0.54 mmol), sodium hydroxide (32.4 mg, 0.81 mmol), and EtOH (10 mL) was stirred at 60 °C for 1 h. The reaction was acidified to pH ~3 with 1M hydrochloric acid and then extracted with EtOAc (3X). The combined organic phase was dried over sodium sulfate, filtered and concentrated in vacuo to provide the crude compound. The crude compound was purified by column chromatography eluting with MeOH/DCM to afford the desired compound **5**. Brown solid 60 mg, yield: 39.9%.^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.01 (s, 1H), 7.52 (d, *J* = 2.2 Hz, 1H), 7.34 – 7.27 (m, 3H), 7.14 (dd, *J* = 6.9, 1.9 Hz, 2H), 7.01 (d, *J* = 8.7 Hz, 1H), 6.62 (dd, *J* = 8.7, 2.3 Hz, 1H), 5.58 (q, *J* = 7.0 Hz, 1H), 1.93 (d, *J* = 7.0 Hz, 3H).



6

5-acrylamido-1-(1-phenylethyl)-1*H*-indole-3-carboxylic acid (6). Added compound **5** (60 mg, 0.214 mmol) and potassium carbonate (88.6 mg, 0.642 mmol) to tetrahydrofuran. Acryloyl chloride (35 μL, 0.428 mmol) was added at 0 °C. The reaction solution was reacted at room temperature for 4 hours. After completion (monitored by TLC), the reaction solution was concentrated under vacuum. Added water and DCM for extraction, adjusted the pH to 4 ~ 5, and collected the organic phase.The crude compound was purified by column chromatography eluting with MeOH/DCM to afford the desired compound. Darkorange solid 45 mg, yield: 58.0%.^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.21 (s, 1H), 8.56 (s, 1H), 8.03 (s, 1H), 7.69 (d, *J* = 8.9 Hz, 1H), 7.30 – 7.20 (m, 3H), 7.09 (t, *J* = 8.3 Hz, 2H), 6.60 (dd, *J* = 16.8, 10.1 Hz, 1H), 6.45 (d, *J* = 16.7 Hz, 1H), 5.68 (d, *J* = 10.1 Hz, 1H), 5.57 (q, *J* = 7.1 Hz, 1H), 1.88 (d, *J* = 7.0 Hz, 3H).

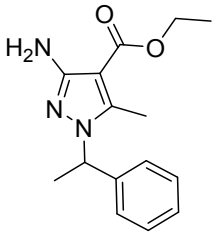


a1

5-acrylamido-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-(1-phenylethyl)-1*H*-indole-3-carboxamide (a1). Compound **6** (45 mg, 0.135 mmol), 3-(aminomethyl)-4,6-dimethylpyridin-2(1*H*)-one (40.98 mg, 0.27 mmol, obtained following the reference procedure[1]), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (46.58 mg, 0.243 mmol), 1-hydroxy-7-azobenzotriazole (HOAT) (33.07 mg, 0.243 mmol), N-methylmorpholine (74 μL, 0.675 mmol) was added to DMSO (5 ml) and reacted at room temperature overnight. After the reaction was completed, the reaction solution was poured into water and DCM for extraction. The organic phase was collected and dried in vacuo to obtain the crude product. The crude compound was purified by column chromatography eluting with MeOH/DCM to afford the desired compound **a1**. Light green solid 50 mg, yield: 79.0%.^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.78 (s, 1H), 8.93 (s, 1H), 7.99 (s, 1H), 7.95 (s, 1H), 7.91 (t, *J* = 5.8 Hz, 1H), 7.51 (d, *J* = 8.9 Hz, 1H), 7.15 (q, *J* = 8.2, 7.5 Hz, 2H), 7.04 (d, *J* = 8.9 Hz, 1H), 7.00 (d, *J* = 7.2 Hz, 2H), 6.01 (d, *J* = 4.3 Hz, 2H), 5.77 (s, 1H), 5.47 (q, *J* = 7.0 Hz, 1H), 5.19 (dd, *J* = 7.6, 4.2 Hz, 1H), 4.52 (d, *J* = 5.7 Hz, 2H), 2.22 (s, 3H), 1.99 (s, 3H), 1.78 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.60, 164.56, 164.02, 149.98, 142.99, 141.43, 134.00, 132.74, 131.04, 130.68, 128.8 (2C), 127.85, 126.16, 125.83 (2C), 124.85, 122.26, 116.68, 111.57, 111.36, 111.27, 110.14, 55.69, 35.87, 21.76, 19.53, 18.52. HRMS *m/z* calculated for $C_{28}H_{28}N_4NaO_3$ $[M + Na]^+$ 491.2059, found 491.2063. t_R (HPLC) = 2.12 min; Purity > 95%.

The representative procedure for the preparation of pyrazole derivatives.

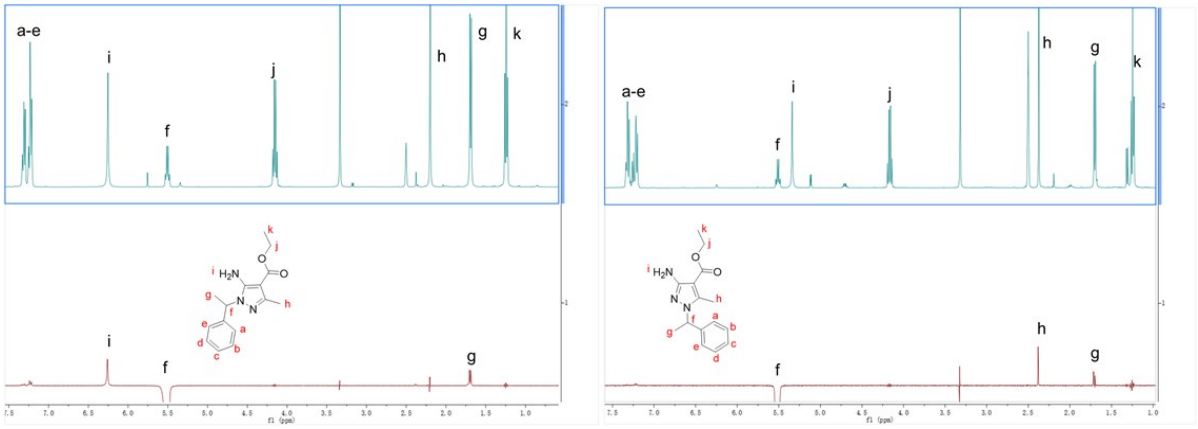
The representative procedure for the preparation of 8a-8f.



8b

ethyl 3-amino-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylate (8b). To a solution of ethyl 5-amino-3-methyl-1*H*-pyrazole-4-carboxylate (338.36 mg, 2 mmol) in DMF (15 mL) was added (1-bromoethyl) benzene (370 μM, 2.4 mmol) and cesium carbonate (1303.28 mg, 4 mmol). The mixture was stirred at 20 °C and stirred for 4 h. After the reaction was completed (monitored by TLC), water / dichloromethane was added for extraction. Dry over anhydrous sodium sulfate, filtered, and column chromatography to to give ethyl 5-amino-3-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylate (Light yellow oily liquid, 240 mg, 43.9%) and ethyl 3-amino-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylate (White solid, 210 mg, 38.4%, desired). The isomers were distinguished

by H-H NOESY spectrum. The spectrums of these compounds were shown below.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33 – 7.29 (m, 2H), 7.25 (d, *J* = 7.2 Hz, 1H), 7.21 (dd, *J* = 7.0, 1.6 Hz, 2H), 5.51 (q, *J* = 6.8 Hz, 1H), 5.34 (s, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.37 (s, 3H), 1.70 (d, *J* = 6.9 Hz, 3H), 1.25 (t, *J* = 7.1 Hz, 3H).



ethyl 3-amino-1-benzyl-5-methyl-1*H*-pyrazole-4-carboxylate (8a). White solid, yield: 41.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.35 – 7.22 (m, 3H), 7.14 – 7.10 (m, 2H), 5.28 (s, 2H), 5.09 (s, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 2.38 (s, 3H), 1.24 (t, *J* = 7.1 Hz, 3H).

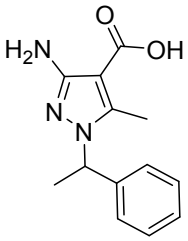
ethyl 1-([1,1'-biphenyl]-4-ylmethyl)-3-amino-5-methyl-1*H*-pyrazole-4-carboxylate (8c). Light yellow solid, yield: 38.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 – 7.58 (m, 4H), 7.44 (dd, *J* = 8.4, 6.9 Hz, 2H), 7.37 – 7.31 (m, 1H), 7.22 (d, *J* = 8.3 Hz, 2H), 5.30 (s, 2H), 5.13 (s, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.41 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H).

ethyl (S)-3-amino-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylate (8d). Using (R)-(1-bromoethyl)benzene (obtained following the reference procedure[2]) as raw material, a similar reaction step as compound **8b** was performed to obtain the target compound. White solid, yield: 34.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (dd, *J* = 8.1, 6.5 Hz, 2H), 7.27 – 7.24 (m, 1H), 7.23 – 7.18 (m, 2H), 5.51 (q, *J* = 6.9 Hz, 1H), 5.33 (s, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.37 (s, 3H), 1.70 (d, *J* = 6.8 Hz, 3H), 1.25 (t, *J* = 7.1 Hz, 3H).

ethyl (R)-3-amino-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylate (8e). Using (S)-(1-bromoethyl)benzene (obtained following the reference procedure[2]) as raw material, a similar reaction step as compound **8b** was performed to obtain the target compound. White solid, yield: 38.1%.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (t, *J* = 7.4 Hz, 2H), 7.27 – 7.24 (m, 1H), 7.24 – 7.20 (m, 2H), 5.51 (q, *J* = 6.9 Hz, 1H), 5.33 (s, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.38 (s, 3H), 1.71 (d, *J* = 6.9 Hz, 3H), 1.25 (t, *J* = 7.0 Hz, 3H).

ethyl 3-amino-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylate (8f). White solid, yield: 56.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.36 – 7.31 (m, 2H), 7.29 – 7.24 (m, 3H), 5.40 – 5.28 (m, 3H), 4.16 (q, *J* = 7.1 Hz, 2H), 1.73 (d, *J* = 7.0 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H).

The representative procedure for the preparation of 9a-9f.



9b

3-amino-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylic acid (9b). 3-amino-5-methyl-1- (1-phenylethyl) -1*H*-pyrazole-4-carboxylic acid ethyl ester (210 mg, 0.77 mmol) and sodium hydroxide (92.4 mg, 2.31 mmol) were added to methanol / water mixed solution (10 ml / 10 ml). The solution was reacted at 80 °C for 16 h. After the reaction was completed (monitored by TLC), the reaction solution was concentrated under vacuum. Added water and DCM for extraction, adjusted the pH to 3 ~ 4, and collected the organic phase. Concentrated and dried in vacuo to obtain the target compound. Light yellow solid 151.2 mg, yield: 80.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (dd, *J* = 8.1, 6.6 Hz, 2H), 7.25 (d, *J* = 7.3 Hz, 1H), 7.23 – 7.18 (m, 2H), 5.49 (q, *J* = 6.8 Hz, 1H), 2.36 (s, 3H), 1.70 (d, *J* = 6.9 Hz, 3H).

3-amino-1-benzyl-5-methyl-1*H*-pyrazole-4-carboxylic acid (9a). Light yellow solid, yield: 85.3%.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39 – 7.24 (m, 3H), 7.19 – 7.10 (m, 2H), 5.10 (s, 2H), 2.38 (s, 3H).

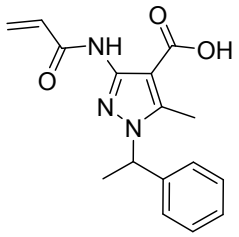
1-([1,1'-biphenyl]-4-ylmethyl)-3-amino-5-methyl-1*H*-pyrazole-4-carboxylic acid (9c). Light yellow solid, yield: 88.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66 – 7.60 (m, 4H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.38 – 7.32 (m, 1H), 7.26 – 7.22 (m, 2H), 5.13 (s, 2H), 2.41 (s, 3H).

(S)-3-amino-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylic acid (9d). White solid, yield: 99.2%.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (dd, *J* = 8.1, 6.5 Hz, 2H), 7.27 – 7.24 (m, 1H), 7.23 – 7.19 (m, 2H), 5.49 (q, *J* = 6.9 Hz, 1H), 2.36 (s, 3H), 1.70 (d, *J* = 6.9 Hz, 3H).

(R)-3-amino-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylic acid (9e). White solid, yield: 95.9%.^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (dd, *J* = 8.1, 6.6 Hz, 2H), 7.25 (d, *J* = 7.2 Hz, 1H), 7.22 – 7.18 (m, 2H), 5.48 (q, *J* = 6.9 Hz, 1H), 2.35 (s, 3H), 1.70 (d, *J* = 6.8 Hz, 3H).

3-amino-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylic acid (9f). Light yellow solid, yield: 99.1%.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.92 (s, 1H), 8.00 (s, 1H), 7.36 – 7.30 (m, 2H), 7.29 – 7.24 (m, 3H), 5.35 (q, *J* = 7.0 Hz, 1H), 1.72 (d, *J* = 7.0 Hz, 3H).

The representative procedure for the preparation of 10a-10f.



10b

3-acrylamido-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylic acid (10b). Added 3-amino-5-methyl-1- (1-phenylethyl) - 1*H*-pyrazole-4-carboxylic acid (80 mg, 0.326 mmol) and potassium carbonate (135.17 mg, 0.978 mmol) to tetrahydrofuran. Acryloyl chloride (52 μL, 0.65 mmol) was added dropwise at 0 °C. The reaction solution was reacted at room temperature for 4 hours. After completion (monitored by TLC), the reaction solution was concentrated under vacuum. Add water and ethyl acetate for extraction, adjust the pH to 4 ~ 5, and collect the organic phase.The crude compound was purified by column chromatography eluting with MeOH/DCM to afford the desired compound. Light yellow solid 67 mg, yield: 68.7%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.19 (s, 1H), 7.32 (t, *J* = 7.2 Hz, 2H), 7.28 – 7.22 (m, 3H), 6.49 (d, *J* = 16.0 Hz, 1H), 5.81 (d, *J* = 11.1 Hz, 1H), 5.66 – 5.60 (m, 1H) 5.45 (q, *J* = 7.0 Hz, 1H), 2.42 (s, 3H), 1.94 (d, *J* = 7.0 Hz, 3H).

3-acrylamido-1-benzyl-5-methyl-1*H*-pyrazole-4-carboxylic acid (10a). Light yellow solid, yield: 45.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H), 10.10 (s, 1H), 7.38 – 7.26 (m, 3H), 7.15 (dd, *J* = 7.1, 1.6 Hz, 2H), 6.08 (dd, *J* = 17.3, 10.2 Hz, 1H), 5.86 (dd, *J* = 10.3, 1.8 Hz, 1H), 5.72 (dd, *J* = 10.2, 1.9 Hz, 1H), 5.29 (s, 2H), 2.44 (s, 3H).

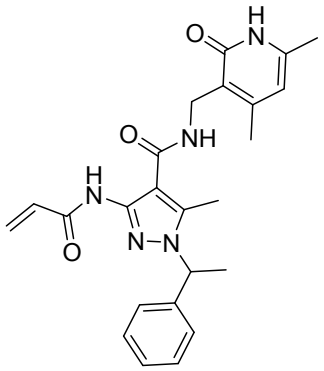
1-([1,1'-biphenyl]-4-ylmethyl)-3-acrylamido-5-methyl-1*H*-pyrazole-4-carboxylic acid (10c). White solid, yield: 71.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (s, 1H), 9.89 (s, 1H), 7.65 (dd, *J* = 7.9, 2.2 Hz, 4H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 1H), 7.26 (dd, *J* = 8.3, 1.9 Hz, 2H), 6.52 – 6.40 (m, 1H), 6.19 (dd, *J* = 17.1, 1.9 Hz, 1H), 5.73 (dd, *J* = 10.2, 1.9 Hz, 1H), 5.35 (d, *J* = 2.9 Hz, 2H), 2.49 (s, 3H).

(S)-3-acrylamido-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylic acid (10d). Light yellow solid, yield: 57.0%.^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 10.18 (s, 1H), 7.41 – 7.25 (m, 5H), 6.45 (dd, *J* = 17.4, 10.0 Hz, 1H), 6.41 – 6.33 (m, 1H), 5.96 (dd, *J* = 10.0, 1.9 Hz, 1H), 5.86 (q, *J* = 6.9 Hz, 1H), 2.58 (s, 3H), 1.83 (d, *J* = 6.9 Hz, 3H).

(R)-3-acrylamido-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylic acid (10e). Light yellow solid, yield: 61.2%.^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 10.49 (s, 1H), 7.33 (dd, *J* = 8.1, 6.5 Hz, 2H), 7.28 – 7.24 (m, 1H), 7.21 (dd, *J* = 7.0, 1.7 Hz, 2H), 6.51 – 6.35 (m, 1H), 6.19 (d, *J* = 16.9 Hz, 1H), 5.72 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.62 (q, *J* = 6.8 Hz, 1H), 2.41 (s, 3H), 1.76 (d, *J* = 6.9 Hz, 3H).

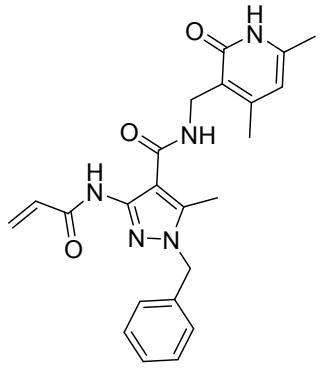
3-acrylamido-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxylic acid (10f). Light yellow solid, yield: 57.5%.^{1}H NMR (400 MHz, DMSO-d_6) δ 12.04 (s, 1H), 11.17 (s, 1H), 7.73 (s, 1H), 7.33 (t, *J* = 7.3 Hz, 2H), 7.29 – 7.20 (m, 3H), 6.29 – 6.09 (m, 2H), 5.73 (d, *J* = 10.1 Hz, 1H), 5.47 (q, *J* = 7.1 Hz, 1H), 1.75 (d, *J* = 7.1 Hz, 3H).

The representative procedure for the preparation of (b1-b15, (S)-b4, (R)-b4, b1', b4').



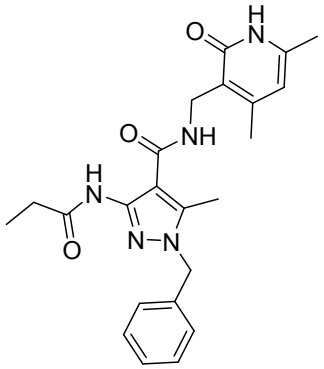
b4

3-acrylamido-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide (b4,SKLB-0335). Compound **10** (67 mg, 0.224 mmol), 3-(aminomethyl)-4,6-dimethylpyridin-2(1*H*)-one (68.13 mg, 0.448 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (77.29 mg, 0.403 mmol), 1-hydroxy-7-azobenzotriazole (HOAT) (54.88 mg, 0.403 mmol), N-methylmorpholine (123 μL, 1.12 mmol) was added to DMSO (5 ml) and reacted at room temperature overnight. After the reaction was completed, the reaction solution was poured into water and DCM for extraction. The organic phase was collected and dried in vacuo to obtain the crude product. The crude compound was purified by column chromatography eluting with MeOH/DCM to afford the desired compound **b4**. Light yellow solid 28 mg, yield: 28.8%.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 10.10 (s, 1H), 7.32 (dd, *J* = 8.1, 6.5 Hz, 2H), 7.26 (d, *J* = 7.2 Hz, 1H), 7.22 – 7.15 (m, 3H), 6.32 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.06 (d, *J* = 17.0 Hz, 1H), 5.80 (s, 1H), 5.68 – 5.58 (m, 2H), 4.15 (d, *J* = 2.2 Hz, 2H), 2.35 (s, 3H), 2.09 (d, *J* = 1.7 Hz, 6H), 1.74 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.37, 163.91, 151.14, 143.88, 142.53, 141.34, 140.11, 130.52, 128.70 (2C), 127.76, 127.64, 125.93 (2C), 125.82, 121.92, 110.08, 107.64, 58.21, 35.16, 21.21, 19.41, 18.48, 10.85. HRMS *m/z* calculated for $C_{24}H_{27}N_5NaO_3$ $[M + Na]^+$ 456.2012, found 456.2014. t_R (HPLC) = 1.91 min; Purity > 97%.



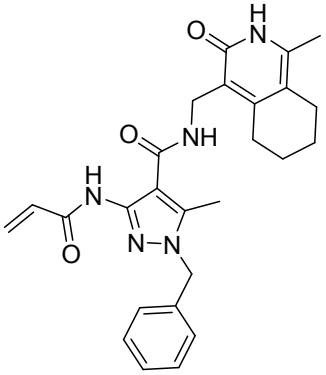
b1

3-acrylamido-1-benzyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1*H*-pyrazole-4-carboxamide (b1). Light yellow solid 32 mg, yield: 40.2%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.21 (s, 1H), 10.11 (s, 1H), 7.40 (s, 1H), 7.27 (d, *J* = 7.8 Hz, 3H), 7.14 – 7.06 (m, 2H), 6.38 (s, 2H), 5.92 (s, 1H), 5.73 – 5.67 (m, 1H), 5.30 (s, 2H), 4.46 (d, *J* = 5.7 Hz, 2H), 2.36 (s, 3H), 2.34 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 168.54, 168.36, 167.84, 155.01, 149.20, 146.65, 143.71, 139.79, 134.67, 132.71 (2C), 131.78, 130.70, 130.62 (2C), 125.72, 114.11, 110.04, 56.98, 39.22, 23.30, 22.33, 14.97. HRMS *m/z* calculated for $C_{23}H_{25}N_5NaO_3$ $[M + Na]^+$ 442,1855, found 442.1858. t_R (HPLC) = 1.89 min; Purity > 95%.



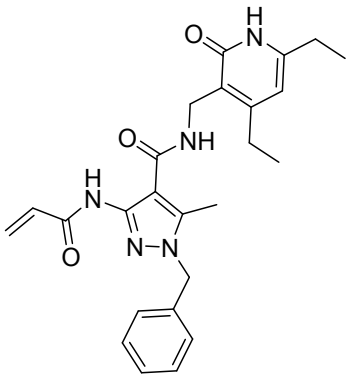
b1'

1-benzyl-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-3-propionamido-1*H*-pyrazole-4-carboxamide (b1'). Using propionyl chloride as raw material, a similar reaction step as compound **b1** was performed to obtain the target compound **b1'**. Light yellow solid 46 mg, yield: 30.8% (the last step). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 9.71 (s, 1H), 7.37 – 7.32 (m, 2H), 7.29 (d, *J* = 7.1 Hz, 1H), 7.14 (d, *J* = 7.2 Hz, 2H), 5.84 (s, 1H), 5.25 (s, 2H), 4.18 (d, *J* = 5.3 Hz, 2H), 2.40 (s, 3H), 2.28 – 2.21 (m, 2H), 2.14 (s, 3H), 2.10 (s, 3H), 0.93 (t, *J* = 7.5 Hz, 3H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.51, 163.87, 150.93, 145.40, 142.61, 139.52, 135.86, 128.78, 128.70 (2C), 127.76, 126.71, 126.62 (2C), 121.84, 110.07, 52.98, 35.19, 30.03, 19.36, 18.39, 11.05, 9.26. HRMS *m/z* calculated for $C_{23}H_{27}N_5NaO_3$ $[M + Na]^+$ 444.2012, found 444.2009. t_R (HPLC) = 1.92 min; Purity > 99%.



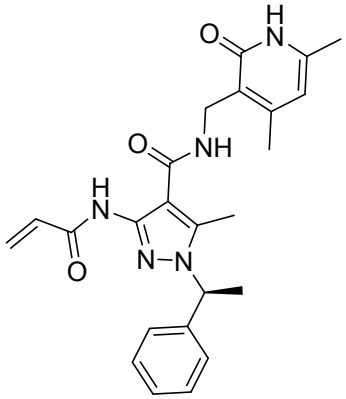
b2

3-acrylamido-1-benzyl-5-methyl-*N*-((1-methyl-3-oxo-2,3,5,6,7,8-hexahydroisoquinolin-4-yl)methyl)-1*H*-pyrazole-4-carboxamide (b2). Light yellow solid 23 mg, yield: 28.4%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.54 (s, 1H), 10.21 (s, 1H), 7.62 (s, 1H), 7.36 – 7.19 (m, 3H), 7.10 (d, *J* = 7.2 Hz, 2H), 6.47 – 6.32 (m, 1H), 5.73 – 5.64 (m, 1H), 5.29 (s, 2H), 5.17 – 5.04 (m, 1H), 4.49 (d, *J* = 5.6 Hz, 2H), 3.48 (s, 3H), 2.42 (s, 4H), 2.17 (d, *J* = 3.7 Hz, 3H), 1.73 (d, *J* = 6.3 Hz, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.88, 163.50, 162.77, 151.59, 140.35, 135.88, 130.87, 128.84, 128.74 (2C), 127.79, 127.66, 126.75, 126.68 (2C), 126.47, 121.18, 115.23, 53.07, 34.82, 27.22, 24.89, 22.13, 22.06, 16.42, 11.12. HRMS *m/z* calculated for $C_{26}H_{29}N_5NaO_3$ $[M + Na]^+$ 482.2168, found 482.2168. t_R (HPLC) = 2.04 min; Purity > 97%.



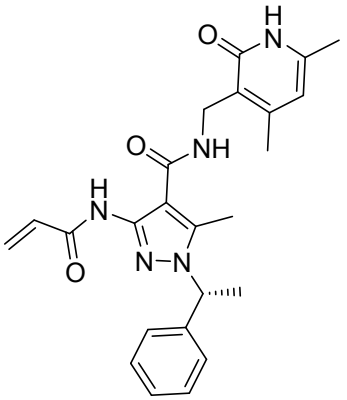
b3

3-acrylamido-1-benzyl-*N*-((4,6-diethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1*H*-pyrazole-4-carboxamide (b3). Light yellow solid 29 mg, yield: 34.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 10.04 (s, 1H), 7.44 (s, 1H), 7.37 – 7.31 (m, 2H), 7.30 – 7.27 (m, 1H), 7.17 – 7.11 (m, 2H), 6.31 (dd, *J* = 17.3, 10.0 Hz, 1H), 6.05 (d, *J* = 17.0 Hz, 1H), 5.87 (s, 1H), 5.64 (dd, *J* = 10.2, 1.9 Hz, 1H), 5.26 (s, 2H), 4.18 (d, *J* = 5.1 Hz, 2H), 2.46 (q, *J* = 7.5 Hz, 2H), 2.40 (q, *J* = 6.2 Hz, 5H), 1.13 (t, *J* = 7.6 Hz, 3H), 1.07 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.02, 164.85, 163.80, 156.45, 148.53, 145.71, 139.17, 135.88, 130.89, 128.73 (2C), 127.78, 127.64, 126.67 (2C), 126.46, 121.25, 106.76, 53.08, 34.88, 26.38, 25.96, 14.48, 12.54, 11.15. HRMS *m/z* calculated for $C_{25}H_{29}N_5NaO_3$ $[M + Na]^+$ 470.2168, found 470.2166. t_R (HPLC) = 2.10 min; Purity > 95%.



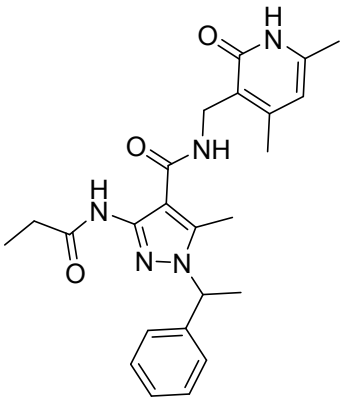
(S)-b4

(S)-3-acrylamido-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide ((S)-b4). Light yellow solid 24 mg, yield: 22.1%.^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.18 (s, 1H), 9.64 (s, 1H), 7.25 (d, *J* = 2.8 Hz, 1H), 7.18 (dd, *J* = 14.5, 6.7 Hz, 2H), 7.07 (d, *J* = 7.5 Hz, 2H), 6.32 – 6.20 (m, 2H), 5.87 (s, 1H), 5.64 – 5.57 (m, 1H), 5.36 (q, *J* = 7.2 Hz, 1H), 4.32 (s, 2H), 2.27 (s, 3H), 2.24 (s, 3H), 2.13 (s, 3H), 1.80 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.37, 163.87, 151.17, 143.84, 142.53, 141.33, 140.02, 130.58, 128.70 (2C), 127.64, 127.57, 125.92 (2C), 125.86, 121.89, 110.10, 107.56, 58.21, 35.13, 21.20, 19.41, 18.46, 10.85.HRMS *m/z* calculated for $C_{24}H_{27}N_5NaO_3$ $[M + Na]^+$ 456.2012, found 456.2007. t_R (HPLC) = 1.96 min; Purity > 98%.



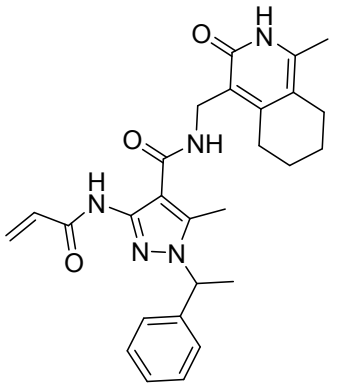
(R)-b4

(R)-3-acrylamido-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide ((R)-b4). Light yellow solid 29 mg, yield: 25.3%.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.39 (s, 1H), 10.09 (s, 1H), 7.37 – 7.29 (m, 2H), 7.28 – 7.25 (m, 1H), 7.21 – 7.17 (m, 2H), 6.32 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.06 (d, *J* = 17.1 Hz, 1H), 5.80 (s, 1H), 5.69 – 5.60 (m, 2H), 4.15 (d, *J* = 2.1 Hz, 2H), 2.35 (s, 3H), 2.09 (d, *J* = 1.6 Hz, 6H), 1.74 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.44, 163.90, 151.19, 143.80, 142.63, 141.33, 140.05, 130.54, 128.70 (2C), 127.80, 127.64, 125.92 (2C), 125.84, 121.85, 110.11, 107.74, 58.19, 35.12, 21.21, 19.42, 18.46, 10.85. HRMS *m/z* calculated for $C_{24}H_{27}N_5NaO_3$ $[M + Na]^+$ 456.2012, found 456.2015. t_R (HPLC) = 1.96 min; Purity > 98%.



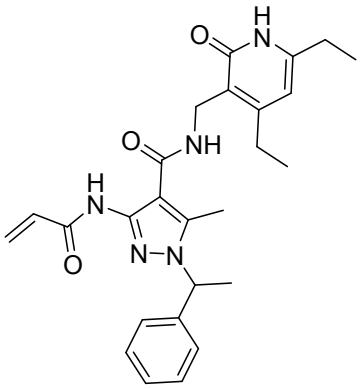
b4'

***N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-(1-phenylethyl)-3-propionamido-1*H*-pyrazole-4-carbox-amide (b4').** Using propionyl chloride as raw material, a similar reaction step as compound **b4** was performed to obtain the target compound **b4'**. Light yellow solid 35 mg, yield: 39.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 9.76 (s, 1H), 7.32 (ddd, *J* = 7.6, 6.4, 1.5 Hz, 3H), 7.28 – 7.22 (m, 1H), 7.20 – 7.15 (m, 2H), 5.84 (s, 1H), 5.63 (q, *J* = 6.8 Hz, 1H), 4.17 (t, *J* = 5.8 Hz, 2H), 2.36 (s, 3H), 2.25 (q, *J* = 7.5 Hz, 2H), 2.13 (s, 3H), 2.12 – 2.08 (m, 3H), 1.73 (d, *J* = 6.8 Hz, 3H), 0.93 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.42, 163.84, 151.11, 143.98, 142.50, 141.39, 140.25, 128.69 (2C), 127.62, 125.90 (2C), 122.03, 121.97,110.03, 107.47, 58.12, 35.10, 29.76, 21.20, 19.41, 18.48, 10.86, 9.10. HRMS *m/z* calculated for $C_{24}H_{29}N_5NaO_3$ $[M + Na]^+$ 458.2168, found 458.2169. t_R (HPLC) = 1.99 min; Purity > 99%.



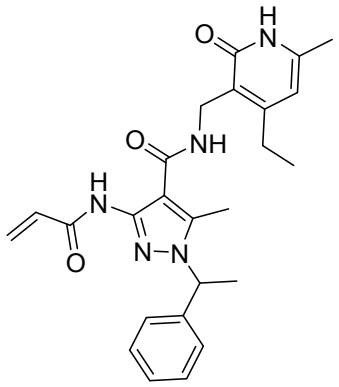
b5

3-acrylamido-5-methyl-*N*-((1-methyl-3-oxo-2,3,5,6,7,8-hexahydroisoquinolin-4-yl)methyl)-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide (b5). Light yellow solid 21 mg, yield: 24.6%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.22 (s, 1H), 11.25 (s, 1H), 9.74 (s, 1H), 7.28 (d, *J* = 8.8 Hz, 3H), 7.19 (d, *J* = 7.5 Hz, 2H), 6.39 (s, 1H), 5.68 (dd, *J* = 8.5, 3.5 Hz, 1H), 5.42 (q, *J* = 7.0 Hz, 1H), 4.49 (d, *J* = 5.2 Hz, 2H), 2.61 (s, 3H), 2.42 (t, *J* = 6.2 Hz, 4H), 2.15 (s, 3H), 1.89 (d, *J* = 6.9 Hz, 3H), 1.77 – 1.66 (m, 2H), 1.61 (d, *J* = 9.6 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.00, 162.66, 161.27, 151.76, 144.30, 141.41, 140.12, 130.77, 128.70 (2C), 127.63, 125.96 (2C), 121.34, 115.16, 58.28, 34.68, 27.18, 24.92, 22.12, 22.05, 21.26, 16.44, 10.91.HRMS *m/z* calculated for $C_{27}H_{31}N_5NaO_3$ $[M + Na]^+$ 496.2325, found 496.2327. t_R (HPLC) = 2.14 min; Purity > 95%.



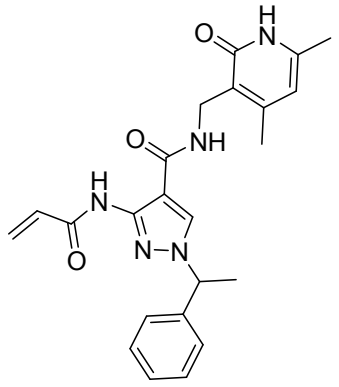
b6

3-acrylamido-*N*-((4,6-diethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide (b6). Light yellow solid 31 mg, yield: 29.3%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.91 (s, 1H), 9.77 (s, 1H), 7.41 (s, 1H), 7.31 – 7.25 (m, 2H), 7.23 (d, *J* = 7.2 Hz, 1H), 7.18 – 7.13 (m, 2H), 6.41 – 6.30 (m, 1H), 5.96 (s, 1H), 5.72 – 5.56 (m, 1H), 5.41 (q, *J* = 6.9 Hz, 1H), 4.49 – 4.41 (m, 2H), 2.68 (q, *J* = 7.8 Hz, 2H), 2.50 (q, *J* = 7.6 Hz, 2H), 2.33 (s, 3H), 1.89 (d, *J* = 7.1 Hz, 3H), 1.20 (q, *J* = 7.5 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.35, 163.78, 163.13, 156.15, 148.71, 145.16, 141.57, 138.46, 131.15, 129.91, 128.74 (2C), 127.63, 126.00 (2C), 121.43, 106.50, 106.36, 58.34, 34.97, 26.42, 26.08, 21.49, 14.58, 12.68, 11.15. HRMS *m/z* calculated for $C_{26}H_{31}N_5NaO_3$ $[M + Na]^+$ 484.2325, found 484.2328. t_R (HPLC) = 2.20 min; Purity > 98%.



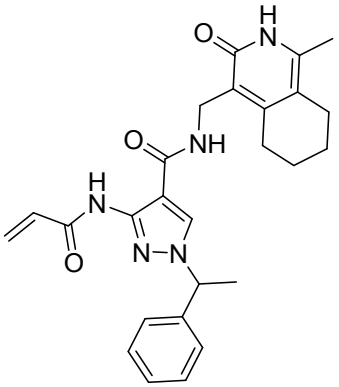
b7

3-acrylamido-*N*-((4-ethyl-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide (b7). Light yellow solid 23 mg, yield: 22.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 10.09 (s, 1H), 7.40 (s, 1H), 7.35 – 7.30 (m, 2H), 7.28 – 7.24 (m, 1H), 7.21 – 7.17 (m, 2H), 6.32 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.05 (d, *J* = 17.2 Hz, 1H), 5.84 (s, 1H), 5.63 (dd, *J* = 4.4, 2.5 Hz, 1H), 4.16 (dd, *J* = 5.0, 1.9 Hz, 2H), 2.44 (q, *J* = 7.6 Hz, 2H), 2.35 (s, 3H), 2.14 – 2.08 (m, 3H), 1.74 (d, *J* = 6.8 Hz, 3H), 1.05 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.25, 163.74, 160.68, 156.20, 145.03, 144.96, 143.10, 141.53, 138.80, 131.08, 128.74 (2C), 127.64, 126.00 (2C), 121.23, 108.14, 106.46, 58.28, 34.89, 26.25, 21.47, 18.76, 14.48, 11.13. HRMS *m/z* calculated for $C_{25}H_{29}N_5NaO_3$ $[M + Na]^+$ 470.2168, found 470.2168. t_R (HPLC) = 2.06 min; Purity > 99%.



b8

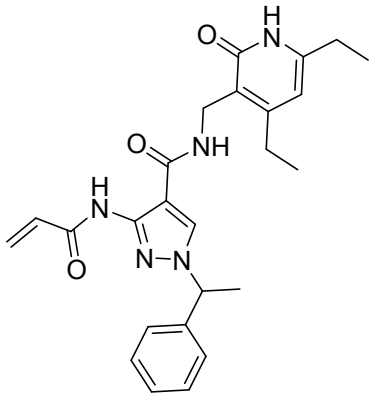
3-acrylamido-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide (b8). Light yellow solid 26 mg, yield: 20.4%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.00 (s, 1H), 10.23 (s, 1H), 7.82 (s, 1H), 7.57 (t, *J* = 5.5 Hz, 1H), 7.19 (dd, *J* = 5.5, 1.9 Hz, 3H), 7.11 (dd, *J* = 7.0, 2.7 Hz, 2H), 6.39 (d, *J* = 16.4 Hz, 1H), 5.90 (s, 1H), 5.76 – 5.64 (m, 1H), 5.40 (d, *J* = 7.0 Hz, 1H), 4.42 (d, *J* = 5.4 Hz, 2H), 2.30 (s, 3H), 2.16 (s, 3H), 1.69 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 164.91, 164.06, 161.78, 151.96, 147.55, 143.27, 140.72, 131.39, 128.59 (2C), 128.25, 127.88, 127.60, 126.52 (2C), 121.40, 110.10, 103.70, 61.54, 34.77, 21.17, 19.63, 18.57. HRMS *m/z* calculated for $C_{23}H_{25}N_5NaO_3$ $[M + Na]^+$ 442.1855, found 442.1851. t_R (HPLC) = 1.98 min; Purity > 99%.



b9

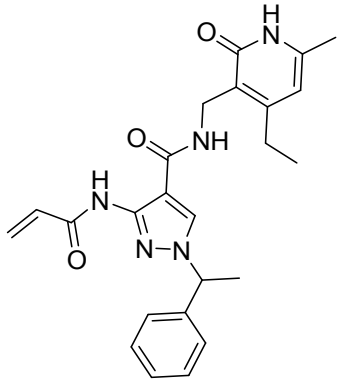
3-acrylamido-*N*-((1-methyl-3-oxo-2,3,5,6,7,8-hexahydroisoquinolin-4-yl)methyl)-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide (b9). Light yellow solid 19 mg, yield: 18.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 10.17 (s, 1H), 8.35 (s, 1H),

7.71 (s, 1H), 7.34 (dd, *J* = 8.1, 6.4 Hz, 2H), 7.30 – 7.27 (m, 1H), 7.26 – 7.22 (m, 2H), 6.14 (d, *J* = 16.9 Hz, 1H), 5.72 (dd, *J* = 10.2, 1.9 Hz, 1H), 5.52 (d, *J* = 7.0 Hz, 1H), 4.23 (d, *J* = 4.3 Hz, 2H), 2.65 (d, *J* = 6.6 Hz, 2H), 2.35 (d, *J* = 6.2 Hz, 2H), 2.10 (s, 3H), 1.76 (d, *J* = 7.1 Hz, 3H), 1.61 (t, *J* = 3.6 Hz, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.65, 162.92, 162.44, 152.41, 147.20, 147.11, 140.71, 140.46, 131.12, 128.67 (2C), 128.01, 127.73, 126.55 (2C), 121.21, 115.51, 104.44, 61.54, 34.23, 27.27, 24.88, 22.12, 22.05, 20.97, 16.45. HRMS *m/z* calculated for $C_{26}H_{29}N_5NaO_3$ [M + Na]$^+$ 470.2168, found 482.2170. t_R (HPLC) = 2.18 min; Purity > 99%.



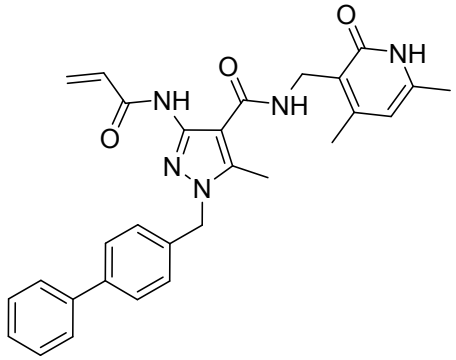
b10

3-acrylamido-*N*-((4,6-diethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide (b10). Light yellow solid 30 mg, yield: 27.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.45 (s, 1H), 10.17 (s, 1H), 8.37 (s, 1H), 7.82 (s, 1H), 7.34 (ddd, *J* = 7.7, 6.2, 1.6 Hz, 2H), 7.31 – 7.27 (m, 1H), 7.26 – 7.21 (m, 2H), 6.15 (d, *J* = 17.0 Hz, 1H), 5.90 (s, 1H), 5.72 (dd, *J* = 10.2, 1.9 Hz, 1H), 5.52 (q, *J* = 7.0 Hz, 1H), 4.22 (d, *J* = 4.8 Hz, 2H), 2.47 (q, *J* = 7.6 Hz, 2H), 2.41 (q, *J* = 7.6 Hz, 2H), 1.76 (d, *J* = 7.1 Hz, 3H), 1.13 (t, *J* = 7.5 Hz, 3H), 1.07 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.23, 163.78, 162.04, 157.14, 148.92, 147.56, 140.60, 131.38, 128.65 (2C), 127.97, 127.58, 127.44, 126.57 (2C), 121.03, 106.66, 103.98, 61.56, 34.50, 26.40, 26.10, 21.17, 14.53, 12.62. HRMS *m/z* calculated for $C_{25}H_{29}N_5NaO_3$ [M + Na]$^+$ 470.2168, found 470.2171. t_R (HPLC) = 2.22 min; Purity > 99%.



b11

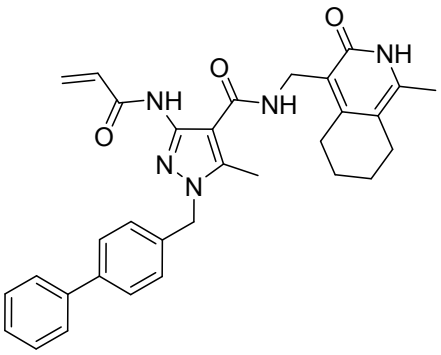
3-acrylamido-*N*-((4-ethyl-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-1-(1-phenylethyl)-1*H*-pyrazole-4-carboxamide (b11). Light yellow solid 25 mg, yield: 21.6%.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 10.16 (s, 1H), 8.36 (s, 1H), 7.80 (s, 1H), 7.34 (dd, *J* = 8.1, 6.5 Hz, 2H), 7.29 (d, *J* = 6.9 Hz, 1H), 7.26 – 7.21 (m, 2H), 6.15 (d, *J* = 16.9 Hz, 1H), 5.88 (s, 1H), 5.72 (dd, *J* = 10.1, 1.9 Hz, 1H), 5.52 (q, *J* = 7.0 Hz, 1H), 4.22 (d, *J* = 5.0 Hz, 2H), 2.49 – 2.44 (m, 2H), 2.12 (s, 3H), 1.76 (d, *J* = 7.0 Hz, 3H), 1.06 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.13, 163.91, 161.82, 157.25, 147.52, 143.76, 140.76, 131.41, 128.60, 128.54 (2C), 127.82, 127.45, 126.44 (2C), 120.54, 108.20, 103.80, 61.50, 34.33, 26.21, 21.18, 18.67, 14.44. HRMS *m/z* calculated for $C_{24}H_{27}N_5NaO_3$ [M + Na]$^+$ 456.2012, found 456.2008. t_R (HPLC) = 2.08 min; Purity > 95%.



b12

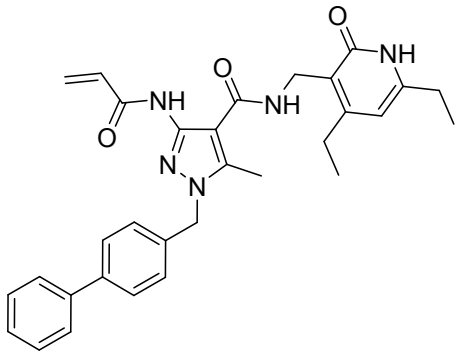
1-([1,1'-biphenyl]-4-ylmethyl)-3-acrylamido-*N*-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1*H*-pyrazole-4-carboxamide (b12). Light yellow solid 34 mg, yield: 30.2%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.11 (s, 1H), 7.52 (dd, *J* = 11.4,

7.9 Hz, 4H), 7.42 (t, *J* = 7.5 Hz, 3H), 7.34 (d, *J* = 7.2 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 2H), 6.40 (s, 1H), 5.89 (s, 1H), 5.77 – 5.64 (m, 1H), 5.32 (d, *J* = 19.2 Hz, 3H), 4.46 (d, *J* = 5.8 Hz, 2H), 2.41 (s, 3H), 2.34 (s, 3H), 2.19 (s, 3H). HRMS *m/z* calculated for $C_{29}H_{29}N_5NaO_3$ [M + Na]$^+$ 518.2168, found 518.2164. t_R (HPLC) = 2.23 min; Purity > 95%.



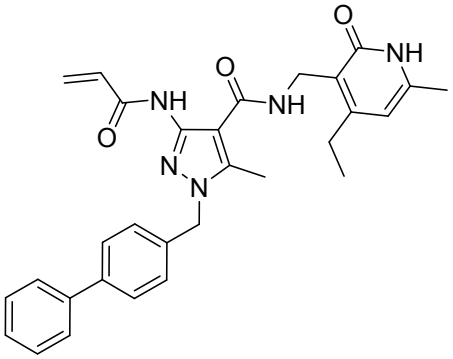
b13

1-([1,1'-biphenyl]-4-ylmethyl)-3-acrylamido-5-methyl-*N*-((1-methyl-3-oxo-2,3,5,6,7,8-hexahydroisoquinolin-4-yl)methyl)-1*H*-pyrazole-4-carboxamide (b13). Light yellow solid 27 mg, yield: 24.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 10.06 (s, 1H), 7.63 (d, *J* = 7.9 Hz, 4H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.37 (d, *J* = 7.4 Hz, 1H), 7.34 (s, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.30 (dd, *J* = 17.0, 10.3 Hz, 1H), 6.03 (d, *J* = 17.1 Hz, 1H), 5.64 (dd, *J* = 10.2, 1.9 Hz, 1H), 5.31 (s, 2H), 4.19 (d, *J* = 5.0 Hz, 2H), 2.62 (d, *J* = 6.2 Hz, 2H), 2.43 (s, 3H), 2.33 (d, *J* = 6.3 Hz, 2H), 2.08 (s, 3H), 1.61 (q, *J* = 4.0, 3.4 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.62, 162.57, 161.96, 150.13, 142.43, 141.99, 140.85, 140.18, 140.01, 136.32, 130.93, 129.40 (2C), 128.29 (2C), 127.98, 127.62, 127.43 (2C), 127.14 (2C), 121.23, 112.07, 111.34, 52.05, 34.58, 26.90, 24.60, 22.56, 22.39, 16.39, 10.92. HRMS *m/z* calculated for $C_{32}H_{33}N_5NaO_3$ [M + Na]$^+$ 558.2481, found 558.2487. t_R (HPLC) = 2.54 min; Purity > 98%.



b14

1-([1,1'-biphenyl]-4-ylmethyl)-3-acrylamido-*N*-((4,6-diethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1*H*-pyrazole-4-carboxamide (b14). Light yellow solid 28 mg, yield: 25.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 10.06 (s, 1H), 7.63 (d, *J* = 7.8 Hz, 4H), 7.45 (t, *J* = 7.5 Hz, 3H), 7.36 (t, *J* = 7.3 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.32 (dd, *J* = 17.1, 10.3 Hz, 1H), 6.06 (d, *J* = 16.4 Hz, 1H), 5.87 (s, 1H), 5.65 (dd, *J* = 10.3, 1.9 Hz, 1H), 5.31 (s, 2H), 4.19 (d, *J* = 5.1 Hz, 2H), 2.46 (d, *J* = 7.9 Hz, 2H), 2.41 (d, *J* = 15.9 Hz, 5H), 1.13 (t, *J* = 7.6 Hz, 3H), 1.07 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.29, 163.96, 162.73, 156.02, 148.42, 140.80, 140.44, 135.05, 131.38, 128.81 (2C), 127.49 (2C), 127.48, 127.28 (2C), 127.01 (2C), 121.45, 106.66, 104.86, 53.11, 35.18, 26.51, 26.15, 14.64, 12.63, 11.54. HRMS *m/z* calculated for $C_{31}H_{33}N_5NaO_3$ [M + Na]$^+$ 546.2481, found 546.2480. t_R (HPLC) = 2.63 min; Purity > 98%.

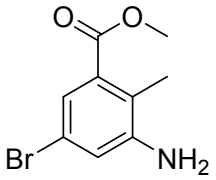


b15

1-([1,1'-biphenyl]-4-ylmethyl)-3-acrylamido-*N*-((4-ethyl-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-methyl-1*H*-pyrazole-4-carboxamide (b15). Light yellow solid 29 mg, yield: 28.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 10.06 (s, 1H),

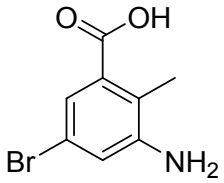
7.63 (d, *J* = 7.8 Hz, 4H), 7.45 (t, *J* = 7.5 Hz, 3H), 7.36 (t, *J* = 7.3 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.32 (dd, *J* = 17.2, 10.2 Hz, 1H), 6.06 (d, *J* = 17.3 Hz, 1H), 5.85 (s, 1H), 5.65 (dd, *J* = 10.2, 1.9 Hz, 1H), 5.31 (s, 2H), 4.18 (d, *J* = 5.1 Hz, 2H), 2.46 (m, 2H), 2.43 (s, 3H), 2.11 (s, 3H), 1.06 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.58, 163.78, 162.46, 154.78, 143.59, 142.54, 142.02, 140.18, 140.01, 136.32, 131.06, 129.40 (2C), 128.28 (2C), 127.98, 127.56, 127.43 (2C), 127.13 (2C), 121.36, 119.64, 106.08, 52.04, 34.57, 25.84, 18.75, 14.72, 10.94. HRMS *m/z* calculated for $C_{30}H_{31}N_5NaO_3$ $[M + Na]^+$ 532.2325, found 532.2327. t_R (HPLC) = 2.40 min; Purity > 97%.

The representative procedure for the preparation of pyrazole derivatives.



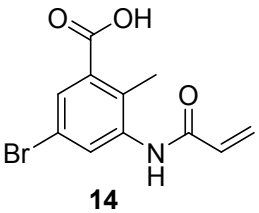
12

methyl 3-amino-5-bromo-2-methylbenzoate (12). methyl 5-bromo-2-methyl-3-nitrobenzoate (2 g, 7.30 mmol), ammonium chloride (1.95 g, 36.5 mmol) was added to a solution of methanol / water (18 ml / 6 ml). While the reaction solution was refluxing, iron powder (2.04 g, 36.5 mmol) was added, and the reaction was continued for 0.5 h. After the reaction was completed (monitored by TLC), added a layer of diatomaceous earth to filter the hot reaction liquid. The diatomaceous earth was washed twice with acetone and the filtrate was collected. The combined organic phase was dried over sodium sulfate, filtered and concentrated in vacuo to provide crude product**.** The crude product was purified by column chromatography eluting with EA/PE to to afford target compound. Orange solid 1.62 g, yield: 90.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.96 (d, *J* = 3.2 Hz, 2H), 5.43 (s, 2H), 3.79 (s, 3H), 2.11 (s, 3H).



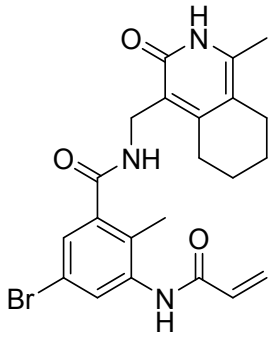
13

3-amino-5-bromo-2-methylbenzoic acid (13). Added 3-amino-5-bromo-2-methylbenzoic acid methyl ester (1.0 g, 4.13 mmol) and sodium hydroxide (0.49 g, 12.39 mmol) to the methanol / water mixed solution (20 ml / 20 ml), The reaction solution was reacted at 80 °C for 3 h. After completion (monitored by TLC), the reaction solution was concentrated under reduced pressure. Add water and ethyl acetate for extraction, adjust the pH to 3 ~ 4, and collect the organic phase. The combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated in vacuo to obtain the target compound 3-amino-5-bromo-2-methylbenzoic acid. Yellow brown solid 0.94 g, yield: 99.02%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (s, 1H), 6.95 (d, *J* = 2.2 Hz, 1H), 6.94 (d, *J* = 2.2 Hz, 1H), 5.38 (s, 2H), 2.13 (s, 3H).



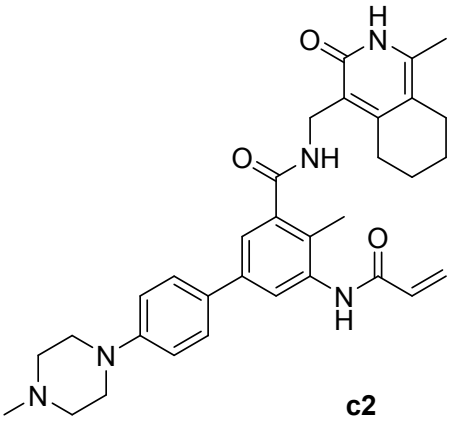
14

3-acrylamido-5-bromo-2-methylbenzoic acid (14). 3-amino-5-bromo-2-methylbenzoic acid (580 mg, 2.52 mmol) and potassium carbonate (1042.8 mg, 11.6 mmol) were added to tetrahydrofuran. Acryloyl chloride (407 μL, 5.04 mmol) was added dropwise at 0 °C. The reaction solution was reacted at room temperature for 4 h. After completion (monitored by TLC), the reaction solution was concentrated under vacuum. Add water and ethyl acetate for extraction, adjust the pH to 3 ~ 4, collect the organic phase and concentrated under vacuum to afford a solid product. The crude product was purified by silica gel column chromatography to afford target compound. White solid 646 mg, yield: 90.34%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.31 (s, 1H), 9.77 (s, 1H), 7.85 (d, *J* = 2.2 Hz, 1H), 7.69 (d, *J* = 2.2 Hz, 1H), 6.54 (dd, *J* = 17.0, 10.2 Hz, 1H), 6.28 (dd, *J* = 17.1, 2.0 Hz, 1H), 5.80 (dd, *J* = 10.2, 2.0 Hz, 1H), 2.31 (s, 3H).



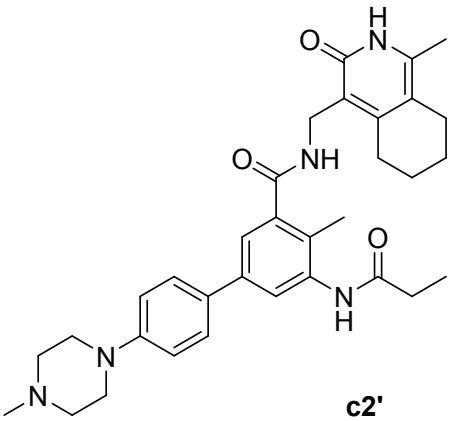
c1

3-acrylamido-5-bromo-2-methyl-*N*-((1-methyl-3-oxo-2,3,5,6,7,8-hexahydroisoquinolin-4-yl)methyl)benzamide (c1). 3-acrylamido-5-bromo-2-methylbenzoic acid (160 mg, 0.56 mmol), 4-(aminomethyl)-1-methyl-5,6,7,8-tetrahydroisoquinoline-3 (2*H*)-one (216.55 mg, 1.13 mmol, obtained following the reference procedure[3]), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (193.23 mg, 1.01 mmol), 1-hydroxy-7-azobenzotriazole (HOAT) (137.47 mg, 1.01 mmol), N-methylmorpholine (307.8 µL, 2.8 mmol) was added to DMSO (5 ml) and reacted at room temperature overnight. After the reaction was completed, the reaction solution was poured into 10 ml of ice water, and a white solid precipitated, and the crude compound was obtained by filtration and drying. Using silica gel column chromatography to afford target compound. White solid 160 mg, yield: 62.3%.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.49 (s, 1H), 9.61 (s, 1H), 8.31 (t, *J* = 4.7 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.19 (d, *J* = 2.2 Hz, 1H), 6.54 (dd, *J* = 17.1, 10.3 Hz, 1H), 6.26 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.78 (dd, *J* = 10.2, 2.0 Hz, 1H), 4.28 (d, *J* = 4.8 Hz, 2H), 2.71 (s, 2H), 2.38 (s, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 1.68-1.60 (m, 4H). HRMS *m/z* calculated for $C_{22}H_{24}BrN_3NaO_3$ $[M + Na]^+$ 480.0899, found 480.0903. t_R (HPLC) = 2.06 min; Purity > 95%.



c2

5-acrylamido-4-methyl-*N*-((1-methyl-3-oxo-2,3,5,6,7,8-hexahydroisoquinolin-4-yl)methyl)-4'-(4-methylpiperazin-1-yl)-[1,1'-biphenyl]-3-carboxamide (c2). To a stirred solution of **c1** (80 mg, 0.17 mmol) in a dioxane–water mixture (10 mL / 2 mL), 4-(4-Methyl-1-piperazinyl)benzene- boronic acid pinacol ester (79.12 mg, 0.26 mmol) was added, followed by the addition of Na_2CO_3 (73.99 mg, 0.70 mmol). The solution was purged with argon for 15 min and then $PdCl_2(dppf)\cdot CH_2Cl_2$ (12.43 mg, 0.017 mmol) was added and the solution was again purged with argon for an additional 10 min. The reaction mixture was stirred at 100 °C for 4 h. After completion (monitored by TLC), the reaction mixture was diluted with water and extracted with 5% MeOH/DCM. The combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The crude compound was purified by column chromatography eluting with MeOH/DCM to afford the desired compound **c2.** Light yellow solid 36 mg, yield: 38.2%.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 9.60 (s, 1H), 8.24 (t, *J* = 4.8 Hz, 1H), 7.68 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.28 (d, *J* = 1.9 Hz, 1H), 7.00 (d, *J* = 8.7 Hz, 2H), 6.55 (dd, *J* = 16.9, 10.2 Hz, 1H), 6.25 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.80 – 5.73 (m, 1H), 4.32 (d, *J* = 4.8 Hz, 2H), 3.18 (t, *J* = 5.1 Hz, 4H), 2.73 (s, 2H), 2.45 (t, *J* = 5.0 Hz, 4H), 2.38 (s, 2H), 2.22 (s, 3H), 2.17 (s, 3H), 2.10 (s, 3H), 1.64 (s, 4H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.32, 164.96, 162.72, 152.05, 150.43, 140.66, 138.68, 138.20, 136.48, 136.38, 130.71, 127.56, 127.39 (2C), 124.46, 124.37, 122.22, 120.88, 116.06 (2C), 115.17, 54.70 (2C), 48.47 (2C), 45.67, 35.38, 29.56, 27.30, 24.76, 22.12, 22.01, 16.32. HRMS *m/z* calculated for $C_{33}H_{39}N_5NaO_3$ $[M + Na]^+$ 576.2951, found 576.2952. t_R (HPLC) = 2.27 min; Purity > 98%.



c2'

4-methyl-*N*-((1-methyl-3-oxo-2,3,5,6,7,8-hexahydroisoquinolin-4-yl)methyl)-4'-(4-methylpiperazin-1-yl)-5-propionamido-[1,1'-biphenyl]-3-carboxamide (c2'). Using propionyl chloride as raw material, a similar reaction step as compound **c2** is performed to obtain the target compound **c2'**. Grey solid 29 mg, yield: 20.01% (the last step).^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 9.30 (s, 1H), 8.21 (t, *J* = 4.8 Hz, 1H), 7.58 (s, 1H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 2.0 Hz, 1H), 7.00 (d, *J* = 8.9 Hz, 2H), 4.32 (d, *J* = 4.8 Hz, 2H), 3.17 (t, *J* = 4.9 Hz, 4H), 2.73 (s, 2H), 2.46 (t, *J* = 5.0 Hz, 4H), 2.36 (m, 4H), 2.23 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 1.65 (d, *J* = 3.8 Hz, 4H), 1.11 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 177.89, 174.37, 166.72, 156.09, 154.33, 144.70, 142.65, 142.16, 140.58, 134.89, 131.59, 131.39 (2C), 128.54, 126.11, 124.88, 120.11 (2C), 119.20, 58.64 (2C), 52.41 (2C), 49.55, 39.37, 33.75, 33.56, 31.31, 28.77, 26.13, 26.02, 20.30, 18.05. HRMS *m/z* calculated for $C_{33}H_{41}N_5NaO_3$ $[M + Na]^+$ 578.3107, found 578.3102. t_R (HPLC) = 2.03 min; Purity > 95%.

Experimental protocols of biological assays

In vitro HMTs inhibition assay.

The enzyme levels of the histone methyltransferase panel were determined using the AlphaLISA immunodetection assay conducted using the enzyme profler service provided by Shanghai ChemPartner (Shanghai, China). Te values were further determined using an AlphaLISA methyltransferase assay kit (PerkinElmer, MA, USA) according to the manufacturer's protocol.

SAM competition inhibition.

100 μL of SKLB-0335, SKLB-0335′ or GSK126 solutions with different concentrations were added to the 384-well detection plate respectively. Two replicate wells were set for each compound concentration. Then add 5 μL of EZH2 enzyme solution (3 μM or 30 μM) to each well, centrifuge at 1000 rpm/min for 1 min and incubate for 15 min or 120 min. Add 5 μL of modified substrate, centrifuge at 1000 rpm / min for 1 min, and incubate at room temperature for 1 h. After the incubation, add 5 μL of acceptor magnetic beads to terminate the enzyme reaction. Similarly, centrifuge at 1000 rpm/min for 1 min, incubate at room temperature for 1 h, and finally add 10 μL of donor magnetic beads under dark conditions, centrifuge at 1000 rpm/min for 1 min, and incubate at room temperature for 30 min. EnSpire's Alpha mode is used to detect signal strength.

K_{off} test on EZH2.

The K_{off} of SKLB-0335 was tested using the spin column method. Prepare Buffer, enzyme mix A (100x (Enzyme-20x IC_{50} cpd. mix)), B (100x (Enzyme-DMSO mix)), preincubation 60 min, prepare substrate mix C (Substrate Mix-GL11 and ^{3}H-SAM) and min control. Take out spin column, flow by gravity, and spin 1300 rpm/min for 1 minute at 4 °C in bucket-swing centrifuge. Balance spin column with 0.7 mL 1x kinase buffer, spin the column 1300 rpm/min for 1 minute. Balance spin column with 0.5 mL 1x kinase buffer, spin the column 1300 rpm/min for 1 minute. Balance spin column with 0.4 mL 1x kinase buffer, spin the column 1300 rpm/min for 1 minute. Put the balanced spin column on new collection tubes. Add Pre-incubation A, B to spin column respectively, spin the column 1300 rpm/min for 1 minute, collect the liquid. Add 10 μL of solution to the prepare substrate mix. Add 10 μL 100x collected solution into 990 uL substrate mixture to iniate the assay. After different assay time (6min, 11min, 15min, 20min, 25min, 30min, 45min, 60min, 90min, 120min, 150min), taking out 20 μL assay product and add into 10 μL stop buffer to stop the assay. Transfer 25 μL of volume per well to Flashplate from assay plate. Incubate for 1 h minimum at room temperature. Wash Flashplate with dH_2O + 0.1% Tween-20 three times. Read plate on Microbeta. Fit the data in GraphPad Prism version 6.0.

Molecular modelling.

The 3D structure of the PRC2 complex was downloaded from the PDB (http://www.rcsb.org/, PDB code 5LS6). Docking of compounds to PRC2 were performed using AutoDock 4.2. Molecule SKLB-0335 was built with Bio^{X} and optimized at molecular mechanical level. For SKLB-0335, the flexible side chain method was performed for covalent docking[4]. The receptor was modified to

remove side chain atoms of Cys663, and the side chain of Cys663 was tethered with the ligand and the C atom type was changed to Z. The C coordinates of Cys663 was used for covalent grid map generation with energy barrier height of 1000 and half-width of 5 Å.

EZH2 Purification and Mass Spectrometry.

The EZH2 catalytic domain (AA 494-737) for the mass spectrometry assay was expressed in *E.coli* and purified. The molecular weight (MW) of proteins and adducts was recorded with the LC-MS. (Shanghai Sangon Co. Ltd.) The LC-MS method is shown in the table below:

LC-MS system	ACQUITY UPLC I Class &XevoG2-XS QTof		
Column	ACQUITY UPLC BEH300 C4 1.7um 2.1*50mm, Waters		
Column temperature	80 °C		
Room temperature	10 °C		
Detection wavelength	280 nm		
Mobile phase	Phase A (0.1% Formic acid in Water), Phase B (0.1% FA in Acetonitrile).		
Flow rate	0.3 mL/min		
Volume	20ul		
Elution gradient	Time(min)	Composition A (%)	Composition B (%)
	0.00	95	5
	1.00	95	5
	7.00	10	90
	7.50	10	90
	7.60	95	5
	8.00	10	90
	8.10	95	5
	10.00	95	5
Detection method	Positive ion, precursor ion scan range: 500-4000 m/z		

Data analysis: Unifi software was used to conduct deconvolution analysis on the original data to obtain accurate molecular weight values. TOF Resolution: 10000; Output range: 10000-50000.

Cell culture.

SU-DHL-6 cell lines used in our study were purchased from the American Type Culture Collection (ATCC). The cells were maintained in DMEM or RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS) and 1% Penicillin-Streptomycin under humidified conditions with 5% CO_2 at 37 °C.

Western blotting analysis.

After treatment with a series of concentrations of SKLB-0335 for various days at 37 °C, cells were harvested, washed in ice-cold PBS, and lysed with RIPA buffer (Beyotime, China). And the protein concentrations were determined by the Bradford method. Proteins were separated by gel electrophoresis on 5 – 10% SDS-PAGE gels and probed with specific antibodies (Cell Signaling Technology, USA) including anti-H3K27me3, anti-EZH2, anti-H3 and anti-GAPDH. All of the antibodies were used at a 1 : 1000 dilution, and the horseradish peroxidase-coupled secondary antibodies (Zhong Shan Golden Bridge Bio-technology, China) were used at 1 : 5000.

Wash-out experiment.

SU-DHL-6 cells were treated with SKLB-0335, SKLB-0335′ or GSK126 at 10 μM for 6 days, then medium containing SKLB-0335, SKLB-0335′ or GSK126 was subsequently removed, and eluent cells with fresh medium every 30 minutes for a total of 5 times to effectively 'wash-out' the compound, and cells were allowed to grow in the absence of SKLB-0335, SKLB-0335′ or GSK126 for 1 – 4 days. Cells were collected and lysed for immunoblotting analysis at last.

Real-time qPCR assay.

Cells were treated with different drugs for 5 days, and then total RNA was extracted with Trizol, according to the manufacturer's instruction. RNA was reverse-transcribed by PrimeScript™RT reagent Kit. RT-qPCR was carried out using the ChamQ Universal SYBR qPCR Master Mix on the CFX96 RT-qPCR system in accordance with the manufacturer's instruction. The reaction procedure was as follows: 95 °C for 30 s followed by 40 cycles of amplification for 5 s at 95 °C, 30 s at 60 °C. The primer sequences used for RT-qPCR are listed as follows.

Gene	Forward	Reverse
GAPDH	CCTTCCGTGTCCCCACT	GCCTGCTTCACCACCTTC
CDX2	AGAAGAGCCGCGAGGAG	GGGAGCAGACCTCACCAT
CCND2	GTGGCCTTGGCATTTCT	ATCTATCGCTCGGGAACA

BMP6	TTCCCAGAAGTCCACAGG	GCACGAACATACAACAGCA
EOMES	GCGCATGTTTCCTTTCTT	ATGTTATTGTCGGCTTTGC

References

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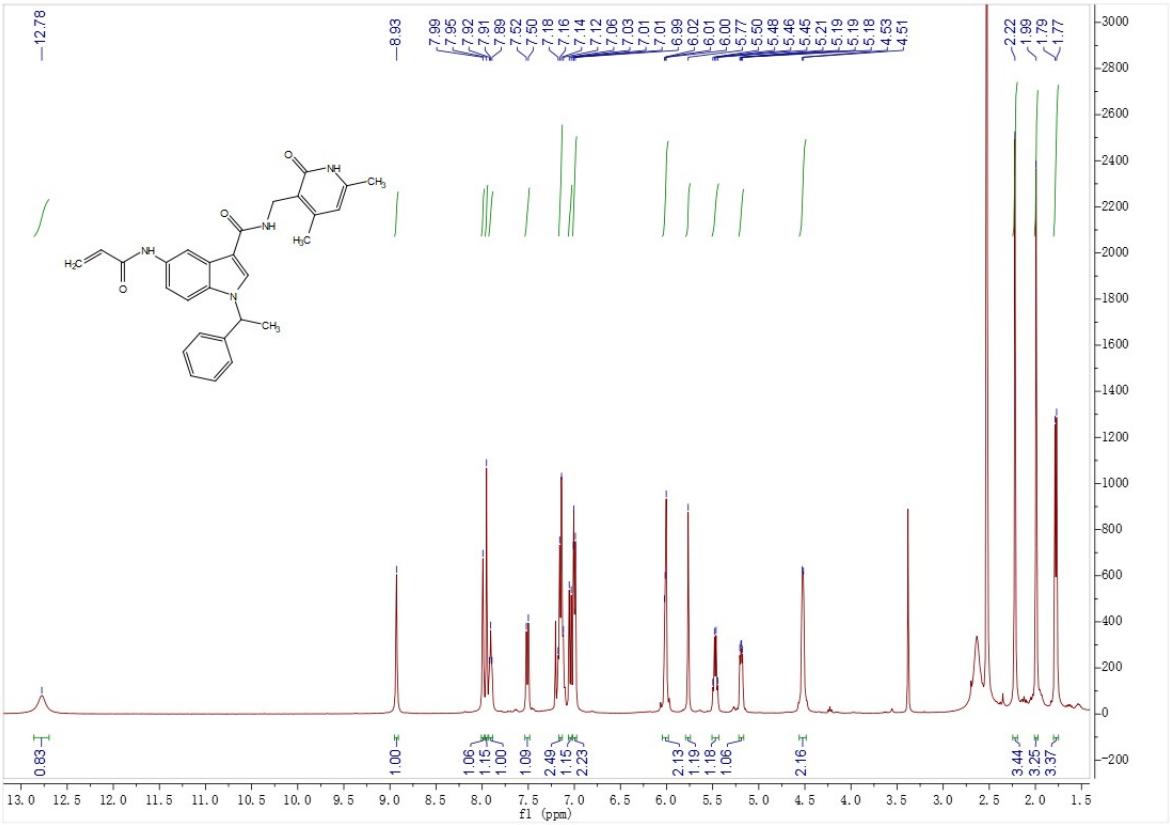
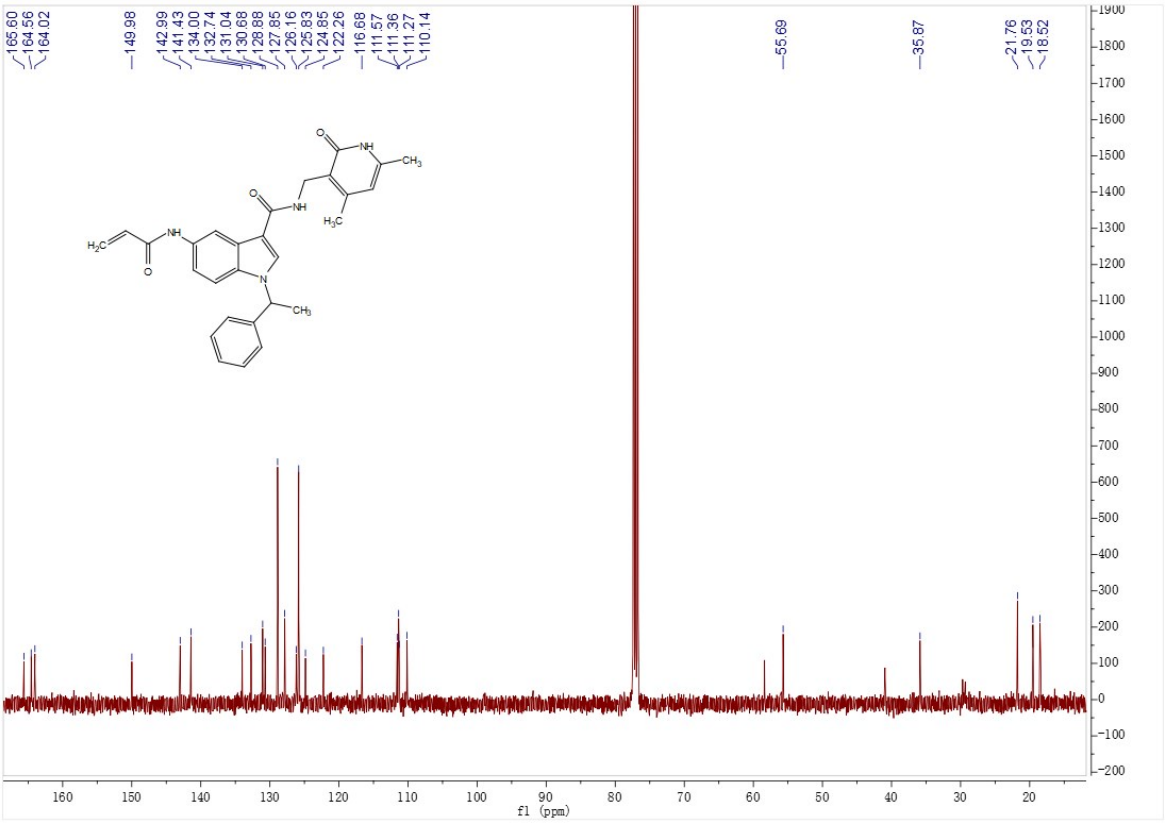
[3] L. Zhang, X. Song, N. Wang, L. Zhao, Q. Feng, X. You, C. Peng, T. Gao, M. Xiong, B. He, C. Gao, Y. Luo, Y. Xu, Q. Zhang, L. Yu, *RSC Advances* **2015**, *5*, 25967-25978.
[4] G. Bianco, S. Forli, D. S. Goodsell, A. J. Olson, *Protein Sci.* **2016**, *25*, 295-301.

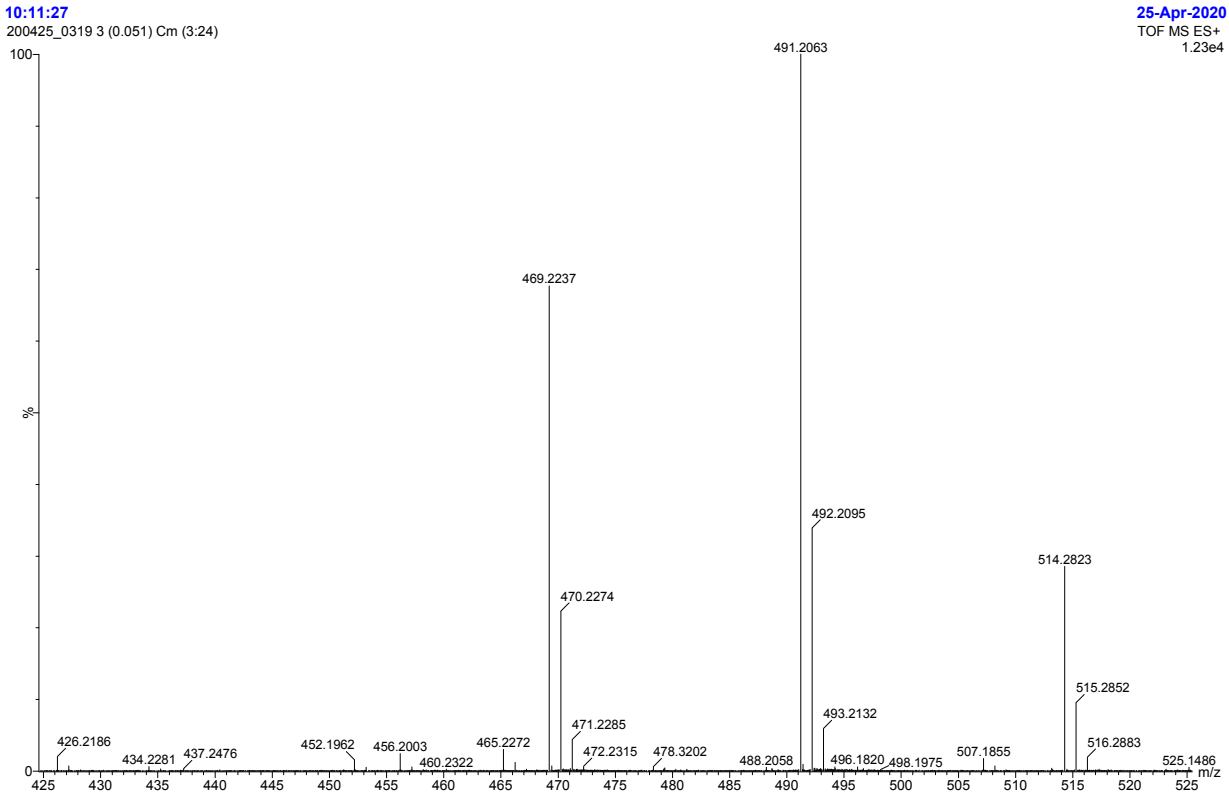
Spectral data

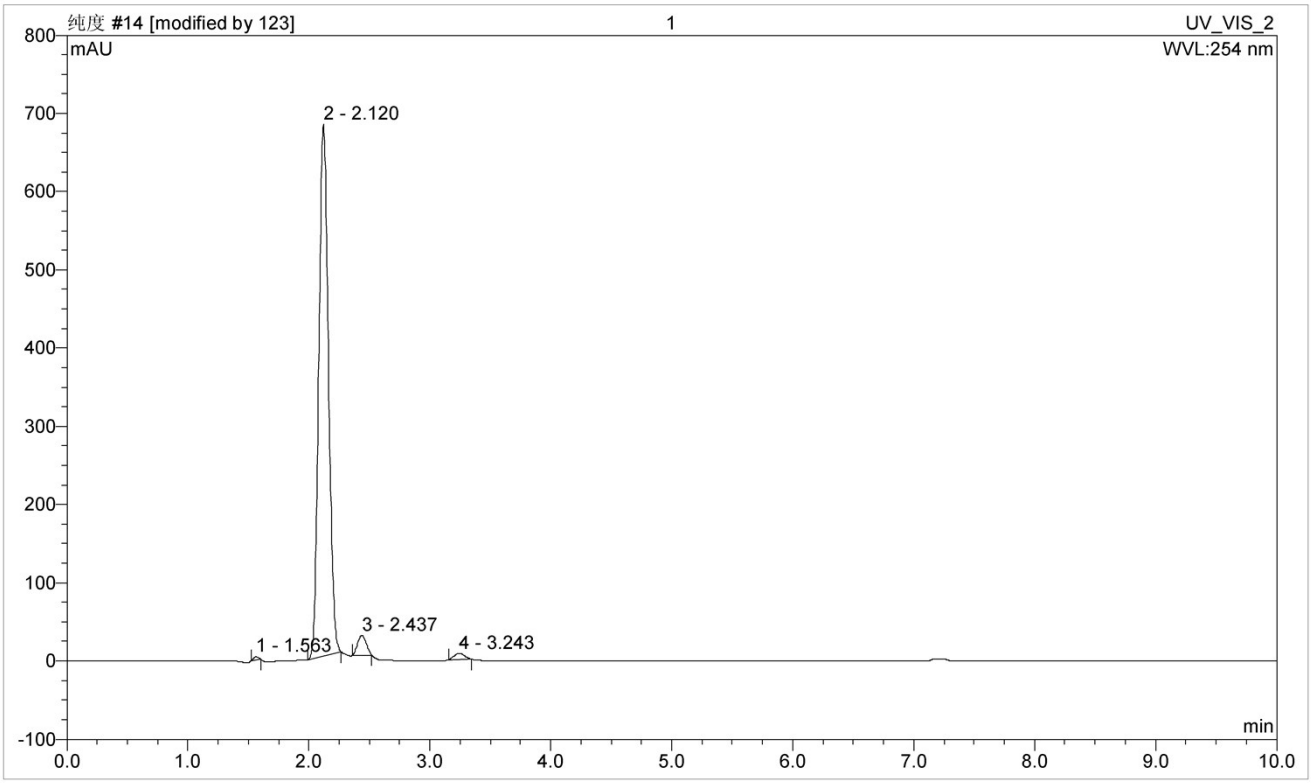
The NMR and HRMS of representative compounds

Compound **a1**



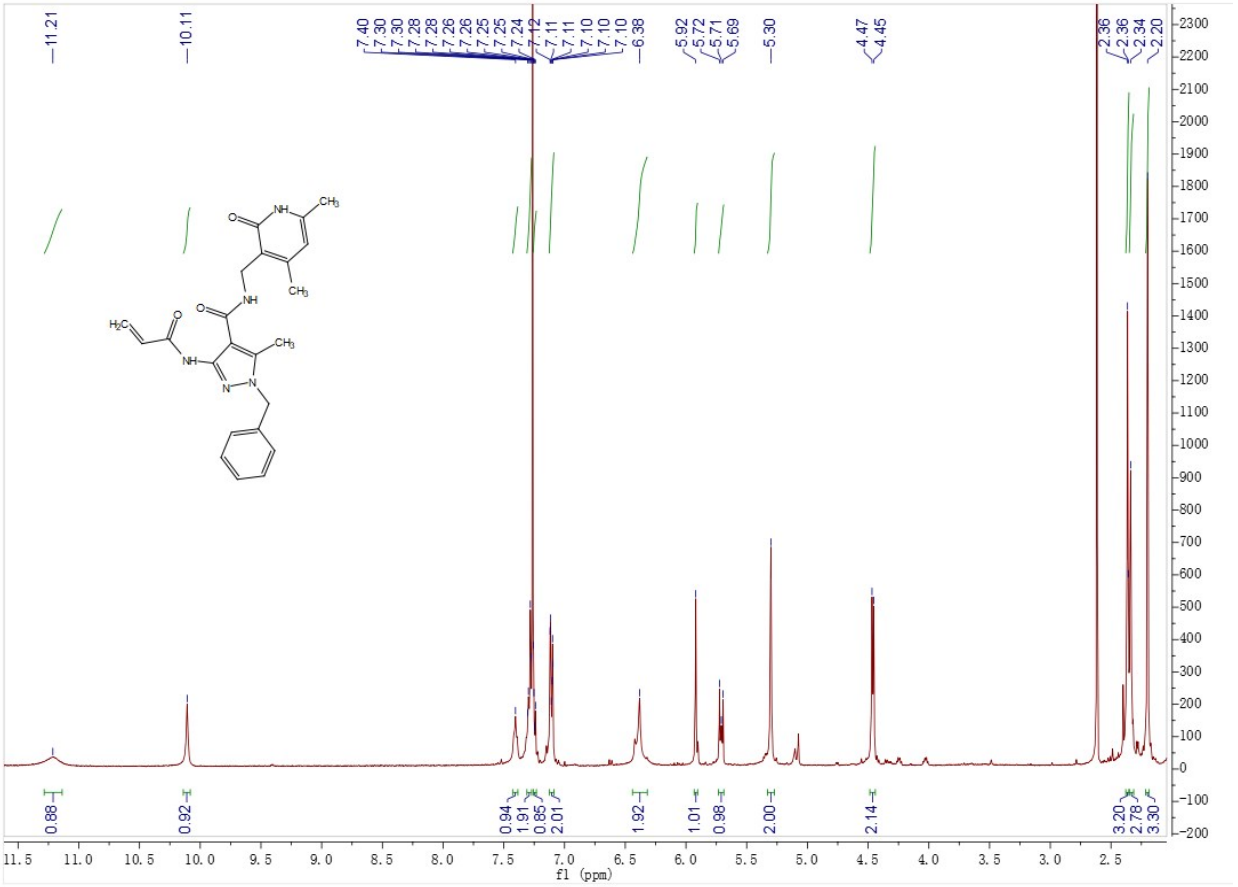






No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.56	n.a.	3.960	0.187	0.28	n.a.	BMB*
2	2.12	n.a.	679.395	62.940	95.29	n.a.	BMB*
3	2.44	n.a.	25.215	2.143	3.24	n.a.	BMB*
4	3.24	n.a.	7.898	0.783	1.19	n.a.	BMB*
Total:			716.468	66.053	100.00	0.000	

Compound **b1**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7.30
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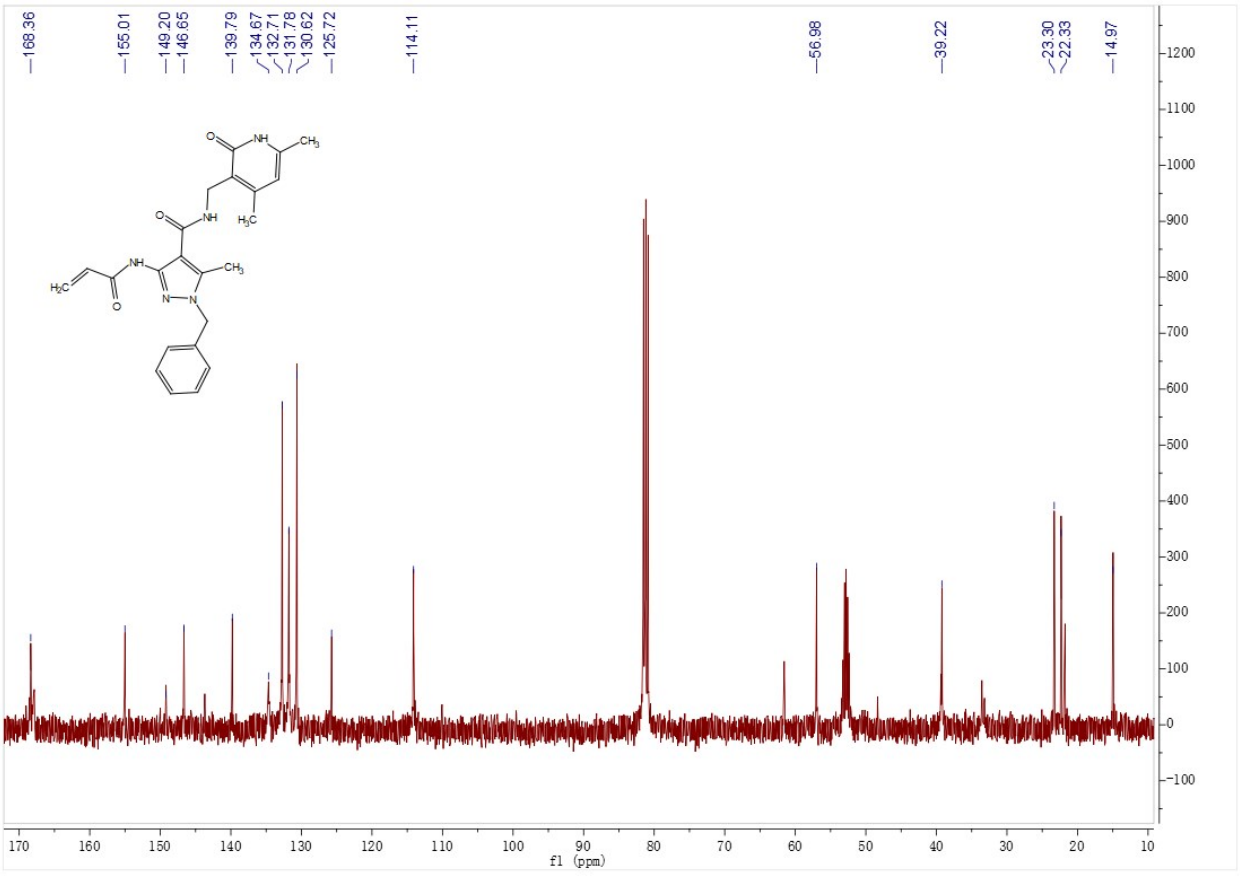
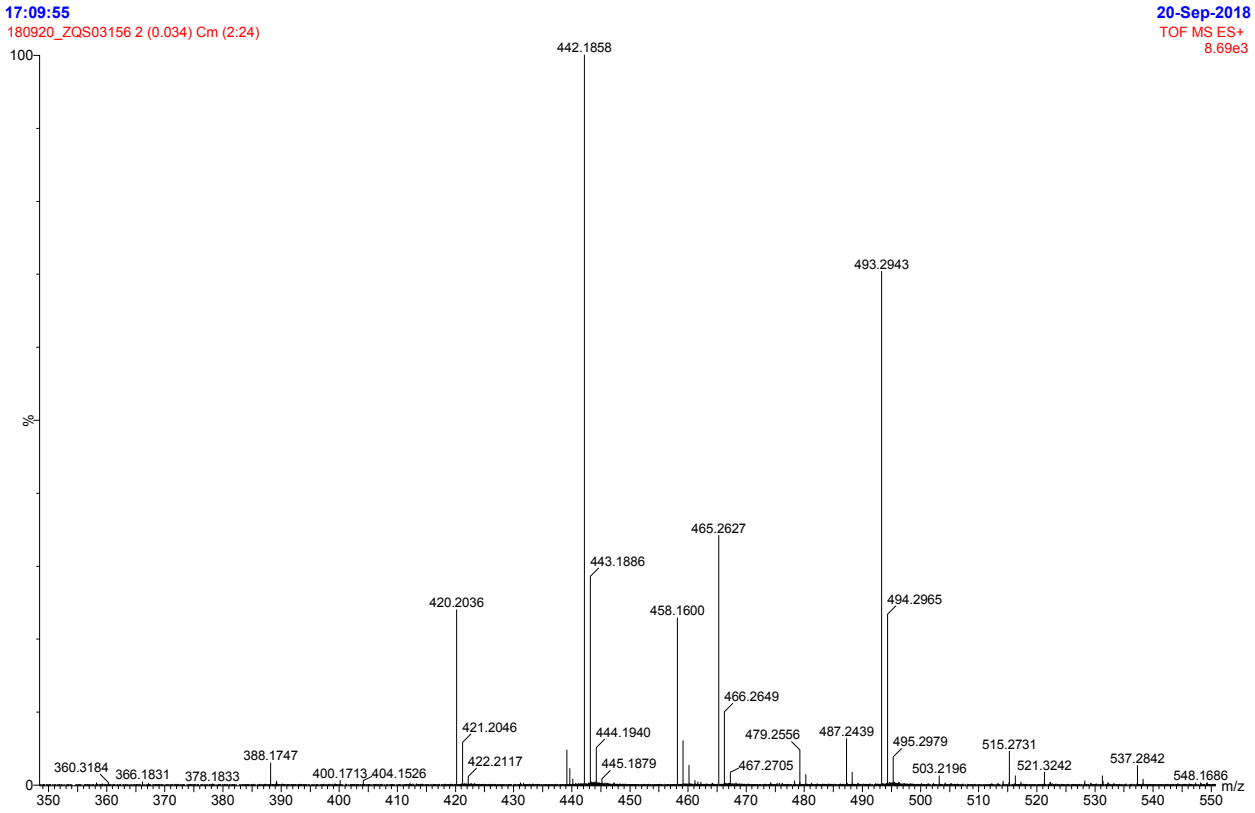
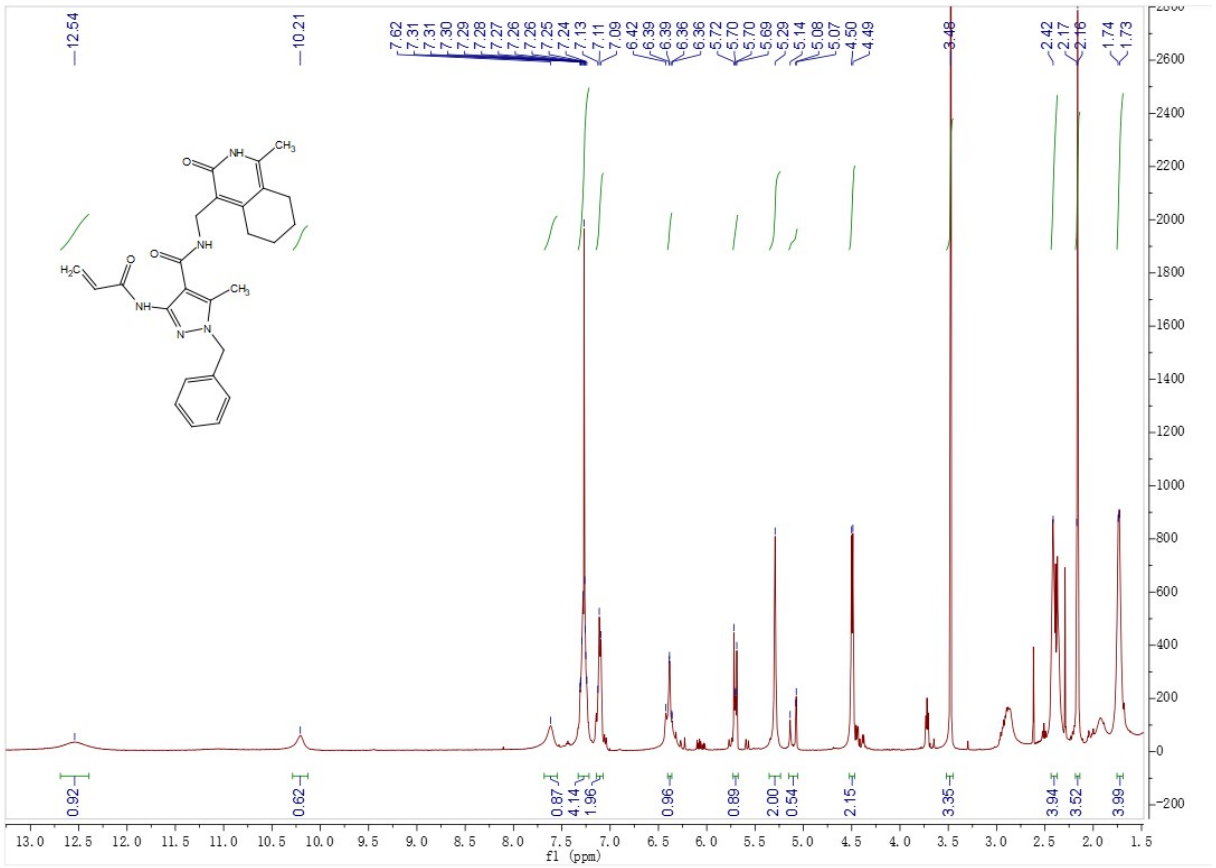
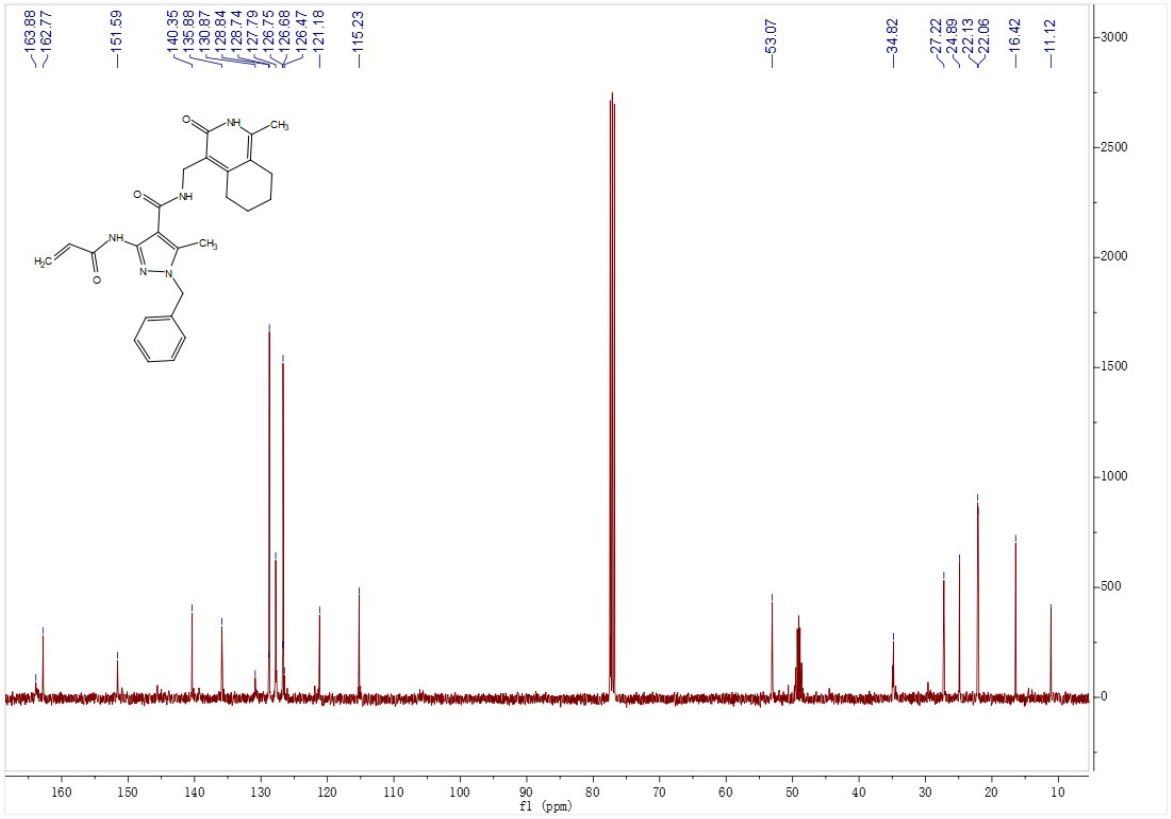
5.72
5.71
5.69
5.30
4.47
4.45
2.36
2.36
2.34
2.20
0.88
0.92
0.94
1.91
0.85
2.01
1.92
1.01
0.98
2.00
2.14
3.20
2.78
3.30
f1 (ppm)

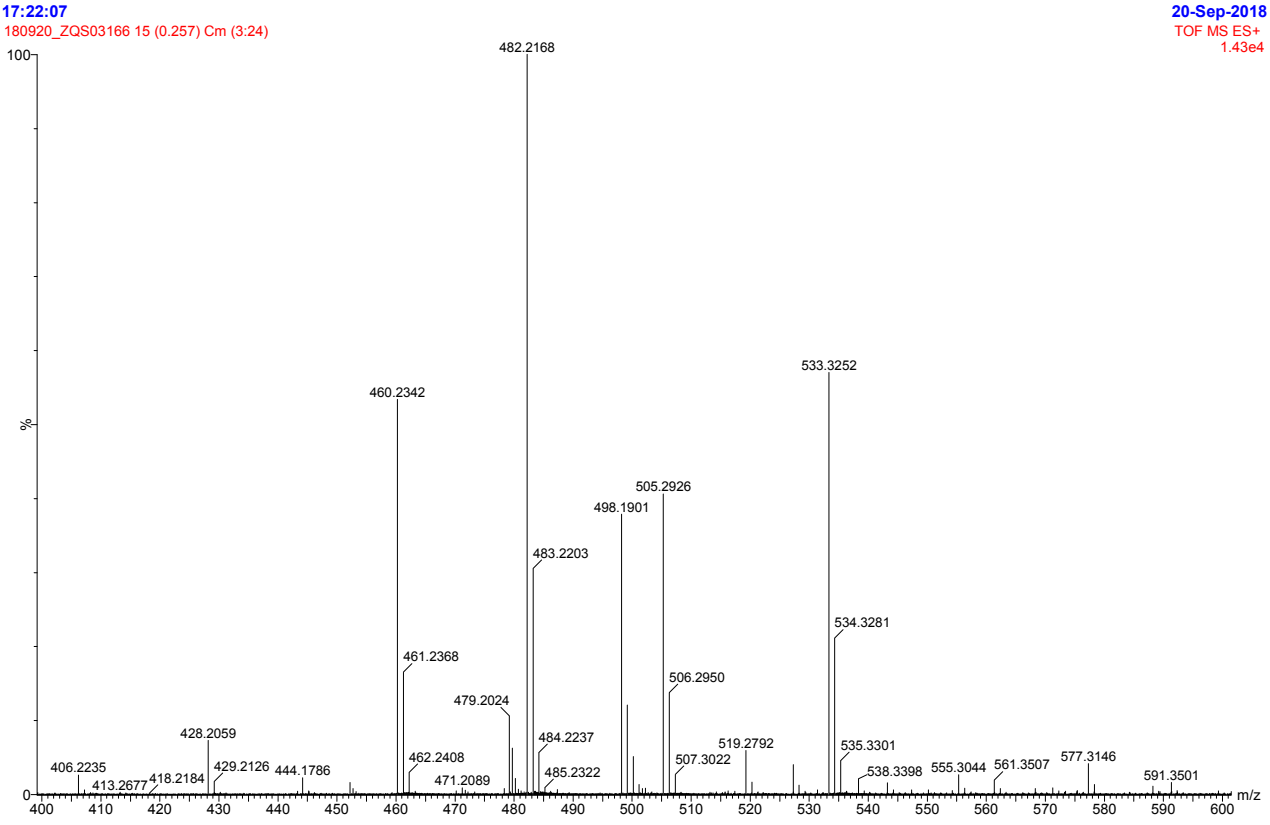

168.36
155.01
149.20
146.65
139.79
134.67
132.71
131.78
130.62
125.72
114.11
56.98
39.22
23.30
22.33
14.97
f1 (ppm)


17:09:55
180920_ZQS03156 2 (0.034) Cm (2:24)
20-Sep-2018
TOF MS ES+
8.69e3
100
%
0
442.1858
493.2943
465.2627
443.1886
420.2036
458.1600
494.2965
466.2649
421.2046
444.1940
479.2556
487.2439
495.2979
515.2731
388.1747
422.2117
445.1879
467.2705
503.2196
521.3242
537.2842
360.3184
366.1831
378.1833
400.1713,404.1526
548.1686
m/z

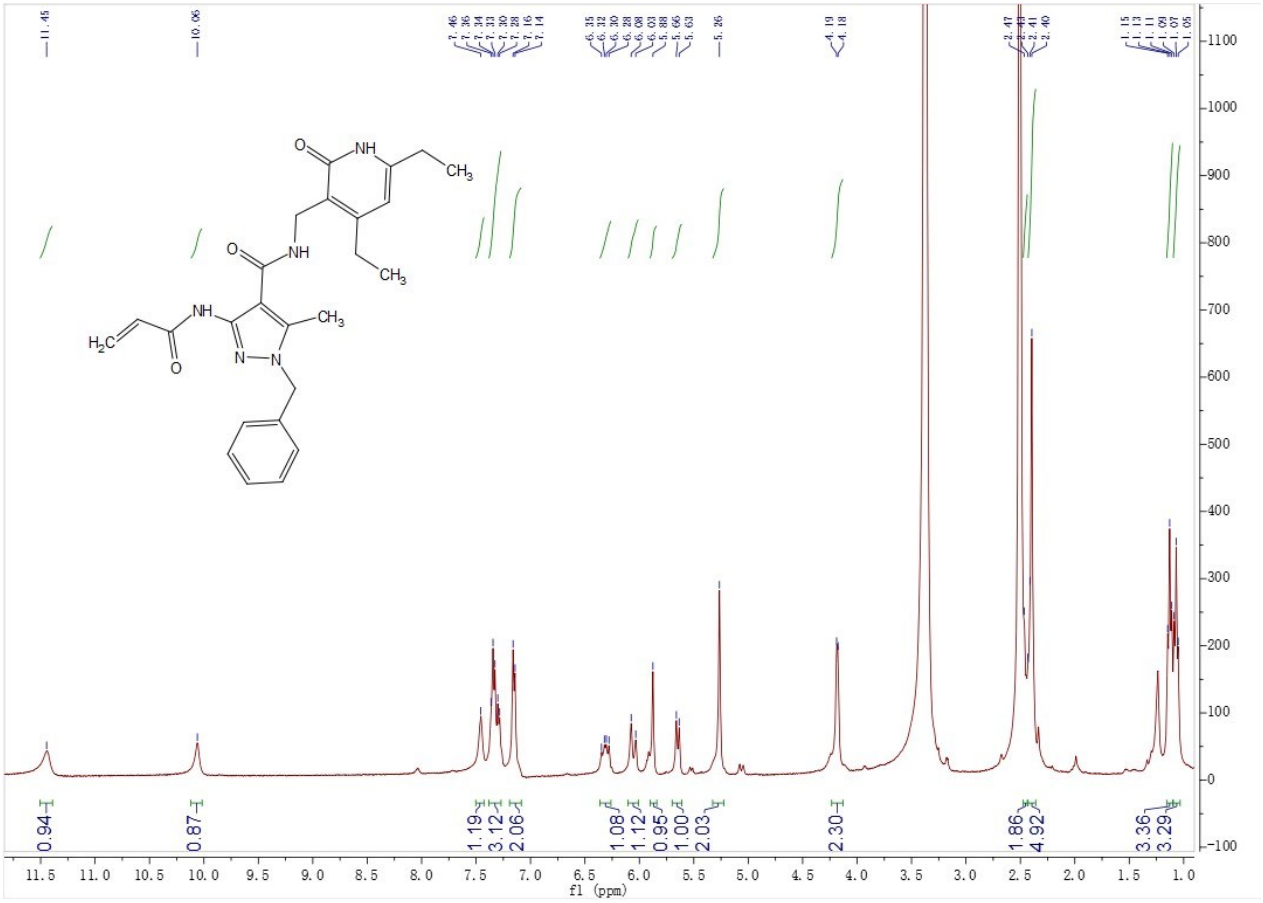
Compound **b2**

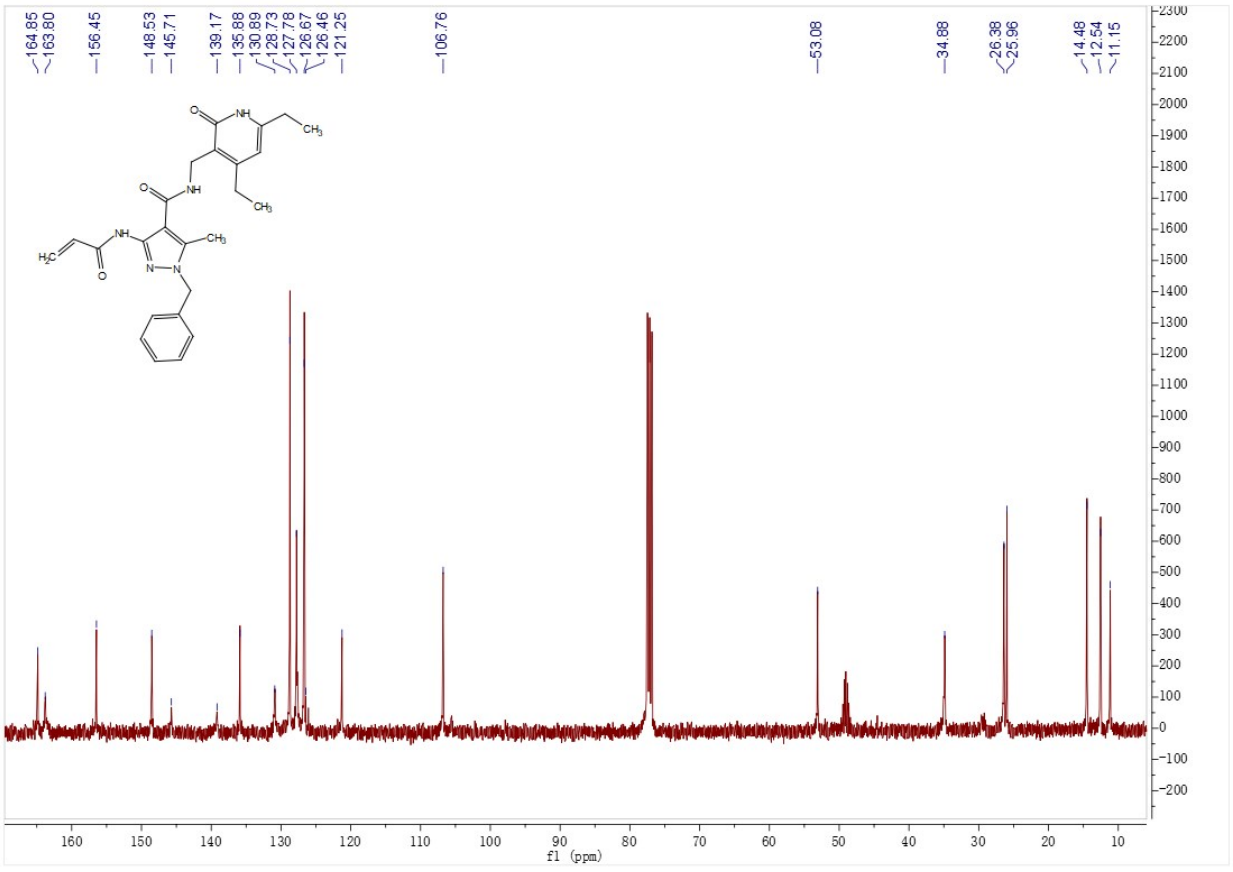

12.54
10.21
7.62
7.31
7.31
7.30
7.29
7.28
7.27
7.26
7.26
7.25
7.24
7.13
7.11
7.09
6.42
6.39
6.39
6.36
6.36
5.72
5.70
5.70
5.69
5.29
5.14
5.08
5.07
4.50
4.49
3.48
2.42
2.17
2.16
1.74
1.73
0.92
0.62
0.87
4.14
1.96
0.96
0.89
2.00
0.54
2.15
3.35
3.94
3.52
3.99
f1 (ppm)

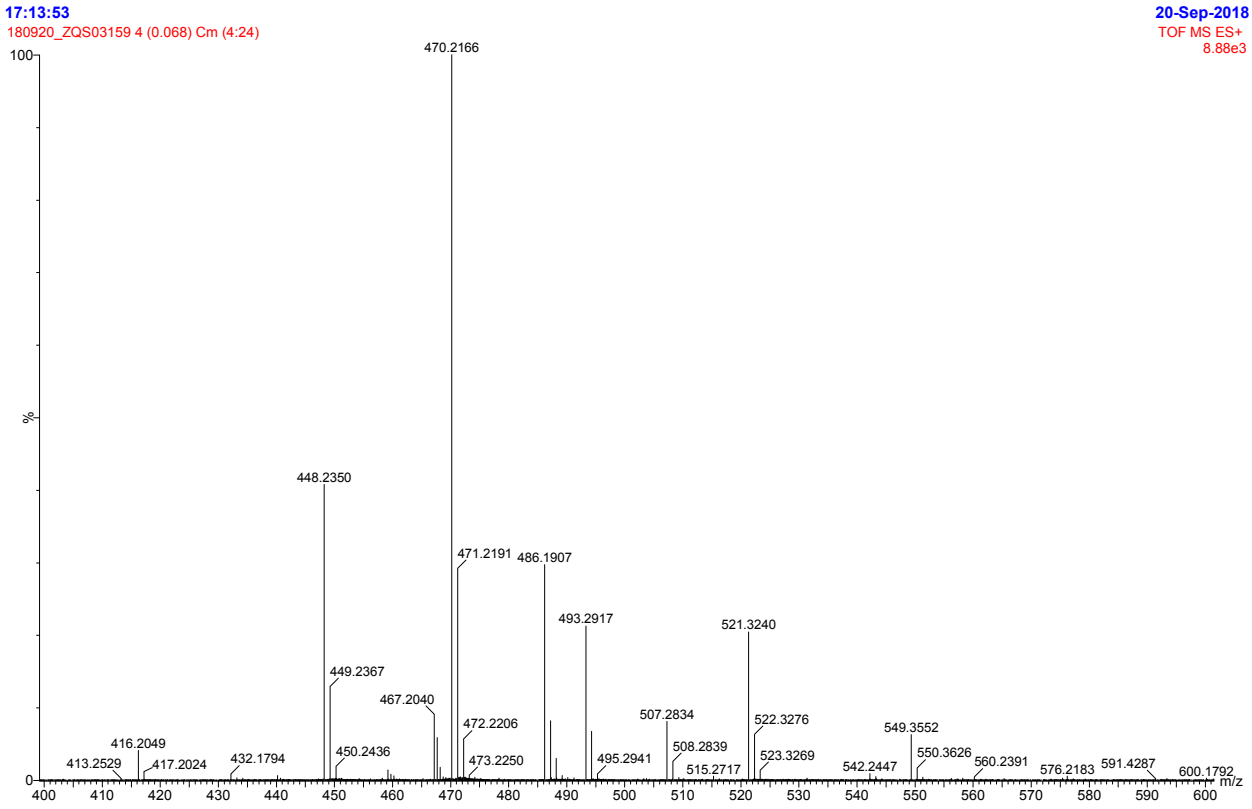

163.88
162.77
151.59
140.35
135.88
130.87
128.84
128.74
127.79
126.75
126.68
126.47
121.18
115.23
53.07
34.82
27.22
24.89
22.13
22.06
16.42
11.12
f1 (ppm)


17:22:07
180920_ZQS03166 15 (0.257) Cm (3:24)
20-Sep-2018
TOF MS ES+
1.43e4
%
m/z
482.2168
460.2342
533.3252
505.2926
498.1901
483.2203
534.3281
461.2368
506.2950
479.2024
484.2237
519.2792
428.2059
462.2408
507.3022
535.3301
406.2235
429.2126
444.1786
471.2089
485.2322
538.3398
555.3044
561.3507
577.3146
591.3501
413.2677
418.2184

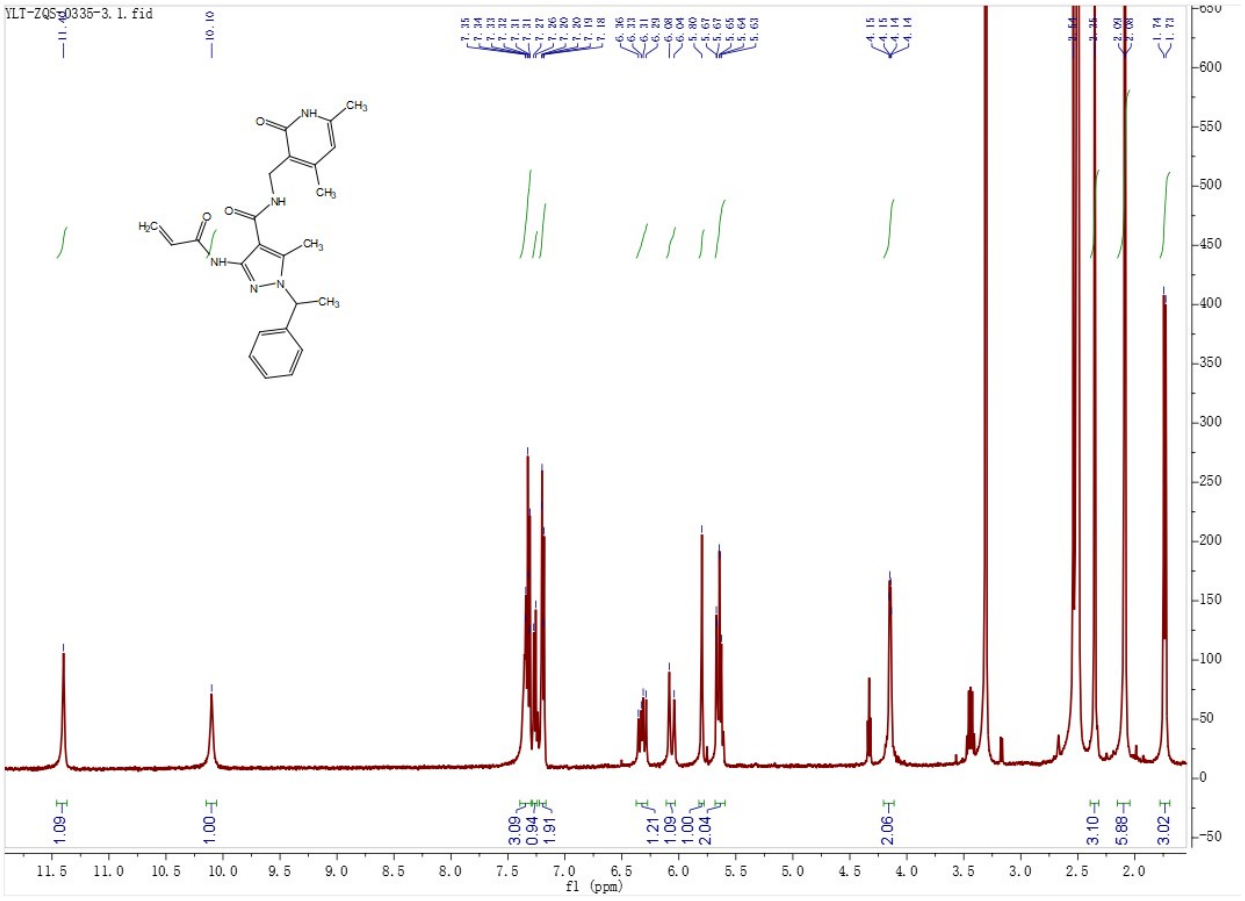
Compound **b3**

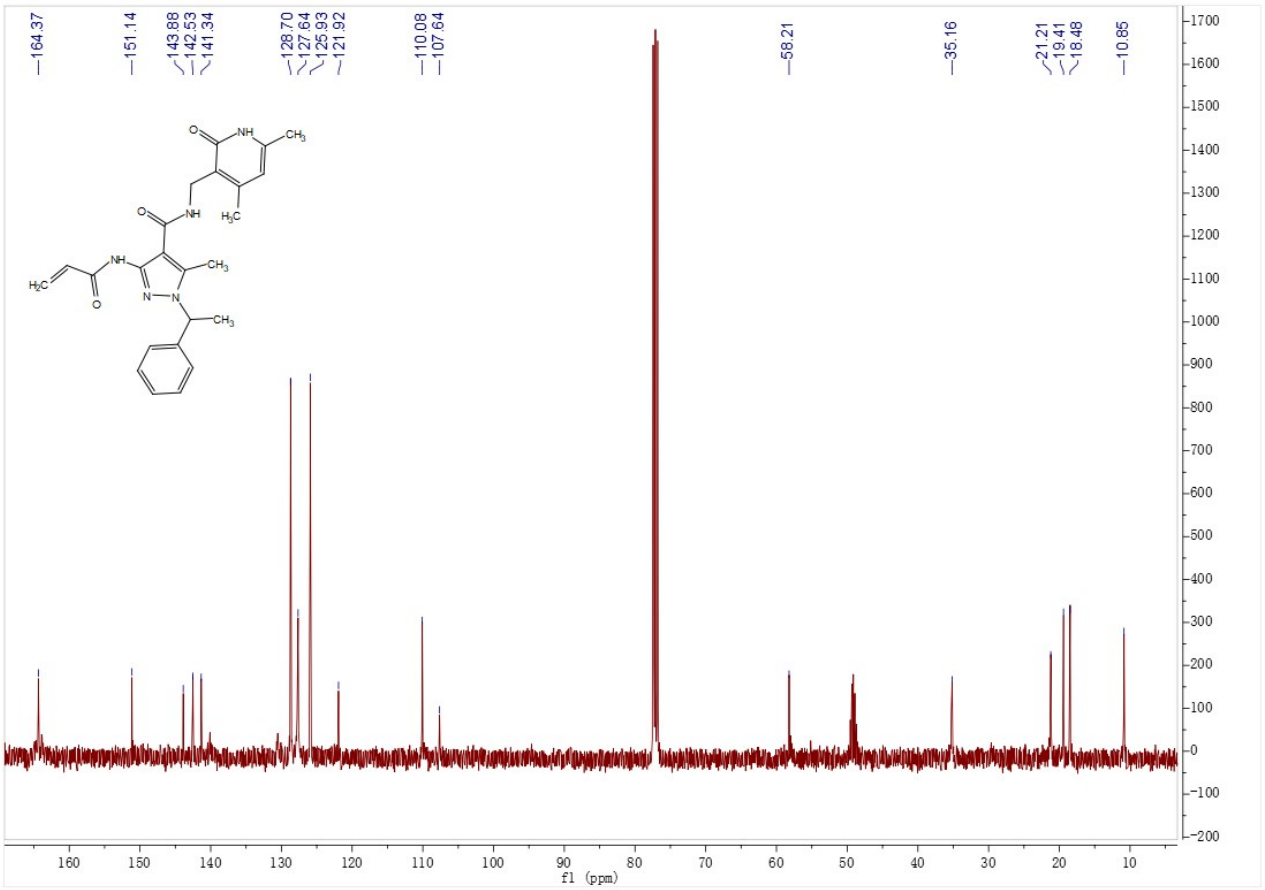

11.46
10.06
7.46
7.35
7.34
7.33
7.30
7.28
7.16
7.14
6.35
6.32
6.30
6.28
6.08
6.03
5.88
5.66
5.63
5.26
4.19
4.18
2.47
2.43
2.41
2.40
1.15
1.13
1.11
1.09
1.07
1.05
0.94
0.87
1.19
3.12
2.06
1.08
1.12
0.95
1.00
2.03
2.30
1.86
4.92
3.36
3.29
f1 (ppm)

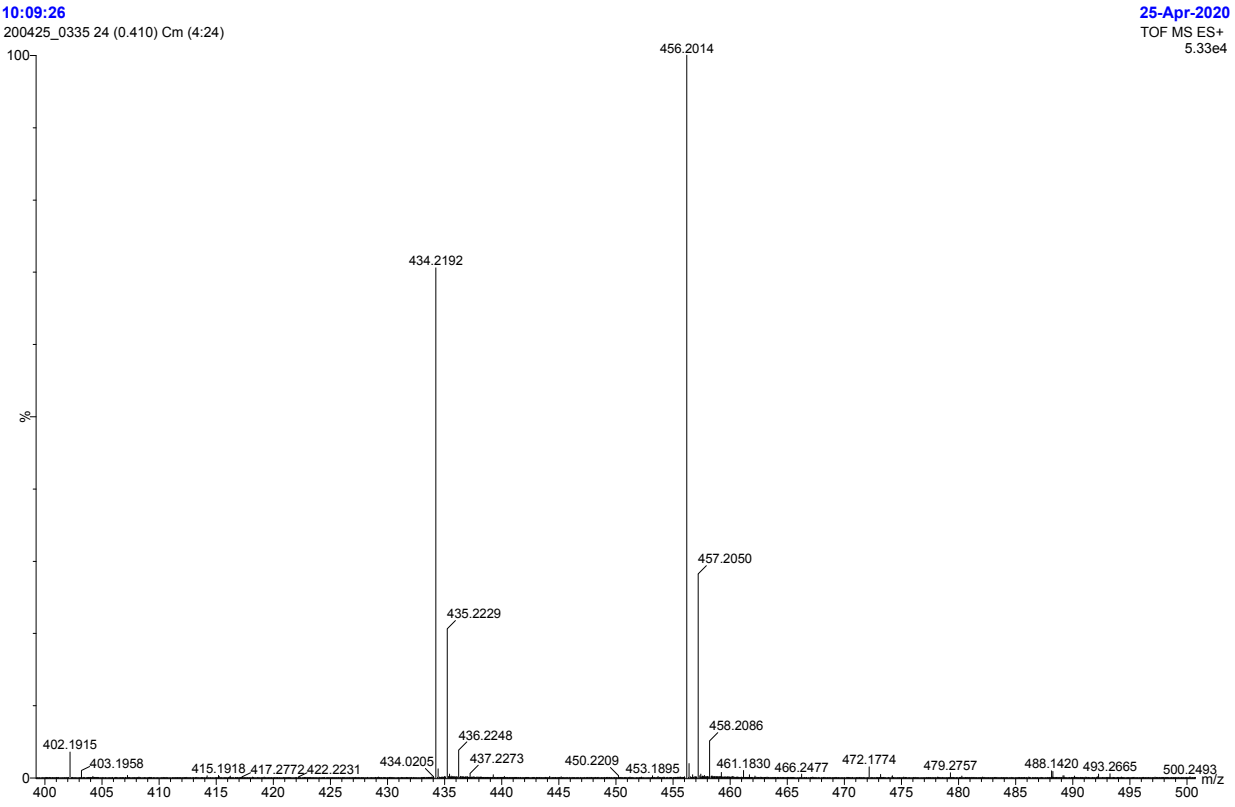

164.85
163.80
156.45
148.53
145.71
139.17
135.88
130.89
128.73
127.78
126.67
126.46
121.25
106.76
53.08
34.88
26.38
25.96
14.48
12.54
11.15
f1 (ppm)

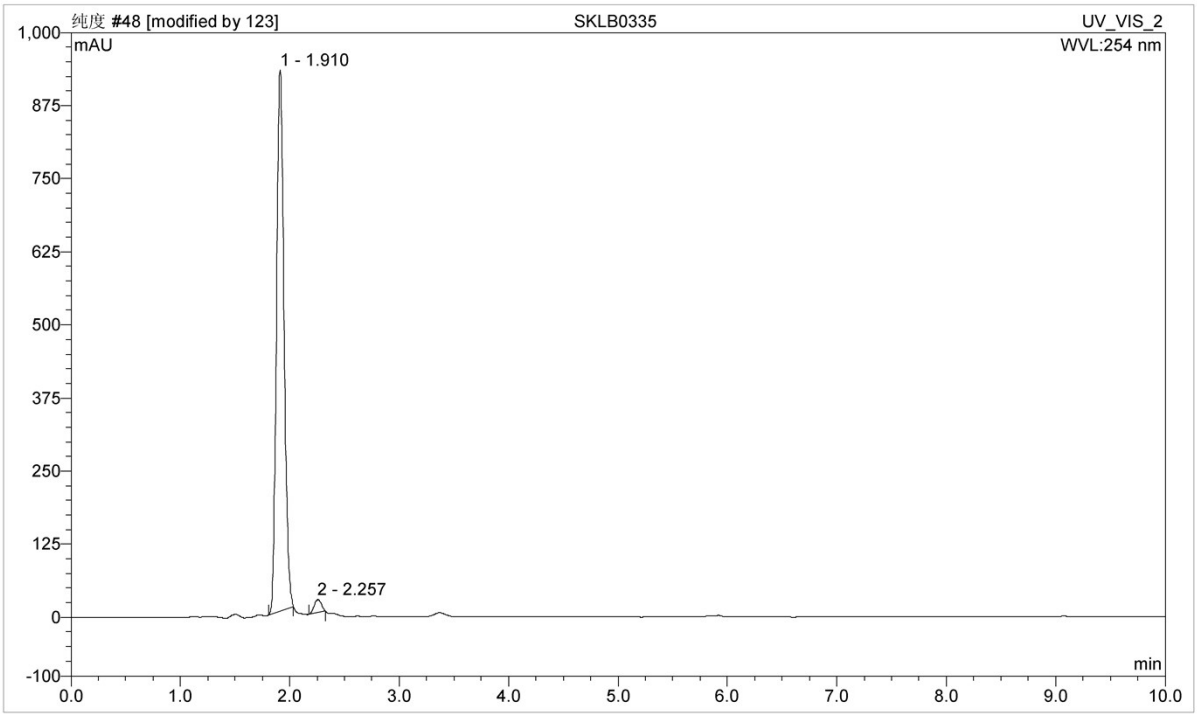

17:13:53
180920_ZQS03159 4 (0.068) Cm (4:24)
20-Sep-2018
TOF MS ES+
8.88e3
100
%
0
470.2166
448.2350
471.2191
486.1907
493.2917
521.3240
449.2367
467.2040
472.2206
507.2834
522.3276
549.3552
416.2049
413.2529
417.2024
432.1794
450.2436
473.2250
495.2941
508.2839
515.2717
523.3269
542.2447
550.3626
560.2391
576.2183
591.4287
600.1792
400 410 420 430 440 450 460 470 480 490 500 510 520 530 540 550 560 570 580 590 600
m/z

Compound **b4**


YLT-ZQS-0335-3.1.fid
11.40
10.10
7.35 7.34 7.33 7.32 7.31 7.27 7.26 7.20 7.19 7.18
6.36 6.31 6.11 6.09 6.08 6.04 5.80 5.67 5.64 5.63
4.15 4.15 4.14 4.14
3.54 3.35 2.09 2.08 1.74 1.73
1.09 1.00 3.09 0.94 1.91 1.21 1.09 1.00 2.04 2.06 3.10 5.88 3.02
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0
f1 (ppm)

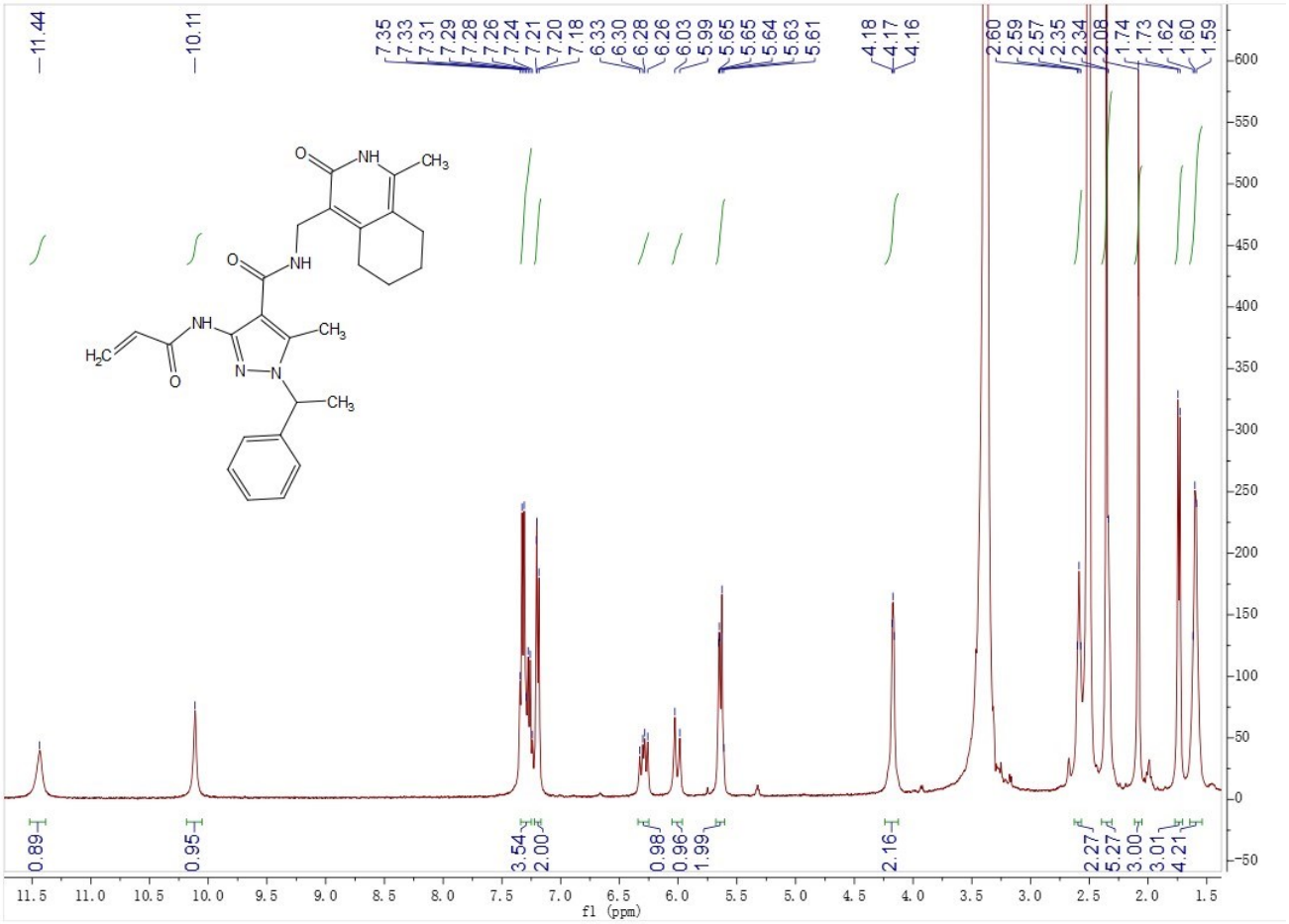

164.37
151.14
143.88
142.53
141.34
128.70
127.64
125.93
121.92
110.08
107.64
58.21
35.16
21.21
19.41
18.48
10.85
f1 (ppm)

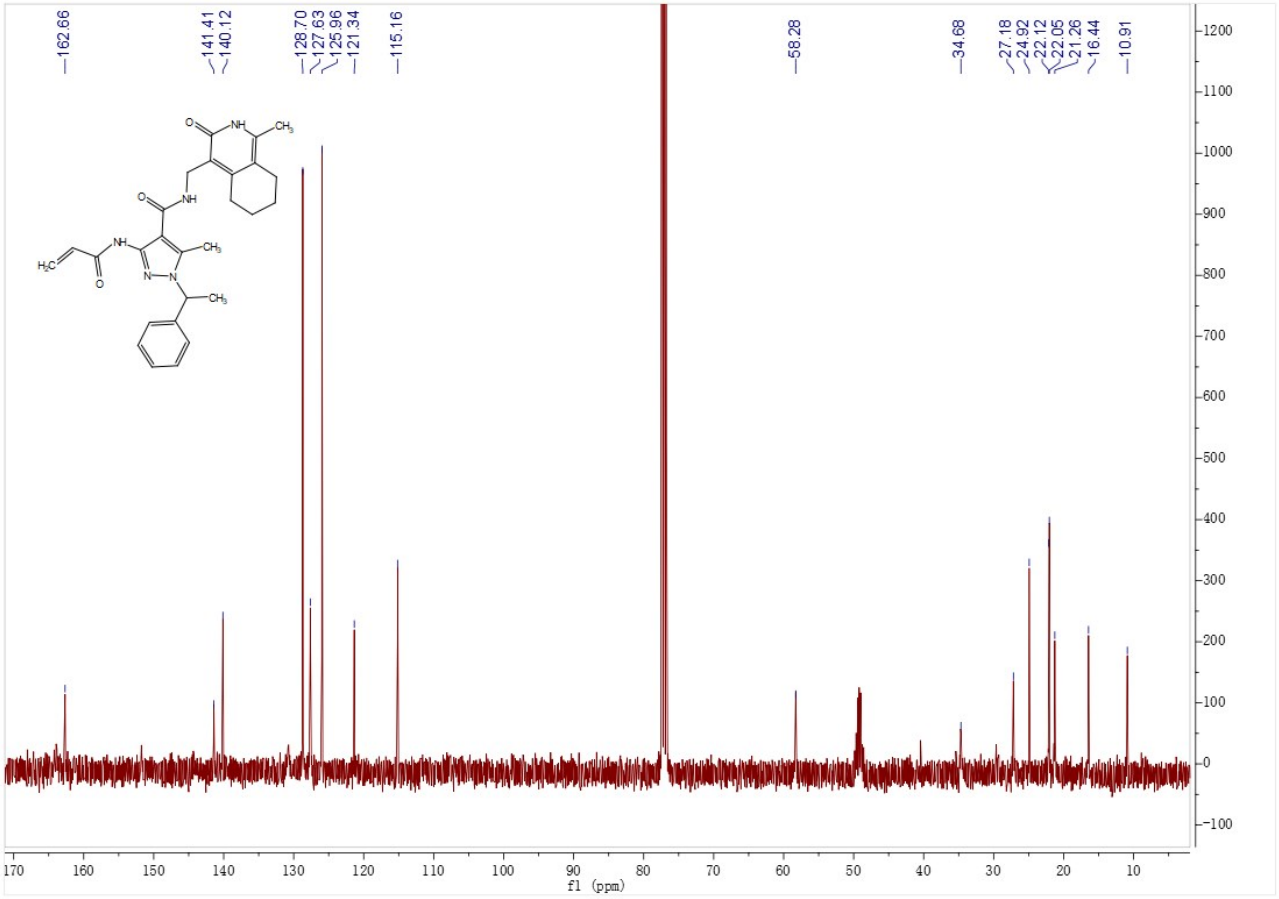

10:09:26
200425_0335 24 (0.410) Cm (4:24)
25-Apr-2020
TOF MS ES+
5.33e4
456.2014
434.2192
457.2050
435.2229
458.2086
436.2248
402.1915
403.1958
415.1918
417.2772
422.2231
434.0205
437.2273
450.2209
453.1895
461.1830
466.2477
472.1774
479.2757
488.1420
493.2665
500.2493
%
m/z

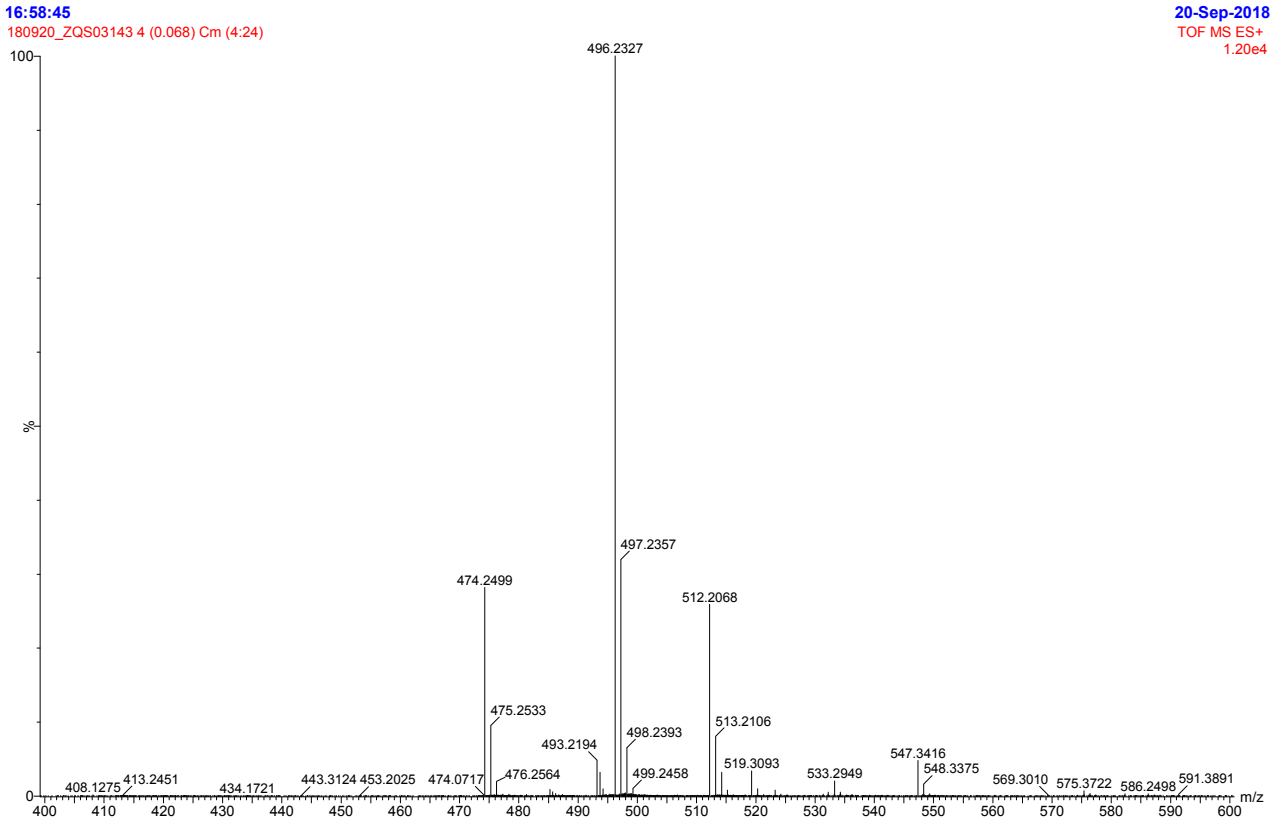


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.91 n.a.		925.134	73.666	97.77	n.a.	BMB*
2	2.26 n.a.		22.146	1.680	2.23	n.a.	BMB*
Total:			947.279	75.346	100.00	0.000	

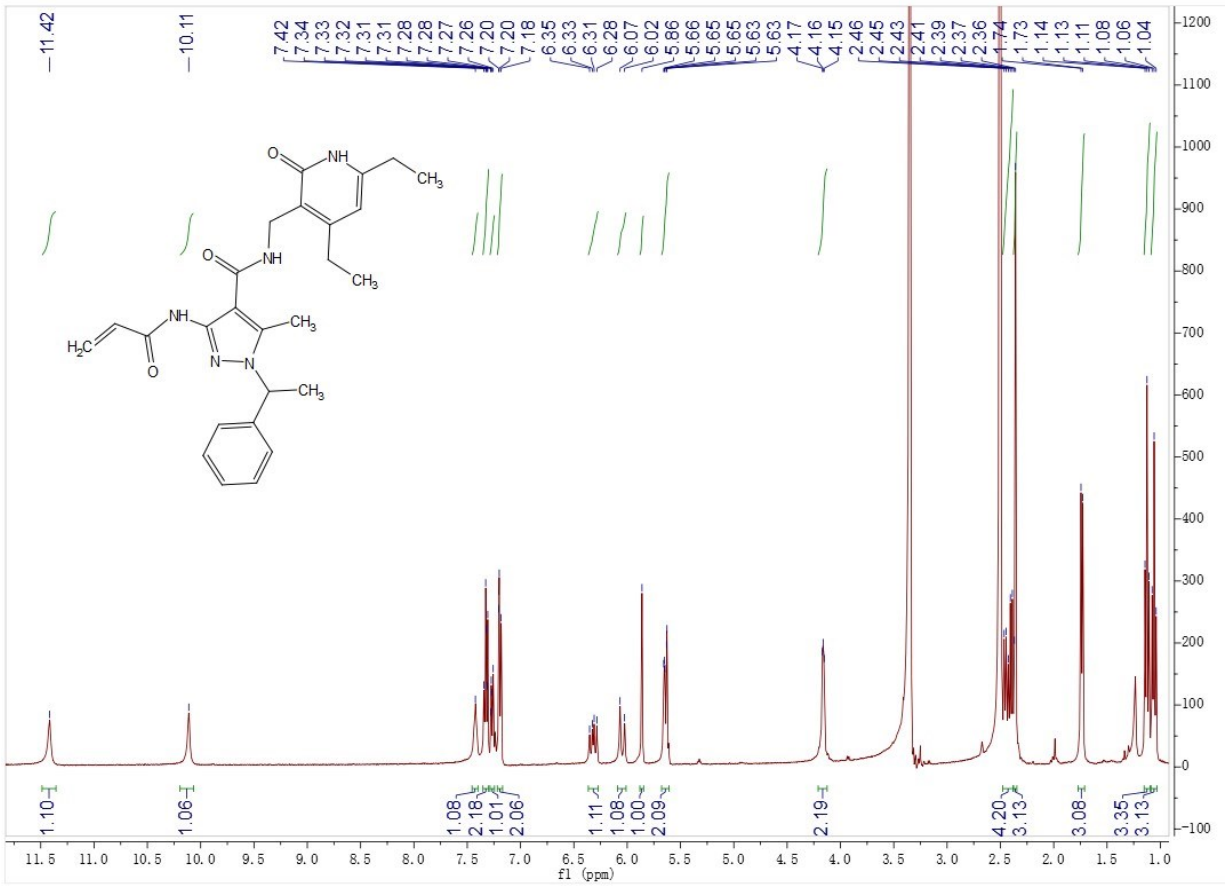
Compound **b5**

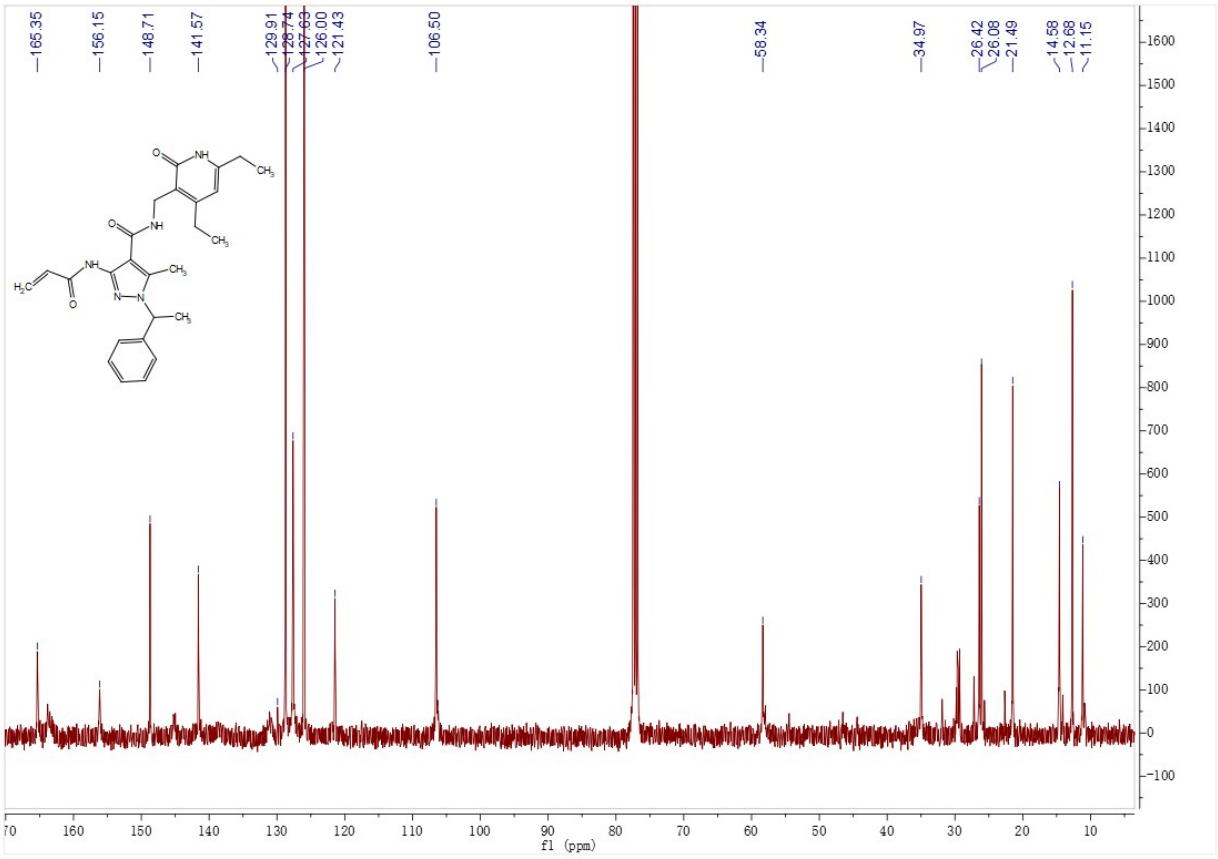


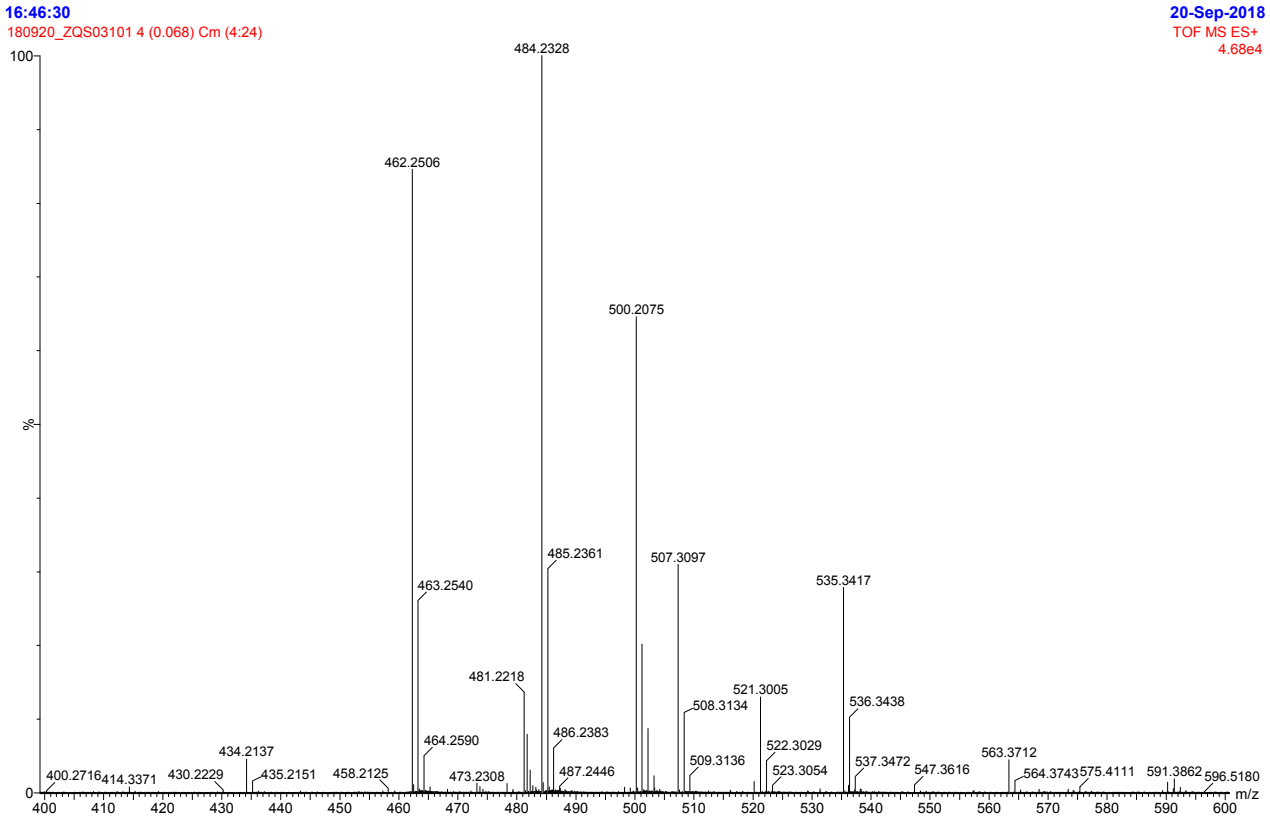

162.66
141.41
140.12
128.70
127.63
125.96
121.34
115.16
58.28
34.68
27.18
24.92
22.12
22.05
21.26
16.44
10.91
f1 (ppm)


16:58:45
180920_ZQS03143 4 (0.068) Cm (4:24)
20-Sep-2018
TOF MS ES+
1.20e4
%
m/z
496.2327
497.2357
474.2499
512.2068
475.2533
513.2106
498.2393
493.2194
519.3093
499.2458
547.3416
548.3375
533.2949
476.2564
474.0717
443.3124
453.2025
434.1721
413.2451
408.1275
569.3010
575.3722
586.2498
591.3891

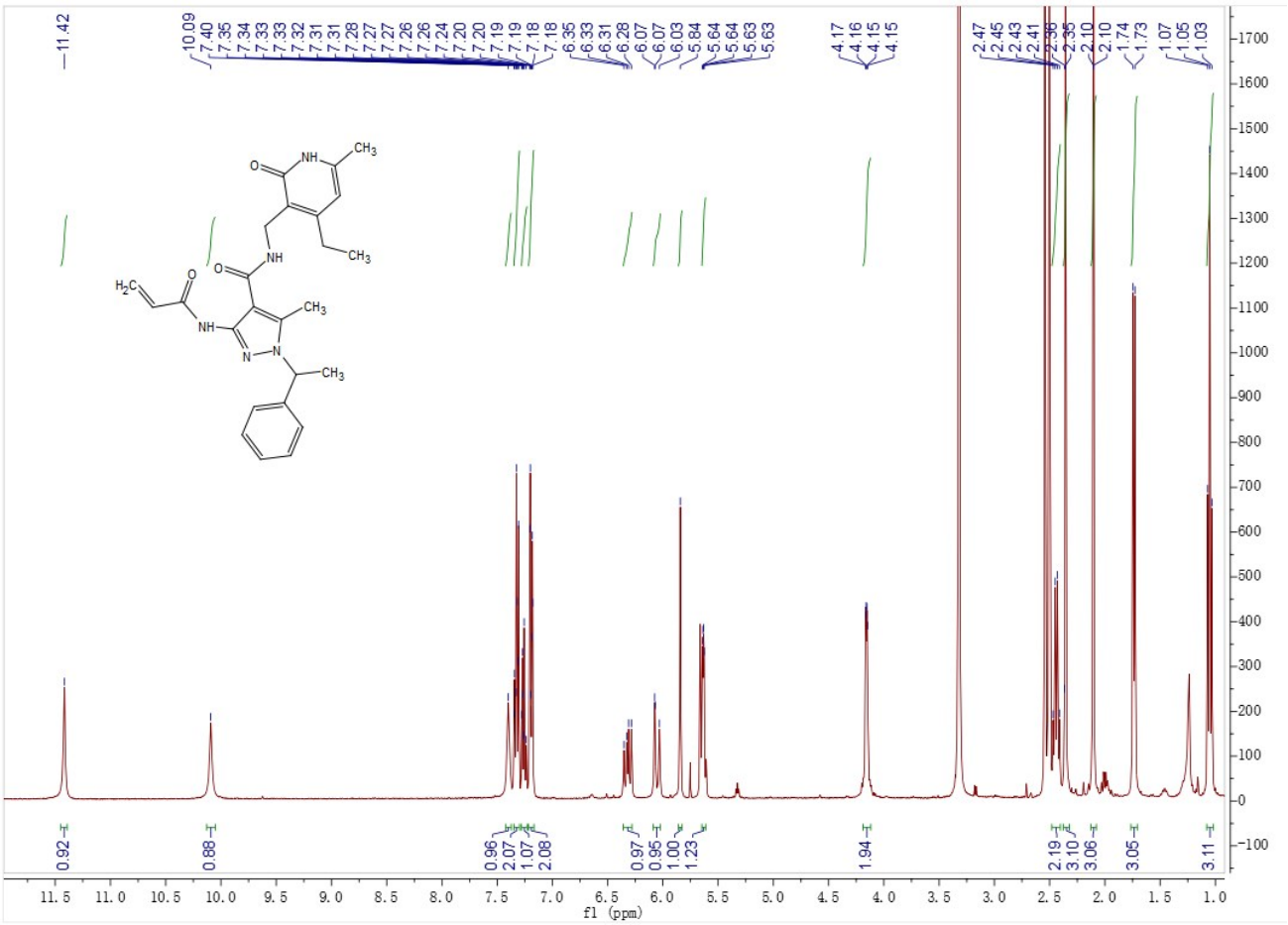
Compound **b6**

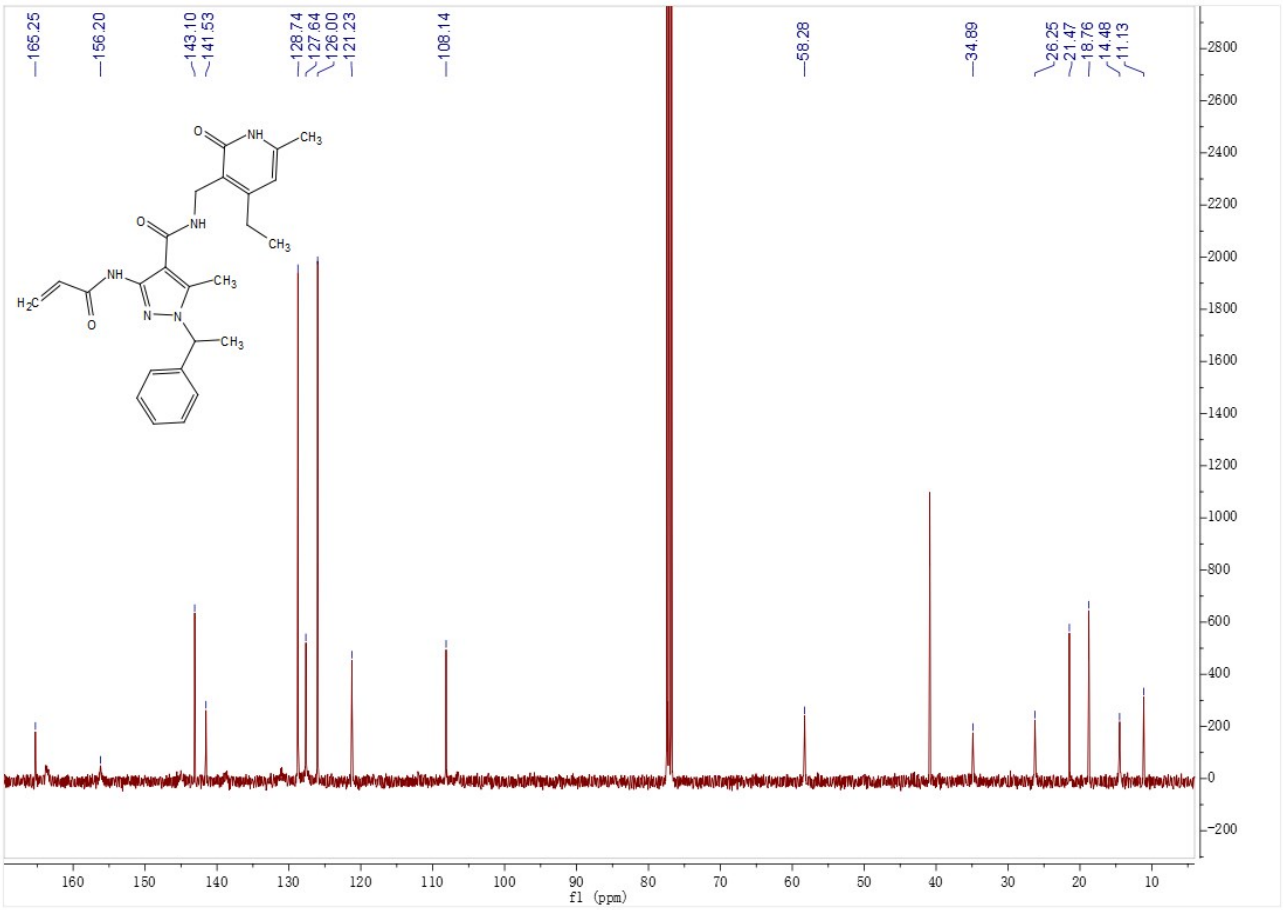

11.42
10.11
7.42
7.34
7.33
7.32
7.31
7.31
7.28
7.28
7.27
7.26
7.20
7.20
7.18
6.35
6.33
6.31
6.28
6.07
6.02
5.86
5.66
5.65
5.65
5.63
5.63
4.17
4.16
4.15
2.46
2.45
2.43
2.41
2.39
2.37
2.36
1.74
1.73
1.14
1.13
1.11
1.08
1.06
1.04
1.10
1.06
1.08
2.18
1.01
2.06
1.11
1.08
1.00
2.09
2.19
4.20
3.13
3.08
3.35
3.13
f1 (ppm)

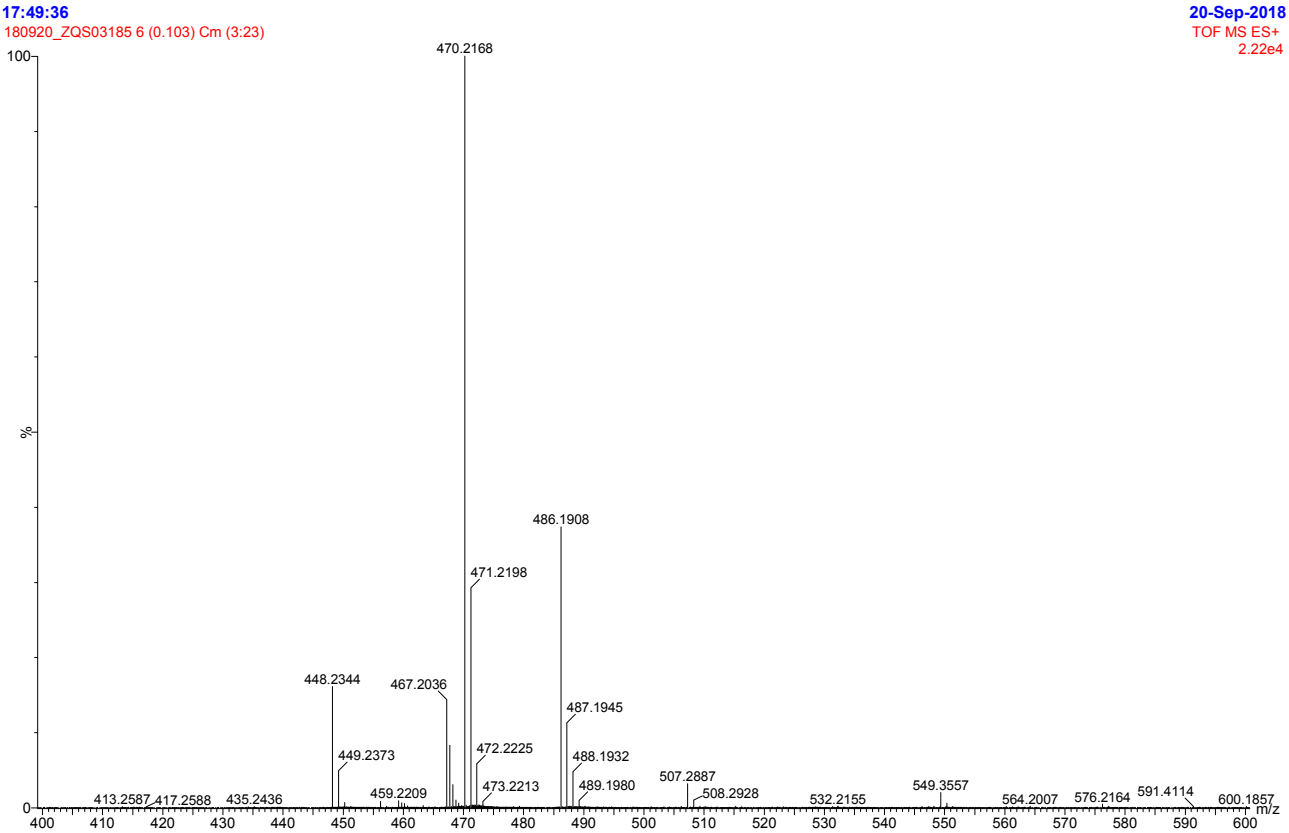

165.35
156.15
148.71
141.57
129.91
128.74
127.63
126.00
121.43
106.50
58.34
34.97
26.42
26.08
21.49
14.58
12.68
11.15
f1 (ppm)


16:46:30
180920_ZQS03101 4 (0.068) Cm (4:24)
20-Sep-2018
TOF MS ES+
4.68e4
100
%
0
484.2328
462.2506
500.2075
485.2361
507.3097
463.2540
535.3417
481.2218
521.3005
508.3134
536.3438
464.2590
486.2383
522.3029
434.2137
509.3136
563.3712
523.3054
537.3472
547.3616
400.2716
414.3371
430.2229
435.2151
458.2125
473.2308
487.2446
564.3743
575.4111
591.3862
596.5180
m/z
400
410
420
430
440
450
460
470
480
490
500
510
520
530
540
550
560
570
580
590
600

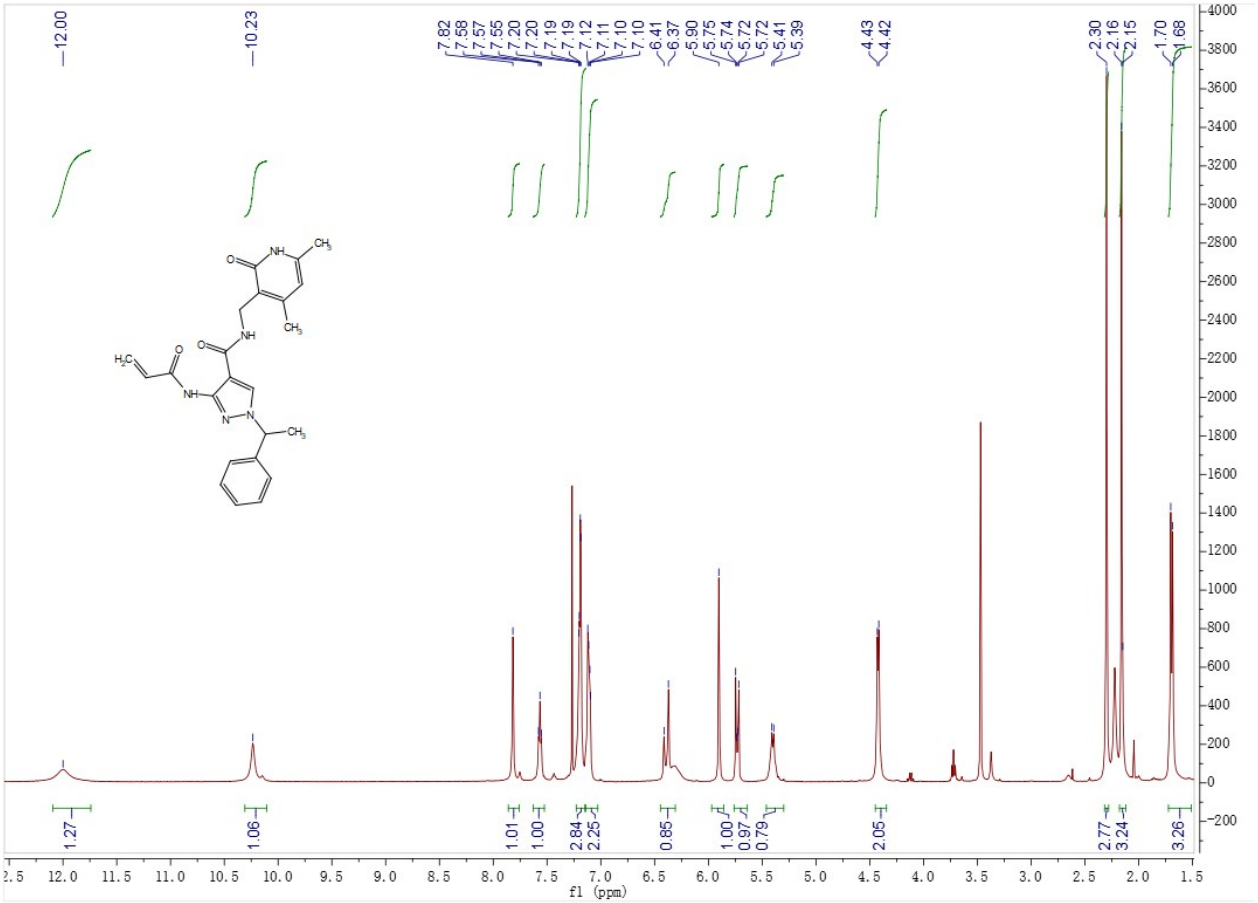
Compound **b7**

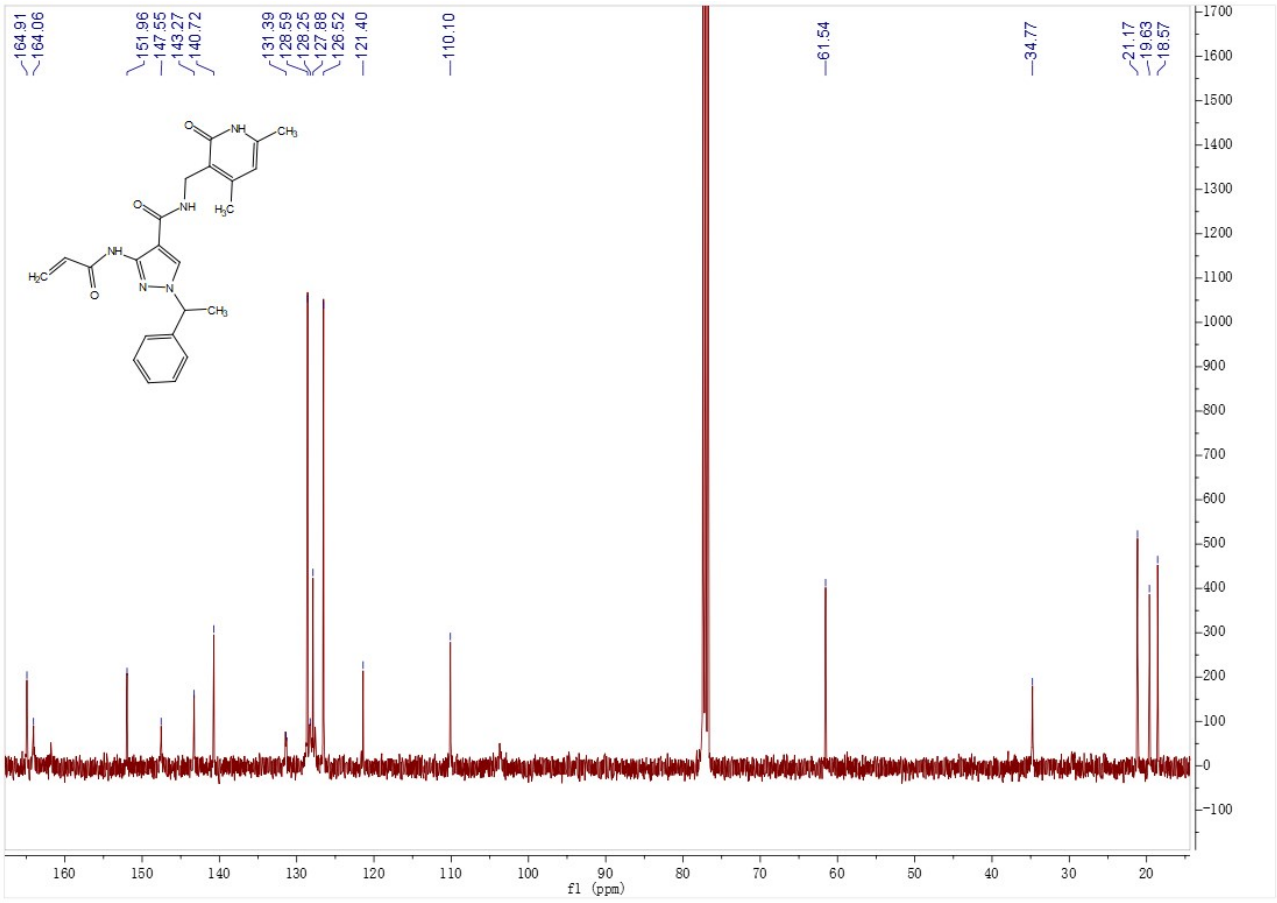

11.42
10.09
7.40
7.35
7.34
7.33
7.33
7.32
7.31
7.31
7.28
7.27
7.27
7.26
7.26
7.24
7.20
7.20
7.19
7.19
7.18
7.18
6.35
6.33
6.31
6.28
6.07
6.03
5.84
5.64
5.64
5.63
5.63
4.17
4.16
4.15
4.15
2.47
2.45
2.43
2.41
2.36
2.35
2.10
2.10
1.74
1.73
1.07
1.05
1.03
0.92
0.88
0.96
2.07
1.07
2.08
0.97
0.95
1.00
1.23
1.94
2.19
3.10
3.06
3.05
3.11
f1 (ppm)

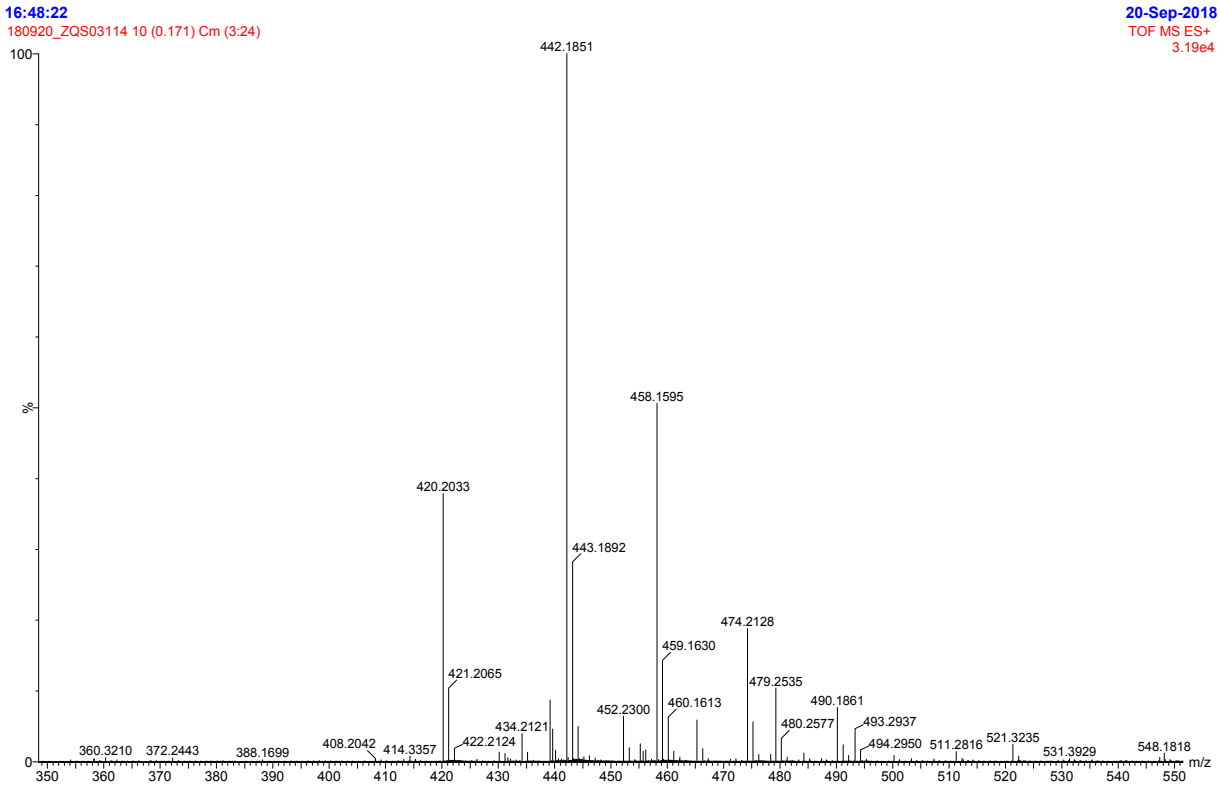

165.25
156.20
143.10
141.53
128.74
127.64
126.00
121.23
108.14
58.28
34.89
26.25
21.47
18.76
14.48
11.13
f1 (ppm)


17:49:36
180920_ZQS03185 6 (0.103) Cm (3:23)
20-Sep-2018
TOF MS ES+
2.22e4
470.2168
486.1908
471.2198
448.2344
467.2036
487.1945
449.2373
472.2225
488.1932
507.2887
413.2587
417.2588
435.2436
459.2209
473.2213
489.1980
508.2928
532.2155
549.3557
564.2007
576.2164
591.4114
600.1857
%
m/z

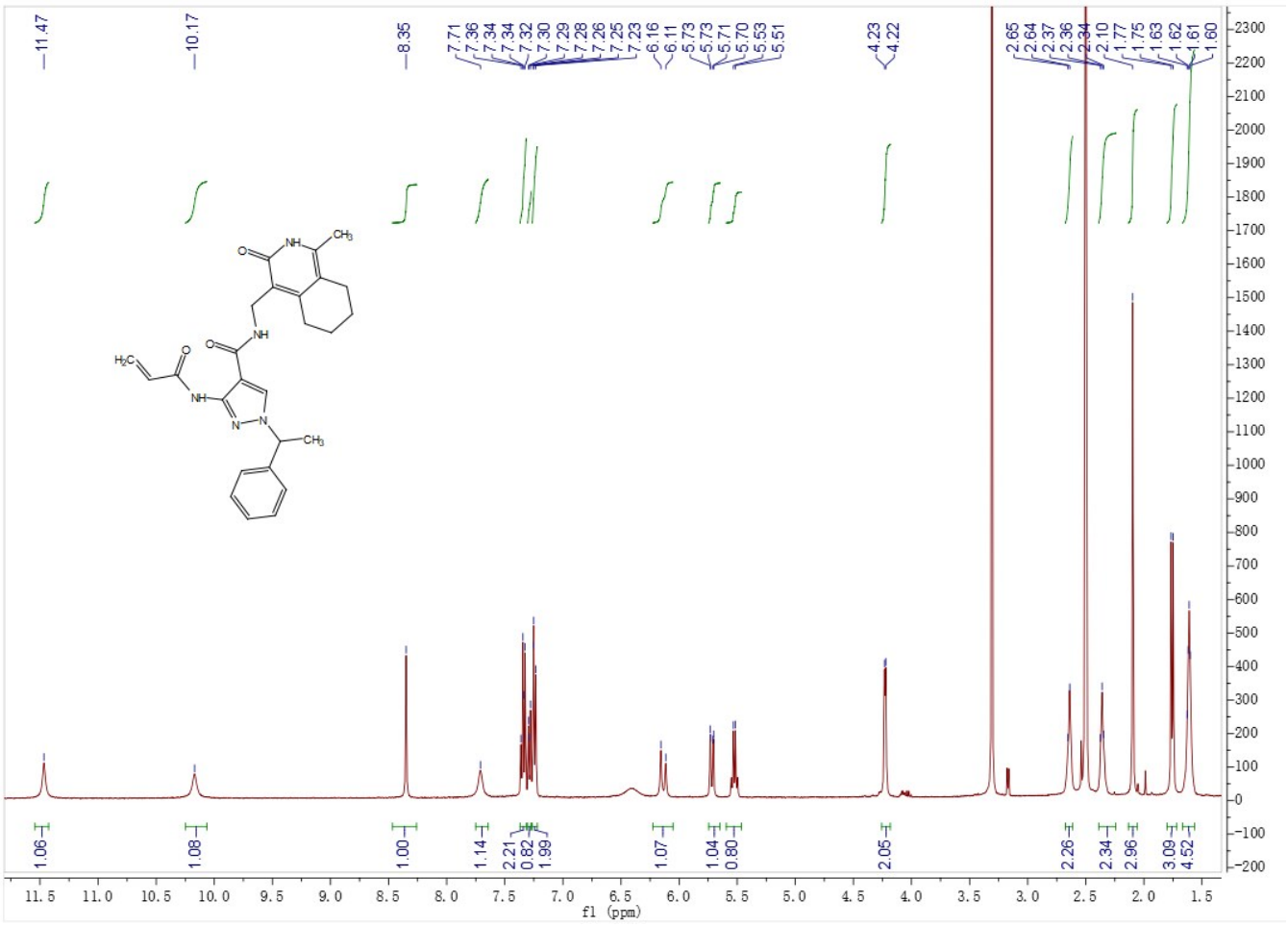
Compound **b8**

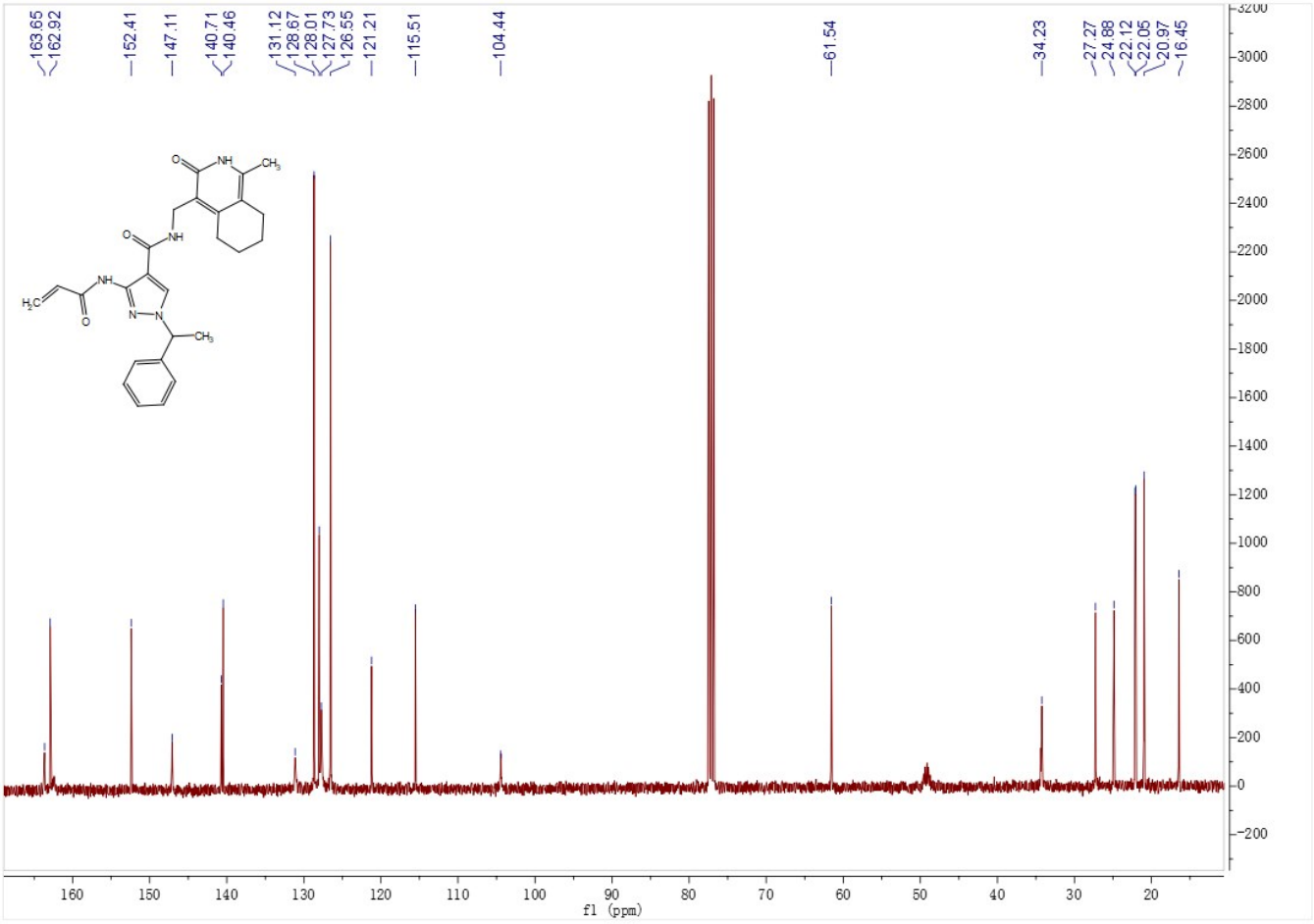

12.00
10.23
7.82
7.58
7.57
7.55
7.20
7.20
7.19
7.19
7.12
7.11
7.10
7.10
6.41
6.37
5.90
5.75
5.74
5.72
5.72
5.41
5.39
4.43
4.42
2.30
2.16
2.15
1.70
1.68
1.27
1.06
1.01
1.00
2.84
2.25
0.85
1.00
0.97
0.79
2.05
2.77
3.24
3.26
f1 (ppm)

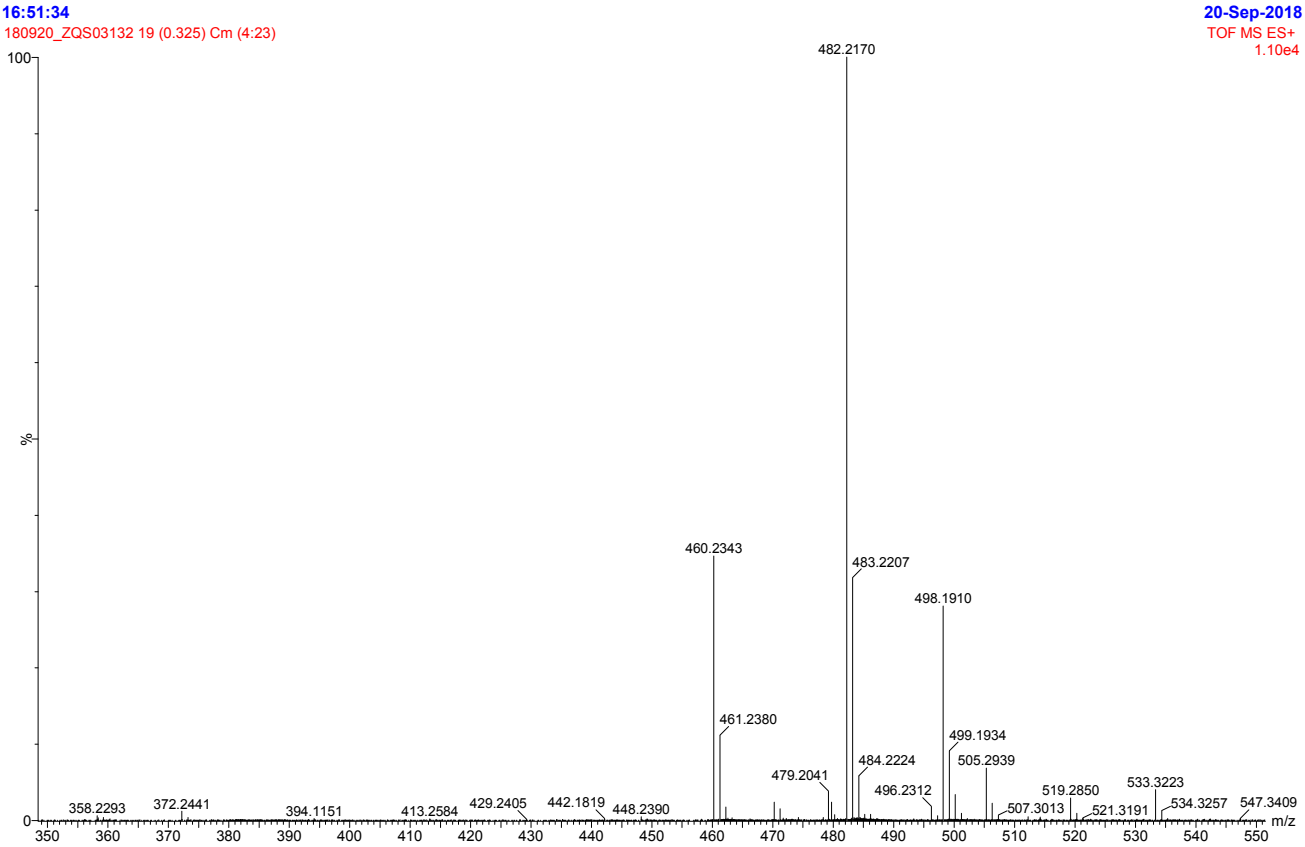

164.91
164.06
151.96
147.55
143.27
140.72
131.39
128.59
128.25
127.88
126.52
121.40
110.10
61.54
34.77
21.17
19.63
18.57
f1 (ppm)


16:48:22
180920_ZQS03114 10 (0.171) Cm (3:24)
20-Sep-2018
TOF MS ES+
3.19e4
442.1851
458.1595
420.2033
443.1892
474.2128
459.1630
421.2065
479.2535
490.1861
452.2300
460.1613
480.2577
493.2937
434.2121
422.2124
494.2950
511.2816
521.3235
360.3210
372.2443
388.1699
408.2042
414.3357
531.3929
548.1818
%
m/z

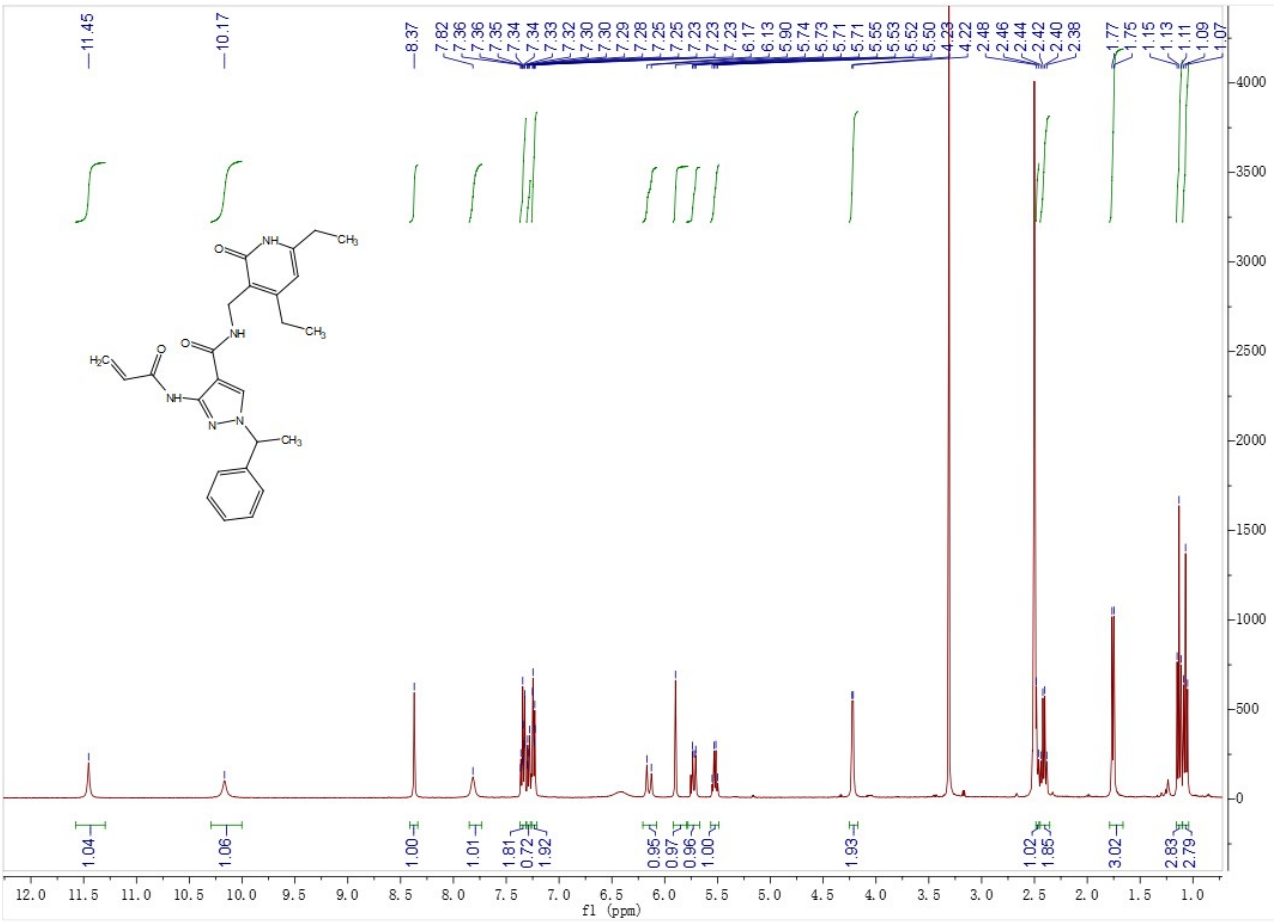
Compound **b9**

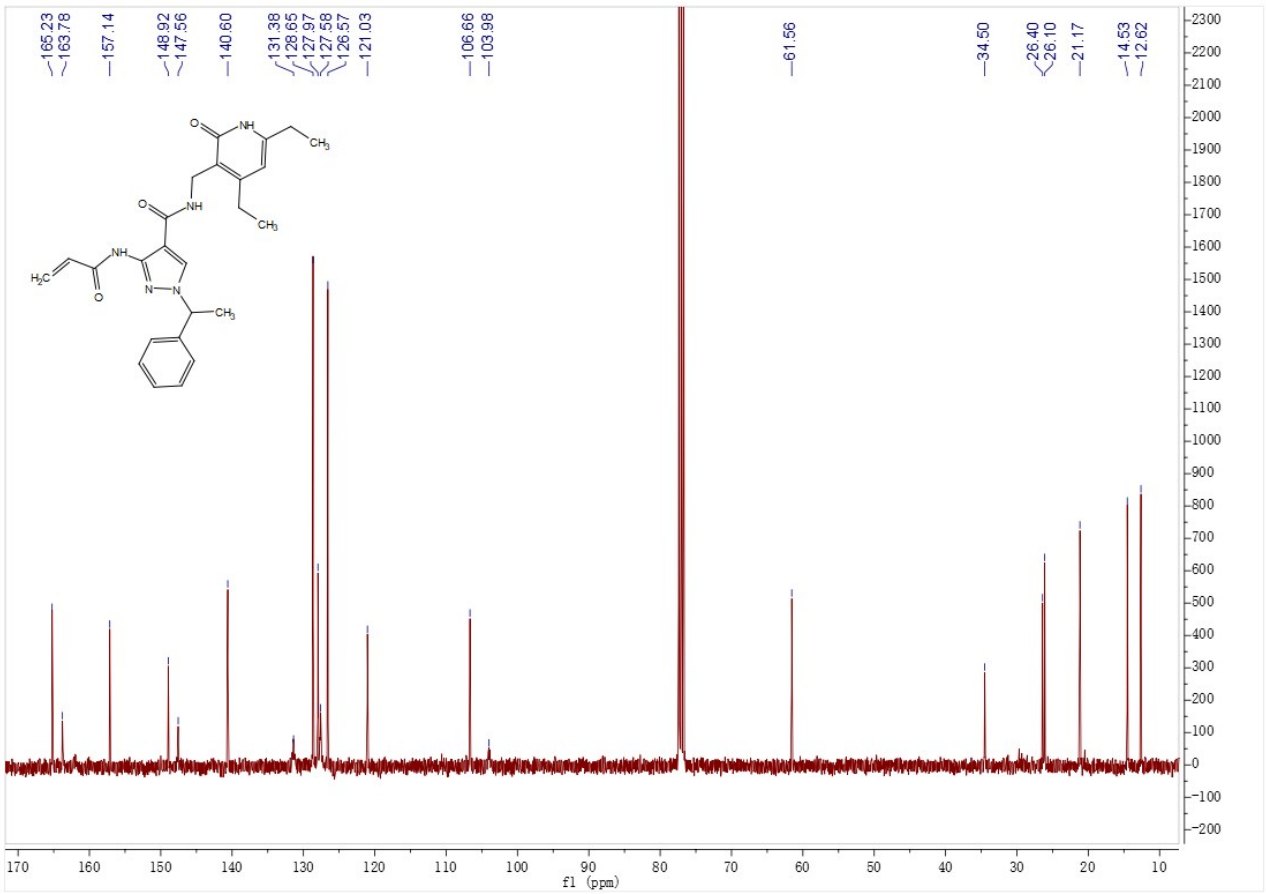

11.47
10.17
8.35
7.71
7.36
7.34
7.34
7.32
7.30
7.29
7.28
7.26
7.25
7.23
6.16
6.11
5.73
5.73
5.71
5.70
5.53
5.51
4.23
4.22
2.65
2.64
2.37
2.36
2.34
2.10
1.77
1.75
1.63
1.62
1.61
1.60
1.06
1.08
1.00
1.14
2.21
0.82
1.99
1.07
1.04
0.80
2.05
2.26
2.34
2.96
3.09
4.52
f1 (ppm)

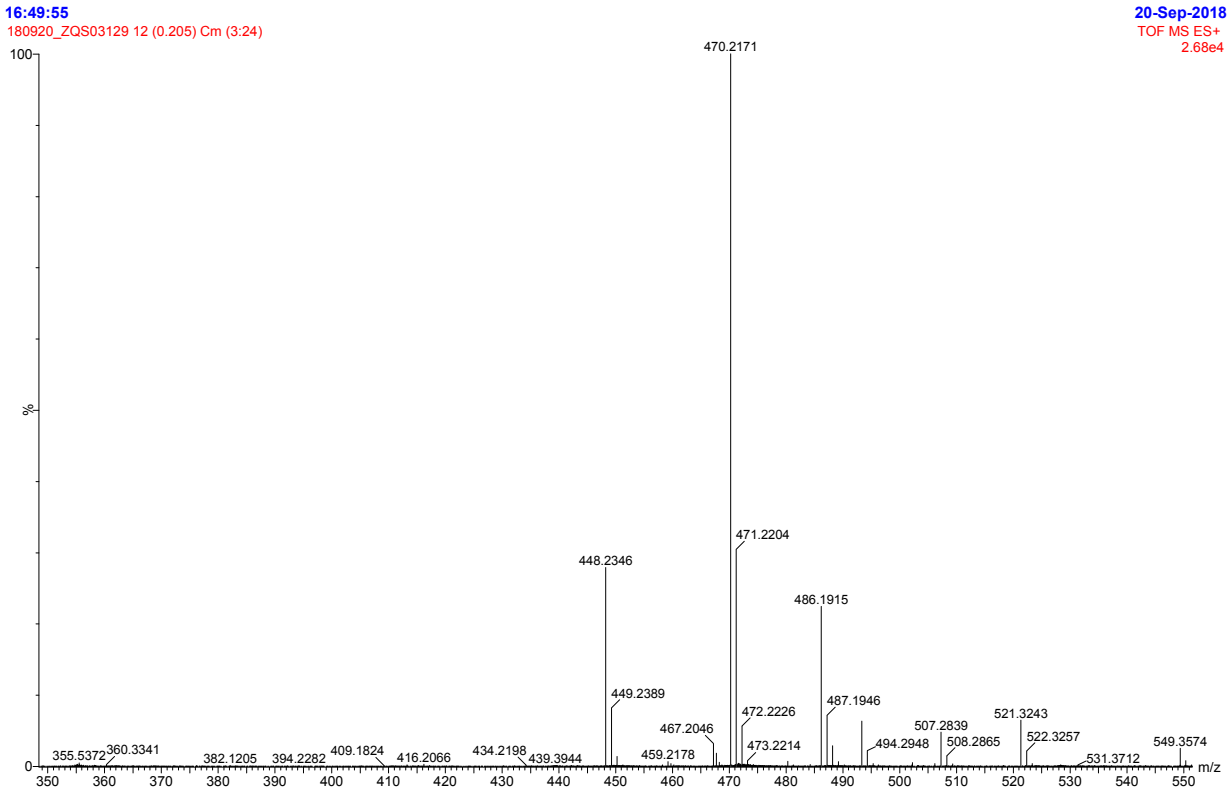

163.65
162.92
152.41
147.11
140.71
140.46
131.12
128.67
128.01
127.73
126.55
121.21
115.51
104.44
61.54
34.23
27.27
24.88
22.12
22.05
20.97
16.45
f1 (ppm)


16:51:34
180920_ZQS03132 19 (0.325) Cm (4:23)
20-Sep-2018
TOF MS ES+
1.10e4
%
m/z
482.2170
460.2343
483.2207
498.1910
461.2380
499.1934
505.2939
484.2224
479.2041
496.2312
519.2850
533.3223
507.3013
521.3191
534.3257
547.3409
358.2293
372.2441
394.1151
413.2584
429.2405
442.1819
448.2390

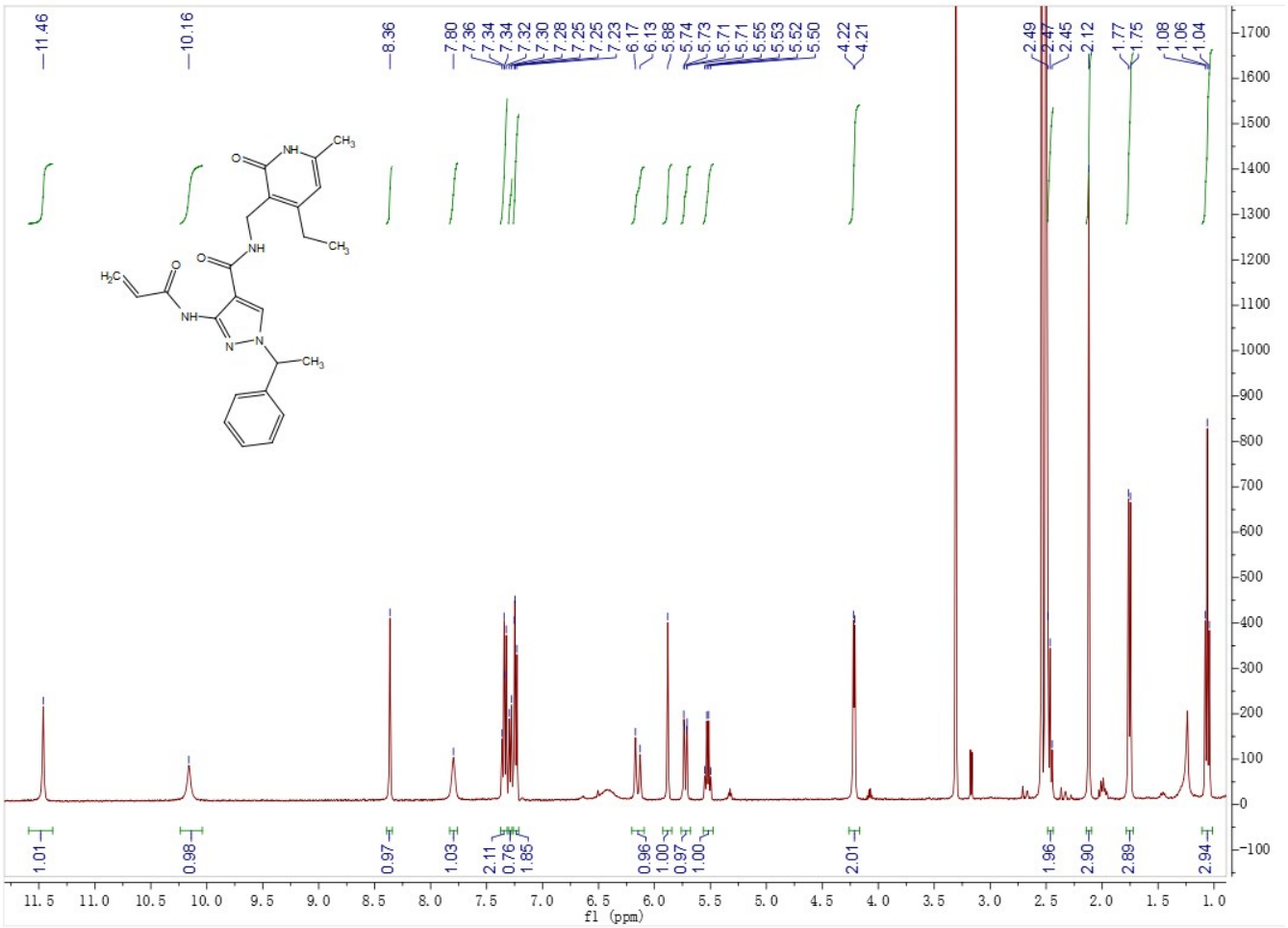
Compound **b10**

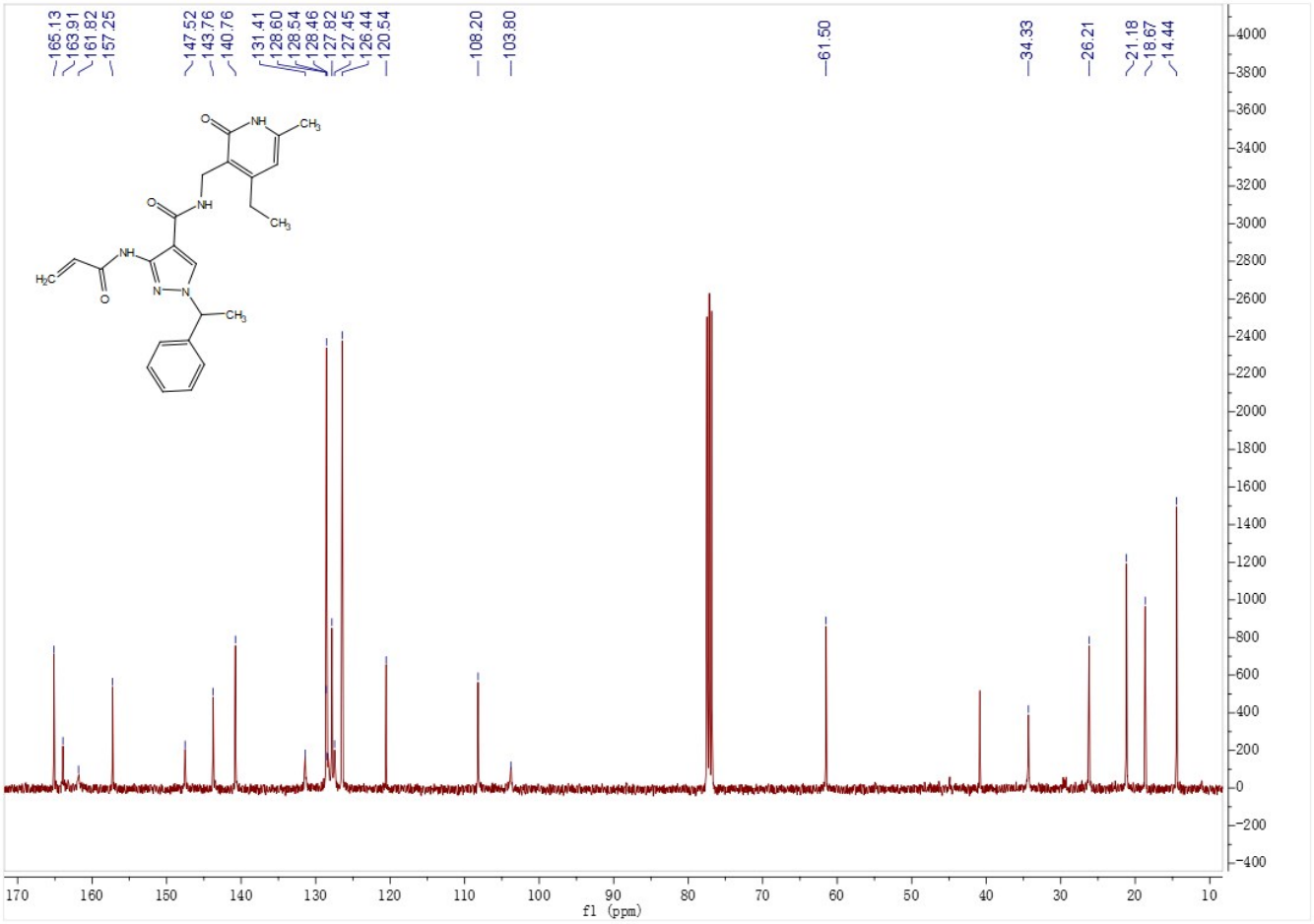






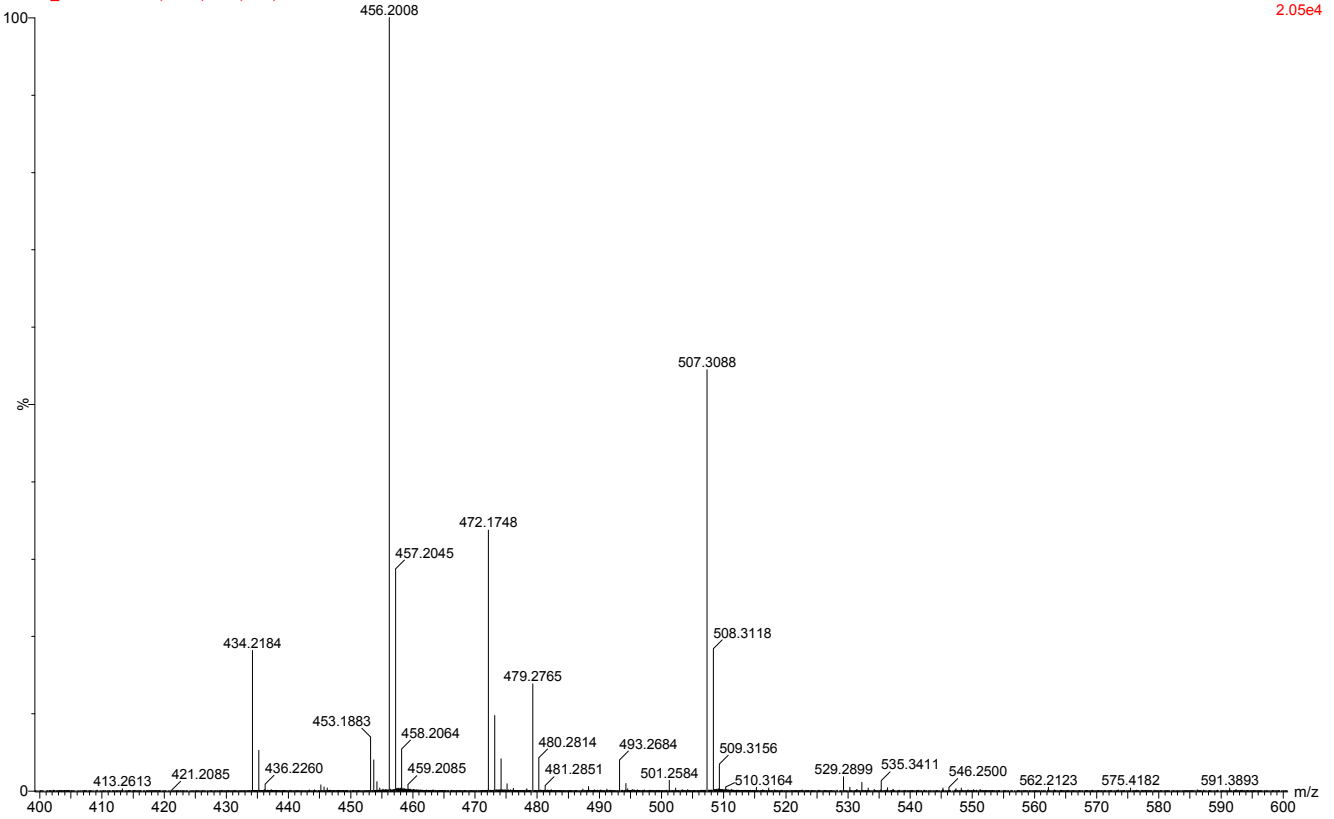
Compound **b11**



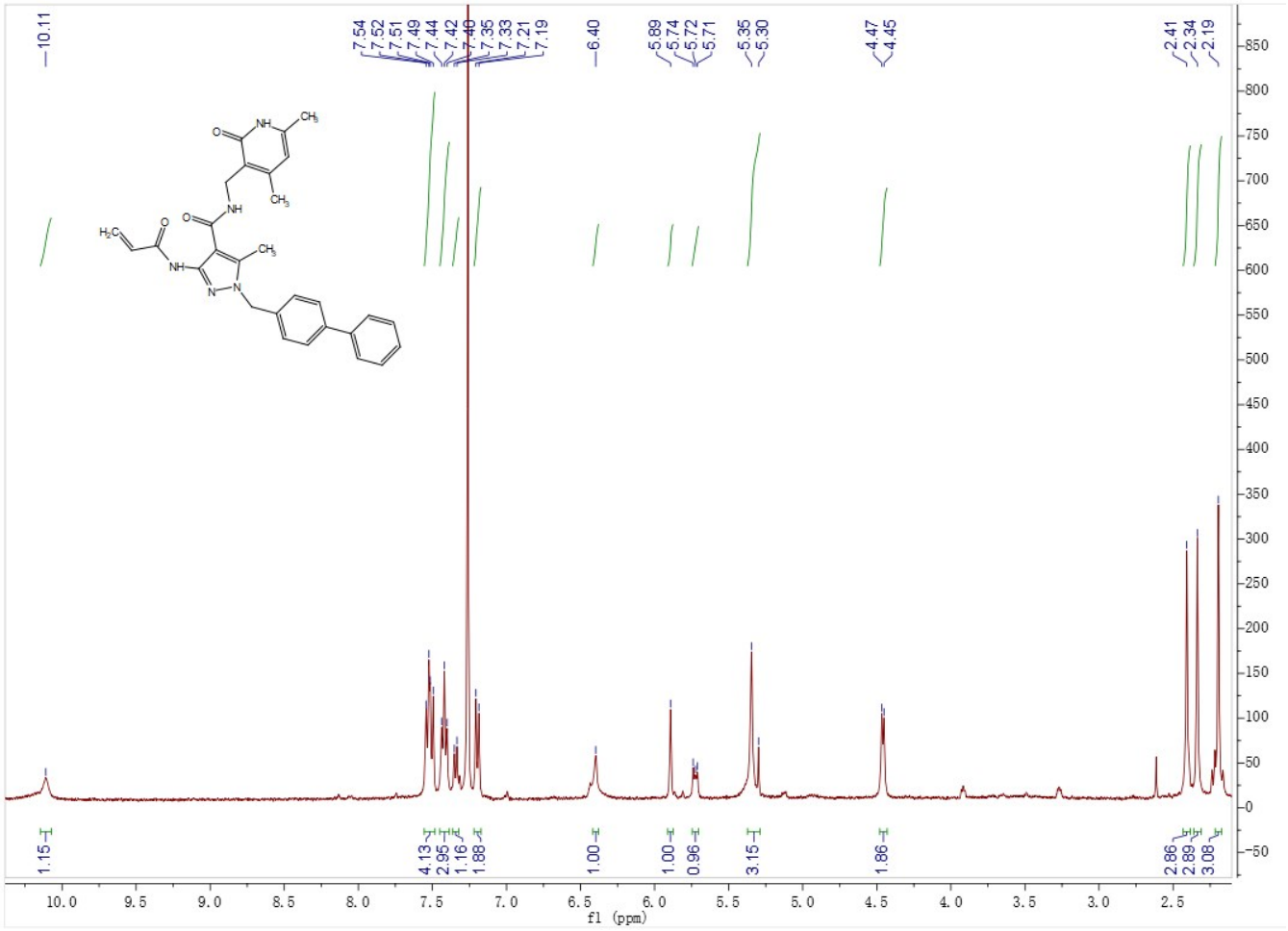


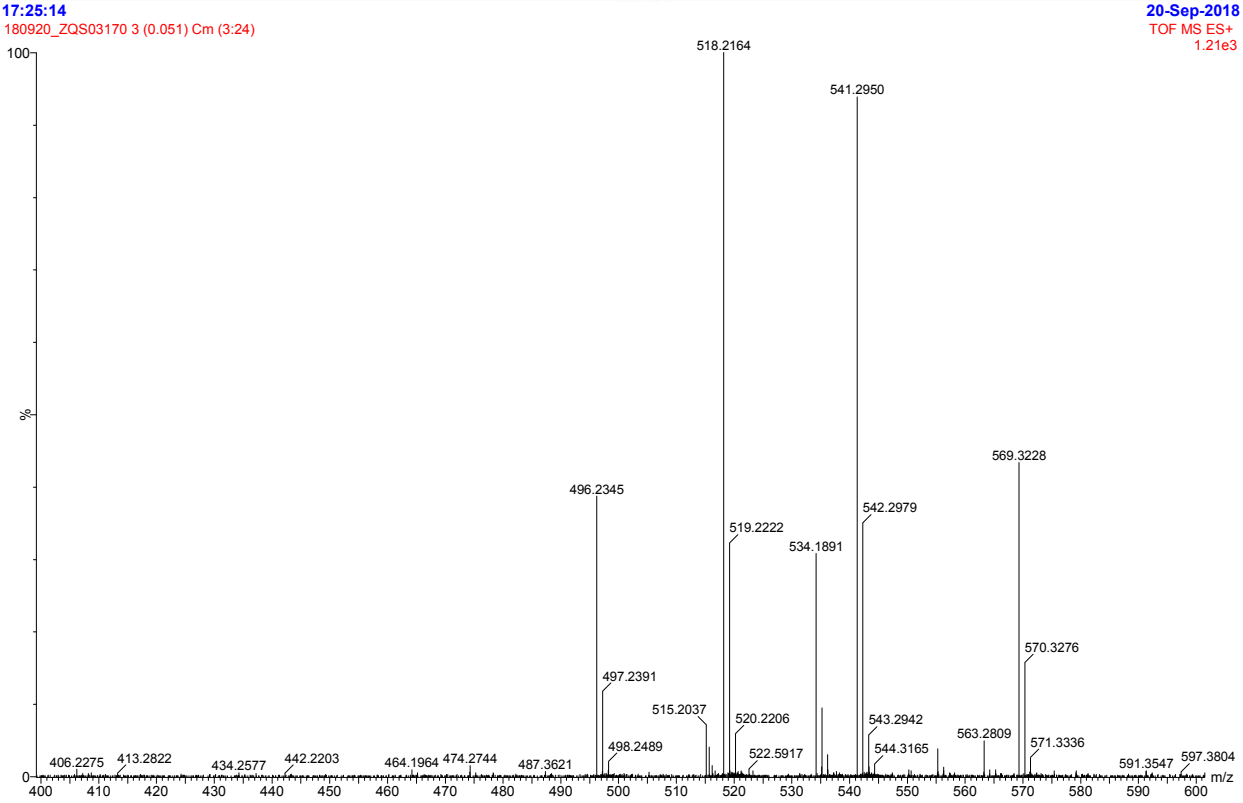
17:48:13
180920_ZQS03183 23 (0.393) Cm (3:24)

20-Sep-2018
TOF MS ES+
2.05e4

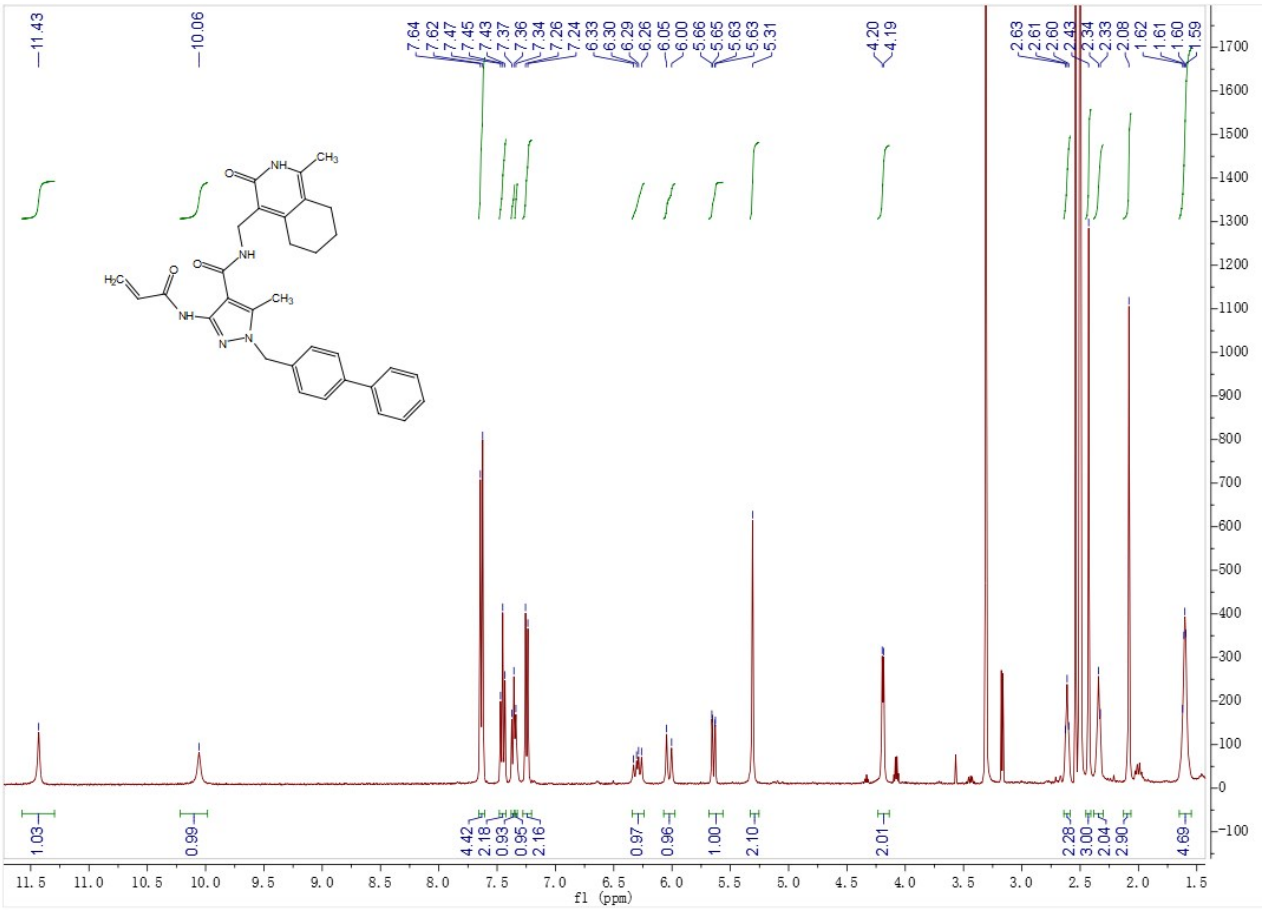


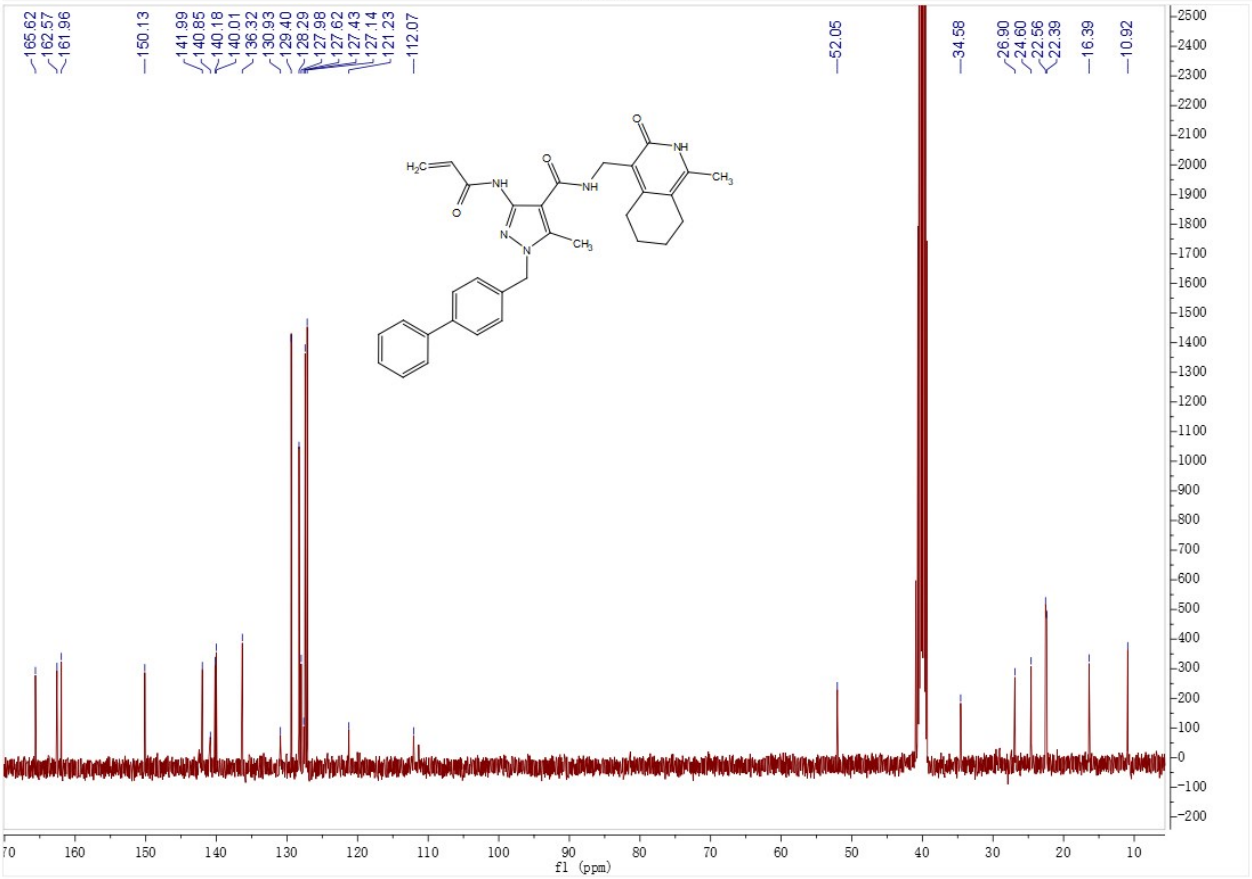
Compound **b12**

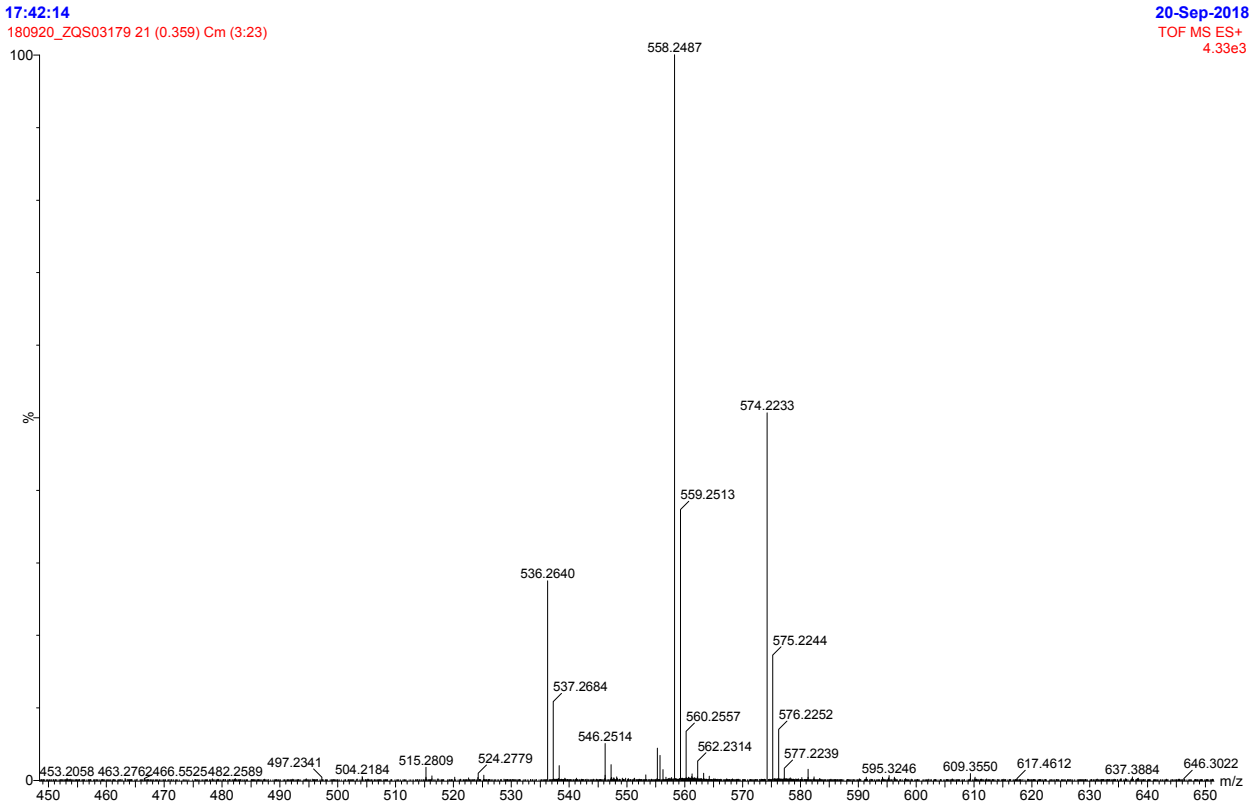




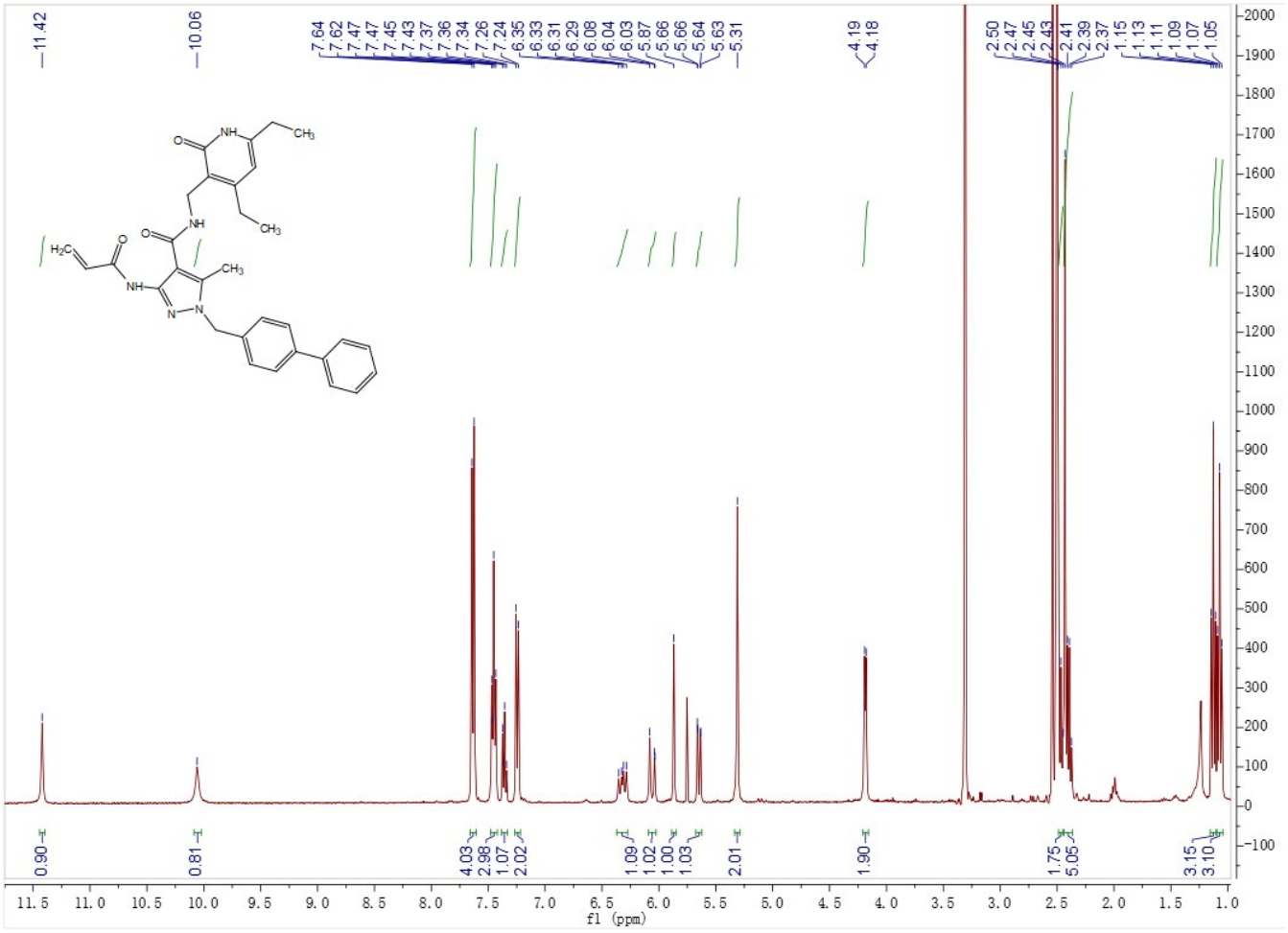
Compound **b13**

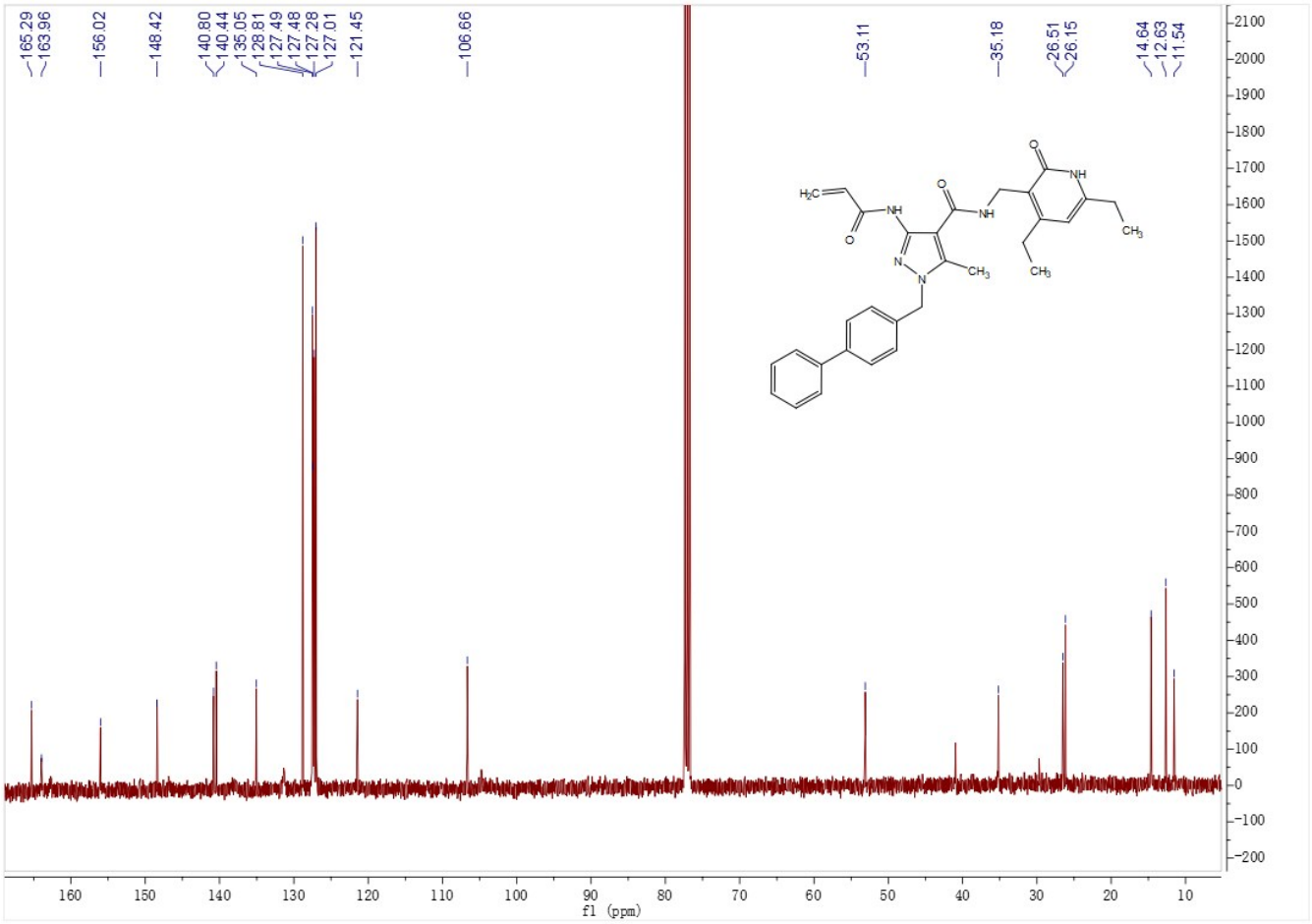






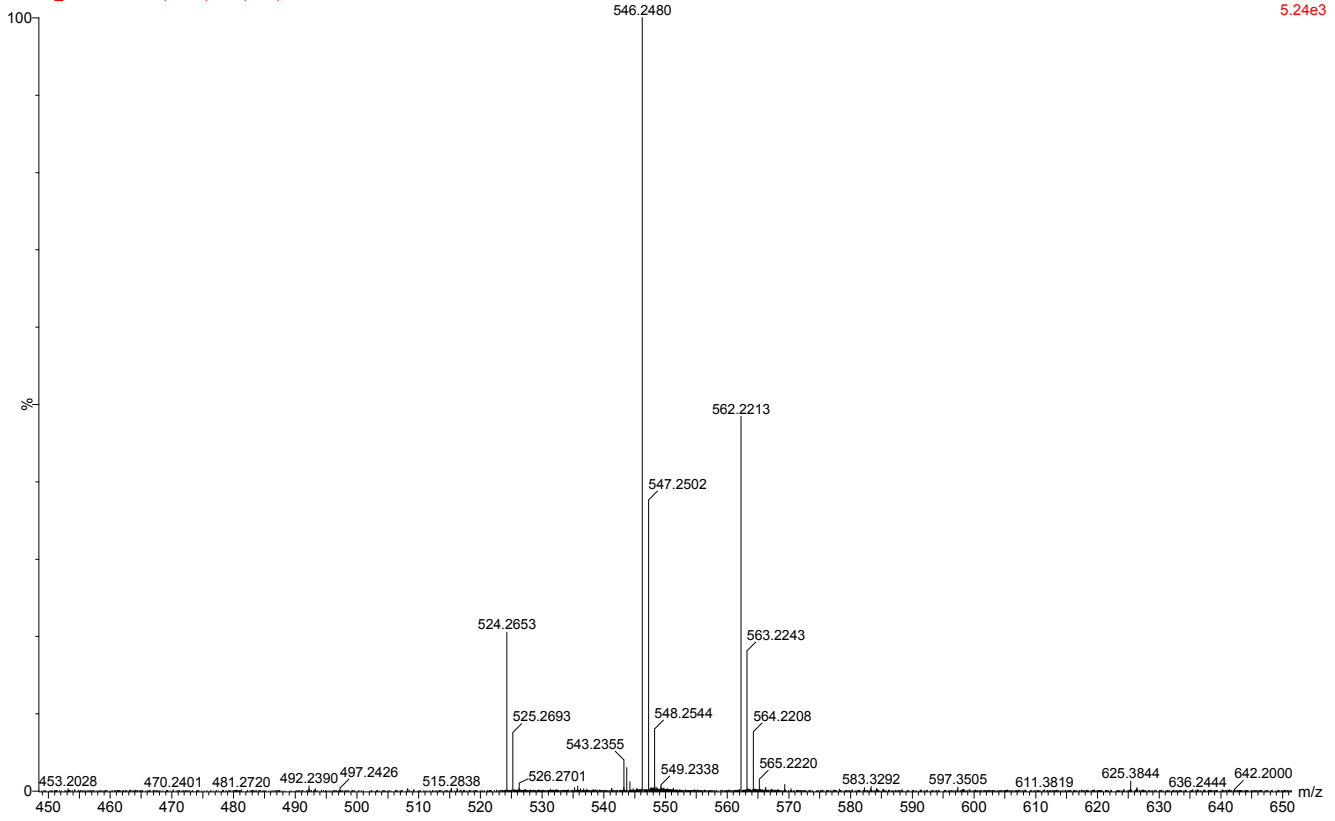
Compound **b14**



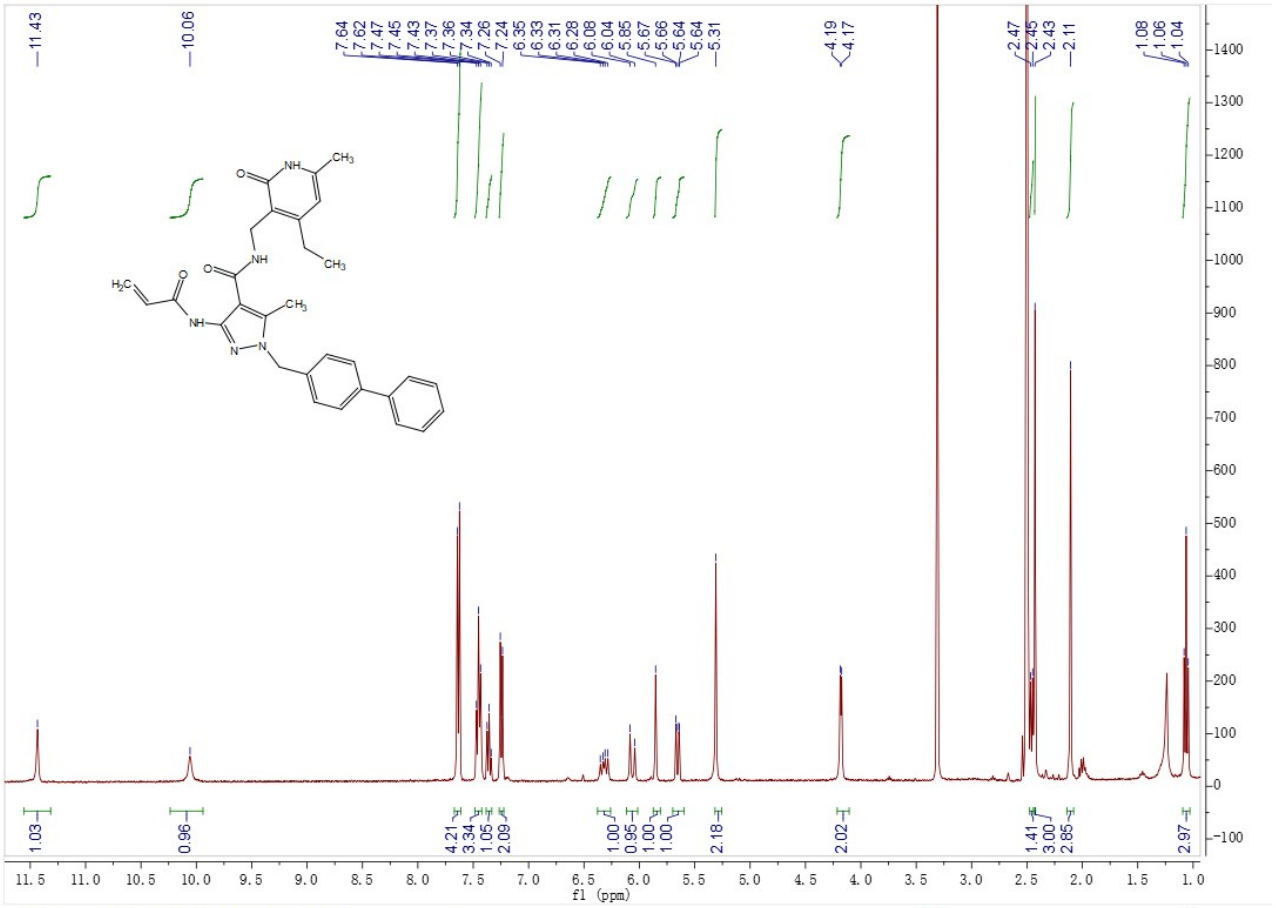


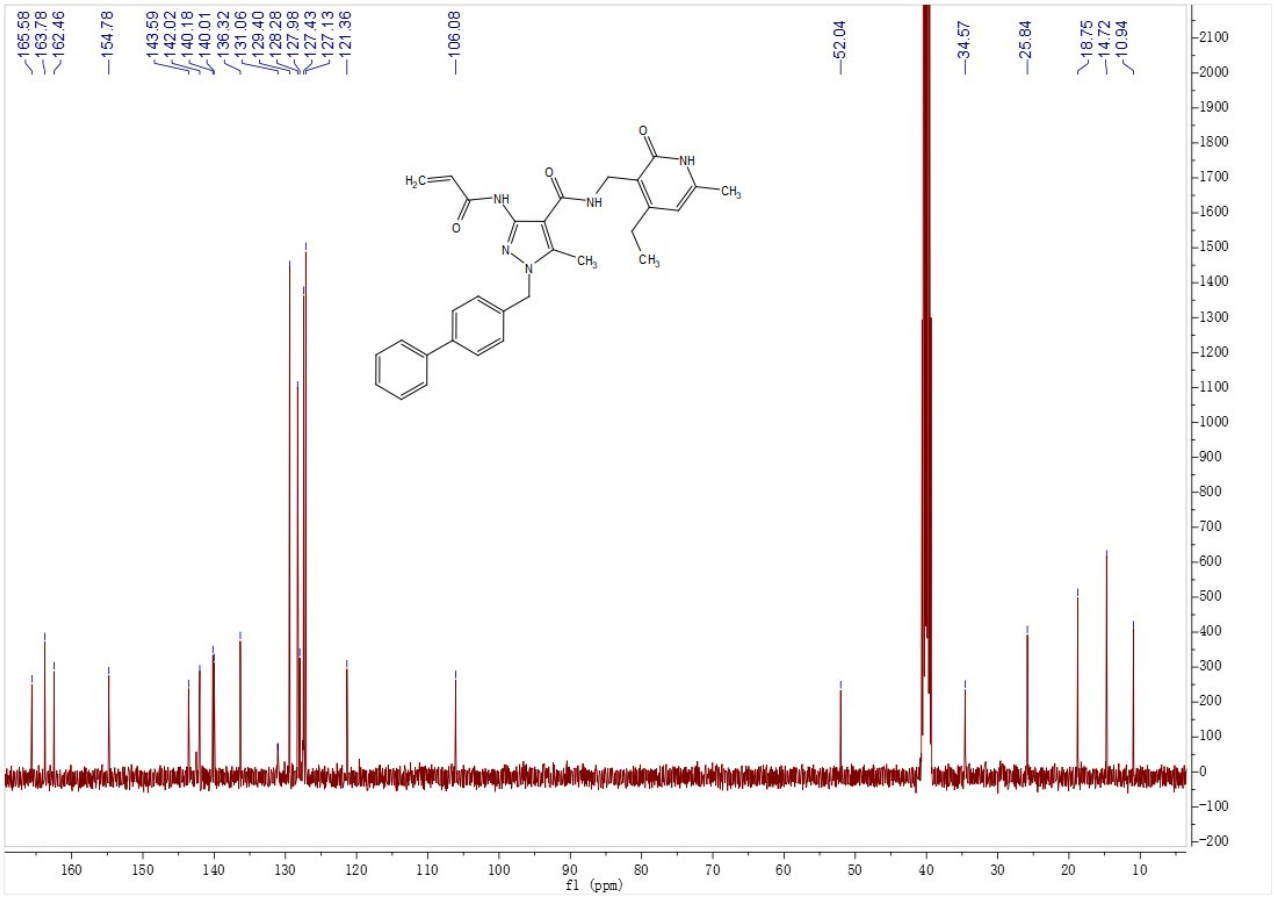
17:39:58
180920_ZQS03178 18 (0.308) Cm (4:23)

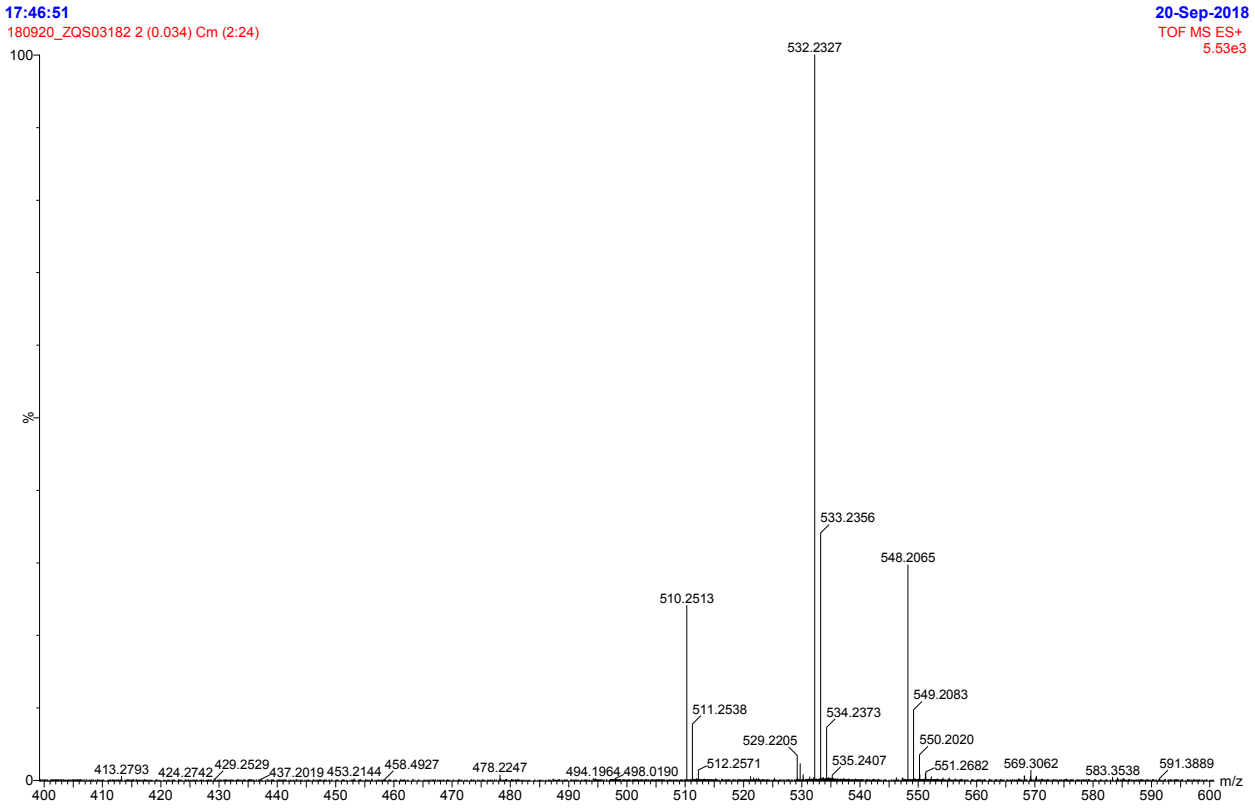
20-Sep-2018
TOF MS ES+
5.24e3



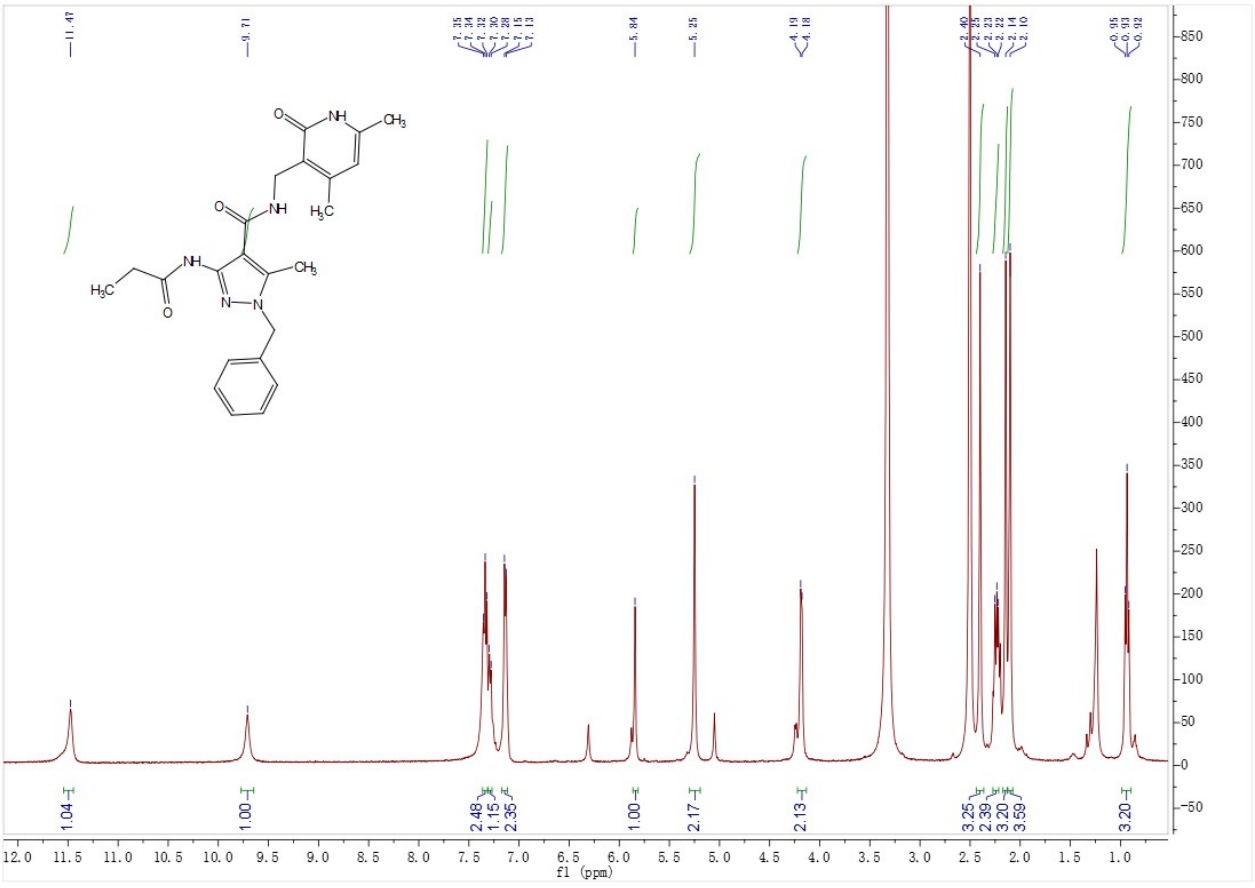
Compound **b15**

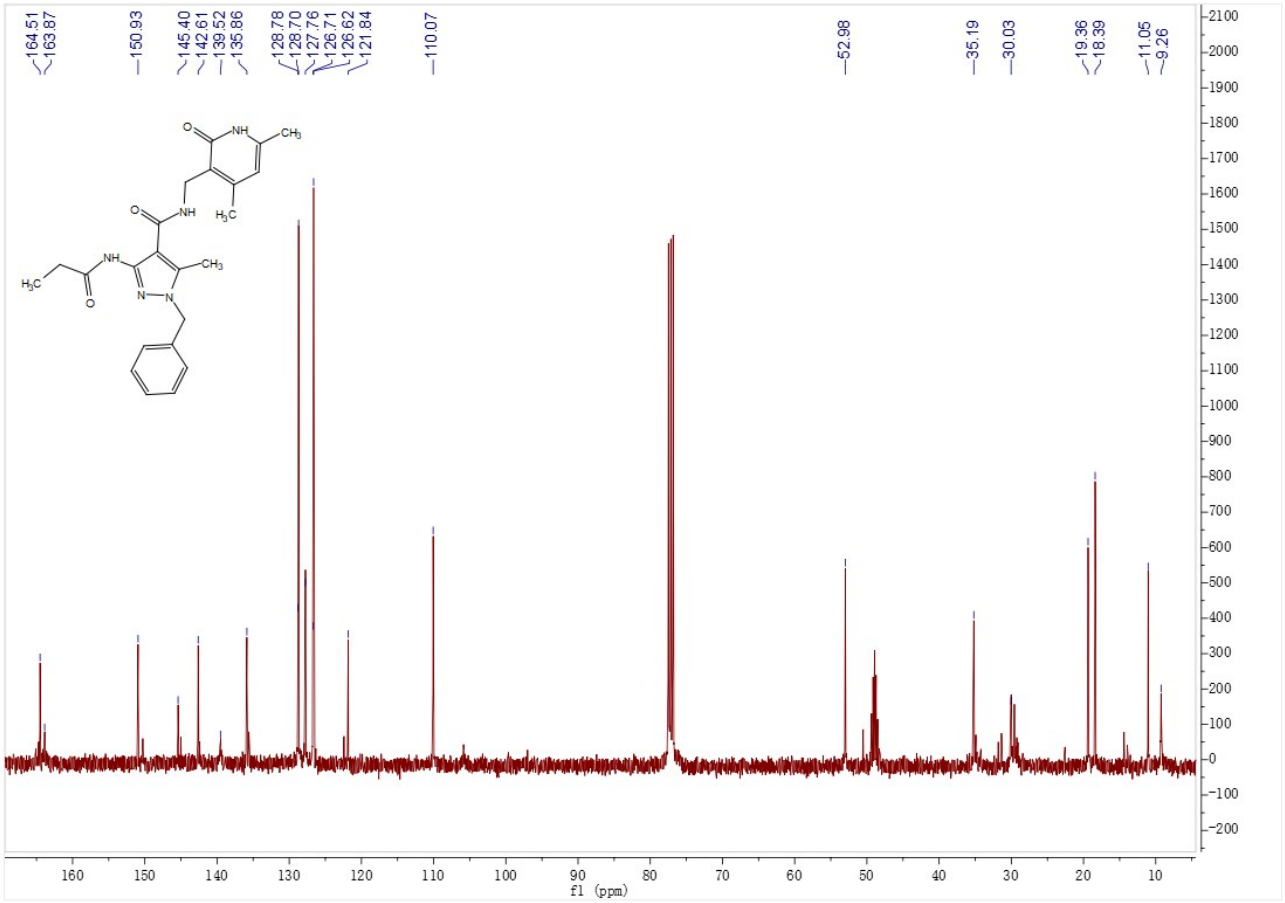


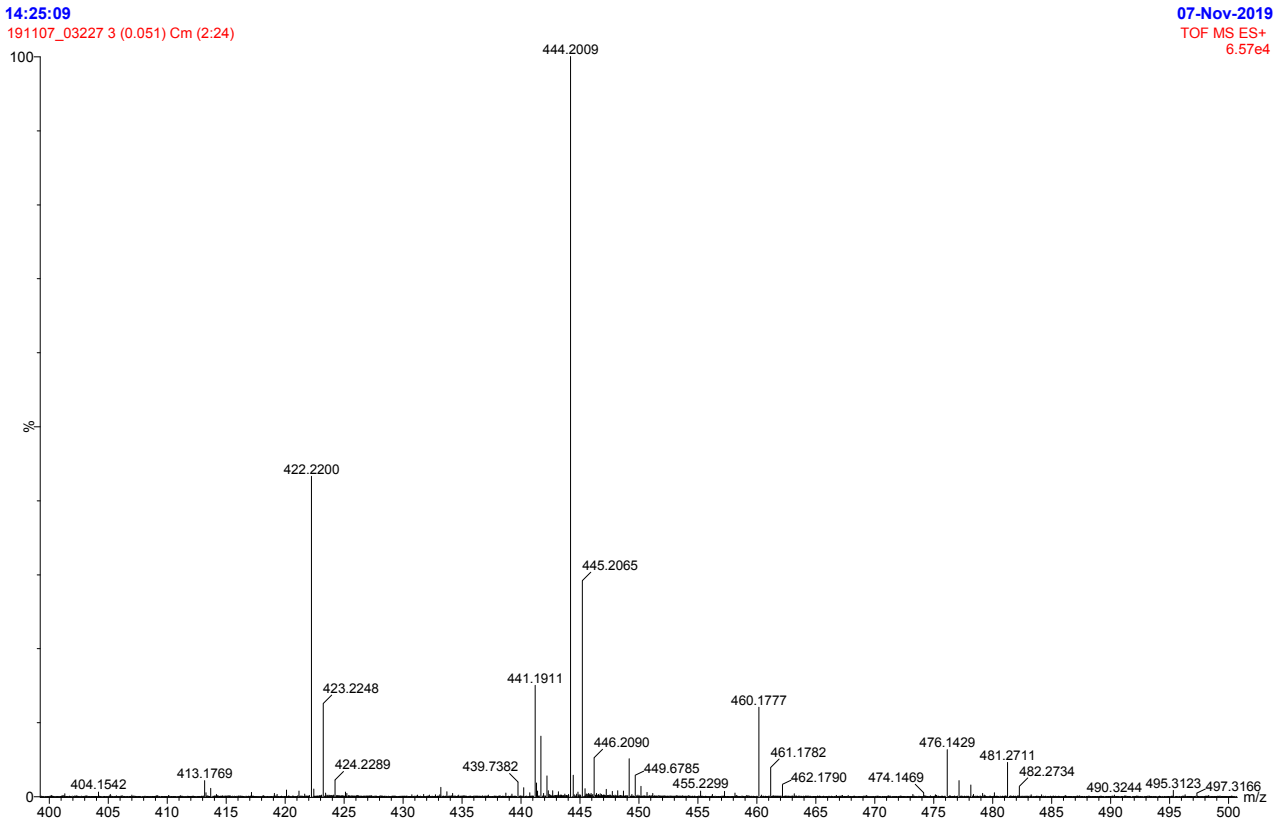



17:46:51
180920_ZQS03182 2 (0.034) Cm (2:24)
20-Sep-2018
TOF MS ES+
5.53e3
100
%
0
532.2327
533.2356
548.2065
510.2513
549.2083
511.2538
534.2373
529.2205
550.2020
535.2407
512.2571
551.2682
569.3062
583.3538
591.3889
413.2793
424.2742
429.2529
437.2019
453.2144
458.4927
478.2247
494.1964
498.0190
m/z

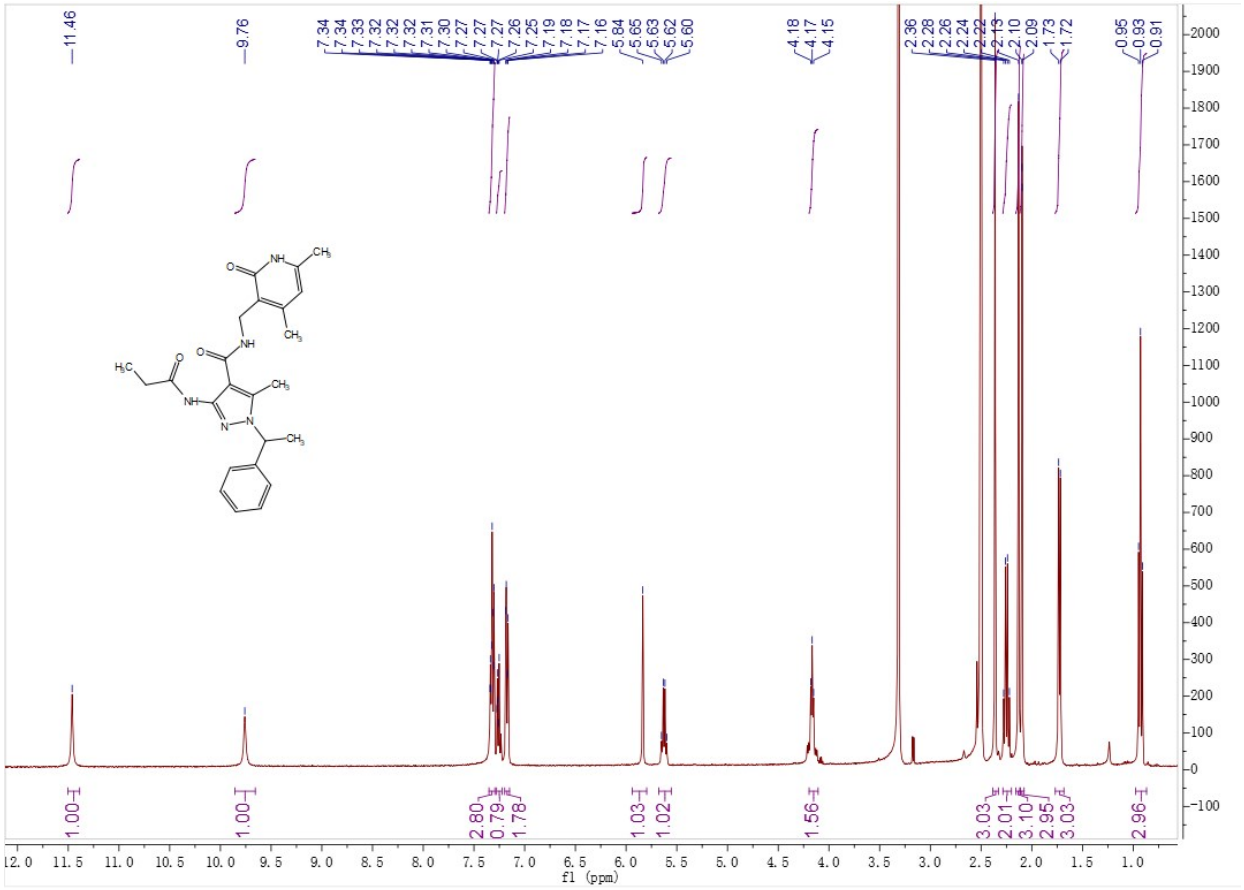
Compound **b1'**

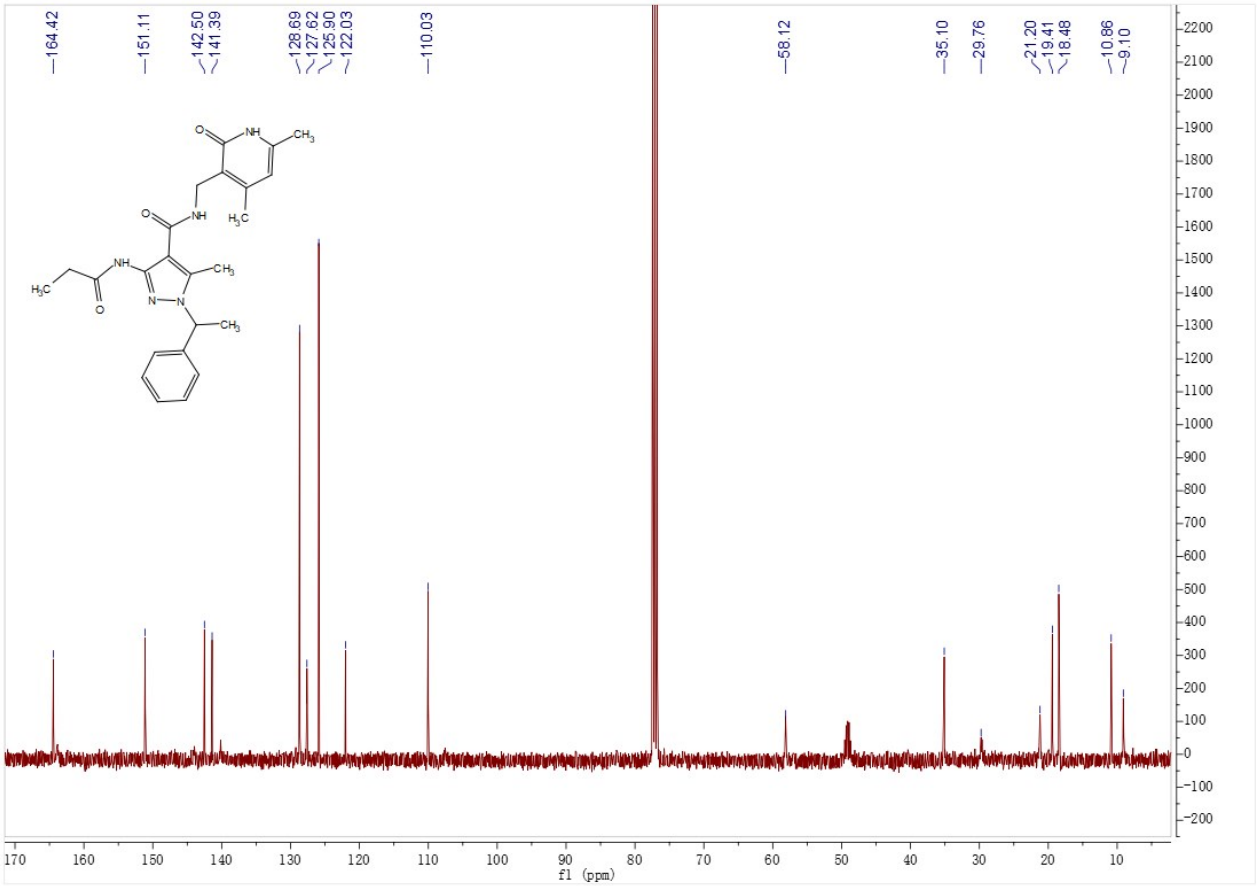

11.47
9.71
7.35
7.34
7.32
7.30
7.28
7.15
7.13
5.84
5.25
4.19
4.18
2.40
2.25
2.23
2.22
2.14
2.10
0.95
0.93
0.92
1.04
1.00
2.48
1.15
2.35
1.00
2.17
2.13
3.25
2.39
3.20
3.59
3.20
f1 (ppm)

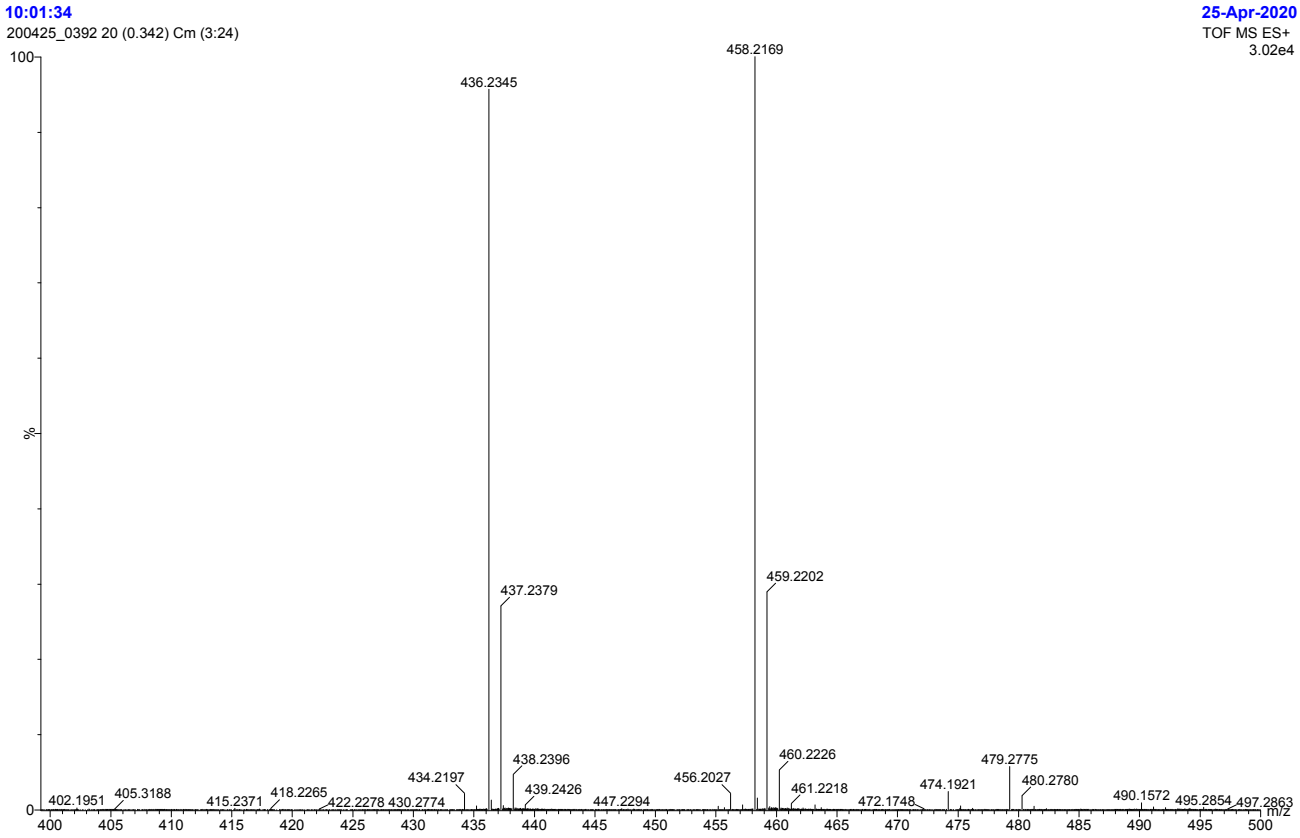

164.51
163.87
150.93
145.40
142.61
139.52
135.86
128.78
128.70
127.76
126.71
126.62
121.84
110.07
52.98
35.19
30.03
19.36
18.39
11.05
9.26
f1 (ppm)

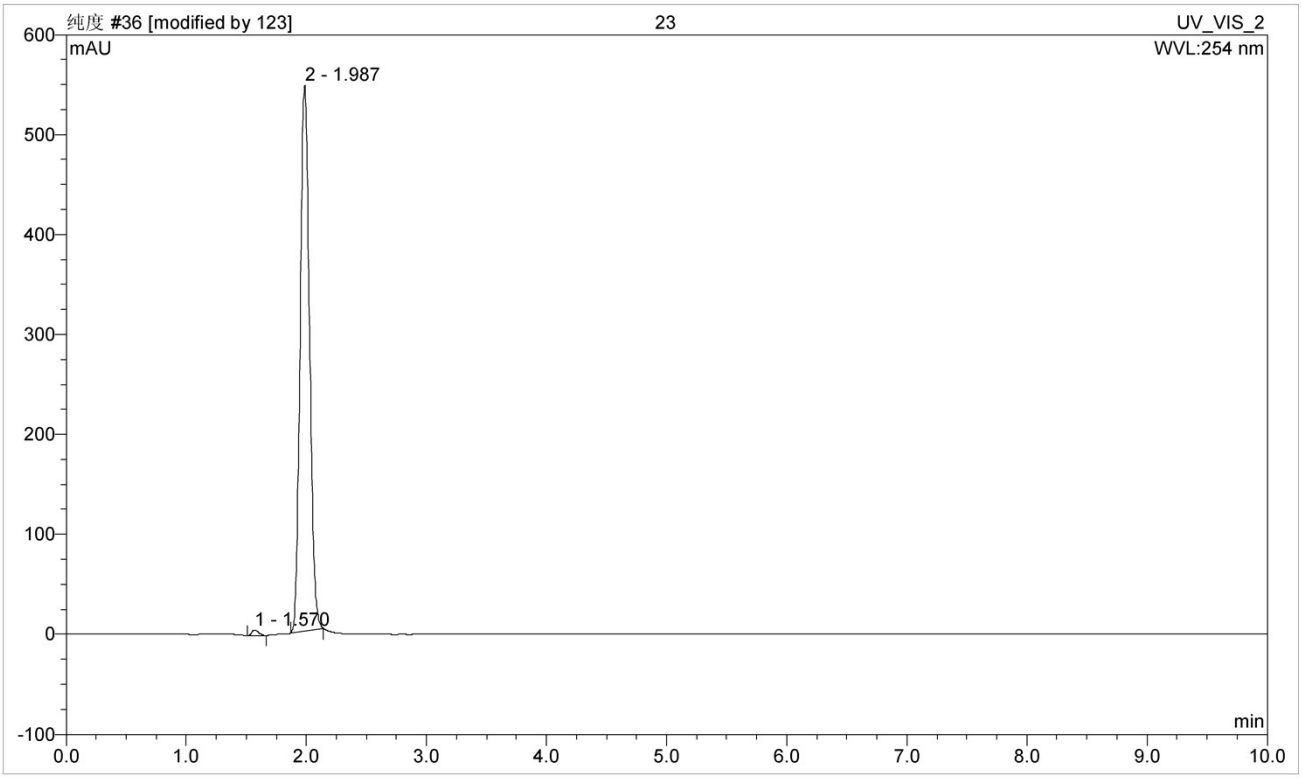

14:25:09
191107_03227 3 (0.051) Cm (2:24)
07-Nov-2019
TOF MS ES+
6.57e4
444.2009
422.2200
445.2065
423.2248
441.1911
460.1777
446.2090
461.1782
476.1429
481.2711
482.2734
424.2289
439.7382
449.6785
455.2299
462.1790
474.1469
404.1542
413.1769
490.3244
495.3123
497.3166
%
m/z

Compound SKLB-0335’



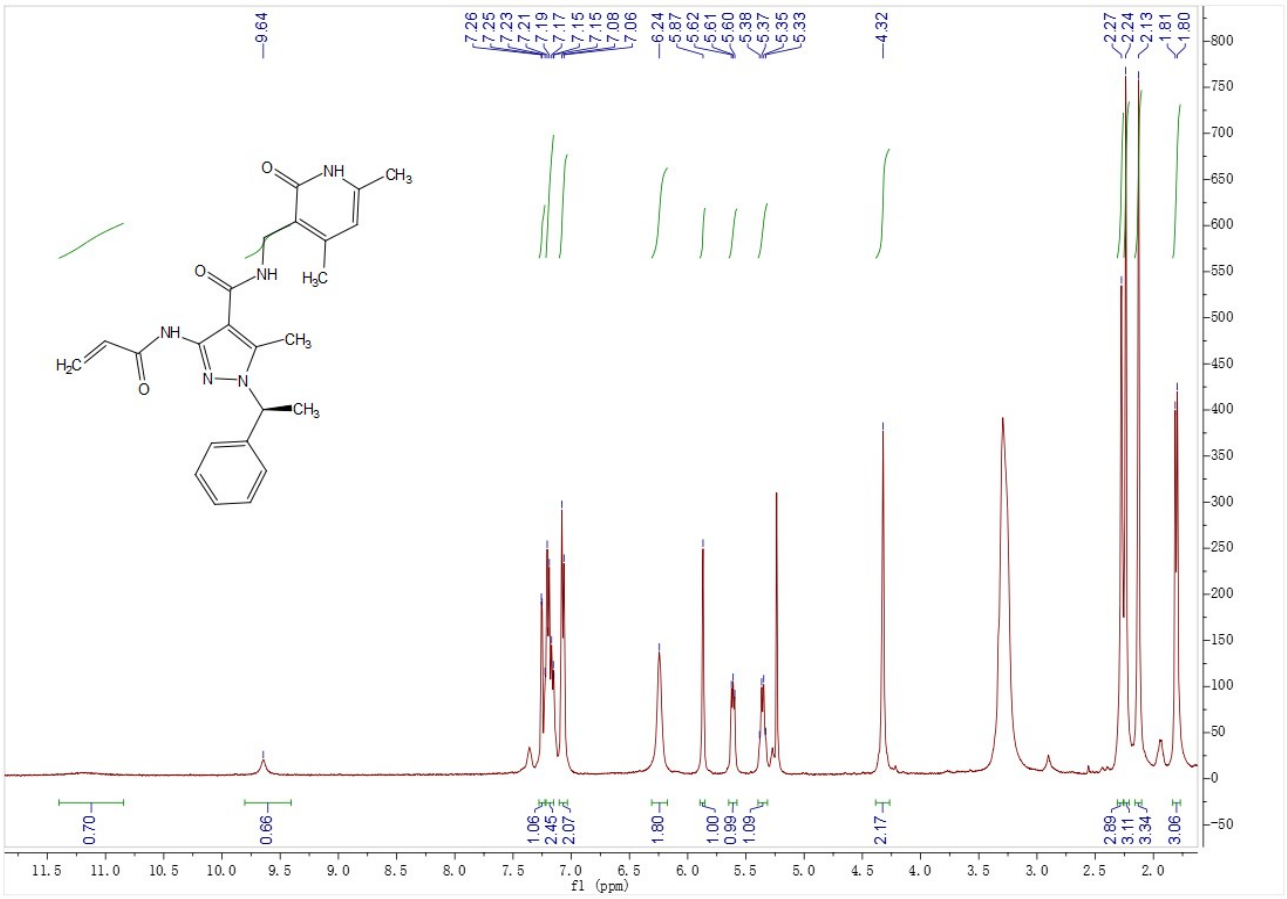


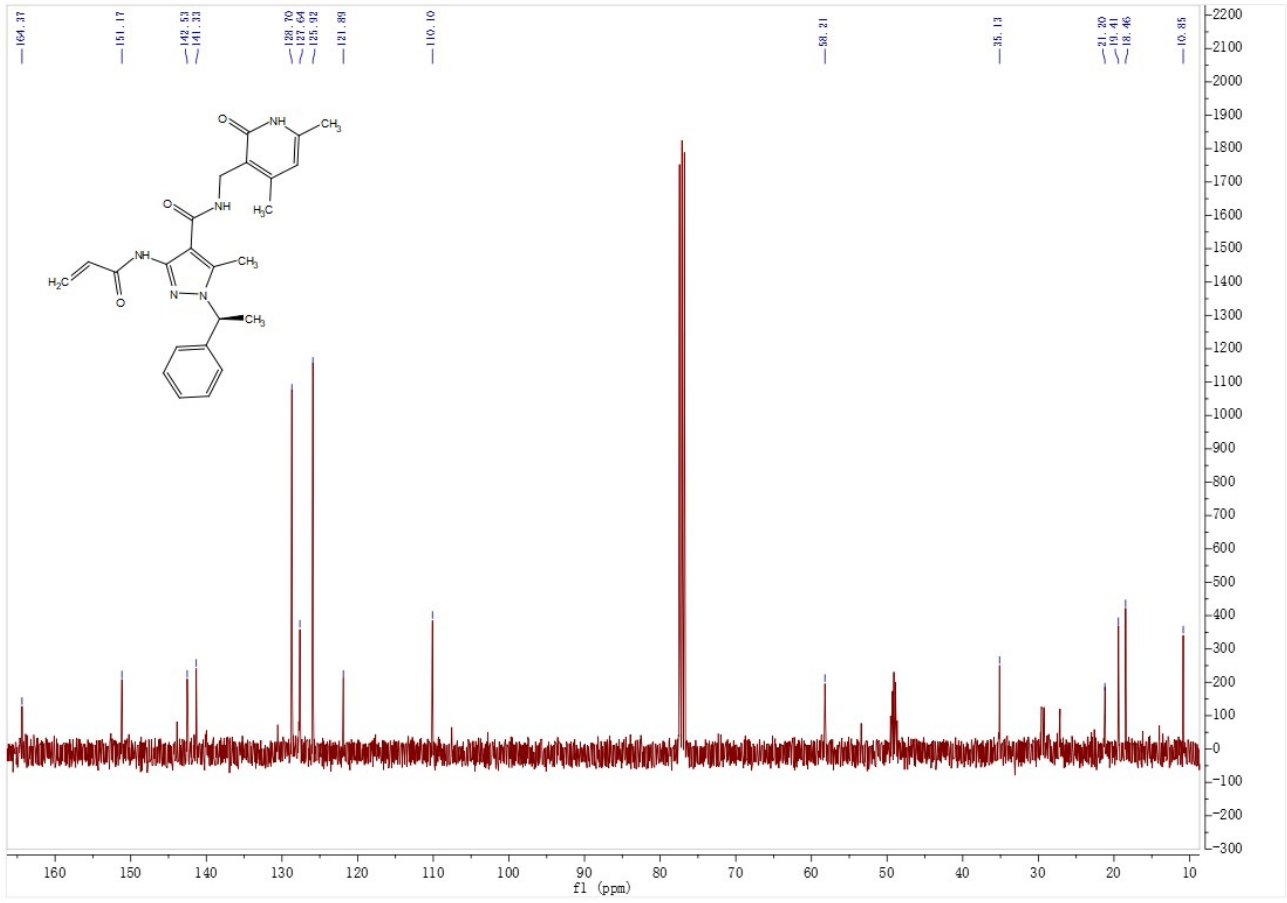


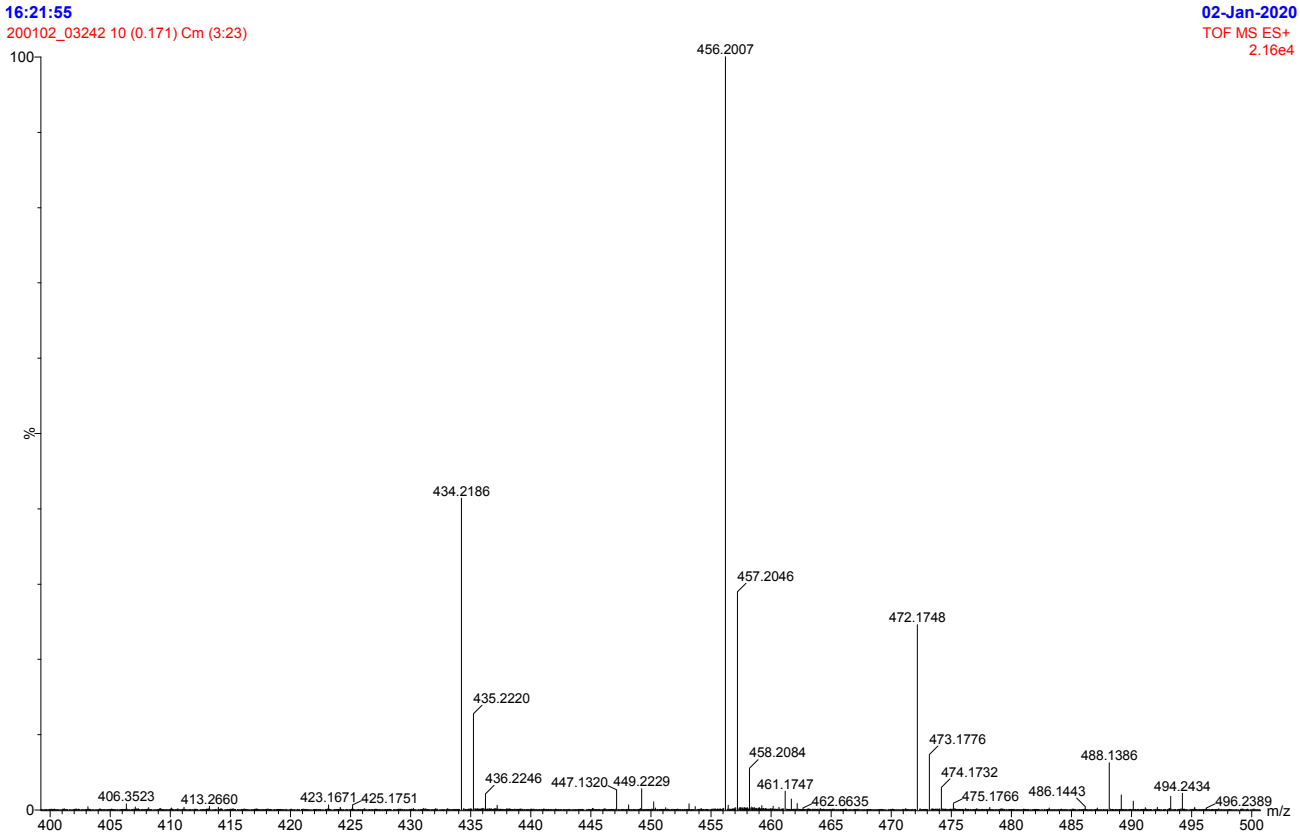


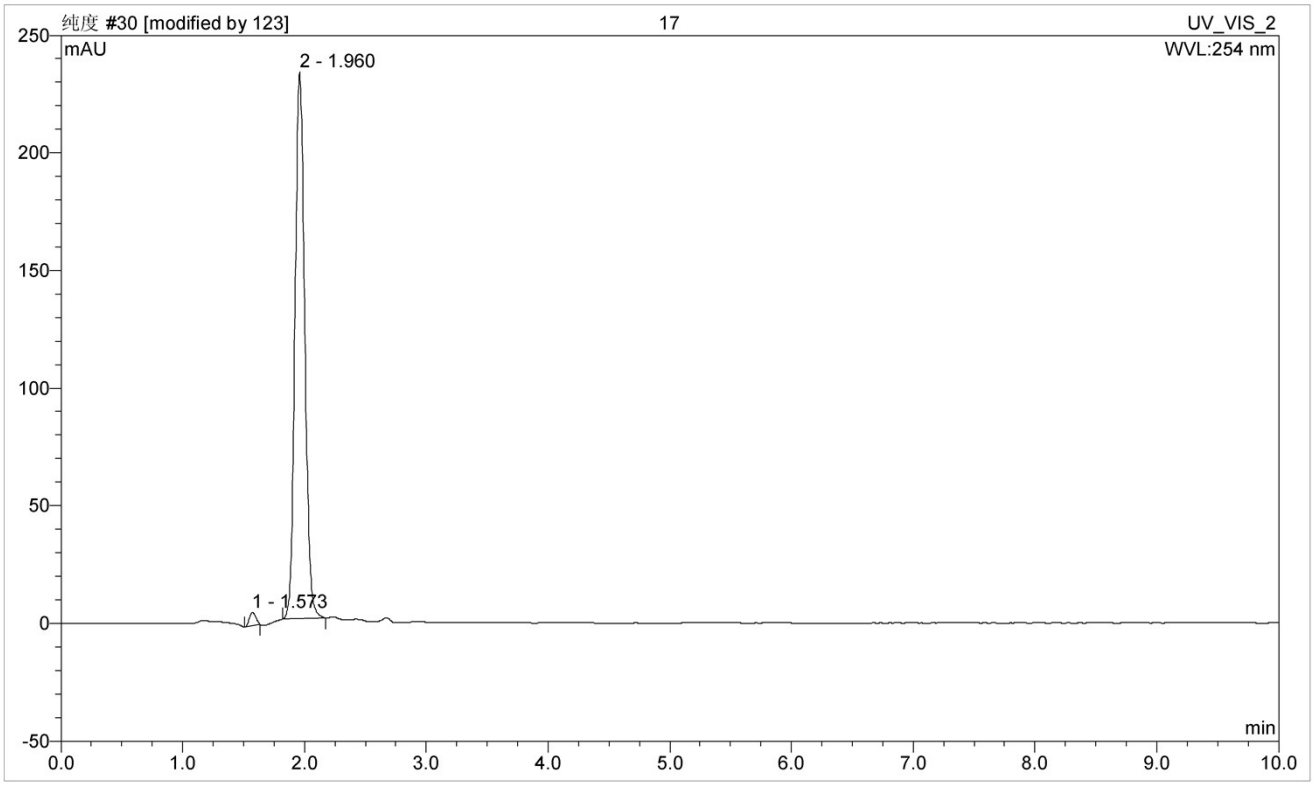
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.57	n.a.	5.726	0.389	0.78	n.a.	BMB*
2	1.99	n.a.	546.127	49.464	99.22	n.a.	BMB*
Total:			551.853	49.853	100.00	0.000	

Compound **(S)-b4**



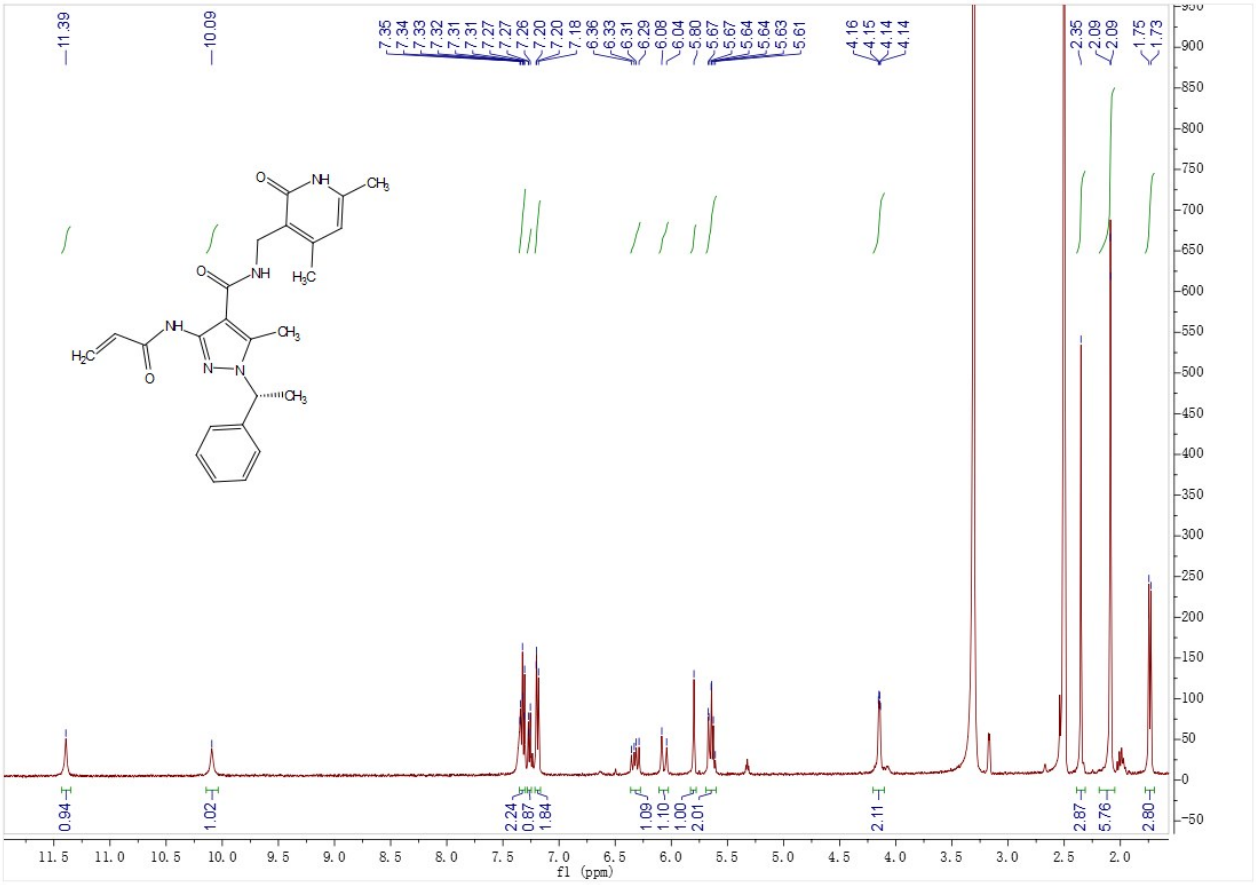


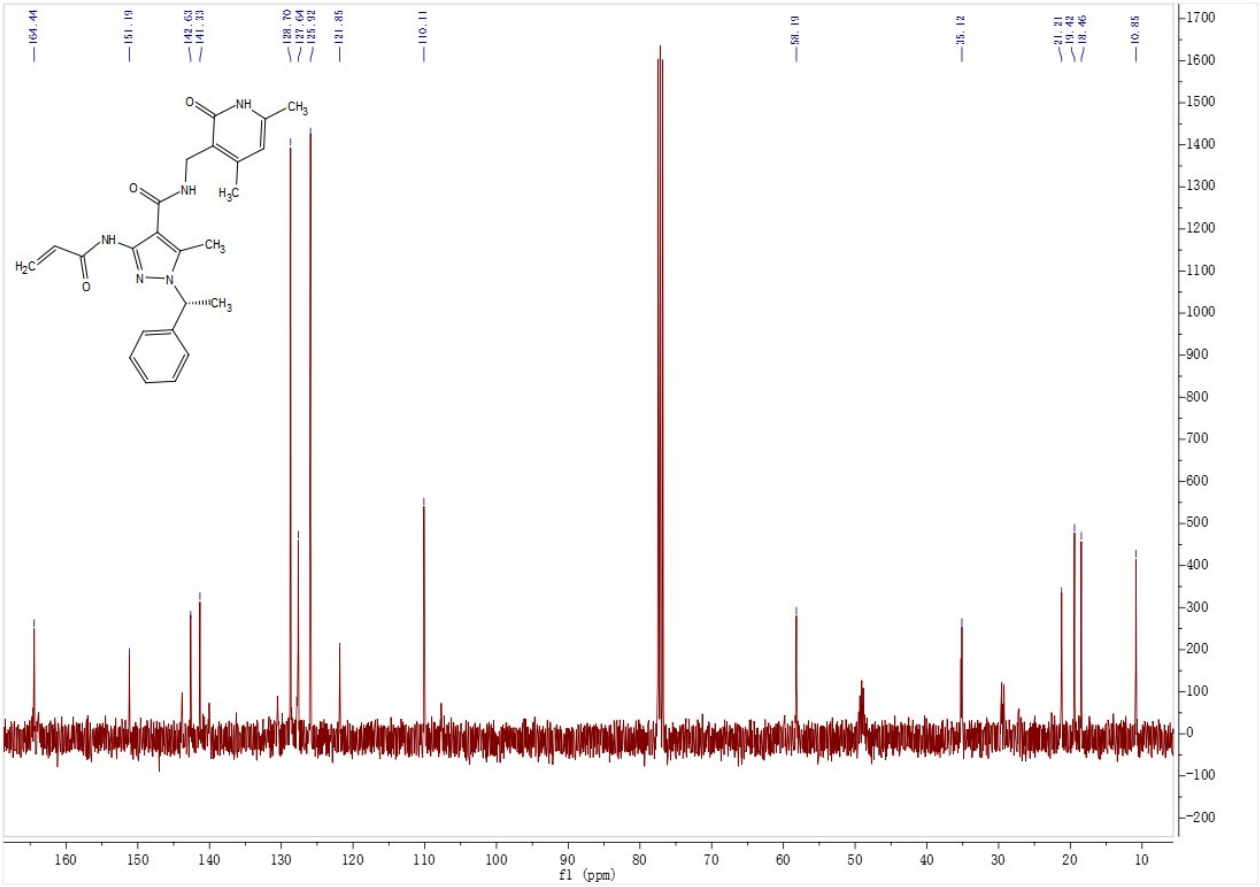


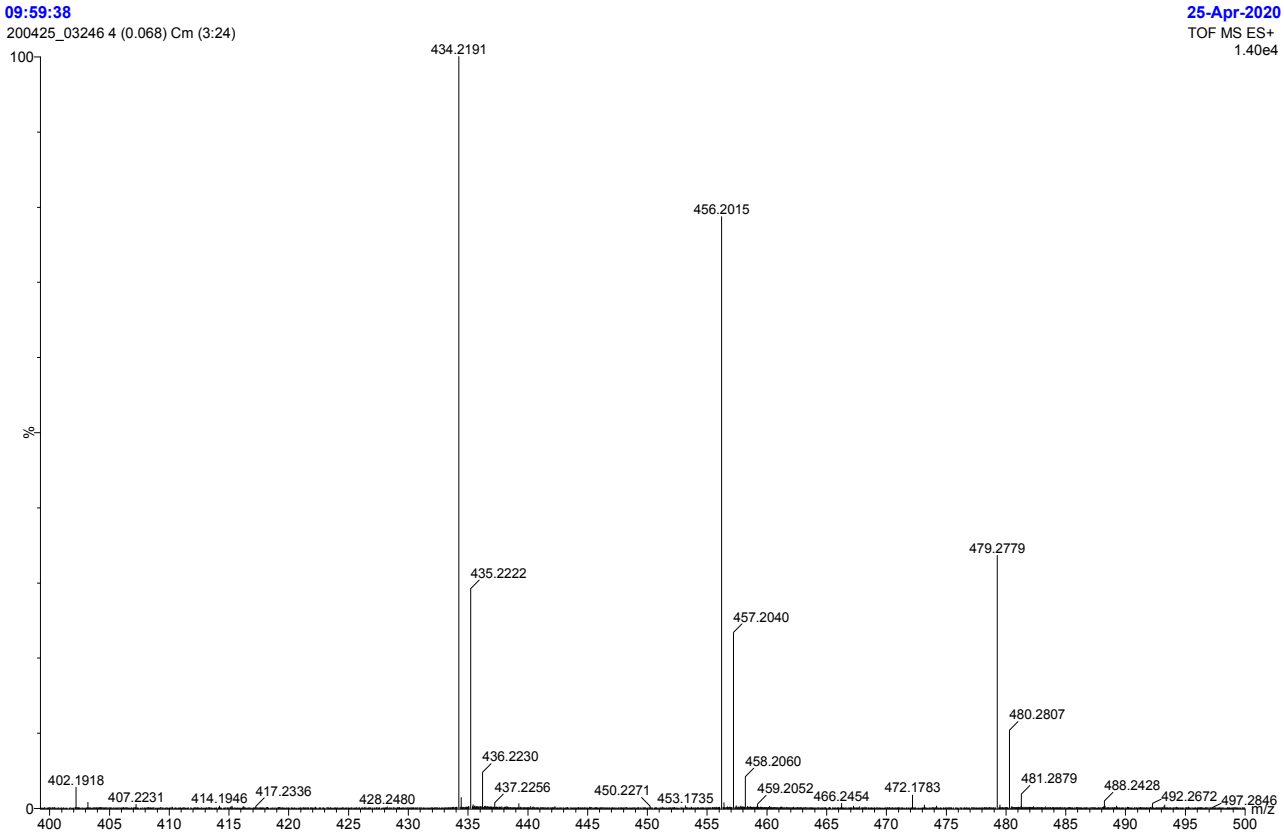


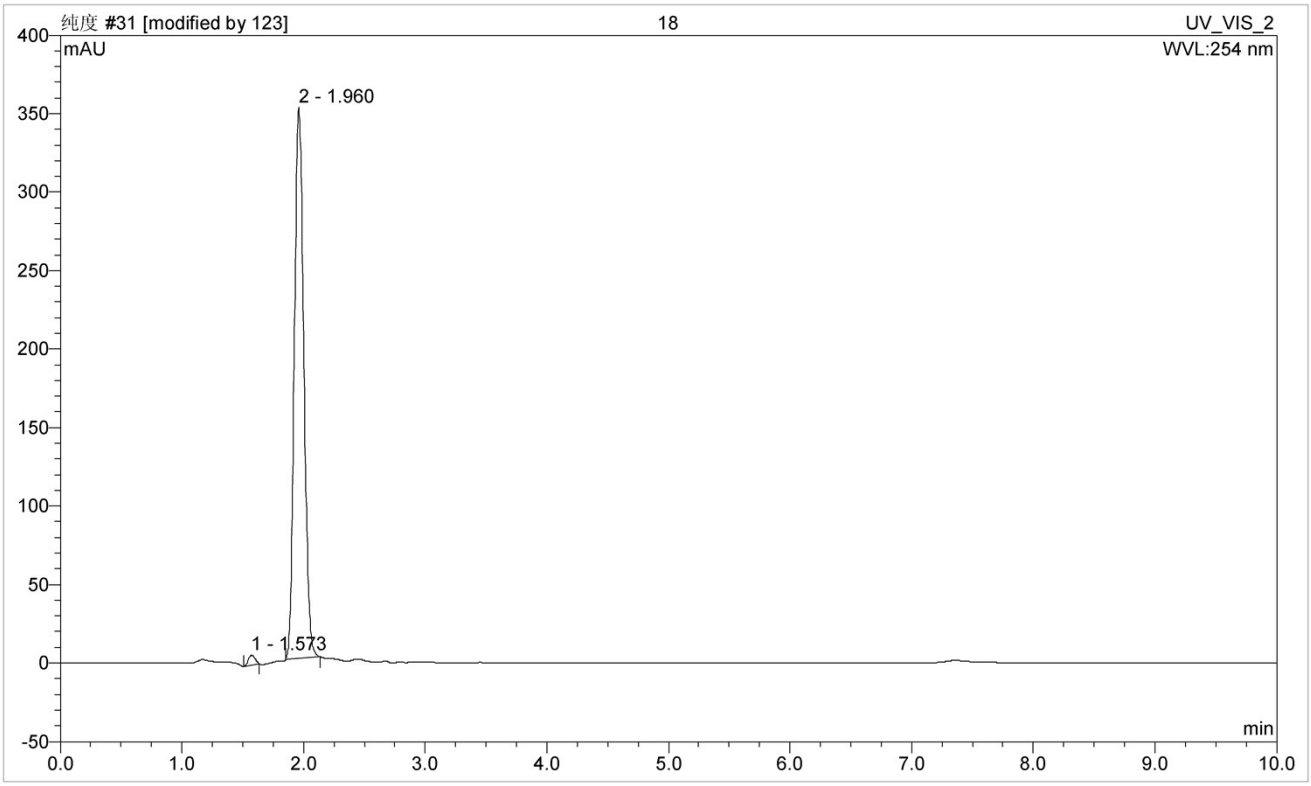
No.	Ret.Time min		Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.57	n.a.		5.496	0.340	1.56	n.a.	BMB*
2	1.96	n.a.		232.423	21.434	98.44	n.a.	BMB*
Total:				237.919	21.774	100.00	0.000	

Compound **(R)-b4**



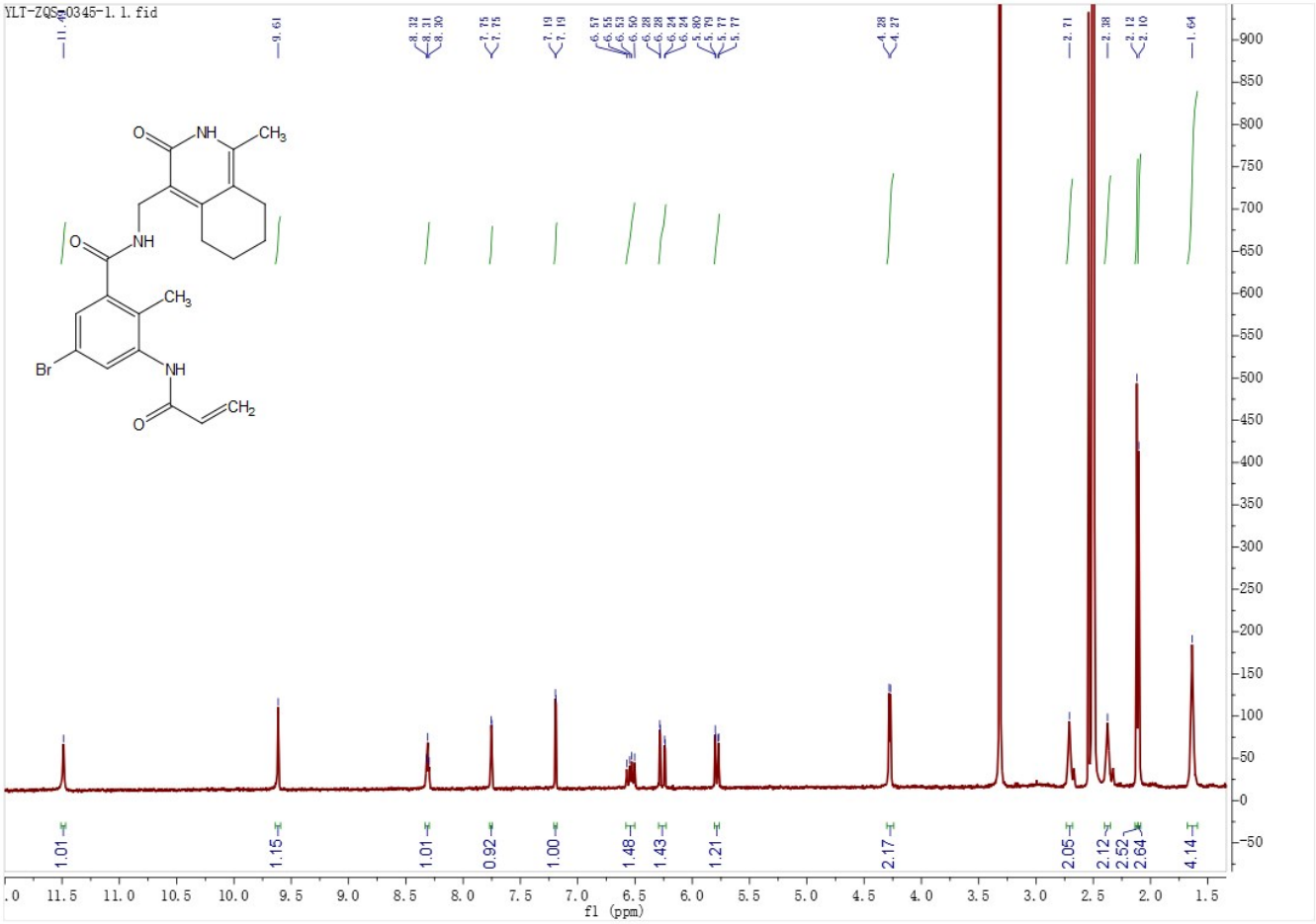


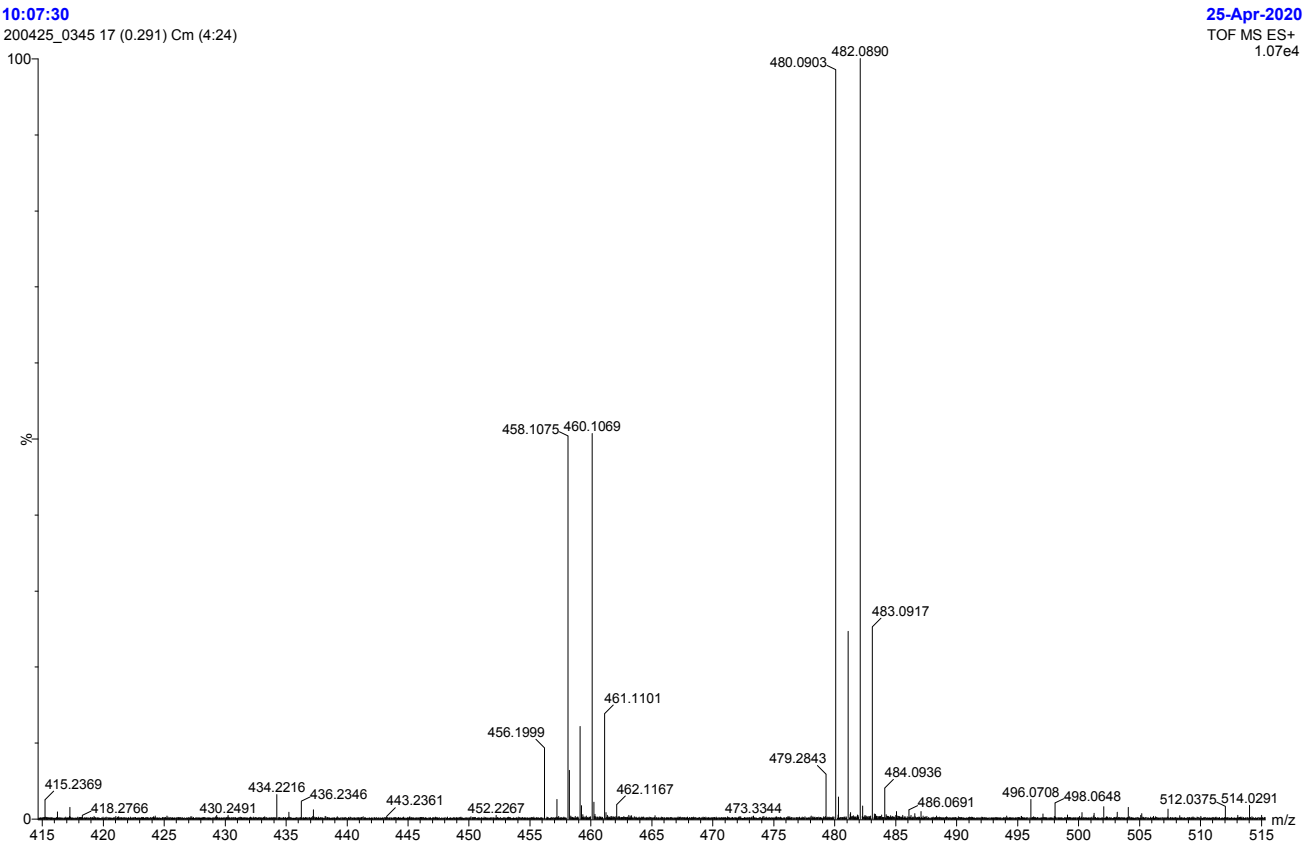




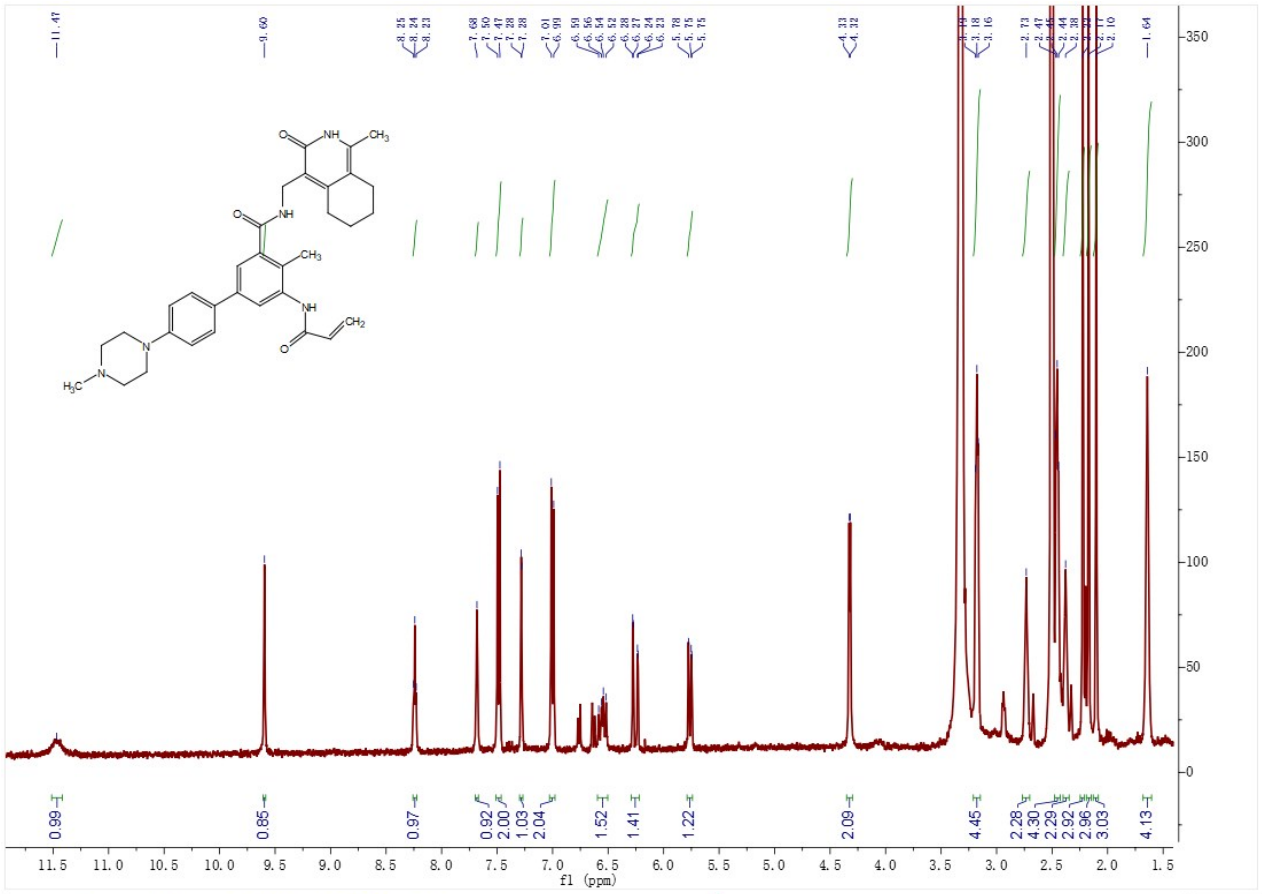
No.	Ret.Time min		Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.57	n.a.		6.242	0.388	1.22	n.a.	BMB*
2	1.96	n.a.		351.049	31.581	98.78	n.a.	BMB*
Total:				357.290	31.969	100.00	0.000	

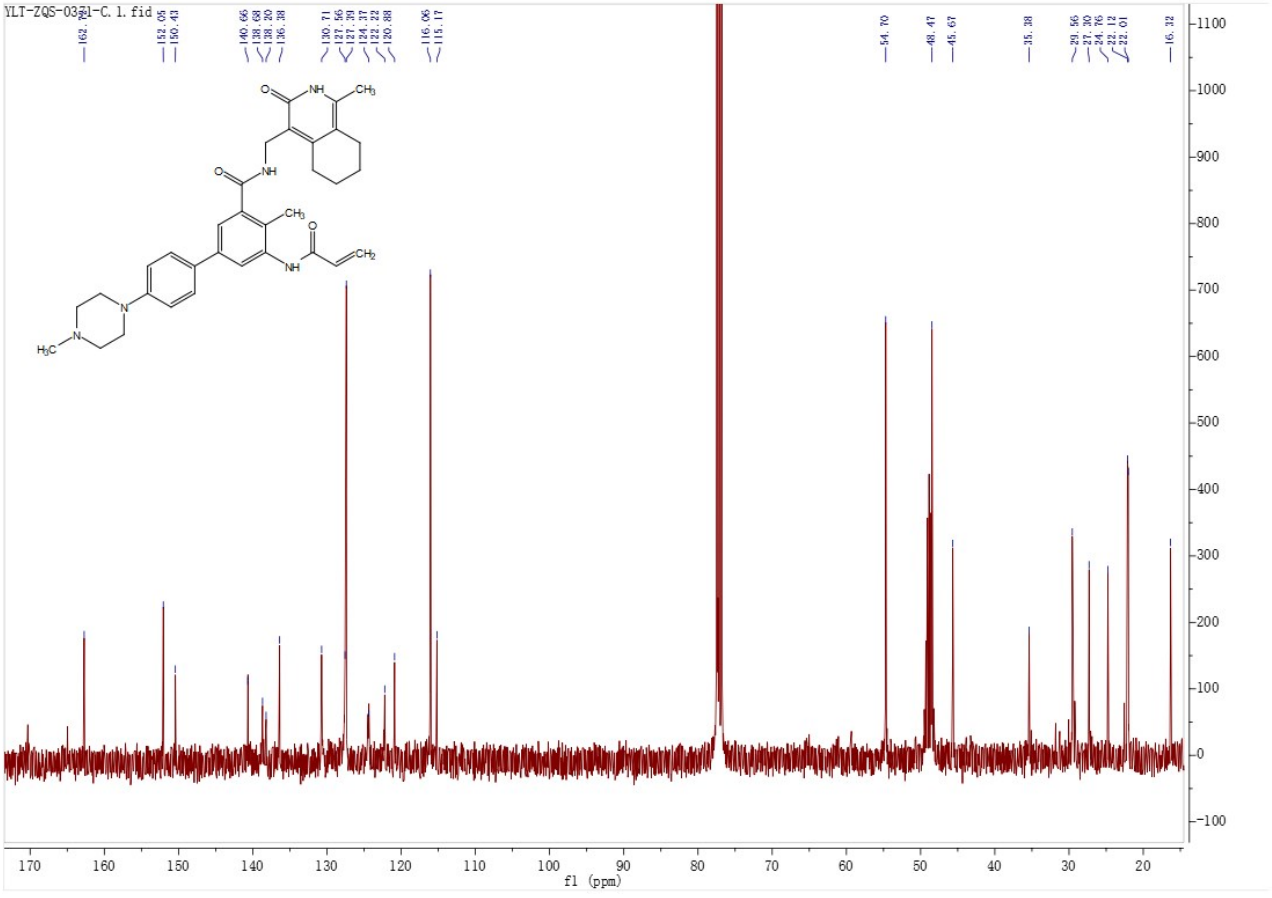
Compound **c1**

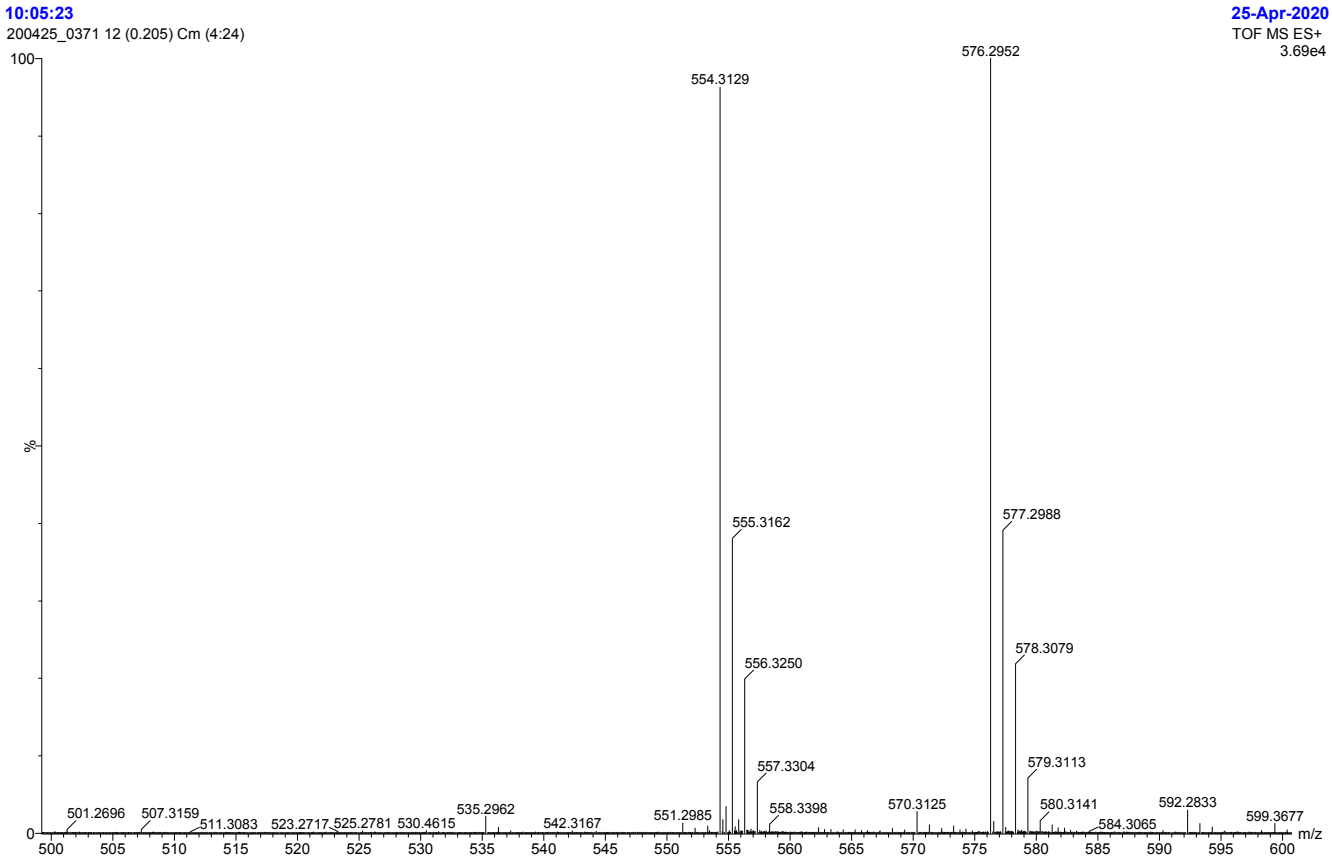

YLT-ZQS-0345-1.1.fid
O
NH
CH3
O
NH
CH3
Br
NH
O
CH2
f1 (ppm)

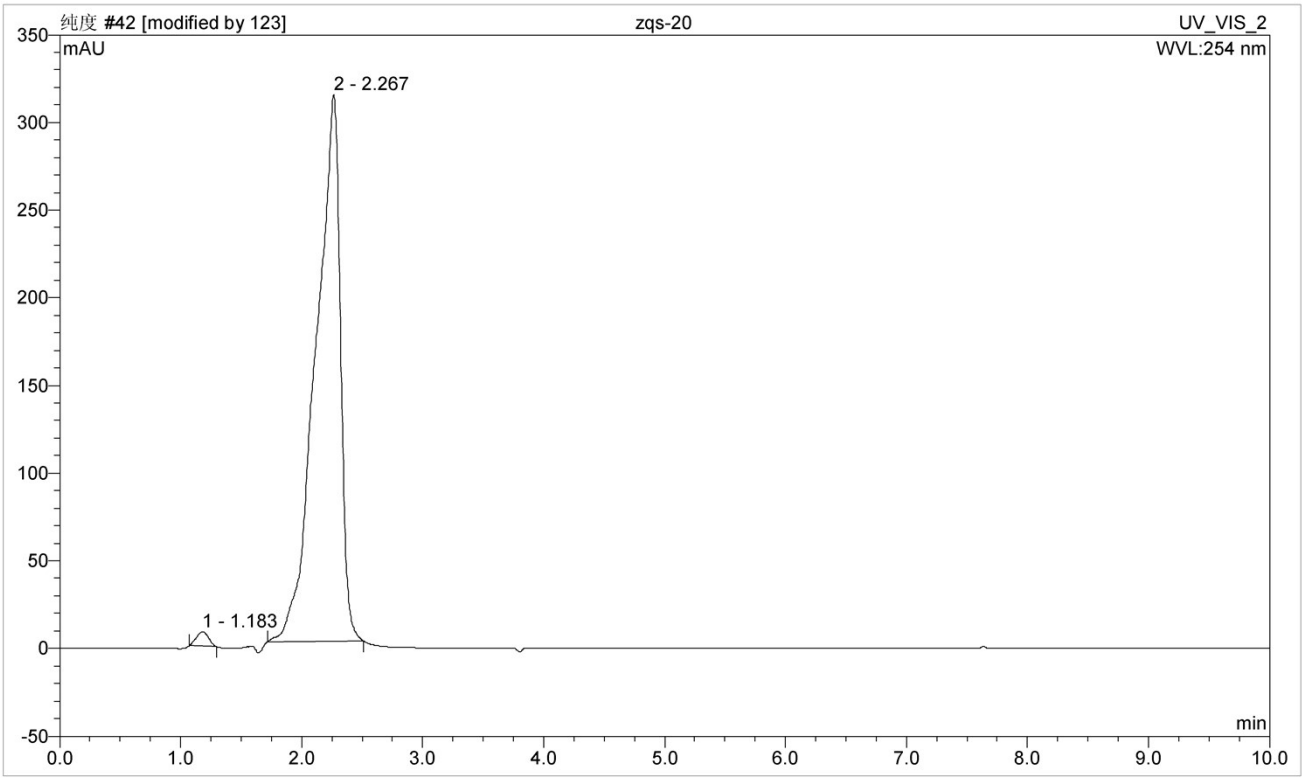

10:07:30
25-Apr-2020
200425_0345 17 (0.291) Cm (4:24)
TOF MS ES+
1.07e4
480.0903
482.0890
458.1075
460.1069
483.0917
461.1101
456.1999
479.2843
484.0936
415.2369
434.2216
436.2346
462.1167
418.2766
430.2491
443.2361
452.2267
473.3344
486.0691
496.0708
498.0648
512.0375
514.0291
m/z

Compound **c2**


11.47
9.60
8.25
8.24
8.23
7.68
7.50
7.47
7.28
7.28
7.01
6.99
6.59
6.56
6.54
6.52
6.28
6.27
6.24
6.23
5.78
5.75
5.75
4.33
4.32
3.19
3.18
3.16
2.73
2.47
2.45
2.44
2.38
2.11
2.10
1.64
0.99
0.85
0.97
0.92
2.00
1.03
2.04
1.52
1.41
1.22
2.09
4.45
2.28
4.30
2.29
2.92
2.96
3.03
4.13
f1 (ppm)

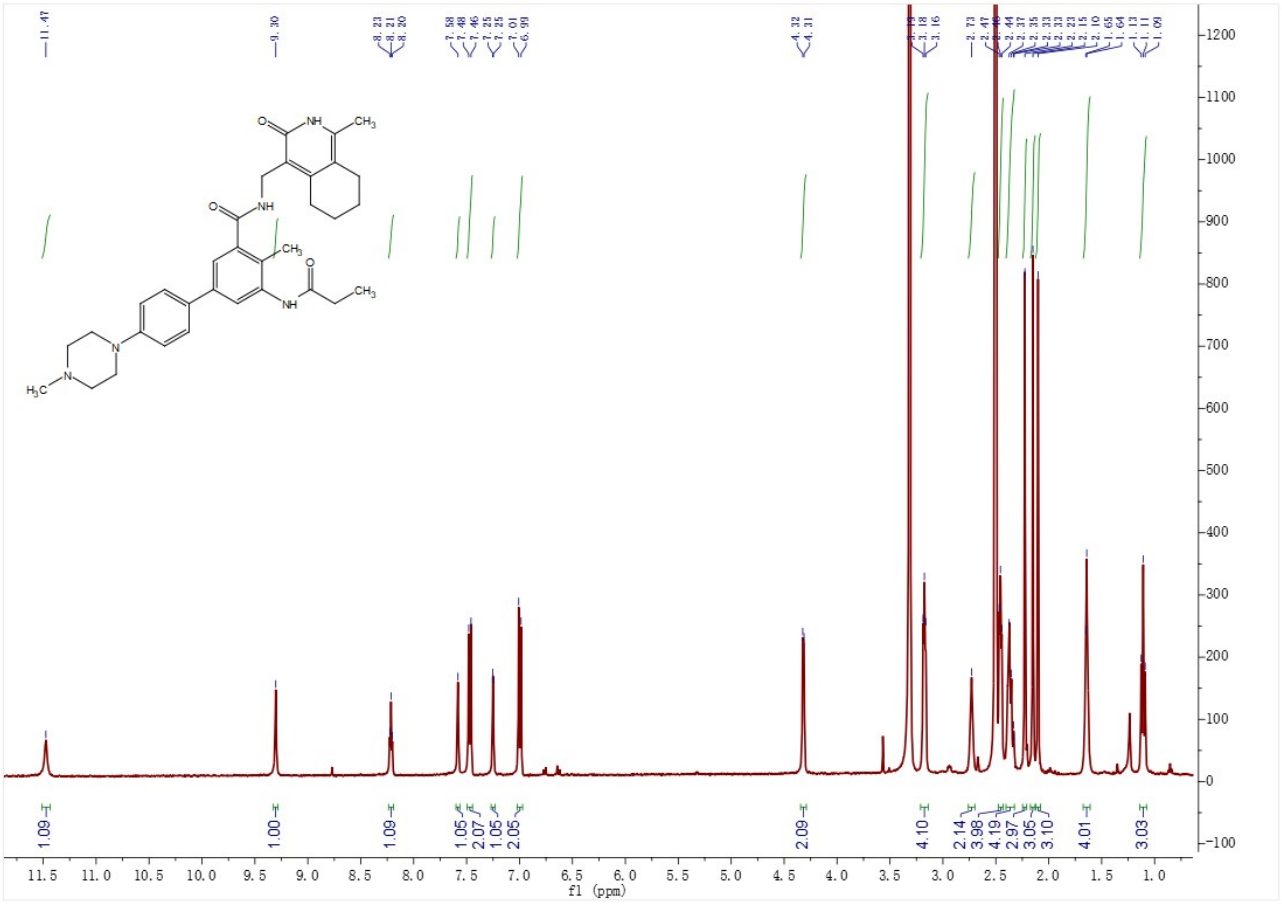

YLT-ZQS-0321-C.1.fid
162.
152.05
150.41
140.66
138.68
138.30
136.38
130.71
127.56
127.39
124.37
122.22
120.88
116.06
115.17
54.70
48.47
45.67
35.38
29.66
27.30
24.76
22.12
22.01
16.32
f1 (ppm)

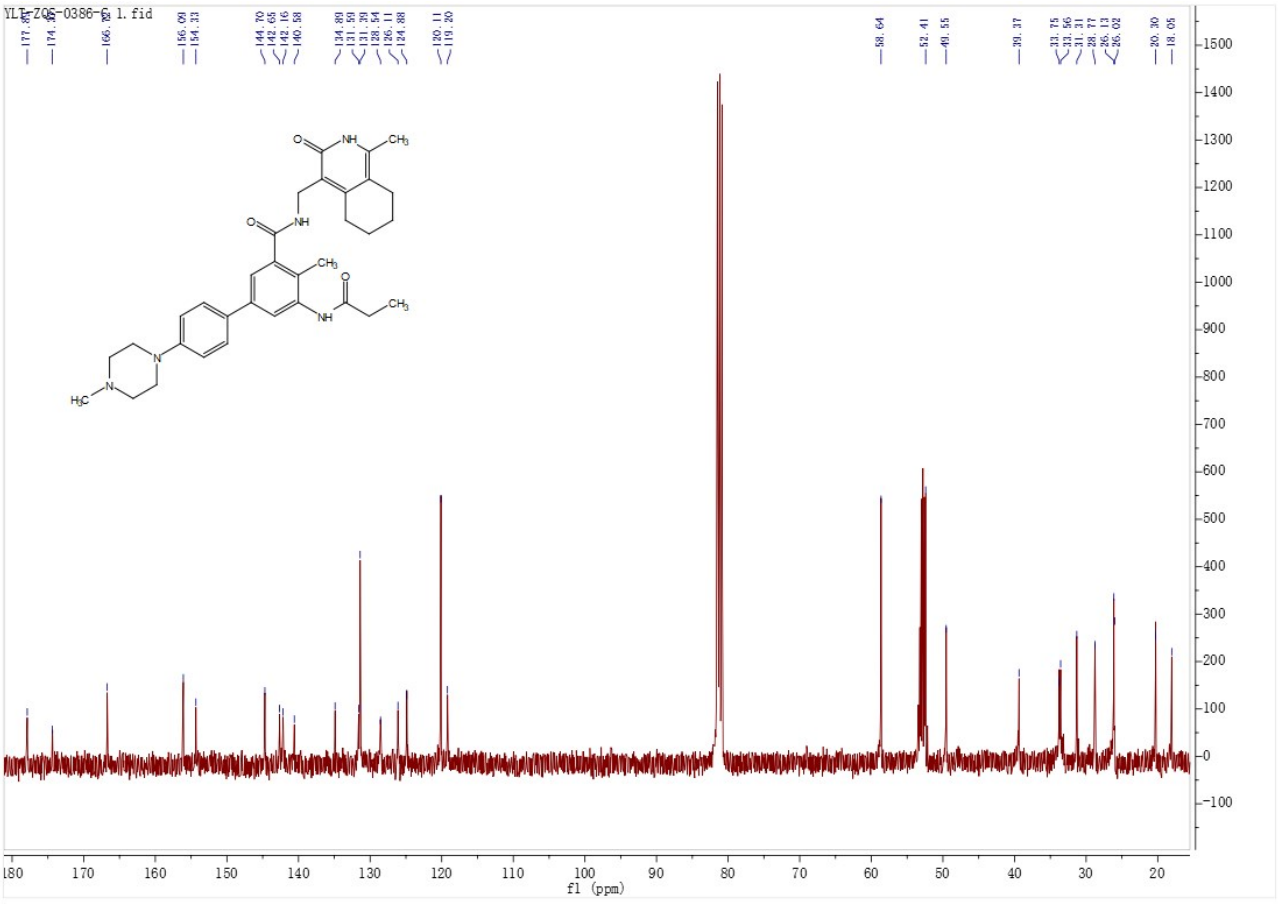


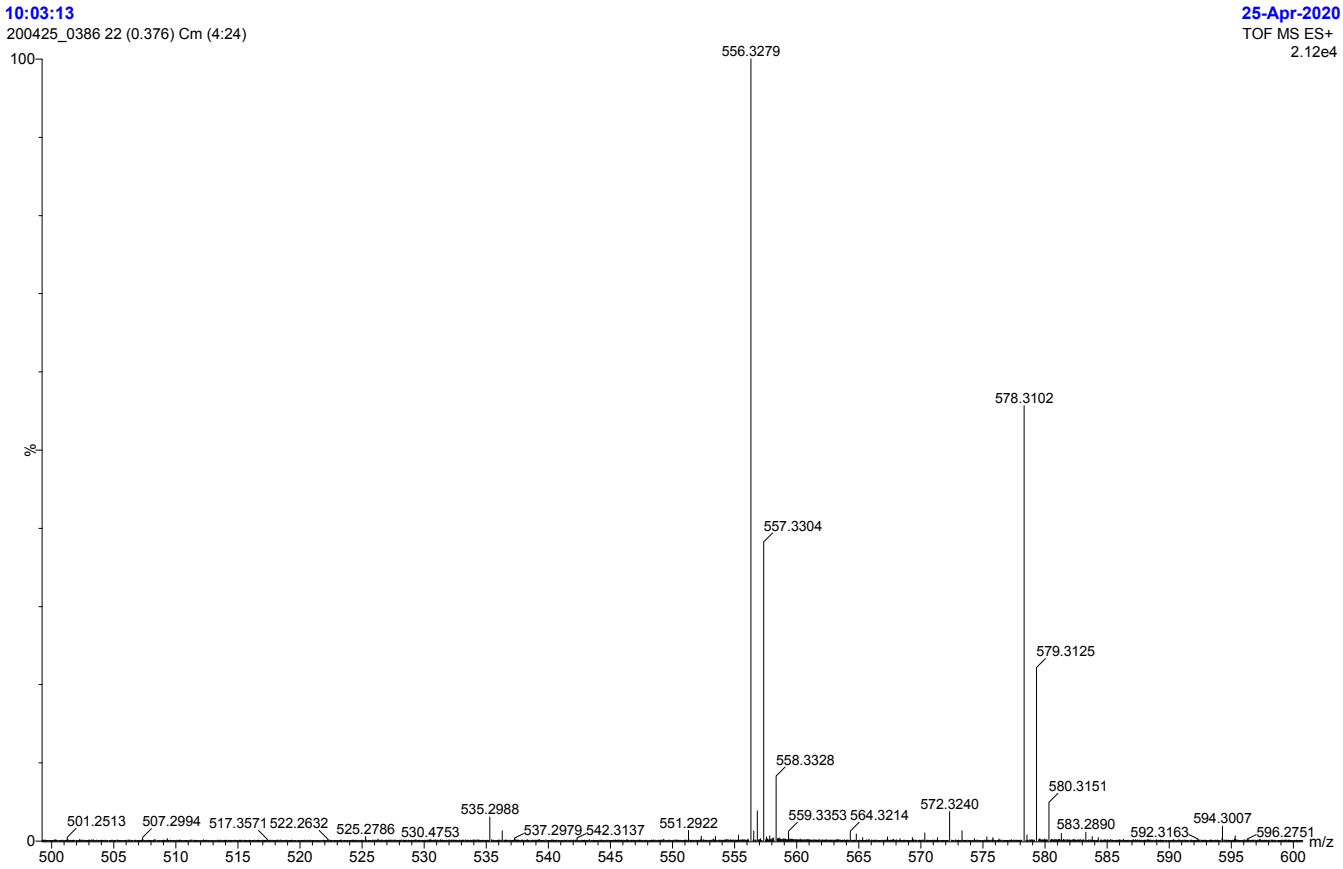


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.18	n.a.	8.137	0.952	1.28	n.a.	BMB*
2	2.27	n.a.	311.563	73.245	98.72	n.a.	BMB*
Total:			319.699	74.197	100.00	0.000	

Compound **c2'**


11.47
9.30
8.23
8.21
8.20
7.58
7.48
7.46
7.25
7.01
6.99
4.32
4.31
3.18
3.16
2.73
2.47
2.44
2.37
2.35
2.33
2.23
2.15
2.10
1.65
1.64
1.11
1.09
1.09
1.00
1.09
1.05
2.07
1.05
2.05
2.09
4.10
2.14
3.98
4.19
2.97
3.05
3.10
4.01
3.03
f1 (ppm)


YLL-ZQS-0386-C 1.fid
177.85
174.46
166.92
156.09
154.33
144.70
142.65
142.16
140.58
134.89
131.59
131.34
128.54
126.11
124.88
120.11
119.20
58.64
52.41
49.55
39.37
33.75
33.56
31.31
28.77
26.13
26.02
20.30
18.05
f1 (ppm)


10:03:13
25-Apr-2020
200425_0386 22 (0.376) Cm (4:24)
TOF MS ES+
2.12e4
100
%
0
556.3279
578.3102
557.3304
579.3125
558.3328
580.3151
572.3240
535.2988
501.2513
507.2994
517.3571
522.2632
525.2786
530.4753
537.2979
542.3137
551.2922
559.3353
564.3214
583.2890
592.3163
594.3007
596.2751
m/z
500
505
510
515
520
525
530
535
540
545
550
555
560
565
570
575
580
585
590
595
600