Supplementary Information for

Pyridine-based small molecule inhibitors of SARM1 alleviate cell death caused by NADase activity.

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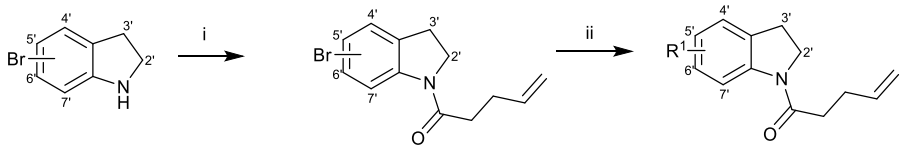
General chemistry methods

NMR spectra were acquired on a Bruker AVANCE III HD 400 nuclear magnetic resonance spectrometer, running at 400 MHz for ^{1}H and 101 MHz for ^{13}C. ^{1}H NMR spectra were recorded in $CHCl_3$-d and $(CH_3)_2SO$-d6, using residual $CHCl_3$ (7.26 ppm) and DMSO (2.50 ppm) as the internal reference. ^{13}C NMR spectra were recorded in $CHCl_3$-d and $(CH_3)_2SO$-d6, using residual $CHCl_3$ (77.16 ppm), DMSO (39.52 ppm) as the internal reference. Mass spectrometry was performed using a Thermo Scientific QExactive mass spectrometer (ESI). Analytical grade solvents and commercially available reagents were used without further purification.

Compounds synthesis and characterization



R^1 is substituted pyridine groups

Scheme S1: synthesis of substituted pyridine derivatives.

Reagents and conditions: (i) pent-4-enoyl chloride, Et_3N, DCM, 0 °C, 30 min, then R.T., 3 h; (ii) pyridineboronic acid pinacol ester, K_2CO_3, $Pd(PPh_3)_4$, dioxane/water = 4:1, 90 °C, overnight

1-(5-(pyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-1)

A round-bottom flask was charged with 5-bromoindoline (1.0 g, 1.0 equiv) and triethylamine (Et_3N, 2.0 mL, 3.0 equiv) under an atmosphere of nitrogen. Anhydrous solvent dichloromethane (20 mL) was added, followed by the dropwise addition of reactant pent-4-enoyl chloride (0.9 ml, 1.5 equiv) under ice-bath condition. After stirring for 30 min under the ice bath, the reaction mixture was allowed to stir for 3 hours at room temperature. Then 80 mL water and ethyl acetate (80 mL×3) were added for extraction. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to give the crude product 1-(5-bromoindolin-1-yl)pent-4-en-1-one as white solid, which was used in the next step without further purification. To the mixture of the intermediate product, potassium carbonate (K_2CO_3, 2.0 g, 3.0 equiv), and 4-pyridineboronic acid pinacol ester (1.0 g, 1.0 equiv) in a solvent mixture of dioxane and water (4:1), the tetrakis(triphenylphosphine)palladium (0.6 g, 0.1 equiv) was added under a nitrogen atmosphere. The reaction was heated to 90°C and stirred for 12 hours, after which it was allowed

to reach room temperature. Then 100 mL water and ethyl acetate (100 mL×3) were added for extraction. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. The residue was purified on silica gel via petroleum ether : ethyl acetate = 1 : 1 to give an white solid (0.97 g, 71%). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.62 (d, J = 5.2 Hz, 2H), 8.33 (d, J = 8.4 Hz, 1H), 7.52 – 7.45 (m, 4H), 5.93 (ddt, J = 16.4, 11.5, 6.0 Hz, 1H), 5.17 – 5.00 (m, 2H), 4.13 (t, J = 8.5 Hz, 2H), 3.27 (t, J = 8.5 Hz, 2H), 2.60 – 2.46 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.76, 150.11, 147.99, 144.06, 137.22, 133.26, 132.14, 126.69, 123.00, 121.22, 117.33, 115.51, 48.25, 35.23, 28.50, 27.96. HRMS (ESI) calculated $C_{18}H_{18}N_2O$, [M+H]+ = 279.1497, and measured [M+H]+: 279.1512

1-(5-(pyridin-3-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-2)

S-2 was prepared in a similar manner described for **S-1**. Yield: 47%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.84 (d, J = 2.4 Hz, 1H), 8.57 (d, J = 4.7 Hz, 1H), 8.35 (d, J = 8.1 Hz, 1H), 7.85 (dt, J = 7.9, 2.0 Hz, 1H), 7.46 – 7.41 (m, 2H), 7.35 (dd, J = 7.9, 4.8 Hz, 1H), 5.95 (ddt, J = 16.2, 9.8, 5.9 Hz, 1H), 5.24 – 4.95 (m, 2H), 4.14 (t, J = 8.5 Hz, 2H), 3.29 (t, J = 8.5 Hz, 2H), 2.64 – 2.46 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.66, 148.12, 148.08, 143.20, 137.29, 136.34, 133.97, 133.17, 132.12, 126.67, 123.51, 123.14, 117.37, 115.48, 48.20, 35.20, 28.53, 28.02. HRMS (ESI) calculated $C_{18}H_{18}N_2O$, [M+H]+ = 279.1497, and measured [M+H]+: 279.1518

1-(5-(4-methoxypyridin-3-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-3)

S-3 was prepared in a similar manner described for **S-1.** Yield: 32%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.53 – 8.39 (m, 2H), 8.31 (d, J = 8.3 Hz, 1H), 7.39 – 7.33 (m, 2H), 6.89 (d, J = 5.6 Hz, 1H), 5.95 (ddt, J = 16.4, 11.7, 5.9 Hz, 1H), 5.19 – 5.01 (m, 2H), 4.11 (t, J = 8.5 Hz, 2H), 3.88 (s, 3H), 3.26 (t, J = 8.5 Hz, 2H), 2.60 – 2.48 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.52, 162.47, 150.70, 150.23, 142.71, 137.33, 131.15, 130.03, 128.99, 126.33, 125.56, 116.72, 115.41, 106.40, 55.38, 48.19, 35.18, 28.58, 28.04. HRMS (ESI) calculated $C_{19}H_{20}N_2O_2$, [M+H]+ = 309.1603, and measured [M+H]+: 309.1606

1-(5-(2-methylpyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-4)

S-4 was prepared in a similar manner described for **S-1.** Yield: 80%. ^{1}H NMR (400 MHz, Chloroform-

d) δ 8.52 (d, J = 5.2 Hz, 1H), 8.34 (d, J = 8.3 Hz, 1H), 7.55 – 7.45 (m, 2H), 7.36 (s, 1H), 7.30 (d, J = 5.3 Hz, 1H), 5.96 (ddt, J = 16.5, 10.2, 6.1 Hz, 1H), 5.19 – 5.01 (m, 2H), 4.14 (t, J = 8.5 Hz, 2H), 3.29 (t, J = 8.5 Hz, 2H), 2.62 (s, 3H), 2.60 – 2.48 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.73, 158.82, 149.58, 148.22, 143.88, 137.26, 133.62, 132.05, 126.67, 123.02, 120.73, 118.44, 117.25, 115.51, 48.23, 35.21, 28.50, 27.97, 24.61. HRMS (ESI) calculated $C_{19}H_{20}N_2O$, [M+H]+ = 293.1654, and measured [M+H]+: 293.1650

1-(5-(2,6-dimethylpyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-5)

S-5 was prepared in a similar manner described for **S-1.** Yield: 67%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.33 (d, J = 8.3 Hz, 1H), 7.50 (d, J = 8.6 Hz, 1H), 7.47 (s, 1H), 7.17 (s, 2H), 5.96 (ddt, J = 16.4, 11.6, 6.0 Hz, 1H), 5.24 – 4.97 (m, 2H), 4.14 (t, J = 5.3 Hz, 2H), 3.28 (t, J = 8.4 Hz, 2H), 2.59 (s, 6H), 2.59 – 2.49 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.82, 158.24, 158.24, 148.67, 137.40, 134.03, 132.07, 126.77, 126.17, 123.15, 118.04, 118.04, 117.31, 115.61, 48.34, 35.32, 28.62, 28.09, 24.74. HRMS (ESI) calculated $C_{20}H_{22}N_2O$, [M+H]+ = 307.1810, and measured [M+H]+: 307.1828

1-(5-(2-(trifluoromethyl)pyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-6)

S-6 was prepared in a similar manner described for **S-1.** Yield: 40%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.71 (d, J = 5.2 Hz, 1H), 8.36 (d, J = 8.3 Hz, 1H), 7.85 (s, 1H), 7.65 (d, J = 5.2 Hz, 1H), 7.52 (d, J = 8.7 Hz, 1H), 7.49 (s, 1H), 5.93 (ddt, J = 16.5, 11.6, 6.2 Hz, 1H), 5.20 – 4.98 (m, 2H), 4.14 (t, J = 8.4 Hz, 2H), 3.29 (t, J = 8.5 Hz, 2H), 2.72 – 2.33 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 171.03, 150.53, 149.86, 148.81, 144.87, 137.28, 132.61, 131.98, 127.04, 123.68, 123.20, 120.47, 117.99 (d, J = 2.8 Hz), 117.59, 115.69, 48.39, 35.37, 28.57, 28.03. HRMS (ESI) calculated $C_{19}H_{17}F_3N_2O$, [M+H]+ = 347.1371, and measured [M+H]+: 347.1370

1-(5-(5-methoxypyridin-3-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-7)

S-7 was prepared in a similar manner described for **S-1.** Yield: 61%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.30 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 5.4 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.42 (s, 1H), 7.06 (dd, J = 5.4, 1.5 Hz, 1H), 6.90 (d, J = 1.5 Hz, 1H), 5.92 (ddt, 1H), 5.22 – 4.93 (m, 2H), 4.09 (t, J = 8.5 Hz, 2H), 3.96 (s, 3H), 3.24 (t, J = 8.5 Hz, 2H), 2.51 (q, J = 5.5, 4.7 Hz, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.70, 164.95, 150.74, 147.17, 143.91, 137.26, 133.43, 131.99, 126.63, 123.01, 117.16, 115.49,

115.04, 107.85, 53.50, 48.22, 35.19, 28.50, 27.94. HRMS (ESI) calculated $C_{19}H_{20}N_2O_2$, [M+H]+ = 309.1603, and measured [M+H]+: 309.1606

1-(5-(2-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-8)

S-8 was prepared in a similar manner described for **S-1.** Yield: 67%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.37 (d, J = 5.2 Hz, 1H), 8.32 (d, J = 8.3 Hz, 1H), 7.49 (d, J = 1.5 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.44 (s, 1H), 7.38 (dd, J = 5.2, 1.5 Hz, 1H), 5.92 (ddt, J = 16.4, 10.1, 6.1 Hz, 1H), 5.16 – 5.01 (m, 2H), 4.13 (t, J = 8.5 Hz, 2H), 3.26 (t, J = 8.5 Hz, 2H), 2.63 – 2.41 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.97, 152.35, 151.20, 150.04, 144.69, 137.29, 132.44, 131.97, 126.96, 123.16, 121.56, 120.10, 117.48, 115.66, 48.37, 35.35, 28.57, 28.02. HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1118

1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (TH-408)

TH-408 was prepared in a similar manner described for **S-1.** Yield: 49%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.64 (s, 1H), 8.48 (d, J = 4.8 Hz, 1H), 8.33 (d, J = 8.4 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.25 (d, J = 4.9 Hz, 1H), 5.93 (ddt, J = 16.3, 11.4, 6.0 Hz, 1H), 5.20 – 4.98 (m, 2H), 4.13 (t, J = 8.5 Hz, 2H), 3.27 (t, J = 8.5 Hz, 2H), 2.64 – 2.42 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.85, 150.24, 147.91, 147.40, 143.80, 137.30, 131.69, 131.42, 130.19, 128.82, 125.34, 125.23, 116.84, 115.58, 48.30, 35.29, 28.59, 28.04. HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1114

1-(5-(3-fluoropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-9)

S-9 was prepared in a similar manner described for **S-1.** Yield: 35%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.50 (d, J = 2.1 Hz, 1H), 8.42 (d, J = 4.8 Hz, 1H), 8.34 (d, J = 8.6 Hz, 1H), 7.49 – 7.43 (m, 2H), 7.37 (dd, J = 4.8, 2.1 Hz, 2H), 5.93 (ddt, J = 16.3, 11.5, 6.0 Hz, 1H), 5.20 – 4.94 (m, 2H), 4.13 (t, J = 8.5 Hz, 2H), 3.27 (t, J = 8.5 Hz, 2H), 2.65 – 2.45 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.96, 158.07, 155.52, 146.12, 146.07, 139.27, 139.01, 137.34, 131.86, 128.67, 125.18, 124.00, 117.24, 115.65, 48.36, 35.37, 28.62, 28.07. HRMS (ESI) calculated $C_{18}H_{17}FN_2O$, [M+H]+ = 297.1403, and measured [M+H]+: 297.141

1-(5-(3-(trifluoromethyl)pyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-10)

S-10 was prepared in a similar manner described for **S-1.** Yield: 79%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.95 (s, 1H), 8.76 (d, J = 5.0 Hz, 1H), 8.31 (d, J = 8.6 Hz, 1H), 7.27 (d, J = 5.0 Hz, 1H), 7.19 – 7.14 (m, 2H), 5.94 (ddt, J = 16.3, 10.0, 6.0 Hz, 1H), 5.20 – 4.90 (m, 2H), 4.14 (t, J = 8.5 Hz, 2H), 3.26 (t, J = 8.5 Hz, 2H), 2.62 – 2.40 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.91, 152.61, 149.47, 147.58 (d, J = 6.0 Hz), 143.81, 137.35, 132.37, 131.30, 128.19, 126.24, 124.62, 124.43, 122.42, 116.67, 115.60, 48.29, 35.34, 28.61, 28.03. HRMS (ESI) calculated $C_{19}H_{17}F_3N_2O$, [M+H]+ = 347.1371, and measured [M+H]+: 347.1375

1-(5-(3-nitropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-11)

S-11 was prepared in a similar manner described for **S-1.** Yield: 55%. ^{1}H NMR (400 MHz, Chloroform-d) δ 9.04 (s, 1H), 8.78 (d, J = 5.0 Hz, 1H), 8.36 (d, J = 8.3 Hz, 1H), 7.41 (d, J = 5.1 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 7.16 (s, 1H), 5.95 (ddt, J = 16.3, 10.2, 6.1 Hz, 1H), 5.23 – 4.99 (m, 2H), 4.15 (t, J = 8.5 Hz, 2H), 3.26 (t, J = 8.5 Hz, 2H), 2.65 – 2.43 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.90, 152.62, 145.64, 145.21, 144.57, 143.56, 137.14, 132.14, 129.27, 127.67, 125.60, 123.78, 117.37, 115.56, 48.23, 35.24, 28.45, 27.85. HRMS (ESI) calculated $C_{18}H_{17}N_3O_3$, [M+H]+ = 324.1348, and measured [M+H]+: 324.1340

1-(5-(3-methoxypyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-12)

S-12 was prepared in a similar manner described for **S-1.** Yield: 57%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.35 (s, 1H), 8.33 – 8.26 (m, 2H), 7.45 – 7.39 (m, 2H), 7.23 (d, J = 4.5 Hz, 1H), 5.93 (ddt, J = 16.5, 11.6, 6.0 Hz, 1H), 5.20 – 4.95 (m, 2H), 4.11 (t, J = 8.5 Hz, 2H), 3.91 (s, 3H), 3.26 (t, J = 8.5 Hz, 2H), 2.61 – 2.46 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.77, 143.36, 143.19, 137.54, 137.42, 134.61, 131.24, 131.05, 128.96, 128.66, 125.49, 124.38, 116.88, 115.59, 56.47, 48.35, 35.34, 28.69, 28.15. HRMS (ESI) calculated $C_{19}H_{20}N_2O_2$, [M+H]+ = 309.1603, and measured [M+H]+: 309.1606

1-(4-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-13)

S-13 was prepared in a similar manner described for **S-1.** Yield: 49%. ^{1}H NMR (400 MHz,

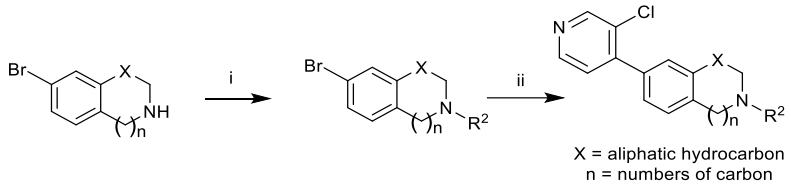
Chloroform-d) δ 8.67 (s, 1H), 8.51 (d, J = 4.7 Hz, 1H), 8.33 (d, J = 8.0 Hz, 1H), 7.28 (t, J = 7.9 Hz, 1H), 7.19 (d, J = 4.8 Hz, 1H), 6.89 (d, J = 7.6 Hz, 1H), 5.91 (ddt, J = 16.3, 11.5, 6.0 Hz, 1H), 5.17 – 4.99 (m, 2H), 4.06 (t, J = 8.5 Hz, 2H), 3.00 (t, J = 8.5 Hz, 2H), 2.67 – 2.32 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.66, 149.86, 147.74, 146.61, 143.36, 137.24, 133.31, 130.71, 129.54, 128.08, 125.12, 123.56, 117.23, 115.44, 47.96, 35.20, 28.48, 27.24. HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1118

1-(6-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-14)

S-14 was prepared in a similar manner described for **S-1.** Yield: 63%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.65 (s, 1H), 8.48 (d, J = 4.4 Hz, 1H), 8.39 (s, 1H), 7.31 – 7.27 (m, 2H), 7.14 (d, J = 7.5 Hz, 1H), 5.92 (ddt, J = 16.2, 9.9, 6.0 Hz, 1H), 5.39 – 4.87 (m, 2H), 4.12 (t, J = 8.6 Hz, 2H), 3.26 (t, J = 8.5 Hz, 2H), 2.71 – 2.38 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.87, 150.11, 147.81, 143.49, 137.35, 136.10, 132.03, 130.35, 128.61, 126.14, 125.60, 124.47, 117.54, 115.57, 48.31, 35.29, 28.53, 28.06. HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1118

1-(7-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-15)

S-15 was prepared in a similar manner described for **S-1.** Yield: 81%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.57 (s, 1H), 8.48 (d, J = 4.9 Hz, 1H), 7.31 (d, J = 7.1 Hz, 1H), 7.25 (d, J = 5.0 Hz, 1H), 7.20 – 7.11 (m, 2H), 5.69 (ddt, J = 16.8, 10.3, 6.4 Hz, 1H), 5.04 – 4.87 (m, 2H), 4.15 (t, J = 7.8 Hz, 2H), 3.14 (t, J = 7.5 Hz, 2H), 2.39 – 2.18 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 150.16, 149.38, 147.93, 147.64, 140.35, 136.97, 134.82, 130.00, 129.50, 126.54, 125.28, 124.84, 124.13, 115.48, 49.70, 34.82, 29.52, 29.12. HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1118



R^2 is substituted carbonyl groups

Scheme S2: synthesis of 3-chloropyridine substituted hydroindole derivatives.

Reagents and conditions: (i) substituted acyl chloride, Et_3N, DCM, 0 °C, 30 min, then R.T., overnight; (ii) 3-chloridepyridine-4-boronic acid pinacol ester, K_2CO_3, $Pd(PPh_3)_4$, dioxane/water = 4:1, 90 °C, overnight

1-(5'-(3-chloropyridin-4-yl)spiro[cyclopentane-1,3'-indolin]-1'-yl)pent-4-en-1-one (S-16)

A round-bottom flask was charged with 5-bromospiro[1,2-dihydroindole-3,1'-cyclopentane] (1.0 g, 1.0 equiv) and triethylamine (Et_3N, 1.6 mL, 3.0 equiv) under an atmosphere of nitrogen. Anhydrous solvent dichloromethane (20 mL) was added, followed by the dropwise addition of reactant pent-4-enoyl chloride (0.7 ml, 1.5 equiv) under ice-bath condition. After stirring for 30 min under the ice bath, the reaction mixture was allowed to stir overnight at room temperature. Then 80 mL water and ethyl acetate (80 mL×3) were added for extraction. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to give the crude product 1-(5'-bromospiro[cyclopentane-1,3'-indolin]-1'-yl)pent-4-en-1-one as white solid, which was used in the next step without further purification. To the mixture of the intermediate product, potassium carbonate (K_2CO_3, 1.6 g, 3.0 equiv), and 3-chloridepyridine-4-boronic acid pinacol ester (0.8 g, 1.0 equiv) in a solvent mixture of dioxane and water (4:1), the tetrakis(triphenylphosphine)palladium (0.5 g, 0.1 equiv) was added under a nitrogen atmosphere. The reaction was heated to 90°C and stirred for 12 hours, after which it was allowed to reach room temperature. Then 100 mL water and ethyl acetate (100 mL×3) were added for extraction. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. The residue was purified on silica gel via petroleum ether : ethyl acetate = 1 : 1 to give a white solid (0.72 g, 57%). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.65 (s, 1H), 8.49 (d, J = 4.9 Hz, 1H), 8.32 (d, J = 8.3 Hz, 1H), 7.37 – 7.24 (m, 4H), 5.94 (ddt, J = 16.4, 11.7, 6.0 Hz, 1H), 5.25 – 4.99 (m, 2H), 3.89 (s, 2H), 2.65 – 2.43 (m, 4H), 1.96 – 1.76 (m, 8H). ^{13}C NMR (101 MHz, Chloroform-d) δ 170.82, 150.31, 147.96, 147.56, 143.17, 139.57, 137.33, 132.01, 130.24, 128.93, 125.40, 123.05, 116.73, 115.67, 63.10, 51.55, 41.36, 35.39, 28.70, 24.95. HRMS (ESI) calculated $C_{22}H_{23}ClN_2O$, [M+H]+ = 367.1577, and measured [M+H]+: 367.1549

1-(6-(3-chloropyridin-4-yl)-1,2,3,4-tetrahydroquinolin-1-yl)pent-4-en-1-one (S-17)

S-17 was prepared in a similar manner described for **S-16.** Yield: 75%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.67 (s, 1H), 8.51 (d, 1H), 7.35 – 7.23 (m, 4H), 5.84 (ddt, J = 16.8, 10.2, 6.5 Hz, 1H), 5.08 – 4.96 (m, 2H), 3.83 (t, J = 6.4 Hz, 2H), 2.81 (t, J = 6.7 Hz, 2H), 2.66 (t, J = 8.5, 6.4 Hz, 2H), 2.46 (dd, J = 8.7, 6.7 Hz, 2H), 2.02 (dt, J = 6.6 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-d) δ 172.21, 150.31, 147.97, 147.00, 139.69, 137.37, 133.06, 129.29, 126.84, 126.18, 126.09, 125.34, 124.67, 115.45, 43.75, 34.15, 29.82, 27.14, 24.08. HRMS (ESI) calculated $C_{19}H_{19}ClN_2O$, [M+H]+ = 327.1264, and measured [M+H]+: 327.1271

1-(6-(3-chloropyridin-4-yl)-1,2,3,4-tetrahydroisoquinolin-2-yl)pent-4-en-1-one (S-18)

S-18 was prepared in a similar manner described for **S-16.** Yield: 78%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.70 (s, 1H), 8.56 (d, J = 4.5 Hz, 1H), 7.37 – 7.14 (m, 3H), 7.07 (t, J = 8.0 Hz, 1H), 5.89 (ddt, J = 15.7, 10.1, 4.5 Hz, 1H), 5.13 – 4.96 (m, 2H), 4.85 – 4.69 (m, 2H), 3.80 – 3.60 (m, 2H), 2.72 – 2.38 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-d) δ 171.24, 149.68, 147.79, 147.34, 137.43, 135.93, 134.45, 132.14, 127.44, 127.03, 126.75, 126.64, 126.38, 115.32, 44.31, 42.93, 32.84, 29.14, 27.37. HRMS (ESI) calculated $C_{19}H_{19}ClN_2O$, [M+H]+ = 327.1264, and measured [M+H]+: 327.1271

1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)hept-6-en-1-one (S-19)

S-19 was prepared in a similar manner described for **S-16.** Yield: 64%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.65 (s, 1H), 8.49 (d, J = 4.3 Hz, 1H), 8.34 (d, J = 8.3 Hz, 1H), 7.33 (s, 2H), 7.26 (d, J = 4.5 Hz, 1H), 5.83 (ddd, J = 16.8, 11.1, 5.6 Hz, 1H), 5.01 (dd, J = 26.0, 13.6 Hz, 2H), 4.12 (t, J = 8.5 Hz, 2H), 3.26 (t, J = 8.6 Hz, 2H), 2.46 (t, J = 7.6 Hz, 2H), 2.13 (dt, J = 7.3 Hz, 2H), 1.79 (tt, J = 7.8 Hz, 2H), 1.52 (tt, J = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-d) δ 171.59, 150.21, 147.88, 147.40, 143.86, 138.54, 131.41, 130.16, 128.78, 125.99, 125.32, 125.19, 116.79, 114.78, 48.29, 35.84, 33.65, 28.65, 28.01, 24.07. HRMS (ESI) calculated $C_{20}H_{21}ClN_2O$, [M+H]+ = 341.1421, and measured [M+H]+: 341.1426

1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)propan-1-one (S-20)

S-20 was prepared in a similar manner described for **S-16.** Yield: 89%. ^{1}H NMR (400 MHz,

Chloroform-d) δ 8.65 (s, 1H), 8.49 (d, J = 4.9 Hz, 1H), 8.34 (d, J = 8.5 Hz, 1H), 7.35 – 7.30 (m, 2H), 7.26 (d, J = 5.0 Hz, 1H), 4.12 (t, J = 8.5 Hz, 2H), 3.27 (t, J = 8.6 Hz, 2H), 2.48 (q, J = 7.4 Hz, 2H), 1.25 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 172.42, 150.29, 147.94, 147.49, 143.96, 131.62, 131.38, 130.25, 128.88, 125.39, 125.25, 116.81, 48.20, 29.31, 28.10, 8.81. HRMS (ESI) calculated $C_{16}H_{15}ClN_2O$, [M+H]+ = 287.0951, and measured [M+H]+: 287.0959

1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pentan-1-one (S-21)

S-21 was prepared in a similar manner described for **S-16.** Yield: 90%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.63 (s, 1H), 8.47 (d, J = 4.9 Hz, 1H), 8.33 (d, J = 8.6 Hz, 1H), 7.34 – 7.28 (m, 2H), 7.24 (d, J = 4.9 Hz, 1H), 4.10 (t, J = 8.5 Hz, 2H), 3.24 (t, J = 8.5 Hz, 2H), 2.43 (t, J = 7.4 Hz, 2H), 1.72 (tt, J = 7.6 Hz, 2H), 1.53 – 1.35 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 171.66, 150.07, 150.07, 147.77, 147.25, 143.79, 131.35, 130.00, 128.61, 125.20, 125.07, 116.60, 48.16, 35.60, 27.87, 26.56, 22.42, 13.89, 13.89. HRMS (ESI) calculated $C_{18}H_{19}ClN_2O$, [M+H]+ = 315.1264, and measured [M+H]+: 315.1271

(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)(tricyclo[3.3.1.13,7]dec-1-yl)methanone (S-22)

S-22 was prepared in a similar manner described for **S-16.** Yield: 51%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.64 (s, 1H), 8.48 (d, J = 5.0 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 7.33 (s, 1H), 7.31 (d, J = 8.6 Hz, 1H), 7.25 (d, J = 5.0 Hz, 1H), 4.40 (t, J = 8.1 Hz, 2H), 3.20 (t, J = 8.1 Hz, 2H), 2.20 – 2.08 (m, 9H), 1.77 (s, 6H). ^{13}C NMR (101 MHz, Chloroform-d) δ 176.46, 150.13, 147.79, 147.42, 145.78, 131.58, 131.58, 130.97, 130.14, 128.48, 125.29, 124.80, 118.38, 49.83, 43.23, 38.30, 36.60, 29.44, 28.40. HRMS (ESI) calculated $C_{24}H_{25}ClN_2O$, [M+H]+ = 393.1734, and measured [M+H]+: 393.1732

1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)prop-2-en-1-one (S-23)

S-23 was prepared in a similar manner described for **S-16.** Yield: 47%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.65 (s, 1H), 8.49 (d, J = 5.0 Hz, 1H), 8.38 (s, 1H), 7.37 – 7.31 (m, 2H), 7.27 (d, J = 5.4 Hz, 1H), 6.64 – 6.49 (m, 2H), 5.83 (dd, 1H), 4.24 (t, J = 8.5 Hz, 2H), 3.28 (t, J = 8.7 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-d) δ 164.19, 150.23, 147.90, 147.36, 143.65, 143.61, 132.12, 131.91, 130.23, 129.52, 128.95, 128.84, 125.36, 117.33, 48.43, 28.01. HRMS (ESI) calculated $C_{16}H_{13}ClN_2O$,

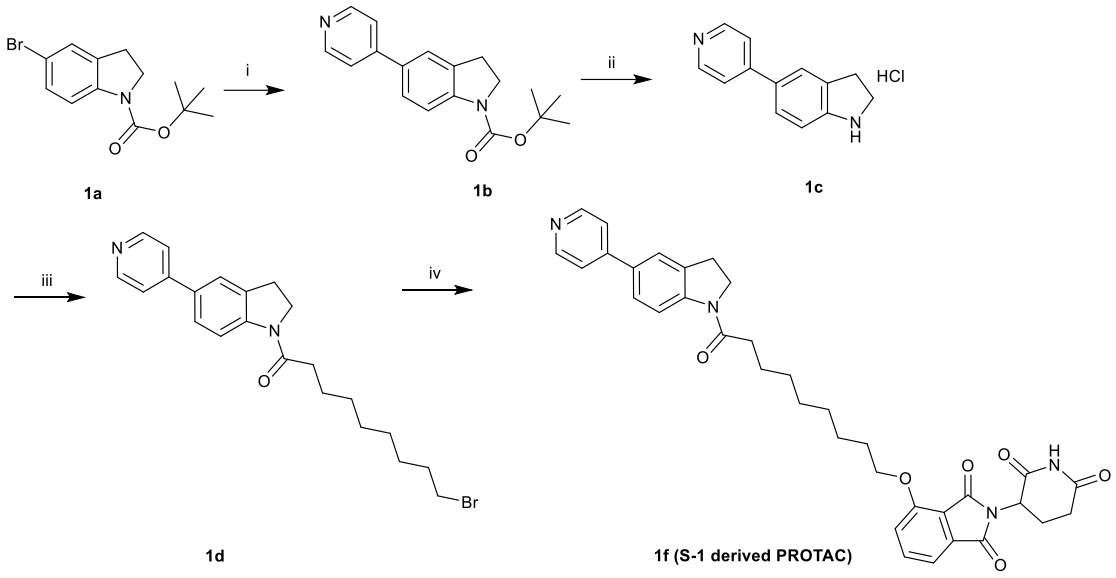
[M+H]+ = 285.0795, and measured [M+H]+: 285.0792

(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)(cyclohexyl)methanone (S-24)

S-24 was prepared in a similar manner described for **S-16.** Yield: 39%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.64 (s, 1H), 8.48 (d, J = 4.9 Hz, 1H), 8.36 (d, J = 7.5 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.25 (d, J = 4.9 Hz, 1H), 4.20 (t, J = 8.5 Hz, 2H), 3.26 (t, J = 8.5 Hz, 2H), 2.49 (t, J = 11.5 Hz, 1H), 1.92 – 1.81 (m, 4H), 1.76 – 1.57 (m, 3H), 1.39 – 1.26 (m, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 175.08, 150.24, 150.24, 147.91, 147.45, 144.12, 131.61, 130.22, 128.79, 125.36, 125.18, 117.17, 48.20, 44.02, 29.16, 28.08, 25.89, 25.87. HRMS (ESI) calculated $C_{20}H_{21}ClN_2O$, [M+H]+ = 341.1421, and measured [M+H]+: 341.1418

4-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)-4-oxobutanenitrile (S-25)

S-25 was prepared in a similar manner described for **S-16.** Yield: 24%. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.66 (s, 1H), 8.50 (d, J = 4.9 Hz, 1H), 8.29 (d, J = 8.2 Hz, 1H), 7.37 – 7.32 (m, 2H), 7.26 (d, J = 4.7 Hz, 1H), 4.12 (t, J = 8.4 Hz, 2H), 3.32 (t, J = 8.4 Hz, 2H), 2.87 – 2.77 (m, 4H). ^{13}C NMR (101 MHz, Chloroform-d) δ 167.18, 150.32, 148.00, 147.26, 143.22, 132.48, 131.39, 130.24, 128.99, 125.44, 125.36, 119.21, 116.93, 48.14, 31.79, 28.10, 12.76. HRMS (ESI) calculated $C_{18}H_{15}ClN_2O$, [M+H]+ = 312.0904, and measured [M+H]+: 312.0898



Scheme S3: synthesis of S-1 derived PROTAC.

Reagents and conditions: (i) pyridine-4-boronic acid pinacol ester, K_2CO_3, $Pd(PPh_3)_4$, dioxane/water = 4:1, 90 °C, overnight; (ii) 1M HCl in dioxane, R.T., 3 h; (iii) 9-bromononanoic acid, HATU, DIPEA, DCM, R.T., 3 h; (iv) 4-hydroxythalidomide, $NaHCO_3$, KI, MeCN, 60 °C, overnight

***tert*-butyl 5-(pyridin-4-yl) -2,3-dihydroindole -1-carboxylate (1b)**

To a mixture of the *tert*-butyl 5-bromo-2,3-dihydroindole-1-carboxylate (**1a**) (1.2 g, 1.0 equiv), potassium carbonate (K_2CO_3, 1.6 g, 3.0 equiv), and pyridine-4-boronic acid pinacol ester (0.8 g, 1.0 equiv) in a solvent mixture of dioxane and water (4:1), triphenylphosphine palladium (0.5 g, 0.1 equiv) was added under a nitrogen atmosphere. The reaction was heated to 90°C and stirred for 12 hours, after which it was allowed to reach room temperature. Then 100 mL water and ethyl acetate (100 mL×3) were added for extraction. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. Concentrated under vacuum to give the crude product *tert*-butyl 5-(pyridin-4-yl) -2,3-dihydroindole -1-carboxylate (**1b**) as yellow solid (1.06g, 90%). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.60 (d, J = 5.3 Hz, 2H), 7.90 (s, 1H), 7.49 – 7.39 (m, 4H), 4.02 (t, J = 8.7 Hz, 2H), 3.13 (t, J = 8.7 Hz, 2H), 1.58 (s, 9H). ^{13}C NMR (101 MHz, Chloroform-d) δ 152.46, 150.19, 150.19, 148.04, 131.86, 126.50, 126.50, 123.21, 121.09, 121.09, 115.03, 115.03, 77.97, 47.94, 29.29, 28.48. HRMS (ESI) calculated $C_{18}H_{20}N_2O_2$, [M+H]+ = 297.1603, and measured [M+H]+: 297.1589

9-bromo-1-(5-(pyridin-4-yl)indolin-1-yl)nonan-1-one (1d)

To a mixture of the intermediate product (**1b**), the 1M hydrochloric acid in dioxane was dropwised to the system for 10 min. Continued stirring the mixture until the yellow color disappeared and a white suspension formed. Evaporated the solvent under reduced pressure and dried the residue under vacuum to get the crude product 5-pyridin-4-yl-2,3-dihydroindole hydrochloride (**1c**) as

yellow solid, which was used in the next step without further purification. To a mixture of the intermediate product (**1c**), HATU (1.6 g, 1.2 equiv) and 9-bromononanoic acid (0.8 g, 1.0 equiv) in a solvent mixture of DCM (20 ml), DIPEA (0.5 g, 3.0 equiv) was added under a nitrogen atmosphere. The reaction was stirred at room temperature for 2 hours. Then 100 mL water and ethyl acetate (100 mL×3) were added for extraction. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. Concentrated under vacuum and purified on silica gel via petroleum ether : ethyl acetate = 2 : 1 to give product 9-bromo-1-(5-(pyridin-4-yl)indolin-1-yl)nonan-1-one (**1d**) as yellow solid (1.13 g, 76%). 1H NMR (400 MHz, Chloroform-d) δ 8.58 (d, J = 5.5 Hz, 2H), 8.30 (d, J = 8.4 Hz, 1H), 7.48 – 7.41 (m, 4H), 4.07 (t, J = 8.5 Hz, 2H), 3.37 (t, J = 6.8 Hz, 2H), 3.22 (t, J = 8.5 Hz, 2H), 2.40 (t, J = 7.4 Hz, 2H), 1.83 (p, J = 6.9 Hz, 2H), 1.72 (p, J = 7.4 Hz, 2H), 1.46 – 1.27 (m, 8H). ^{13}C NMR (101 MHz, Chloroform-d) δ 171.63, 150.25, 150.25, 147.87, 144.17, 133.11, 132.22, 126.62, 122.99, 121.15, 121.15, 117.27, 48.28, 35.93, 34.05, 32.81, 29.33, 29.28, 28.64, 28.14, 27.97, 24.46. HRMS (ESI) calculated $C_{22}H_{27}BrN_2O$, [M+H]+ = 415.1385, and measured [M+H]+: 415.1383

2-(2,6-dioxopiperidin-3-yl)-4-((9-oxo-9-(5-(pyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)nonyl)oxy)-1H-isoindole-1,3(2H)-dione (S-1 derived PROTAC, 1f)

To a mixture of the 9-bromo-1-(5-(pyridin-4-yl)indolin-1-yl)nonan-1-one (**1d**) (0.31 g, 1.0 equiv), $NaHCO_3$ (186 mg, 3 equiv) and KI (24 mg, 0.2 equiv) in a solvent mixture of MeCN (20 ml), 4-hydroxythalidomide (220 mg, 1.1 equiv) weas added slowly. The reaction was stirred at 60 °C overnight. Then 100 mL water and ethyl acetate (100 mL×3) were added for extraction. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. Concentrated under vacuum and purified on silica gel via petroleum ether : ethyl acetate = 1 : 1 to give product 9-bromo-1-(5-(pyridin-4-yl)indolin-1-yl)nonan-1-one (**1d**) as yellow solid (0.27 g, 61%). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.10 (s, 1H), 8.58 (d, J = 6.1 Hz, 2H), 8.17 (d, J = 8.4 Hz, 1H), 7.81 (dd, J = 8.5, 7.2 Hz, 1H), 7.70 (s, 1H), 7.68 – 7.66 (m, 2H), 7.64 (dd, J = 8.4, 2.0 Hz, 1H), 7.51 (d, J = 8.6 Hz, 1H), 7.44 (d, J = 7.3 Hz, 1H), 5.08 (dd, J = 12.9, 5.4 Hz, 1H), 4.21 (t, J = 6.4 Hz, 2H), 4.14 (t, J = 8.6 Hz, 2H), 3.21 (t, J = 8.6 Hz, 2H), 2.65 – 2.52 (m, 2H), 2.52 – 2.50 (m, 4H), 2.46 (t, J = 7.3 Hz, 2H), 1.77 (tt, J = 10.9, 6.7, 5.1 Hz, 2H), 1.61 (t, J = 7.0 Hz, 2H), 1.48 (t, J = 7.4 Hz, 2H), 1.37 – 1.34 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d6) δ 172.74, 171.30, 169.91, 166.83, 165.28, 156.02, 150.10, 146.62, 144.14, 136.99, 133.23, 132.95, 131.53, 125.89, 123.04, 120.58, 119.78, 116.22, 116.07, 115.10, 68.81, 48.73, 47.70, 34.87, 30.93, 28.83, 28.59, 28.57, 28.39, 27.31, 25.24, 23.90, 21.99. HRMS (ESI) calculated $C_{35}H_{36}N_4O_6$, [M+H]+ = 609.2713, and measured [M+H]+: 609.2714

Materials and Methods

Cell Culture and CCK-8 Assay Methods

MO3.13 and HMC3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine. Cells were maintained in a humidified incubator at 37°C with 5% CO2. The medium was changed every 2-3 days. For the Cell Counting Kit-8 (CCK-8) assay, MO3.13 and HMC3 cells were seeded into 96-well plates at a density of 5,000 cells per well in 100 µL of culture medium. After an overnight incubation to allow cell attachment, various treatments were applied according to the experimental design. Following the treatment period, 10 µL of CCK-8 solution (Dojindo Molecular Technologies) was added to each well. The plates were then incubated for an additional 2 hours at 37°C with 5% CO2. Absorbance was measured at 450 nm using a microplate reader. All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD).

SARM1 Protein Expression Method

The active truncated human SARM1 protein (412-724) was cloned into the pET-28a(+) vector, resulting in a construct with an N-terminal 6xHis-tag. This construct was transformed into E. coli BL21 (DE3) cells, which were grown at 37°C until the optical density at 600 nm (OD600) reached 0.6-0.8. The culture temperature was then reduced to 16°C, and protein expression was induced by adding 0.5 mM IPTG. The cells were grown overnight for approximately 18 hours before being harvested by centrifugation at 4,000 x g for 10 minutes at 4°C. The cell pellets were resuspended in lysis buffer (50 mM hepes, pH 8.0, 300 mM NaCl, , 1 mM phenylmethylsulfonyl fluoride (PMSF), and 5% Triton X-100) . The cells were lysated and then clarified by centrifugation at 20,000 x g for 30 minutes at 4°C. The supernatant was then applied to a nickel-nitrilotriacetic acid (Ni-NTA) affinity column pre-equilibrated with the lysis buffer.The column was washed with 10 column volumes (CVs) of wash buffer (50 mM hepes, pH 8.0, 300 mM NaCl, 5% Triton X-100, and 50 mM imidazole) to remove non-specifically bound proteins. The SARM1 protein was eluted using an elution buffer containing 50 mM hepes, pH 8.0, 300 mM NaCl, 5% Triton X-100 and 300 mM imidazole. Elution fractions containing the SARM1 protein were identified by SDS-PAGE. The protein was then subjected to size-exclusion chromatography (SEC) using a Superdex 200 Increase 10/300 GL column (GE Healthcare) pre-equilibrated with the elution buffer. Peak fractions containing the SARM1 protein were collected, and stored at -80°C in 10ml aliquots.

Supplementary Tables and Figures

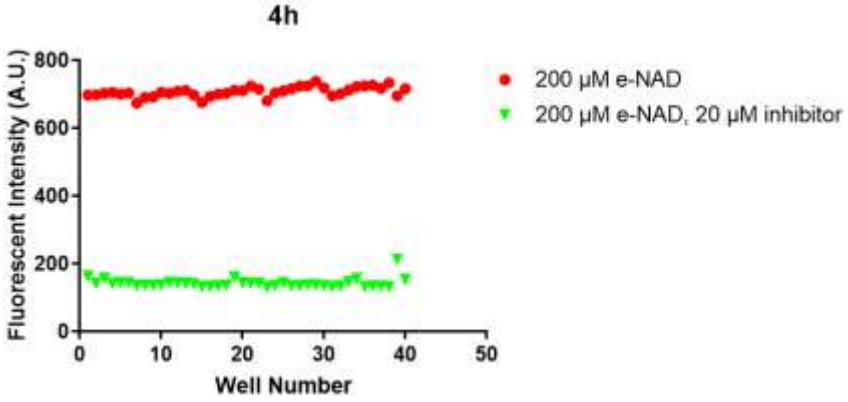


Figure S1A: High-throughput screening methodology. 200 µM 1,N6-Etheno-NAD (e-NAD) and 40 µg/ml active truncated human SARM1 protein (412-724) was incubated for 2h as positive control. 200 µM 1,N6-Etheno-NAD (e-NAD), 20 µM DSRM-3716 and 40 µg/ml active truncated human SARM1 protein (412-724) was incubated for 2h as negative control.

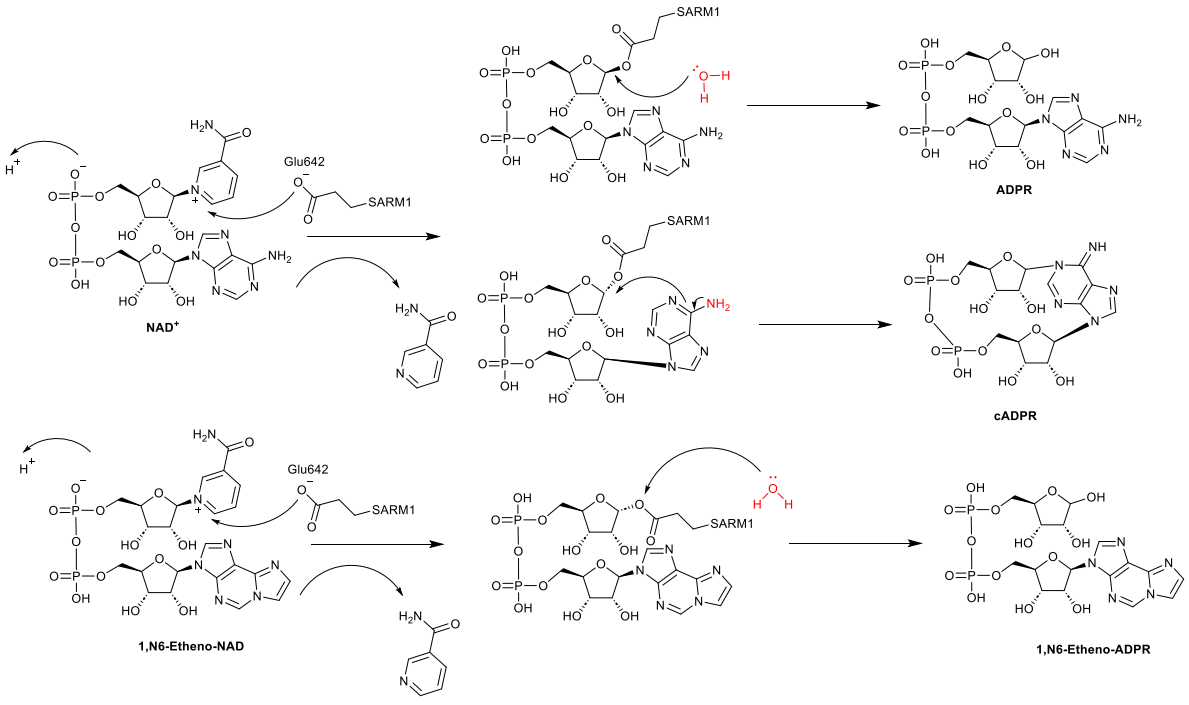


Figure S1B: Enzymatic Assay Details and Reaction Scheme of ADPR, cADPR, and 1,N6-Etheno-NAD Production

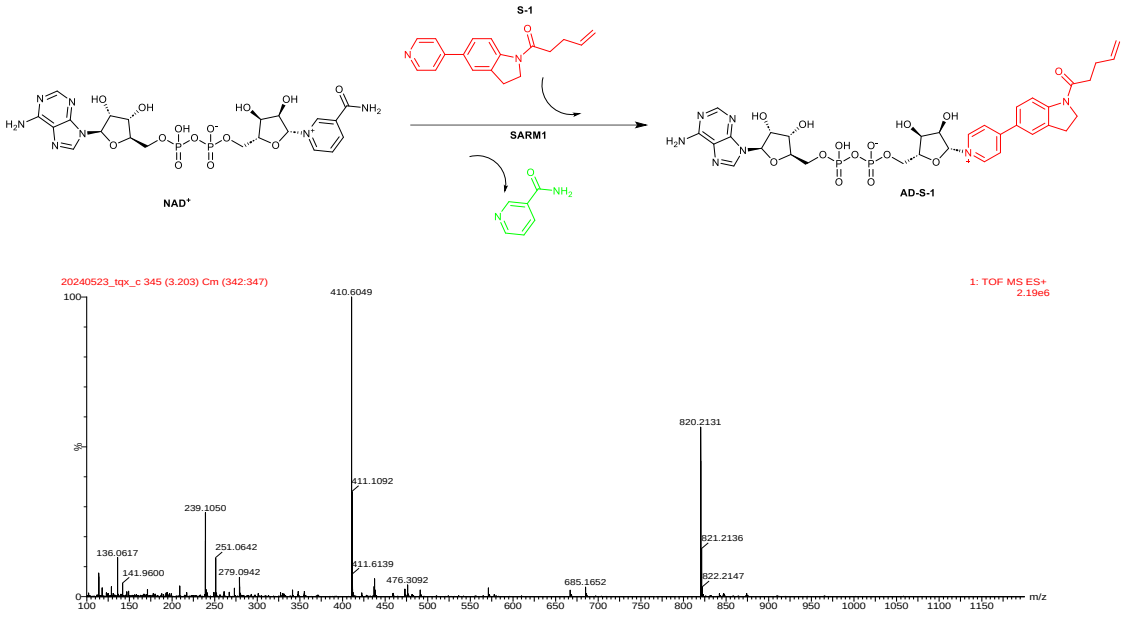


Figure S2: Schematic Diagram and High-Resolution Mass Spectrometry Analysis of the Reaction Product AD-S-1. HRMS (ESI) calculated $C_{33}H_{39}N_7O_{14}P_2$, [M+H]+ = 820.2108, and measured [M+H]+: 820.2131

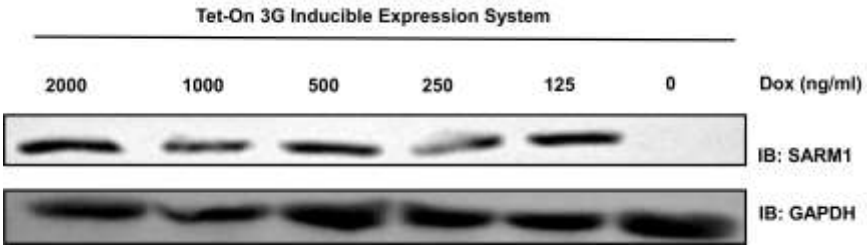


Figure S3: Expression Levels of SARM1 in a Tet-On 3G Inducible Expression System Across Different Doxycycline (Dox) Concentrations

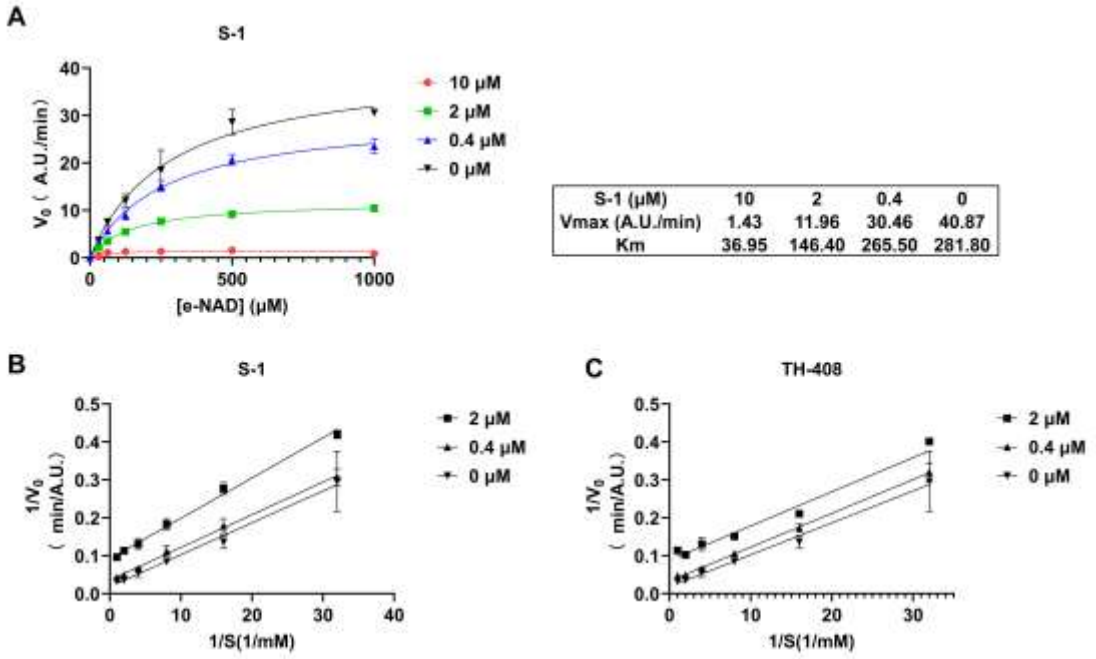


Figure S4: Enzyme Kinetic Studies of **S-1** and **TH408** Showing Uncompetitive Inhibition Mechanism. (A) Michaelis-Menten curves for the lead compound **S-1** at various concentrations (0, 0.4, 2, and 10 µM) are shown. (B) Lineweaver-Burk plot for **S-1** at concentrations of 0, 0.4, and 2 µM. (C) Lineweaver-Burk plot for **TH408** at concentrations of 0, 0.4, and 2 µM.

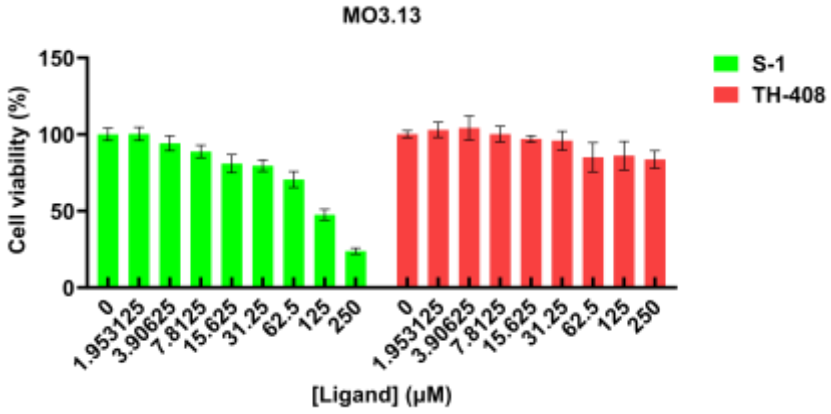


Figure S5: Cytotoxicity evaluation of the molecules.

Table S1: Compound SMILES strings

S-1	**O=C(CCC=C)N1C2=CC=C(C3=CC=NC=C3)C=C2CC1**
S-2	**O=C(CCC=C)N1C2=CC=C(C3=CC=CN=C3)C=C2CC1**
S-3	**O=C(CCC=C)N1C2=CC=C(C3=C(OC)C=CN=C3)C=C2CC1**
S-4	**O=C(CCC=C)N1C2=CC=C(C3=CC(C)=NC=C3)C=C2CC1**
S-5	**O=C(CCC=C)N1C2=CC=C(C3=CC(C)=NC(C)=C3)C=C2CC1**
S-6	**O=C(CCC=C)N1C2=CC=C(C3=CC(C(F)(F)F)=NC=C3)C=C2CC1**
S-7	**O=C(CCC=C)N1C2=CC=C(C3=CC(OC)=CN=C3)C=C2CC1**
S-8	**O=C(CCC=C)N1C2=CC=C(C3=CC(Cl)=NC=C3)C=C2CC1**
TH-408	**O=C(CCC=C)N1C2=CC=C(C3=C(Cl)C=NC=C3)C=C2CC1**
S-9	**FC(C=NC=C1)=C1C2=CC=C(N(C(CCC=C)=O)CC3)C3=C2**
S-10	**O=C(CCC=C)N1C2=CC=C(C3=C(C(F)(F)F)C=NC=C3)C=C2CC1**
S-11	**O=C(CCC=C)N1C2=CC=C(C3=C([N+]([O-])=O)C=NC=C3)C=C2CC1**
S-12	**O=C(CCC=C)N1C2=CC=C(C3=C(OC)C=NC=C3)C=C2CC1**
S-13	**O=C(N1C2=CC=CC(C3=C(Cl)C=NC=C3)=C2CC1)CCC=C**
S-14	**O=C(N1C2=CC(C3=C(Cl)C=NC=C3)=CC=C2CC1)CCC=C**
S-15	**ClC(C=NC=C1)=C1C2=C(N(C(CCC=C)=O)CC3)C3=CC=C2**
S-16	**C=CCCC(N1CC2(C3=CC(C4=CC=NC=C4Cl)=CC=C31)CCCC2)=O**
S-17	**O=C(CCC=C)N1C2=CC=C(C=C2CCC1)C3=C(C=NC=C3)Cl**
S-18	**O=C(N1CC2=CC=C(C3=C(Cl)C=NC=C3)C=C2CC1)CCC=C**
S-19	**O=C(N1C2=CC=C(C3=C(Cl)C=NC=C3)C=C2CC1)CCCCC=C**
S-20	**O=C(CC)N1C2=CC=C(C3=C(Cl)C=NC=C3)C=C2CC1**
S-21	**O=C(CCCC)N1C2=CC=C(C3=C(Cl)C=NC=C3)C=C2CC1**
S-22	**O=C(C12CC3CC(C2)CC(C1)C3)N4C5=CC=C(C6=C(Cl)C=NC=C6)C=C5CC4**
S-23	**O=C(C=C)N1C2=CC=C(C3=C(Cl)C=NC=C3)C=C2CC1**
S-24	**O=C(C1CCCCC1)N2C3=CC=C(C4=C(Cl)C=NC=C4)C=C3CC2**
S-25	**O=C(C1CCCCC1)N2C3=CC=C(C4=C(Cl)C=NC=C4)C=C3CC2**
1b	**O=C(OC(C)(C)C)N1C2=CC=C(C3=CC=NC=C3)C=C2CC1**
1d	**O=C(N1C2=CC=C(C=C2CC1)C3=CC=NC=C3)CCCCCCCCBr**
PROTAC	**O=C(N1C2=CC=C(C=C2CC1)C3=CC=NC=C3)CCCCCCCCOC4=CC=CC(C(N5C6CCC(NC6=O)=O)=O)=C4C5=O**

Table S2: Introduction of Life Chemicals- Pre-plated Diversity Sets

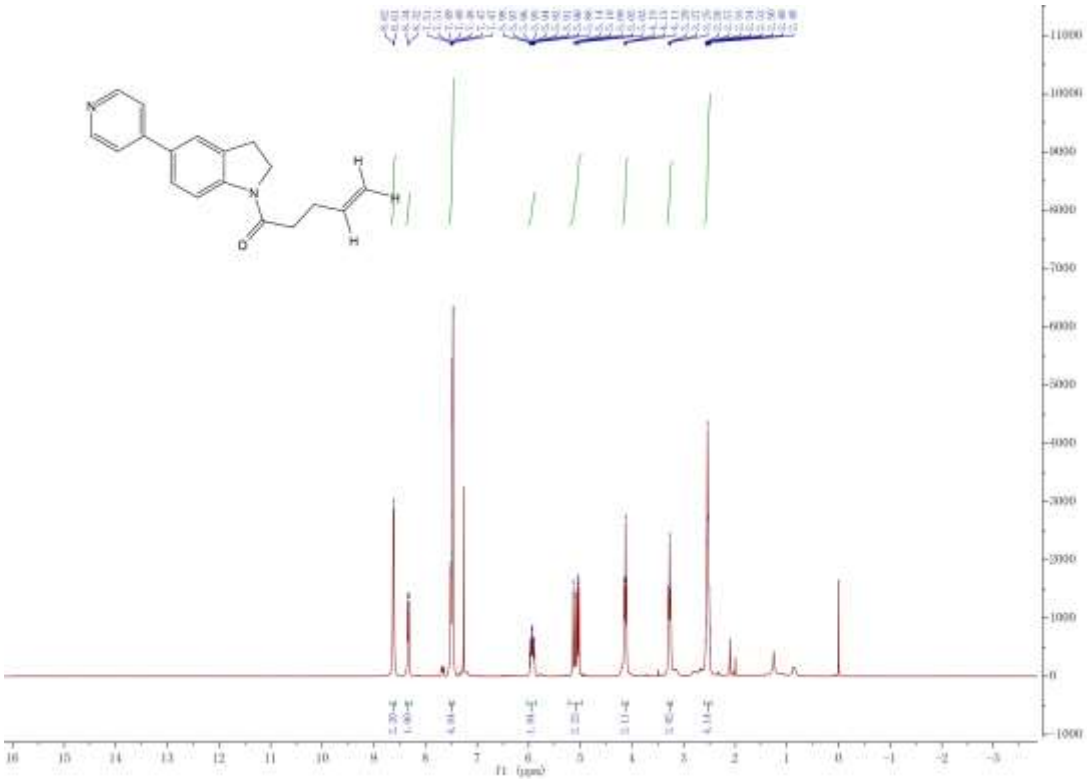
Diversity Set	Average values							
	MW	HbD	HbAc	cLogP	RotB	Fsp3	PSA	Tanimoto
50K	357.29	1.38	3.77	2.51	4.91	0.35	93.67	0.79
20K	355.04	1.39	3.77	2.42	4.78	0.35	93.06	0.74
15K	357.53	1.40	3.77	2.48	4.94	0.36	91.34	0.76
10K	359.44	1.38	3.76	2.59	5.02	0.35	90.06	0.78
5K	361.31	1.34	3.78	2.76	5.11	0.33	88.52	0.80

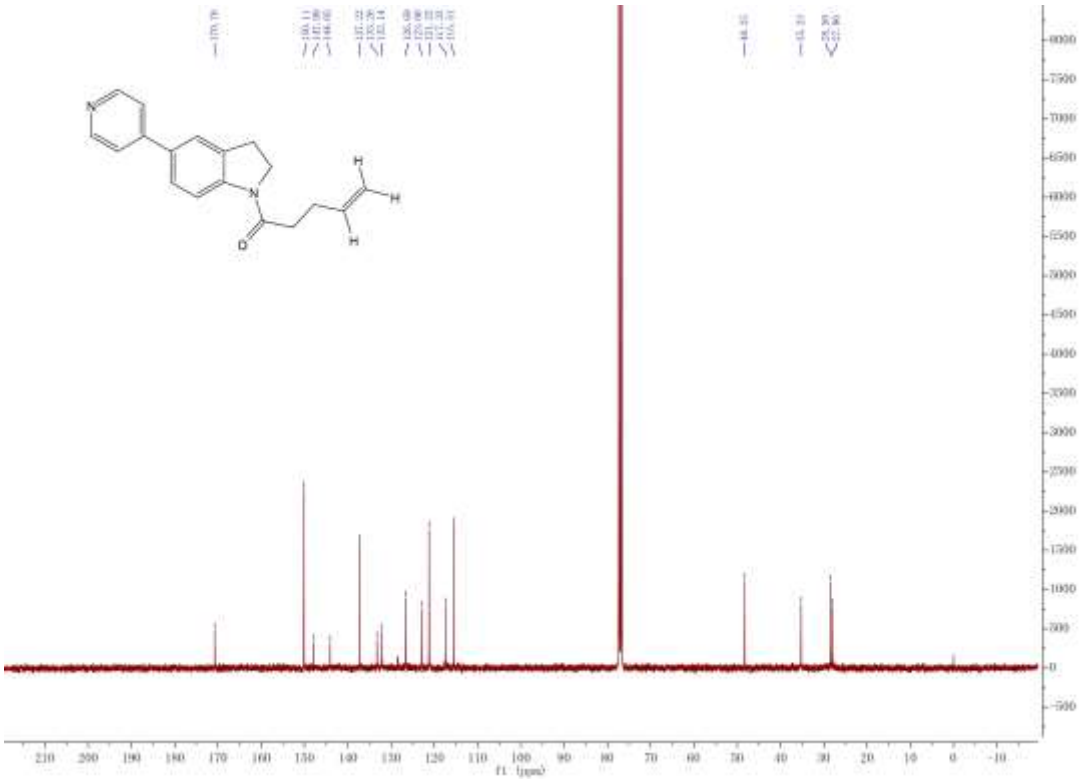
Diversity Set	Number of compounds	Number of Scaffolds	Number of Scaffold-based compounds	Number of Singletones
50K	50,240	2,242	42,283	7,957
20K	20,160	1,847	17,974	2,186
15K	15,040	1,947	12,904	2,136
10K	9,920	1,813	7,893	2,027
5K	5,120	1,413	3,378	1,742

The "life chemicals pre-plated diversity sets" originate from the Active Screening Platform at the Center for Pharmaceutical Technology, Tsinghua University, which is a commercial library. Provided by Life Chemicals, these sets are a series of pre-formulated compound diversity collections. These collections encompass up to 50,000 novel compounds, selected for their optimal physicochemical properties, making them a rich resource for high-throughput screening initiatives.

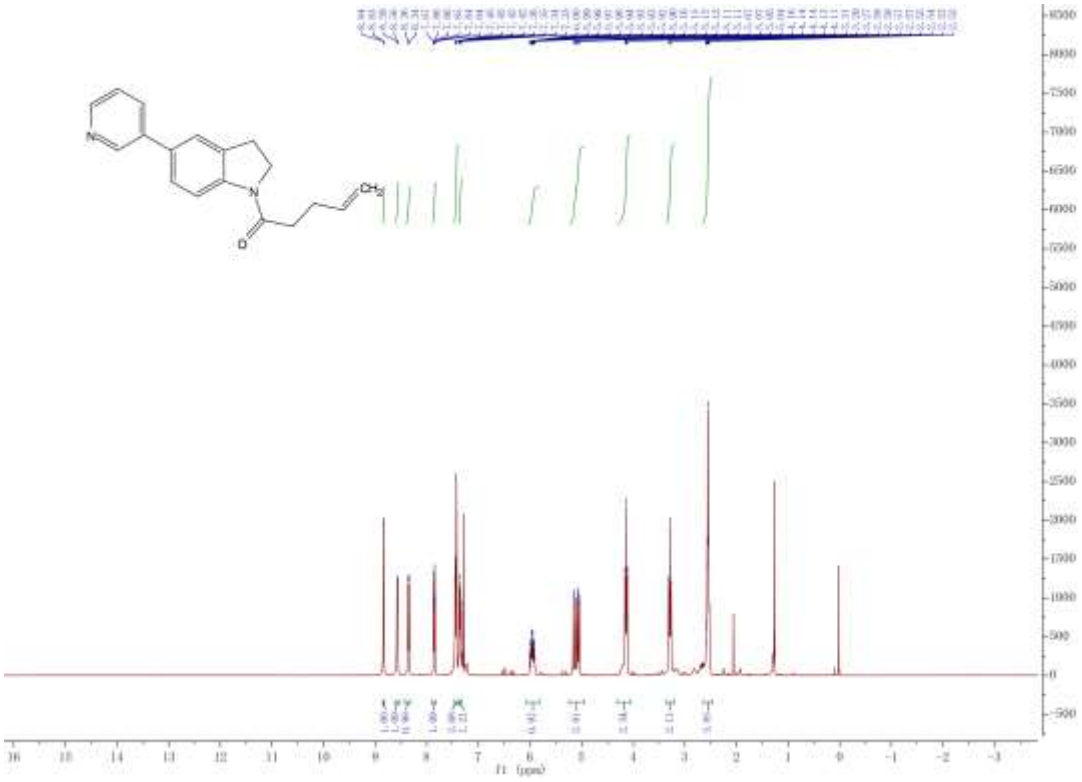
4. NMR Spectra

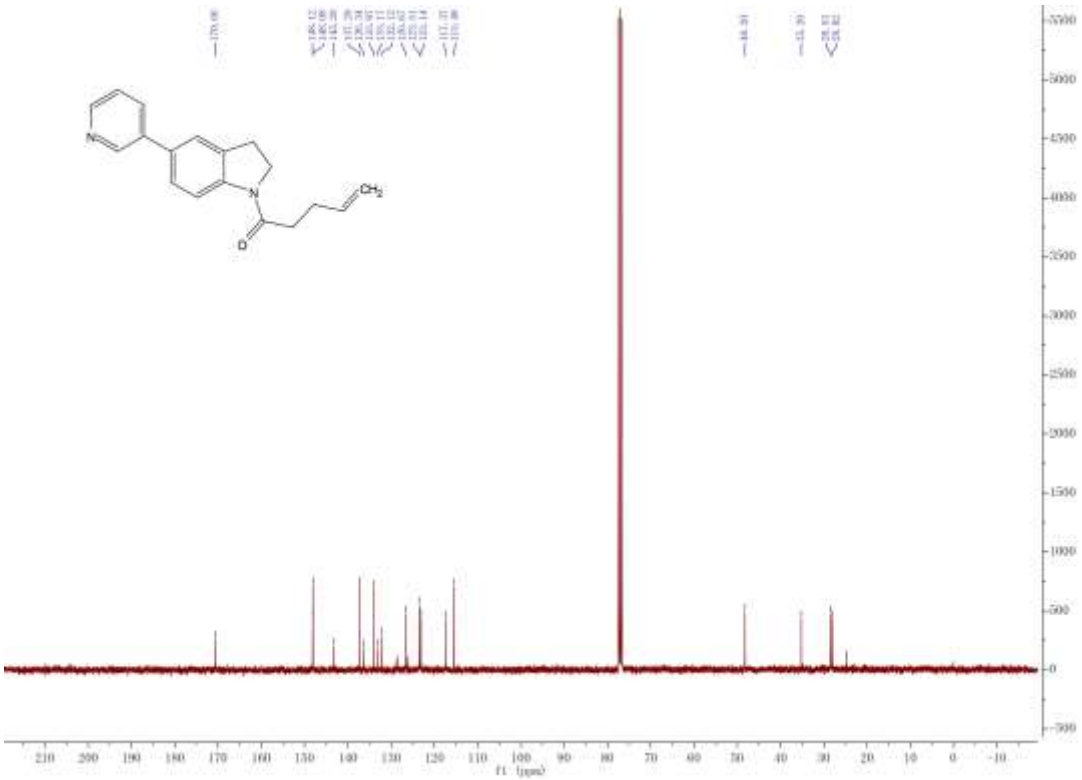
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(pyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-1)



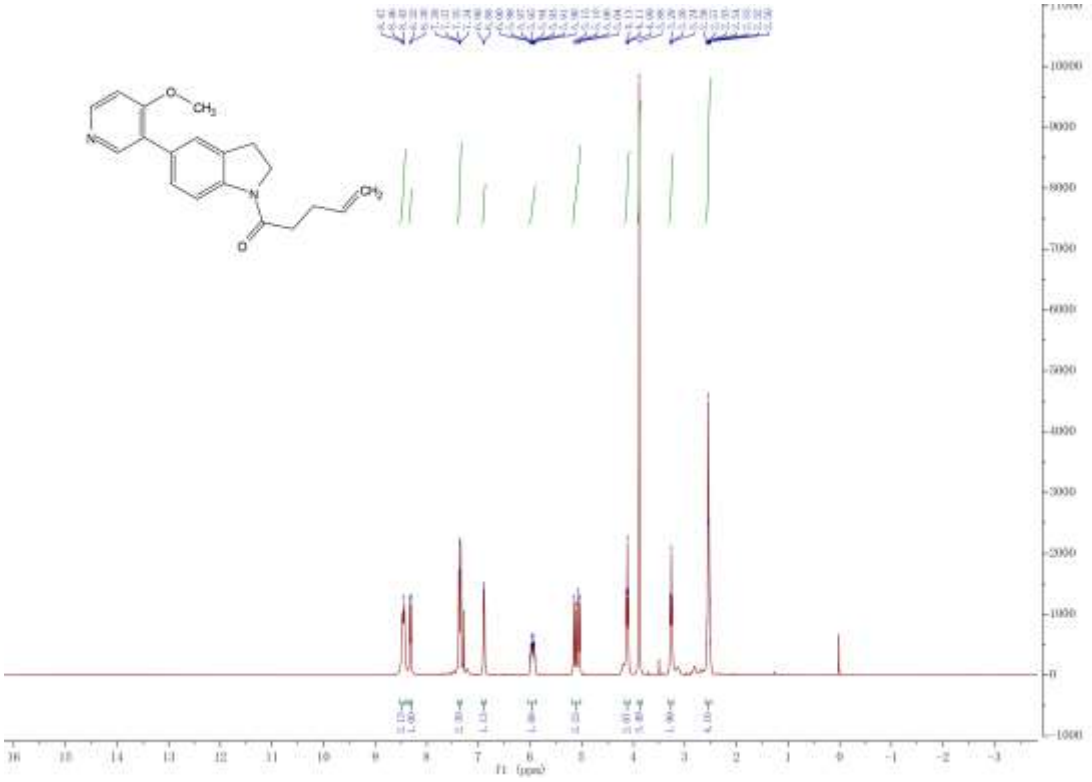


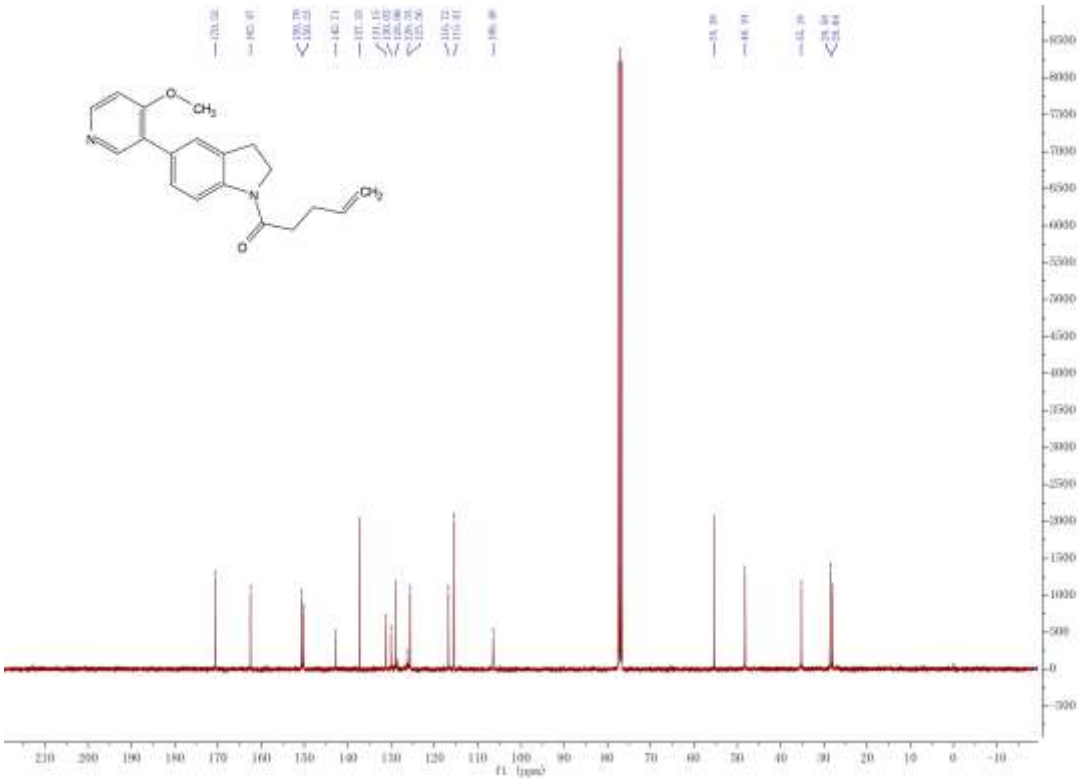
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(pyridin-3-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-2)



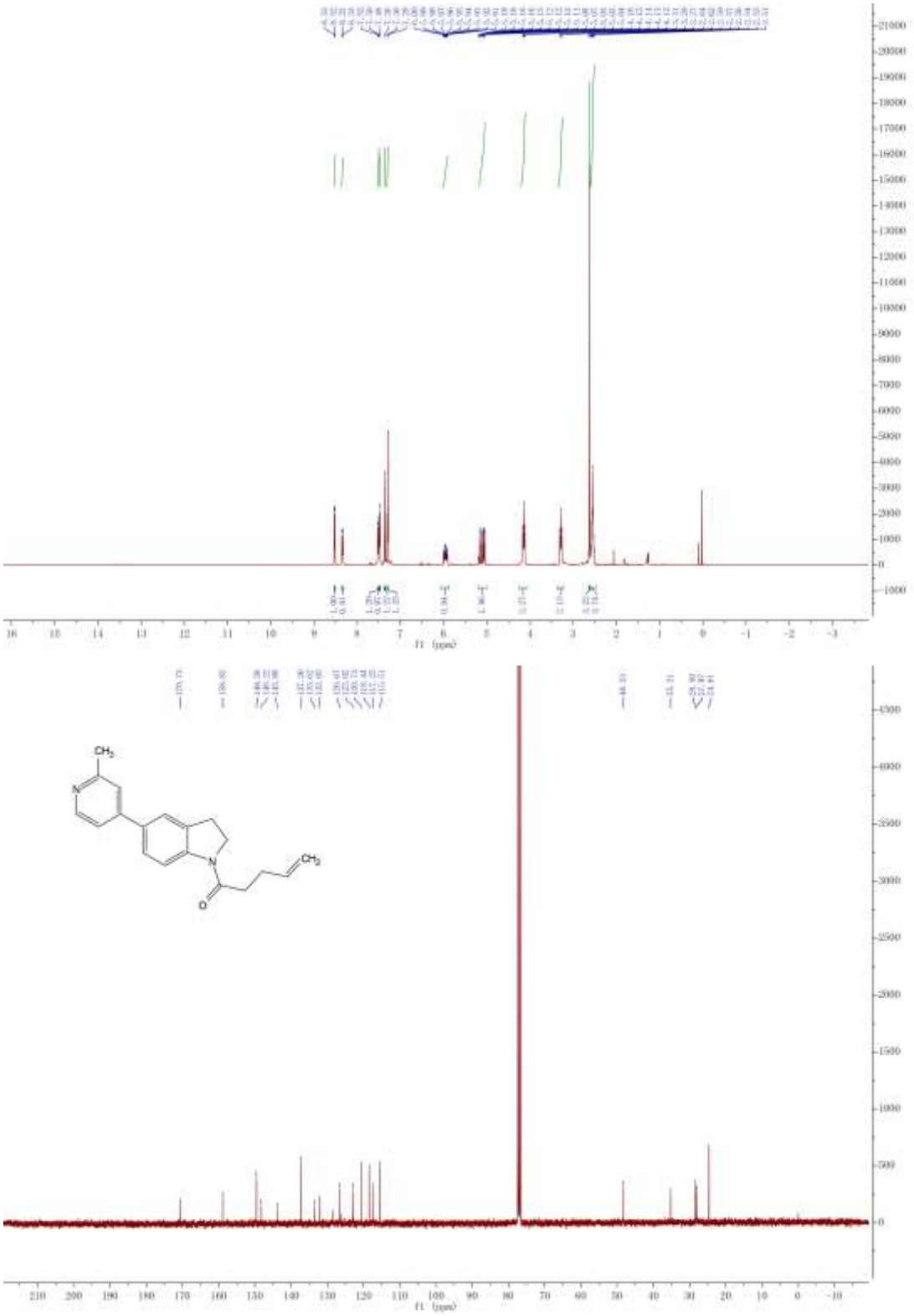


Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(4-methoxypyridin-3-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-3)

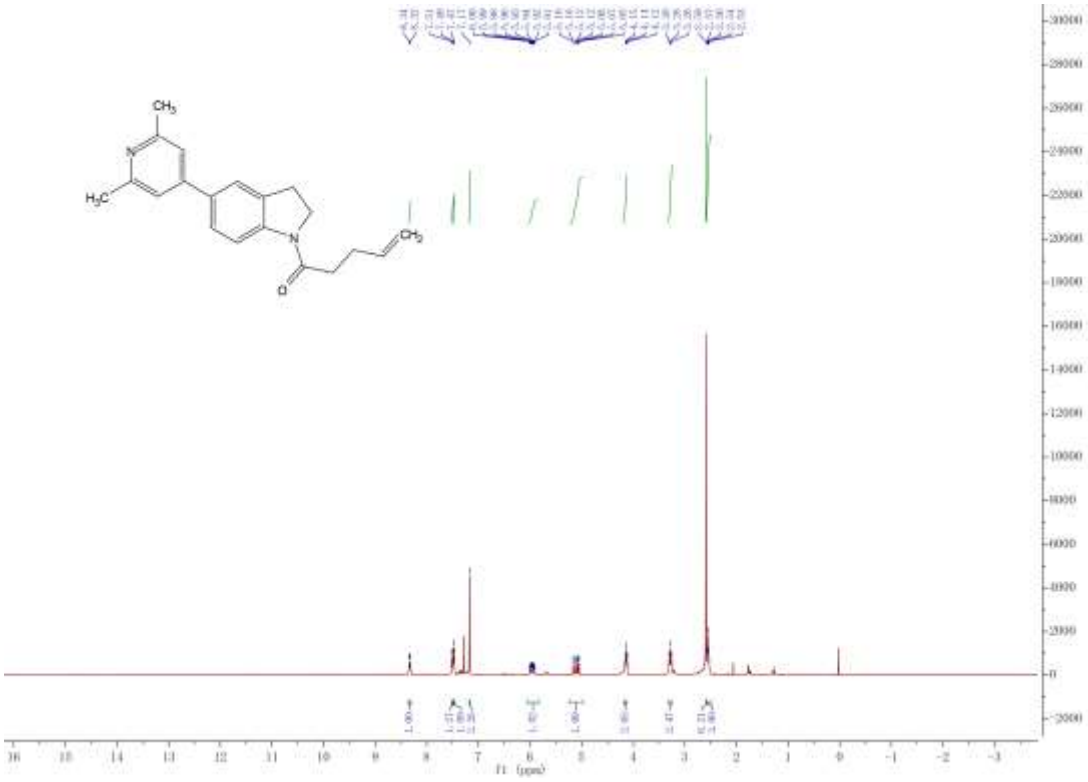


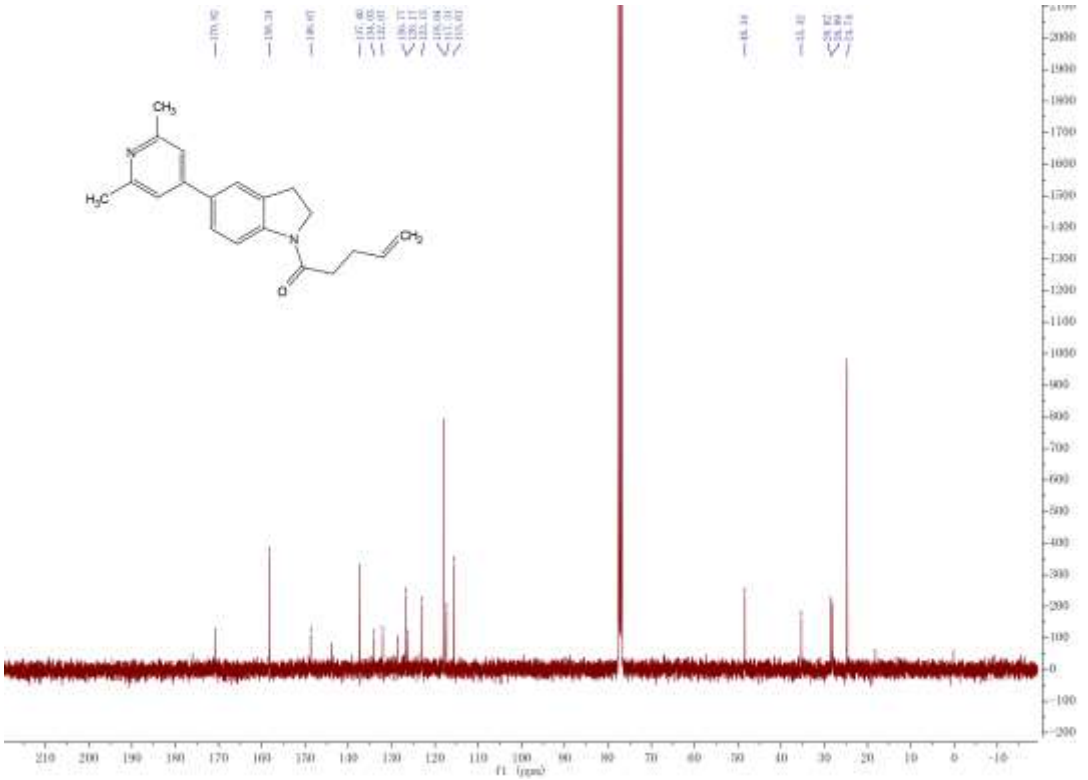


Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(2-methylpyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-4)

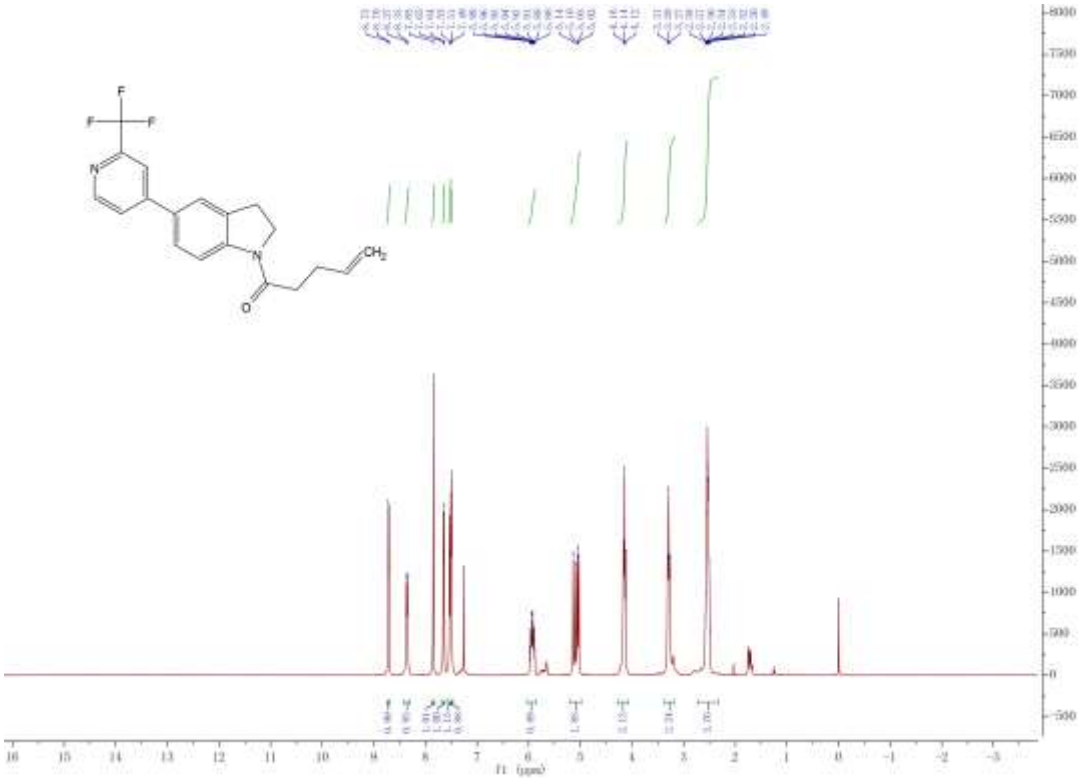


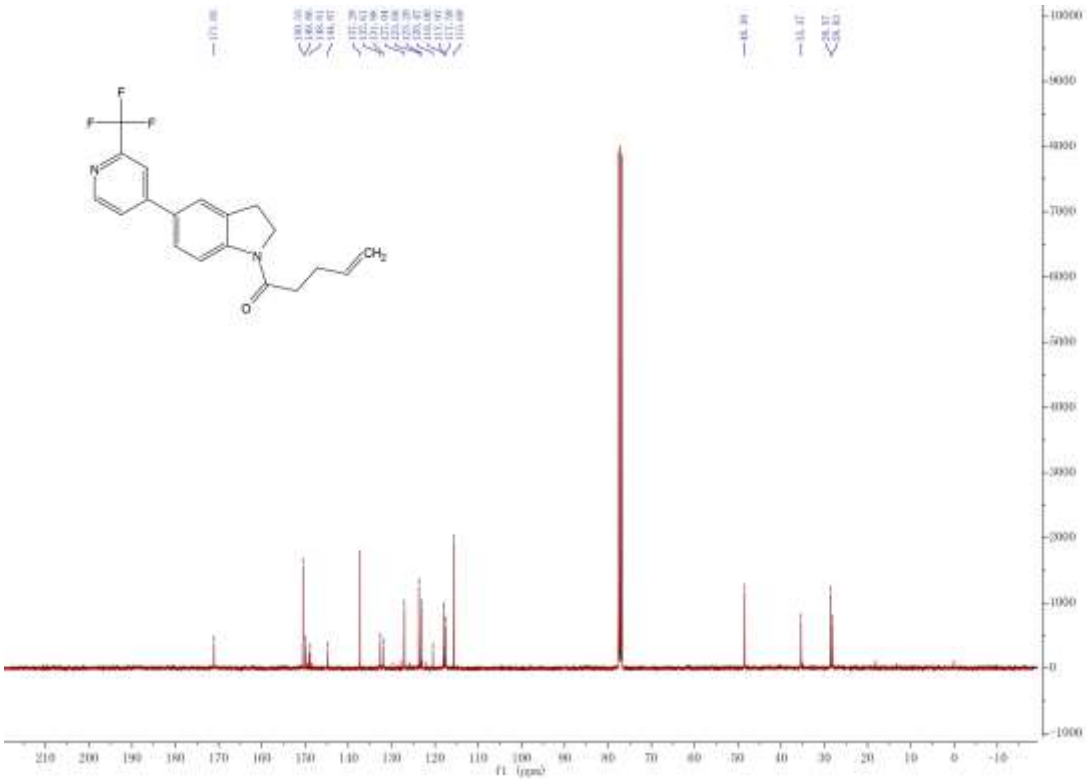
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(2,6-dimethylpyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-5)



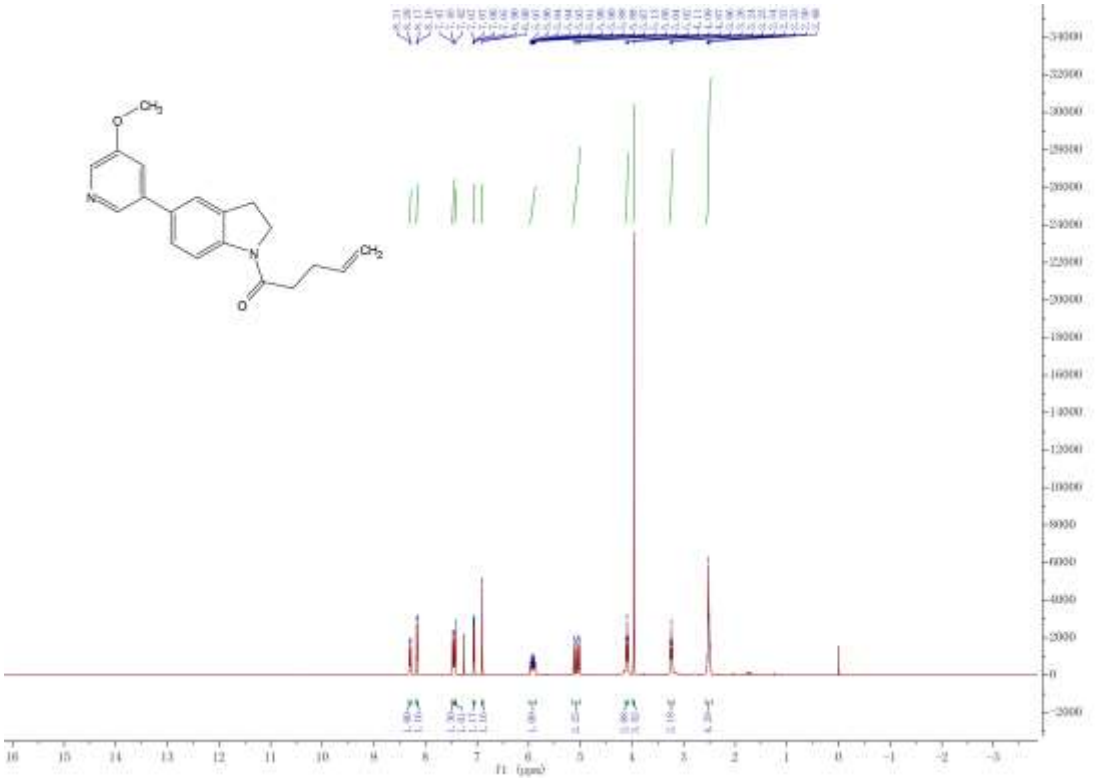


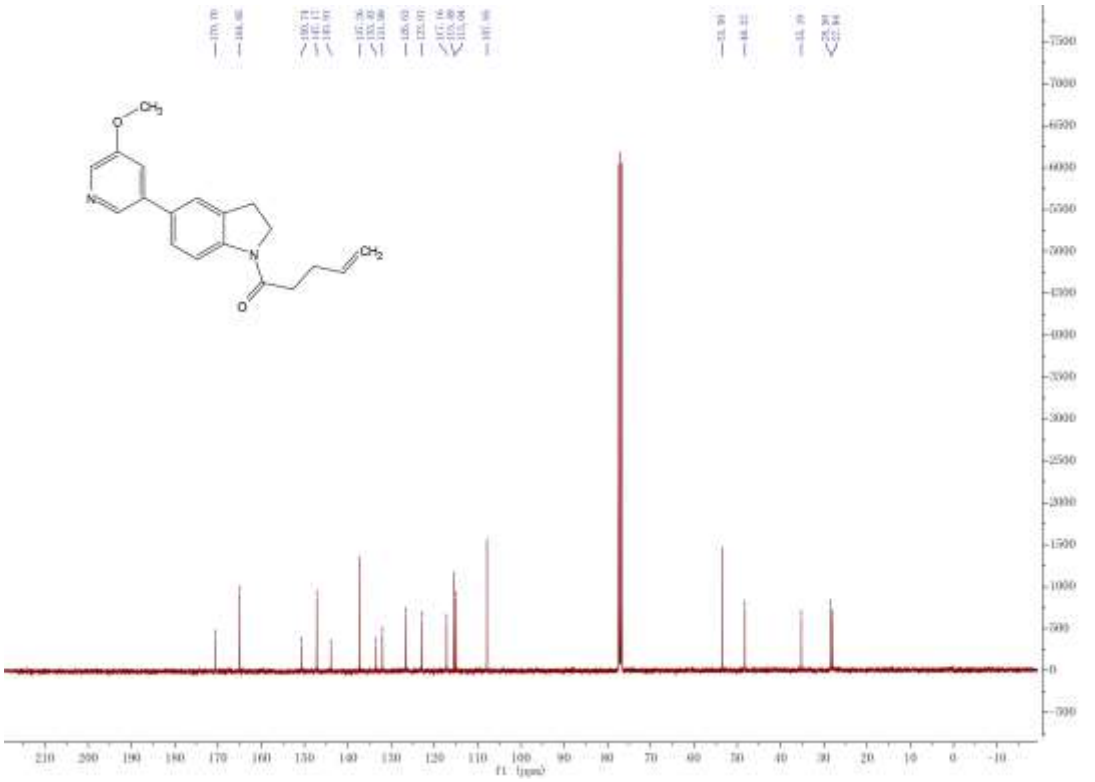
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(2-(trifluoromethyl)pyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-6)



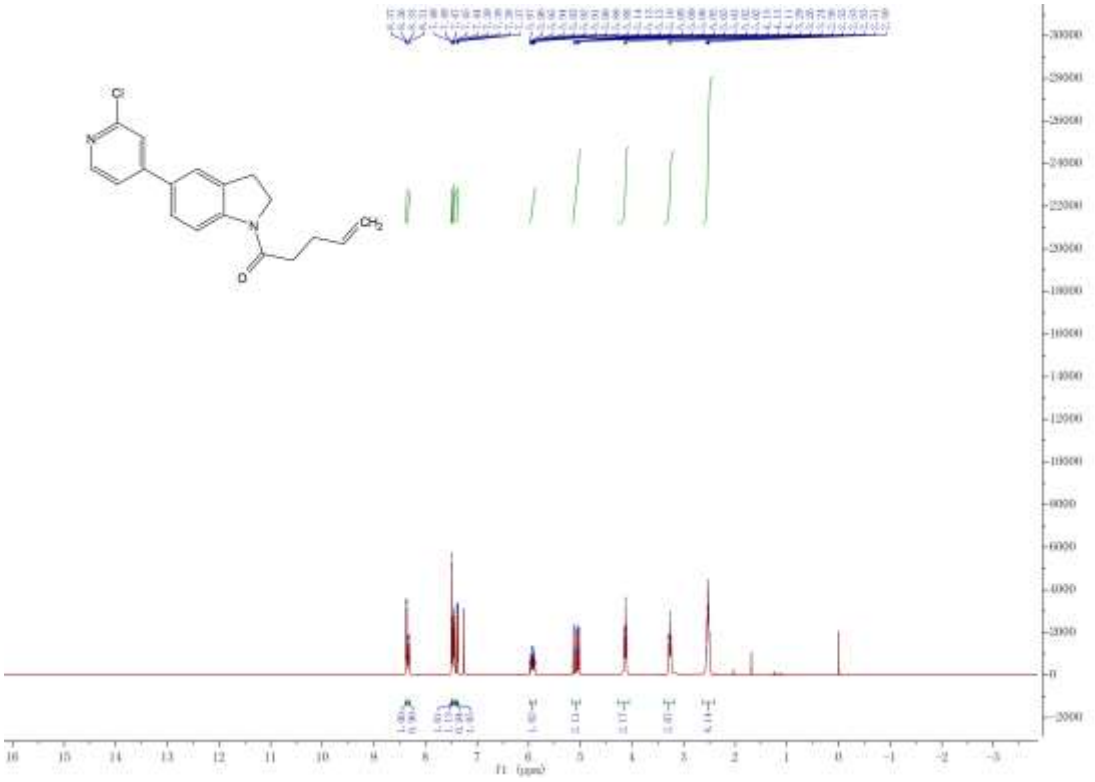


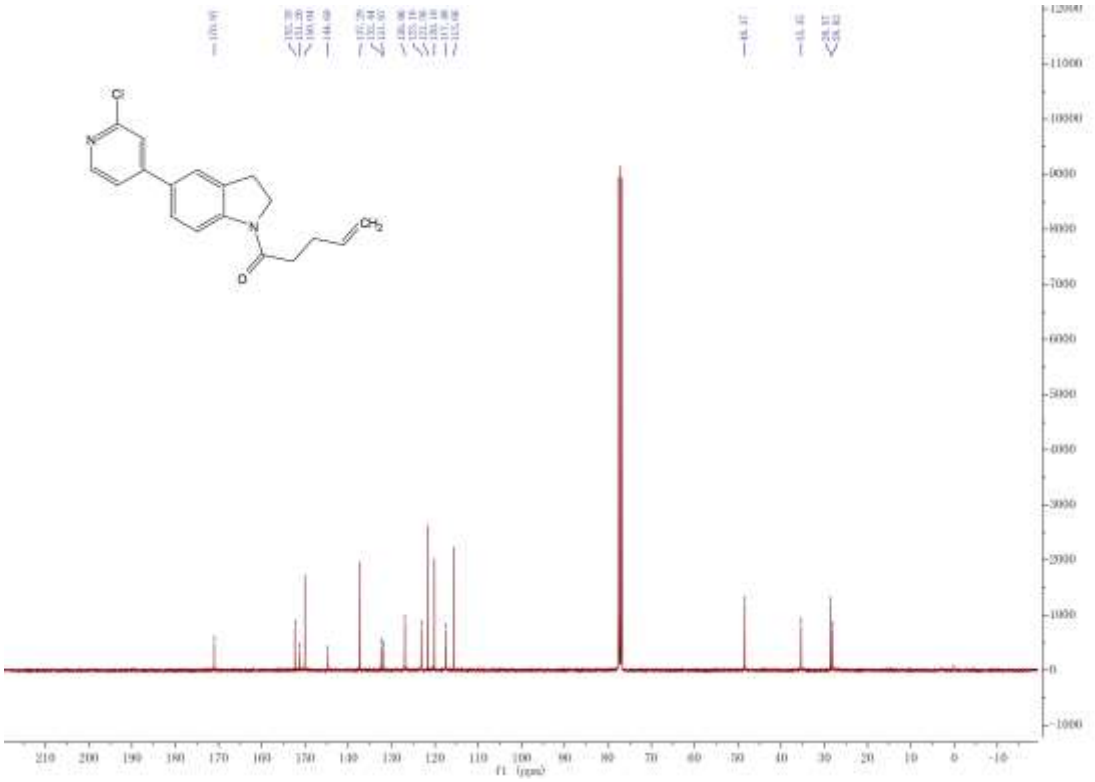
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(5-methoxypyridin-3-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-7)



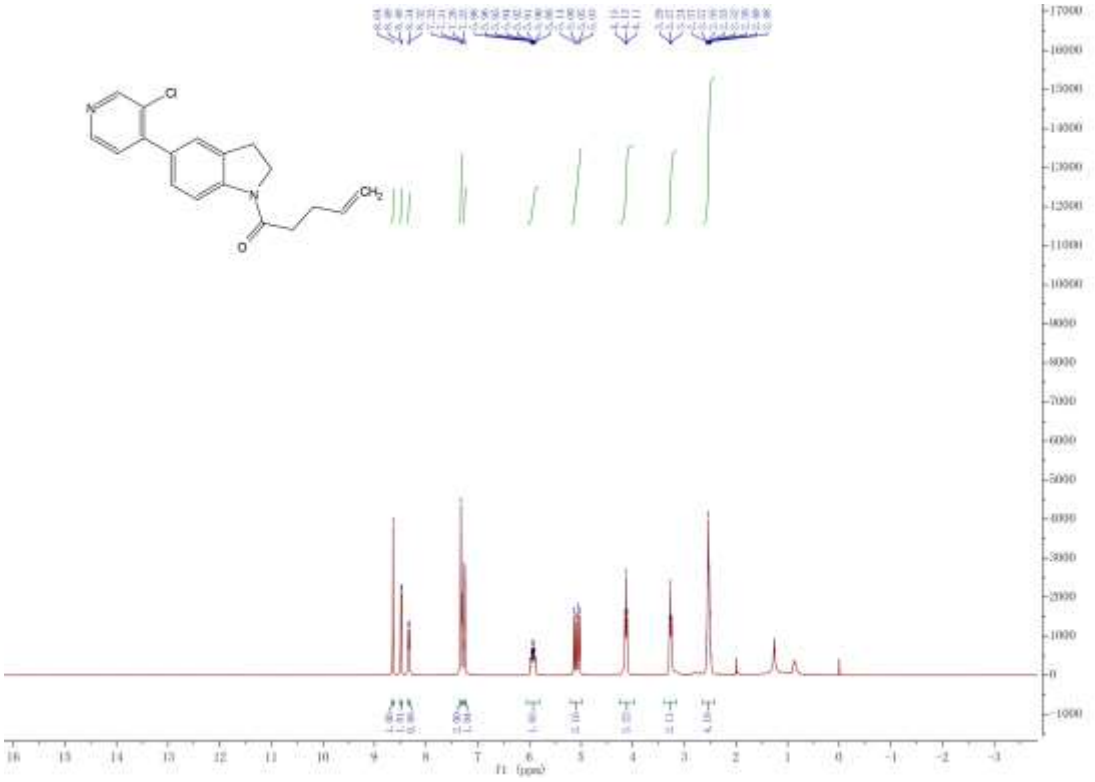


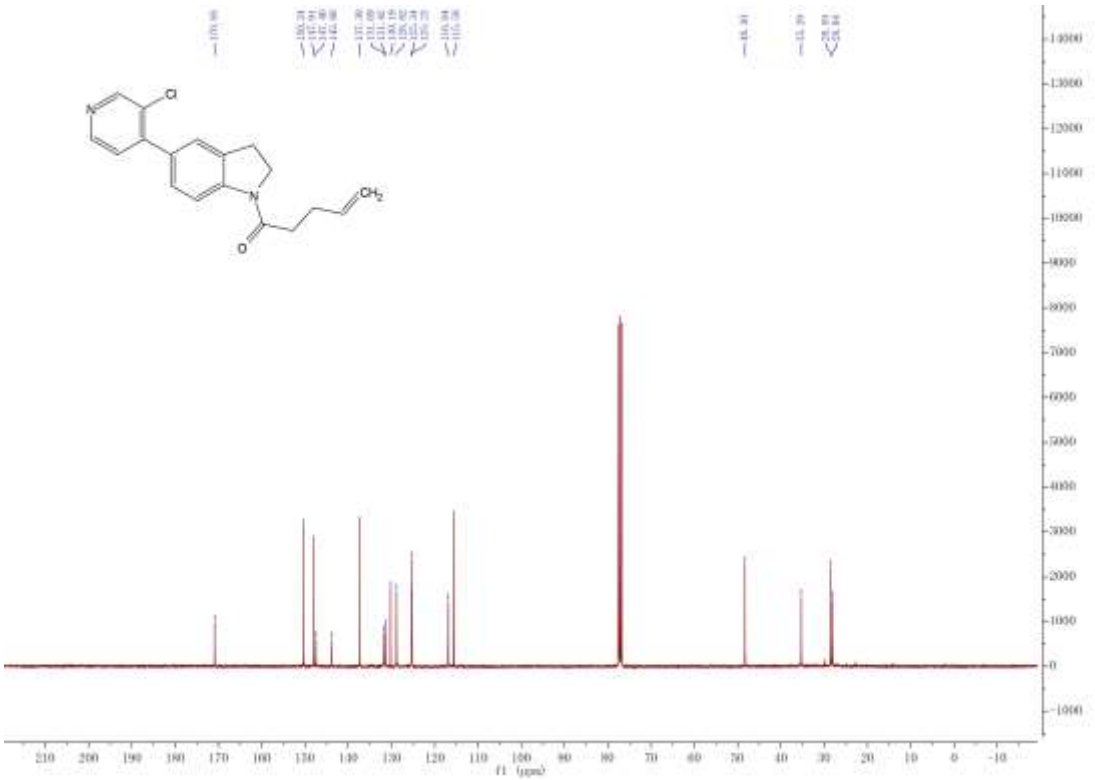
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(2-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-8)



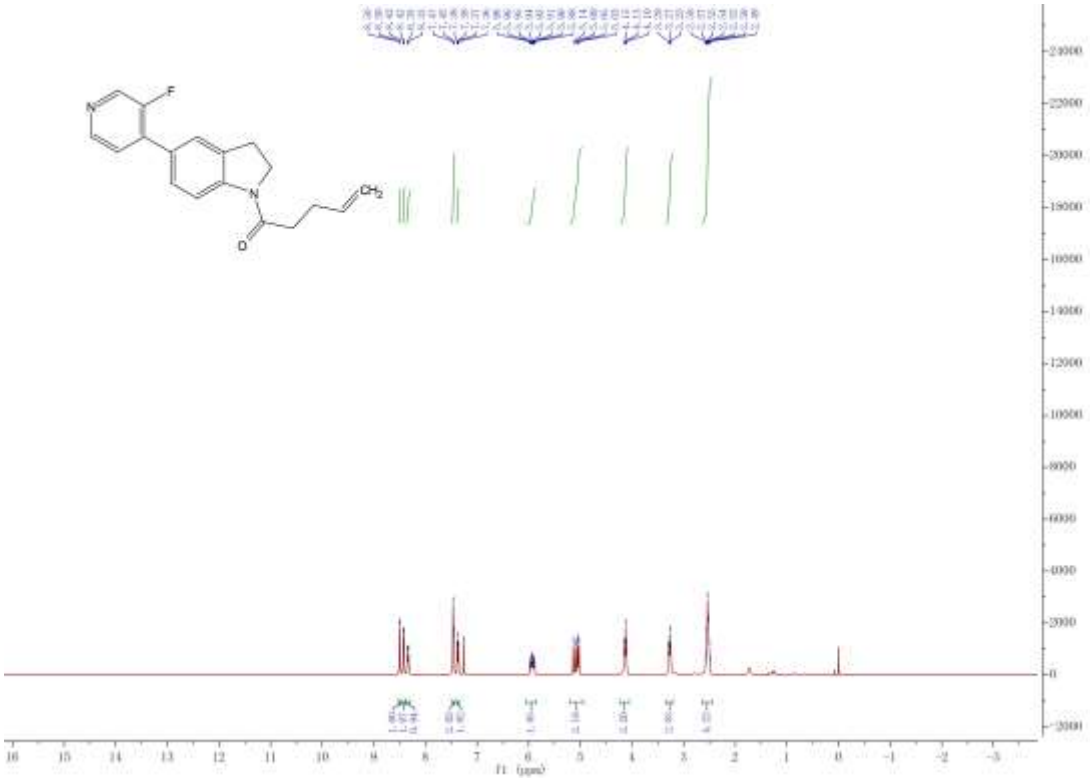


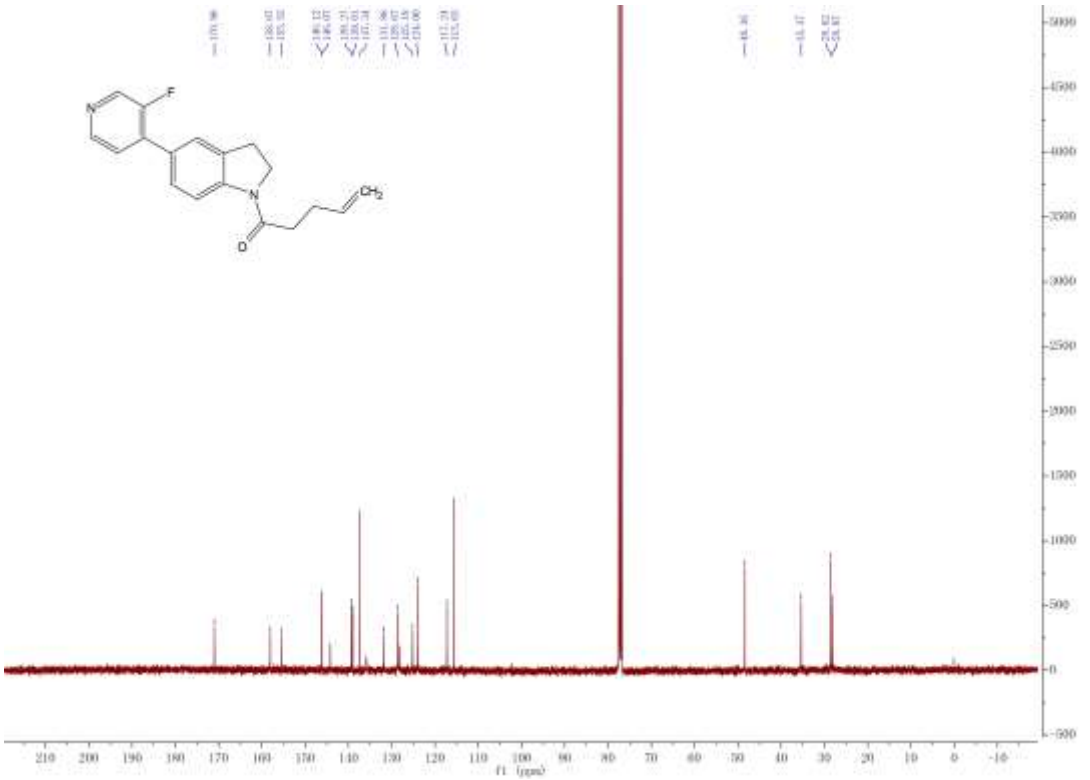
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (TH-408)



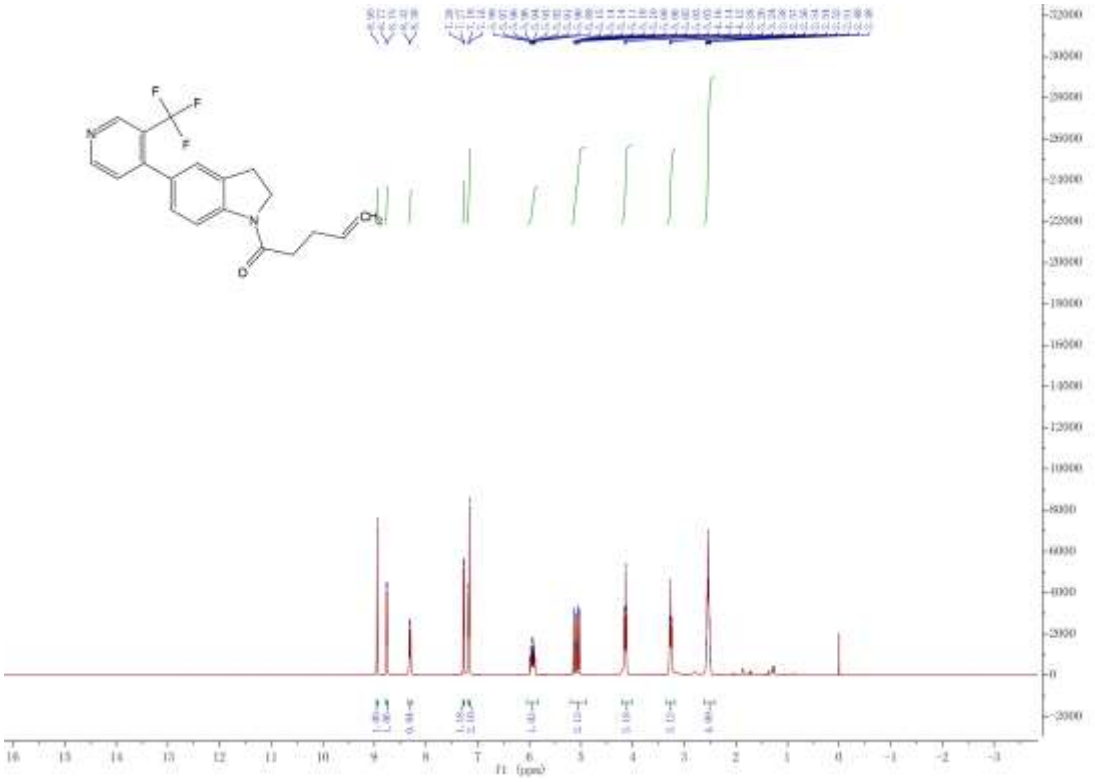


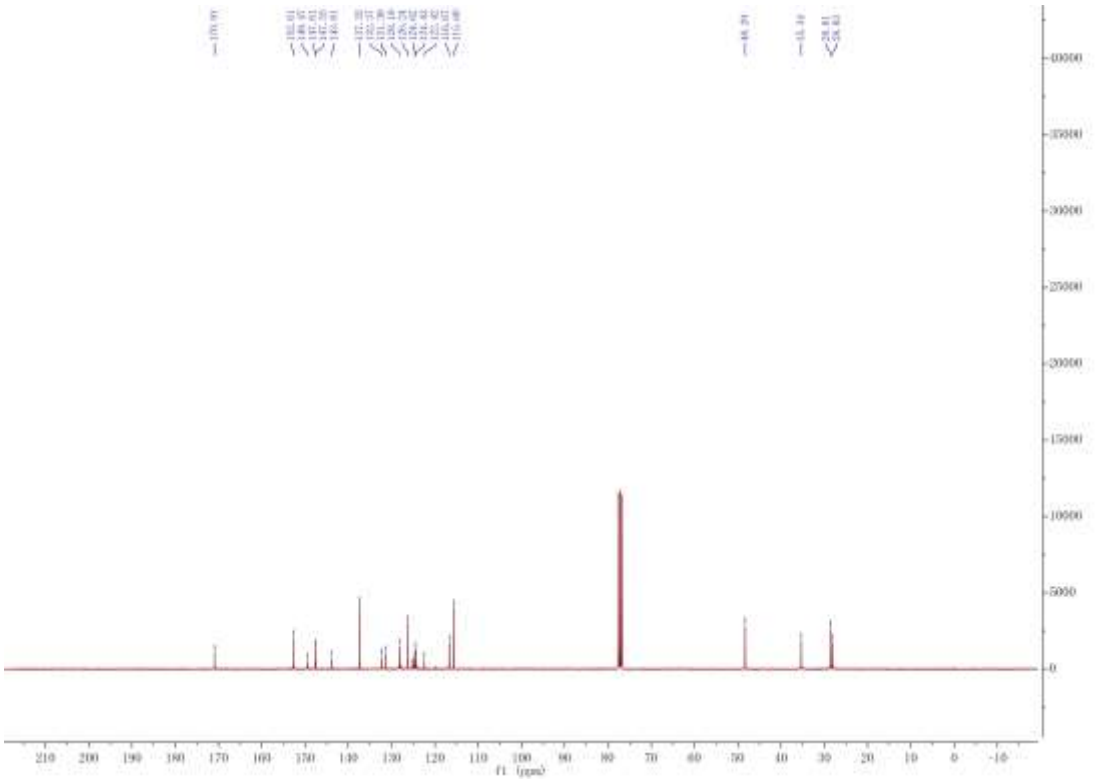
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-fluoropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-9)



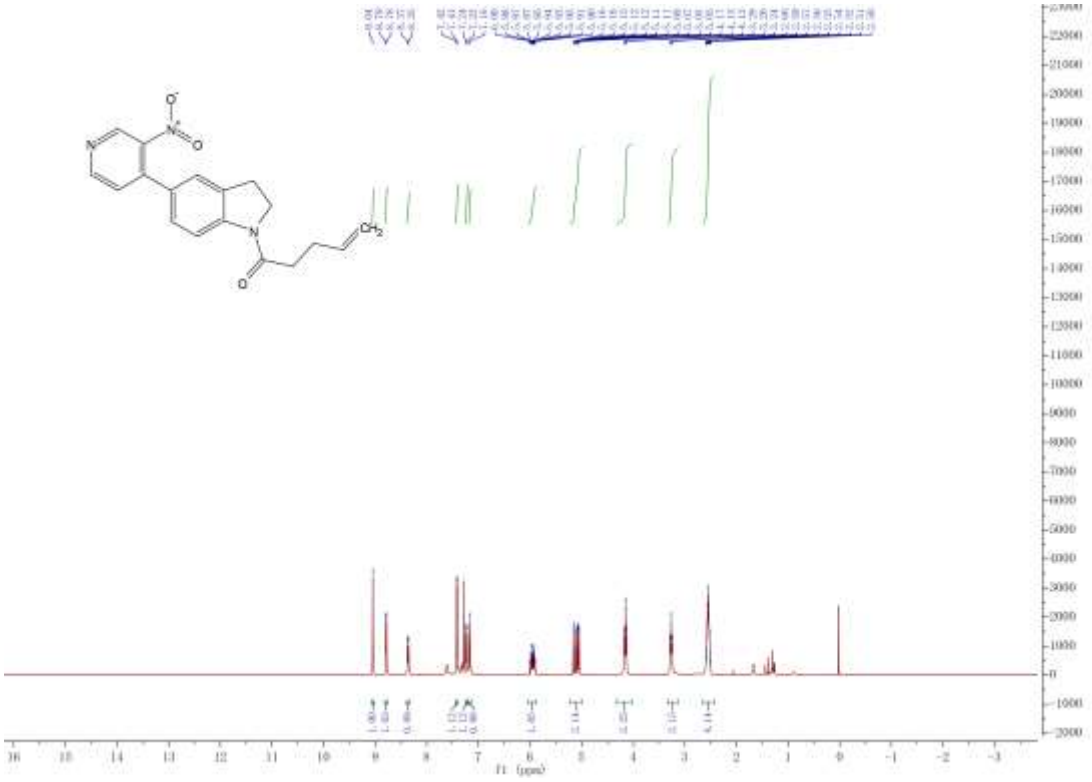


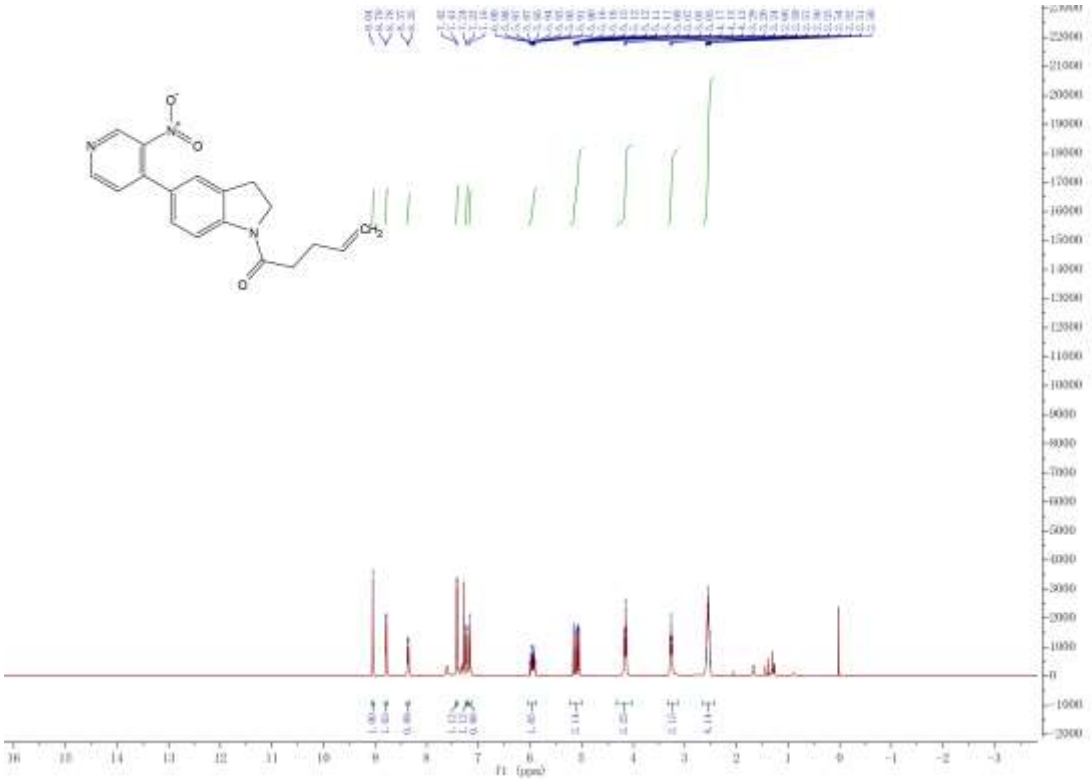
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-(trifluoromethyl)pyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-10)



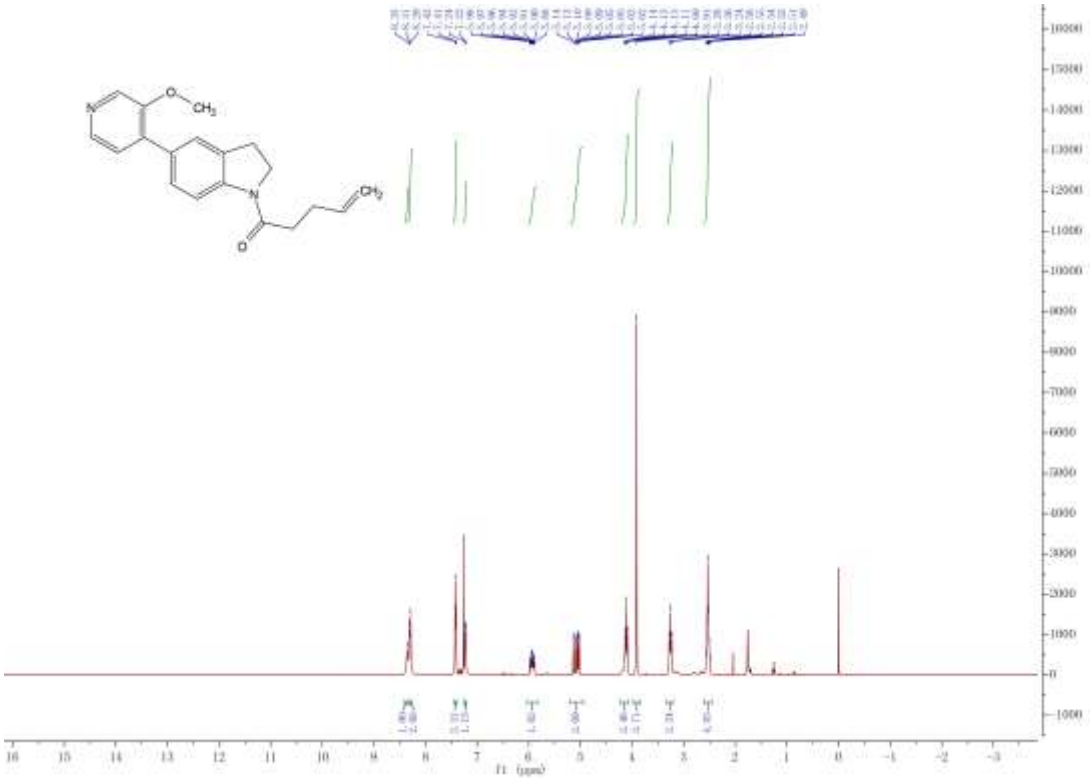


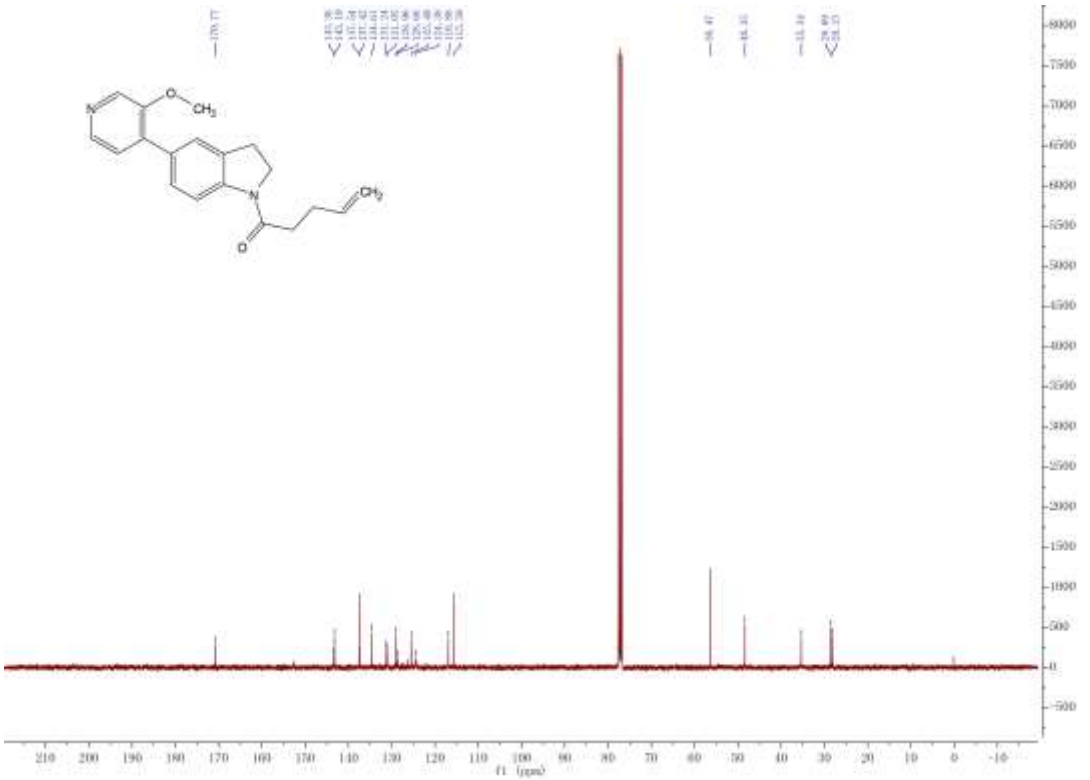
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-nitropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-11)



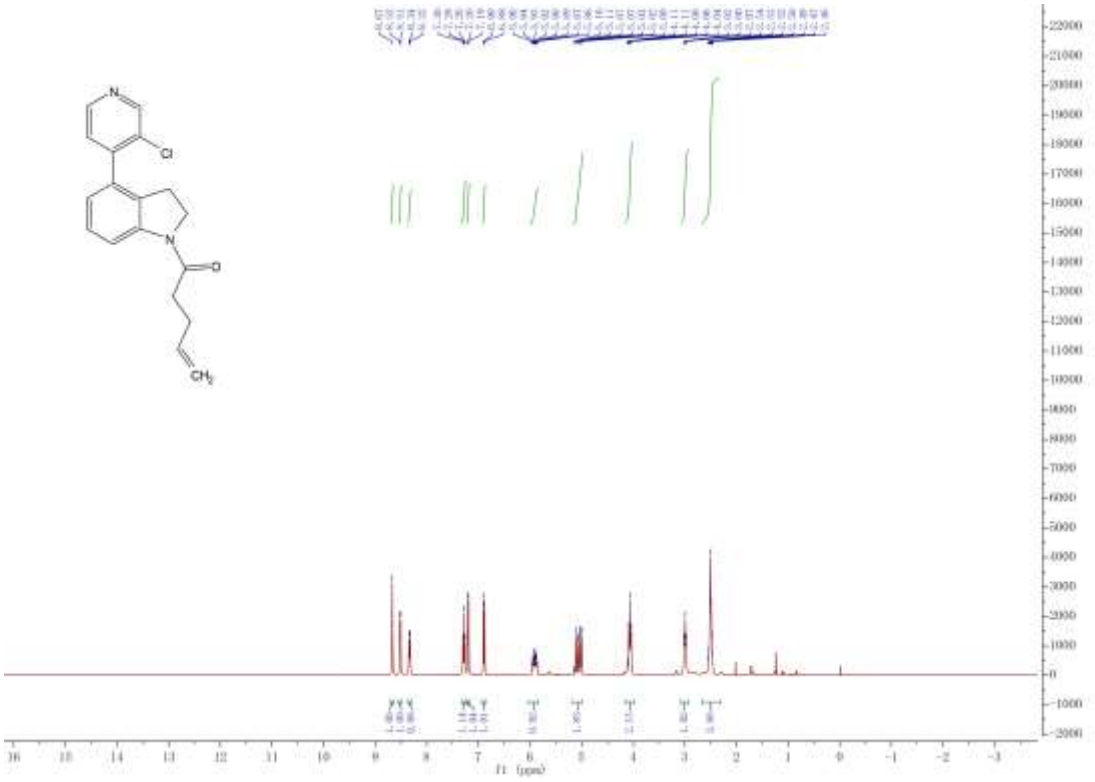


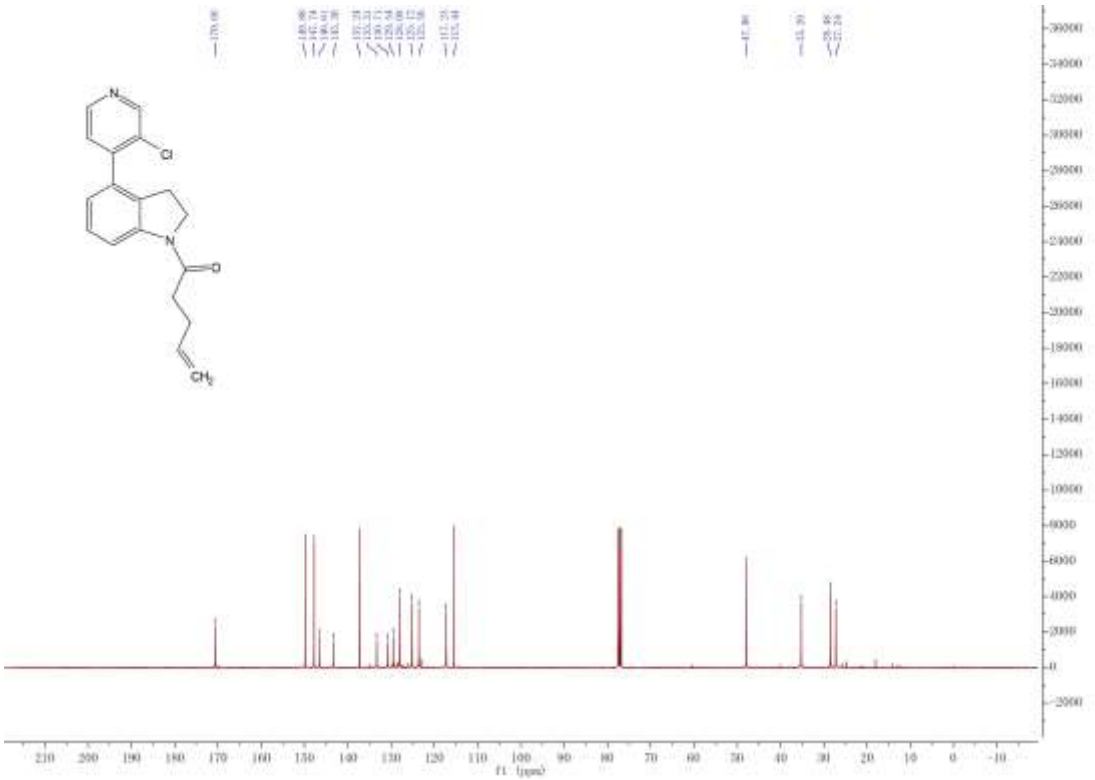
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-methoxypyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-12)



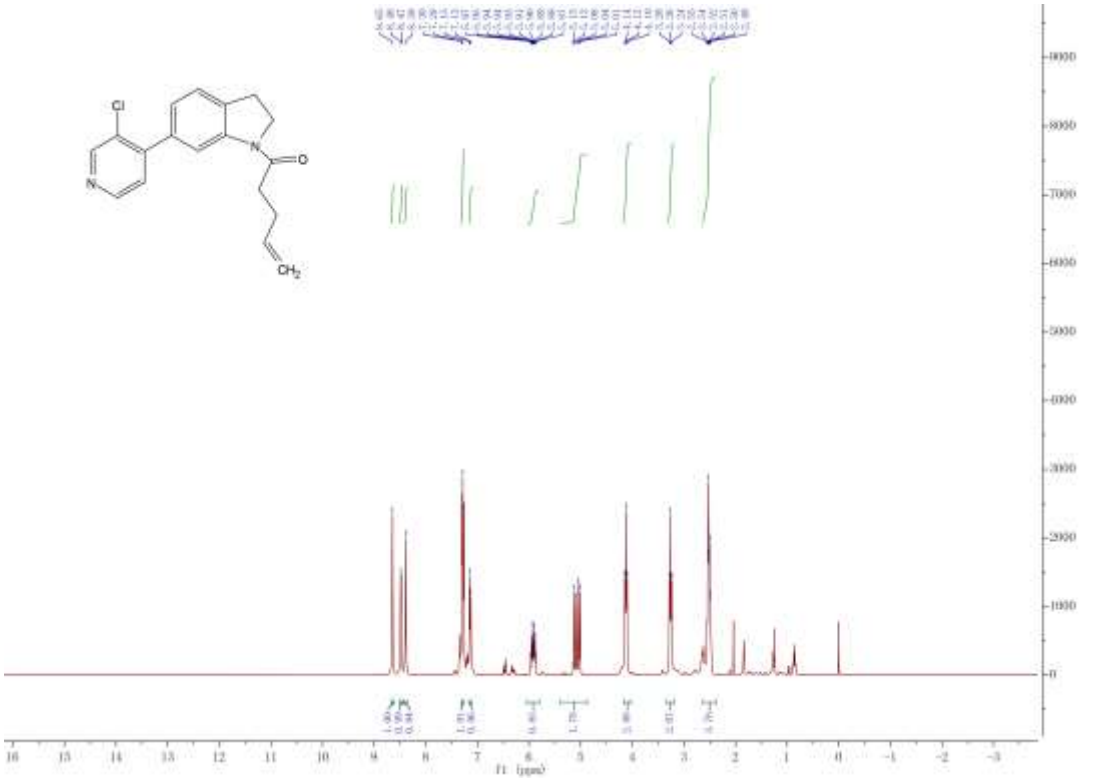


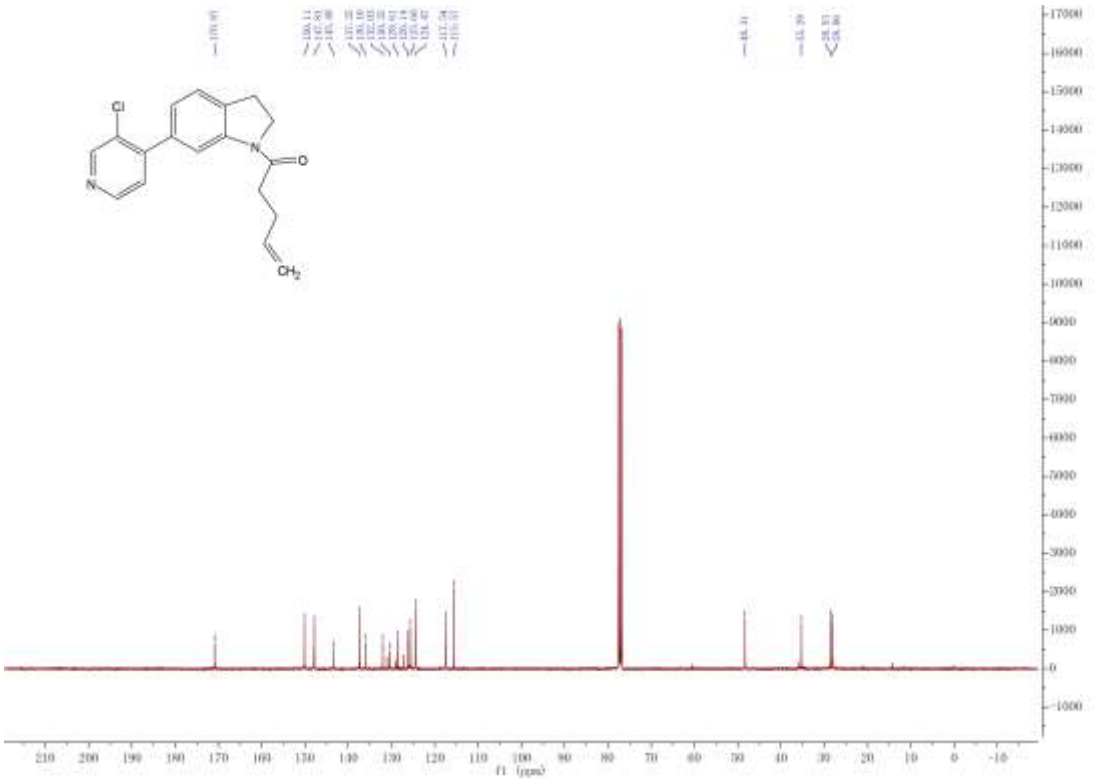
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(4-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-13)



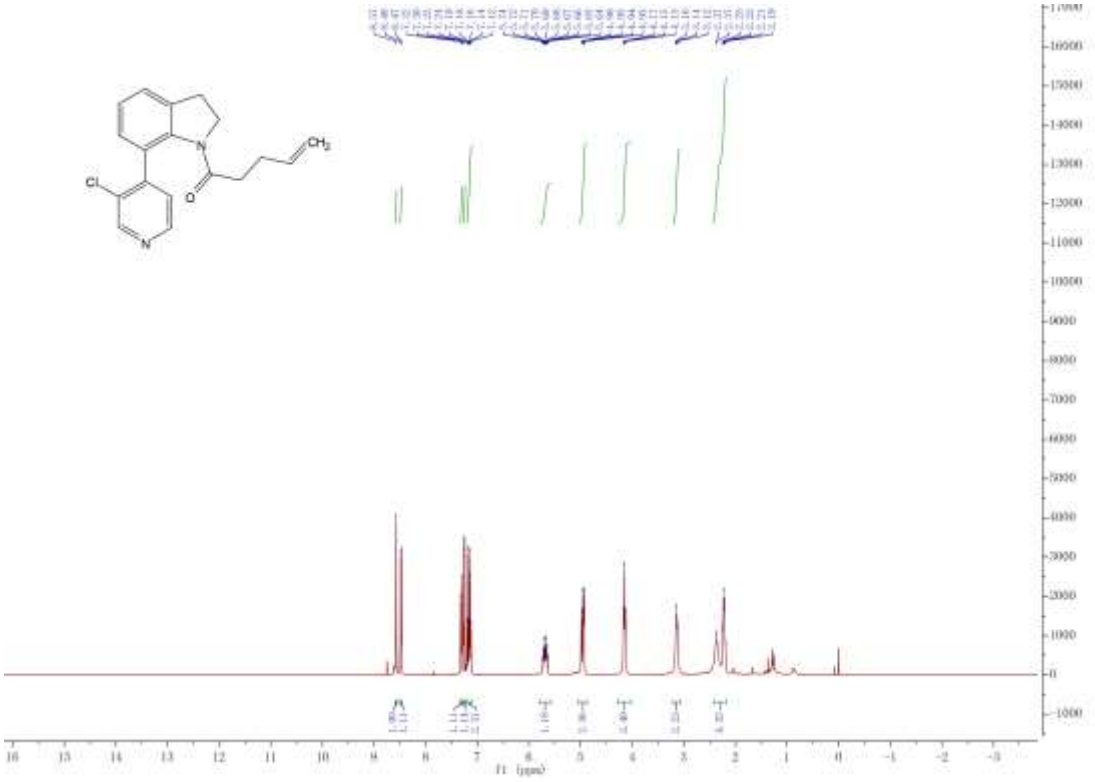


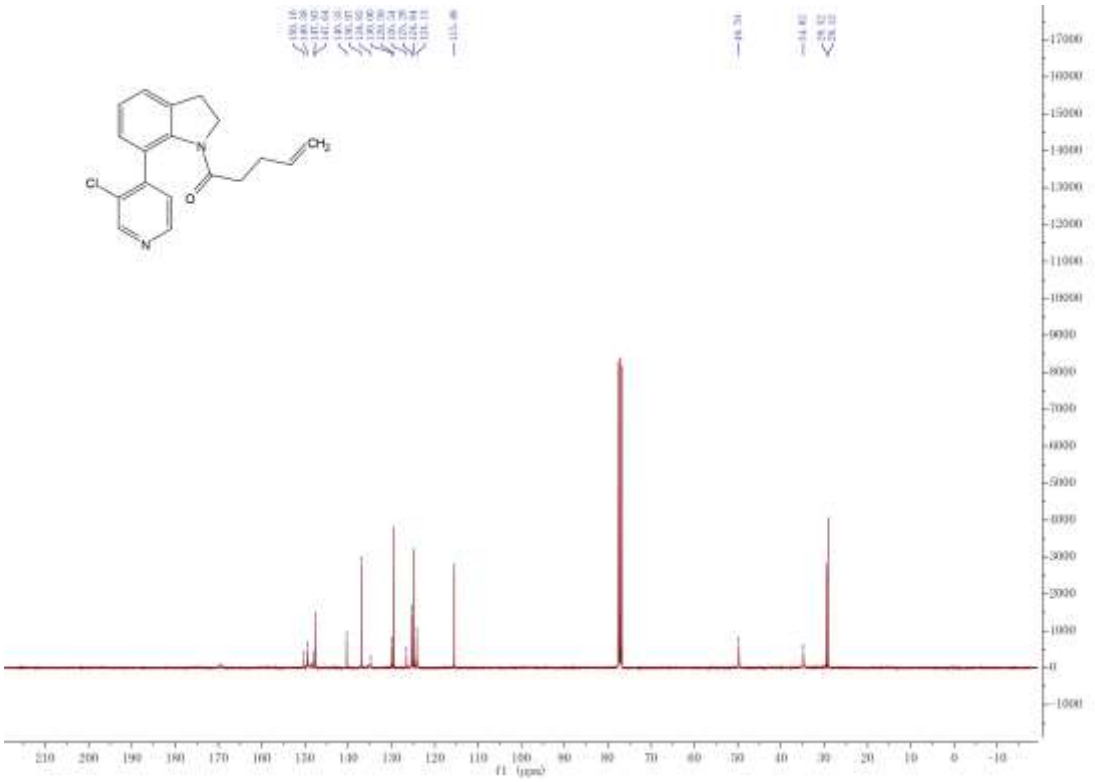
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(6-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-14)



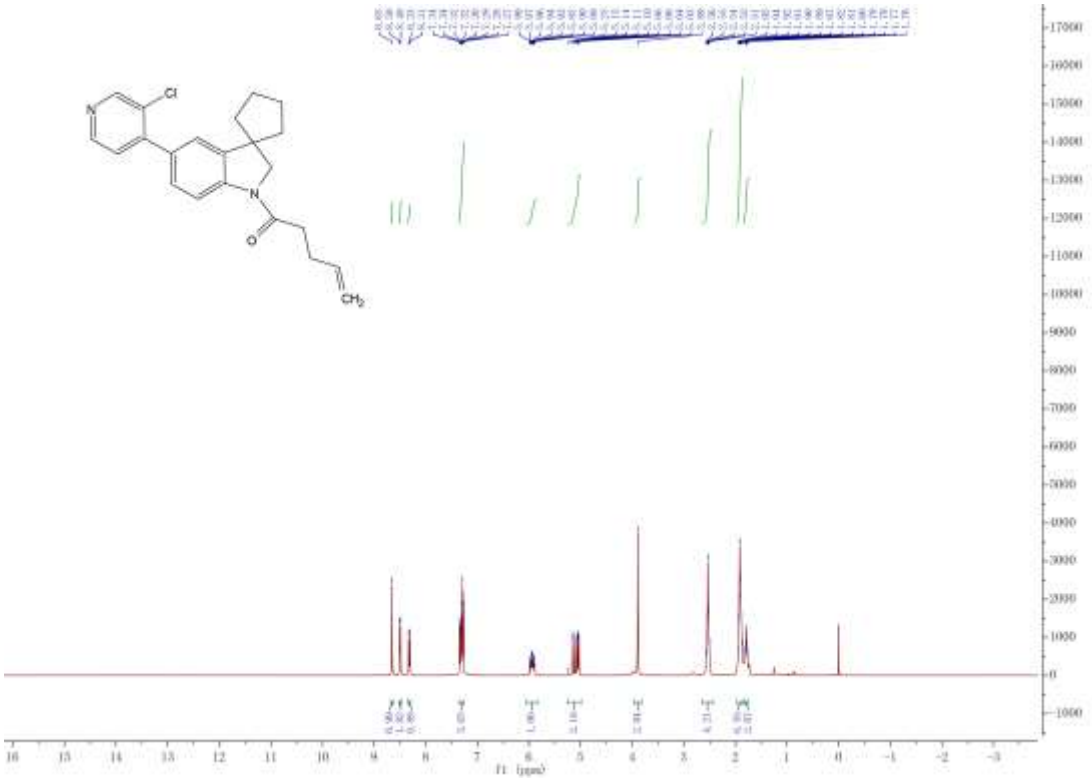


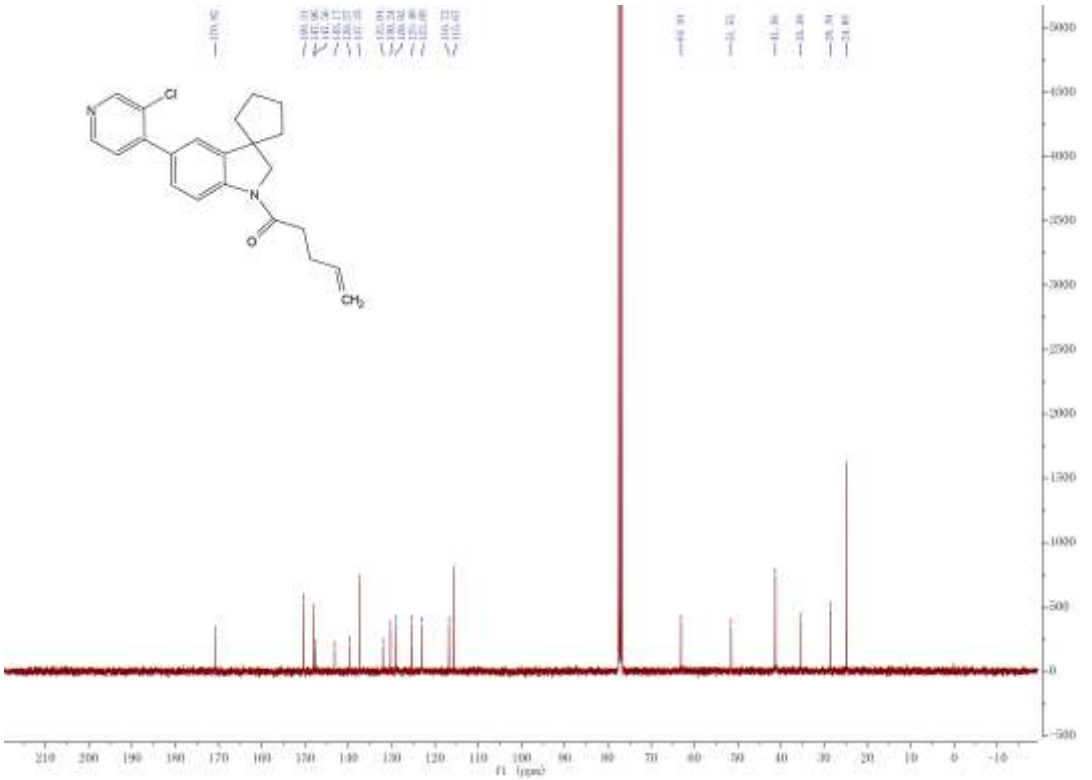
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(7-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pent-4-en-1-one (S-15)



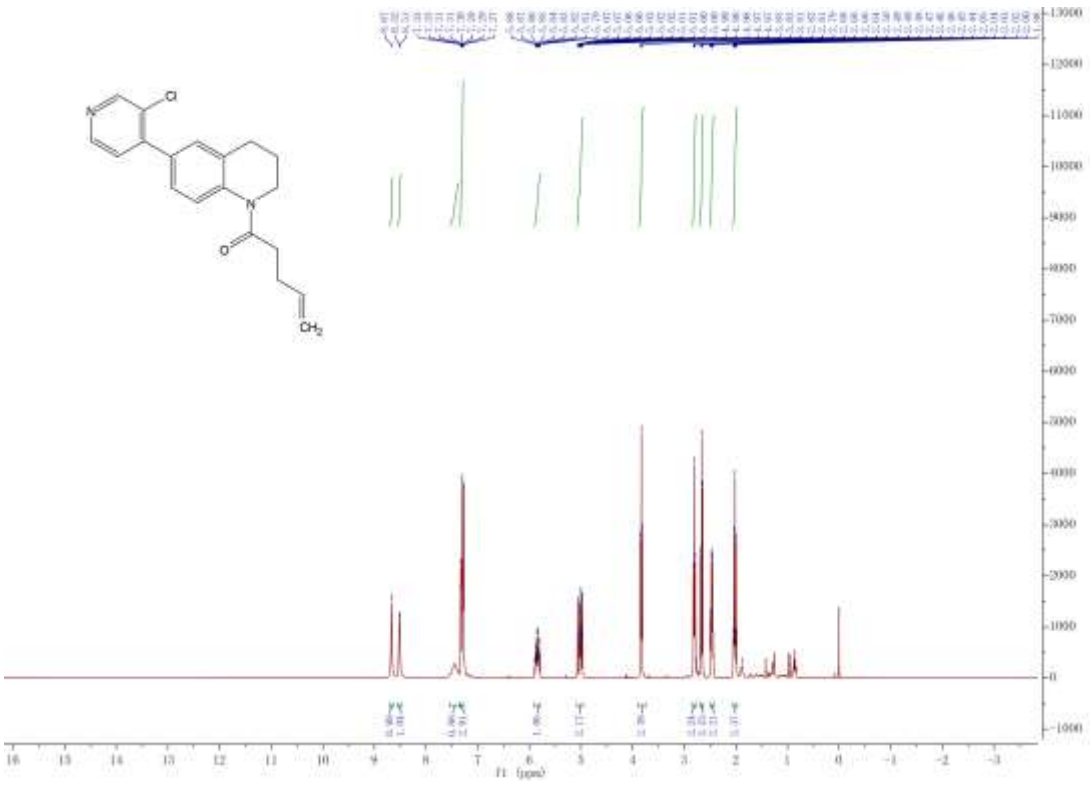


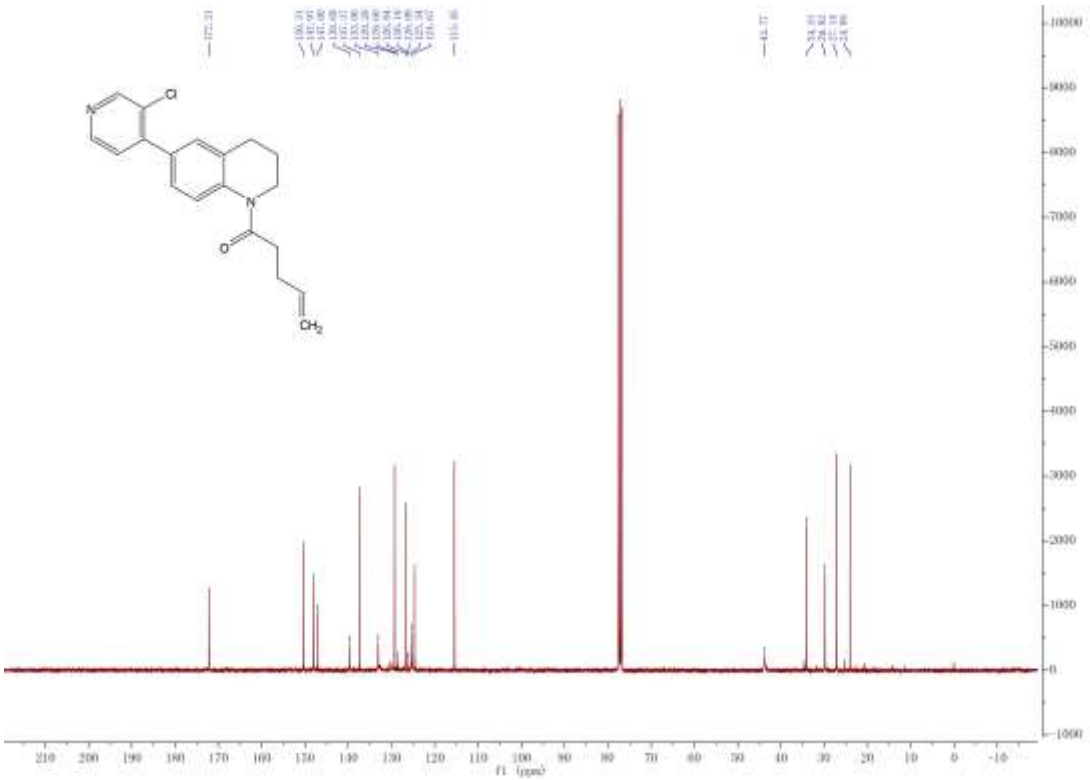
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5'-(3-chloropyridin-4-yl)spiro[cyclopentane-1,3'-indolin]-1'-yl)pent-4-en-1-one (S-16)



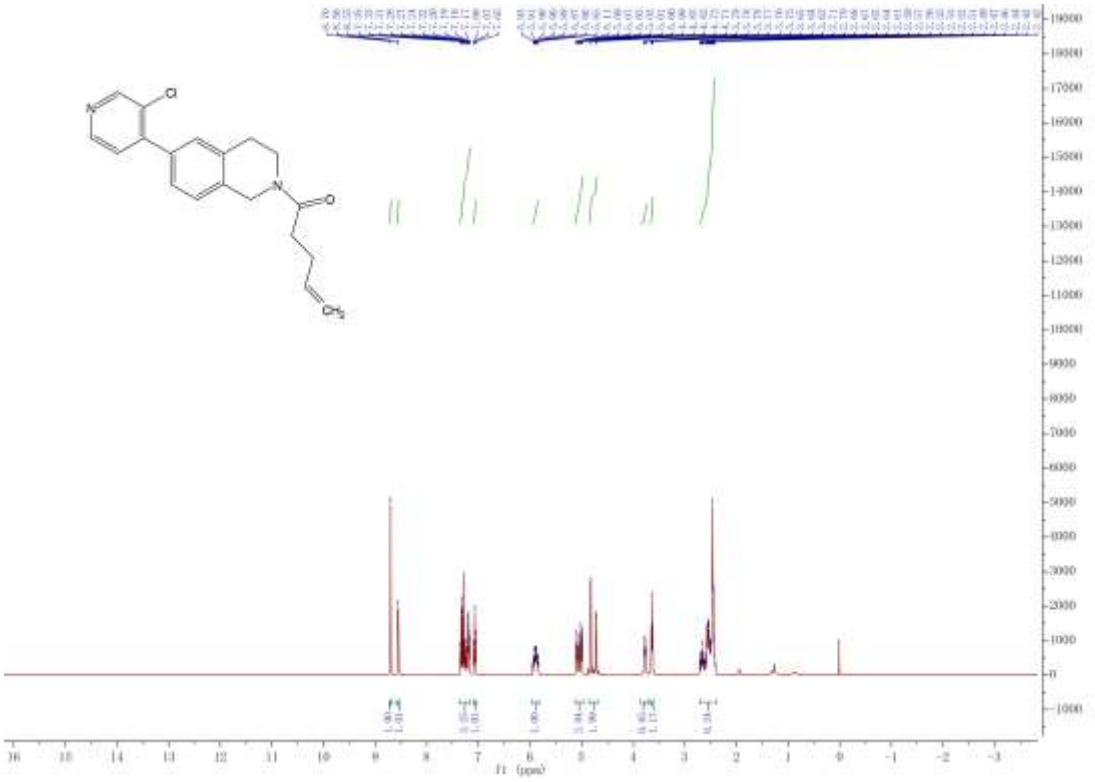


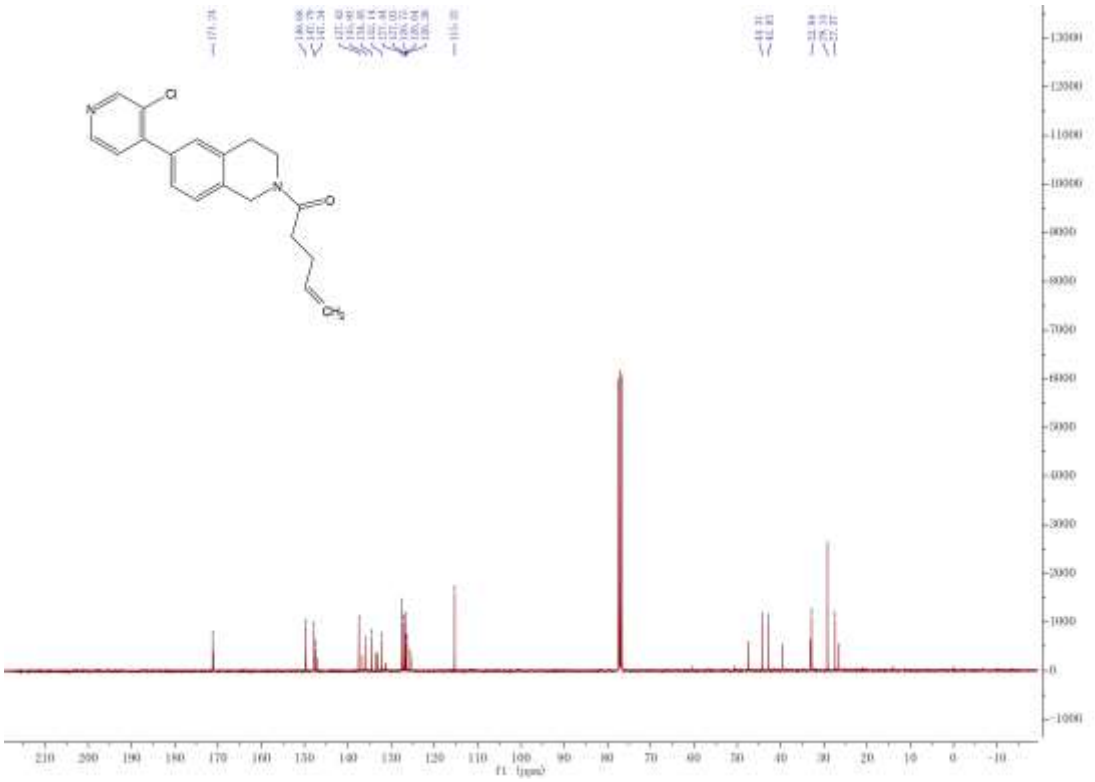
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(6-(3-chloropyridin-4-yl)-1,2,3,4-tetrahydroquinolin-1-yl)pent-4-en-1-one (S-17)



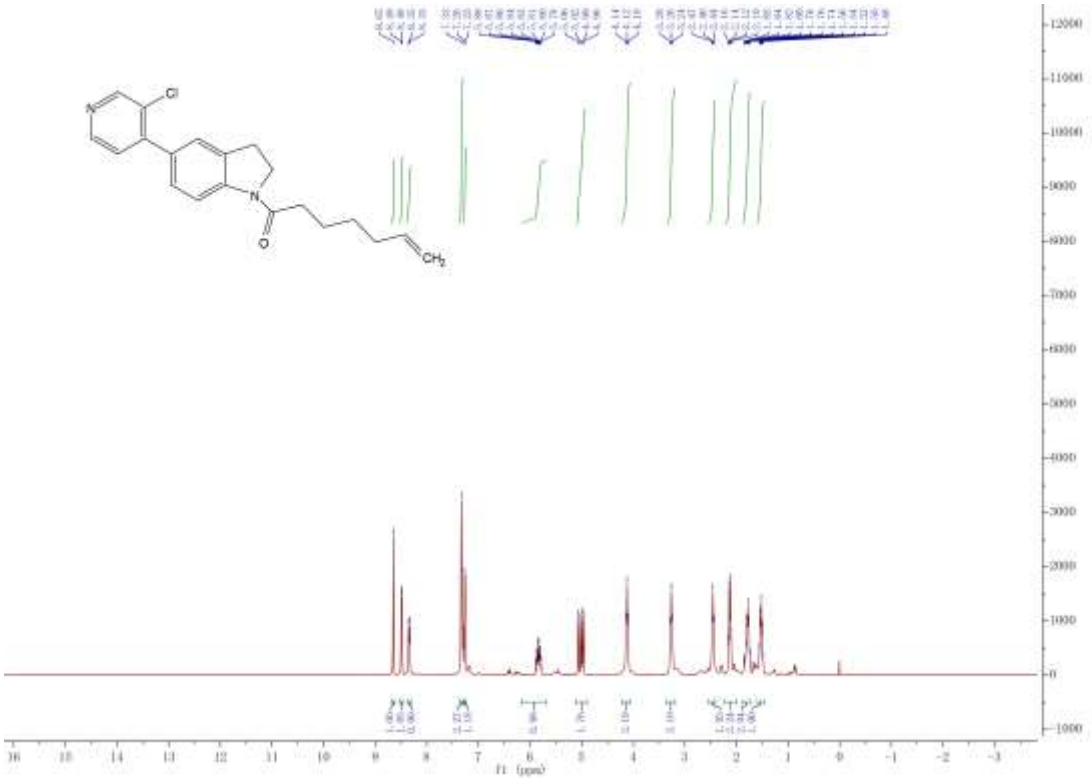


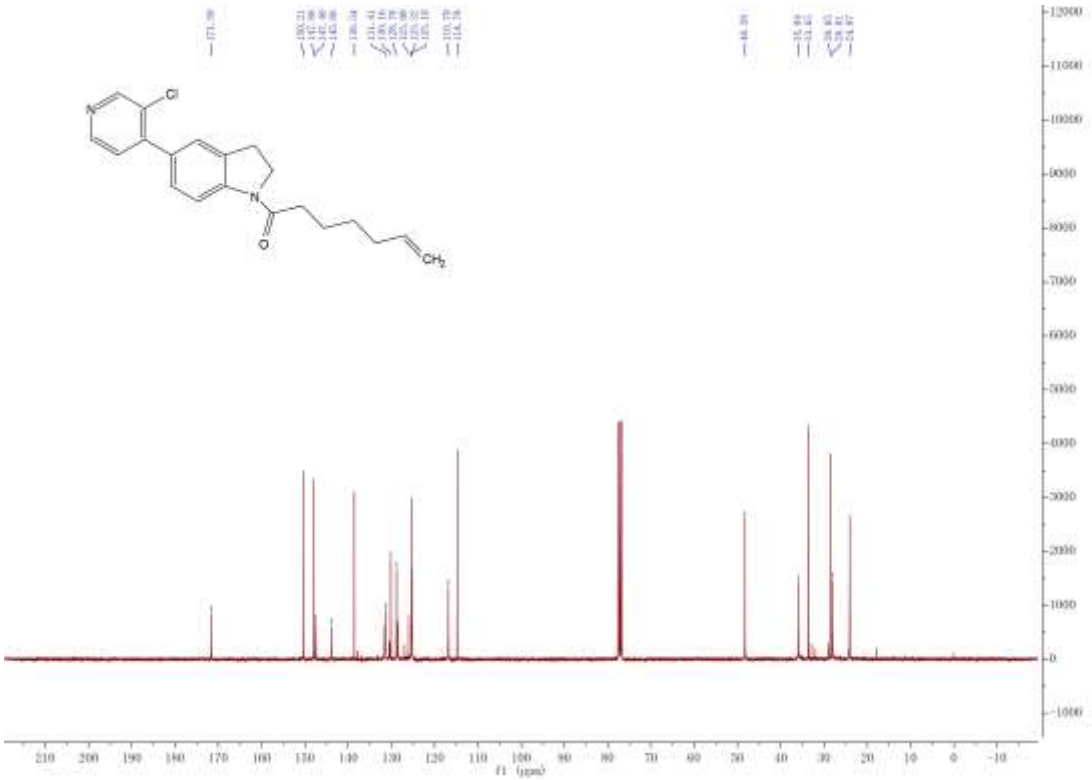
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(6-(3-chloropyridin-4-yl)-1,2,3,4-tetrahydroisoquinolin-2-yl)pent-4-en-1-one (S-18)



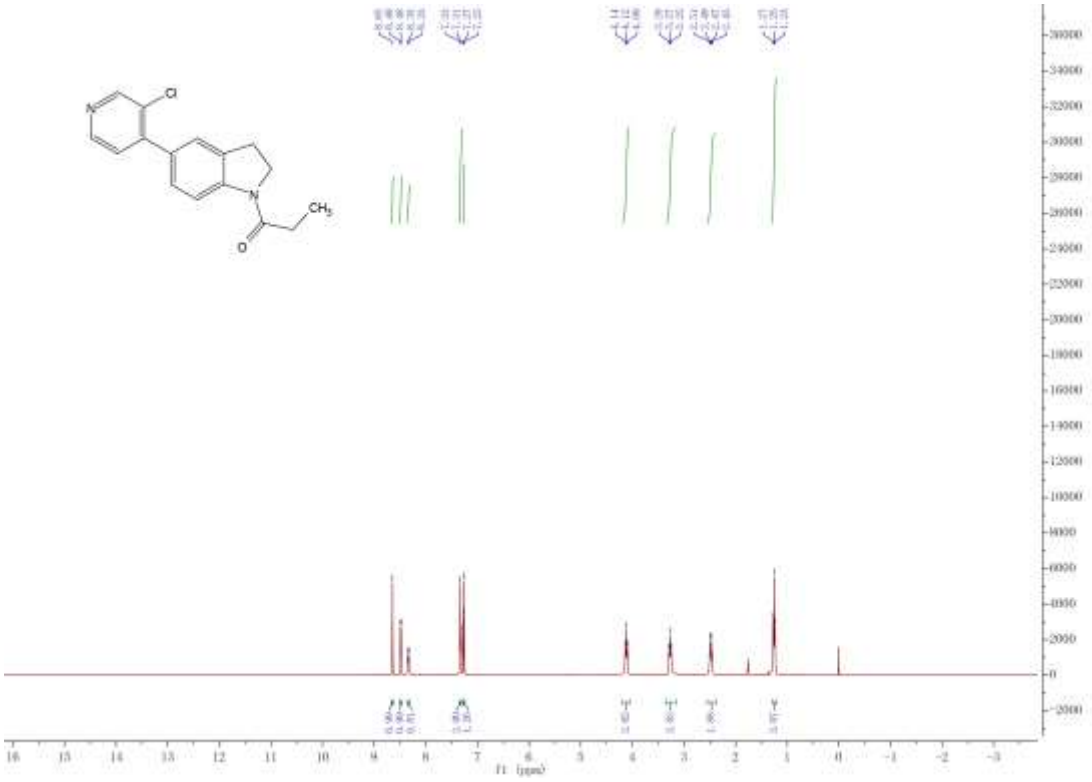


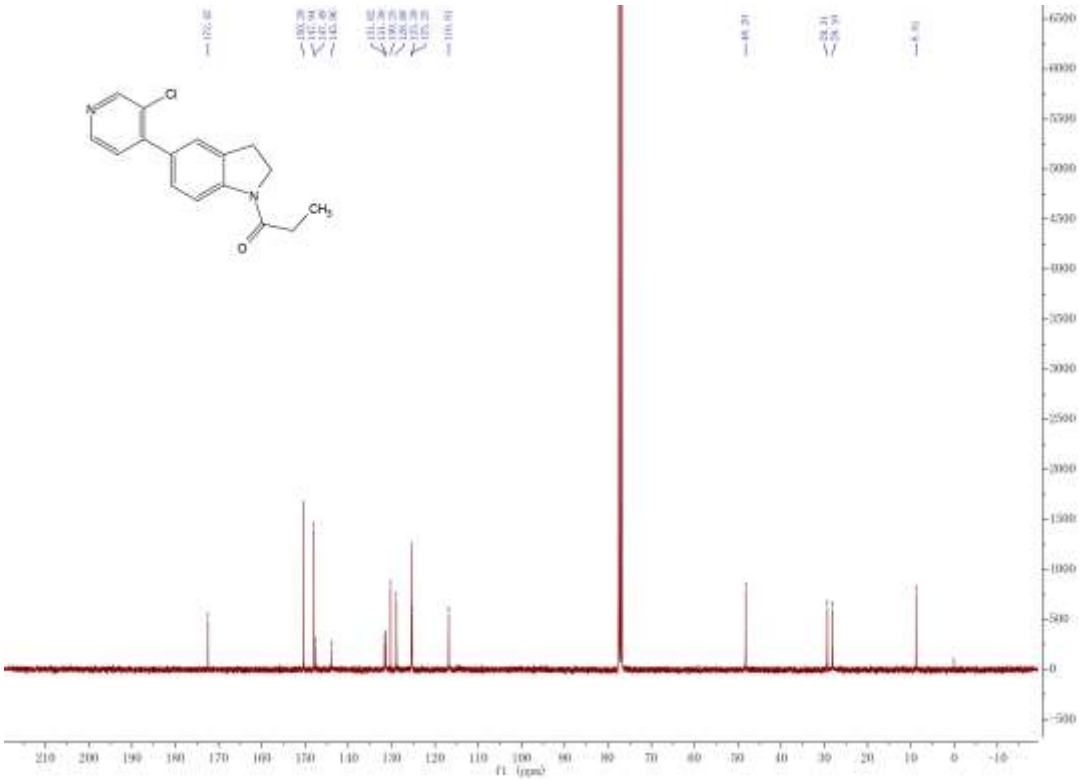
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)hept-6-en-1-one (S-19)



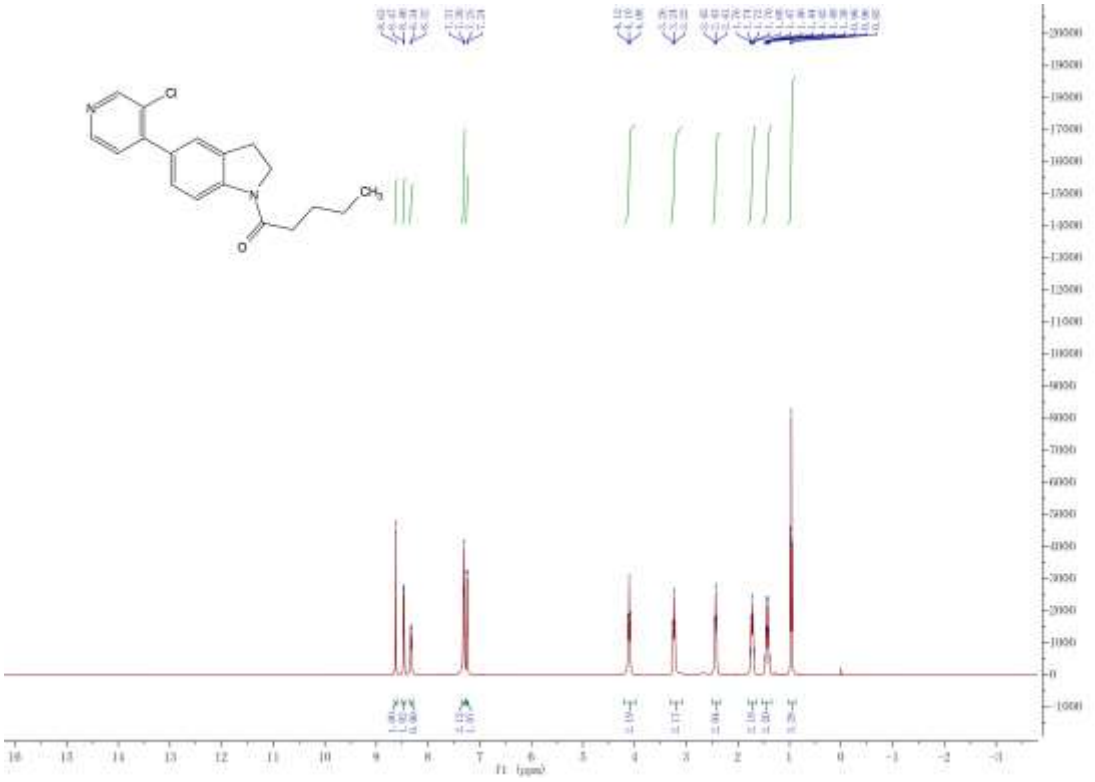


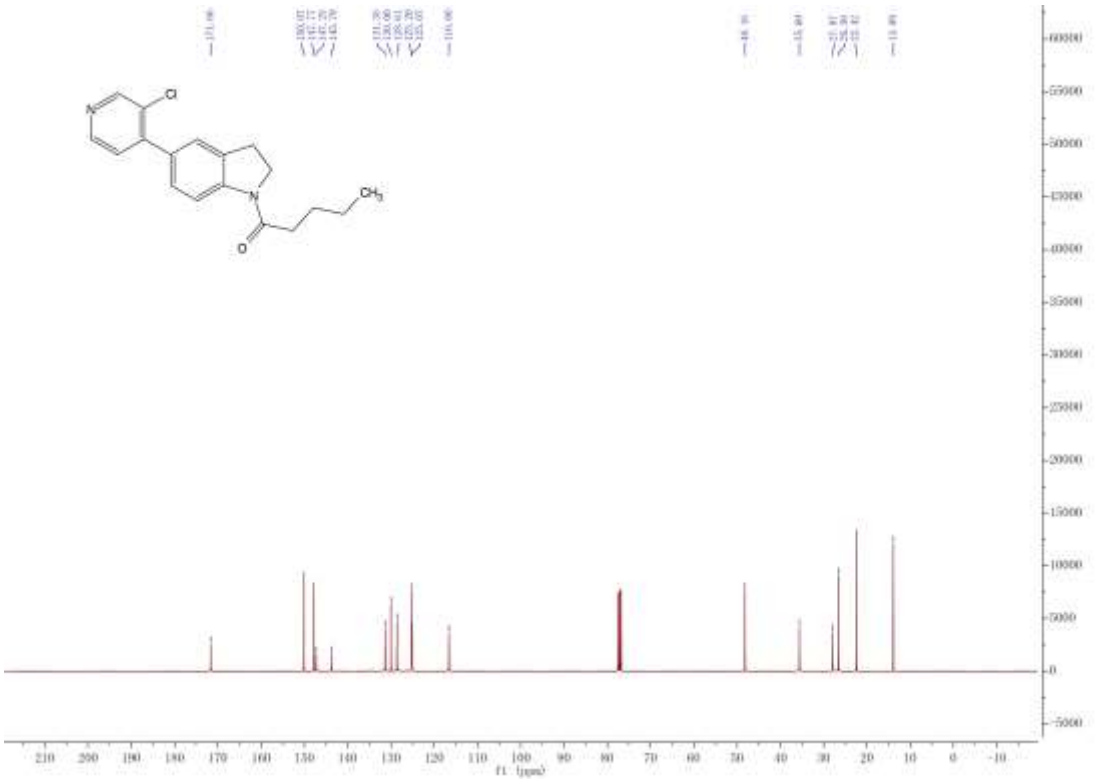
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)propan-1-one (S-20)



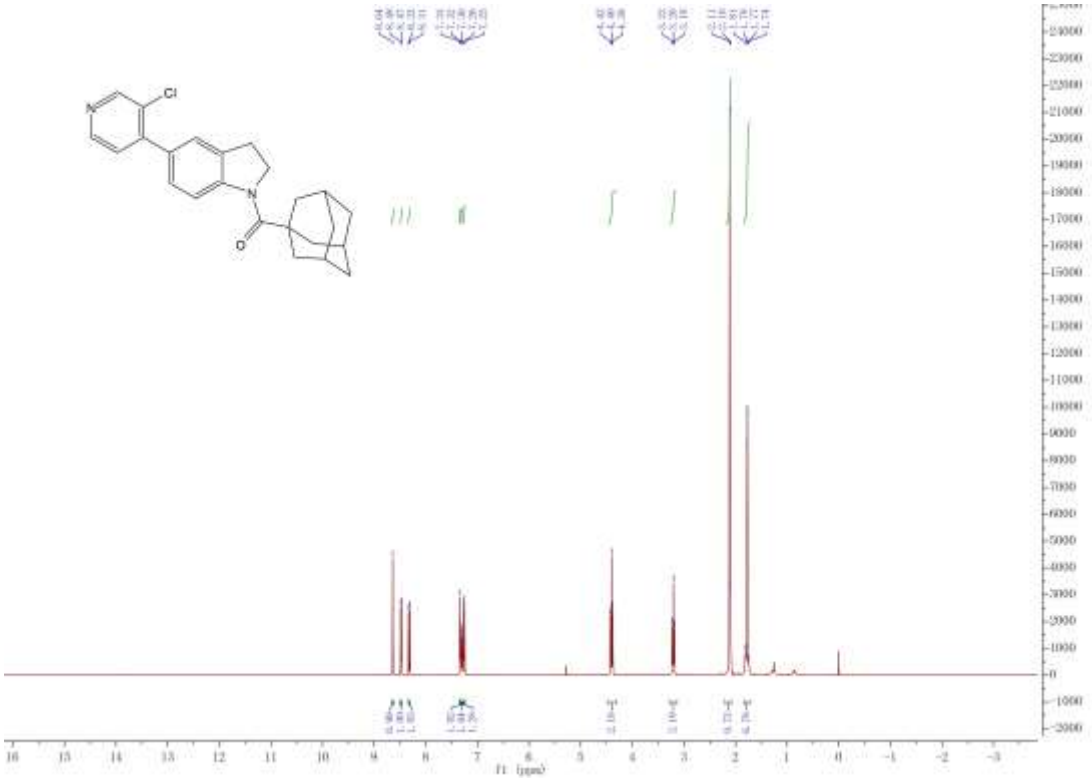


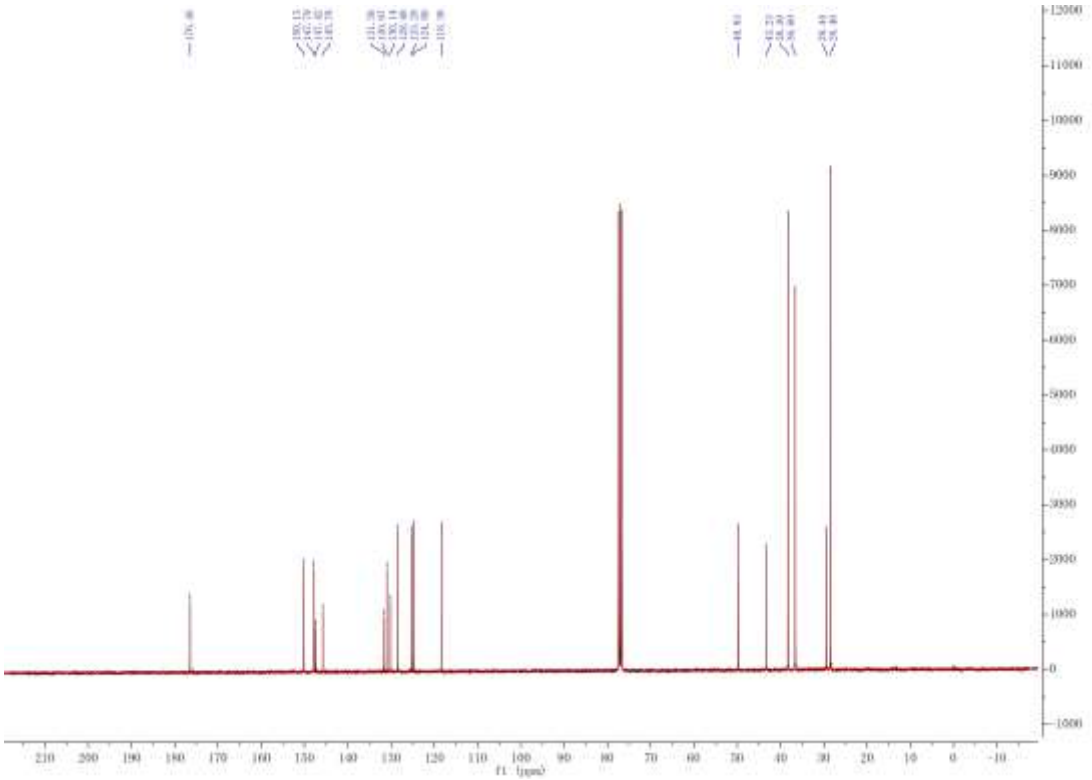
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)pentan-1-one (S-21)



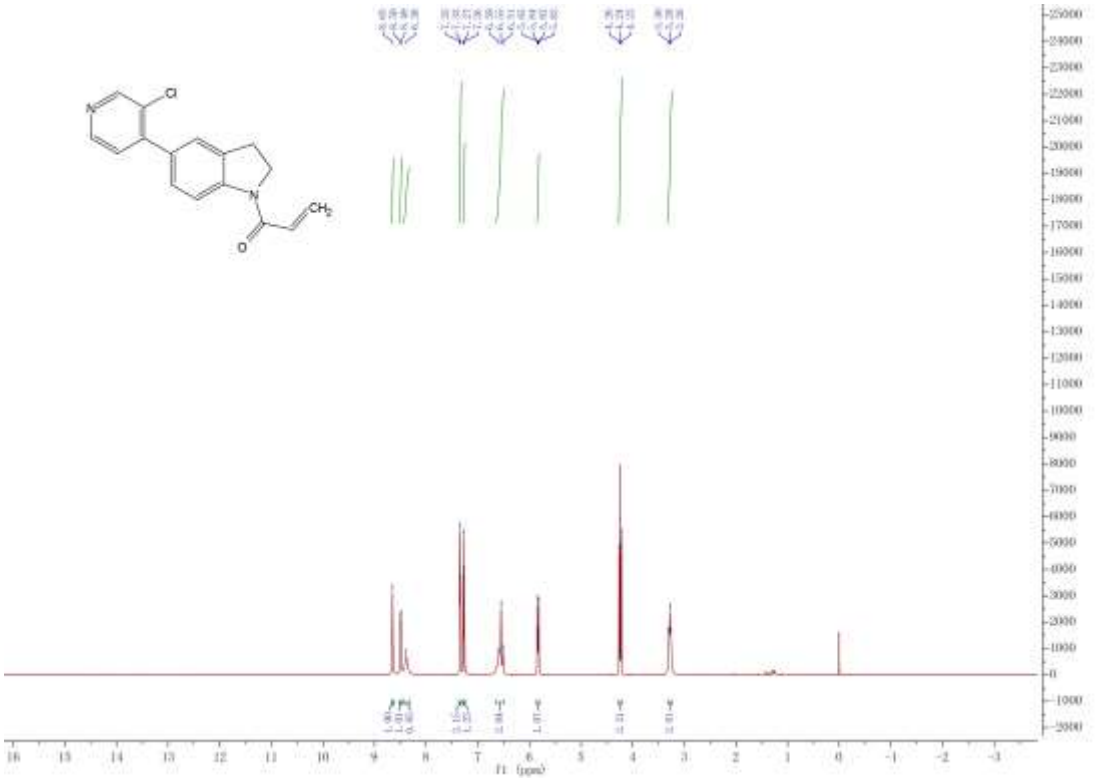


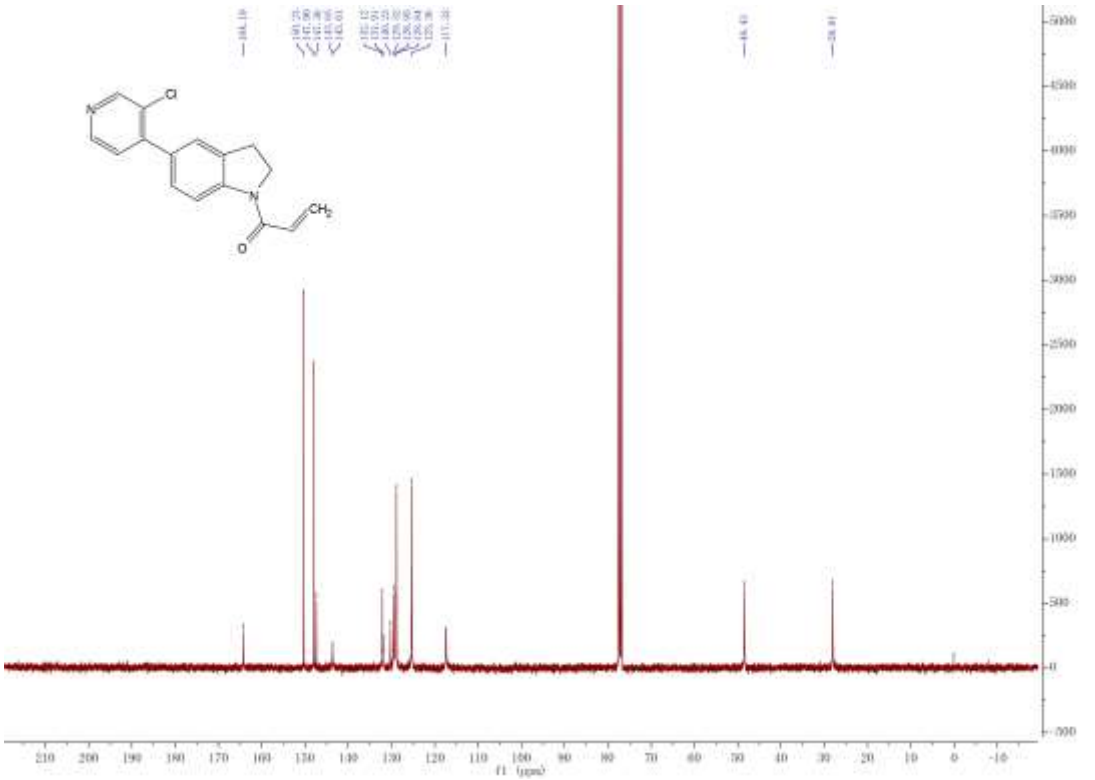
Spectra of ^{1}H-NMR and ^{13}C-NMR of (5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)(tricyclo[3.3.1.13,7]dec-1-yl)methanone (S-22)



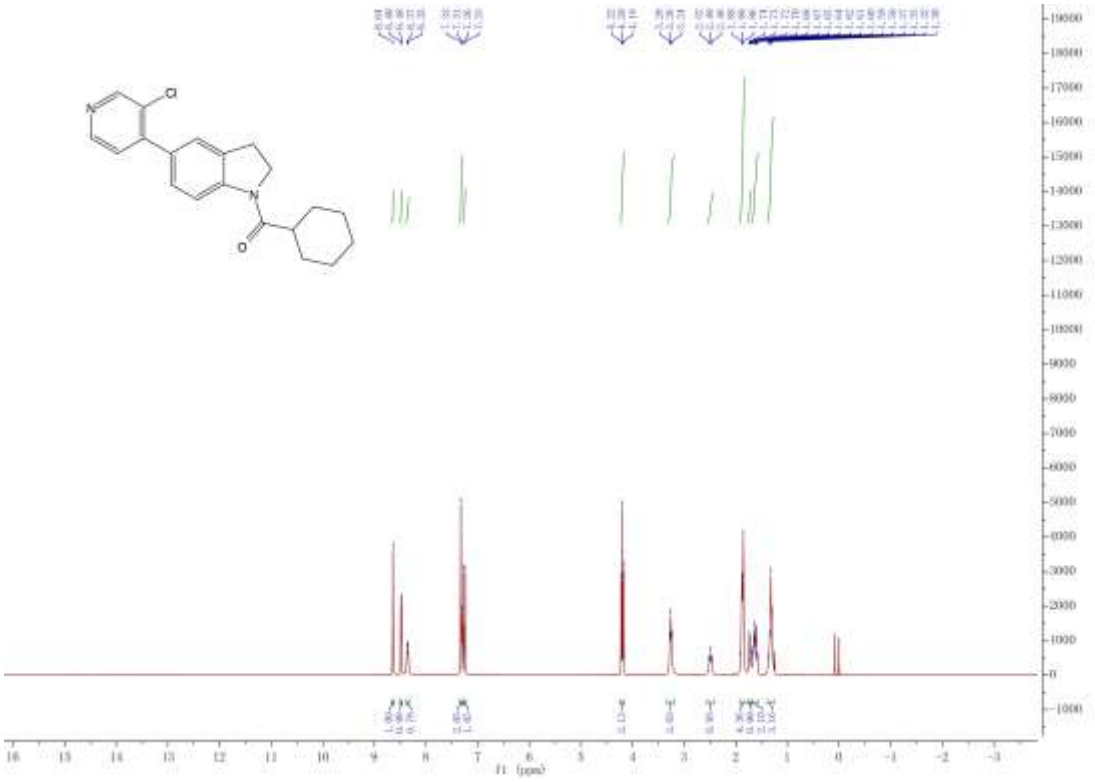


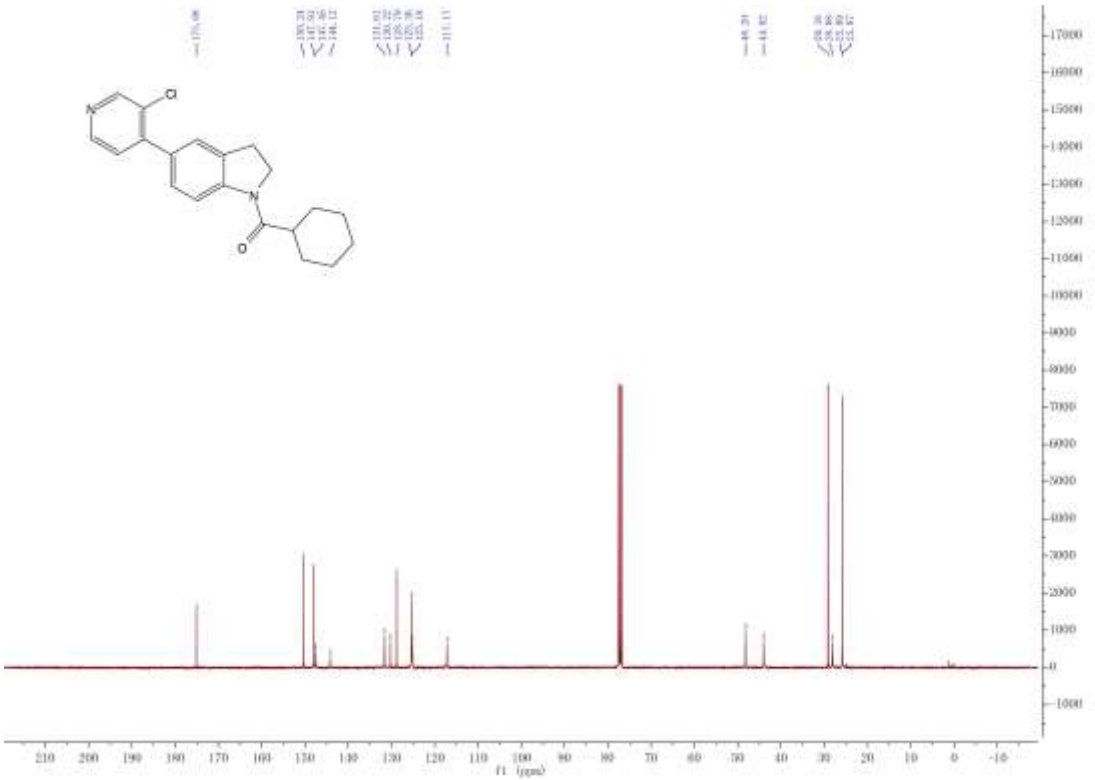
Spectra of ^{1}H-NMR and ^{13}C-NMR of 1-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)prop-2-en-1-one (S-23)



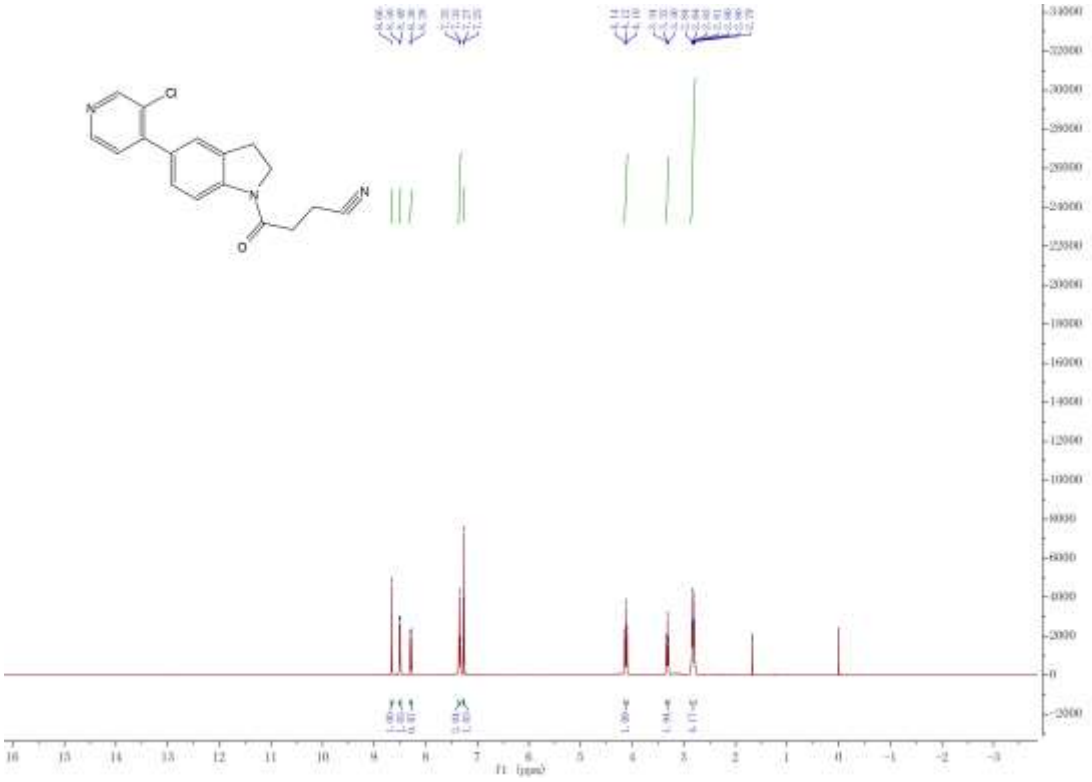


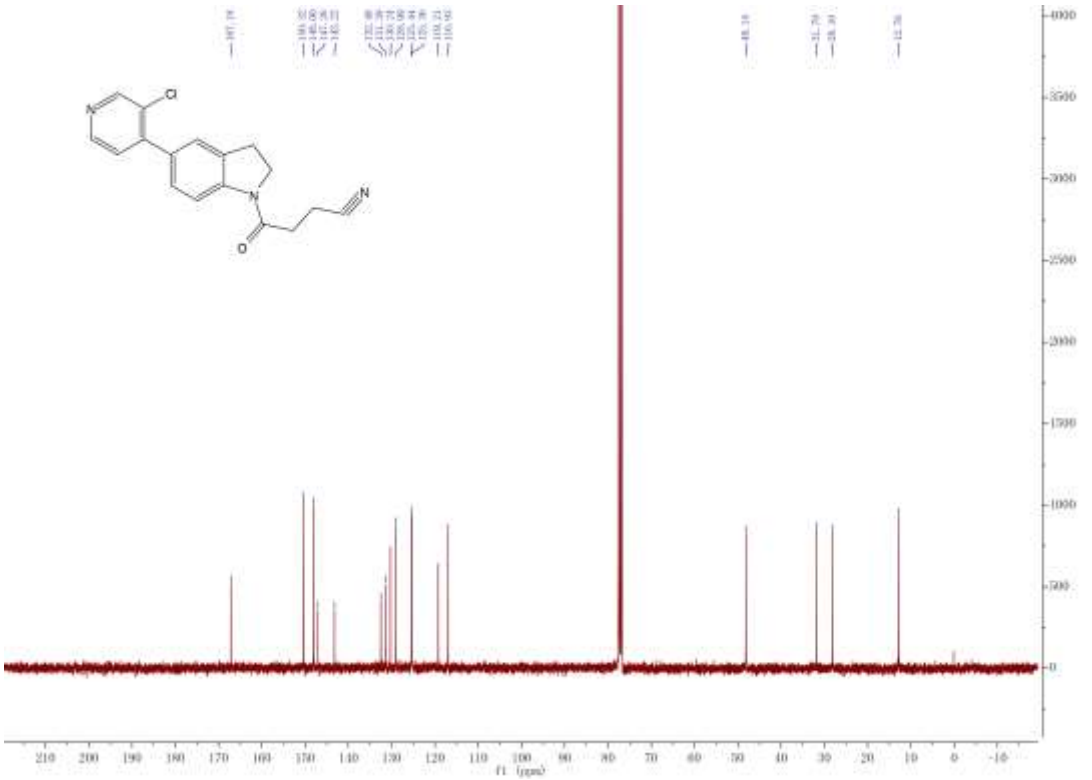
Spectra of ^{1}H-NMR and ^{13}C-NMR of (5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)(cyclohexyl)methanone (S-24)



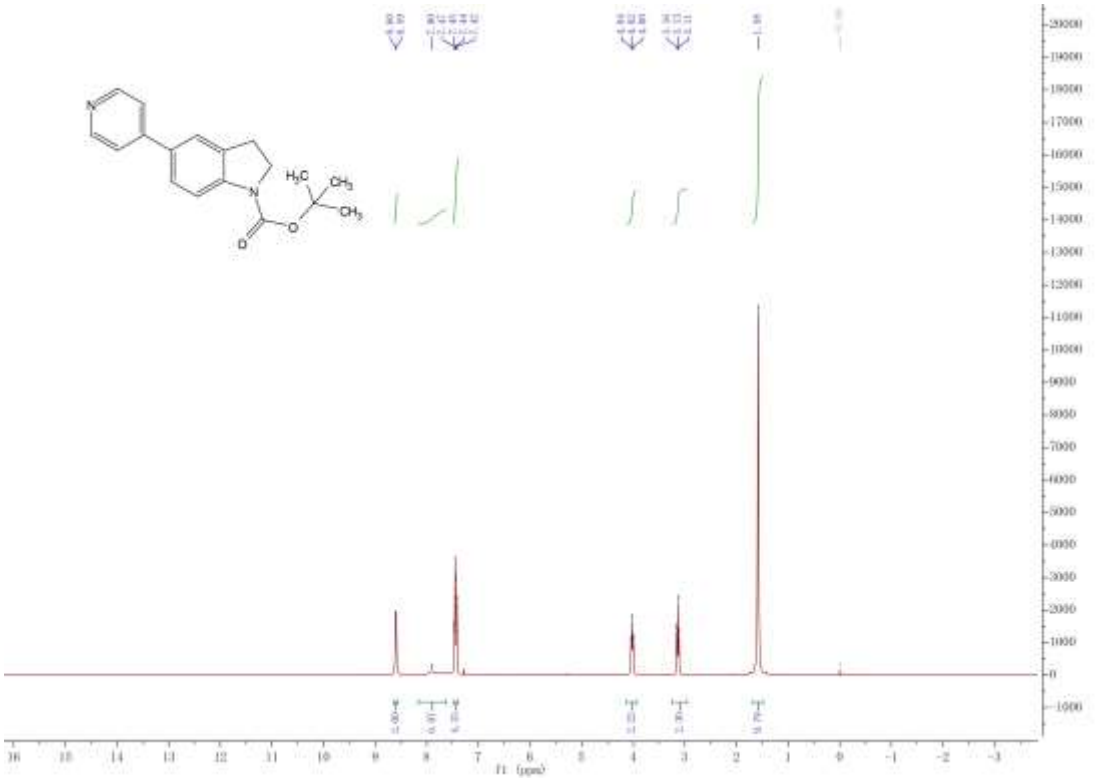


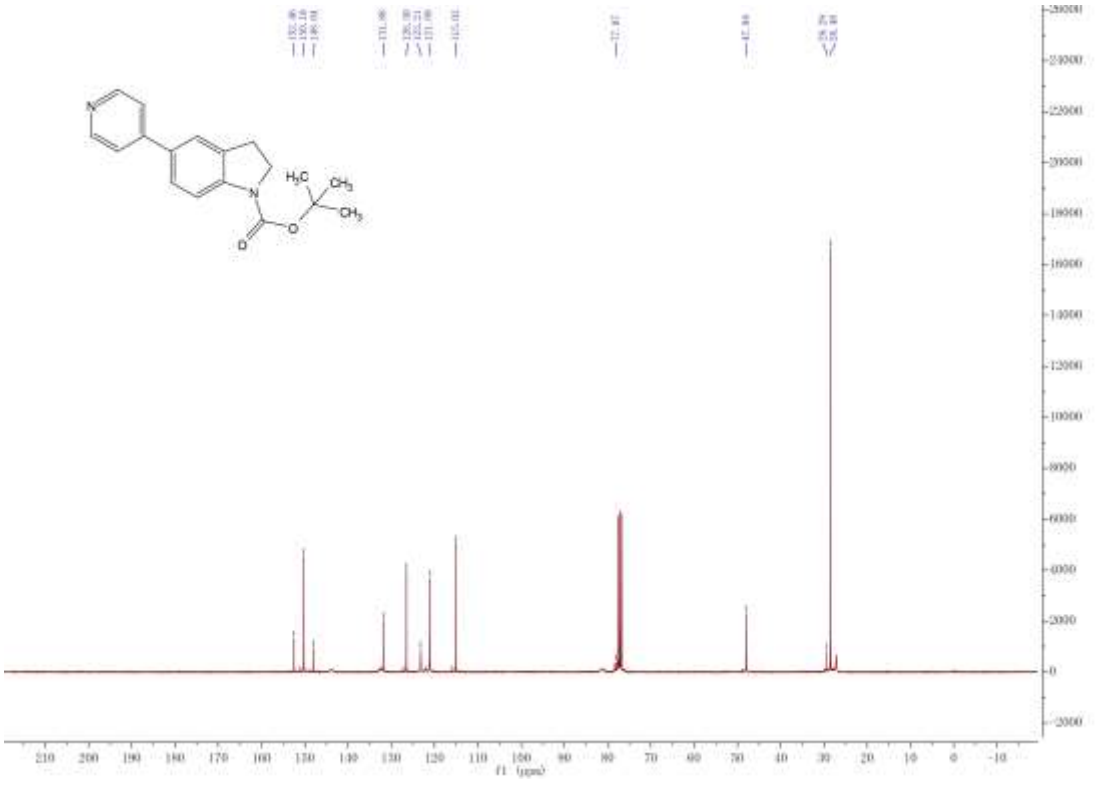
Spectra of ^{1}H-NMR and ^{13}C-NMR of 4-(5-(3-chloropyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)-4-oxobutanenitrile (S-25)



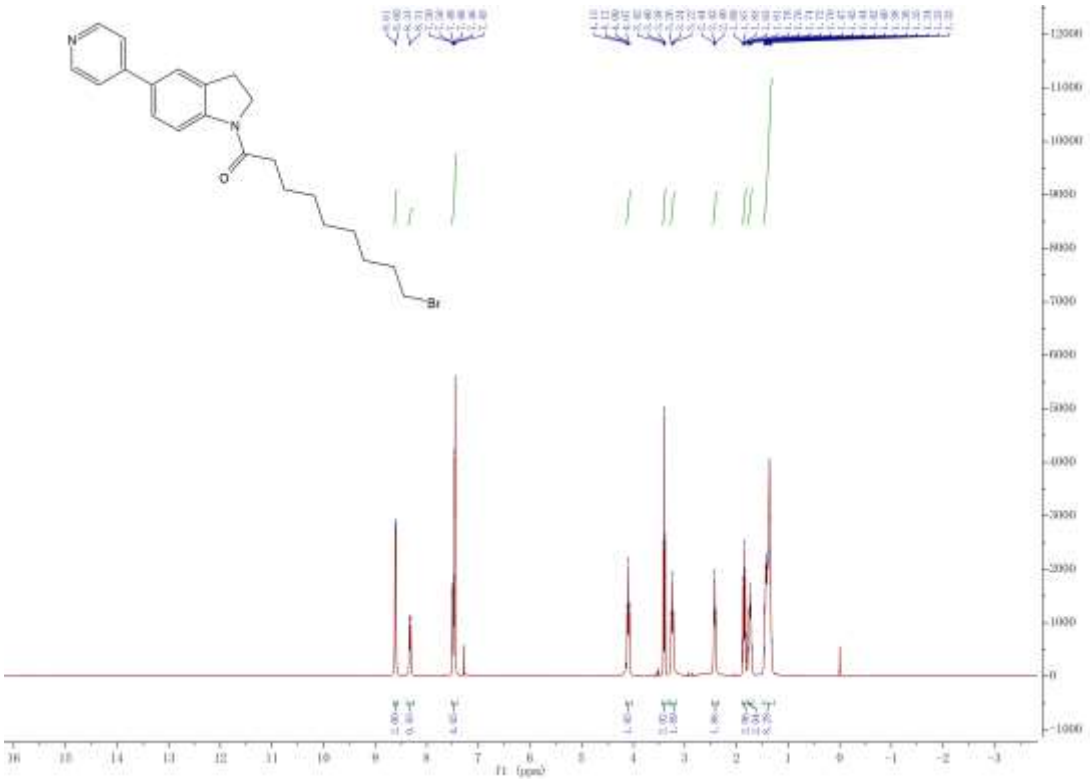


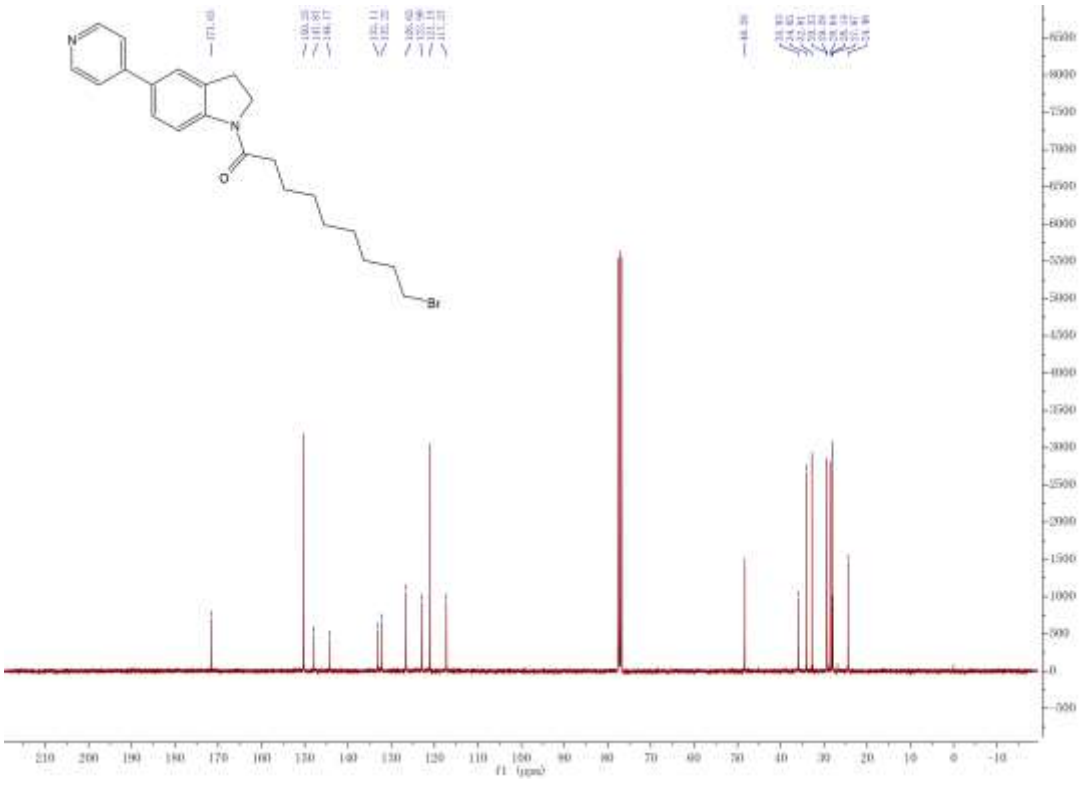
Spectra of ^{1}H-NMR and ^{13}C-NMR of *tert*-butyl 5-(pyridin-4-yl) -2,3-dihydroindole -1-carboxylate (1b)



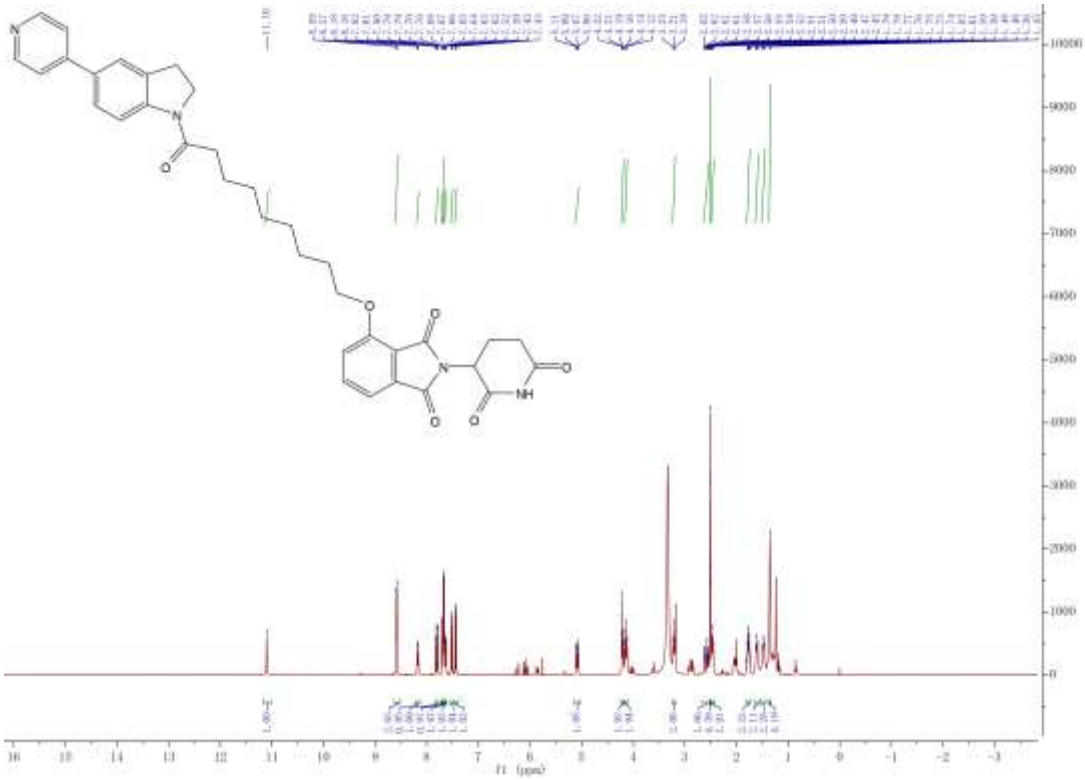


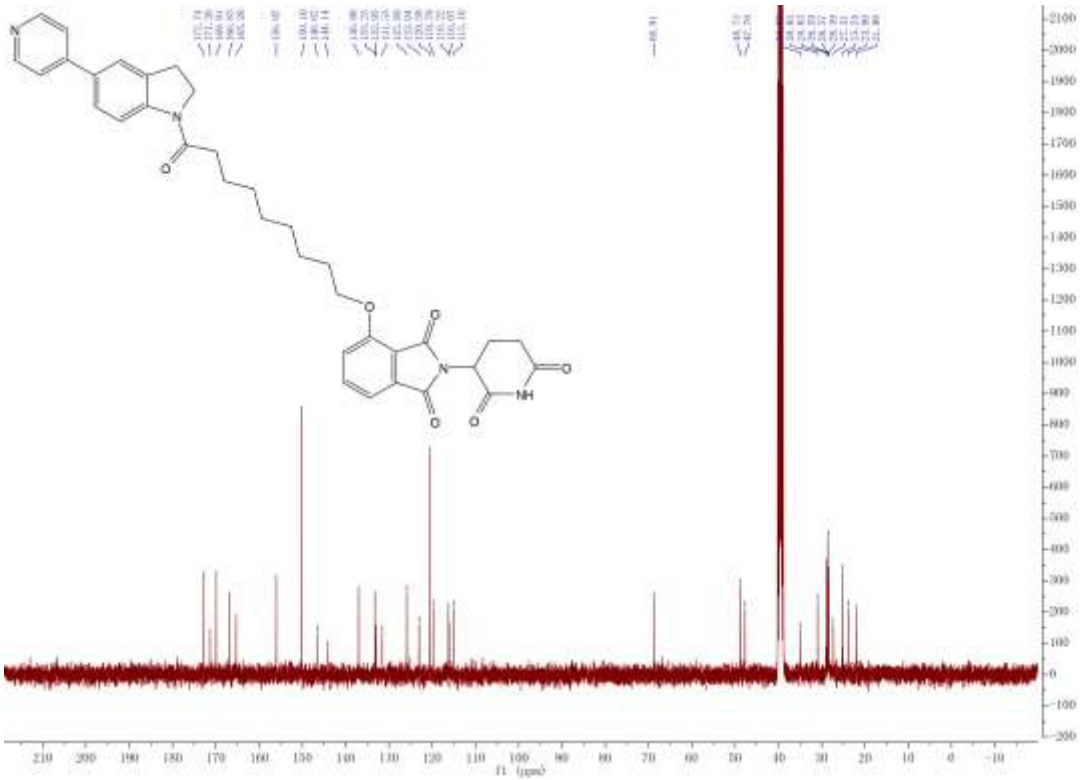
Spectra of ^{1}H-NMR and ^{13}C-NMR of 9-bromo-1-(5-(pyridin-4-yl)indolin-1-yl)nonan-1-one (1d)





2-(2,6-dioxopiperidin-3-yl)-4-((9-oxo-9-(5-(pyridin-4-yl)-2,3-dihydro-1H-indol-1-yl)nonyl)oxy)-1H-isoindole-1,3(2H)-dione (1f, S-1 derived PROTAC)





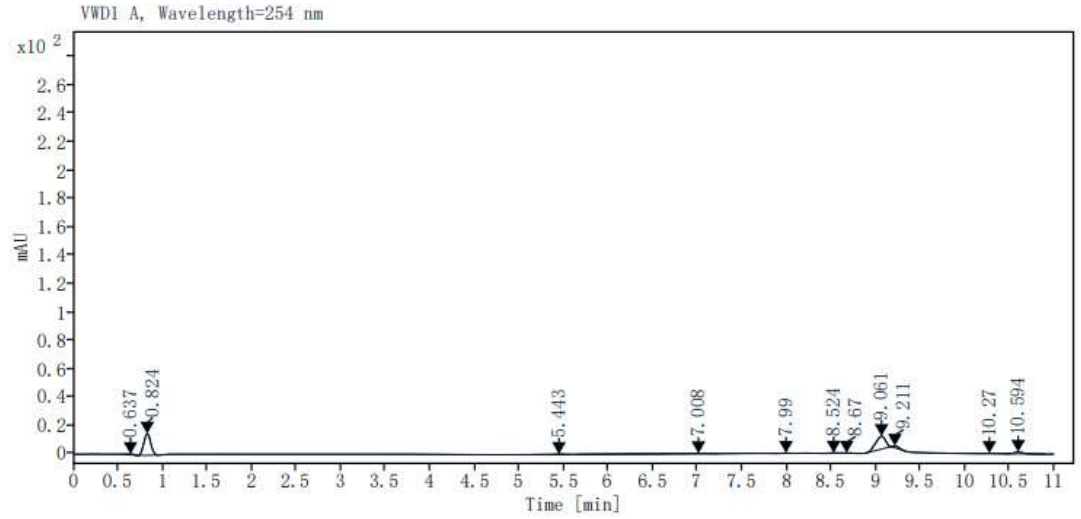
HRMS spectra and HPLC trace

Instrument information and method

HRMS spectra were obtained from a XEVO G2 QTOF spectrometer (Waters Ltd.). HPLC analysis was performed on Agilent 1260 Inffnity using a C18 column [(Agilent Ltd.) SB-C18, 50 × 2.1 mm, 1.8 µm, 0.3 mL/min] and SHIMADZU LC-20AR (Shimadzu Ltd.) using a C8 column (CAPCELL PAK C8 DD, 250 × 10 mm, 5 µm, 4.0 mL/min).

HPLC conditions	Mobile phase A: 0.1% Formic acid in water Mobile phase B: acetonitrile			
Method A	Time (min)	Solvent A (%)	Solvent B (%)	Flow (mL/min)
	0.00	95.0	5.0	0.300
	0.25	95.0	5.0	0.300
	9.00	0.0	100.0	0.300
	10.00	0.0	100.0	0.300
	10.10	95.0	5.0	0.300
	11.00	95.0	5.0	0.300
	Column: Agilent SB-C18 1.8μm (2.1*50 mm)			
Method B	Time (min)	Solvent A (%)	Solvent B (%)	Flow (mL/min)
	0.00	80.0	20.0	1.000
	0.25	80.0	20.0	1.000
	12.00	0.0	100.0	1.000
	16.00	0.0	100.0	1.000
	16.10	80.0	20.0	1.000
	17.00	80.0	20.0	1.000
	Column: CAPCELL PAK C8 DD (250*10 mm)			

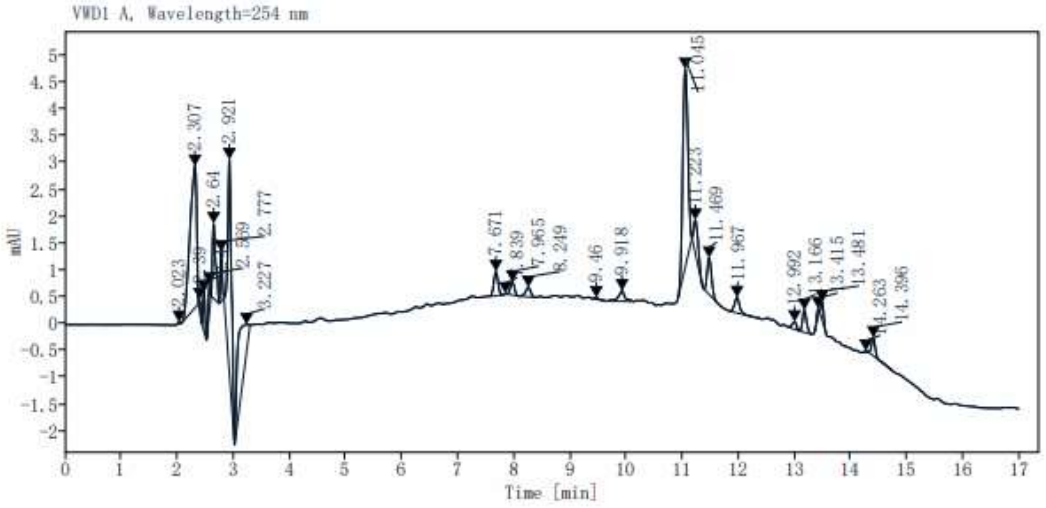
HPLC trace for blank of sequence for all compounds except for S-22 and S-25 (method A)



Signal: VWD1 A, Wavelength=254 nm

Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
0.637	0.0881	2.7417	0.4837	1.4393
0.824	0.0864	83.3077	15.0812	43.7342
5.443	0.1162	1.8926	0.2503	0.9935
7.008	0.8498	11.7658	0.1747	6.1767
7.990	0.1162	1.0452	0.1483	0.5487
8.524	0.0881	1.1260	0.2115	0.5911
8.670	0.0921	0.8708	0.1538	0.4572
9.061	0.1196	69.5674	9.0610	36.5209
9.211	0.0852	6.3460	1.2097	3.3315
10.270	0.1820	2.4627	0.1818	1.2929
10.594	0.1135	9.3605	1.2768	4.9140

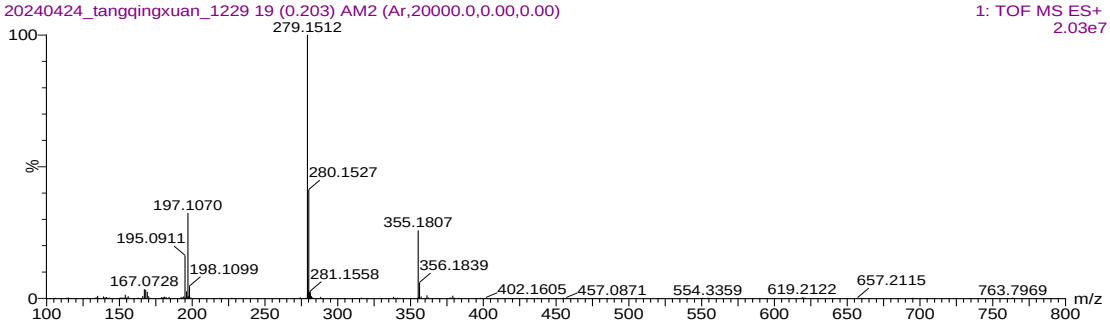
HPLC trace for blank of sequence for S-22 and S-25 (method B)



Signal: VWD1 A, Wavelength=254 nm

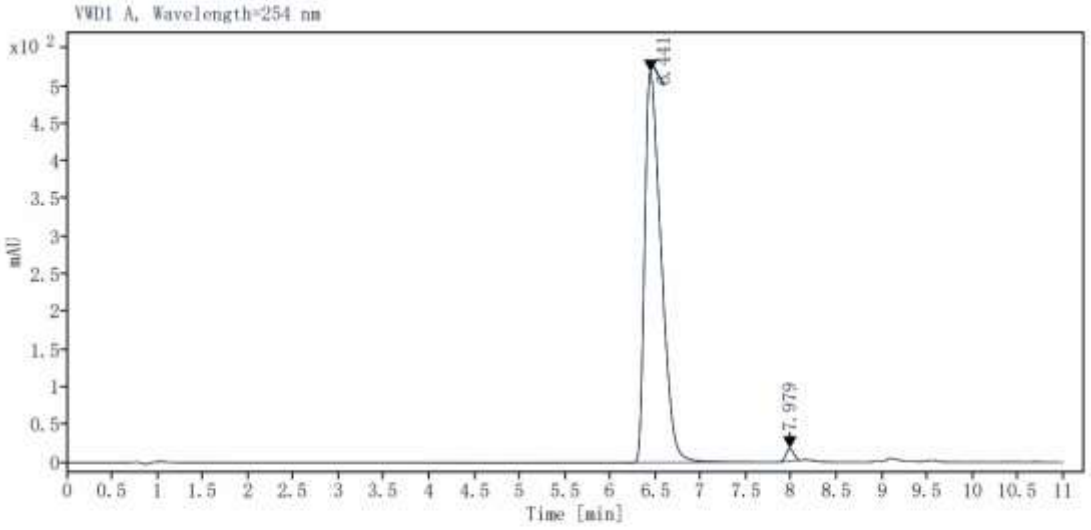
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
2.023	0.0422	0.0768	0.0296	0.0690
2.307	0.1181	21.9700	2.6406	19.7561
2.390	0.0259	0.2930	0.1811	0.2635
2.470	0.0402	1.8652	0.7444	1.6773
2.569	0.0401	1.1557	0.4792	1.0392
2.640	0.0606	5.6854	1.3932	5.1125
2.777	0.0394	2.2351	0.9179	2.0099
2.921	0.0697	19.2747	3.9645	17.3324
3.227	0.3758	13.4375	0.5111	12.0834
7.671	0.0837	2.3798	0.4499	2.1400
7.839	0.0444	0.0385	0.0143	0.0346
7.965	0.0830	1.2480	0.2425	1.1223
8.249	0.1000	1.1359	0.1744	1.0214
9.460	0.0813	0.1601	0.0295	0.1440
9.918	0.1124	1.5805	0.1953	1.4212
11.045	0.1033	23.4655	3.6848	21.1009
11.223	0.0948	3.4986	0.6113	3.1461
11.469	0.0885	3.7918	0.6755	3.4097
11.967	0.1002	1.9301	0.2881	1.7356

HRMS spectra for S-1



HRMS (ESI) calculated $C_{18}H_{18}N_2O$, [M+H]+ = 279.1497, and measured [M+H]+: 279.1512.

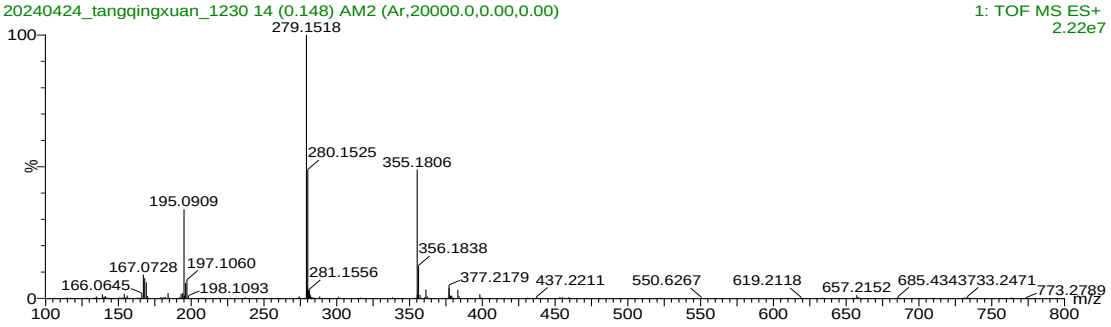
HPLC trace for S-1 (purity, 98.49%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

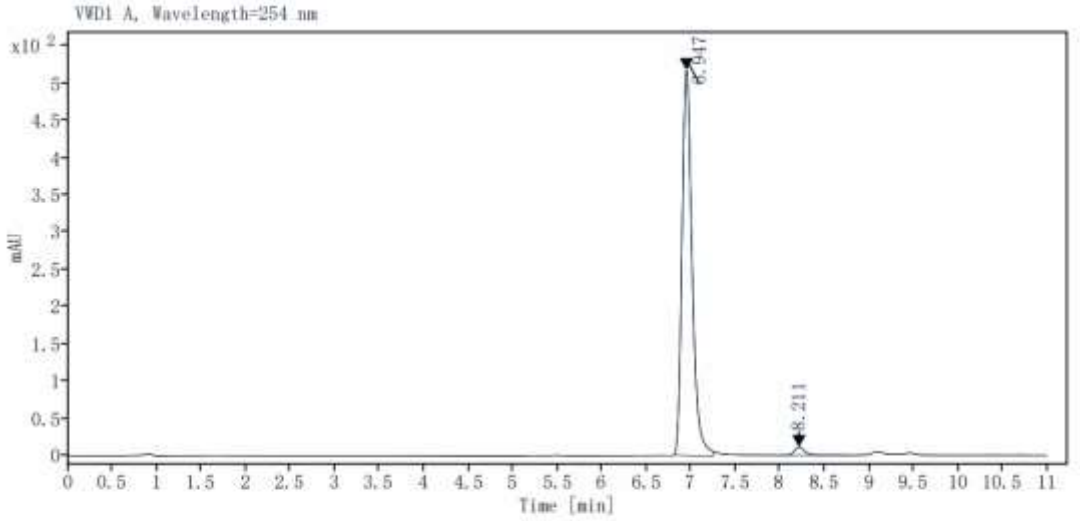
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
6.441	0.1917	6570.4482	519.1284	98.4889
7.979	0.0960	100.8076	16.8079	1.5111

HRMS spectra for S-2



HRMS (ESI) calculated $C_{18}H_{18}N_2O$, [M+H]+ = 279.1497, and measured [M+H]+: 279.1518

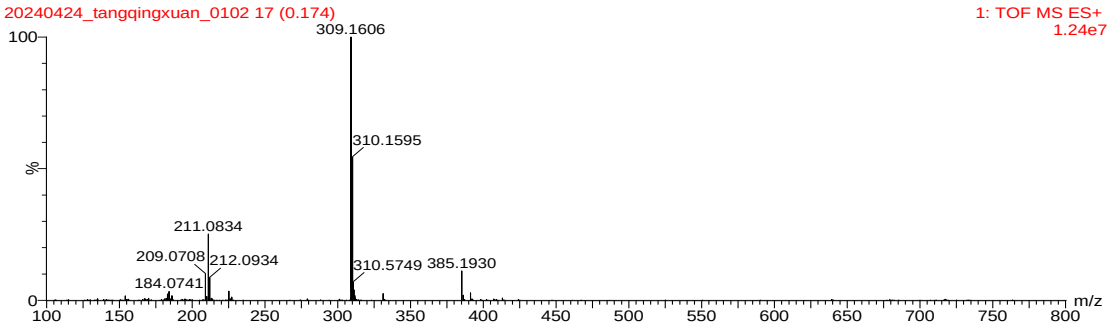
HPLC trace for S-2 (purity, 97.92%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

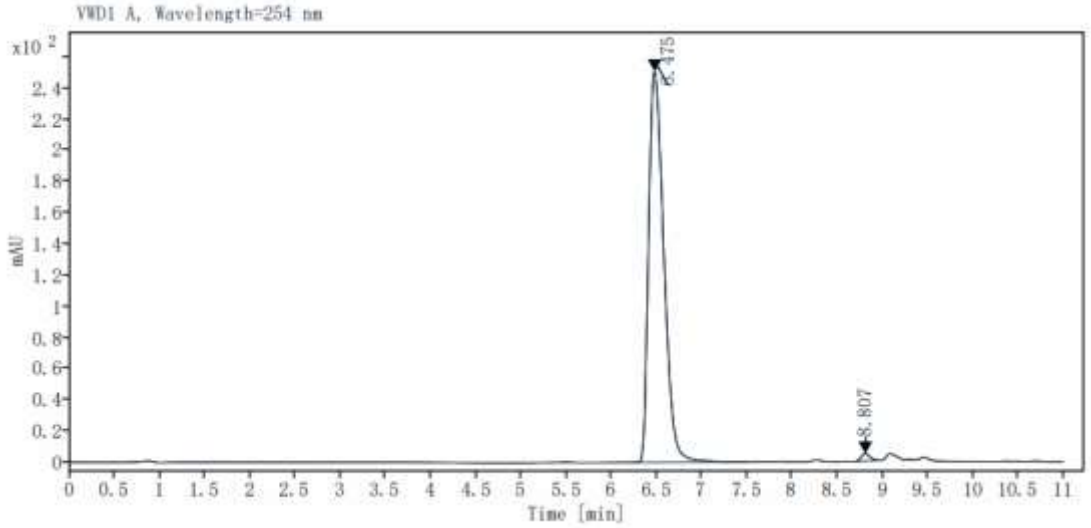
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
6.947	0.1319	4097.6777	517.7788	97.9214
8.211	0.1226	86.9807	10.9589	2.0786

HRMS spectra for S-3



HRMS (ESI) calculated $C_{19}H_{20}N_2O_2$, [M+H]+ = 309.1603, and measured [M+H]+: 309.1606

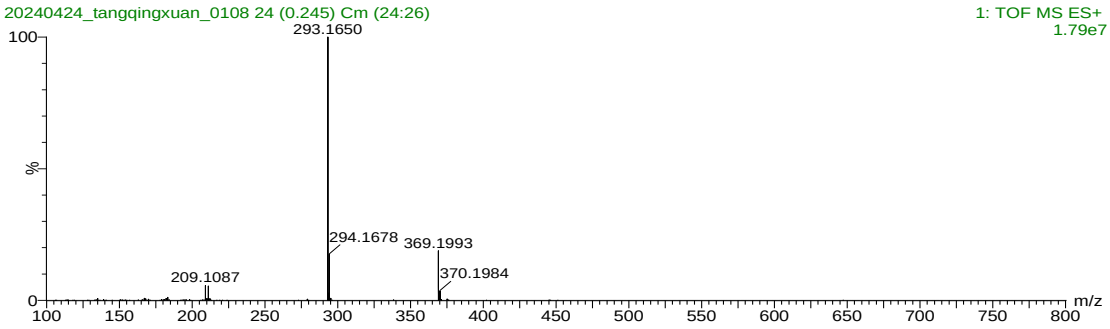
HPLC trace for S-3 (purity, 98.90%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

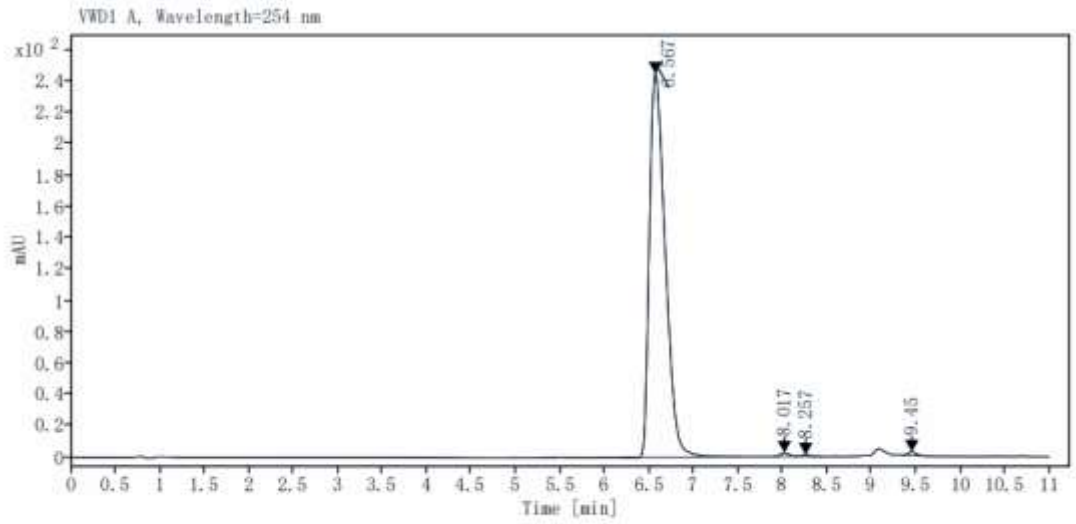
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
6.475	0.1835	2913.4114	250.7777	98.9067
8.807	0.1091	32.2036	4.6312	1.0933

HRMS spectra for S-4



HRMS (ESI) calculated $C_{19}H_{20}N_2O$, [M+H]+ = 293.1654, and measured [M+H]+: 293.1650

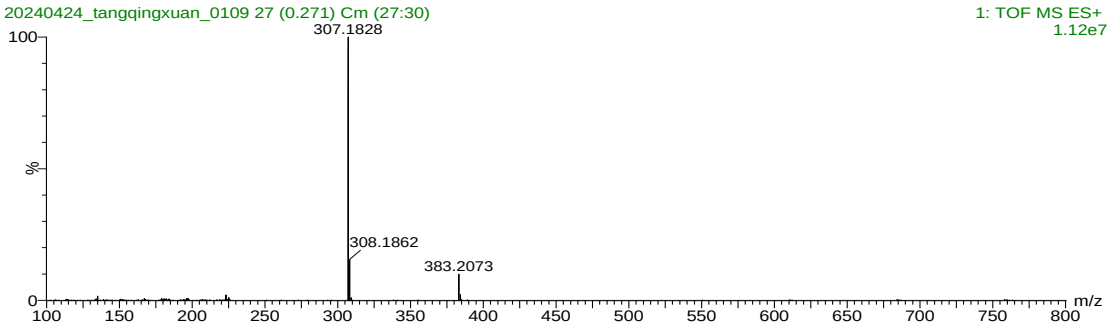
HPLC trace for S-4 (purity, 98.83%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

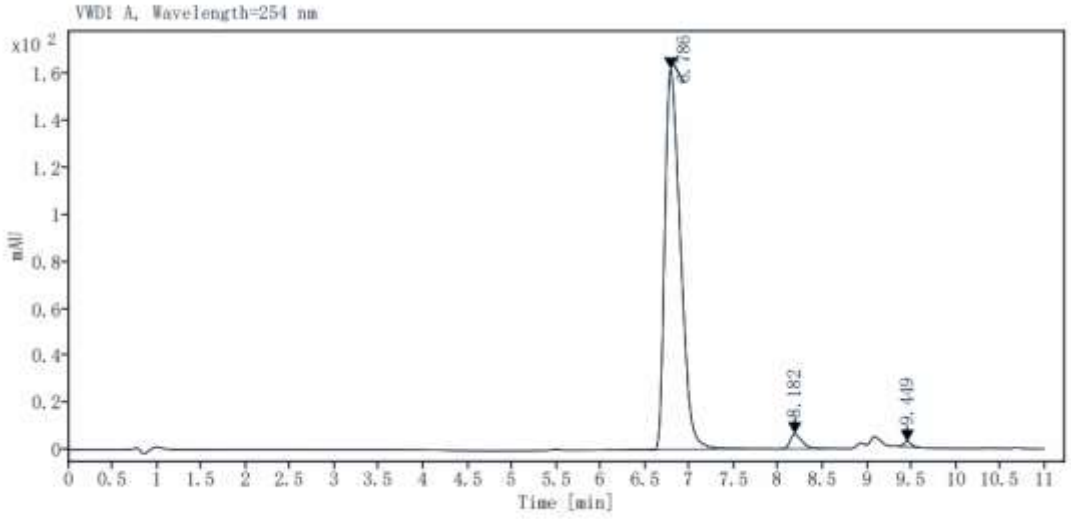
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
6.567	0.1960	3020.9558	244.7359	98.8358
8.017	0.1059	15.4118	2.3091	0.5042
8.257	0.0950	6.2359	1.0241	0.2040
9.450	0.0957	13.9377	2.2678	0.4560

HRMS spectra for S-5



HRMS (ESI) calculated $C_{20}H_{22}N_2O$, [M+H]+ = 307.1810, and measured [M+H]+: 307.1828

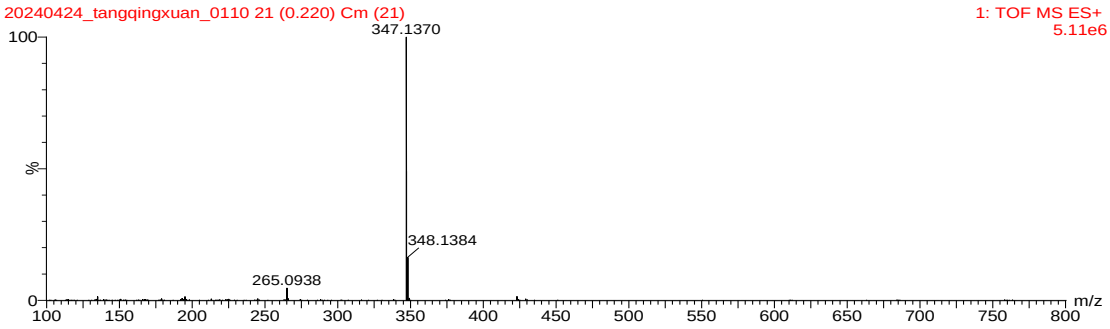
HPLC trace for S-5 (purity, 96.62%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

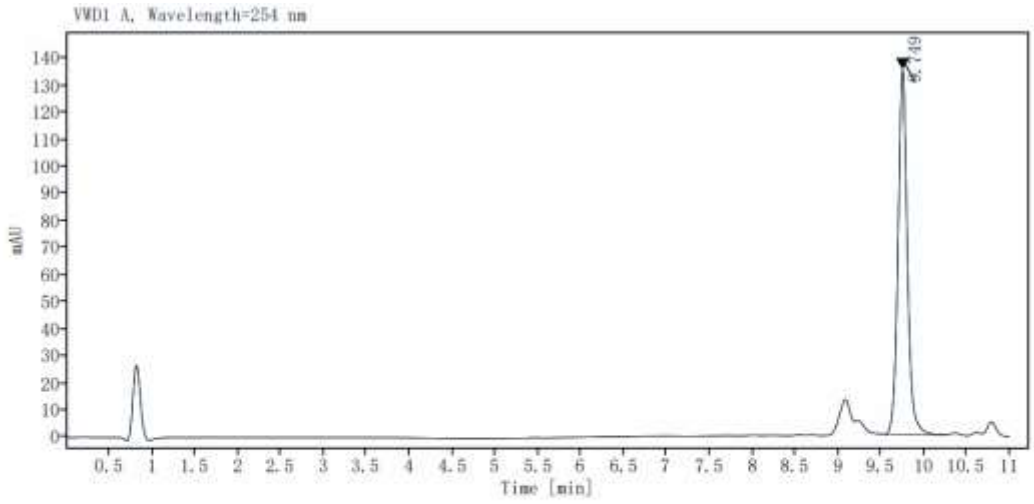
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
6.786	0.1946	1978.7377	161.8880	96.6163
8.182	0.1329	55.9496	6.2221	2.7319
9.449	0.0956	13.3501	2.1766	0.6518

HRMS spectra for S-6



HRMS (ESI) calculated $C_{19}H_{17}F_3N_2O$, [M+H]+ = 347.1371, and measured [M+H]+: 347.1370

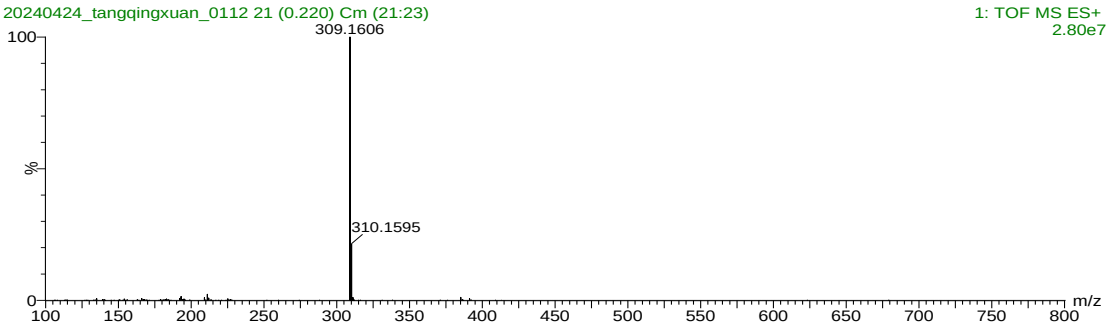
HPLC trace for S-6 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

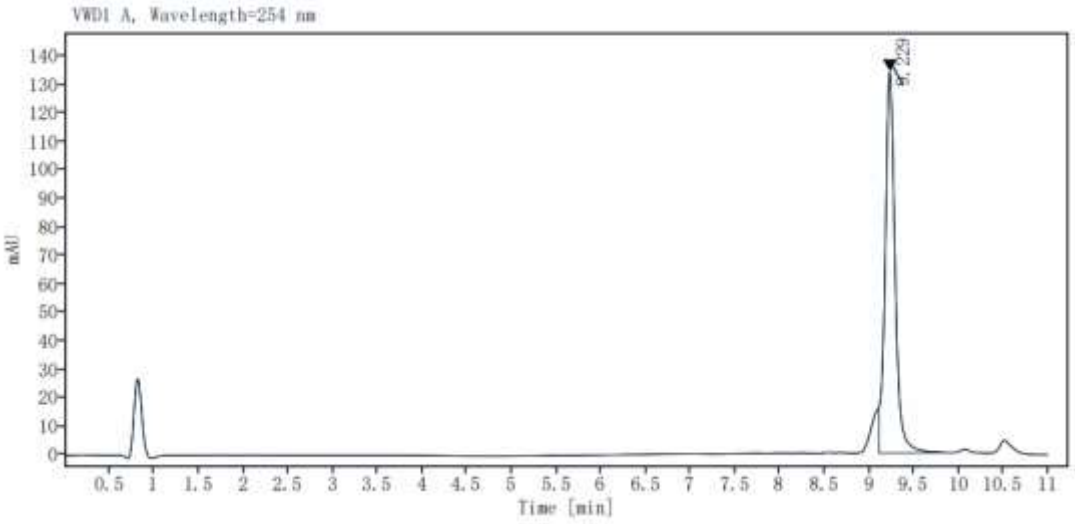
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.749	0.1168	1025.7622	134.7071	100.0000

HRMS spectra for S-7



HRMS (ESI) calculated $C_{19}H_{20}N_2O_2$, [M+H]+ = 309.1603, and measured [M+H]+: 309.1606

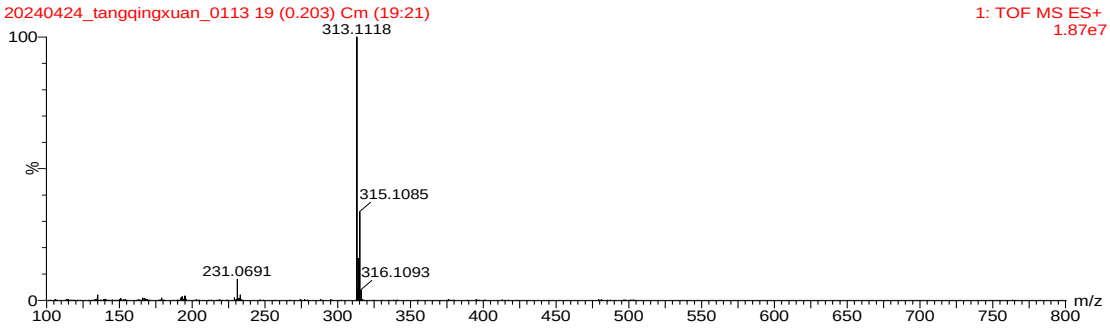
HPLC trace for S-7 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

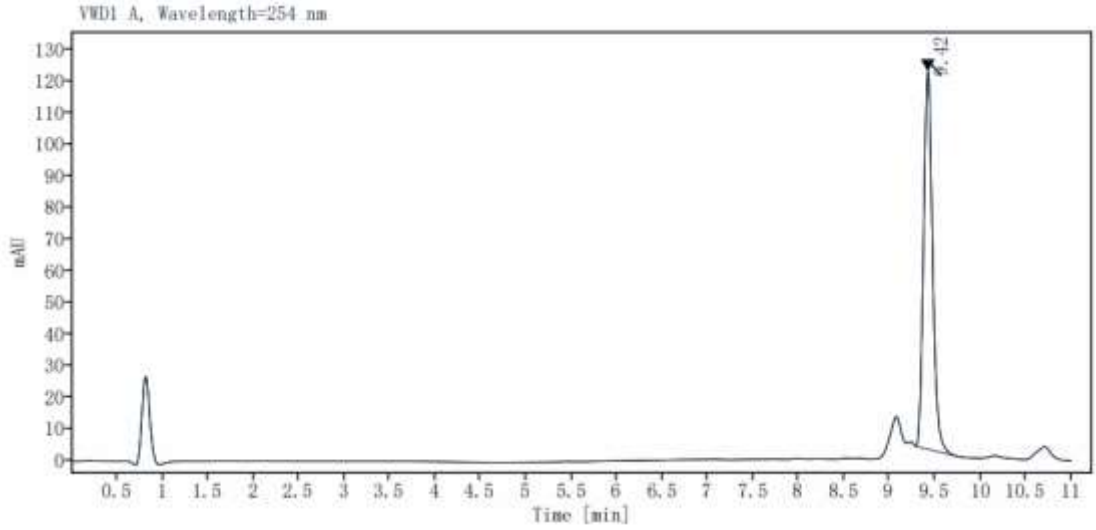
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.229	0.1299	1043.2941	133.8300	100.0000

HRMS spectra for S-8



HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1118

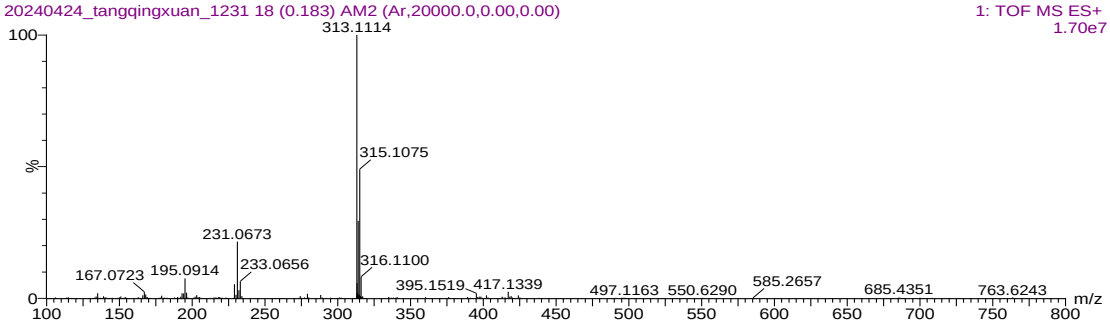
HPLC trace for S-8 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

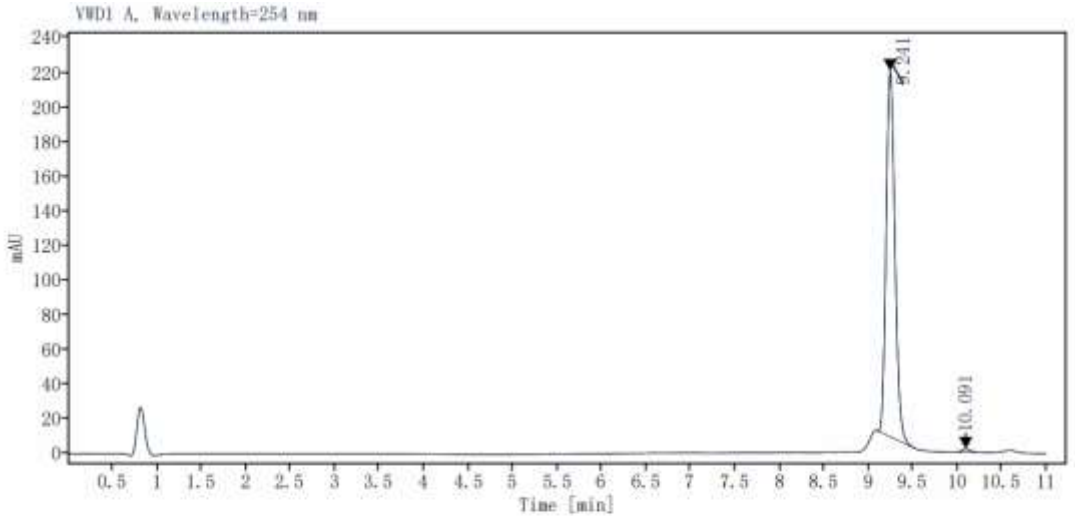
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.420	0.1071	828.1421	119.1893	100.0000

HRMS spectra for TH-408



HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1114

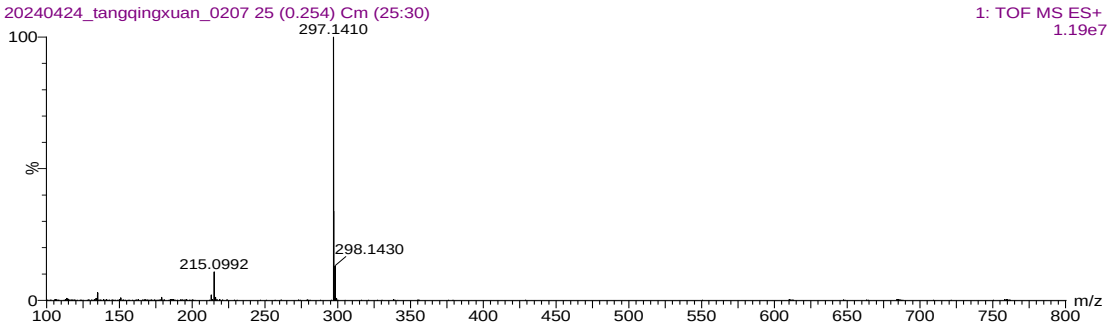
HPLC trace for TH-408 (purity, 99.03%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

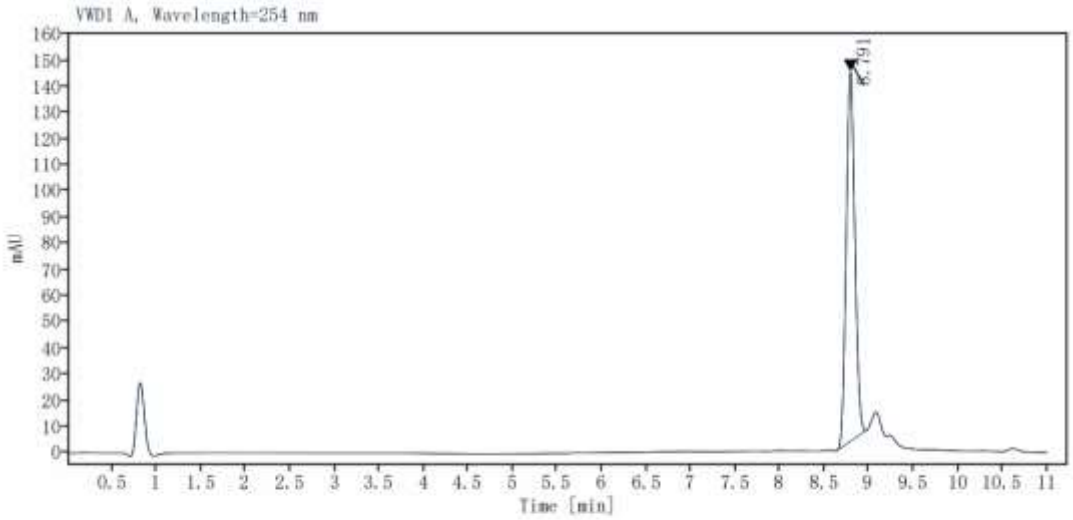
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.241	0.1061	1450.0017	211.0805	99.0325
10.091	0.1133	14.1652	1.9370	0.9675

HRMS spectra for S-9



HRMS (ESI) calculated $C_{18}H_{17}FN_2O$, [M+H]+ = 297.1403, and measured [M+H]+: 297.141

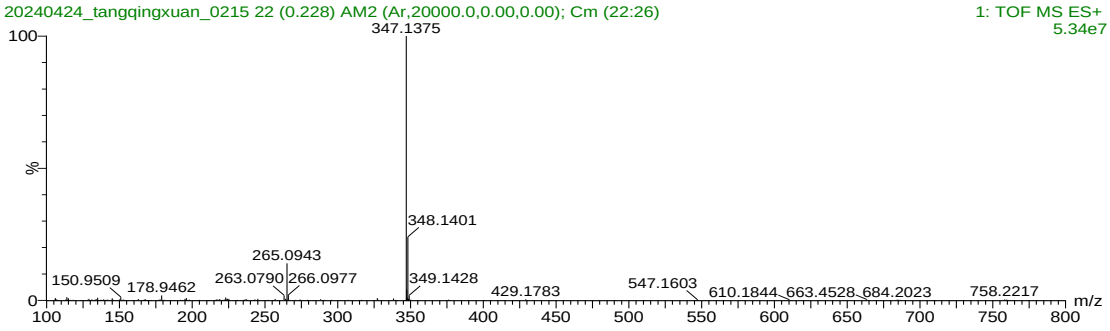
HPLC trace for S-9 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

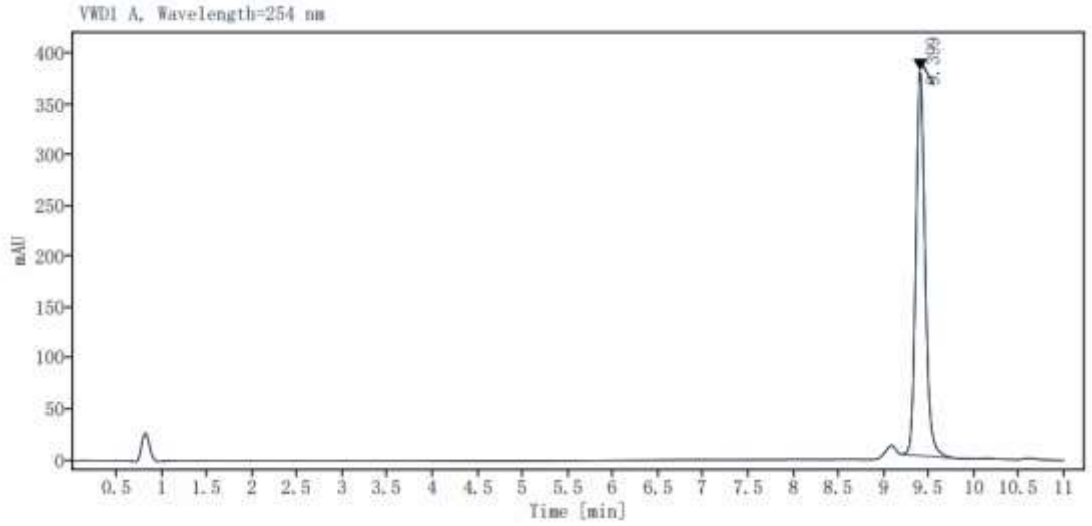
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
8.791	0.1073	960.6519	141.3400	100.0000

HRMS spectra for S-10



HRMS (ESI) calculated $C_{19}H_{17}F_3N_2O$, [M+H]+ = 347.1371, and measured [M+H]+: 347.1375

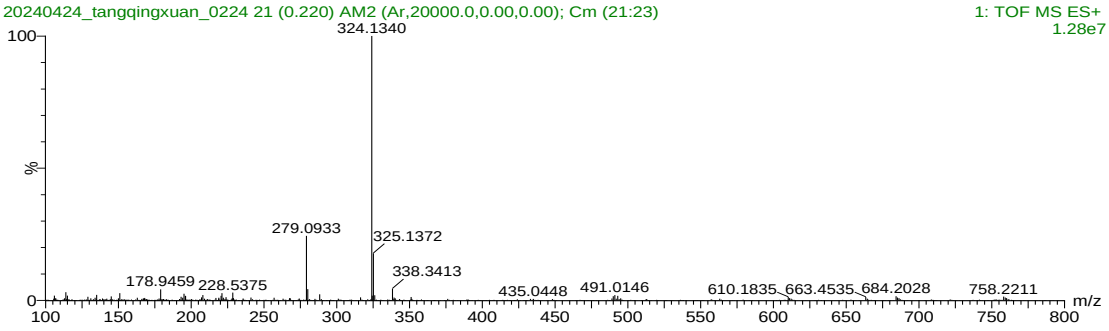
HPLC trace for S-10 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

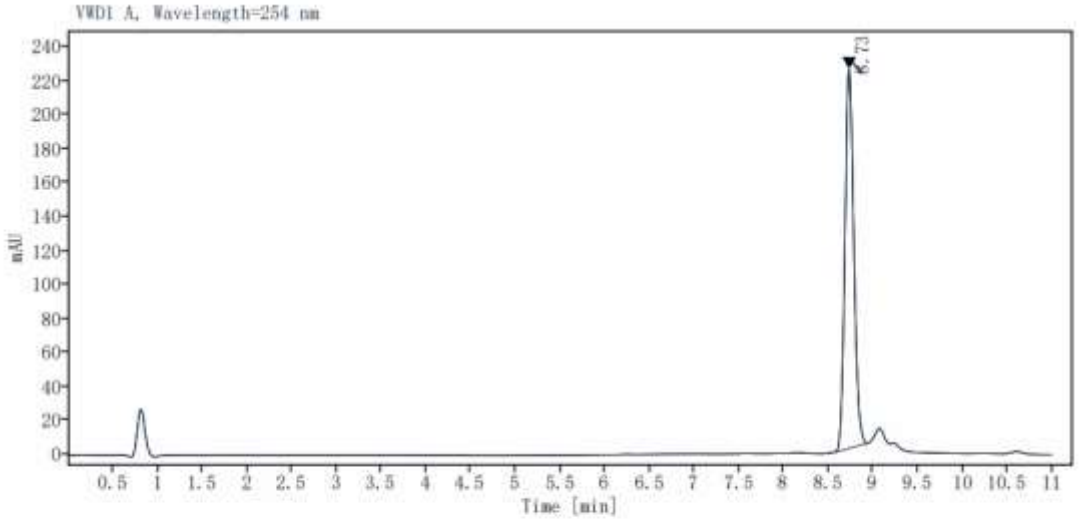
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.399	0.1123	2732.7261	378.3625	100.0000

HRMS spectra for S-11



HRMS (ESI) calculated $C_{18}H_{17}N_3O_3$, [M+H]+ = 324.1348, and measured [M+H]+: 324.1340

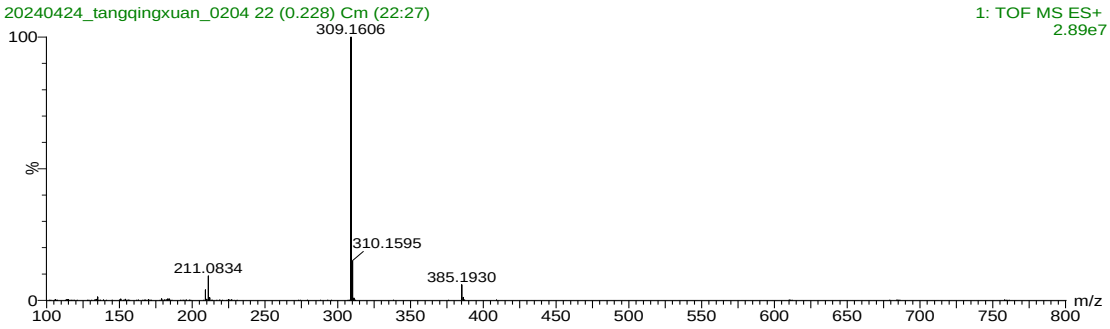
HPLC trace for S-11 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

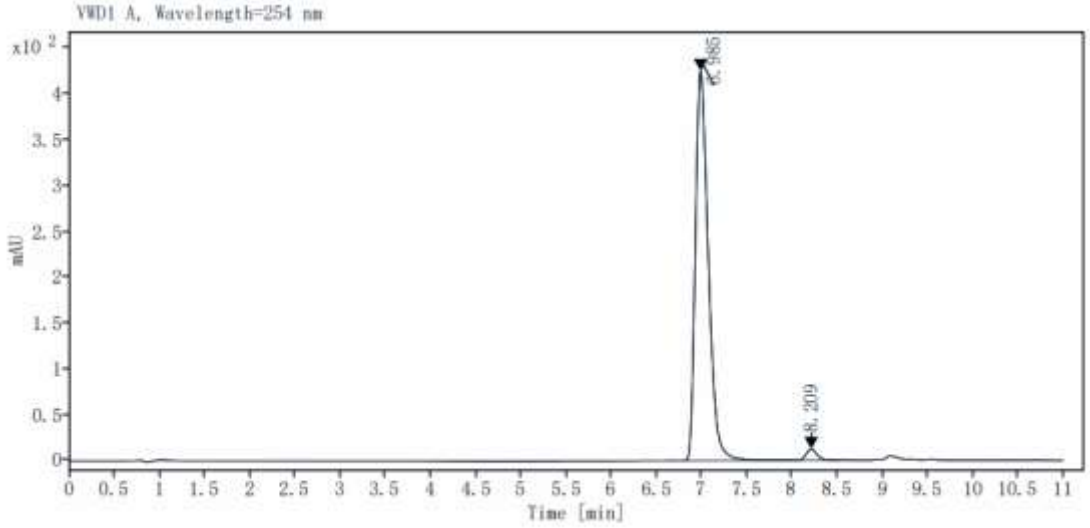
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
8.730	0.1091	1549.6306	222.8377	100.0000

HRMS spectra for S-12



HRMS (ESI) calculated $C_{19}H_{20}N_2O_2$, [M+H]+ = 309.1603, and measured [M+H]+: 309.1606

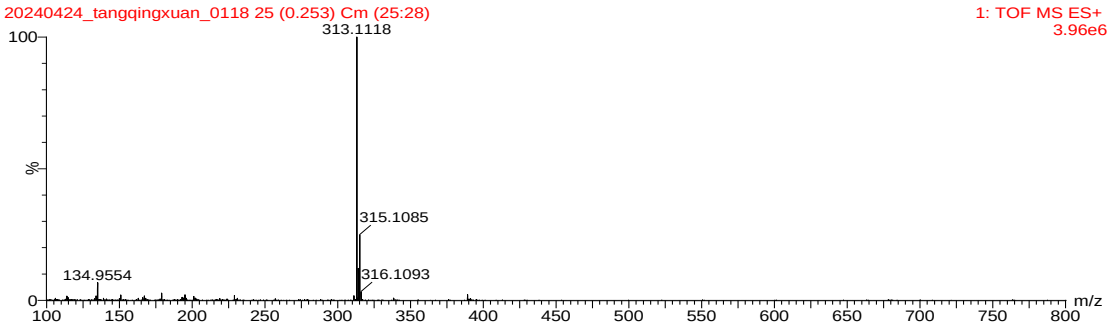
HPLC trace for S-12 (purity, 97.73%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

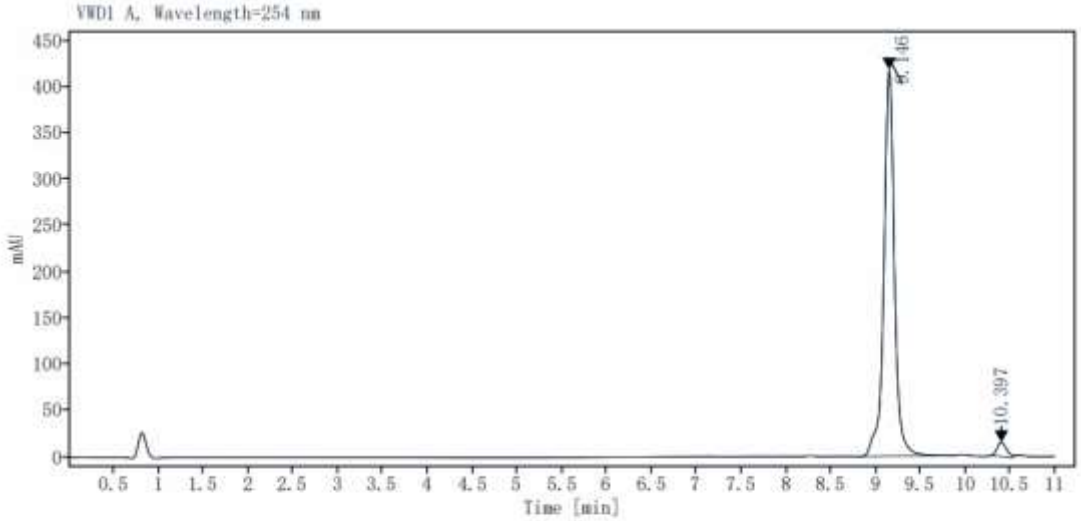
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
6.985	0.1544	4238.8691	424.8307	97.7300
8.209	0.1249	98.4573	12.1083	2.2700

HRMS spectra for S-13



HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1118

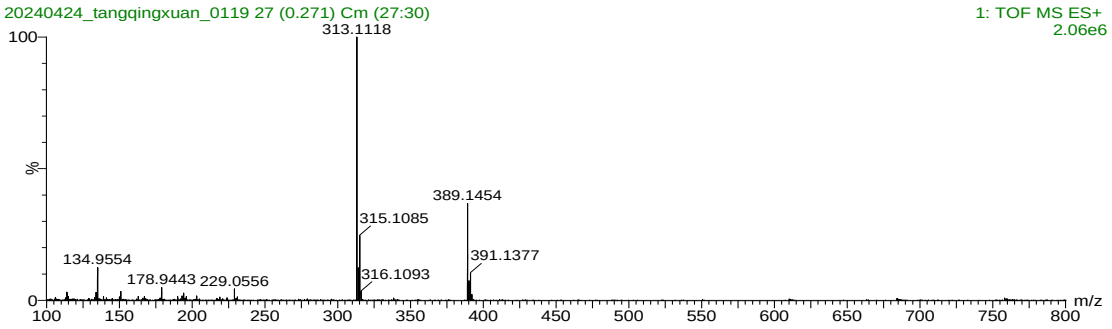
HPLC trace for S-13 (purity, 96.83%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

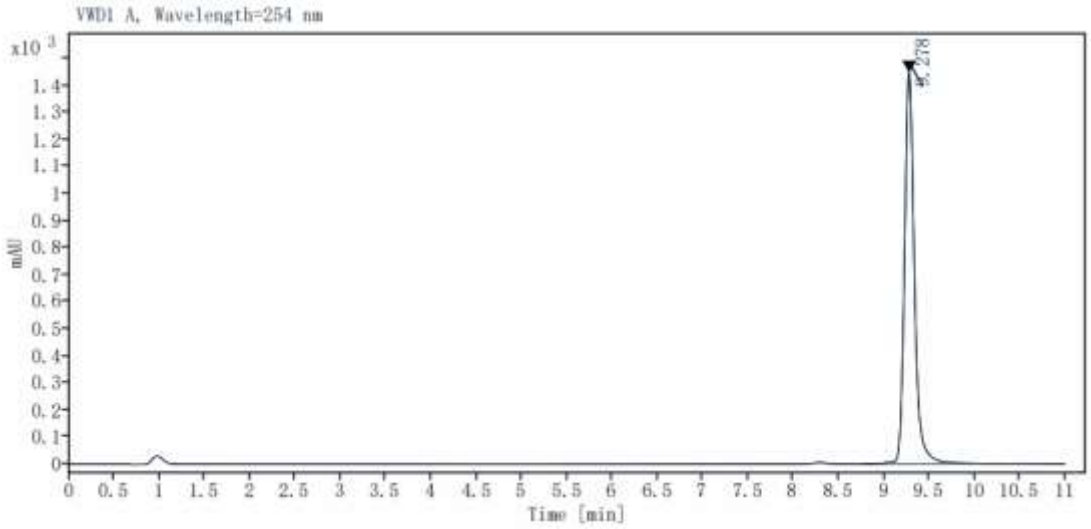
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.146	0.1240	3436.3147	417.4023	96.8279
10.397	0.1238	112.5757	15.1561	3.1721

HRMS spectra for S-14



HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1118

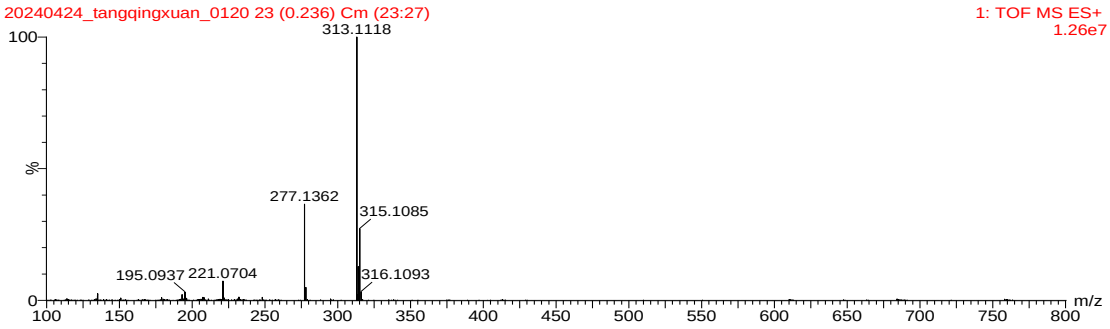
HPLC trace for S-14 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

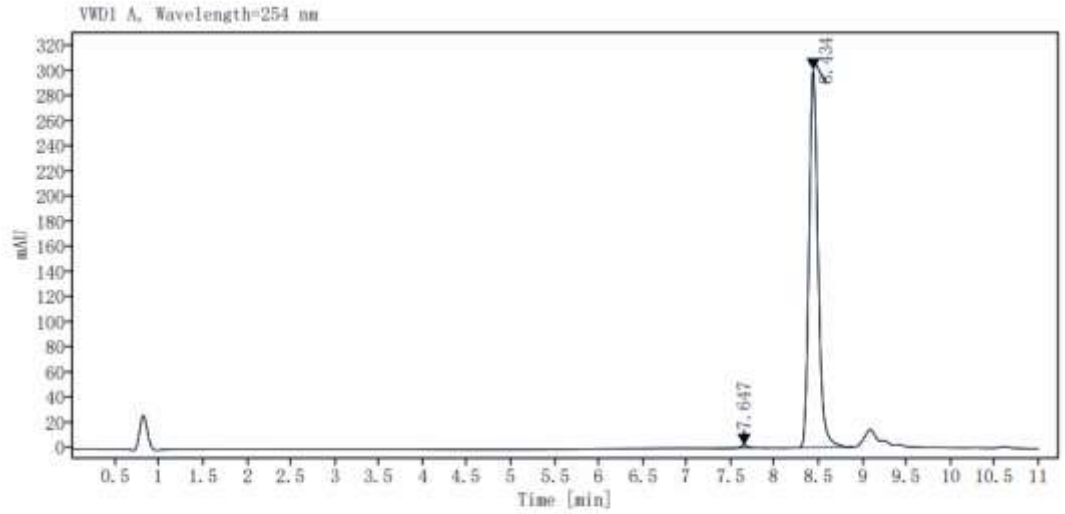
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.278	0.1175	11090.8857	1445.0328	100.0000

HRMS spectra for S-15



HRMS (ESI) calculated $C_{18}H_{17}ClN_2O$, [M+H]+ = 313.1108, and measured [M+H]+: 313.1118

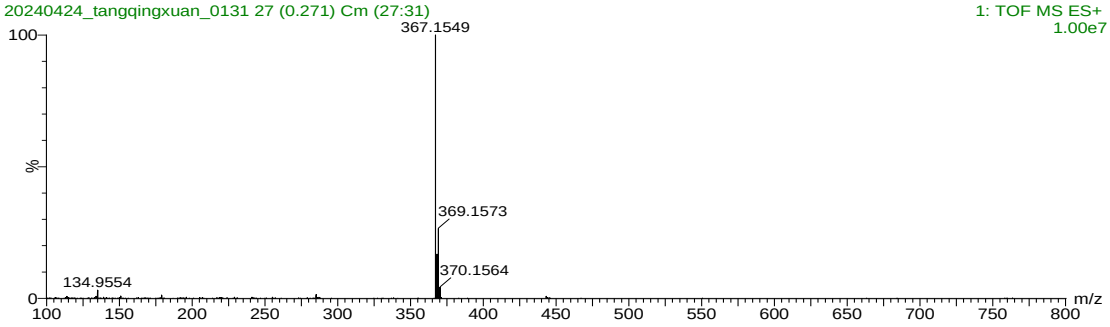
HPLC trace for S-15 (purity, 99.25%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

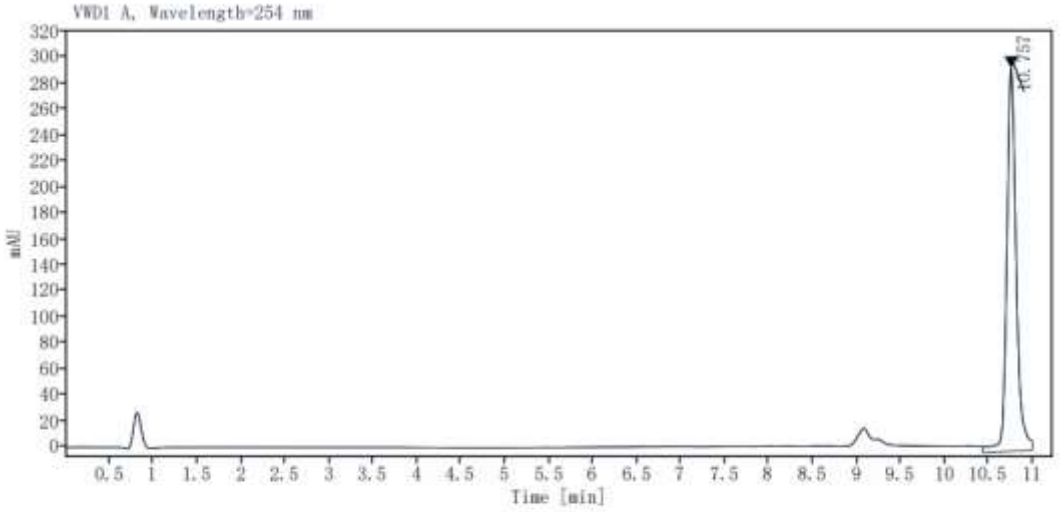
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
7.647	0.1197	16.4733	2.0522	0.7545
8.434	0.1122	2166.9690	300.3223	99.2455

HRMS spectra for S-16



HRMS (ESI) calculated $C_{22}H_{23}ClN_2O$, [M+H]+ = 367.1577, and measured [M+H]+: 367.1549

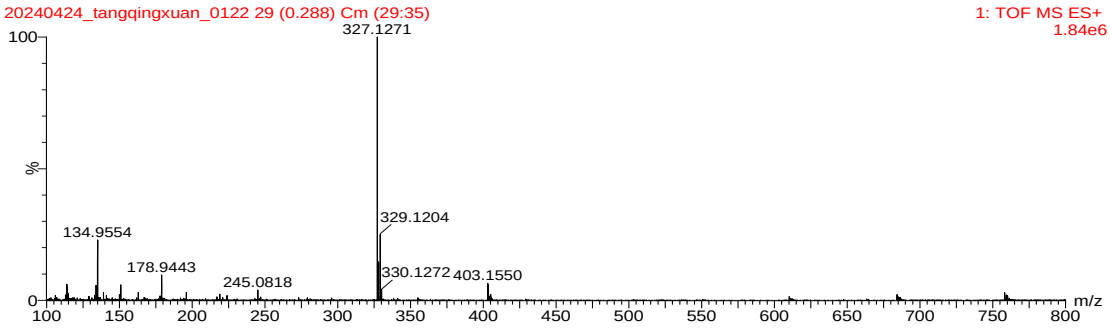
HPLC trace for S-16 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

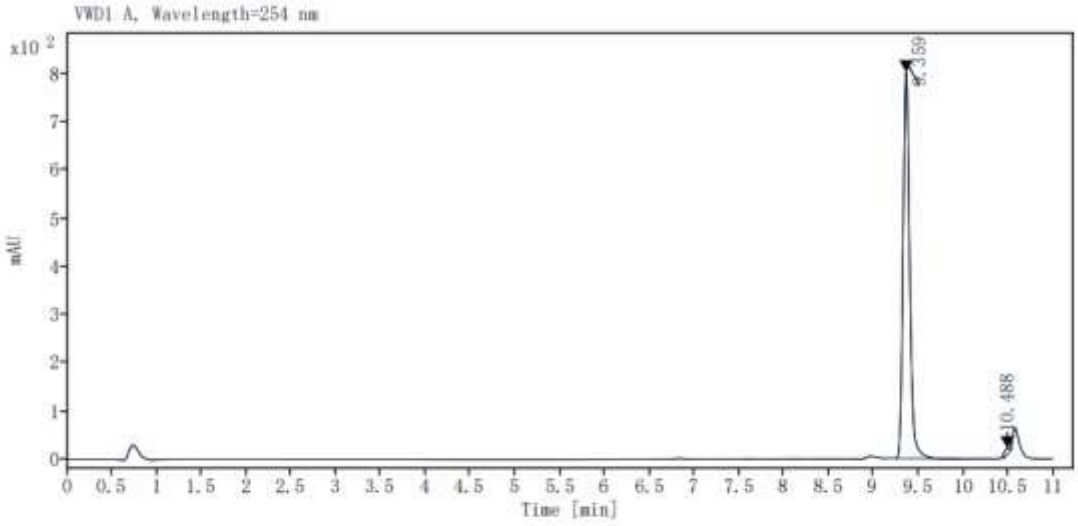
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
10.757	0.1250	2206.5828	294.3026	100.0000

HRMS spectra for S-17



HRMS (ESI) calculated $C_{19}H_{19}ClN_2O$, [M+H]+ = 327.1264, and measured [M+H]+: 327.1271

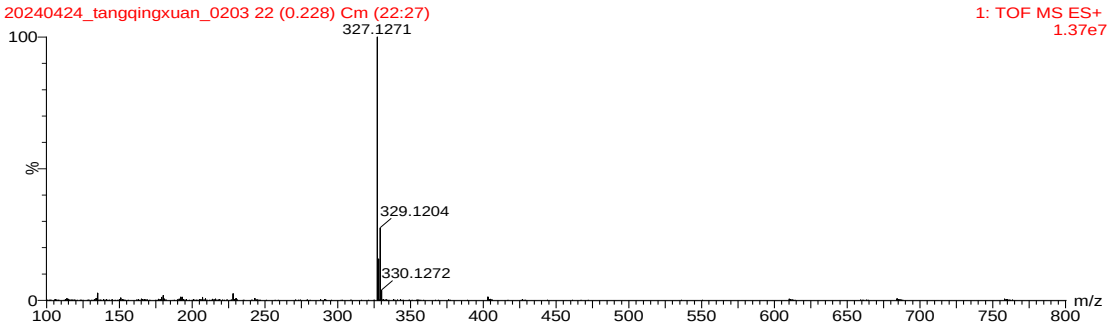
HPLC trace for S-17 (purity, 98.51%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

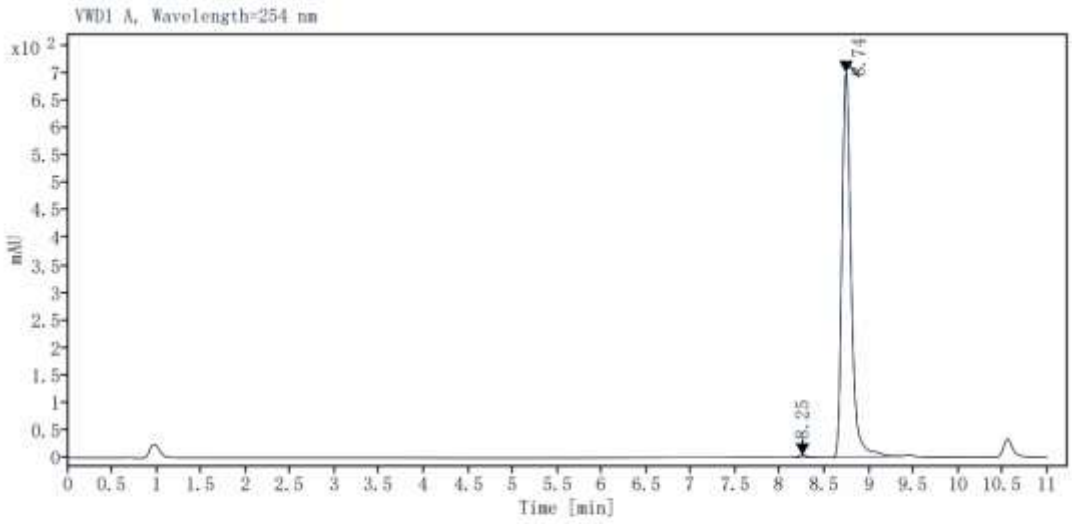
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.359	0.0774	4082.6995	801.7144	98.5140
10.488	0.0793	61.5830	12.9384	1.4860

HRMS spectra for S-18



HRMS (ESI) calculated $C_{19}H_{19}ClN_2O$, [M+H]+ = 327.1264, and measured [M+H]+: 327.1271

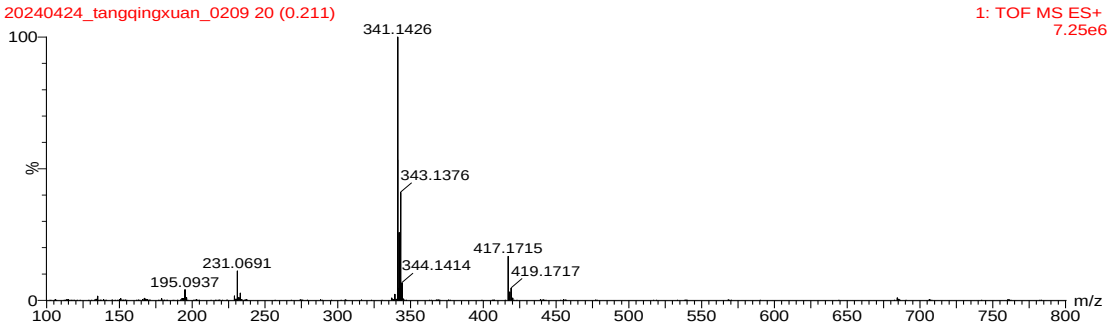
HPLC trace for S-18 (purity, 99.48%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

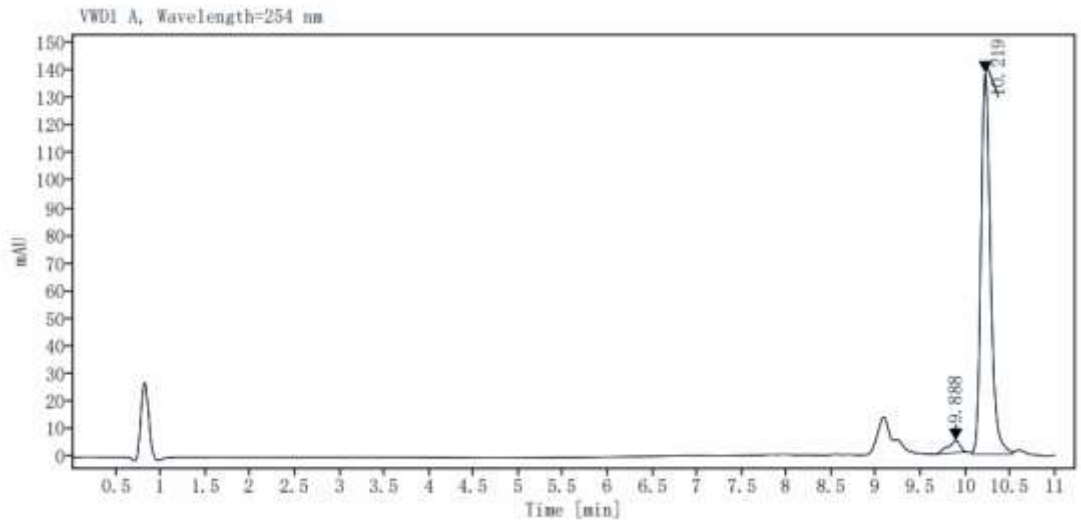
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
8.250	0.1085	26.3020	3.7193	0.5225
8.740	0.1095	5007.5469	699.9510	99.4775

HRMS spectra for S-19



HRMS (ESI) calculated $C_{20}H_{21}ClN_2O$, [M+H]+ = 341.1421, and measured [M+H]+: 341.1426

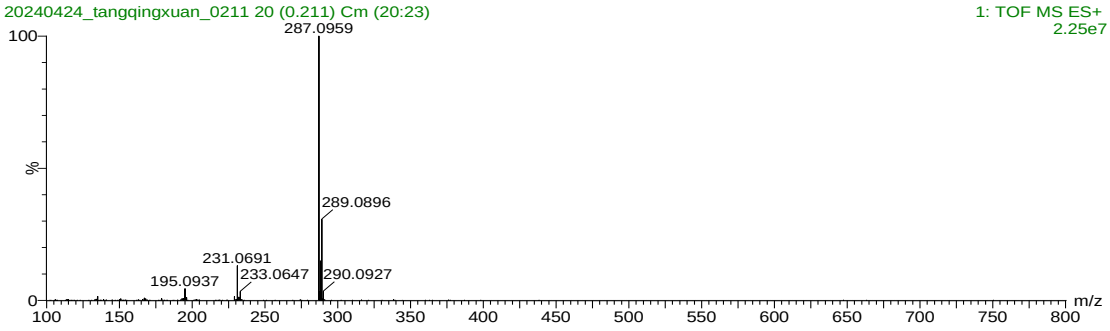
HPLC trace for S-19 (purity, 96.13%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

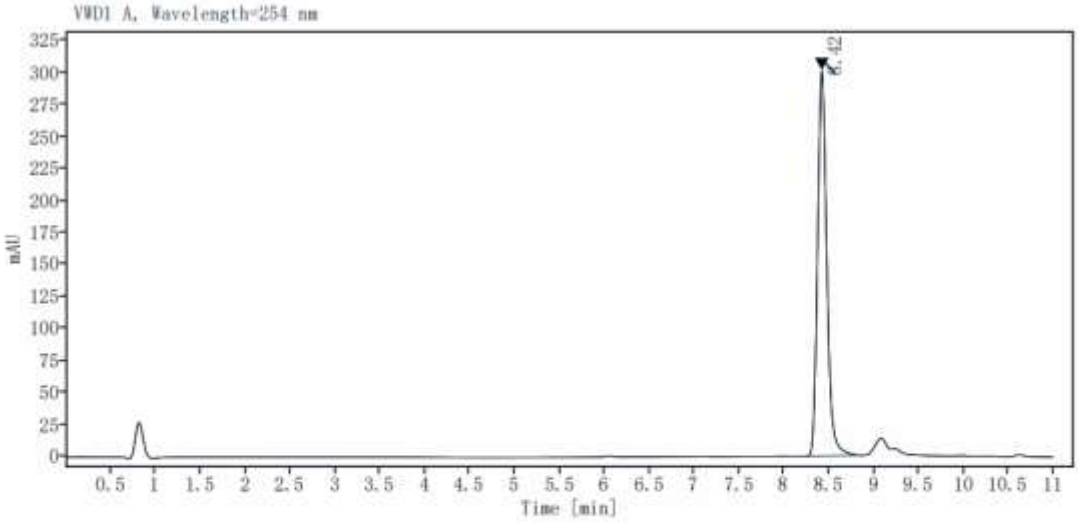
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.888	0.1358	41.4052	4.3985	3.8714
10.219	0.1242	1028.1101	137.9924	96.1286

HRMS spectra for S-20



HRMS (ESI) calculated $C_{16}H_{15}ClN_2O$, [M+H]+ = 287.0951, and measured [M+H]+: 287.0959

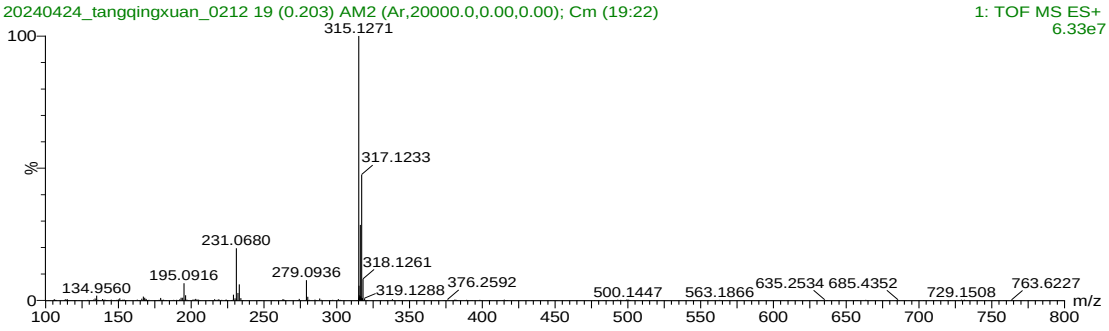
HPLC trace for S-20 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

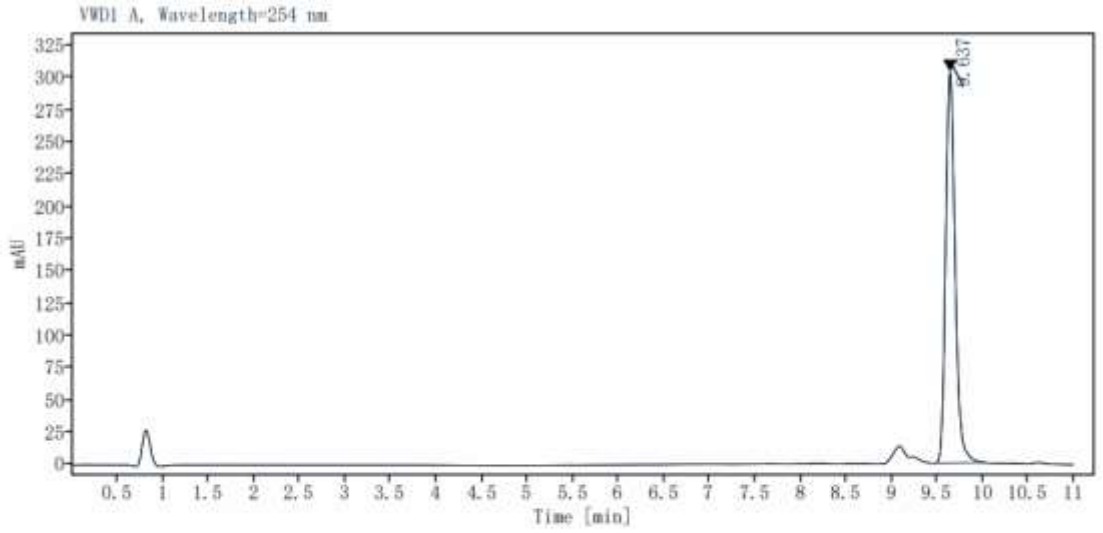
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
8.420	0.1124	2176.6836	300.9221	100.0000

HRMS spectra for S-21



HRMS (ESI) calculated $C_{18}H_{19}ClN_2O$, [M+H]+ = 315.1264, and measured [M+H]+: 315.1271

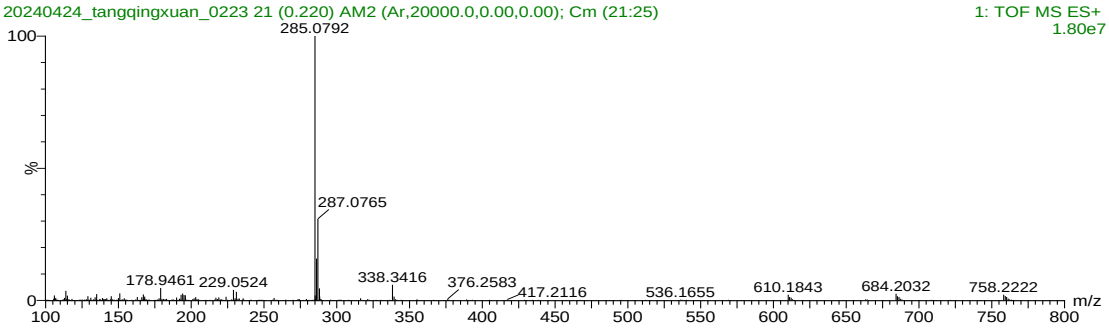
HPLC trace for S-21 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

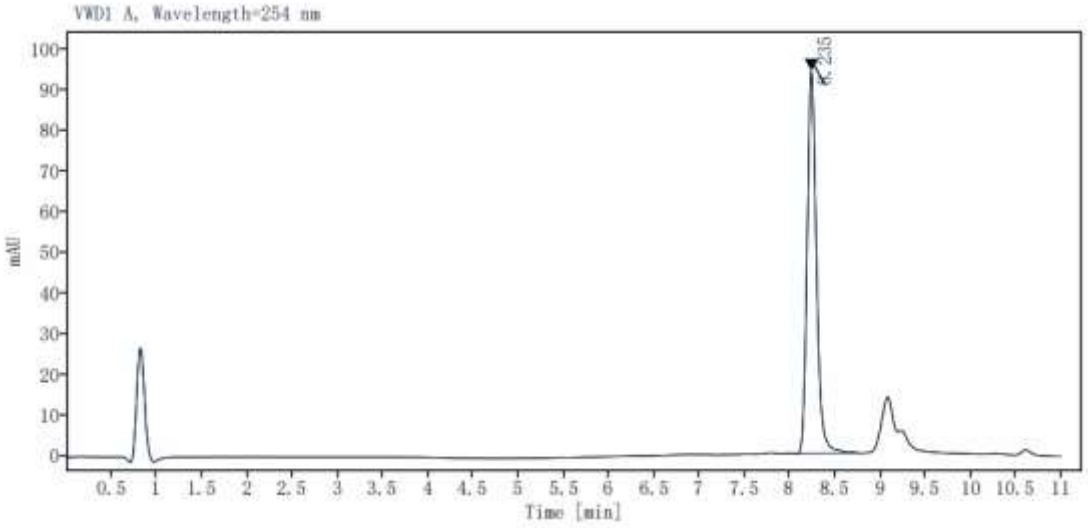
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.637	0.1119	2178.3867	302.7588	100.0000

HRMS spectra for S-23



HRMS (ESI) calculated $C_{16}H_{13}ClN_2O$, [M+H]+ = 285.0795, and measured [M+H]+: 285.0792

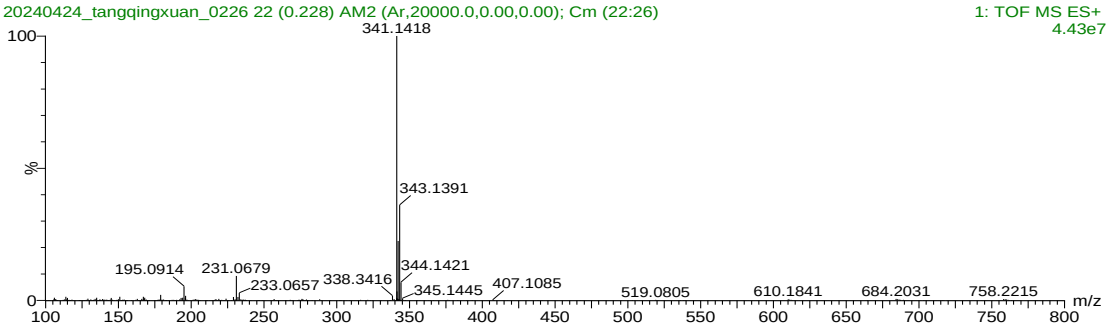
HPLC trace for S-23 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

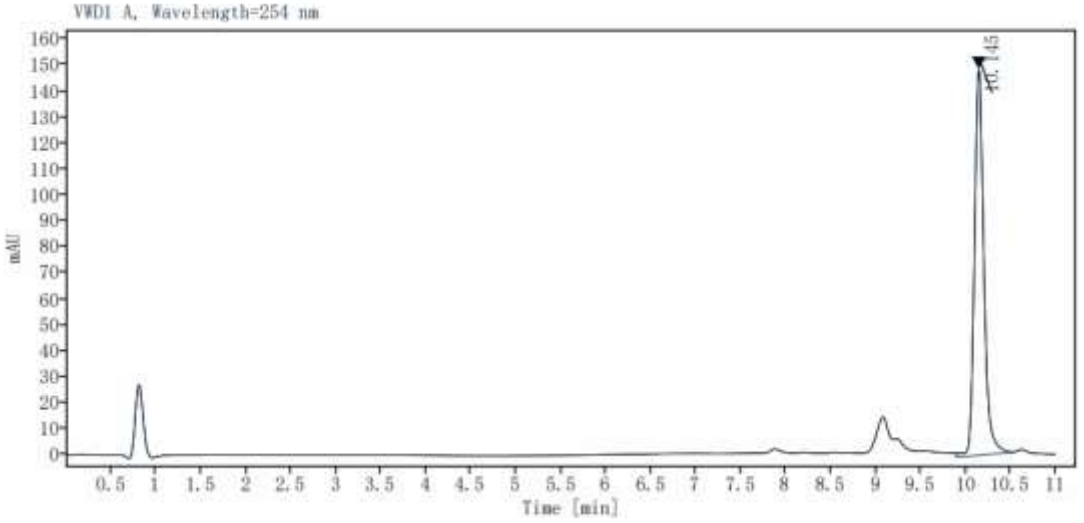
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
8.235	0.1126	681.9696	94.0080	100.0000

HRMS spectra for S-24



HRMS (ESI) calculated $C_{20}H_{21}ClN_2O$, [M+H]+ = 341.1421, and measured [M+H]+: 341.1418

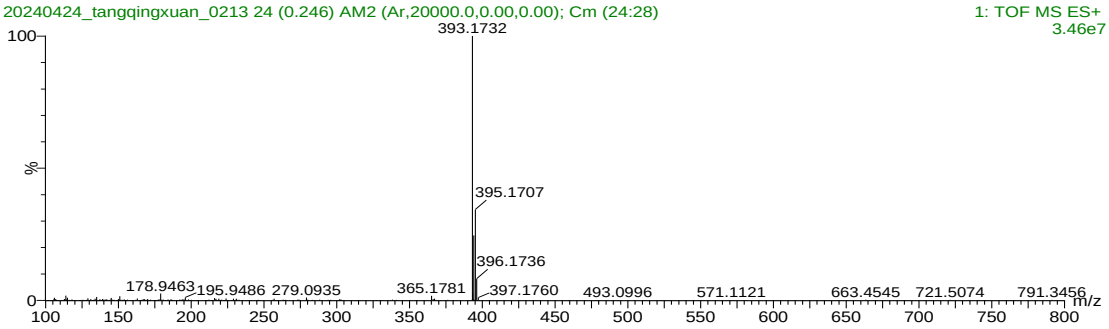
HPLC trace for S-24 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

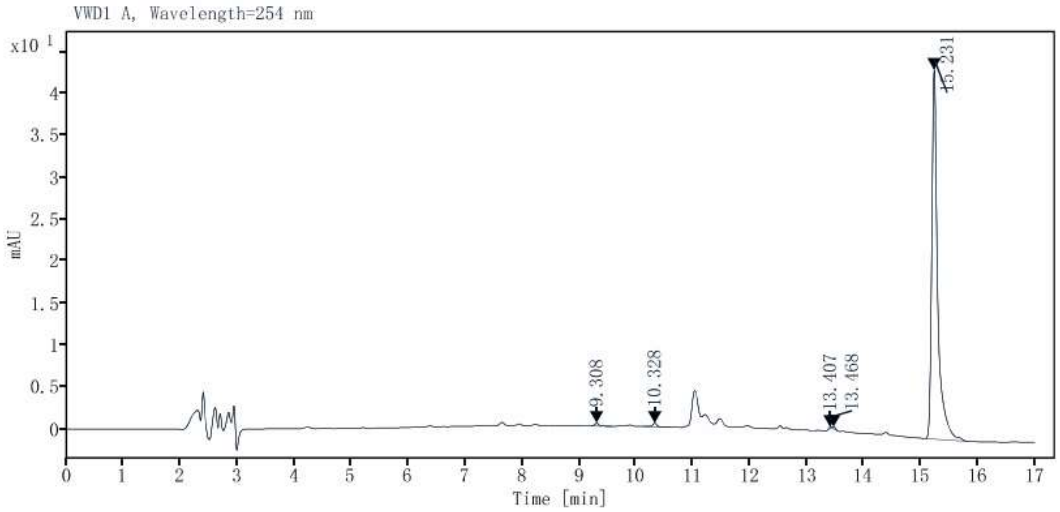
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
10.145	0.1226	1094.2311	148.7195	100.0000

HRMS spectra for S-22



HRMS (ESI) calculated $C_{24}H_{25}ClN_2O$, [M+H]+ = 393.1734, and measured [M+H]+: 393.1732

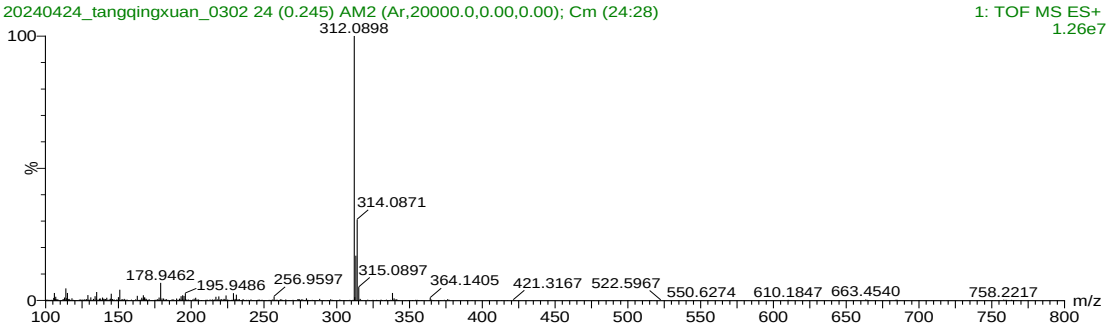
HPLC trace for S-22 (purity, 98.35%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

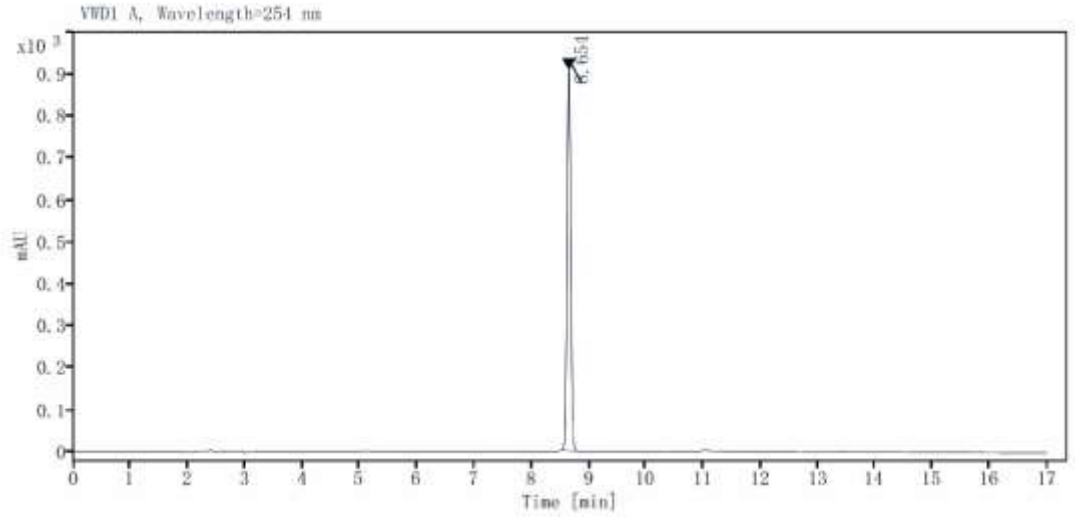
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
9.308	0.0751	1.7604	0.3597	0.5365
10.328	0.0824	2.4012	0.4283	0.7318
13.407	0.0485	0.5058	0.1616	0.1542
13.468	0.0553	0.7580	0.2248	0.2310
15.231	0.1095	322.6840	44.0537	98.3465

HRMS spectra for S-25



HRMS (ESI) calculated $C_{18}H_{15}ClN_2O$, [M+H]+ = 312.0904, and measured [M+H]+: 312.0898

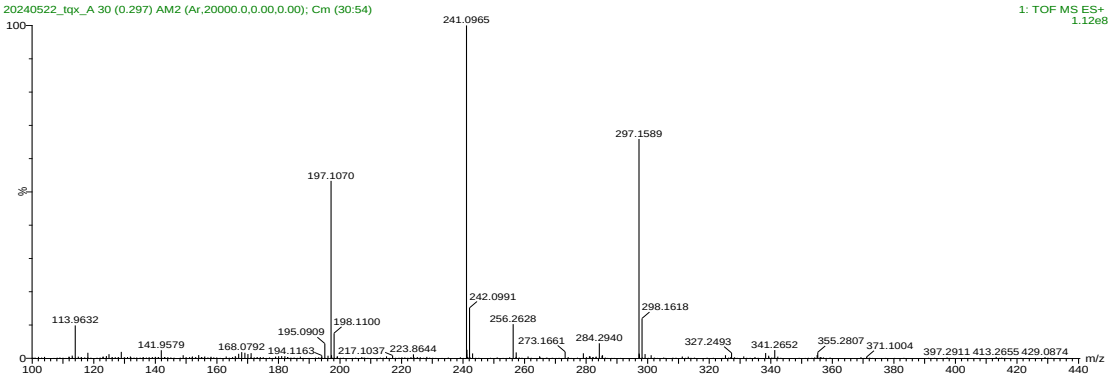
HPLC trace for S-25 (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

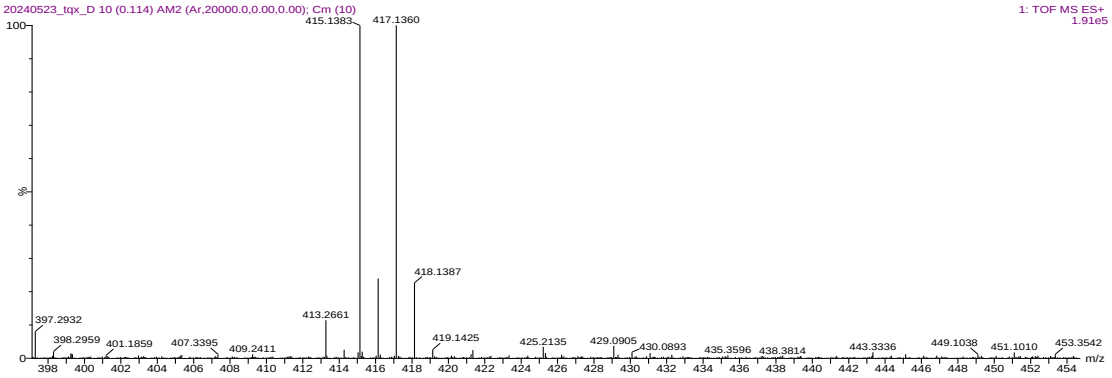
Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
8.654	0.0687	4024.3447	907.8820	100.0000

HRMS spectra for 1b



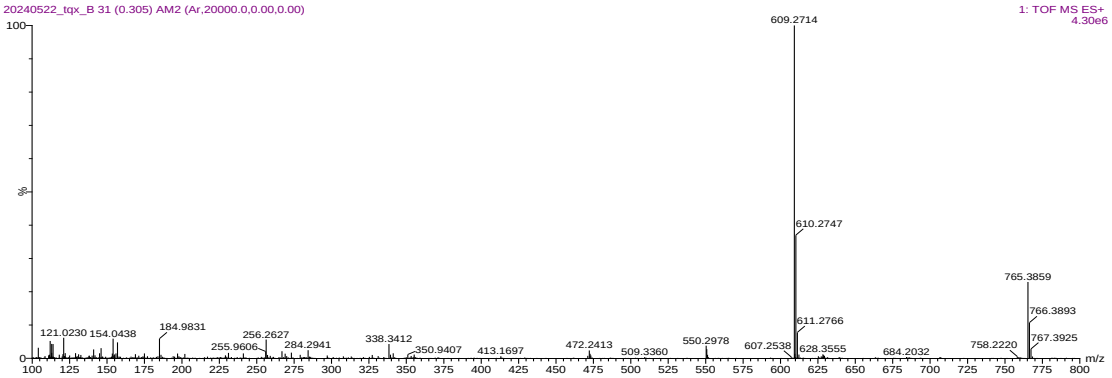
HRMS (ESI) calculated $C_{18}H_{20}N_2O_2$, [M+H]+ = 297.1603, and measured [M+H]+: 297.1589

HRMS spectra for 1d



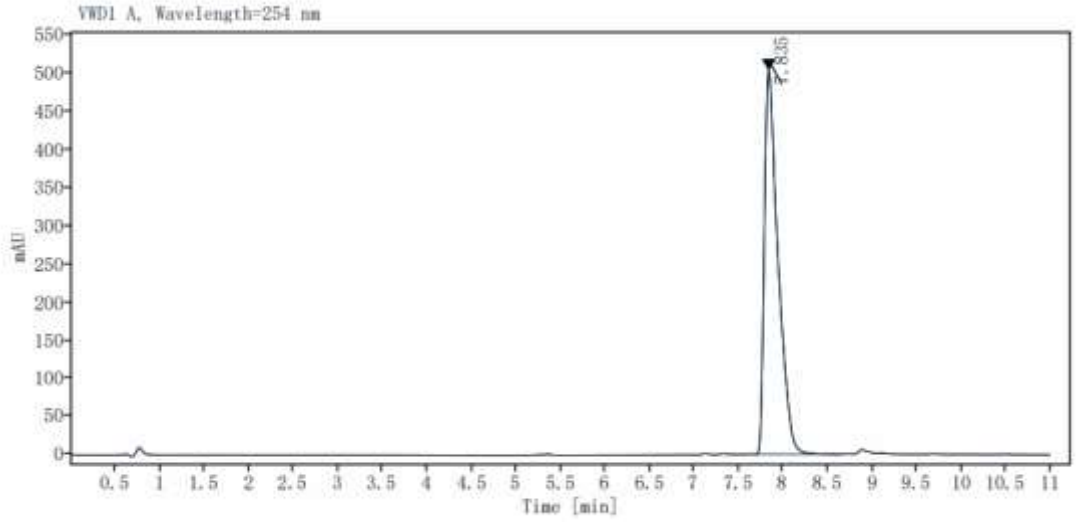
HRMS (ESI) calculated $C_{22}H_{27}BrN_2O$, [M+H]+ = 415.1385, and measured [M+H]+: 415.1383

HRMS spectra for S-1 derived PROTAC



HRMS (ESI) calculated $C_{35}H_{36}N_4O_6$, [M+H]+ = 609.2713, and measured [M+H]+: 609.2714

HPLC trace for S-1 derived PROTAC (purity, 100%, detection at 254nm)



Signal: VWD1 A, Wavelength=254 nm

Retention Time [min]	Peak Width [min]	Peak Area	Peak Height	Area %
7.835	0.1567	5365.6885	502.1758	100.0000