Supporting information

1 Experimental protocols

1.1 Chemistry and chemical methods

Most chemicals and solvents were of analytical grade and, when necessary, were purified and dried by standard methods. Reactions were monitored by thin-layer chromatography (TLC) using precoated silica gel plates (silica gel GF/UV 254), and spots were visualized under UV light (254 nm). Melting points (uncorrected) were determined on a Mel-TEMP II melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K, using TMS as an internal standard. MS spectra were recorded on a Shimadzu GC-MS 2050 (ESI) or an Agilent 1946A-MSD (ESI) Mass Spectrum. Column chromatography was performed with silica gel (200-300 mesh). Chemical shifts (d) are expressed in parts per million relative to tetramethylsilane, which was used as an internal standard, coupling constants (*J*) are in hertz (Hz), and the signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet.

*1.1.1 The preparation of 4-Nitrobenzonitrile (**2**)*

To dissolved 4-Nitrobenzaldehyde **1** (5 g, 33.11 mmol), hydroxylamine hydrochloride（4.24 g，66 mmol）in 50 ml DMSO. The mixture was heated to 100℃ for 0.5 h, The residue was poured into water and the resulting precipitate was recovered by filtration, and dried at infrared to give pale yellow solid **2** as crude(4 g, 82%). MS (ESI, m/z):149.1$[M+H]^{+}$.

*1.1.2 4-Nitrobenzimidamide hydrochloride (**3**)*

4-Nitrobenzonitrile **3** (1.8 g，12.1mmol),Sodium methanolate (0.07 g，1.3 mmol) were added into 20 ml Methanol and stirred for 5 h at room temperature. After that, ammonium chloride（0.65 g, 12.2 mmol）was added and stirred for 4 h at room temperature. The mixture was filtrated to collect the filtrate and then concentration. The residue was washed with diethyl ether to give pale yellow solid **3** (1.1 g, 97%). MS (ESI, m/z):187.3$[M+H]^{+}$.

*1.1.3 2-(4-nitrophenyl)pyrimidine-4,6-diol (**4**)*

A solution of Sodium methanolate in (5.1 g，102.6 mmol) in 30 ml dry methanol was dropped into the mixture of 4-Nitrobenzimidamide hydrochloride **3,** diethyl malonate dissolved in 70 ml dry methanol and then heated to 70℃ for 4h under N_2. After reaction completed, the reaction liquid was poured into 150 ml H_2O, and adjusted the pH to 4~5 with 6N HCl. The resulting precipitate was

recovered by filtration, and dried at infrared to give yellow solid **4** (9.5g, 87.4%). MS (ESI, m/z):234.3$[M+H]^+$.

*1.1.4 4,6-dichloro-2-(4-nitrophenyl)pyrimidine (**5**)*

To the suspension of 2-(4-nitrophenyl)pyrimidine-4,6-diol **4** (9.5g, 40.5 mmol) in $POCl_3$ 20 ml, was added a few drops of DMF, then the reaction mixture was stirred at 100℃ for 8 h. The reaction liquid was dropped slowly into 200mL ice water. The resulting precipitate was filtered and purify by flash chromatography to give yellow solid **5** (5.0 g, 46.3%). MS (ESI, m/z) 270.3$[M+H]^+$.

*1.1.5 4-(4,6-dichloropyrimidin-2-yl)aniline (**6**)*

A suspension of 4,6-dichloro-2-(4-nitrophenyl)pyrimidine **5** (2.8g, 10.37mmol), Fe (1.75 g, 31.1 mmol), NH_4Cl (1.69 g, 31.1 mmol) in30 ml CH_3OH: H_2O (1:1) was heated to reflux for 2h. The hot mixture was then filtered through a Celite pad, and the filtrate was evaporated under vacuum. The residue was dissolved in EtOAc and washed with H_2O, and the aqueous phase was further extracted with ethyl acetate (2×20 mL). The organic extracts were combined, dried over Na_2SO_4, filtered, and evaporated under vacuum to obtain yellow solid 6 (1.5, 60.7%). MS (ESI, m/z):240.2$[M+H]^+$.

*1.1.6 The preparation of N-(4-(4,6-dichloropyrimidin-2-yl)phenyl)acrylamide (**7**)*

To a solution of compound **6** in 5 ml dry DMF at 0℃was subsequently added triethylamine (0.64 ml, 4.58 mmol), then dropwise added the acyl chloride (0.38 ml,4.58 mmol) dissolved in 5 ml dry DMF. The solution was stirred for 1h at 0℃. The aqueous phase was further extracted with ethyl acetate (2×20 mL). The organic extracts were combined, dried over Na_2SO_4, filtered and concentrated, the residue was subjected to column purification to give yellow solid **7** (0.3 g, 56.5%). MS (ESI, m/z):294.1$[M+H]^+$.

*1.1.7 N,N-dimethyl-4-nitrobenzamide (**9a**)*

A suspension of 4-Nitrobenzoyl chloride **8** (0.5 g, 2.7 mmol), dimethylamine hydrochloride (0.33 g, 4.05 mmol) in dry THF was stirred for 1h at 0℃. After that, the mixture was then poured into water. The resulting precipitate was recovered by filtration and purified by column chromatography to give white solid **9a** (0.48 g, 91.5%). MS (ESI, m/z):195.2$[M+H]^+$.

*1.1.8 N,N-diethyl-4-nitrobenzamide (**9b**)*

Compound **9b** was synthesized from **8** and diethylamine hydrochloride following the similar procedure described above for the preparation of **9a**.（yield, 85.1%）. MS (ESI，m/z):223.2[M+H]$^+$.

*1.1.9 (4-nitrophenyl)(pyrrolidin-1-yl)methanone (**9c**)*

Compound **9c** was synthesized from **8** and tetrahydro pyrrole following the similar procedure described above for the preparation of **9a**. (yield, 45.2%). MS (ESI,m/z):221.2[M+H]$^+$.

*1.1.10 morpholino(4-nitrophenyl)methanone (**9d**)*

Compound **9d** was synthesized from **8** and morpholine following the similar procedure described above for the preparation of **9a**. (yield, 94.1%). MS (ESI, m/z):237.1[M+H]$^+$.

*1.1.11 N-(4-fluorophenyl)-4-nitrobenzamide (**9e**)*

Compound **9e** was synthesized from **8** and 4-Fluoroaniline following the similar procedure described above for the preparation of **9a**. (yield, 69.5%). MS (ESI, m/z):231.1[M+H]$^+$.

*1.1.12 N-(3,5-bis(trifluoromethyl)phenyl)-4-nitrobenzamide (**9f**)*

Compound **9f** was synthesized from **8** and 3,5-Bis(trifluoromethyl)aniline following the similar procedure described above for the preparation of **9a**. (yield, 91.8%. MS (ESI, m/z):379.2[M+H]$^+$.

*1.1.13 4-nitro-N-(m-tolyl)benzamide (**9g**)*

Compound **9g** was synthesized from 8 and m-Toluidine following the similar procedure described above for the preparation of **9a**. (yield, 96.2%). MS (ESI, m/z):257.3[M+H]$^+$.

*1.1.14 N-(4-methoxyphenyl)-4-nitrobenzamide (**9h**)*

Compound **9h** was synthesized from **8** and p-Anisidine following the similar procedure described above for the preparation of **9a**. (yield, 96.2%). MS (ESI, m/z):273.3[M+H]$^+$.

*1.1.15 The preparation of N-(2,5-dimethylphenyl)-4-nitrobenzamide (**9i**)*

Compound **9i** was synthesized from **8** and 2,5-Dimethylaniline following the similar procedure described above for the preparation of **9a**. (yield, 92.6%). MS (ESI, m/z):271.1[M+H]$^+$.

*1.1.16 4-amino-N,N-dimethylbenzamide (**10a**)*

A suspension of compound **9a** (0.5, 2.3mmol), Fe (0.38g, 6.9mmol), NH_4Cl (0.37g, 6.9mmol) in CH_3OH : H_2O (1:1) was heated to reflux for 1h. After that, the mixture was then poured into water. The resulting precipitate was recovered by filtration and purified by column chromatography to give white solid **10a** (0.34 g, 78.2%). MS (ESI, m/z):193.1[M+ H]$^+$.

*1.1.17 4-amino-N,N-diethylbenzamide (**10b**)*

Compound **10b** was synthesized from **9b** following the similar procedure described above for the preparation of **10a**. (yield, 78.2%). MS (ESI, m/z):193.1[M+ H]$^+$.

*1.1.18 (4-aminophenyl)(pyrrolidin-1-yl)methanone (**10c**)*

Compound **10c** was synthesized from **9c** following the similar procedure described above for the preparation of **10a**. (yield, 89.4%). MS (ESI,m/z):191.1[M+H]$^+$.

*1.1.19 (4-aminophenyl)(morpholino)methanone (**10d**)*

Compound **10d** was synthesized from **9d** following the similar procedure described above for the preparation of **10a**. (yield, 78.7%). MS (ESI, m/z):207.3[M+H]$^+$.

*1.1.20 4-amino-N-(4-fluorophenyl)benzamide (**10e**)*

Compound **10e** was synthesized from **9e** following the similar procedure described above for the preparation of **10a**. (yield, 87.6%). MS (ESI, m/z):206.3[M+H]$^+$.

*1.1.21 4-amino-N-(3,5-bis(trifluoromethyl)phenyl)benzamide (**10f**)*

Compound **10f** was synthesized from **9f** following the similar procedure described above for the preparation of **10a**. (yield, 84.0%). MS (ESI, m/z):349.1[M+ H]$^+$.

*1.1.22 4-amino-N-(m-tolyl)benzamide (**10g**)*

Compound **10g** was synthesized from **9g** following the similar procedure described above for the preparation of **10a**. (yield, 60.8%). MS (ESI, m/z):227.2[M+H]$^+$.

*1.1.23 4-amino-N-(4-methoxyphenyl)benzamide (**10h**)*

Compound **10h** was synthesized from **9h** following the similar procedure described above for the preparation of **10a**. (yield, 71.2%). MS (ESI, m/z):243.2[M+H]$^+$.

*1.1.24 4-amino-N-(2,5-dimethylphenyl)benzamide (**10i**)*

Compound **10i** was synthesized from **9i** following the similar procedure described above for the preparation of **10a**. (yield, 68.0%). MS (ESI, m/z):241.3[M+ H]$^+$.

*1.1.25 4-((2-(4-acrylamidophenyl)-6-chloropyrimidin-4-yl)amino)-N,N-dimethylbenzamide (**11a**)*

A suspension of compound **7** (0.2 g, 0.68 mmol), compound **10a** (0.08 g, 0.45 mmol), K_2CO_3 (0.25 g, 1.8 mmol), $Pd(Ph_3P)_2Cl_2$ (0.03g, 0.045mmol) in 10 ml dry 1,4-dioxane was heated to reflux for 10 h. After that, the mixture was filtrated to collect the filtrate, The organic phase was further extracted with ethyl acetate (3×20 mL). The organic extracts were combined, dried over Na_2SO_4, filtered and concentrated, the residue was subjected to column purification to give white solid **11a** (70 mg, 34%). mp:238-240℃.^{1}H NMR (300 MHz, d_6-DMSO) δ 10.44 (s, 1 H), 10.09 (s, 1 H), 8.29 (d, J = 8.8 Hz, 2 H), 7.83 (t, J = 9.0 Hz, 4 H), 7.50 (d, J = 8.5 Hz, 2 H), 6.76 (s, 1 H), 6.49 (dd, J = 17.1, 10.2 Hz, 1 H), 6.31 (d, J = 17.0 Hz, 1 H), 5.81 (d, J = 9.7 Hz, 1 H), 2.99 (s, 6 H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 169.84, 163.38, 163.30, 161.15, 158.41, 141.85, 140.31, 131.64, 131.17, 130.32, 128.81, 128.31, 127.46, 119.11, 119.02, 103.15, 22.07. HRMS（ESI）m/z calcd for $C_{22}H_{20}ClN_5O_2$ [M+H]$^+$ 422.1392, found 422.1387.

*1.1.26 4-((2-(4-acrylamidophenyl)-6-chloropyrimidin-4-yl)amino)-N,N-diethylbenzamide (**11b**)*

Compound **11b** was synthesized from **7** and **10b** following the similar procedure described above for the preparation of **11a**. (yield, 20.5%). mp:158-161℃.^{1}H NMR (300 MHz, d_6-DMSO) δ 10.71 (s, 1 H), 10.53 (s, 1 H), 8.27 (d, J = 8.7 Hz, 2 H), 7.98 – 7.79 (m, 4 H), 7.42 (d, J = 8.6 Hz, 2 H), 6.90 (s, 1 H), 6.58 (dd, J = 16.9, 10.2 Hz, 1 H), 6.29 (d, J = 17.1 Hz, 1 H), 5.79 (d, J = 13.6 Hz, 1 H), 1.20 (d, J = 16.6 Hz, 4 H), 1.13 (d, J = 6.7 Hz, 6 H) . ^{13}C NMR (75 MHz, d_6-DMSO) δ 169.76, 163.38, 161.18, 158.39, 141.84, 139.96, 131.66, 131.36, 131.17, 128.81, 127.45, 127.29, 119.37, 119.01, 103.06, 30.32, 28.97. HRMS（ESI）m/z calcd for $C_{24}H_{24}ClN_5O_2$ $[M+H]^+$ 450.1703, found 450.1697.

*1.1.27 N-(4-(4-chloro-6-((4-(pyrrolidine-1-carbonyl)phenyl)amino)pyrimidin-2-yl)phenyl) acrylamide (**11c**)*

Compound **11c** was synthesized from **7** and **10c** following the similar procedure described above for the preparation of **11a**. (yield, 32.3 %). mp:234-236℃.^{1}H NMR (400 MHz, d_6-DMSO) δ 10.45 (s, 1 H), 10.11 (s, 1 H), 8.29 (d, J = 8.8 Hz, 2 H), 7.90 – 7.78 (m, 4 H), 7.63 (d, J = 8.6 Hz, 2 H), 6.81 – 6.72 (m, 1 H), 6.49 (dd, J = 24.0, 12.0 Hz, 1 H), 6.31(d, J = 23.1, 1 H), 5.80 (d, J = 12.31 H), 3.46 (q, J = 24.1 Hz, 4 H), 1.85 (q, J = 4.8 Hz, 4 H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 167.83, 163.40, 163.33, 161.16, 158.44, 141.85, 140.64, 131.68, 131.21, 131.07, 128.79, 128.35, 127.34, 119.02, 103.17, 49.00, 46.04, 26.04, 23.88. HRMS（ESI）m/z calcd for $C_{24}H_{22}ClN_5O_2$ $[M+H]^+$ 448.1551, found 448.1544.

*1.1.28 N-(4-(4-chloro-6-((4-(morpholine-4-carbonyl)phenyl)amino)pyrimidin-2-yl)phenyl) acrylamide (**11d**)*

Compound **11d** was synthesized from **7** and **10d** following the similar procedure described above for the preparation of **11**a. (yield, 23.5%). mp:215-217℃.^{1}H NMR (400 MHz, d_6-DMSO) δ 10.69 (s, 1 H), 10.51 (s, 1 H), 8.27 (t, J = 8.7 Hz, 2 H), 7.89 (dd, J = 8.6, 3.7 Hz, 4 H), 7.50 (d, J = 8.6 Hz, 2 H), 6.90 (s, 1 H), 6.58 (dd, J = 17.0, 10.2 Hz, 1 H), 6.29 (d, J = 17.0,1 H), 5.79 (d, J = 11.4, 1 H), 3.59 (t, 4 H), 3.50 (t, 4 H).^{13}C NMR (101 MHz, d_6-DMSO) δ 169.47, 163.97, 163.82, 161.72, 158.80, 142.43, 141.28, 132.28, 131.70, 129.71, 129.24, 128.87, 127.74, 119.67, 119.56, 104.12, 66.62. HRMS（ESI）m/z calcd for $C_{24}H_{22}ClN_5O_3$ $[M+H]^+$ 464.1494, found 464.1486.

1.1.29 4-((2-(4-acrylamidophenyl)-6-chloropyrimidin-4-yl)amino)-N-(4-fluorophenyl)benzamide (**11e**)

Compound **11e** was synthesized from **7** and **10e** following the similar procedure described above for the preparation of **11a.** (yield, 22.6%). mp:199-201℃.^{1}H NMR (400 MHz, d_6-DMSO) δ 10.68 (s, 1 H), 10.58 (s, 1 H), 10.27 (s, 1 H), 8.31 (d, *J* = 8.7 Hz, 2 H), 8.08 (d, *J* = 8.7 Hz, 2 H), 7.98 (d, *J* = 8.7 Hz, 2 H), 7.90 (d, *J* = 8.8 Hz, 2 H), 7.83 (d, *J* = 5.2 Hz, 2 H),7.20 (d, *J* = 8.9 Hz, 2 H), 6.92 (s, 1 H), 6.57 (dd, *J* = 16.9, 10.1 Hz, 1 H), 6.31 (d, *J* = 16.9 Hz, 1 H), 5.80 (d, *J* = 11.9 Hz, 1 H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 164.79, 163.51, 163.31, 161.18, 158.35, 142.62, 142.04, 131.81, 131.14, 128.76, 128.72, 128.16, 127.22, 122.14, 122.06, 119.05, 118.81, 115.16, 114.95, 103.68. HRMS（ESI）m/z calcd for $C_{26}H_{19}ClFN_5O_2$[M+H]$^+$ 488.1295, found 488.1288.

*1.1.30 4-((2-(4-acrylamidophenyl)-6-chloropyrimidin-4-yl)amino)-N-(3,5-bis(trifluoromethyl) phenyl)benzamide (**11f**)*

Compound **11f** was synthesized from **7** and **10f** following the similar procedure described above for the preparation of **11a**. (yield, 24.7%). mp:221-223℃.^{1}H NMR (300 MHz, d_6-DMSO) δ 10.66 (s, 1 H), 10.42 (s, 1 H), 10.19 (s, 1 H), 8.53 (s, 2 H), 8.26 (d, *J* = 8.5 Hz, 2 H), 8.06 (d, *J* = 8.4 Hz, 2 H), 7.98 – 7.86 (m, 2 H), 7.83 (d, *J* = 8.5 Hz, 2 H), 7.73 (s, 1 H), 6.73 (s, 1 H), 6.47 (dd, *J* = 16.7, 9.9 Hz, 1 H), 6.29 (d, *J* = 16.7 Hz, 1 H), 5.78 (d, *J* = 10.2 Hz, 1 H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 165.36, 163.37, 163.27, 160.97, 158.52, 143.04, 141.90, 141.28, 131.65, 131.10, 130.74, 130.31, 128.97, 128.75, 127.34, 127.07, 125.09, 121.48, 119.59, 118.94, 118.78, 103.66.HRMS（ESI）m/z calcd for $C_{28}H_{18}ClF_6N_5O_2$ [M+H]$^+$ 606.1136, found 606.1133.

*1.1.31 4-((2-(4-acrylamidophenyl)-6-chloropyrimidin-4-yl)amino)-N-(m-tolyl)benzamide (**11g**)*

Compound **11g** was synthesized from **7** and **10f** following the similar procedure described above for the preparation of **11a.** (yield, 23.8%). mp:143-145℃.^{1}H NMR (300 MHz, d_6-DMSO) δ 10.44 (s, 1 H), 10.17 (s, 1 H), 10.08 (s, 1 H), 8.30 (d, *J* = 8.6 Hz, 2 H), 8.06 (d, *J* = 8.5 Hz, 2 H), 7.96 – 7.88 (m, 2 H), 7.85 (d, *J* = 8.6 Hz, 2 H), 7.64 (s, 1 H), 7.59 (d, *J* = 7.7 Hz, 1 H), 7.21 (t, *J* =

7.8 Hz, 1 H), 6.89 (d, *J* = 7.4 Hz, 1 H), 6.76 (s, 1 H), 6.49 (dd, *J* = 16.9, 10.0 Hz, 1 H), 6.30 (d, *J* = 16.1 Hz, 1 H), 5.79 (d, *J* = 11.1 Hz, 1 H), 2.30 (s, 3 H). ^{13}C NMR (75MHz,d_6-DMSO) δ 165.27, 163.91, 163.83, 161.58, 159.03, 142.82, 142.41, 139.75, 138.14, 132.17, 131.69, 129.29, 129.12, 128.85, 127.89, 124.60, 121.32, 119.52, 119.39, 117.96, 104.01, 40.81, 40.52, 40.24, 39.97, 39.69, 39.41, 39.14, 21.71. HRMS（ESI）m/z calcd for $C_{27}H_{22}ClN_5O_2$ $[M+H]^+$ 484.1544, found 484.1539.

*1.1.32 4-((2-(4-acrylamidophenyl)-6-chloropyrimidin-4-yl)amino)-N-(4-methoxyphenyl) benzamide (**11h**)*

Compound **11h** was synthesized from **7** and **10h** following the similar procedure described above for the preparation of **11a.** (yield, 22.0%). mp:191-193℃.^{1}H NMR (400 MHz, d_6-DMSO) δ 10.57 (s, 1 H), 10.40 (s, 1 H), 10.07 (s, 1 H), 8.31 (d, *J* = 8.8 Hz, 2 H), 8.06 (d, *J* = 8.7 Hz, 2 H), 7.95 (d, *J* = 8.7 Hz, 2 H), 7.88 (d, *J* = 8.8 Hz, 2 H), 7.71 (d, *J* = 9.0 Hz, 2 H), 6.94 (d, *J* = 9.1 Hz, 2 H), 6.86 (s, 1 H), 6.57 – 6.47 (dd,1H), 6.30 (d,1 H), 5.81 (d, 1 H), 3.76(s, 3 H).^{13}C NMR(101 MHz,d_6-DMSO) δ 164.96, 163.97, 163.85, 161.63, 159.01, 155.93, 142.72, 142.41, 132.87, 132.19, 131.72, 129.88, 129.27, 129.15, 127.87, 122.43, 119.57, 119.43, 114.19, 103.99, 55.66. HRMS（ESI）m/z calcd for $C_{27}H_{22}ClN_5O_3$ $[M+H]^+$ 500.1494, found 500.1487.

*1.1.33 4-((2-(4-acrylamidophenyl)-6-chloropyrimidin-4-yl)amino)-N-(2,5-dimethylphenyl) Benzamide (**11i**)*

Compound **11i** was synthesized from **7** and **10i** following the similar procedure described above for the preparation of **11a.** (yield, 27.1%). mp:141-143℃.^{1}H NMR (400 MHz, d_6-DMSO) δ 10.53 (s, 1 H), 10.31 (s, 1 H), 9.77 (s, 1 H), 8.33 (d, *J* = 8.8 Hz, 2 H), 8.10 (d, *J* = 8.7 Hz, 2 H), 7.96 (d, *J* = 8.6 Hz, 2 H), 7.90 (d, *J* = 8.7 Hz, 2 H), 7.21 (s, 1 H), 7.16 (d, *J* = 7.7 Hz, 1 H), 6.99 (d, *J* = 7.5 Hz, 1 H), 6.84 (s, 1 H), 6.54 (dd, *J* = 16.9, 10.2 Hz, 1 H), 6.34 (d, *J* = 16.9 HZ,.1 H), 5.82 (d, *J* = 10.2 Hz, 1 H), 2.30 (s, 3 H), 2.23 (s, 3 H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 164.71, 163.42, 163.34, 161.12, 160.23, 158.50, 142.31, 141.93, 136.36, 134.92, 131.70, 131.19, 130.42, 130.04, 128.80, 128.74, 128.33, 127.35, 127.04, 126.42, 119.04, 118.97, 40.09, 39.88, 39.67, 39.46, 39.25, 39.04, 38.84, 20.50, 17.51. HRMS（ESI）m/z calcd for $C_{28}H_{24}ClN_5O_2$ $[M+H]^+$ 498.1698, found 498.1695.

1.2 Biological assay method

1.2.1 Cell culture

The human cell lines Raji, HL60, Ramos cells were obtained from Chinese academy of sciences cell bank. Cancer cell lines were maintained as a monolayer culture in PRAM1640 or IMDM (Keygentech, CN), supplemented with 10% FBS (Gibco) in a humidified atmosphere (5% CO_2) at 37°C.

1.2.2 Antiproliferative assays

Cellular chemosensitivity was determined by using a modified MTT method assay in vitro. In brief, Raji and HL60 cells in 200 ml culture medium were seeded into 96-well microplates at 3000-5000 cells per well respectively and cultured in PRAM1640 or IMDM with 10% FBS, incubated at 37 °C for 12-24 h prior to drug exposure. Cell numbers were titrated to keep control cells growing in the exponential phase throughout the 48 h incubation period. Cells were treated with final concentrations of 40, 20, 10, 5 and 1μM of tested compounds simultaneously and incubated for 48 h and then 20 ml of MTT solution (5 mg/ml in medium) was added to each well and incubated for 4 h. The formed blue formazan crystals were pelleted to the bottom of the well by tablet centrifugation, separated from the supernatant, and dissolved in 150 ml of DMSO. The optical density at 490 nm was determined by Varioskan Flash Multimode Reader. The IC_{50} value, that is, the concentration (μM) of a compound was able to cause 50% cell death with respect to the control culture, was calculated according to the inhibition ratios.

1.2.3 In vitro kinase enzymatic assay

The BTK kinase enzyme assay system (Catalog: V9071, Promega). Concentrations consisting of suitable 100 nM were used for all of the tested compounds. The experiments were performed according to the instructions of the manufacturer. The more detailed and complete protocols can be seen the ADP-Glo™ kinase Assay Technica Manual availableat:http://cn.promega.com/resources/protocols/product-inf-ormationsheets/n/btk-kinase-enzyme-system-protocol/. The test of Btk was performed in a 384-well plate, through the steps below:

(1) perform a 5 mL kinase reaction using 1× kinase buffer (e.g., 1× reaction buffer A), (2) incubate at room temperature for 60 min, (3) add 5 mL of ADP-Glo™ Reagent to stop the kinase reaction and deplete the unconsumed ATP, leaving only ADP and a very low background of ATP, (4) incubate at room temperature for 40 min, (5) add 10 mL of Kinase Detection, (6) reagent to convert ADP to ATP and introduce luciferase and luciferin to detect ATP, (7) incubate at room temperature for 30 min, (8) plate was measured on POLARstar Omega to detect the luminescence (Integration time 0.5-1s).

1.2.4 Western Blot

Cells with different treatments for 24h were washed twice with PBS, then collected and lysed in lysis buffer (100 mM of Tris–Cl, pH 6.8, 4% (m/v) SDS, 20% (v/v) glycerol, 200 mM of β-mercaptoethanol, 1mM of PMSF, 0.1 mM NaF and DTT) for 0.5 h on the ice. The lysates were then subjected to centrifugation (13,000 rpm) at 4 °C for 20 min. Protein concentration in the supernatants was detected by BCA protein assay (Thermo, Waltham, MA). Then equal amount of protein was separated with 12% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA) using a semi-dry transfer system (Bio-rad, Hercules, CA). Proteins were detected using specific antibodies overnight at 4 °C followed by HRP-conjugated secondary antibodies for 1 h at 37 °C. All of the antibodies were diluted in PBST containing 1% BSA. Enhanced chemi-luminescent reagents (Beyotime, Jiangsu, China) were used to detect the HRP on the immunoblots, and the visualized bands were captured by film. The bands were quantified by Quantity One software (Vision 4.62, Bio-rad, Hercules, CA), and the relative protein level were normalized to β-actin.

1.2.5 Flow Cytometric Analysis.

The Ramos cells were treated with 5 μM **11g,** DMSO and Ibrutinib for 48 h. After treatment, the cells were washed twice with ice-cold PBS, collected by centrifugation, and fixed in ice-cold 70% (v/v) ethanol, washed with PBS, re-suspended with 0.1 mg/mL RNase, stained with 40 mg/mL PI, and analyzed by flow cytometry using FACScalibur (Becton Dickinson). The cell cycle distributions were calculated using Flowjo 7.6.1 software.

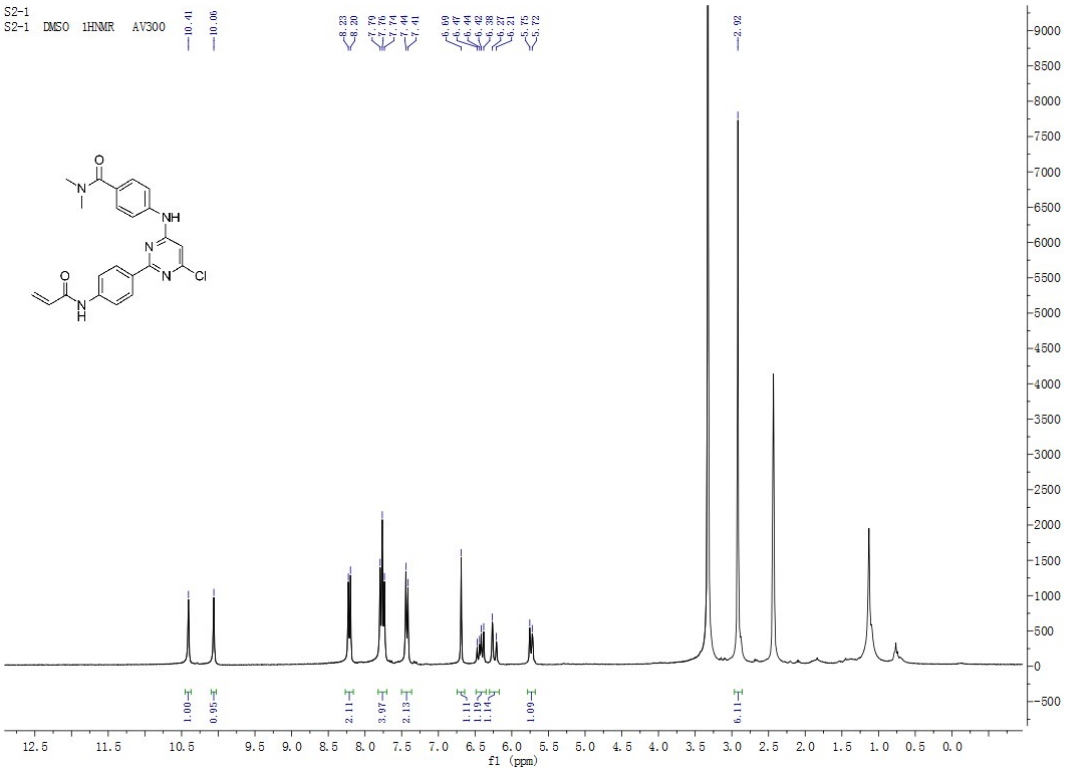
1.2.6 Docking study

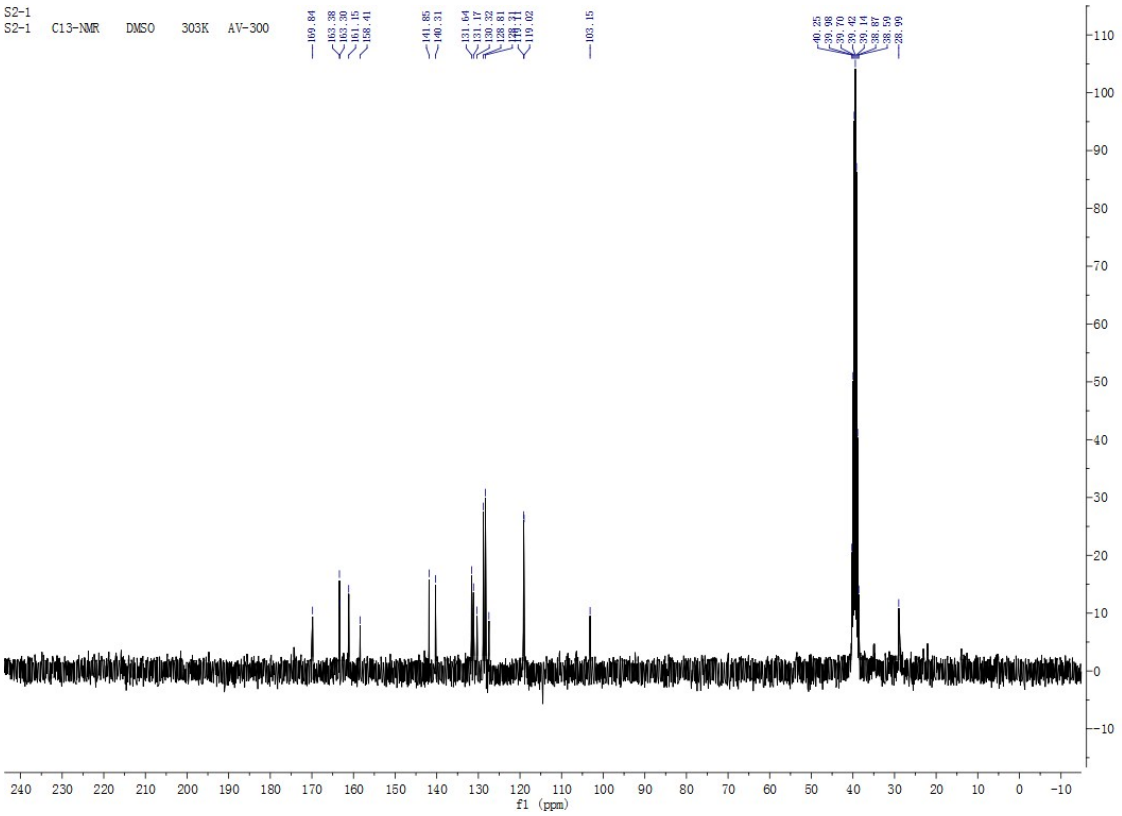
The docking study was used with the CDOCKER model of Discovery Studio 3.0. The general step

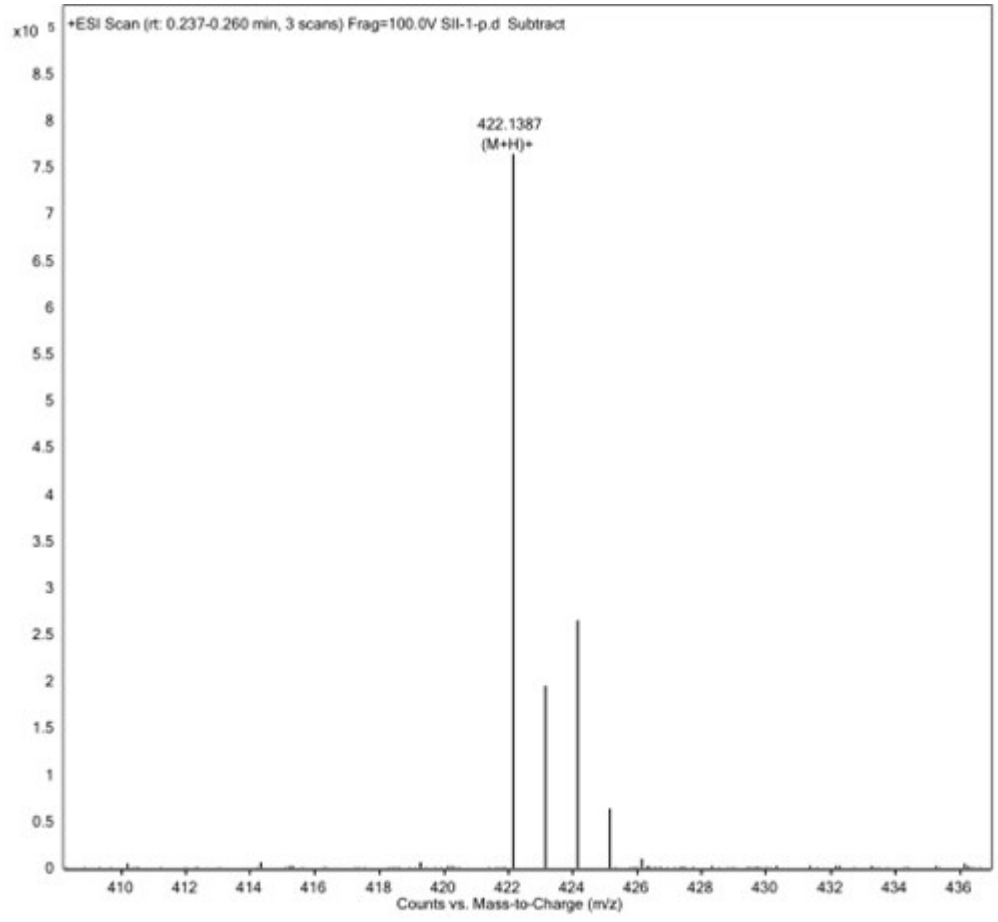
was step by step according to the course of Discovery Studio software. And the protein surface was simulated with surface model in structure of Discovery Studio 3.0. At last, the docking results were presented with Discovery Studio visualization.

3 Spectral data

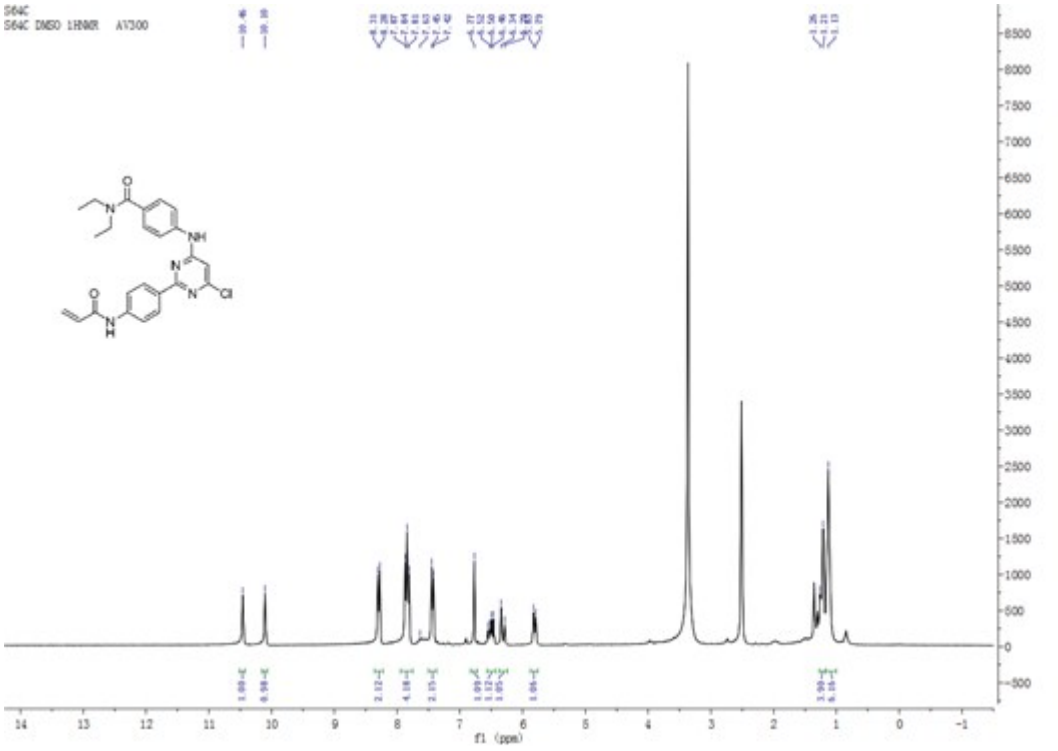
11a

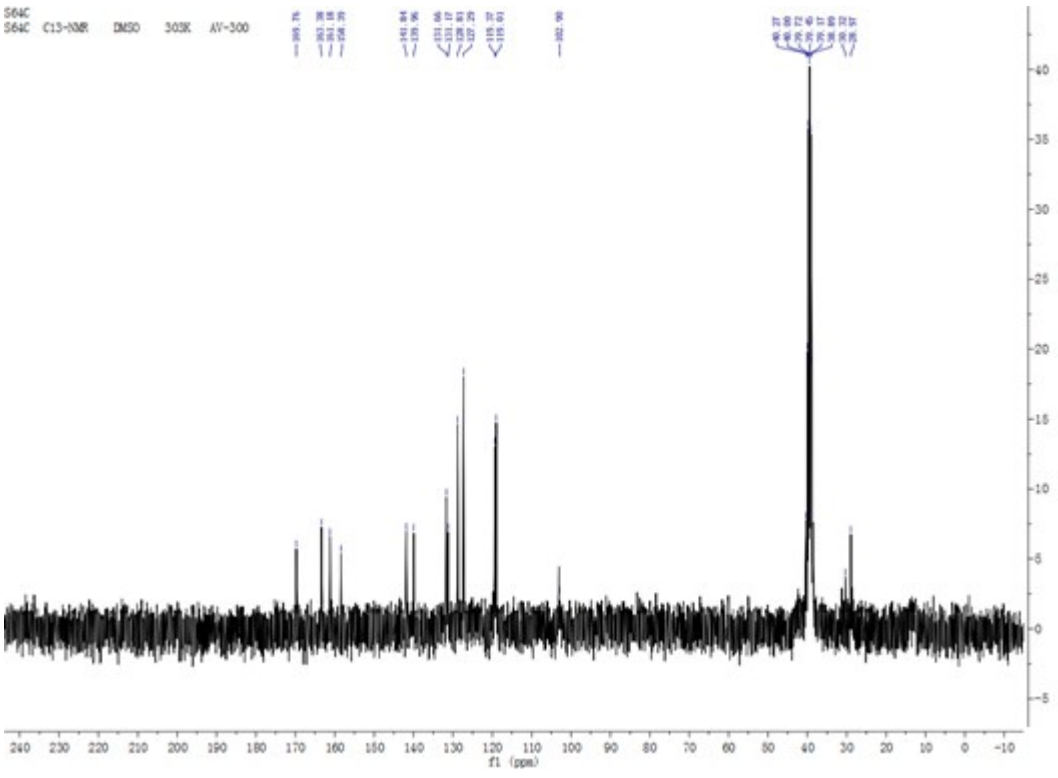


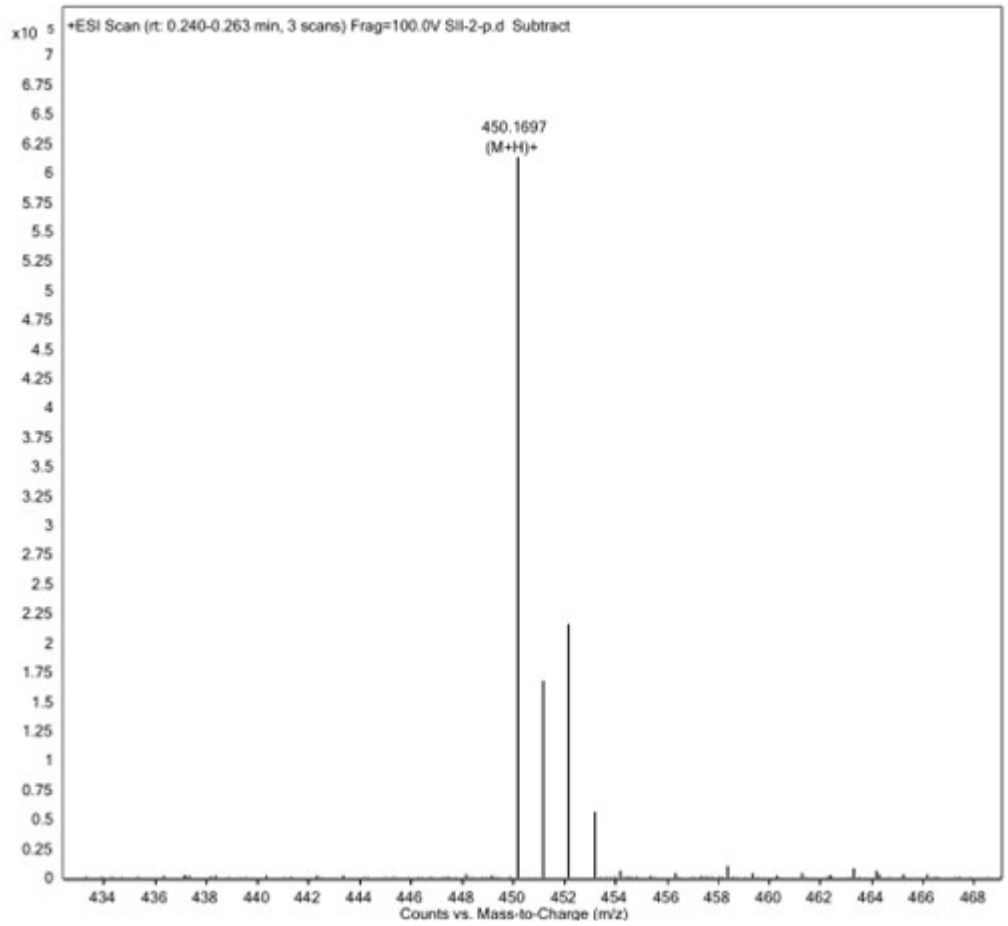

S2-1
S2-1 C13-NMR DMSO 303K AV-300
169.84
163.38
163.39
161.15
158.41
141.85
140.31
131.64
131.17
130.32
128.81
119.02
103.15
40.25
39.98
39.70
39.42
39.14
38.87
38.59
28.99
f1 (ppm)


x10 5
+ESI Scan (rt: 0.237-0.260 min, 3 scans) Frag=100.0V SII-1-p.d Subtract
422.1387
(M+H)+
Counts vs. Mass-to-Charge (m/z)

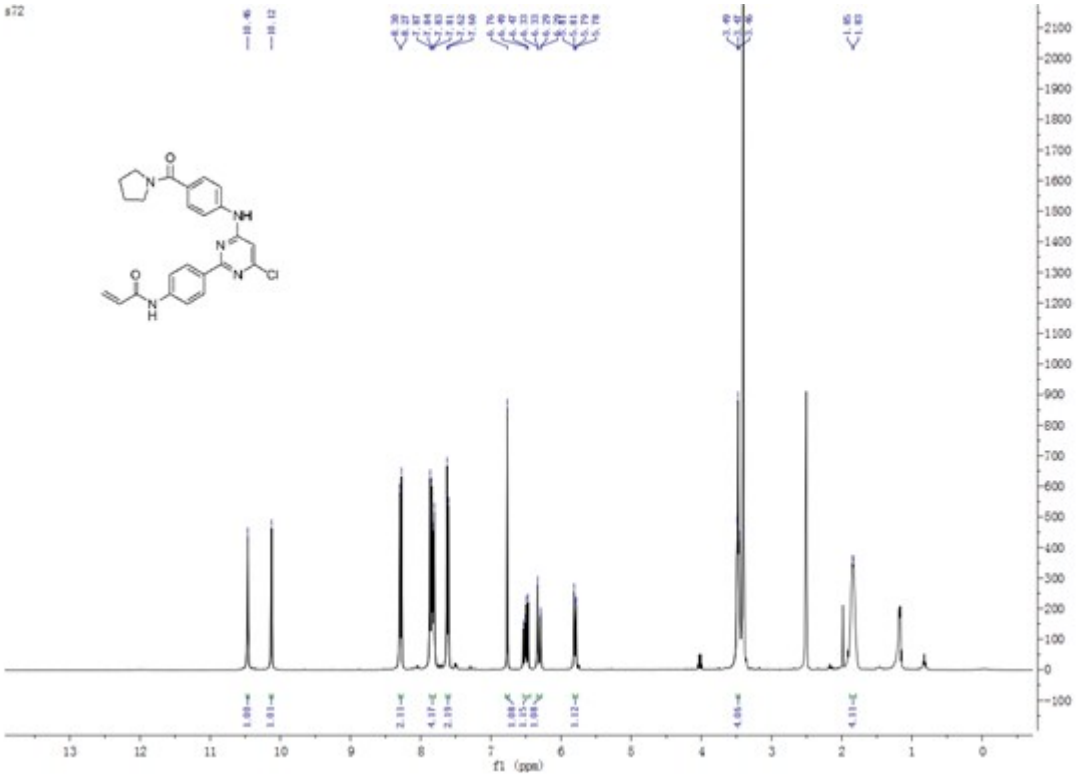
11b

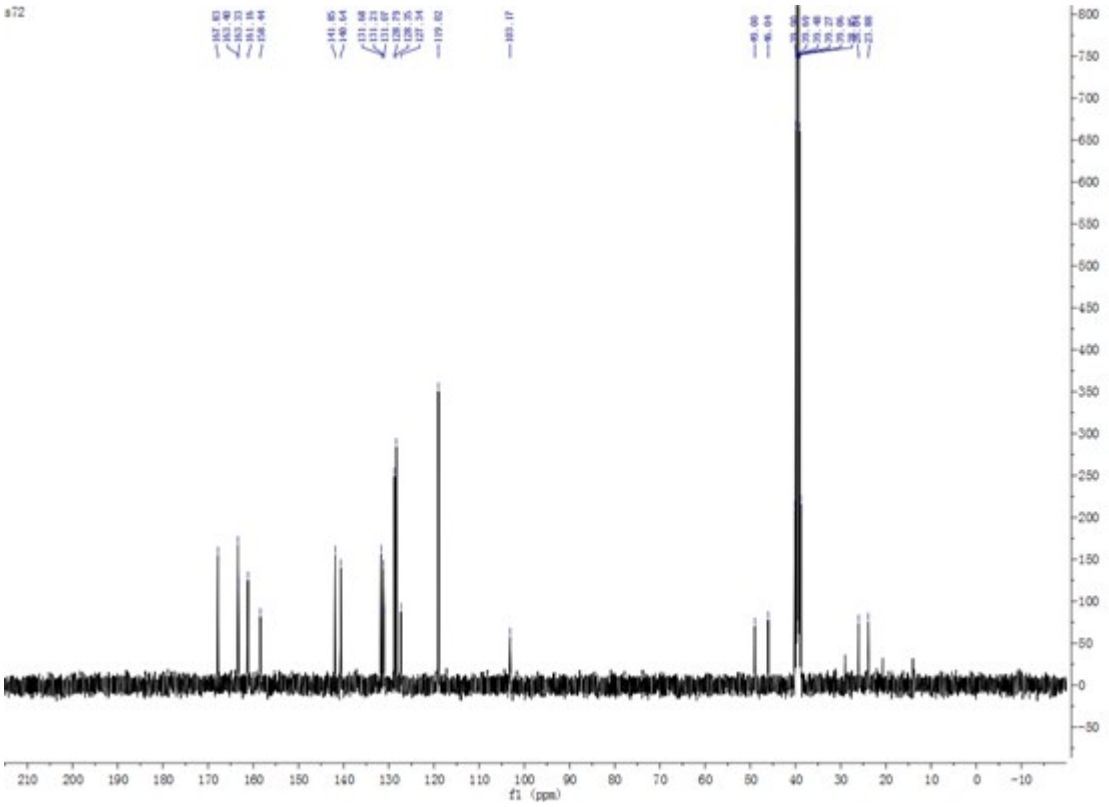

S64C
S64C DMSO 1HNMR AV300
f1 (ppm)

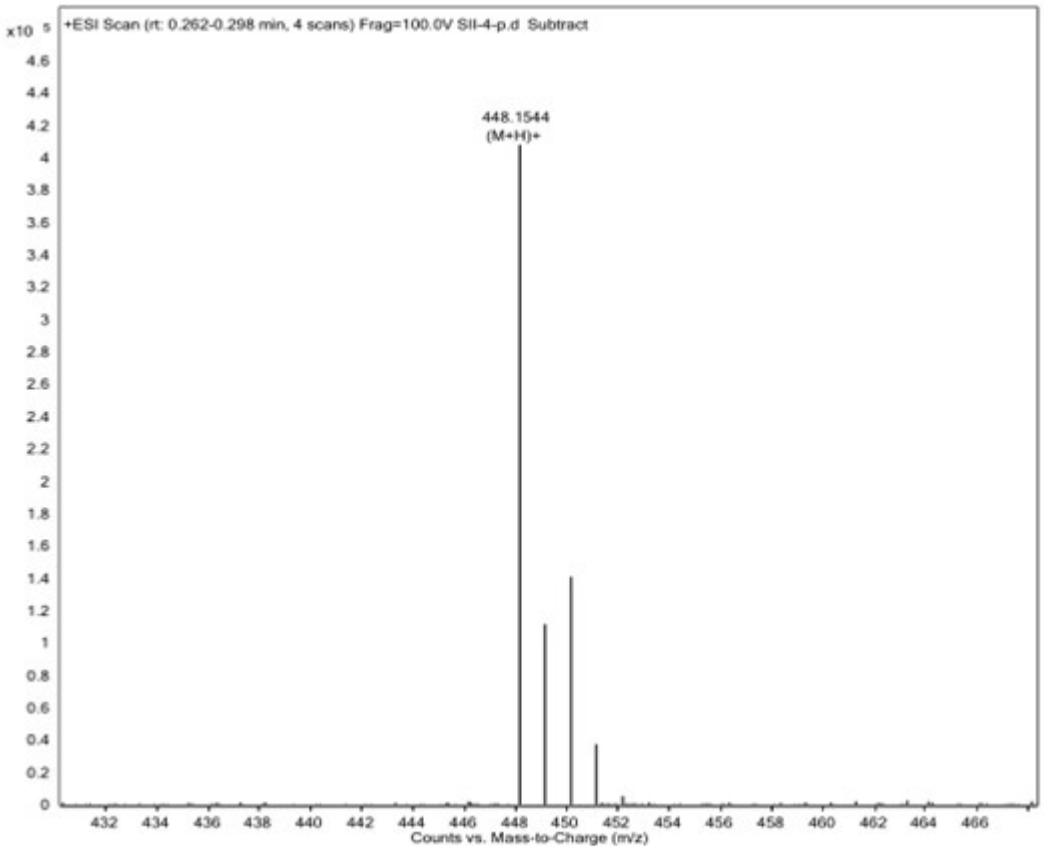

S64C
S64C C13-NMR DMSO 303K AV-300
f1 (ppm)


+ESI Scan (rt: 0.240-0.263 min, 3 scans) Frag=100.0V SII-2-p.d Subtract
x10 5
450.1697
(M+H)+
Counts vs. Mass-to-Charge (m/z)

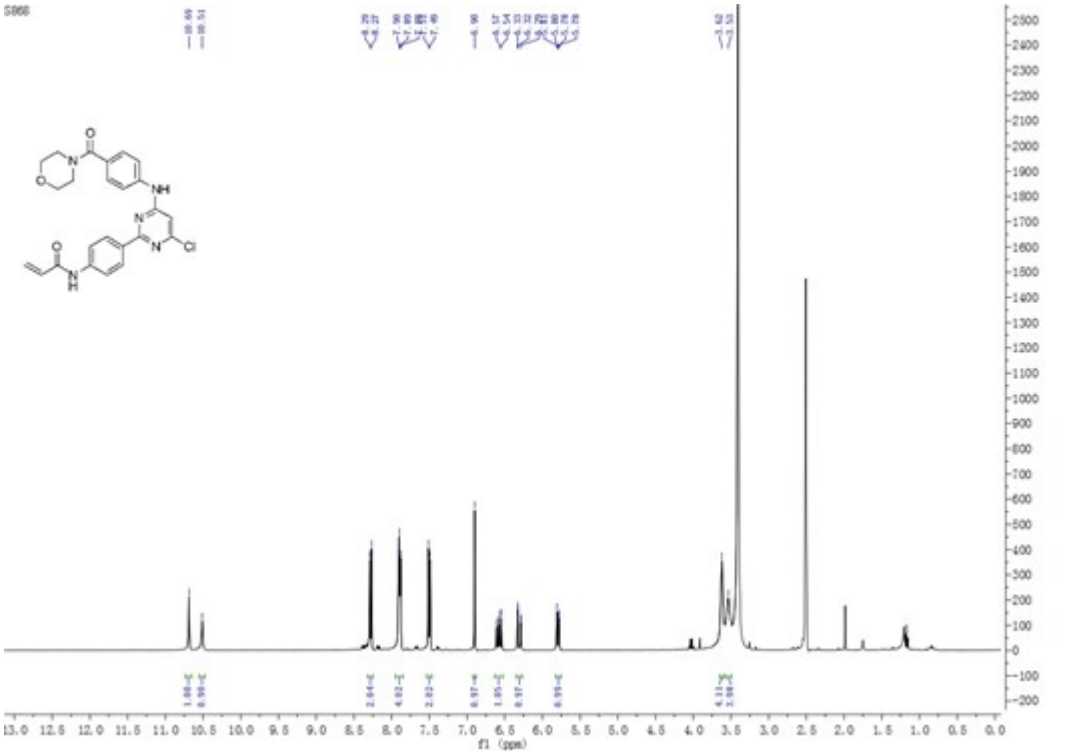
11c

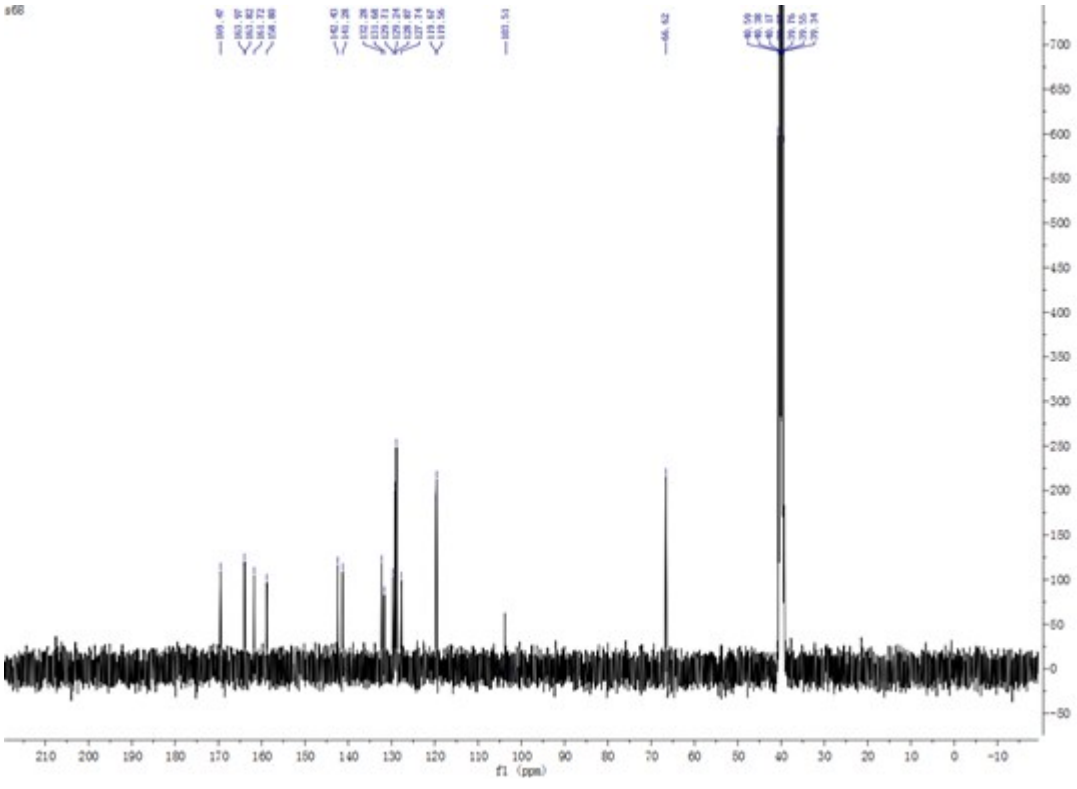

s72
f1 (ppm)

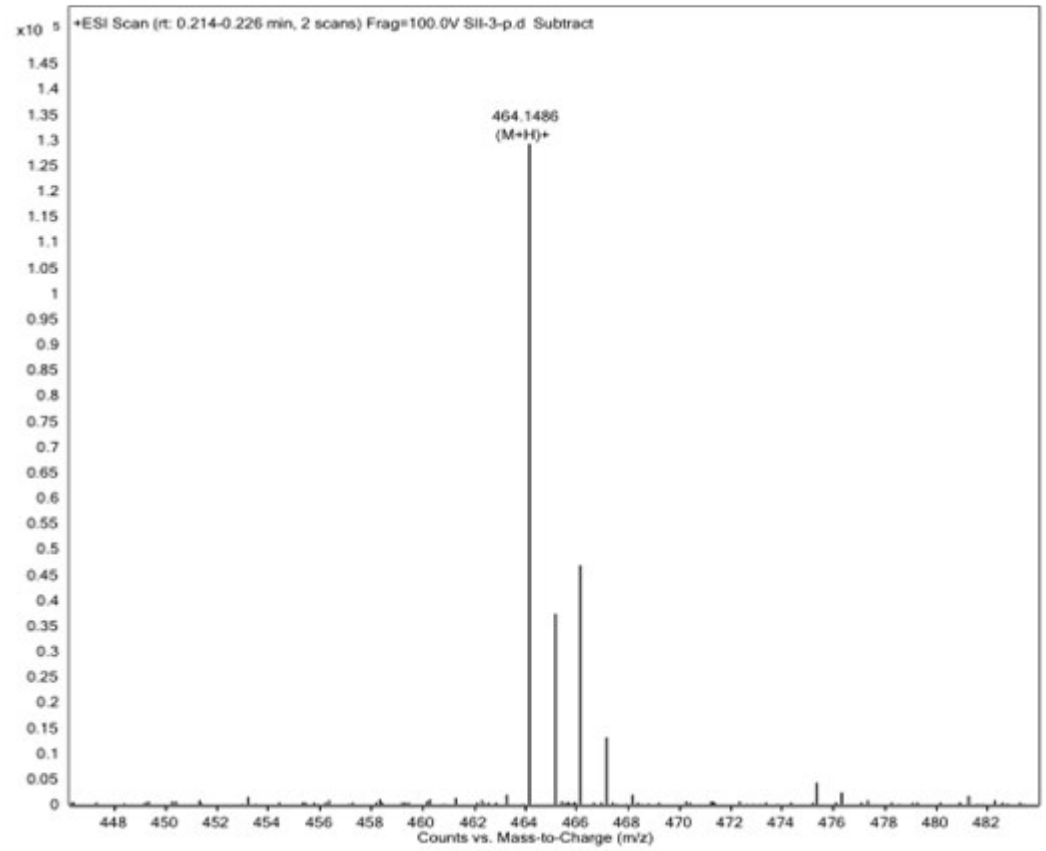

s72
f1 (ppm)


+ESI Scan (rt: 0.262-0.298 min, 4 scans) Frag=100.0V SII-4-p.d Subtract
x10 5
448.1544
(M+H)+
Counts vs. Mass-to-Charge (m/z)

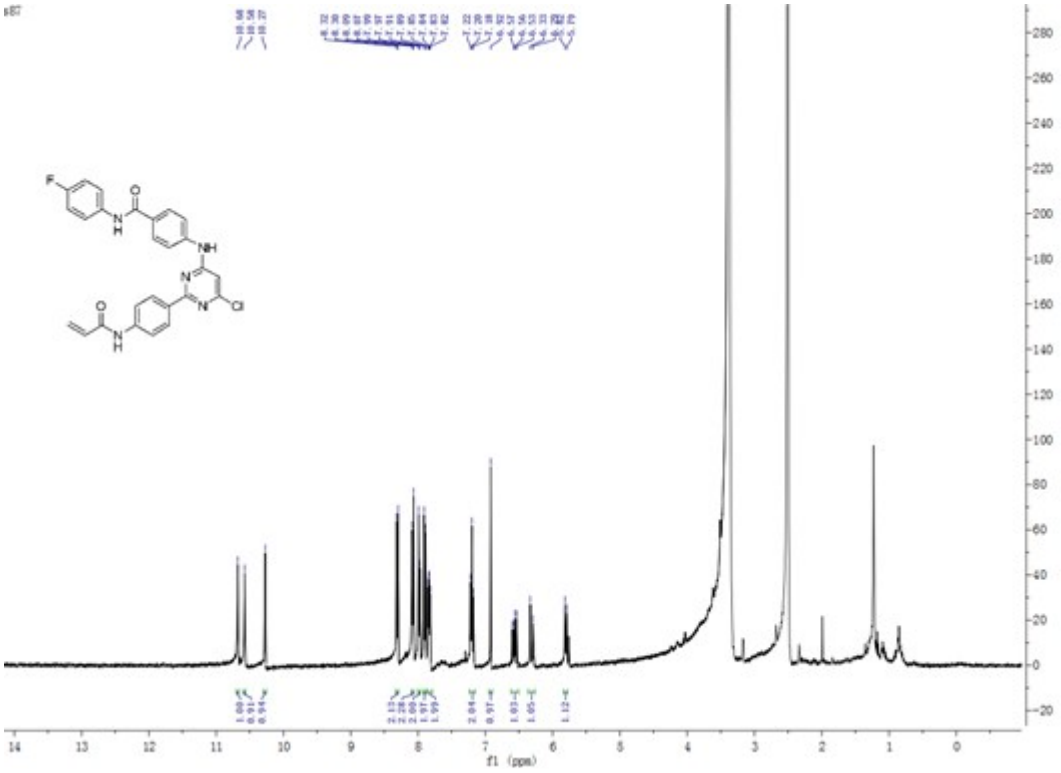
11d

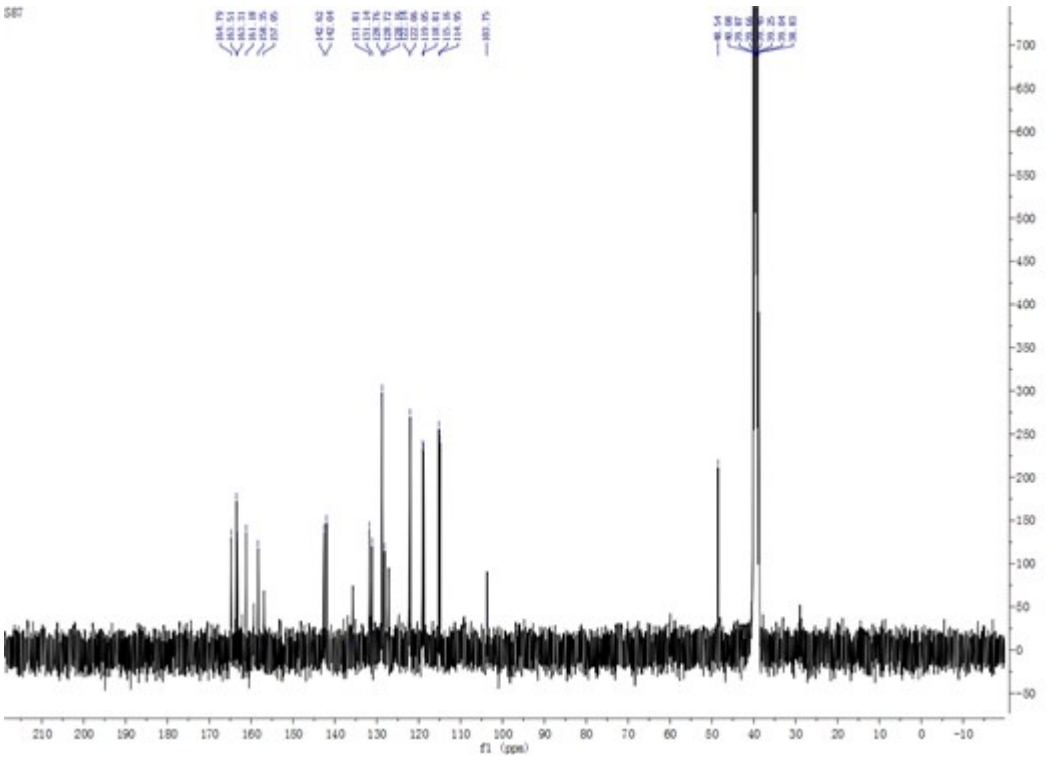

S868
f1 (ppm)

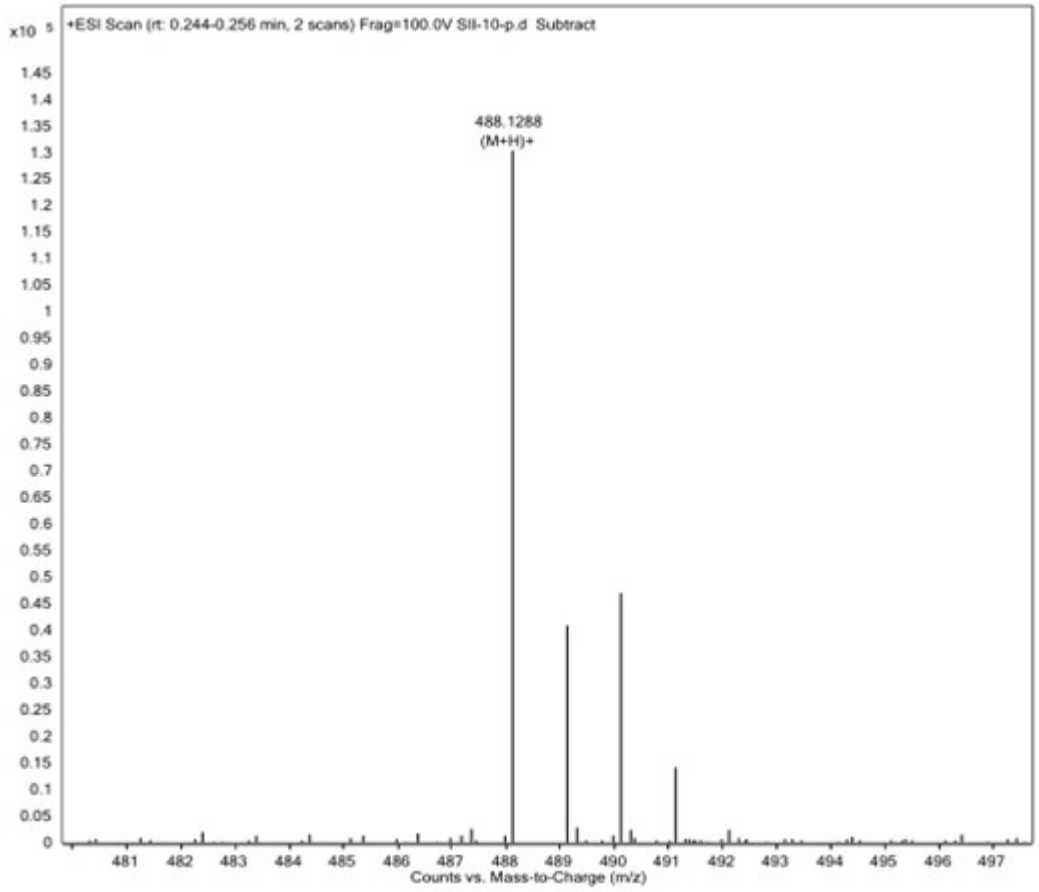

s68
f1 (ppm)


+ESI Scan (rt: 0.214-0.226 min, 2 scans) Frag=100.0V SII-3-p.d Subtract
x10 5
464.1486
(M+H)+
Counts vs. Mass-to-Charge (m/z)

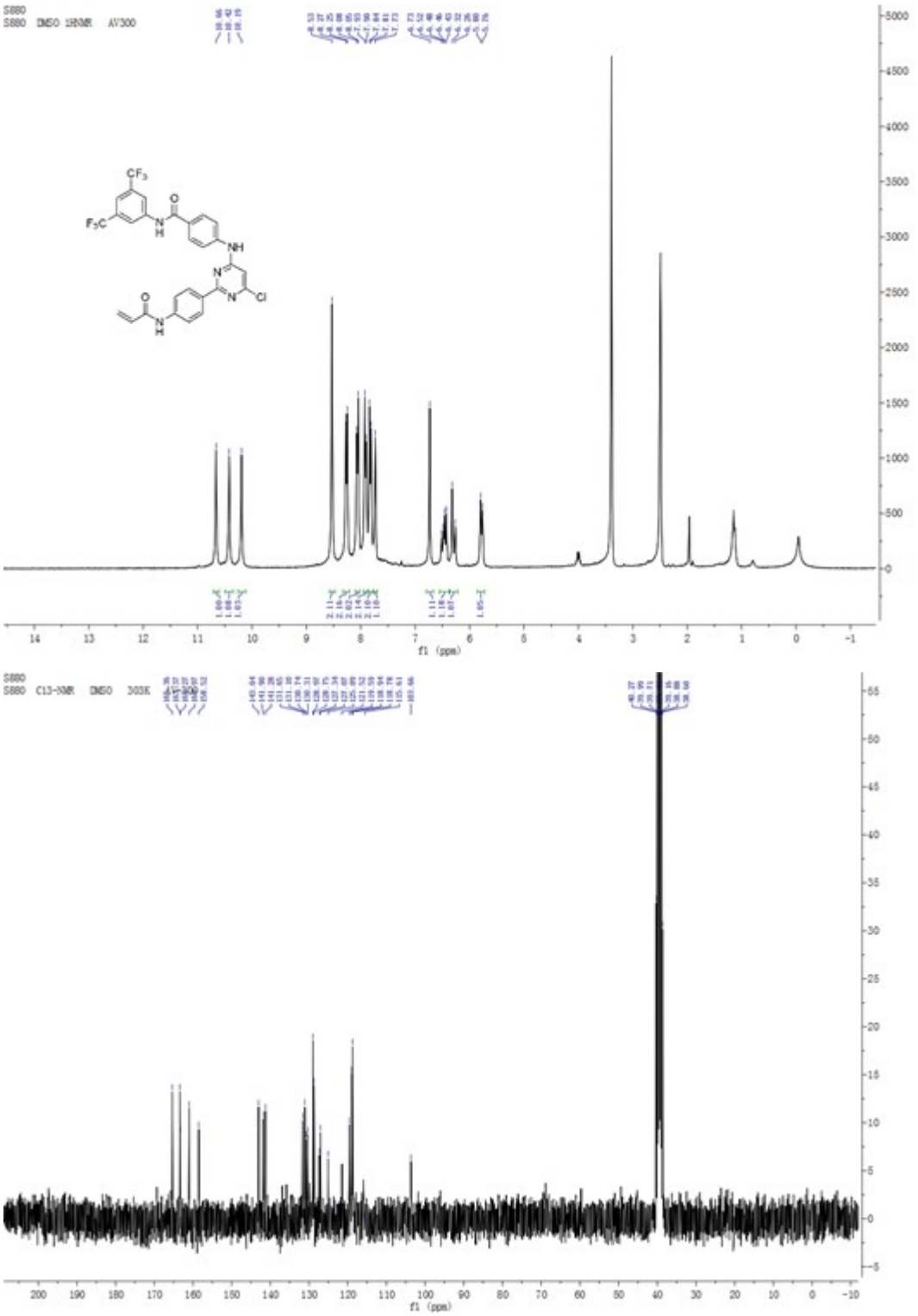
11e

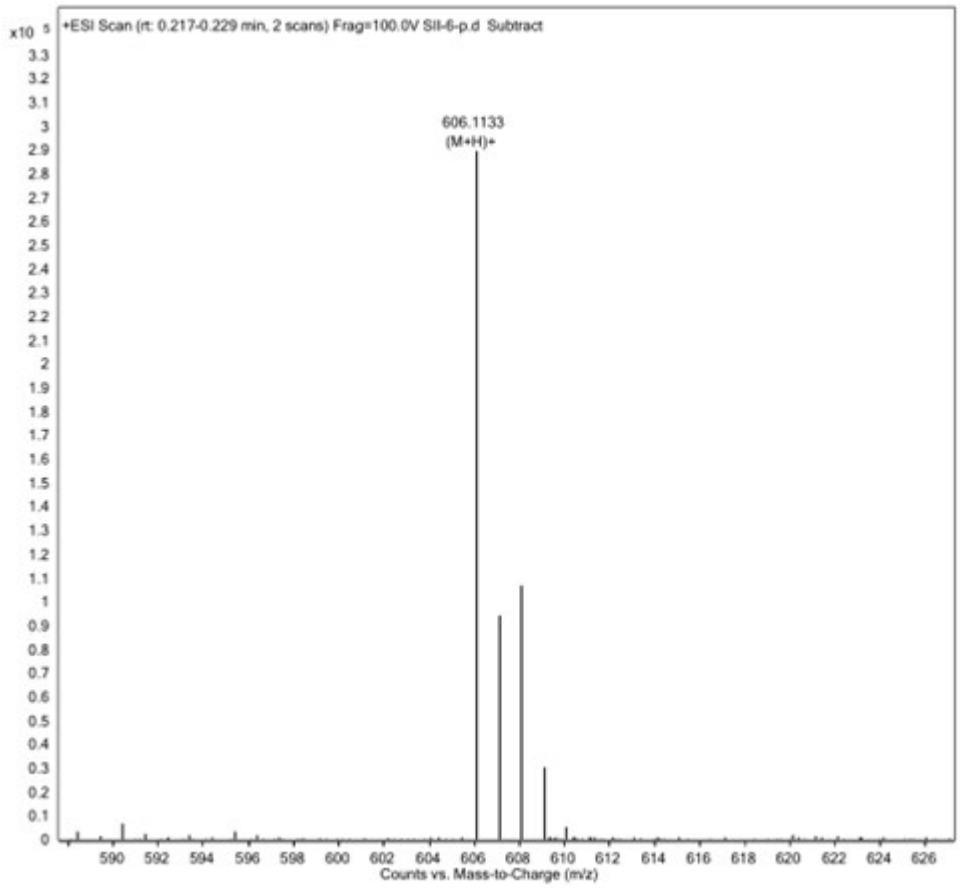

f1 (ppm)


f1 (ppm)

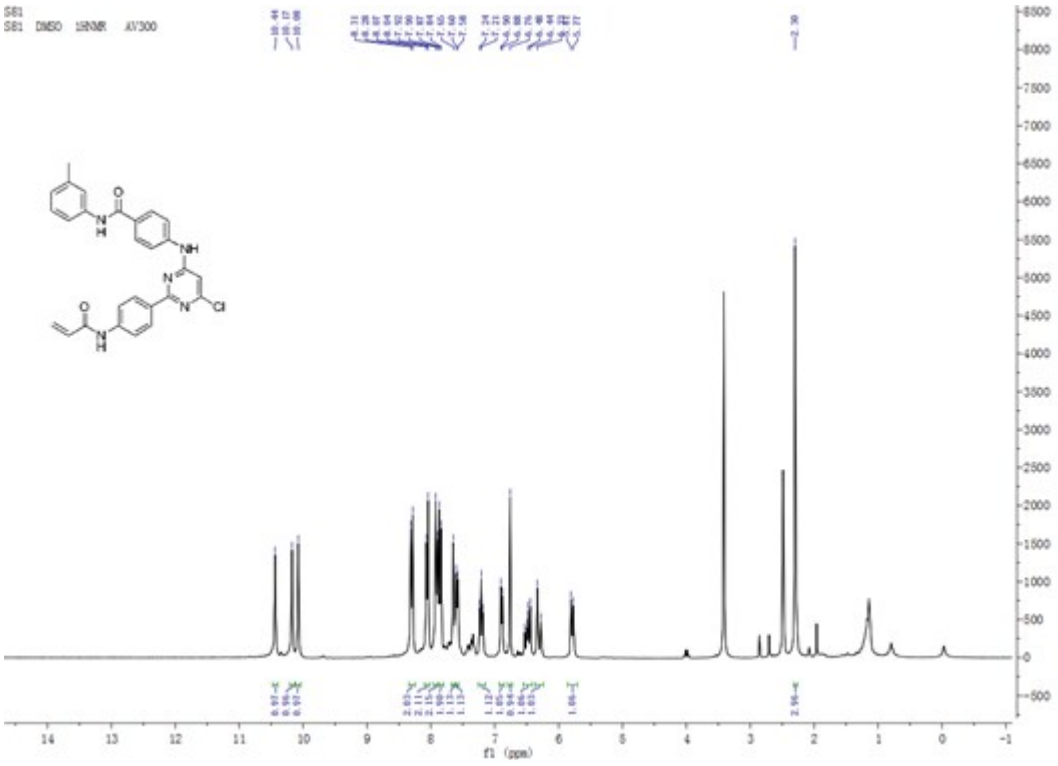

+ESI Scan (rt: 0.244-0.256 min, 2 scans) Frag=100.0V SII-10-p.d Subtract
x10 5
488.1288
(M+H)+
Counts vs. Mass-to-Charge (m/z)

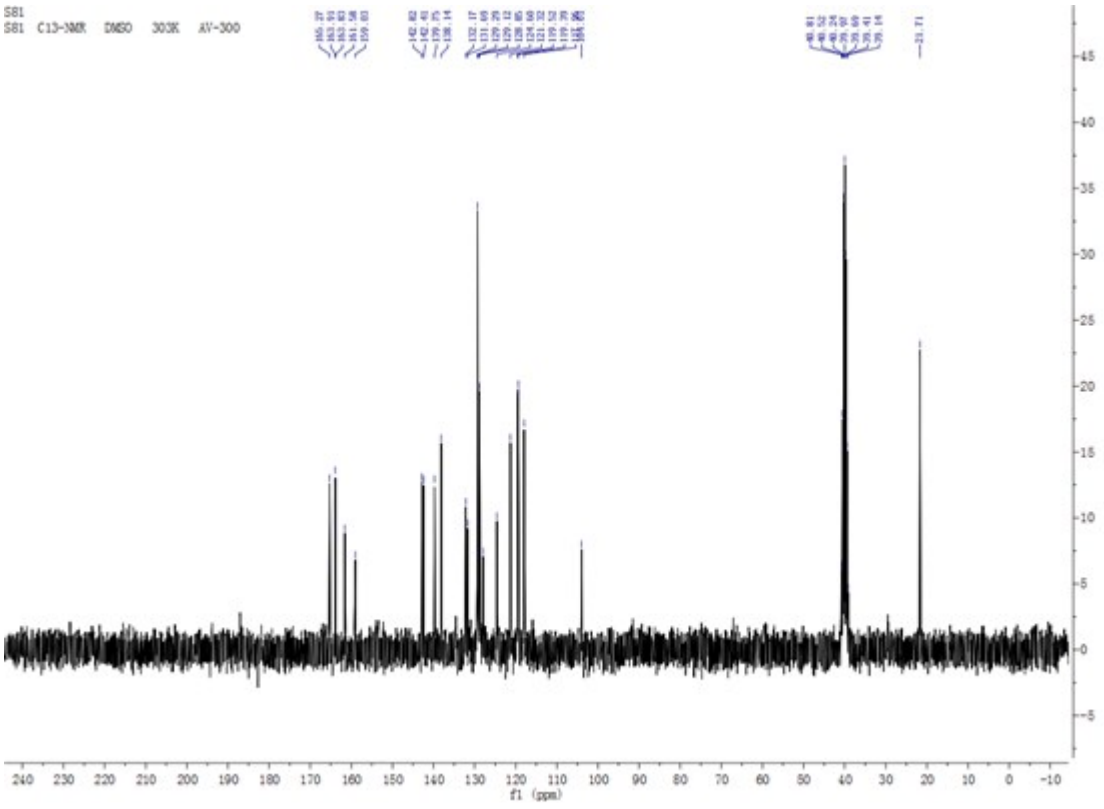
11f

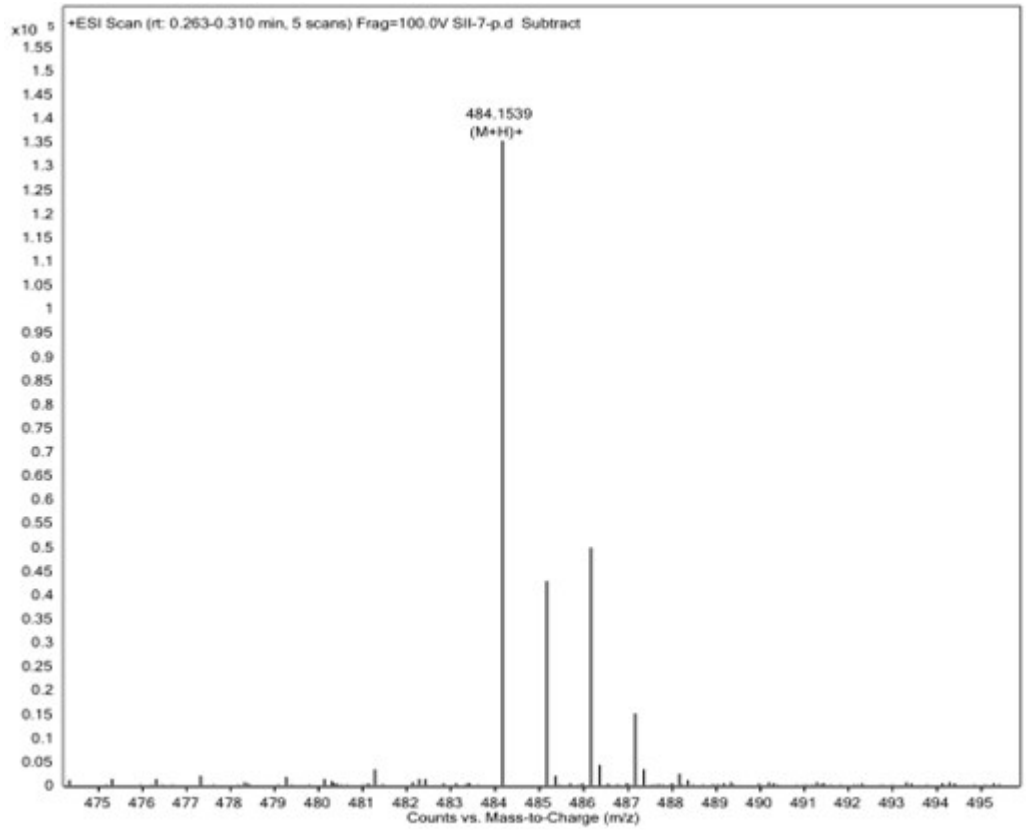



+ESI Scan (rt: 0.217-0.229 min, 2 scans) Frag=100.0V SII-6-p.d Subtract
x10 5
606.1133
(M+H)+
Counts vs. Mass-to-Charge (m/z)

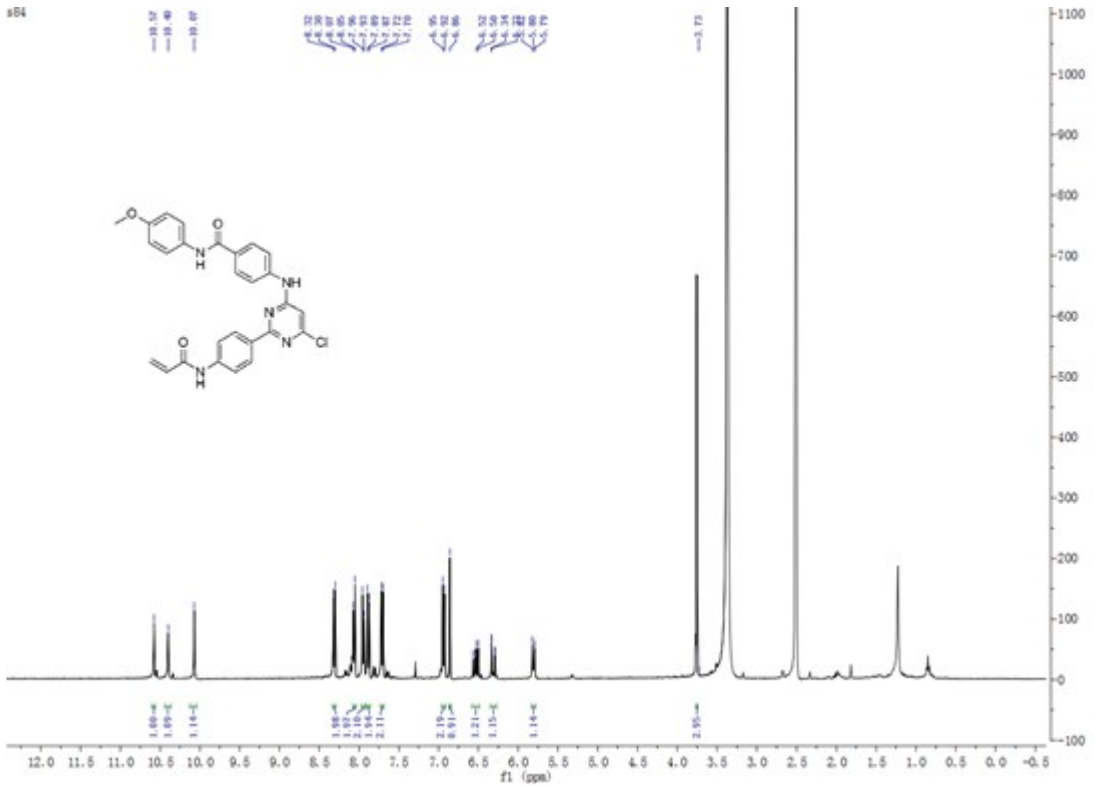
11g

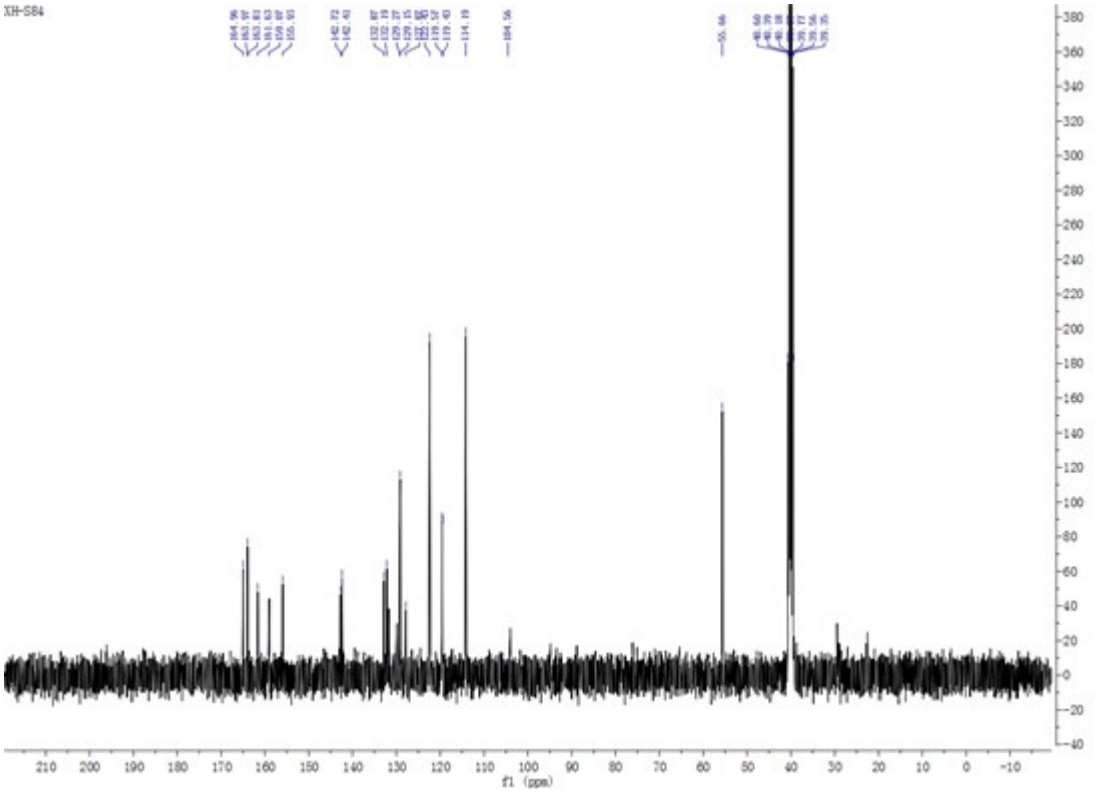

SII
SII DMSO 1HNMR AV300
f1 (ppm)

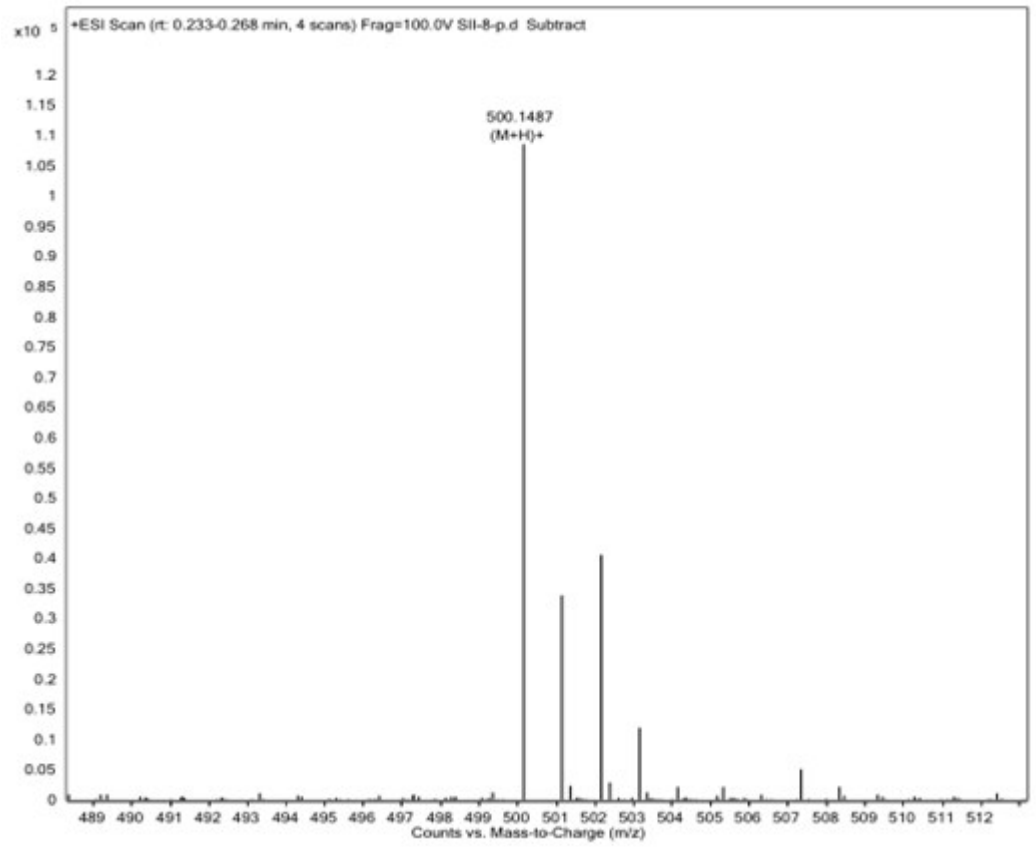

S81
S81 C13-NMR DMSO 303K AV-300
f1 (ppm)


+ESI Scan (rt: 0.263-0.310 min, 5 scans) Frag=100.0V SII-7-p.d Subtract
x10 5
484.1539
(M+H)+
Counts vs. Mass-to-Charge (m/z)

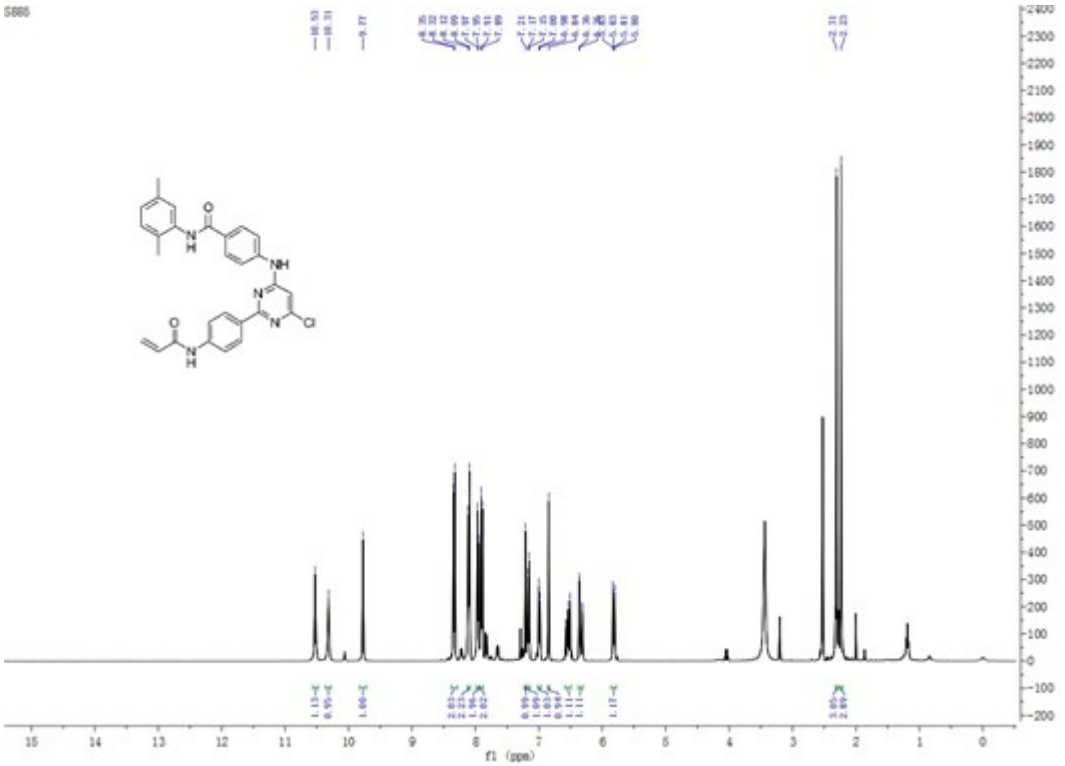
11h

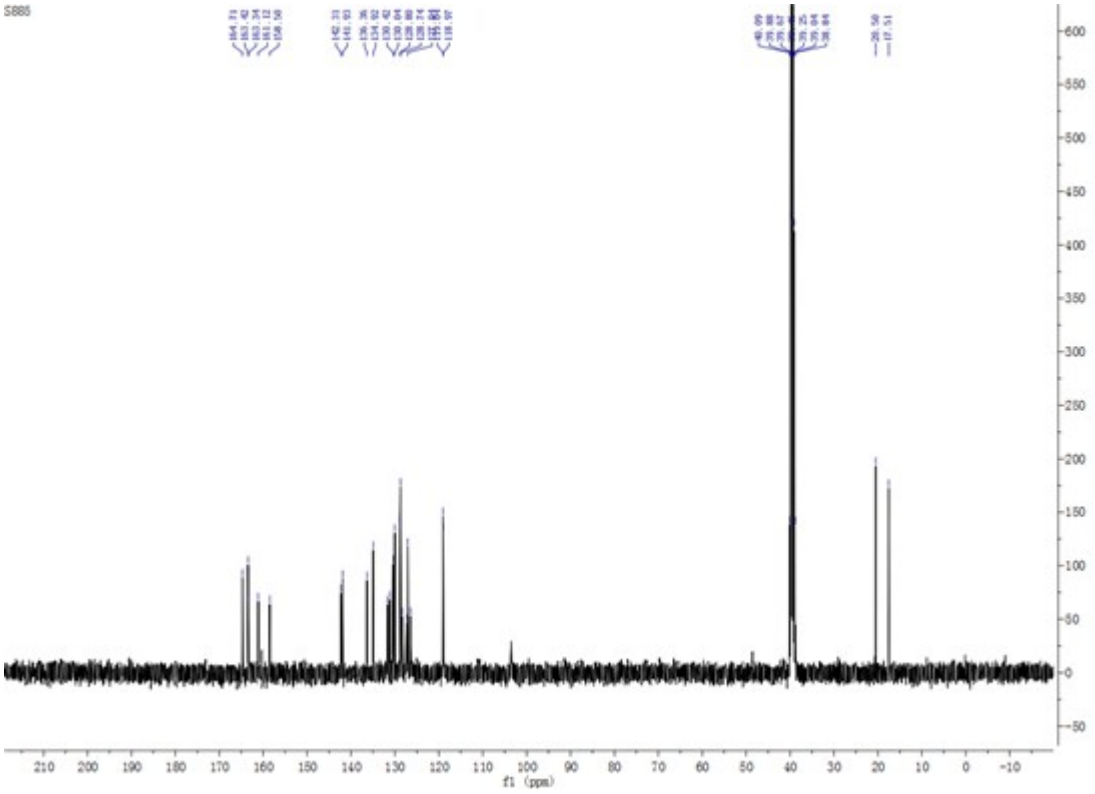

s84
f1 (ppm)

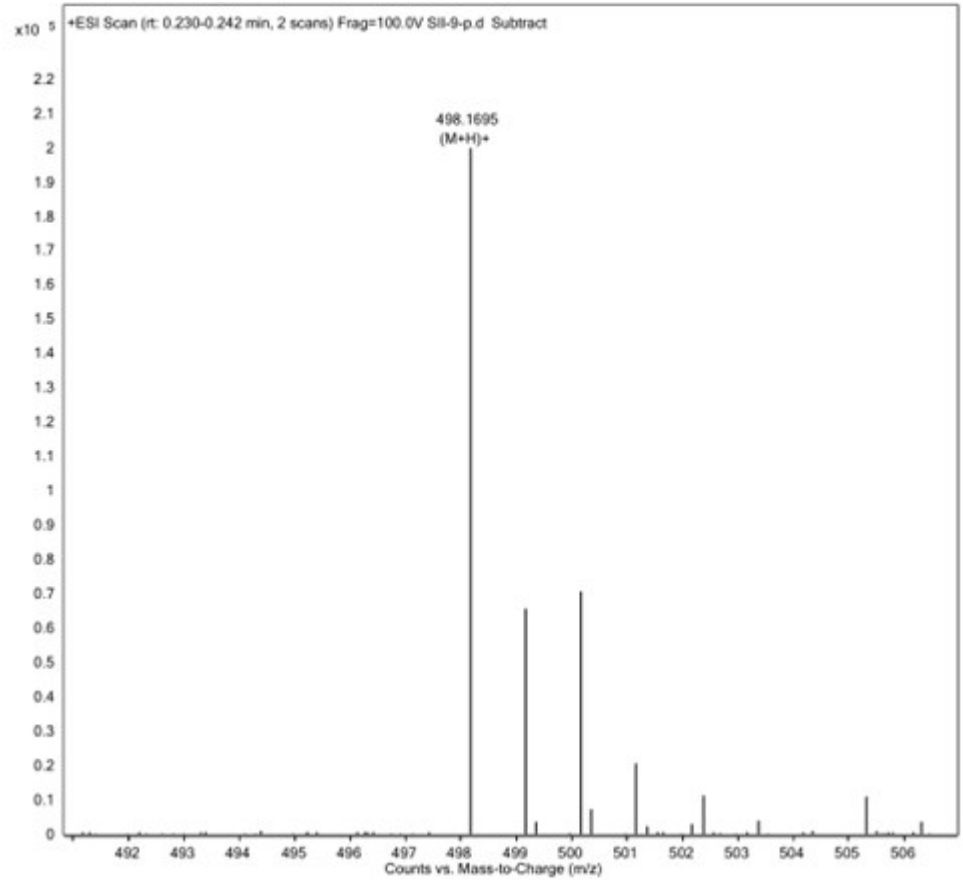

XH-S84
f1 (ppm)


+ESI Scan (rt: 0.233-0.268 min, 4 scans) Frag=100.0V SII-8-p.d Subtract
x10 5
500.1487
(M+H)+
Counts vs. Mass-to-Charge (m/z)

11i


f1 (ppm)


S885
f1 (ppm)


+ESI Scan (rt: 0.230-0.242 min, 2 scans) Frag=100.0V SII-9-p.d Subtract
x10 5
498.1695
(M+H)+
Counts vs. Mass-to-Charge (m/z)