

Electronic Supplementary Information (ESI)

The discovery of novel imidazo[1,2-a]pyridine derivatives as covalent anticancer agents

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1. Synthesis of compounds

1-(4-(3-(tert-butylamino)-2-(naphthalen-1-yl)imidazo[1,2-a]pyridin-8-yl)piperazin-1-yl)prop-2-en-1-one (**I-2**)

Following the synthetic method of **I-1**, compound **I-2** was obtained as a yellow solid in 56% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.03 (d, *J* = 7.5 Hz, 1H), 7.96 – 7.85 (m, 3H), 7.67 (dd, *J* = 7.0, 1.1 Hz, 1H), 7.55 (dd, *J* = 8.1, 7.1 Hz, 1H), 7.51 – 7.44 (m, 2H), 6.74 (t, *J* = 7.1 Hz, 1H), 6.60 (dd, *J* = 16.8, 10.5 Hz, 1H), 6.46 (d, *J* = 6.9 Hz, 1H), 6.31 (dd, *J* = 16.8, 1.9 Hz, 1H), 5.71 (dd, *J* = 10.5, 1.9 Hz, 1H), 4.01 – 3.88 (m, 2H), 3.85 – 3.76 (m, 2H), 3.72 – 3.65 (m, 2H), 3.51 (s, 2H), 0.74 (s, 9H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 165.38, 139.97, 137.52, 137.03, 133.82, 131.97, 128.51, 128.40, 128.21, 128.00, 127.40, 126.34, 125.69, 125.36, 117.70, 55.81, 50.13, 49.21, 45.88, 41.76, 29.74. ESI-LCMS (m/z): [M+H]⁺ calculated for C₂₈H₃₂N₅O 454.25; found 454.08.

1-(4-(3-(tert-butylamino)-2-(1*H*-indazol-7-yl)imidazo[1,2-a]pyridin-8-yl)piperazin-1-yl)prop-2-en-1-one (**I-3**)

Following the synthetic method of **I-1**, compound **I-3** was obtained as a yellow solid in 55% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.31 (s, 1H), 8.11 (s, 1H), 8.03 (d, *J* = 8.7 Hz, 1H), 7.93 (d, *J* = 6.7 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 6.69 (t, *J* = 7.1 Hz, 1H), 6.63 (dd, *J* = 16.8, 10.6 Hz, 1H), 6.41 (d, *J* = 7.3 Hz, 1H), 6.33 (dd, *J* = 16.8, 1.8 Hz, 1H), 5.73 (dd, *J* = 10.5, 1.8 Hz, 1H), 3.96 (s, 2H), 3.84 (s, 2H), 3.66 (s, 2H), 3.50 (s, 2H), 1.01 (s, 9H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 165.50, 139.90, 137.98, 137.31, 135.19, 128.11, 127.89, 127.45, 123.66, 120.25, 117.56, 111.49, 109.55, 55.18, 50.12, 49.28, 45.90, 41.81, 30.40. ESI-LCMS (m/z): [M+H]⁺ calculated for C₂₅H₃₀N₇O 444.24; found 444.08.

1-(4-(3-(tert-butylamino)-2-(2-fluoro-6-hydroxyphenyl)imidazo[1,2-a]pyridin-8-yl)piperazin-1-yl)prop-2-en-1-one (**I-4**)

Following the synthetic method of **I-1**, compound **I-4** was obtained as a yellow solid in 39% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.09 (d, *J* = 6.8 Hz, 1H), 7.23 – 7.14 (m, 1H), 6.89 – 6.82 (m, 1H), 6.77 (t, *J* = 7.1 Hz, 1H), 6.70 – 6.65 (m, 1H), 6.64 – 6.57 (m, 1H), 6.50 (d, *J* = 7.3 Hz, 1H), 6.34 (dd, *J* = 16.8, 1.8 Hz, 1H), 5.74 (dd, *J* = 10.5, 1.8 Hz, 1H), 3.96 (s, 2H), 3.84 (s, 2H), 3.57 (s, 2H), 3.40 (s, 2H), 1.00 (s, 9H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 165.14, 160.85, 157.42, 138.96, 136.42, 129.38 – 128.54 (m), 127.65, 127.12, 125.75, 117.69, 112.47, 112.17 – 111.48 (m),

108.29, 106.02 (d, $J = 24.0$ Hz), 55.92, 49.76, 48.93, 45.52, 41.46, 29.32. ESI-LCMS (m/z): $[M]^+$ calculated for $C_{24}H_{28}FN_5O_2$ 437.22; found 437.93.

1-(4-(3-((2,6-dimethylphenyl)amino)-2-(2-fluoro-6-hydroxyphenyl)imidazo[1,2-a]pyridin-8-yl)piperazin-1-yl)prop-2-en-1-one (**I-5**)

Following the synthetic method of **I-1**, compound **I-5** was obtained as a yellow solid in 41% yield. 1H NMR (400 MHz, Chloroform-*d*) δ 7.31 (d, $J = 6.7$ Hz, 1H), 7.15 (q, $J = 8.2$ Hz, 1H), 6.90 (d, $J = 7.5$ Hz, 2H), 6.83 (d, $J = 8.1$ Hz, 1H), 6.77 (t, $J = 7.5$ Hz, 1H), 6.69 – 6.65 (m, 1H), 6.64 – 6.56 (m, 2H), 6.48 (d, $J = 7.4$ Hz, 1H), 6.34 (dd, $J = 16.8, 1.7$ Hz, 1H), 5.75 (dd, $J = 10.5, 1.7$ Hz, 1H), 5.68 (d, $J = 6.1$ Hz, 1H), 3.97 (s, 2H), 3.84 (s, 2H), 3.60 (s, 2H), 3.42 (s, 2H), 1.90 (s, 6H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 165.61, 161.05, 158.62, 157.46, 139.64, 139.14, 135.46, 132.02, 129.66 (d, $J = 11.7$ Hz), 129.57, 128.37, 127.26, 126.69, 123.82, 121.59, 116.34, 113.22, 112.60, 107.97, 106.57, 106.33, 50.09, 49.08, 45.89, 41.76, 18.32. ESI-LCMS (m/z): $[M]^+$ calculated for $C_{28}H_{28}FN_5O_2$ 485.22; found 485.93.

Ethyl{[2-(6-fluoro-2-hydroxyphenyl)-8-[4-(1-oxoprop-2-enyl)piperazin-1-yl]imidazo[3,2-*a*]pyridin-3-yl]amino}acetate (**I-6**)

Following the synthetic method of **I-1**, compound **I-6** was obtained as a yellow solid in 49% yield. 1H NMR (400 MHz, Chloroform-*d*) δ 7.93 (d, $J = 6.7$ Hz, 1H), 7.18 (q, $J = 7.7$ Hz, 1H), 6.82 (dd, $J = 11.0, 7.8$ Hz, 2H), 6.72 – 6.66 (m, 1H), 6.60 (dd, $J = 16.7, 10.6$ Hz, 1H), 6.51 (d, $J = 7.4$ Hz, 1H), 6.32 (d, $J = 16.8$ Hz, 1H), 5.75 – 5.69 (m, 1H), 4.08 (q, $J = 7.1$ Hz, 2H), 3.93 (s, 2H), 3.82 (s, 2H), 3.69 (d, $J = 3.4$ Hz, 2H), 3.54 (s, 2H), 3.38 (s, 2H), 1.17 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.87, 165.54, 139.55, 135.87, 129.62, 129.51 (d, $J = 14.7$ Hz), 128.25, 127.32, 126.91, 116.81, 113.09, 113.06, 108.61, 106.45, 106.21, 61.24, 49.37, 49.12, 14.05. ESI-LCMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{27}FN_5O_4$ 468.20; found 468.17.

1-(4-(3-(tert-butylamino)-2-(2-fluoro-6-hydroxyphenyl)imidazo[1,2-a]pyridin-8-yl)piperazin-1-yl)-2-chloroethan-1-one (**I-7**)

Intermediate **9** (0.07 g, 0.14 mmol) was dissolved in anhydrous DCM, and then $NaHCO_3$ (0.012 g, 0.14 mmol) was added as follows. The chloroacetyl chloride (23 μ L, 0.14 mmol) was added to the above system at low temperature. The mixture was stirred for 0.5 h. Pour the mixture into water (10 mL) and extract with ethyl acetate (10 mL \times 3), the combined organic phase was

washed with water (10 mL × 3), dried over anhydrous Na₂SO₄, and concentrated under vacuum. Purification of the residue by flash column chromatography using DCM/MeOH as the gradient elution to afford compound **I-7** as a yellow solid in 50% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.12 (d, *J* = 6.7 Hz, 1H), 7.22 – 7.15 (m, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.79 (t, *J* = 7.1 Hz, 1H), 6.68 (dd, *J* = 11.2, 8.3 Hz, 1H), 6.54 (d, *J* = 7.3 Hz, 1H), 4.13 (s, 2H), 3.92 – 3.87 (m, 2H), 3.83 – 3.78 (m, 2H), 3.60 (s, 2H), 3.42 – 3.36 (m, 2H), 1.00 (s, 9H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 165.30, 158.79, 157.75, 139.12, 136.54, 129.56 (d, *J* = 11.7 Hz), 118.30, 112.95, 112.23, 106.59, 106.35, 56.42, 49.98, 49.14, 46.45, 42.04, 40.85, 29.71. ESI-LCMS (m/z): [M]⁺ calculated for C₂₃H₂₇ClFN₅O₂ 459.18; found 459.95.

1-(4-(3-(tert-butylamino)-2-(2-fluoro-6-hydroxyphenyl)imidazo[1,2-a]pyridin-8-yl)piperazin-1-yl)prop-2-yn-1-one (**I-8**)

Intermediate **9** (0.07 g, 0.14 mmol) was dissolved in anhydrous DCM and pre-cooled in an ice-water bath, then DIPEA (27 μL, 0.28 mmol) was added to another reaction vial. The HBTU (0.011 g, 0.03 mmol), and propargylate (8 μL, 0.12 mmol) was added dropwise slowly. After activation for 3 h, the mixture of incorporating propargylate was added dropwise into the pre-cooled reaction vial. The reaction was partitioned between DCM and water. The organic layer was separated, washed with water and brine, and concentrated in vacuo. Purification by flash column chromatography on silica gel using DCM/MeOH as the gradient elution to obtain **I-8** as a yellow solid in 43% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 6.4 Hz, 1H), 7.22 – 7.16 (m, 1H), 6.86 (d, *J* = 8.2 Hz, 1H), 6.78 (t, *J* = 7.1 Hz, 1H), 6.68 (dd, *J* = 11.5, 8.0 Hz, 1H), 6.52 (d, *J* = 7.2 Hz, 1H), 4.08 – 4.03 (m, 2H), 3.94 – 3.89 (m, 2H), 3.58 (s, 2H), 3.42 – 3.37 (m, 2H), 3.16 (s, 1H), 1.01 (s, 9H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 160.80, 157.33, 151.48, 138.76, 136.34, 129.39, 129.01 (d, *J* = 11.7 Hz), 117.78, 112.41, 111.81, 108.47, 105.95, 79.61, 74.84, 55.83, 49.59, 48.64, 46.56, 40.92, 29.52. ESI-LCMS (m/z): [M]⁺ calculated for C₂₄H₂₆FN₅O₂ 435.21; found 435.99.

1-(4-(3-(tert-butylamino)-2-(naphthalen-1-yl)imidazo[1,2-a]pyridin-8-yl)piperazin-1-yl)prop-2-yn-1-one (**I-9**)

Following the synthetic method of **I-8**, compound **I-9** was obtained as a pale green solid in 41% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.04 (d, *J* = 6.7 Hz, 1H), 7.97 – 7.89 (m, 2H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.67 (d, *J* = 6.9 Hz, 1H), 7.57 – 7.53 (m, 1H), 7.51 – 7.45 (m, 2H), 6.74 (t, *J* = 7.1

Hz, 1H), 6.46 (d, $J = 7.3$ Hz, 1H), 4.06 – 3.99 (m, 2H), 3.93 – 3.87 (m, 2H), 3.73 – 3.68 (m, 2H), 3.57 – 3.51 (m, 2H), 3.14 (s, 1H), 0.75 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 151.81, 139.88, 137.56, 133.87, 132.01, 128.56, 128.42, 128.25, 126.38, 125.82, 125.72, 125.40, 117.89, 111.43, 79.44, 75.40, 55.87, 50.03, 49.07, 47.03, 41.39, 29.78. ESI-LCMS (m/z): $[\text{M}]^+$ calculated for $\text{C}_{28}\text{H}_{29}\text{N}_5\text{O}$ 451.24; found 451.99.

1-(4-(3-((2,6-dimethylphenyl)amino)-2-(2-fluoro-6-hydroxyphenyl)imidazo[1,2-*a*]pyridin-8-yl)piperazin-1-yl)prop-2-yn-1-one (**I-10**)

Following the synthetic method of **I-8**, compound **I-10** was obtained as a pale green solid in 53% yield. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.32 (d, $J = 6.6$ Hz, 1H), 7.15 (q, $J = 8.2$ Hz, 1H), 6.90 (d, $J = 7.5$ Hz, 2H), 6.83 (d, $J = 8.2$ Hz, 1H), 6.77 (t, $J = 7.5$ Hz, 1H), 6.68 (t, $J = 7.1$ Hz, 1H), 6.63 – 6.57 (m, 1H), 6.49 (d, $J = 7.4$ Hz, 1H), 5.69 (d, $J = 6.1$ Hz, 1H), 4.08 – 4.03 (m, 2H), 3.94 – 3.89 (m, 2H), 3.63 – 3.58 (m, 2H), 3.44 – 3.39 (m, 2H), 3.17 (s, 1H), 1.90 (s, 6H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 158.60, 157.43, 151.91, 139.49, 139.09, 135.41, 129.68 (d, $J = 11.6$ Hz), 129.56, 126.70, 123.88, 121.62, 116.52, 113.16, 112.60, 108.16, 106.57, 106.33, 79.69, 75.23, 50.00, 48.89, 46.98, 41.31, 29.70. ESI-LCMS (m/z): $[\text{M}]^+$ calculated for $\text{C}_{28}\text{H}_{26}\text{FN}_5\text{O}_2$ 483.21; found 483.74.

1-(4-(2-(2-fluoro-6-hydroxyphenyl)-3-((2-(trifluoromethyl)benzyl)amino)imidazo[1,2-*a*]pyridin-8-yl)piperazin-1-yl)prop-2-yn-1-one (**I-11**)

Following the synthetic method of **I-8**, compound **I-11** was obtained as a yellow solid in 50% yield. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.95 – 7.90 (m, 1H), 7.50 (d, $J = 7.7$ Hz, 1H), 7.29 (t, $J = 7.5$ Hz, 1H), 7.19 (t, $J = 7.6$ Hz, 1H), 7.12 – 7.05 (m, 1H), 7.03 (d, $J = 7.6$ Hz, 1H), 6.84 (t, $J = 7.1$ Hz, 1H), 6.73 (d, $J = 8.1$ Hz, 1H), 6.58 – 6.52 (m, 2H), 4.16 (s, 2H), 4.06 – 4.03 (m, 2H), 3.93 – 3.89 (m, 2H), 3.60 – 3.56 (m, 2H), 3.43 – 3.39 (m, 2H), 3.17 (s, 1H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 160.57, 157.63, 151.93, 139.51, 136.71, 136.13, 131.63, 130.58, 129.30 (d, $J = 11.9$ Hz), 127.68, 127.03, 125.95 (d, $J = 5.6$ Hz), 116.70, 113.03, 112.78, 108.54, 106.28, 106.04, 79.67, 75.27, 50.00, 48.90, 47.02, 41.35, 29.72. ESI-HRMS (m/z): $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{28}\text{H}_{24}\text{F}_4\text{N}_5\text{O}_2$ 538.1866; found 538.1861.

1-{8-[2-(6-fluoro-2-hydroxyphenyl)-3-({[2-(trifluoromethyl)phenyl]methyl}amino)imidazo[3,2-*a*]pyridin-8-yl]-3,8-diazabicyclo[3.2.1]octan-3-yl}prop-2-yn-1-one (**I-12**)

Following the synthetic method of **I-8**, compound **I-12** was obtained as a yellow solid in 43% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.91 (d, *J* = 7.4 Hz, 1H), 7.51 (d, *J* = 7.7 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.09 (q, *J* = 8.3, 7.5 Hz, 2H), 6.84 – 6.79 (m, 1H), 6.74 (d, *J* = 8.0 Hz, 1H), 6.55 (dd, *J* = 11.9, 8.8 Hz, 1H), 6.46 (d, *J* = 7.3 Hz, 1H), 4.86 (d, *J* = 6.5 Hz, 1H), 4.68 (d, *J* = 5.3 Hz, 1H), 4.32 (d, *J* = 11.3 Hz, 1H), 4.18 – 4.09 (m, 2H), 3.81 (d, *J* = 13.0 Hz, 1H), 3.14 (d, *J* = 10.3 Hz, 1H), 3.06 (d, *J* = 13.5 Hz, 2H), 2.33 – 2.26 (m, 1H), 2.23 – 2.12 (m, 2H), 2.11 – 2.04 (m, 1H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 160.58, 158.16, 157.62, 148.71, 139.66, 136.86, 136.74, 136.16, 131.65, 130.56, 129.28 (d, *J* = 11.9 Hz), 127.68, 127.02, 125.97 (d, *J* = 5.7 Hz), 116.25, 113.11, 112.71, 108.40, 106.33, 106.10, 77.26, 75.97, 56.92, 55.95, 54.33, 51.96, 48.70, 27.81, 27.27. ESI-LCMS (m/z): [M]⁺ calculated for C₃₀H₂₅F₄N₅O₂ 563.19; found 563.75.

1-{7-[2-(6-fluoro-2-hydroxyphenyl)-3-({[2-(trifluoromethyl)phenyl]methyl}amino)imidazo[3,2-*a*]pyridin-8-yl]-2,7-diazaspiro[3.5]nonan-2-yl}prop-2-yn-1-one (**I-13**)

Following the synthetic method of **I-8**, compound **I-13** was obtained as a yellow solid in 48% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.89 (d, *J* = 6.6 Hz, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.30 (t, *J* = 7.3 Hz, 1H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.12 – 7.02 (m, 2H), 6.81 (t, *J* = 7.1 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.54 (dd, *J* = 11.2, 8.2 Hz, 2H), 4.16 (d, *J* = 4.7 Hz, 2H), 3.99 (s, 2H), 3.84 (s, 2H), 3.48 – 3.42 (m, 2H), 3.37 (dt, *J* = 11.9, 5.3 Hz, 2H), 3.00 (s, 1H), 2.05 (t, *J* = 5.4 Hz, 4H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 158.17, 157.83, 153.28, 140.24, 136.81, 136.27, 131.61, 130.55, 129.15 (d, *J* = 12.0 Hz), 127.63, 126.86, 125.93 (d, *J* = 5.6 Hz), 116.08, 113.10, 112.80, 108.29, 106.18, 105.94, 77.86, 74.77, 60.29, 57.91, 46.81, 35.25, 34.38, 29.71. ESI-LCMS (m/z): [M+H]⁺ calculated for C₃₁H₂₈F₄N₅O₂ 578.21; found 578.83.

1-(4-(2-(2-fluoro-6-hydroxyphenyl)-3-((4-isopropylbenzyl)amino)imidazo[1,2-*a*]pyridin-6-yl)piperazin-1-yl)prop-2-yn-1-one (**I-14**)

Following the synthetic method of **I-8**, compound **I-14** was obtained as a reddish brown solid in 33% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.56 (s, 1H), 7.44 (d, *J* = 9.6 Hz, 1H), 7.15 – 7.06 (m, 6H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.65 – 6.58 (m, 1H), 3.96 (d, *J* = 10.3 Hz, 4H), 3.83 (s, 2H), 3.19 (s, 1H), 3.05 (d, *J* = 18.4 Hz, 4H), 2.88 – 2.81 (m, 1H), 1.20 (d, *J* = 6.8 Hz, 6H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 165.15, 148.46, 139.20, 136.64, 128.38, 126.80, 126.29, 125.96, 121.85 (d, *J* = 30.6 Hz), 116.39, 108.98, 79.81, 75.11, 50.65, 49.93, 46.07, 41.84, 40.77, 33.75, 24.02. ESI-

LCMS (m/z): [M]⁺ calculated for C₃₀H₃₀FN₅O₂ 511.24; found 511.90.

1-(4-(3-((4-isopropylbenzyl)amino)-2-(thiophen-2-yl)imidazo[1,2-a]pyridin-6-yl)piperazin-1-yl)prop-2-yn-1-one (**I-15**)

Following the synthetic method of **I-8**, compound **I-15** was obtained as a yellow solid in 42% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.73 (d, *J* = 2.2 Hz, 1H), 7.67 (d, *J* = 5.0 Hz, 1H), 7.44 (d, *J* = 9.6 Hz, 1H), 7.39 (dd, *J* = 4.9, 2.9 Hz, 1H), 7.27 (s, 1H), 7.23 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 8.1 Hz, 2H), 6.96 (dd, *J* = 9.6, 1.9 Hz, 1H), 4.16 (s, 2H), 3.95 – 3.89 (m, 2H), 3.82 – 3.77 (m, 2H), 3.19 (s, 1H), 3.00 – 2.95 (m, 2H), 2.93 – 2.90 (m, 2H), 2.90 – 2.85 (m, 1H), 1.23 (d, *J* = 6.9 Hz, 6H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 165.51, 136.80, 128.63, 128.52, 127.25, 126.97, 126.39, 126.06, 125.96, 109.02, 52.40, 51.05, 50.22, 45.68, 33.91, 24.16. ESI-LCMS (m/z): [M+H]⁺ calculated for C₂₈H₃₀N₅OS 484.21; found 484.01.

1-(4-(2-(2-hydroxynaphthalen-1-yl)-3-((4-isopropylbenzyl)amino)imidazo[1,2-a]pyridin-6-yl)piperazin-1-yl)prop-2-yn-1-one (**I-16**)

Following the synthetic method of **I-8**, compound **I-16** was obtained as a yellow solid in 36% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.81 (d, *J* = 8.0 Hz, 1H), 7.75 – 7.69 (m, 2H), 7.52 (s, 1H), 7.46 (t, *J* = 9.2 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.23 (d, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 11.6 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 2H), 6.75 (d, *J* = 8.0 Hz, 2H), 3.98 – 3.92 (m, 2H), 3.85 – 3.80 (m, 2H), 3.68 (s, 2H), 3.20 (s, 1H), 3.04 (dt, *J* = 20.7, 4.9 Hz, 4H), 2.80 (s, 1H), 2.79 – 2.73 (m, 1H), 1.16 (d, *J* = 6.9 Hz, 6H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 154.04, 139.40, 135.97, 130.19, 128.77, 127.87, 126.93, 126.37, 123.48, 123.00, 118.84, 116.78, 109.27, 79.84, 51.74, 51.00, 50.22, 46.69, 41.24, 33.63, 29.71, 23.93. ESI-LCMS (m/z): [M+H]⁺ calculated for C₃₄H₃₄N₅O₂ 544.26; found 544.06.

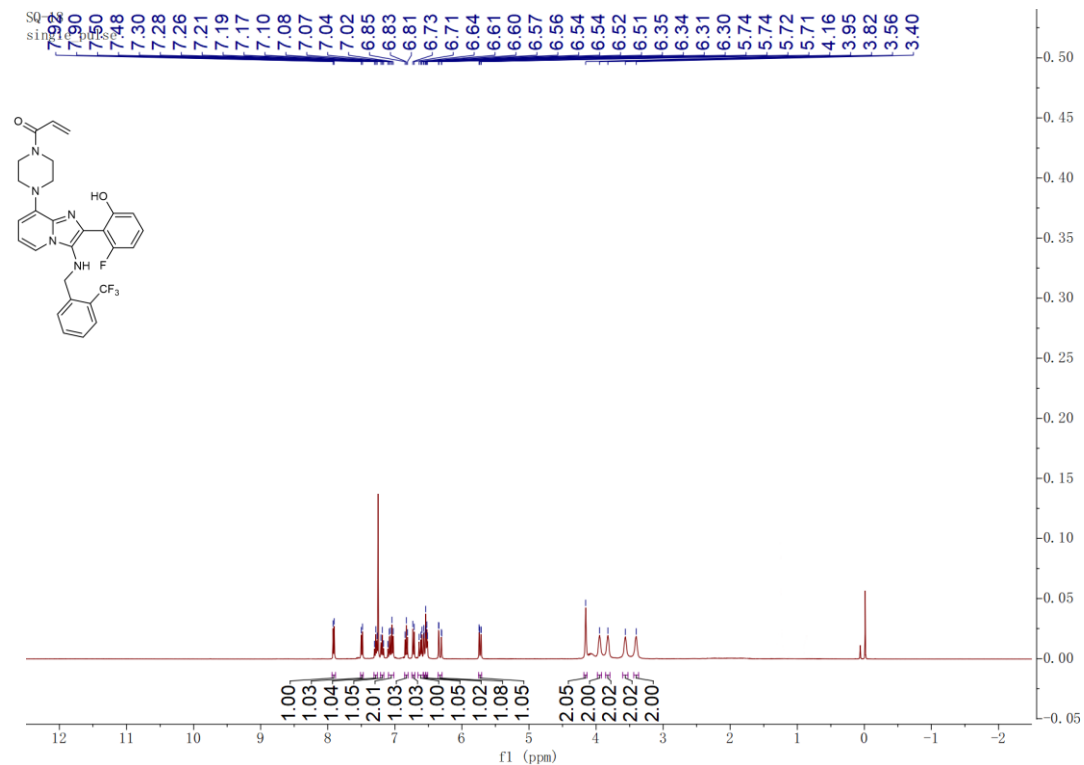
1-(4-(3-(tert-butylamino)-2-(thiophen-2-yl)imidazo[1,2-a]pyridin-6-yl)piperazin-1-yl)prop-2-yn-1-one (**I-17**)

Following the synthetic method of **I-8**, compound **I-17** was obtained as a yellow solid in 40% yield. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.75 (s, 1H), 7.68 (s, 1H), 7.64 (d, *J* = 4.6 Hz, 1H), 7.46 (d, *J* = 9.5 Hz, 1H), 7.35 (s, 1H), 7.01 (d, *J* = 9.4 Hz, 1H), 3.97 (s, 2H), 3.84 (s, 2H), 3.18 (s, 1H), 3.12 (s, 2H), 3.06 (s, 2H), 1.11 (s, 9H). ¹³C NMR (100 MHz, Chloroform-*d*) δ 138.83, 127.19, 125.33, 123.82, 122.59, 121.83, 116.91, 110.50, 79.75, 56.51, 51.22, 50.41, 46.80, 41.32, 30.57.

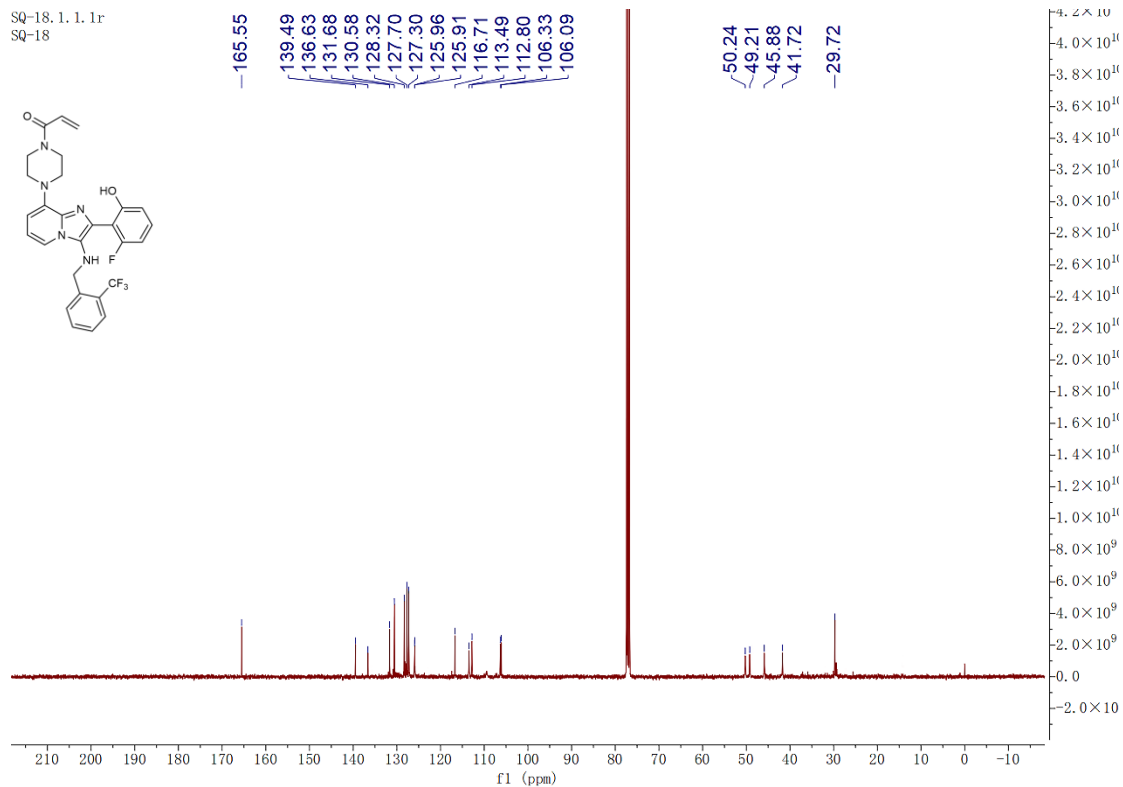
ESI-LCMS (m/z): [M+H]⁺ calculated for C₂₂H₂₆N₅OS 408.18; found 407.85.

2. ¹H and ¹³C NMR spectra of compound I-1 ~ 17

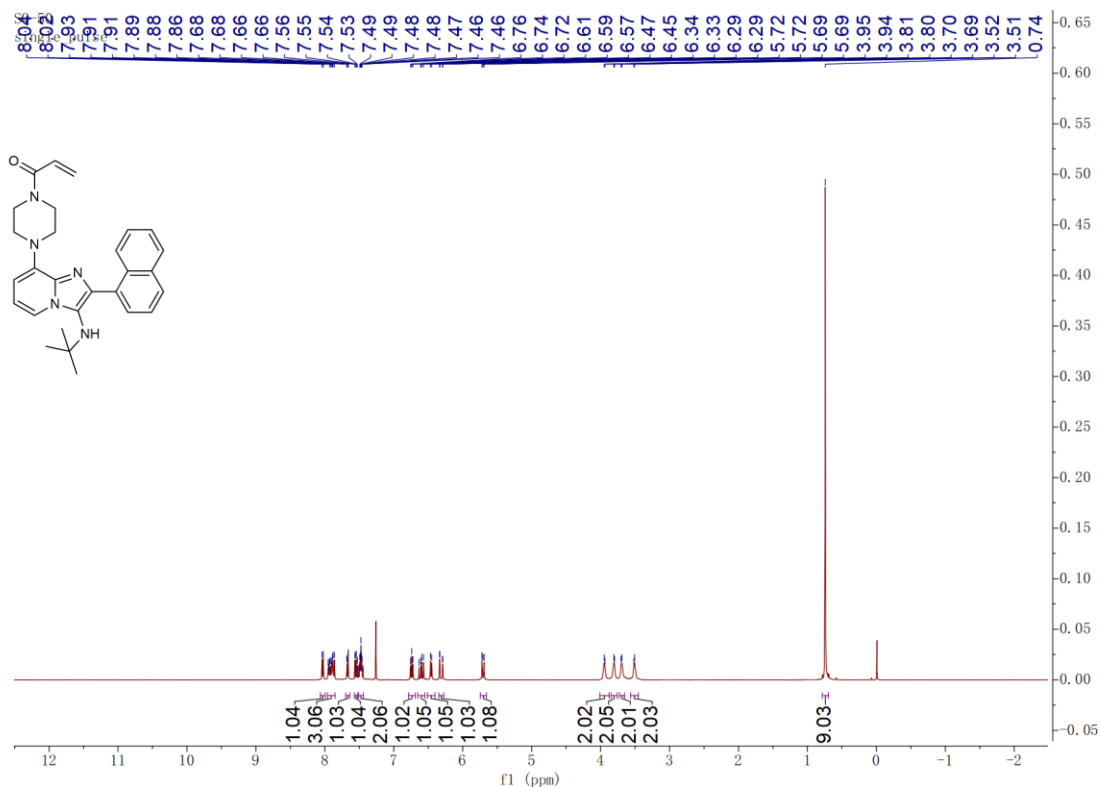
¹H NMR spectrum of compound I-1 (400 MHz, Chloroform-*d*)



¹³C NMR spectrum of compound I-1 (100 MHz, Chloroform-*d*)



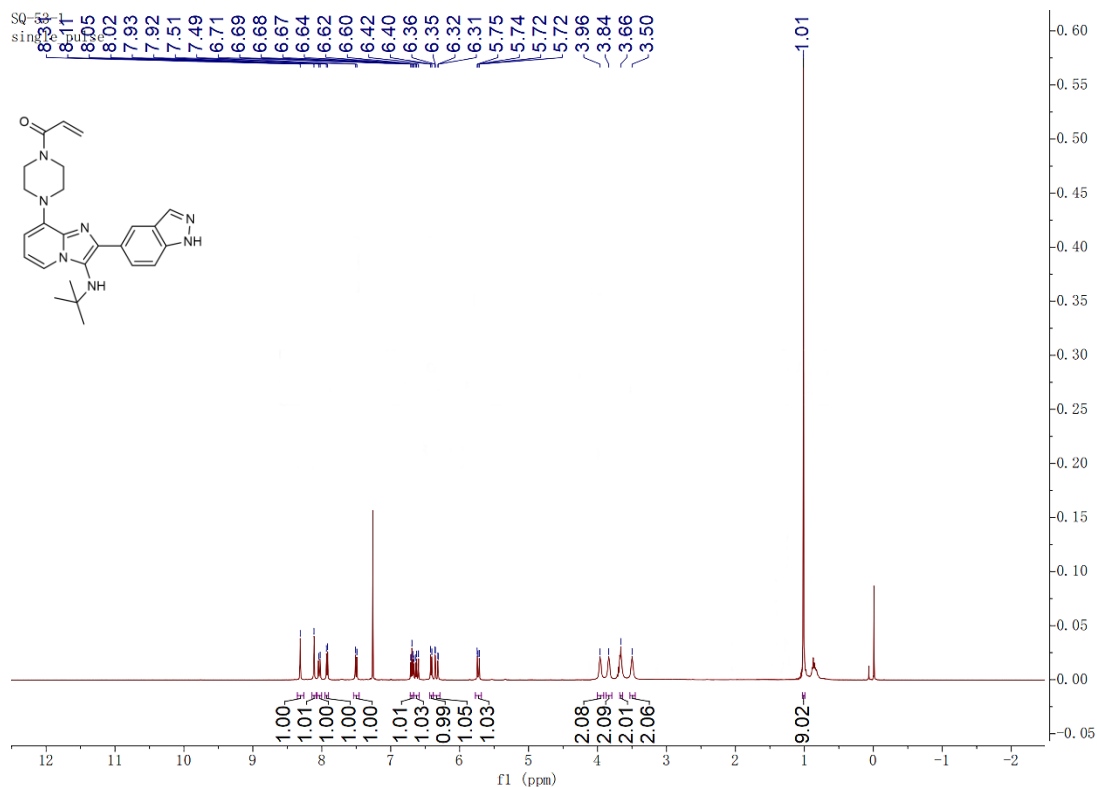
¹H NMR spectrum of compound I-2 (400 MHz, Chloroform-*d*)



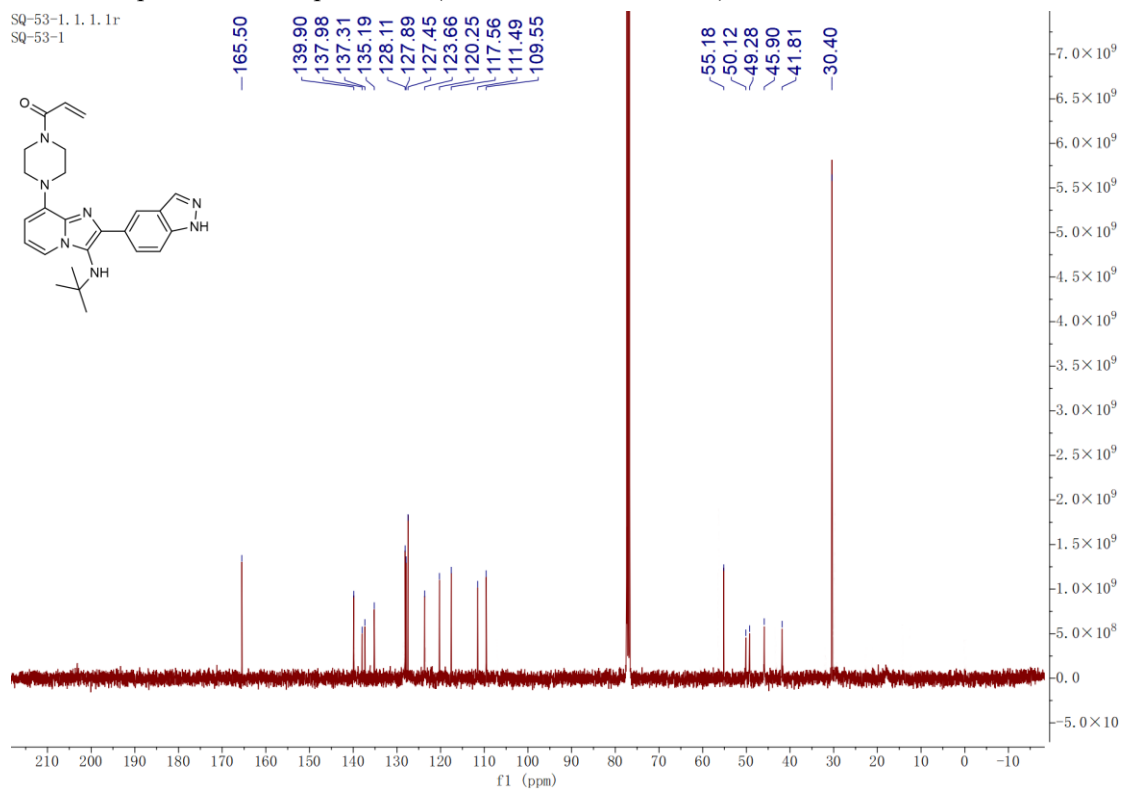
¹³C NMR spectrum of compound I-2 (100 MHz, Chloroform-*d*)



¹H NMR spectrum of compound I-3 (400 MHz, Chloroform-*d*)

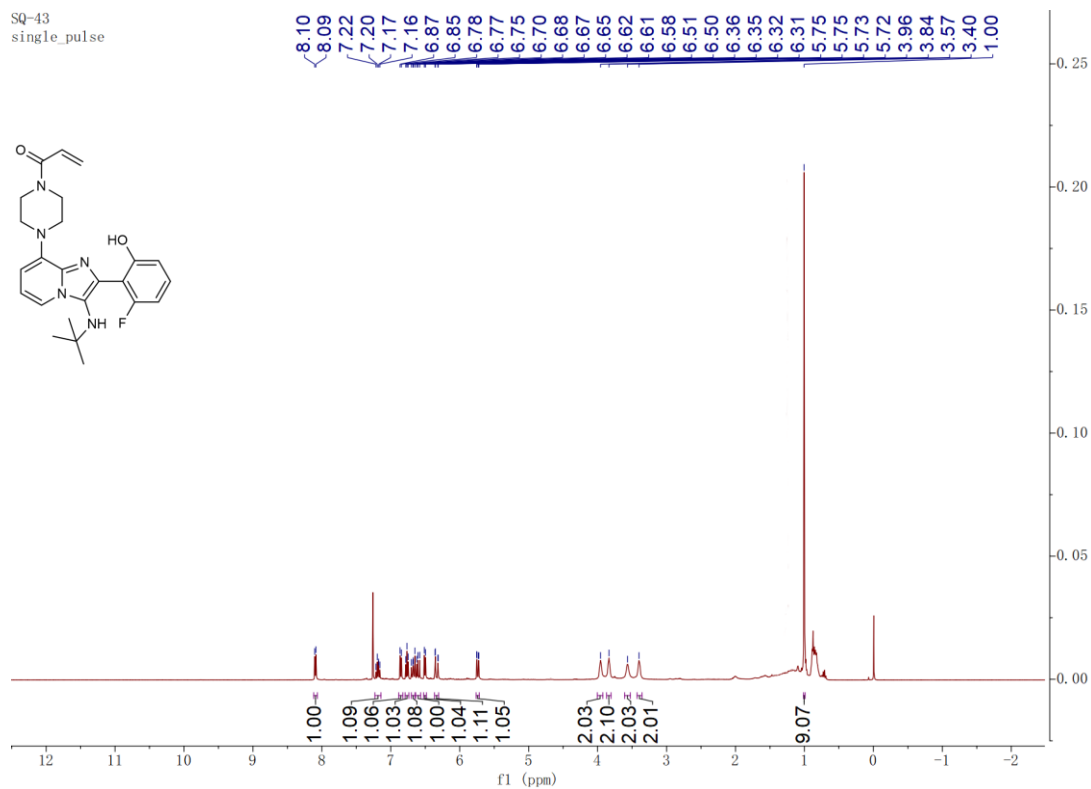


¹³C NMR spectrum of compound I-3 (100 MHz, Chloroform-*d*)



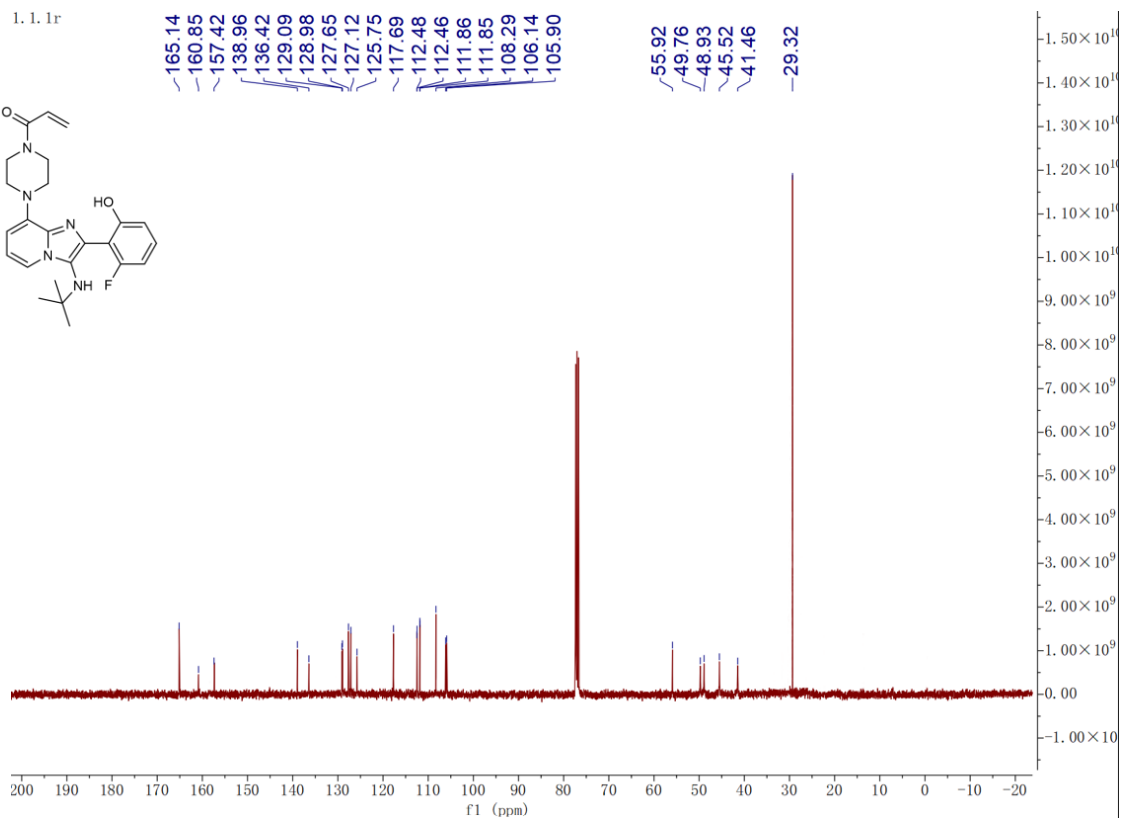
¹H NMR spectrum of compound I-4 (400 MHz, Chloroform-*d*)

SQ-43
single_pulse

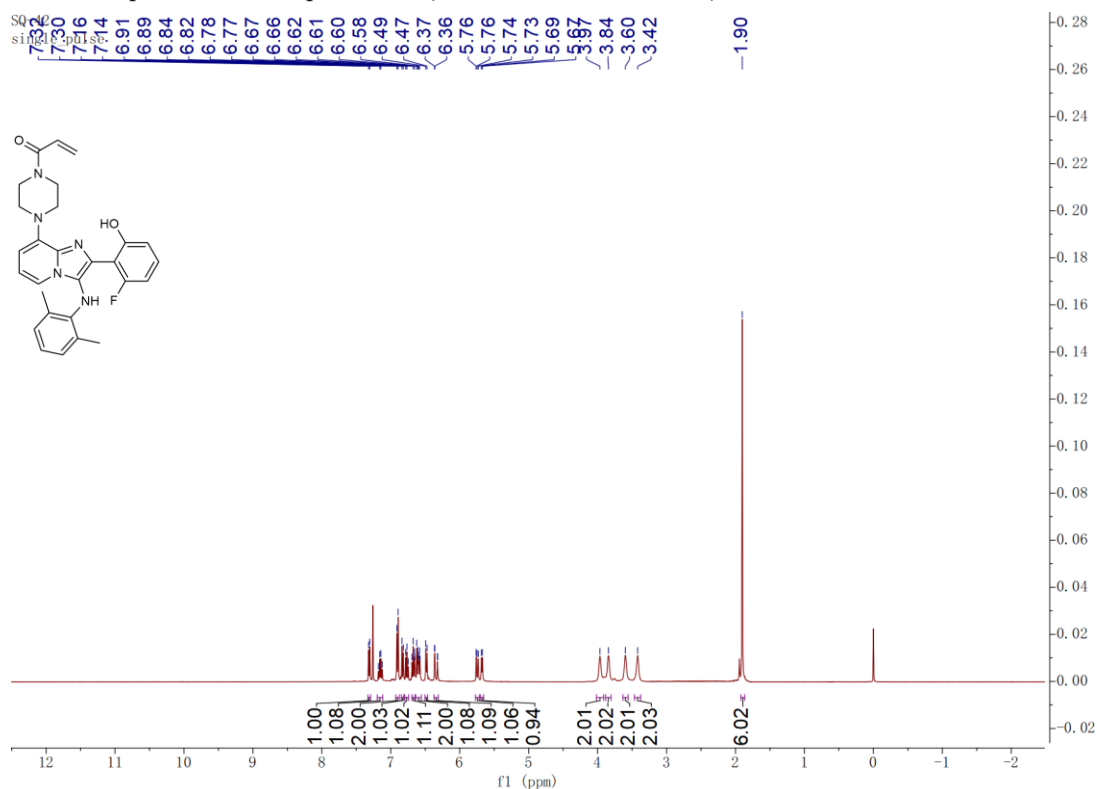


¹³C NMR spectrum of compound I-4 (100 MHz, Chloroform-*d*)

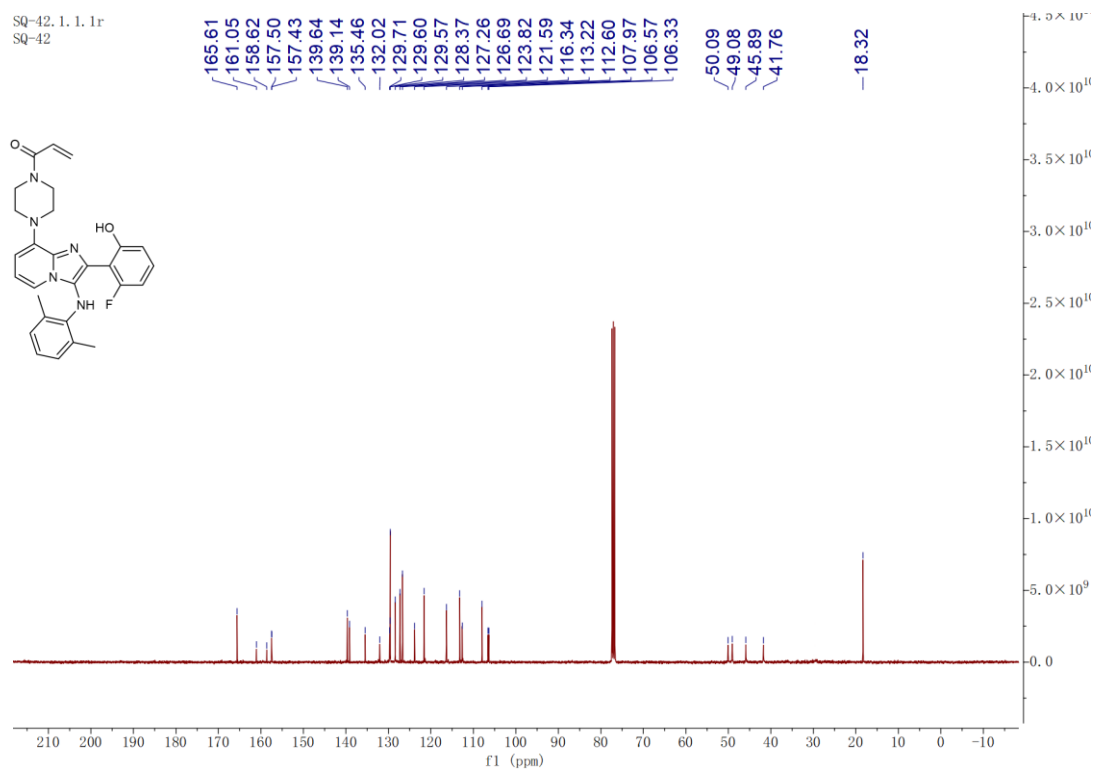
1. 1. 1r



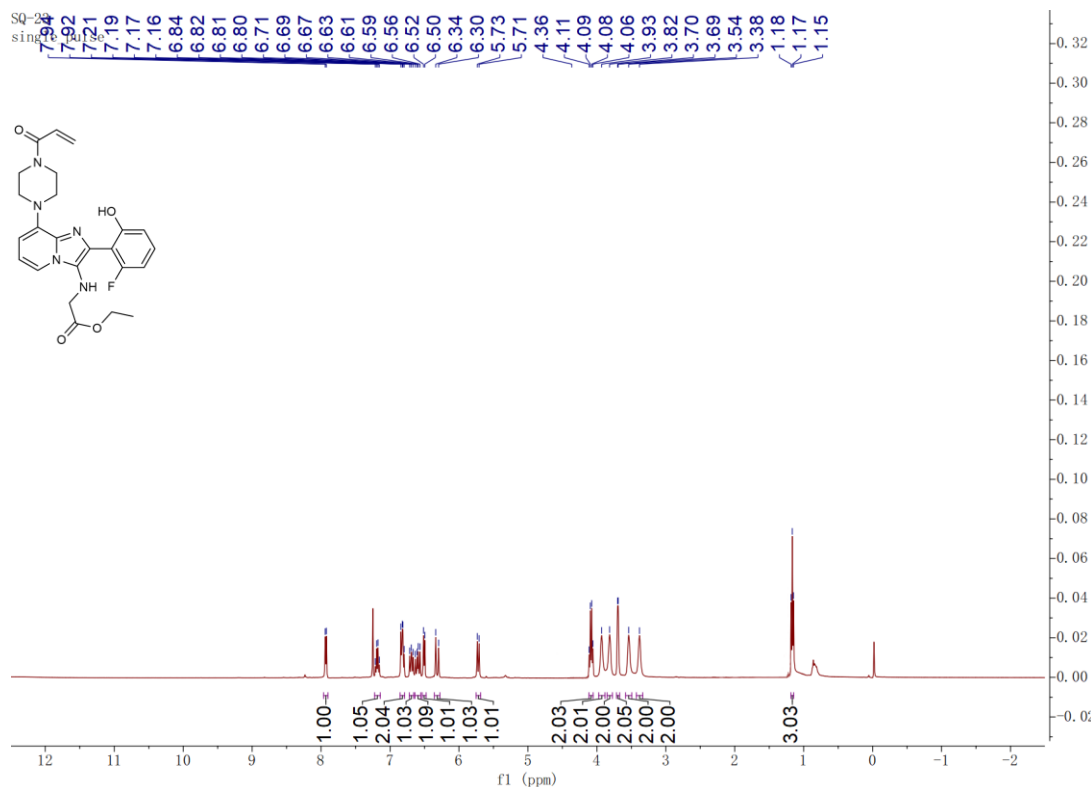
¹H NMR spectrum of compound I-5 (400 MHz, Chloroform-*d*)



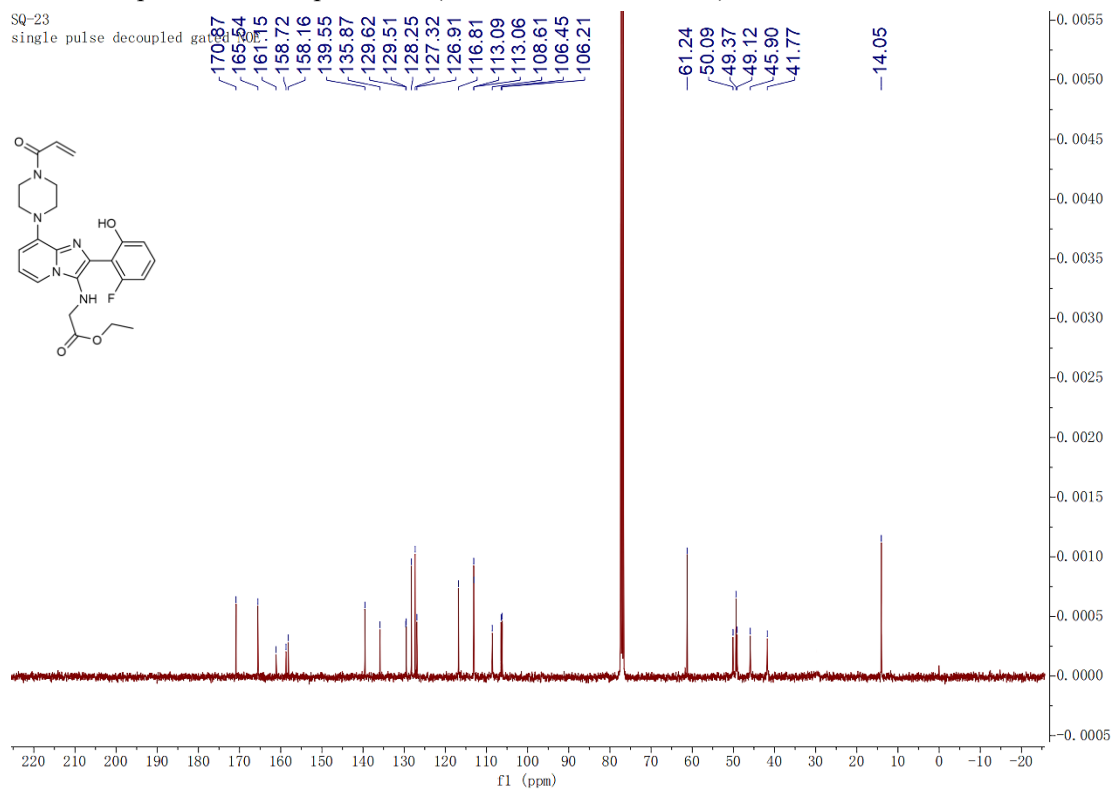
¹³C NMR spectrum of compound I-5 (100 MHz, Chloroform-*d*)



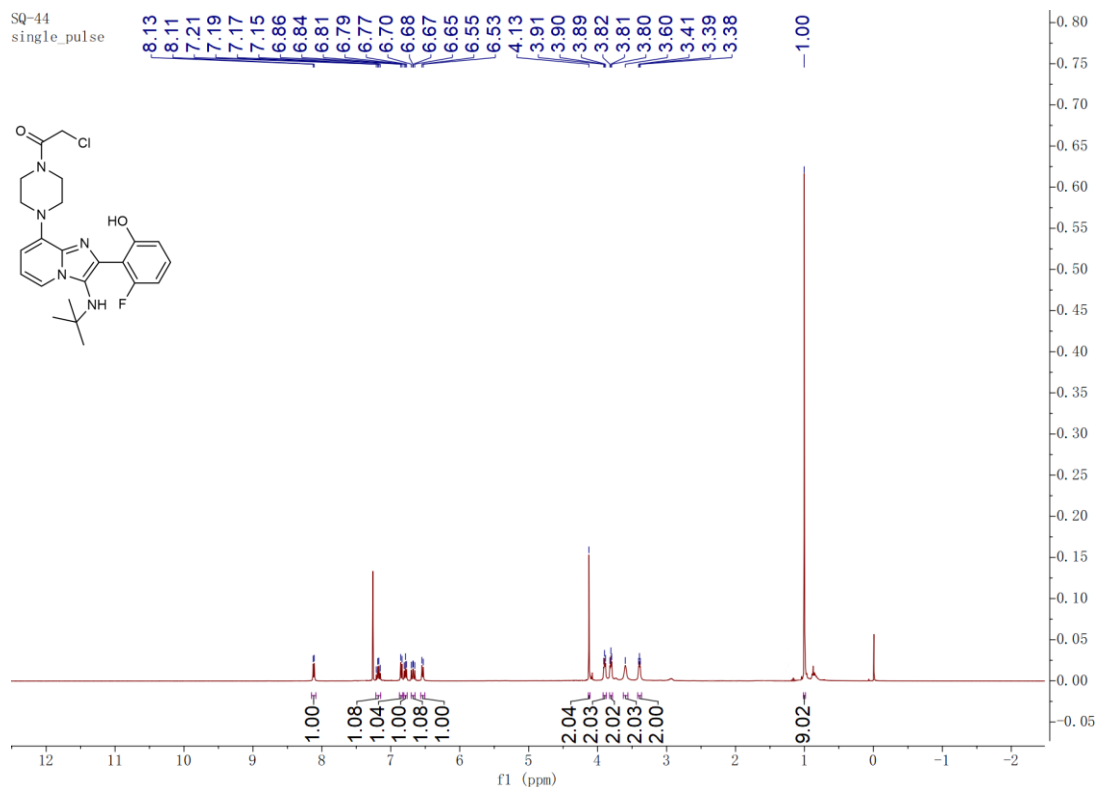
¹H NMR spectrum of compound I-6 (400 MHz, Chloroform-*d*)



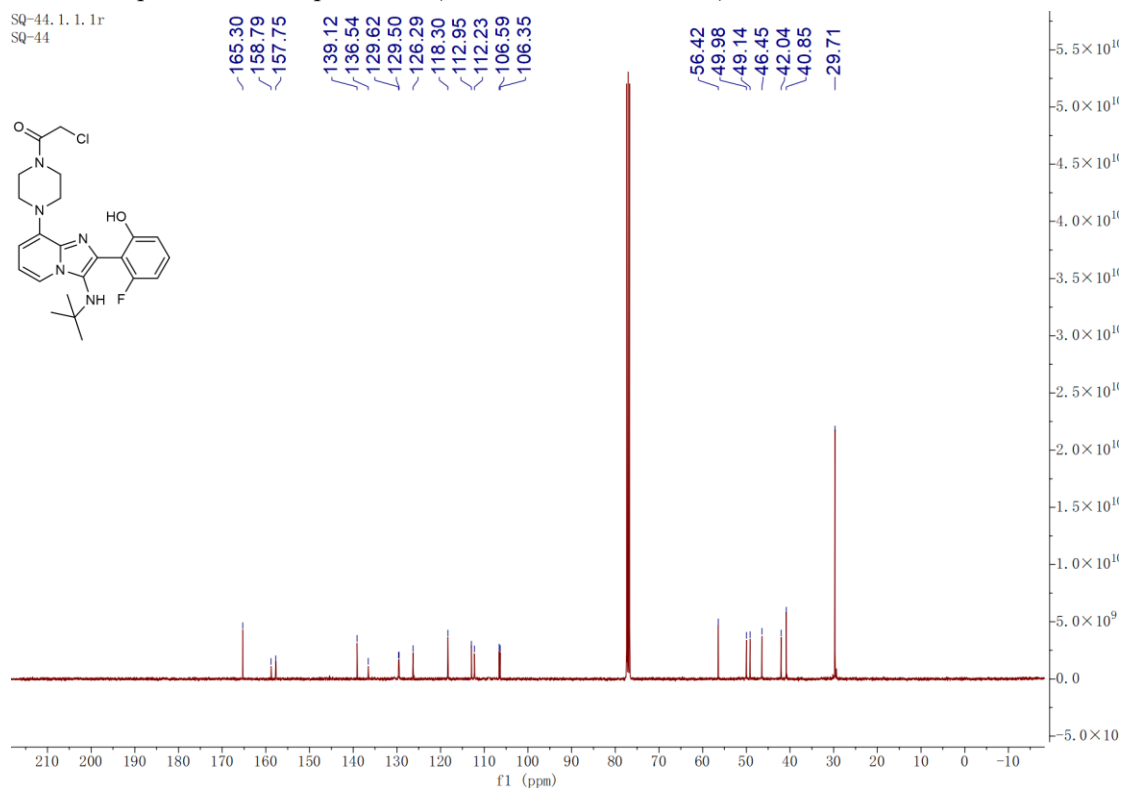
¹³C NMR spectrum of compound I-6 (100 MHz, Chloroform-*d*)



¹H NMR spectrum of compound I-7 (400 MHz, Chloroform-*d*)

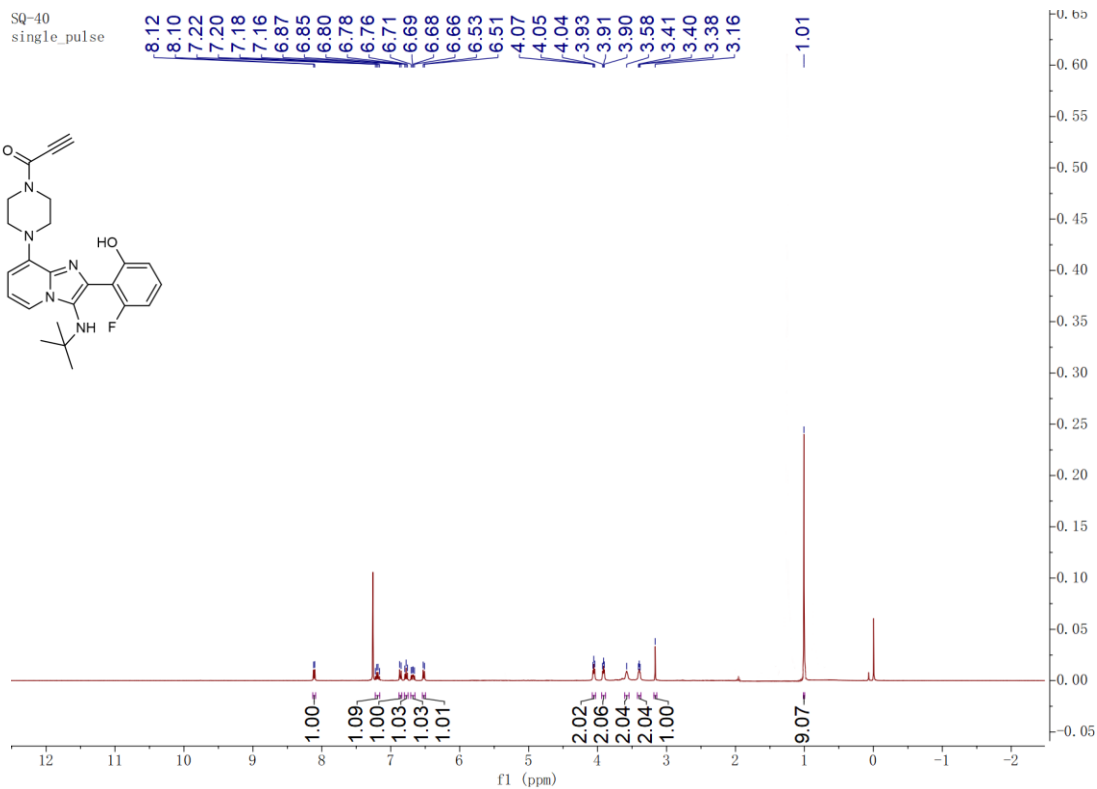


¹³C NMR spectrum of compound I-7 (100 MHz, Chloroform-*d*)



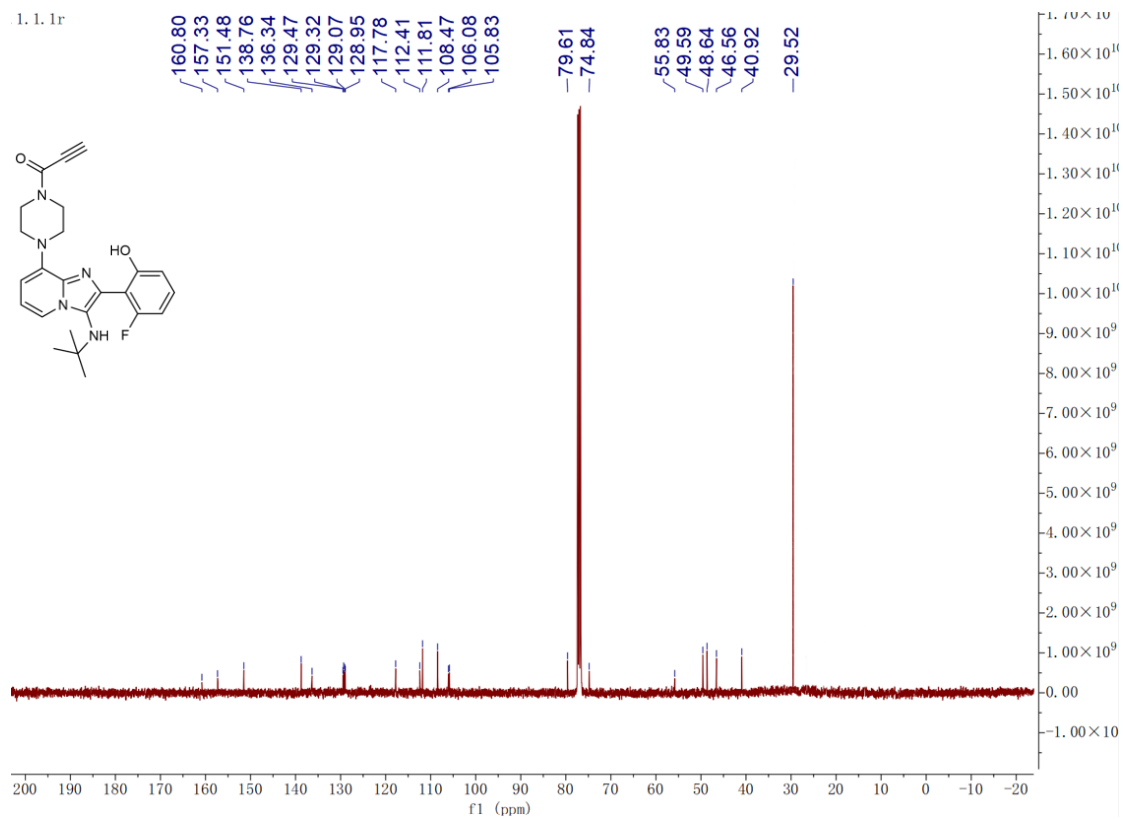
¹H NMR spectrum of compound **I-8** (400 MHz, Chloroform-*d*)

SQ-40
single_pulse

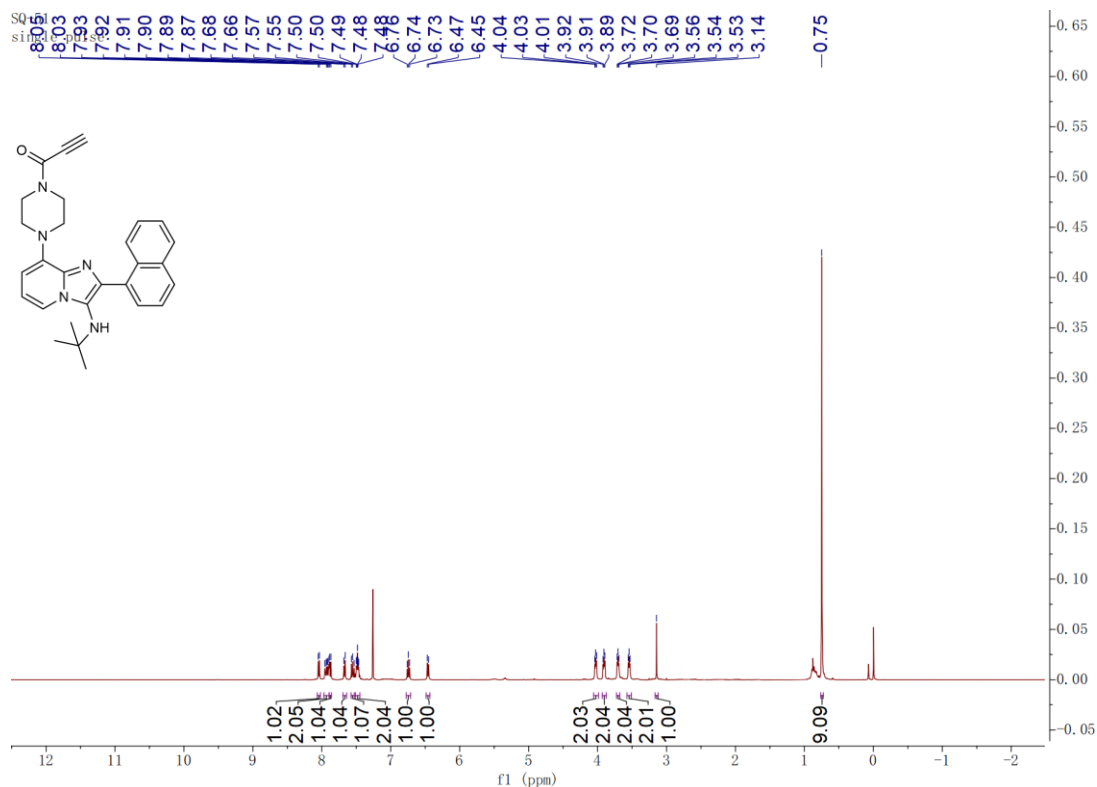


¹³C NMR spectrum of compound **I-8** (100 MHz, Chloroform-*d*)

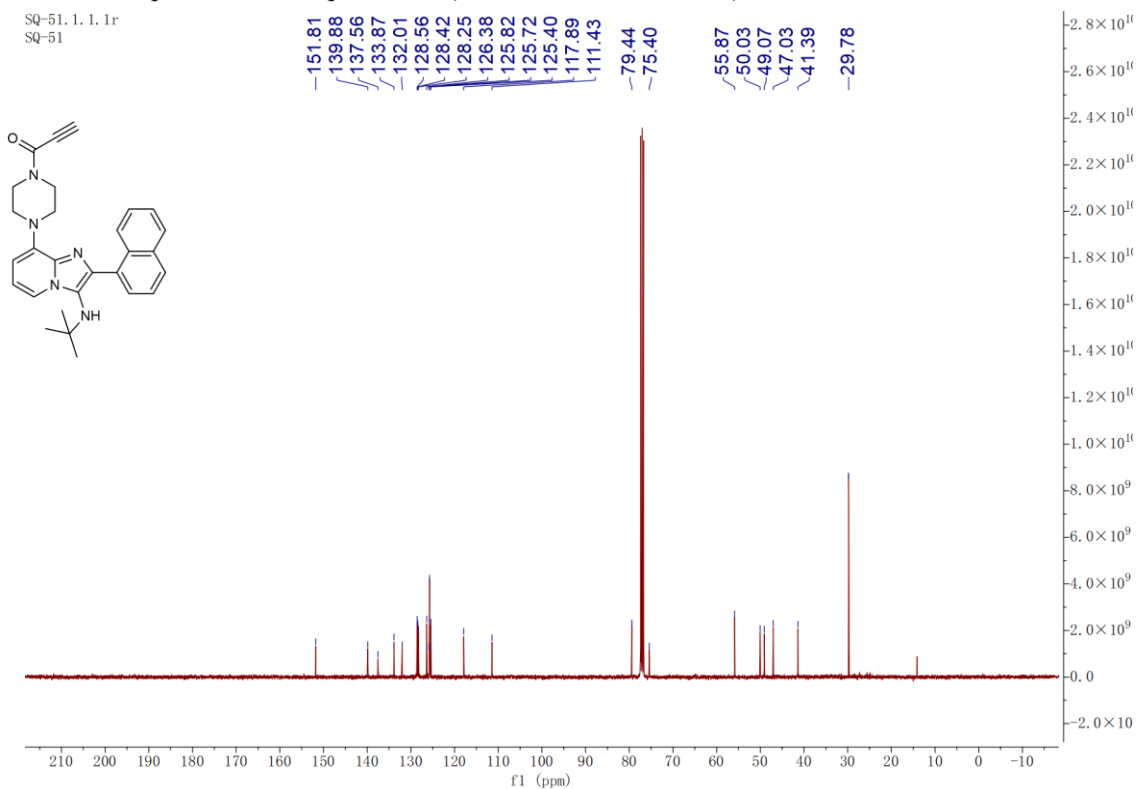
1. 1. 1r



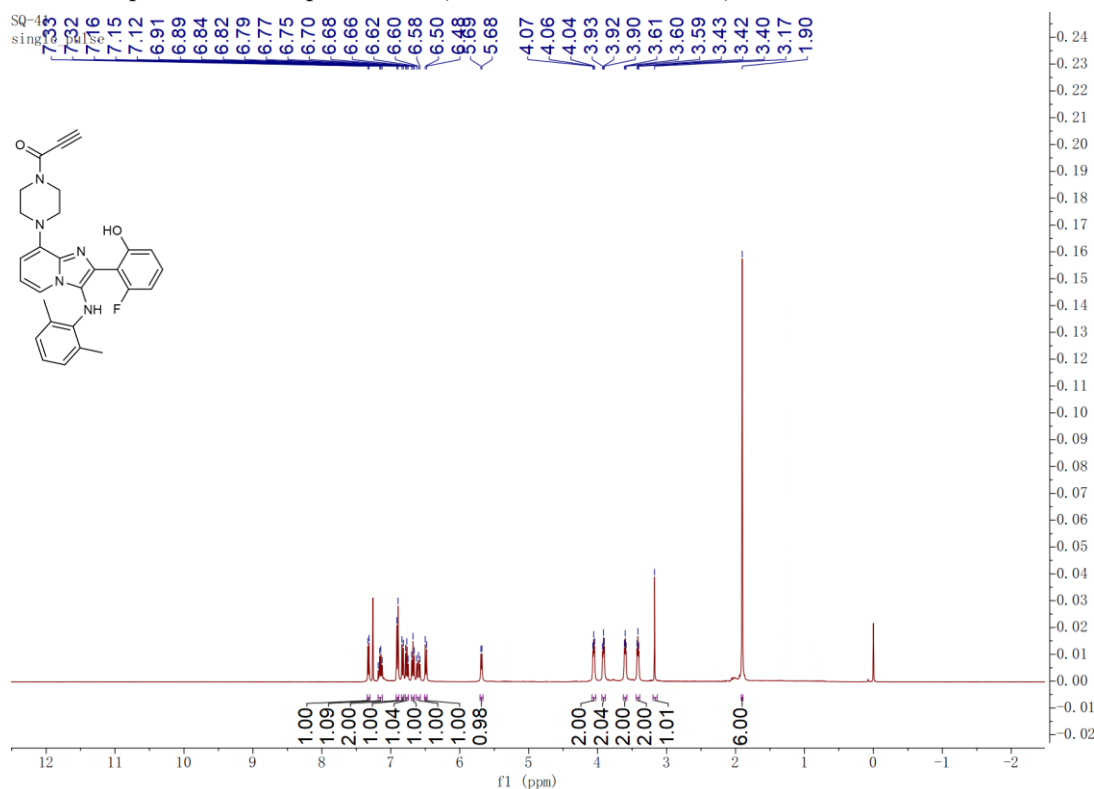
¹H NMR spectrum of compound I-9 (400 MHz, Chloroform-*d*)



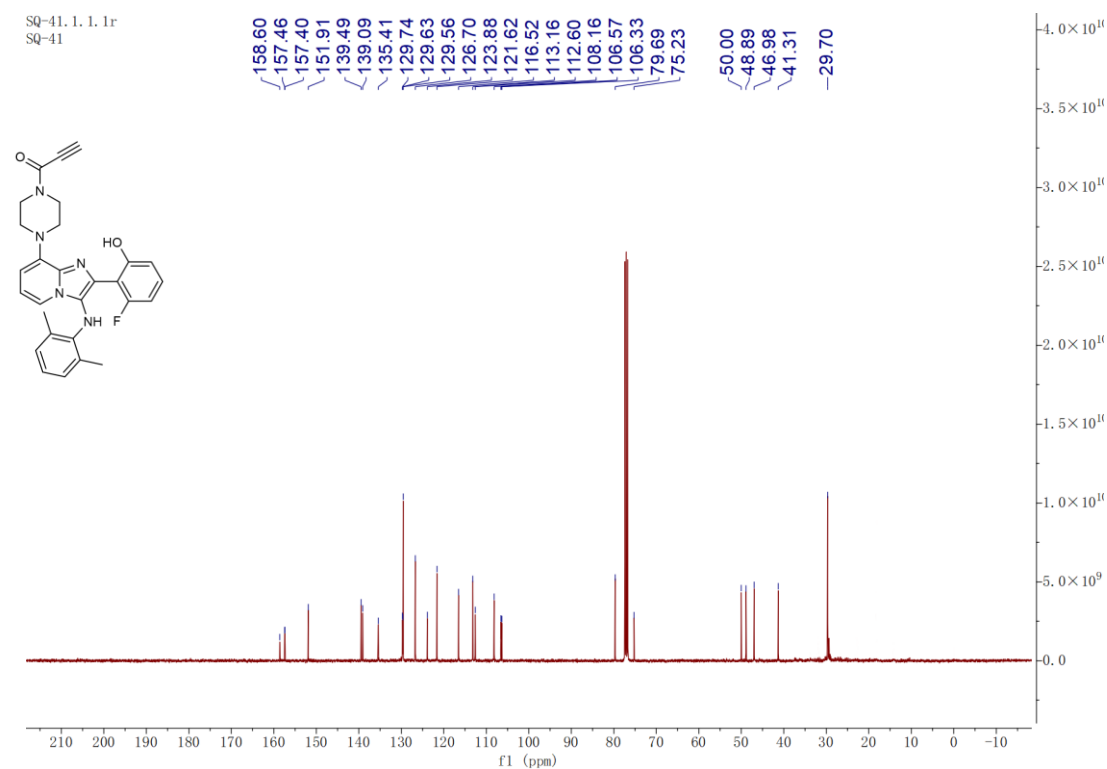
¹³C NMR spectrum of compound I-9 (100 MHz, Chloroform-*d*)



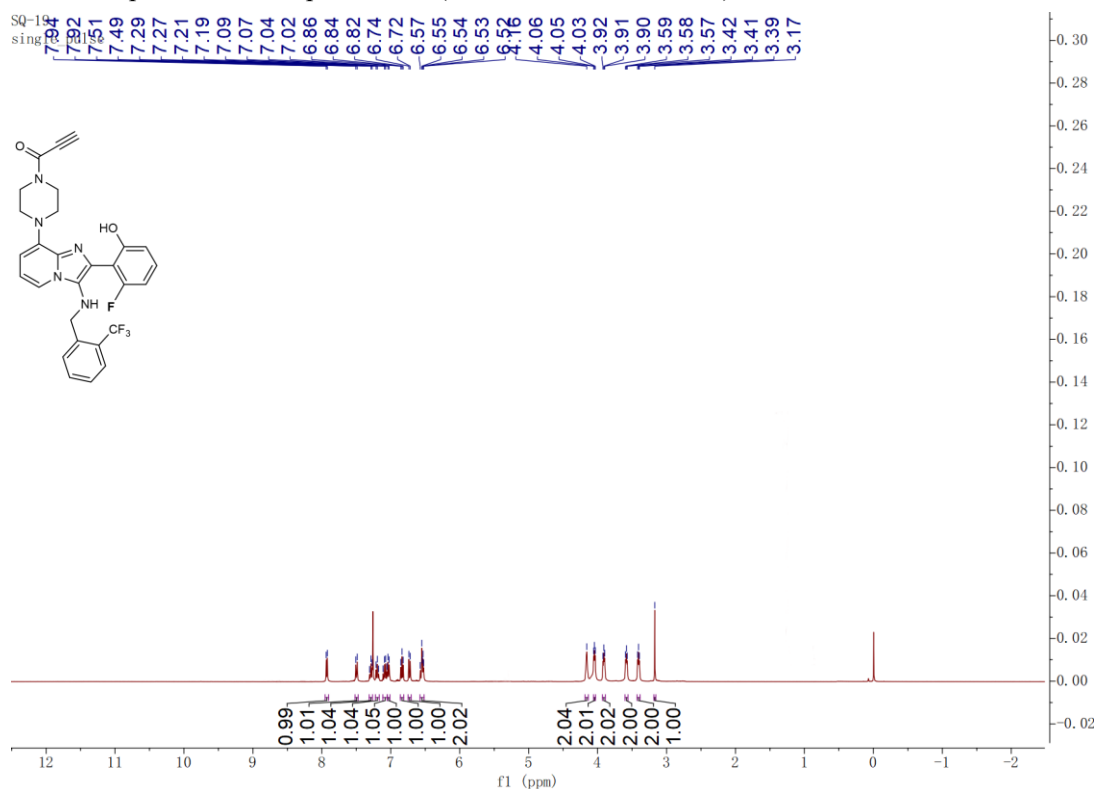
¹H NMR spectrum of compound **I-10** (400 MHz, Chloroform-*d*)



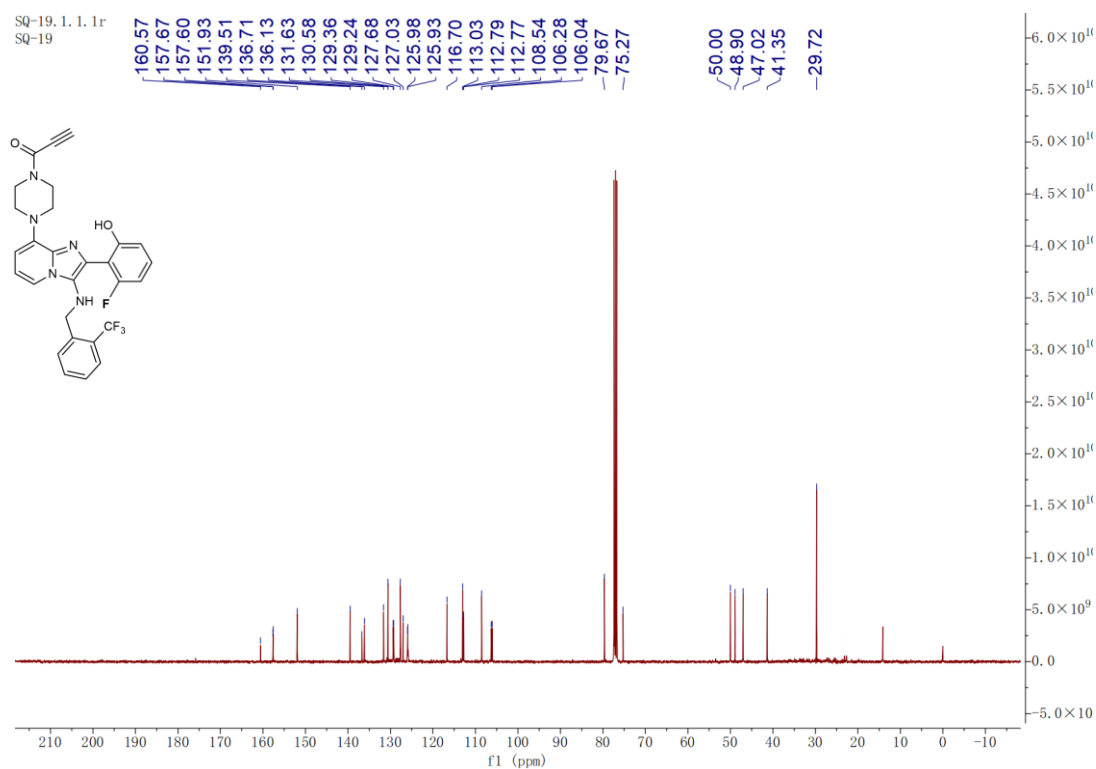
¹³C NMR spectrum of compound **I-10** (100 MHz, Chloroform-*d*)



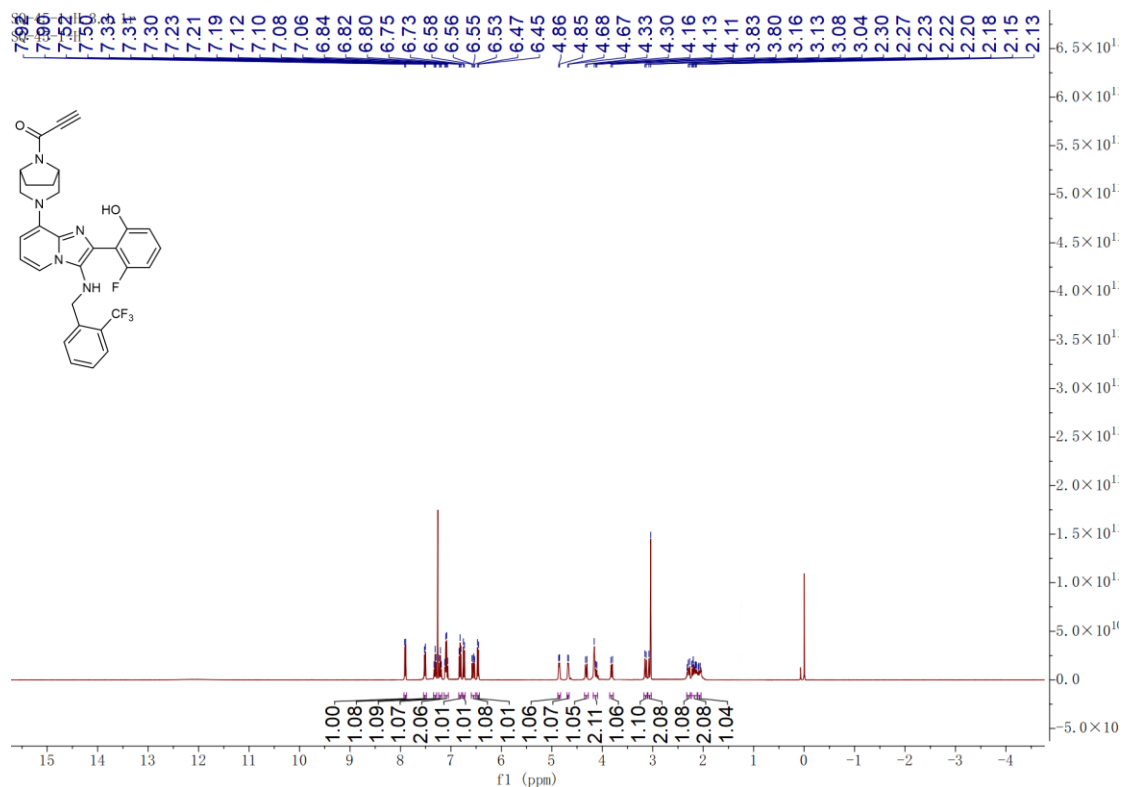
¹H NMR spectrum of compound **I-11** (400 MHz, Chloroform-*d*)



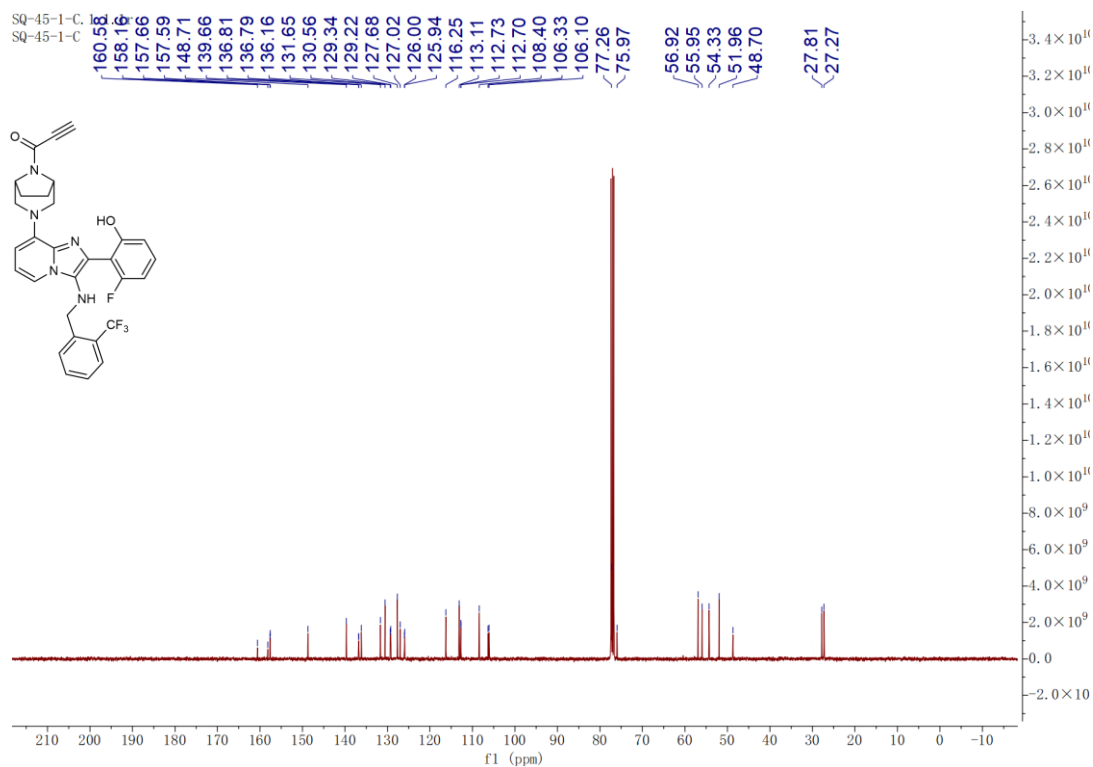
¹³C NMR spectrum of compound **I-11** (100 MHz, Chloroform-*d*)



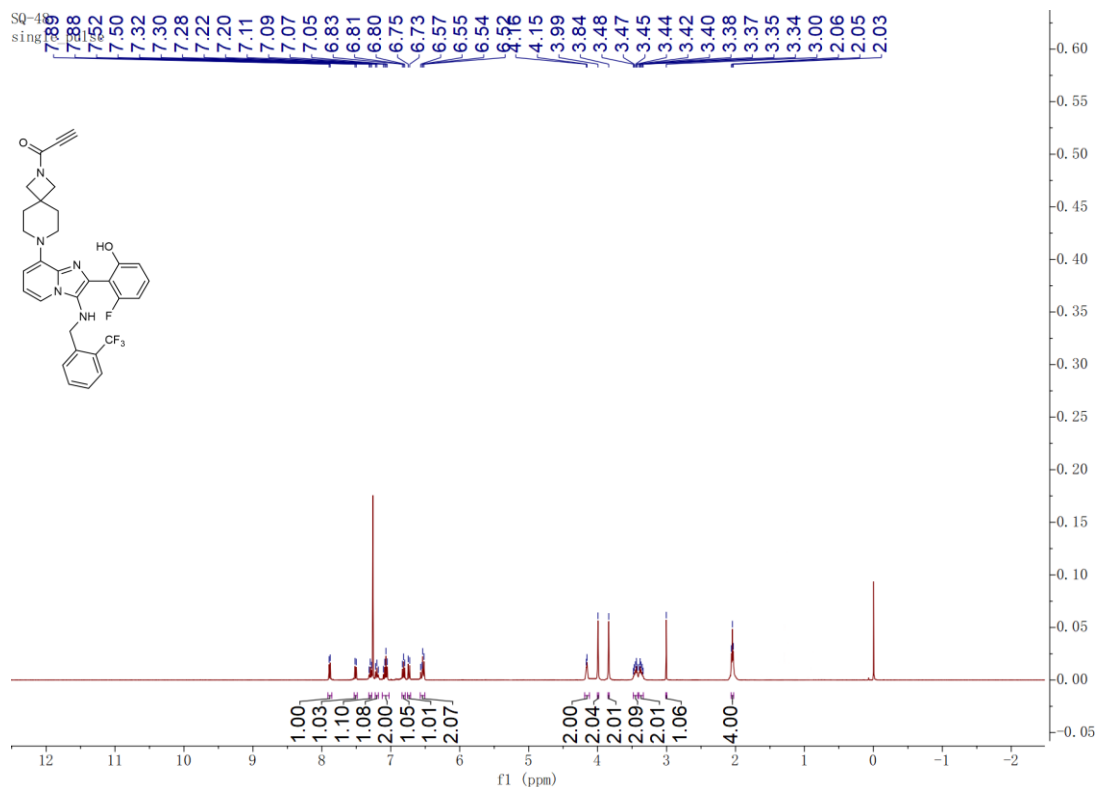
¹H NMR spectrum of compound **I-12** (400 MHz, Chloroform-*d*)



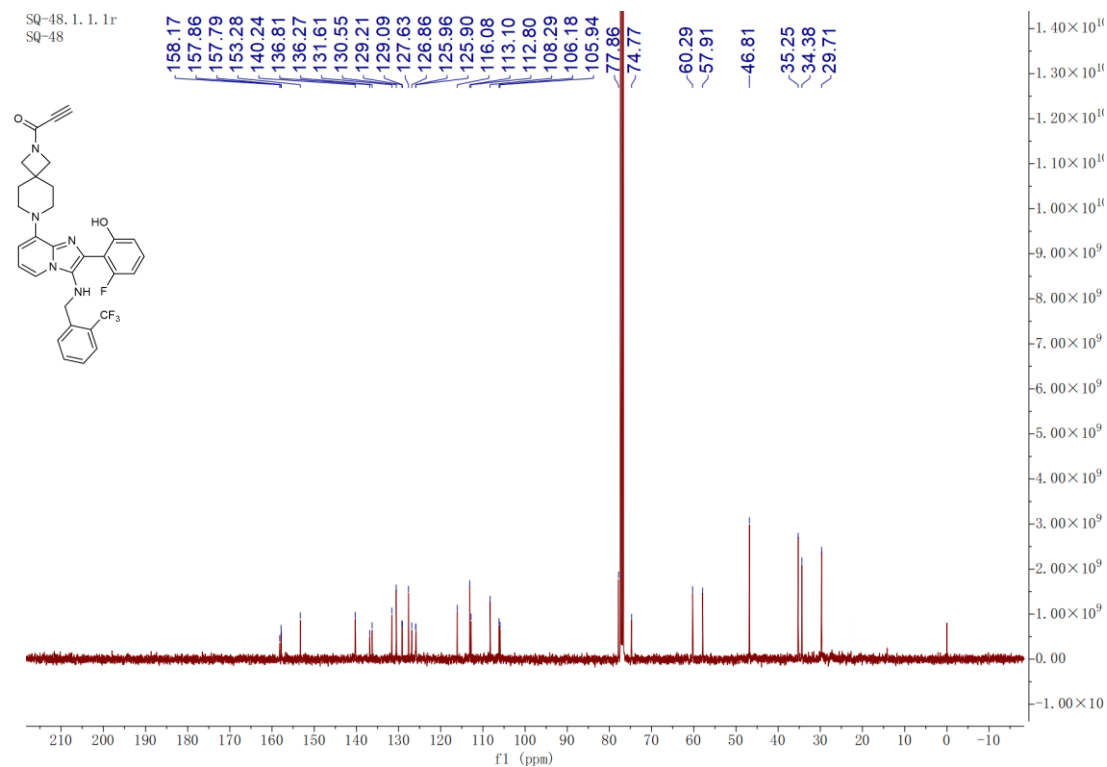
¹³C NMR spectrum of compound **I-12** (100 MHz, Chloroform-*d*)



¹H NMR spectrum of compound **I-13** (400 MHz, Chloroform-*d*)

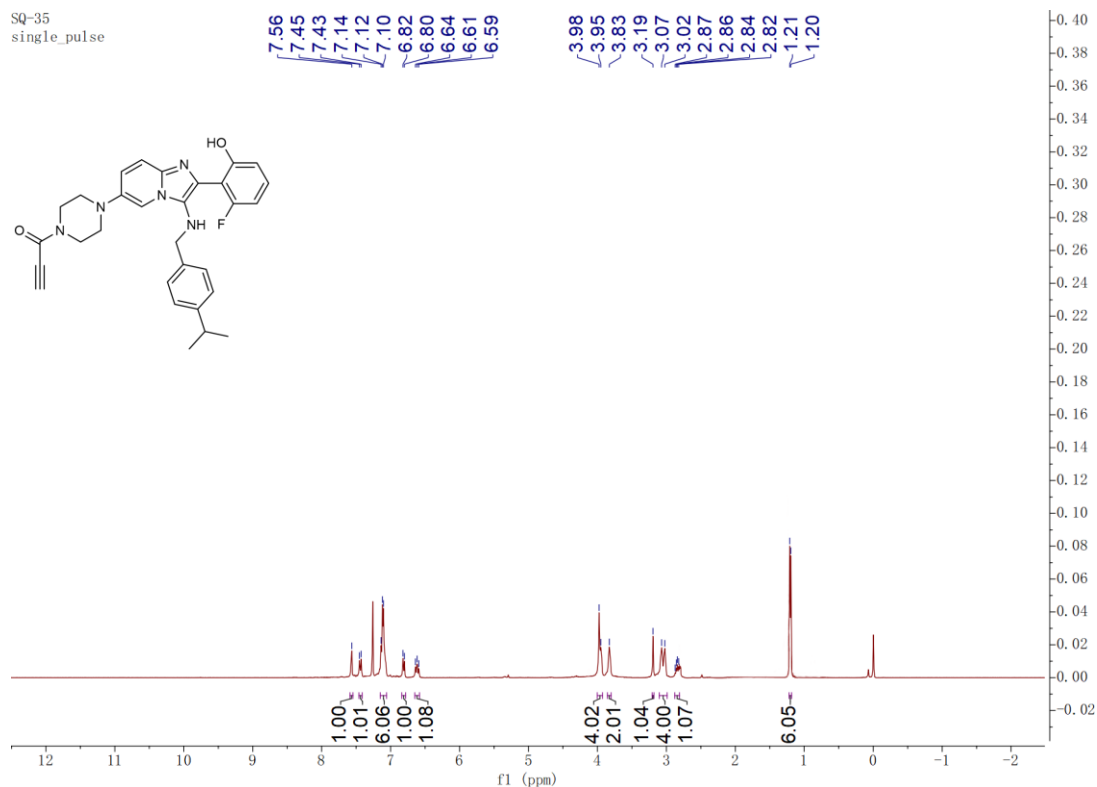


¹³C NMR spectrum of compound **I-13** (100 MHz, Chloroform-*d*)

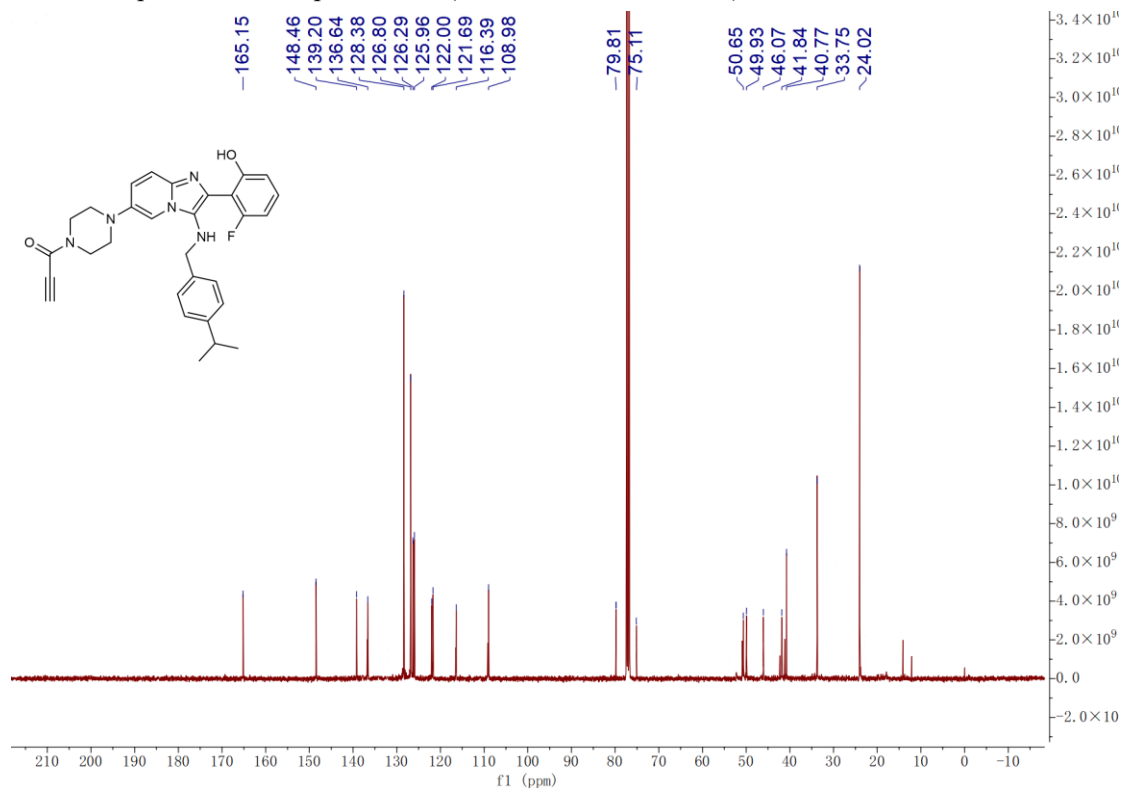


¹H NMR spectrum of compound **I-14** (400 MHz, Chloroform-*d*)

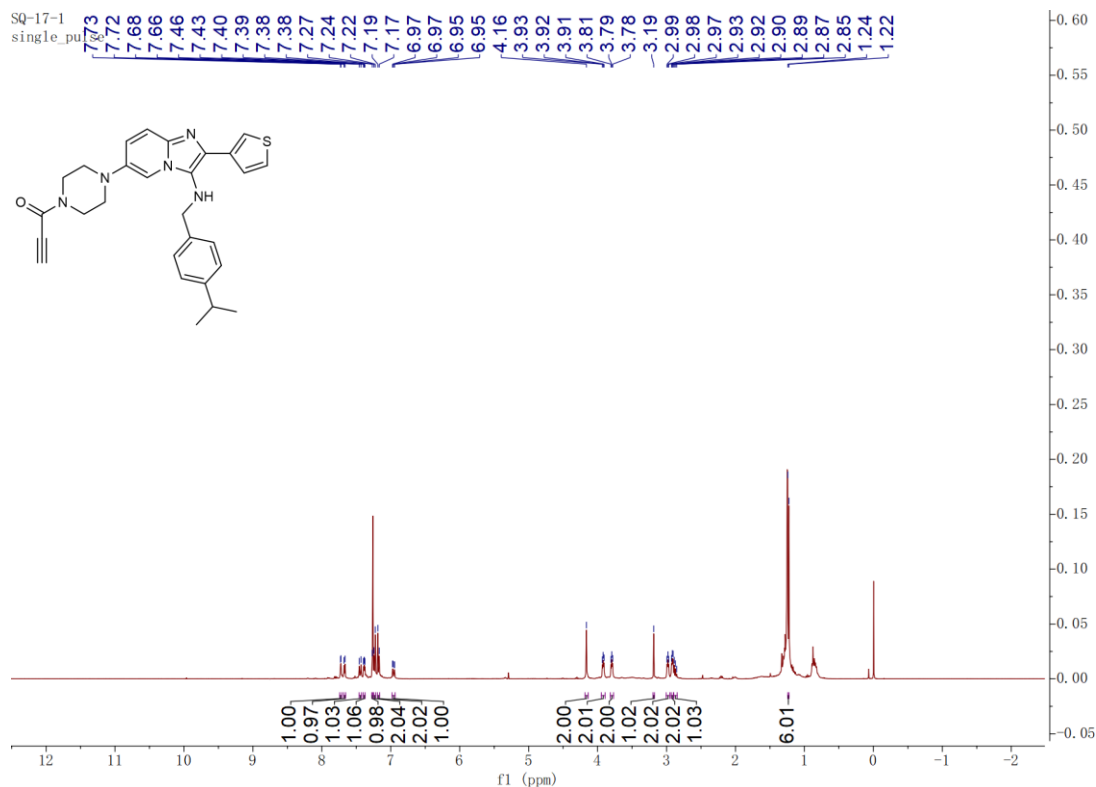
SQ-35
single_pulse



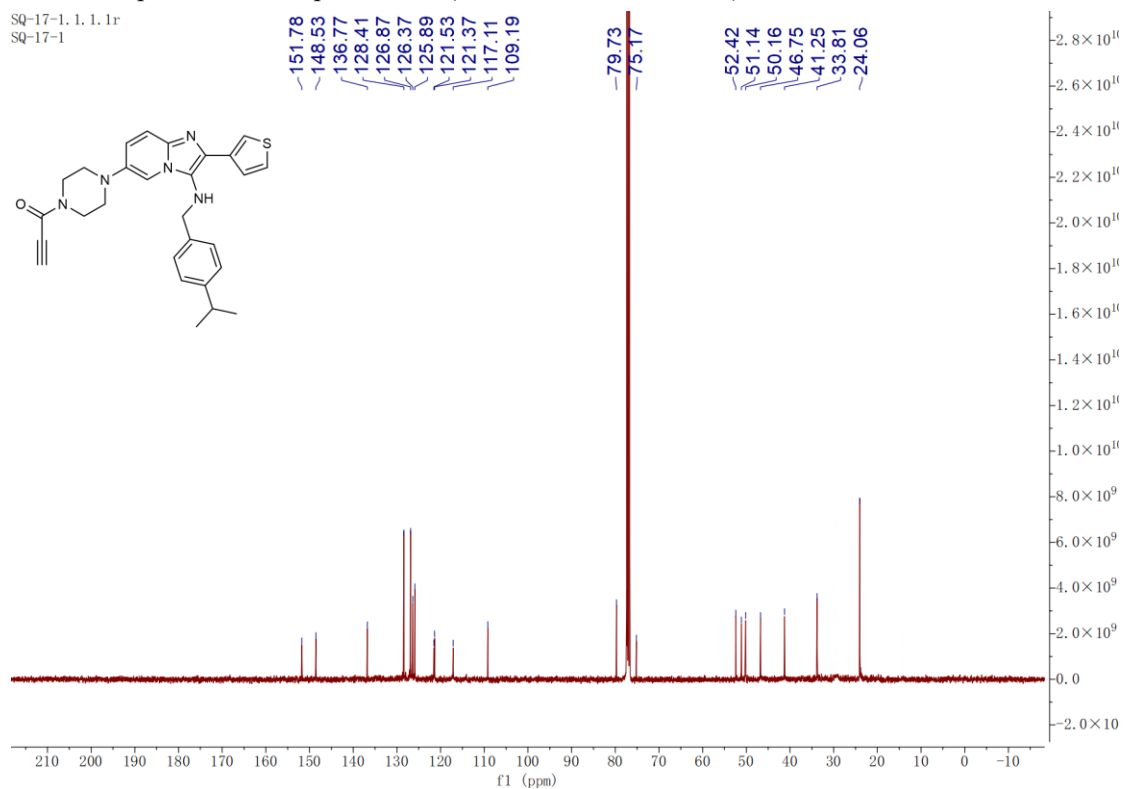
¹³C NMR spectrum of compound **I-14** (100 MHz, Chloroform-*d*)



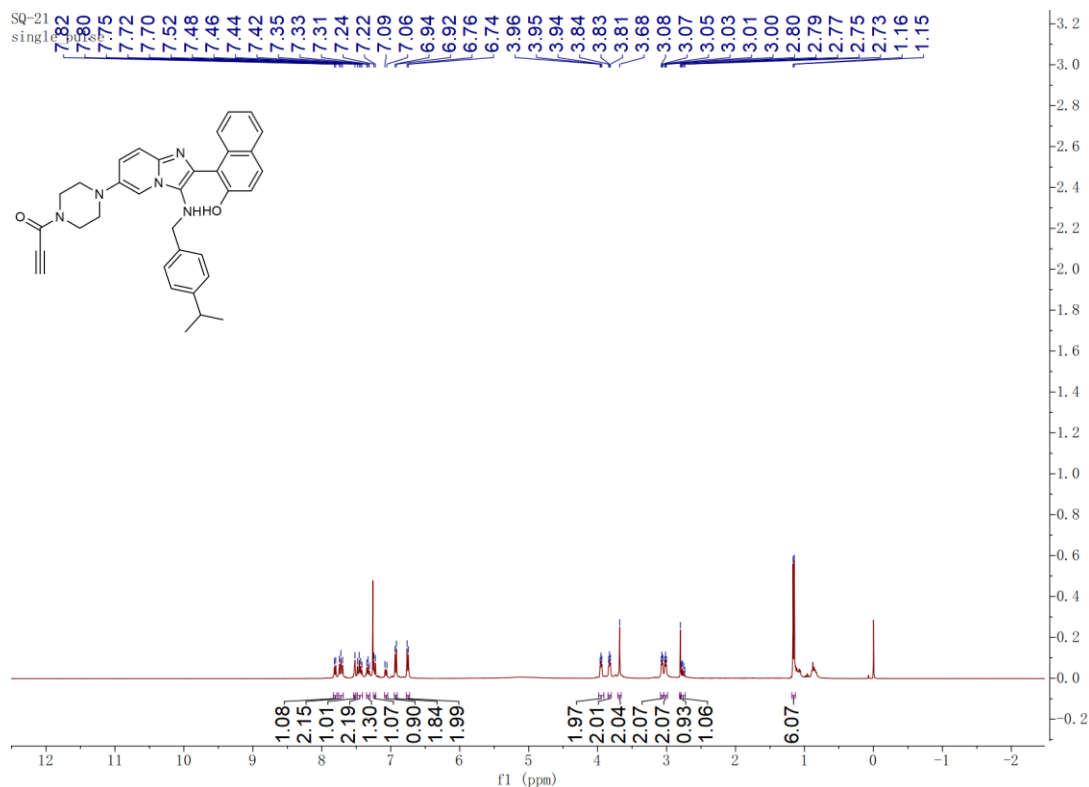
¹H NMR spectrum of compound I-15 (400 MHz, Chloroform-*d*)



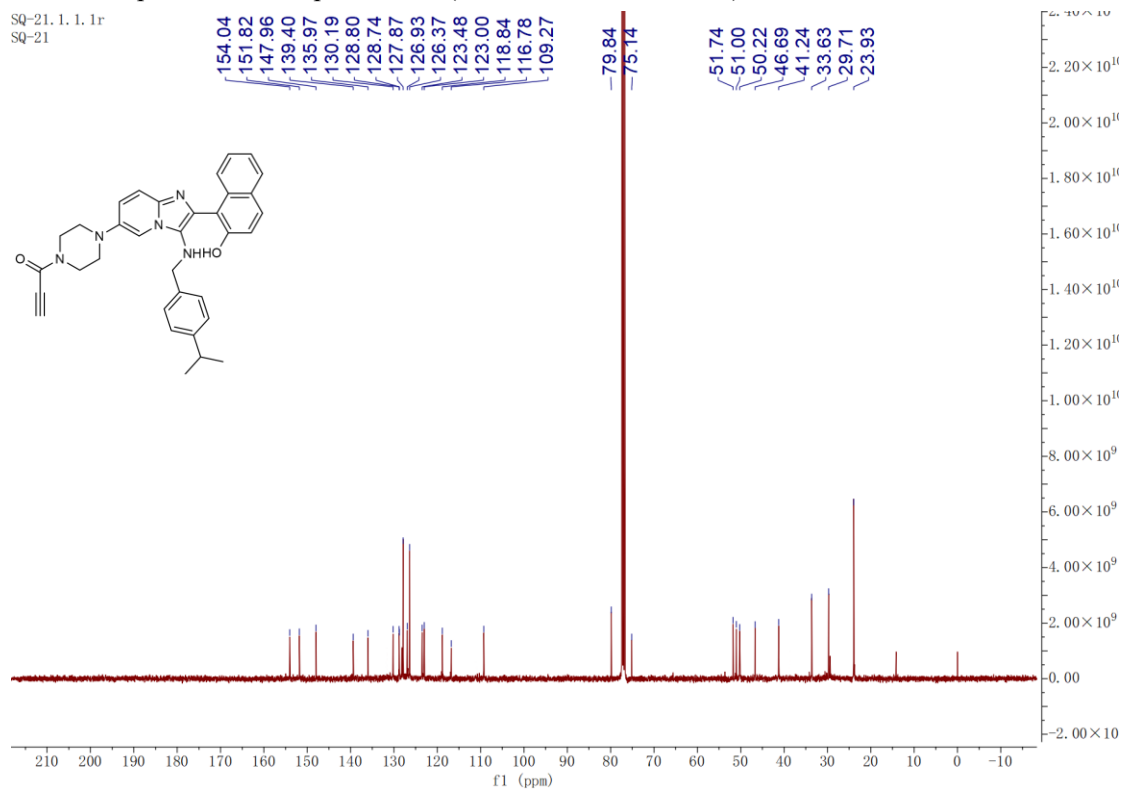
¹³C NMR spectrum of compound I-15 (100 MHz, Chloroform-*d*)



¹H NMR spectrum of compound **I-16** (400 MHz, Chloroform-*d*)

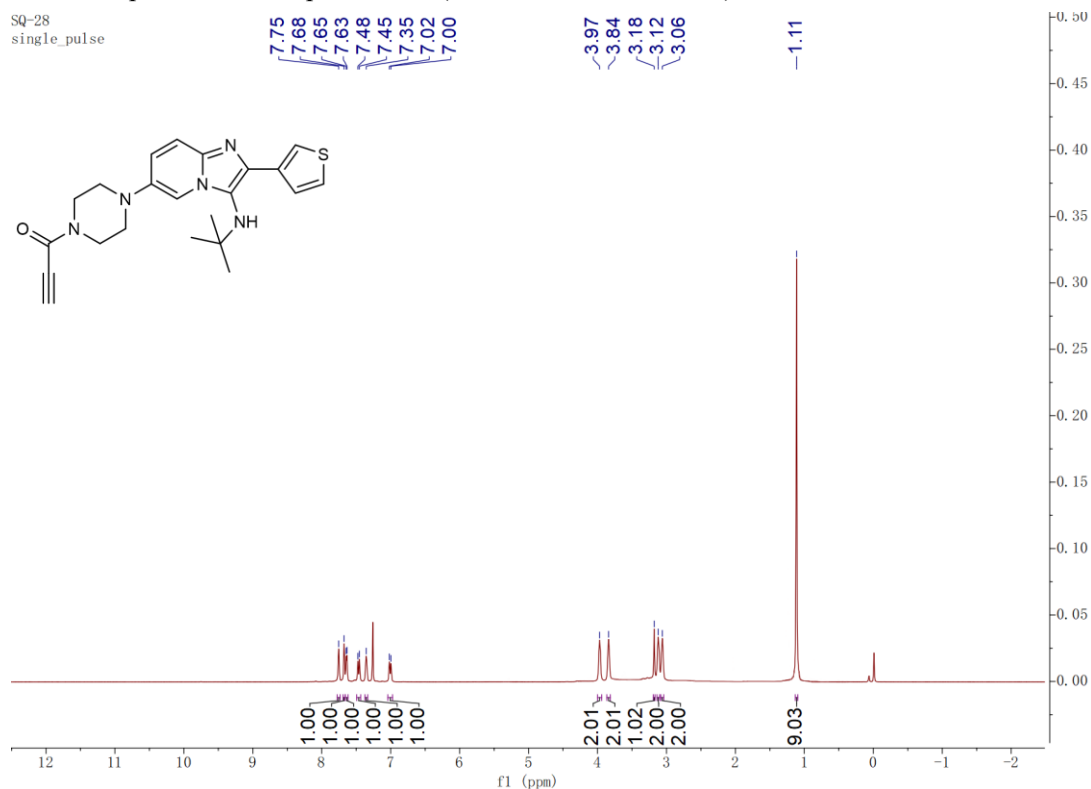


¹³C NMR spectrum of compound **I-16** (100 MHz, Chloroform-*d*)



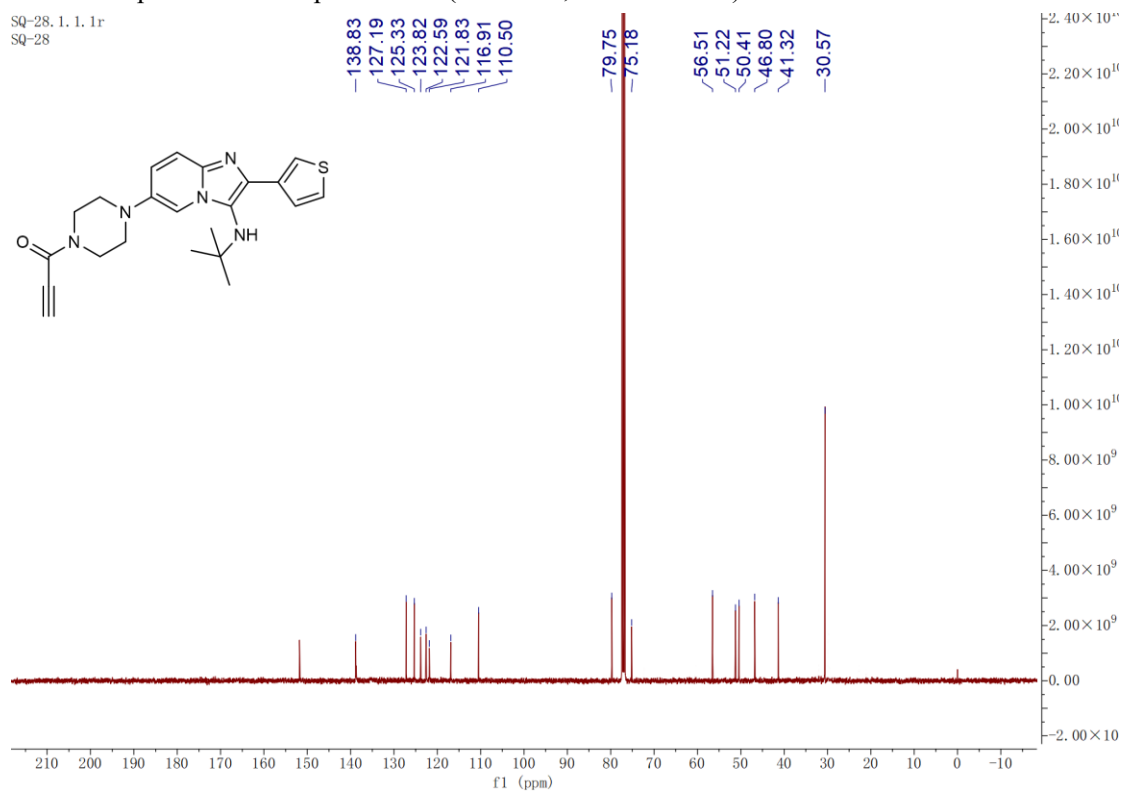
¹H NMR spectrum of compound I-17 (400 MHz, Chloroform-*d*)

SQ-28
single_pulse



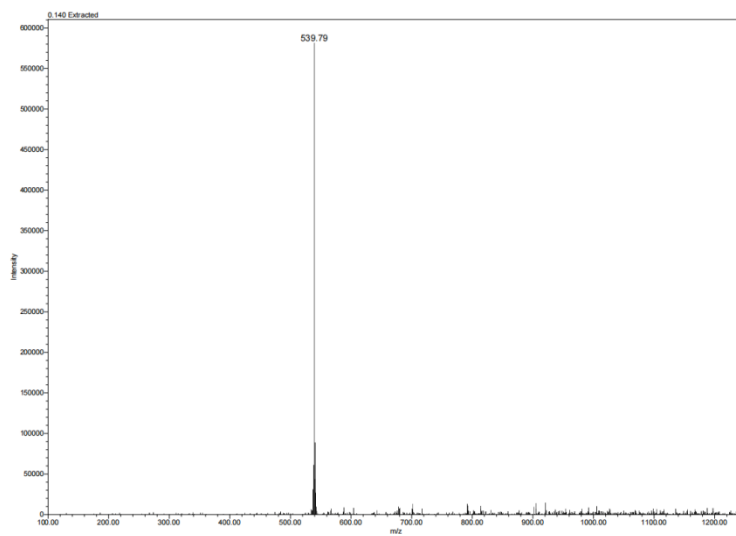
¹³C NMR spectrum of compound I-17 (100 MHz, Chloroform-*d*)

SQ-28.1.1.1r
SQ-28

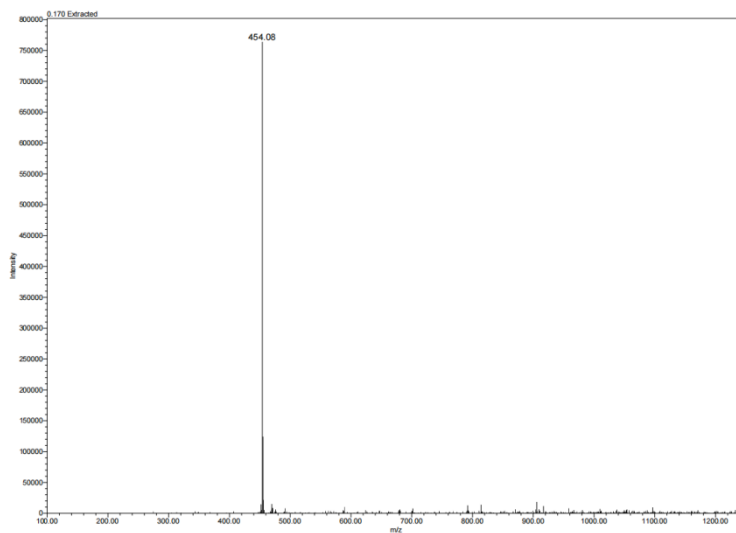


3. MS and HRMS spectra of compounds synthesized

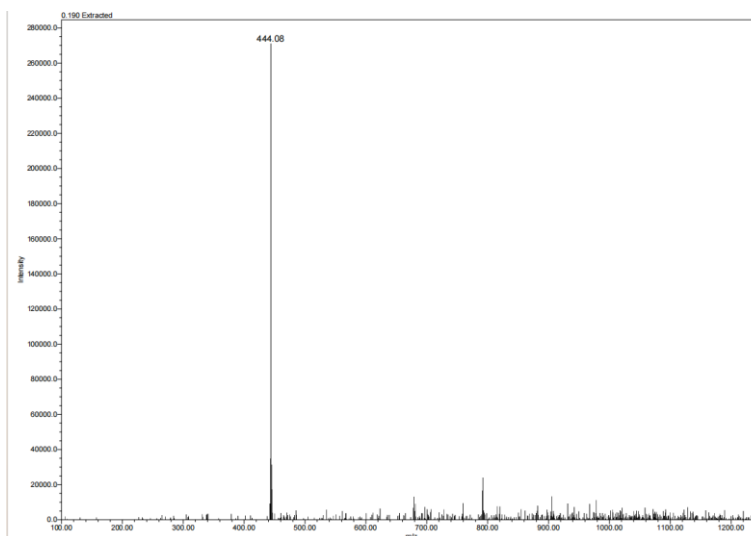
I-1 MS



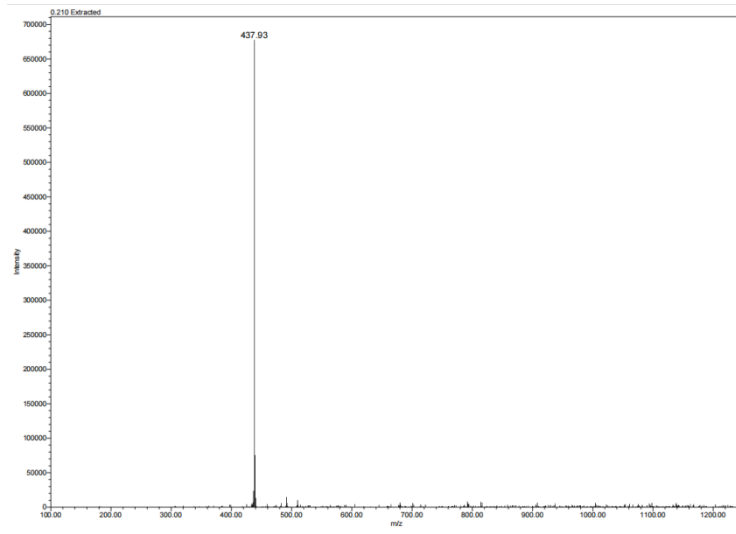
I-2 MS



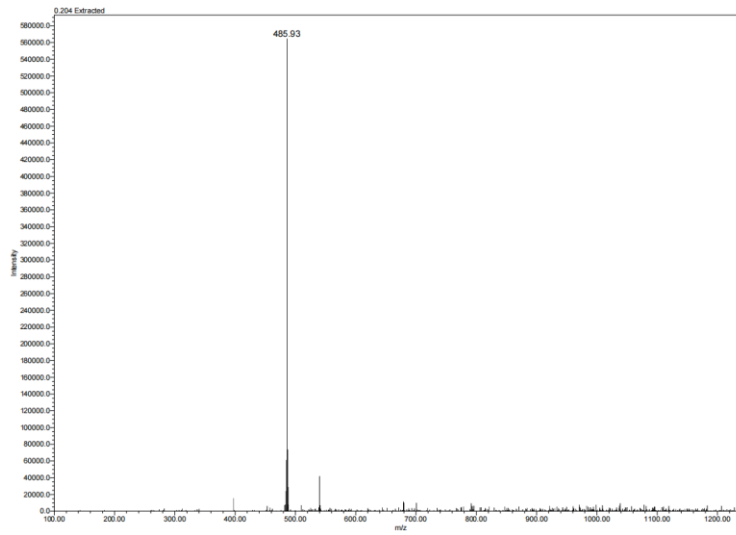
I-3 MS



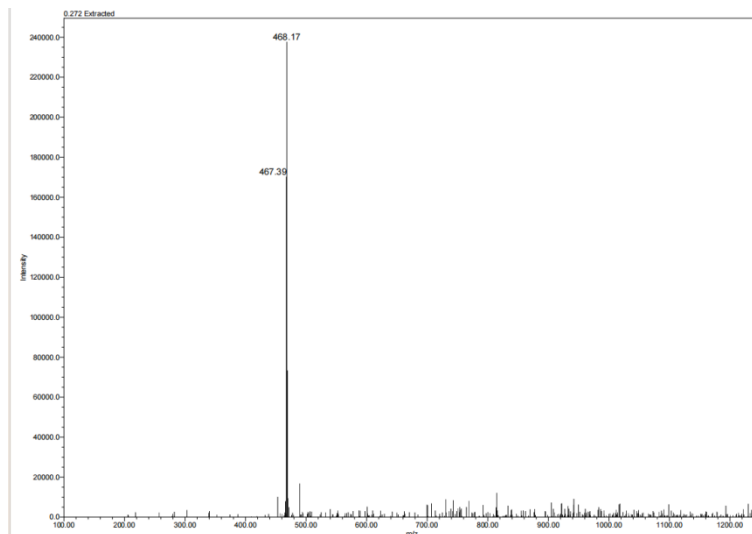
I-4 MS



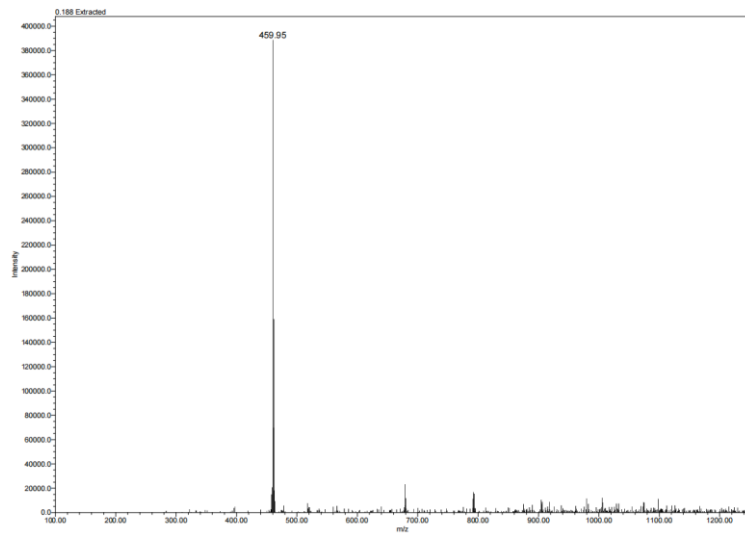
I-5 MS



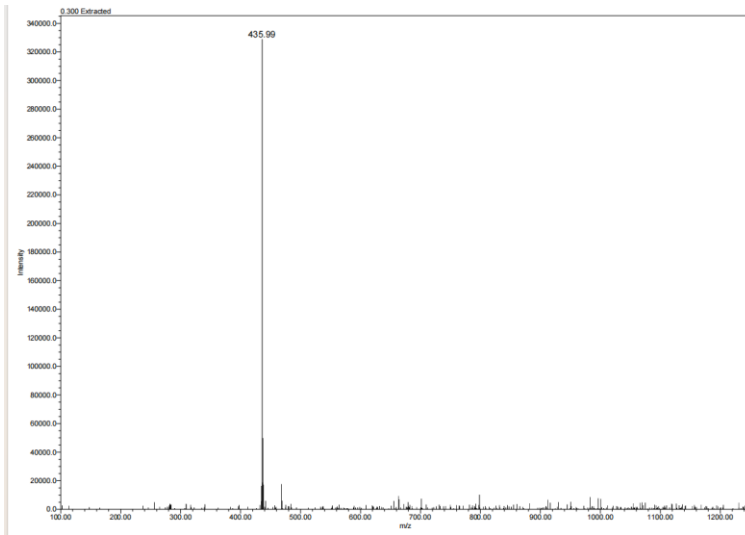
I-6 MS



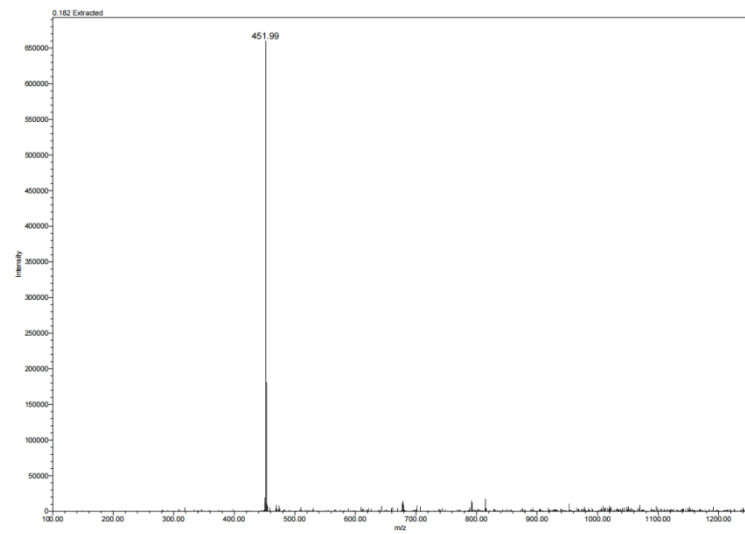
I-7 MS



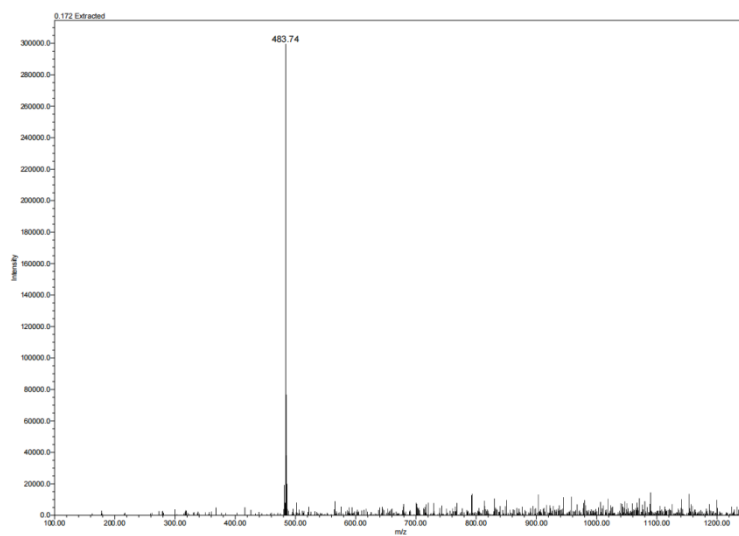
I-8 MS



I-9 MS

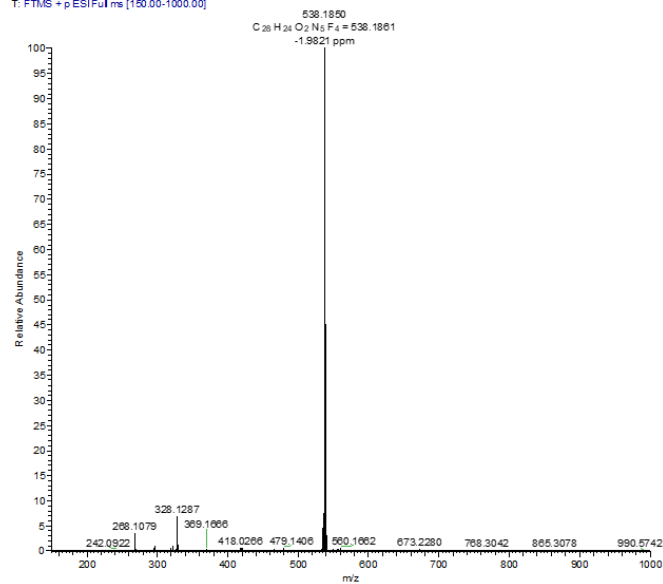


I-10 MS

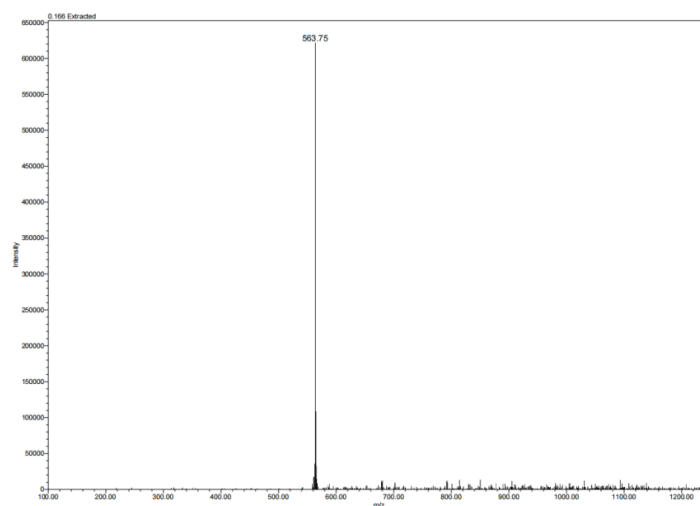


I-11 HRMS

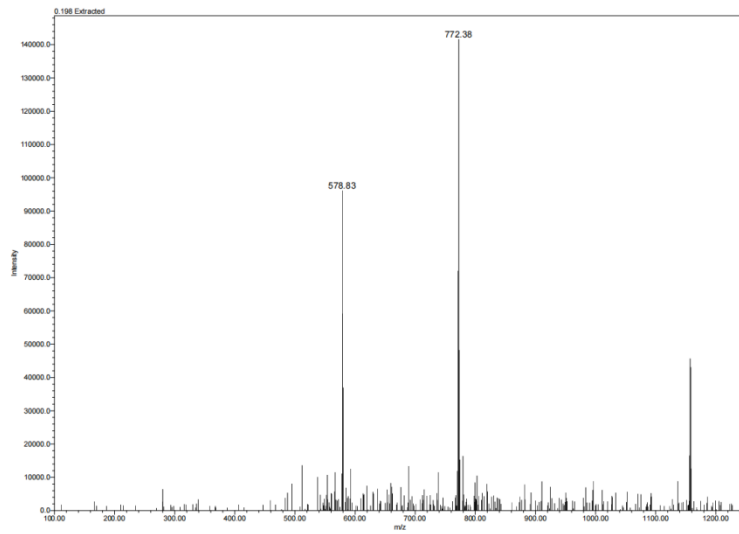
20231010-SQ-19_231010083137 #25 RT: 0.20 AV: 1 NL: 2.05E8
T: FTMS + p ESIFul ms [150.00-1000.00]



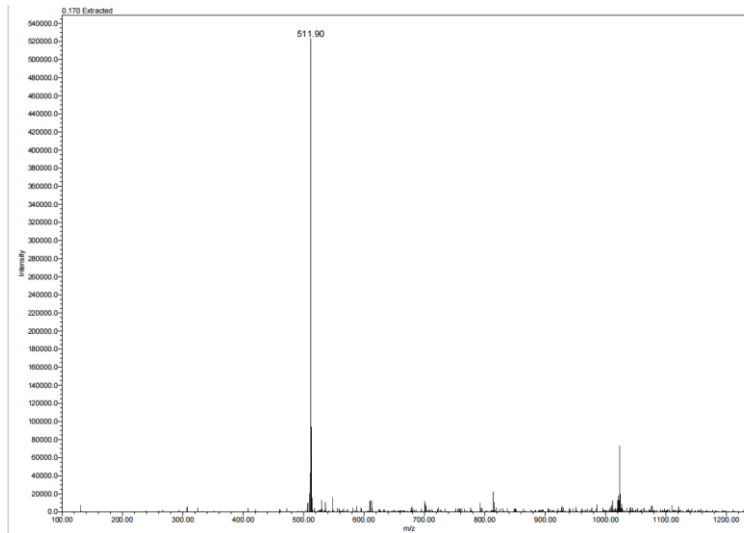
I-12 MS



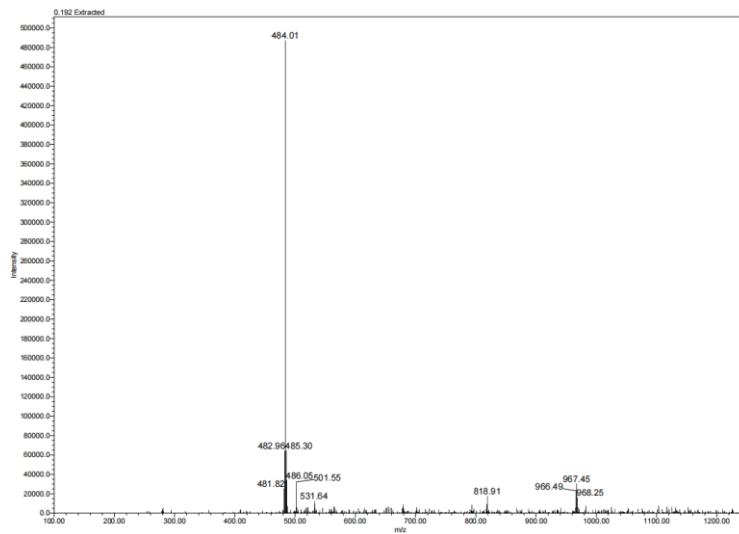
I-13 MS



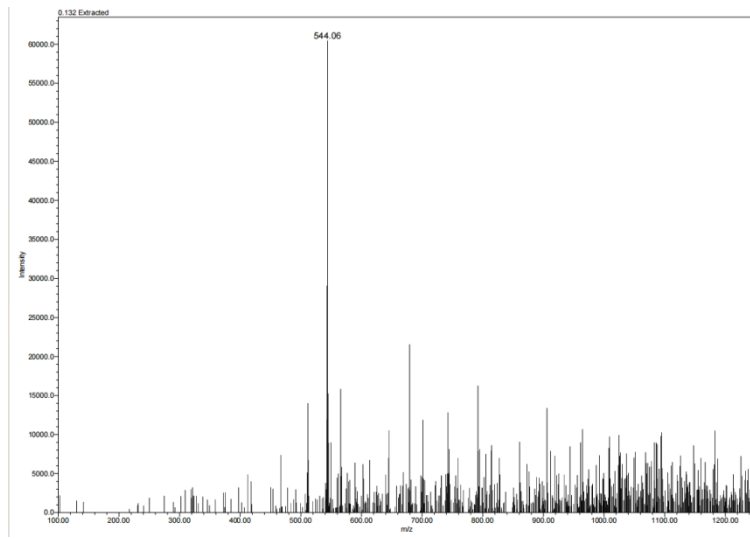
I-14 MS



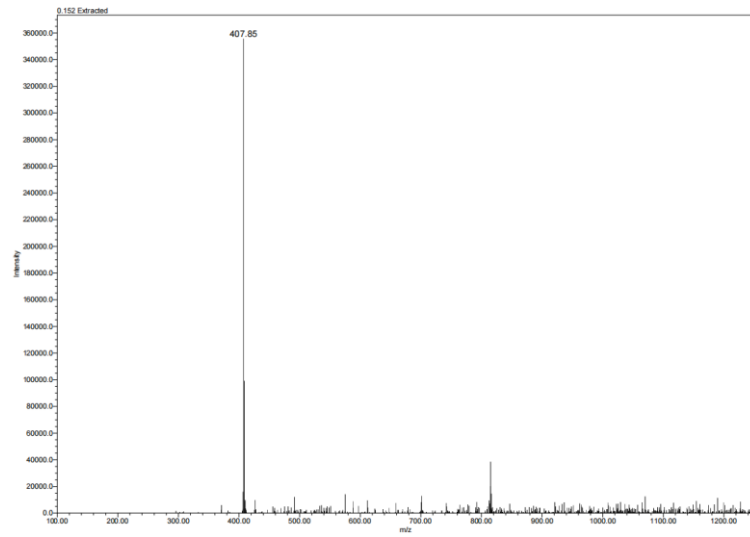
I-15 MS



I-16 MS



I-17 MS

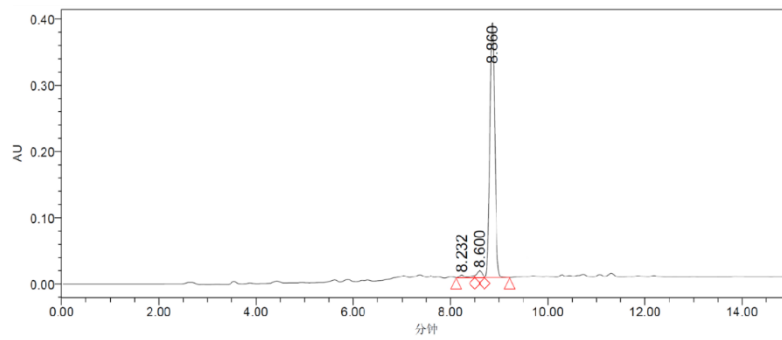


4. HPLC analysis for target compounds

Compound I-1

Result

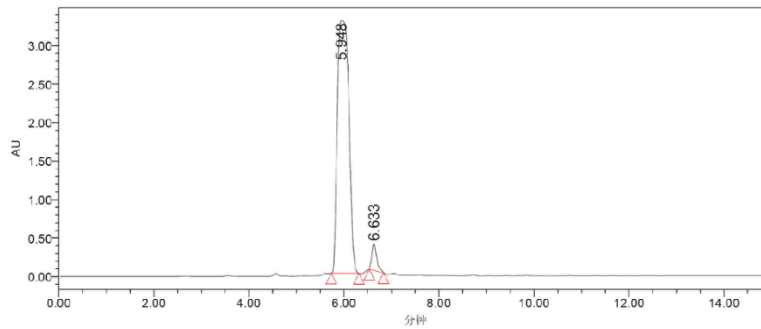
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|---------|---------------|
| 1 | 8.232 | 52715 | 1.85 |
| 2 | 8.6 | 81430 | 2.85 |
| 3 | 8.86 | 2721148 | 95.3 |



Compound I-2

Result

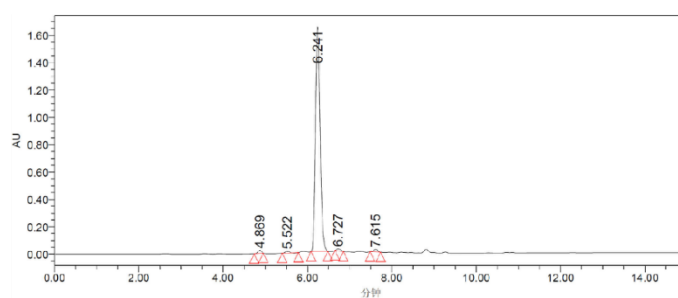
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 5.948 | 55431431 | 95.72 |
| 2 | 6.633 | 2481496 | 4.28 |



Compound I-3

Result

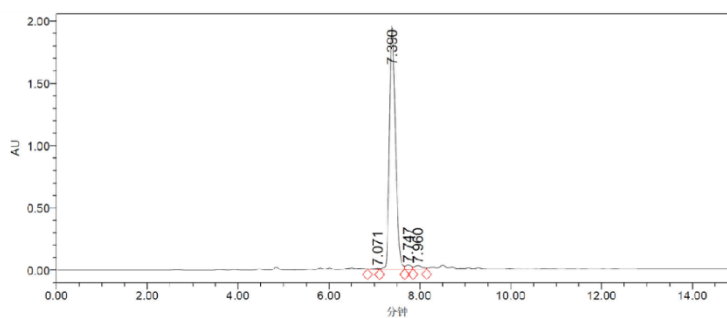
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 4.869 | 127630 | 0.96 |
| 2 | 5.522 | 97082 | 0.73 |
| 3 | 6.241 | 12802185 | 96.64 |
| 4 | 6.727 | 100173 | 0.76 |
| 5 | 7.615 | 120128 | 0.91 |



Compound I-4

Result

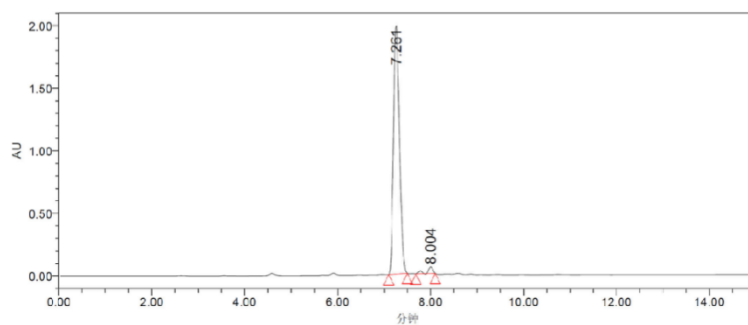
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 7.071 | 80589 | 0.43 |
| 2 | 7.39 | 18131720 | 96.22 |
| 3 | 7.747 | 291545 | 1.55 |
| 4 | 7.96 | 340022 | 1.8 |



Compound I-5

Result

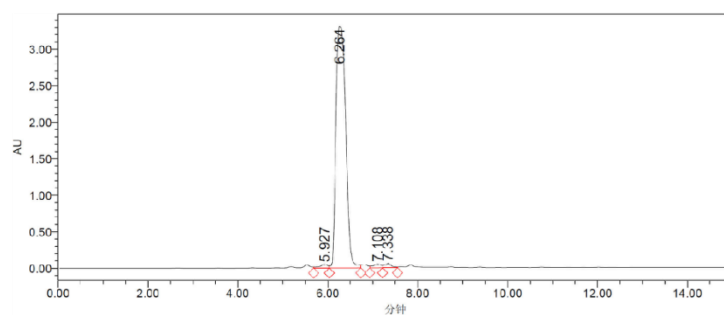
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 7.261 | 17763681 | 97.45 |
| 2 | 8.004 | 465606 | 2.55 |



Compound I-6

Result

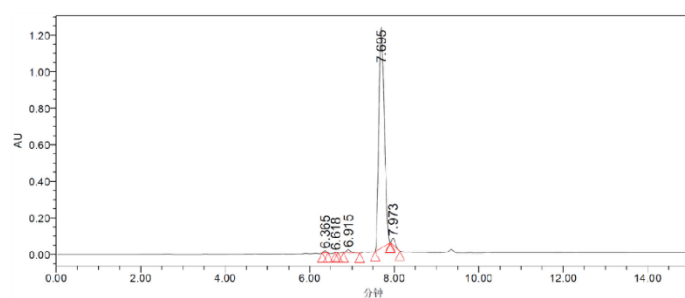
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 5.927 | 580374 | 1.12 |
| 2 | 6.264 | 49847386 | 96.5 |
| 3 | 7.108 | 596980 | 1.16 |
| 4 | 7.338 | 630957 | 1.22 |



Compound I-7

Result

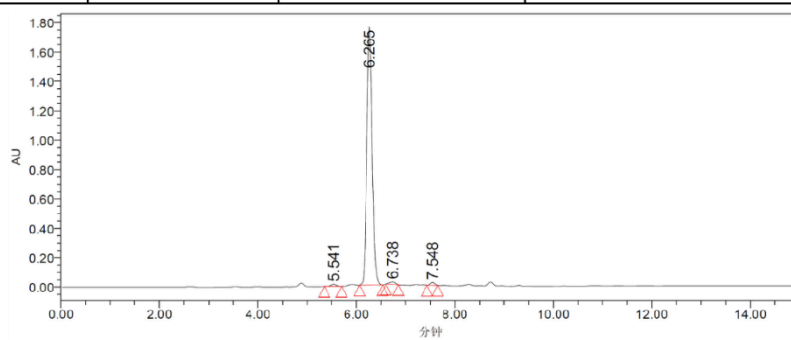
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 6.365 | 53760 | 0.48 |
| 2 | 6.618 | 2550 | 0.02 |
| 3 | 6.915 | 118437 | 1.05 |
| 4 | 7.695 | 10938125 | 96.74 |
| 5 | 7.973 | 193868 | 1.71 |



Compound I-8

Result

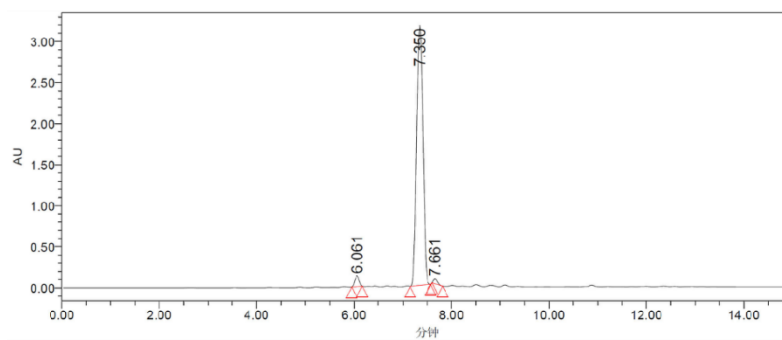
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 5.541 | 113418 | 0.83 |
| 2 | 6.265 | 13332173 | 97.29 |
| 3 | 6.738 | 138315 | 1.01 |
| 4 | 7.548 | 119413 | 0.87 |



Compound I-9

Result

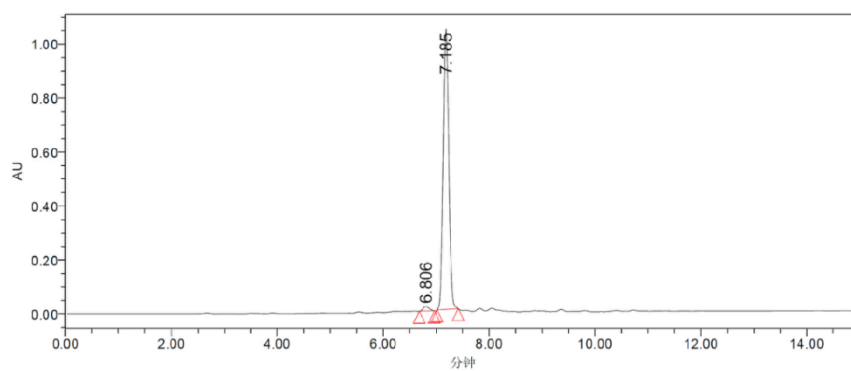
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 6.061 | 803336 | 2.74 |
| 2 | 7.35 | 28183458 | 96.17 |
| 3 | 7.661 | 317647 | 1.08 |



Compound I-10

Result

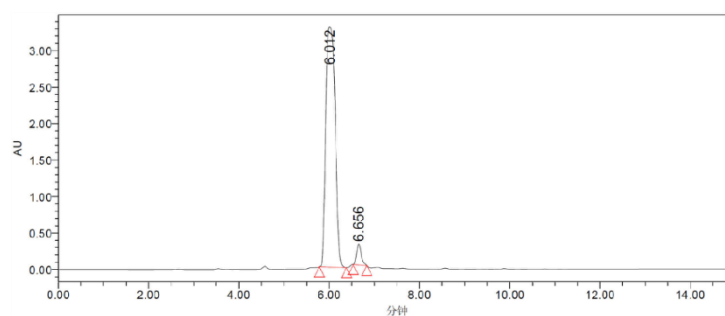
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|---------|---------------|
| 1 | 6.806 | 140404 | 1.83 |
| 2 | 7.185 | 7526950 | 98.17 |



Compound I-11

Result

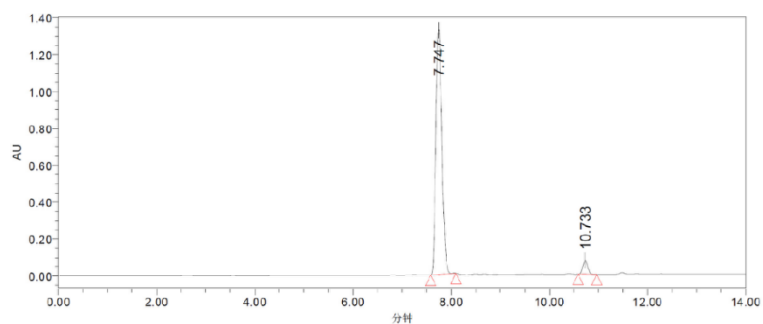
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 6.012 | 47594008 | 96.01 |
| 2 | 6.656 | 1975372 | 3.99 |



Compound I-12

Result

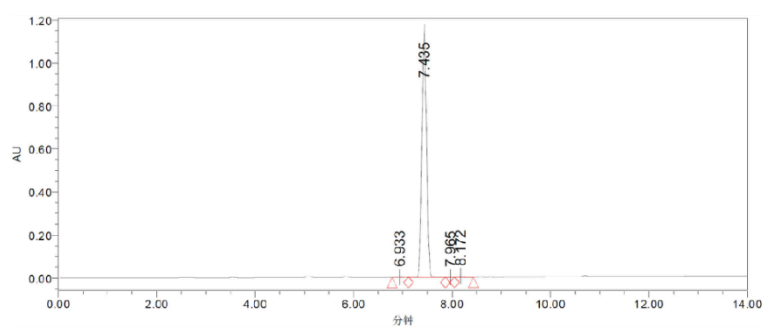
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 7.747 | 11145736 | 95.54 |
| 2 | 10.733 | 520194 | 4.46 |



Compound I-13

Result

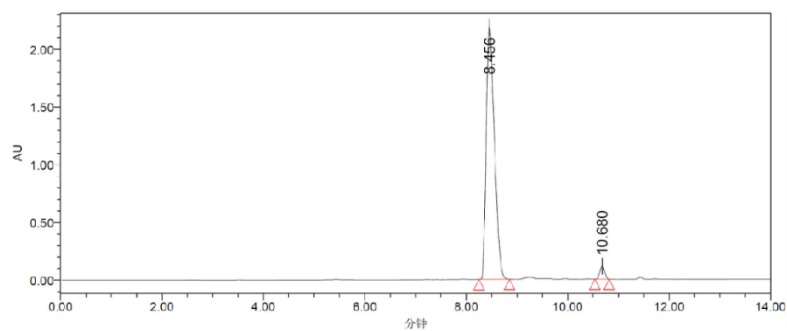
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|---------|---------------|
| 1 | 6.933 | 16293 | 0.21 |
| 2 | 7.435 | 7652243 | 99.32 |
| 3 | 7.965 | 2607 | 0.03 |
| 4 | 8.172 | 33171 | 0.43 |



Compound I-14

Result

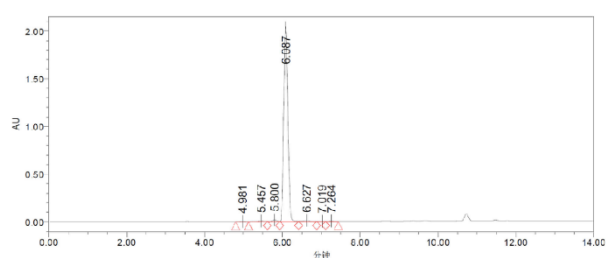
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 8.456 | 24552481 | 97.05 |
| 2 | 10.68 | 745183 | 2.95 |



Compound I-15

Result

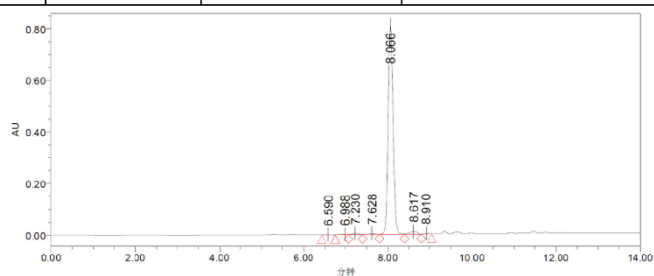
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|----------|---------------|
| 1 | 4.981 | 4194 | 0.03 |
| 2 | 5.457 | 62518 | 0.41 |
| 3 | 5.8 | 124360 | 0.81 |
| 4 | 6.087 | 14947789 | 97.66 |
| 5 | 6.627 | 124493 | 0.81 |
| 6 | 7.019 | 14154 | 0.09 |
| 7 | 7.264 | 28478 | 0.19 |



Compound I-16

Result

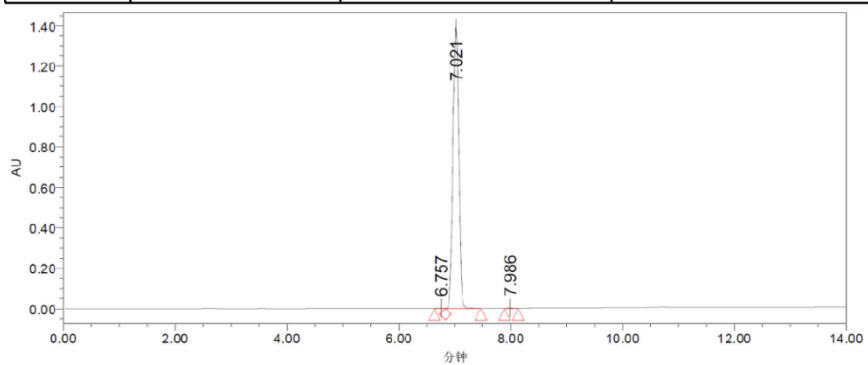
| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|---------|---------------|
| 1 | 6.59 | 2548 | 0.04 |
| 2 | 6.988 | 3770 | 0.06 |
| 3 | 7.23 | 43023 | 0.65 |
| 4 | 7.628 | 51274 | 0.77 |
| 5 | 8.066 | 6385534 | 96.13 |
| 6 | 8.617 | 135128 | 2.03 |
| 7 | 8.91 | 21628 | 0.33 |



Compound I-17

Result

| | Ret. Time | Area | Rel. Area (%) |
|---|-----------|---------|---------------|
| 1 | 6.757 | 916 | 0.01 |
| 2 | 7.021 | 9872017 | 99.96 |
| 3 | 7.986 | 2674 | 0.03 |



5. 2D docking model of I-11

