Supplementary information

Reactive oxygen species-responsive Pre-PROTAC for tumor-specific protein degradation

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Material and methods: Chemstry

Material and instruments

All chemicals were obtained from commercial suppliers (Adamas and Alfa, bidepharm, ChemShuttl), and used without further purification. Flash chromatography was carried out on silica gel (200-300 mesh). All new compounds were characterized by ^{1}H NMR, ^{13}C NMR,HRMS. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE III 500 MHZ (operating at 500 MHz for ^{1}H and ^{13}C NMR), chemical shifts were reported in ppm relative to the residual *d*6-DMSO (2.50 ppm 1H), MeOD (3.31 ppm 1H) and coupling constants (J) are given in Hz. Multiplicities of signals are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiple. High Resolution Mass spectra were recorded on AB Triple 4600 spectrometer with CH_3CN and H_2O as solvent. All the final compounds were all purified by C18 reverse phase preparative HPLC column with solvent A (0.05% HCl in H_2O) and solvent B (CH_3CN) as eluents.

High-performance liquid chroma (HPLC) (SHIMADZU) analysis was carried out at a flow rate of 0.4 mL/min under 254 nm wavelength detection, with a linear gradient elution using the mobile phase A (distilled water with 0.1%TFA) and the mobile phase B (acetonitrile with 0.1% TFA) from 10% B to 90% B in 12 min.

H_2O_2 responsive of pre-PROTACs

To determine whether the cleavage of pre-PROTACs is related to generation of ROS, 10 μL pre-PROTACs (10 mM in DMSO) were treated with 20 μL H_2O_2 (50 mM in PBS buffer) in 50 μL PBS buffer (pH = 7.4) and 20 μL CH_3CN for 8 h at 37 ℃ and analyzed by HPLC. The time dependent experiment of Pre-PROTAC **7** with H_2O_2. 10 μL pre-PROTAC **7** (10 mM in DMSO) was incubated with the indicated ratio of H_2O_2 (20 μL 50 mM in PBS buffer) in 50 μL PBS buffer (pH = 7.4) and 20 μL CH_3CN at 37 ℃ for 2 h, 4 h, 6 h, 8 h and each sample was analyzed by HPLC.

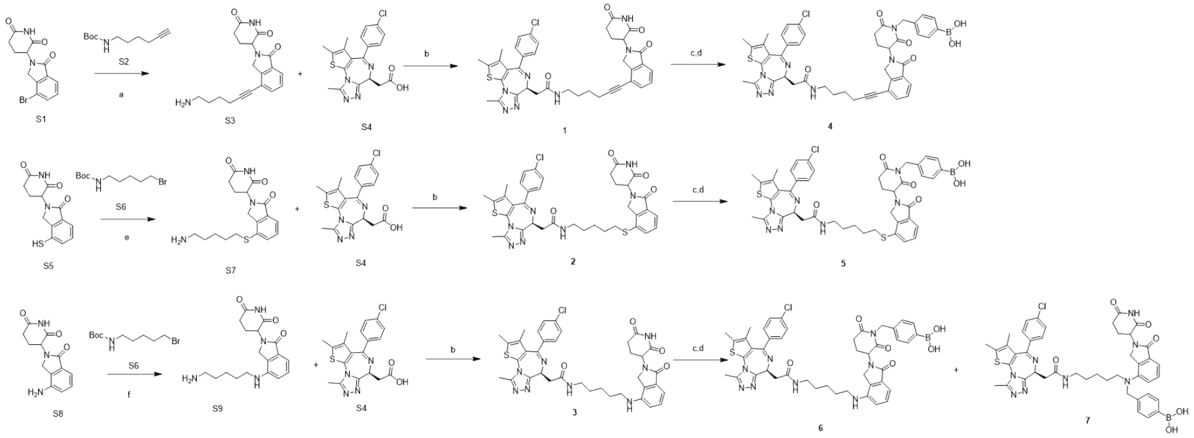
Pre-PROTAC 7 with different concentration of H_2O_2

Pre-PROTAC **7** (2.5 μL 10 mM in DMSO) was incubated in 12.5 μL PBS buffer (pH = 7.4) and 5 μL CH_3CN separately with of 1 μL, 3 μL, 9 μL, 27 μL H_2O_2 (1 mM in PBS buffer) at 37℃ for 8 h and each sample was analyzed by HPLC.

The stability of pre-PROTAC 7

Pre-PROTAC **7** (30 μL 10 mM in DMSO) was incubated in 120 μL PBS buffer (pH 7.4) and 50 μL CH_3CN at 37℃ for 0 h, 12 h, 24 h, 36 h, 48 h and each sample was analyzed by HPLC.

Synthesis route and produre of pre-PROTACs



Reagents and conditions:

a) i, $Pd(PPh_3)_2Cl_2$, CuI, DMF, TEA, 80°C, 8 h; ii, TFA, DCM, RT, 12 h;

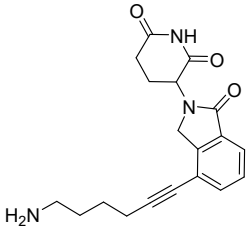
b) HATU, DIPEA, DMF, RT, 12h;

c) 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, NaHMDs, DCM, -78℃ to RT,12 h; d) 1 M HCl (aq), CH_3CN/H_2O, RT, 12h; e) K_2CO_3, DMF, RT, 1 h; f) DIPEA, 110℃, 2 h; g) TFA, DCM, RT, 12 h.

Scheme S1. Synthesis of ROS-activated pre-PROTACs **4-7**.

The route of chemical Synthesis.

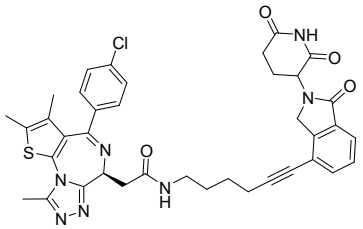
3-(4-(6-aminohex-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**S3**)



The compound **S1** (200 mg, 0.62 mmol) **S2** (244 mg,1.24 mmol), $Pd(PPh_3)_2Cl_2$ (21.7 mg, 0.031 mmol), CuI (11.8 mg, 0.062 mmol) and anhydrous DMF (10 mL) were added into a 25 mL two-neck-bottle and stirred 5 min at room temperature under N_2 atmosphere. After the reaction mixture was slowly added 5 mL anhydrous Et_3N, the reaction was stirred at 80 °C for 12 h, Then the reaction mixture was condensed under the vacuum to remove the solution and was purified by column chromatography (5% DCM in CH_3OH) to get the intermediate. (207 mg, crude yield 76%) as brown solid.

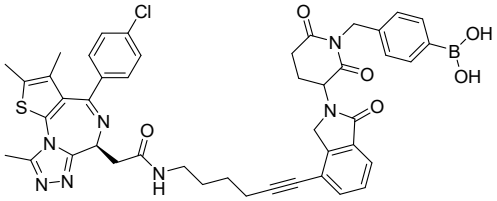
Then the intermediate was added into the TFA solution (4 mL TFA in 15 mL DCM) in a 25 mL single bottle and was stirred at room temperature for overnight. Then the reaction mixture was purified by preparative HPLC (10% to 90% acetonitrile/0.05% HCl in H_2O) to obtain the compound **S3**. White solid, 146 mg (yield 91%).^{1}H NMR (500 MHz, Methanol-d_4) δ 9.32 (d, J = 7.6 Hz, 1H), 9.18 (d, J = 7.8 Hz, 1H), 9.07 (t, J = 7.6 Hz, 1H), 6.74 (dd, J = 13.4, 5.2 Hz, 1H), 6.09 (d, J = 17.5 Hz, 1H), 6.03 (d, J = 17.5 Hz, 1H), 4.62 - 4.53 (m, 2H), 4.52 - 4.42 (m, 1H), 4.38 - 4.33 (m, 1H), 4.15 (t, J = 6.9 Hz, 2H), 4.13 - 4.02 (m, 1H), 3.78 - 3.73 (m, 1H), 3.45 - 4.39 (m, 2H), 3.35 - 3.25 (m, 2H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 174.61, 172.22, 170.97, 145.31, 135.86, 133.00, 129.65, 123.90, 120.70, 96.11, 77.88, 57.47, 53.72, 40.38, 32.36, 27.87, 26.54, 24.10, 19.67. HRMS(ESI): calcd for $C_{19}H_{22}N_3O_3$ $[M+H]^+$, 340.1656; found, 340.1663.

2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(6-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hex-5-yn-1-yl)acetamide (**1**)



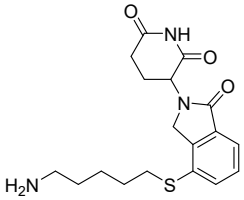
The compound **S4** (35 mg, 0.089 mmol), **S3** (30 mg, 0.089 mmol), DIPEA (34.7 mg, 0.26 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in 5 mL DMF to stir at room temperature for overnight. Then the reaction mixture was diluted with 30 mL H_2O, extracted with EA, washed with saturated salt solution, dried over Na_2SO_4, and was purified by column chromatography (7%DCM in CH_3OH) to get the compound **1**. White solid, 37 mg (yield 58%).^{1}H NMR (500 MHz, DMSO-d_6) δ 10.99 (d, J = 11.0 Hz, 1H), 8.30 - 8.23 (m, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.61 (ddd, J = 7.6, 3.9, 1.1 Hz, 1H), 7.51 (td, J = 7.7, 1.4 Hz, 1H), 7.46 (dd, J = 8.9, 2.4 Hz, 2H), 7.42 (d, J = 8.6 Hz, 2H), 5.13 (dd, J = 12.5, 4.7 Hz, 1H), 4.50 (ddd, J = 8.6, 5.8, 4.2 Hz, 1H), 4.44 (d, J = 17.7 Hz, 1H), 4.36 - 4.24 (m, 1H), 3.29 - 3.14 (m, 4H), 2.93 - 2.84 (m, 1H), 2.65 - 2.56 (m, 4H), 2.55 - 2.52 (m, 1H), 2.47 - 2.42 (m, 1H), 2.40 (s, 3H), 2.01 - 1.90 (m, 1H), 1.64 (br, 4H), 1.58 (s, 3H).^{13}C NMR (126 MHz, Methanol-d_4) δ 174.57, 171.99, 170.89, 167.47, 156.46, 153.15, 145.33, 138.95 (d, J = 6.2 Hz), 136.62, 135.72, 134.99, 132.96, 132.67, 132.02, 131.80, 129.98 (d, J = 7.1 Hz), 129.62, 123.72, 120.87, 97.04, 77.61, 54.47, 53.62, 40.08, 37.87, 32.29, 29.84, 27.00, 23.95, 19.90, 14.42 (d, J = 2.1 Hz), 13.04, 11.49.HRMS(ESI): calcd for $C_{38}H_{37}ClN_7O_4S$ $[M+H]^+$, 722.2311; found, 722.2320.

(4-((3-(4-(6-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)hex-1-yn-1-yl)-1-oxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl)phenyl)boronic acid (**4**)



The compound **1** (20 mg, 0.03 mmol) was added to a 25 mL 2-neck-bottle with 8 mL DCM (Dried by CaH). Then NaHMDs (50 μL, 0.05 mmol) was slowly added into the mixture to stir at -78 ℃ for 1 h under N_2 atmosphere. After that the compound 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (12 mg, 0.04 mmol) was dissolved in 1mL DCM (Dried by CaH) to slowly add into the reaction and stirred at room temperature overnight. After the reaction was completed, the mixture was condensed under the vacuum followed with 2 mL CH_3CN/H_2O solution (CH_3CN/H_2O = 1:1), then 1 mL 1 M HCl (aq) was added to stir at room temperature for overnight. Then the reaction mixture was purified by preparative HPLC (10% to 90% acetonitrile/0.05% HCl in H_2O) to obtain the compound **4**. White solid, 5.2 mg (yield 20%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (t, *J* = 5.7 Hz, 1H), 7.73 - 7.66 (m, 3H), 7.60 (dd, *J* = 7.6, 4.3 Hz, 1H), 7.50 (td, *J* = 7.6, 2.2 Hz, 1H), 7.46 - 7.38 (m, 4H), 7.17 (d, *J* = 6.2 Hz, 2H), 5.29 (dt, *J* = 13.5, 4.3 Hz, 1H), 4.85 - 4.72 (m, 2H), 4.53 - 4.43 (m, 2H), 4.28 (dd, *J* = 17.7, 8.6 Hz, 1H), 3.25 - 3.13 (m, 4H), 3.10 - 3.03 (m, 1H), 2.78 - 2.71 (m, 1H), 2.58 (s, 3H), 2.54 - 2.51(m, 3H), 2.37 (d, *J* = 2.6 Hz, 3H), 2.05 - 1.99 (m, 1H), 1.64 - 1.60 (m, 4H), 1.55 (d, *J* = 3.4 Hz, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 171.89, 170.52, 169.55, 167.82, 155.12, 150.02, 143.78, 136.71, 135.35, 134.48, 134.15, 132.26, 130.92, 130.20, 129.92, 128.51, 126.81, 126.25, 122.73, 118.88, 96.29, 83.67, 76.63, 73.61, 53.93, 45.65, 28.56, 25.52, 25.02, 18.60, 14.04, 12.72, 11.35, 8.62. HRMS(ESI): calcd for $C_{45}H_{44}BClN_7O_6S$ $[M+H]^+$, 856.2850; found, 856.2854.

3-(4-((5-aminopentyl)thio)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**S7**)

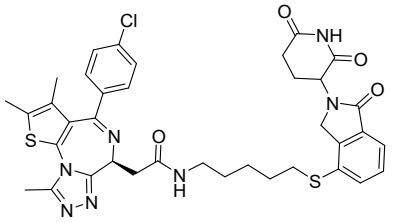


The compound **S5** (1 g, 3.62 mmol), **S6** (1.1 g, 4.34 mmol) and K_2CO_3 (1.5 g, 10.86 mmol) were dissolved in 10 mL DMF to stir for 3h at room temperature. Then the reaction mixture was diluted with 50 mL H_2O, extracted with EA, washed with saturated salt solution, dried over Na_2SO_4, and was purified by column chromatography (3% DCM in CH_3OH) to get the intermediate.

Then the intermediate was added into the TFA solution (2 mL TFA in 15 mL DCM) in a 25 mL single bottle and was stirred at room temperature for overnight. Then the reaction mixture was purified by preparative HPLC (10% to 90% acetonitrile/0.05% HCl in H_2O) to obtain the compound **S7**. White solid,600 mg (yield 46%).^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.79 (s, 2H), 7.63 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.59 - 7.50 (m, 2H), 5.13 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.35 (d, *J* = 17.4 Hz, 1H), 4.21 (d, *J* = 17.4 Hz, 1H), 3.08 (t, *J* = 7.2 Hz, 2H), 2.95 - 2.88 (m, 1H), 2.82 - 2.74 (m, 2H), 2.62 - 2.57 (m, 1H), 2.47 - 2.41 (m, 1H), 2.03 - 1.99 (m, 1H), 1.68 - 1.51 (m, 4H), 1.48 - 1.39 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.96, 171.08, 167.85, 140.96, 132.05 (d, *J* = 13.1 Hz), 130.20, 129.23, 120.27,

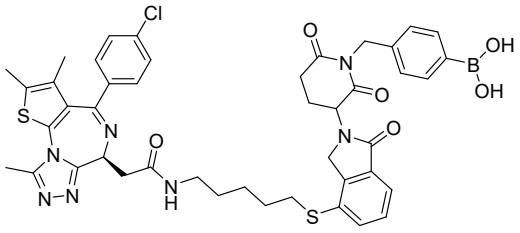
51.68, 46.71, 31.22 (d, J = 9.2 Hz), 28.08, 26.52, 24.89, 22.39. HRMS(ESI): calcd for $C_{18}H_{24}N_3O_3S$ $[M+H]^+$, 362.1533; found, 362.1539.

2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(5-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)thio)pentyl)acetamide (**2**)



The compound **S4** (473 mg, 1.25 mmol), **S7** (501 mg, 1.25 mmol), DIPEA (487.5 mg, 3.75 mmol) and HATU (544 mg, 1.5 mmol) were dissolved in 10 mL DMF to stir at room temperature for overnight. Then the reaction mixture was diluted with 50 mL H_2O, extracted with EA, washed with saturated salt solution, dried over Na_2SO_4, and was purified by column chromatography (7%DCM in CH_3OH) to get the compound **2**. Yellow solid,595 mg (yield 64%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.97 (s, 1H), 8.18 (t, J = 5.7 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 7.3 Hz, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 8.3 Hz, 2H), 7.43 - 7.40 (m, 2H), 5.12 (dd, J = 13.3, 5.0 Hz, 1H), 4.55- 4.45 (m, 1H), 4.35 (dd, J = 17.4, 3.0 Hz, 1H), 4.20 (dd, J = 17.2, 4.4 Hz, 1H), 3.27 - 3.02 (m, 6H), 2.95 - 2.86 (m, 1H), 2.59 (s, 3H), 2.56 (br, 1H), 2.47 - 2.40 (m, 1H), 2.40 (s, 3H), 2.02 - 1.96 (m, 1H), 1.64 - 1.59 (m, 5H), 1.46 (br, 4H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 174.63, 172.71, 172.09, 171.19, 166.24, 157.02, 152.22, 143.10, 138.04 (d, J = 8.4 Hz), 133.95, 133.51, 133.25, 133.10, 132.54 (d, J = 9.6 Hz), 132.02, 131.31, 130.34, 129.79 (d, J = 6.4 Hz), 121.74, 55.25, 53.69 (d, J = 6.7 Hz), 43.53, 40.13, 38.85, 33.53 (d, J = 5.8 Hz), 32.33, 29.88 (d, J = 16.3 Hz), 26.86, 24.05, 14.40, 12.92, 11.61, 11.56. HRMS(ESI): calcd for $C_{37}H_{39}ClN_7O_4S_2$ $[M+H]^+$, 744.2188; found, 744.2194.

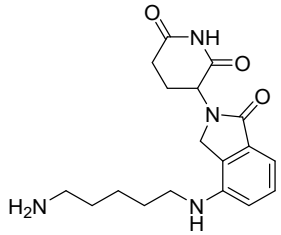
(4-((3-(4-((5-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)pentyl)thio)-1-oxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl)phenyl)boronic acid (**5**)



The compound **5** was obtained from the substitution of **2** and 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane according the similar process of **4** . White solid, 6.5 mg (yield 10%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (t, J = 5.7 Hz, 1H), 7.71 (d, J = 7.6 Hz, 2H), 7.64 - 7.54 (m, 2H), 7.53 - 7.39 (m, 5H), 7.19 (d, J = 7.3 Hz, 2H), 5.30 (dd, J = 13.4, 5.1 Hz, 1H), 4.83 (qd, J = 15.1, 3.6 Hz, 2H), 4.54 (t, J = 7.1 Hz, 1H), 4.38 (d, J = 17.3 Hz, 1H), 4.19 (d, J = 17.3 Hz, 1H), 3.27 - 3.20 (m, 2H), 3.08 (dt, J = 23.3, 7.0 Hz, 4H), 2.80 (d, J = 16.5 Hz, 1H), 2.61 (s, 3H), 2.56 - 2.52 (m,

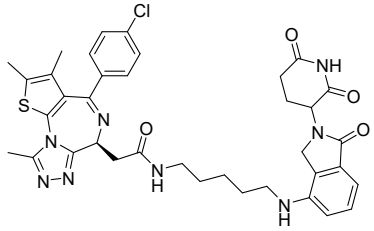
1H), 2.40 (s, 3H), 2.09 - 2.05 (m, 1H), 1.67 - 1/59 (m, 1H), 1.60 (d, *J* = 3.5 Hz, 2H), 1.49 - 1.43 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.87, 170.57, 169.36, 167.94, 163.32, 155.06, 150.14, 140.80, 139.00, 136.52, 135.45, 134.14, 132.17 (d, *J* = 8.5 Hz), 132.01, 131.13, 130.25, 130.07, 129.95, 129.72, 129.24, 128.52, 126.21, 120.18, 53.82, 52.38, 46.88, 42.92, 38.24, 37.53, 34.28, 31.41, 28.72, 28.31, 25.48, 21.70, 14.07, 12.73, 11.31. HRMS(ESI): calcd for $C_{44}H_{46}BClN_7O_6S_2$ $[M+H]^+$, 878.2727; found, 878.2734.

3-(4-((5-aminopentyl)-λ^2-azaneyl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**S9**)



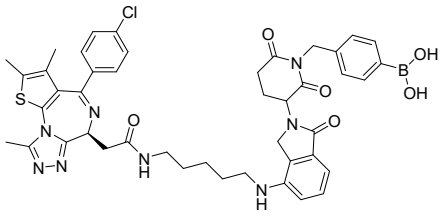
The synthesis of compound **S9** was according the route of compound **9c** in *Org. Lett.* 2019, 21, 3838−3841. Yellow liquid, 234 mg (yield 68%).^{1}H NMR (500 MHz, DMSO) δ 11.01 (s,1H), 7.29 (t, *J* = 7.7 Hz, 1H), 6.94 (d, *J* = 7.1 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 5.12 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.27 - 4.07 (m, 2H), 3.13 (t, *J* = 7.0 Hz, 2H), 2.98 - 2.88 (m, 1H), 2.84 - 2.75 (m, 2H), 2.67 - 2.57 (m, 1H), 2.35 - 2.22 (m, 1H), 2.08 - 1.99 (m, 1H), 1.65 - 1.51 (m, 4H), 1.45 - 1.37 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 173.38, 171.71, 169.34, 159.39, 159.09, 158.80, 158.50, 143.88, 132.56, 129.70, 127.18, 119.36, 117.05, 114.74, 112.52, 112.44, 110.81, 51.97, 46.18, 43.04, 39.26, 31.69, 28.30, 27.26, 23.85, 23.27. HRMS(ESI): calcd for $C_{18}H_{24}N_4O_3$ $[M+H]^+$, 344.1843; found, 344.1847.

2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(5-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)pentyl)acetamide (**3**)



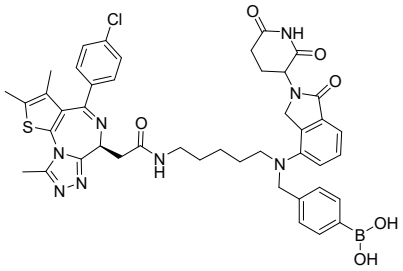
The synthesis of compound **3** was according the route of compound **SIAIS213110** in *Org. Lett.* 2019, 21, 3838−3841. Yellow solid, 112 mg (yield 60%).^{1}H NMR (500 MHz, Methanol-d_4) δ 7.50 (d, *J* = 8.6 Hz, 2H), 7.46 - 7.39 (m, 3H), 7.30 (d, *J* = 6.7 Hz, 1H), 7.07 (d, *J* = 7.3 Hz, 1H), 5.13 (dd, *J* = 13.3, 5.2 Hz, 1H), 4.78 (dd, *J* = 8.6, 5.7 Hz, 1H), 4.42 (d, *J* = 17.1 Hz, 1H), 4.37 (d, *J* = 17.1 Hz, 1H), 3.43 (dd, *J* = 15.3, 8.7 Hz, 1H), 3.34 (d, *J* = 5.8 Hz, 1H), 3.30 - 3.27 (m, 4H), 2.93 - 2.84 (m, 1H), 2.81 (s, 3H), 2.78 - 2.73 (m, 1H),2.46 (s, 3H) 2.44 - 2.41 (m, 1H), 2.18 - 2.14 (m, 1H), 1.78 - 1.72 (m, 2H), 1.68 (s, 3H), 1.66 - 1.61 (m, 2H), 1.55 - 1.48 (m, 2H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 174.60, 172.18, 171.94, 171.60, 167.59, 156.44, 153.17, 139.15, 136.53, 135.08, 133.76, 132.70, 131.98, 130.94, 130.43, 130.03, 115.84, 54.41, 53.61, 47.40, 46.50, 40.09, 37.64, 32.34, 30.13, 28.85, 25.13, 24.19, 14.41, 13.03, 11.47. HRMS(ESI): calcd for $C_{37}H_{40}ClN_8O_4S$ $[M+H]^+$, 727.2576; found, 727.2573.

(4-((3-(4-((5-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)pentyl)amino)-1-oxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl)phenyl)boronic acid (**6**)



The compound **3** (60 mg, 0.08 mmol) was added to a 25 mL 2-neck-bottle with 10 mL DCM (Dried by CaH). Then NaHMDs (120 μL, 0.12 mmol) was slowly added into the mixture to stir at -78℃ for 1 h under N_2 atmosphere. After that the compound 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (36 mg, 0.12 mmol) was dissolved in 1mL DCM (Dried by CaH) to slowly add into the reaction and stirred at room temperature overnight. After the reaction was completed, the mixture was condensed under the vacuum followed with 2 mL CH_3CN/H_2O solution (CH_3CN/H_2O = 1:1), then 1 mL 1 M HCl (aq) was added to stir at room temperature for overnight. Then the reaction mixture was purified by preparative HPLC (10% to 90% acetonitrile/0.05% HCl in H_2O) to obtain the compound **6**. White solid, 6.3 mg (yield 9%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (d, J = 4.7 Hz, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.67 (d, J = 7.5 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 7.36 (dd, J = 16.3, 7.8 Hz, 2H), 7.24 (d, J = 7.5 Hz, 3H), 4.74 (s, 1H), 4.72 - 4.68 (m, 1H), 4.58 (t, J = 7.2 Hz, 1H), 4.50 (s, 3H), 3.29 (br, 2H), 3.23 - 3.17 (m, 3H), 3.06 (d, J = 6.6 Hz, 2H), 2.66 (s, 3H), 2.41 (s, 3H), 2.27 - 2.23 (m, 1H), 2.17 - 2.12 (m, 1H), 2.09 - 2.02 (m, 1H), 1.59 (s, 3H), 1.53 - 1.46 (m, 2H), 1.42 - 1.37 (m, 2H), 1.32 - 1.19 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.67, 173.31, 172.30, 171.97, 169.11, 163.96, 154.81, 150.77, 139.33, 136.03, 135.82, 134.47, 134.26, 133.67, 131.89, 130.54, 130.22, 129.97, 128.61, 127.89, 53.48, 47.24, 46.26, 38.41, 37.09, 31.61, 30.67, 28.97, 24.79 (d, J = 26.2 Hz), 23.71, 14.11, 12.82. HRMS(ESI): calcd for $C_{44}H_{47}BClN_8O_6S$ $[M+H]^+$, 861.3115; found, 861.3123.

(4-(((5-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)pentyl)(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)methyl)phenyl)boronic acid (**7**)



The compound **7** was obtained through the same route of compound **6**. White solid, 7.4 mg (yield

10%).^{1}H NMR (500 MHz, DMSO-d_6) δ 10.96 (d, J = 8.6 Hz, 1H), 8.18 (t, J = 5.7 Hz, 1H), 7.69 (d, J = 7.7 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.34 (t, J = 7.7 Hz, 1H), 7.22 (d, J = 7.4 Hz, 3H), 7.11 (d, J = 8.1 Hz, 1H), 5.06 (dd, J = 13.3, 5.2 Hz, 1H), 4.54 (t, J = 7.1 Hz, 1H), 4.47 - 4.31 (m, 4H), 3.24 - 3.16 (m, 4H), 3.11 - 3.04 (m, 2H), 2.92 - 2.81 (m, 1H), 2.62 (s, 3H), 2.60 - 2.55 (m, 1H), 2.46 - 2.43 (m, 1H), 2.41 (s, 3H), 2.00 - 1.95 (m, 1H), 1.59 (s, 3H), 1.50 (p, J = 7.7 Hz, 2H), 1.41 (p, J = 7.1 Hz, 2H), 1.27 (p, J = 8.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.01, 171.08, 169.19, 168.05, 163.83, 154.89, 150.65, 136.18, 135.74, 134.26, 133.45, 131.74, 130.49, 130.18, 129.94, 128.61, 127.15, 53.58, 51.72, 47.61, 38.39, 37.23, 31.24, 29.00, 26.32, 23.71, 22.48, 14.12, 12.82, 11.33. HRMS(ESI): calcd for $C_{44}H_{47}BClN_8O_6S$ $[M+H]^+$, 861.3115; found, 861.3117.

Material and methods: Biology

Cell culture

293T cell line was cultured in 10% FBS and 1% P/S DMEM culture medium. T47D was cultured in 10% FBS, 1% P/S and insulin RPMI-1640 culture medium. All cell lines were examined as mycoplasma free.

ROS detection

Cell lines were cultured in 96-well plates with 8,000 cells per well. ROS detected using Meilun Reactive Oxygen Species Assay Kit (MA0219). Fluorescence images were taken using inverted fluorescence microscope. Fluorescence intensity was quantified using ImageJ.

Western blotting

T47D or 293T were seeded in 1 ml culture medium in 24-well plate at the density of 1.5 x 10^5 cell/ml. After treatment of elevated concentrations of compounds, cells were collected and then lysed in SDS lysis buffer with phosphatase inhibitors (TargetMol: C0004) and proteosome inhibitors (Merck: 539134). Antibodies in the study include beta-actin (CST: 5125), BRD3 (Santa Cruse: sc-81202), BRD4 (CST: 13440), Anti-Rabbit IgG,HRP-linked (CST: 7074), Anti-rat IgG,HRP-linked (CST: 7077).

Cell growth inhibition

T47D or 293T were seeded in 96-well plates with 4,000 cells per well. After cells attached to the bottom, increasing concentrations of different compounds were added into each well. Cell viability were measured via CCK-8 kit (TargetMol, C0005). And IC_{50} was calculated using Prism Graphad. All the experiments were repeated three times.

Degradation signaling study

10 mM NAC (N-Acetyl-L-Cysteine, aladdin) or 2 uM MG132 (Meilun, MB5137) or 0.5 uM MLN4924 (MCE, HY-70062) was pretreated for 1 hour in T47D cell line, and then 100 nM pre-PROTAC(7) was added into cell line. Cells were collected and lysed in SDS lysis buffer after treatment of 6 hours.

Supplementary Figures

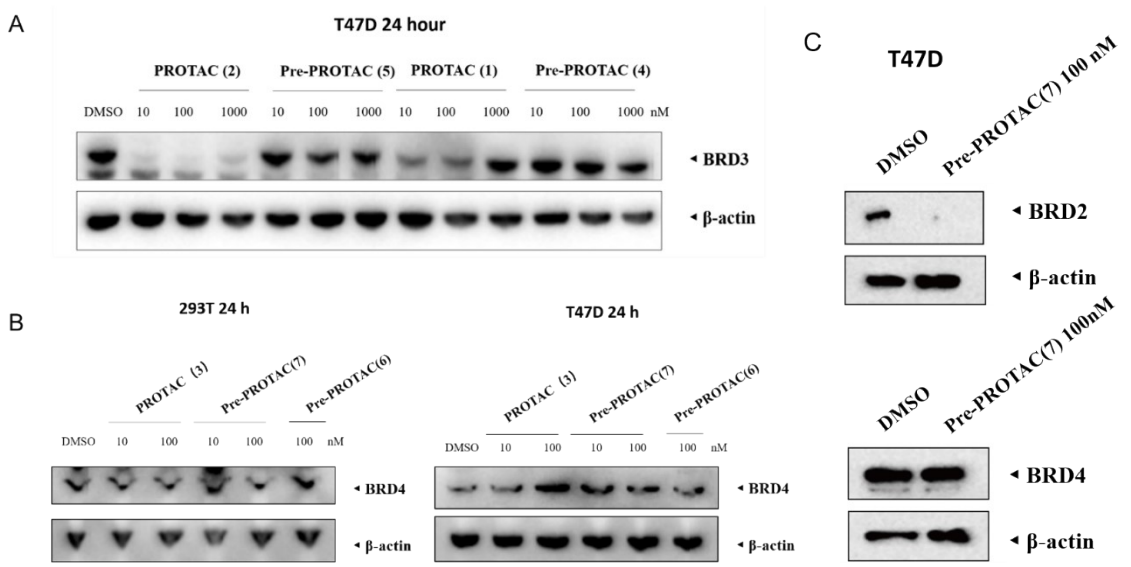
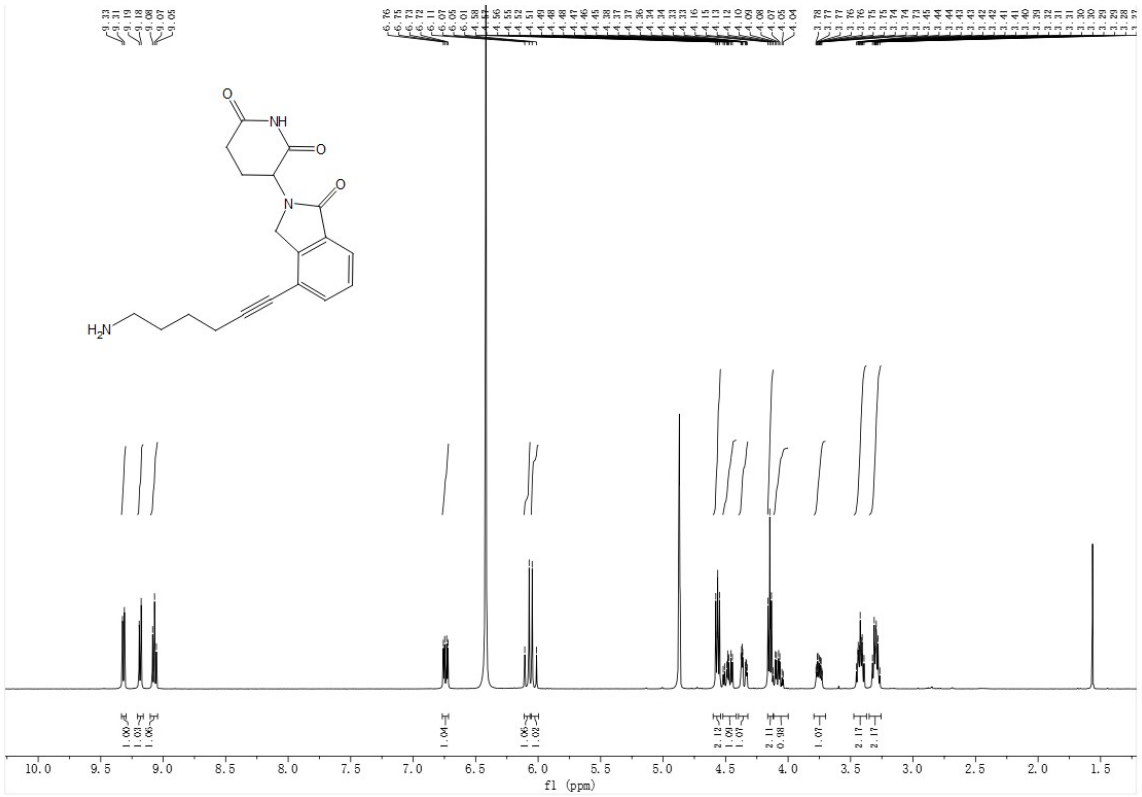
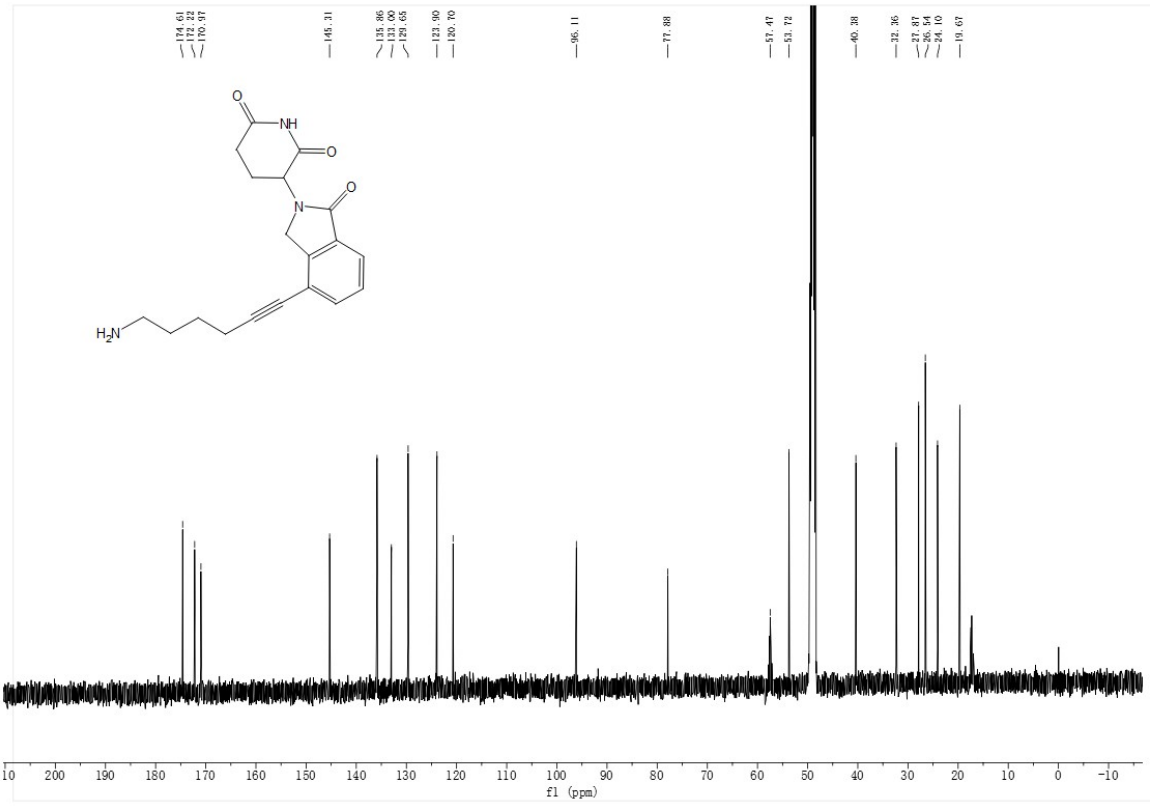


Figure S1. Western blotting of BRD2/3/4 abundance after treatment of different compounds (A) Degradation of BRD3 after treatment of PROTAC (1,2) or Pre-PROTAC (4,5) in T47D cell line; (B) Degradation of BRD4 after treatment of PROTAC(3) or Pre-PROTAC(6,7) in 293T or T47D cell lines. (C) Degradation of BRD2 or BD4 after treatment of Pre-PROTAC(7).

^{1}H and ^{13}C spectrum

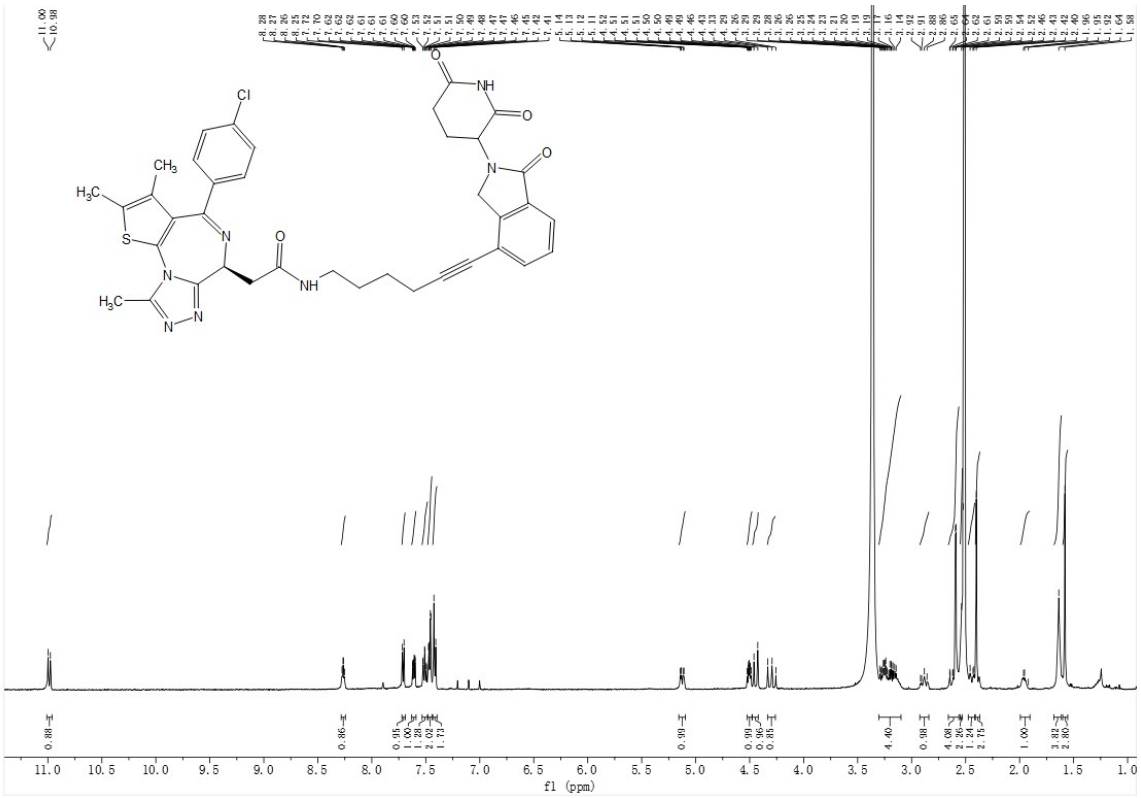
3-(4-(6-aminohex-1-yn-1-yl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**S3**)

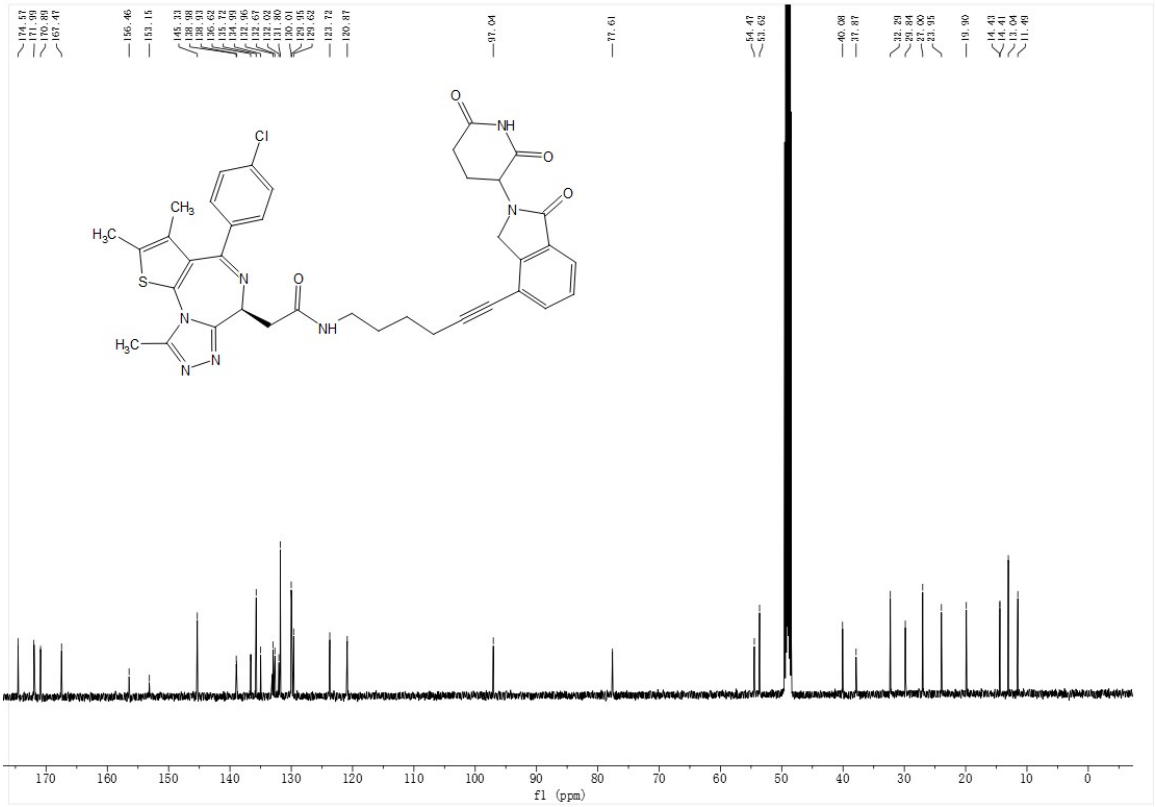




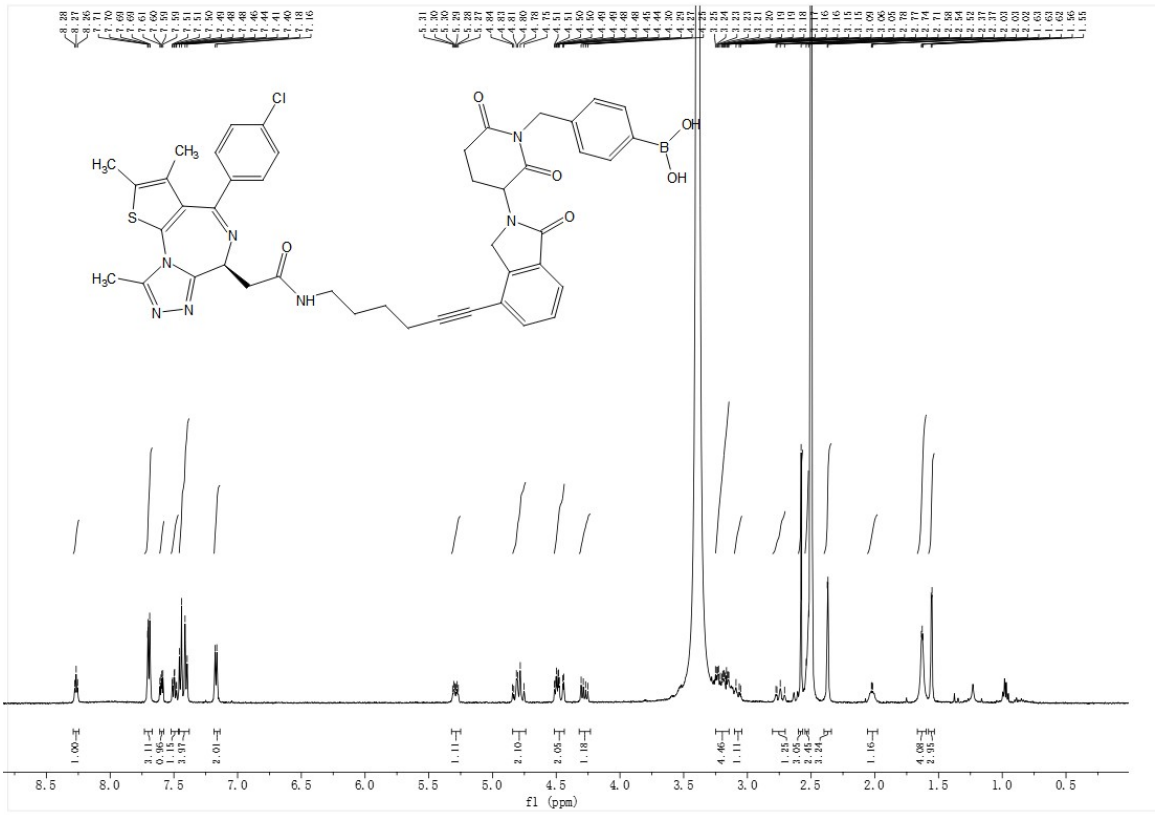
2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-

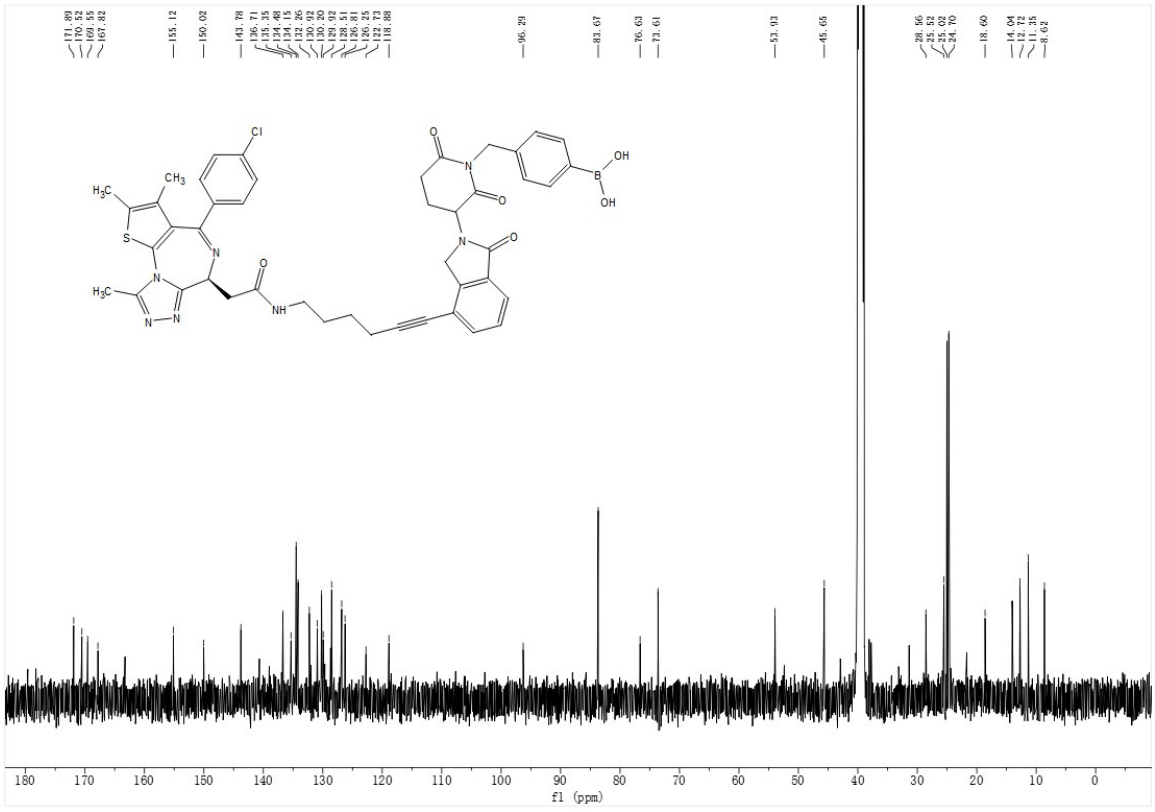
(6-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hex-5-yn-1-yl)acetamide (**1**)



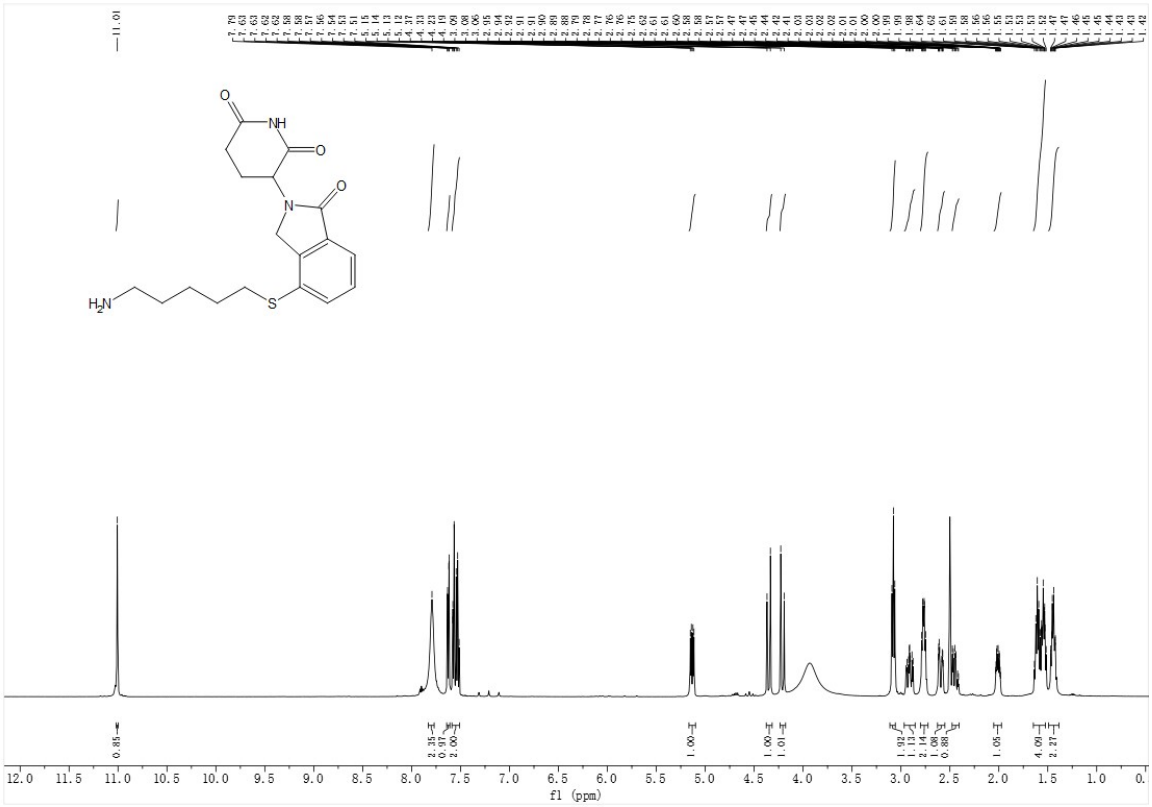


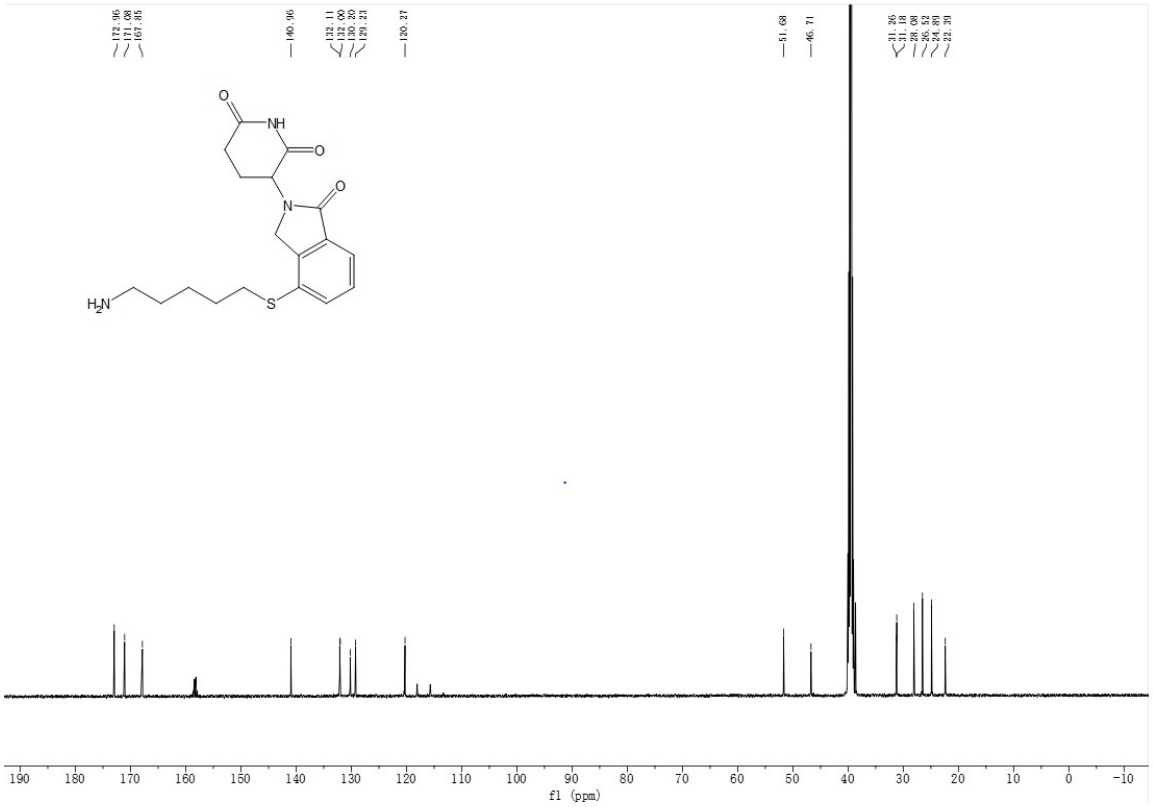
(4-((3-(4-(6-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)hex-1-yn-1-yl)-1-oxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl)phenyl)boronic acid (**4**)



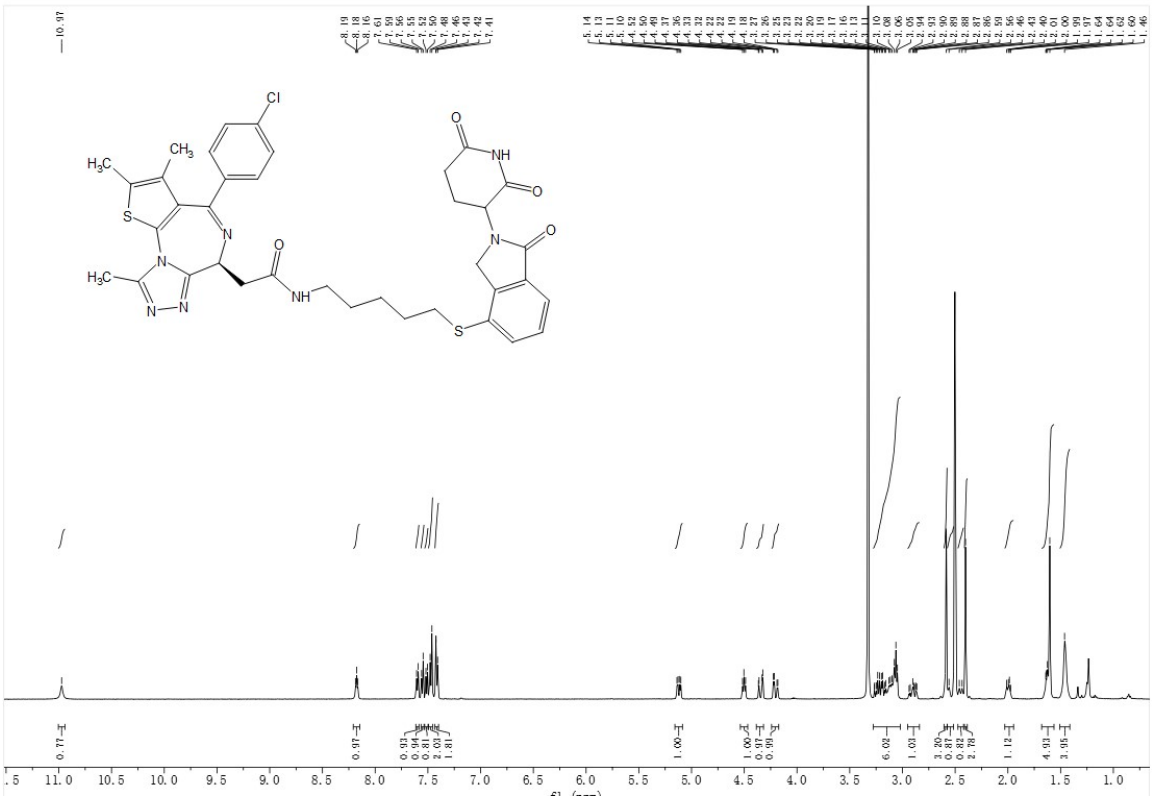


3-(4-((5-aminopentyl)thio)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**S7**)





2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(5-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)thio)pentyl)acetamide (**2**)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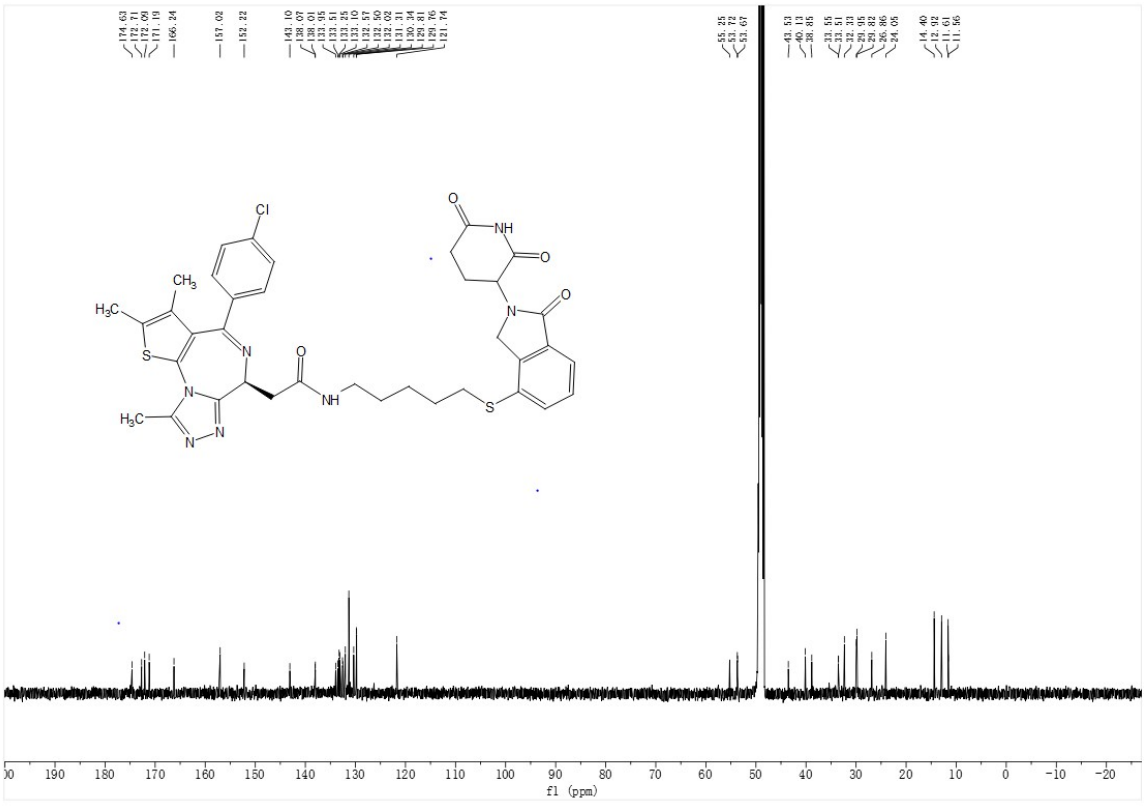
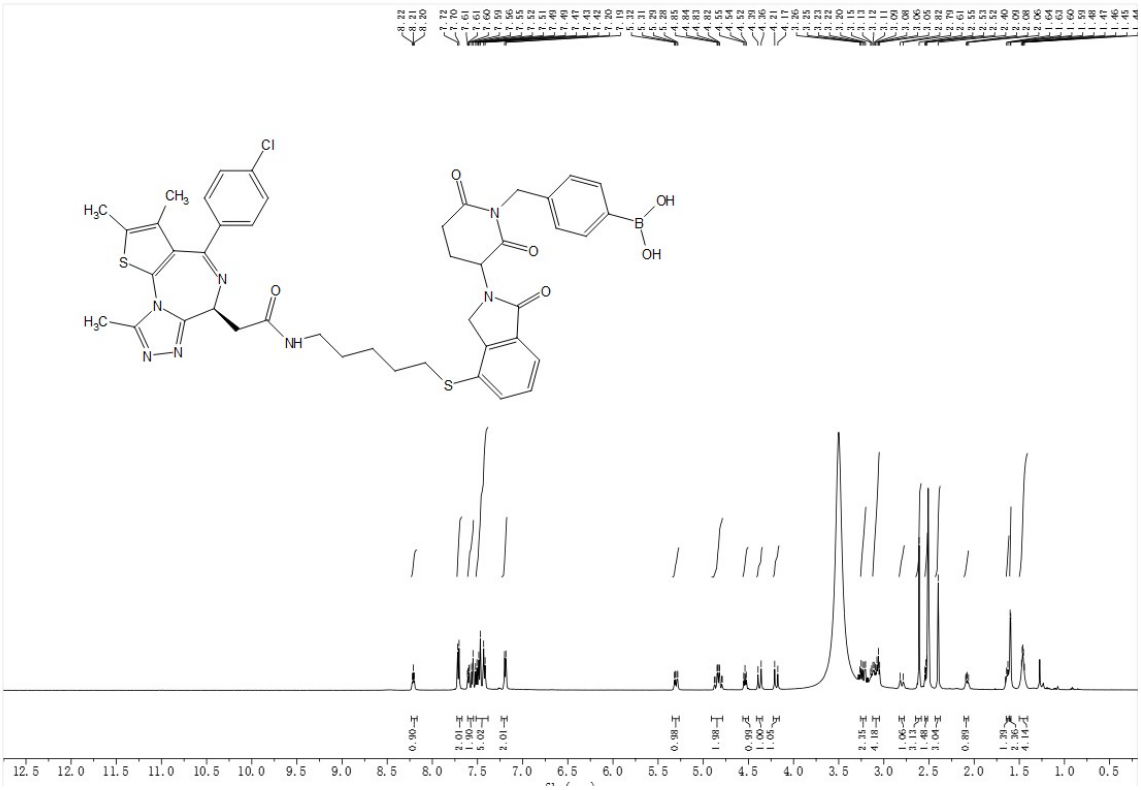
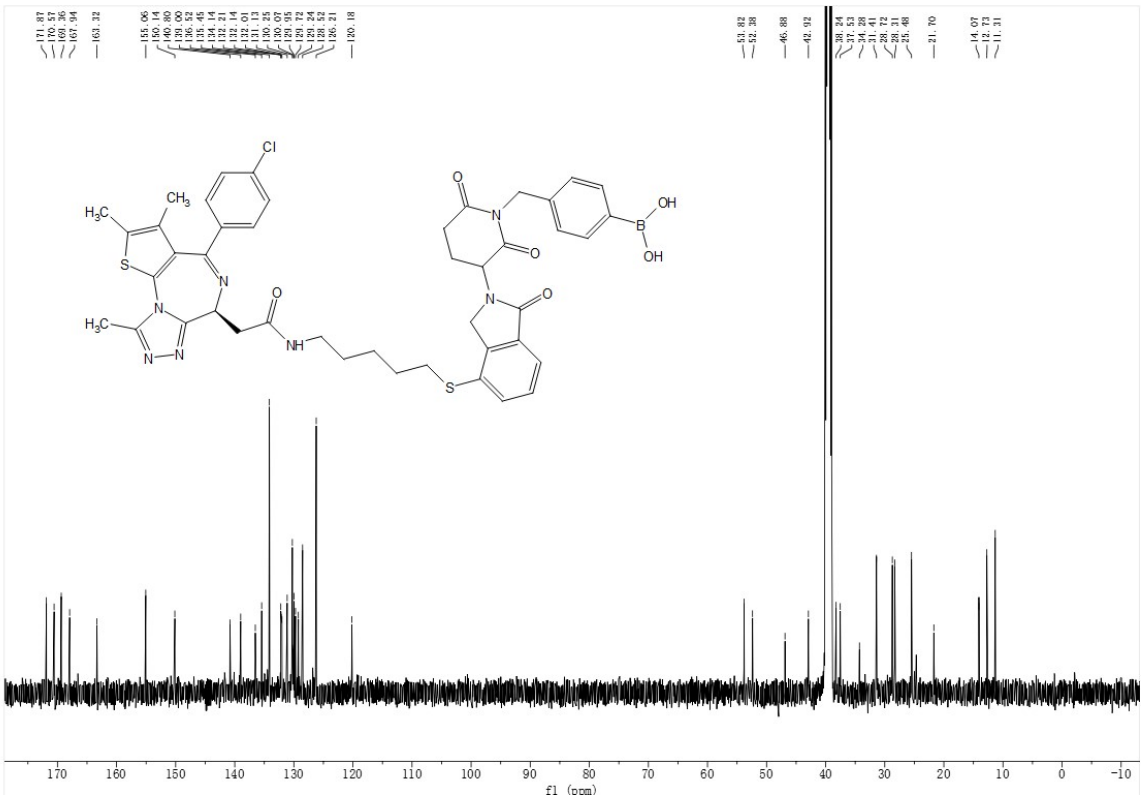
S
N
O
NH
H3C
N
N
O
NH
O
N
O
S

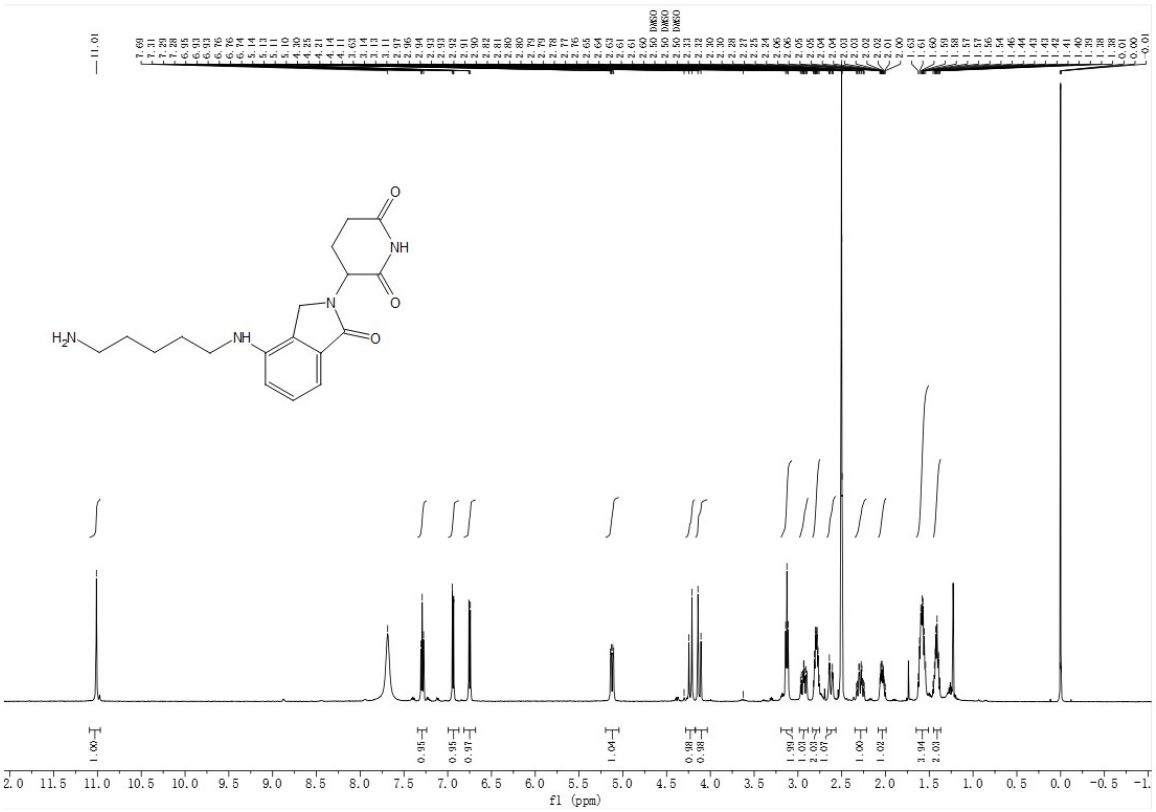

Cl
CH3
H3C
S
N
O
NH
H3C
N
N
O
NH
O
N
O
S
f1 (ppm)

(4-((3-(4-((5-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)pentyl)thio)-1-oxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl)phenyl)boronic acid (**5**)





3-(4-((5-aminopentyl)-λ^2-azaneyl)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**S9**)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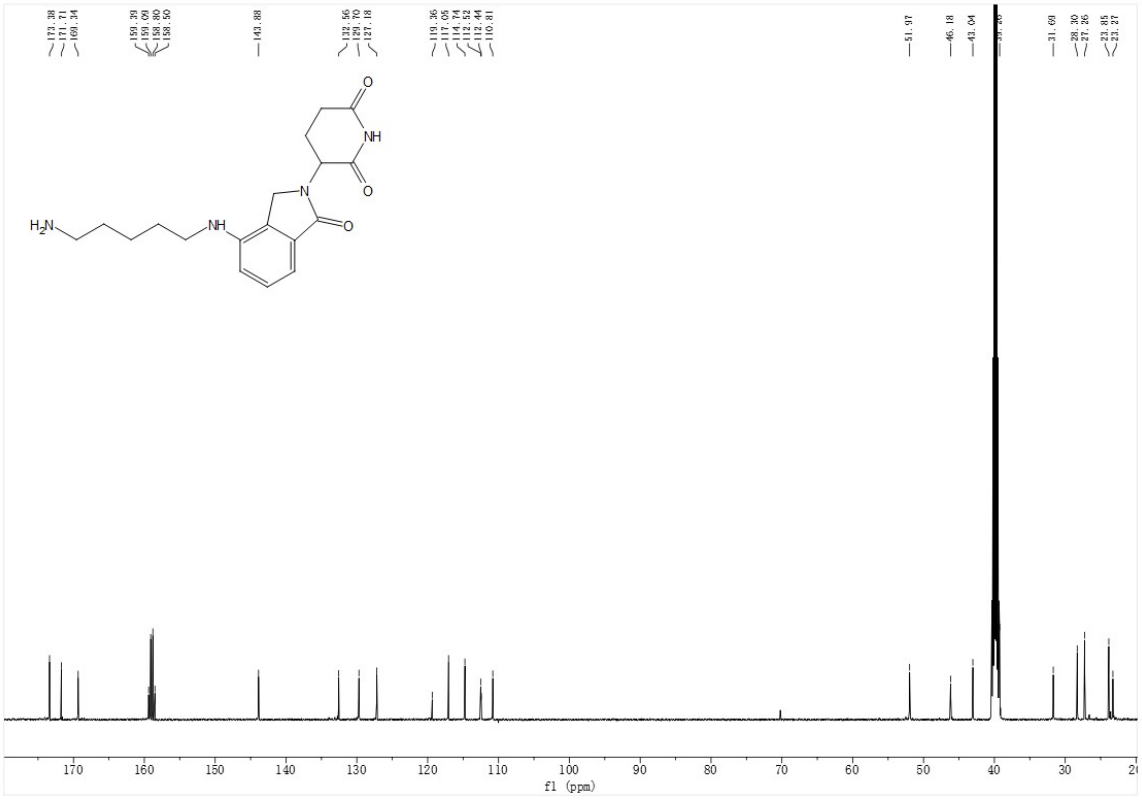
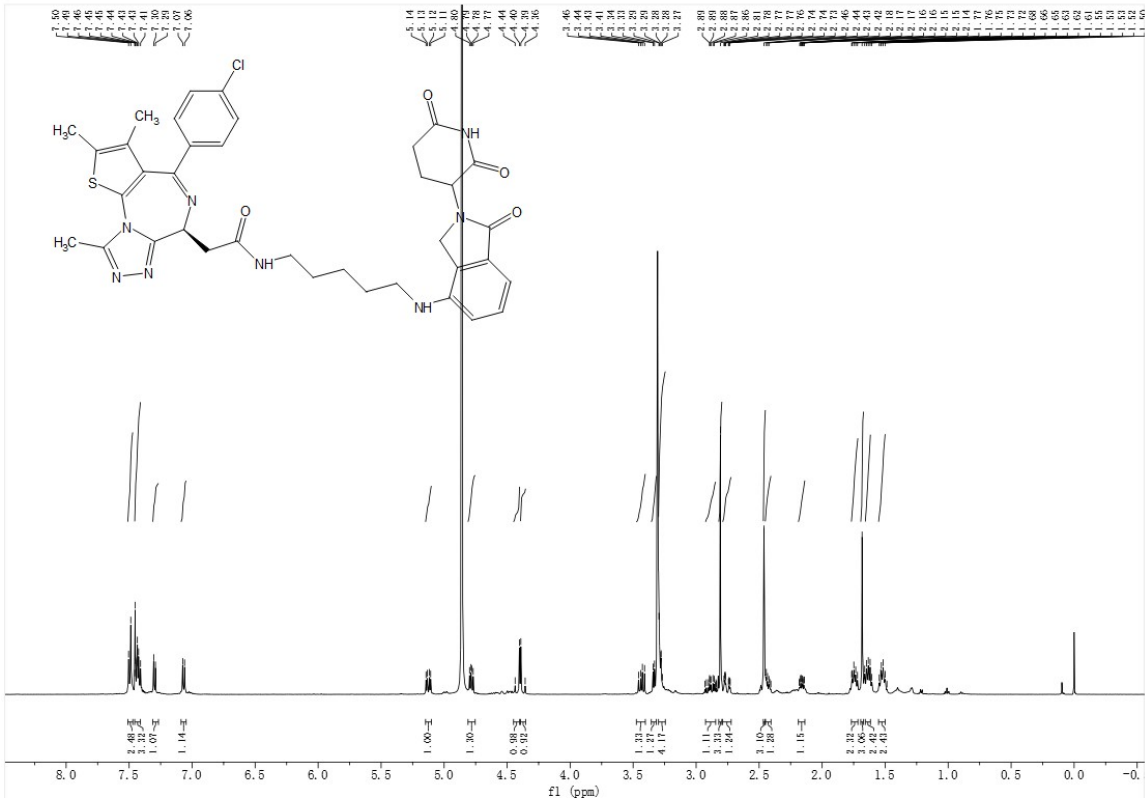
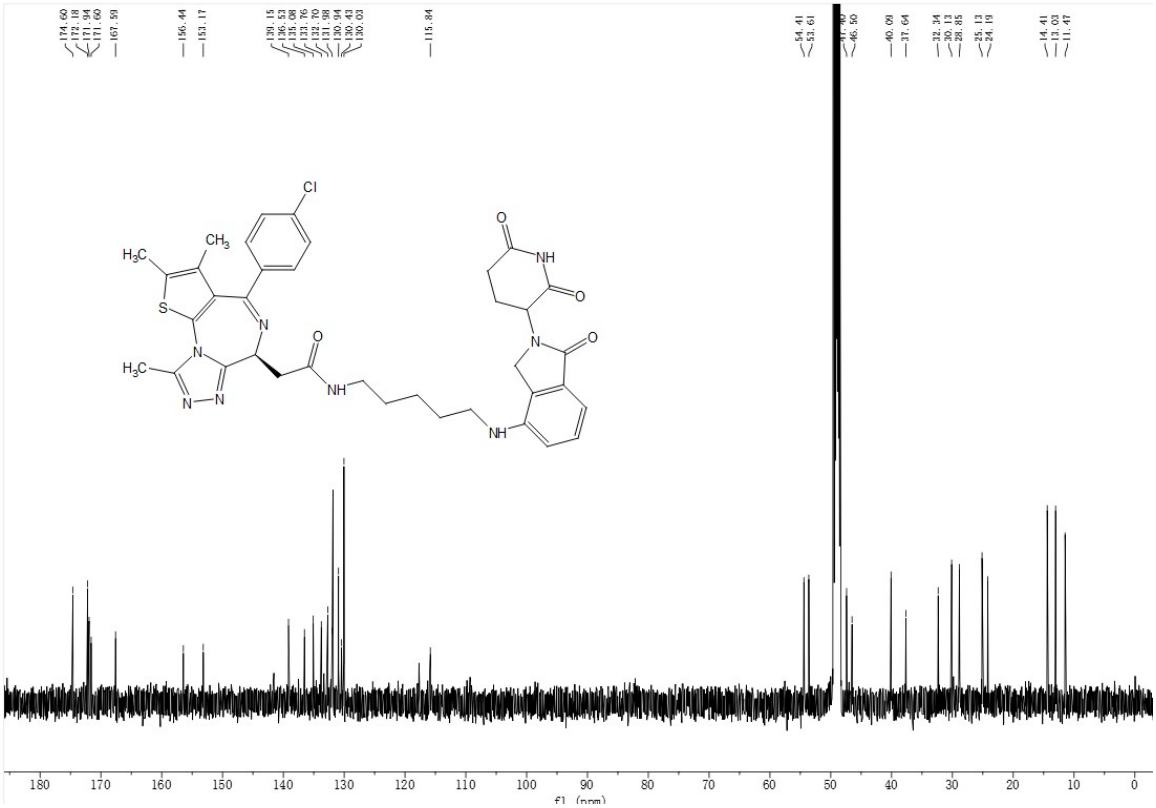
NH
O
N
O
H2N
NH
fl (ppm)


O
NH
O
N
O
H2N
NH
fl (ppm)

2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(5-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)pentyl)acetamide (**3**)





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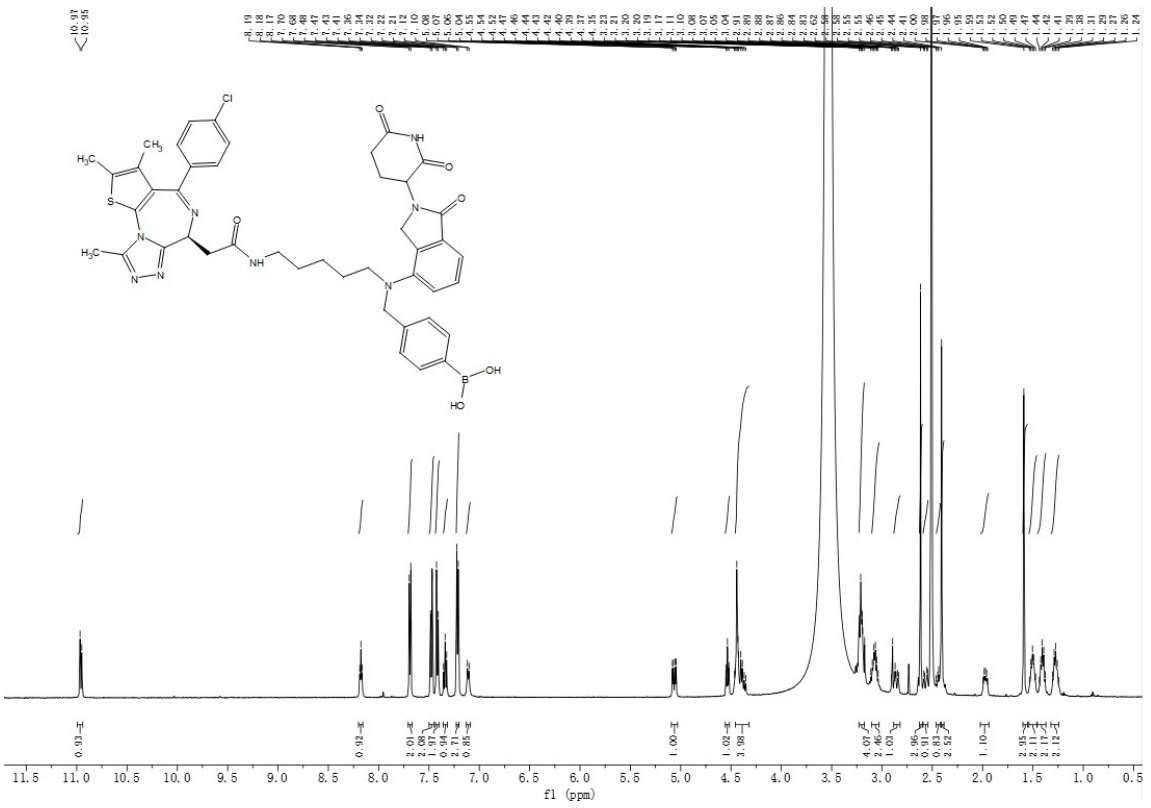

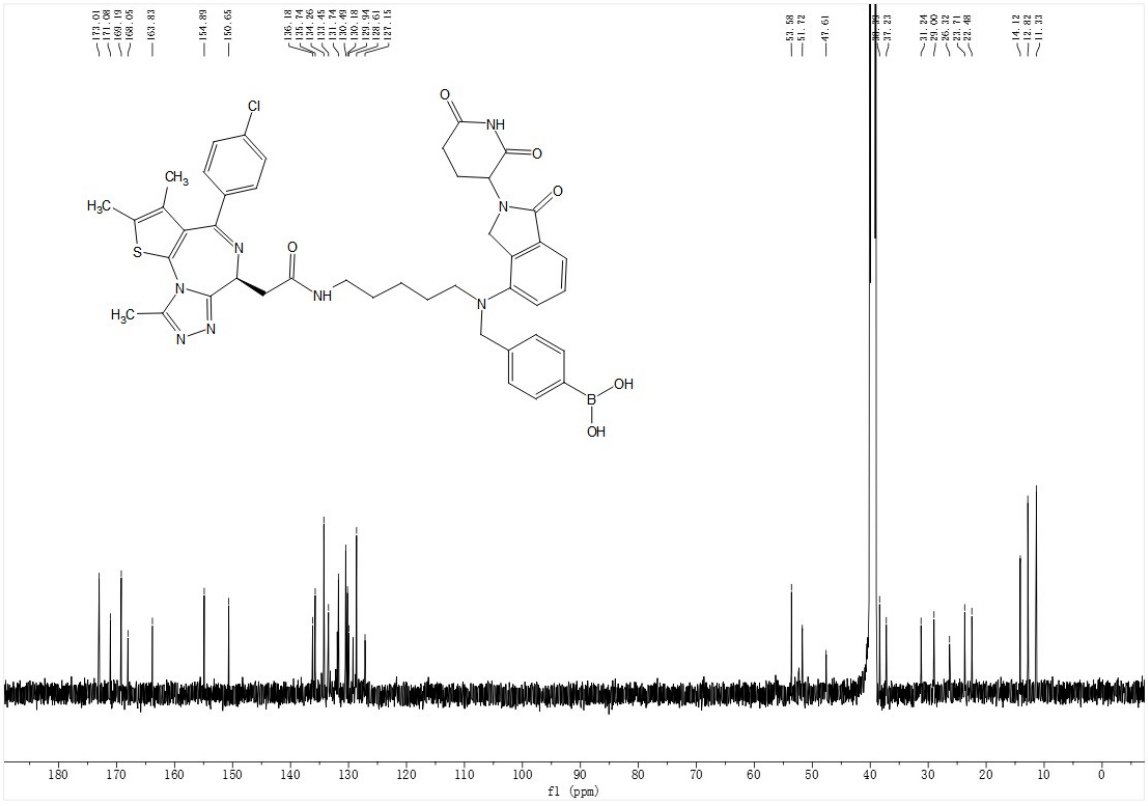

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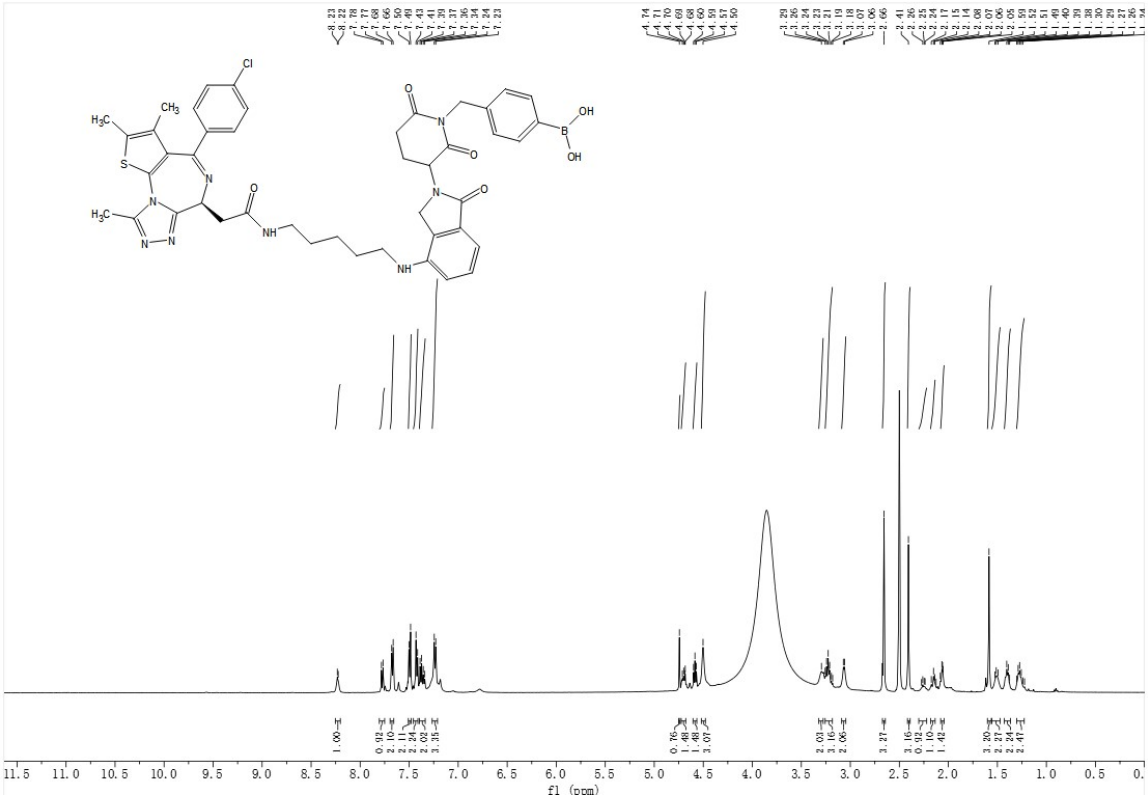
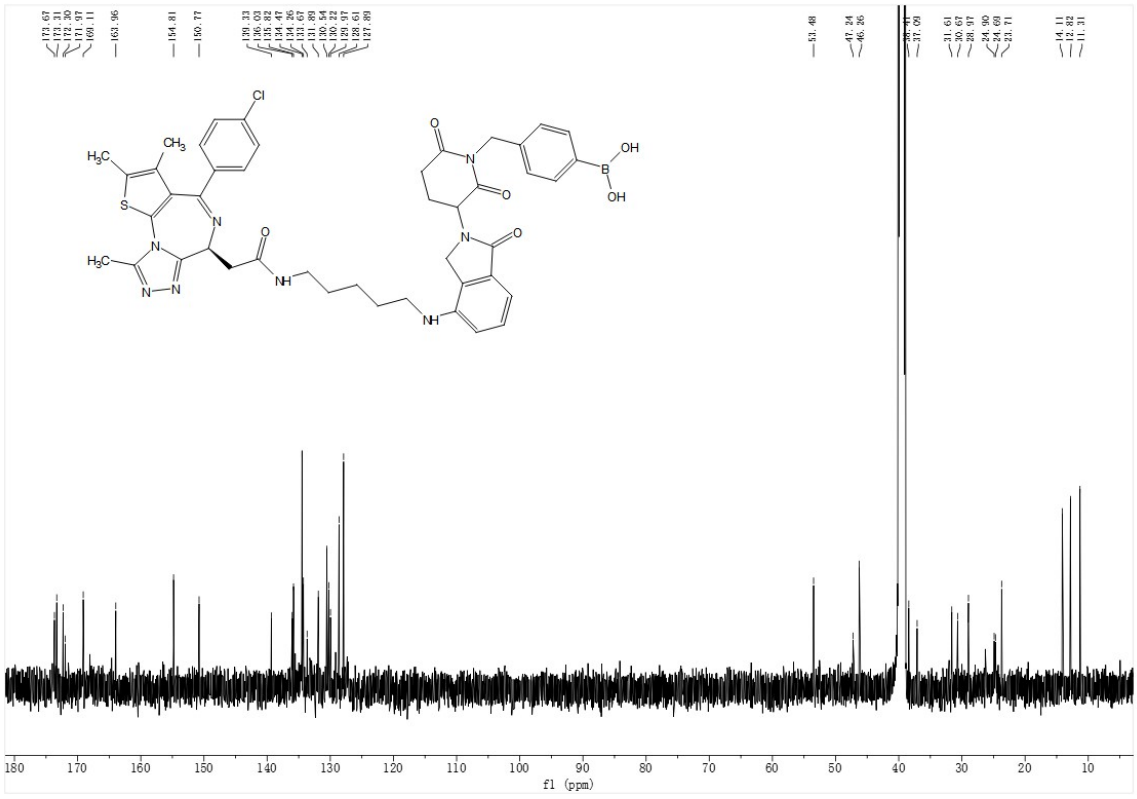
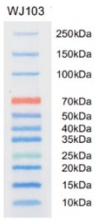
a][1,4]diazepin-6-yl)acetamido)pentyl)amino)-1-oxoisoindolin-2-yl)-2,6-dioxopiperidin-1-yl)methyl)phenyl)boronic acid (**6**)

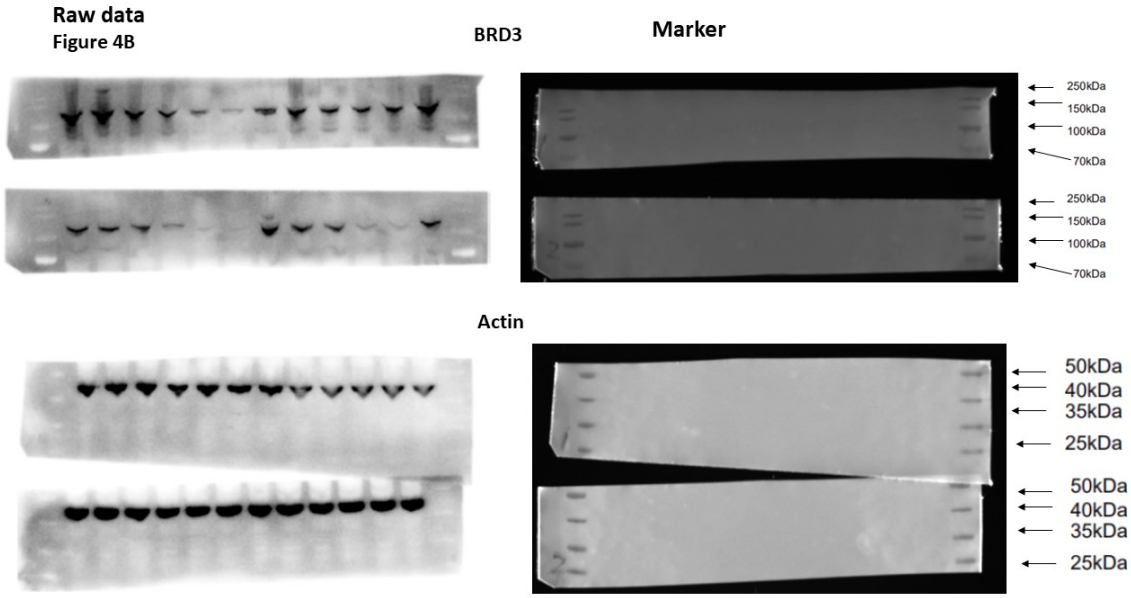



Cl
CH3
H3C
S
N
N
N
N
H3C
O
NH
O
N
O
O
N
O
B
OH
OH
NH
f1 (ppm)

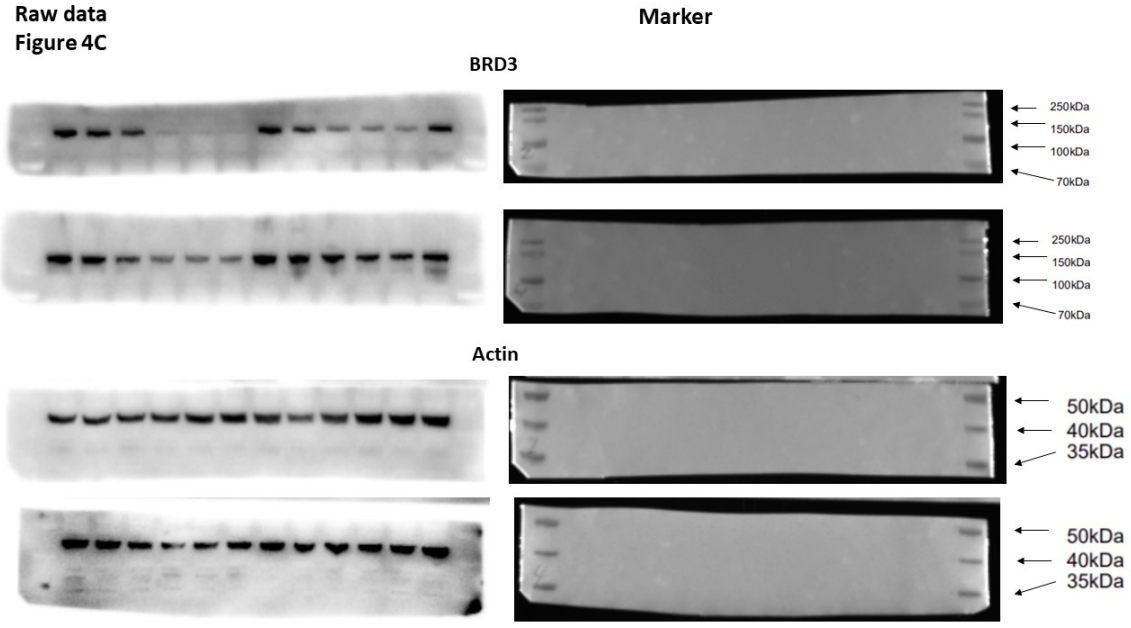
Raw western blotting data



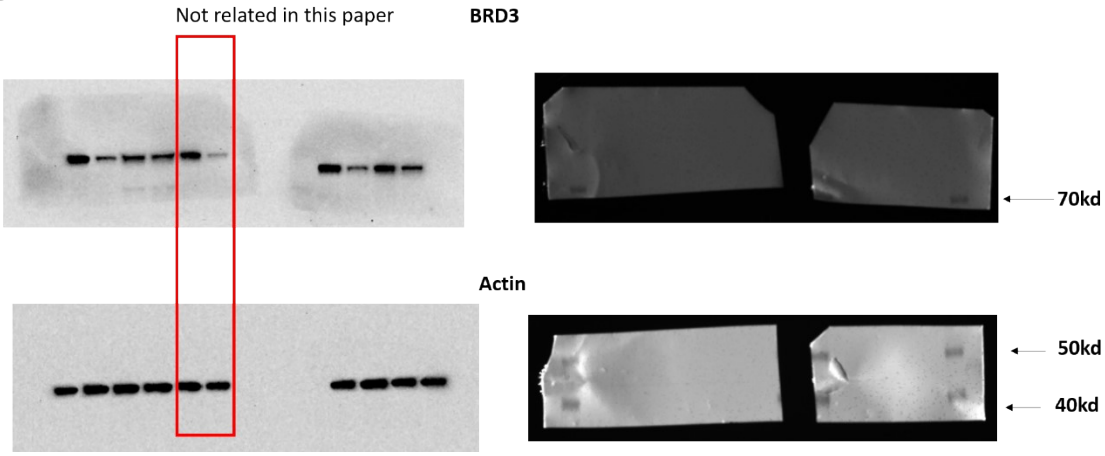
Raw data
Figure 4B



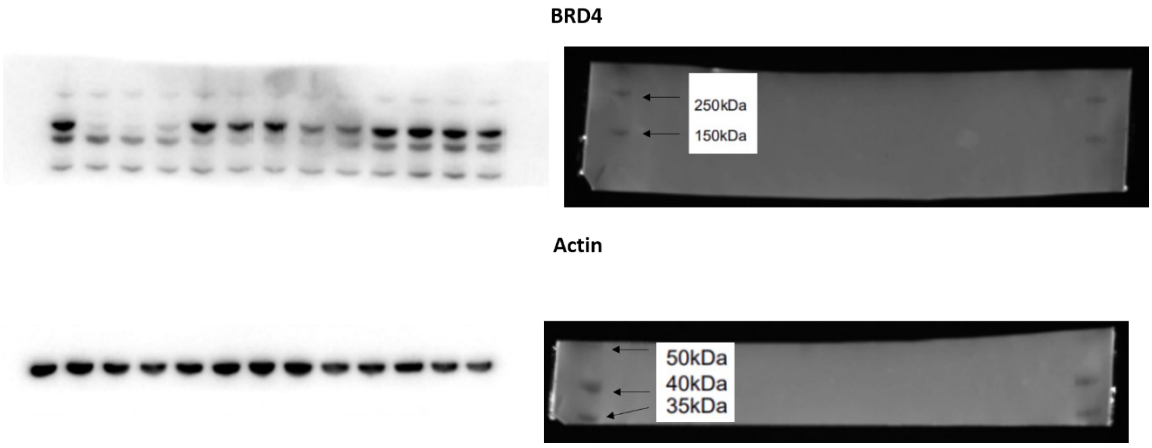
Raw data
Figure 4C



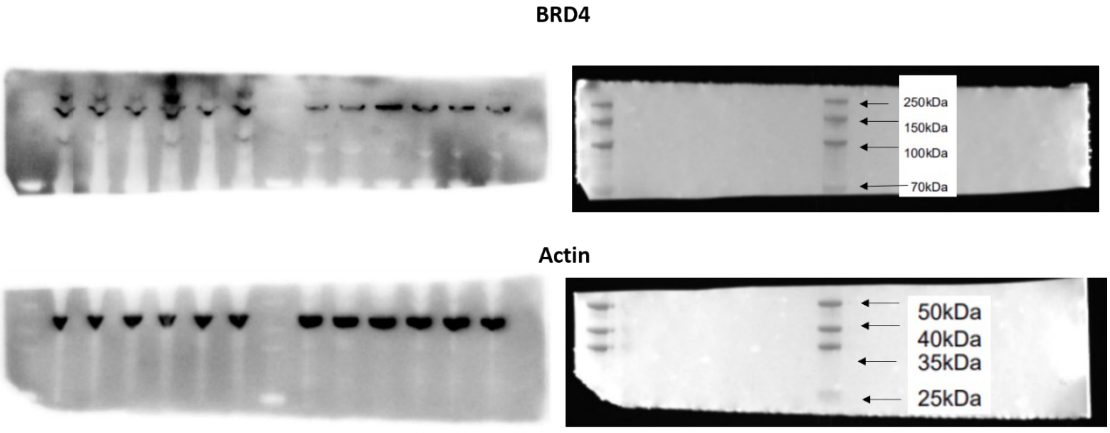
Raw data
Figure 5



Raw data
Figure s1A



Raw data
Figure s1B



Raw data
Figure s1C