

Supporting Information

Pharmacokinetic Studies

The pharmacokinetic properties of compounds in rats were determined as follows. The compound was administered intravenously to the femoral vein of male Sprague Dawley rats as a solution in NMP/PEG200 (1:9, v/v) at 2 mg/kg, or as a 10 mg/kg NMP/PEG200 (1:9, v/v) solution for gavage. Serial plasma samples were collected by sublingual vein bleeding for 24 h (0.083, 0.25, 0.5, 1, 2, 4, 8, 24 h) from one group of rats (n = 3). Samples were extracted and the concentration of compound was determined by LC–MS/MS. LOQ was set to 2.5 ng/mL.

Chemistry General Procedure

All chemicals were obtained from commercial suppliers (Adamas and Alfa, bidepharm, ChemShuttle), and used without further purification, unless otherwise indicated. Flash chromatography was carried out on silica gel (200–300 mesh). Analytical TLC was performed on Haiyang ready-to-use plates with silica gel 60 (F₂₅₄). All new compounds were characterized by ¹H NMR, ¹³C NMR, HRMS. ¹H NMR, ¹³C NMR spectra were recorded on Bruker AVANCE III 500 MHz (operating at 500 MHz for ¹H NMR, ¹³C NMR), chemical shifts were reported in ppm relative to the residual *d*₆-DMSO (2.50 ppm ¹H, 39.52 ppm ¹³C), *d*₄-Methanol(3.30 ppm ¹H, 49 ppm ¹³C), and coupling constants (*J*) are given in Hz. Multiplicities of signals are described as follows: s --- singlet, br.--- broad singlet, d --- doublet, t --- triplet, m --- multiple. High Resolution Mass spectra were recorded on AB Triple 4600 spectrometer with acetonitrile and water as solvent. The final compounds were all purified by C18 reverse phase preparative HPLC column with solvent A (0.5% HCl in H₂O) and solvent B (MeCN) as eluents.

(E)-N-(4-(piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide (3)

The compound (*E*)-3-(pyridin-3-yl)acrylic acid (500 mg, 3.35 mmol) **1**, *tert*-butyl 4-(4-aminobutyl)piperidine-1-carboxylate (860 mg, 3.35 mmol) **2**, HATU (1.5 g, 4.0 mmol) and DIPEA (1.3 g, 10 mmol), DMF (10 mL) were added to a 50 mL single bottle. After being stirred overnight at 60 °C, the crude mixture was cooled down to room temperature, diluted with 70% brine aqueous (50 mL), extracted with EtOAc. After that, the combined organic layer was washed with water and brine, then dried over Na₂SO₄ and concentrated in vacuum to afford crude oil. After solvent evaporation, the residue was purified by column chromatography on silica gel (5% CH₃OH in DCM) to give the intermediate (880 mg, crude yield 68%) as yellow solid.

NMR spectra shows in Figures S3.

The obtained intermediate was then added to a 25 mL single bottle with TFA solution (4 mL TFA in 10 mL DCM) and stirred at room temperature for 12 h. When the reaction was completed, the mixture was purified by preparative HPLC (10 to 90% acetonitrile/0.05% TFA in H₂O) to obtain the target compound (600 mg, 92%) as yellow oily liquid. ¹H NMR (500 MHz, Methanol-*d*₄) δ 9.12 (s, 1H), 8.85 - 8.80 (m, 2H), 8.12 - 7.98 (m, 1H), 7.63 (d, *J* = 15.8 Hz, 1H), 7.02 (d, *J* = 15.9 Hz, 1H), 3.44 - 3.27 (m, 5H), 3.05 - 2.95 (m, 2H), 1.95 (dd, *J* = 13.6, 3.4 Hz, 2H), 1.60 (p, *J* = 7.1 Hz, 3H), 1.45 - 1.27 (m, 7H). ¹³C NMR (126 MHz, Methanol-*d*₄) δ 166.63, 144.34, 143.27, 142.88, 136.57, 134.02, 129.05, 128.60, 45.29, 40.56, 36.60, 34.72, 30.31, 29.95, 24.73, 18.74, 17.30. MS(ESI) *m/z*: 288.2074 [M+H]⁺.

(E)-N-(4-(1-(4-(piperazin-1-yl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide (5)

The title compound (yellow oily liquid, 600 mg, 60% yield over two steps) was synthesized according to procedures for the preparation of **3**. ¹H NMR (500 MHz, Methanol-*d*₄) δ 9.13 (s, 1H), 8.87 (dd, *J* = 18.8, 6.9 Hz, 2H), 8.15 (dd, *J* = 8.2, 5.7 Hz, 1H), 7.64 (d, *J* = 15.8 Hz, 1H), 7.50 - 7.42 (m, 2H), 7.26 - 7.16 (m, 2H), 7.05 (d, *J* = 15.8 Hz, 1H), 4.98 (br, 4H), 3.63 (s, 4H), 3.43 (s, 4H), 3.34 (t, *J* = 7.0 Hz, 2H), 1.94 - 1.75 (m, 2H), 1.70 - 1.56 (m, 3H), 1.49

- 1.40 (m, 2H), 1.40 – 1.32 (m, 2H), 1.31 – 1.19 (m, 2H). ¹³C NMR (126 MHz, Methanol-*d*₄) δ 170.98, 165.15, 151.30, 144.11, 141.01, 140.71, 135.51, 132.25, 128.93, 128.19, 127.49, 115.81, 45.55, 42.96, 39.30, 35.39 (d, *J* = 10.0 Hz), 29.05, 23.64. MS(ESI) *m/z*: 476.3024 [M+H]⁺.

NMR spectra shows in Figures S4.

7-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-4-yl)hept-6-yn-1-yl methanesulfonate (7)

The compound 3-(4-bromo-1-oxoisindolin-2-yl)piperidine-2,6-dione (800 mg, 2.5 mmol) **6**, hept-6-yn-1-ol (560 mg, 5 mmol), Pd(PPh₃)₂Cl₂ (175 mg, 0.25 mmol), CuI (95 mg, 0.5 mmol) and anhydrous DMF (5 mL) were added to a 50 mL single bottle and stirred 5 min at room temperature under Ar atmosphere. After the mixture was added 2.5 mL anhydrous Et₃N, the reaction was stirred at 80 °C for 12 h, Then the crude mixture was cooled down to room temperature, diluted with H₂O (50 mL), extracted with EtOAc. After that, the combined organic layer was washed with water and brine, then dried over Na₂SO₄ and concentrated in vacuum to afford crude oil. After solvent evaporation, the residue was purified by column chromatography on silica gel (5% CH₃OH in DCM) to give the compound (800 mg, crude yield 90%) as white solid.

The obtained intermediate dissolved in 30 mL DCM was added to a 25 mL single bottle with Et₃N (676 mg, 6.7 mmol) and stirred at 0 °C for 15 min. When the reaction was completed, the mixture was purified by column chromatography on silica gel (5% CH₃OH in DCM) to obtain target compound (887 mg, 91%) as white solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.99 (s, 1H), 7.71 (dd, *J* = 7.5, 1.1 Hz, 1H), 7.64 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 5.13 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.45 (d, *J* = 17.6 Hz, 1H), 4.31 (d, *J* = 17.6 Hz, 1H), 4.22 (t, *J* = 6.4 Hz, 2H), 3.15 (s, 3H), 2.96 - 2.86 (m, 1H), 2.64 – 2.55 (m, 1H), 2.52 (br, 2H), 2.47 - 2.40 (m, 1H), 2.06 - 1.97 (m, 1H), 1.81 – 1.66 (m, 2H), 1.62 (p, *J* = 7.0 Hz, 2H), 1.55 - 1.47 (m, 2H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 172.88, 170.99, 167.67, 143.74, 134.10, 131.99, 128.60, 122.63, 118.80, 96.12, 76.53, 70.40, 51.65, 46.97, 36.55, 31.20, 28.02, 27.46, 24.29, 22.34, 18.64. MS(ESI) *m/z*: 433 [M+H]⁺.

NMR spectra shows in Figures S5.

8-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-4-yl)oct-7-yn-1-yl methanesulfonate (8)

The title compound (white solid, 914mg, 82% yield over two steps) was synthesized according to procedures for the preparation of 7. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.00 (s, 1H), 7.70 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.63 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 5.14 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.45 (d, *J* = 17.6 Hz, 1H), 4.31 (d, *J* = 17.7 Hz, 1H), 4.20 (t, *J* = 6.4 Hz, 2H), 3.15 (s, 3H), 2.95 - 2.86 (m, 1H), 2.64 - 2.56 (m, 1H), 2.48 (d, *J* = 6.9 Hz, 2H), 2.47 - 2.41 (m, 1H), 2.04 - 1.97 (m, 1H), 1.73 - 1.64 (m, 2H), 1.59 (p, *J* = 7.0 Hz, 2H), 1.51 - 1.35 (m, 4H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 172.89, 171.00, 167.67, 143.74, 134.08, 131.97, 128.60, 122.61, 118.84, 96.27, 76.44, 70.41, 51.64, 46.96, 36.54, 31.20, 28.43, 27.81 (d, *J* = 21.7 Hz), 24.43, 22.37, 18.67. MS(ESI) *m/z*: 447 [M+H]⁺.

NMR spectra shows in Figures S6.

(E)-N-(4-(1-(4-(4-(7-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-4-yl)hept-6-yn-1-yl)piperazin-1-yl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide (SIAIS630120)

The compound (E)-N-(4-(1-(4-(piperazin-1-yl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide (20 mg, 0.04 mmol) **5**, 7-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-4-yl)hept-6-yn-1-yl methanesulfonate (20.7mg, 0.048mmol) **7**, DIPEA(15.6 mg,0.12 mmol), NaI(17.8 mg,0.12 mmol)and DMF (2 mL) were added to a 25 mL single bottle. After being stirred 6 h at 60 °C, the resulting mixture was purified by preparative HPLC (10 to 90% acetonitrile/0.05% HCl in H₂O) to obtain target compound (5.8mg, 25%) as white solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.00 (s, 1H), 8.89 (s, 1H), 8.70 - 8.66 (m, 1H), 8.28 - 8.25 (m, 2H), 7.71 (d, *J* = 7.5 Hz, 1H), 7.65 (d, *J* = 7.5 Hz, 1H), 7.56 - 7.45 (m, 2H), 7.28 (d, *J* = 8.5 Hz, 2H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.82 (d, *J* = 15.9 Hz, 2H), 5.16 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.47 (d, *J* = 17.7 Hz, 1H), 4.33 (d, *J* = 17.7 Hz, 1H), 3.89 (d, *J* = 12.9 Hz, 2H), 3.55 (br, 4H), 3.24 - 3.16 (m, 5H), 3.14 - 3.04 (m, 5H), 2.98 - 2.88 (m, 1H), 2.65 - 2.56 (m, 1H), 2.55 - 2.51 (m, 2H), 2.49 - 2.43 (m, 1H), 2.07 - 1.97 (m, 1H), 1.84 - 1.75 (m, 2H), 1.69 -

1.58 (m, 4H), 1.53 - 1.42 (m, 5H), 1.35 - 1.29 (m, 2H), 1.26 - 1.22 (m, 3H), 1.12 - 0.97 (m, 2H). ¹³C NMR (126 MHz, DMSO-d₆) δ 172.91, 171.03, 168.96, 167.64, 164.08, 150.17, 143.77, 134.09, 133.84, 131.99, 128.63, 128.44, 127.03, 125.94, 125.27, 122.68, 118.76, 114.79, 96.00, 76.62, 55.16, 51.64, 50.39, 47.00, 44.69, 38.70, 35.53, 35.41, 31.20, 29.24, 27.43, 25.28, 23.49, 22.43 (d, *J* = 9.8 Hz), 18.49. MS(ESI) *m/z*: 812.4496 [M+H]⁺.

NMR spectra shows in Figures S7.

(E)-N-(4-(1-(4-(4-(8-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-4-yl)oct-7-yn-1-yl)piperazin-1-yl)benzoyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide (SIAIS630121)

The title compound (white solid, 5.7mg, 24% yield) was synthesized according to procedures for the preparation of **SIAIS630120**. ¹H NMR (500 MHz, Methanol-d₄) δ 8.84 (d, *J* = 2.2 Hz, 1H), 8.63 (dd, *J* = 5.2, 1.5 Hz, 1H), 8.37 - 8.32 (m, 1H), 7.75 (d, *J* = 6.6 Hz, 1H), 7.71 (dd, *J* = 8.1, 5.2 Hz, 1H), 7.63 - 7.55 (m, 2H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 8.7 Hz, 2H), 7.07 (d, *J* = 8.9 Hz, 2H), 6.81 (d, *J* = 15.9 Hz, 1H), 5.19 (dd, *J* = 13.3, 5.2 Hz, 1H), 4.53 (d, *J* = 17.4 Hz, 1H), 4.47 (d, *J* = 17.4 Hz, 1H), 3.95 (br, 1H), 3.67 (br, 2H), 3.39 - 3.32 (m, 6H), 3.25 - 3.19 (m, 4H), 3.18 - 3.11 (m, 2H), 2.95 - 2.86 (m, 1H), 2.84 - 2.75 (m, 1H), 2.57 - 2.49 (m, 3H), 2.24 - 2.15 (m, 1H), 1.86 - 1.80 (m, 2H), 1.70 (p, *J* = 6.9 Hz, 3H), 1.65 - 1.55 (m, 5H), 1.53 - 1.39 (m, 4H), 1.37 - 1.27 (m, 3H), 1.24 - 1.07 (m, 2H). ¹³C NMR (126 MHz, Methanol-d₄) δ 174.69, 172.32 (d, *J* = 11.1 Hz), 171.01, 167.28, 152.17, 147.08, 145.28, 139.35, 136.11, 135.78, 132.92, 129.69 (d, *J* = 4.6 Hz), 128.84, 126.69, 126.40, 123.76, 120.93, 116.85, 97.12, 77.40, 58.00, 53.70, 52.85, 47.10, 40.58, 37.11 (d, *J* = 10.7 Hz), 32.35, 30.53, 29.39 (d, *J* = 3.7 Hz), 27.12, 24.99 (d, *J* = 3.4 Hz), 24.11, 19.94. MS(ESI) *m/z*: 826.4655 [M+H]⁺.

NMR spectra shows in Figures S8.

Figure Legends

Figure S1. Construction and verification of NAMPT knock-in HEK293T cell line using Tild-CRISPR.

(A) Genomic DNA sequence map from Snap Gene software of 293FT KI cell line. (B)

Different fields of fluorescence microscope imaging. Magnification: 10 X, ruler: 100 μm .

Figure S2. Drug metabolism and pharmacokinetic/pharmacodynamic profile of 630120 and 630121.

Plasma concentration-time profiles of (A) **630120** and (B) **630121** in rats.

Figure S3. ^1H and ^{13}C NMR spectra for compound 3

Figure S4. ^1H and ^{13}C NMR spectra for compound 5

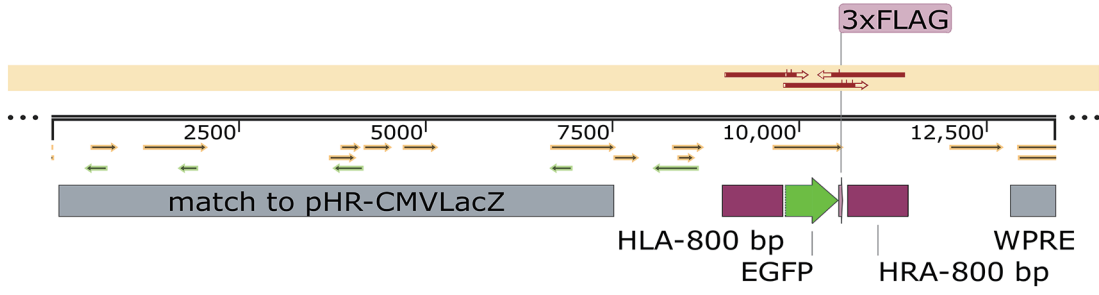
Figure S5. ^1H and ^{13}C NMR spectra for compound 7

Figure S6. ^1H and ^{13}C NMR spectra for compound 8

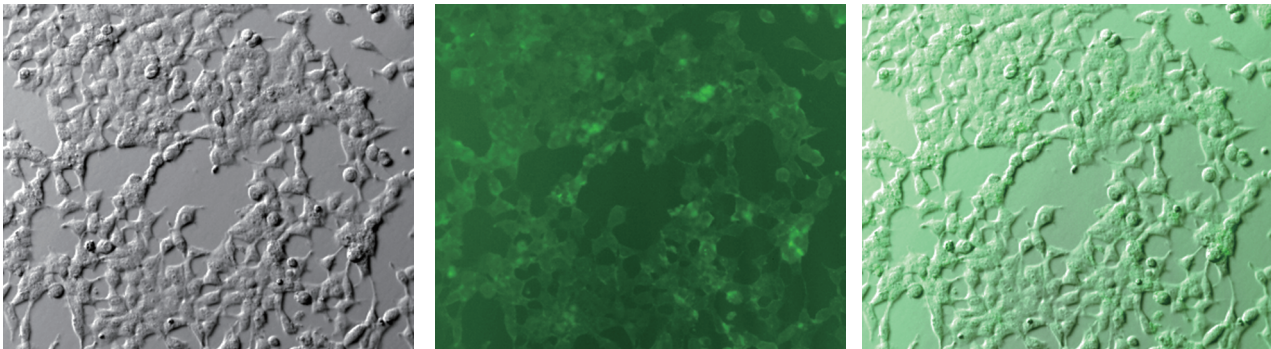
Figure S7. ^1H and ^{13}C NMR spectra for compound 630120

Figure S8. ^1H and ^{13}C NMR spectra for compound 630121

A



B

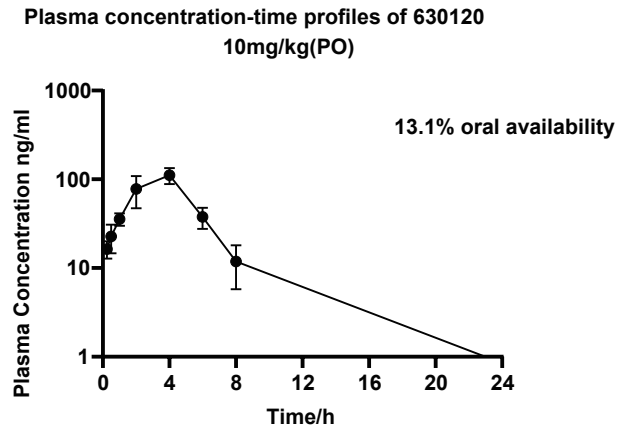
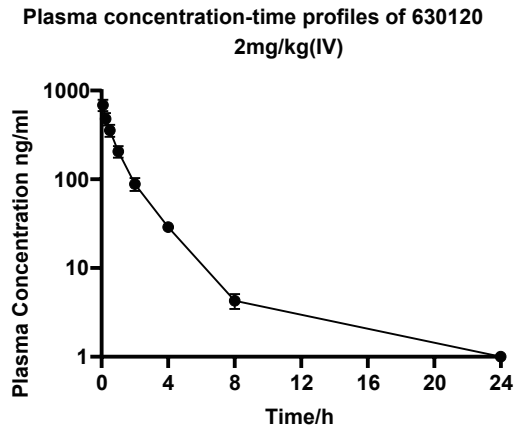


Bright field

FITC field

Merged field

A



B

