Supporting Information

Design, Synthesis and Bioevaluation of Pyrido[2,3-*d*]pyrimidin-7-ones as Potent SOS1 Inhibitors

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1. Biological assays

Cell culture

SW620 (CCL-227), MIA PaCa-2 (CRM-CRL-1420), and A549 (CRM-CCL-185) were purchased from the American Type Culture Collection (ATCC). NCI-H358 (TCHu151), AsPC-1 (TCHu 8), PANC-1 (SCSP-535), and A-375 (SCSP-533) were kindly provided by Cell Bank/Stem Cell Bank, Chinese Academy of Sciences. SW620 was cultured in Leibovitz's L-15 medium (BasalMedia, L620KJ). MIA PaCa-2, PANC-1 and A-375 were cultured in DMEM medium (BasalMedia, L110KJ). A549 was cultured in Ham's F-12K medium (BasalMedia, L450KJ). NCI-H358 and AsPC-1 were cultured in RPMI 1640 medium (BasalMedia, L220KJ). All mediums were supplemented with 10% fetal bovine serum (Meilunbio, PWL001), 100 U/mL of penicillin, and 0.1 mg/mL of streptomycin (Meilunbio, PWL062). MIA PaCa-2, A549, NCI-H358, AsPC-1, PANC-1, and A-375 cells were grown at 37 °C in a humidified atmosphere of 5% (v/v) CO_2. SW620 was incubated at 37 °C under 100% air. All cell lines were identified using short tandem repeat analysis (STR).

Protein expression and purification

For 6×His-tagged $KRAS^{G12D}$ (residues 1-169) and 6×His-tagged SOS1 (residues 564-1049) recombinant protein expression and purification, the coding sequence of the indicated $KRAS^{G12D}$ and SOS1 gene were cloned into the pET28A vector with 6×His tag. $KRAS^{G12D}$ and SOS1 plasmids were transformed into BL21 (DE3) gold-competent cells, and then cells were grown in lysogeny broth (LB) medium and induced by isopropyl β-D-1-thiogalactopyranoside (IPTG) with a final concentration of 0.2 mM for 12 h at 16 °C. Cells were harvested and lysed in buffer containing 20 mM HEPES pH 7.6, 150 mM NaCl, 1 mM tris (2-carboxyethyl) phosphine (TCEP), and 20 mM imidazole. The supernatant was coupled to a HisTrap HP column (17524802, GE Healthcare) and eluted with buffer containing 20 mM HEPES pH 7.6, 150 mM NaCl, 1 mM TCEP, and 300 mM imidazole. The elution was further purified by gel filtration using Superdex 200 Increase 10/300 GL column (28990944, GE Healthcare) eluted with buffer containing 20 mM HEPES pH 7.4,150 mM NaCl, 1 mM TCEP. The purified proteins were concentrated and stored at -80 °C.

For GST-tagged $KRAS^{G12D}$ (residues 1-169) recombinant protein expression and purification, the coding sequence of the indicated $KRAS^{G12D}$ gene was cloned into the pGEX6p-1 vector with GST tag. GST-tagged $KRAS^{G12D}$ plasmids were transformed into BL21 (DE3) gold-competent cells, and then cells were grown in terrific broth (TB) medium and induced by IPTG with a final concentration of 0.2 mM for 12 h at 16 °C. Cells were harvested and lysed in buffer containing 20 mM HEPES pH 7.6, 150 mM NaCl, 1 mM TCEP. The supernatant was coupled to a GSTtrap HP column (17528202, GE Healthcare) and eluted with buffer containing 20 mM HEPES pH 7.6, 150 mM NaCl, 1 mM TCEP, and 20 mM glutathione. The elution was further purified by gel filtration using Superdex 200 Increase 10/300 GL column eluted with buffer containing 20 mM HEPES pH 7.4, 150 mM NaCl, and 1 mM TCEP. The protein was concentrated and stored at -80 °C.

Thermal Shift Assay (TSA)

6×His-tagged SOS1 and 6×His-tagged $KRAS^{G12D}$ were used for TSA. SOS1 (final concentration 1 μM) or $KRAS^{G12D}$ (final concentration 10 μM) recombinant protein was incubated with SOS1 inhibitors (final concentration 10 μM) and 5×SYPRO orange dye (S5692, Sigma) in a volume of 20 μL buffer containing 20 mM HEPES pH 7.8, 150 mM NaCl. The thermostability of SOS1 or $KRAS^{G12D}$ was tested using Bio-Rad CFX96 RT-PCR system, heated from 25°C to 90°C at 0.5°C/10 s, and the fluorescence signal was recorded to calculate the melting temperature offset.

Homogeneous Time-Resolved Fluorescence (HTRF) Assay

6×His-tagged SOS1 and GST-tagged $KRAS^{G12D}$ were used for the HTRF assay. HTRF assay was carried out in an assay buffer containing 10 mM HEPES pH 7.4, 150 mM NaCl, 1 mM TCEP, 5 mM $MgCl_2$, and 0.05% bovine serum albumin. The HTRF assay was divided into compound group, DMSO group, and blank control group. The total volume of the assay was 21 μL. 1 μL of the indicated compound or DMSO was added to the OptiPate-384 assay plate (6007299, PerkinElmer). For the compound group and DMSO group, SOS1 protein (final concentration 50 nM) and fluorescent GTP analogue EDA–GTP–DY-647P1 (NU-820-647P1, Jena Bioscience) (final concentration 50 nM) were mixed in the assay buffer and added to the OptiPate-384 assay plate in a volume of 10 μL. Blank control was added with 10 μL of a diluted solution containing EDA-GTP-DY-647P1 (final concentration 50 nM). After 15 min incubation at room temperature, 10 μL of GST-tagged $KRAS^{G12D}$ (final concentration 10 nM) and anti-GST-terbium (61GSTTLB, Cisbio) (dilution ratio 1:200) solution diluted with assay buffer was added to each well. After 30 min incubation at room temperature, the HTRF signal was measured on TECAN Spark. The excitation was set at 320 nm and emissions were measured at 620 and 665 nm. Data were analyzed using GraphPad Prism software.

3-D proliferation assay

Cells were seeded at 500 cells per well in low adsorption 96-well Nunclon Sphera plates (174929, Thermo Scientific). The indicated concentrations of compounds or DMSO were added to each well and incubated for 7 days. Cell viability was quantified by CellTiter-Glo 3D cell viability assay (G9863, Promega). Cell growth inhibition ratio = $(1- (Value_{DMSO}-Value_{compound}/ Value_{DMSO}))*100\%$. The IC_{50} was calculated by fitting a dose-response curve (variable slope) by nonlinear regression.

Western Blot

Cells are lysed in ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (P0013C, Beyotime) containing protease inhibitors (B14001, Bimake) and phosphatase inhibitors (B15001, Bimake). After sonication and centrifugation, total protein concentration was determined by a BCA kit (23225, Thermo Scientific). The heated denatured proteins were electrophoresed through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins on the gel were transferred to nitrocellulose (NC) membranes by constant current, then the NC

membranes were blocked with 5% skim milk for 1 h, and incubated with primary antibody overnight at 4 °C. p-ERK (Thr202/Tyr204) antibody (4370), total-ERK antibody (4695), p-AKT(Ser473) antibody (4060), total-AKT antibody (4685) and β-Tubulin (15115) antibodies were purchased from Cell Signaling Technology. After incubation with a secondary antibody (W4011, Promega) for 1 h at room temperature, NC membranes were scanned by GenGnome XRQ NPC instrument with ECL luminous liquid (MA0186, Meilunbio) and quantified by grayscale analysis.

Kinase inhibition assay

The inhibitory profiles of compound **8u** against a small panel of kinases including FGFR1, EGFR, HER2, PDGFRα, CDK2, and CDK4 were determined through the kinase profiling service from Shanghai ChemPartner Co., Ltd.

2. Molecular docking study

The X-ray crystal structure of the complex of between SOS1 and inhibitor BI-3406 (PDB ID: 6SCM) was used as a model for docking studies. Structure was prepared using the Protein Preparation Wizard in the module from the Schrödinger Small Molecular Drug Discovery Suite (Maestro), and water molecules were removed. The grid file was generated by using Receptor Grid Generation, picking BI-3406 in the crystal structure as the center of the box, and keeping the rest of the parameters as default. The 3D conformations of small molecules 20u and 20p was generated by using the LigPrep. They were docked using Glide with SP mode in Ligand Docking module.

3. Figures S1-4

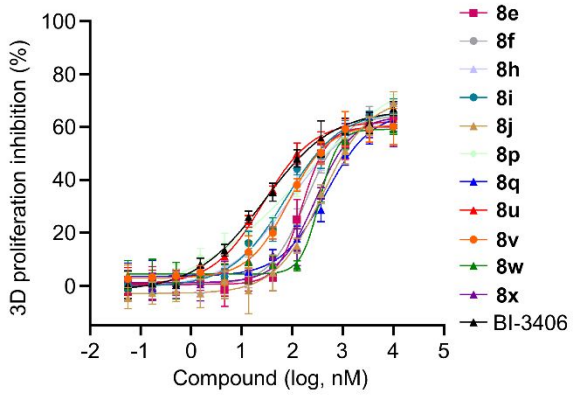


Figure S1. 3-D proliferation inhibitory activities of selected SOS1 inhibitors in MIA PaCa-2 cells. Error bar represents mean ± SD from four replicate experiments.

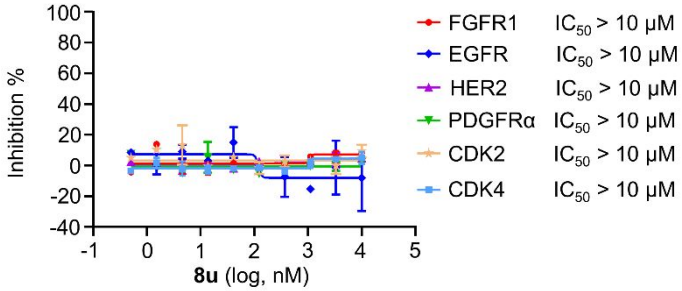


Figure S2. The kinases inhibitory activities of compound **8u** against FGFR1, EGFR, HER2, PDGFRα, CDK2 and CDK4. Error bar represents mean ± SD from duplicate experiments.

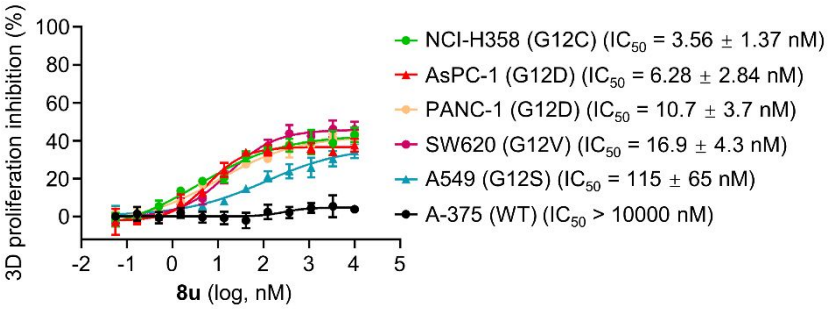


Figure S3. 3-D cell growth inhibition of **8u** against a panel of cancer cell lines with various KRAS mutation statuses. Error bar represents mean ± SD from four replicate experiments.

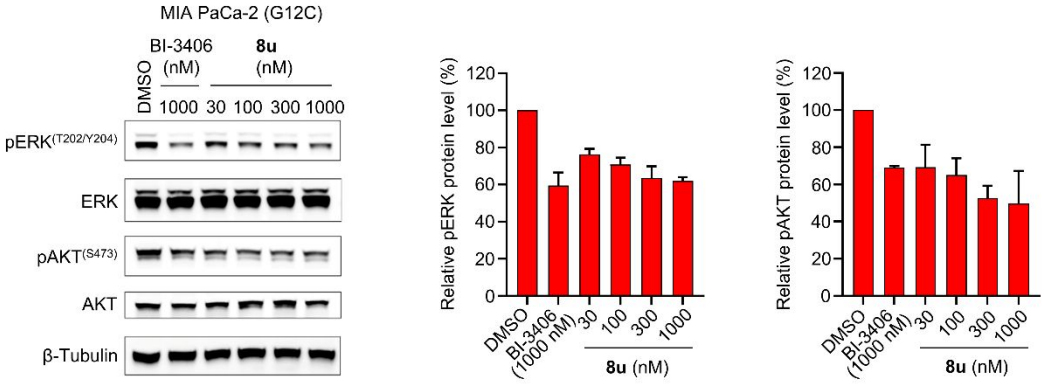


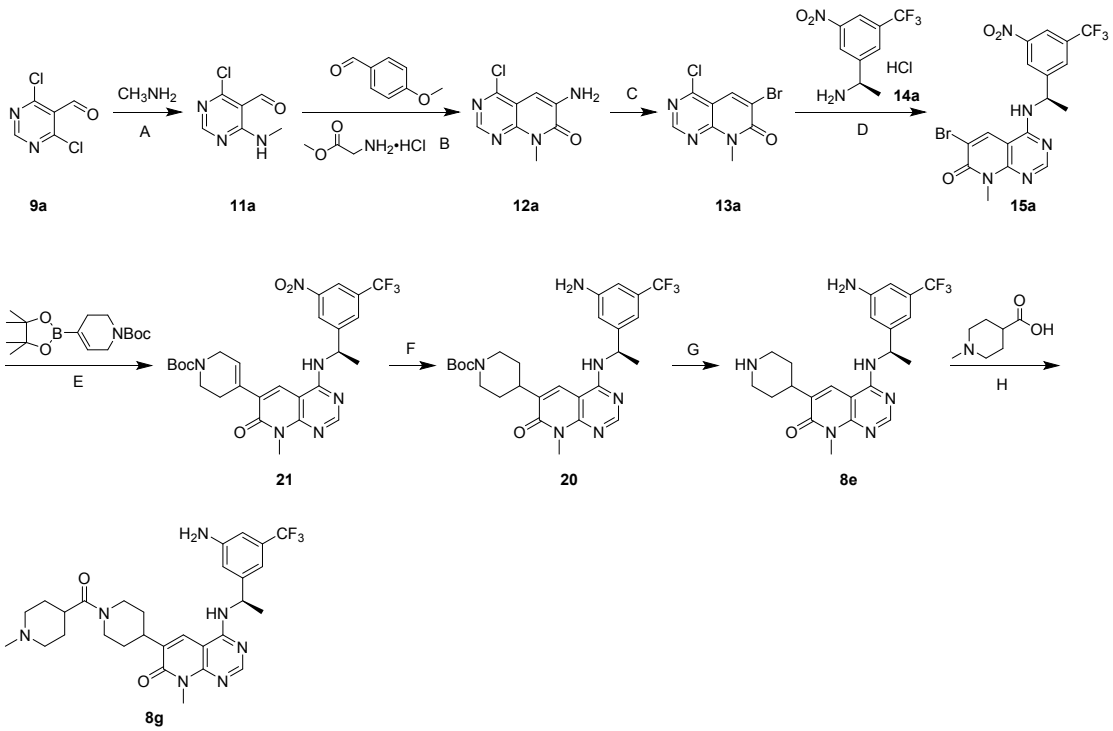
Figure S4. Representative immunoblots of pERK and pAKT in MIA PaCa-2 (KRAS G12C) cells that were treated with the indicated concentration of 8u for 2 h. pERK and pAKT levels were quantified (Right panel). Quantified data represent the mean ± SD from two independent biological replicates.

4. Synthetic details

General information of Chemistry

All chemicals were purchased from commercial available suppliers and used directly without further purification. No unexpected or unusually high safety hazards were encountered in this study. The reactions were monitored by thin-layer chromatography (TLC) on prefabricated plate visualized with UV light. Column chromatography was performed on silica gel (200-300 mesh). The preparative high-performance liquid chromatography (HPLC) was performed on a Shimadzu CBM-20Alite HPLC system with the UV detector set to 254 nm. HPLC parameters: PDA: SPD-20A; PUMP: LC-20AP; C18 colum: 5 μm, 250×20 mm, SHIMADZU Shim-pack GIST; flow rate: 10 mL/min; eluent: A (water with 0.1% TFA) and B (acetonitrile with 0.1% TFA). The purities of the final compounds **8a**-**x** were at least 90%, which were measured by analytical HPLC on a Shimadzu CBM-20Alite HPLC system with UV detection at 254 nM. Analytical HPLC parameters: PDA: SPD-M20A; pump: LC-20AD; C18 column: 5 μm, 150 × 4.6 mm, SHIMADZU Shim-pack GIST; colum oven: CTO-10ASvp; flow rate: 1mL/min, a gradient program set to 10% of acetonitrile in water containing 0.1% TFA progressing to 100% of acetonitrile in 15 minutes. ^{1}H NMR and ^{13}C NMR were recorded on Bruker AVANCE III 400 or 500 MH_Z spectrometers. Chemical shifts are reported in units of parts per million (ppm, δ), and coupling constants (J) are reported in Hz. The chemical shifts of ^{1}H NMR and ^{13}C NMR are calibrated to residual solvent peak as below: $CDCl_3$, 7.26 ppm (H), 77.16 ppm (C), DMSO-d_6, 2.50 ppm (H), 39.52 ppm (C), CD_3OD, 3.31 ppm (H), 49.00 ppm (C). The following abbreviations are used to report spin multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br s (broad singlet). The high resolution electrospray mass spectra (HRMS) were carried out on an Agilent G6520 Q-TOF instrument with electrospray ionization (ESI).

Scheme S1. Synthesis of compounds 8e and 8g[a]



[a]Reagents and conditions: (A) $CHCl_3$, TEA, 0 ℃ - rt, overnight, 61.9%. (B) i) TEA, MeOH, rt, overnight; ii) 70% AcOH, 50 ℃, 8h, 72.2% (two steps). (C) CuBr, NaBr, *t*-BuONO, CH_3CN, 0 ℃ - rt, overnight, 50.6%. (D) DIPEA, CsF, DMSO, 80 ℃, overnight, 81.3%. (E) Cs_2CO_3, $Pd(dppf)Cl_2$, 1,4-dioxane, 80 ℃, overnight, 76.5%. (F) Pd/C, H_2, EtOAc, rt, overnight, 87.7%. (G) HCl (4 M in 1,4-dioxane), DCM, 0 ℃ - rt, overnight, 51.0%. (H) HATU, DIPEA, DMF, rt, overnight. 43.2%.

Step A: 4-chloro-6-(methylamino)pyrimidine-5-carbaldehyde (11a)

To a solution of 4,6-dichloropyrimidine-5-carbaldehyde (2.0 g, 11.30 mmol) in $CHCl_3$ (30 mL) was added methylamine hydrochloride (0.8 g，12.42 mmol) and Et_3N (4.8 mL, 33.90 mmol). The reaction mixture was stirred at room temperature overnight. The reaction was extracted with DCM, washed with brine and dried over anhydrous sodium sulfate (Na_2SO_4). The organic layer was concentrated under reduced pressure, and the residue was purified by silica gel chromatography to afford **11a** as a white solid (1.2 g, 61.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.25 (s, 1H), 9.17 (s, 1H), 8.48 (s, 1H), 3.02 (d, J = 4.9 Hz, 3H).

Step B: 6-amino-4-chloro-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (12a)

To a solution of compound **11a** (1.6 g, 9.21 mmol) in MeOH (20 ml) was added p-methoxybenzaldehyde (1.1 mL, 9.21 mmol), glycine methyl ester hydrochloride (1.3 g, 10.13 mmol) and Et_3N (2.8 mL, 20.26 mmol). The reaction mixture was stirred at room temperature overnight, and then a 70% solution of AcOH was added to the mixture, stirred at 50 °C for another 8 hours. The resulted suspension was cooled to room temperature, filtered to afford yellow solid, and the crude compound was triturated by ethyl acetate to afford **12a** as an off-white solid (1.4 g, 72.2%). ^{1}H NMR (500 MHz,

DMSO-d_6) δ 8.59 (s, 1H), 6.79 (s, 1H), 6.39 (s, 2H), 3.71 (s, 3H).

Step C: 6-bromo-4-chloro-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (13a)

To a suspension of CuBr (0.6 g, 2.85 mmol) and NaBr (0.7 g, 7.12 mmol) in MeCN (30 mL) was added *t*-BuONO (0.5 mL, 4.15 mmol) at 0°C under nitrogen. The ice bath was removed, and the mixture was stirred at room temperature for 30 minutes before being cooled to 0 °C again. A solution of **12a** (0.5 g, 2.37 mmol) in MeCN (10 mL) was slowly added to the reaction mixture. The reaction mixture was allowed to warm from 0 °C to room temperature and stirred overnight. The reaction was then diluted with water, extracted with ethyl acetate, washed with brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by silica gel chromatography to afford compound **13a** an off-white solid (0.33 g, 50.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.99 (s, 1H), 8.56 (s, 1H), 3.71 (s, 3H).

Step D: (*R*)-6-bromo-8-methyl-4-((1-(3-nitro-5-(trifluoromethyl) phenyl) ethyl) amino) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (15a)

To a solution of **13a** (200 mg, 0.73 mmol) in DMSO (5 ml) was added (*R*)-1-(3-nitro-5-(trifluoromethyl)phenyl)ethan-1-amine hydrochloride (237 mg, 0.87 mmol), CsF (332 mg, 2.19 mmol) and DIPEA (0.6 mL, 3.64mmol). The reaction mixture was stirred at 80 °C under nitrogen for 12 h, then the mixture was extracted with ethyl acetate, washed with brine and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure and purified by silica gel chromatography to give compound **15a** an off-white solid (320 mg, 88.0%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.93 (s, 1H), 8.56 (t, J = 1.9 Hz, 1H), 8.51 (d, J = 7.2 Hz, 1H), 8.38 (s, 1H), 8.35 (d, J = 2.1 Hz, 1H), 8.28 (d, J = 1.7 Hz, 1H), 5.67-5.61 (m, 1H), 3.61 (s, 3H), 1.61 (d, J = 7.1 Hz, 3H).

Step E: *tert*-butyl (*R*)-4-(8-methyl-4-((1-(3-nitro-5-(trifluoromethyl)phenyl)ethyl)amino)-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-6-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (21)

To a solution of **15a** (80 mg, 0.18 mmol) in 1,4-dioxane (8 ml) was added *N*-Boc-5,6-dihydro-2H-pyridine-4-boronic acid (314 mg, 1.02 mmol), Cs_2CO_3 (662 mg, 2.03 mmol), Pd(dppf)Cl_2 (25 mg, 0.03 mmol) and H_2O (0.8 mL). The reaction mixture was purged with nitrogen three times, and heated at 80 °C under nitrogen for 12 hours. The resulting mixture was extracted with ethyl acetate, washed with brine and dried over anhydrous Na_2SO_4. The organic layer was concentrated and purified by silica gel chromatography to provide **21** an off-white solid (297 mg, 76.5%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 (s, 1H), 8.46 (d, J = 7.3 Hz, 1H), 8.35 (s, 1H), 8.33 (s, 1H), 8.28 (s, 1H), 8.23 (s, 1H), 6.39 (s, 1H), 5.70-5.64 (m, 1H), 4.07 – 4.00 (m, 2H), 3.57 (s, 3H), 3.55-3.52 (m, 2H), 2.60-2.53 (m, 1H), 2.48-2.44 (m, 1H), 1.62 (d, J = 7.1 Hz, 3H), 1.44 (s, 9H).

Step F: *tert*-butyl(*R*)-4-(4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-6-yl)piperidine-1-carboxylate

(20)

To a solution of **21** (120 mg, 0.21 mmol) in ethyl acetate (30 mL) was added 10% Pd/C (200 mg). The suspension was stirred under hydrogen atmosphere (balloom) at room temperature for 12 hours. The mixture was filtered through Celite, washed with ethyl acetate, the filtrate was concentrated under reduced pressure and purified by silica gel chromatography to afford **20** an off-white solid (100 mg, 87.7%).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.22 (d, J = 7.6 Hz, 1H), 8.15 (s, 1H), 6.80 (d, J = 8.3 Hz, 2H), 6.69 (s, 1H), 5.56 (s, 2H), 5.47-5.42 (m, 1H), 4.13 (s, 2H), 3.59 (s, 3H), 3.03-2.97 (m, 1H), 2.82 (s, 2H), 1.82-1.75 (m, 2H), 1.52 (d, J = 7.0 Hz, 3H), 1.51-1.46 (m, 2H), 1.41 (s, 9H).

Step G: (*R*)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methyl-6-(piperidin-4-yl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8e)

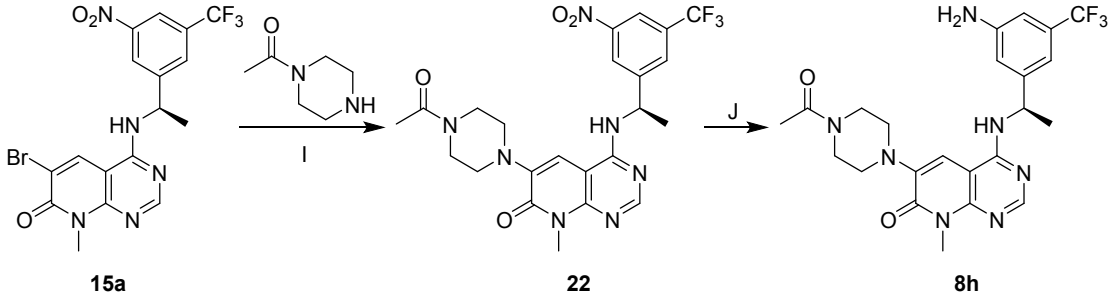
To a solution of **20** (120 mg, 0.22 mmol) in DCM (10 mL) was slowly added HCl (4M in 1,4-dioxane, 3 mL). The reaction mixture was stirred at room temperature for 12 hours, and then concentrated under reduced pressure. The residue was neutralized with saturated aqueous sodium bicarbonate solution, and extracted with ethyl acetate, washed with brine and dried over anhydrous Na_2SO_4. The resulting residue was further purified by preparative HPLC to afford **8e** as a TFA salt and a white solid (50 mg, 51.0%). Purity = 98.2%, retention time: 10.35 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.69 (d, J = 11.6 Hz, 1H), 8.40-8.28 (m, 3H), 8.11 (s, 1H), 6.83 (d, J = 8.8 Hz, 2H), 6.73 (s, 1H), 5.50-5.44 (m, 2H), 3.59 (s, 3H), 3.41 (d, J = 12.8 Hz, 2H), 3.11-3.01 (m, 3H), 2.04 (d, J = 12.9 Hz, 2H), 1.85-1.73 (m, 2H), 1.54 (d, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.75, 158.00, 157.24 (d, J = 11.3 Hz), 153.00, 149.05, 146.75, 133.08, 129.86 (q, J = 30.2 Hz), 126.94 (d, J = 8.8 Hz), 124.63 (q, J = 272.2 Hz), 115.28, 109.52, 108.44, 96.85, 49.38, 43.56, 34.94, 28.42, 27.42 (d, J = 13.9 Hz), 22.04. HRMS (ESI) for $C_{22}H_{26}F_3N_6O$ $[M + H]^+$, calc: 447.2115, found: 447.212.

Step H: (*R*)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methyl-6-(1-(1-methylpiperidine-4-carbonyl)piperidin-4-yl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8g).

To a solution of compound **8e** (35 mg, 0.08 mmol) in DMF (8 mL) was added 1-methylpiperidine-4-carboxylic acid hydrochloride (16 mg, 0.09 mmol), HATU (45 mg, 0.12 mmol) and DIPEA (52 μL, 0.31 mmol). The reaction mixture was stirred at room temperature for 12 hours. The mixture was extracted with ethyl acetate, washed with brine and dried over anhydrous Na_2SO_4. The collected organic layer was concentrated under reduced pressure and further purified by preparative HPLC to afford **8g** as a TFA salt and a white solid (20 mg, 43.2%). Purity = 99.4%, retention time: 10.99 min. ^{1}H NMR (500 MHz, DMSO- d_6) δ 9.28 (s, 1H), 8.35 (s, 1H), 8.23 (t, J = 6.7 Hz, 1H), 8.13 (d, J = 2.9 Hz, 1H), 6.81 (s, 1H), 6.79 (s, 1H), 6.70 (s, 1H), 5.49-5.38 (m, 1H), 4.61 (d, J = 12.4 Hz, 1H), 4.10 (d, J = 13.3 Hz, 1H), 3.59 (s, 3H), 3.47 (br s, 3H), 3.23-3.14 (m, 2H), 3.13-3.06 (m, 2H), 3.00-2.89 (m, 3H), 2.67-2.63 (m, 2H), 2.03-1.84 (m, 4H), 1.77-1.69 (m, 1H), 1.67-1.56 (m, 1H), 1.52 (d, J = 5.3 Hz, 3H), 1.48-1.37 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.90, 161.95, 157.90, 157.12, 152.81, 149.22, 146.77,

134.25, 129.71, 126.88, 124.64 (q, J = 272.2 Hz), 115.19, 109.44, 108.29, 96.92, 52.96 (d, J = 5.0 Hz), 49.37, 45.41, 42.93, 41.79, 36.43 (d, J = 7.6 Hz), 34.51, 28.45, 26.19 (d, J = 7.6 Hz), 22.08 (d, J = 7.6 Hz). HRMS (ESI) for $C_{29}H_{37}F_3N_7O_2$ $[M + H]^+$, calc: 572.2955, found: 572.2955.

Scheme S2. Synthesis of compounds 8h[a]



[a]Reagents and conditions: (I) $Pd(AcO)_2$, Xantphos, Cs_2CO_3, 1,4-dioxane, 90 ℃, overnight; 70.4%. (J) Fe, NH_4Cl, EtOH, 90 ℃, overnight, 53.2%.

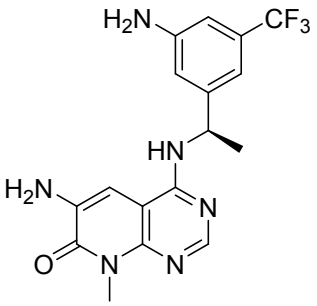
Step I: (*R*)-6-(4-acetylpiperazin-1-yl)-8-methyl-4-((1-(3-nitro-5-(trifluoromethyl) phenyl) ethyl)amino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (22)

To a solution of **15a** (130 mg, 0.28 mmol) in 1,4-dioxane (6 ml) was added 1-Acetylpiperazine (71 mg, 0.55 mmol), Cs_2CO_3 (179 mg, 0.55 mmol), $Pd(OAc)_2$ (6 mg, 0.03 mmol) and Xantphos (32 mg, 0.06 mmol). The reaction mixture was purged with nitrogen three times, and heated at 90 °C under nitrogen for 12 hours. The mixture was extracted with DCM, washed with brine and dried over anhydrous Na_2SO_4. The collected organic layer was concentrated under reduced pressure and purified by silica gel chromatography to give **22** an off-white solid (100 mg, 70.4 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.54 (s, 1H), 8.36 (s, 1H), 8.27 (s, 1H), 8.27-8.24 (m, 2H), 7.50 (s, 1H), 5.70-5,64 (m, 1H), 3.64-3.61 (m, 4H), 3.60 (s, 3H), 3.22-3.19 (m, 2H), 3.14-3.11 (m, 2H), 2.06 (s, 3H), 1.63 (d, J = 7.1 Hz, 3H).

Step J: (*R*)-6-(4-acetylpiperazin-1-yl)-4-((1-(3-amino-5-(trifluoromethyl) phenyl)ethyl)amino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8h).

To a solution of compound **22** (100 mg, 0.19 mmol) in EtOH (10 mL) was added Fe powder (107 mg, 1.92 mmol), NH_4Cl (103 mg, 1.92 mmol) and H_2O (2 mL). The suspension was stirred at 90 °C under nitrogen for 12 hours. The resulting mixture was filtered through Celite, washed with DCM, the filtrate was then washed with brine and dried over anhydrous Na_2SO_4. The collected organic layer was concentrated under reduced pressure and further purified by preparative HPLC to afford **8h** as a TFA salt and a white solid (50 mg, 53.2%). Purity = 97.2%, retention time: 11.60 min.^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (s, 1H), 8.11 (d, J = 7.7 Hz, 1H), 7.52 (s, 1H), 6.84 (d, J = 13.1 Hz, 2H), 6.72 (s, 1H), 5.48-5.42 (m, 1H), 3.90 (br s, 2H), 3.63-3.59 (m, 7H), 3.21-3.14 (m, 2H), 3.13-3.05 (m, 2H), 2.05 (s, 3H), 1.53 (d, J = 7.0 Hz, 3H). - NH_2 in water. ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.37, 159.10, 157.27, 154.89, 149.96, 148.71, 147.08, 139.94, 129.83 (q, J = 30.2 Hz), 125.62 (q, J = 272.2 Hz), 115.37,

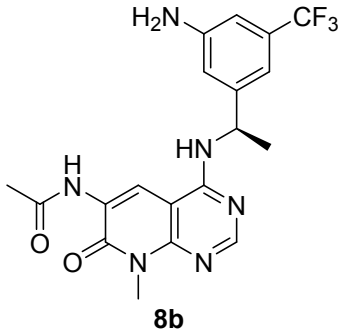
112.04, 109.94, 108.55, 97.29, 49.34 (t, J = 18.9 Hz), 45.65, 40.71, 28.58, 22.24, 21.26. HRMS (ESI) for $C_{23}H_{27}F_3N_7O_2$ $[M + H]^+$, calc: 490.2173, found: 490.2180.



8a

(*R*)-6-amino-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8a)

Compound **8a** was prepared from **12a** via two subsequent steps according to step D and step F for the preparation of **8g**. The crude product was further purified by preparative HPLC to give **8a** as a TFA salt and a white solid. Purity = 96.0%, retention time: 11.09 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.24 (s, 1H), 6.98 (s, 1H), 6.91 (s, 1H), 6.80 (s, 1H), 6.54 (br s, 4H), 5.37 (p, J = 6.9 Hz, 1H), 3.65 (s, 3H), 1.52 (d, J = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.64, 155.83, 151.71, 147.41, 146.96, 146.91, 135.95, 129.91 (q, J = 30.2 Hz), 124.55 (q, J = 273.3 Hz), 116.59, 111.42, 109.65, 100.23, 99.09, 49.67, 28.72, 22.41. HRMS (ESI) for $C_{17}H_{18}F_3N_6O$ $[M + H]^+$, calc: 379.1489, found: 379.1492.

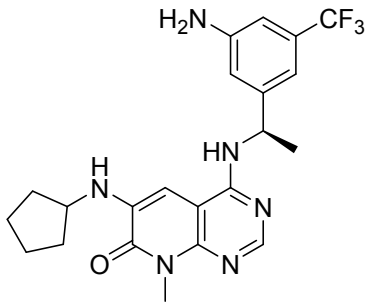


8b

(*R*)-*N*-(4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-6-yl)acetamide (8b)

Compound **8b** was prepared from **18** via two subsequent steps according to step D and step F for the preparation of **8g**. The crude product was purified by silica gel chromatography to give **8b** as a white solid. Purity = 96.7%, retention time: 12.40 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.85 (s, 1H), 8.36 (d, J = 7.7 Hz, 1H), 8.31 (s, 1H), 6.86 (s, 1H), 6.81 (d, J = 1.8 Hz, 1H), 6.68 (d, J = 1.9 Hz, 1H), 5.53 (s, 2H), 5.42 (p, J = 7.2 Hz, 1H), 3.65 (s, 3H), 2.18 (s, 3H), 1.52 (d, J = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.26, 158.39, 157.89, 155.92, 150.10, 149.41, 147.06, 129.75 (q, J = 30.2 Hz), 126.51, 124.72 (q, J = 273.3 Hz), 115.58, 115.21, 109.44 (d, J = 3.0 Hz), 107.97 (q, J = 3.0 Hz), 97.15, 49.65, 28.87, 24.05, 22.04. HRMS (ESI) for

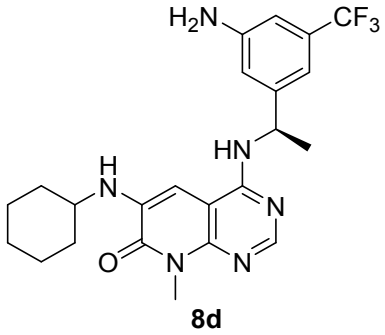
$C_{19}H_{20}F_3N_6O_2$ [M + H]$^+$, calc: 421.1594, found: 421.1595



8c

(*R*)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-6-(cyclopentylamino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8c)

Compound **8c** was prepared from **13a** via two subsequent steps according to step I and step F for the preparation of **22** and **8g**, respectively. The crude product was further purified by preparative HPLC to give **8c** as a TFA salt and a white solid. Purity = 95.3%, retention time: 14.83 min.1H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.09 (br s, 3H), 6.96 (s, 1H), 6.95 (s, 1H), 6.90 (s, 1H), 6.80 (s, 1H), 5.46 (p, J = 7.1 Hz, 1H), 3.92 (p, J = 6.4 Hz, 1H), 3.65 (s, 3H), 2.14 – 2.00 (m, 2H), 1.79 – 1.67 (m, 2H), 1.65 – 1.45 (m, 7H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.46, 155.91, 151.41, 147.60, 147.42, 145.99, 136.14, 129.88 (q, J = 30.2 Hz), 124.58 (q, J = 273.3 Hz), 116.03, 110.83, 109.24, 99.34, 95.38, 53.40, 49.41, 32.12 (d, J = 9.1 Hz, 2C), 28.75, 23.89 (q, J = 3.0 Hz, 2C), 22.41. HRMS (ESI) for $C_{22}H_{26}F_3N_6O$ [M + H]+, calc: 447.2115, found: 447.2115

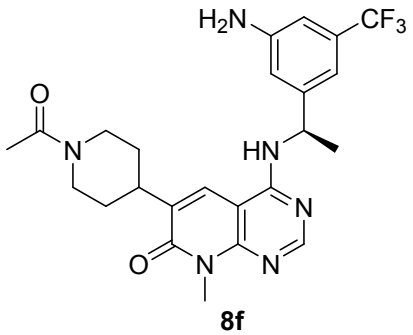


8d

(*R*)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-6-(cyclohexylamino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8d)

Compound **8d** was prepared from **13a** via the similar route for **8c**. The title compound was obtained as a TFA salt and a white solid. Purity = 98.04%, retention time: 15.41 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.81 (d, J = 7.9 Hz, 1H), 6.96 (s, 1H), 6.91 (s, 1H), 6.87 (s, 1H), 6.77 (s, 1H), 6.24 (br s, 3H), 5.46 (p, J = 7.1 Hz, 1H), 3.65 (s, 3H), 3.52 – 3.42 (m, 1H), 2.01 – 1.90 (m, 2H), 1.79 – 1.68 (m, 2H), 1.66 – 1.15 (m, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 158.56, 156.05, 151.60, 148.09, 147.37, 145.91, 135.24, 129.85 (q, J = 30.2 Hz), 124.61 (q, J = 273.3 Hz), 115.68, 110.40, 108.87, 99.31, 95.00, 49.88, 49.35, 31.80 (d, J = 24.2 Hz, 2C), 28.79, 25.42, 24.62 (d,

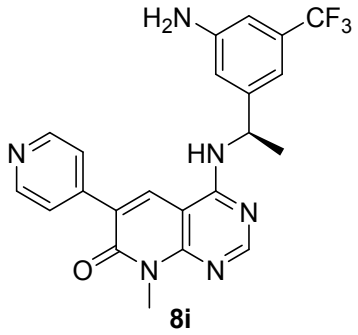
J = 16.6 Hz, 2C), 22.41. HRMS (ESI) for $C_{23}H_{28}F_3N_6O$ $[M + H]^+$, calc: 461.2271, found: 461.2265.



8f

(*R*)-6-(1-acetylpiperidin-4-yl)-4-((1-(3-amino-5-(trifluoromethyl) phenyl) ethyl) amino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8f)

Compound **8f** was prepared from **13a** and 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridin-1(2*H*)-yl)ethan-1-one via two subsequent steps according to step E and step F for the preparation of **8g**. The crude product was further purified by preparative HPLC to give **8f** as a TFA salt and a white solid. Purity = 96.5%, retention time: 12.19 min.^{1}H NMR (500 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.22 (dd, J = 7.8, 3.1 Hz, 1H), 8.14 (d, J = 2.6 Hz, 1H), 6.79 (d, J = 7.2 Hz, 2H), 6.69 (s, 1H), 5.55 (s, 2H), 5.47- 5.41 (m, 1H), 4.59 (d, J = 12.9 Hz, 1H), 3.95 (d, J = 13.6 Hz, 1H), 3.59 (s, 3H), 3.18 - 3.04 (m, 2H), 2.61 (t, J = 12.9 Hz, 2H), 2.03 (s, 3H), 1.88 (t, J = 12.5 Hz, 1H), 1.80 - 1.75 (m, 1H), 1.58 - 1.44 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.92, 161.96, 157.84, 157.01, 152.75, 147.70, 147.04 (d, J = 3.8 Hz), 134.52 (d, J = 2.5 Hz), 129.9 (q, J = 30.2 Hz), 126.81, 124.54 (q, J = 272.2 Hz), 115.97, 110.81, 109.32, 96.97, 49.44, 46.44, 41.37, 36.08 (d, J = 7.6 Hz), 32.07 (d, J = 16.4 Hz), 30.78 (d, J = 27.7 Hz), 28.47, 22.17 (d, J = 5.0 Hz), 21.40. HRMS (ESI) for $C_{24}H_{28}F_3N_6O_2$ $[M + H]^+$, calc: 489.2220, found: 489.2229.

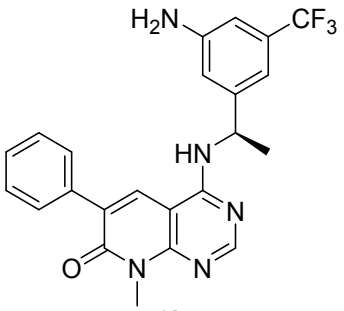


8i

(*R*)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methyl-6-(pyridin-4-yl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8i)

Compound **8i** was prepared from **13a** and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine via two subsequent steps according to step E and step J for the preparation of **21** and **8h**, respectively. The crude product was purified by silica gel chromatography to give **8i** as a white solid. Purity = 99.4%, retention time: 10.99 min.^{1}H NMR (400 MHz,

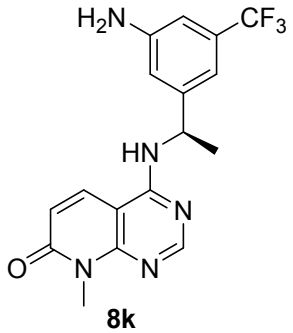
DMSO-d_6) δ 8.76 (s, 1H), 8.68-8.65 (m, 2H), 8.51 (d, J = 7.7 Hz, 1H), 8.42 (s, 1H), 7.83-7.79 (m, 2H), 6.82 (d, J = 6.3 Hz, 2H), 6.69 (s, 1H), 5.57 (s, 2H), 5.50-5.43 (m, 1H), 3.65 (s, 3H), 1.54 (d, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.86, 158.46, 158.40, 154.07, 149.54, 149.03 (d, J = 7.6 Hz), 146.54, 144.31, 132.25, 129.87 (q, J = 30.2 Hz), 125.53, 124.63 (q, J = 272.2 Hz), 123.38, 114.80, 109.29 (d, J = 5.0 Hz), 108.13 (d, J = 3.8 Hz), 97.36, 49.56, 28.64, 22.19. HRMS (ESI) for $C_{22}H_{20}F_3N_6O$ $[M + H]^+$, calc: 441.1645, found: 441.1651.



8j

(*R*)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methyl-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8j)

Compound **8j** was prepared from **13a** and 4,4,5,5-tetramethyl-2-phenyl-1,3,2-dioxaborolane via two subsequent steps according to step E and step F for the preparation of **8g**. The crude product was further purified by preparative HPLC to give **8j** as a TFA salt and a white solid. Purity = 99.20%, retention time: 15.25 min. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.41 (s, 1H), 8.34 (s, 1H), 7.71 – 7.65 (m, 2H), 7.43 – 7.32 (m, 3H), 7.25 (s, 1H), 7.19 (t, J = 1.8 Hz, 1H), 7.04 (s, 1H), 5.50 (q, J = 7.0 Hz, 1H), 3.72 (s, 3H), 1.62 (d, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 163.83, 159.61, 158.05, 154.50, 148.48, 144.50, 137.52, 132.69 (q, J = 31.5 Hz), 132.56, 131.52, 129.90, 128.98, 128.93, 126.39, 124.23, 119.71, 116.26, 113.25,99.50, 51.35, 29.22, 22.05. HRMS (ESI) for $C_{23}H_{21}F_3N_5O$ $[M + H]^+$, calc: 440.1693, found: 440.1699.



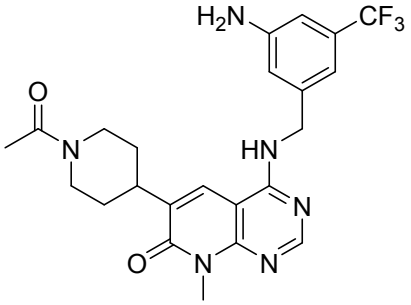
8k

(*R*)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8k)

Compound **8k** was prepared from **19** via two subsequent steps according to step D and step F for the preparation of **8g**. The crude product was purified by silica gel chromatography to give **8p** as a white solid. Purity = 96.8%, retention time: 10.94 min.

^{1}H NMR (400 MHz, MeOD-d_4) δ 8.36 (s, 1H), 8.27 (d, J = 9.6 Hz, 1H), 6.92 (s, 2H), 6.80 (s, 1H), 6.58 (d, J = 9.6 Hz, 1H), 5.47 (q, J = 7.2 Hz, 1H), 3.69 (s, 3H), 1.59 (d, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.19, 158.08 (d, J=9.8 Hz), 157.83, 154.15, 149.30, 146.70, 132.71, 129.78 (q, J= 30.6 Hz), 129.66 (q, J = 273.0 Hz), 118.48, 114.83, 109.27, 107.99, 97.03 (d, J = 6.7 Hz), 49.38 (d, J = 11.5 Hz), 27.88, 22.15 (d, J = 4.3 Hz). HRMS (ESI) for $C_{17}H_{17}F_3N_5O$ $[M + H]^+$, calc: 364.1380, found: 364.1380.

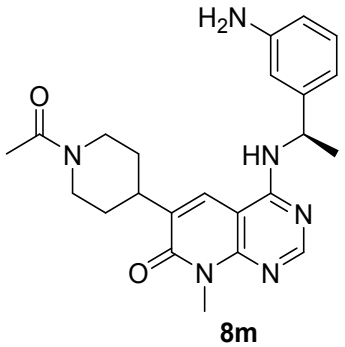
The following compounds were prepared from desired starting materials via the similar route as preparing compound **20**.



8l

6-(1-acetylpiperidin-4-yl)-4-((3-amino-5-(trifluoromethyl)benzyl)amino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8l)

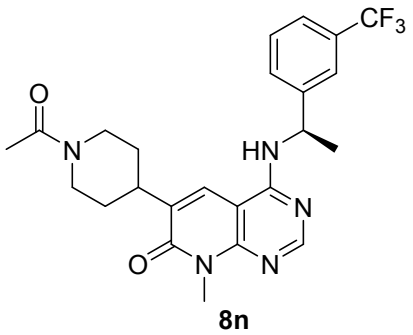
The crude product was purified by silica gel chromatography to give **8l** as a white solid. Purity = 97.2%, retention time: 11.69 min.^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58 (t, J = 6.0 Hz, 1H), 8.39 (s, 1H), 8.05 (s, 1H), 6.75 (s, 1H), 6.71 (d, J = 5.0 Hz, 2H), 5.58 (s, 2H), 4.65 (d, J = 5.8 Hz, 2H), 4.58 -4.51 (m, 1H), 3.93 (d, J = 13.4 Hz, 1H), 3.60 (s, 3H), 3.18-3.12 (m, 1H), 3.10-3.02 (m, 1H), 2.65-2.56 (m, 1H), 2.02 (s, 3H), 1.87 (d, J = 13.1 Hz, 1H), 1.77 (d, J = 12.9 Hz, 1H), 1.48-1.23 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.88, 161.98, 158.66, 157.27 (d, J = 11.3 Hz), 152.72, 149.65, 141.55, 134.47, 129.81 (q, J = 30.2 Hz), 126.71, 124.61 (q, J = 272.2 Hz), 115.63, 110.46 (d, J = 3.8 Hz), 108.22 (d, J = 2.5 Hz), 96.95, 46.41, 43.56, 41.39, 35.92, 31.99, 30.79, 28.44, 21.36. HRMS (ESI) for $C_{23}H_{26}F_3N_6O_2$ $[M + H]^+$, calc: 475.2064, found: 475.2067.



8m

(*R*)-6-(1-acetylpiperidin-4-yl)-4-((1-(3-aminophenyl)ethyl)amino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8m)

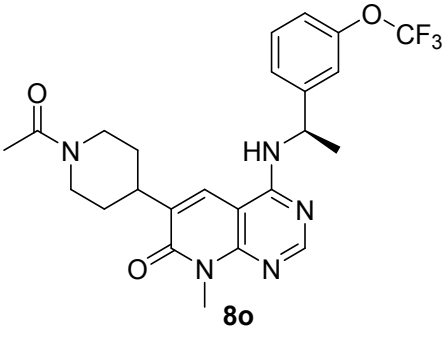
The crude product was further purified by preparative HPLC to give **8m** as a TFA salt and a white solid. Purity = 98.0%, retention time: 9.16 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (br s, 2H), 8.33 (dd, J = 7.2, 2.5 Hz, 1H), 8.31 (s, 1H), 8.16 (d, J = 2.7 Hz, 1H), 7.45 – 7.31 (m, 2H), 7.23 (s, 1H), 7.11 (d, J = 7.7 Hz, 1H), 5.50 (p, J = 7.1 Hz, 1H), 4.66 – 4.54 (m, 1H), 4.03 – 3.89 (m, 1H), 3.59 (s, 3H), 3.22 – 3.03 (m, 2H), 2.67 – 2.55 (m, 1H), 2.03 (s, 3H), 1.93 – 1.73 (m, 2H), 1.56 (d, J = 7.0 Hz, 3H), 1.55 – 1.38 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.93 (d, J = 4.5 Hz), 161.97, 157.89, 157.02, 152.75, 146.97, 134.58 (d, J = 6.0 Hz), 133.53, 129.90, 126.82, 124.71, 120.66, 119.66, 97.02, 49.54 (d, J = 1.5 Hz), 46.45, 41.38, 36.08 (d, J = 16.6 Hz), 32.11 (d, J = 10.6 Hz), 30.82 (d, J = 30.2 Hz), 28.49, 22.39, 21.43. HRMS (ESI) for $C_{23}H_{29}N_6O_2$ [M + H]$^+$, calc: 421.2347, found: 421.2353.



8n

(*R*)-6-(1-acetylpiperidin-4-yl)-8-methyl-4-((1-(3-(trifluoromethyl) phenyl) ethyl) amino) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8n)

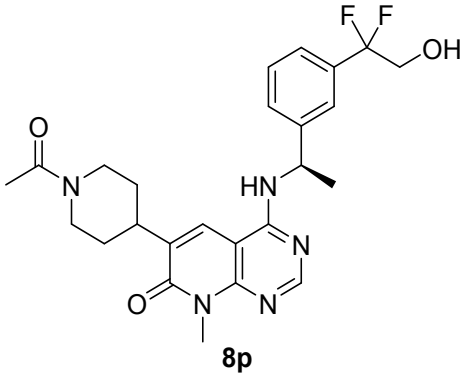
The crude product was purified by silica gel chromatography to give **8n** as a white solid. Purity = 97.5%, retention time: 14.50 min.^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (s, 1H), 8.29 (d, J = 7.5 Hz, 1H), 8.13 (s, 1H), 7.73 (s, 1H), 7.70 (d, J = 7.3 Hz, 1H), 7.61-7.55 (m, 2H), 5.62-5.56 (m, 1H), 4.62-4.56 (m, 1H), 3.99-3.91 (m, 1H), 3.58 (s, 3H), 3.21-3.12 (m, 1H), 3.12-3.03 (m, 1H), 2.64-2.58 (m, 1H), 2.03 (s, 3H), 1.92-1.84 (m, 1H), 1.81-1.75 (m, 1H), 1.59 (d, J = 7.0 Hz, 3H), 1.57-1.43 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.89, 161.93, 157.83, 156.98, 152.74, 146.34, 134.58, 130.29, 129.53, 129.09 (q, J = 30.2 Hz), 126.68, 124.36 (q, J = 272.2 Hz), 123.57 (d, J = 3.8 Hz), 122.58 (d, J = 3.8 Hz), 97.00, 49.45, 46.40, 41.34, 36.08 (d, J = 3.8 Hz), 32.03 (d, J = 13.9 Hz), 30.73 (d, J = 21.4 Hz), 28.42, 22.36, 21.37. HRMS (ESI) for $C_{24}H_{27}F_3N_5O_2$ [M + H]$^+$, calc: 474.2111, found: 474.2118.



8o

(*R*)-6-(1-acetylpiperidin-4-yl)-8-methyl-4-((1-(3-(trifluoromethoxy)phenyl)ethyl) amino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8o).

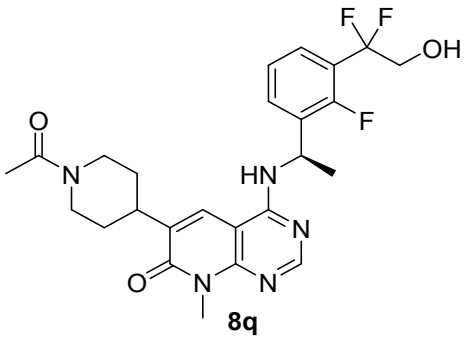
The crude product was purified by silica gel chromatography to give **8o** as a white solid. Yield 40 mg, 69.0%. Purity = 99.2%, retention time: 14.82 min.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H), 8.28 (d, J = 7.6 Hz, 1H), 8.13 (s, 1H), 7.49 – 7.39 (m, 2H), 7.36 (s, 1H), 7.22 (d, J = 7.7 Hz, 1H), 5.59 – 5.32 (m, 1H), 4.58 (d, J = 12.0 Hz, 1H), 3.95 (d, J = 13.4 Hz, 1H), 3.58 (s, 3H), 3.20 – 3.03 (m, 2H), 2.64 – 2.57 (m, 1H), 2.03 (s, 3H), 1.88 (d, J = 13.3 Hz, 1H), 1.78 (d, J = 12.9 Hz, 1H), 1.57 (d, J = 7.1 Hz, 3H), 1.54 – 1.41 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.89, 161.95, 157.84, 157.02, 156.93, 152.75, 148.52, 147.76, 134.56, 130.38, 126.73, 125.18, 119.12, 118.65, 97.00, 49.27, 46.41, 41.35, 36.07, 32.05 (d, J = 13.9 Hz), 30.74 (d, J = 18.9 Hz), 28.44, 22.27, 21.39. HRMS (ESI) for $C_{24}H_{27}F_3N_5O_3$ $[M + H]^+$, calc: 490.2061, found: 490.2068.



8p

(*R*)-6-(1-acetylpiperidin-4-yl)-4-((1-(3-(1,1-difluoro-2-hydroxyethyl) phenyl) ethyl)amino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8p)

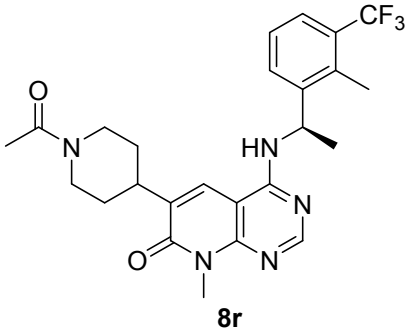
The crude product was further purified by preparative HPLC to give **8p** as a TFA salt and a white solid. Purity = 99.3%, retention time: 11.77 min.^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 8.30 (d, J = 7.2 Hz, 1H), 8.14 (s, 1H), 7.55 (s, 1H), 7.51 (d, J = 7.7 Hz, 1H), 7.44 (t, J = 7.7 Hz, 1H), 7.38 (d, J = 7.7 Hz, 1H), 5.63 (t, J = 6.4 Hz, 1H), 5.58 (t, J = 7.2 Hz, 1H), 4.59 (d, J = 12.9 Hz, 1H), 3.95 (d, J = 13.5 Hz, 1H), 3.82 (td, J = 14.2, 6.3 Hz, 2H), 3.58 (s, 3H), 3.19-3.12 (m, 1H), 3.08 (td, J = 12.0, 3.1 Hz, 1H), 2.60 (td, J = 12.8, 2.7 Hz, 1H), 2.03 (s, 3H), 1.90-1.85 (m, 1H), 1.82-1.73 (m, 1H), 1.57 (d, J = 7.0 Hz, 3H), 1.54-1.41 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.90, 161.96, 157.87, 157.04 (d, J = 11.3 Hz), 152.76, 145.10, 135.02 (t, J = 5.2 Hz), 134.50, 128.60, 127.86, 126.79, 124.14, 123.11, 121.52 (t, J = 243.2 Hz), 96.96, 63.94 (t, J = 32.8 Hz), 49.47, 46.42, 41.36, 36.07 (d, J = 3.8 Hz), 32.07 (d, J = 11.3 Hz), 30.77 (d, J = 18.9 Hz), 28.45, 22.36, 21.40. HRMS (ESI) for $C_{25}H_{30}F_2N_5O_3$ $[M + H]^+$, calc: 486.2311, found: 486.2316.



8q

(*R*)-6-(1-acetylpiperidin-4-yl)-4-((1-(3-(1,1-difluoro-2-hydroxyethyl)-2-fluorophenyl)ethyl)amino)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8q)

The crude product was further purified by preparative HPLC to give **8q** as a TFA salt and a white solid. Purity = 98.9%, retention time: 12.14 min. ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.29 (d, J = 1.6 Hz, 1H), 8.18 (s, 1H), 7.53 (t, J = 7.2 Hz, 1H), 7.45 (t, J = 7.2 Hz, 1H), 7.20 (t, J = 8.0 Hz, 1H), 5.76 (qd, J = 6.8, 2.4 Hz, 1H), 4.72 (dd, J = 13.2, 2.4 Hz, 1H), 4.11 – 3.95 (m, 3H), 3.70 (s, 3H), 3.26 (dd, J = 13.2, 2.4 Hz, 1H), 3.18 (tt, J = 12.4, 3.6 Hz, 1H), 2.75 (td, J = 13.2, 2.4 Hz, 1H), 2.15 (d, J = 1.6 Hz, 3H), 2.07 – 1.90 (m, 2H), 1.71 – 1.53 (m, 5H). ^{13}C NMR (126 MHz, MeOD-d_4) δ 171.40, 164.42, 159.34, 157.77 (2C), 154.05, 136.08, 133.70 (d, J = 14.1 Hz), 130.56, 128.36, 127.88 (t, J = 6.1 Hz), 125.12 (d, J = 3.3 Hz), 123.72 (td, J = 25.8, 12.9 Hz), 121.20 (t, J = 244.7 Hz), 99.24, 65.11 (td, J = 33.4, 3.5 Hz), 48.30, 46.44(d, J = 4.3 Hz), 43.47, 37.82, 33.01 (d, J = 2.4 Hz), 31.98 (d, J = 3.0 Hz), 29.28, 21.25 (2C). HRMS (ESI) for $C_{25}H_{29}F_3N_5O_3$ $[M + H]^+$, calc: 504.2217, found: 504.2217.

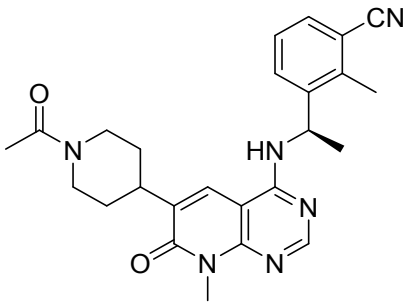


8r

(*R*)-6-(1-acetylpiperidin-4-yl)-8-methyl-4-((1-(2-methyl-3-(trifluoromethyl)phenyl)ethyl)amino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8r)

The crude product was further purified by preparative HPLC to give **8r** as a white solid. Purity = 99.5%, retention time: 15.59 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 – 8.28 (m, 2H), 8.16 (s, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 5.69 (p, J = 6.6 Hz, 1H), 4.65 – 4.53 (m, 1H), 4.01 – 3.88(m, 1H), 3.60 (s, 3H), 3.56 (s, 3H), 3.20 – 3.01 (m, 2H), 2.66 – 2.54 (m, 1H), 2.03 (s, 3H), 1.92 – 1.73 (m, 2H), 1.59 – 1.44 (m, 5H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.87, 161.91, 157.57, 157.01, 152.68, 145.78, 134.53, 133.52, 129.26, 127.73 (q, J = 27.7 Hz), 126.76, 126.42, 124.19, 123.70, 97.01, 46.44 (d, J = 10.1 Hz), 41.33, 36.09, 32.05, 31.96, 30.81, 30.66,

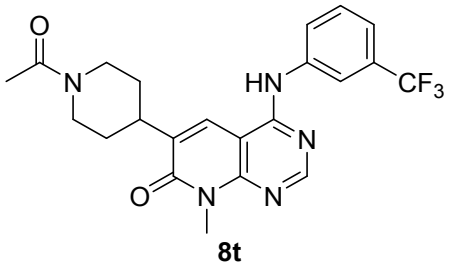
28.40, 21.37, 21.32, 14.17. HRMS (ESI) for $C_{25}H_{29}F_3N_5O_2$ $[M + H]^+$, calc: 488.2268, found: 488.2266.



8s

(*R*)-3-(1-((6-(1-acetylpiperidin-4-yl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-4-yl)amino)ethyl)-2-methylbenzonitrile (8s)

The crude product was further purified by preparative HPLC to give **8s** as a white solid. Purity = 99.2%, retention time: 13.38 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (d, J = 6.4 Hz, 1H), 8.30 (s, 1H), 8.14 (s, 1H), 7.71 (d, J = 7.9 Hz, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 5.58 (p, J = 6.9 Hz, 1H), 4.67 – 4.52 (m, 1H), 4.01 – 3.89 (m, 1H), 3.56 (s, 3H), 3.23 – 3.00 (m, 2H), 2.67 – 2.56 (m, 4H), 2.03 (s, 3H), 1.92 – 1.73 (m, 2H), 1.62 – 1.35 (m, 5H). ^{13}C NMR (201 MHz, DMSO-d_6) δ 168.29, 162.31, 157.95, 157.37, 153.05, 145.35 (d, J = 4.0 Hz), 139.06 (d, J = 8.0 Hz), 134.97, 131.60, 130.23, 127.57 (d, J = 2.0 Hz), 127.13 (d, J = 6.0 Hz), 118.88, 112.90, 97.43, 47.20 (d, J = 4.0 Hz), 46.81, 41.74 (d, J = 2.0 Hz), 36.47 (d, J = 4.0 Hz), 32.44 (d, J = 20.1 Hz), 31.14 (d, J = 30.2 Hz), 28.82, 21.79, 21.52 (d, J = 4.0 Hz), 17.22. HRMS (ESI) for $C_{25}H_{29}N_6O_2$ $[M + H]^+$, calc: 445.2347, found: 445.2347.

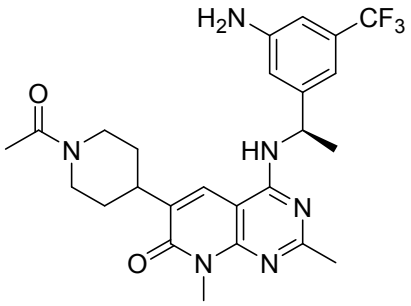


8t

6-(1-acetylpiperidin-4-yl)-8-methyl-4-((3-(trifluoromethyl) phenyl) amino) pyrido [2,3-*d*]pyrimidin-7(8*H*)-one (8t)

The crude product was purified by silica gel chromatography to give **8t** as a white solid. Purity = 97.3%, retention time: 14.56 min.^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.56 (s, 1H), 8.22 (s, 1H), 8.08 (s, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.62 (t, J = 8.0 Hz, 1H), 7.46 (d, J = 7.8 Hz, 1H), 4.59 (d, J = 13.2 Hz, 1H), 3.96 (d, J = 14.1 Hz, 1H), 3.65 (s, 3H), 3.21-3.09 (m, 2H), 2.63 (t, J = 13.1 Hz, 1H), 2.04 (s, 3H), 1.90 (d, J = 12.8 Hz, 1H), 1.81 (d, J = 12.4 Hz, 1H), 1.55-1.49 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.92, 161.87, 156.66, 156.56, 153.29, 139.93, 135.63, 129.72, 129.32 (q, J = 30.2 Hz), 126.46 (d, J = 8.8 Hz), 125.92, 124.26 (q, J = 272.2 Hz), 119.92, 118.30,

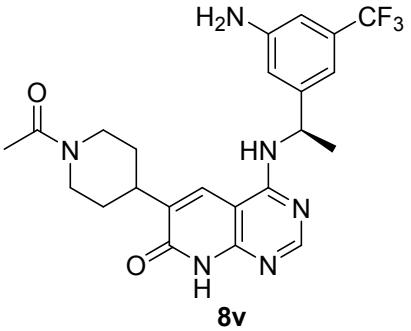
98.28, 46.39, 41.36, 36.12, 31.90, 30.69, 28.53, 21.39. HRMS (ESI) for $C_{22}H_{23}F_3N_5O_2$ $[M + H]^+$, calc: 446.1798, found: 446.1804.



8u

(*R*)-6-(1-acetylpiperidin-4-yl)-4-((1-(3-amino-5-(trifluoromethyl)phenyl) ethyl)amino)-2,8-dimethylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8u)

The crude product was further purified by preparative HPLC to give **8u** as a TFA salt and a white solid. Purity = 96.9%, retention time: 13.64 min. ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.12 (s, 1H), 7.38 (d, J = 9.6 Hz, 1H), 7.29 (t, J = 2.4 Hz, 1H), 7.15 (d, J = 6.4 Hz, 1H), 5.56 (q, J = 7.2 Hz, 1H), 4.76 – 4.65 (m, 1H), 4.11 – 4.01 (m, 1H), 3.71 (s, 3H), 3.26 (dd, J = 13.2, 2.4 Hz, 1H), 3.17 (td, J = 13.2, 2.4 Hz, 1H), 2.74 (td, J = 12.8, 2.4 Hz, 1H), 2.46 (d, J = 2.4 Hz, 3H), 2.14 (d, J = 2.4 Hz, 3H), 2.06 – 1.87 (m, 2H), 1.66 (d, J = 6.8 Hz, 3H), 1.64 - 1.52 (m, 2H). ^{13}C NMR (126 MHz, MeOD-d_4) δ 171.42, 166.81(d, J = 6.0 Hz), 164.40, 159.01, 153.95, 148.70 (d, J = 10.1 Hz), 142.83, 135.20, 132.95 (q, J = 32.0 Hz), 128.26, 125.4 (q, J = 270.9 Hz), 121.00 (d, J = 10.6 Hz), 118.11 (d, J = 40.8 Hz), 114.72 (d, J = 20.2 Hz), 96.88, 51.54, 48.28, 43.45, 37.78 (d, J = 4.9 Hz), 33.00 (d, J = 3.7 Hz), 32.00 (d, J = 9.3 Hz), 29.32, 25.60 (d, J = 2.8 Hz), 21.79 (d, J = 2.9 Hz), 21.24. HRMS (ESI) for $C_{25}H_{30}F_3N_6O_2$ $[M + H]^+$, calc: 503.2377, found: 503.2380.

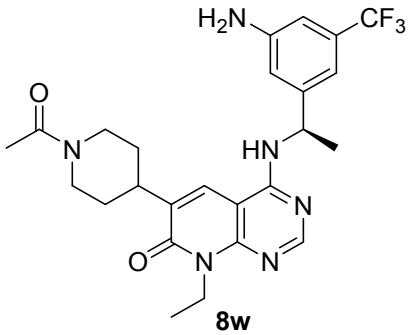


8v

(*R*)-6-(1-acetylpiperidin-4-yl)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl) amino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8v)

The crude product was further purified by preparative HPLC to give **8v** as a TFA salt and a white solid. Purity = 98.1%, retention time: 10.75min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 8.28 – 8.19 (m, 2H), 8.11 (d, J = 2.3 Hz, 1H), 6.89 – 6.84 (m, 2H), 6.76 (s, 1H), 6.17 (br s, 2H), 5.42 (p, J = 7.1 Hz, 1H), 4.64 – 4.52 (m, 1H), 3.98 – 3.87 (m, 1H),3.19 – 3.08 (m, 1H), 3.06 – 2.97(m, 1H), 2.64 – 2.55 (m, 1H), 2.03

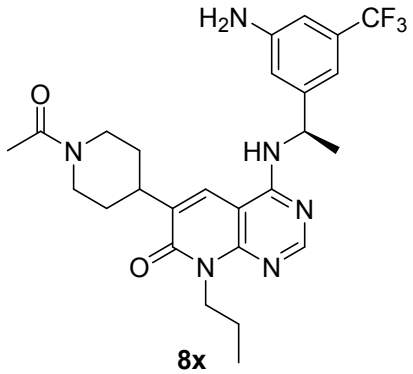
(s, 3H), 1.92 – 1.69 (m, 2H), 1.56 – 1.38 (m, 5H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.89, 162.58, 158.47, 158.18, 157.40, 157.21, 152.70, 148.47, 146.87, 135.80, 129.86 (q, J = 30.2 Hz), 128.18, 124.57 (d, J = 272.2 Hz), 115.49, 110.15, 108.79, 96.42, 49.25, 46.41, 41.36, 35.37(d, J = 6.3 Hz), 32.04(d, J = 11.3Hz), 30.77(d, J = 22.7 Hz), 22.14, 22.09, 21.36. HRMS (ESI) for $C_{23}H_{26}F_3N_6O_2$ $[M + H]^+$, calc: 475.2064, found: 475.2063.



8w

(*R*)-6-(1-acetylpiperidin-4-yl)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl) amino)-8-ethylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8w)

The crude product was further purified by preparative HPLC to give **8w** as a TFA salt and a white solid. Purity = 97.9%, retention time: 13.17 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.29 – 8.23 (m, 1H), 8.13 (d, J = 2.4 Hz, 1H), 7.30 (br s, 2H), 6.95 (s, 1H), 6.91 (s, 1H), 6.82 (s, 1H), 5.44 (p, J = 7.1 Hz, 1H), 4.63 – 4.54 (m, 1H), 4.40 – 4.28 (m, 2H), 4.00 – 3.90 (m, 1H), 3.21 – 3.03 (m, 2H), 2.65 – 2.54 (m, 1H), 2.03 (s, 3H), 1.93 – 1.73 (m, 2H), 1.53 (d, J = 7.1 Hz, 3H), 1.52 – 1.40 (m, 2H), 1.15 (t, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.95, 161.38, 157.92, 157.16, 152.22, 147.33 (d, J = 9.1 Hz), 147.17 (d, J = 4.5 Hz), 134.79 (d, J = 4.5 Hz), 129.94 (q, J = 30.2 Hz), 126.94 (d, J = 3.0 Hz), 124.55 (q, J = 273.3 Hz), 116.20, 111.17, 109.57, 97.00, 49.48, 46.48, 41.41, 36.22, 36.04 (d, J = 10.6 Hz), 32.13 (d, J = 21.1 Hz), 30.88 (d, J = 34.7 Hz), 22.25 (d, J = 6.0 Hz), 21.47, 13.32. HRMS (ESI) for $C_{25}H_{30}F_3N_6O_2$ $[M + H]^+$, calc: 503.2377, found: 503.2381.



8x

(*R*)-6-(1-acetylpiperidin-4-yl)-4-((1-(3-amino-5-(trifluoromethyl)phenyl) ethyl)amino)-8-propylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (8x)

The crude product was further purified by preparative HPLC to give **8x** as a TFA salt

and a white solid. Purity = 97.0%, retention time: 14.03 min.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.25 (d, J = 7.6 Hz, 1H), 8.13 (s, 1H), 6.86 (d, J = 12.6 Hz, 2H), 6.75 (s, 1H), 5.47-5.39 (m, 1H), 4.61 (br s, 2H), 4.59 (d, J = 13.0 Hz, 1H), 4.25 (t, J = 7.7 Hz, 2H), 3.95 (d, J = 13.5 Hz, 1H), 3.18-3.07 (m, 2H), 2.60 (t, J = 12.8 Hz, 1H), 2.03 (s, 3H), 1.90-1.76 (m, 3H), 1.64-1.44 (m, 6H), 0.87 (t, J = 7.4 Hz, 3H). -NH_2 in water. ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.90, 161.55, 157.90, 157.07, 152.42, 148.28, 146.96, 134.67, 129.86 (q, J = 30.2 Hz), 126.87, 124.56 (q, J = 272.2 Hz), 115.62, 110.32, 108.91, 96.88, 49.39, 46.43, 42.46, 41.37, 36.09 (d, J = 7.6 Hz), 32.03 (d, J = 13.9 Hz), 30.75 (d, J = 25.2 Hz), 22.14 (q, J = 5.0 Hz), 21.37, 20.96, 11.32. HRMS (ESI) for $C_{26}H_{32}F_3N_6O_2$ $[M + H]^+$, calc: 517.2533, found: 517.2541.

5. The ^{1}H and ^{13}C NMR spectra of compound 8u