Pyrimidinone nicotinamide mimetics as selective tankyrase and Wnt pathway inhibitors suitable for in vivo pharmacology

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Section 1: Supplemental Data

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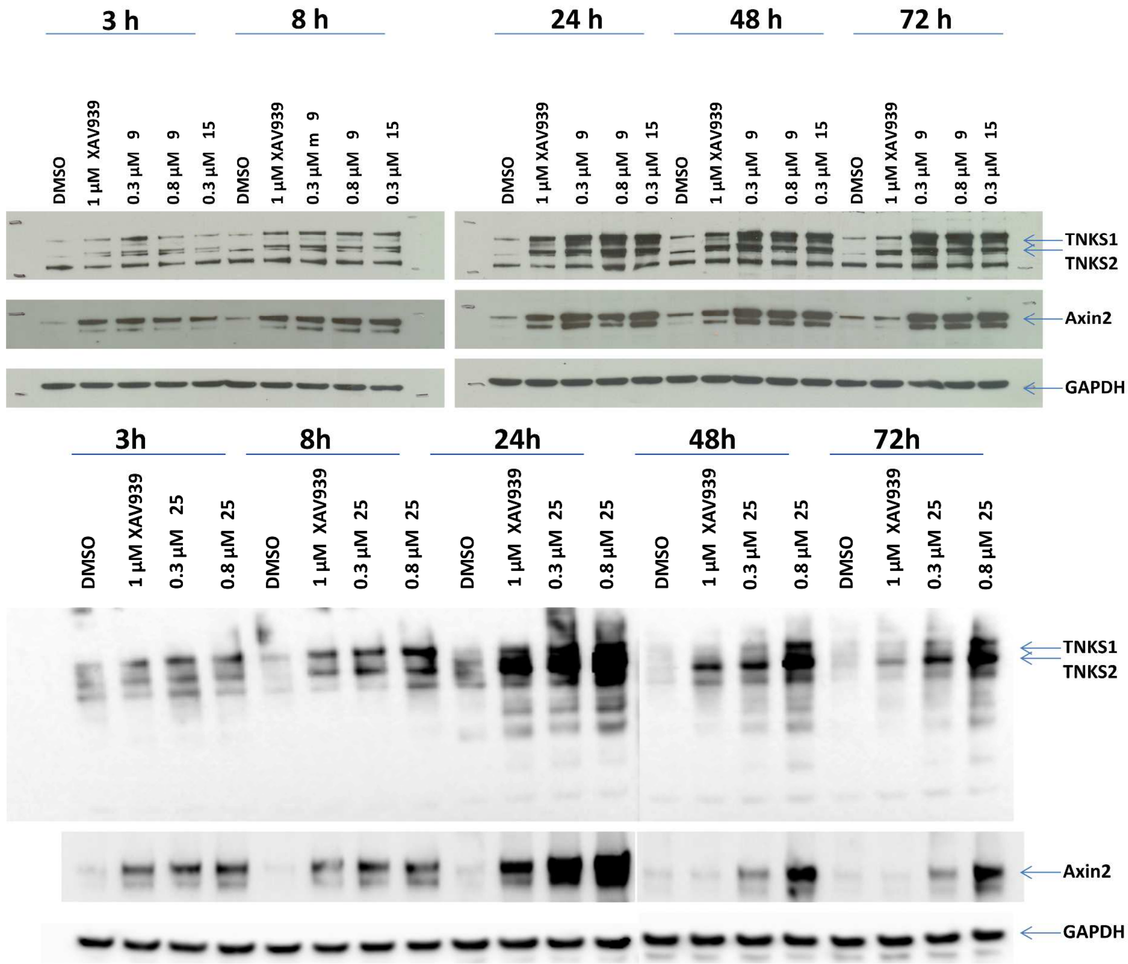
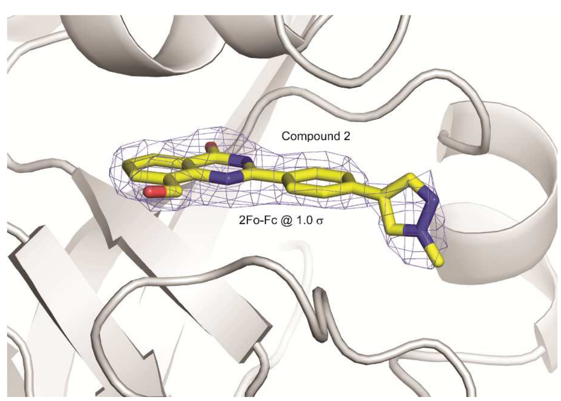
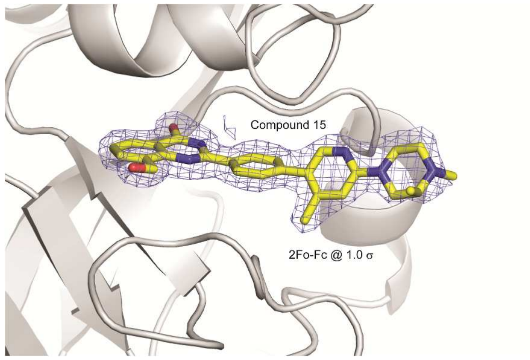


Figure S2: Electron density for ligands 2a and 15 bound to TNKS1

4W5S: Electron density for compound 2a



4W6E: Electron density for compound 15



Section 2: Experimental Conditions

TNKS1 enzyme Scintillation Proximity Assay (SPA) presented in Tables 1-5

Inhibitory potency (IC_{50}, concentration of a compound inhibiting 50% of Tankyrase-1 enzymatic activity) of compounds on Tankyrase-1 enzymatic activity was evaluated using a Scintillation Proximity Assay (SPA). The assay was designed to measure compound inhibition of Tankyrase-1 autoPARsylation (Tankyrase-1 was both enzyme and substrate in this assay). Truncated recombinant human Tankyrase-1 protein (amino acids E1023 – T1327; molecular weight, 37,111 Dalton) was purified from SF9 cells. The assay was conducted using 0.11 µM of Tankyrase-1 protein and 3 µM Nicotinamide Adenine Dinucleotide (NAD^+, 2.12 µM 3H-NAD^+ with a specific radioactivity of 1690 Ci/mol, 0.88 µM biotin- NAD^+), in pH 7.5 Tris buffer (60 mM Tris, 1 mM DTT, 0.01% (v/v) Tween-20®, 2.5 mM $MgCl_2$, 0.3 mg/mL BSA). For IC_{50} determination, 10 mM DMSO stock solution of a compound was sequentially diluted by two-fold in DMSO, and aliquots of the diluted solutions were transferred to 384-well assay plates and mixed with Tankyrase-1 solution. Normally, ten compound concentrations were included varying from 8.3 µM to 0.3 nM Reactions were initiated by adding the NAD^+ mixture, and run at room temperature for 90 minutes. Subsequently the reactions were quenched by adding a detection mixture made of 100 µg/well scintillant coated Streptavidin beads (PerkinElmer) in pH7.4 Tris buffer (60 mM Tris, 50 mM NaCl), incubated at 25 °C for overnight, and read with a radioactivity counter (TopCount 384, PerkinElmer). Percentage inhibition numbers were calculated for each compound concentration using the equation % inh = (Signal – Min)/ (Max – Min), where "Signal" is the reading for each reaction; "Min" is the reading of negative control in which the Tankyrase-1 activity was fully inhibited; and "Max" is the reading of reactions without inhibitors. IC_{50}'s were calculated by fitting the percentage inhibition data to a four-parameter equation using ActivityBase (IDBS). Positive control XAV939 has an IC_{50} of 0.006 µM in this TNKS1 enzyme assay. Differences in enzyme IC_{50}'s less than 55 nM may not be meaningful because the TNKS1 enzyme assay reaches its theoretical detection limit of 55 nM, which is half of the enzyme concentration (110 nM) used under the assay conditions. The IC_{50} for compound **9** is an average of 4 tests. The IC_{50} reported for compounds **1a** and **16** is an average of two tests. The IC_{50}'s for all remaining compounds are the result of one test. The following table provides the standard deviations for several control compounds:

Control Compound	Avg IC_{50} (µM)	Standard Deviation	N Tests
q	0.060	0.018	4
r	0.151	0.012	4
s	0.0098	0.0013	4
t	0.019	0.0072	3

PARP1 enzyme SPA presented in Tables 1-5

The scintillation proximity assay was used to evaluate compounds for their inhibition to the full length human PARP1 enzyme purified from SF9 cells (in-house made, molecular weight 114 kDa). Testing compounds were dissolved in DMSO to 3.6 mM and 10-fold serially diluted in DMSO. Diluted compounds were added to 384-well assay plates (Greiner 784075). The final compound testing concentrations ranged from 30 µM to 3 nM. For 100% inhibition, the reaction contained 1 µM of an AstraZeneca proprietary PARP1 inhibitor (IC_{50} = 4.6 nM). Reagent 1 contained 5 nM of the PARP1 enzyme and 10 nM of a double-strand oligo DNA (custom made, Fisher Scientific) in the assay buffer as described in the TNKS1 SPA section. Reagent 2 contained 2 µM of NAD, 0.88 µM of Biotinylated NAD (VWR International), and 0.125 µM of [Adenine-2,8-3H]-NAD (Perkin Elmer NET443H, Specific activity 27.3 Ci/mmol), and was also made in the assay buffer. Reaction was initiated by adding 6 µL of each of the reagent 1 and reagent 2 to the compound plate. The plate was allowed to incubate at room temperature for 30 minutes. At the end of the incubation, the reaction was quenched by the addition of 5 µL of a detection mixture containing 60 mM Tris pH 7.5, 50 mM NaCl, 5.9 µg/µL streptavidin coated PVT SPA beads (Perkin Elmer RPNQ0066), and 1 µM of the PARP1 inhibitor. Following an overnight incubation at room temperature, the assay plate was read on a TopCount (Perkin Elmer) for radioactivity. IC_{50} values were calculated in the similar fashion as described in the TNKS1 SPA section. Olaparib has an IC_{50} of <0.003 µM in this assay. The IC_{50} reported for compounds **9** and **16** is an average of two tests. The IC_{50}'s for all remaining compounds are the result of one test. The following table provides the standard deviations for several control compounds:

Control Compound	Avg IC_{50} (µM)	Standard Deviation	N Tests
u	0.0026	0.00076	7
v	0.011	0.0030	7
w	0.012	0.0032	7
x	0.0054	0.0022	7
y	0.0046	0.0030	7

PARP Panel Assay conditions performed at BPS biosciences (presented in Table 6)

Enzymes and Substrates

Assay	Catalog # (Lot #)	Amino acids in construct	Enzyme Used per Reaction (nM)	Substrate	Activated DNA
PARP1	80501 (120206-1)	Full length	1.43	2.5 µM NAD^+/2.5 µM NAD^+-Biotin	0.026 mg/mL
PARP2	80502 (130207C)	2-583	6.52	2.5 µM AD^+/2.5 µM NAD^+-Biotin	0.026 mg/mL
TNKS1	80504 (131030)	1001-1327	8.0	25 µM NAD^+/2.5 µM NAD^+-Biotin	N/A
TNKS2	80505 (130314G2)	667-1166	3.73	25 µM NAD^+/2.5 µM NAD^+-Biotin	N/A
PARP6	80506 (120816)	Full length	81.6	50 µM NAD^+/5 µM NAD^+-Biotin	0.026 mg/mL

Assay Conditions

In general, all assays were done by following the BPS PARP assay kit protocols. The enzymatic reactions were conducted in duplicate at room temperature for 1 hour in a 96 well plate coated with histone substrate. 50 µL of reaction buffer (Tris•HCl, pH 8.0) contains NAD^+, biotinylated NAD^+, activated DNA, a PARP enzyme, and the test compound. After enzymatic reactions, 50 µL of Streptavidin-horseradish peroxidase was added to each well and the plate was incubated at room temperature for an additional 30 min. 100 µL of developer reagents were added to wells and luminescence was measured using a BioTek Synergy 2 microplate reader.

Data Analysis

PARP activity assays were performed in duplicates. The luminescence data were analyzed using the computer software, Graphpad Prism. In the absence of the compound, the luminescence (Lt) in each data set was defined as 100% activity. In the absence of the PARP, the luminescence (Lb) in each data set was defined as 0% activity. The percent activity in the presence of each compound was calculated according to the following equation: % activity = [(L- Lb)/(Lt - Lb)]×100, where L= the luminescence in the presence of the compound, L_b = the luminescence in the absence of the PARP, and L_t = the luminescence in the absence of the compound. The percent inhibition was calculated according to the following equation: % inhibition = 100 - % activity.

TNKS1, TNKS2, and Axin2 Western Blot data Experimental Conditions

DLD1 cells were treated with various compounds at indicated time points. The cell lysates were generated using 1X RIPA buffer (50 mM Tris-HCl at pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 1 mM EDTA) supplemented with protease inhibitor (Sigma, P8340) and phosphatase inhibitor cocktails (Millipore, 17-317). The lysates were normalized for protein concentration, resolved by SDS–polyacrylamide gel electrophoresis (PAGE) and probed with the anti-Axin2 antibody (CST #2151) and anti-TNKS1/2 antibody (abcam #ab13587).

DLD-1 Wnt reporter assay protocol

To identify potential inhibitors of the Wnt pathway, a reporter assay was used as primary screen to measure the decrease in activity of a constitutively active β-Catenin pathway in the DLD-1 TOPFlash / EF1a Renilla reporter cell line. On day 1, 7500 DLD-1 TOPFlash / EF1a Renilla cells/well were seeded in 30 µL of assay media (DMEM+2%FBS+1%L-Glu) in flat-bottom 384 white plate, and incubated at 37 °C overnight. On the next day, the cells were dosed directly using a Labcyte Echo® Liquid Handler (7 point dilution starting with 3 µM and going down 3-fold to 0.0025 µM). After 24 h of treatment, 30 µL of Steady-Glo Luciferase reagent was added into each well except wells for "min" control. The plates were incubated at room temperature for 15 min in the dark. Plates were read on Tecan Ultra for luciferase signal. IC_{50} was calculated using an Activity Base template (DMSO control as "Max", no Luciferase reagent as "Min"). All concentration points were run in duplicate for all compounds on each test occasion. Reported IC_{50}'s for all compounds are the average of at least two separate test occasions.

Caco-2 Apparent Permeability and Efflux Ratio

HTS Transwell-96 Well Permeable Supports were purchased from Corning Corporation (Cambridge, MA). The Caco-2 cells were seeded into transwell plates. When the 14-day Caco-2 cultured cells had reached confluence and were differentiated, they were ready to be used for transport studies. The cell monolayer integrity was assessed by Trans-epithelial electrical resistance (TEER) measurement using a Millicell Epithelial Volt-Ohm measuring system (Millipore). The monolayer was washed using pre-warmed Hank's balanced salt solution (HBSS) (25 mM HEPES, pH 7.4), then pre-incubated at 37 °C for 30 minutes.
A 10 µM working solution of test compounds was prepared in HBSS (25 mM HEPES, pH 7.4) from a DMSO stock solution. The final concentration of DMSO in the incubation system was less than 1%. To determine the rate of drug transport in the apical to basolateral direction, 10 µM of working solution was added to the transwell insert (apical compartment), and the wells in the receiver plate (basolateral compartment) were filled with HBSS (25 mM HEPES, pH 7.4). To determine the rate of drug transport in the basolateral to apical direction, 10 µM of working solution was added to the basolateral compartment, and the apical compartment was filled with HBSS. After incubation at 37 °C for 2 h, aliquots of samples at t=0 and at t=2 h were taken from the apical and basolateral compartments and were diluted 10-fold in the buffer and then mixed with 3 volumes of cold acetonitrile containing an appropriate analytical internal standard. After vortex and centrifugation, an aliquot of the supernatant was used for LC/MS/MS analysis. All incubations were performed in duplicate. The Caco2 monolayer integrity after 2-hour transport period was assessed by Lucifer yellow

leakage. 100 µM Lucifer yellow solution was added to the transwell insert (apical compartment) and the wells in the receiver plate (basolateral compartment) were filled with HBSS (25 mM HEPES, pH 7.4). After incubation at 37 °C for 30 minutes, aliquots were taken from the apical and basolateral wells to measure Lucifer Yellow fluorescence in a fluorescence plate reader. The apparent permeability (P_{app}) in units of 10^{-6}cm/s was calculated as P_{app} = (Vol $_{acceptor\ well}$ / (Area × Time)) × Drug]$_{acceptor}$/[Drug] $_{initial\ donor}$). The efflux ratio was determined as Efflux ratio = $P_{app(B\text{-}A)}$ / $P_{app(A\text{-}B)}$, where $P_{app(B\text{-}A)}$ indicates the apparent permeability coefficient in basolateral to apical direction, and $P_{app(A\text{-}B)}$ indicates the apparent permeability coefficient in apical to basolateral direction. Metoprolol, cimetidine, and erythromycin that have no, moderate, and high efflux were used as quality control compounds, showing mean efflux ratios of 0.67, 5.7, and 73, respectively, in the Caco-2 assay.

Solution Formulations

IV formulation for compound 9 for in vivo IV/IP/PO solution studies (up to 0.4 mg/mL)

Compound **9** can be formulated for IV/IP/PO use in tetraethylene glycol (TEG) / 40% SBECD solution 50/50 at a concentration of up to 0.4 mg/mL (based on freebase).

Vehicle Preparation, 100 mL (prepare all solutions aseptically if possible)

Weigh 40 grams SBECD into a 100 mL volumetric flask. Add half the required volume of water-for-injection and sonicate or stir to dissolve. Once dissolved make up to volume using water-for-injection.

Formulation Preparation of up to a 0.4 mg/mL IV/IP/PO Solution

1. Weigh the required amount of compound **9** freebase into a volumetric flask or pre-volume marked vial.
2. Add 50% of the final volume of TEG and stir to dissolve the compound. The compound will dissolve with 24-hour stirring.
3. After the compound dissolves, bring to volume (quantum satis) with 50% of the required final volume of 40% SBECD solution and stir.
4. Make sure the formulation is a clear solution with a possible haze.
5. Measure and record the solution pH. The pH should be about pH 5.

IV formulation for compound 25 for in vivo IV studies (up to 20 mg/mL)

Compound **25** can be formulated for IV use in 20% SBECD solution adjusted to pH 4.0 at a concentration of up to 20 mg/mL (based on parent form). The maximum-dosing volume for the vehicle is 10 mL/kg for a single dose to mouse, twice daily for up to 14 days.

Vehicle Preparation (prepare the solution aseptically, if possible)
20% SBECD Solution

Weigh 20 grams SBECD into a 100 mL volumetric flask. Add about half the volume of water-for-injection and sonicate or stir to dissolve. Make the solution up to volume with water-for-injection. Mix well.

Formulation Preparation of up to a 20 mg/mL IV Solution (prepare the formulation aseptically if possible)

1. Weigh the required amount of compound **25** parent form into a volumetric flask or pre-volume marked vial.
2. Add 85% of the final volume of 20% SBECD solution and stir.
3. Add 1 N methanesulfonic acid and reduce the pH to about 4.0.
4. After the compound dissolves, measure the solution pH.
5. Adjust the pH to 4.0 with sodium hydroxide or methanesulfonic acid solution, if necessary.
6. Stir and make sure the formulation is a clear solution with a possible slight haze.
7. Make up to volume with 20% SBECD solution.
8. Measure and record the solution pH.
9. Sterile filter the solution through the appropriate filter (0.22 µm) prior to administration.

X-ray Crystallography Methods, PDB Codes, and Crystallographic Table

Human tankyrase 1 PARP catalytic domain was expressed and purified as described from the literature.[1] For compound **2a**, apo tankyrase was crystallized using standard conditions.[1] The crystals were soaked overnight in mother liquor and the presence of 1 mM of compound **2a** and 1% DMSO. For compound **15**, human tankyrase catalytic domain and 1 mM of compound **15** were co-crystallized in 1.4 M sodium formate and 0.1 M sodium acetate pH 5.0. Crystals for both compounds were flash frozen in liquid nitrogen in 20% butanediol and mother liquor. X-ray diffraction data was collected on a Rigaku FRE+ Superbright rotating anode. Data was processed and scaled using XDS[2] and Scala.[3] Phases were solved using molecular replacement with the previously solved structure in Phaser.[4] Model building was completed using COOT.[5] AutoBUSTER was used for refinement of the models.[6] Statistics and library files can be obtained from the PDB.

The structures have been deposited in the PDB for compound **2a** and **15** under the codes 4W5S and 4W6E, respectively.

Crystallographic Table

	Compound **2a**	Compound **15**

[1] Lehtiö, L.; Collins, R.; van den Berg, S.; Johansson, A.; Dahlgren, L. G.; Hammarström, M.; Helleday, T.; Holmberg-Schiavone, L.; Karlberg, T.; Weigelt, J. Zinc binding catalytic domain of human tankyrase 1. *J. Mol. Biol.* **2008,** *379*, 136-45

[2] Kabsch, W. (2010). XDS. *Acta Crystallogr D Biol Crystallogr* **66**,125-132.

[3] Kabsch, W. (1988). Evaluation of single-crystal X-ray diffraction data from a position-sensitive detector. *J. Appl. Cryst.***21**, 915-924.

[4] McCoy, A.J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). Phaser crystallographic software. *J. Appl. Cryst.* **40**, 658-674.

[5] Emsley, P., Lohkamp, B., Scott, W.G. & Cowtan, K. (2010). Features and development of Coot. *Acta Crystallogr D Biol Crystallogr* **66**, 486-501.

[6] Bricogne, G., et al. (2011). Buster V2.11.5 (Global Phasing Ltd., Cambridge, United Kingdom).

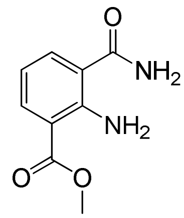
PDB ID	4W5S	4W6E
Space Group	$I2_12_12_1$	$I2_12_12_1$
Cell Constants a; b; c; (Å)	81.01; 81.93; 82.17	80.77; 81.85; 83.31
Cell Constants α; β; γ (°)	90; 90; 90	90; 90; 90
Resolution Limit (Å)	2.8	1.95
Resolution Range (Å)	58.02-2.8 [2.9-2.8]	58.38-1.95 [2.02-1.95]
Completeness (%)	99.8 [100]	100 [100]
Reflections, unique	6993 [688]	20488 [2015]
Multiplicity	6.95 [7.02]	6.82 [5.31]
R_{Sym} (%)	21.4 [48.3]	6.8 [46.7]
I/Sigma	7.6 [2.3]	11.2 [2.4]
R-Work(%)	19.7	20.5
R-Free (%)	26.3	25.4
Non-Hydrogen Protein Atoms	1678	1689
Non-Hydgrogen Ligand Atoms	25	34
Solvent Molecules	89	226

Values in brackets are for the highest resolution shell.

Synthetic procedures and compound characterization data

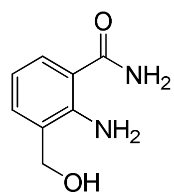
All reagents and solvents used were purchased from commercial sources and were used without further purification. ^{1}H NMR spectra were obtained using a Bruker 300 MHz, 400 MHz, or 500 MHz spectrometer at temperatures ranging from 23 °C to 100 °C; chemical shifts are expressed in parts per million (ppm, δ units) and are referenced to the residual protons in the deuterated solvent used. Coupling constants are given in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br s (broad singlet). Mass spectrometry analyses were performed with an Agilent 1100 equipped with Waters columns (Atlantis T3, 2.1x50 mm, 3 µm; or Atlantis dC18, 2.1 x 50 mm, 5 µm) eluted with a gradient mixture of water and acetonitrile with either formic acid or ammonium acetate or ammonium hydroxide added as a modifier. Reverse-phase chromatography was performed as specified for individual compounds. Thin layer chromatography was performed using EMD silica gel 60 F_{254} plates. Column chromatography was performed using SiliCycle Silia*Sep* preloaded silica gel cartridges on Teledyne ISCO CombiFlash Companion automated purification systems. Unless otherwise indicated, all final compounds were purified to ≥ 95% purity as assessed by analytical HPLC using an Agilent 1100 equipped with Waters columns (Atlantis

T3, 2.1x50 mm, 3 µm; or Atlantis dC18, 2.1 x 50 mm, 5 µm) eluted for > 10 minutes with a gradient mixture of water and acetonitrile with either formic acid or ammonium acetate added as a modifier, monitored at wavelengths of 220, 254, and 280 nm.



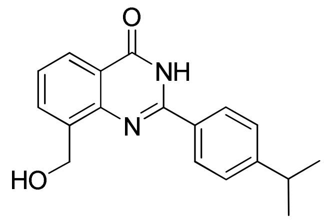
Methyl 2-amino-3-carbamoylbenzoate

HBTU (214.36 g, 0.563 mol) was added to a stirred solution of 2-amino-3-(methxycarbonyl) benzoic acid (100 g, 0.512 mol) and Hunig's base (132.43 g, 1.025 mol)in DMF (400 mL). The resulting clear brown solution was stirred at room temperature for 10 minutes. Ammonium hydroxide (21.53 g, 0.615 mol) was added drop-wise to the above reaction mixture. The reaction mixture was stirred at room temperature for 2 hours. The mixture turned into a brown suspension. At this stage, HPLC indicated complete loss of the starting material and formation of product. Water (800 mL) was added to the reaction mixture. The precipitate was filtered and washed with water to yield the desired product as a light yellow solid (91.0 g, 90.8%) *m/z*: 194.9 $[M+H]^+$. ^1H NMR (300 MHz, DMSO) δ ppm 8.0 (s, 2H), 7.87-7.90 (q, 2H), 7.77-7.80 (q, 1H), 7.3 (s, 1H), 6.52-6.57 (t, 1H), 3.8 (s, 3H).



2-Amino-3-(hydroxymethyl) benzamide

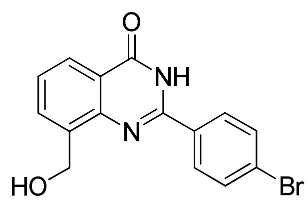
A dark yellow suspension of methyl 2-amino-3-carbamoylbenzoate (90 g, 0.463 mol) in THF (1350 mL) was cooled to 0 °C and treated with $LiAlH_4$ (2M in THF) drop-wise under nitrogen. The reaction mixture turned bright yellow suspension. The reaction mixture was allowed to warm to room temperature and stirred for 4 hours. At this stage most of the starting material was converted into the product. The reaction mixture was cooled in an ice bath and quenched with saturated ammonium chloride (450 mL) which was then passed through a celite bed. The celite bed was washed with 3:1 dichloromethane/tetrahydrofuran. The combined filtrate was extracted with ethyl acetate (270 mL). Combined extracts were dried over sodium sulfate, filtered and concentrated to give crude material. The crude material was triturated with ethyl acetate (180 mL)/ether (90 mL) mixture. The solid was filtered and washed with ether (90 mL) to give 2-amino-3-(hydroxymethyl) benzamide (63.9 g, 84 %) as a white solid with 93.7 % purity. HPLC: 2.47 min, 93.7 %; *m/z*: 167.0 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d6) δ ppm 7.75 (s, 1H), 7.50 (d, 1H), 7.21 (d, 1H), 7.09 (d, 1H), 6.52 (t, 1H), 6.46 (s, 2H), 5.12(t, 1H), 4.41(d, 2H).



Compound 1a

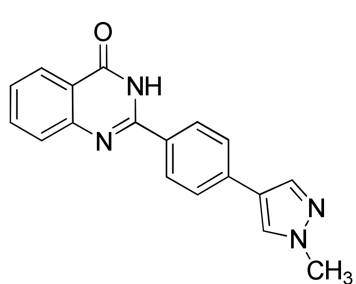
8-(hydroxymethyl)-2-(4-isopropylphenyl)quinazolin-4(3H)-one

A 40 mL vial was charged with 2-amino-3-(hydroxymethyl)benzamide (80 mg, 0.48 mmol), 4-isopropylbenzaldehyde (78 mg, 0.53 mmol), iron(III) chloride (156 mg, 0.96 mmol) and Water (4 mL). The resulting suspension was refluxed (bath temperature 120 °C) for 2.5 h. After cooling to room temperature, the product was precipitated out from water, filtered, and washed with water and ether to give crude material. The solid material was dissolved in 2.0 mL of DMSO and purified by reverse phase HPLC (C18 column, 20-80% ammonium acetate/water/acetonitrile, 10 minutes) to give 8-(hydroxymethyl)-2-(4-isopropylphenyl)quinazolin-4(3H)-one (12 mg, 8.5 %) as a white solid. *m/z*: 295 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-*d6*) δ ppm 12.55 (broad s, 1H), 8.15 (d, *J*=8.34 Hz, 2 H), 8.00 - 8.06 (m, 1 H), 7.89 (s, 1 H), 7.49 (t,*J*=7.58 Hz, 1 H), 7.43 (d, *J*=8.34 Hz, 2 H), 5.27 (br. s., 1 H), 5.04 (s, 2 H), 2.99 (quin, *J*=6.95 Hz, 1 H), 1.26 (d, *J*=7.07 Hz, 6 H).



2-(4-Bromophenyl)-8-(hydroxymethyl)quinazolin-4(3*H*)-one

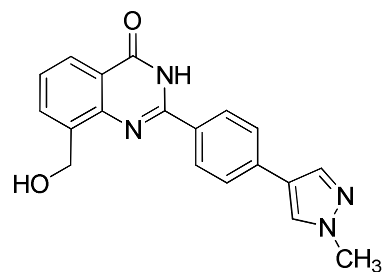
A 40 mL vial was charged with 2-amino-3-(hydroxymethyl)benzamide (235 mg, 1.41 mmol), 4-bromobenzaldehyde (288 mg, 1.56 mmol), iron (III) chloride (459 mg, 2.83 mmol) and water (10 mL). The resulting suspension was refluxed (bath temperature 116 °C) for 2.5 h. After cooling to room temperature, the product precipitated from water. The precipitate was filtered, washed with water and ether to give crude material. The crude material was dissolved in DMSO (8.0 mL) and was purified by reverse phase HPLC (C18 column, 10-80% ammonium acetate/water/acetonitrile, 10 minutes) to give the title compound as a white solid (310 mg, 66.2 %). *m/z*: 331 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-*d6*) δ ppm 12.62 (s, 1 H), 8.16 (d, *J*=8.59 Hz, 2 H), 8.04 (d, *J*=7.07 Hz, 1 H), 7.92(d, *J*=7.33 Hz, 1 H), 7.78 (d, *J*=8.34 Hz, 2 H), 7.53 (t, *J*=7.71 Hz, 1 H), 5.26 (br s, 1 H), 5.04 (s, 2 H).



Compound 2

2-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)quinazolin-4(3H)-one

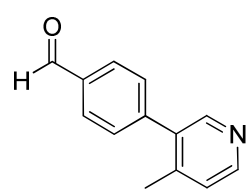
A suspension of 2-(4-bromophenyl)quinazolin-4(3H)-one (100 mg, 0.33 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (69 mg, 0.33 mmol), $Pd(PPh_3)_4$ (38.4 mg, 0.03 mmol) and cesium carbonate (433 mg, 1.33 mmol) was mixed in dioxane (2 mL) and water (0.5 mL). The suspension was degassed, filled with nitrogen, and heated at 95 °C for 16 h. LCMS showed the reaction was complete. The resulting precipitate was collected via filtration and purified by reverse phase HPLC (C18 column, 10-90% ammonium acetate/water/acetonitrile) to give 2-(4-(1-methyl-1H-pyrazol-4-yl)phenyl)quinazolin-4(3H)-one (37 mg, 37 %). *m/z*: 303 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm 12.49 (s, 1 H), 8.30 (s, 1 H), 8.11 - 8.25 (m, 3 H), 8.01 (s, 1 H), 7.79 - 7.88 (m, 1 H), 7.69 - 7.79 (m, 3 H), 7.51 (t, J=7.44 Hz, 1 H), 3.89 (s, 3 H).



Compound 2a

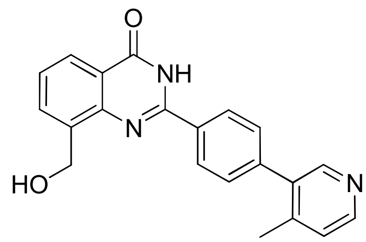
8-(Hydroxymethyl)-2-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)quinazolin-4(3*H*)-one

A 10 mL microwave vial was charged with 2-(4-bromophenyl)-8-(hydroxymethyl)quinazolin-4(3*H*)-one (70 mg, 0.21 mmol),1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (52.8 mg, 0.25 mmol), cesium carbonate (138 mg, 0.42 mmol) and tetrakis(triphenylphosphine)palladium(0) (12.21 mg, 10.57 µmol). Degassed dioxane (3.0 mL) and water (0.5 mL) were added. The reaction vial was capped and heated to 150 °C in microwave for 25 minutes. At this stage LC-MS indicated clean product formation and loss of starting material. After cooling, the solvent was evaporated under reduced pressure and the residue was partitioned between water (3 mL) and EtOAc (25 mL). The organic layer was separated and dried over sodium sulfate and the solvent was evaporated. The residue was dissolved in DMSO (2.0 mL) and was purified by reverse phase HPLC chromatography (10% to 85% CH_3CN/H_2O/ammonium acetate, C18 column, 10 minutes) to give 8-(hydroxymethyl)-2-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)quinazolin-4(3*H*)-one as a white solid (18.0 mg, 25.6 %). *m/z*: 333 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-*d6*): δ ppm 12.50 (s, 1 H), 8.29 - 8.35 (m, 1 H), 8.23 (d, *J*=8.59 Hz, 2 H), 8.00 -8.07 (m, 2 H), 7.87 - 7.95 (m, 1 H), 7.76 (d, *J*=8.59 Hz, 2 H), 7.50 (t, *J*=7.58 Hz, 1 H), 5.26 (br s, 1 H), 5.06 (d, *J*=2.02 Hz, 2 H), 3.90 (s, 3 H).



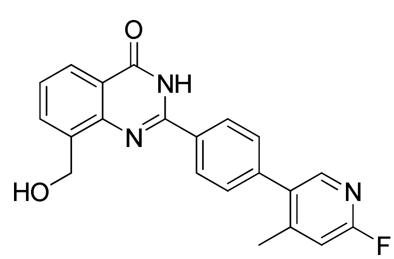
4-(4-Methylpyridin-3-yl)benzaldehyde

A 20 mL microwave vial was charged with 4-bromobenzaldehyde (200 mg, 1.08 mmol), 4-methylpyridin-3-ylboronic acid (178 mg, 1.30 mmol), cesium carbonate (528 mg, 1.62 mmol) and tetrakis(triphenylphosphine)palladium(0) (18.74 mg, 0.02 mmol). Degassed dioxane (8.0 mL) and water (1.0 mL) were added. The reaction vial was capped and heated to 150 °C in microwave for 25 minutes. At this stage LC/MS indicated clean product formation and loss of starting material. After cooling, the solvent was evaporated under reduced pressure and the residue was partitioned between water (5 mL) and EtOAc (40 mL). The organic layer was separated, dried over sodium sulfate, and concentrated to give 4-(4-methylpyridin-3-yl)benzaldehyde (148 mg, 69.4 %). The crude material was used without further purification. *m/z*: 198 $[M+H]^+$.



Compound 3

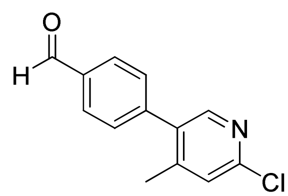
<u>8-(Hydroxymethyl)-2-(4-(4-methylpyridin-3-yl)phenyl)quinazolin-4(3H)-one</u>
A 10 mL microwave vial was charged with 2-amino-3-(hydroxymethyl)benzamide (80 mg, 0.48 mmol), 4-(4-methylpyridin-3-yl)benzaldehyde (95 mg, 0.48 mmol), sodium bisulfite (100 mg, 0.96 mmol), and DMA (3 mL). The vial was capped and heated to 150 °C in microwave for 1.5 hours. After cooling to room temperature, sodium bisulfite was filtered off, and the filter cake was washed with DMA. The DMA solution (4 mL) was directly purified by reverse phase HPLC (Atlantis, OBD column, 30mm x 50mm 5 µm), 10-70% /water (0.1% TFA) /acetonitrile 0.1%), 10 minutes). The fractions were concentrated to give 8-(hydroxymethyl)-2-(4-(4-methylpyridin-3-yl)phenyl)quinazolin-4(3H)-one (22 mg, 13 %) as a white solid. Product was suspended in 1.0 mL of 1% aqueous ammonium hydroxide solution, sonicated 10 seconds, the precipitate was filtered off and washed with water to afford the title compound as a free base. *m/z*: 344 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 12.64 (s, 1 H), 8.43 - 8.54 (m, 2 H), 8.34 (m, *J*=8.59 Hz, 2 H), 8.06 (d, *J*=6.57 Hz, 1 H), 7.94 (d, *J*=7.07 Hz, 1 H), 7.62 (m,*J*=8.34 Hz, 2 H), 7.54 (t, *J*=7.71 Hz, 1 H), 7.40 (d, *J*=5.05 Hz, 1 H), 5.27 (br. s., 1 H), 5.07 (br. s., 2 H), 2.33 (s, 3 H).



Compound 4

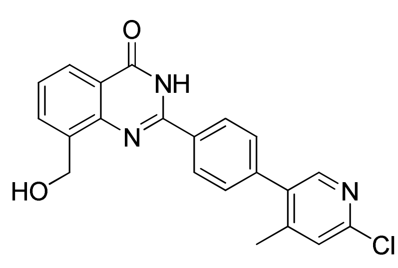
<u>2-(4-(6-Fluoro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one</u>

A 10 mL microwave vial was charged with 2-(4-bromophenyl)-8 (hydroxymethyl)quinazolin-4(3H)-one (75 mg, 0.23 mmol), 6-fluoro-4-methylpyridin-3-ylboronic acid (35.1 mg, 0.23 mmol), cesium carbonate (148 mg, 0.45 mmol) and tetrakis(triphenylphosphine)palladium(0) (7.85 mg, 6.79 µmol). Degassed dioxane (3.5 mL) and water (0.5 mL) were added. The reaction vial wsa capped and heated to 150 °C in microwave for 30 minutes. At this stage LC/MS indicated product formation and loss of starting material. After cooling, the solvent was evaporated under reduced pressure and the residue was suspended in 3.0 mL of water. The precipitate was filtered off and washed with water. The solid material was dissolved in DMSO (4.0 mL), filtered (0.45 µm filter) and purified by reverse phase HPLC (Atlantis, OBD column, 30mm x 50mm 5µm) water (0.1% TFA) /acetonitrile (0.1% TFA), 20-80%, 10 minutes. The fractions were concentrated to give 2-(4-(6-fluoro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (25 mg, 23 %) as a white solid. *m/z* : 362 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 12.65 (s, 1 H), 8.33 (m, *J*=8.34 Hz, 2 H), 8.13 (s, 1 H), 8.06 (d, *J*=6.82 Hz, 1 H), 7.93 (d, *J*=6.32 Hz, 1 H), 7.62(m, *J*=8.34 Hz, 2 H), 7.54 (t, *J*=7.71 Hz, 1 H), 7.24 (s, 1 H), 5.22 - 5.34 (m, 1 H), 5.07 (d, *J*=5.56 Hz, 2 H), 2.37 (s, 3 H).



4-(6-Chloro-4-methylpyridin-3-yl)benzaldehyde

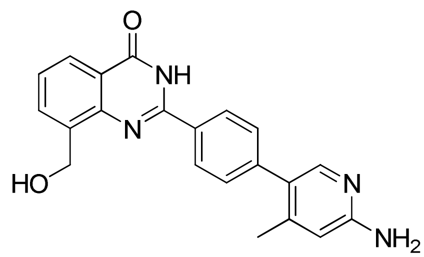
A 3.0 L four-neck round bottomed flask was charged with 4-bromo benzaldehyde (178.12 g, 0.962 mol), 6-chloro-4-methylpyridin-3-ylboronic acid (165 g, 0.962 mol), cesium carbonate (470.5 g, 1.44 mol) and tetrakis(triphenylphosphine)palladium(0) (55.62 g, 0.0481 mol). Degassed dioxane (825 mL) and water (165 mL) were added. The reaction mixture was degassed with argon (5 times) and heated to 120 °C for 20 minutes. At this stage HPLC indicated product formation and loss of starting material. After cooling, water (650 mL) and EtOAc (3300 mL) were added. The organic layer was collected and concentrated. The obtained crude mass (300 g) was subjected to column chromatography to afford a white solid (170 g, 76.2 %). *m/z*: 232.0 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO) δ ppm 10.1 (s, 1H), 8.3 (s, 1H), 7.90-8.01 (d, 2H), 7.63-7.65 (d, 2H), 7.5 (s, 1H), 2.3 (s, 3H).



Compound 5

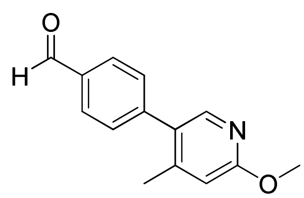
2-[4-(6-Chloro-4-methyl-pyridin-3-yl)-phenyl]-8-hydroxymethyl-3H-quinazolin-4-one

A mixture of 2-amino-3-(hydroxymethyl) benzamide (155 g, 0.9327 mol) 4-(6-chloro-4-methylpyridin-3-yl) benzaldehyde (216.09 g, 0.9327 mol) and copper (II) chloride (150 g, 1.119 mol) in DMSO (3.1 L) was heated to 100 °C for 1 hour. HPLC indicated clean product formation. The reaction mass was slowly quenched with water (15.3 L) and stirred for 2 h. The solid was filtered through a Buchner funnel, washed with water (6.2 L). Crude solid was purified by column chromatography, the solid was dried in a vacuum tray drier at 80 °C under vacuum for 14-16 h to yield a yellow solid (230.0 g, 65 %). *m/z*: 376.5 $(M-H)^-$. ^1H NMR (300 MHz, DMSO) δ ppm 7.8 (s, 1H), 7.5 (d, 1H), 7.2 (d, 1H), 7.1 (d, 1H), 6.5 (t, 1H), 6.5 (s, 2H), 5.1 (t, 1H), 4.4 (d, 2H) ppm.

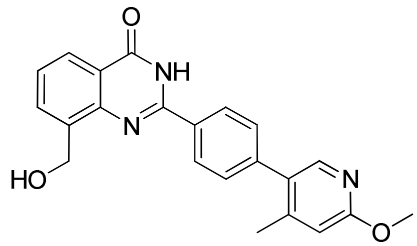


Compound 6

<u>2-(4-(6-Amino-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one</u>
2-(4-bromophenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (100 mg, 0.30 mmol), 4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine (85 mg, 0.36 mmol), cesium carbonate (197 mg, 0.60 mmol), tetrakis(triphenylphosphine)palladium(0) (13.96 mg, 0.01 mmol) in dioxane (1 mL) and water (0.167 mL) was degassed and heated in microwave at 150 °C for 20 min. The precipitate was filtered, washed with EtOAc, MeOH, and DCM to give the final product as a white solid. *m/z*: 359 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-*d*6) δ ppm 12.56 (s, 1 H) 8.26 (d, *J*=8.29 Hz, 2 H) 8.05 (d, *J*=7.91 Hz, 1 H) 7.92 (d, *J*=6.97 Hz, 1 H) 7.82 (s, 1 H) 7.39 -7.58 (m, 3 H) 6.41 (s, 1 H) 6.01 (br. s., 2 H) 5.17 - 5.36 (m, 1 H) 5.06 (d, *J*=5.27 Hz, 2 H) 2.19 (s, 3 H).



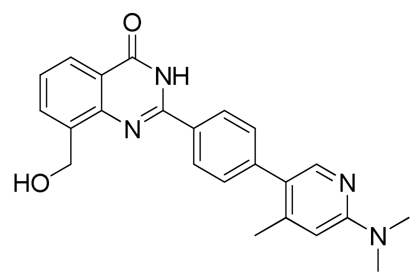
<u>4-(6-Methoxy-4-methylpyridin-3-yl)benzaldehyde</u>
A 10 mL microwave vial was charged with 4-bromobenzaldehyde (250 mg, 1.35 mmol), 6-methoxy-4-methylpyridin-3-ylboronic acid (226 mg, 1.35 mmol), cesium carbonate (660 mg, 2.03 mmol) and tetrakis(triphenylphosphine)palladium(0) (15.61 mg, 0.01 mmol). Degassed dioxane (3.5 mL) and water (0.5 mL) were added. The reaction vial was capped and heated to 150 °C in microwave for 25 minutes. At this stage LC/MS indicated clean product formation and loss of starting material. After cooling, the solvent was evaporated under reduced pressure and the residue was partitioned between water (5 mL) and EtOAc (40 mL), organic layer separated, dried over sodium sulfate and concentrated under reduced pressure. The crude material was triturated with ether and the product was precipitated out, filtered off, and washed with ether to give 4-(6-methoxy-4-methylpyridin-3-yl)benzaldehyde (260 mg, 85 %) as a white solid which was used without further purification. *m/z*: 228 $[M+H]^+$.



Compound 7

<u>8-(Hydroxymethyl)-2-(4-(6-methoxy-4-methylpyridin-3-yl)phenyl)quinazolin-4(3H)-one</u>

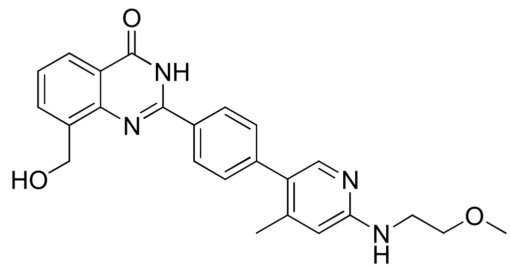
A 20 mL microwave vial charged with 2-amino-3-(hydroxymethyl)benzamide (150 mg, 0.90 mmol), 4-(6-methoxy-4-methylpyridin-3-yl)benzaldehyde (205 mg, 0.90 mmol), sodium bisulfite (188 mg, 1.81 mmol) and DMA (10 mL). The vial was capped and heated to 150 °C in microwave for 2.3 h. After cooling to room temperature, the sodium bisulfite was filtered off, and the filter cake was washed with DMA. The DMA solution (12 mL) was directly purified by reverse phase HPLC (Atlantis, OBD column, 30mm x 50mm 5µm), 10-70% water (0.1% TFA) /acetonitrile (0.1% TFA), 10 minutes).The fractions were concentrated to give 8-(hydroxymethyl)-2-(4-(6-methoxy-4-methylpyridin-3-yl)phenyl)quinazolin-4(3H)-one (56.0 mg, 16.61 %) as a light yellow solid. *m/z* : 374 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 12.61 (s, 1 H), 8.30 (d, *J*=8.34 Hz, 2 H), 8.00 - 8.11 (m, 2 H), 7.93 (d, *J*=7.33 Hz, 1 H), 7.44 - 7.65 (m, 3H), 5.27 (t, *J*=5.56 Hz, 1 H), 5.07 (d, *J*=5.81 Hz, 2 H), 3.89 (s, 3 H), 2.29 (s, 3 H).



Compound 8

<u>2-(4-(6-(Dimethylamino)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one</u>

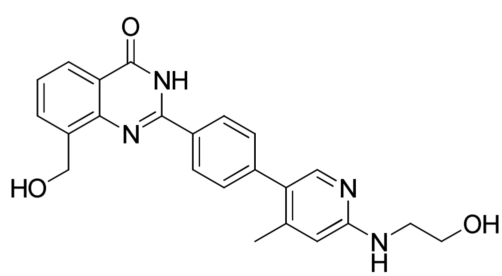
A solution of 2-(4-methylpiperazin-1-yl)ethanamine (400 mg, 2.79 mmol) and 2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (100 mg, 0.26 mmol) in DMA (1 mL) was degassed and heated in microwave at 180 °C for 1 h, then 190 °C for 2 h. LCMS showed the formation of 2-(4-(6-(dimethylamino)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one as the major product. The mixture was cooled to room temperature and filtered. The solid was washed with 10 mL of 20% iPrOH/ EtOAc three times. Water (20 mL) was added to the filtrate. The organic layer was separated. The aq. layer was extracted with 20% iPrOH/ EtOAc (3 x 50 mL). The extracts were combined and concentrated. Purification by reverse phase HPLC (Atlantis T3, 19 x 100 mm, 5µM,20-30% ACN in 0.1% TFA buffer, 6 min) gave the product 2-(4-(6-(dimethylamino)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (6 mg, 5.68 %). It was basified by adding 1 mL NH_4OH , and the solid was filtered to give the product as a yellow solid. *m/z*: 387 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 12.44 (br. s., 1 H) 8.28 (s, 2 H) 8.06 (s, 1 H) 8.00 (s, 1 H) 7.91 (s, 1 H) 7.54 (s, 3 H) 6.62 (s, 1 H) 5.28 (br. s.,1 H) 5.07 (br. s., 2 H) 3.07 (s, 6 H) 2.28 (s, 3 H).



Compound 9

8-Hydroxymethyl-2-{4-[6-(2-methoxy-ethylamino)-4-methyl-pyridin-3-yl]-phenyl}-3H-quinazolin-4-one

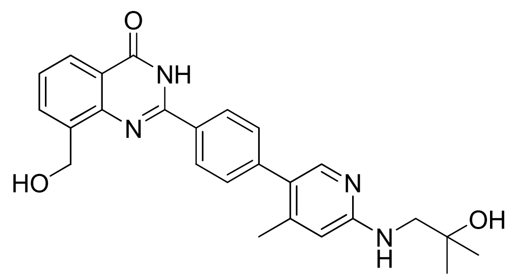
A mixture of 2-methoxyethanamine (475 mL, 5.56 mol) and 2-(4-(6-chloro-4- methylpyridin-3-yl) phenyl)-8-(hydroxymethyl) quinazolin-4(3H)-one (210 g, 0.556 mol) was heated at 150 °C in an autoclave for 24 h. The mixture was cooled to room temperature. The reaction was quenched with water (2.1 L), stirred for 2 h and the precipitated solid was filtered. The filtered cake was washed with water (630 mL), purified by column chromatography and slurry with MTBE. The obtained slurry was filtered and dried in a vacuum tray dryer at 90 °C under vacuum for 14-16 h to yield a pale yellow solid (90 g, 36.6%). *m/z*: 417.0 $[M+H]^+$. HRMS (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{24}H_{26}N_4O_3$ 417.1921; Found 417.1924. ^{1}H NMR (400 MHz, DMSO) δ ppm 12.6 (s, 1H), 8.3 (d, 2H), 8.1 (d, 1H), 7.85-7.96 (t, 2H), 7.46-7.57 (t, 3H), 6.57-6.65 (s, 1H), 6.5 (s, 1H), 5.20-5.33 (t, 1H), 5.1 (d, 2H), 3.42-3.55 (q, 4H), 3.3 (s, 3H), 2.2 (s, 3H).



Compound 10

2-(4-(6-(2-Hydroxyethylamino)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one

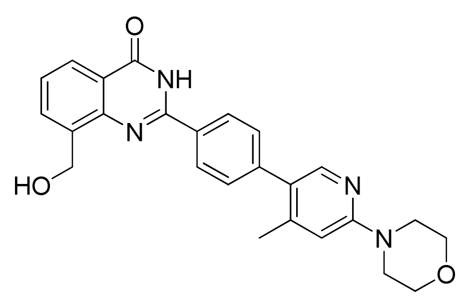
A mixture of 2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (80 mg, 0.21 mmol) and 2-aminoethanol (64.7 mg, 1.06 mmol) hydrochloride in DMSO (2 mL) was heated in a microwave reactor at 165 °C for 4 hours. LCMS indicated the formation of the desired product. Reverse phase HPLC (Atlantis T3, 19x100 mm, 5 µm, 15-85% MeCN/H2O with 0.1% TFA) gave 2-(4-(6-(2-hydroxyethylamino)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (33 mg, 39%). *m/z*: 403.1 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.30 (s, 3 H) 3.45 (br. s., 2 H) 3.63 (t, *J*=5.31 Hz, 2 H) 5.06 (s, 2 H) 6.88 - 7.10 (m, 1 H) 7.42 - 7.64 (m, 3 H) 7.85 (s, 1 H) 7.93 (d, *J*=7.58 Hz, 1 H) 8.06 (d, *J*=7.83 Hz, 1 H) 8.32 (d, *J*=8.34 Hz, 2 H) 12.64 (s, 1 H).



Compound 11

<u>2-(4-(6-(2-Hydroxy-2-methylpropylamino)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one</u>

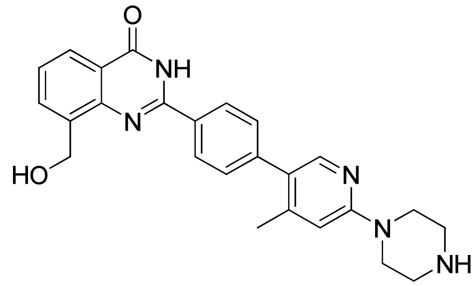
A mixture of chloro[2-(dicyclohexylphosphino)-3,6-dimethoxy-2′,4′, 6′-triisopropyl-1,1′-biphenyl][2-(2-aminoethyl)phenyl]palladium(II) (31.7 mg, 0.04 mmol), 2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (150 mg, 0.40 mmol), 1-amino-2-methylpropan-2-ol (70.8 mg, 0.79 mmol) and sodium tert-butoxide (153 mg, 1.59 mmol) in 1,4-dioxane (15 mL) was stirred at 95 °C for 1 h. LCMS indicated the completion of reaction. The reaction mixture was filtered through a pad of celite, washed with methanol, and the filtrate was concentrated. The residue was taken into methanol and pre-purified with a silica gel column (eluted with ethyl acetate/methanol 4:1). The fractions were combined and concentrated, the residue was purified with reverse phase chromatography column (eluted with 5% to 50% ACN/water/0.1%TFA) to yield 2-(4-(6-(2-hydroxy-2-methylpropylamino)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (77 mg, 35.6 %) as a yellow solid. *m/z*: 431.4 $[M+H]^+$. ^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm 0.98 - 1.23 (m, 6 H) 2.23 (s, 3 H), 3.35 (s, 2H), 4.99 (s, 2 H) 7.03 (s, 1 H) 7.35 - 7.60 (m, 3 H) 7.79 (s, 1 H) 7.81 - 7.92 (m, 1 H) 7.99 (dd, *J*=7.91, 1.51 Hz, 1 H) 8.24 (d, *J*=8.48 Hz, 2 H) 12.55 (br. s., 1 H).



Compound 12

<u>8-(Hydroxymethyl)-2-(4-(4-methyl-6-morpholinopyridin-3-yl)phenyl)quinazolin-4(3H)-one</u>

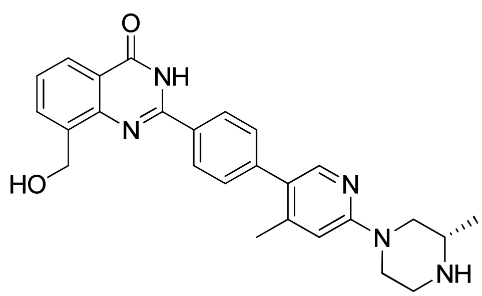
2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (100 mg, 0.26 mmol) in morpholine (23.06 mg, 0.26 mmol) was heated to 160 °C in a microwave for 4 h. Water was added and the precipitate was filtered. The solid was redissolved in DMSO (6 mL) and purified by Gilson HPLC (Atlantis T3, 19 x 100 mm, 5 µm, 20-39% ACN in 0.1% TFA buffer, 6 min) to give 8-(hydroxymethyl)-2-(4-(4-methyl-6-morpholinopyridin-3-yl)phenyl)quinazolin-4(3H)-one (25 mg, 22.04 %) as a white solid. *m/z*: 429$[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm 12.55 (br. s., 1 H) 8.29 (s, 2 H) 8.04 (s, 2 H) 7.92 (s, 1 H) 7.54 (s, 3 H) 6.83 (s, 1 H) 5.26 (s, 1 H) 5.07 (br. s.,2 H) 3.71 (br. s., 4 H) 3.52 (br. s., 4 H) 2.28 (s, 3 H)



Compound 13

8-(Hydroxymethyl)-2-(4-(4-methyl-6-(piperazin-1-yl)pyridin-3-yl)phenyl)quinazolin-4(3H)-one

A mixture of 2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (80 mg, 0.21 mmol) and piperazine (1824 mg, 21.17 mmol) was heated in a microwave reactor at 165 °C for 5 hours. LCMS indicated the formation of the desired product. Reverse phase HPLC (Atlantis T3, 19x100 mm, 5 µm, 10-70% MeCN/H2O with 0.1% TFA) gave 8-(hydroxymethyl)-2-(4-(4-methyl-6-(piperazin-1-yl)pyridin-3-yl)phenyl)quinazolin-4(3H)-one (6 mg, 6%). *m/z* $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d6) δ ppm 2.29 (s, 3 H) 3.22 (br. s., 4 H) 3.67 - 3.84 (m, 4 H) 4.95 - 5.13 (m, 2 H) 6.83 - 7.02 (m, 1 H) 7.41 - 7.62 (m, 3 H) 7.92 (d, J=7.33 Hz, 1 H) 8.01 - 8.12 (m, 2 H) 8.25 - 8.35 (m, 2 H) 8.83 (br. s., 2 H) 12.60 (br. s., 1 H).



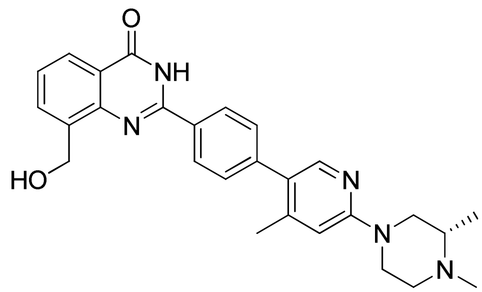
Compound 14

(S)-8-(hydroxymethyl)-2-(4-(4-methyl-6-(3-methylpiperazin-1-yl)pyridin-3-yl)phenyl)quinazolin-4(3H)-one

Condition 1: A mixture of 2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (100 mg, 0.26 mmol) and (S)-2-methylpiperazine (106 mg, 1.06 mmol) in 1 mL pyridine was heated in microwave for 1 h at 230 °C. Condition 2: A mixture of 2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (100 mg, 0.26 mmol) and (S)-2-methylpiperazine (106 mg, 1.06 mmol) and 3 drops of 1 M aq. HCl in 1 mL n-butanol was heated in microwave for 1 h at 230 °C.

Both reaction mixtures were combined and partitioned between brine and $CHCl_3$/isopropanol (5/1), the layers were separated, the water layer was extracted with $CHCl_3$/isopropanol (5/1), the organics were combined, dried (anhydrous Na_2SO_4), concentrated, the residue was purified via reverse phase chromatography (eluted with 5% to 70% ACN/water/TFA) to yield (S)-8-(hydroxymethyl)-2-(4-(4-methyl-6-(3-methylpiperazin-1-yl)pyridin-3-yl)phenyl)quinazolin-4(3H)-one (76 mg, 26 %) *m/z*: 442.3 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-*d6*) δ ppm 1.12 - 1.35 (d, 3 H), 2.30 (s, 3 H), 2.50 (m, 1 H), 2.95 (dd, *J* =

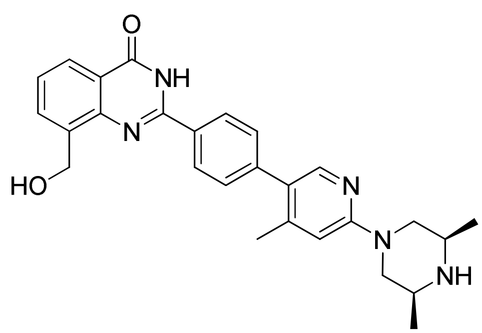
13.93, 10.67 Hz, 1 H), 3.01 - 3.26 (m, 2 H), 3.34-3.43 (m, 2 H), 4.41 (m, 1 H), 5.06 (s, 2 H), 6.98 (s, 1 H), 7.34 - 7.62 (m, 3 H), 7.93 (d, *J*=7.53 Hz, 1 H), 7.99 - 8.12 (m, 2 H), 8.29 (d, *J*=8.28 Hz, 2 H,) 8.69 (d, *J*=9.03 Hz, 1 H), 9.03 (br. s., 1 H), 12.60 (br. s., 1 H).



Compound 15

(S)-2-(4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one

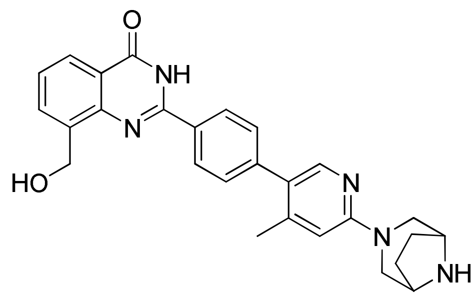
2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (150 mg, 0.40 mmol) and (S)-1,2-dimethylpiperazine (299 mg, 1.99 mmol)in Hunig's base (1 mL, 0.40 mmol) and *n*-BuOH (1 mL) was heated to 180 °C in a microwave for 7 h. Water was added and the precipitate was filtered. The solid was redissolved in DMSO (6 mL) and purified by reverse phase HPLC (Atlantis T3, 19 x 100 mm, 5 µm, 20-31% ACN in 0.1% TFA buffer) to give (S)-2-(4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one as a white solid (29 mg, 16%). *m/z*: 456 $[M+H]^+$. HRMS (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{27}H_{31}N_5O_2$ 456.2394; Found 456.2395. ^1H NMR (400 MHz, DMSO-d6) δ ppm 12.51(s, 1H) 8.27 (d, J=8.59 Hz, 2 H) 7.96 - 8.12 (m, 2 H) 7.86 - 7.95 (m, 1 H) 7.39 - 7.60 (m, 3 H) 6.82 (s, 1 H) 5.26 (br.s., 1 H) 5.06 (d, J=4.04 Hz, 2 H) 4.04 - 4.22 (m, 2 H) 2.94 (td, J=12.00, 3.03 Hz, 1 H) 2.77 - 2.87 (m, 1 H) 2.57 (dd, J=12.63, 10.11 Hz, 1 H) 2.27(s, 3 H) 2.22 (s, 3 H) 2.11 - 2.20 (m, 1 H) 2.01 - 2.10 (m, 1 H) 1.07 (d, J=6.32 Hz, 3 H).



Compound 16

2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one

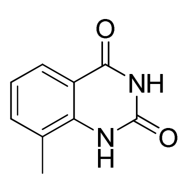
To a suspension of 2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (150 mg, 0.32 mmol) and (2S,6R)-2,6-dimethylpiperazine (91 mg, 0.79 mmol) in BuOH (1.5 ml) was added three drops of HCl (0.032 ml, 0.03 mmol) (1M in water). The mixture was stirred in a microwave for 1 h at 230 °C. The mixture was directly purified on a reverse phase column (eluted with 5% to 70% acetonitrile/water/TFA) to yield 2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (110 mg, 60.6 %) as a white solid. *m/z*: 456.3 $[M+H]^+$. HRMS (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{27}H_{31}N_5O_2$ 456.2394; Found 456.2396. ^1H NMR (400 MHz, methanol-*d*4) δ ppm 1.33 (d, *J*=6.53 Hz, 6 H) 2.28 (s, 3 H) 2.92 (dd, *J*=14.31, 11.54 Hz, 2 H) 3.32 - 3.48 (m, 2 H) 4.43 (dd, *J*=14.05, 2.26 Hz, 2 H) 5.10 (s, 2 H) 7.05 (s, 1 H) 7.35 - 7.49 (m, 3 H) 7.85 (dd, *J*=7.40, 1.13 Hz, 1 H) 7.93 (s, 1 H) 8.07 (dd, *J*=7.91, 1.38 Hz, 1 H) 8.13 - 8.21 (m, 2 H).



Compound 17

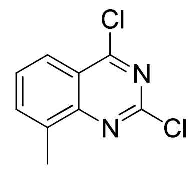
<u>2-(4-(6-(3,8-Diazabicyclo[3.2.1]octan-3-yl)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one</u>

A mixture of tert-butyl 3,8-diazabicyclo[3.2.1]octane-8-carboxylate (100 mg, 0.47 mmol), chloro(2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II) (74.1 mg, 0.09 mmol), 2-(4-(6-chloro-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (178 mg, 0.47 mmol) and sodium tert-butoxide (226 mg, 2.36 mmol) in THF (15 mL) was stirred at 82 °C for 2.5 h. LCMS indicated the completion of reaction. The hot mixture was filtered through a bed of celite, washed with DCM, the filtrate was concentrated, the residue was purified on Gilson (5% to 90% ACN/Water/0.1%TFA) to yield tert-butyl 3-(5-(4-(8-(hydroxymethyl)-4-oxo-3,4-dihydroquinazolin-2-yl)phenyl)-4-methylpyridin-2-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate. The solid was taken into 5 mL of 4 M HCl in dioxane and the suspension was stirred at room temperature for 15 min, concentrated, the residue was purified on Gilson (5% to 75% ACN/water/ammonium acetate) to yield 2-(4-(6-(3,8-diazabicyclo[3.2.1]octan-3-yl)-4-methylpyridin-3-yl)phenyl)-8-(hydroxymethyl)quinazolin-4(3H)-one (16 mg, 7.5 %) as a white solid. MS+ found 454.2. 1H NMR (400 MHz, METHANOL-*d*4) δ ppm 1.82 (s, 2 H) 1.84 - 2.04 (m, 4 H) 2.21 (s, 3 H) 3.06 (d, *J*=11.80 Hz, 2 H) 3.85 (br. s., 2 H) 4.00 (d, *J*=11.04 Hz, 2 H) 5.10 (s, 2 H) 6.65 (s, 1 H) 7.26 - 7.50 (m, 3 H) 7.84 (d, *J*=7.28 Hz, 1 H) 7.90 (s, 1 H) 8.07 (d, *J*=7.78 Hz, 1 H) 8.12 (d, *J*=8.28 Hz, 2 H).



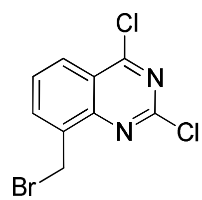
<u>8-Methylquinazoline-2,4(1*H*,3*H*)-dione</u>

A suspension of 2-amino-3-methylbenzoic acid (9.9 g, 65.49 mmol) and urea (9.28 mL, 204.34 mmol) in N-methyl-2-pyrrolidinone (50 mL) was heated to 150 °C over night. The resulting solution was cooled to room temperature and poured into ice-water (300 mL) and stirred until the ice melted. The white solid was filtered off and washed with water (30 mL x 3), then dried overnight to afford 8-methylquinazoline-2,4(1H,3H)-dione (7.50 g, 65.0 %) as a beige solid. *m/z*: 175 $(M-H)^-$. ^1H NMR (400 MHz, DMSO-*d*6, 22°C) δ ppm 2.34 (3H, s), 7.09 (1H, t), 7.35 - 7.6 (1H, m), 7.77 (1H, d), 10.41 (1H, s), 11.34 (1H, s).



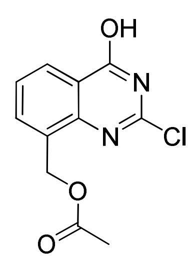
2,4-Dichloro-8-methylquinazoline

A mixture of 8-methylquinazoline-2,4(1H,3H)-dione (9.99 g, 56.71 mmol) and phosphorus oxychloride (80 mL, 858.27 mmol) was heated at 100 °C for 4.5 hours, then stirred at ambient temperature overnight. The reaction was heated for an additional 5 h. The mixture was cooled and added dropwise to warm water at 45 °C. The solid formed was filtered off and triturated with ether/heptane (1:1) for 30 minutes before filtering off and drying in a vacuum oven at 45 °C overnight to give 2,4-dichloro-8-methylquinazoline (10.95 g, 91 %) as a fluffy cream solid. 1H NMR (400 MHz, $CDCl_3$, 30 °C) δ ppm 2.75 (3H, s), 7.54 - 7.65 (1H, m), 7.82 (1H, d), 8.04 - 8.15 (1H, m).



8-(Bromomethyl)-2,4-dichloroquinazoline

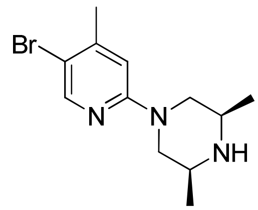
N-bromosuccinimide (5.23 mL, 61.67 mmol) and 2,2'-azobis(2-methylpropionitrile) (1.688 g, 10.28 mmol) were added portionwise to a suspension of 2,4-dichloro-8-methylquinazoline (10.95 g, 51.39 mmol) in degassed ethyl acetate (100 mL) at 65 °C under nitrogen. The resulting solution was stirred at reflux for 2.25 h. The mixture was concentrated to provide an orange solid. The solid was triturated with MeOH (50 mL) and filtered to afford 8-(bromomethyl)-2,4-dichloroquinazoline (13.32 g, 89 %) as a pale yellow crystalline solid. 1H NMR (400 MHz, DMSO, 30 °C) δ ppm 5.14 (2H, d), 7.89 (1H, td), 8.26 - 8.36 (2H, m).



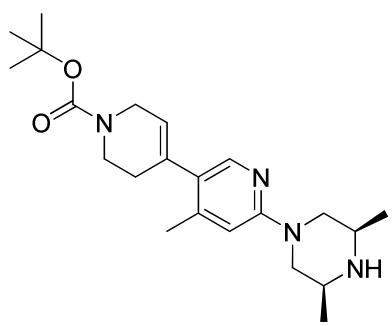
(2-Chloro-4-hydroxyquinazolin-8-yl)methyl acetate

A mixture of 8-(bromomethyl)-2,4-dichloroquinazoline (3 g, 10.28 mmol) and potassium acetate (7.46 g, 76.04 mmol) in DMF (60 mL) was stirred at 70 °C for 4 h. EtOAc (100 mL) was added, and water (60 mL x 4) was used to wash the mixture. The organic phase was concentrated and the crude product was purified by flash silica chromatography, elution

gradient 0 to 50% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford (2-chloro-4-hydroxyquinazolin-8-yl)methyl acetate (1.310 g, 50.5 %) as a white solid.
A second set of fractions was contaminated with succinimide. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in isohexane. Pure fractions were evaporated to dryness to afford (2-chloro-4-hydroxyquinazolin-8-yl)methyl acetate (0.81 g, 31.2%) as a white solid. Total product: (2-chloro-4-hydroxyquinazolin-8-yl)methyl acetate (1.310 g, 50.5 %).*m/z*: 251 $(M-H)^-$. ^1H NMR (400 MHz, DMSO, 21°C) δ ppm 2.02 (3H, s), 5.32 (2H, s), 7.47 (1H, t), 7.77 (1H, d), 7.99 (1H, dd), 13.31 (1H, s).

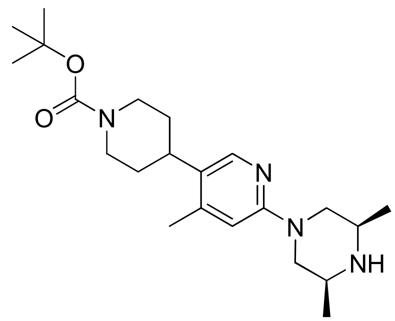


<u>(3S,5R)-1-(5-bromo-4-methylpyridin-2-yl)-3,5-dimethylpiperazine</u>
A mixture of 5-bromo-2-chloro-4-methylpyridine (4.5 g, 21.80 mmol), Hunig's Base (3.81 mL, 21.80 mmol) and (2R,6S)-2,6-dimethylpiperazine (7.47 g, 65.39 mmol) in DMSO (45 mL) was stirred at 100 °C for 16 hours. The reaction mixture was diluted with water (500 mL), and extracted with DCM (2x 200 mL). The organic layer was washed with brine (200 mL) and dried over $MgSO_4$, filtered and evaporated. The crude product was purified by flash silica chromatography, elution gradient 0 to 5% methanolic NH_3 in DCM. Pure fractions were evaporated to dryness to afford (3S,5R)-1-(5-bromo-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (4.50 g, 72.6 %) as a yellow oil which crystallized on standing. The mixed fractions were repurified using the above conditions to provide (3S,5R)-1-(5-bromo-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (0.91 g, 14.7 %) as a yellow oil which crystallized on standing. *m/z*: 285.97 $[M+H]^+$. ^1H NMR (400 MHz, CDCl3, 30 °C) δ ppm 1.14 (6H, d), 2.29 - 2.32 (3H, m), 2.35 (2H, dd), 2.92 (2H, dtt), 3.97 - 4.14 (2H, m), 6.51 (1H, s), 8.16 (1H, s).



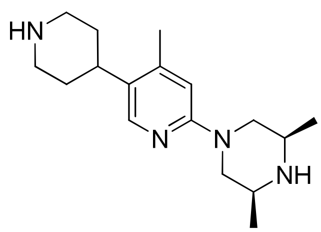
<u>tert-Butyl 6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate</u>
A mixture of tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (1.088 g, 3.52 mmol), chloro(2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II) (0.277 g, 0.35 mmol), (3S,5R)-1-(5-bromo-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (1 g, 3.52 mmol) and sodium carbonate (1.119 g, 10.56 mmol) in 1,4-dioxane (10 mL) and water (2.5 mL) was stirred at 70 °C for 2 h. LCMS indicated the completion of reaction. The mixture was directly

pre-loaded onto silica gel and purified via normal phase chromatography (eluted with 100% ethyl acetate to 35% methanol in ethyl acetate) to yield tert-butyl 6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate (1.360 g, 100 %) as an off white solid. *m/z*: 387 $[M+H]^+$. ^1H NMR (400 MHz, CDCl3, 30 °C) δ ppm 1.14 (3H, s), 1.16 (3H, s), 1.50 (9H, s), 2.21 (3H, s), 2.26 - 2.41 (4H, m), 2.95 (2H, dqd), 3.60 (2H, t), 4.02 (2H, d), 4.11 (2H, dd), 5.55 (1H, s), 6.44 (1H, s), 7.88 (1H, s).



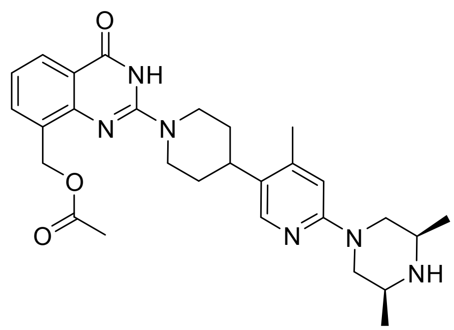
<u>tert-Butyl 4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidine-1-carboxylate</u>

tert-butyl 6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate (1.366 g, 3.53 mmol) and 10% Pd/C (0.376 g, 0.35 mmol) in MeOH (25 mL) were stirred under a hydrogen balloon at room temperature for 36 hours. LCMS indicated only half the product formation. The atmosphere of the reaction was replaced with N_2, and a second batch of Pd/C (0.376 g, 0.35 mmol) was added, then the N_2 was replaced with H_2 via an H_2 balloon. The resulting mixture was stirred for further 5 h. LCMS indicated the completion of reaction. The reaction mixture was filtered through a bed of celite and the filtrate was concentrated to dryness to afford tert-butyl 4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidine-1-carboxylate (1.373 g, 100 %) as a white solid. *m/z*: 389 $[M+H]^+$. 1H NMR (400 MHz, CDCl3, 30 °C) δ ppm 1.13 (3H, s), 1.15 (3H, s), 1.48 (9H, s), 1.61 (2H, qd), 1.73 (2H, d), 2.27 (3H, s), 2.34 (2H, dd), 2.68 (1H, ddd), 2.78 (2H, t), 2.94 (2H, dtt), 4.08 (2H, dd), 4.24 (2H, d), 6.44 (1H, s), 7.97 (1H, s).



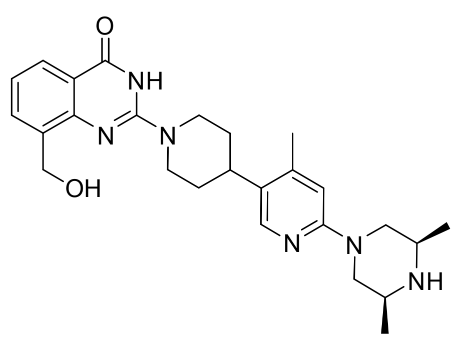
<u>(3S,5R)-3,5-dimethyl-1-(4-methyl-5-(piperidin-4-yl)pyridin-2-yl)piperazine</u>

To a solution of tert-butyl 4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidine-1-carboxylate (1.373 g, 3.53 mmol) in MeOH (10 ml) was added 4 M HCl in dioxane (8 mL, 230.38 mmol) at room temperature. The mixture was stirred at room temperature for 30 min. The solvent was removed via rotary evaporator. The white solid was taken into 50 mL of methanol, to the mixture was added polymer supported carbonate (tetraalkylammonium carbonate, polymer-bound, 3.6 grams, 2.5-3.5mmol/g) and the suspension was stirred at room temperature for 2 h. The solid was filtered off, and the filtrate was concentrated to yield (3S,5R)-3,5-dimethyl-1-(4-methyl-5-(piperidin-4-yl)pyridin-2-yl)piperazine (1.019 g, 100 %). ^{1}H NMR (400 MHz, CDCl3, 30 °C) δ ppm 1.13 (3H, s), 1.15 (3H, s), 1.65 (2H, td), 1.74 (2H, d), 2.27 (3H, s), 2.34 (2H, dd), 2.62 - 2.78 (3H, m), 2.95 (2H, dqd), 3.19 (2H, d), 4.07 (2H, dd), 6.44 (1H, s), 8.04 (1H, s).



(2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidin-1-yl)-4-oxo-3,4-dihydroquinazolin-8-yl)methyl acetate

DIEA (1.851 ml, 10.60 mmol) was added in one portion to (2-chloro-4-oxo-3,4-dihydroquinazolin-8-yl)methyl acetate (0.893 g, 3.53 mmol) and (3S,5R)-3,5-dimethyl-1-(4-methyl-5-(piperidin-4-yl)pyridin-2-yl)piperazine (1.019 g, 3.53 mmol) in DMSO (15 mL). The resulting mixture was stirred at 100 °C for 1.5 hours under nitrogen. LCMS indicated the completion of reaction. To the mixture was added water (200 mL) and brine (20 mL). The resulting solid was collected by filtration, washed with water, and air dried to yield a brown solid as (2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidin-1-yl)-4-oxo-3,4-dihydroquinazolin-8-yl)methyl acetate (1.780 g, 100 %) which was used without further purification.

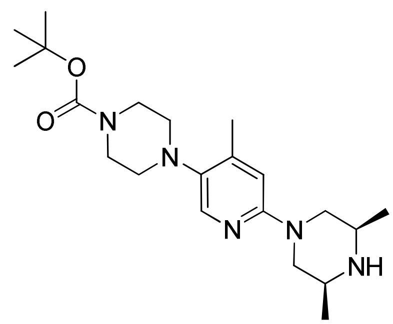


Compound 18

2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidin-1-yl)-8-(hydroxymethyl)quinazolin-4(3H)-one

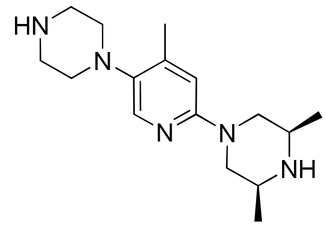
To a stirred solution of (2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidin-1-yl)-4-oxo-3,4-dihydroquinazolin-8-yl)methyl acetate (1.78 g, 3.53 mmol) in MeOH (50 mL) was added potassium carbonate (0.975 g, 7.05 mmol). The suspension was stirred at 50 °C for 1.5 h. LCMS indicated the completion of reaction, the solid was filtered off through a bed of celite, the filtrated was concentrated, the residue was purified by silica gel chromatography (eluted with 10% to 40% methanol in ethyl acetate) to yield the product (93% purity, 1.26 g) as a light brown solid. The solid was repurified via reverse phase chromatography (eluted with 5% to 50% ACN/water/0.1%TFA) to yield the pure product as

TFA salt. The product was dissolved into 150 mL of $CHCl_3$/isopropanol (5/1), then washed with sat. aq.Na_2CO_3 and brine. The organic layer was dried (anhydrous Na_2SO_4), filtered, and concentrated until about 2 mL of isopropanol was left in the flask and the solid started to appear. The residue was left to stand still for 1 h until a large amount of crystals started to appear, then was diluted with hexane (100 mL), the solid was collected by filtration and dried to yield 2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidin-1-yl)-8-(hydroxymethyl)quinazolin-4(3H)-one (0.820 g, 50.3 %) as a white solid. *m/z*: 463 $[M+H]^+$. ^1H NMR (500 MHz, DMSO, 30 °C) δ ppm 1.00 (3H, s), 1.02 (3H, s), 1.64 (2H, qd), 1.77 (2H, d), 2.15 (2H, dd), 2.27 (3H, s), 2.72 (2H, dqd), 2.81 - 2.9 (1H, m), 2.98 (2H, t), 3.31 (2H, s), 4.05 (2H, dd), 4.56 (2H, d), 4.79 (2H, s), 6.61 (1H, s), 7.08 - 7.14 (1H, m), 7.65 (1H, dd), 7.80 (1H, dd), 7.88 (1H, s).



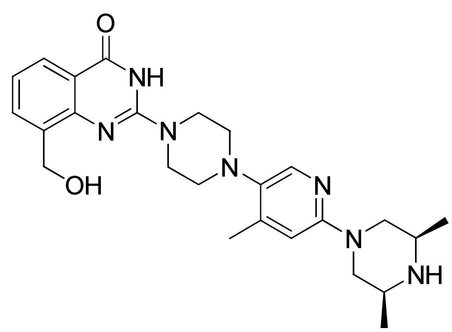
<u>tert-Butyl 4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazine-1-carboxylate</u>

$Pd_2(dba)_3$ (0.128 g, 0.14 mmol) was added to a degassed mixture of tert-butyl piperazine-1-carboxylate (0.573 g, 3.08 mmol), dicyclohexyl(2',6'-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (0.230 g, 0.56 mmol) and (3S,5R)-1-(5-bromo-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (0.795 g, 2.80 mmol) and sodium 2-methylpropan-2-olate (0.376 g, 3.92 mmol) in toluene (20 mL). The resulting suspension was stirred at 80 °C for 14 hours under nitrogen. The reaction mixture was filtered through diatomaceous earth and the filtrate was diluted with EtOAc (20 mL), and washed sequentially with water (2 x 20 mL) and saturated brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 5% MeOH in DCM. Pure fractions were evaporated to dryness to afford tert-butyl 4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazine-1-carboxylate (0.892 g, 82 %) as an orange oil. *m/z*: 390 $[M+H]^+$. ^1H NMR (400 MHz, CDCl3, 30 °C) δ ppm 1.14 (3H, s), 1.16 (3H, s), 1.48 (9H, s), 2.25 (3H, s), 2.34 (2H, dd), 2.78 - 2.85 (4H, m), 2.96 (2H, dtt), 3.51 - 3.55 (4H, m), 4.03 (2H, dd), 6.51 (1H, s), 7.88 (1H, s).



(3S,5R)-3,5-dimethyl-1-(4-methyl-5-(piperazin-1-yl)pyridin-2-yl)piperazine

2,2,2-trifluoroacetic acid (1.000 ml, 13.06 mmol) was added dropwise to tert-butyl 4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazine-1-carboxylate (885 mg, 1.82 mmol) in DCM (20 mL). The resulting solution was stirred at room temperature for 2 h. The crude product was purified by ion exchange chromatography using an SCX column. The desired product was eluted from the column using 7 M NH_3/MeOH and fractions were evaporated to dryness to afford crude product. The crude product was then purified by flash silica chromatography, using an elution gradient of 0 to 10% 7 N ammonia/MeOH in DCM. Pure fractions were evaporated to dryness to afford (3S,5R)-3,5-dimethyl-1-(4-methyl-5-(piperazin-1-yl)pyridin-2-yl)piperazine (403 mg, 77 %) as a pale yellow oil which crystallized on standing. *m/z* 290 $[M+H]^+$. ^{1}H NMR (400 MHz, CDCl3, 30 °C) δ ppm 1.14 (6H, d), 2.25 (3H, s), 2.28-2.37 (2H, m), 2.85 (4H, dd), 2.89-3.01 (6H, ddt), 4.03 (2H, dd), 6.50 (1H, s), 7.92 (1H, s).

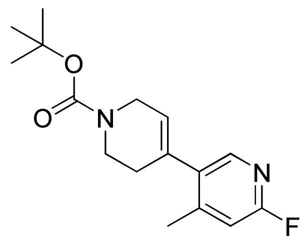


Compound 19

2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazin-1-yl)-8-(hydroxymethyl)quinazolin-4(3H)-one

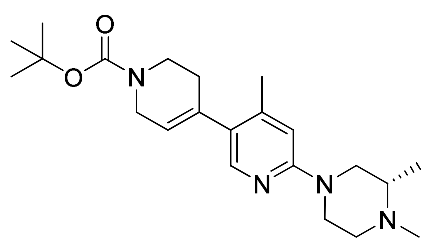
N,N-diisopropylethylamine (0.338 mL, 2.04 mmol) was added in one portion to (2-chloro-4-oxo-3,4-dihydroquinazolin-8-yl)methyl acetate (120 mg, 0.47 mmol) and (3S,5R)-3,5-dimethyl-1-(4-methyl-5-(piperazin-1-yl)pyridin-2-yl)piperazine (137 mg, 0.47 mmol) in DMF (3 mL). The resulting solution was stirred at 100 °C for 60 minutes. The reaction mixture was allowed to cool to 50 °C. Potassium carbonate (328 mg, 2.37 mmol) and MeOH (5 mL) were added and the mixture was stirred for 2 h at 50 °C. The mixture was allowed to cool to room temperature and concentrated under vacuum and the crude product was dissolved in DMF (6 mL) and this was filtered. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5μ silica, 50 mm diameter, 100 mm length), using decreasingly polar mixtures of water containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazin-1-yl)-8-(hydroxymethyl)quinazolin-

4(3H)-one (175 mg, 79 %) as a white solid. *m/z*: 464 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO, 33°C) δ ppm 1.01 (3H, s), 1.02 (3H, s), 2.15 (2H, t), 2.26 (3H, s), 2.74 (2H, ddd), 2.84 - 2.89 (4H, m), 3.76 (4H, s), 4.02 (2H, dd), 4.77 (2H, s), 6.67 (1H, s), 6.93 (1H, t), 7.42 (1H, d), 7.75 (1H, d), 7.83 (1H, s).



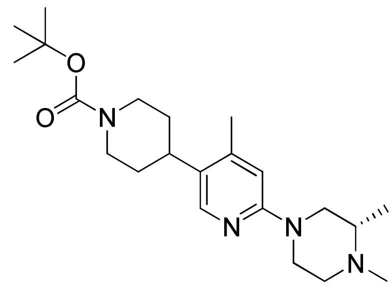
<u>tert-Butyl 6-fluoro-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate</u>

In a 25 mL sealed tube was added tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (1.790 g, 5.79 mmol), 5-bromo-2-fluoro-4-methylpyridine (1 g, 5.26 mmol), and $Pd(PPh_3)_4$ (0.304 g, 0.26 mmol) in 1,4-dioxane (10 mL) to give a suspension. K_2CO_3 (2.182 g, 15.79 mmol) was added. The mixture was heated in a microwave at 140 °C for 20 min. The precipitate was removed by suction filtration. The filtrate was concentrated and extracted with EtOAc and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was subjected to silica gel chromatography to give tert-butyl 6-fluoro-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate (1.17 g, 76 %).



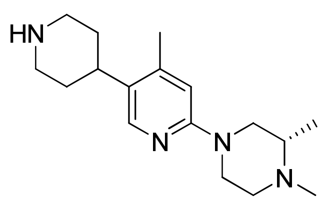
<u>(S)-tert-butyl 6-(3,4-dimethylpiperazin-1-yl)-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate</u>

In a 25 mL sealed tube was added tert-butyl 6-fluoro-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate (3.6 g, 12.31 mmol), (S)-1,2-dimethylpiperazine (2.76 g, 14.78 mmol), and K_2CO_3 (8.51 g, 61.57 mmol) in DMSO (20 mL) to give a colorless suspension. The mixture was stirred at 120 °C overnight. The mixture was extracted with EtOAc and the organics were washed with brine. The combined organic layers were dried over Na_2SO_4, concentrated, and then purified by silica gel chromatography to give (S)-tert-butyl 6-(3,4-dimethylpiperazin-1-yl)-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate (3.5 g, 73%).



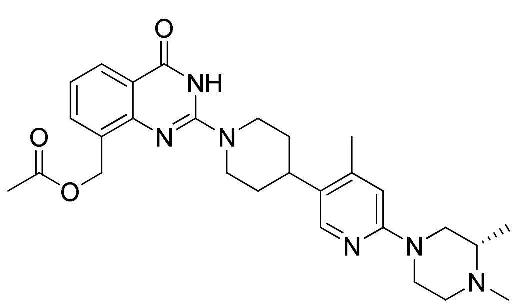
<u>(S)-tert-butyl 4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidine-1-carboxylate</u>

In a 250 mL pear shaped flask was added (S)-tert-butyl 6-(3,4-dimethylpiperazin-1-yl)-4-methyl-5',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylate (3.5 g, 9.05 mmol) and 10% Pd-C (0.964 g, 0.91 mmol) in MeOH (100 mL) to give a black suspension. The mixture was stirred at room temperature under a balloon of H_2 for 24 h. The precipitate was filtered off. The filtrate was concentrated to give (S)-tert-butyl 4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidine-1-carboxylate (3.0 g, 85%). The product was used without further purification.



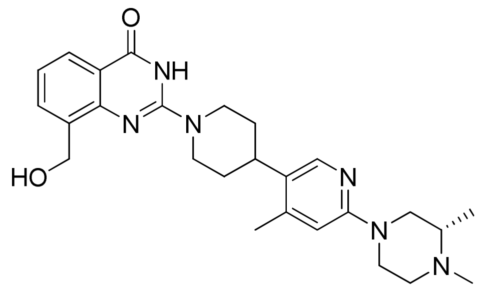
<u>(S)-1,2-dimethyl-4-(4-methyl-5-(piperidin-4-yl)pyridin-2-yl)piperazine</u>

In a 100 mL pear shaped flask was added (S)-tert-butyl 4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperidine-1-carboxylate (3.5 g, 9.01 mmol) and TFA (30 mL, 389.39 mmol) in DCM (30 mL) to give a yellow solution. The mixture was stirred at room temperature for 2 h, and then concentrated. The product was purified by prep-HPLC (CH_3CN-H_2O-5 mM aq. NH_4HCO_3, 5%-95%, 1.5-3.6 min) to give the title compound (2.5 g, 96 %). *m/z*: 289 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, 25°C) δ ppm 1.02 (3H, d), 1.42-1.70 (4H, m), 1.93-2.03 (1H, m), 2.10 (1H, t), 2.18 (3H, s), 2.22 (3H, s), 2.42 (1H, t), 2.61-2.86 (5H, m), 3.03-3.13 (1H, m), 3.93-4.18 (3H, m), 6.62 (1H, s), 7.88 (1H, s).



<u>[2-(4-{6-[(3*S*)-3,4-dimethylpiperazin-1-yl]-4-methylpyridin-3-yl}piperidin-1-yl)-4-oxo-3,4-dihydroquinazolin-8-yl]methyl acetate</u>

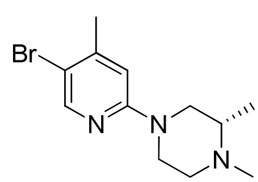
Into a 8 mL sealed tube was placed a solution of (2-chloro-4-oxo-3,4-dihydroquinazolin-8-yl)methyl acetate (150 mg, 0.59 mmol, 1.00 equiv) in DMSO (3 mL), DIEA (768 mg, 5.94 mmol, 10.00 equiv), (2S)-1,2-dimethyl-4-[4-methyl-5-(piperidin-4-yl)pyridin-2-yl]piperazine tris(trifluoroacetic acid) (751 mg, 1.19 mmol, 1.00 equiv). The resulting solution was stirred for 2 h at 100 ^{o}C. The resulting solution was diluted with 50 mL of DCM. The resulting solution was extracted with 3 x 10 mL of DCM and the organic layers were combined and dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 180 mg (60%) of [2-(4-[6-[(3S)-3,4-dimethylpiperazin-1-yl]-4-methylpyridin-3-yl]piperidin-1-yl)-4-oxo-3,4-dihydroquinazolin-8-yl]methyl acetate as a yellow solid which was used without further purification. *m/z*: 505 $[M+H]^+$.



Compound 20

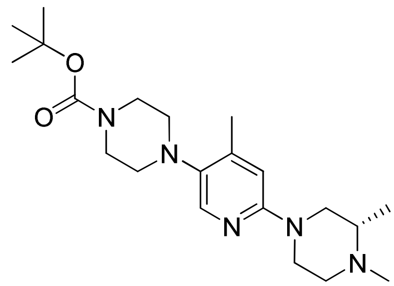
2-(4-{6-[(3*S*)-3,4-dimethylpiperazin-1-yl]-4-methylpyridin-3-yl}piperidin-1-yl)-8-(hydroxymethyl)quinazolin-4(3*H*)-one

Into a 100 mL round-bottom flask, was placed a solution of [2-(4-[6-[(3S)-3,4-dimethylpiperazin-1-yl]-4-methylpyridin-3-yl]piperidin-1-yl)-4-oxo-3,4-dihydroquinazolin-8-yl]methyl acetate (180 mg, 0.36 mmol, 1.00 equiv) in methanol (50 mL), potassium carbonate (148 mg, 1.07 mmol, 3.00 equiv). The resulting solution was stirred for 1 h at room temperature. The solids were filtered out. The filtrate was concentrated. The crude product was purified by Prep-HPLC (Gemini-NX 150*21.20mm C18 AXIA Packed, 5 µm 110A; mobile phase, H_2O with 0.05% formic acid and MeCN, 8.0% MeCN up to 22.0% in 8 min, detector UV 254/220 nm). This resulted in 68.9 mg (35%) of 2-(4-[6-[(3S)-3,4-dimethylpiperazin-1-yl]-4-methylpyridin-3-yl]piperidin-1-yl)-8-(hydroxymethyl)-3,4-dihydroquinazolin-4-one (bis formic acid salt) as a yellow solid. *m/z*: 463 $[M+H]^+$. ^{1}H NMR (400MHz, DMSO-d_6, 25°C): δ ppm 1.01-1.09(d, 3H), 1.50-1.70(m, 2H), 1.70-1.80(m, 2H), 2.11-2.32(m, 8H), 2.45-2.55(m, 1H), 2.79-2.90(m, 3H), 2.90-3.02(m, 2H), 3.98-4.01(m, 2H), 4.52-4.55(d, 2H), 4.79(s, 2H), 6.66(s, 1H), 4.79(s, 2H), 6.66(s, 1H), 7.11-7.14(m, 1H), 7.67-7.69(d, 1H), 7.79-7.81(d, 1H), 7.90(s, 1H), 8.18(s, 2H).



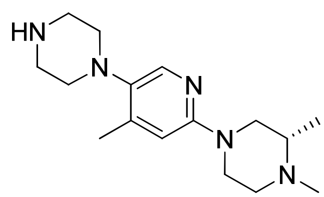
(S)-4-(5-bromo-4-methylpyridin-2-yl)-1,2-dimethylpiperazine

A mixture of 5-bromo-2-fluoro-4-methylpyridine (800 mg, 4.21 mmol), (S)-1,2-dimethylpiperazine bis HCl salt (788 mg, 4.21 mmol), Hunig's Base (2.206 ml, 12.63 mmol) in DMSO (10 ml) was stirred at 100 °C for 24 h. The reaction mixture was partitioned between water and DCM, the layers were separated. The organic layer was dried (anhydrous Na_2SO_4), filtered and concentrated, the residue was purified via silica gel chromatography (eluted with 100% ethyl acetate to 35% methanol in ethyl acetate) to yield (S)-4-(5-bromo-4-methylpyridin-2-yl)-1,2-dimethylpiperazine (1050 mg, 88 %) as a yellow solid which was used without further purification. *m/z*: 286.0 $[M+H]^+$.



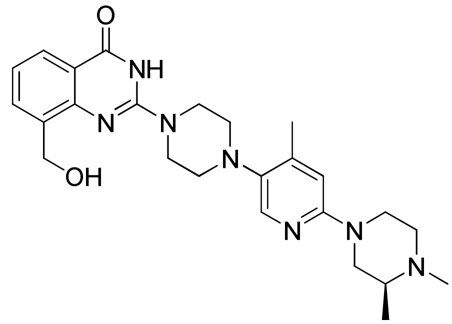
<u>(S)-tert-butyl 4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazine-1-carboxylate</u>

$Pd_2(dba)_3$ (0.169 g, 0.18 mmol) was added to a degassed mixture of tert-butyl piperazine-1-carboxylate (0.757 g, 4.06 mmol), (S)-4-(5-bromo-4-methylpyridin-2-yl)-1,2-dimethylpiperazine (1.05 g, 3.69 mmol), dicyclohexyl(2',6'-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (0.303 g, 0.74 mmol) and sodium tert-butoxide (0.533 g, 5.54 mmol) in toluene (40 mL). The resulting suspension was stirred at 80 °C for 14 h under N_2. The reaction mixture was filtered through celite and the filtrate was diluted with EtOAc (100 mL), and washed sequentially with water (20 mL), brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 5% methanol in DCM. Pure fractions were evaporated to dryness to afford (S)-tert-butyl 4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazine-1-carboxylate (0.700 g, 48.6 %) as an orange oil. *m/z*: 390.2 $[M+H]^+$.



<u>(S)-1,2-dimethyl-4-(4-methyl-5-(piperazin-1-yl)pyridin-2-yl)piperazine</u>

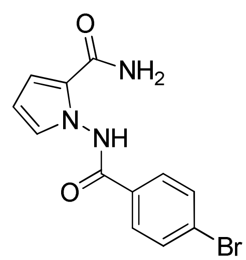
To a solution of (S)-tert-butyl 4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazine-1-carboxylate (700 mg, 1.80 mmol) in MeOH (4 ml) was added 4 M HCl in dioxane (5 mL, 143.99 mmol) at room temperature. The reaction mixture was stirred at room temperature for 30 min. The solvent was removed by concentration. To the residue was added 20 mL methanol, followed by addition of tetraalkyammonium carbonate, polymer bound (2.5-3.5 mmol/g loading, 4.5 g), and the resulting suspension was stirred at room temperature for 5 h. The solid was filtered off, washed with methanol, the filtrate was concentrated, the residue (light brown oil) was used in the next step without further purification. *m/z*: 290.2 $[M+H]^+$.



Compound 21

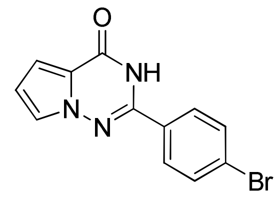
(S)-2-(4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazin-1-yl)-8-(hydroxymethyl)quinazolin-4(3H)-one

DIEA (0.387 ml, 2.22 mmol) was added in one portion to (2-chloro-4-oxo-3,4-dihydroquinazolin-8-yl)methyl acetate (140 mg, 0.55 mmol) and (S)-1,2-dimethyl-4-(4-methyl-5-(piperazin-1-yl)pyridin-2-yl)piperazine (160 mg, 0.55 mmol) in DMF (3 mL). The resulting solution was stirred at 100 °C for 1 h. The reaction mixture was allowed to cool to 50 °C. Potassium carbonate (383 mg, 2.77 mmol) and methanol (5 mL) were added and the mixture was stirred for 1 h at 50 °C. The mixture was allowed to cool to room temperature and concentrated under vacuum and the crude product was dissolved in DMF and this was filtered and the filtrate was purified via reverse phase chromatography (CH_3CN, H_2O (0.1%TFA), 254 nm) to yield the product (86 mg) which was repurified via reverse phase HPLC (Xbridge C18 19mm x 100mm 5µm, 20 to 30% CH_3CN in H_2O (0.2 % NH_4OH) over 5 min, 5 mL/min, 254 nm) to yield (S)-2-(4-(6-(3,4-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)piperazin-1-yl)-8-(hydroxymethyl)quinazolin-4(3H)-one (46.0 mg, 17.91 %) as a white solid. *m/z*: 464.3 $[M+H]^+$. ^1H NMR (400 MHz, methanol-*d*4) δ ppm 1.18 (d, J=6.27 Hz, 3 H) 2.15 - 2.30 (m, 1 H) 2.31 - 2.42 (m, 7 H) 2.60 (dd, J=12.67, 10.42 Hz, 1 H) 2.82 - 2.99 (m, 2 H) 3.00 - 3.09 (m, 4 H) 3.74 - 3.90 (m, 4 H) 3.90 - 4.04 (m, 2 H) 4.97 (s, 2 H) 6.76 (s, 1 H) 7.20 (t, J=7.65 Hz, 1 H) 7.57 - 7.76 (m, 1 H) 7.85 (s, 1 H) 7.97 (dd, J=8.03, 1.25 Hz, 1 H).

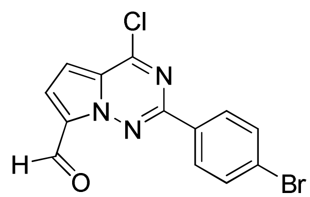


1-(4-Bromobenzamido)-1H-pyrrole-2-carboxamide

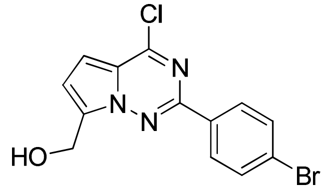
To a solution of 1-amino-1H-pyrrole-2-carboxamide (1.74 g, 13.91 mmol), and triethylamine (1.938 mL, 13.91 mmol) in acetonitrile (20 mL) was added portionwise, 4-bromobenzoyl chloride (3.05 g, 13.91 mmol). The mixture solution was stirred at 20 °C for 16 hours under nitrogen. The mixture was evaporated to dryness and stirred in water (100 mL) for 1 h before filtering off the solid to give 1-(4-bromobenzamido)-1H-pyrrole-2-carboxamide (3.80 g, 89 %) as a white solid. *m/z*: 308 $[M+H]^+$. ^1H NMR (400 MHz, DMSO, 30 °C) δ ppm 6.10 (1H, dd), 6.86 (1H, dd), 6.95 - 7.04 (1H, m), 7.75 (3H, p), 7.83 - 7.92 (3H, m), 11.59 (1H, s).



2-(4-Bromophenyl)pyrrolo[2,1-f][1,2,4]triazin-4(3H)-one
A mixture of 1-(4-bromobenzamido)-1H-pyrrole-2-carboxamide (3.80 g, 12.33 mmol), and 30% ammonia in water (10 mL, 12.33 mmol) in a 20 mL sealed tube was heated at 80 °C overnight in the microwave. Additional IPA (2 mL) was added and the reaction mixture was heated at 100 °C for 16 hours in the microwave. The mixture was filtered under suction and washed with methanol to give 2-(4-bromophenyl)pyrrolo[2,1-f][1,2,4]triazin-4(3H)-one (1.890 g, 52.8 %) as a pale yellow crystalline solid. *m/z*: 288 $[M-H]^-$. ^1H NMR (400 MHz, DMSO, 30 °C) δ ppm 6.61 (1H, dd), 6.96 (1H, dd), 7.68 (1H, dd), 7.73 - 7.81 (2H, d), 7.9 - 7.96 (2H, d), 11.98 (1H, bs).

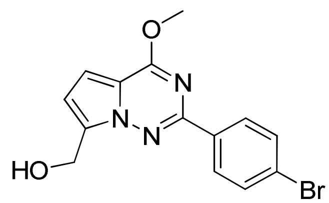


2-(4-Bromophenyl)-4-chloropyrrolo[2,1-f][1,2,4]triazine-7-carbaldehyde
A mixture of $POCl_3$ (5.69 ml, 61.01 mmol), and DMF (2.347 ml, 30.51 mmol) in a cooled 5 mL sealed tube was stirred for 10 minutes before adding 2-(4-bromophenyl)pyrrolo[2,1-f][1,2,4]triazin-4(3H)-one (1.77 g, 6.10 mmol). The mixture was heated at 95 °C for 4 h. The reaction mixture was diluted with water (200 mL), and extracted with DCM (200 mL x 3). The organic was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution 50% DCM in heptane. Pure fractions were evaporated to dryness to afford 2-(4-bromophenyl)-4-chloropyrrolo[2,1-f][1,2,4]triazine-7-carbaldehyde (1.870 g, 91 %) as a cream solid. *m/z*: 337 $[M+H]^+$. ^1H NMR (400 MHz, DMSO, 30 °C) δ ppm 7.21 - 7.26 (1H, m), 7.65 (1H, d), 7.76 - 7.83 (2H, m), 8.25 - 8.31 (2H, m), 10.53 (1H, s).

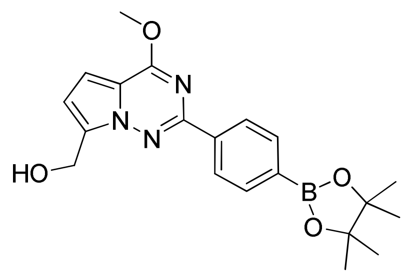


(2-(4-Bromophenyl)-4-chloropyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol
A solution of 2-(4-bromophenyl)-4-chloropyrrolo[2,1-f][1,2,4]triazine-7-carbaldehyde (1.87 g, 5.56 mmol) in THF (20 mL) was cooled to 0 °C before adding $NaBH_4$ (0.210 g, 5.56 mmol). The mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with water (200 mL), and extracted with DCM (200 mL). The organic was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica

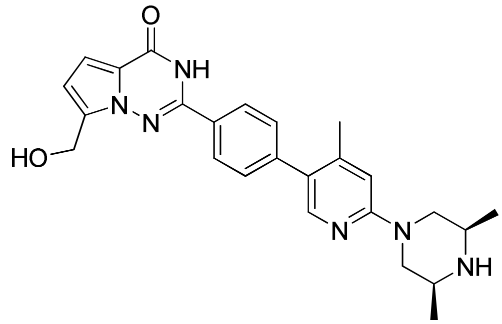
chromatography, elution 30% ether in heptane to 100% ether. Pure fractions were evaporated to dryness to afford (2-(4-bromophenyl)-4-chloropyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol (1.600 g, 85 %) as a yellow waxy solid. *m/z*: 339 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO, 30 °C) δ ppm 4.94 (2H, d), 5.46 (1H, t), 7.09 - 7.17 (2H, m), 7.73 - 7.8 (2H, d), 8.18 - 8.27 (2H, d).



<u>(2-(4-Bromophenyl)-4-methoxypyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol</u>
(2-(4-bromophenyl)-4-chloropyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol (1.28 g, 3.78 mmol) and 5.4 M sodium methoxide in methanol (1.40 mL, 7.56 mmol) were dissolved in MeOH (10 mL). The resulting solution was stirred at 60 °C for 1 hour. The reaction mixture was diluted with water (200 mL), and extracted with ethyl acetate (200 mL). The organic was dried over MgSO4, filtered and evaporated to afford crude (2-(4-bromophenyl)-4-methoxypyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol (1.240 g, 98 %) as a white solid. *m/z*: 334 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO, 30 °C) δ ppm 4.22 (3H, s), 4.88 (2H, d), 5.27 (1H, t), 6.83 (1H, d), 6.87 (1H, d), 7.75 (2H, d), 8.28 (2H, d).



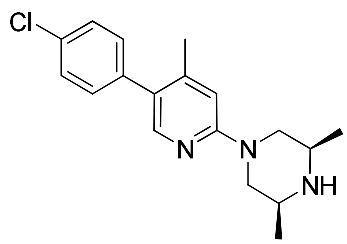
<u>(4-Methoxy-2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol</u>
Bis(pinacolato)diboron (780 mg, 3.07 mmol), KOAc (792 mg, 8.07 mmol), and palladium (II) chloride dppf complex (94 mg, 0.128 mmol) was added to (2-(4-bromophenyl)-4-methoxypyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol (0.8 g, 2.39 mmol) in DME (30 mL) under nitrogen. The resulting mixture was degassed and stirred at 85 °C for 20 h. The reaction mixture was pre-absorbed onto silica and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution 20% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford (4-methoxy-2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol (0.950 g, 104 %) as a foamy tan solid. *m/z*: 382 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO, 30 °C) δ ppm 1.34 (12H, s), 4.23 (3H, s), 4.90 (2H, d), 5.26 (1H, t), 6.77 - 6.9 (2H, m), 7.81 - 7.89 (2H, m), 8.32 - 8.42 (2H, m).



Compound 22

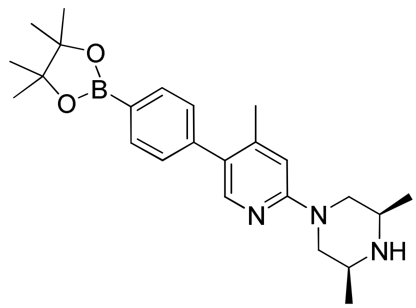
2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-7-(hydroxymethyl)pyrrolo[2,1-f][1,2,4]triazin-4(3H)-one

A mixture of (3S,5R)-1-(5-bromo-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (0.298 g, 1.05 mmol), (4-methoxy-2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyrrolo[2,1-f][1,2,4]triazin-7-yl)methanol (0.4 g, 1.05 mmol), potassium phosphate tribasic (446 mg, 2.1 mmol), and [1,1'-bis(di-*tert*-butylphosphino)ferrocene]dichloropalladium(II) (34 mg, 0.0525 mmol) in DMF (4 mL) and water (1.0 mL) was degassed before heating at 105 °C for 1.5 hours in the microwave. The crude reaction was purified by flash silica chromatography, elution 100% DCM to 10% MeOH in DCM containing 1% triethylamine. Pure fractions were evaporated to dryness to afford crude intermediate. The dark brown oil was dissolved in DMF (5 mL) and sodium thiomethoxide (0.221 g, 3.15 mmol) was added. The mixture was stirred at 50 °C for 1 h before excess sodium thiomethoxide was removed via filtration through a pad of silica. The crude product was purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5μ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-7-(hydroxymethyl)pyrrolo[2,1-f][1,2,4]triazin-4(3H)-one (0.300 g, 64.3 %) as a white solid. *m/z*: 445 $[M+H]^+$. ^1H NMR (500 MHz, DMSO, 30 °C) δ ppm 1.05 (6H, d), 2.21 - 2.32 (5H, m), 2.68 - 2.83 (2H, m), 4.21 (2H, dd), 4.78 (2H, s), 5.21 (1H, m), 6.57 (1H, d), 6.79 (1H, s), 6.92 (1H, d), 7.47 - 7.56 (2H, d), 7.98 (1H, s), 8.04 - 8.11 (2H, d).



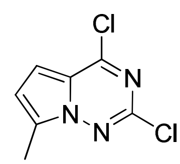
(3S,5R)-1-(5-(4-chlorophenyl)-4-methylpyridin-2-yl)-3,5-dimethylpiperazine

(4-chlorophenyl)boronic acid (2.97 g, 19.00 mmol), $Pd(Ph_3)_4$ (2.74 g, 2.38 mmol), sodium carbonate (23.75 mL, 47.50 mmol) and (3S,5R)-1-(5-bromo-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (4.5 g, 15.83 mmol) in toluene (90 mL) and ethanol (45.0 mL) was heated to 80 °C overnight. The reaction mixture was diluted with water (200 mL), and extracted with ethyl acetate (200 mL). The organic was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution 5% methanolic NH_3 in DCM. Pure fractions were evaporated to dryness to afford (3S,5R)-1-(5-(4-chlorophenyl)-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (4.00 g, 80 %) as a brown oil which crystallized on standing. *m/z*: 316.06 $[M+H]^+$. ^1H NMR (400 MHz, CDCl3, 30 °C) δ ppm 1.16 (6H, d), 2.21 (3H, s), 2.41 (2H, dd), 2.97 (2H, dtt), 4.16 (2H, dd), 6.53 (1H, s), 7.17 - 7.24 (2H, m), 7.33 - 7.42 (2H, m), 8.00 (1H, s).



<u>(3S,5R)-3,5-dimethyl-1-(4-methyl-5-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyridin-2-yl)piperazine</u>

Pd(dba)$_2$ (0.273 g, 0.47 mmol) was added in one portion to (3S,5R)-1-(5-(4-chlorophenyl)-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (2.5 g, 7.92 mmol), bis(pinacolato)diboron (2.412 g, 9.50 mmol), potassium acetate (2.331 g, 23.75 mmol) and tricyclohexylphosphine (0.533 g, 1.90 mmol) in dioxane (44 mL) at 21 °C. The resulting solution was stirred under nitrogen at 100 °C for 2 h. Water (0.570 mL, 31.66 mmol) was added and the reaction heated overnight. The reaction mixture was filtered through celite washed with DCM (50 mL).The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM. Pure fractions were evaporated to dryness to afford (3S,5R)-3,5-dimethyl-1-(4-methyl-5-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyridin-2-yl)piperazine (2.0 g, 62.0 %) as a cream foam. *m/z*: 408.15 $[M+H]^+$. ^1H NMR (400 MHz, CDCl3, 30 °C) δ ppm 1.24 (6H, d), 1.36 (12H, s), 2.23 (3H, s), 2.59 (2H, dd), 3.07 (2H, dtt), 4.21 (2H, dd), 6.54 (1H, s), 7.28 - 7.32 (2H, m), 7.79 - 7.9 (2H, m), 8.03 (1H, s).

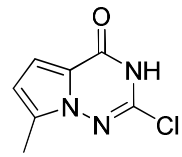


<u>2,4-Dichloro-7-methylpyrrolo[2,1-f][1,2,4]triazine</u>

A mixture of 7-methylpyrrolo[2,1-f][1,2,4]triazine-2,4(1H,3H)-dione[7] (4.7 g, 31.1mmol), $POCl_3$ (8.81 mL, 3 eq) and DIPEA (10.8 mL, 2 eq) in 20 mL toluene in a pressure vessel was heated to 150 °C for 24 h. The reaction mixture was cooled to room temperature., concentrated, then cooled to 0 °C, and 40 mL water was added. The pH was adjusted to 8 with aq. $NaHCO_3$. The resulting solid was collected via filtration. Purification with a silica gel

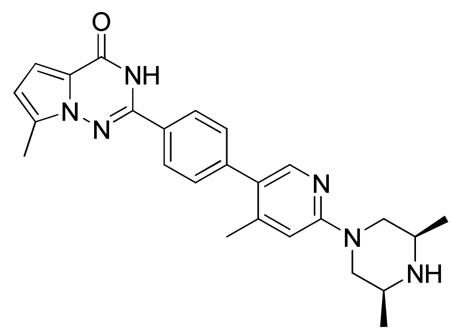
[7] Migliara, O.; Petruso, S.; Sprio, V. Synthesis of a new bridgehead nitrogen heterocyclic system. Pyrrolo [2,1-f]-1,2,4-triazine derivatives. *J. Heterocyclic Chem.* **1979,** 16, 833-834.

column (PE:EA=4:1) gave 2 g of 2,4-dichloro-7-methylpyrrolo[2,1-f][1,2,4]triazine as yellow solid.[8] ^{1}H NMR (400 MHz, DMSO, 20°C) δ ppm 7.17 (1H, d), 7.01 (1H, d), 2.51 (3H, s).



2-Chloro-7-methylpyrrolo[2,1-f][1,2,4]triazin-4(3H)-one

2,4-dichloro-7-methylpyrrolo[2,1-f][1,2,4]triazine (0.8 g) was added to a mixture of 3 g KOH in 20 mL water. The reaction was refluxed for 20 min. The reaction mixture was cooled to room temperature, then acidified with 2 N aq. HCl. The resulting white solid was collected via filtration to give 0.57 g of 2-chloro-7-methylpyrrolo[2,1-f][1,2,4]triazin-4(3H)-one. *m/z*: 182.51 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO, 20°C) δ ppm 12.68 (1H, s), 6.85 (1H, d), 6.35 (1H, d), 2.3 (3H, s).

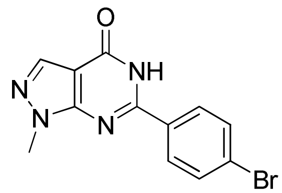


Compound 23

2-(4-(6-((3R,5S)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-7-methylpyrrolo[2,1-f][1,2,4]triazin-4(3H)-one

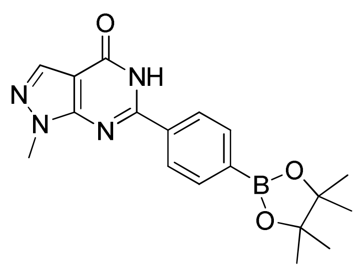
(3R,5S)-3,5-dimethyl-1-(4-methyl-5-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyridin-2-yl)piperazine (0.111 g, 0.27 mmol), 2-chloro-7-methylpyrrolo[2,1-f][1,2,4]triazin-4(3H)-one (0.05 g, 0.27 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II) (0.021 g, 0.03 mmol) and Cs_2CO_3 (0.266 g, 0.82 mmol) were stirred at 100 °C for 1.5 h. LC/MS indicated reaction completion. The residue was purified by reverse phase HPLC (acetonitrile/water w/ 0.1% TFA) using an Atlantis Prep T3 OBD column to give 2-(4-(6-((3R,5S)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-7-methylpyrrolo[2,1-f][1,2,4]triazin-4(3H)-one (0.012 g, 10.37 %). *m/z*: 429 $[M+H]^+$. ^{1}H NMR (500 MHz, DMSO, 20°C) δ ppm 1.04 (6H, d), 2.26 (4H, m), 2.48 (3H, s), 2.52 - 2.54 (2H, m), 2.62 - 2.66 (1H, m), 2.75 (2H, ddd), 4.20 (2H, dd), 6.36 - 6.46 (1H, m), 6.79 (1H, s), 6.87 (1H, d), 7.51 (1H, d), 7.98 (1H, s), 8.07 (1H, d).

[8] Mastalerz, H.; Vyas, D.M.; Trainor, G.L.; Gavai, A.V. Pyrrolotriazine Compounds Useful As Kinase Inhibitors And Methods Of Treating Kinase-Associated Conditions Therewith. US 2007/0004731, January 4, 2007.



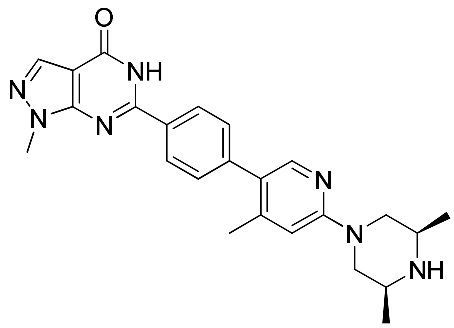
6-(4-Bromophenyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one

A 250 mL round bottom flask was charged with 5-amino-1-methyl-1H-pyrazole-4-carboxamide (1 g, 7.14 mmol), 4-bromobenzaldehyde (1.320 g, 7.14 mmol), pTsOH (2.71 g, 14.27 mmol) and toluene (71.4 mL). The flask was fixed with a Dean-Stark trap and heated to 140 °C for 18 h. LCMS confirmed the formation of the product. The reaction mixture was concentrated in vacuo leaving an orange solid. This material was then suspended in 20 mL of 1 N NaOH and diluted with EtOAc. A solid remained suspended in the aqueous layer. The aqueous layer was drained into a flask and the organic layer was washed twice more with water (total aqueous volume: 200 mL). The aqueous layers were all combined and filtered leaving a peach solid (690 mg). The organic layer was concentrated in vacuo leaving a yellow solid (1.065 g). LCMS showed this material to be a 50:50 mixture of product and bi-product. This material was recrystallized out of hot MeCN and the precipitate was collected via filtration leaving a peach solid (137 mg). The two solids were combined to give 6-(4-bromophenyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (827 mg). m/z: 305 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 12.42 (s, 1 H) 7.98 - 8.21 (m, 3 H) 7.77 (d, J=8.59 Hz, 2 H) 3.96 (s, 3 H).



1-Methyl-6-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one

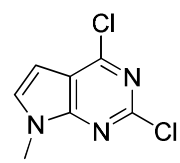
To a 25 mL sealed tube was addd 6-(4-bromophenyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (1 g, 3.28 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (0.999 g, 3.93 mmol), and $PdCl_2$(dppf) (0.240 g, 0.33 mmol) in DMF (10 mL) to give a brown suspension. Potassium acetate (0.643 g, 6.55 mmol) was added and the mixture was stirred at 80 °C overnight. The precipitate was filtered off. The filtrate was concentrated in vacuo and subjected to column chromatography eluted with EtOAc and petroleum ether to give 1-methyl-6-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (0.3 g, 26%).



Compound 24

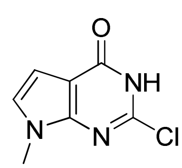
<u>6-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one</u>

In a 25 mL sealed tube was added 1-methyl-6-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (300 mg, 0.85 mmol), (3S,5R)-1-(5-bromo-4-methylpyridin-2-yl)-3,5-dimethylpiperazine (242 mg, 0.85 mmol), and tetrakis(triphenylphosphine)palladium(0) (98 mg, 0.09 mmol) in dioxane (5 mL) and H_2O (1 mL) to give a yellow suspension. Sodium carbonate (181 mg, 1.70 mmol) was added. The mixture was stirred at 100 °C overnight. The precipitate was filtered off, and the filtrate was concentrated. The product was purified by reverse phase HPLC (Xbridge C18 19*150 mm; mobile phase, water with 0.1% formic acid and CH_3CN, 4% CH_3CN up to 27% in 10 min, 27% CH_3CN up to 100% in 1 min, down to 4% in 1 min; Detector, 254/220 nm) to give the title compound (30 mg, 8 %). *m/z*: 429 $[M+H]^+$. ^1H NMR (400 MHz, D_2O) δ ppm 1.29 (6H, d), 1.78 (3H, s), 2.58 (2H, t), 3.16 (2H, m), 3.46 (3H, s), 3.94 (2H, d), 6.33 (2H, d), 6.85 (1H, s), 6.96 (2H, d), 7.38 (2H, d), 7.52 (1H, s), 8.36 (1H, s).



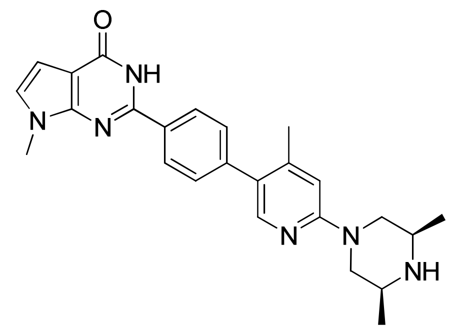
<u>2,4-Dichloro-7-methyl-7H-pyrrolo[2,3-d]pyrimidine</u>

A 250 mL round-bottomed flask was charged with 2,4-dichloro-7H-pyrrolo[2,3-d]pyrimidine (2.16 g, 11.49 mmol), 15% aq. NaOH (6.13 g, 22.98 mmol) (40mL) and DCM (40 mL). The flask was stirred vigorously and then N-benzyl-N,N-diethylethanaminium chloride (0.262 g, 1.15 mmol) was added. The mixture was stirred at room temperature overnight. The reaction mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (1x). The combined organics were dried (Na_2SO_4), filtered, and concentrated to give 2,4-dichloro-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (2.31 g, 99 %) as a yellow solid. *m/z*: 201.9 $[M+H]^+$. ^1H NMR (400 MHz, chloroform-*d*) δ ppm 3.80 (s, 3 H) 6.53 (d, *J*=3.51 Hz, 1 H) 7.12 (d, *J*=3.51 Hz, 1 H).



<u>2-Chloro-7-methyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one</u>

A mixture of 2,4-dichloro-7-methyl-7H-pyrrolo[2,3-d]pyrimidine (2.32 g, 11.48 mmol) and KOH (68.9 ml, 137.79 mmol) (2 M aq.) was stirred at reflux (bath temperature 130 °C) for 20 min. The reaction mixture was cooled in an ice bath for 20 min, and then HCl (138 ml, 137.79 mmol) (1 M aq.) was added dropwise with stirring. After 5 min, the precipitate was collected via filtration, washed with water, and air dried to give 2-chloro-7-methyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (2.108 g, 100 %) as a white solid. *m/z*: 183.9 $[M+H]^+$.



Compound 25

2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-7-methyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one

A mixture of 2-chloro-7-methyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (171 mg, 0.93 mmol), chloro(2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II) (73.4 mg, 0.09 mmol), (3S,5R)-3,5-dimethyl-1-(4-methyl-5-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyridin-2-yl)piperazine (760 mg, 0.93 mmol) and sodium carbonate (297 mg, 2.80 mmol) in 1,4-dioxane (8 mL) and water (2.00 mL) was stirred for overnight under N_2 at 80 °C. To the mixture was added water and ethyl acetate, the layers were separated, the water layer was extracted with ethyl acetate (50 mL x 2), and the organics were combined, dried (anhydrous Na_2SO_4), filtered and concentrated. The residue was purified on silica gel (eluted with 100% ethyl acetate to 35% methanol in ethyl acetate), the fractions were combined, concentrated, the residue was taken into 2 mL of methanol, and a fine solid started to appear. To the suspension was added 35 mL of hexane. After sonication the solid in hexane turned sticky. Then 2 mL of ether was added to the suspension, and after sonication the fine solid was collected by filteration and air dried to yield 2-(4-(6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-4-methylpyridin-3-yl)phenyl)-7-methyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (200 mg, 50.0 %) as an off white solid. *m/z*: 429.2 $[M+H]^+$. HRMS (ESI+) *m/z*: $[M+H]^+$ Calcd for $C_{25}H_{30}N_6O_1$ 429.2397; Found 429.2392. ^1H NMR (500 MHz, DMSO, 30 °C) δ ppm 1.04 (6H, d), 2.26 (5H, m), 2.76 (2H, ddd), 3.79 (3H, s), 4.19 (2H, dd), 6.48 (1H, d), 6.78 (1H, s), 7.12 (1H, d), 7.48 (2H, d), 7.98 (1H, s), 8.23 (2H, d), 12.01 (1H, m).