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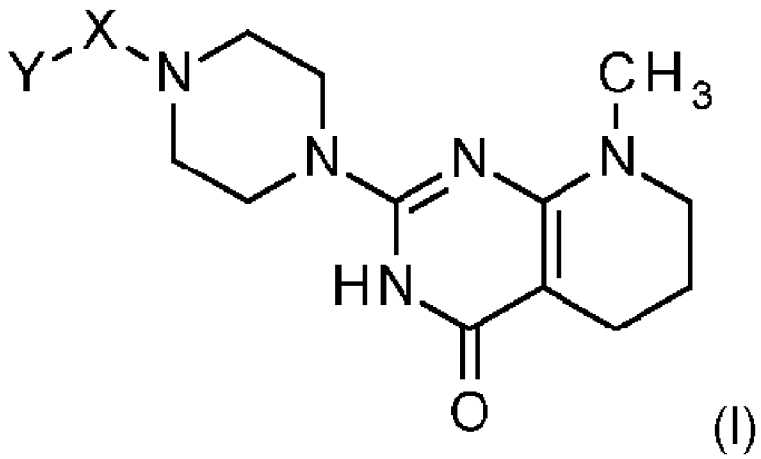
(54) Title: PYRIDO[2,3-d]PYRIMIDIN-4-ONE COMPOUNDS AS TANKYRASE INHIBITORS



(I)

(57) Abstract: Pyrido[2,3-d]pyrimidin-4-one compounds, formulations containing those compounds, and their use as tankyrase 1 and 2 inhibitors Formula (I).

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— *with sequence listing part of description (Rule 5.2(a))*

PYRIDO[2,3-d]PYRIMIDIN-4-ONE COMPOUNDS AS TANKYRASE INHIBITORS

Wnt signaling triggers three intracellular signaling cascades which include the β-catenin-mediated canonical pathway, the non-canonical planar cell polarity and the Wnt/calcium pathway. The evolutionarily conserved canonical Wnt signaling pathway regulates many cellular processes including cell proliferation, differentiation, adhesion and maintenance. The canonical pathway, which regulates β-catenin protein levels within cells, is initiated when Wnt ligands bind to cell surface Frizzled and lipoprotein receptor-related protein (LRP)5/6 co-receptors, which in turn promote the displacement of the kinase GSK3 from the APC/Axin/GSK-3 (adenomatous polyposis coli (APC), Axin, glycogen synthase kinase 3α/β (GSK3)) destruction complex. In the presence of Wnt binding (On-state), Dishevelled (a protein in the Wnt pathway) is activated which, in turn, recruits GSK3 away from the destruction complex leading to the accumulation of cytosolic β-catenin, translocation of β-catenin to the nucleus, interaction with T-cell factor/lymphoid enhancer factor (TCF/LEF) family transcription factors and transcription of canonical Wnt pathway responsive genes. In the absence of Wnt ligands (Off-state), cytosolic β-catenin is constitutively phosphorylated and targeted for ubiquitination and degradation by the proteasome.

The two highly homologous human tankyrase isoforms, tankyrase 1 and 2 (TNKS1 and TNKS2) are members of the poly(ADP-ribose)polymerase (PARP) enzyme family that catalyze the post-translational modification of proteins using β NAD+ as a substrate to successively add ADP ribose moieties onto target proteins (parylation or parsylation). One of the protein substrates for tankyrases is Axin, a concentration-limiting component of the β- catenin destruction complex; parsylation marks Axin for degradation and tankyrase inhibition leads to Axin stabilization, Wnt signaling inhibition and β catenin degradation.

Wnt signaling pathway activating mutations are found in a broad range of cancers and are believed to contribute to tumor initiation, maintenance, and/or progression. Therefore, inhibition of tankyrase activity appears to be a promising approach in the treatment of cancers such as colorectal cancer, gastric cancer, liver cancer, breast cancer (including triple negative breast cancer), ovarian cancer, medulloblastoma, melanoma,

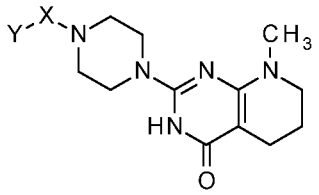
lung cancer (including non-small cell lung cancer), pancreatic cancer, prostate cancer, glioblastoma, T-cell lymphoma, T-lymphoblastic lymphoma, T-cell acute lymphocytic leukemia (T-ALL)), mantle cell lymphoma, multiple myeloma, chronic myeloid leukemia, and acute myeloid leukemia.

Considerable efforts have been made to identify pharmaceutical agents that inhibit the canonical Wnt/β-catenin signaling pathway. TNKS1 and TNKS2 inhibitors such as WO 2013/117288 are known.

Despite WO 2013/117288, there is a need to find compounds having TNKS1 and TNKS2 inhibitory activity. There is a further need to find compounds having selective inhibition of TNKS1 and TNKS2 over other PARPs.

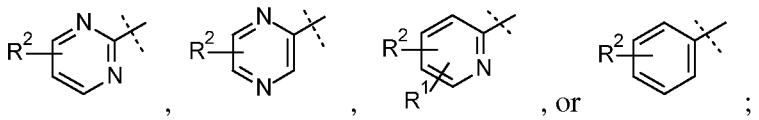
Figure 1 is a representative XRPD pattern for 8-Methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one 4-methylbenzenesulfonic acid salt, the compound of Example 6.

One aspect of the present invention are compounds of Formula I:



wherein:

Y is:



X is $-CH_2-$, $-C(CH_3)H-$, or $-C(CH_2CH_3)H-$;

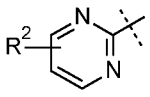
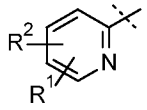
R^1 is hydrogen, hydroxy, or halo;

R^2 is hydrogen, halo, -CN, $-CH_3$, CF_3, or $-OCH_3$;

or a pharmaceutically acceptable salt thereof.

Preferably, a further aspect of the present invention provides compounds of Formula I wherein:

Y is:



or ;

X is $-CH_2-$, $-C(CH_3)H-$, or $-C(CH_2CH_3)H-$;

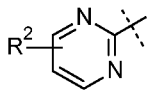
R^1 is hydrogen, hydroxy, or halo;

R^2 is hydrogen, halo, -CN, $-CH_3$, CF_3, or $-OCH_3$;

or a pharmaceutically acceptable salt thereof.

Preferably, another aspect of the present invention provides compounds of Formula I wherein:

Y is


;

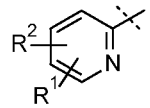
X is $-CH_2-$, $-C(CH_3)H-$, or $-C(CH_2CH_3)H-$;

R^2 is hydrogen, halo, -CN, $-CH_3$, CF_3, or $-OCH_3$;

or a pharmaceutically acceptable salt thereof.

Another preferred aspect of the present invention provides compounds of Formula I wherein:

Y is:


;

X is $-CH_2-$, $-C(CH_3)H-$, or $-C(CH_2CH_3)H-$;

R^1 is hydrogen, hydroxy, or halo;

R^2 is hydrogen, halo, -CN, $-CH_3$, CF_3, or $-OCH_3$;

or a pharmaceutically acceptable salt thereof.

A preferred aspect of the present invention is a compound:

8-Methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof;

8-Methyl-2-[4-(1-pyrimidin-2-ylethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof;

2-[4-[(4-Chloropyrimidin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof; or 2-[4-[(4-methoxypyrimidin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof.

Another preferred aspect of the present invention is a compound: 2-[4-[(3-Bromo-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof; 2-[4-[(3-Chloro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof; 2-[4-[(3-Fluoro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof; or 2-[[4-(8-Methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazin-1-yl]methyl]pyridine-3-carbonitrile, or a pharmaceutically acceptable salt thereof.

A further aspect of the present invention is a pharmaceutical composition comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier.

Still a further aspect of the present invention provides a method of inhibiting Tankyrase 1 and 2 in a cancer patient in need thereof, comprising administering a therapeutically effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof, to said patient.

Another aspect of the present invention provides a method of treating a cancer which is colorectal cancer, gastric cancer, liver cancer, breast cancer, triple negative breast cancer, ovarian cancer, medulloblastoma, melanoma, lung cancer, non-small cell lung cancer, pancreatic cancer, prostate cancer, glioblastoma, T-cell lymphoma, T-lymphoblastic lymphoma, T-cell acute lymphocytic leukemia (T-ALL), mantle cell lymphoma, multiple myeloma, chronic myeloid leukemia, or acute myeloid leukemia in a patient comprising administering to a patient in need thereof a therapeutically effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof.

A still further aspect of the present invention provides a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in therapy.

Another aspect of the present invention provides a compound of Formula I, or a pharmaceutically acceptable salt thereof, for use in the treatment of a cancer which is colorectal cancer, gastric cancer, liver cancer, breast cancer, triple negative breast cancer, ovarian cancer, medulloblastoma, melanoma, lung cancer, non-small cell lung cancer, pancreatic cancer, prostate cancer, glioblastoma, T-cell lymphoma, T-lymphoblastic lymphoma, T-cell acute lymphocytic leukemia (T-ALL), mantle cell lymphoma, multiple myeloma, chronic myeloid leukemia, or acute myeloid leukemia.

A further aspect of the present invention provides use of a compound of Formula I, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for treatment of a cancer which is colorectal cancer, gastric cancer, liver cancer, breast cancer, triple negative breast cancer, ovarian cancer, medulloblastoma, melanoma, lung cancer, non-small cell lung cancer, pancreatic cancer, prostate cancer, glioblastoma, T-cell lymphoma, T-lymphoblastic lymphoma, T-cell acute lymphocytic leukemia (T-ALL), mantle cell lymphoma, multiple myeloma, chronic myeloid leukemia, or acute myeloid leukemia.

The term “patient” means mammal and “mammal” includes, but is not limited to, a human.

The term “triple negative breast cancer” refers to a breast cancer characterized by a tumor sample having tested negative for estrogen receptors (ER), progesterone receptors (PR), and hormone epidermal growth factor receptors 2 (HER2/neu).

"Therapeutically effective amount" or "effective amount" means the dosage of a compound, or pharmaceutically acceptable salt thereof, or pharmaceutical composition containing a compound, or pharmaceutically acceptable salt thereof, necessary to inhibit Wnt/β-catenin signaling pathway in a cancer patient, and either destroy the target cancer cells or slow or arrest the progression of the cancer in a patient. Anticipated dosages of a compound or a pharmaceutically acceptable salt thereof are in the range of 0.1 to 200 mg/patient/day. Preferred dosages are anticipated to be in the range of 1 to 175 mg/patient/day. Most preferred dosages are anticipated to be in the range of 5 to 150 mg/patient/day. The exact dosage required to treat a patient and the length of treatment time will be determined by a physician in view of the stage and severity of the disease as well as the specific needs and response of the individual patient. Although expressed as

dosage on a per day basis, the dosing regimen may be adjusted to provide a more optimal therapeutic benefit to a patient and to manage and/or ameliorate undesirable pharmacodynamic effects. In addition to daily dosing, dosing every other day (Q2D); every other day over a five day period followed by two days without dosing (T.I.W.); or every third day (Q3D) may be appropriate.

The terms "treatment," "treat," and "treating," are meant to include the full spectrum of intervention for the cancer from which the patient is suffering, such as administration of the active compound to alleviate to slow or reverse one or more of the symptoms and to delay progression of the cancer even if the cancer is not actually eliminated. The patient to be treated is a mammal, in particular a human being.

A compound of the present invention is preferably formulated as a pharmaceutical composition using a pharmaceutically acceptable carrier and administered by a variety of routes. Preferably, such compositions are for oral administration. Such pharmaceutical compositions and processes for preparing them are well known in the art. *See, e.g.*, REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY (A. Gennaro, *et al.*, eds., 19th ed., Mack Publishing Co., 1995). In a particular embodiment, the pharmaceutical composition comprises 8-methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-4(3H)-one or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier and optionally other therapeutic ingredients particularly for treatment of a specific cancer type.

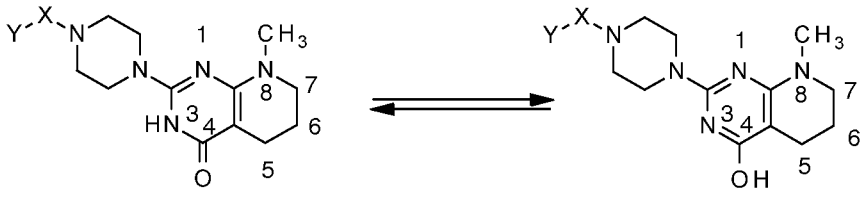
A compound of the present invention is capable of reaction with a number of inorganic and organic acids to form pharmaceutically acceptable acid addition salts. Such pharmaceutically acceptable salts and common methodology for preparing them are well known in the art. *See, e.g.*, P. Stahl, *et al.*, HANDBOOK OF PHARMACEUTICAL SALTS: PROPERTIES, SELECTION AND USE, (VCHA/Wiley-VCH, 2002); S.M. Berge, *et al.*, "Pharmaceutical Salts, " *Journal of Pharmaceutical Sciences*, Vol. 66, No. 1, January 1977.

A compound of the present invention, such as Example 1, is named: 8-methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-4(3H)-one (IUPAC) ; and may also be named: pyrido[2,3-d]pyrimidin-4(3H)-one,

5,6,7,8-tetrahydro-8-methyl-2-[4-(2-pyrimidinylmethyl)-1-piperazinyl]- (CAS); and other names may be used to unambiguously identify a compound of the present invention.

It will be understood compounds of Formula I are depicted as a single stereoisomer. A particular defined substituent may give rise to a chiral center affording a racemic mixture or two stereoisomers. As used herein, unless otherwise designated, references to a specific compound are meant to include individual stereoisomers and racemic mixtures including the named compound. Specific stereoisomers can be prepared by stereospecific synthesis using enantiomerically pure or enriched starting materials. The specific stereoisomers of either starting materials, intermediates, or racemic mixtures can be resolved by techniques well known in the art, such as those found in Stereochemistry of Organic Compounds, E. I. Eliel and S. H. Wilen (Wiley 1994) and Enantiomers, Racemates, and Resolutions, J., Jacques, A. Collet, and S. H. Wilen (Wiley 1991), including chromatography on chiral stationary phases, enzymatic resolutions, or fractional crystallization or chromatography of diastereomers formed for that purpose, such as diastereomeric salts. Where a chiral compound is isolated or resolved into its isomers, but absolute configurations or optical rotations are not determined, the isomers are arbitrarily designated as isomer 1 and isomer 2 corresponding to the order each elutes from chiral chromatography and if chiral chromatography is initiated early in the synthesis, the same designation is applied to subsequent intermediates and examples.

One of ordinary skill in the art will recognize the compounds of Formula I can exist in tautomeric equilibrium. For illustrative purposes, the equilibrium is shown below:



For convenience, the 4-oxo form is depicted in Formula I, and the corresponding nomenclature is used throughout this specification. However, such depictions include the corresponding tautomeric hydroxy form.

The compounds employed as initial starting materials in the synthesis of compounds of the present invention are well known and, to the extent not commercially available, are readily synthesized using specific references provided, by standard procedures commonly employed by those of ordinary skill in the art, or are found in general reference texts.

Examples of known procedures and methods include those described in general reference texts such as Comprehensive Organic Transformations, VCH Publishers Inc, 1989; Compendium of Organic Synthetic Methods, Volumes 1-10, 1974-2002, Wiley Interscience; Advanced Organic Chemistry, Reactions Mechanisms, and Structure, 5th Edition, Michael B. Smith and Jerry March, Wiley Interscience, 2001; Advanced Organic Chemistry, 4th Edition, Part B, Reactions and Synthesis, Francis A. Carey and Richard J. Sundberg, Kluwer Academic / Plenum Publishers, 2000, etc., and references cited therein.

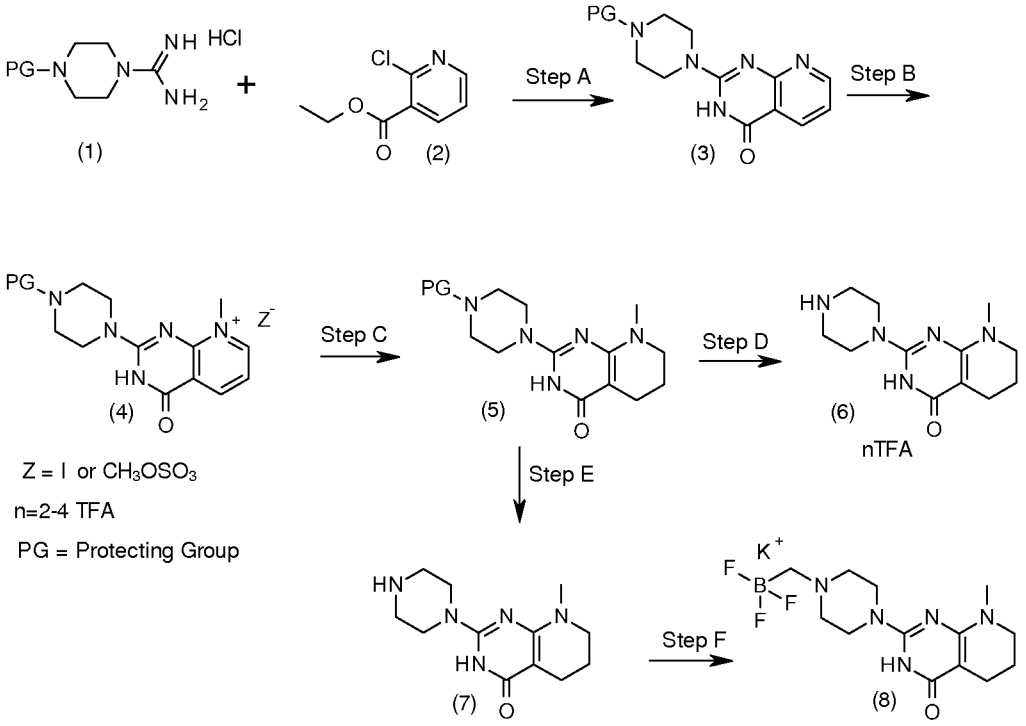
Additionally, certain intermediates described in the following schemes may contain one or more nitrogen protecting groups. The variable protecting group may be the same or different in each occurrence depending on the particular reaction conditions and the particular transformations to be performed. The protection and deprotection conditions are well known to the skilled artisan and are described in the literature (See for example "*Greene's Protective Groups in Organic Synthesis*", Fourth Edition, by Peter G.M. Wuts and Theodora W. Greene, John Wiley and Sons, Inc. 2007).

Abbreviations used herein are defined according to *Aldrichimica Acta*, Vol. 17, No. 1, 1984. Other abbreviations are defined as follows: "APC" refers to adenomatous polyposis coli; "BID" refers to twice daily dosing; "biotinylated NAD+" refers to 6-biotin-17-nicotinamide-adenine-dinucleotide; "BOC" refers to refers to *tert*-butyloxycarbonyl; "DCM" refers to dichloromethane; "DMF" refers to dimethylformamide; "DMAP" refers to 4-dimethylaminopyridine; "DMEM" refers to Dulbecco's Modified Eagle's Medium; "DMSO" refers to dimethyl sulfoxide; "DPBS" refers to Dulbecco's Phosphate Buffered Saline; "DTT" refers to dithiothreitol; "EDCI"

refers to 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; "EGFP" refers to Enhanced Green Fluorescent Protein; "EtOAc" refers to ethyl acetate; "FBS" refers to Fetal Bovine Serum; "Flag tag" refers to Flag peptide DYKDDDDK, N-terminus to C-terminus (SEQ ID NO: 6); "HEK" refers to human embryonic kidney; "HEPES" refers to 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; "HOAc" refers to acetic acid; "HRP" refers to horseradish peroxidase; "IPAm" refers to isopropylamine; "MEM" refers to Minimum Essential Medium; "MeOH" refers to methanol; "MOI" refers to multiplicity of infection; "NCBI" refers to National Center for Biotechnology Information; "PBS" refers to Phosphate Buffered Saline; "RPMI" refers to Roswell Park Memorial Institute; "SCX" refers to a purification column of strong cation exchange phenyl sulfonic acid bound to silica; "SCX-2" refers to a purification column of strong cation exchange propylsulfonic acid bound to silica; "SFC" refers to supercritical fluid chromatography; "TBS" refers to Tris buffered saline; "THF" refers to tetrahydrofuran; "TBME" refers to *tert*-butyl methyl ether; "TMB peroxidase" refers to 3,3',5,5'-tetramethylbenzidine; Tris" refers to tris(hydroxymethyl)aminomethane; and "X-Phos" refers to 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl.

The compounds of the present invention, or salts thereof, may be prepared by a variety of procedures known in the art, some of which are illustrated in the Schemes, Preparations, and Examples below. The specific synthetic steps for each of the routes described may be combined in different ways, or in conjunction with steps from different schemes, to prepare compounds of Formula I, or pharmaceutically acceptable salts thereof. The products of each step in the schemes below can be recovered by conventional methods well known in the art, including extraction, evaporation, precipitation, chromatography, filtration, trituration, and crystallization. In the schemes below, all substituents unless otherwise indicated, are as previously defined. The reagents and starting materials are readily available to one of ordinary skill in the art.

Scheme 1



Scheme 1 depicts the formation of 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one (6) as a TFA salt, the neutral material (7) and the trifluoroboranate methyl salt of (7).

In Step A, a protected 2-piperazine-1-yl-3H-pyrido[2,3-d]pyrimidin-4-one (3) is obtained from cyclization of a protected piperazine-1-carboxamide hydrochloride salt (1) with ethyl 2-chloronicotinate (2). The reaction proceeds in an inert solvent, such as DMF, in the presence of a strong base, such as potassium *tert*-butoxide, at a temperature of 50 – 120 °C. The preferred protecting group is a *tert*-butyloxycarbonyl but other carbamate protecting groups could be used.

In Step B, a protected 2-piperazine-1-yl-3H-pyrido[2,3-d]pyrimidin-4-one (3) is methylated using methyl iodide to give the quaternary salt, protected 8-methyl-2-piperazin-1-yl-3H-pyrido[2,3-d]pyrimidin-8ium-4-one iodide (4) (Z is I, Scheme 1). The reaction proceeds in an autoclave in an inert solvent, such as THF or dioxane, at a temperature of 50 – 120 °C for 4 to 24 h. Alternatively the methyl sulfate salt can be

formed by adding dimethylsulfate to compound (3) in a polar aprotic solvent such as DMF with heating at about 60-80 °C. The solution is transferred at room temperature to a vessel containing TMBE to precipitate the product (4) for Z is CH_3OSO_3.

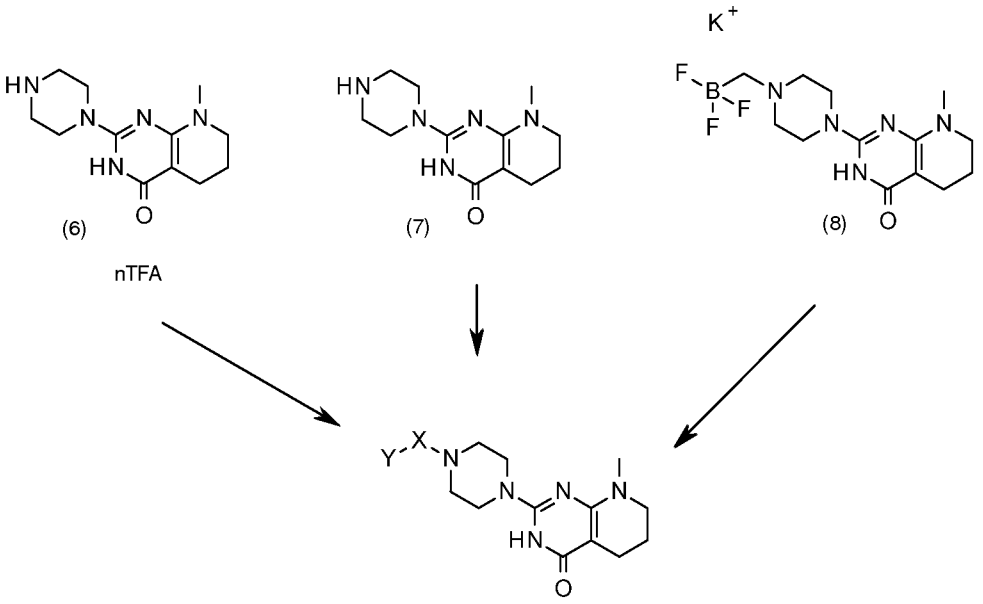
In Scheme 1, Step C, the quaternary salt (4, Z is I) is reduced to provide protected 8-methyl-2-piperzin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one (5). The reduction can be accomplished using a reducing agent, such as sodium cyanoborohydride in a mixture of DMF/TFA of about 10/1. The TFA and reducing agent are added at a temperature of 0 – 5 °C with the temperature not allowed to rise above 15 °C. The reaction is then allowed to go about 12 to 24 h at RT. Additional amounts of TFA and reducing agent can be added with cooling to drive the reaction to completion.

Alternatively, Step C can be accomplished by reducing the methyl sulfate salt (4, Z is CH_3OSO_3) with a reducing agent such as platinum oxide and hydrogenating at about 155 psi in a polar solvent such as MeOH to give compound (5).

In Step D, the piperazinyl ring is deprotected to provide 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one (6). Acidic conditions for removal of protecting groups such as BOC are well known in the art. Preferred conditions use a 1/1 mixture of DCM and TFA at RT for a period of 2 to 24 h to give the product as a TFA salt with 2-4 equivalents of TFA present. The salt form is not characterized but is calculated by weight.

Step E shows the deprotection of the protecting group as discussed above to give the TFA salt which can be neutralized with an ion exchange resin or an SCX column using ammonia/MeOH to give the neutral material (7). The nitrogen of the pyrazine ring can be converted to the methyl trifluoroboranate salt using potassium bromomethyl trifluoroborate in a solvent such as THF to give a salt form as shown in Step F, compound 8.

Scheme 2



I

Scheme 2 illustrates the formation of compounds of Formula I from the salt form (6), the neutral material (7), or the methyl trifluoro boranate salt (8).

There are many ways to form compounds of Formula I from the intermediates (6), (7), and (8) such as alkylation, reductive alkylation, or Suzuki couplings depending on the availability or synthesis of appropriate aldehydes or ketones for reductive alkylations, appropriate halide reagents for alkylations or boronic acid reagents for Suzuki couplings. The TFA amine salt (6) or neutral amine (7) can be used in reductive alkylations or alkylation to give compounds of Formula I. Reductive alkylations are well known in the art and involve the reaction of an aldehyde with an amine using a reducing agent such as sodium cyanoborohydride in an appropriate solvent such as DMF at room temperature or other reducing agents such as sodium triacetoxyborohydride in an appropriate solvent such as DCM at room temperature. A catalytic amount of methanol can be used if desired with DMF and sodium cyanoborohydride to push the reaction forward faster. An appropriate aryl methyl halide can be used to alkylate the TFA amine salt (6) or neutral material (7) using an appropriate organic base such as triethylamine or an inorganic base such as potassium carbonate in a solvent such as acetonitrile or DCM. Sodium iodide can be used in situ to convert a chloromethyl substrate to a more reactive iodo methyl

substrate to complete the alkylation of the appropriate pyrazine, pyridine, or pyrimidine. The reactions can be stirred at room temperature for 1-3 days to give compounds of Formula I.

Compound (8) can be coupled with aryl halides under Suzuki palladium catalyzed cross coupling conditions to form N-methyl heteroaryl substituted products. Compound 8 the methyl trifluoroboranate salt, provides another variation to prepare compounds of Formula I. The skilled artisan will recognize that there are a variety of conditions useful for facilitating such cross-coupling reactions. Accordingly, a suitable palladium reagent includes palladium(II) acetate and a suitable organophosphorus reagent such as X-phos with a base such as cesium carbonate, sodium carbonate, potassium carbonate, or potassium phosphate tribasic monohydrate. Other suitable organophosphorus and palladium reagents include bis(triphenylphosphine)palladium(II) chloride, tris(dibenzylideneacetone)dipalladium (0) with tricyclohexylphosphine, (1,1'-bis(diphenylphosphino)ferrocene)palladium(II) chloride, or palladium tetrakistriphenylphosphine.

In an optional step, a pharmaceutically acceptable salt of a compound of Formula I, such as a hydrochloride salt, can be formed by reaction of a free base compound of Formula I with an appropriate acid in a suitable solvent under standard conditions. Additionally, the formation of such salts can occur simultaneously upon deprotection of a nitrogen protecting group.

The following preparations and examples further illustrate the invention. Unless noted to the contrary, the compounds illustrated herein are named and numbered using Accelrys® Draw version 4.0 (Accelrys, Inc., San Diego, CA), IUPACNAME ACDLABS, or ChemDraw® Ultra 12.0.

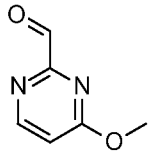
General Method Description of Reverse Phase Purification

System: Agilent 1200 LCM/MS equipped with Mass Selective Detector (MSD) mass spectrometer and Leap autosampler/fraction collector; Column: 75 × 30 mm Phenomenex Gemini-NX, 5μ particle size column with 10 × 20 mm guard; Solvent System: A: 10 mM ammonium bicarbonate, pH 10 as the aqueous phase, B: ACN as the organic phase; Flow rate: 85 mL/min; Method: The gradient for each method is designated in the method name as the beginning %B-ending %B (e.g. as in High pH 13-

48). The gradient is set as follows: 0-1 min (hold at begin %B), 1-8 min (gradient from begin %B to end %B), 8-8.1 min (ramp from end %B to 100%B), 8.1-9 min (hold 100% B).

Preparation 1

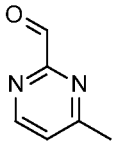
4-Methoxypyrimidine-2-carbaldehyde



Combine 4-methoxypyrimidine-2-methanol (300 mg, 2.14 mmol) and oxalyl chloride (1 mL, 2.0 M in DCM) in DCM (5 mL) and cool to -78 °C. Add DMSO (0.33 mL, 4.71 mmol) and stir the mixture for 2 h. Add triethylamine (0.66 mL, 4.71 mmol) and warm the mixture to room temperature overnight. Add water (5 mL), stir 1 h and extract with DCM (2 × 50 mL). Dry the combined organic extracts over sodium sulfate, filter, and concentrate the filtrate under reduced pressure to give a light brown solid and used without further purification. ^{1}H NMR(400 MHz, $CDCl_3$) δ aldehyde peak 9.94 (s, 1H), remaining peaks not assignable due to the mixture of more than one compound.

Preparation 2

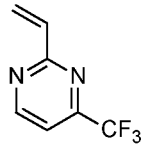
4-Methylpyrimidine-2-carbaldehyde



Prepare 4-methylpyrimidine-2-carboxaldehyde essentially as described in Preparation 1 using (4-methylpyrimidin-2-yl)methanol to obtain the title compound and use without further purification. ^{1}H NMR, 400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.79 (d, 1H, J = 5.2 Hz), 7.33 (d, 1H, J = 4.8 Hz), 2.65 (s, 3H)

Preparation 3

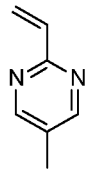
4-(Trifluoromethyl)-2-vinyl-pyrimidine



Combine 2-chloro-4-(trifluoromethyl)pyrimidine (250 mg, 1.37 mmol), potassium vinyltrifluoroborate (184 mg, 1.37 mmol), cesium carbonate (1.34 g, 4.11 mmol), THF (5 mL) and water (0.5 mL) in a vial. Degas with a stream of nitrogen for 1 min. Add bis(triphenylphosphine)palladium(II) chloride (48 mg, 0.069 mmol), seal the vial, and heat the mixture at 85 °C for 3 days. Cool, dilute the mixture with DCM, filter through diatomaceous earth, and concentrate the filtrate under reduced pressure to give the title compound (0.200 g, 84%) which is used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (d, 1H, J = 5.0 Hz), 7.43 (d, 1H, J = 5.0 Hz), 6.94 (ABX, 1H, J_{AX} = 17.3, J_{BX} = 10.47), 6.74 (ABX, 1H, J_{AB} = 1.54, J_{AX} = 17.3), 5.84 (ABX, 1H, J_{AB} = 1.54, J_{BX} = 10.47).

Preparation 4

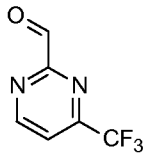
5-Methyl-2-vinyl-pyrimidine



Prepare the title compound essentially as described in Preparation 3 using 2-choro-5-methylpyrimidine and use without further purification (220 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 2H), 6.73 (ABX, 1H, J_{AX} = 17.4, J_{BX} = 10.7), 6.41 (ABX, 1H, J_{AB} = 1.8, J_{AX} = 17.4), 5.54 (ABX, 1H, J_{AB} = 1.8, J_{BX} = 10.7), 2.16 (s, 3H).

Preparation 5

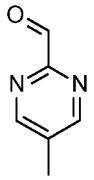
4-(Trifluoromethyl)pyrimidine-2-carbaldehyde



Combine 4-(trifluoromethyl)-2-vinyl-pyrimidine (200 mg, 0.84 mol) with sodium periodate (737 mg, 3.45 mmol) and osmium tetroxide, polymer bound (292 mg, 0.057 mmol) in dioxane (3 mL) and water(1 mL) and stir mixture at room temperature overnight. Dilute the mixture with EtOAc (5 mL) and water (5 mL). Filter the mixture through glass wool and separate the layers. Dry the organic layer over magnesium sulfate, filter, and concentrate the filtrate to give the title compound (40 mg, 20%). Use the crude material without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ aldehyde 10.16 (s, 1H), remaining peaks not assignable due to the mixture of more than one compound.

Preparation 6

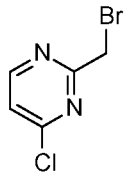
5-Methylpyrimidine-2-carbaldehyde



Prepare 5-methylpyrimidine-2-carbaldehyde essentially as described in Preparation 5, using 2-chloro-5-methyl-pyrimidine to give the title compound (90 mg, 44%) and use without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.79 (s, 2H), 2.42 (s, 3H).

Preparation 7

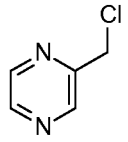
2-(Bromomethyl)-4-chloro-pyrimidine



Combine 2-methyl-4-chloropyrimidine (200 mg, 1.55 mmol), carbon tetrachloride (5 mL), N-bromosuccinimide (304 mg, 1.71 mmol), and benzoyl peroxide (38 mg, 1.6 mmol) in a vial, flush with nitrogen for 2 min, seal and heat at 80 °C overnight. Use the crude reaction mixture without further purification. GC-MS *m/e*: (^{79}BR/^{81}Br 205/207 (M^+).

Preparation 8

2-(Chloromethyl)pyrazine



Dissolve 2-pyrazinylmethanol (2.0 g, 18.1 mmol) in DCM (200 mL) and cool to 0 °C while stirring under nitrogen. Add thionyl chloride (4.63 mL, 63.6 mmol) drop wise over 10 min, and allow to warm to 25 °C. Stir at room temperature for 16 h. Concentrate the mixture and then dilute with DCM. Wash the crude solution with saturated $NaHCO_3$, dry over $MgSO_4$, filter, and concentrate. Dissolve the crude residue in DCM and purify by silica gel flash chromatography (hexane/EtOAc, 95:5 to 100% EtOAc gradient) to give the title compound as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.67 (s = 2H), 8.50-8.55 (m, 2H), 8.73 (s, 1H).

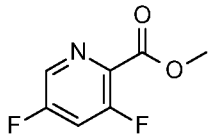
Preparation 9

2-(Bromomethyl)-3-chloro-pyrazine

Add together 2-chloro-3-methyl-pyrazine (5.0 g, 38.9 mmol), N-bromosuccinimide (6.92 g, 38.9 mmol), benzoyl peroxide (0.471 g, 1.94 mmol), and carbon tetrachloride (50 mL) and heat at reflux under nitrogen for 16 h. Cool the reaction mixture to 25 °C and dilute with hexanes. Filter the mixture, concentrate the liquid under reduced pressure, and purify by silica gel flash chromatography (hexane/EtOAc, 95:5 with gradient to 40:60) to give the title compound as a brown oil (3.93 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (s = 2H), 8.32 (d, J = 2.4 Hz, 1H), 8.47 (d, J = 2.4 Hz, 1H).

Preparation 10

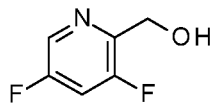
Methyl 3,5-difluoropyridine-2-carboxylate



Add together 3,5-difluoropyridine-2-carboxylic acid (1.4 g, 8.8 mmol) and DCM (30 mL) and cool to 0 °C. Add MeOH (3 mL), DMAP, (1.32 g, 10.6 mmol) and EDCI (2.1 g, 10.6 mmol). Allow mixture to warm to room temperature and stir overnight. Concentrate the reaction mixture under reduced pressure and purify the residue by chromatography on silica gel (elution with 10/1 petroleum ether/EtOAc) to give the title compound (1.03 g, 72%). LC-ES/MS m/z 174 $(M+H)^+$.

Preparation 11

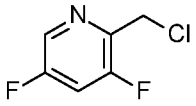
(3,5-Difluoro-2-pyridyl)methanol



Add together methyl 3,5-difluoropyridine-2-carboxylate (1.0 g, 4.6 mmol), THF (10 mL) and 2 M lithium borohydride in THF (15 mL, 23 mmoL). Stir the mixture at room temperature for 2 days. Concentrate under reduced pressure and purify the residue by silica gel chromatography eluting with DCM to give the title compound (0.448 g, 60%).

Preparation 12

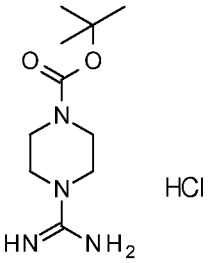
2-(Chloromethyl)-3,5-difluoropyridine



Add together (3,5-difluoro-2-pyridyl)methanol (200 mg, 1.4 mmol), DCM (4 mL), DMF (0.1 mL) and thionyl chloride (1 mL) and stir the mixture for 4 hours. Adjust the pH to about 7.0 with 4 M aqueous sodium bicarbonate and extract the mixture with DCM (3 × 50 mL). Combine the organic portions, dry over anhydrous sodium sulfate, filter, and concentrate to give the title compound (182 mg, 81%) as an orange oil.

Preparation 13

tert-Butyl 4-carbamimidoylpiperazine-1-carboxylate hydrochloride

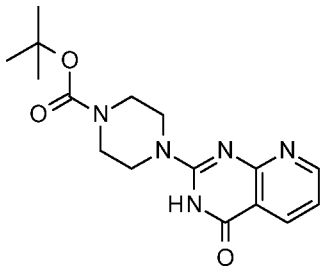


Charge a 10 L jacketed reactor with *tert*-butyl piperazine-1-carboxylate (1500 g, 8.054 mol) and DMF (4.05 L). Stir until a solution is obtained. Add 1H-pyrazole-1-carboxamide HCl (1180 g, 8.054 mol) followed by diisopropylamine (1041 g, 8.054 mol) over 15 min at 22 to 28 °C. Heat at 55 to 60 °C for 5 h and then cool to 20 °C. Transfer the reaction mixture to a 50 L jacketed reactor containing TBME (30 L) over 15 to 20 minutes and rinse the transfer lines with DMF (400 mL). Stir the suspension for 1 hour at 20 °C and then filter through three separate 26 cm Buchner funnels. Wash each product

cake with TMBE (2 × 1000 mL) and dry in a vacuum oven at 60 °C overnight to give the title compound (1898 g, 89%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (s, 9H), 3.42-3.50 (m, 4H), 3.33-3.42 (m, 4H), 7.77 (s, 4H).

Preparation 14

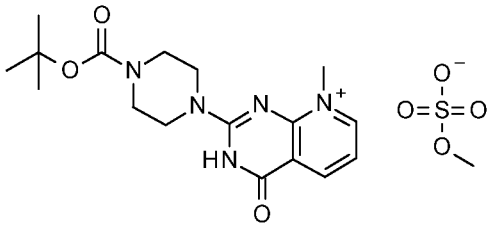
tert-Butyl 4-(4-oxo-3H-pyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate



Charge a 22 L round bottom flask with *tert*-butyl 4-carbamimidoylpiperazine-1-carboxylate hydrochloride (1897 g, 7.165 mol), DMF (10.1 L), and ethyl 2-chloronicotinate (1266 g, 6.824 mol). Add potassium *tert*-butoxide (1293 g, 11.52 mol) portion-wise over 55 min while allowing the temperature to rise gradually from 17 to 62 °C. Heat the reaction at 100 °C for 2.5 h. -Cool the reaction mixture to 20 °C and transfer to a 30 L jacketed reactor containing water (15.2 L) using DMF (350 mL) to rinse the reactor and transfer lines. Introduce 3 N hydrochloric acid (1.52 L) until a pH of 5 to 6 is obtained and stir the suspension for 2 hours at 20 °C. Collect the product by filtration through three separate 26 cm Buchner funnels and wash each cake with water (3 × 1.5 L). Combine the cakes, suspend them in water (15.0 L), and stir for 60 min at 20 °C. Collect the product by filtration through two separate 26 cm Buchner funnel and wash the cake with water (2 × 1.5 L). Dry the solid in a vacuum oven at 60 °C for 24 hours to give the title compound (1332 g, 59%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (s, 9H), 3.36-3.49 (m, 4H), 3.62-3.76 (m, 4H), 7.13-7.20 (m, 1H), 8.22-8.27 (m, 1H), 8.62-8.70 (m, 1H).

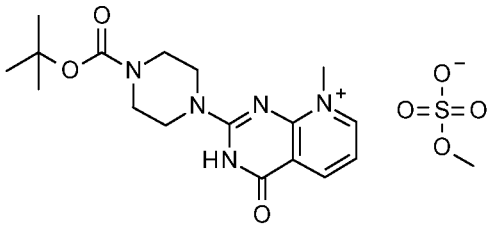
Preparation 15

tert-Butyl 4-(8-methyl-4-oxo-3H-pyrido[2,3-d]pyrimidin-8-ium-2-yl)piperazine-1-carboxylate, methyl sulfate



Charge a 20 L jacketed reactor with *tert*-butyl 4-(4-oxo-3H-pyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate (1331 g, 4.017 mol) and DMF (6.66 L). Add dimethylsulfate (583 g, 2.83 mol) over 5-10 min and observe a slight exotherm to 38 °C. Heat the solution to 63 to 73 °C for 30 minutes. Then introduce additional dimethylsulfate (50.7 g, 0.402 mol) and heat at 68 to 73 °C for 30 min. Sample of the reaction shows 2.0% starting material remaining. Add additional dimethylsulfate (50.7 g, 0.402 mol) and heat at 68 to 73 °C. Sample again to show 1.3% staring material remaining. Cool the reaction to 20 °C and transfer over 20 to 30 minutes to a 50 L jacket reactor containing TBME (26.6 L), *tert*-butyl 4-(8-methyl-4-oxo-3H-pyrido[2,3-d]pyrimidin-8-ium-2-yl)piperazine-1-carboxylate and seeds (1 g) using DMF (300 mL) to rinse the reactor and transfer lines. Stir the suspension overnight at 20 °C overnight and collect the product by filtration using three separate 26 cm Buchner funnels. Rinse each cake with TBME (3 × 1.3 L), Combine the three portions and suspend the material in TMBE (13.3 L), stir for 1 h at 20 °C, and collect the product on two separate 26 cm Buchner funnel. Wash each cake with TBME (3 × 2.0 L) and dry in a vacuum oven at 55 to 60 °C for 16 hours to give the title compound (1812 g, 98%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (s, 9H), 3.37 (s, 3H), 3.45-3.55 (m, 4H), 3.80-3.95 (m, 4H), 4.08 (s, 3H), 7.40-7.45 (m, 1H), 8.72-8.76 (m, 1H), 8.86-8.91 (m, 1H)

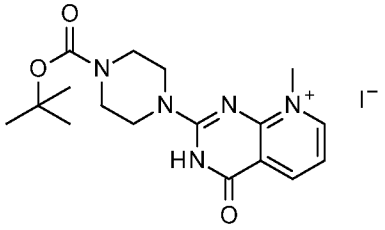
Preparation 15 Seed Crystal Formation



Charge a 250 mL reactor with *tert*-butyl 4-(4-oxo-3H-pyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate (15 g, 45.3 mmol) and dimethylformamide (75 mL). Add dimethylsulfate (6.28 g, 49.8 mmol) in one portion to the stirring mixture (observe slight exotherm to 34 °C). Heat the resulting solution at 68 to 73 °C for 3 hours. Add dimethylsulfate (0.57 g, 4.5 mmol) and stir at 68 to 73 °C for an additional 1 hour. Cool to 20 °C and transfer drop wise to a 1 L flask containing TBME (300 mL). Observe the sticky solid change to stirrable suspension, stir overnight at 20 °C and filter on a Buchner funnel. Wash the product cake with TBME (3 x 35 mL) and suspend in TBME (100 mL). Stir the suspension for 1 hour at 20 °C, filter on a Buchner funnel and wash the cake with TBME (2 x 35 mL). Dry the solids in a vacuum oven at 55 to 60 °C for 16 hours to give *tert*-Butyl 4-(8-methyl-4-oxo-3H-pyrido[2,3-d]pyrimidin-8-ium-2-yl)piperazine-1-carboxylate, methyl sulfate (18.84 g, 91% yield) (HPLC purity 95%).

Preparation 16

tert-Butyl 4-(8-methyl-4-oxo-3H-pyrido[2,3-d]pyrimidin-8-ium-2-yl)piperazine-1-carboxylate iodide

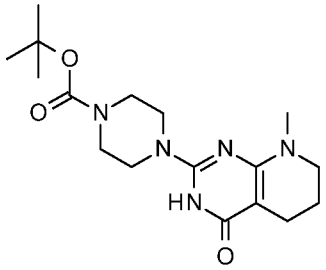


Combine *tert*-butyl 4-(4-oxo-3H-pyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate (115.43 g), THF (1.4 L), and methyl iodide (24 mL) in a 2 L Parr autoclave with mechanical stirrer. Seal the autoclave and stir the reaction with heating at 70 °C for 22 hours. Cool the reaction to room temperature and transfer the resulting bright yellow

slurry to a round bottom flask using MeOH. Concentrate the slurry and dry the resulting solid in a vacuum oven at 50-60 °C to give the title compound as a yellow solid (167.44 g, 100%). LC-ES/MS m/z 346.2 $[M+H]^+$, T_R = 1.16 min.

Preparation 17

tert-Butyl 4-(8-methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate



Charge a 2 gallon pressure vessel with *tert*-butyl 4-(8-methyl-4-oxo-3H-pyrido[2,3-d]pyrimidin-8-ium-2-yl)piperazine-1-carboxylate, methyl sulfate (881 g, 1.927 mol) and MeOH (4.9 L). Add platinum oxide (1% w/w, 8.81 g) and pressurize with hydrogen to 155 psi. Stir for 4 hours at 22-28 °C. Filter through a filter cartridge to collect the catalyst. Set aside the filtrate to be combined with the filtrate of a second hydrogenation. Repeat the reaction on another portion of *tert*-butyl 4-(8-methyl-4-oxo-3H-pyrido[2,3-d]pyrimidin-8-ium-2-yl)piperazine-1-carboxylate, methyl sulfate (925 g, 2.023 mol), MeOH (5.0 L) and platinum oxide (1% w/w, 9.25g) as before filter and combine the two filtrates and concentrate to an oily residue. Dissolve the residue in DCM (9.3 L) and wash with 0.1 N aqueous HOAc (2 × 2.0 L and 2 × 1.1L) and 0.5 M NaOH (8.0 L). Add water (4.0 L) and adjust to pH 6-7 with 0.1 N aqueous HOAc (about 1.9 L). Separate the layers and dry the organic layer over magnesium sulfate, filter, and concentrate to a volume of about 4.5 L and add EtOAc (16.0 L) to crystallize the product. Concentrate the suspension to a volume of about 4.5 L, cool to 0 to 5 °C and collect the product by filtration. Wash the cake with EtOAc (2 × 2.0 L and 2 x 1.1 L) and dry in a vacuum oven at 55 to 65 °C for 16 h to give the title compound (1185 g, 77%) as a white solid (very electrostatic). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.76-1.88 (m, 2H), 2.53 (t, J = 6.3 Hz, 2h), 3.04 (s, 3H), 3.20-3.26 (m, 2H), 3.40-3.52 (m, 4 H), 3.60-3.70 (m,

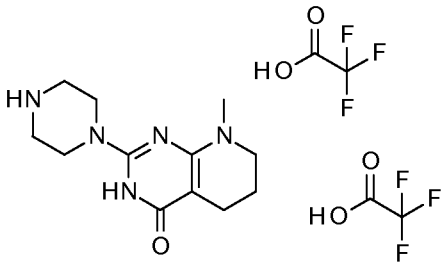
4H), 5.01 (s, 1H), 12.1-12.3 (br s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 20.00, 21.63, 28.81, 36.55, 44.99, 50.20, 80.38, 85.85, 152.38, 155.15, 160.14, 164.03. ESI/ MS m/z 350.0 $[M+H]^+$.

Preparation 17 Alternative Synthesis

Charge a round bottom flask with *tert*-butyl 4-(8-methyl-4-oxo-3H-pyrido[2,3-d]pyrimidin-8-ium-2-yl)piperazine-1-carboxylate iodide (167.44 g, 353.76 mmol) and DMF (815 mL) and cool in an ice bath to an internal temperature of 0-5 °C. Add trifluoroacetic acid (80.25 mL, 1.06 mol) at a rate to maintain the internal temperature below 15 °C (45 min). Cool the reaction to an internal temperature of 0-5 °C and add sodium cyanoborohydride (66.69 g, 1.06 mol) at a rate to maintain the temperature below 15 °C (about 50 min). Stir the reaction for 18 h while warming to 25 °C. Cool the reaction back down to 0-5 °C and add trifluoroacetic acid (80.25 mL, 1.06 mol) at a rate to maintain the temperature below 15 °C (10 min) followed by sodium cyanoborohydride (66.69 g, 1.06 mol) at a rate to maintain the temperature below 15 °C (10 min). Stir the reaction overnight while warming to 25 °C. Cool the reaction back down to 0-5 °C and add trifluoroacetic acid (26.6 mL, 0.345 mol) at a rate to maintain the temperature below 10 °C (5 min) followed by sodium cyanoborohydride (22.3 g, 0.345 mol) in two portions over 10 min. Stir overnight while warming to 25 °C. Fit a 12 L bucket with an overhead stirrer and charge it with sodium bicarbonate (297.18 g, 3.54 mol) and water (7.2 L). Add the crude reaction mixture to the bicarbonate solution using a separatory funnel over a period of 30 min and observe gas evolution. Stir the mixture for 2 hours and then let sit at 25 °C overnight. Collect the resulting solid by filtration and rinse with water and diethyl ether. Dry the solid in a vacuum oven at about 60 °C overnight to give the title compound (111.34 g, 90%) as a beige solid. LC-ES/MS m/z 350.0 $[M+H]^+$, T_R = 1.93 min.

Preparation 18

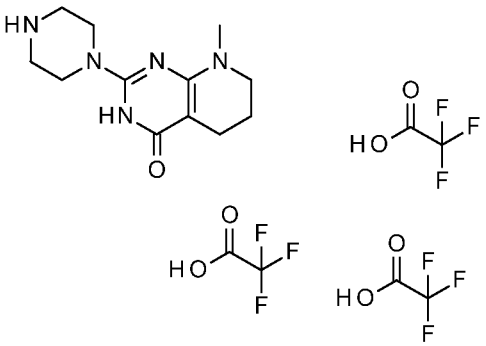
8-Methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) salt



Add together *tert*-butyl 4-(8-methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate (46.8 g, 133.9 mmol) and DCM (100 mL). Add trifluoroacetic acid (80 mL, 1.09 moles) over 15 min and stir at 25 °C overnight. Concentrate under reduced pressure, add DCM (200 mL), and re-concentrate under reduced pressure four times to give the title compound as an off white solid (69.685 g, 100% crude). LC-ES/MS m/z 250.0 $[M+H]^+$, T_R = 0.53 min.

Preparation 19

8-Methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one; tri-2,2,2-trifluoroacetic acid

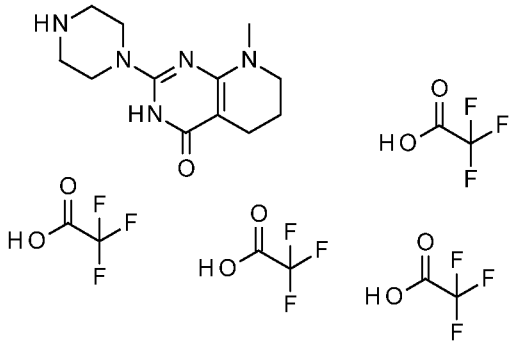


Add together *tert*-butyl 4-(8-methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate (196.0 g, 560.8 mmol) and DCM (783 mL). Cool the cloudy suspension in an ice bath to an internal temperature of 4 °C and add trifluoroacetic acid (254 mL) over 10 min while stirring. Stir for 42 hours at room temperature. Remove the volatiles under reduced pressure and dissolve in DCM.

Remove the volatiles under reduced pressure and dry under vacuum at 40 °C overnight to give the title compound (431.5 g, 99%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 8.96 (br s, 2H), 7.49 (br s, TFA/water, 9H), 3.78 (t, 4H, J = 5 Hz), 3.29 (t, 2H, J = 5 Hz), 3.15 (br s, 4H), 3.04 (s, 3H), 2.37 (t, 2H , J = 6.3 Hz), 1.76 (pt, 2H, J = 5.8 Hz

Preparation 20

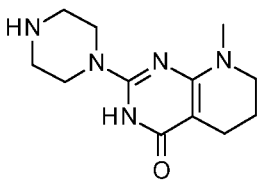
8-Methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one; tetra-2,2,2-trifluoroacetic acid



Add together *tert*-butyl 4-(8-methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate (0.29 g, 0.83 mmol), DCM (3 mL) and trifluoroacetic acid (3 mL). Stir the reaction at room temperature for 4 hrs. Concentrate the mixture and dissolve the residue in DCM (2x) and concentrate the mixture. Dry under vacuum for 1 hour to give the title compound (0.556 g, 95%) which is used without purification or characterization.

Preparation 21

8-Methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Charge a round bottom flask with *tert*-butyl 4-(8-methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazine-1-carboxylate (730 mg, 1.53 mmol), DCM (15 mL) and trifluoroacetic acid (15 mL) and stir the reaction at room temperature

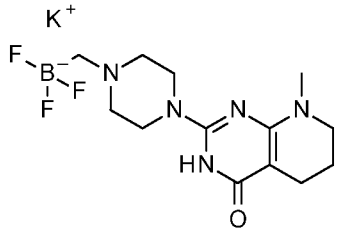
for 3 hours. Concentrate under reduced pressure, dissolve the residue in methanol, add ion-exchange resin Dowex® 50WX4-400 (5.5 g), and stir the mixture at room temperature overnight. Filter the mixture and wash the ion exchange resin with 7 M ammonia in methanol. Combine the filtrate and washings, and concentrate under reduce pressure to give the title compound as an orange solid (385 mg, 95%). LC-ES/MS m/z 250 $[M+H]^+$.

Alternate Preparation 21

Prewash a 10 g SCX-2 column with DCM (30 mL) and load with a solution of 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) (0.5 g, 1.05 mmol) in DCM (10 mL) and MeOH (1 mL). Wash the column with DCM (30 mL), followed by MeOH (30 mL), and elute with 2 M NH_3/MeOH (60 mL). Concentrate the fractions containing the product under reduced pressure to give the title compound (0.26 g, 100%).

Preparation 22

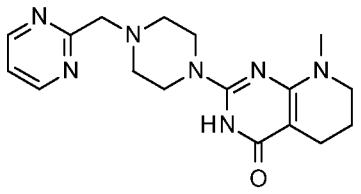
Potassium trifluoro-[[4-(8-methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl) piperazin-1-yl]methyl]boranuide



Dissolve 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one (250 mg, 1.00 mmol) in THF (4 mL) and add potassium bromomethyl trifluoroborate (208 mg, 1.00 mmol). Heat at 80 °C for 90 min and then concentrate under a stream of nitrogen. Add acetone (30 mL) and potassium bicarbonate (100 mg, 1.00 mmol) and stir at room temperature overnight. Remove the solids by filtration and concentrate the filtrate under reduced pressure to give the title compound (273 mg, 73%) as an off-white solid. ES/MS m/z 330 $[M-K^+]^-$.

Example 1

8-Methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Dissolve 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one; tri-2,2,2-trifluoroacetic acid (409 g, 531 mmol) in DCM (2.0 L) in a 5 L extraction funnel. Purge the funnel with nitrogen and equip it with an overhead mechanical stirrer. Add pyrimidine-2-carboxaldehyde (66.6 g, 616 mmol) and stir for 40 min. Then add sodium triacetoxyborohydride (225.1 g, 1060 mmol) portion wise and observe an exotherm from 20 to 36 °C immediately after the addition. Stir the reaction at room temperature overnight. Pour the reaction carefully into 1 M NaOH (2.32 L) at 20 °C in a 10 L reactor while controlling the resulting exotherm with a chiller. Adjust the pH to 8 to 9 with 1 N NaOH (2.55 L). Collect the organic layer and extract the aqueous layer with DCM (2.32 L). Combine the organic layers, concentrate and dry under a stream of nitrogen overnight to give the title compound (193 g, 100% crude product). ^{1}H NMR (300 MHz, DMSO-d6) δ (br s, 1H), 8.78 (d, 2H, J = 5.5 Hz), 7.41 (t, 1H, J = 4.9 Hz), 3.76 (s, 2H), 3.52 (br s, 4H), 3.187 (t, 2H , J = 5.2 Hz), 2.96 (s, 3H), 2.54 (m, 2H), 2.28 (t, 2H, J = 6Hz), 1.72(pt, 2H, J = 5.2 Hz). This lot is combined with 4 other lots for purification (203 g). Purify by silica filtration (20 cm diameter by 8 cm high) and elute with 90% DCM / 10% 2 M NH_3 in MeOH to give the title compound (162 g, 84%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.74 (d, *J* = 5.1 Hz, 2H), 7.20 (t, *J* = 5.1 Hz, 1H), 3.85 (s, 2H), 3.78-3.69 (m, 4H), 3.27-3.19 (m, 2H), 3.03 (2, 3H), 2.68-2.59 (m, 4H), 2.47-2.38 (m, 2H), 1.88-1.76 (m, 2H).

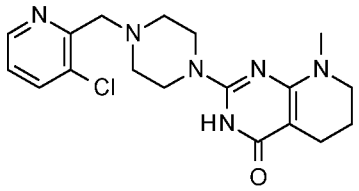
Example 1 Alternate Synthesis

Combine 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one tri-2,2,2-trifluoroacetic acid (203.23 g, 344 mmol), pyrimidine-2-carbaldehyde (40 g, 370 mmol) and DMF (343 mL) and stir until a homogeneous solution is obtained (30

min). Cool the solution in an ice bath and add sodium cyanoborohydride (25 g, 397 mmol) in a 5 g portion followed by a 10 g portion 5 minutes later. Stir for 1.5 hr and add the final 10 g of cyanoborohydride. Stir 30 minutes while maintaining the temperature below 25 °C. Stir the reaction overnight and allow to warm 25 °C. Pour the reaction mixture into a beaker (4L) fitted with an overhead stirrer containing sodium bicarbonate (115 g, 1.37 mol) and water (350 mL). Stir the mixture for 20 min and then add EtOAc (1L) and stir for 30 min. Pour the mixture through diatomaceous earth (700 g) over about 30 min and gravity filter for about 1 hour. Then apply vacuum and rinse the diatomaceous earth with EtOAc (2 × 1 L). Combine and concentrate the organic layer to give a viscous yellow oil with a crude weight of 117 g. Purify the oil by silica gel flash chromatography (DCM to 90% DCM/MeOH, gradient). Dry the resulting material in a vacuum oven at 50 °C to give the title compound as a white foamy solid (44.22 g, 38%). LC-ES/MS m/z 342.3 $[M+H]^+$, T_R = 0.96 min.

Example 2

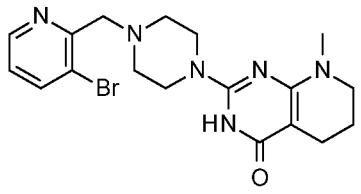
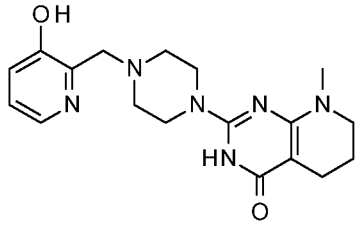
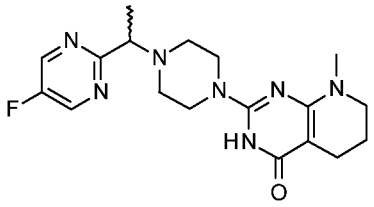
2-[4-[(3-Chloro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Add together 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one; tetra 2,2,2-trifluoroacetic acid (0.556 g, 0.788 mmol), 3-chloropicolinaldehyde (557.87 mg, 3.94 mmol) in DMF (3 mL) followed by sodium cyanoborohydride (148.6 mg, 2.36 mmol). The reaction is stirred at room temperature for 5 days. Dilute the reaction with $CHCl_3$ (75 mL) and wash with saturated $NaHCO_3$ and brine. Dry over Na_2SO_4, filter, and concentrate to dryness. The crude product is purified by silica gel chromatography eluting with DCM to 90% DCM/MeOH to give the title product (214 mg, 72%). LC ES/MS m/z 375.3 (^{35}Cl) $(M+H)^+$.

The following Examples 3, 4 and 5 are prepared essentially following the procedure described in Example 2, using the appropriate aldehyde or ketone, stirring at room temperature for 2 hrs up to 48 hrs and monitoring reaction for completion, adding more sodium cyanoborohydride if needed and stirring for a further 24 hours.

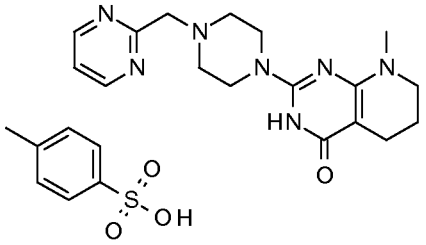
Table 1

Ex. No.	Chemical name	Structure	LC-ES/MS m/z
3[a]	2-[4-[(3-Bromo-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		419.1 (^{79}Br) $(M+H)^+$
4[b]	2-[4-[(3-Hydroxy-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		357.2 $(M+H)^+$
5[c]	8-Methyl-2-[4-[1-[5-(trifluoromethyl)pyrimidin-2-yl]ethyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		374.2 $(M+H)^+$

[a]MeOH (1 mL) added in addition to DMF as solvent.
[b] Dichlormethane is solvent and sodium triacetoxyborohydride is used instead of sodium borohydride.
[c] MeOH is solvent.

Example 6

8-Methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one 4-methylbenzenesulfonic acid salt



Add 8-methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one (274.5 mg, 0.804 mmol) to ethanol (0.5 mL) and add toluenesulfonic acid monohydrate (160 mg). Sonicate to give a dark amber red solution. Add heptane (5 mL), cap the mixture, stir, and heat the mixture to 80 °C. The mixture solidifies to a tan-brown solid. Stir mixture for 30 minutes, collect the solid by vacuum filtration and air dry to give the title compound (387 mg, 94%).

Example 6 X-Ray Powder Diffraction

The XRPD patterns of crystalline solids are obtained on a Bruker D4 Endeavor X-ray powder diffractometer, equipped with a CuKa source (λ = 1.54060 Å) and a Vantec detector, operating at 35 kV and 50 mA. The sample is scanned between 4 and 40° in 2θ, with a step size of 0.0087° in 2θ and a scan rate of 0.5 seconds/step, and with 0.6 mm divergence, 5.28mm fixed anti-scatter, and 9.5 mm detector slits. The dry powder is packed on a quartz sample holder and a smooth surface is obtained using a glass slide. It is well known in the crystallography art that, for any given crystal form, the relative intensities of the diffraction peaks may vary due to preferred orientation resulting from factors such as crystal morphology and habit. Where the effects of preferred orientation are present, peak intensities are altered, but the characteristic peak positions of the polymorph are unchanged. See, *e.g.* The U. S. Pharmacopeia 35 - National Formulary 30 Chapter <941> Characterization of crystalline and partially crystalline solids by X-ray powder diffraction (XRPD) Official December 1, 2012-May 1, 2013. Furthermore, it is also well known in the crystallography art that for any given crystal form the angular peak positions may vary slightly. For example, peak positions can shift due to a variation

in the temperature or humidity at which a sample is analyzed, sample displacement, or the presence or absence of an internal standard. In the present case, a peak position variability of ± 0.2 in 2θ will take into account these potential variations without hindering the unequivocal identification of the indicated crystal form. Confirmation of a crystal form may be made based on any unique combination of distinguishing peaks (in units of ° 2θ), typically the more prominent peaks. The crystal form diffraction patterns, collected at ambient temperature and relative humidity, were adjusted based on NIST 675 standard peaks at 8.85 and 26.77 degrees 2-theta.

A prepared sample of Example 6 is characterized by an XRPD pattern using CuKa radiation as having diffraction peaks (2-theta values) as described in Table 1 below. Specifically the pattern contains a peak at 7.68 in combination with one or more of the peaks selected from the group consisting of 12.02, 12.93, 15.17, 19.24 and 23.21 with a tolerance for the diffraction angles of 0.2 degrees.

X-ray powder diffraction peaks of Example 6

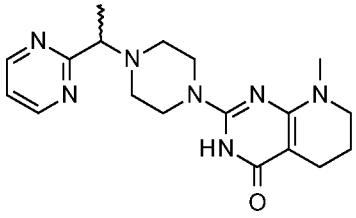
Table 2

Peak	Angle (2-Theta °)	Relative Intensity (%)
1	7.28	20
2	7.68	100
3	9.88	15
4	12.02	46
5	12.42	16
6	12.93	69
7	13.11	36
8	13.76	33
9	15.17	45
10	17.02	32
11	17.24	17
12	19.24	83
13	19.88	19
14	20.27	27
15	21.85	36
16	22.06	47
17	22.52	23

18	23.21	75
19	23.42	27
20	24.36	38
21	24.80	33
22	25.76	21
23	25.87	23
24	28.58	28

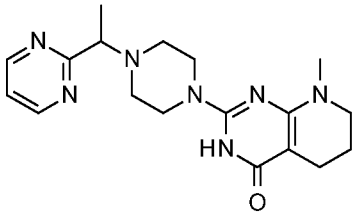
Example 7

8-Methyl-2-[4-(1-pyrimidin-2-ylethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



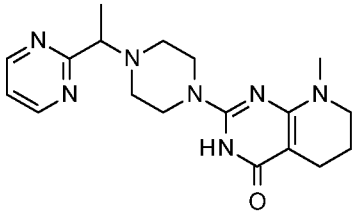
Example 8

8-Methyl-2-[4-(1-pyrimidin-2-ylethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, Isomer 1



Example 9

8-Methyl-2-[4-(1-pyrimidin-2-ylethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, Isomer 2



Combine 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) salt (1.0 g, 2.1 mmol), 2-acetylpyrimidine (0.38 g, 3.14 mmol), DMF (3 mL), MeOH (1 mL), sodium cyanoborohydride (0.20 g, 3.14 mmol) and stir at room temperature for 20 hours. Add additional 2-acetylpyrimidine (0.38 g, 3.14 mmol) and sodium cyanoborohydride (0.20 g, 3.14 mmol) and heat at 80 °C overnight. Evaporate the DMF under a stream of nitrogen for 4 days and purify by silica gel flash chromatography eluting with DCM to 90% DCM/MeOH to give the title compound, Example 7 (330 mg, 44%) as the racemate.

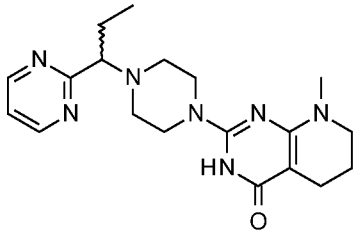
Separate 8-Methyl-2-[4-(1-pyrimidin-2-ylethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one using SFC on a Phenomene® Lux® Cellulose-2 column (2.1 × 25 cm, 5 µm). Mobile Phase: 40% EtOH (0.2% IPAm)/carbon dioxide. Flow rate: 70 mL/min. Detection: 225 nm. Obtain the first eluting peak as Isomer 1 and the second eluting peak as Isomer 2.

Example 8, Isomer 1: 135 mg, 99.25% pure with 0.75% of Isomer 2 as an impurity (T_R = 4.59 min; Phenomenex® Lux® Cellulose-2 column (2.1 × 25 cm, 5 µm), mobile phase: 40% EtOH (0.2% IPAm)/carbon dioxide, flow rate: 5 mL/min. detection: 225 nm). LC-ES/MS m/z 356.3 $(M+H)^+$.

Example 9, Isomer 2: 121 mg, 96.63% pure with 3.36% of Isomer 1 as an impurity (T_R = 5.91 min, Phenomenex® Lux® Cellulose-2 column (2.1 × 25 cm, 5 µm), mobile phase: 40% EtOH (0.2% IPAm)/carbon dioxide, flow rate: 5 mL/min, detection: 225 nm). LC-ES/MS m/z 356.3 $(M+H)^+$.

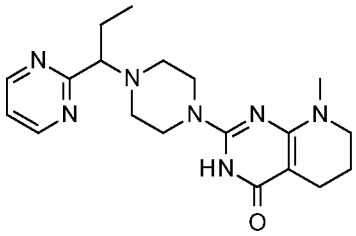
Example 10

8-Methyl-2-[4-(1-pyrimidin-2-ylpropyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



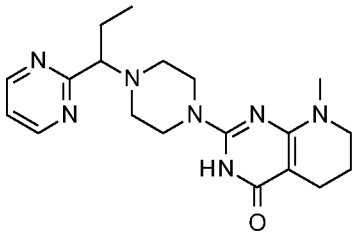
Example 11

8-Methyl-2-[4-(1-pyrimidin-2-ylpropyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, Isomer 1



Example 12

8-Methyl-2-[4-(1-pyrimidin-2-ylpropyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, Isomer 2



Prepare Examples 10, 11, and 12 essentially following the procedure as described in Examples 7, 8, and 9 using 1-(2-pyrimidinyl)-1-propanone and purifying by silica gel flash chromatography eluting with a gradient of DCM to 90% DCM/MeOH. Purify a second time by silica gel chromatography using the same conditions. Then purify further by high pH prep HPLC (Column: 150 g High resolution C18 Gold; Initial: 5% acetonitrile/95% 10 mM ammonium bicarbonate w/5% MeOH with gradient to 60% acetonitrile/40% ammonium bicarbonate w/5% MeOH over 30 min., detection wavelengths 239, 254, 280 and 290 nm) to give the title compound, Example 10 (140 mg, 18%). Separate the resulting enantiomeric mixture using SFC using the conditions described for Examples 8 and 9 with the exception that a Lux® Cellulose-4 column is used.

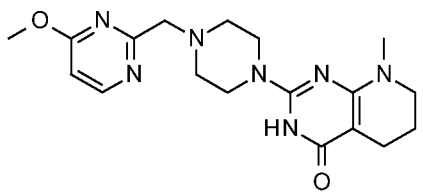
Example 11, Isomer 1: 50 mg, >99.9% purity (T_R = 4.26 min; Phenomenex® Lux® Cellulose-4 column (2.1 × 25 cm, 5 µm), mobile phase: 40% EtOH (0.2%

IPAm)/carbon dioxide, flow rate: 5 mL/min, detection: 225 nm). LC-ES/MS m/z 370.2 $(M+H)^+$.

Example 12, Isomer 2: 47 mg, >99.9% purity (T_R = 5.67 min; Phenomenex® Lux® Cellulose-4 column (2.1 × 25 cm, 5 µm). Mobile Phase: 40% EtOH (0.2% IPAm)/carbon dioxide. Flow rate: 5 mL/min. Detection: 225 nm), LC-ES/MS m/z 370.2 $(M+H)^+$.

Example 13

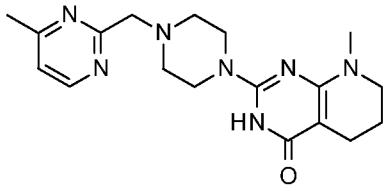
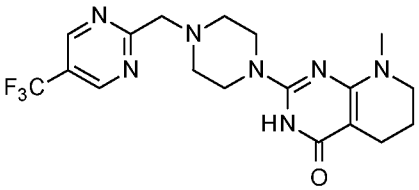
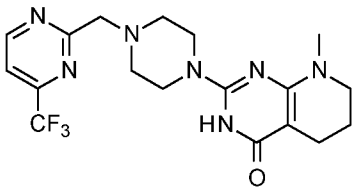
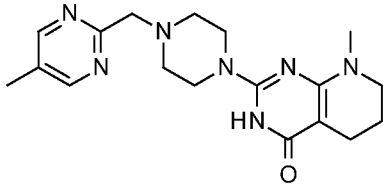
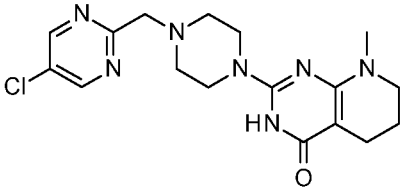
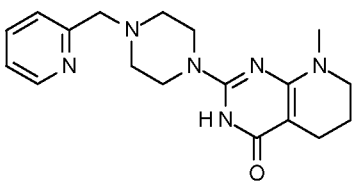
2-[4-[(4-methoxypyrimidin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Combine 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) salt (0.5 g, 1.0 mmol), 4-methoxypyrimidine-2-carbaldehyde (150 mg, 1.08 mmol), DMF (10 mL), MeOH (1 mL) and sodium cyanoborohydride (136 mg, 2.17 mmol) and stir at room temperature for 3 days. Dilute with MeOH and absorb onto an SCX-2 column, rinse with MeOH and then elute with 2 M NH_3 in MeOH, concentrate and purify by silica gel flash chromatography eluting with a gradient of DCM to 90% DCM/MeOH to give the title compound (43 mg, 11%). LC-ES/MS m/z 372.1 $(M+H)^+$ T_R = 1.519 min.

The following Examples are prepared essentially following the procedure of Example 13, using the appropriate aldehyde and the appropriate chromatography.

Table 3

Ex. No.	Chemical Name	Structure	LC-ES/MS m/z
14[1]	8-Methyl-2-[4-[(4-methylpyrimidin-2-yl)methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		356.1 $(M+H)^+$
15[2]	8-Methyl-2-[4-[[5-(trifluoromethyl)pyrimidin-2-yl]methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		410.1 $(M+H)^+$
16	8-Methyl-2-[4-[[4-(trifluoromethyl)pyrimidin-2-yl]methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		410.2 $(M+H)^+$
17[3]	8-Methyl-2-[4-[(5-methylpyrimidin-2-yl)methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		356.3 $(M+H)^+$
18[4]	2-[4-[(5-Chloropyrimidin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		376.0 (^{35}Cl) $(M+H)^+$
19[5]	8-Methyl-2-[4-(2-pyridylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		341.2 $(M+H)^+$

[1] Stir reaction for 3 days.

[2] Add additional aldehyde (1 eq) and sodium cyanoborohydride (1.5 eq); then heat at 80 °C for 6 h. Further purify by general method of reverse phase chromatography (High pH 5-100).

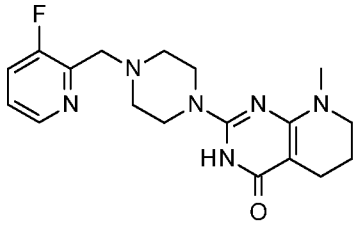
[3] Further purify by general method of reverse phase chromatography (High pH 9-24).

[4] Further purify by general method of reverse phase chromatography (Low pH 0.1% TFA/ACN) followed by chromatography on an SCX-2 column.

[5] Stir reaction at rt for 12 hours and further purify by general method of reverse phase chromatography (High pH 13-48).

Example 20

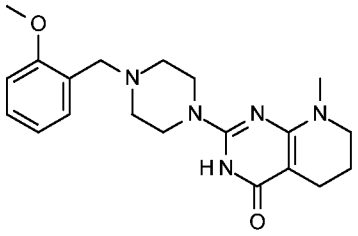
2-[4-[(3-Fluoro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Suspend 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) (20.31 g, 34.34 mmol) in DCM (114 mL) and purge with nitrogen. Add 3-fluoropyridine-2-carbaldehyde (5.26 g, 41.2 mmol) and stir for 60 minutes. Then add sodium triacetoxyborohydride (14.56 g, 68.68 mmol) in one portion and observe an exotherm to reflux. Stir and allow the reaction to cool to room temperature over 1 hour. Pour into 0.5 M NaOH (200 mL) and adjust the pH : 7-8 with 2 M NaOH. Collect the organic layer and extract the aqueous layer with DCM (2 × 200 mL). Combine and dry the organic layers over sodium sulfate, filter, and concentrate to give an oil. Purify by silica gel chromatography (400 g), eluting with 95% DCM/ 5% MeOH for 8 column volumes and with 90% DCM/10% MeOH for 8 column volumes to give the title compound (7.59 g, 61.7%) with >97% HPLC purity and also 2.64 g (21.5%) of additional product with 87% HPLC purity. LC ES/MS m/z 359.1 $(M+1)^+$.

Example 21

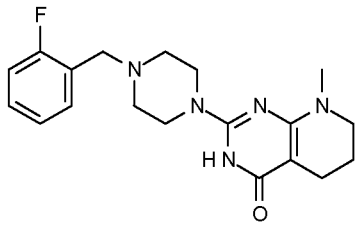
2-[4-[(2-Methoxyphenyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one

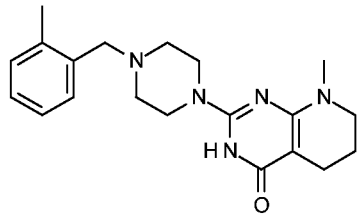
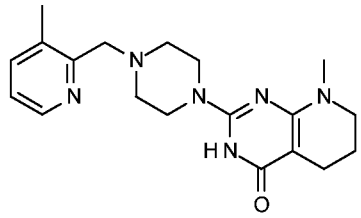
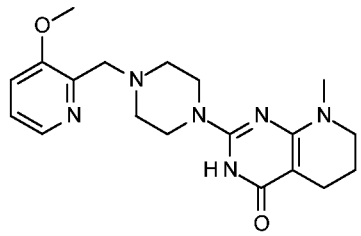
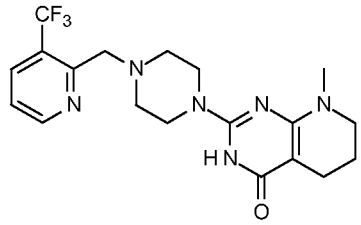
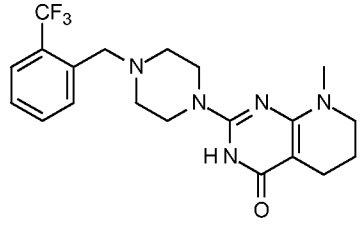


Combine 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) salt (0.25 g, 0.52 mmol), 2-methoxybenzaldehyde (0.21 g, 1.57 mmol), DMF (15 mL), MeOH (5 mL) and sodium cyanoborohydride (100 mg, 1.57 mmol) and stir at room temperature for12 hours. Dilute with methanol and absorb onto an SCX-2 column, rinse with MeOH and then elute with 2 M NH_3 in MeOH, concentrate and purify by reverse phase chromatography, see general method for reverse chromatography (High pH 21-55) to give the title compound (223 mg, 59%). LC-ES/MS m/z 370.2 $(M+H)^+$.

The following compounds are prepared essentially as described in Example 21 using the appropriate aldehyde.

Table 4

Ex. No.	Chemical name	Structure	LC-ES/MS m/z
22	2-[4-[(2-Fluorophenyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		358.2 $(M+H)^+$

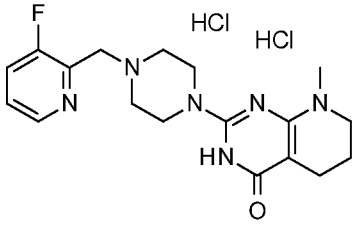
23[a]	8-Methyl-2-[4-(o-tolylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		354.2 $(M+H)^+$
24[b]	8-Methyl-2-[4-[(3-methyl-2-pyridyl)methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		355.2 $(M+H)^+$
25[c]	2-[4-[(3-Methoxy-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		371.2 $(M+H)^+$
26[b]	8-Methyl-2-[4-[[3-(trifluoromethyl)-2-pyridyl]methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		409.2 $(M+H)^+$
27[d]	8-Methyl-2-[4-[[2-(trifluoromethyl)phenyl]methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		408.2 $(M+H)^+$

[a] Reverse phase chromatography (High pH 30-64).
[b] Reverse phase chromatography (High pH 23-57).
[c] Reverse phase chromatography (High pH 13-48).
[d] Reverse phase chromatography (High pH 39-73).

5

Example 28

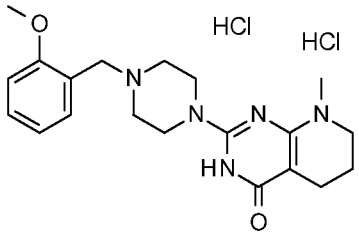
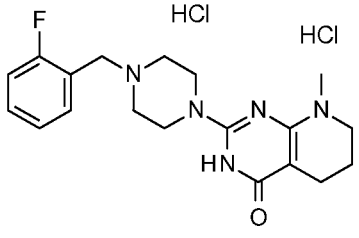
2-[4-[(3-Fluoro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, dihydrochloride

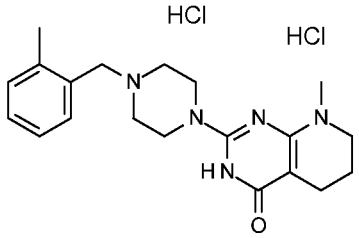
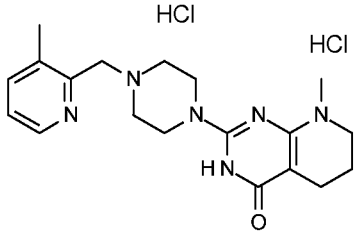
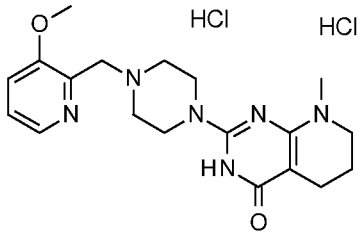
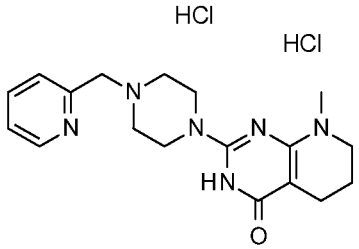
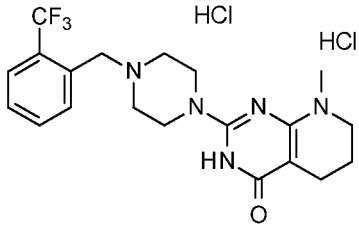


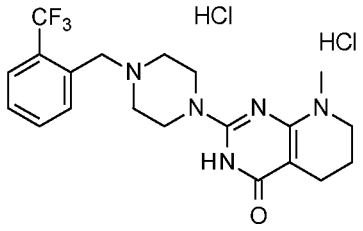
In 2 vials add to each DCM (5 mL) 4M HCl in dioxane (1 mL, 4 mmol), and 2-[4-[(3-fluoro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one (0.117 g, 0.65 mmol). Shake the vials for 1 hour, combine into 1 vial and then evaporate under a stream of nitrogen overnight. Dry the material in a vacuum oven overnight to give the title compound (0.28 g, 99%). LC-ES/MS m/z 359.2 $[M+H]^+$, T_R = 1.04 min.

The following Examples are prepared essentially as described in Example 28 using the appropriate compound of Examples 21-27.

Table 5

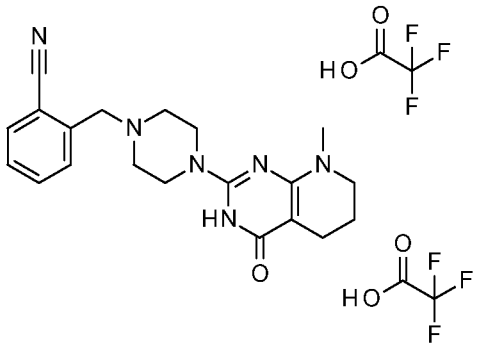
Ex. No.	Chemical name	Structure	LC-ES/MS m/z
29	2-[4-[(2-Methoxyphenyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one dihydrochloride		370.2 $(M+H)^+$
30	2-[4-[(2-Fluorophenyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one dihydrochloride		358.2 $(M+H)^+$

31	8-Methyl-2-[4-(o-tolylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one dihydrochloride		354.2 $(M+H)^+$
32	8-Methyl-2-[4-[(3-methyl-2-pyridyl)methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one dihydrochloride		355.2 $(M+H)^+$
33	2-[4-[(3-Methoxy-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one dihydrochloride		371.2 $(M+H)^+$
34	8-Methyl-2-[4-(2-pyridylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one dihydrochloride		341.2 $(M+H)^+$
35	8-Methyl-2-[4-[[3-(trifluoromethyl)-2-pyridyl]methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one dihydrochloride		409.2 $(M+H)^+$

36	8-Methyl-2-[4-[[2-(trifluoromethyl)phenyl]methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one dihydrochloride		408.2 $(M+H)^+$

Example 37

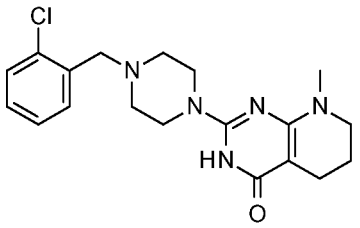
2-[[4-(8-Methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazin-1-yl]methyl]benzonitrile; bis-2,2,2-trifluoroacetic acid



Combine a solution of 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) (0.364 g, 0.76 mmol) in DMF (3.2 mL) with 2-cyanobenzaldehyde (0.499 g, 3.18 mmol) and stir at room temperature for 1 hour. Add sodium cyanoborohydride (0.143 g, 2.28 mmol) and stir at room temperature overnight. Pour into water and attempt collection by filtration. Observe clogging of filter and then add 2 N NaOH and NaCl (aqueous) and extract the crude product into ethyl acetate (3×). Combine the organic layers, dry over sodium sulfate, filter, and concentrate. Purify by silica gel chromatography eluting with 98% DCM /2% ethanol isocratic for 10 min with step gradient to 95% DCM /5% ethanol and hold for 45 min. Purify further by preparative reverse phase chromatography eluting with 5% acetonitrile (0.1% TFA)/95% water (0.1%TFA) gradient to 54% acetonitrile (0.1% TFA)/46% water (0.1%TFA) to give the title compound (48.1 mg, 10.7%). LC-ES/MS m/z 365.2 $[M+H]^+$

Example 38

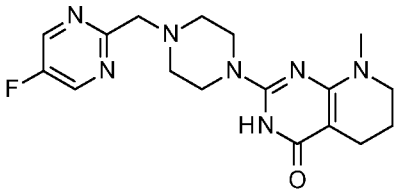
2-[4-[(2-Chlorophenyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Prepare the title compound essentially as described in Example 37 using 2-chloro-5-methyl-pyrimidine 2-chlorobenzaldehyde. Purify the crude material by silica gel flash chromatography (5% EtOH/$CHCl_3$ to 10% EtOH/$CHCl_3$ gradient). Recrystallize the material from DMSO/MeOH to obtain a white solid (83 mg, 29%). LC-ES/MS m/z 374.3 (^{35}Cl) $[M+H]^+$.

Example 39

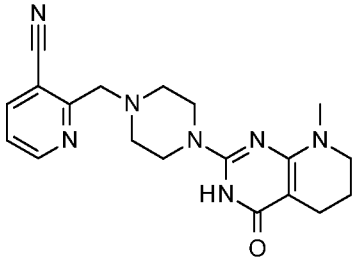
2-[4-[(5-Fluoropyrimidin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Combine potassium trifluoro-[[4-(8-methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazin-1-yl]methyl]boranuide (273 mg, 0.74 mmol), 2-chloro-5-fluoropyrimidine (98 mg, 0.74 mmol), cesium carbonate (723 mg, 2.22 mmol), X-Phos (71 mg, 0.15 mmol), THF (10 mL) and water (1 mL) and degas with a stream of nitrogen for 2 min. Add palladium (II) acetate (17 mg, 0.074 mmol) and heat at 80 °C overnight. Partition the mixture between ethyl acetate and water and separate the organic layer. Dry over magnesium sulfate, filter, and concentrate the filtrate under reduced pressure. Purify the resulting residue by silica gel flash chromatography eluting with DCM to 90% DCM/MeOH to give the title compound (20 mg, 7.5%). LC-ES/MS m/z 360.2 $[M+H]^+$.

Example 40

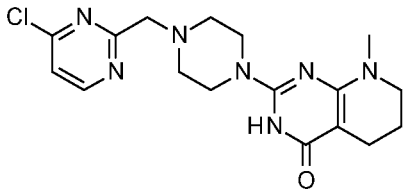
2-[[4-(8-Methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazin-1-yl]methyl]pyridine-3-carbonitrile



Dissolve 2-[4-[(3-bromo-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one (167 mg, 0.398 mmol) in DMF (10 mL) and degas with a stream of nitrogen for 1 min. Add tetrakis(triphenylphosphine)palladium (92 mg, 0.080 mmol) and zinc cyanide (187 mg, 1.59 mmol) and heat the mixture at 120 °C overnight. Cool the reaction and dilute with MeOH. Transfer the mixture to a 10 g SCX-2 column and elute with 2 M NH_3 in MeOH. Concentrate and further purify the resulting residue reverse phase chromatography (High pH 9-29) to give the title compound (35 mg, 24%). LC-ES/MS m/z 366.2 $(M+H)^+$

Example 41

2-[4-[(4-Chloropyrimidin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one

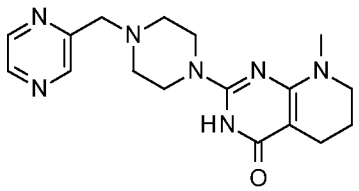


Combine 2-(bromomethyl)-4-chloro-pyrimidine (322 mg, 1.55 mmol) as a crude solution in carbon tetrachloride (5 mL), DCM (2 mL), triethylamine (0.65 mL, 4.65 mmol), and 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) (739 mg, 1.55 mmol) and stir at 25 °C for 3 days. Partition the reaction mixture between DCM and water and extract the aqueous layer with DCM. Combine the organic layers, dry over $MgSO_4$, filter, and concentrate. Purify the resulting

residue by mass guided SFC (column: 4-nitrobenzene sulfonamide, Princeton Chromatography, 150 × 30 mm, flow rate = 100 mL/min; method: 95% CO_2/14 mM ammonia in MeOH isocratic for 30 sec. then gradient to 60% CO_2/14 mM ammonia in MeOH gradient over 330 sec., then ramp to 50% CO_2/14 mM ammonia in MeOH over 10 sec. and hold for 30 sec.) to give 71 mg of desired product with ammonia salt impurities. The product is further purified by silica gel flash chromatography (CH_2Cl_2/MeOH, 90:10) to give the title compound (28 mg, 5%). LC-ES/MS m/z 376.0 $[M+H]^+$

Example 42

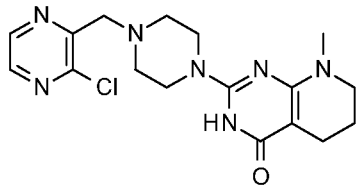
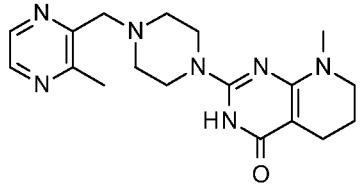
8-Methyl-2-[4-(pyrazin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Combine 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one bis-(2,2,2-trifluoroacetic acid) (500 mg, 1.05 mmol), triethylamine (0.73 mL, 5.2 mmol), sodium iodide (78.5 mg, 0.52 mmol) and acetonitrile (7 mL). Add 2-(chloromethyl)pyrazine (201 mg, 1.57 mmol) and stir at 25 °C for 72 h. Dilute the reaction with DCM and water and extract the product into DCM. Wash the organic portion with saturated $NaHCO_3$ and brine, dry over Na_2SO_4, filter, and concentrate. Purify the resulting residue by silica gel flash chromatography (DCM / 2M NH_3 in MeOH, 90:10) to give the title compound as a yellow solid (249 mg, 0.72 mmol,70%). LC-ES/MS m/z 342.0 $[M+H]^+$, T_R = 0.99 min.

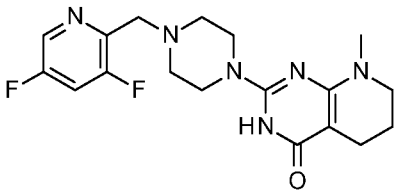
The following Examples are prepared essentially following the procedure described in Example 42, using the appropriate halo-methylpyrazine.

Table 6

Ex. No.	Chemical name	Structure	LC-ES/MS m/z
43	2-[4-[(3-Chloropyrazin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		376.0 $[M+H]^+$, T_R = 1.17 min.
44	8-Methyl-2-[4-[(3-methylpyrazin-2-yl)methyl]piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one		356.0 $[M+H]^+$, T_R = 1.03 min.

Example 45

2-[4-[(3,5-Difluoro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one



Add 2-(chloromethyl)-3,5-difluoropyridine (182 mg, 1.11 mmol), 8-methyl-2-piperazin-1-yl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one (260 mg, 1.04 mmol), potassium carbonate (2.2 g, 15 mmol) and acetonitrile (5 mL) and stir the mixture at room temperature overnight. Concentrate the reaction mixture under reduced pressure and purify the residue by silica gel chromatography (elution with MeOH/DCM, 1/20) to give the title compound (160 mg, 43%). LC-ES/MS m/z 377.2 $(M+H)^+$, T_R = 1.11 min.

Cancer is increasingly recognized as a heterogeneous collection of diseases whose initiation and progression are induced by the aberrant activation or function of one or more genes that regulate DNA repair, genome stability, cell proliferation, cell death,

adhesion, angiogenesis, invasion, and metastasis in cell and tissue microenvironments. Variant or aberrant function of the "cancer" genes may result from naturally occurring DNA polymorphism, changes in genome copy number (through amplification, deletion, chromosome loss, or duplication), changes in gene and chromosome structure (through chromosomal translocation, inversion, or other rearrangement that leads to deregulated gene expression), and point mutations. Cancerous neoplasms may be induced by one aberrant gene function, and maintained by the same aberrant gene function, or maintenance and progression exacerbated by additional aberrant gene activations or functions.

Beyond the genetic chromosomal aberrations mentioned above, each of the cancers may also include epigenetic modifications of the genome including DNA methylation, genomic imprinting, and histone modification by acetylation, methylation, or phosphorylation. An epigenetic modification may play a role in the induction and/or maintenance of the malignancy.

Diagnosis of cancerous malignancies by biopsy, immunophenotyping and other tests are known and routinely used. In addition to high resolution chromosome banding and advanced chromosomal imaging technologies, chromosome aberrations in suspected cases of cancer can be determined through cytogenetic analysis such as fluorescence in situ hybridization (FISH), karyotyping, spectral karyotyping (SKY), multiplex FISH (M-FISH), comparative genomic hybridization (CGH), single nucleotide polymorphism arrays (SNP Chips) and other diagnostic and analysis tests known and used by those skilled in the art.

An important part of the Wnt/β-catenin signaling pathway is the regulated proteolysis of the downstream effector β-catenin by the β-catenin destruction complex. The principal constituents of the β-catenin destruction complex are adenomatous polyposis coli (APC), Axin, and GSK3α/β. In the absence of Wnt pathway activation, cytosolic β-catenin is constitutively phosphorylated and targeted for degradation. Upon Wnt stimulation, the β-catenin destruction complex disassociates, which leads to the accumulation of cytosolic β-catenin, translocation to the nucleus, and transcription of Wnt canonical pathway responsive genes.

Considerable efforts have been made to identify pharmaceutical agents that inhibit the canonical Wnt/β-catenin signaling pathway. TNKS1 and TNKS2 inhibitors such as XAV939, Huang et al., *Nature*, 2009, *461*, 614; JW55, Waaler et al., *Cancer Res.,* 2012, *72*(11), 2822; G007-LK, Lau et al., *Cancer Res.,* 2013, *73*(10), 3132; TNKS656, Shultz et al., *J. Med. Chem.* published online July 11, 2013, DOI: 10.1021/jm400807n; and WO 2013/117288 are known. Despite these efforts, no clinical TNKS1 and TNKS2 inhibitor therapeutic agents have emerged at this time.

Aberrant activation of the pathway, mediated by over expression of Wnt proteins or mutations affecting components of the β-catenin destruction complex, thus leading to stabilization of β-catenin, has been observed in cancers. Notably, truncating mutations of APC are the most prevalent genetic alterations in colorectal carcinomas (Miyaki, M. et al. *Cancer Res.* 1994, 54, 3011-20; Miyoshi, Y. et al. *Hum. Mol. Genet.* 1992, 1, 229-33; and Powell, S. M. et al. *Nature* 1992, 359, 235-7). In addition, Axin1 and Axin2 mutations, negative regulators of the Wnt signaling pathway, have been identified in patients with hepatocarcinomas and colorectal cancer respectively (Taniguchi, K. et al. *Oncogene* 2002, 21, 4863-71; Liu, W. et al. *Nat. Genet.* 2000, 26, 146-7; Lammi, L. et al. *Am. J. Hum. Genet.* 2004, 74, 1043-50). These somatic mutations result in Wnt-independent stabilization of β-catenin and constitutive activation of β-catenin-mediated transcription.

Aberrant Wnt signaling pathway activity has been implicated in several cancers (Waaler et al. *Cancer Res.* 2012, 72, 2822-2832; Busch et al. *BMC Cancer* 2013, 13, 211; Yang et al. *Oncogene* 2011, 30, 4437-4446; De Robertis et al. *Mol. Cancer Ther.* 2013, 12, 1180-1189; Polakis, P. *Curr. Opin. Genet. Dev.* 2007, 17, 45-51; and Barker, N. et al. *Nat. Rev. Drug Discov.* 2006, 5, 997-1014), including colorectal, gastric, liver, breast, triple negative breast cancer, ovarian, medulloblastoma, melanoma, lung, non-small cell lung, pancreas, prostate cancers and glioblastomas. Aberrant Wnt/β-catenin pathway signaling activity has been implicated in T-cell lymphoma, T-lymphoblastic lymphoma, T-cell acute lymphocytic leukemia (T-ALL) Groen et al. *Cancer Res.* 2008, 68, 6969-6977; multiple myeloma Qiang et al. *Oncogene* 2003, 22, 1536-1545, and Chim et al. *Leukemia* 2007, 21, 2527-2536; mantle cell lymphoma Gelebart et al. *Blood* 2008, 112, 5171-5179; chronic myeloid leukemia (CML), Heidel et al. *Cell Stem Cell* 2012,

10(4):412-424, and acute myeloid leukemia (AML), Ysebaert et al. *Leukemia* 2006, 20, 1211-1216.

It has been found that β-catenin degradation can be promoted by stabilizing the Axin/APC/GSK3α/β destruction complex through the inhibition of the poly-ADP-ribose polymerase (PARP) enzymes tankyrase 1 and tankyrase 2, Huang et al. *Nature* 2009, 461, 614-620.

The following *in vitro* and *in vivo* studies demonstrate the Wnt/β-catenin signaling pathway inhibitory activity and efficacy of exemplified and tested compounds of Formula I, or a pharmaceutically acceptable salt thereof, in inhibiting hTNKS1 and hTNKS2, stabilization of Axin2 in HEK293 cells, selectivity against PARP1 inhibition, reduce the expression of Wnt-inducible genes expression, and *in vivo* antitumor activity. These assays are generally recognized by those skilled in the art as indicative of human clinical chemotherapeutic activity. Inhibition of TNKS 1 and TNKS 2 are believed to be effective against aberrant activation of the Wnt/β-catenin signaling pathway. Assays evidencing Wnt/β-catenin signaling pathway inhibitory activity and efficacy may be carried out substantially as follows or by similar assays affording similar data.

Assays

Generally, cell lines are generated using commercially available materials and by procedures known to and routinely used by those skilled in the art.

Biochemical assay to demonstrate compound inhibition of hTNKS enzyme activity

The enzymatic activity of hTNKS1 and hTNKS2 is assessed using an enzyme-linked immunosorbent assay (ELISA) which detects poly ADP ribose incorporated into plate-bound Telomeric repeat binding factor 1 (TRF1) protein (NCBI, Accession number NP_059523.2 (SEQ ID NO: 1) in a 384-well format. Using recombinant hTNKS1 (NCBI, Accession number NP_003738.2 (SEQ ID NO: 2) or hTNKS2 (NCBI, Accession number NP_079511.1 (SEQ ID NO: 3) enzyme, this assay uses biotinylated NAD+ and measures its incorporation into recombinant hTRF1 using a streptavidin-horseradish peroxidase (HRP) conjugate and TMB peroxidase substrate to generate a colorimetric signal. Recombinant Flag-tagged hTRF1 protein is generated by expressing full length human TRF1 protein with an N-terminal Flag tag in E. coli. Recombinant Flag-hTNKS1

(with a change of Q83P) protein is generated by expressing full length human TNKS1 with an N-terminal Flag tag in Baculovirus according to the manufacturer's protocol of Bac-to-Bac Baculovirus Expression system (Invitrogen™; See also Invitrogen™ User Manual, Version F, dated 04 September 2010; and Invitrogen™ Instruction Manual dated 27 February 2002). The Flag tags on hTRF1 and hTNK1 are used only for purification of the enzymes and are not otherwise involved in the ELISA assay.

Flag-TRF1 is diluted to 5 µg/ml using TBS coating buffer (50 mM Tris, pH 8.0, 150 mM NaCl) and 25 µl is added to each well of a Corning 3700 plate (Tewksbury, MA #CLS3700). The plates are incubated overnight at 4 °C. The next day the plates are washed 3 times with 50 µl/well wash buffer (PBS (prepared from 10× concentrate using Hyclone, Logan, UT #SH30258.01) with 0.1% Tween-20 (Sigma, St. Louis, MO #7949)) followed with blocking for 1.5 hours at room temperature using 50 µl 1% Casein block buffer (Thermo Scientific, Waltham, MA #37528) in 1× PBS (Roche, Indianapolis, IN #11666789001). After blocking, the plates are washed 3 times with 50 µl/well wash buffer. The enzyme assay is set up using 2 µg/ml of Flag-hTNKS1, 9.5 µM NAD+ (Sigma, St. Louis, MO #N0632), 0.5 µM biotin-NAD+ (Trevigen, Gaithersburg, MD #4670-500-01), and compounds diluted from 10 µM to 4 nM final concentration in 50 mM Tris, pH 8.0 (Invitrogen™, Grand Island, NY #15568-025), 4 mM $MgCl_2$, 0.2 mM DTT, 0.5% Triton X-100 (Roche, Indianapolis, IN #11332481001) and 1.0 % DMSO in a total volume of 25 µl. The reaction is incubated for 120 minutes at room temperature and stopped by washing the plate 3 times with 50 µl/well wash buffer. Detection of the biotin-NAD+ incorporation is done using 25 µl/well of Strepavidin-HRP (GE Life Sciences Pittsburgh, PA #RPN1231V) diluted 1:3000 with wash buffer and incubated for 60 minutes at room temperature. The plate is then washed 3 times using 50 µl/well wash buffer. This is followed by incubation with 25 µl/well TMB peroxidase substrate kit (KPL, Gaithersburg, MD #50-76-02 and #50-65-02) for 15 minutes at room temperature and stopping the reaction using 25 µl/well of 2 N H_2SO_4. The absorbance is read at 450 nm using an Envision model 2103.

By substantially following the procedures described above for hTNKS1, and using hTNKS2, an essentially similar ELISA assay is prepared.

Activity of those compounds tested against both hTNKS isoforms is shown in Table 7.

Table 7

Example No.	hTNKS1 IC_{50} (nM)	hTNKS2 IC_{50} (nM)
1	19.1 (± 17.7, n=13)	13.7 (± 7.77, n=13)
2	10.7 (±7.13, n=6/7)	6.25 (± 1.20, n=5)
3	10.9 (± 10.5, n=4)	6.58 (±4.48, n=5)
4	26.4 (± 11.2, n=5)	11.6 (±6.88, n=5)
5	85.2 (± 35.5, n=8)	69.4 (±17.6, n=8)
6	17.5 (± 6.48, n=3)	9.95
7	41.9 (± 19.9, n=5)	37.1 (±8.12, n=5)
8	94.8 (± 2.99, n=3/4)	68.7 (± 8.48, n=3/4)
9	83.3 (± 89.4, n=4)	54.9 (± 44.7, n=4)
10	158 (± 89.0, n=4)	121 (± 11.3, n=4)
11	32.7 (± 29.9, n=5)	24.7 (± 20.0, n=5)
12	27.5 (± 32.7, n=5)	22.0 (± 15.1, n=5)
13	14.9 (± 9.89, n=6/7)	12.4 (±3.67, n=6)
14	22.8 (± 13.0, n=7)	11.7 (± 7.32, n=6)
15	25.3 (± 5.05, n=6/7)	16.7 (±5.16, n=5)
16	55.1 (± 32.5, n=6)	43.4 (± 23.8, n=4)
17	67.6 (±64.7, n=5)	81.5 (± 9.15, n=4)
18	114 (±84.2, n=6)	85.1 (± 6.72, n=5)
19	19.9 (± 6.75, n=5)	11.8 (± 6.55, n=5)
20	31.8 (±38.2, n=6)	11.3 (± 4.26, n=5)
28	29.5 (± 47.3, n=5)	35.8 (± 19.1, n=4/5)
29	18.4 (±13.3, n=5)	14.1 (± 3.57, n=5)
30	41.7 (±40.2, n=5)	24.5 (±22.6, n=5)
31	15.2 (±5.04, n=5)	12.2 (± 4.59, n=5)
32	37.2 (±39.7, n=6)	22.7 (±24.2, n=6)
33	35.8 (± 32.5, n=6)	26.6 (± 20.8, n=6)

34	47.6 (±36.6, n=5)	11.6 (± 4.30, n=4)
35	21.6	7.54
36	26.2 (± 2.55, n=5)	17.6 (±11.0, n=5)
37	19.8 (± 13.3, n=4)	10.8 (± 1.34, n=4)
38	45.3 (± 32.0, n=4)	30.2 (± 5.13, n=4)
39	30.3 (± 26.6, n=6)	15.9 (± 8.48, n=6)
40	14.3 (± 4.89, n=5)	8.41 (± 1.99, n=5)
41	14.5 (± 10.7, n=5)	10.6 (± 3.51, n=5)
42	19.8 (± 15.9, n=5)	13.0 (±7.41, n=5)
43	34.3 (± 12.7, n=4)	12.7 (± 6.54, n=4)
44	17.6 (± 8.49, n=6)	12.7 (± 2.49, n=6)
45	37.3 (± 11.1, n=6)	19.6 (± 9.93, n=6)

Mean ± SEM; SEM = standard error of the mean

Data in Table 7 provides evidence that the compounds tested have *in vitro* inhibitory activity against both isoforms of human tankyrases.

EGFP-Axin2 Stabilization Assay to demonstrate cell-based activity of tankyrase inhibitors

Enhanced Green Fluorescent Protein-Axin2 cells (EGFP-Axin2) are prepared by stable transfection of HEK293 cells with an Axin2 construct containing an N-terminal truncated EGFP tag (amino acids 228-466; SEQ ID NO:7). Changes in Axin2 levels, specifically Axin2 stabilization, are monitored by quantitation of the level of EGFP in the stable cell line after various treatments in a 384-well format. Increases in fluorescence, reflecting the stabilization of the EGFP-Axin2 fusion protein as a consequence of tankyrase inhibition, are monitored in an Acumen Laser Scanning Cytometer.

HEK293 cells (ATCC, Manassas, VA #CRL-1573) are maintained in complete medium of DMEM:F12 (Invitrogen™, Grand Island, NY #93-0152DK) containing 5% FBS (Invitrogen™, Grand Island, NY #10082-147), 20 mM HEPES (Hyclone, Logan, UT #SH30237.01), and Glutamax (Invitrogen™, Grand Island, NY #35050-061). EGFP-Axin2 cells are generated by transfecting HEK293 cells with full length human Axin2 (NCBI, Accession number NP_004646.3 (SEQ ID NO: 4)) containing a truncated

(amino acids 228-466) EGFP N-terminal tag (full length EGFP, NCBI, Accession number ABG78037.1 (SEQ ID NO: 5) in pcDNA3.1+ (according to the manufacturer's protocol, Invitrogen™, Grand Island, NY #V79020). Stable EGFP-Axin2 cells are maintained in HEK293 complete medium above with the addition of 800 µg/ml G418 (Invitrogen™, Grand Island, NY #10131-035). The EGFP-Axin2 stabilization assay is done by plating 2000 EGFP-Axin2 cells/well in a poly-D-lysine coated BD 384-well plate (BD Biosciences, San Jose, CA #356663) and incubating in 30 µl/well complete HEK293 medium and grown overnight at 37 °C, 5% CO_2. Compounds in 100% DMSO are added directly to the cell media at 100 nl/well. Final concentration of compounds tested in the assay is 33 µM -1.7 nM with final concentration of DMSO in the assay being 0.33%. Cells are incubated with compound for 24 hours at 37 °C, 5% CO_2 and the cells fixed using 2% final concentration of formaldehyde for 15 minutes at room temperature. The fixed cells are washed twice for 20 minutes each in 40 µl/well PBS (Hyclone, Logan, UT #SH30264.01) containing 0.1% Triton X-100 (Thermo Fisher Scientific, Waltham, MA #BP151-500). They are then stained using 30 µl/well PBS containing 10 µg/ml propidium iodide (Invitrogen™, Grand Island, NY #P3566) and 50 µg/ml RNaseA (Sigma, St. Louis, MO #R6513). EGFP intensity is measured using an Acumen model eX3 Acumen Laser Scanning Cytometer gated to have 10% EGFP/cell.

Table 8

Example No.	Axin2 Stabilization EC_{50} (nM)
1	65.9 (± 22.7, n=11)
2	32.0 (± 9.34, n=4)
3	18.4 (± 15.8, n=3)
4	26.9 (± 11.5, n=3)
5	115 (± 78.3, n=4)
6	59.5 (± 6.94, n=4)
7	121 (± 21.9, n=3)
8	71.5 (± 28.7, n=5)
9	70.9 (± 30.6, n=4)

10	590 (± 228, n=3)
11	119 (± 26.6, n=4)
12	122 (± 31.2, n=4)
13	101 (± 26.8, n=4)
14	52.7 (± 23.9, n=4)
15	115 (± 17.5, n=4)
16	509 (± 130, n=3)
17	198 (± 57.4, n=3)
18	901 (± 138, n=3)
19	62.6 (± 14.6, n=4)
20	77.9 (± 29.6, n=3)
28	130 (± 79.4, n=4)
29	41.7 (± 12.9, n=3)
30	57.7 (± 27.1, n=3)
31	38.5 (± 6.69, n=3)
32	136 (± 84.8, n=4)
33	122 (± 73.6, n=4)
34	156 (± 141, n=3)
35	27.8
36	29.1(± 8.88, n=3)
37	23.9 (± 4.48, n=3)
38	34.8 (± 4.46, n=3)
39	181 (± 64.9, n=5)
40	39.8 (± 16.2, n=6)
41	61.6 (± 10.5, n=4)
42	141 (± 44.0, n=3)
43	74.5 (± 20.0, n=4)
44	118 (± 33.2, n=4)
45	144 (± 1.88 n=3)

Mean ± SEM; SEM = standard error of the mean

Data in Table 8 provides evidence that the compounds tested stabilize Axin2 in HEK293 cells.

Human PARP1 Enzyme Assay to assess selectivity of tankyrase inhibitors (vs. PARP1)

The Poly ADP-ribose polymerase 1(PARP1) assay is an ELISA which detects poly ADP ribose incorporated into plate-bound histone protein in a 384-well format. Using recombinant hPARP1 enzyme, this assay uses biotinylated NAD+ and measures the incorporation into histone using a streptavidin-horseradish peroxidase (HRP) conjugate and TMB peroxidase substrate to generate a colorimetric signal.

Histone is diluted to 0.1 mg/ml in coating buffer (50 mM Na_2CO_3, pH 9.4, Mallinckrodt, St. Louis, MO) and 25 µl is added to each well of a Corning 3700 plate. The plates are incubated overnight at 4 °C. The next day the plates are washed 3× with 50 µl/well wash buffer (PBS (prepared from 10× concentrate) with 0.1% Tween-20) followed with blocking for 1.5 hours at room temperature using 50 µl 1% Casein block buffer in 1× PBS (Roche, Indianapolis, IN #11666789001). After blocking, the plates are washed 3 times with 50 µl/well wash buffer. The PARP1 enzyme assay is set up by using 0.01 U/µl hPARP1 (Trevigen, Gaithersburg, MD #4668-500-01), 0.5X PARP cocktail, activated DNA (Trevigen, Gaithersburg, MD #4671-096-03 and #4671-096-06) and compounds diluted from 10 µM to 4 nM final concentration in Assay buffer containing 50 mM Tris, pH 8.0, 10 mM $MgCl_2$, 1 mM DTT (Invitrogen™, Grand Island, NY #15508-013), 0.5% Triton X-100 and 1.0 % DMSO. The reaction is incubated for 60 minutes at room temperature and stopped by washing the plate 3 times with 50 µl/well wash buffer. Detection of the biotin-NAD+ incorporation is done using 25 µl/well of Strepavidin-HRP diluted 1:3000 with wash buffer and incubated for 60 minutes at room temperature. The plate is then washed 3 times using 50 µl/well wash buffer. This is followed by incubation with 25 µl/well TMB peroxidase substrate kit (KPL, Gaithersburg, MD #50-76-02 and #50-65-02) for 15 minutes at room temperature and stopping the reaction using 25 µl/well of 2 N H_2SO_4. The absorbance is read at 450 nm using an Envision model 2103.

Table 9

Example No.	PARP1 Inhibition IC_{50} (nM)
1	4,510 (± 3030, n=2)
2	919 (n=1/2)
3	2,330 (± 234, n=2)
4	3,020 (± 197, n=2)
5	7,660 (± 1450, n=2)
6	2,670 (± 433, n=2)
7	5,930 (± 647, n=2)
8	5470
9	3940
10	33,700 (± 3130, n=2)
11	13,500
12	5,050
13	3,470 (± 318, n=2)
14	5,660 (± 698, n=2)
15	6,910 (± 746, n=2)
16	187 (± 183, n=2)
17	7,220 (± 1790, n=2)
18	77,600 (± 24000, n=2)
19	5390
20	8,240 (±10800, n=4)
29	6,070 (± 1730, n=2)
30	8,800 (± 1720, n=2)
31	17,400 (± 48.1, n=2)
32	21,500 (± 2340, n=2)
33	15,300 (± 2230, n=2)
34	16,200 (± 5740, n=2)
36	95,700 (± 6420, n=2)

37	2,440 (± 598, n=2)
38	46,700 (± 23700, n=2)
39	7,740
40	2,840
41	4,660
42	2,650 (± 143, n=2)
43	6,390 (± 1430, n=2)
44	5,830 (± 229, n=2)
45	3,530 (± 2030, n=2)

Mean ± SEM; SEM = standard error of the mean

Data in Table 9 provides evidence as to each tested compound's selective inhibition of tankyrases when compared to PARP1 inhibition.

DLD-1 TOPFlash Assay to determine the ability of tankyrase inhibitors to reduce the expression of Wnt-inducible genes

DLD-1 cells contain a mutation in the adenoma polyposis coli (APC) gene which encodes a truncated APC protein. This protein is incapable of binding the destruction complex and causes a constitutively activated Wnt pathway by allowing β catenin to translocate to the nucleus and activate the TCF/LEF transcription factors. DLD-1 TOPFlash is a reporter cell line derived from DLD-1 (human colorectal adenocarcinoma) cells by stable transfection of a TCF4 promoter linked to a luciferase reporter. The amount of luciferase in the cell lysates is quantitated by measuring luminescence in a 96 well format.

DLD-1 cells (ATCC, Manassas, VA, #CCL-221) are maintained in complete medium of RPMI (Invitrogen™, Grand Island, NY #11875-093) containing 10% FBS (Hyclone, Logan, UT #SH30070.03). DLD-1 TOPFlash cells are generated according to the manufacturer's protocol by infecting DLD-1 cells with Cignal Lenti TCF/LEF Reporter (Luc) (Qiagen, Valencia, CA #CLS-018L-8, lot#BX16) at an MOI of 10. Polybreen (Sigma, St. Louis, MO #H9268) is added to a final concentration of 8 μg/ml and the cells are incubated overnight at 37 °C, 5% CO_2. The next day the medium is

changed to fresh growth medium containing 10 µg/ml puromycin (Clontech, Mountain View, CA #631305) and the cells are incubated for an additional 3 days at 37 °C, 5% CO_2 to generate a stable cell line. The TOPFlash assay is done by plating DLD-1 TOPFlash cells at 10,000 cells/well in a Corning 96 well white/clear plate (Corning Tewksbury, MA #3610) and incubating in 30 µl/well complete medium containing RPMI (Hyclone, Logan, UT #SH30027.01), 10% FBS and 10 µg/ml puromycin and grown overnight at 37 °C, 5% CO_2. The compounds in 100% DMSO are diluted 28.5 fold in OptiMEM® (Invitrogen™, Grand Island, NY #31985-062) containing 0.2% BSA (diluted from 7.5% BSA Invitrogen™, Grand Island, NY #15260-037) and 5 µl diluted compound added to the 30 µl cell culture medium/well. Final concentration of compounds tested in the assay is 50 µM -1.5 nM with final concentration of DMSO in the assay being 0.48%. Cells are incubated with compound for 24 hours at 37 °C, 5% CO_2 and the plates removed from the incubator and placed at room temperature for 30 minutes. BugLite™ (3×) reagent is prepared by dissolving 2.296g DTT (Sigma, St. Louis, MO #D0632), 1.152g CoA (Sigma, St. Louis, MO #C3019), 0.248g ATP (Sigma, St. Louis, MO #A7699), and 0.42g Luciferin (Biosynth AG, Itasca, IL #L-8240) in 1 liter of Triton-X100 lysis buffer which contains 150 mM Tris (108.15 ml 1M Tris HCl and 41.85 ml 1M Tris Base (Sigma, St. Louis, MO #T3253 and T-1503)), 3 mM $MgCl_2$ and 3% Triton X-100. After the plates are equilibrated to room temperature, 18 µl of 3× BugLite reagent is added to each well and the plates are incubated at room temperature for 30 minutes with shaking. Luminescence is measured using an Envision model 2103.

Table 10

Example No.	Wnt-inducible gene expression inhibition IC_{50} (µM)
1	0.0132 (± 0.00595, n=6)
2	0.00817 (± 0.00587, n=4)
3	0.00866 (± 0.00190, n=3)
4	0.00671 (± 0.00248, n=3)
5	0.0211 (± 0.00437, n=4)
6	0.00720 (± 0.00120, n=2)

7	0.0146 (± 0.0128, n=3)
8	0.0184 (± 0.0110, n=3)
9	0.0282 (± 0.0257, n=4)
10	0.171 (± 0.0474, n=3)
11	0.0281 (± 0.00945, n=3)
12	0.0195 (± 0.0111, n=3)
13	0.0230 (± 0.0126, n=3)
14	0.00935 (± 0.00872, n=3)
15	0.0154 (± 0.00630, n=3)
16	0.102 (± 0.0268, n=3)
17	0.0373 (± 0.0103, n=3)
18	0.156 (± 0.0724, n=3)
19	0.0126 (± 0.00325, n=3)
20	0.0222 (± 0.00155, n=2)
28	0.0228 (± 0.0105, n=3)
29	0.0177 (± 0.0138, n=3)
30	0.0168 (± 0.0124, n=3)
31	0.0178 (± 0.0119, n=3)
32	0.0201 (± 0.0178, n=3)
33	0.0268 (± 0.0229, n=3)
34	0.0236 (± 0.0246, n=3)
35	0.0058
36	0.0208 (± 0.0168, n=3)
37	0.00622 (± 0.00286, n=3)
38	0.0222 (± 0.00824, n=3)
39	0.0206 (± 0.0129, n=4)
40	0.00695 (± 0.00456, n=4)
41	0.0138 (± 0.00253, n=3)
42	0.0220 (± 0.00397, n=3)
43	0.0110 (± 0.00217, n=3)

44	0.0112 (± 0.00315, n=3)
45	0.0188 (± 0.00510, n=4)

Mean ± SEM; SEM = standard error of the mean

Data in Table 10 demonstrates that the compounds tested are inhibitors of Wnt inducible genes as measured by the TOPFlash Wnt reporter assay.

Assessment of in vivo antitumor activity of tankyrase inhibitors:

C57BL/6J-*Apc*^{Min}/J strain mice carry a truncating mutation at codon 850 of the *Apc* gene and develop intestinal polyps and colorectal neoplasms at the age of 3-6 months. Truncation in the APC gene leads to activation of the Wnt signaling pathway and elevated levels of β catenin are frequently detected in pre-neoplastic/neoplastic lesions in these mice.

In order to assess the *in vivo* antitumor activity of tankyrase inhibitors, C57BL/6J-*Apc*^{Min}/J strain mice are purchased from Jackson Laboratories (stock number 002020) and acclimated for 1 week. Animals are divided into 2 groups and treated with either 25 mg/kg (BID) of Example 1 or vehicle for 60 consecutive days. At the end of the treatment period, the animals are sacrificed and the number of polyps in the small intestine is counted under a dissection microscope. As shown in Table 11, C57BL/6J-*Apc*^{Min}/J strain mice treated with the compound of Example 1 has a statistically significant lower number of tumors in the small intestine when compared to vehicle-treated animals.

Table 11

Treatment	**Number of Animals per Group**	**Average Number of polyps per Animal**	**p Value**
Vehicle	13	7.3 ± 0.53	
Example 1	9	4.3 ± 0.70	0.0028

Data in Table 11 provides evidence that *in vivo* treatment with Example 1 reduces the number of intestinal polyps in C57BL/6J-*Apc*^{Min}/J strain mice.

Colony Formation Assay

The compound of Example 1 is also tested in an *in vitro* colony formation assay against four different human tumor cell lines, three derived from pancreatic tumors (Capan-2, HPAF-II, and Panc 04.03) and one derived from non-small cell lung cancer (A549). This assay is carried out using commercially available materials by procedures known and routinely used by those skilled in the art.

A549 cells (ATCC, Manassas, VA # CCL-185) are maintained in complete medium of F12K (Hyclone, Logan, UT #SH30526) containing 10% FBS (Invitrogen, Grand Island, NY #16000-044). Capan-2 cells (ATCC, Manassas, VA # HTB-80) are maintained in complete medium of McCoys 5A (Hyclone, Logan, UT #SH30200) containing 10% FBS (Invitrogen, Grand Island, NY #16000-044). HPAF-II cells (ATCC, Manassas, VA #CRL-1997) are maintained in complete medium of EMEM (Hyclone, Logan, UT #SH30024) containing 10% FBS (Invitrogen, Grand Island, NY #16000-044). Panc 04.03 cells (ATCC, Manassas, VA #CRL-2555) are maintained in complete medium of RPMI (Hyclone, Logan, UT #SH30255) containing 15% FBS (Invitrogen, Grand Island, NY #16000-044) and 20 µg/ml Insulin (Sigma, St. Louis, MO #I9278). Colony formation assays are done by plating A549 cells at 250 cells/well; Capan-2 cells at 2000 cells/well; HPAF-II cells at 1000 cells/well or Panc 04.03 cells at 2000 cells/well each in a 6-well plate (Corning Life Sciences, Tewksbury, MA #353046), incubating in 2 ml complete medium and grown overnight at 37°C, 5% CO_2. The next day the medium is removed and replaced with 2 ml fresh respective growth medium for each cell line. Test compounds in 100% DMSO are diluted in 100% DMSO to 1000X concentration and 2 µl added to 2 ml of medium in the well to achieve a final concentration of 0.03 to 3 µM. Cells are incubated at 37°C, 5% CO_2. Every three to four days, the medium is removed and replaced with 2 ml fresh complete medium for each cell line as before. After the medium change, fresh compound is added as above. Cells are incubated a total of 11 days in the presence of compound. On day 11, the medium is removed and the cells washed once with 5 ml DPBS (Hyclone, Logan, UT #SH30028). Crystal violet stain (0.5% crystal violet (Sigma, St Louis, MO, #C3886) in 20% Methanol (EMD Millipore, Billerica, MA #MX0490-4) is added to each well (0.4 ml)and incubated at room

temperature for 15 min. The wells are then washed twice with DPBS and the plates photographed using the Fuji LAS4000 (FujiFilm, Tokyo, Japan). Analysis of the colony area within each well is done using FujiFilm Colony Version 1.1 Software (FujiFilm, Tokyo, Japan).

Table 12

Effect of Compound of Example 1 on Colony Formation

	% Inhibition of Colony Formation			
Compound of Example 1 Concentration	**A549**	**Capan-2**	**HPAF-II**	**Panc 04.03**
Control	0	0	0	0
0.03 μM	25.46	-20.82	2.44	-34.35
0.11 μM	46.50	18.04	40.47	51.39
0.33 μM	54.23	55.90	61.65	92.79
1 μM	74.35	63.92	85.22	92.51
3 μM	72.07	70.73	88.23	90.03

The data in Table 12 evidences the compound of Example 1 inhibits colony formation when compared to control against each of the cell lines tested.

Table 13

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Polypeptide Used in Assays	**Amino Acid Sequences**
hTRF1 NCBI, Accession number NP_059523.2	(SEQ ID NO: 1)
hTNKS1 NCBI, Accession number NP_003738.2	(SEQ ID NO: 2)
hTNKS2 NCBI, Accession number NP_079511.1	(SEQ ID NO: 3)

hAxin2 NCBI, Accession number NP_004646.3	(SEQ ID NO: 4)
EGFP NCBI, Accession number ABG78037.1	(SEQ ID NO: 5)
Flag Peptide Sigma-Aldrich	(SEQ ID NO: 6)
EGFP Amino acids 228-466 NCBI, Accession number ABG78037.1	(SEQ ID NO: 7)

Sequences

SEQ ID NO: 1 - hTRF1 - protein

MAEDVSSAAPSPRGCADGRDADPTEEQMAETERNDEEQFECQELLECQVQVGA
PEEEEEEEEDAGLVAEAEAVAAGWMLDFLCLSLCRAFRDGRSEDFRRTRNSAEAI
IHGLSSLTACQLRTIYICQFLTRIAAGKTLDAQFENDERITPLESALMIWGSIEKEH
DKLHEEIQNLIKIQAIAVCMENGNFKEAEEVFERIFGDPNSHMPFKSKLLMIISQKD
TFHSFFQHFSYNHMMEKIKSYVNYVLSEKSSTFLMKAAAKVVESKRTRTITSQDK
PSGNDVEMETEANLDTRKSVSDKQSAVTESSEGTVSLLRSHKNLFLSKLQHGTQ
QQDLNKKERRVGTPQSTKKKKESRRATESRIPVSKSQPVTPEKHRARKRQAWLW
EEDKNLRSGVRKYGEGNWSKILLHYKFNNRTSVMLKDRWRTMKKLKLISSDSE
D

SEQ ID NO: 2 - hTNKS1 - protein

MAASRRSQHHHHHHQQQLQPAPGASAPPPPPPPLSPGLAPGTTPASPTASGLAPF
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AAGVAPNPAGSGSNNSPSSSSSPTSSSSSSPSSPGSSLAESPEAAGVSSTAPLGPGA
AGPGTGVPAVSGALRELLEACRNGDVSRVKRLVDAANVNAKDMAGRKSSPLHF
AAGFGRKDVVEHLLQMGANVHARDDGGLIPLHNACSFGHAEVVSLLLCQGADP
NARDNWNYTPLHEAAIKGKIDVCIVLLQHGADPNIRNTDGKSALDLADPSAKAV
LTGEYKKDELLEAARSGNEEKLMALLTPLNVNCHASDGRKSTPLHLAAGYNRV
RIVQLLLQHGADVHAKDKGGLVPLHNACSYGHYEVTELLLKHGACVNAMDLW
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KGHSLLQAAREADLAKVKKTLALEIINFKQPQSHETALHCAVASLHPKRKQVTEL
LLRKGANVNEKNKDFMTPLHVAAERAHNDVMEVLHKHGAKMNALDTLGQTA
LHRAALAGHLQTCRLLLSYGSDPSIISLQGFTAAQMGNEAVQQILSESTPIRTSDV
DYRLLEASKAGDLETVKQLCSSQNVNCRDLEGRHSTPLHFAAGYNRVSVVEYLL
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AAAKGKYEICKLLLKHGADPTKKNRDGNTPLDLVKEGDTDIQDLLRGDAALLD
AAKKGCLARVQKLCTPENINCRDTQGRNSTPLHLAAGYNNLEVAEYLLEHGAD
VNAQDKGGLIPLHNAASYGHVDIAALLIKYNTCVNATDKWAFTPLHEAAQKGR

TQLCALLLAHGADPTMKNQEGQTPLDLATADDIRALLIDAMPPEALPTCFKPQAT
VVSASLISPASTPSCLSAASSIDNLTGPLAELAVGGASNAGDGAAGTERKEGEVA
GLDMNISQFLKSLGLEHLRDIFETEQITLDVLADMGHEELKEIGINAYGHRHKLIK
GVERLLGGQQGTNPYLTFHCVNQGTILLDLAPEDKEYQSVEEEMQSTIREHRDG
GNAGGIFNRYNVIRIQKVVNKKLRERFCHRQKEVSEENHNHHNERMLFHGSPFIN
AIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPTHKDRSCYIC
HRQMLFCRVTLGKSFLQFSTMKMAHAPPGHHSVIGRPSVNGLAYAEYVIYRGEQ
AYPEYLITYQIMKPEAPSQTATAAEQKT

SEQ ID NO: 3 - hTNKS2 - protein

MSGRRCAGGGAACASAAAEAVEPAARELFEACRNGDVERVKRLVTPEKVNSRD
TAGRKSTPLHFAAGFGRKDVVEYLLQNGANVQARDDGGLIPLHNACSFGHAEV
VNLLLRHGADPNARDNWNYTPLHEAAIKGKIDVCIVLLQHGAEPTIRNTDGRTA
LDLADPSAKAVLTGEYKKDELLESARSGNEEKMMALLTPLNVNCHASDGRKSTP
LHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVTELLVKHG
ACVNAMDLWQFTPLHEAASKNRVEVCSLLLSYGADPTLLNCHNKSAIDLAPTPQ
LKERLAYEFKGHSLLQAAREADVTRIKKHLSLEMVNFKHPQTHETALHCAAASP
YPKRKQICELLLRKGANINEKTKEFLTPLHVASEKAHNDVVEVVVKHEAKVNAL
DNLGQTSLHRAAYCGHLQTCRLLLSYGCDPNIISLQGFTALQMGNENVQQLLQE
GISLGNSEADRQLLEAAKAGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYN
RVSVVEYLLQHGADVHAKDKGGLVPLHNACSYGHYEVAELLVKHGAVVNVAD
LWKFTPLHEAAAKGKYEICKLLLQHGADPTKKNRDGNTPLDLVKDGDTDIQDLL
RGDAALLDAAKKGCLARVKKLSSPDNVNCRDTQGRHSTPLHLAAGYNNLEVAE
YLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNATDKWAFTPL
HEAAQKGRTQLCALLLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPSAL
PSCYKPQVLNGVRSPGATADALSSGPSSPSSLSAASSLDNLSGSFSELSSVVSSSGT
EGASSLEKKEVPGVDFSITQFVRNLGLEHLMDIFEREQITLDVLVEMGHKELKEIG
INAYGHRHKLIKGVERLISGQQGLNPYLTLNTSGSGTILIDLSPDDKEFQSVEEEM
QSTVREHRDGGHAGGIFNRYNILKIQKVCNKKLWERYTHRRKEVSEENHNHANE
RMLFHGSPFVNAIIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGC

PVHKDRSCYICHRQLLFCRVTLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLA
LAEYVIYRGEQAYPEYLITYQIMRPEGMVDG

SEQ ID NO: 4 - hAxin2 - protein

MSSAMLVTCLPDPSSSFREDAPRPPVPGEEGETPPCQPGVGKGQVTKPMPVSSNT
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WFACNGFRQMNLKDTKTLRVAKAIYKRYIENNSIVSKQLKPATKTYIRDGIKKQ
QIDSIMFDQAQTEIQSVMEENAYQMFLTSDIYLEYVRSGGENTAYMSNGGLGSLK
VVCGYLPTLNEEEEWTCADFKCKLSPTVVGLSSKTLRATASVRSTETVDSGYRSF
KRSDPVNPYHIGSGYVFAPATSANDSEISSDALTDDSMSMTDSSVDGIPPYRVGS
KKQLQREMHRSVKANGQVSLPHFPRTHRLPKEMTPVEPATFAAELISRLEKLKLE
LESRHSLEERLQQIREDEEREGSELTLNSREGAPTQHPLSLLPSGSYEEDPQTILDD
HLSRVLKTPGCQSPGVGRYSPRSRSPDHHHHHHSQYHSLLPPGGKLPPAAASPGA
CPLLGGKGFVTKQTTKHVHHHYIHHHAVPKTKEEIEAEATQRVHCFCPGGSEYY
CYSKCKSHSKAPETMPSEQFGGSRGSTLPKRNGKGTEPGLALPAREGGAPGGAG
ALQLPREEGDRSQDVWQWMLESERQSKPKPHSAQSTKKAYPLESARSSPGERAS
RHHLWGGNSGHPRTTPRAHLFTQDPAMPPLTPPNTLAQLEEACRRLAEVSKPPK
QRCCVASQQRDRNHSATVQTGATPFSNPSLAPEDHKEPKKLAGVHALQASELVV
TYFFCGEEIPYRRMLKAQSLTLGHFKEQLSKKGNYRYYFKKASDEFACGAVFEEI
WEDETVLPMYEGRILGKVERID

SEQ ID NO: 5 – EGFP – full length protein

MDRKFVFLVSILSIVVASVTGETTRAPTPTPTPTPTPTPTPTPTPTPTPTPTPTP
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VQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV
NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGS
VQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGI
TLGMDELYK

SEQ ID NO: 6 - Flag Peptide

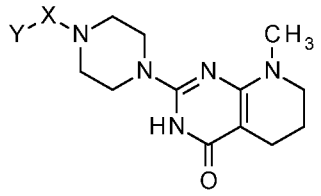
DYKDDDDK

SEQ ID NO: 7 – EGFP – truncated protein amino acids 228-466

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLP
VPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKT
RAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIK
VNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDH
MVLLEFVTAAGITLGMDELYK

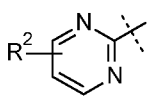
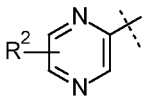
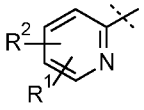
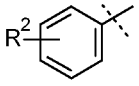
We claim:

1. A compound having the formula:



wherein:

Y is:


, 
, 
, or 
;

X is $-CH_2-$, $-C(CH_3)H-$, or $-C(CH_2CH_3)H-$;

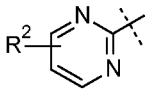
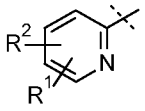
R^1 is hydrogen, hydroxy, or halo;

R^2 is hydrogen, halo, -CN, $-CH_3$, CF_3, or $-OCH_3$;

or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 wherein:

Y is:


or 
;

X is $-CH_2-$, $-C(CH_3)H-$, or $-C(CH_2CH_3)H-$;

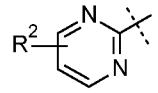
R^1 is hydrogen, hydroxy, or halo;

R^2 is hydrogen, halo, -CN, $-CH_3$, CF_3, or $-OCH_3$;

or a pharmaceutically acceptable salt thereof.

3. A compound of claim 1 or 2 wherein:

Y is



;

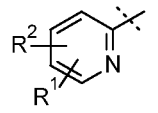
X is $-CH_2-$, $-C(CH_3)H-$, or $-C(CH_2CH_3)H-$;

R^2 is hydrogen, halo, -CN, $-CH_3$, CF_3, or $-OCH_3$;

or a pharmaceutically acceptable salt thereof.

4. A compound of claim 1 or 2 wherein:

Y is:



;

X is $-CH_2-$, $-C(CH_3)H-$, or $-C(CH_2CH_3)H-$;

R^1 is hydrogen, hydroxy, or halo;

R^2 is hydrogen, halo, -CN, $-CH_3$, CF_3, or $-OCH_3$;

or a pharmaceutically acceptable salt thereof.

5. A compound of any one of claims 1, 2, or 3 which is:

8-Methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof;

8-Methyl-2-[4-(1-pyrimidin-2-ylethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof thereof;

2-[4-[(4-Chloropyrimidin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof; or

2-[4-[(4-methoxypyrimidin-2-yl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, or a pharmaceutically acceptable salt thereof.

6. A compound of any one of claims 1, 2, or 4 which is:

2-[4-[(3-Bromo-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one or a pharmaceutically acceptable salt thereof;

2-[4-[(3-Chloro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, , or a pharmaceutically acceptable salt thereof;
2-[4-[(3-Fluoro-2-pyridyl)methyl]piperazin-1-yl]-8-methyl-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one, , or a pharmaceutically acceptable salt thereof; or
2-[[4-(8-Methyl-4-oxo-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-2-yl)piperazin-1-yl]methyl]pyridine-3-carbonitrile, or a pharmaceutically acceptable salt thereof.

7. A compound of any one of claims 1, 2, 3 or 5 which is 8-methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4(3H)-one, or a pharmaceutically acceptable salt thereof.

8. A compound of any one of claims 1, 2, 3, 5 or 7 which is 8-Methyl-2-[4-(pyrimidin-2-ylmethyl)piperazin-1-yl]-3,5,6,7-tetrahydropyrido[2,3-d]pyrimidin-4-one 4-methylbenzenesulfonic acid salt.

9. A pharmaceutical composition comprising a compound of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable carrier.

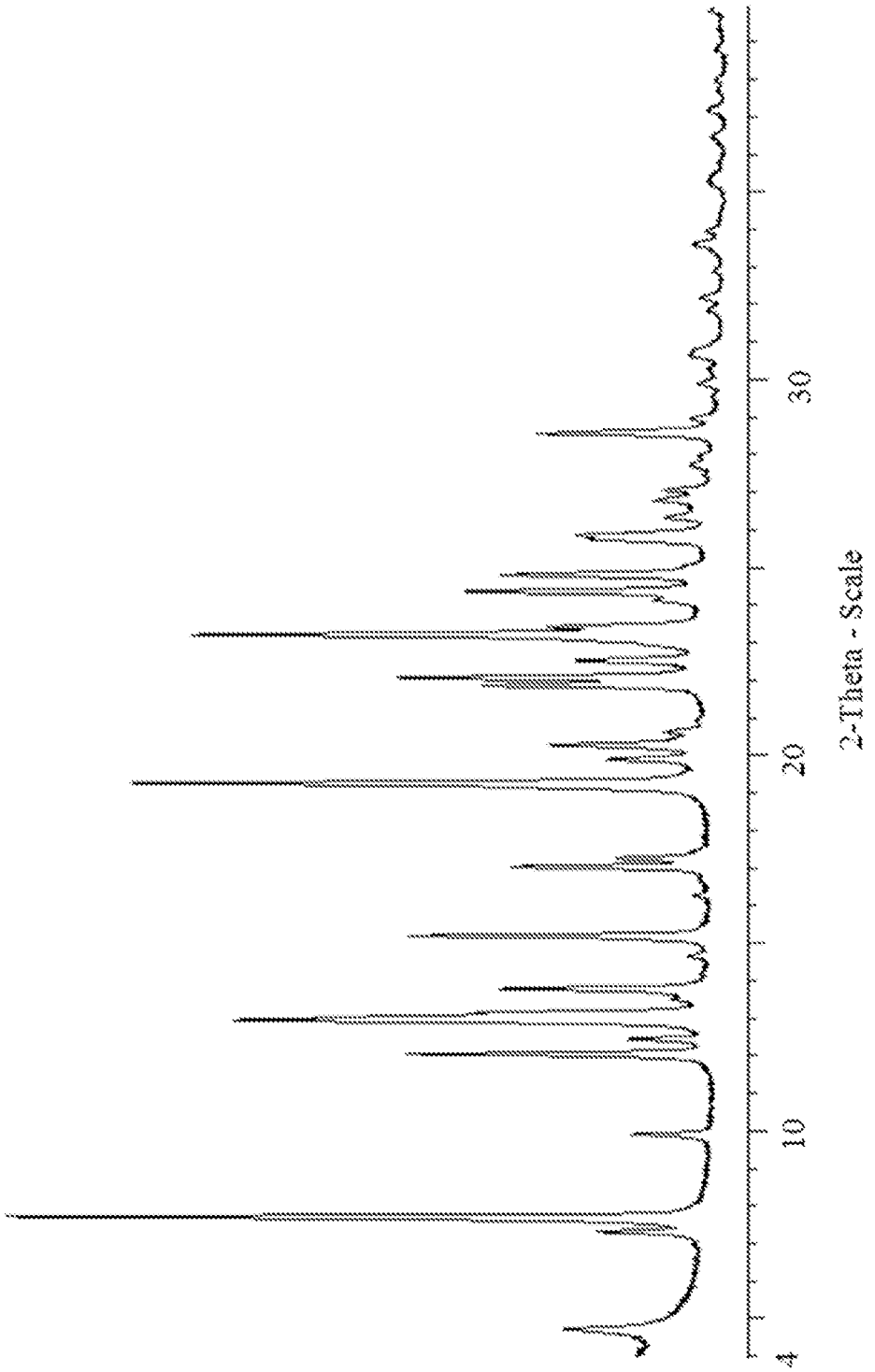
10. A method of treating a cancer which is colorectal cancer, gastric cancer, liver cancer, breast cancer, triple negative breast cancer, ovarian cancer, medulloblastoma, melanoma, lung cancer, non-small cell lung cancer, pancreatic cancer, prostate cancer, glioblastoma, T-cell lymphoma, T-lymphoblastic lymphoma, T-cell acute lymphocytic leukemia (T-ALL), mantle cell lymphoma, multiple myeloma, chronic myeloid leukemia, or acute myeloid leukemia in a patient comprising administering to a patient in need thereof a therapeutically effective amount of a compound of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof.

11. A compound of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, for use in therapy.

12. A compound of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, for use in the treatment of a cancer which is colorectal cancer, gastric cancer,

liver cancer, breast cancer, triple negative breast cancer, ovarian cancer, medulloblastoma, melanoma, lung cancer, non-small cell lung cancer, pancreatic cancer, prostate cancer, glioblastoma, T-cell lymphoma, T-lymphoblastic lymphoma, T-cell acute lymphocytic leukemia (T-ALL), mantle cell lymphoma, multiple myeloma, chronic myeloid leukemia, or acute myeloid leukemia.

Fig. 1



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/062832

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D471/04 A61K31/519 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/117288 A1 (MERCK PATENT GMBH [DE]) 15 August 2013 (2013-08-15) cited in the application claim 1	1-12
A	GIANLUCA PAPEO ET AL: "PARP inhibitors in cancer therapy: an update", CURRENT OPINION IN THERAPEUTIC PATENTS,, vol. 23, no. 4, 1 April 2013 (2013-04-01), pages 503-514, XP008163073, ISSN: 0962-2594, DOI: 10.1517/13543776.2013.768615 figure 1; compound 3	1-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 2015	13/02/2015

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/062832

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