TREATMENT OF B-CELL MALIGNANCIES BY A COMBINATION JAK AND PI3K INHIBITORS

This application claims the benefit of priority of 61/976,815, filed April 8, 2014, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

This invention relates to methods of treating B-cell malignancies using a combination of inhibitors of JAK1 and/or JAK2 and inhibitors of PI3Kδ.

BACKGROUND

The B-cell receptor (BCR) is present on both normal and most malignant B-cells. Engagement of the BCR provides important survival signals, and interruption of the BCR signal can lead to B-cell death. Studies performed with siRNA to inhibit BCR expression have shown that constitutive signaling by the BCR is critical for the survival and proliferation of human B-cell lymphomas. The primary role of BCR signaling in these cells appears to be activation of spleen tyrosine kinase (Syk) which in turn leads to several downstream events that promote cell survival, including activation of Bruton tyrosine kinase (BTK), phosphatidylinositol 3 kinase (PI3K), and AKT. A number of B-cell malignancies, including diffuse large B-cell lymphomas (DLBCL), have been shown to be particularly dependent upon BCR survival signals as evidenced by their sensitivity to genetic and pharmacological inhibition of BCR signaling components in vitro. It has been shown that DLBCL cells engage PI3K, augmenting anti-apoptotic NF-kB signaling and survival signals and that inhibition of the PI3K/AKT pathway synergizes with NF-kB inhibition in killing DLBCL cell lines *in vitro*.

Aberrant activation of JAKs, through production of cytokines and growth factors, has also been associated with increased malignant cell proliferation and survival in a number of tumor types. JAKs activate a number of downstream pathways implicated in the proliferation and survival of malignant cells including the STATs, a family of important latent transcription factors. Of clinical relevance, levels of serum IL-10 and IL-6, which signal through the JAKs, have been found to be elevated in patients with

DLBCL compared to normal controls (Gupta et al, 2012). Further, patients with high serum IL-10 levels were shown to have a shorter event-free survival (Gupta et al, 2012). Within the JAK family of kinases, JAK1 has been shown to cooperate with JAK2, JAK3, and TYK2 and to play a dominant role in mediating the signaling of a number of inflammatory cytokines including IL-6, IL-10 and interferon.

In DLBCL, JAK pathway activation occurs through both autocrine and paracrine mechanisms. In the tumor cells, BCR signaling leads to increased IL-6 and IL-10 production through activation of the NF-kB pathway (Lam et al, 2008). A subset of DLBCLs has been characterized as having high expression of STAT3, IL-6, and/or IL-10 and it has been shown that JAK inhibition is cytotoxic in these DLBCL cell lines and synergizes with NF-kB inhibitors. In addition to JAK/STAT pathway activation through autocrine pathways, the stromal compartment can also provide a source of these cytokines in a paracrine manner (Hodge et al, 2005).

For these reasons, there is a need to develop new therapies that can be used to treat B-cell malignancies, such as DLBCL. This invention is directed to this need and others.

DESCRIPTION OF THE DRAWINGS

FIG. 1A depicts Western blot analysis probing for IL6 and IL10 for various DLBCL cell lines.

FIG. 1B depicts Western blot analysis for actin and p-Stat3 for Pfeiffer cells treated with IL6 or IL10.

FIG. 2A depicts % inhibition in the cellular proliferation assay in Pfeiffer cells as a function of the concentration of Compound 28 with vehicle (DMSO), DMSO+IL10, and DMSO+IL10+ruxolitinib.

FIG. 2B depicts % inhibition in the cellular proliferation assay in Pfeiffer cells as a function of the concentration of Compound 28 with vehicle (DMSO), DMSO+IL10, and DMSO+IL10+Compound 7.

FIG. 3 depicts % inhibition in the cellular proliferation assay in HBL-1 cells as a function of the concentration of Compound 28 with vehicle (DMSO), DMSO+IL10, and DMSO+IL10+ruxolitinib.

FIG. 4 depicts Western blot analysis of Pfeiffer cells after treatment with vehicle (DMSO), ruxolitinib, Compound 28, or Compound 28 and ruxolitinib with or without IL10.

FIG. 5 depicts Western blot analysis of Pfeiffer cells after treatment with vehicle (DMSO), Compound 7, Compound 28, or Compound 28 and Compound 7 with or without IL10.

FIG. 6 depicts % inhibition in the cellular proliferation assay in Pfeiffer cells as a function of the concentration of Compound 28 with vehicle (DMSO), DMSO+IL10, and DMSO+IL10+Compound 16.

FIG. 7 depicts Annexin-V staining of Pfeiffer cells treated with Compound 28 +/- Compound 16, showing a synergistic apoptosis induction from the combination therapy.

FIG. 8 depicts Western blot analysis of Pfeiffer cells after treatment with Compound 28 +/- Compound 16 showing effect on STAT3 and pAKT.

SUMMARY

The present application provides a method of treating a B-cell malignancy in a patient in need thereof, comprising administering to said patient: (a) an inhibitor of JAK1 and/or JAK2; and (b) an inhibitor of PI3Kδ.

The present application further provides a method of treating a disease selected from diffuse large B-cell lymphoma, chronic lymphocytic leukemia (CLL), Non-Hodgkin lymphoma, hairy cell leukemia, Mantle cell lymphoma, small lymphocytic lymphoma, follicular lymphoma, lymphoplasmacytic lymphoma, extranodal marginal zone lymphoma, Hodgkin's lymphoma, Burkitt lymphoma, Waldenstrom's macroglobulinemia, prolymphocytic leukemia, acute lymphoblastic leukemia, myelofibrosis, mucosa-associated lymphatic tissue (MALT) lymphoma, mediastinal (thymic) large B-cell lymphoma, lymphomatoid granulomatosis, splenic marginal zone lymphoma, primary

effusion lymphoma, intravascular large B-cell lymphoma, plasma cell leukemia, extramedullary plasmacytoma, smouldering myeloma (aka asymptomatic myeloma), monoclonal gammopathy of undetermined significance (MGUS), activated B-cell like (ABC) diffuse large B cell lymphoma (ABC-DLBCL), and germinal center B cell (GCB) diffuse large B cell lymphoma (GCB-DLBCL) in a patient in need thereof, comprising administering to said patient: (a) an inhibitor of JAK1 and/or JAK2; and (b) an inhibitor of PI3Kδ.

In some embodiments of the methods, the inhibitor of JAK1 and/or JAK2 is selected from:

3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

3-[1-(6-chloropyridin-2-yl)pyrrolidin-3-yl]-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile;

4-[(4-{3-cyano-2-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrrol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile;

{1-{1-[3-Fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-[4-fluoro-2-(trifluoromethyl)phenyl]piperidine-1-carboxamide;

[3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-1-(1-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperidin-4-yl)azetidin-3-yl]acetonitrile;

[trans-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-3-(4-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperazin-1-yl)cyclobutyl]acetonitrile;

{trans-3-(4-{[4-[(3-hydroxyazetidin-1-yl)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{trans-3-(4-{[4-{[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{trans-3-(4-{[4-{[(2R)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

4-(4-{3-[(dimethylamino)methyl]-5-fluorophenoxy}piperidin-1-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]butanenitrile;

5-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide;

4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide;

5-{3-(cyanomethyl)-3-[4-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide;

{1-(cis-4-{[6-(2-hydroxyethyl)-2-(trifluoromethyl)pyrimidin-4-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{1-(cis-4-{[4-[(ethylamino)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{1-(cis-4-{[4-(1-hydroxy-1-methylethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{1-(cis-4-{[4-{[(3R)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{1-(cis-4-{[4-{[(3S)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{trans-3-(4-{[4-({[(1S)-2-hydroxy-1-methylethyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{trans-3-(4-{[4-({[(2R)-2-hydroxypropyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{trans-3-(4-{[4-({[(2S)-2-hydroxypropyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{trans-3-(4-{[4-(2-hydroxyethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

((2R,5S)-5-{2-[(1R)-1-hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile;

4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide;

and pharmaceutically acceptable salts of any of the aforementioned.

In some embodiments of the methods, the inhibitor of PI3Kδ is selected from:

7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one;

(S)-7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one;

4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-{1-[(2S)-2-hydroxypropyl]azetidin-3-yl}-3-methoxybenzonitrile;

4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-[1-(2-hydroxyethyl)azetidin-3-yl]-3-methoxybenzonitrile;

5-{3-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-cyano-2-ethoxy-5-methylphenyl}-N,N-dimethylpyridine-2-carboxamide;

4-{3-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-5-chloro-2-ethoxy-6-fluorophenyl}pyrrolidin-2-one; and

N-{1-[5-chloro-8-(3-fluorophenyl)cinnolin-7-yl]ethyl}-9H-purin-6-amine;

4-chloro-3'-fluoro-3-methyl-6-[1-(9H-purin-6-ylamino)ethyl]biphenyl-2-carbonitrile;

and pharmaceutically acceptable salts of any of the aforementioned.

The present application also provides an inhibitor of JAK1 and/or JAK2 for use in combination with PI3Kδ inhibitor for the treatment of a B-cell malignancy or any of the diseases embodied herein.

The present application further provides use of an inhibitor of JAK1 and/or JAK2 and an PI3Kδ inhibitor for preparation of a medicament for treatment of a B-cell malignancy or any of the diseases embodied herein.

DETAILED DESCRIPTION

The present application provides, *inter alia*, a method of treating a disease selected from diffuse large B-cell lymphoma, chronic lymphocytic leukemia (CLL), Non-Hodgkin lymphoma, hairy cell leukemia, Mantle cell lymphoma, small lymphocytic lymphoma, follicular lymphoma, lymphoplasmacytic lymphoma, extranodal marginal zone lymphoma, Hodgkin's lymphoma, Burkitt lymphoma, Waldenstrom's macroglobulinemia, prolymphocytic leukemia, acute lymphoblastic leukemia, myelofibrosis, mucosa-associated lymphatic tissue (MALT) lymphoma, mediastinal (thymic) large B-cell lymphoma, lymphomatoid granulomatosis, splenic marginal zone lymphoma, primary effusion lymphoma, intravascular large B-cell lymphoma, plasma cell leukemia, extramedullary plasmacytoma, smouldering myeloma (aka asymptomatic myeloma), monoclonal gammopathy of undetermined significance (MGUS), activated B-cell like (ABC) diffuse large B cell lymphoma (ABC-DLBCL), and germinal center B cell (GCB) diffuse large B cell lymphoma (GCB-DLBCL) in a patient in need thereof, comprising administering to said patient: (a) an inhibitor of JAK1 and/or JAK2; and (b) an inhibitor of PI3Kδ.

In some embodiments, the non-Hodgkin lymphoma is non-Hodgkin lymphoma (NHL) is relapsed or refractory NHL or recucurrent follicular NHL.

In some embodiments, the disease is diffuse large B cell lymphoma (DLBCL).

In some embodiments, the disease is activated B-cell like (ABC) diffuse large B cell lymphoma (ABC-DLBCL) or germinal center B cell (GCB) diffuse large B cell lymphoma (GCB-DLBCL).

In some embodiments, the inhibitor of JAK1 and/or JAK2 and the inhibitor of PI3Kδ are administered simultaneously.

In some embodiments, the inhibitor of JAK1 and/or JAK2 and the inhibitor of PI3Kδ are administered sequentially.

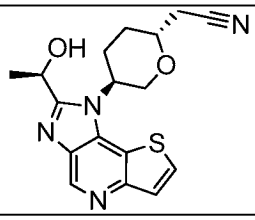
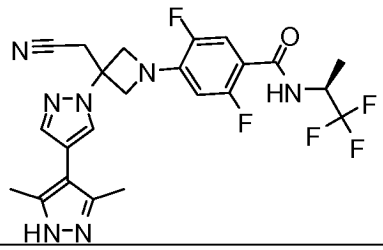
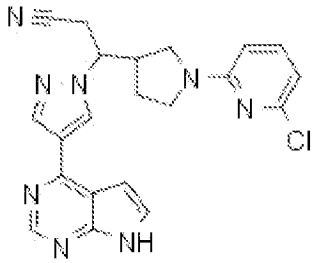
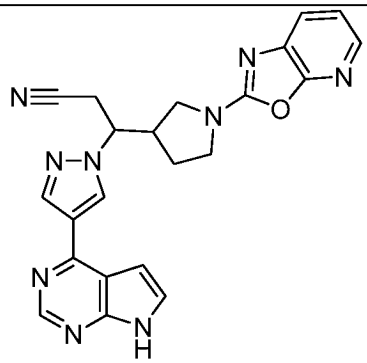
In some embodiments, the inhibitor of JAK1 and/or JAK2 is selective for JAK1 and JAK1 over JAK3 and TYK2. In some embodiments, the inhibitor of JAK1 and/or JAK2 is selective for JAK1 over JAK2, JAK3, and TYK2. For example, some of the compounds described herein, or a pharmaceutically acceptable salt thereof, preferentially inhibit JAK1 over one or more of JAK2, JAK3, and TYK2. In some embodiments, the compounds inhibit JAK1 preferentially over JAK2 (e.g., have a JAK1/JAK2 IC_{50} ratio >1). In some embodiments, the compounds or salts are about 10-fold more selective for JAK1 over JAK2. In some embodiments, the compounds or salts are about 3-fold, about 5-fold, about 10-fold, about 15-fold, or about 20-fold more selective for JAK1 over JAK2 as calculated by measuring IC_{50} at 1 mM ATP (e.g., see Example A).

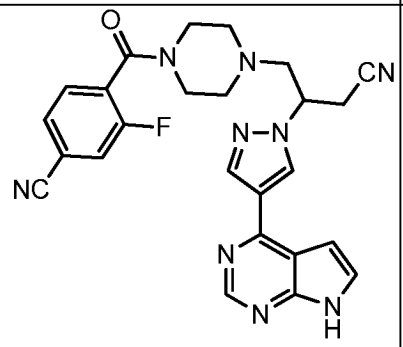
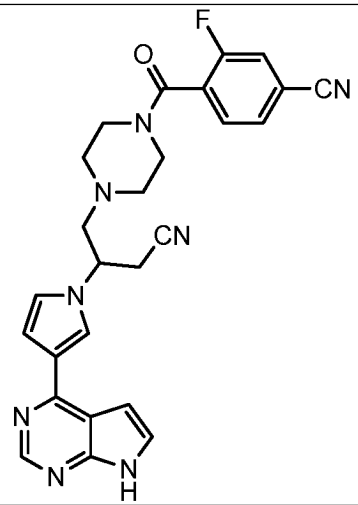
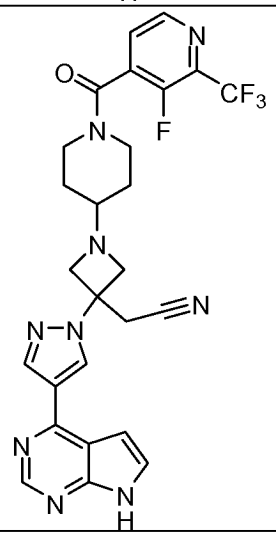
In some embodiments, the inhibitor of JAK1 and/or JAK2 is 3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile. In some embodiments, the inhibitor of JAK1 and/or JAK2 is (3*R*)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile (ruxolitinib; also known as INCB018424). Ruxolitinib has an IC_{50} of less than 10 nM at 1 mM ATP (assay A) at JAK1 and JAK2. 3-Cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile and ruxolitinib can be made by the procedure described in US 7,598,257 (Example 67), filed December 12, 2006, which is incorporated herein by reference in its entirety. In some embodiments, the inhibitor of JAK1 and/or JAK2 is (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile phosphoric acid salt.

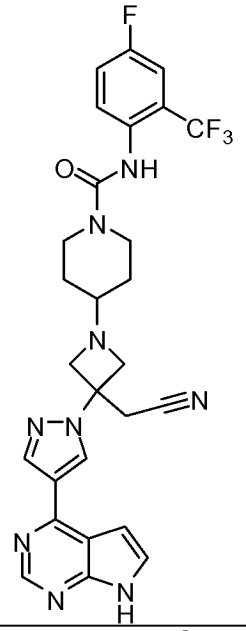
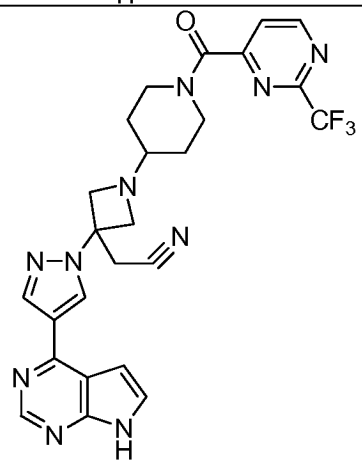
In some embodiments, the inhibitor of JAK1 and/or JAK2 is a compound of Table 1, or a pharmaceutically acceptable salt thereof. The compounds in Table 1 are selective

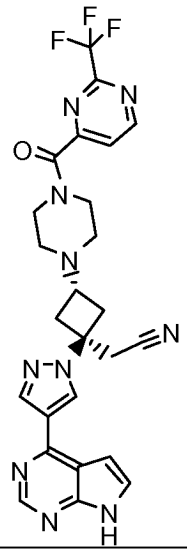
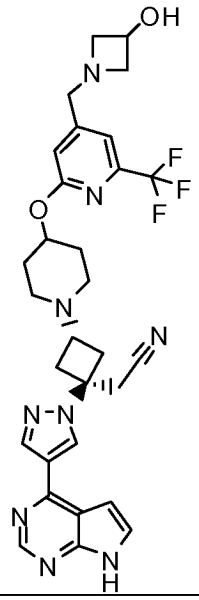
JAK1 inhibitors (selective over JAK2, JAK3, and TYK2). The IC_{50}s obtained by the method of Assay A at 1 mM ATP are shown in Table 1.

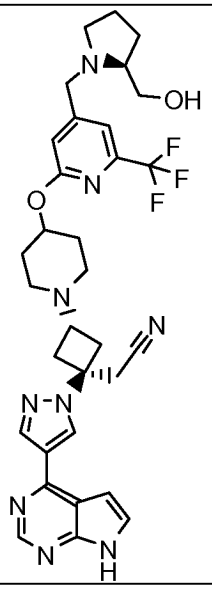
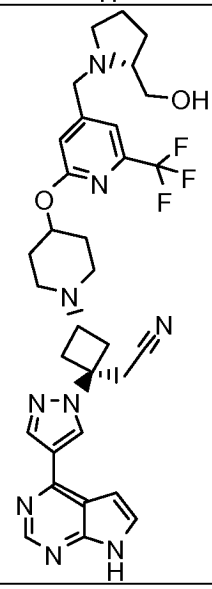
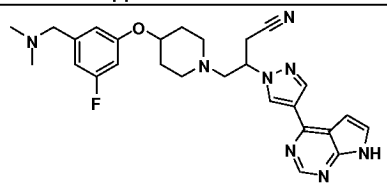
Table 1

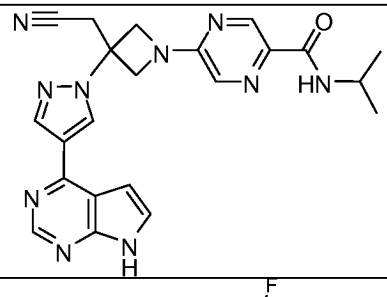
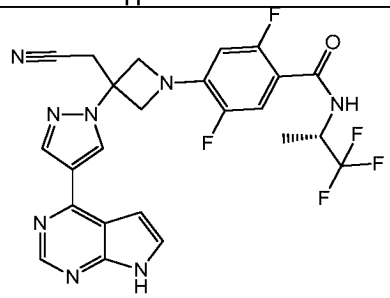
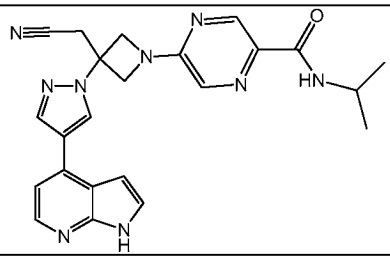
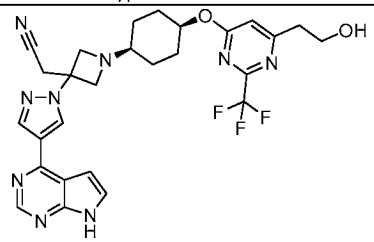
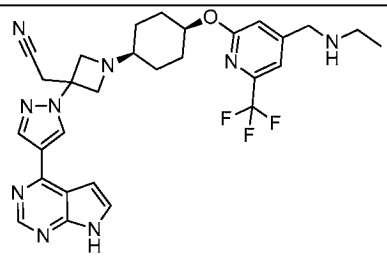
#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
1	Example 1 herein	((2R,5S)-5-{2-[(1R)-1-hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile		++	>10
2	Example 2 herein	4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide		+++	>10
3	US 2010/ 0298334 (Example 2)[a]	3-[1-(6-chloropyridin-2-yl)pyrrolidin-3-yl]-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile		+	>10
4	US 2010/ 0298334 (Example 13c)	3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile		+	>10

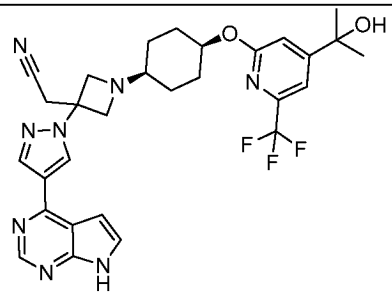
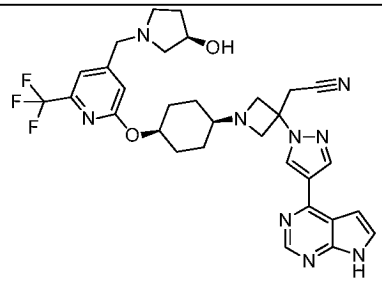
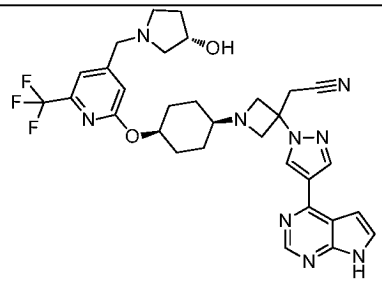
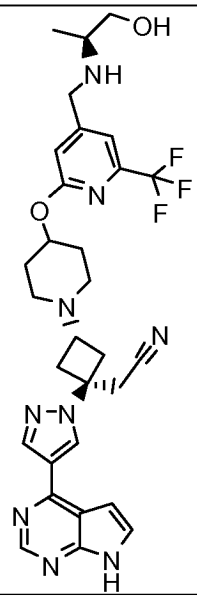
#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
5	US 2011/ 0059951 (Example 12)	4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile		+	>10
6	US 2011/ 0059951 (Example 13)	4-[(4-{3-cyano-2-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrrol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile		+	>10
7	US 2011/ 0224190 (Example 1)	{1-{1-[3-Fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile		+	>10

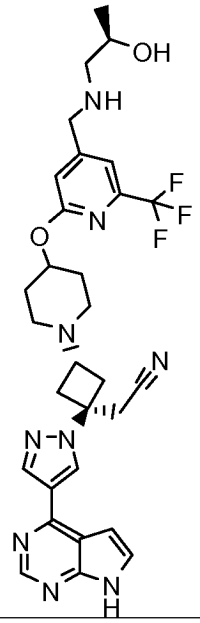
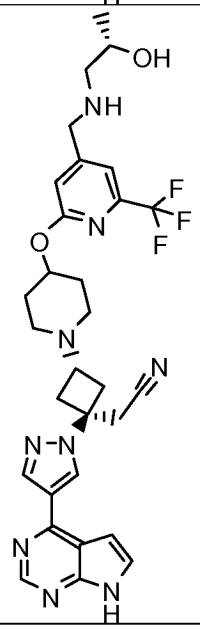
#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
8	US 2011/ 0224190 (Example 154)	4-{3-(Cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-[4-fluoro-2-(trifluoromethyl)phenyl] piperidine-1-carboxamide		+	>10
9	US 2011/ 0224190 (Example 85)	[3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-1-(1-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperidin-4-yl)azetidin-3-yl]acetonitrile		+	>10

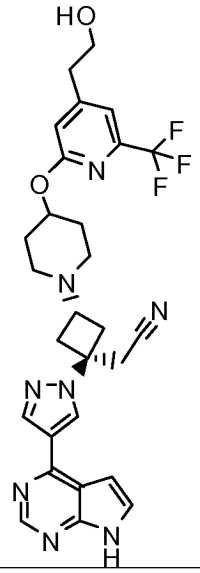
#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
10	US 2012/ 0149681 (Example 7b)	[*trans*-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-3-(4-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperazin-1-yl)cyclobutyl]acetonitrile		+	>10
11	US 2012/ 0149681 (Example 157)	{*trans*-3-(4-{[4-[(3-hydroxyazetidin-1-yl)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile		+	>10

#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
12	US 2012/ 0149681 (Example 161)	{*trans*-3-(4-{[4-{[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile		+	>10
13	US 2012/ 0149681 (Example 162)	{*trans*-3-(4-{[4-{[(2R)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile		+	>10
14	US 2012/ 0149682 (Example 20)[b]	4-(4-{3-[(dimethylamino)methyl]-5-fluorophenoxy}piperidin-1-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]butanenitrile		+	>10

#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
15	US 2013/ 0018034 (Example 18)	5-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide		+	>10
16	US 2013/ 0018034 (Example 28)	4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide		+	>10
17	US 2013/ 0018034 (Example 34)	5-{3-(cyanomethyl)-3-[4-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide		+	>10
18	US 2013/ 0045963 (Example 45)	{1-(*cis*-4-{[6-(2-hydroxyethyl)-2-(trifluoromethyl)pyrimidin-4-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile		+	>10
19	US 2013/ 0045963 (Example 65)	{1-(*cis*-4-{[4-[(ethylamino)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile		+	>10

#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
20	US 2013/ 0045963 (Example 69)	{1-(*cis*-4-{[4-(1-hydroxy-1-methylethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile		+	>10
21	US 2013/ 0045963 (Example 95)	{1-(*cis*-4-{[4-{[(3R)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile		+	>10
22	US 2013/ 0045963 (Example 95)	{1-(*cis*-4-{[4-{[(3S)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile		+	>10
23	US 2014/ 0005166 (Example 1)	{*trans*-3-(4-{[4-({[(1S)-2-hydroxy-1-methylethyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile		+	>10

#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
24	US 2014/ 0005166 (Example 14)	{*trans*-3-(4-{[4-({[(2R)-2-hydroxypropyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile		+	>10
25	US 2014/ 0005166 (Example 15)	{*trans*-3-(4-{[4-({[(2S)-2-hydroxypropyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile		+	>10

#	Prep.	Name	Structure	JAK1 IC_{50} (nM)	JAK2/ JAK1
26	US 2014/ 0005166 (Example 20)	{*trans*-3-(4-{[4-(2-hydroxyethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile		+	>10

+ means <10 nM (see Example A for assay conditions)
++ means ≤ 100 nM (see Example A for assay conditions)
+++ means ≤ 300 nM (see Example A for assay conditions)
[a]Data for enantiomer 1
[b]Data for enantiomer 2

In some embodiments, the inhibitor of JAK1 and/or JAK2 is {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile, or a pharmaceutically acceptable salt thereof.

In some embodiments, the inhibitor of JAK1 and/or JAK2 is {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile adipic acid salt.

In some embodiments, the inhibitor of JAK1 and/or JAK2 is 4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide, or a pharmaceutically acceptable salt thereof.

In some embodiments, the inhibitor of JAK1 and/or JAK2 is selected from (R)-3-[1-(6-chloropyridin-2-yl)pyrrolidin-3-yl]-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile, (R)-3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-

[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile, (R)-4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile, (R)-4-[(4-{3-cyano-2-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrrol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile, or (R)-4-(4-{3-[(dimethylamino)methyl]-5-fluorophenoxy}piperidin-1-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]butanenitrile, (S)-3-[1-(6-chloropyridin-2-yl)pyrrolidin-3-yl]-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile, (S)-3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile, (S)-4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile, (S)-4-[(4-{3-cyano-2-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrrol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile, (S)-4-(4-{3-[(dimethylamino)methyl]-5-fluorophenoxy}piperidin-1-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]butanenitrile; and pharmaceutically acceptable salts of any of the aforementioned.

In some embodiments, the compounds of Table 1 are prepared by the synthetic procedures described in US Patent Publ. No. 2010/0298334, filed May 21, 2010, US Patent Publ. No. 2011/0059951, filed August 31, 2010, US Patent Publ. No. 2011/0224190, filed March 9, 2011, US Patent Publ. No. 2012/0149681, filed November 18, 2011, US Patent Publ. No. 2012/0149682, filed November 18, 2011, US Patent Publ. 2013/0018034, filed June 19, 2012, US Patent Publ. No. 2013/0045963, filed August 17, 2012, and US Patent Publ. No. 2014/0005166, filed May 17, 2013, each of which is incorporated herein by reference in its entirety.

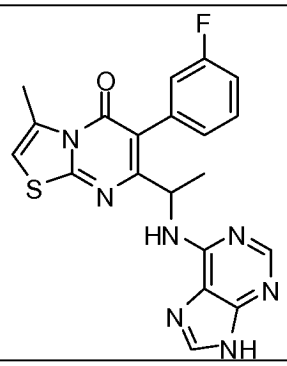
In some embodiments, the inhibitor of JAK1 and/or JAK2 is selected from the compounds of US Patent Publ. No. 2010/0298334, filed May 21, 2010, US Patent Publ. No. 2011/0059951, filed August 31, 2010, US Patent Publ. No. 2011/0224190, filed March 9, 2011, US Patent Publ. No. 2012/0149681, filed November 18, 2011, US Patent Publ. No. 2012/0149682, filed November 18, 2011, US Patent Publ. 2013/0018034, filed June 19, 2012, US Patent Publ. No. 2013/0045963, filed August 17, 2012, and US Patent Publ. No. 2014/0005166, filed May 17, 2013, each of which is incorporated herein by

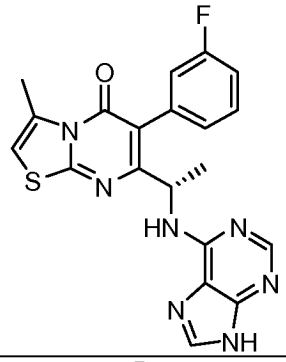
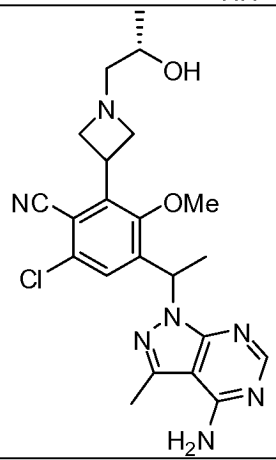
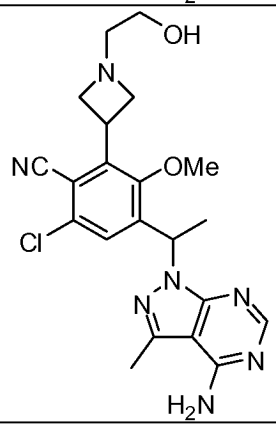
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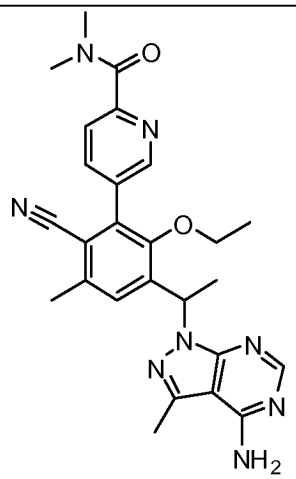
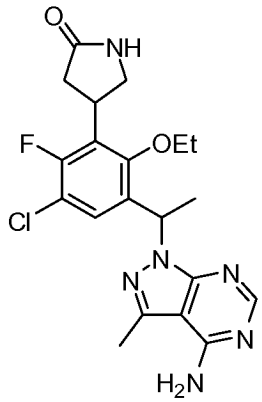
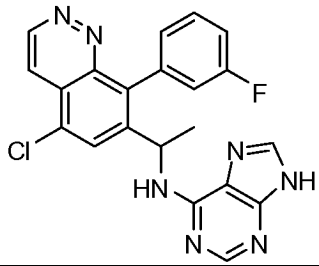
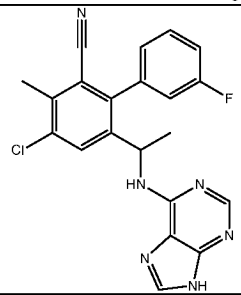
The inhibitors of PI3Kδ described herein can be selective. By "selective" is meant that the compound binds to or inhibits a kinase with greater affinity or potency, respectively, compared to at least one other kinase. In some embodiments, the compounds described herein are selective inhibitors of PI3Kδ (*e.g.*, over PI3Kα, PI3Kβ and PI3Kγ). In some embodiments, selectivity can be at least about 2-fold, 5-fold, 10-fold, at least about 20-fold, at least about 50-fold, at least about 100-fold, at least about 200-fold, at least about 500-fold or at least about 1000-fold. Selectivity can be measured by methods routine in the art. In some embodiments, selectivity can be tested at the K_m ATP concentration of each enzyme. In some embodiments, the selectivity of compounds described herein can be determined by cellular assays associated with particular PI3K kinase activity.

In some embodiments, the inhibitor of PI3Kδ is a compound shown in Table 2. The compounds of Table 2 have been tested in Assay B and shown to be inhibitors of PI3Kδ with the IC_{50}s in Table 2.

Table 2

#	Prep.	Name	Structure	PI3Kδ IC_{50} (nM)
27	US 2011/0015212 (Example 10)	7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one		+

#	Prep.	Name	Structure	PI3Kδ IC_{50} (nM)
28	US 2011/0015212 (Example 15)	(S)-7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one		+
29	US 2013/0059835 (Example 269)	4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-{1-[(2S)-2-hydroxypropyl]azetidin-3-yl}-3-methoxybenzonitrile		+
30	US 2013/0059835 (Example 268)	4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-[1-(2-hydroxyethyl)azetidin-3-yl]-3-methoxybenzonitrile		+

#	Prep.	Name	Structure	PI3Kδ IC_{50} (nM)
31	US 2013/ 0059835 (Example 314)	5-{3-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-cyano-2-ethoxy-5-methylphenyl}-N,N-dimethylpyridine-2-carboxamide		+
32a, 32b, 32c, 32d	US 2013/ 0059835 (Example 345-348 (four diastereomers)) Compound 32a, 32b, 32c, and 32d are Examples 345, 346, 347, and 348 respectively	4-{3-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-5-chloro-2-ethoxy-6-fluorophenyl}pyrrolidin-2-one		32a (++), 32b (+) 32c (+) 32d (++)
33	US 2011/0183985 (Example 17-single enantiomer)	N-{1-[5-chloro-8-(3-fluorophenyl)cinnolin-7-yl]ethyl}-9H-purin-6-amine		+
34	US 2012/ 0157430	4-chloro-3'-fluoro-3-methyl-6-[1-(9H-purin-6-ylamino)ethyl]biphenyl-2-carbonitrile		+++

+ means <50 nM

++ means 50 nM to 200 nM
+++ means 50 nM to 100 nM

In some embodiments, the inhibitor of PI3Kδ is selected from:

(S)-4-(3-((S)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-chloro-2-ethoxy-6-fluorophenyl)pyrrolidin-2-one;

(R)-4-(3-((S)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-chloro-2-ethoxy-6-fluorophenyl)pyrrolidin-2-one;

(S)-4-(3-((R)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-chloro-2-ethoxy-6-fluorophenyl)pyrrolidin-2-one;

(R)-4-(3-((R)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-chloro-2-ethoxy-6-fluorophenyl)pyrrolidin-2-one;

N-{(1S)-1-[5-chloro-8-(3-fluorophenyl)cinnolin-7-yl]ethyl}-9H-purin-6-amine;

and pharmaceutically acceptable salts of any of the aforementioned.

In some embodiments, the inhibitor of PI3Kδ is (S)-7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

In some embodiments, the inhibitor of PI3Kδ is 4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-{1-[(2S)-2-hydroxypropyl]azetidin-3-yl}-3-methoxybenzonitrile, or a pharmaceutically acceptable salt thereof

In some embodiments, the inhibitor of PI3Kδ is 4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-[1-(2-hydroxyethyl)azetidin-3-yl]-3-methoxybenzonitrile, or a pharmaceutically acceptable salt thereof

In some embodiments, the inhibitor of PI3Kδ is 5-{3-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-cyano-2-ethoxy-5-methylphenyl}-N,N-dimethylpyridine-2-carboxamide, or a pharmaceutically acceptable salt thereof

In some embodiments, the inhibitor of PI3Kδ is selected from:

4-[(R)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-{1-[(2S)-2-hydroxypropyl]azetidin-3-yl}-3-methoxybenzonitrile;

4-[1(R)-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-[1-(2-hydroxyethyl)azetidin-3-yl]-3-methoxybenzonitrile;

5-{3-[1(R)-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-cyano-2-ethoxy-5-methylphenyl}-N,N-dimethylpyridine-2-carboxamide;

4-[(S)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-{1-[(2S)-2-hydroxypropyl]azetidin-3-yl}-3-methoxybenzonitrile;

4-[1(S)-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-[1-(2-hydroxyethyl)azetidin-3-yl]-3-methoxybenzonitrile;

5-{3-[1(S)-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-cyano-2-ethoxy-5-methylphenyl}-N,N-dimethylpyridine-2-carboxamide;

and pharmaceutically acceptable salts of any of the aforementioned.

In some embodiments, the inhibitor of PI3Kδ is a compound of US Patent Publ. No. US 2011/0015212, filed June 28, 2010, US Patent Publ. No. 2013/0059835, filed August 31, 2013, US Patent Publ. No. 2011/0183985, filed December 17, 2010, or US Patent Publ. No. 2012/0157430, filed December 19, 2011, each of which is incorporated herein by reference in its entirety.

In some embodiments, the compounds of Table 2 are prepared by the methods in US Patent Publ. No. US 2011/0015212, filed June 28, 2010, US Patent Publ. No. 2013/0059835, filed August 31, 2013, US Patent Publ. No. 2011/0183985, filed December 17, 2010, or US Patent Publ. No. 2012/0157430, filed December 19, 2011, each of which is incorporated herein by reference in its entirety.

In some embodiments, the inhibitor of JAK1 and/or JAK2 is (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile, or a pharmaceutically acceptable salt thereof; and (7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

In some embodiments, the inhibitor of JAK1 and/or JAK2 is {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile, or pharmaceutically acceptable salt thereof;

and the inhibitor of PI3Kδ is 7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

In some embodiments, the inhibitor of JAK1 and/or JAK2 is 4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide, or pharmaceutically acceptable salt thereof; and the inhibitor of PI3Kδ is 7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

In some embodiments, the present application provides a method of treating diffuse large B-cell lymphoma in a patient in need thereof, comprising administering to said patient (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile, or a pharmaceutically acceptable salt thereof; and the inhibitor of PI3Kδ is (7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

In some embodiments, the present application provides a method of treating diffuse large B-cell lymphoma in a patient in need thereof, comprising administering to said patient {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile, or pharmaceutically acceptable salt thereof; and 7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

In some embodiments, the present application provides a method of treating diffuse large B-cell lymphoma in a patient in need thereof, comprising administering to said patient 4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide, or pharmaceutically acceptable salt thereof; and 7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

The compounds described herein can be asymmetric (*e.g.*, having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended

unless otherwise indicated. Compounds that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically inactive starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. *Cis* and *trans* geometric isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms.
In some embodiments, the compound has the *(R)*-configuration. In some embodiments, the compound has the *(S)*-configuration.

Resolution of racemic mixtures of compounds can be carried out by any of numerous methods known in the art. An example method includes fractional recrystallizaion using a chiral resolving acid which is an optically active, salt-forming organic acid. Suitable resolving agents for fractional recrystallization methods are, for example, optically active acids, such as the D and L forms of tartaric acid, diacetyltartaric acid, dibenzoyltartaric acid, mandelic acid, malic acid, lactic acid or the various optically active camphorsulfonic acids such as β-camphorsulfonic acid. Other resolving agents suitable for fractional crystallization methods include stereoisomerically pure forms of α-methylbenzylamine (*e.g.*, *S* and *R* forms, or diastereomerically pure forms), 2-phenylglycinol, norephedrine, ephedrine, N-methylephedrine, cyclohexylethylamine, 1,2-diaminocyclohexane, and the like.

Resolution of racemic mixtures can also be carried out by elution on a column packed with an optically active resolving agent (*e.g.*, dinitrobenzoylphenylglycine). Suitable elution solvent composition can be determined by one skilled in the art.

Compounds described herein also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond together with the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Example prototropic tautomers include ketone – enol pairs, amide - imidic acid pairs, lactam – lactim pairs, enamine – imine pairs, and annular forms where a proton can

occupy two or more positions of a heterocyclic system, for example, 1H- and 3H-imidazole, 1H-, 2H- and 4H- 1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution.

Compounds described herein can also include all isotopes of atoms occurring in the intermediates or final compounds. Isotopes include those atoms having the same atomic number but different mass numbers. For example, isotopes of hydrogen include tritium and deuterium.

The term, "compound," as used herein is meant to include all stereoisomers, geometric iosomers, tautomers, and isotopes of the structures depicted. Compounds herein identified by name or structure as one particular tautomeric form are intended to include other tautomeric forms unless otherwise specified.

All compounds, and pharmaceutically acceptable salts thereof, can be found together with other substances such as water and solvents (e.g. hydrates and solvates) or can be isolated.

In some embodiments, the compounds described herein, or salts thereof, are substantially isolated. By "substantially isolated" is meant that the compound is at least partially or substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the compounds described herein. Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of the compounds described herein, or salt thereof. Methods for isolating compounds and their salts are routine in the art.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The expressions, "ambient temperature" and "room temperature" or "rt" as used herein, are understood in the art, and refer generally to a temperature, *e.g.* a reaction temperature, that is about the temperature of the room in which the reaction is carried out, for example, a temperature from about 20 °C to about 30 °C.

The present invention also includes pharmaceutically acceptable salts of the compounds described herein. As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts of the present invention include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, non-aqueous media like ether, ethyl acetate, alcohols (e.g., methanol, ethanol, iso-propanol, or butanol) or acetonitrile (ACN) are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and *Journal of Pharmaceutical Science*, 66, 2 (1977), each of which is incorporated herein by reference in its entirety.

As used herein, the term "individual" or "patient," used interchangeably, refers to any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and most preferably humans.

In some embodiments, the inhibitors are administered in a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" refers to the amount of active compound or pharmaceutical agent that elicits the biological or medicinal response that is being sought in a tissue, system, animal, individual or human by a researcher, veterinarian, medical doctor or other clinician. In some embodiments,

the dosage of the compound, or a pharmaceutically acceptable salt thereof, administered to a patient or individual is about 1 mg to about 2 g, about 1 mg to about 1000 mg, about 1 mg to about 500 mg, about 1 mg to about 200 mg, about 1 mg to about 100 mg, about 1 mg to 50 mg, or about 50 mg to about 500 mg.

As used herein, the term "treating" or "treatment" refers to one or more of (1) inhibiting the disease; for example, inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology); and (2) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology) such as decreasing the severity of disease.

Combination Therapies

One or more additional pharmaceutical agents such as, for example, chemotherapeutics, anti-inflammatory agents, steroids, immunosuppressants, as well as Bcr-Abl, Flt-3, EGFR, HER2, c-MET, VEGFR, PDGFR, cKit, IGF-1R, RAF, FAK,Akt mTOR, PIM, and AKT (e.g., AKT1, AKT2, or AKT3) kinase inhibitors such as, for example, those described in WO 2006/056399, or other agents such as, therapeutic antibodies can be used in combination with the compounds of the present invention for treatment of PI3K-associated diseases, disorders or conditions. The one or more additional pharmaceutical agents can be administered to a patient simultaneously or sequentially.

Example antibodies for use in combination therapy include but are not limited to Trastuzumab (e.g. anti-HER2), Ranibizumab (e.g. anti-VEGF-A), Bevacizumab (trade name Avastin, e.g. anti-VEGF, Panitumumab (e.g. anti-EGFR), Cetuximab (e.g. anti-EGFR), Rituxan (anti-CD20) and antibodies directed to c-MET.

One or more of the following agents may be used in combination with the compounds of the present invention and are presented as a non limiting list: a cytostatic agent, cisplatin, doxorubicin, taxotere, taxol, etoposide, irinotecan, camptostar, topotecan,

paclitaxel, docetaxel, epothilones, tamoxifen, 5-fluorouracil, methoxtrexate, temozolomide, cyclophosphamide, SCH 66336, R115777, L778,123, BMS 214662, Iressa, Tarceva, antibodies to EGFR, Gleevec™, intron, ara-C, adriamycin, cytoxan, gemcitabine, Uracil mustard, Chlormethine, Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylenemelamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, Streptozocin, Dacarbazine, Floxuridine, Cytarabine, 6-Mercaptopurine, 6-Thioguanine, Fludarabine phosphate, oxaliplatin, leucovirin, ELOXATIN™, Pentostatine, Vinblastine, Vincristine, Vindesine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Mithramycin, Deoxycoformycin, Mitomycin-C, L-Asparaginase, Teniposide 17.alpha.-Ethinylestradiol, Diethylstilbestrol, Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Methylprednisolone, Methyltestosterone, Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine, Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, goserelin, Cisplatin, Carboplatin, Hydroxyurea, Amsacrine, Procarbazine, Mitotane, Mitoxantrone, Levamisole, Navelbene, Anastrazole, Letrazole, Capecitabine, Reloxafine, Droloxafine, Hexamethylmelamine, Avastin, herceptin, Bexxar, Velcade, Zevalin, Trisenox, Xeloda, Vinorelbine, Porfimer, Erbitux, Liposomal, Thiotepa, Altretamine, Melphalan, Trastuzumab, Lerozole, Fulvestrant, Exemestane, Fulvestrant, Ifosfomide, Rituximab, C225, Campath, Clofarabine, cladribine, aphidicolon, rituxan, sunitinib, dasatinib, tezacitabine, Sml1, fludarabine, pentostatin, triapine, didox, trimidox, amidox, 3-AP, MDL-101,731, bendamustine (Treanda), ofatumumab, or GS-1101 (also known as CAL-101).

Example chemotherapeutics include proteosome inhibitors (*e.g.*, bortezomib), thalidomide, revlimid, and DNA-damaging agents such as melphalan, doxorubicin, cyclophosphamide, vincristine, etoposide, carmustine, and the like.

Example steroids include coriticosteroids such as dexamethasone or prednisone.

Example Bcr-Abl inhibitors include the compounds, and pharmaceutically acceptable salts thereof, of the genera and species disclosed in U.S. Pat. No. 5,521,184, WO 04/005281, and U.S. Ser. No. 60/578,491.

Example suitable Flt-3 inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 03/037347, WO 03/099771, and WO 04/046120.

Example suitable RAF inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 00/09495 and WO 05/028444.

Example suitable FAK inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 04/080980, WO 04/056786, WO 03/024967, WO 01/064655, WO 00/053595, and WO 01/014402.

Example suitable mTOR inhibitors include compounds, and their pharmaceutically acceptable salts, as disclosed in WO 2011/025889.

In some embodiments, the compounds of the invention can be used in combination with one or more other kinase inhibitors including imatinib, particularly for treating patients resistant to imatinib or other kinase inhibitors.

In some embodiments, the compounds of the invention can be used in combination with a chemotherapeutic in the treatment of cancer, such as multiple myeloma, and may improve the treatment response as compared to the response to the chemotherapeutic agent alone, without exacerbation of its toxic effects. Examples of additional pharmaceutical agents used in the treatment of multiple myeloma, for example, can include, without limitation, melphalan, melphalan plus prednisone [MP], doxorubicin, dexamethasone, and Velcade (bortezomib). Further additional agents used in the treatment of multiple myeloma include Bcr-Abl, Flt-3, RAF and FAK kinase inhibitors. Additive or synergistic effects are desirable outcomes of combining a PI3K inhibitor of the present invention with an additional agent. Furthermore, resistance of multiple myeloma cells to agents such as dexamethasone may be reversible upon treatment with the PI3K inhibitor of the present invention. The agents can be combined with the present compound in a single or continuous dosage form, or the agents can be administered simultaneously or sequentially as separate dosage forms.

In some embodiments, a corticosteroid such as dexamethasone is administered to a patient in combination with the compounds of the invention where the dexamethasone is administered intermittently as opposed to continuously.

In some further embodiments, combinations of the compounds of the invention with other therapeutic agents can be administered to a patient prior to, during, and/or after a bone marrow transplant or stem cell transplant.

Pharmaceutical Formulations and Dosage Forms

When employed as pharmaceuticals, the compounds described herein can be administered in the form of pharmaceutical compositions. These compositions can be prepared in a manner well known in the pharmaceutical art, and can be administered by a variety of routes, depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including transdermal, epidermal, ophthalmic and to mucous membranes including intranasal, vaginal and rectal delivery), pulmonary (*e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal or intranasal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal intramuscular or injection or infusion; or intracranial, *e.g.*, intrathecal or intraventricular, administration. Parenteral administration can be in the form of a single bolus dose, or may be, for example, by a continuous perfusion pump. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. This invention also includes pharmaceutical compositions which contain, as the active ingredient, the compound described herein or a pharmaceutically acceptable salt thereof, in combination with one or more pharmaceutically acceptable carriers (excipients). In some embodiments, the composition is suitable for topical administration. In making the compositions of the invention, the active ingredient is typically mixed with an excipient, diluted by an excipient or enclosed within such a carrier in the form of, for example, a capsule, sachet, paper, or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges,

sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, the active compound can be milled to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it can be milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size can be adjusted by milling to provide a substantially uniform distribution in the formulation, *e.g.* about 40 mesh.

The compounds described herein may be milled using known milling procedures such as wet milling to obtain a particle size appropriate for tablet formation and for other formulation types. Finely divided (nanoparticulate) preparations of the compounds described herein can be prepared by processes known in the art, e.g., see International App. No. WO 2002/000196.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions can be formulated in a unit dosage form, each dosage containing from about 5 to about 1000 mg (1 g), more usually about 100 to about 500 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

In some embodiments, the compositions of the invention contain from about 5 to about 50 mg of the active ingredient. One having ordinary skill in the art will appreciate that this embodies compositions containing about 5 to about 10, about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50 mg of the active ingredient.

In some embodiments, the compositions of the invention contain from about 50 to about 500 mg of the active ingredient. One having ordinary skill in the art will appreciate that this embodies compositions containing about 50 to about 100, about 100 to about 150, about 150 to about 200, about 200 to about 250, about 250 to about 300, about 350 to about 400, or about 450 to about 500 mg of the active ingredient.

In some embodiments, the compositions of the invention contain from about 500 to about 1000 mg of the active ingredient. One having ordinary skill in the art will appreciate that this embodies compositions containing about 500 to about 550, about 550 to about 600, about 600 to about 650, about 650 to about 700, about 700 to about 750, about 750 to about 800, about 800 to about 850, about 850 to about 900, about 900 to about 950, or about 950 to about 1000 mg of the active ingredient.

Similar dosages may be used of the compounds described herein in the methods and uses of the invention.

The active compound can be effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It will be understood, however, that the amount of the compound actually administered will usually be determined by a physician, according to the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, the active ingredient is

typically dispersed evenly throughout the composition so that the composition can be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, about 0.1 to about 1000 mg of the active ingredient of the present invention.

The tablets or pills of the present invention can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the compounds and compositions of the present invention can be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions can be nebulized by use of inert gases. Nebulized solutions may be breathed directly from the nebulizing device or the nebulizing device can be attached to a face mask, tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions can be administered orally or nasally from devices which deliver the formulation in an appropriate manner.

Topical formulations can contain one or more conventional carriers. In some embodiments, ointments can contain water and one or more hydrophobic carriers selected from, for example, liquid paraffin, polyoxyethylene alkyl ether, propylene glycol, white Vaseline, and the like. Carrier compositions of creams can be based on water in combination with glycerol and one or more other components, e.g. glycerinemonostearate, PEG-glycerinemonostearate and cetylstearyl alcohol. Gels can be formulated using isopropyl alcohol and water, suitably in combination with other components such as, for example, glycerol, hydroxyethyl cellulose, and the like. In some embodiments, topical formulations contain at least about 0.1, at least about 0.25, at least about 0.5, at least about 1, at least about 2, or at least about 5 wt % of the compound described herein. The topical formulations can be suitably packaged in tubes of, for example, 100 g which are optionally associated with instructions for the treatment of the select indication, e.g., psoriasis or other skin condition.

The amount of compound or composition administered to a patient will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions can be administered to a patient already suffering from a disease in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. Effective doses will depend on the disease condition being treated as well as by the judgment of the attending clinician depending upon factors such as the severity of the disease, the age, weight and general condition of the patient, and the like.

The compositions administered to a patient can be in the form of pharmaceutical compositions described above. These compositions can be sterilized by conventional sterilization techniques, or may be sterile filtered. Aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the compound preparations typically will be between 3 and 11, more preferably from 5 to 9 and most preferably from 7 to 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of pharmaceutical salts.

The therapeutic dosage of a compound of the present invention can vary according to, for example, the particular use for which the treatment is made, the manner of administration of the compound, the health and condition of the patient, and the judgment of the prescribing physician. The proportion or concentration of a compound described herein in a pharmaceutical composition can vary depending upon a number of factors including dosage, chemical characteristics (*e.g.*, hydrophobicity), and the route of administration. For example, the compounds described herein can be provided in an aqueous physiological buffer solution containing about 0.1 to about 10% w/v of the compound for parenteral administration. Some typical dose ranges are from about 1 μg/kg to about 1 g/kg of body weight per day. In some embodiments, the dose range is from about 0.01 mg/kg to about 100 mg/kg of body weight per day. The dosage is likely to depend on such variables as the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, formulation of the excipient, and its route of administration. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The compositions of the invention can further include one or more additional pharmaceutical agents such as a chemotherapeutic, steroid, anti-inflammatory compound, or immunosuppressant, examples of which are listed herein.

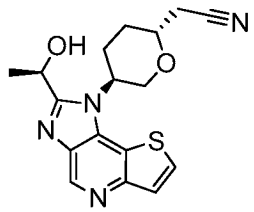
Kits

The present invention also includes pharmaceutical kits useful, for example, in the treatment or prevention of PI3K-associated diseases or disorders, such as cancer, which include one or more containers containing a pharmaceutical composition comprising a therapeutically effective amount of a compound described herein. Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers, etc., as will be readily apparent to those skilled in the art. Instructions, either as inserts or as labels, indicating quantities of the components to be

administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit.

EXAMPLES

Example 1. ((2R,5S)-5-{2-[(1R)-1-Hydroxyethyl]-1*H*-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2*H*-pyran-2-yl)acetonitrile



Step 1. tert-Butyl (4S)-2,2-dimethyl-4-vinyl-1,3-oxazolidine-3-carboxylate

To a suspension of methyl triphenylphosphonium bromide (5.63 g, 15.8 mmol) in tetrahydrofuran (140 mL) was added 2.5 M n-butyllithium in hexane (7.35 mL, 18.4 mmol). The deep red solution was stirred at 0 °C for 1 h. Then a solution of *tert*-butyl (4R)-4-formyl-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (from Aldrich, 3.01 g, 13.1 mmol) in tetrahydrofuran (7.3 mL) was added drop wise at 0 °C. The red solution was warmed to room temperature and stirred for 12 h. Hexanes was added to the reaction mixture in 4:1 (v/v) ratio. The suspension was filtered through Celite and the filtrate concentrated. The resultant residue was purified by flash chromatography (eluting with 10% ethyl acetate in hexanes) to give the desired compound as colorless oil (1.92 g, 64%).

Step 2. tert-Butyl [(1S)-1-(hydroxymethyl)prop-2-en-1-yl]carbamate

To a solution of tert-butyl (4S)-2,2-dimethyl-4-vinyl-1,3-oxazolidine-3-carboxylate (1.90 g, 8.36 mmol) in methanol (83 mL) was added *p*-toluenesulfonic acid monohydrate (0.80 g, 4.2 mmol) at 0 °C. The mixture was slowly warmed to room temperature overnight. The reaction mixture was diluted with saturated $NaHCO_3$ solution, concentrated, and then diluted with ethyl acetate. The organic layer was washed with *sat.* $NaHCO_3$ (2x) and brine, dried over Na_2SO_4, filtered and concentrated to give

the desired product as colorless oil (1.187 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (1H, m), 5.25 (2H, m), 4.90 (1H, m), 4.25 (1H, br s), 3.67 (2H, m), 1.45 (9H, s) ppm.

Step 3. tert-Butyl [(1S)-1-({[1-(hydroxymethyl)prop-2-en-1-yl]oxy}methyl)prop-2-en-1-yl]carbamate

To a flask was charged with *tert*-butyl [(1S)-1-(hydroxymethyl)prop-2-en-1-yl]carbamate (0.401 g, 2.14 mmol), tris(dibenzylideneacetone)dipalladium(0) (59 mg, 0.064 mmol), *N,N'*-(1S,2S)-cyclohexane-1,2-diylbis[2-(diphenylphosphino)-1-naphthamide] (150 mg, 0.19 mmol), and 4-dimethylaminopyridine (78 mg, 0.64 mmol). The reaction mixture was purged with N_2 three times, and then methylene chloride (21.3 mL), and 1.0 M triethylborane in THF (130 μL, 0.13 mmol) was added sequentially. After stirring for 10 min, 2-vinyloxirane (0.150 g, 2.14 mmol) was added and the resulting mixture was stirred overnight. The reaction was diluted with dichloromethane and *sat.* $NaHCO_3$ solution. The organic layer was separated and dried over Na_2SO_4, filtered and concentrated. The crude residue was purified with flash chromatography (eluting with 0-50% ethyl acetate/hexanes) to give the desired product (0.271 g, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.85 (1H, m), 5.67 (1H, m), 5.84~5.17 (4H, m), 4.83 (1H, m), 4.30 (1H, br s), 3.83 (1H, m), 3.69 (1H, dd, J = 4.5 and 6.9 Hz), 3.54 (2H, m), 3.36 (1H, dd, J = 4.5 and 6.9 Hz), 1.45 (9H, s) ppm.

Step 4. 2-({(2S)-2-[(tert-Butoxycarbonyl)amino]but-3-en-1-yl}oxy)but-3-en-1-yl acetate

To a mixture of *tert*-butyl [(1S)-1-({[1-(hydroxymethyl)prop-2-en-1-yl]oxy}methyl)prop-2-en-1-yl]carbamate (268 mg, 1.04 mmol) in methylene chloride (10 mL) was added with triethylamine (435 μL, 3.12 mmol). The mixture was cooled to 0 °C, and acetyl chloride (150 μL, 2.1 mmol) was added drop wise. The reaction was stirred at room temperature for 2 h, then quenched with water. The organic layer was concentrated and the resultant residue purified on silica gel (eluting with 20% ethyl acetate/hexanes) to give the desired product (0.26 g, 85%). LCMS calculated for $C_{10}H_{18}NO_3$ $(M-100+H)^+$: m/z = 200.1; Found: 200.1.

Step 5. {(5S)-5-[(tert-Butoxycarbonyl)amino]-5,6-dihydro-2H-pyran-2-yl}methyl acetate

To a 500 mL 2-neck round bottom flask, benzylidene(dichloro)(1,3-dimesitylimidazolidin-2-id-2-yl)(tricyclohexylphosphoranyl)ruthenium (38 mg, 0.044 mmol) was added. After purged with nitrogen for 3 times, dichloromethane (anhydrous, 8 mL) was added followed by 2-({(2S)-2-[(tert-butoxycarbonyl)amino]but-3-en-1-yl}oxy)but-3-en-1-yl acetate (265 mg, 0.885 mmol). The reaction mixture was stirred at room temperature for 15 h. The mixture was concentrated *in vacuo*. The residue was purified via flash chromatography (eluting with hexanes to 25% EtOAc in hexanes) to give the desired product as a brown oil (0.205 g, 85%). LCMS calculated for $C_9H_{14}NO_5$ $(M+H\text{-}Bu+H)^+$: m/z = 216.1; Found: 216.1. ^1H NMR (300 MHz, $CDCl_3$) δ 5.94 (0.17H, m), 5.84 (0.83H, m), 5.69 (1H, m), 4.89 (0.13H, m), 4.70 (0.83H, m), 4.25 (1H, m), 4.05 (4H, m), 3.56 (0.13H, m), 3.38 (0.87H, m), 2.04 (2.49H, s), 2.03 (0.51H, m), 1.38 (9H, s) ppm (The product was a ~5:1 mixture of trans- and cis-isomers).

Step 6. [(5S)-5-Amino-5,6-dihydro-2H-pyran-2-yl]methyl acetate

To a solution of {(5S)-5-[(*tert*-butoxycarbonyl)amino]-5,6-dihydro-2*H*-pyran-2-yl}methyl acetate (205 mg, 0.756 mmol) in methylene chloride (5.2 mL) was added 4.0 M hydrogen chloride in dioxane (1.5 mL, 6.0 mmol). The reaction solution was stirred at room temperature for 6 h. The solvent was removed under reduced pressure to give the desired product as white solid. LCMS calculated for $C_8H_{14}NO_3$ $(M+H)^+$: m/z = 172.1; Found: 172.1.

Step 7. {(5S)-5-[(6-Nitrothieno[3,2-b]pyridin-7-yl)amino]-5,6-dihydro-2H-pyran-2-yl}methyl acetate

A mixture of 7-chloro-6-nitrothieno[3,2-b]pyridine (156 mg, 0.727 mmol), [(5S)-5-amino-5,6-dihydro-2*H*-pyran-2-yl]methyl acetate (129 mg, 0.754 mmol) and *N,N*-diisopropylethylamine (0.26 mL, 1.5 mmol) in isopropyl alcohol (1.7 mL) was heated at 90 °C for 2 h. The reaction mixture was concentrated and purified with flash chromatography to give the desired product (0.21 g 83%). LCMS calculated for $C_{15}H_{16}N_3O_5S$ $(M+H)^+$: m/z = 350.1; Found: 350.0.

Step 8. {(5S)-5-[(6-Aminothieno[3,2-b]pyridin-7-yl)amino]tetrahydro-2H-pyran-2-yl}methyl acetate

A mixture of {(5S)-5-[(6-nitrothieno[3,2-b]pyridin-7-yl)amino]-5,6-dihydro-2*H*-pyran-2-yl}methyl acetate (210 mg, 0.600 mmol) and 10% palladium on carbon (0.21 g) in methanol (4.0 mL) was subjected to balloon pressure of H_2 at room temperature for 2 h. The mixture was filtered, and the filtrate was concentrated and purified with flash chromatography (eluting with 15% methanol in dichloromethane) to give the desired product (145 mg, 75%). LCMS calculated for $C_{15}H_{20}N_3O_3S$ $(M+H)^+$: m/z = 322.1; Found: 322.0.

Step 9. (1R)-1-{1-[(3S)-6-(Hydroxymethyl)tetrahydro-2H-pyran-3-yl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-2-yl}ethanol

A mixture of (2R)-2-hydroxypropanamide (131 mg, 1.47 mmol) and triethyloxonium tetrafluoroborate (263 mg, 1.38 mmol) in THF (2 mL) was stirred at room temperature for 2 h. The solvent was removed and the residue dissolved in ethanol (0.85 mL) and added to a suspension of {(5S)-5-[(6-aminothieno[3,2-b]pyridin-7-yl)amino]tetrahydro-2*H*-pyran-2-yl}methyl acetate (145 mg, 0.451 mmol) in ethanol (3.1 mL). The mixture was stirred at 80 °C for 1 h. The reaction was cooled to room temperature and diluted with water (1.0 mL). Lithium hydroxide (32.4 mg, 1.35 mmol) was added, and the mixture was stirred for 2 h. The reaction mixture was diluted with methanol and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% ammonium hydroxide, at flow rate of 60 mL/min) to give the desired product as white solid (95 mg, 63%). LCMS calculated for $C_{16}H_{20}N_3O_3S$ $(M+H)^+$: m/z = 334.1; Found: 334.0.

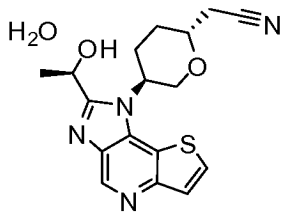
Step 10: ((2R,5S)-5-{2-[(1R)-1-Hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)methyl 4-methylbenzenesulfonate and ((2S,5S)-5-{2-[(1R)-1-hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)methyl 4-methylbenzenesulfonate

To a solution of (1R)-1-{1-[(3S)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl]-1*H*-imidazo[4,5-d]thieno[3,2-b]pyridin-2-yl}ethanol (100 mg, 0.300 mmol) (previous step) in methylene chloride (3.4 mL) and pyridine (0.146 mL, 1.80 mmol) was added *p*-toluenesulfonyl chloride (57.2 mg, 0.300 mmol) and 4-dimethylaminopyridine (1.8 mg, 0.015 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was concentrated, diluted with methanol, and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% ammonium hydroxide, at flow rate of 60 mL/min) to give two peaks. On analytic HPLC (Waters SunFire C18, 2.1 x 50 mm, 5 μM; Flow rate 3 mL/min; Injection volume 2 μL; At gradient from 2 to 80% B in 3 minutes (A = water with 0.025% TFA, B = acetonitrile)): First peak (45.3 mg, 31%) retention time 1.81 min, LCMS calculated for $C_{23}H_{26}N_3O_5S_2$ $(M+H)^+$: m/z = 488.1; Found: 488.1. Second peak (8.5 mg, 5.8%) retention time 1.88 min, LCMS calculated for $C_{23}H_{26}N_3O_5S_2$ $(M+H)^+$: m/z = 488.1; Found: 488.1.

Step 11. ((2R,5S)-5-{2-[(1R)-1-Hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile

A mixture of ((2R,5S)-5-{2-[(1R)-1-hydroxyethyl]-1*H*-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2*H*-pyran-2-yl)methyl 4-methylbenzenesulfonate (from 1st peak of previous step, 27 mg, 0.055 mmol) and sodium cyanide (4.5 mg, 0.092 mmol) in dimethyl sulfoxide (0.4 mL) was stirred at 50 °C for 4 h. After cooling, the mixture was diluted with methanol and purified with prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% ammonium hydroxide, at flow rate of 30 mL/min) to give the desired product (14.5 mg, 76%). LCMS calculated for $C_{17}H_{19}N_4O_2S$ $(M+H)^+$: m/z = 343.1; Found: 343.0. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.51 (1H, s), 8.45 (1H, d, *J* = 5.5 Hz), 7.97 (1H, d, *J* = 5..5 Hz), 5.31 (1H, m), 5.20 (1H, m), 4.31 (1H, m), 4.23 (1H, m), 4.02 (1H, m), 2.96 (1H, dd, *J* = 17.0 and 4.5 Hz), 2.85 (1H, dd, *J* = 17.0 and 4.5 Hz), 2.66 (1H, m), 2.26 (1H, m), 2.09 (1H, m), 1.73 (1H, m), 1.69 (3H, d, *J* = 6.5 Hz) ppm.

Example 1a. ((2R,5S)-5-{2-[(1R)-1-Hydroxyethyl]-1*H*-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2*H*-pyran-2-yl)acetonitrile hydrate



((2R,5S)-5-{2-[(1R)-1-Hydroxyethyl]-1*H*-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2*H*-pyran-2-yl)acetonitrile (52 mg, 0.15 mmol) from Example 25 was crystallized from a mixture of acetonitrile (8 mL) and water (4 mL). The resulting colorless prism crystal collected was suitable for X-ray crystal structure analysis.

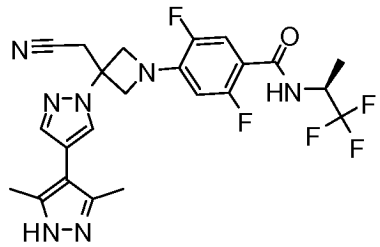
Crystal data shows: ~0.520 x 0.180 x 0.100mm, orthorhombic, P212121, a = 6.962(3) Å, b = 11.531(4) Å, c = 20.799(7) Å, Vol = 1669.6(10) $Å^3$, Z = 4, T = -100.°C, Formula weight = 359.42, Density = 1.430g/cm^3, μ(Mo) = 0.22mm^{-1}.

Data collection was done on a Bruker SMART APEX-II CCD system, MoKalpha radiation, standard focus tube, anode power = 50kV x 42mA, crystal to plate distance = 5.0cm, 512 x 512 pixels/frame, beam center = (256.13,253.14), total frames = 1151, oscillation/frame = 0.50°, exposure/frame = 10.1 sec/frame, SAINT integration, hkl min/max = (-9, 9, -15, 15, -27, 27), data input to shelx = 17025, unique data = 3975, two-theta range = 3.92 to 55.72°, completeness to two-theta 55.72 = 99.80%, R(int-xl) = 0.0681, SADABS correction applied.

Structure was solved using XS(Shelxtl), refined using shelxtl software package, refinement by full-matrix least squares on F^2, scattering factors from Int. Tab. Vol C Tables 4.2.6.8 and 6.1.1.4, number of data = 3975, number of restraints = 0, number of parameters = 235, data/parameter ratio = 16.91, goodness-of-fit on F^2 = 1.04, R indices[I>4sigma(I)] R1 = 0.0505, wR2 = 0.1242, R indices(all data) R1 = 0.0769, wR2 = 0.1401, max difference peak and hole = 0.724 and -0.277 e/$Å^3$, refined flack parameter = -0.12(13), All of the CH hydrogen atoms were refined using a riding model. The OH hydrogens were found from a difference map and fully refined.

Results showed that the asymmetric unit contains one molecule and one water as shown with thermal ellipsoids drawn to the 50% probability level. The stereochemistry at each of three stereocenters (as indicated in the name and structure of the compound above) was confirmed. The flack parameter refined to 0.28(24) indicating the correct enantiomeric setting.

Example 2. 4-[3-(Cyanomethyl)-3-(3',5'-dimethyl-1*H*,1'*H*-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-*N*-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide



Step 1: 2,4,5-Trifluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide

To a solution of 2,4,5-trifluorobenzoic acid (5.00 g, 28.4 mmol) in acetonitrile (50 mL) was added *N,N*-dimethylformamide (40 μL) followed by addition of oxalyl chloride (3.60 mL, 42.6 mmol). After 90 min, the volatiles were removed under reduced pressure. The residue was co-evaporated with acetonitrile (50 mL). The residue was then dissolved in methylene chloride (50 mL). This solution was added drop-wise into a cooled (ice bath) mixture of (2S)-1,1,1-trifluoropropan-2-amine hydrochloride (5.52 g, 36.9 mmol) (from Synquest, 98% ee) in toluene (100 mL) and 0.5 M sodium hydroxide aqueous solution (142 mL, 71.0 mmol). After addition, the ice bath was removed, and the reaction was allowed to warm to rt. The reaction was stirred overnight. The organic layer was separated. The aqueous layer was extracted with methylene chloride (50 mL). The combined organic layers were washed with 20% brine (75 mL) and water (2 x 75 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the desired product (6.49 g, 84%) which was directly used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.01 (d, J = 7.6 Hz, 1H), 7.92 – 7.50 (m, 2H), 4.76 (m, 1H), 1.31 (d, J = 7.0 Hz, 3H) ppm. LCMS *cacld.* for $C_{10}H_8F_6NO$ $(M+1)^+$: m/z = 272.0; Found: 272.0.

Step 2: 2,5-Difluoro-4-(3-hydroxyazetidin-1-yl)-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide

A mixture of 2,4,5-trifluoro-*N*-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide (6.39 g, 23.6 mmol), azetidin-3-ol hydrochloride (3.19 g, 28.3 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (8.81 mL, 58.9 mmol) in acetonitrile (25 mL) was stirred at 80 °C for 2 h. The reaction mixture was diluted with EtOAc (75 mL) and washed with 1N HCl (50 mL), 1N $NaHCO_3$ (60 mL), 20% brine (50 mL) and water (75 mL). The aqueous layers were extracted with EtOAc (100 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield the desired product (7.59 g, 91.8%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.38 (dd, J = 8.9, 1.9 Hz, 1H), 7.27 (dd, J = 12.8, 6.5 Hz, 1H), 6.38 (dd, J = 12.3, 7.5 Hz, 1H), 5.71 (d, J = 6.4 Hz, 1H), 4.74 (dp, J = 15.3, 7.6 Hz, 1H), 4.62 – 4.46 (m, 1H), 4.30 – 4.15 (m, 2H), 3.71 (m, 2H), 1.29 (d, J = 7.1 Hz, 3H) ppm. LCMS *cacld.* for $C_{13}H_{14}F_5N_2O_2$ $(M+1)^+$: m/z = 325.1; Found: 325.1.

Step 3: 2,5-Difluoro-4-(3-oxoazetidin-1-yl)-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide

To a solution of 2,5-difluoro-4-(3-hydroxyazetidin-1-yl)-*N*-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide (7.57 g, 23.3 mmol) in methylene chloride (93 mL) was added iodobenzene diacetate (9.40 g, 29.2 mmol) and 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (1.82 g, 11.7 mmol) (TEMPO) at room temperature. The reaction mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc (100 mL), washed with 0.5N $NaHCO_3$ (2x80 mL), 20% brine (100 mL) and water (100 mL). The aqueous layers were extracted with ethyl acetate (75 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column eluting with 0% to 5% ethyl acetate in methylene chloride to afford the crude product which was recrystallized from MTBE (50 mL) and heptane (100 mL) to give the desired product (5.44g, 72%) as colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.52 (d, J = 8.0 Hz,

1H), 7.36 (dd, J = 12.5, 6.5 Hz, 1H), 6.63 (dd, J = 12.1, 7.6 Hz, 1H), 4.90 (d, J = 2.1 Hz, 4H), 4.86 – 4.68 (m, 1H), 1.31 (d, J = 7.1 Hz, 3H) ppm. LCMS *cacld.* for $C_{13}H_{12}F_5N_2O_2$ $(M+1)^+$: m/z = 323.1; Found: 323.0.

Step 4: 4-[3-(Cyanomethylene)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide

Diethyl cyanomethylphosphonate (1.95 mL, 11.8 mmol) was added drop-wise to a cooled (ice bath) solution of 1.0 M potassium *tert*-butoxide in THF (11.8 mL, 11.8 mmol) which was diluted with tetrahydrofuran (12 mL). The bath was removed and the reaction was warmed to room temperature, and stirred for 90 min. The reaction solution was cooled with an ice bath again. The above prepared solution was then added over 12 min to a cooled (ice-bath) solution of 2,5-difluoro-4-(3-oxoazetidin-1-yl)-*N*-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide (4.00 g, 12.4 mmol) in tetrahydrofuran (50 mL). The reaction mixture was stirred for 30 min. The ice bath was removed, and the reaction was stirred at room temperature overnight, then quenched by the addition of 20% brine (75 mL) and ethyl acetate (75 mL). The organic layer was separated. The aqueous layer was extracted with ethyl acetate (50 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column with ethyl acetate in hexanes (0% to 30%) to yield the desired product (2.6g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 – 8.37 (m, 1H), 7.33 (dd, J = 12.5, 6.4 Hz, 1H), 6.59 (dd, J = 12.0, 7.4 Hz, 1H), 5.88 (m, 1H), 4.94 – 4.75 (m, 4H), 4.76 (m, 1H), 1.31 (d, J = 7.1 Hz, 3H) ppm. LCMS *cacld.* for $C_{15}H_{13}F_5N_3O$ $(M+1)^+$: m/z = 346.1; Found: 346.1.

Step 5: 4-{3-(Cyanomethyl)-3-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide

A mixture of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (1.00 g, 5.15 mmol), 4-[3-(cyanomethylene)azetidin-1-yl]-2,5-difluoro-*N*-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide (1.78 g, 5.15 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.31 mL, 2.1 mmol) in acetonitrile (20.2 mL) was heated at 50 °C overnight. After

cooling, the solvent was removed under reduced pressure. The residue was used in the next step without further purification. LCMS *cacld.* for $C_{24}H_{28}BF_5N_5O_3$ $(M+1)^+$: m/z = 540.2; Found: 540.1.

Step 6: 4-[3-(Cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide

A mixture of 4-{3-(cyanomethyl)-3-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-*N*-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide (329 mg, 0.610 mmol), 4-bromo-3,5-dimethyl-1*H*-pyrazole (206 mg, 1.18 mmol), tetrakis(triphenylphosphine)palladium(0) (110 mg, 0.098 mmol) and sodium carbonate (320 mg, 3.0 mmol) in 1,4-dioxane (10 mL)/water (5 mL) was purged with nitrogen and stirred at 110 °C for 1 h. The reaction mixture was diluted with EtOAc, washed with water and brine, concentrated. The residue was purified first with silica gel (eluting with 0-100% EtOAc/hexanes followed by 10% methanol/dichloromethane), and then by prep-LCMS (XBridge C18 column, eluting with a gradient of acetonitrile/water containing 0.1% ammonium hydroxide, at flow rate of 60 mL/min) to give the desired product (30 mg, 9.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.17 (1H, s), 8.45 (1H, d, J = 8.0 Hz), 8.10 (1H, s), 7.70 (1H, s), 7.34 (1H, m), 6.61 (1H, s), 4.77 (1H, m), 4.62 (2H, d, J = 9.0 Hz), 4.39 (1H, d, J = 9.0 Hz), 3.64 (2H, s), 2.22 (6H, s), 1.31 (6H, d, J = 7.0 Hz) ppm. LCMS calculated for $C_{23}H_{23}F_5N_7O$ $(M+H)^+$: m/z = 508.2; Found: 508.0.

Example A: *In vitro* JAK Kinase Assay

Compounds herein were tested for inhibitory activity of JAK targets according to the following *in vitro* assay described in Park *et al.*, *Analytical Biochemistry* **1999**, *269*, 94-104. The catalytic domains of human JAK1 (a.a. 837-1142), JAK2 (a.a. 828-1132) and JAK3 (a.a. 781-1124) were expressed using baculovirus in insect cells and purified. The catalytic activity of JAK1, JAK2 or JAK3 was assayed by measuring the phosphorylation of a biotinylated peptide. The phosphorylated peptide was detected by homogenous time resolved fluorescence (HTRF). IC_{50}s of compounds were measured for each kinase in the 40 μL reactions that contain the enzyme, ATP and 500 nM peptide in

50 mM Tris (pH 7.8) buffer with 100 mM NaCl, 5 mM DTT, and 0.1 mg/mL (0.01%) BSA. For the 1 mM IC_{50} measurements, ATP concentration in the reactions was 1 mM. Reactions were carried out at room temperature for 1 hour and then stopped with 20 μL 45 mM EDTA, 300 nM SA-APC, 6 nM Eu-Py20 in assay buffer (Perkin Elmer, Boston, MA). Binding to the Europium labeled antibody took place for 40 minutes and HTRF signal was measured on a PHERA star plate reader (BMG, Cary, NC). The data for the JAK1 and/or JAK2 inhibitors were obtained by testing the compounds in the Example A assay at 1 mM ATP.

Example B: PI3Kδ scintillation proximity assay

Materials

[γ-^{33}P]ATP (10mCi/mL) was purchased from Perkin–Elmer (Waltham, MA). Lipid kinase substrate, D-myo-Phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2)D (+)-sn-1,2-di-O-octanoylglyceryl, 3-O-phospho linked (PIP2), CAS 204858-53-7, was purchased from Echelon Biosciences (Salt Lake City, UT). PI3Kδ (p110δ /p85α) was purchased from Millipore (Bedford, MA). ATP, $MgCl_2$, DTT, EDTA, MOPS and CHAPS were purchased from Sigma–Aldrich (St. Louis, MO). Wheat Germ Agglutinin (WGA) YSi SPA Scintillation Beads was purchased from GE healthcare life sciences (Piscataway, NJ).

The kinase reaction was conducted in polystyrene 384-well matrix white plate from Thermo Fisher Scientific in a final volume of 25 μL. Inhibitors were first diluted serially in DMSO and added to the plate wells before the addition of other reaction components. The final concentration of DMSO in the assay was 0.5%. The PI3K assays were carried out at room temperature in 20 mM MOPS, pH 6.7, 10 mM $MgCl_2$, 5 mM DTT and CHAPS 0.03%. Reactions were initiated by the addition of ATP, the final reaction mixture consisted of 20 μM PIP2, 20 μM ATP, 0.2 μCi [γ-^{33}P] ATP, 4 nM PI3Kδ. Reactions were incubated for 210 min and terminated by the addition of 40 μL SPA beads suspended in quench buffer: 150mM potassium phosphate pH 8.0, 20% glycerol. 25 mM EDTA, 400 μM ATP. The final concentration of SPA beads was

1.0mg/mL. After the plate sealing, plates were shaken overnight at room temperature and centrifuged at 1800 rpm for 10 minutes, the radioactivity of the product was determined by scintillation counting on Topcount (Perkin–Elmer). IC_{50} determination was performed by fitting the curve of percent control activity versus the log of the inhibitor concentration using the GraphPad Prism 3.0 software. The data for the PI3Kδ inhibitors were obtained by testing the compounds in the Example B assay.

Example C: Pfeiffer Model of Lymphoma

Methods:

Female SCID mice, (5 to 8 weeks of age, Charles River Laboratories, Wilmington, MA) were inoculated with 1 × 107 tumor cells (Pfeiffer, ATCC #CRL-2632, Manassas, VA) and matrigel (BD Biosciences #354234) in 0.2 mL sterile saline. The inoculation was performed subcutaneously on the flank. Tumor tissue fragments (approximately 3 mm × 3 mm) were collected 3 to 6 weeks after the inoculation of cultured cells and implanted subcutaneously in lieu of cellular inoculation. Tissue fragments were implanted as solid pieces using blunt-tip forceps. The treatment of tumor bearing mice was started 15 to 25 days after tumor inoculation, depending upon the tumor size. Animals were sorted to obtain roughly equivalent mean tumor volumes in each group. Minimum mean tumor volume in all groups was 150 mm3 on the first day of treatment and groups consisted of 7 animals. Experimental therapeutic agent, Example 347, was administered to mice orally (PO). Treatment frequency was 2 times daily for a minimum of 14 days for efficacy. The size of subcutaneous tumors was measured 2 to 3 times weekly using a digital caliper. The tumor volume was calculated by measuring the tumor in 2 dimensions and utilizing the equation: Volume = [Length × (Width2)]/2; where the larger number was length, and the smaller number width. If multiple tumors were formed, the final volume was the sum of the individual tumors subject to the same equation: eg, 2 tumors; Volume = {[L1 × (W1)2]/2} + {[L2 x (W2)2]/2}. Effects on tumor growth were reported as percent tumor growth inhibition (%TGI). Percent TGI was calculated with the equation: (1- (Tx vol. / control vol.))*100, where control volume was the vehicle or untreated tumor volume on a given day, and Tx volume was any

treatment group tumor volume on that same day. Statistical differences between treatment and vehicle controls were assessed using ANOVA: Single Factor test.

Results:

Compound 32c (Table 2 *supra*) was evaluated as a single agent in the Pfeiffer human tumor xenograft model of diffuse large B-cell lymphoma, a subtype of NHL. Pfeiffer cancer cells were shown to be sensitive to the anti-proliferative effects of Example 347*in vitro*. Therefore, a tumor model was established based on subcutaneous inoculation of tumor cells into immune compromised SCID mice and tumor-bearing mice received twice daily oral doses of vehicle or Compound 32c at 0.3, 1, 3, or 10 mg/kg for 14 days. Compound 32c treatment inhibited tumor growth by 22%, 24%, 36%, and 58% (percent tumor growth inhibition) with increasing dose.

Example D. Western-Blot analysis

The following materials and methods were used in the Western blot analysis *infra*. Cells (5 million) were lysed in a 300 µl volume of lysis buffer. Soluble fractions were collected by centrifugation. 25 µl of cell lysate were loaded into Tris-Glycine polyacrylamide gels and subjected to electrophoresis. The proteins were transferred into nitrocellulose membrane and probed with antibodies from Cell Signaling Technology for the following proteins : phospho-Stat3 Y705, phospho-Akt S473, pim1, pim2, pim3, c-myc, phospho-p70S6K, phosho-S6, phospho-Bad S112 and actin.

Example E. Levels of IL6 and IL10 in cell lines

High levels of IL6 and IL10 were seen in various DLBCL cell lines (FIG. 1A). IL6 and IL10 were also shown to activate JAK/STAT signaling with IL10 being a stronger activator of JAK/STAT signaling than IL6 across a panel of DLBCL cell lines (FIG. 1B). High levels of IL6 and IL10 are present in serum from DLBCL patients and correlate with shorter event free survival and a higher International Prognostic Index score.

Example F. IL10 makes Pfeiffer cells resistant to PI3Kδ inhibition and can be reversed by JAK1/2 or JAK1 blockade

Cell proliferation assay

Diffuse large B cell lymphoma cells were seeded at 2000 cell/well in 96 well culture plates in the absence or presence of 10 ng/ml IL10. Compounds were added to these cells after dilution in DMSO first, followed by dilution in culture medium (4x concentration). The cells were cultured in an incubator for 3 days with 5% CO_2. Cell proliferation was assessed using the Cell titer-glow assay (Promega, Madison, WI). The cell proliferation assay was carried out first in Pfeiffer cells (germinal center B cell (GCB) diffuse large B cell lymphoma (GCB-DLBCL) cells) and HBL-1 cells (activated B-cell like (ABC) diffuse large B cell lymphoma (ABC-DLBCL)).

FIG. 2A depicts % inhibition in the cellular proliferation assay in Pfeiffer cells as a function of the concentration of Compound 28 (a PI3Kδ inhibitor) with vehicle (DMSO), DMSO+IL10, and DMSO+IL10+ruxolitinib (a JAK1/JAK2 inhibitor). FIG. 2B depicts % inhibition in the cellular proliferation assay in Pfeiffer cells as a function of the concentration of Compound 28 (a PI3Kδ inhibitor) with vehicle (DMSO), DMSO+IL10, and DMSO+IL10+Compound 7 (a selective JAK1 inhibitor). The results show that IL10 makes Pfeiffer cells resistant to PI3Kδ inhibition, but that this resistance can be reversed by blocking JAK1 and/or JAK2 signaling. Hence, a synergistic effect on Pfeiffer cell proliferation when a PI3Kδ inhibitor and a JAK1 and/JAK2 inhibitor are used in combination. Synergy was also observed without the IL10. Induction of apoptosis was also observed with this combination.

Similar results were observed in HBL-1 cells with ruxolitinib. Accordingly, FIG. 3 depicts % inhibition in the cellular proliferation assay in HBL-1 cells as a function of the concentration of Compound 28 (a PI3Kδ inhibitor) with vehicle (DMSO), DMSO+IL10, and DMSO+IL10+ruxolitinib (a JAK1/JAK2 inhibitor).

Example G. IL10-induced Expression Of Pim2 Is Blocked By a JAK1/2 Inhibitor

Pfeiffer cells were treated for 24 hours with vehicle (DMSO), ruxolitinib (18424), Compound 28, or Compound 28 and ruxolitinib (18424) with or without IL10 and then

subjected to Western blot analysis to probe for the following proteins: phospho-Stat3 Y705, phospho-Akt S473, Pim2, c-Myc, phospho-p70S6K, phosho-S6, phospho-Bad S112 and actin. FIG. 4 shows that IL10-induced expression of Pim2 is blocked by ruxolitinib (a JAK1/JAK2 inhibitior). IL6 and IL10 promote cell survival through the expression of Pim2, which is dependent on JAK1 activity. FIG. 4 also shows a synergistic reduction of c-Myc and P-S6 in the presence of combined Compound 28 and ruxolitinib treatment. The reduction of c-Myc protein may be responsible for the synergistic effect for the combination treatment.

Example H. IL10-induced Expression Of Pim2 Is Blocked By a Selective JAK1 Inhibitor

Pfeiffer cells were treated for 24 hours with vehicle (DMSO), Compound 7, Compound 28, or Compound 28 and Compound 7 with or without IL10 and then subjected to Western blot analysis to probe for the following proteins: phospho-Stat3 Y705, phospho-Akt S473, Pim2, c-Myc, phospho-p70S6K, phosho-S6, phospho-Bad S112 and actin. FIG. 5 shows that IL10-induced expression of Pim2 is blocked by a selective JAK1 inhibitor (Compound 7).

Example I. Increase in Potency of PI3Kδ Inhibitor by Selective JAK1 Inhibitor

To test the effects of IL-10 on cell growth and sensitivity to BCR pathway inhibition, the Pfeiffer cell line was used as a model system of DLBCL. Pfeiffer cells are of the germinal center B-cell (GCB) subtype of DLBCL, have been shown to express PI3Kδ, are sensitive to PI3Kδ inhibition, and activate the JAK/STAT pathway in response to multiple cytokines as shown above. Pfeiffer cells were treated for 3 days with various concentrations of Compound 28 in the presence or absence of IL-10 and 1 μM of Compound 16, and cell growth was measured using an ATP readout (see table below). As shown in FIG. 6, the presence of IL-10 shifted the potency of Compound 28 by ~10-fold (IC_{50}=0.67 μM, - IL-10; IC_{50}=6.36 μM, + IL-10). Addition of the JAK1 inhibitor, Compound 16, reversed this effect so that the combination was ~50-fold more potent. In this system, the JAK1 inhibitor alone had no effect (IC_{50} > 1 μM). Further, as

shown in FIG. 7 (showing Annexin-V staining of Pfeiffer cells treated for 3 days in 10% FBS + IL10; Compound 16 was tested at 1 μM), inhibition of PI3Kδ together with JAK1 signaling led to increased apoptosis whereas neither agent alone had a significant effect.

	10%FBS+IL10		
	Compound 28	Compound 16	Compound 28+Compound 16
IC_{50} (μM)	6.36	>1	0.035

Example J. Effect of Combined JAK1 and PI3Kδ Treatment on STAT3 Phosphorylation and pAKT Inhibition

To assess the effects on downstream signaling pathways, Pfeiffer cells were treated with Compound 28 +/- Compound 16 for 4 hours and then stimulated with IL-10 for 15 minutes. Extracts were analyzed by Western blot for pAKT and pSTAT3. As shown in FIG. 8, the AKT pathway was constitutively activated in Pfeiffer cells. Blockade of PI3Kδ signaling led to complete inhibition of pAKT whereas treatment with the JAK1 inhibitor had no effect. In contrast, Compound 16 led to inhibition of STAT3 phosphorylation whereas the PI3Kδ inhibitor did not. The combination of both compounds was required to block both pathways.

All patents, patent publications, and journal articles referred to *supra* are incorporated herein by reference in their entireties.

WHAT IS CLAIMED IS:

1. A method of treating a disease selected from diffuse large B-cell lymphoma, chronic lymphocytic leukemia (CLL), Non-Hodgkin lymphoma, hairy cell leukemia, Mantle cell lymphoma, small lymphocytic lymphoma, follicular lymphoma, lymphoplasmacytic lymphoma, extranodal marginal zone lymphoma, Hodgkin's lymphoma, Burkitt lymphoma, Waldenstrom's macroglobulinemia, prolymphocytic leukemia, acute lymphoblastic leukemia, myelofibrosis, mucosa-associated lymphatic tissue (MALT) lymphoma, mediastinal (thymic) large B-cell lymphoma, lymphomatoid granulomatosis, splenic marginal zone lymphoma, primary effusion lymphoma, intravascular large B-cell lymphoma, plasma cell leukemia, extramedullary plasmacytoma, smouldering myeloma (aka asymptomatic myeloma), monoclonal gammopathy of undetermined significance (MGUS), activated B-cell like (ABC) diffuse large B cell lymphoma (ABC-DLBCL), and germinal center B cell (GCB) diffuse large B cell lymphoma (GCB-DLBCL) in a patient in need thereof, comprising administering to said patient: (a) an inhibitor of JAK1 and/or JAK2; and (b) an inhibitor of PI3Kδ; wherein:

(a) said inhibitor of JAK1 and/or JAK2 is selected from:

3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

3-[1-(6-chloropyridin-2-yl)pyrrolidin-3-yl]-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile;

4-[(4-{3-cyano-2-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrrol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile;

{1-{1-[3-Fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-[4-fluoro-2-(trifluoromethyl)phenyl]piperidine-1-carboxamide;

[3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-1-(1-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperidin-4-yl)azetidin-3-yl]acetonitrile;

[*trans*-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-3-(4-{[2-(trifluoromethyl)pyrimidin-4-yl]carbonyl}piperazin-1-yl)cyclobutyl]acetonitrile;

{*trans*-3-(4-{[4-[(3-hydroxyazetidin-1-yl)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{*trans*-3-(4-{[4-{[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{*trans*-3-(4-{[4-{[(2R)-2-(hydroxymethyl)pyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

4-(4-{3-[(dimethylamino)methyl]-5-fluorophenoxy}piperidin-1-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]butanenitrile;

5-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide;

4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide;

5-{3-(cyanomethyl)-3-[4-(1H-pyrrolo[2,3-b]pyridin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-N-isopropylpyrazine-2-carboxamide;

{1-(*cis*-4-{[6-(2-hydroxyethyl)-2-(trifluoromethyl)pyrimidin-4-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{1-(*cis*-4-{[4-[(ethylamino)methyl]-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{1-(*cis*-4-{[4-(1-hydroxy-1-methylethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{1-(*cis*-4-{[4-{[(3R)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{1-(*cis*-4-{[4-{[(3S)-3-hydroxypyrrolidin-1-yl]methyl}-6-(trifluoromethyl)pyridin-2-yl]oxy}cyclohexyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile;

{*trans*-3-(4-{[4-({[(1S)-2-hydroxy-1-methylethyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{*trans*-3-(4-{[4-({[(2R)-2-hydroxypropyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{*trans*-3-(4-{[4-({[(2S)-2-hydroxypropyl]amino}methyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

{*trans*-3-(4-{[4-(2-hydroxyethyl)-6-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)-1-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]cyclobutyl}acetonitrile;

((2R,5S)-5-{2-[(1R)-1-hydroxyethyl]-1H-imidazo[4,5-d]thieno[3,2-b]pyridin-1-yl}tetrahydro-2H-pyran-2-yl)acetonitrile;

4-[3-(cyanomethyl)-3-(3',5'-dimethyl-1H,1'H-4,4'-bipyrazol-1-yl)azetidin-1-yl]-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide;

and pharmaceutically acceptable salts of any of the aforementioned; and

(b) said inhibitor of PI3Kδ is selected from:

7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one;

(S)-7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one;

4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-{1-[(2S)-2-hydroxypropyl]azetidin-3-yl}-3-methoxybenzonitrile;

4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-[1-(2-hydroxyethyl)azetidin-3-yl]-3-methoxybenzonitrile;

5-{3-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-cyano-2-ethoxy-5-methylphenyl}-N,N-dimethylpyridine-2-carboxamide;

4-{3-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-5-chloro-2-ethoxy-6-fluorophenyl}pyrrolidin-2-one; and

N-{1-[5-chloro-8-(3-fluorophenyl)cinnolin-7-yl]ethyl}-9H-purin-6-amine;

4-chloro-3'-fluoro-3-methyl-6-[1-(9H-purin-6-ylamino)ethyl]biphenyl-2-carbonitrile;

and pharmaceutically acceptable salts of any of the aforementioned.

2. The method of claim 1, wherein said inhibitor of JAK1 and/or JAK2 is selected from:

(R)-3-[1-(6-chloropyridin-2-yl)pyrrolidin-3-yl]-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

(R)-3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

(R)-4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile;

(R)-4-[(4-{3-cyano-2-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrrol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile;

(R)-4-(4-{3-[(dimethylamino)methyl]-5-fluorophenoxy}piperidin-1-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]butanenitrile;

(S)-3-[1-(6-chloropyridin-2-yl)pyrrolidin-3-yl]-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

(S)-3-(1-[1,3]oxazolo[5,4-b]pyridin-2-ylpyrrolidin-3-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile;

(S)-4-[(4-{3-cyano-2-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile;

(S)-4-[(4-{3-cyano-2-[3-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrrol-1-yl]propyl}piperazin-1-yl)carbonyl]-3-fluorobenzonitrile;

(S)-4-(4-{3-[(dimethylamino)methyl]-5-fluorophenoxy}piperidin-1-yl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]butanenitrile;

and pharmaceutically acceptable salts of any of the aforementioned.

3. The method of claim 1, wherein said inhibitor of JAK1 and/or JAK2 is (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile, or a pharmaceutically acceptable salt thereof.

4. The method of claim 1, wherein said inhibitor of JAK1 and/or JAK2 is (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile phosphoric acid salt.

5. The method of claim 1, wherein said inhibitor of JAK1 and/or JAK2 is {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile, or pharmaceutically acceptable salt thereof.

6. The method of claim 1, wherein said inhibitor of JAK1 and/or JAK2 is {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile adipic acid salt.

7. The method of claim 1, wherein said inhibitor of JAK1 and/or JAK2 is 4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide, or a pharmaceutically acceptable salt thereof.

8. The method of claim 1, wherein said inhibitor of PI3Kδ is selected from:

(S)-4-(3-((S)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-chloro-2-ethoxy-6-fluorophenyl)pyrrolidin-2-one;

(R)-4-(3-((S)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-chloro-2-ethoxy-6-fluorophenyl)pyrrolidin-2-one;

(S)-4-(3-((R)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-chloro-2-ethoxy-6-fluorophenyl)pyrrolidin-2-one;

(R)-4-(3-((R)-1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-chloro-2-ethoxy-6-fluorophenyl)pyrrolidin-2-one;

(N-{(1S)-1-[5-chloro-8-(3-fluorophenyl)cinnolin-7-yl]ethyl}-9H-purin-6-amine;

and pharmaceutically acceptable salts of any of the aforementioned.

9. The method of claim 1, wherein said inhibitor of PI3Kδ is (S)-7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

10. The method of claim 1, wherein said inhibitor of PI3Kδ is 4-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-chloro-2-[1-(2-hydroxyethyl)azetidin-3-yl]-3-methoxybenzonitrile, or a pharmaceutically acceptable salt thereof.

11. The method of claim 1, wherein said inhibitor of PI3Kδ is 5-{3-[1-(4-amino-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl]-6-cyano-2-ethoxy-5-methylphenyl}-N,N-dimethylpyridine-2-carboxamide, or a pharmaceutically acceptable salt thereof.

12. The method of claim 1, wherein said inhibitor of PI3Kδ is (N-{(1S)-1-[5-chloro-8-(3-fluorophenyl)cinnolin-7-yl]ethyl}-9H-purin-6-amine, or a pharmaceutically acceptable salt thereof.

13. The method of claim 1, wherein said disease is diffuse large B-cell lymphoma.

14. The method of claim 1, wherein said disease is activated B-cell like (ABC) diffuse large B cell lymphoma (ABC-DLBCL) or germinal center B cell (GCB) diffuse large B cell lymphoma (GCB-DLBCL).

15. The method of claim 1, wherein said inhibitor of JAK1 and/or JAK2 and said inhibitor of PI3Kδ are administered simultaneously.

16. The method of claim 1, wherein said inhibitor of JAK1 and/or JAK2 and said inhibitor of PI3Kδ are administered sequentially.

17. A method of treating diffuse large B-cell lymphoma in a patient in need thereof, comprising administering to said patient (3R)-3-cyclopentyl-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]propanenitrile, or a pharmaceutically acceptable salt thereof; and (7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

18. A method of treating diffuse large B-cell lymphoma in a patient in need thereof, comprising administering to said patient {1-{1-[3-fluoro-2-(trifluoromethyl)isonicotinoyl]piperidin-4-yl}-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-3-yl}acetonitrile, or pharmaceutically acceptable salt thereof; and 7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.

19. A method of treating diffuse large B-cell lymphoma in a patient in need thereof, comprising administering to said patient 4-{3-(cyanomethyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]azetidin-1-yl}-2,5-difluoro-N-[(1S)-2,2,2-trifluoro-1-methylethyl]benzamide, or pharmaceutically acceptable salt thereof; and 7-(1-(9H-purin-6-ylamino)ethyl)-6-(3-fluorophenyl)-3-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, or a pharmaceutically acceptable salt thereof.