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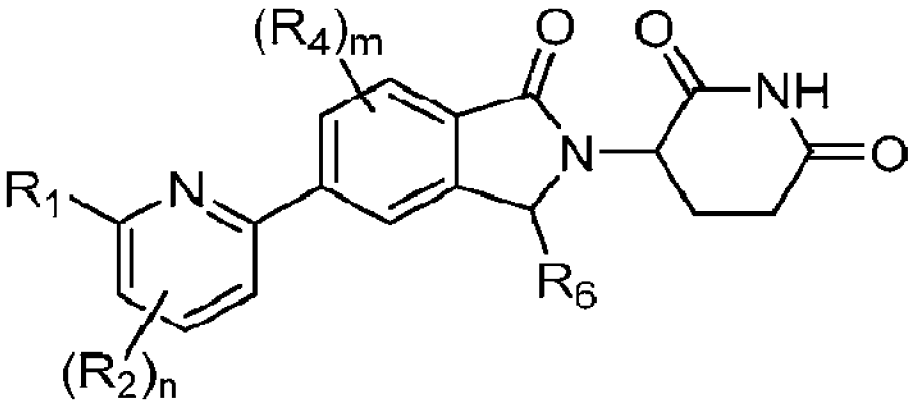
(54) **Titre : COMPOSES OXOISOINDOLINE SUBSTITUES PAR PYRIDINYLE**
(54) **Title: PYRIDINYL SUBSTITUTED OXOISOINDOLINE COMPOUNDS**



(I)

(57) **Abrégé/Abstract:**
Disclosed are compounds of Formula (I): or a salt thereof, wherein R1, R2, R4, R6, m, and n are defined herein. Also disclosed are methods of using such compounds to inhibit Helios protein, and pharmaceutical compositions comprising such compounds. These compounds are useful in the treatment of viral infections and proliferative disorders, such as cancer.

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Abstract:

Disclosed are compounds of Formula (I): or a salt thereof, wherein R1, R2, R4, R6, m, and n are defined herein. Also disclosed are methods of using such compounds to inhibit Helios protein, and pharmaceutical compositions comprising such compounds. These compounds are useful in the treatment of viral infections and proliferative disorders, such as cancer.

PYRIDINYL SUBSTITUTED OXOISOINDOLINE COMPOUNDS

CROSS REFERENCE

This application claims the benefit of Indian Provisional Application Serial No. 202111016193 filed April 6, 2021 and Indian Provisional Application Serial No. 202111022098, filed May 17, 2021, each incorporated herein in its entirety.

DESCRIPTION

The present invention generally relates to pyridinyl substituted oxoisoindoline compounds that inhibit Helios protein. Provided herein are pyridinyl substituted oxoisoindoline compounds, compositions comprising such compounds, and methods of their use. The invention further pertains to pharmaceutical compositions comprising at least one compound according to the invention that are useful for the treatment of proliferative disorders, such as cancer, and viral infections.

BACKGROUND OF THE INVENTION

Regulatory T cells (Tregs) play an essential role in controlling self-tolerance and immune homeostasis via maintenance of inhibitory activity and anergy in the face of vigorous immune and inflammatory responses. Through the preservation of a stable, anergic and suppressive phenotype, Tregs attenuate excessive immune responses and prevent or ameliorate autoimmunity. A number of reports have documented the presence of Tregs within human tumor tissues. Studies demonstrated a clear negative correlation between the number of Tregs and T cell infiltration into the tumor and survival (Curiel et al., 2004, *Nat. Med.* 10: 942-949; Viguier et al., 2004, *J Immuno.* 1173:1444-1453; Beyer et al., 2006, *Blood* 108: 804-811; Zou et al., 2006, *Nat. Rev. Immunol.* 6: 295-307), implying a potential critical role of Tregs in preventing the development of effective anti-tumor immunity. Accumulated evidence indicates that Foxp3+CD25+CD4+Tregs dominantly infiltrate into tumors and apparently hinder immune responses to tumor cells in rodents and humans. Once activated by a specific antigen, Tregs suppress responder T cells in an antigen-nonspecific and bystander manner *in vitro* (Takahashi et al., 1998, *Int Immunol.* 10:1969–80; Thornton et al., 1998, *J Exp. Med.* 188:287–96). Foxp3+CD25+CD4+Tregs are apparently capable of suppressing a wide range of

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antitumor immune responses involving CD4+ helper T cells, CD8+ T cells, natural killer cells, and natural killer T cells (Tanaka et al., 2017, *Cell Research* 27:109-118). Intratumoral depletion of CD25+CD4+Tregs induced regression of established tumors with a change in the cytokine milieu at tumor sites (Yu et al., 2005, *J Exp Med.* 201: 779–91). In addition, transfer of Treg-depleted CD4+ T cells markedly augmented antitumor immune responses compared with Tregs containing T-cell transfer (Antony et al., 2005, *J Immunol* 174:2591–601). Tumor-infiltrating Tregs activated by either tumor-derived self-antigens or tumor-associated antigens can similarly suppress specific antitumor immune responses. Modulation of the activities of key factors to control Treg differentiation could represent a potential therapeutic strategy for the treatment of certain diseases, including cancer and viral infections.

FoxP3+CD4 Tregs are remarkably stable. Studies are still evolving to understand the genetic mechanisms that ensure their phenotypic stability after expansion during inflammation, infection or autoimmunity. Transcription factors (TF) responsible for maintaining the stable immunosuppressive phenotype of Tregs likely contribute to this process. The Helios (IKZF2) gene, a member of the Ikaros family of TFs, differs from other Ikaros family members based on its selective expression by thymocytes undergoing negative selection, as well as by regulatory lineages of CD4 and CD8 T cells. Helios is expressed by two regulatory T-cell lineages, FoxP3+CD4+ and Ly49+CD8+ Tregs, which are essential to maintain self-tolerance (Kim et al., 2015, *Science* 350:334–339; Sebastian et al., 2016, *J Immunol* 196:144–155). Interestingly, recent studies suggest that although Helios is largely dispensable for Treg activity in the steady state, control of the genetic program of FoxP3+ CD4 Tregs by Helios in the context of inflammation is essential to maintain a stable phenotype and potentiate suppressive function (Thornton et al., 2010, *J Immunol.* 184:3433–3441; Kim et al., 2015). Helios expression by Tregs was demonstrated to be crucial in their capability to maintain a suppressive and anergic phenotype in the face of intense inflammatory responses. Activation of the IL-2Rα–STAT5 pathway was demonstrated to be a key contributor by ensuring Treg survival and stability (Kim et al., 2015). Helios plays an indispensable role in maintaining the phenotype of FoxP3+ CD4 Tregs by exerting dominant, lymphocyte-intrinsic inhibition to prevent autoimmune disease in the presence of highly activated self-reactive T cells from scurfy mice, which have no FoxP3 fork head domain. Bone marrow (BM) chimeras

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reconstituted with Helios–/–/Scurfy BM but not Helios+/+/Scurfy BM cells rapidly developed autoimmunity (Kim et al., 2015). These observations indicate the critical contribution of Helios to self-reactive T cell selection, differentiation, and function. Immune suppression exerted by Tregs can impede antitumor immune responses. A selective deficiency of Helios in FoxP3+ CD4 Tregs results in increased Treg instability and conversion of intratumoral CD4 Treg to effector T cells (Teff). Instability of intratumoral Tregs may increase the numbers of Teff cells within tumors as a combined result of Treg conversion and reduced Treg suppressive activities. In addition, defective IL-2 responses were observed in Helios-deficient intratumoral Tregs, which results in decreased numbers of activated Tregs and may also contribute to the increased intratumoral Teff activities. Interaction between tumor cells and infiltrating immune cells leads to secretion of inflammatory mediators, including TNF-α, IL-6, IL-17, IL-1, and TGF-β, and the formation of a local inflammatory environment (Kim et al., 2015).

Lineage instability of Helios-deficient Tregs is also accompanied by diminished FoxP3 expression and results in the acquisition of an effector phenotype by producing proinflammatory cytokines. Effector cell conversion of Helios-deficient Tregs within the tumor-tissue microenvironment is associated with increased expression of genes that control Teff phenotype (Yates et al., 2018, *PNAS*, 2018, 115: 2162-2167). Acquisition of an unstable phenotype by Helios deficiency only occurs within the tumor microenvironment (TME), but not in peripheral lymphoid organs (Nakagawa et al., 2016, *PNAS* 113: 6248-6253). Within the chronic inflammatory TME, Helios deficiency in Tregs could drastically alleviate the repressed genetic programs associated with T helper cell differentiation by up-regulating T helper cell associated TFs and effector cytokines. These genetic changes of Helios-deficient Tregs are most apparent in a Treg subpopulation with high affinity for self-antigens, as shown by enhanced GITR/PD-1 expression and increased responsiveness to self-antigens. Their combined effects may promote a phenotype conversion of Tregs into Teff within the TME with increased T-cell receptor (TCR) engagement and costimulatory receptor expression by Tregs, suggesting that the alterations in gene expression, as a central feature of Treg conversion, are immune milieu dependent (Yates et al., 2018).

Reduced Helios expression in FoxP3+ CD4 Tregs may allow conversion of memory Tregs into Teff cells that express self-reactive T-cell receptors with specificity

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for tumor antigens. An altered Treg signature might be selectively induced within the chronic inflammatory conditions of growing tumor. Helios-deficient Tregs may display a TCR repertoire skewed toward high-affinity against self-peptides/MHC, which can promote robust activation in TME (Yates et al., 2018). In view of the increased self-reactivity of TCR in CD4 Tregs compared with conventional T cells, conversion of Tregs could generate highly potent effector CD4 T cells accompanied by attenuated Treg-mediated suppression within the TME. A more effective strategy may depend on approaches that selectively convert intratumoral Tregs into Teff cells without affecting the systemic Treg population. As a key player in the maintenance of Treg size and functional stability in response to diverse immunological perturbations, pharmacological intervention of Helios could be relevant to the strategies that strengthen current tumor immunotherapy. Since Treg to Teff conversion may be confined to inflammatory intratumoral microenvironments, antibody or small molecule-based approaches that target Helios may lead to improved Treg dependent cancer immunotherapy. Importantly, conversion of Helios-deficient Tregs only occurs within the local inflammatory environment of the tumor. This approach may not provoke the autoimmune side effects associated with systemic reduction of Tregs. Therefore, strategies that specifically harness Helios-dependent control of the intratumoral Treg phenotype represent a significant promise to improve cancer immunotherapy. Furthermore, removal of Foxp3+Tregs was also reported to enhance vaccine-induced antitumor T-cell responses (Nishikawa et al., 2010, *Int. J. Cancer* 127: 759–767), suggesting that decreasing Helios levels could be beneficial in boosting the efficacy of cancer vaccines.

Besides anti-tumor immunotherapy, during viral infections, Treg cells can limit the immunopathology resulting from excessive inflammation, yet potentially inhibit effective antiviral T cell responses and promote virus persistence (Schmitz et al., 2013, *PLOS Pathogens* 9: e1003362). Chronic, but not acute, infection of mice with lymphocytic choriomeningitis virus results in a marked expansion of Foxp3+ Tregs, implying a potential mechanism that certain infectious agents could evade host immune responses by activation and expansion of Tregs (Punkosdy et al., 2011, *PNAS* 108: 3677–3682). Treatment benefits could be achieved by decreasing Helios levels in activated Tregs in the context relevant to chronic viral infections.

There is a need for compounds useful as inhibitors of Helios protein.

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SUMMARY OF THE INVENTION

The present invention provides pyridinyl substituted oxoisoindoline compounds of Formula (I) or salts thereof, which are useful to decrease Helios protein levels, decrease Helios activity levels and/or inhibit Helios expression levels in the cells.

The present invention also provides pharmaceutical compositions comprising a compound of Formula (I) and/or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.

The present invention also provides a method of treating a disease or disorder by decreasing the activity of Helios protein, the method comprising administering to a patient a compound of Formula (I) and/or a pharmaceutically acceptable salt thereof.

The present invention also provides processes and intermediates for making the compounds of Formula (I) and/or salts thereof.

The present invention also provides a compound of Formula (I) and/or a pharmaceutically acceptable salt thereof, for use in therapy.

The present invention also provides the use of the compounds of Formula (I) and/or pharmaceutically acceptable salts thereof, for the manufacture of a medicament to decrease Helios protein levels, decrease Helios activity levels and/or inhibit Helios expression levels in cells to control Treg differentiation, for the treatment of certain diseases, including cancer and viral infections.

The compounds of Formula (I) and compositions comprising the compounds of Formula (I) may be used in treating, preventing, or curing viral infections and various proliferative disorders, such as cancer. Pharmaceutical compositions comprising these compounds are useful in treating, preventing, or slowing the progression of diseases or disorders in a variety of therapeutic areas, such as viral infections and cancer.

These and other features of the invention will be set forth in expanded form as the disclosure continues.

DETAILED DESCRIPTION

Applicants have found substituted oxoisoindoline compounds that inhibit Helios protein by facilitating the interaction of Helios protein and the corresponding E3 ubiquitin ligase complex (Cullin4-Cereblon, CUL4-CRBN). These compounds decrease Helios

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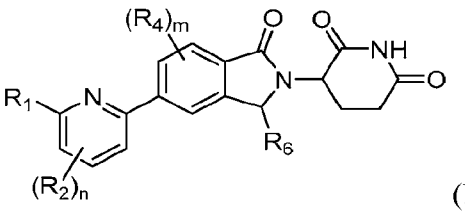
protein levels, decrease Helios activity levels and/or inhibit Helios expression levels in the cells to control Treg differentiation. These compounds are useful for the treatment of certain diseases, including cancer and viral infections. The compounds are provided to be useful as pharmaceuticals with desirable stability, bioavailability, therapeutic index, and toxicity values that are important to their drugability.

The first aspect of the present invention provides at least one compound of Formula (I):



(I)

or a salt thereof, wherein:

R_1 is $-NH_2$ or $-NH(CH_3)$;

each R_2 is independently F, Cl, –CN, C_{1-4} alkyl, $-CH_2F$, $-CHF_2$, $-CF_3$, $-OCH_3$, or cyclopropyl;

each R_4 is independently F, Cl, $-CH_3$, $-CH_2F$, $-CHF_2$, $-CF_3$, or $-OCH_3$;

R_6 is hydrogen, C_{1-2} alkyl, or C_{1-2} fluoroalkyl;

m is zero, 1, 2, or 3; and

n is zero, 1, 2, or 3;

with the proviso that when R_6 is hydrogen, m is 1, 2, or 3.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_1 is $-NH_2$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_1 is $-NH(CH_3)$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently F, Cl, –CN, $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH_2CH_2CH_3$, $-CH_2F$, $-CHF_2$, $-CF_3$, $-OCH_3$, or cyclopropyl.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently F, Cl, –CN, $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH_2CH_2CH_3$, $-CH_2F$, $-CHF_2$, or $-CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein

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each R_2 is independently F, Cl, –CN, $–CH_3$, $–CH_2CH_3$, $–CH_2F$, $–CHF_2$, or $–CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently F, Cl, –CN, $–CH_3$, or $–CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently –CN, $–CH_3$, or $–CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently F, –CN, $–CH_3$, or $–CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently F, –CN, or $–CH_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein n is zero, 1, or 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein n is zero or 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein n is 1 or 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein n is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein n is 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein n is 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently –CN, $–CH_3$, or $–CF_3$; and n is zero, 1, or 2. Included in this embodiment are compounds in which n is 1 or 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently –CN, $–CH_3$, or $–CF_3$; and n is zero or 1. Included in this embodiment are compounds in which n is 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently –CN, $–CH_3$, or $–CF_3$; and n is 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is independently –CN or $–CH_3$; and n is 1 or 2. Included in this embodiment are compounds in which n is 1. Also included in this embodiment are compounds in which n

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is 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_2 is $-CH_3$; and n is 1 or 2. Included in this embodiment are compounds in which n is 1. Also included in this embodiment are compounds in which n is 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_4 is independently F, Cl, $-CH_3$, $-CH_2F$, $-CHF_2$, or $-CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_4 is independently F, $-CH_3$, $-CH_2F$, $-CHF_2$, or $-CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_4 is independently F, $-CH_3$, $-CHF_2$, or $-CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_4 is independently F or $-CH_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_4 is F_3. Included in this embodiment are compounds in which m is 1. Also included in this embodiment are compounds in which m is 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein each R_4 is $-CH_3$. Included in this embodiment are compounds in which m is 1. Also included in this embodiment are compounds in which m is 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein m is zero, 1, or 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein m is zero or 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein m is 1 or 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein m is 1, 2, or 3.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein m is 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; and m is 1, 2, or 3.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; and m is 1 or 2.

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One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; and m is 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; and m is 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; and m is 3.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; m is 1; and R_4 is F, Cl, $-CH_3$, $-CH_2F$, $-CHF_2$, or $-CF_3$. Included in this embodiment are compounds in which R_4 is F, $-CH_3$, $-CHF_2$, or $-CF_3$. Also included in this embodiment are compounds in which R_4 is F, $-CH_3$ or $-CF_3$. Additionally, included in this embodiment are compounds in which R_4 is F or $-CH_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; m is 1; and R_4 is F.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; m is 1; and R_4 is $-CH_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is hydrogen; m is 1 or 2; and R_4 is F or $-CH_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl or C_{1-2} fluoroalkyl.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl, $-CH_2F$, $-CF_2H$, $-CF_3$, or $-CH_2CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl, $-CH_2F$, $-CF_2H$, or $-CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl, or $-CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is $-CH_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is $-CH_2F$, $-CF_2H$, $-CF_3$, or $-CH_2CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein

R_6 is $-CH_2F$, $-CF_2H$, or $-CF_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl or C_{1-2} fluoroalkyl; and m is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl, $-CH_2F$, $-CF_2H$, $-CF_3$, or $-CH_2CF_3$; and m is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl, $-CH_2F$, $-CF_2H$, or $-CF_3$; and m is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl, or $-CF_3$; and m is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is C_{1-2} alkyl; and m is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is $-CH_3$; and m is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is $-CH_2F$, $-CF_2H$, $-CF_3$, or $-CH_2CF_3$; and m is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_6 is $-CH_2F$, $-CF_2H$, or $-CF_3$; and m is zero.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_1 is $-NH_2$ or $-NH(CH_3)$; each R_2 is independently $-CN$, $-CH_3$, or $-CF_3$; each R_4 is independently F or $-CH_3$; R_6 is hydrogen or $-CH_3$; m is zero or 1; and n is 1 or 2; with the proviso that when R_6 is hydrogen, m is 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_1 is $-NH_2$; each R_2 is independently $-CN$, $-CH_3$, or $-CF_3$; R_4 is F or $-CH_3$; R_6 is hydrogen or $-CH_3$; m is zero or 1; and n is 1 or 2; with the proviso that when R_6 is hydrogen, m is 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_1 is $-NH(CH_3)$; each R_2 is independently $-CN$ or $-CH_3$; R_4 is F or $-CH_3$; R_6 is hydrogen or $-CH_3$; m is zero or 1; and n is 1 or 2; with the proviso that when R_6 is hydrogen, m is 1.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_1 is $-NH_2$; each R_2 is independently $-CN$, $-CH_3$, or $-CF_3$; each R_4 is independently F or

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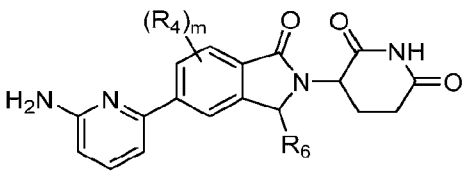
$-CH_3$; R_6 is $-CH_3$; m is 1; and n is 1 or 2. Included in this embodiment are compounds in which each R_2 is $-CH_3$.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_1 is $-NH_2$; each R_2 is independently $-CN$ or $-CH_3$; R_4 is F or $-CH_3$; R_6 is hydrogen; m is 1; and n is 1, or 2.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein R_1 is $-NH_2$ or $-NH(CH_3)$; each R_2 is independently $-CH_3$; R_4 is F; R_6 is hydrogen or $-CH_3$; m is zero or 1; and n is 1, or 2; with the proviso that when R_6 is hydrogen, m is 1.

One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:



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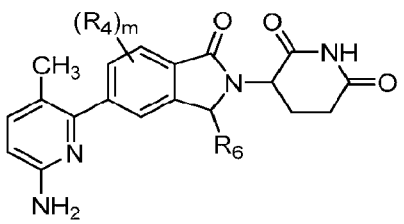

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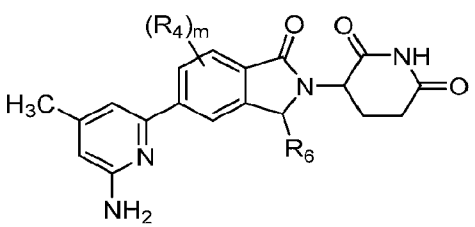

One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:

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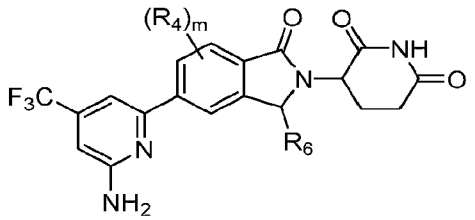

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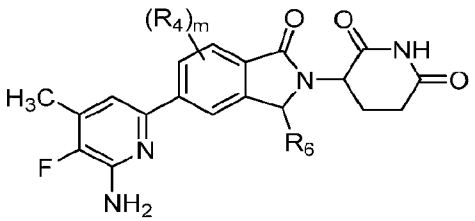
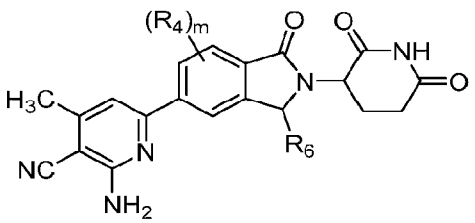
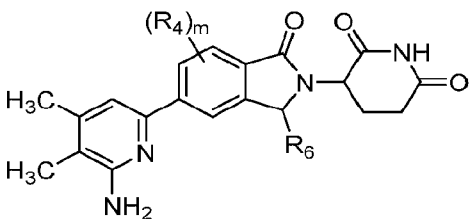


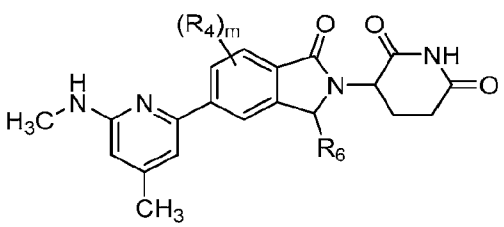
One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:



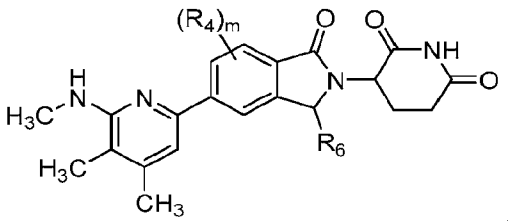
One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:



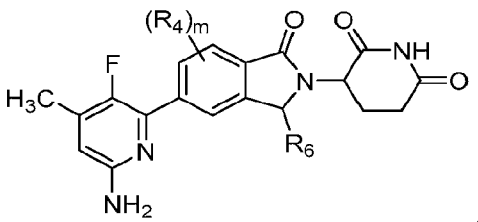
One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:



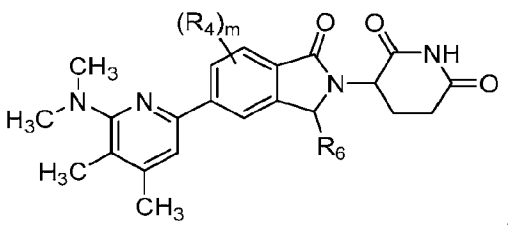
One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:



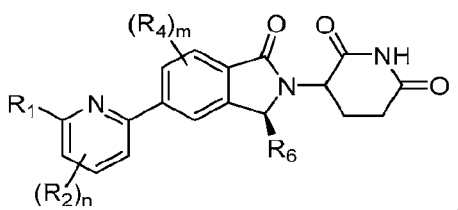
One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:



One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:



One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:



One embodiment provides a compound of Formula (I) or a salt thereof, having the structure:

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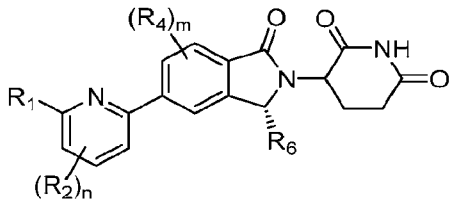

One embodiment provides a compound of Formula (I) or a salt thereof, wherein said compound is: 2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-4-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (1); 2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-6-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (2); 2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-4-fluoro-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (3); 3-(5-(6-amino-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)piperidine-2,6-dione (4); 3-(5-(6-amino-4-(trifluoroethyl) pyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)piperidine-2,6-dione (5); 3-(4-fluoro-5-(4-methyl-6-(methylamino)pyridin-2-yl)-1-oxoisoindolin-2-yl) piperidine-2,6-dione (6); 3-(5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (7); 3-((*S*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (8); 3-((*R*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (9); 2-amino-6-((3S)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (10); 2-amino-6-((3R)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (11); 3-((S)-5-(4,5-dimethyl-6-(methylamino) pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (12); 3-((R)-5-(4,5-dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (13); 6-((3S)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2-(methylamino) nicotinonitrile (14); 6-((3R)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2-(methylamino)nicotinonitrile (15); 3-((*S*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (16); 3-((*R*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (17); 3-(5-(6-amino-3-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (18); 3-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (19); 3-(5-(6-amino-3-fluoro-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (20); 3-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (21-22); 2-amino-6-((3*R*)-2-(2,6-dioxopiperidin-3-yl)-4-fluoro-3-methyl-1-oxoisoindolin-5-

yl)-4-methylnicotinonitrile (23); 3-((*R*)-5-(6-amino-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (24); 3-((*R*)-5-(6-amino-4-(trifluoromethyl)pyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (25); 3-((*R*)-5-(6-amino-3-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (26); 3-((*R*)-5-(6-aminopyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (27); (*R*)-3-((*R*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione 28); 3-((R)-5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (29); or 3-((*S*)-5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (30).

One embodiment provides a compound of Formula (I) or a salt thereof, wherein said compound is 3-((R)-5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione.

One embodiment provides a compound of Formula (I) or a salt thereof, wherein said compound is 3-((S)-5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione.

The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof. This invention encompasses all combinations of the aspects and/or embodiments of the invention noted herein. It is understood that any and all embodiments of the present invention may be taken in conjunction with any other embodiment or embodiments to describe additional embodiments. It is also to be understood that each individual element of the embodiments is meant to be combined with any and all other elements from any embodiment to describe an additional embodiment.

The features and advantages of the invention may be more readily understood by those of ordinary skill in the art upon reading the following detailed description. It is to be appreciated that certain features of the invention that are, for clarity reasons, described above and below in the context of separate embodiments, may also be combined to form a single embodiment. Conversely, various features of the invention that are, for brevity reasons, described in the context of a single embodiment, may also be combined so as to form sub-combinations thereof. Embodiments identified herein as exemplary or preferred are intended to be illustrative and not limiting.

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Unless specifically stated otherwise herein, references made in the singular may also include the plural. For example, “a” and “an” may refer to either one, or one or more.

As used herein, the phrase “compounds and/or salts thereof” refers to at least one compound, at least one salt of the compounds, or a combination thereof. For example, compounds of Formula (I) and/or salts thereof includes a compound of Formula (I); two compounds of Formula (I); a salt of a compound of Formula (I); a compound of Formula (I) and one or more salts of the compound of Formula (I); and two or more salts of a compound of Formula (I).

Unless otherwise indicated, any atom with unsatisfied valences is assumed to have hydrogen atoms sufficient to satisfy the valences.

The definitions set forth herein take precedence over definitions set forth in any patent, patent application, and/or patent application publication incorporated herein by reference.

Listed below are definitions of various terms used to describe the present invention. These definitions apply to the terms as they are used throughout the specification (unless they are otherwise limited in specific instances) either individually or as part of a larger group.

Throughout the specification, groups and substituents thereof may be chosen by one skilled in the field to provide stable moieties and compounds.

In accordance with a convention used in the art,

is used in structural formulas herein to depict the bond that is the point of attachment of the moiety or substituent to the core or backbone structure.

The terms “halo” and “halogen,” as used herein, refer to F, Cl, Br, and I.

The term “cyano” refers to the group -CN.

The term “amino” refers to the group $-NH_2$.

The term "oxo" refers to the group =O.

The term “alkyl” as used herein, refers to both branched and straight-chain saturated aliphatic hydrocarbon groups containing, for example, from 1 to 12 carbon atoms, from 1 to 6 carbon atoms, and from 1 to 4 carbon atoms. Examples of alkyl

groups include, but are not limited to, methyl (Me), ethyl (Et), propyl (*e.g.*, n-propyl and i-propyl), butyl (*e.g.*, n-butyl, i-butyl, sec-butyl, and *t*-butyl), and pentyl (*e.g.*, n-pentyl, isopentyl, neopentyl), n-hexyl, 2-methylpentyl, 2-ethylbutyl, 3-methylpentyl, and 4-methylpentyl. When numbers appear in a subscript after the symbol "C", the subscript defines with more specificity the number of carbon atoms that a particular group may contain. For example, "C_{1-4} alkyl" denotes straight and branched chain alkyl groups with one to four carbon atoms.

The term "fluoroalkyl" as used herein is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups substituted with one or more fluorine atoms. For example, "C_{1-4} fluoroalkyl" is intended to include C_1, C_2, C_3, and C_4 alkyl groups substituted with one or more fluorine atoms. Representative examples of fluoroalkyl groups include, but are not limited to, $-CF_3$ and $-CH_2CF_3$.

The compounds of the present invention include all isotopes of atoms occurring in the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include deuterium (D) and tritium (T). Isotopes of carbon include ^{13}C and ^{14}C. Isotopically-labeled compounds of the invention can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described herein, using an appropriate isotopically-labeled reagent in place of the non-labeled reagent otherwise employed.

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 compounds of Formula (I) can form salts which are also within the scope of this invention. Unless otherwise indicated, reference to an inventive compound is understood to include reference to one or more salts thereof. The term "salt(s)" denotes acidic and/or basic salt(s) formed with inorganic and/or organic acids and bases. In addition, the term "salt(s) may include zwitterions (inner salts), *e.g.*, when a compound of Formula (I) contains both a basic moiety, such as an amine or a pyridine or imidazole ring, and an acidic moiety, such as a carboxylic acid. Pharmaceutically acceptable (*i.e.*,

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non-toxic, physiologically acceptable) salts are preferred, such as, for example, acceptable metal and amine salts in which the cation does not contribute significantly to the toxicity or biological activity of the salt. However, other salts may be useful, *e.g.*, in isolation or purification steps which may be employed during preparation, and thus, are contemplated within the scope of the invention. Salts of the compounds of the formula (I) may be formed, for example, by reacting a compound of the Formula (I) with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

Exemplary acid addition salts include acetates (such as those formed with acetic acid or trihaloacetic acid, for example, trifluoroacetic acid), adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides (formed with hydrochloric acid), hydrobromides (formed with hydrogen bromide), hydroiodides, maleates (formed with maleic acid), 2-hydroxyethanesulfonates, lactates, methanesulfonates (formed with methanesulfonic acid), 2-naphthalenesulfonates, nicotinates, nitrates, oxalates, pectinates, persulfates, 3-phenylpropionates, phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates (such as those formed with sulfuric acid), sulfonates (such as those mentioned herein), tartrates, thiocyanates, toluenesulfonates such as tosylates, undecanoates, and the like.

Exemplary basic salts include ammonium salts, alkali metal salts such as sodium, lithium, and potassium salts; alkaline earth metal salts such as calcium and magnesium salts; barium, zinc, and aluminum salts; salts with organic bases (for example, organic amines) such as trialkylamines such as triethylamine, procaine, dibenzylamine, N-benzyl-β-phenethylamine, 1-ephenamine, N,N′-dibenzylethylene-diamine, dehydroabietylamine, N-ethylpiperidine, benzylamine, dicyclohexylamine or similar pharmaceutically acceptable amines and salts with amino acids such as arginine, lysine and the like. Basic nitrogen-containing groups may be quaternized with agents such as lower alkyl halides (*e.g.*, methyl, ethyl, propyl, and butyl chlorides, bromides and iodides), dialkyl sulfates (*e.g.*, dimethyl, diethyl, dibutyl, and diamyl sulfates), long chain halides (*e.g.*, decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides), aralkyl halides (*e.g.*, benzyl

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and phenethyl bromides), and others.

The compounds of Formula (I) can be provided as amorphous solids or crystalline solids. Lyophilization can be employed to provide the compounds of Formula (I) as a solid.

It should further be understood that solvates (e.g., hydrates) of the Compounds of Formula (I) are also within the scope of the present invention. The term "solvate" means a physical association of a compound of Formula (I) with one or more solvent molecules, whether organic or inorganic. This physical association includes hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolable solvates. Exemplary solvates include hydrates, ethanolates, methanolates, isopropanolates, acetonitrile solvates, and ethyl acetate solvates. Methods of solvation are known in the art.

Various forms of prodrugs are well known in the art and are described in Rautio, J. et al., *Nature Review Drug Discovery,* 17, 559-587 (2018).

In addition, compounds of Formula (I), subsequent to their preparation, can be isolated and purified to obtain a composition containing an amount by weight equal to or greater than 99% of a compound of Formula (I) ("substantially pure"), which is then used or formulated as described herein. Such "substantially pure" compounds of Formula (I) are also contemplated herein as part of the present invention.

"Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent. The present invention is intended to embody stable compounds.

The term "Helios inhibitor" refers to an agent capable of decreasing Helios protein levels, decreasing Helios activity level and/or inhibiting Helios expression level in the cells to control Treg differentiation. The Helios inhibitor may be a reversible or irreversible inhibitor.

As used herein, "Helios" protein refers a protein that is a member of the Ikaros family of zinc finger proteins. In humans, Helios is encoded by the IKZF2 gene. Helios is also known as IKAROS family zinc finger 2, ANF1A2, ZNF1A2, ZNFN1A2, zinc finger protein, subfamily 1A, 2, and Ikaros family zinc finger protein 2. The members of

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this protein family include Ikaros, Helios, Aiolos, Eos, and Pegasus. As used herein Helios protein includes various isoform, which includes the isoforms 1-5 listed below.

Isoform 1 (UniProt Q9UKS7-1)

METEAIDGYITCDNELSPEREHSNMAIDLTSSTPNGQHASPSHMTSTNSVKLEMQSDEEC
DRKPLSREDEIRGHDEGSSLEEPLIESSEVADNRKVQELQGEGGIRLPNGKLKCDVCGMV
CIGPNVLMVHKRSHTGERP**FHCNQCGASFTQKGNLLRHIKLH**SGEKPFKCPFCSYACRRR
DALTGHLRTHSVGKPHKCNYCGRSYKQRSSLEEHKERCHNYLQNVSMEAAGQVMSHHVPP
MEDCKEQEPIMDNNISLVPFERPAVIEKLTGNMGKRKSSTPQKFVGEKLMRFSYPDIHFD
MNLTYEKEAELMQSHMMDQAINNAITYLGAEALHPLMQHPPSTIAEVAPVISSAYSQVYH
PNRIERPISRETADSHENNMDGPISLIRPKSRPQEREASPSNSCLDSTDSESSHDDHQSY
QGHPALNPKRKQSPAYMKEDVKALDTTKAPKGSLKDIYKVFNGEGEQIRAFKCEHCRVLF
LDHVMYTIHMGCHGYRDPLECNICGYRSQDRYEFSSHIVRGEHTFH (SEQ ID NO: 1)

Isoform 2 (UniProt Q9UKS7-2)

METEAIDGYITCDNELSPEREHSNMAIDLTSSTPNGQHASPSHMTSTNSVKLEMQSDEEC
DRKPLSREDEIRGHDEGSSLEEPLIESSEVADNRKVQELQGEGGIRLPNGERP**FHCNQCG**
ASFTQKGNLLRHIKLHSGEKPFKCPFCSYACRRRDALTGHLRTHSVGKPHKCNYCGRSYK
QRSSLEEHKERCHNYLQNVSMEAAGQVMSHHVPPMEDCKEQEPIMDNNISLVPFERPAVI
EKLTGNMGKRKSSTPQKFVGEKLMRFSYPDIHFDMNLTYEKEAELMQSHMMDQAINNAIT
YLGAEALHPLMQHPPSTIAEVAPVISSAYSQVYHPNRIERPISRETADSHENNMDGPISL
IRPKSRPQEREASPSNSCLDSTDSESSHDDHQSYQGHPALNPKRKQSPAYMKEDVKALDT
TKAPKGSLKDIYKVFNGEGEQIRAFKCEHCRVLFLDHVMYTIHMGCHGYRDPLECNICGY
RSQDRYEFSSHIVRGEHTFH (SEQ ID NO: 2)

Isoform 4 (UniProt Q9UKS7-4)

METEAIDGYITCDNELSPEREHSNMAIDLTSSTPNGQHASPSHMTSTNSVKLEMQSDEEC
DRKPLSREDEIRGHDEGSSLEEPLIESSEVADNRKVQELQGEGGIRLPNGERP**FHCNQCG**
ASFTQKGNLLRHIKLHSGEKPFKCPFCSYACRRRDALTGHLRTHSVGKPHKCNYCGRSYK
QRSSLEEHKERCHNYLQNVSMEAAGQVMSHHGEKLMRFSYPDIHFDMNLTYEKEAELMQS
HMMDQAINNAITYLGAEALHPLMQHPPSTIAEVAPVISSAYSQVYHPNRIERPISRETAD
SHENNMDGPISLIRPKSRPQEREASPSNSCLDSTDSESSHDDHQSYQGHPALNPKRKQSP
AYMKEDVKALDTTKAPKGSLKDIYKVFNGEGEQIRAFKCEHCRVLFLDHVMYTIHMGCHG
YRDPLECNICGYRSQDRYEFSSHIVRGEHTFH (SEQ ID NO: 3)

Isoform 6 (UniProt Q9UKS7-6)

METEAIDGYITCDNELSPEREHSNMAIDLTSSTPNGQHASPSHMTSTNSVKLEMQSDEEC
DRKPLSREDEIRGHDEGSSLEEPLIESSEVADNRKVQELQGEGGIRLPNGKLKCDVCGMV
CIGPNVLMVHKRSHTGERP**FHCNQCGASFTQKGNLLRHIKLH**SGEKPFKCPFCSYACRRR
DALTGHLRTHSVGKPHKCNYCGRSYKQRSSLEEHKERCHNYLQNVSMEAAGQVMSHHDS
(SEQ ID NO: 4)

Isoform 7 (UniProt Q9UKS7-7)

METEAIDGYITCDNELSPEREHSNMAIDLTSSTPNGQHASPSHMTSTNSVKLEMQSDEEC
DRKPLSREDEIRGHDEGSSLEEPLIESSEVADNRKVQELQGEGGIRLPNGERP**FHCNQCG**
ASFTQKGNLLRHIKLHSGEKPFKCPFCSYACRRRDALTGHLRTHSVPPMEDCKEQEPIMD
NNISLVPFERPAVIEKLTGNMGKRKSSTPQKFVGEKLMRFSYPDIHFDMNLTYEKEAELM
QSHMMDQAINNAITYLGAEALHPLMQHPPSTIAEVAPVISSAYSQVYHPNRIERPISRET
ADSHENNMDGPISLIRPKSRPQEREASPSNSCLDSTDSESSHDDHQSYQGHPALNPKRKQ
SPAYMKEDVKALDTTKAPKGSLKDIYKVFNGEGEQIRAFKCEHCRVLFLDHVMYTIHMGC
HGYRDPLECNICGYRSQDRYEFSSHIVRGEHTFH (SEQ ID NO: 5)

The "Helios" isoforms 1, 2, 4, 6, and 7 listed above includes the degron FHCNQCGASFTQKGNLLRHIKLH (SEQ ID NO: 6)(bold and underlined). A degron is a portion of a protein that plays a role in regulating protein degradation rates.

As used herein, "Eos" protein is encoded by the IKZF4 gene, and is also known as IKAROS family zinc finger 4, ZNFN1A4, zinc finger protein, subfamily 1A, 4, Ikaros family zinc finger protein 4, and KIAA1782. "Eos" protein includes isoforms encoded by the following two human isoforms 1 (Q9H2S9-1) and 2 (Q9H2S9-2):

Isoform 1 (UniProt Q9H2S9-1)

MHTPPALPRRFQGGGRVRTPGSHRQGKDNLERDPSGGCVPDFLPQAQDSNHFIMESLFCE
SSGDSSLEKEFLGAPVGPSVSTPNSQHSSPSRSLSANSIKVEMYSDEESSRLLGPDERLL
EKDDSVIVEDSLSEPLGYCDGSGPEPHSPGGIRLPNGKLKCDVCGMVCIGPNVLMVHKRS
HTGERP**FHCNQCGASFTQKGNLLRHIKLH**SGEKPFKCPFCNYACRRRDALTGHLRTHSVS
SPTVGKPYKCNYCGRSYKQQSTLEEHKERCHNYLQSLSTEAQALAGQPGDEIRDLEMVPD
SMLHSSSERPTFIDRLANSLTKRKRSTPQKFVGEKQMRFSLSDLPYDVNSGGYEKDVELV
AHHSLEPGFGSSLAFVGAEHLRPLRLPPTNCISELTPVISSVYTQMQPLPGRLELPGSRE
AGEGPEDLADGGPLLYRPRGPLTDPGASPSNGCQDSTDTESNHEDRVAGVVSLPQGPPPQ
PPPTIVVGRHSPAYAKEDPKPQEGLLRGTPGPSKEVLRVVGESGEPVKAFKCEHCRILFL
DHVMFTIHMGCHGFRDPFECNICGYHSQDRYEFSSHIVRGEHKVG (SEQ ID NO: 7)

Isoform 2 (UniProt Q9H2S9-2)

MDSRYLQLQLYLPSCSLLQGSGDSSLEKEFLGAPVGPSVSTPNSQHSSPSRSLSANSIKV
EMYSDEESSRLLGPDERLLEKDDSVIVEDSLSEPLGYCDGSGPEPHSPGGIRLPNGKLKC
DVCGMVCIGPNVLMVHKRSHTGERP**FHCNQCGASFTQKGNLLRHIKLH**SGEKPFKCPFCN
YACRRRDALTGHLRTHSVSSPTVGKPYKCNYCGRSYKQQSTLEEHKERCHNYLQSLSTEA
QALAGQPGDEIRDLEMVPDSMLHSSSERPTFIDRLANSLTKRKRSTPQKFVGEKQMRFSL
SDLPYDVNSGGYEKDVELVAHHSLEPGFGSSLAFVGAEHLRPLRLPPTNCISELTPVISS
VYTQMQPLPGRLELPGSREAGEGPEDLADGGPLLYRPRGPLTDPGASPSNGCQDSTDTES
NHEDRVAGVVSLPQGPPPQPPPTIVVGRHSPAYAKEDPKPQEGLLRGTPGPSKEVLRVVG
ESGEPVKAFKCEHCRILFLDHVMFTIHMGCHGFRDPFECNICGYHSQDRYEFSSHIVRGE
HKVG (SEQ ID NO: 8)

The "Eos" protein isoforms 1 and 2 listed above includes the degron FHCNQCGASFTQKGNLLRHIKLH (SEQ ID NO: 6) (bold and underlined), which is

the same as the degron for the "Helios" protein.

As used herein, "Ikaros" protein is encoded by the IKZF1 gene. Ikaros is also known as IKAROS family zinc finger 1, ZNFN1A1, zinc finger protein, subfamily 1A, 1, Ikaros family zinc finger protein 1, IK1, lymphoid transcription factor LyF-1, Hs.54452, PPP1R92, protein phosphatase 1, regulatory subunit 92, PRO0758, CVID13, and CLL-associated antigen KW-6. Ikaros protein includes isoforms encoded by amino acid sequences Q13422-1, Q13422-2, Q13422-3, Q13422-4, Q13422-7, and Q13422-8. Ikaros protein also includes isoforms encoded by amino acid sequences Q13422-5 and Q13422-6.

As used herein, "Aiolos" protein is encoded by the IKZF3 gene. Aiolos protein is also known as IKAROS family zinc finger 3, ZNFN1A3, zinc finger protein, subfamily 1A, 3, Ikaros family zinc finger protein 3, and AIO. Aiolos protein includes isoforms encoded by amino acid sequences Q9UKT9-1, Q9UKT9-3, Q9UKT9-4, Q9UKT9-6, Q9UKT9-7, Q9UKT9-8, Q9UKT9-9, and Q9UKT9-14. Aiolos protein also includes isoforms encoded by amino acid sequences Q9UKT9-2, Q9UKT9-5, Q9UKT9-10, Q9UKT9-11, Q9UKT9-12, and Q9UKT9-13, Q9UKT9-15, and Q9UKT9-16.

As used herein, "Pegasus" protein is also known as IKAROS family zinc finger 5, ZNFN1A5, zinc finger protein, subfamily 1A, 5, and Ikaros family zinc finger protein 5. Pegasus is encoded by the IKZF5 gene.

As used herein, the term "contacting" refers to the bringing together of indicated moieties in an *in vitro* system or an *in vivo* system. For example, "contacting" Helios protein with a compound of Formula (I) includes the administration of a compound of the present invention to an individual or patient, such as a human, having Helios protein, as well as, for example, introducing a compound of Formula (I) into a sample containing a cellular or purified preparation containing Helios protein.

The terms "treat," "treating," and "treatment," as used herein, refer to any type of intervention or process performed on, or administering an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, or slowing down or preventing the progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease. In contrast, "prophylaxis" or "prevention" refers to administration to a subject who does not have a disease to prevent the disease from occurring. "Treat," "treating," and "treatment" does

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not encompass prophylaxis or prevention.

"Therapeutically effective amount" is intended to include an amount of a compound of the present invention alone or an amount of the combination of compounds claimed or an amount of a compound of the present invention in combination with other active ingredients effective to decrease Helios protein levels, decrease Helios activity levels and/or inhibit Helios expression levels in the cells, or effective to treat or prevent viral infections and proliferative disorders, such as cancer.

As used herein, the term "cell" is meant to refer to a cell that is *in vitro*, *ex vivo* or *in vivo*. In some embodiments, an *ex vivo* cell can be part of a tissue sample excised from an organism such as a mammal. In some embodiments, an *in vitro* cell can be a cell in a cell culture. In some embodiments, an *in vivo* cell is a cell living in an organism such as a mammal.

The term "patient" includes human and other mammalian subjects that receive either therapeutic or prophylactic treatment.

The term "subject" includes any human or non-human animal. For example, the methods and compositions herein disclosed can be used to treat a subject having cancer. A non-human animal includes all vertebrates, *e.g.*, mammals and non-mammals, including non-human primates, sheep, dogs, cows, chickens, amphibians, reptiles, etc. In one embodiment, the subject is a human subject.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including, *i.e.*, adjuvant, excipient or vehicle, such as diluents, preserving agents, fillers, flow regulating agents, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms; and not injurious to the patient.

The term "pharmaceutical composition" means a composition comprising a

compound of the invention in combination with at least one additional pharmaceutically acceptable carrier.

UTILITY

The compounds of Formula (I) are useful for the treatment of cancer.

In one embodiment, the present invention provides a combined preparation of a compound of Formula (I), and/or a pharmaceutically acceptable salt thereof, a stereoisomer thereof or a tautomer thereof, and additional therapeutic agent(s) for simultaneous, separate or sequential use in the treatment and/or prophylaxis of multiple diseases or disorders associated with the activity of Helios protein. The combined preparation can be used to decrease Helios protein level, Helios activity level and/or Helios expression level in the cells to control Treg differentiation.

The compounds for Formula (I) and pharmaceutical compositions comprising at least one compound of Formula (I) are useful in treating or preventing any diseases or conditions that are associated with the activity of Helios protein. These include viral and other infections (*e.g.*, skin infections, GI infection, urinary tract infections, genito-urinary infections, systemic infections), and proliferative diseases (*e.g.*, cancer). The compounds of Formula (I) and pharmaceutical compositions comprising in at least one compound of Formula (I) may be administered to animals, preferably mammals (*e.g.*, domesticated animals, cats, dogs, mice, rats), and more preferably humans. Any method of administration may be used to deliver the compound or pharmaceutical composition to the patient. In certain embodiments, the compound of Formula (I) or pharmaceutical composition comprising at least compound of Formula (I) is administered orally. In other embodiments, the Formula (I) or pharmaceutical composition comprising at least compound of Formula (I) is administered parenterally.

The compounds of Formula (I) can selectively decrease Helios protein levels, decrease Helios activity levels and/or inhibit Helios expression levels in the cells to control Treg differentiation. For example, the compounds of Formula (I) can be used to selectively decrease Helios activity levels and/or inhibit Helios expression levels in the cells to control Treg differentiation in a cell or in an individual in need of a decrease in Helios protein levels, decrease in Helios activity levels and/or inhibition of Helios expression level by administering an inhibiting amount of a compound of Formula (I) or a

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salt thereof.

In one aspect, the compound(s) of Formula (I) are sequentially administered prior to administration of the immuno-oncology agent. In another aspect, compound(s) of Formula (I) are administered concurrently with the immuno-oncology agent. In yet another aspect, compound(s) of Formula (I) are sequentially administered after administration of the immuno-oncology agent.

In another aspect, compounds of Formula (I) may be co-formulated with an immuno-oncology agent.

Immuno-oncology agents include, for example, a small molecule drug, antibody, or other biologic or small molecule. Examples of biologic immuno-oncology agents include, but are not limited to, cancer vaccines, antibodies, and cytokines. In one aspect, the antibody is a monoclonal antibody. In another aspect, the monoclonal antibody is humanized or human.

In one aspect, the immuno-oncology agent is (i) an agonist of a stimulatory (including a co-stimulatory) receptor or (ii) an antagonist of an inhibitory (including a co-inhibitory) signal on T cells, both of which result in amplifying antigen-specific T cell responses (often referred to as immune checkpoint regulators).

Certain of the stimulatory and inhibitory molecules are members of the immunoglobulin super family (IgSF). One important family of membrane-bound ligands that bind to co-stimulatory or co-inhibitory receptors is the B7 family, which includes B7-1, B7-2, B7-H1 (PD-L1), B7-DC (PD-L2), B7-H2 (ICOS-L), B7-H3, B7-H4, B7-H5 (VISTA), and B7-H6. Another family of membrane bound ligands that bind to co-stimulatory or co-inhibitory receptors is the TNF family of molecules that bind to cognate TNF receptor family members, which includes CD40 and CD40L, OX-40, OX-40L, CD70, CD27L, CD30, CD30L, 4-1BBL, CD137 (4-1BB), TRAIL/Apo2-L, TRAILR1/DR4, TRAILR2/DR5, TRAILR3, TRAILR4, OPG, RANK, RANKL, TWEAKR/Fn14, TWEAK, BAFFR, EDAR, XEDAR, TACI, APRIL, BCMA, LTβR, LIGHT, DcR3, HVEM, VEGI/TL1A, TRAMP/DR3, EDAR, EDA1, XEDAR, EDA2, TNFR1, Lymphotoxin α/TNFβ, TNFR2, TNFα, LTβR, Lymphotoxin α 1β2, FAS, FASL, RELT, DR6, TROY, NGFR.

In one aspect, T cell responses can be stimulated by a combination of a compound of Formula (I) and one or more of (i) an antagonist of a protein that inhibits T cell

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activation (e.g., immune checkpoint inhibitors) such as CTLA-4, PD-1, PD-L1, PD-L2, LAG-3, TIM-3, Galectin 9, CEACAM-1, BTLA, CD69, Galectin-1, TIGIT, CD113, GPR56, VISTA, 2B4, CD48, GARP, PD1H, LAIR1, TIM-1, and TIM-4, and (ii) an agonist of a protein that stimulates T cell activation such as B7-1, B7-2, CD28, 4-1BB (CD137), 4-1BBL, ICOS, ICOS-L, OX40, OX40L, GITR, GITRL, CD70, CD27, CD40, DR3 and CD28H.

Other agents that can be combined with compounds of Formula (I) for the treatment of cancer include antagonists of inhibitory receptors on NK cells or agonists of activating receptors on NK cells. For example, compounds of Formula (I) can be combined with antagonists of KIR, such as lirilumab.

Yet other agents for combination therapies include agents that inhibit or deplete macrophages or monocytes, including but not limited to CSF-1R antagonists such as CSF-1R antagonist antibodies including RG7155 (WO11/70024, WO11/107553, WO11/131407, WO13/87699, WO13/119716, WO13/132044) or FPA-008 (WO11/140249; WO13169264; WO14/036357).

In another aspect, compounds of Formula (I) can be used with one or more of agonistic agents that ligate positive costimulatory receptors, blocking agents that attenuate signaling through inhibitory receptors, antagonists, and one or more agents that increase systemically the frequency of anti-tumor T cells, agents that overcome distinct immune suppressive pathways within the tumor microenvironment (e.g., block inhibitory receptor engagement (e.g., PD-L1/PD-1 interactions), deplete or inhibit Tregs (e.g., using an anti-CD25 monoclonal antibody (e.g., daclizumab) or by ex vivo anti-CD25 bead depletion), inhibit metabolic enzymes such as IDO, or reverse/prevent T cell anergy or exhaustion) and agents that trigger innate immune activation and/or inflammation at tumor sites.

In one aspect, the immuno-oncology agent is a CTLA-4 antagonist, such as an antagonistic CTLA-4 antibody. Suitable CTLA-4 antibodies include, for example, YERVOY (ipilimumab) or tremelimumab.

In another aspect, the immuno-oncology agent is a PD-1 antagonist, such as an antagonistic PD-1 antibody. Suitable PD-1 antibodies include, for example, OPDIVO™ (nivolumab), KEYTRUDA™ (pembrolizumab), or MEDI-0680 (AMP-514; WO2012/145493). The immuno-oncology agent may also include pidilizumab (CT-011),

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though its specificity for PD-1 binding has been questioned. Another approach to target the PD-1 receptor is the recombinant protein composed of the extracellular domain of PD-L2 (B7-DC) fused to the Fc portion of IgG1, called AMP-224.

In another aspect, the immuno-oncology agent is a PD-L1 antagonist, such as an antagonistic PD-L1 antibody. Suitable PD-L1 antibodies include, for example, MPDL3280A (RG7446; WO2010/077634), durvalumab (MEDI4736), BMS-936559 (WO207/005874), and MSB0010718C (WO2013/79174).

In another aspect, the immuno-oncology agent is a LAG-3 antagonist, such as an antagonistic LAG-3 antibody. Suitable LAG3 antibodies include, for example, BMS-986016 (WO10/19570, WO14/08218), or IMP-731 or IMP-321 (WO08/132601, WO09/44273).

In another aspect, the immuno-oncology agent is a CD137 (4-1BB) agonist, such as an agonistic CD137 antibody. Suitable CD137 antibodies include, for example, urclumab and PF-05082566 (WO12/32433).

In another aspect, the immuno-oncology agent is a GITR agonist, such as an agonistic GITR antibody. Suitable GITR antibodies include, for example, BMS-986153, BMS-986156, TRX-518 (WO06/105021, WO09/009116) and MK-4166 (WO11/028683).

In another aspect, the immuno-oncology agent is an IDO antagonist. Suitable IDO antagonists include, for example, INCB-024360 (WO206/122150, WO07/75598, WO08/36653, WO08/36642), indoximod, or NLG-919 (WO09/73620, WO09/1156652, WO11/56652, WO12/142237).

In another aspect, the immuno-oncology agent is an OX40 agonist, such as an agonistic OX40 antibody. Suitable OX40 antibodies include, for example, MEDI-6383 or MEDI-6469.

In another aspect, the immuno-oncology agent is an OX40L antagonist, such as an antagonistic OX40 antibody. Suitable OX40L antagonists include, for example, RG-7888 (WO06/029879).

In another aspect, the immuno-oncology agent is a CD40 agonist, such as an agonistic CD40 antibody. In yet another embodiment, the immuno-oncology agent is a CD40 antagonist, such as an antagonistic CD40 antibody. Suitable CD40 antibodies include, for example, lucatumumab or dacetuzumab.

In another aspect, the immuno-oncology agent is a CD27 agonist, such as an

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agonistic CD27 antibody. Suitable CD27 antibodies include, for example, varlilumab.

In another aspect, the immuno-oncology agent is MGA271 (to B7H3) (WO11/109400).

The combination therapy is intended to embrace administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner. Substantially simultaneous administration can be accomplished, for example, by administering to the subject a single dosage form having a fixed ratio of each therapeutic agent or in multiple, single dosage forms for each of the therapeutic agents. Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination selected may be administered by intravenous injection while the other therapeutic agents of the combination may be administered orally. Alternatively, for example, all therapeutic agents may be administered orally or all therapeutic agents may be administered by intravenous injection. Combination therapy also can embrace the administration of the therapeutic agents as described above in further combination with other biologically active ingredients and non-drug therapies (*e.g.*, surgery or radiation treatment.) Where the combination therapy further comprises a non-drug treatment, the non-drug treatment may be conducted at any suitable time so long as a beneficial effect from the co-action of the combination of the therapeutic agents and non-drug treatment is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the non-drug treatment is temporally removed from the administration of the therapeutic agents, perhaps by days or even weeks.

Types of cancers that may be treated with the compound of Formula (I) include, but are not limited to, brain cancers, skin cancers, bladder cancers, ovarian cancers, breast cancers, gastric cancers, pancreatic cancers, prostate cancers, colon cancers, blood cancers, lung cancers and bone cancers. Examples of such cancer types include neuroblastoma, intestine carcinoma such as rectum carcinoma, colon carcinoma, familiar

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adenomatous polyposis carcinoma and hereditary non-polyposis colorectal cancer, esophageal carcinoma, labial carcinoma, larynx carcinoma, hypopharynx carcinoma, tongue carcinoma, salivary gland carcinoma, gastric carcinoma, adenocarcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, renal carcinoma, kidney parenchymal carcinoma, ovarian carcinoma, cervix carcinoma, uterine corpus carcinoma, endometrium carcinoma, chorion carcinoma, pancreatic carcinoma, prostate carcinoma, testis carcinoma, breast carcinoma, urinary carcinoma, melanoma, brain tumors such as glioblastoma, astrocytoma, meningioma, medulloblastoma and peripheral neuroectodermal tumors, Hodgkin lymphoma, non-Hodgkin lymphoma, Burkitt lymphoma, acute lymphatic leukemia (ALL), chronic lymphatic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), adult T-cell leukemia lymphoma, diffuse large B-cell lymphoma (DLBCL), hepatocellular carcinoma, gall bladder carcinoma, bronchial carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, multiple myeloma, basalioma, teratoma, retinoblastoma, choroid melanoma, seminoma, rhabdomyosarcoma, craniopharyngioma, osteosarcoma, chondrosarcoma, myosarcoma, liposarcoma, fibrosarcoma, Ewing sarcoma and plasmocytoma.

One or more additional pharmaceutical agents or treatment methods such as, for example, anti-viral agents, chemotherapeutics or other anti-cancer agents, immune enhancers, immunosuppressants, radiation, anti-tumor and anti-viral vaccines, cytokine therapy (*e.g.*, IL2 and GM-CSF), and/or tyrosine kinase inhibitors can be optionally used in combination with the compounds of Formula (I) for treatment of Helios protein associated diseases, disorders or conditions. The agents can be combined with the present compounds in a single dosage form, or the agents can be administered simultaneously or sequentially as separate dosage forms.

Suitable chemotherapeutic or other anti-cancer agents include, for example, alkylating agents (including, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes) such as uracil mustard, chlormethine, cyclophosphamide (CYTOXAN™), ifosfamide, melphalan, chlorambucil, pipobroman, triethylene-melamine, triethylenethiophosphoramine, busulfan, carmustine, lomustine, streptozocin, dacarbazine, and temozolomide.

In the treatment of melanoma, suitable agents for use in combination with the compounds of Formula (I) include: dacarbazine (DTIC), optionally, along with other

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chemotherapy drugs such as carmustine (BCNU) and cisplatin; the "Dartmouth regimen", which consists of DTIC, BCNU, cisplatin and tamoxifen; a combination of cisplatin, vinblastine, and DTIC, temozolomide or YERVOY™. Compounds of Formula (I) may also be combined with immunotherapy drugs, including cytokines such as interferon alpha, interleukin 2, and tumor necrosis factor (TNF) in the treatment of melanoma.

Compounds of Formula (I) may also be used in combination with vaccine therapy in the treatment of melanoma. Antimelanoma vaccines are, in some ways, similar to the anti-virus vaccines which are used to prevent diseases caused by viruses such as polio, measles, and mumps. Weakened melanoma cells or parts of melanoma cells called antigens may be injected into a patient to stimulate the body's immune system to destroy melanoma cells.

Melanomas that are confined to the arms or legs may also be treated with a combination of agents including one or more compounds of Formula (I), using a hyperthermic isolated limb perfusion technique. This treatment protocol temporarily separates the circulation of the involved limb from the rest of the body and injects high doses of chemotherapy into the artery feeding the limb, thus providing high doses to the area of the tumor without exposing internal organs to these doses that might otherwise cause severe side effects. Usually the fluid is warmed to 38.9 °C to 40 °C. Melphalan is the drug most often used in this chemotherapy procedure. This can be given with another agent called tumor necrosis factor (TNF).

Suitable chemotherapeutic or other anti-cancer agents include, for example, antimetabolites (including, without limitation, folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors) such as methotrexate, 5-fluorouracil, floxuridine, cytarabine, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, pentostatine, and gemcitabine.

Suitable chemotherapeutic or other anti-cancer agents further include, for example, certain natural products and their derivatives (for example, vinca alkaloids, antitumor antibiotics, enzymes, lymphokines and epipodophyllotoxins) such as vinblastine, vincristine, vindesine, bleomycin, dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, ara-C, paclitaxel (Taxol), mithramycin, deoxyco-formycin, mitomycin-C, L-asparaginase, interferons (especially IFN-a), etoposide, and teniposide.

Other cytotoxic agents include navelbene, CPT-11, anastrazole, letrazole,

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capecitabine, reloxafine, and droloxafine.

Also suitable are cytotoxic agents such as epidophyllotoxin; an antineoplastic enzyme; a topoisomerase inhibitor; procarbazine; mitoxantrone; platinum coordination complexes such as cisplatin and carboplatin; biological response modifiers; growth inhibitors; antihormonal therapeutic agents; leucovorin; tegafur; and haematopoietic growth factors.

Other anti-cancer agent(s) include antibody therapeutics such as trastuzumab (HERCEPTIN®), antibodies to costimulatory molecules such as CTLA-4, 4-1BB and PD-1, or antibodies to cytokines (IL-1O or TGF-β).

Other anti-cancer agents also include those that block immune cell migration such as antagonists to chemokine receptors, including CCR2 and CCR4.

Other anti-cancer agents also include those that augment the immune system such as adjuvants or adoptive T cell transfer.

Anti-cancer vaccines include dendritic cells, synthetic peptides, DNA vaccines and recombinant viruses.

The pharmaceutical composition of the invention may optionally include at least one signal transduction inhibitor (STI). A "signal transduction inhibitor" is an agent that selectively inhibits one or more vital steps in signaling pathways, in the normal function of cancer cells, thereby leading to apoptosis. Suitable STIs include, but are not limited to: (i) bcr/abl kinase inhibitors such as, for example, STI 571 (GLEEVEC™); (ii) epidermal growth factor (EGF) receptor inhibitors such as, for example, kinase inhibitors (IRESSA™, SSI-774) and antibodies (Imclone: C225 [Goldstein et al., *Clin. Cancer Res.*, 1:1311-1318 (1995)], and Abgenix: ABX-EGF); (iii) her-2/neu receptor inhibitors such as farnesyl transferase inhibitors (FTI) such as, for example, L-744,832 (Kohl et al., *Nat. Med.*, 1(8):792-797 (1995)); (iv) inhibitors of Akt family kinases or the Akt pathway, such as, for example, rapamycin (see, for example, Sekulic et al., *Cancer Res.*, 60:3504-3513 (200)); (v) cell cycle kinase inhibitors such as, for example, flavopiridol and UCN-O1 (see, for example, Sausville, *Curr. Med. Chem. Anti-Canc. Agents*, 3:47-56 (203)); and (vi) phosphatidyl inositol kinase inhibitors such as, for example, LY294002 (see, for example, Vlahos et al., *J. Biol. Chem.*, 269:5241-5248 (1994)). Alternatively, at least one STI and at least one compound of Formula (I) may be in separate pharmaceutical compositions. In a specific embodiment of the present invention, at least one compound

of Formula (I) and at least one STI may be administered to the patient concurrently or sequentially. In other words, at least one compound of Formula (I) may be administered first, at least one STI may be administered first, or at least one compound of Formula (I) and at least one STI may be administered at the same time. Additionally, when more than one compound of Formula (I) and/or STI is used, the compounds may be administered in any order.

The present invention further provides a pharmaceutical composition for the treatment of a chronic viral infection in a patient comprising at least one compound of Formula (I), optionally, at least one chemotherapeutic drug, and, optionally, at least one antiviral agent, in a pharmaceutically acceptable carrier.

Also provided is a method for treating a chronic viral infection in a patient by administering an effective amount of the above pharmaceutical composition.

In a specific embodiment of the present invention, at least one compound of Formula (I) and at least one chemotherapeutic agent are administered to the patient concurrently or sequentially. In other words, at least one compound of Formula (I) may be administered first, at least one chemotherapeutic agent may be administered first, or at least one compound of Formula (I) and the at least one STI may be administered at the same time. Additionally, when more than one compound of Formula (I) and/or chemotherapeutic agent is used, the compounds may be administered in any order. Similarly, any antiviral agent or STI may also be administered at any point in comparison to the administration of the compound of Formula (I).

Chronic viral infections that may be treated using the present combinatorial treatment include, but are not limited to, diseases caused by: hepatitis C virus (HCV), human papilloma virus (HPV), cytomegalovirus (CMV), herpes simplex virus (HSV), Epstein-Barr virus (EBV), varicella zoster virus, coxsackie virus, human immunodeficiency virus (HIV).

Suitable antiviral agents contemplated for use in combination with the compound of Formula (I) can comprise nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors and other antiviral drugs.

Examples of suitable NRTIs include zidovudine (AZT); didanosine (ddl); zalcitabine (ddC); stavudine (d4T); lamivudine (3TC); abacavir (1592U89); adefovir

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dipivoxil [bis(POM)-PMEA]; lobucavir (BMS-180194); BCH-I0652; emitricitabine [(-)-FTC]; beta-L-FD4 (also called beta-L-D4C and named beta-L-2′,3′-dicleoxy-5-fluoro-cytidene); DAPD, ((-)-beta-D-2,6-diamino-purine dioxolane); and lodenosine (FddA). Typical suitable NNRTIs include nevirapine (BI-RG-587); delaviradine (BHAP, U-90152); efavirenz (DMP-266); PNU-142721; AG-1549; MKC-442 (1-(ethoxy-methyl)-5-(1-methylethyl)-6-(phenylmethyl)-(2,4(1H,3H)-pyrimidinedione); and (+)-calanolide A (NSC-675451) and B. Typical suitable protease inhibitors include saquinavir (Ro 31-8959); ritonavir (ABT-538); indinavir (MK-639); nelfnavir (AG-1343); amprenavir (141W94); lasinavir (BMS-234475); DMP-450; BMS-2322623; ABT-378; and AG-1549. Other antiviral agents include hydroxyurea, ribavirin, IL-2, IL-12, pentafuside and Yissum Project No.11607.

The combination therapy is intended to embrace administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner. Substantially simultaneous administration can be accomplished, for example, by administering to the subject a single dosage form having a fixed ratio of each therapeutic agent or in multiple, single dosage forms for each of the therapeutic agents. Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination selected may be administered by intravenous injection while the other therapeutic agents of the combination may be administered orally. Alternatively, for example, all therapeutic agents may be administered orally or all therapeutic agents may be administered by intravenous injection. Combination therapy also can embrace the administration of the therapeutic agents as described above in further combination with other biologically active ingredients and non-drug therapies (*e.g.*, surgery or radiation treatment). Where the combination therapy further comprises a non-drug treatment, the non-drug treatment may be conducted at any suitable time so long as a beneficial effect from the co-action of the combination of the therapeutic agents and non-drug treatment is achieved. For example,

in appropriate cases, the beneficial effect is still achieved when the non-drug treatment is temporally removed from the administration of the therapeutic agents, perhaps by days or even weeks.

PHARMACEUTICAL COMPOSITIONS

The invention also provides pharmaceutically compositions which comprise a therapeutically effective amount of one or more of the compounds of Formula (I), formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents, and optionally, one or more additional therapeutic agents described above.

The compounds of Formula (I) may be administered by any suitable route, preferably in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended. The compounds and compositions of the compound of Formula (I) can be administered for any of the uses described herein by any suitable means, for example, orally, such as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions (including nanosuspensions, microsuspensions, spray-dried dispersions), syrups, and emulsions; sublingually; bucally; parenterally, such as by subcutaneous, intravenous, intramuscular, or intrasternal injection, or infusion techniques (*e.g.*, as sterile injectable aqueous or non-aqueous solutions or suspensions); nasally, including administration to the nasal membranes, such as by inhalation spray; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories. They can be administered alone, but generally will be administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, liquid capsule, suspension, or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. For example, the pharmaceutical composition may be provided as a tablet or capsule comprising an amount of active ingredient in the range of from about 0.1 to 1000 mg, preferably from about 0.25 to 250 mg, and more preferably from about 0.5 to 100 mg. A suitable daily dose for a human or other mammal may vary

widely depending on the condition of the patient and other factors, but, can be determined using routine methods.

Any pharmaceutical composition contemplated herein can, for example, be delivered orally via any acceptable and suitable oral preparations. Exemplary oral preparations, include, but are not limited to, for example, tablets, troches, lozenges, aqueous and oily suspensions, dispersible powders or granules, emulsions, hard and soft capsules, liquid capsules, syrups, and elixirs. Pharmaceutical compositions intended for oral administration can be prepared according to any methods known in the art for manufacturing pharmaceutical compositions intended for oral administration. In order to provide pharmaceutically palatable preparations, a pharmaceutical composition in accordance with the invention can contain at least one agent selected from sweetening agents, flavoring agents, coloring agents, demulcents, antioxidants, and preserving agents.

A tablet can, for example, be prepared by admixing at least one compound of Formula (I) and/or at least one pharmaceutically acceptable salt thereof with at least one non-toxic pharmaceutically acceptable excipient suitable for the manufacture of tablets. Exemplary excipients include, but are not limited to, for example, inert diluents, such as, for example, calcium carbonate, sodium carbonate, lactose, calcium phosphate, and sodium phosphate; granulating and disintegrating agents, such as, for example, microcrystalline cellulose, sodium crosscarmellose, corn starch, and alginic acid; binding agents, such as, for example, starch, gelatin, polyvinyl-pyrrolidone, and acacia; and lubricating agents, such as, for example, magnesium stearate, stearic acid, and talc. Additionally, a tablet can either be uncoated, or coated by known techniques to either mask the bad taste of an unpleasant tasting drug, or delay disintegration and absorption of the active ingredient in the gastrointestinal tract thereby sustaining the effects of the active ingredient for a longer period. Exemplary water soluble taste masking materials, include, but are not limited to, hydroxypropyl-methylcellulose and hydroxypropyl-cellulose. Exemplary time delay materials, include, but are not limited to, ethyl cellulose and cellulose acetate butyrate.

Hard gelatin capsules can, for example, be prepared by mixing at least one compound of Formula (I) and/or at least one salt thereof with at least one inert solid diluent, such as, for example, calcium carbonate; calcium phosphate; and kaolin.

Soft gelatin capsules can, for example, be prepared by mixing at least one

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compound of Formula (I) and/or at least one pharmaceutically acceptable salt thereof with at least one water soluble carrier, such as, for example, polyethylene glycol; and at least one oil medium, such as, for example, peanut oil, liquid paraffin, and olive oil.

An aqueous suspension can be prepared, for example, by admixing at least one compound of Formula (I) and/or at least one pharmaceutically acceptable salt thereof with at least one excipient suitable for the manufacture of an aqueous suspension. Exemplary excipients suitable for the manufacture of an aqueous suspension, include, but are not limited to, for example, suspending agents, such as, for example, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, alginic acid, polyvinyl-pyrrolidone, gum tragacanth, and gum acacia; dispersing or wetting agents, such as, for example, a naturally-occurring phosphatide, e.g., lecithin; condensation products of alkylene oxide with fatty acids, such as, for example, polyoxyethylene stearate; condensation products of ethylene oxide with long chain aliphatic alcohols, such as, for example heptadecaethylene-oxycetanol; condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol, such as, for example, polyoxyethylene sorbitol monooleate; and condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, such as, for example, polyethylene sorbitan monooleate. An aqueous suspension can also contain at least one preservative, such as, for example, ethyl and n-propyl p-hydroxybenzoate; at least one coloring agent; at least one flavoring agent; and/or at least one sweetening agent, including but not limited to, for example, sucrose, saccharin, and aspartame.

Oily suspensions can, for example, be prepared by suspending at least one compound of Formula (I) and/or at least one pharmaceutically acceptable salt thereof in either a vegetable oil, such as, for example, arachis oil; olive oil; sesame oil; and coconut oil; or in mineral oil, such as, for example, liquid paraffin. An oily suspension can also contain at least one thickening agent, such as, for example, beeswax; hard paraffin; and cetyl alcohol. In order to provide a palatable oily suspension, at least one of the sweetening agents already described hereinabove, and/or at least one flavoring agent can be added to the oily suspension. An oily suspension can further contain at least one preservative, including, but not limited to, for example, an anti-oxidant, such as, for example, butylated hydroxyanisol, and alpha-tocopherol.

Dispersible powders and granules can, for example, be prepared by admixing at

least one compound of Formula (I) and/or at least one pharmaceutically acceptable salt thereof with at least one dispersing and/or wetting agent; at least one suspending agent; and/or at least one preservative. Suitable dispersing agents, wetting agents, and suspending agents are as already described above. Exemplary preservatives include, but are not limited to, for example, anti-oxidants, e.g., ascorbic acid. In addition, dispersible powders and granules can also contain at least one excipient, including, but not limited to, for example, sweetening agents; flavoring agents; and coloring agents.

An emulsion of at least one compound of Formula (I) and/or at least one pharmaceutically acceptable salt thereof can, for example, be prepared as an oil-in-water emulsion. The oily phase of the emulsions comprising compounds of Formula (I) may be constituted from known ingredients in a known manner. The oil phase can be provided by, but is not limited to, for example, a vegetable oil, such as, for example, olive oil and arachis oil; a mineral oil, such as, for example, liquid paraffin; and mixtures thereof. While the phase may comprise merely an emulsifier, it may comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Suitable emulsifying agents include, but are not limited to, for example, naturally-occurring phosphatides, e.g., soy bean lecithin; esters or partial esters derived from fatty acids and hexitol anhydrides, such as, for example, sorbitan monooleate; and condensation products of partial esters with ethylene oxide, such as, for example, polyoxyethylene sorbitan monooleate. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make-up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations. An emulsion can also contain a sweetening agent, a flavoring agent, a preservative, and/or an antioxidant. Emulsifiers and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate, sodium lauryl sulfate, glyceryl distearate alone or with a wax, or other materials well known in the art.

The compounds of Formula (I) and/or at least one pharmaceutically acceptable salt thereof can, for example, also be delivered intravenously, subcutaneously, and/or intramuscularly via any pharmaceutically acceptable and suitable injectable form.

Exemplary injectable forms include, but are not limited to, for example, sterile aqueous solutions comprising acceptable vehicles and solvents, such as, for example, water, Ringer's solution, and isotonic sodium chloride solution; sterile oil-in-water microemulsions; and aqueous or oleaginous suspensions.

Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules using one or more of the carriers or diluents mentioned for use in the formulations for oral administration or by using other suitable dispersing or wetting agents and suspending agents. The compounds may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art. The active ingredient may also be administered by injection as a composition with suitable carriers including saline, dextrose, or water, or with cyclodextrin (i.e. Captisol), cosolvent solubilization (i.e. propylene glycol) or micellar solubilization (i.e. Tween 80).

The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

A sterile injectable oil-in-water microemulsion can, for example, be prepared by 1) dissolving at least one compound of Formula (I) in an oily phase, such as, for example, a mixture of soybean oil and lecithin; 2) combining the Formula (I) containing oil phase with a water and glycerol mixture; and 3) processing the combination to form a microemulsion.

A sterile aqueous or oleaginous suspension can be prepared in accordance with methods already known in the art. For example, a sterile aqueous solution or suspension can be prepared with a non-toxic parenterally-acceptable diluent or solvent, such as, for

example, 1,3-butane diol; and a sterile oleaginous suspension can be prepared with a sterile non-toxic acceptable solvent or suspending medium, such as, for example, sterile fixed oils, e.g., synthetic mono- or diglycerides; and fatty acids, such as, for example, oleic acid.

Pharmaceutically acceptable carriers are formulated according to a number of factors well within the purview of those of ordinary skill in the art. These include, without limitation: the type and nature of the active agent being formulated; the subject to which the agent-containing composition is to be administered; the intended route of administration of the composition; and the therapeutic indication being targeted. Pharmaceutically acceptable carriers include both aqueous and non-aqueous liquid media, as well as a variety of solid and semi-solid dosage forms. Such carriers can include a number of different ingredients and additives in addition to the active agent, such additional ingredients being included in the formulation for a variety of reasons, *e.g.*, stabilization of the active agent, binders, etc., well known to those of ordinary skill in the art. Descriptions of suitable pharmaceutically acceptable carriers, and factors involved in their selection, are found in a variety of readily available sources such as, for example, Allen, L. V. Jr. *et al*. *Remington: The Science and Practice of Pharmacy (2 Volumes)*, 22nd Edition (2012), Pharmaceutical Press.

Pharmaceutically acceptable carriers, adjuvants, and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-alpha-tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens, polyethoxylated castor oil such as CREMOPHOR™ surfactant (BASF), or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as alpha-, beta-, and gamma-cyclodextrin, or chemically modified derivatives such as

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hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compounds of the formulae described herein.

The pharmaceutically active compounds of this invention can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals. The pharmaceutical compositions may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc. Tablets and pills can additionally be prepared with enteric coatings. Such compositions may also comprise adjuvants, such as wetting, sweetening, flavoring, and perfuming agents.

For therapeutic purposes, the active compounds of this invention are ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered orally, the compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain a controlled-release formulation as may be provided in a dispersion of active compound in hydroxypropylmethyl cellulose.

The amounts of compounds that are administered and the dosage regimen for treating a disease condition with the compounds and/or compositions of this invention depends on a variety of factors, including the age, weight, sex, the medical condition of the subject, the type of disease, the severity of the disease, the route and frequency of administration, and the particular compound employed. Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods. A daily dose of about 0.001 to 100 mg/kg body weight, preferably between about 0.0025 and about 50 mg/kg body weight and most preferably between about 0.005 to 10 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day. Other dosing schedules include one dose per week and one dose per two day cycle.

Pharmaceutical compositions of this invention comprise at least one compound of Formula (I) and/or at least one pharmaceutically acceptable salt thereof, and optionally an

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additional agent selected from any pharmaceutically acceptable carrier, adjuvant, and vehicle. Alternate compositions of this invention comprise a compound of the Formula (I) described herein, or a prodrug thereof, and a pharmaceutically acceptable carrier, adjuvant, or vehicle.

The present invention also includes pharmaceutical kits useful, for example, in the treatment or prevention of Helios protein-associated diseases or disorders, and other diseases referred to herein which include one or more containers containing a pharmaceutical composition comprising a therapeutically effective amount of a compound of Formula (I). 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, 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.

The dosage regimen for the compounds of the present invention will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the species, age, sex, health, medical condition, and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; the route of administration, the renal and hepatic function of the patient, and the effect desired.

By way of general guidance, the daily oral dosage of each active ingredient, when used for the indicated effects, will range between about 0.001 to about 5000 mg per day, preferably between about 0.01 to about 1000 mg per day, and most preferably between about 0.1 to about 250 mg per day. Intravenously, the most preferred doses will range from about 0.01 to about 10 mg/kg/minute during a constant rate infusion. Compounds of Formula (I) may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three, or four times daily.

The compounds are typically administered in admixture with suitable pharmaceutical diluents, excipients, or carriers (collectively referred to herein as pharmaceutical carriers) suitably selected with respect to the intended form of administration, *e.g.*, oral tablets, capsules, elixirs, and syrups, and consistent with conventional pharmaceutical practices.

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Dosage forms (pharmaceutical compositions) suitable for administration may contain from about 1 milligram to about 200 milligrams of active ingredient per dosage unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.1-95% by weight based on the total weight of the composition.

A typical capsule for oral administration contains at least one of the compounds of Formula (I) (250 mg), lactose (75 mg), and magnesium stearate (15 mg). The mixture is passed through a 60 mesh sieve and packed into a No. 1 gelatin capsule.

A typical injectable preparation is produced by aseptically placing at least one of the compounds of Formula (I) (250 mg) into a vial, aseptically freeze-drying and sealing. For use, the contents of the vial are mixed with 2 mL of physiological saline, to produce an injectable preparation.

The present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, a therapeutically effective amount of at least one of the compounds of Formula (I), alone or in combination with a pharmaceutical carrier. Optionally, compounds of Formula (I) can be used alone, in combination with other compounds of Formula (I), or in combination with one or more other therapeutic agent(s), *e.g.*, an anticancer agent or other pharmaceutically active material.

Regardless of the route of administration selected, the compounds of Formula (I), which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound of Formula (I) employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the rate and extent of absorption, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition,

general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of Formula (I) employed in the pharmaceutical composition at levels lower than that required in order to achieve the therapeutic effect and gradually increase the dosage until the effect is achieved.

In general, a suitable daily dose of a compound of Formula (I) will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, oral, intravenous, intracerebroventricular and subcutaneous doses of the compounds of Formula (I) for a patient will range from about 0.01 to about 50 mg per kilogram of body weight per day.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In certain aspects of the invention, dosing is one administration per day.

While it is possible for a compound of Formula (I) to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

The above other therapeutic agents, when employed in combination with the compounds of Formula (I), may be used, for example, in those amounts indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art. In the methods of the present invention, such other therapeutic agent(s) may be administered prior to, simultaneously with, or following the administration of the inventive compounds.

METHODS OF PREPARATION

The compounds of the present invention can be prepared in a number of ways well known to one skilled in the art of organic synthesis. The compounds of the present invention can be synthesized using the methods described below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as

appreciated by those skilled in the art. Preferred methods include, but are not limited to, those described below. All references cited herein are hereby incorporated by reference in their entirety.

The compounds of this invention may be prepared using the reactions and techniques described in this section. The reactions are performed in solvents appropriate to the reagents and materials employed and are suitable for the transformations being effected. Also, in the description of the synthetic methods described below, it is to be understood that all proposed reaction conditions, including choice of solvent, reaction atmosphere, reaction temperature, duration of the experiment and work up procedures, are chosen to be the conditions standard for that reaction, which should be readily recognized by one skilled in the art. It is understood by one skilled in the art of organic synthesis that the functionality present on various portions of the molecule must be compatible with the reagents and reactions proposed. Such restrictions to the substituents that are compatible with the reaction conditions will be readily apparent to one skilled in the art and alternate methods must then be used. This will sometimes require a judgment to modify the order of the synthetic steps or to select one particular process scheme over another in order to obtain a compound of the invention. It will also be recognized that another major consideration in the planning of any synthetic route in this field is the judicious choice of the protecting group used for protection of the reactive functional groups present in the compounds described in this invention. An authoritative account describing the many alternatives to the trained practitioner is Greene and Wuts (*Protective Groups In Organic Synthesis*, Fourth Edition, Wiley and Sons, 207).

Compounds of Formula (I) may be prepared by reference to the methods illustrated in the following Schemes. As shown therein the end product is a compound having the same structural formula as Formula (I). It will be understood that any compound of Formula (I) may be produced by the schemes by the suitable selection of reagents with appropriate substitution. Solvents, temperatures, pressures, and other reaction conditions may readily be selected by one of ordinary skill in the art. Starting materials are commercially available or readily prepared by one of ordinary skill in the art. Constituents of compounds are as defined herein or elsewhere in the specification.

General routes to compounds described in the invention are illustrated in Schemes 1–2, where the R_1, R_2, R_4 and R_6 are defined previously in the text or a functional group

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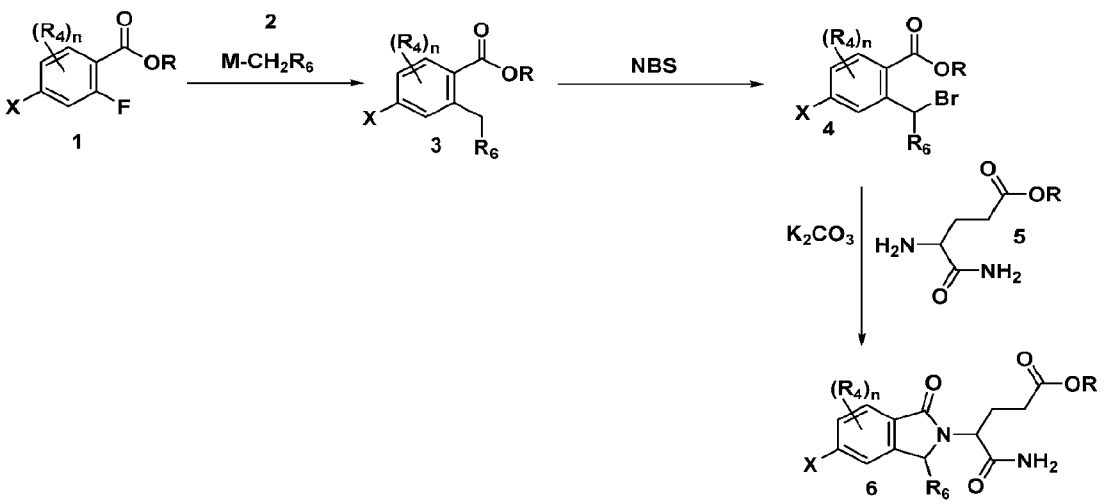
that can be converted to the final substituent. The substituent X is a leaving group such as a halide (preferably I, Br, or Cl) or a triflate. The substituent M is a suitable coupling partner, such as boronic acid, boronic ester or stannane. The substituent R is a carboxylic acid protecting group such as *tert*-butyl, methyl, ethyl, or benzyl. As shown in Scheme 1, a general procedure for the preparation of compounds of the invention involves starting with a suitably substituted aryl fluoride **1**. When treated with a suitable nucleophile, such as intermediate **2** where M can be MgBr or Li, substituted aryl halides such as **3** can be produced. Halogenation, preferably bromination, can be accomplished by treating **3** with reagents such as N-bromosuccinimide, to afford the bromide **4**. Treatment of **4** with an amine **5** under basic conditions such as potassium carbonate, will result in initial displacement of the secondary bromide followed by cyclization to afford the lactam **6**.

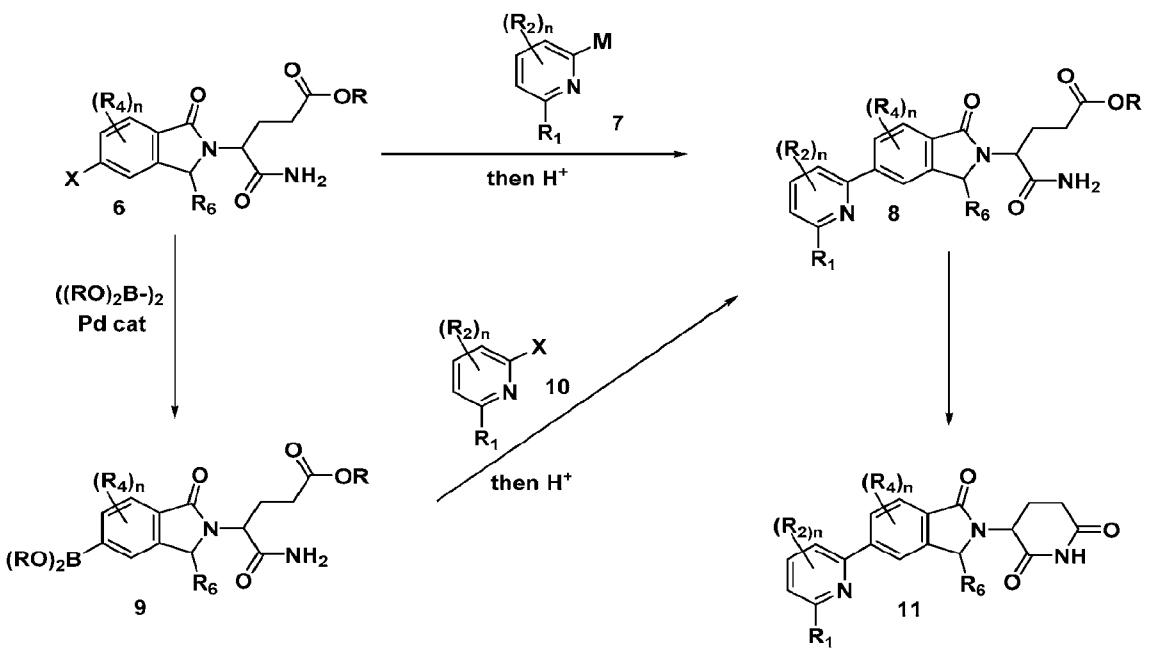
SCHEME 1



When M is a stannane, **6** can be united with a suitably substituted heterocycle **7** in a Stille coupling reaction using a suitable catalyst system (e.g. $Pd(PPh_3)_4$ or bis(triphenylphosphine)dichloropalladium(II)/CuI) to give **8.** Alternatively, **6** can be converted to the boronic acid or boronic ester **9** by conditions well-known to one skilled in the art. The boronic acid or boronate ester, **9** can be united with a suitably substituted heterocycle **10** in a Suzuki-Miyaura coupling reaction using a suitable palladium catalyst (e.g. $Pd(PPh_3)_4$ or 1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II)) in the presence of a suitable base (e.g. cesium carbonate, potassium phosphate, or sodium bicarbonate) to give **8**.

Depending on the specific selection of acid protecting group R in intermediate **8**, different conditions may be required to convert it into compound **11** (Scheme 2). For instance where R = methyl, ethyl, or benzyl, base-induced cyclization of **8** may be preferred for the direct conversion **8** to **11** using a suitable base (e.g. LiHMDS) in a suitable solvent (e.g. tetrahydrofuran). Where R = *tert*-butyl, acid-induced cyclization of **12** may be preferred for direct conversion of **8** to **11** using a suitable acid (e.g. benzenesulfonic acid) in a suitable solvent (e.g. acetonitrile). In some cases, it may be preferable to use a twostep procedure, first liberating free carboxylic acid corresponding to **8** using conditions which are appropriate to the specific acid protecting group R. Such methods are well known to one of ordinary skill in the art of organic synthesis. For instance where R=*tert*-butyl, acid hydrolysis using a suitable acid (e.g. trifluoroacetic acid or hydrochloric acid) may be preferred. Where R=methyl, ethyl, or benzyl, basic hydrolysis using a suitable base (e.g. LiOH) may be preferred. In other cases, where R=benzyl, it may be advantageous to deprotect by the action of palladium-catalyzed hydrogenolysis. Once liberated, the carboxylic acid can be activated toward intramolecular attack by the pendant primary amide by the action of thionyl chloride/dimethylformamide or carbonyldiimidazole/dimethylaminopyridine to afford **11**.

SCHEME 2



EXAMPLES

The following examples illustrate the particular embodiments of the present invention and do not limit the scope of the present invention. Chemical abbreviations and symbols as well as scientific abbreviations and symbols have their usual and customary meanings unless otherwise specified. Additional abbreviations employed in the Examples and elsewhere in this application are defined above. Common intermediates are generally useful for the preparation of more than one Example. Compounds of the Examples are identified by the example and step in which they were prepared (e.g., "1-A" denotes the Example 1, step A), or by the example only where the compound is the title compound of the example (for example, "1" denotes the title compound of Example 1). In some instances alternate preparations of intermediates or examples are described. Frequently chemists skilled in the art of synthesis may devise alternative preparations which may be desirable based on one or more considerations such as shorter reaction time, less expensive starting materials, ease of operation or isolation, improved yield, amenable to catalysis, avoidance of toxic reagents, accessibility of specialized instrumentation, and decreased number of linear steps, etc. The intent of describing alternative preparations is to further enable the preparation of the examples of this invention. In some instances some functional groups in the outlined examples and claims may be replaced by well-known bioisosteric replacements known in the art, for example, replacement of a carboxylic acid group with a tetrazole or a phosphate moiety.

ABBREVIATIONS

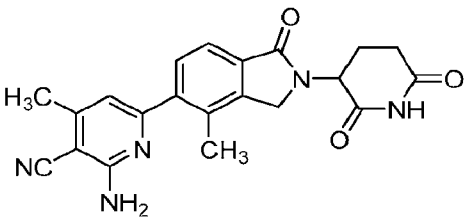
ACN	acetonitrile
AIBN	2,2-azobisiosbutyronitrile
n-BuLi	n-butyl lithium
DCE	dichloroethane
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
dppf	bis(diphenylphosphino)ferrocene

EtOH	ethanol
EtOAc	ethyl acetate
Hex	hexanes
H-Glu(OtBu)-NH_2 HCl	tert-butyl (4S)-4,5-diamino-5-oxopentanoate hydrochloride
HPLC	High Performance Liquid Chromatography
Hunig's base	*N*,*N*-diisopropylethylamine
LiHMDS	lithium bis(trimethylsilyl)amide
MeCN	acetonitrile
min	minute(s)
mL	milliliter(s)
NBS	n-bromosuccinimide
$Pd(dppf)_2Cl_2$	[1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II)
$Pd(dtbpf)Cl_2$	[1,1′-Bis(di-*tert*-butylphosphino)ferrocene]dichloropalladium(II)
$Pd(PPh_3)_4$	*tetrakis*(triphenylphosphine)palladium
$PhSO_3H$	benzenesulfonic acid
PTSOH	para-toluenesulfonic acid
TEA	triethylamine
THF	tetrahydrofuran
XPhos Pd G2	chloro(2-dicyclohexylphosphino-2′,4′,6′-triisopropyl-1,1′-biphenyl)[2-(2′-amino-1,1′-biphenyl)]palladium(II)

Preparative HPLC Method 1: XBridge C18, 200 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: a 0-min hold at 15% B, 15-50% B over 25 min, then a 6-min hold at 100% B; Flow Rate: 20 mL/min; Column Temperature: 25 °C. Fraction collection was triggered by MS signals.

EXAMPLE 1

2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-4-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile



(1)

Preparation 1A: *tert*-butyl (S)-5-amino-4-(5-bromo-4-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate.

To a suspension of tert-butyl (S)-4,5-diamino-5-oxopentanoate hydrochloride (2.162 g, 6.06 mmol) in acetonitrile (30 ml) at 0 °C was added DIPEA (3.018 ml, 17.28 mmol). After stirring for 20 min, the reaction mixture was treated with a solution of methyl 4-bromo-2-(bromomethyl)-3-methylbenzoate (2.650 g, 8.22 mmol) in MeCN (10 mL). The reaction mixture was stirred at 0 °C for 5 min. The ice bath was removed and the reaction mixture was allowed to warm to room temperature and stirred at room temperature for 1 h. The reaction mixture was warmed to 60 °C and held at that temperature overnight. The reaction mixture was concentrated, dissolved in EtOAc, washed with water twice, then 1.5 M K_2HPO_4, and also with brine, and then dried over $MgSO_4$, filtered, and concentrated. The material was purified using silica gel and eluting with 30-100% EtOAc/Hex. The resulting material was triturated with 8 mL ether to obtain 33.7 % yield of the titled product. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 7.70 (d, J=8.1 Hz, 1H), 7.56 (d, J=8.1 Hz, 1H), 6.23 (br s, 1H), 5.31 (br s, 1H), 4.92 (dd, J=8.6, 6.4 Hz, 1H), 4.55-4.47 (m, 1H), 4.45-4.36 (m, 1H), 2.41 (s, 3H), 2.41-2.14 (m, 4H), 1.45 (s, 9H).

Preparation 1B: *tert*-butyl (S)-5-amino-4-(4-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate

A dry flask was charged with tert-butyl (S)-5-amino-4-(5-bromo-4-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (300 mg, 0.730 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (278 mg, 1.094 mmol), and potassium acetate (214 mg, 2.188 mmol) and flushed with nitrogen. The solids were suspended in dioxane (10 mL) and degassed with a stream of nitrogen for 5 min with stirring. The reaction mixture was treated with $Pd(dppf)Cl_2$ (16.02 mg, 21.88 μmol). The flask was degassed for 5 min, sealed, and heated to 60 °C for 18 h under nitrogen. The reaction mixture was

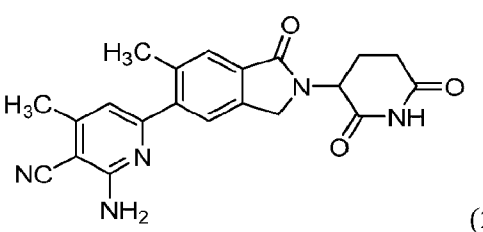
diluted with EtOAc, washed with brine, and dried over $MgSO_4$. It was concentrated and purified by 40 g silica gel column by ISCO, eluting with 0-40% B/DCM (where B= 15% EtOH/EtOAc+0.1% TEA), to afford the product in 99% yield. MS (ES): m/z = 459.3 $[M+H]^+$.

Example 1:

A 2 mL microwave vial was charged with 2-amino-6-bromo-4-methylnicotinonitrile (12 mg, 0.057 mmol), tert-butyl (S)-5-amino-4-(4-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (28.5 mg, 0.062 mmol), $Pd(dtbpf)Cl_2$ (1.106 mg, 1.698 μmol), dioxane (1.5 mL) and aqueous K_3PO_4 (0.075 mL, 0.226 mmol). The vial was sealed and the air was replaced with nitrogen. The reaction mixture was microwaved for 15 min at 120 °C. After cooling to room temperature, the reaction mixture was diluted with EtOAc, washed with brine, and the organic layer separated and concentrated. The residue obtained was dissolved in 1 mL of $PhSO_3H$ solution in MeCN (1.44 g/40 mL) and microwaved for 10 min at 120 °C. The material was concentrated to dryness, and the residue dissolved in 1.9 mL of DMSO, and purified by Preparative HPLC Method 1 to afford the titled compound in 49 % yield. MS (ES): m/z = 390.3 $[M+H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 11.03 (s, 1 H) 7.66 (d, *J*=7.63 Hz, 1 H) 7.53 (d, *J*=7.93 Hz, 1 H) 6.91 (s, 2 H) 6.78 (s, 1 H) 5.18 (br dd, *J*=13.43, 5.19 Hz, 1 H) 4.52 (br d, *J*=17.09 Hz, 1 H) 4.35 (br d, *J*=17.40 Hz, 1 H) 2.91-3.01 (m, 1 H) 2.65 (br d, *J*=16.78 Hz, 1 H) 2.47 (br dd, *J*=13.12, 4.27 Hz, 1 H) 2.43 (s, 3 H) 2.33 (s, 3 H) 2.03-2.10 (m, 1 H).

EXAMPLE 2

2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-6-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile



(2)

Preparation 2A: Methyl 4-bromo-2-(bromomethyl)-5-methylbenzoate.

To a solution of 4-bromo-2,5-dimethylbenzoic acid (5.0 g, 21.82 mmol) at room temperature was added $SOCl_2$ (31.66 mL, 436 mmol), and stirred for 2 h. LCMS was used to confirm the formation of the acid chloride, and then concentrated to dryness. Methanol (30 mL) was added at room temperature and stirred for 0.5 h and then concentrated to dryness again. To the obtained methyl ester was added CCl_4 (180 mL), followed by 1-bromopyrrolidine-2,5-dione (4.08 g, 22.92 mmol) and then AIBN (0.108 g, 0.654 mmol). The resulting mixture was heated to 80 °C with stirring overnight. After cooling to room temperature, the mixture was concentrated, dissolved in EtOAc, washed with brine, and dried over $MgSO_4$. LCMS showed two major peaks. The mixture of regioisomers obtained (5.4 g) was used for the next step.

Preparation 2B: *tert*-butyl (S)-5-amino-4-(5-bromo-6-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate

To a suspension of methyl 4-bromo-2-(bromomethyl)-5-methylbenzoate (5.4 g, 16.76 mmol) in acetonitrile (100 mL) was added tert-butyl (S)-4,5-diamino-5-oxopentanoate, HCl (4.00 g, 16.76 mmol), followed by Hunig's base (5.84 mL, 33.52 mmol). After stirring for 1 h at room temperature, the reaction mixture was placed into a 40 °C bath and stirred at that temperature for 6 days. The reaction mixture was cooled to room temperature and diluted with EtOAc, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by ISCO [120 g column], eluting with EtOAc/DCM from 0-100% to obtain 2.80 g of the desired compound. In addition, two other side products (methyl (S)-5-(((1-amino-5-(tert-butoxy)-1,5-dioxopentan-2-yl) amino)methyl)-4-bromo-2-methylbenzoate and tert-butyl (S)-5-amino-4-(((2-((R)-1-amino-5-(tert-butoxy)-1,5-dioxopentan-2-yl)-6-bromo-3-oxoisoindolin-5-yl)methyl) amino)-5-oxopentanoate) were isolated. MS (ES): m/z = 411.0 $[M+H]^+$. ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 7.70 (d, *J*=6.44 Hz, 2 H) 6.31 (br s, 1 H) 5.36 (br s, 1 H) 4.90 (dd, *J*=8.68, 6.34 Hz, 1 H) 4.51 (d, *J*=16.98 Hz, 1 H) 4.41 (d, *J*=16.98 Hz, 1 H) 2.51 (s, 3 H) 2.21-2.43 (m, 3 H) 2.12-2.19 (m, 1 H) 1.44 (s, 9 H).

Preparation 2C: *tert*-butyl (S)-5-amino-4-(6-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate

A 40 mL pressure vial was charged with tert-butyl (S)-5-amino-4-(5-bromo-6-

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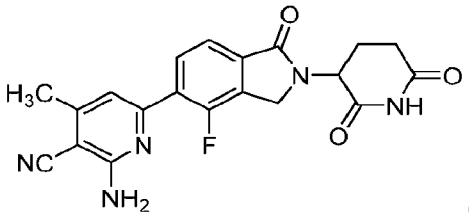
methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (600 mg, 1.458 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (556 mg, 2.188 mmol), and potassium acetate (430 mg, 4.376 mmol) and flushed with nitrogen. The solids were suspended in dioxane (20 mL) and degassed with a stream of nitrogen for 5 min with stirring. The reaction mixture was treated with $Pd(dppf)Cl_2$ (32.02 mg, 0.044 mmol) and degassed for 5 min. the vial was sealed, and heated to 95 °C for 3 h under nitrogen. The reaction mixture was cooled to room temperature, diluted with EtOAc, washed with brine, and dried over $MgSO_4$. The filtrate was concentrated and purified by 40 g silica gel column by ISCO, eluting with (0%-40% B/DCM, where B= 15% EtOH/EtOAc+0.1% TEA) to give the titled compound in 95 % yield. MS (ES): m/z = 459.3 $[M+H]^+$.

Example 2:

A 2 mL microwave vial was charged with 2-amino-6-bromo-4-methylnicotinonitrile (12 mg, 0.057 mmol), tert-butyl (S)-5-amino-4-(6-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (28.5 mg, 0.062 mmol), $Pd(dtbpf)Cl_2$ (1.106 mg, 1.698 μmol), dioxane (1.5 mL) and aqueous K_3PO_4 (0.075 mL, 0.226 mmol). The vial was sealed and the air was replaced with nitrogen. The reaction mixture was microwaved for 15 min at 120 °C. After cooling to room temperature. The reaction mixture was diluted with EtOAc, washed with brine, and the organic layer separated and concentrated. The residue obtained was dissolved in 1 mL of $PhSO_3H$ solution in MeCN (1.44 g/40 mL) and microwaved for 10 min at 120 °C. The mixture was concentrated to dryness, and the residue dissolved in 1.7 mL of DMSO, and purified by Preparative HPLC Method 1 to afford the title compound in 35 % yield. MS (ES): m/z = 390.3 $[M+H]^+$. ^1H NMR (500 MHz, DMSO-d_6) δ ppm 10.97-11.02 (m, 1 H) 7.65 (s, 1 H) 7.55 (s, 1 H) 6.76 (s, 1 H) 5.13 (dd, J=13.31, 5.18 Hz, 1 H) 4.39-4.51 (m, 1 H) 4.28-4.39 (m, 1 H) 2.87-2.98 (m, 1 H) 2.58-2.65 (m, 1 H) 2.51 (d, J=1.74 Hz, 3 H) 2.42-2.46 (m, 1 H) 2.41 (br s, 3 H) 2.39-2.40 (m, 1 H) 1.99-2.06 (m, 1 H).

EXAMPLE 3

2-Amino-6-(2-(2,6-dioxopiperidin-3-yl)-4-fluoro-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile



(3)

Intermediate 3A: 5-Bromo-4-fluoro-3-hydroxyisobenzofuran-1(3*H*)-one

To a stirred solution of 2,2,6,6-tetramethylpiperidine (7.07 mL, 41.6 mmol) in THF (150 mL) was added 2.5 M solution of n-BuLi in hexanes (16 mL, 40.0 mmol) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. To this, a solution of 4-bromo-3-fluorobenzoic acid (3.5 g, 15.98 mmol) in anhydrous THF (100 mL) was added dropwise at -50 °C. The reaction mixture was stirred for 3 h at the same temperature. Anhydrous DMF (2.48 mL, 32.0 mmol) was added at -50 °C and the reaction mixture was allowed to attain room temperature and stirred for 16 h. The reaction was quenched with 1.5 N HCl (100 mL). The reaction mixture was extracted with ethyl acetate (3 x 30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 120 g column, 0-50% EtOAc/pet-ether) to give 5-bromo-4-fluoro-3-hydroxyisobenzofuran-1(3*H*)-one (1.0 g, 23 % yield) as a yellow solid. LCMS (Method A): retention time 0.48 min, $[M+H]^+$ 245.1, 247.1; ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.93 (dd, J = 8.0, 5.5 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 6.74 (br s, 1H), 5.94 (br s, 1H).

Intermediate 3B: *tert*-Butyl (*S*)-5-amino-4-(5-bromo-4-fluoro-1-oxoisoindolin-2-yl)-5-oxopentanoate

To a stirred solution of 5-bromo-4-fluoro-3-hydroxyisobenzofuran-1(3*H*)-one (1.7 g, 6.88 mmol) and *tert*-butyl (*S*)-4,5-diamino-5-oxopentanoate HCl (1.67 g, 8.26 mmol) in DMF (30 mL) was added sodium triacetoxyborohydride (3.65 g, 17.21 mmol) at 0 °C. The reaction mixture was allowed to attain room temperature and stirred for 48 h. The reaction mixture was diluted with ice water (50 mL) and the resulting white solid was filtered and dried under reduced pressure to give *tert*-butyl (*S*)-5-amino-4-(5-bromo-4-fluoro-1-oxoisoindolin-2-yl)-5-oxopentanoate (1.6 g, 50 % yield) as a white solid. LCMS (Method A): retention time 1.39 min, $[M+H]^+$ 413.9, 415.3; ^{1}H NMR (400 MHz, DMSO-

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d_6) δ 7.85 (dd, J = 8.0, 6.0 Hz, 1H), 7.59 (br s, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.23 (br s, 1H), 4.77-4.59 (m, 3H), 2.26-2.13 (m, 3H), 2.08-1.96 (m, 1H), 1.34 (s, 9H).

Intermediate 3C: *tert*-Butyl (*S*)-5-amino-4-(5-(6-amino-5-cyano-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)-5-oxopentanoate

A stirred solution of 2-amino-6-chloro-4-methylnicotinonitrile (50 mg, 0.30 mmol) and hexamethylditin (0.093 mL, 0.45 mmol) in toluene (2 mL) was purged with argon for five minutes followed by the addition of [1,1'-bis(di-*tert*-butylphosphino) ferrocene]dichloropalladium(II) (9.72 mg, 0.015 mmol). The reaction mixture was stirred for 2 h at 100 °C, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give crude product. $[M+H]^+$ 298.2. To a solution of the crude product in dioxane (2 mL), was added *tert*-butyl(*S*)-5-amino-4-(5-bromo-4-fluoro-1-oxoisoindolin-2-yl)-5-oxopentanoate (98 mg, 0.24 mmol). The reaction mixture was purged with argon for five minutes, bis(triphenylphosphine)palladium(II) chloride (16.60 mg, 0.024 mmol) was added and the reaction mixture was heated at 100 °C for 16 h. The reaction mixture was cooled to room temperature, filtered and the filtrate was concentrated under reduced pressure to give the crude product which was purified by flash chromatography (SiO_2, 80 g column, 0-100% B (B = 15% EtOH in EtOAc, 0.5%TEA)/chloroform) to give *tert*-butyl (*S*)-5-amino-4-(5-(6-amino-5-cyano-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)-5-oxopentanoate (60 mg, 40.7 % yield). LCMS (Method A): retention time 1.35 min, $[M+H]^+$ 468.3.

Example 3:

To a stirred solution of Intermediate 3C (60 mg, 0.13 mmol) in acetic acid (2 mL), benzene sulfonic acid (20.30 mg, 0.13 mmol) was added. The reaction mixture was heated to 150 °C under micro wave irradiation for 10 minutes. Volatiles were removed under reduced pressure and the resulting crude product was purified via preparative LC-MS (column: Waters XBridge C18, 19 x 150 mm, 5 μm particles; mobile phase A: 0.1% trifluoroacetic acid in water; mobile phase B: acetonitrile; gradient: 10-40% B over 20 minutes, then a 5-minute hold at 100% B; flow: 15 mL/min). The desired fractions were lyophilized to dryness to give the 2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-4-fluoro-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (15 mg, 29% yield). LCMS (Method G):

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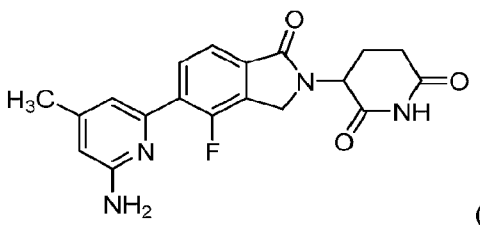
retention time 1.62 min, $[M+H]^+$ 394.2; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (br s, 1H), 8.01 (t, J=7.3 Hz, 1H), 7.70 (d, J=7.9 Hz, 1H), 7.06 (d, J=1.5 Hz, 1H), 6.99 (s, 2H), 5.14 (dd, J=13.3, 5.1 Hz, 1H), 4.74-4.38 (m, 2H), 3.05-2.85 (m, 1H), 2.75-2.57 (m, 2H), 2.46 (s, 3H), 2.13-1.95 (m, 1H).

General procedure 2: A stirred solution of Intermediate 3B (1 eq.) and aryl stannane reagent (1 eq.) in dioxane (5 mL/mmol) was purged with argon for five minutes. Bis(triphenylphosphine) palladium(II) chloride (0.1 eq.) was added and the reaction mixture was heated at 100 °C for 16 h. The reaction mixture was cooled to room temperature, filtered through celite pad and the filtrate was concentrated under reduced pressure. The crude material was transferred to a microwave vial, $PhSO_3H$ (2 eq.) and acetic acid (5 mL/mmol) were added and the mixture was heated at 150 °C for 10 min in a microwave reactor. The mixture was concentrated and the residue was purified by prep-HPLC.

EXAMPLE 4

3-(5-(6-Amino-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)piperidine-2,6-dione



(4)

Intermediate 4A: 4-Methyl-6-(trimethylstannyl)pyridin-2-amine

A stirred solution of 6-bromo-4-methylpyridin-2-amine (75 mg, 0.382 mmol) and hexamethylditin (0.119 mL, 0.57 mmol) in toluene (5 mL) was purged with argon for five minutes followed by the addition of [1,1'-bis(di-*tert*-butylphosphino)ferrocene] dichloropalladium(II) (12.4 mg, 0.02 mmol). The reaction mixture was stirred for 2 h at 100 °C, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give 4-methyl-6-(trimethylstannyl)pyridin-2-amine (130 mg, 89 % yield). LCMS (Method A): retention time 1.62 min, $[M+H]^+$ 273.1.

Example 4:

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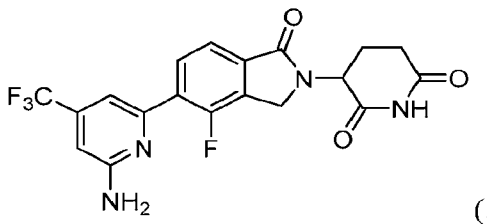
The Stille coupling and cyclization were accomplished by following general procedure 2 with Preparation 4A and Preparation 3B. MS (ES): m/z = $[M+H]^+$ 369.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.02 (s, 1H), 8.02 (t, *J* = 7.2 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 1H), 6.84 (s, 1H), 6.34 (s, 1H), 6.03 (s, 2H), 5.15 (dd, *J* = 5.0, 13.3 Hz, 1H), 4.66-4.57 (m, 1H), 4.49-4.38 (m, 1H), 3.02-2.85 (m, 1H), 2.62 (br d, *J* = 17.4 Hz, 1H), 2.49-2.40 (m, 1H), 2.22 (s, 3H), 2.07-1.98 (m, 1H).

EXAMPLE 5

3-(5-(6-Amino-4-(trifluoroethyl) pyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione



(5)

Intermediate 5A:

A stirred solution of 6-chloro-4-(trifluoromethyl)pyridin-2-amine (75 mg, 0.382 mmol) and hexamethylditin (0.119 mL, 0.57 mmol) in toluene (5 mL) was purged with argon for five minutes followed by the addition of [1,1'-bis(di-*tert*-butylphosphino) ferrocene]dichloropalladium(II) (12.4 mg, 0.02 mmol). The reaction mixture was stirred for 2 h at 100 °C, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give 4-(trifluoromethyl)-6-(trimethylstannyl)pyridin-2-amine (130 mg, 89 % yield). LCMS (Method A): retention time 1.92 min, $[M+H]^+$ 327.1.

Example 5:

The Stille coupling and cyclization were accomplished by following general procedure 1 with Intermediate 5A and Intermediate 3B. MS (ES): m/z = $[M+H]^+$ 423.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.02 (s, 1H), 8.05 (t, *J* = 7.3 Hz, 1H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.15 (s, 1H), 6.78 (d, *J* = 11.8 Hz, 3H), 5.15 (dd, *J* = 5.0, 13.3 Hz, 1H), 4.68-4.57 (m, 1H), 4.52-4.39 (m, 1H), 2.99-2.82 (m, 1H), 2.67-2.58 (m, 1H), 2.48-2.39 (m, 1H), 2.12-1.93 (m, 1H).

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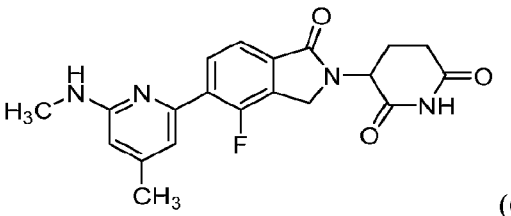
EXAMPLE 6

3-(4-Fluoro-5-(4-methyl-6-(methylamino)pyridin-2-yl)-1-oxoisoindolin-2-yl) piperidine-2,6-dione



(6)

Intermediate 6A: *N*,4-Dimethyl-6-(trimethylstannyl)pyridin-2-amine

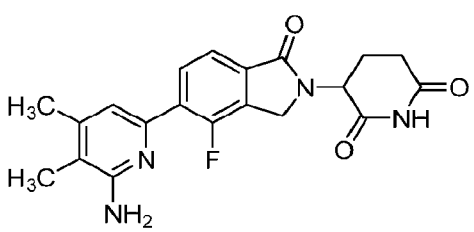
A stirred solution of 6-chloro-*N*,4-dimethylpyridin-2-amine (100 mg, 0.64 mmol) and hexamethylditin (0.199 mL, 0.96 mmol) in toluene (10 mL) was purged with argon for five minutes followed by the addition of [1,1'-bis(di-*tert*-butylphosphino)ferrocene] dichloropalladium(II) (20.8 mg, 0.032 mmol). The reaction mixture was stirred for 2 h at 100 °C, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give *N*,4-dimethyl-6-(trimethylstannyl)pyridin-2-amine (250 mg, 55.0 % yield). LCMS (Method A): retention time 1.29 min, $[M+H]^+$ 287.1.

Example 6:

The Stille coupling and cyclization were accomplished by following general procedure 2 with Intermediate 6A and Intermediate 3B. MS (ES): m/z = $[M+H]^+$ 383.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (s, 1H), 8.02 (s, 1H), 7.72 (d, J = 7.5 Hz, 1H), 6.93 (s, 1H), 6.58 (br s, 1H), 5.16 (dd, J = 13.1, 5.0 Hz, 1H), 4.82-4.40 (m, 3H), 2.89 (s, 3H), 2.75-2.63 (m, 1H), 2.64-2.57 (m, 1H), 2.46-2.41 (m, 1H), 2.36-2.28 (m, 3H), 2.06-2.00 (m, 1H). 19/19

EXAMPLE 7

3-(5-(6-Amino-4,5-dimethylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)piperidine-2,6-dione



(7)

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Intermediate 7A: 3,4-Dimethyl-6-(trimethylstannyl)pyridin-2-amine

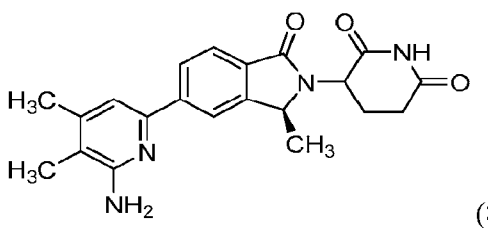
A stirred solution of 6-chloro-3,4-dimethylpyridin-2-amine (75 mg, 0.48 mmol) and hexamethylditin (0.149 mL, 0.72 mmol) in toluene (3 mL) was purged with argon for five minutes followed by the addition of [1,1'-bis(di-*tert*-butylphosphino)ferrocene] dichloropalladium(II) (15.6 mg, 0.024 mmol). The reaction mixture was stirred for 2 h at 100 °C, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give 3,4-dimethyl-6-(trimethylstannyl)pyridin-2-amine (150 mg, 55.0 % yield). LCMS (Method A): retention time 1.16 min, $[M+H]^+$ 287.2.

Example 7:

The Stille coupling and cyclization were accomplished by following general procedure 1 with Intermediate 7A and Intermediate 3B. MS (ES): m/z = $[M+H]^+$ 383.0; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (br s, 1H), 8.05 (t, J = 7.3 Hz, 1H), 7.65 (d, J = 7.9 Hz, 1H), 6.93 (d, J = 1.5 Hz, 1H), 5.81 (s, 2H), 5.14 (dd, J = 13.3, 5.1 Hz, 1H), 4.71-4.34 (m, 2H), 3.01-2.88 (m, 1H), 2.67-2.58 (m, 2H), 2.46-2.41 (m, 1H), 2.23 (s, 3H), 2.04 (s, 3H).

EXAMPLES 8 AND 9

3-((*S*)-5-(6-Amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione and 3-((*R*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione



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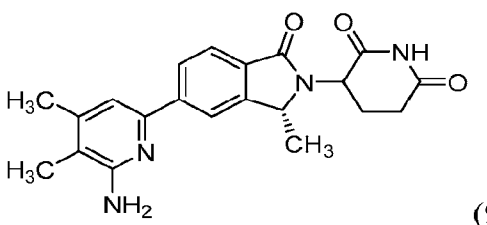

(9)

Intermediate 8A: 4-Bromo-2-ethylbenzoic acid

To a solution of 4-bromo-2-fluorobenzoic acid (5 g, 22.83 mmol) in anhydrous THF (100 mL) was added 1 M solution of ethyl magnesium bromide in THF (22.83 mL, 68.5 mmol) at -78 °C in 15 min. The reaction mixture was slowly warmed to room temperature and stirred under nitrogen atmosphere for 12 h. The reaction mixture was cooled to 0 °C. The reaction was quenched with dropwise addition of MeOH (15 mL).

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The reaction mixture was concentrated under reduced pressure. The resulting residue is partitioned between EtOAc and 2 M aqueous HCl. The layers were separated and the aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give crude product. The crude product was purified by flash chromatography (SiO_2, 80 g column, 0-30% EtOAc/pet-ether) to give 4-bromo-2-ethylbenzoic acid (4 g, 76 % yield). LCMS (Method D): retention time 2.49 min, $[M+H]^+$ 228.8, 230.0; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.04 (br s, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.55 (d, J = 2.0 Hz, 1H), 7.49 (dd, J = 8.0, 2.0 Hz, 1H), 2.92 (q, J = 7.2 Hz, 2H), 1.15 (t, J = 7.5 Hz, 3H).

Intermediate 8B: Methyl 4-bromo-2-ethylbenzoate

To a stirred mixture of 4-bromo-2-ethylbenzoic acid (4.0 g, 17.46 mmol) and Cs_2CO_3 (11.38 g, 34.9 mmol) in DMF (40 mL) was added methyl iodide (2.18 mL, 34.9 mmol). The reaction mixture was stirred at room temperature for 14 h, filtered through celite pad and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 80 g column, 0-50% EtOAc/Pet-ether) to give methyl 4-bromo-2-ethylbenzoate (3.3 g, 78 % yield) as a colorless oil. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 7.76 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 1.5 Hz, 1H), 7.40 (dd, J = 8.5, 2.0 Hz, 1H), 3.91 (s, 3H), 2.99 (q, J = 7.5 Hz, 2H), 1.26 (t, J = 7.5 Hz, 3H).

Intermediate 8C: Methyl 4-bromo-2-(1-bromoethyl)benzoate

To a stirred solution of methyl 4-bromo-2-ethylbenzoate (3.0 g, 12.34 mmol) in benzene (40 mL) was added NBS (3.29 g, 18.51 mmol) followed by AIBN (0.405 g, 2.47 mmol). The reaction mixture was heated at 85 °C for 15 h. The reaction mixture was cooled to room temperature, diluted with EtOAc, washed with saturated 10% sodium thiosulfate solution and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 40 g column, 0-30% EtOAc/pet-ether) to give methyl 4-bromo-2-(1-bromoethyl)benzoate (2.1 g, 53% yield) as a white solid. ^{1}H NMR (400 MHz, chloroform-d) δ 7.96 (d, J = 2.0 Hz, 1H), 7.73 (s, 1H), 7.49 (dd, J = 8.5, 2.0 Hz, 1H), 6.28 (q, J = 7.0 Hz, 1H), 3.95 (s, 3H).

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Intermediate 8D: *tert*-Butyl (4*S*)-5-amino-4-(5-bromo-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate

To a stirred solution of methyl 4-bromo-2-(1-bromoethyl) benzoate (1.0 g, 3.11 mmol) and H-Glu(OtBu)-NH_2 HCl (0.754 g, 3.73 mmol) in acetonitrile (30 mL) was added DIPEA (2.71 mL, 15.53 mmol). The resulting reaction mixture was heated at 85 °C for 15 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 40 g column, 0-10% MeOH/DCM) to give *tert*-butyl (4*S*)-5-amino-4-(5-bromo-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (650 mg, 51 % yield) as a semi-solid. LCMS (Method A): retention time 1.45 min, $[M+H]^+$ 411.3, 413.3.

Intermediate 8E: *tert*-butyl 5-amino-4-(5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate

A stirred solution of 6-chloro-3,4-dimethylpyridin-2-amine (200 mg, 1.28 mmol), and hexamethylditin (544 mg, 1.66 mmol) in toluene (6 mL) was purged with argon for five minutes followed by the addition of [1,1'-bis(di-*tert*-butylphosphino)ferrocene] dichloropalladium(II) (83 mg, 0.13 mmol). The reaction mixture was stirred for 2 h at 100 °C, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give crude product. $[M+H]^+$ 287.0. To a solution of this crude product in dioxane (4 mL), was added *tert*-butyl 5-amino-4-(5-bromo-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (0.289 g, 0.70 mmol). The reaction mixture was purged with argon for five minutes. Bis(1,2-bis(diphenylphosphino)ethane)palladium(0) (0.063 g, 0.07 mmol) was added and the reaction mixture was heated at 100 °C for 16 h. The reaction mixture was cooled to room temperature, filtered and the filtrate was concentrated under reduced pressure to give the crude product which was purified by flash chromatography (SiO_2, 80 g column, 0-100% B (B = 15% EtOH in EtOAc + 0.5%TEA)/chloroform) to give *tert*-butyl 5-amino-4-(5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (110 mg, 34.6 % yield). LCMS (Method A): retention time 1.40 min, $[M+H]^+$ 453.3.

Intermediate 8F: 3-(5-(6-Amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione

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To a stirred solution of tert-butyl 5-amino-4-(5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (110 mg, 0.243 mmol, 34.6 % yield) in acetonitrile (5 mL), p-toluene sulfonic acid (84 mg, 0.442 mmol) was added. The reaction mixture was heated to 120 °C under micro wave irradiation for 30 minutes. Volatiles were removed under reduced pressure and the crude product was purified via preparative HPLC (Column: Hypersil gold c18 (19 X 250 mm), 5 μm Mobile Phase A- 10 mM ammonium acetate in water, Mobile Phase B: ACN FLOW: 20 mL T/B%:0/20, 18/80, 19/100, 21/100, 22/20, 24/20). The desired fractions were lyophilized to dryness to give the 3-(5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione. LCMS (Method D): retention time 2.15 min, $[M+H]^+$ 379.0.

Examples 8 and 9:

3-(5-(6-Amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (100 mg) was subjected to SFC separation, (Column Welk-01(R,R)(250*4.6) mm, 5 μm; % CO_2: 45%; % cosolvent: 5 mM ammonium acetate in methanol and acetonitrile (1:1); flow: 4 g/min; temperature: 30 °C; UV: 237 nm), the first peak fractions eluted at 2.64 min retention time were concentrated and lyophilized to afford 3-((S)-5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (12 mg, 15% yield). LCMS (Method D): retention time 2.112 min, $[M+H]^+$ 379.0; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10-10.71 (s, 1H), 8.19 (s, 1H), 8.12 (d, J=7.9 Hz, 1H), 7.69 (d, J=8.0 Hz, 1H), 7.14 (s, 1H), 5.73 (s, 2H), 4.93-4.67 (m, 2H), 2.85-2.71 (m, 2H), 2.25 (s, 3H), 2.04 (s, 3H), 1.51 (d, J=6.6 Hz, 3H) and the second peak fractions eluted at 4.03 min retention time were concentrated to lyophilized to afford 3-((R)-5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (8 mg, 10% yield). LCMS (Method D): retention time 2.162 min, $[M+H]^+$ 379.0; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00-10.89 (s, 1H), 8.18 (s, 1H), 8.13 (d, J=8.0 Hz, 1H), 7.70 (d, J=8.0 Hz, 1H), 7.13 (s, 1H), 5.73 (s, 2H), 4.84-4.68 (m, 2H), 2.91-2.65 (m, 3H), 2.25 (s, 3H), 2.04 (s, 3H), 1.51-1.46 (m, 3H).

EXAMPLES 10 AND 11

2-Amino-6-((3S)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile and 2-amino-6-((3R)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-

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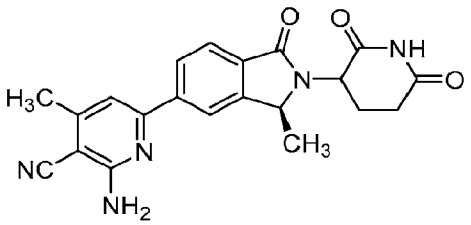
oxoisoindolin-5-yl)-4-methylnicotinonitrile



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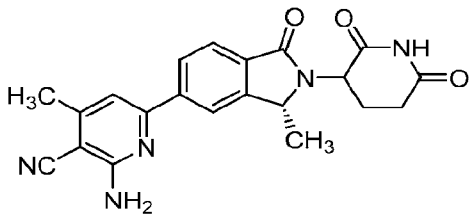

(11)

Intermediates 10A and 11A:

A stirred solution of 2-amino-6-chloro-4-methylnicotinonitrile (300 mg, 1.790 mmol), and 1,1,1,2,2,2-hexamethyldistannane (762 mg, 2.327 mmol) in toluene (10 mL) was purged with argon for five minutes followed by the addition of [1,1'-bis(di-*tert*-butylphosphino)ferrocene]dichloropalladium(II) (117 mg, 0.179 mmol). The reaction mixture was stirred for 2 h at 100 °C, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to give crude product. $[M+H]^+$ 298.0 The crude material was dissolved in dioxane, Intermediate 8D (0.695 g, 1.689 mmol) was added. The reaction mixture was purged with argon for five minutes and bis(diphenylphosphino)ethane) palladium(0) (0.153 g, 0.169 mmol) was added. The reaction mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature, filtered and the filtrate was concentrated under reduced pressure to give the crude product which was purified by flash chromatography (SiO_2, 80 g column, 0-100% B (B = 15% EtOH in EtOAc, 0.5%TEA)/chloroform) to give *tert*-butyl 5-amino-4-(5-(6-amino-5-cyano-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (200 mg, 0.431 mmol, 25.5 % yield). LCMS (Method A): retention time 1.47 min, $[M+H]^+$ 464.3.

Examples 10 and 11:

To a stirred solution of *tert*-butyl 5-amino-4-(5-(6-amino-5-cyano-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (200 mg, 0.431 mmol) in acetonitrile (10 mL), p-toluene sulfonic acid (164 mg, 0.863 mmol) was added. The reaction mixture was heated to 120 °C under microwave irradiation for 30 minutes. Volatiles were removed under reduced pressure and the resulting crude product was purified preparative HPLC (Column: Hypersil gold c18 (19 X 250 mm), 5 μm Mobile Phase A- 10 mM ammonium acetate in water; Mobile Phase B: ACN Flow: 20 mL T/B%:

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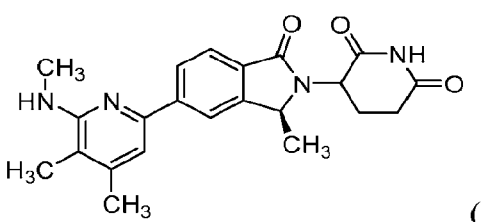
0/20,18/80, 19/100, 21/100, 22/20, 24/20). The desired fractions were lyophilized to dryness to give the 2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile LCMS (Method D): retention time 1.66 min, $[M+H]^+$ 390.2 as a mixture of diastereomers. The diastereomers were separated by SFC (Column Welk-01(R,R)(250*4.6) mm, 5 μm; %CO_2: 45%; % cosolvent: 5 mM ammonium acetate in methanol and acetonitrile (1:1); flow: 4 g/min; temperature: 30 °C; UV: 237 nm), first eluting isomers fractions at 2.28 min retention time were concentrated to dryness and lyophilized to afford 2-amino-6-((3S)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (20 mg, 12% yield). LCMS (Method D): retention time 1.64 min, $[M+H]^+$ 390.0; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01-10.72 (s, 1H), 8.28 (s, 1H), 8.19 (dd, J=8.0, 1.3 Hz, 1H), 7.76 (d, J=8.0 Hz, 1H), 7.35 (s, 1H), 6.92 (s, 2H), 4.94-4.68 (m, 2H), 2.65-2.58 (m, 2H), 2.44 (s, 3H), 2.14-1.91 (m, 2H), 1.51 (d, J=6.6 Hz, 3H); and the second eluting isomer fractions at 3.25 min retention time were concentrated to dryness and lyophilized to afford 2-amino-6-((3R)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (20 mg, 12% yield). LCMS (Method D): retention time 1.66 min, $[M+H]^+$ 390.0; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01-10.72 (s, 1H), 8.27 (s, 1H), 8.20 (d, J=8.0 Hz, 1H), 7.78 (d, J=8.0 Hz, 1H), 7.34 (s, 1H), 6.90 (s, 2H), 4.84-4.70 (m, 2H), 2.84-2.67 (m, 2H), 2.44 (s, 3H), 2.14-1.91 (m, 2H), 1.49 (br d, J=6.6 Hz, 3H).

EXAMPLES 12 AND 13

3-((S)-5-(4,5-Dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione and 3-((R)-5-(4,5-dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione



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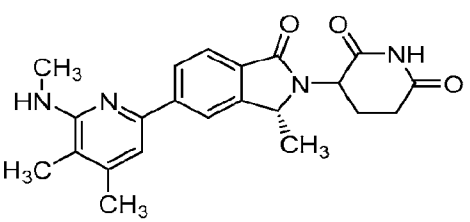

(13)

Intermediates 12A and 13A:

To a solution of Intermediate 8D (350 mg, 0.764 mmol) in dioxane (8 mL) was added 6-chloro-N,3,4-trimethylpyridin-2-amine (130 mg, 0.764 mmol) followed by 3 M

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aqueous potassium phosphate, dibasic (0.764 mL, 2.291 mmol) solution. The reaction mixture was purged with nitrogen for 15 min at room temperature. $PdCl_2(dppf)$-CH_2Cl_2 adduct (62.4 mg, 0.076 mmol) was added under nitrogen and the reaction mixture was heated at 100 °C for 12 h. The reaction mixture was cooled to room temperature, filtered through celite pad and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 24 g column, 0-10% MeOH\DCM) to afford *tert*-butyl (4S)-5-amino-4-(5-(4,5-dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (230 mg, 0.493 mmol, 64.6 % yield) as an off-white solid. LCMS (method A): retention time 1.81 min, $[M+H]^+$ 467.4.

Examples 12 and 13:

To a stirred solution of tert-butyl 5-amino-4-(5-(4,5-dimethyl-6-(methylamino) pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (200 mg, 0.429 mmol) in acetic acid (1 mL), benzenesulfonic acid (67.8 mg, 0.429 mmol) was added. The reaction mixture was heated to 120 °C under microwave irradiation for 30 minutes. Volatiles were removed under reduced pressure and the resulting crude product was purified via preparative HPLC (Column: X Select CSH C18 (250*20 mm) 5 μm Mobile phase: A: 10 mM ammonium acetate B: CAN T/B: 0/20, 18/85, 20/100, 21/20, 23/20 Flow: 20 mL/min). The desired fractions were lyophilized to dryness to give the 3-(5-(4,5-dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione LCMS (Method A): retention time 1.47 min, $[M+H]^+$ 393.4 as a mixture of diastereomers. The diastereomers were separated by SFC (Column Chiralpak IC (250*4.6) mm. 5 μm; % CO_2: 45%; % cosolvent: 5 mM ammonium acetate in methanol and acetonitrile (1:1); flow: 4 g/min; temperature: 30 °C; UV: 237 nm), first eluting isomers fractions at 8.65 min retention time were concentrated to dryness and lyophilized to afford 3-((S)-5-(4,5-dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (3 mg, 2% yield), LCMS (Method D): retention time 1.743 min, $[M+H]^+$ 393.4; ^1H NMR (400 MHz, DMSO-d_6) δ 11.07-10.81 (m, 1H), 8.35-8.12 (m, 2H), 7.71 (d, J=8.0 Hz, 1H), 7.14 (s, 1H), 6.03 (br d, J=4.0 Hz, 1H), 4.88-4.69 (m, 2H), 2.96 (d, J=4.5 Hz, 3H), 2.78-2.63 (m, 3H), 2.26 (s, 3H), 2.03 (s, 4H), 1.49 (d, J=6.5 Hz, 3H) and the second peak fractions eluted at 11.18 min retention time were concentrated to lyophilized to afford 3-((R)-5-(4,5-dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-

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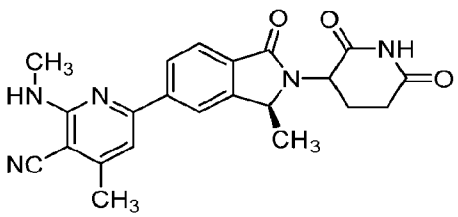
oxoisoindolin-2-yl)piperidine-2,6-dione (3 mg, 2% yield) LCMS (Method D): retention time 1.669 min, $[M+H]^+$ 393.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.07-10.81 (m, 1H), 8.35-8.12 (m, 2H), 7.71 (d, J=8.0 Hz, 1H), 7.14 (s, 1H), 6.03 (br d, J=4.0 Hz, 1H), 4.88-4.69 (m, 2H), 2.96 (d, J=4.5 Hz, 3H), 2.78-2.63 (m, 3H), 2.26 (s, 3H), 2.03 (s, 4H), 1.49 (d, J=6.5 Hz, 3H).

EXAMPLES 14 AND 15

6-((3S)-2-(2,6-Dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2-(methylamino) nicotinonitrile and 6-((3R)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2-(methylamino)nicotinonitrile



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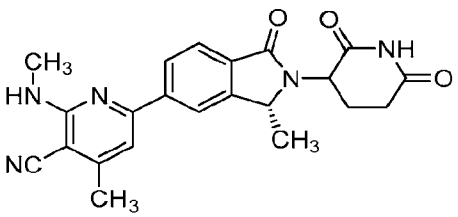

(15)

Intermediates 14A and 15A:

To a solution of Intermediate 8D (200 mg, 0.436 mmol) in dioxane (8 mL) was added 6-chloro-4-methyl-2-(methylamino) nicotinonitrile (79 mg, 0.436 mmol), followed by K_3PO_4 (0.291 mL, 0.873 mmol 3 M aqueous solution). The reaction mixture was purged with nitrogen for 15 min at room temperature. Xphos Pd G4 (37.6 mg, 0.044 mmol) was added under nitrogen atmosphere and the reaction mixture was heated at 80 °C for 12 h. The reaction mixture was cooled to room temperature, filtered through celite pad and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 24 g column, 0-10% MeOH\DCM) to afford tert-butyl (4S)-5-amino-4-(5-(5-cyano-4-methyl-6-(methylamino) pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (75 mg, 0.157 mmol, 36.0 % yield) as an off-white solid. LCMS (method A): retention time 1.59 min, $[M+H]^+$ 478.2.

Examples 14 and 15:

To a solution of tert-butyl (4S)-5-amino-4-(5-(5-cyano-4-methyl-6-(methylamino) pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (60 mg, 0.126 mmol) in acetonitrile (3 mL) was added benzene sulfonic acid (19.87 mg, 0.126 mmol) at room

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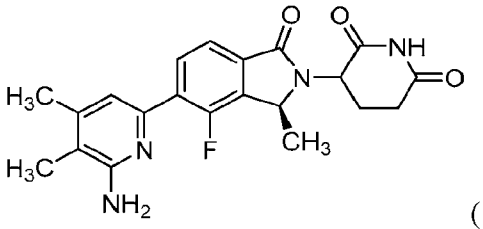
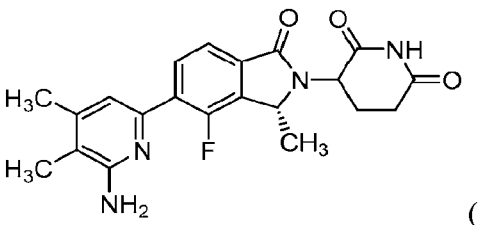
temperature. The reaction mixture was heated to 120 °C for 45 min in a microwave reactor. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to get crude product. The crude product was purified by flash chromatography (SiO_2, 24 g column, 0-10% MeOH/DCM) to afford 6-(2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2- (methylamino) nicotinonitrile as an off-white solid as a mixture of diastereomers. LCMS (method A): retention time 1.22 min, $[M+H]^+$ 404.1. The diastereomers were separated by SFC (Column Welk-01(R,R)(250*4.6) mm, 5 μm; % CO_2: 45%; % cosolvent: 5 mM ammonium acetate in methanol and acetonitrile (1:1); flow: 4 g/min; temperature: 30 °C; UV: 237 nm), first eluting isomers fractions at 2.91 min retention time were concentrated to dryness and lyophilized to afford 6-((3S)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2-(methylamino) nicotinonitrile (6 mg, 12% yield), LCMS (Method D): retention time 2.071 min, $[M+H]^-$ 404.2; ^1H NMR (400 MHz, DMSO-d_6) δ 11.27-10.74 (m, 1H), 8.35 (s, 1H), 8.28 (d, J=8.0 Hz, 1H), 7.77 (d, J=8.5 Hz, 1H), 7.37 (s, 1H), 7.12 (br d, J=4.5 Hz, 1H), 4.87 (q, J=6.2 Hz, 1H), 4.81-4.71 (m, 1H), 3.00 (d, J=4.5 Hz, 3H), 2.68 (s, 1H), 2.44 (s, 3H), 1.72 (s, 3H), 1.52 (d, J=7.0 Hz, 3H) and the second peak fractions eluted at 4.74 min retention time were concentrated to lyophilized to afford 6-((3R)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2-(methylamino)nicotinonitrile (8 mg, 16% yield). LCMS (Method D): retention time 2.063 min, $[M+H]^+$ 404.2; 1H NMR (400 MHz, DMSO-d_6) δ 11.08-10.87 (m, 1H), 8.34 (s, 1H), 8.28 (d, J=8.3 Hz, 1H), 7.77 (d, J=8.0 Hz, 1H), 7.36 (s, 1H), 7.19-7.05 (m, 1H), 4.91-4.71 (m, 2H), 2.99 (d, J=4.5 Hz, 3H), 2.76-2.57 (m, 3H), 2.44 (s, 3H), 1.71 (s, 1H), 1.49 (d, J=6.8 Hz, 3H).

EXAMPLES 16 AND 17

3-((*S*)-5-(6-Amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione and 3-((*R*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione



(16)



(17)

Intermediate 16A: 4-Bromo-2-ethyl-3-fluorobenzoic acid

A solution of 4-bromo 2,3-difluorobenzoic acid (2.0 g, 8.44 mmol) in tetrahydrofuran (40 mL) was cooled to -78° C and 1 M solution of ethyl magnesium bromide in THF (8.44 mL, 25.3 mmol) was added dropwise. The reaction mixture was allowed to attain room temperature and stirred under nitrogen atmosphere for 12 h. The reaction was quenched by the addition of MeOH (15 mL) dropwise at 0 °C. Volatiles were removed under reduced pressure and the residue was partitioned between EtOAc and 2 M aqueous HCl. The layers were separated and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give crude product. The crude product was purified by flash chromatography (SiO_2, 40 g column, 0-80% EtOAc/Pet-ether) to afford 4-bromo-2-ethyl-3-fluorobenzoic acid (1 g, 48 % yield). LCMS (Method A): retention time 0.69 min, $[M-H]^+$ 245.1, 247.1; ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.36 (s, 1H), 7.68-7.53 (m, 2H), 2.95 (qd, J = 7.4, 2.6 Hz, 2H), 1.24-1.06 (m, 3H).

Intermediate 16B: Methyl 4-bromo-2-ethyl-3-fluorobenzoate

To a stirred mixture of 4-bromo-2-ethyl-3-fluorobenzoic acid (0.7 g, 2.83 mmol) and K_2CO_3 (0.783 g, 5.67 mmol) in acetone (15 mL) was added dimethyl sulfate (0.541 mL, 5.67 mmol) dropwise at room temperature. The reaction mixture was stirred at 50 °C for 14 h and filtered through celite pad. The filtrate was concentrated under vacuum and purified by flash chromatography (SiO_2, 24 g column, 0-50% EtOAc/Pet-ether) to afford methyl 4-bromo-2-ethyl-3-fluorobenzoate (0.51 g, 69 % yield) as a colorless oil. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.58-7.51 (m, 1H), 7.49-7.37 (m, 1H), 3.90 (s, 3H), 3.01 (qd, J = 7.4, 2.6 Hz, 2H), 1.23 (t, J = 7.4 Hz, 3H).

Intermediate 16C: Methyl 4-bromo-2-(1-bromoethyl)-3-fluorobenzoate

To a stirred solution of methyl 4-bromo-2-ethyl-3-fluorobenzoate (0.515 g, 1.972

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mmol) in DCE (10 mL) was added NBS (0.386 g, 2.170 mmol) followed by AIBN (0.065 g, 0.394 mmol). The reaction mixture was heated at 85 °C for 15 h. The reaction mixture was diluted with ethyl acetate, washed with saturated 10% sodium thiosulfate solution and brine solution. The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum. The resulting residue was purified by flash chromatography (SiO_2, 24 g column, 0-30% EtOAc/Pet-ether) to afford methyl 4-bromo-2-(1-bromoethyl)-3-fluorobenzoate (0.6 g, 89 % yield) as a white solid. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.70-7.53 (m, 1H), 7.52-7.44 (m, 1H), 6.16-5.87 (m, 1H), 3.93 (s, 3H), 1.95 (dd, J = 7.0, 1.3 Hz, 3H).

Intermediate 16D: *tert*-Butyl (4S)-5-amino-4-(5-bromo-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate

To a stirred solution of methyl 4-bromo-2-(1-bromoethyl)-3-fluorobenzoate (0.86 g, 2.53 mmol) and H-Glu(OtBu)-NH_2 HCl (0.845 g, 3.54 mmol) in acetonitrile (15 mL) was added DIPEA (1.325 mL, 7.59 mmol). The reaction mixture was heated at 85 °C for 15 h. Volatiles were removed under reduced pressure and purified by flash chromatography (SiO_2, 40 g column, 0-10% MeOH/DCM) to afford *tert*-butyl (4S)-5-amino-4-(5-bromo-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (0.23 g, 21 % yield) as a pale yellow solid. LCMS (Method A): retention time 1.19 min, $[M+23H]^+$ 451.3, 453.4.

Intermediate 16E: *tert*-Butyl (4*S*)-5-amino-4-(5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate

To a stirred solution of *tert*-butyl (4*S*)-5-amino-4-(5-bromo-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (350 mg, 0.815 mmol) in 1,4-dioxane (20 mL), was added 3,4-dimethyl-6-(trimethylstannyl) pyridin-2-amine (232 mg, 0.815 mmol). The mixture was purged with argon for five minutes and bis(triphenylphosphine)palladium(II) chloride (57.2 mg, 0.082 mmol) was added. The reaction mixture was heated at 100 °C for 16 h, cooled to room temperature and diluted with ethyl acetate. The mixture was washed with brine solution and the organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum afforded the crude compound. The crude compound was purified by flash chromatography (SiO_2, 24 g column, 0-5%

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MeOH/DCM), combined product fractions were concentrated under reduced pressure to afford *tert*-butyl (4*S*)-5- amino-4-(5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (270 mg, 70.4 % yield) as a pale brown solid. LCMS (Method A): retention time 1.64 min, $[M+H]^+$ 471.2 ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (t, J=6.9 Hz, 1H), 7.59 (s, 1H), 7.47 (br s, 1H), 7.30-7.13 (m, 2H), 6.90 (s, 1H), 5.74 (br d, J=5.3 Hz, 2H), 4.89 (q, J=6.1 Hz, 1H), 4.50-4.37 (m, 1H), 2.31-2.19 (m, 6H), 2.04 (s, 3H), 1.55-1.45 (m, 3H), 1.37 (d, J=2.9 Hz, 9H).

Examples 16 and 17:

To a stirred solution of *tert*-butyl (4S)-5-amino-4-(5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl- 1-oxoisoindolin-2-yl)-5-oxopentanoate (270 mg, 0.574 mmol) in acetonitrile (10 mL), PTSOH (218 mg, 1.148 mmol) was added. The reaction mixture was heated at 120 °C under microwave irradiation for 30 minutes. Volatiles were removed under reduced pressure and the resulting crude product was purified via prep-HPLC using polar organic method (CELLULOSE-2 [250 x 4.6 mm], 10 mM ammonium acetate in MeOH, Flow: 23 mL/min (Isocratic gradient)), the first eluting isomer fractions at 9.73 min retention time were concentrated to dryness and lyophilized to give 3-(*(S)*-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (60 mg, 27% yield). LCMS (Method G): retention time 1.83 min, $[M+H]^+$ 397.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10-10.87 (m, 1H), 8.01 (t, J=7.3 Hz, 1H), 7.57 (d, J=8.0 Hz, 1H), 6.92 (d, J=2.0 Hz, 1H), 5.78 (s, 2H), 5.01 (q, J=6.5 Hz, 1H), 4.73 (br dd, J=12.3, 4.8 Hz, 1H), 2.82-2.67 (m, 1H), 2.64-2.55 (m, 2H), 2.23 (s, 3H), 2.04 (s, 3H), 1.54 (d, J=7.0 Hz, 3H). The second eluted peak fractions at 13.96 min retention time were concentrated to dryness and lyophilized to give 3-((*R*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (70 mg, 31% yield). LCMS (Method G): retention time 1.84 min, $[M+H]^+$ 397.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.07-10.89 (m, 1H), 8.01 (t, J=7.1 Hz, 1H), 7.58 (d, J=8.0 Hz, 1H), 6.91 (s, 1H), 5.79 (s, 2H), 4.89 (q, J=6.5 Hz, 1H), 4.80 (dd, J=12.6, 5.1 Hz, 1H), 2.94-2.78 (m, 1H), 2.73-2.59 (m, 2H), 2.22 (s, 3H), 2.03 (s, 3H), 1.51 (d, J=6.8 Hz, 3H).

Method A: ACQUITY UPLC® BEH C18 (3.0 x 50 mm) 1.7 μm; Mobile Phase A: 95:5 water:acetonitrile with 2.5 mM NH_4OAc; Mobile Phase B: 5:95 water:acetonitrile

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with 2.5 mM NH_4OAc; Temperature: 40 °C; Gradient: 20 %B to 100 %B over 2 min; flow: 0.7 mL/min; Detection: MS and UV (220 nm).

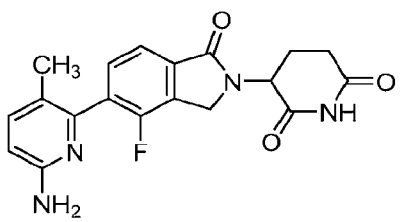
Method B: Column: XBridge BEH XP C18 (50 x 2.1) mm, 2.5 μm; Mobile Phase A: 95:5 water:acetonitrile with 10 mM NH_4OAc; Mobile Phase B: 5:95 water:acetonitrile with 10 mM NH_4OAc; Temperature: 50 °C; Gradient: 0 %B to 100 %B over 3 min; Flow: 1.1 mL/min; Detection: MS and UV (220 nm).

Method D: Column-Kinetex XB-C18 (75 X 3 mm-2.6 μm); Mobile Phase A: 5 mM NH_4COOH in water; Mobile Phase B: Acetonitrile; Gradient: 10 %B to 50 %B over 3 min, Flow: 1.0 mL/min; 50 %B to 100 %B up to 4.1 min, Flow: 1.0 mL/min; hold till 4.5 min; 4.5 min to 5.0 min 90 %B Flow: 1.5 mL/min; Detection: MS and UV (220 nm).

Method G: Column-Kinetex XB-C18 (75 X 3 mm-2.6 μm); Mobile Phase A: 5 mM NH_4CO_2H in water; Mobile Phase B: Acetonitrile; Gradient: 20 %B to 100 %B over 4 min, Flow: 1.0 mL/min; hold to 4.6 min, Flow: 1.5 mL/min; hold till 4.7 min ; 4.7 min to 5.0 min 20 %B, Flow: 1.0 mL/min; Detection: MS and UV (220 nm).

EXAMPLE 18

3-(5-(6-Amino-3-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)piperidine-2,6-dione



(18)

Example 18 was synthesized from 6-chloropyridin-2-amine and Intermediate 3B using General Procedure 2. The crude product was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 0.98 min, $[M+H]^+$ 369.1; ^1H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 7.77 (br d, J = 7.6 Hz, 2H), 7.74-7.68 (m, 1H), 6.93-6.87 (m, 1H), 5.17 (dd, J = 13.3, 5.1 Hz, 1H), 4.67 (d, J = 17.6 Hz, 1H), 4.55-4.47 (m, 1H), 3.01-2.89 (m, 2H), 2.67-2.61 (m, 1H), 2.47-2.39 (m, 1H), 2.04 (s, 3H).

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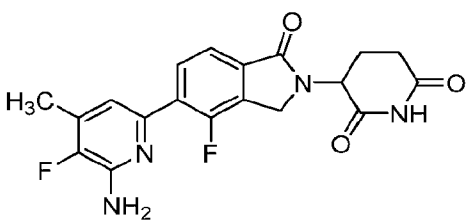
General Procedure 4:

A mixture of aryl halide (1 eq.), aryl boronic acid pinacol ester (1.0 eq.), potassium carbonate (1.5 eq.), dioxane (4 mL/mmol) and water (0.4 mL/mmol) was purged with argon for 5 min at room temperature. [1,1'-Bis(diphenylphosphino) ferrocene]palladium(II) dichloride dichloromethane complex (0.05 eq.) was added and the reaction mixture was heated at 110 °C for 2 h. The reaction mixture was cooled to room temperature, diluted with EtOAc and filtered through celite pad. The filtrate was washed with brine solution, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography. The isolated product was dissolved in acetonitrile, pTSA•H_2O (2 eq.) was added and the mixture was heated at 120 °C for 1.5 h in a microwave reactor. The reaction mixture was cooled to room temperature and concentrated under reduced pressure and the crude product was purified by prep-HPLC to afford the desired product.

EXAMPLE 19

3-(5-(6-Amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)piperidine-2,6-dione



(19)

Intermediates 19A and 19B: 6-Bromo-3-fluoro-4-methylpyridin-2-amine and 6-bromo-5-fluoro-4-methylpyridin-2-amine

To a solution of 6-bromo-4-methylpyridin-2-amine (2.6 g, 13.90 mmol) in chloroform (100 mL) and water (100 mL) was added Selectfluor (2.462 g, 6.95 mmol). The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was diluted with DCM (200 mL), washed with brine solution, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography (SiO_2, 80 g column, 0-30% EtOAc/pet ether) to afford Intermediate 19A: 6-bromo-3-fluoro-4-methylpyridin-2-amine (600 mg, 19.6 % yield); LCMS

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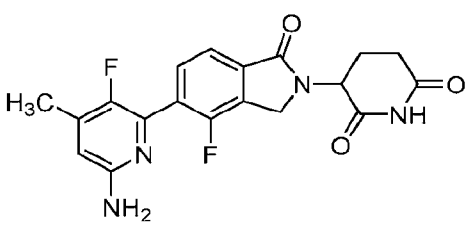
(Method A): retention time 1.21 min, $[M+H]^+$ 207.1. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.60 (d, J = 4.2 Hz, 1H), 6.55 (s, 2H), 2.13 (d, J = 1.9 Hz, 3H); and Intermediate 19B: 6-bromo-5-fluoro-4-methylpyridin-2-amine (500 mg, 4.4 % yield); LCMS (Method A): retention time 1.14, $[M+H]^-$ 207.1; ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 6.25 (d, J = 4.2 Hz, 1H), 4.52-4.22 (m, 2H), 2.23 (d, J = 1.1 Hz, 3H).

Example 19:

Example 19 was synthesized by using General Procedure 4 with 6-bromo-3-fluoro-4-methylpyridin-2-amine and *tert*-butyl (S)-5-amino-4-(4-fluoro-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (synthesized by procedures shown in Example 2 starting with 4-bromo-3-fluoro-2-methylbenzoic acid). The crude product was purified by preparative LCMS (Column: YMC EXRS 250 mm x 21 mm, Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min LCMS (Method D): retention time 1.56 min, $[M+H]^+$ 387.15; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H), 8.09-7.92 (m, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.32-6.78 (m, 1H), 5.14(dd, J = 13.8, 5.8 Hz,1H), 4.74-4.33 (m, 2H), 2.98-2.86 (m, 1H), 2.68-2.56 (m, 2H), 2.24 (s, 3H), 22.09-2.05(m, 1H).

EXAMPLE 20

3-(5-(6-Amino-3-fluoro-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)piperidine-2,6-dione



(20)

Example 20 was synthesized by using General Procedure 4 with 6-bromo-5-fluoro-4-methylpyridin-2-amine and *tert*-butyl (S)-5-amino-4-(4-fluoro-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (synthesized by procedures shown in Example 2 starting with 4-bromo-3-fluoro-2-methylbenzoic acid).

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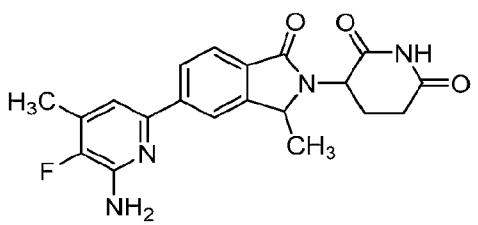
The crude product was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.03 min, $[M+H]^+$ 387.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 7.74-7.57 (m, 2H), 6.46 (d, J = 4.5 Hz, 1H), 5.97 (s, 2H), 5.16 (dd, J = 5.0, 13.3 Hz, 1H), 4.65 (d, J = 17.5Hz, 1H), 4.52-4.42 (m, 1H), 3.03-2.86 (m, 1H), 2.68-2.59 (m, 1H), 2.49-2.41 (m, 1H), 2.21 (s, 3H), 2.12-1.99 (m, 1H).

EXAMPLES 21 AND 22

3-(5-(6-Amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione



(21-22)

Intermediate 21A: *tert*-Butyl (4*S*)-5-amino-4-(3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate

A mixture of Intermediate 8D (1.0 g, 2.43 mmol), potassium acetate (0.239 g, 2.43 mmol) and Bispin (0.617 g, 2.43 mmol) in anhydrous DME (15 mL) was purged with argon for 10 min at room temperature. $PdCl_2$(dppf)-CH_2Cl_2 adduct (0.159 g, 0.195 mmol) was added under argon atmosphere. The vial was sealed and the mixture was heated at 90 °C for 12 h. The reaction mixture was cooled to room temperature, filtered through celite pad and the filtrate was concentrated under reduced pressure. The residue was dissolved in diethyl ether and filtered through celite pad. The filtrate was concentrated under reduced pressure to afford *tert*-butyl (4S)-5-amino-4-(3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (1.0 g, 90 % yield). LCMS (Method A): retention time 1.70 min, $[M+H]^+$ 459.1.

Preparation of 21B and 22B: *tert*-Butyl (4*S*)-5-amino-4-(5-(6-amino-5-fluoro-4-

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methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate

To a solution of *tert*-butyl (4*S*)-5-amino-4-(3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (0.241 g, 0.527 mmol) in dioxane (10 mL) was added 6-bromo-3-fluoro-4-methylpyridin-2-amine (0.09 g, 0.44 mmol) followed by sodium bicarbonate (0.5 M solution, 2.195 mL, 1.097 mmol). The reaction mixture was purged with nitrogen for 15 min at room temperature, bis(triphenylphosphine)palladium(II) chloride (0.031 g, 0.044 mmol) was added under nitrogen and heated at 100 °C for 12 h. The reaction mixture was cooled to room temperature, filtered through celite pad and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 24 g column, 50-100% EtOAc/DCM) to afford *tert*-butyl (4S)-5-amino-4-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (150 mg) as a mixture of diastereomers. The diastereomers were separated by SFC (Column Chiral Pak IG (250*4.6) mm, 5 μm; %CO_2: 45%; % co solvent: 5 mM ammonium acetate in methanol and acetonitrile (1:1); flow: 4 g/min; temperature: 30 °C; UV: 237 nm), first eluting isomers fractions at 3.4 min retention time were concentrated to dryness to afford *tert*-butyl (4*S*)-5-amino-4-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (40 mg, 20 % yield). LCMS (Method A): retention time 1.41, $[M+H]^+$ 457.1 and the second peak fractions eluted at 4.6 min retention time were concentrated to afford *tert*-butyl (4S)-5-amino-4-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (50 mg, 25 % yield). LCMS (Method A): retention time 1.40, $[M+H]^+$ 457.4.

Example 21:

To a stirred solution of (*4S*)-5-amino-4-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (0.04 g, 0.088 mmol) in acetonitrile (10 mL), benzene sulfonic acid (0.028 g, 0.175 mmol) was added. The reaction mixture was heated at 120 °C under microwave irradiation for 30 minutes. The reaction mixture was cooled to room temperature, concentrated under reduced pressure and purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10%

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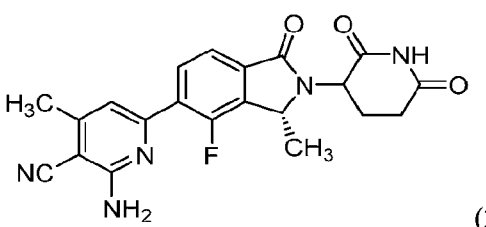
B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.19 min, $[M+H]^+$ 383.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.14 (s, 1H), 8.07 (dd, *J* = 1.1, 8.1 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 4.4 Hz, 1H),4.82-4.67 (m, 2H), 2.93-2.79 (m, 1H), 2.71-2.59 (m, 2H), 2.26 (s, 3H), 2.05-1.96 (m, 1H), 1.48 (d, *J* = 6.6 Hz, 3H).

Example 22:

To a stirred solution of (*4S*)-5-amino-4-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl)-5-oxopentanoate (0.04 g, 0.088 mmol) in acetonitrile (10 mL), benzene sulfonic acid (0.028 g, 0.175 mmol) was added. The reaction mixture was heated at 120 °C under microwave irradiation for 30 minutes. The reaction mixture was cooled to room temperature, concentrated under reduced pressure and the residue was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.19 min, $[M+H]^+$ 383.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 1H), 8.16 (s, 1H), 8.07 (dd, *J* = 1.1, 8.0 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 4.3 Hz, 1H),6.26 (br s, 2H), 4.83 (q, *J* = 6.6 Hz, 1H), 4.74 (br dd, *J* = 4.3, 11.3 Hz, 1H), 2.83-2.71 (m, 1H), 2.64-2.55 (m, 2H), 2.25 (d, *J* = 1.5 Hz, 3H), 2.09 -1.96 (m, 1H), 1.50 (d, *J* = 6.6 Hz, 3H).

EXAMPLE 23

2-Amino-6-((3*R*)-2-(2,6-dioxopiperidin-3-yl)-4-fluoro-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile



(23)

Example 23 was synthesized from 2-amino-6-chloro-4-methylnicotinonitrile and *tert*-butyl *(S)*-5-amino-4-(*(R)*-4-fluoro-3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-

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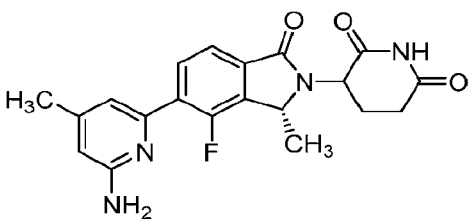
dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (synthesized by procedures shown in Example 2 starting with 4-bromo-3-fluoro-2-methylbenzoic acid) using general procedure 4. The crude product was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.19 min, $[M+H]^+$ 408.0; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.17-7.85 (m, 1H), 7.73-7.52 (m, 1H), 7.05 (s, 1H), 6.99 (s, 2H), 5.08-4.89 (m, 1H), 4.86-4.72 (m, 1H), 2.90-2.75 (m, 1H), 2.74-2.58 (m, 2H), 2.42 (s, 3H), 2.12-2.00 (m, 1H), 1.57-1.47 (m, 3H).

EXAMPLE 24

3-((*R*)-5-(6-Amino-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione



(24)

Example 24 was synthesized from 6-chloro-4-methylpyridin-2-amine and *tert*-butyl *(S)*-5-amino-4-(*(R)*-4-fluoro-3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (synthesized by procedures shown in Example 2 starting with 4-bromo-3-fluoro-2-methylbenzoic acid) using General Procedure 4. The crude product was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.20 min, $[M+H]^+$ 383.2; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (d, J = 0.8 Hz, 1H), 8.01-7.84 (m, 1H), 7.71 (d, J = 7.8 Hz, 1H), 6.96 (br d, J = 1.0 Hz, 1H), 6.71-6.54 (m, 1H), 4.99-4.91 (m, 1H), 4.87-4.78 (m, 1H), 2.86 (br dd, J = 4.4, 3.4 Hz, 1H), 2.75-2.62 (m, 2H), 2.47-2.44 (m, 1H), 2.34 (s, 4H), 1.94 (d, J = 16.0 Hz, 1H), 1.53 (d, J = 6.8 Hz,

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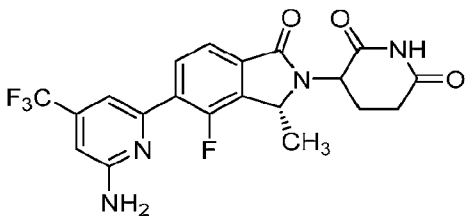
3H).

EXAMPLE 25

3-((*R*)-5-(6-Amino-4-(trifluoromethyl)pyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione

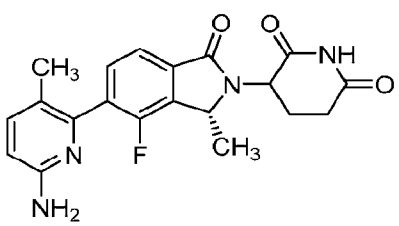


(25)

Example 25 was synthesized from 6-chloro-4-(trifluoromethyl)pyridin-2-amine and *tert*-butyl *(S)*-5-amino-4-(*(R)*-4-fluoro-3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (synthesized by procedures shown in Example 2 starting with 4-bromo-3-fluoro-2-methylbenzoic acid) using general procedure 4. The crude product was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.39 min, $[M+H]^+$ 438.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H), 8.13-7.95 (m, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.15 (s, 1H), 6.79 (s, 2H), 4.98-4.88 (m, 1H), 4.86-4.77 (m, 1H), 2.85 (br s, 1H), 2.93-2.78 (m, 1H), 2.72-2.59 (m, 2H), 2.08-2.00 (m, 1H), 1.52 (d, J = 6.8 Hz, 3H).

EXAMPLE 26

3-((*R*)-5-(6-Amino-3-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione



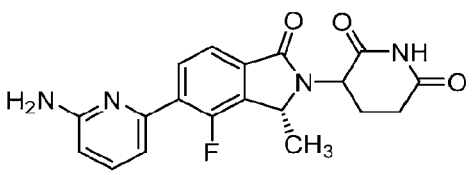
(26)

Example 26 was synthesized from 6-chloro-5-methylpyridin-2-amine and *tert*-

butyl *(S)*-5-amino-4-(*(R)*-4-fluoro-3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (synthesized by procedures shown in Example 2 starting with 4-bromo-3-fluoro-2-methylbenzoic acid) using general procedure 4. The crude product was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.07 min, $[M+H]^+$ 383.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.90-7.77 (m, 1H), 7.74-7.61 (m, 2H), 6.86 (br dd, J = 5.5, 3.9 Hz, 1H), 4.96 (q, J = 6.5 Hz, 1H), 4.84 (dd, J = 12.6, 5.1 Hz, 1H), 3.01-2.82 (m, 2H), 2.71-2.60 (m, 2H), 2.12-2.01 (m, 5H), 1.51 (d, J = 6.6 Hz, 3H).

EXAMPLE 27

3-(*(R)*-5-(6-Aminopyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione



(27)

Example 27 was synthesized from 6-chloropyridin-2-amine and *tert*-butyl *(S)*-5-amino-4-(*(R)*-4-fluoro-3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) isoindolin-2-yl)-5-oxopentanoate (synthesized by procedures shown in Example 2 starting with 4-bromo-3-fluoro-2-methylbenzoic acid) using General Procedure 4. The crude product was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.01 min, $[M+H]^+$ 369.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (t, J = 7.1 Hz, 1H), 7.81-7.62 (m, 2H), 7.22 (s, 1H), 7.09 (s, 1H), 7.04 (br d, J = 7.0 Hz, 1H), 6.97 (s, 1H), 6.78-6.72 (m, 1H), 4.93 (q, J = 6.5 Hz, 1H), 4.82 (dd, J = 12.4, 5.3 Hz, 1H), 2.91-2.83 (m, 1H), 2.75-2.60 (m, 2H), 2.36-2.32

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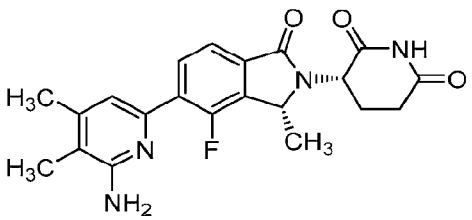
(m, 1H), 1.52 (d, J = 6.8 Hz, 3H).

EXAMPLE 28

(R)-3-(*(R)*-5-(6-Amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione

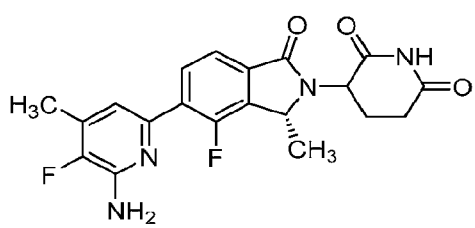


(28)

To a stirred solution of Example 17 (250 mg, 0.531 mmol) in acetonitrile (4 mL) was added TFA (1 mL, 13.81 mmol). The reaction mixture was heated at 90 °C for 2 h. The reaction mixture was concentrated under reduced pressure and the crude product was purified by preparative SFC (Chiral Pak IC (250 X 50) mm, 5 µm; %CO_2: 50%; % co solvent: 50 % of 5 mM ammonium acetate in ACN:MEOH (50:50); flow: 300.0 g/min; temperature: 40 °C; UV: 240 nm), first eluting isomers fractions at 6.9 min retention time were concentrated to dryness and lyophilized to afford *(R)*-3-(*(R)*-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (15 mg, 7 % yield). LCMS (Method D): retention time 1.28 min, $[M+H]^+$ 397.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97-10.89 (m, 1H), 7.99 (br d, J = 6.8 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 6.91 (s, 1H), 5.80 (s, 2H), 5.00 (q, J = 6.7 Hz, 1H), 4.76-4.67 (m, 1H), 2.82-2.70 (m, 1H), 2.67-2.55 (m, 2H), 2.22 (s, 3H), 2.03 (s, 4H), 1.53 (d, J = 6.8 Hz, 3H).

EXAMPLE 29

3-((R)-5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione



(29)

Example 29 was synthesized from 6-chloro-3-fluoro-4-methylpyridin-2-amine and *tert*-butyl *(S)*-5-amino-4-(*(R)*-4-fluoro-3-methyl-1-oxo-5-(4,4,5,5-tetramethyl-1,3,2-

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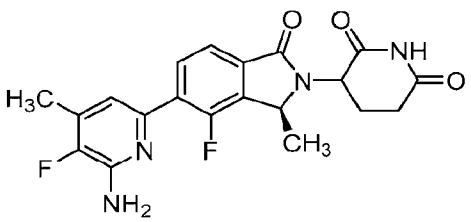
dioxaborolan-2-yl)isoindolin-2-yl)-5-oxopentanoate (synthesized by procedures shown in Example 2 starting with 4-bromo-3-fluoro-2-methylbenzoic acid) using General Procedure 4. The crude product was purified by preparative LCMS (Column: Waters XBridge C18, 150 mm x 19 mm, 5-µm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: a 0-minute hold at 10% B, 10-30% B over 20 minutes, then a 5-minute hold at 100% B; Flow Rate: 20 mL/min. LCMS (Method B): retention time 1.18 min, $[M+H]^+$ 401.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 1H), 7.95 (t, J = 7.1 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 6.93 (d, J = 3.3 Hz, 1H), 4.90 (q, J = 6.6 Hz, 1H), 4.80 (dd, J = 4.9, 12.9 Hz, 1H), 2.89-2.78 (m, 1H), 2.70-2.60 (m, 2H), 2.24 (s, 3H), 2.07-1.97 (m, 1H), 1.51 (d, J = 6.5 Hz, 3H).

EXAMPLE 30

3-((*S*)-5-(6-Amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione



(30)

Preparation of 30A: 3-Fluoro-4-methyl-6-(trimethylstannyl)pyridin-2-amine

A stirred solution of 6-bromo-3-fluoro-4-methylpyridin-2-amine (0.05 g, 0.24 mmol) and hexamethylditin (0.076 mL, 0.366 mmol) in toluene (3 mL) was purged with argon for five minutes followed by the addition of [1,1'-bis(di-*tert*-butylphosphino) ferrocene]dichloropalladium(II) (0.016 g, 0.024 mmol). The reaction mixture was stirred for 15 h at 100 °C, cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure to afford 3-fluoro-4-methyl-6-(trimethylstannyl)pyridin-2-amine (69 mg, 82 % yield). LCMS (Method A): retention time 1.68, $[M+H]^+$ 289.2.

Example 30:

The Stille coupling and cyclization were accomplished by following General Procedure 2 with Intermediate 30A and Intermediate 16D. LCMS (Method D): retention

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time 1.17 min, $[M+H]^+$ 401.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 7.94 (t, J = 7.3 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 3.3 Hz, 1H), 6.31 (br s, 2H), 5.01 (q, J = 6.6 Hz, 1H), 4.72 (dd, J = 4.9, 11.9 Hz, 1H), 2.79-2.67 (m, 1H), 2.64-2.55 (m, 2H), 2.24 (s, 3H), 2.07-1.98 (m, 1H), 1.53 (d, J =6.8 Hz, 3H).

BIOLOGICAL ASSAYS

The pharmacological properties of the compounds of this invention may be confirmed by a number of biological assays. The exemplified biological assays, which follow, have been carried out with compounds of the invention.

Helios Cellular Degradation Assay

Jurkat cells were plated at 80,000 cells/well in 40 μL RPMI + 10% FBS in a 384 well cell culture plate prior to using acoustic dispensing technology for adding compound of interest. Cell cultures were incubated for 72 h at 37 °C and 5% CO_2. In order to facilitate analysis, cell cultures were spun down at 200 rpm for 5 min and the supernatant was discarded. After shaking the plate to dislodge the cell pellet, cells were resuspended in 50 μL of Fixation Buffer (eBioScience FoxP3 buffer set 00-5523-00) for 60 min at room temperature. After centrifuging and discarding the supernatant, cells were permeabilized with 50 μL of Permeabilization buffer (eBioScience FoxP3 buffer set 00-5523-00) for 10 min at room temperature. Following permeabilization, cells were spun down and the supernatant was replaced with 20 μL fluorescently labelled antibodies against Helios, Ikaros and Aiolos or corresponding Isotype controls in 1x Permeabilization buffer (Ikaros-Alexa488 [Biolegend, Cat #368408 , 1:50], Helios-PE [CST, Cat #29360, 1:50], Aiolos-Alexa647 [Biolegend, Cat #371106Biolegend, 1:25]) and staining reactions were incubated for 1 h at room temperature; protected from light. Subsequently, 30 μL of 1x Permeabilization buffer was added prior to centrifuging the cells and discarding the supernatant. Stained cells were resuspended in 25 μL of flow cytometry staining buffer (PBS + 0.2%BSA) and analyzed using an Intellicyt Ique Plus flow cytometer.

TABLE 4

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Ex. No.	Helios Jurkat IC_{50} (uM)	Ikaros Jurkat IC_{50} (uM)
1	1.4	>10
3	0.003	>10
7	0.001	>10
9	0.003	>10
10	0.31	>10
11	0.006	>10
12	0.033	>10
13	2.8	>10
14	1.0	>10
15	0.057	>10
16	0.56	>10
17	0.007	>10
19	0.0035	>10
20	490	>10
21	0.0058	>10
22	0.20	>10
23	0.035	>10
24	0.16	>10
25	1.3	>10
29	0.024	>10
30	0.70	>10

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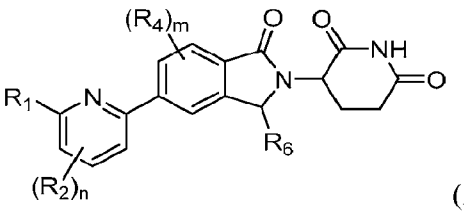
CLAIMS

What is claimed is:

1. A compound of Formula (I):



(I)

or a salt thereof, wherein:

R_1 is $-NH_2$ or $-NH(CH_3)$;

each R_2 is independently F, Cl, –CN, C_{1-4} alkyl, $-CH_2F$, $-CHF_2$, $-CF_3$, $-OCH_3$, or cyclopropyl;

each R_4 is independently F, Cl, $-CH_3$, $-CH_2F$, $-CHF_2$, $-CF_3$, or $-OCH_3$;

R_6 is hydrogen, C_{1-2} alkyl, or C_{1-2} fluoroalkyl;

m is zero, 1, 2, or 3; and

n is zero, 1, 2, or 3;

with the proviso that when R_6 is hydrogen, m is 1, 2, or 3.

2. The compound according to claim 1 or a salt thereof, wherein R_1 is $-NH_2$.

3. The compound according to claim 1 or a salt thereof, wherein R_1 is $-NH(CH_3)$.

4. The compound according to any one of claims 1-3 or a salt thereof, wherein each R_2 is independently F, –CN, $-CH_3$, or $-CF_3$.

5. The compound according to any one of claims 1-5 or a salt thereof, wherein R_6 is C_{1-2} alkyl, $-CH_2F$, $-CF_2H$, $-CF_3$, or $-CH_2CF_3$.

6. The compound according to any one of claims 1-5 or a salt thereof, wherein R_6 is $-CH_3$.

7. The compound according to any one of claims 1-5 or a salt thereof, wherein:
R_6 is C_{1-2} alkyl, $-CH_2F$, $-CF_2H$, $-CF_3$, or $-CH_2CF_3$; and
m is zero.

8. The compound according to any one of claims 1-5 or a salt thereof, wherein:
R_6 is $-CH_3$; and
m is zero.

9. The compound according to any one of claims 1-5 or a salt thereof, wherein each R_4 is independently F, $-CH_3$, $-CHF_2$, or $-CF_3$.

10. The compound according to any one of claims 1-5 or a salt thereof, wherein:
each R_4 is F, $-CH_3$, $-CHF_2$, or $-CF_3$;
R_6 is hydrogen; and
m is 1 or 2.

11. The compound according to any one of claims 1-5 or a salt thereof, wherein:
each R_4 is F or $-CH_3$;
R_6 is hydrogen; and
m is 1 or 2.

12. The compound according to any one of claims 1-5 or a salt thereof, wherein:
R_4 is F or $-CH_3$;
R_6 is hydrogen; and
m is 1.

13. The compound according to claim 1 or a salt thereof, wherein said compound is:
2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-4-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (1);
2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-6-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (2);

2-amino-6-(2-(2,6-dioxopiperidin-3-yl)-4-fluoro-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (3);

3-(5-(6-amino-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl)piperidine-2,6-dione (4);

3-(5-(6-amino-4-(trifluoroethyl) pyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (5);

3-(4-fluoro-5-(4-methyl-6-(methylamino)pyridin-2-yl)-1-oxoisoindolin-2-yl) piperidine-2,6-dione (6);

3-(5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (7);

3-((*S*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (8);

3-((*R*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (9);

2-amino-6-((3S)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (10);

2-amino-6-((3R)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (11);

3-((S)-5-(4,5-dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (12);

3-((R)-5-(4,5-dimethyl-6-(methylamino)pyridin-2-yl)-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (13);

6-((3S)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2-(methylamino) nicotinonitrile (14);

6-((3R)-2-(2,6-dioxopiperidin-3-yl)-3-methyl-1-oxoisoindolin-5-yl)-4-methyl-2-(methylamino)nicotinonitrile (15);

3-((*S*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (16);

3-((*R*)-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (17);

3-(5-(6-amino-3-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (18);

3-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (19);

3-(5-(6-amino-3-fluoro-4-methylpyridin-2-yl)-4-fluoro-1-oxoisoindolin-2-yl) piperidine-2,6-dione (20);

3-(5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (21-22);

2-amino-6-((3*R*)-2-(2,6-dioxopiperidin-3-yl)-4-fluoro-3-methyl-1-oxoisoindolin-5-yl)-4-methylnicotinonitrile (23);

3-(*(R)*-5-(6-amino-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (24);

3-(*(R)*-5-(6-amino-4-(trifluoromethyl)pyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (25);

3-(*(R)*-5-(6-amino-3-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (26);

3-(*(R)*-5-(6-aminopyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl) piperidine-2,6-dione (27);

(R)-3-(*(R)*-5-(6-amino-4,5-dimethylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione 28);

3-((R)-5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (29); or

3-((*S*)-5-(6-amino-5-fluoro-4-methylpyridin-2-yl)-4-fluoro-3-methyl-1-oxoisoindolin-2-yl)piperidine-2,6-dione (30).

14. A pharmaceutical composition comprising a compound according to any one of claims 1-11 or a pharmaceutically-acceptable salt thereof; and a pharmaceutically acceptable carrier.

15. Use of a compound according to any one of claims 1-11 for the treatment of cancer.

16. The use of claim 13, wherein said cancer is selected from cancer of the colon, gastric, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, cervical cancer, renal cancer, cancer of the head and neck, lymphoma, leukemia and melanoma.

17. A method of decreasing Helios protein levels, Helios activity level, or Helios expression level in the cells comprising contacting said Helios protein with a compound according to any one of claims 1-11, or a pharmaceutically acceptable salt thereof.

18. The method according to Claim 15, wherein Helios protein is the amino acid sequence encoded by SEQ ID NOs: 1, 2, 3, 4, or 5.

19. A method of decreasing Eos protein levels, Eos activity level, or Eos expression level in the cells comprising contacting said Eos protein with a compound according to any one of claims 1-11, or a pharmaceutically acceptable salt thereof.

20. The method according to Claim 17, wherein Eos protein is the amino acid sequence encoded by SEQ ID NOs: 7 or 8.

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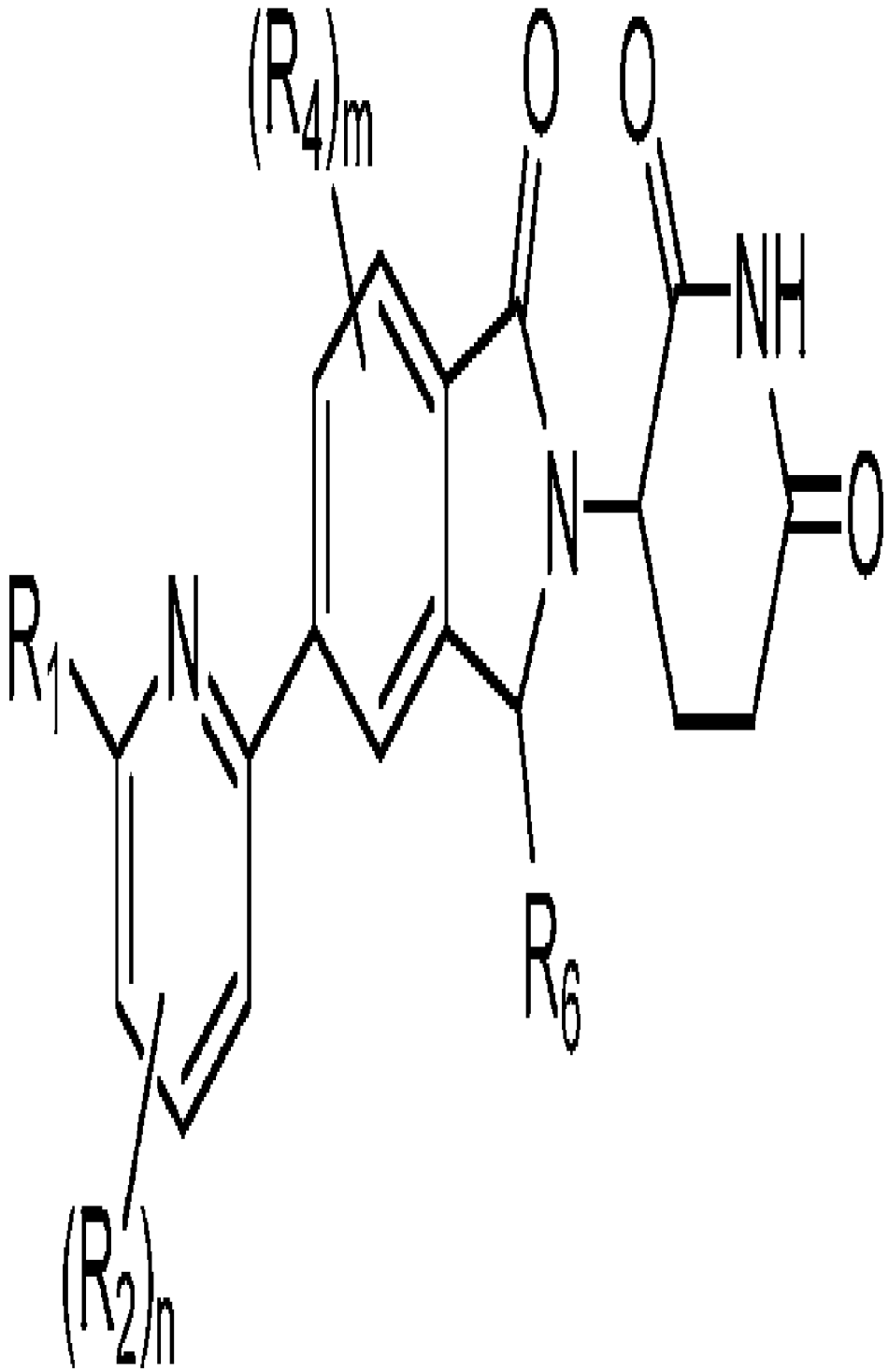

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