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CANADIAN PATENT APPLICATION**

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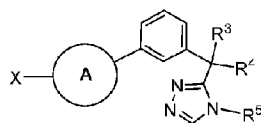
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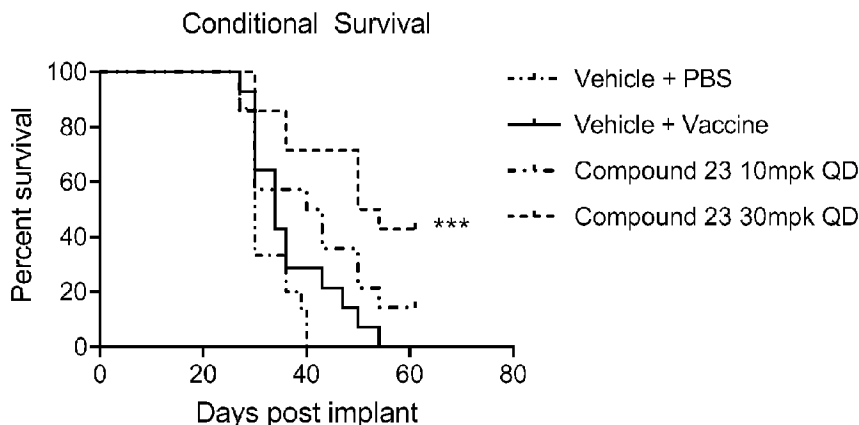
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(54) Titre : COMPOSES BENZYL-TRIAZOLES SUBSTITUES POUR L'INHIBITION DE CBL-B ET AUTRES
UTILISATIONS DE CES COMPOSES
(54) Title: SUBSTITUTED BENZYL-TRIAZOLE COMPOUNDS FOR CBL-B INHIBITION, AND FURTHER USES
THEREOF



(I)

FIG. 1



(57) Abrégé/Abstract:

Compounds of formula (I), compositions, and methods for use in inhibiting the E3 enzyme Cbl-b in the ubiquitin proteasome pathway are disclosed. The compounds, compositions, and methods can be used to modulate the immune system, to treat diseases amenable to immune system modulation, and for treatment of cells in vivo, in vitro, or ex vivo. Also disclosed are pharmaceutical compositions comprising a Cbl-b inhibitor and a cancer vaccine, as well as methods for treating cancer using a Cbl-b inhibitor and a cancer vaccine; and pharmaceutical compositions comprising a Cbl-b inhibitor and an oncolytic virus, as well as methods for treating cancer using a Cbl-b inhibitor and an oncolytic virus.

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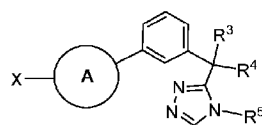
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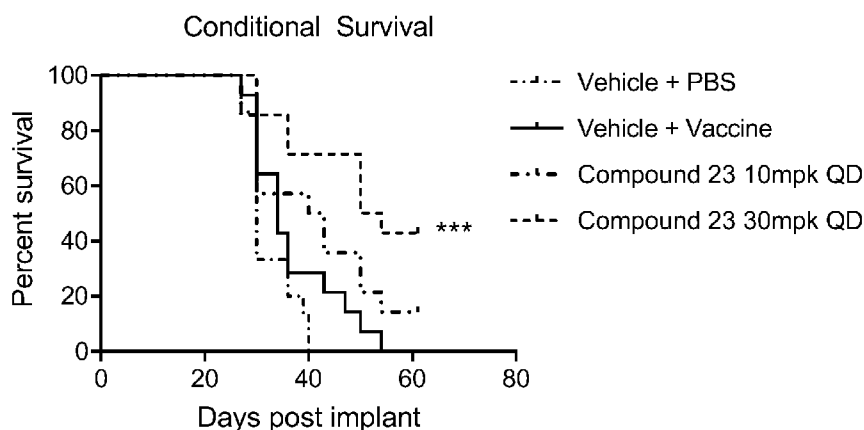
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(54) Title: SUBSTITUTED BENZYL-TRIAZOLE COMPOUNDS FOR CBL-B INHIBITION, AND FURTHER USES THEREOF



(I)

FIG. 1



(57) Abstract: Compounds of formula (I), compositions, and methods for use in inhibiting the E3 enzyme Cbl-b in the ubiquitin proteasome pathway are disclosed. The compounds, compositions, and methods can be used to modulate the immune system, to treat diseases amenable to immune system modulation, and for treatment of cells *in vivo*, *in vitro*, or *ex vivo*. Also disclosed are pharmaceutical compositions comprising a Cbl-b inhibitor and a cancer vaccine, as well as methods for treating cancer using a Cbl-b inhibitor and a cancer vaccine; and pharmaceutical compositions comprising a Cbl-b inhibitor and an oncolytic virus, as well as methods for treating cancer using a Cbl-b inhibitor and an oncolytic virus.

[Continued on next page]



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**SUBSTITUTED BENZYL-TRIAZOLE COMPOUNDS
FOR CBL-B INHIBITION, AND FURTHER USES THEREOF**

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims the benefit under 35 U.S.C. § 119 of U.S. provisional application numbers: 62/866,914, filed June 26, 2019; 62/880,285, filed July 30, 2019; 62/888,845, filed August 19, 2019; and 62/888,870, filed August 19, 2019, wherein the contents of each are incorporated herein by reference in their entirety.

FIELD

[2] Provided herein are compounds and compositions for inhibition of the Cbl-b enzyme and methods of use thereof in modulating the immune system, treatment of diseases, and treatment of cells *in vivo*, *in vitro*, or *ex vivo*. Also provided herein are pharmaceutical compositions, kits, and methods of treating cancer comprising a combination of an inhibitor of the Cbl-b enzyme and a cancer vaccine; and pharmaceutical compositions, kits, and methods of treating cancer comprising a combination of an inhibitor of the Cbl-b enzyme and an oncolytic virus.

BACKGROUND

[3] The ubiquitin proteasome pathway is a complex system involved in the regulation of protein function and catabolism. Proteins in eukaryotic cells are conjugated with ubiquitin, a 76 amino acid, 8.5 kilodalton protein. This conjugation, known as ubiquitination, results in altered function or degradation of the target protein. Ubiquitination of the target protein occurs via a coupled series of reactions involving ubiquitin and a set of enzymes known as E1, E2, and E3 enzymes. Ubiquitin is activated by the ubiquitin-activating enzyme, or E1 enzyme. Ubiquitin is then transferred to a ubiquitin-conjugating enzyme, or E2 enzyme. Finally, a ubiquitin ligase, or E3 enzyme, promotes the transfer of ubiquitin from the E2 enzyme to the target protein. Polyubiquitination of the target protein predominantly serves as a signal leading to degradation of the ubiquitin-conjugated protein by the proteasome, where it undergoes proteolysis. Ubiquitination by E3 ligases can also result in altered protein activity, interactions, or localization. Ubiquitination regulates diverse biology including cell division, DNA repair, and cellular signaling.

[4] The synthesis and degradation of proteins in the cell is critical for cell cycle regulation, cell proliferation, apoptosis, and many other cellular processes. Thus, the ability to modulate the ubiquitin proteasome pathway offers a wealth of opportunities to intervene in disease processes. Mechanisms for intervention can include enhanced degradation of oncogene products, reduced degradation of tumor-suppressor proteins, modulation of immune cell response, and modulation of anti-tumor immune responses.

[5] Therapeutic cancer vaccines have been evaluated in numerous clinical trials. However, only two therapeutic cancer vaccines have been licensed for use in the United States. In particular, the Bacillus of Calmette and Guerin strain of *Mycobacterium bovis* has been approved for treatment of bladder cancer, and an ex vivo-activated, autologous cell vaccine has been approved for treatment of prostate cancer. Even so, the response rates and overall survival of patients treated with cancer vaccines are considerably lower than desirable. Thus, what is needed in the art are methods of improving the efficacy of cancer vaccines.

[6] Although numerous clinical trials employing an oncolytic virus to treat cancer have been conducted, only one oncolytic virus has been licensed for use in the United States and Europe. In particular, talimogene laherparepvec is a genetically modified herpes simplex virus approved for treatment of melanoma. However, even talimogene laherparepvec has not been shown to improve overall survival or to benefit patients with visceral metastases. Thus, what is needed in the art are methods of improving the efficacy of oncolytic virus therapy.

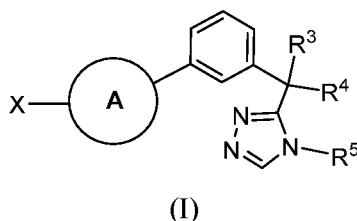
[7] Approximately 35 E2 enzymes and over 500 E3 enzymes are encoded in the human genome. Casitas B-lineage lymphoma proto-oncogene-b (Cbl-b) is an E3 ubiquitin ligase that negatively regulates T-cell activation (Wallner *et al.*, Clin Dev Immunol, 2012: 692639). Discovery of agents that modulate E2 or E3 enzymes accordingly provides the potential for therapies directed against disease processes involving a particular E2 or E3 enzyme. This patent application is directed to agents that inhibit one such E3 enzyme, Casitas B-lineage lymphoma proto-oncogene-b (Cbl-b); agents that inhibit Cbl-b, for use in combination with cancer vaccines, and to pharmaceutical compositions comprising Cbl-b inhibitors and cancer vaccines; and agents that inhibit Cbl-b, for use in combination with oncolytic viruses, and to pharmaceutical compositions comprising Cbl-b inhibitors and oncolytic viruses.

SUMMARY

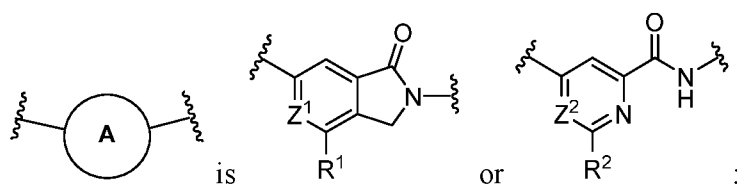
[8] Disclosed herein are compounds and compositions for inhibition of the Cbl-b enzyme and methods of use thereof in modulating the immune system, treatment of diseases, and treatment of cells *in vivo*, *in vitro*, or *ex vivo*. Also disclosed herein are methods for use of a

Cbl-b inhibitor in treating cancer. In brief, the Cbl-b inhibitor may be administered to an individual with cancer, either alone or as part of a combination therapy with one or more of an immune checkpoint inhibitor, an anti-neoplastic agent, and radiation therapy. Additionally, cells treated *in vivo* and/or *in vitro* with a compound or composition as disclosed herein may be used in adoptive cell therapy for treating cancer.

[9] Disclosed herein are compounds of Formula (I):



or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing, wherein:



Z¹ is CH or N;

Z² is CH or N;

R¹ is -CF₃ or cyclopropyl;

R² is -CF₃ or cyclopropyl;

R³ is H, C₁-C₂ alkyl, or C₁-C₂ haloalkyl;

R⁴ is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, 4- to 8-membered heterocyclyl, or C₃-C₆ cycloalkyl,

wherein the heterocyclyl or cycloalkyl groups are optionally substituted by 1-5 **R⁶** groups;

or **R³** and **R⁴** are taken together with the carbon atom to which they are attached to form C₃-

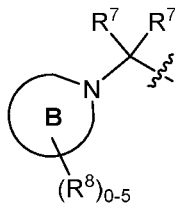
C₅ cycloalkyl or 4- to 6-membered heterocyclyl, each of which is optionally substituted by 1-5 **R⁶** groups;

R⁵ is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₃-C₆ cycloalkyl;

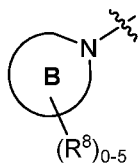
each **R⁶** is independently C₁-C₆ alkyl, halo, hydroxy, -O(C₁-C₆ alkyl), -CN, C₁-C₆ alkyl-CN, C₁-C₆ alkyl-OH, or C₁-C₆ haloalkyl;

or two **R⁶** groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro C₃-C₆ cycloalkyl or spiro 4- to 6-membered heterocyclyl;

X is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, C₁-C₆ alkyl-OH, C₁-C₆ alkyl-CN, C₃-C₆ cycloalkyl



optionally substituted by 1-5 **R**⁸ groups, or



is 4- to 7-membered heterocyclyl or 5- to 8- membered heteroaryl, each of which heterocyclyl or heteroaryl optionally contains 1-2 additional heteroatoms selected from the group consisting of N, O, and S, and each of which heterocyclyl or heteroaryl is optionally substituted by 1-5 **R**⁸ groups;

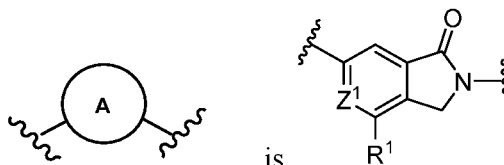
each **R**⁷ is independently H, C₁-C₆ alkyl, C₁-C₆ alkyl-OH, or C₁-C₆ haloalkyl;

or two **R**⁷ groups are taken together with the carbon atom to which they are attached to form a C₃-C₅ cycloalkyl or 3- to 5- membered heterocyclyl; and

each **R**⁸ is independently halo, C₁-C₆ alkyl, C₁-C₆ alkyl-CN, C₁-C₆ alkyl-OH, C₁-C₆ haloalkyl, -CN, oxo, or -O(C₁-C₆ alkyl);

or two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused C₃-C₅ cycloalkyl or 3- to 5-membered heterocyclyl.

[10] In some embodiments,

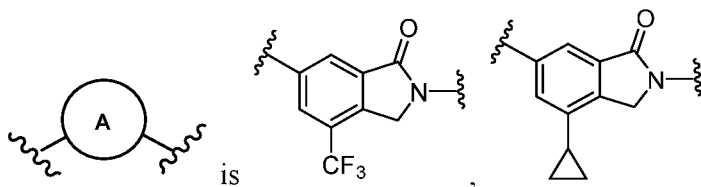


is

. In some

embodiments, **Z**¹ is CH. In some embodiments, **Z**¹ is N.

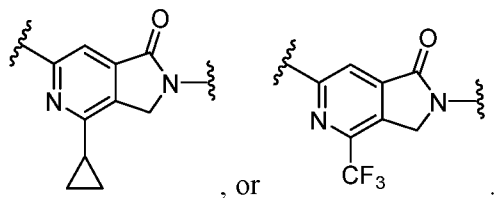
[11] In some embodiments,



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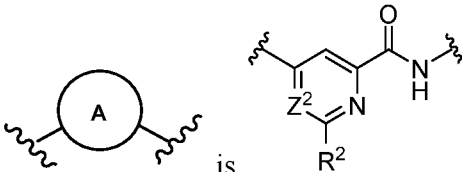
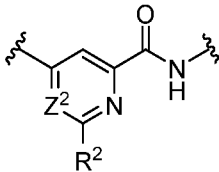
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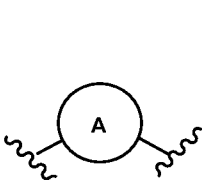
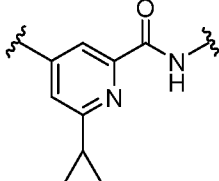
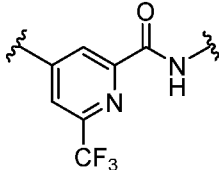
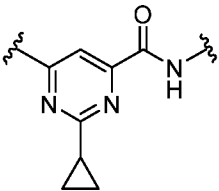
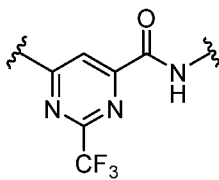
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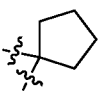
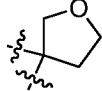
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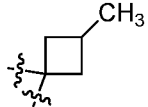
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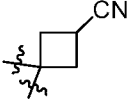
[12] In some embodiments,  is . In some embodiments, Z^2 is CH. In some embodiments, Z^2 is N.

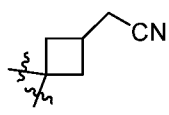
[13] In some embodiments,  is , , , or .

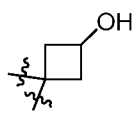
[14] In some embodiments, R^3 is H, C_1 - C_2 alkyl, or C_1 - C_2 haloalkyl; R^4 is H, C_1 - C_3 alkyl, C_1 - C_3 haloalkyl, 4- to 6-membered heterocyclyl, or C_4 - C_5 cycloalkyl, wherein the heterocyclyl or cycloalkyl groups are optionally substituted by 1-3 R^6 groups; or R^3 and R^4 are taken together with the carbon atom to which they are attached to form C_4 - C_5 cycloalkyl or 4- to 6-membered heterocyclyl, each of which is optionally substituted by 1-3 R^6 groups. In some

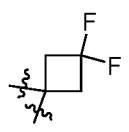
embodiments, R^3 is H, $-CH_3$, or $-CF_3$; and R^4 is H, $-CH_3$, $-CF_3$, cyclobutyl, or ; or R^3 and R^4 are taken together with the carbon atom to which they are attached to form , , , or , each of which is optionally substituted by 1-3 R^6 groups. In

some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are attached, and are substituted by 1 R^6 group which is methyl, to form . In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are attached,

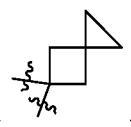
and are substituted by 1 R^6 group, to form . In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are attached, and are substituted by 1 R^6

group, to form . In some embodiments, $\mathbf{R}^3$ and $\mathbf{R}^4$ are taken together with the

carbon atom to which they are attached, and are substituted by 1 $\mathbf{R}^6$ group, to form . In some embodiments, $\mathbf{R}^3$ and $\mathbf{R}^4$ are taken together with the carbon atom to which they are

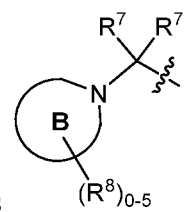
attached, and are substituted by 2 $\mathbf{R}^6$ groups, to form .

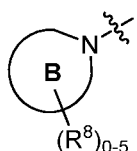
[15] In some embodiments, each $\mathbf{R}^6$ is independently $\text{C}_1\text{-C}_3$ alkyl, halo, hydroxy, $-\text{O}(\text{C}_1\text{-C}_3 \text{ alkyl})$, $-\text{CN}$, $\text{C}_1\text{-C}_3$ alkyl-CN, $\text{C}_1\text{-C}_3$ alkyl-OH, or $\text{C}_1\text{-C}_3$ haloalkyl; or two $\mathbf{R}^6$ groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro $\text{C}_3\text{-C}_6$ cycloalkyl or spiro 4- to 5-membered heterocyclyl. In some embodiments, each $\mathbf{R}^6$ is independently $-\text{CH}_3$, F, hydroxy, $-\text{OCH}_3$, $-\text{CN}$, $-\text{CH}_2\text{CN}$, $-\text{CH}_2\text{OH}$, or $-\text{CF}_3$; or two $\mathbf{R}^6$ groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro cyclopropyl. In some embodiments, $\mathbf{R}^3$ and $\mathbf{R}^4$ are taken together with the carbon atom to which they are attached to form cyclobutyl, which is substituted by 2 $\mathbf{R}^6$ groups which are taken together with the carbon atom to which

they are attached to form a spiro cyclopropyl, to form .

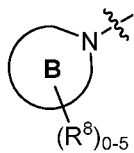
[16] In some embodiments, $\mathbf{R}^5$ is H, $\text{C}_1\text{-C}_3$ alkyl, $\text{C}_1\text{-C}_3$ haloalkyl, or $\text{C}_3\text{-C}_4$ cycloalkyl. In some embodiments, $\mathbf{R}^5$ is H, $-\text{CH}_3$, $-\text{CHF}_2$, or cyclopropyl.

[17] In some embodiments, $\mathbf{X}$ is H, $\text{C}_1\text{-C}_3$ alkyl, $\text{C}_1\text{-C}_3$ haloalkyl, $\text{C}_1\text{-C}_3$ alkyl-OH, $\text{C}_1\text{-C}_3$ alkyl-CN, or $\text{C}_3\text{-C}_5$ cycloalkyl optionally substituted by 1-3 $\mathbf{R}^8$ groups. In some

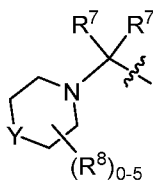
embodiments, $\mathbf{X}$ is H or $-\text{CH}_3$. In some embodiments, $\mathbf{X}$ is .

[18] In some embodiments,  is 4- to 6-membered heterocyclyl or 5- to 6-membered heteroaryl, each of which heterocyclyl or heteroaryl optionally contains 1-2

additional heteroatoms selected from the group consisting of N, O, and S, and each of which heterocyclyl or heteroaryl is optionally substituted by 1-5 R^8 groups. In some embodiments,



is 4- to 5-membered heterocyclyl or 5- to 6- membered heteroaryl, each of which heterocyclyl or heteroaryl optionally contains one additional heteroatom selected from the group consisting of N and O, and each of which heterocyclyl or heteroaryl is optionally substituted by 1-5 R^8 groups.



[19] In some embodiments, **X** is $(R^8)_{0-5}$.

[20] In some embodiments, **Y** is O. In some embodiments, **Y** is CH_2 , CHR^8 , or $C(R^8)_2$.

[21] In some embodiments, each R^7 is independently H, C_1 - C_3 alkyl, C_1 - C_3 alkyl-OH, or C_1 - C_3 haloalkyl; or two R^7 groups are taken together with the carbon atom to which they are attached to form a C_3 - C_5 cycloalkyl or 3- to 5- membered heterocyclyl. In some embodiments, each R^7 is independently H, $-CH_3$, $-CH_2OH$, or $-CF_3$; or two R^7 groups are taken together with the carbon atom to which they are attached to form cyclopropyl or oxetanyl.

[22] In some embodiments, each R^8 is independently halo, C_1 - C_3 alkyl, C_1 - C_3 alkyl-CN, C_1 - C_3 alkyl-OH, C_1 - C_3 haloalkyl, $-CN$, oxo, or $-O(C_1$ - C_3 alkyl); or two R^8 groups are taken together with the carbon atoms or atoms to which they are attached to form a spiro or fused C_3 - C_5 cycloalkyl or a spiro or fused 3- to 5-membered heterocyclyl. In some embodiments, each R^8 is independently F, $-CH_3$, $-CH_2CH_3$, $-CH_2CN$, $-CH_2OH$, $-CF_3$, $-CN$, oxo, or $-OCH_3$; or two R^8 groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused cyclopropyl or oxetanyl.

[23] Also disclosed herein is a compound selected from the compounds in Table 1, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing. Also disclosed herein is a compound selected from any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing, and a pharmaceutically acceptable excipient.

[24] Also disclosed herein is a method of modulating activity of an immune cell, the method comprising contacting the immune cell with an effective amount of any compound disclosed

above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[25] Also disclosed herein is a method of treating a cancer responsive to inhibition of Cbl-b activity in an individual in need thereof, the method comprising administering an effective amount of any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing, to the individual.

[26] Also disclosed herein is a method of inhibiting Cbl-b activity in an individual in need thereof, the method comprising administering an effective amount of any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing, to the individual.

[27] Also disclosed herein is a method for treating or preventing a disease or condition associated with Cbl-b activity in an individual in need thereof, the method comprising administering any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing, to the individual.

[28] Also disclosed herein is a method of producing a modified immune cell, the method comprising culturing a cell population containing an immune cell in the presence of an effective amount of any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[29] Also disclosed herein is a modified immune cell comprising a Cbl-b inhibitor, wherein the Cbl-b inhibitor is any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[30] Also disclosed herein is an isolated modified immune cell, wherein the immune cell has been contacted or is in contact with any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[31] Also disclosed herein is a composition comprising a cell population containing an isolated modified immune cell, wherein the immune cell has been contacted or is in contact with any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[32] Also disclosed herein is a method of inhibiting abnormal cell proliferation, the method comprising administering an effective amount of an isolated modified immune cell, wherein the immune cell has been contacted or is in contact with any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing, to an individual in need thereof.

[33] Also disclosed herein is a method of inhibiting abnormal cell proliferation, the method comprising administering a composition comprising a cell population containing an isolated modified immune cell, wherein the immune cell has been contacted or is in contact with any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing, to an individual in need thereof.

[34] Also disclosed herein is a method of inhibiting abnormal cell proliferation, the method comprising administering an effective amount of any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[35] Also disclosed herein is a cell culture composition comprising a cell population containing an immune cell and a Cbl-b inhibitor, wherein the Cbl-b inhibitor is any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[36] Also disclosed herein is a pharmaceutical composition comprising a Cbl-b inhibitor and one or both of an adjuvant and an antigen, wherein the Cbl-b inhibitor is any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[37] Also disclosed herein is an article of manufacture comprising any modified immune cell as disclosed herein, any composition comprising a cell population as disclosed herein, any cell culture composition as disclosed herein, or any pharmaceutical composition as disclosed herein.

[38] Also disclosed herein is a kit comprising any modified immune cell as disclosed herein or any composition comprising a cell population as disclosed herein.

[39] Also disclosed herein is the use of a Cbl-b inhibitor in the manufacture of a medicament for treating or preventing a disease or condition associated with Cbl-b activity, wherein the Cbl-b inhibitor is any compound disclosed above or herein, or a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[40] In any of the embodiments disclosed herein, the Cbl-b protein can be a mammalian Cbl-b, or a human Cbl-b.

[41] Disclosed herein are Cbl-b inhibitor compounds, vaccines, and compositions comprising Cbl-b inhibitors and vaccines, as well as methods of use thereof in treating cancer. The Cbl-b inhibitor and the cancer vaccine may be administered to an individual with cancer.

[42] Also, disclosed herein are Cbl-b inhibitor compounds, oncolytic viruses, and compositions comprising Cbl-b inhibitors and oncolytic viruses, as well as methods of use

thereof in treating cancer. The Cbl-b inhibitor and the oncolytic virus may be administered to an individual with cancer.

[43] Provided herein is a method of immunizing, the method comprising administering to an individual in need thereof an effective amount of a small molecule Cbl-b inhibitor, and administering to the individual an effective amount of a vaccine.

[44] Provided herein is a method of treating cancer, the method comprising administering to an individual with cancer an effective amount of a small molecule Cbl-b inhibitor, and administering to the individual an effective amount of an oncolytic virus.

[45] Provided herein is a method of treating cancer, the method comprising administering to an individual with cancer an effective amount of an agent capable of lowering activation threshold of an immune cell, and administering to the individual an effective amount of a therapeutic cancer vaccine; or administering to the individual an effective amount of an oncolytic virus.

[46] Provided herein is a pharmaceutical composition comprising a cancer vaccine and a small molecule Cbl-b inhibitor, optionally wherein the composition further comprises a pharmaceutically acceptable excipient.

[47] Provided herein is a kit for treating cancer, the kit comprising (a) a small molecule Cbl-b inhibitor; (b) a therapeutic cancer vaccine; and (c) instructions for administration of an effective amount of the Cbl-b inhibitor and the therapeutic cancer vaccine to treat cancer in an individual.

[48] Provided herein is a kit for treating cancer, the kit comprising (a) a pharmaceutical composition comprising a small molecule Cbl-b inhibitor and a therapeutic cancer vaccine; and (b) instructions for administration of an effective amount of the pharmaceutical composition comprising the Cbl-b inhibitor and the therapeutic cancer vaccine to treat cancer in an individual.

[49] Provided herein is a pharmaceutical composition comprising an oncolytic virus and a small molecule Cbl-b inhibitor, optionally wherein the composition further comprises a pharmaceutically acceptable excipient.

[50] Provided herein is a kit for treating cancer, the kit comprising (a) a small molecule Cbl-b inhibitor; (b) an oncolytic virus; and (c) instructions for administration of an effective amount of the Cbl-b inhibitor and the oncolytic virus to treat cancer in an individual.

[51] Provided herein is a kit for treating cancer, the kit comprising (a) a pharmaceutical composition comprising a small molecule Cbl-b inhibitor and an oncolytic virus; and (b)

instructions for administration of an effective amount of the pharmaceutical composition comprising the small molecule Cbl-b inhibitor and the oncolytic virus to treat cancer in an individual.

[52] And, provided herein is a method of treating a disease via cell therapy in a subject in need thereof, the method comprising administering to the individual an effective amount of one or more therapeutic cells to treat the disease; and administering to the subject an effective amount of a Cbl-b inhibitor, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31, and wherein treatment with the therapeutic cells is enhanced by the combination with the Cbl-b inhibitor.

BRIEF DESCRIPTION OF THE DRAWING

[53] FIG. 1 shows percent conditional survival of mice depicted by the Kaplan-Meier plot.

[54] FIG. 2 shows tumor volume of individual mice.

[55] FIGS. 3 and 4 show tumor volume of individual tumors.

DETAILED DESCRIPTION

[56] Provided herein are compounds and pharmaceutical compositions that inhibit the Cbl-b enzyme, as well as methods of treatment using such compounds and pharmaceutical compositions. The compounds and compositions can be used in methods of modulating the immune system, for treatment of diseases, and for treatment of cells *in vivo*, *in vitro*, or *ex vivo*. Also, provided herein are pharmaceutical compositions comprising cancer vaccines and compounds that inhibit the Cbl-b enzyme, as well as methods of treatment using such compounds, cancer vaccines, and pharmaceutical compositions. Also provided herein are pharmaceutical compositions comprising oncolytic viruses and compounds that inhibit the Cbl-b enzyme, as well as methods of treatment using such compounds, oncolytic viruses, and pharmaceutical compositions.

[57] T-cell activation and T-cell tolerance are tightly controlled processes regulating the immune response to tumors while preventing autoimmunity. Tolerance prevents the immune system from attacking cells expressing “self” antigens. During peripheral tolerance, T-cells that recognize “self” antigens (*i.e.*, self-reactive T-cells) become functionally unresponsive or are deleted after encountering “self” antigens outside of the thymus. Peripheral tolerance processes therefore are important for preventing autoimmune diseases. Normally, cancer cells are removed by activated T-cells that recognize tumor antigens expressed on the surface of the cancer cells. However, in cancer, the tumor microenvironment can support T-cell tolerance to cancer cells, which allows cancer cells to avoid recognition and removal by the immune

system. The ability of cancer cells to avoid tumor immunosurveillance can contribute to uncontrolled tumor growth. Therefore, T-cell tolerance can be a form of T-cell dysfunction. General principles of T-cell dysfunction are well known in the art (*see* Schietinger *et al.*, Trends Immunol., 35: 51-60, 2014). Additional types of T-cell dysfunction that can contribute to uncontrolled tumor growth include T-cell exhaustion, T-cell senescence, and/or T-cell anergy. Therefore, treating T-cell dysfunction, for example, by increasing T-cell activation, increasing T-cell proliferation, decreasing T-cell tolerance, and/or decreasing T-cell exhaustion, is beneficial for preventing or treating cancer. Additional cells of the immune system are important for recognition and removal of cancer cells during immune surveillance. For example, Natural Killer (NK)-cells are lymphocytes of the innate immune system that are able to identify and kill cancer cells (*see* Martinez-Losato *et al.*, Clin Cancer Res., 21: 5048-5056, 2015). Recent studies have also shown that B-cell subsets with distinct phenotypes and functions exhibit diverse roles in the anti-tumor response (*see* Saravaria *et al.*, Cell Mol Immunol., 14: 662-674, 2017). Due to their role in tumor surveillance, NK-cells and B-cells may also be amenable as therapeutic targets for the prevention or treatment of cancer.

[58] Cbl-b is a RING-type E3 ligase that plays an important role in the immune system due to its function as a negative regulator of immune activation. Cbl-b has an essential role in decreasing the activation of T-cells, thereby enhancing T-cell tolerance. Studies have found that cbl-b-deficient T-cells display lower thresholds for activation by antigen recognition receptors and co-stimulatory molecules (*e.g.*, CD28). For example, loss of Cbl-b in T-cells uncouples the requirement for CD28 costimulation during T-cell activation and proliferation (*see* Bachmaier *et al.*, Nature, 403: 211-216, 2000). Such *cbl-b*^{-/-} T-cells are largely resistant to T-cell anergy, a tolerance mechanism in which T-cells are functionally inactivated and T-cell proliferation is greatly impaired (*see* Jeon *et al.*, Immunity, 21: 167-177, 2004; and Schwartz *et al.*, Annu Rev Immunol., 21: 305-34, 2003). In support of this, loss of Cbl-b in *cbl-b* knockout mice resulted in impaired induction of T-cell tolerance and exacerbated autoimmunity (*see* Jeon *et al.*, Immunity, 21: 167-177, 2004). Importantly, loss of Cbl-b in mice also resulted in a robust anti-tumor response that depends primarily on cytotoxic T-cells. One study showed that *cbl-b*^{-/-} CD8⁺ T-cells are resistant to T regulatory cell-mediated suppression and exhibit enhanced activation and tumor infiltration. Therapeutic transfer of naive *cbl-b*^{-/-} CD8⁺ T-cells was sufficient to mediate rejection of established tumors (*see* Loeser *et al.*, J Exp Med., 204: 879-891, 2007). Recent studies have shown that Cbl-b also plays a role in NK-cell activation. Genetic deletion of Cbl-b or targeted inactivation of its E3

ligase activity allowed NK-cells to spontaneously reject metastatic tumors in a mouse model (see Paolino *et al.*, Nature, 507: 508-512, 2014).

[59] Provided herein are compounds and compositions that are potent inhibitors of Cbl-b and can be used in novel approaches to treat diseases such as cancer. In some embodiments, the compounds and compositions provided herein can be used in methods of modulating the immune system, such as increasing activation of T-cells, NK-cells, and B-cells, as well as in the treatment of such cells *in vivo*, *in vitro*, or *ex vivo*.

I. Definitions

[60] An “effective amount” of an agent disclosed herein is an amount sufficient to carry out a specifically stated purpose. An “effective amount” may be determined empirically and in a routine manner, in relation to the stated purpose. An “effective amount” or an “amount sufficient” of an agent is that amount adequate to produce a desired biological effect, such as a beneficial result, including a beneficial clinical result. In some embodiments, the term “effective amount” refers to an amount of an agent effective to “treat” a disease or disorder in an individual (*e.g.*, a mammal such as a human).

[61] The term “Cbl-b” as used herein refers to a Cbl-b protein. The term also includes naturally occurring variants of Cbl-b, including splice variants or allelic variants. The term also includes non-naturally occurring variants of Cbl-b, such as a recombinant Cbl-b protein or truncated variants thereof, which generally preserve the binding ability of naturally occurring Cbl-b or naturally occurring variants of Cbl-b (*e.g.*, the ability to bind to an E2 enzyme).

[62] The terms “pharmaceutical formulation” and “pharmaceutical composition” refer to preparations that are in such form as to permit the biological activity of the active ingredient to be effective, and that contain no additional components that are unacceptably toxic to an individual to which the formulation or composition would be administered. Such formulations or compositions may be sterile. Such formulations or compositions may be sterile, with the exception of the inclusion of an oncolytic virus.

[63] “Excipients” as used herein include pharmaceutically acceptable excipients, carriers, vehicles, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable excipient is an aqueous pH buffered solution.

[64] Reference to a compound as described in a pharmaceutical composition, or to a compound as described in a claim to a pharmaceutical composition, refers to the compound

described by the formula recited in the pharmaceutical composition, without the other elements of the pharmaceutical composition, that is, without carriers, excipients, etc.

[65] The terms “treating” or “treatment” of a disease refer to executing a protocol, which may include administering one or more therapeutic agent to an individual (human or otherwise), in an effort to obtain beneficial or desired results in the individual, including clinical results. Beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total). “Treatment” also can mean prolonging survival as compared to expected survival of an individual not receiving treatment. Further, “treating” and “treatment” may occur by administration of one dose of a therapeutic agent or therapeutic agents, or may occur upon administration of a series of doses of a therapeutic agent or therapeutic agents. “Treating” or “treatment” does not require complete alleviation of signs or symptoms, and does not require a cure. “Treatment” also can refer to clinical intervention, such as administering one or more therapeutic agents to an individual, designed to alter the natural course of the individual or cell being treated (*i.e.*, to alter the course of the individual or cell that would occur in the absence of the clinical intervention). The term “therapeutic agent” can refer to a Cbl-b inhibitor, a modified immune cell, or compositions thereof.

[66] As used herein, an “individual” or a “subject” is a mammal. A “mammal” for purposes of treatment includes humans; non-human primates; domestic and farm animals; and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, etc. In some embodiments, the individual or subject is human.

[67] As used herein, the term “T-cell dysfunction” refers to a state of reduced immune responsiveness to antigenic stimulation. The term “T-cell dysfunction” includes common elements of both T-cell exhaustion and/or T-cell anergy in which antigen recognition may occur, but the ensuing immune response is ineffective to control tumor growth. The term “T-cell dysfunction” also includes being refractory or unresponsive to antigen recognition, such as, impaired capacity to translate antigen recognition to downstream T-cell effector functions, such as proliferation, cytokine production, and/or target cell killing.

[68] The term “T-cell anergy” refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T-cell receptor. “T-cell anergy” can also result upon stimulation with antigen in the absence of co-stimulation, resulting

in the cell becoming refractory to subsequent activation by the antigen even in the context of co-stimulation.

[69] The term “T-cell exhaustion” refers to a state of T-cell dysfunction that arises from sustained TCR signaling that can occur during cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. It is defined by poor effector function, sustained expression of inhibitory receptors, and a transcriptional state distinct from that of functional effector or memory T-cell.

[70] A “T-cell dysfunction disorder” is a disorder or condition characterized by decreased responsiveness of T-cells to antigenic stimulation. Decreased responsiveness may result in ineffective control of a tumor. In some embodiments, the term “T-cell dysfunction disorder” encompasses cancer such as a hematologic cancer or a non-hematologic cancer. In some embodiments, a “T-cell dysfunctional disorder” is one in which T-cells are anergic or have decreased ability to secrete cytokines, proliferate, or execute cytolytic activity.

[71] “Enhancing T-cell function” means to induce, cause, or stimulate a T-cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T-cells. Examples of enhanced T-cell function include increased T-cell activation (*e.g.*, increased cytokine production, increased expression of T-cell activation markers, etc.), increased T-cell proliferation, decreased T-cell exhaustion, and/or decreased T-cell tolerance relative to the state of the T-cells before treatment with a Cbl-b inhibitor. Methods of measuring enhancement of T-cell function are known in the art.

[72] “Proliferation” is used herein to refer to the proliferation of a cell. Increased proliferation encompasses the production of a greater number of cells relative to a baseline value. Decreased proliferation encompasses the production of a reduced number of cells relative to a baseline value. In some embodiments, the cell is an immune cell such as a T-cell and increased proliferation is desired. In some embodiments, the cell is a cancer cell and reduced proliferation is desired.

[73] “Alkyl” as used herein refers to a saturated linear (*i.e.*, unbranched) or branched univalent hydrocarbon chain or combination thereof. Particular alkyl groups are those having a designated number of carbon atoms, for example, an alkyl group having 1 to 20 carbon atoms (a “C₁-C₂₀ alkyl”), having 1 to 10 carbon atoms (a “C₁-C₁₀” alkyl), having 1 to 8 carbon atoms (a “C₁-C₈ alkyl”), having 1 to 6 carbon atoms (a “C₁-C₆ alkyl”), having 2 to 6 carbon atoms (a “C₂-C₆ alkyl”), or having 1 to 4 carbon atoms (a “C₁-C₄ alkyl”). Examples of alkyl groups include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl,

n-butyl, t-butyl, isobutyl, sec-butyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like.

[74] “Alkenyl” as used herein refers to an unsaturated linear (*i.e.*, unbranched) or branched univalent hydrocarbon chain or combination thereof, having at least one site of olefinic unsaturation (*i.e.*, having at least one moiety of the formula C=C). Particular alkenyl groups are those having a designated number of carbon atoms, for example, an alkenyl group having 2 to 20 carbon atoms (a “C₂-C₂₀ alkenyl”), having 2 to 10 carbon atoms (a “C₂-C₁₀ alkenyl”), having 2 to 8 carbon atoms (a “C₂-C₈ alkenyl”), having 2 to 6 carbon atoms (a “C₂-C₆ alkenyl”), or having 2 to 4 carbon atoms (a “C₂-C₄ alkenyl”). The alkenyl group may be in “cis” or “trans” configurations or, alternatively, in “E” or “Z” configurations. Examples of alkenyl groups include, but are not limited to, groups such as ethenyl (or vinyl), prop-1-enyl, prop-2-enyl (or allyl), 2-methylprop-1-enyl, but-1-enyl, but-2-enyl, but-3-enyl, buta-1,3-dienyl, 2-methylbuta-1,3-dienyl, homologs and isomers thereof, and the like.

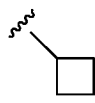
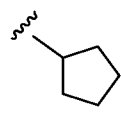
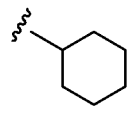
[75] “Alkynyl” as used herein refers to an unsaturated linear (*i.e.*, unbranched) or branched univalent hydrocarbon chain or combination thereof, having at least one site of acetylenic unsaturation (*i.e.*, having at least one moiety of the formula C≡C). Particular alkynyl groups are those having a designated number of carbon atoms, for example, an alkynyl group having 2 to 20 carbon atoms (a “C₂-C₂₀ alkynyl”), having 2 to 10 carbon atoms (a “C₂-C₁₀ alkynyl”), having 2 to 8 carbon atoms (a “C₂-C₈ alkynyl”), having 2 to 6 carbon atoms (a “C₂-C₆ alkynyl”), or having 2 to 4 carbon atoms (a “C₂-C₄ alkynyl”). Examples of alkynyl groups include, but are not limited to, groups such as ethynyl (or acetylenyl), prop-1-ynyl, prop-2-ynyl (or propargyl), but-1-ynyl, but-2-ynyl, but-3-ynyl, homologs, and isomers thereof, and the like.

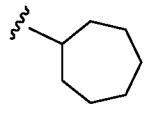
[76] “Alkylene” as used herein refers to the same residues as alkyl, but having bivalency. Particular alkylene groups are those having 1 to 6 carbon atoms (a “C₁-C₆ alkylene”), 1 to 5 carbon atoms (a “C₁-C₅ alkylene”), 1 to 4 carbon atoms (a “C₁-C₄ alkylene”), or 1 to 3 carbon atoms (a “C₁-C₃ alkylene”). Examples of alkylene groups include, but are not limited to, groups such as methylene (-CH₂-), ethylene (-CH₂CH₂-), propylene (-CH₂CH₂CH₂-), butylene (-CH₂CH₂CH₂CH₂-), and the like.

[77] “Cycloalkyl” as used herein refers to non-aromatic, saturated or unsaturated, cyclic univalent hydrocarbon structures. Particular cycloalkyl groups are those having a designated number of annular (*i.e.*, ring) carbon atoms, for example, a cycloalkyl group having from 3 to 12 annular carbon atoms (a “C₃-C₁₂ cycloalkyl”). A particular cycloalkyl is a cyclic hydrocarbon having from 3 to 8 annular carbon atoms (a “C₃-C₈ cycloalkyl”), or having 3 to 6

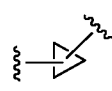
annular carbon atoms (a “C₃-C₆ cycloalkyl”). Cycloalkyl can consist of one ring, such as cyclohexyl, or multiple rings, such as adamantyl, but excludes aryl (*i.e.*, aromatic) groups. A cycloalkyl comprising more than one ring may be fused, spiro, or bridged, or combinations

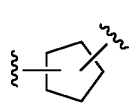
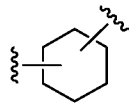
thereof. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl ,

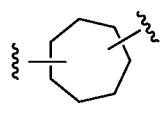
cyclobutyl , cyclopentyl , cyclohexyl , 1-cyclohexenyl, 3-

cyclohexenyl, cycloheptyl , norbornyl, and the like.

[78] “Cycloalkylene” as used herein refers to the same residues as cycloalkyl, but having bivalency. Particular cycloalkylene groups are those having 3 to 12 annular carbon atoms (a “C₃-C₁₂ cycloalkylene”), having from 3 to 8 annular carbon atoms (a “C₃-C₈ cycloalkylene”), or having 3 to 6 annular carbon atoms (a “C₃-C₆ cycloalkylene”). Examples of cycloalkylene

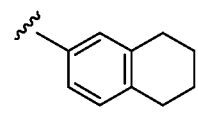
groups include, but are not limited to, cyclopropylene , cyclobutylene ,

cyclopentylene , cyclohexylene , 1,2-cyclohexenylene, 1,3-

cyclohexenylene, 1,4-cyclohexenylene, cycloheptylene , norbornylene, and the like.

[79] “Aryl” as used herein refers to an aromatic carbocyclic group having a single ring (*e.g.*, phenyl), or multiple condensed rings (*e.g.*, naphthyl or anthryl) where one or more of the condensed rings may not be aromatic. Particular aryl groups are those having from 6 to 14 annular (*i.e.*, ring) carbon atoms (a “C₆-C₁₄ aryl”). An aryl group having more than one ring where at least one ring is non-aromatic may be connected to the parent structure at either an aromatic ring position or at a non-aromatic ring position. In one variation, an aryl group having more than one ring where at least one ring is non-aromatic is connected to the parent structure at an aromatic ring position. Examples of aryls include, but are not limited to, groups such as

phenyl, naphthyl, 1-naphthyl, 2-naphthyl, 1,2,3,4-tetrahydronaphthalen-6-yl and the like.

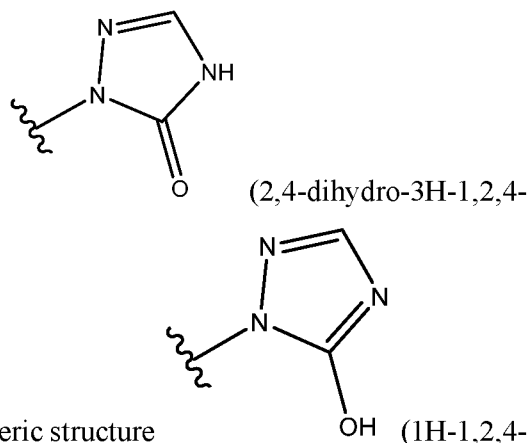


[80] “Carbocyclyl” or “carbocyclic” refers to an aromatic or non-aromatic univalent cyclic group in which all of the ring members are carbon atoms, such as cyclohexyl, phenyl, 1,2-dihydronaphthyl, etc.

[81] “Arylene” as used herein refers to the same residues as aryl, but having bivalency. Particular arylene groups are those having from 6 to 14 annular carbon atoms (a “C₆-C₁₄ arylene”). Examples of arylene include, but are not limited to, groups such as phenylene, o-phenylene (*i.e.*, 1,2-phenylene), m-phenylene (*i.e.*, 1,3-phenylene), p-phenylene (*i.e.*, 1,4-phenylene), naphthylene, 1,2-naphthylene, 1,3-naphthylene, 1,4-naphthylene, 2,7-naphthylene, 2,6-naphthylene, and the like.

[82] “Heteroaryl” as used herein refers to an unsaturated aromatic cyclic group having from 1 to 14 annular carbon atoms and at least one annular heteroatom, including, but not limited to, heteroatoms such as nitrogen (N), oxygen (O), and sulfur (S). A heteroaryl group may have a single ring (*e.g.*, pyridyl or imidazolyl) or multiple condensed rings (*e.g.*, indoliziny, indolyl, or quinoliny) where at least one of the condensed rings is aromatic. Particular heteroaryl groups are 5- to 14-membered rings having 1 to 12 annular carbon atoms and 1 to 6 annular heteroatoms independently selected from the group consisting of nitrogen (N), oxygen (O), and sulfur (S) (a “5- to 14- membered heteroaryl”); 5- to 10-membered rings having 1 to 8 annular carbon atoms and 1 to 4 annular heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur (a “5- to 10- membered heteroaryl”); or 5-, 6-, or 7-membered rings having 1 to 5 annular carbon atoms and 1 to 4 annular heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur (a “5- to 7- membered heteroaryl”). In one variation, heteroaryl includes monocyclic aromatic 5-, 6-, or 7-membered rings having from 1 to 6 annular carbon atoms and 1 to 4 annular heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. In another variation, heteroaryl includes polycyclic aromatic rings having from 1 to 12 annular carbon atoms and 1 to 6 annular heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. A heteroaryl group having more than one ring where at least one ring is non-aromatic may be connected to the parent structure at either an aromatic ring position or at a non-aromatic ring position. Examples of heteroaryl include, but are not limited to, groups such as pyridyl, benzimidazolyl, benzotriazolyl, benzo[b]thienyl, quinoliny, indolyl, benzothiazolyl, and the

like. “Heteroaryl” also includes moieties such as



triazol-3-one-2-yl), which has the aromatic tautomeric structure triazol-5-ol-1-yl).

[83] “Heterocyclyl” and “heterocyclic groups” as used herein refer to non-aromatic saturated or partially unsaturated cyclic groups having the number of atoms and heteroatoms as specified, or if no number of atoms or heteroatoms is specified, having at least three annular atoms, from 1 to 14 annular carbon atoms, and at least one annular heteroatom, including, but not limited to, heteroatoms such as nitrogen, oxygen, and sulfur. A heterocyclic group may have a single ring (*e.g.*, tetrahydrothiophenyl, oxazolidinyl) or multiple condensed rings (*e.g.*, decahydroquinoliny, octahydrobenzo[d]oxazolyl). Multiple condensed rings include, but are not limited to, bicyclic, tricyclic, and quadacyclic rings, as well as bridged or spirocyclic ring systems. Examples of heterocyclic groups include, but are not limited to, aziridinyl, azetidiny, pyrrolidinyl, piperidinyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, oxazolidinyl, piperazinyl, morpholinyl, dioxanyl, 3,6-dihydro-2H-pyranyl, 2,3-dihydro-1H-imidazolyl, and the like.

[84] “Heteroarylene” as used herein refers to the same residues as heteroaryl, but having bivalency. Particular heteroarylene groups are 5- to 14-membered rings having 1 to 12 annular carbon atoms and 1 to 6 annular heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur (a “5- to 14- membered heteroarylene”); 5- to 10-membered rings having 1 to 8 annular carbon atoms and 1 to 4 annular heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur (a “5- to 10- membered heteroarylene”); or 5-, 6-, or 7-membered rings having 1 to 5 annular carbon atoms and 1 to 4 annular heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur (a “5- to 7-membered heteroarylene”). Examples of heteroarylene include, but are not limited to, groups such as pyridylene, benzimidazolylene, benzotriazolylene, benzo[b]thienylene, quinoliny, indolylene, benzothiazolylene, and the like.

[85] “Halo” or “halogen” refers to elements of the Group 17 series having atomic number 9 to 85. Halo groups include fluoro (F), chloro (Cl), bromo (Br), and iodo (I).

[86] “Haloalkyl,” “haloalkylene,” “haloaryl,” “haloarylene,” “haloheteroaryl,” and similar terms refer to a moiety substituted with at least one halo group. Where a haloalkyl moiety or other halo-substituted moiety is substituted with more than one halogen, it may be referred to by using a prefix corresponding to the number of halogen moieties attached. For example, dihaloaryl, dihaloalkyl, trihaloaryl, trihaloalkyl, etc., refer to aryl and alkyl substituted with two (“di”) or three (“tri”) halo groups, which may be, but are not necessarily, the same halo; thus, for example, the haloaryl group 4-chloro-3-fluorophenyl is within the scope of dihaloaryl. The subset of haloalkyl groups in which each hydrogen (H) of an alkyl group is replaced with a halo group is referred to as a “perhaloalkyl.” A particular perhaloalkyl group is trifluoroalkyl ($-\text{CF}_3$). Similarly, “perhaloalkoxy” refers to an alkoxy group in which a halogen takes the place of each hydrogen (H) in the hydrocarbon making up the alkyl moiety of the alkoxy group. An example of a perhaloalkoxy group is trifluoromethoxy ($-\text{OCF}_3$). “Haloalkyl” includes monohaloalkyl, dihaloalkyl, trihaloalkyl, perhaloalkyl, and any other number of halo substituents possible on an alkyl group; and similarly for other groups such as haloalkylene, haloaryl, haloarylene, haloheteroaryl, etc.

[87] “Amino” refers to the group $-\text{NH}_2$.

[88] “Oxo” refers to the group $=\text{O}$, that is, an oxygen atom doubly bonded to carbon or another chemical element.

[89] “Optionally substituted,” unless otherwise specified, means that a group is unsubstituted or substituted by one or more (*e.g.*, 1, 2, 3, 4, or 5) of the substituents listed for that group, in which the substituents may be the same or different. In one embodiment, an optionally substituted group is unsubstituted. In one embodiment, an optionally substituted group has one substituent. In another embodiment, an optionally substituted group has two substituents. In another embodiment, an optionally substituted group has three substituents. In another embodiment, an optionally substituted group has four substituents. In some embodiments, an optionally substituted group has 1 to 2, 1 to 3, 1 to 4, or 1 to 5 substituents. When multiple substituents are present, each substituent is independently chosen unless indicated otherwise. For example, each ($\text{C}_1\text{-C}_4$ alkyl) substituent on the group $-\text{N}(\text{C}_1\text{-C}_4 \text{ alkyl})(\text{C}_1\text{-C}_4 \text{ alkyl})$ can be selected independently from the other, so as to generate groups such as $-\text{N}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, etc.

[90] In addition to the disclosure herein, the term “substituted,” when used to modify a specified group or radical, can also mean that one or more hydrogen atoms (H) of the specified group or radical are each, independently of one another, replaced with the same or different substituent groups as defined herein. In some embodiments, a group that is substituted has 1, 2, 3, or 4 substituents, 1, 2, or 3 substituents, 1 or 2 substituents, or one substituent.

[91] Substituents can be attached to any chemically possible location on the specified group or radical, unless indicated otherwise. Thus, in one embodiment, -C₁-C₈ alkyl-OH includes, for example, -CH₂CH₂OH, -CH(OH)-CH₃, -CH₂C(OH)(CH₃)₂, and the like. By way of further example, in one embodiment, -C₁-C₆ alkyl-OH includes, for example, -CH₂CH₂OH, -CH(OH)-CH₃, -CH₂C(OH)(CH₃)₂, and the like. By way of further example, in one embodiment, -C₁-C₆ alkyl-CN includes, for example, -CH₂CH₂CN, -CH(CN)-CH₃, -CH₂C(CN)(CH₃)₂, and the like.

[92] Unless a specific isotope of an element is indicated in a formula, the disclosure includes all isotopologues of the compounds disclosed herein, such as, for example, deuterated derivatives of the compounds (where H can be ²H, *i.e.*, deuterium (D)). Deuterated compounds may provide favorable changes in pharmacokinetic (ADME) properties. Isotopologues can have isotopic replacements at any or at all locations in a structure, or can have atoms present in natural abundance at any or all locations in a structure.

[93] A “small molecule” as used herein refers to a compound of 1,000 daltons or less in molecular weight.

[94] Hydrogen atoms can also be replaced with close bioisosteres, such as fluorine, provided that such replacements result in stable compounds.

[95] The disclosure also includes any or all of the stereochemical forms, including any enantiomeric or diastereomeric forms of the compounds described herein, and cis/trans or E/Z isomers. Unless stereochemistry is explicitly indicated in a chemical structure or name, the structure or name is intended to embrace all possible stereoisomers of a compound depicted. In addition, where a specific stereochemical form is depicted, it is understood that all other stereochemical forms are also described and embraced by the disclosure, as well as the general non-stereospecific form and mixtures of the disclosed compounds in any ratio, including mixtures of two or more stereochemical forms of a disclosed compound in any ratio, such that racemic, non-racemic, enantioenriched, and scalemic mixtures of a compound are embraced. Compositions comprising a disclosed compound also are intended, such as a composition of a substantially pure compound, including a specific stereochemical form thereof. Compositions

comprising a mixture of disclosed compounds in any ratio also are embraced by the disclosure, including compositions comprising mixtures of two or more stereochemical forms of a disclosed compound in any ratio, such that racemic, non-racemic, enantioenriched, and scalemic mixtures of a compound are embraced by the disclosure. If stereochemistry is explicitly indicated for one portion or portions of a molecule, but not for another portion or portions of a molecule, the structure is intended to embrace all possible stereoisomers for the portion or portions where stereochemistry is not explicitly indicated.

[96] This disclosure also embraces any and all tautomeric forms of the compounds described herein.

[97] The disclosure is intended to embrace all salts of the compounds described herein, as well as methods of using such salts of the compounds. In one embodiment, the salts of the compounds comprise pharmaceutically acceptable salts. Pharmaceutically acceptable salts are those salts that can be administered as drugs or pharmaceuticals to humans and/or animals and that, upon administration, retain at least some of the biological activity of the free compound (i.e., neutral compound or non-salt compound). The desired salt of a basic compound may be prepared by methods known to those of skill in the art by treating the compound with an acid. Examples of inorganic acids include, but are not limited to, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, and phosphoric acid. Examples of organic acids include, but are not limited to, formic acid, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, sulfonic acids, and salicylic acid. Salts of basic compounds with amino acids, such as aspartate salts and glutamate salts, also can be prepared. The desired salt of an acidic compound can be prepared by methods known to those of skill in the art by treating the compound with a base. Examples of inorganic salts of acid compounds include, but are not limited to, alkali metal and alkaline earth salts, such as sodium salts, potassium salts, magnesium salts, and calcium salts; ammonium salts; and aluminum salts. Examples of organic salts of acid compounds include, but are not limited to, procaine, dibenzylamine, N-ethylpiperidine, N,N'-dibenzylethylenediamine, and triethylamine salts. Salts of acidic compounds with amino acids, such as lysine salts, also can be prepared. For lists of pharmaceutically acceptable salts, see, for example, P. H. Stahl and C. G. Wermuth (eds.) "Handbook of Pharmaceutical Salts, Properties, Selection and Use" Wiley-VCH, 2011 (ISBN: 978-3-90639-051-2). Several pharmaceutically acceptable salts are also disclosed in Berge, J. Pharm. Sci. 66:1 (1977).

[98] As described in Biological Example 1, 8, and/or 12 the Cbl-b activity assay (Cbl-b inhibition assay) used to measure the IC₅₀ values for Cbl-b inhibition uses a mixture comprising an N-terminal biotinylated Avi-tagged Cbl-b, a fluorescently-labeled inhibitor probe tagged with BODIPY FL (Example 54), and assay buffer. In one embodiment, the Cbl-b activity assay (Cbl-b inhibition assay) used to measure IC₅₀ for inhibition of Cbl-b uses the conditions described in Biological Example 1, 8, and/or 12, with 0.5 nM Cbl-b (“High” final concentration). In another embodiment, the Cbl-b activity assay (Cbl-b inhibition assay) used to measure IC₅₀ for inhibition of Cbl-b uses the conditions described in Biological Example 1, 8, and/or 12, with 0.125 nM Cbl-b (“Low” final concentration).

[99] It is appreciated that certain features disclosed herein, which are, for clarity, described in the context of separate embodiments, also may be provided in combination in a single embodiment. Conversely, various features disclosed herein, which are, for brevity, described in the context of a single embodiment, also may be provided separately or in any suitable subcombination. All combinations of the embodiments pertaining to the chemical groups represented by the variables are specifically embraced by this disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace compounds that are stable compounds (*i.e.*, compounds that can be isolated, characterized, and tested for biological activity). In addition, all subcombinations of the chemical groups listed in the embodiments describing such variables also are specifically embraced by this disclosure and are disclosed herein just as if each and every such subcombination of chemical groups was individually and explicitly disclosed herein.

[100] It is understood that aspects and embodiments described herein as “comprising” include “consisting of” and “consisting essentially of” embodiments.

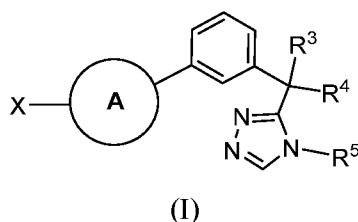
[101] As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless otherwise indicated or clear from context. For example, “an” excipient includes one or more excipients.

[102] Reference to “about” a value, encompasses from 90% to 110% of that value. For instance, about 50 billion cells refers to 45 to 55 billion cells, and includes 50 billion cells. For instance, a temperature of “about 100 degrees” refers to a temperature of about 90 degrees to about 110 degrees.

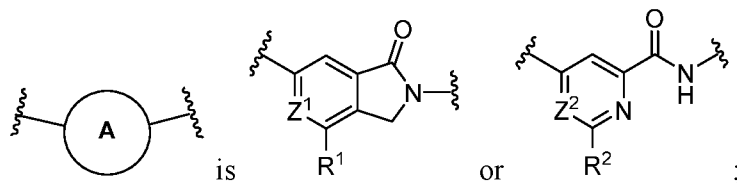
[103] When numerical ranges of compounds are given, all compounds within those numerical limits designated “a” and “b” are included, unless expressly excluded. For example, reference to compounds 9-13 refers to compounds 9, 10, 11, 12, and 13.

II. Compounds

[104] In one aspect, provided is a compound of Formula (I):



or a tautomer thereof, or a pharmaceutically acceptable salt thereof,
wherein:



Z¹ is CH or N;

Z² is CH or N;

R¹ is -CF₃ or cyclopropyl;

R² is -CF₃ or cyclopropyl;

R³ is H, C₁-C₂ alkyl, or C₁-C₂ haloalkyl;

R⁴ is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, 4- to 8-membered heterocyclyl, or C₃-C₆ cycloalkyl,
wherein the heterocyclyl or cycloalkyl groups are optionally substituted by 1-5 **R⁶** groups;

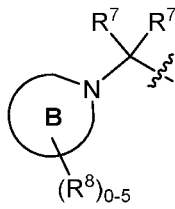
or **R³** and **R⁴** are taken together with the carbon atom to which they are attached to form
C₃-C₅ cycloalkyl or 4- to 6-membered heterocyclyl, each of which is optionally substituted by
1-5 **R⁶** groups;

R⁵ is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₃-C₆ cycloalkyl;

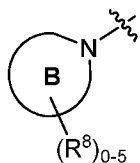
each **R⁶** is independently C₁-C₆ alkyl, halo, hydroxy, -O-(C₁-C₆ alkyl), -CN, C₁-C₆ alkyl-CN,
C₁-C₆ alkyl-OH, or C₁-C₆ haloalkyl;

or two **R⁶** groups attached to the same carbon atom are taken together with the carbon atom to
which they are attached to form a spiro C₃-C₆ cycloalkyl or spiro 4- to 6-membered
heterocyclyl;

X is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, C₁-C₆ alkyl-OH, C₁-C₆ alkyl-CN, C₃-C₆ cycloalkyl



optionally substituted by 1-5 **R**⁸ groups, or



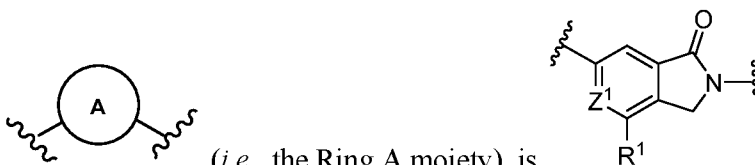
is 4- to 7-membered heterocyclyl or 5- to 8- membered heteroaryl, each of which heterocyclyl or heteroaryl optionally contains 1-2 additional heteroatoms selected from the group consisting of N, O, and S, and each of which heterocyclyl or heteroaryl is optionally substituted by 1-5 **R**⁸ groups;

each **R**⁷ is independently H, C₁-C₆ alkyl, C₁-C₆ alkyl-OH, or C₁-C₆ haloalkyl;

or two **R**⁷ groups are taken together with the carbon atom to which they are attached to form a C₃-C₅ cycloalkyl or 3- to 5- membered heterocyclyl; and

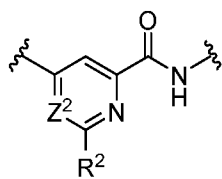
each **R**⁸ is independently halo, C₁-C₆ alkyl, C₁-C₆ alkyl-CN, C₁-C₆ alkyl-OH, C₁-C₆ haloalkyl, -CN, oxo, or -O(C₁-C₆ alkyl);

or two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused C₃-C₅ cycloalkyl or 3- to 5-membered heterocyclyl.

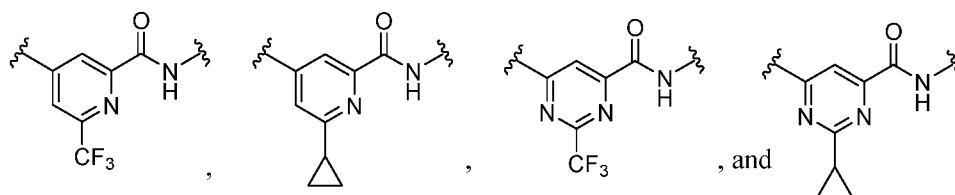
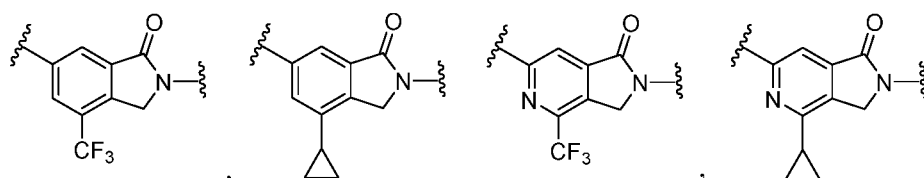


[105] In some embodiments, (i.e., the Ring A moiety), is

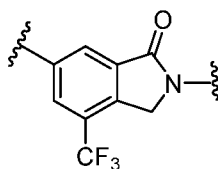
. In some embodiments, **Z**¹ is CH. In other embodiments, **Z**¹ is N. In some embodiments, **R**¹ is -CF₃. In other embodiments, **R**¹ is cyclopropyl. In some embodiments, the Ring A moiety



is . In some embodiments, **Z**² is CH. In other embodiments, **Z**² is N. In some embodiments, **R**² is -CF₃. In other embodiments, **R**² is cyclopropyl. In some embodiments, the Ring A moiety is selected from the group consisting of:

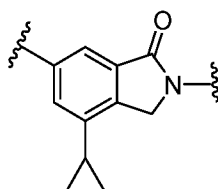


In some



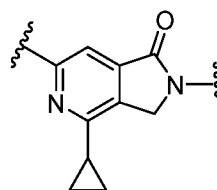
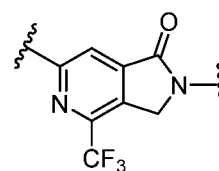
embodiments, the Ring A moiety is

. In some embodiments, the Ring A



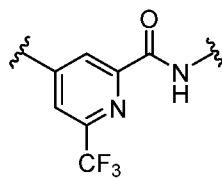
moiety is

. In some embodiments, the Ring A moiety is



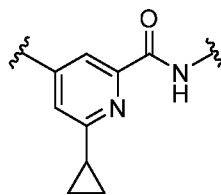
In some embodiments, the Ring A moiety is

. In some embodiments, the

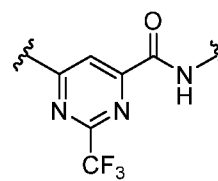


Ring A moiety is

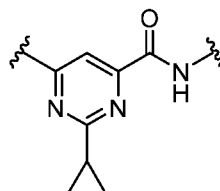
. In some embodiments, the Ring A moiety is



. In some embodiments, the Ring A moiety is



. In some



embodiments, the Ring A moiety is

[106] In some embodiments, R^3 is H, C_1 - C_2 alkyl, or C_1 - C_2 haloalkyl. In some embodiments, R^3 is H, $-CH_3$, or $-CF_3$.

[107] In some embodiments, R^3 is H.

[108] In some embodiments, R^3 is C_1 - C_2 alkyl. In some embodiments, R^3 is methyl. In some embodiments, R^3 is ethyl.

[109] In some embodiments, R^3 is C_1 - C_2 haloalkyl. In some embodiments, R^3 is C_1 - C_2 haloalkyl containing 1-5 halogen atoms. In some embodiments, R^3 is C_1 - C_2 haloalkyl containing 1-3 halogen atoms. In some embodiments, R^3 is C_1 haloalkyl. In some embodiments, R^3 is C_2 haloalkyl. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro, bromo, and fluoro atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro and fluoro atoms. In some embodiments, the halogen atoms are all fluoro atoms. In some embodiments, the halogen atoms are a combination of chloro and fluoro atoms. In some embodiments, R^3 is $-CF_3$, $-CCl_3$, $-CF_2Cl$, $-CFCl_2$, $-CHF_2$, $-CH_2F$, $-CHCl_2$, $-CH_2Cl$, or $-CHFCl$. In some embodiments, R^3 is $-CF_3$.

[110] In some embodiments, R^4 is H, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl, 4- to 8-membered heterocyclyl, or C_3 - C_6 cycloalkyl, wherein the heterocyclyl or cycloalkyl groups are optionally substituted by 1-5 R^6 groups. In some embodiments, R^4 is H, C_1 - C_3 alkyl, C_1 - C_3 haloalkyl, 4- to 6-membered heterocyclyl, or C_4 - C_5 cycloalkyl, wherein the heterocyclyl or cycloalkyl groups are optionally substituted by 1-3 R^6 groups. In some embodiments, R^4 is H, $-CH_3$, -

CF_3 , cyclobutyl, or .

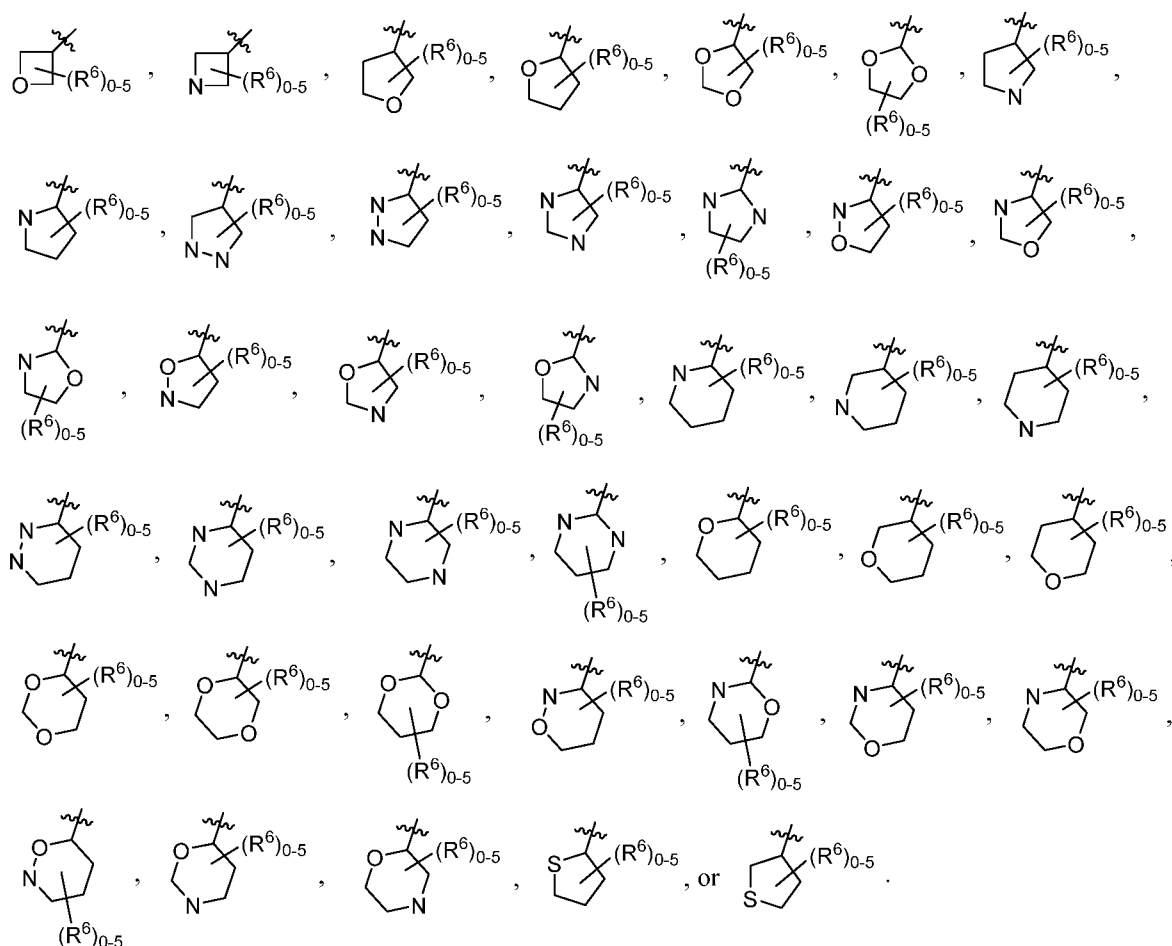
[111] In some embodiments, R^4 is H.

[112] In some embodiments, R^4 is C_1 - C_6 alkyl. In some embodiments, R^4 is C_1 - C_3 alkyl. In some embodiments, R^4 is methyl, ethyl, *n*-propyl, or isopropyl. In some embodiments, R^4 is $-CH_3$.

[113] In some embodiments, R^4 is C_1 - C_6 haloalkyl. In some embodiments, R^4 is C_1 - C_6 haloalkyl containing 1-7 halogen atoms. In some embodiments, R^4 is C_1 - C_3 haloalkyl. In some embodiments, R^4 is C_1 - C_3 haloalkyl containing 1-7 halogen atoms. In some embodiments, R^4 is C_1 - C_2 haloalkyl containing 1-5 halogen atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro, bromo, and fluoro atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro and fluoro atoms. In some embodiments, the halogen atoms are all

fluoro atoms. In some embodiments, the halogen atoms are a combination of chloro and fluoro atoms. In some embodiments, R^4 is $-CF_3$, $-CCl_3$, $-CF_2Cl$, $-CFC_2$, $-CHF_2$, $-CH_2F$, $-CHCl_2$, $-CH_2F$, or $-CHFCl$. In some embodiments, R^4 is $-CF_3$.

[114] In some embodiments, R^4 is 4- to 8-membered heterocyclyl optionally substituted by 1-5 R^6 groups. In some embodiments, R^4 is 4- to 6-membered heterocyclyl optionally substituted by 1-3 R^6 groups. In some embodiments, R^4 is a 4-membered heterocyclyl optionally substituted by 1-2 R^6 groups. In some embodiments, the heterocyclyl is substituted by 5 R^6 groups. In some embodiments, the heterocyclyl is substituted by 4 R^6 groups. In some embodiments, the heterocyclyl is substituted by 3 R^6 groups. In some embodiments, the heterocyclyl is substituted by 2 R^6 groups. In some embodiments, the heterocyclyl is substituted by 1 R^6 group. In some embodiments, the heterocyclyl is unsubstituted. In some embodiments, the heterocyclyl contains 1-3 heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the heterocyclyl contains one nitrogen atom. In some embodiments, the heterocyclyl contains two nitrogen atoms. In some embodiments, the heterocyclyl contains one oxygen atom. In some embodiments, the heterocyclyl contains two oxygen atoms. In some embodiments, the heterocyclyl contains one oxygen atom and one nitrogen atom. In some embodiments, the heterocyclyl contains one sulfur atom. In some embodiments, the heterocyclyl contains one nitrogen atom and one sulfur atom. In some embodiments, R^4 is oxetanyl, azetidiny, tetrahydrofuranyl, dioxolanyl, pyrrolidinyl, pyrazolidinyl, piperidinyl, isoxazolidinyl, or tetrahydropyranyl, each of which is optionally substituted by 1-5 R^6 groups. In some embodiments, R^4 is:



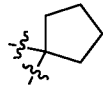
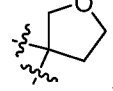
In some embodiments, $\mathbf{R}^4$ is .

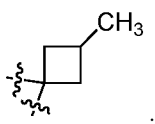
[115] In some embodiments, $\mathbf{R}^4$ is C₃-C₆ cycloalkyl optionally substituted by 1-5 $\mathbf{R}^6$ groups. In some embodiments, $\mathbf{R}^4$ is C₄-C₅ cycloalkyl optionally substituted by 1-3 $\mathbf{R}^6$ groups. In some embodiments, the cycloalkyl is substituted by 5 $\mathbf{R}^6$ groups. In some embodiments, the cycloalkyl is substituted by 4 $\mathbf{R}^6$ groups. In some embodiments, the cycloalkyl is substituted by 3 $\mathbf{R}^6$ groups. In some embodiments, the cycloalkyl is substituted by 2 $\mathbf{R}^6$ groups. In some embodiments, the cycloalkyl is substituted by 1 $\mathbf{R}^6$ group. In some embodiments, the cycloalkyl is unsubstituted. In some embodiments, $\mathbf{R}^4$ is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, each of which is optionally substituted by 1-5 $\mathbf{R}^6$ groups. In some embodiments, $\mathbf{R}^4$ is cyclopropyl or cyclobutyl. In some embodiments, $\mathbf{R}^4$ is cyclobutyl.

[116] In some embodiments, $\mathbf{R}^3$ and $\mathbf{R}^4$ are taken together with the carbon atom to which they are attached to form C₃-C₅ cycloalkyl or 4- to 6-membered heterocyclyl, each of which is optionally substituted by 1-5 $\mathbf{R}^6$ groups. In some embodiments, $\mathbf{R}^3$ and $\mathbf{R}^4$ are taken together with the carbon atom to which they are attached to form C₄-C₅ cycloalkyl or 4- to 6-membered

heterocyclyl, each of which is optionally substituted by 1-3 R^6 groups. In some embodiments,

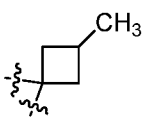
R^3 and R^4 are taken together with the carbon atom to which they are attached to form ,

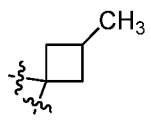
, , or , each of which is optionally substituted by 1-3 R^6 groups. In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are

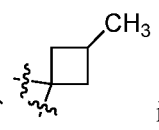
attached, and are substituted by 1 R^6 group which is methyl, to form .

[117] In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are attached to form C₃-C₅ cycloalkyl optionally substituted by 1-5 R^6 groups. In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are attached to form C₄-C₅ cycloalkyl optionally substituted by 1-3 R^6 groups. In some embodiments, the cycloalkyl is substituted by 5 R^6 groups. In some embodiments, the cycloalkyl is substituted by 4 R^6 groups. In some embodiments, the cycloalkyl is substituted by 3 R^6 groups. In some embodiments, the cycloalkyl is substituted by 2 R^6 groups. In some embodiments, the cycloalkyl is substituted by 1 R^6 group. In some embodiments, the cycloalkyl is unsubstituted. In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are

attached to form  or , each of which is optionally substituted by 1-3 R^6 groups. In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are

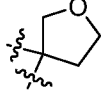
attached, and are substituted by 1 R^6 group which is methyl, to form . In some embodiments, the absolute stereochemistry at the carbon atom to which the methyl group of

 is attached is (*R*)- (using the Cahn–Ingold–Prelog rules). In some embodiments,

the absolute stereochemistry at the carbon atom to which the methyl group of  is attached is (*S*)-.

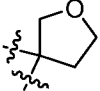
[118] In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are attached to form 4- to 6-membered heterocyclyl optionally substituted by 1-5 R^6 groups.

In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are attached to form 4- to 6-membered heterocyclyl optionally substituted by 1-3 R^6 groups. In some embodiments, the heterocyclyl is substituted by 5 R^6 groups. In some embodiments, the heterocyclyl is substituted by 4 R^6 groups. In some embodiments, the heterocyclyl is substituted by 3 R^6 groups. In some embodiments, the heterocyclyl is substituted by 2 R^6 groups. In some embodiments, the heterocyclyl is substituted by 1 R^6 group. In some embodiments, the heterocyclyl is unsubstituted. In some embodiments, the heterocyclyl contains 1-3 heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the heterocyclyl contains one nitrogen atom. In some embodiments, the heterocyclyl contains two nitrogen atoms. In some embodiments, the heterocyclyl contains one oxygen atom. In some embodiments, the heterocyclyl contains two oxygen atoms. In some embodiments, the heterocyclyl contains one oxygen atom and one nitrogen atom. In some embodiments, the heterocyclyl contains one sulfur atom. In some embodiments, the heterocyclyl contains one nitrogen atom and one sulfur atom. In some embodiments, R^4 is oxetanyl, azetidiny, tetrahydrofuranyl, dioxolanyl, pyrrolidinyl, pyrazolidinyl, piperidinyl, isoxazolidinyl, or tetrahydropyranyl, each of which is optionally substituted by 1-5 R^6 groups. In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are

attached to form  or , each of which is optionally substituted by 1-3 R^6 groups.

In some embodiments, R^3 and R^4 are taken together with the carbon atom to which they are

attached to form . In some embodiments, R^3 and R^4 are taken together with the carbon

atom to which they are attached to form .

[119] In some embodiments, each R^6 is independently C_1 - C_6 alkyl, halo, hydroxy, -O-(C_1 - C_6 alkyl), -CN, C_1 - C_6 alkyl-CN, C_1 - C_6 alkyl-OH, or C_1 - C_6 haloalkyl. In some embodiments, each R^6 is independently C_1 - C_3 alkyl, halo, hydroxy, -O-(C_1 - C_3 alkyl), -CN, C_1 - C_3 alkyl-CN, C_1 - C_3 alkyl-OH, or C_1 - C_3 haloalkyl. In some embodiments, each R^6 is independently -CH₃, fluoro (F), hydroxy, -OCH₃, -CN, -CH₂CN, -CH₂OH, or -CF₃.

[120] In some embodiments, R^6 is C_1 - C_6 alkyl. In some embodiments, R^6 is C_1 - C_3 alkyl. In some embodiments, R^6 is methyl, ethyl, *n*-propyl, or isopropyl. In some embodiments, R^6 is -CH₃.

[121] In some embodiments, R^6 is halo. In some embodiments, R^6 is chloro, fluoro, or bromo. In some embodiments, R^6 is chloro or fluoro. In some embodiments, R^7 is fluoro.

[122] In some embodiments, R^6 is hydroxyl.

[123] In some embodiments, R^6 is $-O(C_1-C_6 \text{ alkyl})$. In some embodiments, R^6 is $-O-(C_1-C_3 \text{ alkyl})$. In some embodiments, R^6 is $-O(\text{methyl})$, $-O(\text{ethyl})$, $-O(n\text{-propyl})$, or $-O(\text{isopropyl})$. In some embodiments, R^6 is $-OCH_3$ or $-OCH_2CH_3$. In some embodiments, R^6 is $-OCH_3$.

[124] In some embodiments, R^6 is $-CN$. In some embodiments, R^6 is $C_1-C_6 \text{ alkyl-CN}$. In some embodiments, R^6 is $C_1-C_3 \text{ alkyl-CN}$. In some embodiments, R^6 is $-CH_2CN$, $-CH_2CH_2-CN$, $-CH_2CH_2CH_2-CN$, or $-C(CH_3)_2-CN$. In some embodiments, R^6 is $-CH_2CN$.

[125] In some embodiments, R^6 is $C_1-C_6 \text{ alkyl-OH}$. In some embodiments, R^6 is $C_1-C_3 \text{ alkyl-OH}$. In some embodiments, R^6 is $-CH_2OH$, $-CH_2CH_2-OH$, $-CH_2CH_2CH_2-OH$, or $-C(CH_3)_2-OH$. In some embodiments, R^6 is $-CH_2OH$.

[126] In some embodiments, R^6 is $C_1-C_6 \text{ haloalkyl}$. In some embodiments, R^6 is $C_1-C_6 \text{ haloalkyl}$ containing 1-7 halogen atoms. In some embodiments, R^6 is $C_1-C_3 \text{ haloalkyl}$. In some embodiments, R^6 is $C_1-C_3 \text{ haloalkyl}$ containing 1-7 halogen atoms. In some embodiments, R^6 is $C_1-C_3 \text{ haloalkyl}$ containing 1-5 halogen atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro, bromo, and fluoro atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro and fluoro atoms. In some embodiments, the halogen atoms are all fluoro atoms. In some embodiments, the halogen atoms are a combination of chloro and fluoro atoms. In some embodiments, R^6 is $-CF_3$, $-CCl_3$, $-CF_2Cl$, $-CFCl_2$, $-CHF_2$, $-CH_2F$, $-CHCl_2$, $-CH_2Cl$, or $-CHFCl$. In some embodiments, R^6 is $-CF_3$.

[127] In some embodiments, two R^6 groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro C_3-C_6 cycloalkyl or spiro 4- to 6-membered heterocyclyl. In some embodiments, two R^6 groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro C_3-C_6 cycloalkyl or spiro 4- to 5-membered heterocyclyl.

[128] In some embodiments, two R^6 groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro C_3-C_6 cycloalkyl. In some embodiments, two R^6 groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro C_3-C_5 cycloalkyl. In some embodiments, two R^6 groups attached to the same carbon atom are taken together with the

carbon atom to which they are attached to form a spiro C₃-C₄ cycloalkyl. In some embodiments, two R⁶ groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro cyclopropyl, cyclobutyl, or cyclopentyl. In some embodiments, two R⁶ groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro cyclopropyl.

[129] In some embodiments, two R⁶ groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro 4- to 6-membered heterocyclyl. In some embodiments, two R⁶ groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro 4- to 5-membered heterocyclyl. In some embodiments, the heterocyclyl contains 1-3 heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the heterocyclyl contains one nitrogen atom. In some embodiments, the heterocyclyl contains two nitrogen atoms. In some embodiments, the heterocyclyl contains one oxygen atom. In some embodiments, the heterocyclyl contains two oxygen atoms. In some embodiments, the heterocyclyl contains one oxygen atom and one nitrogen atom. In some embodiments, the heterocyclyl contains one sulfur atom. In some embodiments, the heterocyclyl contains one nitrogen atom and one sulfur atom. In some embodiments, two R⁶ groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro oxetanyl, azetidiny, tetrahydrofuranyl, dioxolanyl, pyrrolidinyl, pyrazolidinyl, piperidinyl, isoxazolidinyl, or tetrahydropyranyl.

[130] In some embodiments, R⁵ is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₃-C₆ cycloalkyl. In some embodiments, R⁵ is H, C₁-C₃ alkyl, C₁-C₃ haloalkyl, or C₃-C₄ cycloalkyl. In some embodiments, R⁵ is H, -CH₃, -CHF₂, or cyclopropyl.

[131] In some embodiments, R⁵ is H.

[132] In some embodiments, R⁵ is C₁-C₆ alkyl. In some embodiments, R⁵ is C₁-C₃ alkyl. In some embodiments, R⁵ is methyl, ethyl, *n*-propyl, or isopropyl. In some embodiments, R⁵ is -CH₃.

[133] In some embodiments, R⁵ is C₁-C₆ haloalkyl. In some embodiments, R⁵ is C₁-C₆ haloalkyl containing 1-7 halogen atoms. In some embodiments, R⁵ is C₁-C₃ haloalkyl. In some embodiments, R⁵ is C₁-C₃ haloalkyl containing 1-7 halogen atoms. In some embodiments, R⁵ is C₁-C₃ haloalkyl containing 1-5 halogen atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro, bromo, and fluoro atoms. In some embodiments, the halogen atoms are independently selected from the

group consisting of chloro and fluoro atoms. In some embodiments, the halogen atoms are all fluoro atoms. In some embodiments, the halogen atoms are a combination of chloro and fluoro atoms. In some embodiments, **R**⁵ is -CF₃, -CCl₃, -CF₂Cl, -CFCl₂, -CHF₂, -CH₂F, -CHCl₂, -CH₂Cl, or -CHFCl. In some embodiments, **R**⁵ is -CHF₂.

[134] In some embodiments, **R**⁵ is C₃-C₆ cycloalkyl. In some embodiments, **R**⁵ is C₃-C₅ cycloalkyl. In some embodiments, **R**⁵ is C₃-C₄ cycloalkyl. In some embodiments, **R**⁵ is cyclopropyl, cyclobutyl, or cyclopentyl. In some embodiments, **R**⁵ is cyclopropyl.

[135] In some embodiments, **X** is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, C₁-C₆ alkyl-OH, C₁-C₆ alkyl-CN, or C₃-C₆ cycloalkyl optionally substituted by 1-5 **R**⁸ groups. In some embodiments, **X** is H, C₁-C₃ alkyl, C₁-C₃ haloalkyl, C₁-C₃ alkyl-OH, C₁-C₃ alkyl-CN, or C₃-C₅ cycloalkyl optionally substituted by 1-3 **R**⁸ groups. In some embodiments, **X** is H or -CH₃.

[136] In some embodiments, **X** is H.

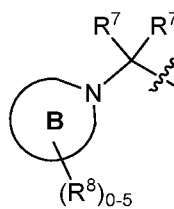
[137] In some embodiments, **X** is C₁-C₆ alkyl. In some embodiments, **X** is C₁-C₃ alkyl. In some embodiments, **X** is methyl, ethyl, *n*-propyl, or isopropyl. In some embodiments, **X** is -CH₃.

[138] In some embodiments, **X** is C₁-C₆ haloalkyl. In some embodiments, **X** is C₁-C₆ haloalkyl containing 1-7 halogen atoms. In some embodiments, **X** is C₁-C₃ haloalkyl. In some embodiments, **X** is C₁-C₃ haloalkyl containing 1-7 halogen atoms. In some embodiments, **X** is C₁-C₃ haloalkyl containing 1-5 halogen atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro, bromo, and fluoro atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro and fluoro atoms. In some embodiments, the halogen atoms are all fluoro atoms. In some embodiments, the halogen atoms are a combination of chloro and fluoro atoms. In some embodiments, **X** is -CF₃, -CCl₃, -CF₂Cl, -CFCl₂, -CHF₂, -CH₂F, -CHCl₂, -CH₂Cl, or -CHFCl. In some embodiments, **X** is -CF₃.

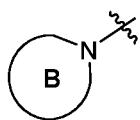
[139] In some embodiments, **X** is C₁-C₆ alkyl-OH. In some embodiments, **X** is C₁-C₃ alkyl-OH. In some embodiments, **X** is -CH₂OH, -CH₂CH₂-OH, -CH₂CH₂CH₂-OH, or -C(CH₃)₂-OH. In some embodiments, **X** is -CH₂OH.

[140] In some embodiments, **X** is C₁-C₆ alkyl-CN. In some embodiments, **X** is C₁-C₃ alkyl-CN. In some embodiments, **X** is -CH₂CN, -CH₂CH₂-CN, -CH₂CH₂CH₂-CN, or -C(CH₃)₂-CN. In some embodiments, **X** is -CH₂CN.

[141] In some embodiments, **X** is C₃-C₆ cycloalkyl optionally substituted by 1-5 **R**⁸ groups. In some embodiments, **X** is C₃-C₅ cycloalkyl optionally substituted by 1-3 **R**⁸ groups. In some embodiments, the cycloalkyl is substituted by 5 **R**⁸ groups. In some embodiments, the cycloalkyl is substituted by 4 **R**⁸ groups. In some embodiments, the cycloalkyl is substituted by 3 **R**⁸ groups. In some embodiments, the cycloalkyl is substituted by 2 **R**⁸ groups. In some embodiments, the cycloalkyl is substituted by 1 **R**⁸ group. In some embodiments, the cycloalkyl is unsubstituted. In some embodiments, **X** is cyclopropyl, cyclobutyl, or cyclopentyl, each of which is optionally substituted by 1-5 **R**⁸ groups. In some embodiments, **X** is cyclopropyl.



[142] In some embodiments, **X** is $(R^8)_{0-5}$, wherein the Ring B moiety, shown as



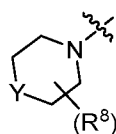
, is 4- to 7-membered heterocyclyl or 5- to 8- membered heteroaryl, each of which heterocyclyl or heteroaryl optionally contains 1-2 additional heteroatoms selected from the group consisting of N, O, and S, and each of which heterocyclyl or heteroaryl is optionally substituted by 1-5 **R**⁸ groups. In some embodiments, the Ring B moiety is 4- to 6-membered heterocyclyl or 5- to 6- membered heteroaryl, each of which heterocyclyl or heteroaryl optionally contains 1-2 additional heteroatoms selected from the group consisting of N, O, and S, and each of which heterocyclyl or heteroaryl is optionally substituted by 1-5 **R**⁸ groups. In some embodiments, the Ring B moiety is 4- to 5-membered heterocyclyl or 5- to 6- membered heteroaryl, each of which heterocyclyl or heteroaryl optionally contains one additional heteroatom selected from the group consisting of N and O, and each of which heterocyclyl or heteroaryl is optionally substituted by 1-5 **R**⁸ groups.

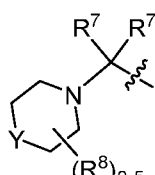
[143] In some embodiments, the Ring B moiety is 4- to 7-membered heterocyclyl optionally containing 1-2 additional heteroatoms selected from the group consisting of N, O, and S, wherein the heterocyclyl is optionally substituted by 1-5 **R**⁸ groups. In some embodiments, the Ring B moiety is 4- to 6-membered heterocyclyl optionally containing 1-2 additional heteroatoms selected from the group consisting of N, O, and S, wherein the heterocyclyl is optionally substituted by 1-5 **R**⁸ groups. In some embodiments, the Ring B moiety is 4- to 5-membered heterocyclyl optionally containing one additional heteroatom selected from the

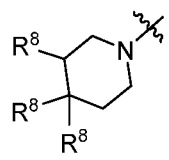
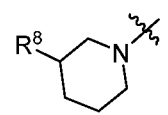
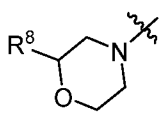
group consisting of N and O, wherein the heterocyclyl is optionally substituted by 1-5 R^8 groups. In some embodiments, the heterocyclyl is substituted by 5 R^8 groups. In some embodiments, the heterocyclyl is substituted by 4 R^8 groups. In some embodiments, the heterocyclyl is substituted by 3 R^8 groups. In some embodiments, the heterocyclyl is substituted by 2 R^8 groups. In some embodiments, the heterocyclyl is substituted by one R^8 group. In some embodiments, the heterocyclyl is unsubstituted. In some embodiments, the heterocyclyl contains 1-2 additional heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the heterocyclyl contains one additional nitrogen atom. In some embodiments, the heterocyclyl contains two additional nitrogen atoms. In some embodiments, the heterocyclyl further contains one oxygen atom. In some embodiments, the heterocyclyl further contains two oxygen atoms. In some embodiments, the heterocyclyl further contains one oxygen atom and one nitrogen atom. In some embodiments, the heterocyclyl further contains one sulfur atom. In some embodiments, the heterocyclyl does not contain additional heteroatoms. In some embodiments, the heterocyclyl is azetidiny, pyrrolidinyl, pyrazolidinyl, piperidinyl, or isoxazolidinyl, each of which is optionally substituted by 1-5 R^8 groups.

[144] In some embodiments, the Ring B moiety is 5- to 8-membered heteroaryl optionally containing 1-2 additional heteroatoms selected from the group consisting of N, O, and S, wherein the heteroaryl is optionally substituted by 1-5 R^8 groups. In some embodiments, the Ring B moiety is 5- to 6-membered heteroaryl optionally containing 1-2 additional heteroatoms selected from the group consisting of N, O, and S, wherein the heteroaryl is optionally substituted by 1-5 R^8 groups. In some embodiments, the Ring B moiety is 5- to 6-membered heteroaryl optionally containing one additional heteroatom selected from the group consisting of N and O, wherein the heteroaryl is optionally substituted by 1-5 R^8 groups. In some embodiments, the heteroaryl is substituted by 5 R^8 groups. In some embodiments, the heteroaryl is substituted by 4 R^8 groups. In some embodiments, the heteroaryl is substituted by 3 R^8 groups. In some embodiments, the heteroaryl is substituted by 2 R^8 groups. In some embodiments, the heteroaryl is substituted by one R^8 group. In some embodiments, the heteroaryl is unsubstituted. In some embodiments, the heteroaryl contains 1-2 additional heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the heteroaryl contains one additional nitrogen atom. In some embodiments, the heteroaryl contains two additional nitrogen atoms. In some embodiments, the heteroaryl further contains one oxygen atom. In some embodiments, the heteroaryl further contains two

oxygen atoms. In some embodiments, the heteroaryl further contains one oxygen atom and one additional nitrogen atom. In some embodiments, the heteroaryl further contains one sulfur atom. In some embodiments, the heteroaryl does not contain additional heteroatoms. In some embodiments, the heteroaryl is pyrrolyl, imidazolyl, pyrazolyl, isoxazolyl, oxazolyl, thiazolyl, isothiazolyl, triazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, or triazyl, each of which is optionally substituted by 1-5 R^8 groups.

[145] In some embodiments, the Ring B moiety is  wherein Y is O, CH_2 , CHR^8 ,

or $C(R^8)_2$, and X is . In some embodiments, Y is oxygen (O). In other embodiments, Y is CH_2 , CHR^8 , or $C(R^8)_2$. In some embodiments, Y is CH_2 . In some embodiments, Y is CHR^8 . In some embodiments, Y is $C(R^8)_2$. In some embodiments, the Ring B moiety is substituted by a total of 1-5 R^8 groups. In some embodiments, the Ring B moiety is substituted by a total of 1-3 R^8 groups. As such, if Y is CHR^8 , then the Ring B moiety can be substituted by up to 4 additional R^8 groups. Similarly, if Y is $CH(R^8)_2$, then the Ring B moiety can be substituted by up to 3 additional R^8 groups. In some embodiments, the Ring B moiety is substituted by 5 R^8 groups. In some embodiments, the Ring B moiety is substituted by 4 R^8 groups. In some embodiments, the Ring B moiety is substituted by 3 R^8 groups. In some embodiments, the Ring B moiety is substituted by 2 R^8 groups. In some embodiments, the Ring B moiety is substituted by one R^8 group. In some embodiments, the Ring B moiety is unsubstituted. In some embodiments, the Ring B moiety is

, , or , wherein each R^8 is independently as described herein.

[146] In some embodiments, each R^7 is independently H, C_1 - C_6 alkyl, C_1 - C_6 alkyl-OH, or C_1 - C_6 haloalkyl. In some embodiments, each R^7 is independently H, C_1 - C_3 alkyl, C_1 - C_3 alkyl-OH, or C_1 - C_3 haloalkyl. In some embodiments, each R^7 is independently H, $-CH_3$, $-CH_2OH$, or $-CF_3$.

[147] In some embodiments, both R^7 groups are hydrogen (H). In some embodiments, one R^7 group is H. In some embodiments, one R^7 group is H, and the other R^7 group is C₁-C₆ alkyl, C₁-C₆ alkyl-OH, or C₁-C₆ haloalkyl. In some embodiments, one R^7 group is H and the other R^7 group is C₁-C₆ alkyl. In some embodiments, one R^7 group is H and the other R^7 group is C₁-C₃ alkyl. In some embodiments, one R^7 group is H and the other R^7 group is -CH₃.

[148] In some embodiments, R^7 is C₁-C₆ alkyl. In some embodiments, R^7 is C₁-C₃ alkyl. In some embodiments, R^7 is methyl, ethyl, *n*-propyl, or isopropyl. In some embodiments, one R^7 group is methyl, ethyl, *n*-propyl, or isopropyl, and the other R^7 group is H. In some embodiments, R^7 is -CH₃.

[149] In some embodiments, R^7 is C₁-C₆ alkyl-OH. In some embodiments, R^7 is C₁-C₃ alkyl-OH. In some embodiments, R^7 is -CH₂OH, -CH₂CH₂-OH, -CH₂CH₂CH₂-OH, or -C(CH₃)₂-OH. In some embodiments, R^7 is -CH₂OH. In some embodiments, one R^7 group is C₁-C₆ alkyl-OH, and the other R^7 group is H. In some embodiments, one R^7 group is C₁-C₃ alkyl-OH, and the other R^7 group is H. In some embodiments, one R^7 group is -CH₂OH, -CH₂CH₂-OH, -CH₂CH₂CH₂-OH, or -C(CH₃)₂-OH, and the other R^7 group is H. In some embodiments, one R^7 group is -CH₂OH, and the other R^7 group is H.

[150] In some embodiments, R^7 is C₁-C₆ haloalkyl. In some embodiments, R^7 is C₁-C₆ haloalkyl containing 1-7 halogen atoms. In some embodiments, R^7 is C₁-C₃ haloalkyl. In some embodiments, R^7 is C₁-C₃ haloalkyl containing 1-7 halogen atoms. In some embodiments, R^7 is C₁-C₃ haloalkyl containing 1-5 halogen atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro, bromo, and fluoro atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro and fluoro atoms. In some embodiments, the halogen atoms are all fluoro atoms. In some embodiments, the halogen atoms are a combination of chloro and fluoro atoms. In some embodiments, R^7 is -CF₃, -CCl₃, -CF₂Cl, -CFCl₂, -CHF₂, -CH₂F, -CHCl₂, -CH₂F, or -CHFCl. In some embodiments, R^7 is -CF₃.

[151] In some embodiments, two R^7 groups are taken together with the carbon atom to which they are attached to form C₃-C₅ cycloalkyl or 3- to 5- membered heterocyclyl. In some embodiments, two R^7 groups are taken together with the carbon atom to which they are attached to form cyclopropyl or oxetanyl.

[152] In some embodiments, two R^7 groups are taken together with the carbon atom to which they are attached to form C₃-C₅ cycloalkyl. In some embodiments, two R^7 groups are taken together with the carbon atom to which they are attached to form cyclopropyl or cyclobutyl.

In some embodiments, two R^7 groups are taken together with the carbon atom to which they are attached to form cyclopropyl.

[153] In some embodiments, two R^7 groups are taken together with the carbon atom to which they are attached to form 3- to 5- membered heterocyclyl. In some embodiments, two R^7 groups are taken together with the carbon atom to which they are attached to form 3- to 4- membered heterocyclyl. In some embodiments, the heterocyclyl contains 1-3 heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the heterocyclyl contains one nitrogen atom. In some embodiments, the heterocyclyl contains two nitrogen atoms. In some embodiments, the heterocyclyl contains one oxygen atom. In some embodiments, the heterocyclyl contains two oxygen atoms. In some embodiments, the heterocyclyl contains one oxygen atom and one nitrogen atom. In some embodiments, the heterocyclyl contains one sulfur atom. In some embodiments, the heterocyclyl contains one nitrogen atom and one sulfur atom. In some embodiments, R^7 is aziridinyl, oxiranyl, oxetanyl, azetidiny, tetrahydrofuranyl, dioxolanyl, pyrrolidinyl, pyrazolidinyl, or isoxazolidinyl.

[154] In some embodiments, each R^8 is independently halo, C_1 - C_6 alkyl, C_1 - C_6 alkyl-CN, C_1 - C_6 alkyl-OH, C_1 - C_6 haloalkyl, -CN, oxo, or -O(C_1 - C_6 alkyl). In some embodiments, each R^8 is independently halo, C_1 - C_3 alkyl, C_1 - C_3 alkyl-CN, C_1 - C_3 alkyl-OH, C_1 - C_3 haloalkyl, -CN, oxo, or -O(C_1 - C_3 alkyl). In some embodiments, each R^8 is independently fluoro (F), -CH₃, -CH₂CH₃, -CH₂CN, -CH₂OH, -CF₃, -CN, oxo, or -OCH₃.

[155] In some embodiments, R^8 is halo. In some embodiments, R^8 is chloro, fluoro, or bromo. In some embodiments, R^8 is chloro or fluoro. In some embodiments, R^8 is fluoro.

[156] In some embodiments, R^8 is C_1 - C_6 alkyl. In some embodiments, R^8 is C_1 - C_3 alkyl. In some embodiments, R^8 is methyl, ethyl, *n*-propyl, or isopropyl. In some embodiments, R^8 is -CH₃ or -CH₂CH₃.

[157] In some embodiments, R^8 is -CN. In some embodiments, R^8 is C_1 - C_6 alkyl-CN. In some embodiments, R^8 is C_1 - C_3 alkyl-CN. In some embodiments, R^8 is -CH₂CN, -CH₂CH₂-CN, -CH₂CH₂CH₂-CN, or -C(CH₃)₂-CN. In some embodiments, R^8 is -CH₂CN.

[158] In some embodiments, R^8 is C_1 - C_6 alkyl-OH. In some embodiments, R^8 is C_1 - C_3 alkyl-OH. In some embodiments, R^8 is -CH₂OH, -CH₂CH₂-OH, -CH₂CH₂CH₂-OH, or -C(CH₃)₂-OH. In some embodiments, R^8 is -CH₂OH.

[159] In some embodiments, R^8 is C_1 - C_6 haloalkyl. In some embodiments, R^8 is C_1 - C_6 haloalkyl containing 1-7 halogen atoms. In some embodiments, R^8 is C_1 - C_3 haloalkyl. In some embodiments, R^8 is C_1 - C_3 haloalkyl containing 1-7 halogen atoms. In some

embodiments, **R**⁸ is C₁-C₃ haloalkyl containing 1-5 halogen atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro, bromo, and fluoro atoms. In some embodiments, the halogen atoms are independently selected from the group consisting of chloro and fluoro atoms. In some embodiments, the halogen atoms are all fluoro atoms. In some embodiments, the halogen atoms are a combination of chloro and fluoro atoms. In some embodiments, **R**⁸ is -CF₃, -CCl₃, -CF₂Cl, -CFCl₂, -CHF₂, -CH₂F, -CHCl₂, -CH₂F, or -CHFCl. In some embodiments, **R**⁸ is -CF₃.

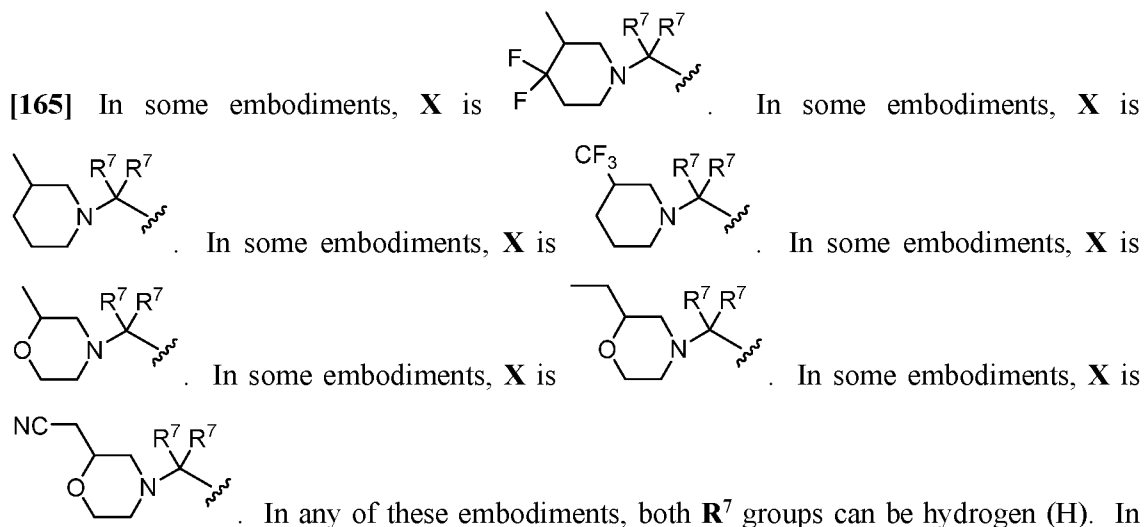
[160] In some embodiments, **R**⁸ is oxo.

[161] In some embodiments, **R**⁸ is -O(C₁-C₆ alkyl). In some embodiments, **R**⁸ is -O-(C₁-C₃ alkyl). In some embodiments, **R**⁸ is -O(methyl), -O(ethyl), -O(*n*-propyl), or -O(isopropyl). In some embodiments, **R**⁸ is -OCH₃ or -OCH₂CH₃. In some embodiments, **R**⁸ is -OCH₃.

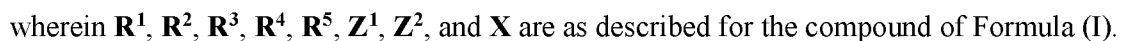
[162] In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused C₃-C₅ cycloalkyl or 3- to 5-membered heterocyclyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused cyclopropyl or oxetanyl.

[163] In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused C₃-C₅ cycloalkyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro C₃-C₅ cycloalkyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro cyclopropyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro cyclobutyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro cyclopentyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a fused C₃-C₅ cycloalkyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a fused cyclopropyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a fused cyclobutyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a fused cyclopentyl. In some embodiments, two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused cyclopropyl.

[164] In some embodiments, two R^8 groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused 3- to 5-membered heterocyclyl. In some embodiments, two R^8 groups are taken together with the carbon atom or atoms to which they are attached to form a spiro 3- to 5-membered heterocyclyl. In some embodiments, two R^8 groups are taken together with the carbon atom or atoms to which they are attached to form a spiro oxetanyl. In some embodiments, two R^8 groups are taken together with the carbon atom or atoms to which they are attached to form a fused 3- to 5-membered heterocyclyl. In some embodiments, two R^8 groups are taken together with the carbon atom or atoms to which they are attached to form a fused oxetanyl. In some embodiments, two R^8 groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused oxetanyl. In some embodiments, the heterocyclyl contains 1-3 heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the heterocyclyl contains one nitrogen atom. In some embodiments, the heterocyclyl contains two nitrogen atoms. In some embodiments, the heterocyclyl contains one oxygen atom. In some embodiments, the heterocyclyl contains two oxygen atoms. In some embodiments, the heterocyclyl contains one oxygen atom and one nitrogen atom. In some embodiments, the heterocyclyl contains one sulfur atom. In some embodiments, the heterocyclyl contains one nitrogen atom and one sulfur atom. In some embodiments, two R^8 groups are taken together with the carbon atom to which they are attached to form a spiro aziridiny, oxiranyl, oxetanyl, azetidiny, tetrahydrofuranyl, dioxolanyl, pyrrolidiny, pyrazolidiny, or isoxazolidiny. In some embodiments, two R^8 groups are taken together with the carbon atoms to which they are attached to form a fused aziridiny, oxiranyl, oxetanyl, azetidiny, tetrahydrofuranyl, dioxolanyl, pyrrolidiny, pyrazolidiny, or isoxazolidiny.



[166] In some embodiments, the compound is of Formula (I-A) or (I-B):



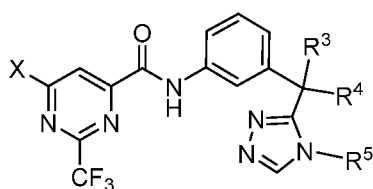
wherein **R**¹, **R**², **R**⁵, **R**⁶, **Z**¹, **Z**², and **X** are as described for the compound of Formula (I).



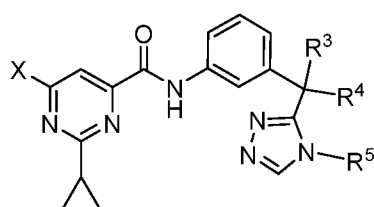
 (I-E)



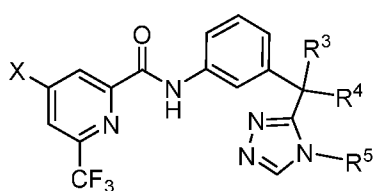
 (I-F)



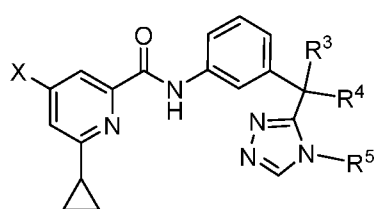
(I-G)



(I-H)



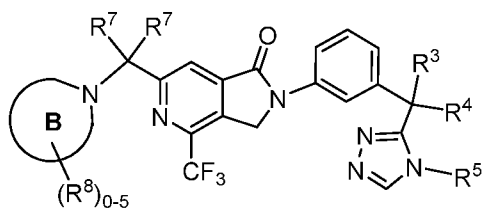
(I-I)



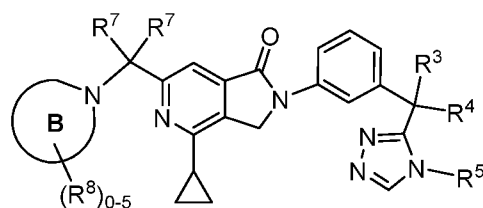
(I-J)

wherein R^3 , R^4 , R^5 , and X are as described for the compound of Formula (I).

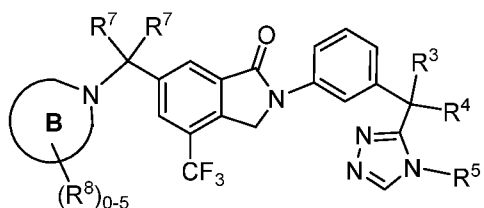
[169] In some embodiments, the compound is of Formula (II-A), (II-B), (II-C), (II-D), (II-E), (II-F), (II-G), or (II-H):



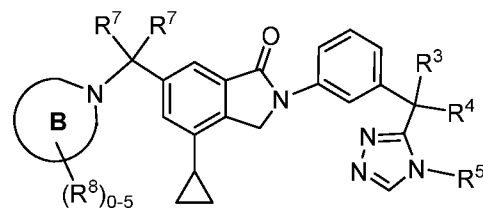
(II-A)



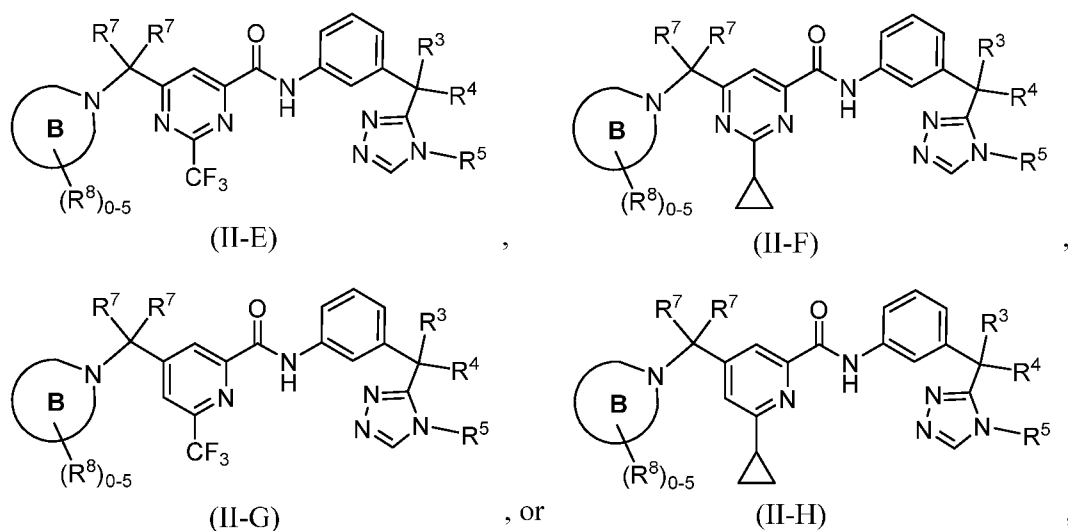
(II-B)



(II-C)

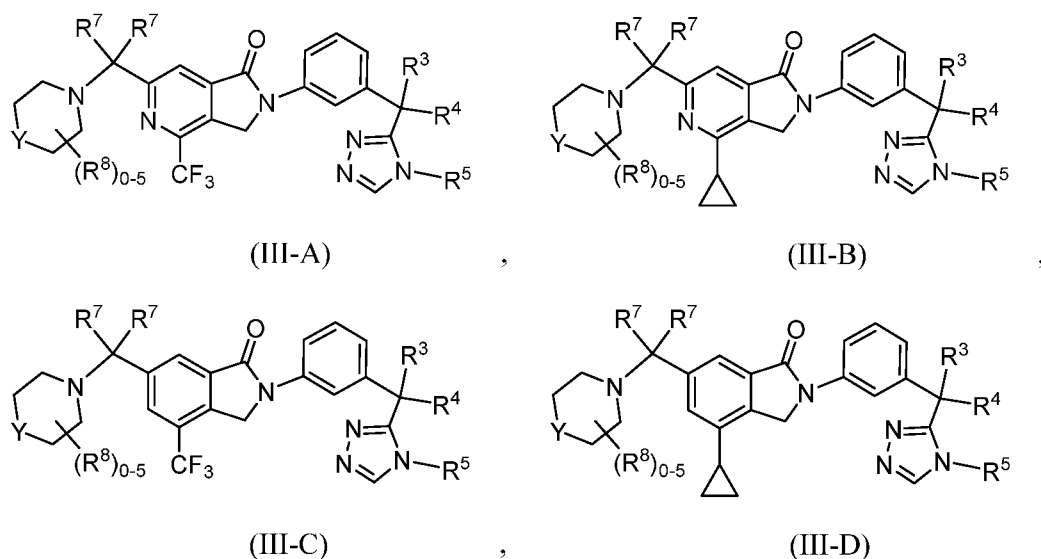


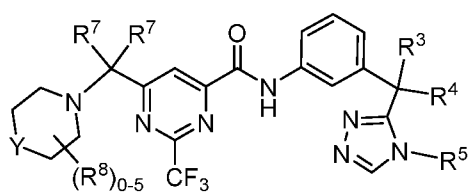
(II-D)



wherein R^3 , R^4 , R^5 , R^7 , R^8 and the Ring B moiety are as described for the compound of Formula (I).

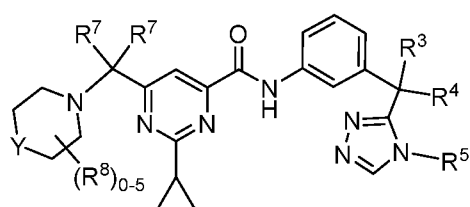
[170] In some embodiments, the compound is of (III-A), (III-B), (III-C), (III-D), (III-E), (III-F), (III-G), or (III-H):





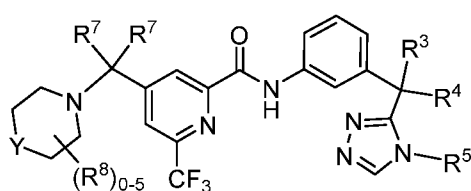
(III-E)

,



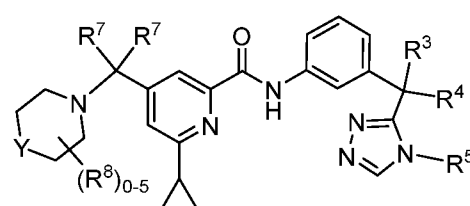
(III-F)

,



(III-G)

, or

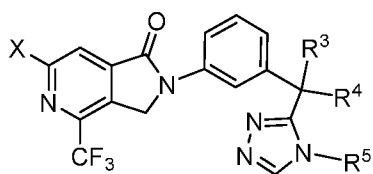


(III-H)

,

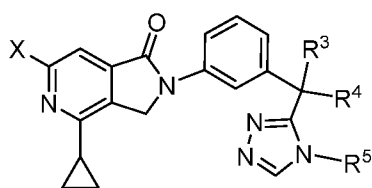
wherein **R**³, **R**⁴, **R**⁵, **R**⁷, **R**⁸, and **Y** are as described for the compound of Formula (I).

[171] In some embodiments, the compound is of (IV-A), (IV-B), (IV-C), (IV-D), (IV-E), (IV-F), (IV-G), or (IV-H):



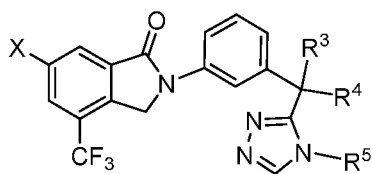
(IV-A)

,



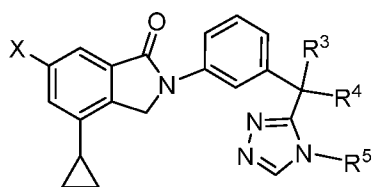
(IV-B)

,



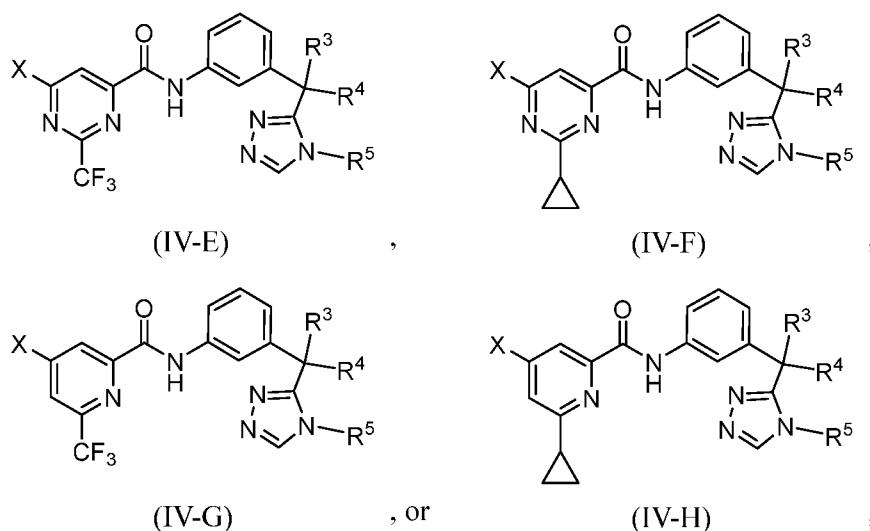
(IV-C)

,



(IV-D)

,



wherein R^3 , R^4 , and R^5 are as described for the compound of Formula (I), and X is H, C₁-C₆ alkyl, C₁-C₆ haloalkyl, C₁-C₆ alkyl-OH, C₁-C₆ alkyl-CN, or C₃-C₆ cycloalkyl optionally substituted by 1-5 R^8 groups. In some embodiments, X is H. In some embodiments, X is C₁-C₆ alkyl. In some embodiments, X is C₁-C₆ haloalkyl. In some embodiments, X is C₁-C₆ alkyl-OH. In some embodiments, X is C₁-C₆ alkyl-CN. In some embodiments, X is C₃-C₆ cycloalkyl optionally substituted by 1-5 R^8 groups.

Table 1. Representative Compounds of This Disclosure

Cmpd No.	Structure	Cmpd No.	Structure
1		2	
3		4	
5		6	

Cmpd No.	Structure	Cmpd No.	Structure
7		8	
9		10	
11		12	
13		14	
15		16	
17		18	
19		20	
21		22	

Cmpd No.	Structure	Cmpd No.	Structure
23		24	
25		26	
27		28	
29		30	
31		32	
33		34	

Cmpd No.	Structure	Cmpd No.	Structure
35		36	
37		38	
39		40	
41		42	
43		44	
45		46	

Cmpd No.	Structure	Cmpd No.	Structure
47		48	
49		50	
51		52	
53			

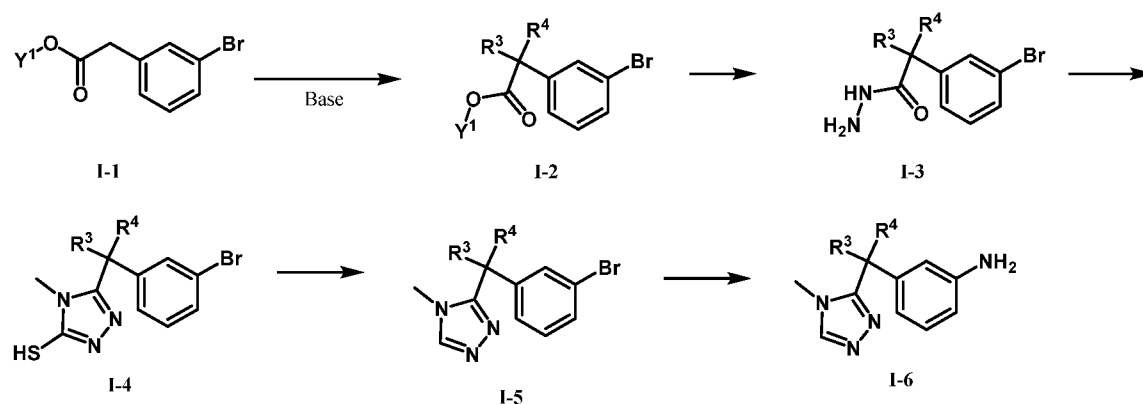
[172] In some embodiments, provided is a compound selected from Compounds Nos. 1-53 in Table 1, or a tautomer thereof, or a pharmaceutically acceptable salt thereof.

[173] In one aspect, provided herein are methods of preparing the compounds of Formula (I) described herein. In another aspect, provided herein are intermediate compounds for preparing the compounds of Formula (I). Also provided herein are compounds which are assay probes, and which are optionally tagged with, for example, a fluorescent label. In a further aspect, provided herein is a method for assaying inhibition of Cbl-b. In one variation, provided herein is a method for assaying inhibition of Cbl-b comprising pre-incubating Cbl-b with an assay probe, such as an assay probe tagged with a fluorescent label, followed by exposing the Cbl-b/assay probe mixture to a candidate compound, and then determining whether and to what

extent the assay probe is displaced by the candidate compound using, for example, FRET signal detection.

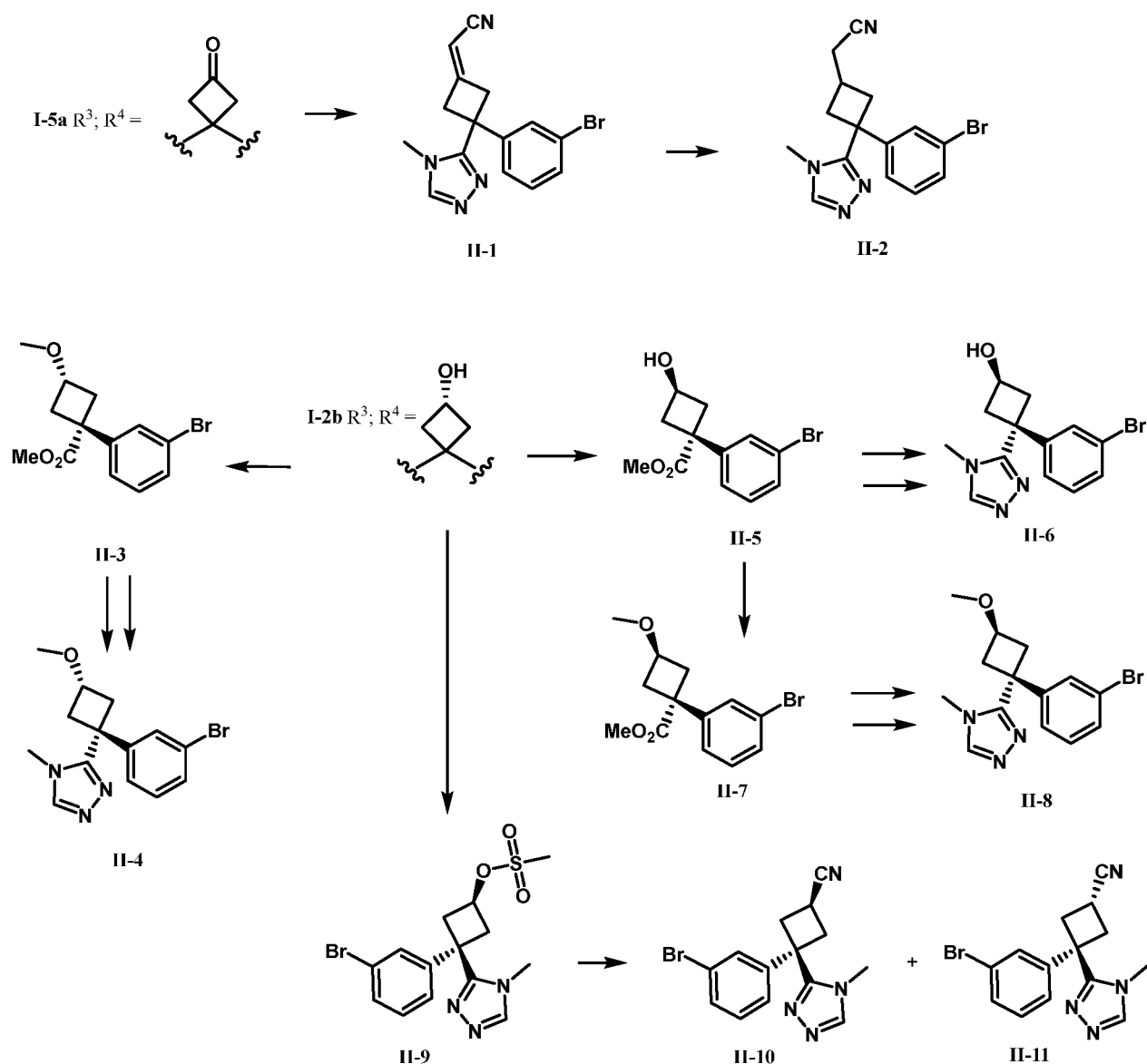
[174] The schemes below describe methods of synthesizing the compounds disclosed herein. Mixtures of stereoisomers produced during synthesis, such as racemic mixtures of final compounds, can be separated into the respective enantiomers using common chromatography methods such as supercritical fluid chromatography in combination with chiral stationary phases, chiral column chromatography, or other methods known in the art.

Scheme I.

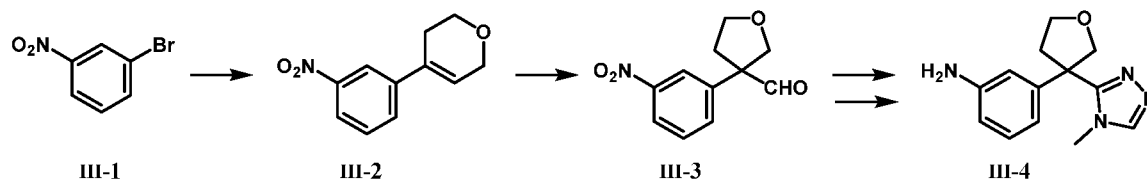


[175] Intermediate compounds of the general formula I-5 or I-6 can be synthesized as outlined in Scheme I, wherein R³ and R⁴ are as defined for the compound of formula (I), and Y¹ is H, forms part of a suitable ester, or is another suitable protecting group for the carboxylic acid. The groups R³ and R⁴ can be installed by deprotonation of an arylacetic acid such as I-1 with a base such as NaH, *i*-PrMgCl, KO-*t*-Bu, or LHMDs, followed by treatment with an electrophile such as an alkyl bromide or formaldehyde, or with a bis-electrophile such as 2-methyl-1,3-dibromo propane or epichlorhydrin (to provide compounds wherein R³ and R⁴ are taken together with the carbon atom to which they are attached to form a cycloalkyl group). Certain compounds of formula I-2, such as wherein R³ and R⁴ are taken together with the carbon atom to which they are attached to form a cyclopentyl group, can be obtained directly from commercial sources. Compounds of formula I-2, wherein R³ and R⁴ are each hydroxymethyl, form an oxetane ring by Mitsunobu cyclization. Triazole assembly can be effected by hydrazide formation, cyclization, and desulphurization to provide compounds of formula I-5. Subsequent conversion of I-5 to compounds of formula I-6 can be achieved by amination using ammonia and copper or Boc carbamate and palladium, followed by deprotection.

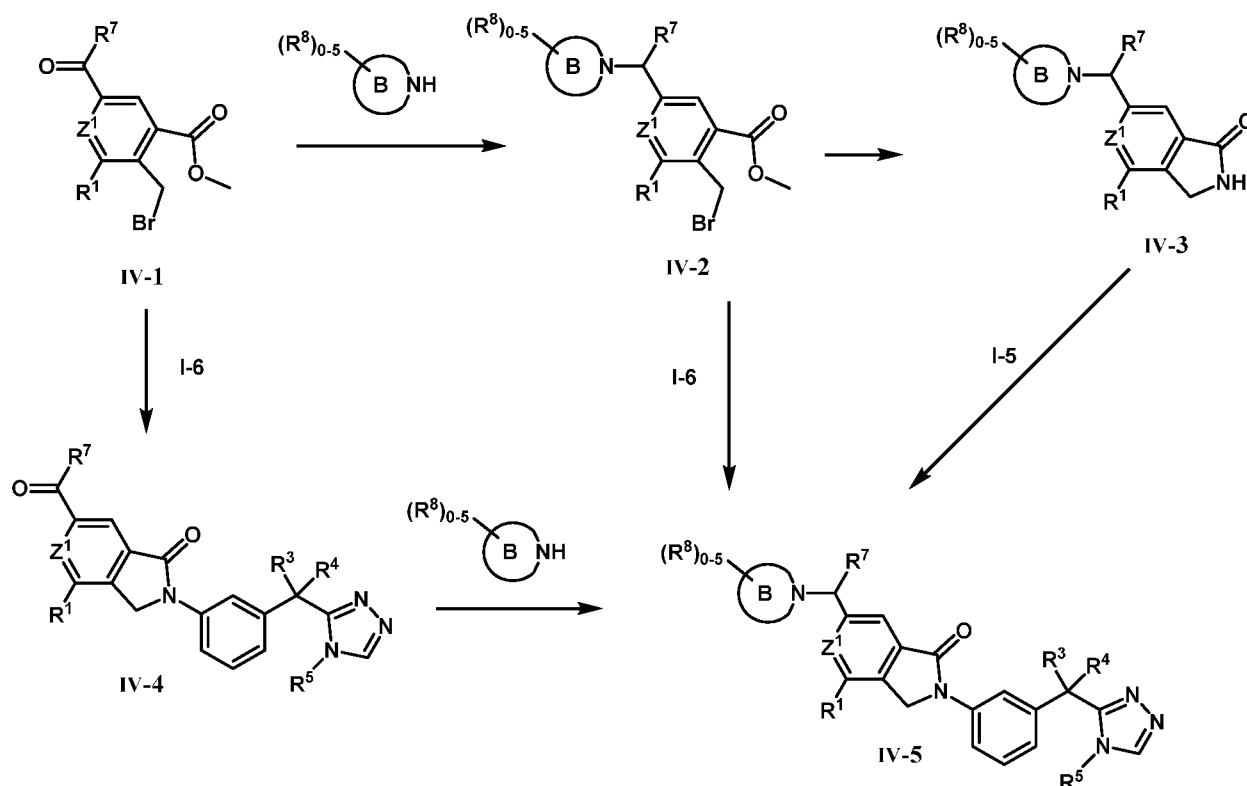
Scheme II.



[176] Intermediates **I-5** can be further elaborated as illustrated in Scheme II. Where R^3 and R^4 form an oxo-substituted ring (**I-5a**), the ketone can be homologated to cyano-olefin **II-1**, and the olefin reduced to form cyanomethyl substituted intermediate **II-2**. Where R^3 and R^4 form a hydroxyl-substituted ring, the hydroxyl group can be alkylated with an electrophile to afford **II-3**, which can then be converted to triazole **II-4**. The stereochemistry can be inverted by Mitsunobu reaction followed by similar alkylation to afford intermediates such as **II-7** and **II-8**. Further substitution can be achieved by converting the hydroxyl group to a nitrile group by mesylation and displacement to afford **II-10** and **II-11**.

Scheme III.

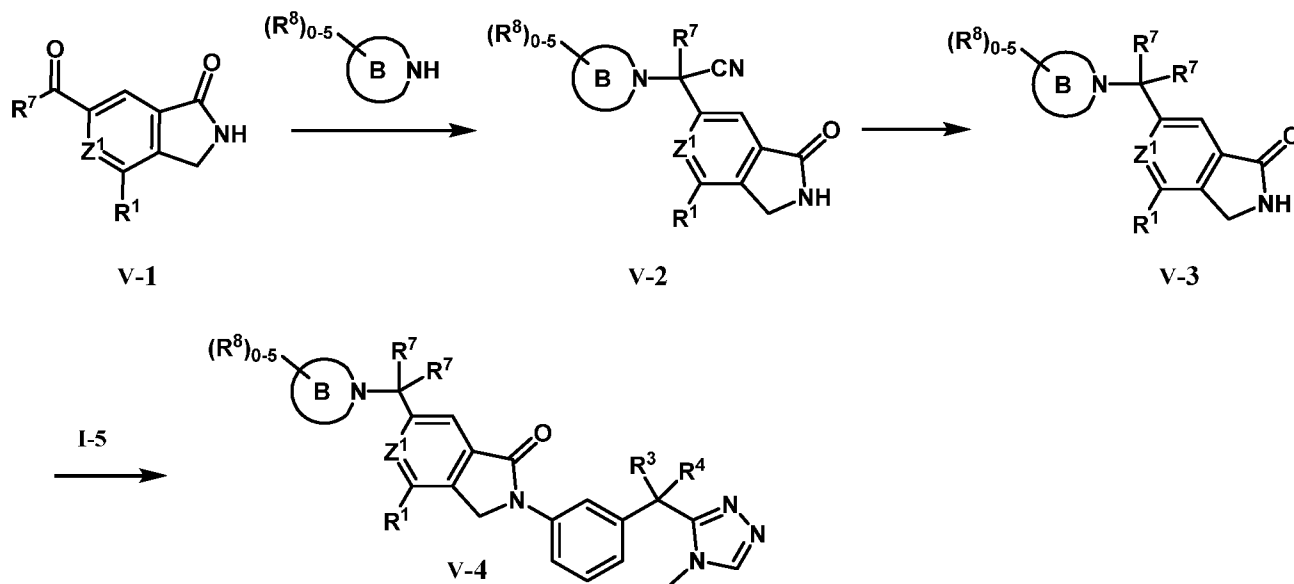
[177] Compounds wherein **R**³ and **R**⁴ are taken together with the carbon atom to which they are attached to form a tetrahydrofuran ring can be assembled as illustrated in Scheme III. Bromide **III-1** can be coupled with an activated dihydropyran under palladium catalysis to afford olefin **III-2**. Olefin **III-2** can be epoxidized and subjected to Lewis-acid promoted ring contraction to form aldehyde **III-3**. Oxidation of aldehyde **III-3** to afford a carboxylic acid, followed by triazole elaboration as outlined in Scheme I, affords intermediate **III-4**.

Scheme IV.

[178] Scheme IV illustrates the assembly of compounds wherein the Ring A moiety is an indolone ring, wherein **R**¹, **R**³, **R**⁴, **R**⁵, **R**⁷, **R**⁸, **Z**¹, and the Ring B moiety are as defined for the compound of formula (I). Formula **IV-1** can be directly condensed with anilines **I-6** to afford intermediates **IV-4**. Compounds of formula **IV-4** are then treated under reductive amination conditions with a heterocycle containing the Ring B moiety to afford compounds of general formula **IV-5**. Alternatively, the order of steps may be switched, and reductive amination with

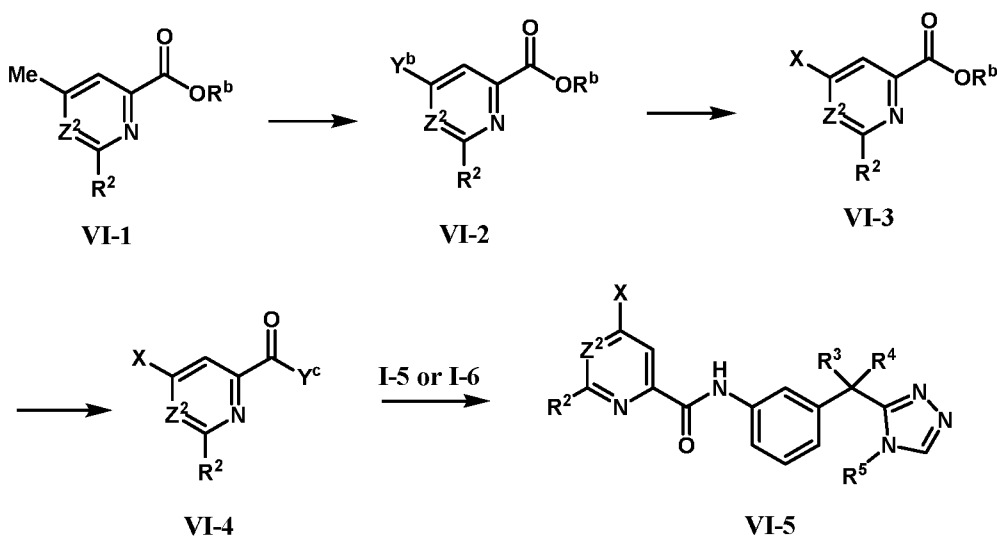
IV-1 and a heterocycle containing the Ring B moiety affords intermediates **IV-2**. The intermediates of formula **IV-2** can then be condensed with anilines **I-6** to afford compounds of general formula **IV-5**. In certain embodiments, formula **IV-2** can be cyclized to form indolones **IV-3**, which are then coupled to bromides **I-5** under palladium catalysis.

Scheme V.



[179] Scheme V illustrates the synthesis of intermediate compounds wherein both R^7 groups are independently C_1 - C_6 alkyl derivatives, and wherein R^1 , R^3 , R^4 , R^7 , R^8 , Z^1 , and the Ring B moieties are as defined for the compound of formula (I). A ketone of the general formula **V-1** is condensed with a heterocycle containing the Ring B moiety using a strong dehydrating agent such as $Ti(OEt)_4$, followed by addition of cyanide to afford Strecker intermediates such as **V-2**. The second R^7 substituent can then be installed by addition of the corresponding Grignard reagent to afford quaternary compounds **V-3**, which can then be coupled to bromides **I-5** to afford compounds of formula **V-4**.

Scheme VI.



[180] Scheme VI outlines a synthesis of compounds of the general formula **VI-5**, wherein **R**², **R**³, **R**⁴, **R**⁵, **Z**², and **X** are as defined for the compound of formula (I); **R**^b forms part of a suitable ester, or is another suitable protecting group for the carboxylic acid; and **Y**^b is an alkyl group such as methyl, a cycloalkyl group such as cyclopropyl, a haloalkyl group such as bromomethyl, or -CHO; and **Y**^c is hydroxyl or NH₂. Methyl pyridines or pyrimidines of formula **VI-1** are carried directly to intermediates **VI-4**, wherein **X** is methyl, for coupling to intermediate compounds of formula **I-5** or **I-6**. Alternatively, the methyl group can be activated by oxidation with SeO₂ to provide an aldehyde, or Br₂ to form a bromomethyl derivative of formula **VI-2**. Amino groups at **X** can then be installed by reductive amination or displacement with a substituted amine to provide compounds **VI-3**, followed by ester hydrolysis under basic conditions to afford compounds **VI-4**, wherein **Y**^c is OH. Acids **VI-4** are then coupled to amines **I-6** with a coupling reagent such as HATU or T3P to afford amides **VI-5**; amides **VI-4**, wherein **Y**^c is NH₂, are coupled with bromides **I-5** under palladium catalysis.

[181] Compounds 1, 3, 4, 17, and 35-53 display IC₅₀ values of 5 nM or less in the Cbl-b inhibition assay of Biological Example 1, and in one embodiment are used for the pharmaceutical compositions and in the methods as disclosed herein. Compounds 8, 15, and 25-34 display IC₅₀ values between greater than 5 nM and 20 nM in the Cbl-b inhibition assay of Biological Example 1, and in one embodiment are used for the pharmaceutical compositions and in the methods as disclosed herein. Compounds 5, 6, 14, 16, and 19-24 display IC₅₀ values of between greater than 20 nM and 100 nM in the Cbl-b inhibition assay of Biological Example 1, and in one embodiment are used for the pharmaceutical compositions and in the methods as disclosed herein. Compounds 2, 7, 9-13, and 18 display IC₅₀ values of greater than 100 nM in

the Cbl-b inhibition assay of Biological Example 1, and in one embodiment are used for the pharmaceutical compositions and in the methods as disclosed herein.

[182] In various embodiments, and as further described herein, compounds as provided herein (as well as compositions comprising compounds described herein, and methods using the compounds or compositions) have IC₅₀ values of 5 nM or less, between greater than 5 nM and 20 nM, between greater than 20 nM and 100 nM, or greater than 100 nM, as determined by the Cbl-b inhibition assay of Biological Example 1. In a further embodiment, and as further described herein, compounds as provided herein (as well as compositions comprising compounds described herein, and methods using the compounds or compositions) have IC₅₀ values of 5 nM or less, as determined by the Cbl-b inhibition assay of Biological Example 1. In a further embodiment, and as further described herein, compounds as provided herein (as well as compositions comprising compounds described herein, and methods using the compounds or compositions) have IC₅₀ values of between greater than 5 nM and 20 nM, as determined by the Cbl-b inhibition assay of Biological Example 1. In a further embodiment, and as further described herein, compounds as provided herein (as well as compositions comprising compounds described herein, and methods using the compounds or compositions) have IC₅₀ values of between greater than 20 nM and 100 nM, as determined by the Cbl-b inhibition assay of Biological Example 1. In a further embodiment, and as further described herein, compounds as provided herein (as well as compositions comprising compounds described herein, and methods using the compounds or compositions) have IC₅₀ values of greater than 100 nM, as determined by the Cbl-b inhibition assay of Biological Example 1.

[183] For IL-2 secretion from an immune cell (*e.g.*, T-cell) co-stimulated with an anti-CD3 antibody and an anti-CD28 antibody, compounds as provided herein (as well as compositions comprising compounds described herein) induce less than 20 fold, between 20 – 35 fold, or greater than 35 fold change over baseline, at inhibitor concentrations of 1 micromolar or 0.3 micromolar.

[184] For IL-2 secretion from an immune cell (*e.g.*, T-cell) stimulated with an anti-CD3 antibody, compounds as provided herein (as well as compositions comprising compounds described herein) induce less than 0.70 fold, between 0.70 – 1.1 fold, or greater than 1.1 fold change over baseline, at inhibitor concentrations of 3 micromolar or 1 micromolar.

[185] For CD25 staining on the cell surface of an immune cell (*e.g.*, T-cell) co-stimulated with an anti-CD3 antibody and an anti-CD28 antibody, compounds as provided herein (as well as compositions comprising compounds described herein) induce less than 1.24 fold, between

1.24 – 1.39 fold, or greater than 1.39 fold change over baseline, at inhibitor concentrations 1 micromolar or 0.3 micromolar.

[186] For CD25 staining on the cell surface of an immune cell (*e.g.*, T-cell) stimulated with an anti-CD3 antibody, compounds as provided herein (as well as compositions comprising compounds described herein) induce less than 1.5 fold, between 1.5 – 2.5 fold, or greater than 2.5 fold change over baseline, at inhibitor concentrations of 3 micromolar or 1 micromolar.

III. Use and Methods

[187] Provided herein are methods for modulating activity of an immune cell (*e.g.*, a T-cell, a B-cell, or a NK-cell) such as by contacting the immune cell with an effective amount of a Cbl-b inhibitor described herein or a composition thereof. Also provided are *in vitro* methods of producing said immune cells with modulated activity, referred to herein as “modified immune cells,” wherein said modified immune cells can be administered to an individual in need thereof (*e.g.*, an individual having cancer) by *ex vivo* methods. Further provided are *in vivo* methods of modulating a response in an individual in need thereof (*e.g.*, an individual with cancer), wherein the method comprises administration of an effective amount of a Cbl-b inhibitor described herein or a composition thereof. Moreover, this disclosure provides *in vitro* methods of producing an expanded population of lymphocytes after *in vivo* lympho-conditioning in an individual with cancer, wherein the lympho-conditioning occurs as a result of administration of an effective amount of a Cbl-b inhibitor described herein or a composition thereof to the individual. The expanded population of lymphocytes can then be administered to the individual with cancer. In some embodiments, the modified immune cells or the expanded population of lymphocytes are produced from a biological sample comprising immune cells obtained from the individual, such as a blood sample comprising peripheral blood mononuclear cells or a tumor biopsy comprising tumor infiltrating lymphocytes (TILs).

[188] Additionally, provided are Cbl-b inhibitors for use as therapeutic active substances. A Cbl-b inhibitor for use in treating or preventing a disease or condition associated with Cbl-b activity is provided. Also, a Cbl-b inhibitor for use in treating cancer is provided. Further provided is the use of a Cbl-b inhibitor in the manufacture of a medicament for treating or preventing a disease or condition associated with Cbl-b activity. Also provided is the use of a Cbl-b inhibitor in the manufacture of a medicament for treating cancer. Moreover, this disclosure provides treatment methods, medicaments and uses comprising a Cbl-b inhibitor as part of a combination therapy for treating cancer involving one or more of an immune checkpoint inhibitor, an antineoplastic agent, and radiation therapy.

[189] In some embodiments of the treatment methods, medicaments, and uses of this disclosure, the cancer is a hematologic cancer such as lymphoma, a leukemia or a myeloma. In other embodiments of the treatment methods, medicaments and uses of this disclosure, the cancer is a non-hematologic cancer such as a sarcoma, a carcinoma, or a melanoma.

[190] Hematologic cancers include, but are not limited to, one or more leukemias such as B-cell acute lymphoid leukemia (“BALL”), T-cell acute lymphoid leukemia (“TALL”), acute lymphoid leukemia (ALL); one or more chronic leukemias including, but not limited to, chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to, B-cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and “preleukemia,” which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells.

[191] Non-hematologic cancers include, but are not limited to, a neuroblastoma, renal cell carcinoma, colon cancer, colorectal cancer, breast cancer, epithelial squamous cell cancer, melanoma, stomach cancer, brain cancer, lung cancer (*e.g.*, NSCLC), pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, prostate cancer, testicular cancer, thyroid cancer, uterine cancer, adrenal cancer, and head and neck cancer.

[192] In some aspects, the effectiveness of administration of a Cbl-b inhibitor in the treatment of a disease or disorder such as cancer is measured by assessing clinical outcome, such as reduction in tumor size or number of tumors, and/or survival. In certain embodiments, “treating cancer” comprises assessing a patient's response to the treatment regimen according to the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) as described (*see, e.g.*, Eisenhauer *et al.*, *Eur J Cancer*, 45:228-247, 2009; and Nishino *et al.*, *Am J Roentgenol*, 195: 281-289, 2010). Response criteria to determine objective anti-tumor responses per RECIST 1.1 include complete response (CR); partial response (PR); progressive disease (PD); and stable disease (SD).

A. Isolation and Processing of Cells

[193] Provided are methods for the preparation and processing of immune cells produced (*e.g.*, modified immune cells) and used in the methods herein. As used herein, the term “modified immune cells” refers to immune cells or a cell population comprising the immune cells which have been cultured, incubated, and/or have been contacted with an effective amount of a Cbl-b inhibitor to modulate the activity of said immune cells. In some embodiments, the modified immune cells can be used for immunotherapy, such as in connection with adoptive immunotherapy methods.

1. Samples

[194] In some embodiments, the immune cells to be modified or cell populations comprising the immune cells to be modified are isolated from a sample, such as a biological sample, *e.g.*, one obtained from or derived from an individual (*e.g.*, a human). In some embodiments, the individual from which the immune cell is isolated is one having a particular disease or condition (*e.g.*, cancer) or in need of a cell therapy or to which cell therapy will be administered. The individual, in some embodiments, is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which immune cells are being isolated, processed, and/or modified. Accordingly, the cells isolated from the individual, in some embodiments, are primary cells (*e.g.*, primary human cells). As used herein, the term “primary cells” refers to cells isolated directly from mammalian biological fluid or tissue (*e.g.*, human biological fluid or tissue).

[195] In some embodiments, the immune cells to be modified are hematopoietic cells, multipotent stem cells, myeloid progenitor cells, lymphoid progenitor cells, T-cells, B-cells, and/or NK-cells. As used herein, the term “hematopoietic cells” includes hematopoietic stem cells and hematopoietic progenitor cells. In some embodiments, the immune cells to be modified are present in a heterogeneous cell population or a composition comprising a heterogeneous cell population. For example, the immune cells to be modified may be hematopoietic cells present in a heterogeneous cell population comprising cells such as differentiated cells derived from a tissue or organ. In some embodiments, the immune cells to be modified are present in a homogenous cell population or a composition comprising a homogenous cell population. For example, the immune cells to be modified may be hematopoietic cells present in a homogenous cell population comprising only hematopoietic cells. In some embodiments, the immune cells to be modified or cell populations comprising

the immune cells to be modified include one or more subsets of immune cells. For example, one or more subsets of immune cells may be CD4⁺ cells, CD8⁺ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, localization, persistence capacities, surface marker profile, cytokine secretion profile, and/or degree of differentiation.

[196] In some embodiments, biological samples described herein include tissue, fluid, and other samples taken directly from the individual, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (*e.g.*, transduction with a viral vector encoding a recombinant chimeric receptor), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, and tissue and organ samples (*e.g.*, sample from a tissue or organ containing a tumor), including processed samples derived therefrom. In some embodiments, the biological sample is a biological fluid sample or a biological tissue sample. In some embodiments, the biological sample is a biological tissue sample.

[197] In some aspects, the biological sample from which the immune cells are derived or isolated is blood or a blood-derived sample, or is derived from an apheresis or leukapheresis product.

[198] Exemplary biological samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Biological samples include, in the context of cell therapy (*e.g.*, adoptive cell therapy) samples from autologous sources (*i.e.*, obtained from or derived from the individual in need of cell therapy) and allogeneic sources (*i.e.*, obtained from or derived from an individual or source other than the individual in need of cell therapy).

[199] In some embodiments, the immune cells to be modified or a cell population comprising the immune cells to be modified are derived from a cell line (*e.g.*, a T-cell line, a B-cell line, a NK-cell line, etc.). In some embodiments, the immune cells to be modified or a cell population comprising the immune cells to be modified are obtained from a xenogeneic source, such as from mouse, rat, non-human primate, or pig.

2. Cell Processing and Separation

[200] In some embodiments, isolation of the immune cells to be modified includes one or more preparation and/or cell separation steps. The one or more cell separation steps can be non-affinity based separation or affinity based separation. As an example, non-affinity based separation can be centrifugation of a composition comprising the immune cells to be modified. In some embodiments, the non-affinity based separation methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient. Affinity based separation methods can include contacting a composition comprising the immune cells to be modified with antibody coated beads. Antibody-coated beads contemplated herein include, but are not limited to, magnetic beads (*e.g.*, Dynabeads® marketed by Life Technologies, Carlsbad, CA, MACS® microbeads marketed by Miltenyi Biotec Inc., Auburn, CA; or EasySep™ Direct RapidSpheres™ marketed by Stemcell Technologies, Vancouver, BC, Canada) coated with an antibody that binds to a marker expressed on the surface of the immune cell to be modified. In some embodiments, specific subpopulations of T-cells, such as cells positive for or otherwise expressing high levels of one or more surface markers, *e.g.*, CD4+, CD8+, etc., are isolated by positive or negative selection techniques. Positive selection can be based on a technique in which the target cells (*e.g.*, immune cells to be modified) have bound to a reagent and are retained for further use. For example, T-cells that are CD3+ can be positively selected using magnetic beads conjugated to anti-CD3 antibodies (*e.g.*, MACS® CD3 human microbeads). Negative selection can be based on a technique in which the target cells (*e.g.*, immune cells to be modified) that have not bound to a reagent are retained. For example, total human primary T-cells can be isolated from peripheral blood mononuclear cells (PBMCs) utilizing negative selection, wherein a cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123 and CD235a are incubated in a sample comprising the PBMCs before passing the sample by magnetic beads for removal of cells expressing those surface markers and retaining the remaining cells in the sample for subsequent processing. In some embodiments, the immune cells or a cell population comprising the immune cells to be modified are washed, centrifuged, and/or incubated in the presence of one or more reagents, for example, to remove unwanted components, enrich for desired components, and/or lyse or remove cells sensitive to particular reagents. In some examples, the immune cells are separated based on one or more property, such as density, adherent properties, size, sensitivity, and/or resistance to particular components. Cell

separation steps do not require 100% enrichment or removal of particular cells. In some embodiments, positive selection of or enrichment for immune cells of a particular type (*e.g.*, CD4⁺ T-cells) refers to increasing the number or percentage of such cells. In some embodiments, removal, or depletion of cells of a particular type that are not of interest such as by negative selection, refers to decreasing the number or percentage of such cells.

[201] In some embodiments, immune cells or a cell population comprising the immune cells are obtained from the circulating blood of an individual, *e.g.*, by apheresis or leukapheresis. In some aspects, a sample comprising the immune cells to be modified contains lymphocytes, including T-cells, B-cells, and NK-cells, as well as monocytes, granulocytes, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets.

[202] In some embodiments, the blood cells collected from the individual are washed such as to remove the plasma fraction and to place the cell population comprising the immune cells to be modified in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cell population comprising the immune cells to be modified is washed with phosphate buffered saline. In some embodiments, the wash solution lacks calcium and/or magnesium. In some aspects, a washing step is accomplished by a semi-automated “flow-through” centrifuge. In some aspects, a washing step is accomplished by tangential flow filtration. In some embodiments, the immune cells to be modified or cell population containing the immune cells to be modified are resuspended in a variety of suitable buffers after washing, such as, for example, calcium and/or magnesium free phosphate buffered saline. In some embodiments, components of a blood cell sample are removed and the immune cells to be modified or a cell population comprising the immune cells to be modified are directly resuspended in a suitable cell culture medium.

[203] Representative methods for processing and/or separating immune cells such as hematopoietic cells from samples containing a cell population containing said hematopoietic cells (*e.g.*, samples comprising PBMCs) are described in Biological Example 2 and Biological Example 3 herein. Methods and techniques for processing and/or separating immune cells such as hematopoietic cells, multipotent stem cells, myeloid progenitor cells, lymphoid progenitor cells, T-cells, B-cells, and/or NK-cells are well known in the art. See for example, U.S. Patent Application No. 2017/0037369; U.S. Patent Application No. 2012/0148553; U.S. Patent No. 6,461,645; U.S. Patent No. 6,352,694; and U.S. Patent No. 7,776,562.

3. Incubation and Treatment

[204] Provided herein are methods for modulating the activity of an immune cell, such as the processed and/or separated immune cells described above, by contacting the immune cell with an effective amount of a Cbl-b inhibitor described herein. Also provided herein are modified immune cells produced by any of the methods described herein such as by culturing a cell population containing an immune cell (*e.g.*, the processed and/or separated immune cells described above) in the presence of an effective amount of a Cbl-b inhibitor to modulate the activity of the immune cell and thereby produce the modified immune cell.

[205] In some embodiments, the immune cells to be modified (*e.g.*, the processed and/or separated immune cells described above) are incubated and/or cultured in a suitable culture medium prior to contacting said immune cells with a Cbl-b inhibitor provided herein. In some embodiments, the immune cells to be modified are incubated and/or cultured in a suitable culture medium simultaneously to contacting said immune cells with a Cbl-b inhibitor provided herein.

[206] The processed and/or separated immune cells to be modified or cell population comprising the immune cells to be modified can be differentiated and/or expanded *in vitro*. In some embodiments, the immune cells to be modified are hematopoietic cells, multipotent stem cells, myeloid progenitor cells, lymphoid progenitor cells, T-cells, B-cells, and/or NK-cells. In some embodiments, the immune cell to be modified is incubated in a suitable cell culture medium comprising a Cbl-b inhibitor described herein before differentiation and/or expansion of the immune cell. In some embodiments, the immune cell to be modified is incubated in a suitable cell culture medium comprising a Cbl-b inhibitor described herein after differentiation and/or expansion of the immune cell. The immune cells become modified (*i.e.*, modified immune cells) upon contact with a Cbl-b inhibitor provided herein in an effective amount to modulate the activity of said immune cells. In some embodiments, the immune cell to be modified is not differentiated and/or expanded *in vitro* and is therefore the same cell type as the modified immune cell that has been contacted with a Cbl-b inhibitor. For example, a T-cell can be incubated in a suitable medium comprising a Cbl-b inhibitor without differentiation of the T-cell. In other embodiments, the immune cell to be modified is differentiated and/or expanded *in vitro* and is therefore a different cell type than the modified immune cell that has been contacted with a Cbl-b inhibitor. For example, a hematopoietic cell can be incubated in a suitable medium comprising a Cbl-b inhibitor as well as other agents that drive differentiation of the hematopoietic cell into a mature hematopoietic cell. Accordingly, in some aspects of

the embodiments herein, the modified immune cells are hematopoietic cells, multipotent stem cells, myeloid progenitor cells, lymphoid progenitor cells, T-cells, B-cells, and/or NK-cells. Methods for expansion and/or differentiation of immune cells are well known in the art. See, for example, International Patent Application No. WO 2017/037083.

[207] An effective amount of a Cbl-b inhibitor is the amount or concentration of the Cbl-b inhibitor that is sufficient to modulate the activity of the immune cell as compared to a reference sample. The reference sample may be immune cells that have not been contacted with the Cbl-b inhibitor. In some embodiments, the concentration of a Cbl-b inhibitor added to a composition (*e.g.*, cell culture medium) comprising the immune cells to be modified is from about 1 pM to about 100 μ M, about 5 pM to about 100 μ M, about 10 pM to about 100 μ M, about 20 pM to about 100 μ M, about 40 pM to about 100 μ M, about 60 pM to about 100 μ M, about 80 pM to about 100 μ M, about 1 nM to about 100 μ M, about 3 nM to about 100 μ M, about 10 nM to about 100 μ M, about 15 nM to about 100 μ M, about 20 nM to about 100 μ M, about 40 nM to about 100 μ M, about 60 nM to about 100 μ M, about 80 nM to about 100 μ M, about 0.1 μ M to about 100 μ M, about 0.1 μ M to about 90 μ M, about 0.1 μ M to about 80 μ M, about 0.1 μ M to about 70 μ M, about 0.1 μ M to about 60 μ M, about 0.1 μ M to about 50 μ M, about 0.1 μ M to about 40 μ M, about 0.1 μ M to about 30 μ M, about 0.1 μ M to about 20 μ M, about 0.1 μ M to about 10 μ M, about 0.2 μ M to about 10 μ M, or about 0.3 μ M to about 8 μ M. In some embodiments, the concentration of a Cbl-b inhibitor added to a composition (*e.g.*, cell culture medium) comprising the immune cells to be modified is about 1 pM, about 2 pM, about 3 pM, about 4 pM, about 5 pM, about 10 pM, about 20 pM, about 30 pM, about 40 pM, about 50 pM, about 60 pM, about 70 pM, about 80 pM, about 90 pM, about 1 nM, about 3 nM, about 5 nM, about 10 nM, about 20 nM, about 40 nM, about 50 nM, about 80 nM, about 0.1 μ M, about 0.2 μ M, about 0.3 μ M, about 0.4 μ M, about 0.5 μ M, about 1 μ M, about 5 μ M, about 10 μ M, about 15 μ M, about 20 μ M, about 25 μ M, about 30 μ M, about 40 μ M, about 50 μ M, about 60 μ M, about 70 μ M, about 80 μ M, about 90 μ M, or about 100 μ M. In some embodiments, the concentration of a Cbl-b inhibitor added to a composition (*e.g.*, cell culture medium) comprising the immune cells to be modified is about 0.3 μ M, about 1 μ M, or about 4 μ M. In some embodiments, the concentration of a Cbl-b inhibitor added to a composition (*e.g.*, cell culture medium) comprising the immune cells to be modified is about 1 μ M or about 8 μ M.

[208] The effective amount of a Cbl-b inhibitor is in contact with the immune cells for a sufficient time to modulate the activity of the immune cell as compared to a reference sample. The reference sample may be immune cells that have not been contacted with the Cbl-b

inhibitor, but are incubated for the same length of time as the composition (*e.g.*, cell culture medium) comprising the immune cells and the Cbl-b inhibitor. In some embodiments, the Cbl-b inhibitor is in contact and/or is incubated with the immune cells from about 1 minute to about 1 hour, about 5 minutes to about 1 hour, about 10 minutes to about 1 hour, about 15 minutes to about 1 hour, about 20 minutes to about 1 hour, about 30 minutes to about 1 hour, about 45 minutes to about 1 hour, about 1 hour to about 2 hours, about 1 hour to about 4 hours, about 1 hour to about 6 hours, about 1 hour to about 8 hours, about 1 hour to about 12 hours, about 1 hour to about 24 hours, about 2 hours to about 24 hours, about 6 hours to about 7 hours, about 6 hours to about 24 hours, about 8 hours to about 24 hours, about 10 hours to about 24 hours, about 15 hours to about 24 hours, about 20 hours to about 24 hours, about 12 hours to about 48 hours, about 24 hours to about 48 hours, or about 36 hours to about 48 hours. In some embodiments, the Cbl-b inhibitor is in contact and/or is incubated with the immune cells for about 1 minute, about 5 minutes, about 10 minutes, about 15 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, or about 24 hours. In some embodiments, the Cbl-b inhibitor is in contact and/or is incubated with the immune cells from about 1 day to about 7 days, about 2 days to about 7 days, about 3 days to about 7 days, about 4 days to about 7 days, about 5 days to about 7 days, or about 6 days to about 7 days. In some embodiments, the Cbl-b inhibitor is in contact and/or is incubated with the immune cells from about 7 days to about 14 days, about 14 days to about 21 days, or about 21 days to about 28 days. In some embodiments, the Cbl-b inhibitor is in contact and/or is incubated with the immune cells for about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days or about 14 days.

[209] In some embodiments, the immune cells or a cell population comprising the immune cells are incubated under a suitable condition to induce proliferation, expansion, activation, and/or survival of the immune cells. Suitable conditions during incubation include, but are not limited to, use of one or more medium of cell culture medium, temperature, incubation time, the presence of a stimulating agent (*e.g.*, anti-CD3 and/or anti-CD28 antibody), and the presence of any other beneficial agents, such as growth factors, cytokines, chemokines, and/or recombinant soluble receptors.

[210] In some embodiments, a suitable condition to induce proliferation, expansion, activation, and/or survival of the immune cells includes the provision of stimulating conditions comprising agents that are capable of activating the immune cell (*e.g.*, NK-cell). For example, a suitable condition to induce proliferation, expansion, activation, and/or survival of a T-cell includes the provision of stimulating conditions that are capable of activating intracellular signaling in the T-cell. Full activation of T-cells generally requires the recognition of antigen by the T-cell receptor, referred to herein as “TCR” (signal one) as well as recognition of costimulators such as CD28 (signal two). In some aspects, one or more agents turn on or initiate a TCR complex-mediated intracellular signaling cascade in a T-cell. For example, a first agent can bind to a component of the TCR complex in order to activate the T-cell and a second agent can bind to a costimulatory molecule on the surface of the T-cell to thereby stimulate the activated T-cell. In some embodiments, the first agent stimulates a TCR/CD3 complex-associated signal in the T-cell by specifically binding to CD3 (*e.g.*, an anti-CD3 antibody). In a further embodiment, the co-stimulatory molecule on the surface of the T-cell may be CD28 and the second agent specifically binds to CD28 (*e.g.*, anti-CD28 antibody). Such agents include, but are not limited to, antibodies, divalent antibody fragments, and binding molecules such as those specific for a TCR complex component (*e.g.*, anti-CD3 antibody) and/or those specific for costimulatory receptor (*e.g.*, anti-CD28 antibody). In some embodiments, an agent that specifically binds to CD3 is an anti-CD3 antibody, a divalent antibody fragment of an anti-CD3 antibody (*e.g.*, (Fab)₂' fragment or a divalent scFv fragment), a monovalent antibody fragment of an anti-CD3 antibody (*e.g.*, a Fab fragment, a Fv fragment, or a scFv fragment), or a CD3 binding molecule (*e.g.*, an aptamer). In some embodiments, an agent that specifically binds to CD28 is an anti-CD28 antibody, a divalent antibody fragment of an anti-CD28 antibody (*e.g.*, (Fab)₂' fragment or a divalent scFv fragment), a monovalent antibody fragment of an anti-CD28 antibody (*e.g.*, a Fab fragment, a Fv fragment, or a scFv fragment), and a CD28 binding molecule (*e.g.*, an aptamer). The one or more agents provided herein (*e.g.*, anti-CD3 antibody and anti-CD28 antibody) for example, can be bound to a solid support such as a bead, or cross-linked with an anti-Fc antibody. In some embodiments, the expansion method step may further comprise the step of adding anti-CD3 antibody and/or anti-CD28 antibody to the culture medium. In some embodiments, the stimulating agents added to the cell culture medium include one or more cytokines such as, but not limited to, one or more of IL-2, IL-7, IL-15, and IL-21. For example, IL-2 can be added at a concentration of at least

about 10 units/mL to a cell culture medium comprising the immune cells and agents such as anti-CD3 antibodies and/or anti-CD28 antibodies.

[211] In some embodiments, a suitable condition to induce proliferation, expansion, activation, and/or survival of a T-cell includes the provision of stimulating conditions or agents which are capable of activating intracellular signaling through the T-cell receptor (TCR) complex, and a Cbl-b inhibitor as described herein. In some embodiments, the immune cells or a cell population comprising the immune cells are incubated with a first agent that stimulates a TCR/CD3 complex-associated signal in the T-cell by specifically binding to CD3 (*e.g.*, an anti-CD3 antibody). In a further embodiment, the immune cells or a cell population comprising the immune cells are incubated with a first agent that stimulates a TCR/CD3 complex-associated signal in the T-cell by specifically binding to CD3 (*e.g.*, an anti-CD3 antibody), with a second agent that binds to the co-stimulatory molecule CD28 (*e.g.*, an anti-CD28 antibody), and with a Cbl-b inhibitor at a concentration of about 1 pM to about 100 μ M (*e.g.*, about 0.3 μ M, about 1 μ M, or about 4 μ M). In some embodiments a suitable condition to induce proliferation, expansion, activation, and/or survival of a T-cell when in the presence of a Cbl-b inhibitor does not require stimulation through a co-stimulatory molecule (*e.g.*, CD28). Contacting T-cells with a Cbl-b inhibitor or a composition thereof can bypass the need for co-stimulation required for T-cells to enter into an activated state. In some embodiments, the immune cells or a cell population comprising the immune cells are incubated with a first agent that stimulates a TCR/CD3 complex-associated signal in the T-cell by specifically binding to CD3 (*e.g.*, an anti-CD3 antibody) and with a Cbl-b inhibitor at a concentration of about 0.001 μ M to about 1,000 μ M, about 0.01 μ M to about 100 μ M, about 0.1 μ M to about 10 μ M, or about 0.1 μ M to about 50 μ M (*e.g.*, about 1 μ M or about 8 μ M).

[212] In some embodiments of the methods for modulating activity of an immune cell, the immune cell is a T-cell and modulating activity of the T-cell comprises increased T-cell activation and/or increased T-cell proliferation. T-cells contemplated in embodiments herein may be in a tolerant state even in the presence of an activating agent that binds to a component of the TCR complex, such as an anti-CD3 antibody, as well as in the presence of a stimulating agent that binds a co-stimulatory molecule, such as an anti-CD28 antibody. In some embodiments, the method of modulating activity of a T-cell comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof in the presence of an anti-CD3 antibody in combination with an anti-CD28 antibody. In some embodiments, the method of modulating activity of a T-cell comprises contacting the T-cell with an effective

amount of a Cbl-b inhibitor or a composition thereof, wherein the T-cell previously has been in contact with an anti-CD3 antibody in combination with an anti-CD28 antibody. In some embodiments, stimulation via the co-stimulatory CD28 molecule is not required for modulating the activity of the T-cell (*e.g.*, increasing T-cell activation and/or increasing T-cell proliferation). In some embodiments, the method of modulating activity of a T-cell comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof in the presence of an anti-CD3 antibody alone. In some embodiments, the method of modulating activity of a T-cell comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof, wherein the T-cell has previously been in contact with one or more agents that activate the T-cell (*e.g.*, an anti-CD3 antibody), wherein said agents do not include an agent that stimulates the CD28 co-stimulatory molecule (*e.g.*, an anti-CD28 antibody).

[213] In some embodiments, the immune cell is a T-cell and modulating activity of the T-cell comprises enhanced T-cell activation and/or enhanced T-cell proliferation. For example, T-cells contemplated in embodiments herein may be in an activated state such as when in the presence of agents that activate the T-cells (*e.g.*, anti-CD3 antibody), and in some further embodiments, in the presence of agents that stimulate the T-cells (*e.g.*, anti-CD28 antibody). Contacting T-cells with a Cbl-b inhibitor or composition thereof can lower the threshold required for activation and therefore enhance activation and/or proliferation of T-cells that are in the presence of an activating agent (*e.g.*, an anti-CD3 antibody) and in some further embodiments, a stimulating agent (*e.g.*, an anti-CD28 antibody). In some embodiments, the method of modulating activity of a T-cell comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof in the presence of an anti-CD3 antibody in combination with an anti-CD28 antibody. In some embodiments, the method of modulating activity of a T-cell comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof, wherein the T-cell has previously been in contact with an anti-CD3 antibody in combination with an anti-CD28 antibody. In some embodiments, stimulation via the co-stimulatory CD28 molecule is not required for modulating the activity of the T-cell (*e.g.*, enhancing T-cell activation and/or enhancing T-cell proliferation). In some embodiments, the method of modulating activity of a T-cell comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof in the presence of an anti-CD3 antibody alone. In some embodiments, the method of modulating activity of a T-cell comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof,

wherein the T-cell has previously been in contact with one or more agents that activate the T-cell (*e.g.*, anti-CD3 antibody).

[214] In some embodiments, the immune cell is a T-cell and modulating activity of the T-cell comprises decreased T-cell dysfunction including decreased T-cell exhaustion, decreased T-cell tolerance, and/or decreased T-cell anergy. General principles of T-cell dysfunction are well known in the art (*see, e.g.*, Schietinger *et al.*, Trends Immunol., 35: 51-60, 2014). Immune tolerance is a process that is part of the normal function of the immune system. Antigen-specific immune tolerance is characterized by a decrease in responsiveness to an antigen, which is induced by previous exposure to that antigen. When specific lymphocytes (*e.g.*, T-cells) encounter antigens, the lymphocytes may be activated, leading to an antigen-specific immune response, or the lymphocytes (*e.g.*, T-cells) may be inactivated or eliminated, leading instead to antigen-specific immune tolerance. In some aspects, tolerance can be caused by clonal anergy, peripheral clonal deletion, suppression of T-cells, and/or other forms of antigen-specific tolerance. In some embodiments, tolerance may result from or be characterized by the induction of anergy. In some aspects, anergy can result from exposure of T-cells to an antigen in the absence of costimulation. Prolonged antigen recognition by the TCR alone, in the absence of the co-stimulatory signal, may lead to anergy (*i.e.*, functional unresponsiveness). Anergic T-cells may be refractory to subsequent antigenic challenge, and may be capable of suppressing other immune responses. Generally, in the natural setting, tolerance is involved in non-reactivity or nonproductive reactivity to self-antigens. In some cases, however, tolerance to a “non-self” antigen can be induced. Thus, in some aspects, the same mechanisms by which mature T-cells that recognize self-antigens in peripheral tissues become incapable of subsequently responding to these antigens also may regulate unresponsiveness to foreign or “non-self” antigens such as those expressed by cancer cells. Accordingly, T-cells contemplated in embodiments herein may be in a tolerant state even in the presence of stimulatory agents such as agents that bind to a co-stimulatory molecule such as CD28. Contacting T-cells with a Cbl-b inhibitor provided herein or a composition thereof can bypass aspects of T-cell dysfunction such as T-cell tolerance, T-cell anergy, and/or T-cell exhaustion. In some embodiments, the method of modulating activity of a T-cell (*e.g.*, decreasing T-cell tolerance, decreasing T-cell anergy and/or decreasing T-cell exhaustion) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof. In some embodiments of the methods herein, modulating activity of a T-cell (*e.g.*, decreasing T-cell tolerance, decreasing T-cell anergy, and/or decreasing T-cell exhaustion) comprises contacting the T-cell

with an effective amount of a Cbl-b inhibitor or a composition thereof in the presence of an anti-CD3 antibody in combination with an anti-CD28 antibody. In some embodiments of the methods herein, the method of modulating activity of a T-cell (*e.g.*, decreasing T-cell tolerance, decreasing T-cell anergy and/or decreasing T-cell exhaustion) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof, wherein the T-cell previously has been in contact with an anti-CD3 antibody in combination with an anti-CD28 antibody. In some embodiments, stimulation via the co-stimulatory CD28 molecule is not required for modulating the activity of the T-cell (*e.g.*, decreasing T-cell tolerance, decreasing T-cell anergy, and/or decreasing T-cell exhaustion). In some embodiments of the methods herein, the method of modulating activity of a T-cell (*e.g.*, decreasing T-cell tolerance, decreasing T-cell anergy, and/or decreasing T-cell exhaustion) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof in the presence of an anti-CD3 antibody alone. In some embodiments, the method of modulating activity of a T-cell (*e.g.*, decreasing T-cell tolerance, decreasing T-cell anergy, and/or decreasing T-cell exhaustion) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof, wherein the T-cell has previously been in contact with one or more agents that activate the T-cell, such as an anti-CD3 antibody alone.

[215] T-cell activation and T-cell tolerance are tightly controlled processes regulating the immune response. Accordingly, provided herein are methods of modulating activity of the T-cell, wherein modulating activity of the T-cell comprises increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and/or decreased T-cell tolerance. In some embodiments, the method of modulating activity of a T-cell (*e.g.*, increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and/or decreased T-cell tolerance) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof. In some embodiments of the methods herein, modulating activity of a T-cell (*e.g.*, increasing T-cell activation, increasing T-cell proliferation, decreasing T-cell exhaustion, and/or decreasing T-cell tolerance) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof in the presence of an anti-CD3 antibody in combination with an anti-CD28 antibody. In some embodiments of the methods herein, the method of modulating activity of a T-cell (*e.g.*, increasing T-cell activation, increasing T-cell proliferation, decreasing T-cell exhaustion, and/or decreasing T-cell tolerance) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof, wherein the T-cell previously has been in contact with an anti-CD3 antibody in combination

with an anti-CD28 antibody. In some embodiments, stimulation via the co-stimulatory CD28 molecule is not required for modulating the activity of the T-cell (*e.g.*, increasing T-cell activation, increasing T-cell proliferation, decreasing T-cell exhaustion, and/or decreasing T-cell tolerance). In some embodiments of the methods herein, the method of modulating activity of a T-cell (*e.g.*, increasing T-cell activation, increasing T-cell proliferation, decreasing T-cell exhaustion, and/or decreasing T-cell tolerance) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor provided herein or a composition thereof in the presence of an anti-CD3 antibody alone. In some embodiments, the method of modulating activity of a T-cell (*e.g.*, increasing T-cell activation, increasing T-cell proliferation, decreasing T-cell exhaustion, and/or decreasing T-cell tolerance) comprises contacting the T-cell with an effective amount of a Cbl-b inhibitor or a composition thereof, wherein the T-cell has previously been in contact with one or more agents that activate the T-cell (*e.g.*, an anti-CD3 antibody).

[216] In some embodiments of the methods herein, increased T-cell activation comprises increased production of one or more cytokines from T-cells or surrounding immune cells in the activated T-cell microenvironment (*e.g.*, myeloid cells). In some embodiments, the one or more cytokines include, but are not limited to, IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-13, IL-18, TNF α , and GM-CSF. In some embodiments, the cytokine is one or more of IL-2, IFN- γ , TNF α , and GM-CSF. In some embodiments, the cytokine is a chemokine. In some embodiments, the one or more chemokines include, but are not limited to, IP-10, Eotaxin, GRO alpha, RANTES, MIP-1 α , MIP-1 β , MIP-2, MCP-1, and MCP-3. Increased expression of cytokines can be measured by ELISA.

[217] In some embodiments of the methods herein, increased T-cell activation comprises increased cell surface expression of one or more T-cell activation markers. In some embodiments, the one or more T-cell activation markers include, but are not limited to, CD25, CD44, CD62L, CD69, CD152 (CTLA4), CD154, CD137, and CD279. In some embodiments, the T-cell activation marker is one or more of CD25, CD69, and CTLA4. Increased expression of cell surface markers can be measured by FACS.

[218] Methods for experimentally determining increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and/or decreased T-cell tolerance are well known in the art. In some embodiments, representative methods of determining T-cell activation can be found in Biological Example 2 provided herein. In some embodiments, representative *in vitro* and *in vivo* methods of determining increased T-cell activation, increased T-cell proliferation,

decreased T-cell exhaustion, and/or decreased T-cell tolerance can be found in Biological Example 3 provided herein.

[219] In some embodiments of the methods for modulating activity of an immune cell, the immune cell is a B-cell and modulating activity of the B-cell comprises increased B-cell activation. In some embodiments, increased B-cell activation comprises increased cell surface expression of one or more B-cell activation markers. In some embodiments, the one or more B-cell activation markers include, but are not limited to, CD69, CD86, and MHC class II (e.g., HLA-DR). In some embodiments, the B-cell activation marker is CD69. Increased expression of cell surface markers can be measured by FACS. In some embodiments, increased B-cell activation comprises increased activation of proteins in signaling pathways such as those mediated by ERK, JNK, and Syk. Increased activation of said proteins can be detected by measurement of levels of phosphorylation on the proteins using reagents such as anti-phospho antibodies available in the art.

[220] In some embodiments of the methods for modulating activity of an immune cell, the immune cell is a NK-cell and modulating activity of the NK-cell comprises increased NK-cell activation. In some embodiments, increased NK-cell activation comprises secretion of one or more cytokines. In some embodiments, the one or more cytokines include, but are not limited to, IFN- γ , TNF α , and MIP-1 β . Increased expression of cytokines can be measured by ELISA. In some embodiments, increased NK-cell activation comprises increased cell surface expression of one or more NK-cell activation markers. In some embodiments, the one or more NK-cell activation markers include, but are not limited to, CD69, and CD107a. Increased expression of cell surface markers can be measured by FACS. In some embodiments, increased NK-cell activation comprises increased killing of target cells such as tumor cells, including primary tumor cells, and cell line derived tumor cells such as the K562 cell line.

[221] Methods for experimentally determining increased B-cell activation and NK-cell activation are well known in the art (*see, e.g.*, Fauriat *et al.*, Blood. 115: 2167-76, 2010; Beano *et al.*, J. Transl. Med. 6: 25, 2008; Claus *et al.*, J. Immunol. Methods, 341: 154-64, 2009; and Fujisaki *et al.*, Cancer Res. 69: 4010-4017, 2009). In some embodiments, representative methods of determining B-cell activation can be found in Biological Example 3 provided herein. In some embodiments, representative methods of determining NK-cell activation can be found in Biological Example 3 provided herein.

[222] Modulation of activity of an immune cell, such as a T-cell, a B-cell, or a NK-cell can be measured by determining a baseline value for a parameter of interest (*e.g.*, cytokine

secretion). For example, T-cell activation, such as in a sample obtained from *in vitro* experiments of cells contacted with a Cbl-b inhibitor, can be measured before contacting or administering said Cbl-b inhibitor to determine a baseline value. A reference value then is obtained for T-cell activation after contacting or administering said Cbl-b inhibitor. The reference value is compared to the baseline value in order to determine the amount of T-cell activation due to contact or administration of the Cbl-b inhibitor or composition thereof. For example, in some embodiments, immune cell (*e.g.*, T-cell) activation is increased by at least 0.1-fold in a sample as compared to a baseline value, wherein the baseline value is obtained before contacting the immune cell (*e.g.*, T-cell) with a Cbl-b inhibitor or a composition thereof. In some embodiments, immune cell (*e.g.*, T-cell) activation is increased by at least about 0.1-fold, about 0.2-fold, about 0.3-fold, about 0.4-fold, about 0.5-fold, about 0.6-fold, about 0.7-fold, about 0.8-fold, about 0.9-fold, about 1-fold, about 2-fold, about 4-fold, about 6-fold, about 8-fold, about 10-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 75-fold, or about 100-fold over a baseline value (*e.g.*, about 0.1-fold to about 100-fold, or about 1-fold to about 100-fold). Immune cell activation can be assessed by measuring biological markers of activation such as increased cytokine secretion, increased cell surface expression of activation markers (*e.g.*, cell surface markers), or increased phosphorylation of proteins in a downstream signaling pathway. The fold over baseline value that indicates immune cell activation can be determined for the parameter being tested and the conditions under which the immune cells are treated. For example, for measuring T-cell activation, a baseline value can be obtained from T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody, wherein the cells are not incubated with a Cbl-b inhibitor. A reference value is then obtained from T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody, wherein the T-cells have been or are in contact with a Cbl-b inhibitor. A positive response for immune cell activation can then be determined by the obtained reference value. Similar reference value measurements can be obtained and compared to a baseline value for assessing T-cell activation, T-cell proliferation, T-cell exhaustion, T-cell tolerance, B-cell activation, and/or NK-cell activation. Measurements for these parameters can be obtained utilizing techniques well known in the art, as well as the techniques provided in Biological Examples 2 and 3.

[223] The terms "baseline" or "baseline value" as used herein can refer to a measurement or characterization before administration of a therapeutic agent as disclosed herein (*e.g.*, a composition comprising a Cbl-b inhibitor as described herein) or at the beginning of

administration of the therapeutic agent. The baseline value can be compared to a reference value in order to determine the increase or decrease of an immune cell function (*e.g.*, increasing T-cell activation, increasing T-cell proliferation, decreasing T-cell exhaustion, and/or decreasing T-cell tolerance). The terms "reference" or "reference value" as used herein can refer to a measurement or characterization after administration of the therapeutic agent as disclosed herein (*e.g.*, a composition comprising a Cbl-b inhibitor as described herein). The reference value can be measured one or more times during an experimental time course, dosage regimen, or treatment cycle, or at the completion of the experimental time course, dosage regimen, or treatment cycle. A "reference value" can be an absolute value, a relative value, a value that has an upper and/or lower limit, a range of values, an average value, a median value, a mean value, or a value as compared to a baseline value. Similarly, a "baseline value" can be an absolute value, a relative value, a value that has an upper and/or lower limit, a range of values, an average value, a median value, a mean value, or a value as compared to a reference value. The reference value and/or baseline value can be obtained from one sample (*e.g.*, one sample obtained from an individual), from two different samples (*e.g.*, a sample obtained from two different individuals) or from a group of samples (*e.g.*, samples obtained from a group of two, three, four, five, or more individuals).

[224] In some embodiments, a positive response for T-cell activation as measured by cytokine secretion (*e.g.*, IL-2 secretion) by T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody in the presence of a Cbl-b inhibitor is at least 2.5-fold over the baseline value for cytokine secretion (*e.g.*, IL-2 secretion) obtained from T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody in the absence of a Cbl-b inhibitor. In some embodiments, a positive response for T-cell activation as measured by surface marker expression (*e.g.*, CD25 surface marker staining) by T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody in the presence of a Cbl-b inhibitor is at least 1.3-fold over the baseline value for surface marker expression (*e.g.*, CD25 surface marker staining) obtained from T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody in the absence of a Cbl-b inhibitor. In some embodiments, a baseline value can be obtained from T-cells stimulated with anti-CD3 antibody alone, wherein the cells are not incubated with a Cbl-b inhibitor. In some embodiments, a positive response for T-cell activation as measured by cytokine secretion (*e.g.*, IL-2 secretion) by T-cells stimulated with anti-CD3 antibody alone in the presence of a Cbl-b inhibitor is at least 0.1-fold over the baseline value for cytokine secretion (*e.g.*, IL-2 secretion) obtained from T-cells stimulated with anti-

CD3 antibody alone in the absence of a Cbl-b inhibitor. In some embodiments, a positive response for T-cell activation as measured by surface marker expression (*e.g.*, CD25 surface marker staining) by T-cells stimulated with anti-CD3 antibody alone in the presence of a Cbl-b inhibitor is at least 0.6-fold over the baseline value for surface marker expression (*e.g.*, CD25 surface marker staining) obtained from T-cells stimulated with anti-CD3 antibody alone in the absence of a Cbl-b inhibitor.

[225] In some aspects, provided herein are methods of producing a modified immune cell, comprising culturing a cell population containing an immune cell in the presence of an effective amount of a Cbl-b inhibitor provided herein or a composition thereof to modulate the activity of the immune cell, thereby producing the modified immune cell. In some embodiments, the immune cell is a T-cell, a B-cell, or a natural killer (NK) cell.

[226] In some embodiments of the methods for producing a modified immune cell, the immune cell that is to be modified is a cell selected from the group consisting of a hematopoietic cell, a multipotent stem cell, a myeloid progenitor cell, a lymphoid progenitor cell, a T-cell, a B-cell, and a NK-cell. In some embodiments, the method further comprises culturing the immune cell with stimulating agents such as cytokines or antibodies that bind to activating proteins expressed by the immune cell (*e.g.*, an anti-CD3 antibody and/or an anti-CD28 antibody). In some embodiments, the immune cell that is to be modified is in a cell population containing the immune cell, wherein the cell population is obtained as a sample from an individual. In some embodiments, the immune cell that is to be modified is in a cell population containing the immune cell, wherein the cell population is obtained from culturing a biological sample (*e.g.*, blood sample, bone marrow sample, etc.) from an individual. In some embodiments, the immune cell is modified by contacting the cell population containing the immune cell with a Cbl-b inhibitor or composition thereof thereby producing a modified immune cell. In some embodiments, the modified immune cell is a cell selected from the group consisting of a hematopoietic cell, a multipotent stem cell, a myeloid progenitor cell, a lymphoid progenitor cell, a T-cell, a B-cell, and a NK-cell. In some embodiments, the immune cell is the same cell type as the modified immune cell. For example, the immune cell can be an inactive T-cell and the modified immune cell can be an activated T-cell. In some embodiments, the immune cell is a different cell type than the modified immune cell. For example, the immune cell can be a hematopoietic stem cell and the modified immune cell can be an NK-cell that has differentiated from the hematopoietic stem cell. In some embodiments of the method of producing the modified immune cell, the method further comprises recovering

the modified immune cell. In some embodiments, the cell population containing the immune cell, the immune cell or the modified immune cell is from an individual (*e.g.*, a human). In some embodiments, the immune cell or modified immune cell is a human immune cell or human modified immune cell, respectively.

[227] Further provided herein are modified immune cells produced by any of the methods described herein such as culturing a cell population containing an immune cell in the presence of an effective amount of a Cbl-b inhibitor to modulate the activity of the immune cell and thereby produce the modified immune cell.

[228] In some embodiments, the Cbl-b inhibitors provided herein are cell membrane permeable. Accordingly, in some embodiments, a modified immune cell provided herein can comprise a Cbl-b inhibitor described herein such as in the cytoplasm of the modified immune cell.

[229] In some aspects, provided herein is an isolated modified immune cell, wherein the modified immune cell has been contacted or is in contact with a Cbl-b inhibitor described herein or a composition thereof. In some embodiments, the modified immune cell is a T-cell, a B-cell, or a natural killer (NK) cell. In some embodiments, the modified immune cell is a hematopoietic cell, a multipotent stem cell, a myeloid progenitor cell, a lymphoid progenitor cell, a T-cell, a B-cell, or a NK-cell.

[230] In some embodiments of the isolated modified immune cell, the modified immune cell is a T-cell, and the T-cell exhibits increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and/or decreased T-cell tolerance. In some embodiments, increased T-cell activation comprises increased production of one or more cytokines from T-cells or surrounding immune cells in the activated T-cell microenvironment (*e.g.*, myeloid cells). In some embodiments, the one or more cytokines include, but are not limited to IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-13, IL-18, TNF α , and GM-CSF. In some embodiments, the one or more cytokines is one or more selected from the group consisting of IL-2, IFN- γ , TNF α , and GM-CSF. In some embodiments, the cytokine is a chemokine. In some embodiments, the one or more chemokines include, but are not limited to IP-10, Eotaxin, GRO α , RANTES, MIP-1 α , MIP-1 β , MIP-2, MCP-1, and MCP-3. In some embodiments, increased T-cell activation comprises increased cell surface expression of one or more T-cell activation markers. In some embodiments, the one or more T-cell activation markers include, but are not limited to, CD25, CD44, CD62L, CD69, CD152 (CTLA4), CD154, CD137, and CD279. In some embodiments, the one or more T-cell activation markers include, but are not limited to, CD25,

CD69, and CTLA4. In some embodiments, the T-cell activation markers are CD25 and/or CD69. In some embodiments, the T-cell has been or is in contact with an anti-CD3 antibody. In some embodiments, the T-cell has been or is in contact with an anti-CD3 antibody in combination with an anti-CD28 antibody.

[231] In some embodiments of the isolated modified immune cell, the modified immune cell is a NK-cell, and the NK-cell exhibits increased NK-cell activation. In some embodiments, increased NK-cell activation comprises increased secretion of one or more cytokines (*e.g.*, IFN- γ , TNF α , and/or MIP-1 β). In some embodiments, increased NK-cell activation comprises increased cell surface expression of one or more NK-cell activation markers (*e.g.*, CD69 and/or CD107a).

[232] In some embodiments of the isolated modified immune cell, the modified immune cell is a B-cell, and the B-cell exhibits increased B-cell activation. In some embodiments, increased B-cell activation comprises increased cell surface expression of one or more B-cell activation markers (*e.g.*, CD69, CD86, and/or HLA-DR).

[233] In some of any embodiments of the methods or modified immune cells provided herein, the immune cell or modified immune cell is a mammalian cell (*e.g.*, human cell). In some embodiments, the immune cell or modified immune cell is a human cell.

[234] In some aspects, incubation is carried out in accordance with techniques such as those described in U.S. Patent No. 6,040,177; Klebanoff *et al.*, J Immunother., 35: 651-660, 2012; Terakura *et al.*, Blood, 119: 72-82, 2012; and Wang *et al.*, J Immunother., 35: 689-701, 2012.

[235] The immune cells to be modified or modified immune cells provided herein can be engineered to express a recombinant chimeric receptor such as a chimeric antigen receptor (CAR). In some embodiments, the CAR comprises from its N terminus to C terminus: an extracellular ligand-binding domain, a transmembrane domain, an intracellular costimulatory domain and an activating cytoplasmic signaling domain. In some embodiments, the CAR comprises from its N terminus to C terminus an extracellular ligand-binding domain, a transmembrane domain, and an activating cytoplasmic signaling domain. The immune cells can be engineered to express the recombinant chimeric receptor (*e.g.*, CAR) before, during, or after contact with a Cbl-b inhibitor provided herein. In some embodiments, an immune cell to be modified is a T-cell (*e.g.*, a CD4⁺ T-cell or a CD8⁺ T-cell). In a further embodiment, the T-cell comprises a recombinant chimeric receptor such as a CAR. In some embodiments, the modified immune cell is a modified T-cell (*e.g.*, a CD4⁺ T-cell or a CD8⁺ T-cell). In a further embodiment, the modified T-cell comprises a recombinant chimeric receptor such as a CAR.

Methods for producing immune cells expressing recombinant chimeric receptors are well known in the art such as by the introduction of a nucleic acid encoding the recombinant chimeric receptor (*e.g.*, CAR) to an immune cell (*e.g.*, T-cell) via a vector (*e.g.*, viral vector). See, for example, International Patent Application No. WO 2017/096329 and U.S. Publication No. US 2017/0204372.

[236] In particular, this disclosure provides methods of producing an expanded population of lymphocytes, the method comprising: (a) obtaining a biological sample comprising lymphocytes from an individual with cancer, wherein the individual has received or is receiving an effective amount of a Cbl-b inhibitor as a monotherapy or as part of a combination therapy, and (b) culturing the lymphocytes in cell culture medium comprising at least one T-cell growth factor to produce an expanded population of lymphocytes. In certain embodiments, the lymphocytes are tumor infiltrating lymphocytes (TILs). In certain embodiments, the lymphocytes are peripheral blood mononuclear cells (PBMCs). In certain embodiments, the at least one T-cell growth factor comprises one or more of the group consisting of IL-2, IL-7, IL-15, and IL-21, optionally wherein the at least one T-cell growth factor comprises IL-2. In some embodiments, the cell culture medium further comprises an anti-CD3 antibody, or both an anti-CD3 antibody and an anti-CD28 antibody. In some embodiments, the cell culture medium further comprises the Cbl-b inhibitor. In some embodiments, the cell culture medium further comprises irradiated feeder cells. In some embodiments, the individual is a human patient. Also provided by this disclosure are compositions comprising the expanded population of TILs produced by the aforementioned methods, and a physiologically acceptable buffer.

[237] In some embodiments, methods for isolation and processing of immune cells to be modified or which have been modified (*i.e.*, modified immune cells) include steps for freezing (*e.g.*, cryopreserving) the cells, either before or after isolation, incubation (*e.g.*, incubation with a Cbl-b inhibitor), and/or engineering (*e.g.*, introduction of a nucleic acid encoding a recombinant chimeric receptor to the immune cell). A variety of freezing solutions and parameters known in the art may be used.

B. Adoptive Cell Therapy

[238] The modified immune cells, such as an expanded population of lymphocytes, or compositions thereof produced by the methods described herein can be used as a therapeutic agent in methods of treatment of an individual in need thereof, such as an individual having cancer. Such methods of treatment include adoptive cell therapy. In some embodiments, the method of treatment includes isolating cells from an individual, preparing, processing,

culturing, and/or engineering them, as described herein, and re-introducing them into the same individual, before or after cryopreservation. In some embodiments, the method of treatment includes isolating cells from an individual, preparing, processing, culturing, and/or engineering them, as described herein, and re-introducing them into a different individual, before or after cryopreservation.

[239] Accordingly, in some aspects, provided herein is a method of modulating the immune response in an individual, the method comprising administering an effective amount of a modified immune cell described herein or a composition thereof to an individual in need thereof (*e.g.*, an individual with a T-cell dysfunction disorder). In some embodiments, the individual has a cancer. In some embodiments, provided herein is a method of treating a cancer responsive to inhibition of Cbl-b activity, the method comprising administering an effective amount of a modified immune cell described herein or a composition thereof to an individual having the cancer responsive to inhibition of Cbl-b activity. In some embodiments, provided herein is a method of inhibiting abnormal cell proliferation, the method comprising administering an effective amount of a modified immune cell described herein or a composition thereof to an individual in need thereof. The term “abnormal cell proliferation” as used herein includes hyperplasia or cancer cell proliferation. The cancer cell may be derived from a hematologic cancer or a non-hematologic cancer. In some embodiments, the cancer is a hematologic cancer, such as lymphoma, a leukemia or a myeloma. In other embodiments, the cancer cell is derived from a non-hematologic cancer, such as a sarcoma, a carcinoma, or a melanoma.

[240] In certain embodiments, an individual in need of treatment, such as an individual having cancer or a T-cell dysfunction disorder, is administered a composition comprising the modified immune cells provided herein at a range of about one million to about 100 billion cells, such as, *e.g.*, 1 million to about 50 billion cells (*e.g.*, about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (*e.g.*, about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (*e.g.*, about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about

650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges.

[241] The modified immune cells and compositions thereof are administered using standard administration techniques, formulations, and/or devices. Provided are formulations and devices, such as syringes and vials, for storage and administration of the compositions. Formulations or pharmaceutical compositions comprising the modified immune cells include those for intravenous, intraperitoneal, subcutaneous, or intramuscular administration. In some embodiments, the modified immune cells are administered parenterally. The term “parenteral,” as used herein, includes, but is not limited to, intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In some embodiments, the cell populations are administered to a subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injections. Compositions of the modified immune cells can be provided as sterile liquid preparations, *e.g.*, isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may in some aspects be buffered to a selected pH. Viscous compositions can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol) and suitable mixtures thereof. Sterile injectable solutions can be prepared by incorporating the modified immune cells in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like.

[242] In some embodiments, the modified immune cells are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. For instance, in some therapeutic regimens of this disclosure, both the modified immune cells and a Cbl-b inhibitor are administered to a mammalian subject in need thereof, wherein the Cbl-b inhibitor is a compound of Formula (I), (I-a), (I-b), (I-A)-(I-J), (II-A)-(II-H), (III-A)-(III-H), or (IV-A)-(IV-H), or any variation thereof. Thus, in some embodiments the therapeutic regimens comprise both adoptive cell therapy and chemotherapy.

[243] After the modified immune cells are administered to an individual (*e.g.*, a human), the biological activity of the modified immune cell populations can be measured by methods known in the art. Parameters to assess include specific binding of modified immune cell or other immune cell to antigen, *in vivo* (*e.g.*, by imaging) or *ex vivo* (*e.g.*, by ELISA or flow

cytometry). In some embodiments, the ability of modified immune cells to destroy target cells can be measured using a cytotoxicity assay (*see, e.g.*, Kochenderfer *et al.*, J. Immunotherapy, 32: 689-702, 2009; and Herman *et al.* J. Immunological Methods, 285: 25-40, 2004. In some embodiments, the biological activity of the modified immune cells also can be measured by assaying expression and/or secretion of certain cytokines, such as IL-2 and IFN γ .

C. Administration of Cbl-b Inhibitor

[244] In some aspects, a Cbl-b inhibitor or composition thereof can be administered directly to an individual to modulate an immune response, treat a disease or condition (*e.g.*, cancer and/or abnormal cell proliferation) and/or inhibit Cbl-b activity in the individual. The Cbl-b inhibitor may be a compound of Table 1, a tautomer thereof, or a pharmaceutically acceptable salt of any of the foregoing.

[245] In some embodiments, provided herein is a method of modulating the immune response, the method comprising administering an effective amount of a Cbl-b inhibitor provided herein or a composition thereof to an individual to modulate the immune response in the individual. In some embodiments, the individual has a cancer such as a hematologic cancer or non-hematological cancer described herein.

[246] In some embodiments, provided herein is a method of treating cancer responsive to inhibition of Cbl-b activity, the method comprising administering an effective amount of a Cbl-b inhibitor provided herein or a composition thereof to an individual to treat the cancer responsive to inhibition of Cbl-b activity. In some embodiments, the cancer is a hematologic cancer or non-hematological cancer such as one described herein.

[247] In some embodiments, provided herein is a method of inhibiting abnormal cell proliferation (*e.g.*, hyperplasia), the method comprising administering an effective amount of a Cbl-b inhibitor provided herein or a composition thereof to an individual to inhibit abnormal cell proliferation in the individual.

[248] In some embodiments, provided herein is a method of inhibiting Cbl-b activity, the method comprising administering an effective amount of a Cbl-b inhibitor provided herein or a composition thereof to an individual to inhibit Cbl-b activity in the individual.

[249] In some embodiments, such as in the modulation of an immune response in an individual in need thereof (*e.g.*, an individual with a T-cell dysfunction disorder), treatment of a disease or condition in an individual (*e.g.*, an individual cancer and/or abnormal cell proliferation) and/or inhibition of Cbl-b activity in an individual, the appropriate dosage of an active agent, will depend on the type of condition, disease, or disorder to be treated, as defined

above, the severity and course of the condition, disease, or disorder, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the Cbl-b inhibitor, and the discretion of the attending physician.

[250] The Cbl-b inhibitor or composition thereof is suitably administered to the individual at one time or over a series of treatments. In some embodiments, the treatment includes multiple administrations of the Cbl-b inhibitor or composition, wherein the interval between administrations may vary. For example, the interval between the first administration and the second administration is about one month, and the intervals between the subsequent administrations are about three months. In some embodiments, a Cbl-b inhibitor is administered at a flat dose. In some embodiments, a Cbl-b inhibitor described herein is administered to an individual at a fixed dose based on the individual's weight (*e.g.*, mg/kg).

[251] In some aspects of this disclosure, the cancer is a hematologic cancer. For example, the hematologic cancer may be a lymphoma, a leukemia, or a myeloma. In other aspects of this disclosure, the cancer is a non-hematologic cancer. In particular, the non-hematologic cancer may be a carcinoma, a sarcoma, or a melanoma.

[252] In some embodiments, the Cbl-b inhibitor is co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. For instance, in some therapeutic regimens of this disclosure, both the Cbl-b inhibitor and modified immune cells are administered to a mammalian subject in need thereof, wherein the Cbl-b inhibitor is a compound of Formula (I), (I-a), (I-b), (I-A)-(I-J), (II-A)-(II-H), (III-A)-(III-H), or (IV-A)-(IV-H), or any variation thereof. Thus, in some embodiments the therapeutic regimens comprise both adoptive cell therapy and chemotherapy.

[253] In some embodiments, the effectiveness of Cbl-b inhibitor administration in the methods herein (*e.g.*, method of modulating an immune response in an individual) can be assessed by measuring the biological activity of immune cells present in a sample (*e.g.*, blood sample) isolated from the treated individual. For example, the ability of immune cells isolated from the individual after treatment with a Cbl-b inhibitor to destroy target cells in a cytotoxicity assay may be measured to assess treatment efficacy. In some embodiments, the biological activity of immune cells present in a sample (*e.g.*, blood sample) can be measured by assaying expression and/or secretion of certain cytokines, such as IL-2 and IFN γ .

[254] This disclosure provides methods of treating cancer, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, and administering to the

individual an effective amount of an additional therapeutic agent. Also provided are methods of treating an individual with cancer, comprising administering to the individual an effective amount of a Cbl-b inhibitor; and administering to the individual an effective amount of an additional therapeutic agent. Additionally, this disclosure provides methods of increasing an anti-cancer immune response, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, and administering to the individual an effective amount of an additional therapeutic agent. Further provided are methods of treating cancer, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, wherein the individual has received or is receiving an effective amount of an additional therapeutic agent.

[255] In some embodiments of the methods of the preceding paragraph, the Cbl-b inhibitor and the additional therapeutic agent are administered consecutively in either order. As used herein, the terms “consecutively,” “serially,” and “sequentially” refer to administration of a Cbl-b inhibitor after an additional therapeutic agent, or administration of the additional therapeutic agent after the Cbl-b inhibitor. For instance, consecutive administration may involve administration of the Cbl-b inhibitor in the absence of the additional therapeutic agent during an induction phase (primary therapy), which is followed by a post-induction treatment phase comprising administration of the additional therapeutic agent. The methods may further comprise a maintenance phase comprising administration of the Cbl-b inhibitor or the additional therapeutic agent. Alternatively, consecutive administration may involve administration of the additional therapeutic agent in the absence of the Cbl-b inhibitor during an induction phase (primary therapy), which is followed by a post-induction treatment phase comprising administration of the Cbl-b inhibitor. The methods may further comprise a maintenance phase comprising administration of the Cbl-b inhibitor or the additional therapeutic agent.

[256] In some embodiments of the combination therapy methods, the Cbl-b inhibitor and the additional therapeutic agent are administered concurrently. As used herein, the terms “concurrently,” “simultaneously,” and “in parallel” refer to administration of a Cbl-b inhibitor and an additional therapeutic agent during the same doctor visit or during the same phase of treatment. For instance, both the Cbl-b inhibitor and the additional therapeutic agent may be administered during one or more of an induction phase, a treatment phase, and a maintenance phase. However, concurrent administration does not require that the Cbl-b inhibitor and the additional therapeutic agent be present together in a single formulation or pharmaceutical

composition, or that the Cbl-b inhibitor and the additional therapeutic agent be administered at precisely the same time.

1. Combination Therapy Comprising a Cbl-b Inhibitor and an Immune Checkpoint Inhibitor

[257] In some embodiments of the combination therapy methods of this disclosure, the additional therapeutic agent comprises an immune checkpoint inhibitor. The term “immune checkpoint” refers to a signaling pathway that prevents activation of immune cells, while the term “immune checkpoint inhibitor” refers to a compound that impedes the immune checkpoint to remove the brake on activation of immune cells. In some embodiments, the immune checkpoint inhibitor is an antagonist of at least one inhibitory checkpoint molecule. In certain embodiments, the inhibitory checkpoint molecule is selected from the group consisting of PD-1 (CD279), PD-L1 (CD274), CTLA-4 (CD125), LAG3 (CD223), PVR (CD155), PVRL2 (CD112), PVRL3 (CD113), TIGIT, TIM3 (CD366), and VISTA. In certain embodiments, the immune checkpoint inhibitor is an antagonist of at least one inhibitory checkpoint molecule selected from the group consisting of PD-1 (CD279), PD-L1 (CD274), and CTLA-4 (CD152).

[258] PD-1 refers to programmed cell death protein 1 (PD-1). PD-1 antagonists suitable for the treatment methods, medicaments, and uses of this disclosure include any chemical compound or biological molecule that blocks binding of PD-L1 expressed on a cancer cell or antigen presenting cell to PD-1 expressed on a lymphocyte (T-cell, B-cell, and/or NK-cell). Alternative names or synonyms for PD-1 and its ligand include CD279, PDCD1, PD1, and SLEB2 for PD-1; and CD274, PDCD1L1, PDL1, B7H1, B7-4, and B7-H for programmed cell death 1 ligand 1 (PD-L1). In some embodiments in which a human subject is being treated, the PD-1 antagonist blocks binding of human PD-L1 to human PD-1. The amino acid sequence of the mature form of human PD-1 is set forth as residues 21-288 in NCBI Locus No. NP_005009. The amino acid sequence of the mature form of human PD-L1 is set forth as residues 19-290 in NCBI Locus No. NP_054862.

[259] CTLA-4 refers to cytotoxic T-lymphocyte associated protein 4. CTLA-4 antagonists suitable for the treatment methods, medicaments and uses of this disclosure include any chemical compound or biological molecule that blocks binding of CTLA-4 expressed on a lymphocyte (T-cell, B-cell, and/or NK-cell) to a ligand (CD80 and/or CD86) expressed on an antigen presenting cell. Alternative names or synonyms for CTLA-4 include CD152, CTLA4, ALPS5, CELIAC3, GRD4, GSE, and IDDM12. In some embodiments in which a human subject is being treated, the CTLA-4 antagonist blocks binding of human CTLA-4 to a human

ligand. The amino acid sequence of the mature form of human CTLA-4 is set forth as residues 36-223 in NCBI Locus No. NP_005205.

[260] LAG3 refers to lymphocyte activating gene 3 protein. LAG3 antagonists suitable for the treatment methods, medicaments, and uses of this disclosure include any chemical compound or biological molecule that blocks binding of LAG3 expressed on a lymphocyte (T-cell, B-cell, and/or NK-cell) to a ligand (MHC class II) expressed on an antigen presenting cell. LAG3 is also known as CD223. In some embodiments in which a human subject is being treated, the LAG3 antagonist blocks binding of human LAG3 to a human ligand. The amino acid sequence of the mature form of human LAG3 is set forth as residues 23-525 in NCBI Locus No. NP_002277.

[261] PVR refers to poliovirus receptor. PVR antagonists suitable for the treatment methods, medicaments, and uses of this disclosure include any chemical compound or biological molecule that blocks binding of PVR expressed on a cancer cell or an antigen presenting cell to TIGIT expressed on a lymphocyte (T-cell, B-cell, and/or NK-cell). Alternative names or synonyms for PVR include CD155, PVS, HVED, NECL5, nectin-like protein 5, and TAGE4. In some embodiments in which a human subject is being treated, the PVR antagonist blocks binding of human PVR to human TIGIT. There are multiple isoforms of human PVR. The amino acid sequence of alpha isoform of human PVR is set forth in NCBI Locus No. NP_006496. The amino acid sequence of beta isoform of human PVR is set forth in NCBI Locus No. NP_001129240. The amino acid sequence of gamma isoform of human PVR is set forth in NCBI Locus No. NP_001129241. The amino acid sequence of delta isoform of human PVR is set forth in NCBI Locus No. NP_001129242.

[262] PVRL2 refers to poliovirus receptor related 2. PVRL2 antagonists suitable for the treatment methods, medicaments, and uses of this disclosure include any chemical compound or biological molecule that blocks binding of PVRL2 expressed on a cancer cell or an antigen presenting cell to TIGIT expressed on a lymphocyte (T-cell, B-cell, and/or NK-cell). Alternative names or synonyms for PVRL2 include CD112, NECTIN2, HVEB, herpesvirus entry mediator B, PRR2, and PVRR2. In some embodiments in which a human subject is being treated, the PVRL2 antagonist blocks binding of human PVRL2 to human TIGIT. The amino acid sequence of the alpha isoform of human PVRL2 is set forth in NCBI Locus No. NP_002847. The amino acid sequence of the delta isoform of human PVRL2 is set forth in NCBI Locus No. NP_001036189.

[263] PVRL3 refers to poliovirus receptor related 3. PVRL3 antagonists suitable for the treatment methods, medicaments, and uses of this disclosure include any chemical compound or biological molecule that blocks binding of PVRL3 expressed on a cancer cell or an antigen presenting cell to TIGIT expressed on a lymphocyte (T-cell, B-cell, and/or NK-cell). Alternative names or synonyms for PVRL3 include CD113, NECTIN3, PRR3, and PVRR3. In some embodiments in which a human subject is being treated, the PVRL3 antagonist blocks binding of human PVRL3 to human TIGIT. The amino acid sequence of isoform 1 of human PVRL3 is set forth in NCBI Locus No. NP_056295. The amino acid sequence of isoform 2 of human PVRL3 is set forth in NCBI Locus No. NP_001230215. The amino acid sequence of isoform 3 of human PVRL3 is set forth in NCBI Locus No. NP_001230217.

[264] TIGIT refers to T-cell immunoreceptor with Ig and ITIM domains protein. TIGIT antagonists suitable for the treatment methods, medicaments, and uses of this disclosure include any chemical compound or biological molecule that blocks binding of TIGIT expressed on a lymphocyte (T-cell, B-cell, or NK-cell) to a ligand (CD112, CD113, and/or CD155) expressed on a cancer cell or an antigen presenting cell. Alternative names or synonyms for TIGIT include VSIG9, V-set and immunoglobulin domain containing 9, VSTM3, V-set and transmembrane domain containing 3, and Washington University cell adhesion molecule (WUCAM). In some embodiments in which a human subject is being treated, the TIGIT antagonist blocks binding of human TIGIT to a human ligand. The amino acid sequence of the mature form of human TIGIT is set forth as residues 22-244 in NCBI Locus No.: NP_776160.

[265] TIM3 refers to T-cell immunoglobulin and mucin-domain containing-3 protein. TIM3 antagonists suitable for the treatment methods, medicaments, and uses of this disclosure include any chemical compound or biological molecule that blocks binding of TIM3 expressed on a lymphocyte (T-cell, B-cell, or NK-cell) to a ligand (galectin-9 phosphatidylserine) expressed on an antigen presenting cell. Alternative names or synonyms for TIM3 include CD366, HAVCR2, hepatitis A virus cellular receptor 2, KIM3, and SPTCL. In some embodiments in which a human subject is being treated, the TIM3 antagonist blocks binding of human TIM3 to a human ligand. The amino acid sequence of the mature form of human TIM3 is set forth as residues 22-301 in NCBI Locus No. NP_116171.

[266] VISTA refers to V-domain Ig suppressor of T-cell activation. VISTA antagonists suitable for the treatment methods, medicaments, and uses of this disclosure include any chemical compound or biological molecule that blocks binding of VISTA expressed on a lymphocyte (T-cell, B-cell, and/or NK-cell) to a ligand expressed on a cancer cell or an antigen

presenting cell. Alternative names or synonyms for VISTA include VSIR, V-set immunoregulatory receptor, PD-1H, B7H5, GI24, PP2135, SISP1, and Dies1. In some embodiments in which a human subject is being treated, the VISTA antagonist blocks binding of human VISTA to a human ligand. The amino acid sequence of the mature form of human VISTA is set forth as residues 33-311 in NCBI Locus No.: NP_071436.

[267] The immune checkpoint inhibitor may be a biological molecule. For instance, the immune checkpoint inhibitor may comprise an antibody or antigen-binding fragment thereof. The antibody or fragment may be a monoclonal antibody (mAb), a human antibody, a humanized antibody, or a chimeric antibody, and may include a human constant region. In some embodiments the human constant region is selected from the group consisting of IgG1, IgG2, IgG3, and IgG4 constant regions, and in certain embodiments, the human constant region is an IgG1 or IgG4 constant region. In some embodiments, the antibody or fragment is a bispecific antibody. In some embodiments, the antigen-binding fragment comprises one of the group consisting of Fab, Fab'-SH, F(ab')₂, scFv, and Fv fragments.

[268] In some embodiments, the at least one inhibitory checkpoint molecule comprises PD-1. In certain embodiments, the immune checkpoint inhibitor is selected from the group consisting of pembrolizumab, nivolumab, cemiplimab, and biosimilars thereof. In one embodiment, the anti-PD-1 antibody is pembrolizumab (MK-3475 marketed as KEYTRUDA® by Merck & Co.). In one embodiment, the anti-PD-1 antibody is nivolumab (BMS-936558 or MDX-1106, marketed as OPDIVO® by Bristol-Myers Squibb). In one embodiment, the anti-PD-1 antibody is cemiplimab (REGN2810, Regeneron). In some embodiments, the immune checkpoint inhibitor is a variant of pembrolizumab, nivolumab, or cemiplimab.

[269] In some embodiments, the at least one inhibitory checkpoint molecule comprises PD-L1. In certain embodiments, the immune checkpoint inhibitor is selected from the group consisting of atezolizumab, avelumab, durvalumab, and biosimilars thereof. In one embodiment, the anti-PD-L1 antibody is atezolizumab (marketed as TECENTRIQ® by Genentech, Inc.). In one embodiment, the anti-PD-L1 antibody is avelumab (marketed as BAVENCIO® by EMD Serono, Inc. and Pfizer, Inc.). In one embodiment, the anti-PD-L1 antibody is durvalumab (MEDI4736 marketed as IMFINZI® by AstraZeneca). In some embodiments, the immune checkpoint inhibitor is a variant of atezolizumab, avelumab, or durvalumab.

[270] In some embodiments, the at least one inhibitory checkpoint molecule comprises CTLA-4. In certain embodiments, the immune checkpoint inhibitor is selected from the group consisting of ipilimumab, tremelimumab, and biosimilars thereof. In one embodiment, the anti-CTLA4 antibody is ipilimumab (MDX-010 or BMS-734016, marketed as YERVOY® by Bristol-Myers Squibb). In one embodiment, the anti-CTLA4 antibody is tremelimumab (ticilimumab, CP-675,206, developed by AstraZeneca). In some embodiments, the immune checkpoint inhibitor is a variant of ipilimumab, or tremelimumab.

[271] In some embodiments, the monoclonal antibody is a “variant” antibody which comprises heavy chain and light chain sequences that are identical to those in the “reference” antibody, except for having three, two, or one conservative amino acid substitutions at positions that are located outside of the light chain CDRs and/or six, five, four, three, two, or one conservative amino acid substitutions that are located outside of the heavy chain CDRs (*e.g.*, the variant positions are located in the framework regions or the constant region). In other words, the reference antibody and the variant antibody comprise identical CDR sequences, but differ from each other due to having a conservative amino acid substitution at no more than three or six other positions in their full length light and heavy chain sequences, respectively. A variant antibody is substantially the same as a reference antibody with respect to the following properties: binding affinity to the inhibitory checkpoint molecule and ability to block the binding of the inhibitory checkpoint molecule to its ligand.

[272] In other embodiments, the immune checkpoint inhibitor may comprise an immunoadhesin comprising the inhibitory checkpoint molecule binding domain of one of its ligands fused to a constant region such as an Fc region of an immunoglobulin molecule.

[273] As used herein the term “biosimilar” refers to a biological product that is similar to but without clinically meaningful differences in safety and effectiveness from a Federal Drug Administration (FDA)-approved reference product. For instance, there may be differences between a biosimilar product and a reference product in clinically inactive components (*e.g.*, differences in excipients of the formulations, minor differences in glycosylation, etc.). Clinically meaningful characteristics can be assessed through pharmacokinetic and pharmacodynamic studies. In some embodiments, the biosimilar product is an interchangeable product as determined by the FDA.

2. Combination Therapy Comprising a Cbl-b Inhibitor and an Antineoplastic Agent

[274] In some embodiments of the combination therapy methods of this disclosure, the additional therapeutic agent comprises an antineoplastic agent. As used herein, the terms “antineoplastic agent” and “antineoplastic agent” refer to a therapeutic agent classified according to the Anatomical Therapeutic Chemical Classification System (ATC) code L01 developed by the World Health Organization. In certain embodiments, the antineoplastic agent is classified as one of the group consisting of a cytotoxic antibiotic (ATC code L01D), a plant alkaloid (ATC code L01C), an antimetabolite (ATC code L01B), an alkylating agent (ATC code L01A), and other antineoplastic agent (ATC code L01X). In some embodiments, the antineoplastic agent is a small molecule drug (*e.g.*, cancer chemotherapeutic agent) as opposed to a biological molecule.

[275] A cytotoxic antibiotic is a suitable antineoplastic agent for the treatment methods, medicaments, and uses of this disclosure. In some embodiments, the cytotoxic antibiotic is selected from the group consisting of ixabepilone, mitomycin, plicamycin, bleomycin, pixantrone, amrubicin, valrubicin, pirarubicin, mitoxantrone, idarubicin, zorubicin, aclarubicin, epirubicin, daunorubicin, doxorubicin, and dactinomycin.

[276] A plant alkaloid is a suitable antineoplastic agent for the treatment methods, medicaments, and uses of this disclosure. In some embodiments, the plant alkaloid is selected from the group consisting of trabectedin, cabazitaxel, paclitaxel poliglumex, docetaxel, paclitaxel, demecolcine, teniposide, etoposide, vintafolide, vinflunine, vinorelbine, vindesine, vincristine, and vinblastine.

[277] An antimetabolite is a suitable antineoplastic agent for the treatment methods, medicaments, and uses of this disclosure. In some embodiments, the antimetabolite is a pyrimidine analog, a purine analog, or a folic acid analog. In some embodiments, the antimetabolite is selected from the group consisting of floxuridine, trifluridine, tegafur, fluorouracil, decitabine, azacitidine, capecitabine, gemcitabine, carmofur, tegafur, fluorouracil, cytarabine, nelarabine, clofarabine, fludarabine, cladribine, tioguanine, mercaptopurine, pralatrexate, pemetrexed, raltitrexed, and methotrexate.

[278] An alkylating agent is a suitable antineoplastic agent for the treatment methods, medicaments, and uses of this disclosure. In some embodiments, the alkylating agent is selected from the group consisting of dacarbazine, temozolomide, pipobroman, mitobronitol, etoglucid, uracil mustard, ranimustine, nimustine, fotemustine, streptozocin, semustine, lomustine, carmustine, carboquone, triaziquone, thiotepe, mannosulfan, treosulfan, busulfan,

bendamustine, prednimustine, trofosfamide, ifosfamide, mechlorethamine, melphalan, chlorambucil, and cyclophosphamide.

[279] In other embodiments, the antineoplastic agent comprises an other antineoplastic agent selected from the group consisting of a platinum compound (ATC Code L01XA), a methylhydrazine (ATC Code L01XB), a sensitizer (ATC Code L01XD), a protein kinase inhibitor (ATC Code L01XE), and an other agent (ATC Code L01XA).

[280] A platinum compound is a suitable antineoplastic agent for the treatment methods, medicaments, and uses of this disclosure. In some embodiments, the platinum compound is selected from the group consisting of cisplatin, carboplatin, oxaliplatin, satraplatin, and polyplatillen.

3. Combination Therapy Comprising a Cbl-b Inhibitor and Radiation Therapy

[281] This disclosure provides methods of treating cancer comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, and administering to the individual an effective amount of radiation therapy. Also provided are methods of treating an individual with cancer, comprising administering to the individual an effective amount of a Cbl-b, and administering to the individual an effective amount of radiation therapy. Additionally, this disclosure provides methods of increasing an anti-cancer immune response, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, and administering to the individual an effective amount of radiation therapy. Further provided are methods of treating cancer, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, wherein the individual has received or is receiving an effective amount of radiation therapy.

[282] In some embodiments, the radiation therapy is external beam radiation therapy. In other embodiments, the radiation therapy is internal radiation therapy. In some embodiments, the radiation therapy is ablative radiation therapy.

[283] In some embodiments, the combination therapy regimen of this disclosure comprises administration of a Cbl-b inhibitor, radiation therapy, and one or both of an immune checkpoint inhibitor and an antineoplastic agent.

[284] Provided herein are methods for treating cancer, comprising administering to an individual with cancer a combination therapy comprising an effective amount of a Cbl-b inhibitor, and an effective amount of a cancer vaccine. Also provided are medicaments comprising a Cbl-b inhibitor for use in combination with a cancer vaccine for treating cancer, and medicaments comprising both a Cbl-b inhibitor and a cancer vaccine for use in treating

cancer. Further provided are uses of a Cbl-b inhibitor in the manufacture of a medicament for treating cancer in an individual when administered in combination with a cancer vaccine. Further provided are uses of a Cbl-b inhibitor and a cancer vaccine in the manufacture of a medicament(s) for treating cancer. Also provided herein are methods for treating cancer, comprising administering to an individual with cancer a combination therapy comprising an effective amount of a Cbl-b inhibitor, and an effective amount of an oncolytic virus. Also provided are medicaments comprising a Cbl-b inhibitor for use in combination with an oncolytic virus for treating cancer, and medicaments comprising both a Cbl-b inhibitor and an oncolytic virus for use in treating cancer. Further provided are uses of a Cbl-b inhibitor in the manufacture of a medicament for treating cancer in an individual when administered in combination with an oncolytic virus. Further provided are uses of a Cbl-b inhibitor and an oncolytic virus in the manufacture of a medicament(s) for treating cancer. In some embodiments, the Cbl-b inhibitor is a “small molecule.”

[285] In some embodiments of the treatment methods, medicaments, and uses of this disclosure, the cancer is a hematologic cancer such as lymphoma, a leukemia, or a myeloma. In other embodiments of the treatment methods, medicaments, and uses of this disclosure, the cancer is a non-hematologic cancer such as a sarcoma, a carcinoma, or a melanoma.

[286] Hematologic cancers include, but are not limited to, one or more leukemias such as B-cell acute lymphoid leukemia (“BALL”), T-cell acute lymphoid leukemia (“TALL”), acute lymphoid leukemia (ALL); one or more chronic leukemias including, but not limited to, chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to, B-cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and “preleukemia,” which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells.

[287] Non-hematologic cancers include but are not limited to, a neuroblastoma, renal cell carcinoma, colon cancer, colorectal cancer, breast cancer, epithelial squamous cell cancer, melanoma, stomach cancer, brain cancer, lung cancer (*e.g.*, NSCLC), pancreatic cancer,

cervical cancer, ovarian cancer, liver cancer, bladder cancer, prostate cancer, testicular cancer, thyroid cancer, uterine cancer, adrenal cancer, and head and neck cancer.

[288] In some aspects, the effectiveness of administration an activation threshold reducer or a costimulation requirement reducer, such as a Cbl-b inhibitor, in the treatment of a cancer is measured by assessing clinical outcome, such as reduction in tumor size or number of tumors, and/or survival. In some embodiments, “treating cancer” comprises assessing a patient’s response to the treatment regimen according to the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) as described (*see, e.g.*, Eisenhauer *et al.*, Eur J Cancer, 45:228-247, 2009; and Nishino *et al.*, Am J Roentgenol, 195: 281-289, 2010). Response criteria to determine objective anti-tumor responses per RECIST 1.1 include complete response (CR); partial response (PR); progressive disease (PD); and stable disease (SD).

[289] Also provided herein are methods for treating cancer, comprising administering to an individual with cancer a combination therapy comprising an effective amount of an agent that lowers activation threshold (activation threshold reducer) of an immune cell (*e.g.*, T-cell, B-cell, and/or NK-cell), and an effective amount of a cancer vaccine. Also provided are medicaments comprising an activation threshold reducer for use in combination with a cancer vaccine for treating cancer, and medicaments comprising both an activation threshold reducer and a cancer vaccine for use in treating cancer. Further provided are uses of an activation threshold reducer in the manufacture of a medicament for treating cancer in an individual when administered in combination with a cancer vaccine. Further provided are uses of an activation threshold reducer and a cancer vaccine in the manufacture of a medicament(s) for treating cancer. Also provided herein are methods for treating cancer, comprising administering to an individual with cancer a combination therapy comprising an effective amount of an agent that lowers activation threshold (activation threshold reducer) of an immune cell (*e.g.*, T-cell, B-cell, and/or NK-cell), and an effective amount of an oncolytic virus. Also provided are medicaments comprising an activation threshold reducer for use in combination with an oncolytic virus for treating cancer, and medicaments comprising both an activation threshold reducer and an oncolytic virus for use in treating cancer. Further provided are uses of an activation threshold reducer in the manufacture of a medicament for treating cancer in an individual when administered in combination with an oncolytic virus. Further provided are uses of an activation threshold reducer and an oncolytic virus in the manufacture of a medicament(s) for treating cancer. In some embodiments, the agent that lowers activation threshold (activation threshold reducer) is an agent that reduces costimulation requirement

(costimulation requirement reducer) of an immune cell (*e.g.*, T-cell, B-cell, and/or NK-cell). In some embodiments, the agent that lowers activation threshold (activation threshold reducer) is an agent that promotes tumor immune-surveillance. In some embodiments, the agent that lowers activation threshold (activation threshold reducer) is a Cbl-b inhibitor. In some embodiments, the agent that reduces costimulation requirement is a Cbl-b inhibitor. In some embodiments, the agent that promotes tumor immune-surveillance is a Cbl-b inhibitor.

[290] In some embodiments, an activation threshold reducer, such as a Cbl-b inhibitor, is capable of increasing T-cell activation and/or T-cell proliferation. In some embodiments, an activation threshold reducer, such as a Cbl-b inhibitor, is capable of decreasing T-cell exhaustion, T-cell tolerance, and/or T-cell anergy.

[291] In some embodiments, an activation threshold reducer, such as a Cbl-b inhibitor, is capable of increasing production of one or more cytokines by T-cells or surrounding immune cells in the activated T-cell microenvironment (*e.g.*, myeloid cells). In some embodiments, the one or more cytokines include, but are not limited to: IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-13, IL-18, TNF α , and GM-CSF. In some embodiments, the cytokine is one or more of: IL-2, IFN- γ , TNF α , and GM-CSF. In some embodiments, the cytokine is a chemokine. In some embodiments, the one or more chemokines include, but are not limited to, IP-10, Eotaxin, GRO alpha, RANTES, MIP-1 α , MIP-1 β , MIP-2, MCP-1, and MCP-3. Increased expression of cytokines can be measured by ELISA.

[292] In some embodiments, an activation threshold reducer, such as a Cbl-b inhibitor, is capable of increasing cell surface expression of one or more T-cell activation markers. In some embodiments, the one or more T-cell activation markers include, but are not limited to, CD25, CD44, CD62L, CD69, CD152 (CTLA4), CD154, CD137, and CD279. In some embodiments, the T-cell activation marker is one or more of CD25, CD69, and CTLA4. Increased expression of cell surface markers can be measured by FACS.

[293] Methods for experimentally determining increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and/or decreased T-cell tolerance are well known in the art. In some embodiments, representative methods of determining T-cell activation can be found in Biological Example 9 and/or Biological Example 12 provided herein. In some embodiments, representative *in vitro* and *in vivo* methods of determining increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and/or decreased T-cell tolerance can be found in Biological Example 10 and/or Biological Example 13.

[294] In some embodiments, an activation threshold reducer, such as a Cbl-b inhibitor, is capable of increasing B-cell activation. In some embodiments, increased B-cell activation comprises increased cell surface expression of one or more B-cell activation markers. In some embodiments, the one or more B-cell activation markers include, but are not limited to, CD69, CD86, and MHC class II (*e.g.*, HLA-DR). In some embodiments, the B-cell activation marker is CD69. Increased expression of cell surface markers can be measured by FACS. In some embodiments, increased B-cell activation comprises increased activation of proteins in signaling pathways, such as those mediated by ERK, JNK, and Syk. Increased activation of proteins can be detected by measurement of levels of phosphorylation on the proteins using reagents, such as anti-phospho antibodies available in the art.

[295] In some embodiments, an activation threshold reducer, such as a Cbl-b inhibitor, is capable of increasing NK-cell activation. In some embodiments, increased NK-cell activation comprises secretion of one or more cytokines. In some embodiments, the one or more cytokines include, but are not limited to, IFN- γ , TNF α , and MIP-1 β . Increased expression of cytokines can be measured by ELISA. In some embodiments, increased NK-cell activation comprises increased cell surface expression of one or more NK-cell activation markers. In some embodiments, the one or more NK-cell activation markers include, but are not limited to, CD69, and CD107a. Increased expression of cell surface markers can be measured by FACS. In some embodiments, increased NK-cell activation comprises increased killing of target cells such as tumor cells, including primary tumor cells, and cell line derived tumor cells, such as the K562 cell line.

[296] Methods for experimentally determining increased B-cell activation and NK-cell activation are well known in the art.

[297] Modulation of activity of an immune cell, such as a T-cell, a B-cell, or a NK-cell can be measured by determining a baseline value for a parameter of interest (*e.g.*, cytokine secretion). For example, T-cell activation, such as in a sample obtained from *in vitro* experiments of cells contacted with a Cbl-b inhibitor, can be measured before contacting or administering said Cbl-b inhibitor to determine a baseline value. A reference value then is obtained for T-cell activation after contacting or administering said Cbl-b inhibitor. The reference value is compared to the baseline value in order to determine the amount of T-cell activation due to contact or administration of the Cbl-b inhibitor or composition thereof. For example, in some embodiments, immune cell (*e.g.*, T-cell) activation is increased by at least 0.1-fold in a sample as compared to a baseline value, wherein the baseline value is obtained

before contacting the immune cell (*e.g.*, T-cell) with a Cbl-b inhibitor or a composition thereof. In some embodiments, immune cell (*e.g.*, T-cell) activation is increased by at least about 0.1-fold, about 0.2-fold, about 0.3-fold, about 0.4-fold, about 0.5-fold, about 0.6-fold, about 0.7-fold, about 0.8-fold, about 0.9-fold, about 1-fold, about 2-fold, about 4-fold, about 6-fold, about 8-fold, about 10-fold, about 20-fold, about 30-fold, but no more than about 50-fold over a baseline value. Immune cell activation can be assessed by measuring biological markers of activation such as increased cytokine secretion, increased cell surface expression of activation markers (*e.g.*, cell surface markers), or increased phosphorylation of proteins in a downstream signaling pathway. The fold over baseline value that indicates immune cell activation can be determined for the parameter being tested and the conditions under which the immune cell were treated. For example, for measuring T-cell activation, a baseline value can be obtained from T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody, wherein the cells are not incubated with a Cbl-b inhibitor. A reference value is then obtained from T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody, wherein the T-cells have been or are in contact with a Cbl-b inhibitor. A positive response for immune cell activation can then be determined by the obtained reference value. Similar reference value measurements can be obtained and compared to a baseline value for assessing T-cell activation, T-cell proliferation, T-cell exhaustion, T-cell tolerance, B-cell activation, and/or NK-cell activation. Measurements for these parameters can be obtained utilizing techniques well known in the art.

[298] The terms “baseline” or “baseline value” as used herein can refer to a measurement or characterization before administration of a therapeutic agent as disclosed herein (*e.g.*, a composition comprising a Cbl-b inhibitor as described herein) or at the beginning of administration of the therapeutic agent. The baseline value can be compared to a reference value in order to determine the increase or decrease of an immune cell function (*e.g.*, increasing T-cell activation, increasing T-cell proliferation, decreasing T-cell exhaustion, and/or decreasing T-cell tolerance). The terms “reference” or “reference value” as used herein can refer to a measurement or characterization after administration of the therapeutic agent as disclosed herein (*e.g.*, a composition comprising a Cbl-b inhibitor as described herein). The reference value can be measured one or more times during an experimental time course, dosage regimen, or treatment cycle, or at the completion of the experimental time course, dosage regimen, or treatment cycle. A “reference value” can be an absolute value, a relative value, a value that has an upper and/or lower limit, a range of values, an average value, a median value,

a mean value, or a value as compared to a baseline value. Similarly, a “baseline value” can be an absolute value, a relative value, a value that has an upper and/or lower limit, a range of values, an average value, a median value, a mean value, or a value as compared to a reference value. The reference value and/or baseline value can be obtained from one sample (*e.g.*, one sample obtained from an individual), from two different samples (*e.g.*, a sample obtained from two different individuals) or from a group of samples (*e.g.*, samples obtained from a group of two, three, four, five or more individuals).

[299] In some embodiments, a positive response for T-cell activation as measured by cytokine secretion (*e.g.*, IL-2 secretion) by T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody in the presence of a Cbl-b inhibitor is at least 2.5-fold over the baseline value for cytokine secretion (*e.g.*, IL-2 secretion) obtained from T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody in the absence of a Cbl-b inhibitor. In some embodiments, a positive response for T-cell activation as measured by surface marker expression (*e.g.*, CD25 surface marker staining) by T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody in the presence of a Cbl-b inhibitor is at least 1.3-fold over the baseline value for surface marker expression (*e.g.*, CD25 surface marker staining) obtained from T-cells stimulated with anti-CD3 antibody in combination with anti-CD28 antibody in the absence of a Cbl-b inhibitor. In some embodiments, a baseline value can be obtained from T-cells stimulated with anti-CD3 antibody alone, wherein the cells are not incubated with a Cbl-b inhibitor. In some embodiments, a positive response for T-cell activation as measured by cytokine secretion (*e.g.*, IL-2 secretion) by T-cells stimulated with anti-CD3 antibody alone in the presence of a Cbl-b inhibitor is at least 0.1-fold over the baseline value for cytokine secretion (*e.g.*, IL-2 secretion) obtained from T-cells stimulated with anti-CD3 antibody alone in the absence of a Cbl-b inhibitor. In some embodiments, a positive response for T-cell activation as measured by surface marker expression (*e.g.*, CD25 surface marker staining) by T-cells stimulated with anti-CD3 antibody alone in the presence of a Cbl-b inhibitor is at least 0.6-fold over the baseline value for surface marker expression (*e.g.*, CD25 surface marker staining) obtained from T-cells stimulated with anti-CD3 antibody alone in the absence of a Cbl-b inhibitor.

[300] This disclosure provides methods of treating cancer, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, and administering to the individual an effective amount of a cancer vaccine. Also provided are methods of treating an individual with cancer, comprising administering to the individual an effective amount of a

Cbl-b inhibitor; and administering to the individual an effective amount of a cancer vaccine. Additionally, this disclosure provides methods of increasing an anti-cancer immune response, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, and administering to the individual an effective amount of a cancer vaccine. Further provided are methods of treating cancer, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, wherein the individual has received or is receiving an effective amount of a cancer vaccine. In addition, this disclosure provides methods of treating cancer, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, and administering to the individual an effective amount of an oncolytic virus. Also provided are methods of treating an individual with cancer, comprising administering to the individual an effective amount of a Cbl-b inhibitor; and administering to the individual an effective amount of an oncolytic virus. Additionally, this disclosure provides methods of increasing an anti-cancer immune response, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, and administering to the individual an effective amount of an oncolytic virus. Further provided are methods of treating cancer, comprising administering to an individual with cancer an effective amount of a Cbl-b inhibitor, wherein the individual has received or is receiving an effective amount of an oncolytic virus.

[301] In some embodiments of the methods of the preceding paragraph, the Cbl-b inhibitor and the cancer vaccine are administered consecutively in either order. In certain embodiments, as used herein, the terms “consecutively”, “serially”, and “sequentially” refer to administration of a Cbl-b inhibitor after a cancer vaccine, or administration of the cancer vaccine after the Cbl-b inhibitor. For instance, consecutive administration may involve administration of the Cbl-b inhibitor in the absence of the cancer vaccine during an induction phase (primary therapy), which is followed by a post-induction treatment phase comprising administration of both the cancer vaccine and the Cbl-b inhibitor. The methods may further comprise a maintenance phase comprising administration of the Cbl-b inhibitor or administration of a further dose of the cancer vaccine. Alternatively, consecutive administration may involve administration of the cancer vaccine in the absence of the Cbl-b inhibitor during an induction phase (primary therapy), which is followed by a post-induction treatment phase comprising administration of the Cbl-b inhibitor. The methods may further comprise a maintenance phase comprising administration of the Cbl-b inhibitor or a further dose of the cancer vaccine. In some embodiments of the methods of the preceding paragraph, the Cbl-b inhibitor and the oncolytic virus are administered consecutively in either order. In certain embodiments, as used

herein, the terms “consecutively”, “serially”, and “sequentially” refer to administration of a Cbl-b inhibitor after an oncolytic virus, or administration of the oncolytic virus after the Cbl-b inhibitor. For instance, consecutive administration may involve administration of the Cbl-b inhibitor in the absence of the oncolytic virus during an induction phase (primary therapy), which is followed by a post-induction treatment phase comprising administration of both the oncolytic virus and the Cbl-b inhibitor. The methods may further comprise a maintenance phase comprising administration of the Cbl-b inhibitor or administration of a further dose of the oncolytic virus. Alternatively, consecutive administration may involve administration of the oncolytic virus in the absence of the Cbl-b inhibitor during an induction phase (primary therapy), which is followed by a post-induction treatment phase comprising administration of the Cbl-b inhibitor. The methods may further comprise a maintenance phase comprising administration of the Cbl-b inhibitor or a further dose of the oncolytic virus.

[302] In some embodiments of the combination therapy methods, the Cbl-b inhibitor and the cancer vaccine are administered concurrently. In certain embodiments, as used herein, the terms “concurrently”, “simultaneously”, and “in parallel” refer to administration of a Cbl-b inhibitor and a cancer vaccine during the same doctor visit or during the same phase of treatment. For instance, both the Cbl-b inhibitor and the cancer vaccine may be administered during one or more of an induction phase, a treatment phase, and a maintenance phase. However, concurrent administration does not require that the Cbl-b inhibitor and the cancer vaccine be present together in a single formulation or pharmaceutical composition, or that the Cbl-b inhibitor and the cancer vaccine be administered at precisely the same time. In some embodiments of the combination therapy methods, the Cbl-b inhibitor and the oncolytic virus are administered concurrently. In certain embodiment, as used herein, the terms “concurrently”, “simultaneously”, and “in parallel” refer to administration of a Cbl-b inhibitor and an oncolytic virus during the same doctor visit or during the same phase of treatment. For instance, both the Cbl-b inhibitor and the oncolytic virus may be administered during one or more of an induction phase, a treatment phase, and a maintenance phase. However, concurrent administration does not require that the Cbl-b inhibitor and the oncolytic virus be present together in a single formulation or pharmaceutical composition, or that the Cbl-b inhibitor and the oncolytic virus be administered at precisely the same time.

[303] In some aspects, the treatment includes multiple administrations of the Cbl-b inhibitor or composition thereof, wherein the interval between administrations may vary. In some embodiments, the Cbl-b inhibitor is administered at a flat dose to an individual (*e.g.*, mg/adult

or mg/child). In some embodiments, the Cbl-b inhibitor is administered to an individual at a fixed dose based in the individual's weight (*e.g.*, mg/kg).

[304] In some embodiments, the effectiveness of the combination therapies disclosed herein can be assessed by measuring the biological activity of immune cells present in a sample isolated from the treated individual. For example, the ability of immune cells, which are isolated from the individual after treatment, to destroy target cells using a cytotoxicity assay can be used to assess treatment efficacy. In some embodiments, the biological activity of immune cells present in a sample can be measured by assaying expression and/or secretion of certain cytokines, such as IL-2 and IFN γ .

[305] The term “cancer vaccine” as used herein, unless otherwise specified, refers to a “therapeutic cancer vaccine” to be administered to an individual with cancer for the purpose of treating cancer (and optionally preventing recurrence of the cancer). In contrast, a “preventative cancer vaccine” (prophylactic cancer vaccine) is to be administered to an individual without cancer for the purpose of preventing cancer or reducing the individual's risk of developing cancer. Examples of preventative cancer vaccines are human papillomavirus vaccines for prevention squamous cell carcinoma and hepatitis B virus vaccines for prevention of hepatocellular carcinoma. Cancer vaccines are immunogenic compositions comprising a pharmaceutically acceptable excipient and at least one tumor antigen, such as a tumor-specific antigen or a tumor-associated antigen. As used herein, the terms “oncolytic virus” and “OV” refer to a virus that infects and kills cancer cells. Death of cancer cells is a result of both direct cytolysis and induction of anti-tumor immunity. In some embodiments, the “oncolytic virus” is a replication-competent virus, which selectively replicates in cancer cells. In other embodiments, the “oncolytic virus” is a replication-deficient virus, which does not replicate in cancer cells either as a consequence of genetic engineering or inactivation (*e.g.*, UV-irradiation or heat) of the oncolytic virus.

[306] In some embodiments, the tumor antigen comprises a “shared tumor antigen” that is common to many cancers of the same type. Non-limiting examples of shared tumor antigens are the breast cancer antigen HER2, the prostate cancer antigens PAP and PSA, and the melanoma antigens MART-1 and MAGE. In other embodiments, the tumor antigen comprises a “neoantigen” that arises as a result of a tumor-specific DNA alteration (*e.g.*, somatic mutation). As such, neoantigens typically possess an amino acid sequence not present in a normal mammalian genome (Schumacher and Schreiber, *Science*, 348: 69-74, 2015). Non-limiting examples of neoantigens are BRAF V600E, KRAS G12D, KRAS G12V, PIK3CA

H1047R, and PIC3CA E545K. A tumor-specific neoantigen database (TSNAdb) is now freely available (Wu *et al.*, Genomics Proteomics Bioinformatics 16: 276-282, 2018).

[307] A variety of techniques are suitable for identification of neoantigens for inclusion in a cancer vaccine as part of a combination therapy comprising an activation threshold reducer, such as a Cbl-b inhibitor. For instance, neoantigens can be identified with methods comprising isolating DNA from a tumor biopsy obtained from an individual, sequencing the DNA, and computational analysis of the sequence to identify one or more neoantigens (Aldous and Dong, Bioorg Med Chem, 26: 2842-2849, 2018). In some embodiments, the computational analysis involves identifying peptides of 8-11 amino acids in length that are predicted to bind to at least one HLA allele expressed by cells of the tumor and which comprise at least one missense mutation (Wu *et al.*, Genomics Proteomics Bioinformatics 16: 276-282, 2018). Neoantigen inclusion in a cancer vaccine is thought to be advantageous for overcoming tolerance and reducing autoimmunity risk.

[308] Cancer vaccine platforms suitable for use in the methods, medicaments, and uses of this disclosure include, but are not limited to, synthetic peptides, recombinant proteins, nucleic acids (DNA or mRNA), microbial vectors, tumor cells, and antigen presenting cells (*see, e.g.*, DeMaria and Bilusic, Hematol Oncol Clin North Am, 33: 199-214, 2019; and Maeng and Berzofsky, F1000Research 2019, 8(F1000 Faculty Rev): 654, 2019).

[309] In some embodiments, the tumor antigen of the cancer vaccine comprises at least one synthetic peptide or recombinant protein. In some embodiments, the synthetic peptide is at least 8 amino acids in length, and in certain embodiments, less than 80 amino acids in length. In some embodiments, the tumor antigen comprises a plurality of synthetic peptides, or the tumor antigen comprises a synthetic peptide or a recombinant protein comprising the amino acid sequence of two, three, or more epitopes. An “epitope” is a portion of an antigen that is bound by an antibody or a B-cell receptor, or that is presented for binding by a T-cell receptor by a major histocompatibility complex molecule (MHC class I or class II) on the surface of a cell, such as a tumor cell or a dendritic cell. In some embodiments, the epitope is a “linear epitope” composed of contiguous amino acids of a tumor antigen sequence (primary structure). In some embodiments, the epitope is a “conformational epitope” composed of non-contiguous amino acids of a tumor antigen (tertiary structure). In some embodiments, the tumor antigen comprises a recombinant protein comprising both linear epitope(s) and conformational epitope(s).

[310] In some embodiments, the tumor antigen is encoded by a DNA or an mRNA molecule. In some embodiments, the tumor antigen is encoded by a nucleic acid of a microbial vector, or said another way, the cancer vaccine comprises a microbial vector. In some embodiments, the microbial vector is a live, attenuated microbial vector. In one embodiment, the live, attenuated microbial vector is TICE® BCG, a live culture preparation of the Bacillus of Calmette and Guérin (BCG) strain of *Mycobacterium bovis*, marketed by Organon USA, Inc., (Roseland, NJ). TICE® BCG is Federal Drug Administration (FDA)-approved for intravesical use upon reconstitution with sterile saline (*e.g.*, pharmaceutically acceptable excipient), and is indicated for the treatment and prophylaxis of carcinoma in situ of the urinary bladder, and for the prophylaxis of primary or recurrent stage Ta and/or T1 papillary tumors following transurethral resection.

[311] In some embodiments, the microbial vector is a recombinant microbial vector, such as a recombinant viral vector or a recombinant bacterial vector. Recombinant viral vectors suitable for use in the combination therapies of this disclosure include, but are not limited to, retroviruses, lentiviruses, adenoviruses, adeno-associated viruses, poxviruses, and herpesviruses (Chulpanova et al., *Biomedicines*, 6: 94, 2018). In some embodiments, the microbial vector is a recombinant bacterial vector. Recombinant bacterial vectors suitable for use in the combination therapies of this disclosure include, but are not limited to, *Clostridium* (*C. novyi*), *Listeria* (*e.g.*, *L. monocytogenes*), *Pseudomonas* (*e.g.*, *P. aeruginosa*), and *Salmonella* (*S. typhimurium*) (Toussant et al., *Expert Rev Vaccines*, 12: 1139-1154, 2013).

[312] In some embodiments, the cancer vaccine comprises an antigen presenting cell (APC), that has been contacted with a tumor antigen, such as a synthetic peptide or a recombinant protein. In some embodiments, the APC is transfected with a nucleic acid encoding a tumor antigen. In some embodiments, the APC is transfected with a nucleic acid encoding a cytokine. In some embodiments, the APCs comprises dendritic cells or mesenchymal stem cells. In one embodiment, the cancer vaccine is PROVENGE® (sipuleucel-T) marketed by Dendreon Corp. (Seattle, WA). PROVENGE® comprises Lactated Ringer's (*e.g.*, pharmaceutically acceptable excipient) and peripheral blood mononuclear cells (PBMC) that have been activated with a PAP-GM-CSF fusion protein consisting of prostatic acid phosphatase linked to granulocyte-macrophage colony-stimulating factor. PROVENGE® is Federal Drug Administration (FDA)-approved for intravenous infusion for the treatment of asymptomatic or minimally symptomatic metastatic prostate cancer.

[313] In some embodiments, the cancer vaccine comprises a killed tumor cell. In some embodiments, the cancer vaccine comprises a tumor cell lysate. In some embodiments, the cancer vaccine comprises an APC that has been contacted with a tumor cell lysate.

[314] Adjuvants of the cancer vaccines suitable for use in the methods, medicaments and uses of this disclosure include, but are not limited to, adjuvants of FDA-approved licensed products. In particular, adjuvants of current FDA-approved licensed products comprise aluminum salts, monophosphoryl lipid A, oil-in-water emulsions (e.g., squalene-in-water emulsions MF59 or AS03), saponins, and CpG oligodeoxynucleotides.

[315] Oncolytic viruses suitable for use in the methods, medicaments, and uses of this disclosure include, but are not limited to, adenovirus, coxsackievirus, echovirus, fowlpox virus, herpes simplex virus, maraba virus, measles virus, myxoma virus, Newcastle disease virus, parvovirus, poliovirus, retrovirus, reovirus, Seneca Valley virus, Semiliki Forest virus, vaccinia virus, and vesicular stomatitis virus (*see, e.g.*, Russell and Peng, *Chin Clin Oncol*, 7: 16, 2018; and Sivanandam *et al.*, *Molecular Therapy Oncolytics*, 13: 93-106).

[316] In some embodiments, the oncolytic virus has not been genetically-engineered (non-recombinant virus). In some embodiments, the non-recombinant virus is an echovirus (*e.g.*, Rigvir), a Newcastle disease virus, a parvovirus, a reovirus, or a Seneca Valley virus.

[317] In some embodiments, the oncolytic virus is a recombinant virus that has been genetically engineered to include one or more gene deletions, one or more gene insertions, or one or more gene deletions and one or more gene insertions. In some embodiments, the recombinant virus has been genetically engineered to alter host cell specificity and/or tumor cell cytotoxicity. In some embodiments, the recombinant oncolytic virus has been genetically engineered by functional deletion of one or more viral genes encoding proteins that suppress a response (*e.g.*, an anti-viral response) of a host cell, and/or by insertion of one or more transgenes encoding proteins that promote a response (*e.g.*, an anti-tumor response) of a host cell (*see, e.g.*, Guo *et al.*, *Frontiers in Immunology*, 8: Article 555, 2017; and Lin *et al.*, *Oncology Letters*, 15: 4053-4060, 2018). In some embodiments, the recombinant virus is further engineered by insertion of a transgene encoding a detectable marker, such as fluorescent protein. Desirable anti-tumor responses include one or both of innate immune response and adaptive immune response.

[318] In some embodiments, the recombinant oncolytic virus is a recombinant herpes simplex virus (HSV), such as HSV type-1. In one embodiment, the recombinant oncolytic virus is IMLYGIC®, also known as talimogene laherparepvec or T-VEC, marketed by Amgen Inc.

(Thousand Oaks, CA). IMLYGIC® is a recombinant HSV-1 that includes functional deletions of ICP34.5 and ICP47 genes, and insertion of a nucleic acid encoding human granulocyte macrophage colony-stimulating factor (GM-CSF). IMLYGIC® is Federal Drug Administration (FDA)-approved for local treatment by intralesional injection (intratumoral administration) of unresectable cutaneous, subcutaneous, and nodal lesions in patients with recurrent melanoma. In addition, IMLYGIC® is European Medicines Agency (EMA)-approved for treatment of adults with unresectable melanomas that is regionally or distantly metastatic (Stages IIIB, IIIC or IVM1a). In particular, the EMA-approved product is to be administered by intralesional injection (intratumoral administration) into cutaneous, subcutaneous, and/or nodal lesions that are visible, palpable or detectable by ultrasound guidance.

[319] In some embodiments, the recombinant oncolytic virus is a recombinant adenovirus, such as a serotype 5 adenovirus. In one embodiment, the recombinant adenovirus is Oncorine (H101), formerly known as Onyx-015. Oncorine is a serotype 5 adenovirus engineered by inactivation (functional deletion) of viral E1B-55k and viral E3 genes. Oncorine is approved by the Chinese State Food and Drug Administration for treating head and neck cancer in combination with chemotherapy (anti-neoplastic agent therapy).

[320] In some embodiments, the recombinant oncolytic virus is a recombinant pox virus. In some embodiments, the poxvirus is a vaccinia virus or a fowlpox virus. In some embodiments, the vaccinia virus is Modified Vaccinia Ankara. In some embodiments, the recombinant vaccinia virus has been genetically engineered by functional deletion of one or more viral genes encoding proteins that suppress a response (e.g., an anti-viral response) of a host cell, and/or by insertion of one or more transgenes encoding proteins that promote a response (e.g., an anti-tumor response) of a host cell (*see, e.g., Guo et al., Journal of Immunotherapy of Cancer, 7: 6, 2019*). In one embodiment, the recombinant vaccinia virus is Pexa-Vec, also known as pexastimogene devacirepvec and JX-594, which is a vaccinia virus engineered by inactivation (functional deletion) of the viral thymidine kinase gene, and by insertion of transgenes encoding human GM-CSF and beta-galactosidase (*Heo et al., Nat Med, 19: 329-336, 2013*). In another embodiment, the recombinant vaccinia virus comprises functional deletion of viral thymidine kinase and vaccinia growth factor genes, and insertion of a transgene encoding the chemokine, CXCL11 (*see, e.g., Liu et al., OncoImmunology, 5: 3, e1091554, 2016; and Liu et al., Nature Communications, 8: 14754, 2017*).

[321] Further embodiments of the combination therapies of this disclosure comprise at least one additional therapeutic agent. In some embodiments, the at least one additional therapeutic agent is selected from the group consisting of an immune checkpoint inhibitor, chemotherapy (antineoplastic agent), radiation therapy, and combinations thereof.

[322] In some embodiments, the immune checkpoint inhibitor is an antagonist of at least one inhibitory immune checkpoint molecule. In some embodiments, the at least one inhibitory immune checkpoint molecule is selected from the group consisting PD-1 (CD279), PD-L1 (CD274), and CTLA4 (CD152). The immune checkpoint inhibitor may be a therapeutic biological product. For instance the immune checkpoint inhibitor may comprise an antibody or antigen-binding fragment thereof. The antibody or fragment may be a monoclonal antibody (mAb), a human antibody, a humanized antibody, or a chimeric antibody, and may include a human constant region. In some embodiments the human constant region is selected from the group consisting of IgG1, IgG2, IgG3, and IgG4 constant regions, and in certain embodiments, the human constant region is an IgG1 or IgG4 constant region. In some embodiments, the antibody or fragment is a bispecific antibody. In some embodiments, the antigen-binding fragment comprises one of the group consisting of Fab, Fab'-SH, F(ab')₂, scFv, and Fv fragments.

[323] In some embodiments, the chemotherapy comprises at least one antineoplastic agent (*i.e.*, WHO ATC code L01). In some embodiments, the at least one antineoplastic agent is selected from the group consisting of a cytotoxic antibiotic, a plant alkaloid, an antimetabolite, an alkylating agent, an other antineoplastic agent, and combinations thereof. As used in reference to chemotherapy, the antineoplastic agent is a “drug”, as opposed to a “therapeutic biological product”.

[324] In some embodiments, the radiation therapy is external beam radiation therapy. In other embodiments, the radiation therapy is internal radiation therapy. In some embodiments, the radiation therapy is ablative radiation therapy.

[325] As used herein the term “biosimilar” refers to a biological product that is similar to but without clinically meaningful differences in safety and effectiveness from a Federal Drug Administration (FDA)-approved reference product. For instance, there may be differences between a biosimilar product and a reference product in clinically inactive components (*e.g.*, differences in excipients of the formulations, minor differences in glycosylation, *etc.*). Clinically meaningful characteristics can be assessed through pharmacokinetic and pharmacodynamic studies. In some embodiments, the biosimilar product is an interchangeable

product as determined by the FDA. In some embodiments, the cancer vaccine is a biosimilar of an FDA-approved product.

IV. Compositions, Formulations and Routes of Administration

[326] Pharmaceutical compositions of any of the compounds disclosed herein, or a salt thereof, or solvate thereof, are embraced by this disclosure. Thus, this disclosure includes pharmaceutical compositions comprising a Cbl-b inhibitor, wherein the Cbl-B inhibitor is a compound of Formula (I), (I-a), (I-b), (I-A)-(I-J), (II-A)-(II-H), (III-A)-(III-H), or (IV-A)-(IV-H), or any variation thereof disclosed herein, or a pharmaceutically acceptable salt or solvate thereof, or tautomers thereof, or stereoisomers or mixtures of stereoisomers thereof, and a pharmaceutically acceptable excipient, such as a pharmaceutically acceptable vehicle or pharmaceutically acceptable carrier. In some embodiments, the compound is a compound selected from Compound Nos. 1-53 in Table 1, or a pharmaceutically acceptable salt or solvate thereof, or tautomers thereof, or stereoisomers or mixtures of stereoisomers thereof. In one aspect, the pharmaceutically acceptable salt is an acid addition salt, such as a salt formed with an inorganic acid or an organic acid.

[327] The compounds and compositions disclosed herein may be administered in any suitable form and by any suitable route that will provide sufficient levels of the compounds for treatment of the disease or disorder. In some embodiments, the Cbl-b inhibitor and/or the additional therapeutic agent are administered by enteral administration. In certain embodiments, the enteral administration is oral administration. In other embodiments, the Cbl-b inhibitor and/or the additional therapeutic agent are administered by parenteral administration. In certain embodiments, the parenteral administration is intratumoral injection. In certain embodiments, the parenteral administration is by a route selected from the group consisting of intravenous, intraperitoneal, and subcutaneous.

[328] Suitable routes of administration include oral administration, enteral administration, parenteral administration including subcutaneous injection, intravenous injection, intraarterial injection, intramuscular injection, intrasternal injection, intraperitoneal injection, intralesional injection, intraarticular injection, intratumoral injection, or infusion techniques. The compounds and compositions also can be administered sublingually, by mucosal administration, by buccal administration, subcutaneously, by spinal administration, by epidural administration, by administration to cerebral ventricles, by inhalation (*e.g.*, as mists or sprays), nasal administration, vaginal administration, rectal administration, topical administration, or transdermal administration, or by sustained release or extended release mechanisms. The

compounds and compositions can be administered in unit dosage formulations containing conventional pharmaceutically acceptable carriers, excipients, adjuvants, and vehicles as desired. The compounds and compositions may be administered directly to a specific or affected organ or tissue. The compounds can be mixed with pharmaceutically acceptable carriers, excipients, adjuvants, and vehicles to form compositions appropriate for the desired route of administration. In some embodiments, the compounds can be mixed with one or both of an antigen and an adjuvant. In some embodiments, the antigen is a cancer antigen.

[329] In certain embodiments disclosed herein, especially those embodiments where a formulation is used for injection or other parenteral administration, including the routes listed herein, but also including any other route of administration described herein (such as oral, enteric, gastric, etc.), the formulations and preparations used in the methods are sterile. Sterile pharmaceutical formulations are compounded or manufactured according to pharmaceutical-grade sterilization standards (United States Pharmacopeia Chapters 797, 1072, and 1211; California Business & Professions Code 4127.7; 16 California Code of Regulations 1751, 21 Code of Federal Regulations 211) known to those of skill in the art. A “sterile” formulation is aseptic, or free or essentially free from all living microorganisms and their spores. Examples of methods of sterilization of pharmaceutical formulations include, but are not limited to, sterile filtration through sterile filtration membranes, exposure to radiation such as gamma radiation, and heat sterilization.

[330] Oral administration is advantageous due to its ease of implementation and patient compliance. If a patient has difficulty swallowing, introduction of medicine via feeding tube, feeding syringe, or gastrostomy can be employed in order to accomplish enteric administration. The active compound, and, if present, other co-administered agents, can be enterally administered in any other pharmaceutically acceptable excipient suitable for formulation for administration via feeding tube, feeding syringe, or gastrostomy.

[331] Intravenous administration also can be used advantageously, for delivery of the compounds or compositions to the bloodstream as quickly as possible and to circumvent the need for absorption from the gastrointestinal tract.

[332] The compounds and compositions described for use herein can be administered in solid form, in liquid form, in aerosol form, or in the form of tablets, pills, caplets, capsules (such as hard gelatin capsules or soft elastic gelatin capsules), powder mixtures, granules, injectables, solutions, suppositories, enemas, colonic irrigations, emulsions, dispersions, food premixes, cachets, troches, lozenges, gums, ointments, cataplasms (poultices), pastes, powders, dressings,

creams, patches, aerosols (*e.g.*, nasal spray or inhalers), gels, suspensions (*e.g.*, aqueous or non-aqueous liquid suspensions, oil-in-water emulsions or water-in-oil liquid emulsions), elixirs, or in other forms suitable for the route of administration. The compounds and compositions also can be administered in liposome formulations. The compounds also can be administered as prodrugs, where the prodrug undergoes transformation in the treated subject to a therapeutically effective form.

[333] In addition, pharmaceutical formulations may contain preservatives, solubilizers, stabilizers, re-wetting agents, emulgators, sweeteners, dyes, adjusters, and salts for the adjustment of osmotic pressure, buffers, coating agents, or antioxidants. Formulations comprising the compound also may contain other substances that have valuable therapeutic properties. Pharmaceutical formulations may be prepared by known pharmaceutical methods. Additional formulations and methods of administration are known in the art. Suitable formulations can be found, *e.g.*, in Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, 21st ed. (2005), which is incorporated herein by reference.

[334] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to methods known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a parenterally acceptable diluent or solvent, for example, as a solution in propylene glycol. Among the acceptable vehicles and solvents that may be employed are water, saline, 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 may be used in the preparation of injectables.

[335] Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose, talc, or starch. Such dosage forms also may comprise additional excipient substances other than inert diluents, *e.g.*, lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms also may comprise buffering agents. Tablets and pills additionally can be prepared with enteric coatings. Acceptable excipients for gel capsules with a soft shell are, for instance, plant oils, wax, fats, semisolid and liquid poly-ols, and so on.

[336] Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions also may comprise additional agents, such as wetting agents, emulsifying and suspending agents, cyclodextrins, and sweetening, flavoring, and perfuming agents. Alternatively, the compound also may be administered in neat form if suitable.

[337] The compounds and compositions also can be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain stabilizers, preservatives, excipients, and the like, in addition to a compound as disclosed herein. Useful lipids include the phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Gregoriadis, G. Ed., *Liposome Technology*, Third Edition: *Liposome Technology: Liposome Preparation and Related Techniques*, CRC Press, Boca Raton, Florida (2006); and Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.W., p. 33 et seq (1976).

[338] The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form can vary depending upon the patient to whom the active ingredient is administered and the particular mode of administration. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the specific compound employed; the age, body weight, body area, body mass index (BMI), general health, sex, and diet of the patient; the time of administration and route of administration used; the rate of excretion; and the drug combination, if any, used. The compounds can be administered in a unit dosage formulation. The pharmaceutical unit dosage chosen is fabricated and administered to provide sufficient concentration of drug in the patient, subject, or individual.

[339] Although the compounds for use as described herein can be administered as the sole active pharmaceutical agent, they also can be used in combination with one or more other agents. When additional active agents are used in combination with the compounds for use as described herein, the additional active agents may generally be employed in therapeutic amounts as indicated in the Physicians' Desk Reference (PDR) 71st Edition (2017), which is

incorporated herein by reference, or such therapeutically useful amounts as would be known to one of ordinary skill in the art, or as are determined empirically for each patient.

[340] Combinations of two or more of the compounds and compositions disclosed herein also can be used. The two or more compounds or compositions can be mixed together shortly before administration and administered together. The two or more compounds or compositions can be administered simultaneously, either by the same route of administration or by different routes of administration. The two or more compounds or compositions can be administered consecutively, either by the same route of administration or by different routes of administration. In one embodiment, a kit form can contain two or more compounds or compositions as individual compounds or compositions, with printed or electronic instructions for administration either as a mixture of compounds or compositions, as separate compounds or compositions administered simultaneously, or as separate compounds or compositions administered consecutively. Where three or more compounds or compositions are administered, they can be administered as a mixture of compounds or compositions, as separate compounds or compositions administered simultaneously, as separate compounds or compositions administered consecutively, as separate compounds or compositions where two or more may be administered simultaneously with the remainder administered consecutively before or after the simultaneous administration, or any other possible combination of mixed administration, simultaneous administration, and consecutive administration.

[341] A compound as disclosed herein may in one aspect be in a purified form and compositions comprising a compound in purified forms are disclosed herein. Compositions comprising a compound as disclosed herein or a salt thereof are provided, such as compositions of substantially pure compounds. In some embodiments, a composition containing a compound as disclosed herein or a salt thereof is in substantially pure form. In one variation, “substantially pure” refers to a composition that contains no more than 35% impurity, wherein the impurity denotes a compound other than the compound (or compounds, if combinations of compounds are used) to be administered in the composition, or a salt or solvate of the compound (or compounds, if combinations are used). The weight of any added vehicle, carrier, or excipient is excluded from such a calculation, and the added vehicle, carrier, or excipient is not considered as an impurity. For example, a composition of a substantially pure compound selected from a compound of Table 1 refers to a composition that contains no more than 35% impurity, wherein the impurity denotes a compound other than the compound or a salt or solvate thereof. In one variation, a composition of substantially pure compound or a salt or

solvate thereof is provided wherein the composition contains no more than 25% impurity. In another variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 20% impurity. In still another variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 10% impurity. In a further variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 5% impurity. In another variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 3% impurity. In still another variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 1% impurity. In a further variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 0.5% impurity. In yet other variations, a composition of substantially pure compound means that the composition contains no more than 15%, no more than 10%, no more than 5%, no more than 3%, or no more than 1% impurity. An impurity may be the compound in a stereochemical form different from the desired stereochemical form. For instance, a composition of substantially pure (*S*)-compound means that the composition contains no more than 15%, no more than 10%, no more than 5%, no more than 3%, or no more than 1% of the (*R*)-form of the compound. Alternatively, as used herein, “enantiomeric excess (ee)” refers to a dimensionless mol ratio describing the purity of chiral substances that contain, for example, a single stereogenic center. For instance, an enantiomeric excess of zero would indicate a racemic (e.g., 50:50 mixture of enantiomers, or no excess of one enantiomer over the other). By way of further example, an enantiomeric excess of ninety-nine would indicate a nearly stereopure enantiomeric compound (i.e., large excess of one enantiomer over the other). The percentage enantiomeric excess, % ee = $\frac{[(R)\text{-compound}] - [(S)\text{-compound}]}{[(R)\text{-compound}] + [(S)\text{-compound}]} \times 100$, where the (*R*)-compound > (*S*)-compound; or % ee = $\frac{[(S)\text{-compound}] - [(R)\text{-compound}]}{[(S)\text{-compound}] + [(R)\text{-compound}]} \times 100$, where the (*S*)-compound > (*R*)-compound. Moreover, as used herein, “diastereomeric excess (de)” refers to a dimensionless mol ratio describing the purity of chiral substances that contain more than one stereogenic center. For example, a diastereomeric excess of zero would indicate an equimolar mixture of diastereoisomers. By way of further example, diastereomeric excess of ninety-nine would indicate a nearly stereopure diastereomeric compound (i.e., large excess of one diastereomer over the other). Diastereomeric excess may be calculated via a similar method to ee. As would

be appreciated by a person of skill, de is usually reported as percent de (% de). % de may be calculated in a similar manner to % ee.

[342] In some aspects, provided herein are compositions comprising a cell population containing a modified immune cell such as those described herein or produced by the methods disclosed herein. In some embodiments, the composition comprises a cell population containing a modified immune cell that has been in contact or is in contact with a Cbl-b inhibitor described herein or a composition thereof. In some embodiments, the modified immune cell has been or is in contact with an anti-CD3 antibody alone. In some embodiments, the modified immune cell has been or is in contact with an anti-CD3 antibody in combination with an anti-CD28 antibody. The provided compositions comprising a cell population containing a modified immune cell described herein may further comprise a pharmaceutical acceptable excipient.

[343] In some aspects, also provided herein is a cell culture composition comprising a cell population containing an immune cell and a Cbl-b inhibitor described herein. In some embodiments, the immune cell is a cell selected from the group consisting of a hematopoietic cell, a multipotent stem cell, a myeloid progenitor cell, a lymphoid progenitor cell, a T-cell, a B-cell, and a NK-cell. In some embodiments, the cell culture composition further comprises an anti-CD3 antibody. In some embodiments, the cell culture composition further comprises an anti-CD3 antibody in combination with an anti-CD28 antibody. Methods for culturing cell compositions containing immune cells are well known in the art and are contemplated herein.

[344] A modified immune cell or compositions as described herein, *e.g.*, a composition comprising a cell population containing the modified immune cell or a pharmaceutical composition, can be provided in a suitable container. Suitable containers include, for example, bottles, vials (*e.g.*, dual chamber vials), syringes (*e.g.*, single or dual chamber syringes), bags (*e.g.*, an intravenous bag), and tubes (*e.g.*, test tubes). The container may be formed from a variety of materials such as glass or plastic.

[345] In some embodiments, a composition comprising a cell population containing a modified immune cell as described herein (*e.g.*, a cell culture composition) is provided in a culture vessel. A culture vessel as provided herein includes, but is not limited to, a tube (*e.g.*, a test tube), a dish (*e.g.*, a tissue culture dish), a bag, a multiwell plate (*e.g.*, a 6-well tissue culture plate), and a flask (*e.g.*, a cell culture flask).

[346] Also provided are the compositions as described herein for any use described herein. In some embodiments, the compositions as described herein are for preparation of a

medicament for treating or preventing a disease or condition associated with Cbl-b activity. In some embodiments, the compositions as described herein are for preparation of a medicament for treating cancer.

[347] Pharmaceutical compositions of any of the compounds disclosed herein, or a salt or solvate thereof, for use in combination with a cancer vaccine are embraced by this disclosure. Thus, the disclosure includes pharmaceutical compositions comprising a Cbl-b inhibitor for use in combination with a cancer vaccine, wherein the Cbl-b inhibitor is a compound of 1-719 (including “a” and “b” variants thereof) of International Patent Appl. WO 2019/148005 or a compound of any of Formula (I-A), Formula (I), Formula (II-A), Formula (II), Formula (III-A), Formula (III), or Formula (IV), or any variation thereof disclosed therein, or a pharmaceutically acceptable salt or solvate thereof, or tautomers thereof, or stereoisomers or mixtures of stereoisomers thereof. In addition, pharmaceutical compositions of any of the compounds disclosed herein, or a salt or solvate thereof, for use in combination with an oncolytic virus are embraced by this disclosure. Thus, the disclosure includes pharmaceutical compositions comprising a Cbl-b inhibitor for use in combination with an oncolytic virus, wherein the Cbl-b inhibitor is a compound of 1-719 (including “a” and “b” variants thereof) of International Patent Appl. WO 2019/148005 or a compound of any of Formula (I-A), Formula (I), Formula (II-A), Formula (II), Formula (III-A), Formula (III), or Formula (IV), or any variation thereof disclosed therein, or a pharmaceutically acceptable salt or solvate thereof, or tautomers thereof, or stereoisomers or mixtures of stereoisomers thereof. The compositions can further comprise a pharmaceutically acceptable excipient, such as a pharmaceutically acceptable vehicle or pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises both a small molecule Cbl-b inhibitor and a cancer vaccine. In addition, in some embodiments, the pharmaceutical composition comprises both a small molecule Cbl-b inhibitor and an oncolytic virus. In some embodiments, the compound is a compound selected from the Cbl-b inhibitors disclosed in the following patent applications: compounds 1-53 of US Patent Appl. No. 62/866,914 or a compound of Formula (I) therein; compounds 1-53 of US Patent Appl. No. 62/880,285 or a compound of Formula (I) therein; or any variation thereof, or a pharmaceutically acceptable salt or solvate thereof, or tautomers thereof, or stereoisomers or mixtures of stereoisomers thereof.

[348] In one aspect, the pharmaceutically acceptable salt is an acid addition salt, such as a salt formed with an inorganic acid or an organic acid.

[349] The compounds, vaccines, and compositions disclosed herein may be administered in any suitable form and by any suitable route that will provide sufficient levels of the compounds, vaccines, or compositions for treatment of the disease or disorder. In some embodiments, the Cbl-b inhibitor and/or the cancer vaccine are administered by enteral administration. The compounds, oncolytic viruses, and compositions disclosed herein may be administered in any suitable form and by any suitable route that will provide sufficient levels of the compounds, oncolytic viruses, or compositions for treatment of the disease or disorder. In some embodiments, the Cbl-b inhibitor and/or the oncolytic virus are administered by enteral administration. In some embodiments, the enteral administration is oral administration. In other embodiments, the Cbl-b inhibitor and/or the cancer vaccine are administered by parenteral administration. In other embodiments, the Cbl-b inhibitor and/or the oncolytic virus are administered by parenteral administration. In some embodiments, the parenteral administration is intratumoral injection. In some embodiments, the parenteral administration is by a route selected from the group consisting of intravenous, intraperitoneal, and subcutaneous.

[350] Suitable routes of administration include oral administration, enteral administration, parenteral administration including subcutaneous injection, intravenous injection, intraarterial injection, intramuscular injection, intrasternal injection, intraperitoneal injection, intralesional injection, intraarticular injection, intratumoral injection, or infusion techniques. The compounds, vaccines, and compositions also can be administered sublingually, by mucosal administration, by buccal administration, subcutaneously, by spinal administration, by epidural administration, by administration to cerebral ventricles, by inhalation (*e.g.*, as mists or sprays), nasal administration, vaginal administration, rectal administration, topical administration, or transdermal administration, or by sustained release or extended release mechanisms. The compounds, vaccines, and compositions can be administered in unit dosage formulations containing conventional pharmaceutically acceptable carriers, excipients, adjuvants, and vehicles as desired. The compounds, vaccines, and compositions may be administered directly to a specific or affected organ or tissue. The compounds and/or vaccines can be mixed with pharmaceutically acceptable carriers, excipients, adjuvants, and vehicles to form compositions appropriate for the desired route of administration. The compounds, oncolytic viruses, and compositions also can be administered sublingually, by mucosal administration, by buccal administration, subcutaneously, by spinal administration, by epidural administration, by administration to cerebral ventricles, by inhalation (*e.g.*, as mists or sprays), nasal

administration, vaginal administration, rectal administration, topical administration, or transdermal administration, or by sustained release or extended release mechanisms. The compounds, oncolytic viruses, and compositions can be administered in unit dosage formulations containing conventional pharmaceutically acceptable carriers, excipients, adjuvants, and vehicles as desired. The compounds, oncolytic viruses, and compositions may be administered directly to a specific or affected organ or tissue. The compounds and/or oncolytic viruses can be mixed with pharmaceutically acceptable carriers, excipients, adjuvants, and vehicles to form compositions appropriate for the desired route of administration.

[351] In certain embodiments disclosed herein, especially those embodiments where a formulation is used for injection or other parenteral administration, including the routes listed herein, but also including any other route of administration described herein (such as oral, enteric, gastric, etc.), the formulations and preparations used in the methods are sterile, except for the presence of the presence of a microbial vector in certain cancer vaccines. To prepare such preparations, all components of the formulations or preparations are prepared in a sterile manner before combining with the microbial vector. In certain embodiments disclosed herein, especially those embodiments where a formulation is used for injection or other parenteral administration, including the routes listed herein, but also including any other route of administration described herein (such as oral, enteric, gastric, etc.), the formulations and preparations used in the methods are sterile, except for the presence of the oncolytic virus. To prepare such preparations, all components of the formulations or preparations are prepared in a sterile manner before combining with the oncolytic virus. Sterile pharmaceutical formulations are compounded or manufactured according to pharmaceutical-grade sterilization standards (United States Pharmacopeia Chapters 797, 1072, and 1211; California Business & Professions Code 4127.7; 16 California Code of Regulations 1751, 21 Code of Federal Regulations 211) known to those of skill in the art. A “sterile” formulation is aseptic, or free or essentially free from all living microorganisms and their spores. Examples of methods of sterilization of pharmaceutical formulations include, but are not limited to, sterile filtration through sterile filtration membranes, exposure to radiation such as gamma radiation, and heat sterilization.

[352] Oral administration is advantageous due to its ease of implementation and patient compliance. If a patient has difficulty swallowing, introduction of medicine via feeding tube, feeding syringe, or gastrostomy can be employed in order to accomplish enteric administration. The active compound, vaccine, or composition, and, if present, other co-administered agents,

can be enterally administered in any other pharmaceutically acceptable excipient suitable for formulation for administration via feeding tube, feeding syringe, or gastrostomy. The active compound, oncolytic virus, or composition, and, if present, other co-administered agents, can be enterally administered in any other pharmaceutically acceptable excipient suitable for formulation for administration via feeding tube, feeding syringe, or gastrostomy.

[353] Intravenous administration also can be used advantageously, for delivery of the compounds, vaccines, or compositions to the bloodstream as quickly as possible and to circumvent the need for absorption from the gastrointestinal tract. Intravenous administration also can be used advantageously, for delivery of the compounds, oncolytic viruses, or compositions to the bloodstream as quickly as possible and to circumvent the need for absorption from the gastrointestinal tract.

[354] The compounds, vaccines, and compositions described for use herein can be administered in solid form, in liquid form, in aerosol form, or in the form of tablets, pills, caplets, capsules (such as hard gelatin capsules or soft elastic gelatin capsules), powder mixtures, granules, injectables, solutions, suppositories, enemas, colonic irrigations, emulsions, dispersions, food premixes, cachets, troches, lozenges, gums, ointments, cataplasms (poultices), pastes, powders, dressings, creams, patches, aerosols (*e.g.*, nasal spray or inhalers), gels, suspensions (*e.g.*, aqueous or non-aqueous liquid suspensions, oil-in-water emulsions or water-in-oil liquid emulsions), elixirs, or in other forms suitable for the route of administration. The compounds, vaccines, and compositions also can be administered in liposome formulations. The compounds, oncolytic viruses, and compositions described for use herein can be administered in solid form, in liquid form, in aerosol form, or in the form of tablets, pills, caplets, capsules (such as hard gelatin capsules or soft elastic gelatin capsules), powder mixtures, granules, injectables, solutions, suppositories, enemas, colonic irrigations, emulsions, dispersions, food premixes, cachets, troches, lozenges, gums, ointments, cataplasms (poultices), pastes, powders, dressings, creams, patches, aerosols (*e.g.*, nasal spray or inhalers), gels, suspensions (*e.g.*, aqueous or non-aqueous liquid suspensions, oil-in-water emulsions or water-in-oil liquid emulsions), elixirs, or in other forms suitable for the route of administration. The compounds, oncolytic viruses, and compositions also can be administered in liposome formulations. The compounds also can be administered as prodrugs, where the prodrug undergoes transformation in the treated subject to a therapeutically effective form.

[355] In addition, pharmaceutical formulations may contain preservatives, solubilizers, stabilizers, re-wetting agents, emulgators, sweeteners, dyes, adjusters, and salts for the

adjustment of osmotic pressure, buffers, coating agents or antioxidants. Formulations comprising the compound, vaccine, or composition also may contain other substances that have valuable therapeutic properties. Formulations comprising the compound, oncolytic virus, or composition also may contain other substances that have valuable therapeutic properties. Pharmaceutical formulations may be prepared by known pharmaceutical methods. Additional formulations and methods of administration are known in the art. Suitable formulations can be found, *e.g.*, in Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, 21st ed. (2005), which is incorporated herein by reference.

[356] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions of the small molecule Cbl-b inhibitor, may be formulated according to methods known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a parenterally acceptable diluent or solvent, for example, as a solution in propylene glycol. Among the acceptable vehicles and solvents that may be employed are water, saline, 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 di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.

[357] Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound, vaccine, or composition may be admixed with at least one inert diluent such as sucrose, lactose, talc, or starch. In such solid dosage forms, the active compound, oncolytic virus, or composition may be admixed with at least one inert diluent such as sucrose, lactose, talc, or starch. Such dosage forms also may comprise additional excipient substances other than inert diluents, *e.g.*, lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms also may comprise buffering agents. Tablets and pills additionally can be prepared with enteric coatings. Acceptable excipients for gel capsules with a soft shell are, for instance, plant oils, wax, fats, semisolid and liquid poly-ols, and so on.

[358] Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions also may comprise additional agents, such as wetting agents, emulsifying and suspending agents, cyclodextrins, and sweetening, flavoring,

and perfuming agents. Alternatively, the compound also may be administered in neat form if suitable.

[359] The compounds, vaccines, and compositions also can be administered in the form of liposomes. The compounds, oncolytic viruses, and compositions also can be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain stabilizers, preservatives, excipients, and the like, in addition to a compound or vaccine as disclosed herein. The present compositions in liposome form can contain stabilizers, preservatives, excipients, and the like, in addition to a compound or oncolytic virus as disclosed herein. Useful lipids include the phospholipids and phosphatidylcholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Gregoriadis, G. Ed., *Liposome Technology*, Third Edition: *Liposome Technology: Liposome Preparation and Related Techniques*, CRC Press, Boca Raton, Florida (2006); and Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.W., p. 33 et seq (1976).

[360] The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form can vary depending upon the patient to whom the active ingredient is administered and the particular mode of administration. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the specific compound or vaccine employed; the age, body weight, body area, body mass index (BMI), general health, sex, and diet of the patient; the time of administration and route of administration used; the rate of excretion; and the drug combination, if any, used. The compounds, vaccines, and compositions can be administered in a unit dosage formulation. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the specific compound or oncolytic virus employed; the age, body weight, body area, body mass index (BMI), general health, sex, and diet of the patient; the time of administration and route of administration used; the rate of excretion; and the drug combination, if any, used. The compounds, oncolytic viruses, and compositions can be administered in a unit dosage formulation. The pharmaceutical unit dosage chosen is fabricated and administered to provide sufficient concentration of drug in the patient, subject, or individual.

[361] Although the compounds, vaccines, and compositions for use as described herein can be administered as the sole active pharmaceutical agents, they also can be used in combination with one or more other agents. When additional active agents are used in combination with the compounds, vaccines, or compositions for use as described herein, the additional active agents may generally be employed in therapeutic amounts as indicated in the Physicians' Desk Reference (PDR) 71st Edition (2017), which is incorporated herein by reference, or such therapeutically useful amounts as would be known to one of ordinary skill in the art, or as are determined empirically for each patient. Although the compounds, oncolytic viruses, and compositions for use as described herein can be administered as the sole active pharmaceutical agents, they also can be used in combination with one or more other agents. When additional active agents are used in combination with the compounds, oncolytic viruses, or compositions for use as described herein, the additional active agents may generally be employed in therapeutic amounts as indicated in the Physicians' Desk Reference (PDR) 71st Edition (2017), which is incorporated herein by reference, or such therapeutically useful amounts as would be known to one of ordinary skill in the art, or as are determined empirically for each patient.

[362] Combinations of two or more of the compounds, vaccines, and compositions disclosed herein also can be used. The two or more compounds, vaccines, or compositions can be mixed together shortly before administration and administered together. The two or more compounds, vaccines, or compositions can be administered simultaneously, either by the same route of administration or by different routes of administration. The two or more compounds, vaccines, or compositions can be administered consecutively, either by the same route of administration or by different routes of administration. In one embodiment, a kit form can contain two or more compounds, vaccines, or compositions as individual compounds, vaccines, or compositions, with printed or electronic instructions for administration either as a mixture of compounds, vaccines, or compositions, as separate compounds, vaccines, or compositions administered simultaneously, or as separate compounds, vaccines, or compositions administered consecutively. Where three or more compounds, vaccines, or compositions are administered, they can be administered as a mixture of compounds, vaccines, or compositions, as separate compounds, vaccines, or compositions administered simultaneously, as separate compounds, vaccines, or compositions administered consecutively, as separate compounds, vaccines, or compositions where two or more may be administered simultaneously with the remainder administered consecutively before or after the simultaneous administration, or any other possible combination of mixed administration, simultaneous administration, and

consecutive administration. Combinations of two or more of the compounds, oncolytic viruses, and compositions disclosed herein also can be used. The two or more compounds, oncolytic viruses, or compositions can be mixed together shortly before administration and administered together. The two or more compounds, oncolytic viruses, or compositions can be administered simultaneously, either by the same route of administration or by different routes of administration. The two or more compounds, oncolytic viruses, or compositions can be administered consecutively, either by the same route of administration or by different routes of administration. In one embodiment, a kit form can contain two or more compounds, oncolytic viruses, or compositions as individual compounds, oncolytic viruses, or compositions, with printed or electronic instructions for administration either as a mixture of compounds, oncolytic viruses, or compositions, as separate compounds, oncolytic viruses, or compositions administered simultaneously, or as separate compounds, oncolytic viruses, or compositions administered consecutively. Where three or more compounds, oncolytic viruses, or compositions are administered, they can be administered as a mixture of compounds, oncolytic viruses, or compositions, as separate compounds, oncolytic viruses, or compositions administered simultaneously, as separate compounds, oncolytic viruses, or compositions administered consecutively, as separate compounds, oncolytic viruses, or compositions where two or more may be administered simultaneously with the remainder administered consecutively before or after the simultaneous administration, or any other possible combination of mixed administration, simultaneous administration, and consecutive administration.

[363] A compound as disclosed herein for use in the pharmaceutical compositions and methods described herein may in one aspect be in a purified form and compositions comprising a compound in purified forms are disclosed herein. Compositions comprising a compound as disclosed herein or a salt thereof are provided, such as compositions of substantially pure compounds. In some embodiments, a composition containing a compound as disclosed herein or a salt thereof is in substantially pure form. In one variation, “substantially pure” refers to a composition that contains no more than 35% impurity, wherein the impurity denotes a compound other than the compound (or compounds, if combinations of compounds are used) to be administered in the composition, or a salt or solvate of the compound (or compounds, if combinations are used). The weight of any added vehicle, carrier, or excipient is excluded from such a calculation, and the added vehicle, carrier, or excipient is not considered as an impurity. For example, a composition of a substantially pure compound selected from a

compound of Table 1 refers to a composition that contains no more than 35% impurity, wherein the impurity denotes a compound other than the compound or a salt or solvate thereof. In one variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 25% impurity. In another variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 20% impurity. In still another variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 10% impurity. In a further variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 5% impurity. In another variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 3% impurity. In still another variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 1% impurity. In a further variation, a composition of substantially pure compound or a salt or solvate thereof is provided wherein the composition contains no more than 0.5% impurity. In yet other variations, a composition of substantially pure compound means that the composition contains no more than 15%, no more than 10%, no more than 5%, no more than 3%, or no more than 1% impurity. An impurity may be the compound in a stereochemical form different from the desired stereochemical form. For instance, a composition of substantially pure (*S*)-compound means that the composition contains no more than 15%, no more than 10%, no more than 5%, no more than 3%, or no more than 1% of the (*R*)- form of the compound. Alternatively, as used herein, “enantiomeric excess (ee)” refers to a dimensionless mol ratio describing the purity of chiral substances that contain, for example, a single stereogenic center. For instance, an enantiomeric excess of zero would indicate a racemic (e.g., 50:50 mixture of enantiomers, or no excess of one enantiomer over the other). By way of further example, an enantiomeric excess of ninety-nine would indicate a nearly stereopure enantiomeric compound (i.e., large excess of one enantiomer over the other). The percentage enantiomeric excess, % ee = $\frac{[(R)\text{-compound}] - [(S)\text{-compound}]}{[(R)\text{-compound}] + [(S)\text{-compound}]} \times 100$, where the (*R*)-compound > (*S*)-compound; or % ee = $\frac{[(S)\text{-compound}] - [(R)\text{-compound}]}{[(S)\text{-compound}] + [(R)\text{-compound}]} \times 100$, where the (*S*)-compound > (*R*)-compound. Moreover, as used herein, “diastereomeric excess (de)” refers to a dimensionless mol ratio describing the purity of chiral substances that contain more than one stereogenic center. For example, a diastereomeric excess of zero would indicate an equimolar mixture of diastereoisomers. By way of further example, diastereomeric

excess of ninety-nine would indicate a nearly stereopure diastereomeric compound (i.e., large excess of one diastereomer over the other). Diastereomeric excess may be calculated via a similar method to ee. As would be appreciated by a person of skill, de is usually reported as percent de (% de). % de may be calculated in a similar manner to % ee.

[364] A compound, vaccine, or composition as disclosed herein can be provided in a suitable container. A compound, oncolytic virus, or composition as disclosed herein can be provided in a suitable container. Suitable containers include, for example, bottles, vials (e.g., dual chamber vials), syringes (e.g., single or dual chamber syringes), bags (e.g., an intravenous bag), and tubes (e.g., test tubes). The container may be formed from a variety of materials such as glass or plastic.

V. Articles of Manufacture or Kits

[365] Also provided are articles of manufacture comprising any of the compounds, pharmaceutical compositions, cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions described herein. The articles of manufacture include suitable containers or packaging materials for the compounds, pharmaceutical compositions, cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions. Examples of a suitable container include, but are not limited to, a bottle, a vial, a syringe, an intravenous bag, or a tube. For cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions, a suitable container can be a culture vessel, including, but not limited to, a tube, a dish, a bag, a multiwell plate, or a flask.

[366] Also provided are kits comprising any of the compounds, pharmaceutical compositions, cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions described herein. The kits can contain the compounds, pharmaceutical compositions, cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions in suitable containers or packaging materials, including, but not limited to, a bottle, a vial, a syringe, an intravenous bag, or a tube. The kits can contain cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions in a culture vessel, including, but not limited to, a tube, a dish, a bag, a multiwell plate, or a flask. The kits can comprise the compounds, pharmaceutical compositions, cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions for administration to an individual in single-dose form or in multiple-dose form. The kits can further comprise instructions or a label for administering the compounds,

pharmaceutical compositions, cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions to an individual according to any of the methods disclosed herein. The kits can further comprise equipment for administering the compounds, pharmaceutical compositions, cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions to an individual, including, but not limited to, needles, syringes, tubing, or intravenous bags. The kits can further comprise instructions for producing any of the compounds, pharmaceutical compositions, cells, modified immune cells, cell populations, cell compositions, cell cultures, or cell culture compositions disclosed herein.

[367] Also provided are articles of manufacture comprising any of the compounds, vaccines, or pharmaceutical compositions described herein. The articles of manufacture include suitable containers or packaging materials for the compounds, vaccines, or pharmaceutical compositions. Also provided are articles of manufacture comprising any of the compounds, oncolytic viruses, or pharmaceutical compositions described herein. The articles of manufacture include suitable containers or packaging materials for the compounds, oncolytic viruses, or pharmaceutical compositions. Examples of a suitable container include, but are not limited to, a bottle, a vial, a syringe, an intravenous bag, or a tube.

[368] Also provided are kits comprising any of the compounds, vaccines, or pharmaceutical compositions described herein. The kits can contain the compounds, vaccines, or pharmaceutical compositions in suitable containers or packaging materials, including, but not limited to, a bottle, a vial, a syringe, an intravenous bag, or a tube. The kits can comprise the compounds, vaccines, or pharmaceutical compositions for administration to an individual in single-dose form or in multiple-dose form. The kits can further comprise instructions or a label for administering the compounds, vaccines, or pharmaceutical compositions to an individual according to any of the methods disclosed herein. The kits can further comprise equipment for administering the compounds, vaccines, or pharmaceutical compositions to an individual, including, but not limited to, needles, syringes, tubing, or intravenous bags. The kits can further comprise instructions for producing any of the compounds, vaccines, or pharmaceutical compositions disclosed herein. Also provided are kits comprising any of the compounds, oncolytic viruses, or pharmaceutical compositions described herein. The kits can contain the compounds, oncolytic viruses, or pharmaceutical compositions in suitable containers or packaging materials, including, but not limited to, a bottle, a vial, a syringe, an intravenous bag, or a tube. The kits can comprise the compounds, oncolytic viruses, or pharmaceutical

compositions for administration to an individual in single-dose form or in multiple-dose form. The kits can further comprise instructions or a label for administering the compounds, oncolytic viruses, or pharmaceutical compositions to an individual according to any of the methods disclosed herein. The kits can further comprise equipment for administering the compounds, oncolytic viruses, or pharmaceutical compositions to an individual, including, but not limited to, needles, syringes, tubing, or intravenous bags. The kits can further comprise instructions for producing any of the compounds, oncolytic viruses, or pharmaceutical compositions disclosed herein.

[369] This disclosure will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of this disclosure. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

EXAMPLES

General Work-up Procedure 1

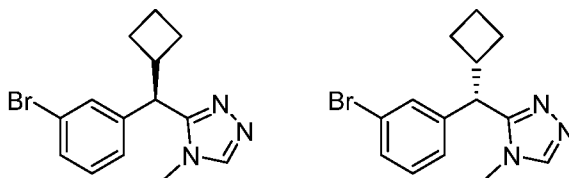
[370] General Work-up Procedure 1 refers to extraction of aqueous solutions with an organic solvent such as EtOAc or DCM 2-3 times. The combined organic extracts were dried over sodium sulfate or anhydrous magnesium sulfate, or were washed with brine, saturated ammonium chloride aqueous solution, or saturated sodium bicarbonate aqueous solution before drying, filtration, and concentration under vacuum.

Purification Procedures

[371] Chromatography A refers to purification over silica gel, typically in pre-packed cartridges, eluting with mixtures of EtOAc in hexanes or petroleum ether; Chromatography B refers to elution with mixtures of MeOH in DCM; Chromatography C refers to use of C18 phase silica gel, eluting with mixtures of acetonitrile in water; Chromatography D refers to elution with mixtures of ethanol, EtOAc, and hexanes. Compounds drawn without stereochemistry were tested as racemic or diastomeric mixtures in the Biological Examples. Abbreviations used in the Examples include the following: DAST: (Diethylamino)sulfur trifluoride; DMF: Dimethylformamide; EDC: N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride; HATU: 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide

hexafluorophosphate; T3P: propylphosphonic anhydride; XPhos: 2-Dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl; Xantphos: 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene; NBS: *N*-bromosuccinimide; NCS: *N*-chlorosuccinimide; BPO: benzoyl peroxide; THF: tetrahydrofuran; EtOAc: ethyl acetate; DCM: dichloromethane; MeOH: methanol; DIEA: *N,N*-diisopropylethylamine; LHMDs: lithium hexamethyldisilazane.

Example A and Example B: (*R*)-3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4*H*-1,2,4-triazole (A) and (*S*)-3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4*H*-1,2,4-triazole (B)



[372] **Step 1: Synthesis of methyl 2-(3-bromophenyl)-2-cyclobutylacetate.** To a mixture of methyl 2-(3-bromophenyl)acetate (10.0 g, 43.7 mmol) in DMF (100 mL) was added *t*-BuOK (6.4 g, 57 mmol) in portions at 0 °C and stirred at 0 °C for 30 min. Bromocyclobutane (7.1 g, 52 mmol) was added to the above solution dropwise at 0 °C. The mixture was stirred at room temperature for 16 h. The mixture was poured into saturated aqueous NH₄Cl and General Work-up Procedure 1 was used. The residue was purified by Chromatography A to afford the title compound (10.2 g, 82%). MS (ESI) calculated for (C₁₃H₁₅BrO₂) [M+H]⁺, 283.0; found, 283.0.

[373] **Step 2: Synthesis of 2-(3-bromophenyl)-2-cyclobutylacetohydrazide.** A mixture of methyl 2-(3-bromophenyl)-2-cyclobutylacetate (10.2 g, 36.0 mmol) in hydrazine (20 mL) and EtOH (80 mL) was stirred at 80 °C for 16 h. The solvents were removed under vacuum. The residue was diluted with ethyl acetate, washed with brine, dried, and concentrated under vacuum to afford the title compound (10.5 g, crude). MS (ESI) calculated for (C₁₂H₁₅BrN₂O) [M+H]⁺, 283.0; found, 283.0.

[374] **Step 3 and Step 4: Synthesis of 5-[(3-bromophenyl)(cyclobutyl)methyl]-4-methyl-1,2,4-triazole-3-thiol.** A mixture of 2-(3-bromophenyl)-2-cyclobutylacetohydrazide (10.5 g, 37.1 mmol) and MeNCS (3.30 g, 45.2 mmol) in THF (100 mL) was stirred at room temperature for 16 h. To the above mixture was added a solution of NaOH (8.0 g) in water (100 mL). The mixture was stirred at 60 °C for 2 h. The mixture was acidified to pH ~ 4 by HCl (1 *N*) and General Work-up Procedure 1 was followed to afford the title compound (11.0 g, crude), which

was used in the next step without purification. MS (ESI) calculated for (C₁₄H₁₆BrN₃S) [M+H]⁺, 338.0; found, 338.0.

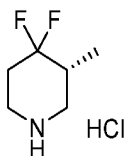
[375] Step 5: Synthesis of 3-[(3-bromophenyl)(cyclobutyl)methyl]-4-methyl-1,2,4-triazole. To a solution of 5-[(3-bromophenyl)(cyclobutyl)methyl]-4-methyl-1,2,4-triazole-3-thiol (2.0 g, 5.9 mmol) in DCM (20.0 mL) were added acetic acid (4.0 mL) and H₂O₂ (3.4 g, 30 mmol) dropwise at room temperature. The reaction was stirred at room temperature for 1 h. The solvent was removed under vacuum and the residue was diluted with water. The aqueous solution was basified by saturated sodium bicarbonate aqueous solution and extracted with DCM. The combined organic layers were washed with brine, dried, and concentrated under vacuum. The residue was purified by Chromatography B to afford the title compound (1.3 g, 72%). MS (ESI) calculated for (C₁₄H₁₆BrN₃) [M+H]⁺, 306.1; found, 306.2.

[376] Step 6: Synthesis of (R)-3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4H-1,2,4-triazole and (S)-3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4H-1,2,4-triazole. The racemic compound, 3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4H-1,2,4-triazole (4.0 g) was separated by prep-chiral-SFC with the following conditions [Column: Lux 5u Cellulose-4, AXIA Packed, 2.12*25 cm, 5 μm; Mobile Phase A: CO₂, Mobile Phase B: MeOH (2 mM NH₃-MeOH); Flow rate: 40 mL/min; Gradient: 30% B; 220 nm] to afford (S)-3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4H-1,2,4-triazole (1.65 g) with shorter retention time on chiral-SFC and (R)-3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4H-1,2,4-triazole (1.68 g) with longer retention time on chiral-SFC.

[377] (S)-3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4H-1,2,4-triazole. MS (ESI) calculated for (C₁₄H₁₆BrN₃) [M+H]⁺, 306.1, found, 306.1. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.35 (s, 1H), 7.59 – 7.36 (m, 2H), 7.34 – 7.20 (m, 2H), 4.23 (d, *J* = 10.8 Hz, 1H), 3.42 (s, 3H), 3.17 – 3.07 (m, 1H), 2.13 – 1.95 (m, 1H), 1.87 – 1.56 (m, 5H).

[378] 3-[(R)-(3-bromophenyl)(cyclobutyl)methyl]-4-methyl-1,2,4-triazole: MS (ESI) calculated for (C₁₄H₁₆BrN₃) [M+H]⁺, 306.1, found, 306.1. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.34 (s, 1H), 7.48 – 7.39 (m, 2H), 7.33 – 7.25 (m, 2H), 4.23 (d, *J* = 10.5 Hz, 1H), 3.42 (s, 3H), 3.21 – 3.01 (m, 1H), 2.1 – 2 (m, 1H), 1.89 – 1.55 (m, 5H).

Example C: (R)-4,4-difluoro-3-methylpiperidine hydrochloride



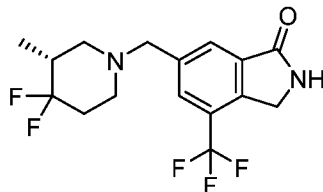
[379] Step 1: Synthesis of 1-benzyl-4,4-difluoro-3-methylpiperidine. To a mixture of 1-benzyl-3-methylpiperidin-4-one (200 g, 983 mmol,) and HF (194 g, 9.68 mol, 176.00 mL, 100% purity) was added SF₄ (240 g, 2.22 mol) in a stainless-steel autoclave at -78 °C. The reaction mixture was then warmed to 15 °C and stirred for 16 h. The reaction mixture was then added dropwise into saturated aq. Na₂CO₃ (2.0 L), extracted with EtOAc (200 mL X 4), filtered and dried, filtered, and then concentrated under vacuum to give the title compound (211 g, crude). ¹H NMR (400 MHz, CDCl₃) δ 7.24 - 7.23 (m, 5H), 3.46 (s, 2H), 2.70 - 2.63 (m, 2H), 2.23 - 2.21 (m, 1H), 1.97- 1.92 (m, 4H), 0.91 (d, *J* = 6.4 Hz, 3H).

[380] Step 2: Synthesis of (R)-1-benzyl-4,4-difluoro-3-methylpiperidine. To a solution of 1-benzyl-4,4-difluoro-3-methylpiperidine (613 g, 2.42 mol) in isopropyl acetate (9 L) was added (2*R*,3*R*)-2,3-bis[(4-methylbenzoyl)oxy] butanedioic acid hydrate (979 g, 2.42 mol) at 30 °C. The mixture was stirred at 30 °C for 2 h, then heated to 70 °C for 2 h. The mixture was cooled to 30 °C for 12 h. The mixture was filtered, and the filtrate was adjusted to pH 8-9 with 1 N sodium hydroxide aqueous solution, then extracted with EtOAc (3 X 1500 mL). The combined organic layers were dried and concentrated under vacuum to give the title compound (377 g, 1.67 mol, 62.3% yield, 90.1% purity). This residue (377 g, 1.67 mol) was dissolved in isopropyl acetate (5.6 L) and to the solution was slowly added (2*S*,3*S*)-2,3-bis[(4-methylbenzoyl)oxy]butanedioic acid (646 g, 1.67 mol) at 30 °C. The mixture was stirred at 30 °C for 2 h, and then heated to 70 °C for 2 h. The mixture was cooled to 30 °C for 12 h. The precipitate was collected by filtration then dissolved in 1 N aqueous sodium hydroxide to pH 8-9 and extracted with EtOAc (3 X 200 mL). The combined organic layers were dried, and concentrated under vacuum to give the title compound (125 g, 33.1% yield, 98.6% purity) which was used in the next step without purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.34 - 7.25 (m, 5H), 3.51 (s, 2H), 2.69 - 2.66 (m, 2H), 2.22 - 2.21 (m, 1H), 2.02 - 1.96 (m, 4H), 0.91 (d, *J* = 6.8 Hz, 3H).

[381] Step 3: Synthesis of (R)-4,4-difluoro-3-methylpiperidine hydrochloride. To a solution of (R)-1-benzyl-4,4-difluoro-3-methylpiperidine (115 g, 510.48 mmol) in MeOH (2.5 L) was added Pd/C (40 g, 10% purity) under N₂. The suspension was degassed under vacuum and purged with H₂ three times. The reaction mixture was stirred under H₂ (50 psi) at 25 °C for 12 h. The reaction mixture was filtered, then cooled to 15 °C and HCl/dioxane (4 M, 114.85 mL) was added, followed by stirring at 15 °C for 2 h. The mixture was concentrated to afford the title compound (50.11 g, 292 mmol, 57.2% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.86

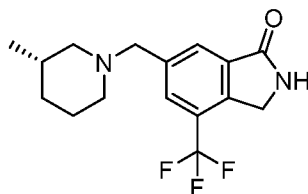
(s, 1H), 9.55 (s, 1H), 3.51 (s, 2H), 3.43 - 3.32 (m, 2H), 3.32 - 3.29 (m, 1H), 2.71 - 2.68 (m, 1H), 2.51 - 2.49 (m, 1H), 2.27 - 2.24 (m, 2H), 0.98 (d, $J = 6.8$, 3H).

Example D: (*R*)-6-((4,4-difluoro-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



[382] To a stirring solution of **Example C** (5.00 g, 29.1 mmol) in DCM (300 mL) was added triethylamine (24.2 mL, 175 mmol) and sodium triacetoxyborohydride (37.1 g, 175 mmol). The suspension was stirred for 10 min and then cooled to 0 °C before **Example K** (9.47 g, 29.1 mmol) in 20 mL of DCM was added. The mixture was stirred at room temperature for about 12 h. General Workup Procedure 1 was used. The residue was dissolved in MeOH (100 mL) and then ammonia (7 N in MeOH, 100 mL) was added to the solution. The mixture was stirred at room temperature for 12 h. The crude reaction mixture was concentrated and then purified by Chromatography B to yield the title compound (7.10 g, 70.0%); ^1H NMR (500 MHz, Chloroform-*d*) δ 8.05 (s, 1H), 7.83 (s, 1H), 7.41 (s, 1H), 4.64 (s, 2H), 3.68 (s, 2H), 2.87 – 2.63 (m, 2H), 2.40 (td, $J = 11.5$, 3.3 Hz, 1H), 2.22 – 1.94 (m, 4H), 1.09 – 0.98 (m, 3H). LCMS: $\text{C}_{16}\text{H}_{17}\text{F}_5\text{N}_2\text{O}$ requires: 348, found: $m/z = 349$ [$\text{M}+\text{H}$] $^+$.

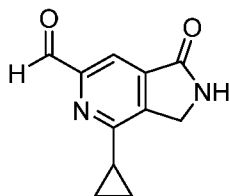
Example E: (*S*)-6-((3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



[383] To a stirring solution of (3*S*)-3-methylpiperidine hydrochloride (4.0 g, 29.4 mmol) in DCM (250 mL) was added triethylamine (25 mL, 176 mmol) and sodium triacetoxyborohydride (37.5 g, 176.9 mmol). The suspension was stirred for 10 min and then cooled to 0 °C. **Example K** (9.6 g, 29.4 mmol) in 20 mL of DCM was added. The mixture was allowed to stir at room temperature for about 12 h. General Workup Procedure 1 was used. The crude product was dissolved in MeOH (100 mL) and then ammonia (7 N in MeOH, 100 mL) was added to the solution. The mixture was stirred at room temperature for 12 h. The solution

was concentrated and purified by Chromatography B to give 6-[[[(3S)-3-methylpiperidin-1-yl]methyl]-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (4.1 g, 45%): ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.18 (s, 1H), 8.14 (s, 1H), 7.16 (s, 1H), 4.58 (s, 2H), 4.04 (s, 2H), 3.14 – 2.98 (m, 3H), 2.43 (s, 1H), 2.18 – 2.10 (m, 1H), 2.04 – 1.73 (m, 2H), 1.34 (t, $J = 7.3$ Hz, 1H), 1.05 (qd, $J = 13.9, 12.9, 4.4$ Hz, 1H), 0.89 (d, $J = 6.6$ Hz, 3H). LCMS: $\text{C}_{16}\text{H}_{19}\text{F}_3\text{N}_2\text{O}$ requires: 312, found: $m/z = 313$ $[\text{M}+\text{H}]^+$.

Example F: 4-cyclopropyl-1-oxo-2H,3H-pyrrolo[3,4-c]pyridine-6-carbaldehyde



[384] Step 1: Synthesis of 3-amino-6-chloro-2-iodopyridine-4-carboxylic acid. To a solution of 5-amino-2-chloropyridine-4-carboxylic acid (100.0 g, 579.47 mmol) in DMF (1.4 L) was added *N*-iodosuccinimide (195.6 g, 869.21 mmol) in portions at 0–10 °C. After stirring for 30 min, the mixture was heated to 80 °C and stirred for 16 h. The mixture was quenched by the addition of water, followed by General Work-up Procedure 1 to afford the title compound (140.0 g, crude), which was used in the next step without purification.

[385] Step 2: Synthesis of methyl 3-amino-6-chloro-2-iodopyridine-4-carboxylate. To a mixture of 3-amino-6-chloro-2-iodopyridine-4-carboxylic acid (70.0 g, 234.80 mmol) in MeOH (90.0 mL) and DCM (900.0 mL) was added TMSCHN_2 (176.1 mL, 2 M in hexane) dropwise at 0 °C. The mixture was stirred at room temperature for 16 h. The organic solvents were removed under vacuum. The residue was triturated with 15% EtOAc in petroleum ether. The solids were collected by filtration and dried to afford the title compound (65.0 g).

[386] Step 3: Synthesis of methyl 3-amino-6-chloro-2-cyclopropylpyridine-4-carboxylate. A degassed mixture of methyl 3-amino-6-chloro-2-iodopyridine-4-carboxylate (45.0 g, 144.23 mmol), cyclopropylboronic acid (49.6 g, 576.92 mmol), K_2CO_3 (59.7 g, 432.69 mmol), and $\text{Pd}(\text{dppf})\text{Cl}_2$ (10.5 g, 14.42 mmol) in dioxane (1.5 L) was stirred at 100 °C for 16 h under N_2 atmosphere. The solids were removed by filtration and the filtrate was concentrated under vacuum. The residue was dissolved in EtOAc and water and General Work-up Procedure 1 was followed before the residue was purified by Chromatography A to afford the title compound (22.1 g, 67% over three steps).

[387] Step 4: Synthesis of methyl 6-chloro-2-cyclopropyl-3-iodopyridine-4-carboxylate. To a mixture of methyl 3-amino-6-chloro-2-cyclopropylpyridine-4-carboxylate (25.0 g, 110.62

mmol) in DCM (1.5 L) were successively added *t*-BuONO (17.0 g, 169.00 mmol) and BF₃·Et₂O (19.0 g, 264.70 mmol) dropwise at 0 °C under N₂ atmosphere. The mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by the addition of hexane. The solids were collected by filtration and dried to afford 6-chloro-2-cyclopropyl-4-(methoxycarbonyl)pyridine-3-diazonium tetrafluoroborate (33.4 g) as a yellow solid, which was added to a mixture of KI (34.1 g, 205.52 mmol) in water (250.0 mL) in portions at 50 °C. The mixture was stirred at 50 °C for 2 h. When the reaction was completed, General Workup Procedure 1 was followed and the residue was purified by Chromatography A to afford the title compound (23.0 g, 62% over two steps).

[388] Step 5: Synthesis of methyl 6-chloro-2-cyclopropyl-3-methylpyridine-4-carboxylate. A degassed mixture of methyl 6-chloro-2-cyclopropyl-3-iodopyridine-4-carboxylate (23.0 g, 68.05 mmol), methylboronic acid (16.3 g, 272.20 mmol), Pd(dppf)Cl₂ (4.9 g, 6.81 mmol) and K₂CO₃ (28.2 g, 204.15 mmol) in dioxane (500 mL) was stirred at 90 °C for 16 h under N₂ atmosphere. The solids were filtered off and the filtrate was concentrated under vacuum. The residue was dissolved in EtOAc and water and General Work-up Procedure 1 was followed before the residue being purified by Chromatography A to afford the title compound (12.5 g, 83%).

[389] Step 6: Synthesis of methyl 3-(bromomethyl)-6-chloro-2-cyclopropylpyridine-4-carboxylate. A mixture of methyl 6-chloro-2-cyclopropyl-3-methylpyridine-4-carboxylate (12.5 g, 55.56 mmol), NBS (19.8 g, 111.11 mmol) and dibenzoyl peroxide (4.0 g, 16.67 mmol) in CCl₄ (150.0 mL) was stirred at 80 °C for 16 h under N₂ atmosphere. The solids were filtered off and the filtrate was concentrated under vacuum. The residue was purified by Chromatography A to afford the title compound (7.8 g, 50%).

[390] Step 7: Synthesis of 6-chloro-4-cyclopropyl-2H,3H-pyrrolo[3,4-c]pyridin-1-one. A mixture of methyl 3-(bromomethyl)-6-chloro-2-cyclopropylpyridine-4-carboxylate (7.8 g, 25.83 mmol) in NH₃ (7 M in MeOH, 80.0 mL) was stirred at room temperature for 16 h under N₂ atmosphere. The mixture was filtered, and the solids were collected and washed with MeOH to afford the title compound (5.0 g, 92.1%).

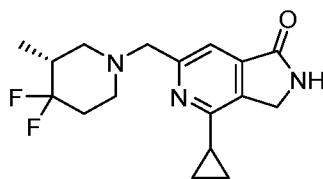
[391] Step 8: Synthesis of 4-cyclopropyl-6-ethenyl-2H,3H-pyrrolo[3,4-c]pyridin-1-one. A degassed mixture of 6-chloro-4-cyclopropyl-2H,3H-pyrrolo[3,4-c]pyridin-1-one (4.3 g, 43.96 mmol), potassium vinyltrifluoroborate (11.8 g, 87.91 mmol), Pd(dppf)Cl₂·CH₂Cl₂ (3.6 g, 4.39 mmol) and Cs₂CO₃ (20.8 g, 131.88 mmol) in THF/water (v/v, 10/1, 220.0 mL) was stirred at 80 °C for 4 h under N₂ atmosphere. The solvent was removed under vacuum. The

residue was dissolved with EtOAc and water, followed by General Work-up Procedure 1, and the resulting residue was purified by Chromatography B to afford the title compound (3.5 g, 87%).

[392] Step 9: Synthesis of 4-cyclopropyl-1-oxo-2H,3H-pyrrolo[3,4-c]pyridine-6-carbaldehyde. To a solution of 4-cyclopropyl-6-ethenyl-2H,3H-pyrrolo[3,4-c]pyridin-1-one (3.5 g, 17.50 mmol) and 4-methylmorpholine *N*-oxide (6.1 g, 52.50 mmol) in THF (50 mL) and water (20 mL) was added $K_2OsO_4 \cdot 2H_2O$ (127.4 mg, 0.35 mmol) at 0 °C. The mixture was stirred at room temperature for 16 h. The reaction mixture was quenched by the addition of $NaHSO_3$ (10.2 g) and stirred for 10 min at room temperature. Then to the mixture was added water (300.0 mL) and the mixture was extracted with EtOAc. The aqueous layer was collected to afford a crude solution of 4-cyclopropyl-6-(1,2-dihydroxyethyl)-2H,3H-pyrrolo[3,4-c]pyridin-1-one (350 mL).

[393] To a three-necked flask charged with silica gel (65.6 g) was added a solution of $NaIO_4$ (7.5 g, 35.00 mmol) in water (32.8 mL) dropwise under vigorously stirring. After stirring for 20 min at room temperature, to the mixture was added the crude solution of 4-cyclopropyl-6-(1,2-dihydroxyethyl)-2H,3H-pyrrolo[3,4-c]pyridin-1-one (350.0 mL in water) while vigorously stirring. The mixture was stirred at room temperature for 4 h. The solids were filtered off and the filtrate was extracted with DCM. The combined organic layers were dried and concentrated under vacuum. The residue was purified by Chromatography A to afford the title compound (1.7 g, 48% over two steps). MS (ESI) calculated for $(C_{11}H_{10}N_2O_2)$ $[M+H]^+$, 203.1; found, 203.2. ^1H NMR (300 MHz, $DMSO-d_6$) δ 9.97 (s, 1H), 9.15 (s, 1H), 7.82 (s, 1H), 4.67 (s, 2H), 2.29 – 2.21 (m, 1H), 1.22 – 1.14 (m, 4H).

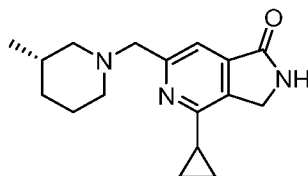
Example G: (R)-4-cyclopropyl-6-((4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2,3-dihydro-1H-pyrrolo[3,4-c]pyridin-1-one



[394] A mixture of **Example C** (840 mg, 4.16 mmol), **Example F** (783 mg, 4.57 mmol), and triethylamine (636 μ L, 4.56 mmol) in DCM (17 mL) was stirred for 10 min at room temperature before sodium triacetoxyborohydride (969 mg, 4.57 mmol) was added. After 1.5 h, additional sodium triacetoxyborohydride (177 mg) was added. The reaction was stirred overnight before being quenched with aq. sodium bicarbonate, followed by General Work-up Procedure 1 using

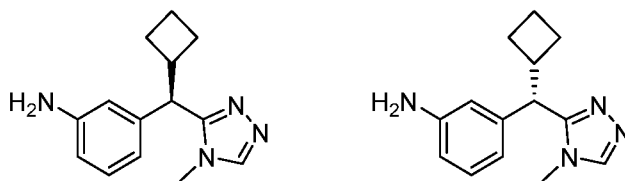
DCM. The residue was purified by Chromatography B, followed by Chromatography C to afford the title compound (626 mg, 47 %). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.90 (s, 1H), 7.41 (s, 1H), 4.52 (s, 2H), 3.68 (d, J = 1.8 Hz, 2H), 2.75 (t, J = 14.5 Hz, 2H), 2.37 – 2.26 (m, 1H), 2.18 – 1.84 (m, 5H), 1.09 – 0.96 (m, 4H), 0.92 (d, J = 6.4 Hz, 3H). LCMS: $\text{C}_{17}\text{H}_{21}\text{F}_2\text{N}_3\text{O}$ requires 321.2, found 322.3 $[\text{M}+\text{H}]^+$.

Example H: (*S*)-4-cyclopropyl-6-((3-methylpiperidin-1-yl)methyl)-2,3-dihydro-1H-pyrrolo[3,4-*c*]pyridin-1-one



[395] A solution 4-cyclopropyl-1-oxo-2H,3H-pyrrolo[3,4-*c*]pyridine-6-carbaldehyde (1.0 g, 4.95 mmol), (3*S*)-3-methylpiperidine hydrochloride (804 mg, 5.93 mmol), and triethylamine (0.69 mL, 4.56 mmol) in DCE (34 mL) was stirred for 15 min at room temperature before sodium triacetoxyborohydride (1.36 g, 6.43 mmol) was added. The reaction was stirred overnight before being quenched with aq. sodium bicarbonate, followed by General Work-up Procedure 1 using DCM. The residue was purified by Chromatography B to afford the title compound (1.1 g, 77 %). LCMS: $\text{C}_{17}\text{H}_{23}\text{N}_3\text{O}$ requires 285.2, found 286.2 $[\text{M}+\text{H}]^+$. ^1H NMR (500 MHz, $\text{Chloroform}-d$) δ 7.68 (s, 1H), 6.45 (s, 1H), 4.57 (s, 2H), 3.69 (s, 2H), 2.83 (t, J = 12.5 Hz, 2H), 2.05 – 1.98 (m, 1H), 1.94 (ddd, J = 12.9, 8.3, 4.7 Hz, 1H), 1.72 (d, J = 9.0 Hz, 3H), 1.33 – 1.18 (m, 3H), 1.06 (dt, J = 8.1, 3.2 Hz, 2H), 0.86 (d, J = 5.7 Hz, 5H).

Example I and Example J: (*R*)-3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline (I) and (*S*)-3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline (J)



[396] **Step 1: Synthesis of *tert*-butyl *N*-[3-(cyclobutyl(4-methyl-1,2,4-triazol-3-yl)methyl)]phenyl]carbamate.** A degassed mixture of 3-[(3-bromophenyl)(cyclobutyl)methyl]-4-methyl-1,2,4-triazole (1.3 g, 4.3 mmol), *tert*-butyl carbamate (547.1 mg, 4.67 mmol), XPhos Pd G3 (718.7 mg, 0.85 mmol), XPhos (404.9 mg, 0.85 mmol), and Cs_2CO_3 (2.8 g, 8.5 mmol) in dioxane (26.0 mL) was stirred at 90 °C for 16 h

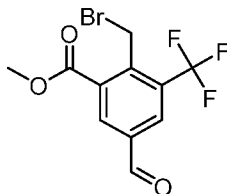
under N₂ atmosphere. The solvent was removed under vacuum and the residue was diluted with water. General Workup Procedure 1 was used. The residue was purified by Chromatography B to afford *tert*-butyl *N*-[3-[cyclobutyl(4-methyl-1,2,4-triazol-3-yl)methyl]phenyl]carbamate (1.0 g, 68%). MS (ESI) calculated for (C₁₉H₂₆N₄O₂) [M+H]⁺, 343; found, 343.

[397] Step 2: Synthesis of (*R*)-3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline and (*S*)-3-(cyclobutyl (4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline. A solution of *tert*-butyl *N*-[3-[cyclobutyl(4-methyl-1,2,4-triazol-3-yl)methyl]phenyl]carbamate (1.0 g, 2.9 mmol) in HCl in 1,4-dioxane (4 M, 10 mL) was stirred at room temperature for 1 h. The solvent was removed under vacuum. The residue was dissolved in MeOH and basified to pH ~10 by ammonia before concentration under vacuum. The residue was purified by Chromatography C to afford 3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline (450 mg), which was further separated by prep-chiral-SFC under the following conditions: [(Column: CHIRAL ART Cellulose-SB, 2*25 cm, 5 μm; Mobile Phase A: CO₂, Mobile Phase B: EtOH; Flow rate: 40 mL/min; Gradient: 40% B; 220 nm] to afford (*S*)-3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline (215.3 mg) with a shorter retention time on chiral-SFC, and (*R*)-3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline (217.6 mg) with a longer retention time on chiral-SFC.

[398] (*S*)-3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline: MS (ESI) calculated for (C₁₄H₁₈N₄) [M+H]⁺, 243, found, 243. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.29 (s, 1H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.41 – 6.33 (m, 3H), 5.01 (s, 2H), 3.91 (d, *J* = 10.5 Hz, 1H), 3.35 (s, 3H), 3.15 – 3.05 (m, 1H), 2.07 (m, 1H), 2.10 – 1.66 (m, 5H).

[399] (*R*)-3-(cyclobutyl (4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)aniline: MS (ESI) calculated for (C₁₄H₁₈N₄) [M+H]⁺, 243, found, 243. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.29 (s, 1H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.41 – 6.33 (m, 3H), 5.01 (s, 2H), 3.91 (d, *J* = 10.5 Hz, 1H), 3.35 (s, 3H), 3.15 – 3.05 (m, 1H), 2.07 (m, 1H), 2.10 – 1.66 (m, 5H).

Example K: Methyl 2-(bromomethyl)-5-formyl-3-(trifluoromethyl)benzoate



[400] Step 1: Synthesis of 3-(methoxycarbonyl)-4-methyl-5-(trifluoromethyl)benzoic acid. To a degassed solution of methyl 5-bromo-2-methyl-3-(trifluoromethyl)benzoate (18.0 g, 60.8 mmol), oxalic acid (11.5 g, 91.2 mmol), acetic anhydride (9.3 g, 91.2 mmol), and *N*-

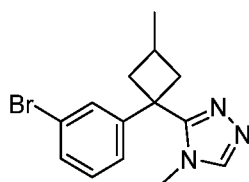
ethyl-*N*-isopropylpropan-2-amine (11.8 g, 91.2 mmol) in dimethylformamide (200 mL) were added Pd(OAc)₂ (1.4 g, 6.1 mmol) and XantPhos (1.8 g, 3.0 mmol). The mixture was stirred at 100 °C for 16 h under nitrogen. The reaction was quenched by the addition of HCl (1 M, 300 mL) to pH ~3. The aqueous solution was extracted with EtOAc (250 mL x 3). The combined organic layers were dried and concentrated. The residue was purified by Chromatography C to afford the title compound (7.5 g, 47%).

[401] Step 2: Synthesis of 4-(bromomethyl)-3-(methoxycarbonyl)-5-(trifluoromethyl)benzoic acid. To a stirred solution of 4-methyl-3-(methoxycarbonyl)-5-(trifluoromethyl)benzoic acid (8 g, 30.5 mmol) and NBS (8.2 g, 46 mmol) in CCl₄ (160 mL) was added BPO (2.2 g, 9 mmol). The solution was stirred at 80 °C for 16 h. The mixture was concentrated. The residue was purified by Chromatography B to afford the title compound (8.0 g, 77%). MS (ESI) calculated for (C₁₁H₈BrF₃O₄) [M-H]⁻, 339; found, 339.

[402] Step 3: Synthesis of methyl 2-(bromomethyl)-5-(hydroxymethyl)-3-(trifluoromethyl)benzoate. To a stirred solution of 4-(bromomethyl)-3-(methoxycarbonyl)-5-(trifluoromethyl)benzoic acid (2.65 g, 7.77 mmol) in tetrahydrofuran (30 mL) was added borane (19.4 mL, 19.4 mmol, 1 M in tetrahydrofuran). The solution was stirred at rt for 6 h. The reaction was then quenched by the addition of MeOH (10 mL). The mixture was concentrated. The residue was purified by Chromatography A to afford the title compound (1.40 g, 84%).

[403] Step 4: Synthesis of methyl 2-(bromomethyl)-5-formyl-3-(trifluoromethyl)benzoate. To a stirred solution of methyl 2-(bromomethyl)-5-(hydroxymethyl)-3-(trifluoromethyl)benzoate (7.1 g, 21.71 mmol) in ethyl acetate (70 mL) was added 2-iodoxybenzoic acid (9.1 g, 32.5 mmol). The reaction was stirred at 70 °C for 3 h. The solids were filtered out, and the filtrate was concentrated in vacuo. The residue was purified by Chromatography A to afford the title compound (6.6 g, 94%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.12 (s, 1H), 8.56 (d, *J* = 1.8 Hz, 1H), 8.45 (d, *J* = 1.8 Hz, 1H), 5.07 (s, 2H), 3.96 (s, 3H).

Example L: 3-[1-(3-bromophenyl)-3-methylcyclobutyl]-4-methyl-1,2,4-triazole



[404] Step 1: Synthesis of methyl 1-(3-bromophenyl)-3-methylcyclobutane-1-carboxylate. To a stirring solution of methyl 2-(3-bromophenyl)acetate (5.0 g, 21.8 mmol) and

1,3-dibromo-2-methylpropane (4.7 g, 21.8 mmol) in DMF (100 mL) was added sodium hydride (60% in paraffin oil) (1.7 g, 43.6 mmol) at 0 °C. After stirring at room temperature for 16 h the reaction mixture was poured into saturated aqueous NH₄Cl and General Workup 1 was used. The residue was purified by Chromatography A to afford the title compound (4.0 g, 65%).

[405] Step 2: Synthesis of 1-(3-bromophenyl)-3-methylcyclobutane-1-carbohydrazide.

To a stirring solution of methyl 1-(3-bromophenyl)-3-methylcyclobutane-1-carboxylate (4.0 g, 14.1 mmol) in ethanol (100 mL) was added hydrazine hydrate (6.9 mL, 70.6 mmol). The mixture was stirred at 80 °C for about 16 h. The reaction mixture was diluted with water and General Workup 1 was used. The residue was used in the next step without purification.

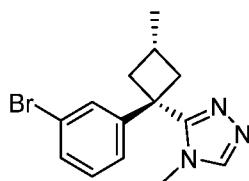
[406] Step 3: Synthesis of 5-[1-(3-bromophenyl)-3-methylcyclobutyl]-4-methyl-1,2,4-triazole-3-thiol.

To a solution of 1-(3-bromophenyl)-3-methylcyclobutane-1-carbohydrazide (3.2 g, 11.3 mmol) in THF (100 mL) was added methyl isothiocyanate (2.5 g, 33.90 mmol). The mixture was stirred at 80 °C for 2 h. The solution was cooled to room temperature and then potassium hydroxide (3.2 g, 56.5 mmol) in water (10 mL) was added. The mixture was stirred at room temperature for 12 h. The solution was neutralized to a pH of around 7 with 1M HCl and then extracted three times with DCM. The layers were dried, filtered, and concentrated. The crude material was used in the next step.

[407] Step 4: Synthesis of 3-[1-(3-bromophenyl)-3-methylcyclobutyl]-4-methyl-1,2,4-triazole.

To a solution of 5-[1-(3-bromophenyl)-3-methylcyclobutyl]-4-methyl-1,2,4-triazole-3-thiol (3.8 g, 11.3 mmol) in methylene chloride (50 mL) and acetic acid (7 mL) at 0 °C was added hydrogen peroxide (3 mL, 30%). The reaction mixture was stirred at room temperature for 16 h and evaporated to dryness. General Workup Procedure 1 was used. The residue was purified by Chromatography B to afford the title compound (2.30 g, 54% over three steps).

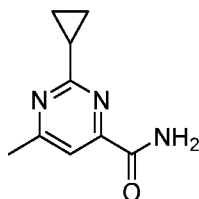
Example M: 3-((1s,3s)-1-(3-bromophenyl)-3-methylcyclobutyl)-4-methyl-4H-1,2,4-triazole



[408] The mixture of 3-[1-(3-bromophenyl)-3-methylcyclobutyl]-4-methyl-1,2,4-triazole was further purified via Chromatography B to afford the title compound. ¹H NMR (500 MHz, Chloroform-*d*) δ 7.99 (s, 1H), 7.56 (d, *J* = 1.8 Hz, 1H), 7.41 (dd, *J* = 7.2, 1.8 Hz, 1H), 7.26 –

7.24 (m, 2H), 3.20 (s, 3H), 2.90 – 2.79 (m, 2H), 2.76 – 2.52 (m, 3H), 1.16 (d, $J = 5.5$ Hz, 3H).
LCMS: $C_{14}H_{16}BrN_3$ requires: 306, found: $m/z = 307$ $[M+H]^+$.

Example N: Synthesis of 2-cyclopropyl-6-methylpyrimidine-4-carboxamide



[409] Step 1: Synthesis of methyl 2-cyclopropyl-6-methylpyrimidine-4-carboxylate.

Cyclopropyl zinc bromide (16.2 mL of 0.5 M solution in tetrahydrofuran, 8.1 mmol, 1.5 equiv) was added to a solution of methyl 2-chloro-6-methylpyrimidine-4-carboxylate (1.0 g, 5.4 mmol, 1 equiv), tetrakis(triphenylphosphine)palladium(0) (500 mg, 0.43 mmol, 0.08 equiv), and tetrahydrofuran (30 mL). The resulting solution was heated at reflux for 24 h. The reaction was allowed to cool to room temperature, and then was poured into saturated NH_4Cl (75 mL) and ethyl acetate (50 mL). The phases were separated and the aq. phase was extracted with ethyl acetate (2x10 mL). The combined organic phases were dried (sodium sulfate), filtered, and concentrated onto Celite. The residue was purified by Chromatography A to afford 510 mg of the title compound.

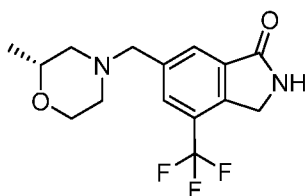
[410] Step 2: Synthesis of 2-cyclopropyl-6-methylpyrimidine-4-carboxylic acid.

A mixture of methyl 2-cyclopropyl-6-methylpyrimidine-4-carboxylate (500 mg, 2.6 mmol, 1 equiv), $LiOH \cdot H_2O$ (330 mg, 7.8 mmol, 3 equiv), tetrahydrofuran (6 mL), and water (2 mL) was stirred vigorously for 17 h. The mixture was poured into 0.1 N HCl (50 mL) saturated with sodium sulfate. The product was extracted with 15% *i*-PrOH/ $CHCl_3$ (3x10 mL) and concentrated to afford 242 mg of the title compound.

[411] Step 3: Synthesis of 2-cyclopropyl-6-methylpyrimidine-4-carboxamide.

To a solution of 2-cyclopropyl-6-methylpyrimidine-4-carboxylic acid (2.0 g, 11.2 mmol) in DCM (20 mL) at 0 °C was added isobutyl chloroformate (1.6 mL, 12.3 mmol). The solution was stirred for 1 h at 0 °C. NH_4OH was added and the solution was stirred at room temperature for 12 h. General Workup 1 was followed to give the title compound (492 mg, 25%).

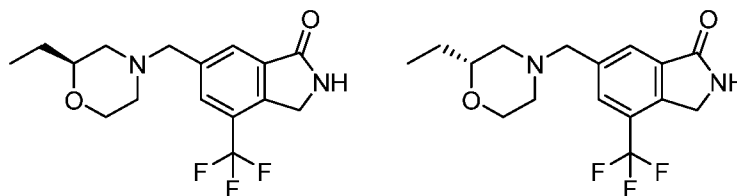
Example O: (R)-6-((2-methylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one



[412] **Step 1: Synthesis of methyl 2-(bromomethyl)-5-[(2R)-2-methylmorpholin-4-yl]methyl]-3-(trifluoromethyl) benzoate:** A solution of **Example K** (1.0 g, 3.1 mmol), (2R)-2-methylmorpholine (311 mg, 3.08 mmol), and titanium ethoxide (1.4 g, 6.1 mmol) in tetrahydrofuran (10 mL) was stirred at 0 °C for 1 h under N₂ atmosphere. Acetic acid (350 µL, 6.15 mmol) and NaBH₃CN (290 mg, 4.61 mmol) were added successively to the above mixture and stirred at room temperature for 16 h. The mixture was concentrated under vacuum. The residue was purified by Chromatography C to afford the title compound (1.0 g, 79%).

[413] **Step 2: Synthesis of (R)-6-((2-methylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one:** A solution of methyl 2-(bromomethyl)-5-[(2R)-2-methylmorpholin-4-yl]methyl]-3-(trifluoromethyl) benzoate (1.0 g, 2.4 mmol) in NH₃ (10 mL, 7 M in MeOH) was stirred at room temperature for 16 h. The mixture was concentrated under vacuum. The residue was purified by Chromatography C to afford the title compound (704 mg, 90%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.88 (s, 1H), 7.89 (s, 1H), 7.85 (s, 1H), 4.53 (s, 2H), 3.78 – 3.67 (m, 1H), 3.78 – 3.60 (m, 2H), 3.60 – 3.42 (m, 2H), 2.72 – 2.55 (m, 2H), 2.13 – 2.03 (m, 1H), 1.82 – 1.72 (m, 1H), 1.02 (d, *J* = 6.3 Hz, 3H). MS C₁₅H₁₇F₃N₂O₂ requires: 315, found: *m/z* = 315 [M+H]⁺.

Example P and Example Q: 6-[(2S)-2-ethylmorpholin-4-yl]methyl]-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (P) and 6-[(2R)-2-ethylmorpholin-4-yl]methyl]-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (Q)



[414] **Step 1: Synthesis of Methyl 2-(bromomethyl)-5-[(2-ethylmorpholin-4-yl)methyl]-3-(trifluoromethyl)benzoate.** A solution of 2-ethylmorpholine (299 mg, 2.60 mmol), **Example K** (845 mg, 2.60 mmol) and titanium ethoxide (1.1 mL, 5.2 mmol) in tetrahydrofuran (10 mL) was stirred at room temperature for 1 h. NaBH₃CN (245 mg, 3.9 mmol) was added and the mixture was stirred at room temperature for 16 h. The solvent was removed under

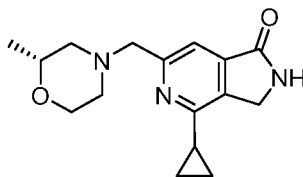
vacuum, and the residue was purified by Chromatography A to afford the title compound (880 mg, 80%). MS: $C_{17}H_{21}BrF_3NO_3$ requires: 423, found: $m/z = 424 [M+H]^+$.

[415] Step 2: Synthesis of 6-[(2*S*)-2-ethylmorpholin-4-yl]methyl]-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (P) and 6-[(2*R*)-2-ethylmorpholin-4-yl]methyl]-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (Q). A solution of methyl 2-(bromomethyl)-5-[(2-ethylmorpholin-4-yl)methyl]-3-(trifluoromethyl) benzoate (1.0 g, 2.36 mmol) in NH_3 (10.0 mL, 7 M in MeOH) was stirred at room temperature for 2 h. The mixture was concentrated under vacuum. The residue was purified by Chromatography C to afford 6-((2-ethylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one (480 mg, 62%). The racemic 6-((2-ethylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one (480 mg) was resolved by SFC (Chiralpak, Mobile Phase A: 8 mmol/L NH_3 in MeOH, Mobile Phase B: IPA) to give the title compounds.

[416] 6-[(2*S*)-2-ethylmorpholin-4-yl]methyl]-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (173 mg). ^1H NMR (300 MHz, $DMSO-d_6$) δ 8.85 (s, 1H), 7.87 (s, 1H), 7.86 (s, 1H), 4.51 (s, 2H), 3.80 – 3.60 (m, 3H), 3.52 – 3.42 (m, 1H), 3.32 – 3.28 (m, 1H), 2.68 – 2.57 (m, 2H), 2.12 – 2.02 (m, 1H), 1.80 (t, $J = 10.5$ Hz, 1H), 1.46 – 1.27 (m, 2H), 0.83 (t, $J = 7.5$ Hz, 3H). MS (ESI): $C_{16}H_{19}F_3N_2O_2$ requires: 328 found: $m/z = 329 [M+H]^+$.

[417] 6-[(2*R*)-2-ethylmorpholin-4-yl]methyl]-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (170 mg). ^1H NMR (300 MHz, $DMSO-d_6$) δ 8.85 (s, 1H), 7.86 (s, 2H), 4.51 (s, 2H), 3.77 – 3.57 (m, 3H), 3.52 – 3.42 (m, 1H), 3.31 – 3.29 (m, 1H), 2.68 – 2.57 (m, 2H), 2.12 – 2.02 (m, 1H), 1.80 (t, $J = 10.5$ Hz, 1H), 1.46 – 1.27 (m, 2H), 0.83 (t, $J = 7.5$ Hz, 3H). MS (ESI): $C_{16}H_{19}F_3N_2O_2$ requires: 328 found: $m/z = 329 [M+H]^+$.

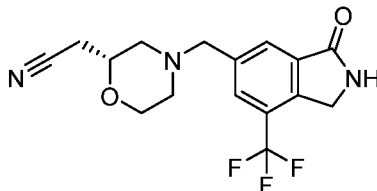
Example R: 4-cyclopropyl-6-[(2*R*)-2-methylmorpholin-4-yl]methyl]-2H,3H-pyrrolo[3,4-*c*]pyridin-1-one



[418] To a stirred mixture of **Example F** (650 mg, 3.33 mmol), (2*R*)-2-methylmorpholine (450 mg, 4.50 mmol), and CH_3COOH (198 mg, 3.33 mmol) in MeOH (24 mL) was added TEA (335 mg, 3.33 mmol) at 0 °C and stirred at room temperature for 16 h under nitrogen atmosphere. Then $NaBH_3CN$ (629 mg, 9.99 mmol) was added to the above mixture at 0 °C and stirred at room temperature for 1 h. General Workup Procedure 1 was used. The residue was

purified by Chromatography C to afford the title compound (509 mg, 53%). MS (ESI) calculated for (C₁₆H₂₁N₃O₂) [M+H]⁺, 288.2; found, 288.2. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.89 (s, 1H), 7.40 (s, 1H), 4.51 (s, 2H), 3.74 – 3.71 (m, 1H), 3.64 (s, 2H), 3.60 – 3.47 (m, 2H), 2.71 – 2.60 (m, 2H), 2.13 – 2.06 (m, 2H), 1.91 – 1.71 (m, 1H), 1.09 – 0.97 (m, 7H).

Example S: (*R*)-2-(4-((3-oxo-7-(trifluoromethyl)isoindolin-5-yl)methyl)morpholin-2-yl)acetonitrile



[419] Step 1: Synthesis of *tert*-butyl (2*S*)-2-[(methanesulfonyloxy)methyl]morpholine-4-carboxylate. To a solution of *tert*-butyl (2*S*)-2-(hydroxymethyl)morpholine-4-carboxylate (3.5 g, 16.1 mmol) and triethylamine (4.6 mL, 32.2 mmol) in DCM (35 mL) was added methanesulfonyl chloride (1.9 mL, 24.16 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h under nitrogen atmosphere. General Workup Procedure 1 was used. The residue was purified by Chromatography B to afford the title compound (4.5 g, 94%).

[420] Step 2: Synthesis of (2*S*)-morpholin-2-ylmethyl methanesulfonate HCl salt. A solution of *tert*-butyl (2*S*)-2-[(methanesulfonyloxy)methyl]morpholine-4-carboxylate (950 mg) in HCl (4 M in dioxane, 10 mL) was stirred at room temperature for 1 h. The mixture was concentrated under vacuum to afford the title compound, which was used in the next step without purification.

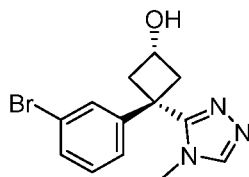
[421] Step 3: Synthesis of methyl 2-(bromomethyl)-5-[(2*S*)-2-[(methanesulfonyloxy)methyl] morpholin-4-yl] methyl]-3-(trifluoromethyl)benzoate. To a solution of (2*S*)-morpholin-2-ylmethyl methanesulfonate HCl salt (600 mg, 3.08 mmol) and methyl 2-(bromomethyl)-5-formyl-3-(trifluoromethyl)benzoate (1.0 g, 3.01 mmol) in tetrahydrofuran (15 mL) was added titanium ethoxide (1.4 g, 6.15 mmol) at 0 °C and stirred at room temperature for 1 h under nitrogen atmosphere. Then to the mixture was added NaBH₃CN (290 mg, 4.61 mmol) and stirred at room temperature for another 16 h under nitrogen atmosphere. The mixture was concentrated under vacuum. The residue was purified by Chromatography C to afford the title compound (800 mg, 51%). MS: C₁₇H₂₁BrF₃NO₆S requires 503; found: *m/z* = 504 [M+H]⁺.

[422] Step 4: Synthesis of (*S*)-4-((3-oxo-7-(trifluoromethyl)isoindolin-5-yl)methyl)morpholin-2-yl)methyl methanesulfonate. A mixture of methyl 2-

(bromomethyl)-5-[[*(2S)*-2-[(methanesulfonyloxy)methyl]morpholin-4-yl]methyl]-3-(trifluoromethyl)benzoate (800 mg, 1.59 mmol) in NH_3 (7 M in MeOH, 8 mL) was stirred at room temperature for 16 h under nitrogen atmosphere. After evaporation, the residue was purified by Chromatography C to afford the title compound which was used in the next step without purification.

[423] Step 5: Synthesis of (*R*)-2-(4-((3-oxo-7-(trifluoromethyl)isoindolin-5-yl)methyl)morpholin-2-yl)methylmethanesulfonate. A mixture of (*S*)-4-((3-oxo-7-(trifluoromethyl)isoindolin-5-yl)methyl)morpholin-2-yl)methylmethanesulfonate (460 mg, 1.13 mmol), KCN (110 mg, 1.69 mmol), and KI (280 mg, 1.69 mmol) in DMF (10 mL) was stirred at 100 °C for 16 h under nitrogen atmosphere. General Workup Procedure 1 was used. The residue was purified by Chromatography C then by Prep-HPLC using acetonitrile in water to afford the title compound (189 mg, 49%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 8.88 (s, 1H), 7.91 – 7.87 (m, 2H), 4.54 (s, 2H), 3.85 – 3.54 (m, 5H), 2.81 – 2.64 (m, 4H), 2.19 – 1.90 (m, 2H). LCMS: $\text{C}_{16}\text{H}_{16}\text{F}_3\text{N}_3\text{O}_2$ requires: 339, found: $m/z = 340$ $[\text{M}+\text{H}]^+$.

Example T: (1*s*,3*s*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol



[424] Step 1: Synthesis of (1*s*,3*s*)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylic acid. To a solution of isopropyl magnesium bromide (2 M in THF, 375 mL) was added a solution of 2-(3-bromophenyl)acetic acid (75 g, 348 mmol) in THF (500 mL) dropwise at 0 °C. 2-(chloromethyl)oxirane (48 g, 523 mmol) was added to the above mixture dropwise at 0 °C under nitrogen. After stirring the reaction mixture at room temperature for 45 min, more isopropyl magnesium bromide (2 M in THF, 375 mL) was added over 30 min. The reaction mixture was slowly heated to 60 °C and stirred for 16 h. The solution was then cooled to room temperature and quenched slowly into 5 *N* HCl. General workup procedure 1 followed by Chromatography B afforded the title compound (36 g, 38%).

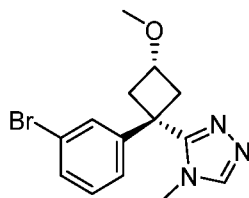
[425] Step 2: Synthesis of 2-((1*s*,3*s*)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carbonyl)-*N*-methylhydrazine-1-carbothioamide. To a solution of (1*s*,3*s*)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylic acid (5.0 g, 14.7 mmol), 4-methyl-3-thiosemicarbazide (1.8 g, 17.5 mmol), and DIEA (5.7 g, 44.2 mmol) in DMF (100 mL) was

added HATU (7.2 g, 19.1 mmol) in portions at 0 °C. The mixture was stirred at room temperature for 16 h. General Workup Procedure 1 was used to afford the title compound which was used in the next step without purification.

[426] Step 3: Synthesis of (1s,3s)-3-(3-bromophenyl)-3-(5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol. A solution of 2-((1s,3s)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carbonyl)-N-methylhydrazine-1-carbothioamide in NaOH (1 M, 160 mL) was stirred at room temperature for 16 h under nitrogen atmosphere. The aqueous phase was acidified by HCl (2 N) to pH ~3. General workup Procedure 1 afforded the title compound, which was used in the next step without purification.

[427] Step 4: Synthesis of (1s,3s)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol. A mixture of (1s,3s)-3-(3-bromophenyl)-3-(5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol (4.5 g, 13.2 mmol) and NaNO₂ (5.4 g, 79.4 mmol) in HNO₃ (40 mL, 1 N) was stirred at room temperature for 16 h under nitrogen atmosphere. General Workup Procedure 1 was used. The residue was purified by Chromatography C to afford the title compound (500 mg, 3% over three steps). ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.29 (s, 1H), 7.47 – 7.29 (m, 4H), 5.30 (d, *J* = 6.9 Hz, 1H), 4.25 – 4.18 (m, 1H), 3.17 (s, 3H), 3.04 – 2.97 (m, 2H), 2.74 – 2.67 (m, 2H). LCMS: C₁₃H₁₄BrN₃O requires: 308, found: *m/z* = 310 [M+H]⁺.

Example U: 3-((1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutyl)-4-methyl-4H-1,2,4-triazole



[428] Step 1: Synthesis of methyl (1s,3s)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylate. To a stirred solution of (1s,3s)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylic acid (20 g, 74 mmol) in DCM (300 mL) and MeOH (30 mL) was added TMSCHN₂ (2 M in hexane, 55 mL) dropwise at 0 °C, and the mixture was stirred at room temperature for 16 h under nitrogen atmosphere. The organic solvents were removed under vacuum. The residue was purified by Chromatography A to afford the title compound (9.5 g, 44%).

[429] Step 2: Synthesis of Methyl (1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carboxylate. To a stirred solution of methyl (1s,3s)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylate (1.53 g, 5.2 mmol) in DMF (25 mL) was added NaH (218 mg, 10.5 mmol, 60%) in portions at 0 °C and stirred at room temperature for 1 h under nitrogen

atmosphere. Then to the mixture was added MeI (1.52 g, 10.5 mmol) dropwise at 0 °C. The mixture was stirred at room temperature for 16 h. General Workup Procedure 1 was used. The residue was purified by Chromatography A to afford the title compound (1.4 g, 90%).

[430] Step 3: Synthesis of (1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carboxylic acid. To a solution of (1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carboxylate (1.4 g, 4.6 mmol) in THF (15 mL) was added a solution of LiOH (0.5 g, 18.7 mmol) in water (5 mL) at 0 °C. The mixture was stirred at room temperature for 3 h under nitrogen atmosphere. The organic solvent was removed under vacuum. The residue was diluted with water and extracted with ethyl acetate. The collected aqueous phase was acidified by HCl (1 N) to pH 3~4 and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated to afford the title compound, which was used in the next step without purification.

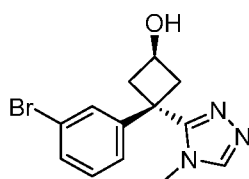
[431] Step 4: Synthesis of 2-((1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carbonyl)-N-methylhydrazine-1-carbothioamide. To a stirred solution of (1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carboxylic acid (1.1 g, 3.8 mmol) and 4-methyl-3-thiosemicarbazide (0.5 g, 4.6 mmol) in DMF (15 mL) were added HATU (2.1 g, 5.5 mmol) and DIEA (1.4 mL, 7.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 16 h under nitrogen atmosphere. The mixture was then quenched by the addition of water. General Workup Procedure 1 afforded the title compound, which was used in the next step without purification.

[432] Step 5: Synthesis of 5-((1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutyl)-4-methyl-4H-1,2,4-triazole-3-thiol. A solution of 2-((1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carbonyl)-N-methylhydrazine-1-carbothioamide (2.4 g, crude) in NaOH (1 N, 24 mL in water) was stirred at room temperature for 16 h under nitrogen atmosphere. The reaction mixture was acidified by HCl (1 N) to pH ~3 and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated to afford the title compound, which was used in the next step without purification.

[433] Step 6: Synthesis of 3-((1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutyl)-4-methyl-4H-1,2,4-triazole. To a mixture of 5-((1s,3s)-1-(3-bromophenyl)-3-methoxycyclobutyl)-4-methyl-4H-1,2,4-triazole-3-thiol (1.4 g, crude) and NaNO₂ (2.7 g, 39.5 mmol) was added HNO₃ (1 N, 40 mL) dropwise at 0 °C, and the mixture was stirred at room temperature for 16 h under nitrogen atmosphere. General Workup Procedure 1 was used. The residue was purified by Chromatography C to afford the title compound (460 mg, 30% over four steps). ¹H NMR

(300 MHz, DMSO-*d*₆) δ 8.31 (s, 1H), 7.49 – 7.43 (m, 2H), 7.36 – 7.32 (m, 2H), 4.09 – 3.99 (m, 1H), 3.19 (s, 3H), 3.12 (s, 3H), 3.07 – 3.01 (m, 2H), 2.79 – 2.72 (m, 2H). MS: C₁₄H₁₆BrN₃O requires: 322, found: m/z = 322 [M+H]⁺.

Example V: (1*r*,3*r*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol



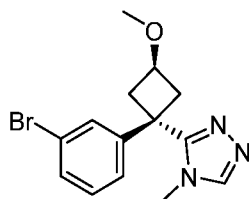
[434] Step 1: Synthesis of 2-((1*r*,3*r*)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carbonyl)-N-methylhydrazine-1-carbothioamide: To a mixture of (1*r*,3*r*)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylic acid (850 mg, 3.32 mmol), 4-methyl-3-thiosemicarbazide (418 mg, 3.98 mmol) and DIEA (1.7 mL, 9.99 mmol) in DMF (40 mL) was added HATU (1651 mg, 4.34 mmol) in portions at 0 °C, and the mixture was stirred at room temperature for 3 h under N₂ atmosphere. The reaction mixture was poured into water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated to afford the title compound, which was used in the next step without purification.

[435] Step 2: Synthesis of (1*r*,3*r*)-3-(3-bromophenyl)-3-(5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol: To a solution of 2-((1*r*,3*r*)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carbonyl)-N-methylhydrazine-1-carbothioamide (1.5 g, 4.19 mmol) was added a solution of NaOH (1 N, 25 mL in water) dropwise at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction mixture was acidified by HCl (1 N) to pH 5~6 and General Workup 1 was followed. The residue was purified by Chromatography A to afford the title compound (410 mg, 34%). MS calculated for C₁₃H₁₄BrN₃OS [M+H]⁺, 340; found, 340.

[436] Step 3: Synthesis of (1*r*,3*r*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol: To a mixture of (1*r*,3*r*)-3-(3-bromophenyl)-3-(5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol (410 mg, 1.20 mmol) and NaNO₂ (510 mg, 7.39 mmol) was added HNO₃ (7 mL, 1 N) dropwise at 0 °C, and the mixture was stirred at room temperature for 16 h. General Workup Procedure 1 was used. After filtration and evaporation, the residue was purified by Chromatography C to afford the title compound (192 mg, 52%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.39 (s, 1H), 7.47 – 7.44 (m, 1H), 7.34 – 7.29 (m, 2H), 7.14 – 7.11 (m, 1H),

5.29 (d, $J = 6.9$ Hz, 1H), 4.06 – 4.01 (m, 1H), 3.32 – 3.19 (m, 2H), 3.19 (s, 3H), 2.47 – 2.40 (m, 2H). MS calculated for $C_{13}H_{14}BrN_3O$ $[M+H]^+$, 308; found, 308.

Example W: 3-((1r,3r)-1-(3-bromophenyl)-3-methoxycyclobutyl)-4-methyl-4H-1,2,4-triazole



[437] Step 1: Synthesis of (1r,3r)-3-(3-bromophenyl)-3-(methoxycarbonyl)cyclobutyl 4-nitrobenzoate: To a stirred solution of methyl (1s,3s)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylate (5.0 g, 17.5 mmol) and 4-nitrobenzoic acid (5.9 g, 35.0 mmol) in THF (50 mL) were successively added PPh_3 (9.2 g, 35.0 mmol) and diethyl azodicarboxylate (8.8 g, 50.5 mmol) dropwise at room temperature, and the mixture was stirred for 16 h under nitrogen atmosphere. The solvent was removed under vacuum. The residue was purified by Chromatography A to afford the title compound (4.8 g, 63%). MS (ESI) calculated for $C_{19}H_{16}BrNO_6$ $[M+H]^+$, 434; found, 434.

[438] Step 2: Synthesis of (1r,3r)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylic acid. To a solution of (1r,3r)-3-(3-bromophenyl)-3-(methoxycarbonyl)cyclobutyl 4-nitrobenzoate (4.8 g, 11.0 mmol) in THF (60 mL) was added LiOH (1.1 g, 44.2 mmol) in H_2O (20 mL). The mixture was stirred at room temperature for 3 h under nitrogen atmosphere. The organic solvent was removed under vacuum and the aqueous phase was extracted with ethyl acetate. The collected aqueous phase was acidified by HCl (1 N) to pH 3~4 and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated under vacuum to provide the title compound, which was used in the next step without purification.

[439] Step 3: Synthesis of methyl (1r,3r)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylate: To a stirred solution of (1r,3r)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylic acid (2.5 g, 9.2 mmol) in DCM (25 mL) and MeOH (2.5 mL) were added $TMSCHN_2$ (2 M in hexane, 7.5 mL) dropwise at 0 °C, and the mixture was stirred at room temperature for 16 h. The solvent was removed under vacuum. The residue was purified by Chromatography A to afford the title compound (1.5 g, 47% over two steps). MS calculated for $C_{12}H_{13}BrO_3$ $[M+H]^+$, 285; found, 285.

[440] Step 4: Synthesis of methyl (1r,3r)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carboxylate: To a stirred solution of methyl (1r,3r)-1-(3-bromophenyl)-3-hydroxycyclobutane-1-carboxylate (1.5 g, 5.2 mmol) in DMF (25 mL) was added NaH (218 mg, 10.5 mmol, 60%) in portions at 0 °C. The mixture was stirred at room temperature for 1 h under nitrogen atmosphere. Then to the mixture was added MeI (1.5 g, 10.5 mmol) dropwise at 0 °C, and the mixture was stirred at room temperature for 16 h under nitrogen atmosphere. General Workup Procedure 1 was used. The residue was purified by Chromatography A to afford the title compound (1.4 g, 90%). MS calculated for C₁₃H₁₅BrO₃ [M+H]⁺, 299; found, 299.

[441] Step 5: Synthesis of (1r,3r)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carboxylic acid: To a solution of (1r,3r)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carboxylate (1.4 g, 4.6 mmol) in THF (15 mL) was added a solution of LiOH (0.45 g, 18.7 mmol) in H₂O (5 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 3 h under nitrogen atmosphere. The organic solvent was removed under vacuum. The residue was diluted with water and extracted with ethyl acetate. The collected aqueous solution was acidified by HCl (1 N) to pH 3~4 and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated under vacuum to provide the title compound, which was used in the next step without purification.

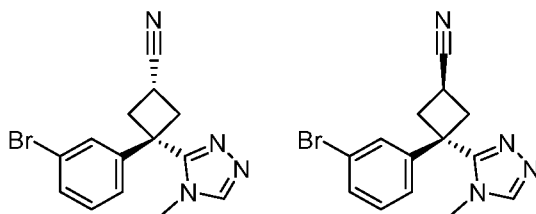
[442] Step 6: Synthesis of 2-((1r,3r)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carbonyl)-N-methylhydrazine-1-carbothioamide: To a stirred solution of (1r,3r)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carboxylic acid (1.1 g, 3.8 mmol) and 4-methyl-3-thiosemicarbazide (0.5 g, 4.6 mmol) in DMF (15 mL) were added HATU (2.1 g, 5.5 mmol) and DIEA (1.0 g, 7.7 mmol) at 0 °C, and the mixture was stirred at room temperature for 16 h under nitrogen atmosphere. The reaction mixture was quenched by the addition of water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated under vacuum to provide the title compound, which was used in the next step without purification.

[443] Step 7: Synthesis of 5-((1r,3r)-1-(3-bromophenyl)-3-methoxycyclobutyl)-4-methyl-4H-1,2,4-triazole-3-thiol: A solution of 2-((1r,3r)-1-(3-bromophenyl)-3-methoxycyclobutane-1-carbonyl)-N-methylhydrazine-1-carbothioamide in NaOH (1 N, 30 mL in water) was stirred at room temperature for 16 h under nitrogen atmosphere. The reaction mixture was acidified by HCl (1 N) to pH ~3 and extracted with ethyl acetate. The combined organic layers were washed with brine and dried. After filtration and evaporation, the residue

was purified by Chromatography C to afford the title compound (430 mg, 23% over three steps). MS calculated for $C_{14}H_{16}BrN_3OS$ $[M+H]^+$, 354; found, 354.

[444] Step 8: Synthesis of 3-((1*r*,3*r*)-1-(3-bromophenyl)-3-methoxycyclobutyl)-4-methyl-4H-1,2,4-triazole: To a mixture of 5-((1*r*,3*r*)-1-(3-bromophenyl)-3-methoxycyclobutyl)-4-methyl-4H-1,2,4-triazole-3-thiol (430 mg, 1.21 mmol) and $NaNO_2$ (838 mg, 12.10 mmol) was added HNO_3 (1 *N*, 13 mL) dropwise at 0 °C, and the mixture was stirred at room temperature for 16 h under nitrogen atmosphere. General Workup Procedure 1 was used. The residue was purified by Chromatography C to afford the title compound (184 mg, 47%). ^1H NMR (300 MHz, $DMSO-d_6$) δ 8.40 (s, 1H), 7.48 – 7.45 (m, 1H), 7.35 – 7.31 (m, 2H), 7.19 – 7.17 (m, 1H), 3.88 – 3.79 (m, 1H), 3.34 – 3.26 (m, 2H), 3.20 (s, 3H), 3.17 (s, 3H), 2.46 – 2.43 (m, 2H). MS calculated for $C_{14}H_{16}BrN_3O$ $[M+H]^+$, 322; found, 322.

Example X and Example Y: (1*s*,3*s*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile (X) and (1*r*,3*r*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile (Y)



[445] Step 1: Synthesis of (1*s*,3*s*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl methanesulfonate. To a mixture of (1*s*,3*s*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutan-1-ol (1.2 g, 3.8 mmol) and triethylamine (788.0 mg, 7.79 mmol) in DCM (10 mL) was added methanesulfonyl chloride (0.74 mL, 4.67 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h and the solvent was removed under vacuum. The residue was purified by Chromatography C to afford the title compound (1.0 g, 66%).

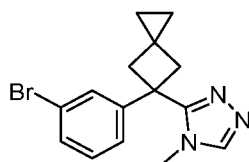
[446] Step 6: Synthesis of (1*s*,3*s*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile (X) and (1*r*,3*r*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile (Y). A mixture of (1*s*,3*s*)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl methanesulfonate (800 mg, 2.07 mmol), KCN (269 mg, 4.14 mmol), and K_2CO_3 (572 mg, 4.14 mmol) in dimethylsulfoxide (20 mL) was stirred at 120 °C for 16 h under nitrogen atmosphere. General Workup Procedure 1 followed by Chromatography C afforded a mixture of products. The mixture was separated by Prep-HPLC

to give the title compounds (1s,3s)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile (187 mg) and (1r,3r)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile (258 mg).

[447] (1s,3s)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.38 (s, 1H), 7.53 – 7.48 (m, 2H), 7.38 – 7.26 (m, 2H), 3.64 – 3.52 (m, 1H), 3.31 – 3.23 (m, 2H), 3.19 (s, 3H), 3.18 – 3.12 (m, 2H). MS(ESI): C₁₄H₁₃BrN₄ requires: 316, found: *m/z* = 317 [M+H]⁺.

[448] (1r,3r)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.43 (s, 1H), 7.52 – 7.47 (m, 1H), 7.42 (t, *J* = 1.5 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.23 – 7.20 (m, 1H), 3.38 – 3.30 (m, 3H), 3.18 (s, 3H), 3.06 – 2.97 (m, 2H). MS(ESI): C₁₄H₁₃BrN₄ requires: 316, found: *m/z* = 317 [M+H]⁺.

Example Z: 3-[5-(3-bromophenyl)spiro[2.3]hexan-5-yl]-4-methyl-1,2,4-triazole



[449] Step 1: Synthesis of 5-(3-bromophenyl)spiro[2.3]hexane-5-carbonitrile. To a suspension of NaH (60% in oil, 1.7 g, 44.0 mmol) in anhydrous DMF (38 mL) at 0 °C was added a solution of 1,1-bis(bromomethyl)cyclopropane (5.0 g, 22 mmol) in anhydrous DMF (2 mL) followed by a solution of 2-(3-bromophenyl)acetonitrile (4.31 g, 22 mmol) in anhydrous DMF (4 mL) and the resulting suspension was stirred at 60 °C for 15 h. The mixture was cooled to 0 °C, and then diluted with water and EtOAc. General Workup Procedure 1 followed by Chromatography A afforded the title compound (4.4 g, 76%). ¹H NMR (500 MHz, CDCl₃) δ 7.68 (t, *J* = 1.9 Hz, 1H), 7.50 – 7.45 (m, 2H), 7.29 (t, *J* = 8.0 Hz, 1H), 3.01 – 2.94 (m, 2H), 2.78 – 2.71 (m, 2H), 0.80 – 0.73 (m, 2H), 0.64 – 0.56 (m, 2H).

[450] Step 2: Synthesis of 5-(3-bromophenyl)spiro[2.3]hexane-5-carboxylic acid. To a solution of 5-(3-bromophenyl)spiro[2.3]hexane-5-carbonitrile (5.0 g, 19.2 mmol) in ethanol (90 mL), was added a solution of NaOH (6.1 g, 154 mmol) in water (20 mL) and the reaction mixture was stirred at reflux for 40 h. The solution was concentrated under reduced pressure, and the residue was neutralized with ice-cold 2 M aqueous HCl. General Workup Procedure 1 afforded the title compound (5.2 g, 97%). ¹H NMR (500 MHz, CDCl₃) δ 11.09 (s, 1H), 7.49 (t, *J* = 2.1 Hz, 1H), 7.40 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.30 – 7.26 (m, 1H), 7.21 (t, *J* = 7.9 Hz, 1H),

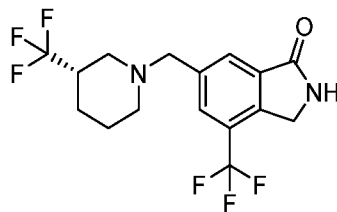
2.96 – 2.89 (m, 2H), 2.74 – 2.65 (m, 2H), 0.56 (dd, $J = 9.6, 6.5$ Hz, 2H), 0.42 (dd, $J = 9.5, 6.5$ Hz, 2H). MS: $C_{13}H_{13}BrO_2$ requires: 280, found: $m/z = 279$ $[M-H]^-$.

[451] Step 3: Synthesis of 5-(3-bromophenyl)spiro[2.3]hexane-5-carbohydrazide. To a solution of 5-(3-bromophenyl)spiro[2.3]hexane-5-carboxylic acid (5.23 g, 18.6 mmol) and Et_3N (6 mL, 43 mmol) in anhydrous DCM (185 mL) at 0 °C was added isobutyl chloroformate (2.7 mL, 21 mmol) and the reaction mixture was warmed to room temperature and stirred for 1 h. The mixture was cooled to 0 °C, hydrazine (55% in water, 6.5 mL, 74 mmol) was added quickly, and the reaction mixture was warmed to room temperature and stirred for 1 h. Water was added, and the phases were separated. The organic phase was washed with brine, then dried, filtered and concentrated under reduced pressure to afford the title compound which was used in the next step without purification.

[452] Step 4: Synthesis of 5-[5-(3-bromophenyl)spiro[2.3]hexan-5-yl]-4-methyl-1,2,4-triazole-3-thiol. To a solution of 5-(3-bromophenyl)spiro[2.3]hexane-5-carbohydrazide (6.1 g, 18.6 mmol) in THF (120 mL), was added methyl isothiocyanate (4.1 g, 56 mmol) and the resulting solution was stirred for 1 h at 65 °C. The mixture was cooled to room temperature, and then a solution of NaOH (5.21 g, 130 mmol) in water (20 mL) was added, and the mixture was stirred at reflux for 12 h. The mixture was cooled to room temperature and the pH was adjusted to ~5 with 3 M aqueous HCl. General Workup Procedure 1 afforded the title product which was used in the next step without purification.

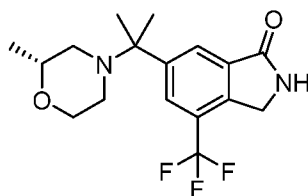
[453] Step 5: Synthesis of 3-[5-(3-bromophenyl)spiro[2.3]hexan-5-yl]-4-methyl-1,2,4-triazole. To a solution of 5-[5-(3-bromophenyl)spiro[2.3]hexan-5-yl]-4-methyl-1,2,4-triazole-3-thiol (7.1 g, 18.6 mmol) in a mixture DCM (90 mL) and AcOH (12 mL) at 0 °C, was added H_2O_2 (30% in water, 5 mL, 49 mmol) and the mixture was warmed to room temperature and stirred for 2.5 h. Additional H_2O_2 (30% in water, 4 mL, 35 mmol) was added and the reaction mixture was stirred for 1 h. The mixture was cooled to 0 °C, and the pH was adjusted to ~13-14 with 2 M aqueous NaOH. General Workup Procedure 1 and Chromatography B was used. The resulting material was suspended in Et_2O and sonicated for 5 min. The resulting suspension was filtered, washed with Et_2O , and air-dried to afford the title compound (2.80 g, 47% over 3 steps). ^1H NMR (500 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.50 – 7.46 (m, 1H), 7.38 (dt, $J = 7.3, 1.9$ Hz, 1H), 7.24 – 7.16 (m, 2H), 3.24 (d, $J = 12.9$ Hz, 2H), 3.21 (s, 3H), 2.71 (d, $J = 12.9$ Hz, 2H), 0.61 – 0.55 (m, 2H), 0.55 – 0.49 (m, 2H). MS: $C_{15}H_{16}BrN_3$ requires: 317, found: $m/z = 318$ $[M+H]^+$.

Example AA: (*S*)-4-(trifluoromethyl)-6-((3-(trifluoromethyl)piperidin-1-yl)methyl)isoindolin-1-one



[454] In a 100 mL round bottom flask at ambient temperature, **Example K** (911 mg, 2.80 mmol) and (*S*)-3-(trifluoromethyl)piperidine hydrochloride (533 mg, 2.81 mmol) were dissolved in DCM (10 mL). Triethylamine (2.0 mL, 14 mmol) was added, followed by sodium triacetoxyborohydride (1.79 g, 8.44 mmol). The reaction was stirred at ambient temperature for 3 h. Aqueous ammonium chloride solution and DCM were added, and General Workup Procedure 1 was followed. To this crude material was added ammonia in MeOH (7 M, 10 mL, 70 mmol) at ambient temperature and the mixture was stirred overnight. Purification by Chromatography B gave the title compound (605 mg, 59%). MS (ESI) calculated for (C₁₆H₁₆F₆N₂O) [M+H]⁺, 367; found, 368.

Example AB: (*R*)-6-(2-(2-methylmorpholino)propan-2-yl)-4-(trifluoromethyl)isoindolin-1-one



[455] **Step 1: Synthesis of methyl 5-bromo-2-methyl-3-(trifluoromethyl)benzoate.** To a mixture of methyl 2-methyl-3-(trifluoromethyl)benzoate (15 g, 73 mmol) in acetic acid (100 mL) was added HNO₃ (46 g) and bromine (12.8 g, 80.1 mmol). Then AgNO₃ (16.1 g, 2.5 M in water) was added over ~30 min. After the mixture was stirred for 16 h at room temperature, General Workup Procedure 1 was used. The residue was purified by Chromatography A to afford the title compound (14.0 g, 70%).

[456] **Step 2: Synthesis of methyl 5-bromo-2-(bromomethyl)-3-(trifluoromethyl)benzoate.** A mixture of methyl 5-bromo-2-methyl-3-(trifluoromethyl)benzoate (14.0 g, 47.1 mmol), NBS (16.8 g, 94.4 mmol), and BPO (2.3 g, 8.9 mmol) in CCl₄ (150 mL) was stirred at 80 °C for 5 h. Then the solids were filtered off. The

filtrate was concentrated under vacuum. The residue was purified by Chromatography A to afford the title compound (11.2 g, 63%).

[457] Step 3: Synthesis of 6-bromo-4-(trifluoromethyl)-2,3-dihydro-1*H*-isoindol-1-one.

To a stirred solution of methyl 5-bromo-2-(bromomethyl)-3-(trifluoromethyl)benzoate (11.2 g, 29.8 mmol) in THF (50 mL) was added NH₃ (7 M in MeOH, 50 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated under vacuum. General Workup Procedure 1 followed by Chromatography A afforded the title compound (8.1 g, 53%). MS (ESI) calc'd for (C₉H₅BrF₃NO) [M+H]⁺, 280.0; found, 280.1.

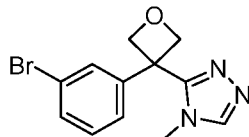
[458] Step 4: Synthesis of 6-acetyl-4-(trifluoromethyl)isoindolin-1-one. A mixture of 6-bromo-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (9.6 g, 34 mmol), tributyl(1-ethoxyethenyl)stannane (18.6 mg, 51.6 mmol) and Pd(dppf)Cl₂·CH₂Cl₂ (2.8 g, 3.4 mmol) in dioxane (100.0 mL) was stirred at 100 °C for 16 h under nitrogen atmosphere. The solvent was removed by evaporation. General Workup Procedure 1 was used. To the residue was added HCl (1 N, 100.0 mL) and THF (200.0 mL). The mixture was stirred at 60 °C for 1 h under nitrogen atmosphere. The organic solvent was removed under vacuum. General Workup Procedure 1 followed by Chromatography A afforded the title compound (4.2 g, 50%). MS (ESI) calculated for (C₁₁H₈F₃NO₂) [M+H]⁺, 244.1; found, 244.0.

[459] Step 5: Synthesis of 2-[(2*R*)-2-methylmorpholin-4-yl]-2-[3-oxo-7-(trifluoromethyl)-1,2-dihydroisoindol-5-yl]propanenitrile. A solution of 6-acetyl-4-(trifluoromethyl)-2,3-dihydroisoindol-1-one (650 mg, 2.67 mmol), (2*R*)-2-methylmorpholine (270 mg, 2.67 mmol), and Ti(OEt)₄ (1.2 g, 5.4 mmol) in tetrahydrofuran (20.0 mL) was stirred at room temperature for 3 h. Trimethylsilyl cyanide (398 mg, 4.01 mmol) was added to the above mixture and stirred at 60 °C for 16 h under nitrogen atmosphere. General Workup Procedure 1 was used. The residue was purified by Chromatography C to afford 2-[(2*R*)-2-methylmorpholin-4-yl]-2-[3-oxo-7-(trifluoromethyl)-1,2-dihydroisoindol-5-yl]propanenitrile (550.0 mg, 58%). MS (ESI) calculated for (C₁₇H₁₈F₃N₃O₂) [M+H]⁺, 354.1; found, 354.0.

[460] Step 6: Synthesis of (*R*)-6-(2-(2-methylmorpholino)propan-2-yl)-4-(trifluoromethyl)isoindolin-1-one. To a degassed solution of 2-[(2*R*)-2-methylmorpholin-4-yl]-2-[3-oxo-7-(trifluoromethyl)-1,2-dihydroisoindol-5-yl] propanenitrile (550 mg, 1.55 mmol) in tetrahydrofuran (20.0 mL) was added methylmagnesium bromide (31.0 mL, 3 M in tetrahydrofuran) dropwise at -60 °C. The reaction mixture was warmed to room temperature and stirred for 2 h under nitrogen atmosphere. The reaction mixture was quenched with saturated NH₄Cl aqueous solution at 0 °C. General Workup Procedure 1 followed by

Chromatography C afforded the title compound (135.3 mg, 33%). MS (ESI) calculated for (C₁₇H₂₁F₃N₂O₂) [M+H]⁺, 343.2; found, 343.2. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.86 (s, 1H), 8.02 (s, 2H), 4.52 (s, 2H), 3.74 (d, *J* = 10.8 Hz, 1H), 3.49 – 3.43 (m, 2H), 2.55 – 2.52 (m, 1H), 2.46 – 2.38 (m, 1H), 2.27 – 2.23 (m, 1H), 1.95 – 1.88 (m, 1H), 1.36 (s, 6H), 1.01 (d, *J* = 6.3 Hz, 3H). ¹⁹F NMR (282 MHz, DMSO-*d*₆) δ -60.11.

Example AC: 3-(3-(3-bromophenyl)oxetan-3-yl)-4-methyl-4H-1,2,4-triazole



[461] Step 1: Synthesis of methyl 2-(3-(3-bromophenyl)-3-hydroxy-2-(hydroxymethyl)propanoate. To a solution of methyl 2-(3-bromophenyl)acetate (12.6 mL, 80.0 mmol) in anhydrous DMF (190 mL) was added paraformaldehyde (9.6 g, 320 mmol) followed by NaOMe (864 mg, 16.0 mmol), and the resulting suspension was stirred for 48 h at room temperature. General Workup Procedure 1 was used. The material was purified by Chromatography A to afford the title compound (5.75 g, 25%).

[462] Step 2: Synthesis of methyl 3-(3-(3-bromophenyl)oxetane-3-carboxylate. To a solution of methyl 2-(3-(3-bromophenyl)-3-hydroxy-2-(hydroxymethyl)propanoate (5.2 g, 17.9 mmol) in anhydrous PhMe (90 mL) was added zinc bis(dimethyldithiocarbamate) (6.0 g, 19.7 mmol) followed by Ph₃P (9.4 g, 36 mmol), and the resulting suspension was cooled to 0 °C. Diethyl azodicarboxylate (5.6 mL, 36 mmol) was then added dropwise, and the mixture was stirred for 24 h at room temperature. The mixture was diluted with DCM, silica gel was added, and the mixture was concentrated under reduced pressure. The material was purified by Chromatography A to afford the title compound (923 mg, 19%).

[463] Step 3: Synthesis of 3-(3-(3-bromophenyl)oxetane-3-carbohydrazide. To a solution of methyl 3-(3-(3-bromophenyl)oxetane-3-carboxylate (762 mg, 2.81 mmol) in MeOH (10 mL) was added hydrazine (55% in H₂O, 1.0 mL, 11 mmol) and the solution was stirred for 16 h. The volatiles were evaporated under reduced pressure and then dried under reduced pressure for 60 h to afford the title compound (765 mg, 100%). MS: C₁₀H₁₁BrN₂O₂ requires: 270, found: *m/z* = 271 [M+H]⁺.

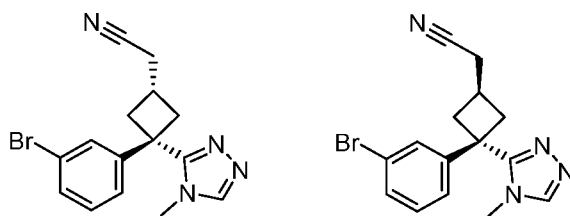
[464] Step 4: Synthesis of 5-[3-(3-(3-bromophenyl)oxetan-3-yl)-4-methyl-1,2,4-triazole-3-thiol. To a solution of 3-(3-(3-bromophenyl)oxetane-3-carbohydrazide (969 mg, 3.57 mmol) in THF (30 mL) was added methyl isothiocyanate (287 mg, 3.93 mmol), and the resulting solution was stirred for 2 h at 65 °C. A solution of KOH (2.41 g, 42.9 mmol) in water (9 mL) was then

added, and the mixture was stirred at reflux for 15 h. The mixture was cooled to room temperature and the pH was adjusted ~2 with 2 M aqueous HCl. The mixture was diluted with EtOAc and the phases were separated. The aqueous phase was extracted with EtOAc, and the combined organic phases were washed with brine, then dried, filtered, and concentrated under reduced pressure to afford the title compound which was used in the next step without purification.

[465] Step 5: Synthesis of 3-(3-(3-bromophenyl)oxetan-3-yl)-4-methyl-4H-1,2,4-triazole.

To a solution of 5-[3-(3-bromophenyl)oxetan-3-yl]-4-methyl-1,2,4-triazole-3-thiol (1.2 g, 3.6 mmol) in DCM (25 mL) and AcOH (2.5 mL) at 0 °C was added H₂O₂ (30% in water, 1.8 mL, 18 mmol) and the mixture was stirred at 0 °C for 1.5 h. The mixture was diluted with DCM and water, and then the pH was adjusted to ~13-14 with 2 M aqueous NaOH. The phases were separated, and the aqueous phase was extracted with DCM twice. The combined organic phases were washed with brine, then dried, filtered, and concentrated under reduced pressure. The material was purified by Chromatography B. The resulting material was suspended in Et₂O and sonicated for 15 min. The resulting suspension was filtered, washed with Et₂O and air-dried to afford the title compound (635 mg, 60% over 2 steps). ¹H NMR (500 MHz, CDCl₃) δ 8.13 (s, 1H), 7.50 (t, J = 1.8 Hz, 1H), 7.48 (ddd, J = 7.9, 1.9, 1.0 Hz, 1H), 7.27 (t, J = 7.8 Hz, 1H), 7.17 (ddd, J = 7.9, 1.9, 1.0 Hz, 1H), 5.52 (d, J = 6.3 Hz, 2H), 5.05 (d, J = 6.3 Hz, 2H), 3.23 (s, 3H). MS: C₁₂H₁₂BrN₃O requires: 293, found: m/z = 294 [M+H]⁺.

Example AD and Example AE: 2-((1s,3s)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile (AD) and 2-((1r,3r)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile (AE)



[466] Step 1: Synthesis of 1-(3-bromophenyl)-3,3-dimethoxy-cyclobutanecarbonitrile.

To a solution of NaH (60% dispersion in oil, 3.7 g, 91 mmol) in DMF (60 mL) at 0 °C was added a solution of 2-(3-bromophenyl)acetonitrile (9.0 g, 46 mmol) in DMF (10 mL) over 15 min, followed by 1,3-dibromo-2,2-dimethoxy-propane (10.0 g, 38 mmol). The reaction mixture was heated to 60 °C and stirred for 18 h. The mixture was cooled, diluted with MeOH, and

then the volatiles were evaporated under reduced pressure. The residue was purified by Chromatography A to afford the title compound (7.3 g, 64%).

[467] Step 2: Synthesis of 1-(3-bromophenyl)-3,3-dimethoxy-cyclobutanecarboxylic acid. To a solution of 1-(3-bromophenyl)-3,3-dimethoxy-cyclobutanecarbonitrile (8.5 g, 29 mmol) in EtOH (43 mL) was added aqueous NaOH (1M in water, 43 mL, 110 mmol) and the mixture was heated to 85 °C for 15 h. The volatiles were evaporated under reduced pressure and then the pH was adjusted to 1-2 using 1 M aqueous HCl. The aqueous layer was extracted with EtOAc four times and the combined organic phases were dried, filtered and concentrated under reduced pressure to afford the title compound (8.6 g, 95%), which was used in the next step without purification.

[468] Step 3: Synthesis of 1-(3-bromophenyl)-3,3-dimethoxycyclobutane-1-carbohydrazide. To a solution of 1-(3-bromophenyl)-3,3-dimethoxy-cyclobutanecarboxylic acid (8.5 g, 27 mmol) in DCM (250 mL) at 0 °C was added TEA (7.5 mL, 54 mmol) followed by isobutyl chloroformate (3.8 mL, 39.7 mmol). The mixture was stirred for 30 min at 0 °C. The mixture was cooled to -30 °C and then hydrazine hydrate (10.5 mL, 108 mmol) was added. The mixture was warmed to room temperature and stirred for 40 min. General Workup Procedure 1 was used. The crude was used in the next step without purification.

[469] Step 4: Synthesis of 5-(1-(3-bromophenyl)-3,3-dimethoxycyclobutyl)-4-methyl-4H-1,2,4-triazole-3-thiol. To a stirring solution of the crude material in THF (230 mL) was added methyl isothiocyanate (5.9 g, 80.9 mmol) and the mixture was stirred at 66 °C for 1 h and then cooled to room temperature. A solution of potassium hydroxide (85% purity, 8.9 g, 135 mmol) in water (30 mL) was then added and the mixture was stirred at 66 °C for 16 h. The pH of the mixture was adjusted to ~7 with 1M aqueous HCl and then diluted with DCM. The phases were separated, and the aqueous phase was extracted with DCM three times. The combined organic layers were washed with brine, dried, filtered, and concentrated under reduced pressure. The crude was used in the next step without purification.

[470] Step 5: Synthesis of 3-(3-bromophenyl)-3-(4-methyl-1,2,4-triazol-3-yl)cyclobutanone. To a stirring solution of the crude material in DCM (100 mL) at 0 °C, was added H₂O₂ (30% in water, 6.9 mL, 67 mmol) followed by acetic acid (15 mL). The mixture was stirred for 1 h. Additional H₂O₂ (30% in water, 4.0 mL, 40 mmol) was added and the mixture was stirred for 30 min. The mixture was diluted with 1M aqueous NaOH and the pH was adjusted to 14. The phases were separated, and the aqueous phase was washed with EtOAc three times. The combined organic layers were washed with brine, dried, filtered, and

concentrated under reduced pressure. The material was purified by Chromatography B to afford the title compound (6.8 g, 72% over 4 steps).

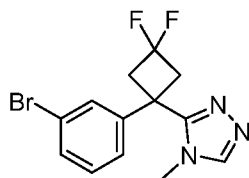
[471] Step 6: Synthesis of 2-(3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutylidene)acetonitrile. To a mixture of 3-(3-bromophenyl)-3-(4-methyl-1,2,4-triazol-3-yl)cyclobutanone (4.9 g, 16 mmol) and 2-diethoxyphosphorylacetonitrile (3.11 mL, 19.2 mmol) in anhydrous THF (82 mL), was added a solution of *t*-BuOK (1M in THF, 19.2 mL, 19.2 mmol) and the reaction mixture was stirred at rt for 2 h. General Workup Procedure 1 afforded the crude title compound, which was used in the next step without purification.

[472] Step 7: Synthesis of 2-((1s,3s)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile and 2-((1r,3r)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile. To the crude 2-(3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutylidene)acetonitrile in THF (160 mL) and *i*-PrOH (30 mL), was added NaBH₄ (1.5 g, 40 mmol) and the reaction mixture was stirred for 18 h. The volatiles were evaporated under reduced pressure and the mixture was diluted with EtOH (100 mL). The solution was stirred for 2 h and then evaporated under reduced pressure. The enantiomers were resolved by SFC over a Lux Cellulose-2 column eluting with IPA in CO₂ at 45 °C to afford 2-((1s,3s)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile (480 mg) and 2-((1r,3r)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile (420 mg).

[473] 2-((1s,3s)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile: ¹H NMR (500 MHz, CDCl₃) δ 8.03 (s, 1H), 7.34 – 7.30 (m, 1H), 7.26 (t, J = 1.7 Hz, 1H), 7.16 (t, J = 7.9 Hz, 1H), 7.10 – 7.06 (m, 1H), 3.18 (s, 3H), 3.17 – 3.10 (m, 2H), 2.82 – 2.69 (m, 1H), 2.55 – 2.43 (m, 4H). MS: C₁₅H₁₅BrN₄ requires: 330, found: m/z = 331 [M+H]⁺.

[474] 2-((1r,3r)-3-(3-bromophenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile: ¹H NMR (500 MHz, C₆D₆) δ 7.58 (s, 1H), 7.56 (t, J = 1.8 Hz, 1H), 7.30 – 7.27 (m, 1H), 6.98 (ddd, J = 7.9, 1.8, 1.0 Hz, 1H), 6.87 (t, J = 7.9 Hz, 1H), 2.62 – 2.55 (m, 2H), 2.46 – 2.39 (m, 2H), 2.37 (s, 3H), 2.20 – 2.09 (m, 1H), 1.86 (d, J = 7.3 Hz, 2H). MS: C₁₅H₁₅BrN₄ requires: 330, found: m/z = 331 [M+H]⁺.

Example AF: 3-(1-(3-bromophenyl)-3,3-difluorocyclobutyl)-4-methyl-4H-1,2,4-triazole



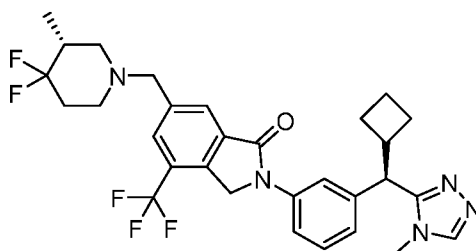
[475] Step 1: Synthesis of methyl 1-(3-bromophenyl)-3,3-difluorocyclobutane-1-carboxylate. To a stirring solution of methyl 1-(3-bromophenyl)-3-oxocyclobutane-1-carboxylate (2.50 g, 8.83 mmol) in methylene chloride (100 mL) was added 1,1,1-trifluoro-N,N-bis(2-methoxyethyl)- λ^4 -sulfanamine (50% in toluene, 11.7 g, 26.49 mmol) at 0 °C. The mixture was allowed to stir at room temperature for about 16 h. General Workup Procedure 1 was used. The residue was purified by Chromatography A to give the title compound (2.50 g, 93%).

[476] Step 2: Synthesis of 1-(3-bromophenyl)-3,3-difluorocyclobutane-1-carbohydrazide. To a stirring solution of methyl 1-(3-bromophenyl)-3,3-difluorocyclobutane-1-carboxylate (2.50 g, 8.19 mmol) in ethanol (80 mL) was added hydrazine hydrate (8. mL, 81.94 mmol). The mixture was stirred at 80 °C for about 16 h. General Workup Procedure 1 was used. The residue was used in the next step without purification.

[477] Step 3: Synthesis of 5-[1-(3-bromophenyl)-3,3-difluorocyclobutyl]-4-methyl-1,2,4-triazole-3-thiol. To a solution of 1-(3-bromophenyl)-3,3-difluorocyclobutane-1-carbohydrazide (2.50 g, 8.19 mmol) in THF (80 mL) was added methyl isothiocyanate (0.60 g, 8.19 mmol). The mixture was stirred at 80 °C for 2 h. The solution was cooled to room temperature, and water (40 mL) and potassium hydroxide (2.30 g, 40.95 mmol) was added. The mixture was stirred at 80 °C for 36 h. General Workup Procedure 1 was used. The crude material was used in the next step.

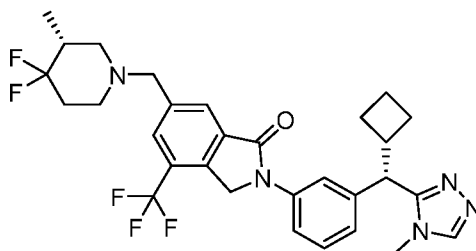
[478] Step 4: Synthesis of 3-[1-(3-bromophenyl)-3,3-difluorocyclobutyl]-4-methyl-1,2,4-triazole. To a stirring solution of 5-[1-(3-bromophenyl)-3,3-difluorocyclobutyl]-4-methyl-1,2,4-triazole-3-thiol (2.9 mg, 8.19 mmol) in methylene chloride (80 mL) and acetic acid (5 mL) was added hydrogen peroxide (1.8 mL, 16.38 mmol). The reaction mixture was stirred at room temperature for 2 h and evaporated to dryness. The residue was purified by Chromatography B to afford the title compound (1.3 g, 44% over three steps). ¹H NMR (500 MHz, Chloroform-*d*) δ 8.19 (s, 1H), 7.47 (dt, *J* = 7.9, 1.4 Hz, 1H), 7.43 (t, *J* = 1.9 Hz, 1H), 7.29 (d, *J* = 1.6 Hz, 1H), 7.27 (d, *J* = 7.9 Hz, 1H), 3.74 (dt, *J* = 14.4, 12.5 Hz, 2H), 3.38 – 3.31 (m, 2H), 3.18 (s, 3H). MS: C₁₃H₁₂BrF₂N₃ requires: 327, found: *m/z* = 328 [M+H]⁺.

Example 1: 2-(3-((*R*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



[479] A mixture of Cs_2CO_3 (639 mg, 1.96 mmol), XantPhos (77.1 mg, 0.133 mmol), **Example D** (231 mg, 0.662 mmol), **Example A** (200 mg, 0.653 mmol), and palladium acetate (15.3 mg, 0.0682 mmol) in dioxane (3.5 mL) was heated in a sealed vessel at 120 °C for 1 hour. After cooling, the reaction was diluted with water and DCM. After filtration, the product was extracted with DCM three times. The combined organic layer was dried, filtered, and concentrated. Silica gel column chromatography using a gradient of MeOH in a mixture of ethyl acetate and DCM, followed by Chromatography C gave the title compound (198 mg, 0.346 mmol, 53% yield). MS (ESI) calculated for $(\text{C}_{30}\text{H}_{32}\text{F}_5\text{N}_5\text{O})$ $[\text{M}+\text{H}]^+$, 574.6; found, 574.5. ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.07 (s, 1H), 8.02 (s, 1H), 7.93 (s, 1H), 7.90 (t, $J = 2.0$ Hz, 1H), 7.77 (dt, $J = 8.3, 1.5$ Hz, 1H), 7.41 (t, $J = 7.9$ Hz, 1H), 7.13 – 7.08 (m, 1H), 5.07 (s, 2H), 4.15 (d, $J = 10.6$ Hz, 1H), 3.74 (s, 2H), 3.43 (s, 3H), 3.33 (ddd, $J = 17.4, 15.2, 7.3$ Hz, 1H), 2.78 (dd, $J = 26.0, 10.2$ Hz, 2H), 2.40 (t, $J = 11.3$ Hz, 1H), 2.24 – 2.21 (m, 1H), 2.14 – 2.11 (m, 3H), 1.90 – 1.83 (m, 5H), 1.81 – 1.73 (m, 1H), 1.00 (d, $J = 6.3$ Hz, 3H).

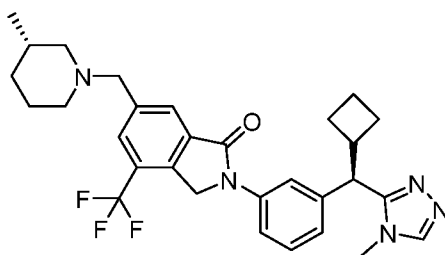
Example 2: 2-(3-((*S*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



[480] This C-N bond formation was performed in a similar manner to Example 1 using **Example B** and **Example D** to give the title compound (171 mg, 0.299 mmol, 46% yield). MS (ESI) calculated for $(\text{C}_{30}\text{H}_{32}\text{F}_5\text{N}_5\text{O})$ $[\text{M}+\text{H}]^+$, 574.6; found, 574.5. ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.07 (s, 1H), 8.02 (s, 1H), 7.93 (s, 1H), 7.90 (t, $J = 2.0$ Hz, 1H), 7.77 (dt, $J = 8.3, 1.5$ Hz, 1H), 7.41 (t, $J = 7.9$ Hz, 1H), 7.13 – 7.08 (m, 1H), 5.07 (s, 2H), 4.15 (d, $J = 10.6$

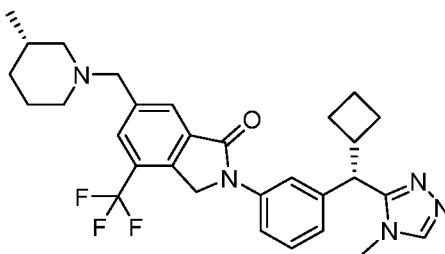
Hz, 1H), 3.74 (s, 2H), 3.43 (s, 3H), 3.33 (ddd, $J = 17.4, 15.2, 7.3$ Hz, 1H), 2.78 (dd, $J = 26.0, 10.2$ Hz, 2H), 2.40 (t, $J = 11.3$ Hz, 1H), 2.24 – 2.20 (m, 1H), 2.14 – 2.04 (m, 3H), 1.92 – 1.83 (m, 5H), 1.81 – 1.73 (m, 1H), 1.00 (d, $J = 6.3$ Hz, 3H).

Example 3: 2-(3-((*R*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(((*S*)-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



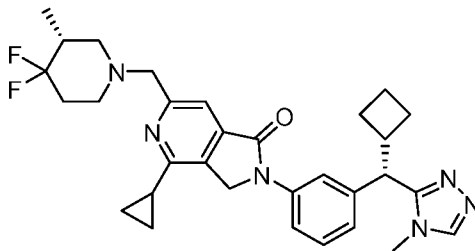
[481] This C-N bond formation was performed in a similar manner to Example 1 using **Example A** and **Example E** to give the title compound (44.8 mg, 0.0833 mmol, 38% yield). MS (ESI) calculated for (C₃₀H₃₄F₃N₅O) [M+H]⁺, 538; found, 539. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.06 (s, 1H), 7.98 (s, 1H), 7.89 (dd, $J = 4.9, 2.9$ Hz, 2H), 7.77 (dd, $J = 8.1, 2.2$ Hz, 1H), 7.40 (t, $J = 7.9$ Hz, 1H), 7.09 (d, $J = 7.7$ Hz, 1H), 5.05 (s, 2H), 4.14 (d, $J = 10.6$ Hz, 1H), 3.64 (s, 2H), 3.42 (s, 3H), 3.37 – 3.24 (m, 1H), 2.77 (t, $J = 9.6$ Hz, 2H), 2.26 – 2.18 (m, 2H), 1.91 – 1.62 (m, 9H), 1.61 – 1.50 (m, 1H), 0.99 – 0.90 (m, 1H), 0.86 (d, $J = 6.1$ Hz, 3H).

Example 4: 2-(3-((*S*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(((*S*)-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



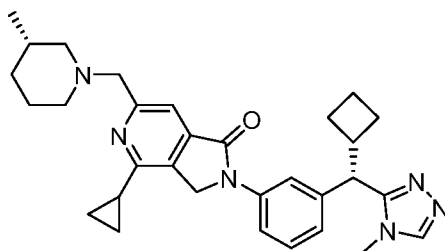
[482] This C-N bond formation was performed in a similar manner to Example 1 using **Example B** and **Example E** to give the title compound (60 mg, 38% yield MS (ESI) calculated for (C₃₀H₃₄F₃N₅O) [M+H]⁺, 538; found, 539. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.06 (s, 1H), 7.98 (s, 1H), 7.89 (dd, $J = 4.9, 2.9$ Hz, 2H), 7.77 (dd, $J = 8.1, 2.2$ Hz, 1H), 7.40 (t, $J = 7.9$ Hz, 1H), 7.09 (d, $J = 7.7$ Hz, 1H), 5.05 (s, 2H), 4.14 (d, $J = 10.6$ Hz, 1H), 3.64 (s, 2H), 3.42 (s, 3H), 3.37 – 3.24 (m, 1H), 2.77 (t, $J = 9.6$ Hz, 2H), 2.26 – 2.18 (m, 2H), 1.91 – 1.62 (m, 9H), 1.61 – 1.50 (m, 1H), 0.99 – 0.90 (m, 1H), 0.86 (d, $J = 6.1$ Hz, 3H).

Example 5: 2-(3-((*S*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-cyclopropyl-6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-1-one



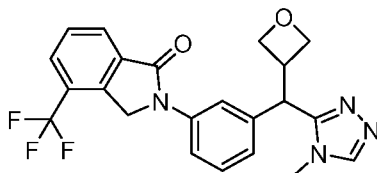
[483] This C-N bond formation was performed in a similar manner to Example 1 using **Example B** and **Example G** to give the title compound (10 mg, 12% yield). MS (ESI) calculated for (C₃₁H₃₆F₂N₆O) [M+H]⁺, 547.3; found, 547.5. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.06 (s, 1H), 7.91 (t, *J* = 2.0 Hz, 1H), 7.78 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.55 (s, 1H), 7.41 (t, *J* = 7.9 Hz, 1H), 7.13 – 7.08 (m, 1H), 5.02 (s, 2H), 4.14 (d, *J* = 10.6 Hz, 1H), 3.71 (s, 2H), 3.42 (s, 3H), 3.32 (ddd, *J* = 16.1, 11.3, 7.8 Hz, 1H), 2.80 (dd, *J* = 23.8, 10.1 Hz, 2H), 2.41 (t, *J* = 10.8 Hz, 1H), 2.05 (td, *J* = 11.0, 9.6, 4.1 Hz, 1H), 1.88 – 1.73 (m, 6H), 1.20 – 1.06 (m, 4H), 0.99 (d, *J* = 6.2 Hz, 3H).

Example 6: 2-(3-((*S*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-cyclopropyl-6-(((*S*)-3-methylpiperidin-1-yl)methyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-1-one



[484] The C-N bond formation was performed in a similar manner as step 4 of Example 1 using **Example B** and **Example H** to give the title compound (2.9 mg, 3.5% yield). MS (ESI) calculated for (C₃₁H₃₈N₆O) [M+H]⁺, 511.3; found, 511.6. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.04 (s, 1H), 7.88 (d, *J* = 2.0 Hz, 1H), 7.77 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.51 (s, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 4.99 (s, 2H), 4.12 (d, *J* = 10.6 Hz, 1H), 3.59 (s, 2H), 3.40 (s, 3H), 3.30 (dq, *J* = 12.8, 8.1 Hz, 1H), 2.76 (d, *J* = 7.9 Hz, 2H), 1.90 – 1.79 (m, 3H), 1.79 – 1.49 (m, 6H), 1.15 – 1.03 (m, 4H), 0.94 – 0.86 (m, 1H), 0.84 (d, *J* = 6.1 Hz, 3H).

Example 7: 2-(3-((4-methyl-4H-1,2,4-triazol-3-yl)(oxetan-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



[485] Step 1: Synthesis of methyl 2-(3-nitrophenyl)-2-(oxetan-3-yl)acetate. Methyl 2-(3-nitrophenyl)acetate (1.00 g, 5.14 mmol) was dissolved in DMF (6 mL) at ambient temperature in a water bath to control any exotherm. 3-Iodooxetane (450 μ L, 5.14 mmol) was added, followed by portion-wise addition of potassium *tert*-butoxide (711 mg, 6.33 mmol). The reaction was stirred for 1 hour and then a solution of saturated ammonium chloride solution, 1N HCl, and DCM were added. The product was extracted with DCM three times. The combined organic layer was dried, filtered, and concentrated. Purification by Chromatography A gave methyl 2-(3-nitrophenyl)-2-(oxetan-3-yl)acetate (227 mg, 0.904 mmol, 18% yield).

[486] Step 2: Synthesis of 2-(3-nitrophenyl)-2-(oxetan-3-yl)acetohydrazide. Methyl 2-(3-nitrophenyl)-2-(oxetan-3-yl)acetate (227 mg, 0.904 mmol) was dissolved in ethanol (1.9 mL). Hydrazine hydrate (385 μ L, 6.18 mmol) was added and the mixture was heated at 40 $^{\circ}$ C overnight. After concentrating the reaction, the product was isolated by silica gel column chromatography using a gradient of MeOH in DCM to give 2-(3-nitrophenyl)-2-(oxetan-3-yl)acetohydrazide (186 mg, 0.742 mmol, 82% yield).

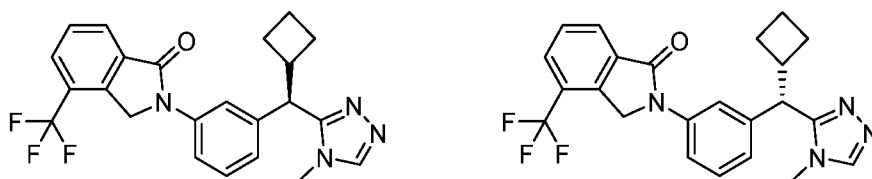
[487] Step 3: Synthesis of 4-methyl-5-((3-nitrophenyl)(oxetan-3-yl)methyl)-4H-1,2,4-triazole-3-thiol. To 2-(3-nitrophenyl)-2-(oxetan-3-yl)acetohydrazide (186 mg, 0.742 mmol) was added THF (2 mL) at ambient temperature. Methyl thioisocyanate (152 μ L, 2.22 mmol) was added and the reaction was heated to 50 $^{\circ}$ C, upon which the reaction mixture became uniform. After stirring for 2 h, the reaction was cooled and 1N NaOH (1.5 mL) was added. The reaction was heated to 50 $^{\circ}$ C for 4 h and then stirred at ambient temperature overnight. The reaction mixture was washed with DCM once, then acidified using 1N HCl. The product was extracted with DCM four times. The combined organic layer was dried, filtered and concentrated. Purification via chromatography gave 4-methyl-5-((3-nitrophenyl)(oxetan-3-yl)methyl)-4H-1,2,4-triazole-3-thiol (171 mg, 0.557 mmol, 75% yield).

[488] Step 4: Synthesis of 3-((4-methyl-4H-1,2,4-triazol-3-yl)(oxetan-3-yl)methyl)aniline. Raney nickel (slurry in water, 1.35 g) was added to 4-methyl-5-((3-nitrophenyl)(oxetan-3-yl)methyl)-4H-1,2,4-triazole-3-thiol (171 mg, 0.557 mmol) at ambient

temperature. Ethanol (4.5 mL) was added and the reaction was heated to 78 °C for 45 minutes. After cooling, the mixture was filtered and concentrated to give the title compound, which was used without purification in the next step.

[489] Step 5: Synthesis of 2-(3-((4-methyl-4*H*-1,2,4-triazol-3-yl)(oxetan-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one. This reaction was performed in a similar manner as step 7 of Example 10 to give 2-(3-((4-methyl-4*H*-1,2,4-triazol-3-yl)(oxetan-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one (38.8 mg, 32% yield). MS (ESI) calculated for (C₂₂H₁₉F₃N₄O₂) [M+H]⁺, 429; found, 429. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.36 (s, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.92 (t, *J* = 1.9 Hz, 1H), 7.85 – 7.76 (m, 2H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 7.7 Hz, 1H), 5.20 (s, 2H), 4.80 – 4.68 (m, 2H), 4.53 – 4.43 (m, 2H), 4.28 (t, *J* = 6.3 Hz, 1H), 3.93 – 3.82 (m, 1H), 3.38 (s, 3H).

Example 8 and 9: (*R*)-2-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one and (*S*)-2-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



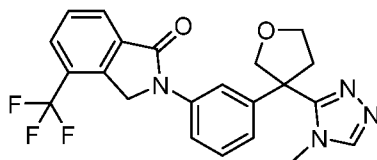
[490] Racemic 2-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one (29 mg) was synthesized following steps 1-6 for Example 7 using bromocyclobutane instead of 3-iodooxetane. This racemic mixture was separated via SFC using an IG column and MeOH and CO₂ as solvents to give (*R*)-2-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one (5.9 mg) with a longer retention time and (*S*)-2-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one (6.1 mg) with a shorter retention time.

[491] (*R*)-2-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one MS (ESI) calculated for (C₂₃H₂₁F₃N₄O) [M+H]⁺, 427; found, 427. ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.32 (s, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.92 (t, *J* = 2.0 Hz, 1H), 7.84 – 7.72 (m, 2H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.04 (d, *J* = 7.7 Hz, 1H), 5.19 (s, 2H), 4.23 (d, *J* = 10.6 Hz, 1H), 3.43 (s, 3H), 3.22 (dt, *J* = 17.9, 8.2 Hz, 1H), 2.18 – 2.04 (m, 1H), 1.90 – 1.64 (m, 5H).

[492] (*S*)-2-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one MS (ESI) calculated for (C₂₃H₂₁F₃N₄O) [M+H]⁺, 427;

found, 427. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.32 (s, 1H), 8.07 (d, $J = 7.6$ Hz, 1H), 8.03 (d, $J = 7.7$ Hz, 1H), 7.91 (d, $J = 1.9$ Hz, 1H), 7.83 – 7.73 (m, 2H), 7.38 (t, $J = 7.9$ Hz, 1H), 7.04 (d, $J = 7.7$ Hz, 1H), 5.19 (s, 2H), 4.23 (d, $J = 10.6$ Hz, 1H), 3.43 (s, 3H), 3.22 (dt, $J = 17.6, 8.6$ Hz, 1H), 2.16 – 2.05 (m, 1H), 1.89 – 1.65 (m, 5H).

Example 10: 2-(3-(3-(4-methyl-4H-1,2,4-triazol-3-yl)tetrahydrofuran-3-yl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



[493] Step 1: Synthesis of 4-(3-nitrophenyl)-3,6-dihydro-2H-pyran. Potassium phosphate hydrate (3.18 g, 15.0 mmol), 1-bromo-3-nitrobenzene (1.01 g, 4.98 mmol), 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.25 g, 5.96 mmol), and PdCl_2dppf (184 mg, 0.251 mmol) were suspended in a mixture of dioxane (14 mL) and water (1 mL). After purging with nitrogen, the mixture was heated at 90 °C overnight. After cooling, the mixture was filtered and rinsed with DCM. The crude filtrate was purified via silica gel column chromatography using a gradient of ethyl acetate in hexanes to give 4-(3-nitrophenyl)-3,6-dihydro-2H-pyran (913 mg, 4.45 mmol, 89% yield).

[494] Step 2: Synthesis of 3-(3-nitrophenyl)tetrahydrofuran-3-carbaldehyde. Sodium carbonate (130 mg, 1.23 mmol) and 4-(3-nitrophenyl)-3,6-dihydro-2H-pyran (104 mg, 0.506 mmol) were suspended in DCM (10 mL) at ambient temperature. mCPBA (77%, 255 mg, 1.14 mmol) was added and the mixture was stirred for 2 h. After addition of saturated sodium carbonate solution, DCM was evaporated and the crude 6-(3-nitrophenyl)-3,7-dioxabicyclo[4.1.0]heptane was extracted with ethyl acetate three times. After drying, filtering, and concentration, the obtained crude material was dissolved in DCM (10 mL) ambient temperature. Boron trifluoride etherate (1.0 mL, 8.1 mmol) was added and the reaction was stirred for 15 minutes. Saturated sodium bicarbonate solution was added and the product was extracted with DCM three times. After drying, filtering, and concentrating, the crude material was purified by Chromatography A to give 3-(3-nitrophenyl)tetrahydrofuran-3-carbaldehyde (59.4 mg, 0.269 mmol, 53% yield).

[495] Step 3: Synthesis of 3-(3-nitrophenyl)tetrahydrofuran-3-carboxylic acid. 3-(3-Nitrophenyl)tetrahydrofuran-3-carbaldehyde (59.4 mg, 0.269 mmol) was dissolved in *tert*-butanol (1 mL) at ambient temperature. 2-Methylbut-2-ene (0.50 mL) was added followed by

a solution of sodium chlorite (~ 80%, 110 mg, 1.21 mmol) and potassium dihydrogen phosphate (95.9 mg, 0.705 mmol) in water (1 mL). After stirring for 30 minutes, the reaction was diluted with water and DCM. The product was extracted with DCM three times. The organic layer was dried, filtered, and concentrated. Purification by Chromatography B gave 3-(3-nitrophenyl)tetrahydrofuran-3-carboxylic acid (48.5 mg, 0.204 mmol, 76% yield).

[496] Step 4: Synthesis of 4-methyl-5-(3-(3-nitrophenyl)tetrahydrofuran-3-yl)-4H-1,2,4-triazole-3-thiol. 3-(3-Nitrophenyl)tetrahydrofuran-3-carboxylic acid (48.5 mg, 0.204 mmol) and HATU (94.4 mg, 0.248 mmol) were dissolved in DMF (0.5 mL) at ambient temperature. DIEA (89 μ L, 0.51 mmol) was added and the reaction was stirred for one minute. *N*-Methylhydrazinecarbothioamide (32.1 mg, 0.305 mmol) was added and the reaction was stirred for one hour. 1N sodium hydroxide solution (0.42 mL) was added and the reaction was stirred overnight. More 1N sodium hydroxide solution (0.44 mL) was added and the reaction was heated at 50 °C overnight. 1N HCl was added and the desired product was extracted with DCM three times. The residue was dried, filtered, and concentrated. Chromatography A afforded 4-methyl-5-(3-(3-nitrophenyl)tetrahydrofuran-3-yl)-4H-1,2,4-triazole-3-thiol (33.6 mg, 0.110 mmol, 54% yield).

[497] Step 5: Synthesis of 4-methyl-3-(3-(3-nitrophenyl)tetrahydrofuran-3-yl)-4H-1,2,4-triazole. 4-methyl-5-(3-(3-nitrophenyl)tetrahydrofuran-3-yl)-4H-1,2,4-triazole-3-thiol (33.6 mg, 0.110 mmol) was dissolved in a mixture of DCM (0.4 mL) and acetic acid (168 μ L) at ambient temperature. Hydrogen peroxide solution (35%, 30 μ L, 0.34 mmol) was added and the reaction was stirred for 2 h. After evaporation of solvents, the material was diluted with DCM and water, and one pellet of potassium hydroxide was added. The product was extracted with DCM four times followed by a 4:1 mixture of dichloromethane and MeOH once. The combined organic layer was dried, filtered, and concentrated to give 4-methyl-3-(3-(3-nitrophenyl)tetrahydrofuran-3-yl)-4H-1,2,4-triazole (25.4 mg, 84% yield) which was used in the next step without purification.

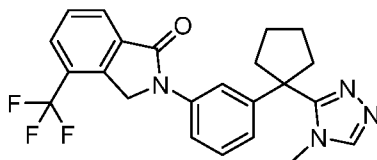
[498] Step 6: Synthesis of 3-(3-(4-methyl-4H-1,2,4-triazol-3-yl)tetrahydrofuran-3-yl)aniline. 4-Methyl-3-(3-(3-nitrophenyl)tetrahydrofuran-3-yl)-4H-1,2,4-triazole (25.4 mg, 0.0926 mmol) was dissolved in a mixture of ethyl acetate (1 mL) and MeOH (1 mL). Palladium on carbon (10%, wet, 5.7 mg) was added and the reaction was hydrogenated using a hydrogen balloon. After stirring at ambient temperature overnight, the reaction was further stirred for 8 h with a fresh hydrogen-filled balloon. After filtration and concentration, the crude 3-(3-(4-

methyl-4*H*-1,2,4-triazol-3-yl)tetrahydrofuran-3-yl)aniline was obtained, which was used directly in the next step.

[499] Step 7: Synthesis of 2-(3-(3-(4-methyl-4*H*-1,2,4-triazol-3-yl)tetrahydrofuran-3-yl)phenyl)-4-(trifluoromethyl)isoindolin-1-one.

3-(3-(4-methyl-4*H*-1,2,4-triazol-3-yl)tetrahydrofuran-3-yl)aniline (24.0 mg, 0.093 mmol) and methyl 2-(bromomethyl)-3-(trifluoromethyl)benzoate (33.0 mg, 0.111 mmol) were dissolved in acetonitrile (0.5 mL) at ambient temperature. Silver nitrate (1M in water, 120 μ L, 0.120 mmol) was added dropwise followed by triethylamine (13 μ L, 0.093 mmol). After stirring overnight, brine, saturated sodium bicarbonate solution, and DCM were added. The reaction was filtered and extracted with DCM three times. The crude material was dried, filtered, and concentrated. Purification using chromatography B, followed by reverse-phase HPLC purification provided the title compound (5.0 mg, 13% yield). MS (ESI) calculated for (C₂₂H₁₉F₃N₄O₂) [M+H]⁺, 429.4; found, 429.3. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.13 (s, 1H), 8.07 (d, *J* = 7.7 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.88 (t, *J* = 2.1 Hz, 1H), 7.79 – 7.69 (m, 2H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.03 (dt, *J* = 8.0, 1.1 Hz, 1H), 5.08 (s, 2H), 4.49 (d, *J* = 8.8 Hz, 1H), 4.33 (d, *J* = 8.8 Hz, 1H), 4.09 (td, *J* = 8.1, 6.6 Hz, 1H), 4.01 (td, *J* = 8.4, 5.7 Hz, 1H), 3.24 (s, 3H), 2.98 (ddd, *J* = 12.7, 8.3, 6.5 Hz, 1H), 2.66 (ddd, *J* = 13.2, 7.8, 5.8 Hz, 1H).

Example 11: 2-(3-(1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclopentyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



[500] Step 1: Synthesis of 1-(3-bromophenyl)-N-[(methylcarbamothioyl)amino]cyclopentane-1-carboxamide.

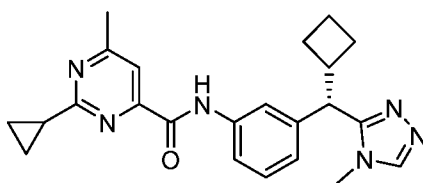
A mixture of 1-(3-bromophenyl)cyclopentane-1-carboxylic acid (2.5 g, 9.3 mmol), 1-amino-3-methylthiourea (1.3 g, 12 mmol), HOBt (1.9 g, 14 mmol), EDC (2.7 g, 14 mmol), and triethylamine (1.9 g, 19 mmol) in DMF (30 mL) was stirred at room temperature for 3 h. The reaction mixture was quenched by the addition of water. The precipitated solids were collected by filtration, washed with water, and dried under vacuum to afford the title compound (2.5 g, crude), which was used in the next step without purification. MS (ESI) calculated for (C₁₄H₁₈BrN₃OS) [M+Na]⁺, 356.3; found, 377.8.

[501] Step 2: Synthesis of 5-[1-(3-bromophenyl)cyclopentyl]-4-methyl-4H-1,2,4-triazole-3-thiol. To a stirred solution of NaOH (1M, 100.0 mL) was added 1-(3-bromophenyl)-*N*-[(methylcarbamothioyl) amino]cyclopentane-1-carboxamide (2.5 g, crude) in portions at room temperature. The mixture was stirred at 40 °C for 16 h. The reaction mixture was quenched by the addition of HCl (1 *N*) at 0 °C to pH ~ 3. The precipitated solids were collected by filtration and washed with water twice. The solids were dried under vacuum to afford the title compound (2.5 g, crude), which was used in the next step without purification. MS (ESI) calculated for (C₁₄H₁₆BrN₃S) [M+H]⁺, 338.3; found, 338.2.

[502] Step 3: Synthesis of 3-(1-(3-bromophenyl)cyclopentyl)-4-methyl-4H-1,2,4-triazole. To a stirred mixture of 5-[1-(3-bromophenyl)cyclopentyl]-4-methyl-4H-1,2,4-triazole-3-thiol (2.5 g, crude) and NaNO₂ (5.1 g, 74 mmol) was added HNO₃ (1 *N*, 75.0 mL) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was quenched by saturated NaHCO₃ aqueous solution at 0 °C and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous Na₂SO₄. After filtration and evaporation, the residue was purified by Chromatography A to afford the title compound (1.9 g, 67% over three steps).

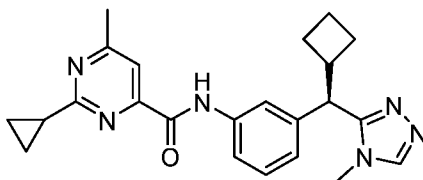
[503] Step 4: Synthesis of 2-(3-(1-(4-methyl-4H-1,2,4-triazol-3-yl)cyclopentyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one. A mixture of sodium *tert*-butoxide (38.7 mg, 0.403 mmol), 3-(1-(3-bromophenyl)cyclopentyl)-4-methyl-4H-1,2,4-triazole (50.1 mg, 0.164 mmol), 4-(trifluoromethyl)isoindolin-1-one (39.0 mg, 0.194 mmol), XantPhos (9.5 mg, 0.0164 mmol), and Pd₂(dba)₃ (10.7 mg, 0.0117 mmol) in dioxane (0.6 mL) were heated at 106 °C overnight. More XantPhos (9.7 mg, 0.017 mmol) and Pd₂(dba)₃ (10.2 mg, 0.0111 mmol) were added and the mixture was heated at 106 °C for another 24 h. The crude reaction mixture was purified by Chromatography A, followed by reverse phase HPLC to give the title compound (14.9 mg, 0.0349 mmol, 21% yield). MS (ESI) calculated for (C₂₃H₂₁F₃N₄O) [M+H]⁺, 427.4; found, 427.5. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.07 – 8.02 (m, 2H), 7.93 (dt, *J* = 7.8, 0.9 Hz, 1H), 7.86 (t, *J* = 2.0 Hz, 1H), 7.75 – 7.69 (m, 1H), 7.64 (ddd, *J* = 8.2, 2.2, 0.9 Hz, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.01 (ddd, *J* = 7.9, 1.9, 0.9 Hz, 1H), 5.05 (d, *J* = 1.5 Hz, 2H), 3.17 (s, 3H), 2.66 – 2.56 (m, 2H), 2.34 – 2.23 (m, 2H), 1.82 (tdd, *J* = 9.9, 6.3, 2.3 Hz, 4H).

Example 12: (*S*)-*N*-(3-(cyclobutyl(4-methyl-4H-1,2,4-triazol-3-yl)methyl)phenyl)-2-cyclopropyl-6-methylpyrimidine-4-carboxamide



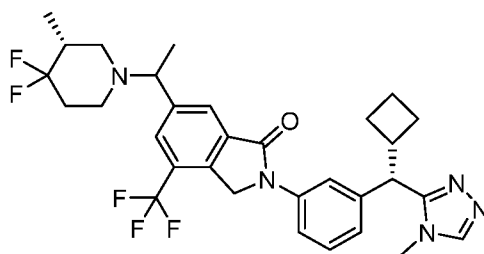
[504] To a mixture of **Example J** (24.4 mg, 0.101 mmol) and 2-cyclopropyl-6-methylpyrimidine-4-carboxylic acid (18.8 mg, 0.101 mmol) in ethyl acetate (0.5 mL) at ambient temperature was added T3P solution (1.47 M in ethyl acetate, 103 μ L, 0.151 mmol), followed by pyridine (42 μ L, 0.52 mmol). The reaction was stirred overnight. After concentration, the crude was purified by reverse phase HPLC to give (*S*)-*N*-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-2-cyclopropyl-6-methylpyrimidine-4-carboxamide (33.5 mg, 0.0832 mmol, 82% yield). MS (ESI) calculated for ($C_{23}H_{26}N_6O$) [$M+H$] $^+$, 403.5; found, 403.4. ^1H NMR (500 MHz, Acetonitrile- d_3) δ 9.93 (s, 1H), 8.06 (s, 1H), 7.76 (s, 1H), 7.73 – 7.64 (m, 2H), 7.35 (t, J = 7.8 Hz, 1H), 7.07 (dd, J = 7.7, 1.4 Hz, 1H), 4.09 (d, J = 10.6 Hz, 1H), 3.39 (s, 3H), 3.34 – 3.23 (m, 1H), 2.54 (s, 3H), 2.33 (tt, J = 8.1, 4.7 Hz, 1H), 2.26 – 2.17 (m, 1H), 1.92 – 1.69 (m, 5H), 1.26 – 1.08 (m, 4H).

Example 13: (*R*)-*N*-(3-(cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-2-cyclopropyl-6-methylpyrimidine-4-carboxamide



[505] This compound was synthesized in a similar fashion to Example 12 using **Example I** to give the title compound (34.6 mg, 0.0860 mmol, 86% yield). MS (ESI) calculated for ($C_{23}H_{26}N_6O$) [$M+H$] $^+$, 403.5; found, 403.4. ^1H NMR (500 MHz, Acetonitrile- d_3) δ 9.93 (s, 1H), 8.06 (s, 1H), 7.76 (s, 1H), 7.73 – 7.65 (m, 2H), 7.35 (t, J = 7.9 Hz, 1H), 7.07 (dt, J = 7.8, 1.3 Hz, 1H), 4.09 (d, J = 10.6 Hz, 1H), 3.39 (s, 3H), 3.28 (ddd, J = 15.2, 10.5, 7.4 Hz, 1H), 2.54 (s, 3H), 2.33 (tt, J = 8.1, 4.7 Hz, 1H), 2.25 – 2.17 (m, 1H), 1.91 – 1.71 (m, 5H), 1.17 (ddt, J = 34.6, 8.2, 2.9 Hz, 4H).

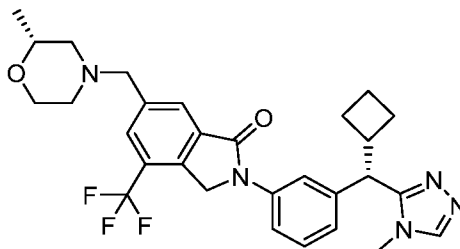
Example 14: 2-(3-((*S*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(1-((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)ethyl)-4-(trifluoromethyl)isoindolin-1-one



[506] Step 1: Synthesis of 6-(1-((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)ethyl)-4-(trifluoromethyl)isoindolin-1-one. Example AB step 4 (49.7 mg, 0.206 mmol), (*R*)-4,4-difluoro-3-methylpiperidine (101 mg, 0.586 mmol), and triethylamine (82 μ L, 0.588 mmol) in MeOH were microwaved at 100 $^{\circ}$ C for one minute. Sodium cyanoborohydride (20.3 mg, 0.323 mmol) was added and the reaction was microwaved at 80 $^{\circ}$ C for 30 minutes followed by 100 $^{\circ}$ C for 12 h. Excess reducing agent was decomposed by the addition of few drops of 1N HCl. General Workup Procedure 1 was used. The crude material was purified by Chromatography B to give the title compound.

[507] Step 2: Synthesis of 2-(3-((*S*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(1-((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)ethyl)-4-(trifluoromethyl)isoindolin-1-one. This coupling reaction was carried out in a similar fashion to Example 1 using 6-(1-((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)ethyl)-4-(trifluoromethyl)isoindolin-1-one (49.7 mg, 0.206 mmol) and **Example B** to afford the title compound (6 mg, 0.010 mmol, 5% over 2 steps). ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.04 (s, 1H), 8.01 (d, J = 2.2 Hz, 1H), 7.92 (s, 1H), 7.88 (d, J = 2.4 Hz, 1H), 7.74 (ddd, J = 8.3, 2.4, 1.0 Hz, 1H), 7.38 (t, J = 7.9 Hz, 1H), 7.07 (d, J = 7.7 Hz, 1H), 5.04 (d, J = 1.6 Hz, 2H), 4.12 (d, J = 10.7 Hz, 1H), 3.88 – 3.74 (m, 1H), 3.40 (s, 3H), 3.33 – 3.20 (m, 1H), 2.81 (m, 2H), 2.68 (m, 2H), 2.32 (dd, J = 25.6, 13.2 Hz, 1H), 2.24 – 1.98 (m, 3H), 1.92 – 1.67 (m, 6H), 1.40 (dd, J = 6.8, 1.8 Hz, 3H), 0.97 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H). MS (ESI) calculated for $\text{C}_{31}\text{H}_{34}\text{F}_5\text{N}_5\text{O}$ $[\text{M}+\text{H}]^+$, 588; found, 588.

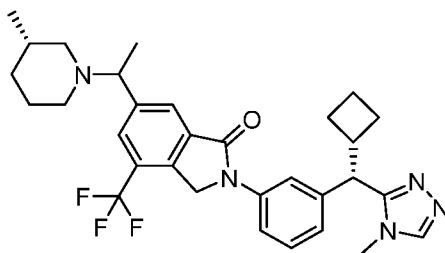
Example 15: 2-(3-((*S*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(((*R*)-2-methylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one



[508] Step 1: 2-{3-[(S)-cyclobutyl(4-methyl-1,2,4-triazol-3-yl)methyl]phenyl}-3-oxo-7-(trifluoromethyl)-1H-isoindole-5-carbaldehyde. 3-[(S)-cyclobutyl(4-methyl-1,2,4-triazol-3-yl)methyl]aniline (148 mg, 0.61 mmol) and methyl 2-(bromomethyl)-5-formyl-3-(trifluoromethyl)benzoate (203 mg, 0.63 mmol) were dissolved in acetonitrile (6 mL) and water (1 mL). Silver nitrate (140 mg, 0.81 mmol) was added followed by triethylamine (86 μ L, 0.62 mmol). General Workup Procedure 1 was used. The crude material was purified via Chromatography B to give the title compound (143 mg, 51%).

[509] Step 2: 2-{3-[(R)-cyclobutyl(4-methyl-1,2,4-triazol-3-yl)methyl]phenyl}-3-oxo-7-(trifluoromethyl)-1H-isoindole-5-carbaldehyde. Sodium triacetoxyborohydride (23 mg, 0.11 mmol) was added to a DCM (1 mL) solution of 2-{3-[(S)-cyclobutyl(4-methyl-1,2,4-triazol-3-yl)methyl]phenyl}-3-oxo-7-(trifluoromethyl)-1H-isoindole-5-carbaldehyde (30 mg, 0.07 mmol) and (2R)-2-methylmorpholine hydrochloride (18 mg, 0.15 mmol). The mixture was stirred at room temperature for 2 h. The suspension was concentrated and purified using Chromatography C to give the title compound (14.4 mg, 0.0267 mmol, 40% yield). MS (ESI) calculated for (C₂₉H₃₂F₃N₅O₂) [M+H]⁺, 540.3; found, 540.5. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.04 (s, 1H), 7.98 (d, *J* = 1.6 Hz, 1H), 7.89 (s, 1H), 7.87 (t, *J* = 2.0 Hz, 1H), 7.75 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.07 (dt, *J* = 7.9, 1.2 Hz, 1H), 5.04 (s, 2H), 4.12 (d, *J* = 10.7 Hz, 1H), 3.78 (ddd, *J* = 11.3, 3.4, 1.7 Hz, 1H), 3.65 (d, *J* = 1.9 Hz, 2H), 3.61 – 3.51 (m, 2H), 3.40 (d, *J* = 1.8 Hz, 3H), 3.35 – 3.23 (m, 1H), 2.70 (dd, *J* = 11.3, 2.1 Hz, 1H), 2.67 – 2.60 (m, 1H), 2.25 – 2.15 (m, 1H), 2.10 – 2.08 (m, 1H), 1.91 – 1.79 (m, 5H), 1.79 – 1.69 (m, 1H), 1.05 (dd, *J* = 6.2, 1.8 Hz, 3H).

Example 16: 2-(3-((S)-cyclobutyl(4-methyl-4H-1,2,4-triazol-3-yl)methyl)phenyl)-6-(1-((S)-3-methylpiperidin-1-yl)ethyl)-4-(trifluoromethyl)isoindolin-1-one

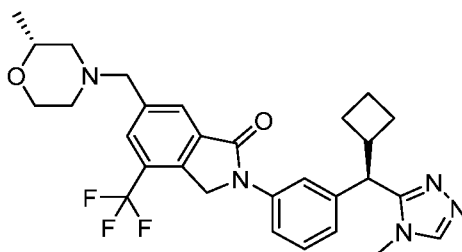


[510] Step 1: Synthesis of (S)-6-acetyl-2-(3-(cyclobutyl(4-methyl-4H-1,2,4-triazol-3-yl)methyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one. Using **Example AB** step 4 (83.1 mg, 0.342 mmol) and (S)-3-((3-bromophenyl)(cyclobutyl)methyl)-4-methyl-4H-1,2,4-triazole

(101 mg, 0.328 mmol), the C-N bond formation was performed in a similar manner to Example 1 to give the title compound (18 mg, 12% yield).

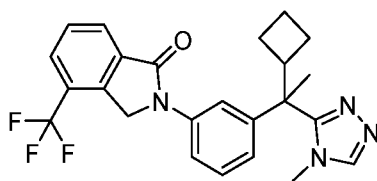
[511] Step 2: Synthesis of 2-(3-((*S*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(1-((*S*)-3-methylpiperidin-1-yl)ethyl)-4-(trifluoromethyl)isoindolin-1-one. This reductive amination reaction was performed in a similar manner to Example 14 step 1 to give the title compound (2.4 mg, 12%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.04 (s, 1H), 7.99 (s, 1H), 7.92 (s, 1H), 7.87 (t, *J* = 2.0 Hz, 1H), 7.75 (ddd, *J* = 8.2, 2.3, 0.9 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.09 – 7.04 (m, 1H), 5.04 (s, 2H), 4.12 (d, *J* = 10.6 Hz, 1H), 3.68 (m, 1H), 3.40 (s, 3H), 3.32 – 3.12 (m, 1H), 2.20 – 2.05 (m, 2H), 2.87 (m, 2H), 2.68 (m, 2H), 1.89 – 1.57 (m, 10H), 1.39 (br s, 3H), 0.86 (m, 1H), 0.86 (d, *J* = 6.0 Hz, 3H), 0.79 (d, *J* = 6.2 Hz, 3H). MS (ESI) calculated for C₃₁H₃₆F₃N₅O [M+H]⁺, 552; found, 553.

Example 17: 2-(3-((*R*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(((*R*)-2-methylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one



[512] This compound was synthesized in a similar manner to Example 15 using 2-{3-[(*R*)-cyclobutyl(4-methyl-1,2,4-triazol-3-yl)methyl]phenyl}-3-oxo-7-(trifluoromethyl)-1*H*-isoindole-5-carbaldehyde (30 mg, 0.066 mmol) and (*R*)-2-methylmorpholine (18 mg, 0.13 mmol) to give 2-(3-((*R*)-cyclobutyl(4-methyl-4*H*-1,2,4-triazol-3-yl)methyl)phenyl)-6-(((*R*)-2-methylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one (14.1 mg, 0.0261 mmol, 39% yield). MS (ESI) calculated for (C₂₉H₃₂F₃N₅O₂) [M+H]⁺, 540.3; found, 540.5. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.04 (s, 1H), 7.98 (s, 1H), 7.89 (q, *J* = 0.8 Hz, 1H), 7.87 (t, *J* = 2.0 Hz, 1H), 7.75 (ddd, *J* = 8.2, 2.3, 1.0 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.07 (dt, *J* = 7.7, 1.3 Hz, 1H), 5.04 (d, *J* = 1.5 Hz, 2H), 4.12 (d, *J* = 10.6 Hz, 1H), 3.78 (ddd, *J* = 11.4, 3.4, 1.7 Hz, 1H), 3.65 (d, *J* = 1.8 Hz, 2H), 3.61 – 3.54 (m, 2H), 3.40 (s, 3H), 3.35 – 3.22 (m, 1H), 2.70 (dt, *J* = 11.2, 2.1 Hz, 1H), 2.63 (dt, *J* = 11.4, 2.0 Hz, 1H), 2.25 – 2.16 (m, 1H), 2.11 – 2.06 (m, 1H), 1.90 – 1.79 (m, 5H), 1.79 – 1.70 (m, 1H), 1.06 (d, *J* = 6.3 Hz, 3H).

Example 18: 2-(3-(1-cyclobutyl-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)ethyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



[513] Step 1: Synthesis of methyl 2-(3-bromophenyl)-2-cyclobutylacetate. This alkylation was performed in a similar manner as step 1 of Example 7 using methyl 2-(3-bromophenyl)-2-cyclobutylacetate (493 μ L, 3.14 mmol) and bromocyclobutane (293 μ L, 3.04 mmol) to give the title compound (454 mg, 1.60 mmol, 53% yield).

[514] Step 2: Synthesis of methyl 2-(3-bromophenyl)-2-cyclobutylpropanoate. Methyl 2-(3-bromophenyl)-2-cyclobutylacetate (454 mg, 1.60 mmol) was dissolved in THF (5 mL) at 0 °C. LHMDS (1M in THF, 2.0 mL, 2.0 mmol) was added and the reaction was stirred for 30 minutes. Methyl iodide (149 μ L, 2.39 mmol) was added as a THF (0.5 mL) solution. The reaction was warmed to ambient temperature and stirred for 2 h. Water and hexanes were added and the product was extracted with hexanes three times. The combined hexanes layer was dried, filtered, and concentrated. Purification by Chromatography A afforded the title compound (293 mg, 0.986 mmol, 62% yield).

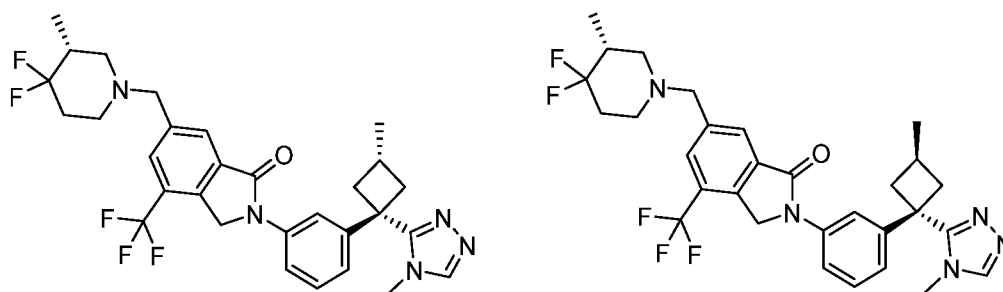
[515] Step 3: Synthesis of 2-(3-bromophenyl)-2-cyclobutylpropanoic acid. Methyl 2-(3-bromophenyl)-2-cyclobutylpropanoate (91.4 mg, 0.308 mmol) was dissolved in THF (1 mL) at ambient temperature. 1N LiOH (0.35 mL, 0.35 mmol) was added followed by MeOH (0.35 mL). The reaction was heated to 64 °C overnight. THF and MeOH were removed under reduced pressure. After neutralization with 1N HCl, the residue was extracted with DCM three times. The combined organic layers were dried, filtered, and concentrated, and the product was used without purification.

[516] Steps 4 and 5: Synthesis of 3-(1-(3-bromophenyl)-1-cyclobutylethyl)-4-methyl-4H-1,2,4-triazole. Following steps 4 and 5 for Example 10 provided the title compound (47.0 mg, 0.147 mmol, 49% yield over 2 steps).

[517] Step 6: Synthesis of 2-(3-(1-cyclobutyl-1-(4-methyl-4H-1,2,4-triazol-3-yl)ethyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one. Following step 4 of Example 11 provided the title compound (14.4 mg, 0.0327 mmol, 22% yield). MS (ESI) calculated for (C₂₄H₂₃F₃N₄O) [M+H]⁺, 441.2; found, 441.6. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.21 (s, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.78 (t, *J* = 2.0 Hz, 1H), 7.75 (t, *J* = 7.8 Hz, 1H), 7.71 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 6.95 (dt, *J* = 7.9, 1.2 Hz, 1H),

5.09 (s, 2H), 3.52 – 3.38 (m, 1H), 3.11 (s, 3H), 2.04 – 1.99 (m, 3H), 1.91 – 1.80 (m, 2H), 1.76 (s, 3H), 1.70 – 1.55 (m, 1H).

Example 19 and Example 20: 6-(((R)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-(((1s,3S)-3-methyl-1-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one (19) and 6-(((R)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-(((1r,3R)-3-methyl-1-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one (20)



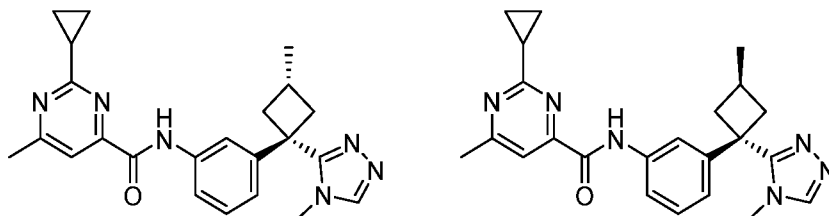
[518] To a solution of **Example D** (200 mg, 0.57 mmol), Xantphos (66 mg, 0.11 mmol), and **Example L** (176 mg, 0.57 mmol) in 1,4-dioxane (5.00 mL) was added Pd(OAc)₂ (12.89 mg, 0.06 mmol) and Cs₂CO₃ (561 mg, 1.72 mmol). The mixture was stirred at 120 °C for 1 h. The solution was filtered through celite and concentrated. The residue was purified by Chromatography C to afford 6-[[[(3R)-4,4-difluoro-3-methylpiperidin-1-yl]methyl]-2-{3-[3-methyl-1-(4-methyl-1,2,4-triazol-3-yl)cyclobutyl]phenyl}-4-(trifluoromethyl)-3H-isoindol-1-one (142 mg, 44%). The isomers were separated via SFC using an IG column with CO₂ and MeOH as mobile phase to afford peak 1 (52 mg) and peak 2 (19 mg).

[519] 6-(((R)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-(((1s,3S)-3-methyl-1-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.05 (t, *J* = 2.1 Hz, 1H), 8.02 (s, 2H), 7.93 (s, 1H), 7.88 (s, 1H), 7.70 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 7.8, 1.3 Hz, 1H), 5.09 (s, 2H), 3.74 (s, 2H), 3.26 (s, 3H), 3.22 (s, 3H), 2.95 – 2.86 (m, 2H), 2.78 (dd, *J* = 26.4, 9.4 Hz, 1H), 2.72 – 2.60 (m, 4H), 2.40 (t, *J* = 11.6 Hz, 1H), 2.12 – 2.02 (m, 2H), 1.20 – 1.11 (m, 3H), 1.00 (d, *J* = 6.4 Hz, 3H). LCMS: C₃₀H₃₂F₅N₃O requires: 573, found: *m/z* = 574 [M+H]⁺.

[520] 6-(((R)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-(((1r,3R)-3-methyl-1-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.10 (s, 1H), 8.03 (s, 1H), 7.94 (s, 1H), 7.88 (s, 1H), 7.72 – 7.65 (m, 1H), 7.48 – 7.41 (m, 1H), 7.11 – 7.05 (m, 1H), 5.08 (s, 2H), 3.74 (s, 2H), 3.26 (s, 3H), 3.20 (s, 3H), 2.78 (d, *J* = 25.0 Hz, 2H), 2.53 – 2.29 (m, 5H), 2.07 (s, 2H), 1.17 (d, *J* = 5.7

Hz, 3H), 1.01 (d, $J = 5.8$ Hz, 3H). LCMS: $C_{30}H_{32}F_5N_5O$ requires: 573, found: $m/z = 574$ $[M+H]^+$.

Example 21 and Example 22: 2-cyclopropyl-6-methyl-N-{3-[(1*r*,3*s*)-3-methyl-1-(4-methyl-1,2,4-triazol-3-yl)cyclobutyl]phenyl}pyrimidine-4-carboxamide (21) and 2-cyclopropyl-6-methyl-N-{3-[(1*s*,3*r*)-3-methyl-1-(4-methyl-1,2,4-triazol-3-yl)cyclobutyl]phenyl}pyrimidine-4-carboxamide (22)

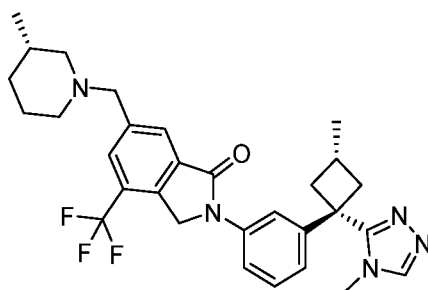


[521] To a solution of **Example L**, Xantphos (57 mg, 0.10 mmol), and **Example N** (87 mg, 0.49 mmol) in 1,4-dioxane (2 mL) was added palladium (II) acetate (11 mg, 0.05 mmol) and CS_2CO_3 (479 mg, 1.47 mmol). The mixture was stirred at 120 °C for 1 h under nitrogen. The residue was filtered through celite and concentrated. The isomers were separated by HPLC (15-98% acetonitrile in water with 0.1% trifluoroacetic acid). The compound was neutralized (PL-HCO₃ MP SPE) to afford Example 21 (42 mg, 19%) and Example 22 (14 mg, 7%).

[522] **2-cyclopropyl-6-methyl-N-{3-[(1*r*,3*s*)-3-methyl-1-(4-methyl-1,2,4-triazol-3-yl)cyclobutyl]phenyl}pyrimidine-4-carboxamide:** ^1H NMR (500 MHz, Acetonitrile- d_3) δ 9.97 (s, 1H), 8.09 (s, 1H), 7.79 – 7.75 (m, 1H), 7.75 – 7.70 (m, 1H), 7.63 (dd, $J = 2.0$ Hz, 1H), 7.38 (dd, $J = 7.9$ Hz, 1H), 7.08 – 6.98 (m, 1H), 3.23 (s, 3H), 3.19 – 3.14 (m, 2H), 2.49 – 2.42 (m, 2H), 2.36 – 2.28 (m, 2H), 1.28 – 1.20 (m, 2H), 1.19 – 1.10 (m, 8H). LCMS: $C_{23}H_{26}N_6O$ requires: 402, found: $m/z = 403$ $[M+H]^+$.

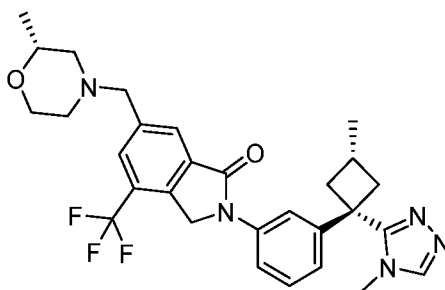
[523] **2-cyclopropyl-6-methyl-N-{3-[(1*s*,3*r*)-3-methyl-1-(4-methyl-1,2,4-triazol-3-yl)cyclobutyl]phenyl}pyrimidine-4-carboxamide.** ^1H NMR (500 MHz, Acetonitrile- d_3) δ 9.99 (s, 1H), 8.02 (s, 1H), 7.85 – 7.73 (m, 3H), 7.41 (dd, $J = 7.9$ Hz, 1H), 7.17 (d, $J = 7.7$, 1.4 Hz, 1H), 3.19 (s, 3H), 2.93 – 2.84 (m, 2H), 2.66 – 2.61 (m, 2H), 2.35 (tt, $J = 8.1$, 4.7 Hz, 1H), 2.13 (s, 1H), 1.21 (dt, $J = 4.7$, 3.0 Hz, 2H), 1.19 – 1.10 (m, 8H). LCMS: $C_{23}H_{26}N_6O$ requires: 402, found: $m/z = 403$ $[M+H]^+$.

Example 23: 2-(3-((1*s*,3*R*)-3-methyl-1-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-6-(((*S*)-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



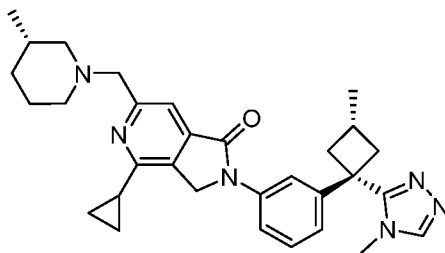
[524] To a solution of **Example M** (385 mg, 1.26 mmol), Xantphos (145 mg, 0.25 mmol), and **Example E** (392.71 mg, 1.26 mmol) in 1,4-dioxane (10.00 mL) were added $\text{Pd}(\text{OAc})_2$ (28.23 mg, 0.13 mmol) and Cs_2CO_3 (1.23 g, 3.77 mmol). The mixture was stirred at 120 °C for 1 h under nitrogen. The residue was filtered through celite and concentrated. The residue was purified by Chromatography B followed by Chromatography C to afford the title compound (250 mg, 37%). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.30 (s, 1H), 8.08 (d, $J = 2.1$ Hz, 1H), 7.98 (s, 1H), 7.92 (s, 1H), 7.69 (dd, $J = 8.2, 2.1$ Hz, 1H), 7.45 (t, $J = 8.0$ Hz, 1H), 7.13 (d, $J = 7.8$ Hz, 1H), 5.21 (s, 2H), 3.66 (s, 2H), 3.23 (s, 3H), 2.91 – 2.83 (m, 2H), 2.73 (t, $J = 11.0$ Hz, 2H), 2.61 – 2.54 (m, 3H), 1.95 (t, $J = 11.1$ Hz, 1H), 1.71 – 1.60 (m, 4H), 1.54 – 1.44 (m, 1H), 1.10 (d, $J = 5.1$ Hz, 3H), 0.94 – 0.85 (m, 1H), 0.83 (d, $J = 6.0$ Hz, 3H). LCMS: $\text{C}_{30}\text{H}_{34}\text{F}_3\text{N}_5\text{O}$ requires: 537, found: $m/z = 538$ $[\text{M}+\text{H}]^+$.

Example 24: 2-(3-((1*S*,3*S*)-3-methyl-1-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-6-(((*R*)-2-methylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one



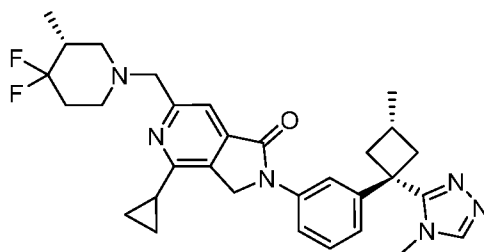
[525] This coupling reaction was carried out in a similar fashion as Example 23 using **Example M** (50 mg, 0.16 mmol) and **Example O** (56 mg, 0.16 mmol) as reactants to afford the title compound (10 mg, 11%). ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.07 – 8.01 (m, 2H), 7.94 (s, 1H), 7.74 – 7.67 (m, 1H), 7.47 (dd, $J = 8.0$ Hz, 1H), 7.40 – 7.37 (m, 1H), 7.21 (d, $J = 7.8$ Hz, 1H), 5.09 (s, 2H), 3.81 (d, $J = 11.5$ Hz, 1H), 3.66 (d, $J = 34.3$ Hz, 6H), 3.22 (s, 3H), 2.96 – 2.83 (m, 3H), 2.70 – 2.58 (m, 4H), 1.18 – 1.13 (m, 3H), 1.09 (d, $J = 6.2$ Hz, 3H). LCMS: $\text{C}_{29}\text{H}_{32}\text{F}_3\text{N}_5\text{O}_2$ requires: 539, found: $m/z = 540$ $[\text{M}+\text{H}]^+$.

Example 25: 4-cyclopropyl-2-(3-((1*s*,3*R*)-3-methyl-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-6-(((*S*)-3-methylpiperidin-1-yl)methyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-1-one



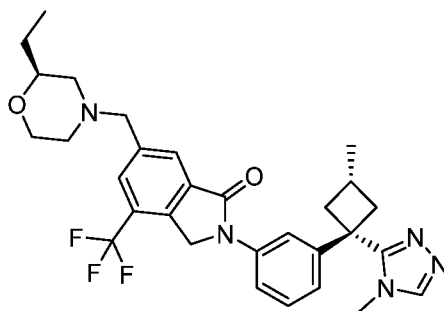
[526] This coupling reaction was carried out in a similar fashion as for Example 23 using **Example M** (50 mg, 0.16 mmol) and **Example H** (47mg, 0.16mmol) as reactants to afford the title compound (10 mg, 11%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.03 (d, *J* = 2.1 Hz, 2H), 7.78 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.55 (s, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.25 – 7.18 (m, 1H), 5.06 (s, 2H), 3.82 (s, 2H), 3.23 (s, 3H), 3.01 – 2.85 (m, 3H), 2.71 – 2.61 (m, 4H), 1.82 – 1.62 (m, 6H), 1.21 – 1.09 (m, 7H), 0.90 (d, *J* = 6.4 Hz, 4H). LCMS: C₃₁H₃₈N₆O requires: 510, found: *m/z* = 511 [M+H]⁺.

Example 26: 4-cyclopropyl-6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-((1*s*,3*S*)-3-methyl-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-1-one



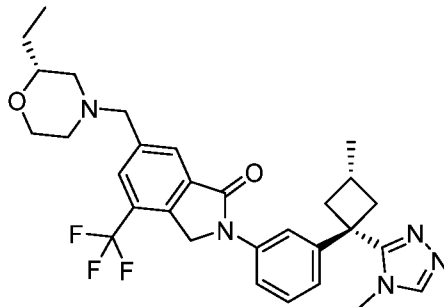
[527] This coupling reaction was carried out in a similar fashion as Example 23 using **Example M** (50 mg, 0.16 mmol) and **Example G** (52 mg, 0.16 mmol) as reactants to afford the title compound (15 mg, 16%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.05 (dd, *J* = 2.1 Hz, 1H), 8.03 (s, 1H), 7.81 – 7.74 (m, 1H), 7.56 (s, 1H), 7.48 (dd, *J* = 8.0 Hz, 1H), 7.25 – 7.19 (m, 1H), 5.05 (s, 2H), 3.72 (s, 2H), 3.23 (s, 3H), 2.95 – 2.75 (m, 6H), 2.66 (d, *J* = 7.3 Hz, 4H), 2.42 (t, *J* = 11.7 Hz, 2H), 2.15 – 2.02 (m, 2H), 1.19 – 1.06 (m, 5H), 1.00 (d, *J* = 6.2 Hz, 4H). LCMS: C₃₁H₃₆F₂N₆O requires: 546, found: *m/z* = 547 [M+H]⁺.

Example 27: 6-(((*S*)-2-ethylmorpholino)methyl)-2-(3-((1*s*,3*R*)-3-methyl-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



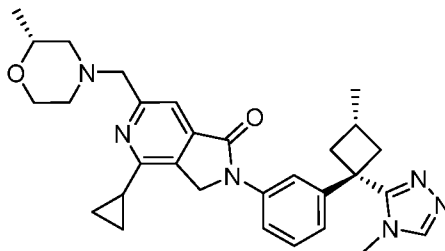
[528] This coupling reaction was carried out in a similar fashion as Example 23 using **Example M** (50 mg, 0.16 mmol) and **Example P** (54 mg, 0.16 mmol) as reactants to afford the title compound (25 mg, 24%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.05 (dd, *J* = 2.0 Hz, 1H), 8.03 (s, 2H), 7.94 (s, 1H), 7.72 – 7.68 (m, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.21 (dt, *J* = 7.9, 1.2 Hz, 1H), 5.09 (s, 2H), 3.83 (d, *J* = 11.2 Hz, 2H), 3.71 (s, 2H), 3.61 (t, *J* = 11.4 Hz, 2H), 3.38 (d, *J* = 23.7 Hz, 1H), 3.22 (s, 3H), 2.97 – 2.85 (m, 2H), 2.78 (d, *J* = 10.6 Hz, 1H), 2.72 – 2.61 (m, 4H), 1.53 – 1.35 (m, 2H), 1.17 – 1.14 (m, 3H), 0.91 (t, *J* = 7.5 Hz, 3H). LCMS: C₃₀H₃₄F₃N₅O₂ requires: 553, found: *m/z* = 554 [M+H]⁺.

Example 28: 6-(((*R*)-2-ethylmorpholino)methyl)-2-(3-((1*S*,3*S*)-3-methyl-1-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



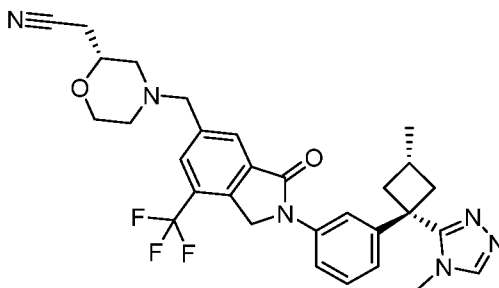
[529] This coupling reaction was carried out in a similar fashion as Example 23 using **Example M** (50 mg, 0.16 mmol) and **Example Q** (54 mg, 0.16 mmol) as reactants to afford the title compound (16 mg, 17%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.05 (dd, *J* = 2.1 Hz, 1H), 8.02 (d, *J* = 4.3 Hz, 2H), 7.93 (s, 1H), 7.74 – 7.69 (m, 1H), 7.47 (dd, *J* = 8.0 Hz, 1H), 7.21 (dd, *J* = 7.7, 1.2 Hz, 1H), 5.08 (d, *J* = 1.4 Hz, 2H), 3.86 – 3.79 (m, 2H), 3.68 (s, 2H), 3.65 – 3.55 (m, 2H), 3.44 – 3.34 (m, 1H), 3.22 (s, 3H), 2.95 – 2.85 (m, 2H), 2.71 – 2.58 (m, 4H), 1.51 – 1.36 (m, 2H), 1.15 (d, *J* = 5.6 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 4H). LCMS: C₃₀H₃₄F₃N₅O₂ requires: 553, found: *m/z* = 554 [M+H]⁺.

Example 29: 4-cyclopropyl-2-(3-((1*S*,3*S*)-3-methyl-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-6-(((*R*)-2-methylmorpholino)methyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-1-one



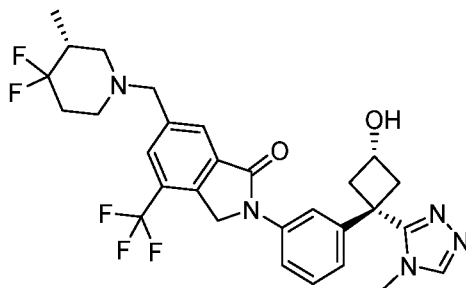
[530] This coupling reaction was carried out in a similar fashion as for Example 23 using **Example M** (51 mg, 0.17 mmol) and **Example R** (48 mg, 0.17 mmol) as reactants to afford the title compound (9 mg, 10%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.05 – 8.04 (m, 1H), 8.03 (s, 1H), 7.81 – 7.75 (m, 1H), 7.55 (s, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.24 – 7.18 (m, 1H), 5.05 (s, 2H), 3.85 – 3.77 (m, 2H), 3.65 (s, 2H), 3.64 – 3.58 (m, 2H), 3.23 (s, 3H), 2.95 – 2.86 (m, 4H), 2.76 (dd, *J* = 11.3, 2.1 Hz, 1H), 2.73 – 2.61 (m, 4H), 1.19 – 0.98 (m, 10H). LCMS: C₃₀H₃₆N₆O₂ requires: 512, found: *m/z* = 513 [M+H]⁺.

Example 30: 2-((*R*)-4-((2-(3-((1*S*,3*S*)-3-methyl-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-3-oxo-7-(trifluoromethyl)isoindolin-5-yl)methyl)morpholin-2-yl)acetonitrile



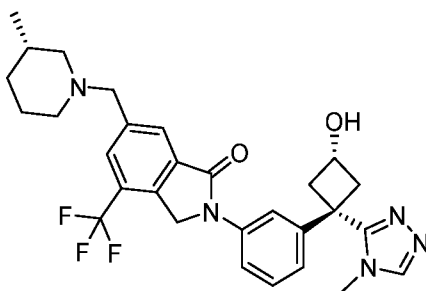
[531] This coupling reaction was carried out in a similar fashion as Example 23 using **Example M** (50 mg, 0.16 mmol) and **Example S** (55 mg, 0.16 mmol) as reactants to afford the title compound (14 mg, 13%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.04 (d, *J* = 2.1 Hz, 1H), 8.03 (s, 2H), 7.93 (s, 1H), 7.70 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.47 (dd, *J* = 8.0 Hz, 1H), 7.24 – 7.19 (m, 1H), 5.09 (s, 2H), 3.94 – 3.87 (m, 1H), 3.85 – 3.77 (m, 1H), 3.77 – 3.64 (m, 3H), 3.22 (s, 3H), 2.96 – 2.78 (m, 3H), 2.75 – 2.53 (m, 6H), 2.27 (t, *J* = 11.3, 3.4 Hz, 1H), 2.12 – 2.03 (m, 1H), 1.14 (d, *J* = 11.6, 5.4 Hz, 3H). LCMS: C₃₀H₃₁F₃N₆O₂ requires: 564, found: *m/z* = 565 [M+H]⁺.

Example 31: 6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-((1*S*,3*S*)-3-hydroxy-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



[532] To a solution of **Example T** (50 mg, 0.16 mmol), Xantphos (18 mg, 0.03 mmol), and **Example D** (56 mg, 0.16 mmol) in 1,4-dioxane (1 mL) were added Pd(OAc)₂ (4 mg, 0.02 mmol) and Cs₂CO₃ (160 mg, 0.49 mmol). The mixture was stirred at 120 °C for 1 h under nitrogen. The residue was filtered through Celite and concentrated. The residue was purified by preparative HPLC with acetonitrile in water to afford the title compound (5 mg, 5.0%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.02 (dd, *J* = 13.7, 3.3 Hz, 3H), 7.93 (s, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.46 (dd, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 5.09 (s, 2H), 4.45 (q, *J* = 7.4 Hz, 1H), 3.74 (s, 2H), 3.37 (d, *J* = 6.9 Hz, 1H), 3.23 (s, 3H), 3.18 – 3.07 (m, 2H), 2.88 (dd, *J* = 11.5, 9.1 Hz, 2H), 2.85 – 2.71 (m, 4H), 2.41 (d, *J* = 12.3 Hz, 1H), 2.10 – 2.04 (m, 2H), 1.00 (d, *J* = 6.3 Hz, 3H). LCMS: C₂₉H₃₀F₅N₅O₂ requires: 575, found: *m/z* = 576 [M+H]⁺.

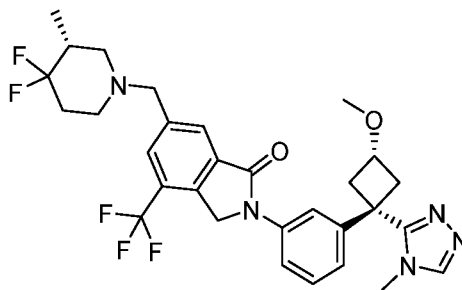
Example 32: 2-(3-((1*S*,3*R*)-3-hydroxy-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-6-(((*S*)-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



[533] This coupling reaction was carried out in a similar fashion as Example 31 using **Example T** (50 mg, 0.16 mmol) and **Example E** (50 mg, 0.16 mmol) as reactants to afford the title compound (8 mg, 9%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.03 (s, 1H), 8.00 (t, *J* = 2.0 Hz, 2H), 7.92 (s, 1H), 7.71 (ddd, *J* = 8.2, 2.2, 0.9 Hz, 1H), 7.46 (dd, *J* = 8.0 Hz, 1H), 7.17 (ddd, *J* = 7.9, 1.9, 0.9 Hz, 1H), 5.08 (d, *J* = 1.6 Hz, 2H), 4.45 (s, 1H), 3.66 (s, 2H), 3.38 (d, *J* = 9.5

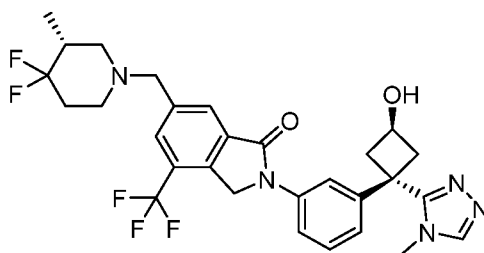
Hz, 1H), 3.23 (s, 3H), 3.12 (ddt, $J = 9.8, 7.3, 2.7$ Hz, 2H), 2.88 (ddd, $J = 9.7, 8.0, 2.8$ Hz, 2H), 2.79 (s, 2H), 1.78 – 1.52 (m, 7H), 0.87 (d, $J = 6.2$ Hz, 3H). LCMS: $C_{29}H_{32}F_3N_5O_2$ requires: 539, found: $m/z = 540$ $[M+H]^+$.

Example 33: 6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-((1*S*,3*S*)-3-methoxy-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



[534] To a solution of **Example U** (50 mg, 0.16 mmol), Xantphos (18 mg, 0.03 mmol), and **Example D** (56 mg, 0.16 mmol) in 1,4-dioxane (1 mL) were added $Pd(OAc)_2$ (4 mg, 0.02 mmol) and Cs_2CO_3 (160 mg, 0.49 mmol). The mixture was stirred at 120 °C for 1 h under nitrogen. The residue was filtered through Celite and concentrated. The residue was purified by preparative HPLC with acetonitrile in water to afford the title compound (4 mg, 4%). ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.04 (s, 2H), 8.00 (t, $J = 2.1$ Hz, 1H), 7.96 (s, 1H), 7.75 – 7.68 (m, 1H), 7.47 (dd, $J = 8.0$ Hz, 1H), 7.24 – 7.17 (m, 1H), 5.09 (s, 2H), 4.16 (q, $J = 7.5$ Hz, 1H), 3.77 (s, 2H), 3.26 (s, 3H), 3.24 (s, 3H), 3.18 – 3.08 (m, 2H), 3.00 – 2.72 (m, 7H), 2.52 – 2.38 (m, 2H), 1.00 (d, $J = 6.3$ Hz, 3H). LCMS: $C_{30}H_{32}F_5N_5O_2$ requires: 589, found: $m/z = 590$ $[M+H]^+$.

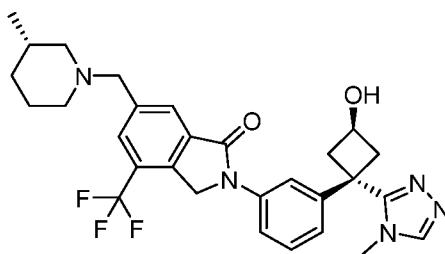
Example 34: 6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-(*trans*-3-hydroxy-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



[535] This coupling reaction was carried out in a similar fashion as Example 31 using **Example D** (57 mg, 0.16 mmol) and **Example V** (51 mg, 0.16 mmol) to give the title

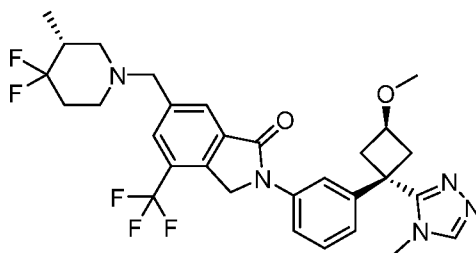
compound (26 mg, 28% yield). MS (ESI) calculated for (C₂₉H₃₀F₅N₅O₂) [M+H]⁺, 576.3; found, 576.5. ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.27 (s, 2H), 8.10 (s, 1H), 7.84 (t, *J* = 2.0 Hz, 1H), 7.67 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 5.09 (s, 2H), 4.20 (p, *J* = 7.4 Hz, 2H), 3.36 (ddt, *J* = 9.5, 7.1, 3.0 Hz, 2H), 3.23 (s, 3H), 3.18 – 3.06 (m, 1H), 3.03 – 2.95 (m, 1H), 2.72 – 2.59 (m, 1H), 2.60 – 2.49 (m, 2H), 2.26 – 2.04 (m, 4H), 1.01 (d, *J* = 5.9 Hz, 4H).

Example 35: 2-(3-((1*r*,3*S*)-3-hydroxy-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-6-(((*S*)-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



[536] This coupling reaction was carried out in a similar fashion as Example 31 using **Example E** (85 mg, 0.27 mmol) and **Example V** (67 mg, 0.22 mmol) to give the title compound (26 mg, 28% yield). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.10 (s, 1H), 7.99 (s, 1H), 7.91 (s, 1H), 7.88 (t, *J* = 2.0 Hz, 1H), 7.70 (ddd, *J* = 8.2, 2.3, 0.9 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.07 (ddd, *J* = 7.8, 1.9, 0.9 Hz, 1H), 5.07 (d, *J* = 1.6 Hz, 2H), 4.23 (p, *J* = 7.5 Hz, 1H), 3.64 (s, 2H), 3.47 – 3.33 (m, 3H), 3.24 (s, 3H), 2.77 (t, *J* = 9.4 Hz, 2H), 2.64 – 2.46 (m, 2H), 1.76 – 1.62 (m, 5H), 1.62 – 1.50 (m, 1H), 1.00 – 0.90 (m, 1H), 0.87 (d, *J* = 6.1 Hz, 3H). LCMS: C₂₉H₃₂F₃N₅O₂ requires 539.3, found 540.5 [M+H]⁺.

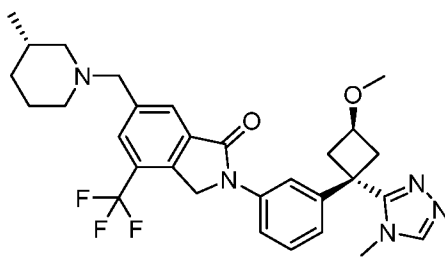
Example 36: 6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-2-(3-(*trans*-3-methoxy-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-4-(trifluoromethyl)isoindolin-1-one



[537] This coupling reaction was carried out in a similar fashion as Example 33 using **Example D** (57 mg, 0.16 mmol) and **Example W** (53 mg, 0.16 mmol) to give the title compound (43 mg, 46% yield). MS (ESI) calculated for (C₃₀H₃₂F₅N₅O₂) [M+H]⁺, 590.3; found,

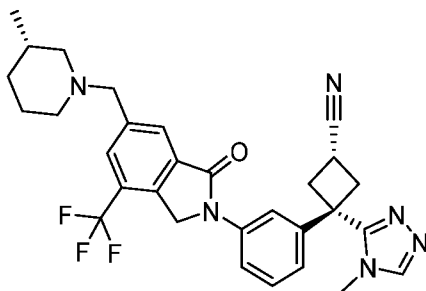
590.5. ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.19 (s, 2H), 8.09 (s, 1H), 7.85 (t, $J = 2.0$ Hz, 1H), 7.70 – 7.66 (m, 1H), 7.43 (t, $J = 8.0$ Hz, 1H), 7.04 (dt, $J = 7.7, 1.2$ Hz, 1H), 5.08 (s, 2H), 4.06 (s, 3H), 3.92 (p, $J = 7.1$ Hz, 1H), 3.37 (ddt, $J = 9.5, 6.9, 2.6$ Hz, 2H), 3.23 (s, 3H), 3.22 (s, 3H), 3.17 – 2.91 (br s, 4H), 2.55 (ddt, $J = 9.9, 7.4, 2.6$ Hz, 2H), 1.00 (d, $J = 6.1$ Hz, 3H).

Example 37: 2-(3-((1*r*,3*S*)-3-methoxy-1-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)phenyl)-6-(((*S*)-3-methylpiperidin-1-yl)methyl)-4-(trifluoromethyl)isoindolin-1-one



[538] This coupling reaction was carried out in a similar fashion as Example 33 using **Example E** (61 mg, 0.19 mmol) and **Example W** (50 mg, 0.16 mmol) to give the title compound (430 mg, 34 % yield). ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.11 (s, 1H), 7.99 (s, 1H), 7.91 (s, 1H), 7.88 (t, $J = 2.1$ Hz, 1H), 7.71 (ddd, $J = 8.2, 2.2, 0.9$ Hz, 1H), 7.45 (t, $J = 8.0$ Hz, 1H), 7.05 (ddd, $J = 7.8, 1.8, 0.9$ Hz, 1H), 5.07 (s, 2H), 3.95 (p, $J = 7.2$ Hz, 1H), 3.64 (s, 2H), 3.39 (ddd, $J = 11.6, 5.8, 2.5$ Hz, 2H), 3.26 (s, 3H), 3.25 (s, 3H), 2.77 (t, $J = 9.4$ Hz, 2H), 2.61 – 2.51 (m, 2H), 1.75 – 1.62 (m, 4H), 1.60 – 1.51 (m, 2H), 0.93 (q, $J = 13.4, 12.5$ Hz, 1H), 0.87 (d, $J = 6.1$ Hz, 3H). LCMS: $\text{C}_{30}\text{H}_{34}\text{F}_3\text{N}_5\text{O}_2$ requires 553.3, found 554.5 $[\text{M}+\text{H}]^+$.

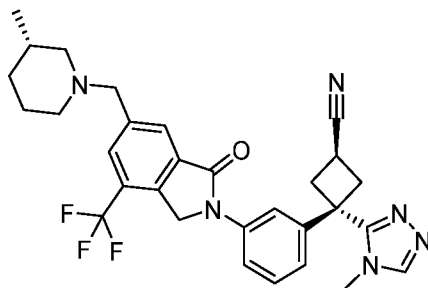
Example 38: (1*R*,3*s*)-3-(4-methyl-4*H*-1,2,4-triazol-3-yl)-3-(3-(6-(((*S*)-3-methylpiperidin-1-yl)methyl)-1-oxo-4-(trifluoromethyl)isoindolin-2-yl)phenyl)cyclobutane-1-carbonitrile



[539] To a solution of **Example X** (50 mg, 0.16 mmol), Xantphos (18 mg, 0.03 mmol), and **Example E** (49 mg, 0.16 mmol) in 1,4-dioxane (1 mL) was added $\text{Pd}(\text{OAc})_2$ (4 mg, 0.02 mmol) and Cs_2CO_3 (160 mg, 0.49 mmol). The mixture was stirred at 120 °C for 1 h under nitrogen.

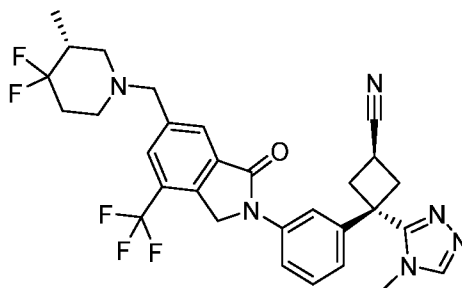
The residue was filtered through Celite and concentrated. The residue was purified by preparative HPLC with acetonitrile in water to afford the title compound (5 mg, 4%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.09 (d, *J* = 4.0 Hz, 1H), 8.00 (s, 1H), 7.95 (dd, *J* = 2.1 Hz, 1H), 7.92 (s, 1H), 7.79 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.53 – 7.49 (m, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 5.09 (s, 2H), 3.64 (s, 2H), 3.52 – 3.31 (m, 4H), 3.27 – 3.21 (m, 3H), 3.21 – 3.08 (m, 4H), 2.77 (dd, *J* = 9.6 Hz, 2H), 1.76 – 1.51 (m, 3H), 0.87 (d, *J* = 6.1 Hz, 4H). LCMS: C₃₀H₃₁F₃N₆O requires: 548, found: *m/z* = 549 [M+H]⁺.

Example 39: (1*S*,3*r*)-3-(4-methyl-4H-1,2,4-triazol-3-yl)-3-(3-(6-(((*S*)-3-methylpiperidin-1-yl)methyl)-1-oxo-4-(trifluoromethyl)isoindolin-2-yl)phenyl)cyclobutane-1-carbonitrile



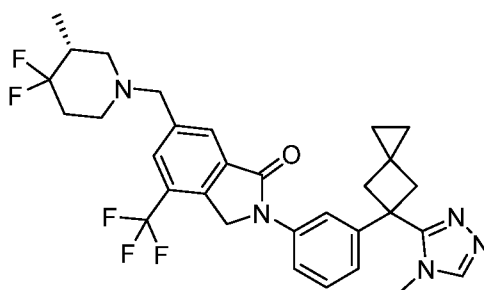
[540] This coupling reaction was carried out in a similar fashion as Example 38 using **Example Y** (50 mg, 0.16 mmol) and **Example E** (50 mg, 0.16 mmol) as reactants to afford the title compound (15 mg, 16%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.14 (s, 1H), 7.99 (s, 1H), 7.92 (s, 1H), 7.87 (dd, *J* = 2.1 Hz, 1H), 7.84 – 7.76 (m, 1H), 7.48 (dd, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 7.8, 1.2 Hz, 1H), 5.09 (s, 2H), 3.64 (s, 2H), 3.49 – 3.41 (m, 2H), 3.38 – 3.29 (m, 1H), 3.22 (s, 4H), 3.08 (td, *J* = 9.2, 2.4 Hz, 2H), 2.77 (dd, *J* = 9.6 Hz, 3H), 1.77 – 1.51 (m, 6H), 0.87 (d, *J* = 6.2 Hz, 4H). LCMS: C₃₀H₃₁F₃N₆O requires: 548, found: *m/z* = 549 [M+H]⁺.

Example 40: (1*R*,3*r*)-3-(3-(6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-1-oxo-4-(trifluoromethyl)isoindolin-2-yl)phenyl)-3-(4-methyl-4H-1,2,4-triazol-3-yl)cyclobutane-1-carbonitrile



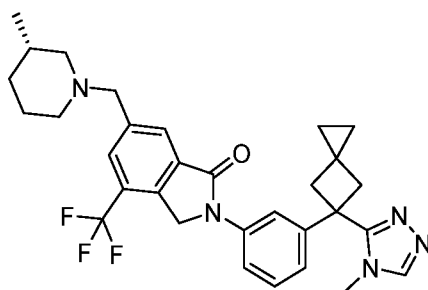
[541] This coupling reaction was carried out in a similar fashion as Example 38 using **Example Y** (50 mg, 0.16 mmol) and **Example D** (55 mg, 0.16 mmol) as reactants to afford the title compound (11 mg, 10%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.23 (s, 2H), 8.15 (s, 1H), 7.87 (t, *J* = 2.0 Hz, 1H), 7.80 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.13 – 7.08 (m, 1H), 5.14 (s, 2H), 4.16 (s, 3H), 3.50 – 3.30 (m, 2H), 3.23 (s, 3H), 3.21 – 3.04 (m, 3H), 2.75 – 2.42 (m, 6H), 1.03 (d, *J* = 6.0 Hz, 3H). LCMS: C₃₀H₂₉F₅N₆O requires: 584, found: *m/z* = 585 [M+H]⁺.

Example 41: 6-[[[(3*R*)-4,4-difluoro-3-methyl-1-piperidyl]methyl]-2-[3-[5-(4-methyl-1,2,4-triazol-3-yl)spiro[2.3]hexan-5-yl]phenyl]-4-(trifluoromethyl)isoindolin-1-one



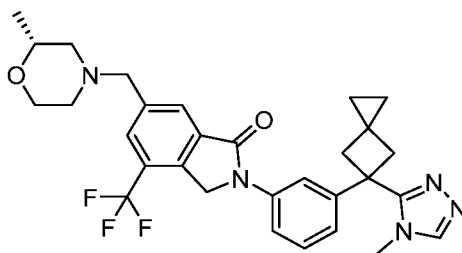
[542] A suspension of **Example D** (104 mg, 0.300 mmol), **Example Z** (105 mg, 0.33 mmol), tris(dibenzylideneacetone)dipalladium(0) (13.7 mg, 0.015 mmol), Xantphos (17 mg, 0.03 mmol), and Cs₂CO₃ (293 mg, 0.9 mmol) in anhydrous 1,4-dioxane (3 mL) was sparged with N₂ for 15 min and then the mixture was stirred at 110 °C for 1 h. The mixture was cooled to room temperature, diluted with DCM, and then silica gel was added. The mixture was concentrated under reduced pressure. The material was purified by Chromatography B and was further purified by preparative HPLC to afford the title compound (89 mg, 51%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.40 (s, 1H), 8.08 (t, *J* = 2.0 Hz, 1H), 8.01 (s, 1H), 7.94 (s, 1H), 7.69 (ddd, *J* = 8.2, 2.2, 0.9 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.03 (ddd, *J* = 7.7, 1.9, 0.9 Hz, 1H), 5.21 (s, 2H), 3.79 – 3.72 (m, 2H), 3.24 (s, 3H), 3.20 (d, *J* = 12.9 Hz, 2H), 2.80 – 2.69 (m, 4H), 2.35 – 2.28 (m, 1H), 2.16 – 1.91 (m, 4H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.65 – 0.55 (m, 2H), 0.52 – 0.44 (m, 2H). MS: C₃₁H₃₂F₅N₅O requires: 585, found: *m/z* = 586 [M+H]⁺.

Example 42: 6-[[[(3*S*)-3-methyl-1-piperidyl]methyl]-2-[3-[5-(4-methyl-1,2,4-triazol-3-yl)spiro[2.3]hexan-5-yl]phenyl]-4-(trifluoromethyl)isoindolin-1-one



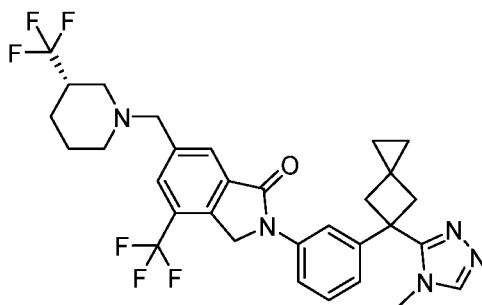
[543] This coupling reaction was carried out in a similar fashion as Example 42 using **Example E** (93 mg, 0.30 mmol) and **Example Z** (105 mg, 0.33 mmol) as reactants to afford the title compound as a formate salt (49 mg, 27%). ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.20 (s, 1H), 8.09 (t, $J = 2.0$ Hz, 1H), 7.98 (s, 1H), 7.92 (s, 1H), 7.70 (ddd, $J = 8.2, 2.2, 0.9$ Hz, 1H), 7.45 (t, $J = 8.0$ Hz, 1H), 7.03 (ddd, $J = 7.9, 1.9, 0.9$ Hz, 1H), 5.21 (s, 2H), 3.66 (s, 2H), 3.25 (s, 3H), 3.20 (d, $J = 12.9$ Hz, 2H), 2.80 – 2.69 (m, 4H), 1.94 (td, $J = 11.0, 2.6$ Hz, 1H), 1.73 – 1.55 (m, 5H), 1.54 – 1.43 (m, 1H), 0.92 – 0.84 (m, 1H), 0.83 (d, $J = 6.3$ Hz, 3H), 0.64 – 0.56 (m, 2H), 0.54 – 0.44 (m, 2H). MS: $\text{C}_{31}\text{H}_{34}\text{F}_3\text{N}_5\text{O}$ requires: 549, found: $m/z = 550$ $[\text{M}+\text{H}]^+$.

Example 43: (*R*)-2-(3-(5-(4-methyl-4*H*-1,2,4-triazol-3-yl)spiro[2.3]hexan-5-yl)phenyl)-6-((2-methylmorpholino)methyl)-4-(trifluoromethyl)isoindolin-1-one



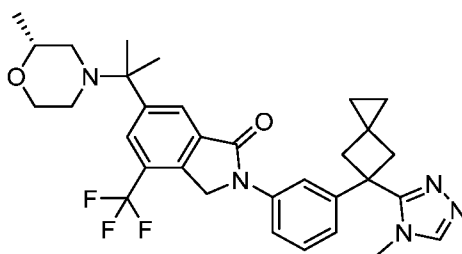
[544] To a solution of **Example Z** (51 mg, 0.16 mmol), Xantphos (18 mg, 0.03 mmol), and **Example O** (51 mg, 0.16 mmol) in 1,4-dioxane (1 mL) were added $\text{Pd}(\text{OAc})_2$ (3 mg, 0.02 mmol) and Cs_2CO_3 (160 mg, 0.49 mmol). The mixture was stirred at 120 °C for 1 h under nitrogen. General Workup Procedure 1 was used. The residue was purified by preparative HPLC with acetonitrile in water to afford the title compound (37 mg, 42%). ^1H NMR (500 MHz, Acetonitrile- d_3) δ 8.13 (br s, 2H), 8.10 (s, 1H), 8.02 (t, $J = 2.1$ Hz, 1H), 7.69 – 7.65 (m, 1H), 7.44 (t, $J = 8.0$ Hz, 1H), 7.13 (dt, $J = 7.8, 1.3$ Hz, 1H), 5.08 (s, 2H), 4.29 – 3.45 (m, 5H), 3.84 (s, 4H), 3.30 – 3.17 (m, 5H), 3.04 – 2.72 (m, 2H), 1.09 (d, $J = 6.3$ Hz, 3H), 0.67 – 0.57 (m, 2H), 0.57 – 0.45 (m, 2H). MS (ESI) calculated for $(\text{C}_{30}\text{H}_{32}\text{F}_3\text{N}_5\text{O}_2)$ $[\text{M}+\text{H}]^+$, 552; found, 552.

Example 44: (*S*)-2-(3-(5-(4-methyl-4*H*-1,2,4-triazol-3-yl)spiro[2.3]hexan-5-yl)phenyl)-4-(trifluoromethyl)-6-((3-(trifluoromethyl)piperidin-1-yl)methyl)isoindolin-1-one



[545] This coupling reaction was carried out in a similar fashion as Example 43 using **Example AA** (63 mg, 0.17 mmol) and **Example Z** (50 mg, 0.16 mmol) as reactants to afford the title compound as a formate salt (32 mg, 33%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.10 (s, 1H), 8.02 (d, *J* = 2.0 Hz, 1H), 7.98 (s, 1H), 7.90 (s, 1H), 7.68 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 1H), 5.06 (s, 2H), 3.70 (s, 2H), 3.24 (d, *J* = 8.2 Hz, 2H), 3.23 (s, 3H), 3.04 – 2.93 (m, 1H), 2.81 (d, *J* = 11.5 Hz, 1H), 2.77 (d, *J* = 12.1 Hz, 2H), 2.46 (q, *J* = 13.9, 12.1 Hz, 1H), 2.11 – 2.00 (m, 3H), 1.75 (d, *J* = 13.7 Hz, 1H), 1.64 – 1.51 (m, 1H), 1.32 (qd, *J* = 12.5, 4.3 Hz, 1H), 0.61 (dd, *J* = 9.7, 6.6 Hz, 2H), 0.51 (dd, *J* = 9.3, 6.4 Hz, 2H). MS (ESI) calculated for (C₃₁H₃₁F₆N₅O) [M+H]⁺, 604; found, 604.

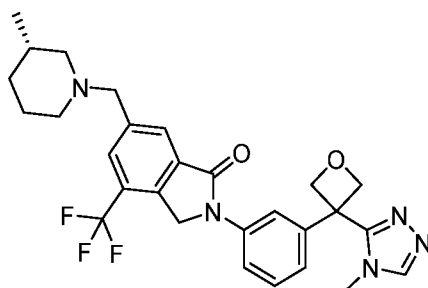
Example 45: (*R*)-2-(3-(5-(4-methyl-4*H*-1,2,4-triazol-3-yl)spiro[2.3]hexan-5-yl)phenyl)-6-(2-(2-methylmorpholino)propan-2-yl)-4-(trifluoromethyl)isoindolin-1-one



[546] This coupling reaction was carried out in a similar fashion as Example 43 using **Example AB** (53 mg, 0.16 mmol) and **Example Z** (50 mg, 0.16 mmol) as reactants to afford the title compound as a formate salt (26 mg, 24%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.13 (s, 2H), 8.08 (s, 1H), 8.00 (d, *J* = 2.1 Hz, 1H), 7.64 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.08 (dd, *J* = 7.8, 1.7 Hz, 1H), 5.03 (s, 2H), 3.73 (dt, *J* = 11.0, 2.4 Hz, 1H), 3.58 – 3.46 (m, 2H), 3.24 – 3.16 (m, 5H), 2.77 – 2.69 (m, 2H), 2.54 (dt, *J* = 11.2, 2.3 Hz, 1H), 2.47 (dt, *J* = 11.3, 2.2 Hz, 1H), 2.27 (td, *J* = 11.2, 3.1 Hz, 1H), 1.37 (s, 3H), 1.37 (s, 3H), 0.99 (d, *J*

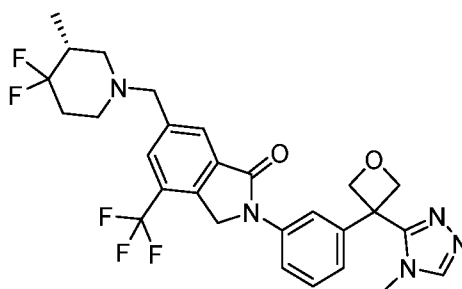
= 6.2 Hz, 3H), 0.58 (dd, J = 9.5, 6.4 Hz, 2H), 0.47 (dd, J = 9.4, 6.4 Hz, 2H). MS (ESI) calculated for (C₃₂H₃₆F₃N₅O₂) [M+H]⁺, 580; found, 581.

Example 46: 6-[[[(3*S*)-3-methyl-1-piperidyl]methyl]-2-[3-[3-(4-methyl-1,2,4-triazol-3-yl)oxetan-3-yl]phenyl]-4-(trifluoromethyl)isoindolin-1-one



[547] A suspension of **Example AC** (78 mg, 0.25 mmol), **Example E** (80 mg, 0.27 mmol), tris(dibenzylideneacetone)dipalladium(0) (11 mg, 0.012 mmol), Xantphos (14.5 mg, 0.025 mmol), and Cs₂CO₃ (244 mg, 0.75 mmol) in anhydrous 1,4-dioxane (2.5 mL) was sparged with N₂ for 15 min and then the mixture was stirred at 110 °C for 17 h. The mixture was cooled to rt, diluted with DCM, and then silica gel was added. The mixture was concentrated under reduced pressure. The material was purified by Chromatography B and was further purified by preparative HPLC to afford the title compound (39 mg, 30%). ¹H NMR (500 MHz, CDCl₃) δ 8.50 (s, 1H), 8.00 – 7.95 (m, 2H), 7.92 (s, 1H), 7.79 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.10 (ddd, J = 7.9, 2.0, 0.9 Hz, 1H), 5.40 (d, J = 6.1 Hz, 2H), 5.21 (s, 2H), 5.11 (d, J = 6.1 Hz, 2H), 3.65 (s, 2H), 3.27 (s, 3H), 2.77 – 2.67 (m, 2H), 1.93 (td, J = 11.3, 2.8 Hz, 1H), 1.70 – 1.55 (m, 4H), 1.54 – 1.42 (m, 1H), 0.92 – 0.83 (m, 1H), 0.82 (d, J = 6.3 Hz, 3H). MS: C₂₈H₃₀F₃N₅O₂ requires: 525, found: m/z = 526 [M+H]⁺.

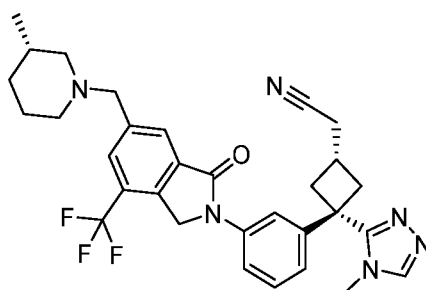
Example 47: 6-[[[(3*R*)-4,4-difluoro-3-methyl-1-piperidyl]methyl]-2-[3-[3-(4-methyl-1,2,4-triazol-3-yl)oxetan-3-yl]phenyl]-4-(trifluoromethyl)isoindolin-1-one



[548] This coupling reaction was carried out in a similar fashion as Example 46 using **Example D** (69 mg, 0.20 mmol) and **Example AC** (64 mg, 0.22 mmol) as reactants to afford

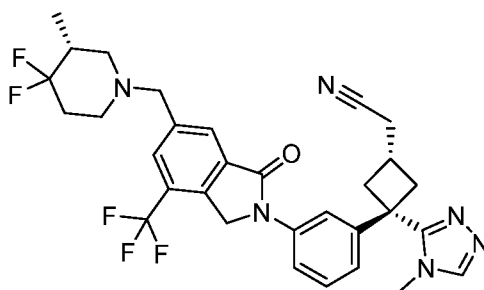
the title compound (74 mg, 66%). ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.50 (s, 1H), 8.01 (s, 1H), 7.97 (t, $J = 2.0$ Hz, 1H), 7.95 (s, 1H), 7.79 (ddd, $J = 8.2, 2.2, 0.9$ Hz, 1H), 7.51 (t, $J = 8.0$ Hz, 1H), 7.11 (ddd, $J = 7.9, 2.0, 0.9$ Hz, 1H), 5.40 (d, $J = 6.1$ Hz, 2H), 5.22 (s, 2H), 5.11 (d, $J = 6.1$ Hz, 2H), 3.82 – 3.69 (m, 2H), 3.27 (s, 3H), 2.83 – 2.67 (m, 2H), 2.32 (t, $J = 10.1$ Hz, 1H), 2.19 – 1.88 (m, 4H), 0.93 (d, $J = 6.6$ Hz, 3H). MS: $\text{C}_{28}\text{H}_{28}\text{F}_5\text{N}_5\text{O}_2$ requires: 561, found: $m/z = 562$ $[\text{M}+\text{H}]^+$.

Example 48: 2-((1*R*,3*s*)-3-(4-methyl-4*H*-1,2,4-triazol-3-yl)-3-(3-(6-(((*S*)-3-methylpiperidin-1-yl)methyl)-1-oxo-4-(trifluoromethyl)isoindolin-2-yl)phenyl)cyclobutyl)acetonitrile



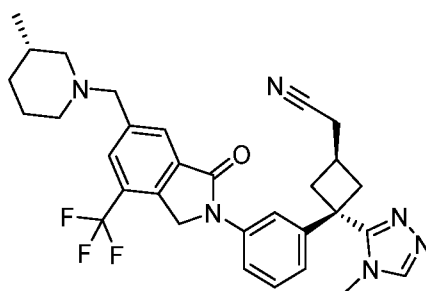
[549] To a mixture of **Example AD** (78 mg, 0.25 mmol), **Example E** (91 mg, 0.28 mmol), tris(dibenzylideneacetone)dipalladium(0) (11 mg, 13 μmol), Xantphos (15 mg, 25 μmol), and Cs_2CO_3 (244 mg, 0.750 mmol) was added degassed dioxane (2.5 mL). The mixture was then heated to 110 $^\circ\text{C}$ for 18 h. The volatiles were evaporated under reduced pressure and the material was purified by Chromatography B followed by preparative HPLC to afford the title compound (36 mg, 26%). ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.41 (s, 1H), 7.97 (s, 1H), 7.92 (s, 1H), 7.87 (t, $J = 1.8$ Hz, 1H), 7.70 (dd, $J = 8.0, 1.5$ Hz, 1H), 7.45 (t, $J = 8.0$ Hz, 1H), 7.04 (d, $J = 7.9$ Hz, 1H), 5.19 (s, 2H), 3.65 (s, 2H), 3.28 (s, 3H), 3.20 – 3.10 (m, 2H), 2.74 (d, $J = 6.4$ Hz, 2H), 2.73 – 2.67 (m, 2H), 2.64 – 2.53 (m, 1H), 2.50 – 2.45 (m, 2H), 1.94 (td, $J = 11.7, 2.6$ Hz, 1H), 1.72 – 1.56 (m, 4H), 1.54 – 1.41 (m, 1H), 0.91 – 0.84 (m, 1H), 0.82 (d, $J = 6.2$ Hz, 3H). MS: $\text{C}_{31}\text{H}_{33}\text{F}_3\text{N}_6\text{O}$ requires: 562, found: $m/z = 563$ $[\text{M}+\text{H}]^+$.

Example 49: 2-((1*S*,3*s*)-3-(3-(6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-1-oxo-4-(trifluoromethyl)isoindolin-2-yl)phenyl)-3-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile



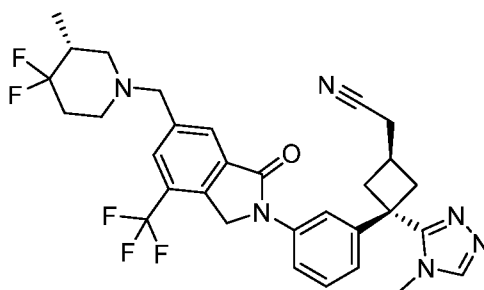
[550] This coupling reaction was carried out in a similar fashion to Example 48 using **Example AD** (78 mg, 0.22 mmol) and **Example D** (82 mg, 0.25 mmol) as reactants to afford the title compound (53 mg, 40%). ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.41 (s, 1H), 8.01 (s, 1H), 7.95 (s, 1H), 7.86 (t, $J = 1.9$ Hz, 1H), 7.70 (dd, $J = 7.9, 1.6$ Hz, 1H), 7.45 (t, $J = 8.0$ Hz, 1H), 7.04 (d, $J = 7.7$ Hz, 1H), 5.20 (s, 2H), 3.81 – 3.69 (m, 2H), 3.28 (s, 3H), 3.19 – 3.13 (m, 2H), 2.80 – 2.70 (m, 4H), 2.63 – 2.54 (m, 1H), 2.50 – 2.44 (m, 2H), 2.36 – 2.28 (m, 1H), 2.18 – 1.88 (m, 4H), 0.93 (d, $J = 6.6$ Hz, 3H). MS: $\text{C}_{31}\text{H}_{31}\text{F}_5\text{N}_6\text{O}$ requires: 598, found: $m/z = 599$ $[\text{M}+\text{H}]^+$.

Example 50: 2-((1*S*,3*r*)-3-(4-methyl-4*H*-1,2,4-triazol-3-yl)-3-(3-(6-(((*S*)-3-methylpiperidin-1-yl)methyl)-1-oxo-4-(trifluoromethyl)isoindolin-2-yl)phenyl)cyclobutyl)acetonitrile



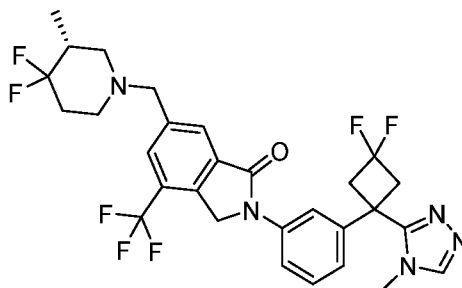
[551] This coupling reaction was carried out in a similar fashion to Example 48 using **Example AE** (78 mg, 0.22 mmol) and **Example E** (91 mg, 0.28 mmol) as reactants to afford the title compound (34 mg, 24%). ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.32 (s, 1H), 8.06 (t, $J = 1.9$ Hz, 1H), 7.98 (s, 1H), 7.92 (s, 1H), 7.72 (ddd, $J = 8.0, 2.0, 0.6$ Hz, 1H), 7.47 (t, $J = 8.0$ Hz, 1H), 7.17 (dd, $J = 7.8, 0.9$ Hz, 1H), 5.21 (s, 2H), 3.66 (s, 2H), 3.25 (s, 3H), 2.96 – 2.86 (m, 2H), 2.84 – 2.67 (m, 7H), 1.94 (t, $J = 10.0$ Hz, 1H), 1.70 – 1.55 (m, 4H), 1.53 – 1.42 (m, 1H), 0.92 – 0.84 (m, 1H), 0.83 (d, $J = 6.2$ Hz, 3H). MS: $\text{C}_{31}\text{H}_{33}\text{F}_3\text{N}_6\text{O}$ requires: 562, found: $m/z = 563$ $[\text{M}+\text{H}]^+$.

Example 51: 2-((1*R*,3*r*)-3-(3-(6-(((*R*)-4,4-difluoro-3-methylpiperidin-1-yl)methyl)-1-oxo-4-(trifluoromethyl)isoindolin-2-yl)phenyl)-3-(4-methyl-4*H*-1,2,4-triazol-3-yl)cyclobutyl)acetonitrile



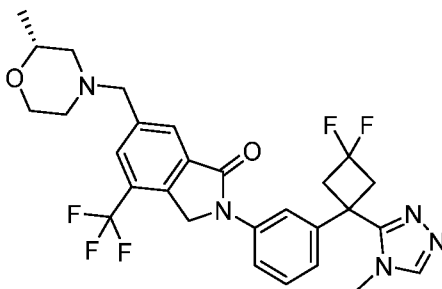
[552] This coupling reaction was carried out in a similar fashion to Example 48 using **Example AE** (78 mg, 0.22 mmol) and **Example D** (82 mg, 0.25 mmol) as reactants to afford the title compound (65 mg, 48%). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.27 (s, 1H), 8.00 (t, J = 1.9 Hz, 1H), 7.96 (s, 1H), 7.90 (s, 1H), 7.67 (dd, J = 8.2, 1.4 Hz, 1H), 7.42 (t, J = 8.0 Hz, 1H), 7.13 (ddd, J = 7.7, 1.7, 0.8 Hz, 1H), 5.17 (s, 2H), 3.79 – 3.65 (m, 2H), 3.19 (s, 3H), 2.93 – 2.81 (m, 2H), 2.77 – 2.61 (m, 7H), 2.27 (t, J = 10.6 Hz, 1H), 2.15 – 1.82 (m, 4H), 0.88 (d, J = 6.5 Hz, 3H). MS: $\text{C}_{31}\text{H}_{31}\text{F}_5\text{N}_6\text{O}$ requires: 598, found: m/z = 599 $[\text{M}+\text{H}]^+$.

Example 52: 2-{3-[3,3-difluoro-1-(4-methyl-1,2,4-triazol-3-yl)cyclobutyl]phenyl}-6-[(3*R*)-4,4-difluoro-3-methylpiperidin-1-yl]methyl}-4-(trifluoromethyl)-3H-isoindol-1-one



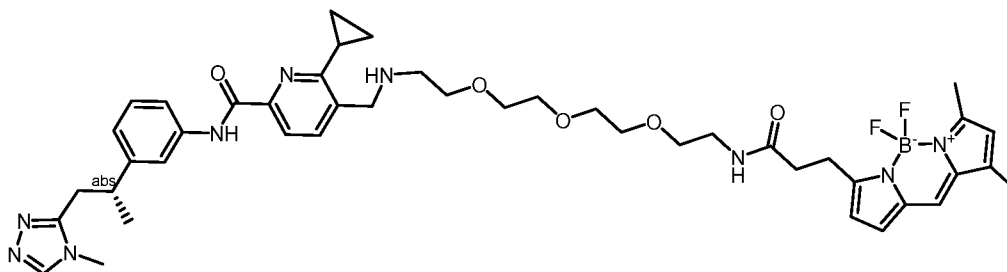
[553] To a solution of **Example AF** (100 mg, 0.30 mmol), Xantphos (35 mg, 0.06 mmol), and **Example D** (106.14 mg, 0.30 mmol) in 1,4-dioxane (1 mL) were added $\text{Pd}(\text{OAc})_2$ (7 mg, 0.03 mmol) and Cs_2CO_3 (0.30 g, 0.91 mmol). The mixture was stirred at 120 °C for 1 h under nitrogen. The residue was filtered through Celite and concentrated. The residue was purified by Chromatography B followed by Chromatography C to afford the title compound (9 mg, 55%). ^1H NMR (500 MHz, $\text{Acetonitrile-}d_3$) δ 8.13 (s, 1H), 8.03 (s, 1H), 8.00 (s, 1H), 7.94 (s, 1H), 7.75 (dd, J = 8.1, 2.2 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.18 (dd, J = 7.7, 1.8 Hz, 1H), 5.09 (s, 2H), 3.83 – 3.68 (m, 4H), 3.42 (td, J = 14.3, 11.0 Hz, 2H), 3.30 (s, 3H), 2.78 (dd, J = 26.0, 10.1 Hz, 2H), 2.40 (t, J = 11.1 Hz, 2H), 2.14 – 1.99 (m, 3H), 1.00 (d, J = 6.2 Hz, 3H). MS: $\text{C}_{29}\text{H}_{28}\text{F}_7\text{N}_5\text{O}$ requires: 595, found: m/z = 596 $[\text{M}+\text{H}]^+$.

**Example 53: 2-{3-[3,3-difluoro-1-(4-methyl-1,2,4-triazol-3-yl)cyclobutyl]phenyl}-6-
{[(2R)-2-methylmorpholin-4-yl]methyl}-4-(trifluoromethyl)-3H-isoindol-1-one**



[554] This coupling reaction was carried out in a similar fashion to Example 52 using **Example AF** (100 mg, 0.30 mmol) and **Example O** (106 mg, 0.30 mmol) as reactants to afford the title compound (9 mg, 55%). ¹H NMR (500 MHz, Acetonitrile-*d*₃) δ 8.13 (s, 1H), 8.03 (s, 1H), 7.99 (t, *J* = 2.1 Hz, 1H), 7.95 (s, 1H), 7.76 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.50 (dd, *J* = 8.0 Hz, 1H), 7.18 (dd, *J* = 7.9, 1.8 Hz, 1H), 5.09 (s, 2H), 3.90 – 3.54 (m, 6H), 3.50 – 3.36 (m, 2H), 3.30 (s, 3H), 2.87 – 2.57 (m, 2H), 2.27 – 2.07 (m, 3H), 1.11 (dd, *J* = 15.3, 6.2 Hz, 3H). MS: C₂₈H₂₈F₅N₅O₂ requires: 561, found: *m/z* = 562 [M+H]⁺.

**Example 54: (R)-6-cyclopropyl-5-(17-(5,5-difluoro-7,9-dimethyl-5H-5λ⁴,6λ⁴-
dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)-15-oxo-5,8,11-trioxa-2,14-
diazahexadecyl)-N-(3-(1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-
yl)phenyl)picolinamide**



[555] **Step 1: Synthesis of methyl 6-cyclopropyl-5-(hydroxymethyl)pyridine-2-carboxylate.** A mixture of methyl 6-chloro-5-(hydroxymethyl)pyridine-2-carboxylate (Gangadasu, B. et al., *Tetrahedron* 2006, 62, 8398-8403) (1.0 g, 5.0 mmol), potassium cyclopropyltrifluoroboranuide (2.1 g, 14.1 mmol), Pd(dppf)Cl₂ (770 mg, 1.05 mmol), and K₃PO₄ (3.8 g, 18.1 mmol) in toluene (40 mL) and water (4 mL) was heated to 100 °C for 16 h under nitrogen. The mixture was cooled to rt and then filtered. The filtrate was evaporated under vacuum. The residue was purified by Chromatography A to afford the title compound (834.0 mg, 81%). LCMS: C₁₁H₁₃NO₃ requires 207.2, found 207.9 [M+H]⁺.

[556] Step 2: Synthesis of 6-cyclopropyl-5-(hydroxymethyl)pyridine-2-carboxylic acid. A mixture of methyl 6-cyclopropyl-5-(hydroxymethyl)pyridine-2-carboxylate (170.0 mg, 0.82 mmol) and LiOH (45.0 mg, 1.88 mmol) in THF (6 mL) and water (2 mL) was stirred at rt for 3 h. The pH of the mixture was adjusted to ~5 with HCl (1 N). The mixture was evaporated under vacuum to afford the title compound (200.0 mg, crude), which was used without purification. MS (ESI) calculated for (C₁₀H₁₁NO₃) [M+H]⁺, 194.1, found, 193.9.

[557] Step 3: Synthesis of 6-cyclopropyl-5-(hydroxymethyl)-N-[3-[(2R)-1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl]phenyl]pyridine-2-carboxamide. To a mixture of 6-cyclopropyl-5-(hydroxymethyl)pyridine-2-carboxylic acid (200.0 mg, crude) in DMF (3 mL) was added DIEA (1 mL, 6.05 mmol), 3-[(2R)-1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl]aniline (173.6 mg, 0.80 mmol), and HATU (883.0 mg, 2.32 mmol). The mixture was stirred at rt for 2 h. The mixture was purified by Chromatography C, then purified by Prep-HPLC to afford the title compound (31.6 mg, 10%). MS (ESI) calculated for (C₂₂H₂₅N₅O₂) [M+H]⁺, 392.2, found, 392.2.

[558] Step 4: Synthesis of (R)-6-cyclopropyl-5-formyl-N-(3-(1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl)phenyl)picolinamide. To a solution of (R)-6-cyclopropyl-5-(hydroxymethyl)-N-(3-(1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl)phenyl)picolinamide (3.1 g, 7.9 mmol) in methylene chloride (30 mL) was added Dess-Martin reagent (4.0 g, 9.5 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, and then quenched by the addition of saturated aqueous NaHCO₃. The aqueous phase was extracted with EtOAc. The organic layers were combined, washed with brine, dried, and filtered. The filtrate was concentrated. The residue was purified by Chromatography B to afford the title compound (1.8 g, 58%). MS (ESI) calculated for (C₂₂H₂₃N₅O₂) [M+H]⁺, 390.2; found 390.2.

[559] Step 5. Synthesis of (R)-5-(13-amino-5,8,11-trioxa-2-azatridecyl)-6-cyclopropyl-N-(3-(1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl)phenyl)picolinamide. Sodium triacetoxyborohydride (0.05 g, 0.23 mmol) was added to a DCM (1.00 mL) solution containing *tert*-butyl N-(2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethyl)carbamate (45 mg, 0.15 mmol) and (R)-6-cyclopropyl-5-formyl-N-(3-(1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl)phenyl)picolinamide (60 mg, 0.15 mmol). The mixture was stirred at room temperature for 3 h. After concentration, the crude reaction mixture was purified by reverse phase preparative HPLC (Waters 5 mM CSH C18 column, 50x50 mm), eluting with acetonitrile in water with 0.1% TFA. The desired fractions were combined and concentrated to give *tert*-butyl

(*R*)-(1-(2-cyclopropyl-6-((3-(1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl)phenyl)carbamoyl)pyridin-3-yl)-5,8,11-trioxa-2-azatridecan-13-yl)carbamate, which was treated with a DCM/TFA 1:1 solution at room temperature. After 1 h the reaction was concentrated to afford the title compound (51 mg). LCMS: $C_{30}H_{43}N_7O_4$ requires $m/z = 565$, found 566 $[M+H]^+$.

[560] Step 6. Synthesis of (*R*)-6-cyclopropyl-5-(17-(5,5-difluoro-7,9-dimethyl-5H-5 λ^4 ,6 λ^4 -dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)-15-oxo-5,8,11-trioxa-2,14-diazaheptadecyl)-N-(3-(1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl)phenyl)picolinamide. Triethylamine (0.01 mL, 6.08 mg, 0.06 mmol) was added to a DMF solution (1 mL) containing HATU (17 mg, 0.05 mmol) and 3-(5,5-difluoro-7,9-dimethyl-5H-5 λ^4 ,6 λ^4 -dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-3-yl)propanoic acid (9 mg, 0.03 mmol). After stirring for 5 min at room temperature, 5-(13-amino-5,8,11-trioxa-2-azatridecan-1-yl)-6-cyclopropyl-N-{3-[(2*R*)-1-(4-methyl-4H-1,2,4-triazol-3-yl)propan-2-yl]phenyl}pyridine-2-carboxamide (17 mg, 0.03 mmol) was added, and the resulting solution was stirred at room temperature for 4 h. The crude reaction mixture was purified by reverse phase preparative HPLC, eluting with acetonitrile in water with 0.1% TFA, to afford the title compound. ^1H NMR (500 MHz, Methanol- d_4) δ 8.00 (s, 2H), 7.67 (t, $J = 2.0$ Hz, 1H), 7.55 – 7.45 (m, 1H), 7.38 (s, 1H), 7.33 (t, $J = 7.9$ Hz, 1H), 7.05 (d, $J = 7.7$ Hz, 1H), 6.97 (d, $J = 4.0$ Hz, 1H), 4.57 (s, 2H), 3.82 (dd, $J = 5.7, 4.2$ Hz, 2H), 3.68 (hd, $J = 3.9, 2.6$ Hz, 4H), 3.65 – 3.62 (m, 3H), 3.61 (s, 3H), 3.60 – 3.56 (m, 2H), 3.48 (t, $J = 5.6$ Hz, 3H), 3.39 (q, $J = 6.2, 5.6$ Hz, 3H), 3.34 (s, 4H), 3.18 (t, $J = 7.8$ Hz, 3H), 2.59 (t, $J = 7.7$ Hz, 2H), 2.47 (s, 3H), 2.35 (tt, $J = 8.3, 4.7$ Hz, 1H), 2.24 (s, 3H), 1.46 (d, $J = 6.7$ Hz, 3H), 1.37 – 1.28 (m, 2H), 1.19 (dt, $J = 8.2, 3.3$ Hz, 3H). LCMS: $C_{44}H_{56}BF_2N_9O_5$ requires $m/z = 840$, found 841 $[M+H]^+$.

BIOLOGICAL EXAMPLES

[561] The following abbreviations apply: ACT (adoptive cell therapy); AUC (area under curve); Cmpd (compound); CP (cell proliferation); E/T (Effector:Target cell ratio); ID (identification); MFI (mean fluorescence intensity); mpk (milligram per kilogram); PBMC (peripheral blood mononuclear cells); TIL (tumor infiltrating lymphocyte); Ub (ubiquitin).

BIOLOGICAL EXAMPLE 1: Evaluation of Cbl-b Inhibition by Candidate Inhibitors

[562] Candidate compounds were evaluated for their ability to bind and inhibit Cbl-b, an E3 ubiquitin-protein ligase, as evidenced by their ability to displace a fluorophore-labeled probe (Example 54) bound to Cbl-b.

Materials and Methods

Cbl-b Displacement Assay (Cbl-b Inhibition Assay)

[563] The ability of candidate compounds to displace a known inhibitor and thereby inhibit Cbl-b activity was measured by monitoring the interaction of Cbl-b with a fluorophore-labeled probe in the presence of the candidate compound. A truncated variant of Cbl-b (UniProt number Q13191; SEQ ID NO:1) containing an Avitag at its N-terminus was co-expressed with BirA biotin ligase and purified using a standard protocol (*see* Dou *et al.*, Nature Structural and Molecular Biology, 8: 982-987, 2013; Avidity LLC).

[564] Cbl-b amino acid residues 36-427:

PKQAAADRRTVEKTWKLMKVVRLCQNPQLQKNSPPYILDILPDITYQHLRLILSKY
DDNQKLAQLSENEYFKIYIDSLMKKSKRAIRLFKEGKERMYYEQSQDRRNLTKLSLIF
SHMLAEIKAIFPNGQFQGDNFRITKADAAEFWRKFFGDKTIVPWKVFRQCLHEVHQI
SSGLEAMALKSTIDLTCDNDYISVFEFDIFTRLFQPWGSILRNWNFLAVTHPGYMAFLT
YDEVKARLQKYSTKPGSYIFRLSCTRLGQWAIGYVTGDGNILQTIPHNKPLFQALIDG
SREGFYLYPDGRSYNPDLTGLCEPTPHDHIKVTQEYELYCEMGSTFQLCKICAEND
KDVKIEPCGHLMTSCLTAWQESDGQGCPFCRCEIKGTEPIIVDPFD (SEQ ID NO:1)

[565] Fluorescently-labeled inhibitor probe was synthesized and tagged with BODIPY FL (Example 54). Cbl-b displacement assays were performed in a 384-well plate at room temperature in a 10 μ L reaction volume by pre-incubating 0.5 nM Cbl-b or 0.125 nM Cbl-b (final concentration, indicated as “High” and “Low”, respectively) in an assay buffer containing 20 mM HEPES pH 7.5, 150 mM NaCl, 0.01% Triton X-100, 0.01% BSA and 0.5 mM TCEP in the presence of a candidate compound in 1% DMSO (final concentration) for one hour. After incubation in the presence of the candidate compound, the plate was incubated for an additional one hour in the presence of an approximate EC₄₀ binding saturation consisting of 150 nM fluorescently-labeled inhibitor probe and 2 nM Streptavidin-Terbium (Cisbio) (final concentrations). Following the one hour incubation, the plates were read for TR-FRET signal at 520/620 nm using an Envision plate reader (Perkin Elmer). The presence of a TR-FRET signal indicated that the probe was not displaced from Cbl-b by the compound candidate. The absence of a FRET signal indicated that the probe was displaced from Cbl-b by the compound candidate.

[566] Compounds were ranked into bins A through D as follows for IC₅₀: A indicates ≤ 5 nM; B indicates $5 \text{ nM} < \text{IC}_{50} \leq 20$ nM; C indicates $20 \text{ nM} < \text{IC}_{50} \leq 100$ nM; D

indicates IC₅₀ > 100 nM.

Table 2. Cbl-b inhibition by tested compounds

Cmpd No.	Cbl-b activity IC ₅₀ (High)	Cbl-b activity IC ₅₀ (Low)	Cmpd No.	Cbl-b activity IC ₅₀ (Low)
1		A	28	B
2		D	29	B
3		A	30	B
4		A	31	B
5		C	32	B
6		C	33	B
7		D	34	B
8	B		35	A
9	D		36	A
10		D	37	A
11	D		38	A
12	D		39	A
13	D		40	A
14		C	41	A
15		B	42	A
16		C	43	A
17		A	44	A
18		D	45	A
19		C	46	A
20		C	47	A
21		C	48	A
22		C	49	A
23		A	50	A
24		C	51	A
25		B	52	A
26		B	53	A
27		B		

Blank cell indicates data not available.

BIOLOGICAL EXAMPLE 2: Evaluation of T-cell Activation by Cbl-b Inhibitors

[567] Loss of Cbl-b function in both T-cells and mice by genetic knockout of the *cbl-b* gene results in loss of the CD28 co-stimulation requirement for T-cell activation and T-cell resistance to anergy (see Bachmaier *et al.*, Nature, 403: 211-216, 2000; and Jeon *et al.*, Immunity, 21: 167-177, 2004). Cbl-b inhibitors described herein were evaluated for their ability to activate T-cells.

Materials and Methods

Purification and assessment of primary human total T-cell activation

[568] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PMBCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3⁺ cells as assessed by flow cytometry. The cells were rested overnight at 37 °C 5% CO₂. The Cbl-inhibitor was added to 1 x 10⁵ cells per well and the plate was incubated for one hour at 37 °C in 5% CO₂ at the concentrations indicated (Table 3) with a final DMSO concentration of <0.1%. For samples stimulated with anti-CD3 antibody and anti-CD28 antibody (anti-CD3/anti-CD28), the Cbl-b inhibitor concentrations tested were 1 μM, and 0.3 μM. For samples stimulated with anti-CD3 antibody alone (anti-CD3), the Cbl-b inhibitor concentrations tested were 3 μM, and 1 μM. Following incubation with the Cbl-b inhibitor, primary human total T-cells were stimulated with either plate bound anti-CD3 antibody (OKT3) alone or plate bound anti-CD3 antibody (OKT3) with soluble anti-CD28 antibody (28.2) (Life Technologies). To prepare plates with plate bound anti-CD3 antibody (OKT3), 96-well round bottom tissue culture plates were coated with 100 μL of anti-CD3 antibody (OKT3) at 10 μg/mL for 4 hours at 37 °C 5% CO₂ in phosphate buffered saline (PBS). The plates were washed with PBS once prior to adding the cells with or without soluble anti-CD28 antibody (28.2) to each well at a final concentration of 5 μg/mL. Cells were stimulated for 48 hours prior to harvesting the cell free supernatant and staining the cell population for surface marker assessment by flow cytometry. Supernatants were analyzed for cytokine secretion, including IL-2 by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies) following the manufacturer's protocol. Cells were stained with anti-CD25 antibody (BD Biosciences) to assess levels of surface marker of activation.

Results

[569] Readouts were reported as fold change over baseline. Baseline for this study was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody and with soluble anti-CD28 antibody, wherein the cells were not incubated with a Cbl-b inhibitor (Table 3). For T-cells stimulated with anti-CD3/anti-CD28, changes greater than 2.5-fold over

baseline for IL-2 secretion and greater than 1.3-fold over baseline for CD25 surface staining were considered significant and a positive response (Table 3). For T-cells stimulated with anti-CD3 alone, changes greater than 0.1-fold over baseline for IL-2 secretion and greater than 0.6-fold over baseline for CD25 surface staining were considered significant and a positive response (Table 3). Compounds were ranked into bins according to their readouts as follows:

[570] For IL-2 secretion with anti-CD3 antibody and anti-CD28 antibody co-stimulation the bins are: C indicates < 20 fold, B indicates 20 – 35 fold, and A indicates >35 fold;

[571] For IL-2 secretion with anti-CD3 antibody stimulation the bins are: C indicates < 0.70 fold, B indicates 0.70 – 1.1 fold, and A indicates >1.1 fold;

[572] For CD25 staining with anti-CD3 antibody and anti-CD28 antibody co-stimulation the bins are: C indicates < 1.24 fold, B indicates 1.24 – 1.39 fold, and A indicates >1.39 fold;

[573] For CD25 staining with anti-CD3 antibody stimulation the bins are: C indicates < 1.5 fold, B indicates 1.5 – 2.5 fold, and A indicates >2.5 fold.

Table 3. T-cell activation as assessed by IL-2 secretion and CD25 surface staining as a consequence of stimulation with anti-CD3, or anti-CD3 and anti-CD28

Compound No.	IL-2 secretion CD3/CD28		IL-2 secretion CD3		CD25 staining CD3/CD28		CD25 staining CD3	
	1 μ M	0.3 μ M	3 μ M	1 μ M	1 μ M	0.3 μ M	3 μ M	1 μ M
1	B	B	A	A	B	B	A	A
2	A	A	B	A	A	A	A	A
3	A	A	A	A	A	A	A	A
5	C	C	C	C	B	B	C	C
11	C	C			C	C	C	C
12	C	C			C	C	C	C
13	C	C			C	C	C	C
14	C	C	C	C	A	B	C	C
15	C	C	C	C	B	B	C	C
16	B	C	B	B	A	A	A	A
17	A	B	A	A	B	B	B	B
18	C	C	C	C	B	B	A	B
19	C	C	C	C	C	C	C	C
20	C	C	A	B	B	B	A	A
21	C	C	B	B	B	B	B	B
22	C	C	C	C	C	C	C	C
23	C	C	A	B	B	B	A	B
24	C	C	C	C	C	C	C	C
25	C	C	B	B	B	B	B	B

Compound No.	IL-2 secretion CD3/CD28		IL-2 secretion CD3		CD25 staining CD3/CD28		CD25 staining CD3	
	1 μ M	0.3 μ M	3 μ M	1 μ M	1 μ M	0.3 μ M	3 μ M	1 μ M
26	B	C	A	A	A	B	A	A
27	B	C	C	C	A	B	C	C
29	B	C	B	A	B	B	B	A
31	C	C	C	C	A	A	B	C
32	A	B	A	A	A	A	A	A
33	B	C	A	A	A	B	A	A
34	A	C	A	A	B	B	B	B
35	B	B	B	B	B	B	A	A
36	C	C	C	C	C	C	C	C
37	B	B	B	A	A	B	A	A
38	B	B	B	A	A	A	A	A
39	A	A	A	A	B	B	A	A
40	A	B	B	B	A	A	A	A
41	A	B	A	A	A	A	A	A
42	A	B	A	A	B	B	A	A
43	A	B	A	A	B	B	A	A
44	A	B	A	A	A	A	A	A
45	B	B	A	A	A	B	A	A
46	A	A	A	A	B	B	A	B
47	A	A	C	B	A	A	B	B
48	A	B	B	A	A	A	B	A
49	A	A	A	A	B	B	A	A
50	A	A	A	A	A	A	A	A
51	A	A	A	A	B	B	A	A
52	B	A	A	A	A	A	A	A
53	A	A	A	A	A	A	A	A

Blank cell indicates data not available.

Conclusions

[574] Cbl-b inhibitors enhanced IL-2 secretion in T-cells stimulated with anti-CD3 antibody alone or in combination with anti-CD28 antibody. Expression of the surface activation marker, CD25, increased in T-cells stimulated with anti-CD3 antibody alone or in combination with anti-CD28 antibody. These results indicate the identified Cbl-b inhibitors have the ability to activate T-cells and that such activation did not require co-stimulation with anti-CD28 antibody.

BIOLOGICAL EXAMPLE 3: Evaluation of Immunomodulatory Effects of Cbl-b

Inhibitors

[575] Cbl-b inhibitors identified from screening assays demonstrated the ability to activate total human T-cells *in vitro* as evidenced by enhanced IL-2 secretion and expression of the CD25 surface activation marker. Further *in vitro* studies are conducted to assess additional

cytokine secretion by T-cells and expression of surface activation markers on T-cells. Additional immunomodulatory effects on T-cells contacted with the Cbl-b inhibitors described herein were assessed, such as the ability of a Cbl-b inhibitor to increase T-cell proliferation, decrease T-cell exhaustion, and decrease T-cell anergy. The ability of Cbl-b inhibitors, such as those described herein, to activate T-cells *in vivo* was also assessed. Other immunomodulatory effects by the Cbl-b inhibitors were assessed, such as the ability of Cbl-b inhibitors to activate B-cells and NK-cells.

Purification and assessment of primary human total T-cell activation

[576] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PMBCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3⁺ cells as assessed by flow cytometry. For measurement of cell proliferation, cells were labeled with Cell Trace Violet (Invitrogen) following the manufacturer's protocol prior to activation by stimulation with anti-CD3 antibody alone or in combination with anti-CD28 antibody. The Cbl-b inhibitor was added to 1×10^5 cells per well at multiple concentrations (*e.g.*, 10 μ M, 1.11 μ M, or 0.123 μ M) with a final DMSO concentration of <0.1%. The plate was incubated for one hour at 37 °C in 5% CO₂. Following incubation with the Cbl-b inhibitor, primary human total T-cells were stimulated with either plate bound anti-CD3 antibody (OKT3) alone or plate bound anti-CD3 antibody (OKT3) with soluble anti-CD28 antibody (28.2) (Life Technologies). To prepare plates with plate bound anti-CD3 antibody (OKT3), 96-well round bottom tissue culture plates were coated with 100 μ L of anti-CD3 antibody (OKT3) at 10 μ g/mL for 4 hours at 37 °C 5% CO₂ in phosphate buffered saline (PBS). The plates were washed with PBS prior to adding the cells with or without soluble anti-CD28 antibody (28.2) to each well at a final concentration of 5 μ g/mL. Cells were stimulated for 48 hours prior to harvesting the cell free supernatant and staining the cell population for surface marker assessment by flow cytometry. Supernatants were analyzed for cytokine secretion (*e.g.*, GM-CSF, IFN γ and TNF α) by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies) following the

manufacturer's protocol. Cells were stained with anti-CD69 (BD Biosciences) to assess levels of surface markers of activation. Proliferation was measured by flow cytometry and data was analyzed with FlowJo v7.6.5 or v10. Readouts were reported as fold change over baseline. In some embodiments, baseline was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody alone, wherein the cells were not incubated with a Cbl-b inhibitor. In some embodiments, baseline was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody and anti-CD28 antibody, where the cells were not incubated with a Cbl-b inhibitor.

[577] Cbl-b inhibitor effects on primary human T-cells were also evaluated in the context of an allogenic mixed lymphocyte reaction (MLR). Allogenic immature dendritic cells were generated under the following conditions. Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Monocytes were isolated from the PMBCs utilizing positive selection with a commercial kit following the manufacturer's protocol (Stemcell Technologies Catalog #17858) to yield >95% CD14+ cells as assessed by flow cytometry. Monocytes were cultured with 30 ng/mL of recombinant human GM-CSF and 20 ng/mL of recombinant human IL-4 for seven days to generate immature dendritic cells. Monocytes and T-cells were either isolated fresh from peripheral blood or thawed from frozen stocks. Human T-cells were isolated, labeled with CFSE and incubated with inhibitors as described above. The Cbl-b inhibitor was added to 1×10^5 T-cells in coculture with 2×10^3 allogenic immature dendritic cells per well at multiple concentrations (*e.g.*, 10 μ M, or 1.11 μ M) with a final DMSO concentration of <0.1% and incubated at 37 °C in 5% CO₂ for 5 days. Proliferation of the T-cells was evaluated by flow cytometry.

[578] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from T-cells (*e.g.*, GM-CSF, IFN γ , and TNF α) and/or surface expression of cell surface markers on T-cells (*e.g.*, CD69) that was indicative of T-cell activation. Cbl-b inhibitors were also tested to determine their ability to induce or enhance T-cell proliferation. Cbl-b inhibitors were tested for their effects on T-cell activation in the presence of costimulation and where conditions were suboptimal for priming.

Human T-cell in vitro models of T-cell exhaustion

[579] T-cell exhaustion was characterized by cells having a poor effector response and a sustained level of inhibitory receptor expression that results in T-cell dysfunction in response

to chronic infections and cancer. *In vitro* models of T-cell exhaustion include allogenic and autologous models. In an autologous model, myeloid cells and SEB (Staphylococcal enterotoxin B, Millipore) were used to stimulate anti-CD3 stimulated T-cells. Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Monocytes were isolated with commercial kits using negative selection with Stemcell Technologies EasySep Human Monocyte Enrichment Kit without CD16 Depletion (Catalog #19058) following the manufacturer's protocol. Isolated monocytes were cultured in complete media (*e.g.*, RPMI 1640 with no additives, 10% HI FBS, 1X Glutamine and 1X β -mercaptoethanol) with 50 ng/mL recombinant human M-CSF (R&D System or Peprotech). Cells were plated at 2×10^6 cells per well (Day 0) and cultured for 5 days and were fed with fresh media and cytokines on Day 2. On Day 5 IFN γ was added at 100 ng/mL and the cells were incubated overnight. Primary human T-cells from the same donor were isolated from PBMCs with a commercial kit using negative selection with Stemcell Technologies EasySep Human T-cell Isolation Kit (Catalog #17951) following the manufacturer's protocol. Purity was confirmed by surface marker detection by flow cytometry for CD4, CD8, CD45RA, CD45RO, CD19, CD14, CD56, and CD3 (BD Biosciences). 3×10^6 cells per/mL T-cells were stimulated with 10 μ g/mL of plate bound anti-CD3 antibody (Clone UCHT-1) for 5 days. This was done in parallel with myeloid cell generation. On Day 6, 2.5×10^4 T-cells were added per well, 12.5×10^3 myeloid cells per well and SEB antigen (0.1 μ g/mL) were added to the wells of a round bottom 96-well plate. Test agents (*e.g.*, Cbl-b inhibitor compounds) or controls (*e.g.*, checkpoint neutralizing antibodies such as anti-PD1 antibody) were added to the wells at the indicated concentrations (*e.g.*, 10 μ M). Cells were cultured for 3 days at which point cell free supernatants were collected and assessed for secreted cytokines (*e.g.*, GM-CSF, IFN γ , and IL-2) by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies). The T-cells were stained for a panel of surface markers including checkpoint inhibitors (*e.g.*, CTLA4) and evaluated by flow cytometry for Cbl-b inhibitor effects.

[580] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from exhausted T-cells (*e.g.*, GM-CSF, IFN γ , and IL-2) in the presence of myeloid cells, which was indicative of decreased T-cell exhaustion. Cbl-b inhibitors were also tested for their effects on checkpoint modulator expression levels following activation of exhausted T-cells.

Human T-cell in vitro models of T-cell Anergy

[581] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3⁺ cells assessed by flow cytometry. The cells were activated with immobilized anti-CD3 antibody (OKT3) and soluble anti-CD28 antibody (28.2) for two days at which time they were washed and allowed to rest for three days in the absence of stimulation. They were then treated with ionomycin (Sigma) for 18-24 hours to induce anergy. Following two washes to remove the ionomycin from the samples, Cbl-b inhibitor compounds were added to the cells at the indicated concentrations (*e.g.*, 10, 1.11, and 0.37 μ M) and incubated for one hour. The cells were then re-challenged with anti-CD3 antibody and anti-CD28 antibody for 24 hours at which point cell free supernatants were collected and assessed for cytokines (*e.g.*, IFN γ) by ELISA (R&D Systems or Peprotech) or Luminex multiplex kits (Procarta Life Technologies) following the manufacturer's protocols.

[582] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from anergic T-cells (*e.g.*, IFN γ), which was indicative of decreased T-cell tolerance.

In vivo activity of Cbl-b inhibitors

[583] A method for determining the pharmacodynamic profile of Cbl-b inhibitors was performed by dosing strains of mice with competent immune systems, such as C57BL/6 or BALB/c mice, with a Cbl-b inhibitor. The Cbl-b inhibitor was dissolved in a suitable formulation and administered by one of various routes, such as intravenous (IV), intraperitoneal (IP), subcutaneous (SC), or oral (PO), at a suitable dose level and frequency (*e.g.*, twice per day BID or thrice per day TID) as informed by prior pharmacokinetic and tolerability studies. Following administration of the Cbl-b inhibitor, T-cells and indirectly other immune cells (*e.g.*, via cytokine production) were stimulated *in vivo* by administration of an anti-CD3 antibody or antigen-binding fragment thereof in PBS at defined amounts such as 2 μ g or 10 μ g per animal by routes such as IV or IP (*See* Hirsh *et al.*, J. Immunol., 1989; Ferran

et al., Eur. J. Immunol., 1990). Additional study control arms included groups of mice treated with a vehicle formulation alone (*i.e.*, formulation without the Cbl-b inhibitor and anti-CD3 antibody), a formulation containing the Cbl-b inhibitor alone, a formulation containing the anti-CD3 antibody alone, PBS alone, or combinations of these agents. The level of immune activation was then assessed by analysis of plasma cytokine levels and/or expression of activation markers on immune cells (*e.g.*, T-cells). Blood or lymphoid organs (*e.g.*, spleen) were collected at defined time points (*e.g.*, 8 hours or 24 hours). Blood samples were processed to collect plasma for determination of cytokine levels using standard methods known in the art. Cytokines measured included IL-2, IFN γ , and TNF α . Additional blood samples and lymphoid tissues were processed for flow cytometric analysis of immune cells (*e.g.*, T-cells) using standard methods to determine expression of cell type-specific markers and activation markers such as CD25 and/or CD69. Augmentation of immune stimulation by Cbl-b inhibitor administration was assessed by comparing the relative concentrations of cytokines in plasma, or the expression levels of activation markers on immune cells between appropriate groups (*e.g.*, mice treated with Cbl-b inhibitor and 2 μ g anti-CD3 antibody versus mice treated with vehicle and 2 μ g anti-CD3 antibody).

[584] Cbl-b inhibitors were tested to determine their ability to induce or enhance the level of cytokines (*e.g.*, IL-2, IFN γ , and TNF α) in blood obtained from treated mice stimulated with an anti-CD3 antibody, which was indicative of modulation of the immune response. Cbl-b inhibitors were also tested to determine their ability to induce or enhance the expression of cell surface markers on T-cells (*e.g.*, CD25 and/or CD69) isolated from treated mice stimulated with an anti-CD3 antibody, which was indicative of modulation of the immune response.

B Cell Activation Assay

[585] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Human primary B-cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Stemcell Technologies Catalog #17954) to yield >95% CD20⁺ cells assessed by flow cytometry. Primary human B-cells were plated at 0.7-1 x 10⁵ per well in a 96-well plate with Cbl-b inhibitors over a dose ranging from 10 μ M to 1 nM and incubated at 37 °C 5% CO₂, with a final DMSO concentration of <0.5%. Cells were stimulated with anti-IgM for 20 hours at 37 °C 5% CO₂. Surface activation markers on mature CD20⁺ IgD⁺ B-cells were monitored by FACS using an anti-CD69 antibody (BD Biosciences).

[586] Cbl-b inhibitors were tested to determine their ability to induce or enhance surface expression of cell surface markers on B-cells (*e.g.*, CD69), which was indicative of B-cell activation.

Purification and activation of primary human NK-cells.

[587] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary NK-cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-092-657 or Stemcell Technologies Catalog #17955) to yield >92% CD56+, CD3- cells as assessed by flow cytometry. The cells were cultured overnight with IL-2 (60 ng/mL) at 37 °C 5% CO₂. Cbl-b inhibitors are added one hour prior to stimulation and incubated at 37 °C 5% CO₂ at a specific concentration (*e.g.*, 10 μM, 1 μM, or 0.1 μM) with a final DMSO concentration of <0.1%. NK-cells were co-cultured with target cells that were engineered to have a red nucleus (K562 NucRed) measurable by flow cytometry. K562 NucRed cells were produced by transduction of K562 cells with IncuCyte NucLight Red Lentivirus reagent (Catalog #4476) and selected for 5 days. Clonal populations were isolated and expanded using standard tissue culture techniques, and individual clones were validated by comparison to wildtype K562 cells in NK-cell killing assays. The cells were mixed at the indicated ratios (*e.g.*, 5:1, 1:1, or 1:5) of NK (effector cells) to K562 NucRed (target cells) for 6 hours. Cell free supernatants were collected and analyzed for cytokine secretion (*e.g.*, TNFα, IFNγ, or MIP1β) by ELISA or Luminex multiplex kits following the manufacturer's protocol. IFNγ secretion was assessed using an R&D Systems ELISA kit (Catalog #DY285), TNFα secretion was assessed using an R&D Systems ELISA kit (Catalog #DY210), and MIP1β secretion was assessed using an R&D Systems ELISA kit (Catalog #DY271).

BIOLOGICAL EXAMPLE 4: Evaluation of a Cbl-b Inhibitor in Combination with an Immune Checkpoint Inhibitor for Treating Cancer

[588] Tumor microenvironments exploit T-cell inhibitory pathways as a mechanism to evade anti-tumor immune responses. The use of immune checkpoint inhibitors such as inhibitors of PD-1, PD-L1, and CTLA-4 have resulted in strikingly efficacious and durable responses against some tumor types (Marshall and Djamgoz, *Front Oncol*, 8:315, 2018). However, the response to immune checkpoint inhibitor monotherapy is not universal and therefore benefits

only a small subset of cancer patients (Lv *et al.*, Journal for ImmunoTherapy of Cancer, 7:159, 2019). This example describes the evaluation of a combination therapy for treating cancer including an immune checkpoint inhibitor and a Cbl-b inhibitor.

[589] In brief, combination therapies were tested in strains of mice with competent immune systems (*e.g.*, C57BL/6 or BALB/c mice) in whom syngeneic tumors were grown. Syngeneic murine tumor cells were injected subcutaneously: CT26 colon cancer cells in BALB/c mice; TC-1 lung cancer cells in C57BL/6 mice; or MC-38 colon cancer cells in C57BL/6 mice. Tumors were allowed to grow to up to 100-200 mm³ at which time the animals were randomized and treatment was initiated. Alternatively, treatment was administered in a prophylactic setting within 1-3 days of tumor cell implant. The Cbl-b inhibitor was dissolved in a suitable formulation and administered at a suitable dose level and frequency as informed by prior pharmacokinetic and tolerability studies. The Cbl-b inhibitor formulation was administered orally (PO) or parenterally (*e.g.*, IV, IP, SC, or intratumorally at one to three injection sites per tumor). The immune checkpoint inhibitor formulation was administered by IP injection every three days (*e.g.*, Days 1, 4, and 7). In addition to the test group of mice who received the combination therapy, the study included control groups of mice who received either the vehicle formulation alone, the Cbl-b inhibitor formulation alone, or the immune checkpoint inhibitor alone.

[590] The level of response was evaluated by measuring tumor growth and comparing tumor growth in the test mice versus the control mice. The level of immune activation was assessed by collecting tumors for analysis of tumor infiltrating lymphocytes (TILs). TILs and lymphoid tissues were processed for flow cytometric analysis using standard methods to determine cell lineage, expression of cell type-specific markers and expression of activation markers such as granzyme B, PD-1, TIM3, and LAG3. Augmentation of the anti-tumor immune response by the combination therapy was assessed by comparing the relative percentage of immune cell populations in the tumor, and the relative levels of expression of activation markers on immune cells in mice of the test and study groups.

BIOLOGICAL EXAMPLE 5: Evaluation of a Cbl-b Inhibitor in Combination with an Anti-Neoplastic Agent for Treating Cancer

[591] Chemotherapy has been reported to have a positive immunologic effect on tumor infiltrating lymphocytes (Lazzari *et al.*, Ther Adv Med Oncol, 10:1-12, 2018), with the balance of regulatory and effector immune cells influencing prognosis. In addition, chemotherapy is contemplated to increase the intratumoral T-cell repertoire by augmenting tumor antigen

presentation. This example describes the evaluation of a combination therapy for treating cancer including an anti-neoplastic agent and a Cbl-b inhibitor.

[592] In brief, combination therapies were tested in strains of mice with competent immune systems (*e.g.*, C57BL/6 or BALB/c mice) in whom syngeneic tumors were grown. Syngeneic murine tumor cells were injected subcutaneously: CT26 colon cancer cells in BALB/c mice; or TC-1 lung cancer cells in C57BL/6 mice. Tumors were allowed to grow up to about 120 mm³ at which time the animals were randomized and treatment was initiated. The Cbl-b inhibitor was dissolved in a suitable formulation and administered at a suitable dose level and frequency as informed by prior pharmacokinetic and tolerability studies. The Cbl-b inhibitor formulation was administered orally (PO) or parenterally (*e.g.*, IV, IP, SC, or intratumorally at one to three injection sites per tumor). The anti-neoplastic agent (*e.g.*, gemcitabine and/or oxaliplatin) was administered by IP injection once every three or four days. In addition to the test group of mice who received the combination therapy, the study included control groups of mice who received either the vehicle formulation alone, the Cbl-b inhibitor formulation alone, or the anti-neoplastic agent alone.

[593] The level of response was evaluated by measuring tumor growth and comparing tumor growth in the test mice versus the control mice. The level of immune activation was assessed by collecting tumors for analysis of tumor infiltrating lymphocytes (TILs). TILs and lymphoid tissues were processed for flow cytometric analysis using standard methods to determine cell lineage, expression of cell type-specific markers, and expression of activation markers such as granzyme B, PD-1, TIM3, and LAG3. Augmentation of the anti-tumor immune response by the combination therapy was assessed by comparing the relative percentage of immune cell populations in the tumor, and the relative levels of expression of activation markers on immune cells in mice of the test and study groups.

BIOLOGICAL EXAMPLE 6: Evaluation of a Cbl-b Inhibitor in Combination with Radiation Therapy for Treating Cancer

[594] Ablative radiation therapy targeting local tumors limits damage to normal tissue and has the ability to enhance the diversity of the T-cell receptor repertoire by increasing the presence of tumor antigens (Lee *et al.*, Blood, 114: 589-595, 2009). Radiotherapy at one site has been reported to lead to regression of distant site tumors that were not irradiated (Ngwa *et al.*, Nat Rev Cancer, 18: 313-322, 2018). The systemic effect of a localized therapy is termed an “abscopal effect,” which in the context of radiation therapy is thought to involve the immune

system. This example describes the evaluation of a combination therapy for treating cancer including radiation therapy and a Cbl-b inhibitor.

[595] In brief, combination therapies were tested in strains of mice with competent immune systems (*e.g.*, C57BL/6 or BALB/c mice) in whom syngeneic tumors were grown. Syngeneic murine tumor cells were injected subcutaneously: CT26 colon cancer cells in BALB/c mice; or B16-F10 melanoma cells in C57BL/6 mice. Tumors were allowed to grow up to about 80 mm³ at which time the animals were randomized and treatment was initiated. In some studies, tumor cells were implanted in both flanks and only one tumor was treated to assess the abscopal effect. The Cbl-b inhibitor was dissolved in a suitable formulation and administered at a suitable dose level and frequency as informed by prior pharmacokinetic and tolerability studies. The Cbl-b inhibitor formulation was administered orally (PO) or parenterally (*e.g.*, IV, IP, SC, or intratumorally at one to three injection sites per tumor). Radiation therapy was administered once at a dose of 20 grays using an X-ray based focal beam irradiator. In addition to the test group of mice who received the combination therapy, the study included control groups of mice who received either the vehicle formulation alone, the Cbl-b inhibitor formulation alone, or radiation therapy alone.

[596] The level of response was evaluated by measuring tumor growth and comparing tumor growth in the test mice versus the control mice. The level of immune activation was assessed by collecting tumors for analysis of tumor infiltrating lymphocytes (TILs). TILs and lymphoid tissues were processed for flow cytometric analysis using standard methods to determine cell lineage, expression of cell type-specific markers, and expression of activation markers such as granzyme B, PD-1, TIM3, and LAG3. Augmentation of the anti-tumor immune response by the combination therapy was assessed by comparing the relative percentage of immune cell populations in the tumor, and the relative levels of expression of activation markers on immune cells in mice of the test and study groups.

BIOLOGICAL EXAMPLE 7: Evaluation Of A Cbl-B Inhibitor In Combination With Adoptive Cell Therapy For Treating Cancer

[597] Adoptive cell therapy (ACT) utilizing autologous tumor-specific T-cells leverages the natural function of T-cells to specifically recognize and eliminate target cells (Hinrichs and Rosenberg, *Immunol Rev*, 257: 56-71, 2014). Specificity of tumor infiltrating lymphocytes (TILs) is due to their ability to recognize tumor-associated antigens, including neoantigens derived from products of mutated genes. This example describes the evaluation of an *in vivo*

lympho-conditioning program with a Cbl-b inhibitor prior to *ex vivo* expansion of TILs for treating cancer with ACT.

[598] Strains of mice with competent immune systems (*e.g.*, C57BL/6 or BALB/c mice) in whom syngeneic tumors were grown were utilized. Syngeneic murine tumor cells were injected subcutaneously or intravenously: 4T1 breast cancer cells in BALB/c mice; RENCA kidney cancer cells in BALB/c mice; B16-F10 melanoma cells in C57BL/6 mice; 3LL lung cancer cells in C57BL/6 mice; or MC-38 colon cancer cells in C57BL/6 mice. Tumors were allowed to grow up to about 50-600 mm³ at which time the animals were randomized and treatment was initiated. The Cbl-b inhibitor was dissolved in a suitable formulation and administered at a suitable dose level and frequency as informed by prior pharmacokinetic and tolerability studies. The Cbl-b inhibitor formulation was administered orally (PO) or parenterally (*e.g.*, IV, IP, SC, or intratumorally at one to three injection sites per tumor). In addition to the test group of mice who received the Cbl-b inhibitor prior to tumor harvest, a control group of mice received either the vehicle formulation alone or was left untreated prior to tumor harvest. Tumor tissue was harvested either from the primary tumor or from tissues with metastases (*e.g.*, lung). The tissues were minced and cultured in medium in the presence or absence of one or more exogenous T-cell growth factors (*e.g.*, IL-2, IL-7, IL-15, and/or IL-21) under conditions suitable for expansion of TILs. Expansion of TILs was done in the presence or absence of the Cbl-b inhibitor. Expanded TILs were assessed for phenotype by flow cytometric analysis by measuring expression of markers for memory, effector, and stemness (*e.g.*, CD95, TCF7, CD62L, CD44, etc.). Upon successful expansion of the TILs, tumor bearing mice were infused with TILs in the presence or absence of the Cbl-b inhibitor to assess the effect of lympho-conditioning and/or subsequent *in vivo* treatment on TIL engraftment and anti-tumor immune responses.

[599] Anti-tumor efficacy of ACT was assessed through tumor measurements to determine the level of tumor growth inhibition by TILs.

BIOLOGICAL EXAMPLE 8: Evaluation of Cbl-b Inhibition by Candidate Inhibitors

[600] Candidate compounds were evaluated for their ability to bind and inhibit Cbl-b, an E3 ubiquitin-protein ligase, as evidenced by their ability to displace a fluorophore-labeled probe bound to Cbl-b.

Materials and Methods

Cbl-b Displacement Assay (Cbl-b Inhibition Assay)

[601] The ability of candidate compounds to displace a known inhibitor and thereby inhibit Cbl-b activity was measured by monitoring the interaction of Cbl-b with a fluorophore-labeled probe in the presence of the candidate compound. A truncated variant of Cbl-b (UniProt number Q13191) containing residues 36-427 and an Avitag at its N-terminus was co-expressed with BirA biotin ligase and purified using a standard protocol (*see* Dou *et al.*, Nature Structural and Molecular Biology 8: 982-987, 2013; Avidity LLC).

[602] Fluorescently-labeled inhibitor probe was synthesized and tagged with BODIPY FL. Cbl-b displacement assays were performed in a 384-well plate at room temperature in a 10 μ L reaction volume by pre-incubating 0.5 nM Cbl-b or 0.125 nM Cbl-b (final concentration, indicated as “High” and “Low”, respectively) in an assay buffer containing 20 mM HEPES pH 7.5, 150 mM NaCl, 0.01% Triton X-100, 0.01% BSA and 0.5 mM TCEP in the presence of a candidate compound in 1% DMSO (final concentration) for one hour. After incubation in the presence of the candidate compound, the plate was incubated for an additional one hour in the presence of an approximate EC₄₀ binding saturation consisting of 150 nM fluorescently-labeled inhibitor probe and 2 nM Streptavidin-Terbium (Cisbio) (final concentrations). Following the one hour incubation, the plates were read for TR-FRET signal at 520/620 nm using an Envision plate reader (Perkin Elmer). The presence of a TR-FRET signal indicated that the probe was not displaced from Cbl-b by the compound candidate. The absence of a FRET signal indicated that the probe was displaced from Cbl-b by the compound candidate.

Results

[603] The resulting data for the Cbl-b activity assays were analyzed using standard methods to determine the IC₅₀ values for the tested compounds. In **Table 8-1**, compounds were ranked into bins as follows for IC₅₀: A indicates < 1 nM; B indicates 1 nM-5 nM; and C indicates > 5nM.

Table 8-1. Cbl-b inhibition by tested compounds

Compound No.	Cbl-b IC ₅₀ (High)	Cbl-b IC ₅₀ (Low)
3		A
23		A
38		B
42		A
17		B

Blank cell indicates data not available.

BIOLOGICAL EXAMPLE 9: Evaluation of T-cell Activation by Cbl-b Inhibitors

[604] Cbl-b inhibitors were evaluated for their ability to activate T-cells.

Materials and Methods

Purification and assessment of primary human total T-cell activation

[605] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PMBCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3+ cells as assessed by flow cytometry. The cells were rested overnight at 37 °C 5% CO₂. The Cbl-inhibitor was added to 1 x 10⁵ cells per well and the plate was incubated for one hour at 37 °C in 5% CO₂ at the concentrations indicated (**Table 9-1**) with a final DMSO concentration of <0.1%. For samples stimulated with anti-CD3 antibody and anti-CD28 antibody (anti-CD3/anti-CD28), the Cbl-b inhibitor concentrations tested were 1 μM, and 0.3 μM. For samples stimulated with anti-CD3 antibody alone (anti-CD3), the Cbl-b inhibitor concentrations tested were 3 μM, and 1 μM. Following incubation with the Cbl-b inhibitor, primary human total T-cells were stimulated with either plate bound anti-CD3 antibody (OKT3) alone or plate bound anti-CD3 antibody (OKT3) with soluble anti-CD28 antibody (28.2) (Life Technologies). To prepare plates with plate bound anti-CD3 antibody (OKT3), 96-well round bottom tissue culture plates were coated with 100 μL of anti-CD3 antibody (OKT3) at 10 μg/mL for 4 hours at 37 °C 5% CO₂ in phosphate buffered saline (PBS). The plates were washed with PBS once prior to adding the cells with or without soluble anti-CD28 antibody (28.2) to each well at a final concentration of 5 μg/mL. Cells were stimulated for 48 hours prior to harvesting the cell free supernatant and staining the cell population for surface marker assessment by flow cytometry. Supernatants were analyzed for cytokine secretion, including IL-2 by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies) following the manufacturer's protocol. Cells were stained with anti-CD25 antibody (BD Biosciences) to assess levels of surface marker of activation.

Results

[606] Readouts were reported as fold change over baseline. Baseline for this study was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody and with soluble anti-CD28 antibody, wherein the cells were not incubated with a Cbl-b inhibitor (**Table 9-1**). For T-cells stimulated with anti-CD3/anti-CD28, changes greater than 2.5-fold over baseline for IL-2 secretion and greater than 1.3-fold over baseline for CD25 surface staining were considered significant and a positive response. For T-cells stimulated with anti-CD3 alone, changes greater than 0.1-fold over baseline for IL-2 secretion and greater than 0.6-fold over baseline for CD25 surface staining were considered significant and a positive response.

[607] Compounds were ranked into bins according to their readouts as follows: For IL-2 secretion with anti-CD3 antibody and anti-CD28 antibody co-stimulation the bins are: C indicates < 10 fold, B indicates 10 – 15 fold, and A indicates > 15 fold; For IL-2 secretion with anti-CD3 antibody stimulation the bins are: C indicates ≤ 0.33 fold, B indicates 0.34 – 0.66 fold, and A indicates > 0.66 fold; For CD25 staining with anti-CD3 antibody and anti-CD28 antibody co-stimulation the bins are: C indicates ≤ 1.24 fold, B indicates 1.25 – 1.39 fold, and A indicates > 1.39 fold; For CD25 staining with anti-CD3 antibody stimulation the bins are: C indicates ≤ 1.04 fold, B indicates 1.05 – 1.14 fold, and A indicates > 1.14 fold.

Table 9-1. T-cell activation as assessed by IL-2 secretion and CD25 surface staining as a consequence of stimulation with anti-CD3, or anti-CD3 and anti-CD28

Cmpd No.	IL-2 secretion CD3/CD28		IL-2 secretion CD3		CD25 staining CD3/CD28		CD25 staining CD3	
	1 μ M	0.3 μ M	3 μ M	1 μ M	1 μ M	0.3 μ M	3 μ M	1 μ M
3	A	A	A	A	A	A	A	A
23	A	A	A	A	A	A	A	A
38	A	A	A	A	A	A	A	A
42	A	A	A	A	A	A	A	A
17	A	B	A	A	C	C	B	A

[608] Cbl-b inhibitors enhanced IL-2 secretion by T-cells stimulated with an anti-CD3 antibody alone or in combination with an anti-CD28 antibody. Expression of the CD25 activation marker on the surface of T cells increased when stimulated with an anti-CD3 antibody alone or in combination with an anti-CD28 antibody. These results indicate the identified Cbl-b inhibitors have the ability to activate T-cells and that such activation did not require co-stimulation with an anti-CD28 antibody.

BIOLOGICAL EXAMPLE 10: Evaluation of Immunomodulatory Effects of Cbl-b Inhibitors

[609] Cbl-b inhibitors identified from screening assays demonstrated the ability to activate total human T-cells *in vitro* as evidenced by enhanced IL-2 secretion and expression of the CD25 surface activation marker.

[610] Further *in vitro* studies were conducted to assess additional cytokine secretion by T-cells and expression of surface activation markers on T-cells. Additional immunomodulatory effects on T-cells contacted with the Cbl-b inhibitors described herein were assessed, such as the ability of a Cbl-b inhibitor to increase T-cell proliferation, decrease T-cell exhaustion, and decrease T-cell anergy. The ability of Cbl-b inhibitors, such as those described herein, to activate T-cells *in vivo* was also assessed. Other immunomodulatory effects by the Cbl-b inhibitors were assessed, such as the ability of Cbl-b inhibitors to activate B-cells and NK-cells.

Purification and assessment of primary human total T-cell activation

[611] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PMBCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3⁺ cells as assessed by flow cytometry. For measurement of cell proliferation, cells were labeled with Cell Trace Violet (Invitrogen) following the manufacturer's protocol prior to activation by stimulation with anti-CD3 antibody alone or in combination with anti-CD28 antibody. The Cbl-b inhibitor was added to 1×10^5 cells per well at multiple concentrations (*e.g.*, 10 μ M, 1.11 μ M, or 0.123 μ M) with a final DMSO concentration of <0.1%. The plate was incubated for one hour at 37 °C in 5% CO₂. Following incubation with the Cbl-b inhibitor, primary human total T-cells were stimulated with either plate bound anti-CD3 antibody (OKT3) alone or plate bound anti-CD3 antibody (OKT3) with soluble anti-CD28 antibody (28.2) (Life Technologies). To prepare plates with plate bound anti-CD3 antibody (OKT3), 96-well round bottom tissue culture plates were coated with 100

μL of anti-CD3 antibody (OKT3) at 10 μg/mL for 4 hours at 37 °C 5% CO₂ in phosphate buffered saline (PBS). The plates were washed with PBS prior to adding the cells with or without soluble anti-CD28 antibody (28.2) to each well at a final concentration of 5 μg/mL. Cells were stimulated for 48 hours prior to harvesting the cell free supernatant and staining the cell population for surface marker assessment by flow cytometry. Supernatants were analyzed for cytokine secretion (*e.g.*, GM-CSF, IFN γ , and TNF α) by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies) following the manufacturer's protocol. Cells were stained with anti-CD69 (BD Biosciences) to assess levels of surface markers of activation. Proliferation was measured by flow cytometry and data was analyzed with FlowJo v7.6.5 or v10. Readouts were reported as fold change over baseline. In some embodiments, baseline was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody alone, wherein the cells were not incubated with a Cbl-b inhibitor. In some embodiments, baseline was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody and anti-CD28 antibody, where the cells were not incubated with a Cbl-b inhibitor.

[612] Cbl-b inhibitor effects on primary human T-cells were also evaluated in the context of an allogenic mixed lymphocyte reaction (MLR). Allogenic immature dendritic cells were generated under the following conditions. Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Monocytes were isolated from the PMBCs utilizing positive selection with a commercial kit following the manufacturer's protocol (StemCells Catalog #17858) to yield >95% CD14⁺ cells as assessed by flow cytometry. Monocytes were cultured with 30 ng/mL of recombinant human GM-CSF and 20 ng/mL of recombinant human IL-4 for seven days to generate immature dendritic cells. Monocytes and T-cells were either isolated fresh from peripheral blood or thawed from frozen stocks. Human T-cells were isolated, labeled with CFSE and incubated with inhibitors as described above. The Cbl-b inhibitor was added to 1 x 10⁵ T-cells in coculture with 2 x 10³ allogenic immature dendritic cells per well at multiple concentrations (*e.g.*, 10 μM, or 1.11 μM) with a final DMSO concentration of <0.1% and incubated at 37 °C in 5% CO₂ for 5 days. Proliferation of the T-cells was evaluated by flow cytometry.

[613] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from T-cells (*e.g.*, GM-CSF, IFN γ , and TNF α) and/or surface expression of cell

surface markers on T-cells (*e.g.*, CD69) that was indicative of T-cell activation. Cbl-b inhibitors were also tested to determine their ability to induce or enhance T-cell proliferation. Cbl-b inhibitors were tested for their effects on T-cell activation in the presence of costimulation and where conditions are suboptimal for priming.

Human T-cell in vitro models of T-cell exhaustion

[614] T-cell exhaustion was characterized by cells having a poor effector response and a sustained level of inhibitory receptor expression that results in T-cell dysfunction in response to chronic infections and cancer. *In vitro* models of T-cell exhaustion include allogenic and autologous models. In an autologous model, myeloid cells and SEB (Staphylococcal enterotoxin B, Millipore) were used to stimulate anti-CD3 stimulated T-cells. Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Monocytes were isolated with commercial kits using negative selection with StemCells EasySep Human Monocyte Enrichment Kit without CD16 Depletion (Catalog #19058) following the manufacturer's protocol. Isolated monocytes were cultured in complete media (*e.g.* RPMI 1640 with no additives, 10% HI FBS, 1X Glutamine and 1X β -mercaptoethanol) with 50 ng/mL recombinant human M-CSF (R&D System or Peprotech). Cells were plated at 2×10^6 cells per well (Day 0) and cultured for 5 days and were fed with fresh media and cytokines on Day 2. On Day 5, IFN γ is added at 100 ng/mL and the cells were incubated overnight. Primary human T-cells from the same donor were isolated from PBMCs with a commercial kit using negative selection (with Stemcell Technologies EasySep Human T-cell Isolation Kit (Catalog #17951) following the manufacturer's protocol. Purity was confirmed by surface marker detection by flow cytometry for CD4, CD8, CD45RA, CD45RO, CD19, CD14, CD56, and CD3 (BD Biosciences). 3×10^6 cells per/mL T-cells were stimulated with 10 μ g/mL of plate bound anti-CD3 antibody (Clone UCHT-1) for 5 days. This was done in parallel with myeloid cell generation. On Day 6, 2.5×10^4 T-cells were added per well, 12.5×10^3 myeloid cells per well and SEB antigen (0.1 μ g/mL) were added to wells of a round bottom 96-well plate. Test agents (*e.g.* Cbl-b inhibitor compounds) or controls (*e.g.*, checkpoint neutralizing antibodies such as anti-PD1 antibody) were added to the wells at the indicated concentrations (*e.g.*, 10 μ M). Cells were cultured for 3 days at which point cell free supernatants were collected and assessed for secreted cytokines (*e.g.*, GM-CSF, IFN γ , and IL-2) by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies). The T-cells were stained for a panel

of surface markers including checkpoint inhibitors (*e.g.*, CTLA4) and evaluated by flow cytometry for Cbl-b inhibitor effects.

[615] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from exhausted T-cells (*e.g.*, GM-CSF, IFN γ , and IL-2) in the presence of myeloid cells, which was indicative of decreased T-cell exhaustion. Cbl-b inhibitors were also tested for their effects on checkpoint modulator expression levels following activation of exhausted T-cells.

Human T-cell in vitro models of T-cell Anergy

[616] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3⁺ cells assessed by flow cytometry. The cells were activated with immobilized anti-CD3 antibody (OKT3) and soluble anti-CD28 antibody (28.2) for two days at which time they were washed and allowed to rest for three days in the absence of stimulation. They were then treated with ionomycin (Sigma) for 18-24 hours to induce anergy. Following two washes to remove the ionomycin from the samples, Cbl-b inhibitor compounds were added to the cells at the indicated concentrations (*e.g.*, 10, 1.11, and 0.37 μ M) and incubated for one hour. The cells were then re-challenged with anti-CD3 antibody and anti-CD28 antibody for 24 hours at which point cell free supernatants were collected and assessed for cytokines (*e.g.*, IFN γ) by ELISA (R&D Systems or Peprotech) or Luminex multiplex kits (Procarta Life Technologies) following the manufacturer's protocols.

[617] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from anergic T-cells (*e.g.*, IFN γ), which was indicative of decreased T-cell tolerance.

In vivo activity of Cbl-b inhibitors

[618] A method of determining the pharmacodynamic profile of Cbl-b inhibitors was performed by dosing strains of mice with competent immune systems such as C57BL/6 or BALB/c mice with a Cbl-b inhibitor. The Cbl-b inhibitor was dissolved in a suitable

formulation and administered by one of various routes such as intravenous (IV), intraperitoneal (IP), subcutaneous (SC), or oral (PO), at a suitable dose level and frequency (*e.g.*, twice per day BID or thrice per day TID) as informed by prior pharmacokinetic and tolerability studies. Following administration of the Cbl-b inhibitor, T-cells and indirectly other immune cells (*e.g.*, via cytokine production) were stimulated *in vivo* by administration of an anti-CD3 antibody or antigen-binding fragment thereof in PBS at defined amounts such as 2 µg or 10 µg per animal by routes such as IV or IP (*see* Hirsh et al., J. Immunol., 1989; Ferran et al., Eur. J. Immunol., 1990). Additional study control arms included groups of mice treated with a vehicle formulation alone (*i.e.*, formulation without the Cbl-b inhibitor and anti-CD3 antibody), a formulation containing the Cbl-b inhibitor alone, a formulation containing the anti-CD3 antibody alone, PBS alone, or combinations of these agents. The level of immune activation was then assessed by analysis of plasma cytokine levels and/or expression of activation markers on immune cells (*e.g.*, T-cells). Blood or lymphoid organs (*e.g.*, spleen) were collected at defined time points (*e.g.*, 8 hours or 24 hours). Blood samples were processed to collect plasma for determination of cytokine levels using standard methods known in the art. Cytokines measured included IL-2, IFN γ , and TNF α . Additional blood samples and lymphoid tissues were processed for flow cytometric analysis of immune cells (*e.g.*, T-cells) using standard methods to determine expression of cell type-specific markers and activation markers such as CD25 and/or CD69. Augmentation of immune stimulation by Cbl-b inhibitor administration was assessed by comparing the relative concentrations of cytokines in plasma, or the expression levels of activation markers on immune cells between appropriate groups (*e.g.*, mice treated with Cbl-b inhibitor and 2 µg anti-CD3 antibody versus mice treated with vehicle and 2 µg anti-CD3 antibody).

[619] Cbl-b inhibitors were tested to determine their ability to induce or enhance the level of cytokines (*e.g.*, IL-2, IFN γ , and TNF α) in blood obtained from treated mice stimulated with an anti-CD3 antibody, which was indicative of modulation of the immune response. Cbl-b inhibitors were also tested to determine their ability to induce or enhance the expression of cell surface markers on T-cells (*e.g.*, CD25 and/or CD69) isolated from treated mice stimulated with an anti-CD3 antibody, which was indicative of modulation of the immune response.

B-Cell Activation Assay

[620] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Human primary B-

cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Stemcell Technologies Catalog #17954) to yield >95% CD20⁺ cells assessed by flow cytometry. Primary human B-cells were plated at 0.7-1 x 10⁵ per well in a 96-well plate with Cbl-b inhibitors over a dose ranging from 10 μ M to 1 nM and incubated at 37 °C 5% CO₂, with a final DMSO concentration of <0.5%. Cells were stimulated with anti-IgM for 20 hours at 37 °C 5% CO₂. Surface activation markers on mature CD20⁺ IgD⁺ B-cells were monitored by FACS using an anti-CD69 antibody (BD Biosciences).

[621] Cbl-b inhibitors were tested to determine their ability to induce or enhance surface expression of cell surface markers on B-cells (e.g., CD69), which was indicative of B-cell activation.

Purification and activation of primary human NK-cells.

[622] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary NK-cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-092-657 or Stemcell Technologies Catalog #17955) to yield >92% CD56⁺, CD3⁺ cells as assessed by flow cytometry. The cells were cultured overnight with IL-2 (60 ng/mL) at 37 °C 5% CO₂. Cbl-b inhibitors were added one hour prior to stimulation and incubated at 37 °C 5% CO₂ at a specific concentration (e.g., 10 μ M, 1 μ M, or 0.1 μ M) with a final DMSO concentration of <0.1%. NK-cells were co-cultured with target cells that were engineered to have a red nucleus (K562 NucRed) measurable by flow cytometry. K562 NucRed cells were produced by transduction of K562 cells with IncuCyte NucLight Red Lentivirus reagent (Catalog #4476) and selected for 5 days. Clonal populations were isolated and expanded using standard tissue culture techniques, and individual clones were validated by comparison to wildtype K562 cells in NK-cell killing assays. The cells were mixed at the indicated ratios (e.g., 5:1, 1:1, or 1:5) of NK (effector cells) to K562 NucRed (target cells) for 6 hours. Cell free supernatants were collected and analyzed for cytokine secretion (e.g., TNF α , IFN γ , or MIP1 β) by ELISA or Luminex multiplex kits following the manufacturer's protocol. IFN γ secretion was assessed using an R&D Systems ELISA kit (Catalog #DY285), TNF α secretion was assessed using an R&D Systems ELISA kit (Catalog #DY210), and MIP1 β secretion was assessed using an R&D Systems ELISA kit (Catalog #DY271).

BIOLOGICAL EXAMPLE 11: Evaluation of Cbl-b Inhibition by Candidate Inhibitors

[623] Candidate compounds were evaluated for their ability to bind and inhibit Cbl-b, an E3 ubiquitin-protein ligase, as evidenced by their ability to displace a fluorophore-labeled probe bound to Cbl-b.

Materials and Methods*Cbl-b Displacement Assay (Cbl-b Inhibition Assay)*

[624] The ability of candidate compounds to displace a known inhibitor and thereby inhibit Cbl-b activity was measured by monitoring the interaction of Cbl-b with a fluorophore-labeled probe in the presence of the candidate compound. A truncated variant of Cbl-b (UniProt number Q13191) containing residues 36-427 and an Avitag at its N-terminus was co-expressed with BirA biotin ligase and purified using a standard protocol (*see* Dou *et al.*, Nature Structural and Molecular Biology 8: 982-987, 2013; Avidity LLC).

[625] Fluorescently-labeled inhibitor probe was synthesized and tagged with BODIPY FL. Cbl-b displacement assays were performed in a 384-well plate at room temperature in a 10 μ L reaction volume by pre-incubating 0.5 nM Cbl-b or 0.125 nM Cbl-b (final concentration, indicated as “High” and “Low”, respectively) in an assay buffer containing 20 mM HEPES pH 7.5, 150 mM NaCl, 0.01% Triton X-100, 0.01% BSA and 0.5 mM TCEP in the presence of a candidate compound in 1% DMSO (final concentration) for one hour. After incubation in the presence of the candidate compound, the plate was incubated for an additional one hour in the presence of an approximate EC₄₀ binding saturation consisting of 150 nM fluorescently-labeled inhibitor probe and 2 nM Streptavidin-Terbium (Cisbio) (final concentrations). Following the one hour incubation, the plates were read for TR-FRET signal at 520/620 nm using an Envision plate reader (Perkin Elmer). The presence of a TR-FRET signal indicated that the probe was not displaced from Cbl-b by the compound candidate. The absence of a FRET signal indicated that the probe was displaced from Cbl-b by the compound candidate.

Results

[626] The resulting data for the Cbl-b activity assays were analyzed using standard methods to determine the IC₅₀ values for the tested compounds. In **Table 11-1**, compounds were ranked into bins as follows for IC₅₀: A indicates < 1 nM; B indicates 1-5 nM; and C indicates > 5nM.

Table 11-1. Cbl-b inhibition by tested compounds

Compound No.	Cbl-b IC ₅₀ (High)	Cbl-b IC ₅₀ (Low)
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3		A
23		A
38		B
42		A
17		B

Blank cell indicates data not available.

BIOLOGICAL EXAMPLE 12: Evaluation of T-cell Activation by Cbl-b Inhibitors

[627] Cbl-b inhibitors were evaluated for their ability to activate T-cells.

Materials and Methods

Purification and assessment of primary human total T-cell activation

[628] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PMBCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3+ cells as assessed by flow cytometry. The cells were rested overnight at 37 °C 5% CO₂. The Cbl-inhibitor was added to 1 x 10⁵ cells per well and the plate was incubated for one hour at 37 °C in 5% CO₂ at the concentrations indicated (**Table 12-1**) with a final DMSO concentration of <0.1%. For samples stimulated with anti-CD3 antibody and anti-CD28 antibody (anti-CD3/anti-CD28), the Cbl-b inhibitor concentrations tested were 1 μM, and 0.3 μM. For samples stimulated with anti-CD3 antibody alone (anti-CD3), the Cbl-b inhibitor concentrations tested were 3 μM, and 1 μM. Following incubation with the Cbl-b inhibitor, primary human total T-cells were stimulated with either plate bound anti-CD3 antibody (OKT3) alone or plate bound anti-CD3 antibody (OKT3) with soluble anti-CD28 antibody (28.2) (Life Technologies). To prepare plates with plate bound anti-CD3 antibody (OKT3), 96-well round bottom tissue culture plates were coated with 100 μL of anti-CD3 antibody (OKT3) at 10 μg/mL for 4 hours at 37 °C 5% CO₂ in phosphate buffered saline (PBS). The plates were washed with PBS once prior to adding the cells with or without soluble anti-CD28 antibody (28.2) to each well at a final concentration of 5 μg/mL. Cells were stimulated for 48 hours prior to harvesting the cell free supernatant and staining the cell population for

surface marker assessment by flow cytometry. Supernatants were analyzed for cytokine secretion, including IL-2 by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies) following the manufacturer's protocol. Cells were stained with anti-CD25 antibody (BD Biosciences) to assess levels of surface marker of activation.

Results

[629] Readouts were reported as fold change over baseline. Baseline for this study was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody and with soluble anti-CD28 antibody, wherein the cells were not incubated with a Cbl-b inhibitor (**Table 12-1**). For T-cells stimulated with anti-CD3/anti-CD28, changes greater than 2.5-fold over baseline for IL-2 secretion and greater than 1.3-fold over baseline for CD25 surface staining were considered significant and a positive response. For T-cells stimulated with anti-CD3 alone, changes greater than 0.1-fold over baseline for IL-2 secretion and greater than 0.6-fold over baseline for CD25 surface staining were considered significant and a positive response.

[630] Compounds were ranked into bins according to their readouts as follows: For IL-2 secretion with anti-CD3 antibody and anti-CD28 antibody co-stimulation the bins are: C indicates ≤ 10 fold, B indicates 11 – 15 fold, and A indicates >15 fold; For IL-2 secretion with anti-CD3 antibody stimulation the bins are: C indicates ≤ 0.33 fold, B indicates 0.34 – 0.66 fold, and A indicates >0.66 fold; For CD25 staining with anti-CD3 antibody and anti-CD28 antibody co-stimulation the bins are: C indicates ≤ 1.24 fold, B indicates 1.25 – 1.39 fold, and A indicates >1.39 fold; For CD25 staining with anti-CD3 antibody stimulation the bins are: C indicates ≤ 1.04 fold, B indicates 1.05 – 1.14 fold, and A indicates >1.14 fold.

Table 12-1. T-cell activation as assessed by IL-2 secretion and CD25 surface staining as a consequence of stimulation with anti-CD3, or anti-CD3 and anti-CD28

Cmpd No.	IL-2 secretion CD3/CD28		IL-2 secretion CD3		CD25 staining CD3/CD28		CD25 staining CD3	
	1 μ M	0.3 μ M	3 μ M	1 μ M	1 μ M	0.3 μ M	3 μ M	1 μ M
3	A	A	A	A	A	A	A	A
23	A	A	A	A	A	A	A	A
38	A	A	A	A	A	A	A	A
42	A	A	A	A	A	A	A	A
17	A	B	A	A	C	C	B	A

[631] Cbl-b inhibitors enhanced IL-2 secretion by T-cells stimulated with an anti-CD3 antibody alone or in combination with an anti-CD28 antibody. Expression of the CD25 activation marker on the surface of T cells increased when stimulated with an anti-CD3 antibody alone or in combination with an anti-CD28 antibody. These results indicate the identified Cbl-b inhibitors have the ability to activate T-cells and that such activation did not require co-stimulation with an anti-CD28 antibody.

BIOLOGICAL EXAMPLE 13: Evaluation of Immunomodulatory Effects of Cbl-b Inhibitors

[632] Cbl-b inhibitors identified from screening assays demonstrated the ability to activate total human T-cells *in vitro* as evidenced by enhanced IL-2 secretion and expression of the CD25 surface activation marker.

[633] Further *in vitro* studies were conducted to assess additional cytokine secretion by T-cells and expression of surface activation markers on T-cells. Additional immunomodulatory effects on T-cells contacted with the Cbl-b inhibitors described herein were assessed, such as the ability of a Cbl-b inhibitor to increase T-cell proliferation, decrease T-cell exhaustion, and decrease T-cell anergy. The ability of Cbl-b inhibitors, such as those described herein, to activate T-cells *in vivo* was also assessed. Other immunomodulatory effects by the Cbl-b inhibitors were assessed, such as the ability of Cbl-b inhibitors to activate B-cells and NK-cells.

Purification and assessment of primary human total T-cell activation

[634] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PMBCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3+ cells as assessed by flow cytometry. For measurement of cell proliferation, cells were labeled with Cell Trace Violet (Invitrogen) following the manufacturer's protocol prior to activation by stimulation with anti-CD3 antibody alone or in combination with anti-CD28 antibody. The Cbl-b inhibitor was added to 1×10^5 cells per well

at multiple concentrations (*e.g.*, 10 μ M, 1.11 μ M, or 0.123 μ M) with a final DMSO concentration of <0.1%. The plate was incubated for one hour at 37 °C in 5% CO₂. Following incubation with the Cbl-b inhibitor, primary human total T-cells were stimulated with either plate bound anti-CD3 antibody (OKT3) alone or plate bound anti-CD3 antibody (OKT3) with soluble anti-CD28 antibody (28.2) (Life Technologies). To prepare plates with plate bound anti-CD3 antibody (OKT3), 96-well round bottom tissue culture plates were coated with 100 μ L of anti-CD3 antibody (OKT3) at 10 μ g/mL for 4 hours at 37 °C 5% CO₂ in phosphate buffered saline (PBS). The plates were washed with PBS prior to adding the cells with or without soluble anti-CD28 antibody (28.2) to each well at a final concentration of 5 μ g/mL. Cells were stimulated for 48 hours prior to harvesting the cell free supernatant and staining the cell population for surface marker assessment by flow cytometry. Supernatants were analyzed for cytokine secretion (*e.g.*, GM-CSF, IFN γ , and TNF α) by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies) following the manufacturer's protocol. Cells were stained with anti-CD69 (BD Biosciences) to assess levels of surface markers of activation. Proliferation was measured by flow cytometry and data was analyzed with FlowJo v7.6.5 or v10. Readouts were reported as fold change over baseline. In some embodiments, baseline was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody alone, wherein the cells were not incubated with a Cbl-b inhibitor. In some embodiments, baseline was the measurement obtained from total human T-cells stimulated with anti-CD3 antibody and anti-CD28 antibody, where the cells were not incubated with a Cbl-b inhibitor.

[635] Cbl-b inhibitor effects on primary human T-cells were also evaluated in the context of an allogenic mixed lymphocyte reaction (MLR). Allogenic immature dendritic cells were generated under the following conditions. Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Monocytes were isolated from the PMBCs utilizing positive selection with a commercial kit following the manufacturer's protocol (StemCells Catalog #17858) to yield >95% CD14⁺ cells as assessed by flow cytometry. Monocytes were cultured with 30 ng/mL of recombinant human GM-CSF and 20 ng/mL of recombinant human IL-4 for seven days to generate immature dendritic cells. Monocytes and T-cells were either isolated fresh from peripheral blood or thawed from frozen stocks. Human T-cells were isolated, labeled with CFSE and incubated with inhibitors as described above. The Cbl-b inhibitor was added to 1 x

10^5 T-cells in coculture with 2×10^3 allogenic immature dendritic cells per well at multiple concentrations (*e.g.*, 10 μ M, or 1.11 μ M) with a final DMSO concentration of <0.1% and incubated at 37 °C in 5% CO₂ for 5 days. Proliferation of the T-cells was evaluated by flow cytometry.

[636] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from T-cells (*e.g.*, GM-CSF, IFN γ , and TNF α) and/or surface expression of cell surface markers on T-cells (*e.g.*, CD69) that was indicative of T-cell activation. Cbl-b inhibitors were also tested to determine their ability to induce or enhance T-cell proliferation. Cbl-b inhibitors were tested for their effects on T-cell activation in the presence of costimulation and where conditions were suboptimal for priming.

Human T-cell in vitro models of T-cell exhaustion

[637] T-cell exhaustion was characterized by cells having a poor effector response and a sustained level of inhibitory receptor expression that results in T-cell dysfunction in response to chronic infections and cancer. *In vitro* models of T-cell exhaustion include allogenic and autologous models. In an autologous model, myeloid cells and SEB (Staphylococcal enterotoxin B, Millipore) were used to stimulate anti-CD3 stimulated T-cells. Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Monocytes were isolated with commercial kits using negative selection with StemCells EasySep Human Monocyte Enrichment Kit without CD16 Depletion (Catalog #19058) following the manufacturer's protocol. Isolated monocytes were cultured in complete media (*e.g.* RPMI 1640 with no additives, 10% HI FBS, 1X Glutamine and 1X β -mercaptoethanol) with 50 ng/mL recombinant human M-CSF (R&D System or Peprotech). Cells were plated at 2×10^6 cells per well (Day 0) and cultured for 5 days and were fed with fresh media and cytokines on Day 2. On Day 5, IFN γ was added at 100 ng/mL and the cells were incubated overnight. Primary human T-cells from the same donor were isolated from PBMCs with a commercial kit using negative selection (with Stemcell Technologies EasySep Human T-cell Isolation Kit (Catalog #17951) following the manufacturer's protocol. Purity was confirmed by surface marker detection by flow cytometry for CD4, CD8, CD45RA, CD45RO, CD19, CD14, CD56, and CD3 (BD Biosciences). 3×10^6 cells per/mL T-cells were stimulated with 10 μ g/mL of plate bound anti-CD3 antibody (Clone UCHT-1) for 5 days. This was done in parallel with myeloid cell generation. On Day 6, 2.5×10^4 T-cells were added per well, 12.5×10^3 myeloid cells per well and SEB antigen (0.1 μ g/mL)

were added to wells of a round bottom 96-well plate. Test agents (*e.g.* Cbl-b inhibitor compounds) or controls (*e.g.*, checkpoint neutralizing antibodies such as anti-PD1 antibody) were added to the wells at the indicated concentrations (*e.g.*, 10 μ M). Cells were cultured for 3 days at which point cell free supernatants were collected and assessed for secreted cytokines (*e.g.*, GM-CSF, IFN γ , and IL-2) by ELISA (R&D Systems, Peprotech or Life Technologies) or Luminex multiplex kits (Procarta Life Technologies). The T-cells were stained for a panel of surface markers including checkpoint inhibitors (*e.g.*, CTLA4) and evaluated by flow cytometry for Cbl-b inhibitor effects.

[638] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from exhausted T-cells (*e.g.*, GM-CSF, IFN γ , and IL-2) in the presence of myeloid cells, which was indicative of decreased T-cell exhaustion. Cbl-b inhibitors were also tested for their effects on checkpoint modulator expression levels following activation of exhausted T-cells.

Human T-cell in vitro models of T-cell Anergy

[639] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary T-cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-096-535 (*i.e.*, cocktail of antibodies against surface markers CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a were incubated with the PBMCs before passing the samples by magnetic beads for removal of cells expressing those surface markers) or Stemcell Technologies Catalog #17951) to yield >95% CD3+ cells assessed by flow cytometry. The cells were activated with immobilized anti-CD3 antibody (OKT3) and soluble anti-CD28 antibody (28.2) for two days at which time they were washed and allowed to rest for three days in the absence of stimulation. They were then treated with ionomycin (Sigma) for 18-24 hours to induce anergy. Following two washes to remove the ionomycin from the samples, Cbl-b inhibitor compounds were added to the cells at the indicated concentrations (*e.g.*, 10, 1.11, and 0.37 μ M) and incubated for one hour. The cells were then re-challenged with anti-CD3 antibody and anti-CD28 antibody for 24 hours at which point cell free supernatants were collected and assessed for cytokines (*e.g.*, IFN γ) by ELISA (R&D Systems or Peprotech) or Luminex multiplex kits (Procarta Life Technologies) following the manufacturer's protocols.

[640] Cbl-b inhibitors were tested to determine their ability to induce or enhance secretion of cytokines from anergic T-cells (*e.g.*, IFN γ), which was indicative of decreased T-cell tolerance.

In vivo activity of Cbl-b inhibitors

[641] A method of determining the pharmacodynamic profile of Cbl-b inhibitors was performed by dosing strains of mice with competent immune systems such as C57BL/6 or BALB/c mice with a Cbl-b inhibitor. The Cbl-b inhibitor was dissolved in a suitable formulation and administered by one of various routes such as intravenous (IV), intraperitoneal (IP), subcutaneous (SC), or oral (PO), at a suitable dose level and frequency (*e.g.*, twice per day BID or thrice per day TID) as informed by prior pharmacokinetic and tolerability studies. Following administration of the Cbl-b inhibitor, T-cells and indirectly other immune cells (*e.g.*, via cytokine production) were stimulated *in vivo* by administration of an anti-CD3 antibody or antigen-binding fragment thereof in PBS at defined amounts such as 2 μ g or 10 μ g per animal by routes such as IV or IP (*see* Hirsh et al., J. Immunol., 1989; Ferran et al., Eur. J. Immunol., 1990). Additional study control arms included groups of mice treated with a vehicle formulation alone (*i.e.*, formulation without the Cbl-b inhibitor and anti-CD3 antibody), a formulation containing the Cbl-b inhibitor alone, a formulation containing the anti-CD3 antibody alone, PBS alone, or combinations of these agents. The level of immune activation was then assessed by analysis of plasma cytokine levels and/or expression of activation markers on immune cells (*e.g.*, T-cells). Blood or lymphoid organs (*e.g.*, spleen) were collected at defined time points (*e.g.*, 8 hours or 24 hours). Blood samples were processed to collect plasma for determination of cytokine levels using standard methods known in the art. Cytokines measured included IL-2, IFN γ , and TNF α . Additional blood samples and lymphoid tissues were processed for flow cytometric analysis of immune cells (*e.g.*, T-cells) using standard methods to determine expression of cell type-specific markers and activation markers such as CD25 and/or CD69. Augmentation of immune stimulation by Cbl-b inhibitor administration was assessed by comparing the relative concentrations of cytokines in plasma, or the expression levels of activation markers on immune cells between appropriate groups (*e.g.*, mice treated with Cbl-b inhibitor and 2 μ g anti-CD3 antibody versus mice treated with vehicle and 2 μ g anti-CD3 antibody).

[642] Cbl-b inhibitors were tested to determine their ability to induce or enhance the level of cytokines (*e.g.*, IL-2, IFN γ , and TNF α) in blood obtained from treated mice stimulated with an anti-CD3 antibody, which was indicative of modulation of the immune response. Cbl-b inhibitors were also tested to determine their ability to induce or enhance the expression of cell

surface markers on T-cells (*e.g.*, CD25 and/or CD69) isolated from treated mice stimulated with an anti-CD3 antibody, which was indicative of modulation of the immune response.

B-Cell Activation Assay

[643] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Human primary B-cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Stemcell Technologies Catalog #17954) to yield >95% CD20+ cells assessed by flow cytometry. Primary human B-cells were plated at $0.7-1 \times 10^5$ per well in a 96-well plate with Cbl-b inhibitors over a dose ranging from 10 μ M to 1 nM and incubated at 37 °C 5% CO₂, with a final DMSO concentration of <0.5%. Cells were stimulated with anti-IgM for 20 hours at 37 °C 5% CO₂. Surface activation markers on mature CD20⁺ IgD⁺ B-cells were monitored by FACS using an anti-CD69 antibody (BD Biosciences).

[644] Cbl-b inhibitors were tested to determine their ability to induce or enhance surface expression of cell surface markers on B-cells (*e.g.*, CD69), which was indicative of B-cell activation.

Purification and activation of primary human NK-cells.

[645] Peripheral blood mononuclear cells (PBMC) were obtained either: 1) by using Ficoll-Paque™ (GE Healthcare) for separation of peripheral blood hematopoietic cells from buffy coats of healthy human donors; or 2) directly from LeukoPak donations. Total human primary NK-cells were isolated from the PBMCs utilizing negative selection with commercial kits following the manufacturer's protocol (Miltenyi Biotec Catalog #130-092-657 or Stemcell Technologies Catalog #17955) to yield >92% CD56⁺, CD3⁺ cells as assessed by flow cytometry. The cells were cultured overnight with IL-2 (60 ng/mL) at 37 °C 5% CO₂. Cbl-b inhibitors were added one hour prior to stimulation and incubated at 37 °C 5% CO₂ at a specific concentration (*e.g.*, 10 μ M, 1 μ M, or 0.1 μ M) with a final DMSO concentration of <0.1%. NK-cells were co-cultured with target cells that were engineered to have a red nucleus (K562 NucRed) measurable by flow cytometry. K562 NucRed cells were produced by transduction of K562 cells with IncuCyte NucLight Red Lentivirus reagent (Catalog #4476) and selected for 5 days. Clonal populations were isolated and expanded using standard tissue culture techniques, and individual clones were validated by comparison to wildtype K562 cells in NK-cell killing assays. The cells were mixed at the indicated ratios (*e.g.*, 5:1, 1:1, or 1:5) of NK

(effector cells) to K562 NucRed (target cells) for 6 hours. Cell free supernatants were collected and analyzed for cytokine secretion (*e.g.*, TNF α , IFN γ , or MIP1 β) by ELISA or Luminex multiplex kits following the manufacturer's protocol. IFN γ secretion was assessed using an R&D Systems ELISA kit (Catalog #DY285), TNF α secretion was assessed using an R&D Systems ELISA kit (Catalog #DY210), and MIP1 β secretion was assessed using an R&D Systems ELISA kit (Catalog #DY271).

BIOLOGICAL EXAMPLE 14: Evaluation of a Cbl-b Inhibitor in Combination with an Oncolytic Virus for Treating Cancer

[646] Oncolytic viruses preferentially infect and kill cancer cells and stimulate host anti-tumor immune responses. This example describes the evaluation of a combination therapy including an oncolytic virus and a Cbl-b inhibitor for treating cancer in mice.

Materials and Methods

[647] In brief, the combination therapy was tested in C57BL/6 mice bearing a syngeneic MC-38 cell tumor. MC-38 cells were derived from a murine adenocarcinoma, which had been induced by subcutaneous injection of dimethylhydrazine (Cameron *et al.*, J Exp Med, 171: 249-263, 1990). MC-38 cells were injected subcutaneously or intraperitoneally. Tumors were allowed to grow for about 5-7 days at which time the animals were randomized and treatment was initiated.

[648] An oncolytic virus, such as vaccinia virus, was administered intraperitoneally at a dose of about 10⁸-10⁹ plaque forming units (Puhlmann *et al.*, Cancer Gene Therapy, 7: 66-73, 2000). The Cbl-b inhibitor was dissolved in a suitable formulation and administered at a suitable dose level and frequency as informed by prior pharmacokinetic and tolerability studies. In initial studies, a Cbl-b inhibitor in a suitable vehicle, or the vehicle alone, was administered orally (PO) at a dose of 180 mg/kg twice per day (BID) beginning on Day 0 (= Day 5-7 post MC-38 injection). In further studies, a Cbl-b inhibitor formulation was administered orally (PO) or parenterally (*e.g.*, IV, IP, SC, or intratumorally at one to three injection sites per tumor). In addition to the test group of mice who received the combination therapy (the Cbl-b inhibitor formulation plus the oncolytic virus), the study included control groups of mice who received: the vehicle formulation plus DPBS, the Cbl-b inhibitor formulation plus DPBS, or the vehicle formulation plus the oncolytic virus.

[649] The level of response was evaluated by measuring tumor growth and comparing tumor growth in the test mice versus the control mice. In addition, the level of immune activation was assessed by collecting tumors for analysis of tumor infiltrating lymphocytes (TILs). In further experiments, PBMCs or splenocytes were also collected. TILs and optionally other lymphocyte samples were processed for flow cytometric analysis using standard methods to determine antigen specificity, expression of cell type-specific markers, and expression of activation markers such as granzyme B, CD25, CD69, PD-1, and LAG3. Augmentation of the anti-tumor immune response by the combination therapy was assessed by comparing the relative percentage of immune cell populations in the tumor, and the relative levels of expression of activation markers on immune cells in mice of the test and study groups.

BIOLOGICAL EXAMPLE 15: Evaluation of Vaccine Combination

Material and Methods

[650] Mice were implanted with 50,000 TC-1 cells/mouse subcutaneously into the right flank at Day Zero (D0). Thirteen days later (D13), when tumors measured approximately 50-100 mm³, mice from appropriate groups (15 mice per group) were injected with HPV16 vaccine (s.c., total 2 doses, at D13 and Day 20 (D20)). Compound **23** was reconstituted in 0.5% Methylcellulose, 0.2% Polysorbate 80 and was administered orally from D13 to Day 47 (D47) at indicated doses. The group of mice that received the vaccine also received an oral vehicle (0.5% Methylcellulose, 0.2% Polysorbate 80). Mice were euthanized when tumor exceeded 1500-2000 mm³, according to IACUC guidelines. Survival was compared using GraphPad Prism using log-rank (Mantel–Cox) test. ** = p<0.001 of treatment vs. Vehicle + Vaccine control group at Day 61.

[651] TC-1 cells were derived by stable transfection of mouse lung epithelial cells with human papillomavirus strain 16 (HPV16) early proteins 6 and 7 (E6 and E7) and activated h-ras oncogene. Cells were obtained from Dr. T-C Wu (Johns Hopkins University). (Lin KY, Guarnieri FG, Staveley-O'Carroll KF, Levitsky HI, August JT, Pardoll DM, *et al.* Treatment of established tumors with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen. *Cancer Res* 1996;56:21–6).

[652] The HPV16 vaccine was prepared as follows: The CTL epitope from HPV16 E749–57 (9 amino acid (aa) peptide, RAHYNIVTF, 100 µg/mouse, SEQ ID NO: 2) was mixed with synthetic T helper epitope PADRE (13 aa peptide, aK-Cha-VAAWTLKAAa, SEQ ID NO: 3, where "a" is D-alanine and Cha is L-cyclohexylalanine, 20 µg/mouse) and with QuilA adjuvant (20 µg/mouse).

Results

[653] To evaluate the antitumor therapeutic response of Compound **23**, the TC-1 syngeneic mouse model was used. This model is poorly immunogenic and requires vaccination to generate an effector CD8+T cell immune response (Cancer Immunol Res. 2017 Sep;5(9):755-766). HPV16 vaccination generates a CD8+T cell response against E7 tumor antigen. FIGS. A and B show the effects of the vaccine in combination with Compound **23** on survival and tumor growth. Although the vaccine alone minimally affected tumor growth and survival of the mice (FIGS. 1 and 2), Compound **23** in combination with vaccine led to significant slowdown of tumor progression (FIG. 2) and was associated with prolonged survival (FIG. 1). These results demonstrate that Compound **23** significantly improved the E7-specific CD8+ T cell response induced by the vaccine, leading to an antitumor response and prolonged survival in tumor bearing mice.

BIOLOGICAL EXAMPLE 16: Single Agent Cancer Therapy

Material and Methods

[654] Mice were implanted with 100,000 colon carcinoma CT26 tumor cells on both flanks of the mice. Eight days later (D8), when tumors measured approximately 50 mm³, mice from appropriate groups (12 mice per group) were treated orally (PO) with Compound **23** (30 mg/kg) or a vehicle control. Treatments were continued daily until Day Twenty Eight (D28). Compound **23** was reconstituted in 0.5% Methylcellulose, 0.2% Polysorbate 80. A group of mice received an oral vehicle (0.5% Methylcellulose, 0.2% Polysorbate 80). Tumor growth inhibition (TGI) was calculated as follows: $(1 - (\text{median tumor volume of drug treated group} / \text{median tumor volume of vehicle treated group})) \times 100$. Vehicle and Compound **23** curves were compared using GraphPad Prism software using Two-way ANOVA Bonferroni's multiple comparison test.

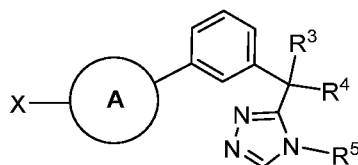
[655] To evaluate the antitumor therapeutic response of Compound **23**, the CT26 syngeneic mouse model was used. FIGS. 3 and 4 show the effects of Compound **23** on CT26 tumor growth, where tumor volumes of individual tumors are shown (tumor volumes from both flanks were included). These results demonstrate that Compound **23** significantly inhibits growth of established tumors (TGI 68%) compared to vehicle control.

[656] The disclosures of all publications, patents, patent applications, and published patent applications referred to herein by an identifying citation are hereby incorporated herein by reference in their entirety.

[657] Although aspects of the foregoing disclosure have been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of this disclosure.

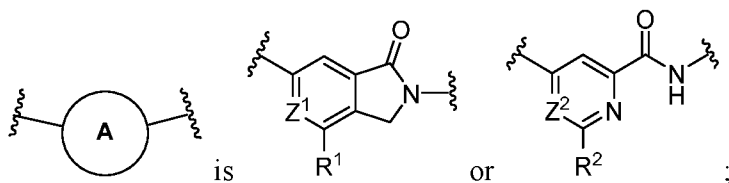
What is claimed is:

1. A compound of Formula (I)



(I)

or a tautomer thereof, or a pharmaceutically acceptable salt thereof, wherein



Z¹ is CH or nitrogen;

Z² is CH or nitrogen;

R¹ is -CF₃ or cyclopropyl;

R² is -CF₃ or cyclopropyl;

R³ is hydrogen, C₁-C₂ alkyl, or C₁-C₂ haloalkyl;

R⁴ is hydrogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, 4- to 8-membered heterocyclyl, or C₃-C₆ cycloalkyl,

wherein the heterocyclyl or cycloalkyl groups are optionally substituted by one to five R⁶ groups;

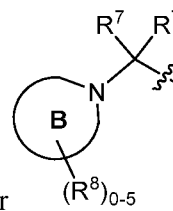
or R³ and R⁴ are taken together with the carbon atom to which they are attached to form a C₃-C₅ cycloalkyl or 4- to 6-membered heterocyclyl, each of which is optionally substituted by one to five R⁶ groups;

R⁵ is hydrogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₃-C₆ cycloalkyl;

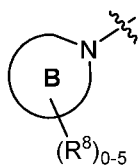
each R^6 is independently C_1 - C_6 alkyl, halo, hydroxy, $-O(C_1$ - C_6 alkyl), $-CN$, C_1 - C_6 alkyl- CN , C_1 - C_6 alkyl- OH , or C_1 - C_6 haloalkyl;

or two R^6 groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro C_3 - C_6 cycloalkyl or spiro 4- to 6-membered heterocyclyl;

X is hydrogen, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl, C_1 - C_6 alkyl- OH , C_1 - C_6 alkyl- CN ,



C_3 - C_6 cycloalkyl optionally substituted by one to five R^8 groups, or



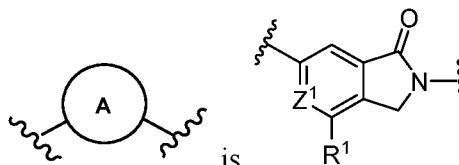
is 4- to 7-membered heterocyclyl or 5- to 8- membered heteroaryl, wherein each heterocyclyl or heteroaryl optionally contains one to two additional heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur, and wherein each heterocyclyl or heteroaryl is optionally substituted by one to five R^8 groups;

each R^7 is independently hydrogen, C_1 - C_6 alkyl, C_1 - C_6 alkyl- OH , or C_1 - C_6 haloalkyl;

or two R^7 groups are taken together with the carbon atom to which they are attached to form a C_3 - C_5 cycloalkyl or 3- to 5- membered heterocyclyl; and

each R^8 is independently halo, C_1 - C_6 alkyl, C_1 - C_6 alkyl- CN , C_1 - C_6 alkyl- OH , C_1 - C_6 haloalkyl, $-CN$, oxo, or $-O(C_1$ - C_6 alkyl);

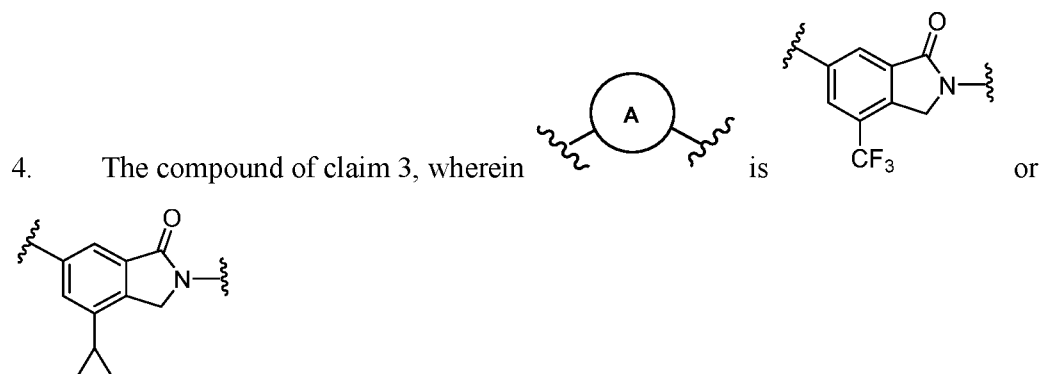
or two R^8 groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused C_3 - C_5 cycloalkyl or 3- to 5-membered heterocyclyl.



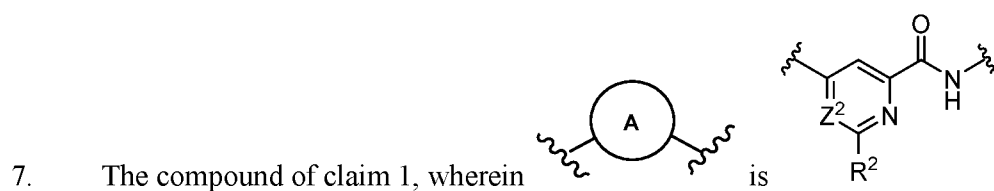
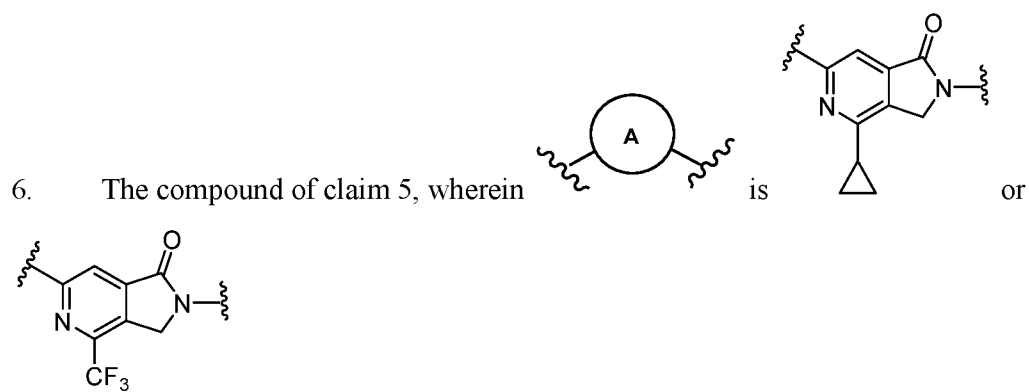
2. The compound of claim 1, wherein

is

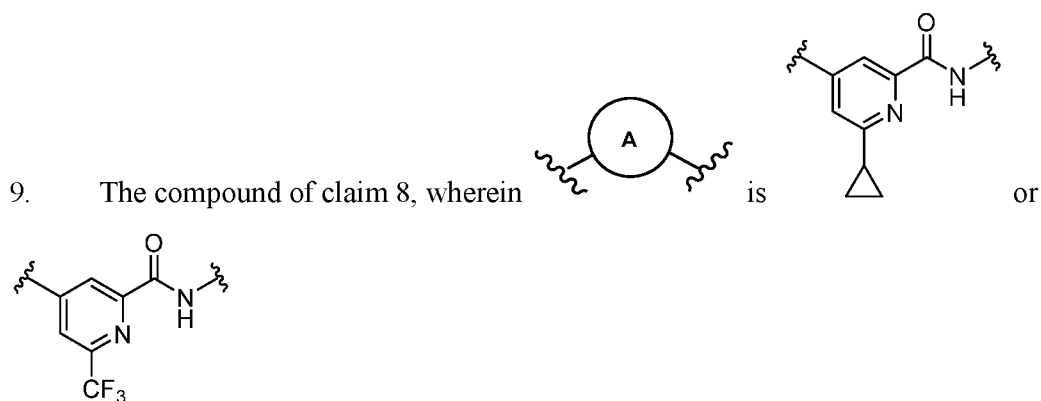
3. The compound of claim 2, wherein Z^1 is CH.



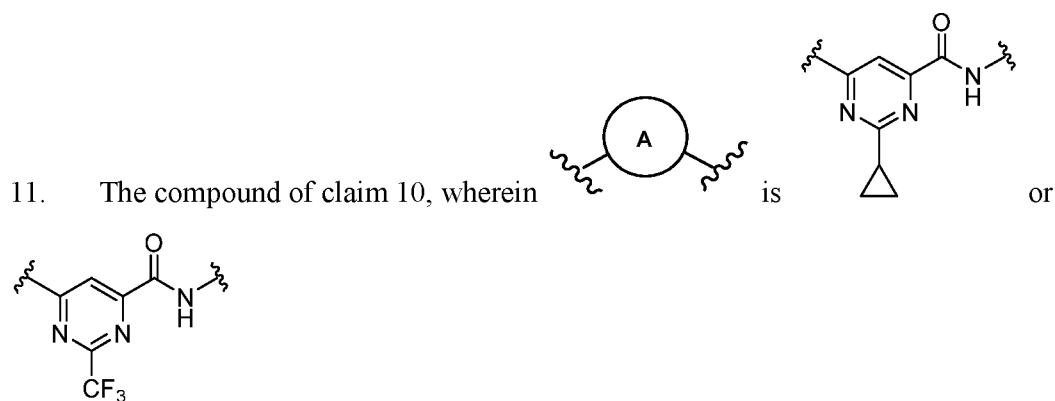
5. The compound of claim 2, wherein Z^1 is N.



8. The compound of claim 7, wherein Z^2 is CH.



10. The compound of claim 7, wherein Z^2 is N.



12. The compound of any one of claims 1-11, wherein

R^3 is hydrogen, C_1 - C_2 alkyl, or C_1 - C_2 haloalkyl;

R^4 is hydrogen, C_1 - C_3 alkyl, C_1 - C_3 haloalkyl, 4- to 6-membered heterocyclyl, or C_4 - C_5 cycloalkyl,

wherein the heterocyclyl or cycloalkyl groups are optionally substituted by one to three R^6 groups;

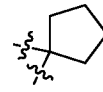
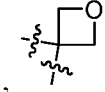
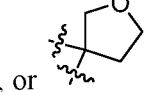
or R^3 and R^4 are taken together with the carbon atom to which they are attached to form a C_4 - C_5 cycloalkyl or 4- to 6-membered heterocyclyl, each of which is optionally substituted by one to three R^6 groups.

13. The compound of claim 12, wherein

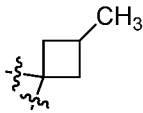
R^3 is hydrogen, $-CH_3$, or $-CF_3$; and

R^4 is hydrogen, $-CH_3$, $-CF_3$, cyclobutyl, or  ;

or R^3 and R^4 are taken together with the carbon atom to which they are attached to form

, , , or , each of which is optionally substituted by one to three R^6 groups.

14. The compound of claim 13, wherein R^3 and R^4 are taken together with the carbon

atom to which they are attached to form .

15. The compound of any one of claims 1-13, wherein

each R^6 is, independently, C_1 - C_3 alkyl, halo, hydroxy, $-O(C_1$ - C_3 alkyl), $-CN$,
 C_1 - C_3 alkyl- CN , C_1 - C_3 alkyl- OH , or C_1 - C_3 haloalkyl;

or two R^6 groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro C_3 - C_6 cycloalkyl or spiro 4- to 5-membered heterocyclyl.

16. The compound of claim 15, wherein

each R^6 is, independently, $-CH_3$, fluoro, hydroxy, $-OCH_3$, $-CN$, $-CH_2CN$, $-CH_2OH$, or $-CF_3$;

or two R^6 groups attached to the same carbon atom are taken together with the carbon atom to which they are attached to form a spiro cyclopropyl.

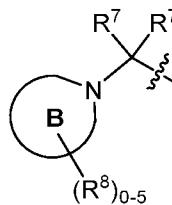
17. The compound of any one of claims 1-16, wherein R^5 is hydrogen, C_1 - C_3 alkyl, C_1 - C_3 haloalkyl, or C_3 - C_4 cycloalkyl.

18. The compound of claim 17, wherein R^5 is hydrogen, $-CH_3$, $-CHF_2$, or cyclopropyl.

19. The compound of any one of claims 1-18, wherein

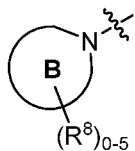
X is hydrogen, C_1 - C_3 alkyl, C_1 - C_3 haloalkyl, C_1 - C_3 alkyl- OH , C_1 - C_3 alkyl- CN , or C_3 - C_5 cycloalkyl optionally substituted by one to three R^8 groups.

20. The compound of claim 19, wherein **X** is hydrogen or -CH₃.



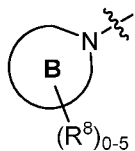
21. The compound of any one of claims 1-18, wherein **X** is

22. The compound of claim 21, wherein

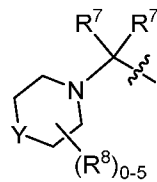


(R⁸)₀₋₅ is a 4- to 6-membered heterocyclyl or 5- to 6- membered heteroaryl, wherein each heterocyclyl or heteroaryl optionally contains one to two additional heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur, and wherein each heterocyclyl or heteroaryl is optionally substituted by one to five **R**⁸ groups.

23. The compound of claim 22, wherein



(R⁸)₀₋₅ is a 4- to 5-membered heterocyclyl or 5- to 6- membered heteroaryl, wherein each heterocyclyl or heteroaryl optionally contains one additional heteroatom selected from the group consisting of nitrogen and oxygen, and wherein each heterocyclyl or heteroaryl is optionally substituted by one to five **R**⁸ groups.



24. The compound of claim 21 or 22, wherein **X** is

25. The compound of claim 24, wherein **Y** is oxygen.

26. The compound of claim 24, wherein **Y** is -CH₂-, -CHR⁸-, or -C(R⁸)₂-.

27. The compound of any one of claims 21-26, wherein

each **R**⁷ is, independently, hydrogen, C₁-C₃ alkyl, C₁-C₃ alkyl-OH, or C₁-C₃ haloalkyl;

or two **R**⁷ groups are taken together with the carbon atom to which they are attached to form a C₃-C₅ cycloalkyl or 3- to 5- membered heterocycl.

28. The compound of claim 27, wherein

each **R**⁷ is, independently, hydrogen, -CH₃, -CH₂OH, or -CF₃;

or two **R**⁷ groups are taken together with the carbon atom to which they are attached to form a cyclopropyl or oxetanyl.

29. The compound of any one of claims 21-28, wherein

each **R**⁸ is, independently, halo, C₁-C₃ alkyl, C₁-C₃ alkyl-CN, C₁-C₃ alkyl-OH, C₁-C₃ haloalkyl, -CN, oxo, or -O(C₁-C₃ alkyl);

or two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused C₃-C₅ cycloalkyl or a spiro or fused 3- to 5-membered heterocycl.

30. The compound of claim 29, wherein

each **R**⁸ is, independently, fluoro, -CH₃, -CH₂CH₃, -CH₂CN, -CH₂OH, -CF₃, -CN, oxo, or -OCH₃;

or two **R**⁸ groups are taken together with the carbon atom or atoms to which they are attached to form a spiro or fused cyclopropyl or oxetanyl.

31. A compound selected from the compounds in Table 1, or a tautomer thereof, or a pharmaceutically acceptable salt thereof.

32. A pharmaceutical composition comprising the compound of any one of claims 1-31, and a pharmaceutically acceptable excipient.

33. A method of modulating activity of an immune cell, the method comprising contacting the immune cell with an effective amount of a Cbl-b inhibitor to modulate activity of the immune cell, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.

34. The method of claim 33, wherein the immune cell comprises a T-cell, a B-cell, or a natural killer (NK)-cell.
35. The method of claim 33 or claim 34, wherein the immune cell has been or is isolated from a blood sample from a mammalian subject.
36. The method of claim 33 or claim 34, wherein the immune cell is a tumor infiltrating lymphocyte (TIL) that has been or is isolated from a tumor of a mammalian subject with cancer.
37. The method of any one of claims 33-36, wherein the immune cell comprises a T-cell, and wherein modulating activity of the T-cell comprises one or more of increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and decreased T-cell tolerance.
38. The method of claim 37, wherein increased T-cell activation comprises increased production of a cytokine.
39. The method of claim 38, wherein the cytokine comprises one or more selected from the group consisting of IL-2, IFN- γ , TNF α , and GM-CSF.
40. The method of claim 37 or 38, wherein increased T-cell activation comprises increased cell surface expression of one or more T-cell activation markers.
41. The method of claim 40, wherein the T-cell activation markers comprise one or more selected from the group consisting of CD25, CD69, and CTLA4.
42. The method of any one of claims 37-41, wherein the T-cell has been or is in contact with an anti-CD3 antibody alone or in combination with an anti-CD28 antibody.
43. The method of any one of claims 37-41, further comprising culturing the immune cell with IL-2 alone or in combination with an anti-CD3 antibody and/or an anti-CD28 antibody.
44. The method of any one of claims 33-36, wherein the immune cell comprises a NK-cell, and wherein modulating activity of an NK-cell comprises increased NK-cell activation.
45. The method of claim 44, wherein increased NK-cell activation comprises increased production of a cytokine.

46. The method of claim 45, wherein the cytokine comprises one or more selected from the group consisting of IFN- γ , TNF α , and MIP1 β .
47. The method of any one of claims 33-46, wherein the immune cell comprises a B-cell, and wherein modulating activity of a B-cell comprises increased B-cell activation, optionally wherein increased B-cell activation comprises increased expression of CD69.
48. The method of any one of claims 33-47, wherein the immune cell is a human immune cell.
49. The method of any one of claims 33-48, wherein the immune cell comprises a recombinant chimeric receptor.
50. The method of claim 49, wherein the recombinant chimeric receptor is a chimeric antigen receptor.
51. A method of producing a modified immune cell, comprising culturing a cell population containing an immune cell in the presence of an effective amount of a Cbl-b inhibitor to modulate activity of the immune cell, thereby producing the modified immune cell, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
52. The method of claim 51, further comprising culturing the immune cell with an anti-CD3 antibody alone or in combination with an anti-CD28 antibody.
53. The method of claim 51, further comprising culturing of the immune cell with IL-2 alone or in combination with an anti-CD3 antibody and/or an anti-CD28 antibody.
54. The method of any one of claims 51-53, further comprising recovering the modified immune cell.
55. The method of any one of claims 51-54, wherein the immune cell has been or is isolated from a blood sample from a mammalian subject, or the immune cell is a tumor infiltrating lymphocyte (TIL) that has been or is isolated from a tumor of a mammalian subject with cancer.

56. The method of any one of claims 51-54, wherein the immune cell is a cell selected from the group consisting of a hematopoietic cell, a multipotent stem cell, a myeloid progenitor cell, a lymphoid progenitor cell, a T-cell, a B-cell, and a NK-cell.
57. The method of any one of claims 51-54, wherein the modified immune cell is a cell selected from the group consisting of a hematopoietic cell, a multipotent stem cell, a myeloid progenitor cell, a lymphoid progenitor cell, a T-cell, a B-cell, and a NK-cell.
58. The method of any one of claims 51-57, wherein the immune cell is a tumor infiltrating lymphocyte (TIL).
59. The method of any one of claims 51-58, wherein the immune cell is a human immune cell.
60. The method of any one of claims 51-59, wherein the immune cell or modified immune cell comprises a recombinant chimeric receptor.
61. The method of claim 60, wherein the recombinant chimeric receptor is a chimeric antigen receptor.
62. A modified immune cell produced by the method of any one of claims 51-61.
63. A modified immune cell comprising a Cbl-b inhibitor, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
64. An isolated modified immune cell, wherein the immune cell has been contacted or is in contact with a Cbl-b inhibitor, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
65. The modified immune cell of claim 63 or claim 64, wherein the immune cell has been or is isolated from a blood sample from a mammalian subject, or the immune cell is a tumor infiltrating lymphocyte (TIL) that has been or is isolated from a tumor of a mammalian subject with cancer.
66. The modified immune cell of claim 64 or 65, wherein the immune cell is a tumor-infiltrating lymphocyte (TIL) isolated from a tumor of a mammalian subject with cancer before the immune cell is contacted with the Cbl-b inhibitor.

67. The modified immune cell of any one of claims 64-66, wherein the modified immune cell is a T-cell, and wherein the T-cell exhibits one or more of increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and decreased T-cell tolerance.

68. The modified immune cell of claim 67, wherein increased T-cell activation comprises increased production of a cytokine.

69. The modified immune cell of claim 68, wherein the cytokine comprises one or more selected from the group consisting of IL-2, IFN- γ , TNF α , and GM-CSF.

70. The modified immune cell of any one of claims 67-69, wherein increased T-cell activation comprises increased cell surface expression of one or more T-cell activation markers.

71. The modified immune cell of claim 70, wherein the T-cell activation markers comprise one or more selected from the group consisting of CD25, CD69, and CTLA4.

72. The modified immune cell of any one of claims 67-71, wherein the T-cell has been or is in contact with an anti-CD3 antibody alone or in combination with an anti-CD28 antibody.

73. The modified immune cell of any one of claims 67-71, wherein the T-cell has been or is in contact with IL-2 alone or in combination with an anti-CD3 antibody and/or an anti-CD28 antibody.

74. The modified immune cell of any one of claims 64-66, wherein the modified immune cell is a NK-cell, and wherein the NK-cell exhibits increased NK-cell activation.

75. The modified immune cell of claim 74, wherein increased NK-cell activation comprises increased production of a cytokine.

76. The modified immune cell of claim 75, wherein the cytokine comprises one or more selected from the group consisting of IFN- γ , TNF α , and MIP1 β .

77. The modified immune cell of any one of claims 64-66, wherein the modified immune cell is a B-cell, and wherein the B-cell exhibits increased B-cell activation, optionally wherein increased B-cell activation comprises increased expression of CD69.

78. The modified immune cell of any one of claims 64-77, wherein the modified immune cell is a human immune cell.
79. The modified immune cell of any one of claims 64-78, wherein the modified immune cell comprises a recombinant chimeric receptor.
80. The modified immune cell of claim 79, wherein the recombinant chimeric receptor is a chimeric antigen receptor.
81. A composition comprising a cell population containing the modified immune cell of any one of claims 62-80.
82. The composition of claim 81, further comprising a pharmaceutically acceptable excipient.
83. The composition of claim 81, wherein the composition is in a culture vessel.
84. The composition of claim 83, wherein the culture vessel is a tube, a dish, a bag, a multiwell plate, or a flask.
85. The composition of claim 81 or 82, wherein the composition is in a suitable container.
86. The composition of claim 85, wherein the suitable container is a bottle, a vial, a syringe, an intravenous bag, or a tube.
87. A method of modulating the immune response, the method comprising administering an effective amount of the modified immune cell of any one of claims 62-80 or an effective amount of the composition of any one of claims 81-86 to an individual in need thereof.
88. The method of claim 87, wherein the individual has a cancer.
89. A method of treating a cancer responsive to inhibition of Cbl-b activity, the method comprising administering an effective amount of the modified immune cell of any one of claims 62-80 or an effective amount of the composition of any one of claims 81-86 to an individual having the cancer responsive to inhibition of Cbl-b activity.
90. The method of claim 88 or 89, wherein the cancer is a hematologic cancer.

91. The method of claim 90, wherein the hematologic cancer is a lymphoma, a leukemia, or a myeloma.
92. The method of claim 88 or 89, wherein the cancer is a non-hematologic cancer.
93. The method of claim 92, wherein the non-hematologic cancer is a sarcoma, a carcinoma, or a melanoma.
94. A method of inhibiting abnormal cell proliferation, the method comprising administering an effective amount of the modified immune cell of any one of claims 62-80 or an effective amount of the composition of any one of claims 81-86 to an individual in need thereof.
95. The method of claim 94, wherein the abnormal cell proliferation is hyperplasia or cancer cell proliferation.
96. The method of claim 95, wherein the cancer cell is from a hematologic cancer.
97. The method of claim 96, wherein the hematologic cancer is a lymphoma, a leukemia, or a myeloma.
98. The method of claim 95, wherein the cancer cell is from a non-hematologic cancer.
99. The method of claim 98, wherein the non-hematologic cancer is a sarcoma, a carcinoma, or a melanoma.
100. A method of modulating the immune response, the method comprising administering an effective amount of a Cbl-b inhibitor to an individual to modulate the immune response in the individual, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
101. A method of inhibiting Cbl-b activity, the method comprising administering an effective amount of a Cbl-b inhibitor to an individual to inhibit Cbl-b activity in the individual, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
102. A method of treating a cancer responsive to inhibition of Cbl-b activity, the method comprising administering an effective amount of a Cbl-b inhibitor to an individual to treat the cancer responsive to inhibition of Cbl-b activity, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.

103. The method of claim 102, wherein the cancer is a hematologic cancer, optionally wherein the hematologic cancer is a lymphoma, a leukemia, or a myeloma.
104. The method of claim 102, wherein the cancer is a non-hematologic cancer, optionally wherein the non-hematologic cancer is a sarcoma, a carcinoma, or a melanoma.
105. The method of any one of claims 100-104, wherein the Cbl-b inhibitor is administered by enteral administration, optionally wherein the enteral administration is oral administration.
106. The method of any one of claims 100-104, wherein the Cbl-b inhibitor is administered by parenteral administration, optionally wherein the parenteral administration is intratumoral administration.
107. The method of any one of claims 102-106, further comprising administering an effective amount of the modified immune cell of any one of claims 62-80 or an effective amount of the composition of any one of claims 81-86 to the individual to treat the cancer.
108. A method of inhibiting abnormal cell proliferation, the method comprising administering an effective amount of a Cbl-b inhibitor to an individual to inhibit abnormal cell proliferation in the individual, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
109. The method of claim 108, wherein the abnormal cell proliferation is hyperplasia or cancer cell proliferation.
110. The method of claim 109, wherein the cancer cell is from a hematologic cancer, optionally wherein the hematologic cancer is a lymphoma, a leukemia, or a myeloma.
111. The method of claim 109, wherein the cancer cell is from a non-hematologic cancer, optionally wherein the non-hematologic cancer is a sarcoma, a carcinoma, or a melanoma.
112. The method of any one of claims 108-111, wherein the Cbl-b inhibitor is administered by enteral administration, optionally wherein the enteral administration is oral administration.
113. The method of any one of claims 108-111, wherein the Cbl-b inhibitor is administered by parenteral administration, optionally wherein the parenteral administration is intratumoral

injection, or the parenteral administration is by a route selected from the group consisting of intravenous, intraperitoneal, and subcutaneous.

114. The method of any one of claims 100-113, wherein the individual has one or more of increased T-cell activation, increased T-cell proliferation, decreased T-cell exhaustion, and decreased T-cell tolerance after administration of the Cbl-b inhibitor.

115. The method of claim 114, wherein increased T-cell activation comprises increased production of a cytokine.

116. The method of claim 115, wherein the cytokine comprises one or more selected from the group consisting of IL-2, IFN- γ , TNF α , and GM-CSF.

117. The method of any one of claims 114-116, wherein increased T-cell activation comprises increased cell surface expression of one or more T-cell activation markers.

118. The method of claim 117, wherein the T-cell activation markers comprise one or more selected from the group consisting of CD25, CD69, and CTLA4.

119. The method of any one of claims 100-118, wherein the individual has increased NK-cell activation after administration of the Cbl-b inhibitor.

120. The method of claim 119, wherein increased NK-cell activation comprises increased production of a cytokine.

121. The method of claim 120, wherein the cytokine comprises one or more selected from the group consisting of IFN- γ , TNF α , and MIP1 β .

122. The method of any one of claims 100-121, wherein the individual has increased B-cell activation after administration of the Cbl-b inhibitor, optionally wherein increased B-cell activation comprises increased expression of CD69.

123. A cell culture composition comprising a cell population containing an immune cell and a Cbl-b inhibitor, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.

124. The cell culture composition of claim 123, wherein the immune cell is a cell selected from the group consisting of a hematopoietic cell, a multipotent stem cell, a myeloid progenitor cell, a lymphoid progenitor cell, a T-cell, a B-cell, and a NK-cell.

125. The cell culture composition of claim 123 or 124, further comprising an anti-CD3 antibody alone or in combination with an anti-CD28 antibody.
126. The cell culture composition of any one of claims 123-125, wherein the immune cell is an engineered immune cell comprising a recombinant chimeric receptor.
127. The cell culture composition of claim 126, wherein the recombinant chimeric receptor is a chimeric antigen receptor.
128. A pharmaceutical composition comprising a Cbl-b inhibitor and one or both of an adjuvant and an antigen, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
129. The pharmaceutical composition of claim 128, wherein the antigen is a cancer antigen.
130. An article of manufacture comprising the modified immune cell of any one of claims 62-80, the composition of any one of claims 81-86, the cell culture composition of any one of claims 123-127, or the pharmaceutical composition of claim 32.
131. The article of manufacture of claim 130, wherein the modified immune cell or cell culture composition is in a tube, a dish, a bag, a multiwell plate, or a flask.
132. The article of manufacture of claim 130, wherein the modified immune cell or pharmaceutical composition is in a bottle, a vial, a syringe, an intravenous bag, or a tube.
133. A kit comprising the modified immune cell of any one of claims 62-80 or the composition of any one of claims 81-86.
134. The kit of claim 133, wherein the modified immune cell is in a tube, a dish, a bag, a multiwell plate, or a flask.
135. The kit of claim 133, wherein the modified immune cell is in a bottle, a vial, a syringe, an intravenous bag, or a tube.
136. The kit of any one of claim 133-135, wherein the kit comprises instructions for administering the modified immune cell or composition to an individual according to the method of any one of claims 87-99.

137. A kit comprising the pharmaceutical composition of claim 32.
138. The kit of claim 137, wherein the kit comprises instructions for administering the pharmaceutical composition to an individual according to the method of any one of claims 100-102.
139. A kit comprising the cell culture composition of any one of claims 123-127.
140. The kit of claim 139, wherein the kit comprises instructions for producing a modified immune cell according to the method of any one of claims 51-61.
141. A method for treating or preventing a disease or condition associated with Cbl-b activity, the method comprising administering a Cbl-b inhibitor to an individual in need thereof, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
142. Use of a Cbl-b inhibitor in the manufacture of a medicament for treating or preventing a disease or condition associated with Cbl-b activity, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
143. Use of a Cbl-b inhibitor in the manufacture of a medicament for treating cancer, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
144. A Cbl-b inhibitor for use in treating or preventing a disease or condition associated with Cbl-b activity, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
145. A Cbl-b inhibitor for use in treating cancer, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31.
146. A method of treating cancer, the method comprising
administering to an individual with cancer an effective amount of a Cbl-b inhibitor,
wherein the Cbl-b inhibitor is a compound of any one of claims 1-31, and
administering to the individual an effective amount of an additional therapeutic agent.
147. The method of claim 146, wherein the Cbl-b inhibitor and the additional therapeutic agent are administered consecutively in either order.
148. The method of claim 146, wherein the Cbl-b inhibitor and the additional therapeutic agent are administered concurrently.

149. The method of any one of claims 146-148, wherein the additional therapeutic agent comprises an immune checkpoint inhibitor.

150. The method of claim 149, wherein the immune checkpoint inhibitor is an antagonist of at least one inhibitory checkpoint molecule selected from the group consisting of PD-1 (CD279), PD-L1 (CD274), CTLA-4 (CD125), LAG3 (CD223), PVR (CD155), PVRL2 (CD112), PVRL3 (CD113), TIGIT, TIM3 (CD366), and VISTA.

151. The method of claim 149, wherein the immune checkpoint inhibitor is an antagonist of at least one inhibitory checkpoint molecule selected from the group consisting of PD-1 (CD279), PD-L1 (CD274), and CTLA-4 (CD152).

152. The method of claim 151, wherein the at least one inhibitory checkpoint molecule comprises PD-1, optionally wherein the immune checkpoint inhibitor is selected from the group consisting of pembrolizumab, nivolumab, cemiplimab, and biosimilars thereof.

153. The method of claim 151, wherein the at least one inhibitory checkpoint molecule comprises PD-L1, optionally wherein the immune checkpoint inhibitor is selected from the group consisting of atezolizumab, avelumab, durvalumab, and biosimilars thereof.

154. The method of claim 151, wherein the at least one inhibitory checkpoint molecule comprises CTLA-4, optionally wherein the immune checkpoint inhibitor is selected from the group consisting of ipilimumab, tremelimumab, and biosimilars thereof.

155. The method of any one of claims 149-151, wherein the immune checkpoint inhibitor comprises an antibody or antigen-binding fragment thereof, optionally wherein the antibody or fragment is human or humanized.

156. The method of any one of claims 146-155, wherein the additional therapeutic agent comprises an antineoplastic agent.

157. The method of claim 156, wherein the antineoplastic agent is classified as one of the group consisting of a cytotoxic antibiotic, a plant alkaloid, an antimetabolite, an alkylating agent, and other antineoplastic agent.

158. The method of claim 157, wherein the antineoplastic agent comprises a cytotoxic antibiotic, optionally wherein the cytotoxic antibiotic is selected from the group consisting of

ixabepilone, mitomycin, plicamycin, bleomycin, pixantrone, amrubicin, valrubicin, pirarubicin, mitoxantrone, idarubicin, zorubicin, aclarubicin, epirubicin, daunorubicin, doxorubicin, and dactinomycin.

159. The method of claim 157, wherein the antineoplastic agent comprises a plant alkaloid, optionally wherein the plant alkaloid is selected from the group consisting of trabectedin, cabazitaxel, paclitaxel poliglumex, docetaxel, paclitaxel, demecolcine, teniposide, etoposide, vintafolide, vinflunine, vinorelbine, vindesine, vincristine, and vinblastine.

160. The method of claim 157, wherein the antineoplastic agent comprises an antimetabolite, optionally wherein the antimetabolite is a pyrimidine analog, a purine analog, or a folic acid analog, optionally wherein the antimetabolite is selected from the group consisting of floxuridine, trifluridine, tegafur, fluorouracil, decitabine, azacitidine, capecitabine, gemcitabine, carmofur, tegafur, fluorouracil, cytarabine, nelarabine, clofarabine, fludarabine, cladribine, tioguanine, mercaptopurine, pralatrexate, pemetrexed, raltitrexed, and methotrexate.

161. The method of claim 157, wherein the antineoplastic agent comprises an alkylating agent, optionally wherein the alkylating agent is selected from the group consisting of dacarbazine, temozolomide, pipobroman, mitobronitol, etoglucid, uracil mustard, ranimustine, nimustine, fotemustine, streptozocin, semustine, lomustine, carmustine, carboquone, triaziquone, thiotepa, mannosulfan, treosulfan, busulfan, bendamustine, prednimustine, trofosfamide, ifosfamide, mechlorethamine, melphalan, chlorambucil, and cyclophosphamide.

162. The method of claim 157, wherein the antineoplastic agent comprises an other antineoplastic agent selected from the group consisting of a platinum compound, a protein kinase inhibitor, and an other agent.

163. The method of claim 162 wherein the antineoplastic agent comprises a platinum compound, optionally wherein the platinum compound is selected from the group consisting of cisplatin, carboplatin, oxaliplatin, satraplatin, and polyplatillen.

164. The method of claim 162, wherein the antineoplastic agent comprises a protein kinase inhibitor.

165. The method of claim 162 wherein the antineoplastic agent comprises an other agent.
166. The method of any one of claims 146-165, further comprising administering to the individual an effective amount of radiation therapy.
167. A method of treating cancer, the method comprising
administering to an individual with cancer an effective amount of a Cbl-b inhibitor,
wherein the Cbl-b inhibitor is a compound of any one of claims 1-31, and
administering to the individual an effective amount of radiation therapy.
168. The method of claim 166 or claim 167, wherein the radiation therapy is external beam radiation therapy.
169. The method of claim 166 or claim 167, wherein the radiation therapy is internal radiation therapy.
170. The method of any one of claims 146-169, wherein the Cbl-b inhibitor is administered by enteral administration, optionally wherein the enteral administration is oral administration.
171. The method of any one of claims 146-169, wherein the Cbl-b inhibitor is administered by parenteral administration, optionally wherein the parenteral administration is intratumoral injection, or the parenteral administration is by a route selected from the group consisting of intravenous, intraperitoneal, and subcutaneous.
172. The method of any one of claims 146-172, wherein the cancer is a hematologic cancer.
173. The method of claim 172, wherein the hematologic cancer is a lymphoma, a leukemia, or a myeloma.
174. The method of any one of claims 146-172, wherein the cancer is a non-hematologic cancer.
175. The method of claim 174, wherein the non-hematologic cancer is a carcinoma, a sarcoma, or a melanoma.
176. A method of producing an expanded population of tumor infiltrating lymphocytes (TILs), the method comprising

(a) obtaining a biological sample comprising TILs from the individual treated according to the method of any one of claims 146-175, and

(b) culturing the TILs in cell culture medium comprising at least one T-cell growth factor to produce an expanded population of TILs.

177. The method of claim 176, wherein the at least one T-cell growth factor comprises IL-2.

178. The method of claim 176 or claim 177, wherein the cell culture medium further comprises an anti-CD3 antibody, or both an anti-CD3 antibody and an anti-CD28 antibody.

179. The method of any one of claims 176-178, wherein the cell culture medium further comprises the Cbl-b inhibitor.

180. The method of any one of claims 176-179, wherein the cell culture medium further comprises irradiated feeder cells.

181. The method of any one of claims 146-180, wherein the individual is a human patient.

182. A composition comprising the expanded population of TILs produced by the method of any one of claims 176-181, and a physiologically acceptable buffer.

183. A method of treating cancer, the method comprising administering an effective amount of the composition of claim 182 to the individual with cancer.

184. The method of claim 183, further comprising continuing to administer an effective amount of the Cbl-b inhibitor to the individual.

185. A method of producing an expanded population of tumor infiltrating lymphocytes (TILs), the method comprising

(a) obtaining a biological sample comprising TILs from an individual having cancer who has received or is receiving an effective amount of a Cbl-b inhibitor, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31; and

(b) culturing the TILs in cell culture medium comprising at least one T-cell growth factor to produce an expanded population of TILs.

186. The method of claim 185, wherein the at least one T-cell growth factor comprises IL-2.

187. The method of claim 185 or claim 186, wherein the cell culture medium further comprises an anti-CD3 antibody, or both an anti-CD3 antibody and an anti-CD28 antibody.

188. The method of any one of claims 185-187, wherein the cell culture medium further comprises the Cbl-b inhibitor.

189. The method of any one of claims 185-188, wherein the cancer is a non-hematologic cancer, optionally wherein the non-hematologic cancer is a sarcoma, a carcinoma, or a melanoma.

190. The method of any one of claims 185-189, wherein the individual is a human patient.

191. A composition comprising the expanded population of TILs produced by the method of any one of claims 185-190, and a physiologically acceptable buffer.

192. A method of treating cancer, the method comprising administering an effective amount of the composition of claim 191 to the individual with cancer.

193. The method of claim 192, further comprising continuing to administer an effective amount of the Cbl-b inhibitor.

194. A method of immunizing, the method comprising
administering to an individual in need thereof an effective amount of a small molecule Cbl-b inhibitor, and
administering to the individual an effective amount of a vaccine.

195. The method of claim 194, wherein the immunizing is a method of treating cancer, comprising
administering to an individual with cancer an effective amount of a small molecule Cbl-b inhibitor, and
administering to the individual an effective amount of a therapeutic cancer vaccine.

196. The method of claim 195, wherein the Cbl-b inhibitor and the cancer vaccine are administered consecutively.

197. The method of claim 195, wherein the Cbl-b inhibitor and the cancer vaccine are administered concurrently.
198. The method of any one of claims 195-197, wherein the cancer vaccine is an immunogenic composition comprising at least one tumor antigen and a pharmaceutically acceptable excipient.
199. The method of claim 198, wherein the at least one tumor antigen comprises at least one synthetic peptide or recombinant protein.
200. The method of claim 198 or claim 199, wherein the immunogenic composition further comprises an adjuvant.
201. The method of claim 200, wherein the adjuvant comprises one or more ingredients of the group consisting of aluminum salts, squalene, and saponins.
202. The method of claim 198 or claim 199, wherein the immunogenic composition further comprises antigen-presenting cells (APCs), optionally wherein the APCs are dendritic cells, optionally wherein the cancer vaccine is PROVENGE.
203. The method of any one of claims 195-198, wherein the cancer vaccine comprises a microbial vector, optionally wherein the microbial vector is TICE-BCG.
204. The method of claim 203, wherein the microbial vector is a recombinant viral vector or a recombinant bacterial vector.
205. The method of any one of claims 195-198, wherein the cancer vaccine comprises killed cancer cells or a cancer cell lysate.
206. A method of treating cancer, the method comprising
administering to an individual with cancer an effective amount of a small molecule Cbl-b inhibitor, and
administering to the individual an effective amount of an oncolytic virus.
207. The method of claim 206, wherein the Cbl-b inhibitor and the oncolytic virus are administered consecutively.

208. The method of claim 206, wherein the Cbl-b inhibitor and the oncolytic virus are administered concurrently.

209. The method of any one of claims 206-208, wherein the oncolytic virus is a virus selected from the group consisting of adenovirus, coxsackievirus, echovirus, fowlpox virus, herpes simplex virus, maraba virus, measles virus, myxoma virus, Newcastle disease virus, parvovirus, poliovirus, retrovirus, reovirus, Seneca Valley virus, Semiliki Forest virus, vaccinia virus, and vesicular stomatitis virus.

210. The method of any one of claims 206-209, wherein the oncolytic virus is a recombinant virus comprising one or both of a functional deletion of at least one viral gene, and an insertion of at least one transgene.

211. The method of claim 210, wherein the recombinant virus comprises a functional deletion of at least one viral gene and an insertion of at least one transgene.

212. The method of claim 210 or claim 211, wherein the transgene encodes human granulocyte macrophage colony-stimulating factor (GM-CSF).

213. The method of any one of claims 206-210, wherein the oncolytic virus is a transgenic serotype 5 adenovirus.

214. The method of any one of claims 206-212, wherein the oncolytic virus is a transgenic herpes simplex virus type-1 (HSV-1), optionally wherein the transgenic HSV-1 is talimogene laherparepvec.

215. The method of any one of claims 206-212, wherein the oncolytic virus is a transgenic vaccinia virus, optionally wherein the transgenic vaccinia virus is pexastimogene devacirepvec.

216. The method of any one of claims 206-210, wherein the oncolytic virus is a non-recombinant oncolytic virus, optionally wherein the non-recombinant oncolytic virus is a virus selected from the group consisting of an echovirus, a Newcastle disease virus, a parvovirus, a reovirus, and a Seneca Valley virus.

217. The method of any one of claims 195-216, wherein the cancer is a hematologic cancer.

218. The method of claim 217, wherein the hematologic cancer is a lymphoma, a leukemia, or a myeloma.
219. The method of 217, wherein the cancer is a non-hematologic cancer.
220. The method of claim 219, wherein the non-hematologic cancer is a carcinoma, a sarcoma, or a melanoma.
221. The method of any one of claims 194-220, wherein the small molecule Cbl-b inhibitor is a compound of any one of claims 1-31.
222. The method of any one of claims 194-221, wherein the small molecule Cbl-b inhibitor is a compound selected from the compounds in Table 1, or a tautomer thereof, stereoisomer thereof, or a pharmaceutically acceptable salt thereof.
223. A method of treating cancer, the method comprising
administering to an individual with cancer an effective amount of an agent capable of lowering activation threshold of an immune cell, and
administering to the individual an effective amount of a therapeutic cancer vaccine; or
administering to the individual an effective amount of an oncolytic virus.
224. The method of claim 223, wherein the agent is further capable of reducing costimulation requirement of an immune cell.
225. The method of claim 223 or claim 224, wherein the agent is further capable of promoting tumor immune-surveillance.
226. The method of any one of claims 223-225, wherein the agent is a small molecule, Cbl-b inhibitor.
227. A pharmaceutical composition comprising a cancer vaccine and a small molecule Cbl-b inhibitor, optionally wherein the composition further comprises a pharmaceutically acceptable excipient.
228. A kit for treating cancer, the kit comprising
(a) a small molecule Cbl-b inhibitor;
(b) a therapeutic cancer vaccine;
(c) instructions for administration of an effective amount of the Cbl-b inhibitor and the therapeutic cancer vaccine to treat cancer in an individual.

229. A kit for treating cancer, the kit comprising
- (a) a pharmaceutical composition comprising a small molecule Cbl-b inhibitor and a therapeutic cancer vaccine; and
 - (b) instructions for administration of an effective amount of the pharmaceutical composition comprising the Cbl-b inhibitor and the therapeutic cancer vaccine to treat cancer in an individual.
230. The method, composition, or kit of any one of claims 223-229, wherein the cancer vaccine is an immunogenic composition comprising at least one tumor antigen and a pharmaceutically acceptable excipient.
231. The method, composition, or kit of claim 230, wherein the at least one tumor antigen comprises at least one synthetic peptide or recombinant protein.
232. The method, composition, or kit of claim 230 or claim 231, wherein the immunogenic composition further comprises an adjuvant.
233. The method, composition, or kit of claim 232, wherein the adjuvant comprises one or more ingredients of the group consisting of aluminum salts, squalene, and saponins.
234. The method, composition, or kit of claim 230 or claim 231 wherein the immunogenic composition further comprises antigen-presenting cells (APCs), optionally wherein the APCs are dendritic cells, optionally wherein the cancer vaccine is PROVENGE.
235. The method, composition, or kit of claim 230, wherein the cancer vaccine comprises a microbial vector, optionally wherein the microbial vector is TICE-BCG.
236. The method, composition, or kit of claim 235, wherein the microbial vector is a recombinant viral vector or a recombinant bacterial vector.
237. The method, composition, or kit of any one of claims 223-230, wherein the cancer vaccine comprises killed cancer cells or a cancer cell lysate.
238. A pharmaceutical composition comprising an oncolytic virus and a small molecule Cbl-b inhibitor, optionally wherein the composition further comprises a pharmaceutically acceptable excipient.

239. A kit for treating cancer, the kit comprising
- (a) a small molecule Cbl-b inhibitor;
 - (b) an oncolytic virus;
 - (c) instructions for administration of an effective amount of the Cbl-b inhibitor and the oncolytic virus to treat cancer in an individual.
240. A kit for treating cancer, the kit comprising
- (a) a pharmaceutical composition comprising a small molecule Cbl-b inhibitor and an oncolytic virus; and
 - (b) instructions for administration of an effective amount of the pharmaceutical composition comprising the small molecule Cbl-b inhibitor and the oncolytic virus to treat cancer in an individual.
241. The method, composition, or kit of any one of claims 223-226, or 238-240, wherein the oncolytic virus is a virus selected from the group consisting of adenovirus, coxsackievirus, echovirus, fowlpox virus, herpes simplex virus, maraba virus, measles virus, myxoma virus, Newcastle disease virus, parvovirus, poliovirus, retrovirus, reovirus, Seneca Valley virus, Semiliki Forest virus, vaccinia virus, and vesicular stomatitis virus.
242. The method, composition, or kit of any one of claims 223-226, or 238-240, wherein the oncolytic virus is a recombinant virus comprising one or both of a functional deletion of at least one viral gene, and an insertion of at least one transgene.
243. The method, composition, or kit of claim 242, wherein the recombinant virus comprises a functional deletion of at least one viral gene and an insertion of at least one transgene.
244. The method, composition, or kit of claim 242 or claim 243, wherein the transgene encodes human granulocyte macrophage colony-stimulating factor (GM-CSF).
245. The method, composition, or kit of any one of claims 223-226 or 238-242, wherein the oncolytic virus is a transgenic serotype 5 adenovirus.
246. The method, composition, or kit of any one of claims 223-226 or 238-244, wherein the oncolytic virus is a transgenic herpes simplex virus type-1 (HSV-1), optionally wherein the transgenic HSV-1 is talimogene laherparepvec.

247. The method, composition, or kit of any one of claims 223-226 or 238-244, wherein the oncolytic virus is a transgenic vaccinia virus, optionally wherein the transgenic vaccinia virus is pexastimogene devacirepvec.

248. The method, composition, or kit of any one of claims 223-226 or 238-244, wherein the oncolytic virus is a non-recombinant oncolytic virus, optionally wherein the non-recombinant oncolytic virus is a virus selected from the group consisting of an echovirus, a Newcastle disease virus, a parvovirus, a reovirus, and a Seneca Valley virus.

249. The method, composition, or kit of any one of claims 223-248, wherein the cancer is a hematologic cancer.

250. The method, composition, or kit of claim 249, wherein the hematologic cancer is a lymphoma, a leukemia, or a myeloma.

251. The method, composition, or kit of any one of claims 223-248, wherein the cancer is a non-hematologic cancer.

252. The method, composition, or kit of claim 251, wherein the non-hematologic cancer is a carcinoma, a sarcoma, or a melanoma.

253. The method, composition or kit of any one of claims 223-252, wherein the small molecule Cbl-b inhibitor is a compound of any one of claims 1-31.

254. The method, composition, or kit of any one of claims 223-253, wherein the small molecule Cbl-b inhibitor is a compound selected from the compounds in Table 1, or a tautomer thereof, stereoisomer thereof, or a pharmaceutically acceptable salt thereof.

255. A method of treating a disease via cell therapy in a subject in need thereof, the method comprising

administering to the individual an effective amount of one or more therapeutic cells to treat the disease; and

administering to the subject an effective amount of a Cbl-b inhibitor, wherein the Cbl-b inhibitor is a compound of any one of claims 1-31, and wherein treatment with the therapeutic cells is enhanced by the combination with the Cbl-b inhibitor.

FIG. 1

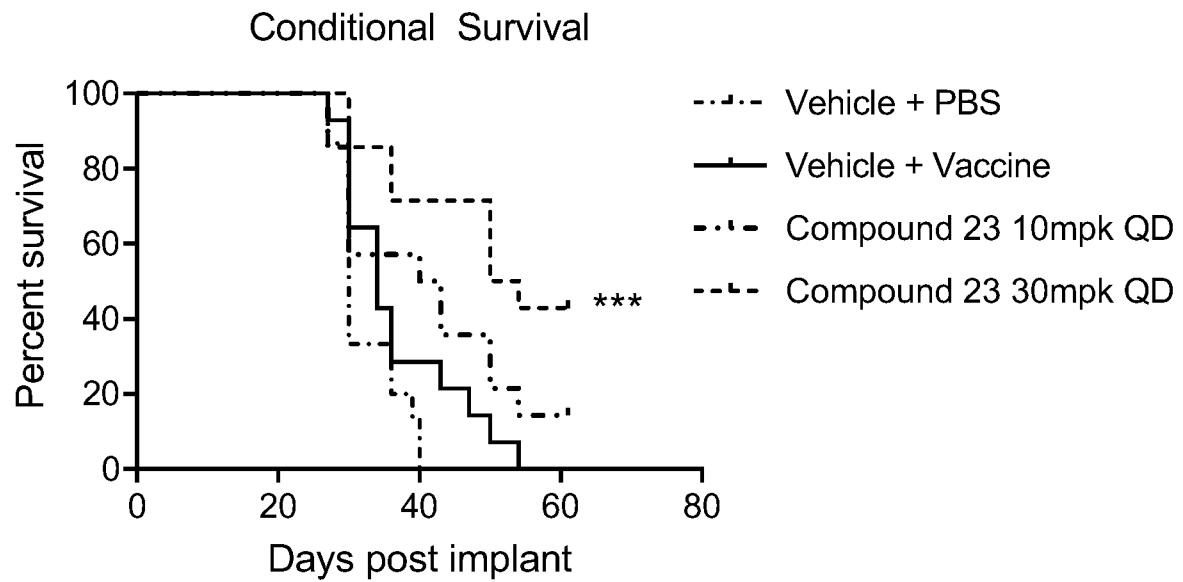


FIG. 2

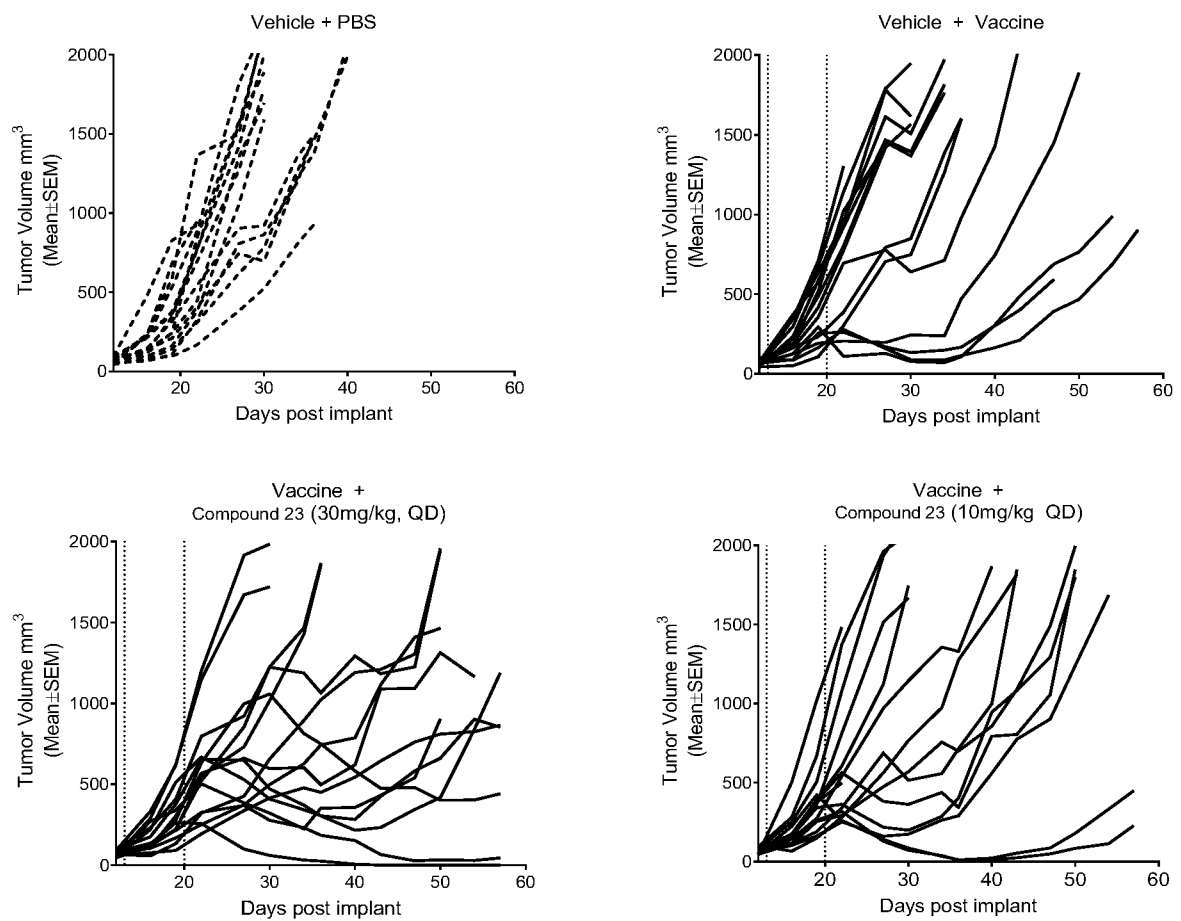


FIG. 3

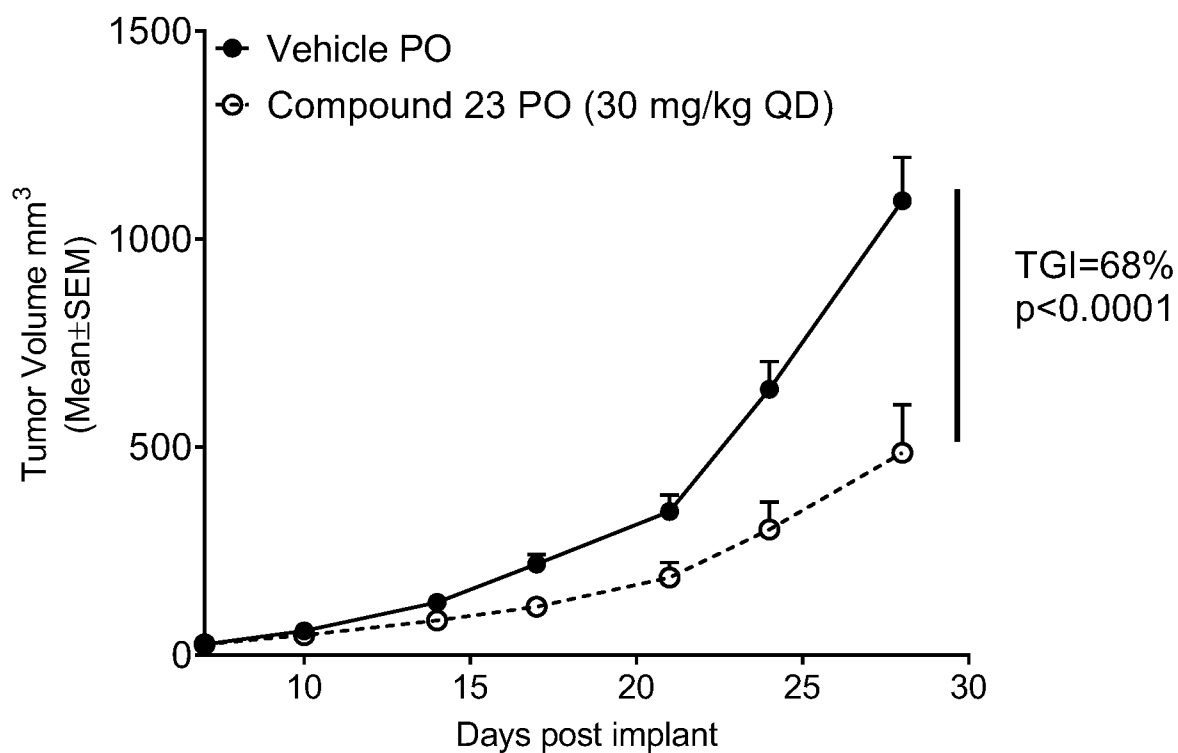
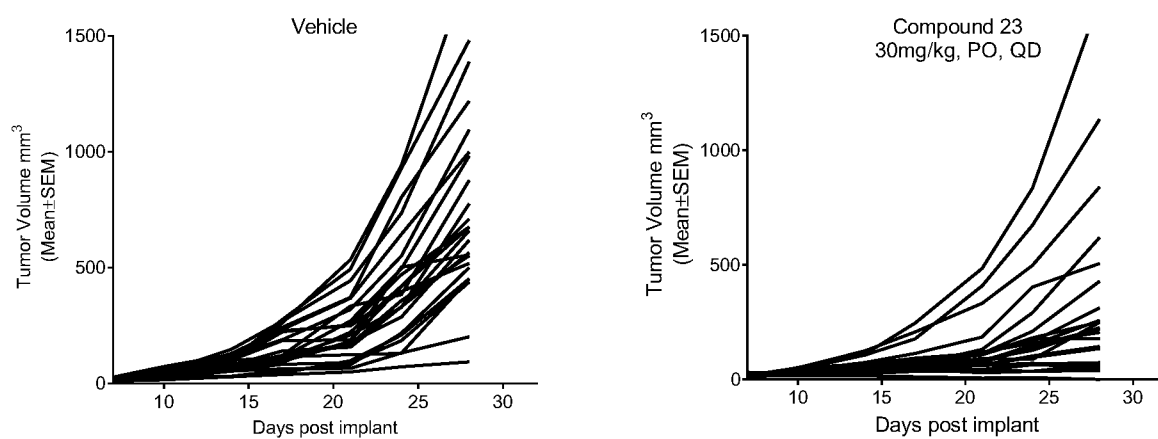
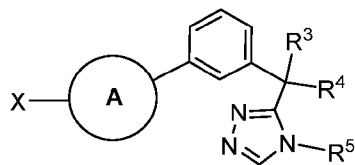


FIG. 4





(I)

FIG. 1

