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(71) Applicants: **BAYLOR COLLEGE OF MEDICINE** [US/US]; One Baylor Plaza, Houston, Texas 77030 (US). **BOARD OF GERENTS, THE UNIVERSITY OF TEXAS SYSTEM** [US/US]; 210 West 7th Street, Austin, Texas 78701 (US).

(72) Inventors: **WANG, Jin**; c/o Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030 (US). **GUO, Wen-Hao**; c/o Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030 (US). **QI, Xiaoli**; c/o Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030 (US). **WANG, Luhua**; c/o The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030 (US). **LIU, Yang**; c/o The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030 (US). **NOMIE, Krys-tle J.**; c/o The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030 (US).

(74) Agent: **DAVIS, Kimberlynn B.** et al.; Kilpatrick Townsend & Stockton LLP, 1100 Peachtree Street NE, Suite 2800, Mailstop: IP Docketing - 22, Atlanta, Georgia 30309 (US).

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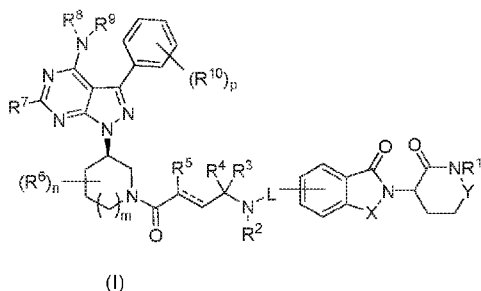
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(54) Title: SMALL MOLECULE PROTEOLYSIS-TARGETING CHIMERAS AND METHODS OF USE THEREOF



(57) Abstract: Novel small molecule proteolysis-targeting chimeras (PRO-TACs) are provided, along with methods for their use as Bruton's tyrosine kinase (BTK) degraders. The small molecule PROTACs described herein are useful in treating and/or preventing BTK-related diseases, such as cancer, neurodegenerative disorders, inflammatory diseases, and metabolic disorders. Also provided are methods for inducing BTK degradation in a cell using the compounds and compositions described herein. Exemplary compounds include: (I).

## Small Molecule Proteolysis-Targeting Chimeras and Methods of Use Thereof

### **CROSS-REFERENCE TO PRIORITY APPLICATION**

5           This application claims priority to U.S. Provisional Application No. 62/860,303, filed June 12, 2019, which is incorporated herein by reference its entirety.

### **STATEMENT REGARDING FEDERALLY FUNDED RESEARCH**

10           This invention was made with government support under Grant No. 5R01CA207701, awarded by the National Institutes of Health. The government has certain rights in the invention.

### **BACKGROUND**

15           Proteolysis-targeting chimeras (PROTACs) are composed of three components, including binders for a targeted protein, an E3 ligase, and a linker bridging these two binders. Formation of the ternary complex is the prerequisite for efficient degradation of the targeted protein. Therefore, enhancing the binding affinity to the targeted protein is a strategy to improve PROTAC potency.

### **SUMMARY**

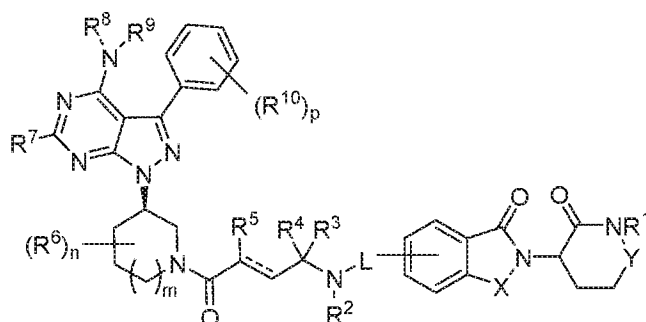
20           Described herein are novel small molecule proteolysis-targeting chimeras (PROTACs) and methods for their use as Bruton's tyrosine kinase (BTK) degraders. The small molecule PROTACs described herein are useful in treating and/or preventing BTK-related diseases, such as cancer, neurodegenerative disorders, inflammatory diseases, and  
25           metabolic disorders. The methods include administering to a subject a compound as described herein.

          A class of small molecule PROTACs as described herein includes compounds of the following formula:

A-L-B

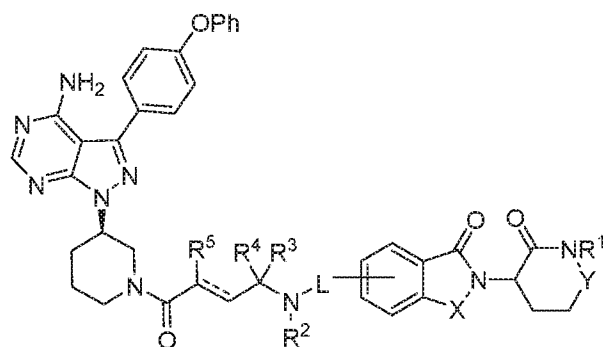
30           and pharmaceutically acceptable salts and prodrugs thereof, wherein A is a BTK binder, L is a linker, and B is an E3 ligase binder. Optionally, L comprises a reversible covalent group. Optionally, B comprises a CRBN ligand, a VHL ligand, a cIAP1 ligand, a MDM2 ligand, a RNF2 ligand, or a DCAF15 ligand.

A class of small molecule PROTACs as described herein includes compounds of the following formula:



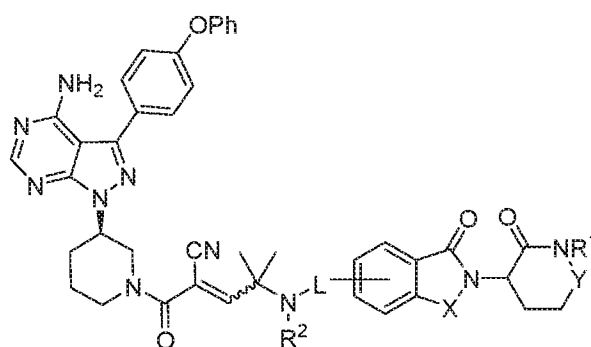
or a pharmaceutically acceptable salt or prodrug thereof, wherein  $\equiv$  is a single bond or a double bond; m is 0-3; n and p are each independently 0-5; L is a linker; X and Y are each independently selected from CH<sub>2</sub>, CHD, CD<sub>2</sub>, CHF, CF<sub>2</sub>, and C(O); R<sup>1</sup>, R<sup>2</sup>, R<sup>8</sup>, and R<sup>9</sup> are each independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted carbonyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl; and R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, each R<sup>6</sup>, R<sup>7</sup>, and each R<sup>10</sup> are each independently selected from hydrogen, halogen, cyano, trifluoromethyl, alkoxy, aryloxy, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl.

A class of small molecule PROTACs as described herein includes compounds of the following formula:

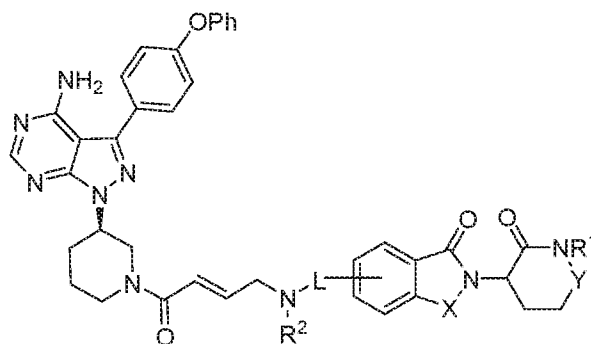


or a pharmaceutically acceptable salt or prodrug thereof, wherein  $\equiv$  is a single bond or a double bond; L is a linker; X and Y are each independently selected from  $\text{CH}_2$ ,  $\text{CHD}$ ,  $\text{CD}_2$ ,  $\text{CHF}$ ,  $\text{CF}_2$ , and  $\text{C(O)}$ ;  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$ , and  $\text{R}^4$  are each independently selected from hydrogen, substituted or unsubstituted alkyl, and substituted or unsubstituted carbonyl; and  $\text{R}^5$  is hydrogen, deuterium, fluoro, chloro, bromo, iodo, cyano,  $-\text{OCH}_3$ ,  $-\text{OCDH}_2$ ,  $-\text{OCD}_2\text{H}$ , or  $-\text{OCD}_3$ . Optionally, L is a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, a substituted or unsubstituted alkynyl, a substituted or unsubstituted alkoxy, or a substituted or unsubstituted amino. In some cases, L contains an amide group. Optionally, X and Y are each  $\text{C(O)}$ . In some cases,  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$ , and  $\text{R}^4$  are each independently selected from hydrogen and methyl.

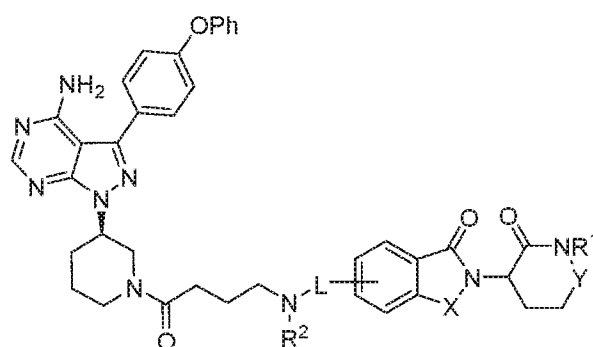
Optionally, the compound has the following formula:



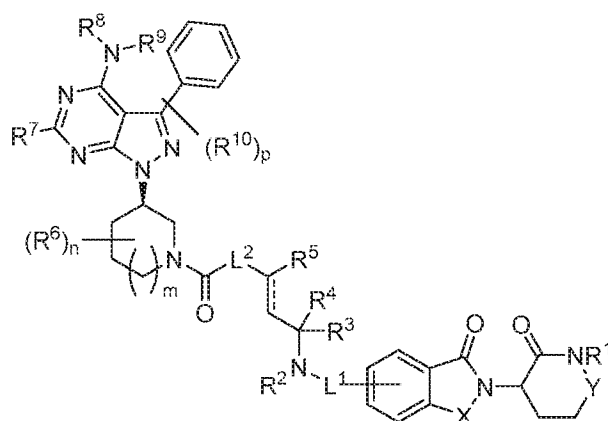
Optionally, the compound has the following formula;



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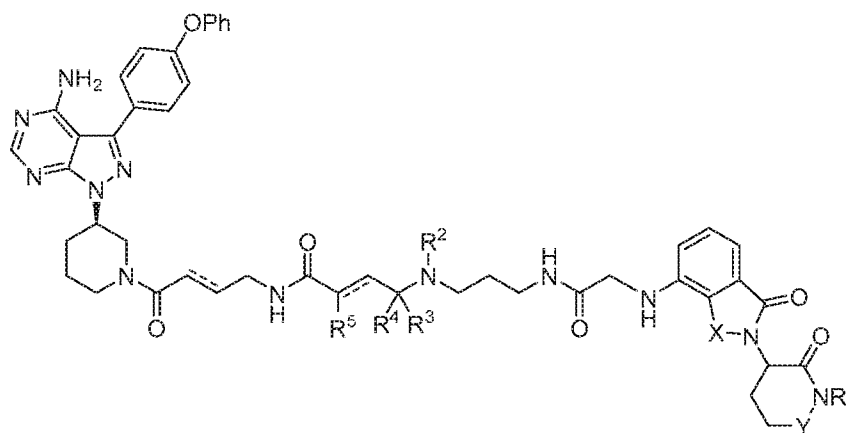


A class of small molecule PROTACs as described herein includes compounds of the following formula:



- 5 or a pharmaceutically acceptable salt or prodrugs thereof, wherein  $\equiv$  is a single bond or a double bond;  $m$  is 0-3;  $n$  and  $p$  are each independently 0-5;  $L^1$  and  $L^2$  are each independently a linker;  $X$  and  $Y$  are each independently selected from  $CH_2$ ,  $CHD$ ,  $CD_2$ ,  $CHF$ ,  $CF_2$ , and  $C(O)$ ;  $R^1$ ,  $R^2$ ,  $R^8$ , and  $R^9$  are each independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted carbonyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl; and  $R^3$ ,  $R^4$ ,  $R^5$ , each  $R^6$ ,  $R^7$ , and each  $R^{10}$  are each independently selected from hydrogen, halogen, cyano, trifluoromethyl, alkoxy, aryloxy, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl.

20 Optionally, the compound has the following formula:



Also described herein is a composition including a compound as described herein and a pharmaceutically acceptable carrier.

Further described herein is a kit including a compound or composition as described  
5 herein.

Methods of treating or preventing a Bruton's tyrosine kinase (BTK)-related disease in a subject are also provided herein. A method of treating or preventing a BTK-related disease in a subject includes administering to the subject an effective amount of a compound or composition as described above. Optionally, the BTK-related disease is cancer (e.g., bladder  
10 cancer, blood cancer, a bone marrow cancer, brain cancer, breast cancer, bronchus cancer, colorectal cancer, cervical cancer, chondrosarcoma, endometrial cancer, gastrointestinal cancer, gastric cancer, genitourinary cancer, head and neck cancer, hepatic cancer, hepatocellular carcinoma, leukemia, liver cancer, lung cancer, lymphoma, melanoma of the skin, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, skin cancer, testicular  
15 cancer, thyroid cancer, or uterine cancer). Optionally, the BTK-related disease is a neurodegenerative disorder or an inflammatory disease.

The methods can further include administering a second compound, biomolecule, or composition. Optionally, the second compound, biomolecule, or composition is a chemotherapeutic agent.

Also described herein are methods of inducing BTK degradation in a cell. A method of inducing BTK degradation in a cell includes contacting a cell with an effective amount of a compound as described herein. The contacting can be performed *in vitro* or *in vivo*.

The details of one or more embodiments are set forth in the drawings and the description below. Other features, objects, and advantages will be apparent from the  
25 description and drawings, and from the claims.

## DESCRIPTION OF DRAWINGS

Fig. 1 shows a demonstration of catalytic degradation of targeted proteins by reversible covalent PROTACs.

Fig. 2A-D depicts BTK degradation induced by PROTACs. Fig. 2A shows chemical structures of BTK degraders and their controls. RC-1, RNC-1 and IRC-1 are BTK degraders with reversible covalent, reversible non-covalent, and irreversible covalent warheads, respectively. RC-Ctrl, RNC-Ctrl, and IRC-Ctrl (i.e., ibrutinib) are the corresponding warhead controls for RC-1, RNC-1 and IRC-1, respectively. Fig. 2B, MOLM-14 cells were incubated with RC-1, RNC-1 and IRC-1 for 24 hours. The BTK levels were quantified by Western blotting. Duplicates were performed with SEM as the error bars. Fig. 2C shows RC-1 dose-dependent BTK degradation in MOLM-14 cells.  $DC_{50}$ : compound concentration inducing 50% of protein degradation. Fig. 2D, MOLM-14 cells were treated with DMSO, RC-1, RC-Ctrl, Pomalidomide, RC-Ctrl + Pomalidomide, and RC-1-Me for 24 hours. All the compound concentrations are 200 nM. Neither RC-Ctrl, nor pomalidomide, nor a combination of both caused BTK degradation, indicating that the bifunctional PROTAC molecule is essential to facilitate the formation of a ternary complex of {BTK-PROTAC-CRBN} in order to induce BTK degradation. Duplicates were performed with SEM as the error bars.

Fig. 3 shows the linker development for reversible covalent BTK degraders. The developed linkers of the RC series of BTK degraders and their corresponding % of BTK degradation in MOLM-14 cells (200 nM, 24 h incubation) are shown.

Fig. 4A-D shows the target engagement for BTK degraders. For Fig. 4A, quantitative Western blot was performed on lysate of  $1 \times 10^6$  MOLM-14 cells using BTK and CRBN recombinant proteins as the standard. Fig. 4B shows the results of a CRBN in-cell target engagement assay. HEK-293 cells were transiently transfected with plasmids expressing a fusion protein of CRBN and nano-luciferase (nLuc) for 24 h and then the cells were treated with a CRBN tracer (0.5  $\mu$ M), which binds to CRBN to induce bioluminescence resonance energy transfer (BRET). The target engagement  $IC_{50}$  values for RC-1, IRC-1, and RNC-1 are 0.25, 0.86, and 1.69  $\mu$ M, respectively. Fig. 4C shows the results of a BTK in-cell target engagement assay. This assay is the same as the CRBN in-cell target engagement assay, except BTK-nLuc fusion plasmid and BTK tracer (1.0  $\mu$ M) were used. The target engagement  $IC_{50}$  values for RC-1, IRC-1, and RNC-1 are 0.043, 0.13 and 1.27  $\mu$ M, respectively. Fig. 4D, the same BTK in-cell target engagement assay as in Fig. 4C was applied to RC-1, RC-Ctrl, DD-03-171 and MT-802. The target engagement  $IC_{50}$  values for

RC-1 and RC-Ctrl are the same within experimental errors, demonstrating that the intracellular accumulation of RC-1 is similar to its parent warhead molecule. In contrast, the target engagement IC<sub>50</sub> values for DD-03-171 and MT-802 are 5 and 11 folds of that of RC-1, respectively. Triplicates were performed with SEM as the error bars.

Fig. 5A-B shows the results from a fluorophore phase separation-based assay for imaging ternary complex formation in living cells. Fig. 5A shows a schematic diagram showing the design of the cellular assay. Fig. 5B contains fluorescence images showing detection of BTK PROTACs-induced interaction between the E3 ligase cereblon and the target protein BTK. A fluorescence histogram of the line across the cells is shown below.

Scale bar: 10  $\mu$ m.

Fig. 6A-B shows that RC-1 overcomes drug resistance. For Fig. 6A, XLA cells overexpressing wild type or C481S mutant BTK were treated with RC-1 for 24 hours. Then Western blotting was performed to evaluate the degradation of BTK. Duplicates were performed. For Fig. 6B, RC-1 induced degradation of BTK, pBTK, IKZF1 and IKZF3 in Mino cells. Duplicates were performed.

Fig. 7A-D contains the results of a proteomic analysis showing RC-1 selectively degrades BTK. MOLM-14 cells were treated with either compounds [200 nM (Fig. 7A) RC-1, (Fig. 7B) RNC-1, (Fig. 7C) IRC-1, or (Fig. 7D) RC-1-Me (RC-1 non-degrader control)] or DMSO for 24 hours. Lysates were treated with a TMT-10plex kit and subjected to mass spec-based proteomics analysis. Datasets represent an average of duplicates. Volcano plot shows protein abundance ( $\log_2$ ) as a function of significance level ( $\log_{10}$ ). Nonaxial vertical lines denote abundance changes from 0.7 to 1.4 (i.e.  $2^{\pm 0.5}$ ), whereas nonaxial horizontal line marks  $P = 0.05$  significance threshold. Downregulated proteins of significance are found in the upper left quadrant of the plots. A total of 7,280 proteins were identified, and only the ones with at least one uniquely identified peptide are displayed.

Fig. 8A-C depicts molecular dynamics simulations for ternary complexes of BTK-PROTAC-CRBN. In Fig. 8A and Fig. 8B, a RNC-1 mediated complex shows a larger structural fluctuation compared with the other two ligands, reflected by (A) Root Mean Square Fluctuation (RMSF) per residue and (B) Root Mean Square Deviation (RMSD) calculated from the simulations of the {BTK-PROTAC-CRBN} ternary complexes. Fig. 8C shows the predicted most stable conformation of {BTK-RC-1-CRBN} ternary complex.

Fig. 9A-B shows a comparison of RC-1 with other reported BTK degraders, including two previously reported BTK degraders DD-03-171 and MT-802. MOLM-14 cells (Fig. 9A) and Mino cells (Fig. 9B) were treated with compounds for 72 hours. Then Alarma blue

assays were performed to quantify the cellular viabilities. Data are presented as means  $\pm$  SEM of 5 replicates.

Fig. 10A-B depicts the PK/PD of RC-1. Fig. 10A is graph showing RC-1 (20 mg/kg, single i.p. injection) in ICR mice plasma (n=3). Fig. 10B shows a representative western blot (left) and quantification (right) of splenic BTK level in mice (n=3) treated with 7 once daily i.p. injections of 20 mg/kg RC-1 (spleens were harvested 24 h after the 7<sup>th</sup> injection).

Fig. 11A-B depicts BTK degradation induced by PROTACs. Fig. 11A shows the chemical structures of BTK degraders. Fig. 11B shows Western blots from MOLM-14 cells treated with the indicated doses of PROTACs for 24 hours. Duplicates were performed.

Fig. 12 is a graph shown the correlation between  $K_d$  towards TBK-1 and  $DC_{50}$ .

Fig. 13A-B depicts the quantification of BTK and CRBN levels in MOLM-14 cells. MOLM-14 cells were maintained in RPMI-1640 complete culture medium.  $1 \times 10^6$  cells were collected and processed for Western blot analysis of BTK and CRBN levels using their corresponding primary and secondary antibodies. BTK and CRBN were quantified by normalizing the sample BTK and CRBN to their standard recombinant BTK and CRBN. The data shown were average of 3 repeat samples.

Fig. 14A shows the complex formation curves of PROTACs for a non-cooperative system ( $\alpha=1$ ). Fig. 14B shows the input parameters and output exact values of PROTACs based on the ternary complex formation modeling.

Fig. 15 shows a 9-point dose response curve for concentrations of the indicated compounds. Each concentration point was performed in duplicate.

Fig. 16A shows the chemical structures of IRC-1-DiMe and Fig. 16B is a graph showing the BTK target engagement for RC-1, IRC-DiMe and RNC-CN-DiMe. For the BTK in-cell target engagement assay, HEK-293 cells were transiently transfected with plasmids expressing a fusion protein of BTK and nano-luciferase (nLuc) for 24 hours and then the cells were treated with a BTK tracer (1.0  $\mu$ M), which binds to BTK to induce energy transfer signals. Adding PROTACs to cells would compete the BTK tracer binding to BTK, thus reducing the NanoBRET signals. The  $IC_{50}$  values for RC-1, IRC-DiMe, and RNC-CN-DiMe are 0.033, 0.38, and 0.93  $\mu$ M, respectively.

Fig. 17 depicts the BTK degradation by three categories of PROTACs in XLA cells overexpressing wild type BTK (XLA-WT) or mutant C481S BTK (XLA-C481S).

Fig. 18A-B contains graphs showing the results of cell viability assays following treatment with BTK PROTAC degraders and their corresponding warhead controls in MOLM-14 cells (Fig. 18A) and Mino cells (Fig. 18B).

Fig. 19A-B shows protein degradation by BTK PROTAC RC-1 in mouse spleen. ICR mice were subjected to single intraperitoneal (IP) injection of RC-1 at the dose of 50 mg or 100 mg per kg body weight (N 3 - 4 per group) and spleens were harvested 24 hours after injection. The splenic p-BTK(Y233), BTK, IKZF1 and IKZF3 levels were measured with Western blot (Fig. 19A) and quantified (Fig. 19B).

Fig. 20 shows BTK degradation induced by RC-1 in mouse cell line. E mu-myc transgenic mouse cells were incubated with RC-1 for 24 hours. The BTK levels were quantified by Western blotting. Duplicates were performed.

Fig. 21A-B show BTK degradation induced by RC-1 and MT-802. MOLM-14 cells were incubated with RC-1 and MT-802 for 24 hours. The BTK levels were quantified by Western blotting. Duplicates were performed with SEM as the error bars

Fig. 22 shows the structures of BTK PROTACs tested.

Fig. 23A-B show BTK degradation by the indicated PROTACs in a MOLM-14 cell line. MOLM-14 cells were treated with PROTACs for 24 hours, followed by Western blotting to measure the BTK levels. The quantification results are shown in Fig. 23A and the Western blots are shown in Fig. 23B.

## DETAILED DESCRIPTION

Described herein are novel small molecule proteolysis-targeting chimeras (PROTACs) and methods for their use as Bruton's tyrosine kinase (BTK) degraders. The small molecule PROTACs described herein are useful in treating and/or preventing cancer, neurodegenerative disorders, inflammatory diseases, metabolic disorders, and other BTK-related diseases.

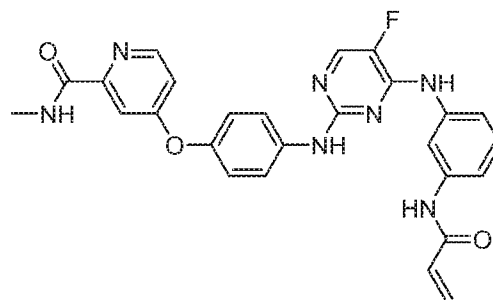
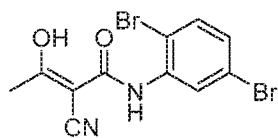
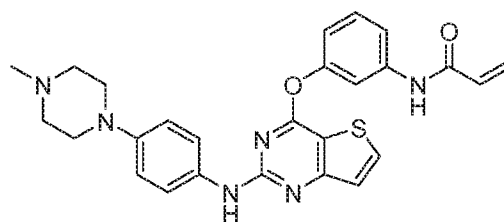
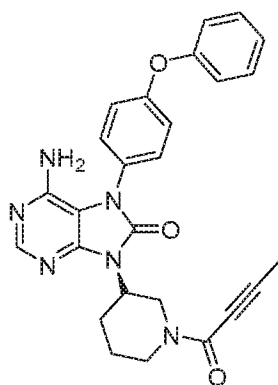
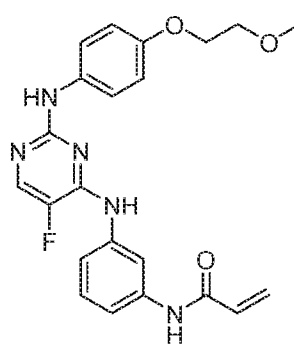
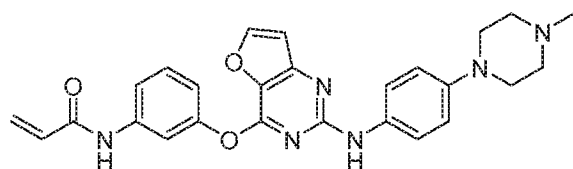
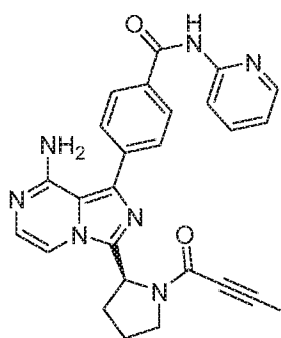
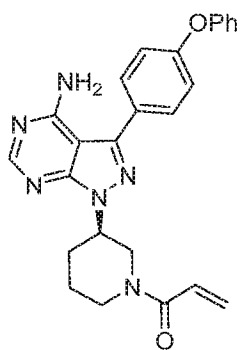
### I. Compounds

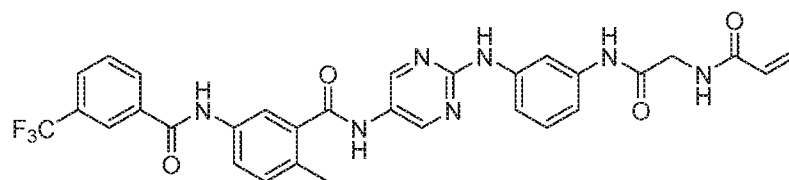
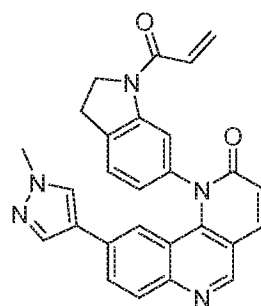
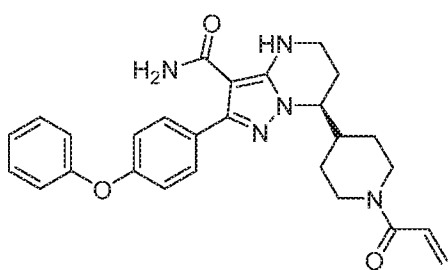
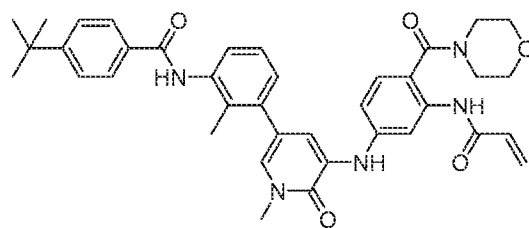
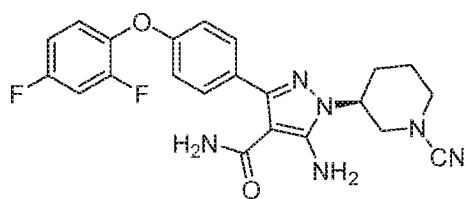
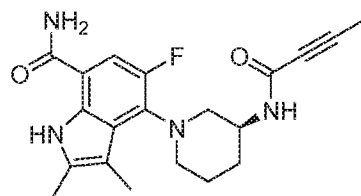
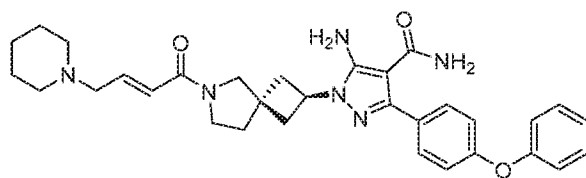
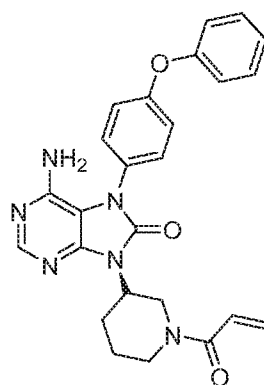
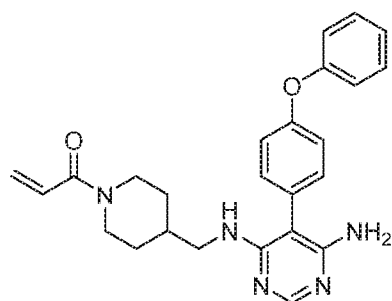
In some cases, the PROTACs described herein are represented by the following formula:

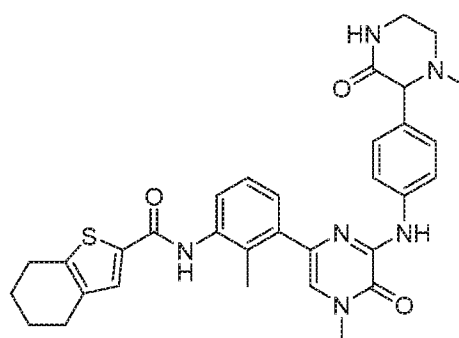
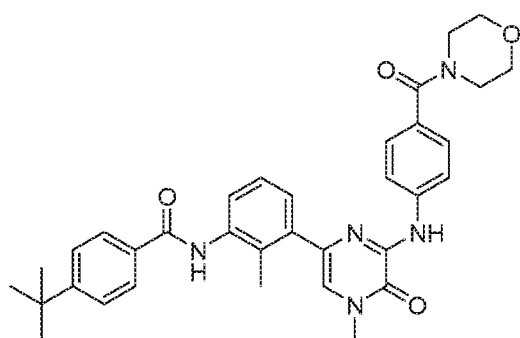
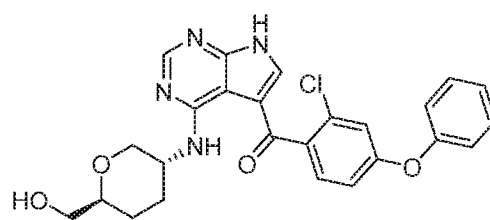
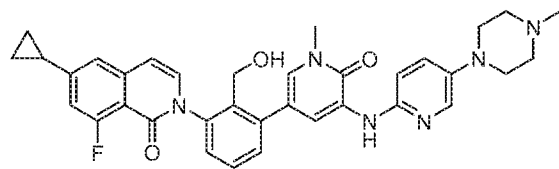
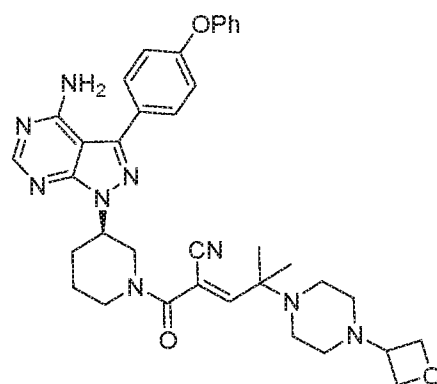
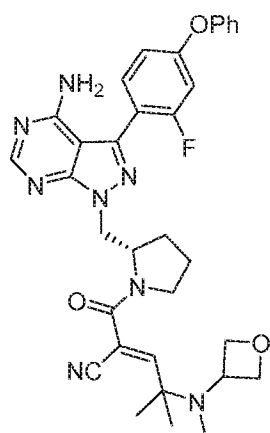
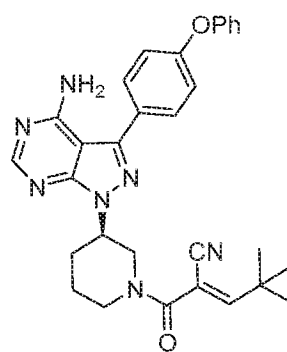
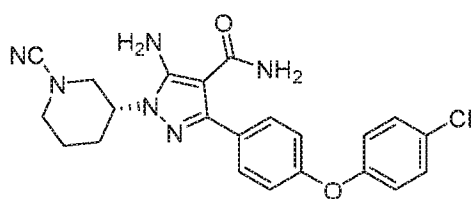


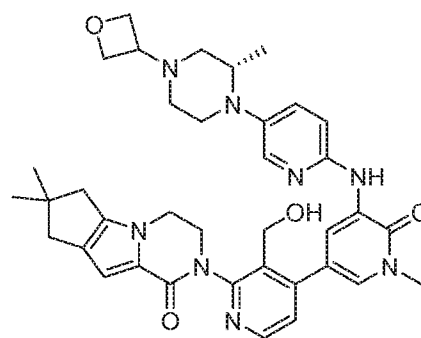
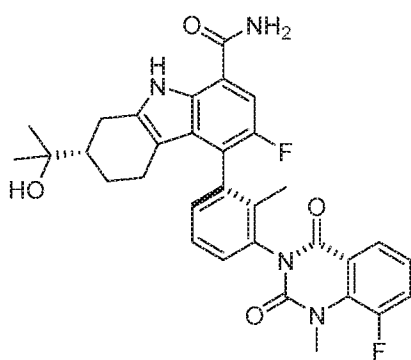
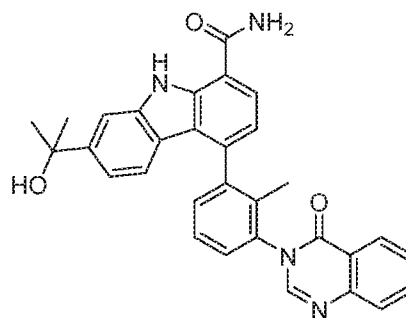
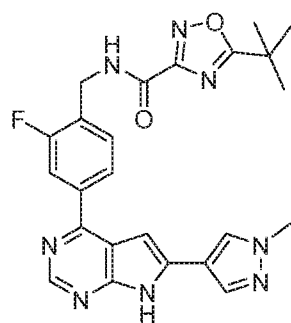
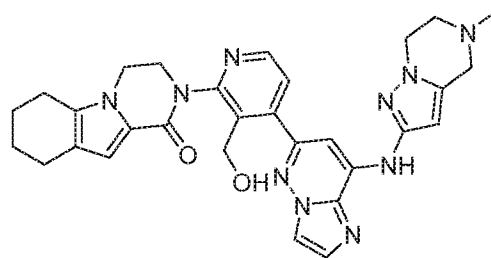
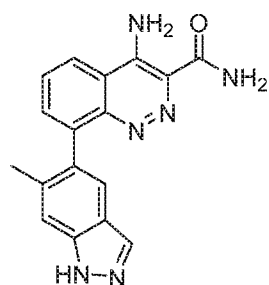
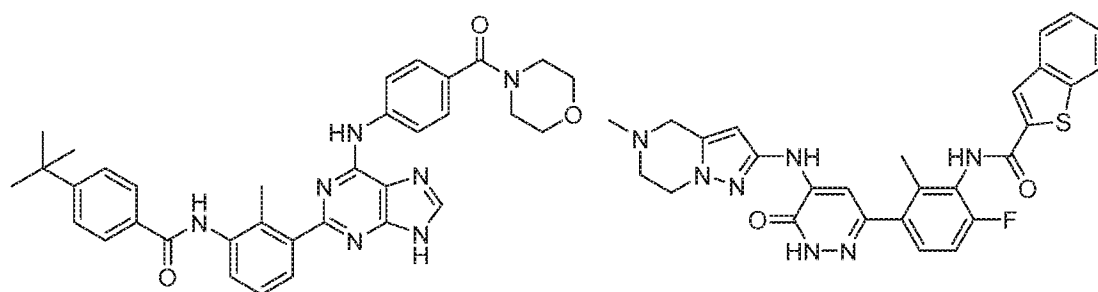
and pharmaceutically acceptable salts and prodrugs thereof.

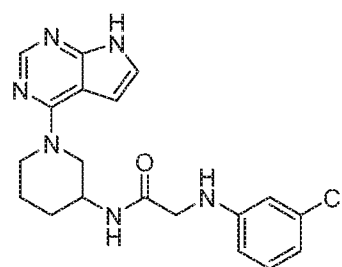
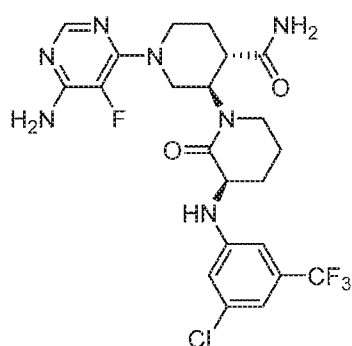
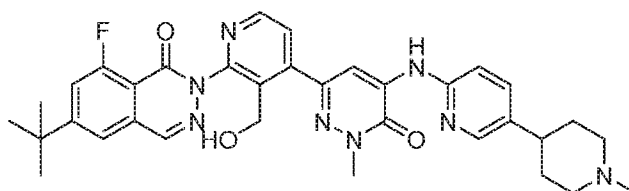
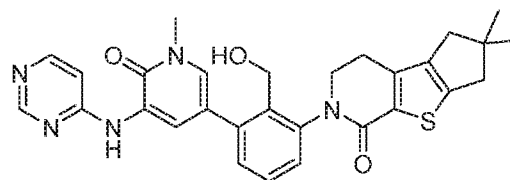
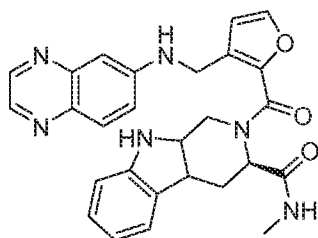
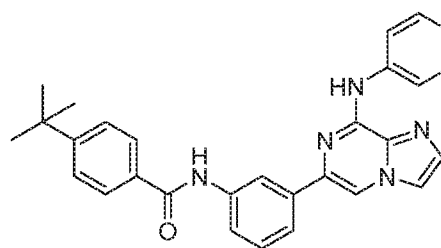
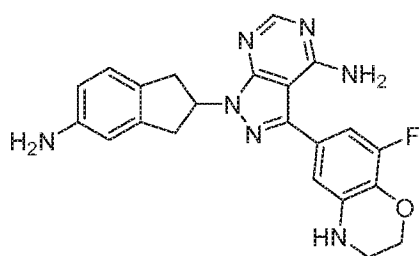
In the formula shown above, A is a BTK binder (also referred to as a BTK inhibitor). In some embodiments, the BTK binders suitable for use as the A group include a Michael acceptor. The L group can form a covalent bond with the A group through the Michael acceptor present in the A group. Exemplary BTK binders for use as the A group are shown below:







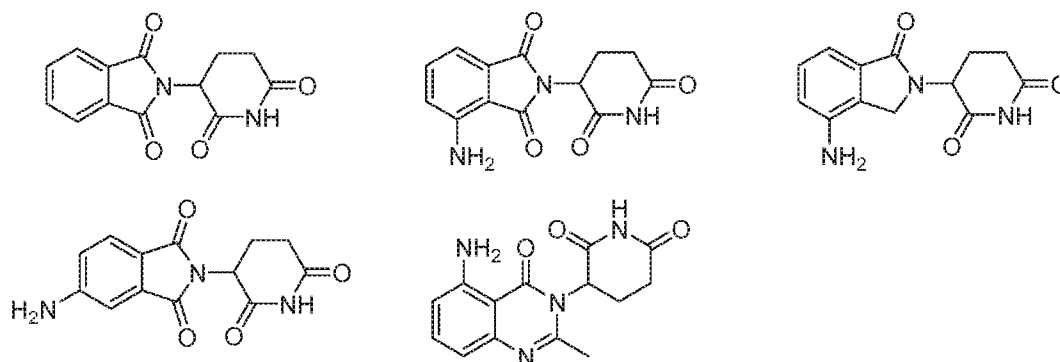




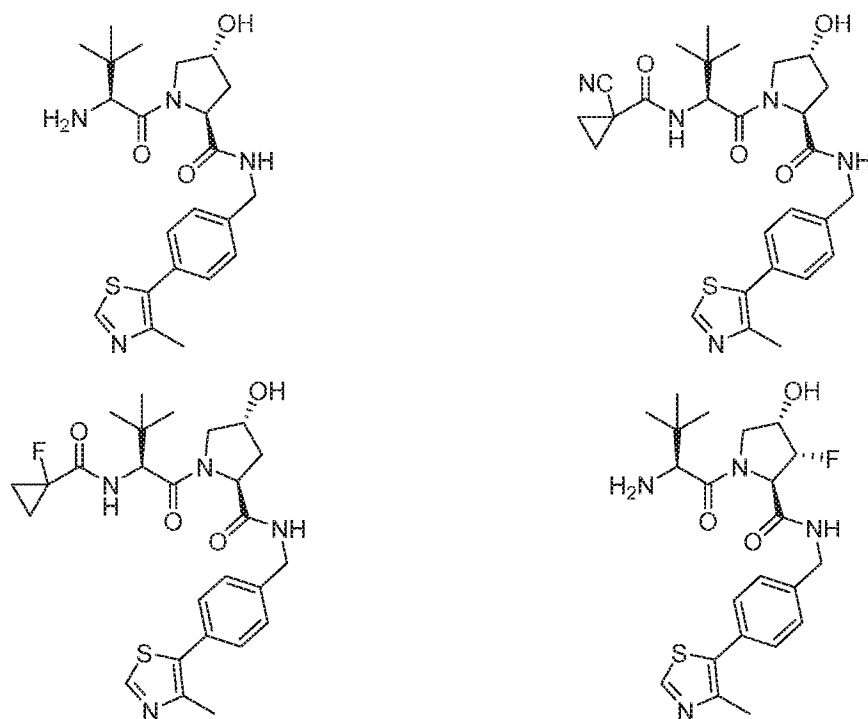
Also, in the formula shown above, L is a linker group. Optionally, the linker group contains a reversible covalent group. Optionally, L is a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, a substituted or unsubstituted alkynyl, a substituted or unsubstituted alkoxy, or a substituted or unsubstituted amino. Optionally, L contains an amide group.

Additionally, in the formula shown above, B is an E3 ligase binder. Exemplary E3 ligase binders for use as the B group include CRBN ligands, VHL ligands, cIAP1 ligands, MDM2 ligands, RNF2 ligands, and DCAF15 ligands.

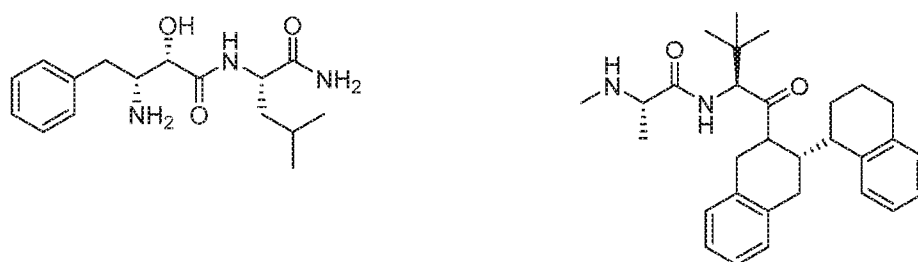
Exemplary CRBN ligands for use as the B group are shown below:

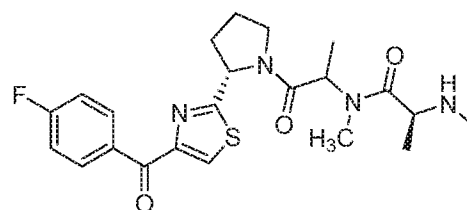
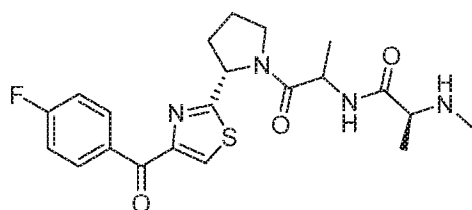


5 Exemplary VHL ligands for use as the B group are shown below:

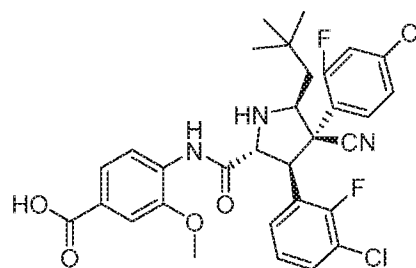
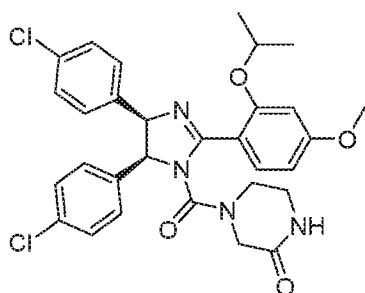


Exemplary cIAP1 ligands for use as the B group are shown below:

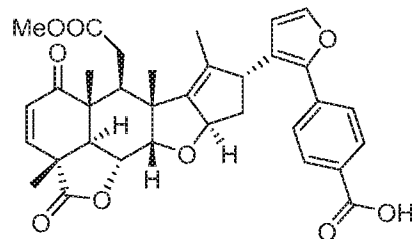
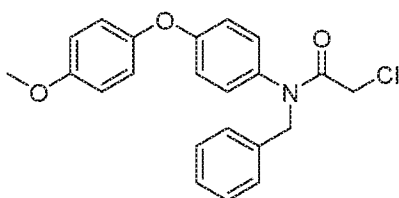




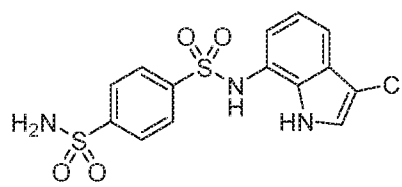
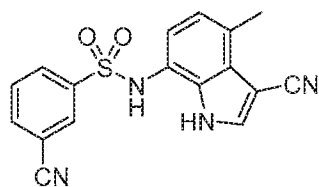
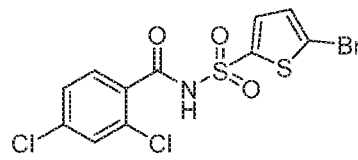
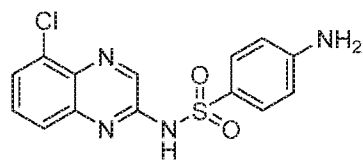
Exemplary MDM2 ligands for use as the B group are shown below:



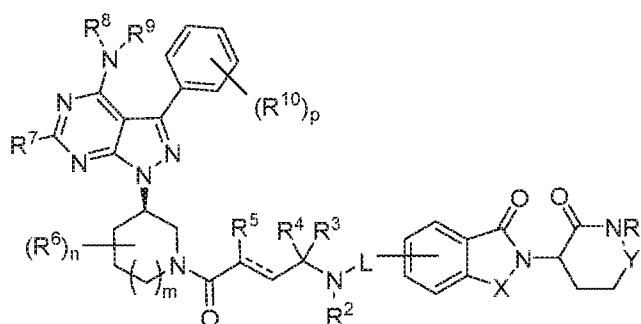
Exemplary RNF4 ligands for use as the B group are shown below:



Exemplary DCAF15 ligands for use as the B group are shown below:



- 5 An exemplary class of PROTACs according to the structure shown above is provided below as **Formula I**:



and pharmaceutically acceptable salts or prodrugs thereof.

In **Formula I**,  $\equiv$  is a single bond or a double bond.

Also, in **Formula I**, m is 0-3.

5 Additionally, in **Formula I**, n and p are each independently 0-5.

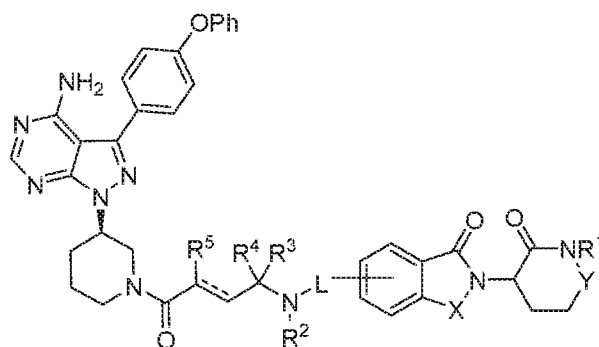
Further, in **Formula I**, L is a linker. Optionally, L is a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, a substituted or unsubstituted alkynyl, a substituted or unsubstituted alkoxy, or a substituted or unsubstituted amino. Optionally, L contains an amide group.

10 Also, in **Formula I**, X and Y are each independently selected from CH<sub>2</sub>, CHD, CD<sub>2</sub>, CHF, CF<sub>2</sub>, and C(O). Optionally X and Y are each C(O).

Additionally, in **Formula I**, R<sup>1</sup>, R<sup>2</sup>, R<sup>8</sup>, and R<sup>9</sup> are each independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted carbonyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl.

Further, in **Formula I**, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, each R<sup>6</sup>, R<sup>7</sup>, and each R<sup>10</sup> are each independently selected from hydrogen, halogen (e.g., fluoro, chloro, bromo, or iodo), cyano, trifluoromethyl, alkoxy, aryloxy, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl.

25 In some cases, the compounds according to **Formula I** are represented by **Structure I-A**:



Structure I-A

In **Structure I-A**,  $\equiv$ , L, X, Y,  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ , and  $R^5$  are as defined above for **Formula I**. For example, in **Structure I-A**,  $\equiv$  is a single bond or a double bond.

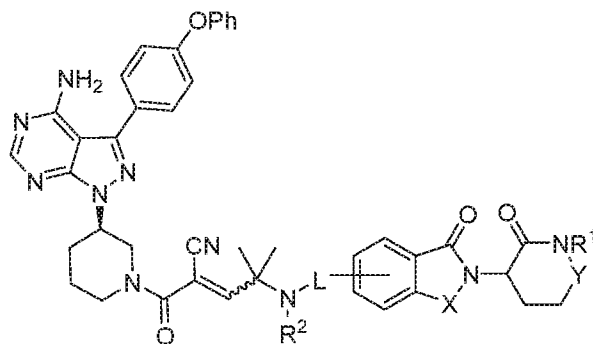
Also, in **Structure I-A**, L is a linker. Optionally, L is a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, a substituted or unsubstituted alkynyl, a substituted or unsubstituted alkoxy, or a substituted or unsubstituted amino. Optionally, L contains an amide group.

Additionally, in **Structure I-A**, X and Y are each independently selected from  $\text{CH}_2$ , CHD,  $\text{CD}_2$ , CHF,  $\text{CF}_2$ , and C(O). Optionally X and Y are each C(O).

Further, in some examples of **Structure I-A**,  $R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are each independently selected from hydrogen, substituted or unsubstituted alkyl (e.g., methyl, ethyl, propyl, or butyl), and substituted or unsubstituted carbonyl.

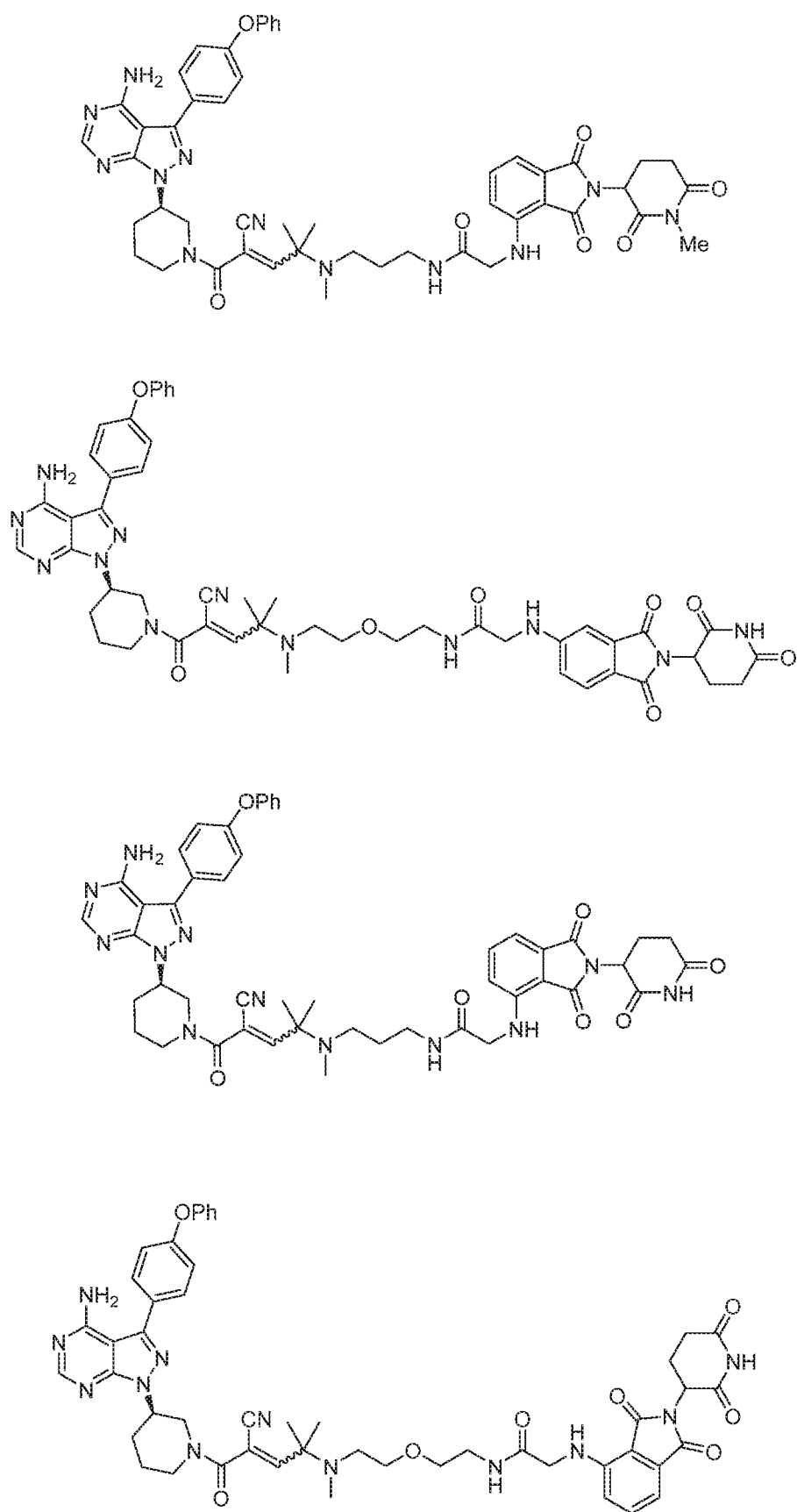
Also, in some examples of **Structure I-A**,  $R^5$  is hydrogen, deuterium, fluoro, chloro, bromo, iodo, cyano,  $-\text{OCH}_3$ ,  $-\text{OCDH}_2$ ,  $-\text{OCD}_2\text{H}$ , or  $-\text{OCD}_3$ .

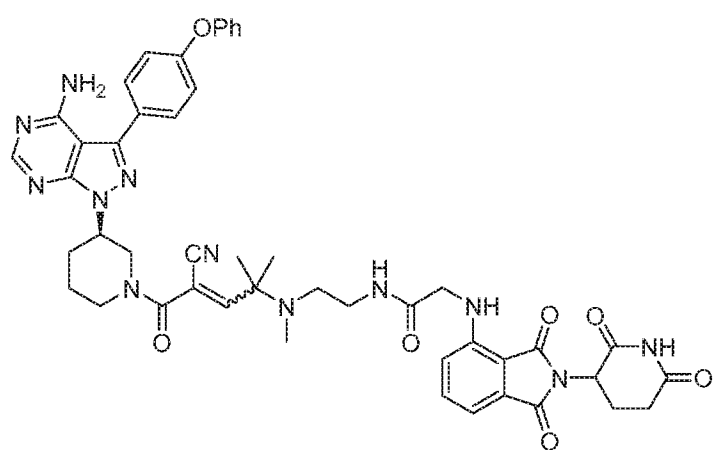
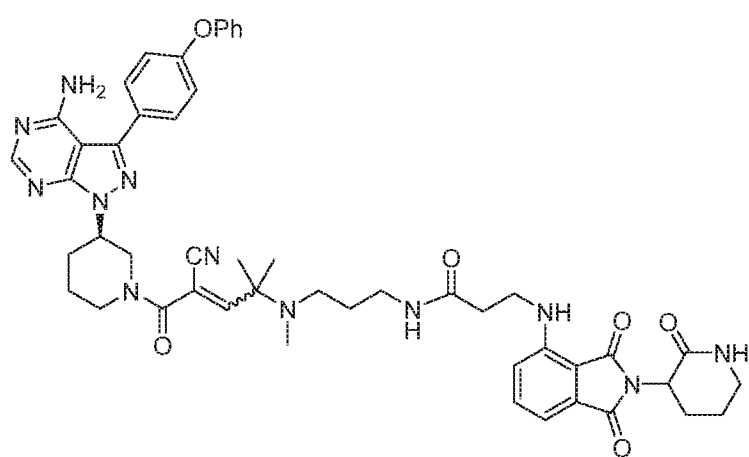
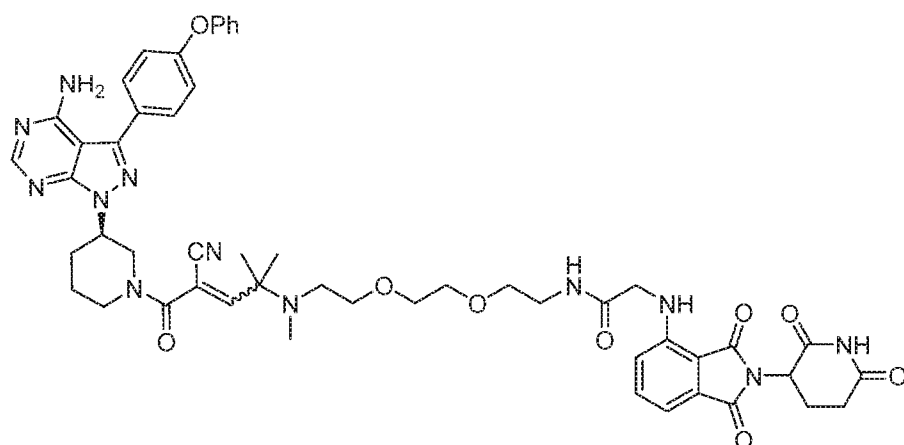
In some cases, the compounds according to **Formula I** are reversible covalent (RC) compounds represented by **Structure I-B**:

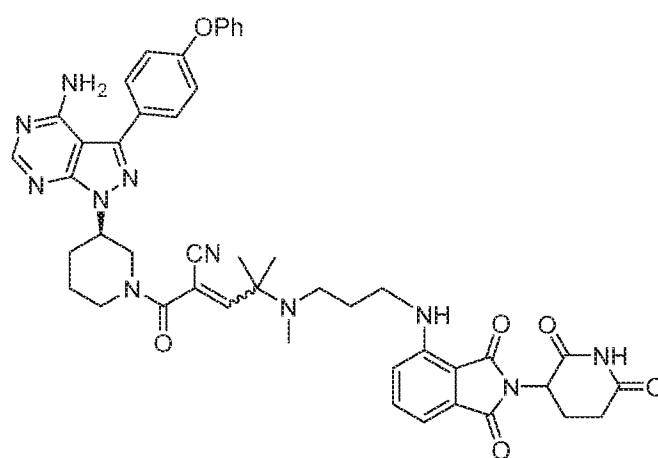
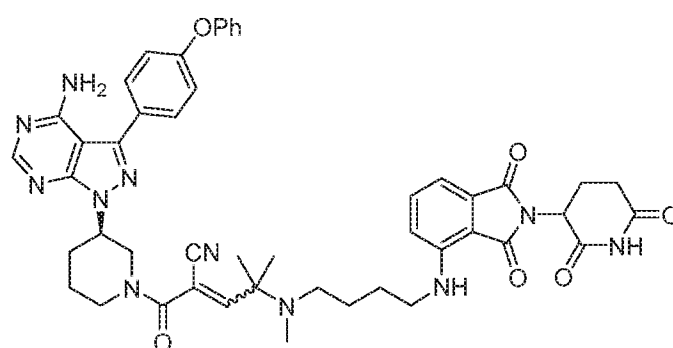
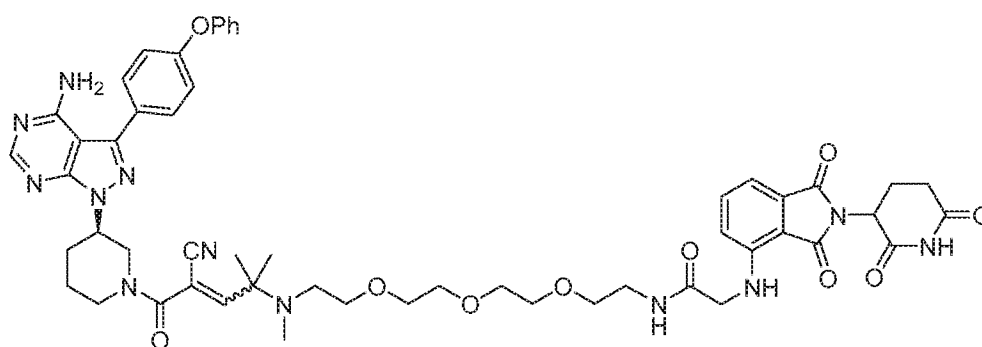


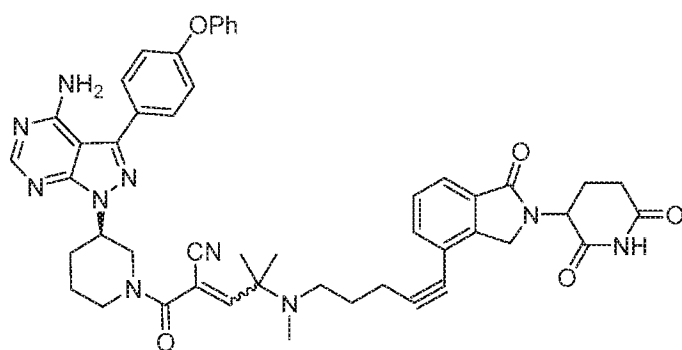
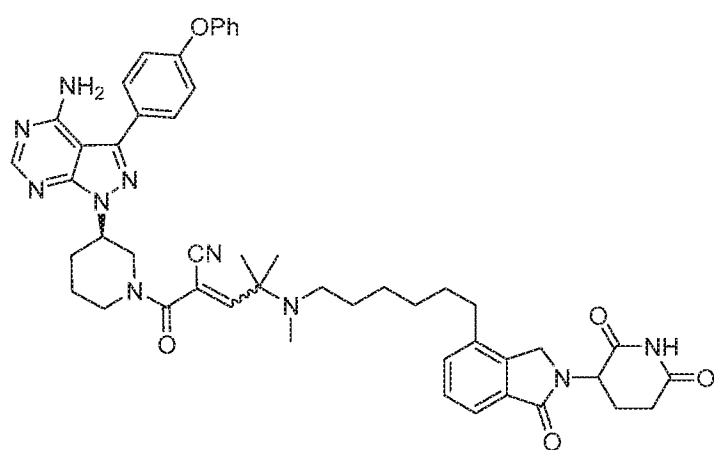
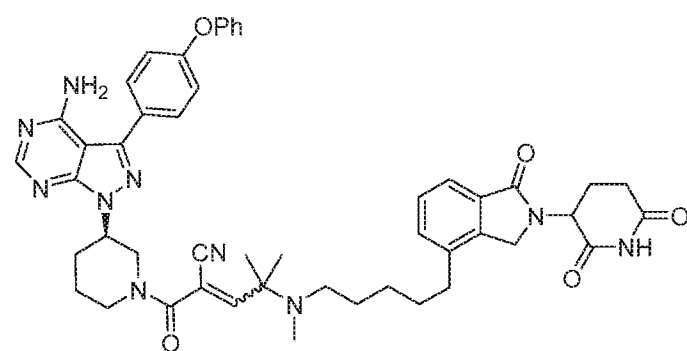
Structure I-B

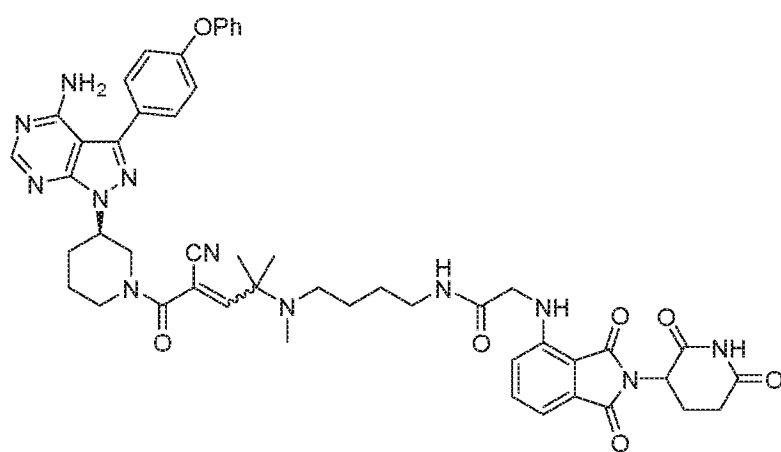
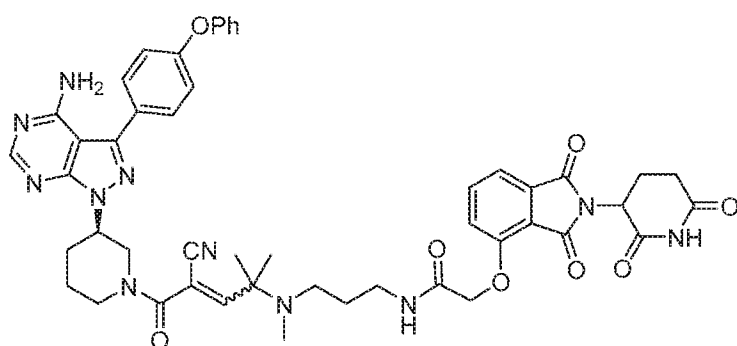
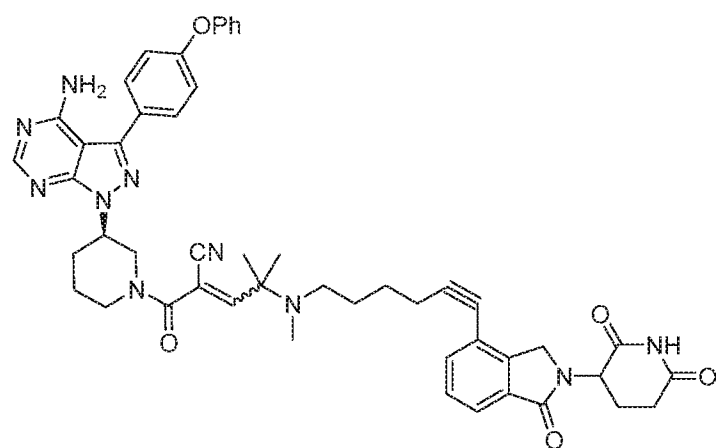
In **Structure I-B**, L, X, Y,  $R^1$ , and  $R^2$  are as defined above for **Formula I**. Examples of **Structure I-B** include the following compounds:

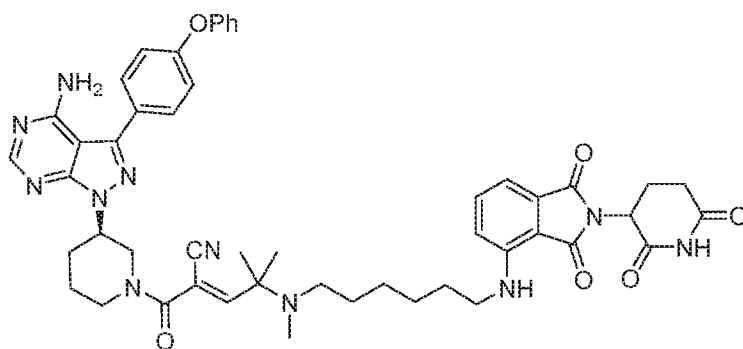
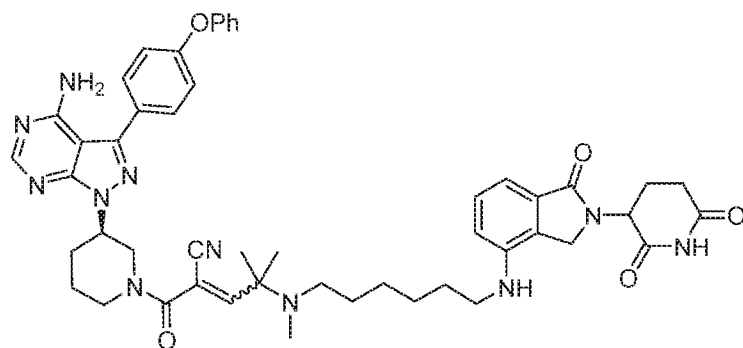
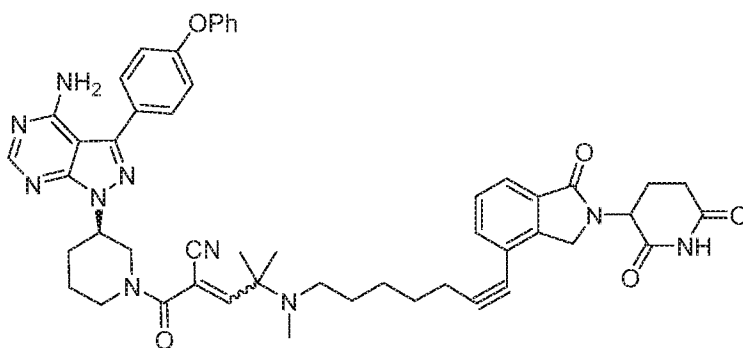
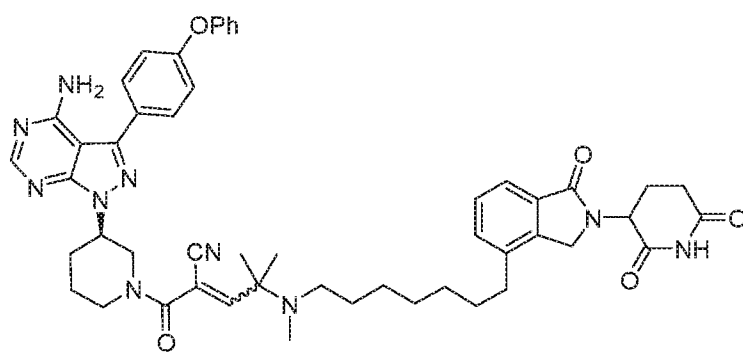


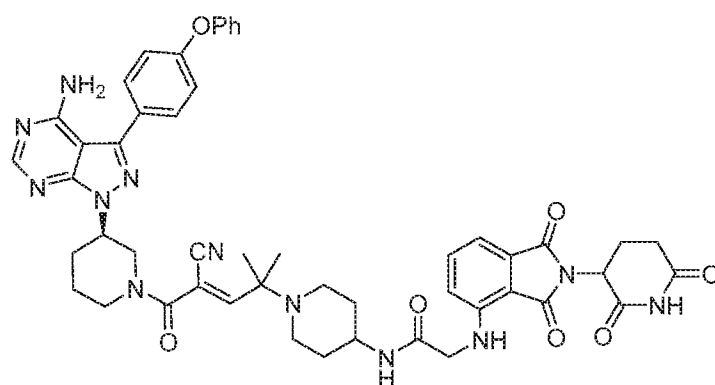
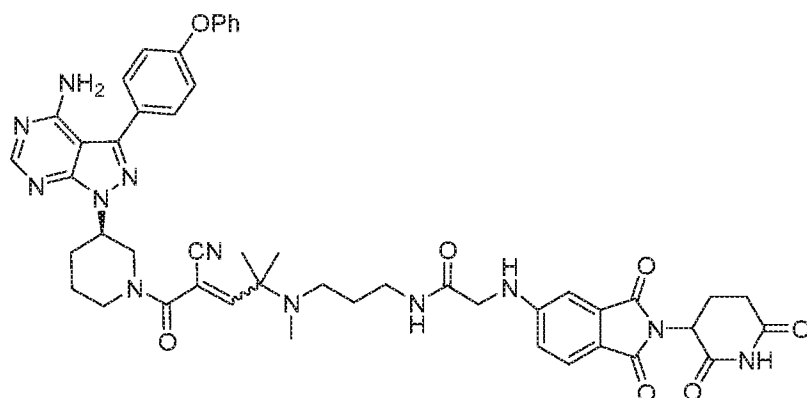




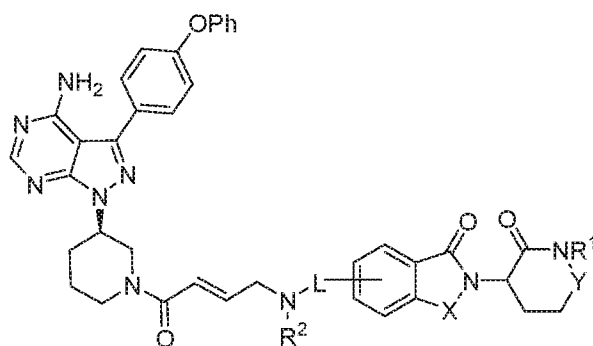






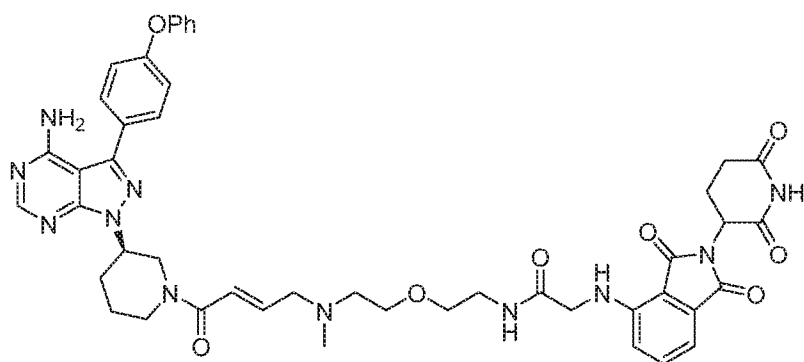
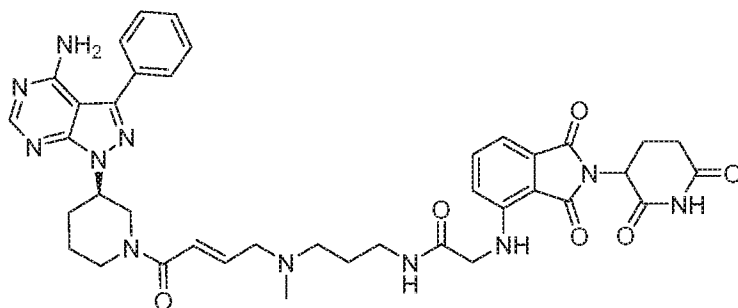
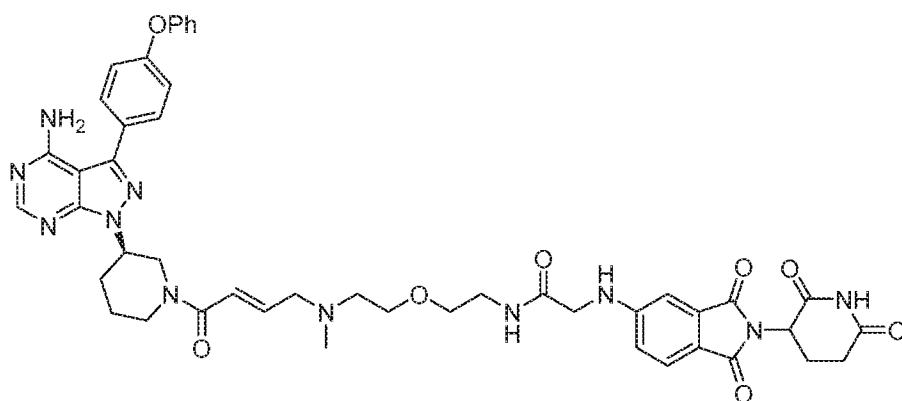


In some cases, the compounds according to **Formula I** are irreversible covalent (IRC) compounds represented by **Structure I-C**:

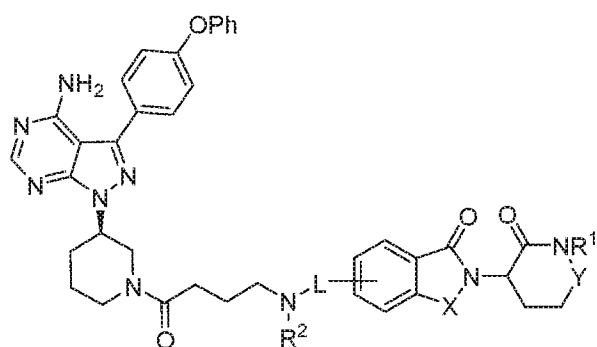


**Structure I-C**

- 5 In **Structure I-C**, L, X, Y, R<sup>1</sup>, and R<sup>2</sup> are as defined above for **Formula I**. Examples of **Structure I-C** include the following compounds:

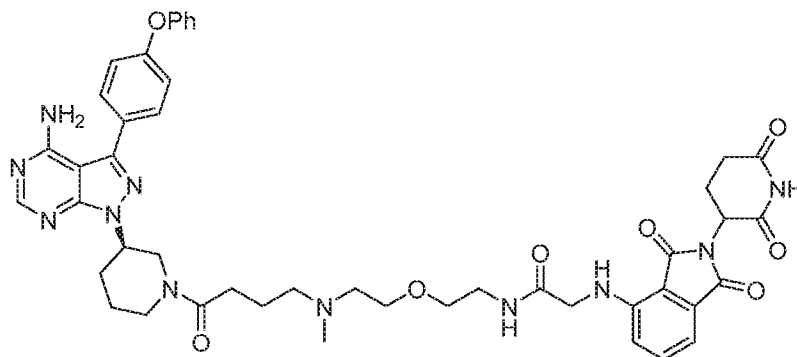
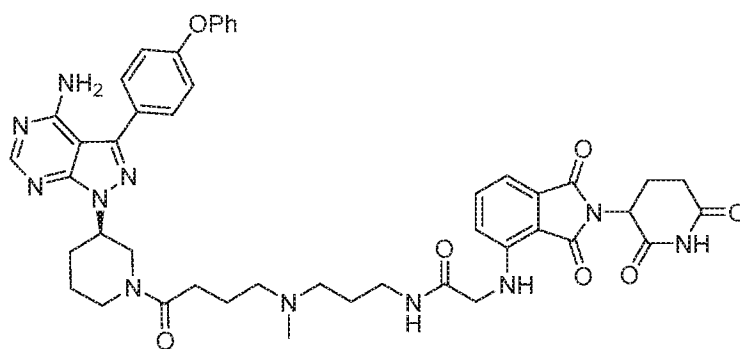
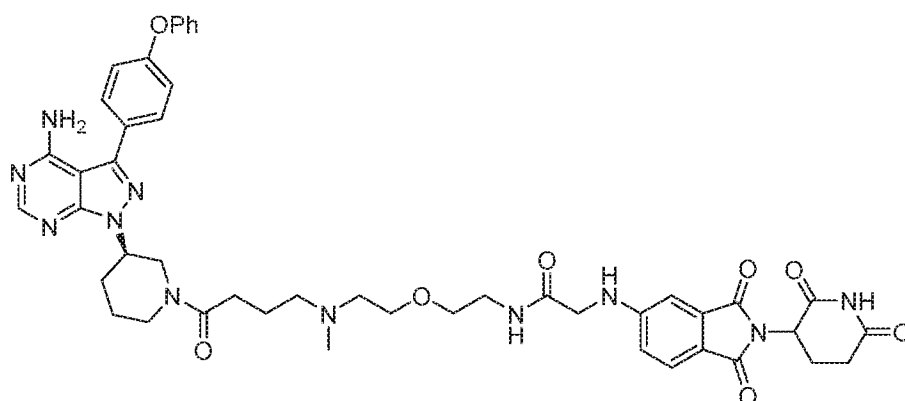


In some cases, the compounds according to **Formula I** are reversible noncovalent (RNC) compounds represented by **Structure I-D**:

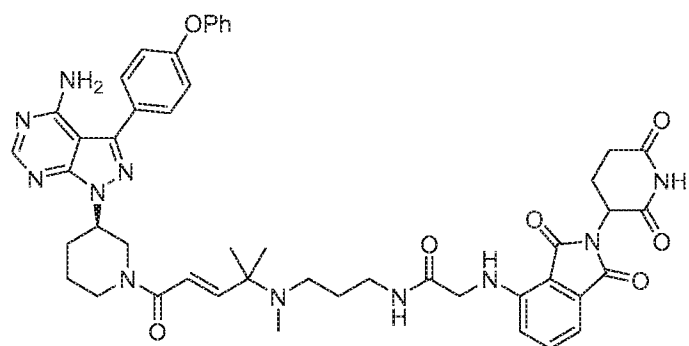
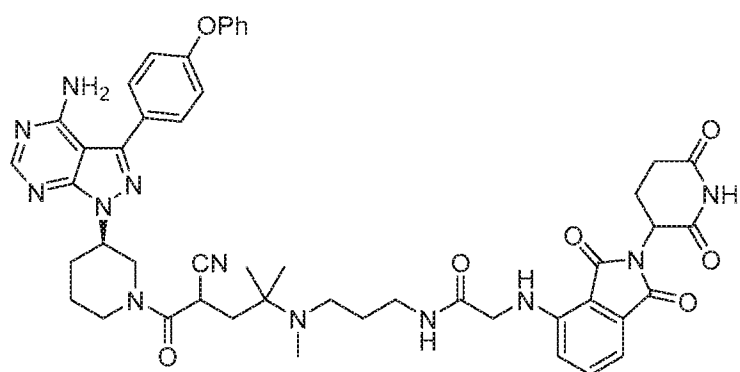


**Structure I-D**

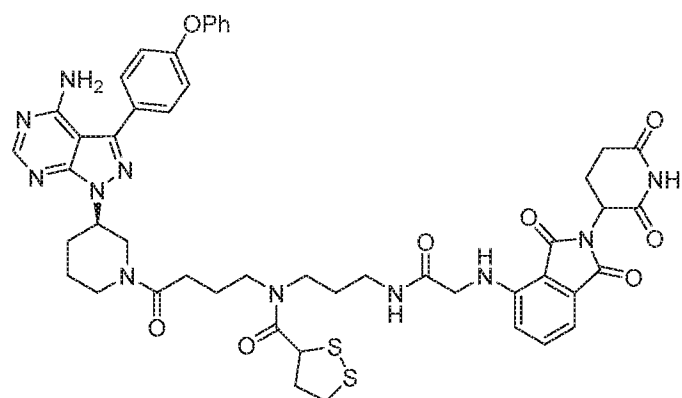
In **Structure I-D**, L, X, Y, R<sup>1</sup>, and R<sup>2</sup> are as defined above for **Formula I**. Examples of **Structure I-D** include the following compounds:



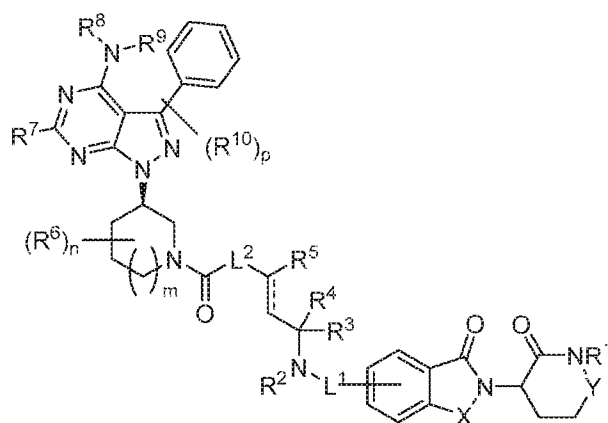
Other examples of **Formula I** include the following compounds:



5



An exemplary class of PROTACs is provided below as **Formula II**:



and pharmaceutically acceptable salts or prodrugs thereof.

In **Formula II**,  $\equiv$  is a single bond or a double bond.

Also, in **Formula II**,  $m$  is 0-3.

5 Additionally, in **Formula II**,  $n$  and  $p$  are each independently 0-5.

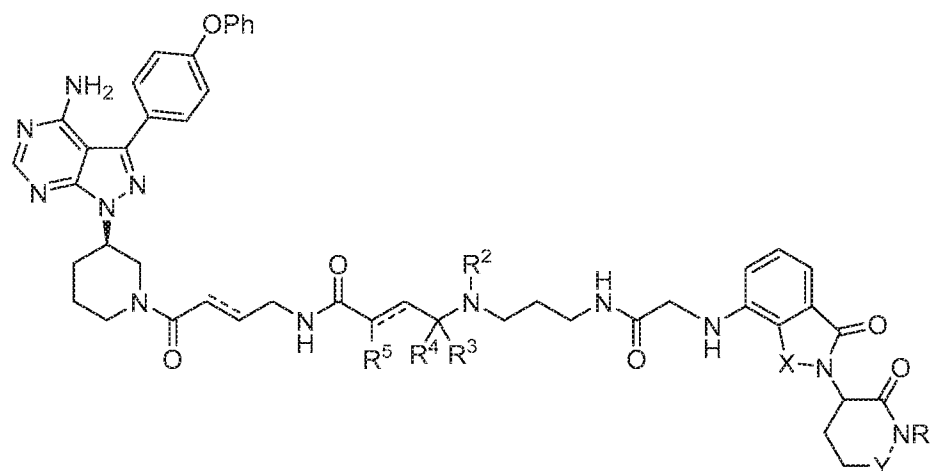
Further, in **Formula II**,  $L^1$  and  $L^2$  are each independently a linker. Optionally,  $L^1$  and/or  $L^2$  is a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, a substituted or unsubstituted alkynyl, a substituted or unsubstituted alkoxy, or a substituted or unsubstituted amino. Optionally,  $L^1$  and/or  $L^2$  contains an amide group.

10 Also, in **Formula II**,  $X$  and  $Y$  are each independently selected from  $CH_2$ ,  $CHD$ ,  $CD_2$ ,  $CHF$ ,  $CF_2$ , and  $C(O)$ . Optionally  $X$  and  $Y$  are each  $C(O)$ .

Additionally, in **Formula II**,  $R^1$ ,  $R^2$ ,  $R^8$ , and  $R^9$  are each independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted carbonyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl.

Further, in **Formula II**,  $R^3$ ,  $R^4$ ,  $R^5$ , each  $R^6$ ,  $R^7$ , and each  $R^{10}$  are each independently selected from hydrogen, halogen, cyano, trifluoromethyl, alkoxy, aryloxy, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl.

25 In some cases, the compounds according to **Formula II** are represented by **Structure II-A**:

**Structure II-A**

In **Structure II-A**,  $\equiv$ , X, Y,  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ , and  $R^5$  are as defined above for **Formula**

**II.**

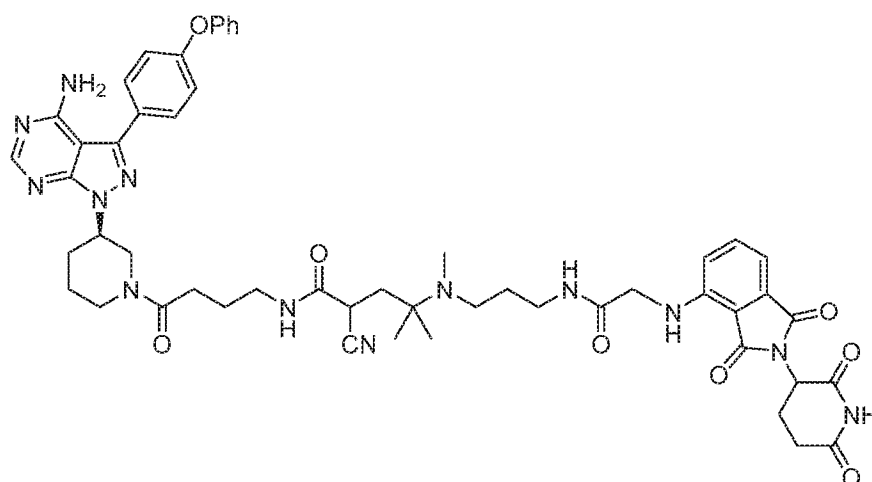
For example, in **Structure II-A**,  $\equiv$  is a single bond or a double bond.

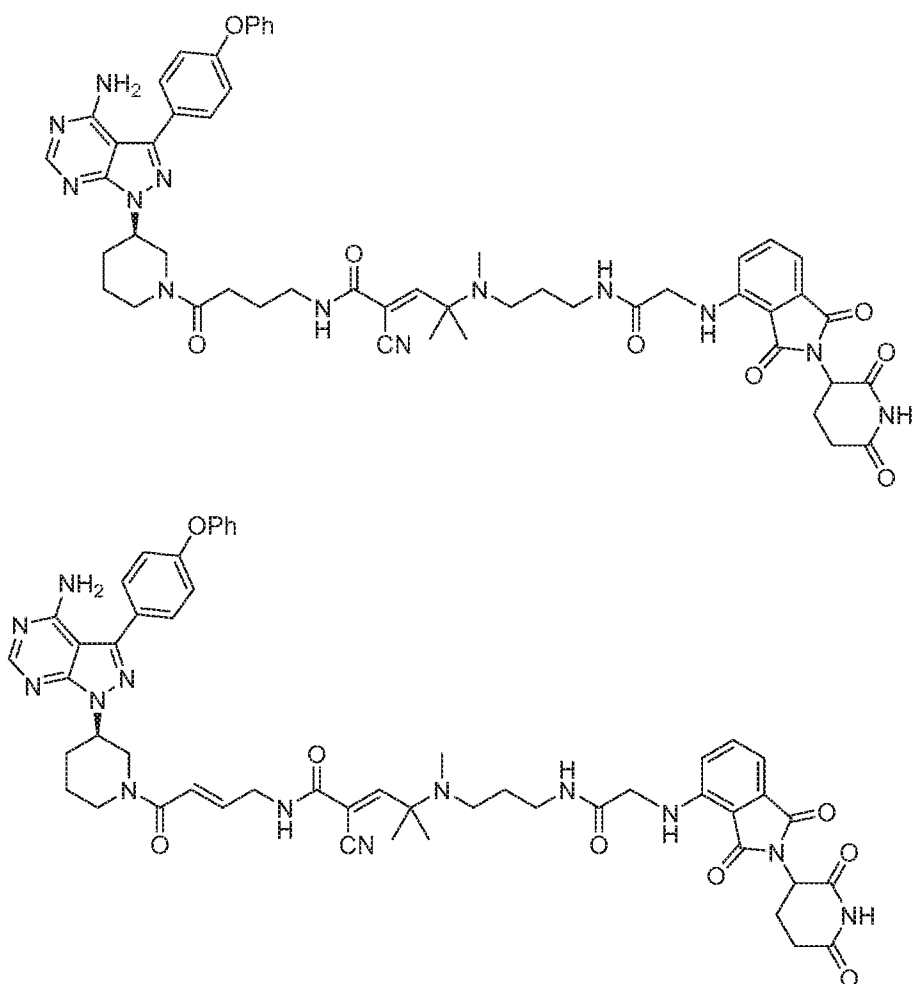
Additionally, in **Structure II-A**, X and Y are each independently selected from  $\text{CH}_2$ ,  $\text{CHD}$ ,  $\text{CD}_2$ ,  $\text{CHF}$ ,  $\text{CF}_2$ , and  $\text{C(O)}$ . Optionally X and Y are each  $\text{C(O)}$ .

Further, in some examples of **Structure II-A**,  $R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are each independently selected from hydrogen, substituted or unsubstituted alkyl (e.g., methyl, ethyl, propyl, or butyl), and substituted or unsubstituted carbonyl.

Also, in some examples of **Structure II-A**,  $R^5$  is hydrogen, deuterium, fluoro, chloro, bromo, iodo, cyano,  $-\text{OCH}_3$ ,  $-\text{OCDH}_2$ ,  $-\text{OCD}_2\text{H}$ , or  $-\text{OCD}_3$ .

Examples of **Structure II-A** include the following compounds:





As used herein, the terms alkyl, alkenyl, and alkynyl include straight- and branched-chain monovalent substituents. Examples include methyl, ethyl, isobutyl, 3-butenyl, and the like. Ranges of these groups useful with the compounds and methods described herein include C<sub>1</sub>-C<sub>20</sub> alkyl, C<sub>2</sub>-C<sub>20</sub> alkenyl, and C<sub>2</sub>-C<sub>20</sub> alkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C<sub>1</sub>-C<sub>12</sub> alkyl, C<sub>2</sub>-C<sub>12</sub> alkenyl, C<sub>2</sub>-C<sub>12</sub> alkynyl, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>2</sub>-C<sub>6</sub> alkenyl, C<sub>2</sub>-C<sub>6</sub> alkynyl, C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>2</sub>-C<sub>4</sub> alkenyl, and C<sub>2</sub>-C<sub>4</sub> alkynyl.

Heteroalkyl, heteroalkenyl, and heteroalkynyl are defined similarly as alkyl, alkenyl, and alkynyl, but can contain O, S, or N heteroatoms or combinations thereof within the backbone. Ranges of these groups useful with the compounds and methods described herein include C<sub>1</sub>-C<sub>20</sub> heteroalkyl, C<sub>2</sub>-C<sub>20</sub> heteroalkenyl, and C<sub>2</sub>-C<sub>20</sub> heteroalkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C<sub>1</sub>-C<sub>12</sub> heteroalkyl, C<sub>2</sub>-C<sub>12</sub> heteroalkenyl, C<sub>2</sub>-C<sub>12</sub> heteroalkynyl, C<sub>1</sub>-C<sub>6</sub> heteroalkyl, C<sub>2</sub>-C<sub>6</sub>

heteroalkenyl, C<sub>2</sub>-C<sub>6</sub> heteroalkynyl, C<sub>1</sub>-C<sub>4</sub> heteroalkyl, C<sub>2</sub>-C<sub>4</sub> heteroalkenyl, and C<sub>2</sub>-C<sub>4</sub> heteroalkynyl.

The terms cycloalkyl, cycloalkenyl, and cycloalkynyl include cyclic alkyl groups having a single cyclic ring or multiple condensed rings. Examples include cyclohexyl, cyclopentylethyl, and adamantanyl. Ranges of these groups useful with the compounds and methods described herein include C<sub>3</sub>-C<sub>20</sub> cycloalkyl, C<sub>3</sub>-C<sub>20</sub> cycloalkenyl, and C<sub>3</sub>-C<sub>20</sub> cycloalkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C<sub>5</sub>-C<sub>12</sub> cycloalkyl, C<sub>5</sub>-C<sub>12</sub> cycloalkenyl, C<sub>5</sub>-C<sub>12</sub> cycloalkynyl, C<sub>5</sub>-C<sub>6</sub> cycloalkyl, C<sub>5</sub>-C<sub>6</sub> cycloalkenyl, and C<sub>5</sub>-C<sub>6</sub> cycloalkynyl.

The terms heterocycloalkyl, heterocycloalkenyl, and heterocycloalkynyl are defined similarly as cycloalkyl, cycloalkenyl, and cycloalkynyl, but can contain O, S, or N heteroatoms or combinations thereof within the cyclic backbone. Ranges of these groups useful with the compounds and methods described herein include C<sub>3</sub>-C<sub>20</sub> heterocycloalkyl, C<sub>3</sub>-C<sub>20</sub> heterocycloalkenyl, and C<sub>3</sub>-C<sub>20</sub> heterocycloalkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C<sub>5</sub>-C<sub>12</sub> heterocycloalkyl, C<sub>5</sub>-C<sub>12</sub> heterocycloalkenyl, C<sub>5</sub>-C<sub>12</sub> heterocycloalkynyl, C<sub>5</sub>-C<sub>6</sub> heterocycloalkyl, C<sub>5</sub>-C<sub>6</sub> heterocycloalkenyl, and C<sub>5</sub>-C<sub>6</sub> heterocycloalkynyl.

Aryl molecules include, for example, cyclic hydrocarbons that incorporate one or more planar sets of, typically, six carbon atoms that are connected by delocalized electrons numbering the same as if they consisted of alternating single and double covalent bonds. An example of an aryl molecule is benzene. Heteroaryl molecules include substitutions along their main cyclic chain of atoms such as O, N, or S. When heteroatoms are introduced, a set of five atoms, e.g., four carbon and a heteroatom, can create an aromatic system. Examples of heteroaryl molecules include furan, pyrrole, thiophene, imadazole, oxazole, pyridine, and pyrazine. Aryl and heteroaryl molecules can also include additional fused rings, for example, benzofuran, indole, benzothiophene, naphthalene, anthracene, and quinoline. The aryl and heteroaryl molecules can be attached at any position on the ring, unless otherwise noted.

The term alkoxy as used herein is an alkyl group bound through a single, terminal ether linkage. The term aryloxy as used herein is an aryl group bound through a single, terminal ether linkage. Likewise, the terms alkenyloxy, alkynyloxy, heteroalkyloxy, heteroalkenyloxy, heteroalkynyloxy, heteroaryloxy, cycloalkyloxy, and heterocycloalkyloxy as used herein are an alkenyloxy, alkynyloxy, heteroalkyloxy, heteroalkenyloxy, heteroalkynyloxy, heteroaryloxy, cycloalkyloxy, and heterocycloalkyloxy group, respectively, bound through a single, terminal ether linkage.

The term hydroxy as used herein is represented by the formula  $\text{---OH}$ .

The terms amine or amino as used herein are represented by the formula  $\text{---NZ}^1\text{Z}^2$ , where  $\text{Z}^1$  and  $\text{Z}^2$  can each be substitution group as described herein, such as hydrogen, an alkyl, halogenated alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The alkoxy, aryloxy, amino, alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, cycloalkyl, or heterocycloalkyl molecules used herein can be substituted or unsubstituted. As used herein, the term substituted includes the addition of an alkoxy, aryloxy, amino, alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, cycloalkyl, or heterocycloalkyl group to a position attached to the main chain of the alkoxy, aryloxy, amino, alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, cycloalkyl, or heterocycloalkyl, e.g., the replacement of a hydrogen by one of these molecules. Examples of substitution groups include, but are not limited to, hydroxy, halogen (e.g., F, Br, Cl, or I), and carboxyl groups. Conversely, as used herein, the term unsubstituted indicates the alkoxy, aryloxy, amino, alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, cycloalkyl, or heterocycloalkyl has a full complement of hydrogens, i.e., commensurate with its saturation level, with no substitutions, e.g., linear decane ( $\text{---(CH}_2\text{)}_9\text{---CH}_3$ ).

The compounds described herein also include isotopic substitutions (e.g., a deuterium or tritium variant) of the compounds. In particular, one or more hydrogen atoms can be substituted by a hydrogen isotope (e.g., a deuterium or a tritium). For example, a methoxy group ( $\text{---OCH}_3$ ) can be substituted with one or more isotopic groups to form, for example,  $\text{---OCDH}_2$ ,  $\text{---OCD}_2\text{H}$ , or  $\text{---OCD}_3$ .

## II. Methods of Making the Compounds

The compounds described herein can be prepared in a variety of ways. The compounds can be synthesized using various synthetic methods. At least some of these methods are known in the art of synthetic organic chemistry. The compounds described herein can be prepared from readily available starting materials. Optimum reaction conditions can vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

Variations on **Formula I** and **Formula II** include the addition, subtraction, or movement of the various constituents as described for each compound. Similarly, when one or more chiral centers are present in a molecule, all possible chiral variants are included. Additionally, compound synthesis can involve the protection and deprotection of various

chemical groups. The use of protection and deprotection, and the selection of appropriate protecting groups can be determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Wuts, Greene's Protective Groups in Organic Synthesis, 5th. Ed., Wiley & Sons, 2014, which is incorporated herein by reference in its entirety.

5           Reactions to produce the compounds described herein can be carried out in solvents, which can be selected by one of skill in the art of organic synthesis. Solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products under the conditions at which the reactions are carried out, i.e., temperature and pressure. Reactions can be carried out in one solvent or a mixture of more than one solvent. Product or  
10       intermediate formation can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (e.g.,  $^1\text{H}$  or  $^{13}\text{C}$ ) infrared spectroscopy, spectrophotometry (e.g., UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

15           Exemplary methods for synthesizing compounds as described herein are provided in Example 1 below.

### **III.     Pharmaceutical Formulations**

          The compounds described herein or derivatives thereof can be provided in a pharmaceutical composition. Depending on the intended mode of administration, the  
20       pharmaceutical composition can be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, or suspensions, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include a therapeutically effective amount of the compound described herein or derivatives thereof in combination with a pharmaceutically acceptable carrier and,  
25       in addition, may include other medicinal agents, pharmaceutical agents, carriers, or diluents. By pharmaceutically acceptable is meant a material that is not biologically or otherwise undesirable, which can be administered to an individual along with the selected compound without causing unacceptable biological effects or interacting in a deleterious manner with the other components of the pharmaceutical composition in which it is contained.

30           As used herein, the term carrier encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations. The choice of a carrier for use in a composition will depend upon the intended route of administration for the composition. The preparation of pharmaceutically acceptable carriers and formulations containing these materials is described

in, e.g., Remington: The Science and Practice of Pharmacy, 22d Edition, Loyd et al. eds.,  
Pharmaceutical Press and Philadelphia College of Pharmacy at University of the Sciences  
(2012). Examples of physiologically acceptable carriers include buffers, such as phosphate  
buffers, citrate buffer, and buffers with other organic acids; antioxidants including ascorbic  
5 acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as  
serum albumin, gelatin, or immunoglobulins; hydrophilic polymers, such as  
polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine;  
monosaccharides, disaccharides, and other carbohydrates, including glucose, mannose, or  
dextrins; chelating agents, such as EDTA; sugar alcohols, such as mannitol or sorbitol; salt-  
10 forming counterions, such as sodium; and/or nonionic surfactants, such as TWEEN® (ICI,  
Inc.; Bridgewater, New Jersey), polyethylene glycol (PEG), and PLURONICS™ (BASF;  
Florham Park, NJ).

Compositions containing the compound described herein or derivatives thereof  
suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or  
15 nonaqueous solutions, dispersions, suspensions or emulsions, and sterile powders for  
reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous  
and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols  
(propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof,  
vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper  
20 fluidity can be maintained, for example, by the use of a coating such as lecithin, by the  
maintenance of the required particle size in the case of dispersions and by the use of  
surfactants.

These compositions may also contain adjuvants, such as preserving, wetting,  
emulsifying, and dispensing agents. Prevention of the action of microorganisms can be  
25 promoted by various antibacterial and antifungal agents, for example, parabens,  
chlorobutanol, phenol, sorbic acid, and the like. Isotonic agents, for example, sugars, sodium  
chloride, and the like may also be included. Prolonged absorption of the injectable  
pharmaceutical form can be brought about by the use of agents delaying absorption, for  
example, aluminum monostearate and gelatin.

30 Solid dosage forms for oral administration of the compounds described herein or  
derivatives thereof include capsules, tablets, pills, powders, and granules. In such solid  
dosage forms, the compounds described herein or derivatives thereof is admixed with at least  
one inert customary excipient (or carrier), such as sodium citrate or dicalcium phosphate, or  
(a) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol, and

silicic acid, (b) binders, as for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, (c) humectants, as for example, glycerol, (d) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate, (e) solution retarders, as for example, paraffin, (f) absorption accelerators, as for example, quaternary ammonium compounds, (g) wetting agents, as for example, cetyl alcohol, and glycerol monostearate, (h) adsorbents, as for example, kaolin and bentonite, and (i) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules, tablets, and pills, the dosage forms may also  
10 comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethyleneglycols, and the like.

Solid dosage forms such as tablets, dragees, capsules, pills, and granules can be  
15 prepared with coatings and shells, such as enteric coatings and others known in the art. They may contain opacifying agents and can also be of such composition that they release the active compound or compounds in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions that can be used are polymeric substances and waxes. The active compounds can also be in micro-encapsulated form, if appropriate, with one or  
20 more of the above-mentioned excipients.

Liquid dosage forms for oral administration of the compounds described herein or derivatives thereof include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents,  
25 and emulsifiers, as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, and fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include additional agents, such  
30 as wetting, emulsifying, suspending, sweetening, flavoring, or perfuming agents.

Suspensions, in addition to the active compounds, may contain additional agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters,

microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances, and the like.

Compositions of the compounds described herein or derivatives thereof for rectal administrations are optionally suppositories, which can be prepared by mixing the compounds with suitable non-irritating excipients or carriers, such as cocoa butter, polyethyleneglycol or a suppository wax, which are solid at ordinary temperatures but liquid at body temperature and, therefore, melt in the rectum or vaginal cavity and release the active component.

Dosage forms for topical administration of the compounds described herein or derivatives thereof include ointments, powders, sprays, inhalants, and skin patches. The compounds described herein or derivatives thereof are admixed under sterile conditions with a physiologically acceptable carrier and any preservatives, buffers, or propellants as may be required. Ophthalmic formulations, ointments, powders, and solutions are also contemplated as being within the scope of the compositions.

Optionally, the compounds described herein can be contained in a drug depot. A drug depot comprises a physical structure to facilitate implantation and retention in a desired site (e.g., a synovial joint, a disc space, a spinal canal, abdominal area, a tissue of the patient, etc.). The drug depot can provide an optimal concentration gradient of the compound at a distance of up to about 0.1 cm to about 5 cm from the implant site. A depot, as used herein, includes but is not limited to capsules, microspheres, microparticles, microcapsules, microfibers particles, nanospheres, nanoparticles, coating, matrices, wafers, pills, pellets, emulsions, liposomes, micelles, gels, antibody-compound conjugates, protein-compound conjugates, or other pharmaceutical delivery compositions. Suitable materials for the depot include pharmaceutically acceptable biodegradable materials that are preferably FDA approved or GRAS materials. These materials can be polymeric or non-polymeric, as well as synthetic or naturally occurring, or a combination thereof. The depot can optionally include a drug pump.

The compositions can include one or more of the compounds described herein and a pharmaceutically acceptable carrier. As used herein, the term pharmaceutically acceptable salt refers to those salts of the compound described herein or derivatives thereof that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds described herein. The term salts refers to the

relatively non-toxic, inorganic and organic acid addition salts of the compounds described herein. These salts can be prepared in situ during the isolation and purification of the compounds or by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate, methane sulphonate, and laurylsulphonate salts, and the like. These may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. (See S.M. Barge et al., J. Pharm. Sci. (1977) 66, 1, which is incorporated herein by reference in its entirety, at least, for compositions taught therein.)

Administration of the compounds and compositions described herein or pharmaceutically acceptable salts thereof can be carried out using therapeutically effective amounts of the compounds and compositions described herein or pharmaceutically acceptable salts thereof as described herein for periods of time effective to treat a disorder. The effective amount of the compounds and compositions described herein or pharmaceutically acceptable salts thereof as described herein may be determined by one of ordinary skill in the art and includes exemplary dosage amounts for a mammal of from about 0.0001 to about 200 mg/kg of body weight of active compound per day, which may be administered in a single dose or in the form of individual divided doses, such as from 1 to 4 times per day. Alternatively, the dosage amount can be from about 0.01 to about 150 mg/kg of body weight of active compound per day, about 0.1 to 100 mg/kg of body weight of active compound per day, about 0.5 to about 75 mg/kg of body weight of active compound per day, about 0.5 to about 50 mg/kg of body weight of active compound per day, about 0.01 to about 50 mg/kg of body weight of active compound per day, about 0.05 to about 25 mg/kg of body weight of active compound per day, about 0.1 to about 25 mg/kg of body weight of active compound per day, about 0.5 to about 25 mg/kg of body weight of active compound per day, about 1 to about 20 mg/kg of body weight of active compound per day, about 1 to about 10 mg/kg of body weight of active compound per day, about 20 mg/kg of body weight of active compound per day, about 10 mg/kg of body weight of active compound per day, about 5 mg/kg of body weight of active compound per day, about 2.5 mg/kg of body weight of active compound per day, about

1.0 mg/kg of body weight of active compound per day, or about 0.5 mg/kg of body weight of active compound per day, or any range derivable therein. Optionally, the dosage amounts are from about 0.01 mg/kg to about 10 mg/kg of body weight of active compound per day.

Optionally, the dosage amount is from about 0.01 mg/kg to about 5 mg/kg. Optionally, the dosage amount is from about 0.01 mg/kg to about 2.5 mg/kg.

Those of skill in the art will understand that the specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors, including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition.

The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. Further, depending on the route of administration, one of skill in the art would know how to determine doses that result in a plasma concentration for a desired level of response in the cells, tissues and/or organs of a subject.

#### IV. Methods of Use

Provided herein are methods to treat, prevent, or ameliorate a BTK-related disease in a subject. The methods include administering to a subject an effective amount of one or more of the compounds or compositions described herein, or a pharmaceutically acceptable salt or prodrug thereof. Effective amount, when used to describe an amount of compound in a method, refers to the amount of a compound that achieves the desired pharmacological effect or other biological effect. The effective amount can be, for example, the concentrations of compounds at which BTK is degraded *in vitro*, as provided herein. Also contemplated is a method that includes administering to the subject an amount of one or more compounds described herein such that an *in vivo* concentration at a target cell in the subject corresponding to the concentration administered *in vitro* is achieved.

The compounds and compositions described herein or pharmaceutically acceptable salts thereof are useful for treating BTK-related diseases in humans, including, without limitation, pediatric and geriatric populations, and in animals, e.g., veterinary applications.

In some embodiments, the BTK-related disease is cancer. Optionally, the cancer is a poor prognosis cancer. The term poor prognosis, as used herein, refers to a prospect of

recovery from a disease, infection, or medical condition that is associated with a diminished likelihood of a positive outcome. In relation to a disease such as cancer, a poor prognosis may be associated with a reduced patient survival rate, reduced patient survival time, higher likelihood of metastatic progression of said cancer cells, and/or higher likelihood of

5 chemoresistance of said cancer cells. Optionally, a poor prognosis cancer can be a cancer associated with a patient survival rate of 50% or less. Optionally, a poor prognosis cancer can be a cancer associated with a patient survival time of five years or less after diagnosis. In some embodiments, the cancer is an invasive cancer.

Optionally, the cancer is a cancer that has an increased expression of BTK as

10 compared to non-cancerous cells of the same cell type. Optionally, the cancer is bladder cancer, brain cancer, breast cancer (e.g., triple negative breast cancer), bronchus cancer, colorectal cancer (e.g., colon cancer, rectal cancer), cervical cancer, chondrosarcoma, endometrial cancer, gastrointestinal cancer, gastric cancer, genitourinary cancer, glioblastoma, head and neck cancer, hepatic cancer, hepatocellular carcinoma, leukemia, liver

15 cancer, lung cancer, lymphoma, melanoma of the skin, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, skin cancer, testicular cancer, thyroid cancer, or uterine cancer. Optionally, the cancer is a cancer that affects one or more of the following sites: oral cavity and pharynx (e.g., tongue, mouth, pharynx, or other oral cavity); digestive system (e.g., esophagus, stomach, small intestine, colon, rectum, anus, anal canal, anorectum, liver and

20 intrahepatic bile duct, gallbladder and other biliary, pancreas, or other digestive organs); respiratory system (e.g., larynx, lung and bronchus, or other respiratory organs); bones and joints; soft tissue (e.g., heart); skin (e.g., melanoma of the skin or other nonepithelial skin); breast; genital system (e.g., uterine cervix, uterine corpus, ovary, vulva, vagina and other female genital areas, prostate, testis, penis and other male genital areas); urinary system (e.g.,

25 urinary bladder, kidney and renal pelvis, and ureter and other urinary organs); eye and orbit; brain and other nervous system; endocrine system (e.g., thyroid and other endocrine); lymphoma (e.g., Hodgkin lymphoma and non-Hodgkin lymphoma); myeloma; or leukemia (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, or other leukemia). Optionally, the cancer is a drug resistant

30 cancer, such as an ibrutinib-resistant cancer.

In some embodiments, the BTK-related disease is a metabolic disorder (e.g., obesity, diabetes, and genetic disorders). Optionally, the BTK-related disease is a neurodegenerative disorder. Optionally, the neurodegenerative disorder is Parkinson's disease. Optionally, the neurodegenerative disorder is Alexander disease, Alper's disease, Alzheimer disease,

amyotrophic lateral sclerosis, ataxia telangiectasia, Batten disease (also known as Spielmeyer-Vogt-Sjogren-Batten disease), Canavan disease, Cockayne syndrome, Corticobasal degeneration, Creutzfeldt-Jakob disease, Huntington's disease, Kennedy's disease, Krabbe disease, Lewy body dementia, Machado-Joseph disease, Spinocerebellar ataxia type 3, multiple sclerosis, multiple system atrophy, Pelizaeus-Merzbacher disease, Pick's disease, Primary lateral sclerosis, Refsum's disease, Sandhoff disease, Schilder's disease, Spielmeyer-Vogt-Sjogren-Batten disease (also known as Batten disease), Spinocerebellar ataxia (multiple types with varying characteristics), Spinal muscular atrophy, Steele-Richardson-Olszewski disease, Tay-Sachs, Transmissible spongiform  
10 encephalopathies (TSE), or Tabes dorsalis.

Optionally, the BTK-related disease is an inflammatory disease. Generally, inflammatory disorders include, but are not limited to, respiratory or pulmonary disorders (including asthma, COPD, chronic bronchitis and cystic fibrosis); cardiovascular related disorders (including atherosclerosis, post-angioplasty, restenosis, coronary artery diseases  
15 and angina); inflammatory diseases of the joints (including rheumatoid and osteoarthritis); skin disorders (including dermatitis, eczematous dermatitis and psoriasis); post transplantation late and chronic solid organ rejection; multiple sclerosis; autoimmune conditions (including systemic lupus erythematosus, dermatomyositis, polymyositis, Sjogren's syndrome, polymyalgia rheumatica, temporal arteritis, Behcet's disease, Guillain  
20 Barre, Wegener's granulomatosis, polyarteritis nodosa); inflammatory neuropathies (including inflammatory polyneuropathies); vasculitis (including Churg-Strauss syndrome, Takayasu's arteritis); inflammatory disorders of adipose tissue; and proliferative disorders (including Kaposi's sarcoma and other proliferative disorders of smooth muscle cells).

Optionally, the BTK-related disease is ischemia, a gastrointestinal disorder, a viral  
25 infection (e.g., human immunodeficiency virus (HIV), including HIV type 1 (HIV-1) and HIV type 2 (HIV-2)), a bacterial infection, a central nervous system disorder, a spinal cord injury, or peripheral neuropathy.

The methods of treating or preventing a BTK-related disease (e.g., cancer) in a subject can further comprise administering to the subject one or more additional agents. The one or  
30 more additional agents and the compounds described herein or pharmaceutically acceptable salts or prodrugs thereof can be administered in any order, including concomitant, simultaneous, or sequential administration. Sequential administration can be administration in a temporally spaced order of up to several days apart. The methods can also include more than a single administration of the one or more additional agents and/or the compounds

described herein or pharmaceutically acceptable salts or prodrugs thereof. The administration of the one or more additional agents and the compounds described herein or pharmaceutically acceptable salts or prodrugs thereof can be by the same or different routes and concurrently or sequentially.

5 Additional therapeutic agents include, but are not limited to, chemotherapeutic agents, anti-depressants, anxiolytics, antibodies, antivirals, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines, chemokines, and/or growth factors. The additional therapeutic agents can be biomolecules.

A chemotherapeutic agent is a compound or composition effective in inhibiting or  
10 arresting the growth of an abnormally growing cell. Thus, such an agent may be used therapeutically to treat cancer as well as other diseases marked by abnormal cell growth. Illustrative examples of chemotherapeutic compounds include, but are not limited to, bexarotene, gefitinib, erlotinib, gemcitabine, paclitaxel, docetaxel, topotecan, irinotecan, temozolomide, carmustine, vinorelbine, capecitabine, leucovorin, oxaliplatin, bevacizumab,  
15 cetuximab, panitumumab, bortezomib, oblimersen, hexamethylmelamine, ifosfamide, CPT-11, deflunomide, cycloheximide, dicarbazine, asparaginase, mitotant, vinblastine sulfate, carboplatin, colchicine, etoposide, melphalan, 6-mercaptopurine, teniposide, vinblastine, antibiotic derivatives (e.g. anthracyclines such as doxorubicin, liposomal doxorubicin, and diethylstilbestrol doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens  
20 (e.g., tamoxifen); antimetabolites (e.g., fluorouracil (FU), 5-FU, methotrexate, floxuridine, interferon alpha-2B, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cisplatin, vincristine and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine  
25 phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chlorambucil, mechlorethamine (nitrogen mustard) and thiotepa); and steroids (e.g., bethamethasone sodium phosphate).

Therapeutic agents further include, but are not limited to, levadopa, a dopamine  
30 agonist, an anticholinergic agent, a monoamine oxidase inhibitor, a COMT inhibitor, amantadine, rivastigmine, an NMDA antagonist, a cholinesterase inhibitor, riluzole, an anti-psychotic agent, an antidepressant, and tetrabenazine.

Any of the aforementioned therapeutic agents can be used in any combination with the compositions described herein. Combinations are administered either concomitantly

(e.g., as an admixture), separately but simultaneously (e.g., via separate intravenous lines into the same subject), or sequentially (e.g., one of the compounds or agents is given first followed by the second). Thus, the term combination is used to refer to concomitant, simultaneous, or sequential administration of two or more agents.

5           Optionally, a compound or therapeutic agent as described herein may be administered in combination with a radiation therapy, an immunotherapy, a gene therapy, or a surgery.

          The methods and compounds as described herein are useful for both prophylactic and therapeutic treatment. For prophylactic use, a therapeutically effective amount of the compounds and compositions or pharmaceutically acceptable salts thereof as described herein  
10           are administered to a subject prior to onset (e.g., before obvious signs of a BTK-related disease), during early onset (e.g., upon initial signs and symptoms of a BTK-related disease), or after the development of a BTK-related disease. Prophylactic administration can occur for several days to years prior to the manifestation of symptoms of a BTK-related disease. Therapeutic treatment involves administering to a subject a therapeutically effective amount  
15           of the compounds and compositions or pharmaceutically acceptable salts thereof as described herein after a BTK-related disease is diagnosed.

          The compounds described herein are also useful in modulating BTK in a cell. Optionally, the compounds and compositions described herein are useful for inducing BTK degradation in a cell. The methods for inducing BTK degradation in a cell includes  
20           contacting a cell with an effective amount of one or more of the compounds as described herein. Optionally, the contacting is performed *in vivo*. Optionally, the contacting is performed *in vitro*.

          The methods herein for prophylactic and therapeutic treatment optionally comprise selecting a subject with or at risk of developing a BTK-related disease. A skilled artisan can  
25           make such a determination using, for example, a variety of prognostic and diagnostic methods, including, for example, a personal or family history of the disease or condition, clinical tests (e.g., imaging, biopsy, genetic tests), and the like. Optionally, the methods herein can be used for preventing relapse of cancer in a subject in remission (e.g., a subject that previously had cancer).

## 30           V.     Kits

          Also provided herein are kits for treating or preventing a BTK-related disease (e.g., cancer, a neurodegenerative disorder, an inflammatory diseases, and/or a metabolic disorder) in a subject. A kit can include any of the compounds or compositions described herein. For example, a kit can include one or more compounds of **Formula I** and/or **Formula II**. A kit

can further include one or more additional agents, such as one or more chemotherapeutic agents. A kit can include an oral formulation of any of the compounds or compositions described herein. A kit can include an intravenous formulation of any of the compounds or compositions described herein. A kit can additionally include directions for use of the kit  
5 (e.g., instructions for treating a subject), a container, a means for administering the compounds or compositions (e.g., a syringe), and/or a carrier.

As used herein the terms treatment, treat, or treating refer to a method of reducing one or more symptoms of a disease or condition. Thus in the disclosed method, treatment can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the  
10 severity of one or more symptoms of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms or signs (e.g., size of the tumor or rate of tumor growth) of the disease in a subject as compared to a control. As used herein, control refers to the untreated condition (e.g., the tumor cells not treated with the compounds and compositions described herein). Thus the  
15 reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition.

As used herein, the terms prevent, preventing, and prevention of a disease or disorder  
20 refer to an action, for example, administration of a composition or therapeutic agent, that occurs before or at about the same time a subject begins to show one or more symptoms of the disease or disorder, which inhibits or delays onset or severity of one or more symptoms of the disease or disorder.

As used herein, references to decreasing, reducing, or inhibiting include a change of  
25 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level. Such terms can include, but do not necessarily include, complete elimination.

As used herein, subject means both mammals and non-mammals. Mammals include, for example, humans; non-human primates, e.g., apes and monkeys; cattle; horses; sheep; rats; mice; pigs; and goats. Non-mammals include, for example, fish and birds.

30 Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application.

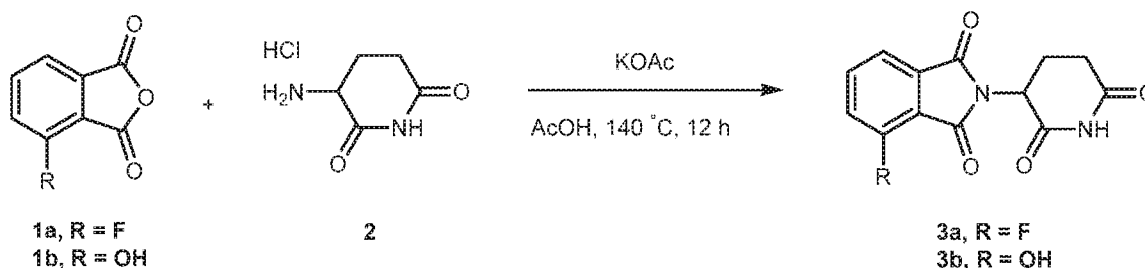
The examples below are intended to further illustrate certain aspects of the methods and compositions described herein, and are not intended to limit the scope of the claims.

## EXAMPLES

## Example 1: Compound Synthesis

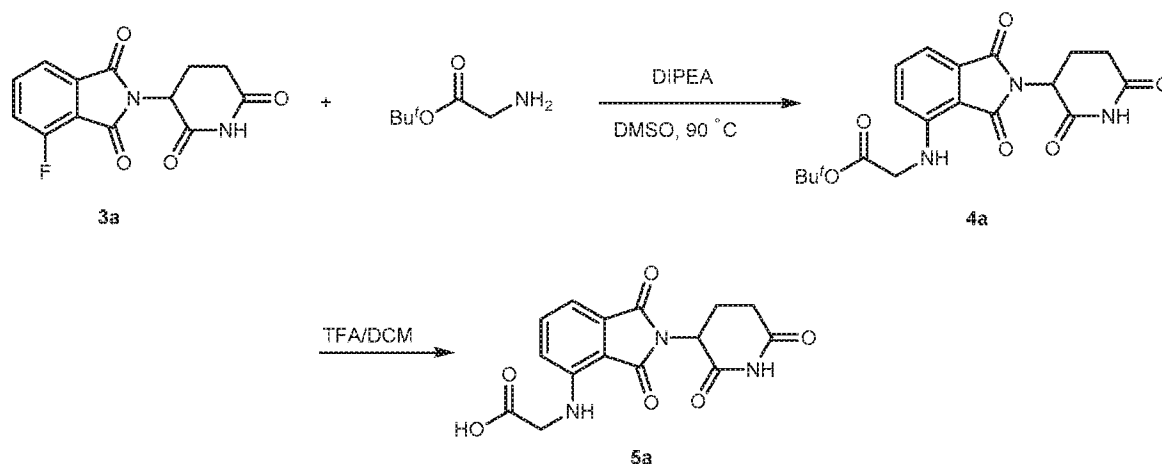
All chemicals were purchased from Sigma-Aldrich (Milwaukee, WI), Combi-Blocks, Inc. (San Diego, CA), or Alfa Aesar (Ward Hill, MA), unless otherwise specified. All solvents and reagents were used as obtained without further purification. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were obtained on a Varian (Palo Alto, CA) 400-MR spectrometer. Chemical shifts (δ) are reported in ppm, and coupling constants (J) are in Hertz (Hz). The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Flash chromatography was performed on a Teledyne ISCO CombiFlash Rf 200 (Teledyne Isco, Inc.; Lincoln, NE). ESI mass spectrometry was measured on an Agilent Mass Spectrometer (Agilent Technologies, Inc.; Santa Clara, CA).

## Scheme 1:



Compound **1a** or **1b** (1 g, 6.1 mmol) and compound **2** (1g, 6.1 mmol) were dissolved in acetic acid (AcOH; 20 mL) and heated to 140 °C overnight. The mixture was then cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash column chromatography (0-10% methanol (MeOH) in dichloromethane (DCM)) to give compound **3a** or **3b** as a white solid (1.3 g, 79%). Compound **3a**. <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 11.16 (s, 1H), 7.95 (m, 1H), 7.79 (d, *J* = 7.3 Hz, 1H), 7.74 (t, *J* = 8.9 Hz, 1H), 5.16 (dd, *J* = 12.8, 5.4 Hz, 1H), 2.89 (m, 1H), 2.57 (m, 2H), 2.07 (M, 1H). Compound **3b**. <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 11.15 (s, 1H), 11.06 (s, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 7.0 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 5.04 (dd, *J* = 12.9, 4.8 Hz, 1H), 2.86 (m, 1H), 2.54 (m, 2H), 1.99 (m, 1H).

Scheme 2:

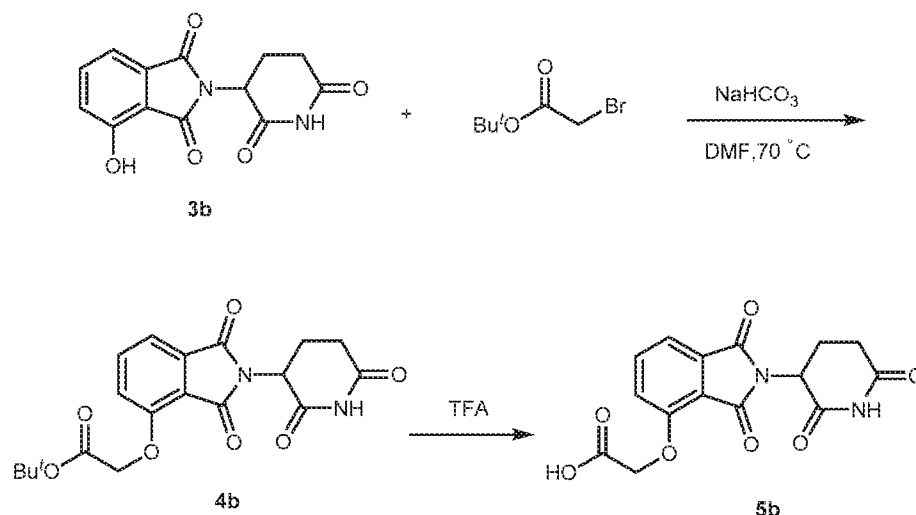


***tert*-Butyl (2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)glycinate (4a).**

Compound 3a (552 mg, 2 mmol), *tert*-butyl glycinate (524 mg, 4 mmol) and N, N-diisopropylethylamine (DIPEA; 516 mg, 4 mmol) were dissolved in dimethylsulfoxide (DMSO; 10 mL) and heated to 90°C. After 4 hours, the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash column chromatography (0-10% MeOH in DCM) to give compound 4a as a yellow solid (542 mg, 70%). <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 11.11 (s, 1H), 7.58 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 6.85 (t, *J* = 6.0 Hz, 1H), 5.07 (dd, *J* = 13.0, 5.4 Hz, 1H), 4.09 (d, *J* = 6.0 Hz, 2H), 2.89 (m, 1H), 2.56 (m, 2H), 2.05 (m, 1H), 1.43 (s, 9H).

**(2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)glycine (5a).** In a 100 mL flask was added compound 4a (542 mg, 1.4 mmol) in trifluoroacetic acid (TFA)/DCM (10 mL, 1/1). The mixture was stirred for 30 minutes at room temperature. LC-MS showed 4a converted into 5a completely. The solvent was then removed *in vacuo* to give product 5a (440 mg, 95%), which was used for for next step without further purification.

Scheme 3:



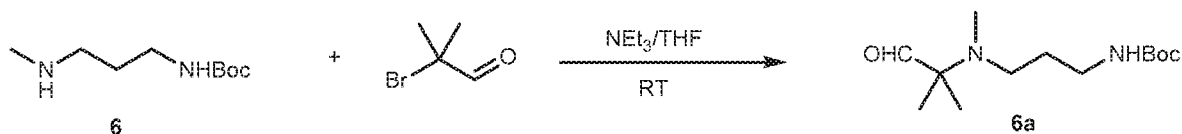
***tert*-Butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetate**

**(4b).** To a solution of compound **3b** (552 mg, 2 mmol) and *tert*-butyl 2-bromoacetate (467 mg, 2.4 mmol) in DMF (10 mL) was added NaHCO<sub>3</sub> (252 mg, 3 mmol). The mixture was heated to 70°C for 4 hours and then the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash column chromatography (0-10% MeOH in DCM) to give compound **4b** as a white solid (582 mg, 75%). <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 8.31 (s, 1H), 7.81 (t, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 7.3 Hz, 1H), 7.38 (d, *J* = 8.6 Hz, 1H), 5.23 (dd, *J* = 13.1, 5.2 Hz, 1H), 4.97 (s, 2H), 3.09 (m, 1H), 2.84 (m, 1H), 2.65 (m, 1H), 2.11 (m, 1H), 1.43 (s, 9H), 1.40 (s, 9H).

**2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetic acid (5b).** In a 100 mL flask was added **4b** (78 mg, 0.2 mmol) in TFA/DCM (10 mL, 1/1). The mixture was stirred for 30 minutes at room temperature. LC-MS showed **4b** converted into **5b** completely. The solvent was then removed *in vacuo* to give product **5b** (66 mg, 95%), which was used in the next step without further purification.

**General Procedure of RC PROTACs Synthesis (RC-1).**

Scheme 4:

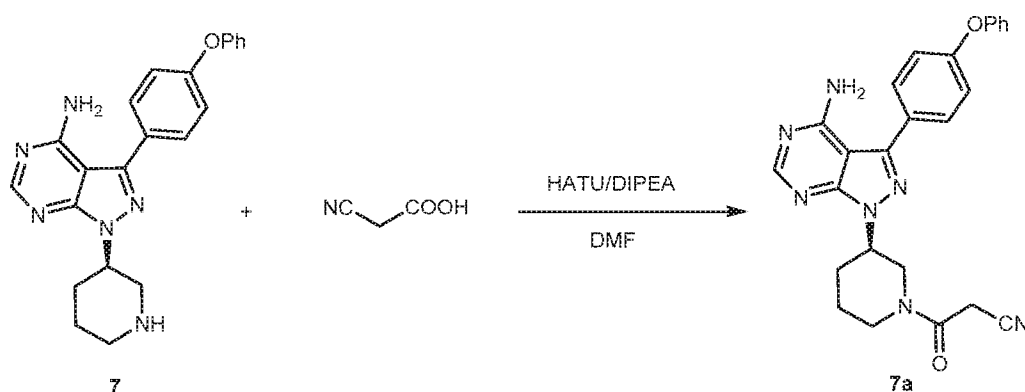


***tert*-Butyl (3-(methyl(2-methyl-1-oxopropan-2-yl)amino)propyl)carbamate (6a).**

To a solution of compound **6** (658 mg, 3.5 mmol) in 20 mL tetrahydrofuran (THF) was added triethylamine (NEt<sub>3</sub>; 707 mg, 7 mmol) and 2-bromo-2-methylpropanal (1.05 g, 7 mmol).

The solution was stirred overnight. Then, the reaction mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (5% MeOH in DCM) to give product **6a** (542 mg, 60%) as a yellow oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.41 (s, 1H), 5.05 (s, 1H), 3.18 (q, *J* = 6.1 Hz, 2H), 2.35 (t, *J* = 6.6 Hz, 2H), 2.20 (s, 3H), 1.65 (m, 2H), 1.43 (s, 9H), 1.07 (s, 6H).

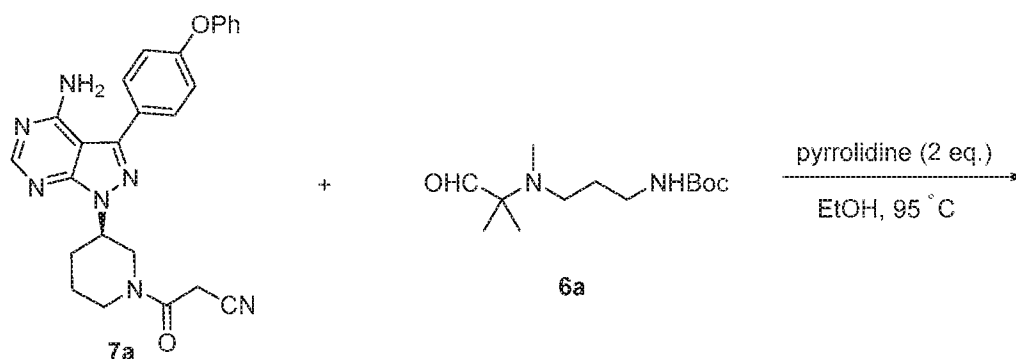
**Scheme 5:**

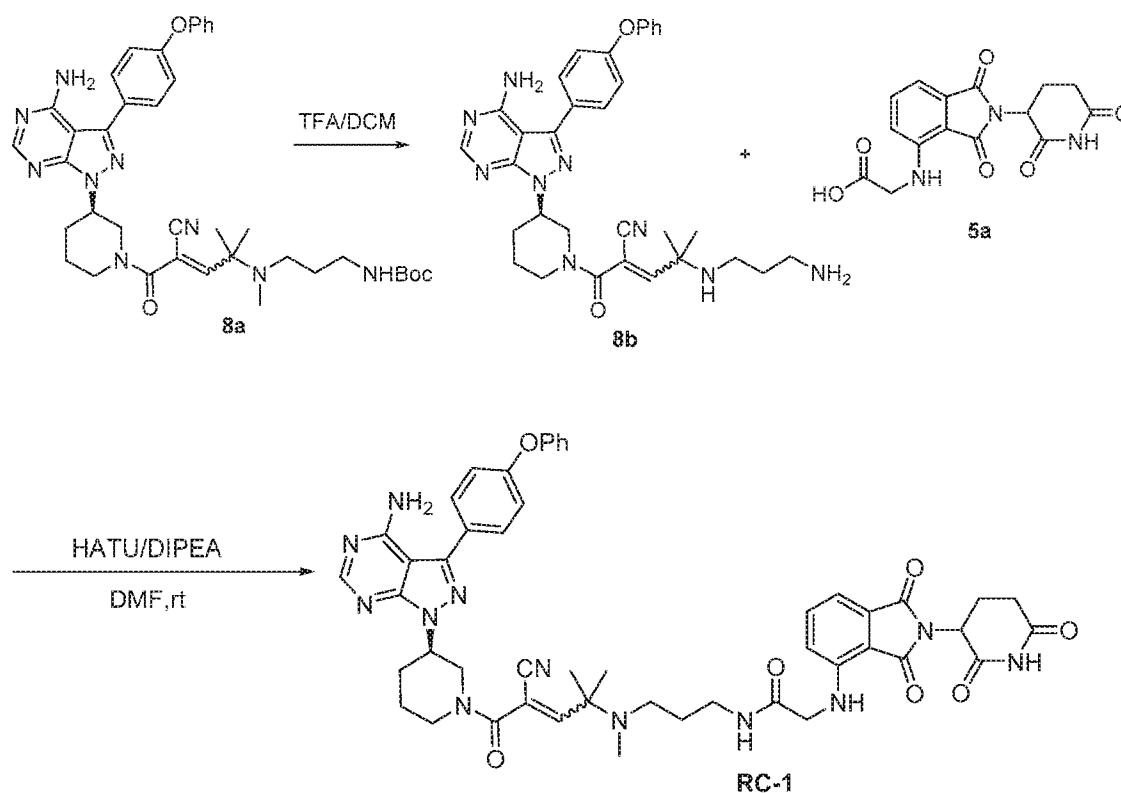


**(R)-3-(3-(4-Amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-3-oxopropanenitrile (7a).**

To a 100 mL flask was added compound **7** (1 g, 2.6 mmol), 2-cyanoacetic acid (330 mg, 3.9 mmol), (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (HATU; 1.48 g, 3.9 mmol) and DIPEA (671 mg, 5.2 mmol) in DMF (20 mL). The reaction mixture was then concentrated *in vacuo* and the residue was purified by flash column chromatography (0~10% MeOH in ethyl acetate) to give product **7a** as a white solid (1.0 g, 90%). <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 8.26 (s, 1H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.43 (m, 2H), 7.15 (m, 5H), 6.77 (s, 1H), 4.78 (m, 1H), 4.44 (m, 1H), 3.89 (m, 4H), 3.27 (m, 2H), 2.23 (m, 1H), 1.72 (m, 2H).

**Scheme 6 -Synthesis of RC-1:**





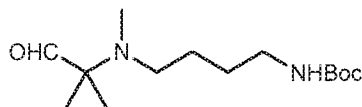
**tert-Butyl (R)-3-((5-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-3-en-2-yl)(methyl)amino)propyl)carbamate (8a).** To a round bottom flask was added compound **7a** (91 mg, 0.2 mmol), pyrrolidine (28 mg, 0.4 mmol), compound **6a** (103 mg, 0.4 mmol) in EtOH (3 mL) and heated to 95°C for 4 hours. TLC showed compound **8a** was generated as a major product. Then the reaction mixture was extracted with ethyl acetate, and the organic layer was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography (0–10% MeOH in Ethyl Acetate) to give product **8a** as a white solid (69 mg, 50%). <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 8.24 (s, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.43 (t, *J* = 7.9 Hz, 2H), 7.15 (m, 5H), 6.78 (br, 2H), 4.87 (s, 1H), 4.29 (s, 1H), 3.97 (s, 1H), 3.77 (s, 1H), 3.44 (m, 1H), 2.93 (m, 2H), 2.30 (s, 3H), 2.13 (m, 3H), 2.02 (m, 2H), 1.71 (m, 1H), 1.48 (m, 2H), 1.35 (s, 9H), 1.17 (d, *s*, 6H).

**N-(3-((5-((R)-3-(4-Amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-3-en-2-yl)(methyl)amino)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (RC-1).** In a 25 mL flask was added **8a** (35 mg, 0.05 mmol) in TFA/DCM (5 mL, 1/1). The mixture was stirred for 30 minutes at room temperature. LC-MS showed **8a** converted into **8b** completely. The solvent was removed *in vacuo* to give compound **8b** (28 mg, 95%), which was used for the

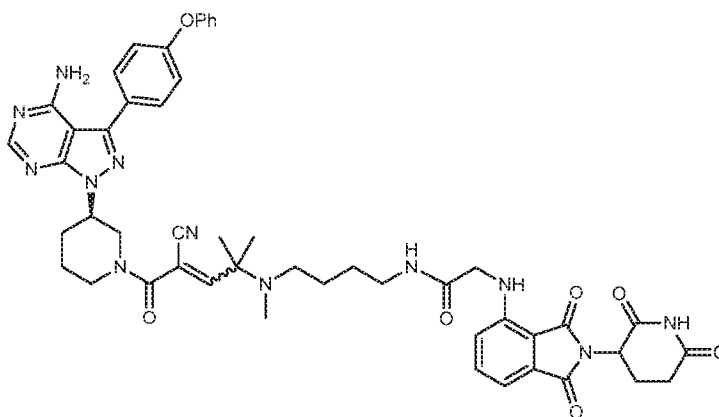
next step without further purification. To compound **8b** was added compound **5a** (33 mg, 0.1 mol), HATU (38 mg, 0.1 mmol) and DIPEA (32 mg, 0.25 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 30 minutes. Then, the reaction mixture was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **RC1** (18 mg, 40%). <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 8.24 (s, 1H), 7.92 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.56 (t, *J* = 7.8 Hz, 1H), 7.43 (t, *J* = 7.8 Hz, 2H), 7.15 (m, 5H), 7.05 (d, *J* = 7.1 Hz, 1H), 6.89 (m, 2H), 6.76 (br, 2H), 5.04 (dd, *J* = 12.7, 5.3 Hz, 1H), 4.86 (m, 1H), 4.17 (m, 1H), 3.91 (d, *J* = 5.4 Hz, 2H), 3.80 (m, 1H), 3.57 (m, 1H), 3.10 (m, 2H), 2.87 (m, 1H), 2.59 (m, 3H), 2.29 (m, 3H), 2.16 (m, 1H), 2.10 (s, 3H), 2.02 (m, 2H), 1.72 (m, 1H), 1.55 (m, *J* = 6.1 Hz, 2H), 1.18 (m, 6H). <sup>13</sup>C NMR (100 MHz, D<sub>6</sub>-DMSO) δ 173.0, 170.2, 169.1, 168.7, 167.7, 163.0, 162.5, 158.7, 157.7, 156.8, 156.0, 154.6, 146.4, 143.8, 136.5, 132.6, 130.5, 128.4, 124.2, 119.44, 119.41, 119.4, 117.8, 115.1, 111.4, 110.5, 109.1, 98.0, 62.5, 59.9, 56.1, 52.3, 49.3, 49.1, 45.8, 37.4, 35.3, 34.7, 31.5, 28.5, 25.9, 23.1, 23.0, 22.7. HRMS (*m/z*): [M+1]<sup>+</sup> calcd. for C<sub>48</sub>H<sub>51</sub>N<sub>12</sub>O<sub>7</sub>, 907.4004; found: 907.4033

Following the general procedure of RC PROTACs Synthesis shown above, additional RC PROTACs were synthesized as shown below, using compound **6b** – **6f** in place of compound **6a**.

#### Synthesis of RC-2:

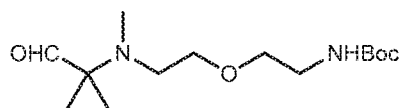


**tert-Butyl (4-(methyl(2-methyl-1-oxopropan-2-yl)amino)butyl)carbamate (6b).** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.44 (s, 1H), 4.98 (s, 1H), 3.11 (m, 2H), 2.28 (t, *J* = 6.0 Hz, 2H), 2.20 (s, 3H), 1.50 (m, 4H), 1.44 (s, 9H).

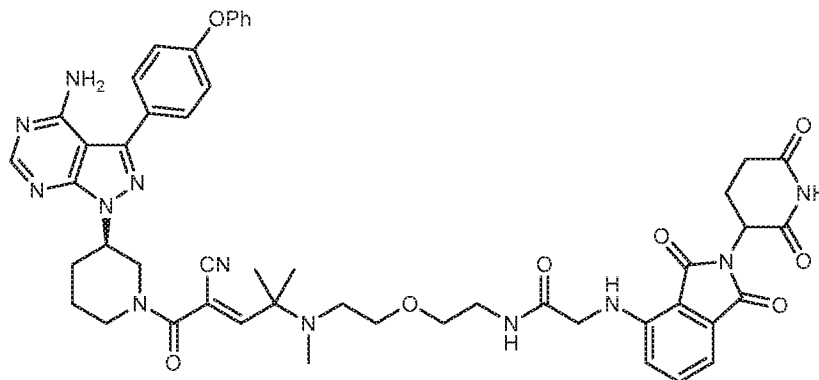


*N*-(4-((5-((*R*)-3-(4-Amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-3-en-2-yl)(methylamino)butyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (RC-2). <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 8.22 (s, 1H), 7.84 (s, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.9 Hz, 2H), 7.13 (m, 5H), 7.04 (d, *J* = 6.8 Hz, 1H), 6.85 (m, 2H), 6.71 (br, 2H), 5.01 (dd, *J* = 12.5, 5.3 Hz, 1H), 4.83 (m, 1H), 4.14 (m, 1H), 3.88 (d, *J* = 5.5 Hz, 2H), 3.80 (m, 1H), 3.61 (m, 1H), 3.31 (m, 3H), 2.86 (m, 1H), 2.56 (m, 2H), 2.22 (m, 3H), 2.07 (m, 3H), 2.013 (m, 2H), 1.68 (m, 1H), 1.35 (m, 4H), 1.18 (m, 6H). <sup>13</sup>C NMR (100 MHz, *D*<sub>6</sub>-DMSO) δ 172.9, 170.2, 169.1, 168.6, 167.7, 163.0, 162.5, 158.7, 157.8, 156.8, 156.1, 154.6, 146.4, 143.8, 136.6, 132.6, 130.5, 130.4, 128.4, 124.1, 119.4, 119.3, 117.8, 115.1, 111.4, 110.6, 109.2, 98.1, 59.9, 52.3, 51.3, 49.3, 49.1, 45.9, 39.0, 35.4, 31.5, 29.4, 27.3, 26.0, 23.3, 22.7. HRMS (*m/z*): [*M*+1]<sup>+</sup> calcd. for C<sub>49</sub>H<sub>53</sub>N<sub>12</sub>O<sub>7</sub>, 921.4160; found: 921.4199.

**Synthesis of RC-3:**



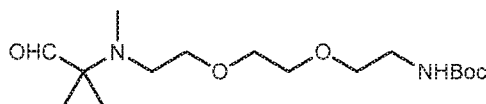
*tert*-Butyl (2-(2-(methyl(2-methyl-1-oxopropan-2-yl)amino)ethoxy)ethyl)carbamate (6c). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.48 (s, 1H), 5.25 (s, 1H), 3.71 (q, *J* = 7.0 Hz, 2H), 3.52 (dd, *J* = 11.0, 5.4 Hz, 4H), 3.31 (m, 2H), 2.51 (t, *J* = 5.7 Hz, 2H), 2.29 (s, 3H), 1.44 (s, 9H), 1.10 (s, 6H).



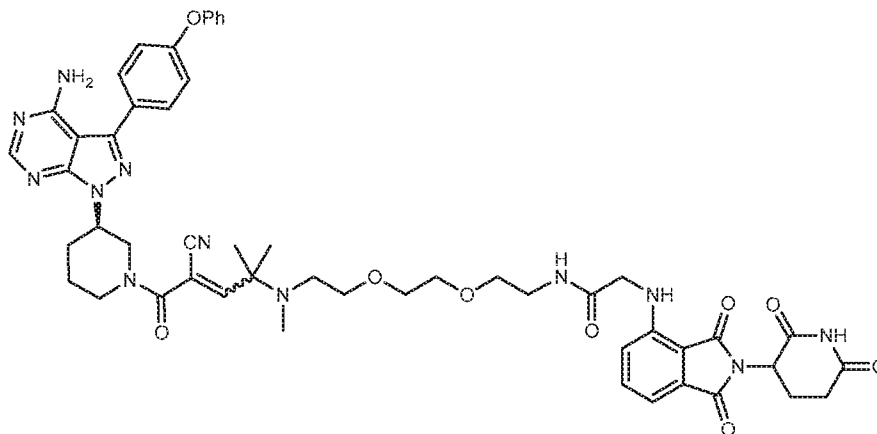
*N*-(2-(2-((5-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-3-en-2-yl)(methylamino)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (RC-3). <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 8.24 (s, 1H), 7.93 (s, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.57 (t, *J* = 7.9 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 2H), 7.16 (m, 5H), 7.06 (d, *J* = 7.1 Hz, 1H), 6.78 (s, 2H), 6.72 (br, 1H), 5.04 (dd, *J* = 12.6, 5.4 Hz, 1H), 4.86 (m, 1H), 4.18 (m, 1H), 3.90 (d, *J* = 18.4 Hz,

2H), 3.63 (m, 1H), 3.40 (m, 6H), 3.25 (m, 4H), 2.87 (m, 1H), 2.65 (m, 3H), 2.31 (m, 1H), 2.18 (s, 3H), 2.04 (m, 2H), 1.72 (m, 1H), 1.20 (m, 6H). HRMS (m/z): [M+1]<sup>+</sup> calcd. for C<sub>49</sub>H<sub>53</sub>N<sub>12</sub>O<sub>8</sub>, 937.4109; found: 937.4137.

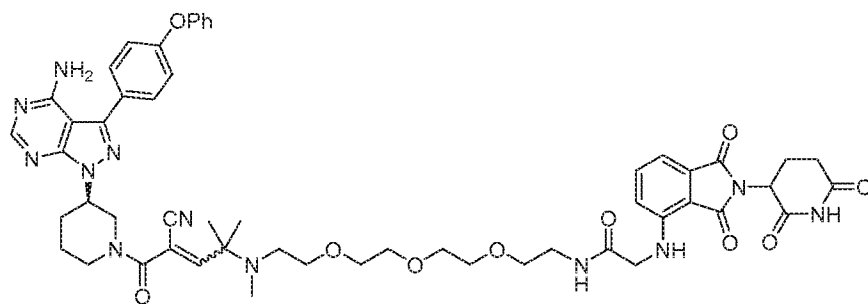
**Synthesis of RC-4:**



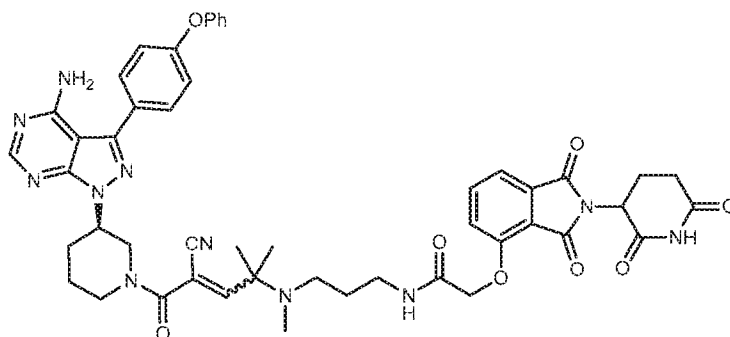
**tert-Butyl (2-(2-(2-(methyl(2-methyl-1-oxopropan-2-yl)amino)ethoxy)ethoxy)ethyl)carbamate (6d).** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.47 (s, 1H), 5.09 (s, 1H), 3.60 (m, 4H), 3.55 (m, 4H), 3.31 (dd, *J* = 5.0 Hz, 2H), 2.54 (t, *J* = 6.1 Hz, 2H), 2.29 (s, 3H), 1.44 (s, 9H), 1.09 (s, 6H).



**N-(2-(2-(2-((5-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-3-en-2-yl)(methyl)amino)ethoxy)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (RC-4).** <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 10.89 (s, 1H), 8.22 (s, 1H), 7.96 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.13 (m, 5H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.86 (m, 2H), 6.75 (s, 1H), 6.70 (br, 1H), 5.01 (dd, *J* = 12.6, 5.2 Hz, 1H), 4.83 (m, 1H), 4.13 (m, 1H), 3.91 (d, *J* = 5.2 Hz, 2H), 3.82 (m, 1H), 3.60 (m, 1H), 3.43 (m, 9H), 3.23 (m, 3H), 2.83 (m, 1H), 2.58 (m, 3H), 2.28 (m, 2H), 2.16 (s, 4H), 2.01 (m, 2H), 1.69 (s, 1H), 1.17 (m, 6H). <sup>13</sup>C NMR (100 MHz, D<sub>6</sub>-DMSO) δ 173.1, 170.3, 169.2, 169.0, 167.7, 163.0, 162.4, 158.7, 157.7, 156.8, 156.1, 154.6, 146.4, 143.8, 136.5, 132.6, 130.5, 128.4, 124.2, 119.4, 117.9, 115.0, 111.4, 110.5, 109.3, 98.1, 70.20, 70.17, 70.1, 69.4, 59.8, 56.0, 51.6, 49.2, 45.8, 39.2, 36.3, 31.5, 29.4, 23.3, 23.2, 22.7. HRMS (m/z): [M+1]<sup>+</sup> calcd. for C<sub>51</sub>H<sub>57</sub>N<sub>12</sub>O<sub>9</sub>, 981.4371; found: 981.4405.

**Synthesis of RC-5:**

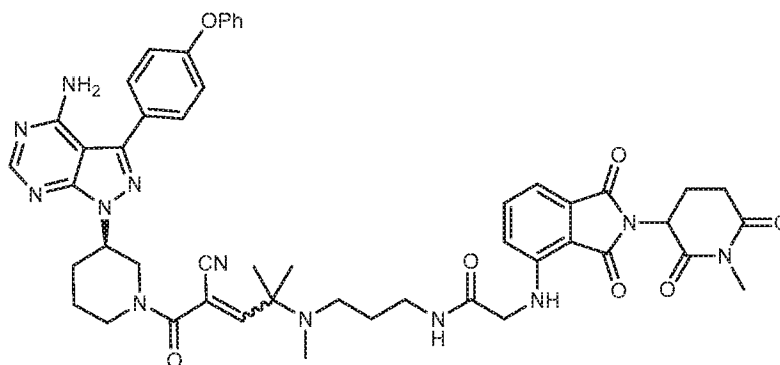
***N*-(16-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-15-cyano-12,13,13-trimethyl-16-oxo-3,6,9-trioxa-12-azahexadec-14-en-1-yl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (RC-5)** <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 10.92 (s, 1H), 8.24 (s, *J* = 8.0 Hz, 1H), 7.99 (s, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.58 (t, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 2H), 7.16 (m, 5H), 7.06 (d, *J* = 7.1 Hz, 1H), 6.88 (m, 2H), 6.78 (s, 1H), 6.73 (br, 1H), 5.04 (dd, *J* = 12.7, 5.4 Hz, 1H), 4.86 (m, 1H), 4.17 (m, 1H), 3.91 (t, *J* = 4.0 Hz, 2H), 3.85 (m, 1H), 3.63 (m, 1H), 3.45 (m, 13H), 3.28 (m, 4H), 2.89 (m, 1H), 2.57 (m, 2H), 2.30 (m, 1H), 2.18 (s, 4H), 2.04 (m, 2H), 1.72 (m, 1H), 1.21 (m, 6H). HRMS (*m/z*): [*M*+1]<sup>+</sup> calcd. for C<sub>53</sub>H<sub>61</sub>N<sub>12</sub>O<sub>10</sub>, 1025.4634; found: 1025.4668.

**Synthesis of RC-9:**

***N*-(3-((5-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-3-en-2-yl)(methyl)amino)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetamide (RC-9).** <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 10.93 (s, 1H), 8.21 (s, 1H), 7.76 (t, *J* = 7.7 Hz, 2H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.41 (m, 4H), 7.13 (m, 5H), 6.68 (br, 3H), 5.06 (dd, *J* = 12.6, 5.3 Hz, 1H), 4.82 (m, 1H), 4.71 (s, 2H), 4.13 (m, 1H), 3.75 (m, 2H), 2.83 (m, 1H), 2.57 (m, 3H), 2.33 (m, 2H), 2.26 (m, *J* = 9.6 Hz, 1H), 2.15 (m, 1H), 2.09 (s, 3H), 2.02 (m, 2H), 1.67 (m, 1H), 1.57 (m, 2H), 1.20 (s, 6H). <sup>13</sup>C NMR (100 MHz, *D*<sub>6</sub>-DMSO) δ 172.9, 170.0, 167.1, 167.0, 165.9, 163.0, 162.5, 158.7, 157.7, 156.8, 156.0, 155.5, 154.6, 143.8, 137.3, 133.6, 130.5, 128.4, 124.2, 121.1,

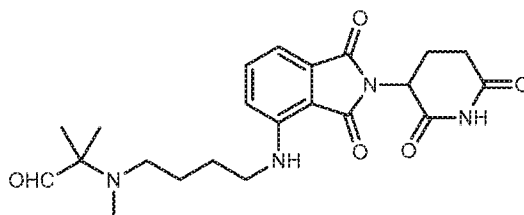
119.4, 117.5, 116.6, 115.1, 109.1, 98.1, 68.5, 60.0, 52.4, 49.5, 49.4, 49.2, 41.1, 37.2, 35.4, 31.4, 29.3, 28.4, 25.9, 23.2, 23.0, 22.5. HRMS (m/z):  $[M+1]^+$  calcd. for  $C_{48}H_{50}N_{11}O_8$ , 908.3844; found: 908.3870.

### Synthesis of RC-1-Me:



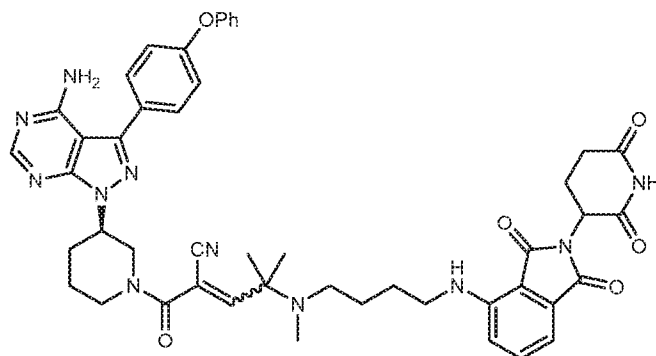
*N*-(3-((5-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-3-en-2-yl)(methylamino)propyl)-2-((1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (RC-1-Me).  $^1H$  NMR (400 MHz,  $D_6$ -DMSO)  $\delta$  8.24 (s, 1H), 7.88 (s, 1H), 7.67 (d,  $J = 7.1$  Hz, 2H), 7.56 (t,  $J = 7.2$  Hz, 1H), 7.43 (t,  $J = 7.1$  Hz, 2H), 7.15 (m, 5H), 7.06 (d,  $J = 7.0$  Hz, 1H), 6.88 (m, 2H), 6.76 (s, 1H), 6.71 (br, 2H), 5.11 (dd,  $J = 12.6, 3.8$  Hz, 1H), 4.86 (m, 1H), 4.17 (m, 1H), 3.91 (d,  $J = 4.2$  Hz, 3H), 3.63 (m, 1H), 3.32 (m, 1H), 3.11 (m, 2H), 3.04 (s, 3H), 2.92 (m, 1H), 2.77 (m, 1H), 2.57 (m, 1H), 2.31 (m, 3H), 2.19 (m, 1H), 2.11 (s, 3H), 2.04 (m, 2H), 1.71 (m, 1H), 1.57 (m, 2H), 1.20 (s, 6H).  $^{13}C$  NMR (100 MHz,  $D_6$ -DMSO)  $\delta$  172.1, 170.0, 169.1, 168.7, 167.7, 163.0, 162.5, 158.7, 157.8, 157.7, 156.8, 156.1, 156.0, 154.6, 146.4, 143.8, 136.6, 136.5, 132.6, 130.5, 130.4, 128.4, 124.2, 119.4, 119.3, 117.9, 115.1, 111.5, 111.4, 110.5, 109.2, 98.1, 59.9, 56.2, 52.4, 52.3, 49.8, 49.7, 49.2, 47.8, 45.9, 37.5, 35.4, 34.8, 31.6, 29.4, 28.5, 26.9, 23.6, 23.1, 21.9. HRMS (m/z):  $[M+1]^+$  calcd. for  $C_{49}H_{53}N_{12}O_7$ , 921.4160; found: 921.4192.

### Synthesis of RC-7:



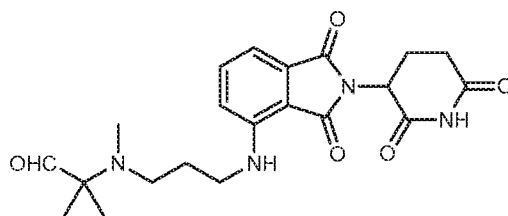
2-((4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)butyl)(methylamino)-2-methylpropanal (6e).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$

9.43 (s, 1H), 7.50 (t,  $J = 7.4$  Hz, 1H), 7.09 (d,  $J = 7.3$  Hz, 1H), 6.88 (d,  $J = 8.3$  Hz, 1H), 6.24 (s, 1H), 4.91 (m, 1H), 3.29 (q,  $J = 6.6$  Hz, 2H), 2.81 (m, 3H), 2.32 (t,  $J = 6.9$  Hz, 2H), 2.22 (s, 3H), 2.11 (m, 1H), 1.69 (m, 2H), 1.60 (m, 2H), 1.08 (s, 6H).

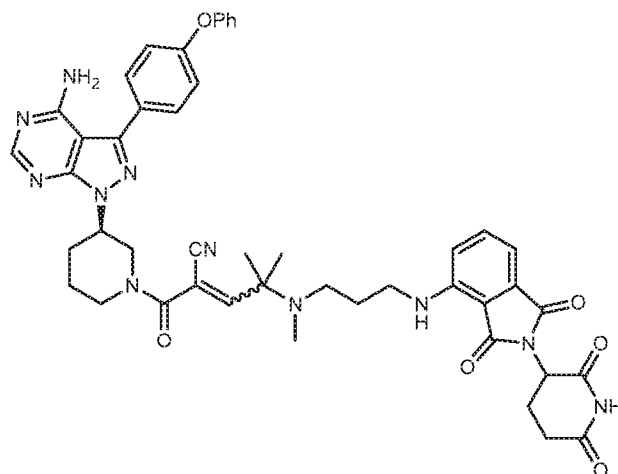


2-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidine-1-carbonyl)-4-(((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)butyl)(methylamino)-4-methylpent-2-enenitrile (RC-7).  $^1\text{H}$  NMR (400 MHz,  $D_6$ -DMSO)  $\delta$  11.09 (s, 1H), 8.24 (s, 1H), 7.65 (m, 2H), 7.54 (m, 1H), 7.43 (t,  $J = 6.9$  Hz, 2H), 7.18 (m, 5H), 7.00 (d,  $J = 6.4$  Hz, 1H), 6.75 (m, 1H), 6.47 (br, 1H), 5.03 (m, 1H), 4.86 (s, 1H), 4.70 (m, 1H), 4.24 (m, 1H), 3.96 (m, 1H), 3.68 (m, 2H), 3.17 (m, 2H), 2.87 (m, 2H), 2.28 (m, 3H), 2.12 (s, 3H), 2.01 (m, 3H), 1.66 (m, 1H), 1.48 (m, 4H), 1.18 (s, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $D_6$ -DMSO)  $\delta$  173.2, 170.5, 169.3, 167.7, 162.9, 162.7, 158.6, 157.6, 156.7, 156.1, 154.4, 146.8, 143.8, 136.7, 132.6, 130.6, 130.5, 128.3, 124.2, 119.41, 119.37, 117.6, 115.2, 110.8, 109.4, 108.9, 97.8, 59.9, 59.8, 52.1, 51.3, 49.0, 48.9, 42.2, 35.2, 31.4, 29.4, 26.9, 25.8, 23.0, 22.6. HRMS ( $m/z$ ):  $[\text{M}+1]^+$  calcd. for  $\text{C}_{47}\text{H}_{50}\text{N}_{11}\text{O}_6$ , 864.3946; found: 864.3976.

#### Synthesis of RC-8:

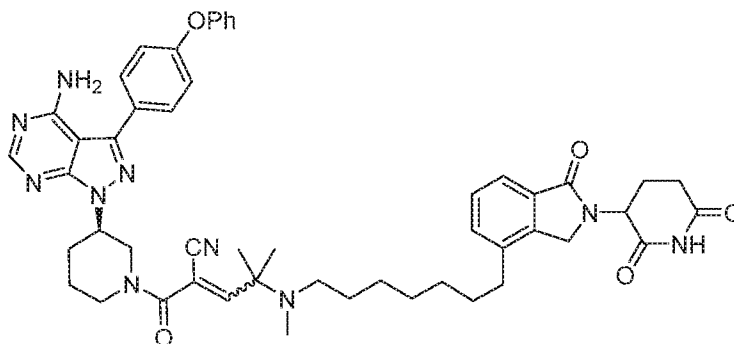


2-(((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)propyl)(methylamino)-2-methylprop-2-enal (6f).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.46 (s, 1H), 7.49 (t,  $J = 7.9$  Hz, 1H), 7.08 (d,  $J = 7.2$  Hz, 1H), 6.88 (d,  $J = 8.8$  Hz, 1H), 6.76 (s, 1H), 4.92 (m, 1H), 3.36 (q,  $J = 5.6$  Hz, 2H), 2.81 (m, 3H), 2.46 (t,  $J = 5.8$  Hz, 2H), 2.27 (s, 3H), 2.12 (m, 1H), 1.82 (m, 2H), 1.10 (s, 6H).

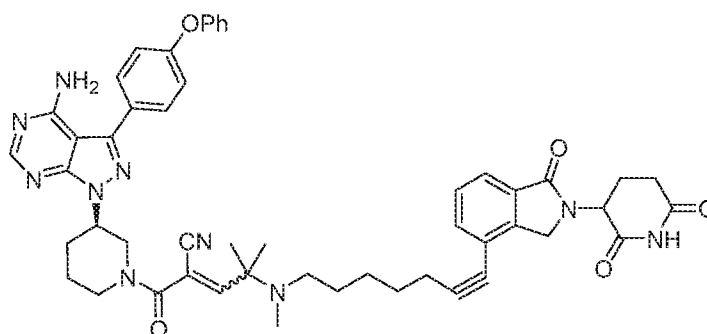


2-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidine-1-carbonyl)-4-((3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)propyl)(methylamino)-4-methylpent-2-enenitrile) (RC-8). <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 11.04 (s, 1H), 8.22 (s, 1H), 7.62 (d, *J* = 6.1 Hz, 2H), 7.51 (s, 1H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.13 (m, 5H), 6.97 (d, *J* = 6.3 Hz, 1H), 6.71 (m, 2H), 5.01 (m, 1H), 4.82 (m, 1H), 4.52 (m, 1H), 4.16 (m, 1H), 4.00 (m, 1H), 3.73 (m, 2H), 3.18 (m, 2H), 2.83 (m, 2H), 2.29 (m, 3H), 2.12 (s, 3H), 1.97 (m, *J* = 5.8 Hz, 3H), 1.66 (m, 3H), 1.16 (s, 6H). <sup>13</sup>C NMR (100 MHz, *D*<sub>6</sub>-DMSO) δ 173.2, 170.5, 169.3, 167.7, 162.9, 162.5, 158.6, 157.6, 156.7, 156.1, 154.4, 146.8, 143.8, 136.7, 132.6, 130.6, 130.5, 128.3, 124.2, 119.42, 119.38, 117.5, 115.3, 110.8, 109.5, 108.9, 97.8, 60.8, 60.1, 52.1, 49.1, 49.0, 40.9, 40.8, 35.4, 31.4, 29.5, 27.3, 23.0, 22.6, 17.5. HRMS (*m/z*): [*M*+1]<sup>+</sup> calcd. for C<sub>46</sub>H<sub>48</sub>N<sub>11</sub>O<sub>6</sub>, 850.3789; found: 850.3811.

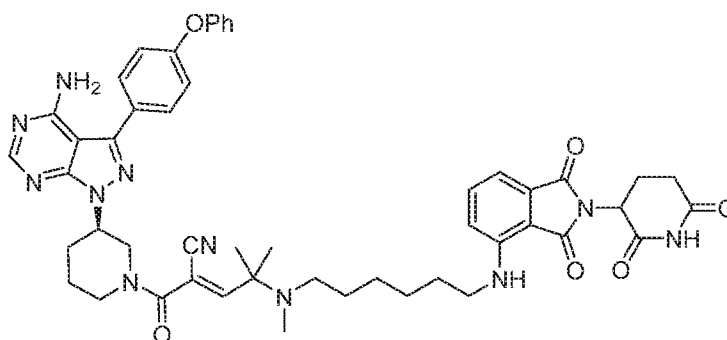
#### Synthesis of RC-11:



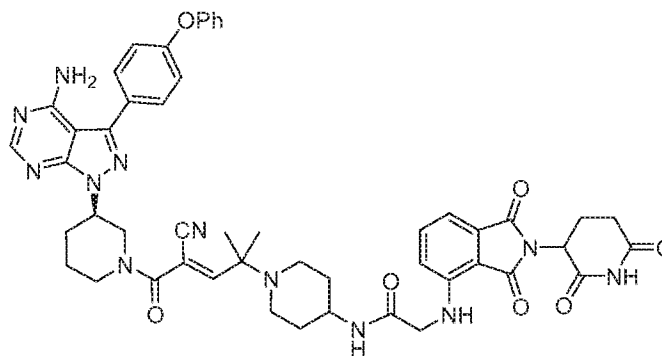
2-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidine-1-carbonyl)-4-((7-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-4-yl)heptyl)(methylamino)-4-methylpent-2-enenitrile) (RC-11) HRMS (*m/z*): [*M*+H]<sup>+</sup> calcd. for C<sub>50</sub>H<sub>57</sub>N<sub>10</sub>O<sub>5</sub>, 877.4513; found: 877.4529.

*Synthesis of RC-12:*

2-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidine-1-carbonyl)-4-((7-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolin-4-yl)hept-6-yn-1-yl)(methyl)amino)-4-methylpent-2-enenitrile (**RC-12**). HRMS (*m/z*): [*M*+*H*]<sup>+</sup> calcd. for C<sub>50</sub>H<sub>53</sub>N<sub>10</sub>O<sub>5</sub>, 873.4200; found: 873.4218.

*Synthesis of RC-13:*

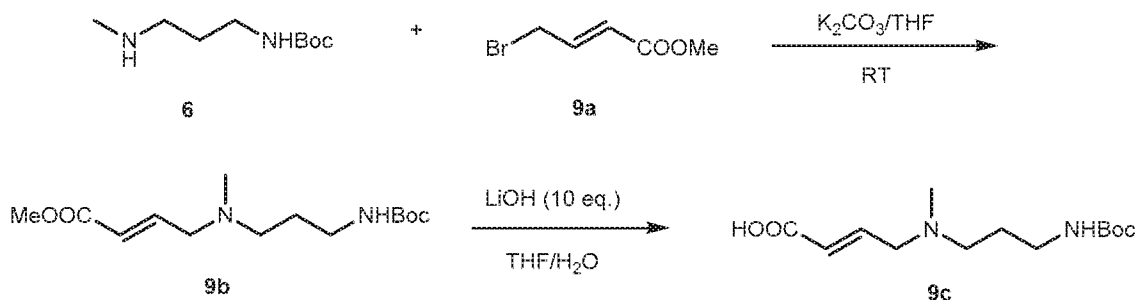
**RC-13** N-(1-((*E*)-5-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-3-en-2-yl)piperidin-4-yl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (**RC-13**) HRMS (*m/z*): [*M*+*H*]<sup>+</sup> calcd. for C<sub>49</sub>H<sub>54</sub>N<sub>11</sub>O<sub>6</sub>, 892.4259; found: 892.4276.

*Synthesis of RC-14:*

(*E*)-2-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidine-1-carbonyl)-4-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)hexyl)(methyl amino)-4-methylpent-2-enenitrile (**RC-14**). <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 11.08 (s, 1H), 8.24 (s, 1H), 8.06 (m, 1H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.22 – 6.98 (m, 6H), 6.85 (m, 3H), 6.66 (m, 1H), 5.04 (dd, *J* = 12.7, 4.8 Hz, 1H), 4.85 (s, 1H), 4.23 (s, 1H), 3.87 (s, 5H), 2.91 – 2.66 (m, 4H), 2.59 – 2.51 (m, 3H), 2.31 – 1.89 (m, 9H), 1.68 (s, 3H), 1.49 (s, 2H), 1.24 – 0.96 (m, 8H). <sup>13</sup>C NMR (100 MHz, *D*<sub>6</sub>-DMSO) δ 173.2, 170.5, 169.2, 167.9, 167.7, 162.9, 158.7, 157.6, 156.7, 156.1, 154.4, 146.2, 143.8, 136.6, 132.5, 130.6, 130.4, 128.3, 124.3, 119.4, 117.9, 115.0, 111.3, 110.1, 108.9, 97.8, 59.4, 51.9, 49.0, 47.1, 45.7, 45.4, 32.0, 31.4, 23.1, 22.8, 22.6. HRMS (*m/z*): [*M*+*H*]<sup>+</sup> calcd. for C<sub>49</sub>H<sub>51</sub>N<sub>12</sub>O<sub>7</sub>, 919.4004; found: 919.4019.

#### General procedure of IRC and RNC PROTACs synthesis (IRC-1 and RNC-1).

##### Scheme 7:

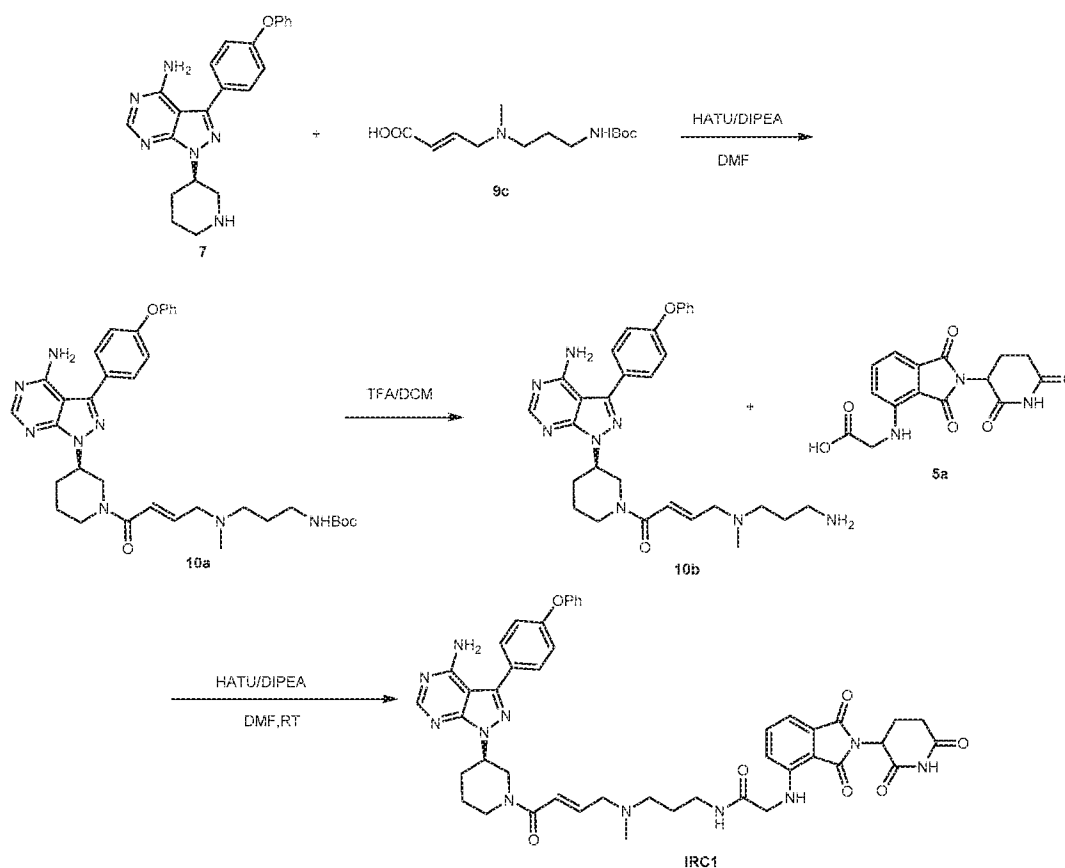


**Methyl (*E*)-4-((3-((tert-butoxycarbonyl)amino)propyl)(methyl)amino)but-2-enoate (**9b**).** To a flask was added compound **6** (376 mg, 2 mmol), K<sub>2</sub>CO<sub>3</sub> (552 mg, 4 mmol) in 20 mL THF. The mixture was stirred for 30 min at room temperature. Then compound **9a** (708 mg, 4 mmol) in 2 mL THF was added dropwise with stirring. The mixture was stirred at room temperature for overnight. The solvent was concentrated *in vacuo* and the residue was purified by flash column chromatography (5% MeOH in DCM) to give product **9b** (354 mg, 62%) as a yellow oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.84 (dt, *J* = 15.7, 6.1 Hz, 1H), 5.88 (dt, *J* = 15.7, 1.4 Hz, 1H), 5.25 (s, 1H), 3.64 (s, 3H), 3.05 (m, 4H), 2.32 (t, *J* = 6.7 Hz, 2H), 2.12 (s, 3H), 1.56 (m, 2H), 1.34 (s, 9H).

**(*E*)-4-((3-((tert-butoxycarbonyl)amino)propyl)(methyl)amino)but-2-enoic acid (**9c**)** Compound **9b** (143 mg, 0.5 mmol) and LiOH (120 mg, 5 mmol) were dissolved in THF (5 mL) and water (5 mL). The mixture was stirred at 40°C for 6 hours. TLC showed **9b** completely disappeared. The mixture was cooled to 0°C and the pH was slowly adjusted to

4-5 with 1N HCl. The solvent was concentrated *in vacuo* and the residue was dissolve in DMF to give a solution **9b** (0.2 mM in DMF), which was used for next step without further purification.

**Scheme 8 - Synthesis of IRC-1:**



5

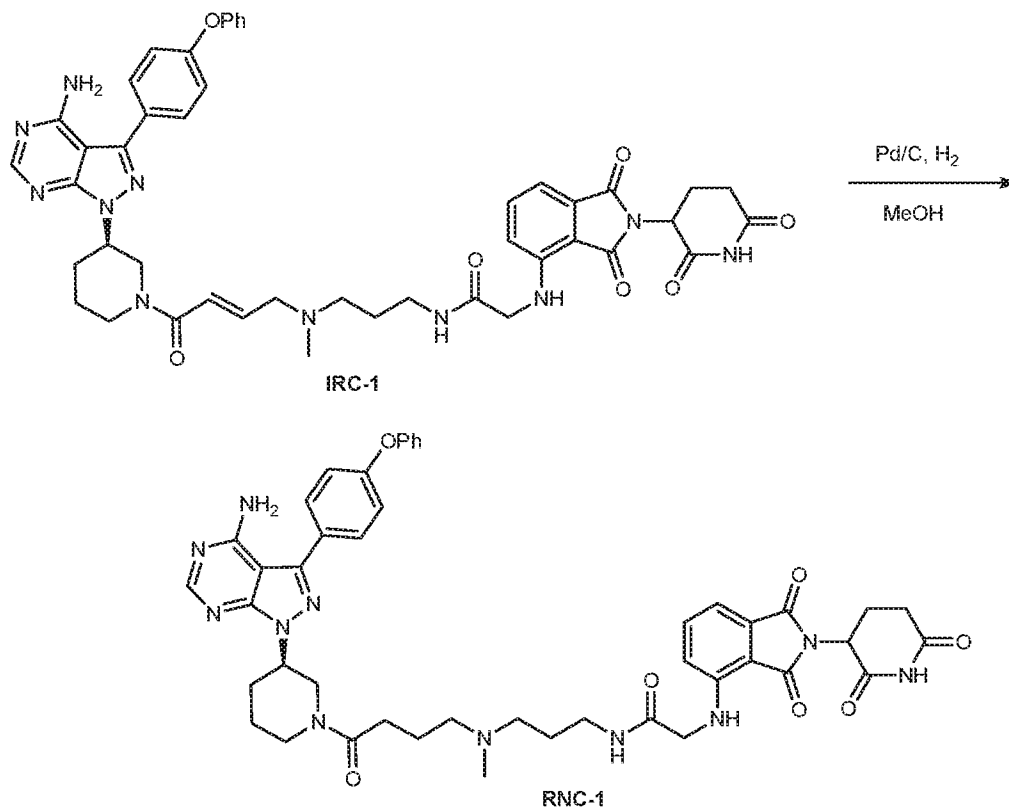
***tert*-Butyl (*R,E*)-3-((4-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobut-2-en-1-yl)(methyl)amino)propyl)-carbamate (**10a**).** To a flask was added compound **7** (77 mg, 0.2 mmol), solution **9c** (1.5 mL, 0.3 mmol), HATU (114 mg, 0.3 mmol) and DIPEA (129 mg, 1 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 30 min. Then the reaction mixture was extracted with ethyl acetate, and the organic layer was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography (0~10% MeOH in Ethyl Acetate) to give product **10a** as a white solid (70 mg, 55%).

15

***N*-(3-(((*E*)-4-((*R*)-3-(4-Amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobut-2-en-1-yl)(methyl)amino)propyl)-2-((2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (**IRC-1**).** In a 25 mL flask was added **10a** (54 mg, 0.1 mmol) in TFA/DCM (5 mL, 1/1). The mixture was stirred

for 30 min at room temperature. LC-MS showed **10a** converted into **10b** completely. Then remove the solvent *in vacuo* to give product **10b** (52 mg, 95%), which was used for next step without further purification. To above **10b** was added compound **5a** (66 mg, 0.2 mol), HATU (78 mg, 0.2 mmol) and DIPEA (64 mg, 0.5 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 30 min. Then the reaction mixture was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **IRC-1** (30 mg, 35%). <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 11.11 (s, 1H), 8.25 (s, 1H), 8.10 (d, *J* = 19.6 Hz, 1H), 7.65 (m, 2H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.16 (m, 5H), 7.06 (d, *J* = 7.0 Hz, 1H), 6.96 (m, 1H), 6.85 (t, *J* = 8.0 Hz 1H), 6.63 (s, 1H), 6.46 (br, 1H), 5.07 (dd, *J* = 12.8, 5.2 Hz, 1H), 4.69 (m, 1H), 4.53 (d, *J* = 11.3 Hz, 1H), 4.10 (m, 1H), 3.91 (s, 2H), 3.76 (m, 1H), 3.15 (m, 5H), 2.88 (m, 2H), 2.61 (m, 2H), 2.26 (m, 3H), 2.11 (s, 3H), 2.01 (m, 2H), 1.94 (m, 1H), 1.56 (m, 3H). <sup>13</sup>C NMR (100 MHz, *D*<sub>6</sub>-DMSO) δ 173.0, 170.2, 169.1, 168.7, 167.7, 165.1, 158.6, 157.7, 156.8, 156.0, 154.5, 146.4, 143.6, 142.2, 136.6, 132.6, 130.49, 130.46, 128.5, 124.2, 122.9, 119.42, 119.39, 117.8, 111.4, 110.5, 98.0, 58.5, 54.8, 53.0, 49.18, 49.14, 45.8, 42.2, 37.6, 31.5, 29.8, 27.2, 25.9, 22.7. HRMS (*m/z*): [*M*+1]<sup>+</sup> calcd. for C<sub>45</sub>H<sub>48</sub>N<sub>11</sub>O<sub>7</sub>, 854.3738; found: 854.3772.

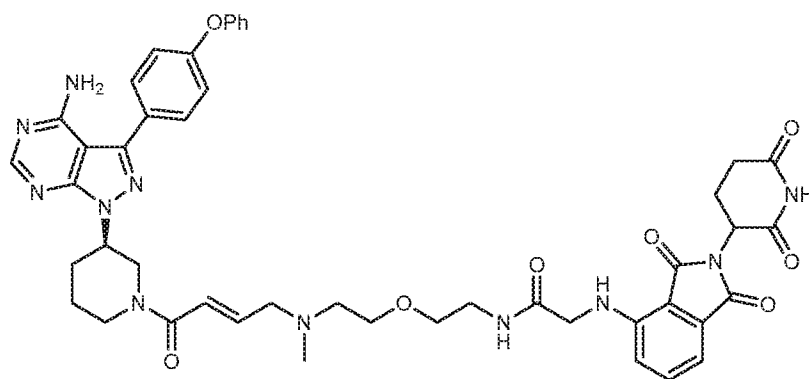
**Scheme 9 - Synthesis of RNC-1:**



*N*-(3-((4-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)(methylamino)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (**RNC-1**). To a flask was added **IRC-1** (17 mg, 0.02 mmol) and Pd/C (1.7 mg, 10%) in MeOH (2 mL). The mixture was stirred under 1 atm H<sub>2</sub> at room temperature overnight. LC-MS showed **IRC-1** converted into **RNC-1** completely. Then the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **RNC-1** (9.4 mg, 55%). <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 11.08 (s, 1H), 8.30 (s, 1H), 8.21 (s, 1H), 8.10 (m, 1H), 7.63 (d, *J* = 7.9 Hz, 2H), 7.53 (t, *J* = 7.4 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.20 – 7.06 (m, 5H), 7.03 (d, *J* = 6.3 Hz, 1H), 6.93 (m, 1H), 6.82 (t, *J* = 7.2 Hz, 1H), 5.04 (d, *J* = 7.8 Hz, 1H), 4.65 (m, 1H), 4.49 (d, *J* = 12.5 Hz, 1H), 4.16 (d, *J* = 13.2 Hz, 1H), 3.99 (d, *J* = 12.5 Hz, 1H), 3.87 (d, *J* = 9.3 Hz, 2H), 3.07 (m, 4H), 2.84 (m, 2H), 2.57 (s, 2H), 2.23 (m, 6H), 2.06 (s, 3H), 2.01 (s, 2H), 1.86 (s, 1H), 1.55 (m, 5H). <sup>13</sup>C NMR (100 MHz, *D*<sub>6</sub>-DMSO) δ 173.2, 171.1, 170.5, 169.1, 168.7, 167.7, 158.6, 157.5, 156.7, 156.1, 154.4, 146.2, 143.6, 136.6, 132.5, 130.6, 130.50, 130.46, 128.4, 124.2, 119.4, 117.8, 111.4, 110.3, 97.8, 56.9, 55.0, 52.5, 49.7, 49.0, 45.7, 42.1, 40.9, 37.6, 31.41, 30.5, 29.7, 27.0, 25.1, 22.8, 22.6. HRMS (*m/z*): [M+1]<sup>+</sup> calcd. for C<sub>45</sub>H<sub>50</sub>N<sub>11</sub>O<sub>7</sub>, 856.3895; found: 856.3920.

Following the general procedure of the IRC and RNC PROTACs Synthesis shown above, additional IRC and RNC PROTACs were synthesized as shown below.

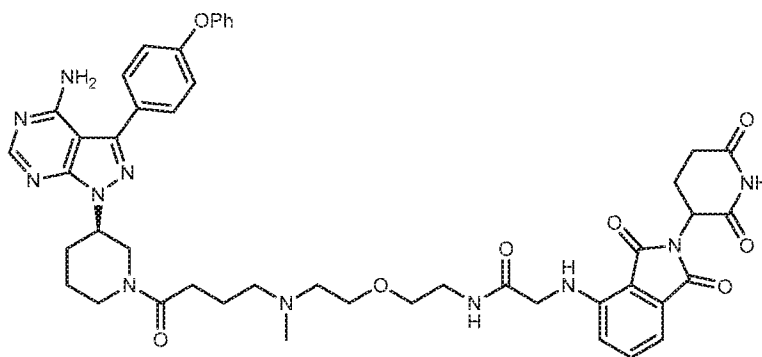
#### Synthesis of IRC-3:



*N*-(2-(2-(((*E*)-4-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-oxobut-2-en-1-yl)(methylamino)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (**IRC-3**) <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 8.25 (s, 1H), 8.01 (s, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.14 (m, 5H), 7.06 (d, *J* = 7.0 Hz, 1H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.73 (s, 1H), 6.56 (s, 2H), 5.11 – 4.95 (m, 1H), 4.71 (s, 1H), 4.04 (s, 1H), 3.93 (d, *J* =

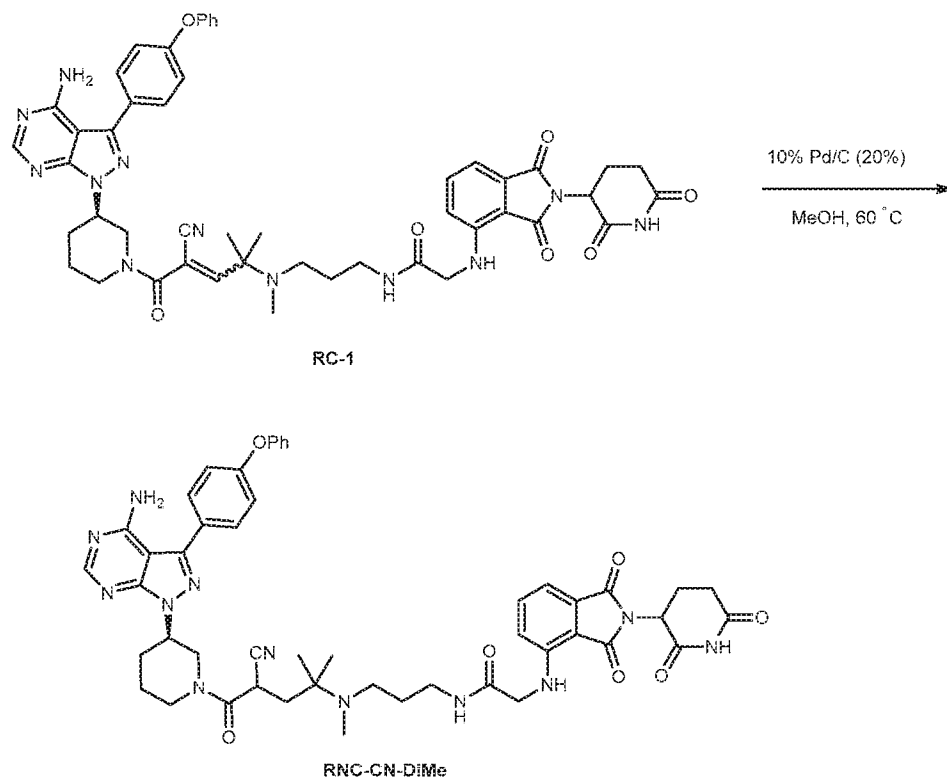
5.0 Hz, 2H), 3.43 (d,  $J = 16.6$  Hz, 5H), 3.18 – 3.02 (m, 6H), 2.87 (dd,  $J = 21.8, 9.3$  Hz, 1H), 2.60 (d,  $J = 18.5$  Hz, 2H), 2.35 – 2.20 (m, 2H), 2.16 (s, 4H), 2.04 (d,  $J = 11.5$  Hz, 1H), 1.94 (s, 1H), 1.59 (d,  $J = 11.3$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $D_6$ -DMSO)  $\delta$  172.9, 170.2, 169.1, 169.0, 167.7, 165.1, 158.7, 157.7, 156.8, 156.04, 156.00, 154.6, 146.4, 143.6, 142.2, 136.6, 136.5, 132.6, 130.5, 130.4, 128.5, 124.2, 122.9, 119.44, 119.35, 117.9, 111.5, 111.4, 110.6, 98.1, 69.3, 69.0, 58.8, 56.5, 53.0, 49.3, 49.1, 45.8, 42.9, 42.8, 41.2, 41.1, 39.2, 31.5, 29.8, 22.7. HRMS ( $m/z$ ):  $[M+1]^+$  calcd. for  $\text{C}_{46}\text{H}_{50}\text{N}_{11}\text{O}_8$ , 884.3833; found: 884.3868.

### Synthesis of RNC-3:

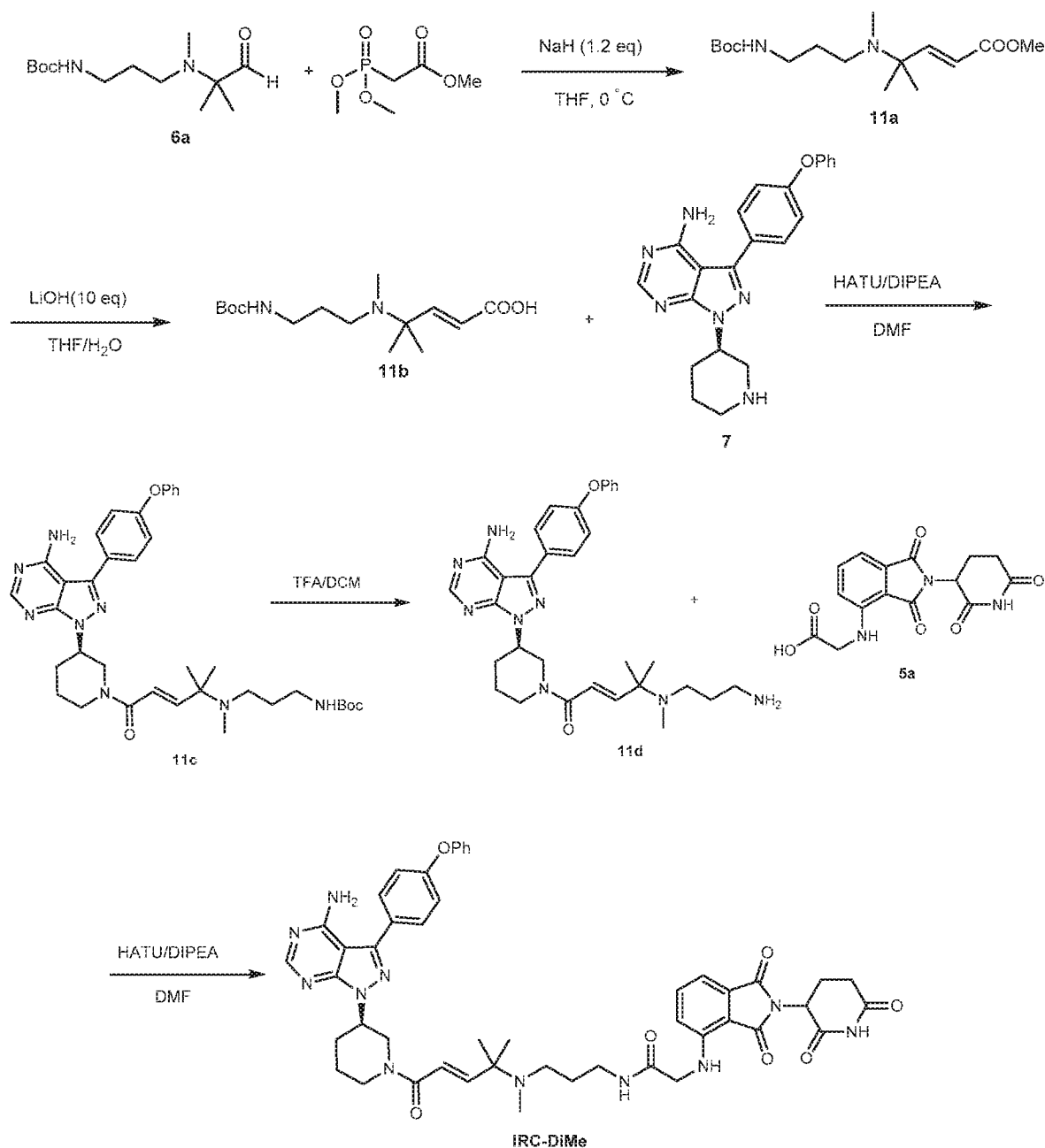


10 *N*-(2-(2-((4-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)(methyl)amino)ethoxy)ethyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amin- o)acetamide (RNC-3).  $^1\text{H}$  NMR (400 MHz,  $D_6$ -DMSO)  $\delta$  8.22 (s, 1H), 7.94 (s, 1H), 7.64 (d,  $J = 8.4$  Hz, 2H), 7.54 (t,  $J = 7.8$  Hz, 1H), 7.40 (t,  $J = 7.8$  Hz, 2H), 7.13 (m, 5H), 7.03 (d,  $J = 7.1$  Hz, 1H), 6.86 (m, 2H), 6.68 (br, 2H), 5.01 (dd,  $J = 12.7, 5.4$  Hz, 1H), 4.65 (m, 1H), 3.91 (d,  $J = 5.3$  Hz, 2H), 3.39 (m, 5H), 2.86 (m, 1H), 2.58 (d,  $J = 19.8$  Hz, 2H), 2.43 (m, 3H), 2.26 (m, 4H), 2.12 (s, 4H), 2.01 (m, 1H), 1.88 (m, 1H), 1.60 (m, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $D_6$ -DMSO)  $\delta$  172.9, 171.3, 170.2, 169.1, 169.0, 167.7, 158.7, 157.7, 156.8, 156.1, 156.0, 154.6, 146.4, 143.6, 136.6, 136.5, 132.6, 130.6, 130.4, 128.5, 124.2, 119.5, 119.4, 117.9, 111.5, 111.4, 110.6, 98.1, 69.3, 69.1, 57.3, 56.8, 49.3, 49.2, 45.9, 42.8, 41.2, 41.1, 39.3, 31.4, 30.5, 29.8, 23.1, 22.7. HRMS ( $m/z$ ):  $[M+1]^+$  calcd. for  $\text{C}_{46}\text{H}_{52}\text{N}_{11}\text{O}_8$ , 886.4000; found: 886.4027.

## Scheme 10 – Synthesis of RNC-CN-DiMe:



*N*-(3-((5-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-cyano-2-methyl-5-oxopent-2-yl)(methylamino)propyl)-2-((2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (**RNC-CN-DiMe**). To a flask was added **RC-1** (18 mg, 0.02 mmol) and Pd/C (3.6 mg, 20%) in MeOH (2 mL). The mixture was stirred under 1 atm H<sub>2</sub> at 60 °C overnight. LC-MS showed **RC-1** converted into **RNC-CN-DiMe** completely. Then the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **RNC-CN-DiMe** (8.5 mg, 47%). <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 11.09 (s, 1H), 8.22 (m, 2H), 8.00 (m, 1H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.52 (t, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 2H), 7.20 – 7.06 (m, 5H), 7.02 (d, *J* = 7.1 Hz, 1H), 6.93 (m, 1H), 6.80 (m, 1H), 5.03 (dd, *J* = 12.9, 5.0 Hz, 1H), 4.67 (m, 1H), 4.34 (m, 1H), 4.18 (m, 2H), 3.89 (m, 6H), 3.32 (m, 1H), 3.02 (m, 4H), 2.54 (m, 2H), 2.24 (m, 2H), 2.11 (m, 2H), 2.05 – 1.85 (m, 6H), 1.48 (m, 2H), 1.08 – 0.80 (m, 6H). <sup>13</sup>C NMR (100 MHz, *D*<sub>6</sub>-DMSO) δ 173.2, 170.5, 169.1, 168.7, 168.6, 167.7, 164.9, 164.6, 158.6, 157.5, 156.7, 156.1, 154.4, 146.2, 143.8, 136.6, 132.5, 130.6, 130.5, 128.3, 124.2, 120.0, 119.7, 119.4, 117.8, 111.4, 110.3, 97.8, 56.2, 52.2, 49.0, 47.5, 46.7, 46.2, 45.7, 40.8, 37.2, 34.7, 31.4, 29.9, 29.4, 28.5, 24.5, 23.6, 23.0, 22.6. LC/MS: *m/z* 909 [M+H]<sup>+</sup>.

**Scheme 11 - IRC-DiMe Synthesis:****Methyl (E)-4-((3-((tert-butoxycarbonyl)amino)propyl)(methyl)amino)-4-**

**methylpent-2-enoate (11a).** To a flask was added NaH (18 mg, 0.43 mmol) in 5 mL THF at 0 °C. Then trimethyl phosphonoacetate (98 mg, 0.54 mmol) in 2 mL THF was added dropwise with stirring. The mixture was stirred at 0 °C for 15 min. Then **6a** (93 mg, 0.36) in 2 mL THF was added dropwise and the mixture was stirred for overnight at room temperature. Remove the solvent *in vacuo* and the residue was purified by flash column chromatography

(5% MeOH in DCM) to give product **11a** (85 mg, 75%) as a light yellow oil. LC/MS:  $m/z$  315  $[M+H]^+$ .

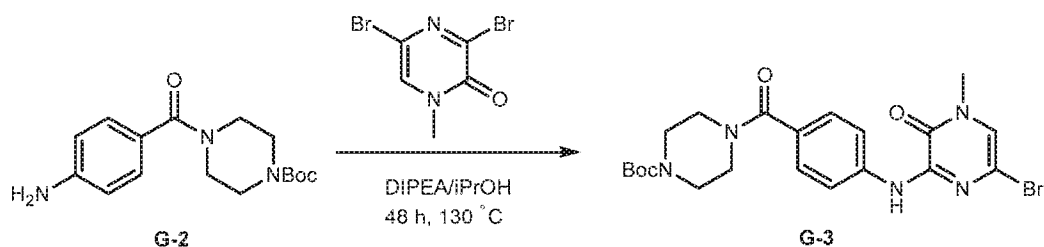
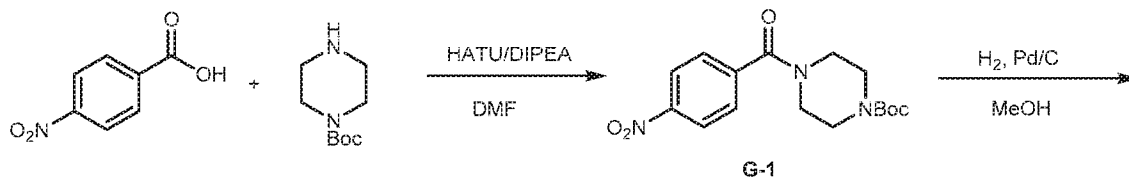
**(E)-4-((3-((tert-butoxycarbonyl)amino)propyl)(methylamino)-4-methylpent-2-enoic acid(11b)**. Compound **11a** (85 mg, 0.27 mmol) and LiOH (65 mg, 2.7 mmol) were dissolved in THF (5 mL) and water (5 mL). The mixture was stirred at 40°C for 6 hours. TLC showed **11a** completely disappeared. The mixture was cooled to 0°C and the pH was slowly adjusted to 4–5 with 1N HCl. The solvent was concentrated *in vacuo* and the residue was dissolve in DMF to give a solution **11b** (0.2 mM in DMF), which was used for next step without further purification. LC/MS:  $m/z$  301  $[M+H]^+$ .

**tert-Butyl(R,E)-3-((5-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-2-methyl-5-oxopent-3-en-2-yl)(methylamino)propyl)carbamate(11c)**. To a flask was added compound **11c** (77 mg, 0.2 mmol), solution **11b** (1.5 mL, 0.3 mmol), HATU (114 mg, 0.3 mmol) and DIPEA (129 mg, 1 mmol) in DMF(3 mL). The mixture was stirred at room temperature for 30 min. Then the reaction mixture was extracted with ethyl acetate, and the organic layer was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography (0–10% MeOH in Ethyl Acetate) to give product **11c** as a white solid (96 mg, 72%). LC/MS:  $m/z$  669  $[M+H]^+$ .

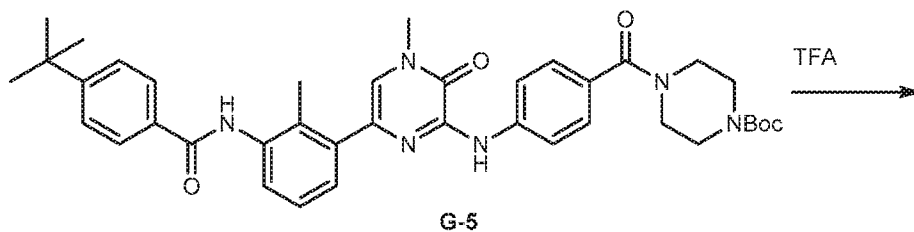
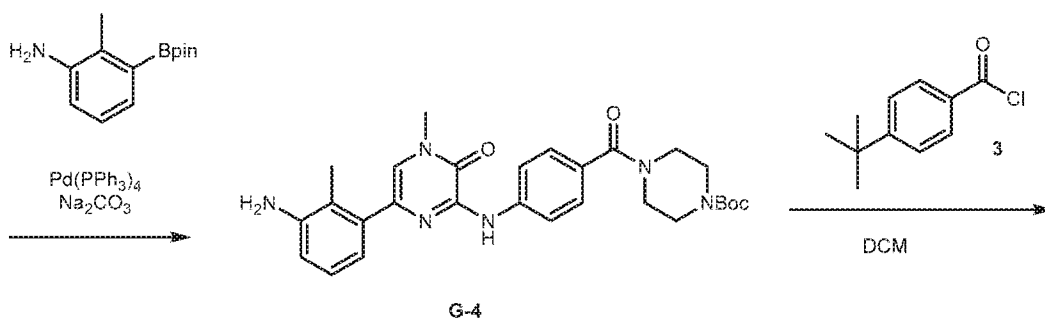
**N-(3-(((E)-5-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-2-methyl-5-oxopent-3-en-2-yl)(methylamino)propyl)-2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamide (IRC-DiMe)**. In a 25 mL flask was added **11c** (28 mg, 0.04 mmol) in TFA/DCM (5 mL, 1/1). The mixture was stirred for 30 min at room temperature. LC-MS showed **11c** converted into **11d** completely. Then remove the solvent *in vacuo* to give product **11d** (22 mg, 95%), which was used for next step without further purification. To above **11d** was added compound **5a** (20 mg, 0.06 mol), HATU (23 mg, 0.06 mmol) and DIPEA (26 mg, 0.2 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 30 min. Then the reaction mixture was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **IRC-DiMe** (10 mg, 29%). <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO)  $\delta$  11.11 (s, 1H), 8.24 (s, 1H), 8.17 (s, 1H), 8.08 (m, 1H), 7.64 (s, 2H), 7.54 (m, 1H), 7.43 (t,  $J$  = 7.5 Hz, 2H), 7.22 – 7.09 (m, 5H), 7.04 (m, 1H), 6.94 (m, 1H), 6.83 (d,  $J$  = 8.5 Hz, 1H), 6.59 (m, 1H), 6.32 (m Hz, 1H), 5.07 (dd,  $J$  = 12.8, 5.2 Hz, 1H), 4.71 (m, 1H), 4.06 (m, 3H), 3.90 (m, 2H), 3.10 (m, 3H), 2.94 – 2.82 (m, 2H), 2.58 (m, 3H), 2.24 (m, 3H), 2.10 (m, 2H), 2.01 (m, 2H), 1.48 (m, 3H), 1.11 (m, 3H), 0.96 (m, 3H). <sup>13</sup>C NMR (100 MHz, D<sub>6</sub>-DMSO)  $\delta$  173.2,

170.5, 169.1, 168.7, 167.7, 165.4, 163.8, 158.6, 157.5, 156.7, 156.0, 154.4, 152.4, 146.2, 136.6, 132.5, 130.6, 130.5, 130.4, 128.4, 119.4, 118.9, 117.8, 110.4, 110.3, 97.9, 58.8, 52.5, 49.8, 49.0, 46.2, 45.7, 37.4, 34.9, 31.4, 30.1, 29.2, 28.2, 25.3, 23.0, 22.6. LC/MS:  $m/z$  882  $[M+H]^+$ .

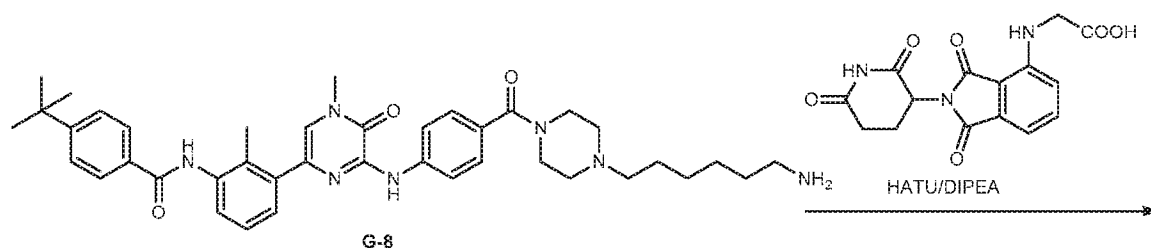
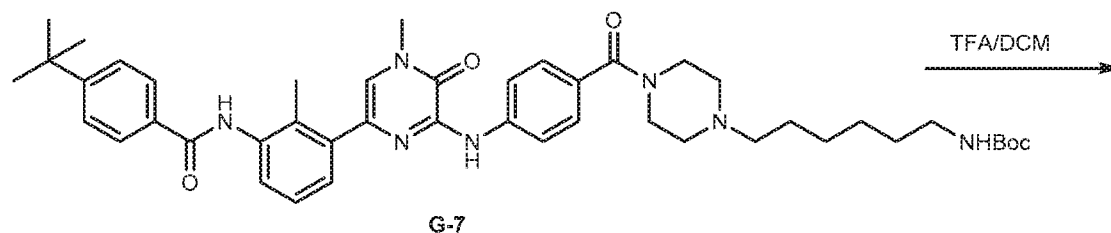
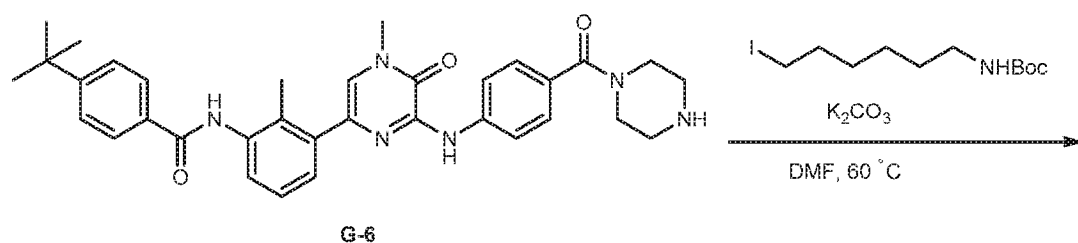
5 **Scheme 12 - DD-03-171 Synthesis:**



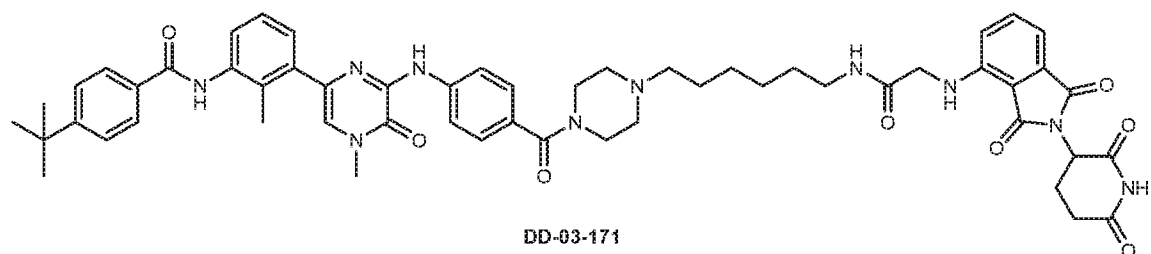
10



15



5



- 10 **tert-Butyl 4-(4-nitrobenzoyl)piperazine-1-carboxylate (G-1).** To a flask was added  
 4-nitrobenzoic acid (835 mg, 5 mmol), *tert*-butyl piperazine-1-carboxylate (930 mg, 5 mmol),  
 HATU (2850 mg, 7.5 mmol) and DIPEA (1935 mg, 15 mmol) in DMF(20 mL). The mixture  
 was stirred at room temperature for 30 minutes. Then the reaction mixture was extracted  
 with ethyl acetate, and the organic layer was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and  
 15 filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column  
 chromatography (0~20% Ethyl Acetate in Hexane) to give product **G-1** as a white solid (1.26

g, 75%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.29 (d, *J* = 8.7 Hz, 2H), 7.57 (d, *J* = 8.7 Hz, 2H), 3.76 (s, 2H), 3.54 (s, 2H), 3.37 (m, 4H), 1.47 (s, 9H). LC/MS: *m/z* 336 [M+H]<sup>+</sup>.

***tert*-Butyl 4-(4-aminobenzoyl)piperazine-1-carboxylate (G-2).** To a flask was added **G-1** (670 mg, 2 mmol) and 10%Pd/C (67 mg, 10%) in MeOH (20 mL). The mixture was stirred under 1 atm H<sub>2</sub> overnight. LC-MS showed **G-1** converted into **G-2** completely. Then the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* and the residue was purified by flash column to give product **G-2** as a white solid (550 mg, 90%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.26 – 7.22 (m, 2H), 6.65 (d, *J* = 8.4 Hz, 2H), 3.89 (s, 2H), 3.58 (s, 4H), 3.44 (s, 4H), 1.46 (s, 9H). LC/MS: *m/z* 306 [M+H]<sup>+</sup>.

***tert*-Butyl 4-(4-((6-bromo-4-methyl-3-oxo-3,4-dihydropyrazin-2-yl)amino)benzoyl)piperazine-1-carboxylate (G-3).** A solution of 3,5-dibromo-1-methylpyrazin-2(1*H*)-one (265 mg, 1 mmol), **G-2** (427 mg, 1.4 mmol), and DIPEA (387 mg, 3 mmol) in isopropanol (5 mL) was stirred in a sealed Schlenk tube at 130 °C for 48 hours. Then the reaction mixture was cooled to room temperature and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography to afford **G-3** (344 mg, 70%) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.38 (s, 1H), 7.80 (d, *J* = 8.3 Hz, 2H), 7.43 (d, *J* = 8.2 Hz, 2H), 6.81 (s, 1H), 3.71 (m, 2H), 3.53 (s, 3H), 3.46 (m, 5H), 3.13 (m, 1H), 1.46 (s, 9H). LC/MS: *m/z* 492 [M+H]<sup>+</sup>.

***tert*-Butyl 4-(4-((6-(3-amino-2-methylphenyl)-4-methyl-3-oxo-3,4-dihydropyrazin-2-yl)amino)benzoyl)piperazine-1-carboxylate(G-4).** To a 25 mL of Schlenk tube equipped with a magnetic stir bar were added 2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (82 mg, 0.35 mmol), **G-3** (172 mg, 0.59 mmol), Na<sub>2</sub>CO<sub>3</sub> (74 mg, 0.7 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (40 mg, 10 mol%). Then dioxane/H<sub>2</sub>O (2.4 mL, v/v=5/1) was added under N<sub>2</sub>. The Schlenk tube was screw capped and heated to 105 °C for 12 hours. Then the reaction mixture was cooled to room temperature, and sat. NH<sub>4</sub>Cl aq. was poured into the reaction mixture and extracted with EtOAc. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography to afford **G-4** (70 mg, 39%) as a light yellow solid. LC/MS: *m/z* 519 [M+H]<sup>+</sup>.

***tert*-Butyl 4-(4-((6-(3-(4-(*tert*-butyl)benzamido)-2-methylphenyl)-4-methyl-3-oxo-3,4-dihydropyrazin-2-yl)amino)benzoyl)piperazine-1-carboxylate(G-5).** To a solution of **G-4** (70 mg, 0.135 mmol) in dry DCM (5 mL) was added pyridine (16 μL, 0.2 mmol) and 4-*tert*-butyl benzoyl chloride (32 mg, 0.162 mmol). After stirring at room temperature for 2 h,

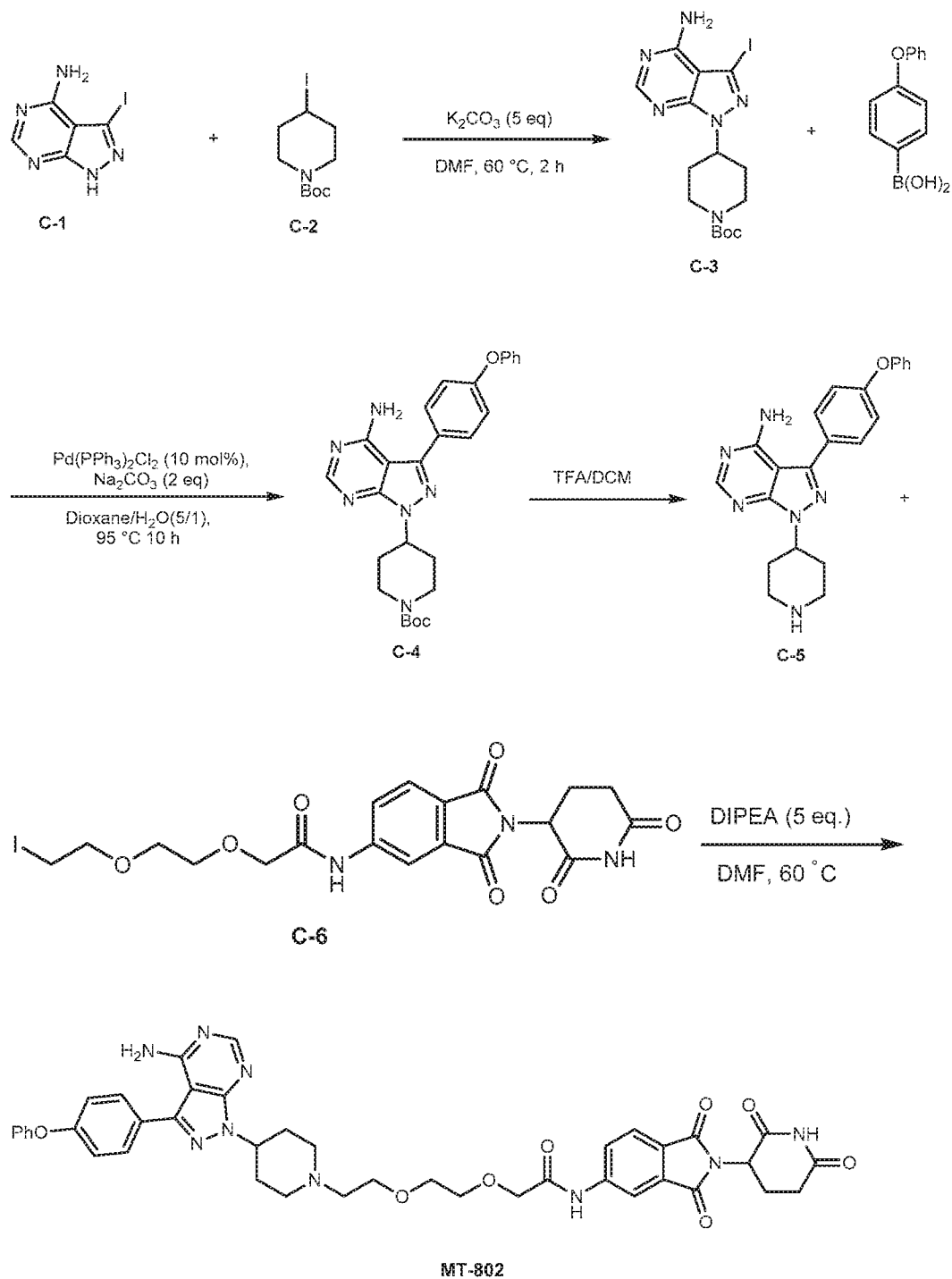
remove the solvent *in vacuo* and the residue was purified by flash column chromatography to afford **G-5** (55 mg, 60%) as a white solid. LC/MS: *m/z* 679 [M+H]<sup>+</sup>.

**tert-Butyl (6-(4-(4-((6-(3-(4-(tert-butyl)benzamido)-2-methylphenyl)-4-methyl-3-oxo-3,4-dihydropyrazin-2-yl)amino)benzoyl)piperazin-1-yl)hexyl)carbamate (G-7).** In a 25 mL flask was added **G-5** (27 mg, 0.04 mmol) in TFA/DCM (3 mL, v/v=1/1). The mixture was stirred for 30 min at room temperature. LC-MS showed **G-5** converted into **C-6** completely. Then remove the solvent *in vacuo* to give product **G-6** (21 mg, 90%), which was used for next step without further purification. To above solution of **G-6** in DMF (2 mL), add K<sub>2</sub>CO<sub>3</sub> (25 mg, 0.18 mmol) and stirred for 15 min, then *tert*-butyl (6-iodohexyl)carbamate (23 mg, 0.07 mmol) was added to the mixture. The mixture was stirred at 60 °C for 2 hour. Then the reaction mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography to afford **G-7** (14 mg, 50%) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.43 (s, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.31 (t, *J* = 7.7 Hz, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 6.77 (s, 1H), 4.52 (m, 1H), 3.64 (m, 2H), 3.63 (s, 3H), 3.48 (m, 1H), 3.09 (m, 2H), 2.49 (m, 4H), 2.40 (m, 2H), 2.38 (s, 3H), 1.43 (s, 9H), 1.36 (s, 9H), 1.52-1.21(m, 6H). LC/MS: *m/z* 778 [M+H]<sup>+</sup>.

**4-(tert-Butyl)-N-(3-(6-((4-(4-(6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamido) hexyl)piperazine-1-carbonyl)phenyl)amino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl) benzamide (DD-03-171).** In a 25 mL flask was added **G-7** (14 mg, 0.018 mmol) in TFA/DCM (5 mL, 1/1). The mixture was stirred for 30 min at room temperature. LC-MS showed **G-7** converted into **G-8** completely. Then remove the solvent *in vacuo* to give product **G-8** (12 mg, 95%), which was used for next step without further purification. To above **G-8** was added compound **5a** (10 mg, 0.03 mol), HATU (12 mg, 0.03 mmol) and DIPEA (13 mg, 0.1 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 30 min. Then the reaction mixture was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **DD-03-171** (7 mg, 42%). <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 11.12 (s, 1H), 9.92 (s, 1H), 9.45 (s, 1H), 8.11 (s, 1H), 8.07 (d, *J* = 8.3 Hz, 2H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.57 (m, 3H), 7.36 (m, 1H), 7.29 (m, 5H), 7.06 (d, *J* = 6.9 Hz, 1H), 6.94 (t, *J* = 5.8 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 5.07 (dd, *J* = 12.7, 5.2 Hz, 1H), 3.91 (d, *J* = 4.6 Hz, 2H), 3.56 (s, 3H), 3.07 (m, 6H), 2.88 (m, 1H), 2.57 (m, 2H), 2.32 (m, 3H), 2.28 (m, 3H), 2.24 (m, 3H), 2.01 (m, 1H), 1.38 (m, 2H), 1.32 (m, 9H), 1.23 (s, 6H). <sup>13</sup>C NMR (100 MHz, D<sub>6</sub>-DMSO) δ 172.9, 170.1, 168.9, 168.7, 168.3, 167.4, 165.3, 154.4, 150.5, 146.3,

145.9, 138.3, 137.2, 136.2, 132.6, 132.1, 131.8, 131.2, 129.0, 127.9, 127.6, 127.2, 126.6, 125.5, 125.2, 120.5, 118.6, 117.5, 111.0, 109.9, 57.7, 52.8, 48.7, 48.6, 45.2, 38.6, 36.7, 34.7, 31.01, 31.00, 29.1, 26.7, 26.3, 26.2, 22.2, 15.6. LC/MS:  $m/z$  991  $[M+H]^+$ .

**Scheme 13 - MT-802 Synthesis:**



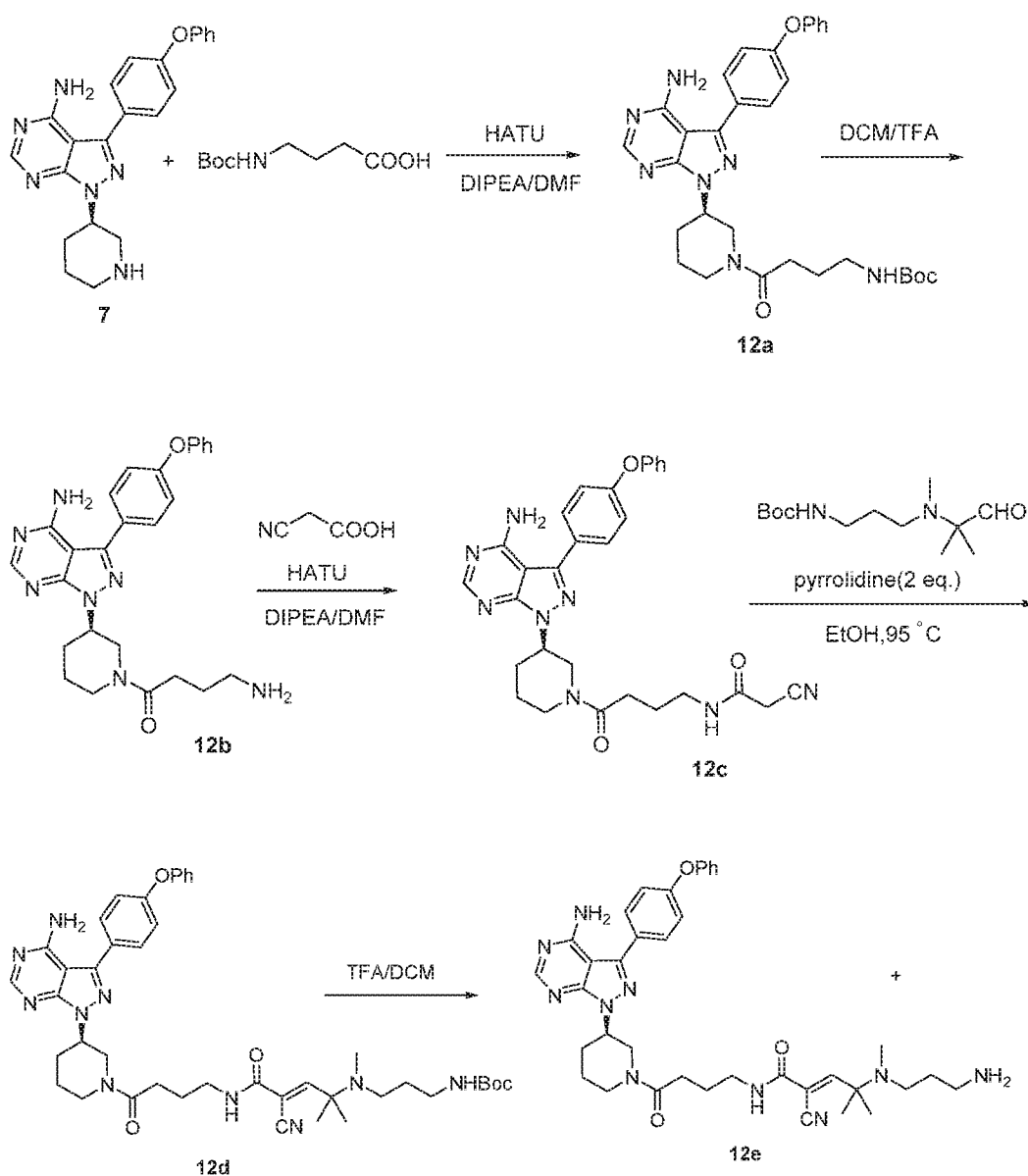
***tert*-Butyl 4-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidine-1-carboxylate (C-3).** To a solution of **C-1** (260 mg, 1 mmol) in 5 mL DMF, add K<sub>2</sub>CO<sub>3</sub> (690 mg, 5 mmol) and stirred for 15 min, then **C-2** (622 mg, 2 mmol) was added to the mixture. The solution was stirred for 2 hours at 60 °C. Then the reaction mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (5% MeOH in DCM) to give product **C-3** (260 mg, 59%) as a white solid. LC/MS: *m/z* 445 [M+H]<sup>+</sup>.

***tert*-Butyl 4-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidine-1-carboxylate (C-4).** To a 25 mL of Schlenk tube equipped with a magnetic stir bar were added (4-phenoxyphenyl)boronic acid (152 mg, 0.71 mmol), **C-3** (260 mg, 0.59 mmol), K<sub>3</sub>PO<sub>4</sub> (250 mg, 1.18 mmol), Pd(dppf)Cl<sub>2</sub> (24 mg, 5 mol%). Then DMF/H<sub>2</sub>O (5 mL, *v/v*=3/2) was added under N<sub>2</sub>. The Schlenk tube was screw capped and heated to 130 °C for 12 hours. Then the reaction mixture was cooled to room temperature, and sat. NH<sub>4</sub>Cl aq. was poured into the reaction mixture and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography to afford **C-4** (88 mg, 80%) as a light yellow solid. <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 8.24 (s, 1H), 7.65 (d, *J* = 8.5 Hz, 2H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.21 – 7.08 (m, 5H), 4.88 (m, 1H), 4.07 (br, 2H), 2.98 (br, 2H), 2.06 – 1.86 (m, 4H), 1.42 (s, 9H).

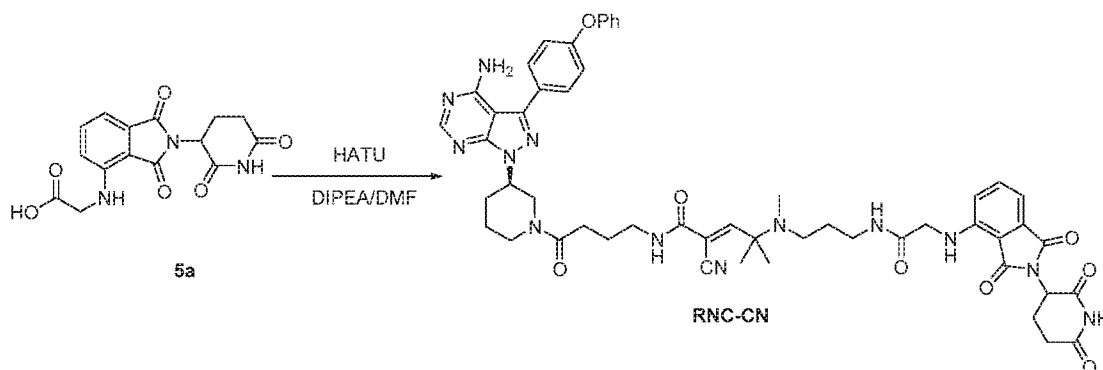
**2-(2-(2-(4-(4-Amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)-N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)acetamide (MT-802).** In a 25 mL flask was added **C-4** (8 mg, 0.02 mmol) in TFA/DCM (3 mL, *v/v*=1/1). The mixture was stirred for 30 min at room temperature. LC-MS showed **C-4** converted into **C-5** completely. Then remove the solvent *in vacuo* to give product **C-5** (7.3 mg, 95%), which was used for next step without further purification. To above solution of **C-5** in DMF, add DIPEA (13 mg, 0.1 mmol) and stirred for 15 min, then **C-6** (16 mg, 0.03 mmol) was added to the mixture. The mixture was stirred at 60 °C for 1 hour. Then the reaction mixture was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a white solid MT-802 (8 mg, 51%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.82 (br, 1H), 9.48 (s, 1H), 8.39 (s, 1H), 8.27 (d, *J* = 8.2 Hz, 1H), 7.99 (s, 1H), 7.82 (d, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.15 (m, 3H), 7.07 (d, *J* = 7.8 Hz, 2H), 4.95 (dd, *J* = 12.1, 5.3 Hz, 1H), 4.86 (m, 1H), 4.16 (m, 2H), 3.85 – 3.70 (m, 6H), 3.32 – 3.17 (m, 2H), 2.93 – 2.72 (m, 5H), 2.49 (m, 4H), 2.20 – 2.12 (m, 1H), 2.05 (d, *J* = 8.6 Hz, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 171.4, 169.0, 168.7, 167.0, 166.8, 158.5, 157.8, 156.3, 155.0, 153.5, 143.8, 143.5, 133.0, 130.0, 129.9, 127.7, 126.5,

125.1, 124.6, 124.0, 119.5, 119.1, 114.8, 98.5, 71.4, 70.5, 70.0, 68.3, 57.1, 53.4, 52.8, 50.9, 49.4, 31.5, 30.5, 30.4, 22.8. LC/MS:  $m/z$  789.3  $[M+H]^+$

**Scheme 14 - Synthesis of RNC-CN:**



10



***Tert*-butyl (*R*)-(4-(3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-  
 d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)carbamate (**12a**).** To a 100 mL flask was  
 5 added compound **7** (78 mg, 0.2 mmol), 4-((*tert*-butoxycarbonyl)amino)butanoic acid (41 mg,  
 0.2 mmol), HATU (114 mg, 0.3 mmol) and DIPEA (129 mg, 1 mmol) in DMF(2 mL). The  
 mixture was stirred at room temperature for 30 min. Then the reaction mixture was extracted  
 with ethyl acetate, and the organic layer was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and  
 filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column  
 10 chromatography (0~10% MeOH in Ethyl Acetate) to give product **12a** as a colorless oil (80  
 mg, 70%).

**(*R*)-4-amino-1-(3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-  
 d]pyrimidin-1-yl)piperidin-1-yl)butan-1-one (**12b**).** In a 25 mL flask was added **12a** (100 mg, 0.175 mmol)  
 in TFA/DCM (5 mL, 1/1). The mixture was stirred for 30 min at room temperature. LC-MS  
 15 showed **12a** converted into **12b** completely. Then remove the solvent *in vacuo* to give  
 product **12b** (78 mg, 95%), which was used for next step without further purification.

**(*R*)-N-(4-(3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-  
 d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)-2-cyanoacetamide (**12c**).** To a 100 mL flask was added  
 compound **12b** (83 mg, 0.175 mmol), 2-cyanoacetic acid (22 mg, 0.263 mmol), HATU (100  
 20 mg, 0.263 mmol) and DIPEA (180  $\mu$ L, 1 mmol) in DMF(2 mL). The mixture was stirred at  
 room temperature for 30 min. Then the reaction mixture was extracted with ethyl acetate, and  
 the organic layer was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was  
 concentrated *in vacuo* and the residue was purified by flash column chromatography (0~10%  
 MeOH in Ethyl Acetate) to give product **12c** as a colorless oil (56 mg, 60%).

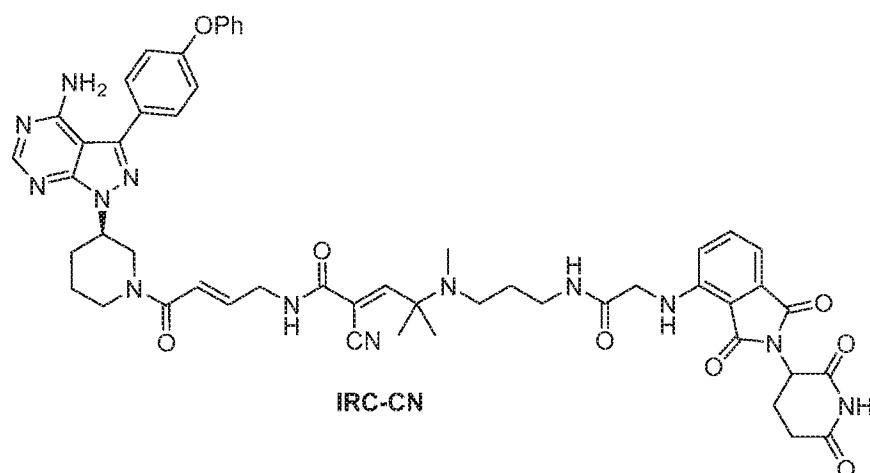
***tert*-Butyl (*R,E*)-(3-((5-((4-(3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-  
 d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)amino)-4-cyano-2-methyl-5-oxopent-3-en-2  
 25 yl)(methyl)amino) propyl)carbamate (**12d**).** To a round bottom flask was added compound

**12c** (53.8 mg, 0.1 mmol), pyrrolidine (14 mg, 0.2 mmol), compound **6a** (52 mg, 0.2 mmol) in EtOH (3 mL) and heated to 95°C for 4 hours. TLC showed compound **12d** was generated as a major product. Then the reaction mixture was extracted with ethyl acetate, and the organic layer was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography (0~10% MeOH in Ethyl Acetate) to give product **12d** as a colorless oil (40 mg, 50%).

**(E)-N-(4-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)-2-cyano-4-((3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamido)propyl)(methyl)amino)-4-methylpent-2-enamide (RNC-CN).** In a 25 mL flask was added **12d** (40 mg, 0.05 mmol) in TFA/DCM (5 mL, 1/1).

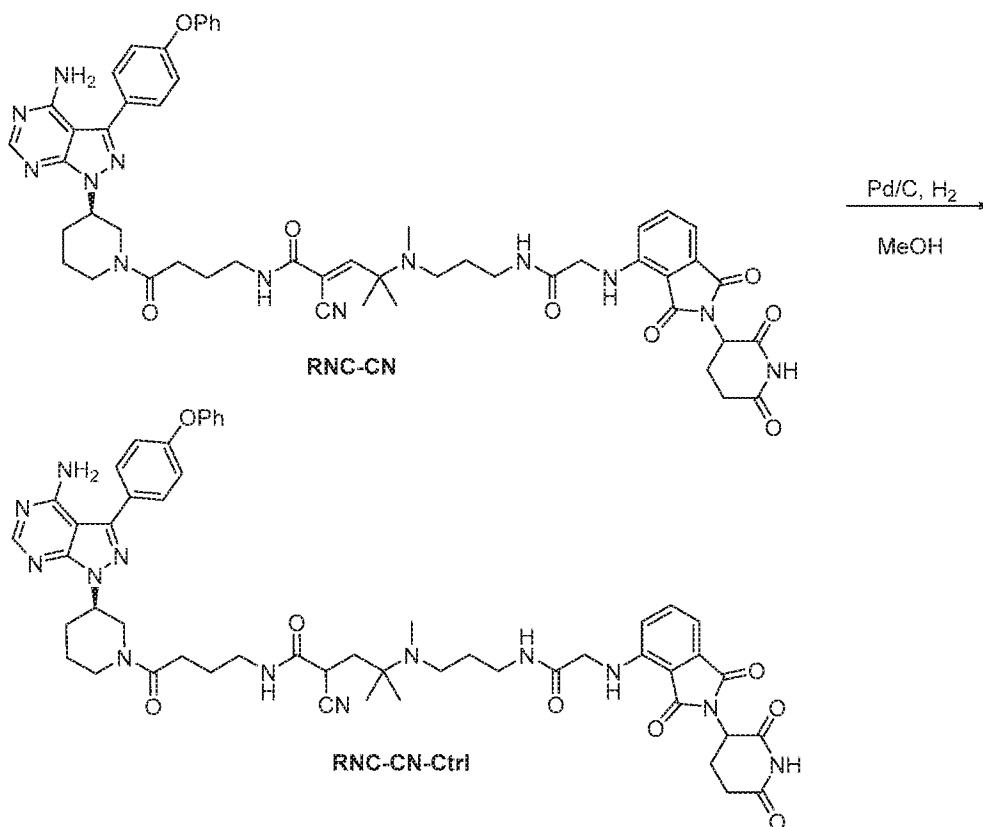
The mixture was stirred for 30 minutes at room temperature. LC-MS showed **12d** converted into **12e** completely. The solvent was removed *in vacuo* to give compound **12e** (32 mg, 95%), which was used for the next step without further purification. To compound **12e** was added compound **5a** (33 mg, 0.1 mol), HATU (38 mg, 0.1 mmol) and DIPEA (32 mg, 0.25 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 30 minutes. Then, the reaction mixture was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **RNC-CN** (20 mg, 40%). <sup>1</sup>H NMR (400 MHz, D<sub>6</sub>-DMSO) δ 8.27 (m, 3H), 8.05 (s, 1H), 7.60 (m, 4H), 7.41 (m, 3H), 7.26 (m, 1H), 7.19 – 6.99 (m, 8H), 6.92 (s, 1H), 6.82 (m, 1H), 5.04 (d, *J* = 8.3 Hz, 1H), 4.58 (m, 2H), 4.24 (s, 1H), 4.02 (m, 1H), 3.08 – 3.02 (m, 4H), 2.85 – 2.68 (m, 3H), 2.58 (m, 1H), 2.38 – 2.18 (m, 7H), 2.03 (m, 3H), 1.81 (m, 2H), 1.68 (m, 5H), 1.53 (m, 3H), 1.16 (m, 6H). <sup>13</sup>C NMR (100 MHz, D<sub>6</sub>-DMSO) δ 173.2, 170.8, 170.5, 169.1, 168.7, 167.7, 165.0, 161.5, 159.6, 158.6, 158.0, 157.5, 156.7, 156.1, 154.4, 146.2, 143.7, 136.6, 132.5, 130.6, 130.5, 128.4, 124.2, 119.4, 119.3, 117.8, 115.5, 111.4, 110.3, 109.8, 97.8, 59.7, 53.1, 52.5, 49.5, 49.2, 49.0, 45.6, 37.4, 35.3, 31.4, 30.2, 29.1, 28.3, 25.0, 24.9, 24.3, 23.9, 23.0, 22.9, 22.6. HRMS (*m/z*): [M+H]<sup>+</sup> calcd. for C<sub>52</sub>H<sub>58</sub>N<sub>13</sub>O<sub>8</sub>, 992.4531; found: 992.4549.

**Synthesis of IRC-CN:**



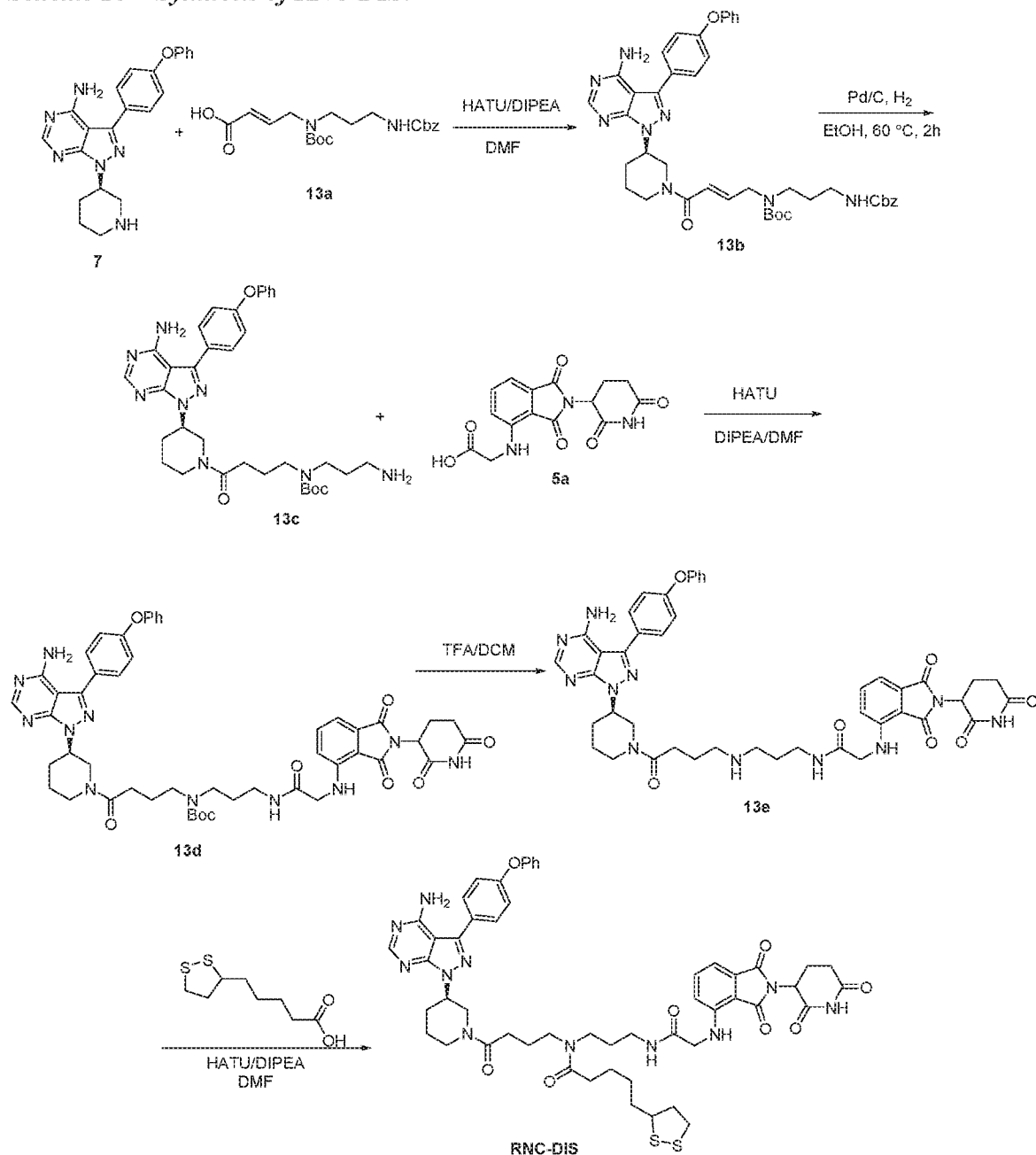
(*E*)-*N*-((*E*)-4-((*R*)-3-(4-amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidin-1-yl)-4-oxobut-2-en-1-yl)-2-cyano-4-((3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamido)propyl)(methyl)amino)-4-methylpent-2-enamide (**IRC-CN**). <sup>1</sup>H NMR (400 MHz, *D*<sub>6</sub>-DMSO) δ 8.60 (m, 1H), 8.22 (s, 1H), 8.05 (s, 1H), 7.60 (m, 3H), 7.45 – 7.21 (m, 3H), 7.06 (m, 5H), 6.98 – 6.75 (m, 2H), 6.54 (m, 2H), 5.04 (d, *J* = 8.4 Hz, 1H), 4.64 (m, 1H), 4.19 (m, 1H), 3.88 (m, 5H), 3.62 (m, 2H), 2.86 (s, 3H), 2.56 (m, 1H), 2.25 (m, 3H), 2.03 (m, 3H), 1.88 (m, 2H), 1.53 (m, 4H), 1.18 (m, 6H). <sup>13</sup>C NMR (100 MHz, *D*<sub>6</sub>-DMSO) δ 173.2, 170.8, 170.5, 169.1, 168.7, 167.7, 165.4, 164.7, 158.6, 167.5, 156.1, 154.4, 146.2, 136.6, 132.5, 130.6, 128.2, 124.2, 119.4, 119.3, 117.8, 111.4, 110.3, 97.8, 62.9, 59.7, 49.0, 45.6, 37.4, 35.3, 31.4, 30.1, 28.3, 23.0, 22.6. HRMS (*m/z*): [M+H]<sup>+</sup> calcd. for C<sub>52</sub>H<sub>56</sub>N<sub>13</sub>O<sub>8</sub>, 990.4375; found: 990.4392.

## Scheme 15 - Synthesis of RNC-CN-Ctrl:



- 5        **N-(4-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)-2-cyano-4-((3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamido)propyl)(methyl)amino)-4-methylpentanamide (RNC-CN-Ctrl).** To a flask was added **RNC-CN** (18 mg, 0.02 mmol) and Pd/C (1.8 mg, 10%) in MeOH (2 mL). The mixture was stirred under 1atm H<sub>2</sub> at room temperature
- 10    overnight. LC-MS showed **RNC-CN** converted into **RNC-CN-Ctrl** completely. Then the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **RNC-CN-Ctrl** (10 mg, 55%). HRMS (m/z): [M+H]<sup>+</sup> calcd. for C<sub>52</sub>H<sub>60</sub>N<sub>13</sub>O<sub>8</sub>, 994.4688; found: 994.4706.

## Scheme 16 - Synthesis of RNC-DIS:



Tert-butyl (R,E)-(4-(3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-

d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobut-2-en-1-yl)(3-  
 (((benzyloxy)carbonyl)amino)propyl)carbamate (**13b**). To a 100 mL flask was added  
 compound **7** (65 mg, 0.17 mmol), **13a** (78 mg, 0.2 mmol), HATU (114 mg, 0.3 mmol) and  
 DIPEA (129 mg, 1 mmol) in DMF (2 mL). The mixture was stirred at room temperature for  
 30 min. Then the reaction mixture was extracted with ethyl acetate, and the organic layer was  
 washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and filtered. The filtrate was concentrated *in vacuo*

and the residue was purified by flash column chromatography (0~10% MeOH in Ethyl Acetate) to give product **13b** as a colorless oil (65 mg, 50%).

**Tert-butyl (4-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)(3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamido)propyl)carbamate (13d).** To a flask was added **13b** (25 mg, 0.039 mmol) and Pd/C (2.5 mg, 10%) in MeOH (2 mL). The mixture was stirred under 1 atm H<sub>2</sub> at room temperature overnight. LC-MS showed **13b** converted into **13c** completely. Then the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* and the residue was used for next step without further purification. To compound **13c** was added compound **5a** (20 mg, 0.059 mol), HATU (23 mg, 0.059 mmol) and DIPEA (26 mg, 0.2 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 30 minutes. Then, the reaction mixture was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **13d** (9.4 mg, 25%).

**N-(4-((R)-3-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-4-oxobutyl)-N-(3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)acetamido) propyl)-5-(1,2-dithiolan-3-yl)pentanamide (RNC-DIS).** In a 25 mL flask was added **13d** (9.4 mg, 0.01 mmol) in TFA/DCM (5 mL, 1/1). The mixture was stirred for 30 minutes at room temperature. LC-MS showed **13d** converted into **13e** completely. The solvent was removed *in vacuo* to give compound **13e** (8.2 mg, 95%), which was used for the next step without further purification. To compound **12e** was added 5-(1,2-dithiolan-3-yl)pentanoic acid (3 mg, 0.015 mol), HATU (6 mg, 0.015 mmol) and DIPEA (6.5mg, 0.05 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 30 minutes. Then, the reaction mixture was concentrated *in vacuo* and the residue was purified by PrepHPLC with a reverse phase C18 column to afford the product as a yellow solid **RNC-DIS** (2 mg, 20%). HRMS (m/z): [M+H]<sup>+</sup> calcd. for C<sub>52</sub>H<sub>60</sub>N<sub>13</sub>O<sub>8</sub>, 1030.4068; found: 1030.4086.

### Example 2:

A proteolysis targeting chimera (PROTAC) is a heterobifunctional molecule that can bind both a target protein and an E3 ubiquitin ligase to facilitate the formation of a ternary complex, leading to ubiquitination and ultimate degradation of the target protein. PROTACs have gained tremendous attention in recent years not only for their numerous applications in chemical biology but also as highly promising therapeutic agents. Compared with oligonucleotide and CRISPR therapeutics that face *in vivo* delivery challenges, PROTACs are

small molecule therapeutics that provide opportunities to achieve broadly applicable body-wide protein knockdown.

Protein degraders have many advantages compared with traditional small molecule inhibitors. First, small molecule inhibitors usually modulate protein functions through stoichiometrically occupying the active sites or binding pockets of targeted proteins; however, PROTACs act as catalysts and are involved in multiple cycles of targeted protein degradation. Therefore, the degradation induced by PROTACs is sub-stoichiometric. Second, due to the competitive nature of small molecule inhibitors, constant systemic drug exposure is necessary to maintain sufficient intracellular concentrations for therapeutic efficacy, usually leading to off-target and side effects. In contrast, optimized PROTACs usually achieve maximal protein degradation in a few hours and maintain the therapeutic effect (even without constant PROTAC exposure) until the targeted protein is re-synthesized in cells. Therefore, the pharmacodynamics (PD) of PROTACs is dependent on not only drug exposure (pharmacokinetic (PK) properties) but also the half-life ( $t_{1/2}$ ) of the targeted protein. Third, small molecules usually interfere with the function of one domain of a multidomain protein. However, this strategy for multidomain kinases, especially in cancer cells, may lead to compensatory feedback activation of its downstream signaling pathways *via* other alternative kinases. In contrast, although PROTACs only target one domain of a multidomain protein, they induce degradation of the full-length protein, reducing the possibility to develop drug resistance through mutations or compensatory protein overexpression and accumulation. Fourth, the specificity of small molecule inhibitors depends solely on the molecular design, which is sometimes difficult to achieve due to the presence of proteins with similar binding pockets, such as kinases. In contrast, the specificity of PROTACs is determined by not only the small molecule binder to targeted proteins but also the protein-protein interactions between the targeted protein and the recruited E3 ligase. Finally, the targets of small molecular inhibitors are usually enzymes and receptors with defined binding pockets or active sites. However, ~75% of the human proteome lacks active sites, such as transcription factors and non-enzymatic proteins, and are considered “undruggable.” In contrast, PROTACs can be designed to bind to any crevice on the surface of the targeted proteins to induce their degradation.

Most PROTACs reported thus far have been based on noncovalent binding to their target proteins. Although irreversible covalent inhibitors, such as ibrutinib, have achieved tremendous clinical success based on their strong target affinities and high target occupancies, it was recently reported that PROTACs with irreversible covalent binders to

targeted proteins failed to induce efficient protein degradation. Although the potential mechanism accounting for the inhibition of protein degradation was not elucidated, it was postulated that irreversible covalent PROTACs are unable to induce protein degradation in a sub-stoichiometric/catalytic manner because they are consumed once they bind to their targeted protein. However, there are also examples arguing against this hypothesis.

Reversible covalent chemistry previously enabled the development of a series of fluorescent probes that can quantify glutathione concentrations in living cells. Studies were designed and are reported herein to determine whether reversible covalent PROTACs can not only enhance the binding affinity to targeted protein but also overcome the “one-shot deal” drawback of irreversible covalent PROTACs (**Fig. 1**). **Fig. 1** shows a demonstration of catalytic degradation of targeted proteins by reversible covalent PROTACs. The premise of this reversible covalent PROTAC design is the weak reactivity ( $mM K_d$ ) between  $\alpha$ -cyano-acrylamide group (the chemical structure shown above) and free thiols. Only when the PROTAC molecule binds to the active site of the targeted protein, the nearby cysteine side chain can react with the  $\alpha$ -cyano-acrylamide group to form a stable covalent bond. Once the targeted protein is degraded, the reversible covalent PROTAC can be regenerated.

To compare how the warhead chemistry of PROTACs with reversible noncovalent (RNC), reversible covalent (RC), and irreversible covalent (IRC) binders affect protein degradation, Bruton's tyrosine kinase (BTK) was chosen as a model target for the studies. Surprisingly, it was discovered that cyano-acrylamide-based reversible covalent binder to BTK can significantly enhance drug accumulation and target engagement in cells. RC-1 was developed as the first reversible covalent BTK PROTAC with a high target occupancy as its corresponding kinase inhibitor. RC-1 is effective as a dual functional inhibitor/degrader, providing a novel mechanism-of-action for PROTACs, and forms a stable ternary complex by reducing protein conformational flexibility compared with the non-covalent PROTAC counterparts. Importantly, it was found that this reversible covalent strategy can be generalized and applied to improve other PROTACs. These studies are detailed below.

### ***Methods and Materials***

**Cell Culture and Treatment.** The MOLM-14 cell line was obtained and the Mino cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA). Both cell lines were cultured in RPMI 1640 medium (Thermo Fisher Scientific; Waltham, MA) supplemented with 10% fetal bovine serum (GE Healthcare; Chicago, IL) and 1% Pen/Strep (Thermo Fisher Scientific). The Wild-type and C481S BTK XLA cell line was obtained and was cultured. HEK 293T/17 cells (ATCC) were maintained in DMEM (Thermo

Fisher Scientific) with 10% fetal bovine serum and 1% Pen-Strep. Cells were grown at 37 °C with 5% CO<sub>2</sub>.

For dose-dependent BTK degradation studies, 1.0 x 10<sup>6</sup> MOLM-14 cells in 2 mL of RPMI 1640 complete media were incubated with indicated doses of BTK PROTAC compounds for 24 hours, with control cells treated with 0.01% DMSO. XLA cells overexpressing wild-type BTK or mutant C481S BTK were plated at the same cell number and density as the above experiments and were treated with RC-1, IRC-1, or RNC-1 at the dose of 1.6, 8.0, 40, 200, and 1000 nM for 24 hours. After completion of treatment, all the cells were collected and processed for Western blot analysis. For proteomic study of the cellular protein profile following BTK degradation, MOLM-14 cells were treated in duplicates with 200 nM of RC-1, RNC-1, IRC-1, or RC-1-Me for 24 hours, and then the cells were collected and processed for proteomics analysis (Sanford Burnham Prebys Medical Discovery Institute; La Jolla, California).

**Immunoblotting.** The collected cells and spleen tissues were lysed in RIPA lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific) immediately before use. Lysates were centrifuged at 15,000 g for 10 minutes at 4 °C and the supernatant was quantified for total protein concentration using the Pierce BCA Protein Assay (Thermo Fisher Scientific). Thirty micrograms of protein were loaded onto sodium dodecyl sulfate–polyacrylamide gel for electrophoresis (Bio-Rad Laboratories; Hercules, CA) and transferred onto PVDF membranes (Millipore; Burlington, MA). The membranes were probed with the specified primary antibodies (Cell Signaling Technology: Anti-BTK, anti-p-BTK (Y223), anti-IKZF1, anti-IKZF3, anti-GAPDH, and anti-β-actin) overnight at 4 °C and the HRP-conjugated secondary antibodies (BIO-RAD) for 1 hour at room temperature. Imaging was performed using the ECL Prime chemiluminescent Western blot detection reagents (GE Healthcare; Chicago, IL) by visualization of the blots to X-Ray film or with an Imager (Kindle Biosciences; Greenwich, CT). All Western blots were subsequently processed and quantified with Imager software. Protein level was normalized to β-actin or GAPDH loading controls.

**AlamarBlue Cell Viability Assay.** MOLM-14 or Mino cells were harvested in the log phase of growth and re-plated into the wells of 96-well plates at the density of 6 x 10<sup>4</sup> cells/ml in 100 μL of complete RPMI-1640 culture medium. After overnight recovery, cells were exposed to serially diluted BTK PROTAC compounds (from 10,000 to 0.64 nM, 5-fold dilution) for 72 hours, which was followed by adding of pre-warmed Resazurin sodium

solution (Sigma; 1mg/mL in PBS) in an amount equal to 10% of the volume in the well. Four hours after incubation, fluorescence signals were collected with a BioTek SYNERGY H1 microplate reader (Winooski, VT) at excitation/emission 544/590 nm from top with gain at 60. The IC<sub>50</sub> was calculated using GraphPad Prism software with a four-parameter dose-response curve fit.

**NanoBRET In-Cell Target Engagement Assay.** The target engagement assay for BTK or CRBN from Promega (Madison, WI) was performed as detailed below. Briefly, HEK 293/T17 cells (ATCC) were transiently transfected with BTK-NanoLuc® fusion vector or NanoLuc®-CRBN fusion vector (Promega) using a calcium phosphate transfection protocol. Forty-eight hours after transfection, the cells were re-suspended in Opti-MEM medium (Life Technologies; Carlsbad, CA) at the density of  $2 \times 10^5$  cells/mL and were plated into 96-well plates (Corning; Corning, NY). Cells were incubated with 1.0  $\mu$ M (BTK) or 0.5  $\mu$ M (CRBN) NanoBRET™ Tracer and serially diluted unlabeled BTK PROTAC compounds (from 10 to 0.0032  $\mu$ M, 5-fold dilution) for 2 hours at 37°C with 5% CO<sub>2</sub> in an incubator or at room temperature outside of the incubator. After 2 hours incubation, the 3X Nano-Glo® Substrate with NanoLuc® extracellular inhibitor were added to cells and developed for 3 minutes at room temperature. BRET signals were collected using a BioTek SYNERGY H1 microplate reader equipped with a 450/80 nm BP filter for donor emission and a 610 nm LP filter for acceptor emission. BRET Ratio was calculated with the equation: [(Acceptor sample / Donor sample) – (Acceptor no tracer control/Donor no tracer control)] x 1000. The IC<sub>50</sub> of the compound against its BTK or CRBN tracer was calculated using GraphPad Prism software.

**In vitro Kinase Assay.** BTK kinase activity inhibition IC<sub>50</sub> was measured by PhosphoSens® Kinase Assay Kit (BioTek). This assay was performed in 384-well, white flat bottom polystyrene NBS microplates (Corning) at room temperature. Active recombinant BTK was purchased from SignalChem (Cat.# B10-10H-10). All compounds (3 warhead control and 4 PROTACs) were dissolved in DMSO (10 mM). Duplicate drug titrations (1000 nM, 200 nM, 40 nM, 8 nM, 1.6 nM, 0.32 nM, 0.64 nM, 0.128 nM and 0.0256 nM) were used to generate each IC<sub>50</sub>. Typical final concentrations of each reaction component were as follows: 2.5 nM BTK, and 10  $\mu$ M PhosphoSens® Substrate, 54 mM HEPES, pH 7.5, 1mM ATP, 1.2 mM DTT, 0.012% Brij-35, 10 mM MgCl<sub>2</sub>, 1% glycerol and 0.2 mg/mL BSA. The mixture was incubated at room temperature for 15 minutes and the fluorescence intensity (RFU) readings (Ex 360 nm/Em 492 nm) were collected for 60 minutes with 3 minute intervals in a BioTek Synergy H1 fluorescence microplate reader. To calculate the IC<sub>50</sub>, the background fluorescence, as determined with the "no kinase" control, was collected for each

time point from the total signal to obtain corrected Relative Fluorescence Units (RFU) values. The corrected RFU vs. time for each inhibitor concentration was collected and the initial reaction rates (slope of the linear portion) for each progress curve were determined for each inhibitor concentration. Then, the velocity (RFU corrected/minute) vs [inhibitor] was plotted and the IC<sub>50</sub> was determined using a 4-parameter logistic fit.

**BTK-SPPIER Assay.** All the plasmid constructs were created by standard molecular biology techniques and confirmed by exhaustively sequencing the cloned fragments. To create the E3 ubiquitin ligase CRBN-EGFP-HOTag3 fusion, full length CRBN was first cloned into pcDNA3 containing EGFP. HOTag3 was then cloned into the pcDNA3 E3 ligase-EGFP construct, resulting in pcDNA3 CRBN-EGFP-HOTag3. Similar procedures were carried out to produce pcDNA3 BTK<sup>KD</sup>-EGFP-HOTag6.

The HEK293T/17 cells were passaged in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), nonessential amino acids, penicillin (100 units/mL), and streptomycin (100 µg/mL).

HEK293T/17 cells were transiently transfected with the plasmid using calcium phosphate transfection reagent or lipofectamine. Cells were grown in 35 mm glass bottom microwell (14 mm) dishes (MatTek Corporation). Transfection was performed when cells were cultured to ~50% confluence. For each transfection, 4.3 µg of plasmid DNA was mixed with 71 µL of 1X Hanks' Balanced Salts buffer (HBS) and 4.3 µL of 2.5 M CaCl<sub>2</sub>. Cells were imaged 24 hours after transient transfection. Time-lapse imaging was performed with the aid of an environmental control unit incubation chamber (InVivo Scientific), which was maintained at 37 °C and 5% CO<sub>2</sub>. Fluorescence images were acquired with an exposure time of 50 ms for EGFP. Chemical reagents, including RC-1, IRC-1 and RNC-1, were carefully added to the cells in the incubation chamber when the time-lapse imaging started. Image acquisition was controlled by the NIS-Elements Ar Microscope Imaging Software (Nikon). Images were processed using NIS-Elements and ImageJ (NIH).

For analysis of the SPPIER signal, images were processed in ImageJ. The sum of droplet pixel fluorescence intensity and cell pixel intensity was scored using the Analyze Particle function in ImageJ.

**Definition for the relative intracellular accumulation coefficient for drug D ( $K'_{P,D}$ ).** The species in a Nano-Luc based target engagement assay includes the target protein nanoLuc fusion (designated as P), the tracer (designated as T), the tracer bound target protein nanoLuc fusion (designated as PT), the drug (designated as D), and the drug bound target protein nanoLuc fusion (designated as PD). In this simplified model, the binding of tracer T

and drug D to intracellular proteins or biomolecules, other than the target protein nanoLuc fusion P, were not considered.

For the tracer T and the target protein nanoLuc fusion P interactions,

$$[P] + [T] \rightleftharpoons [PT] \quad (\text{eq. 1})$$

$$K_{d,T} = \frac{[P][T]}{[PT]} \quad (\text{eq. 2})$$

$$C_T = [T] + [PT] \quad (\text{eq. 3})$$

wherein  $K_{d,T}$  is the dissociation equilibrium constant for the binding between the target protein nanoLuc fusion and the tracer, [P] is the equilibrium concentration of the target protein nanoLuc fusion, [T] is the equilibrium concentration of the free tracer T, [PT] is the equilibrium concentration of the tracer bound form of the target protein nanoLuc fusion, and  $C_T$  is the total intracellular concentration of the tracer T.

For the drug D and the target protein nanoLuc fusion P interactions,

$$[P] + [D] \rightleftharpoons [PD] \quad (\text{eq. 4})$$

$$K_{d,D} = \frac{[P][D]}{[PD]} \quad (\text{eq. 5})$$

$$C_{D,in} = [D] + [PD] \quad (\text{eq. 6})$$

wherein  $K_{d,D}$  is the dissociation equilibrium constant for the binding between the target protein nanoLuc fusion and the drug, [P] is the equilibrium concentration of the target protein nanoLuc fusion, [D] is the equilibrium concentration of the free drug D, [PD] is the equilibrium concentration of the drug bound form of the target protein nanoLuc fusion, and  $C_{D,in}$  is the total intracellular concentration of the drug D.

Based on the fact that the target protein nanoLuc fusion P is either unbound or bound to T or D, it can be deduced that

$$C_P = [P] + [PD] + [PT] \quad (\text{eq. 7})$$

wherein  $C_P$  is the total concentration of the target protein nanoLuc fusion P.

Without the addition of drug D, the tracer bound nanoLuc fusion P (PT) accounts for 100% of all the nanoLuc fusion P and corresponds to the concentration  $C_P$ . When the addition of drug D competes off the tracer to leave only 50% of PT, it can be deduced that:

$$[PT] = \frac{1}{2} C_P$$

$$\text{Based on Eq. 3,} \quad [T] = C_T - [PT] = C_T - \frac{1}{2} C_P$$

$$\text{Based on Eq. 2,} \quad [P] = K_{d,T} \frac{[PT]}{[T]} = K_{d,T} \frac{C_P}{2C_T - C_P} \quad (\text{eq. 8})$$

Based on Eq. 7,  $[PD] = C_P - [P] - [PT] = \frac{1}{2} C_P - K_{a,T} \frac{C_P}{2C_T - C_P}$  (eq. 9)

Based on Eq. 5,  $[D] = K_{a,D} \frac{[PD]}{[P]}$

The intracellular accumulation coefficient for drug D ( $K_{P,D}$ ) can be defined as

$$K_{P,D} = \frac{C_{D,in}}{C_{D,ex}}$$

5 wherein  $C_{D,in}$  is the total intracellular concentration of drug D and  $C_{D,ex}$  is the total extracellular concentration of drug D.

When 50% of target engagement is reached with the addition of drug D to the medium (i.e.  $C_{D,ex} = IC_{50,TE}$  assuming intracellular drug accumulation does not significantly change the extracellular drug concentration), it can reasonably be assumed that  $[D] \gg [PD]$  due to the limited amount of the target protein nanoLuc fusion P in cells. Because  $C_{D,in} = [D] + [PD]$ , then it can be approximated that  $C_{D,in} = [D]$ . Therefore,

$$K_{P,D} = \frac{C_{D,in}}{C_{D,ex}} = \frac{[D]}{IC_{50,TE}} = \frac{K_{a,D}}{IC_{50,TE}} \frac{[PD]}{[P]}$$

Under the same assay conditions,  $C_P$ ,  $C_T$  and  $K_{a,T}$  are constants. Therefore,  $[PD]/[P]$  is a constant, which can be defined as A. It was then be deduced that

15 
$$K_{P,D} = A \frac{K_{a,D}}{IC_{50,TE}}$$

The relative intracellular accumulation coefficient for drug D ( $K'_{P,D}$ ) is defined as

$$K'_{P,D} = \frac{K_{P,D}}{A} = \frac{K_{a,D}}{IC_{50,TE}}$$

Therefore, under the same assay conditions, a greater  $K'_{P,D}$  value for a drug reflects its higher propensity to accumulate inside cells.

20 **Animals.** Female ICR mice (weighing 22-28 g) were obtained and were housed 2-4 per cage in an American Animal Association Laboratory Animal Care accredited facility and maintained under standard conditions of temperature ( $22^\circ\text{C} \pm 2^\circ\text{C}$ ), relative humidity (50%) and light and dark cycle (12/12 hours), and had access to food and water ad libitum. Mice were allowed to acclimate to their environment for one week before the experiments.

25 **Pharmacokinetic and Pharmacodynamic Studies of RC-1.** The pharmacokinetic profile of the BTK PROTAC RC-1 was evaluated in female ICR mice (weighing 22-28 g, n = 3). RC-1 was formulated in a non-aqueous solvent (30% PEG-400, 5% Tween 80, and 5% DMSO in deionized water) and was administered in a single intraperitoneal (IP) injection (20 mg/kg). Blood samples (25  $\mu\text{L}$ ) were withdrawn from the tail vein at the time points of 10

minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and up to 24 hours after dosing. The blood samples were collected into 1.5 mL centrifuge tubes coated with 0.5 M EDTA and were immediately centrifuged at 12,000 x g at 4 °C for 15 minutes. The resultant plasma was extracted with 9 volumes of acetonitrile and then were centrifuged at the same conditions described above. The supernatant was stored at -80 °C for liquid chromatography-mass spectrometry (LC-MS) analysis. The standard curve was established by serially diluting RC-1 in plasma collected from naïve mice (from 5.0 to 0.003 µg/mL, 4-fold dilution). The pharmacokinetic (PK) parameters were calculated with the Microsoft Excel PK Solver.

To investigate the protein degradation effect of RC-1 *in vivo*, mice were subjected to a single RC-1 administration at the dose of 50 mg/kg or 100 mg/kg (i.p.) or seven once-daily RC-1 administration at the dose of 20 mg/kg (i.p.) (n = 2-3). Twenty-four hours after the one-time RC-1 injection or 24 hours after the 7<sup>th</sup> injection, mice were anesthetized with isoflurane and the whole spleen was dissected and stored at -80 °C for Western blot analysis of protein level.

**Molecular Dynamics Modeling: Construction of initial model for ternary complexes of {BTK-Ligands-CRBN}.** To construct the ternary complexes of BTK/ligands/CRBN, the binary complexes of BTK/CRBN were firstly modeled. The structure files of BTK/ibrutinib(PDB code: 5P9J) and that of CRBN/ lenalidomide (PDB code: 4TZ4) were obtained from RCSB. Multiple residues at the entrance of the protein BTK (L408, N484 and K558) and CRBN (I371, H353 and E377) were randomly selected as the indication of active binding site, and constrained protein-protein docking simulations were performed by using ZDOCK. Three candidate binding conformations were reported and downloaded from the ZDOCK server. Since the protein-protein binding interface of the second conformation is in between the entrance of active sites of BTK and CRBN, this predicted BTK/CRBN complex was selected for ligand docking. To select a proper ligand conformation for the follow-up MD simulation, the low-energetic molecular conformations of the linker designed for PROTACs were sampled. The linker structure whose length fit without steric clashes in between BTK and CRBN of the chosen protein complex was selected. Finally, the linker was artificially attached to the ibrutinib and lenalidomide for constructing the initial ternary complexes. A total of 3 complexes were constructed for BTK/RNC-1/CRBN, BTK/IRC-1/CRBN and BTK/RC-1/CRBN, respectively.

**Refining ternary complexes of BTK/ Ligands/ CRBN/ by molecular dynamic simulations.** To further refine the structures of the selected BTK/ligand/CRBN complexes and to assess their stability under a physically motivated force field, the initial complex

structure was solvated in an all-atom explicit-solvent environment for molecular dynamics simulation. An appropriate number of counter ions was added to neutralize the system. A cubic box was used with periodic boundary conditions to ensure no artificial contacts between solute and its images, which resulted in a total of ~270K atoms for each of the three simulation systems. Energy minimization was performed using the steepest decent method until the maximum force was smaller than 1000.0 kJ mol<sup>-1</sup>nm<sup>-1</sup>. The system was further equilibrated for 1 ns under NVT and NPT ensemble, respectively, before continuing with a production run of more than 200 ns. During the equilibration run, the protein and ligand positions are constrained by a harmonic potential. For both the equilibration and production runs, the temperature was set to 300K using the V-rescale thermostat separately for protein-ligand complex and solution. The coupling constant of the external thermal bath was set to 0.1 ps. Also, the pressure of the system was set to 1 atm (Parrinello-Rahman coupling). Since one covalent bond is formed in between the residue C481 of BTK and the ligands RC-1 and IRC-1, a harmonic potential with the spring constant of 167360 kJ mol<sup>-1</sup>nm<sup>-2</sup> was added in between the corresponding sulfur and carbon atoms to mimic this additional constraint. All simulations were performed using the CHARMM36 force field and with molecular simulation package GROMACS-2019.

## Results

### Comparison of Different PROTAC Warhead Chemistry for BTK Degradation.

As the first FDA-approved covalent kinase inhibitor, ibrutinib irreversibly reacts with the free cysteine residue (C481) in the active site of BTK to form a covalent bond. Ibrutinib can still bind to the C481S BTK mutant mostly through hydrogen bonding, but with >40 folds lower affinity. Ibrutinib is >6 folds more potent than its Michael acceptor saturated ibrutinib analog in a kinase inhibition assay for wild type BTK (IC<sub>50</sub> 0.72 nM vs 4.9 nM), while both compounds are equally potent towards BTK C481S mutant (IC<sub>50</sub> 4.6 nM vs 4.7 nM).

To compare how different warhead chemistry may affect the target protein degradation, three PROTAC molecules were designed, RC-1, RNC-1 and IRC-1, which form reversible covalent (RC), reversible noncovalent (RNC), and irreversible covalent (IRC) binding to BTK (Fig. 2A). Pomalidomide was chosen as the CRBN E3 ligase binder. RC-1, RNC-1 and IRC-1 share the same linker and E3 ligand binder, and differ only by the binding moieties to BTK. MOLM-14, an acute myeloid leukemia cell line, was chosen as a model system to study PROTAC-mediated BTK degradation.

To test whether the three PROTACs can induce BTK degradation, MOLM-14 cells were treated with RC-1, RNC-1 and IRC-1 for 24 hours, followed by Western blot to quantify the

total BTK levels (**Fig. 2B**). IRC-1 induced inefficient BTK degradation. Comparing RC-1 and RNC-1, it was found that RC-1 was more potent for BTK degradation than RNC-1 at lower concentrations (8 nM and 40 nM) and comparable to RNC-1 at 200 nM. RC-1 potently induced BTK degradation in MOLM-14 cells ( $DC_{50} = 6.6$  nM, **Fig. 2C**), as one of the most potent BTK degraders reported so far. Additionally, neither the RC-1 warhead, nor pomalidomide, nor a combination of both caused BTK degradation (**Fig. 2D**), indicating that the bifunctional PROTAC molecule is essential to facilitate the formation of a ternary complex of {BTK-PROTAC-CRBN} to induce BTK degradation.

**Linker Development for Reversible Covalent BTK PROTAC.** Based on the promising result for RC-1, the development of suitable linkers between the BTK covalent reversible binder and pomalidomide (**Fig. 3**) was studied. Common knowledge in the industry is that increasing PROTAC linker length alleviates steric clashes between BTK and CRBN and improves the efficacy of BTK degradation, indicating the absence of thermodynamic cooperativity in the formation of a ternary complex of {BTK-PROTAC-CRBN}. RC-2, RC-3, RC-4 and RC-5, were synthesized, which are 1, 2, 5, and 8 atoms longer in the linker length, respectively, as compared with RC-1. Surprisingly and in direct contrast with the understanding in the field, it was found that RC-1 is the most efficacious for BTK degradation compared with the PROTACs possessing longer linkers, suggesting cooperative ternary complex formation (**Fig. 11**).

To test whether the BTK degradation efficacy of RC-1 can be improved through further reducing the linker length, RC-6, RC-7 and RC-8, were designed, which are 1, 2, and 3 atoms shorter in the linker length compared with RC-1. Unfortunately, RC-6 had an intramolecular reaction between the amide and the Michael acceptor. Comparing the BTK degradation capability in MOLM-14 cells, it was found herein that RC-7 and RC-8 are inferior to RC-1, possibly due to unfavorable steric clashes between BTK and CRBN. It was also determined in the studies described herein that the efficacy of BTK degradation decreases significantly through only a single atom change of the thalidomide aryl amine nitrogen to an oxygen (RC-9) (**Fig. 11**).

In a recent study, a BTK PROTAC MT-802 has the linker placed at the C5 position on the phthalimide ring of pomalidomide instead of the C4 position as in RC-1. RC-10 was synthesized by placing the linker at the C5 position of the phthalimide ring and it was found that RC-10 cannot induce any BTK degradation (**Fig. 11**). Therefore, RC-1 has the optimal linker length and position for BTK degradation with the BTK and CRBN binders used.

**Quantitative Measurements of BTK and CRBN Concentrations in Cells.** In a previous study in the field, a series of PROTACs for TANK-Binding Kinase 1 (TBK-1) were developed with different binding affinities towards TBK-1 and von Hippel-Lindau (VHL), the E3 ligase recruited. The data from the study were re-analyzed and a strong correlation was found between binding affinities towards TBK-1 and the  $DC_{50}$  values in cells (**Fig. 12**), suggesting that tighter binding to the target protein leads to more efficient PROTAC-induced protein degradation in cells. Therefore, it is reasonable to assume that the binding affinity of RC-1 to BTK is higher than RNC-1 due to the covalent bond formed between RC-1 and BTK. It could be taken for granted that tighter binding to BTK leads to more formation of the ternary complex, resulting in more efficient BTK degradation. However, the formation of the {BTK-PROTAC-CRBN} ternary complex depends on not only the binding affinities of PROTAC towards BTK and CRBN but also the absolute concentrations of BTK, CRBN and PROTAC in cells. Previous studies on PROTACs only focus on the biochemical measurements for binding affinities of PROTACs to target proteins and E3 ligases without knowing the intracellular concentrations of these proteins.

Quantitative Western blots were performed to measure the levels of BTK and CRBN in MOLM-14 cells using a series of concentrations of their corresponding recombinant proteins as the standards. Lysate from a million MOLM-14 cells usually produces 250  $\mu$ g of protein as determined by BCA assays. Because proteins usually occupy 20-30% of cell volume (assuming 25% w/v, i.e., 250 mg/mL), the volume of 1 million MOLM-14 cells was calculated to be  $\sim 1$   $\mu$ L (i.e., the volume of each MOLM-14 cell is  $\sim 1000$   $\mu$ m<sup>3</sup>). Based on quantitative Western blots, the total amount of BTK and CRBN in 1 million MOLM-14 cells was determined to be 60 ng and 0.72 ng, respectively. Considering the molecular weights of BTK and CRBN are 77 kD and 55 kD, it can be deduced that the absolute concentrations for BTK and CRBN in MOLM-14 cells are 780 nM and 13 nM, respectively (**Fig. 4A and Fig. 13A-B**). Based on biochemical binding assays, the  $K_d$  values between PROTACs and BTK are in the range of 3.0–6.4 nM (**Table 1**), while the  $K_d$  values between the PROTACs and CRBN are in the range of 1.5–4.2  $\mu$ M (**Table 2**).

Table 1

| Compound | $K_d$ (nM) | BTK Inhibition $IC_{50}$ (nM) | Cell Viability $IC_{50}$ ( $\mu$ M) | BTK TE $IC_{50,TE}$ ( $\mu$ M) | $K_{PD}$             |
|----------|------------|-------------------------------|-------------------------------------|--------------------------------|----------------------|
| RC-1     | 6.4        | 1.8                           | 0.31                                | 0.043                          | $1.5 \times 10^{-1}$ |
| IRC-1    | 3.0        | 0.8                           | 2.7                                 | 0.13                           | $2.3 \times 10^{-2}$ |
| RNC-1    | 4.7        | 21.2                          | 4.1                                 | 1.27                           | $3.7 \times 10^{-3}$ |

|             |     |      |      |       |                      |
|-------------|-----|------|------|-------|----------------------|
| RC-1-Me     |     | 6.8  | 0.21 |       |                      |
| RC-Ctrl     | 9.1 | 2.2  | 0.50 | 0.054 | $1.7 \times 10^{-1}$ |
| IRC-Ctrl    |     | 0.3  | 0.33 |       |                      |
| RNC-Ctrl    |     | 13.6 | 0.42 |       |                      |
| RNC-CN-DiMe | 29  |      |      | 0.93  | $3.1 \times 10^{-2}$ |
| IRC-DiMe    | 11  |      |      | 0.38  | $2.9 \times 10^{-2}$ |

The data shown in **Table 1** were obtained as follows: The dissociation equilibrium constant  $K_d$  was measured using the full-length BTK protein by Eurofins DiscoverX. The reported  $K_d$  values for RC-1, IRC-1 and RC-Ctrl were measured in the absence of DTT in the buffer. DTT (6 mM) used in the standard assay condition increases the  $K_d$  values of RC-1 and RC-crtl by 2-3 folds, while it does not affect the measurement for IRC-1. The reported  $K_d$  value for RNC-1 was measured in the standard assay buffer with the presence of DTT. Duplicates were performed. The biochemical BTK inhibition (BTK Inhibition  $IC_{50}$ ) was measured using the BTK assay kit from AssayQuant Technologies Inc. Duplicates were performed. Cell viability  $IC_{50}$  was performed by treating MOLM-14 cells with compounds for 72 hours, followed by Alamar Blue assays. BTK Target Engagement  $IC_{50}$  is the concentration of an unlabeled compound that results in a half-maximal inhibition binding of the BTK tracer. The target engagement of compounds was assessed following Promega's assay protocol. Triplicates were performed.  $K'_{P,D}$  is a relative intracellular accumulation coefficient for drug D and calculated as  $K_d/IC_{50,TE}$ .  $K'_{P,D}$  is an assay-dependent parameter to quantify the tendency of intracellular accumulation of a drug. Under the same assay conditions, a greater  $K'_{P,D}$  value for a drug reflects its higher tendency to accumulate inside cells. Please refer to the supporting information for detailed explanation.

**Table 2**

| Compound     | $K_d$ ( $\mu$ M) | $IC_{50,TE}$ ( $\mu$ M) | $K'_{P,D}$ |
|--------------|------------------|-------------------------|------------|
| RC-1         | 4.2              | 0.25                    | 16.8       |
| IRC-1        | 1.5              | 0.86                    | 1.7        |
| RNC-1        | 1.8              | 1.69                    | 1.1        |
| Pomalidomide | 1.4              | 0.30                    | 4.7        |

The data shown in **Table 2** were obtained as follows: The dissociation equilibrium constant  $K_d$  was measured using a truncated Cereblon protein. Triplicates were performed. CRBN Target Engagement  $IC_{50}$  is the concentration of an unlabeled compound that results in a half-maximal inhibition binding of the CRBN tracer. The target engagement of compounds

was assessed using Promega's assay. Triplicates were performed.  $K'_{P,D}$  is a relative intracellular accumulation coefficient for drug D and calculated as  $K_d/IC_{50,TE}$ .  $K'_{P,D}$  is an assay-dependent parameter to quantify the tendency of intracellular accumulation of a drug. Under the same assay conditions, a greater  $K'_{P,D}$  value for a drug reflects its higher tendency to accumulate inside cells. Further details are provided in the Methods and Materials section above.

Assuming there is no cooperativity effect in the formation of the ternary complex {BTK-PROTAC-CRBN}, it can be predicted that BTK PROTACs binding to CRBN is the determining factor for ternary complex formation due to the low abundance of CRBN and the weak binding between pomalidomide and CRBN. Therefore, under this situation, further increasing PROTAC binding affinities to BTK, such as comparing RC-1 and RNC-1, would not lead to a meaningful increase of ternary complex formation. This conclusion is also supported by the mathematical model for three-body binding equilibria (Fig. 14A-B).

#### Fluorophore Phase Transition-Based Imaging of Ternary Complex Formation in

**Live Cells.** To visualize small molecule-induced protein-protein interactions (PPIs), the fluorophore phase transition-based principle was applied and a PPI assay named SPPIER (separation of phases-based protein interaction reporter) was designed. A SPPIER protein design includes three domains, a protein-of-interest, an enhanced GFP (EGFP) and a homo-oligomeric tag (HOTag). Upon small molecule-induced PPI between two proteins-of-interest, multivalent PPIs from HOTags drive EGFP phase separation, forming brightly fluorescent droplets. Here, to detect PROTAC-induced PPI between BTK and CRBN, the kinase domain of BTK (amino acid residues 382 – 659, referred to as BTK<sup>KD</sup>) was engineered into SPPIER to produce a BTK<sup>KD</sup>-EGFP-HOTag6 construct, which forms tetramers when expressing in cells (Fig. 5A). The previously reported CRBN-EGFP-HOTag3 fusion construct, which forms hexamers in cells, was used as the E3 ligase SPPIER (Fig. 5A). If PROTACs can induce {BTK-PROTAC-CRBN} ternary complex formation in cells, they will crosslink the BTK<sup>KD</sup>-EGFP-HOTag6 tetramers and the CRBN-EGFP-HOTag3 hexamers to produce EGFP phase separation, which can be conveniently visualized with a fluorescence microscope. This assay is named as BTK-SPPIER. HEK293 T/17 cells were transiently transfected with both constructs. Twenty-four hours after transfection, the cells were incubated with 10  $\mu$ M of RC-1, IRC-1 or RNC-1. Live cell fluorescence imaging revealed that RC-1, but not IRC-1 nor RNC-1, induced appreciable green fluorescent droplets (Fig. 5B). This imaging result indicated that RC-1 is more efficient to induce {BTK-PROTAC-CRBN} ternary complex formation in living cells than RNC-1 and IRC-1 under the same

experimental conditions. It should be noted that the concentration needed for RC-1 to induce appreciable droplet formation in this assay is much higher than its  $DC_{50}$  in MOLM-14 cells potentially due to the high overexpression of the target proteins and the sensitivities of the assay.

5        **Inhibition of Cell Viabilities by BTK Degraders.** The potencies of inhibiting cell growth for RC-1, IRC-1, RNC-1, RC-1-Me (RC-1 non-degrader control), and their corresponding BTK binder controls, RC-Ctrl, IRC-Ctrl (i.e. ibrutinib), and RNC-Ctrl in MOLM-14 cells were then examined. All the chemical structures and  $IC_{50}$  values are found in **Fig. 2A** and **Table 1**, respectively. Differing by a methyl group, RC-1 can but RC-1-Me cannot degrade BTK in cells (**Fig. 2D**). Interestingly, both compounds have similar  $IC_{50}$  values (0.31 vs 0.21  $\mu$ M), showing that BTK inhibition but not degradation accounts for the toxicity in MOLM-14 cells. The  $IC_{50}$  values for RC-Ctrl, IRC-Ctrl, and RNC-Ctrl are also similar in the range of 0.3–0.5  $\mu$ M, suggesting that these three warheads inhibit BTK to a similar extent in cells. Surprisingly, both the  $IC_{50}$  values for IRC-1 and RNC-1 are in the  $\mu$ M range (2.7 and 4.1  $\mu$ M). A biochemical BTK kinase inhibition assay showed that IRC-1 and RNC-1 have slightly diminished inhibitory activities (< 3-fold) compared with their corresponding warhead controls (**Table 1**). However, the difference between enzymatic activities is insufficient to explain the >10-fold difference in  $IC_{50}$  values in cells, suggesting that IRC-1 and RNC-1 may have poorer intracellular accumulation than their corresponding warhead controls. In contrast, RC-1 and RC-Ctrl have similar  $IC_{50}$  values in both biochemical BTK inhibition and cellular growth inhibition assays (**Table 1**), suggesting similar compound exposure in cells. The growth inhibition assay for the BTK degraders prompted the investigation of alternative mechanisms to explain the high potency of RC-1.

25        **Comparison of Intracellular Concentrations of BTK Degraders.** Since the binding affinities between BTK and its PROTACs cannot explain the difference in BTK degradation between RC-1 and RNC-1 and potencies for cell growth inhibition among RC-1, RNC-1 and IRC-1, studies were designed to determine whether the intracellular concentration of RC-1 may be higher than those of RNC-1 and IRC-1, leading to more potent pharmacological effects. To test this possibility, the Nano-luciferase based bioluminescence resonance energy transfer (NanoBRET) assay developed by Promega was used. HEK-293 cells were transiently transfected with plasmids expressing a fusion protein of Cereblon and nano-luciferase (nLuc) for 24 hours and then the cells were treated with a Cereblon tracer, which binds to Cereblon to induce NanoBRET signals. Adding PROTACs to cells would compete the CRBN tracer binding to CRBN, thus reducing the NanoBRET signals. The

target engagement  $IC_{50}$  is defined as the concentration of an unlabeled compound that results in a half-maximal inhibition binding between the fluorescent tracer and the nanoLuc fusion protein. The NanoBRET assay is ratiometric and independent of the expression level of the nanoLuc fusion protein. Based on this assay, it was found that the CRBN target engagement  $IC_{50}$  value of RC-1 is 3 and 7-fold as low as those of IRC-1 and RNC-1, respectively (**Fig. 4B**). It should be noted that target engagement  $IC_{50}$  values are dependent on assay conditions, including the tracer concentration and the expression level of the nanoLuc fusion protein. Therefore, the target engagement  $IC_{50}$  values for different compounds can only meaningfully be compared under the same assay conditions.

To quantitatively compare the intracellular concentrations of these PROTACs, a parameter  $K_{P,D}$  was defined as the intracellular accumulation coefficient for drug D, in which P and D denote partition and drug, respectively.  $K_{P,D}$  is defined as the ratio between the total intracellular and extracellular concentrations of drug D and calculated as  $K_{P,D} = \frac{C_{D,in}}{C_{D,ex}}$ , where  $C_{D,in}$  and  $C_{D,ex}$  are the total intracellular and extracellular concentrations of drug D, respectively. When 50% of target engagement is reached with the addition of drug D to the medium (i.e.,  $C_{D,ex} = IC_{50,TE}$  assuming intracellular drug accumulation does not significantly change the extracellular drug concentration;  $C_{D,in} = [D]$  assuming the total intracellular concentration of drug D is much greater than that of the target protein P), it is deduced that  $K_{P,D} = \frac{C_{D,in}}{C_{D,ex}} = \frac{[D]}{IC_{50,TE}} = \frac{K_{d,D}}{IC_{50,TE}} \frac{[PD]}{[P]}$ , where  $K_{d,D}$  is the dissociation equilibrium constant for the binding between the target protein or its nanoLuc fusion and the drug, [P] is the equilibrium concentration of the target protein nanoLuc fusion, [D] is the equilibrium concentration of the free drug D, [PD] is the equilibrium concentration of the drug bound form of the target protein nanoLuc fusion, and  $IC_{50,TE}$  is the target engagement  $IC_{50}$  in the NanoBRET assay. Under the same assay conditions, [PD] and [P] are constants and  $[PD]/[P]$  can be defined as a constant A. Based on this, it is deduced that  $K_{P,D} = A \frac{K_{d,D}}{IC_{50,TE}}$ . The relative intracellular accumulation coefficient for drug D ( $K'_{P,D}$ ) is further defined as  $K'_{P,D} = \frac{K_{P,D}}{A} = \frac{K_{d,D}}{IC_{50,TE}}$ . Under the same assay conditions, a greater  $K'_{P,D}$  value for a drug reflects its higher tendency to accumulate inside cells. It should be noted that  $K_{P,D}$  is independent of assays and assay conditions but requires quantification of [P] and [PD]. In contrast,  $K'_{P,D}$  is an assay condition dependent parameter but provides a convenient approach to quantitatively compare the tendency of intracellular accumulation of drugs.

Based on the  $K'_{P,D}$  values calculated by dividing the  $K_d$  to CRBN with the in-cell target engagement  $IC_{50}$  (Table 2), it was deduced that the intracellular concentration of RC-1 is 10 and 16-fold as the levels of IRC-1 and RNC-1, respectively. Additionally, the calculated CLogP and polar surface area (PSA) values for these three compounds are similar (Table 3), weighing against the possibility that the physicochemical properties of RC-1 are the cause of its high intracellular concentration. Not to be bound by theory, it was therefore concluded that the efficient BTK degradation and potent cell growth inhibition induced by RC-1 is achieved mostly through its high intracellular concentration, due to the reversible covalent structural moiety in RC-1.

Table 3

| Compound      | CLogP | tPSA  |
|---------------|-------|-------|
| RC-1          | 4.26  | 247.6 |
| IRC-1         | 4.71  | 223.8 |
| RNC-1         | 4.55  | 223.8 |
| RNC-1-CN-DiMe | 3.81  | 247.6 |
| IRC-1-DiMe    | 5.42  | 223.8 |

**RC-1 is a unique BTK degrader with high target occupancy.** Although the PROTACs are characterized as BTK degraders, they have warheads that can bind and inhibit BTK, essentially as dual-functional BTK inhibitors and degraders. Biochemical BTK kinase inhibition assays were performed to measure the  $IC_{50}$  values for RC-1, RNC-1 and IRC-1 and their corresponding warhead controls (Table 1 and Fig. 15). IRC-Ctrl (i.e. Ibrutinib) forms a covalent bond with BTK and is expected to be the most potent BTK inhibitor ( $IC_{50} = 0.3$  nM). In comparison, RC-Ctrl and RNC-Ctrl have reduced BTK inhibition activities by 7 and 45 folds, respectively. The BTK PROTACs, RC-1, RNC-1, and IRC-1, have similar BTK inhibitory activities to their corresponding warheads (Table 1 and Fig. 15).

A similar NanoBRET based live-cell target engagement assay was performed for BTK. Consistent with the CRBN target engagement assay, it was found that the BTK target engagement  $IC_{50}$  value of RC-1 is 3 and 30 folds of the values for IRC-1 and RNC-1, respectively (Fig. 4C). Based on the  $K'_{P,D}$  values calculated by dividing the  $K_d$  to BTK with the in-cell target engagement  $IC_{50}$  (Table 1), the intracellular concentration of RC-1 is 6 and 40 folds as high as those of IRC-1 and RNC-1, respectively, similar to the trend observed in the CRBN target engagement assay (Fig. 4B).

To further rule out the possibility that the enhanced intracellular accumulation and target engagement of RC-1 is due to its physical properties, two additional compounds, RNC-1-CN-DiMe and IRC-1-DiMe, were synthesized (**Fig. 16A**). RNC-1-CN-DiMe can be viewed as direct reduction of the C=C double bond in the Michael acceptor of RC-1 to a single bond. IRC-1-DiMe can be viewed as RC-1 only lacking the cyano group but maintaining the dimethyl moiety. The BTK target engagement IC<sub>50</sub> value of RC-1 is at least an order of magnitude smaller than the values for IRC-1-DiMe and RNC-1-CN-DiMe, respectively (**Table 1** and **Fig. 16B**). Calculating the  $K_{PD}$  values, the intracellular concentration of RC-1 is 5 folds of those of IRC-1-DiMe and RNC-1-CN-DiMe (**Table 1**), showing that the enhanced intracellular accumulation of RC-1 may not be attributed to the physical property changes caused by the additional cyano or dimethyl groups in RC-1 compared with IRC-1 and RNC-1.

Due to poor intracellular accumulation, most PROTACs have low target occupancy (**Fig. 4D**) and rely on the sub-stoichiometric protein degradation to achieve maximal efficacy. Using the NanoBRET-based BTK in-cell target engagement assay, it was found that RC-1 can achieve 50% and 90% of target engagements at 40 nM and 200 nM, respectively. Therefore, RC-1 can function as both a BTK inhibitor and degrader.

**RC-1 degrades BTK regardless of its mutation status.** Mutations in BTK, C481S in particular, confer ibrutinib resistance in clinic. It was determined whether BTK degradation induced by the PROTACs described herein is affected by BTK mutation status. XLA cells overexpressing wild type BTK or C481S mutant BTK were treated with RC-1, RNC-1 and IRC-1 for 24 hours, followed by Western blot to compare the BTK levels (**Fig. 6A** and **Fig. 17**). Dose-dependent BTK degradation induced by RC-1 was observed regardless of its mutation status with comparable potency. This observation is consistent with the conclusion herein that altering PROTAC binding affinities to BTK within a range does not significantly change the ternary complex formation efficiency (**Fig. 14A-B**). The potency of RC-1 is weaker in XLA cells than in MOLM-14 cells possibly because BTK is overexpressed in XLA cells. It is also interesting to note that IRC-1 induces much more effective degradation of the BTK C481S mutant than its wild type in XLA cells (**Fig. 17**), suggesting that the irreversible covalent bond formation between IRC-1 and BTK causes the inefficient protein degradation.

**RC-1 degrades BTK with higher specificity than IRC-1 and RNC-1.** To explore the effects of the degraders described herein on the whole proteome, MOLM-14 cells were treated with RC-1, RNC-1, IRC-1, RC-1-Me (non-degrader control), or DMSO and a

quantitative multiplexed proteomic approach was employed to measure the whole cellular protein levels (**Fig. 7A-D**). The result showed that both in IRC-1 and RNC-1-treated cells, seven kinases were degraded, including BTK. However, for RC-1-treated cells, only two kinases (BTK and CSK) can be degraded, showing that RC-1 has more selectivity than IRC-1 and RNC-1 for kinase degradation. However, no degradation is observed in RC-1-Me-treated cells, indicating that the degradation observed for RC-1 is CRBN dependent. In addition, immunomodulatory imide drugs (IMiD)-dependent substrates, including IKZF1, ZFP91 and ZNF692, are also specifically degraded by RC-1, RNC-1 and IRC-1.

**Molecular dynamics simulations predict a stable ternary complex for RC-1.** To better understand the ternary complex formation, molecular dynamics simulations were applied to evaluate the global rearrangement of the ternary structures for BTK, CRBN, and RC-1 or IRC-1 or RNC-1, and compared the binding stabilities of these three ligands to BTK and CRBN. The residue-based RMSF (root mean square fluctuations) was calculated based on the entire simulation trajectory (>200 ns) to assess the average fluctuation of each residue of the three simulated complexes. It is clear that RNC-1 mediated complex {BTK-RNC-1-CRBN} has the largest fluctuation compared with the other two complexes {BTK-RC-1-CRBN} and {BTK-IRC-1-CRBN} (**Fig. 8A**), suggesting that covalent binding could help to overcome the unfavorable entropy penalties during the ternary complex formation. Additionally, the RMSD (Root-mean-square deviation) values were calculated based on the C $\alpha$  atoms to reflect the conformation changes from the input structures based on x-ray crystallography. Consistent with the RMSF analyses, the RMSD changes for the {BTK-RC-1-CRBN} and {BTK-IRC-1-CRBN} complexes are much smaller than the one for the {BTK-RNC-1-CRBN} complex, suggesting that covalent bonding can help to stabilize the ternary complexes (**Fig. 8B**).

Low energy conformations of the protein complex can shed light on the detailed molecular interactions which stabilize the binding interface. The conformation with the lowest energy along the trajectories was extracted and shown in **Fig. 8C**. The binding mode and critical molecular interactions, i.e., hydrogen bonds and  $\pi$ - $\pi$  interactions formed between the ligands and BTK and CRBN, are conserved compared to the crystal structure of BTK/ibrutinib and CRBN/lenalidomide. From the predicted binding mode, several hydrogen bonds and the covalent bond between RC-1 and the residue C481 of BTK anchors the ligand to the binding site of BTK. Additionally, a hydrogen bond between Y355 of CRBN and anion- $\pi$  interaction formed between H353, hence, further greatly stabilizing the binding of

RC-1 with proteins, and helping to hold the orientation of RC-1 to adjust and stabilize the ternary complexes (Fig. 8C).

**RC-1 outperforms other reported BTK degraders in cell viability and target engagement assays.** The goal of blocking BTK signaling with either BTK inhibitors or

5 degraders is to inhibit the growth of cancer cells. To this end, previously reported BTK degraders DD-03-171 and MT-802 were compared head-to-head with RC-1, RNC-1 and IRC-1 in their abilities to inhibit cancer cell growth (Fig. 9A and 9B, and Fig. 18A and 18B). RNC-1 was used as a surrogate for comparison. In MOLM-14 cells, RC-1 has the most potent inhibitory effect among all the PROTACs compared (Fig. 9A and Fig. 18A).  
10 RC-1 and RC-1-Me, which does not induce BTK degradation, have similar IC<sub>50</sub> values in inhibiting MOLM-14 cell growth, indicating that the growth inhibitory effect induced by PROTACs in MOLM-14 cells is due to BTK inhibition instead of its degradation. The high potency of RC-1 can be due to the combinatorial effects of its high intracellular concentration and tight binding to BTK.

15 The potency of BTK degraders in Mino cells, a BTK dependent MCL cell line, was also compared. In Mino cells, RC-1 and DD-03-171 have comparable potency for inhibiting cell growth and outperform all the other BTK PROTACs tested (Fig. 9B and Fig. 18B). Additionally, RC-1 can degrade not only BTK and phosphorylated BTK but also IKZF1 and IKZF3 (Fig. 6B), similar to DD-03-171. RC-1 is more potent than ibrutinib in Mino cells,  
20 but have similar potencies in Jeko-1, Rec-1 and Maver-1 cells (Table 4).

**Table 4**

| Compound  | Mino | Jeko-1 | Rec-1 | Maver-1 |
|-----------|------|--------|-------|---------|
| Ibrutinib | 3.8  | 0.3    | 6.2   | 5.4     |
| RC-Ctrl   | >5   | 0.6    | >5    | >5      |
| RC-1-Me   | 3.3  | 0.6    | >5    | 4.2     |
| RC-1      | 0.4  | 0.2    | 4.1   | 3.3     |

Triplicates were performed.

Additionally, BTK in-cell target engagement among RC-1, RC-Ctrl (RC-1 warhead) and reported BTK degraders was compared (Fig. 4D). The target engagement IC<sub>50</sub> values for  
25 RC-1 and RC-Ctrl (59 vs 47 nM) are the same within experimental errors, demonstrating that the intracellular accumulation of RC-1 is essentially the same as its parent warhead molecule, although their molecular weights differ by 1.7 folds. In contrast, the target engagement IC<sub>50</sub>

values for DD-03-171 and MT-802 are 5 and 11 folds as high as that of RC-1, respectively.

In terms of BTK degradation, RC-1 is more potent than MT-802 at a low concentration (8 nM) and has a comparable potency at a high concentration (200 nM) (**Fig. 21A-B**). However, RC-1 is unique compared with other BTK degraders because it not only degrades BTK efficiently but also shows high target engagement to inhibit BTK in case BTK is not completely degraded. RC-1 demonstrates a novel dual mechanism of action (MOA) for BTK inhibition and degradation.

**RC-1 has an ideal plasma half-life and degrades BTK in vivo.** The plasma half-life of RC-1 (20 mg/kg, i.p. injection) in ICR mice (female, 5-6 weeks, n=3) using LC-MS/MS. The PK data were fitted into a non-compartmental model using PK solver. RC-1 has a plasma half-life ( $t_{1/2}$ ) of 3.4 hours,  $C_{max}$  of 20  $\mu$ M, and AUC of 72  $\mu$ M·h (**Fig. 10A**). To further test the pharmacodynamics of RC-1 *in vivo*, ICR mice (n=3) were treated with 20 mg/kg of RC-1 (i.p. injection). After 7 days injection, the mice were sacrificed, and their spleens, the locations of the majority of B cells, were collected. Western blotting showed that RC-1-treated mice had ~50% BTK level reduction in the spleens compared with the vehicle-treated mice (**Fig. 10B** and **Fig. 19A-B**).

For confirmation of the inhibition, a mouse B cell lymphoma cell line derived from Eu-Myc mice was treated with RC-1 *in vitro*. It was found that the maximum BTK degradation is only 30-40% even dosed up to 25  $\mu$ M of RC-1 (**Fig. 20**), indicating that RC-1 is much less potent for BTK degradation in mouse cells than in human cells. Therefore, this preliminary study showed that RC-1 has desirable PK/PD properties *in vivo*.

**BTK Degradation using PROTACS.** Additional compounds as described herein, including RC-11, RC-12, RC-13, RC-14, IRC-1-DiMe, RNC-1-CN-DiMe, RNC-DIS, IRC-CN, RNC-CN, and RNC-CN-Ctrl were analyzed for BTK degradation using the methods described above in this example. The compounds were compared to two BTK inhibitors known in the art, labeled as Comparative 1 and Comparative 2. The structures of the compounds are shown in **Fig. 22**. BTK degradation of the compounds in a MOLM-14 cell line was tested as described above. Specifically, the MOLM-14 cells were treated with the PROTACs for 24 hours, followed by Western blotting to measure the BTK levels. The quantification results are shown in **Fig. 23A** and the Western blots are shown in **Fig. 23B**.

The compounds and methods of the appended claims are not limited in scope by the specific compounds and methods described herein, which are intended as illustrations of a few aspects of the claims and any compounds and methods that are functionally equivalent

are within the scope of this disclosure. Various modifications of the compounds and methods in addition to those shown and described herein are intended to fall within the scope of the appended claims. Further, while only certain representative compounds, methods, and aspects of these compounds and methods are specifically described, other compounds and methods are intended to fall within the scope of the appended claims. Thus, a combination of 5 steps, elements, components, or constituents can be explicitly mentioned herein; however, all other combinations of steps, elements, components, and constituents are included, even though not explicitly stated.

**WHAT IS CLAIMED IS:**

1. A compound of the following formula:



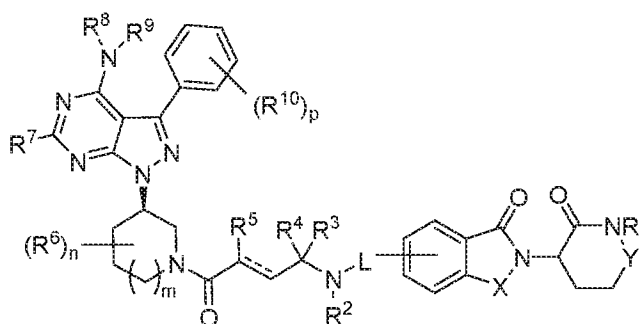
or a pharmaceutically acceptable salt or prodrug thereof, wherein:

A is a BTK binder;

L is a linker; and

B is an E3 ligase binder.

2. The compound of claim 1, wherein L comprises a reversible covalent group.
3. The compound of claim 1 or 2, wherein B comprises a CRBN ligand, a VHL ligand, a cIAP1 ligand, a MDM2 ligand, a RNF2 ligand, or a DCAF15 ligand.
4. A compound of the following formula:



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

== is a single bond or a double bond;

m is 0-3;

n and p are each independently 0-5;

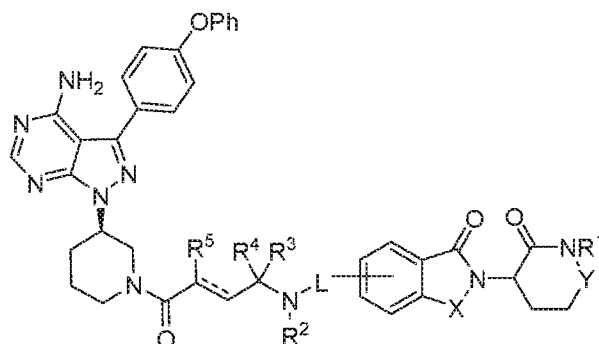
L is a linker;

X and Y are each independently selected from CH<sub>2</sub>, CHD, CD<sub>2</sub>, CHF, CF<sub>2</sub>, and C(O);

R<sup>1</sup>, R<sup>2</sup>, R<sup>8</sup>, and R<sup>9</sup> are each independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted carbonyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl; and

$R^3$ ,  $R^4$ ,  $R^5$ , each  $R^6$ ,  $R^7$ , and each  $R^{10}$  are each independently selected from hydrogen, halogen, cyano, trifluoromethyl, alkoxy, aryloxy, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl.

5. A compound of the following formula:



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

$\equiv$  is a single bond or a double bond;

L is a linker;

X and Y are each independently selected from  $\text{CH}_2$ ,  $\text{CHD}$ ,  $\text{CD}_2$ ,  $\text{CHF}$ ,  $\text{CF}_2$ , and  $\text{C(O)}$ ;

$R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are each independently selected from hydrogen, substituted or unsubstituted alkyl, and substituted or unsubstituted carbonyl; and

$R^5$  is hydrogen, deuterium, fluoro, chloro, bromo, iodo, cyano,  $-\text{OCH}_3$ ,  $-\text{OCDH}_2$ ,  $-\text{OCD}_2\text{H}$ , or  $-\text{OCD}_3$ .

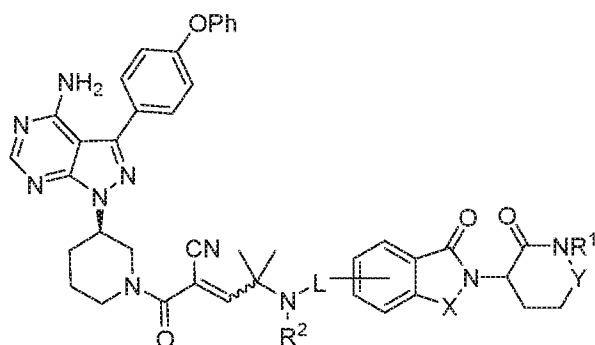
6. The compound of claim 5, wherein L is a substituted or unsubstituted alkyl, a substituted or unsubstituted alkenyl, a substituted or unsubstituted alkynyl, a substituted or unsubstituted alkoxy, or a substituted or unsubstituted amino.

7. The compound of claim 5 or 6, wherein L contains an amide group.

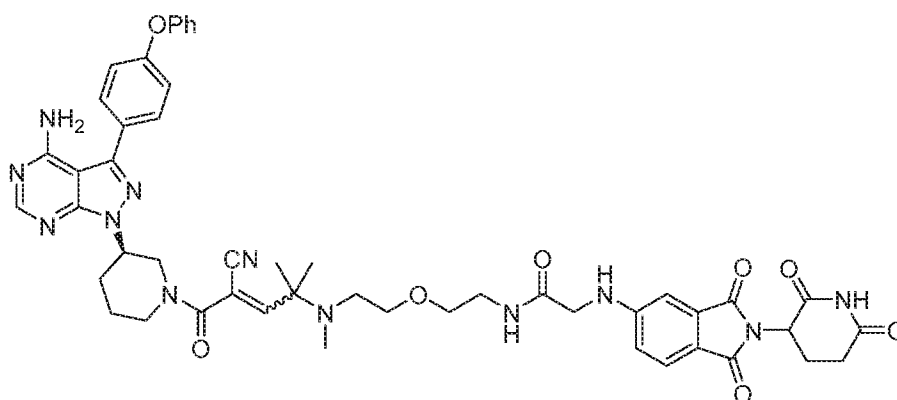
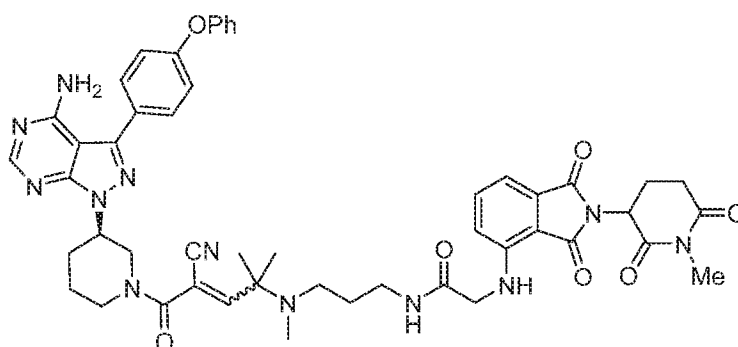
8. The compound of any one of claims 5-7, wherein X and Y are each  $\text{C(O)}$ .

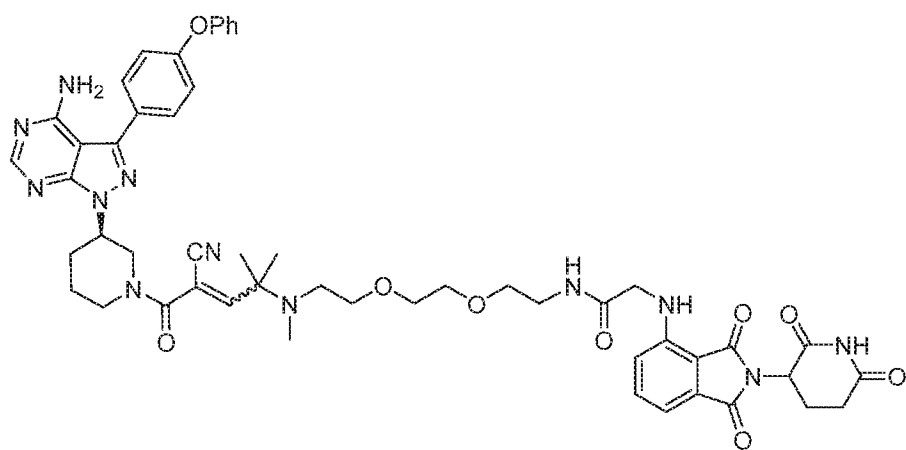
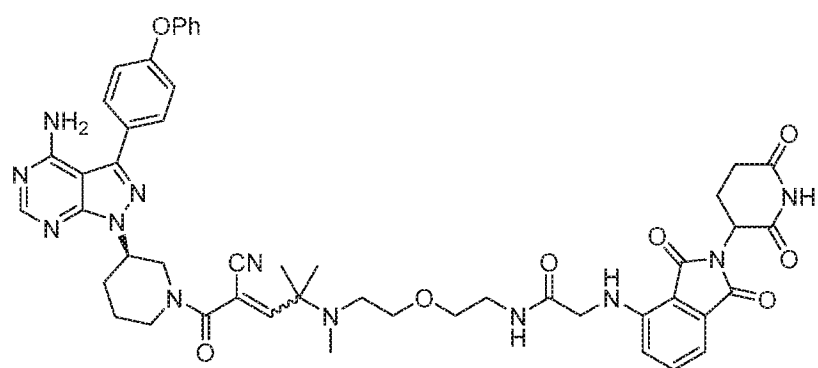
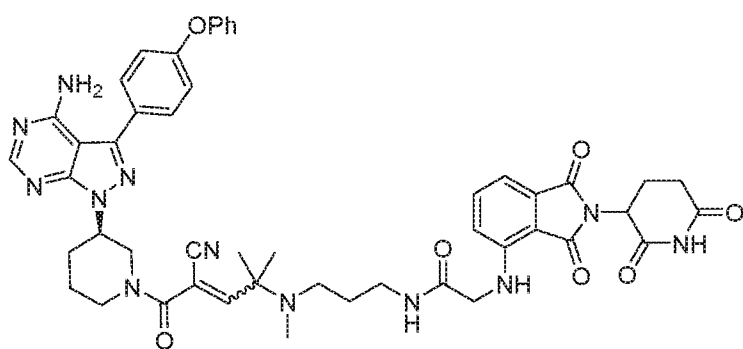
9. The compound of any one of claims 5-8, wherein  $R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are each independently selected from hydrogen and methyl.

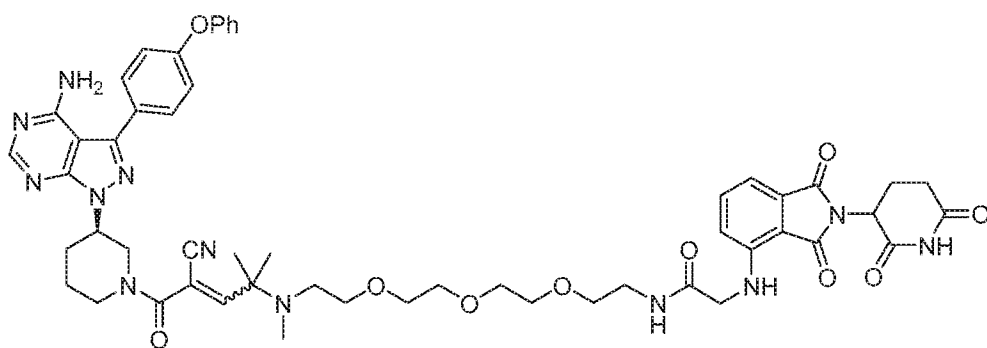
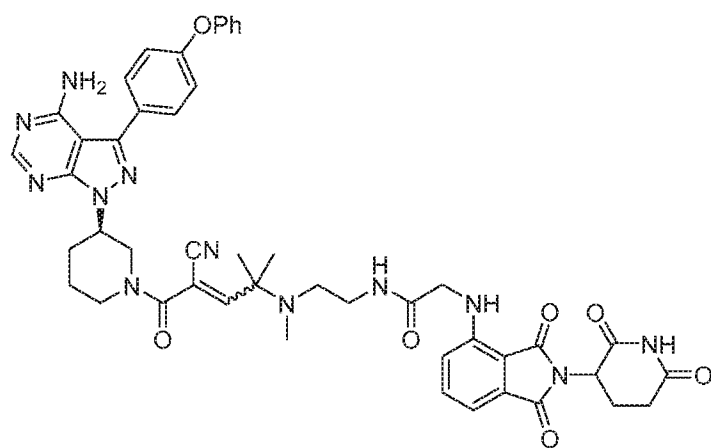
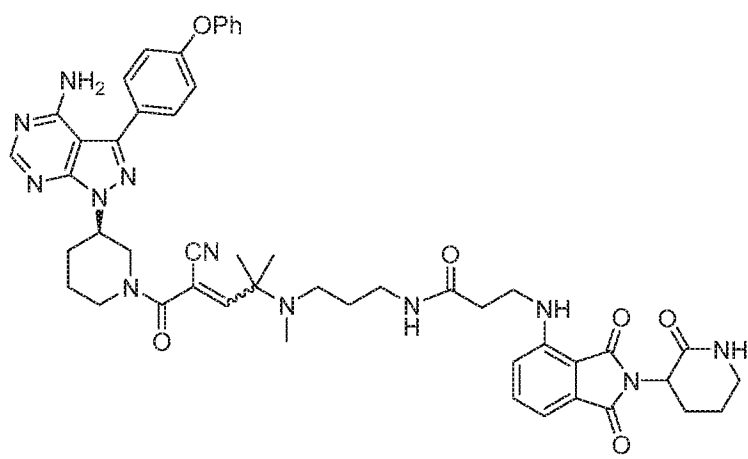
10. The compound of claim 5, wherein the compound has the following formula:

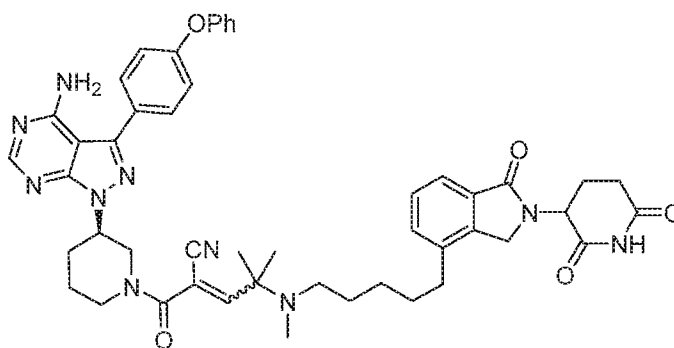
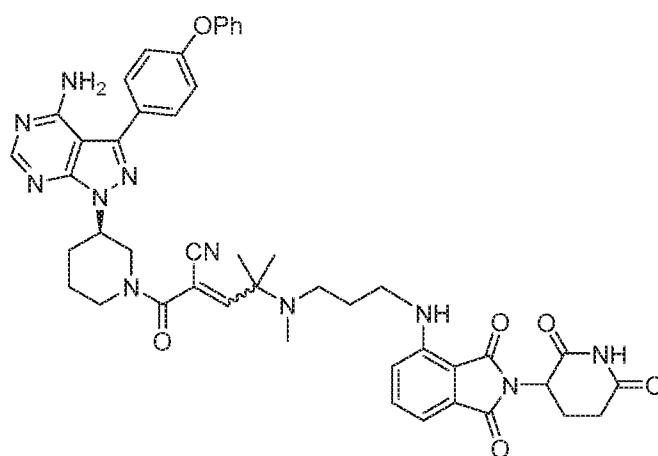
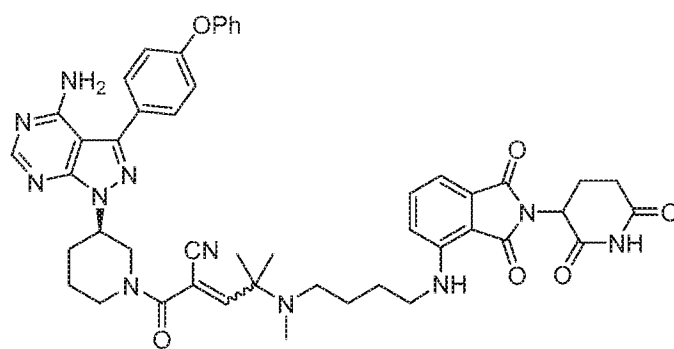


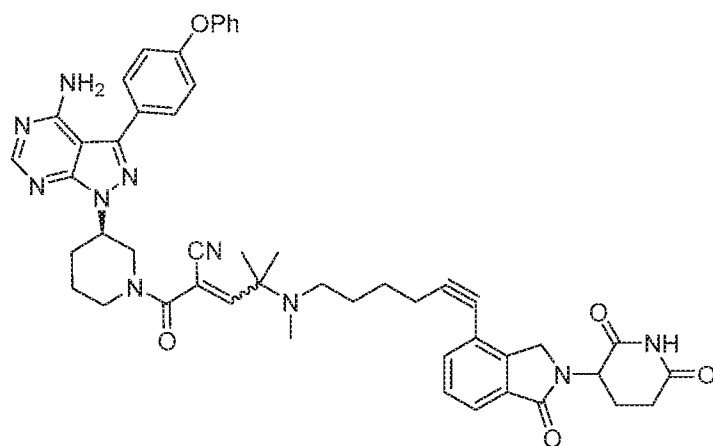
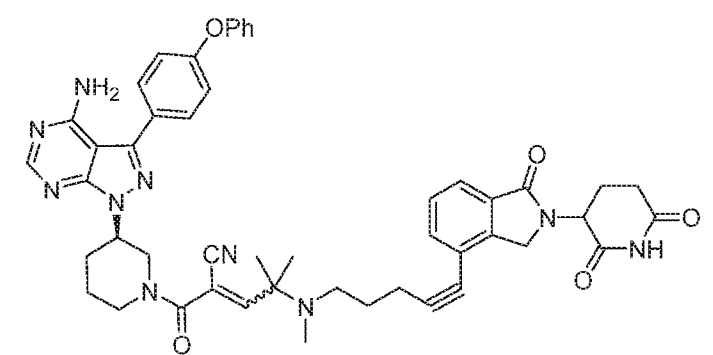
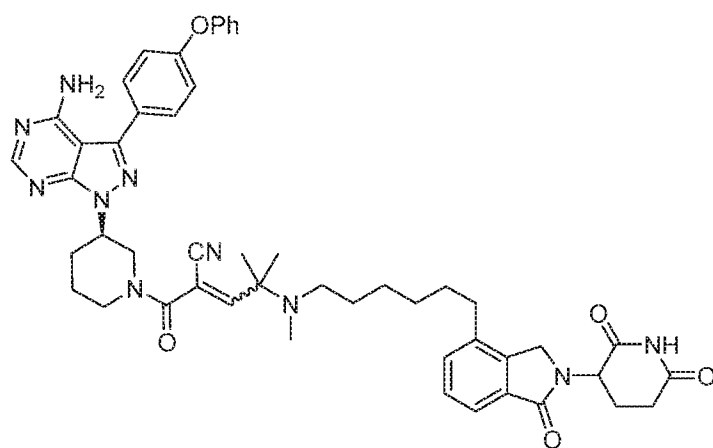
11. The compound of claim 10, wherein the compound is selected from the group consisting of:

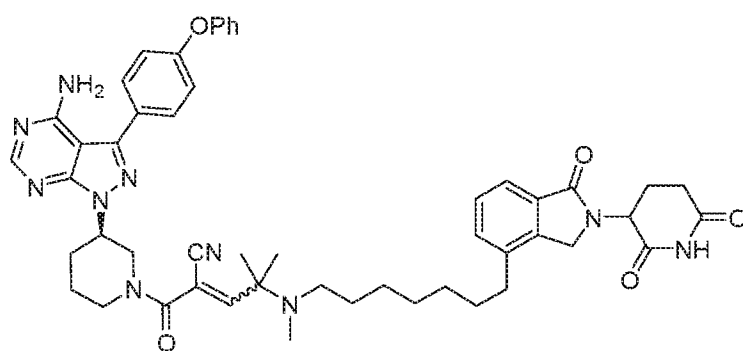
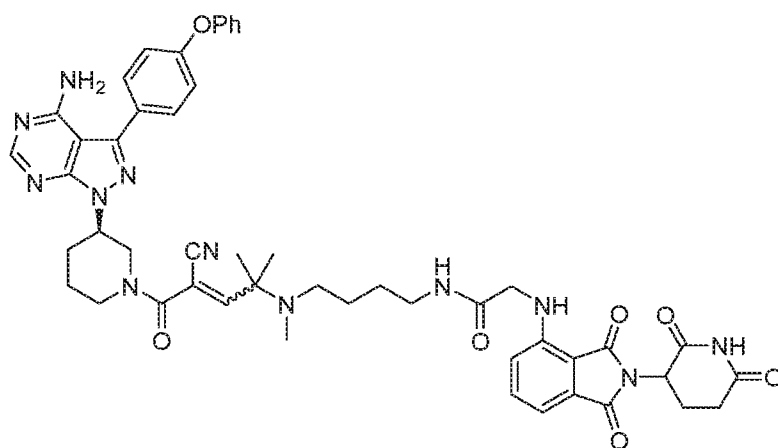
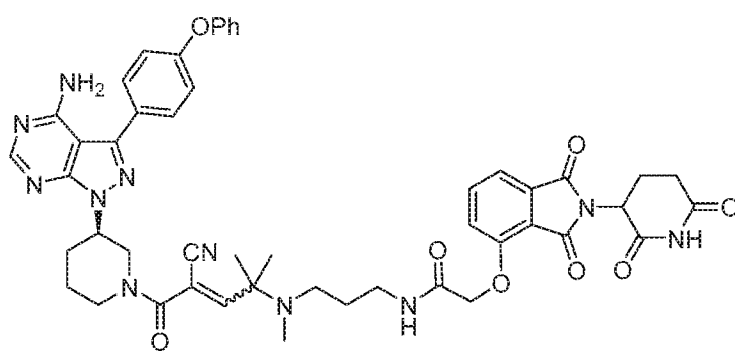


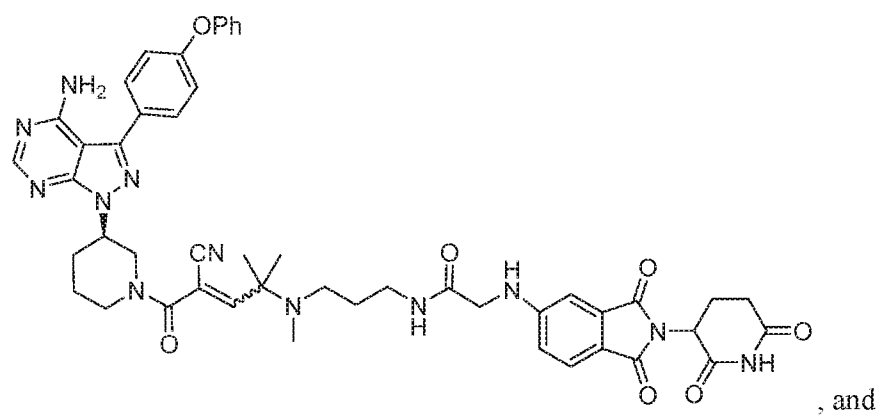
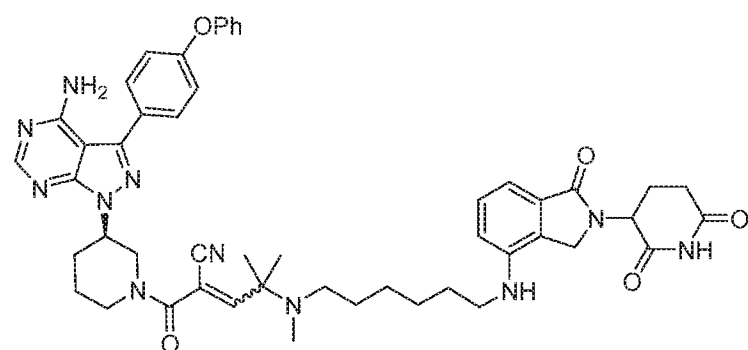
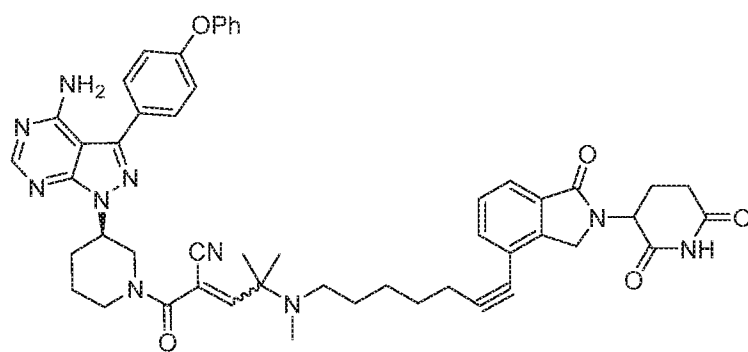


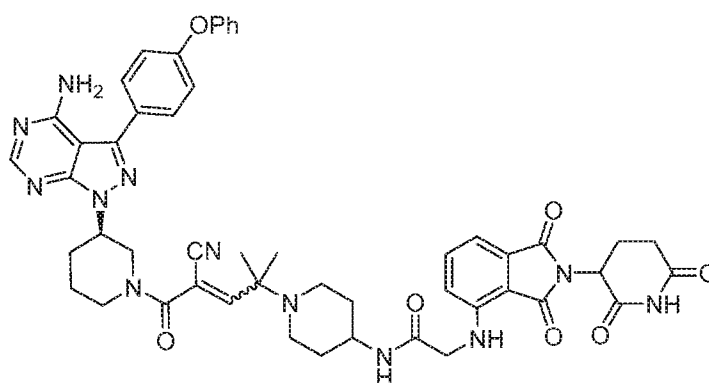




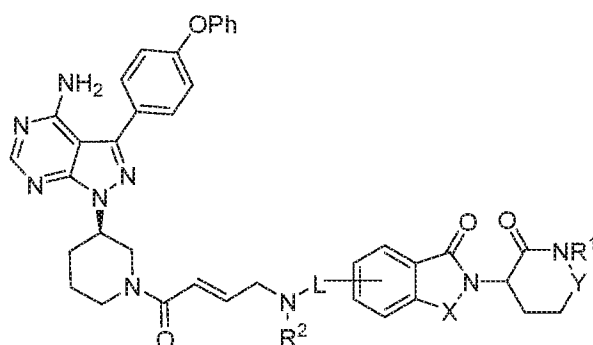




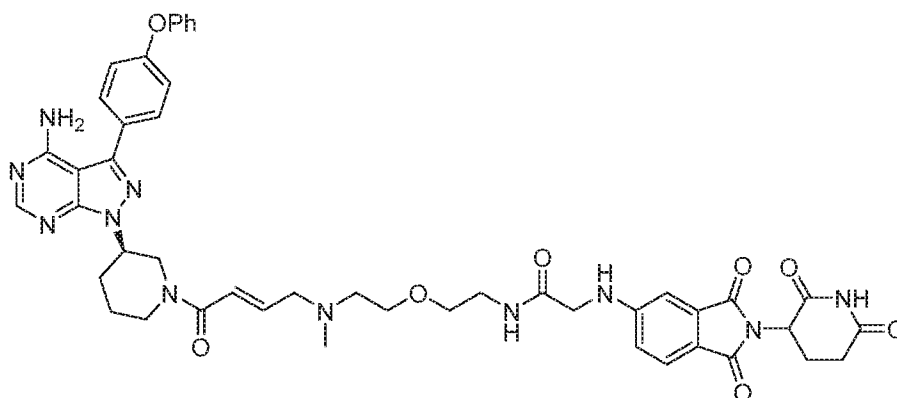


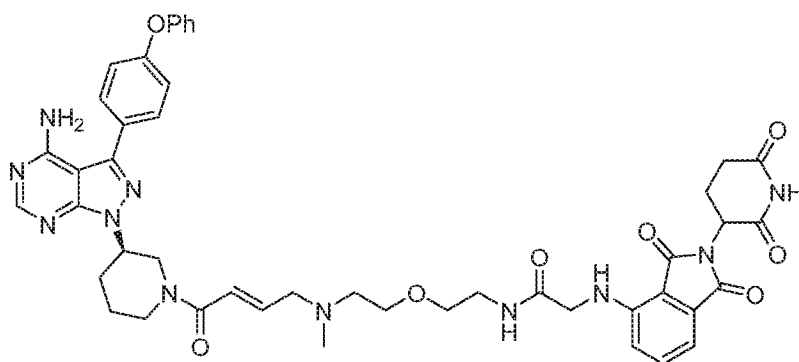
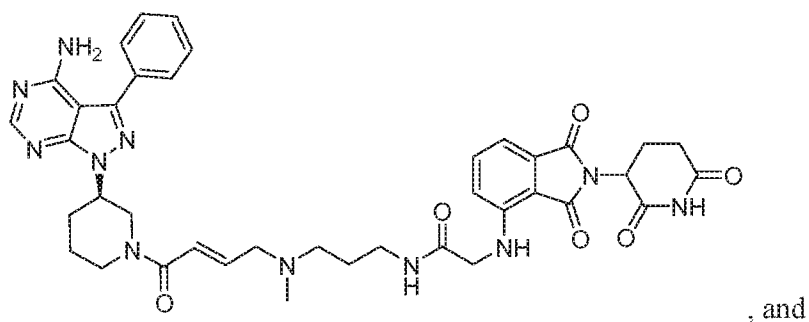


12. The compound of claim 5, wherein the compound has the following formula:

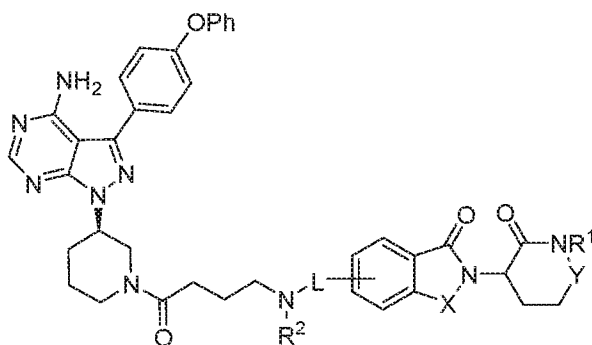


13. The compound of claim 12, wherein the compound is selected from the group consisting of:

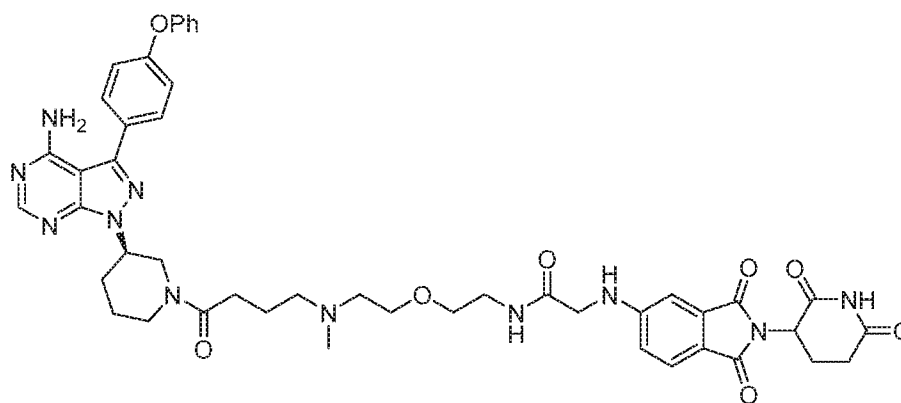


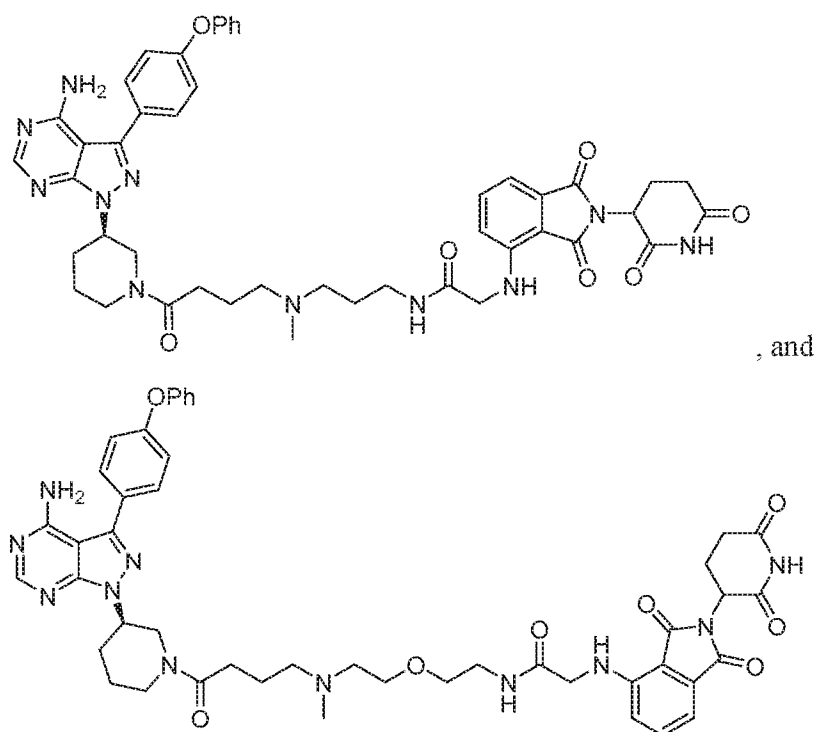


14. The compound of claim 5, wherein the compound has the following formula:

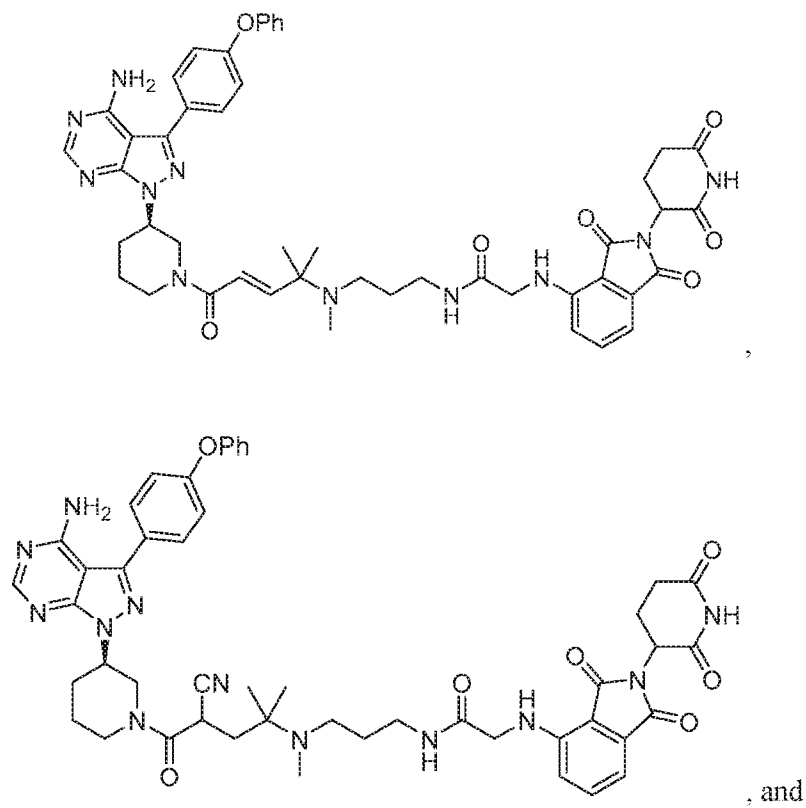


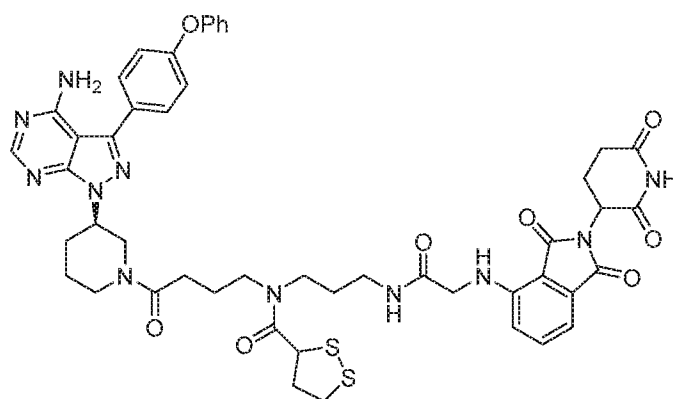
15. The compound of claim 14, wherein the compound is selected from the group consisting of:



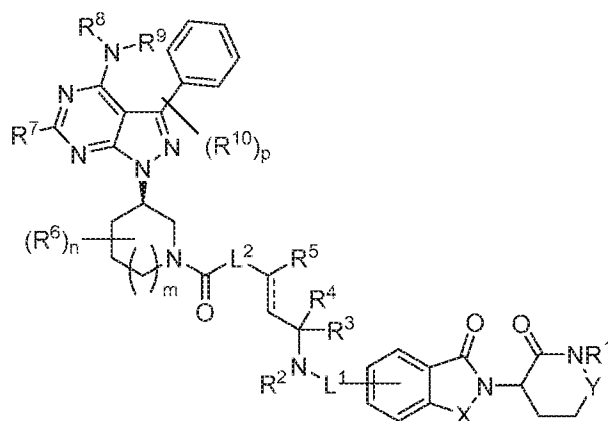


16. The compound of claim 5, wherein the compound is selected from the group consisting of:





17. A compound of the following formula:



or a pharmaceutically acceptable salt or prodrugs thereof, wherein:

=== is a single bond or a double bond;

m is 0-3;

n and p are each independently 0-5;

$L^1$  and  $L^2$  are each independently a linker;

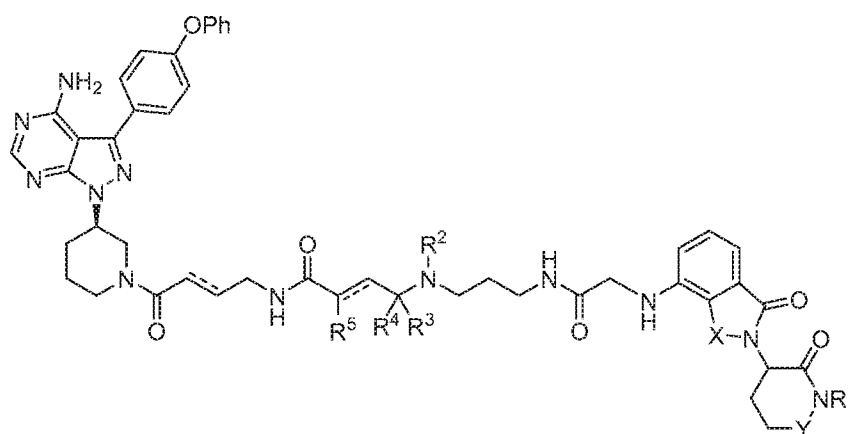
X and Y are each independently selected from CH<sub>2</sub>, CHD, CD<sub>2</sub>, CHF, CF<sub>2</sub>, and C(O):

R<sup>1</sup>, R<sup>2</sup>, R<sup>8</sup>, and R<sup>9</sup> are each independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted carbonyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl; and

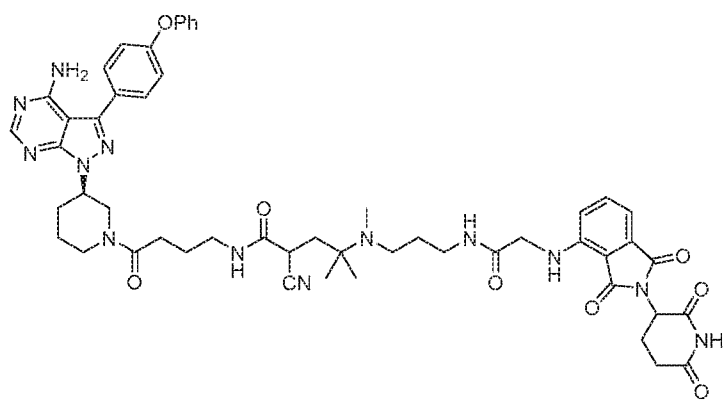
R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, each R<sup>6</sup>, R<sup>7</sup>, and each R<sup>10</sup> are each independently selected from hydrogen, halogen, cyano, trifluoromethyl, alkoxy, aryloxy, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or

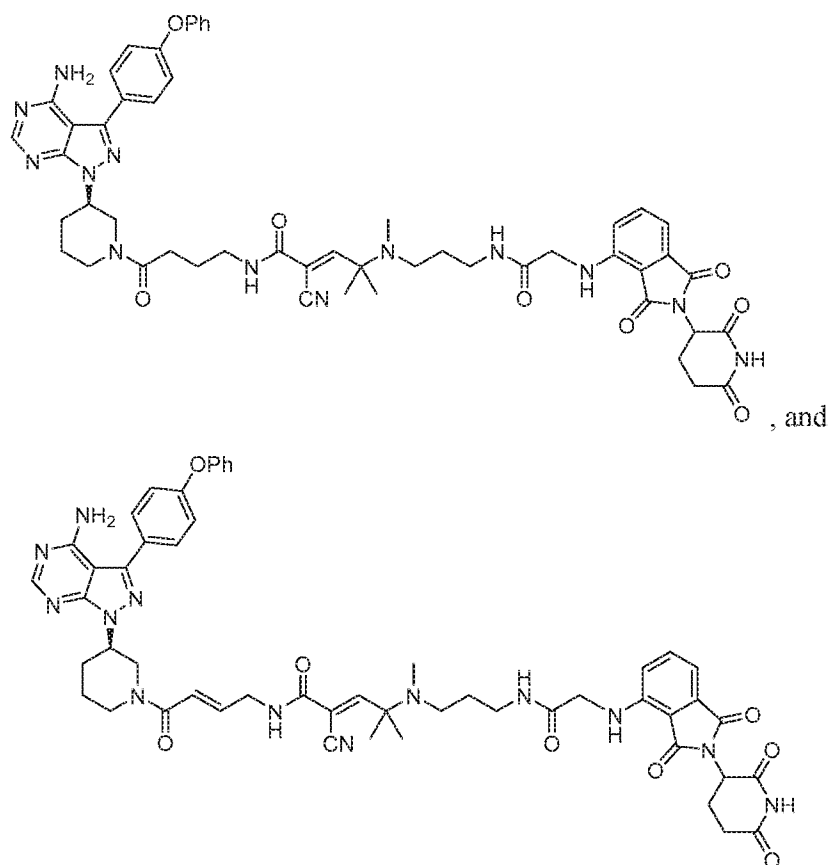
unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocycloalkyl.

18. The compound of claim 17, wherein the compound has the following formula:



19. The compound of claim 18, wherein the compound is selected from the group consisting of:





20. A composition comprising a compound of any one of claims 1-19 and a pharmaceutically acceptable carrier.
21. A kit comprising a compound of any one of claims 1-19 or a composition of claim 20.
22. A method of treating or preventing a BTK-related disease in a subject, comprising:  
administering to the subject an effective amount of a compound or composition of any of claims 1-20.
23. The method of claim 22, wherein the BTK-related disease is cancer.
24. The method of claim 23, wherein the cancer is bladder cancer, blood cancer, a bone marrow cancer, brain cancer, breast cancer, bronchus cancer, colorectal cancer, cervical cancer, chondrosarcoma, endometrial cancer, gastrointestinal cancer, gastric cancer, genitourinary cancer, head and neck cancer, hepatic cancer, hepatocellular carcinoma, leukemia, liver cancer, lung cancer, lymphoma, melanoma of the skin, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, skin cancer, testicular cancer, thyroid cancer, or uterine cancer.

25. The method of claim 22, wherein the BTK-related disease is a neurodegenerative disorder.
26. The method of claim 22, wherein the BTK-related disease is an inflammatory disease.
27. The method of any one of claims 22-26, further comprising administering a second compound, biomolecule, or composition.
28. The method of claim 27, wherein the second compound, biomolecule, or composition comprises a chemotherapeutic agent.
29. A method of inducing BTK degradation in a cell, comprising:  
contacting a cell with an effective amount of a compound of any one of claims 1-19.
30. The method of claim 29, wherein the contacting is performed *in vitro* or *in vivo*.

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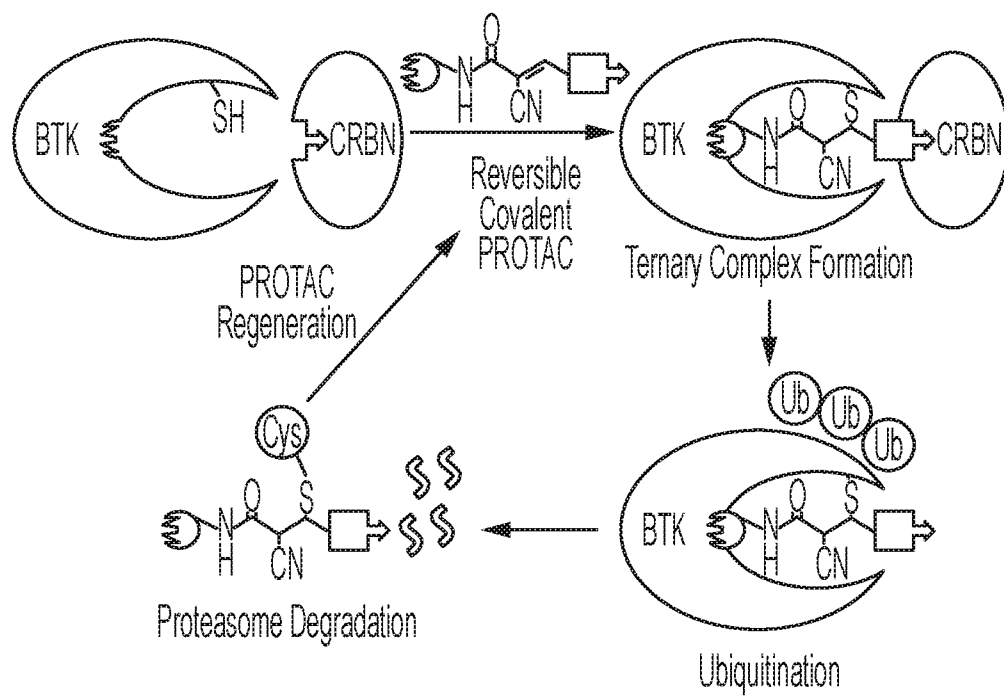


FIG. 1

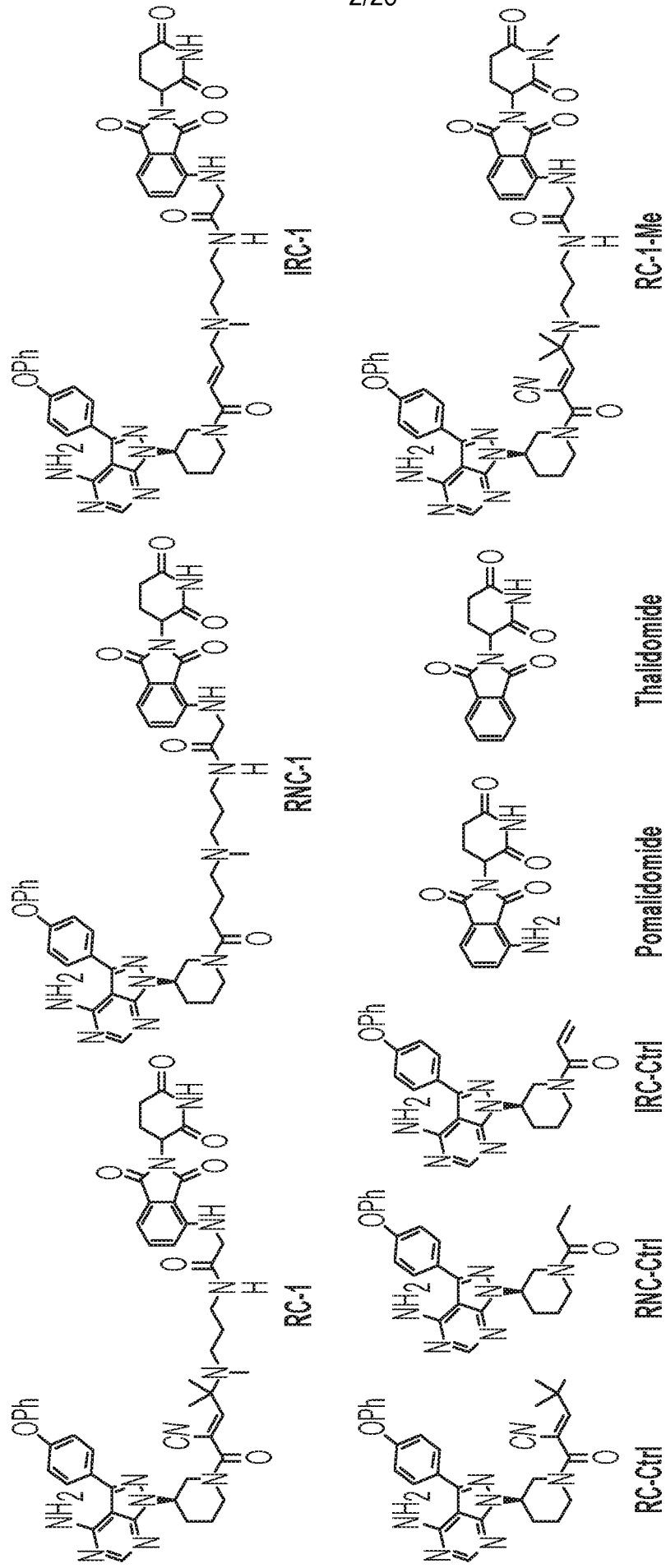


FIG. 2A

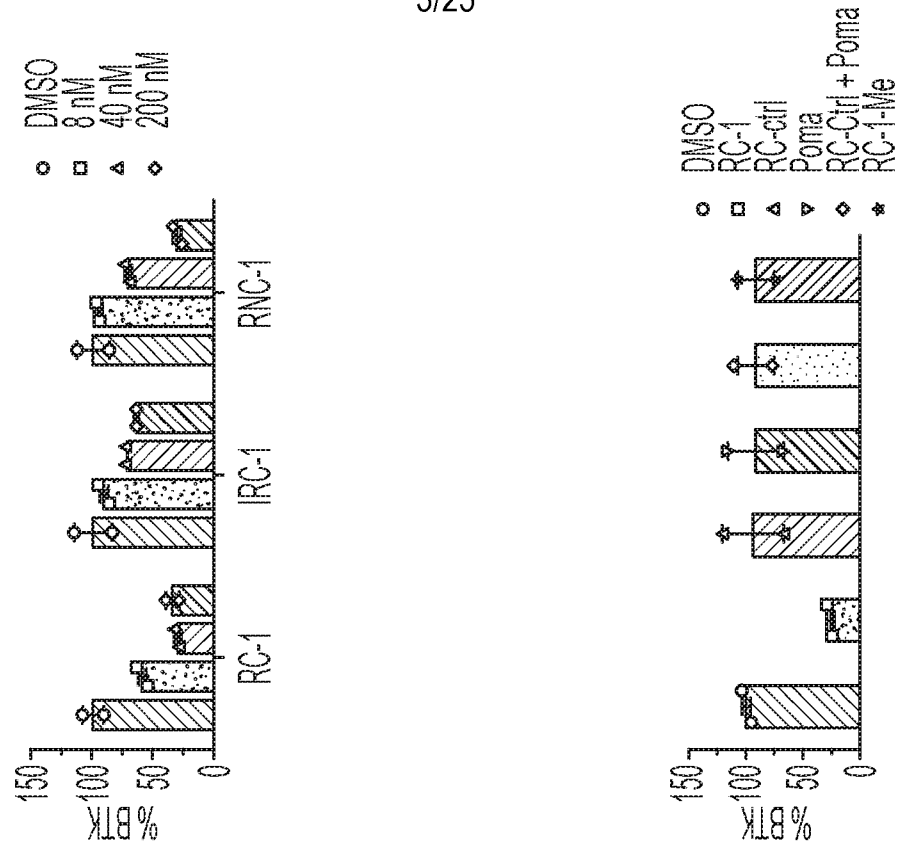


FIG. 2B

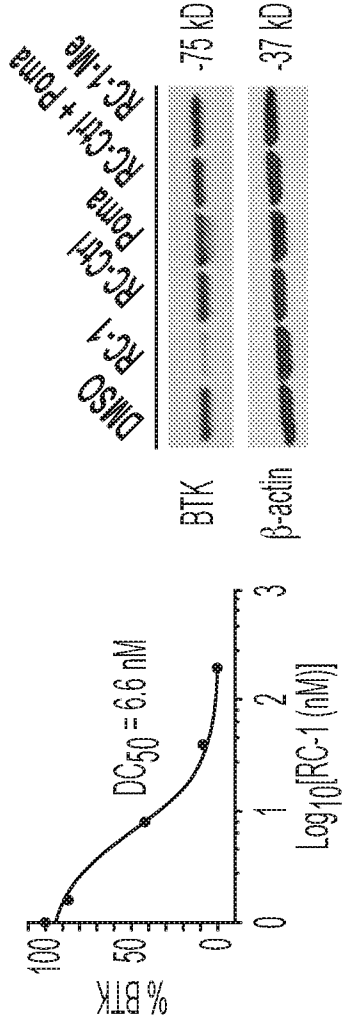
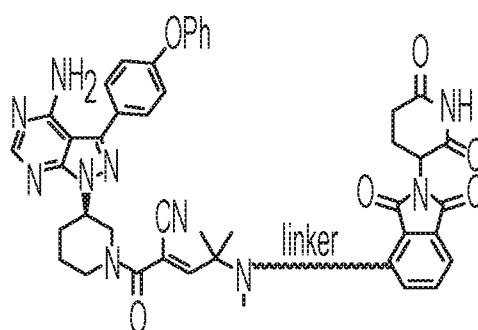


FIG. 2C

FIG. 2D

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| Compound | Linker | Degradation<br>at 200 nM |
|----------|--------|--------------------------|
| RC-1     |        | 81%                      |
| RC-2     |        | 49%                      |
| RC-3     |        | 39%                      |
| RC-4     |        | 43%                      |
| RC-5     |        | 56%                      |
| RC-6     |        | --                       |
| RC-7     |        | 6%                       |
| RC-8     |        | 15%                      |
| RC-9     |        | 30%                      |

FIG. 3

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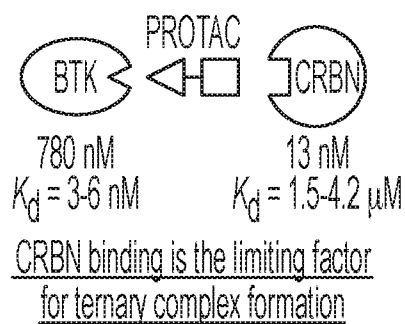


FIG. 4A

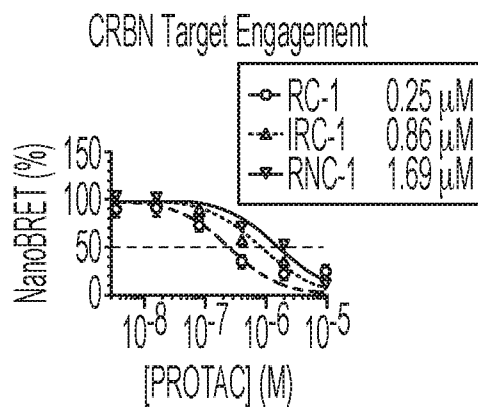


FIG. 4B

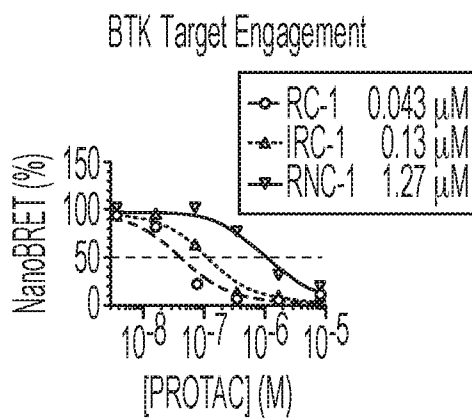


FIG. 4C

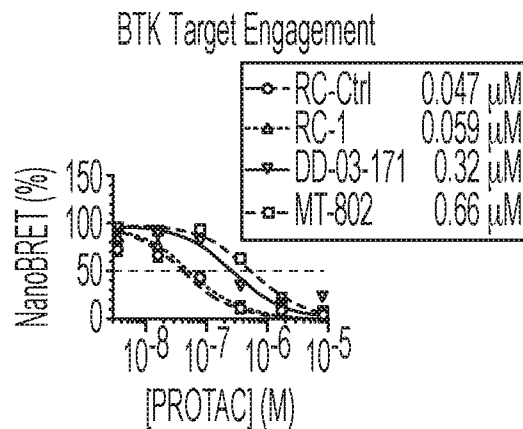


FIG. 4D

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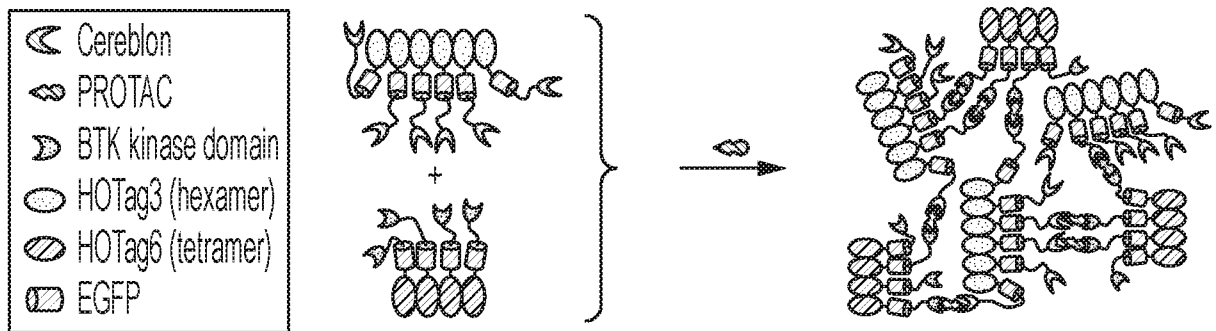


FIG. 5A

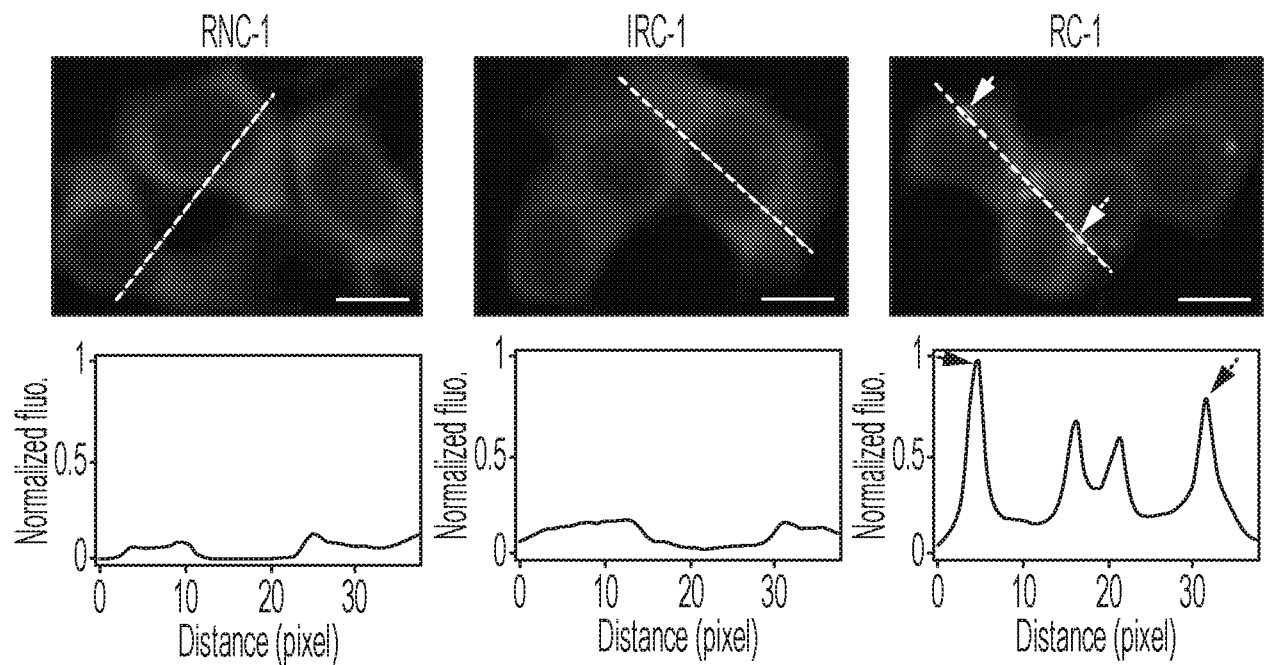


FIG. 5B

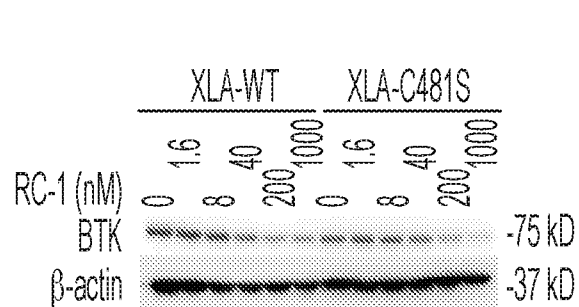


FIG. 6A

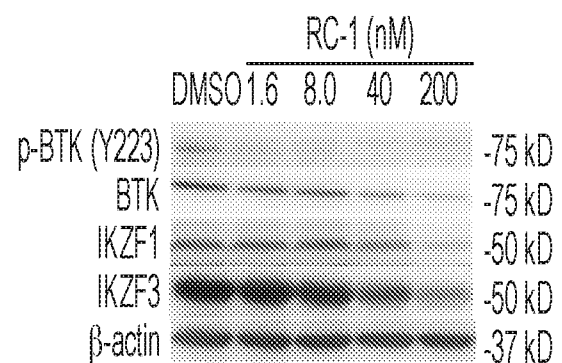


FIG. 6B

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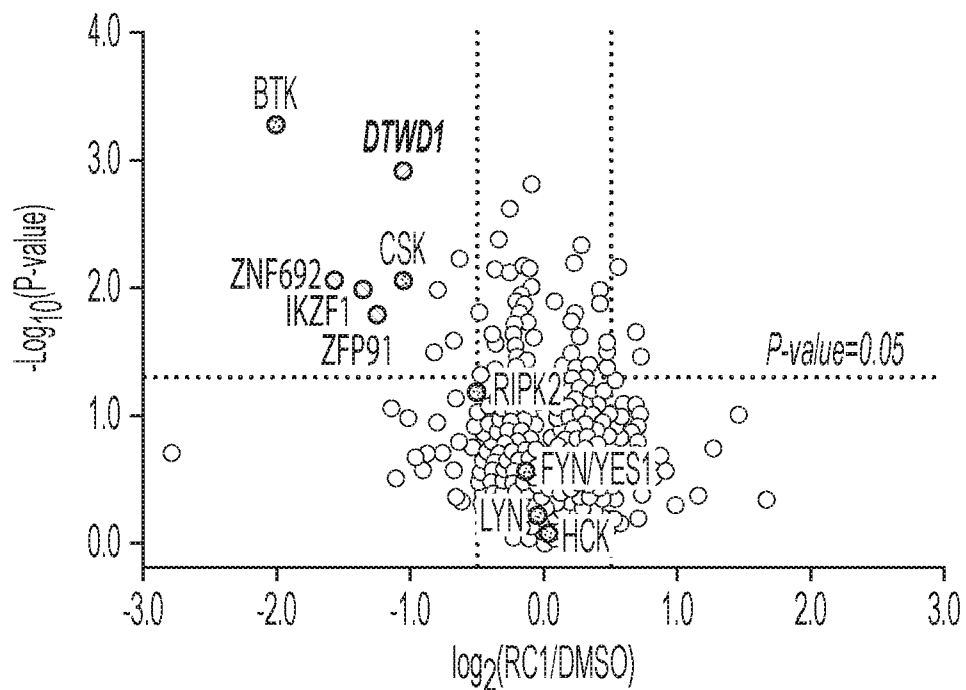


FIG. 7A

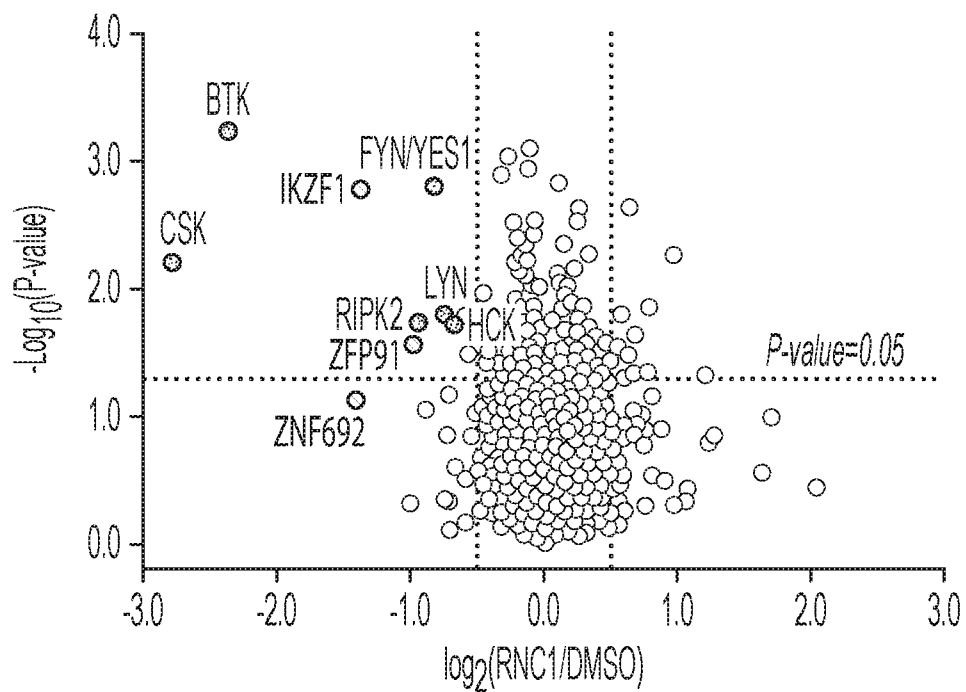


FIG. 7B

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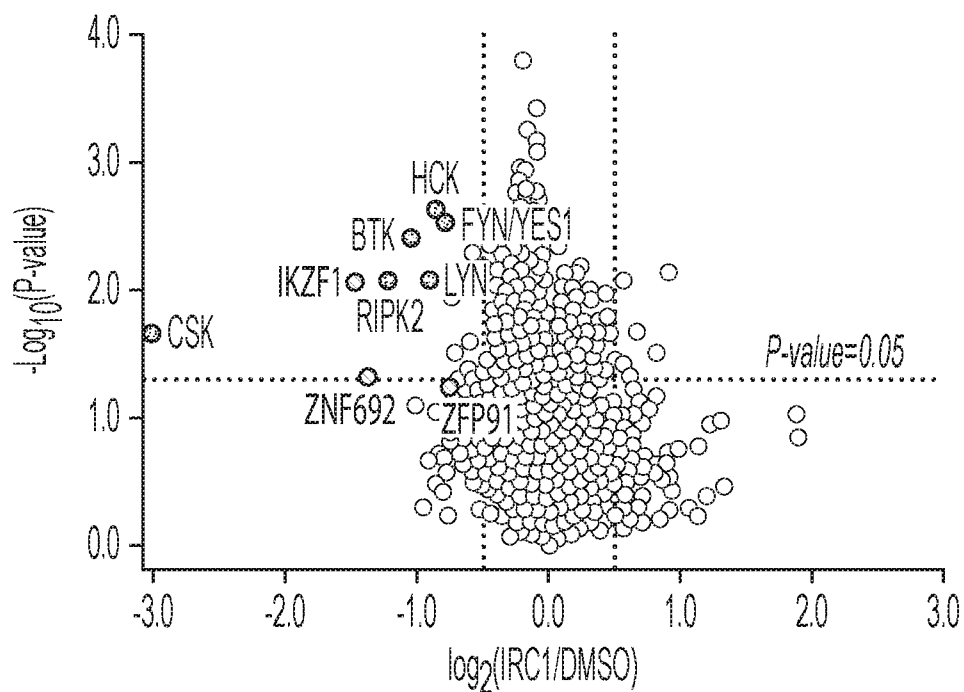


FIG. 7C

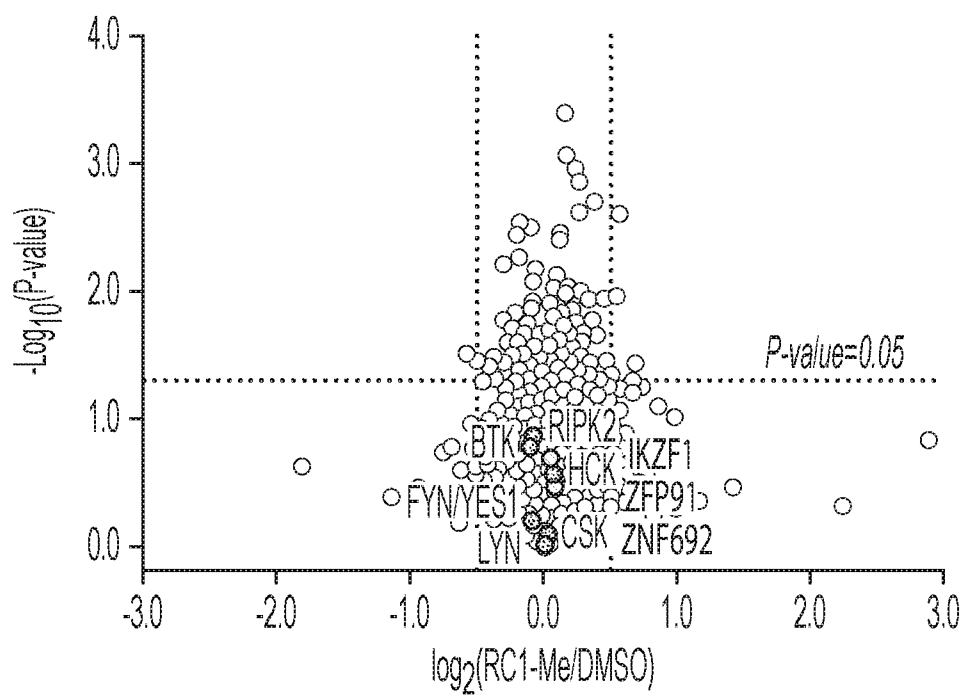


FIG. 7D

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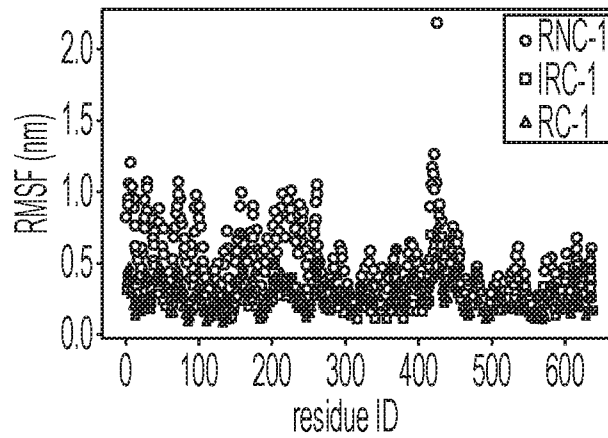


FIG. 8A

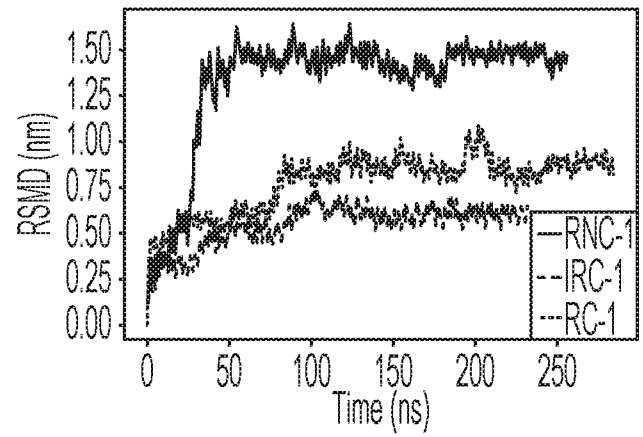


FIG. 8B

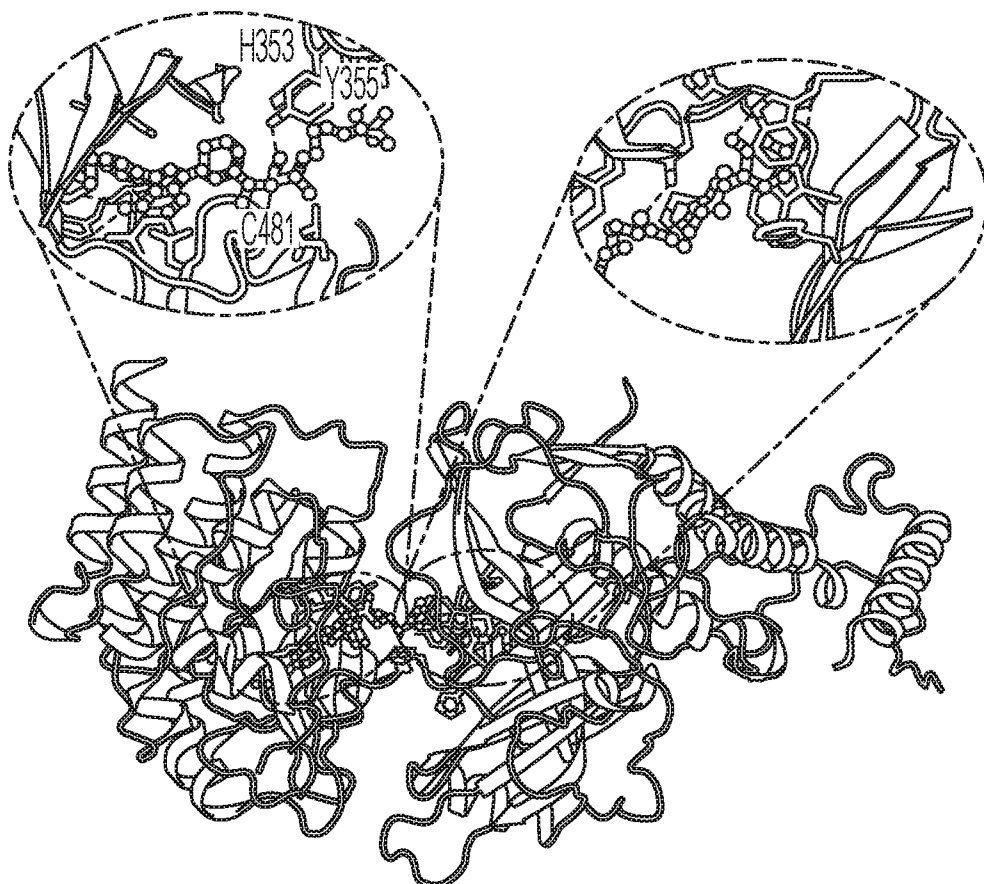


FIG. 8C

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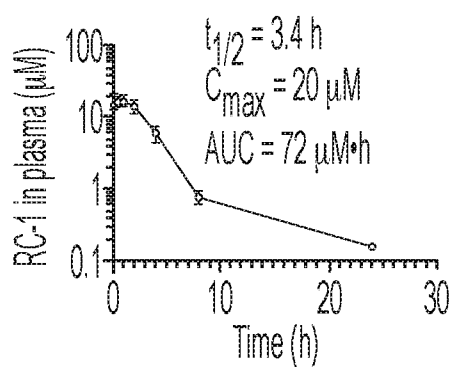
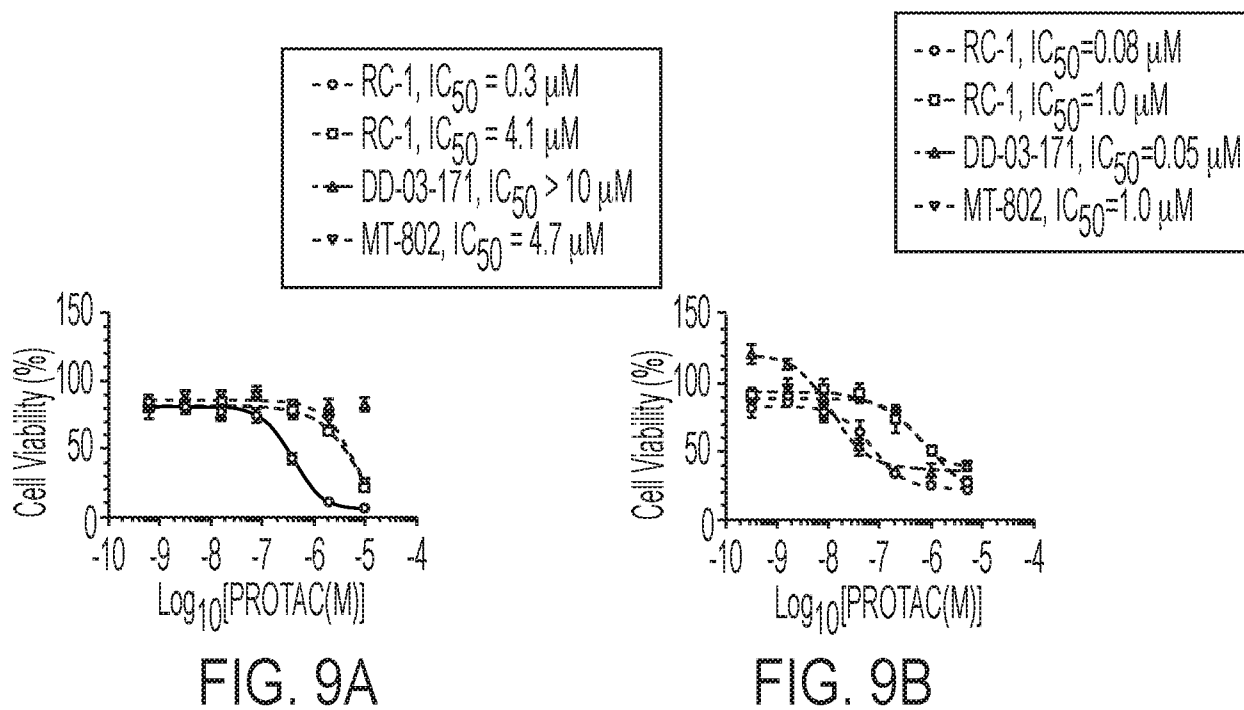


FIG. 10A

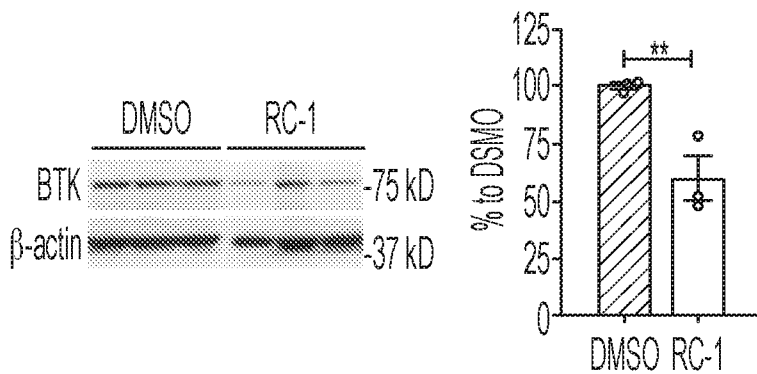


FIG. 10B

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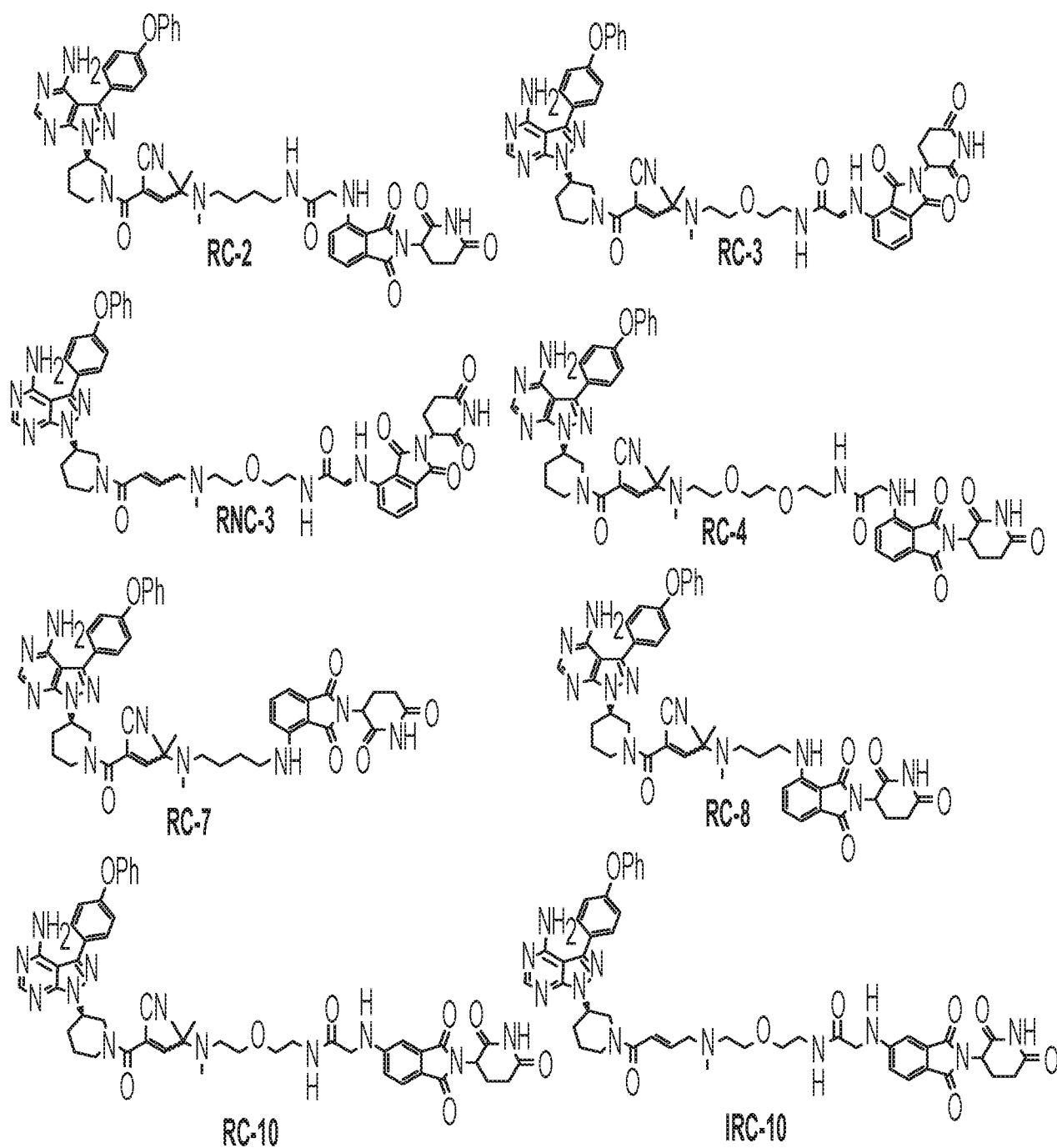


FIG. 11A

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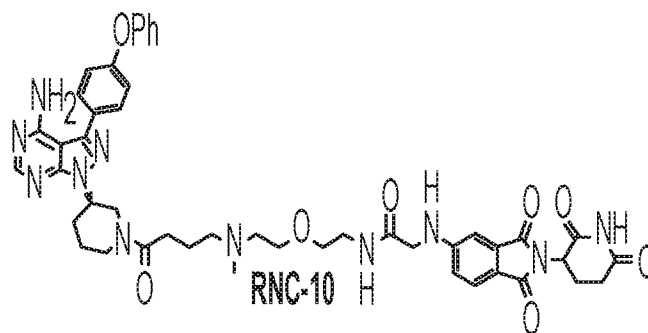
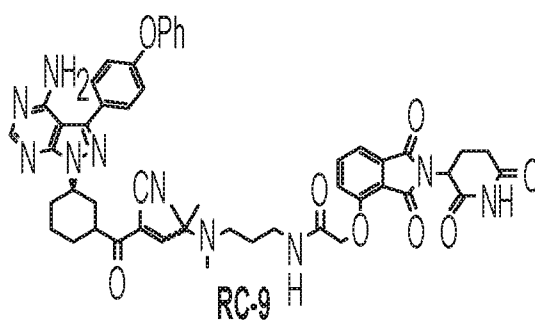
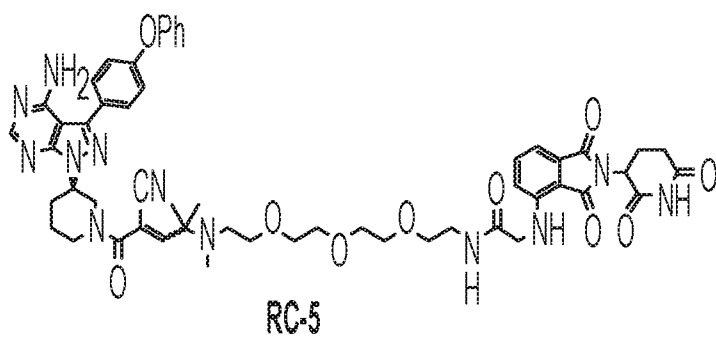
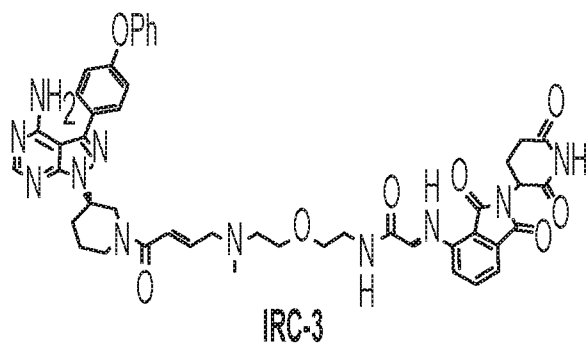


FIG. 11A  
CONTINUED

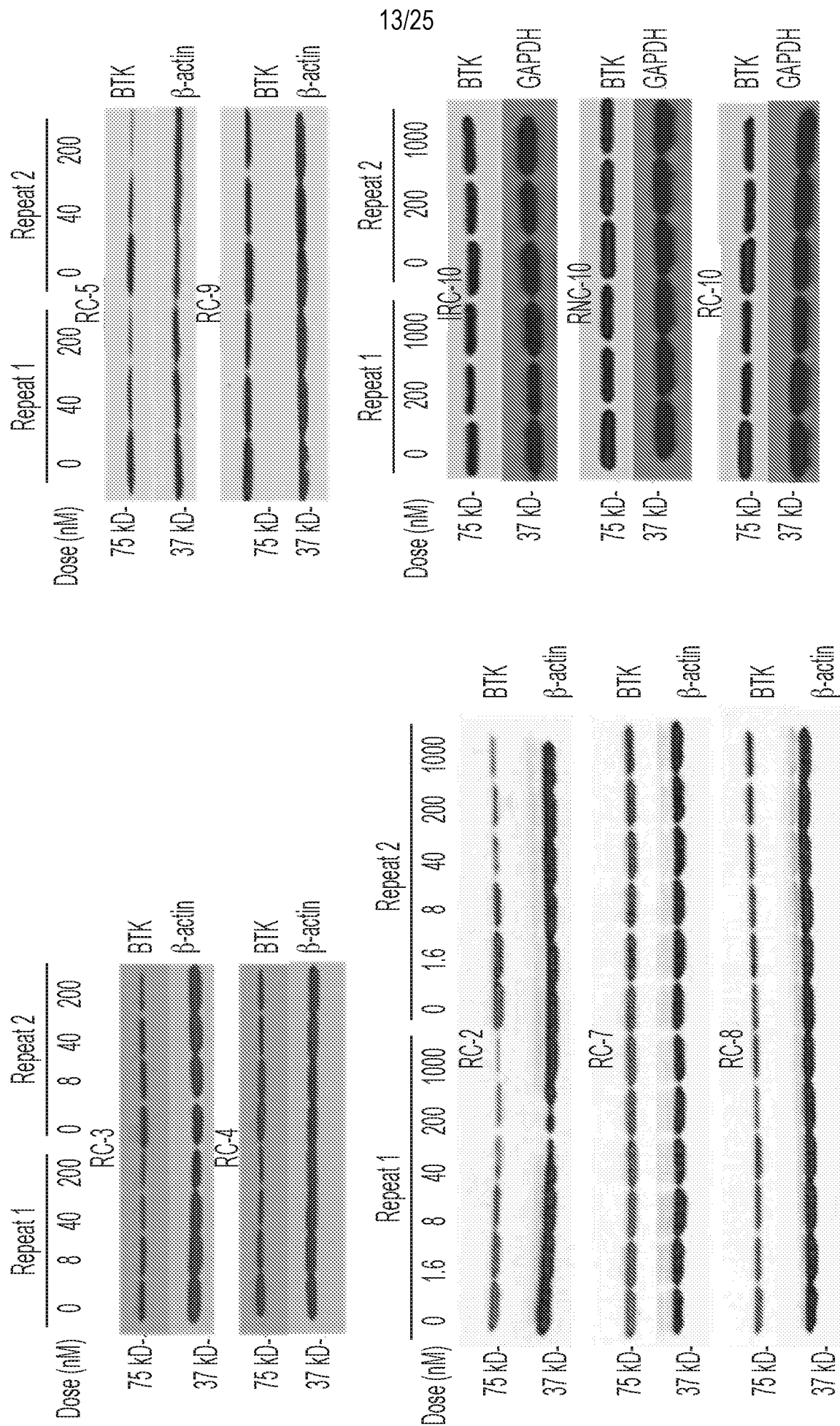


FIG. 11B

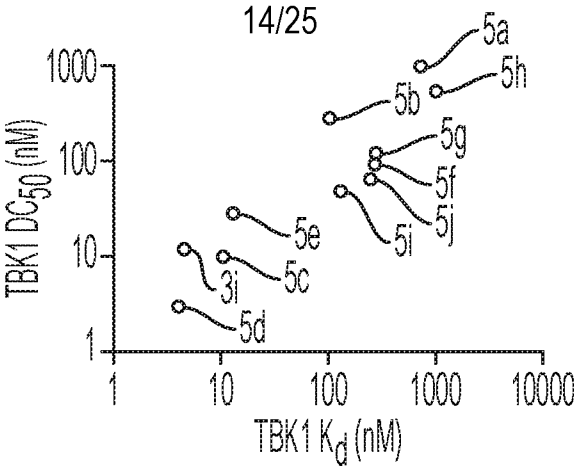


FIG. 12

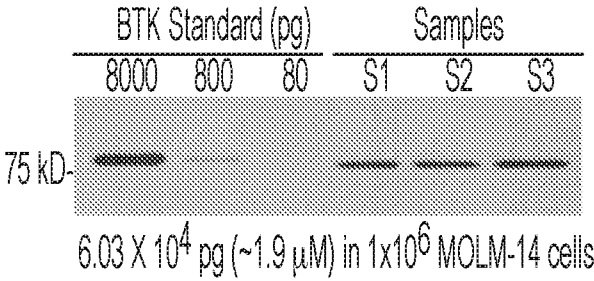


FIG. 13A

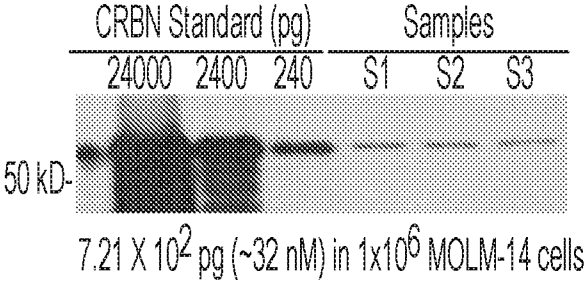


FIG. 13B

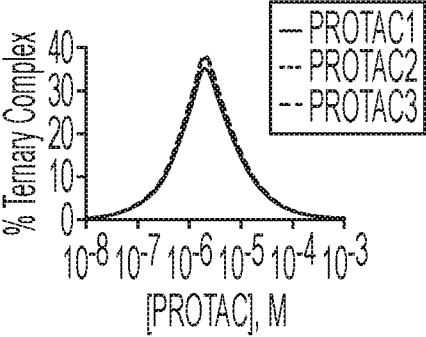


FIG. 14A

| Input Parameters    |          |          |          | Output Exact Values                            |          |          |          |
|---------------------|----------|----------|----------|------------------------------------------------|----------|----------|----------|
|                     | PROTAC1  | PROTAC2  | PROTAC3  |                                                | PROTAC1  | PROTAC2  | PROTAC3  |
| [A] <sub>t</sub> /M | 1.90E-06 | 1.90E-06 | 1.90E-06 | [B] <sub>t,max</sub> /M                        | 2.00E-06 | 1.93E-06 | 1.91E-06 |
| k <sub>ab</sub> /M  | 2.00E-08 | 2.00E-09 | 2.00E-10 | [ABC] <sub>max,noncoop</sub>                   | 1.12E-08 | 1.20E-08 | 1.22E-08 |
| [C] <sub>t</sub> /M | 3.20E-08 | 3.20E-08 | 3.20E-08 | [ABC] <sub>max,noncoop</sub> /[L] <sub>t</sub> | 34.99%   | 37.42%   | 38.24%   |
| k <sub>bc</sub> /M  | 3.00E-06 | 3.00E-06 | 3.00E-06 | TF <sub>50(exact)</sub> /M                     | 6.54E-07 | 6.98E-07 | 7.15E-07 |
| α                   | 1.0      | 1.0      | 1.0      | Tl <sub>50(exact)</sub> /M                     | 7.82E-06 | 7.14E-06 | 6.93E-06 |

FIG. 14B

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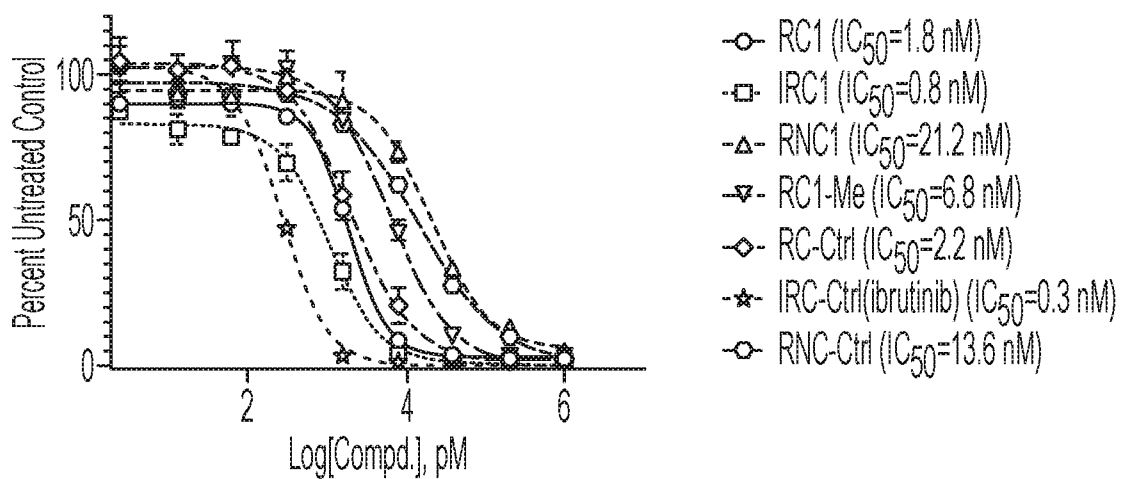


FIG. 15

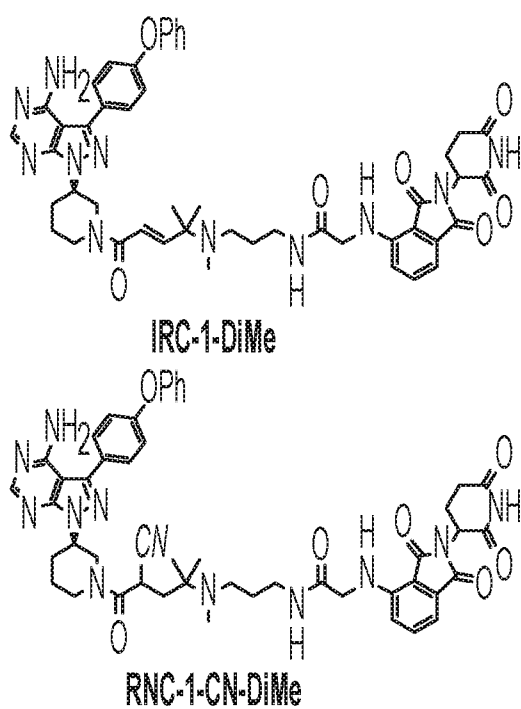


FIG. 16A

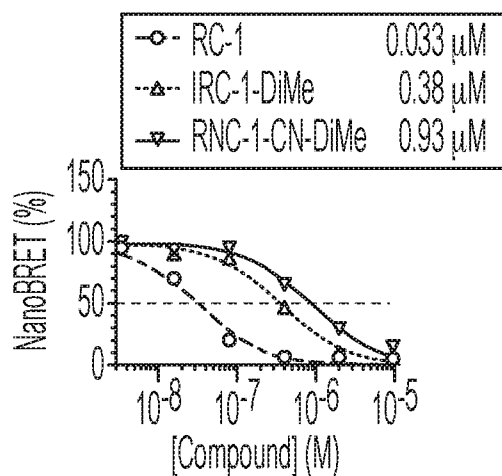


FIG. 16B

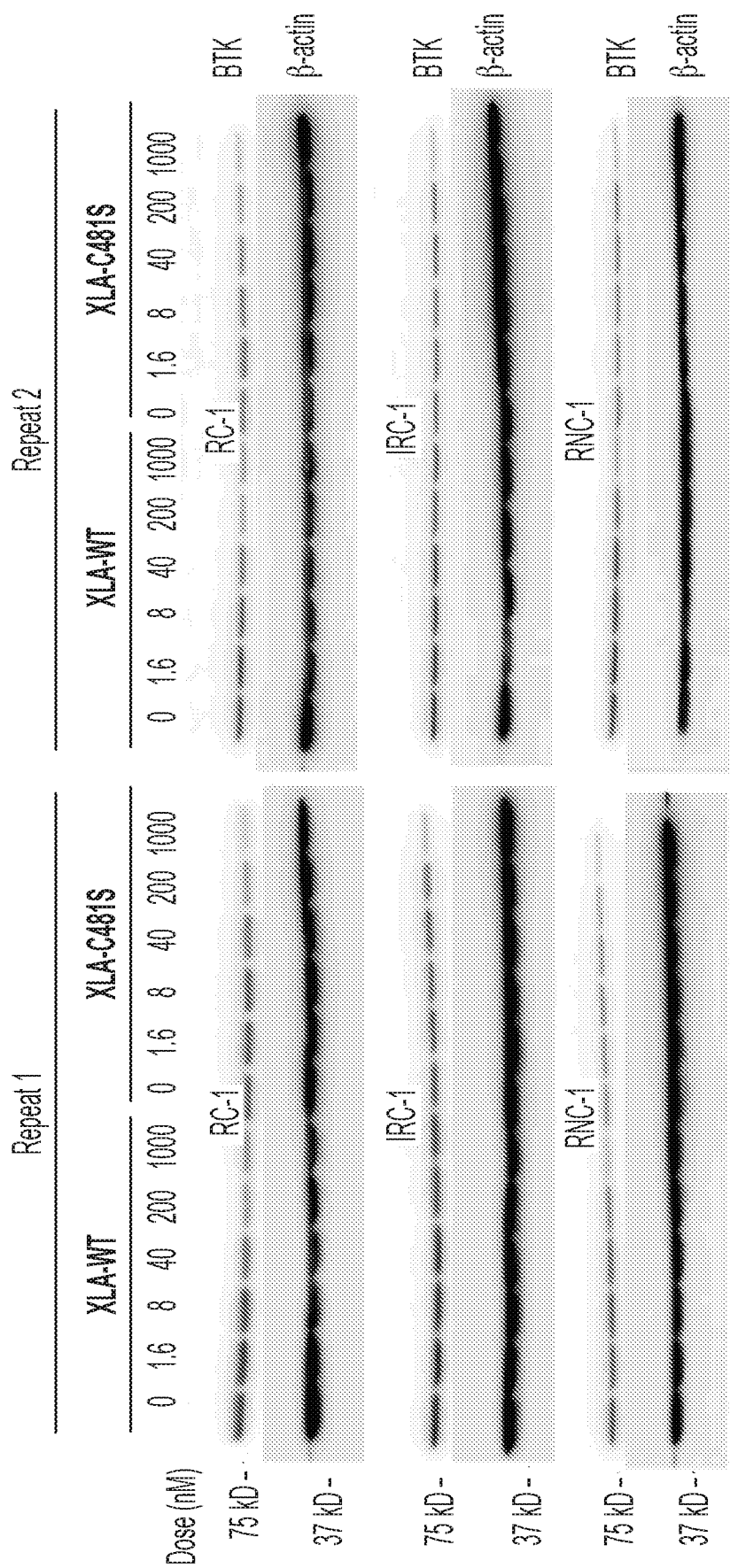


FIG. 17

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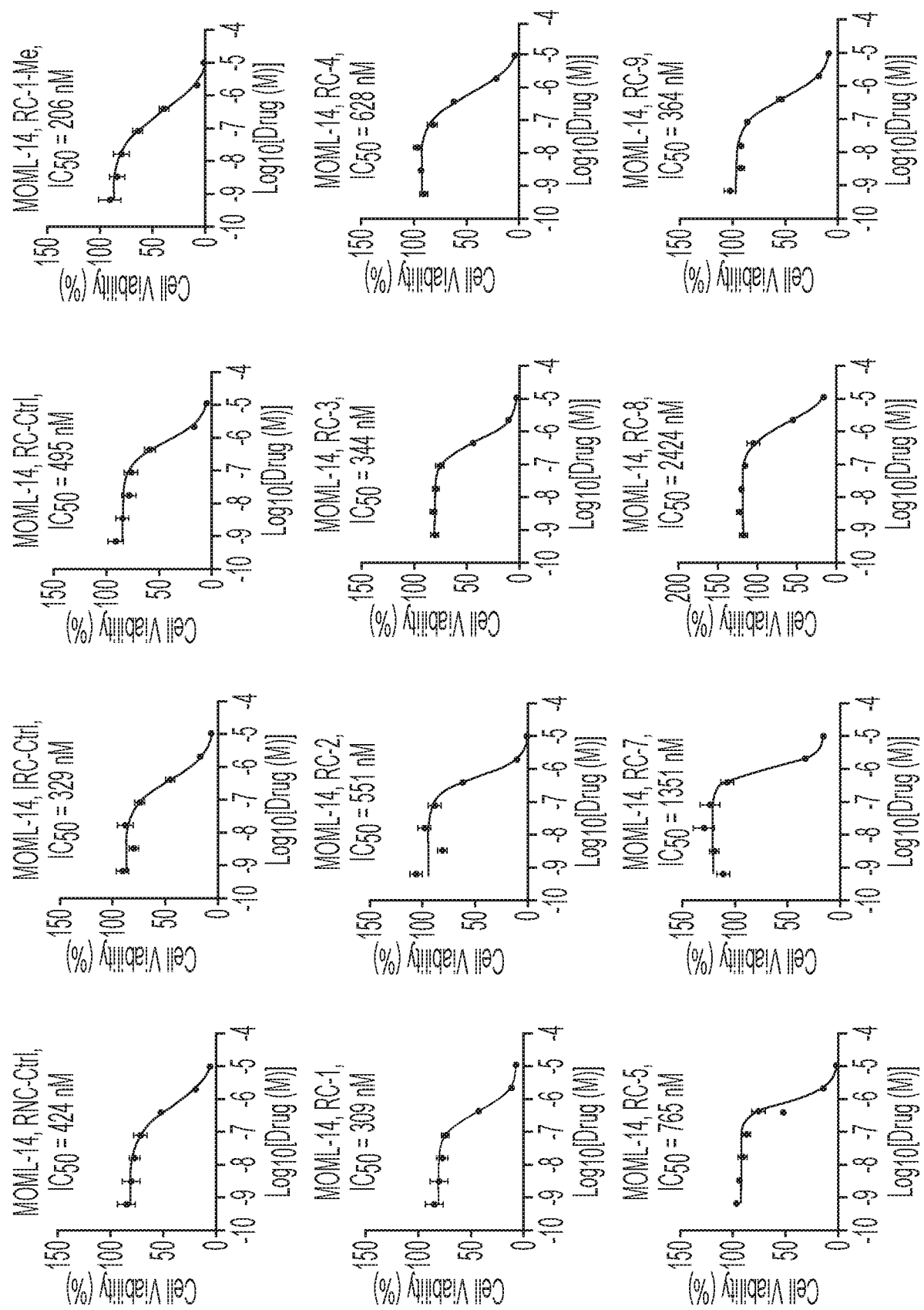


FIG. 18A

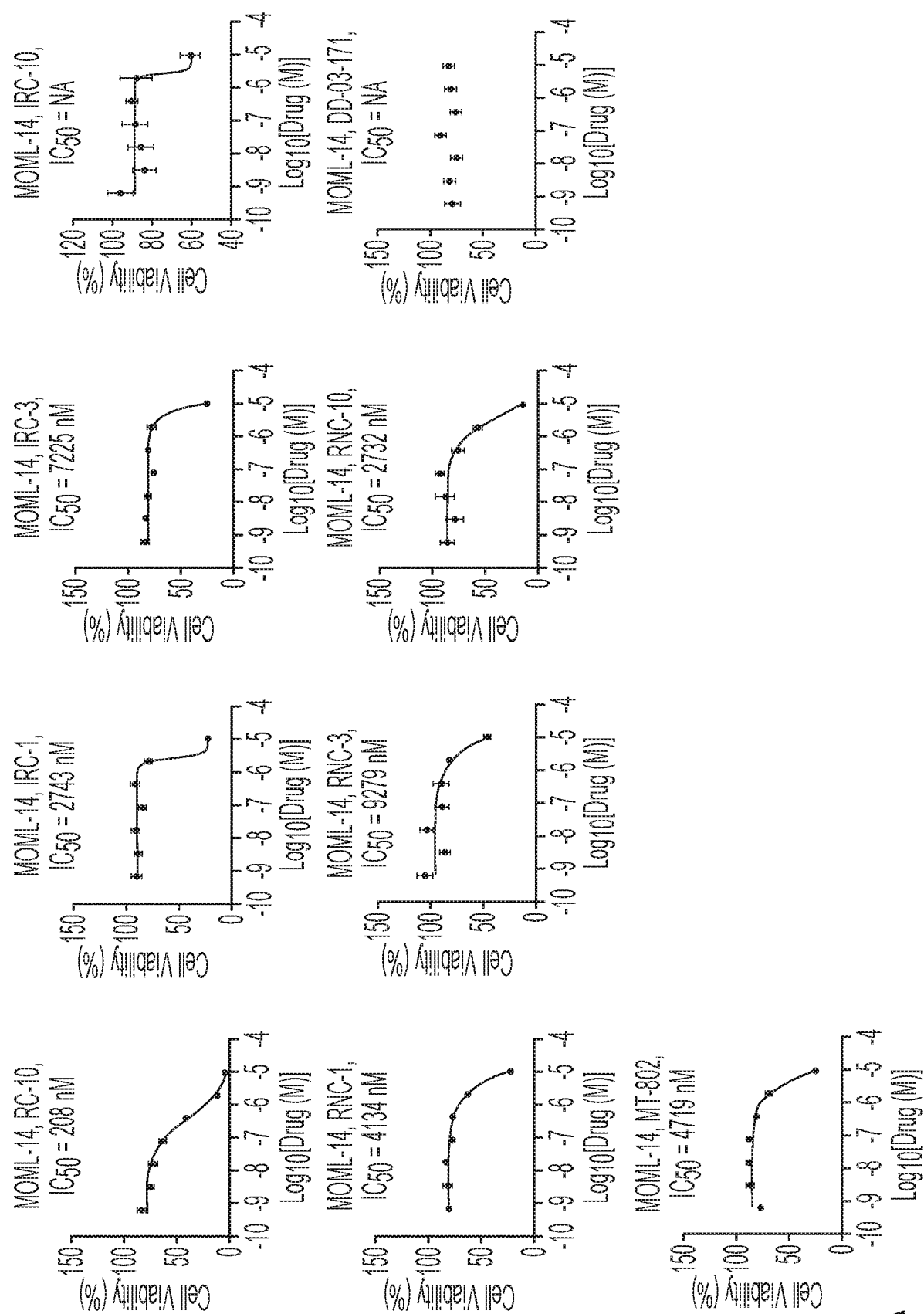


FIG. 18A  
CONTINUED

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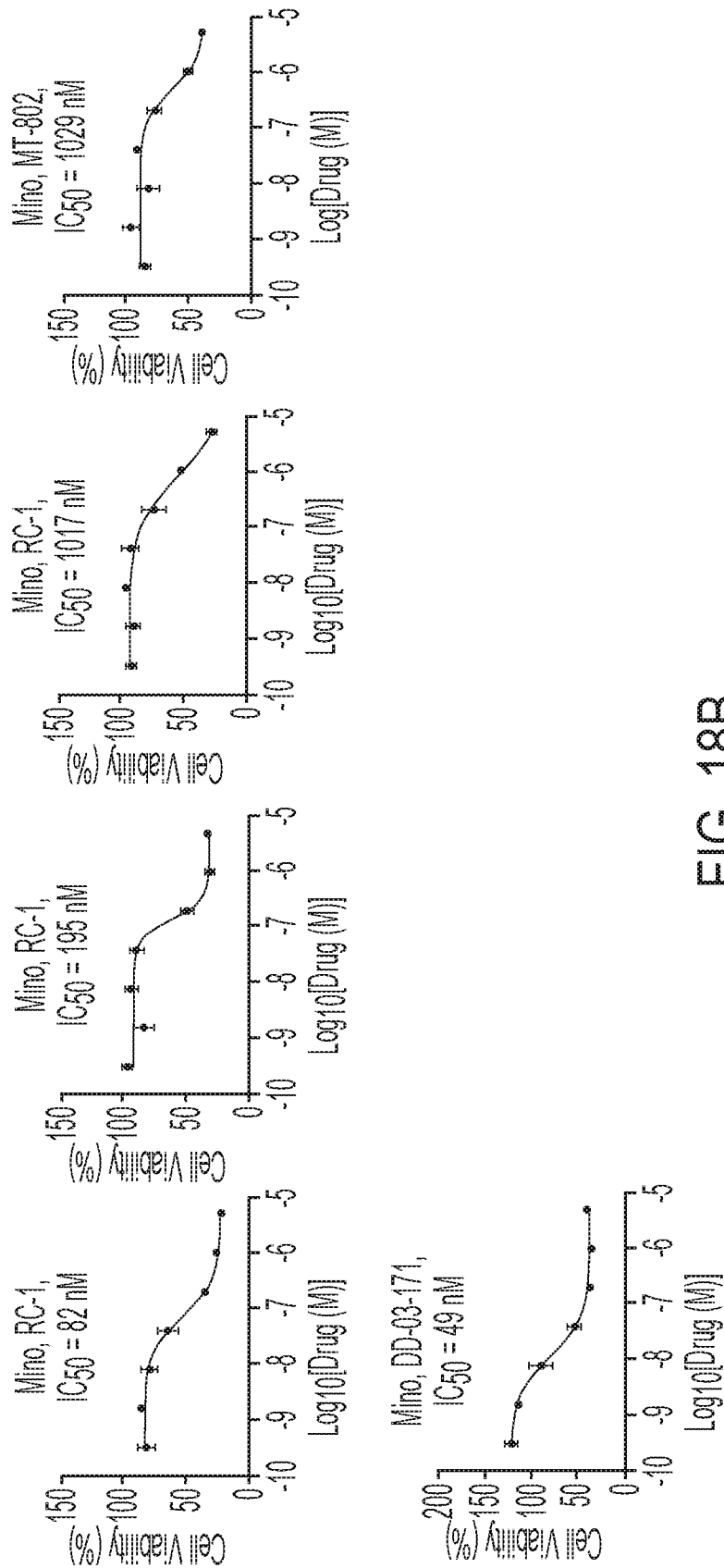


FIG. 18B

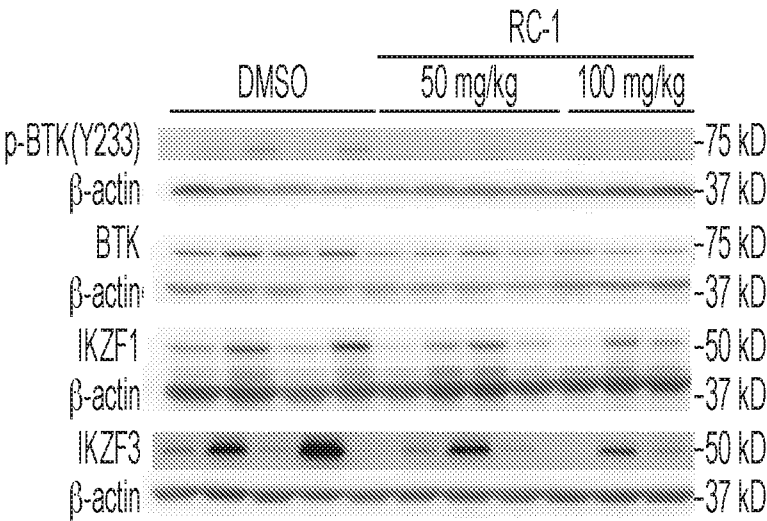


FIG. 19A

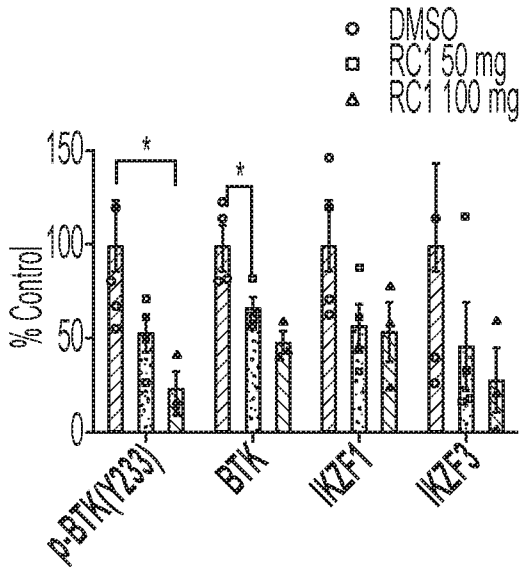


FIG. 19B

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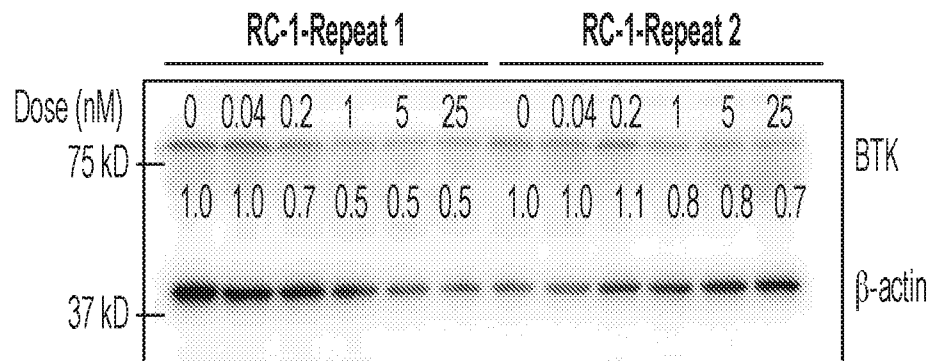


FIG. 20

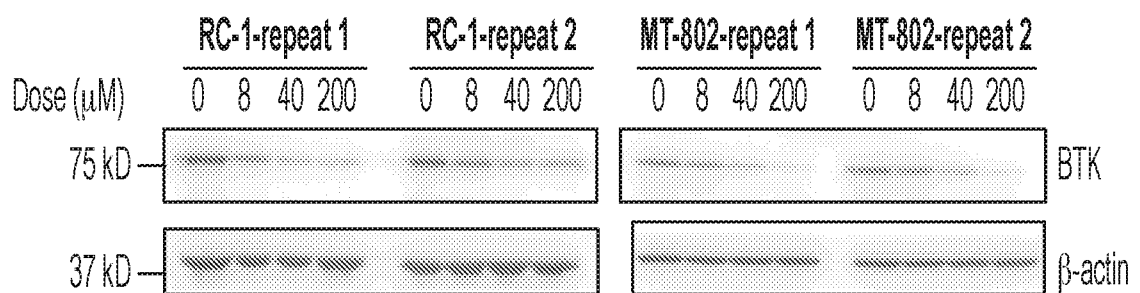


FIG. 21A

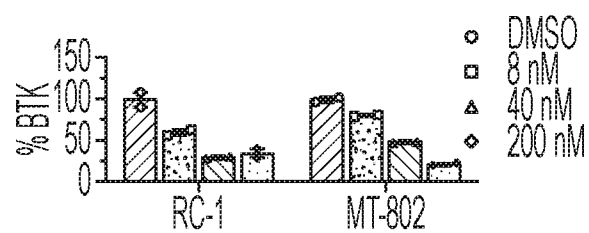


FIG. 21B

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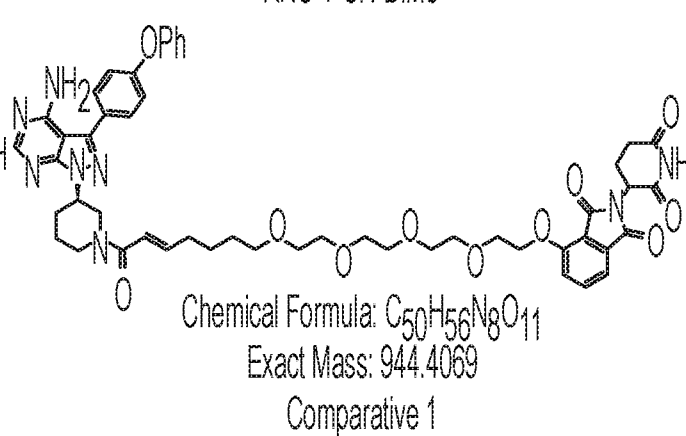
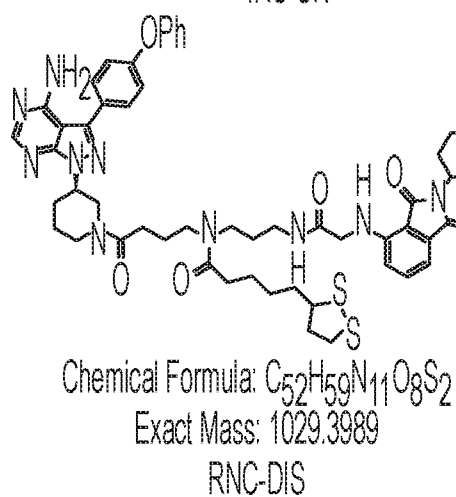
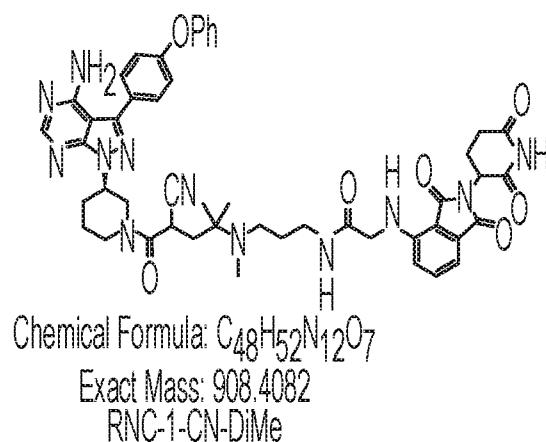
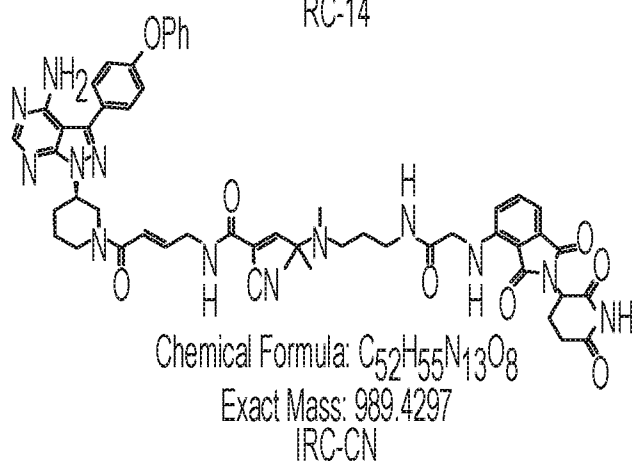
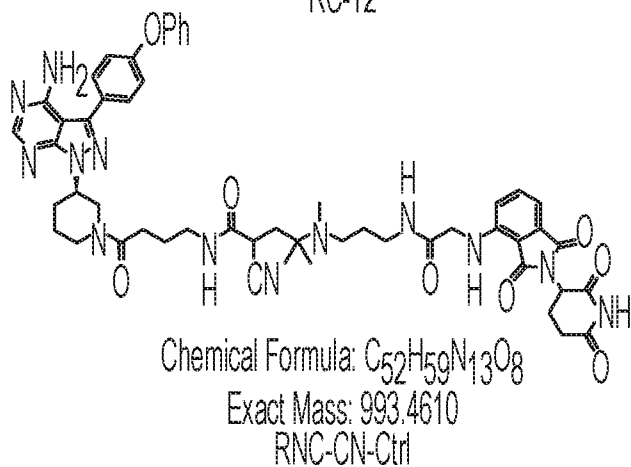
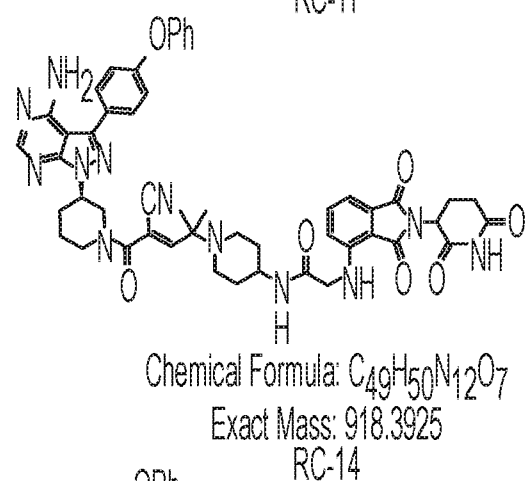
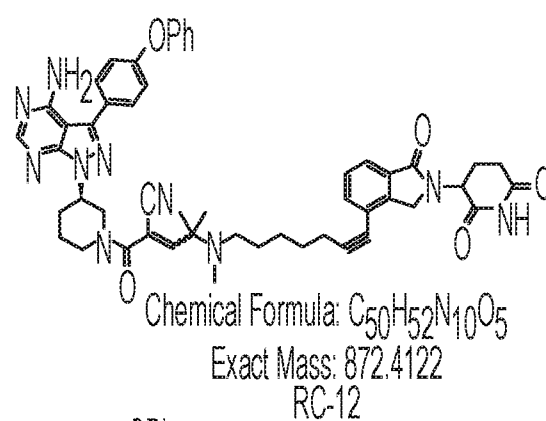
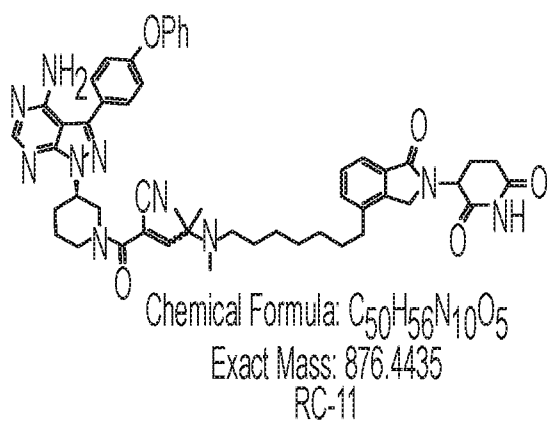


FIG. 22

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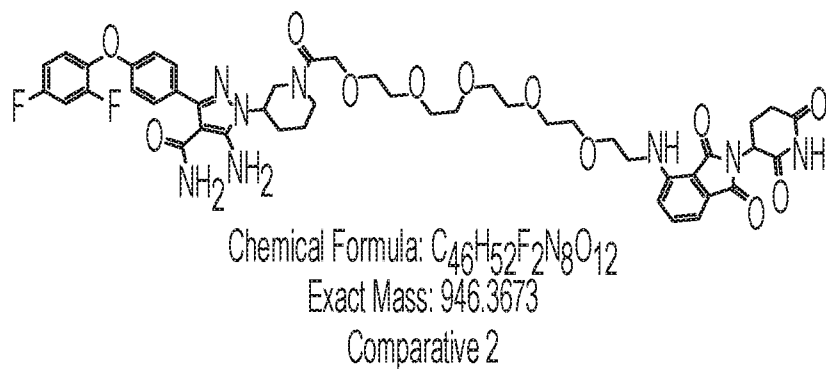
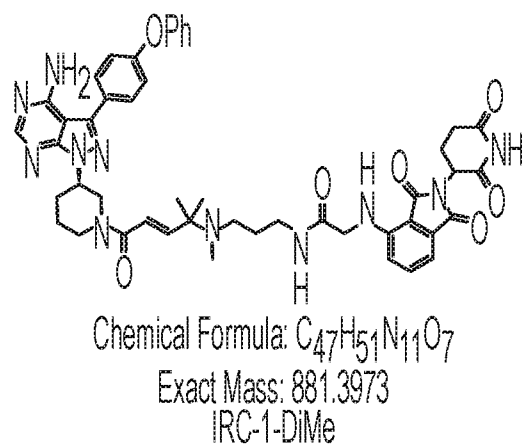
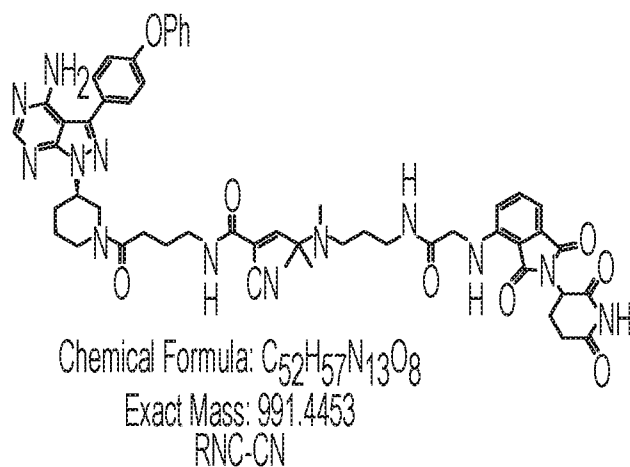
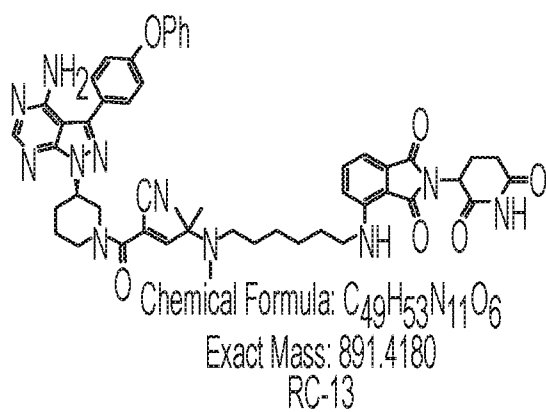


FIG. 22  
CONTINUED

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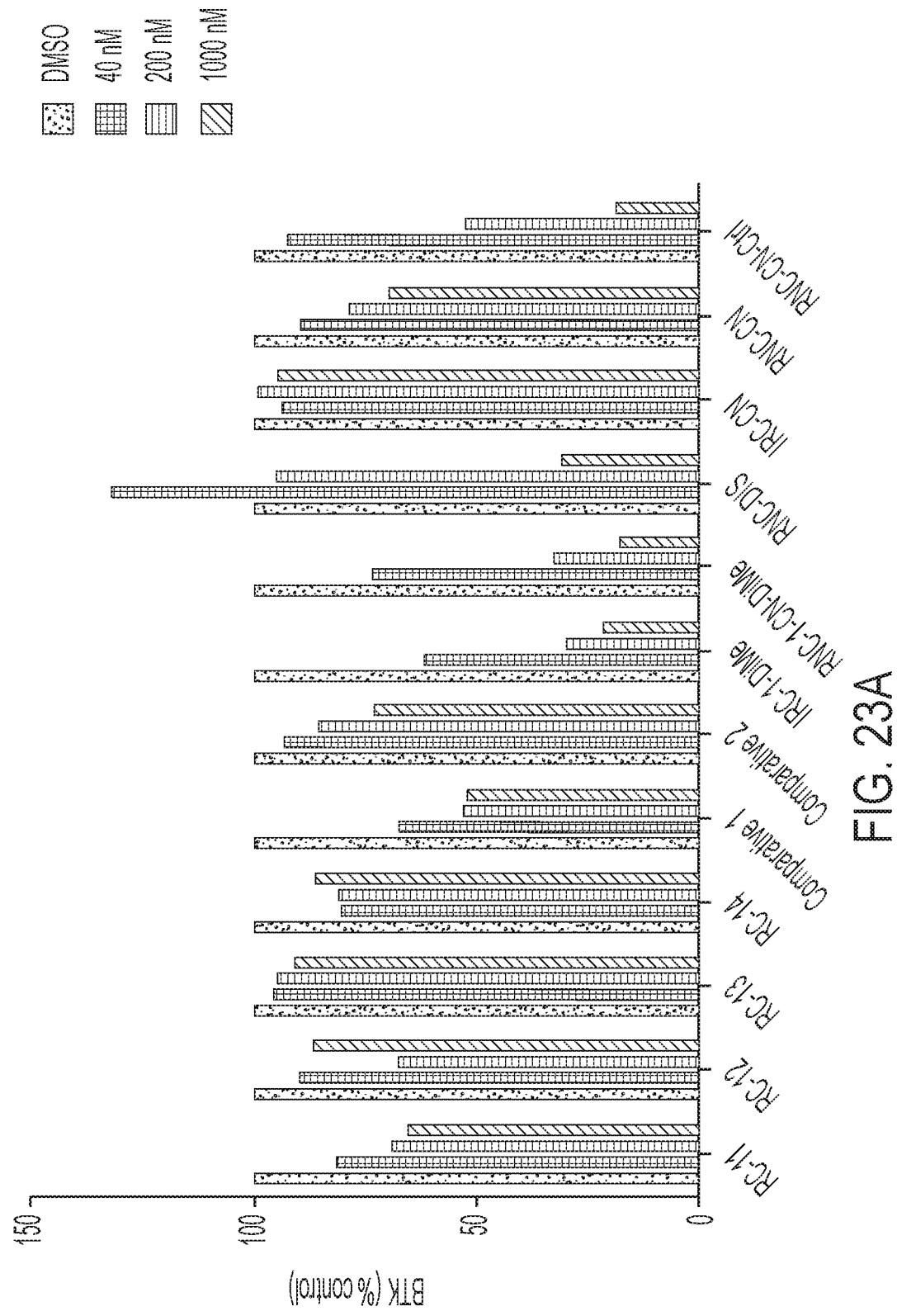


FIG. 23A

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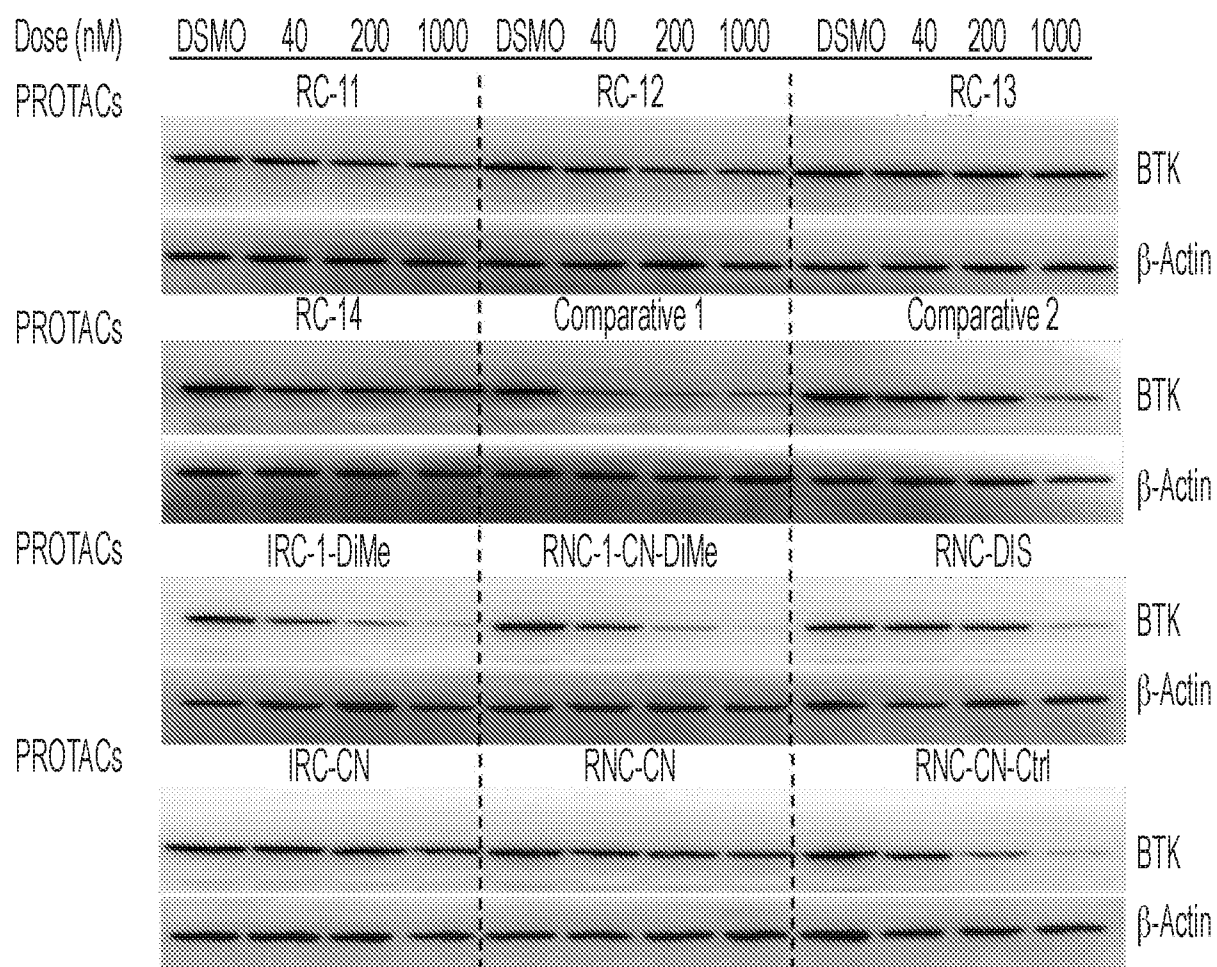


FIG. 23B

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2020/037607

A. CLASSIFICATION OF SUBJECT MATTER  
INV. C07D487/04 A61K31/519 A61P25/00 A61P29/00 A61P35/00  
ADD.

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

## B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                     | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | WO 2008/121742 A2 (PHARMACYCLICS INC [US];<br>HONIGBERG LEE [US] ET AL.)<br>9 October 2008 (2008-10-09)<br>paragraph [0004]; claim 1; table 2<br>----- | 4-30                  |
| X         | WO 2010/009342 A2 (PHARMACYCLICS INC [US];<br>HONIGBERG LEE [US] ET AL.)<br>21 January 2010 (2010-01-21)<br>claim 2<br>-----                           | 4-30                  |
| A         | WO 2015/048689 A1 (PHARMACYCLICS INC [US])<br>2 April 2015 (2015-04-02)<br>paragraph [0005]; claim 1<br>-----                                          | 4-30                  |
| A         | WO 2017/156495 A1 (CORVUS PHARMACEUTICALS<br>INC [US]) 14 September 2017 (2017-09-14)<br>claims 1,5,48<br>-----<br>-/-                                 | 4-30                  |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

4 September 2020

Date of mailing of the international search report

15/09/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Gettins, Marc

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/037607

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                 |                       |
|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                              | Relevant to claim No. |
| A                                                    | ZHENGYING PAN ET AL: "Discovery of Selective Irreversible Inhibitors for Brutons Tyrosine Kinase", CHEMMEDCHEM, WILEY-VCH, DE, vol. 2, no. 1, 15 January 2007 (2007-01-15), pages 58-61, XP008118842, ISSN: 1860-7179, DOI: 10.1002/CMDC.200600221 [retrieved on 2006-12-12] the whole document | 4-30                  |
| A                                                    | WO 2018/144649 A1 (ARVINAS INC [US]) 9 August 2018 (2018-08-09) claim 1                                                                                                                                                                                                                         | 4-30                  |
| X                                                    | CHRISTOPHER P. TINWORTH ET AL: "PROTAC-Mediated Degradation of Bruton's Tyrosine Kinase Is Inhibited by Covalent Binding", ACS CHEMICAL BIOLOGY, vol. 14, no. 3, 26 February 2019 (2019-02-26), pages 342-347, XP055708652, ISSN: 1554-8929, DOI: 10.1021/acschembio.8b01094 figure 5           | 4-30                  |
| X,P                                                  | XIUYUN SUN ET AL: "A chemical approach for global protein knockdown from mice to non-human primates", CELL DISCOVERY, vol. 5, no. 1, 1 December 2019 (2019-12-01), XP055727415, GB ISSN: 2056-5968, DOI: 10.1038/s41421-018-0079-1 figure 7; compound P13IS                                     | 4-30                  |
| X,P                                                  | CN 110 724 143 A (UNIV TSINGHUA) 24 January 2020 (2020-01-24) claims 1,10 & DATABASE WPI Week 202001 Thomson Scientific, London, GB; AN 2020-10549N & CN 110 724 143 A (UNIV TSINGHUA) 24 January 2020 (2020-01-24) abstract                                                                    | 4-30                  |
| X,P                                                  | WO 2019/148150 A1 (DANA FARBER CANCER INST INC [US]) 1 August 2019 (2019-08-01) claims 37,49,63; table A1                                                                                                                                                                                       | 4-30                  |
|                                                      | -/--                                                                                                                                                                                                                                                                                            |                       |

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/037607

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                            |                       |
|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                         | Relevant to claim No. |
| X                                                    | WO 2019/042445 A1 (SHANGHAI MEIZER<br>PHARMACEUTICALS CO LTD [CN])<br>7 March 2019 (2019-03-07)<br>page 8; claim 1; compound 30<br>& EP 3 677 584 A1 (SHANGHAI MEIZER<br>PHARMACEUTICALS CO LTD [CN])<br>8 July 2020 (2020-07-08)<br>----- | 4-30                  |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2020/037607

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1, 2(completely); 20-30(partially)  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1, 2(completely); 20-30(partially)

Claim 1 is directed to compounds A-L-B where none of the components is defined adequately in structural terms. A and B are defined by a result to be achieved (BTK binder or E3 ligase binder) and L is a linker (defined by its role). In claim 2 L comprises (i.e. is not limited to only being) a reversible covalent group which encompasses a vast range of compounds and is not a meaningful limitation of claim 1. In claim 3 B comprises one of 6 classes of ligands which is again an attempt to define a structural feature by a result to be achieved. Present claims 1-3 therefore relate to an extremely large number of possible compounds. Present claims 1-3 also encompass compounds defined, in part, only by their desired function, contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained. The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed, except for the compounds disclosed in the description. Undue experimentation would be required to screen compounds randomly. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search for the claims.

The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of the claims (PCT Guidelines 9.19 and 9.23).

The search of the claims was consequently restricted to compounds having a significant, clearly defined structural moiety in common and which represent a generalisation of the examples/preferred embodiments which are derivable from the application. It is considered that support and disclosure in the sense of Article 6 and 5 PCT is to be found for only a very small proportion of the compounds claimed, see the compounds of claims 4-19. This appears to cover all of the actual embodiments and Formulae I and II as defined on pages 17 and 29 in the description.

The search of the claims was restricted to those claimed compounds and their uses since these appear to be supported and a generalisation of their structural formulae. Claims 1-3 were not searched. Claims 4-19 were all fully searched and claims 20-30 were only (partially) searched in so far as they are directed to the scope of claims 4-19.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/037607

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/037607

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |            |
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