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(54) Title: METHODS AND COMPOUNDS FOR TREATING DISORDERS

(57) Abstract: The present invention relates to methods and compositions for the treatment of BAF-related disorders such as cancers and viral infections.

WO 2019/152440 A1

METHODS AND COMPOUNDS FOR TREATING DISORDERS

Background

Disorders can be affected by the BAF complex. BRD9 is a component of the BAF complex. The present invention relates to useful methods and compositions for the treatment of BAF-related disorders, such as cancer and infection.

Summary of the Invention

Bromodomain-containing protein 9 (BRD9) is a protein encoded by the BRD9 gene on chromosome 5. BRD9 is a component of the BAF (BRG1- or BRM-associated factors) complex, a SWI/SNF ATPase chromatin remodeling complex, and belongs to family IV of the bromodomain-containing proteins. BRD9 is present in several SWI/SNF ATPase chromatin remodeling complexes and is upregulated in multiple cancer cell lines. Accordingly, agents which reduce the levels and/or activity of BRD9 may provide new methods for the treatment of disease and disorders, such as cancer. The inventors have found that depleting BRD9 in cells results in the depletion of the SS18-SSX fusion protein in those cells. The SS18-SSX fusion protein has been detected in more than 95% of synovial sarcoma tumors and is often the only cytogenetic abnormality in synovial sarcoma. Thus, agents that degrade BRD9, e.g., antibodies, enzymes, polynucleotides, and compounds, are useful in the treatment of cancers related to BRD9 or SS18-SSX expression such as soft tissue sarcomas, e.g., synovial sarcoma.

The present disclosure features useful methods to treat cancer, e.g., in a subject in need thereof. In some embodiments, the methods described herein are useful in the treatment of disorders associated with BRD9 expression, e.g., adult soft tissue sarcomas. In some embodiments, the methods described herein are useful in the treatment of disorders associated with SS18-SSX fusion protein.

In one aspect, the invention features a method of treating adult soft tissue sarcoma in a subject in need thereof, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in the sarcoma.

In another aspect, the invention features a method of treating adult soft tissue sarcoma in a subject in need thereof, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of a BAF complex (e.g., GBAF) in the sarcoma.

In another aspect, the invention features a method of reducing tumor growth of an adult soft tissue sarcoma in a subject in need thereof, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in the tumor.

In another aspect, the invention features a method of inducing apoptosis in an adult soft tissue sarcoma cell, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell.

In another aspect, the invention features a method of reducing the level of BRD9 in an adult soft tissue sarcoma cell, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell.

In some embodiments of any of the above aspects, the adult soft tissue sarcoma cell is in a subject. In some embodiments, the subject or cell has been identified as expressing SS18-SSX fusion protein or BRD9 fusion protein.

In another aspect, the invention features a method of modulating the level of an SS18-SSX fusion protein, SS18 wild-type protein, or SSX wild-type protein in a cell or subject, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in a cell or subject. In some embodiments, the cell is in a subject.

In another aspect, the invention features a method of treating a disorder related to an SS18-SSX fusion protein, SS18 wild-type protein, or SSX wild-type protein in a subject in need thereof, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in an SS18-SSX fusion protein-expressing cell in the subject.

In some embodiments of any of the above aspects, the effective amount of the agent reduces the level and/or activity of BRD9 by at least 5% (e.g., 6%, 7%, 8%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) as compared to a reference. In some embodiments, the effective amount of the agent that reduces the level and/or activity of BRD9 by at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) as compared to a reference. In some embodiments, the effective amount of the agent that reduces the level and/or activity of BRD9 by at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%).

In some embodiments, the effective amount of the agent reduces the level and/or activity of BRD9 by at least 5% (e.g., 6%, 7%, 8%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) as compared to a reference for at least 12 hours (e.g., 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 30 hours, 36 hours, 48 hours, 72 hours, or more). In some embodiments, the effective amount of the agent that reduces the level and/or activity of BRD9 by at least 5% (e.g., 6%, 7%, 8%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) as compared to a reference for at least 4 days (e.g., 5 days, 6 days, 7 days, 14 days, 28 days, or more).

In some embodiments, the subject has cancer. In some embodiments, the cancer expresses SS18-SSX fusion protein and/or the cell or subject has been identified as expressing SS18-SSX fusion protein. In some embodiments, the disorder is synovial sarcoma or Ewing's sarcoma. In some embodiments, the disorder is synovial sarcoma.

In one aspect, the invention features a method of modulating the activity of a BAF complex in a cell or subject, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

In another aspect, the invention features a method of increasing the level of BAF47 in a cell or subject, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

In one aspect, the invention features a method of decreasing Wnt/β-catenin signaling in a cell or subject, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

In one aspect, the invention features a method treating a disorder related to BAF47 in a subject in need thereof, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in the subject.

In some embodiments, the disorder related to BAF47 is a cancer or viral infection. In some embodiments, the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma,

bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, or colorectal cancer.

In some embodiments, the viral infection is an infection with a virus of the Retroviridae family, Hepadnaviridae family, Flaviviridae family, Adenoviridae family, Herpesviridae family, Papillomaviridae family, Parvoviridae family, Polyomaviridae family, Paramyxoviridae family, or Togaviridae family.

In an aspect, the invention features a method for treating cancer in a subject in need thereof, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in a cancer cell, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

In an aspect, the invention features a method of reducing tumor growth of a cancer in a subject in need thereof, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in a tumor cell, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

In one aspect, the invention features a method of inducing apoptosis in a cancer cell, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

In one aspect, the invention features a method of reducing the level of BRD9 in a cancer cell, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

In some embodiments of any of the above aspects, the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, or colorectal cancer. In some embodiments, the cancer is non-small cell lung cancer, stomach cancer, or breast cancer.

In one aspect, the invention features a method of modulating the activity of a BRD9 fusion protein in a cell or subject, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

In an aspect, the invention features a method of modulating the level of a BRD9 fusion protein in a cell or subject, the method including contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject. In some embodiments, the cell is in a subject.

In an aspect, the invention features a method of treating a disorder related to a BRD9 fusion protein in a subject in need thereof, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in a BRD9 fusion protein-expressing cell.

In some embodiments of any of the above aspects, the subject has cancer. In some embodiments, the cancer expresses a BRD9 fusion protein and/or the cell or subject has been identified as expressing a BRD9 fusion protein. In some embodiments, the method further includes administering to the subject or contacting the cell with an anticancer therapy. In some embodiments, the anticancer therapy is a chemotherapeutic or cytotoxic agent or radiotherapy. In some embodiments, the chemotherapeutic or cytotoxic agent is doxorubicin or ifosfamide. In some embodiments, the anticancer therapy and the agent that reduces the level and/or activity of BRD9 in a cell are administered within 28 days of each other and each in an amount that together are effective to treat the subject. In some embodiments, the subject or cancer has been identified as having an elevated level of an SS18-SSX fusion protein or a BRD9 fusion protein as compared to a reference. In some embodiments, the subject or cancer has been identified as having a decreased level of SS18 wild-type protein or SSX wild-type protein as compared to a reference.

In one aspect, the invention features a method of treating a viral infection, the method including administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in a cell of the subject.

In some embodiments, the disorder is a viral infection is an infection with a virus of the Retroviridae family such as the lentiviruses (e.g., Human immunodeficiency virus (HIV) and deltaretroviruses (e.g., human T cell leukemia virus I (HTLV-I), human T cell leukemia virus II (HTLV-II)), Hepadnaviridae family (e.g., hepatitis B virus (HBV)), Flaviviridae family (e.g., hepatitis C virus (HCV)), Adenoviridae family (e.g., Human Adenovirus), Herpesviridae family (e.g., Human cytomegalovirus (HCMV), Epstein-Barr virus, herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human herpesvirus 6 (HHV-6), Herpesvitus K*, CMV, varicella-zoster virus), Papillomaviridae family (e.g., Human Papillomavirus (HPV, HPV E1)), Parvoviridae family (e.g., Parvovirus B19), Polyomaviridae family (e.g., JC virus and BK virus), Paramyxoviridae family (e.g., Measles virus), Togaviridae family (e.g., Rubella virus). In some embodiments, the disorder is Coffin Siris, Neurofibromatosis (e.g., NF-1, NF-2, or Schwannomatosis), or Multiple Meningioma. In some embodiments, the viral infection is an infection with a virus of the Retroviridae family, Hepadnaviridae family, Flaviviridae family, Adenoviridae family, Herpesviridae family, Papillomaviridae family, Parvoviridae family, Polyomaviridae family, Paramyxoviridae family, or Togaviridae family.

In some embodiments of any of the above aspects, the agent that reduces the level and/or activity of BRD9 in a cell is a small molecule compound, an antibody, an enzyme, and/or a polynucleotide. In some embodiments, the agent that reduces the level and/or activity of BRD9 in a cell is an enzyme. In some embodiments, the enzyme is a clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), or a meganuclease. In some embodiments, the CRISPR-associated protein is CRISPR-associated protein 9 (Cas9).

In some embodiments of any of the above aspects, the agent that reduces the level and/or activity of BRD9 in a cell is a polynucleotide. In some embodiments, the polynucleotide is an antisense

nucleic acid, a short interfering RNA (siRNA), a short hairpin RNA (shRNA), a micro RNA (miRNA), a CRISPR/Cas 9 nucleotide (e.g., a guide RNA (gRNA)), or a ribozyme. In some embodiments, the polynucleotide has a sequence having at least 70% sequence identity (e.g., 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9% identity, or more) to the nucleic acid sequence of any one of SEQ ID NOs: 3-202. In some embodiments, the polynucleotide has a sequence having at least 70% sequence identity (e.g., 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9% identity, or more) to the nucleic acid sequence of any one of SEQ ID NOs: 3-139.

In some embodiments of any of the above aspects, the agent that reduces the level and/or activity of BRD9 in a cell is a small molecule compound, or a pharmaceutically acceptable salt thereof.

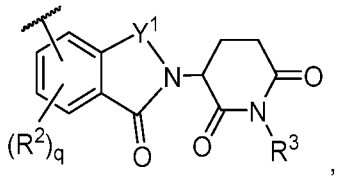
In some embodiments, the small molecule compound, or a pharmaceutically acceptable salt thereof is a degrader. In some embodiments, the degrader has the structure of **Formula I**:

A-L-B

Formula I

wherein A is a BRD9 binding moiety; L is a linker; and B is a degradation moiety, or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation moiety is a ubiquitin ligase moiety. In some embodiments, the ubiquitin ligase binding moiety includes Cereblon ligands, IAP (Inhibitors of Apoptosis) ligands, mouse double minute 2 homolog (MDM2), hydrophobic tag, or von Hippel-Lindau ligands, or derivatives or analogs thereof.

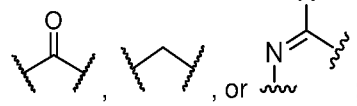
In some embodiments, the degradation moiety has the structure of **Formula A-1**:



,

Formula A-1

where

Y^1 is  ;

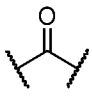
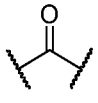
each of R^3 and R^4 is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

q is 0, 1, 2, 3, or 4; and

each R^2 is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino, or a pharmaceutically acceptable salt thereof.

In some embodiments, R^3 is H or optionally substituted C_1-C_6 alkyl.

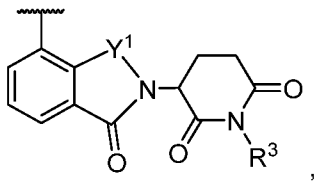
In some embodiments, R^3 is H or CH_3. In some embodiments, R^3 is H. In some embodiments, R^3 is CH_3.

In some embodiments, Y^1 is  or  . In some embodiments, Y^1 is  .

In some embodiments, R^2 is, independently, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, hydroxyl, or optionally substituted amino.

In some embodiments, q is 0 or 1. In some embodiments, q is 0. In some embodiments, q is 1.

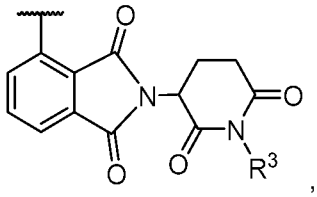
In some embodiments, the degradation moiety has the structure of **Formula A-1a**:



Formula A-1a

or a pharmaceutically acceptable salt thereof.

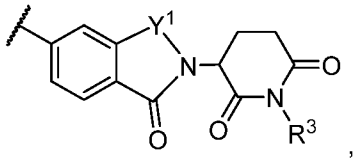
In some embodiments, the degradation moiety has the structure of **Formula A-1b**:



Formula A-1b

or a pharmaceutically acceptable salt thereof.

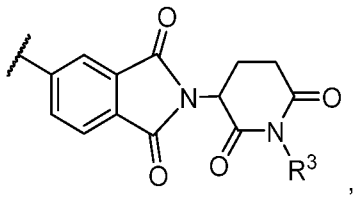
In some embodiments, the degradation moiety has the structure of **Formula A-1c**:



Formula A-1c

or a pharmaceutically acceptable salt thereof.

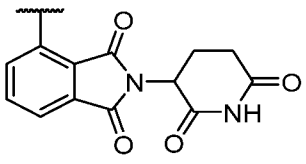
In some embodiments, the degradation moiety has the structure of **Formula A-1d**:



Formula A-1d

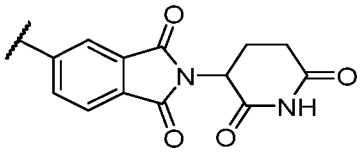
or a pharmaceutically acceptable salt thereof.

In some embodiments, the degradation moiety has the structure:



1a

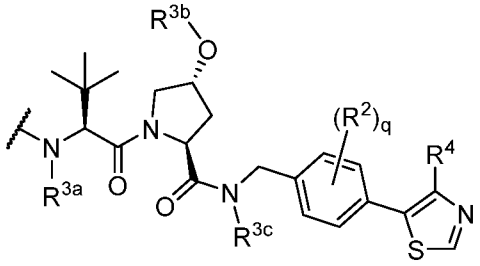
or



1b

or is a derivative or an analog thereof.

In some embodiments, the degradation moiety has the structure of



Formula B-1,

where

q is 0, 1, 2, 3, or 4;

each R^2 is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

each of R^{3a}, R^{3b}, and R^{3c} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl; and

R^4 is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl,

or a pharmaceutically acceptable salt thereof.

In some embodiments, each R^2 is, independently, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, hydroxyl, or optionally substituted amino.

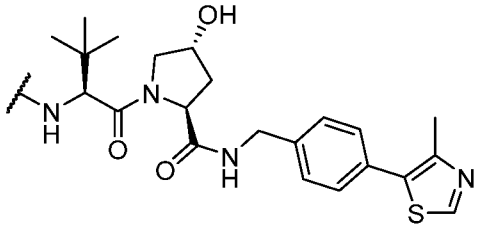
In some embodiments, q is 0 or 1.

In some embodiments, q is 0.

In some embodiments, each of R^{3a}, R^{3b}, and R^{3c} is, independently, H or optionally substituted C_1-C_6 alkyl.

In some embodiments, R^{3a} is H. In some embodiments, R^{3b} is H. In some embodiments, R^{3c} is H.

In some embodiments, the degradation moiety has the structure:



2a

or is a derivative or an analog thereof.

In some embodiments, the linker has the structure of **Formula II**:

A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$-(D)-$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2

Formula II

where

A^1 is a bond between the linker and A;

A^2 is a bond between B and the linker;

each of B^1, B^2, B^3, and B^4 is, independently, optionally substituted C_1-C_2 alkyl, optionally substituted C_1-C_3 heteroalkyl, O, S, $S(O)_2$, or NR^N;

each R^N is, independently, H, optionally substituted C_{1-4} alkyl, optionally substituted C_{2-4} alkenyl, optionally substituted C_{2-4} alkynyl, optionally substituted C_{2-6} heterocyclyl, optionally substituted C_{6-12} aryl, or optionally substituted C_{1-7} heteroalkyl;

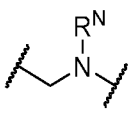
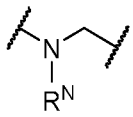
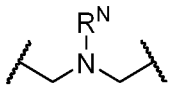
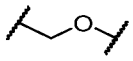
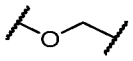
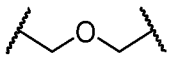
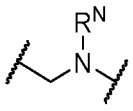
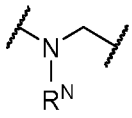
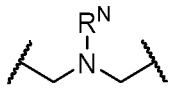
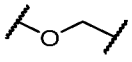
each of C^1 and C^2 is, independently, carbonyl, thiocarbonyl, sulphonyl, or phosphoryl;

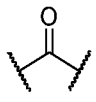
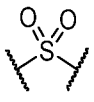
each of f, g, h, i, j, and k is, independently, 0 or 1; and

D is optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} alkynyl, optionally substituted C_{2-6} heterocyclyl, optionally substituted C_{6-12} aryl, optionally substituted C_2-C_{10} polyethylene glycol, or optionally substituted C_{1-10} heteroalkyl, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2.

In some embodiments, each of B^1, B^2, B^3, and B^4 is, independently, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 heteroalkyl, or NR^N.

In some embodiments, each R^N is, independently, H or optionally substituted C_{1-4} alkyl. In some embodiments, each R^N is, independently, H or CH_3.

In some embodiments, each of B^1 and B^4 is, independently,  ,  ,  ,  ,  , or  . In some embodiments, B^1 is  ,  ,  , or  .

In some embodiments, each of C^1 and C^2 is, independently,  or  . In some embodiments, C^1 is  .

In some embodiments, B^2 is NR^N.

In some embodiments, B^2 is optionally substituted C_1-C_4 alkyl.

In some embodiments, f is 0. In some embodiments, f is 1.

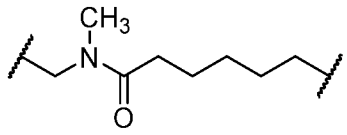
In some embodiments, g is 0. In some embodiments, g is 1.

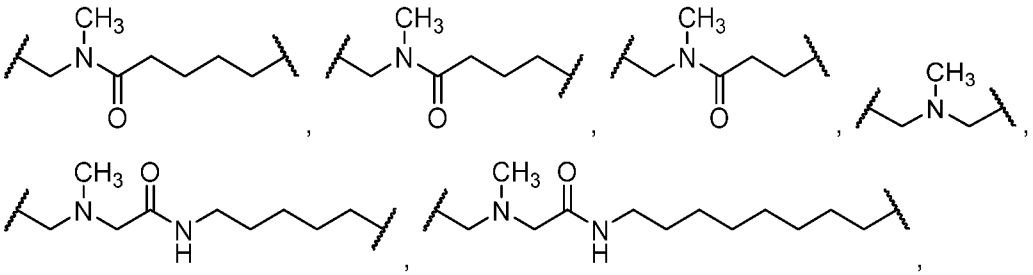
In some embodiments, h is 0. In some embodiments, h is 1.

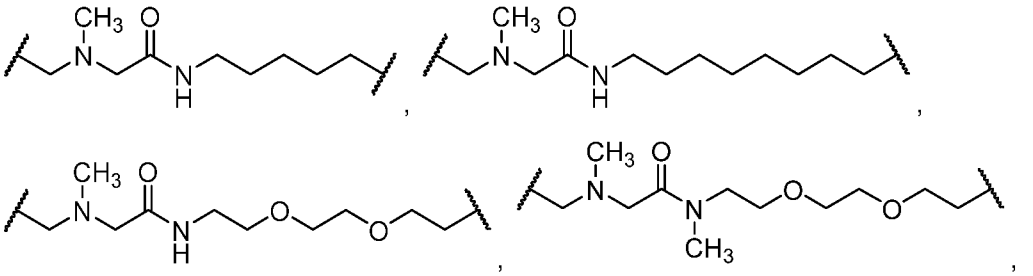
In some embodiments, i is 0. In some embodiments, i is 1.

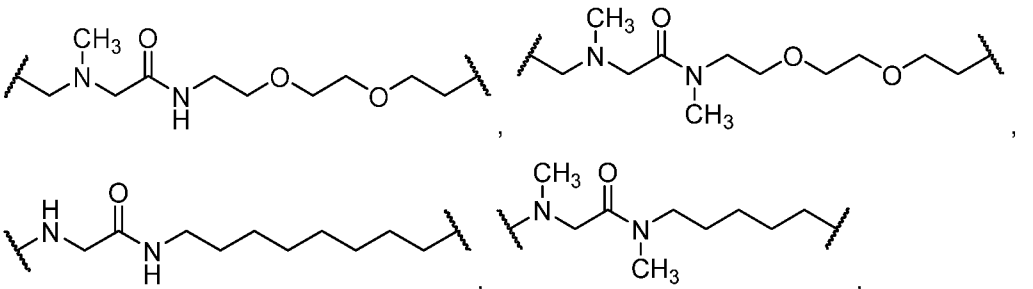
In some embodiments, j is 0. In some embodiments, j is 1.

In some embodiments, k is 0. In some embodiments, k is 1.

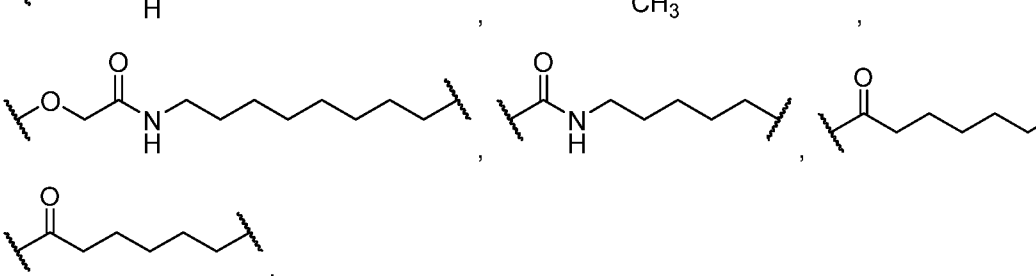
In some embodiments, the linker has the structure of  ,

 ,

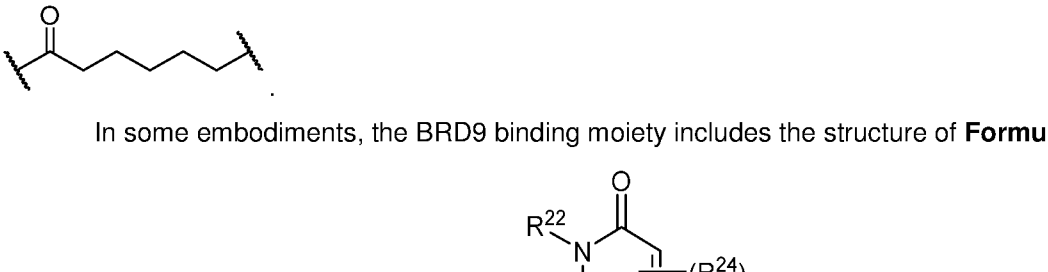
 ,

 ,

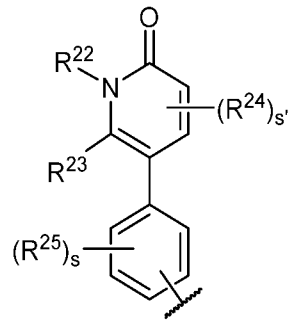
,

 ,

, or

 .

In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-a**:



Formula E-a,

where

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s' is 0, 1, or 2;

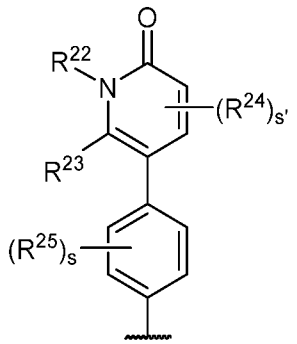
each R^{24} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino, or two

R^{24} combine with the carbon atoms to which they are attached to form an optionally substituted C_6-C_{10} aryl or optionally substituted C_2-C_9 heteroaryl;

s is 0, 1, 2, 3, or 4; and

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino, or a pharmaceutically acceptable salt thereof.

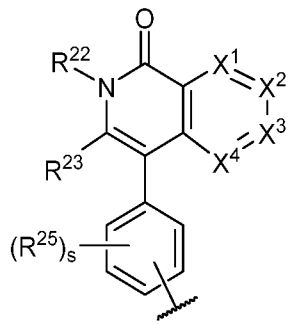
In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-b**:



Formula E-b,

or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure **Formula E-1a**:



Formula E-1a,

where

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

X^1 is N or CR^{24a};

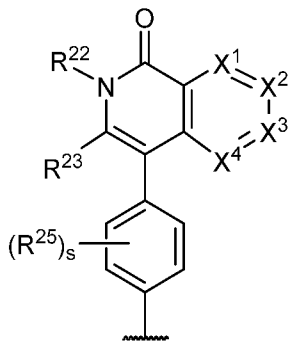
X^2 is N or CR^{24b};

X^3 is N or CR^{24c};

X^4 is N or CR^{24d}; and

each of R^{24a}, R^{24b}, R^{24c}, and R^{24d} is, independently, H, halogen, hydroxyl, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino, or a pharmaceutically acceptable salt thereof.

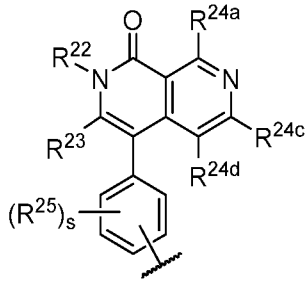
In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-1b**:



Formula E-1b,

or a pharmaceutically acceptable salt thereof.

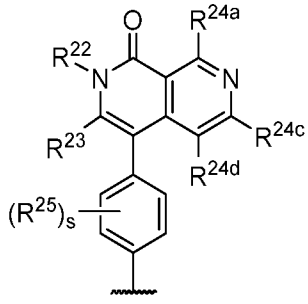
In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-2a**:



Formula E-2a,

or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-2b**:



Formula E-2b,

or a pharmaceutically acceptable salt thereof.

In some embodiments, R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_3-C_6 carbocyclyl.

In some embodiments, R^{22} is H or CH_3.

In some embodiments, R^{23} is H or optionally substituted C_1-C_6 alkyl.

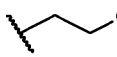
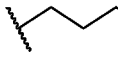
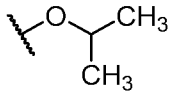
In some embodiments, R^{23} is H.

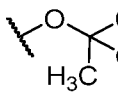
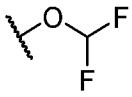
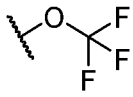
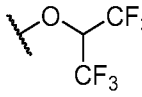
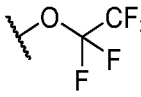
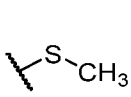
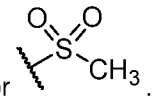
In some embodiments, s is 0, 1, or 2. In some embodiments, s is 1 or 2. In some embodiments, s is 1. In some embodiments, s is 2.

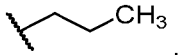
In some embodiments, each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl.

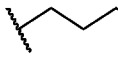
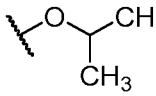
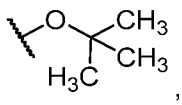
In some embodiments, each R^{25} is, independently, optionally substituted C_1-C_6 alkyl or optionally substituted C_1-C_6 heteroalkyl.

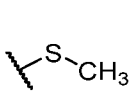
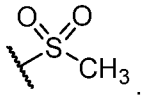
In some embodiments, each R^{25} is, independently, F,  ,  ,

 ,  ,  ,  ,  ,  ,

 ,  ,  ,  ,  ,  ,  .

In some embodiments, each R^{25} is, independently,  ,  ,  ,

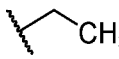
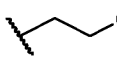
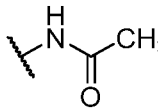
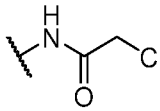
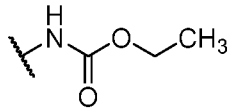
 ,  ,  ,  ,  ,  ,

 ,  .

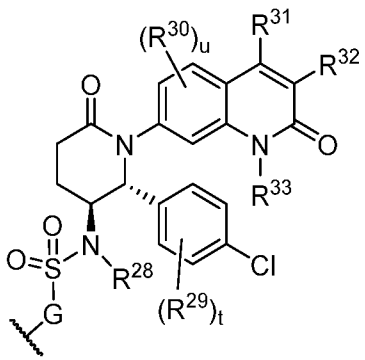
In some embodiments, s' is 1.

In some embodiments, each of R^{24a}, R^{24b}, R^{24c}, and R^{24d} is, independently, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted amino.

In some embodiments, each of R^{24a}, R^{24b}, R^{24c}, and R^{24d} is, independently, $-NH_2$,  ,

 ,  ,  ,  ,  .

In some embodiments, the BRD9 binding moiety includes the structure of **Formula F-a**:



Formula F-a,

where

each of R^{28} and R^{33} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

t is 0, 1, 2, 3, or 4;

each R^{29} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

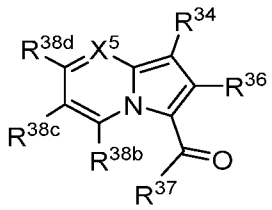
u is 0, 1, 2, 3, or 4;

each R^{30} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

each of R^{31} and R^{32} is, independently, selected form the group consisting of H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl; and

G is optionally substituted C_1-C_6 alkylene, optionally substituted C_6-C_{10} arylene, or optionally substituted C_3-C_6 carbocyclylene, or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula G**:



Formula G,

where

R^{34} is optionally substituted C_6-C_{10} aryl or C_2-C_9 heteroaryl;

R^{36} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

R^{37} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

X^5 is CR^{38a} or N;

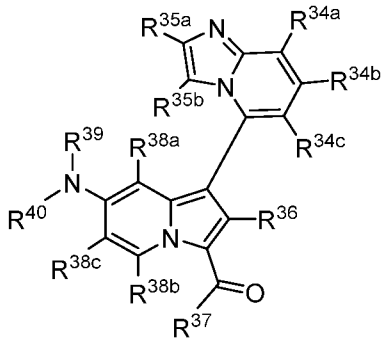
each of R^{38a}, R^{38b}, and R^{38c} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino; and

R^{38d} is hydrogen or $-NR^{39}R^{40}$; and

each of R^{39} and R^{40} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl, or R^{39} and R^{40} combine to form an optionally substituted C_2-C_9 heterocyclyl,

where at least one of R^{34}, R^{39}, or R^{40} includes a bond to the linker, or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula G-1**:



Formula G-1,

where

each of R^{34a}, R^{34b}, and R^{34c} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

each R^{35a} and R^{35b} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

R^{36} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

R^{37} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

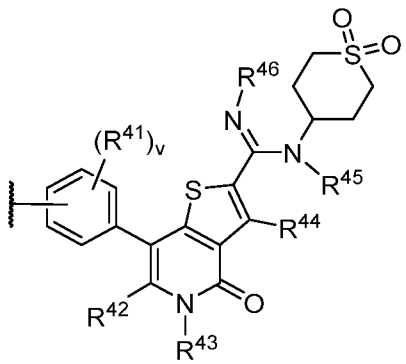
each R^{38a}, R^{38b}, and R^{38c} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino; and

R^{38d} is hydrogen or $-NR^{39}R^{40}$; and

each R^{39} and R^{40} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl, or R^{39} and R^{40} combine to form an optionally substituted C_2-C_9 heterocyclyl,

where at least one of R^{34}, R^{39}, or R^{40} includes a bond to the linker, or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula H-a**:



Formula H-a

where

v is 0, 1, 2, 3, or 4;

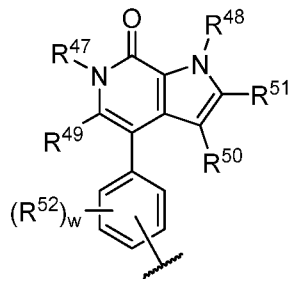
each R^{41} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

R^{42} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

R^{44} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl; and

each R^{43}, R^{45}, and R^{46} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl, or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula J-a**:



Formula J-a,

where

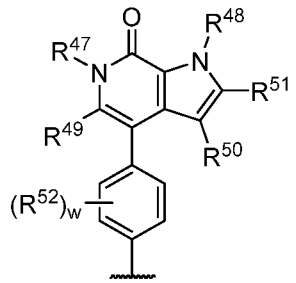
each of R^{47} and R^{48} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

each of R^{49}, R^{50}, and R^{51} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

w is 0, 1, 2, 3, or 4; and

each R^{52} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino, or a pharmaceutically acceptable salt thereof.

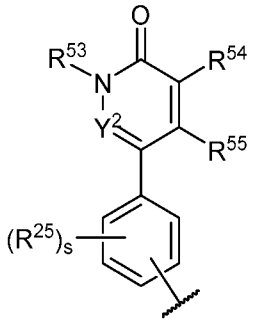
In some embodiments, the BRD9 binding moiety includes the structure of **Formula J-b**:



Formula J-b,

or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-3**:



Formula E-3,

where

Y^2 is N or CR^{23};

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

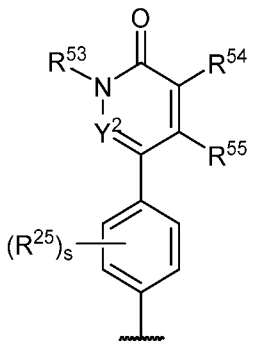
R^{53} is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{54} is H or optionally substituted C_2-C_9 heteroaryl; and

R^{55} is H or NR^a, where R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl,

where if R^{53} is H and R^{54} is H, then R^{55} is NR^a; if R^{54} is H and R^{55} is H, then R^{53} is optionally substituted C_3-C_{10} carbocyclyl; and if R^{53} is H and R^{55} is H, then R^{54} is optionally substituted C_2-C_9 heteroaryl, or a pharmaceutically acceptable salt thereof.

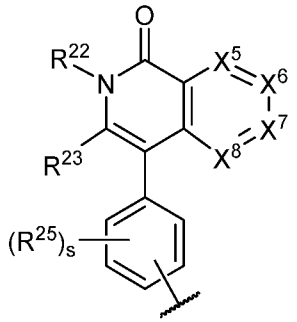
In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-3a**:



Formula E-3a,

or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-4**:



Formula E-4,

where

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

X^5 is N or CR^{56a};

X^6 is N or CR^{56b};

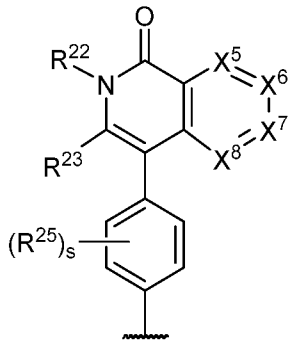
X^7 is N or CR^{56c};

X^8 is N or CR^{56d}; and

each of R^{56a}, R^{56b}, R^{56c}, and R^{56d} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, optionally substituted sulfonamide, or optionally substituted amino, or a pharmaceutically acceptable salt thereof.

In some embodiments, X^7 is N or CH. In some embodiments, X^8 is N or CH.

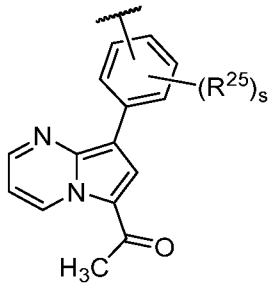
In some embodiments, the BRD9 binding moiety includes the structure of **Formula E-4a**:



Formula E-4a,

or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula G-2**:



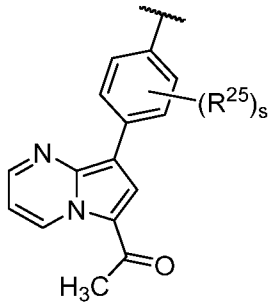
Formula G-2,

where

s is 0, 1, 2, 3, or 4; and

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino, or a pharmaceutically acceptable salt thereof.

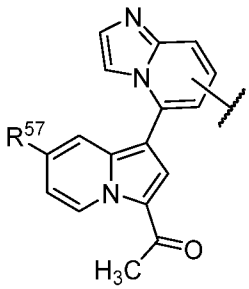
In some embodiments, the BRD9 binding moiety includes the structure of **Formula G-2a**:



Formula G-2a,

or a pharmaceutically acceptable salt thereof.

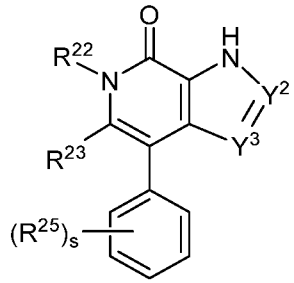
In some embodiments, the BRD9 binding moiety includes the structure of **Formula G-3**:



Formula G-3,

where R^{57} is optionally substituted C_2-C_{10} heterocyclyl, or a pharmaceutically acceptable salt thereof.

In some embodiments, the BRD9 binding moiety includes the structure of **Formula J-1**:



Formula J-1,

where

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

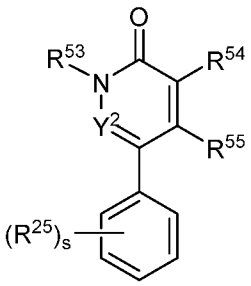
each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

Y^2 is N or CR^{58a};

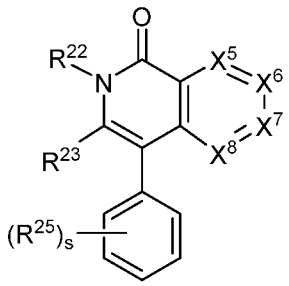
Y^3 is N or CR^{58b}; and

each of R^{58a} and R^{58b} is, independently, H or optionally substituted C_1-C_6 alkyl, or a pharmaceutically acceptable salt thereof.

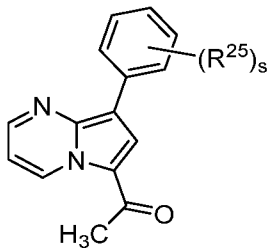
In another aspect, the disclosure features a compound having the structure of **Formula K-1, Formula K-2, Formula M-2**, **Formula M-3**, or **Formula O-1**:



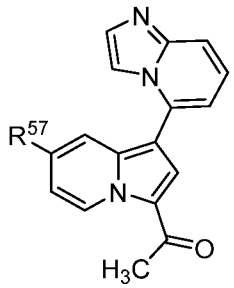
Formula K-1



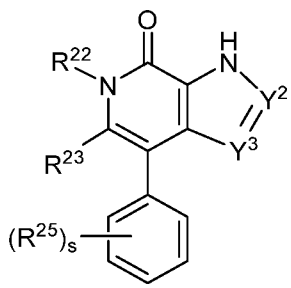
Formula K-2



Formula M-2



Formula M-3



Formula O-1

where

Y^2 is N or CR^{23};

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

R^{53} is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{54} is H or optionally substituted C_2-C_9 heteroaryl;

R^{55} is H or NR^a, where R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

X^5 is N or CR^{56a};

X^6 is N or CR^{56b};

each of X^7 and X^8 is, independently, N or CH;

each of R^{56a} and R^{56b} is, independently, H or NR^a, where each R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{57} is optionally substituted C_2-C_{10} heterocyclyl;

Y^2 is N or CR^{58a};

Y^3 is N or CR^{58b}; and

each of R^{58a} and R^{58b} is, independently, H or optionally substituted C_1-C_6 alkyl,

where if R^{53} is H and R^{54} is H, then R^{55} is NR^a; if R^{54} is H and R^{55} is H, then R^{53} is optionally substituted C_3-C_{10} carbocyclyl; and if R^{53} is H and R^{55} is H, then R^{54} is optionally substituted C_2-C_9 heteroaryl, or a pharmaceutically acceptable salt thereof.

In some embodiments, the compound has the structure of **Formula K-1**.

In some embodiments, the compound has the structure of **Formula K-2**.

In some embodiments, the compound has the structure of **Formula M-2**.

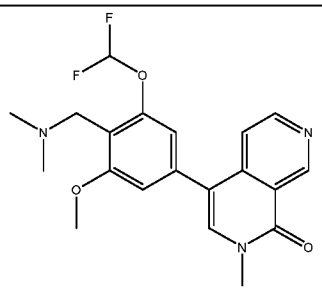
In some embodiments, the compound has the structure of **Formula M-3**.

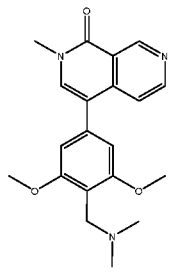
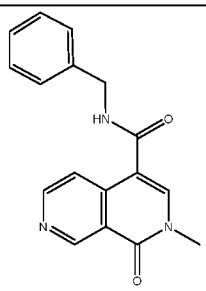
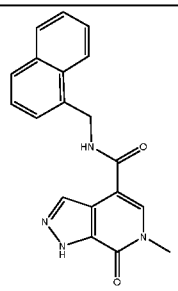
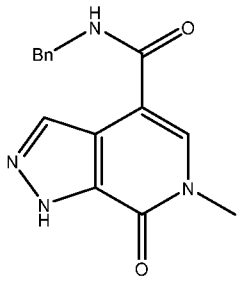
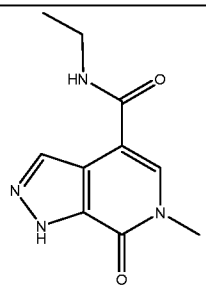
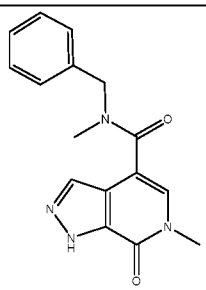
In some embodiments, the compound has the structure of **Formula O-1**.

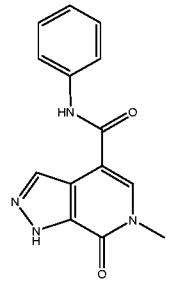
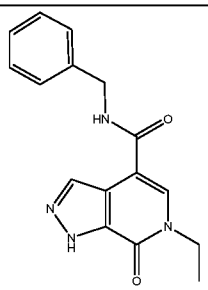
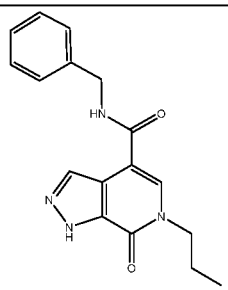
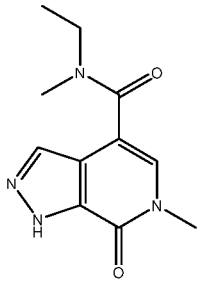
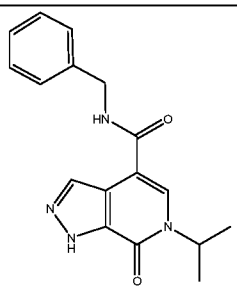
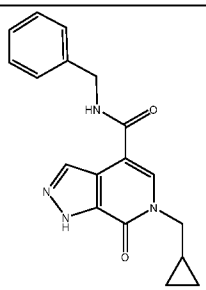
In some embodiments, s is 0, 1, or 2.

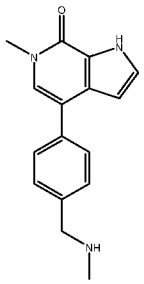
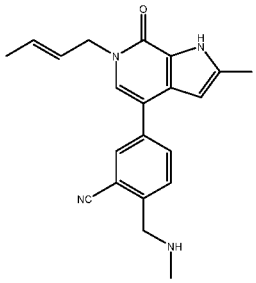
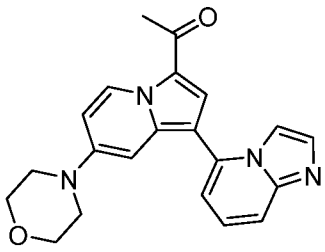
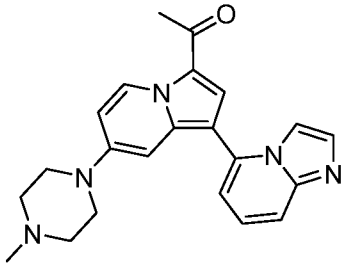
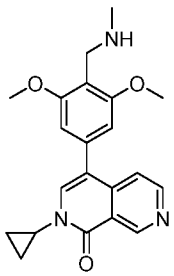
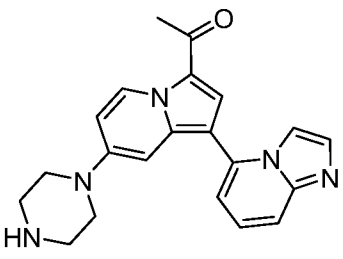
In some embodiments, the compound has the structure of compounds B1-B65 in Table 1. In some embodiments, the compound has the structure of compounds B1, B3-B13, B16-B22, and B28-B67 in Table 1.

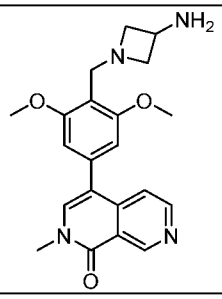
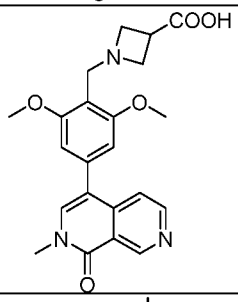
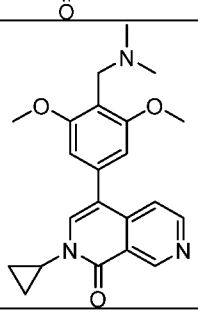
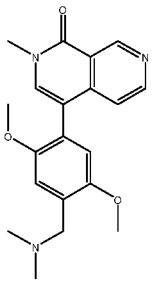
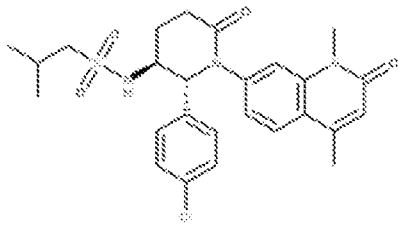
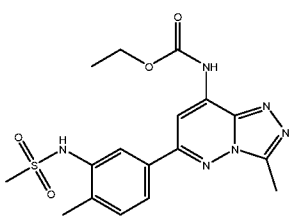
Table 1. Compounds B1-B68 of the Disclosure

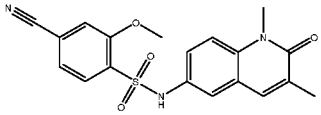
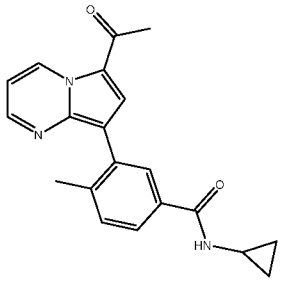
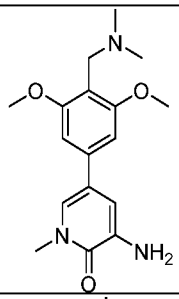
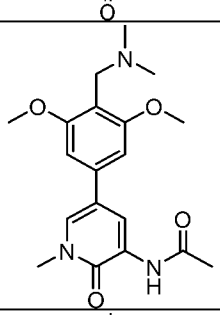
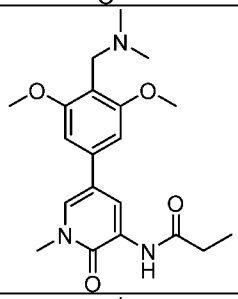
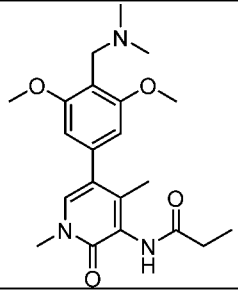
Compound No.	Structure
B1	

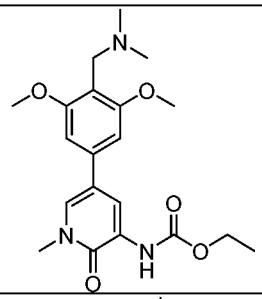
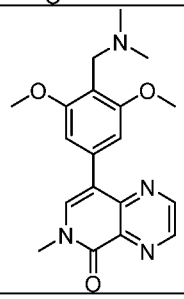
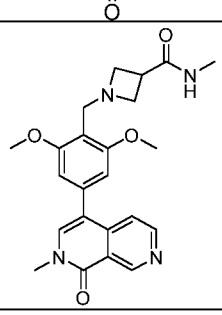
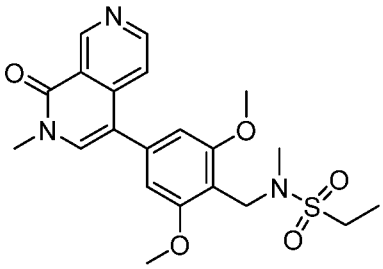
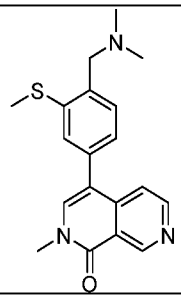
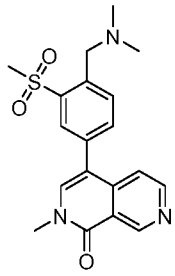
Compound No.	Structure
B2	
B3	
B4	
B5	
B6	
B7	

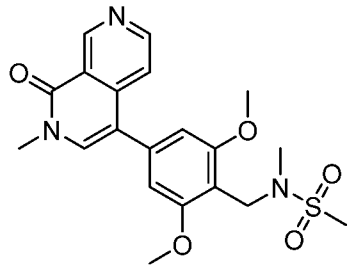
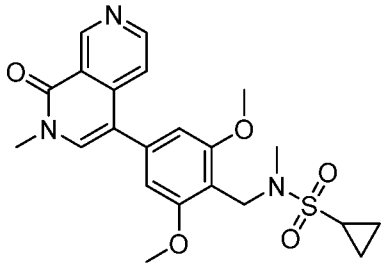
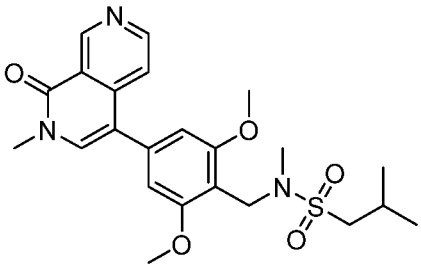
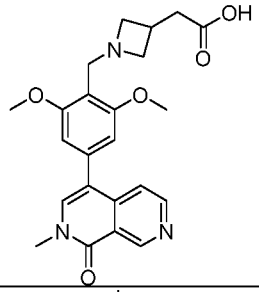
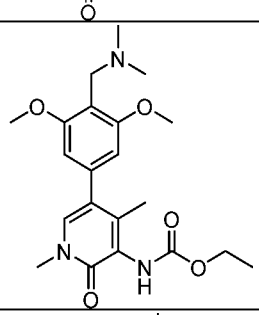
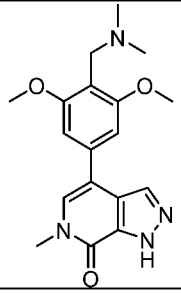
Compound No.	Structure
B8	
B9	
B10	
B11	
B12	
B13	

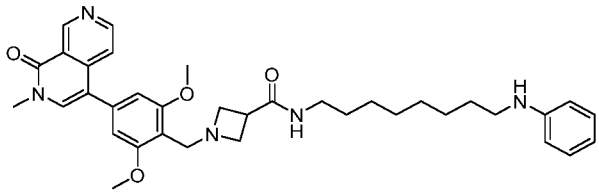
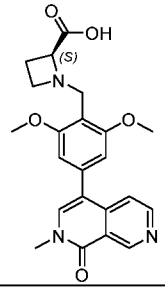
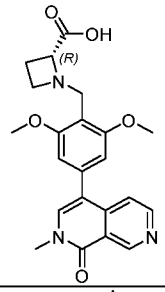
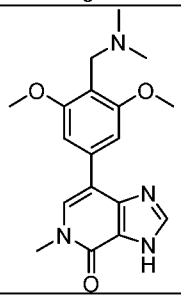
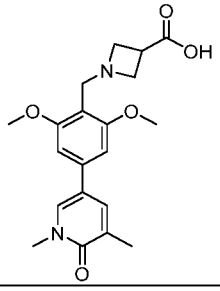
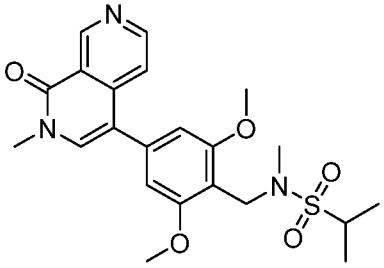
Compound No.	Structure
B14	
B15	
B16	
B17	
B18	
B19	

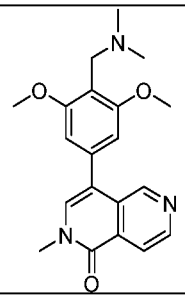
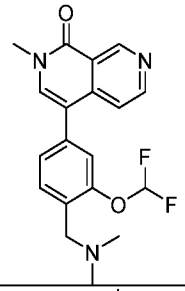
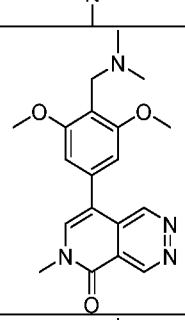
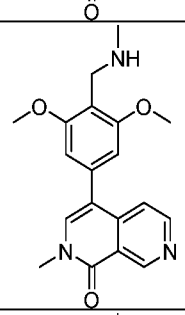
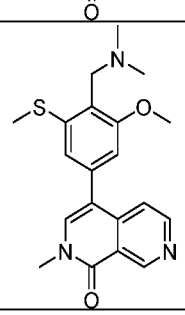
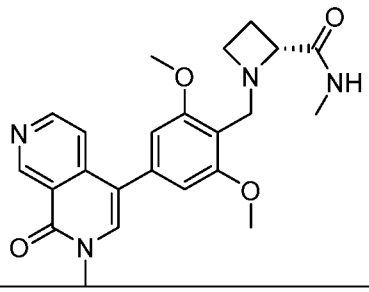
Compound No.	Structure
B20	
B21	
B22	
B23	
B24	
B25	

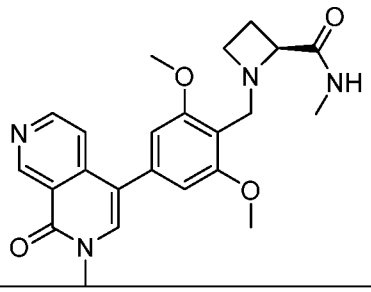
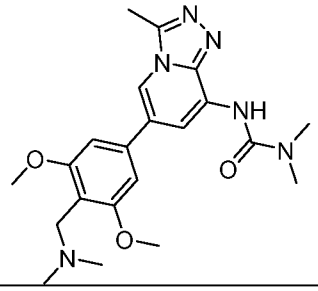
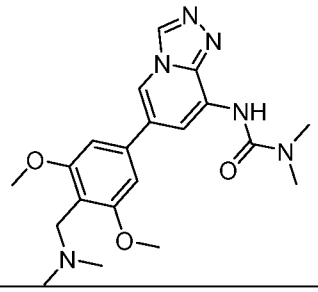
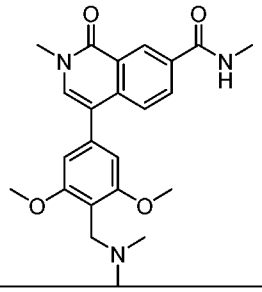
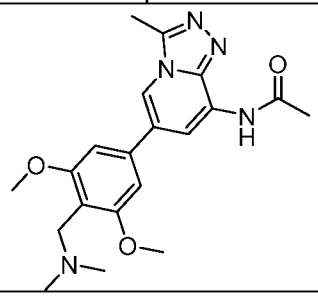
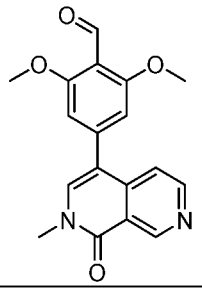
Compound No.	Structure
B26	
B27	
B28	
B29	
B30	
B31	

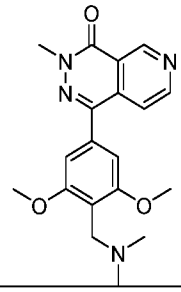
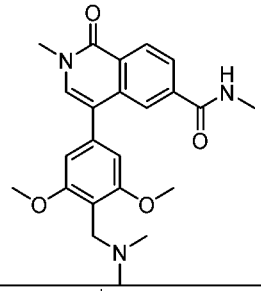
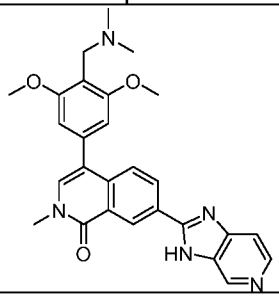
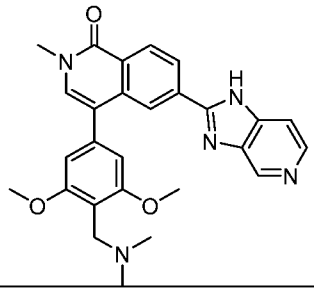
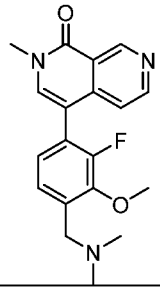
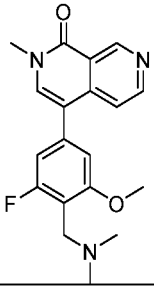
Compound No.	Structure
B32	
B33	
B34	
B35	
B36	
B37	

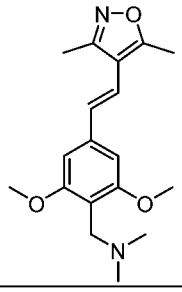
Compound No.	Structure
B38	
B39	
B40	
B41	
B42	
B43	

Compound No.	Structure
B44	
B45	
B46	
B47	
B48	
B49	

Compound No.	Structure
B50	
B51	
B52	
B53	
B54	
B55	

Compound No.	Structure
B56	
B57	
B58	
B59	
B60	
B61	

Compound No.	Structure
B62	
B63	
B64	
B65	
B66	
B67	

Compound No.	Structure
B68	

In another aspect, the disclosure features a pharmaceutical composition including any of the foregoing compounds and a pharmaceutically acceptable excipient.

In yet another aspect, the disclosure features a method of treating a cancer in a subject in need thereof, the method including administering to the subject an effective amount of any of the foregoing compounds or any of the foregoing pharmaceutical compositions.

In another aspect, the disclosure features a method of treating a cancer related to BRD9 inhibition in a subject in need thereof, the method including administering to the subject an effective amount of any of the foregoing compounds or any of the foregoing pharmaceutical compositions.

In yet another aspect, the disclosure features a compound having the structure of **Formula I**:

A-L-B

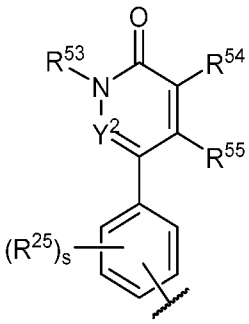
Formula I,

where

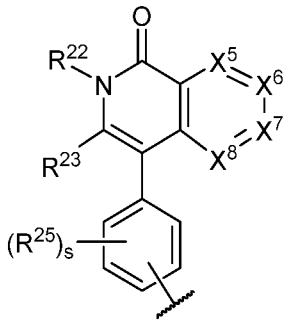
L is a linker;

B is a degradation moiety; and

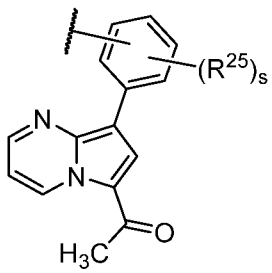
A has the structure of **Formula E-3, Formula E-4, Formula G-2**, **Formula G-3**, or **Formula E-5**:



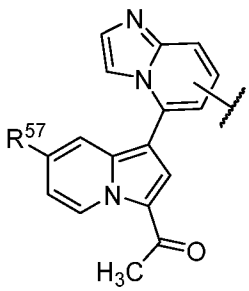
Formula E-3



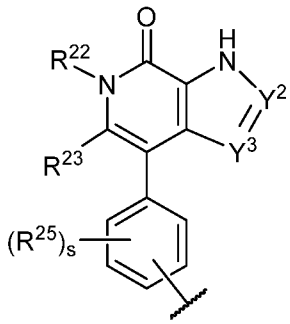
Formula E-4



Formula G-2



Formula G-3



Formula E-5

where

Y^2 is N or CR^{23};

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

R^{53} is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{54} is H or optionally substituted C_2-C_9 heteroaryl;

R^{55} is H or NR^a, where R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

each of X^5 and X^6 is, independently, N or CR^{56};

each of X^7 and X^8 is, independently, N or CH;

each R^{56} is, independently, H or NR^a, where R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{57} is optionally substituted C_2-C_{10} heterocyclyl;

each of Y^2 and Y^3 is, independently, N or CR^{58}; and

each R^{58} is, independently, H or optionally substituted C_1-C_6 alkyl,

where if R^{53} is H and R^{54} is H, then R^{55} is NR^a; if R^{54} is H and R^{55} is H, then R^{53} is optionally substituted C_3-C_{10} carbocyclyl; and if R^{53} is H and R^{55} is H, then R^{54} is optionally substituted C_2-C_9 heteroaryl, or a pharmaceutically acceptable salt thereof.

In some embodiments, A has the structure of **Formula E-3**.

In some embodiments, A has the structure of **Formula E-4**.

In some embodiments, A has the structure of **Formula G-2**.

In some embodiments, A has the structure of **Formula G-3**.

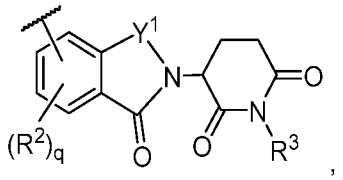
In some embodiments, A has the structure of **Formula E-5**.

In some embodiments, s is 0, 1, or 2.

In some embodiments, the degradation moiety is a ubiquitin ligase binding moiety.

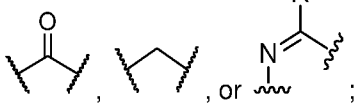
In some embodiments, the ubiquitin ligase binding moiety includes Cereblon ligands, IAP (Inhibitors of Apoptosis) ligands, mouse double minute 2 homolog (MDM2), or von Hippel-Lindau ligands, or derivatives or analogs thereof.

In some embodiments, the degradation moiety has the structure of **Formula A-1**:



Formula A-1

where

Y^1 is  ;

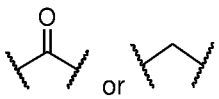
R^3 and R^4 are, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

q is 0, 1, 2, 3, or 4; and

each R^2 is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino, or a pharmaceutically acceptable salt thereof.

In some embodiments, R^3 is H or optionally substituted C_1-C_6 alkyl.

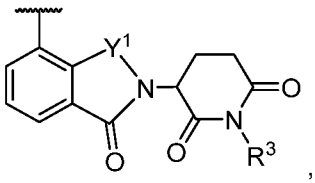
In some embodiments, R^3 is H or CH_3. In some embodiments, R^3 is H. In some embodiments, R^3 is CH_3.

In some embodiments, Y^1 is  . In some embodiments, Y^1 is  .

In some embodiments, each R^2 is, independently, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, hydroxyl, or optionally substituted amino.

In some embodiments, q is 0 or 1. In some embodiments, q is 0.

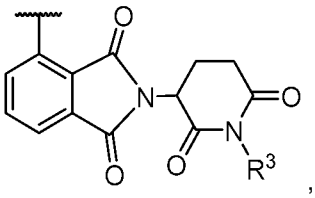
In some embodiments, the structure of **Formula A-1** has the structure of **Formula A-1a**:



Formula A-1a

or a pharmaceutically acceptable salt thereof.

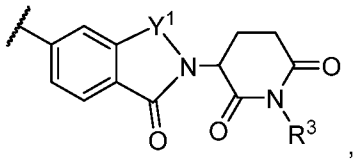
In some embodiments, the structure of **Formula A-1** has the structure of **Formula A-1b**:



Formula A-1b

or a pharmaceutically acceptable salt thereof.

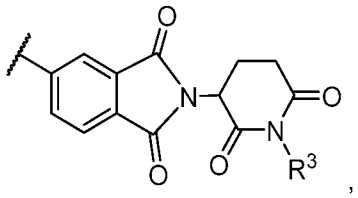
In some embodiments, the structure of **Formula A-1c** has the structure of **Formula A-1c**:



Formula A-1c

or a pharmaceutically acceptable salt thereof.

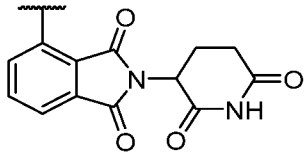
In some embodiments, the structure of **Formula A-1** has the structure of **Formula A-1d**:



Formula A-1d

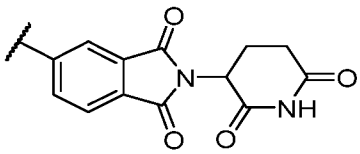
or a pharmaceutically acceptable salt thereof.

In some embodiments, the degradation moiety has the structure:



1a

or



1b

or is a derivative or an analog thereof.

In some embodiments, the linker has the structure of **Formula II**:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where

A^1 is a bond between the linker and A;

A^2 is a bond between B and the linker;

each of B^1, B^2, B^3, and B^4 is, independently, optionally substituted C_1-C_2 alkyl, optionally substituted C_1-C_3 heteroalkyl, O, S, $S(O)_2$, or NR^N;

R^N is H, optionally substituted C_{1-4} alkyl, optionally substituted C_{2-4} alkenyl, optionally substituted C_{2-4} alkynyl, optionally substituted C_{2-6} heterocyclyl, optionally substituted C_{6-12} aryl, or optionally substituted C_{1-7} heteroalkyl;

each of C^1 and C^2 is, independently, carbonyl, thiocarbonyl, sulphonyl, or phosphoryl;

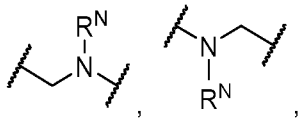
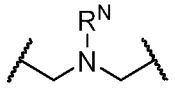
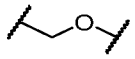
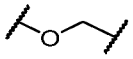
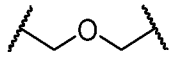
f, g, h, I, j, and k are each, independently, 0 or 1; and

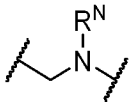
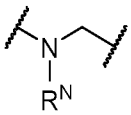
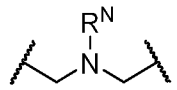
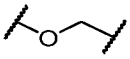
D is optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} alkynyl, optionally substituted C_{2-6} heterocyclyl, optionally substituted C_{6-12} aryl, optionally substituted C_2-C_{10} polyethylene glycol, or optionally substituted C_{1-10} heteroalkyl, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2.

In some embodiments, each of B^1, B^2, B^3, and B^4 is, independently, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 heteroalkyl, or NR^N.

In some embodiments, R^N is H or optionally substituted C_{1-4} alkyl.

In some embodiments, R^N is H or CH_3.

In some embodiments, each of B^1 and B^4 is, independently,  , ,  ,  ,  , or  .

In some embodiments, B^1 is  ,  ,  , or  .

In some embodiments, each of C^1 and C^2 is, independently,  or  . In some embodiments, C^1 is  .

In some embodiments, B^2 is NR^N.

In some embodiments, B^2 is optionally substituted C_1-C_4 alkyl.

In some embodiments, f is 0. In some embodiments, f is 1.

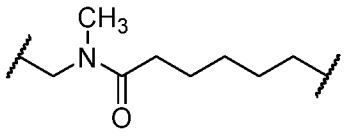
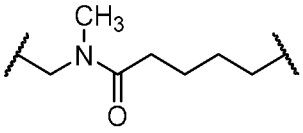
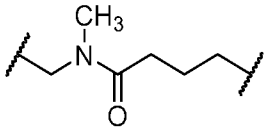
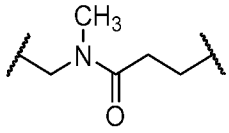
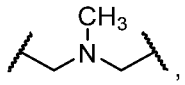
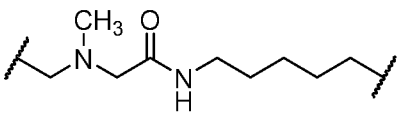
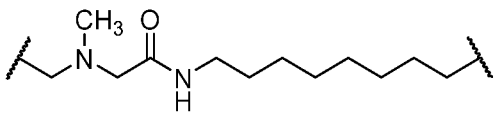
In some embodiments, g is 0. In some embodiments, g is 1.

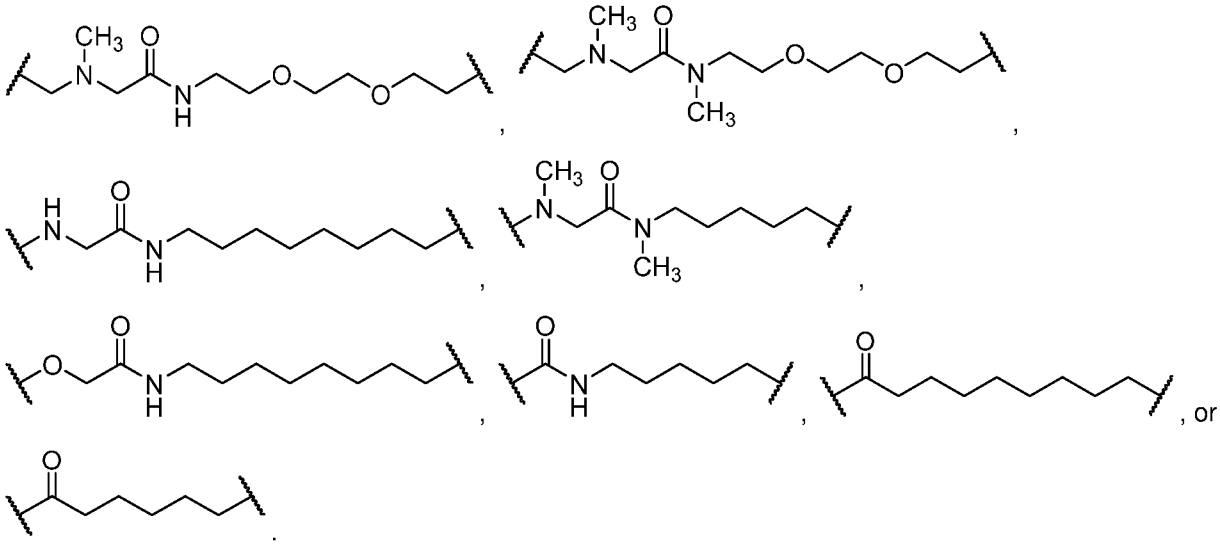
In some embodiments, h is 0. In some embodiments, h is 1.

In some embodiments, i is 0. In some embodiments, i is 1.

In some embodiments, j is 0. In some embodiments, j is 1.

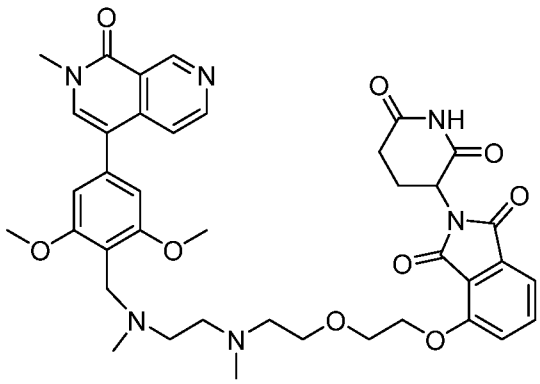
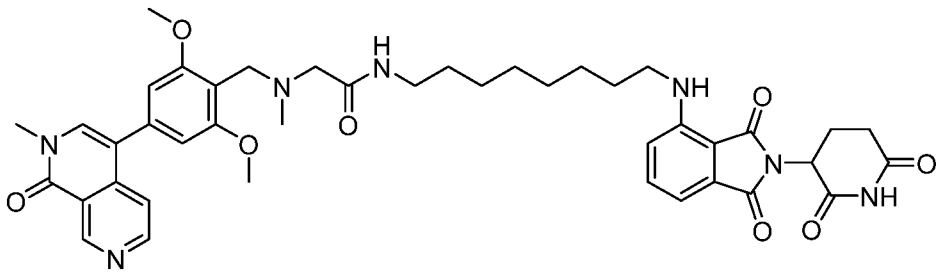
In some embodiments, k is 0. In some embodiments, k is 1.

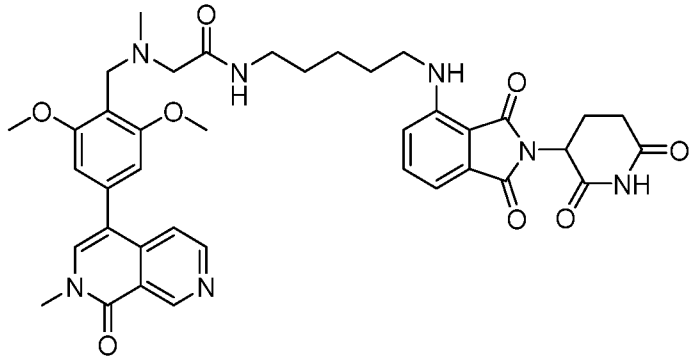
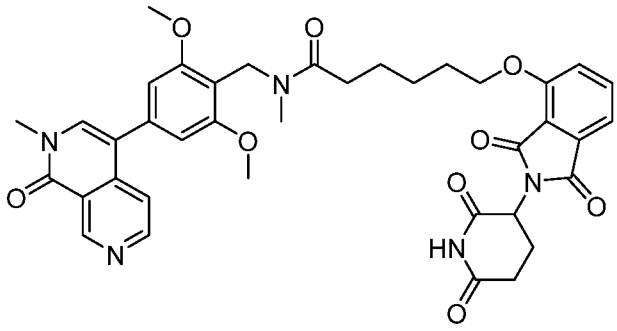
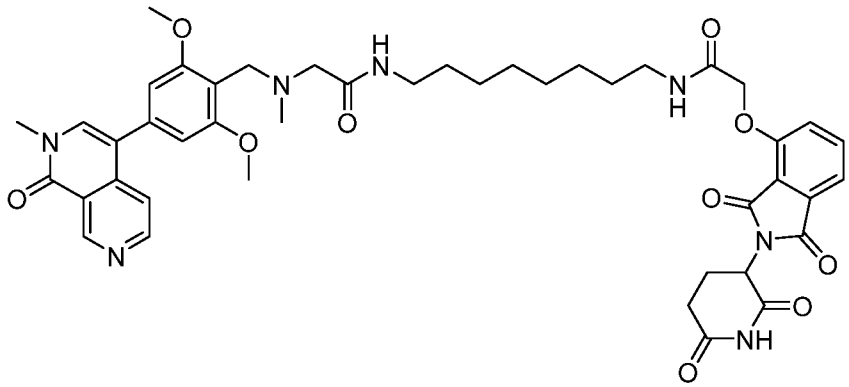
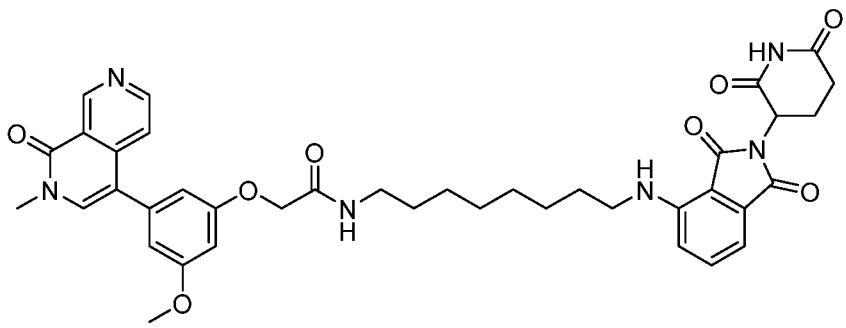
In some embodiments, the linker has the structure of  ,  ,  ,  ,  ,  ,  ,

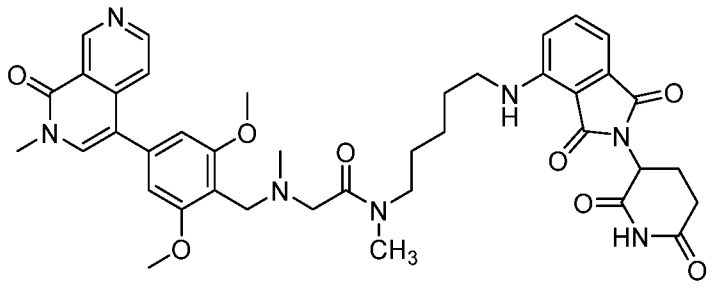
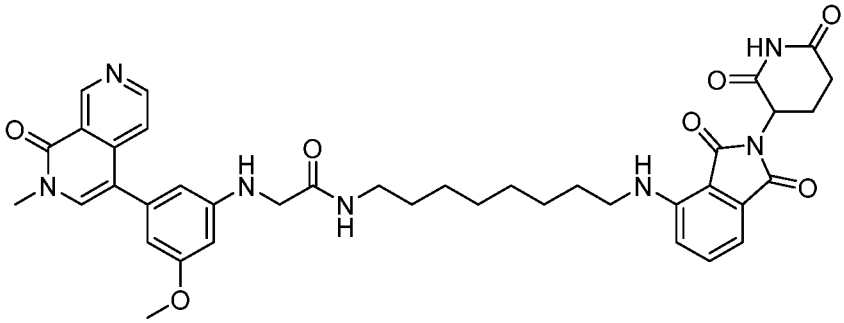
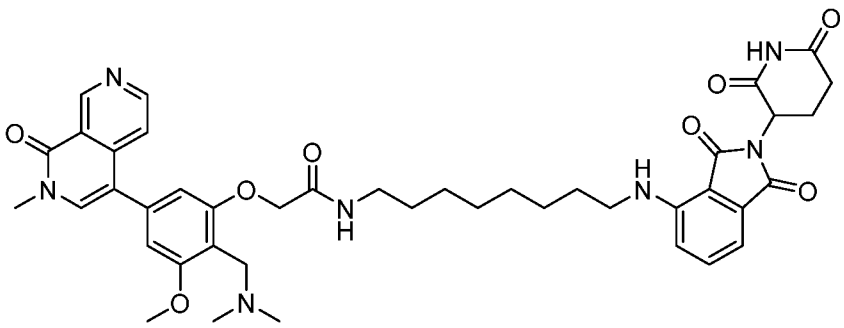
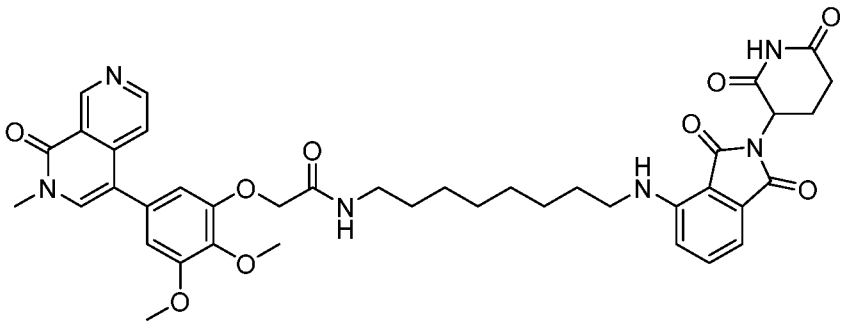


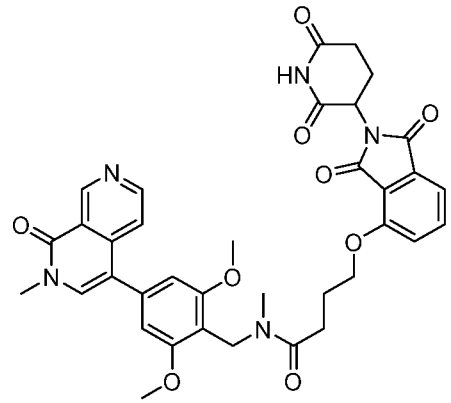
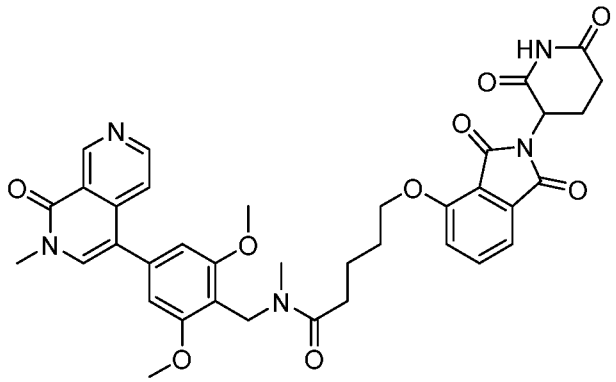
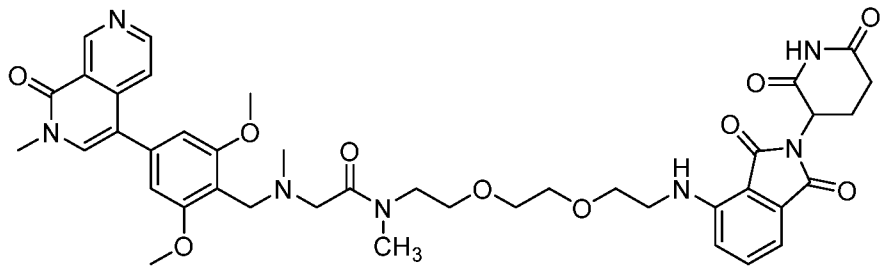
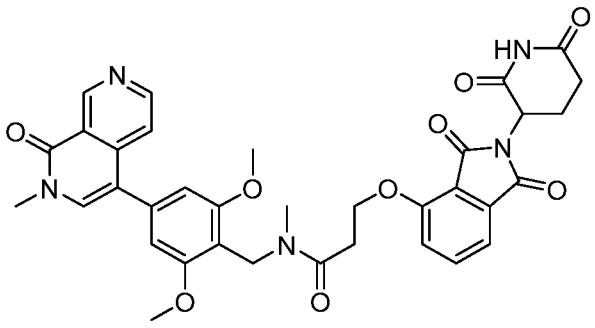
In some embodiments, the compound has the structure of any of compounds D1-D20 in Table 2. In some embodiments, the compound has the structure of any of compounds D1-D17 in Table 2. In some embodiments, the compound has the structure of any of compounds D18-D20 in Table 2.

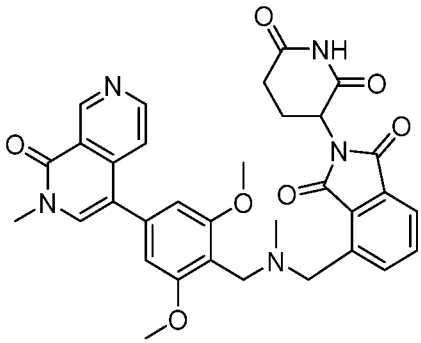
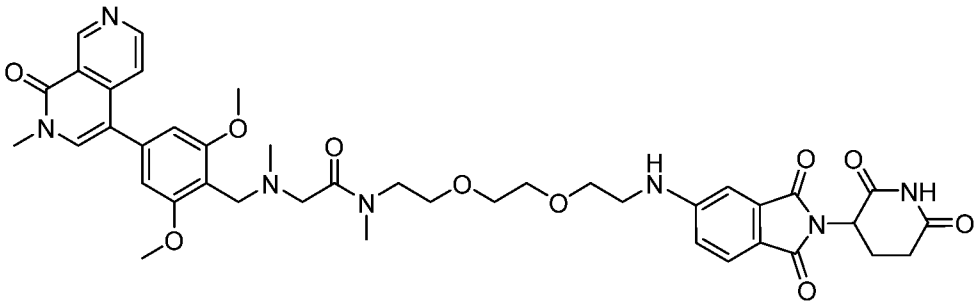
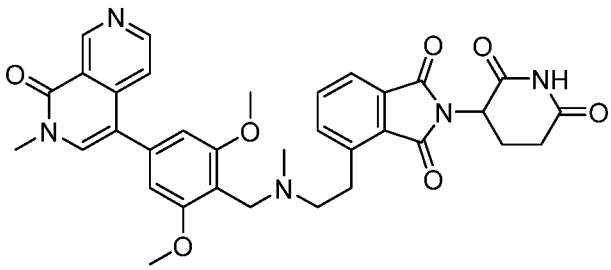
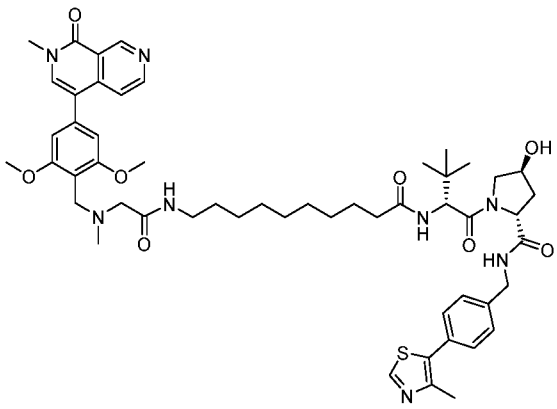
Table 2. Compounds D1-D20 of the Disclosure

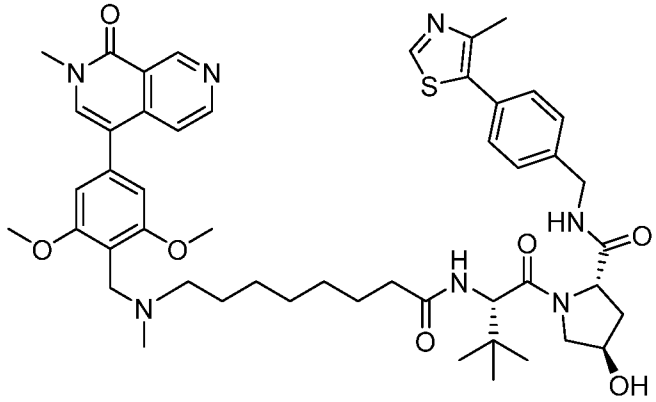
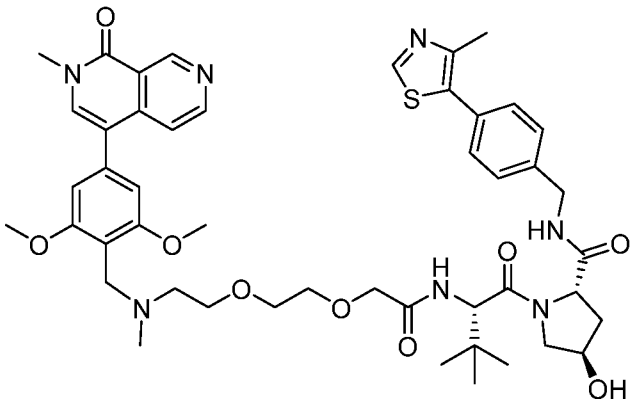
Compound No.	Structure
D1	
D2	

Compound No.	Structure
D3	
D4	
D5	
D6	

Compound No.	Structure
D7	
D8	
D9	
D10	

Compound No.	Structure
D11	
D12	
D13	
D14	

Compound No.	Structure
D15	
D16	
D17	
D18	

Compound No.	Structure
D19	
D20	

In another aspect, the disclosure features a pharmaceutical composition including any of the foregoing compounds and a pharmaceutically acceptable excipient.

In yet another aspect, the disclosure features a method of treating a cancer in a subject in need thereof, the method including administering to the subject an effective amount of any of the foregoing compounds or any of the foregoing pharmaceutical compositions.

In another aspect, the disclosure features a method of treating a cancer related to BRD9 inhibition in a subject in need thereof, the method including administering to the subject an effective amount of any of the foregoing compounds or any of the foregoing pharmaceutical compositions.

In some embodiments, the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

Chemical Terms

For any of the following chemical definitions, a number following an atomic symbol indicates that total number of atoms of that element that are present in a particular chemical moiety. As will be understood, other atoms, such as hydrogen atoms, or substituent groups, as described herein, may be present, as necessary, to satisfy the valences of the atoms. For example, an unsubstituted C_2 alkyl group has the formula $–CH_2CH_3$. When used with the groups defined herein, a reference to the number of carbon atoms includes the divalent carbon in acetal and ketal groups but does not include the carbonyl

carbon in acyl, ester, carbonate, or carbamate groups. A reference to the number of oxygen, nitrogen, or sulfur atoms in a heteroaryl group only includes those atoms that form a part of a heterocyclic ring.

The term "acyl," as used herein, represents a hydrogen or an alkyl group that is attached to a parent molecular group through a carbonyl group, as defined herein, and is exemplified by formyl (i.e., a carboxyaldehyde group), acetyl, trifluoroacetyl, propionyl, and butanoyl. Exemplary unsubstituted acyl groups include from 1 to 6, from 1 to 11, or from 1 to 21 carbons.

The term "alkyl," as used herein, refers to a branched or straight-chain monovalent saturated aliphatic hydrocarbon radical of 1 to 20 carbon atoms (e.g., 1 to 16 carbon atoms, 1 to 10 carbon atoms, or 1 to 6 carbon atoms).

An alkylene is a divalent alkyl group. The term "alkenyl," as used herein, alone or in combination with other groups, refers to a straight chain or branched hydrocarbon residue having a carbon-carbon double bond and having 2 to 20 carbon atoms (e.g., 2 to 16 carbon atoms, 2 to 10 carbon atoms, 2 to 6, or 2 carbon atoms).

The term "alkynyl," as used herein, alone or in combination with other groups, refers to a straight chain or branched hydrocarbon residue having a carbon-carbon triple bond and having 2 to 20 carbon atoms (e.g., 2 to 16 carbon atoms, 2 to 10 carbon atoms, 2 to 6, or 2 carbon atoms).

The term "amino," as used herein, represents $-N(R^{N1})_2$, wherein each R^{N1} is, independently, H, OH, NO_2, $N(R^{N2})_2$, SO_2OR^{N2}, SO_2R^{N2}, SOR^{N2}, an *N*-protecting group, alkyl, alkoxy, aryl, arylalkyl, cycloalkyl, acyl (e.g., acetyl, trifluoroacetyl, or others described herein), wherein each of these recited R^{N1} groups can be optionally substituted; or two R^{N1} combine to form an alkylene or heteroalkylene, and wherein each R^{N2} is, independently, H, alkyl, or aryl. The amino groups of the compounds described herein can be an unsubstituted amino (i.e., $-NH_2$) or a substituted amino (i.e., $-N(R^{N1})_2$).

The term "aryl," as used herein, refers to an aromatic mono- or polycarbocyclic radical of 6 to 12 carbon atoms having at least one aromatic ring. Examples of such groups include, but are not limited to, phenyl, naphthyl, 1,2,3,4-tetrahydronaphthyl, 1,2-dihydronaphthyl, indanyl, and 1H-indenyl.

The term "arylalkyl," as used herein, represents an alkyl group substituted with an aryl group. Exemplary unsubstituted arylalkyl groups are from 7 to 30 carbons (e.g., from 7 to 16 or from 7 to 20 carbons, such as C_1-C_6 alkyl C_6-C_{10} aryl, C_1-C_{10} alkyl C_6-C_{10} aryl, or C_1-C_{20} alkyl C_6-C_{10} aryl), such as, benzyl and phenethyl. In some embodiments, the alkyl and the aryl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective groups.

The term "azido," as used herein, represents a $-N_3$ group.

The term "bridged cyclyl," as used herein, refers to a bridged polycyclic group of 5 to 20 atoms, containing from 1 to 3 bridges. Bridged cyclyl includes bridged carbocyclyl (e.g., norbornyl) and bridged heterocyclyl (e.g., 1,4-diazabicyclo[2.2.2]octane).

The term "cyano," as used herein, represents a –CN group.

The term "carbocyclyl," as used herein, refers to a non-aromatic C_3-C_{12} monocyclic or polycyclic (e.g., bicyclic or tricyclic) structure in which the rings are formed by carbon atoms. Carbocyclyl structures include cycloalkyl groups and unsaturated carbocyclyl radicals. Polycyclic carbocyclyl includes spirocyclic carbocyclyl, bridged carbocyclyl, and fused carbocyclyl.

The term "cycloalkyl," as used herein, refers to a saturated, non-aromatic, monovalent mono- or polycarbocyclic radical of 3 to 10, preferably 3 to 6 carbon atoms. This term is further exemplified by radicals such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, norbornyl, and adamantyl.

The term "halogen," as used herein, means a fluorine (fluoro), chlorine (chloro), bromine (bromo), or iodine (iodo) radical.

The term "heteroalkyl," as used herein, refers to an alkyl group, as defined herein, in which one or more of the constituent carbon atoms have been replaced by nitrogen, oxygen, or sulfur. In some embodiments, the heteroalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups. Examples of heteroalkyl groups are an "alkoxy" which, as used herein, refers alkyl–O– (e.g., methoxy and ethoxy). A heteroalkylene is a divalent heteroalkyl group. The term "heteroalkenyl," as used herein, refers to an alkenyl group, as defined herein, in which one or more of the constituent carbon atoms have been replaced by nitrogen, oxygen, or sulfur. In some embodiments, the heteroalkenyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkenyl groups. Examples of heteroalkenyl groups are an "alkenoxy" which, as used herein, refers alkenyl–O–. A heteroalkenylene is a divalent heteroalkenyl group. The term "heteroalkynyl," as used herein, refers to an alkynyl group, as defined herein, in which one or more of the constituent carbon atoms have been replaced by nitrogen, oxygen, or sulfur. In some embodiments, the heteroalkynyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkynyl groups. Examples of heteroalkynyl groups are an "alkynoxy" which, as used herein, refers alkynyl–O–. A heteroalkynylene is a divalent heteroalkynyl group.

The term "heteroaryl," as used herein, refers to an aromatic monocyclic or polycyclic structure of 5 to 12 atoms having at least one aromatic ring containing 1, 2, or 3 ring atoms selected from nitrogen, oxygen, and sulfur, with the remaining ring atoms being carbon. One or two ring carbon atoms of the heteroaryl group may be replaced with a carbonyl group. Examples of heteroaryl groups are pyridyl, pyrazoyl, benzooxazolyl, benzoimidazolyl, benzothiazolyl, imidazolyl, oxaxolyl, and thiazolyl.

The term "heteroarylalkyl," as used herein, represents an alkyl group substituted with a heteroaryl group. Exemplary unsubstituted heteroarylalkyl groups are from 7 to 30 carbons (e.g., from 7 to 16 or from 7 to 20 carbons, such as C_1-C_6 alkyl C_2-C_9 heteroaryl, C_1-C_{10} alkyl C_2-C_9 heteroaryl, or C_1-C_{20} alkyl C_2-C_9 heteroaryl). In some embodiments, the alkyl and the heteroaryl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective groups.

The term "heterocyclyl," as used herein, refers a monocyclic or polycyclic (e.g., bicyclic or tricyclic) structure having 3 to 12 atoms having at least one ring containing 1, 2, 3, or 4 ring atoms selected from N, O or S, wherein no ring is aromatic. Polycyclic heterocyclyl includes spirocyclic heterocyclyl, bridged heterocyclyl, and fused heterocyclyl. Examples of heterocyclyl groups include, but are not limited to, morpholinyl, thiomorpholinyl, furyl, piperazinyl, piperidinyl, pyranyl, pyrrolidinyl, tetrahydropyranyl, tetrahydrofuranyl, and 1,3-dioxanyl.

The term "heterocyclylalkyl," as used herein, represents an alkyl group substituted with a heterocyclyl group. Exemplary unsubstituted heterocyclylalkyl groups are from 7 to 30 carbons (e.g., from 7 to 16 or from 7 to 20 carbons, such as C_1-C_6 alkyl C_2-C_9 heterocyclyl, C_1-C_{10} alkyl C_2-C_9 heterocyclyl, or C_1-C_{20} alkyl C_2-C_9 heterocyclyl). In some embodiments, the alkyl and the heterocyclyl

each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective groups.

The term "hydroxyalkyl," as used herein, represents alkyl group substituted with an –OH group.

The term "hydroxyl," as used herein, represents an –OH group.

The term "*N*-protecting group," as used herein, represents those groups intended to protect an amino group against undesirable reactions during synthetic procedures. Commonly used *N*-protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis," 3rd Edition (John Wiley & Sons, New York, 1999). *N*-protecting groups include, but are not limited to, acyl, aryloyl, or carbamyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, α-chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and chiral auxiliaries such as protected or unprotected D, L, or D, L-amino acids such as alanine, leucine, and phenylalanine; sulfonyl-containing groups such as benzenesulfonyl, and p-toluenesulfonyl; carbamate forming groups such as benzyloxycarbonyl, p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4- 20 dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenylyl)-1-methylethoxycarbonyl, α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxy carbonyl, t-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2,-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxy carbonyl, fluorenyl-9-methoxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, and phenylthiocarbonyl, arylalkyl groups such as benzyl, triphenylmethyl, and benzyloxymethyl, and silyl groups, such as trimethylsilyl. Preferred *N*-protecting groups are alloc, formyl, acetyl, benzoyl, pivaloyl, t-butylacetyl, alanyl, phenylsulfonyl, benzyl, t-butyloxycarbonyl (Boc), and benzyloxycarbonyl (Cbz).

The term "nitro," as used herein, represents an $–NO_2$ group.

The term "thiol," as used herein, represents an –SH group.

The alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl (e.g., cycloalkyl), aryl, heteroaryl, and heterocyclyl groups may be substituted or unsubstituted. When substituted, there will generally be 1 to 4 substituents present, unless otherwise specified. Substituents include, for example: alkyl (e.g., unsubstituted and substituted, where the substituents include any group described herein, e.g., aryl, halo, hydroxyl), aryl (e.g., substituted and unsubstituted phenyl), carbocyclyl (e.g., substituted and unsubstituted cycloalkyl), halogen (e.g., fluoro), hydroxyl, heteroalkyl (e.g., substituted and unsubstituted methoxy, ethoxy, or thioalkoxy), heteroaryl, heterocyclyl, amino (e.g., NH_2 or mono- or dialkyl amino), azido, cyano, nitro, or thiol. Aryl, carbocyclyl (e.g., cycloalkyl), heteroaryl, and heterocyclyl groups may also be substituted with alkyl (unsubstituted and substituted such as arylalkyl (e.g., substituted and unsubstituted benzyl)).

Compounds described herein can have one or more asymmetric carbon atoms and can exist in the form of optically pure enantiomers, mixtures of enantiomers such as, for example, racemates, optically pure diastereoisomers, mixtures of diastereoisomers, diastereoisomeric racemates, or mixtures of diastereoisomeric racemates. The optically active forms can be obtained for example by resolution of the racemates, by asymmetric synthesis or asymmetric chromatography (chromatography with a chiral

adsorbent or eluant). That is, certain of the disclosed compounds may exist in various stereoisomeric forms. Stereoisomers are compounds that differ only in their spatial arrangement. Enantiomers are pairs of stereoisomers whose mirror images are not superimposable, most commonly because they contain an asymmetrically substituted carbon atom that acts as a chiral center. "Enantiomer" means one of a pair of molecules that are mirror images of each other and are not superimposable. Diastereomers are stereoisomers that are not related as mirror images, most commonly because they contain two or more asymmetrically substituted carbon atoms and represent the configuration of substituents around one or more chiral carbon atoms. Enantiomers of a compound can be prepared, for example, by separating an enantiomer from a racemate using one or more well-known techniques and methods, such as, for example, chiral chromatography and separation methods based thereon. The appropriate technique and/or method for separating an enantiomer of a compound described herein from a racemic mixture can be readily determined by those of skill in the art. "Racemate" or "racemic mixture" means a compound containing two enantiomers, wherein such mixtures exhibit no optical activity; i.e., they do not rotate the plane of polarized light. “Geometric isomer" means isomers that differ in the orientation of substituent atoms in relationship to a carbon-carbon double bond, to a cycloalkyl ring, or to a bridged bicyclic system. Atoms (other than H) on each side of a carbon- carbon double bond may be in an E (substituents are on opposite sides of the carbon- carbon double bond) or Z (substituents are oriented on the same side) configuration. "R," "S," "S*," "R*," "E," "Z," "cis," and "trans," indicate configurations relative to the core molecule. Certain of the disclosed compounds may exist in atropisomeric forms. Atropisomers are stereoisomers resulting from hindered rotation about single bonds where the steric strain barrier to rotation is high enough to allow for the isolation of the conformers. The compounds described herein may be prepared as individual isomers by either isomer-specific synthesis or resolved from an isomeric mixture. Conventional resolution techniques include forming the salt of a free base of each isomer of an isomeric pair using an optically active acid (followed by fractional crystallization and regeneration of the free base), forming the salt of the acid form of each isomer of an isomeric pair using an optically active amine (followed by fractional crystallization and regeneration of the free acid), forming an ester or amide of each of the isomers of an isomeric pair using an optically pure acid, amine or alcohol (followed by chromatographic separation and removal of the chiral auxiliary), or resolving an isomeric mixture of either a starting material or a final product using various well known chromatographic methods. When the stereochemistry of a disclosed compound is named or depicted by structure, the named or depicted stereoisomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by weight relative to the other stereoisomers. When a single enantiomer is named or depicted by structure, the depicted or named enantiomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by weight optically pure. When a single diastereomer is named or depicted by structure, the depicted or named diastereomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by weight pure. Percent optical purity is the ratio of the weight of the enantiomer or over the weight of the enantiomer plus the weight of its optical isomer. Diastereomeric purity by weight is the ratio of the weight of one diastereomer or over the weight of all the diastereomers. When the stereochemistry of a disclosed compound is named or depicted by structure, the named or depicted stereoisomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by mole fraction pure relative to the other stereoisomers. When a single enantiomer is named or depicted by structure, the depicted or named enantiomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by mole fraction pure. When a single

diastereomer is named or depicted by structure, the depicted or named diastereomer is at least 60%, 70%, 80%, 90%, 99%, or 99.9% by mole fraction pure. Percent purity by mole fraction is the ratio of the moles of the enantiomer or over the moles of the enantiomer plus the moles of its optical isomer. Similarly, percent purity by moles fraction is the ratio of the moles of the diastereomer or over the moles of the diastereomer plus the moles of its isomer. When a disclosed compound is named or depicted by structure without indicating the stereochemistry, and the compound has at least one chiral center, it is to be understood that the name or structure encompasses either enantiomer of the compound free from the corresponding optical isomer, a racemic mixture of the compound, or mixtures enriched in one enantiomer relative to its corresponding optical isomer. When a disclosed compound is named or depicted by structure without indicating the stereochemistry and has two or more chiral centers, it is to be understood that the name or structure encompasses a diastereomer free of other diastereomers, a number of diastereomers free from other diastereomeric pairs, mixtures of diastereomers, mixtures of diastereomeric pairs, mixtures of diastereomers in which one diastereomer is enriched relative to the other diastereomer(s), or mixtures of diastereomers in which one or more diastereomer is enriched relative to the other diastereomers. The invention embraces all of these forms.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Definitions

In this application, unless otherwise clear from context, (i) the term "a" may be understood to mean "at least one"; (ii) the term "or" may be understood to mean "and/or"; and (iii) the terms "including" and "including" may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps.

As used herein, the terms "about" and "approximately" refer to a value that is within 10% above or below the value being described. For example, the term "about 5 nM" indicates a range of from 4.5 to 5.5 nM.

As used herein, the term "administration" refers to the administration of a composition (e.g., a compound or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intratumoral, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal, and vitreal.

As used herein, the term "adult soft tissue sarcoma" refers to a sarcoma that develops in the soft tissues of the body, typically in adolescent and adult subjects (e.g., subjects who are at least 10 years

old, 11 years old, 12 years old, 13 years old, 14 years old, 15 years old, 16 years old, 17 years old, 18 years old, or 19 years old). Non-limiting examples of adult soft tissue sarcoma include, but are not limited to, synovial sarcoma, fibrosarcoma, malignant fibrous histiocytoma, dermatofibrosarcoma, liposarcoma, leiomyosarcoma, hemangiosarcoma, Kaposi's sarcoma, lymphangiosarcoma, malignant peripheral nerve sheath tumor/neurofibrosarcoma, extraskeletal chondrosarcoma, extraskeletal osteosarcoma, extraskeletal myxoid chondrosarcoma, and extraskeletal mesenchymal.

As used herein, the term "BAF complex" refers to the BRG1- or HRBM-associated factors complex in a human cell.

As used herein, the terms "GBAF complex" and "GBAF" refer to a SWI/SNF ATPase chromatin remodeling complex in a human cell. GBAF complex subunits may include, but are not limited to, ACTB, ACTL6A, ACTL6B, BICRA, BICRAL, BRD9, SMARCA2, SMARCA4, SMARCC1, SMARCD1, SMARCD2, SMARCD3, and SS18.The term "cancer" refers to a condition caused by the proliferation of malignant neoplastic cells, such as tumors, neoplasms, carcinomas, sarcomas, leukemias, and lymphomas.

As used herein, a "combination therapy" or "administered in combination" means that two (or more) different agents or treatments are administered to a subject as part of a defined treatment regimen for a particular disease or condition. The treatment regimen defines the doses and periodicity of administration of each agent such that the effects of the separate agents on the subject overlap. In some embodiments, the delivery of the two or more agents is simultaneous or concurrent and the agents may be co-formulated. In some embodiments, the two or more agents are not co-formulated and are administered in a sequential manner as part of a prescribed regimen. In some embodiments, administration of two or more agents or treatments in combination is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one agent or treatment delivered alone or in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive (e.g., synergistic). Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination may be administered by intravenous injection while a second therapeutic agent of the combination may be administered orally.

As used herein, the term "BRD9" refers to bromodomain-containing protein 9, a component of the BAF (BRG1- or BRM-associated factors) complex, a SWI/SNF ATPase chromatin remodeling complex, and belongs to family IV of the bromodomain-containing proteins. BRD9 is encoded by the *BRD9* gene, the nucleic acid sequence of which is set forth in SEQ ID NO: 1. The term "BRD9" also refers to natural variants of the wild-type BRD9 protein, such as proteins having at least 85% identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9% identity, or more) to the amino acid sequence of wild-type BRD9, which is set forth in SEQ ID NO: 2.

As used herein, the term "degrader" refers to a small molecule compound including a degradation moiety, wherein the compound interacts with a protein (e.g., BRD9) in a way which results in degradation of the protein, e.g., binding of the compound results in at least 5% reduction of the level of the protein, e.g., in a cell or subject.

As used herein, the term "degradation moiety" refers to a moiety whose binding results in degradation of a protein, e.g., BRD9. In one example, the moiety binds to a protease or a ubiquitin ligase that metabolizes the protein, e.g., BRD9.

By "determining the level of a protein" is meant the detection of a protein, or an mRNA encoding the protein, by methods known in the art either directly or indirectly. "Directly determining" means performing a process (e.g., performing an assay or test on a sample or "analyzing a sample" as that term is defined herein) to obtain the physical entity or value. "Indirectly determining" refers to receiving the physical entity or value from another party or source (e.g., a third-party laboratory that directly acquired the physical entity or value). Methods to measure protein level generally include, but are not limited to, western blotting, immunoblotting, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, immunofluorescence, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, liquid chromatography (LC)-mass spectrometry, microcytometry, microscopy, fluorescence activated cell sorting (FACS), and flow cytometry, as well as assays based on a property of a protein including, but not limited to, enzymatic activity or interaction with other protein partners. Methods to measure mRNA levels are known in the art.

By "modulating the activity of a BAF complex," is meant altering the level of an activity related to a BAF complex (e.g., GBAF), or a related downstream effect. The activity level of a BAF complex may be measured using any method known in the art, e.g., the methods described in Kadoch et al, Cell 153:71-85 (2013), the methods of which are herein incorporated by reference.

By "reducing the activity of BRD9," is meant decreasing the level of an activity related to an BRD9, or a related downstream effect. A non-limiting example of inhibition of an activity of BRD9 is decreasing the level of a BAF complex (e.g., GBAF) in a cell. The activity level of BRD9 may be measured using any method known in the art. In some embodiments, an agent which reduces the activity of BRD9 is a small molecule BRD9 inhibitor. In some embodiments, an agent which reduces the activity of BRD9 is a small molecule BRD9 degrader.

By "reducing the level of BRD9," is meant decreasing the level of BRD9 in a cell or subject. The level of BRD9 may be measured using any method known in the art.

By "level" is meant a level of a protein, or mRNA encoding the protein, as compared to a reference. The reference can be any useful reference, as defined herein. By a "decreased level" or an "increased level" of a protein is meant a decrease or increase in protein level, as compared to a reference (e.g., a decrease or an increase by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 150%, about 200%, about 300%, about 400%, about 500%, or more; a decrease or an increase of more than about 10%, about 15%, about 20%, about 50%, about 75%, about 100%, or about 200%, as compared to a reference; a decrease or an increase by less than about 0.01-fold, about 0.02-fold, about 0.1-fold, about 0.3-fold, about 0.5-fold, about 0.8-fold, or less; or an increase by more than about 1.2-fold, about 1.4-fold, about 1.5-fold, about 1.8-fold, about 2.0-fold, about 3.0-fold, about 3.5-fold, about 4.5-fold, about 5.0-fold, about 10-fold, about 15-fold, about 20-fold, about 30-fold, about 40-fold, about 50-fold, about 100-fold,

about 1000-fold, or more). A level of a protein may be expressed in mass/vol (e.g., g/dL, mg/mL, µg/mL, ng/mL) or percentage relative to total protein or mRNA in a sample.

As used herein, the term "inhibitor" refers to any agent which reduces the level and/or activity of a protein (e.g., BRD9). Non-limiting examples of inhibitors include small molecule inhibitors, degraders, antibodies, enzymes, or polynucleotides (e.g., siRNA).

As used herein, the terms "effective amount," "therapeutically effective amount," and "a "sufficient amount" of an agent that reduces the level and/or activity of BRD9 (e.g., in a cell or a subject) described herein refer to a quantity sufficient to, when administered to the subject, including a human, effect beneficial or desired results, including clinical results, and, as such, an "effective amount" or synonym thereto depends on the context in which it is being applied. For example, in the context of treating cancer, it is an amount of the agent that reduces the level and/or activity of BRD9 sufficient to achieve a treatment response as compared to the response obtained without administration of the agent that reduces the level and/or activity of BRD9. The amount of a given agent that reduces the level and/or activity of BRD9 described herein that will correspond to such an amount will vary depending upon various factors, such as the given agent, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, and/or weight) or host being treated, and the like, but can nevertheless be routinely determined by one of skill in the art. Also, as used herein, a "therapeutically effective amount" of an agent that reduces the level and/or activity of BRD9 of the present disclosure is an amount which results in a beneficial or desired result in a subject as compared to a control. As defined herein, a therapeutically effective amount of an agent that reduces the level and/or activity of BRD9 of the present disclosure may be readily determined by one of ordinary skill by routine methods known in the art. Dosage regimen may be adjusted to provide the optimum therapeutic response.

The term "inhibitory RNA agent" refers to an RNA, or analog thereof, having sufficient sequence complementarity to a target RNA to direct RNA interference. Examples also include a DNA that can be used to make the RNA. RNA interference (RNAi) refers to a sequence-specific or selective process by which a target molecule (e.g., a target gene, protein, or RNA) is down-regulated. Generally, an interfering RNA ("iRNA") is a double-stranded short-interfering RNA (siRNA), short hairpin RNA (shRNA), or single-stranded micro-RNA (miRNA) that results in catalytic degradation of specific mRNAs, and also can be used to lower or inhibit gene expression.

The terms "short interfering RNA" and "siRNA" (also known as "small interfering RNAs") refer to an RNA agent, preferably a double-stranded agent, of about 10-50 nucleotides in length, the strands optionally having overhanging ends comprising, for example 1, 2 or 3 overhanging nucleotides (or nucleotide analogs), which is capable of directing or mediating RNA interference. Naturally-occurring siRNAs are generated from longer dsRNA molecules (e.g., >25 nucleotides in length) by a cell's RNAi machinery (e.g., Dicer or a homolog thereof).

The term "shRNA", as used herein, refers to an RNA agent having a stem-loop structure, comprising a first and second region of complementary sequence, the degree of complementarity and orientation of the regions being sufficient such that base pairing occurs between the regions, the first and second regions being joined by a loop region, the loop resulting from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region.

The terms "miRNA" and "microRNA" refer to an RNA agent, preferably a single-stranded agent, of about 10-50 nucleotides in length, preferably between about 15-25 nucleotides in length, which is capable of directing or mediating RNA interference. Naturally-occurring miRNAs are generated from stem-loop precursor RNAs (i.e., pre-miRNAs) by Dicer. The term "Dicer" as used herein, includes Dicer as well as any Dicer ortholog or homolog capable of processing dsRNA structures into siRNAs, miRNAs, siRNA-like or miRNA-like molecules. The term microRNA ("miRNA") is used interchangeably with the term "small temporal RNA" ("stRNA") based on the fact that naturally-occurring miRNAs have been found to be expressed in a temporal fashion (e.g., during development).

The term "antisense," as used herein, refers to a nucleic acid comprising a polynucleotide that is sufficiently complementary to all or a portion of a gene, primary transcript, or processed mRNA, so as to interfere with expression of the endogenous gene (e.g., BRD9). "Complementary" polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. It is understood that two polynucleotides may hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other.

The term "antisense nucleic acid" includes single-stranded RNA as well as double-stranded DNA expression cassettes that can be transcribed to produce an antisense RNA. "Active" antisense nucleic acids are antisense RNA molecules that are capable of selectively hybridizing with a primary transcript or mRNA encoding a polypeptide having at least 80% sequence identity (e.g., 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9% identity, or more) with the targeted polypeptide sequence (e.g., a BRD9 polypeptide sequence). The antisense nucleic acid can be complementary to an entire coding strand, or to only a portion thereof. In some embodiments, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence. The term "coding region" refers to the region of the nucleotide sequence comprising codons that are translated into amino acid residues. In some embodiments, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence. The term "noncoding region" refers to 5′ and 3′ sequences that flank the coding region that are not translated into amino acids (i.e., also referred to as 5′ and 3′ untranslated regions). The antisense nucleic acid molecule can be complementary to the entire coding region of mRNA, or can be antisense to only a portion of the coding or noncoding region of an mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides in length.

"Percent (%) sequence identity" with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software.

Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

The term "pharmaceutical composition," as used herein, represents a composition containing a compound described herein formulated with a pharmaceutically acceptable excipient, and manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (e.g., a tablet, capsule, caplet, gelcap, or syrup); for topical administration (e.g., as a cream, gel, lotion, or ointment); for intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); or in any other pharmaceutically acceptable formulation.

A "pharmaceutically acceptable excipient," as used herein, refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a patient. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

As used herein, the term "pharmaceutically acceptable salt" means any pharmaceutically acceptable salt of the compound of any of the compounds described herein. For example, pharmaceutically acceptable salts of any of the compounds described herein include those that are within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and are commensurate with a reasonable benefit/risk

ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., J. Pharmaceutical Sciences 66:1-19, 1977 and in Pharmaceutical Salts: Properties, Selection, and Use, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting a free base group with a suitable organic acid.

The compounds described herein may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds described herein, be prepared from inorganic or organic bases. Frequently, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and bases and methods for preparation of the appropriate salts are well-known in the art. Salts may be prepared from pharmaceutically acceptable non-toxic acids and bases including inorganic and organic acids and bases. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, and valerate salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, and magnesium, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, and ethylamine.

By a "reference" is meant any useful reference used to compare protein or mRNA levels. The reference can be any sample, standard, standard curve, or level that is used for comparison purposes. The reference can be a normal reference sample or a reference standard or level. A "reference sample" can be, for example, a control, e.g., a predetermined negative control value such as a "normal control" or a prior sample taken from the same subject; a sample from a normal healthy subject, such as a normal cell or normal tissue; a sample (e.g., a cell or tissue) from a subject not having a disease; a sample from a subject that is diagnosed with a disease, but not yet treated with a compound described herein; a sample from a subject that has been treated by a compound described herein; or a sample of a purified protein (e.g., any described herein) at a known normal concentration. By "reference standard or level" is meant a value or number derived from a reference sample. A "normal control value" is a pre-determined value indicative of non-disease state, e.g., a value expected in a healthy control subject. Typically, a normal control value is expressed as a range ("between X and Y"), a high threshold ("no higher than X"), or a low threshold ("no lower than X"). A subject having a measured value within the normal control value for a particular biomarker is typically referred to as "within normal limits" for that biomarker. A normal reference standard or level can be a value or number derived from a normal subject not having a disease or disorder (e.g., cancer); a subject that has been treated with a compound described herein. In preferred embodiments, the reference sample, standard, or level is matched to the sample subject sample by at

least one of the following criteria: age, weight, sex, disease stage, and overall health. A standard curve of levels of a purified protein, e.g., any described herein, within the normal reference range can also be used as a reference.

As used herein, the term "subject" refers to any organism to which a composition in accordance with the invention may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include any animal (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans). A subject may seek or be in need of treatment, require treatment, be receiving treatment, be receiving treatment in the future, or be a human or animal who is under care by a trained professional for a particular disease or condition.

As used herein, the terms "treat," "treated," or "treating" mean both therapeutic treatment and prophylactic or preventative measures wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disorder, or disease, or obtain beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of a condition, disorder, or disease; stabilized (i.e., not worsening) state of condition, disorder, or disease; delay in onset or slowing of condition, disorder, or disease progression; amelioration of the condition, disorder, or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder, or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

As used herein, the terms "variant" and "derivative" are used interchangeably and refer to naturally-occurring, synthetic, and semi-synthetic analogues of a compound, peptide, protein, or other substance described herein. A variant or derivative of a compound, peptide, protein, or other substance described herein may retain or improve upon the biological activity of the original material.

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

Brief Description of the Drawings

FIG. 1 is a series of graphs illustrating the effect of specific guide RNA (sgRNA) targeting of the BRD9 BAF complex subunit on synovial sarcoma cell growth. The Y-axis indicated the dropout ratio. The X-axis indicates the nucleotide position of the BRD9 gene. The grey box indicates the range of the negative control sgRNAs in the screen. The SYO1 cell line carries SS18-SSX2 fusion protein. The breakpoint joining the N-terminal region of SS18 to the C-terminal region of SSX2 are indicated by the black lines in their respective panel. The linear protein sequence is show with BRD9 PFAM domains annotated from the PFAM database.

FIG. 2 is an image illustrating dose dependent depletion of BRD9 levels in a synovial sarcoma cell line (SYO1) in the presence of a BRD9 degrader.

FIG. 3 is an image illustrating sustained suppression of BRD9 levels in a synovial sarcoma cell line (SYO1) in the presence of a BRD9 degrader over 72 hours.

FIG. 4 is an image illustrating sustained suppression of BRD9 levels in two cell lines (293T and SYO1) in the presence of a BRD9 degrader over 5 days.

FIG. 5 is an image illustrating sustained suppression of BRD9 levels in three synovial sarcoma cell lines (293T, SYO1, and Yamato) in the presence of a BRD9 degrader over 7 days compared to the levels in cells treated with CRISPR reagents.

FIG. 6 is an image illustrating the effect on cell growth of six cell lines (SYO1, Yamato, A549, HS-SY-II, ASKA, and 293T) in the presence of a BRD9 degrader and a BRD9 inhibitor.

FIG. 7 is an image illustrating the effect on cell growth of two cell lines (SYO1 and G401) in the presence of a BRD9 degrader.

FIG. 8 is an image illustrating the effect on cell growth of three synovial sarcoma cell lines (SYO1, HS-SY-II, and ASKA) in the presence of a BRD9 degrader, BRD9 binder and E3 ligase binder.

FIG. 9 is an image illustrating the effect on cell growth of three non-synovial sarcoma cell lines (RD, HCT116, and Calu6) in the presence of a BRD9 degrader, BRD9 binder and E3 ligase binder.

FIG. 10 is a graph illustrating the percentage of SYO1 in various cell cycle phases following treatment with DMSO, Compound 1 at 200nM, or Compound 1 at 1μM for 8 or 13 days.

FIG. 11 is a series of contour plots illustrating the percentage of SYO1 cells in various cell cycle phases following treatment with DMSO, Compound 1 at 200nM, Compound 1 at 1μM, or lenalidomide at 200nM for 8 days. Numerical values corresponding to each contour plot are found in the table below.

FIG. 12 is a series of contour plots illustrating the percentage of SYO1 cells in various cell cycle phases following treatment with DMSO, Compound 1 at 200nM, Compound 1 at 1μM, or lenalidomide at 200nM for 13 days. Numerical values corresponding to each contour plot are found in the table below.

FIG. 13 is a series of contour plots illustrating the percentage of early- and late-apoptotic SYO1 cells following treatment with DMSO, Compound 1 at 200nM, Compound 1 at 1μM, or lenalidomide at 200nM for 8 days. Numerical values corresponding to each contour plot are found in the table below.

FIG. 14 is a graph illustrating the proteins present in BAF complexes including the SS18-SSX fusion protein.

Detailed Description

The present inventors have found that depletion of BRD9 in cancer cells results in the depletion of the SS18-SSX fusion protein and further inhibits the proliferation of the cancer cells.

Accordingly, the invention features methods and compositions useful for the inhibition of the activity of the SS18-SSX fusion proteins, e.g., for the treatment of cancer such as adult soft tissue sarcomas. The invention further features methods and compositions useful for inhibition of the activity of the BRD9 protein, e.g., for the treatment of cancer such as adult soft tissue sarcomas, e.g., in a subject in need thereof. Exemplary methods are described herein.

Compounds

Agents described herein that reduce the level and/or activity of BRD9 in a cell may be an antibody, a protein (such as an enzyme), a polynucleotide, or a small molecule compound. The agents reduce the level of an activity related to BRD9, or a related downstream effect, or reduce the level of BRD9 in a cell or subject.

Small Molecule Compounds

In some embodiments of the invention, the agent that reduces the level and/or activity of BRD9 in a cell is a small molecule compound. In some embodiments, the small molecule compound is a structure of **Formula I**:

A-L-B

Formula I

where A is a BRD9 binding moiety; L is a linker; and B is a degradation moiety, or a pharmaceutically acceptable salt thereof. In some embodiments, the degradation moiety is a ubiquitin ligase moiety. In some embodiments, the ubiquitin ligase binding moiety includes Cereblon ligands, IAP (Inhibitors of Apoptosis) ligands, mouse double minute 2 homolog (MDM2), hydrophobic tag, or von Hippel-Lindau ligands, or derivatives or analogs thereof.

Pharmaceutical Uses

The compounds described herein are useful in the methods of the invention and, while not bound by theory, are believed to exert their desirable effects through their ability to modulate the level, status, and/or activity of a BAF complex, e.g., by inhibiting the activity or level of the BRD9 protein in a cell within the BAF complex in a mammal.

An aspect of the present invention relates to methods of treating disorders related to BRD9 such as cancer in a subject in need thereof. In some embodiments, the compound is administered in an amount and for a time effective to result in one of (or more, e.g., two or more, three or more, four or more of): (a) reduced tumor size, (b) reduced rate of tumor growth, (c) increased tumor cell death (d) reduced tumor progression, (e) reduced number of metastases, (f) reduced rate of metastasis, (g) decreased tumor recurrence (h) increased survival of subject, and (i) increased progression free survival of a subject.

Treating cancer can result in a reduction in size or volume of a tumor. For example, after treatment, tumor size is reduced by 5% or greater (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater) relative to its size prior to treatment. Size of a tumor may be measured by any reproducible means of measurement. For example, the size of a tumor may be measured as a diameter of the tumor.

Treating cancer may further result in a decrease in number of tumors. For example, after treatment, tumor number is reduced by 5% or greater (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or greater) relative to number prior to treatment. Number of tumors may be measured by any reproducible means of measurement, e.g., the number of tumors may be measured by counting tumors visible to the naked eye or at a specified magnification (e.g., 2x, 3x, 4x, 5x, 10x, or 50x).

Treating cancer can result in a decrease in number of metastatic nodules in other tissues or organs distant from the primary tumor site. For example, after treatment, the number of metastatic nodules is reduced by 5% or greater (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater) relative to number prior to treatment. The number of metastatic nodules may be measured by any reproducible means of measurement. For example, the number of metastatic nodules may be measured

by counting metastatic nodules visible to the naked eye or at a specified magnification (e.g., 2x, 10x, or 50x).

Treating cancer can result in an increase in average survival time of a population of subjects treated according to the present invention in comparison to a population of untreated subjects. For example, the average survival time is increased by more than 30 days (more than 60 days, 90 days, or 120 days). An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with the compound described herein. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with a pharmaceutically acceptable salt of a compound described herein.

Treating cancer can also result in a decrease in the mortality rate of a population of treated subjects in comparison to an untreated population. For example, the mortality rate is decreased by more than 2% (e.g., more than 5%, 10%, or 25%). A decrease in the mortality rate of a population of treated subjects may be measured by any reproducible means, for example, by calculating for a population the average number of disease-related deaths per unit time following initiation of treatment with a pharmaceutically acceptable salt of a compound described herein. A decrease in the mortality rate of a population may also be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following completion of a first round of treatment with a pharmaceutically acceptable salt of a compound described herein.

Combination Therapies

A method of the invention can be used alone or in combination with an additional therapeutic agent, e.g., other agents that treat cancer or symptoms associated therewith, or in combination with other types of therapies to treat cancer. In combination treatments, the dosages of one or more of the therapeutic compounds may be reduced from standard dosages when administered alone. For example, doses may be determined empirically from drug combinations and permutations or may be deduced by isobolographic analysis (e.g., Black et al., *Neurology* 65:S3-S6 (2005)). In this case, dosages of the compounds when combined should provide a therapeutic effect.

In some embodiments, the second therapeutic agent is a chemotherapeutic agent (e.g., a cytotoxic agent or other chemical compound useful in the treatment of cancer). These include alkylating agents, antimetabolites, folic acid analogs, pyrimidine analogs, purine analogs and related inhibitors, vinca alkaloids, epipodopyyllotoxins, antibiotics, L-Asparaginase, topoisomerase inhibitors, interferons, platinum coordination complexes, anthracenedione substituted urea, methyl hydrazine derivatives, adrenocortical suppressant, adrenocorticosteroides, progestins, estrogens, antiestrogen, androgens, antiandrogen, and gonadotropin-releasing hormone analog. Also included is 5-fluorouracil (5-FU), leucovorin (LV), irenotecan, oxaliplatin, capecitabine, paclitaxel, and doxetaxel. Non-limiting examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine;

acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., *Agnew, Chem. Intl. Ed Engl.* 33:183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin, including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5- FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL® (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, NJ), ABRAXANE®, cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, IL), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (e.g., CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Two or more

chemotherapeutic agents can be used in a cocktail to be administered in combination with the first therapeutic agent described herein. Suitable dosing regimens of combination chemotherapies are known in the art and described in, for example, Saltz et al., *Proc. Am. Soc. Clin. Oncol.* 18:233a (1999), and Douillard et al., *Lancet* 355(9209):1041-1047 (2000).

In some embodiments, the second therapeutic agent is a therapeutic agent which is a biologic such a cytokine (e.g., interferon or an interleukin (e.g., IL-2)) used in cancer treatment. In some embodiments the biologic is an anti-angiogenic agent, such as an anti-VEGF agent, e.g., bevacizumab (AVASTIN®). In some embodiments the biologic is an immunoglobulin-based biologic, e.g., a monoclonal antibody (e.g., a humanized antibody, a fully human antibody, an Fc fusion protein or a functional fragment thereof) that agonizes a target to stimulate an anti-cancer response, or antagonizes an antigen important for cancer. Such agents include RITUXAN® (rituximab); ZENAPAX® (daclizumab); SIMULECT® (basiliximab); SYNAGIS® (palivizumab); REMICADE® (infliximab); HERCEPTIN® (trastuzumab); MYLOTARG® (gemtuzumab ozogamicin); CAMPATH® (alemtuzumab); ZEVALIN® (ibritumomab tiuxetan); HUMIRA® (adalimumab); XOLAIR® (omalizumab); BEXXAR® (tositumomab-I-131); RAPTIVA® (efalizumab); ERBITUX® (cetuximab); AVASTIN® (bevacizumab); TYSABRI® (natalizumab); ACTEMRA® (tocilizumab); VECTIBIX® (panitumumab); LUCENTIS® (ranibizumab); SOLIRIS® (eculizumab); CIMZIA® (certolizumab pegol); SIMPONI® (golimumab); ILARIS® (canakinumab); STELARA® (ustekinumab); ARZERRA® (ofatumumab); PROLIA® (denosumab); NUMAX® (motavizumab); ABTHRAX® (raxibacumab); BENLYSTA® (belimumab); YERVOY® (ipilimumab); ADCETRIS® (brentuximab vedotin); PERJETA® (pertuzumab); KADCYLA® (ado-trastuzumab emtansine); and GAZYVA® (obinutuzumab). Also included are antibody-drug conjugates.

The second agent may be a therapeutic agent which is a non-drug treatment. For example, the second therapeutic agent is radiation therapy, cryotherapy, hyperthermia, and/or surgical excision of tumor tissue.

The second agent may be a checkpoint inhibitor. In one embodiment, the inhibitor of checkpoint is an inhibitory antibody (e.g., a monospecific antibody such as a monoclonal antibody). The antibody may be, e.g., humanized or fully human. In some embodiments, the inhibitor of checkpoint is a fusion protein, e.g., an Fc-receptor fusion protein. In some embodiments, the inhibitor of checkpoint is an agent, such as an antibody, that interacts with a checkpoint protein. In some embodiments, the inhibitor of checkpoint is an agent, such as an antibody, that interacts with the ligand of a checkpoint protein. In some embodiments, the inhibitor of checkpoint is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of CTLA-4 (e.g., an anti-CTLA4 antibody or fusion a protein such as ipilimumab/YERVOY® or tremelimumab). In some embodiments, the inhibitor of checkpoint is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of PD-1 (e.g., nivolumab/OPDIVO®; pembrolizumab/KEYTRUDA®; pidilizumab/CT-011). In some embodiments, the inhibitor of checkpoint is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of PDL1 (e.g., MPDL3280A/RG7446; MEDI4736; MSB0010718C; BMS 936559). In some embodiments, the inhibitor of checkpoint is an inhibitor (e.g., an inhibitory antibody or Fc fusion or small molecule inhibitor) of PDL2 (e.g., a PDL2/Ig fusion protein such as AMP 224). In some embodiments, the inhibitor of checkpoint is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of B7-H3 (e.g., MGA271), B7-H4, BTLA, HVEM, TIM3,

GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, B-7 family ligands, or a combination thereof.

In some embodiments, the anti-cancer therapy is a T cell adoptive transfer (ACT) therapy. In some embodiments, the T cell is an activated T cell. The T cell may be modified to express a chimeric antigen receptor (CAR). CAR modified T (CAR-T) cells can be generated by any method known in the art. For example, the CAR-T cells can be generated by introducing a suitable expression vector encoding the CAR to a T cell. Prior to expansion and genetic modification of the T cells, a source of T cells is obtained from a subject. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available in the art, may be used. In some embodiments, the T cell is an autologous T cell. Whether prior to or after genetic modification of the T cells to express a desirable protein (e.g., a CAR), the T cells can be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

In any of the combination embodiments described herein, the first and second therapeutic agents are administered simultaneously or sequentially, in either order. The first therapeutic agent may be administered immediately, up to 1 hour, up to 2 hours, up to 3 hours, up to 4 hours, up to 5 hours, up to 6 hours, up to 7 hours, up to, 8 hours, up to 9 hours, up to 10 hours, up to 11 hours, up to 12 hours, up to 13 hours, 14 hours, up to hours 16, up to 17 hours, up 18 hours, up to 19 hours up to 20 hours, up to 21 hours, up to 22 hours, up to 23 hours up to 24 hours or up to 1-7, 1-14, 1-21 or 1-30 days before or after the second therapeutic agent.

Pharmaceutical Compositions

The pharmaceutical compositions described herein are preferably formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form suitable for administration in vivo.

The compounds described herein may be used in the form of the free base, in the form of salts, solvates, and as prodrugs. All forms are within the methods described herein. In accordance with the methods of the invention, the described compounds or salts, solvates, or prodrugs thereof may be administered to a patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compounds described herein may be administered, for example, by oral, parenteral, buccal, sublingual, nasal, rectal, patch, pump, intratumoral, or transdermal administration and the pharmaceutical compositions formulated accordingly. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal, and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time.

A compound described herein may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic

administration, a compound described herein may be incorporated with an excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, and wafers. A compound described herein may also be administered parenterally. Solutions of a compound described herein can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO, and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (2012, 22nd ed.) and in The United States Pharmacopeia: The National Formulary (USP 41 NF36), published in 2018. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that may be easily administered via syringe. Compositions for nasal administration may conveniently be formulated as aerosols, drops, gels, and powders. Aerosol formulations typically include a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomizing device. Alternatively, the sealed container may be a unitary dispensing device, such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form includes an aerosol dispenser, it will contain a propellant, which can be a compressed gas, such as compressed air or an organic propellant, such as fluorochlorohydrocarbon. The aerosol dosage forms can also take the form of a pump-atomizer. Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, where the active ingredient is formulated with a carrier, such as sugar, acacia, tragacanth, gelatin, and glycerine. Compositions for rectal administration are conveniently in the form of suppositories containing a conventional suppository base, such as cocoa butter. A compound described herein may be administered intratumorally, for example, as an intratumoral injection. Intratumoral injection is injection directly into the tumor vasculature and is specifically contemplated for discrete, solid, accessible tumors. Local, regional, or systemic administration also may be appropriate. A compound described herein may advantageously be contacted by administering an injection or multiple injections to the tumor, spaced for example, at approximately, 1 cm intervals. In the case of surgical intervention, the present invention may be used preoperatively, such as to render an inoperable tumor subject to resection. Continuous administration also may be applied where appropriate, for example, by implanting a catheter into a tumor or into tumor vasculature.

The compounds described herein may be administered to an animal, e.g., a human, alone or in combination with pharmaceutically acceptable carriers, as noted herein, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration, and standard pharmaceutical practice.

Dosages

The dosage of the compounds described herein, and/or compositions including a compound described herein, can vary depending on many factors, such as the pharmacodynamic properties of the

compound; the mode of administration; the age, health, and weight of the recipient; the nature and extent of the symptoms; the frequency of the treatment, and the type of concurrent treatment, if any; and the clearance rate of the compound in the animal to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. The compounds described herein may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response. In general, satisfactory results may be obtained when the compounds described herein are administered to a human at a daily dosage of, for example, between 0.05 mg and 3000 mg (measured as the solid form). Dose ranges include, for example, between 10-1000 mg (e.g., 50-800 mg). In some embodiments, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1000 mg of the compound is administered.

Alternatively, the dosage amount can be calculated using the body weight of the patient. For example, the dose of a compound, or pharmaceutical composition thereof, administered to a patient may range from 0.1-50 mg/kg (e.g., 0.25-25 mg/kg). In exemplary, non-limiting embodiments, the dose may range from 0.5-5.0 mg/kg (e.g., 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0 mg/kg) or from 5.0-20 mg/kg (e.g., 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg/kg).

Kits

The invention also features kits including (a) a pharmaceutical composition including an agent that reduces the level and/or activity of BRD9 in a cell or subject described herein, and (b) a package insert with instructions to perform any of the methods described herein. In some embodiments, the kit includes (a) a pharmaceutical composition including an agent that reduces the level and/or activity of BRD9 in a cell or subject described herein, (b) an additional therapeutic agent (e.g., an anti-cancer agent), and (c) a package insert with instructions to perform any of the methods described herein.

Examples

Example 1 – High density tiling sgRNA screen against human BAF complex subunits in synovial sarcoma cell line SYO1

The following example shows that BRD9 sgRNA inhibits cell growth in synovial sarcoma cells.

Procedure: To perform high density sgRNA tiling screen, an sgRNA library against BAF complex subunits was custom synthesized at Cellecta (Mountain View, CA). Sequences of DNA encoding the BRD9-targeting sgRNAs used in this screen are listed in Table 3. Negative and positive control sgRNA were included in the library. Negative controls consisted of 200 sgRNAs that do not target human genome. The positive controls are sgRNAs targeting essential genes (CDC16, GTF2B, HSPA5, HSPA9, PAFAH1B1, PCNA, POLR2L, RPL9, and SF3A3). DNA sequences encoding all positive and negative control sgRNAs are listed in Table 4. Procedures for virus production, cell infection, and performing the sgRNA screen were previously described (Tsherniak et al, *Cell* 170:564-576 (2017); Munoz et al, *Cancer Discovery* 6:900-913 (2016)). For each sgRNA, 50 counts were added to the sequencing counts and for each time point the resulting counts were normalized to the total number of counts. The log2 of the ratio between the counts (defined as dropout ratio) at day 24 and day 1 post-infection was calculated. For negative control sgRNAs, the 2.5 and 97.5 percentile of the log2 dropout ratio of all non-targeting sgRNAs

was calculated and considered as background (grey box in the graph). Protein domains were obtained from PFAM regions defined for the UNIPROT identifier: Q9H8M2.

Results: As shown in FIG. 1, targeted inhibition of the GBAF complex component BRD9 by sgRNA resulted in growth inhibition of the SYO1 synovial sarcoma cell line. sgRNAs against other components of the BAF complexes resulted in increased proliferation of cells, inhibition of cell growth, or had no effect on SYO1 cells. These data show that targeting various subunits of the GBAF complex represents a therapeutic strategy for the treatment of synovial sarcoma.

Table 3: BRD9 sgRNA Library

SEQ ID NO	Nucleic Acid Sequence		SEQ ID NO	Nucleic Acid Sequence
203	CAAGAAGCACAAGAAGCACA		319	GCTCTACAGCGTGGTCAACA
204	CTTGTGCTTCTTGCCCATGG		320	CGGGAGCCTGCTCTACAGCG
205	CTTCTTGTGCTTCTTGCCCA		321	CGTGGTCAACACGGCCGAGC
206	ACAAGAAGCACAAGGCCGAG		322	CCCACCATCAGCGTCCGGCT
207	CTCGTAGGACGAGCGCCACT		323	ACGGCCGAGCCGGACGCTGA
208	CGAGTGGCGCTCGTCCTACG		324	GGGCACCCACCATCAGCGTC
209	GAGTGGCGCTCGTCCTACGA		325	GCCGAGCCGGACGCTGATGG
210	AGGCTTCTCCAGGGGCTTGT		326	CCATGTCCGTGTTGCAGAGG
211	AGATTATGCCGACAAGCCCC		327	CCGAGCCGGACGCTGATGGT
212	ACCTTCAGGACTAGCTTTAG		328	CGAGCTCAAGTCCACCGGGT
213	AGCTTTAGAGGCTTCTCCAG		329	GCGAGCTCAAGTCCACCGGG
214	CTAGCTTTAGAGGCTTCTCC		330	AGAGCGAGCTCAAGTCCACC
215	TAGCTTTAGAGGCTTCTCCA		331	GAGAGCGAGCTCAAGTCCAC
216	CTAAAGCTAGTCCTGAAGGT		332	GAAGCCTGGGAGTAGCTTAC
217	GCCTCTAAAGCTAGTCCTGA		333	CTCTCCAGTAAGCTACTCCC
218	CTTCACTTCCTCCGACCTTC		334	AGCCCAGCGTGGTGAAGCCT
219	AAGCTAGTCCTGAAGGTCGG		335	AAGCCCAGCGTGGTGAAGCC
220	AGTGAAGTGACTGAACTCTC		336	ACTCCCAGGCTTCACCACGC
221	GTGACTGAACTCTCAGGATC		337	CTCCCAGGCTTCACCACGCT
222	ATAGTAACTGGAGTCGTGGC		338	CTCGTCTTTGAAGCCCAGCG
223	CATCATAGTAACTGGAGTCG		339	CACTGGAGAGAAAGGTGACT
224	TGACCTGTCATCATAGTAAC		340	GCACTGGAGAGAAAGGTGAC
225	ACTCCAGTTACTATGATGAC		341	AGTAGTGGCACTGGAGAGAA
226	CTTTGTGCCTCTCTCGCTCA		342	CGAAAGCGCAGTAGTGGCAC
227	GGTCAGACCATGAGCGAGAG		343	CTGCATCGAAAGCGCAGTAG
228	GAAGAAGAAGAAGTCCGAGA		344	ATGCAGAATAATTCAGTATT
229	GTCCAGATGCTTCTCCTTCT		345	AGTATTTGGCGACTTGAAGT
230	GTCCGAGAAGGAGAAGCATC		346	CGACTTGAAGTCGGACGAGA

SEQ ID NO	Nucleic Acid Sequence		SEQ ID NO	Nucleic Acid Sequence
231	GGAGAAGCATCTGGACGATG		347	GAGCTGCTCTACTCAGCCTA
232	TGAGGAAAGAAGGAAGCGAA		348	CACGCCTGTCTCATCTCCGT
233	ATCTGGACGATGAGGAAAGA		349	TCAGCCTACGGAGATGAGAC
234	AGAAGAAGCGGAAGCGAGAG		350	CAGGCGTGCAGTGTGCGCTG
235	GAAGAAGCGGAAGCGAGAGA		351	CCGCGGCCCCTCTAGCCTGC
236	CCGCCCAGGAAGAGAAGAAG		352	CATCCTTCACAAACTCCTGC
237	AGAGAGGGAGCACTGTGACA		353	TAGCCTGCAGGAGTTTGTGA
238	AGGGAGCACTGTGACACGGA		354	CAGGAGTTTGTGAAGGATGC
239	GAGGGAGCACTGTGACACGG		355	AGGAGTTTGTGAAGGATGCT
240	GCACTGTGACACGGAGGGAG		356	TGGGAGCTACAGCAAGAAAG
241	GAGGCTGACGACTTTGATCC		357	GAGCTACAGCAAGAAAGTGG
242	AGGCTGACGACTTTGATCCT		358	GAAAGTGGTGGACGACCTCC
243	TCCACCTCCACCTTCTTCCC		359	CGCCTGTGATCTGGTCCAGG
244	CGACTTTGATCCTGGGAAGA		360	CTCCGCCTGTGATCTGGTCC
245	CTTTGATCCTGGGAAGAAGG		361	GACCTCCTGGACCAGATCAC
246	TGATCCTGGGAAGAAGGTGG		362	CTCCTGGACCAGATCACAGG
247	TCCTGGGAAGAAGGTGGAGG		363	GCTGGAAGAGCGTCCTAGAG
248	CGGACTGGCCGATCTGGGGG		364	TGCAGCCCACCTGCTTCAGC
249	ACGCTCGGACTGGCCGATCT		365	GACGCTCTTCCAGCTGAAGC
250	AGGTGGAGCCGCCCCCAGAT		366	CTCTTCCAGCTGAAGCAGGT
251	CGCTCGGACTGGCCGATCTG		367	GCTCTTCCAGCTGAAGCAGG
252	GCTCGGACTGGCCGATCTGG		368	CCTCCAGATGAAGCCAAGGT
253	CACGCTCGGACTGGCCGATC		369	GCTTCATCTGGAGGCTTCAT
254	TGTGTCCGGCACGCTCGGAC		370	GGCTTCATCTGGAGGCTTCA
255	CTGGCTGTGTCCGGCACGCT		371	CTTACCTTGGCTTCATCTGG
256	ATCGGCCAGTCCGAGCGTGC		372	AAACTTACCTTGGCTTCATC
257	CACCCTTGCCTGGCTGTGTC		373	GAAGCCTCCAGATGAAGCCA
258	CGAGCGTGCCGGACACAGCC		374	TCCTAGGGTGTCCCCAACCT
259	TGTTCCAGGAGTTGCTGAAT		375	CCTAGGGTGTCCCCAACCTG
260	CACACCTATTCAGCAACTCC		376	GTGTCTGTCTCCACAGGTTG
261	GCTGGCGGAGGAAGTGTTCC		377	TGTGTCTGTCTCCACAGGTT
262	TTTACCTCTGAAGCTGGCGG		378	CCACAGGTTGGGGACACCCT
263	CCCCGGTTTACCTCTGAAGC		379	AGAGCTGCTGCTGTCTCCTA
264	ACTTCCTCCGCCAGCTTCAG		380	CAGAGCTGCTGCTGTCTCCT
265	CAGGAAAAGCAAAAAATCCA		381	AGACAGCAGCAGCTCTGTTC
266	GCTTTCAGAAAAGATCCCCA		382	ATCCACAGAAACGTCGGGAT
267	AGGAAAAGCAAAAAATCCAT		383	GAGATATCCACAGAAACGTC

SEQ ID NO	Nucleic Acid Sequence		SEQ ID NO	Nucleic Acid Sequence
268	GGAAAAGCAAAAAATCCATG		384	GGAGATATCCACAGAAACGT
269	GGAGCAATTGCATCCGTGAC		385	GTCCTATCCCGACGTTTCTG
270	GTCACGGATGCAATTGCTCC		386	TCTCCATGCTCAGCTCTCTG
271	TTTATTATCATTGAATATCC		387	CTCACCCAGAGAGCTGAGCA
272	AATGATAATAAAACATCCCA		388	ATCTCCATGCTCAGCTCTCT
273	ATAAAACATCCCATGGATTT		389	TATCTCCATGCTCAGCTCTC
274	TTCATGGTGCCAAAATCCAT		390	ATGTCCTGTTTACACAGGGA
275	TTTCATGGTGCCAAAATCCA		391	TTACACAGGGAAGGTGAAGA
276	TAATGAATACAAGTCAGTTA		392	AGTTCAAATGGCTGTCGTCA
277	CAAGTCAGTTACGGAATTTA		393	TGACGACAGCCATTTGAACT
278	ATAATGCAATGACATACAAT		394	AAGTTCAAATGGCTGTCGTC
279	AACTTGTAGTACACGGTATC		395	TCGTCTCATCCAAGTTCAAA
280	CTTCGCCAACTTGTAGTACA		396	TGAGACGACGAAGCTCCTGC
281	AGATACCGTGTACTACAAGT		397	GTGCTTCGTGCAGGTCCTGC
282	GCGAAGAAGATCCTTCACGC		398	GCAGGACCTGCACGAAGCAC
283	TCATCTTAAAGCCTGCGTGA		399	GCTCCGCCTGTGCTTCGTGC
284	TTCTCAGCAGGCAGCTCTTT		400	GGACCTGCACGAAGCACAGG
285	CAATGAAGATACAGCTGTTG		401	CACGAAGCACAGGCGGAGCG
286	ACTGGTACAACTTCAGGGAC		402	AGGCGGAGCGCGGCGGCTCT
287	CTTGTACTGGTACAACTTCA		403	AGGGAGCTGAGGTTGGACGA
288	ACTTGTACTGGTACAACTTC		404	GTTGGACAGGGAGCTGAGGT
289	TTGGCAGTTTCTACTTGTAC		405	AGGCGTTGGACAGGGAGCTG
290	TACCTGATAACTTCTCTACT		406	CCCTCTCGGAGGCGTTGGAC
291	AGCCGAGTAGAGAAGTTATC		407	CCTCTCGGAGGCGTTGGACA
292	AGCTGCATGTTTGAGCCTGA		408	CTGGTCCCTCTCGGAGGCGT
293	GCTGCATGTTTGAGCCTGAA		409	CCCTGTCCAACGCCTCCGAG
294	AAGCTGCAGGCATTCCCTTC		410	CCTGTCCAACGCCTCCGAGA
295	GGTACTGTCCGTCAAGCTGC		411	GTGGTGCTGGTCCCTCTCGG
296	AGGGAATGCCTGCAGCTTGA		412	CAGGTGGTGCTGGTCCCTCT
297	CTTGACGGACAGTACCGCAG		413	GCATCTCACCCAGGTGGTGC
298	CGCCAGCACGTGCTCCTCTG		414	CGAGAGGGACCAGCACCACC
299	TACCGCAGAGGAGCACGTGC		415	GAGAGGGACCAGCACCACCT
300	AGAGGAGCACGTGCTGGCGC		416	GTGGGGGCATCTCACCCAGG
301	GGAGCACGTGCTGGCGCTGG		417	CCCCGACACTCAGGCGAGAA
302	AGCACGCAGCTGACGAAGCT		418	TCCCCGACACTCAGGCGAGA
303	GCACGCAGCTGACGAAGCTC		419	AGCCCTTCTCGCCTGAGTGT
304	CAGCTGACGAAGCTCGGGAC		420	CTGGCTGCTCCCCGACACTC

SEQ ID NO	Nucleic Acid Sequence		SEQ ID NO	Nucleic Acid Sequence
305	AAGCTCGGGACAGGATCAAC		421	CCCTTCTCGCCTGAGTGTCG
306	CCTTGCCGCCTGGGAGGAAC		422	GCCCTTCTCGCCTGAGTGTC
307	AGGATCAACCGGTTCCTCCC		423	TAGGGGTCGTGGGTGACGTC
308	ATCAACCGGTTCCTCCCAGG		424	AAGAAACTCATAGGGGTCGT
309	GCACTACCTTGCCGCCTGGG		425	GAAGAAACTCATAGGGGTCG
310	AGAGCACTACCTTGCCGCCT		426	GAGACTGAAGAAACTCATAG
311	CCGGTTCCTCCCAGGCGGCA		427	GGAGACTGAAGAAACTCATA
312	TCCTCTTCAGATAGCCCATC		428	TGGAGACTGAAGAAACTCAT
313	ATGGGCTATCTGAAGAGGAA		429	TCTTCAGTCTCCAGAGCCTG
314	GGGCTATCTGAAGAGGAACG		430	TTGGCAGAGGCCGCAGGCTC
315	TGGGCTATCTGAAGAGGAAC		431	TAGGTCTTGGCAGAGGCCGC
316	TATCTGAAGAGGAACGGGGA		432	CTAGAGTTAGGTCTTGGCAG
317	ATCTGAAGAGGAACGGGGAC		433	GGTGGTCTAGAGTTAGGTCT
318	TGTTGACCACGCTGTAGAGC			

Table 4: Control sgRNA Library

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
434	1|sg_Non_Targeting_Human_0001|Non_Targeting_Human	Non_Targeting_Human	GTAGCGAACGTGTCCGGCGT
435	1|sg_Non_Targeting_Human_0002|Non_Targeting_Human	Non_Targeting_Human	GACCGGAACGATCTCGCGTA
436	1|sg_Non_Targeting_Human_0003|Non_Targeting_Human	Non_Targeting_Human	GGCAGTCGTTCGGTTGATAT
437	1|sg_Non_Targeting_Human_0004|Non_Targeting_Human	Non_Targeting_Human	GCTTGAGCACATACGCGAAT
438	1|sg_Non_Targeting_Human_0005|Non_Targeting_Human	Non_Targeting_Human	GTGGTAGAATAACGTATTAC
439	1|sg_Non_Targeting_Human_0006|Non_Targeting_Human	Non_Targeting_Human	GTCATACATGGATAAGGCTA
440	1|sg_Non_Targeting_Human_0007|Non_Targeting_Human	Non_Targeting_Human	GATACACGAAGCATCACTAG
441	1|sg_Non_Targeting_Human_0008|Non_Targeting_Human	Non_Targeting_Human	GAACGTTGGCACTACTTCAC
442	1|sg_Non_Targeting_Human_0009|Non_Targeting_Human	Non_Targeting_Human	GATCCATGTAATGCGTTCGA
443	1|sg_Non_Targeting_Human_0010|Non_Targeting_Human	Non_Targeting_Human	GTCGTGAAGTGCATTCGATC
444	1|sg_Non_Targeting_Human_0011|Non_Targeting_Human	Non_Targeting_Human	GTTCGACTCGCGTGACCGTA
445	1|sg_Non_Targeting_Human_0012|Non_Targeting_Human	Non_Targeting_Human	GAATCTACCGCAGCGGTTCG

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
446	1|sg_Non_Targeting_Human_0013|Non_Targeting_Human	Non_Targeting_Human	GAAGTGACGTCGATTCGATA
447	1|sg_Non_Targeting_Human_0014|Non_Targeting_Human	Non_Targeting_Human	GCGGTGTATGACAACCGCCG
448	1|sg_Non_Targeting_Human_0015|Non_Targeting_Human	Non_Targeting_Human	GTACCGCGCCTGAAGTTCGC
449	1|sg_Non_Targeting_Human_0016|Non_Targeting_Human	Non_Targeting_Human	GCAGCTCGTGTGTCGTACTC
450	1|sg_Non_Targeting_Human_0017|Non_Targeting_Human	Non_Targeting_Human	GCGCCTTAAGAGTACTCATC
451	1|sg_Non_Targeting_Human_0018|Non_Targeting_Human	Non_Targeting_Human	GAGTGTCGTCGTTGCTCCTA
452	1|sg_Non_Targeting_Human_0019|Non_Targeting_Human	Non_Targeting_Human	GCAGCTCGACCTCAAGCCGT
453	1|sg_Non_Targeting_Human_0020|Non_Targeting_Human	Non_Targeting_Human	GTATCCTGACCTACGCGCTG
454	1|sg_Non_Targeting_Human_0021|Non_Targeting_Human	Non_Targeting_Human	GTGTATCTCAGCACGCTAAC
455	1|sg_Non_Targeting_Human_0022|Non_Targeting_Human	Non_Targeting_Human	GTCGTCATACAACGGCAACG
456	1|sg_Non_Targeting_Human_0023|Non_Targeting_Human	Non_Targeting_Human	GTCGTGCGCTTCCGGCGGTA
457	1|sg_Non_Targeting_Human_0024|Non_Targeting_Human	Non_Targeting_Human	GCGGTCCTCAGTAAGCGCGT
458	1|sg_Non_Targeting_Human_0025|Non_Targeting_Human	Non_Targeting_Human	GCTCTGCTGCGGAAGGATTC
459	1|sg_Non_Targeting_Human_0026|Non_Targeting_Human	Non_Targeting_Human	GCATGGAGGAGCGTCGCAGA
460	1|sg_Non_Targeting_Human_0027|Non_Targeting_Human	Non_Targeting_Human	GTAGCGCGCGTAGGAGTGGC
461	1|sg_Non_Targeting_Human_0028|Non_Targeting_Human	Non_Targeting_Human	GATCACCTGCATTCGTACAC
462	1|sg_Non_Targeting_Human_0029|Non_Targeting_Human	Non_Targeting_Human	GCACACCTAGATATCGAATG
463	1|sg_Non_Targeting_Human_0030|Non_Targeting_Human	Non_Targeting_Human	GTTGATCAACGCGCTTCGCG
464	1|sg_Non_Targeting_Human_0031|Non_Targeting_Human	Non_Targeting_Human	GCGTCTCACTCACTCCATCG
465	1|sg_Non_Targeting_Human_0032|Non_Targeting_Human	Non_Targeting_Human	GCCGACCAACGTCAGCGGTA
466	1|sg_Non_Targeting_Human_0033|Non_Targeting_Human	Non_Targeting_Human	GGATACGGTGCGTCAATCTA
467	1|sg_Non_Targeting_Human_0034|Non_Targeting_Human	Non_Targeting_Human	GAATCCAGTGGCGGCGACAA
468	1|sg_Non_Targeting_Human_0035|Non_Targeting_Human	Non_Targeting_Human	GCACTGTCAGTGCAACGATA

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
469	1|sg_Non_Targeting_Human_0036|Non_Targeting_Human	Non_Targeting_Human	GCGATCCTCAAGTATGCTCA
470	1|sg_Non_Targeting_Human_0037|Non_Targeting_Human	Non_Targeting_Human	GCTAATATCGACACGGCCGC
471	1|sg_Non_Targeting_Human_0038|Non_Targeting_Human	Non_Targeting_Human	GGAGATGCATCGAAGTCGAT
472	1|sg_Non_Targeting_Human_0039|Non_Targeting_Human	Non_Targeting_Human	GGATGCACTCCATCTCGTCT
473	1|sg_Non_Targeting_Human_0040|Non_Targeting_Human	Non_Targeting_Human	GTGCCGAGTAATAACGCGAG
474	1|sg_Non_Targeting_Human_0041|Non_Targeting_Human	Non_Targeting_Human	GAGATTCCGATGTAACGTAC
475	1|sg_Non_Targeting_Human_0042|Non_Targeting_Human	Non_Targeting_Human	GTCGTCACGAGCAGGATTGC
476	1|sg_Non_Targeting_Human_0043|Non_Targeting_Human	Non_Targeting_Human	GCGTTAGTCACTTAGCTCGA
477	1|sg_Non_Targeting_Human_0044|Non_Targeting_Human	Non_Targeting_Human	GTTCACACGGTGTCGGATAG
478	1|sg_Non_Targeting_Human_0045|Non_Targeting_Human	Non_Targeting_Human	GGATAGGTGACCTTAGTACG
479	1|sg_Non_Targeting_Human_0046|Non_Targeting_Human	Non_Targeting_Human	GTATGAGTCAAGCTAATGCG
480	1|sg_Non_Targeting_Human_0047|Non_Targeting_Human	Non_Targeting_Human	GCAACTATTGGAATACGTGA
481	1|sg_Non_Targeting_Human_0048|Non_Targeting_Human	Non_Targeting_Human	GTTACCTTCGCTCGTCTATA
482	1|sg_Non_Targeting_Human_0049|Non_Targeting_Human	Non_Targeting_Human	GTACCGAGCACCACAGGCCG
483	1|sg_Non_Targeting_Human_0050|Non_Targeting_Human	Non_Targeting_Human	GTCAGCCATCGGATAGAGAT
484	1|sg_Non_Targeting_Human_0051|Non_Targeting_Human	Non_Targeting_Human	GTACGGCACTCCTAGCCGCT
485	1|sg_Non_Targeting_Human_0052|Non_Targeting_Human	Non_Targeting_Human	GGTCCTGTCGTATGCTTGCA
486	1|sg_Non_Targeting_Human_0053|Non_Targeting_Human	Non_Targeting_Human	GCCGCAATATATGCGGTAAG
487	1|sg_Non_Targeting_Human_0054|Non_Targeting_Human	Non_Targeting_Human	GCGCACGTATAATCCTGCGT
488	1|sg_Non_Targeting_Human_0055|Non_Targeting_Human	Non_Targeting_Human	GTGCACAACACGATCCACGA
489	1|sg_Non_Targeting_Human_0056|Non_Targeting_Human	Non_Targeting_Human	GCACAATGTTGACGTAAGTG
490	1|sg_Non_Targeting_Human_0057|Non_Targeting_Human	Non_Targeting_Human	GTAAGATGCTGCTCACCGTG
491	1|sg_Non_Targeting_Human_0058|Non_Targeting_Human	Non_Targeting_Human	GTCGGTGATCCAACGTATCG

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
492	1|sg_Non_Targeting_Human_0059|Non_Targeting_Human	Non_Targeting_Human	GAGCTAGTAGGACGCAAGAC
493	1|sg_Non_Targeting_Human_0060|Non_Targeting_Human	Non_Targeting_Human	GTACGTGGAAGCTTGTGGCC
494	1|sg_Non_Targeting_Human_0061|Non_Targeting_Human	Non_Targeting_Human	GAGAACTGCCAGTTCTCGAT
495	1|sg_Non_Targeting_Human_0062|Non_Targeting_Human	Non_Targeting_Human	GCCATTCGGCGCGGCACTTC
496	1|sg_Non_Targeting_Human_0063|Non_Targeting_Human	Non_Targeting_Human	GCACACGACCAATCCGCTTC
497	1|sg_Non_Targeting_Human_0064|Non_Targeting_Human	Non_Targeting_Human	GAGGTGATCGATTAAGTACA
498	1|sg_Non_Targeting_Human_0065|Non_Targeting_Human	Non_Targeting_Human	GTCACTCGCAGACGCCTAAC
499	1|sg_Non_Targeting_Human_0066|Non_Targeting_Human	Non_Targeting_Human	GCGCTACGGAATCATACGTT
500	1|sg_Non_Targeting_Human_0067|Non_Targeting_Human	Non_Targeting_Human	GGTAGGACCTCACGGCGCGC
501	1|sg_Non_Targeting_Human_0068|Non_Targeting_Human	Non_Targeting_Human	GAACTGCATCTTGTTGTAGT
502	1|sg_Non_Targeting_Human_0069|Non_Targeting_Human	Non_Targeting_Human	GATCCTGATCCGGCGGCGCG
503	1|sg_Non_Targeting_Human_0070|Non_Targeting_Human	Non_Targeting_Human	GGTATGCGCGATCCTGAGTT
504	1|sg_Non_Targeting_Human_0071|Non_Targeting_Human	Non_Targeting_Human	GCGGAGCTAGAGAGCGGTCA
505	1|sg_Non_Targeting_Human_0072|Non_Targeting_Human	Non_Targeting_Human	GAATGGCAATTACGGCTGAT
506	1|sg_Non_Targeting_Human_0073|Non_Targeting_Human	Non_Targeting_Human	GTATGGTGAGTAGTCGCTTG
507	1|sg_Non_Targeting_Human_0074|Non_Targeting_Human	Non_Targeting_Human	GTGTAATTGCGTCTAGTCGG
508	1|sg_Non_Targeting_Human_0075|Non_Targeting_Human	Non_Targeting_Human	GGTCCTGGCGAGGAGCCTTG
509	1|sg_Non_Targeting_Human_0076|Non_Targeting_Human	Non_Targeting_Human	GAAGATAAGTCGCTGTCTCG
510	1|sg_Non_Targeting_Human_0077|Non_Targeting_Human	Non_Targeting_Human	GTCGGCGTTCTGTTGTGACT
511	1|sg_Non_Targeting_Human_0078|Non_Targeting_Human	Non_Targeting_Human	GAGGCAAGCCGTTAGGTGTA
512	1|sg_Non_Targeting_Human_0079|Non_Targeting_Human	Non_Targeting_Human	GCGGATCCAGATCTCATTCG
513	1|sg_Non_Targeting_Human_0080|Non_Targeting_Human	Non_Targeting_Human	GGAACATAGGAGCACGTAGT
514	1|sg_Non_Targeting_Human_0081|Non_Targeting_Human	Non_Targeting_Human	GTCATCATTATGGCGTAAGG

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
515	1|sg_Non_Targeting_Human_0082|Non_Targeting_Human	Non_Targeting_Human	GCGACTAGCGCCATGAGCGG
516	1|sg_Non_Targeting_Human_0083|Non_Targeting_Human	Non_Targeting_Human	GGCGAAGTTCGACATGACAC
517	1|sg_Non_Targeting_Human_0084|Non_Targeting_Human	Non_Targeting_Human	GCTGTCGTGTGGAGGCTATG
518	1|sg_Non_Targeting_Human_0085|Non_Targeting_Human	Non_Targeting_Human	GCGGAGAGCATTGACCTCAT
519	1|sg_Non_Targeting_Human_0086|Non_Targeting_Human	Non_Targeting_Human	GACTAATGGACCAAGTCAGT
520	1|sg_Non_Targeting_Human_0087|Non_Targeting_Human	Non_Targeting_Human	GCGGATTAGAGGTAATGCGG
521	1|sg_Non_Targeting_Human_0088|Non_Targeting_Human	Non_Targeting_Human	GCCGACGGCAATCAGTACGC
522	1|sg_Non_Targeting_Human_0089|Non_Targeting_Human	Non_Targeting_Human	GTAACCTCTCGAGCGATAGA
523	1|sg_Non_Targeting_Human_0090|Non_Targeting_Human	Non_Targeting_Human	GACTTGTATGTGGCTTACGG
524	1|sg_Non_Targeting_Human_0091|Non_Targeting_Human	Non_Targeting_Human	GTCACTGTGGTCGAACATGT
525	1|sg_Non_Targeting_Human_0092|Non_Targeting_Human	Non_Targeting_Human	GTACTCCAATCCGCGATGAC
526	1|sg_Non_Targeting_Human_0093|Non_Targeting_Human	Non_Targeting_Human	GCGTTGGCACGATGTTACGG
527	1|sg_Non_Targeting_Human_0094|Non_Targeting_Human	Non_Targeting_Human	GAACCAGCCGGCTAGTATGA
528	1|sg_Non_Targeting_Human_0095|Non_Targeting_Human	Non_Targeting_Human	GTATACTAGCTAACCACACG
529	1|sg_Non_Targeting_Human_0096|Non_Targeting_Human	Non_Targeting_Human	GAATCGGAATAGTTGATTCG
530	1|sg_Non_Targeting_Human_0097|Non_Targeting_Human	Non_Targeting_Human	GAGCACTTGCATGAGGCGGT
531	1|sg_Non_Targeting_Human_0098|Non_Targeting_Human	Non_Targeting_Human	GAACGGCGATGAAGCCAGCC
532	1|sg_Non_Targeting_Human_0099|Non_Targeting_Human	Non_Targeting_Human	GCAACCGAGATGAGAGGTTC
533	1|sg_Non_Targeting_Human_0100|Non_Targeting_Human	Non_Targeting_Human	GCAAGATCAATATGCGTGAT
534	1|sg_Non_Targeting_Human_GA_0101|Non_Targeting_Human	Non_Targeting_Human	ACGGAGGCTAAGCGTCGCAA
535	1|sg_Non_Targeting_Human_GA_0102|Non_Targeting_Human	Non_Targeting_Human	CGCTTCCGCGGCCCGTTCAA
536	1|sg_Non_Targeting_Human_GA_0103|Non_Targeting_Human	Non_Targeting_Human	ATCGTTTCCGCTTAACGGCG
537	1|sg_Non_Targeting_Human_GA_0104|Non_Targeting_Human	Non_Targeting_Human	GTAGGCGCGCCGCTCTCTAC

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
538	1|sg_Non_Targeting_Human_GA_0105|Non_Targeting_Human	Non_Targeting_Human	CCATATCGGGGCGAGACATG
539	1|sg_Non_Targeting_Human_GA_0106|Non_Targeting_Human	Non_Targeting_Human	TACTAACGCCGCTCCTACAG
540	1|sg_Non_Targeting_Human_GA_0107|Non_Targeting_Human	Non_Targeting_Human	TGAGGATCATGTCGAGCGCC
541	1|sg_Non_Targeting_Human_GA_0108|Non_Targeting_Human	Non_Targeting_Human	GGGCCCGCATAGGATATCGC
542	1|sg_Non_Targeting_Human_GA_0109|Non_Targeting_Human	Non_Targeting_Human	TAGACAACCGCGGAGAATGC
543	1|sg_Non_Targeting_Human_GA_0110|Non_Targeting_Human	Non_Targeting_Human	ACGGGCGGCTATCGCTGACT
544	1|sg_Non_Targeting_Human_GA_0111|Non_Targeting_Human	Non_Targeting_Human	CGCGGAAATTTTACCGACGA
545	1|sg_Non_Targeting_Human_GA_0112|Non_Targeting_Human	Non_Targeting_Human	CTTACAATCGTCGGTCCAAT
546	1|sg_Non_Targeting_Human_GA_0113|Non_Targeting_Human	Non_Targeting_Human	GCGTGCGTCCCGGGTTACCC
547	1|sg_Non_Targeting_Human_GA_0114|Non_Targeting_Human	Non_Targeting_Human	CGGAGTAACAAGCGGACGGA
548	1|sg_Non_Targeting_Human_GA_0115|Non_Targeting_Human	Non_Targeting_Human	CGAGTGTTATACGCACCGTT
549	1|sg_Non_Targeting_Human_GA_0116|Non_Targeting_Human	Non_Targeting_Human	CGACTAACCGGAAACTTTTT
550	1|sg_Non_Targeting_Human_GA_0117|Non_Targeting_Human	Non_Targeting_Human	CAACGGGTTCTCCCGGCTAC
551	1|sg_Non_Targeting_Human_GA_0118|Non_Targeting_Human	Non_Targeting_Human	CAGGAGTCGCCGATACGCGT
552	1|sg_Non_Targeting_Human_GA_0119|Non_Targeting_Human	Non_Targeting_Human	TTCACGTCGTCTCGCGACCA
553	1|sg_Non_Targeting_Human_GA_0120|Non_Targeting_Human	Non_Targeting_Human	GTGTCGGATTCCGCCGCTTA
554	1|sg_Non_Targeting_Human_GA_0121|Non_Targeting_Human	Non_Targeting_Human	CACGAACTCACACCGCGCGA
555	1|sg_Non_Targeting_Human_GA_0122|Non_Targeting_Human	Non_Targeting_Human	CGCTAGTACGCTCCTCTATA
556	1|sg_Non_Targeting_Human_GA_0123|Non_Targeting_Human	Non_Targeting_Human	TCGCGCTTGGGTTATACGCT
557	1|sg_Non_Targeting_Human_GA_0124|Non_Targeting_Human	Non_Targeting_Human	CTATCTCGAGTGGTAATGCG
558	1|sg_Non_Targeting_Human_GA_0125|Non_Targeting_Human	Non_Targeting_Human	AATCGACTCGAACTTCGTGT
559	1|sg_Non_Targeting_Human_GA_0126|Non_Targeting_Human	Non_Targeting_Human	CCCGATGGACTATACCGAAC
560	1|sg_Non_Targeting_Human_GA_0127|Non_Targeting_Human	Non_Targeting_Human	ACGTTCGAGTACGACCAGCT

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
561	1|sg_Non_Targeting_Human_GA_0128|Non_Targeting_Human	Non_Targeting_Human	CGCGACGACTCAACCTAGTC
562	1|sg_Non_Targeting_Human_GA_0129|Non_Targeting_Human	Non_Targeting_Human	GGTCACCGATCGAGAGCTAG
563	1|sg_Non_Targeting_Human_GA_0130|Non_Targeting_Human	Non_Targeting_Human	CTCAACCGACCGTATGGTCA
564	1|sg_Non_Targeting_Human_GA_0131|Non_Targeting_Human	Non_Targeting_Human	CGTATTCGACTCTCAACGCG
565	1|sg_Non_Targeting_Human_GA_0132|Non_Targeting_Human	Non_Targeting_Human	CTAGCCGCCCAGATCGAGCC
566	1|sg_Non_Targeting_Human_GA_0133|Non_Targeting_Human	Non_Targeting_Human	GAATCGACCGACACTAATGT
567	1|sg_Non_Targeting_Human_GA_0134|Non_Targeting_Human	Non_Targeting_Human	ACTTCAGTTCGGCGTAGTCA
568	1|sg_Non_Targeting_Human_GA_0135|Non_Targeting_Human	Non_Targeting_Human	GTGCGATGTCGCTTCAACGT
569	1|sg_Non_Targeting_Human_GA_0136|Non_Targeting_Human	Non_Targeting_Human	CGCCTAATTTCCGGATCAAT
570	1|sg_Non_Targeting_Human_GA_0137|Non_Targeting_Human	Non_Targeting_Human	CGTGGCCGGAACCGTCATAG
571	1|sg_Non_Targeting_Human_GA_0138|Non_Targeting_Human	Non_Targeting_Human	ACCCTCCGAATCGTAACGGA
572	1|sg_Non_Targeting_Human_GA_0139|Non_Targeting_Human	Non_Targeting_Human	AAACGGTACGACAGCGTGTG
573	1|sg_Non_Targeting_Human_GA_0140|Non_Targeting_Human	Non_Targeting_Human	ACATAGTCGACGGCTCGATT
574	1|sg_Non_Targeting_Human_GA_0141|Non_Targeting_Human	Non_Targeting_Human	GATGGCGCTTCAGTCGTCGG
575	1|sg_Non_Targeting_Human_GA_0142|Non_Targeting_Human	Non_Targeting_Human	ATAATCCGGAAACGCTCGAC
576	1|sg_Non_Targeting_Human_GA_0143|Non_Targeting_Human	Non_Targeting_Human	CGCCGGGCTGACAATTAACG
577	1|sg_Non_Targeting_Human_GA_0144|Non_Targeting_Human	Non_Targeting_Human	CGTCGCCATATGCCGGTGGC
578	1|sg_Non_Targeting_Human_GA_0145|Non_Targeting_Human	Non_Targeting_Human	CGGGCCTATAACACCATCGA
579	1|sg_Non_Targeting_Human_GA_0146|Non_Targeting_Human	Non_Targeting_Human	CGCCGTTCCGAGATACTTGA
580	1|sg_Non_Targeting_Human_GA_0147|Non_Targeting_Human	Non_Targeting_Human	CGGGACGTCGCGAAAATGTA
581	1|sg_Non_Targeting_Human_GA_0148|Non_Targeting_Human	Non_Targeting_Human	TCGGCATACGGGACACACGC
582	1|sg_Non_Targeting_Human_GA_0149|Non_Targeting_Human	Non_Targeting_Human	AGCTCCATCGCCGCGATAAT
583	1|sg_Non_Targeting_Human_GA_0150|Non_Targeting_Human	Non_Targeting_Human	ATCGTATCATCAGCTAGCGC

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
584	1|sg_Non_Targeting_Human_GA_0151|Non_Targeting_Human	Non_Targeting_Human	TCGATCGAGGTTGCATTCGG
585	1|sg_Non_Targeting_Human_GA_0152|Non_Targeting_Human	Non_Targeting_Human	CTCGACAGTTCGTCCCGAGC
586	1|sg_Non_Targeting_Human_GA_0153|Non_Targeting_Human	Non_Targeting_Human	CGGTAGTATTAATCGCTGAC
587	1|sg_Non_Targeting_Human_GA_0154|Non_Targeting_Human	Non_Targeting_Human	TGAACGCGTGTTTCCTTGCA
588	1|sg_Non_Targeting_Human_GA_0155|Non_Targeting_Human	Non_Targeting_Human	CGACGCTAGGTAACGTAGAG
589	1|sg_Non_Targeting_Human_GA_0156|Non_Targeting_Human	Non_Targeting_Human	CATTGTTGAGCGGGCGCGCT
590	1|sg_Non_Targeting_Human_GA_0157|Non_Targeting_Human	Non_Targeting_Human	CCGCTATTGAAACCGCCCAC
591	1|sg_Non_Targeting_Human_GA_0158|Non_Targeting_Human	Non_Targeting_Human	AGACACGTCACCGGTCAAAA
592	1|sg_Non_Targeting_Human_GA_0159|Non_Targeting_Human	Non_Targeting_Human	TTTACGATCTAGCGGCGTAG
593	1|sg_Non_Targeting_Human_GA_0160|Non_Targeting_Human	Non_Targeting_Human	TTCGCACGATTGCACCTTGG
594	1|sg_Non_Targeting_Human_GA_0161|Non_Targeting_Human	Non_Targeting_Human	GGTTAGAGACTAGGCGCGCG
595	1|sg_Non_Targeting_Human_GA_0162|Non_Targeting_Human	Non_Targeting_Human	CCTCCGTGCTAACGCGGACG
596	1|sg_Non_Targeting_Human_GA_0163|Non_Targeting_Human	Non_Targeting_Human	TTATCGCGTAGTGCTGACGT
597	1|sg_Non_Targeting_Human_GA_0164|Non_Targeting_Human	Non_Targeting_Human	TACGCTTGCGTTTAGCGTCC
598	1|sg_Non_Targeting_Human_GA_0165|Non_Targeting_Human	Non_Targeting_Human	CGCGGCCCACGCGTCATCGC
599	1|sg_Non_Targeting_Human_GA_0166|Non_Targeting_Human	Non_Targeting_Human	AGCTCGCCATGTCGGTTCTC
600	1|sg_Non_Targeting_Human_GA_0167|Non_Targeting_Human	Non_Targeting_Human	AACTAGCCCGAGCAGCTTCG
601	1|sg_Non_Targeting_Human_GA_0168|Non_Targeting_Human	Non_Targeting_Human	CGCAAGGTGTCGGTAACCCT
602	1|sg_Non_Targeting_Human_GA_0169|Non_Targeting_Human	Non_Targeting_Human	CTTCGACGCCATCGTGCTCA
603	1|sg_Non_Targeting_Human_GA_0170|Non_Targeting_Human	Non_Targeting_Human	TCCTGGATACCGCGTGGTTA
604	1|sg_Non_Targeting_Human_GA_0171|Non_Targeting_Human	Non_Targeting_Human	ATAGCCGCCGCTCATTACTT
605	1|sg_Non_Targeting_Human_GA_0172|Non_Targeting_Human	Non_Targeting_Human	GTCGTCCGGGATTACAAAAT
606	1|sg_Non_Targeting_Human_GA_0173|Non_Targeting_Human	Non_Targeting_Human	TAATGCTGCACACGCCGAAT

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
607	1|sg_Non_Targeting_Human_GA_0174|Non_Targeting_Human	Non_Targeting_Human	TATCGCTTCCGATTAGTCCG
608	1|sg_Non_Targeting_Human_GA_0175|Non_Targeting_Human	Non_Targeting_Human	GTACCATACCGCGTACCCTT
609	1|sg_Non_Targeting_Human_GA_0176|Non_Targeting_Human	Non_Targeting_Human	TAAGATCCGCGGGTGGCAAC
610	1|sg_Non_Targeting_Human_GA_0177|Non_Targeting_Human	Non_Targeting_Human	GTAGACGTCGTGAGCTTCAC
611	1|sg_Non_Targeting_Human_GA_0178|Non_Targeting_Human	Non_Targeting_Human	TCGCGGACATAGGGCTCTAA
612	1|sg_Non_Targeting_Human_GA_0179|Non_Targeting_Human	Non_Targeting_Human	AGCGCAGATAGCGCGTATCA
613	1|sg_Non_Targeting_Human_GA_0180|Non_Targeting_Human	Non_Targeting_Human	GTTCGCTTCGTAACGAGGAA
614	1|sg_Non_Targeting_Human_GA_0181|Non_Targeting_Human	Non_Targeting_Human	GACCCCCGATAACTTTTGAC
615	1|sg_Non_Targeting_Human_GA_0182|Non_Targeting_Human	Non_Targeting_Human	ACGTCCATACTGTCGGCTAC
616	1|sg_Non_Targeting_Human_GA_0183|Non_Targeting_Human	Non_Targeting_Human	GTACCATTGCCGGCTCCCTA
617	1|sg_Non_Targeting_Human_GA_0184|Non_Targeting_Human	Non_Targeting_Human	TGGTTCCGTAGGTCGGTATA
618	1|sg_Non_Targeting_Human_GA_0185|Non_Targeting_Human	Non_Targeting_Human	TCTGGCTTGACACGACCGTT
619	1|sg_Non_Targeting_Human_GA_0186|Non_Targeting_Human	Non_Targeting_Human	CGCTAGGTCCGGTAAGTGCG
620	1|sg_Non_Targeting_Human_GA_0187|Non_Targeting_Human	Non_Targeting_Human	AGCACGTAATGTCCGTGGAT
621	1|sg_Non_Targeting_Human_GA_0188|Non_Targeting_Human	Non_Targeting_Human	AAGGCGCGCGAATGTGGCAG
622	1|sg_Non_Targeting_Human_GA_0189|Non_Targeting_Human	Non_Targeting_Human	ACTGCGGAGCGCCCAATATC
623	1|sg_Non_Targeting_Human_GA_0190|Non_Targeting_Human	Non_Targeting_Human	CGTCGAGTGCTCGAACTCCA
624	1|sg_Non_Targeting_Human_GA_0191|Non_Targeting_Human	Non_Targeting_Human	TCGCAGCGGCGTGGGATCGG
625	1|sg_Non_Targeting_Human_GA_0192|Non_Targeting_Human	Non_Targeting_Human	ATCTGTCCTAATTCGGATCG
626	1|sg_Non_Targeting_Human_GA_0193|Non_Targeting_Human	Non_Targeting_Human	TGCGGCGTAATGCTTGAAAG
627	1|sg_Non_Targeting_Human_GA_0194|Non_Targeting_Human	Non_Targeting_Human	CGAACTTAATCCCGTGGCAA
628	1|sg_Non_Targeting_Human_GA_0195|Non_Targeting_Human	Non_Targeting_Human	GCCGTGTTGCTGGATACGCC
629	1|sg_Non_Targeting_Human_GA_0196|Non_Targeting_Human	Non_Targeting_Human	TACCCTCCGGATACGGACTG

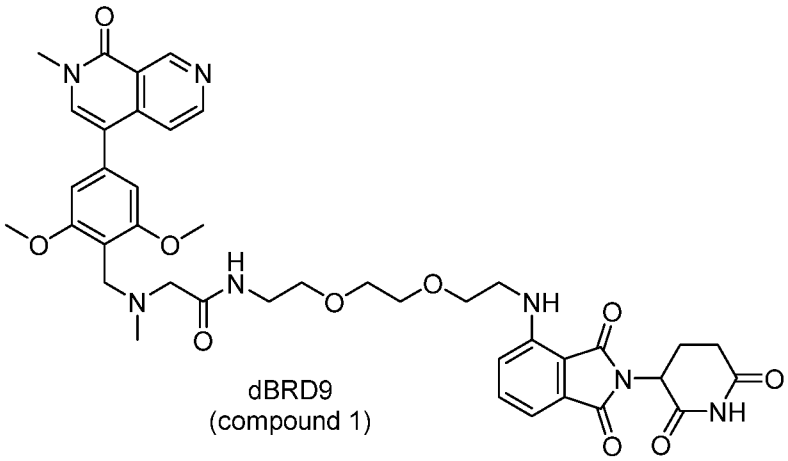
SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
630	1|sg_Non_Targeting_Human_GA_0197|Non_Targeting_Human	Non_Targeting_Human	CCGTTGGACTATGGCGGGTC
631	1|sg_Non_Targeting_Human_GA_0198|Non_Targeting_Human	Non_Targeting_Human	GTACGGGGCGATCATCCACA
632	1|sg_Non_Targeting_Human_GA_0199|Non_Targeting_Human	Non_Targeting_Human	AAGAGTAGTAGACGCCCGGG
633	1|sg_Non_Targeting_Human_GA_0200|Non_Targeting_Human	Non_Targeting_Human	AAGAGCGAATCGATTTCGTG
634	3|sg_hCDC16_CC_1|CDC16	CDC16	TCAACACCAGTGCCTGACGG
635	3|sg_hCDC16_CC_2|CDC16	CDC16	AAAGTAGCTTCACTCTCTCG
636	3|sg_hCDC16_CC_3|CDC16	CDC16	GAGCCAACCAATAGATGTCC
637	3|sg_hCDC16_CC_4|CDC16	CDC16	GCGCCGCCATGAACCTAGAG
638	3|sg_hGTF2B_CC_1|GTF2B	GTF2B	ACAAAGGTTGGAACAGAACC
639	3|sg_hGTF2B_CC_2|GTF2B	GTF2B	GGTGACCGGGTTATTGATGT
640	3|sg_hGTF2B_CC_3|GTF2B	GTF2B	TTAGTGGAGGACTACAGAGC
641	3|sg_hGTF2B_CC_4|GTF2B	GTF2B	ACATATAGCCCGTAAAGCTG
642	3|sg_hHSPA5_CC_1|HSPA5	HSPA5	CGTTGGCGATGATCTCCACG
643	3|sg_hHSPA5_CC_2|HSPA5	HSPA5	TGGCCTTTTCTACCTCGCGC
644	3|sg_hHSPA5_CC_3|HSPA5	HSPA5	AATGGAGATACTCATCTGGG
645	3|sg_hHSPA5_CC_4|HSPA5	HSPA5	GAAGCCCGTCCAGAAAGTGT
646	3|sg_hHSPA9_CC_1|HSPA9	HSPA9	CAATCTGAGGAACTCCACGA
647	3|sg_hHSPA9_CC_2|HSPA9	HSPA9	AGGCTGCGGCGCCCACGAGA
648	3|sg_hHSPA9_CC_3|HSPA9	HSPA9	ACTTTGACCAGGCCTTGCTA
649	3|sg_hHSPA9_CC_4|HSPA9	HSPA9	ACCTTCCATAACTGCCACGC
650	3|sg_hPAFAH1B1_CC_1|PAFAH1B1	PAFAH1B1	CGAGGCGTACATACCCAAGG
651	3|sg_hPAFAH1B1_CC_2|PAFAH1B1	PAFAH1B1	ATGGTACGGCCAAATCAAGA
652	3|sg_hPAFAH1B1_CC_3|PAFAH1B1	PAFAH1B1	TCTTGTAATCCCATACGCGT

SEQ ID NO.	gRNA Label	Gene	Nucleic Acid Sequence
653	3|sg_hPAFAH1B1_CC_4|PAFAH1B1	PAFAH1B1	ATTCACAGGACACAGAGAAT
654	3|sg_hPCNA_CC_1|PCNA	PCNA	CCAGGGCTCCATCCTCAAGA
655	3|sg_hPCNA_CC_2|PCNA	PCNA	TGAGCTGCACCAAAGAGACG
656	3|sg_hPCNA_CC_3|PCNA	PCNA	ATGTCTGCAGATGTACCCCT
657	3|sg_hPCNA_CC_4|PCNA	PCNA	CGAAGATAACGCGGATACCT
658	3|sg_hPOLR2L_CC_1|POLR2L	POLR2L	GCTGCAGGCCGAGTACACCG
659	3|sg_hPOLR2L_CC_2|POLR2L	POLR2L	ACAAGTGGGAGGCTTACCTG
660	3|sg_hPOLR2L_CC_3|POLR2L	POLR2L	GCAGCGTACAGGGATGATCA
661	3|sg_hPOLR2L_CC_4|POLR2L	POLR2L	GCAGTAGCGCTTCAGGCCCA
662	3|sg_hRPL9_CC_1|RPL9	RPL9	CAAATGGTGGGGTAACAGAA
663	3|sg_hRPL9_CC_2|RPL9	RPL9	GAAAGGAACTGGCTACCGTT
664	3|sg_hRPL9_CC_3|RPL9	RPL9	AGGGCTTCCGTTACAAGATG
665	3|sg_hRPL9_CC_4|RPL9	RPL9	GAACAAGCAACACCTAAAAG
666	3|sg_hSF3A3_CC_1|SF3A3	SF3A3	TGAGGAGAAGGAACGGCTCA
667	3|sg_hSF3A3_CC_2|SF3A3	SF3A3	GGAAGAATGCAGAGTATAAG
668	3|sg_hSF3A3_CC_3|SF3A3	SF3A3	GGAATTTGAGGAACTCCTGA
669	3|sg_hSF3A3_CC_4|SF3A3	SF3A3	GCTCACCGGCCATCCAGGAA
670	3|sg_hSF3B3_CC_1|SF3B3	SF3B3	ACTGGCCAGGAACGATGCGA
671	3|sg_hSF3B3_CC_2|SF3B3	SF3B3	GCAGCTCCAAGATCTTCCCA
672	3|sg_hSF3B3_CC_3|SF3B3	SF3B3	GAATGAGTACACAGAACGGA
673	3|sg_hSF3B3_CC_4|SF3B3	SF3B3	GGAGCAGGACAAGGTCGGGG

Example 2 – BRD9 degrader depletes BRD9 protein

The following example demonstrates the depletion of the BRD9 protein in synovial sarcoma cells treated with a BRD9 degrader.

Procedure: Cells were treated with DMSO or the BRD9 degrader, Compound 1 (also known as dBRD9, see Remillard et al, *Angew. Chem. Int. Ed. Engl.* 56(21):5738-5743 (2017); see structure of compound 1 below), for indicated doses and timepoints.



dBRD9
(compound 1)

Whole cell extracts were fractionated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane using a transfer apparatus according to the manufacturer's protocols (Bio-Rad). After incubation with 5% nonfat milk in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.5% Tween 20) for 60 min, the membrane was incubated with antibodies against BRD9 (1:1,000, Bethyl laboratory A303-781A), GAPDH (1:5,000, Cell Signaling Technology), and/or MBP (1:1,000, BioRad) overnight at 4℃. Membranes were washed three times for 10 min and incubated with anti-mouse or anti-rabbit antibodies conjugated with either horseradish peroxidase (HRP, FIGS. 2-3) or IRDye (FIG. 4, 1:20,000, LI-COR) for at least 1h. Blots were washed with TBST three times and developed with either the ECL system according to the manufacturer's protocols (FIGS. 2-3) or scanned on an Odyssey CLx Imaging system (FIG. 4).

Results: Treatment of SYO1 synovial sarcoma cells with the BRD9 degrader Compound 1 results in dose dependent (FIG. 2) and time dependent (FIG. 3) depletion of BRD9 in the cells. Further, as shown in FIG. 4, the depletion of BRD9 by Compound 1 is replicated in a non-synovial sarcoma cell line (293T) and may be sustained for at least 5 days.

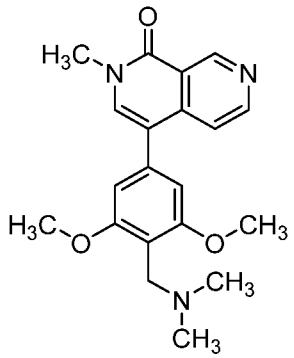
Example 3 – Inhibition of growth of synovial cell lines by BRD9 inhibitors and BRD9 degraders

The following example demonstrates that BRD9 degraders and inhibitors selectively inhibit growth of synovial sarcoma cells.

Procedures:

Cells were treated with DMSO or the BRD9 degrader, Compound 1, at indicated concentrations, and proliferation was monitored from day 7 to day 14 by measuring confluency over time using an IncuCyte live cell analysis system (FIG. 5). Growth medium and compounds were refreshed every 3-4 days.

Cells were seeded into 12-well plates and treated with DMSO, 1μM BRD9 inhibitor, Compound 2 (also known as BI-7273, see Martin et al, *J Med Chem.* 59(10):4462-4475 (2016); see structure of compound 2 below), or 1 μM BRD9 degrader, Compound 1.



BI-7273
(compound 2)

The number of cells was optimized for each cell line. Growth medium and compounds were refreshed every 3-5 days. SYO1, Yamato, A549, 293T and HS-SY-II cells were fixed and stained at day 11. ASKA cells were fixed and stained at day 23. Staining was done by incubation with crystal violet solution (0.5 g Crystal Violet, 27 ml 37% Formaldehyde, 100 mL 10X PBS, 10 mL Methanol, 863 dH20 to 1L) for 30min followed by 3x washes with water and drying the plates for at least 24h at room temperature. Subsequently plates were scanned on an Odyssey CLx Imaging system (FIG. 6).

Cells were seeded into 96-well ultra low cluster plate (Costar, #7007) in 200 μL complete media and treated at day 2 with DMSO, Staurosporin, or BRD9 degarder, Compound 1, at indicated doses (FIG. 7). Media and compounds were changed every 5 d and cell colonies were imaged at day 14.

Results: As shown in FIGS. 5, 6, and 7, treatment of synovial sarcoma cell lines (SYO1, Yamato, HS-SY-II, and ASKA) with a BRD9 inhibitor, Compound 2, or a BRD9 degrader, Compound 1, results in inhibition of the growth of the cells, but does not result in inhibition of the growth of non-synovial control cancer cell lines (293T, A549, G401).

Example 4 – Selective inhibition of growth of synovial cell lines by BRD9 degraders and BRD9 binders

The following example demonstrates that BRD9 degraders and binders selectively inhibit growth of synovial sarcoma cells.

Procedure: Cells were seeded into 6-well or 12-well plates and were treated daily with a BRD9 degrader (Compound 1), a bromo-domain BRD9 binder (Compound 2), E3 ligase binder (lenalidomide), DMSO, or staurosporin (positive control for cell killing), at indicated concentrations. The number of cells was optimized for each cell line. Growth media was refreshed every 5 days. By day 14, medium was removed, cells were washed with PBS, and stained using 500 μL of 0.005% (w/v) crystal violet solution in 25% (v/v) methanol for at least 1 hour at room temperature. Subsequently plates were scanned on an Odyssey CLx Imaging system.

Results: As shown in FIGS. 8 and 9, treatment of synovial sarcoma cell lines (SYO1, HS-SY-II, and ASKA) with Compound 1 or Compound 2 resulted in inhibition of the growth of the cells, but did not result in inhibition of the growth of non-synovial control cancer cell lines (RD, HCT116, and Calu6). Overall, Compound 1 showed most significant growth inhibition in all synovial cell lines.

Example 5- Inhibition of cell growth in synovial sarcoma cells

The following example shows that BRD9 degraders inhibit cell growth and induce apoptosis in synovial sarcoma cells.

Procedure: SYO1 cells were treated for 8 or 13 days with DMSO, a BRD9 degrader (Compound 1) at 200nM or 1μM, or an E3 ligase binder (lenalidomide) at 200nM. Compounds were refreshed every 5 days. Cell cycle analysis was performed using the Click-iT™ Plus EdU Flow Cytometry Assay (Invitrogen). The apoptosis assay was performed using the Annexin V-FITC Apoptosis Detection Kit (Sigma A9210). Assays were performed according to the manufacturer's protocol.

Results: As shown in FIGS. 10-13, treatment with Compound 1 for 8 or 13 days resulted in reduced numbers of cells in the S-phase of the cell cycle as compared to DMSO and lenalidomide. Treatment with Compound 1 for 8 days also resulted in increased numbers of early- and late-apoptotic cells as compared to DMSO controls.

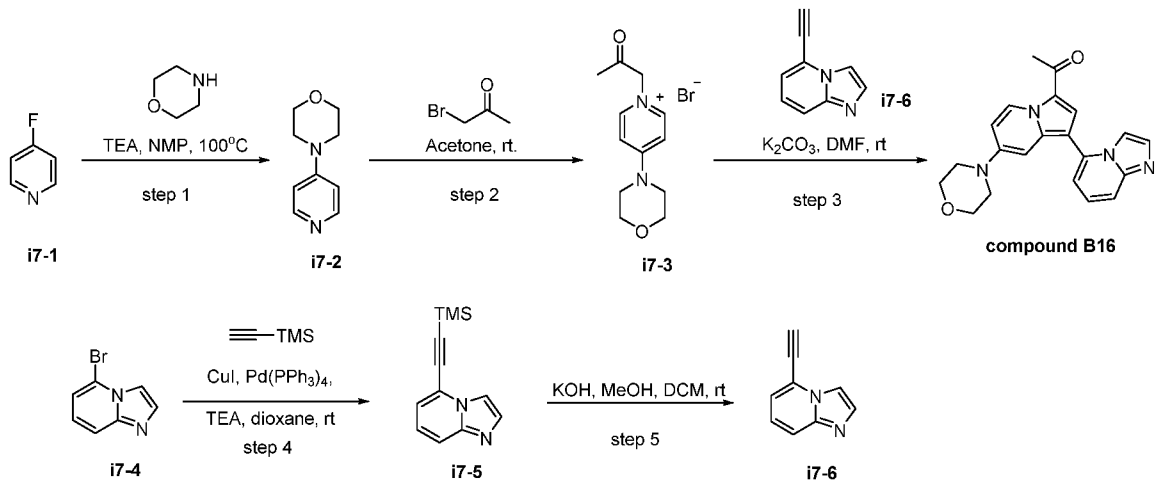
Example 6 – Composition for SS18-SSX1-BAF

The following example shows the identification of BRD9 as a component of SS18-SSX containing BAF complexes.

Procedure: A stable 293T cell line expressing HA-SS18SSX1 was generated using lentiviral integration. SS18-SSX1 containing BAF complexes were subject to affinity purification and subsequent mass spectrometry analysis revealed SS18-SSX1 interacting proteins.

Results: As shown in FIG. 14, BAF complexes including the SS18-SSX fusion protein also included BRD9. More than 5 unique peptides were identified for ARID1A (95 peptides), ARID1B (77 peptides), SMARCC1 (69 peptides), SMARCD1 (41 peptides), SMARCD2 (37 peptides), DPF2 (32 peptides), SMARCD3 (26 peptides), ACTL6A (25 peptides), BRD9 (22 peptides), DPF1 Isoform 2 (18 peptides), DPF3 (13 peptides), and ACTL6B (6 peptides).

Example 7 – Preparation of 1-(1-[imidazo[1,2-a]pyridin-5-yl]-7-(morpholin-4-yl)indolizin-3-yl)ethan-1-one (compound B16)



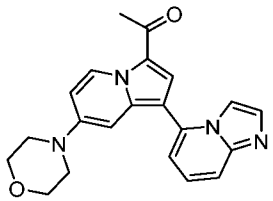
Step 1: Preparation of 4-(pyridin-4-yl)morpholine (i7-2)

To the solution of 4-chloropyridine (1 g, 8.807 mmol, 1 equiv) in toluene (25 mL) was added morpholine (920.79 mg, 10.569 mmol, 1.2 equiv), $Pd(OAC)_2$ (197.74 mg, 0.881 mmol, 0.1 equiv), BINAP (1.10 g, 1.761 mmol, 0.2 equiv), and *tert*-BuONa (2.54 g, 26.422 mmol, 3 equiv). The resulting solution was stirred at 110 ℃ for 12 hours under nitrogen atmosphere. The resulting solution was concentrated. The residue was purified by Flash column chromatography with EtOAc/PE (0~100%), to give compound 4-(pyridin-4-yl)morpholine (600 mg, 41.49%) as yellow solid. LCMS (ESI) m/z: [M+H]+ = 165.

Step 2: Preparation of 4-(morpholin-4-yl)-1-(2-oxopropyl)pyridin-1-ium bromide (i7-3)

1-bromopropan-2-one (275.27 mg, 2.010 mmol, 1.1 equiv) was added slowly to a solution of 4-(pyridin-4-yl)morpholine (300 mg, 1.827 mmol, 1 equiv) in ACN (5 mL), and the resulting mixture was stirred at room temperature for 3 hour. The solid was collected by filtration, washed, and dried in vacuo to give pure 4-(morpholin-4-yl)-1-(2-oxopropyl)pyridin-1-ium bromide (181 mg, 32.89%). LCMS (ESI) m/z: [M+H]+ = 221.

Step 3: Preparation of 1-(1-[imidazo[1,2-a]pyridin-5-yl]-7-(morpholin-4-yl)indolizin-3-yl)ethan-1-one (compound B16)



compound B16

To the solution of 4-(morpholin-4-yl)-1-(2-oxopropyl)pyridin-1-ium bromide (181 mg, 0.601 mmol, 1 equiv) in DMF (5 mL) was added 5-ethynylimidazo[1,2-a]pyridine (256.30 mg, 1.803 mmol, 3 equiv) K_2CO_3 (249.17 mg, 1.803 mmol, 3 equiv). The resulting solution was stirred at 90 ℃ for 3 hours. The resulting solution was concentrated. The residue was purified by reverse flash chromatography (conditions: column, C18 silica gel; mobile phase, MeOH in water, 10% to 100% gradient in 45 minutes; detector, UV 254 nm). This resulted in 1-(1-[imidazo[1,2-a]pyridin-5-yl]-7-(morpholin-4-yl)indolizin-3-yl)ethan-1-one (105.7 mg, 46.51%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 9.66 (d, 1H), 8.19 (d, 1H), 8.11 (d, 2H), 8.03 – 7.85 (m, 2H), 7.57 (d, 1H), 7.21 – 7.12 (m, 1H), 6.72 (d, 1H), 3.72 (t, 4H), 3.29 (d, 4H), 2.52 (s, 3H). LCMS (ESI) m/z: [M+H]+ = 361.10.

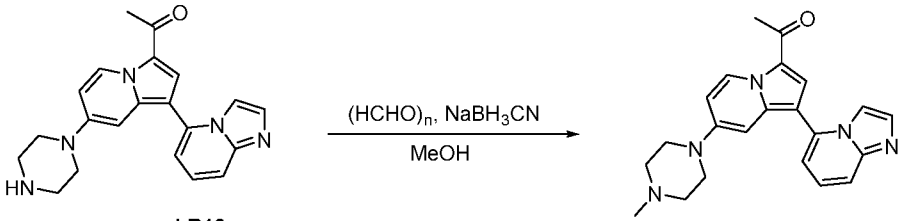
Step 4: Preparation of 5-[2-(trimethylsilyl)ethynyl]imidazo[1,2-a]pyridine (i7-5)

To the solution of 5-bromoimidazo[1,2-a]pyridine (2 g, 10.150 mmol, 1 equiv) in dioxane (30 mL) was added ethynyltrimethylsilane (1196.38 g, 12.181 mmol, 1.2 equiv), CuI (386.63 mg, 2.030 mmol, 0.2 equiv), $Pd(PPh_3)_4$ (1172.95 g, 1.015 mmol, 0.1 equiv), and TEA (3081.38 g, 30.451 mmol, 3.0 equiv). The resulting solution was stirred at room temperature for 3 hours under nitrogen atmosphere. The resulting solution was concentrated. The residue was purified by Flash column chromatography with EtOAc/PE (0~100%), to give compound 5-[2-(trimethylsilyl)ethynyl]imidazo[1,2-a]pyridine (1.13 g, 51.94%) as yellow solid. LCMS (ESI) m/z: [M+H]+ = 215.

Step 5: Preparation of 5-ethynylimidazo[1,2-a]pyridine (i7-6)

To the solution of 5-[2-(trimethylsilyl)ethynyl]imidazo[1,2-a]pyridine (1.127 g, 5.258 mmol, 1 equiv) in MeOH (20 mL), DCM (10 mL) was added NaOH (420.60 mg, 10.516 mmol, 2 equiv). The resulting solution was stirred at room temperature for 1 hour. The resulting solution was concentrated. The residue was purified by Flash column chromatography with EtOAc/PE (0~100%), to give compound 5-ethynylimidazo[1,2-a]pyridine (578 mg, 77.33%) as yellow solid. LCMS (ESI) m/z: [M+H]+ = 143.

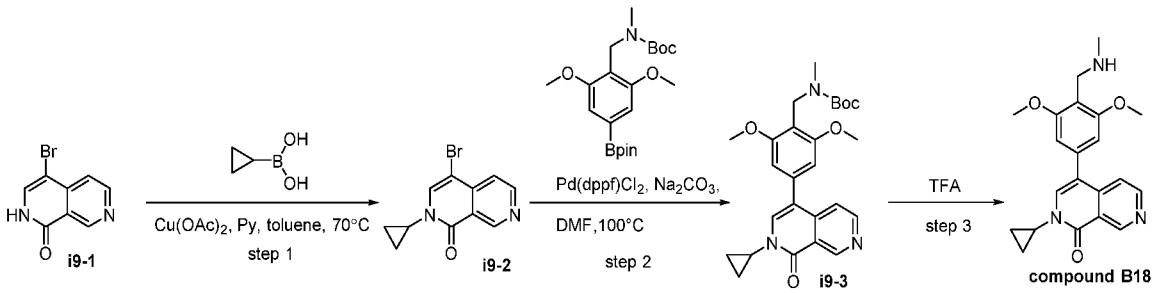
Example 8 – Preparation of 1-(1-[imidazo[1,2-a]pyridin-5-yl]-7-(4-methylpiperazin-1-yl)indolizin-3-yl)ethan-1-one (compound B17)



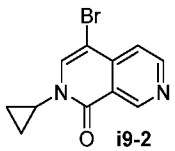
compound B19 compound B17

To a stirred mixture of 1-(1-[imidazo[1,2-a]pyridin-5-yl]-7-(piperazin-1-yl)indolizin-3-yl)ethan-1-one (50 mg, 0.139 mmol, 1 equiv) and $(HCHO)_n$ (21.07 mg, 0.696 mmol, 5 equiv) in MeOH (2 mL) was added $NaBH_3CN$ (17.48 mg, 0.278 mmol, 2 equiv) in portions, and the resulting mixture was stirred for 2 hours at room temperature. The reaction mixture was then concentrated under reduced pressure. The crude product (50 mg) was purified by Prep-HPLC (conditions: X Bridge Shield RP18 OBD Column, 19*250 mm, 10 μm; mobile phase, Water (10 mmol NH_4HCO_3) and ACN (35% Phase B up to 68% in 8 minutes); Detector, UV). This resulted in 1-(1-[imidazo[1,2-a]pyridin-5-yl]-7-(4-methylpiperazin-1-yl)indolizin-3-yl)ethan-1-one (19.5 mg, 37.54%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 9.64 (d, *J* = 8.0 Hz, 1H), 8.06 (s, 1H), 7.81 (s, 1H), 7.66 – 7.52 (m, 2H), 7.35 (dd, *J* = 9.1, 6.9 Hz, 1H), 7.12 (dd, *J* = 7.9, 2.6 Hz, 1H), 7.03 (d, *J* = 6.7 Hz, 1H), 6.60 (d, *J* = 2.5 Hz, 1H), 3.27 (t, *J* = 5.0 Hz, 4H), 2.55 (s, 3H), 2.42 (t, *J* = 5.1 Hz, 4H), 2.21 (s, 3H). LCMS (ESI) m/z: [M+H]+ = 374.10.

Example 9 – Preparation of 2-cyclopropyl-4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2,7-naphthyridin-1-one (compound B18)

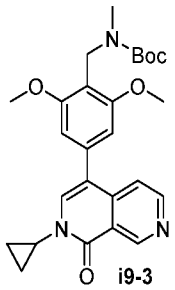


Step 1: Preparation of 4-bromo-2-cyclopropyl-2,7-naphthyridin-1-one (i9-2)



To a solution of 4-bromo-2H-2,7-naphthyridin-1-one (400.00 mg, 1.777 mmol, 1.00 equiv), cyclopropylboronic acid (229.02 mg, 2.666 mmol, 1.5 equiv) and pyridine (281.19 mg, 3.555 mmol, 2.00 equiv) in toluene (20.00 mL) was added $Cu(OAc)_2$ (645.68 mg, 3.555 mmol, 2.00 equiv). The resulting mixture was stirred at 70 degrees C for 16 hours. The solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel eluted with MeOH/DCM from 0% to 10% to give 4-bromo-2-cyclopropyl-2,7-naphthyridin-1-one (260 mg, 55.18%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 265.

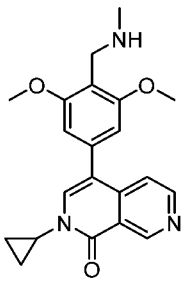
Step 2: Preparation of N-[[4-(2-cyclopropyl-1-oxo-2,7-naphthyridin-4-yl)-2,6-dimethoxyphenyl]methyl]-N-methylcarbamate (i9-3)



i9-3

To a solution of 4-bromo-2-cyclopropyl-2,7-naphthyridin-1-one (260.00 mg, 0.981 mmol, 1.00 equiv) and *tert*-butyl *N*-[[2,6-dimethoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl]-*N*-methylcarbamate (439.41 mg, 1.079 mmol, 1.10 equiv) in DMF (5.00 mL) was added $Pd(dppf)Cl_2$ (71.76 mg, 0.098 mmol, 0.10 equiv) and Na_2CO_3 (207.89 mg, 1.961 mmol, 2.00 equiv). After stirring at 100 degrees C for 1 hour under nitrogen atmosphere, water (100mL) was added, and the mixture was extracted with EtOAc (50 mL x 4). The organic layer was washed with water (2 x 30 mL) and saturated brine (1 x 30 mL), then dried over anhydrous sodium sulfate, filtered, and concentrated to give crude product, which was purified by chromatography on silica gel eluted with PE/EA from 0% to 80% to give *tert*-butyl *N*-[[4-(2-cyclopropyl-1-oxo-2,7-naphthyridin-4-yl)-2,6-dimethoxyphenyl]methyl]-*N*-methylcarbamate (140 mg, 30.66%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 466.

Step 3: Preparation of 2-cyclopropyl-4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2,7-naphthyridin-1-one (compound B18)

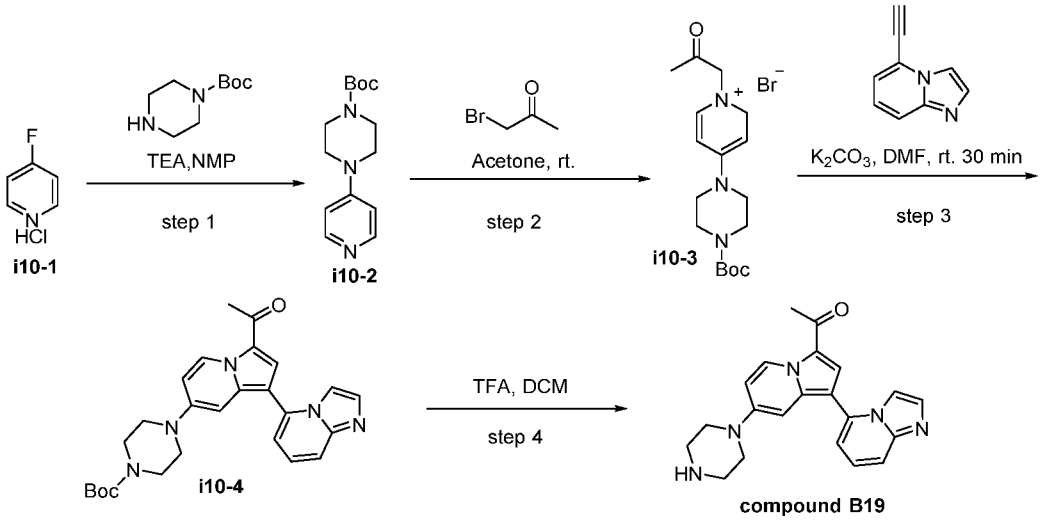


compound B18

To a solution of *tert*-butyl *N*-[[4-(2-cyclopropyl-1-oxo-2,7-naphthyridin-4-yl)-2,6-dimethoxyphenyl]methyl]-*N*-methylcarbamate (140.00 mg, 0.301 mmol, 1.00 equiv) in DCM (1 .00 mL) was added TFA (1.00 mL). The resulting mixture was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure. The crude product was purified by Prep-HPLC (conditions:

XBridge Shield RP18 OBD Column, 5um, 19*150mm; Mobile Phase A:Water (10 mmol/L NH_4HCO_3), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 17% B to 23% B in 8 min; 254 nm; Rt: 6.2 min) to afford 2-cyclopropyl-4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2,7-naphthyridin-1-one (45.1 mg, 41.04%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 9.44 (s, 1H), 8.72 (d, *J* = 5.7 Hz, 1H), 7.59 (s, 1H), 7.51 (d, *J* = 5.7 Hz, 1H), 6.73 (s, 2H), 3.82 (s, 6H), 3.72 (s, 2H), 3.46 - 3.38(m, 1H), 2.28 (s, 3H), 1.10 - 0.97 (m, 4H). LCMS (ESI) m/z: $[M+H]^+$ = 366.25.

Example 10 – Preparation of *1-(1-[imidazo[1,2-a]pyridin-5-yl] -7-(piperazin-1-yl)indolizin-3-yl)ethan-1-one (compound B19)*



Step 1: Preparation of tert-butyl-4-(pyridin-4-yl)piperazine-1-carboxylate (i10-2)

To a stirred mixture of 4-fluoropyridine hydrochloride(4.78 g, 35.792 mmol, 1 equiv) and tert-butylpiperazine-1-carboxylate(8.00 g, 42.950 mmol, 1.2 equiv) in NMP (25 mL) was added TEA (10.87 g, 107.376 mmol, 3 equiv) at room temperature. The mixture was stirred for 3 hours at 100 degrees C. To the mixture was added EA (50 mL) and washed with water (3 x 20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum, and the crude product was used in the next step directly without further purification. LCMS (ESI) m/z: [M+H]+ = 264.2.

Step 2: Preparation of 4-[4-[(tert-butoxy)carbonyl]piperazin-1-yl]-1-(2-oxopropyl)pyridin-1-ium bromide (i10-3)

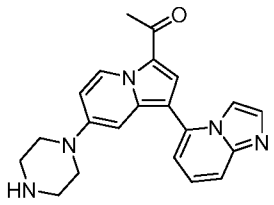
A mixture of tert-butyl 4-(pyridin-4-yl)piperazine-1-carboxylate (526 mg, 1.997 mmol, 1 equiv) and 1-bromopropan-2-one (820.79 mg, 5.992 mmol, 3.00 equiv) in acetone(10 mL) was stirred for 3 hours at room temperature. The precipitated solids were collected by filtration and washed with acetone (3 x 5 mL), and the crude product was used in the next step directly without further purification. LCMS (ESI) m/z: [M+H]+ = 322.

Step 3: Preparation of tert-butyl-4-(3-acetyl-1-[imidazo[1,2-a]pyridin-5-yl]indolizin-7-yl)piperazine-1-carboxylate (i10-4)

To a stirred mixture of 4-[4-[(tert-butoxy)carbonyl]piperazin-1-yl]-1-(2-oxopropyl)pyridin-1-ium bromide (1 g, 2.498 mmol, 1 equiv) and 5-ethynylimidazo[1,2-a]pyridine(0.43 g, 2.998 mmol, 1.2 equiv) in

DMF(16 mL) was added K_2CO_3 (0.69 g, 4.996 mmol, 2 equiv), and the resulting mixture was stirred for 15 hours at room temperature. The resulting mixture was diluted with water and extracted with EtOAc (2 x 20 mL). The organic layers were washed with water (3 x 10 mL) and dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford tert-butyl 4-(3-acetyl-1-[imidazo[1,2-a]pyridin-5-yl]indolizin-7-yl)piperazine-1-carboxylate (178mg, 14.27%). LCMS (ESI) m/z: [M+H]+ = 460.

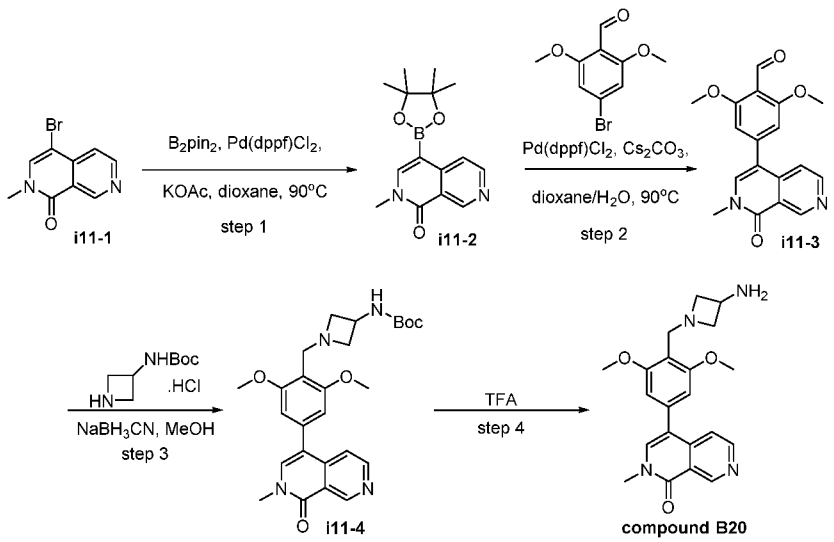
Step 4: Preparation of 1-(1-[imidazo[1,2-a]pyridin-5-yl] -7-(piperazin-1-yl)indolizin-3-yl)ethan-1-one (compound B19)



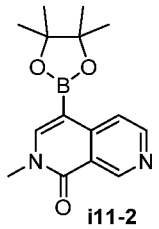
compound B19

To a stirred solution of tert-butyl 4-(3-acetyl-1-[imidazo[1,2-a]pyridin-5-yl]indolizin-7-yl)piperazine-1-carboxylate (60 mg, 0.131 mmol, 1 equiv) in DCM (5 mL) was added TFA (3.00 mL, 40.389 mmol, 309.35 equiv). The resulting mixture was stirred for 2 hours at room temperature, and then was concentrated under reduced pressure. The crude product was purified by Prep-HPLC (conditions: X Bridge Shield RP18 OBD Column, 5 μm,19*150 mm; mobile phase, Water (0.05% NH_3H_2O) and ACN (35% Phase B up to 58% in 8 minutes); Detector, UV). This resulted in 1-(1-[imidazo[1,2-a]pyridin-5-yl] -7-(piperazin-1-yl)indolizin-3-yl)ethan-1-one (43.6 mg, 30.06%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.81 (d, *J* = 7.8 Hz, 1H), 8.18 – 8.03 (m, 4H), 7.92 (d, *J* = 8.9 Hz, 1H), 7.63 (d, *J* = 7.3 Hz, 1H), 7.12 (d, *J* = 8.0,1H), 6.87 (s, 1H), 3.69 – 3.61 (m, 4H), 3.42 – 3.29 (m, 4H), 2.61 (d, *J* = 1.5 Hz, 3H). LCMS (ESI) m/z: [M+H]+ = 360.05.

Example 11 – Preparation of 4-[4-[(3-aminoazetidin-1-yl)methyl]-3,5-dimethoxyphenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (compound B20)

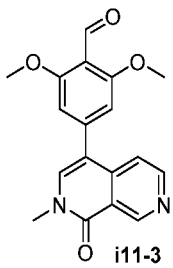


Step 1: Preparation of 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydro-2,7-naphthyridin-1-one (i11-2)



To the solution of 4-bromo-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (2.7 g, 11.294 mmol, 1 equiv) in dioxane (15 mL) was added 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (3.44 g, 13.552 mmol, 1.2 equiv), $Pd(dppf)Cl_2$ (0.83 g, 1.129 mmol, 0.1 equiv), and AcOK (3.33 g, 33.881 mmol, 3 equiv). The resulting solution was stirred at 90 ℃ for 2 hours under nitrogen atmosphere. The resulting solution was concentrated. The residue was purified by Flash column chromatography with EtOAc/PE (0~100%) to give compound 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydro-2,7-naphthyridin-1-one (1.62 g, 50.13%) as light yellow solid. LCMS (ESI) m/z: [M+H]+ = 287.

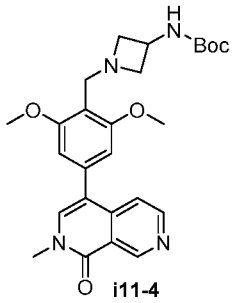
Step 2: Preparation of 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (i11-3)



To the solution of 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydro-2,7-naphthyridin-1-one (1.62 g, 5.662 mmol, 1 equiv) in dioxane (30 mL) was added 4-bromo-2,6-dimethoxybenzaldehyde (1.39 g, 5.662 mmol, 1 equiv), $Pd(dppf)Cl_2$ (414.26 mg, 0.566 mmol, 0.1 equiv), Cs_2CO_3 (5.53 g, 16.985 mmol, 3 equiv), and H_2O (3 mL). The resulting solution was stirred at 90 ℃ for 2 hours under nitrogen atmosphere. The resulting solution was concentrated. The residue was purified by

Flash column chromatography with EtOAc/PE (0~100%) to give compound 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (1.02 g, 55.55%) as yellow solid. LCMS (ESI) m/z: [M+H]+ = 325.

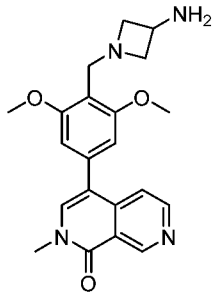
Step 3: Preparation of tert-butyl N-(1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)carbamate (i11-4)



i11-4

To a stirred solution of 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (450.00 mg, 1.387 mmol, 1.00 equiv) in MeOH (10.00 mL) was added $NaBH_3CN$ (261.57 mg, 4.162 mmol, 3.00 equiv), *tert*-butyl *N*-(azetidin-3-yl)carbamate hydrochloride (347.46 mg, 1.665 mmol, 1.20 equiv). The resulting mixture was stirred for 1 hour at room temperature. The residue was purified by Flash column chromatography with EtOAc/PE (0~100%) to afford *tert*-butyl *N*-(1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)carbamate (475 mg, 71.24%) as a light yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 481.

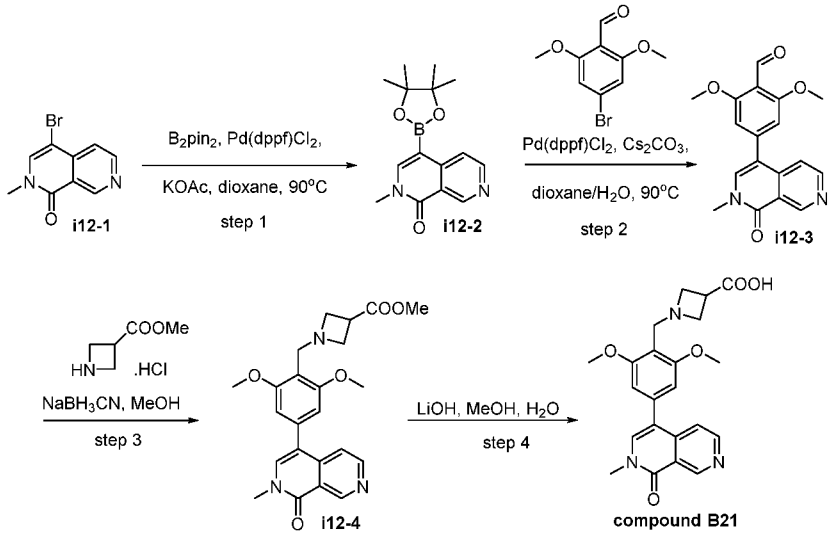
Step 4: Preparation of 4-[4-[(3-aminoazetidin-1-yl)methyl]-3,5-dimethoxyphenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (compound B20)



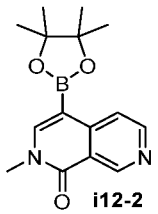
compound B20

To the solution of *tert*-butyl *N*-(1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)carbamate(50.00 mg, 0.104 mmol, 1.00 equiv) in DCM (2.00 mL) was added TFA (2.00 mL, 26.926 mmol, 258.79 equiv). The resulting solution was stirred at room temperature for 1 hour. The resulting solution was concentrated. The crude product was purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um, 19*150mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 35% B to 65% B in 8 min; 254 nm; Rt: 7.38 min) to afford 4-[4-[(3-aminoazetidin-1-yl)methyl]-3,5-dimethoxyphenyl]-2-methyl-2,7-naphthyridin-1-one(9.8mg,24.76%) as a yellow solid. ^1H NMR (400 MHz, MeOD) δ 9.57 (s, 1H), 8.70 (d, 1H), 7.87 (s, 1H), 7.72 (d, 1H), 6.88 (s, 2H), 4.63 (s, 2H), 4.54 (t, 2H), 4.45 (t, 2H), 4.38 (d, 1H), 3.98 (d, 6H), 3.73 (d, 3H), 2.69 (s, 1H). LCMS (ESI) m/z: [M+H]+ = 381.25.

Example 12 – Preparation of 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylic acid (compound B21)

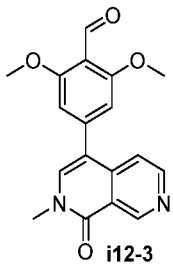


Step 1: Preparation of 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydro-2,7-naphthyridin-1-one (i12-2)



To the solution of 4-bromo-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (2.7 g, 11.294 mmol, 1 equiv) in dioxane (15 mL) was added 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (3.44 g, 13.552 mmol, 1.2 equiv), $Pd(dppf)Cl_2$ (0.83 g, 1.129 mmol, 0.1 equiv), and AcOK (3.33 g, 33.881 mmol, 3 equiv). The resulting solution was stirred at 90 ℃ for 2 hours under nitrogen atmosphere. The resulting solution was concentrated. The residue was purified by Flash column chromatography with EtOAc/PE (0-100%) to give compound 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydro-2,7-naphthyridin-1-one (1.62 g, 50.13%) as light yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 287.

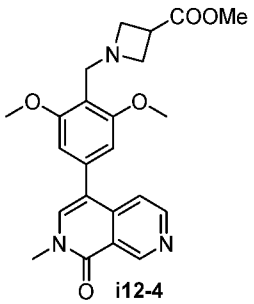
Step 2: Preparation of 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (i12-3)



To the solution of 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydro-2,7-naphthyridin-1-one (1.62 g, 5.662 mmol, 1 equiv) in dioxane (30 mL) was added 4-bromo-2,6-dimethoxybenzaldehyde (1.39 g, 5.662 mmol, 1 equiv), $Pd(dppf)Cl_2$ (414.26 mg, 0.566 mmol, 0.1 equiv),

and Cs_2CO_3 (5.53 g, 16.985 mmol, 3 equiv),H_2O (3 mL). The resulting solution was stirred at 90 ℃ for 2 hours under nitrogen atmosphere. The resulting solution was concentrated. The residue was purified by Flash column chromatography with EtOAc/PE (0~100%) to give compound 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (1.02 g, 55.55%) as yellow solid. LCMS (ESI) m/z: [M+H]+ = 325.

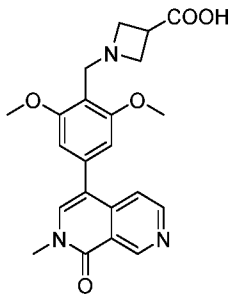
Step 3: Preparation of methyl 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylate (i12-4)



i12-4

To a stirred solution of 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (600.00 mg, 1.850 mmol, 1.00 equiv) in DCM (15.00 mL) was added methyl azetidine-3-carboxylate hydrochloride (336.52 mg, 2.220 mmol, 1.20 equiv) and $NaBH_3CN$ (348.76 mg, 5.550 mmol, 3.00 equiv). The resulting mixture was stirred for 1 hour at room temperature. The residue was purified by Flash column chromatography with EtOAc/PE (0~100%) to afford methyl 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylate (800 mg, 102.12%) as a light yellow solid. LCMS (ESI) m/z: [M+H]+ = 424.

Step 4: Preparation of 1-*[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylic acid (compound B21)*

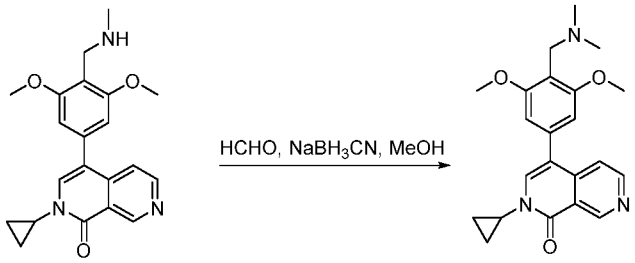


compound B21

To the solution of methyl 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylate (50 mg, 0.118 mmol, 1 equiv) in MeOH (10 mL, 0.312 mmol, 2.64 equiv) was added LiOH (28.28 mg, 1.181 mmol, 10 equiv). The resulting solution was stirred at room temperature for 12 hours. The resulting solution was concentrated. The crude product was purified by Prep-HPLC (conditions: SunFire C18 OBD Prep Column, 19 mm X 250 mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 4% B to 4% B in 2 min; 254 nm; Rt: 9.83 min) to afford 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylic acid (8.6 mg, 16.91%) as a yellow solid. ^1H NMR (400 MHz,

MeOD) δ 9.55 (s, 1H), 8.70 (d, 1H), 7.80 (s, 1H), 7.64 (d, 1H), 6.88 (d, 2H), 4.56 (s, 2H), 4.39 (d, 4H), 3.99 (d, 6H), 3.72 (d, 4H). LCMS (ESI) m/z: $[M+H]^+$ = 410.10.

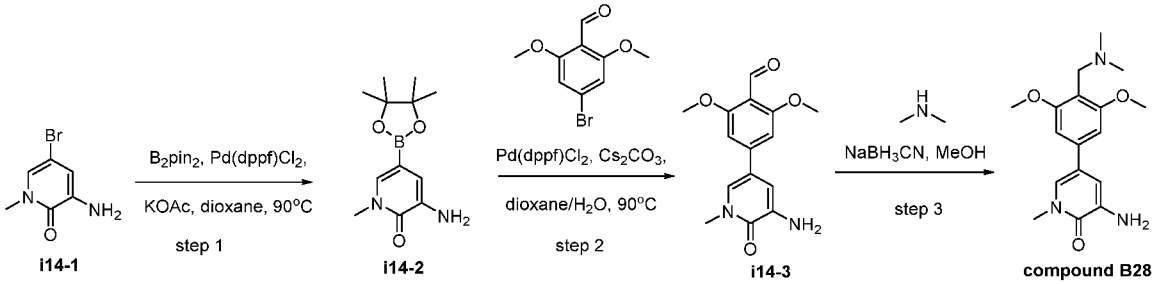
Example 13 – Preparation of 2-cyclopropyl-4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-2,7-naphthyridin-1-one (compound B22)



compound B18 compound B22

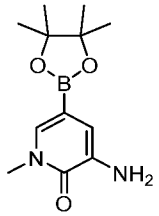
To a solution of 2-cyclopropyl-4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2,7-naphthyridin-1-one (40.00 mg, 0.109 mmol, 1.00 equiv) and a solution of formaldehyde in water (0.20 mL, 37%) was added $NaBH_3CN$ (20.64 mg, 0.328 mmol, 3.00 equiv). The resulting mixture was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure. The crude product was purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um, 19*150mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 32% B to 68% B in 8 min; 254 nm; Rt: 7.38 min) to afford 2-cyclopropyl-4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-2,7-naphthyridin-1-one(10mg, 24.08%) as a white solid. ^1H NMR (400 MHz, Methanol-d4) δ 9.53 (s, 1H), 8.68 (d, J = 5.7 Hz, 1H), 8.57 (s, 1H), 7.66 (s, 1H), 7.58 (d, J = 5.7 Hz, 1H), 6.81 (s, 2H), 4.07 (s, 2H), 3.93 (s, 6H), 3.43 (s, 1H), 3.33 (s, 3H), 2.63 (s, 6H), 1.19 (t, J = 6.8 Hz, 2H), 1.08 – 1.00 (m, 2H). LCMS (ESI) m/z: $[M+H]^+$ = 380.25.

Example 14 – Preparation of 3-amino-5-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-1-methylpyridin-2(1H)-one (compound B28)



i14-1 i14-2 i14-3 compound B28

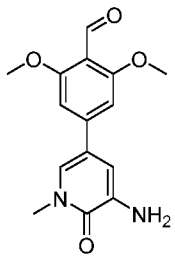
Step 1: Preparation of 3-amino-1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (i14-2)



i14-2

To a stirred solution of 3-amino-5-bromo-1-methylpyridin-2-one (3.00 g, 14.775 mmol, 1.00 equiv) in 1,4-dioxane (50 mL) was added 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (4.46 g, 17.556 mmol, 1.2 equiv), $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (1.19 g, 1.463 mmol, 0.1 equiv), and AcOK (4.31 g, 43.891 mmol, 3 equiv). The mixture was stirred for 1.5 hours at 90 degrees C under N_2 atmosphere. Then the solvent was evaporated, and the resulting residue was purified by flash chromatography eluting with PE/EA (1:2) to afford 3-amino-1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (3.25 g, 88.82%) as a brown yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 251.

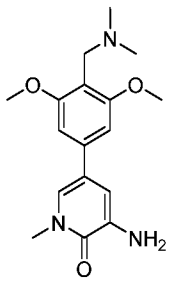
Step 2: Preparation of 4-(5-amino-1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-2,6-dimethoxybenzaldehyde (i14-3)



i14-3

To a stirred solution of 3-amino-1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (3.69 g, 14.754 mmol, 1 equiv) in 1,4-dioxane (80 mL) and H_2O (8 mL) was added 4-bromo-2,6-dimethoxybenzaldehyde (3.25 g, 13.278 mmol, 0.90 equiv), $Pd(dppf)Cl_2 \bullet CH_2Cl_2$ (1.20 g, 1.475 mmol, 0.1 equiv), and Cs_2CO_3 (14.42 g, 44.261 mmol, 3 equiv). The solution was stirred for 1 hour at 90 degrees C under N_2 atmosphere. Then the mixture was diluted with water and extracted with EtOAc, and the combined organic layer was concentrated. The residue was purified by silica gel chromatography eluting with PE/EtOAc (1:3) to afford 4-(5-amino-1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-2,6-dimethoxybenzaldehyde (3.0 g, 70.53%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 289.

Step 3: Preparation of 3-amino-5-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-1-methylpyridin-2(1H)-one (compound B28)

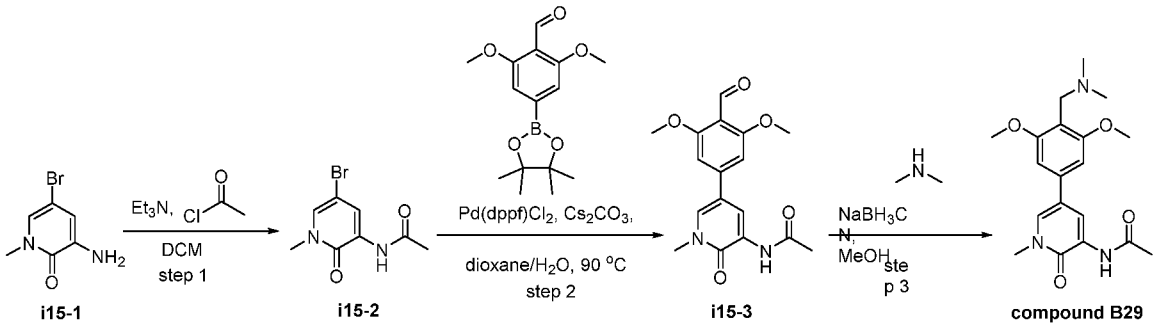


compound B28

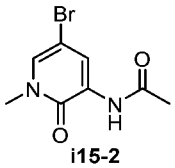
To a stirred solution of dimethylamine hydrochloride(1.22 g, 14.984 mmol, 1.5 equiv) in MeOH (50 mL) was added 4-(5-amino-1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-2,6-dimethoxybenzaldehyde (2.88 g, 9.989 mmol, 1 equiv). After 30 minutes of stirring, $NaBH_3CN$ (1.26 g, 19.979 mmol, 2 equiv) was added in portions, and the mixture was stirred for 1 hour at 25 degrees C. Then MeOH was evaporated, and the residue was purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um, 19*150mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 5%

B to 22% B in 8 min; 254 nm; Rt: 7.52 min). This resulted in 3-amino-5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-1,2-dihydropyridin-2-one (500 mg, 15.75%) as light green salt. ^{1}H NMR (300 MHz, Methanol-d4) δ 8.56 (s, 1H), 7.39 (d, *J* = 2.2 Hz, 1H), 7.06 (d, *J* = 2.3 Hz, 1H), 6.84 (s, 2H), 4.22 (s, 2H), 3.97 (s, 6H), 3.67 (s, 3H), 2.76 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 318.15.

Example 15 – Preparation of N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (compound B29)



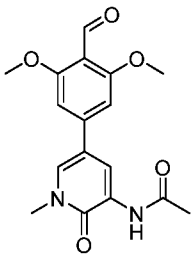
Step 1: preparation of N-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (i15-2)



i15-2

To a stirred solution of 3-amino-5-bromo-1-methyl-1,2-dihydropyridin-2-one (406.08 mg, 2.000 mmol, 1 equiv) and Et_3N (1619.05 mg, 16.000 mmol, 8 equiv) in DCM (5 mL) was added acetyl chloride (628.00 mg, 8.00 mmol, 4 equiv) dropwise at 0 degrees C. The mixture was stirred for 1 hour at room temperature. Then the solvent was evaporated, and the residue was purified by flash chromatography, eluted with EtOAc/PE (0-100%) to give the *N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (487 mg, 99.36%). LCMS (ESI) m/z: $[M+H]^+$ = 245.

Step 2: Preparation of N-[5-(4-formyl-3,5-dimethoxyphenyl)-1-methylidene-2-oxo-1,2-dihydro-1lambda4-pyridin-3-yl]acetamide (i15-3)

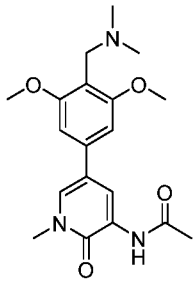


i15-3

To a stirred solution of 2,6-dimethoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (292 mg, 1.000 mmol, 1 equiv), and *N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (244.96 mg, 1.000 mmol, 1 equiv) in 1,4-dioxane (5 mL) and H_2O (0.5 mL) was added $Pd(dppf)Cl_2•CH_2Cl_2$ (81.62 mg, 0.100 mmol, 0.1 equiv) and Cs_2CO_3 (976.99 mg, 2.999 mmol, 3 equiv). The mixture was reacted for 1 hour at 90 degrees C under N_2 atmosphere. After cooling, the mixture was concentrated, and the residue was purified by flash chromatography, eluted with EtOAc/PE (0-10 %) to

afford *N*-[5-(4-formyl-3,5-dimethoxyphenyl)-1-methylidene-2-oxo-1,2-dihydro-1lambda4-pyridin-3-yl]acetamide (280 mg, 85.06%) as a white solid. LCMS (ESI) m/z: $[M+H]^+$ = 331.

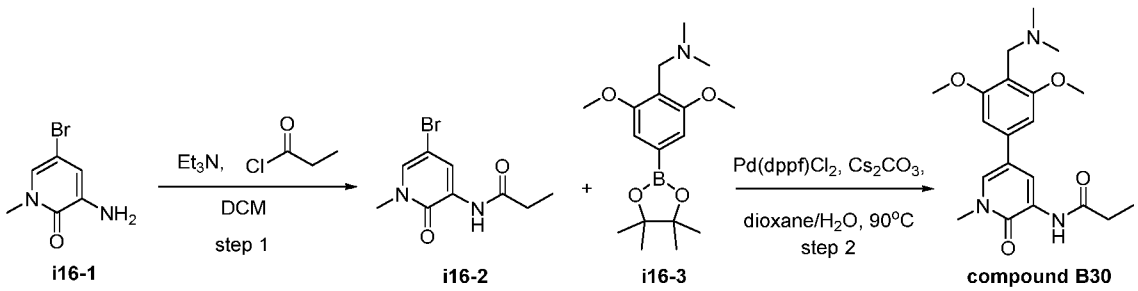
Step 3: Preparation of N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (compound B29)



compound B29

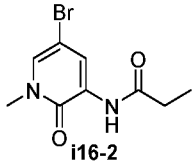
To a stirred solution of dimethylamine hydrochloride (98.73 mg, 1.211 mmol, 1.6 equiv) in MeOH (5 mL) was added *N*-[5-(4-formyl-3,5-dimethoxyphenyl)-1-methyl-2-oxo-1,2-dihydropyridin-3-yl]acetamide (250 mg, 0.757 mmol, 1 equiv). The mixture was stirred for 30 minutes at 25 degrees C. Then $NaBH_3CN$ (95.12 mg, 1.514 mmol, 2 equiv) was added in portions, and the reaction mixture was stirred for another 1 hour at 25 degrees C. The mixture was quenched with addition of water and extracted with DCM. The organic layer was concentrated, and the residue was purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um, 19*150mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 12% B to 25% B in 8 min; 254 nm; Rt: 5.68 min), to give *N*-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (26.4 mg, 9.32%) as white solid. ^{1}H NMR (300 MHz, Methanol-d4) δ 8.69 (d, *J* = 2.5 Hz, 1H), 8.56 (s, 0.3H, FA), 7.81 (d, *J* = 2.5 Hz, 1H), 6.88 (s, 2H), 4.23 (s, 2H), 3.99 (s, 6H), 3.72 (s, 3H), 2.77 (s, 6H), 2.25 (s, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 360.25.

Example 16 – Preparation of N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (compound B30)



i16-1 **i16-2** **i16-3** **compound B30**

Step 1: preparation of N-(5-bromo-1-methyl-2-oxopyridin-3-yl)propanamide (i16-2)

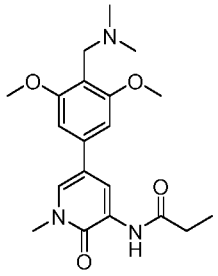


i16-2

To a stirred solution of 3-amino-5-bromo-1-methylpyridin-2-one (150.00 mg, 0.738 mmol, 1.00 equiv) and Et_3N (300 mg, 2.95 mmol, 4 equiv) in DCM (3.00 mL) was added propanoyl chloride (348.60 mg, 3.69 mmol, 5 equiv) dropwise at 0 degrees C, and the solution was stirred for 1 hour. Then the

solvent was evaporated, and the residue was purified by silica gel column chromatography, eluted with EtOAc/PE (0-100 %) to afford *N*-(5-bromo-1-methyl-2-oxopyridin-3-yl)propanamide (168 mg, 87.77%) as a purple solid. LCMS (ESI) m/z: [M+H]+ = 259.

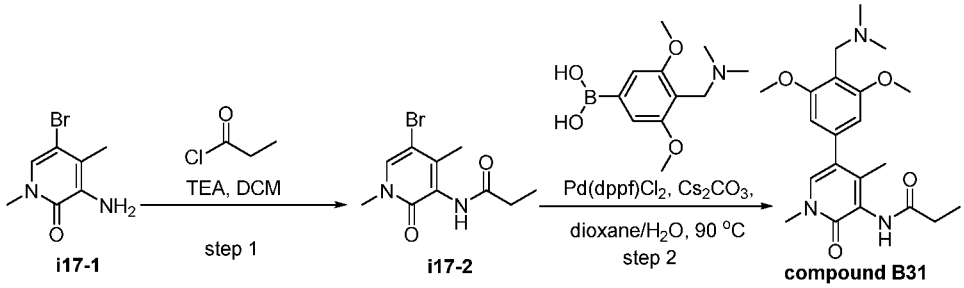
Step 2: Preparation of N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (compound B30)



compound B30

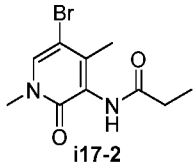
To a solution of *N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (100 mg, 0.386 mmol, 1 equiv) and [[2,6-dimethoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl] dimethylamine (123.97 mg, 0.386 mmol, 1 equiv) in 1,4-dioxane (3 mL) and H_2O (0.3 mL) was added Cs_2CO_3 (377.25 mg, 1.158 mmol, 3 equiv) and $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (31.52 mg, 0.039 mmol, 0.1 equiv). The mixture was stirred for 1 hour at 90 degrees C under N_2 atmosphere. After the solvent was evaporated, the mixture was purified by flash chromatography, eluted with DCM/MeOH (0-10%) to afford 270 mg of crude product, which was further purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um,19*150mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 12% B to 22% B in 8 min; 254 nm; Rt: 4.95 min) to afford *N*-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (26mg,18.04%) as a white solid. ^{1}H NMR (300 MHz, Chloroform-d) δ 12.14 (s, 1H), 8.73 (d, *J* = 2.4 Hz, 1H), 8.48 (s, 1H), 7.26 (d, *J* = 2.5 Hz, 1H), 6.64 (s, 2H), 4.29 (s, 2H), 3.92 (s, 6H), 3.72 (s, 3H), 2.80 (s, 6H), 2.51 (q, *J* = 7.5 Hz, 2H), 1.28 (t, *J* = 7.5 Hz, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 374.40.

Example 17 – Preparation of N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (compound B31)



i17-1 **i17-2** **compound B31**

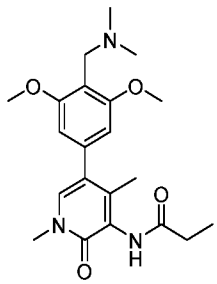
Step 1: Preparation of N-(5-bromo-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (i17-2)



i17-2

To a solution of 5-bromo-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-aminium (400 mg, 1.83 mmol, 1.0 eq.) and TEA (742.43 mg, 7.34 mmol, 4.0 eq.) in DCM (5 mL) was added propanoyl chloride (186.68 mg, 2.02 mmol, 1.1 eq.) at 0 ℃. The resulting solution was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with CH_2Cl_2/MeOH (10:1) to afford *N*-(5-bromo-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (460 mg, 73%) as a yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 273.1.

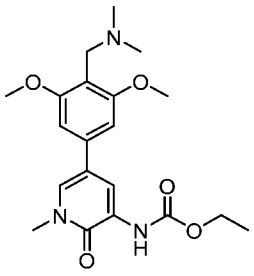
Step 2: Preparation of N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (compound B35)



compound B31

To a solution of *N*-(5-bromo-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (100 mg, 0.37 mmol, 1.0 eq.) and [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]boronic acid (87.53 mg, 0.37 mmol, 1.0 eq.) in dioxane (2.5 mL) and H_2O (0.5 mL) was added Cs_2CO_3 (238.58 mg, 0.73 mmol, 2.0 eq.) and Pd(dppf)Cl_2·CH_2Cl_2 (29.90 mg, 0.037 mmol, 0.1 eq.). The resulting solution was stirred at 90 degree C for 1 hour (under N_2 atmosphere). LCMS indicated that the reaction was completed. The mixture was allowed to cool down to room temperature. The resulting mixture was diluted water (30 mL) and extracted with EtOAc (30 mL x 2). After dry over Na_2SO_4, the filtrate was concentrated in vacuo. The crude product was purified by Prep-HPLC (conditions: Xselect CSH F-Phenyl OBD Column 19*150 mm 5umn; Mobile Phase A:Water (0.1% FA), Mobile Phase B: EtOH-HPLC; Flow rate: 25 mL/min; Gradient: 5% B to 11% B in 10 min; 220 nm; Rt: 7.60 min) to afford formate of *N*-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1,4-dimethyl-2-oxo-1,2-dihydropyridinyl)propanamide (8.6 mg, 6%) as light brown solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.56 (s, 1H), 7.59 (s, 1H), 6.77 (s, 2H), 4.36 (s, 2H), 3.96 (s, 6H), 3.64 (s, 3H), 3.33 (s, 5H), 2.87 (s, 6H), 2.51 (q, *J* = 7.7 Hz, 2H), 2.06 (s, 3H), 1.26 (t, *J* = 7.6 Hz, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 388.35.

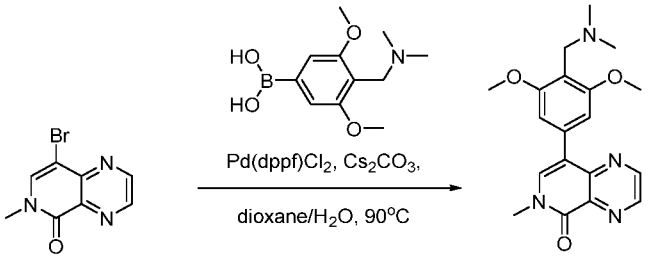
Example 18 – Preparation of N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (compound B32)



compound B32

Compound B32 was prepared in a similar manner as described for compound B42. N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)acetamide (40 mg, 27.27%) was obtained as a white solid. ^{1}H NMR (300 MHz, Chloroform-d) δ 8.64 (s, 0.35H, FA), 8.34 (d, J = 2.3 Hz, 1H), 7.88 (s, 1H), 7.20 (d, J = 2.4 Hz, 1H), 6.64 (s, 2H), 4.26 (q, J = 7.1 Hz, 2H), 4.16(s, 2H), 3.92 (s, 6H), 3.71 (s, 3H), 2.67 (s, 6H), 1.35 (t, J = 7.1 Hz, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 390.20.

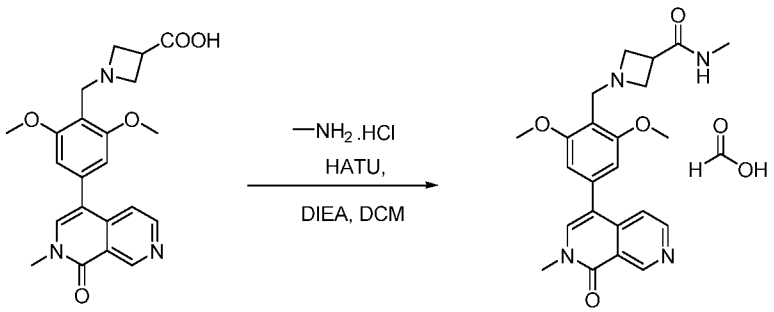
Example 19 – Preparation of 8-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-6-methylpyrido[3,4-b]pyrazin-5-one (compound B33)



compound B33

To a stirred mixture of 8-bromo-6-methylpyrido [3,4-b]pyrazin-5-one (81.0 mg, 0.34 mmol, 1.0 equiv) and 4-[(dimethylamino)methyl]-3,5-dimethoxyphenylboronic acid (96.80 mg, 0.41 mmol, 1.2 equiv) in 1,4-dioxane (4 mL) and H_2O (1 mL) was added $Pd(dppf)Cl_2$ (49.38 mg, 0.067 mmol, 0.2 equiv) and Cs_2CO_3 (274.84 mg, 0.84 mmol, 2.5 equiv), and the reaction was stirred at 90 degrees C under nitrogen atmosphere. After completion of the reaction, the mixture was allowed to cool down to room temperature. The reaction was diluted with water (25 mL) and extracted with EtOAc (3 x 20 mL). The filtrate was concentrated under reduced pressure. The crude product was purified by Prep-HPLC (conditions: Xselect CSH F-Phenyl OBD Column 19*150 mm 5 μm; Mobile Phase A: Water (0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 6% B to 10% B in 6 minutes; 220 nm; R_T: 4.37 minutes) to afford formate of 8-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-6-methylpyrido [3,4-b]pyrazin-5-one (15.1 mg, 12.54%) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.98 (s, 1H), 8.87 (s, 1H), 8.58 (s, 0.75H, FA), 7.97 (s, 1H), 7.02 (d, J = 1.6 Hz, 2H), 4.30 (s, 2H), 3.96 (d, J = 1.5 Hz,6H), 3.79 (s, 3H), 2.82 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 355.4.

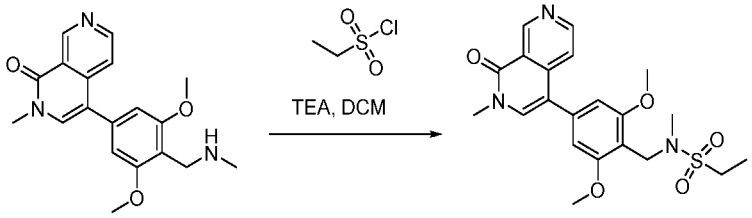
Example 20 – Preparation of 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl] methyl]-N-methylazetidine-3-carboxamide formic acid (compound B34 formic acid)



compound B21 compound B34 formic acid

To a stirred mixture of 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylic acid (127.87 mg, 0.24 mmol, 1.0 equiv) and DIEA (189.43 mg, 1.47 mmol, 6.0 equiv) in DCM (3 mL) was added methanamine hydrochloride(19.79 mg, 0.29 mmol, 1.20 equiv). The mixture was stirred at room temperature for 5 minutes, then HATU (139.32 mg, 0.37 mmol, 1.50 equiv) was added. The mixture was stirred for another 2 hours at room temperature. The residue was directly purified by Prep-HPLC (conditions: Xselect CSH F-Phenyl OBD Column 19*150 mm 5 μm; Mobile Phase A: Water (0.1% FA), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 7% B to 7% B in 7 minutes; 220 nm; R_T: 5.17 minutes) to afford 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methylazetidine-3-carboxamide formic acid (13.7 mg, 11%) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.54 (s, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 8.55 (brs, 1.3H, FA), 7.77 (s, 1H), 7.62 (d, *J* = 5.8 Hz, 1H), 6.86 (s, 2H), 4.45 (s, 2H), 4.24-4.16 (m, 4H), 3.97 (s, 6H), 3.72 (s, 3H), 3.56 (p, *J* = 7.9 Hz, 1H), 2.79 (s, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 423.25.

Example 21 – Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]m ethyl]-N-methylethane-1-sulfonamide (compound B35)

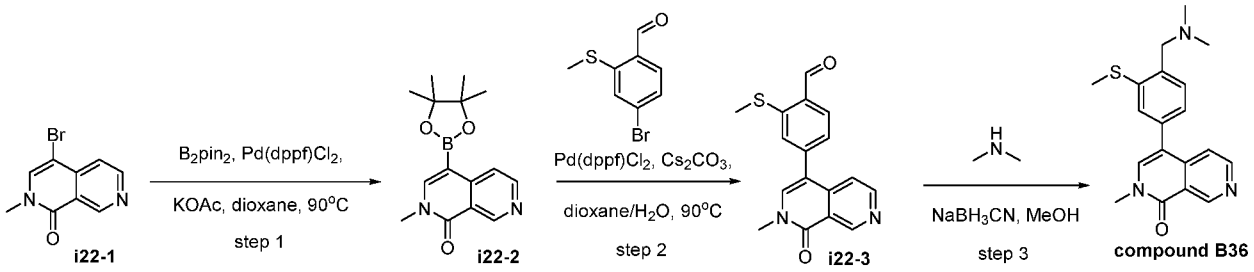


compound B35

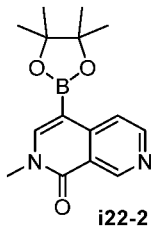
To a stirred mixture of 4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (65 mg, 1.0 equiv) and TEA (58.29 mg, 3.0 equiv) in DCM (1 mL) was added propane-2-sulfonyl chloride (37.2 mg, 1.5 equiv). The mixture was stirred at 25 degrees C for 2 hours. The resulting mixture was concentrated under vacuum. The crude product was purified by Prep-HPLC (conditions: XBridge Shield RP18 OBD Column, 5 μm,19*150 mm; Mobile Phase A: Water (0.05% NH_3H_2O), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 27% B to 46% B in 8 minutes; 220 nm; R_T: 7.8 minutes) to afford N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methylethane-1-sulfonamide (8.4 mg, 10%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.54 (d, *J* = 0.9 Hz, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 7.77 (s, 1H), 7.65 (dd, *J* = 5.8, 1.0 Hz, 1H), 6.79 (s, 2H), 4.52 (s, 2H),3.91 (s, 6H), 3.72 (s, 3H), 3.17 (q, *J* = 7.3 Hz, 2H), 2.77 (s, 3H), 1.34 (q, *J* = 8.4, 7.9 Hz, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 432.25.

Example 22 – Preparation of 4-[4-[(dimethylamino)methyl]-3-(methylsulfanyl)phenyl]-2-methyl-1,2-

dihydro-2,7-naphthyridin-1-one (compound B36)

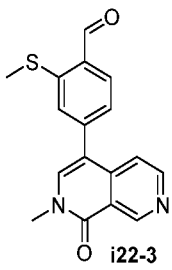


Step 1: Preparation of 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydro-2,7-naphthyridin-1-one (i22-2)



To a solution of 4-bromo-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (239 mg, 1.000 mmol, 1 equiv) and 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (380.80 mg, 1.500 mmol, 1.5 equiv) in dioxane (3.00 mL) was added CH_3COOK (294.34 mg, 2.999 mmol, 3 equiv) and $Pd(dppf)Cl_2$ (36.57 mg, 0.050 mmol, 0.05 equiv). The resulting solution was stirred at 80 degree C for 1 hour. The resulting mixture was concentrated under reduced pressure. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:1). This resulted in 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydro-2,7-naphthyridin-1-one (228 mg, 79.71%) as a white solid. LCMS (ESI) m/z: [M+H]+ = 287.1.

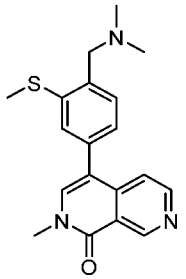
Step 2: Preparation of 4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)-2-(methylsulfanyl)benzaldehyde (i22-3)



To a solution of 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,7-naphthyridin-1-one (228.00 mg, 0.797 mmol, 1.00 equiv), 4-bromo-2-(methylsulfanyl)benzaldehyde (184.15 mg, 0.797 mmol, 1.00 equiv), and Cs_2CO_3 (776.89 mg, 2.390 mmol, 3 equiv) in 1,4-dioxane (3.00 mL) was added $Pd(dppf)Cl_2$ (58.30 mg, 0.080 mmol, 0.10 equiv) and H_2O (1.00 mL) at 25 degrees C. The resulting solution was stirred for 2 hours at 80 degrees C. The resulting solution was diluted with 10 mL of water. The resulting solution was extracted with ethyl acetate (2 x 20 mL) and the organic layers combined and dried over anhydrous sodium sulfate and concentrated. The residue was applied onto a silica gel column

with ethyl acetate/petroleum ether (1:1). This resulted in 4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)-2-(methylsulfanyl)benzaldehyde (200 mg, 80.87%) as a yellow solid. LCMS (ESI) m/z: [M+H]+ = 311.1.

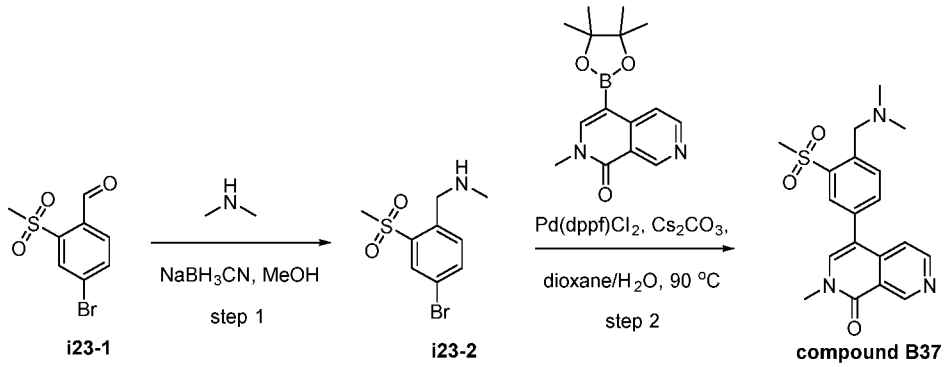
Step 3: Preparation of 4-[4-[(dimethylamino)methyl]-3-(methylsulfanyl)phenyl]-2-methyl-2,7-naphthyridin-1-one (compound B36)



compound B36

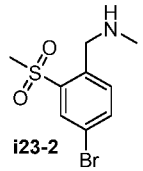
To a solution of 4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)-2-(methylsulfanyl)benzaldehyde (200.00 mg, 0.644 mmol, 1.00 equiv) and dimethylamine (34.86 mg, 0.773 mmol, 1.20 equiv) in MeOH (3.00 mL) was added $NaBH_3CN$ (80.99 mg, 1.289 mmol, 2.00 equiv) at 0 degrees C. The resulting solution was stirred for 1 hours at 0 degrees C. The resulting solution was diluted with 10 mL of water and extracted with ethyl acetate (2 x 20 mL), and the organic layers combined and dried over anhydrous sodium sulfate and concentrated. The crude product was purified by Prep-HPLC (conditions: Atlantis HILIC OBD Column, 19 mm X 150 mm; mobile phase, Water(0.1%FA) and ACN (hold 5% PhaseB in 2 minutes, up to 17% in 8 minutes); Detector, UV). This resulted in 4-[4-[(dimethylamino)methyl]-3-(methylsulfanyl)phenyl]-2-methyl-2,7-naphthyridin-1-one (150 mg, 68.57%) as a white solid. ^1H NMR (400 MHz, Methanol-d4) δ 9.54 (s, 1H), 8.68 (d, *J* = 5.8 Hz, 1H), 7.73 (s, 1H), 7.56 (d, *J* = 5.7 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 1.7 Hz, 1H), 7.26(dd, *J* = 7.8, 1.7 Hz, 1H), 3.71 (s, 3H), 3.64 (s, 2H), 2.52 (s, 3H), 2.33 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 340.20.

Example 23 – Preparation of 4-[4-[(dimethylamino)methyl]-3-methanesulfonylphenyl]-2-methyl-2,7-naphthyridin-1-one (compound B37)



i23-1 i23-2 compound B37

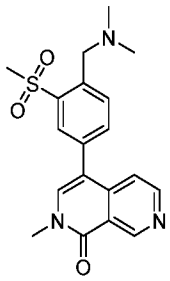
Step 1: Preparation of [(4-bromo-2-methanesulfonylphenyl)methyl]dimethylamine *(i23-2)*



i23-2

To a solution of 4-bromo-2-methanesulfonylbenzaldehyde (263 mg, 1.000 mmol, 1 equiv) and dimethylamine (135.20 mg, 2.999 mmol, 3 equiv) in MeOH (3.00 mL) was added $NaBH_3CN$ (125.63 mg, 1.999 mmol, 2 equiv) at 0 degrees C. The resulting solution was stirred for 1 hour at 0 degrees C. The resulting solution was diluted with 10 mL of water. The resulting solution was extracted with ethyl acetate (2 x 20 mL), and the organic layers combined, dried over anhydrous sodium sulfate, and concentrated. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0-50%). This resulted in [(4-bromo-2-methanesulfonylphenyl)methyl]dimethylamine (175 mg, 59.92%) as a yellow solid. LCMS (ESI) m/z: [M+H]+ = 278.0.

Step 2: Preparation of 4-[4-[(dimethylamino)methyl]-3-methanesulfonylphenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (compound B37)

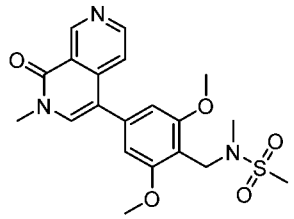


compound B37

To a solution of [(4-bromo-2-methanesulfonylphenyl)methyl]dimethylamine (175 mg, 0.599 mmol, 1.00 equiv) and 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,7-naphthyridin-1-one (205.65 mg, 0.719 mmol, 1.20 equiv) in 1,4-dioxane (3.00 mL) was added Cs_2CO_3 (585.43 mg, 1.797 mmol, 3.00 equiv), $Pd(dppf)Cl_2$ (43.82 mg, 0.060 mmol, 0.10 equiv), and H_2O (1.00 mL) at 25 degrees C. The resulting solution was stirred for 2 hours at 80 degrees C. The resulting solution was diluted with 10 mL of water and extracted with ethyl acetate (2 x 20 mL), and the organic layers combined and dried over anhydrous sodium sulfate and concentrated. The crude product was purified by Prep-HPLC (conditions: Atlantis HILIC OBD Column,19 mm X 250 mm; mobile phase, Water(0.1%FA) and ACN (hold 5% PhaseB

in 2 minutes, up to 17% in 8 minutes); Detector, UV). This resulted in 4-[4-[(dimethylamino)methyl]-3-methanesulfonylphenyl]-2-methyl-2,7-naphthyridin-1-one (120 mg, 53.94%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.55 (s, 1H), 8.71 (d, *J* = 5.7 Hz, 1H), 8.16 (d, *J* = 1.9 Hz, 1H), 7.80 (d, *J* = 9.0 Hz, 2H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 5.8 Hz,1H), 3.96 (s, 2H), 3.72 (s, 3H), 3.47 (s, 3H), 2.33 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 372.10.

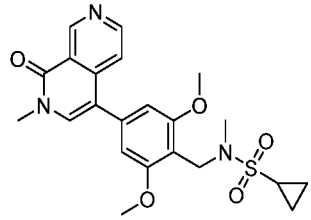
Example 24 – Preparation N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methylmethanesulfonamide (compound B38)



compound B38

Compound B38 was prepared in a similar manner as described for compound B35. N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methylmethane sulfonamide (14.2 mg, 16%) was obtained as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.57 (s, 1H), 8.70 (d, *J* = 6.1 Hz, 1H), 7.90 (s, 1H), 7.79 (d, *J* = 6.2 Hz, 1H), 6.80 (s, 2H), 4.50 (s, 2H), 3.92 (s, 6H), 3.74 (s, 3H),2.94 (s, 3H), 2.75 (s, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 418.10.

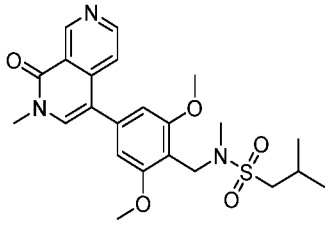
Example 25 – Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methyl cyclopropanesulfonamide (compound B39)



compound B39

Compound B39 was prepared in a similar manner as described for compound B35. N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methylcyclopropanesulfonamide (14.2 mg, 31%) was obtained as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.54 (d, *J* = 0.9 Hz, 1H), 8.70 (d, *J* = 5.7 Hz, 1H), 7.77 (s, 1H), 7.65 (d, *J* = 5.6 Hz, 1H), 6.79 (s, 2H), 4.54 (s, 2H), 3.91 (s, 6H),3.72 (s, 3H), 3.29 (s, 1H), 2.77 (s, 3H), 1.14 -1.05 (m, 4H). LCMS (ESI) m/z: $[M+H]^+$ = 444.20.

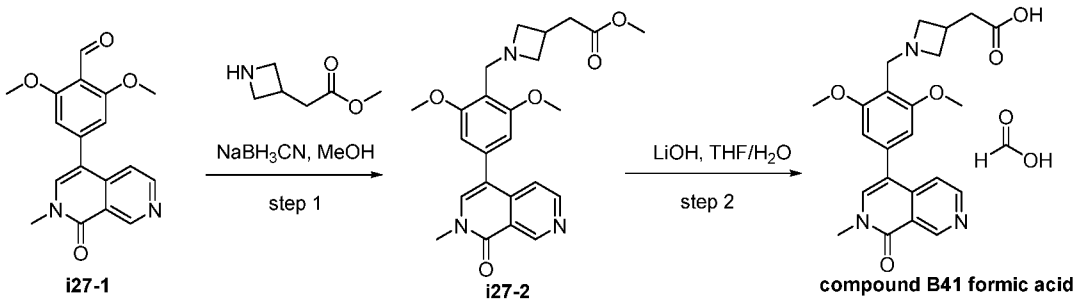
Example 26 – Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N,2-dim ethylpropane-1-sulfonamide (compound B40)



compound B40

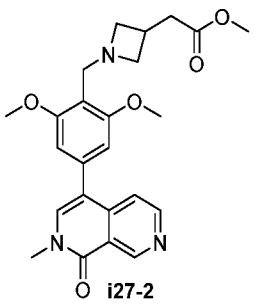
Compound B40 was prepared in a similar manner as described for compound B35. N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N,2-dimethylpropane-1-sulfonamide (10.3 mg, 8%) was obtained as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.54 (d, *J* = 0.9 Hz, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 7.77 (s, 1H), 7.65 (dd, *J* = 5.8, 0.9 Hz, 1H), 6.79 (s, 2H), 4.52 (s, 2H), 3.91 (s, 6H),3.72 (s, 3H), 2.99 (d, *J* = 6.5 Hz, 2H), 2.76 (s, 3H), 2.26 (hept, *J* = 6.6 Hz, 1H), 1.14 (d, *J* = 6.7 Hz, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 460.15.

Example 27 – Preparation of 2-(1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)acetic acid formic acid (compound B41 formic acid)



i27-1 **i27-2** **compound B41 formic acid**

Step 1: Preparation of 2-(1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)acetate (i27-2)

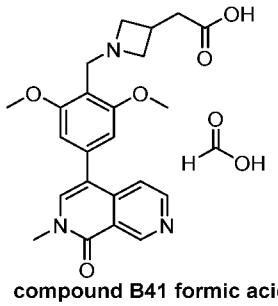


i27-2

To a solution of 2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)benzaldehyde (100.00 mg, 0.308 mmol, 1.00 equiv) and methyl 2-(azetidin-3-yl)acetate; trifluoroacetic acid (82.48 mg, 0.339 mmol, 1.10 equiv) in MeOH (3.00 mL) was added $NaBH_3CN$ (38.75 mg, 0.617 mmol, 2.00 equiv). The resulting mixture was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM. Pure fractions were evaporated to dryness to afford methyl 2-(1-[[2,6-dimethoxy-4-

(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)acetate (110 mg, 81.55%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 438.

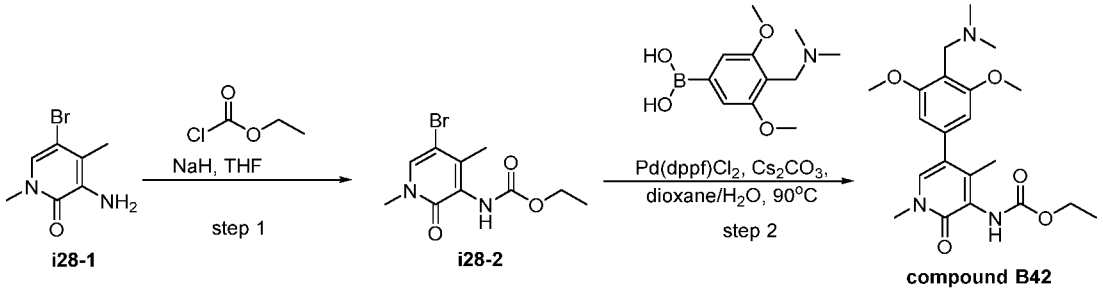
Step 2: Preparation of 2-(1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)acetic acid formic acid (compound B41 formic acid)



compound B41 formic acid

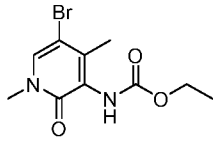
To a solution of methyl 2-(1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)acetate (120 mg, 0.274 mmol, 1 equiv) in MeOH (5 mL) and H_2O (1 mL) was added LiOH (65.69 mg, 2.743 mmol, 10 equiv). The resulting mixture was stirred at room temperature for 3 hours. The solvent was removed under reduced pressure, the residue was dissolved in water (10 mL). The mixture was acidified to pH 3 with 1 N HCl (aq.). The solvent was removed under reduced pressure. The crude product was purified by preparative HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um,19*150mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 5% B to 35% B in 8 min; 254 nm; Rt: 7.25 min). Fractions containing the desired compound were evaporated to dryness to afford 2-(1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidin-3-yl)acetic acid formic acid (8.1 mg, 6.16 %) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.44 (s, 1H), 8.72 (d, *J* = 5.6 Hz, 1H), 8.26 (s, 0.67H, FA), 7.86 (s, 1H), 7.56 (d, *J* = 5.6 Hz, 1H), 6.79 (d, *J* = 11.8 Hz, 0H), 6.74 (s, 2H), 4.36 (dd, *J* = 8.8, 7.1 Hz, 1H), 4.04 (dd, *J* = 8.8, 5.8 Hz, 1H), 3.90 (s, 6H), 3.76 (s, 2H), 3.60 (s, 3H), 2.72 (p, *J* = 7.0 Hz, 1H), 2.65 – 2.52 (m, 3H), 2.28 (dd, *J* =17.3, 6.4 Hz, 1H). LCMS (ESI) m/z: $[M+H]^+$ = 424.25.

Example 28 – Preparation ethyl N-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)carbamate (compound B42)



i28-1 **i28-2** **compound B42**

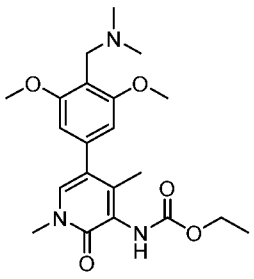
Step 1: Preparation of N-(5-bromo-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)propanamide (i28-2)



i28-2

To a solution of 3-amino-5-bromo-1,4-dimethylpyridin-2-one (300.00 mg, 1.382 mmol, 1.00 equiv) in THF(3.00 mL) was added NaH (66.33 mg, 2.764 mmol, 2 equiv) and ethyl chloroformate (179.98 mg, 1.658 mmol, 1.2 equiv). The resulting solution was stirred for 1 hour at room temperature. Then the reaction was quenched with saturated NH_4Cl (aq.) and purified by silica gel column chromatography, eluted with PE/EtOAc (5:1) to afford ethyl N-(5-bromo-1,4-dimethyl-2-oxopyridin-3-yl)carbamate (287mg, 71.82%). LCMS (ESI) m/z: [M+H]+ = 289.1.

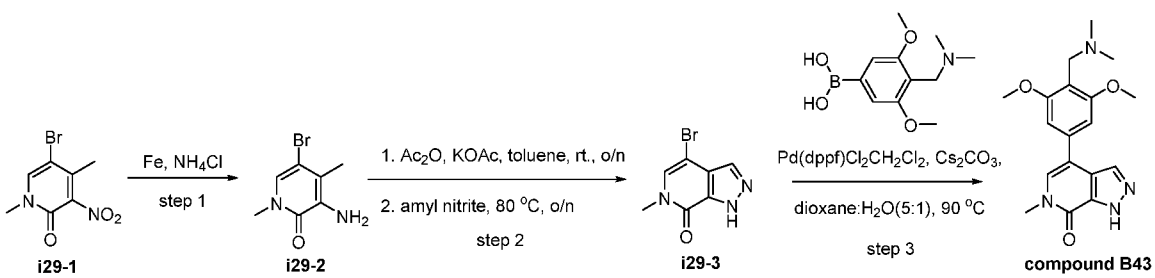
Step 2: Preparation of ethyl N-(5-[4-[(dimethylamino) methyl]-3, 5-dimethoxyphenyl]-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)carbamate (compound B42)



compound B42

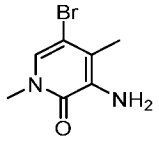
To a solution of ethyl *N*-(5-bromo-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)carbamate (25 mg, 0.086 mmol, 1 equiv) and [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]boronic acid (20.67 mg, 0.086 mmol, 1 equiv) in solvent dioxane (2 mL) and H_2O (0.5 mL) was added Cs_2CO_3 (84.52 mg, 0.259 mmol, 3 equiv) and $Pd(dppf)Cl_2$ (9.49 mg, 0.013 mmol, 0.15 equiv). The resulting solution was stirred at 90 degree C for 2 hours (under N_2 atmosphere). The crude product (140 mg) was purified by Prep-HPLC (conditions: X Bridge Shield RP18 OBD Column, 5um,19*150mm; Mobile Phase A:Water(0.05% NH_3H_2O), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 30% B to 65% B in 8 minutes; 220 nm; Rt: 8.2 minutes) to afford ethyl *N*-(5-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-1,4-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)carbamate (9.1 mg, 4.46%) as a light brown solid. ^{1}H NMR (300 MHz, Methanol-d4) δ 7.56 (s, 1H), 6.65 (s, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.88 (s, 6H), 3.79 (s, 2H), 3.64 (s, 3H), 2.41 (s, 6H), 2.12 (s, 3H), 1.32 (t, *J* = 7.1 Hz, 3H). LCMS (ESI) m/z: [M+H]+ = 404.3.

Example 29 – Preparation of 4-(4-((dimethyllamino) methyl) -3, 5-dimethoxyphenyl)-6-methyl-1, 6-dihydro-7H-pyrazolo[3,4-c]pyridin-7-one (compound B43)



i29-1 **i29-2** **i29-3** **compound B43**

Step 1: Preparation of 3-amino-5-bromo-1,4-dimethylpyridin-2(1H)-one (i29-2)



i29-2

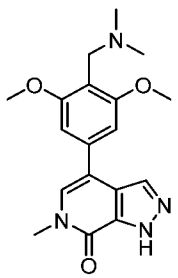
To a stirred mixture of NH_4Cl (17.3 g, 323.42 mmol, 10.0 equiv) in H_2O/EtOH (1/1, 400 mL) was added 5-bromo-1, 4-dimethyl-3-nitro-1, 2-dihydropyridin-2-one (8.0 g, 32.38 mmol, 1.0 equiv) and Fe (18.1 g, 324.11 mmol, 10.0 equiv) at room temperature. The mixture was stirred at rt. for 2 hours, the solid was filtered off. The filtrate was diluted with water (100 mL) and extracted with ethyl acetate (200 mL x 2). The organic layers were combined and washed with saturated NaCl (aq.), dried over with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. This resulted in 6.56 g (93%) 3-amino-5-bromo-1,4-dimethyl- pyridin-2(1H)-one as a red solid that was used directly without further purification. LCMS (ESI) m/z: $[M+H]^+ = 217$.

Step 2: Preparation of 4-bromo-6-methyl-1,6-dihydro-7H-pyrazolo[3,4-c]pyridin-7 -one (i29-3)

i29-3

To a solution of acetyl acetate (2.82 g, 27.62 mmol, 3.0 equiv) in toluene (50 mL) was added KOAc (1.01 g,11.11 mmol,1.20 equiv) at 25 degrees C. After stirring for 24 hours, to the yellow mixture was added 3-methylbutyl nitrite (174.86 mg, 1.49 mmol, 1.50 equiv), the resulting mixture was stirred at 110 degrees C for another 18 hours. Then it was allowed to cool down and mixture was concentrated under vacuum, the residue was purified by silica gel column chromatography (EtOAc/PE from 1/2 to 1/1). This resulted in 1.15 g (38%) of 4-bromo-6-methyl-1, 6-dihydro-7H-pyrazolo [3, 4-c]pyridin-7 -one as a yellow solid. LCMS (ESI) m/z: $[M+H]^+ = 228$.

Step 3: Preparation of 4-(4-((dimethyllamino) methyl) -3, 5-dimethoxyphenyl)-6-methyl-1, 6-dihydro- 7H-pyrazolo [3, 4-c] pyridin-7-one (compound B43)

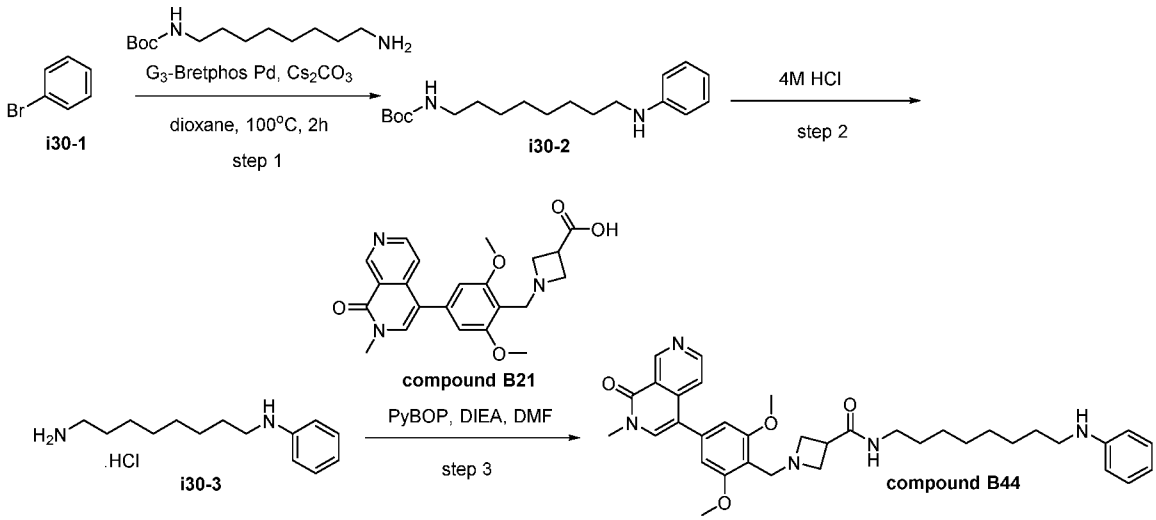


compound B43

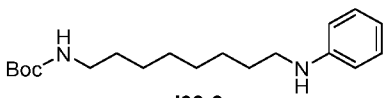
To a stirred mixture of 4-bromo-6-methyl-1H,6H,7H-pyrazolo[3,4-c]pyridin-7-one (220.34 mg, 0.97 mmol, 1.10 equiv) and [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]boronic acid (210 mg, 0.88 mmol, 1.0 equiv) in dioxane (5 mL) and H_2O (1 mL), was added Cs_2CO_3 (858.57 mg, 2.64 mmol, 3.0 equiv) and $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (107.6 mg, 0.13 mmol, 0.15 equiv) at 25 degrees C. The resulting mixture was heated to 90 degrees C under nitrogen atmosphere. After 16 hours, it was cooled down and diluted with water

(10 mL), then extracted with ethyl acetate (20 mL x 2). The organic layers were combined and washed with saturated NaCl (aq.), dried over with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure, then the crude product was purified by preparative-HPLC Column (XBridge Shield RP18 OBD Column, 5um, 19*150mm; Mobile Phase A: Water (0.1%FA), Mobile Phase B: MeOH--HPLC; Flow rate: 25 mL/min; Gradient: 5% B to 35% B in 8 min; 254 nm; Rt: 3.87 min). This resulted in 19.1 mg (3%) formate of 4-(4-((dimethyllamino) methyl) -3, 5-dimethoxyphenyl)-6-methyl-1,6-dihydro-7H-pyrazolo[3,4-c]pyridin-7-one as a yellow solid. ^{1}H NMR (300 MHz, Methanol-*d4*) δ 8.51 (s, 1H, FA), 8.16 (s, 1H), 7.51 (s, 1H), 7.02 (s, 2H), 4.40 (s, 2H), 4.03 (s, 6H), 3.74 (s, 3H), 2.90 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 343.3.

Example 30 – Preparation of 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl] methyl]-N-[8-(phenylamino)octyl]azetidine-3-carboxamide (compound B44)

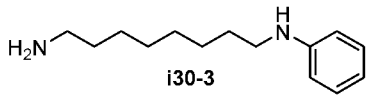


Step 1: Preparation of tert-butyl (8-(phenylamino)octyl)carbamate (i30-2)



To a stirred mixture of bromobenzene (25.42 mg, 0.162 mmol, 0.23 equiv) and tert-butyl N-(8-aminooctyl)carbamate (172.05 mg, 0.70 mmol, 1.0 equiv) in dioxane was added Cs_2CO_3 (314.26 mg, 0.97 mmol, 1.37 equiv) and G_3-Bretphos Pd (53.63 mg, 0.063 mmol, 0.09 equiv). The mixture was stirred at 100 degrees C for 2 h under nitrogen atmosphere. After cooling, the mixture was diluted with water (20 mL) and extracted with DCM (30 mL x 3). The organic layers were combined and dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product. The residue was purified by silica gel column chromatography, eluted with (PE/EtOAc 10:1) to afford tert-butyl N-[8-(phenylamino)octyl]carbamate (150 mg, 53%) as an off-white solid. LCMS (ESI) m/z: $[M+H]^+$ = 321.

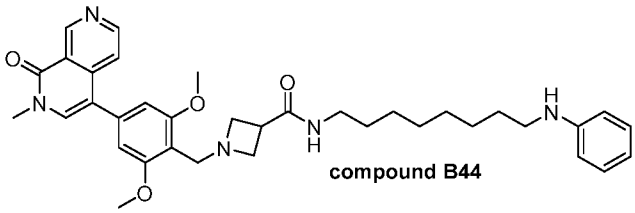
Step 2: Preparation of N1-phenyloctane-1,8-diamine (i30-3)



i30-3

To a solution of tert-butyl N-[8-(phenylamino)octyl]carbamate (110.0 mg, 0.34 mmol, 1.0 equiv) in DCM (8 mL) was added TFA (2 mL), and the mixture was stirred 2 h at room temperature. Then it was concentrated under reduced pressure to afford N1-phenyloctane-1,8-diamine (105 mg, 95%) as a yellow solid, that was used directly without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 221.

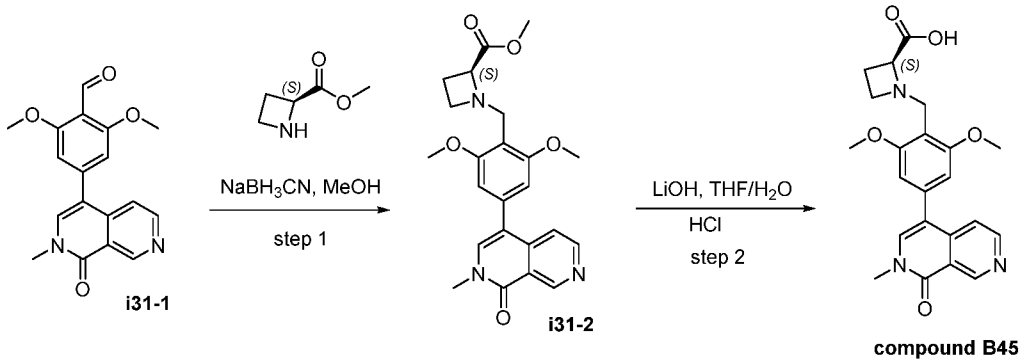
Step 4: 1-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)-N-(8-(phenylamino)octyl)azetidine-3-carboxamide (compound B44)



compound B44

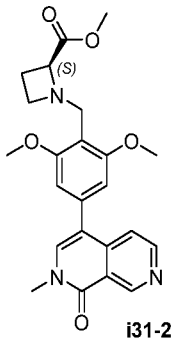
To a stirred solution of 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl) phenyl]methyl]azetidine-3-carboxylic acid (95 mg, 0.23 mmol, 1.0 equiv) and N1-phenyloctane-1,8-diamine (102.26 mg, 0.46 mmol, 2.0 equiv) in DMF (2 mL), was added EDCI (53.38 mg, 0.278 mmol, 1.20 equiv) and HOBT (37.62 mg, 0.278 mmol, 1.20 equiv). The resulting mixture was stirred at room temperature under nitrogen atmosphere. The reaction was quenched by the addition of water (5 mL) and extracted with DCM (30 mL x 3). The organic layers were combined and dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product. The crude product was purified by Prep-HPLC (conditions: XBridge Shield RP18 OBD Column, 5um, 19*150 mm; Mobile Phase A: Water ($0.05\%NH_3{\cdot}H_2O$), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 49% B to 69% B in 8 min; 220nm; Rt: 7.8 min) to afford 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-N-[8-(phenyl- amino)octyl]azetidine-3-carboxamide (6.0 mg, 4%) as a light yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.53 (s, 1H), 8.68 (d, *J* = 5.8 Hz, 1H), 7.73 (s, 1H), 7.63 (d, *J* = 5.8 Hz, 1H), 7.09 (t, *J* = 7.8 Hz, 2H), 6.74 (s, 2H), 6.66 – 6.56 (m, 3H), 3.88 (s, 6H), 3.80 (s, 2H), 3.71 (s, 3H), 3.50 (d, *J* = 8.0 Hz, 4H), 3.19 (dt, *J* = 11.1, 7.6 Hz, 3H), 3.06 (t, *J* = 7.2 Hz, 2H), 1.61 (p, *J* = 7.1 Hz, 2H), 1.51 (q, *J* = 6.9 Hz, 2H), 1.46 – 1.37 (m, 2H), 1.37 – 1.28 (m, 8H). LCMS (ESI) m/z: [M+H]+ = 612.50.

Example 31 – Preparation of (2S)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl] azetidine-2-carboxylic acid (compound B45)



i31-1 i31-2 compound B45

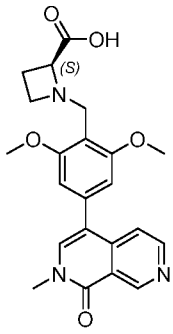
Step 1: Preparation of methyl (2S)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-2-carboxylate (i31-2)



i31-2

To a solution of 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (40.00 mg, 0.123 mmol, 1.00 equiv) and methyl (2*S*)-azetidine-2-carboxylate (15.62 mg, 0.136 mmol, 1.10 equiv) in MeOH (3.00 mL) was added Et_3N (14.98 mg, 0.148 mmol, 1.20 equiv) and $NaBH_3CN$ (23.25 mg, 0.370 mmol, 3.00 equiv) at 0 ℃. The resulting solution was stirred for 1 hour at 0 ℃. The resulting solution was diluted with 10 mL of water. The resulting solution was extracted with ethyl acetate (2 x 20 mL) and the organic layers combined and dried over anhydrous sodium sulfate and concentrated. The residue was applied onto a silica gel column with CH_2Cl_2/MeOH (50:1), which resulted in 42 mg (80.42%) of methyl (2*S*)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-2-carboxylate as a yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 423.2.

Step 2: Preparation of (2S)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-2-carboxylic acid (compound B45)

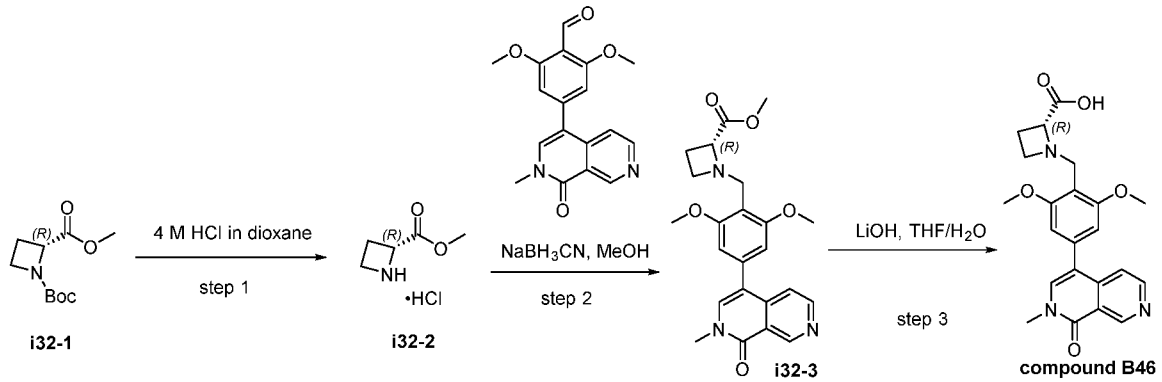


compound B45

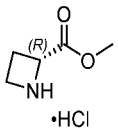
To a solution of methyl (2*S*)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl] methyl]azetidine-2-carboxylate (42 mg, 0.099 mmol, 1.00 equiv) in THF(1.50 mL) was added LiOH (11.88 mg, 0.496 mmol, 5.00 equiv) and H_2O (1.00 mL) at 0 ℃. The resulting solution was stirred for 2 hours at

25 °C. The resulting solution was diluted with 10 mL of water. Then HCl (6 M) (0.50 mL, 16.456 mmol, 165.92 equiv) was added, and the resulting solution was extracted with ethyl acetate (2 x 20 mL). The organic layers combined and dried over anhydrous sodium sulfate and concentrated. The crude product was purified by Prep-HPLC (conditions: Atlantis HILIC OBD Column, 19 mm X 250 mm; mobile phase, Water (0.1% FA) and ACN (hold 5% PhaseB in 2 minutes, up to 17% in 8 minutes); Detector, uv). This resulted in 25 mg (61.65%) (2*S*)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-2-carboxylic acid as a white solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.55 (s, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 7.82 (s, 1H), 7.66 (d, *J* = 5.8 Hz, 1H), 6.85 (s, 2H), 5.00 (t, *J* = 9.5 Hz, 1H), 4.53 (s, 2H), 4.14 (q, *J* = 9.6 Hz, 1H), 3.98 (s, 7H), 3.72 (s, 3H), 2.72 (d, *J* = 10.6 Hz, 1H), 2.61 (q, *J* = 10.1 Hz, 1H). LCMS (ESI) m/z: $[M+H]^+$ = 410.15

Example 32 – Preparation of 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylic acid (compound B46)



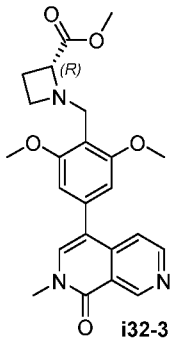
Step 1: preparation of methyl (2R)-azetidine-2-carboxylate hydrochloride (i32-2)



i32-2

A mixture of 1-*tert*-butyl 2-methyl (2R)-azetidine-1,2-dicarboxylate (40.30 mg, 0.187 mmol, 1.00 equiv) and HCl (4M) in 1,4-dioxane (3.00 mL) was stirred at room temperature for 1 hour. Then the solvent was evaporated, and the result crude methyl (2*R*)-azetidine-2-carboxylate hydrochloride was used directly in the next step without further purification. LCMS (ESI) m/z: [M+H]+ = 116.

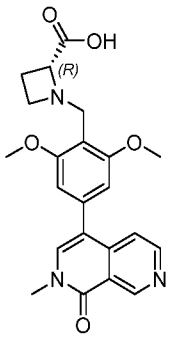
Step 2: Preparation of methyl (2R)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl] methyl]azetidine-2-carboxylate (i32-3)



i32-3

To a stirred solution of methyl (2*R*)-azetidine-2-carboxylate hydrochloride (28.23 mg, 0.186 mmol, 1.00 equiv) and Et_3N (37.69 mg, 0.372 mmol, 2 equiv) in MeOH (2.00 mL) was added 2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)benzaldehyde (60.40 mg, 0.186 mmol, 1.00 equiv), and the mixture was stirred for 0.5 hours before $NaBH_3CN$ (23.41 mg, 0.372 mmol, 2 equiv) was added in portions at room temperature under ambient atmosphere. Then the reaction was quenched by the addition of water (10 mL) at 0 ℃, and the mixture extracted with EtOAc (20 mL x 2). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to give crude product that was purified by chromatography on silica gel, eluted with MeOH/DCM (0-10 %) to afford methyl (2*R*)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-2-carboxylate (68 mg, 86.23%) as a yellow solid. LCMS (ESI) m/z: [M+H]+ = 424.

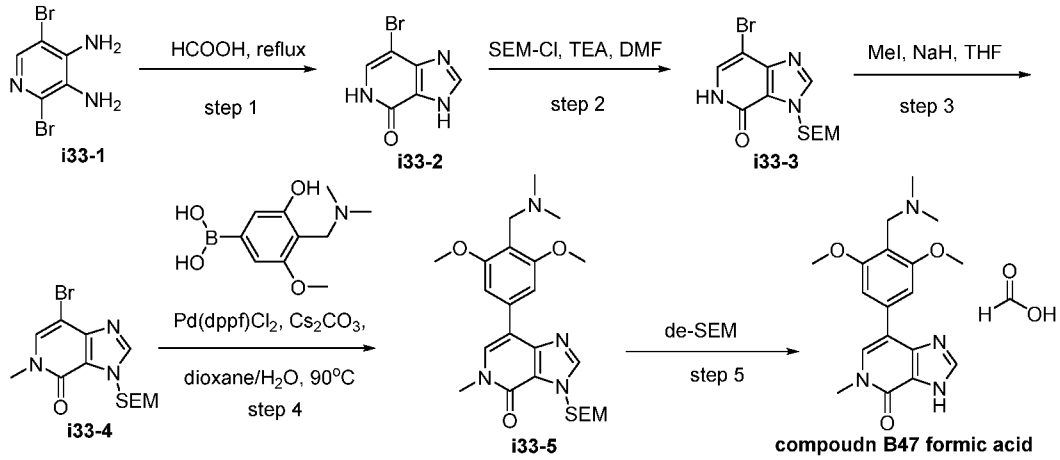
Step 3: Preparation of 1-[[2, 6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl] methyl]azetidine-3-carboxylic acid (compound B46)



compound B46

To a stirred solution of methyl1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylate (840 mg, 1.984 mmol, 1 equiv) in the mixed solvent of THF (4 mL) and H_2O (2 mL) was added LiOH (475.04 mg, 19.836 mmol, 10.00 equiv), and the solution was stirred for 1 hour at ambient atmosphere. The mixture was purified by reverse phase flash to get a crude product, and the result residue was further purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5 μm,19*150 mm; Mobile Phase A: Water (0.1% FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 38% B to 58% B in 8 minutes; 254 nm; R_t: 7.35 minutes) to afford 1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-3-carboxylic acid (421 mg, 51.84%) as a light yellow semi-solid. ^{1}H NMR (300 MHz, Methanol-*d4*) δ 9.62 (s, 1H), 8.72 (d, *J* = 6.4 Hz, 1H), 8.08 (s, 1H), 7.92 (d, *J* = 6.3 Hz, 1H), 6.88 (s, 2H), 5.18 (t, *J* = 9.7 Hz, 1H), 4.56 (s, 2H), 4.19 (q, *J* = 9.6 Hz, 1H), 3.98 (s, 7H), 3.75 (s, 3H), 2.72 – 2.59 (m, 2H). LCMS (ESI) m/z: [M+H]+ = 410.20.

Example 33 – Preparation of 7-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-5-methyl-3H-imidazo[4,5-c]pyridin-4-one formic acid (compound B47 formic acid)

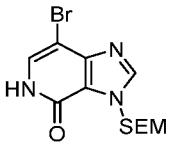


Step 1: Preparation of 7-bromo-3,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one (i33-2)

i33-2

A solution of 2,5-dibromopyridine-3,4-diamine (1.06 g, 3.97 mmol, 1.0 equiv) in HCOOH (3 mL) was refluxed at 100 degrees C for 6 hours. The resulting mixture was concentrated under reduced pressure. The crude product was purified by flash silica chromatography, elution gradient 0 to 60% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford 7-bromo-3,5-dihydro-4*H*-imidazo[4,5-c]pyridin-4-one (594 mg, 70%) as an off-white solid. LCMS (ESI) m/z: $[M+H]^+$ = 214.

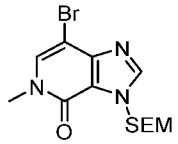
Step 2: Preparation of 7-bromo-3-[[2-(trimethylsilyl)ethoxy]methyl]-5H-imidazo[4,5-c]pyridin-4-one (i33-3)



i33-3

To a stirred mixture of 7-bromo-3,5-dihydro-4*H*-imidazo[4,5-c]pyridin-4-one (400.0 mg, 1.87 mmol, 1.0 equiv) and [2-(chloromethoxy)ethyl]trimethylsilane (467.39 mg, 2.80 mmol, 1.50 equiv) in DMF was added TEA (567.36 mg, 5.61 mmol, 3.0 equiv) at room temperature. The resulting mixture was stirred for 3h at 80 degrees C. After cooling, the solution was diluted with DCM (50 mL) and washed with water (3 x 20 mL), dried over anhydrous sodium sulfate, filtered and concentrated to give 7-bromo-3-[[2-(trimethylsilyl)ethoxy]methyl]-5H-imidazo- [4,5-c]pyridin-4-one(711.6 mg, 88%) as a light-yellow syrup that was used directly without further purification. LCMS (ESI) m/z: $[M+H]^+$ =344.

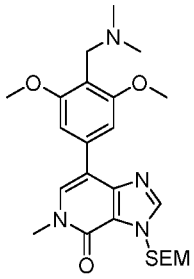
Step 3: Preparation of 7-bromo-5-methyl-3-[[2-(trimethylsilyl)ethoxy]methyl]imidazo[4,5-c]pyridin-4-one (i33-4)



i33-4

A mixture of 7-bromo-3-[[2-(trimethylsilyl)ethoxy]methyl]-5H-imidazo[4,5-c]pyridin-4-one (643.0 mg, 1.87 mmol, 1.0 equiv) in THF (10.0 mL) was cooled to 0 degrees C, then NaH (53.78 mg, 2.24 mmol, 1.20 equiv) was added in portions. The mixture was stirred for 20 min, and then CH_3I (795.27 mg, 5.60 mmol, 3.0 equiv) was added. After stirring for 1 h at room temperature under nitrogen atmosphere, the reaction was quenched with water (100 mL) and the mixture extracted with EA (4 x 100 mL). The organic layers were combined and dried over anhydrous sodium sulfate, filtered and concentrated to give 7-bromo-5-methyl-3-[[2-(trimethylsilyl)ethoxy]methyl]imidazo[4,5-c]pyridin-4-one (660 mg, 83%) as a brown-yellow syrup, that was used directly without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 358.

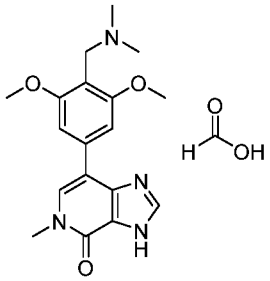
Step 4: Preparation of 7-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-5-methyl-3-[[2-(trimethylsilyl) ethoxy]methyl]imidazo[4,5-c]pyridin-4-one (i33-5)



i33-5

To a solution of 7-bromo-5-methyl-3-[[2-(trimethylsilyl)ethoxy]methyl]imidazo[4,5-c]pyridin-4-one (200.0 mg, 0.56 mmol, 1.0 equiv) and 4-[(dimethylamino)methyl]-3,5-dimethoxyphenylboronic acid (160.14 mg, 0.67 mmol, 1.20 equiv) in dioxane (10 mL) and H_2O(2 mL) was added Cs_2CO_3 (545.59 mg, 1.68 mmol, 3.0 equiv) and $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (40.84 mg, 0.056 mmol, 0.10 equiv). The mixture was stirred for 2 hours at 90 degrees C under a nitrogen atmosphere. After cooling, the mixture was diluted with water (20 mL) and extracted with EtOAc (30 mL x 3). The organic layers were combined and dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 7% CH_3OH in CH_2Cl_2. Pure fractions were evaporated to dryness to afford 7-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-5-methyl-3-[[2-(trimethylsilyl)ethoxy]methyl]imidazo[4,5-c]pyridin-4-one (140 mg, 53%). LCMS (ESI) m/z: $[M+H]^+$ = 473

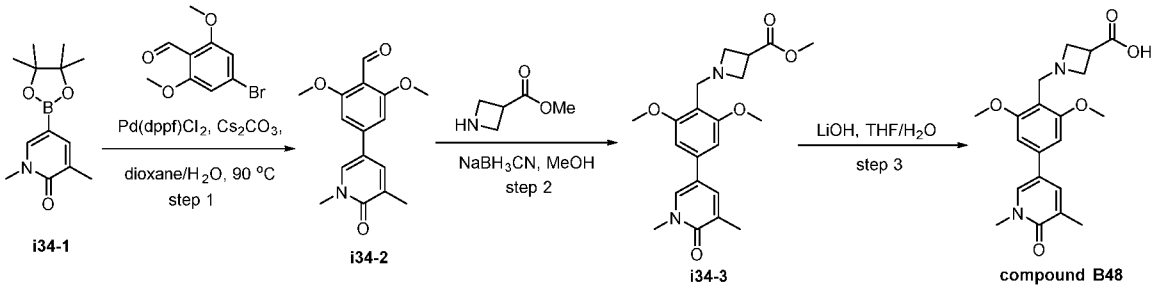
Step 5: Preparation of 7-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-5-methyl-3H-imidazo[4,5-c]pyridin-4-one formic acid (compound B47 formic acid)



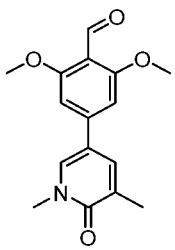
compoudn B47 formic acid

A mixture of 7-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-5-methyl-3-[[2-(trimethylsilyl)ethoxy] methyl]imidazo[4,5-c]pyridin-4-one (120.0 mg, 0.25 mmol, 1.0 equiv) in 2M HCl-1,4-dioxane (5 mL) was stirred for 2 hours at 70 degrees C. After cooling, the mixture was concentrated under reduced pressure. The crude product was purified by Prep-HPLC with the following conditions : Column: SunFire C18 OBD Prep Column, 100Å, 5 µm, 19 mm X 250 mm; Mobile Phase A:Water (0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 7% B to 26% B in 8 min; 254 nm; Rt: 6.25 min. to afford formate of 26 mg (25%) of 7-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-5-methyl-3H-imidazo [4,5-c]pyridin-4-one as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.55 (s, 1H), 8.24 (s, 1H), 7.78 (s, 1H), 7.20 (s, 2H), 4.39 (s, 2H), 4.03 (s, 6H), 3.77 (s, 3H), 2.89 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 343.15.

Example 34 – Preparation of 1-[[4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxyphenyl]methyl]azetidine-3-carboxylic acid (compound B48)



Step 1: Preparation of 4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxybenzaldehyde (i34-2)

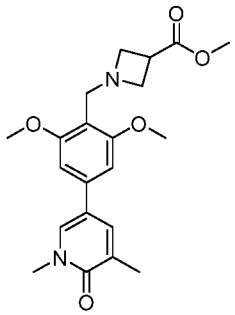


i34-2

To a stirred solution of 1,3-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-one (430 mg, 1.73 mmol, 1.0 equiv) and 4-bromo-2,6-dimethoxybenzaldehyde (634.52 mg, 2.59 mmol, 1.50 equiv) in 1,4-dioxane(25 mL)/H_2O (5 mL), was added $Pd(dppf)Cl_2$ (126.3 mg, 0.17 mmol, 0.10 equiv) and Cs_2CO_3 (1 124.78 mg, 3.45 mmol, 2.0 equiv). The resulting solution was stirred at 90 degrees C for 2 h under nitrogen atmosphere. Then the mixture was allowed to cool down to room temperature, the mixture

was diluted with water (25 mL) and extracted with EtOAc (3 x 25 mL). The organic layers were combined and dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product. The residue was purified by silica gel column chromatography, eluted with CH_2Cl_2/MeOH (20:1) to afford 4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxybenzaldehyde (313 mg, 51.42%) as an off-white solid. LCMS (ESI) m/z: $[M+H]^+$ = 288

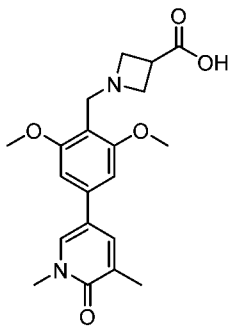
Step 2: Preparation of methyl 1-[[4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxyphenyl]methyl]azetidine-3-carboxylate (i34-3)



i34-3

To a stirred mixture of 4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxybenzaldehyde (313.00 mg, 1.09 mmol, 1.0 equiv) and methyl azetidine-3-carboxylate (188.14 mg, 1.63 mmol, 1.50 equiv) in MeOH was added $NaBH_3CN$ (136.92 mg, 2.18 mmol, 2.0 equiv), the mixture was stirred at room temperature under nitrogen atmosphere. Then the mixture was diluted with water (25 mL) and extracted with EtOAc (3 x 25 mL). The organic layers were combined and dried over anhydrous sodium sulfate, filtered and concentrated to give a crude product. The residue was purified by silica gel column chromatography, eluted with $CHCl_3$/MeOH (10:1) to afford methyl 1-[[4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxyphenyl] methyl]- azetidine-3-carboxylate (172.5 mg, 35%) as a off-white solid. LCMS (ESI) m/z: $[M+H]^+$ = 386.4

Step 3: Preparation of 1-[[4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxyphenyl]methyl]azetidine-3-carboxylic acid (compound B48)

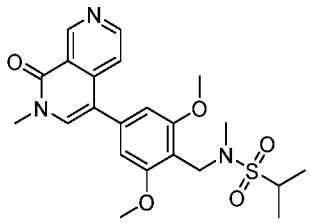


compound B48

A mixture of methyl 1-[[4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxyphenyl]methyl]azetidine-3-carboxylate (169.0 mg, 0.48 mmol, 1.0 equiv) and LiOH (52.36 mg, 2.19 mmol, 5.0 equiv) in THF(3 mL) and H_2O (3 mL) was stirred for 1 h at room temperature. Then the mixture was acidified with 12 N HCl until pH 4. The mixture was extracted with DCM (30 mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated to give the crude product which was purified by Prep-HPLC with the following

conditions (Column: SunFire C18 OBD Prep Column , 100Å, 5 µm, 19 mm X 250 mm;Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 7% B to 24% B in 8 min; 254 nm; Rt: 7.85 min) to afford 1-[[4-(1,5-dimethyl-6-oxopyridin-3-yl)-2,6-dimethoxyphenyl]methyl]azetidine-3-carboxylic acid (48 mg, 29%) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 7.98 (d, *J* = 2.5 Hz, 1H), 7.84 (s, 1H), 6.91 (s, 2H), 4.48 (s, 2H), 4.30 (d, *J* = 9.8 Hz, 4H), 4.01 (s, 6H), 3.69 (s, 3H), 3.59 (s, 1H), 2.23 (s, 3H) . LCMS (ESI) m/z: $[M+H]^+$ = 373.20.

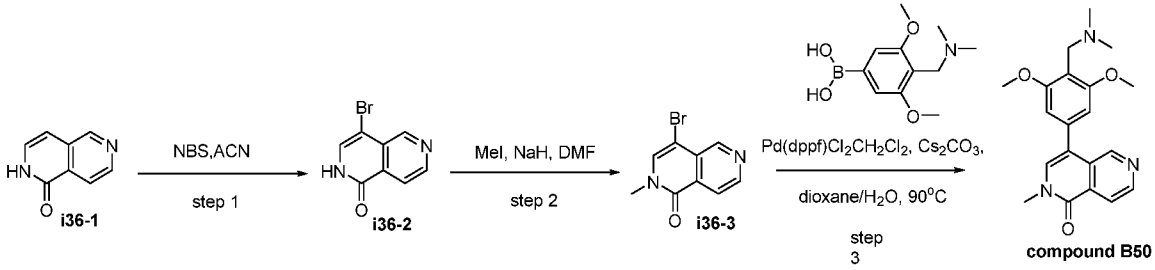
Example 35 – Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methyl propane-2-sulfonamide (compound B49)



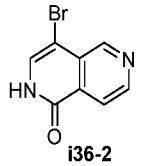
compound B49

Compound B49 was prepared in a similar manner as described for compound B35. N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl) phenyl] methyl]-N-methylpropane-2-sulfonamide (19.1 mg, 9%) was obtained as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.56 (s, 1H), 8.70 (d, *J* = 5.9 Hz, 1H), 7.85 (s, 1H), 7.72 (d, *J* = 6.0 Hz, 1H), 6.80 (s, 2H), 4.53 (s, 2H), 3.91 (s, 6H), 3.73 (s, 3H), 3.62-3.49 (m, 1H), 2.77 (s, 3H), 1.35 (d, *J* = 6.8 Hz, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 446.25.

Example 36 – Preparation of 4-(4-((dimethylamino) methyl)-3, 5-dimethoxyphenyl)-2methyl-2,6-naphthyridin-1(2H)-one (compound B50)



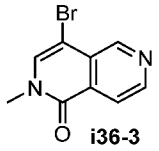
Step 1: Preparation of 4-bromo-2, 6-naphthyridin-1(2H)-one (i36-2)



i36-2

To a stirred solution of 1,2-dihydro-2,6-naphthyridin-1-one (500 mg, 3.421 mmol, 1.00 equiv) in CH_3CN (10 mL) was added 1-bromopyrrolidine-2,5-dione (669.81 mg, 3.763 mmol, 1.10 equiv) at room temperature. The resulting mixture was stirred for 3 hours at room temperature. The mixture was concentrated under vacuum. The residue was purified by flash silica gel column chromatography, eluted with ethyl acetate/petroleum ether from 50% to 100%. This resulted in 4-bromo-2, 6-naphthyridin-1(2*H*)-one (760 mg, 99.08%) of as a yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 225.

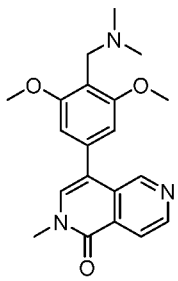
Step 2: Preparation of 4-bromo-2-methyl-2, 6-naphthyridin-1(2H)-one (i36-3)



i36-3

To a stirred solution of 4-bromo-2, 6-naphthyridin-1(2H)-one (396.0 mg, 1.76 mmol, 1.0 equiv) in DMF (6 mL) was added NaH (59.12 mg, 2.464 mmol, 1.40 equiv) at 0 degrees C. After 10 minutes of stirring, iodomethane (499.53 mg, 3.519 mmol, 2.00 equiv) was added to the solution. The solution was stirred at 25 degrees C for 10 hours. Then water (50 mL) was added, and the reaction mixture was then extracted with DCM (50 mL x 3). The combined organic layers were washed with saturated brine (30 mL x 2), dried over anhydrous sodium sulfate, filtered, and concentrated to give 331 mg of crude product. This material was used directly in the next step without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 239.

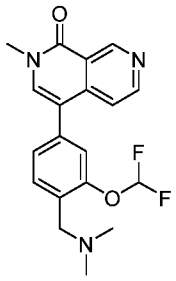
Step 3: Preparation of 4-(4-((dimethylamino) methyl) -3, 5-dimethoxyphenyl)-2-methyl-2,6-naphthyridin-1 (2H)-one (compound B50)



compound B50

To a stirred mixture of 4-bromo-2-methyl-1,2-dihydro-2,6-naphthyridin-1-one (80.30 mg, 0.336 mmol, 1.10 equiv) and [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]boronic acid (73 mg, 0.305 mmol, 1.00 equiv) in dioxane (5 mL) and H_2O (1 mL) was added Cs_2CO_3 (298.45mg, 0.916mmol, 3.00 equiv) and $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (37.40 mg, 0.046 mmol, 0.15 equiv). The resulting reaction mixture was stirred for 5 hours at 90 degrees C under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure, and then the residue was diluted with DCM (100 mL) and filtered through a short pad of Celite. The solvent was evaporated and the crude product was purified by preparative HPLC (conditions: XBridge Shield RP18 OBD Column, 5um, 19*150 mm; Mobile Phase A: Water (10 mmol/L NH_4HCO_3), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 35% B to 75% B in 8 mintues; 220 nm; R_t: 7.9 minutes). This resulted in 4-(4-((dimethylamino) methyl) -3, 5-dimethoxyphenyl)-2 –methyl -2, 6-naphthyridin-1 (2H)-one (9.6 mg, 8.90%) as a yellow solid. ^{1}H NMR (300 MHz, Methanol-d4) δ 9.02 (s, 1H), 8.69 (d, *J* = 5.3 Hz, 1H), 8.28 (d, *J* = 5.4 Hz, 1H), 7.60 (s, 1H), 6.81 (s, 2H), 3.89 (s, 6H), 3.74 (d, *J* = 4.1 Hz, 5H), 2.36 (s,6H). LCMS (ESI) m/z: $[M+H]^+$ = 354.15.

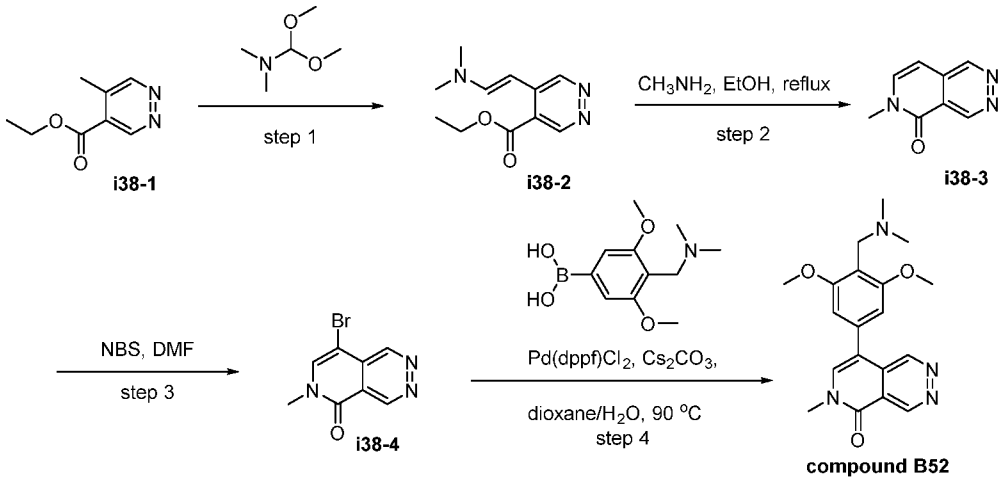
Example 37 – Preparation of 4-(3-(difluoromethoxy)-4-((dimethylamino)methyl)phenyl)-2-methyl-2,7-naphthyridin-1(2H)-one (compound B51)



compound B51

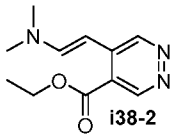
Compound B51 was prepared in a similar manner as described for compound B50. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (d, J = 0.9 Hz, 1H), 8.71 (d, J = 5.6 Hz, 1H), 7.85 (s, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.45 (dd, J = 5.6, 0.9 Hz, 1H), 7.34 (dd, J = 7.8, 1.7 Hz, 1H), 7.28 – 7.24 (m, 1H), 3.57 (s, 3H), 3.47 (s, 2H), 2.19 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 360.2.

Example 38 – Preparation of 8-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-6-methyl-5H,6H-pyrido[3,4-d]pyridazin-5-one (compound B52)



i38-1 i38-2 i38-3 i38-4 compound B52

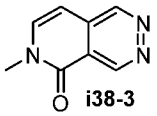
Step 1: Preparation of ethyl 5-[(E)-2-(dimethylamino)ethenyl]pyridazine-4-carboxylate (i38-2)



i38-2

A mixture of ethyl 5-methylpyridazine-4-carboxylate (200.0 mg, 1.20 mmol, 1.0 equiv) in (dimethoxymethyl)dimethylamine (5.00 mL) was stirred at 80 degrees C for 3 hours. After completion of the reaction, the solvent was removed under reduced pressure to afford ethyl 5-[(*E*)-2-(dimethylamino)ethenyl]pyridazine-4-carboxylate (320 mg) as a black solid. The crude was not further purification and directly used in next step. LCMS (ESI) m/z: $[M+H]^+$ = 222.1.

Step 3: Preparation of 6-methylpyrido[3,4-d]pyridazin-5-one (i38-3)

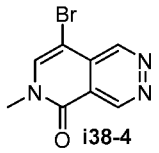


i38-3

To a stirred mixture of ethyl 5-[(*E*)-2-(dimethylamino)ethenyl]pyridazine-4-carboxylate (300.0 mg, 1.36 mmol, 1.0 equiv) in EtOH (5 mL) was added methanamine hydrochloride (915.48 mg,

1.56 mmol, 10.0 equiv). The reaction was stirred for 3 hours at 75 degrees C. After cooling, the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography eluted with DCM/MeOH (20:1) to give 6-methylpyrido[3,4-d]pyridazin-5-one (200 mg, 91%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 162.2.

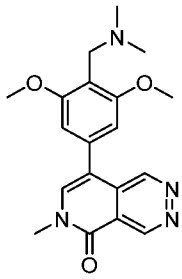
Step 4: Preparation of 8-bromo-6-methylpyrido[3,4-d]pyridazin-5-one (i38-4)



i38-4

To a stirred mixture of 6-methylpyrido[3,4-d]pyridazin-5-one (170.0 mg, 1.06 mmol, 1.0 equiv) in DMF (1 mL) was added NBS (226.42 mg, 1.27 mmol, 1.2 equiv). The reaction was stirred room temperature for 2 hours. The reaction mixture was diluted with EA (50 mL), washed with water (3 x 30 mL) and saturated brine (1 x 30 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to afford crude product. The residue was purified by silica gel column chromatography, eluted with DCM/MeOH (10:1) to afford 8-bromo-6-methylpyrido[3,4-d]pyridazin-5-one (82 mg, 32%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 240.1.

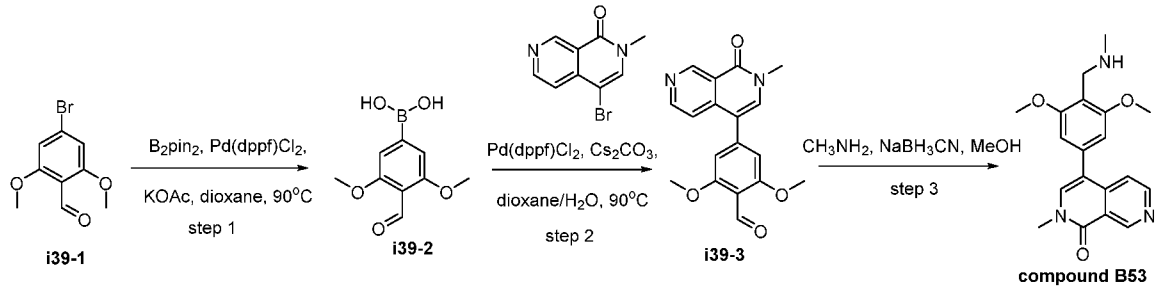
Step 4: Preparation of 8-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-6-methyl-5H,6H-pyrido[3,4-d]pyridazin-5-one (compound B52)



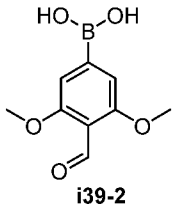
compound B52

To a solution of 8-bromo-6-methyl-5H,6H-pyrido[3,4-d]pyridazin-5-one (60.0 mg, 0.25 mmol, 1.0 equiv), [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl] boronic acid (59.76 mg, 0.25 mmol, 1.0 equiv), Cs_2CO_3 (162.87 mg, 0.5 mmol, 2.0 equiv) in dioxane (3 mL) and H_2O (0.8 mL) was added $Pd(dppf)Cl_2$ (18.29 mg, 0.025 mmol, 0.1 equiv). The resulting mixture was stirred at 90 degrees C for 1 hour under nitrogen atmosphere. After cooling, the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel eluted with DCM/MeOH (20:1) to give the crude product. The crude product was further purified by Prep-HPLC (conditions: XBridge Shield RP18 OBD Column, 5 μm, 19*150 mm; Mobile Phase A: Water (10 mmol/L NH_4HCO_3), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 17% B to 47% B in 8 minutes; 220 nm; Rt: 7.8 minutes) to afford 8-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-6-methyl-5*H*,6*H*-pyrido[3,4-d]pyridazin-5-one (17.3 mg, 19.5%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 9.72 (d, *J* = 1.4 Hz, 1H), 9.51 (d, *J* = 1.4 Hz, 1H), 8.18 (s, 1H), 6.81 (s, 2H), 3.82 (s, 6H), 3.66 (s, 3H), 3.47 (s, 2H), 2.14 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 355.20.

Example 39 – Preparation of 4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (compound B53)



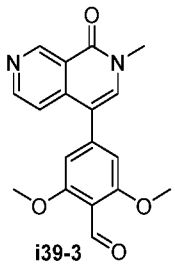
Step 1: Preparation of (4-formyl-3,5 -dimethoxyphenyl)boronic acid (i39-2)



i39-2

To a solution of 4-bromo-2,6-dimethoxybenzaldehyde (4.00 g, 16.322 mmol, 1.00 equiv) and KOAc (4.81 g, 48.965 mmol, 3.00 equiv) in 1,4-dioxane(30.00 ml) was added Bis(pinacolato)diboron (4.97 g, 19.586 mmol, 1.20 equiv) and Pd(dppf)Cl_2 CH_2Cl_2 (1.33 g, 1.632 mmol, 0.10 equiv). The resulting solution was stirred for 3 hours at 90 ℃. The solids were filtered out. The resulting mixture was concentrated. This resulted in 2.5 g (72.94%) of (4-formyl-3,5 -dimethoxyphenyl)boronic acid as a brown solid.

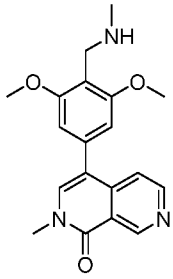
Step 2: Preparation of 2, 6 -dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (i39-3)



i39-3

To a solution of (4-formyl-3,5-dimethoxyphenyl)boronic acid (2.80 g, 13.334 mmol, 1.00 equiv), 4-bromo-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (4.78 g, 20.001 mmol, 1.50 equiv), and Cs_2CO_3 (13.03 g, 40.002 mmol, 3 equiv) in 1,4-dioxane (17.50 mL, 198.599 mmol, 15.49 equiv) was added Pd(dppf)Cl_2•CH_2Cl_2 (1.09 g, 1.333 mmol, 0.1 equiv) and H_2O (3.50 mL, 194.276 mmol, 14.57 equiv). The resulting solution was stirred for 2 hours at 80 ℃. The resulting solution was diluted with 20 mL of water. The resulting solution was extracted with ethyl acetate (2 x 20 mL) and the organic layers combined and dried over anhydrous sodium sulfate and concentrated. The residue was applied onto a silica gel column with dichloromethane/methanol (2:1). This resulted in 3 g (69.37%) of 2,6 -dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde as a yellow solid.

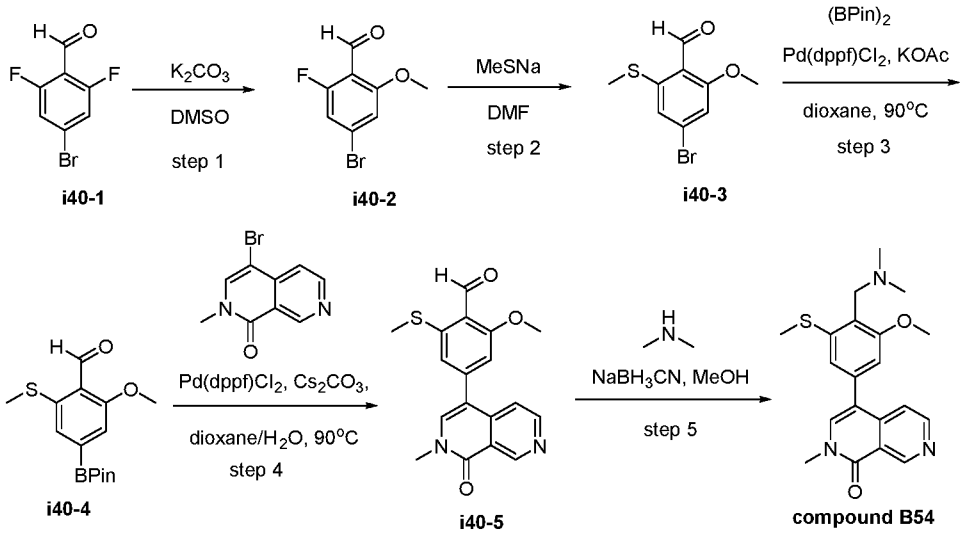
Step 3: Preparation of 4-[3,5 -dimethoxy-4- [(methylamino)methyl]phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (compound B53)



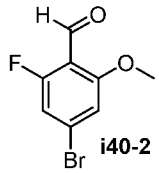
compound B53

To a solution of 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (200.00 mg, 0.617 mmol, 1.00 equiv) in MeOH(5.00 mL) was added methylamine (28.73 mg, 0.925 mmol, 1.50 equiv) at 25 ℃ and the reaction mixture was stirred for 10 minutes. Then $NaBH_3CN$ (116.25 mg, 1.850 mmol, 3.00 equiv) was added to the reaction mixture. The resulting solution was stirred for 1 hour at 25 ℃. The resulting mixture was concentrated. The crude product was purified by Prep-HPLC (conditions: XBridge Prep C18 OBD Column, 5 μm,19*150mm; mobile phase, Water (0.1% FA) and ACN (hold 5% PhaseB in 2 minutes, up to 26% in 8 minutes); Detector, uv). This resulted in 70 mg (33.45%) of 4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one as a white solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.55 (s, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 8.54 (s, 1H), 7.78 (s, 1H), 7.61 (dd, *J* = 5.7, 0.9 Hz, 1H), 6.87 (s, 2H), 4.33 (s, 2H), 3.97 (s, 6H), 3.72 (s, 3H), 2.74 (s, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 340.4.

Example 40 – Preparation of 4-[4-[(dimethylamino)methyl]-3-methoxy-5-(methylsulfanyl)phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (compound B54)

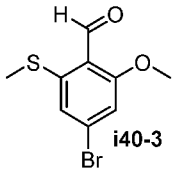


Step 1: Preparation of 4-bromo-2-fluoro-6-methoxybenzaldehyde (i40-2)



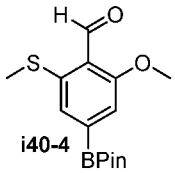
To the solution of 4-bromo-2,6-difluorobenzaldehyde (5.00 g, 22.624 mmol, 1.00 equiv) in MeOH (43.00 mL) was added sodium methoxide (1.83 g, 33.936 mmol, 1.5 equiv). The resulting solution was stirred at 65 ℃ for 12 hours. The resulting solution was concentrated. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (1:1) to afford 4-bromo-2-fluoro-6-methoxybenzaldehyde (2.87 g, 54.44%) as a light yellow solid. LCMS (ESI) m/z: [M+H]+ = 233.

Step 2: Preparation of 4-bromo-2-methoxy-6-(methylsulfanyl)benzaldehyde (i40-3)



To the solution of 4-bromo-2-fluoro-6-methoxybenzaldehyde (1.00 g, 4.291 mmol, 1.00 equiv) in DMSO (20.00 mL) was added (methylsulfanyl)sodium (0.45 g, 6.437 mmol, 1.50 equiv). The resulting solution was stirred at room temperature for 12 hours. The reaction was quenched with water at room temperature. The resulting mixture was extracted with EtOAc (3 x 75 mL). The combined organic layers were washed with water (3 x 75 mL) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (1:1) to afford 4-bromo-2-methoxy-6-(methylsulfanyl)benzaldehyde (988 mg, 88.17%) as a light yellow solid. LCMS (ESI) m/z: [M+H]+ = 261.

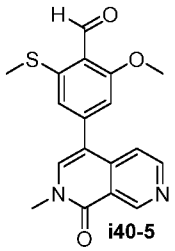
Step 3: Preparation of 2-methoxy-6-(methylsulfanyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (i40-4)



i40-4

To the solution of 4-bromo-2-methoxy-6-(methylsulfanyl)benzaldehyde (400.00 mg, 1.532 mmol, 1.00 equiv) in dioxane (15.00 mL) was added KOAc (451.00 mg, 4.595 mmol, 3 equiv), $Pd(dppf)Cl_2$ (112.08 mg, 0.153 mmol, 0.1 equiv), and 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi-1,3,2-dioxaborolane. The resulting solution was stirred at 90 ℃ for 6 hours under nitrogen atmosphere. The resulting mixture was filtered, the filter cake was washed with EtOAc (3 x 15 mL). The filtrate was concentrated under reduced pressure. This resulted in crude 2-methoxy-6-(methylsulfanyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (300 mg, 63.55%) as a light yellow oil, that was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 309.

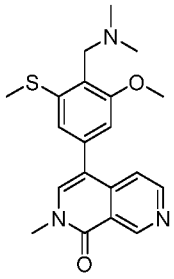
Step 4: Preparation of 2-methoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)-6-methylsulfanyl)benzaldehyde (i40-5)



i40-5

To the solution of 4-bromo-2-methyl-2,7-naphthyridin-1-one (442.15 mg, 1.849 mmol, 1.2 equiv) and 2-methoxy-6-(methylsulfanyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde in dioxane (15.00 mL) was added H_2O (1.50 mL), $Pd(dppf)Cl_2$ (112.77 mg, 0.154 mmol, 0.1 equiv), and Cs_2CO_3 (1.51 g, 4.624 mmol, 3 equiv). The resulting solution was stirred at 90 ℃ for 3 hours. The crude was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with CH_2Cl_2 / MeOH (10:1) to afford 2-methoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)-6-(methylsulfanyl)benz aldehyde (70 mg, 13.34%) as a light yellow solid. LCMS (ESI) m/z: [M+H]+ = 341.

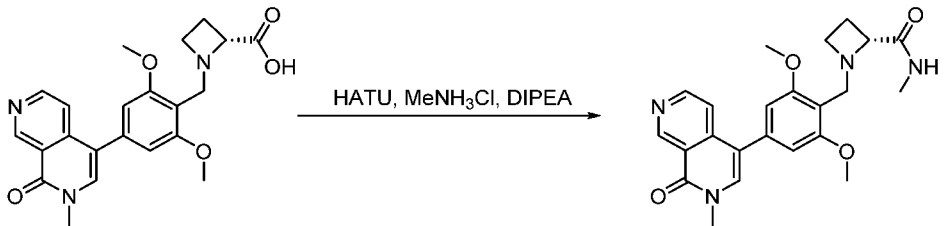
Step 5: Preparation of 4-[4-[(dimethylamino)methyl]-3-methoxy-5-(methylsulfanyl)phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (compound B54)



compound B54

To the solution of 2-methoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)-6-(methylsulfanyl)benzaldehyde (70 mg, 0.206 mmol, 1 equiv) in MeOH (2 mL) was added dimethylamine (13.91 mg, 0.308 mmol, 1.5 equiv) and $NaBH_3CN$ (38.77 mg, 0.617 mmol, 3 equiv). The resulting solution was stirred at room temperature for 1 hour. The resulting solution was concentrated. The crude product was purified by Prep-HPLC (conditions: SunFire C18 OBD Prep Column, 100 mm, 5 μm, 19 mm X 250 mm; Mobile Phase A: Water(0.1% FA), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 9% B to 15% B in 8 minutes; 254 nm; Rt: 8.68 minutes) to afford 4-[4-[(dimethylamino)methyl]-3-methoxy-5-(methylsulfanyl)phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 9.54 (d, 1H), 8.69 (d, 1H), 7.75 (s, 1H), 7.60 (dd, 1H), 7.02 (d, 1H), 6.92 (d, 1H), 3.89 (s, 3H), 3.73 (d, 5H), 2.51 (s, 3H), 2.34 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 370.20.

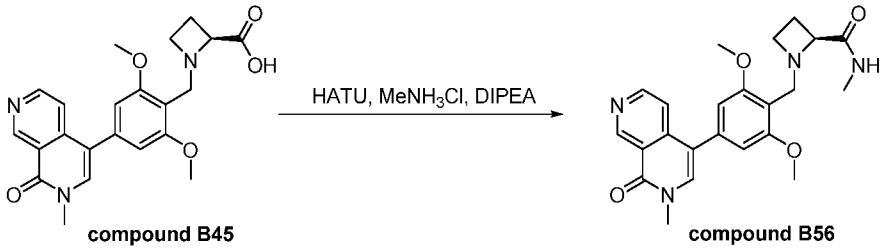
Example 41 – Preparation of (2R)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methylazetidine-2-carboxamide (compound B55)



compound B46 **compound B55**

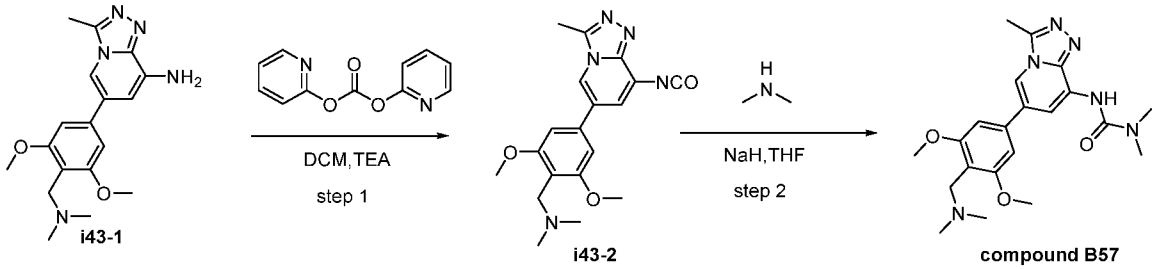
To a stirred solution of (2*R*)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]azetidine-2-carboxylic acid (40.9 mg, 0.100 mmol, 1.00 equiv) and DIPEA (64.6 mg, 0.499 mmol, 5.00 equiv) in DMF (0.5 mL) was added HATU (76 mg, 0.200 mmol, 2.00 equiv) and methylamine (12.4 mg, 0.400 mmol, 4 equiv). The solution was stirred for 2 hours at room temperature. The resulting mixture was purified directly by Prep-HPLC (conditions: XBridge Shield RP18 OBD Column, 5 μm,19*150mm;Mobile Phase A:Water (0.05% NH_3H_2O), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 45% B to 75% B in 8 minutes; 220 nm; Rt: 8.2 minutes) to afford (2*R*)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-*N*-methylazetidine-2-carboxamide (6 mg) as a white solid. ^{1}H NMR (300 MHz, Methanol-*d4*) δ 9.53 (s, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 7.74 (s, 1H), 7.61 (dd, *J* = 5.8, 0.9 Hz, 1H), 6.75 (s, 2H), 3.89 (s, 6H), 3.94 – 3.80 (m, 1H), 3.78 – 3.66 (m, 1H), 3.72 (s, 4H), 3.31 – 3.14 (m, 2H), 2.76 (s, 3H), 2.31 – 2.20 (m, 1H), 2.07 – 1.89 (m, 1H). LCMS (ESI) m/z: [M+H]+ = 423.15.

Example 42 – Preparation of (2S)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methylazetidine-2-carboxamide (compound B56)

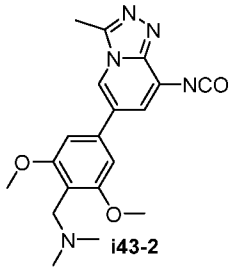


To a solution of (2*S*)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl] azetidine-2-carboxylic acid (40 mg, 0.098 mmol, 1.00 equiv) in DMF(2.00 mL) was added methylamine hydrochloride (7.92 mg, 0.117 mmol, 1.20 equiv), HATU (74.29 mg, 0.195 mmol, 2.00 equiv), and DIEA (37.88 mg, 0.293 mmol, 3.00 equiv) at 0 ℃. The resulting solution was stirred for 2 hours at 25 ℃. The resulting solution was diluted with 10 mL of water. The resulting solution was extracted with ethyl acetate (2 x 20 mL) and the organic layers combined and dried over anhydrous sodium sulfate and concentrated. The crude product was purified by Prep-HPLC (conditions: Atlantis HILIC OBD Column,19 mm X 250 mm; mobile phase, Water(0.1% FA) and ACN (hold 5% PhaseB in 2 minutes, up to 17% in 8 minutes); Detector, uv). This resulted in 30 mg (72.68%) (2*S*)-1-[[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]-N-methylazetidine-2-carboxamide as a yellow solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.59 (s, 1H), 8.71 (d, *J* = 6.1 Hz, 1H), 7.93 (s, 1H), 7.77 (dd, *J* = 6.1, 0.8 Hz, 1H), 6.86 (s, 2H), 5.01 (t, *J* = 9.3 Hz, 1H), 4.51 (d, *J* = 1.6 Hz, 2H), 4.20 (q, *J* = 9.6 Hz, 1H), 4.03 (t, *J* = 9.4Hz, 1H), 3.98 (s, 6H), 3.74 (s, 3H), 2.78 (s, 3H), 2.74 – 2.61 (m, 1H), 2.61 – 2.46 (m, 1H). LCMS (ESI) m/z: $[M+H]^+$ = 423.20.

Example 43 – Preparation of 1-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl)-3,3-dimethylurea (compound B57)



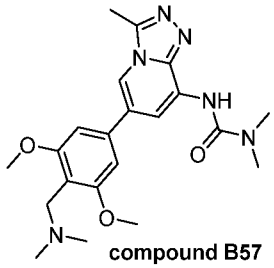
Step 1: Preparation of [(4-[8-isocyanato-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-6-yl]-2,6-dimethoxyphenyl) methyl]dimethylamine (i43-2)



To a solution of 6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-amine (50.00 mg, 0.146 mmol, 1.00 equiv) and TEA (44.46 mg, 0.439 mmol, 3.00 equiv) in

DCM (5.00 mL) was added bis(pyridin-2-yl) carbonate (31.66 mg, 0.146 mmol, 1.00 equiv) at 25 ℃. The resulting solution was stirred for 1 overnight at 25 ℃. The resulting mixture was concentrated. This resulted in 30 mg (55.76%) of [(4-[8-isocyanato-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-6-yl]-2,6-dimethoxyphenyl)methyl]dimethylamine as a brown crude solid.

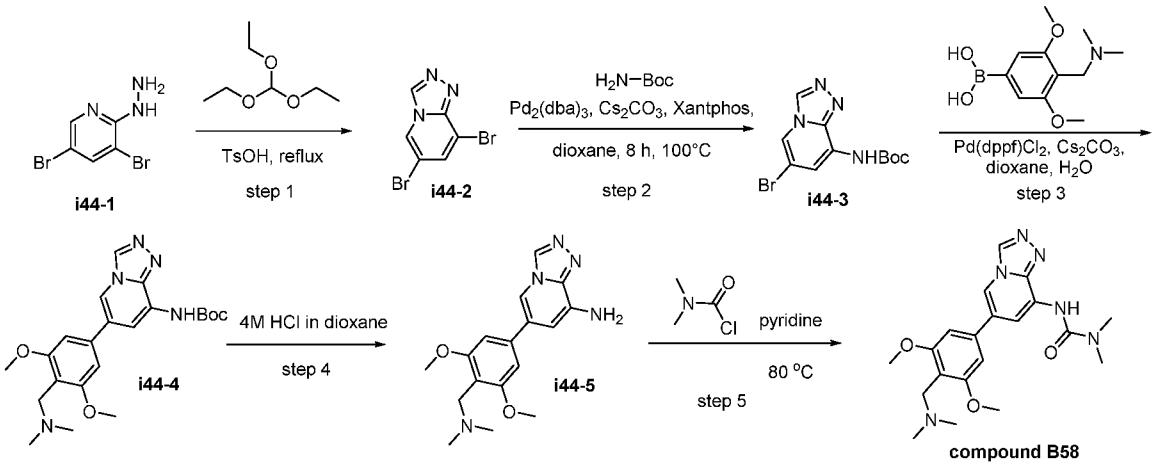
Step 2: Preparation of 1-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl)-3,3-dimethylurea (compound B57)



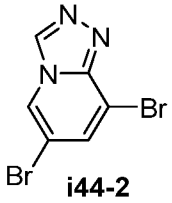
compound B57

To a solution of [(4-[8-isocyanato-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-6-yl]-2,6-dimethoxyphenyl)methyl]dimethylamine (20.00 mg, 0.054 mmol, 1.00 equiv) in THF (5.00 mL) was added dimethylamine (2.45 mg, 0.054 mmol, 1.00 equiv) and NaH (3.92 mg, 0.163 mmol, 3.00 equiv). The resulting solution was stirred for 2 hours at 25 ℃. The reaction was then quenched by the addition of 2 mL of MeOH. The resulting mixture was concentrated. The crude product was purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 19*250 mm,5 μm; mobile phase, Water (0.1% FA) and ACN (hold 5% PhaseB in 2 minutes,up to 22% in 6 minutes); Detector, UV). This resulted in 2 mg (8.91%) of 1-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl)-3,3-dimethylurea as a brown solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 8.56 (s, 1.3H, FA), 8.29 (d, *J* = 1.4 Hz, 1H), 8.23 (d, *J* = 1.4 Hz, 1H), 7.08 (s, 2H), 4.23 (s, 2H), 4.03 (s, 6H), 3.18 (s, 6H), 2.86 (s, 3H), 2.75 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 413.30.

Example 44 – Preparation Preparation of 1-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-[1,2,4]triazolo[4,3-a]pyridin-8-yl)-3,3- dimethylurea (compound B58)



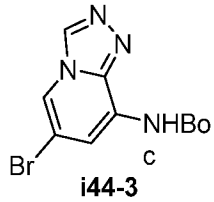
Step 1: Preparation of 6,8-dibromo-[1,2,4]triazolo[4,3-a]pyridine (i44-2)



i44-2

A mixture of 3,5-dibromo-2-hydrazinylpyridine (1 g, 3.746 mmol, 1 eq.) and TsOH (0.02 g, 0.112 mmol, 0.03 eq.) in triethoxymethane (25 mL) was stirred at 110 ℃ for 4 hours. The mixture was cooled and quenched by the addition of 20 mL of water. It was extracted with ethyl acetate (3 x 20 mL), and the organic layers combined and dried, concentrated under reduced pressure to afford the 6,8-dibromo-[1,2,4] triazolo[4,3-a]pyridine (801 mg crude) as a white solid, which was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 277.

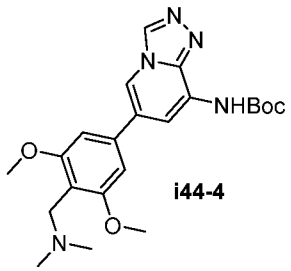
Step 2: Preparation of tert-butyl N-[6-bromo-[1,2,4]triazolo[4,3-a]pyridin-8-yl]carbamate (i44-3)



i44-3

To a solution of 6,8-dibromo-[1,2,4]triazolo[4,3-a]pyridine (800 mg, 2.889 mmol, 1 eq.) and *tert*-butyl carbamate (507.65 mg, 4.333 mmol, 1.5 eq.) in dioxane (16 mL) was added $Pd_2(dba)_3$ (132.27 mg, 0.144 mmol, 0.05 eq.), Cs_2CO_3 (1882.54 mg, 5.778 mmol, 2.0 eq.) ,and XantPhos (250.74 mg, 0.433 mmol, 0.15 eq.). The resulting solution was stirred for 8 hours at 100 ℃ under a nitrogen atmosphere. The reaction was then quenched by the addition of 20 mL of water and extracted with ethyl acetate (3 x 30 mL), and the organic layers were combined and dried over anhydrous sodium sulfate. The residue was applied onto a silica gel column with dichloromethane/methanol (10:1) to afford *tert*-butyl (6-bromo-[1,2,4]triazolo[4,3-a] pyridin-8-yl)carbamate (400 mg,44.0%) as a gray solid. LCMS (ESI) m/z: [M+H]+ = 315.

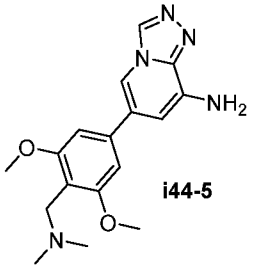
Step3: Preparation of tert-butyl N-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-[1,2,4]triazolo[4,3-a] pyridin-8-yl) carbamate (i44-4)



i44-4

To a solution of *tert*-butyl (6-bromo-[1,2,4]triazolo[4,3-a]pyridin-8-yl)carbamate (400 mg, 1.277 mmol, 1 eq.) and [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]boronic acid (244.31 mg, 1.022 mmol, 0.8 eq.) in H_2O (2.00 mL) and dioxane (8.00 mL) was added $PdCl_2$(dppf) (93.46 mg, 0.128 mmol, 0.1 eq.) and Cs_2CO_3 (832.35 mg, 2.555 mmol, 2.0 eq.). The resulting solution was stirred for 13 hours at 80 ℃ under a nitrogen atmosphere. The reaction was cooled and then quenched by the addition of 20 mL of water and extracted with ethyl acetate (3x 20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was applied onto a silica gel column with dichloromethane/methanol (5:1) to afford *tert*-butyl (6-(4- ((dimethylamino)methyl)-3,5-dimethoxyphenyl)-[1,2,4]triazolo[4,3-a]pyridin-8-yl)carbamate (216 mg, 16.8%) as a gray solid. LCMS (ESI) m/z: [M+H]+ = 428.

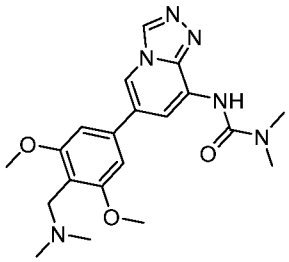
Step 4: Preparation of 6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-[1,2,4]triazolo[4,3-a]pyridin-8-amine (i44-5)



i44-5

A solution of *tert*-butyl (6-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-[1,2,4]triazolo[4,3-a]pyridin-8-yl)carbamate (216 mg, 1 equiv) in HCl (4 M) in 1,4-dioxane (8 mL) was stirred for 4 hours at room temperature. The resulting mixture was concentrated to afford 6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-[1,2,4]triazolo[4,3-a]pyridin-8-amine (120 mg, 73.7%) as a gray solid, which was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 328.

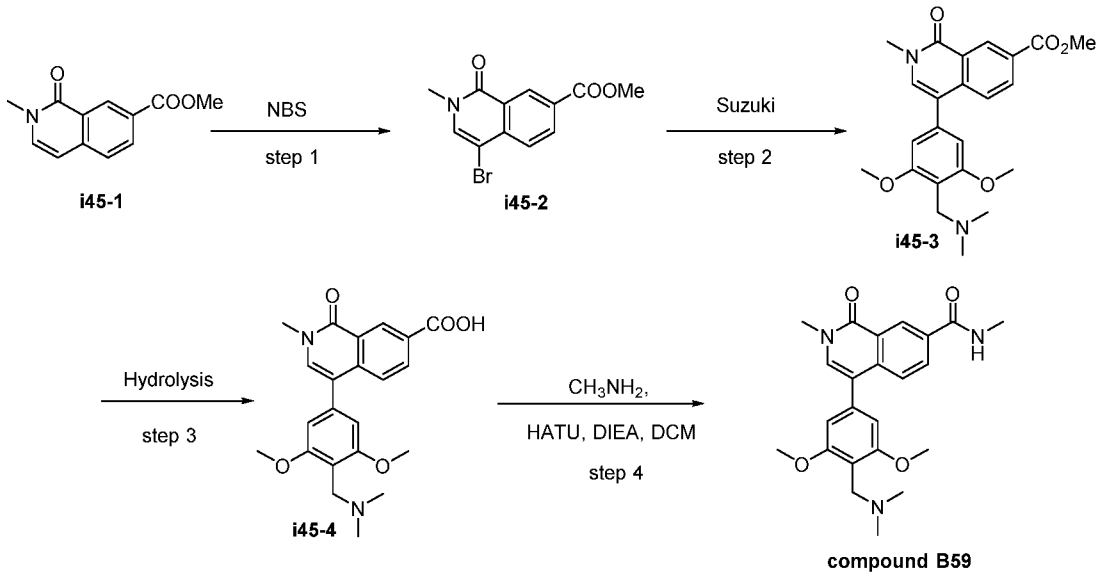
Step 5: Preparation of 1-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-[1,2,4]triazolo[4,3-a] pyridin-8-yl)-3,3-dimethylurea (compound B58)



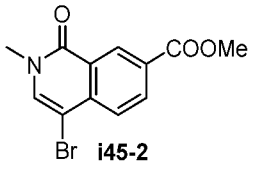
compound B58

To a solution of 6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-[1,2,4]triazolo[4,3-a]pyridin-8-amine (120 mg, 0.367 mmol, 1 equiv) in pyridine (5 mL, 62.118 mmol, 169.47 equiv) was added dimethylcarbamic chloride (78.83 mg, 0.733 mmol, 2.0 equiv) The resulting solution was stirred for 2 hours at 80 ℃. The mixture was cooled and concentrated, the crude product was purified by Prep-HPLC (conditions: Atlantis HILIC OBD Column19*150 mm*5 μm ; mobile phase, Phase A: Water(0.05% TFA) Phase B: MeOH-HPLC; Detector, uv: 254/220 nm). This resulted in 14 mg (9.59%) of 1-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-[1,2,4]triazolo[4,3-a]pyridine -8-yl)-3,3-dimethylurea as a grey solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 8.75 (d, *J* = 2.0 Hz, 1H), 8.49 (s, 1H), 8.42 (d, *J* = 2.0 Hz, 1H), 7.09 (s, 2H), 4.42 (s, 2H), 4.05 (s, 6H), 3.23 (q, *J* = 7.3 Hz, 1H), 3.13 (s, 6H), 2.91 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 399.1.

Example 45 – Preparation of 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-N,2-dimethyl-1-oxoisoquinoline-7-carboxamide (compound B59)



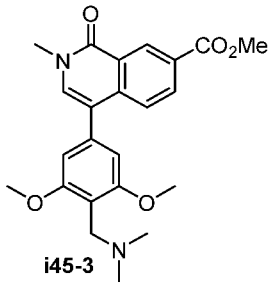
Step 1: Preparation of methyl 4-bromo-2-methyl-1-oxoisoquinoline-7-carboxylate (i45-2)



To a solution of methyl 2-methyl-1-oxoisoquinoline-7-carboxylate (200.00 mg, 0.921 mmol, 1.00 equiv) in solvent CH_2Cl_2 (3.00 mL) was added NBS (327.74 mg, 1.841 mmol, 2.00 equiv), and the

resulting solution was stirred at 0 ℃ for 1 hour. The resulting mixture was concentrated under reduced pressure. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0~50%). This resulted in 210 mg (77.02%) of methyl 4-bromo-2-methyl-1-oxoisoquinoline-7-carboxylate as a yellow solid. LCMS (ESI) m/z: [M+H]+ = 296.1.

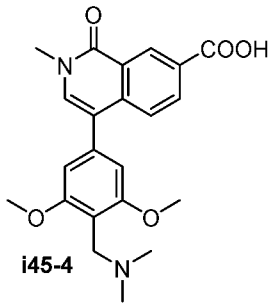
Step 2: Preparation of methyl 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-2-methyl-1-oxoisoquinoline-7-carboxylate (i45-3)



i45-3

To a solution of methyl 4-bromo-2-methyl-1-oxoisoquinoline-7-carboxylate (210.00 mg, 0.709 mmol, 1.00 equiv), 4-[(dimethylamino)methyl]-3,5-dimethoxyphenylboronic acid (203.46 mg, 0.851 mmol, 1.20 equiv), and Cs_2CO_3 (693.19 mg, 2.128 mmol, 3.00 equiv) in 1,4-dioxane (3.00 mL) was added $Pd(dppf)Cl_2$ (51.89 mg, 0.071 mmol, 0.10 equiv) and H_2O (1.00 mL) at 25 ℃. The resulting solution was stirred for 2 hours at 80 ℃. The resulting solution was diluted with 10 mL of water. The resulting solution was extracted with ethyl acetate (2 x 20 mL) and the organic layers combined and dried over anhydrous sodium sulfate and concentrated. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0-100%). This resulted in 195 mg (66.99%) of methyl 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-2-methyl-1-oxoisoquinoline-7-carboxylate as a yellow solid. LCMS (ESI) m/z: [M+H]+ = 411.2.

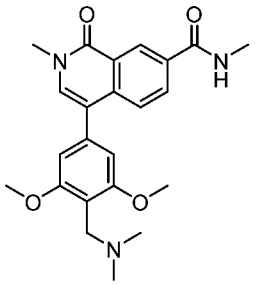
Step 3: Preparation of 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-2-methyl-1-oxoisoquinoline-7-carboxylic acid (i45-4)



i45-4

To a solution of methyl methyl 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-2-methyl-1-oxoisoquinoline-7-carboxylate (195.00 mg, 0.475 mmol, 1.00 equiv) in Hydrochloric acid 37% solution in water (3.00 mL) at 25 ℃. The resulting solution was stirred for 2 hours at 90 ℃. The resulting mixture was concentrated under vacuum. This resulted in 185 mg (98.23%) of 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-2-methyl-1-oxoisoquinoline-7-carboxylic acid as a yellow solid, that was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 397.1.

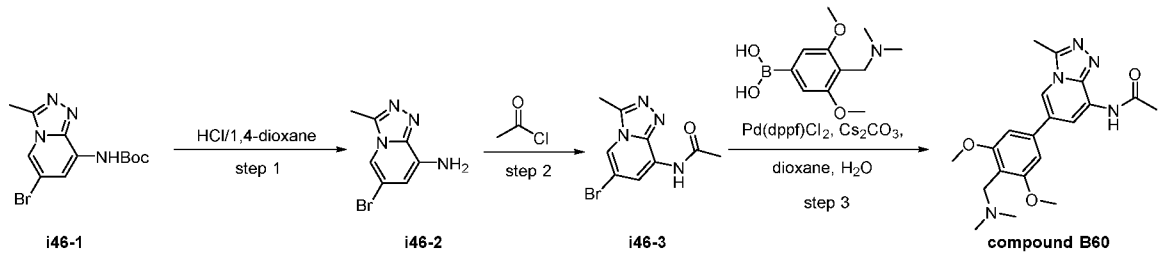
Step 4: Preparation of4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-N,2-dimethyl-1-oxoisoquinoline-7-carboxamide (compound B59)



compound B59

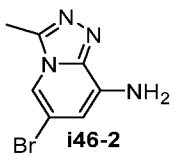
To a solution of 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-2-methyl-1-oxoisoquinoline-7-carboxylic acid (180 mg, 0.454 mmol, 1.00 equiv) in CH_2Cl_2 (3.00 mL) was added HATU (345.28 mg, 0.908 mmol, 2.00 equiv). After that, CH_3NH_2 (28.20 mg, 0.908 mmol, 2.00 equiv) and DIEA (293.41 mg, 2.270 mmol, 5.00 equiv) was added at 0 ℃. The resulting solution was stirred for 2 hours at 25 ℃. The resulting solution was diluted with 10 mL of water and extracted with ethyl acetate (2 x 20 mL) and the organic layers combined and dried over anhydrous sodium sulfate and concentrated. The crude product was purified by Prep-HPLC (conditions: Atlantis HILIC OBD Column, 19 mm X 250 mm; mobile phase, Water(0.1% FA) and ACN (hold 5% PhaseB in 2 minutes, up to 17% in 8 minutes); Detector, uv). This resulted in 150 mg (80.65%) 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-N,2-dimethyl-1-oxoisoquinoline-7-carboxamide as a yellow solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 8.89 (d, *J* = 2.0 Hz, 1H), 8.12 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.75 (d, *J* = 8.6 Hz, 1H), 7.57 (s, 1H), 6.88 (s, 2H), 4.44 (s, 2H), 3.97 (s, 6H), 3.71 (s, 3H), 2.98 (s, 3H), 2.93 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 410.30.

Example 46 – Preparation of *N*-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl)acetamide (compound B60)



i46-1 **i46-2** **i46-3** **compound B60**

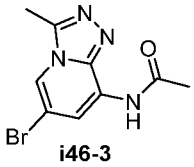
Step 1: Preparation of 6-bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-amine (i46-2)



i46-2

Into a 50-mL round-bottom flask, was placed tert-butyl *N*-[6-bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl]carbamate (300.00 mg, 0.917 mmol, 1.00 equiv), HCl (gas) in 1,4-dioxane (7.50 mL, 205.696 mmol, 269.20 equiv). The resulting solution was stirred for 2 hours at 25 ℃. The resulting mixture was concentrated. This resulted in 180 mg (86.46%) of 6-bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-amine as a white solid.

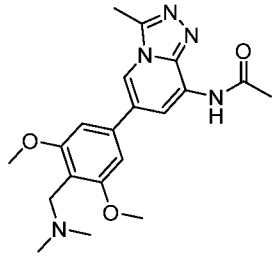
Step 2: Preparation of N-[6-bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl]acetamide (i46-3)



i46-3

To a solution of 6-bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-amine (180.00 mg, 0.793 mmol, 1.00 equiv) in THF (10.00 mL) was added NaH (38.05 mg, 1.585 mmol, 2.00 equiv) and acetyl chloride (74.67 mg, 0.951 mmol, 1.20 equiv) at 25 ℃. The resulting solution was stirred for 2 hours at 25 ℃. The reaction was then quenched by the addition of 5 mL of MeOH. The resulting mixture was concentrated. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:1). This resulted in 100 mg (46.88%) of *N*-[6-bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl]acetamide as a yellow solid.

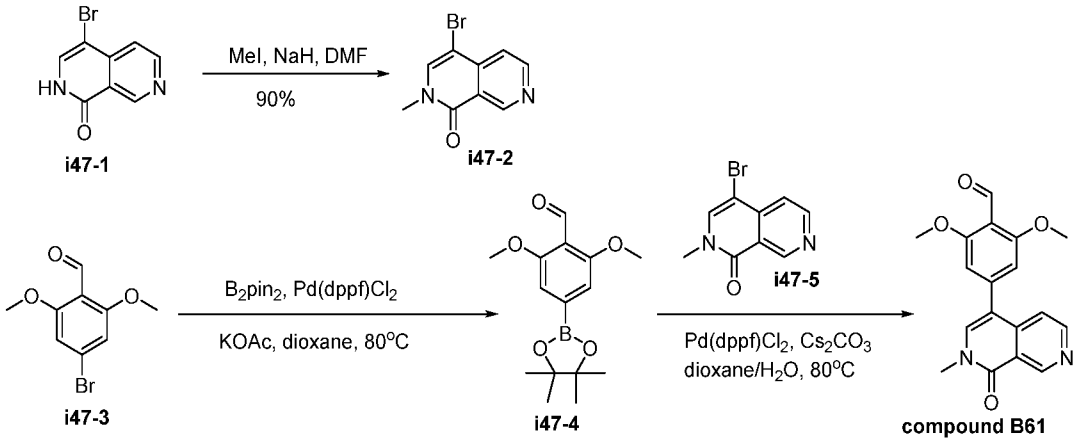
Step 3: Preparation of N-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl)acetamide (compound B60)



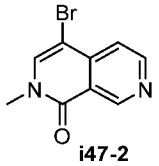
compound B60

To a solution of *N*-[6-bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl]acetamide (60.00 mg, 0.223 mmol, 1.00 equiv), [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]boronic acid (53.31 mg, 0.223 mmol, 1.00 equiv), and Cs_2CO3 (145.29 mg, 0.446 mmol, 2.00 equiv) in 1,4-dioxane (5.00 mL) was added Pd(dppf)Cl2 CH_2Cl_2 (18.21 mg, 0.022 mmol, 0.10 equiv) and H_2O (1.00 mL) at 25 ℃. The resulting solution was stirred for 2 hours at 80 ℃. The resulting solution was diluted with 10 mL of water. The resulting solution was extracted with ethyl acetate (2 x 20 mL) and the organic layers were combined and dried over anhydrous sodium sulfate and concentrated. The crude product was purified by Prep-HPLC (conditions: SunFire C18 OBD Prep Column, 19 mm X 250 mm; mobile phase, Water (0.1% FA (formic acid)) and ACN (hold 5% PhaseB in 2 minutes,up to 17% in 8 minutes); Detector, uv). This resulted in 10.3 mg (12.05%) of *N*-(6-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-yl)acetamide as a brown semi-solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 8.55 (s, 1H, FA), 8.48 (d, *J* = 1.4 Hz, 1H), 8.35 (d, *J* = 1.5 Hz, 1H), 7.08 (s, 2H), 4.38 (s, 2H), 4.05 (s, 6H), 2.88 (s, 6H), 2.85 (s, 3H), 2.33 (s, 3H). LCMS (ESI) m/z: $[M+H]^+$ = 384.25.

Example 47 – Preparation of 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (compound B61)

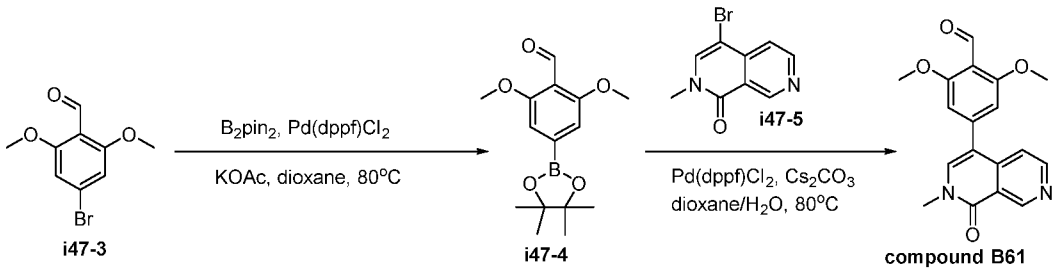


Step 1: Preparation of 4-bromo-2-methyl-2,7-naphthyridin-1(2H)-one (i47-2)



To a solution of 4-bromo-1,2-dihydro-2,7-naphthyridin-1-one (20.00 g, 88.871mmol, 1 equiv) in DMF (150 mL) was added NaH (8.80 g, 60%, 222.178 mmol, 2.5 equiv) in portions at 0 ℃, and the resulting mixture was stirred at 0 ℃ for 30 minutes. Then CH_3I (1.90 g, 133.307mmol, 1.5 equiv) was added dropwise. The resulting mixture was stirred at 0 ℃ for 1 hour. The mixture was poured into ice water (200 mL) and stirred for 30 minutes. The mixture was filtered and the solid was dried to afford 4-bromo-2-methyl-2,7-naphthyridin-1(2H)-one (20.00 g, 94.13 %) as light grey solid. LCMS (ESI, *m/z*): $[M+H]^+ = 239.1$, $[M+H+2]^+ = 241.1$.

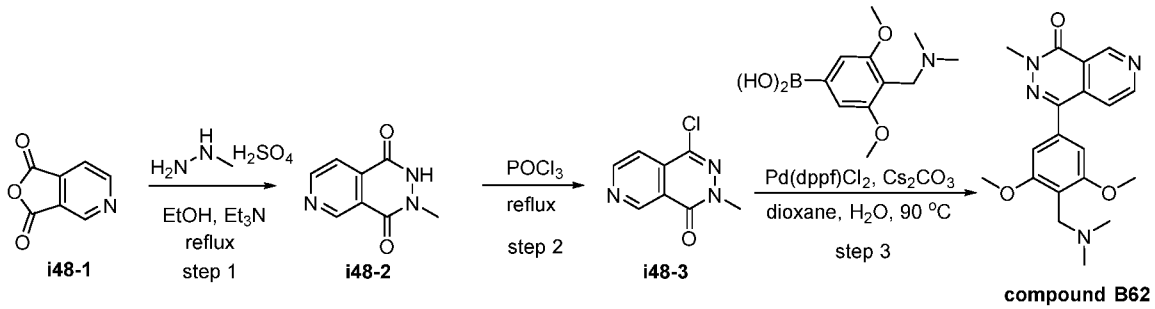
Step 2: Preparation of 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (compound B61)



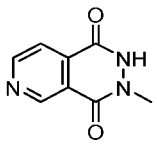
To a solution of 4-bromo-2,6-dimethoxybenzaldehyde (5.00 g, 20.402 mmol, 1 equiv) in 1,4-dioxane (300 mL) was added 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) -1,3,2-dioxaborolane (6.22 g, 24.483 mmol, 1.2 equiv), $Pd(dppf)Cl_2$ (298.57 mg, 0.408 mmol, 0.02 equiv), and KOAc (4.00 g, 40.804 mmol, 2 equiv) at 25 ℃. The resulting mixture was stirred at 80 ℃ for 1 hour under N_2 atmosphere. The mixture was cooled to 60 ℃. Then 4-bromo-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (4.91 g, 20.538 mmol, 1 equiv), Cs_2CO_3 (13.38 g, 41.076 mmol, 2 equiv), $Pd(dppf)Cl_2$ (298.57 mg, 0.408 mmol, 0.02 equiv), and water (60 mL) were added. The resulting mixture was stirred at 80 ℃ for 1 hour. The mixture was filtered and activated charcoal (5 g) was added to the filtrate and

refluxed at 100 °C for 1 hour. The mixture was filtered and concentrated to get crude product, which was slurried in EA, EtOH, and water respectively to afford light brown solid. This solid was dissolved in DCM and MeOH (200 mL, v/v=20/1), and then precipitated with EA (200 mL) dropwise under stirring. The solid was filtered and dried to afford 2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzaldehyde (2.3 g, 34.63%) as light grey solid. LCMS (ESI, *m/z*): $[M+H]^+$ = 325.2. ^{1}H NMR (300 MHz, DMSO) δ 10.41 (s, 1H), 9.46 (s, 1H), 8.74 (d, *J* = 5.7 Hz, 1H), 7.99 (s, 1H), 7.64 (d, *J* = 5.7Hz, 1H), 6.85 (s, 2H), 3.89 (s, 6H), 3.62 (s, 3H).

Example 48 – Preparation of 1-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-3-methylpyrido[3,4-d]pyridazin-4(3H)-one 2,2,2-trifluoroacetate (compound B62)



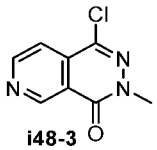
Step 1: Preparation of 3-methyl-2,3-dihydropyrido[3,4-d]pyridazine-1,4-dione (i48-2)



i48-2

To a stirred mixture of furo[3,4-c]pyridine-1,3-dione (2.00 g, 13.413 mmol, 1.00 equiv) and methylhydrazine sulfate (5.80 g, 40.240 mmol, 3 equiv) in EtOH (20.00 mL) was added Et_3N (8.14 g, 80.480 mmol, 6.00 equiv), the resulting solution was stirred at 80 degrees C under nitrogen atmosphere for 10 hours. Then the resulting mixture was concentrated under reduced pressure to give 4.2 g of crude product. This material was used directly in the next step without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 178.

Step 2: Preparation of N 1-chloro-3-methylpyrido[3,4-d]pyridazin-4(3H)-one (i48-3)

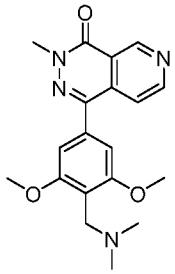


i48-3

Into $POCl_3$ (20.00 mL, 214.567 mmol, 9.05 equiv) was added 3-methyl-2H-pyrido[3,4-d]pyridazine-1,4-dione (4.20 g, 23.707 mmol, 1.00 equiv), and then it was stirred for 8 h at 105 degrees C under nitrogen atmosphere. The resulting mixture was concentrated to remove $POCl_3$, then neutralized with the saturated solution of $NaHCO_3$ (200 mL), extracted with EA (300 mL x 3). The combined organic layers were washed with the solution of saturated NaCl, dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure, then purified by flash chromatography (ethyl acetate/petroleum ether from 1:4 to 1:1). This resulted -chloro-3-methylpyrido[3,4-d]pyridazin-4(3H)-one.

This material was used directly in the next step without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 196.

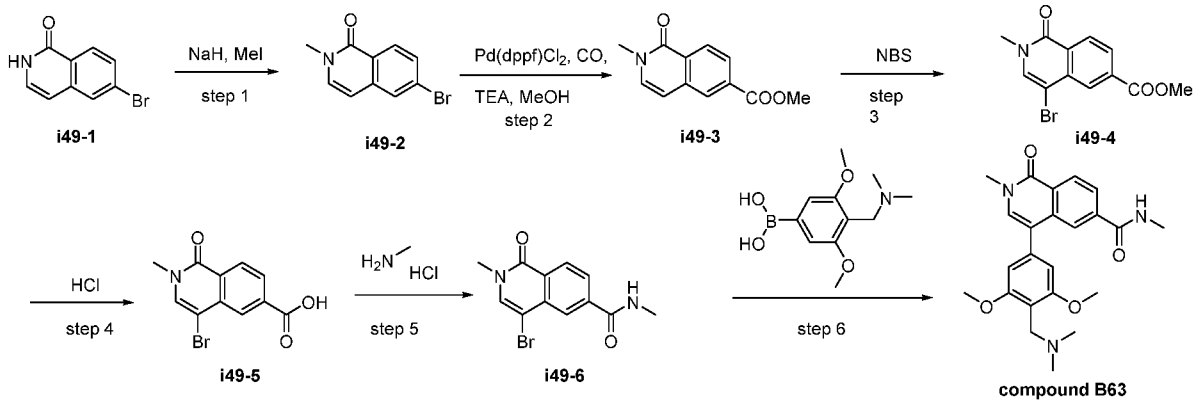
Step 3: Preparation of 1-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-3-methylpyrido[3,4-d]pyridazin-4(3H)-one 2,2,2-trifluoroacetate (PH-FOG-P3-B87).



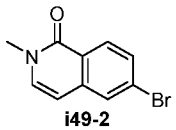
compound B62

Into a stirred mixture of 1-chloro-3-methyl-3H,4H-pyrido[3,4-d]pyridazin-4-one(150.00 mg, 0.767 mmol, 1.00 equiv) and [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]boronic acid(275.00 mg, 1.150 mmol, 1.50 equiv) in dioxane (5.00 mL) and H_2O (0.50 mL) was added $Pd(dppf)Cl_2.CH_2Cl_2$(62.62 mg, 0.077 mmol, 0.10 equiv) and Cs_2CO_3(999.40 mg, 3.067 mmol, 4.00 equiv) at 25 degrees C under N_2 atmosphere. Then the reaction was stirred at 90 degrees C for 12 h. The resulting mixture was extracted with EtOAc (2 x 40mL). The combined organic layers were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by Prep-HPLC (conditions: Sunfire C18 OBD Prep Column, 5um, 19mm*250mm; Mobile Phase A: Water (0.05% TFA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 2% B to 2% B in 2 min; 254 nm; Rt: 13.78 min). This resulted in 20.3 mg (5.57%) of 1-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-3-methylpyrido[3,4-d]pyridazin-4(3H) as a white solid. ^{1}H NMR (300 MHz, Methanol-d4) δ 9.66 (s, 1H), 8.99 (d, *J* = 5.6 Hz, 1H), 7.79 (d, *J* = 5.6 Hz, 1H), 7.04 (s, 2H), 4.46 (s, 2H), 4.00 (s, 6H), 3.93 (s, 3H), 2.94 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 355.15.

Example 49 – Preparation of 4-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-N,2-dimethyl-1-oxo-1,2-dihydroisoquinoline-6- carboxamide (compound B63)



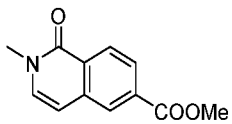
Step 1: Preparation of 6-bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridin-8-amine (i49-2)



i49-2

To a solution of 6-bromo-2H-isoquinolin-1-one (5.0 g, 22.316 mmol, 1.00 equiv) in DMF was added sodium hydride (60% in oil, 803.3 mg) at 0 ℃. The mixture was stirred for 15 minutes. MeI (9.5 g, 66.947 m mol, 3.00 equiv) was added, and the mixture was allowed to warm to room temperature and stirred for additional 1 hour. The reaction mixture was quenched by water and extracted with DCM (3 x 100 mL). The DCM layer was concentrated under vacuum. This resulted in 6-bromo-2-methylisoquinolin-1-one as a white solid (6.45 g, crude) that was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 238.

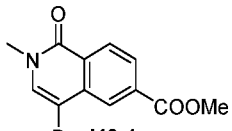
Step 2: Preparation of methyl 2-methyl-1-oxo-1,2-dihydroisoquinoline-6-carboxylate (i49-3)



i49-3

To a solution of 6-bromo-2-methylisoquinolin-1-one (1 g, 4.200 mmol, 1.00 equiv) and $PdCl_2$(dppf) (307.3 mg, 0.420 m mol, 0.10 equiv) in MeOH (9 mL) was added Et_3N (7.00 mL, 50.361 mmol, 11.99 equiv) in a pressure tank. The mixture was purged with nitrogen for 20 minutes and then was pressurized to 50 atm with carbon monoxide. The mixture was then stirred at 100 ℃ for 15 hours. The reaction mixture was cooled to room temperature and filtered to remove insoluble solids. The resulting mixture was concentrated under vacuum. The residue was purified by silica gel column chromatography, eluted with PE (petroleum ether) / EtOAc (ethyl acetate) (2:1) to afford methyl 2-methyl-1-oxoisoquinoline-6-carboxylate as yellow solid (501 mg, 55%). LCMS (ESI) m/z: [M+H]+ = 218.

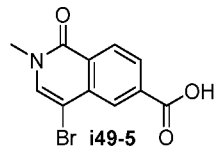
Step 3: Preparation of methyl 4-bromo-2-methyl-1-oxo-1,2-dihydroisoquinoline-6-carboxylate (i49-4)



i49-4

To a stirred solution of methyl 2-methyl-1-oxoisoquinoline-6-carboxylate (200 mg, 0.921 mmol, 1.00 equiv) in THF (10 mL) was added NBS (245.8 mg, 1.381 mmol, 1.50 equiv) in portions over 25 minutes at 0 ℃. The resulting mixture was stirred for additional 2 hours at room temperature. The resulting mixture was diluted with 10 mL of water and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with saturated NaCl (20 mL) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The crude product (161 mg) was used in the next step directly without further purification.

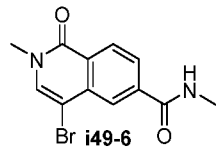
Step 4: Preparation of 4-bromo-2-methyl-1-oxo-1,2-dihydroisoquinoline-6-carboxylic acid (i49-5)



Br **i49-5**

A solution of methyl 4-bromo-2-methyl-1-oxois oquinoline-6-carboxylate (161 mg, 0.544 mmol, 1.00 equiv) in conc. HCl (5 mL) was stirred for 4 hours at 100 ℃. The resulting mixture was concentrated under vacuum. The crude product (177 mg) was used in the next step directly without further purification. LCMS (ESI) m/z: [M+H]+ =283.

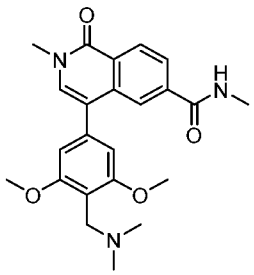
Step 5: Preparation of 4-bromo-N,2-dimethyl-1-oxo-1,2-dihydroisoquinoline-6-carboxamide (i49-6)



Br **i49-6**

A solution of 4-bromo-2-methyl-1-oxoisoquinoline-6-carboxylic acid (85 mg, 0.301 mmol, 1.00 equiv) in DMF was treated with HATU (137.5 mg, 0.362 m mol, 1.20 equiv) for 30 minutes at room temperature followed by the addition of DIEA (194.7 mg, 1.507 mmol, 5.00 equiv) and methylamine (9.4 mg, 0.301 mmol, 1.00 equiv) at room temperature. The resulting mixture was stirred for 2 hours at room temperature under nitrogen atmosphere. The resulting mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with CH_2Cl_2 / MeOH (10:1) to afford 4-bromo-*N*,2-dimethyl-1-oxoisoquinoline-6-carboxamide (81 mg, 91%) as a white solid. LCMS (ESI) m/z: [M+H]+ = 296.

Step 6: Preparation of 4-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-N,2-dimethyl-1-oxo-1,2-dihydroisoquinoline-6-carboxamide (compound B63)

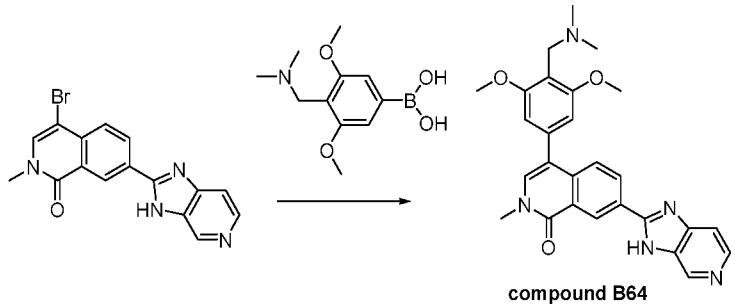


compound B63

To a solution of 4-bromo-N,2-dimethyl-1-oxo-1,2-dihydroisoquinoline-6-carboxamide (80 mg, 0.271 mmol, 1.00 equiv) and [4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]boronic acid (97.2 mg, 0.407 mmol, 1.50 equiv) in dioxane (2 mL) and water (0.4 mL) was added Cs_2CO_3 (264.95 mg, 0.813 mmol, 3.00 e.q.) and $Pd(dppf)Cl_2$ (19.8 mg, 0.027 mmol, 0.10 equiv). After stirring for 2 hours at 75 ℃ under nitrogen atmosphere, the resulting mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography and eluted with CH_2Cl_2 / MeOH (5:1). The crude product was further purified by Prep-HPLC (conditions:Atlantis HILIC OBD Column 19*150 mm*5 μm; mobile phase, Phase A: Water(10 mmol/L NH_4HCO_3); Phase B: ACN, Gradient; Detector, uv 254/220 nm). This gave 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-*N*,2-dimethyl-1-oxo-1,2-

dihydroisoquinoline-6-carboxamide (10.1 mg, 9.1%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.64 (d, *J* = 4.9 Hz, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 1.6 Hz, 1H), 7.93 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.65 (s, 1H), 6.73 (s, 2H), 3.79 (s, 6H), 3.60 (s, 3H), 3.47 (s, 2H), 2.78 (d, *J* = 4.5 Hz, 3H), 2.15 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 410.20.

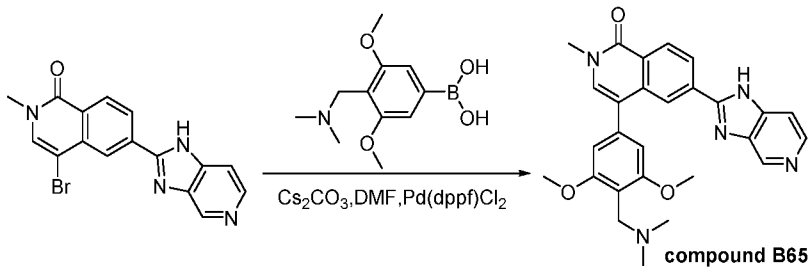
Example 50 – Preparation of 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-7-[1*H*-imidazo[4,5-c]pyridin-2-yl]-2-methylisoquinolin-1-one (compound B64)



compound B64

To a solution of 4-bromo-7-[3*H*-imidazo[4,5-c]pyridin-2-yl]-2-methylisoquinolin-1-one (80.00 mg, 0.225 mmol, 1.00 equiv) and [[2,6-dimethoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl]dimethylamine (108.52 mg, 0.338 mmol, 1.5 equiv) in mixed DMF (3.00 mL) and H_2O (0.30 mL) was added Cs_2CO_3 (220.15 mg, 0.676 mmol, 3 equiv) and $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (18.39 mg, 0.023 mmol, 0.10 equiv). After stirring for 2 hours at 90 ℃ under a nitrogen atmosphere, the resulting mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with CH_2Cl_2 / MeOH (12:1) to afford a crude product. The crude product was further purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5 μm,19*150mm; Mobile Phase A: Water(10 mmol/L NH_4HCO_3), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 40% B to 55% B in 8 minutes; 254/220 nm; R_t (retention time): 6.50 minutes) to afford 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-7-[1*H*-imidazo[4,5-c]pyridin-2-yl]-2-methylisoquinolin-1-one (7 mg, 6.62%) as a grey solid. ^{1}H NMR (300 MHz, Methanol-*d4*) δ 9.21 (d, *J* = 2.0 Hz, 1H), 8.91 (d, *J* = 1.0 Hz, 1H), 8.48 (dd, *J* = 8.6, 2.0 Hz, 1H), 8.32 (d, *J* = 5.8 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.68 (dd, *J* = 5.8, 1.0 Hz, 1H), 7.55 (s, 1H), 6.80 (s, 2H), 3.91 (s, 6H), 3.76 (d, *J* = 18.1 Hz, 5H), 2.41 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 470.20.

Example 51 – Preparation of 4-(4-((dimethylamino)methyl)-3,5-dimethoxyphenyl)-6-(1H-imidazo[4,5-c]pyridin-2-yl)-2-methylisoquinolin-1(2H)-one (compound B65)

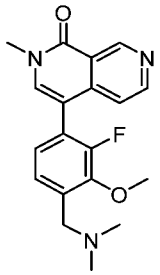


compound B65

To a solution of 4-bromo-6-[1H-imidazo[4,5-c]pyridin-2-yl]-2-methylisoquinolin-1-one (100.00 mg, 0.282 m mol, 1.00 e.q.) and 4-[(dimethylamino)methyl]-3,5-dimethoxyphenylboronic acid (100.96 mg, 0.422 mmol, 1.50 e.q.) in DMF(2 mL) and water(0.4mL), was added Cs_2CO_3 (275.19 mg, 0.845 mmol,

3.00 e.q.) and Pd(dppf)Cl_2 (20.60 mg, 0.028 mmol, 0.10 e.q.). After stirring for 2 h at 80 degrees C under a nitrogen atmosphere, the resulting mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with CH_2Cl_2 / MeOH (5:1). The crude product was purified by Prep-HPLC with the following conditions (2#SHIMADZU (HPLC-01)): Column, Atlantis HILIC OBD Column, 19 mm X 250 mm X5um; mobile phase, Water (0.1%FA) and ACN (hold 5% Phase B in 5 min ,up to 10% in 10.5 min); Detector, uv,254. This resulted in 5.0 mg (11.35%) of to afford 4-[4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl]-6-[1H-imidazo[4,5-c]pyridin-2-yl]-2-methy-lisoquinolin-1-one as a yellow solid. ^{1}H NMR (300 MHz, Methanol-d4) δ 8.90 (s, 1H), 8.64 (d, *J* = 8.5 Hz, 1H), 8.59 (s, 1H), 8.55 (br s, 1H, FA), 8.33 (d, *J* = 6.5 Hz, 2H), 7.70 (d, *J* = 5.9 Hz, 1H), 7.58 (s, 1H), 6.96 (s, 2H), 4.44 (s, 2H), 3.99 (s, 6H), 3.75 (s, 3H), 2.95 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 470.45.

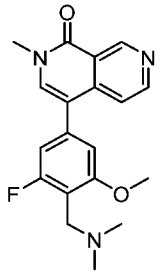
Example 52 – Preparation of 4-(4-((dimethylamino)methyl)-2-fluoro-3-methoxyphenyl)-2-methyl-2,7-naphthyridin-1(2H)-one (compound B66)



compound B66

Compound B66 was prepared in a similar manner as described for compound B50. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (d, *J* = 0.8 Hz, 1H), 8.69 (d, *J* = 5.7 Hz, 1H), 7.88 (s, 1H), 7.28 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.19 – 7.09 (m, 2H), 3.86 (d, *J* = 1.2 Hz, 3H), 3.57 (s, 3H), 3.48 (s, 2H), 2.20 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 342.2.

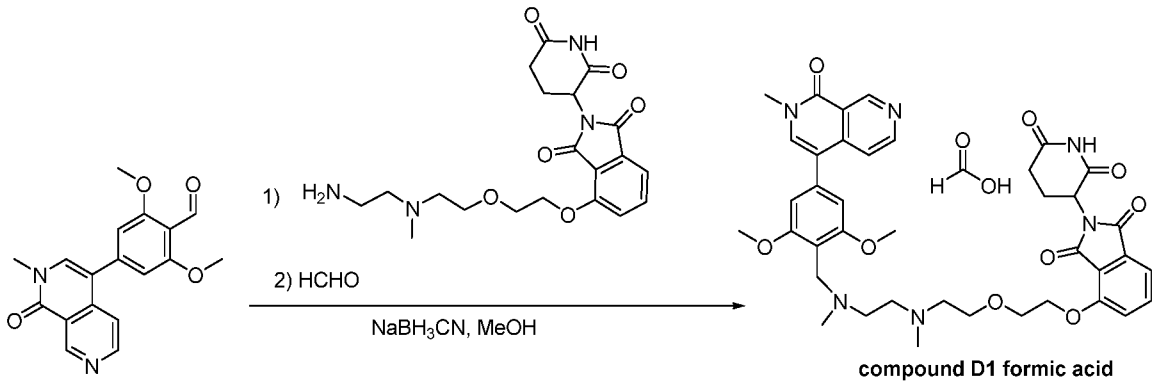
Preparation of 4-(4-((dimethylamino)methyl)-3-fluoro-5-methoxyphenyl)-2-methyl-2,7-naphthyridin-1(2H)-one (compound B67)



compound B67

Compound B67 was prepared in a similar manner as described for compound B50. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (d, *J* = 0.9 Hz, 1H), 8.71 (d, *J* = 5.7 Hz, 1H), 7.89 (s, 1H), 7.52 (dd, *J* = 5.7, 0.9 Hz, 1H), 6.94 (d, *J* = 1.8 Hz, 1H), 6.90 (dd, *J* = 10.0, 1.5 Hz, 1H), 3.84 (s, 3H), 3.57 (s, 4H), 3.48 (d, *J* = 1.8 Hz, 2H), 2.15 (s, 6H). LCMS (ESI) m/z: [M+H]+ = 342.2.

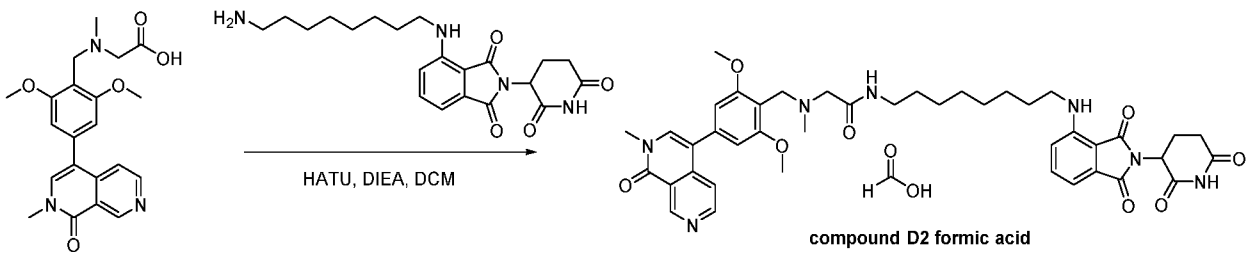
Example 53 – Preparation of 4-[2-(2-[[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)(methyl)amino)ethyl](methyl)amino]ethoxy)ethoxy]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione formic acid (compound D1 formic acid)



compound D1 formic acid

A solution of 2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)benzaldehyde (30.00 mg, 0.092 mmol, 1.00 equiv) and 4-(2-[2-[(2-aminoethyl)(methyl)amino]ethoxy]ethoxy)-2- (2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (38.71 mg, 0.093 mmol, 1.00 equiv) in MeOH (1 mL) was stirred for 3 hours at room temperature under nitrogen atmosphere. To the above mixture was added $NaBH_3CN$ (11.63 mg, 0.185 mmol, 2.00 equiv) and stirred for 1h at room temperature under nitrogen atmosphere. Then HCHO (27.77 mg, 0.925 mmol, 10.00 equiv) was added. After 1 hour. The above mixture was added $NaBH_3CN$ (11.63 mg, 0.185 mmol, 2.00 equiv) and stirred for 1h at room temperature under nitrogen atmosphere. The crude product (30mg) was purified by Prep-HPLC (conditions: SunFire Prep C18 OBD Column, 19×150 mm 5 μm 10 nm; Mobile Phase A:Water (0.1% FA), Mobile Phase B:ACN; Flow rate:25 mL/min; Gradient:7 B to 17 B in 12 min; 254/220 nm; R_T: 7.68 minutes) to afford 4-[2-(2-[[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin -4-yl)phenyl]methyl](methyl)amino)ethyl](methyl)amino]ethoxy)ethoxy]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione; formic acid (10.1 mg) as a white solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 2.03 – 2.14 (1H, m), 2.42 (3H, s), 2.60 – 2.77 (3H, m), 2.82 (6H, d), 2.96 (5H, s), 3.28 (3H, s), 3.70 (3H, s), 3.80 (2H, s), 3.93 (8H, s), 4.34 (4H, d), 5.04 – 5.13 (1H, m), 6.79 (2H, s), 7.31 – 7.38 (2H, m), 7.57 (1H, d), 7.66 (1H, t), 7.73 (1H, s), 8.44 (1H, s), 8.66 (1H, d), 9.52 (1H, s). LCMS (ESI) m/z: [M+H]+ = 741.45.

Example 54 – Preparation of 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-N-(8-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]amino]octyl)acetamide formic acid (compound D2 formic acid)

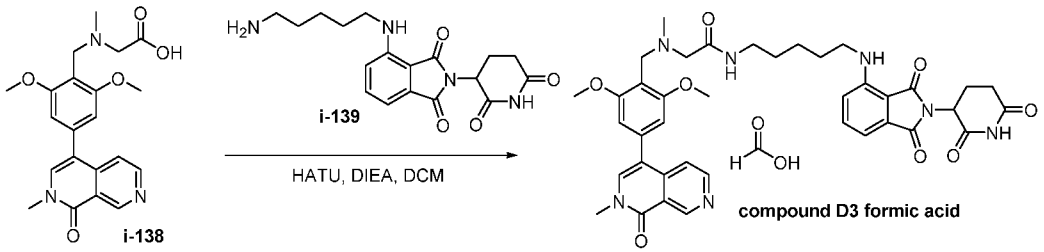


compound D2 formic acid

To the solution of 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl] methyl](methyl)amino)acetic acid (40 mg, 0.101 mmol, 1 equiv) in DCM (2 mL) was added 4-[(8-aminooctyl)amino]-2-(2,6-dioxopiperidin-3-yl)-2,3-dihydro-1*H*-isoindole-1,3-dione (48.37 mg, 0.121 mmol, 1.2 equiv), HATU (57.40 mg, 0.151 mmol, 1.5 equiv), and DIEA (39.02 mg, 0.302 mmol, 3 equiv). The

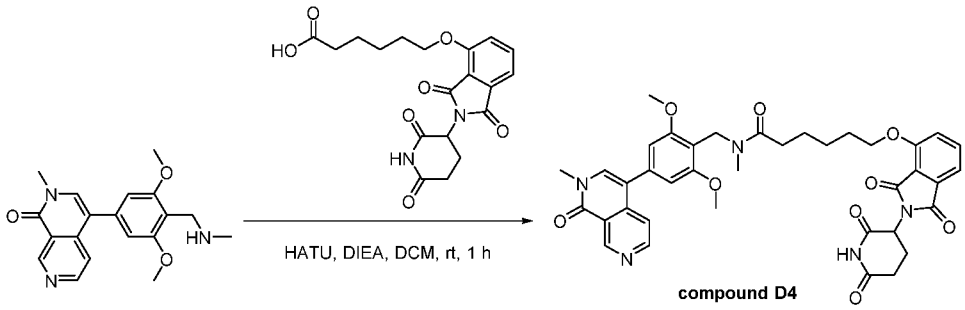
resulting solution was stirred at room temperature for 1 hour. The mixture was concentrated. The crude product was purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um, 19*150 mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 15% B to 40% B in 8 min; 254 nm; Rt: 7.04 min) to afford 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-*N*-(8-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]amino]octyl)acetamide formic acid (14.9 mg, 17.13%) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.51 (d, *J* = 0.8 Hz, 1H), 8.67 (d, *J* = 5.7 Hz, 1H), 7.73 (s, 1H), 7.62 (d, *J* = 5.7 Hz, 1H), 7.52 (dd, *J* = 8.6, 7.1 Hz, 1H), 6.99(dd, *J* = 12.1, 7.8 Hz, 2H), 6.76 (s, 2H), 5.05 (dd, *J* = 12.4, 5.5 Hz, 1H), 3.90 (s, 6H), 3.81 (s, 2H), 3.69 (s, 3H), 3.24 (dt, *J* = 9.7, 7.0 Hz, 4H), 3.14 (s, 2H), 2.87(ddd, *J* = 19.0, 14.1, 5.2 Hz, 1H), 2.81 – 2.64 (m, 2H), 2.38 (s, 3H), 2.17 – 2.07 (m, 1H), 1.59 (q, *J* = 6.9 Hz, 2H), 1.53 (d, *J* = 7.3 Hz, 2H), 1.36 (s, 8H). LCMS (ESI) m/z: $[M+H]^+$ = 780.40.

Example 55 – Preparation of 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl] methyl](methyl)amino)-N-(5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]amino]pentyl)acetamide formic acid (compound D3 formic acid)



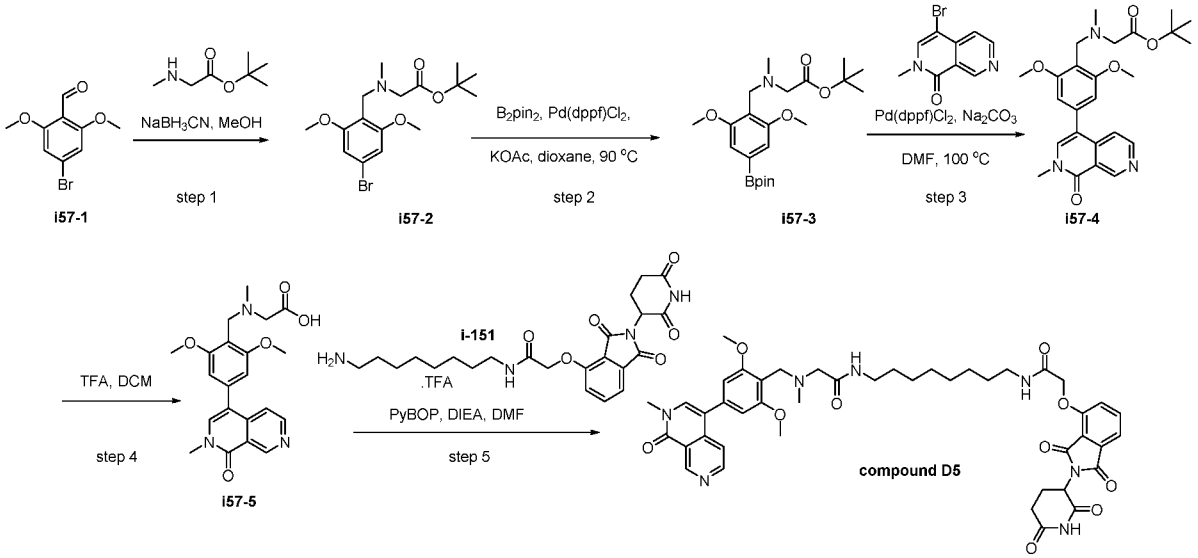
To the solution of 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)acetic acid (40 mg, 0.101 mmol, 1 equiv) in DCM (2 mL) was added 4-[(5-aminopentyl)amino]-2-(2,6-dioxopiperidin-3-yl)-2,3-dihydro-1H-isoindole-1,3-dione (43.29 mg, 0.121 mmol, 1.2 equiv), HATU (57.40 mg, 0.151 mmol, 1.5 equiv), and DIEA (39.02 mg, 0.302 mmol, 3 equiv). The resulting solution was stirred at room temperature for 1 hour. The mixture was concentrated. The crude product was purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um, 19*150mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 12% B to 30% B in 8 min; 254 nm; Rt: 7.15 min) to afford 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-*N*-(5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]amino]pentyl)acetamide formic acid (15.2 mg, 18.44%) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.49 (d, *J* = 0.9 Hz, 1H), 8.67 (d, *J* = 5.7 Hz, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 5.7 Hz, 1H), 7.46 (dd, *J* = 8.5, 7.1 Hz, 1H), 6.96 (dd, *J* = 12.3, 7.8 Hz, 2H), 6.75 (s, 2H), 5.04 (dd, *J* = 12.4, 5.4 Hz, 1H), 3.89 (s, 6H), 3.78 (s, 2H), 3.69 (s, 3H), 3.27 (q, *J* = 6.5 Hz, 4H), 3.13 (s, 2H), 2.87 (ddd, *J* = 18.8, 14.1, 5.2 Hz, 1H), 2.81 – 2.63 (m, 2H), 2.38 (s, 3H), 2.17 – 2.09 (m, 1H), 1.67 (p, *J* = 7.0 Hz, 2H), 1.58 (p, *J* = 6.8 Hz, 2H), 1.45 (q, *J* = 8.0 Hz, 2H). LCMS (ESI) m/z: $[M+H]^+$ = 738.30.

Example 56 – Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl] -6-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]-N-methylhexanamide (compound D4)

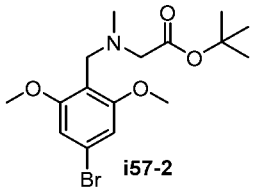


To a solution of 6-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy]hexanoic acid (50.00 mg, 0.13 mmol, 1 eq.) and DIPEA (49.92 mg, 0.39 mmol, 3 eq.) in DCM (2 mL) was added PyBOP (100.49 mg, 0.19 mmol, 1.5 eq.) and 4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2-methyl -1,2-dihydro-2,7-naphthyridin-1-one (43.69 mg, 0.13 mmol, 1.00 eq.). The resulting solution was stirred at room temperature for 1 hour. The solution was concentrated. The crude product was purified by Prep-HPLC (conditions: X Select CSH Prep C18 OBD Column, 5um,19*150mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 15% B to 32% B in 8 min; 254 nm; Rt: 6.45 min) to afford *N*-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-6-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]-N-methylhexanamide (8.4 mg, 9.16%) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.54 (d, *J* = 7.5 Hz, 1H), 8.71 – 8.64 (m, 1H), 7.84 (d, *J* = 5.5 Hz, 1H), 7.79 – 7.70 (m, 1H), 7.74 – 7.63 (m, 1H), 7.47 – 7.33 (m,2H), 6.76 (d, *J* = 15.9 Hz, 2H), 5.08 (dd, *J* = 12.5, 5.5 Hz, 1H), 4.76 (s, 1H), 4.69 (d, *J* = 6.7 Hz, 1H), 4.24 (dt, *J* = 11.6, 6.1 Hz, 2H), 3.89 (d, *J* = 16.9 Hz, 6H), 3.71(d, *J* = 9.7 Hz, 3H), 2.88 (s, 3H), 2.70 (td, *J* = 16.0, 14.2, 6.7 Hz, 4H), 2.11 (s, 1H), 1.90 (dt, *J* = 14.7, 7.6 Hz, 2H), 1.80 – 1.57 (m, 4H). LCMS (ESI) m/z: $[M+H]^+$ = 710.30.

Example 57 – Preparation of N-[8-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)acetamido]octyl]-2-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]acetamide (compound D5)



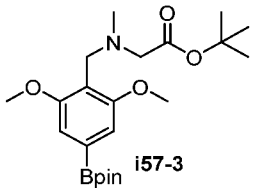
Step 1: preparation of tert-butyl 2-[[(4-bromo-2,6-dimethoxyphenyl)methyl](methyl)amino]acetate (i57-2)



i57-2

To a stirred solution of tert-butyl 2-(methylamino)acetate hydrochloride (5.93 g, 32.643 mmol, 1.60 equiv) in MeOH (60 mL) was added 4-bromo-2,6-dimethoxybenzaldehyde (5 g, 20.402 mmol, 1 equiv), and the mixture was stirred for 30 minutes before $NaBH_3CN$ (2.56 g, 40.737 mmol, 2.00 equiv) was added in portions. The resulting solution was stirred for another 3 hours at 25 degrees C. Then the mixture was concentrated. The residue was purified by silica gel column chromatography, eluted with EA/PE (1:1) to afford *tert*-butyl 2-[[(4-bromo-2,6-dimethoxyphenyl)methyl] (methyl)amino]acetate (4.97 g, 65.09%) as a white solid. LCMS (ESI) m/z: $[M+H]^+$ = 374.

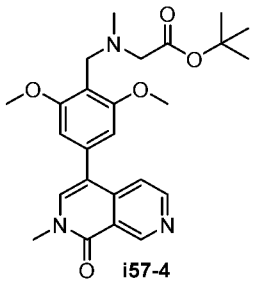
Step 2: Preparation of tert-butyl 2-([[2,6-dimethoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl] (methyl)amino)acetate (i57-3)



i57-3

To a stirred solution of *tert*-butyl 2-[[(4-bromo-2,6-dimethoxyphenyl)methyl](methyl)amino] acetate (4.5 g, 12.023 mmol, 1 equiv) and $Pd(dppf)Cl_2•CH_2Cl_2$ (981.87 mg, 1.202 mmol, 0.1 equiv) in 1,4-dioxane (60 mL) was added AcOK (3.6 g, 36.681 mmol, 3.05 equiv) and 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborol-ane (3.7 g, 14.570 mmol, 1.21 equiv). The mixture was stirred at 90 degrees C under N_2 atmosphere for 3 hours. Then the reaction was cooled to room temperature and filtered. The filter cake was washed with EtOAc, and the filtrate was concentrated. The residue was used directly in the next step. LCMS (ESI) m/z: $[M+H]^+$ = 422.

Step 3: Preparation of tert-butyl 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl) phenyl]methyl](methyl)amino)acetate (i57-4)

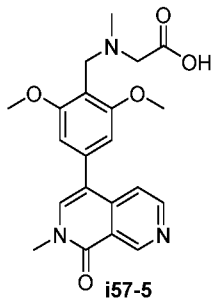


i57-4

To a stirred solution of *tert*-butyl 2-([[2,6-dimethoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl](methyl)amino)acetate (7.28 g, 17.278 mmol, 1 equiv) and $Pd(dppf)Cl_2•CH_2Cl_2$ (1.41 g, 1.728 mmol, 0.1 equiv) in 1,4-dioxane (80 mL)/H_2O(4 mL) was added Cs_2CO_3 (16.89 g, 51.835 mmol, 3 equiv) and 4-bromo-2-methyl-1,2-dihydro-2,7-naph-thyridin-1-one (4.13 g, 17.278 mmol, 1 equiv). The resulting mixture was stirred at 90 degrees C under N_2 atmosphere for 3.5 hours. The resulting solution

was concentrated, and the residue was purified by silica gel column chromatography, eluted with DCM/MeOH (0-10 %) to afford *tert*-butyl 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)acetate (4.43 g, 56.53%) as a yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 454.

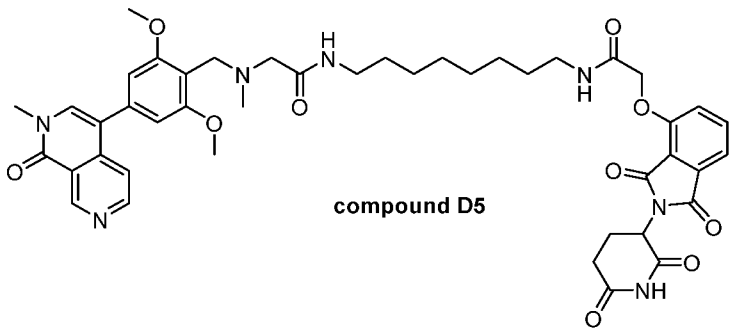
Step 4: Preparation of 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl] (methyl)amino)acetic acid (i57-5)



i57-5

A mixture of *tert*-butyl 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)acetate (4.23 g, 9.327 mmol, 1 equiv) in TFA (17 mL) and DCM (50 mL) was stirred for 17 hours at 25 degrees C. The resulting solution was concentrated, and the residue was purified by reverse flash chromatography (conditions: column, C18 silica gel; mobile phase, MeCN in water (0.1% FA), 10% to 50% gradient in 10 min; detector, UV 254 nm, to afford the 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl] (methyl)amino)acetic acid (3.20 g, 86.48%) as a yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 398.

Step 5: Preparation of N-[8-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)acetamido]octyl]-2-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]acetamide (compound D5)

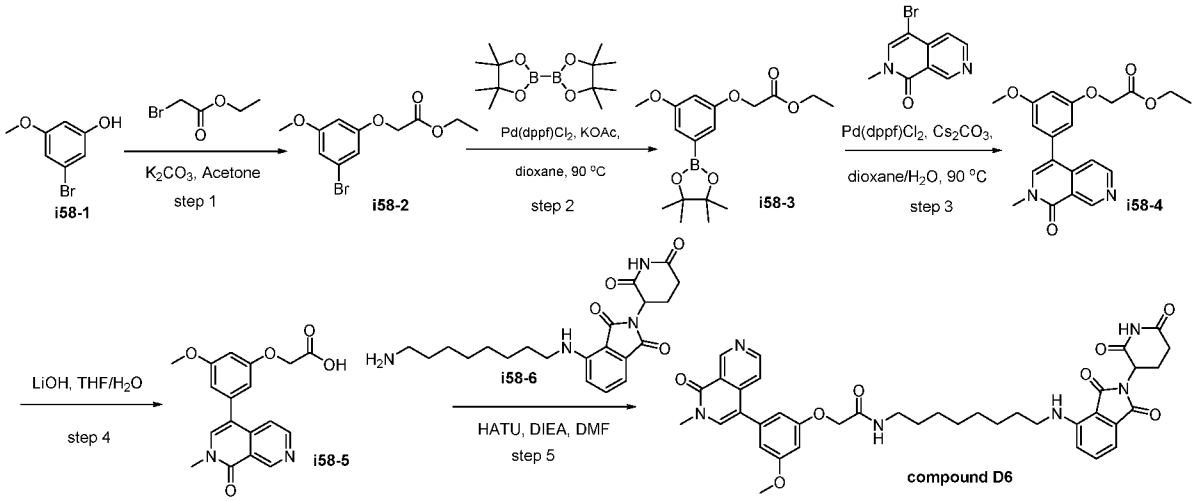


compound D5

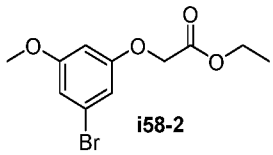
To a solution of 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl] methyl](methyl)amino)acetic acid (20.82 mg, 0.052 mmol, 1 equiv) in DMF (1 mL) was added DIEA (20.32 mg, 0.157 mmol, 3 equiv), PyBOP (29.89 mg, 0.079 mmol, 1.50 equiv), and *N*-(8-aminooctyl)-2-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]acetamide trifluoro-acetic acid (30 mg, 0.052 mmol, 1 equiv). The mixture was stirred for 2 hours at room temperature under ambient atmosphere. The resulting solution was purified by Prep-HPLC (conditions: XSelect CSH Prep C18 OBD Column, 5um,19*150 mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 20% B to 55% B in 8 min; 254 nm; Rt: 5.75 min) to afford *N*-[8-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)acetamido]octyl]-2-[[2-(2,6-

dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy]acetamide (24.6 mg, 56.03%) as a white solid. ^{1}H NMR (300 MHz, Methanol-d4) δ 9.50 (s, 1H), 8.68 (d, *J* = 5.7 Hz, 1H), 7.87 – 7.72 (m, 2H), 7.63 (d, *J* = 5.8 Hz, 1H), 7.54 (d, *J* = 7.4 Hz, 1H), 7.41 (d, *J* = 8.4 Hz,1H), 6.79 (s, 2H), 5.13 (dd, *J* = 12.4, 5.4 Hz, 1H), 4.73 (s, 2H), 3.92 (s, 8H), 3.70 (s, 3H), 3.29 - 3.20 (m, 6H), 2.93 – 2.71 (m, 3H), 2.50 (s, 3H), 2.120 - 2.10 (m, 1H),1.52 (d, *J* = 8.2 Hz, 4H), 1.32 (s, 8H). LCMS (ESI) m/z: $[M+H]^+$ = 838.35.

Example 58 – Preparation of N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)-2-(3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy)acetamide (compound D6)

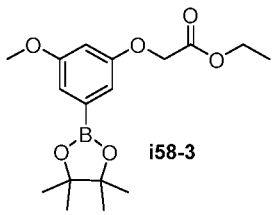


Step 1: Preparation of ethyl 2-(3-bromo-5-methoxyphenoxy)acetate (i58-2)



To a solution of 3-bromo-5-methoxyphenol (1 g, 4.925 mmol, 1 equiv) and ethyl 2-bromoacetate (0.99 g, 5.928 mmol, 1.20 equiv) in acetone (10 mL, 136.021 mmol, 27.62 equiv) was added K_2CO_3 (1.36 g, 9.851 mmol, 2 equiv), and the resulting solution was stirred at 25 ℃ for 1 hour. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with EA/PE (1:4) to afford methyl 2-(3-bromo-5-methoxyphenoxy)acetate (1.23 g, 90.78%) as a colorless liquid. LCMS (ESI) m/z: [M+H]+ = 289.

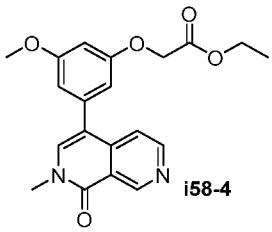
Step 2: Preparation of ethyl 2-(3-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)acetate (i58-3)



To a solution of ethyl 2-(3-bromo-5-methoxyphenoxy)acetate (630 mg, 2.179 mmol, 1 equiv) and 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (664.00 mg, 2.615

mmol, 1.2 equiv) in dioxane (6mL) was added $Pd(dppf)Cl_2$ (159.44 mg, 0.218 mmol, 0.1 equiv) and KOAC (427.70 mg, 4.358 mmol, 2 equiv. The resulting solution was stirred at 90 ℃ for 2 hours (under N_2 atmosphere). After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with EA/PE (1:4) to afford ethyl 2-[3-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy]acetate (550 mg, 78.0%) as an off-white solid. LCMS (ESI) m/z: [M+H]+ = 337.

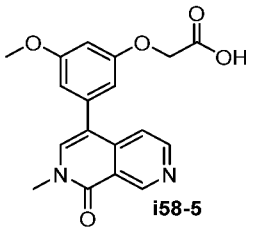
Step 3: Preparation of ethyl 2-(3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy) acetate (i58-4)



i58-4

To a solution of ethyl 2-[3-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy] acetate (550 mg, 1.636 mmol, 1.40 equiv) and 4-bromo-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (280 mg, 1.171 mmol, 1 equiv) in dioxane (16 mL) and H_2O (4 mL) was added Cs_2CO_3 (763.20 mg, 2.342 mmol, 2 equiv) and $Pd(dppf)Cl_2$ (85.70 mg, 0.117 mmol, 0.1 equiv), and the resulting solution was stirred at 80 ℃ for 1 hour (under N_2 atmosphere). After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with EA/PE (60:40) to afford ethyl 2-[3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy]acetate (303 mg, 70.23%) as a brown solid. LCMS (ESI) m/z: [M+H]+ = 369.

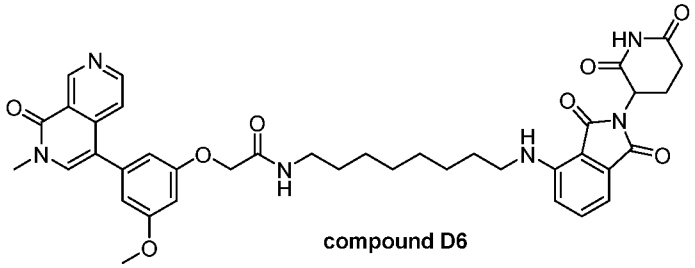
Step 4: Preparation of 2-(3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy)acetic acid (i58-5)



i58-5

A solution of ethyl 2-[3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy]acetate (50 mg) in HCl (12 M, 2 mL) was stirred at 90 ℃ for 40 minutes. The resulting mixture was cooled and was concentrated under reduced pressure to give 2-[3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy]acetic acid (86.8 mg) as an off-white solid, which was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 341.

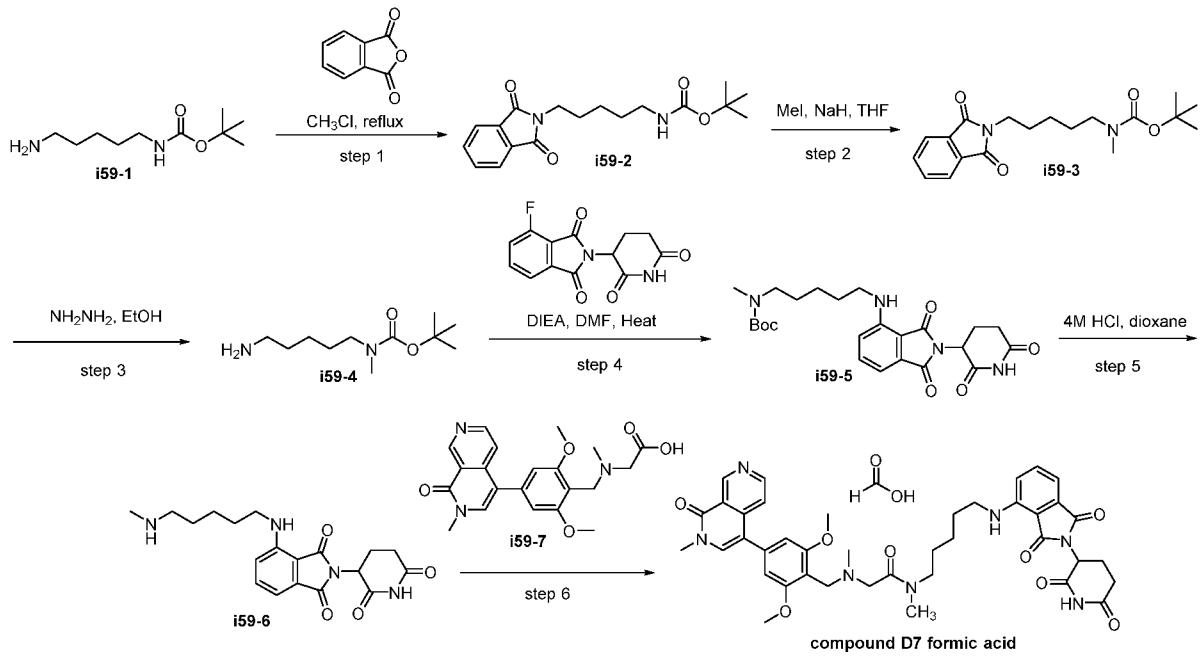
Step 5: Preparation of N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)-2-(3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy)acetamide (compound D6)



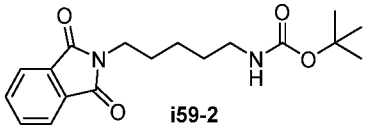
compound D6

To a solution of 2-[3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy]acetic acid (42.49 mg, 0.125 mmol, 1.00 equiv) and 4-[(8-aminooctyl)amino]-2-(2,6-dioxopiperidin-3-yl)-2,3-dihydro-1*H*-isoindole-1,3-dione (50 mg, 0.125 mmol, 1 equiv) in DMF (2 mL) was added HATU (71.21 mg, 0.187 mmol, 1.50 equiv) and DIEA (96.82 mg, 0.749 mmol, 6.00 equiv). The resulting solution was stirred at 25 ℃ for 1 hour. The crude product was purified by Prep-HPLC (conditios: XBridge Prep C18 OBD Column, 5 μm,19*150mm; mobile phase, Water (0.1% FA) and ACN; Detector, uv 254nm) to give *N*-(8-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino]octyl)-2-[3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy]acetamide (36 mg, 39.89%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 11.10 (s, 1H), 9.46 (s, 1H), 8.72 (d, *J* = 5.8 Hz, 1H), 8.08 (t, *J* = 5.8 Hz, 1H), 7.88 (d, *J* = 2.3 Hz, 1H), 7.57 (dd, *J* = 8.6, 7.0 Hz, 2H), 7.08 (d, *J* = 8.6 Hz, 1H), 7.01 (d, *J* = 7.0 Hz, 1H), 6.64 (q, *J* = 1.8 Hz, 3H), 6.51 (s, 1H), 5.05 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.52 (s, 2H), 3.80 (s, 3H), 3.59 (s, 3H), 3.27 (d, *J* = 5.8 Hz, 2H), 3.12 (q, *J* = 6.6 Hz, 2H), 2.98 – 2.80 (m, 1H), 2.65 – 2.52 (m, 2H), 2.11 – 1.98 (m, 1H), 1.55 (t, *J* = 7.0 Hz, 2H), 1.40 (d, *J* = 6.5 Hz, 2H), 1.24 (t, *J* = 7.4 Hz, 8H).; LCMS (ESI) m/z: [M+H]+ = 723.15.

Example 59 – Preparation of 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-N-(5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]amino]pentyl)-N-methylacetamide formic acid (compound D7 formic acid)



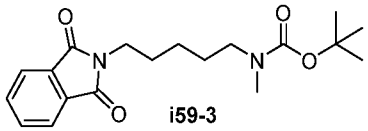
Step 1: Preparation of tert-butyl N-[5-(1,3-dioxoisoindol-2-yl)pentyl]carbamate (i59-2)



i59-2

A mixture of *tert*-butyl N-(5-aminopentyl)carbamate(4.00 g, 19.773 mmol, 1.00 equiv) and phthalic anhydride (3.22 g, 21.750 mmol, 1.1 equiv) in toluene(50.00 mL, 469.945 mmol, 23.77 equiv) was stirred at reflux for 3 hours. The solvent was removed under reduced pressure, and the crude product was purified by flash silica chromatography, elution gradient 0 to 40% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford *tert*-butyl *N*-[5-(1,3-dioxoisoindol-2-yl)pentyl]carbamate (5.8 g, 88.25%) as a white solid. LCMS (ESI) m/z: $[M+H]^+$ = 333.

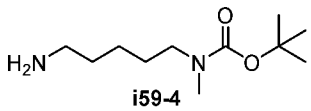
Step 2: Preparation of tert-butyl N-[5-(1,3-dioxoisoindol-2-yl)pentyl]-N-methylcarbamate (i59-3)



i59-3

To a solution of *tert*-butyl *N*-[5-(1,3-dioxoisoindol-2-yl)pentyl]carbamate (2.00 g, 6.017 mmol, 1.00 equiv) in DMF (30.00 mL) was added NaH (0.48 g, 12.034 mmol, 2.00 equiv, 60%) at 0 ℃, the resulting mixture was stirred at 0 ℃ for 30 minutes, and methyl iodide(1.28 g, 9 mmol, 1.50 equiv) was added to the reaction mixture at 0 ℃. After stirring at room temperature for 16 hours, and the reaction was quenched by the addition of water (50 mL) at 0 ℃. The mixture was extracted with EtOAc (100 mL x 4). The organic layer was washed with water (100 mL) and saturated brine (100 mL), and then dried over anhydrous sodium sulfate, filtered, and concentrated to give crude product that was purified by flash silica chromatography, elution gradient 0 to 60% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford *tert*-butyl N-[5-(1,3-dioxoisoindol-2-yl)pentyl]-N-methylcarbamate(1.8 g, 86.36%) as a colorless oil. LCMS (ESI) m/z: $[M+H]^+$ = 347.

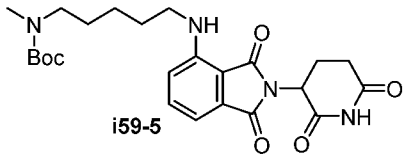
Step 3: Preparation of tert-butyl N-(5-aminopentyl)-N-methylcarbamate (i59-4)



i59-4

A mixture of *tert*-butyl *N*-[5-(1,3-dioxoisoindol-2-yl)pentyl]-N-methylcarbamate(1.00 g, 2.887 mmol, 1.00 equiv) and $NH_2NH_2.H_2O$(0.36 g, 0.006 mmol, 2.00 equiv, 80%) in EtOH (10.00 mL) was stirred at reflux for 1 hour. The solid was filtered out, and the filtrate was concentrated under reduced pressure to afford *tert*-butyl N-(5-aminopentyl)-*N*-methylcarbamate(372 mg, 59.57%) as a yellow oil that was used directly without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 217.

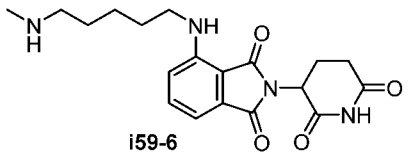
Step 4: Preparation of N-(5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]amino]pentyl)-N-methylcarbamate (i59-5)



i59-5

To a solution of *tert*-butyl *N*-(5-aminopentyl)-*N*-methylcarbamate(215.37 mg, 0.996 mmol, 1.10 equiv) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindole-1,3-dione(250.00 mg, 0.905 mmol, 1.00 equiv) in NMP (3.00 mL) was added DIEA(233.95 mg, 1.810 mmol, 2.00 equiv). The resulting mixture was stirred at 90 ℃ for 4 hours. The reaction mixture was diluted with EA (50 mL). The resulting mixture was washed with water (3 x 30 mL) and saturated brine (30 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 75% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford *tert*-butyl *N*-(5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]amino]pentyl)-*N*-methylcarbamate(198mg,46.30%) as a yellow oil. LCMS (ESI) m/z: $[M+H]^+$ = 473.

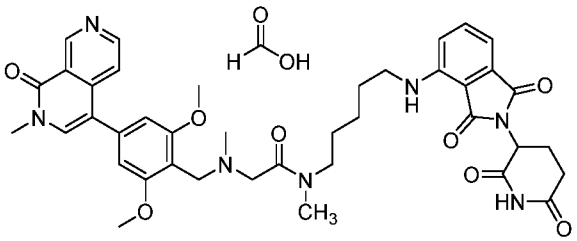
Step 5: Preparation of 2-(2,6-dioxopiperidin-3-yl)-4-[[5-(methylamino)pentyl]amino]isoindole-1,3-dione (i59-6)



i59-6

A solution of *tert*-butyl *N*-(5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]amino]pentyl)-*N*-methylcarbamate (198.00 mg) in a solution of HCl in 1,4-dioxane (5.00 mL, 4 M) was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure to afford 2-(2,6-dioxopiperidin-3-yl)-4-[[5-(methylamino)pentyl]amino]isoindole-1,3-dione (153 mg, 97.8%) as a yellow solid that was used directly without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 373.

Step 6: Preparation of 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-N-(5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]amino]pentyl)-N-methylacetamide formic acid (compound D7 formic acid)

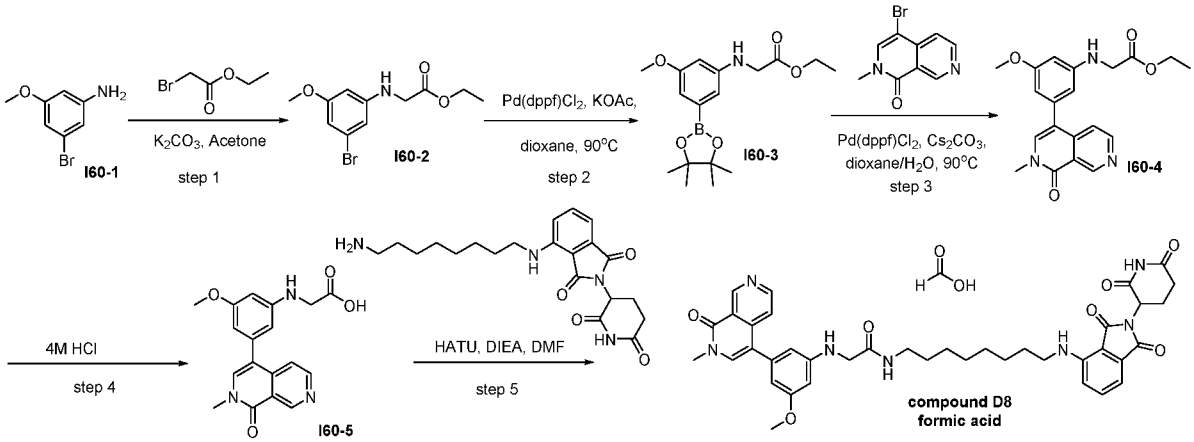


compound D7 formic acid

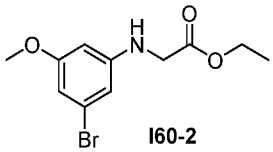
To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-[[5-(methylamino)pentyl]amino]-2,3-dihydro-1*H*-isoindole-1,3-dione (60.00 mg, 0.161 mmol, 1.00 equiv), 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)acetic acid (64.03 mg, 0.161 mmol, 1.00 equiv), and DIEA (41.64 mg, 0.322 mmol, 2.00 equiv) in DMF (1.00 mL) was added HATU (91.89 mg, 0.242 mmol, 1.50 equiv). The resulting mixture was stirred at room temperature for 16 hours. The crude product (mg) was purified by Prep-HPLC (conditions: XBridge Shield RP18 OBD Column, 5 μm, 19*150mm; Mobile Phase A: Water (0.1% FA), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient:

10% B to 40% B in 8 minutes; 254/220 nm; R_t: 7.32 minutes) to afford 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-*N*-(5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino]pentyl)-*N*-methylacetamide formic acid (99.4 mg) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.51 (s, 0.5H), 9.41 (s, 0.5H), 8.67 (dd, *J* = 14.1, 5.7 Hz, 1H), 8.55 (s, 0.28H, FA), 7.74 (d, *J* = 15.6 Hz, 1H), 7.63 (dd, *J* = 18.0, 5.8, 1H), 7.51 (dd, *J* = 8.6, 7.1 Hz, 0.5H), 7.30 (dd, *J* = 8.5, 7.1 Hz, 0.5H), 7.00 (dd, *J* = 7.9, 3.5 Hz, 1H), 6.92 (d, *J* = 7.0 Hz, 1H), 6.78 (d, *J* = 17.9 Hz, 2H), 5.05 (dd, *J* = 12.8, 5.6, 1H), 4.23 (s, 1H), 3.91 (d, *J* = 5.4 Hz, 8H), 3.86 (s, 1.5H), 3.70 (s, 1.5H), 3.61 (s, 2H), 3.49 – 3.41 (m, 1H), 3.30 (s, 1H), 3.13 (d, *J* = 6.8 Hz, 1H), 3.02 (s, 1.5H), 2.93 (s, 1.5H), 2.89 – 2.80 (m, 1H), 2.80 – 2.71 (m, 4H), 2.54 (s, 2H), 2.12 (td, *J* = 8.0, 2.7 Hz, 1H), 1.76 – 1.53 (m, 4H), 1.45 (q, *J* = 8.1 Hz, 1H), 1.22 (d, *J* = 7.9 Hz, 1H). LCMS (ESI) m/z: $[M+H]^+$ = 752.20.

Example 60 – Preparation of 2-cyclopropyl-4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2,7-naphthyridin-1-one (compound D8)

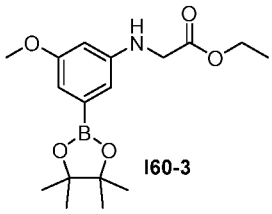


Step 1: Preparation of ethyl 2-[(3-bromo-5-methoxyphenyl)amino]acetate (i60-2)



To a solution of 3-bromo-5-methoxyaniline (5.00 g, 24.746 mmol, 1.00 equiv) and K_2CO_3 (5.13 g, 37.119 mmol, 1.50 equiv) in acetone (100.00 mL) was added ethyl bromoacetate (4.96 g, 29.695 mmol, 1.20 equiv). The resulting mixture was stirred at reflux for 3 days. The reaction mixture was filtered, and the filtrate was evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford ethyl 2-[(3-bromo-5-methoxyphenyl)amino]acetate (1.8 g, 25.24%) as a yellow gum. LCMS (ESI) m/z: [M+H]+ = 288.

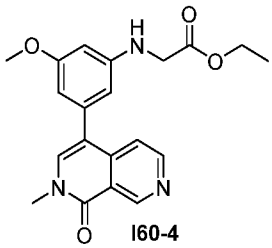
Step 2: Preparation of 2-[[3-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]amino]acetate (i60-3)



I60-3

To a solution of bis(pinacolato)diboron(528.78 mg, 2.082 mmol, 1.2 equiv), ethyl 2-[(3-bromo-5-methoxyphenyl)amino]acetate(500.00 mg, 1.735 mmol, 1.00 equiv) and KOAc(340.61 mg, 3.471 mmol, 2 equiv) in dioxane (10.00 mL) was added Pd(dppf)Cl_2(126.97 mg, 0.174 mmol, 0.1 equiv). The resulting mixture was stirred at 90 ℃ for 2 hours under a nitrogen atmosphere. The resulting mixture was diluted with ethyl acetate (100 mL), washed with water (3 x 100 mL) and saturated brine (100 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford ethyl 2-[[3-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]amino]acetate (365 mg, 62.75%) as a yellow gum. LCMS (ESI) m/z: $[M+H]^+$ = 336.

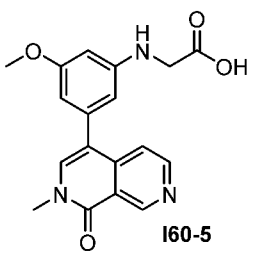
Step 3: Preparation of 2-[[3-methoxy-5-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]amino]acetate (i60-4



I60-4

To a solution of ethyl 2-[[3-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]amino]acetate (100.00 mg, 0.298 mmol, 1.00 equiv), 4-bromo-2-methyl-2,7-naphthyridin-1-one (71.32 mg, 0.298 mmol, 1.00 equiv) and Cs_2CO_3 (194.40 mg, 0.597 mmol, 2.00 equiv) in dioxane (4.00 mL) and H_2O (1.00 mL) was added Pd(dppf)Cl_2 (21.83 mg, 0.030 mmol, 0.10 equiv). The resulting mixture was stirred at 80 ℃ for 2 hours under a nitrogen atmosphere. The reaction mixture was diluted with EA (100 mL) and washed with water (3 x 100 mL) and saturated brine (100 mL) The organic layer was dried over Na_2SO_4, filtered, and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 80% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford ethyl 2-[[3-methoxy-5-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]amino]acetate (70 mg, 63.87%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 368.

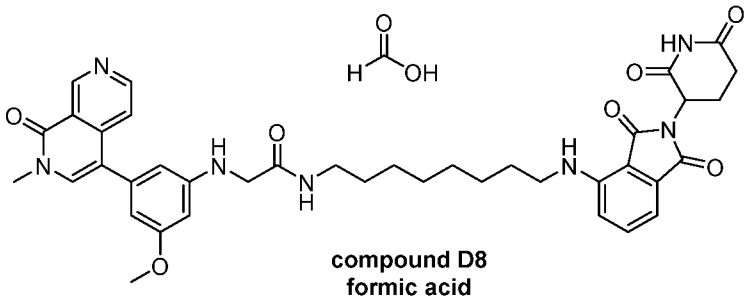
Step 4: Preparation of [[3-methoxy-5-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]amino]acetic acid (i60-5)



I60-5

Ethyl 2-[[3-methoxy-5-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]amino]acetate (70.00 mg, 0.191 mmol, 1.00 equiv) was added to a solution of HCl in water (2.00 mL, 12 N). The resulting mixture was stirred at 90 ℃ for 1 hour. The solvent was removed to afford [[3-methoxy-5-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]amino]acetic acid (65 mg) as a brown solid that was used directly without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 340.

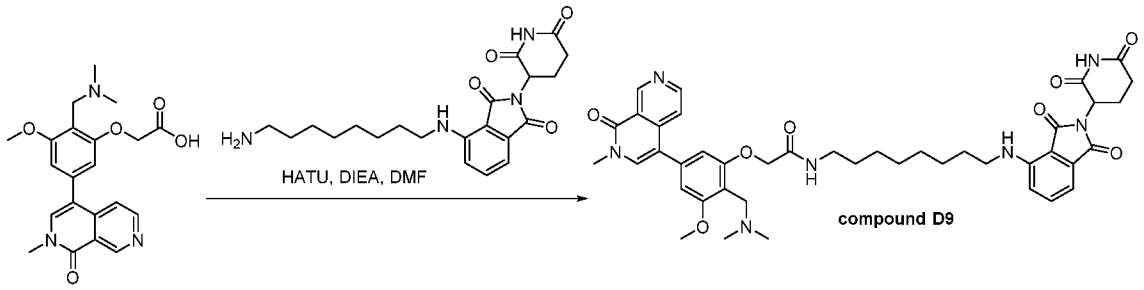
Step 5: Preparation of N-(8-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]amino]octyl)-2-[[3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]amino]acetamide formic acid (compound D8 formic acid)



compound D8 formic acid

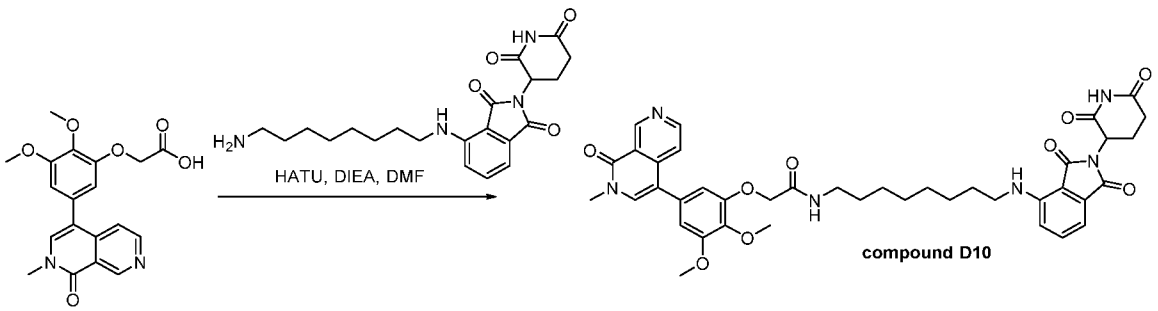
To a solution of 2-[[3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]amino]acetic acid (65.00 mg, 0.192 mmol, 1.00 equiv), 4-[(8-aminooctyl)amino]-2-(2,6-dioxopiperidin-3-yl)-2,3-dihydro-1*H*-isoindole-1,3-dione (76.71 mg, 0.192 mmol, 1.00 equiv) and DIEA (49.51 mg, 0.383 mmol, 2.00 equiv) in DMF (1.00 mL, 12.922 mmol, 67.46 equiv) was added HATU (109.25 mg, 0.287 mmol, 1.50 equiv). The resulting mixture was stirred at room temperature for 16 hours. The crude product was purified by Prep-HPLC (conditions: XBridge Shield RP18 OBD Column 30*150mm, 5 μm; Mobile Phase A: Water (0.1% FA), Mobile Phase B: ACN; Flow rate: 40 mL/minute; Gradient: 18% B to 18% B in 2 minutes; 254/220 nm; R_t: 11.43 minutes) to afford *N*-(8-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]amino]octyl)-2-[[3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]amino]acetamide formic acid (36.8 mg) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.49 (d, *J* = 0.8 Hz, 1H), 8.64 (d, *J* = 5.9 Hz, 1H), 7.94 (t, *J* = 6.0 Hz, 1H), 7.73 – 7.67 (m, 2H), 7.51 (dd, *J* = 8.6, 7.1 Hz, 1H), 6.99 (s, 1H), 6.99 (dd, *J* = 16.0, 7.8 Hz, 2H), 6.36 (t, *J* = 1.8 Hz, 1H), 6.26 (d, *J* = 1.8 Hz, 2H), 5.06 (dd, *J* = 12.5, 5.4 Hz, 1H), 3.79 (d, *J* = 7.7 Hz, 5H), 3.67 (s, 3H), 3.24 (dt, *J* = 8.4, 6.5 Hz, 4H), 2.87 (ddd, *J* = 17.7, 14.1, 5.0 Hz, 1H), 2.81 – 2.64 (m, 2H), 2.17 – 2.07 (m, 1H), 1.60 (p, *J* = 7.0 Hz, 2H), 1.47 (d, *J* = 13.8 Hz, 2H), 1.34 (d, *J* = 7.2 Hz, 2H), 1.25 (s, 6H). LCMS (ESI) m/z: $[M+H]^+$ = 722.30.

Example 61 – Preparation of 2-(2-((dimethylamino)methyl)-3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy)-N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)acetamide (compound D9)



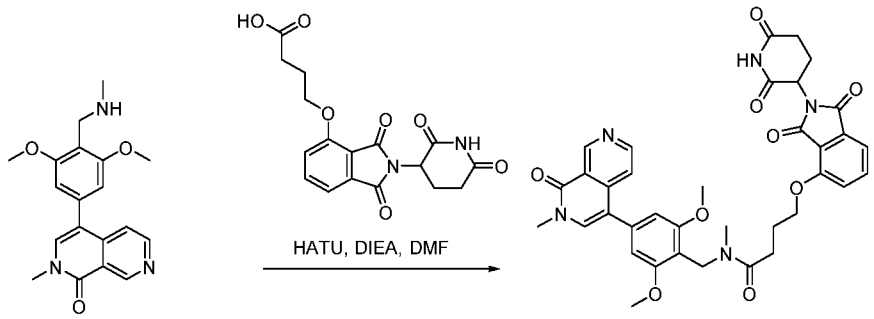
Compound D9 was prepared in a similar manner as described for compound D8. 2-(2-((dimethylamino)methyl)-3-methoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy)-N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)acetamide (17.8 mg, 11.3%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.52 (s, 1H), 8.67 (d, *J* = 5.8 Hz, 1H), 8.56 (s, 0.83H, FA), 7.75 (s, 1H), 7.60 – 7.50 (m, 2H), 7.02 (dd, *J* = 9.5, 7.8 Hz, 2H), 6.90 (d, *J* = 1.3 Hz, 1H), 6.78 (d, *J* = 1.3 Hz, 1H), 5.06 (dd, *J* = 12.5, 5.4 Hz, 1H), 4.81 (s, 2H), 4.29 (s, 2H), 3.96 (s, 3H), 3.68 (s, 3H), 3.27 (dt, *J* = 14.0, 6.9 Hz, 4H), 2.87 - 2.65 (m, 9H), 2.12 (dtd, *J* = 13.0, 5.0, 2.3 Hz, 1H), 1.61 (p, *J* = 6.9 Hz, 2H), 1.48 (t, *J* = 6.9 Hz, 2H), 1.37 (t, *J* = 7.6 Hz, 2H), 1.27 (d, *J* = 3.8 Hz, 6H). LCMS (ESI) m/z: [M+H]+ = 780.40.

Example 62 – Preparation of 2-(2,3-dimethoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy)-N-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)acetamide (compound D10)



Compound D10 was prepared in a similar manner as described for compound D8. 2-(2,3-dimethoxy-5-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenoxy)-*N*-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)acetamide (32.7mg,32.17%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.51 (s, 1H), 8.67 (d, *J* = 5.9 Hz, 1H), 7.73 (s, 1H), 7.63 (d, *J* = 5.9 Hz, 1H), 7.53 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.00 (dd, *J* = 12.0, 7.8 Hz, 2H), 6.85 (d, *J* = 1.9 Hz, 1H), 6.77 (d, *J* = 1.9 Hz, 1H), 5.06 (dd, *J* = 12.5, 5.4 Hz, 1H), 4.62 (s, 2H), 3.92 (d, *J* = 2.9 Hz, 6H), 3.68 (s, 3H), 3.28 (q, *J* = 6.9 Hz, 4H), 2.92 - 2.65(m, 3H), 2.16 – 2.03 (m, 1H), 1.62 (p, *J* = 6.9 Hz, 2H), 1.53 (t, *J* = 7.0 Hz, 2H), 1.38 (d, *J* = 8.0 Hz, 2H), 1.31 (s, 8H). LCMS (ESI) m/z: [M+H]+ = 753.35.

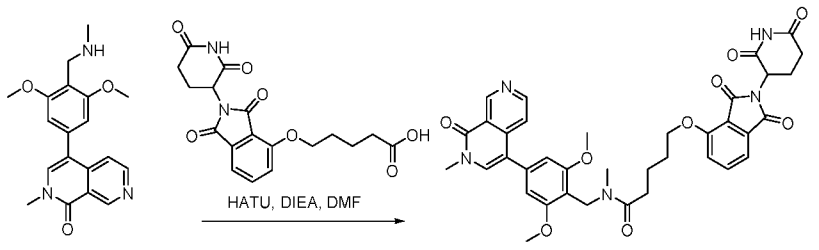
Example 63 – Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-4-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]-N-methylbutanamide (compound D11)



compound D11

To a solution of 4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (50 mg, 0.147 mmol, 1 equiv) and 4-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy]butanoic acid (53.08 mg, 0.147 mmol, 1 equiv) in DMF (1 mL) was added DIEA (38.08 mg, 0.295 mmol, 2 equiv). The resulting mixture was stirred for 10 minutes at 25 ℃. Then HATU (84.02 mg, 0.221 mmol, 1.5 equiv) was added to the reaction mixture. The resulting solution was stirred for 2 hours at 25 ℃. The crude product was purified by Prep-HPLC (conditions: SunFire C18 OBD Prep Column, 19 mm X 250 mm; mobile phase, Water (0.1% FA) and ACN (24% PhaseB up to 48% in 8 minutes); Detector, uv). This resulted in 27 mg (26.88%) of *N*-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-4-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]-*N*-methylbutanamide as a white solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.54 (s, 1H), 8.69 (d, *J* = 6.0 Hz, 1H), 7.84 – 7.74 (m, 2H), 7.67 (s, 1H), 7.54 – 7.41 (m, 2H), 6.77 (s, 1H), 6.72 (s, 1H), 5.13 – 5.02 (m, 1H), 4.76 (dd, *J* = 10.7, 2.5 Hz, 2H), 4.33 (dt, *J* = 19.9, 5.9 Hz, 2H), 3.85 (d, *J* = 17.8 Hz, 6H), 3.71 (d, *J* = 12.1 Hz, 4H), 3.06 – 2.97 (m, 1H), 2.89 (s, 2H), 2.80 (s, 3H), 2.74 – 2.59 (m, 3H), 2.27 – 2.13 (m, 3H). LCMS (ESI) m/z: [M+H]+ = 682.25.

Example 64 – Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]-N-methylpentanamide (compound D12)

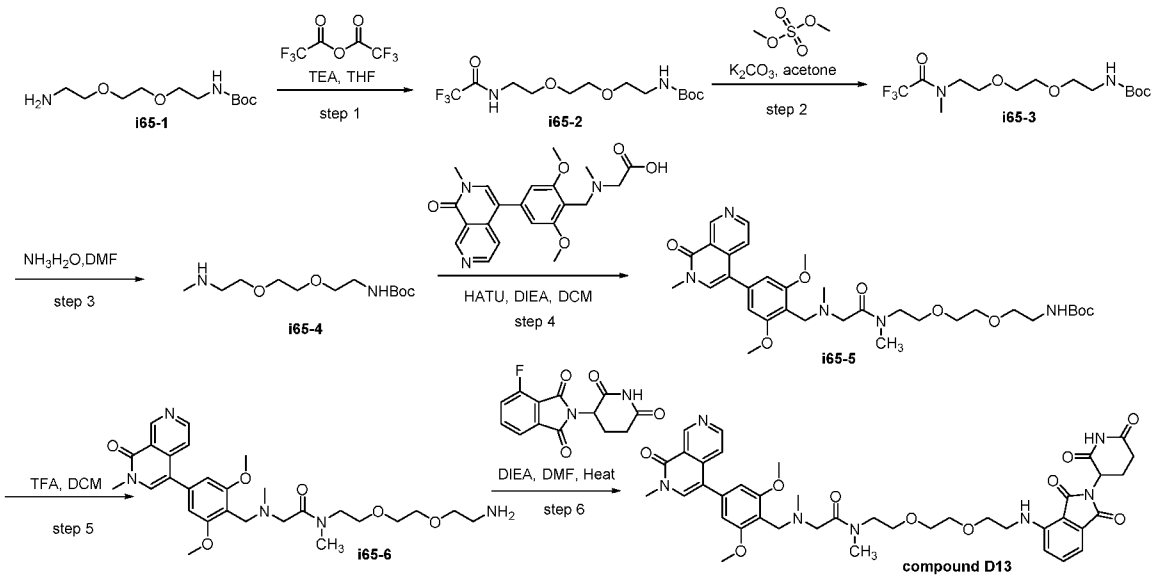


compound D12

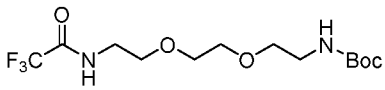
To a stirred solution of 4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (50 mg, 0.147 mmol, 1 equiv) and 5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]pentanoic acid (55.15 mg, 0.147 mmol, 1 equiv) in DMF (2 mL) was added DIEA (57.12 mg, 0.442 mmol, 3 equiv) at 25 ℃. The resulting mixture was stirred for 10 minutes at 25 ℃. Then HATU (84.02 mg, 0.221 mmol, 1.5 equiv) was added to the reaction mixture. The resulting solution was stirred for 2 hours at 25 ℃. The crude product was purified by Prep-HPLC (conditions: SunFire C18 OBD Prep Column, 19 mm X 250 mm; mobile phase, Water (0.1% FA) and ACN (24% PhaseB up to 53% in 8 minutes); Detector, uv). This resulted in 48.1 mg (46.9%) of *N*-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-5-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-

2,3-dihydro-1*H*-isoindol-4-yl]oxy]-*N*-methylpentanamide as a white solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.55 (s, 1H), 8.71 – 8.64 (m, 1H), 7.85 –7.71 (m, 3H), 7.45 (dd, *J* = 9.6, 7.9 Hz, 2H), 6.76 (d, *J* = 9.2 Hz, 2H), 5.05 (dd, *J* = 12.6, 5.7 Hz, 1H), 4.74 (d, *J* = 16.1 Hz, 2H), 4.30 (q, *J* = 6.8, 6.2 Hz, 2H), 3.86 (s, 6H), 3.72 (dd, *J* = 2.7, 1.1 Hz, 3H), 2.85 (s, 2H), 2.84 (d, *J* = 40.1 Hz, 4H), 2.76 – 2.52 (m, 2H), 2.08 – 1.87 (m, 5H). LCMS (ESI) m/z: [M+H]+ = 696.4.

Example 65 – Preparation of 2-((2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)(methyl)amino)-N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-N-methylacetamide (compound D13)



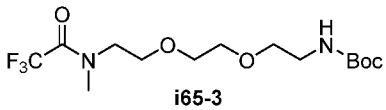
Step 1: Preparation of tert-butyl (2-(2-(2-(2,2,2-trifluoroacetamido)ethoxy)ethoxy)ethyl)carbamate (i65-2)



i65-2

To a solution of *tert*-butyl *N*-[2-[2-(2-aminoethoxy) ethoxy] ethyl] carbamate (1.15 g, 4.631 mmol, 1.00 equiv) and TEA (0.94 g, 9.262 mmol, 2.00 equiv) in THF (12.00 mL) at 0 degree was added trifluoroacetic anhydride (1.46 g, 6.947 mmol, 1.50 equiv). The resulting solution was stirred at 25 degree for 12 hours. The resulting mixture was concentrated. The residue was applied onto a silica gel column eluted with THF/PE (40/60). Fractions containing the desired compound were evaporated to dryness to afford *tert*-butyl *N*-(2-[2-[2-(2,2,2-trifluoroacetamido)ethoxy]ethoxy]ethyl) carbamate (1.347 g, 80.0%) as a colorless oil. LCMS (ESI) m/z: [M+H]+ = 345.

Step 2: Preparation of tert-butyl (2-(2-(2-(2,2,2-trifluoro-N-methylacetamido)ethoxy)ethoxy)ethyl) carbamate (i65-3)

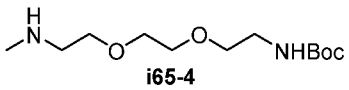


i65-3

A solution of *tert*-butyl N-(2-[2-[2-(2,2,2-trifluoroacetamido)ethoxy]ethoxy]ethyl) carbamate (1.347 g, 3.912 mmol, 1.00 equiv) and K_2CO_3 (0.65 g, 4.694 mmol, 1.20 equiv) in acetone (15.00 mL) was stirred at 0 degree. Then dimethyl sulfate (0.74 g, 5.867 mmol, 1.51 equiv) was added to the

mixture, and the resulting solution was stirred at 25 degree for 12 hours. The resulting solution was diluted with of EtOAc, and it was washed with water (3 x 50 mL). The organic layer was dried and evaporated to dryness to afford *tert*-butyl *N*-(2-[2-[2-(2,2,2-trifluoro-*N*-methylacetamido)ethoxy]ethoxy] ethyl)carbamate (1.75 g, 98.91%) as a colorless oil, which was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 359.

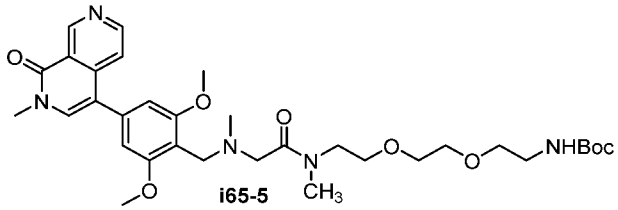
Step 3: Preparation of tert-butyl (2-(2-(2-(methylamino)ethoxy)ethoxy)ethyl)carbamate (i65-4)



i65-4

A solution of *tert*-butyl *N*-(2-[2-[2-(2,2,2-trifluoro-*N*-methylacetamido)ethoxy]ethoxy]ethyl) carbamate (1.65 g) in DMF (16.00 mL) was stirred at 0 degree. Then ammonium hydroxide (16.00 mL) was added to the mixture, and the resulting solution was stirred at 25 degrees for 12 hours. The mixture was evaporated to dryness to afford crude *tert*-butyl N-(2-[2-[2-(methylamino) ethoxy]ethoxy] ethyl)carbamate as a colorless oil, which was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 263.

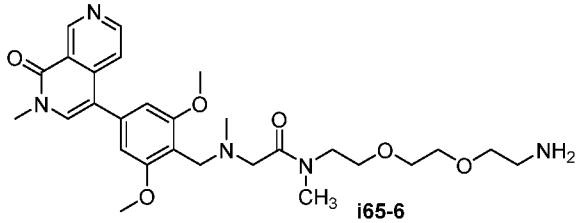
Step 4: Preparation of tert-butyl (1-(2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl)-2,5-dimethyl-4-oxo-8,11-dioxa-2,5-diazatridecan-13-yl)carbamate (i65-5)



i65-5

To a solution of *tert*-butyl *N*-(2-[2-[2-(methylamino)ethoxy]ethoxy]ethyl)carbamate (600.00 mg, 2.287 mmol, 1.00 equiv) and ([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl) amino)acetic acid (1.09 g, 2.744 mmol, 1.2 equiv) in DCM (5.00 mL) was added HATU (1.30 g, 3.431 mmol, 1.5 equiv) and DIEA (886.75 mg, 6.861 mmol, 3 equiv). The resulting solution was stirred at 25 degree for 1 hour. The mixture was added H_2O (100 mL) and extracted with DCM (100 mL x 4). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to give crude product that was purified by a silica gel column eluted with MeOH/DCM (5.4/94.6) to afford *tert*-butyl *N*-[2-(2-[2-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-*N*-methylacetamido] ethoxy]ethoxy) ethyl]carbamate (536 mg, 36.52%) as an off-white solid. LCMS (ESI) m/z: [M+H]+ = 642.

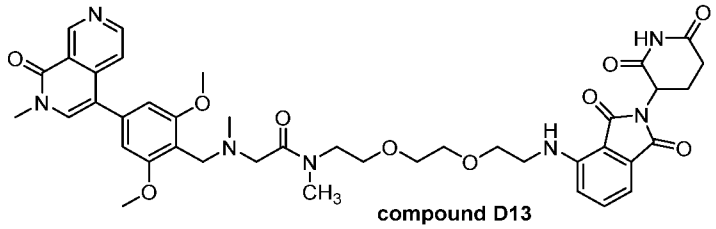
Step 5: Preparation of N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-2-((2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)(methyl)amino)-N-methylacetamide (i65-5)



i65-6

A solution of *tert*-butyl *N*-[2-(2-[2-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl] methyl](methyl)amino)-*N*-methylacetamido]ethoxy]ethoxy)ethyl]carbamate (536.00 mg, 0.835 mmol, 1.00 equiv) and TFA (1.10 mL, 9.673 mmol, 17.78 equiv) in DCM (5.00 mL) was stirred at 25 degree for 1 hour. The resulting mixture were evaporated to dryness to afford *N*-[2-[2-(2-aminoethoxy)ethoxy]ethyl]-2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-*N*-methylacetamide (670 mg, crude) as a yellow oil, which was used directly without further purification. LCMS (ESI) m/z: [M+H]+ = 542.

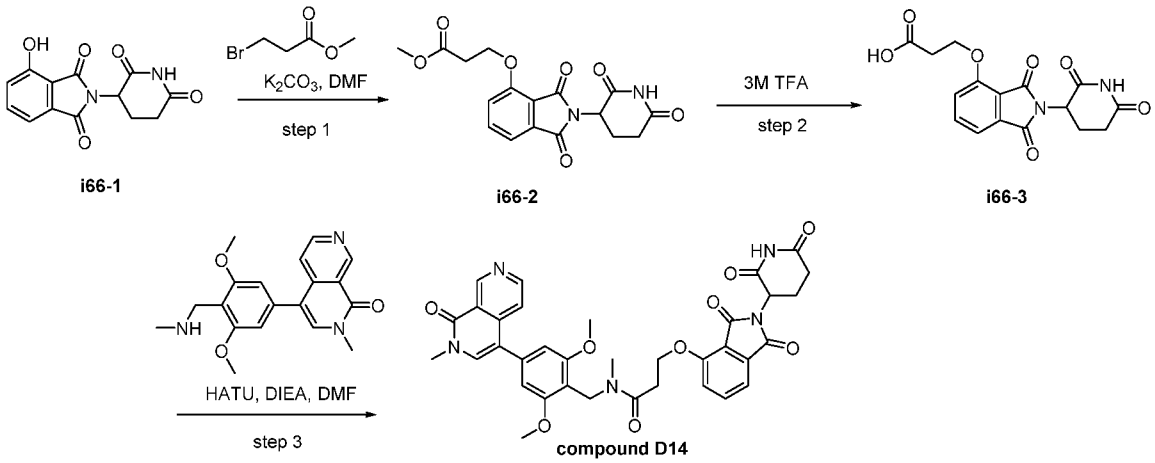
Step 6: Preparation of 2-((2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)(methyl)amino)-N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-N-methylacetamide (compound D13)



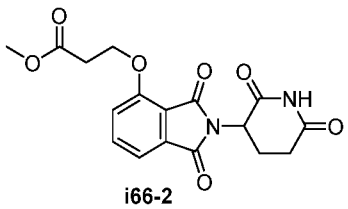
compound D13

To a solution of *N*-[2-[2-(2-aminoethoxy)ethoxy]ethyl]-2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-*N*-methylacetamide (169.42 mg, 0.313 mmol, 1.20 equiv) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoro-2,3-dihydro-1*H*-isoindole-1,3-dione (72.00 mg, 0.261 mmol, 1.00 equiv) in NMP (2.00 mL) was added DIEA (168.44 mg, 1.303 mmol, 5.00 equiv). The resulting solution was stirred at 90 degree for 5 hours. Without any additional work-up, the mixture was purified by prep-HPLC (conditions: SunFire C18 OBD Prep Column , 100Å, 5 μm, 19 mm X 250 mm; Mobile Phase A: Water(0.1% FA), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 16% B to 22% B in 10 minutes; 254 nm; R_t: 9.3 minutes) to give 2-((2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)(methyl)amino)-*N*-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy) ethoxy)ethyl)-*N*-methylacetamide (7 mg, 3.37%) as a yellow solid. ^{1}H NMR (400 MHz, Acetonitrile-*d3*) δ 9.52 (d, *J* = 3.2 Hz, 1H), 9.09 (s, 1H), 8.70 (d, *J* = 5.7 Hz, 1H), 7.67 – 7.39 (m, 3H), 7.02 (dt, *J* = 8.5, 5.1 Hz, 2H), 6.73 (d, *J* = 1.7 Hz, 2H), 6.52 – 6.38 (m, 1H), 4.94 (dd, *J* = 12.5, 5.4 Hz, 1H), 3.97 (d, *J* = 9.0 Hz, 2H), 3.86 (s, 6H), 3.67 (q, *J* = 3.8, 2.3 Hz, 2H), 3.65 – 3.60 (m, 4H), 3.60 – 3.52 (m, 6H), 3.52 – 3.46 (m, 2H), 3.46 – 3.37 (m, 2H), 2.99 (s, 1H), 2.91 (s, 2H), 2.84 – 2.57 (m, 3H), 2.48 (d, *J* = 3.4 Hz, 3H), 2.15 – 2.03 (m, 1H). LCMS (ESI) m/z: [M+H]+ = 798.40.

Example 66 – Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]-N-methylpropanamide (compound D14)



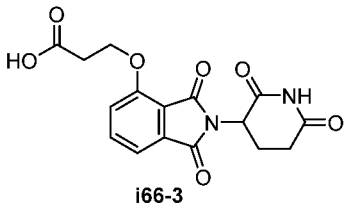
Step 1: Preparation of methyl 3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]oxy]propanoate (i66-2)



i66-2

To a stirred solution of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindole-1,3-dione (500.00 mg, 1.823 mmol, 1.00 equiv) and methyl 3-bromopropanoate (395.84 mg, 2.370 mmol, 1.30 equiv) in DMF was added K_2CO_3 (755.96 mg, 5.470 mmol, 3.00 equiv). The resulting solution was stirred for 2 hours at 25 ℃. The solids were filtered out. The filtrate was concentrated. The residue was applied onto a silica gel column with dichloromethane/methanol (10:1). This resulted in 400 mg (60.89%) of methyl 3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]oxy]propanoate as a green solid. LCMS (ESI) m/z: $[M-H]^+$ = 361.

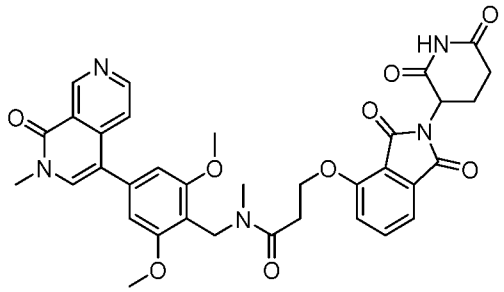
Step 2: Preparation of 3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]oxy]propanoic (i66-3)



i66-3

Into a 8-mL sealed tube was added methyl 3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]oxy]propanoate (100.00 mg, 0.278 mmol, 1.00 equiv) and TFA (3.00 mL, 3M in water). The resulting solution was stirred for 2 hours at 70 ℃. The resulting mixture was concentrated. This resulted in 70 mg (72.84%) of 3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]oxy]propanoic acid as a yellow solid, which was used directly without further purification. LCMS (ESI) m/z: $[M+H]^+$ = 347.

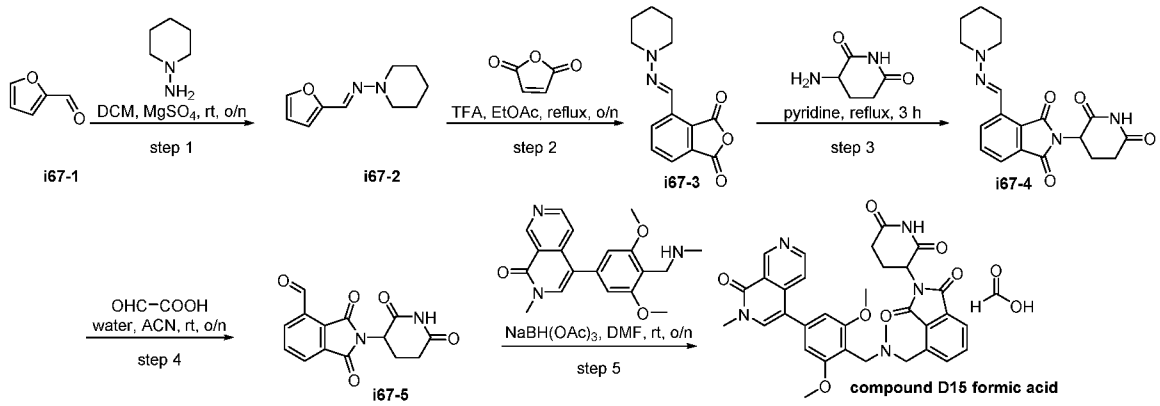
Step 3: Preparation of N-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]-N-methylpropanamide (compound D14)



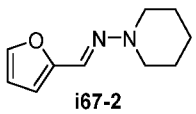
compound D14

To a solution of 3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy]propanoic acid (60.00 mg, 0.173 mmol, 1.00 equiv) and DIEA (44.79 mg, 0.347 mmol, 2.00 equiv) in DMF (2.00 mL) was added HATU (98.82 mg, 0.260 mmol, 1.50 equiv). The reaction mixture was stirred for 10 minutes at 25 ℃. Then 4-[3,5-dimethoxy-4-[(methylamino)methyl]phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (58.80 mg, 0.173 mmol, 1.00 equiv) was added to the reaction mixture. The resulting solution was stirred for 2 hours at 25 ℃. The crude product was purified by Prep-HPLC (conditions: SunFire C18 OBD Prep Column, 19 mm X 250 mm; mobile phase, Water (0.1% FA) and ACN (26% PhaseB up to 44% in 8 minutes); Detector, UV). This resulted in 28.1 mg (24.29%) of *N*-[[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl]-3-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy]-N-methylpropanamide as a green solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.53 (s, 1H), 8.67 (d, *J* = 5.9 Hz, 1H), 7.84 (s, 1H), 7.72 (d, *J* = 6.0 Hz, 1H), 7.62 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.33 (d, *J* = 7.1 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.77 (s, 2H), 5.17 (dd, *J* = 12.7, 5.5 Hz, 1H), 4.77 – 4.59 (m, 2H), 4.16 – 4.01 (m, 2H), 3.91 (s, 5H), 3.88 (s, 1H), 3.72 (s, 3H), 3.01 – 2.85 (m, 4H), 2.81 (s, 2H), 2.80 – 2.62 (m, 2H), 2.20 – 2.10 (m, 1H). LCMS (ESI) m/z: $[M+H]^+$ = 668.25.

Example 67 – Preparation of 4-(((2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl) benzyl)(methyl)amino)methyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione formic acid (compound D15 formic acid)



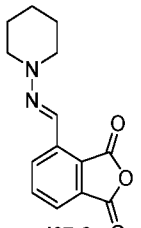
Step 1: Preparation of (E)-1-(furan-2-yl)-N-(piperidin-1-yl)methanimine (i67-2)



i67-2

To a mixture of furan-2-carbaldehyde (1.00 g, 10.407 mmol, 1.00 equiv) and piperidin-1-amine (1.04 g, 10.407 mmol, 1.00 equiv) in DCM (25.00 mL) was added $MgSO_4$ (2.51 g, 20.815 mmol, 2.00 equiv). The resulting mixture was stirred overnight at room temperature. The resulting mixture was concentrated under vacuum. The residue was purified by silica gel column chromatography, eluted with MeOH in DCM from 0% to 10% to afford the desired product (*E*)-1-(furan-2-yl)-N-(piperidin-1-yl)methanimine (1.70 g, 9.551 mmol, 82.48%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 179.

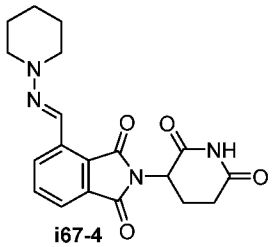
Step 2: Preparation of (E)-4-((piperidin-1-ylimino)methyl)isobenzofuran-1,3-dione (i67-3)



i67-3

To a solution of furan-2,5-dione (1.12 g, 11.446 mmol, 1.20 equiv) and (*E*)-1-(furan-2-yl)-N-(piperidin-1-yl)methanimine (1.70 g, 9.538 mmol, 1.00 equiv) in EtOAc (30 mL) was added TFA (0.20 mL). The resulting mixture was stirred overnight under reflux. The resulting mixture was concentrated under vacuum to afford the crude product (*E*)-4-((piperidin-1-ylimino)methyl)isobenzofuran-1,3-dione (3.10 g, crude) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 259.

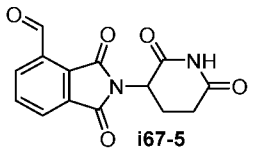
Step 3: Preparation of (E)-2-(2,6-dioxopiperidin-3-yl)-4-((piperidin-1-ylimino)methyl)isoindoline -1,3-dione (i67-4)



i67-4

To a mixture of 3-aminopiperidine-2,6-dione (1.54 g, 12.016 mmol, 1.00 equiv) in pyridine (15.00 mL) was added (*E*)-4-((piperidin-1-ylimino)methyl)isobenzofuran-1,3-dione (3.1 g, 12.016 mmol, 1.00 equiv). The resulting mixture was stirred for 3 hours under reflux. The resulting mixture was concentrated under vacuum. The residue was purified by silica gel column chromatography, eluted with EA in PE from 0% to 50% to afford (*E*)-2-(2,6-dioxopiperidin-3-yl)-4-((piperidin-1-ylimino)methyl)isoindoline-1,3-dione (1.00 g, 2.717 mmol, 22.62%) as a yellow solid. LCMS (ESI) m/z: $[M+H]^+$ = 369.

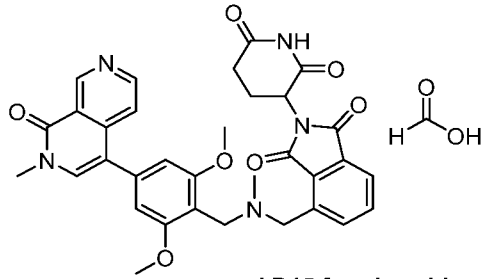
Step 3: Preparation of 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindoline-4-carbaldehyde (i67-5)



i67-5

2-oxoacetic acid (5.00 g, 0.068 mmol, 0.02 equiv) in H_2O (5.00 mL) was added to a solution of 2-(2,6-dioxopiperidin-3-yl)-4-[(1*E*)-[(piperidin-1-yl)imino]methyl]-2,3-dihydro-1H-isoindole-1,3-dione (1.00 g, 2.714 mmol, 1.00 equiv) in ACN (2.00 mL). The resulting mixture was stirred overnight at room temperature. The resulting mixture was extracted with EtOAc (3 x 100 mL). The combined organic layer was washed with saturated sodium bicarbonate solution (2 x 100 mL) and brine (1x100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography, eluted with EtOAc in PE from 0% to 50% to afford 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo -2,3-dihydro-1*H*-isoindole-4-carbaldehyde (460 mg, 1.608 mmol, 59.20%) as a brown solid. LCMS (ESI) m/z: $[M+H]^+$ = 287.

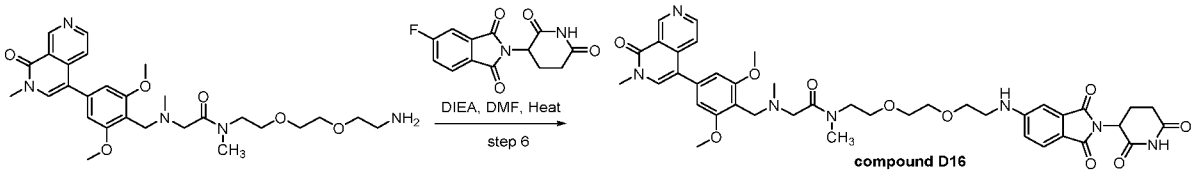
Step 4: Preparation of 4-(((2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin -4-yl)benzyl)(methyl)amino)methyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione formic acid (compound D4 formic acid)



compound D15 formic acid

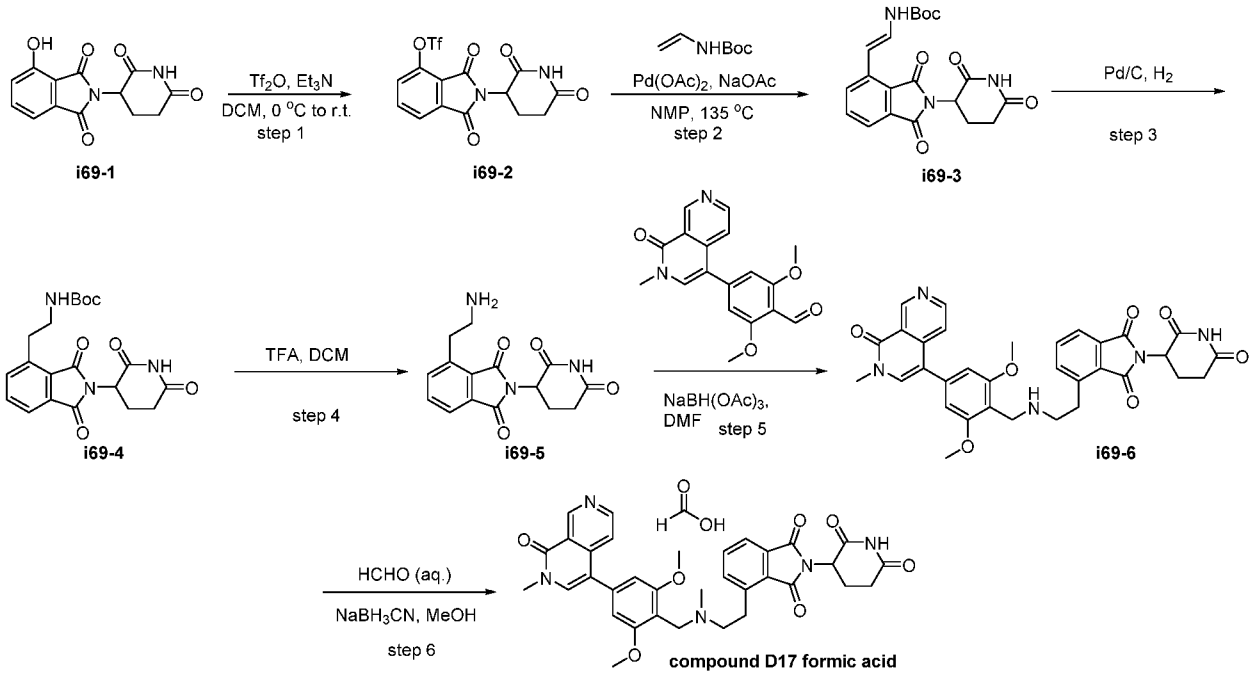
To a solution of 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-4-carbaldehyde (67.47 mg, 0.236 mmol, 1.00 equiv) in DMF (3.00 mL) was added 4-[3,5-dimethoxy-4-[(methylaminomethyl]phenyl]-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (80.00 mg, 0.236 mmol, 1.00 equiv). The mixture was stirrred overnight at room temperature, and then $NaBH_3CN$ (99.91 mg, 0.472 mmol, 2.00 equiv) was added. The resulting mixture was stirred for one hour at room temperature. The mixture was filtered, and the filtrate was purified by prep-HPLC (conditions: SunFire C_{18} OBD Prep Column , 100Å, 5 µm, 19 mm X 250 mm; Mobile Phase A: Water (0.1% FA), Mobile Phase B: ACN; Flow rate: 25 mL/minute; Gradient: 10% B to 16% B in 14 minutes; 254 nm; Rt: 12.7 minutes) to afford 4-(((2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)(methyl)amino)methyl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione;formate (30.9 mg, 0.0472 mmol, 19.67%) as a light yellow solid. ^{1}H NMR (300 MHz, Acetonitrile-*d3*) δ 9.53 (d, *J* = 0.9 Hz, 1H), 9.03 (s, 1H), 8.70 (d, *J* = 5.7 Hz, 1H), 8.18 (s, 0.3H, FA), 8.02 (dd, *J* = 6.9, 1.9 Hz, 1H), 7.91 – 7.78 (m, 2H), 7.57 – 7.49 (m, 2H), 6.67 (s, 2H), 5.05 (dd, *J* = 12.2, 5.3 Hz, 1H), 4.51 (d, *J* = 4.5 Hz, 2H), 4.16 (s, 2H), 3.83 (s, 6H), 3.62 (s, 3H), 2.85 – 2.59 (m, 6H), 2.20 – 2.07 (m, 1H). LCMS (ESI) m/z: $[M+H]^+$ = 610.35.

Example 68 – Preparation of 2-((2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)benzyl)(methyl)amino)-N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)amino)ethoxy)ethoxy)ethyl)-N-methylacetamide (compound D16)

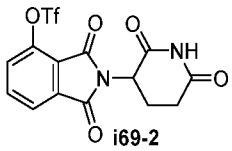


Compound D16 was prepared in a similar manner as described for compound D13. 2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-1,2-dihydro-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)-*N*-[2-[2-(2-[[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-5-yl]amino]ethoxy)ethoxy]ethyl]-*N*-methylacetamide (10 mg, 3.39%) was obtained as a yellow solid. ^{1}H NMR (400 MHz, Methanol-*d4*) δ 9.52 (s, 1H), 8.68 (dd, *J* = 5.7, 2.3 Hz, 1H), 8.55 (s, 0.5H, FA), 7.74 (d, *J* = 6.3 Hz, 1H), 7.62 (dd, *J* = 5.9, 2.7 Hz, 1H), 7.48 (dd, *J* = 10.8, 8.4 Hz, 1H), 6.97 (d, *J* = 2.2 Hz, 1H), 6.85 – 6.71 (m, 3H), 5.01 (dt, *J* = 12.7, 4.9 Hz, 1H), 4.61 (s, 2H), 4.29 (s, 2H), 4.11 (s, 1H), 3.92 (d, *J* = 2.9 Hz, 6H), 3.71 (d, *J* = 1.8 Hz, 3H), 3.66 (dd, *J* = 7.1, 3.9 Hz, 7H), 3.62 – 3.52 (m, 2H), 3.42 – 3.34 (m, 2H), 3.03 (d, *J* = 7.0 Hz, 3H), 2.91 – 2.73 (m, 2H), 2.73 – 2.62 (m, 4H), 2.12 – 2.00 (m, 1H). LCMS (ESI) m/z: [M+H]+ = 798.40.

Example 69 – Preparation of 4-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl) amino)ethyl]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (compound D17)



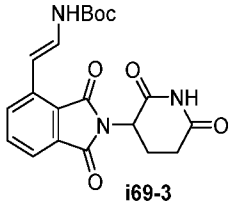
Step 1: 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl trifluoromethanesulfonate (i69-2)



i69-2

To a stirred solution of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindole-1,3-dione (0.50 g, 1.823 mmol, 1.00 equiv) in DCM (7.00 mL) was added Et_3N (0.76 mL, 7.513 mmol, 3.00 equiv) and pyridine (0.76 mL, 9.611 mmol, 5.18 equiv) at 0 ℃. Tf_2O (0.77 g, 2.735 mmol, 1.50 equiv) was then added dropwise at 0 ℃, and the mixture was stirred at this temperature for 30 minutes and then warmed to room temperature for 1 hour. The reaction was quenched by addition of sat. aq. NH_4Cl (5 mL). The resulting mixture was extracted with DCM (2 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was suspended in 5 mL of DCM and then filtered. The light brown residue was dissolved in 40 mL of MeCN and filtered. The filtrate was concentrated in vacuo to afford 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl trifluoromethanesulfonate (610 mg, 82.35%) as a light brown solid. LCMS (ESI) m/z: [M+H]+ = 407.

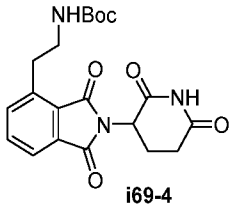
Step 2: tert-butyl N-[(E)-2-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]ethenyl]carbamate (i69-3)



i69-3

To a mixture of 2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl trifluoromethanesulfonate (580 mg, 1.428 mmol, 1.00 equiv), AcONa (257.6 mg, 3.141 mmol, 2.20 equiv), and $Pd(OAc)_2$ (32.1 mg, 0.143 mmol, 0.10 equiv) was added *tert*-butyl *N*-ethenylcarbamate (572.3 mg, 3.997 mmol, 2.80 equiv) and NMP (5 mL) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 5 hours at 130 ℃ under nitrogen atmosphere. It was then diluted with EtOAc (30 mL). The resulting mixture was washed with water (3 x 20 mL). The organic layer was dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC (PE/EtOAc 1:1) to afford *tert*-butyl *N*-[(E)-2-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]ethenyl]carbamate (140 mg, 25%) as a yellow solid. LCMS (ESI) m/z: [M+H]+ = 400.

Step 3: tert-butyl N-[2-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]ethyl]carbamate (i69-4)

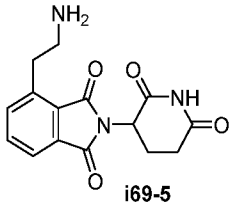


i69-4

A mixture of *tert*-butyl *N*-[(E)-2-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]ethenyl]carbamate(135 mg, 0.338 mmol, 1.00 equiv) and 10% Pd/C (30 mg) in MeOH (5 mL) was stirred under an atmosphere of hydrogen at room temperature for 2 hours. The solution was filtered

through a Celite pad and the pad was washed with methanol (20 mL). The filtrate was evaporated to dryness to give *tert*-butyl N-[2-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]ethyl]carbamate (135 mg, quant.) as a light yellow oil. LCMS (ESI) m/z: [M+H]+ = 402.

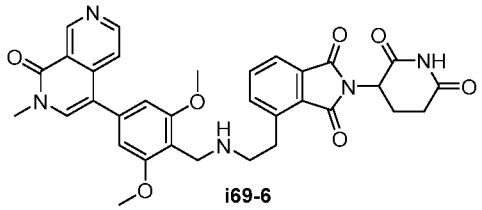
Step 4: 4-(2-aminoethyl)-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione; trifluoroacetic acid (i69-5)



i69-5

To a stirred solution of *tert*-butyl *N*-[2-[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindol-4-yl]ethyl]carbamate (125 mg, 0.311 mmol, 1.00 equiv) in DCM (3 mL) was added TFA (1 mL, 13.463 mmol, 43.23 equiv) at room temperature. The reaction solution was stirred for 30 minutes at room temperature and then concentrated in vacuo to give 4-(2-aminoethyl)-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione trifluoroacetic acid (129 mg, quant.) as a light brown oil. LCMS (ESI) m/z: [M+H]+ = 302.

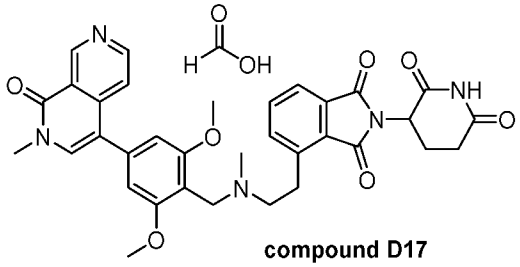
Step 5: 4-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]amino)ethyl]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (i69-6)



i69-6

A solution of 4-(2-aminoethyl)-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione trifluoroacetic acid (120 mg, 0.289 mmol, 1.00 equiv) and 2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)benzaldehyde (79.7 mg, 0.246 mmol, 0.85 equiv) in DMF (2 mL) was stirred for 45 minutes at room temperature. To the above mixture was added $NaBH(OAc)_3$ (122.47 mg, 0.578 mmol, 2.00 equiv) at room temperature. The resulting mixture was stirred for additional 1 hour at room temperature. Without any additional work-up, the mixture was purified by reverse phase flash (conditions: C18 column; mobile phase, MeCN in water (0.1% FA), 5% to 80% gradient in 30 min; detector, UV 254 nm) to afford 4-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl]amino)ethyl]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (60 mg, 34%) as a colorless oil. LCMS (ESI) m/z: [M+H]+ = 610.

Step 6: 4-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)ethyl]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (compound D17)



compound D17

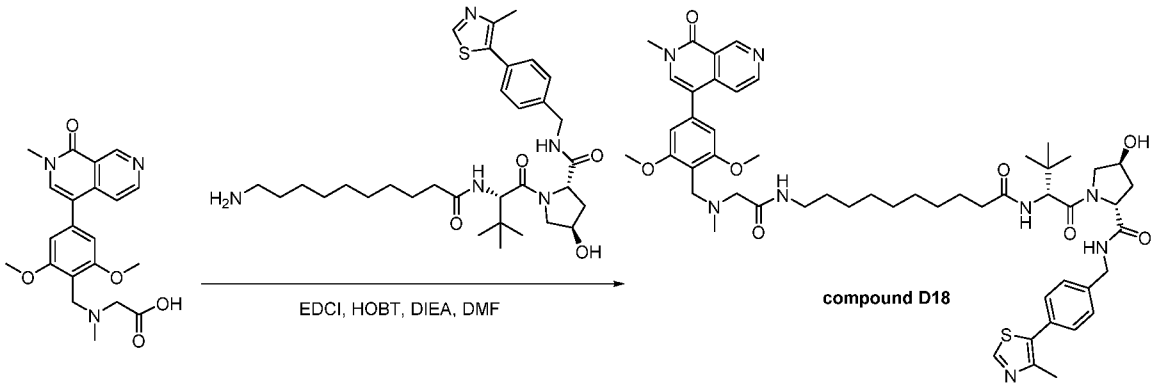
To a stirred solution of 4-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl] amino)ethyl]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione (50 mg, 0.082 mmol, 1.00 equiv) in MeOH (1.00 mL) was added HCHO (37% in water) (0.1 mL) at room temperature. The solution was stirred for 10 minutes at room temperature. Then to the above mixture was added $NaBH_3CN$ (15.0 mg, 0.238 mmol, 2.90 equiv), and the resulting mixture was stirred for additional 1 hour at room temperature. The crude solution was directly purified by Prep-HPLC (conditions: SunFire C18 OBD Prep Column , 100 Å, 5 μm, 19 mm X 250 mm; Mobile Phase A:Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 9% B to 19% B in 12 min; 254 nm) to afford three isomers 4-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)ethyl]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione formic acid (first peak, isomer A, 3.1 mg, 5.76%), 4-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)ethyl]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione formic acid (second peak, isomer B, 5.4 mg, 9.56%), and 4-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)ethyl]-2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione formic acid (third peak, isomer C, 6.7 mg, 11.94%) each as a white solid.

Isomer A: ^{1}H NMR (300 MHz, Methanol-d4) δ 9.52 (d, *J* = 0.9 Hz, 1H), 8.67 (d, *J* = 5.8 Hz, 1H), 8.10 (t, *J* = 4.9 Hz, 1H), 7.91 (d, *J* = 4.6 Hz, 2H), 7.68 (s, 1H), 7.56 (dd, *J* = 5.9, 0.9 Hz, 1H), 6.70 (s, 2H), 5.33 (br s, 1H), 5.21 (dd, *J* = 12.5, 5.4 Hz, 1H), 4.19 (s, 2H), 3.86 (s, 6H), 3.70 (s, 3H), 2.94 – 2.65 (m, 6H), 2.25 – 2.12 (m, 1H), 1.74 (br s, 3H). LCMS (ESI) m/z: [M+H]+ = 624.30.

Isomer B: ^{1}H NMR (300 MHz, Methanol-d4) δ 9.53 (d, *J* = 0.9 Hz, 1H), 8.67 (d, *J* = 5.8 Hz, 1H), 8.10 (dd, *J* = 6.1, 2.9 Hz, 1H), 7.97 (d, *J* = 6.1 Hz, 2H), 7.72 (s, 1H), 7.58 (dd, *J* = 5.8, 0.9 Hz, 1H), 6.76 (s, 2H), 5.46 (br s, 1H), 5.22 (dd, *J* = 12.5, 5.4 Hz, 1H), 4.41 (br s, 2H), 3.89 (s, 6H), 3.71 (s, 3H), 2.84 (s, 4H), 2.78 – 2.65 (m, 2H), 2.24 – 2.11 (s, 1H), 1.84 (d, *J* = 6.9 Hz, 3H). LCMS (ESI) m/z: [M+H]+ = 624.35.

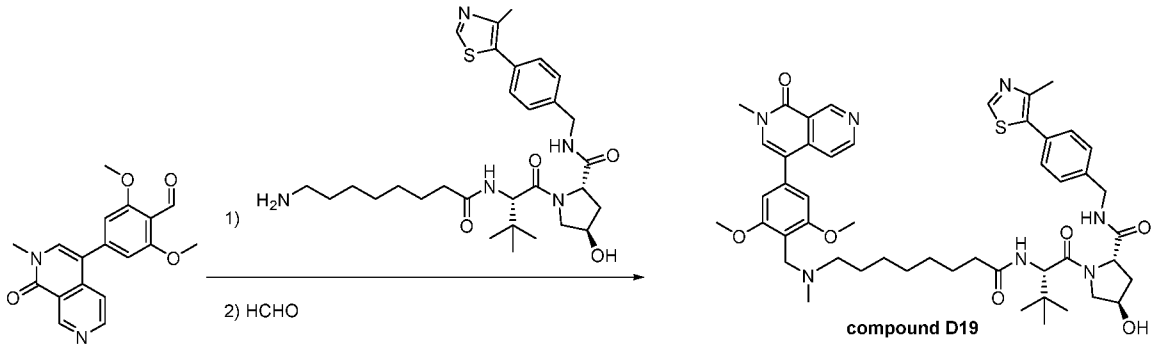
Isomer C: ^{1}H NMR (300 MHz, Methanol-d4) δ 9.53 (d, *J* = 0.9 Hz, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 8.55 (s, 0.5H, FA), 7.87 – 7.70 (m, 4H), 7.64 (dd, *J* = 5.8, 0.9 Hz, 1H), 6.82 (s, 2H), 5.15 (dd, *J* = 12.2, 5.3 Hz, 1H), 4.26 (s, 2H), 3.94 (s, 6H), 3.72 (s, 3H), 3.61 – 3.49 (m, 2H), 3.27 (s, 2H), 2.96 – 2.67 (m, 6H), 2.21 – 2.09 (m, 1H). LCMS (ESI) m/z: [M+H]+ = 624.35.

Example 70 – Preparation of (2S,4R)-1-[(2S)-2-[10-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)ph enyl]methyl](methyl)amino)acetamido]decanamido]-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-met hyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (compound D18)



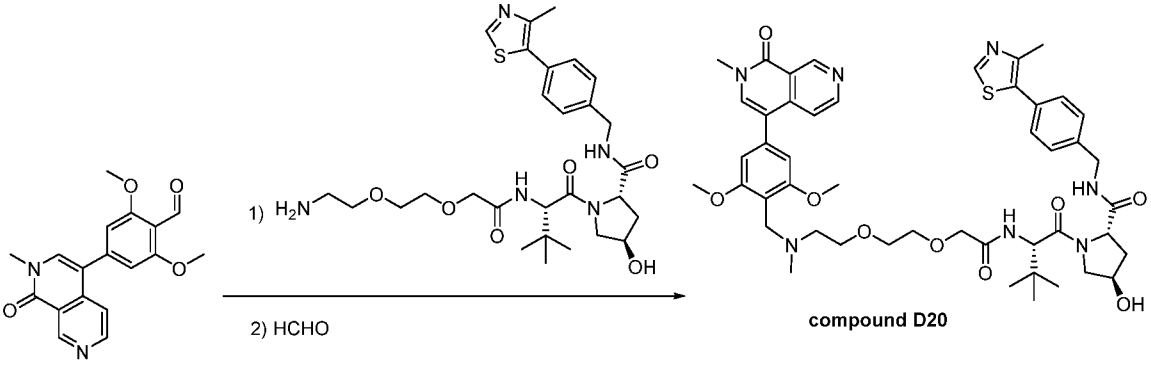
To a stirred solution of ([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)acetic acid(39.70 mg, 0.100 mmol, 1.00 equiv), EDCI(76.60 mg, 0.400 mmol, 4 equiv), HOBT (53.99 mg, 0.400 mmol, 4 equiv) and DIEA (129.10 mg, 0.999 mmol, 10 equiv) in DMF(1.00 mL) was added (2S,4R)-1-[(2S)-2-(10-aminodecanamido)-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (59.92 mg, 0.100 mmol, 1 equiv) . The mixture was stirred for 5 h at room temperature under air atmosphere. Then, without any additional work-up, the resulting solution was purified by Prep-TLC (Column: XBridge Prep OBD C18 Column, 19*250mm,5um; Mobile Phase A:Water(10MMOL/L NH_4HCO_3), Mobile Phase B:ACN; Flow rate:25 mL/min; Gradient:35 B to 55 B in 15 min; 254/220 nm; R_T: 11.08 minutes) to afford (2S,4R)-1-[(2S)-2-[10-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl) amino)acetamido]decanamido]-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl] methyl]pyrrolidine-2-carboxamide (43 mg, 43.21%) as a white solid. ^{1}H NMR (300 MHz, Methanol-d4) δ 9.54 (d, *J* = 0.9 Hz, 1H), 8.88 (s, 1H), 8.69 (d, *J* = 5.8 Hz, 1H), 7.75 (s, 1H), 7.63 (d, *J* = 5.8, 1H), 7.53 – 7.39 (m, 4H), 6.76 (s, 2H), 4.68 – 4.48 (m, 4H), 4.37 (d, *J* = 15.5 Hz, 1H), 3.93 (s, 1H), 3.90 (s, 6H), 3.81 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.76 (s, 2H), 3.71 (s, 3H), 3.21 (t, *J* = 7.0 Hz, 2H), 3.09 (s, 2H), 2.48 (s, 3H), 2.35 (s, 3H), 2.31 – 2.18 (m, 3H), 2.15 – 2.04 (m, 1H), 1.65 – 1.44 (m, 4H), 1.41 - 1.25 (m, 10H), 1.04 (s, 9H). LCMS (ESI) m/z: [M+H]+ = 979.60.

Example 71 – Preparation of (2S,4R)-1-[(2S)-2-[8-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)ph enyl]methyl](methyl)amino)octanamido]-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (compound D19)



To a stirred solution of (2S,4R)-1-[(2S)-2-[2-[2-(2-aminoethoxy)ethoxy]acetamido]-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide(53.25mg, 0.092 mmol, 1 equiv) in methanol was added 2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)benza ldehyde(30.00 mg, 0.092 mmol, 1.00 equiv) dropwise in portions at room temperature under nitrogen atmosphere. The resulting mixture was stirred for additional 2h at room temperature. To the above mixture was added $NaBH_3CN$ (7.75 mg, 0.123 mmol, 2.00 equiv) at room temperature. The resulting mixture was stirred for additional 1h at room temperature. To the above mixture was added $NaBH_3CN$ (7.75 mg, 0.123 mmol, 2.00 equiv) and CH_2O at room temperature. The resulting mixture was stirred for additional 1h at room temperature. The crude product (50.2mg) was purified by Prep-HPLC (conditions: Xselect CSH F-Phenyl OBD column, 19*250, 5 μm; Mobile Phase A: Water (0.05% TFA), Mobile Phase B:ACN; Flow rate:25 mL/min; Gradient:20 B to 30 B in 12 min; 254/220 nm; R_T: 11.48 minutes) to afford (2S,4R)-1-[(2S)-2-[8-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)octanamido]-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide as a white solid. ^{1}H NMR (400 MHz , Methanol-d4) δ 1.05 (9H, s), 1.44 (6H, s), 1.67 (2H, s), 1.87 (2H, s), 2.05 – 2.16 (1H, m), 2.19 – 2.40 (3H, m), 2.50 (3H, s), 2.86 (3H, s), 3.12 – 3.24 (1H, m), 3.24 – 3.32 (1H, m), 3.76 (3H, s), 3.79 – 3.87 (1H, m), 3.92 (1H, d), 3.98 (6H, s), 4.31 – 4.42 (2H, m), 4.48 – 4.64 (4H, m), 4.66 (1H, s), 6.91 (2H, s), 7.40 – 7.52 (4H, m), 7.94 (1H, d), 8.08 (1H, s), 8.72 (1H, d), 8.99 (1H, s), 9.62 (1H, s). LCMS (ESI) m/z: [M+H]+ = 894.55.

Example 72 – Preparation of (2S,4R)-1-[(2S)-2-(2-[2-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)p henyl]methyl](methyl)amino)ethoxy]ethoxy]acetamido)-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (compound D24)



To a stirred solution of (2S,4R)-1-[(2S)-2-[2-[2-(2-aminoethoxy)ethoxy]acetamido]-3,3-dimeth ylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide(35.50mg, 0.062 mmol, 1.00 equiv) in methanol was added 2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)benzaldehyde (20.00 mg, 0.062 mmol, 1.00 equiv) dropwise in portions at room temperature under nitrogen atmosphere. The resulting mixture was stirred for additional 2 hours at room temperature. To the above mixture was added $NaBH_3CN$ (7.75 mg, 0.123 mmol, 2.00 equiv) at room temperature. The resulting mixture was stirred for additional 1h at room temperature. To the above mixture was added $NaBH_3CN$ (7.75 mg, 0.123 mmol, 2.00 equiv) and CH_2O at room temperature. The resulting mixture was stirred for additional 1h at room temperature. The crude product (30.5mg) was purified by Prep-HPLC (conditions: Xselect CSH F-Phenyl OBD column, 19*250, 5 μm; Mobile Phase A:Water(0.05%TFA), Mobile Phase B:ACN; Flow rate:25 mL/min; Gradient:18 B to 27 B in 12 min; 254/220 nm; R_T: 10.97 minutes) to afford (2S,4R)-1-[(2S)-2-(2-[2-[2-([[2,6-dimethoxy-4-(2-methyl-1-oxo-2,7-naphthyridin-4-yl)phenyl]methyl](methyl)amino)ethoxy]ethoxy]acetamido)-3,3-dimethylbutanoyl] -4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide as a white solid. ^1H NMR (400 MHz , Methanol-d4) δ 1.04 – 1.08 (9H, m), 2.05 – 2.14 (1H, m), 2.25 (1H, s), 2.49 (3H, t), 2.94 (3H, d), 3.70 – 3.92 (9H, m), 3.96 (8H, d), 4.02 – 4.17 (2H, m), 4.31 – 4.67 (6H, m), 4.74 - 4.82 (1H, m), 6.90 (2H, d), 7.39 – 7.49 (4H, m), 7.85 - 7.95 (1H, m), 8.01 – 8.07 (1H, m), 8.71 (1H, d), 8.92 – 8.99 (1H, m), 9.61 (1H, s) LCMS (ESI) m/z: [M+H]+ = 898.50.

Example 73 – BRD9 bromodomain TR-FRET Competition Binding Assay

This example demonstrates the ability of the compounds of the disclosure to biochemically inhibit BRD9 bromodomain in a competition binding assay.

Procedure: His-Flag-BRD9 (P133-K239; Swiss Prot Q9H8M2; SEQ ID NO:1 mgsshhhhhhenlyfq/gdykddddkgslevlfqg/PAENESTPIQQLLEHFLRQLQRKDPHGFFAFPVTDAIAPGYSMII KHPMDFGTMKDKIVANEYKSVTEFKADFKLMCDNAMTYNRPDTVYYKLAKKILHAGFKMMSK) was cloned, expressed, purified, and then treated with TEV protease. Cleaved His tag was removed by purification. The binding of a biotinylated small molecule ligand of BRD9 was assessed via the LANCE® TR-FRET platform (PerkinElmer), and the compounds were assayed for inhibitory activity against this interaction.

Results: A mixture of biotinylated-ligand and SureLight™ Allophycocyanin-Streptavidin (APC-SA, PerkinElmer AD0201) in 50 mM HEPES (pH 7.4), 50 mM NaCl, 1 mM TCEP (pH 7), 0.01% (v/v) Tween-20, 0.01% (w/v) bovine serum albumin was added to a white 384-well PerkinElmer Proxiplate Plus plate. DMSO or 3-fold serially diluted compounds were then added to the Proxiplate followed by addition of Flag-BRD9. After a 10 minute incubation at room temperature, Eu-W1024 anti-FLAG (PerkinElmer, AD0273) was added. The final reaction mixture that contained 3.75 nM biotinylated ligand, 3 nM Flag-BRD9, 7.5 nM SureLight™ Allophycocyanin-Streptavidin, and 0.2 nM Eu-W1024 anti-FLAG was incubated at room temperature for 90 minutes.

The plates were then read on a PerkinElmer Envision plate reader to determine the ratio of emission at 665 nm over 615 nm. Data was normalized to a DMSO control (100%) and a no protein control (0%) and then fit to a four parameter, non-linear curve fit to calculate an IC_{50} (μM) as shown in Table 5. As shown by the results in Table 5, a number of compounds of the present disclosure exhibit an IC_{50} value of < 1 μM for BRD9 binding, indicating their affinity for targeting BRD9.

Table 5. Bromodomain TR-FRET Binding

Compound No.	Bromodomain TR-FRET BRD9 IC_{50} (nM)
B1	NT
B2	+++
B3	+
B4	+
B5	+
B6	+
B7	+
B8	+
B9	+
B10	+
B11	+
B12	+
B13	+
B14	+++
B15	+++
B16	++
B17	++
B18	+++
B19	++
B20	++++
B21	+++
B22	+++
B23	+++

Compound No.	Bromodomain TR-FRET BRD9 IC_{50} (nM)
B24	++
B25	++
B26	+
B27	++
B28	+++
B29	++
B30	++
B31	+
B32	+++
B33	++
B34	++++
B35	++++
B36	+++
B37	++
B38	++++
B39	++++
B40	++++
B41	+++
B42	+
B43	++++
B44	++++
B45	+++
B46	+++
B47	+++
B48	++
B49	+++
B50	+++
B51	NT
B52	+++
B53	+++
B54	++++
B55	**NT**
B56	+++
B57	+
B58	+
B59	+++
B60	+++

Compound No.	Bromodomain TR-FRET BRD9 IC_{50} (nM)
B61	NT
B62	+++
B63	+++
B64	+++
B65	+++
B66	++
B67	+++
B68	+
Compound 1	+++
D1	+++
D2	+++
D3	+++
D4	++++
D5	++++
D6	+++
D7	+++
D8	+++
D9	+++
D10	+++
D11	++++
D12	++++
D13	+++
D14	++++
D15	+++
D16	+++
D17	NT
D18	++++
D19	+++
D20	+++

"+" indicates inhibitory effect of > 1000 nM;
"++" indicates inhibitory effect of 100-1000 nM;
"+++" indicates inhibitory effect of 10-100 nM;
"++++" indicates inhibitory effect of < 10 nM;
"NT" indicates not tested

Example 74 – SYO1 BRD9 NanoLuc Degradation Assay

This example demonstrates the ability of the compounds of the disclosure to degrade a Nanoluciferase-BRD9 fusion protein in a cell-based degradation assay.

Procedure: A stable SYO-1 cell line expressing 3xFLAG-NLuc-BRD9 was generated. On day 0 cells were seeded in 30 µL media into each well of 384-well cell culture plates. The seeding density was

8000 cells/well. On day 1, cells were treated with 30 nL DMSO or 30 nL of 3-fold serially DMSO-diluted compounds (10 points in duplicates with 1 µM as final top dose). Subsequently plates were incubated for 6 hours in a standard tissue culture incubator and equilibrated at room temperature for 15 minutes. Nanoluciferase activity was measured by adding 15 µL of freshly prepared Nano-Glo Luciferase Assay Reagent (Promega N1130), shaking the plates for 10 minutes and reading the bioluminescence using an EnVision reader.

Results: The Inhibition% was calculated using the following formula: %Inhibition = 100 x (Lum_{HC} – Lum_{Sample}) / (Lum_{HC} –Lum_{LC}). DMSO treated cells are employed as High Control (HC) and 1 µM of a known BRD9 degrader standard treated cells are employed as Low Control (LC). The data was fit to a four parameter, non-linear curve fit to calculate IC_{50} (µM) values as shown in Table 6. As shown by the results in Table 6, a number of compounds of the present disclosure exhibit an IC_{50} value of < 1 µM for the degradation of BRD9, indicating their use as compounds for reducing the levels and/or activity of BRD9 and their potential for treating BRD9-related disorders.

Table 6. SYO1 BRD9-NanoLuc Degradation

Compound No.	SYO1 BRD9-NanoLuc degradation IC50 (nM)
Compound 1	++++
D1	++
D2	++++
D3	+++
D4	++++
D5	++++
D6	++++
D7	+++
D8	++++
D9	++++
D10	++++
D11	++++
D12	++++
D13	+++
D14	+
D15	+
D16	+++
D17	+
D18	++++

Compound No.	SYO1 BRD9-NanoLuc degradation IC50 (nM)
D19	+
D20	+

"+" indicates inhibitory effect of > 1000 nM;
"++" indicates inhibitory effect of 100-1000 nM;
"+++" indicates inhibitory effect of 10-100 nM;
"++++" indicates inhibitory effect of < 10 nM;
"NT" indicates not tested

Other Embodiments

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present application is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

While the invention has been described in connection with specific embodiments thereof, it will be understood that invention is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are in the claims.

What is claimed is:

Claims

1. A method of treating adult soft tissue sarcoma in a subject in need thereof, the method comprising administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in the sarcoma.

2. A method of reducing tumor growth of an adult soft tissue sarcoma in a subject in need thereof, the method comprising administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in the tumor.

3. A method of inducing apoptosis in an adult soft tissue sarcoma cell, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell.

4. A method of reducing the level and/or activity of BRD9 in an adult soft tissue sarcoma cell, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell.

5. The method of claim 3 or 4, wherein the adult soft tissue sarcoma cell is in a subject.

6. The method of any one of claims 1 to 5, wherein the subject or cell has been identified as expressing SS18-SSX fusion protein or BRD9 fusion protein.

7. The method of any one of claims 1 to 6, wherein the effective amount of the agent reduces the level and/or activity of BRD9 by at least 5% as compared to a reference.

8. The method of any one of claims 1 to 7, wherein the effective amount of the agent reduces the level and/or activity of BRD9 by at least 5% as compared to a reference for at least 12 hours.

9. The method of any one of claims 1 to 8, wherein the level and/or activity of SS18-SSX or BRD9 fusion protein is reduced in the subject or cell.

10. The method of any one of claims 1 to 9, wherein the adult soft tissue sarcoma is synovial sarcoma.

11. A method of modulating the activity of an SS18-SSX fusion protein, SS18 wild-type protein, or SSX wild-type protein in a cell, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell.

12. A method of modulating the level and/or activity of an SS18-SSX fusion protein, SS18 wild-type protein, or SSX wild-type protein in a cell or subject, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in a cell or subject.

13. The method of claim 11 or 12, wherein the cell is in a subject.

14. A method of treating a disorder related to an SS18-SSX fusion protein, SS18 wild-type protein, or SSX wild-type protein in a subject in need thereof, the method comprising administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in an SS18-SSX fusion protein-expressing cell in the subject.

15. The method of any one of claims 11 to 14, wherein the subject has cancer.

16. The method of claim 15, wherein the cancer expresses SS18-SSX fusion protein and/or the cell or subject has been identified as expressing SS18-SSX fusion protein.

17. The method of any one of claims 14 to 16, wherein the disorder is synovial sarcoma or Ewing's sarcoma.

18. The method of claim 17, wherein the disorder is synovial sarcoma.

19. A method of modulating the activity of a BAF complex in a cell or subject, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

20. A method of increasing the level and/or activity of BAF47 in a cell or subject, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

21. A method of decreasing Wnt/β-catenin signaling in a cell or subject, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

22. A method treating a disorder related to BAF47 in a subject in need thereof, the method comprising administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in the subject.

23. The method of claim 22, wherein the disorder related to BAF47 is a cancer or viral infection.

24. The method of claim 23, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, or colorectal cancer.

25. The method of claim 23, wherein the viral infection is an infection with a virus of the Retroviridae family, Hepadnaviridae family, Flaviviridae family, Adenoviridae family, Herpesviridae family, Papillomaviridae family, Parvoviridae family, Polyomaviridae family, Paramyxoviridae family, or Togaviridae family.

26. A method for treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in a cancer cell, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

27. A method of reducing tumor growth of a cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in a tumor cell, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

28. A method of inducing apoptosis in a cancer cell, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

29. A method of reducing the level and/or activity of BRD9 in a cancer cell, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, colorectal cancer, non-small cell lung cancer, stomach cancer, or breast cancer.

30. The method of any one of claims 26 to 29, wherein the cancer is a CD8+ T-cell lymphoma, endometrial carcinoma, ovarian carcinoma, bladder cancer, stomach cancer, pancreatic cancer, esophageal cancer, prostate cancer, renal cell carcinoma, melanoma, or colorectal cancer.

31. The method of any one of claims 26 to 30, wherein the cancer is non-small cell lung cancer, stomach cancer, or breast cancer.

32. A method of modulating the activity of a BRD9 fusion protein in a cell or subject, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

33. A method of modulating the level and/or activity of a BRD9 fusion protein in a cell or subject, the method comprising contacting the cell with an effective amount of an agent that reduces the level and/or activity of BRD9 in the cell or subject.

34. The method of claim 32 or 33, wherein the cell is in a subject.

35. A method of treating a disorder related to a BRD9 fusion protein in a subject in need thereof, the method comprising administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in a BRD9 fusion protein-expressing cell.

36. The method of any one of claims 32 to 35, wherein the subject has cancer.

37. The method of claim 36, wherein the cancer expresses a BRD9 fusion protein and/or the cell or subject has been identified as expressing a BRD9 fusion protein.

38 The method of any one of claims 35 to 37, wherein the disorder related to a BRD9 fusion protein is Ewing's sarcoma, lung cancer, or renal cancer.

39. The method of any one of claims 1 to 38, wherein the method further comprises administering to the subject or contacting the cell with an anticancer therapy.

40. The method of claim 39, wherein the anticancer therapy is a chemotherapeutic or cytotoxic agent or radiotherapy.

41. The method of claim 40, wherein the chemotherapeutic or cytotoxic agent is doxorubicin or ifosfamide.

42. The method of claim 40 or 41, wherein the anticancer therapy and the agent that reduces the level and/or activity of BRD9 in a cell are administered within 28 days of each other and each in an amount that together are effective to treat the subject.

43. The method of any one of claims 1 to 42, wherein the subject or cancer has been identified as having an elevated level of an SS18-SSX fusion protein or a BRD9 fusion protein as compared to a reference.

44. The method of any one of claims 1 to 43, wherein the subject or cancer has been identified as having a decreased level of SS18 wild-type protein or SSX wild-type protein as compared to a reference.

45. A method of treating a viral infection, the method comprising administering to the subject an effective amount of an agent that reduces the level and/or activity of BRD9 in a cell of the subject.

46. The method of claim 45, wherein the viral infection is an infection with a virus of the Retroviridae family, Hepadnaviridae family, Flaviviridae family, Adenoviridae family, Herpesviridae family, Papillomaviridae family, Parvoviridae family, Polyomaviridae family, Paramyxoviridae family, or Togaviridae family.

47. The method of any one of claims 1 to 46, wherein the agent that reduces the level and/or activity of BRD9 in a cell is a small molecule compound, an antibody, an enzyme, and/or a polynucleotide.

48. The method of claim 47, wherein the agent that reduces the level and/or activity of BRD9 in a cell is an enzyme.

49. The method of claim 48, wherein the enzyme is a clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), or a meganuclease.

50. The method of claim 49, wherein the CRISPR-associated protein is CRISPR-associated protein 9 (Cas9).

51. The method of claim 47, wherein the agent that reduces the level and/or activity of BRD9 in a cell is a polynucleotide.

52. The method of claim 51, wherein the polynucleotide is an antisense nucleic acid, a short interfering RNA (siRNA), a short hairpin RNA (shRNA), a micro RNA (miRNA), a CRISPR/Cas 9 nucleotide, or a ribozyme.

53. The method of claim 51, wherein the polynucleotide comprises a sequence having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 3-202.

54. The method of claim 53, wherein the polynucleotide comprises a sequence having at least 85% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 3-139.

55. The method of claim 47, wherein the agent that reduces the level and/or activity of BRD9 in a cell is a small molecule compound.

56. The method of claim 55, wherein the small molecule compound is a small molecule BRD9 inhibitor.

57. The method of claim 55 or 56, wherein the small molecule compound is a degrader.

58. The method of claim 57, wherein the degrader has the structure of **Formula I**:

A-L-B

Formula I

wherein

A is a BRD9 binding moiety;

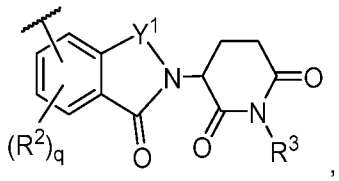
L is a linker; and

B is a degradation moiety.

59. The method of claim 58, wherein the degradation moiety is a ubiquitin ligase binding moiety.

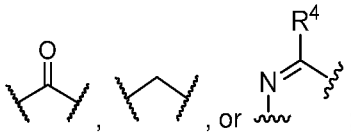
60. The method of claim 59, wherein the ubiquitin ligase binding moiety comprises Cereblon ligands, IAP (Inhibitors of Apoptosis) ligands, mouse double minute 2 homolog (MDM2), or von Hippel-Lindau ligands, or derivatives or analogs thereof.

61. The method of claim 59 or 60, wherein the degradation moiety has the structure of **Formula A-1**:



Formula A-1

wherein

Y^1 is  ;

each of R^3 and R^4 is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

q is 0, 1, 2, 3, or 4; and

each R^2 is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino,

or a pharmaceutically acceptable salt thereof.

62. The method of claim 61, wherein R^3 is H or optionally substituted C_1-C_6 alkyl.

63. The method of claim 62, wherein R^3 is H or CH_3.

64. The method of claim 63, wherein R^3 is H.

65. The method of claim 63, wherein R^3 is CH_3.

66. The method of any one of claims 61 to 65, wherein Y^1 is  or  .

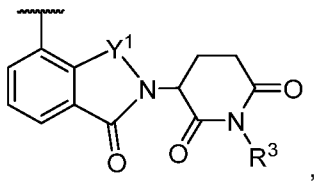
67. The method of claim 66, wherein Y^1 is  .

68. The method of any one of claims 61 to 67, wherein each R^2 is, independently, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, hydroxyl, or optionally substituted amino.

69. The method of any one of claims 61 to 68, wherein q is 0 or 1.

70. The method of claim 69, wherein q is 0.

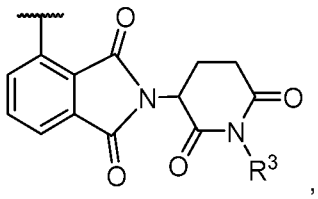
71. The method of any one of claims 61 to 70, wherein the degradation moiety has the structure of **Formula A-1a**:



Formula A-1a

or a pharmaceutically acceptable salt thereof.

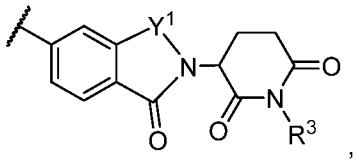
72. The method of any one of claims 61 to 70, wherein the degradation moiety has the structure of **Formula A-1b**:



Formula A-1b

or a pharmaceutically acceptable salt thereof.

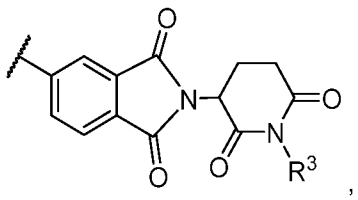
73. The method of any one of claims 61 to 70, wherein the degradation moiety has the structure of **Formula A-1c**:



Formula A-1c

or a pharmaceutically acceptable salt thereof.

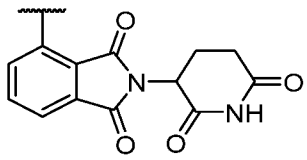
74. The method of any one of claims 61-70, wherein the degradation moiety has the structure of **Formula A-1d**:



Formula A-1d

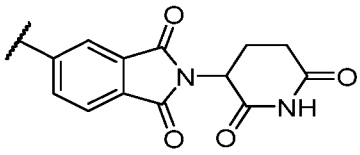
or a pharmaceutically acceptable salt thereof.

75. The method of claim 59 or 60, wherein the degradation moiety has the structure:



1a

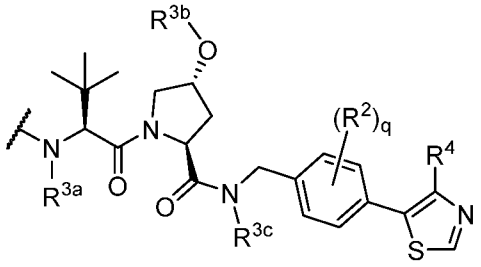
or



1b

or is a derivative or an analog thereof.

76. The method of claim 59 or 60, wherein the degradation moiety has the structure of



Formula B-1,

wherein

q is 0, 1, 2, 3, or 4;

each R^2 is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

each of R^{3a}, R^{3b}, and R^{3c} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl; and

R^4 is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl,

or a pharmaceutically acceptable salt thereof.

77. The method of claim 76, wherein each R^2 is, independently, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, hydroxyl, or optionally substituted amino.

78. The method of claims 76 or 77, wherein q is 0 or 1.

79. The method of claim 78, wherein q is 0.

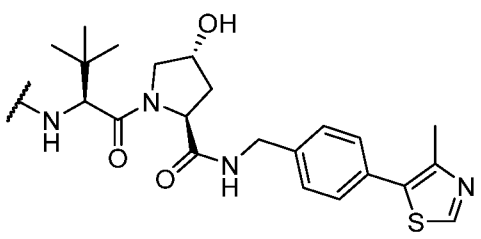
80. The method of any one of claims 76 to 79, wherein each of R^{3a}, R^{3b}, and R^{3c} is, independently, H or optionally substituted C_1-C_6 alkyl.

81. The method of claim 80, wherein R^{3a} is H.

82. The method of claim 80, wherein R^{3b} is H.

83. The method of claim 80, wherein R^{3c} is H.

84. The method of claim 59 or 60, wherein the degradation moiety has the structure:



2a

or is a derivative or an analog thereof.

85. The method of any one of claims 58 to 84, wherein the linker has the structure of **Formula II**:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

wherein

A^1 is a bond between the linker and A;

A^2 is a bond between B and the linker;

each of B^1, B^2, B^3, and B^4 is, independently, optionally substituted C_1-C_2 alkyl, optionally substituted C_1-C_3 heteroalkyl, O, S, $S(O)_2$, or NR^N;

each R^N is, independently, H, optionally substituted $C_{1–4}$ alkyl, optionally substituted $C_{2–4}$ alkenyl, optionally substituted $C_{2–4}$ alkynyl, optionally substituted $C_{2–6}$ heterocyclyl, optionally substituted $C_{6–12}$ aryl, or optionally substituted $C_{1–7}$ heteroalkyl;

each of C^1 and C^2 is, independently, carbonyl, thiocarbonyl, sulphonyl, or phosphoryl;

each of f, g, h, i, j, and k is, independently, 0 or 1; and

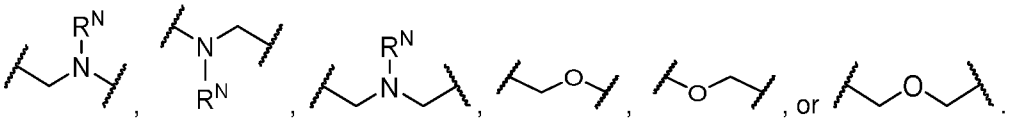
D is optionally substituted $C_{1–10}$ alkyl, optionally substituted $C_{2–10}$ alkenyl, optionally substituted $C_{2–10}$ alkynyl, optionally substituted $C_{2–6}$ heterocyclyl, optionally substituted $C_{6–12}$ aryl, optionally substituted C_2-C_{10} polyethylene glycol, or optionally substituted $C_{1–10}$ heteroalkyl, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2.

86. The method of claim 85, wherein each of B^1, B^2, B^3, and B^4 is, independently, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 heteroalkyl, or NR^N.

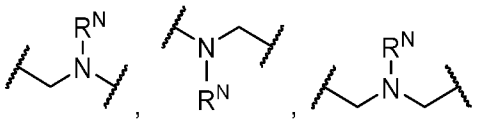
87. The method of claim 85 or 86, wherein each R^N is, independently, H or optionally substituted $C_{1–4}$ alkyl.

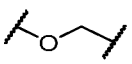
88. The method of any one of claims 85 to 87, wherein each R^N is, independently, H or CH_3.

89. The method of any one of claims 85 to 88, wherein each of B^1 and B^4 is, independently,

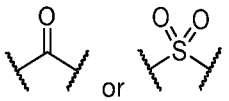


90. The method of claim 89 wherein B^1 is





91. The method of any one of claims 85 to 90, wherein each of C^1 and C^2 is, independently,

 or .

92. The method of claim 91, wherein C^1 is  .

93. The method of any one of claims 85 to 83, wherein B^2 is NR^N.

94. The method of any one of claims 85 to 84, wherein B^2 is optionally substituted C_1-C_4 alkyl.

95. The method of any one of claims 85 to 94, wherein f is 0.

96. The method of any one of claims 85 to 94, wherein f is 1.

97. The method of any one of claims 85 to 96, wherein g is 1.

98. The method of any one of claims 85 to 97, wherein h is 0.

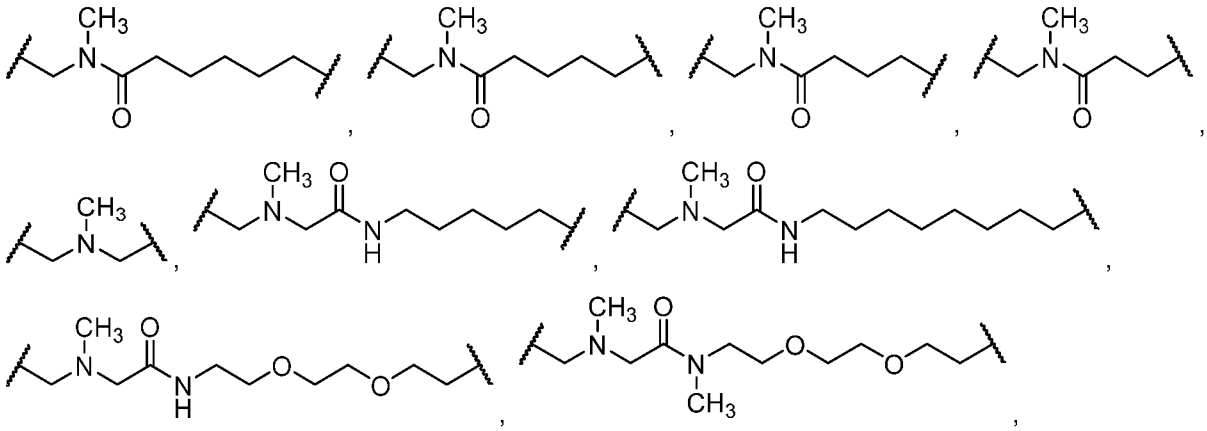
99. The method of any one of claims 85 to 97, wherein h is 1.

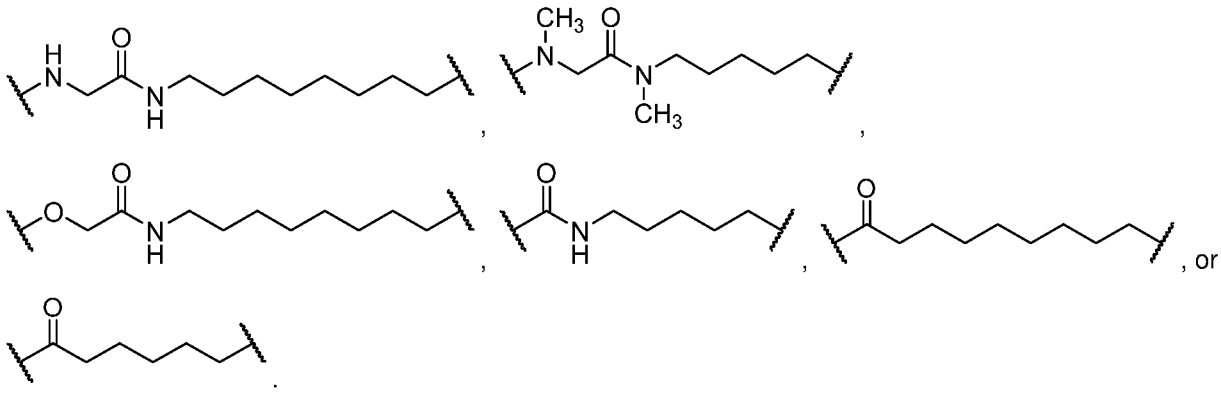
100. The method of any one of claims 85 to 99, wherein i is 0.

101. The method of any one of claims 85 to 100, wherein j is 0.

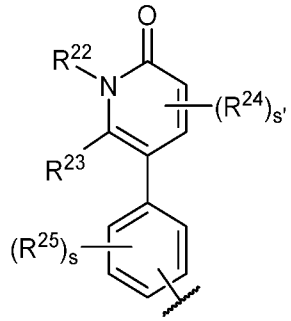
102. The method of any one of claims 85 to 101, wherein k is 0.

103. The method of any one of claims 85 to 102, wherein the linker has the structure of





104. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula E-a**:



Formula E-a,

wherein

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s' is 0, 1, or 2;

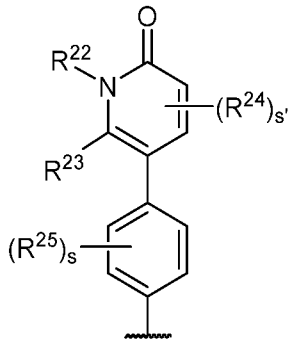
each R^{24} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino, or two R^{24} combine with the carbon atoms to which they are attached to form an optionally substituted C_6-C_{10} aryl or optionally substituted C_2-C_9 heteroaryl;

s is 0, 1, 2, 3, or 4; and

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino,

or a pharmaceutically acceptable salt thereof.

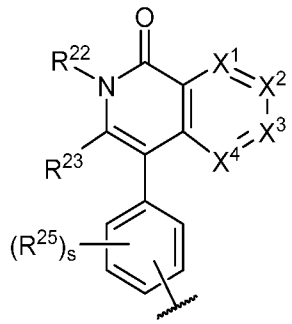
105. The method of claim 104, wherein the BRD9 binding moiety comprises the structure of **Formula E-b**:



Formula E-b,

or a pharmaceutically acceptable salt thereof.

106. The method of any one of claims 58 to 105, wherein the BRD9 binding moiety comprises the structure **Formula E-1a**:



Formula E-1a,

wherein

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

X^1 is N or CR^{24a};

X^2 is N or CR^{24b};

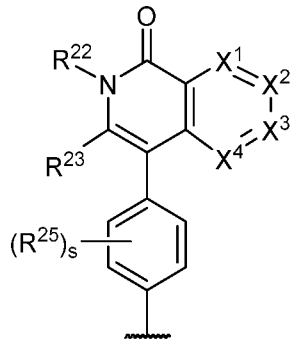
X^3 is N or CR^{24c};

X^4 is N or CR^{24d}; and

each of R^{24a}, R^{24b}, R^{24c}, and R^{24d} is, independently, H, halogen, hydroxyl, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino,

or a pharmaceutically acceptable salt thereof.

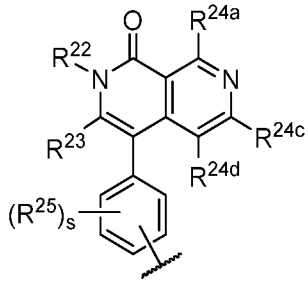
107. The method of claim 106, wherein the BRD9 binding moiety comprises the structure of **Formula E-1b**:



Formula E-1b,

or a pharmaceutically acceptable salt thereof.

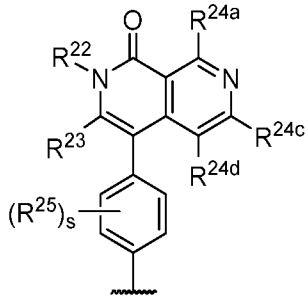
108. The method of claim 106, wherein the BRD9 binding moiety comprises the structure of **Formula E-2a**:



Formula E-2a,

or a pharmaceutically acceptable salt thereof.

109. The method of claim 108, wherein the BRD9 binding moiety comprises the structure of **Formula E-2b**:



Formula E-2b,

or a pharmaceutically acceptable salt thereof.

110. The method of any one of claims 95 to 109, wherein R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_3-C_6 carbocyclyl.

111. The method of claim 110, wherein R^{22} is H or CH_3.

112. The method of any one of claims 104 to 111, wherein R^{23} is H or optionally substituted C_1-C_6 alkyl.

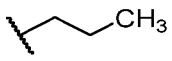
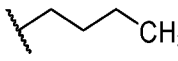
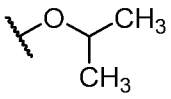
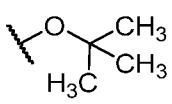
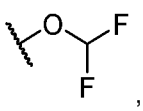
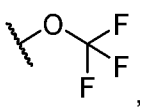
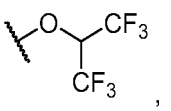
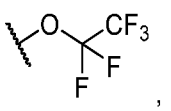
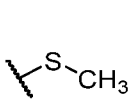
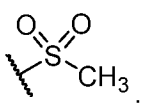
113. The method of claim 112, wherein R^{23} is H.

114. The method of any one of claims 104 to 113, wherein s is 0, 1, or 2.

115. The method of claim 114, wherein s is 1 or 2.

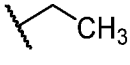
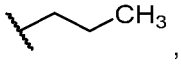
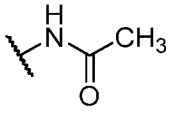
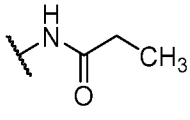
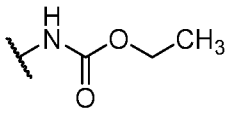
116. The method of claim 115, wherein s is 2.

117. The method of any one of claims 104 to 116, wherein each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl.

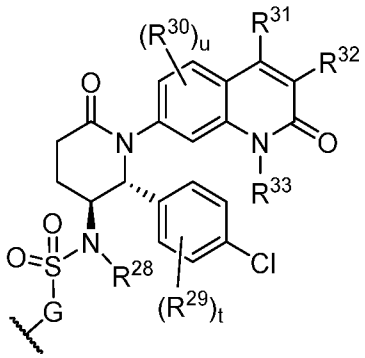
118. The method of claim 117, wherein each R^{25} is, independently, F,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , or  .

119. The method of any one of claims 104 to 118, wherein s' is 1.

120. The method of any one of claims 104 to 119, wherein each of R^{24a}, R^{24b}, R^{24c}, and R^{24d} is, independently, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted amino.

121. The method of claim 120, wherein each of R^{24a}, R^{24b}, R^{24c}, and R^{24d} is, independently, $-NH_2$,  ,  ,  ,  ,  , or  .

122. The method of any one of claims 58 to 103, the BRD9 binding moiety comprises the structure of **Formula F-a**:



Formula F-a,

wherein

each of R^{28} and R^{33} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

t is 0, 1, 2, 3, or 4;

each R^{29} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

u is 0, 1, 2, 3, or 4;

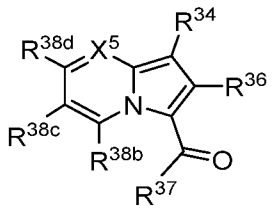
each R^{30} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

each of R^{31} and R^{32} is, independently, selected form the group consisting of H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl; and

G is optionally substituted C_1-C_6 alkylene, optionally substituted C_6-C_{10} arylene, or optionally substituted C_3-C_6 carbocyclylene,

or a pharmaceutically acceptable salt thereof.

123. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula G**:



Formula G,

wherein

R^{34} is optionally substituted C_6-C_{10} aryl or C_2-C_9 heteroaryl;

R^{36} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

R^{37} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

X^5 is CR^{38a} or N;

each of R^{38a}, R^{38b}, and R^{38c} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino; and

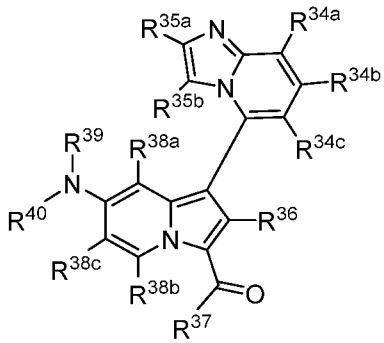
R^{38d} is hydrogen or $-NR^{39}R^{40}$; and

each of R^{39} and R^{40} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl, or R^{39} and R^{40} combine to form an optionally substituted C_2-C_9 heterocyclyl,

wherein at least one of R^{34}, R^{39}, or R^{40} includes a bond to the linker,

or a pharmaceutically acceptable salt thereof.

124. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula G-1**:



Formula G-1,

wherein

each of R^{34a}, R^{34b}, and R^{34c} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

each R^{35a} and R^{35b} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

R^{36} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

R^{37} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

each R^{38a}, R^{38b}, and R^{38c} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino; and

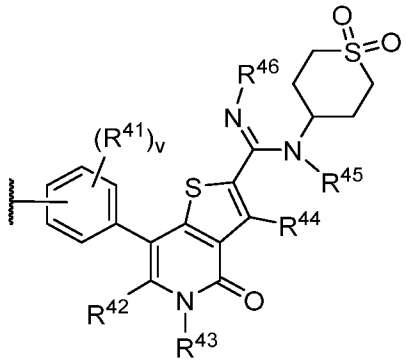
R^{38d} is hydrogen or $-NR^{39}R^{40}$; and

each R^{39} and R^{40} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl, or R^{39} and R^{40} combine to form an optionally substituted C_2-C_9 heterocyclyl,

wherein at least one of R^{34}, R^{39}, or R^{40} includes a bond to the linker,

or a pharmaceutically acceptable salt thereof.

125. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula H-a**:



Formula H-a

wherein

v is 0, 1, 2, 3, or 4;

each R^{41} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

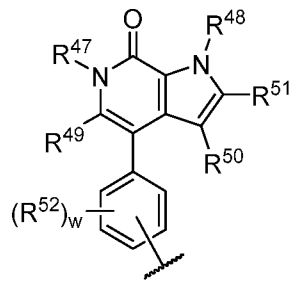
R^{42} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

R^{44} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl; and

each R^{43}, R^{45}, and R^{46} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl,

or a pharmaceutically acceptable salt thereof.

126. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula J-a**:



Formula J-a,

wherein

each of R^{47} and R^{48} is, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

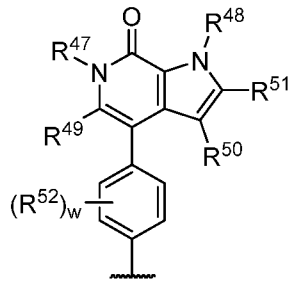
each of R^{49}, R^{50}, and R^{51} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

w is 0, 1, 2, 3, or 4; and

each R^{52} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino,

or a pharmaceutically acceptable salt thereof.

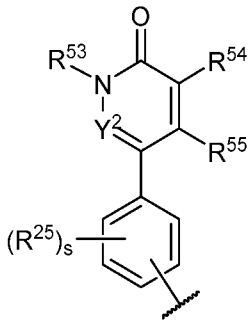
127. The method of claim 126, wherein the BRD9 binding moiety comprises the structure of **Formula J-b**:



Formula J-b,

or a pharmaceutically acceptable salt thereof.

128. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula E-3**:



Formula E-3,

wherein

Y^2 is N or CR^{23};

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

R^{53} is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

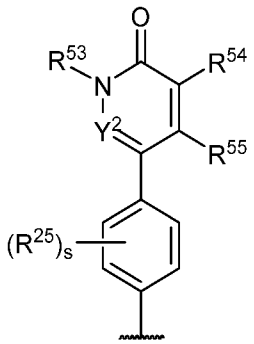
R^{54} is H or optionally substituted C_2-C_9 heteroaryl; and

R^{55} is H or NR^a, wherein R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl,

wherein if R^{53} is H and R^{54} is H, then R^{55} is NR^a; if R^{54} is H and R^{55} is H, then R^{53} is optionally substituted C_3-C_{10} carbocyclyl; and if R^{53} is H and R^{55} is H, then R^{54} is optionally substituted C_2-C_9 heteroaryl,

or a pharmaceutically acceptable salt thereof.

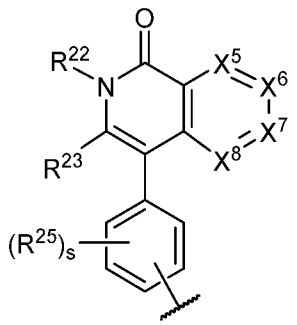
129. The method of claim 128, wherein the method of **Formula E-3** has the structure of **Formula E-3a**:



Formula E-3a,

or a pharmaceutically acceptable salt thereof.

130. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula E-4**:



Formula E-4,

wherein

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

X^5 is N or CR^{56a};

X^6 is N or CR^{56b};

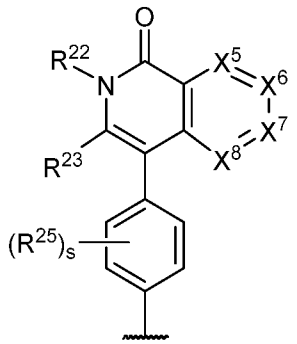
X^7 is N or CR^{56c};

X^8 is N or CR^{56d}; and

each of R^{56a}, R^{56b}, R^{56c}, and R^{56d} is, independently, H, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, optionally substituted sulfonamide, or optionally substituted amino,

or a pharmaceutically acceptable salt thereof.

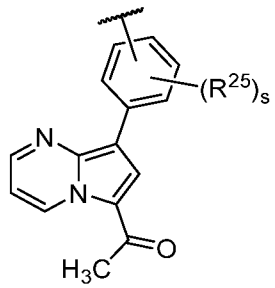
131. The method of claim 130, wherein the BRD9 binding moiety comprises the structure of **Formula E-4a**:



Formula E-4a,

or a pharmaceutically acceptable salt thereof.

132. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula G-2**:



Formula G-2,

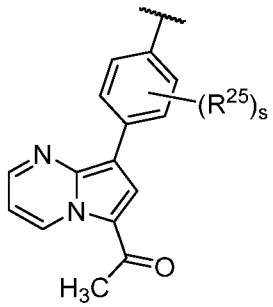
wherein

s is 0, 1, 2, 3, or 4; and

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino,

or a pharmaceutically acceptable salt thereof.

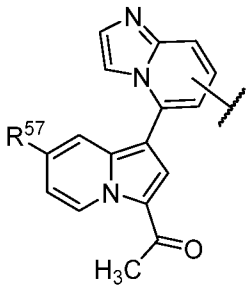
133. The method of claim 132, wherein the BRD9 binding moiety comprises the structure of **Formula G-2a**:



Formula G-2a,

or a pharmaceutically acceptable salt thereof.

134. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula G-3**:



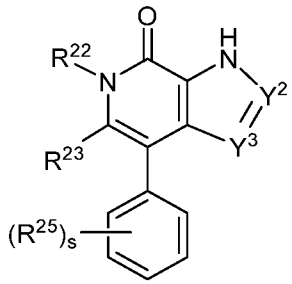
Formula G-3,

wherein

R^{57} is optionally substituted C_2-C_{10} heterocyclyl,

or a pharmaceutically acceptable salt thereof.

135. The method of any one of claims 58 to 103, wherein the BRD9 binding moiety comprises the structure of **Formula J-1**:



Formula J-1,

wherein

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

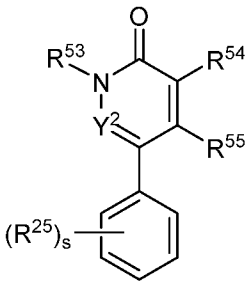
Y^2 is N or CR^{58a};

Y^3 is N or CR^{58b}; and

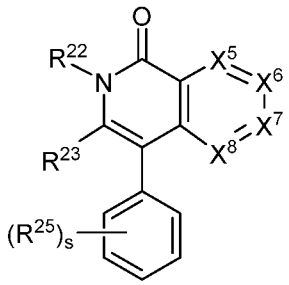
each of R^{58a} and R^{58b} is, independently, H or optionally substituted C_1-C_6 alkyl,

or a pharmaceutically acceptable salt thereof.

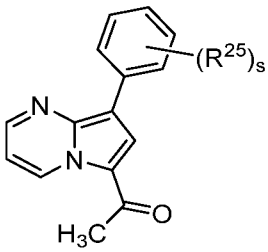
136. A compound having the structure of **Formula K-1, Formula K-2, Formula M-2**, **Formula M-3**, or **Formula O-1**:



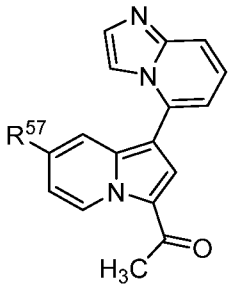
Formula K-1



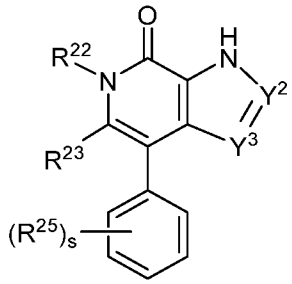
Formula K-2



Formula M-2



Formula M-3



Formula O-1

wherein

Y^2 is N or CR^{23};

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

R^{53} is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{54} is H or optionally substituted C_2-C_9 heteroaryl;

R^{55} is H or NR^a, wherein R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

X^5 is N or CR^{56a};

X^6 is N or CR^{56b};

each of X^7 and X^8 is, independently, N or CH;

each of R^{56a} and R^{56b} is, independently, H or NR^a, wherein each R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{57} is optionally substituted C_2-C_{10} heterocyclyl;

Y^2 is N or CR^{58a};

Y^3 is N or CR^{58b}; and

each of R^{58a} and R^{58b} is, independently, H or optionally substituted C_1-C_6 alkyl,

wherein if R^{53} is H and R^{54} is H, then R^{55} is NR^a; if R^{54} is H and R^{55} is H, then R^{53} is optionally substituted C_3-C_{10} carbocyclyl; and if R^{53} is H and R^{55} is H, then R^{54} is optionally substituted C_2-C_9 heteroaryl,

or a pharmaceutically acceptable salt thereof.

137. The compound of claim 136, wherein the compound has the structure of **Formula K-1**.

138. The compound of claim 136, wherein the compound has the structure of **Formula K-2**.

139. The compound of claim 136, wherein the compound has the structure of **Formula M-2**.

140. The compound of claim 136, wherein the compound has the structure of **Formula M-3**.

141. The compound of claim 136, wherein the compound has the structure of **Formula O-1**.

142. The compound of any one of claims 136 to 141, wherein s is 0, 1, or 2.

143. The compound of any one of claims 136 to 142, wherein the compound has the structure of any of compounds B1-B65 in Table 1.

144. A pharmaceutical composition comprising the compound of any one of claims 136 to 143 and a pharmaceutically acceptable excipient.

145. A method of treating a cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of any one of claims 136 to 143, or a pharmaceutical composition of claim 144.

146. A method of treating a cancer related to BRD9 inhibition in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of any one of claims 136 to 143, or a pharmaceutical composition of claim 144.

147. A compound having the structure of **Formula I**:

A-L-B

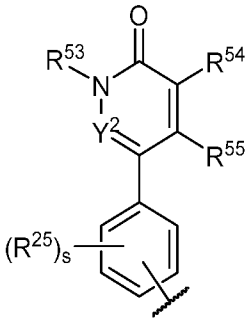
Formula I,

wherein

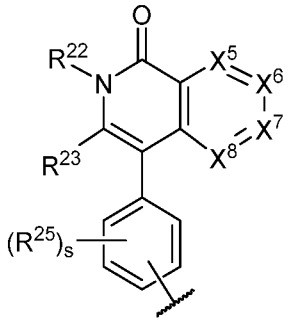
L is a linker;

B is a degradation moiety; and

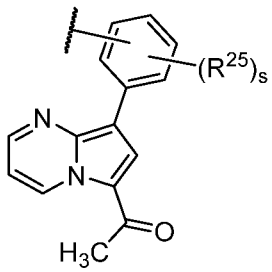
A has the structure of **Formula E-3, Formula E-4, Formula G-2**, **Formula G-3**, or **Formula E-5**:



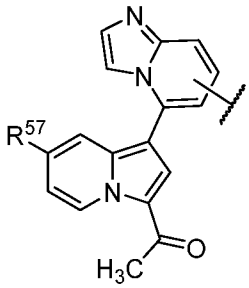
Formula E-3



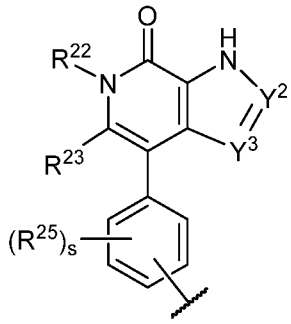
Formula E-4



Formula G-2



Formula G-3



Formula E-5

wherein

Y^2 is N or CR^{23};

R^{22} is H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

R^{23} is H, halogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_6-C_{10} aryl;

s is 0, 1, 2, 3, or 4;

each R^{25} is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino;

R^{53} is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{54} is H or optionally substituted C_2-C_9 heteroaryl;

R^{55} is H or NR^a, wherein R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

each of X^5 and X^6 is, independently, N or CR^{56};

each of X^7 and X^8 is, independently, N or CH;

each R^{56} is, independently, H or NR^a, wherein R^a is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted C_3-C_{10} carbocyclyl;

R^{57} is optionally substituted C_2-C_{10} heterocyclyl;

each of Y^2 and Y^3 is, independently, N or CR^{58}; and

each R^{58} is, independently, H or optionally substituted C_1-C_6 alkyl,

wherein if R^{53} is H and R^{54} is H, then R^{55} is NR^a; if R^{54} is H and R^{55} is H, then R^{53} is optionally substituted C_3-C_{10} carbocyclyl; and if R^{53} is H and R^{55} is H, then R^{54} is optionally substituted C_2-C_9 heteroaryl,

or a pharmaceutically acceptable salt thereof.

148. The compound of claim 147, wherein A has the structure of **Formula E-3**.

149. The compound of claim 147, wherein A has the structure of **Formula E-4**.

150. The compound of claim 147, wherein A has the structure of **Formula G-2**.

151. The compound of claim 147, wherein A has the structure of **Formula G-3**.

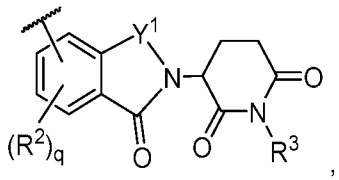
152. The compound of claim 147, wherein A has the structure of **Formula E-5**.

153. The compound of any one of claims 147to 152, wherein s is 0, 1, or 2.

154. The compound of any one of claims 147 to 153, wherein the degradation moiety is a ubiquitin ligase binding moiety.

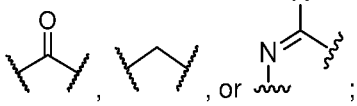
155. The compound of claim 154, wherein the ubiquitin ligase binding moiety comprises Cereblon ligands, IAP (Inhibitors of Apoptosis) ligands, mouse double minute 2 homolog (MDM2), or von Hippel-Lindau ligands, or derivatives or analogs thereof.

156. The compound of claim 154 or 155, wherein the degradation moiety has the structure of **Formula A-1**:



Formula A-1

wherein

Y^1 is 

;

R^3 and R^4 are, independently, H, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl;

q is 0, 1, 2, 3, or 4; and

each R^2 is, independently, halogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted C_3-C_{10} carbocyclyl, optionally substituted C_2-C_9 heterocyclyl, optionally

substituted C_6-C_{10} aryl, optionally substituted C_2-C_9 heteroaryl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 heteroalkenyl, hydroxyl, thiol, or optionally substituted amino,

or a pharmaceutically acceptable salt thereof.

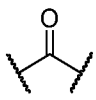
157. The compound of claim 156, wherein R^3 is H or optionally substituted C_1-C_6 alkyl.

158. The compound of claim 157, wherein R^3 is H or CH_3.

159. The compound of claim 158, wherein R^3 is H.

160. The compound of claim 158, wherein R^3 is CH_3.

161. The compound of any one of claims 156 to 160, wherein Y^1 is  or  .

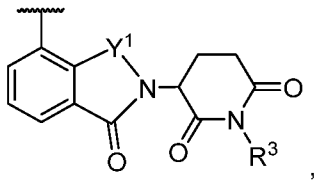
162. The compound of claim 161, wherein Y^1 is  .

163. The compound of any one of claims 156 to 162, wherein each R^2 is, independently, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, hydroxyl, or optionally substituted amino.

164. The compound of any one of claims 156 to 163, wherein q is 0 or 1.

165. The compound of claim 164, wherein q is 0.

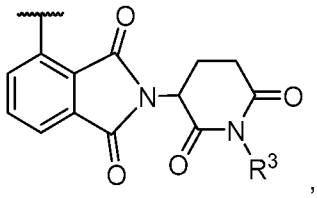
166. The compound of any one of claims 156 to 165, wherein the degradation moiety has the structure of **Formula A-1a**:



Formula A-1a

or a pharmaceutically acceptable salt thereof.

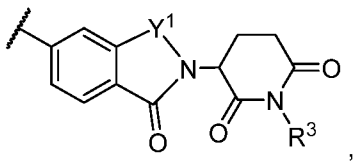
167. The compound of any one of claims 156 to 166, wherein the degradation moiety has the structure of **Formula A-1b**:



Formula A-1b

or a pharmaceutically acceptable salt thereof.

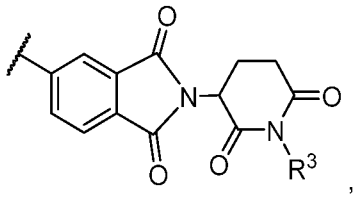
168. The compound of any one of claims 156 to 167, wherein the degradation moiety has the structure of **Formula A-1c**:



Formula A-1c

or a pharmaceutically acceptable salt thereof.

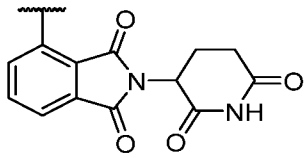
169. The compound of any one of claims 156 to 167, wherein the degradation moiety has the structure of **Formula A-1d**:



Formula A-1d

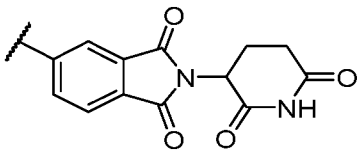
or a pharmaceutically acceptable salt thereof.

170. The compound of any one of claims 156 to 167, wherein the degradation moiety has the structure:



1a

or



1b

or is a derivative or an analog thereof.

171. The compound of any one of claims 149 to 170, wherein the linker has the structure of **Formula II**:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

wherein

A^1 is a bond between the linker and A;

A^2 is a bond between B and the linker;

each of B^1, B^2, B^3, and B^4 is, independently, optionally substituted C_1-C_2 alkyl, optionally substituted C_1-C_3 heteroalkyl, O, S, $S(O)_2$, or NR^N;

R^N is H, optionally substituted $C_{1–4}$ alkyl, optionally substituted $C_{2–4}$ alkenyl, optionally substituted $C_{2–4}$ alkynyl, optionally substituted $C_{2–6}$ heterocyclyl, optionally substituted $C_{6–12}$ aryl, or optionally substituted $C_{1–7}$ heteroalkyl;

each of C^1 and C^2 is, independently, carbonyl, thiocarbonyl, sulphonyl, or phosphoryl;

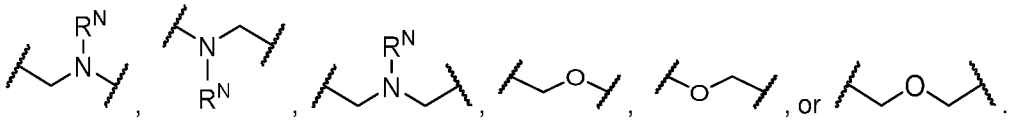
f, g, h, I, j, and k are each, independently, 0 or 1; and

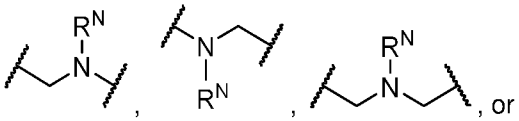
D is optionally substituted $C_{1–10}$ alkyl, optionally substituted $C_{2–10}$ alkenyl, optionally substituted $C_{2–10}$ alkynyl, optionally substituted $C_{2–6}$ heterocyclyl, optionally substituted $C_{6–12}$ aryl, optionally substituted C_2-C_{10} polyethylene glycol, or optionally substituted $C_{1–10}$ heteroalkyl, or a chemical bond linking $A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h$- to -$(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$.

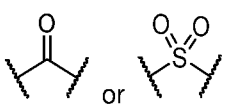
172. The compound of claim 171, wherein each of B^1, B^2, B^3, and B^4 is, independently, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 heteroalkyl, or NR^N.

173. The compound of claim 171 or 172, wherein R^N is H or optionally substituted $C_{1–4}$ alkyl.

174. The compound of any one of claims 171 to 173, wherein R^N is H or CH_3.

175. The compound of any one of claims 171 to 174, wherein each of B^1 and B^4 is, independently,  .

176. The compound of claim 175 wherein B^1 is  .

177. The compound of any one of claims 171 to 176, wherein each of C^1 and C^2 is, independently,  .

178. The compound of claim 177, wherein C^1 is  .

179. The compound of any one of claims 171 to 178, wherein B^2 is NR^N.

180. The compound of any one of claims 171 to 179, wherein B^2 is optionally substituted C_1-C_4 alkyl.

181. The compound of any one of claims 171 to 180, wherein f is 0.

182. The compound of any one of claims 171 to 180, wherein f is 1.

183. The compound of any one of claims 171 to 182, wherein g is 1.

184. The compound of any one of claims 171 to 183, wherein h is 0.

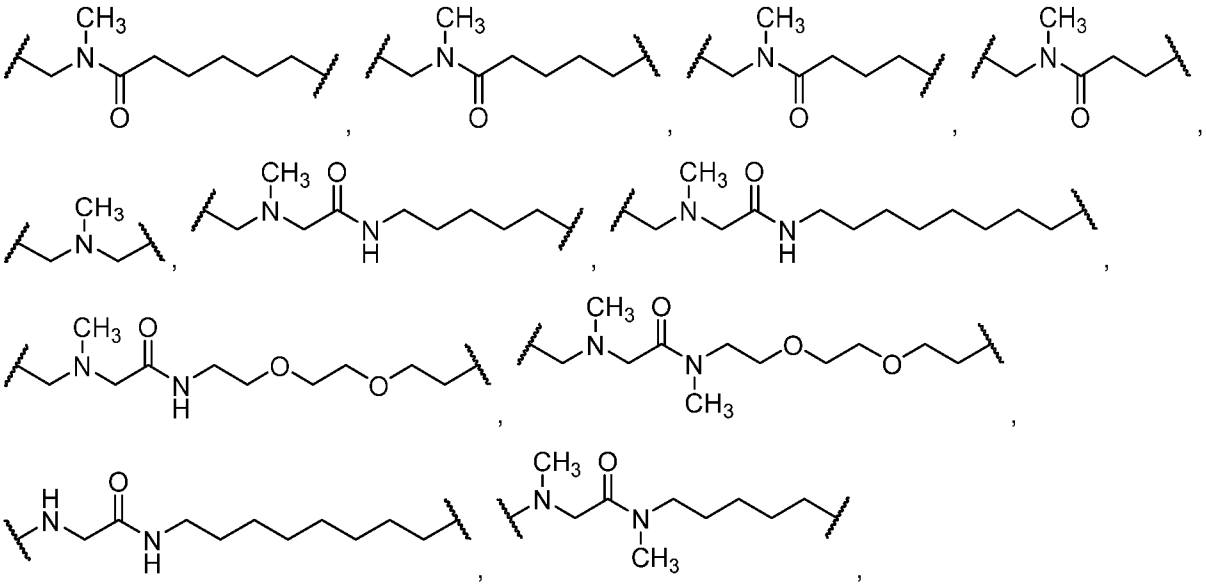
185. The compound of any one of claims 171 to 183, wherein h is 1.

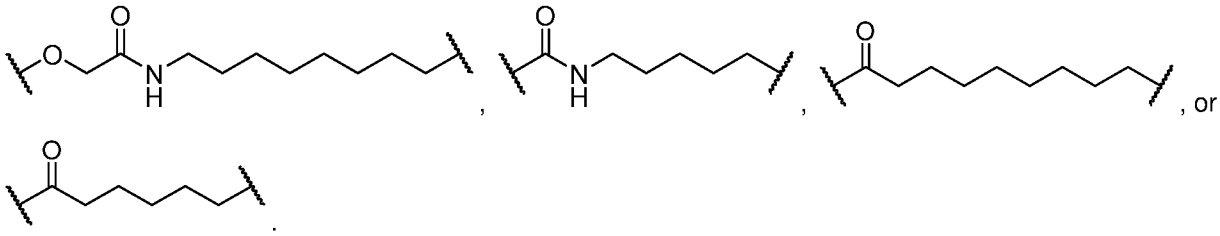
186. The compound of any one of claims 171 to 185, wherein i is 0.

187. The compound of any one of claims 171 to 186, wherein j is 0.

188. The compound of any one of claims 171 to 187, wherein k is 0.

189. The compound of any one of claims 171 to 188, wherein the linker has the structure of





190. The compound of any one of claims 136 to 142, wherein the compound has the structure of any of compounds D1-D20 in Table 2.

191. A pharmaceutical composition comprising the compound of any one of claims 138 to 190 and a pharmaceutically acceptable excipient.

192. A method of treating a cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of any one of claims 149 to 190, or a pharmaceutical composition of claim 191.

193. A method of treating a cancer related to BRD9 inhibition in a subject in need thereof, the method comprising administering to the subject an effective amount of a compound of any one of claims 149 to 190, or a pharmaceutical composition of claim 191.

FIG. 1

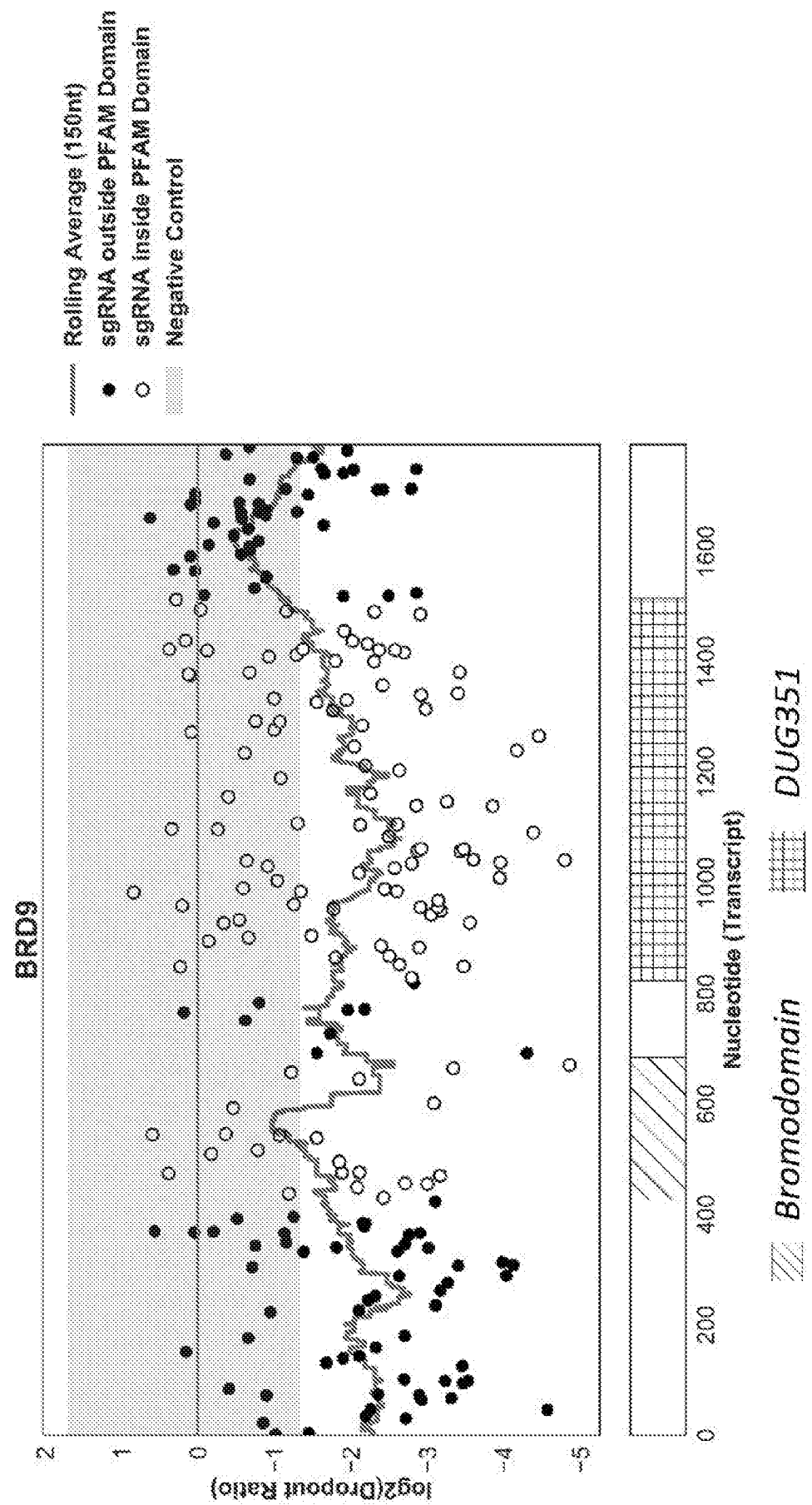

BRD9
Rolling Average (150nt)
sgRNA outside PFAM Domain
sgRNA inside PFAM Domain
Negative Control
log2(Dropout Ratio)
2
1
0
-1
-2
-3
-4
-5
0
200
400
600
800
1000
1200
1400
1600
Nucleotide (Transcript)
Bromodomain
DUG351

FIG. 2

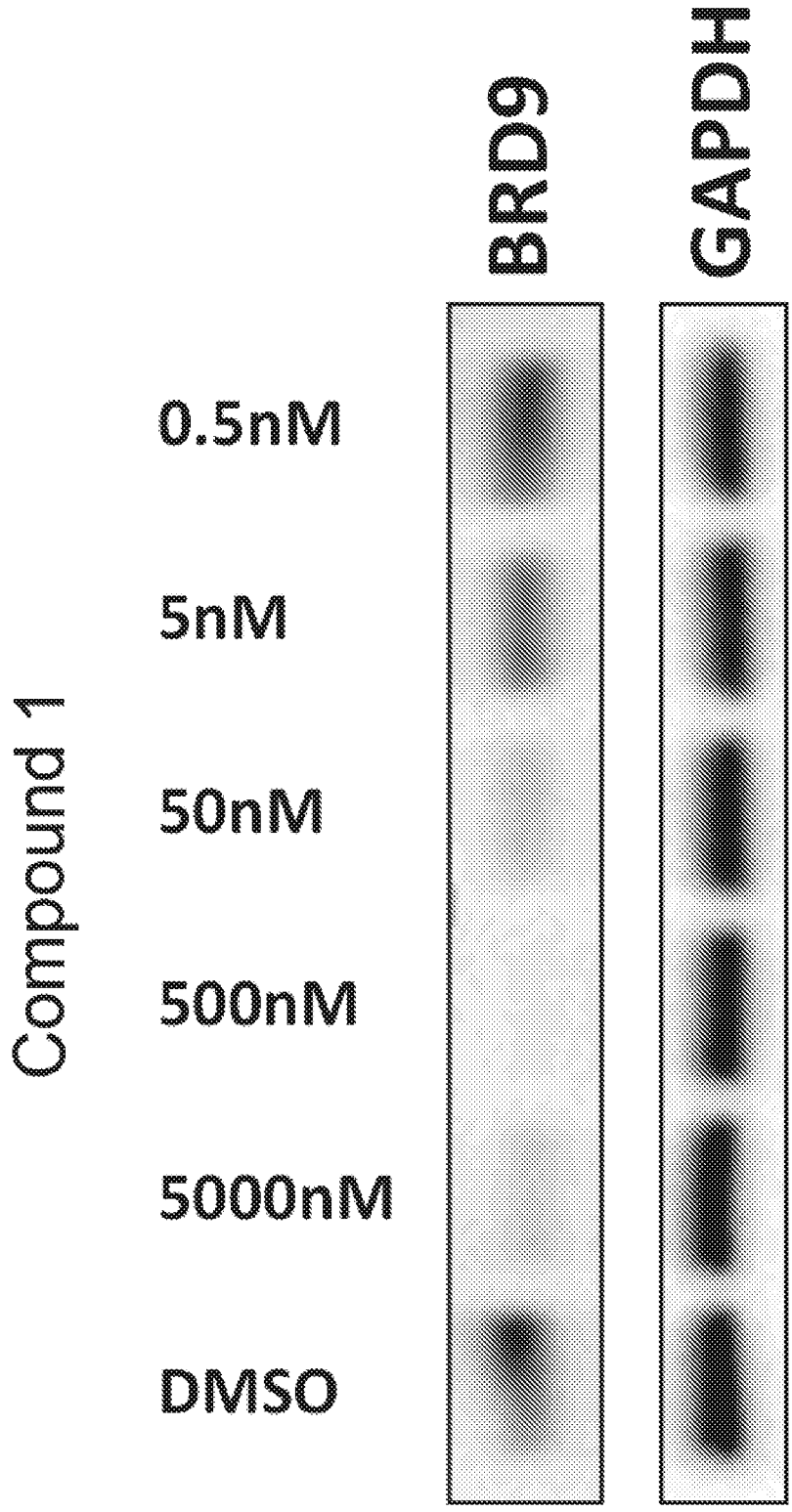


FIG. 3

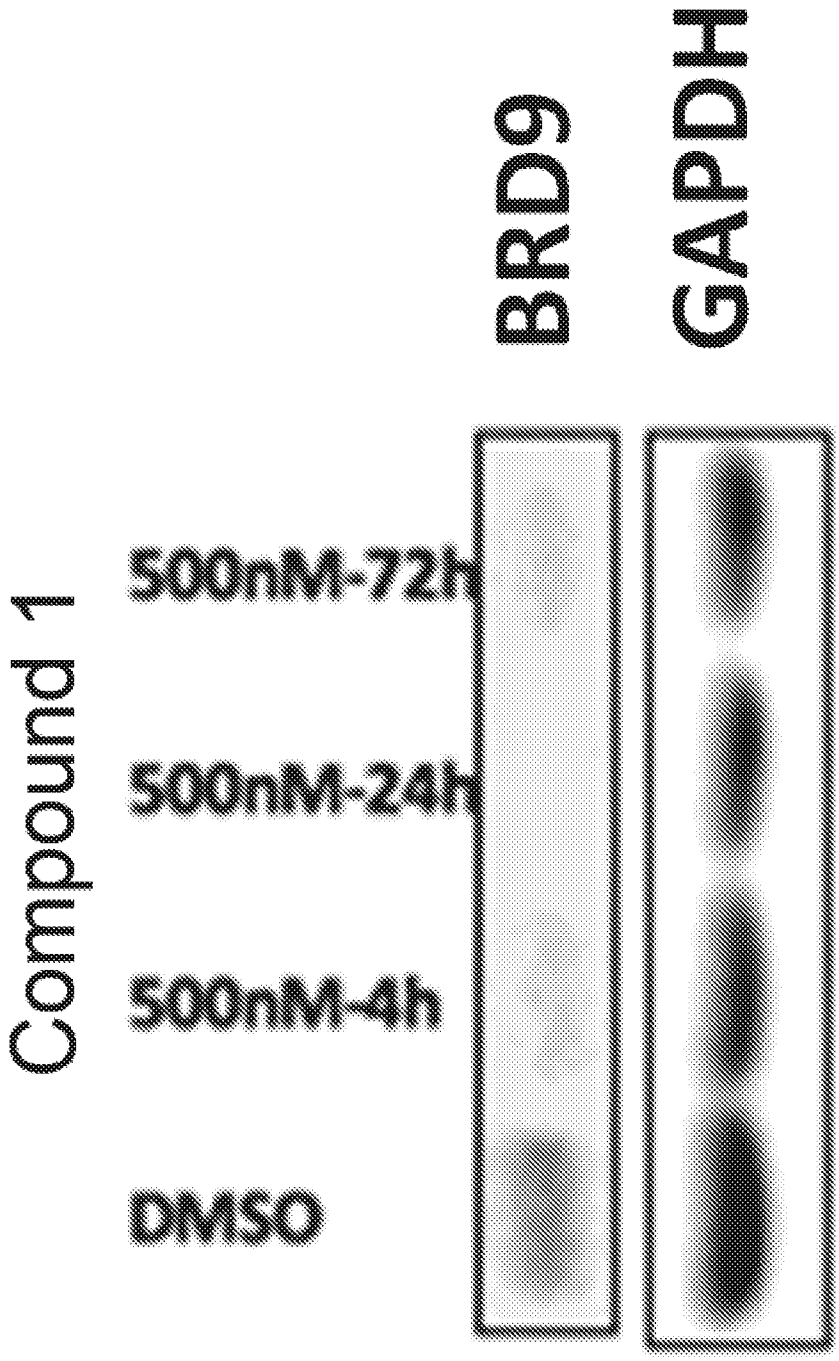


FIG. 4

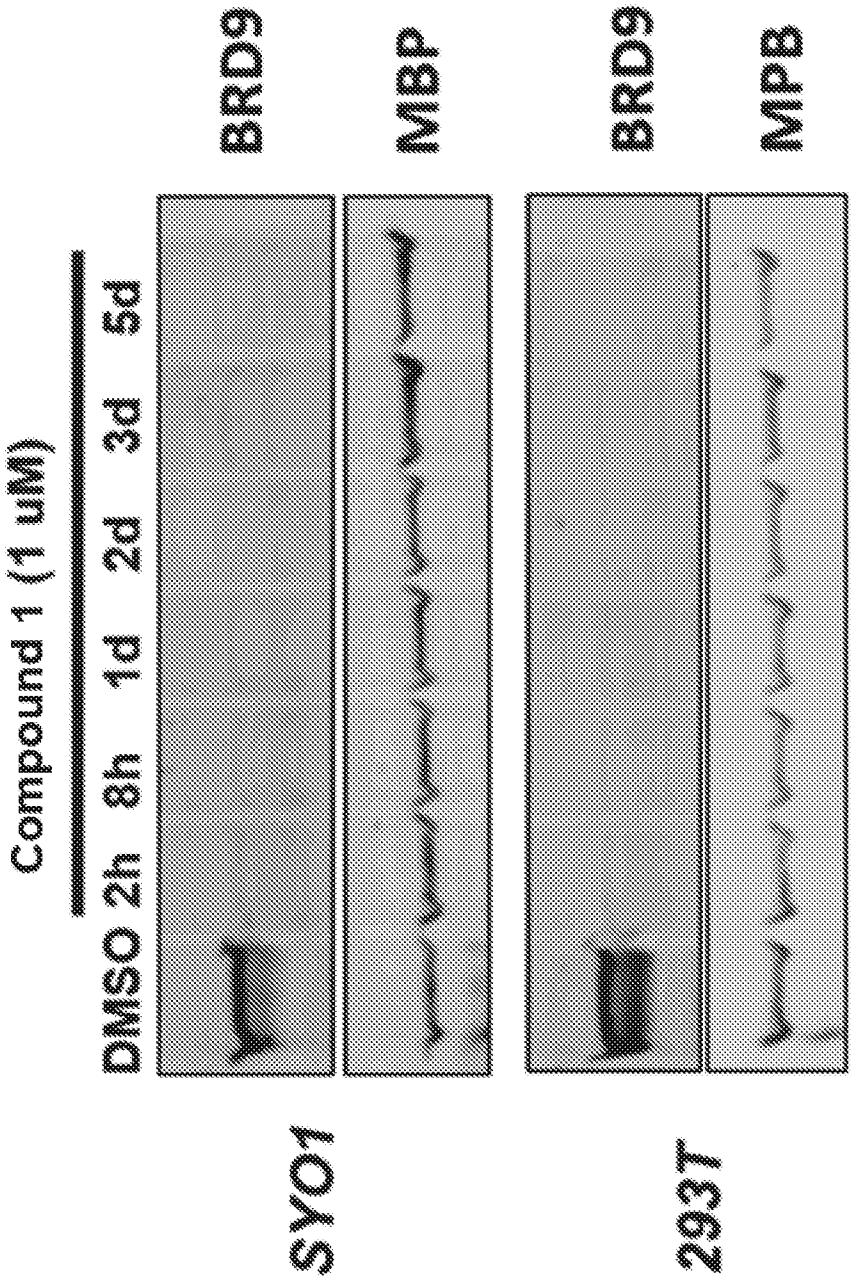

Compound 1 (1 uM)
DMSO 2h 8h 1d 2d 3d 5d
BRD9
MBP
BRD9
MPB
SYO1
293T

FIG. 5

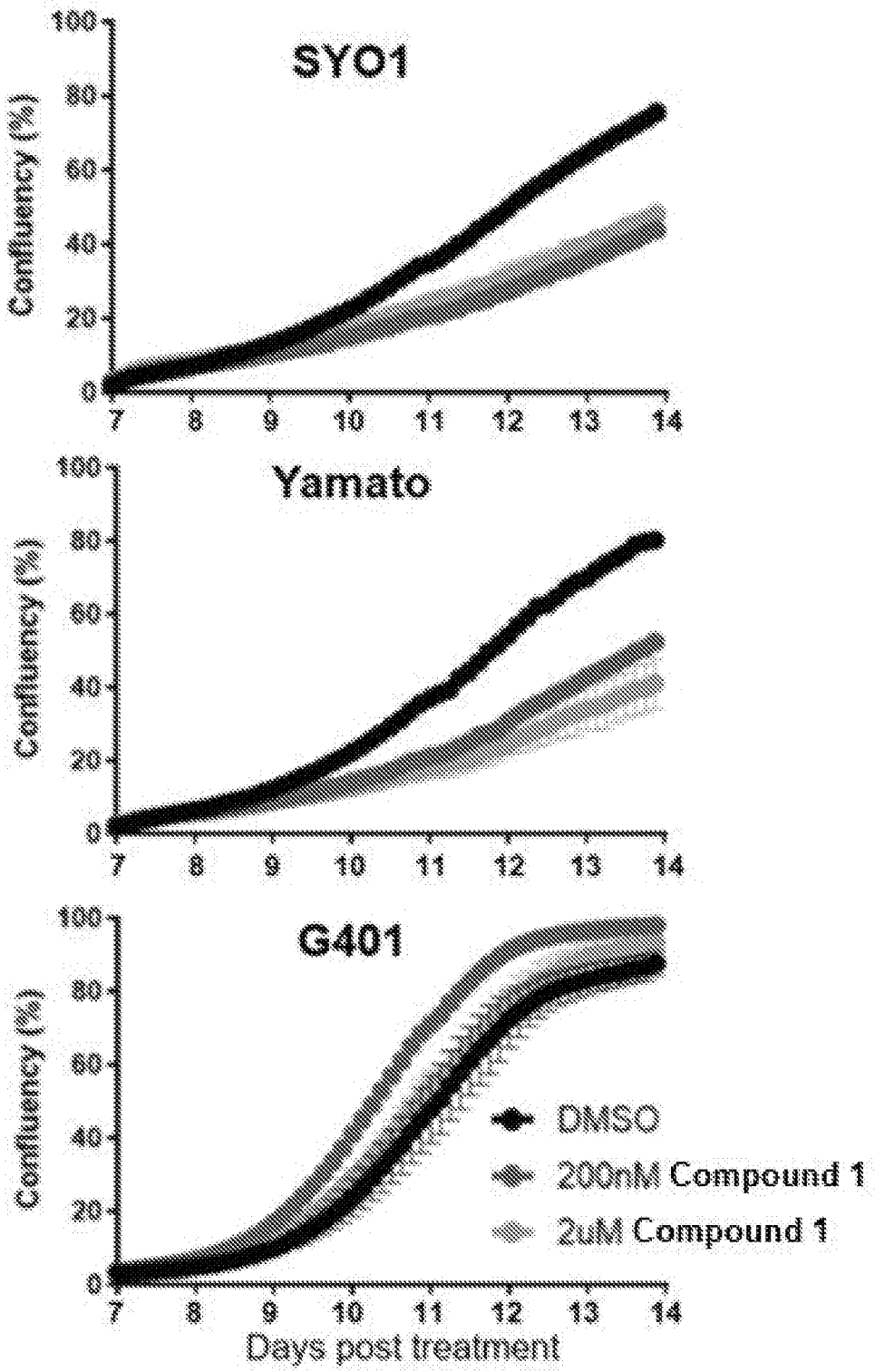

SYO1
Yamato
G401
Confluency (%)
100
80
60
40
20
0
7
8
9
10
11
12
13
14
Days post treatment
DMSO
200nM Compound 1
2uM Compound 1

FIG. 6

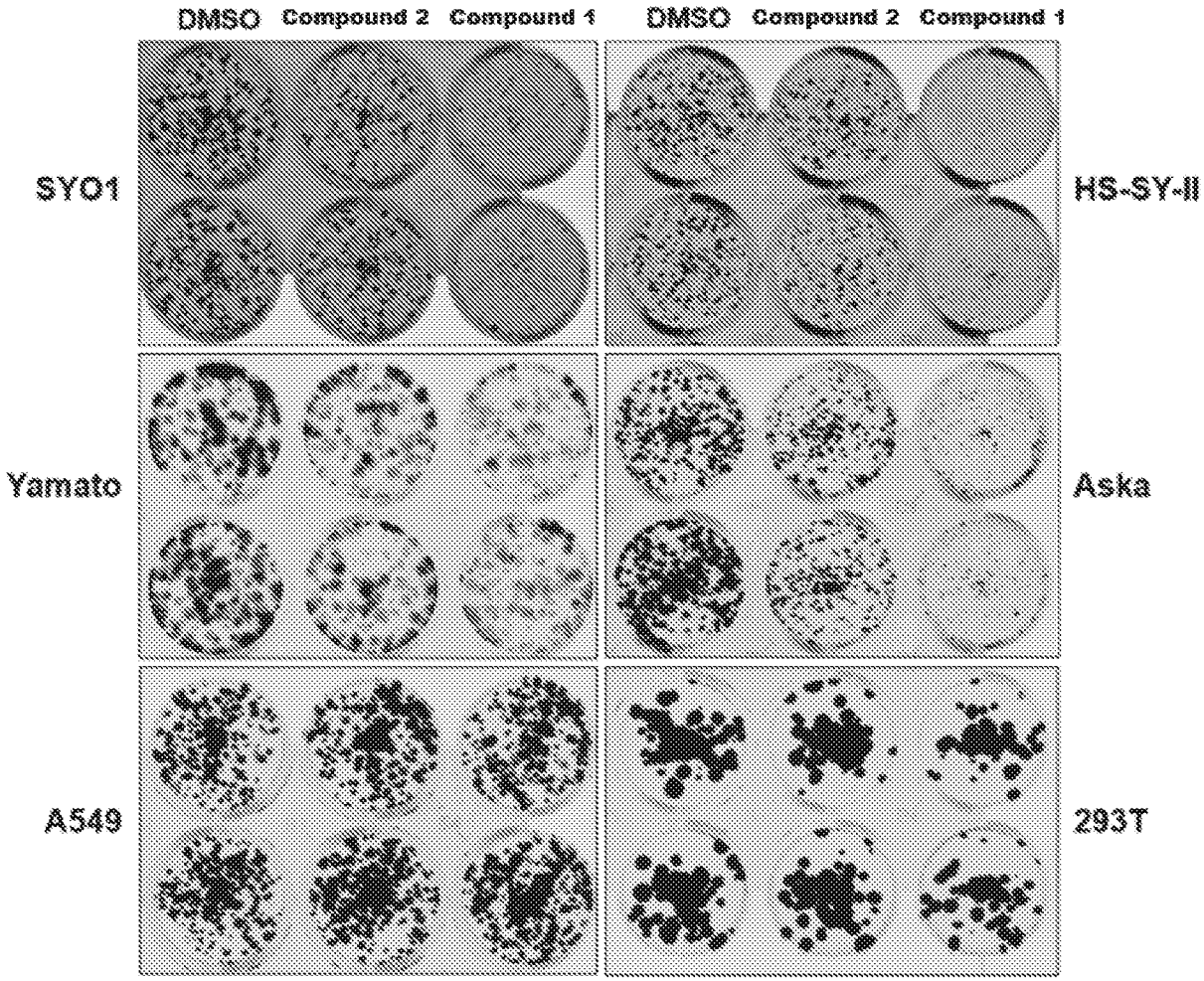

DMSO
Compound 2
Compound 1
DMSO
Compound 2
Compound 1
SYO1
HS-SY-II
Yamato
Aska
A549
293T

FIG. 7

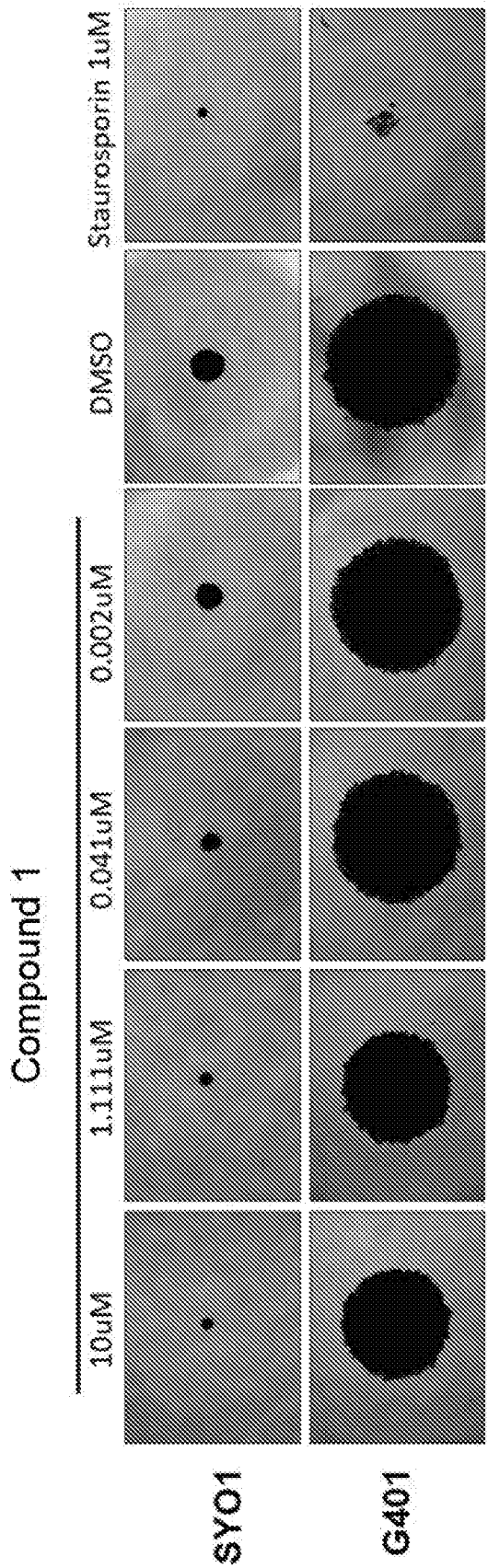
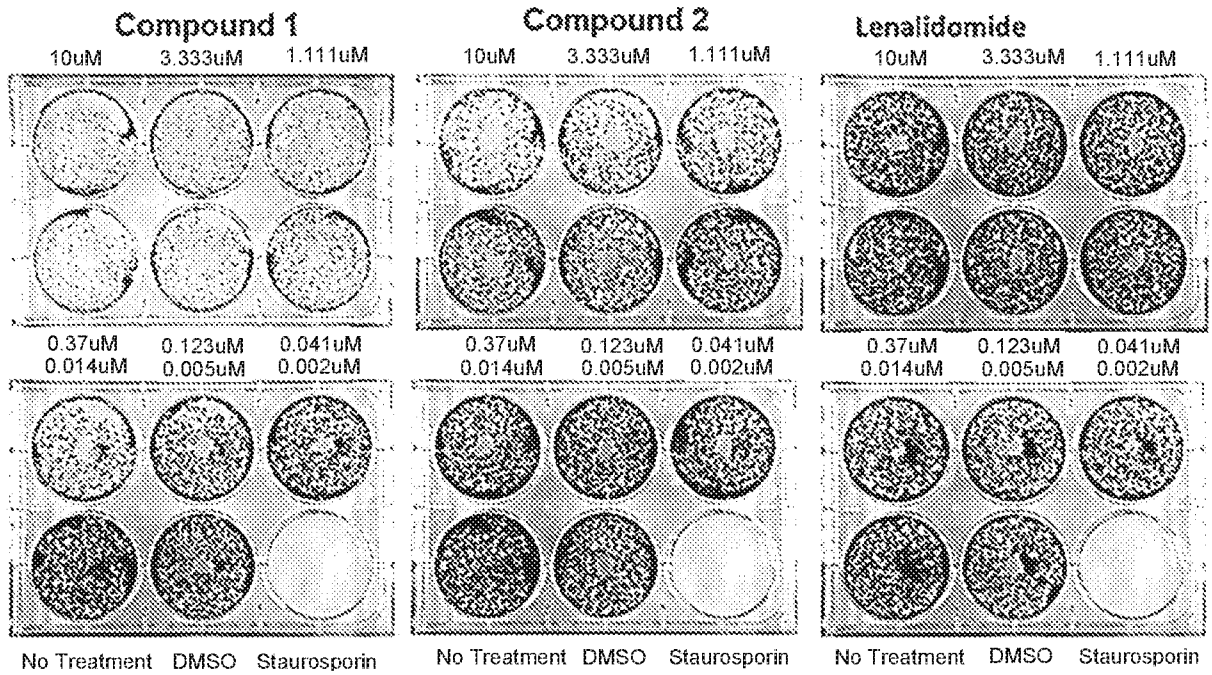
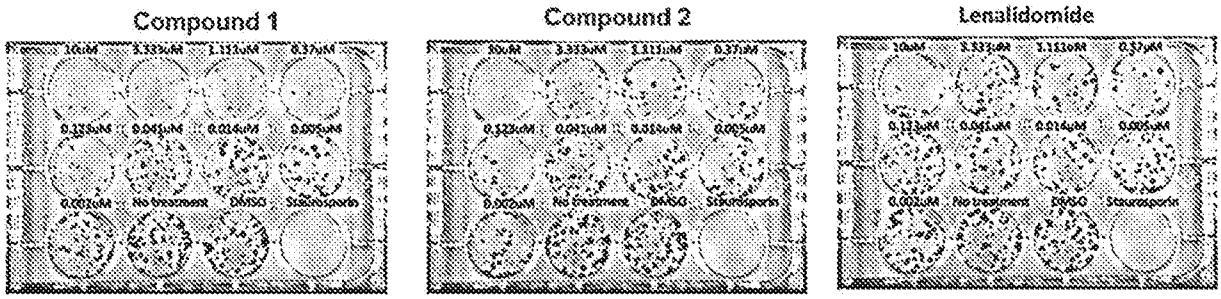

Compound 1
10uM
1.111uM
0.041uM
0.002uM
DMSO
Staurosporin 1uM
SYO1
G401

FIG. 8

SYO1



HS-SY-II



ASKA

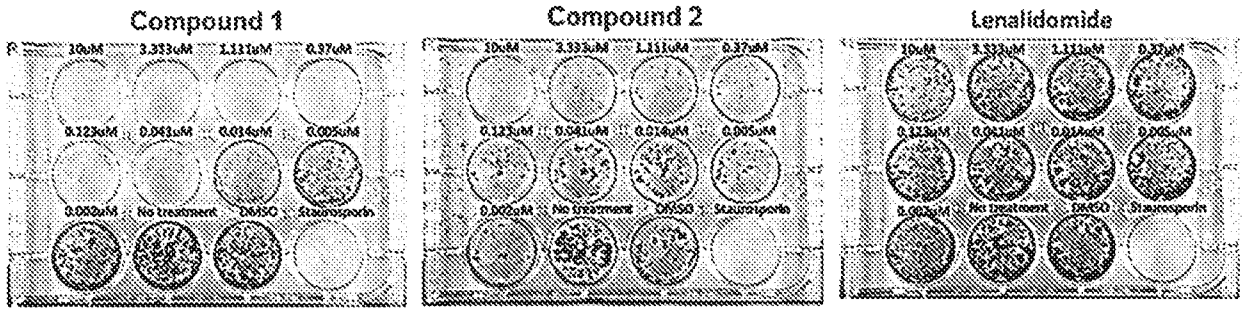
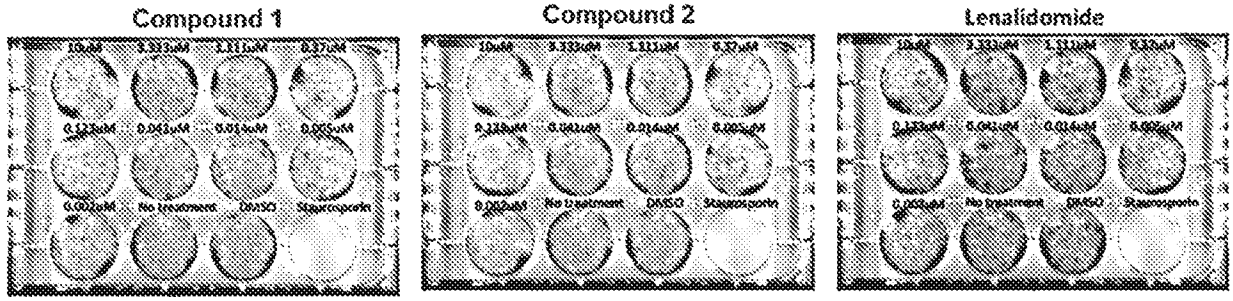
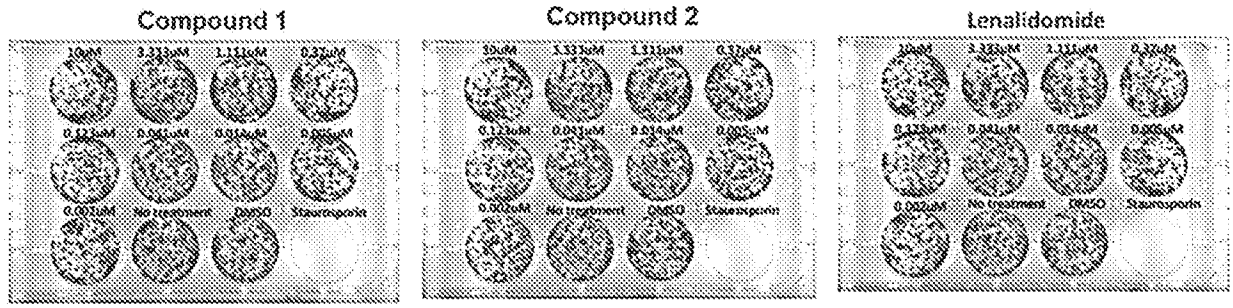


FIG. 9

RD



HCT116



Calu6

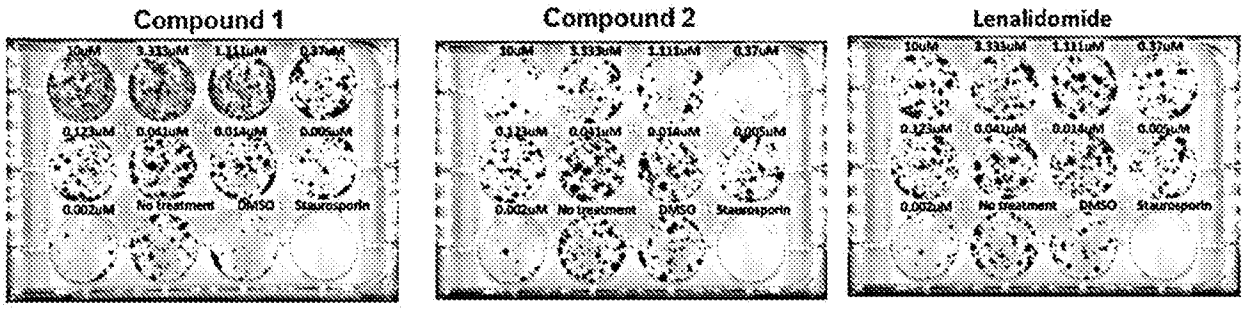


FIG. 10

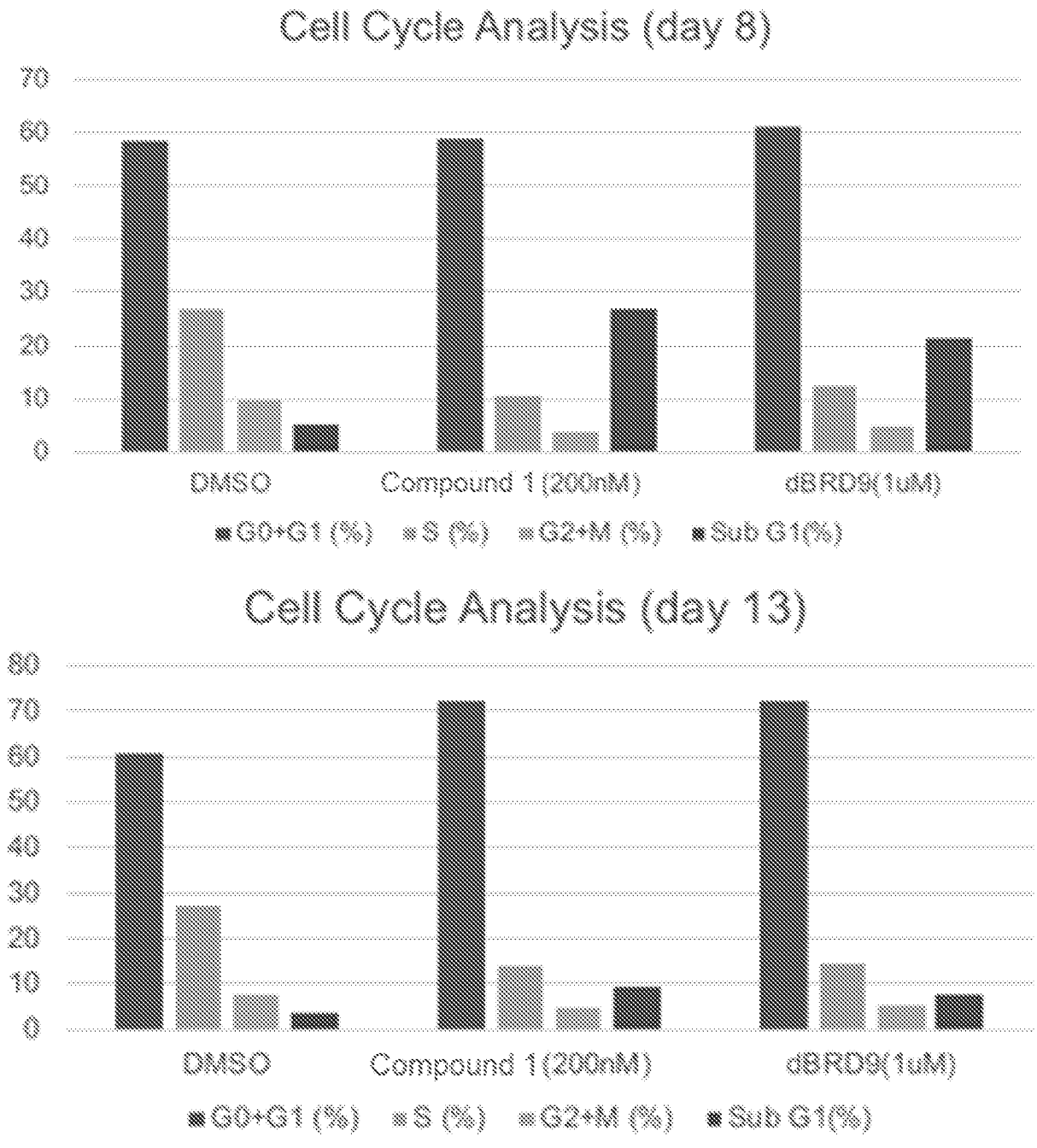
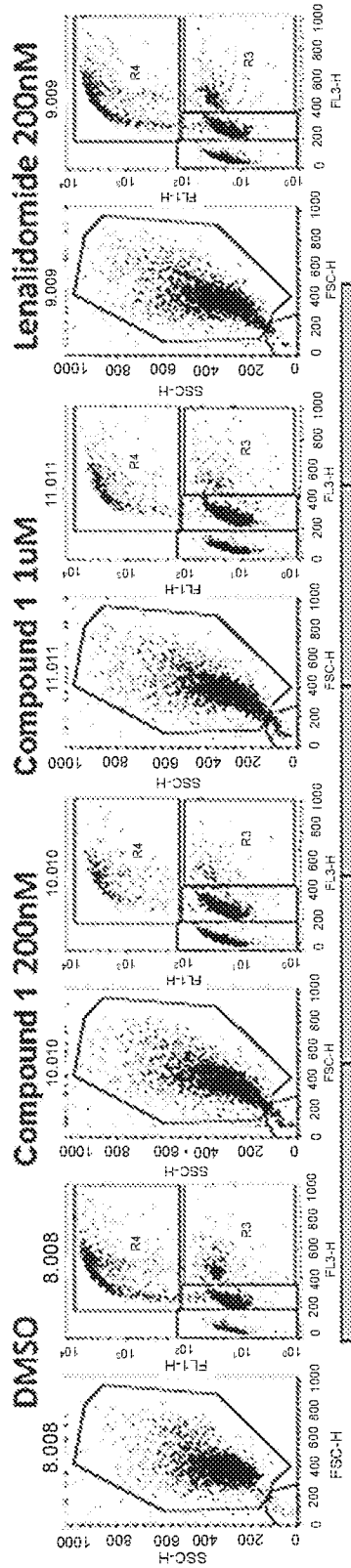
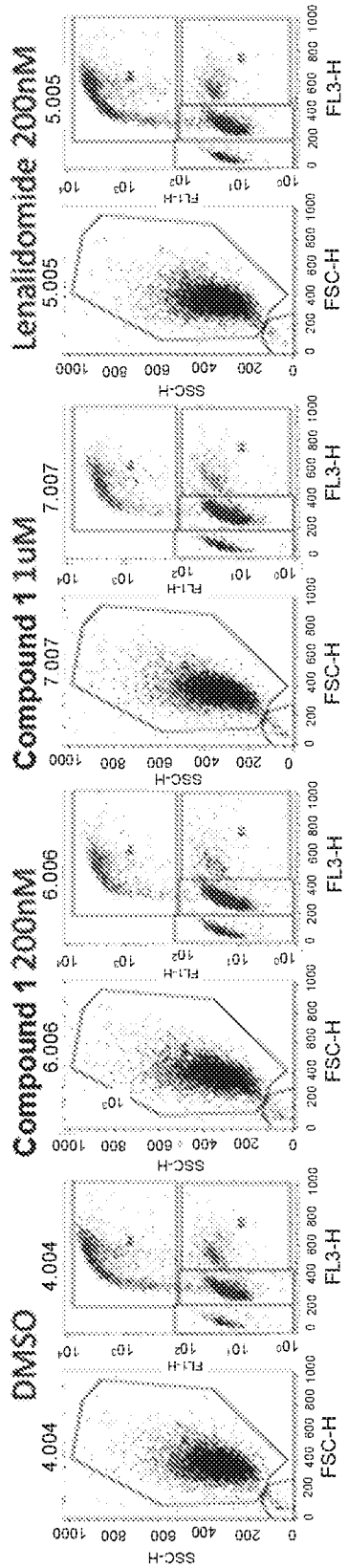

Cell Cycle Analysis (day 8)
70
60
50
40
30
20
10
0
DMSO
Compound 1 (200nM)
dBRD9(1uM)
G0+G1 (%)
S (%)
G2+M (%)
Sub G1(%)
Cell Cycle Analysis (day 13)
80
70
60
50
40
30
20
10
0
DMSO
Compound 1 (200nM)
dBRD9(1uM)
G0+G1 (%)
S (%)
G2+M (%)
Sub G1(%)

FIG. 11



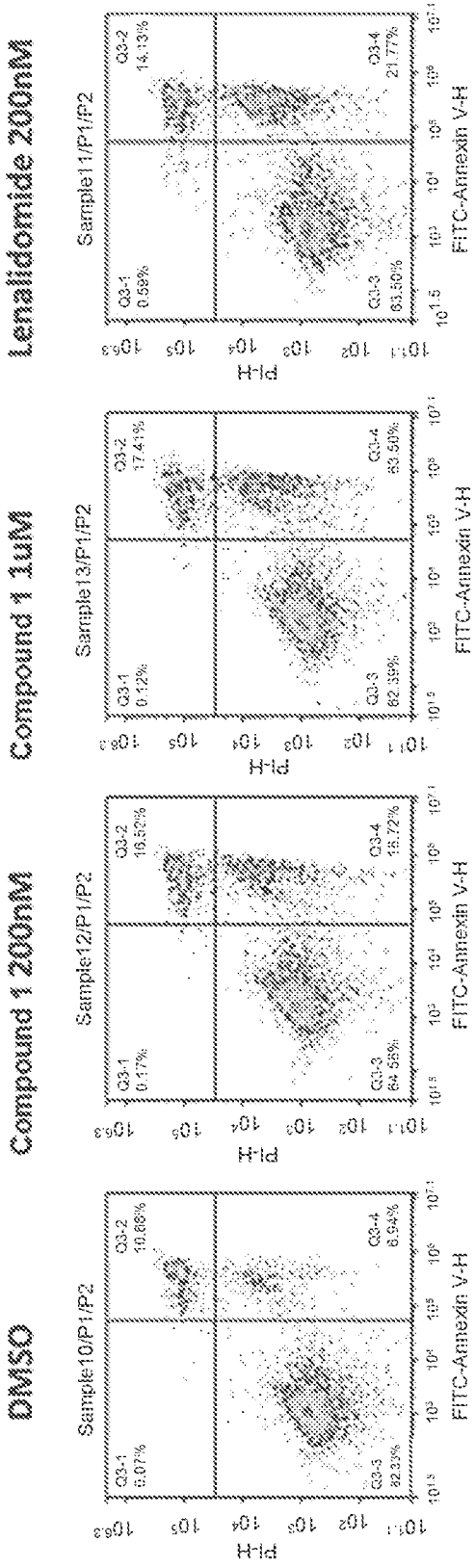
	G0+G1 (%)	S (%)	G2+M (%)	Sub G1(%)
DMSO	58.22	27.01	9.43	5.17
Compound 1 (200nM)	58.9	10.3	3.95	26.61
Compound 1 (1μM)	61.08	12.36	4.8	21.46
Lenalidomide (200nM)	50.45	22.27	8.61	18.43

FIG. 12



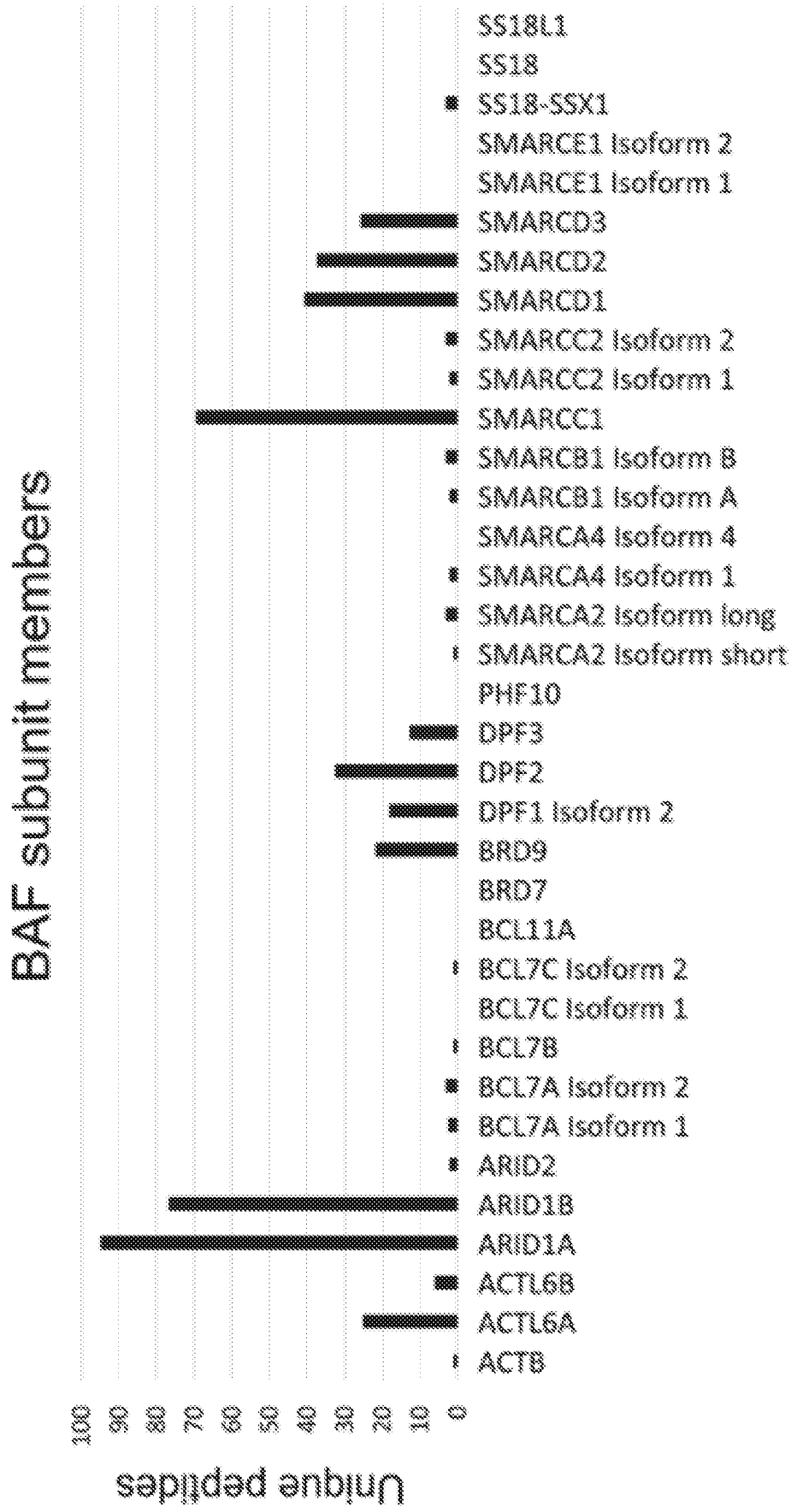
	G0-G1 (%)	S (%)	G2-M (%)	Sub G1(%)
DMSO	60.83	27.29	7.53	3.88
Compound 1 (200nM)	71.9	14.06	4.76	9.1
Compound 1 (1μM)	72.21	14.53	5.08	7.91
Lenalidomide(200nM)	58.67	27.28	6.71	7.11

FIG. 13



	DMSO	Compound 1 (200nM)	Compound 1 (1μM)	Lenalidomide(200nM)
Early Apoptosis Cell	6.94	18.72	20.08	21.77
Late Apoptosis Cell	10.66	16.52	17.41	14.13

FIG. 14


BAF subunit members
SS18L1
SS18
SS18-SSX1
SMARCE1 Isoform 2
SMARCE1 Isoform 1
SMARCD3
SMARCD2
SMARCD1
SMARCC2 Isoform 2
SMARCC2 Isoform 1
SMARCC1
SMARCB1 Isoform B
SMARCB1 Isoform A
SMARCA4 Isoform 4
SMARCA4 Isoform 1
SMARCA2 Isoform long
SMARCA2 Isoform short
PHF10
DPF3
DPF2
DPF1 Isoform 2
BRD9
BRD7
BCL11A
BCL7C Isoform 2
BCL7C Isoform 1
BCL7B
BCL7A Isoform 2
BCL7A Isoform 1
ARID2
ARID1B
ARID1A
ACTL6B
ACTL6A
ACTB
100 90 80 70 60 50 40 30 20 10 0
Unique peptides

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/15733

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 47/50, C07D 471/04, C07D 519/00 (2019.01)
CPC - A61K 47/55, C07D 471/04, C07D 495/04, C07D 519/00, A61K 31/551

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)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2017/223452 A1 (Dana-Farber Cancer Institute, Inc.) 28 December 2017 (28.12.2017) pg 5, ln 23-27, pg 88, ln 14-16, pg 101, ln 3-10, pg 101, ln 12-34 pg 105, ln 16-18, pg 167, ln 33 to pg 168, ln 8, pg 168, ln 16-32, Claim 1, Claim 33, Claim 35, Claim 36, Claim 37	1-5, 11-13, 19-21, 26-30, 32-35, 45-46 ---- 14, 22-25
Y	US 2017/0014491 A1 (The Board of Trustees of the Leland Stanford Junior University) 19 January 2017 (19.01.2017) para [0008], [0054], [0139]	14, 22-25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 June 2019	01 JUL 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/15733

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.:
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:

3. ☒ Claims Nos.: 6-10, 15-18, 31, 36-44, 47-135, 143-146, 154-193
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:

- see extra sheet for Box No. III Observations where unity of invention is lacking -

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 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.:
1-5, 11-14, 19-30, 32-35, 45-46

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/15733

Continuation of:
Box No. III. Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: claims 1-5, 11-14, 19-30, 32-35, 45-46, drawn to a method of treating adult soft tissue sarcoma, cancers or viral infection in a subject in need thereof.

Groups II+: Claims 136-142, drawn to a composition comprising a compound having the structure of Formula K-1, Formula K-2, Formula M-2, Formula M-3, or Formula O-1. Group II+ will be searched upon payment of additional fees. The composition may be searched, for example, to the extent that the the compound encompass of a compound of Formula K-1, wherein Y2 is N, s is 0, R53 is H, R54 is H, R55 is H; for an additional fee and election as such. It is believed that claims 136, 137, 142(in part) read on this exemplary invention. Additional compound(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected compound(s). Failure to clearly identify how any paid additional invention fees are to be applied to the"+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be a compound of Formula K-2, wherein R22 is H, R23 is H, s is 0, X5 is N, X6 is CR58a, X7 is CH, X8 is CH, R58a is H (Claims 136, 138, 142(in part)).

Groups III+: Claims 147-153, drawn to a composition comprising a compound having the structure of Formula I (A-L-B) L is a linker; B is a degradation moiety; and A has the structure of Formula E-3, Formula E-4, Formula G-2, Formula G-3, or Formula E-5. Group III+ will be searched upon payment of additional fees. The formula A-L-B may be searched, for example, to the extent that the the A encompass a compound of Formula E-3, wherein Y2 is N, s is 0, R53 is H, R54 is H, R55 is H; for an additional fee and election as such. It is believed that claims 147, 138, 153(in part) read on this exemplary invention. Additional compound(s) having the formula A-L-B will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected compound(s) having the formula A-L-B. Failure to clearly identify how any paid additional invention fees are to be applied to the"+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be a compound having the formula A-L-B wherein A comprises a compound of Formula E4, R22 is H, R23 is H, s is 0, X5 is N, X6 is CR56, X7 is CH, X8 is CH, R56 is H (Claims 147, 149, 153(in part)).

The inventions listed as Groups I, II+ and III+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I includes the special technical feature of a method which differ from the special technical feature of a composition as disclosed by Groups II+ and III+.

Groups II+ include the special technical feature of a compound having the structure of Formula K-1, Formula K-2, Formula M-2, Formula M-3, or Formula O-1, not required by Groups III+.

Groups III+ include the special technical feature of a compound having the structure of Formula A-L-B, not required by Groups II+.

The inventions of Groups II+ each include the special technical feature of a compound having the structure of Formula K-1, Formula K-2, Formula M-2, Formula M-3, or Formula O-1, not required by any of the other inventions of Group II+.

The inventions of Groups III+ each include the special technical feature of a compound having the structure of Formula A-L-B, not required by any of the other inventions of Group III+.

Common Technical Features

The inventions of Groups I, II+ and III+ share the technical feature of inhibiting the activity of bromodomain-containing proteins.
The inventions of Groups II+ and III+ share the technical feature of a compound comprising the activity to inhibit bromodomain-containing proteins.
The inventions of Groups II+ and III+ share the technical feature of claims 136 and 147, respectively.

However, these shared technical features do not represent a contribution over prior art in view of WO 2017/223452 A1 to Dana-Farber Cancer Institute, Inc. (hereafter 'DFCI').

DFCI teaches (instant claim 136) a compound having the structure of Formula K-2, wherein R22 is C1-C6 alkyl, R23 is H, s is 1, R25 is C1-C6 heteroalkyl, X5 is CR58a, X6 is N, X7 is H, X8 is H (pg 31, compound TL-1).

DFCI teaches (instant claim 147) a compound having the structure of Formula I (A-L-B) L is a linker;
B is a degradation moiety (pg 2, ln 13-20, bifunciional compounds, which function to recruit targeted proteins to E3 ubiquitin ligase for degradation, methods of preparation and use thereof. The bifunction compound is of Formula X: (Targeting Ligand ? Linker ? Degron); wherein: the Targeting Ligand is capable of binding to a targeted protein such as a bromodomain-containing protein (e.g. , BRD9); the Linker is a group that covalently binds to the Targeting Ligand and the Degron; the Degron is capable of binding to a ubiquitin ligase, such as an E3 ubiquitin ligase (e.g. cereblon).); A has the structure of Formula E-4, wherein R22 is C1-C6 alkyl, R23 is H, s is 1, R25 is C1-C6 heteroalkyl, X5 is CR58a, X6 is N, X7 is H, X8 is H (pg 31, compound TL-1).

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I, II+, and III+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.
Claims 6-10, 15-18, 31, 36-44, 47-135, 143-146, 154-189, 190-193 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).