

Description

Title of Invention: NOVEL GSPT1 DEGRADER AND USE THEREOF

Technical Field

- [1] The present invention relates to a novel GSPT1 degrader and a use thereof. Specifically, the present invention relates to a compound represented by Formula I, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof; a pharmaceutical composition for treating a disorder of uncontrolled cell proliferation comprising the same; and a method of treating a disorder of uncontrolled cell proliferation by administering the same to a mammal.

Background Art

- [2] The traditional drug development strategy is to directly regulate the activity of the protein by binding the drug to the specific active site of disease-related proteins such as enzymes, receptors, transmitters, and membrane proteins. Therefore, transcription factors, scaffold proteins, aggregates, and the like in the nucleus remain undruggable targets for drug development due to the lack of a docking pocket structure. According to the Human Protein Atlas, there are FDA-approved drugs for only about 15% of the approximately 4,500 known disease-related proteins.
- [3] In order to overcome these limitations of traditional drug development strategies, research on targeted protein degradation techniques has been actively conducted for targets that are difficult to develop drugs.
- [4] Targeted protein degradation techniques seek to treat diseases by removing disease-related proteins using the protein degradation system existing in the body. Intracellular protein degradation is accomplished by lysosomes and proteasomes, and approximately 80% of cellular proteins are labeled with ubiquitin by the ubiquitin-proteasome system (UPS) and then degraded by proteasome.
- [5] Ubiquitin is a protein composed of 76 amino acids. E1, E2, and E3 ubiquitin ligases are involved in the process of labeling ubiquitin for protein degradation by UPS (ubiquitination), and the labeled protein is degraded by 26S proteasome, which is an ATP-dependent protein degradation enzyme complex. E3 ubiquitin ligase binds to both E2 ligase and substrate proteins and is responsible for recognizing substrate proteins to be labeled with ubiquitin.
- [6] Drugs using the TPD strategy can be classified into PROTAC (proteolysis targeting chimera) and molecular glues depending on the compound structure. PROTAC is a heterobifunctional compound in which the ligand of E3 ligase, a component of the UPS, and the ligand of the target protein are connected through a linker. On the other

hand, the molecular glues use the characteristic of small molecule compounds to induce the formation of specific protein complexes to promote the interaction between the target protein and E3 ligase involved in protein degradation, thereby degrading the target protein. The molecular glues have a much lower molecular weight than PROTAC, and therefore have the advantage of enabling the development of therapeutic agents with excellent pharmacokinetic properties.

- [7] Previous studies have shown that immunomodulatory imide (IMiD) molecules, including thalidomide, lenalidomide, and pomalidomide, bind to cereblon (CRBN), a substrate receptor for CRL4 E3 ubiquitin ligase (Ito et al., Science 327:1345-1350 (2010)). It has been reported that when IMiD binds to CRBN, it generates a new surface that causes neo-interaction between CRBN and Ikaros (IKZF1) and Aiolos (IKZF3), thereby allowing the IMiD drug to cause CRBN-dependent ubiquitination and subsequent protein degradation of Ikaros and Aiolos (Kronke et al., Science 343:301-305 (2014); Lu et al., Science 343:305-309 (2014)).
- [8] Meanwhile, International Publication No. WO 2022/066835 discloses an IMiD-based compound that selectively degrades GSPT1 compared to Ikaros and Aiolos. However, this literature lacks research on characteristics essential for successful anticancer agent development, such as the compound's persistence in GSPT1 degradation, safety, stability, and the like.

Disclosure of Invention

Technical Problem

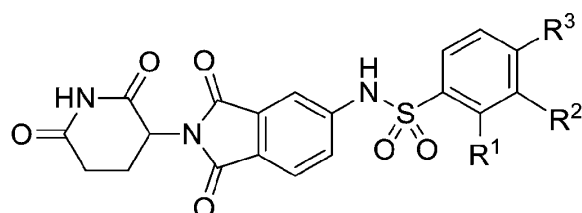
- [9] Accordingly, through extensive characterization studies of IMiD-based compounds, the present inventors found that when a specific substituent is present at each position of the phenylsulfonamide ring bound to the pomalidomide structure, the persistence in GSPT1 degradation and pH stability, as well as anticancer efficacy and safety, are significantly improved, and excellent anticancer efficacy could be achieved against a cancer with a neuroendocrine phenotype.

Solution to Problem

- [10] In one aspect of the present invention, there is provided a compound represented by Formula I below, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof:

[11] [Formula I]

[12]



- [13] in Formula I,
- [14] R¹ is halogen, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, or C₁-C₆ haloalkoxy;
- [15] R² is C₁-C₆ alkyl optionally substituted with R^{2a};
- [16] R^{2a} is hydroxy, halogen, C₁-C₆ alkoxy, C₁-C₆ haloalkoxy, or -NR'R'';
- [17] R' and R'' are each hydrogen or C₁-C₆ alkyl, or R' and R'' may be taken together with the nitrogen atom to which they are attached to form a 3 to 8-membered heterocyclic ring optionally comprising one additional heteroatom selected from N, O, and S; and
- [18] R³ is hydrogen or halogen.
- [19] In another aspect of the present invention, there is provided a pharmaceutical composition for treating a disorder of uncontrolled cell proliferation in a mammal, comprising a compound represented by Formula I, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof.
- [20] In another aspect of the present invention, there is provided a method of regulating a cereblon activity or a GSPT1 activity in at least one cell, comprising contacting the at least one cell *in vitro* with a compound represented by Formula I, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof.
- [21] In another aspect of the present invention, there is provided a method of treating a disorder of uncontrolled cell proliferation in a mammal, comprising administering a compound represented by Formula I, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof to a mammal.

Advantageous Effects of Invention

- [22] The compound according to the present invention exhibits high selectivity and sustained degradation activity against GSPT1, excellent pH stability, and low cytotoxicity against normal cells, and thus has excellent anticancer efficacy and a high therapeutic index. In addition, the compound according to the present invention can exhibit excellent anticancer activity against a cancer with a neuroendocrine phenotype, such as small cell lung cancer (SCLC), lung neuroendocrine cancer (NEC), and neuroendocrine prostate cancer (NEPC).

Brief Description of Drawings

- [23] Figure 1 illustrates a result obtained by measuring the GSPT1 degradation activity of Reference Compound 1 and example compounds on various neosubstrates by concentration.
- [24] Figure 2 illustrates a result obtained by observing the change in GSPT1 expression level over time when treated with Reference Compound 1 and example compounds (100 nM).
- [25] Figure 3 illustrates a result obtained by measuring the real time proliferation of cancer cells (NCI-H1155 cells) after treatment with Reference Compound 1 and

example compounds.

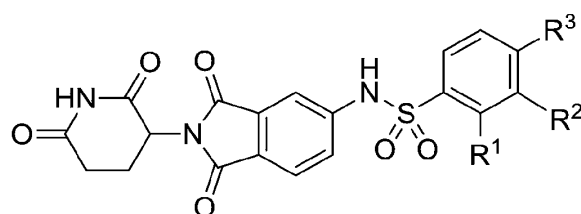
- [26] Figure 4 illustrates a result obtained by performing TMT labeling proteomics analysis after treating the HL60 cell line with example compounds.
- [27] Figure 5 illustrates a result obtained by observing the GSPT1 expression level and the protein translation rate over time after treating the NCI-H1155 cells with 1 μ M of Compound 3.
- [28] Figure 6 illustrates a result obtained by observing the GSPT1 expression level, the N-MYC expression level, and the protein translation rate (left); and a result obtained by measuring the expression levels of GSPT1, N-MYC, and ATF-4 and the degree of activation of caspase 3 (right), over time after treating the NCI-H1155 cells with 1 μ M of Compound 3.
- [29] Figure 7 illustrates a result obtained by measuring the GSPT1 expression level, the ATF-4 expression level, and the degree of activation of caspase 3 over time after treating the HL60 cells with 0.3 μ M of Compound 3.
- [30] Figure 8 illustrates a result obtained by observing the GSPT1 expression level and the protein translation rate over time after treating the NCI-H2023 cells with 1 μ M of Reference Compound 1 and Compound 3.
- [31] Figure 9a illustrates a result obtained by measuring the cell viability after treating various small cell lung cancer (SCLC) and lung adenocarcinoma (LUAD) cell lines with Compound 3 and Compound 4.
- [32] Figure 9b illustrates a result obtained by calculating the EC₅₀ of Compound 3 and Compound 4 for lung adenocarcinoma (LUAD), small cell lung cancer (SCLC), lung neuroendocrine cancer (NEC), and neuroendocrine prostate cancer (NEPC) cell lines.
- [33] Figure 10 illustrates the therapeutic index (TI) of each compound calculated based on the cell viability of Reference Compound 1, Compound 3, and Compound 4 measured in the NCI-H1155, HL60 and HeKa cells.
- [34] Figure 11 illustrates the change in bioluminescence (Figure 11a) and the change in body weight (Figure 11b) over time after administration of Compound 3 in the HL-60-Luc AML animal model.
- [35] Figure 12a illustrates a result obtained by observing the expression levels of GSPT1 and N-MYC 6 and 24 hours after administration of Compound 4 in the NCI-H1155 lung cancer animal model (Figure 12a); and a result obtained by measuring the change in tumor volume over time after administration of Compound 4 (Figure 12b).
- [36] Figure 13 illustrates a result obtained by measuring the change in tumor volume (Figure 13a) and the change in body weight (Figure 13b) over time after administration of Compound 3 at various dosage and administration cycles.

Mode for the Invention

- [37] Hereinafter, the present invention will be described in more detail.
- [38] Each description and embodiment disclosed herein can also be applied to each of other descriptions and embodiments. That is, all combinations of the various elements disclosed herein fall within the scope of the present application. In addition, the scope of the present application is not to be construed as being limited by the specific descriptions set forth below.
- [39] In one aspect of the present invention, there is provided a compound represented by Formula I below, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof:

[40] [Formula I]

[41]



- [42] in Formula I,
- [43] R¹ is halogen, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, or C₁-C₆ haloalkoxy;
- [44] R² is C₁-C₆ alkyl optionally substituted with R^{2a};
- [45] R^{2a} is hydroxy, halogen, C₁-C₆ alkoxy, C₁-C₆ haloalkoxy, or -NR'R'';
- [46] R' and R'' are each hydrogen or C₁-C₆ alkyl, or R' and R'' may be taken together with the nitrogen atom to which they are attached to form a 3 to 8-membered heterocyclic ring optionally comprising one additional heteroatom selected from N, O, and S; and
- [47] R³ is hydrogen or halogen.
- [48] In Formula I above, R¹ is halogen, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, or C₁-C₆ haloalkoxy. In one embodiment, R¹ is halogen, C₁-C₃ alkyl, C₁-C₃ alkoxy, C₁-C₃ haloalkyl, or C₁-C₃ haloalkoxy. In one embodiment, R¹ may be halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₁-C₆ haloalkoxy. In one embodiment, R¹ may be halogen, C₁-C₃ alkyl, C₁-C₃ haloalkyl, or C₁-C₃ haloalkoxy. In one embodiment, R¹ may be halogen or C₁-C₆ alkyl. In one embodiment, R¹ may be halogen or C₁-C₃ alkyl. In one embodiment, R¹ may be a halogen (F, Cl, Br, or I). For example, R¹ includes F, Cl, Br, I, -OCH₂F, -OCHF₂, -OCF₃, -CH₂F, -CHF₂, -CF₃, or -CH₃, but is not limited thereto.
- [49] In Formula I above, R² is C₁-C₆ alkyl or C₁-C₆ alkyl substituted with R^{2a}. In one embodiment, R² may be C₁-C₆ alkyl. In one embodiment, R² may be C₁-C₃ alkyl. In one embodiment, R² may be C₁-C₆ alkyl substituted with R^{2a}. In one embodiment, R² may be C₁-C₃ alkyl substituted with R^{2a}. In this case, R^{2a} may be hydroxy, halogen, C₁-C₆ alkoxy, or C₁-C₆ haloalkoxy. In one embodiment, R^{2a} may be hydroxy, halogen, C₁-C₃ alkoxy, or C₁-C₃ haloalkoxy. In one embodiment, R^{2a} may be hydroxy or C₁-C₆

haloalkoxy. In one embodiment, R^{2a} may be hydroxy or C₁-C₃ haloalkoxy. For example, R² includes -CH₃, -CH₂CH₃, -CH₂OCF₃, -CH₂OCHF₂, -CH₂OCH₂F, -CH₂OH, or -CH₂CH₂OH, but is not limited thereto.

[50] Alternatively, R² may be C₁-C₆ alkyl substituted with R^{2a}, and R^{2a} may be -NR'R". In one embodiment, R² may be C₁-C₃ alkyl substituted with R^{2a}. R' and R" may be each hydrogen or C₁-C₆ alkyl. In one embodiment, said R' and R" may be each hydrogen or C₁-C₃ alkyl.

[51] In addition, said R' and R" may be taken together with the nitrogen atom to which they are attached to form a 3 to 8-membered heterocyclic ring optionally comprising one additional heteroatom selected from N, O, and S. In one embodiment, said R' and R" may be taken together with the nitrogen atom to which they are attached to form a morpholinyl, thiomorpholinyl, piperazinyl, or piperidinyl ring.

[52] For example, R² includes -CH₂-morpholinyl, -CH₂-CH₂-morpholinyl, -CH₂-NH₂, -CH₂-NH(CH₃), -CH₂-N(CH₃)₂, -CH₂CH₂-NH₂, -CH₂-CH₂NH(CH₃), or -CH₂-CH₂-N(CH₃)₂, but is not limited thereto.

[53] In Formula I above, R³ is hydrogen or halogen. In one embodiment, R³ is hydrogen. In one embodiment, R³ is halogen. For example, R³ may be F, Cl, Br, or I. For example, R³ may be F.

[54] The present inventors have found the problem that the compounds disclosed in the prior document (WO 2022/066835) do not exhibit a sustained GSPT1 degradation efficacy, and after a certain period of time, for example, 48 hours after treatment, the GSPT1 expression level is restored and the tumor cells whose proliferation was suppressed grow again. Without intending to be bound by theory, the present inventors have found that the compounds disclosed in the prior document are unstable at physiological pH (pH 7), and the present inventors believe that this pH instability of the compounds disclosed in the above prior document is one of the reasons why the GSPT1 degradation activity and tumor inhibition activity are transient.

[55] Accordingly, in order to obtain a compound that has improved pH stability, exhibits sustained and selective degradation activity against GSPT1, and has excellent tumor proliferation inhibition activity, the present inventors conducted extensive research on the properties of a compound in which phenylsulfonamide is bound to the pomalidomide structure by modifying its structure. As a result, it was found that a compound having an alkyl group or an alkyl group substituted with a specific substituent at the meta position based on the position at which the sulfonamide group is bonded exhibits excellent pH stability, sustained and selective degradation activity against GSPT1, and excellent tumor proliferation inhibition activity. In particular, the present inventors have found that a compounds which has, in addition to the above substitution at the meta position, a halogen or an alkyl or alkoxy group optionally sub-

stituted with halogen at the adjacent ortho position, and which is unsubstituted or substituted with halogen (preferably, F) at the adjacent para position, exhibits significantly improved properties in terms of pH stability, GSPT1 degradation activity, and tumor proliferation inhibition activity.

[56] In one embodiment, the compound represented by Formula I of the present invention may have the following combination of substituents:

[57] R¹ is halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₁-C₆ haloalkoxy;

[58] R² is C₁-C₆ alkyl optionally substituted with R^{2a};

[59] R^{2a} is hydroxy or C₁-C₆ haloalkoxy; and

[60] R³ is hydrogen or halogen.

[61] In one embodiment, the compound represented by Formula I of the present invention may have the following combination of substituents:

[62] R¹ is halogen or C₁-C₆ alkyl;

[63] R² is C₁-C₆ alkyl; and

[64] R³ is hydrogen or halogen.

[65] For example, in Formula I above, R¹ may be halogen or C₁-C₃ alkyl; R² may be C₁-C₃ alkyl; and R³ may be hydrogen or halogen. For example, in Formula I above, R¹ may be F, Cl, Br, I, -CH₃, or -CH₂CH₃; R² may be -CH₃ or -CH₂CH₃; and R³ may be hydrogen or F.

[66] In one embodiment, the compound represented by Formula I of the present invention may have the following combination of substituents:

[67] R¹ is C₁-C₆ haloalkyl or C₁-C₆ haloalkoxy;

[68] R² is C₁-C₆ alkyl; and

[69] R³ is hydrogen or halogen.

[70] For example, in Formula I above, R¹ may be C₁-C₃ haloalkyl or C₁-C₃ haloalkoxy; R² may be C₁-C₃ alkyl; and R³ may be hydrogen or halogen. For example, in Formula I above, R¹ may be -OCH₂F, -OCHF₂, -OCF₃, -CH₂F, -CHF₂, or -CF₃; R² may be -CH₃ or -CH₂CH₃; and R³ may be hydrogen or F.

[71] In one embodiment, the compound represented by Formula I of the present invention may have the following combination of substituents:

[72] R¹ is halogen;

[73] R² is C₁-C₆ alkyl optionally substituted with R^{2a};

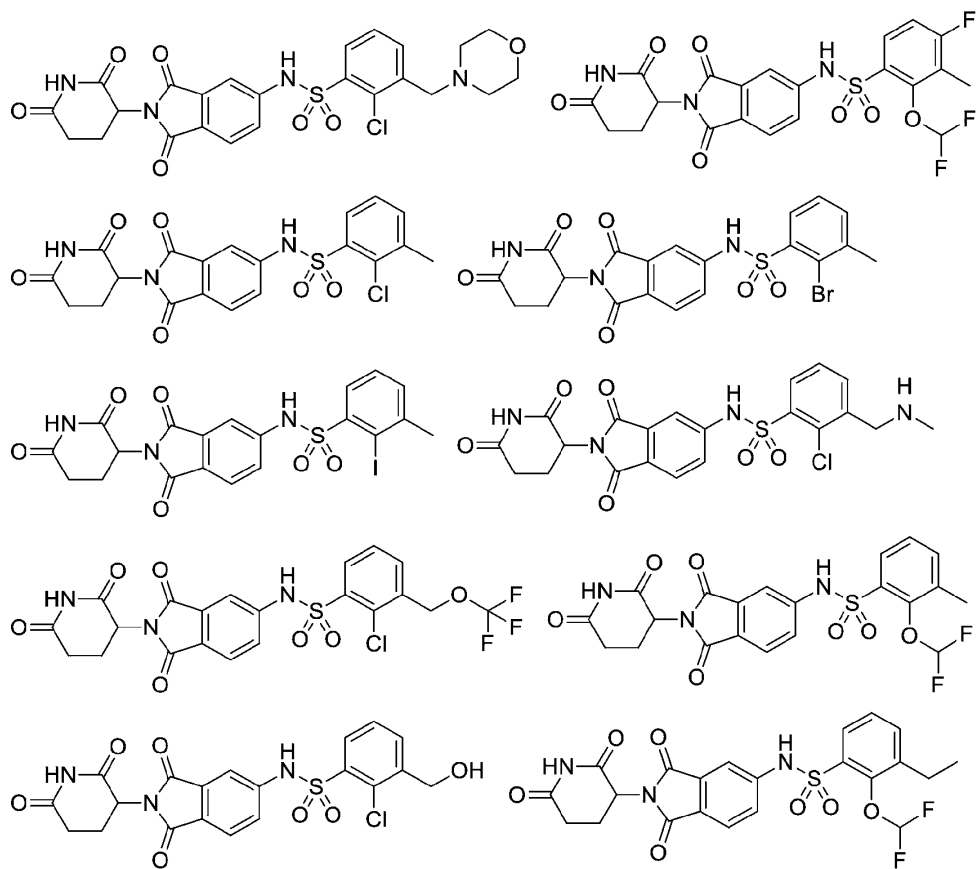
[74] R^{2a} is hydroxy or C₁-C₆ haloalkoxy; and

[75] R³ is hydrogen.

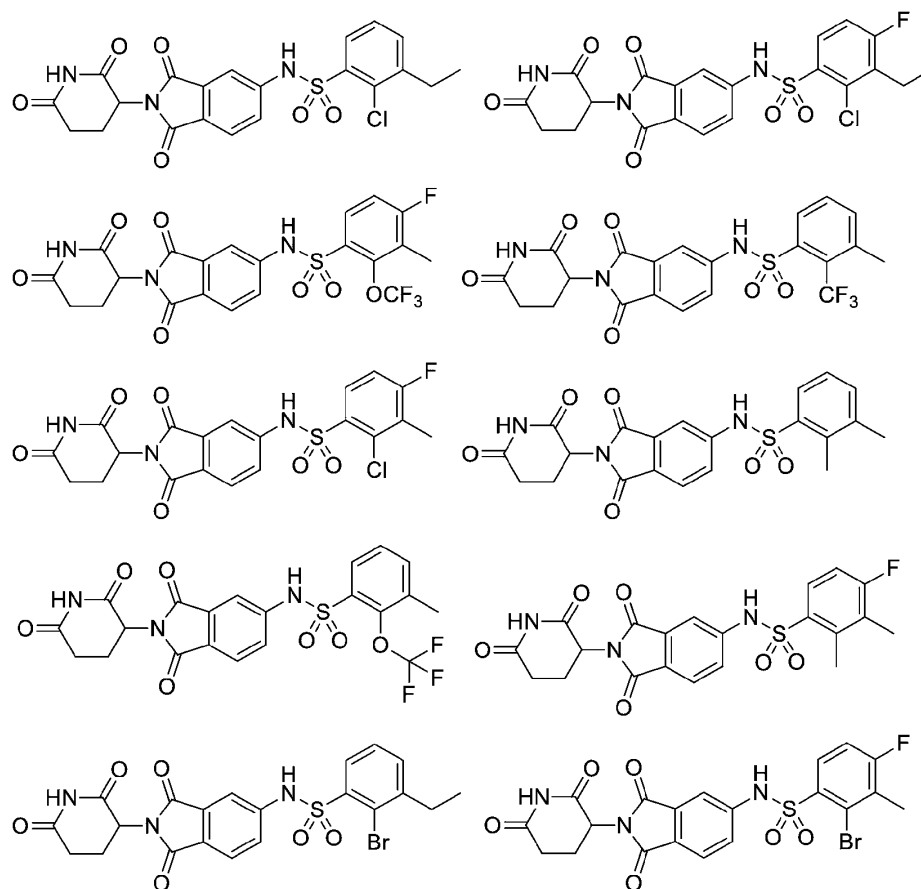
[76] For example, in Formula I above, R¹ may be halogen, R² may be C₁-C₃ alkyl optionally substituted with hydroxy or C₁-C₃ haloalkoxy; and R³ may be hydrogen. For example, in Formula I above, R¹ may be Cl, Br, or I; and R² may be -CH₂OCHF₂, -CH₂OCH₂F, -CH₂OH, or -CH₂CH₂OH.

- [77] In one embodiment, in the compound represented by Formula I of the present invention, R¹ may be halogen, R² may be C₁-C₆ alkyl, and R³ may be hydrogen. For example, in Formula I above, R¹ may be Cl, Br, or I; R² may be C₁-C₃ alkyl, such as methyl or ethyl; and R³ may be hydrogen.
- [78] In one embodiment, the compound represented by Formula I of the present invention has the following combination of substituents:
- [79] R¹ is halogen;
- [80] R² is C₁-C₆ alkyl substituted with NR'R'';
- [81] R' and R'' are each hydrogen, C₁-C₆ alkyl, or C₁-C₃ alkyl; and
- [82] R³ is hydrogen.
- [83] For example, in the compound represented by Formula I of the present invention, R¹ may be halogen (for example, Cl); R² may be methylaminomethyl; and R³ may be hydrogen.
- [84] In one embodiment, the compound represented by Formula I of the present invention may have the following combination of substituents:
- [85] R¹ is halogen;
- [86] R² is C₁-C₆ alkyl substituted with NR'R'';
- [87] R' and R'' may be taken together with the nitrogen atom to which they are attached to form a 3 to 8-membered heterocyclic ring optionally comprising one additional heteroatom selected from N, O, and S; and
- [88] R³ is hydrogen.
- [89] In one embodiment, the compound represented by Formula I of the present invention may have the following combination of substituents:
- [90] R¹ is halogen;
- [91] R² is C₁-C₆ alkyl substituted with NR'R'';
- [92] R' and R'' may be taken together with the nitrogen atom to which they are attached to form a morpholinyl, thiomorpholinyl, piperazinyl, or piperidinyl ring; and
- [93] R³ is hydrogen.
- [94] For example, in the compound represented by Formula I of the present invention, R¹ may be halogen (for example, Cl); R² may be morpholinylmethyl, and R³ may be hydrogen.
- [95] Regarding the above combinations of R¹, R², and R³, the specific examples previously described for each of R¹, R², and R³ can also be applied.
- [96] The compound represented by Formula I may be a compound represented by any one of the following formulas:

[97]

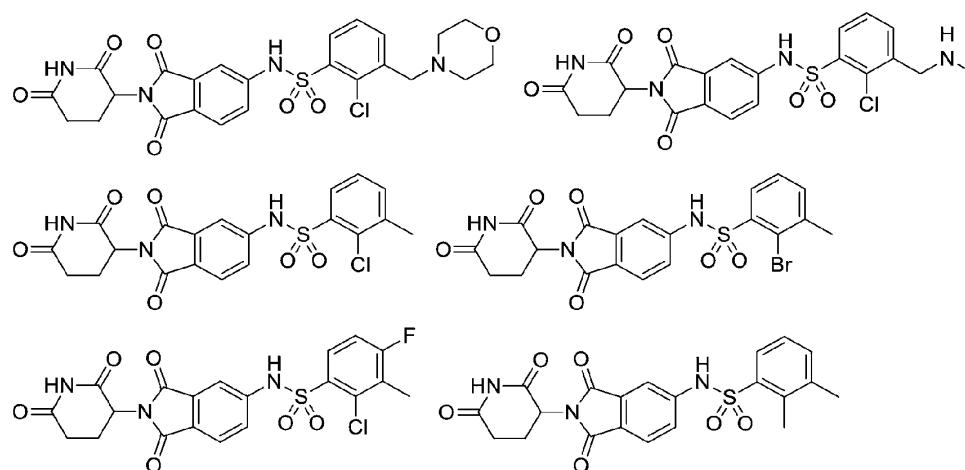


[98]



[99] In one embodiment, the compound represented by Formula I according to the present invention may be a compound having any one of the following structures:

[100]

[101] **Definition**

[102] All technical and scientific terms used herein have meanings commonly understood

by those of ordinary skill in the art and, unless otherwise stated, conventional measurement methods, manufacturing methods, and conventional ingredients or substances are used based on conventional techniques such as pharmacology, pharmaceutical chemistry, mass spectrometry, NMR, HPLC, and biochemistry.

- [103] Individual features and components of each implementation described and illustrated herein may be combined with features and components of any other implementation without departing from the scope or spirit of the present disclosure.
- [104] Unless otherwise specified, in the present specification and the appended claims, "or" and "and" mean "and/or". The terms "include" and "included" are open-ended and mean that a compound, composition or method may include additional features or ingredients in addition to the specific features or ingredients listed.
- [105] In the present specification, the numerical range indicated using the term "to" refers to a range that includes the numerical values described before and after the term "to" as the lower limit and the upper limit, respectively.
- [106] As used herein, the term "optional" or "optionally" means that a subsequently described event or circumstance may or may not occur, and that the description includes instances in which said event or circumstance occurs and instances in which it does not occur. For example, the term "optionally substituted" means both substitution and unsubstitution with the specified substituents.
- [107] **Compound**
- [108] As used herein, unless otherwise stated, the term "alkyl", whether used alone or as a part of a substituent, refers to a saturated straight chain and branched carbon chain having 1 to 6 carbon atoms, 1 to 4 carbon atoms, or 1 to 3 carbon atoms. Non-limiting examples of the alkyl group include methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, and the like.
- [109] The term "alkoxy" refers to the -O-alkyl group. Non-limiting examples of the alkoxy group include methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, sec-butoxy, iso-butoxy, tert-butoxy, and the like.
- [110] The term "heteroatom" means nitrogen, oxygen, or sulfur, and includes any oxidized form of nitrogen, such as N(O) (N⁺-O⁻) and sulfur, such as S(O) and S(O)₂, and any basic quaternized forms of nitrogen.
- [111] The term "heterocycloalkyl", "heterocyclic", or "heterocycle" refers to a saturated or partially unsaturated cyclic group containing one or more heteroatoms, with the remaining ring atoms being carbon. The heterocycloalkyl group may contain, for example, 1 or 2 heteroatoms. The heterocycloalkyl group may contain 3 to 8 ring elements, 5 to 7 ring elements, or 5 or 6 ring elements. The heterocycloalkyl group includes, for example, morpholinyl, thiomorpholinyl, piperazinyl, piperidinyl, pyrrolidinyl, azetidiny, and the like, but is not limited thereto.

- [112] The term "oxo" refers to the (=O) group.
- [113] As used herein, the term "halogen" refers to an atom belonging to group 17 of the periodic table. The halogen atom includes fluorine, chlorine, bromine, and iodine, etc., and may be used interchangeably with the term "halo", which refers to a monovalent functional group composed of halogen.
- [114] As used herein, the term "hydroxy" refers to the -OH functional group (hydroxyl group).
- [115] As used herein, the term "amino" refers to -NH₂.
- [116] As used herein, the term "alkylamino" refers to a group in which one of two Hs in an amino group is substituted with an alkyl group. Examples of the alkylamino group include, but are not limited thereto, methylamino, ethylamino, and propylamino.
- [117] As used herein, the term "dialkylamino" refers to -N(alkyl)₂. In this case, the two alkyls may be the same or different from each other. Examples of the dialkylamino substituent include, but are not limited thereto, dimethylamino, diethylamino, ethylmethylamino, and dipropylamino.
- [118] As used herein, the term "haloalkyl" refers to an alkyl group substituted by one or more halogen atoms. The halogens may be the same (e.g., CHF₂, -CF₃) or different (e.g., CF₂Cl). Where specified, the haloalkyl group may be optionally substituted with one or more substituents other than halogen. Examples of haloalkyl groups may include fluoromethyl, dichloroethyl, trifluoromethyl, trichloromethyl, pentafluoroethyl, and pentachloroethyl groups, but are not limited thereto.
- [119] As used herein, the term "haloalkoxy" refers to an alkoxy group substituted with one or more halogen atoms, where alkoxy is as defined above. Non-limiting examples of the haloalkoxy group may include fluoromethoxy, dichloroethoxy, trifluoromethoxy, trichloromethoxy, and the like.
- [120] As used herein, the term "hydroxyalkyl" refers to an alkyl group substituted with one or more -OH groups, where alkyl is as defined above.
- [121] In the present specification, "⋈", "*", or "-" is used to indicate a position at which a substituent is bonded to the remaining moieties of the compound. For example, if - is indicated at the end of a substituent, it means that the end is attached to the remaining moieties of the compound. In addition, when two or more substituents are linked by "-," it means that the substituent immediately before "-" is bonded to a substitutable atom of the substituent immediately after "-."
- [122] As used herein, the term "solvate" may refer to a compound of the present invention or a salt thereof comprising a stoichiometric or non-stoichiometric amount of a solvent bound by non-covalent intermolecular forces. Preferred solvents therefor may be solvents that are volatile, non-toxic, and/or suitable for administration to humans. The

solvent may be water, in which case the "solvate" is referred to as "hydrate". The compound of the present invention may exist as a hydrate, which can be obtained, for example, by crystallization from a solvent or aqueous solution. In this regard, one, two, three or any number of solvent or water molecules may combine with the compound according to the present disclosure to form a solvate and a hydrate. Unless stated to the contrary, the present disclosure includes all such possible solvates.

- [123] In addition, the compound according to the present invention may exist in different crystal polymorphic forms, which makes it possible for certain modifications to become metastable. Unless otherwise stated, the compound of the present invention includes all of these possible polymorphic forms.
- [124] As used herein, the term "stereoisomer" may refer to a compound of the present invention or a salt thereof that has the same chemical formula or molecular formula but is optically or sterically different, and specifically, may be a diastereomer, an enantiomer, or a geometric isomer.
- [125] In some embodiments, the compound of the present invention contains one or more asymmetric centers and may be in the form of a racemate, a single enantiomer, a mixture of enantiomers, a single diastereomer, a mixture of diastereomers, etc. In one embodiment, due to the nature of the asymmetric center or limited rotation, the compound of the present invention may exist in the form of an enantiomer or a diastereomer.
- [126] When two or more asymmetric centers are present in the compound of the present invention, multiple diastereomers and enantiomers of the chemical structures disclosed herein may exist. Pure isomers, separated isomers, partially pure isomers, or racemic mixtures are all intended to be within the scope of the present invention.
- [127] Purification of the isomers and separation of isomer mixtures can be achieved by standard techniques known in the art. For example, a diastereomeric mixture can be separated into its respective diastereomers by a chromatographic process or crystallization, and a racemate can be separated into its respective enantiomers by a chromatographic process or resolution of the chiral phase.
- [128] The compound of the present invention may be used in the form of a pharmaceutically acceptable salt derived from an acid or a base.
- [129] The term "pharmaceutically acceptable salt" refers to a salt of an active pharmaceutical ingredient prepared with an acid or base that is tolerated by the biological system, or is tolerated by the subject, or is tolerated by the biological system and tolerated by the subject when administered in a therapeutically effective amount. When the compound of the present invention contains a relatively acidic functional group, a base addition salt can be obtained by contacting the neutral form of this compound with a sufficient amount of the desired base, either in a pure solvent or in a suitable

inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, magnesium salts, lithium salts, strontium salts or similar salts, but are not limited thereto.

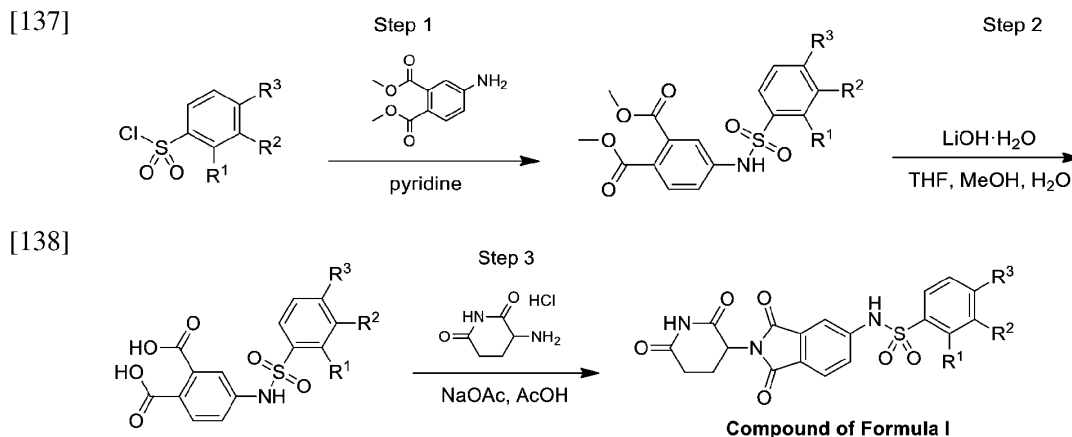
- [130] When the compound of the present disclosure contains a relatively basic functional group, an acid addition salt can be obtained by contacting the neutral form of this compound with a sufficient amount of the desired acid, either in a pure solvent or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, carbonic acid, monohydrogen carbonic acid, phosphoric acid, monohydrogen phosphoric acid, dihydrogen phosphoric acid, sulfuric acid, monohydrogen sulfuric acid, hydroiodic acid, or phosphorous acid, as well as those derived from relatively non-toxic organic acids such as acetic acid, propionic acid, isobutyric acid, maleic acid, malonic acid, benzoic acid, succinic acid, suberic acid, fumaric acid, lactic acid, mandelic acid, phthalic acid, benzenesulfonic acid, p-tolylsulfonic acid, citric acid, tartaric acid, or methanesulfonic acid, but are not limited thereto. In addition, salts of amino acids such as alginate and salts of organic acids such as glucuronic acid or galacturonic acid are also included.

[131] **Method for preparing compound**

- [132] The compound according to the present invention can be easily prepared from commercially available starting materials, compounds known in the literature, or intermediates easily prepared therefrom through standard synthetic methods and procedures in the related field.
- [133] The methods described herein can be monitored according to any suitable method known in the art. For example, the formation of a product can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (e.g., ^1H or ^{13}C), infrared spectroscopy, spectrophotometry (e.g., UV-visible rays), mass spectrometry, or chromatography, such as high performance liquid chromatography (HPLC), gas chromatography (GC), gel permeation chromatography (GPC), or thin layer chromatography (TLC).
- [134] The following general reaction scheme generally illustrates a representative method for preparing a compound represented by Formula I. Those of ordinary skill in the art can easily prepare a compound represented by Formula I by appropriately selecting starting materials, reaction temperature, reaction conditions, catalyst, solvent, processing method, and the like, suitable for the desired compound based on the preparation method specifically disclosed in the examples herein. Hereinafter, unless otherwise limited, the designation of each substituent in Formula I in the reaction scheme is the same as that of the substituent at the corresponding position in Formula I.

[135] In one aspect, the compound represented by Formula I according to the present invention can be prepared by reacting a phenylsulfonylchloride compound substituted with R¹, R² and R³ as shown in Reaction Scheme A below:

[136] [Reaction Scheme A]



[138]

[139] The final target phenylsulfonylchloride compound substituted with R¹, R², and R³ corresponding to the substituents R¹, R², and R³ of a compound represented by Formula I can be purchased as a commercially available compound or can be easily manufactured based on known techniques in the field of medicinal chemistry and examples herein.

[140] In Step 1, the phenylsulfonylchloride compound and dimethyl 4-aminobenzene-1,2-dicarboxylate are reacted in the presence of an appropriate solvent, such as pyridine at an appropriate temperature (for example, 20 °C to 30 °C, such as about 25 °C) for a period of time (for example, about 1 hour).

[141] In Step 2, the dicarboxylate compound obtained in Step 1 is hydrolyzed with LiOH in an appropriate solvent (for example, a mixed solution of THF, MeOH, and water) to obtain a phthalic acid compound. The reaction can be performed at an appropriate temperature (for example, about 20 °C to about 80 °C, such as about 50 °C) for an appropriate period of time (for example, about 8 hours to about 15 hours, such as about 12 hours).

[142] In Step 3, the phthalic acid compound obtained in Step 2 can be reacted with 3-aminopiperidine-2,6-dione or an appropriate salt thereof (for example, HCl salt) in the presence of an appropriate solvent and reagent, such as acetic acid and sodium acetate to obtain a compound represented by Formula I. The reaction can be performed at an appropriate temperature (for example, 80°C to 120 °C, such as 100 °C) for a certain period of time (for example, about 8 hours to about 15 hours, such as about 12 hours).

[143] **Medicinal use, pharmaceutical composition, and administration method**

- [144] In another aspect of the present invention, there is provided a pharmaceutical composition for treating a disorder of uncontrolled cell proliferation, comprising the compound represented by Formula I, stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof. The compound represented by Formula I, stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof is as described above.
- [145] As used herein, the term "treating" or "treatment" refers to inhibiting a disease, for example, inhibiting a disease, condition or disorder in a subject who experiences or exhibits the pathology or signs of the disease, condition or disorder, i.e., preventing further development of the pathology and/or signs, or ameliorating the disease, for example, ameliorating the disease, condition or disorder in a subject who experiences or exhibits the pathology or signs of the disease, condition or disorder, i.e., reversing the pathology and/or signs, for example, reducing the severity of the disease.
- [146] In one embodiment, the disorder of uncontrolled cell proliferation is cancer.
- [147] In one embodiment, the cancer may be pediatric cancer, such as pediatric acute leukemia or medulloblastoma. In one embodiment, the cancer may be selected from brain cancer, lung cancer, blood cancer, bladder cancer, colon cancer, cervical cancer, endometrial cancer, ovarian cancer, squamous cell cancer, kidney cancer, peritoneal cancer, breast cancer, stomach cancer, large intestine cancer, prostate cancer, pancreatic cancer, urogenital tract cancer, lymphatic cancer, laryngeal cancer, skin cancer, malignant melanoma, large intestine cancer, endometrial carcinoma, thyroid cancer, rhabdomyosarcoma, liver cancer, and a combination thereof. For example, the cancer may be lung cancer, prostate cancer, breast cancer, ovarian cancer, endometrial cancer, bladder cancer, or skin cancer.
- [148] The lung cancer may be small cell lung cancer, non-small cell lung cancer, or lung neuroendocrine cancer. The prostate cancer may be androgen receptor-positive prostate cancer (AR-positive prostate cancer; ARPC), castration-resistant prostate cancer (CRPC), double-negative prostate cancer (DNPC) (not expressing androgen receptor nor neuroendocrine markers), or neuroendocrine prostate cancer (NEPC). The brain cancer may be glioblastoma, medulloblastoma, glioma, or a combination thereof. The kidney cancer may be kidney clear cell carcinoma. The bladder cancer may be bladder urothelial carcinoma. The blood cancer may be selected from chronic myeloid leukemia (CML), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), hairy cell leukemia, chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), large granular lymphocytic leukemia (LGL), acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell-lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma, Burkitt lymphoma, Hodgkin's lymphoma,

non-Hodgkin's lymphoma, and a combination thereof.

- [149] In one embodiment, the disorder of uncontrolled cell proliferation may be related to cereblon (CRBN) dysfunction.
- [150] As used herein, "cereblon" and "CRBN" can be used interchangeably, and refer to a protein encoded by the human CRBN gene at the cytogenetic location of 3p26.2 and the molecular location between base pairs 3,148,489 and 3,179,716 of chromosome 3 (UCSC Genome Browser for Humans, December 2013 (GRCh38/hg38) assembly). CRBN is the substrate recognition element of the DCX (DDB1-CUL4-X-box) E3 protein ligase complex that mediates ubiquitination and subsequent proteasome degradation of target proteins. The DCX (DDB1-CUL4-X-box) E3 protein ligase complex is composed of at least CRBN, CUL4A, DDB1, and RBX1. The CRBN protein has two isoforms generated by alternative splicing: Isoform 1 has 442 amino acids and a molecular weight of 50,546 Da; and Isoform 2 has 441 amino acids and a molecular weight of 50,475 Da.
- [151] The disorder of uncontrolled cell proliferation may be related to GSPT1 dysfunction. The disorder of uncontrolled cell proliferation may be a disorder related to GSPT1 or caused by GSPT1. For example, the disorder of uncontrolled cell proliferation may be cancer related to GSPT1.
- [152] Herein, GSPT1 (G1 to S phase transition 1), also called eRF3, is a translation termination factor that binds to eRF1 and mediates stop codon recognition and release of nascent proteins from ribosomes. GSPT1 plays a key role in maintaining high-fidelity protein synthesis (*Cell*. 2011 Oct 14; 147(2): 396-408). Ubiquitination and degradation of GSPT1 are known to induce inhibition of the expression level of cancer-inducing proteins (translationally addicted oncoproteins, for example, c-MYC, N-MYC, L-MYC, BCL-2, MCL-1, etc.) whose expression levels remain high depending on protein translation in cancer cells (Mullard, *Nat Rev Drug Discov*. 2022, 21: 865-867). In addition, the degradation of GSPT1 is known to activate a cell death mechanism called "integrated stress response" (Surka et al., *Blood* 2021, 137(5): 661-677). This response is known to be mediated by ATF-4 and ultimately cause cell death in a caspase 3-dependent manner.
- [153] The compound represented by Formula I according to the present invention can be useful for the treatment of MYC-driven cancer, such as cancer driven by cancer-inducing proteins whose expression level is maintained at a high level depending on protein translation, such as c-MYC, n-MYC, and l-MYC. The MYC-driven cancer includes, for example, prostate cancer, breast cancer, liver cancer, colorectal cancer, and the like, but is not limited thereto. In one embodiment, the compound represented by Formula I according to the present invention can be useful for the treatment of cancers exhibiting high n-Myc expression level.

- [154] In addition, the compound represented by Formula I according to the present invention can be useful for the treatment of cancers sensitive to the integrated stress response, such as blood cancer. The blood cancer includes acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), and the like, but is not limited thereto. For example, the blood cancer is acute myeloid leukemia.
- [155] The compound represented by Formula I according to the present invention is a molecular glue with a structure based on pomalidomide, one of the immunomodulatory imide drugs, and can bind to CRBN protein and induce selective GSPT1 degradation. A compound represented by Formula I with a specific combination of substituents can have excellent selectivity, especially for GSPT1, compared to various neosubstrates such as IKAROS, AIOLOS, and CK1 α . The compound represented by Formula I of the present invention has excellent selectivity and degradation activity against GSPT1 compared to the compound disclosed in the prior document WO 2022/066835. In particular, the compounds disclosed in the above prior document only exhibit temporary degradation activity against GSPT1, and then restore GSPT1 expression level and show reproliferation of tumor cells, whereas the compound represented by Formula I according to the present invention exhibits sustained and stable GSPT1 degradation activity and excellent tumor cell proliferation inhibitory activity.
- [156] Furthermore, the compound represented by Formula I according to the present invention has excellent inhibitory activity against tumor cell proliferation and significantly reduced cytotoxicity against normal cells, and, thus, it has a much larger therapeutic index than the compounds disclosed in the above prior document.
- [157] In one embodiment, the cancer is a cancer with a neuroendocrine phenotype. The present inventors have found that a compound represented by Formula I exhibits particularly excellent anticancer activity, especially against a cancer with a neuroendocrine phenotype, such as lung cancer or prostate cancer.
- [158] A cancer with a neuroendocrine phenotype have been reported to share morphology and marker-based histology, such as high nuclear-to-cytoplasm ratio, frequent mitosis characteristic, and granular chromatin (Am. Soc. Clin. Oncol. Educ. Book. 2015; 35: 92-103). In addition, it has been reported that a cancer with a neuroendocrine phenotype essentially has TP53 and RB1 loss and/or inactivation mutations at the molecular level and expresses common neuroendocrine markers such as chromogranin A (CHGA) and synaptophysin (SYP) (Nat. Med. 2016; 22: 298-305; Lancet Oncol. 2015; 16: e435-e446).
- [159] In one embodiment, a cancer with a neuroendocrine phenotype includes neuroendocrine prostate cancer (NEPC), castration-resistant prostate cancer, and lung neuroendocrine tumor, but is not limited thereto.

- [160] In one embodiment, the pharmaceutical composition may comprise conventional pharmaceutically acceptable carriers, excipients, or additives. The pharmaceutically acceptable carrier, excipient, or additive may include one or more pharmaceutically acceptable diluents, preservatives, antioxidants, solubilizers, emulsifiers, coloring agents, releasing agents, coating agents, sweetener, flavoring agents and fragrances, and adjuvants, but is not limited thereto. The disclosed pharmaceutical composition may conveniently be presented in a unit dosage form and may be prepared by any method well known in the fields of pharmaceuticals and pharmaceutical sciences.
- [161] The pharmaceutical composition may be formulated according to a conventional method, and may be prepared as various oral formulations such as tablets, pills, powders, capsules, syrups, emulsions, and microemulsions, or parenteral formulations such as intramuscular, intravenous or subcutaneous formulation, or formulations for topical application to the skin. The pharmaceutical composition may be a single composition or separate compositions. The pharmaceutical composition comprises a compound, stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt according to one aspect as an active ingredient of the pharmaceutical composition.
- [162] It may be particularly advantageous to formulate the aforementioned pharmaceutical compositions into a unit dosage form for ease of administration and uniformity of dosage. As used herein, the term "unit dosage form" refers to a physically discrete unit suitable as unit dosages, wherein each unit contains a predetermined amount of an active ingredient calculated to produce the desired therapeutic effect in relation to the required pharmaceutical carrier. Typical examples of unit dosage forms include tablets (including divided or coated tablets), capsules, or pills for oral administration; single-dose vials for solutions or suspensions for injection; suppositories for rectal administration; powder packet; wafer; and separated multiple batches thereof.
- [163] When the pharmaceutical composition is prepared in the form of an oral formulation, examples of additives or carriers used may include cellulose, calcium silicate, corn starch, lactose, sucrose, dextrose, calcium phosphate, stearic acid, magnesium stearate, calcium stearate, gelatin, talc, surfactant, suspending agent, emulsifying agent, diluent, and the like. When the pharmaceutical composition of the present invention is prepared in the form of an injection, the additive or carrier may include water, saline, aqueous glucose solution, similar aqueous sugar solution, alcohol, glycol, ether (for example, polyethylene glycol 400), oil, fatty acid, fatty acid ester, glyceride, surfactant, suspending agent, emulsifying agent, and the like.
- [164] In one embodiment, the pharmaceutical composition of the present invention may be prepared in a liquid dosage form, wherein the liquid dosage form may comprise preservatives, stabilizers, buffering substances, flavor modifiers, sweeteners, coloring agents, antioxidants, and complex formers, etc. For example, the complex forming

agent may include a chelating agent such as ethylenediamine tetraacetic acid, nitrilotriacetic acid, diethylenetriamine pentacetic acid, and salts thereof. It may optionally be necessary to stabilize the liquid dosage form with a physiologically acceptable base or buffer to a pH range of approximately 9 or less. To the extent possible, it is desirable to prepare stable dosage forms at neutral or slightly basic pH values (pH 8 or less). In this respect, the compound of the present invention having excellent stability at neutral pH may also be advantageous in terms of formulation stability. In order to enhance the solubility and/or stability of the disclosed compounds in liquid dosage forms, parenteral injection forms, or intravenous injection forms, if necessary, it may be advantageous to use α -, β - or γ -cyclodextrins or derivatives thereof, especially hydroxyalkyl substituted cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin. In addition, co-solvents such as alcohols can improve the solubility and/or stability of the compound according to the present disclosure in a pharmaceutical composition.

[165] The dosage of the pharmaceutical composition is an amount effective for treatment of a subject or patient, and may be administered orally or parenterally as desired. It may be administered in one to several divided doses to be administered in an amount of 0.01 to 1000 mg, more specifically 0.1 to 300 mg per kg of body weight daily based on the active ingredient when administered orally, or in an amount of 0.01 to 100 mg, more specifically 0.1 to 50 mg per kg of body weight daily based on the active ingredient when administered parenterally. The dose to be administered to a specific subject or patient should be determined in light of several related factors such as body weight, age, sex, and health condition of the patient, the diet, the administration time, the administration method, the severity of the disease, and the like, and it should be understood that it may be appropriately increased or decreased by a specialist. The above dosage is not intended to limit the scope of the present invention in any way. A physician or veterinarian of ordinary skill in the art can readily determine and prescribe the required effective amount of the pharmaceutical composition. For example, by a physician or veterinarian, a dose of the compound of the present invention used in a pharmaceutical composition may start at a level lower than that required to achieve the desired therapeutic effect, and may gradually increase until the desired effect is achieved.

[166] In one embodiment, the pharmaceutical composition includes within its scope a pharmaceutical composition comprising, as an active ingredient, a therapeutically effective amount of at least one of the disclosed compounds, alone or in combination with a pharmaceutically acceptable carrier. The term "therapeutically effective amount" or "effective amount" refers to an amount sufficient to produce a beneficial or desired clinical result, for example, an amount sufficient to alleviate, ameliorate, stabilize,

reverse, slow or delay the progression of a disease.

- [167] Depending on the mode of administration, the pharmaceutical composition comprises 0.05 to 99% by weight, preferably 0.1 to 70% by weight, more preferably 0.1 to 50% by weight of an active ingredient, and 1 to 99.95% by weight, preferably 30 to 99.9% by weight, more preferably 50 to 99.9% by weight of a pharmaceutically acceptable carrier, with all percentages based on the total weight of the composition.
- [168] In one embodiment, the pharmaceutical composition may further comprise at least one agent known to treat cancer.
- [169] The at least one agent may be selected from uracil mustard, chlormethine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, carmustine, lomustine, streptozocin, dacarbazine, temozolomide, thiotepa, altretamine, methotrexate, 5-fluorouracil, floxuridine, cytarabine, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, pentostatin, bortezomib, vinblastine, vincristine, vinorelbine, vindesine, bleomycin, dactinomycin, daunorubicin, doxorubicin, epirubicin, dexamethasone, clofarabine, cladribine, pemetrexed, idarubicin, paclitaxel, docetaxel, ixabepilone, mithramycin, topotecan, irinotecan, deoxycoformycin, mitomycin-C, L-asparaginase, interferon, etoposide, teniposide 17 α -ethinyl estradiol, diethylstilbestrol, testosterone, prednisone, fluoxymesterone, drostanolone propionate, testolactone, megestrol acetate, tamoxifen, methylprednisolone, methyltestosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, flutamide, toremifene, goserelin, cisplatin, carboplatin, hydroxyurea, amsacrine, procarbazine, mitotane, mitoxantrone, levamisole, navelbine, anastrozole, letrozole, capecitabine, raloxifene, droloxifene, hexamethylmelamine, oxaliplatin, gefitinib, capecitabine, erlotinib, azacitidine, temozolomide, gemcitabine, vasostatin, and a combination thereof.
- [170] In addition, the at least one agent may be selected from a DNA methyltransferase inhibitor, a HDAC-inhibitor, a glucocorticoid, a mTOR inhibitor, a cytotoxic agent, or a combination thereof.
- [171] The DNA methyltransferase inhibitor may be 5-aza-2'-deoxycytidine, 5-azacitidine, zebularine, epigallocatechin-3-gallate, procaine, or a combination thereof.
- [172] The HDAC-inhibitor may be vorinostat, entinostat, panobinostat, trichostatin A, mocetinostat, belinostat, dacinostat, givinostat, tubastatin A, pracinostat, droxinostat, quisinostat, romidepsin, valproic acid, AR-42 (OSU-HDAC42), tacedinaline, ricolinostat, apicidin, or a combination thereof.
- [173] The glucocorticoid may be dexamethasone, prednisolone, methylprednisolone, betamethasone, triamcinolone, fludrocortisone, beclomethasone, or a combination thereof.

- [174] The mTor inhibitor may be BEZ235, everolimus, sirolimus, temsirolimus, rapamycin, AZD8055, or a combination thereof.
- [175] The cytotoxic agent may be selected from an alkylating agent, an antimetabolite, an antitumor antibiotic, a mitosis inhibitor, or a chemotherapeutic agent selected from anthracycline, cytarabine, purine analog, sorafenib, gemtuzumab ozogamicin, rituximab, or a combination thereof.
- [176] The alkylating agent may be selected from carboplatin, cisplatin, cyclophosphamide, chlorambucil, melphalan, carmustine, busulfan, lomustine, dacarbazine, oxaliplatin, ifosfamide, mechlorethamine, temozolomide, thiotepa, bendamustine, and streptozocin.
- [177] The antimetabolite may be selected from gemcitabine, 5-fluorouracil, capecitabine, hydroxyurea, mercaptopurine, pemetrexed, fludarabine, nelarabine, cladribine, clofarabine, cytarabine, decitabine, pralatrexate, floxuridine, methotrexate, and thioguanine.
- [178] The antitumor antibiotic may be selected from doxorubicin, mitoxantrone, bleomycin, daunorubicin, dactinomycin, epirubicin, idarubicin, plicamycin, mitomycin, pentostatin, and valrubicin.
- [179] The mitosis inhibitor may be selected from irinotecan, topotecan, rubitecan, cabazitaxel, docetaxel, paclitaxel, etoposide, vincristine, ixabepilone, vinorelbine, vinblastine, and teniposide.
- [180] The at least one agent may be a BCL2 inhibitor, a FLT3 inhibitor, an IDH 1/2 inhibitor, a CDK (cyclin-dependent kinase) inhibitor, a transcription inhibitor, a HSP inhibitor, or a combination thereof.
- [181] The BCL2 (B-cell lymphoma 2) inhibitor may include venetoclax, navitoclax, obatoclax mesylate, sabutoclax, lisaftoclax, and the like.
- [182] The FLT3 (FMS-like tyrosine kinase 3) inhibitor may include midostaurin, quizartinib, gilteritinib, sorafenib, crenolanib, pexidartinib, and the like.
- [183] The IDH (isocitrate dehydrogenase) 1/2 inhibitor may include ivosidenib, enasidenib, vorasidenib, olutasidenib, AGI-6780, AGI-5198, GSK321, and the like.
- [184] The CDK inhibitor may include a CDK7 inhibitor, a CDK9 inhibitor, a CDK 12 inhibitor, and the like, and may include, for example, samuraciclib, alvociclib, fadraciclib, seliciclib, zotiraciclib, atuvaciclib, enitociclib, voruciclib, SY5609, XL201, Q-901, KRLS-017, GTAEXS-617, TGN-1062, THZ1, THZ2, SY-1365, YKL-5-124, ICEC0942, LY3405105, LDC4297, BS-181, SNS-32, AT-7519, AZD-4573, KB-0742, AU-07, BTXA-51, GFH-009, JS-101, PRT-2527, QHRD-107, TP-1287, SYHX-1903, CTX-439, KIN-004, SY-12882, THZ-531, CT-7439, AU-003, AU-004, and the like.
- [185] The transcription inhibitor includes any agent capable of inhibiting the transcription of a cancer-inducing protein. The compound of the present invention can exhibit an-

anticancer effects by inhibiting the translation process from RNA to protein based on GSPT1 degradation activity. Therefore, combined use with a transcription inhibitor that inhibits the transcription process from DNA to RNA can exhibit enhanced anticancer effects. Such transcription inhibitors may include, for example, lurbinectedin, which is known to inhibit transcription by covalently binding to residues present in the minor groove of DNA.

- [186] The HSP (heat shock protein) inhibitor includes a HSP70 inhibitor, a HSP90 inhibitor, and the like, and may include, for example, pimitespib, luminespib, tanespimycin, alvespimycin, ganetespib, onalespib, geldanamycin, rocaglamide, and the like.
- [187] In one embodiment, the at least one agent may be co-packaged, co-formulated, and/or co-transferred with the compound represented by Formula I according to the present invention, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof.
- [188] In one embodiment, the at least one agent may be provided in the form of a kit comprising the compound represented by Formula I according to the present invention, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof. By means of the kit, two or more ingredients, which may be active or inactive ingredients, carriers, diluents, etc., may be provided along with instructions for the preparation of the actual dosage form by the patient or the individual administering the drug to the patient.
- [189] In another aspect, there is provided a method of regulating a cereblon activity or a GSPT1 activity in at least one cell, comprising contacting the at least one cell *in vitro* with a compound represented by Formula I, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof. Among the terms or elements mentioned in the description of the method, those that are the same as those mentioned above are as described above.
- [190] As used herein, the term "contacting" refers to bringing together the disclosed compound or pharmaceutical composition in close proximity to a cell, target protein, or other biological entity, in a manner that allows the disclosed compound or pharmaceutical composition to directly affect the activity of the cell, target protein, or other biological entity; that is, by interacting with the cell, target protein, or other biological entity itself, or in a manner that allows the disclosed compound or pharmaceutical composition to indirectly affect the activity of the cell, target protein, or other biological entity; that is, by interacting with other molecules, cofactors, factors, or proteins on which the activity of the cell, target protein, or other biological entity itself depends.
- [191] The compound represented by Formula I according to the present invention or a pharmaceutical composition comprising the same has activity as a modulator of

cereblon protein. In addition, the compound represented by Formula I according to the present invention or a pharmaceutical composition comprising the same has activity as a modulator of GSPT1 expression and/or activity. In addition, the compound represented by Formula I according to the present invention or a pharmaceutical composition comprising the same has activity as a cell proliferation inhibitor. Accordingly, the compound represented by Formula I according to the present invention can be used to evaluate new compounds. For example, the test compound can be evaluated by comparing the analytical values obtained by performing a biological analysis with the test compound and the compound of the present invention, respectively. The biological analysis includes cereblon binding analysis, GSPT1 degradation activity analysis, or cell proliferation test, etc., but is not limited thereto.

[192] In another aspect, there is provided a method of treating a disorder of uncontrolled cell proliferation, comprising administering a compound represented by Formula I, a stereoisomer, hydrate, solvate, or pharmaceutically acceptable salt thereof to a subject.

[193] Among the terms or elements mentioned in the description of the method, those that are the same as those mentioned above are as described above. As used herein, the term "subject" refers to a subject in need of treatment for a disease, and more specifically means a mammal such as a human or non-human primate, a mouse, a dog, a cat, a horse, and a cow.

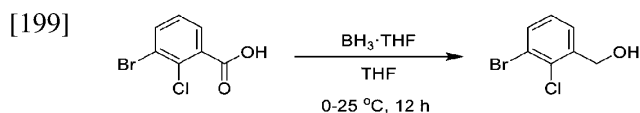
[194] In another aspect, there is provided a medicinal use of the compound represented by Formula I, stereoisomer, hydrate, or solvate thereof, for the treatment of a disorder of uncontrolled cell proliferation, such as cancer; or a use of the compound represented by Formula I, stereoisomer, hydrate, or solvate thereof, for manufacturing a medicament for the treatment of a disorder of uncontrolled cell proliferation, such as cancer. The terms or elements mentioned in the description of the use are as described above.

[195] Hereinafter, the present invention will be described in detail by way of examples. However, the following examples are only for illustrating the present invention, and the scope of the present invention is not limited to the following examples.

[196] **[Preparation Example]**

[197] **Preparation Example 1: (3-bromo-2-chlorobenzyl)oxy)(tert-butyl)diphenylsilane (Intermediate A1)**

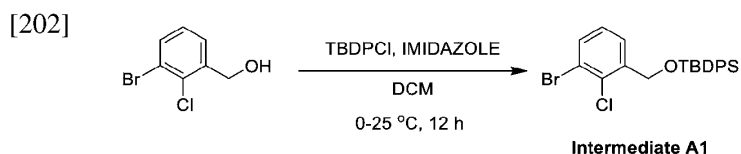
[198] Step 1: (3-bromo-2-chloro-phenyl)methanol



[200] $\text{BH}_3\cdot\text{THF}$ (1 M, 42.47 mL) was slowly added dropwise to a solution of 3-bromo-2-chloro-benzoic acid (5 g, 21.23 mmol) in THF (50 mL) at 0 °C under N_2 at-

mosphere. The mixture was stirred at 25 °C for 12 hours under N₂ atmosphere. The mixture was neutralized by slowly adding water (20 mL) and K₂CO₃ at 0 °C under N₂ atmosphere. Thereafter, it was extracted with EtOAc (50 mL*3). The organic layer was washed with brine (100 mL*3), dried over anhydrous Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain (3-bromo-2-chloro-phenyl)methanol (4.4 g, 94% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 4.80 (2H, s), 7.16 (1H, t, *J* = 8.0 Hz), 7.47 (1H, dd, *J* = 7.6, 0.8 Hz), 7.57 (1H, dd, *J* = 8.0, 1.2 Hz).

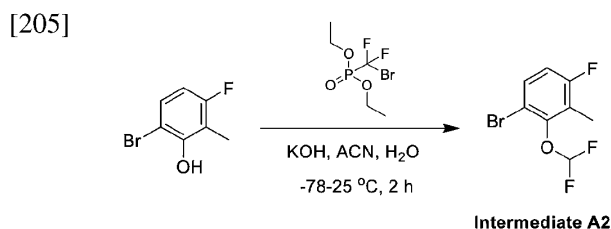
[201] Step 2: (3-bromo-2-chlorobenzyl)oxy)(tert-butyl)diphenylsilane



[203] To a solution of (3-bromo-2-chloro-phenyl)methanol (4.4 g, 19.87 mmol) and imidazole (1.62 g, 23.84 mmol) in DCM (20 mL) was added tert-butyl-chloro-diphenylsilane (TBDPCL; 6.55 g, 23.84 mmol) at 0 °C. The mixture was stirred at 25 °C for 12 hours. The mixture was concentrated under reduced pressure, diluted with water (300 mL), and then extracted with EtOAc (200 mL*3). The organic layer was washed with brine (400 mL), dried over anhydrous Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (PE : EtOAc = 100:0 to 100:1) to obtain **Intermediate A1** (8.8 g, 96% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 1.13 (9H, s), 4.83 (2H, s), 7.22 (1H, t, *J* = 8.0 Hz), 7.37-7.46 (6H, m), 7.52-7.58 (1H, m), 7.69 (4H, dd, *J* = 8.0, 1.6 Hz), 7.74 (1H, dd, *J* = 7.6, 1.2 Hz).

[204] **Preparation Example 2:**

1-bromo-2-(difluoromethoxy)-4-fluoro-3-methyl-benzene (Intermediate A2)

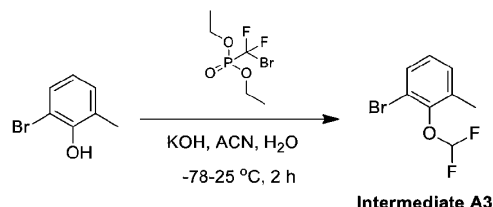


[206] To a solution of KOH (5.47 g, 97.55 mmol) in water (20 mL) and ACN (20 mL) was added 6-bromo-3-fluoro-2-methyl-phenol (1 g, 4.88 mmol) at 25 °C. Thereafter, 1-[[bromo(difluoro)methyl]-ethoxy-phosphoryl]oxyethane (2.60 g, 9.75 mmol) was added at -78 °C. The mixture was stirred at -78 °C for 0.3 hours and stirred at 25 °C for 1.7 hours. The mixture was diluted with DCM (50 mL) and water (50 mL). Thereafter, it was extracted with DCM (50 mL*3). The organic layer was washed with brine (100 mL*3), dried over anhydrous Na₂SO₄, filtered, and then concentrated under reduced

pressure to obtain **Intermediate A2** (1.2 g, 96% yield) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 2.30 (3H, d, $J = 2.0$ Hz), 6.22-6.76 (1H, m), 6.91 (1H, t, $J = 8.8$ Hz), 7.43 (1H, dd, $J = 8.8, 5.6$ Hz).

[207] **Preparation Example 3: 1-bromo-2-(difluoromethoxy)-3-methylbenzene (Intermediate A3)**

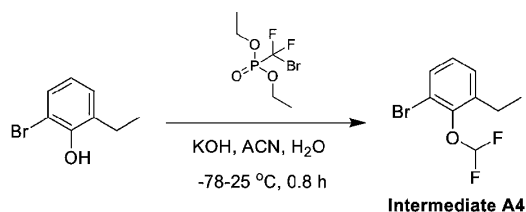
[208]



[209] To a solution of KOH (24 g, 427.73 mmol) in water (15 mL) and ACN (15 mL) was added 2-bromo-6-methylphenol (4 g, 21.39 mmol) at 25 °C. Thereafter, 1-[[bromo(difluoro)methyl]-ethoxy-phosphoryl]oxyethane (11.42 g, 42.77 mmol) was added at -78 °C. The mixture was stirred at -78 °C for 0.3 hours and stirred at 25 °C for 1.7 hours. The mixture was diluted with water (100 mL). Thereafter, it was extracted with DCM (50 mL*3). The organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain **Intermediate A3** (4 g, 79% yield) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 2.38 (3H, s), 6.31-6.75 (1H, m), 7.00-7.08 (1H, m), 7.20 (1H, d, $J = 7.6$ Hz), 7.45 (1H, d, $J = 8.0$ Hz).

[210] **Preparation Example 4: 1-bromo-2-(difluoromethoxy)-3-ethylbenzene (Intermediate A4)**

[211]

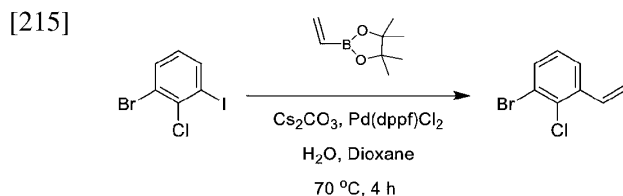


[212] To a solution of KOH (11.16 g, 198.95 mmol) in water (8 mL) and ACN (8 mL) was added 2-bromo-6-ethylphenol (2 g, 9.95 mmol) at 25 °C. Thereafter, 1-[[bromo(difluoro)methyl]-ethoxy-phosphoryl]oxyethane (5.31 g, 19.90 mmol) was added at -78 °C. The mixture was stirred at -78 °C for 0.3 hours and stirred at 25 °C for 0.5 hours. The mixture was diluted with water (20 mL). Thereafter, it was extracted with DCM (20 mL*3). The organic layer was washed with brine (30 mL*3), dried over anhydrous Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain **Intermediate A4** (1.6 g, 64% yield) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 1.24-1.26 (3H, m), 2.75-2.82 (2H, m), 6.31-6.75 (1H, m), 7.03-7.12 (1H, m), 7.22-7.28

(1H, m), 7.43-7.48 (1H, m).

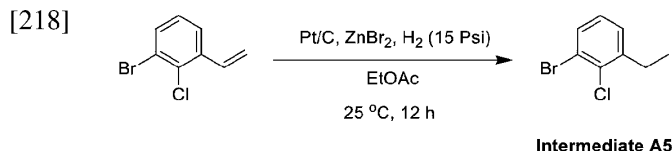
[213] **Preparation Example 5: 1-bromo-2-chloro-3-ethyl-benzene (Intermediate A5)**

[214] Step 1: 1-bromo-2-chloro-3-vinyl-benzene



[216] A mixture of 1-bromo-2-chloro-3-iodo-benzene (450 mg, 1.42 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (225 mg, 1.46 mmol), Cs_2CO_3 (1.16 g, 3.54 mmol) and $\text{Pd}(\text{dppf})\text{Cl}_2$ (104 mg, 142.04 μmol) in water (1 mL) and dioxane (10 mL) was degassed and purged three times with N_2 , and then the mixture was stirred at 70°C for 4 hours under N_2 atmosphere. The mixture was diluted with water (30 mL) and extracted with EtOAc (15 mL*3). The organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (PE : EtOAc = 100 : 0 to 99 : 1) to obtain 1-bromo-2-chloro-3-vinyl-benzene (0.2 g, 65% yield) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 5.42 (1H, d, $J = 11.2$ Hz), 5.72 (1H, d, $J = 17.2$ Hz), 7.07-7.19 (2H, m), 7.49-7.58 (2H, m).

[217] Step 2: 1-bromo-2-chloro-3-ethyl-benzene

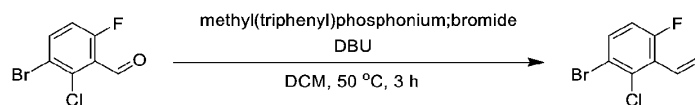


[219] To a solution of 1-bromo-2-chloro-3-vinyl-benzene (0.2 g, 919.58 μmol) in EtOAc (5 mL) were added Pt/C (773 mg, 183.95 μmol , 5% purity) and ZnBr_2 (42 mg, 186.50 μmol) under N_2 atmosphere. The mixture was degassed and purged three times with H_2 (15 psi), and then the mixture was stirred at 25°C for 12 hours under H_2 (15 psi) atmosphere. The mixture was filtered and filtered under reduced pressure to obtain **Intermediate A5** (220 mg, crude product) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 1.24 (3H, t, $J = 7.6$ Hz), 2.76-2.87 (2H, m) 7.01-7.10 (1H, m), 7.18 (1H, dd, $J = 7.6, 1.2$ Hz), 7.48 (1H, dd, $J = 8.0, 1.6$ Hz).

[220] **Preparation Example 6: 1-bromo-2-chloro-3-ethyl-4-fluorobenzene (Intermediate A6)**

[221] Step 1: 1-bromo-2-chloro-4-fluoro-3-vinyl-benzene

[222]



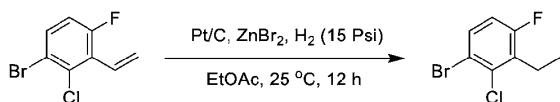
[223]

To a solution of methyl(triphenyl)phosphonium bromide (3 g, 8.40 mmol) in DCM (20 mL) was added DBU (1.42 g, 9.36 mmol). The reaction mixture was stirred at 50 °C for 30 minutes. Thereafter, a solution of 3-bromo-2-chloro-6-fluorobenzaldehyde (1 g, 4.21 mmol) in DCM (10 mL) was added to the reaction mixture. The mixture was stirred at 50 °C for 3 hours. The mixture was diluted with DCM (50 mL) and washed with HCl solution (0.1 M, 50 mL*2). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to obtain a yellow residue. The residue was purified by silica column chromatography (PE : EtOAc = 99 : 1) to obtain 1-bromo-2-chloro-4-fluoro-3-vinylbenzene (160 mg, 16% yield) as a white liquid. ¹H NMR (400 MHz, CDCl₃) δ ppm 5.69 (1H, d, *J* = 12.0 Hz), 5.94 (1H, d, *J* = 18.0 Hz), 6.79 (1H, dd, *J* = 18.0, 12.0 Hz), 6.93 (1H, t, *J* = 9.6 Hz), 7.49 (1H, dd, *J* = 8.8, 5.2 Hz).

[224]

Step 2: 1-bromo-2-chloro-3-ethyl-4-fluorobenzene

[225]



Intermediate A6

[226]

To a solution of 1-bromo-2-chloro-4-fluoro-3-vinylbenzene (510 mg, 2.17 mmol) in EtOAc (5 mL) were added ZnBr₂ (102 mg, 453 μmol) and Pt/C (2.04 g, 971 μmol, 10% purity) under N₂ atmosphere. The reaction mixture was degassed, purged three times with H₂ (15 psi), and then stirred at 25 °C for 12 hours under H₂ (15 psi) atmosphere. The mixture was filtered and concentrated under reduced pressure to obtain **Intermediate A6** (500 mg, crude product) as a white oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 1.19 (3H, t, *J* = 7.6 Hz), 2.87 (2H, d, *J* = 7.6, 2.4 Hz), 6.88 (1H, t, *J* = 8.8 Hz), 7.45 (1H, dd, *J* = 8.8, 5.6 Hz).

[227]

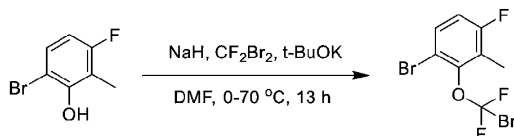
Preparation Example 7:

1-bromo-4-fluoro-3-methyl-2-(trifluoromethoxy)benzene (Intermediate A7)

[228]

Step 1: 1-bromo-2-[bromo(difluoro)methoxy]-4-fluoro-3-methylbenzene

[229]



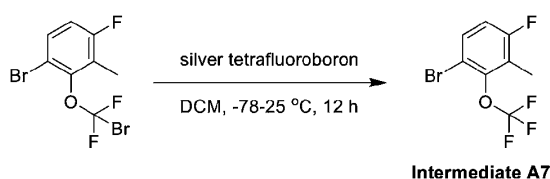
[230]

To a solution of 6-bromo-3-fluoro-2-methylphenol (1 g, 4.88 mmol) in DMF (10 mL) was added NaH (595 mg, 14.88 mmol, 60% purity) at 0 °C under N₂ atmosphere.

The mixture was stirred at 0 °C for 10 minutes. Thereafter, *t*-BuOK (606 mg, 5.40 mmol) and dibromo(difluoro)methane (18.96 mmol, 1.75 mL) were added. The reaction mixture was stirred at 70 °C for 12 hours 50 minutes under N₂ atmosphere. The mixture was slowly poured into water (30 mL) and extracted with DCM (15 mL *3). The organic layer was washed with brine (30 mL*3), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (PE : EtOAc = 1 : 0 to 100 : 1) to obtain 1-bromo-2-[bromo(difluoro)methoxy]-4-fluoro-3-methyl-benzene (690 mg, crude product) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.30 (3H, s), 6.95 (1H, t, *J* = 8.8 Hz), 7.46 (1H, dd, *J* = 8.8, 5.6 Hz).

[231] Step 2: 1-bromo-4-fluoro-3-methyl-2-(trifluoromethoxy)benzene

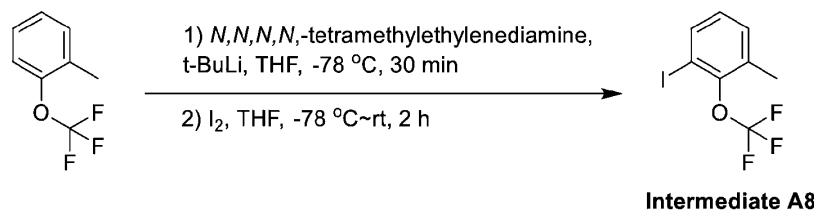
[232]



[233] To a solution of 1-bromo-2-[bromo(difluoro)methoxy]-4-fluoro-3-methyl-benzene (690 mg, 2.07 mmol) in DCM (8 mL) was added AgBF₄ (402 mg, 2.07 mmol) at -78 °C. The mixture was stirred at 25 °C for 12 hours. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica column chromatography (PE : EtOAc = 1 : 0) to obtain **Intermediate A7** (300 mg, 53% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.30 (3H, d, *J* = 1.6 Hz), 6.95 (1H, t, *J* = 8.8 Hz), 7.46 (1H, dd, *J* = 8.82, 6.0 Hz).

[234] **Preparation Example 8: 1-iodo-3-methyl-2-(trifluoromethoxy)benzene (Intermediate A8)**

[235]



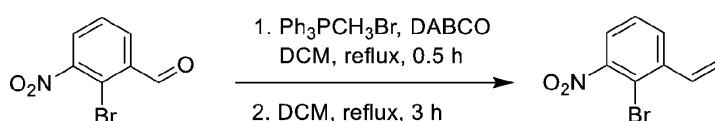
[236] To a solution of 1-methyl-2-(trifluoromethoxy)benzene (2.0 g, 11.35 mmol) and N,N,N',N'-tetramethylethylenediamine (1.32 g, 11.36 mmol, 1.71 mL) in THF (10 mL) was added *tert*-butyl lithium (1.3 M, 14.00 mL) at -78 °C under N₂ atmosphere and stirred for 30 minutes. I₂ (3.44 g, 13.55 mmol, 2.73 mL) was added to the mixture and stirred at -78 °C for 2 hours. The mixture was quenched with saturated NH₄Cl (30 mL) and extracted with DCM (3x20 mL). The organic layer was washed with brine (2x20 mL) and concentrated to obtain a yellow residue. The residue was purified by

silica column chromatography (PE : EtOAc = 100 : 0) and purified twice by prep-HPLC (PE : EtOAc = 1 : 0). However, the two compounds were not separated, so they were further purified using prep-HPLC (column: Waters Xbridge C18 150x50 mmx10 mm; mobile phase: [water (NH₄HCO₃)-ACN]; gradient: 64%-94% B over 10 min). The resulting compound was extracted with PE (3*30 mL), dried over anhydrous Na₂SO₄, and then concentrated under reduced pressure to obtain **Intermediate A8** (0.2 g, 662.18 μ mol, 6% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.30 (3H, s), 6.84 (1H, t, *J*=7.6 Hz), 7.14 (1H, d, *J*=7.6 Hz), 7.63 (1H, d, *J*=7.6 Hz).

[237] **Preparation Example 9: 2-bromo-3-ethyl-aniline (Intermediate B1)**

[238] **Step 1: 2-bromo-1-nitro-3-vinyl-benzene**

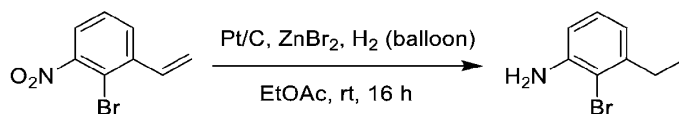
[239]



[240] To a solution of methyl(triphenyl)phosphonium bromide (5.3 g, 14.84 mmol) in DCM (50 mL) was added DBU (2.5 g, 16.42 mmol, 2.48 mL). The reaction mixture was stirred at 50 °C for 30 minutes. Thereafter, a solution of 2-bromo-3-nitro-benzaldehyde (1.7 g, 7.39 mmol) in DCM (30 mL) was added to the reaction mixture. The mixture was stirred at 50 °C for 3 hours. The mixture was diluted with DCM (100 mL) and washed with HCl solution (0.1 M, 50 mL*2). The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to obtain a yellow residue. The residue was purified by silica column chromatography (PE : EtOAc = 50 : 1 to 20 : 1) to obtain 2-bromo-1-nitro-3-vinyl-benzene (0.8 g, 3.51 mmol, 47% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 5.39-5.51 (1H, m), 5.65-5.76 (1H, m), 6.97-7.08 (1H, m), 7.32-7.38 (1H, m), 7.47-7.54 (1H, m), 7.62-7.67 (1H, m).

[241] **Step 2: 2-bromo-3-ethyl-aniline**

[242]



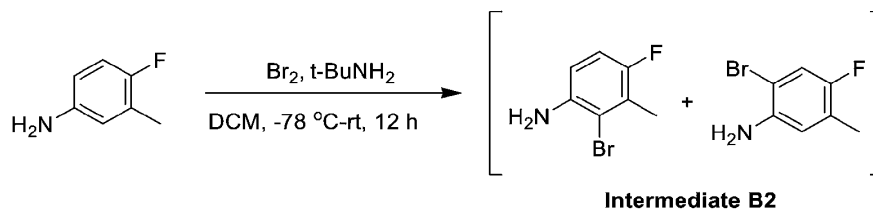
Intermediate B1

[243] To a solution of 2-bromo-1-nitro-3-vinyl-benzene (0.1 g, 438.51 μ mol) in EtOAc (10 mL) were added Pt/C (50 mg, 11.90 μ mol, 5% purity) and ZnBr₂ (20 mg, 88.81 μ mol, 4.44 μ L) under N₂ atmosphere. The mixture was degassed and purged several times with H₂ (15 psi), and then the mixture was stirred at 25 °C for 16 hours under H₂ (15 psi) atmosphere. The mixture was filtered through Celite and washed with EtOAc (3*20 mL). The filtrate was washed with water (2*15 mL), and the organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated to obtain **Intermediate B1**

(0.16 g, 799.70 μmol , 91% yield) as a yellow oil. The final compound was used in the next reaction without further purification. ^1H NMR (400 MHz, CDCl_3) δ ppm 1.24 (3H, t, $J = 7.6$ Hz), 2.75 (2H, q, $J = 7.2$ Hz), 4.1 (2H, s), 6.64-6.67 (2H, m), 7.06 (1H, t, $J = 8.0$ Hz).

[244] **Preparation Example 10: 2-bromo-4-fluoro-5-methylaniline (Intermediate B2)**

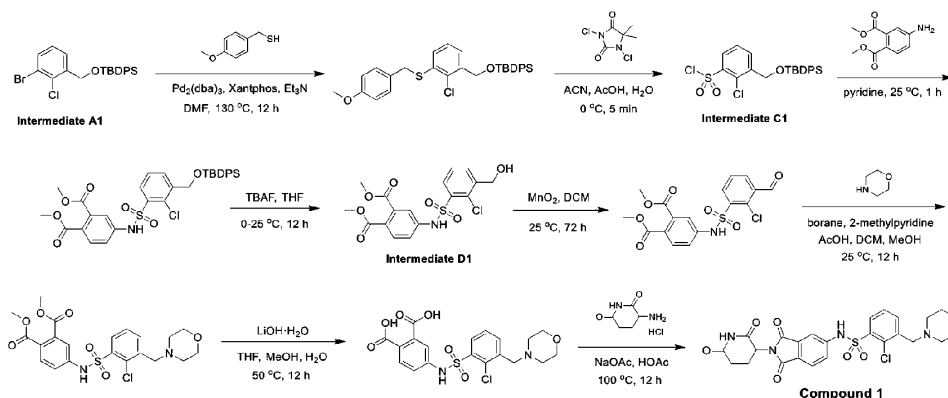
[245]



[246] To a solution of tert-butylamine (2.37 g, 32.35 mmol, 3.40 mL) in DCM (40 mL) was slowly added dropwise Br_2 (2.58 g, 16.14 mmol, 832.12 μL) in DCM (20 mL) at -78°C . The mixture was stirred at -78°C for 1 hour. 4-fluoro-3-methylaniline (2.0 g, 15.98 mmol, 205.76 μL) in DCM (20 mL) was added dropwise to the reaction mixture at -78°C , and slowly heated to 25°C , and stirred for 12 hours. The mixture was diluted with DCM (200 mL) and washed with saturated Na_2SO_3 (100 mL*2) and brine (1*100 mL). The organic layer was dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to obtain a yellow residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 150*40 mm* 15 μm ; mobile phase: [water (TFA)-ACN]; B%: 35%-65%, 10 minutes) to obtain **Intermediate B2** (4.0 g, 9.80 mmol, 61% yield) as a purple solid. The ratio of 2-bromo-4-fluoro-3-methylaniline to 2-bromo-4-fluoro-5-methylaniline was about 1:2. ^1H NMR (400 MHz, CDCl_3) δ ppm 2.33-2.36 (3H, m), 6.61-6.64 (1H, m), 6.86 (1H, t, $J = 8.8$ Hz); LC/MS (ESI) $m/z = 204.0$ $[\text{M}+2]^+$. LC/MS $t_R = 0.75$ min.

[247] **Reaction Scheme 1: Synthesis of 2-chloro-N - (2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)-3-(morpholinomethyl)benzenesulfonamide**

[248]



- [249] Step 1: tert-butyl((2-chloro-3-((4-methoxybenzyl)thio)benzyl)oxy)diphenylsilane
- [250] To a solution of **Intermediate A1** (8.8 g, 19.14 mmol) in DMF (100 mL) were added Xantphos (1.12 g, 1.93 mmol), Et₃N (2.94 g, 29.05 mmol) and Pd₂(dba)₃ (1.76 g, 1.92 mmol) at 25 °C under N₂ atmosphere. Thereafter, the mixture was stirred at 130 °C for 12 hours. The mixture was diluted with water (300 mL) and extracted with EtOAc (200 mL*3). The organic layer was washed with brine (200 mL*3), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (PE: EtOAc = 100 : 0 to 19 : 1) to obtain tert-butyl((2-chloro-3-((4-methoxybenzyl)thio)benzyl)oxy)diphenylsilane (8.2 g, 80% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 1.12 (9H, s), 3.78 (3H, s), 4.09 (2H, s), 4.83 (2H, s), 6.83 (2H, d, *J* = 8.4 Hz), 7.15-7.21 (1H, m), 7.22-7.30 (3H, m), 7.35-7.46 (6H, m), 7.61 (1H, d, *J* = 7.2 Hz), 7.70 (4H, dd, *J* = 7.6, 1.2 Hz).
- [251] Step 2: 3-(((tert-butyl)diphenylsilyl)oxy)methyl)-2-chlorobenzenesulfonylchloride (Intermediate C1)
- [252] To a solution of tert-butyl((2-chloro-3-((4-methoxybenzyl)thio)benzyl)oxy)diphenylsilane (2 g, 3.75 mmol) in ACN (30 mL), AcOH (1.3 mL) and water (0.8 mL) was added 1,3-dichloro-5,5-dimethyl-imidazolidine-2,4-dione (1.47 g, 7.45 mmol) at 0 °C. The mixture was stirred at 0 °C for 5 minutes. The reaction mixture was neutralized to pH = 7 with saturated NaHCO₃. The mixture was concentrated under reduced pressure, diluted with water (200 mL), and then extracted with DCM (150 mL*3). The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (PE : EtOAc = 100 : 0 to 19 : 1) to obtain **Intermediate C1** (1.7 g, 95% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 1.15 (9H, s), 4.91 (2H, s), 7.42 (4H, d, *J* = 7.2 Hz), 7.45-7.49 (2H, m), 7.57 (1H, t, *J* = 8.0 Hz), 7.69 (4H, dd, *J* = 8.0, 2.0 Hz), 8.10 (1H, d, *J* = 7.6 Hz), 8.17 (1H, d, *J* = 7.6 Hz).
- [253] Step 3: Dimethyl 4-((3-(((tert-butyl)dimethylsilyl)oxo)methyl)-2-chlorophenyl)sulfinamido)phthalate
- [254] To a solution of dimethyl 4-aminobenzene-1,2-dicarboxylic acid (0.5 g, 2.39 mmol) in pyridine (20 mL) was added **Intermediate C1** (1.7 g, 3.55 mmol). The mixture was stirred at 25 °C for 1 hour. The mixture was concentrated under reduced pressure, diluted with water (200 mL), and then extracted with EtOAc (300 mL*3). The organic layer was washed with brine (300 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (PE = EtOAc = 10:1 to 4:1) to obtain dimethyl 4-((3-(((tert-butyl)dimethylsilyl)oxo)methyl)-2-chlorophenyl)sulfinamido)phthalate (1.2

g, 44% yield, 57% purity) as a white solid. LC/MS (ESI) m/z = 652.3 $[M+H]^+$. LC/MS t_R = 0.753 min.

[255] Step 4: Dimethyl 4-((2-chloro-3-(hydroxymethyl)phenyl)sulfonamido)phthalate (Intermediate D1)

[256] To a solution of dimethyl 4-((3-(((tert-butyldimethylsilyl)oxo)methyl)-2-chlorophenyl)sulfonamido)phthalate (1.2 g, 1.05 mmol) in THF (10 mL) was added TBAF (1 M, 4.19 mL) at 0 °C. The mixture was stirred at 25 °C for 12 hours. The mixture was filtered under reduced pressure, diluted with water (50 mL), and then extracted with EtOAc (30 mL*3). The organic layer was washed with HCl (1 M, 50 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure to obtain a residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 150*40 mm* 15 μ m; mobile phase: [water (TFA)-ACN]; gradient: 30%-60% B over 10 min) to obtain **Intermediate D1** (0.35 g, 81% yield) as a white solid. 1H NMR (400 MHz, $DMSO-d_6$) δ ppm 3.76 (6H, d, J = 14.0 Hz), 4.57 (2H, s), 7.27-7.34 (2H, m), 7.58 (1H, s), 7.67 (1H, d, J = 9.2 Hz), 7.78-7.86 (1H, m), 8.04 (1H, dd, J = 8.0, 2.0 Hz), 11.36 (1H, s); LC/MS (ESI) m/z = 382.1 $[M - 32 + H]^+$. LC/MS t_R = 0.533 min.

[257] Step 5: Dimethyl

4-[(2-chloro-3-formyl-phenyl)sulfonamido]benzene-1,2-dicarboxylic acid

[258] MnO_2 (1.47 g, 16.92 mmol) was added to a solution of **Intermediate D1** (0.35 g, 845.76 μ mol). The mixture was stirred at 25 °C for 72 hours. The mixture was filtered and concentrated under reduced pressure to obtain dimethyl 4-[(2-chloro-3-formyl-phenyl)sulfonamido]benzene-1,2-dicarboxylic acid (0.44 g, crude product) as a white solid. LC/MS (ESI) m/z = 434.1 $[M+Na]^+$. LC/MS t_R = 0.558 min.

[259] Step 6: Dimethyl 4-((2-chloro-3-(morpholinomethyl)phenyl)sulfonamido)phthalate

[260] To a solution of dimethyl 4-[(2-chloro-3-formyl-phenyl)sulfonamido]benzene-1,2-dicarboxylic acid (100 mg, 242.83 μ mol) in MeOH (1 mL) and DCM (1 mL) were added borane;2-methylpyridine (50 mg, 467.46 μ mol) and AcOH (1 mg, 24.28 μ mol). The mixture was stirred at 25 °C for 12 hours. The mixture was concentrated under reduced pressure, and the residue was purified by prep-TLC (PE : EtOAc = 1 : 2) to obtain dimethyl 4-((2-chloro-3-(morpholinomethyl)phenyl)sulfonamido)phthalate (70 mg, 58% yield, 97% purity) as a yellow solid. LC/MS (ESI) m/z = 483.2 $[M+H]^+$. LC/MS t_R = 0.479 min.

[261] Step 7: 4-((2-chloro-3-(morpholinomethyl)phenyl)sulfonamido)phthalic acid

[262] To a solution of dimethyl 4-((2-chloro-3-(morpholinomethyl)phenyl)sulfonamido)phthalate (70 mg, 140.60

μmol) in THF (1 mL), MeOH (1 mL) and H_2O (1 mL) was added LiOH· H_2O (27 mg, 643.47 μmol). The mixture was stirred at 50 °C for 12 hours. The reaction mixture was filtered and concentrated under reduced pressure to obtain

4-((2-chloro-3-(morpholinomethyl)phenyl)sulfinamido)phthalic acid (80 mg, crude product) as a white solid. LC/MS (ESI) m/z = 455.1 $[\text{M}+\text{H}]^+$. LC/MS t_R = 0.417 min.

[263] Step 8: 2-chloro-*N*-

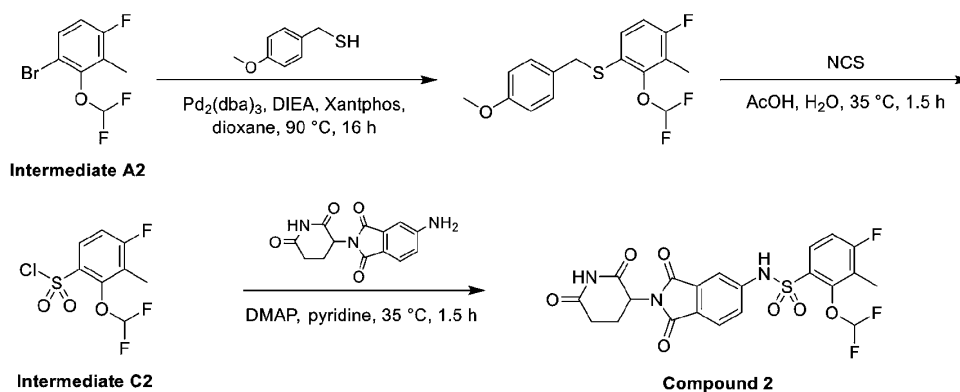
(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)-3-(morpholinomethyl)benzenesulfonamide (Compound 1)

[264] To a solution of 3-aminopiperidine-2,6-dione (43 mg, 261.26 μmol , HCl salt) and 4-((2-chloro-3-(morpholinomethyl)phenyl)sulfinamido)phthalic acid (80 mg, 175.87 μmol) in AcOH (2 mL) was added NaOAc (32 mg, 390.10 μmol). The mixture was stirred at 100 °C for 12 hours. The mixture was filtered and concentrated under reduced pressure to obtain a residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 150*25 mm* 10 μm ; mobile phase: [water (FA)-ACN]; gradient: 6%-36% B over 10 min) to obtain **Compound 1** (35.84 mg, 37% yield, 99.01% purity) as a yellow solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.88-2.06 (1H, m), 2.35 (4H, br s), 2.57-2.69 (2H, m), 2.77-2.91 (1H, m), 3.49-3.62 (6H, m), 5.07 (1H, dd, J = 13.2, 5.2 Hz), 7.47 (2H, s), 7.56 (1H, s), 7.79 (2H, s), 8.07-8.16 (1H, m), 11.11 (1H, s), 11.46-11.89 (1H, m); LC/MS (ESI) m/z = 547.2 $[\text{M}+\text{H}]^+$. LC/MS t_R = 1.528 min.

[265] **Reaction Scheme 2: 2-(difluoromethoxy)-*N*-**

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isindolin-5-yl]-4-fluoro-3-methyl-benzenesulfonamide

[266]



[267] Step 1:

3-(difluoromethoxy)-1-fluoro-4-[(4-methoxyphenyl)methylsulfanyl]-2-methyl-benzene

[268] A mixture of **Intermediate A2** (400 mg, 1.57 mmol), (4-methoxyphenyl)methanethiol (242 mg, 1.57 mmol), $\text{Pd}_2(\text{dba})_3$ (144 mg, 0.157 mmol), xantphos (182 mg, 0.314 mmol) and DIEA (608 mg, 4.71 mmol) in

1,4-dioxane (6 mL) was degassed and purged three times with N₂, and then stirred at 90 °C under N₂ atmosphere for 16 hours. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (30 mL X 3). The organic layer was washed with brine (20 mL X 2), dried over anhydrous Na₂SO₄, and concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (0 to 10% EtOAc/ PE gradient) to obtain

3-(difluoromethoxy)-1-fluoro-4-[(4-methoxyphenyl)methylsulfanyl]-2-methyl-benzene (350 mg, 68.0% yield) as a colorless oil. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.16 (d, *J* = 2.0 Hz, 3H), 3.74-3.71 (m, 3H), 4.14 (s, 2H), 6.72 (s, 1H), 6.89-6.83 (m, 2H), 6.91 (s, 1H), 7.20-7.14 (m, 1H), 7.27-7.21 (m, 2H), 7.36-7.33 (m, 1H), 7.37 (s, 1H).

[269] Step 2: 2-(difluoromethoxy)-4-fluoro-3-methyl-benzenesulfonyl chloride (**Intermediate C2**)

[270] To a solution of 3-(difluoromethoxy)-1-fluoro-4-[(4-methoxyphenyl)methylsulfanyl]-2-methyl-benzene (200 mg, 0.001 mmol) in AcOH (3 mL) and H₂O (1 mL) was added NCS (325 mg, 2.44 mmol). The mixture was stirred at 35 °C for 1.5 hours. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (20 mL X 3). The organic layer was washed with brine (10 mL X 2), dried over anhydrous Na₂SO₄, and concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (0 to 10% EtOAc/ PE gradient) to obtain **Intermediate C2** (100 mg, 59.8% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.39 (3H, s), 6.52-6.90 (1H, m), 7.20 (1H, t, *J* = 8.4 Hz), 7.98 (1H, dd, *J* = 8.8, 5.6 Hz).

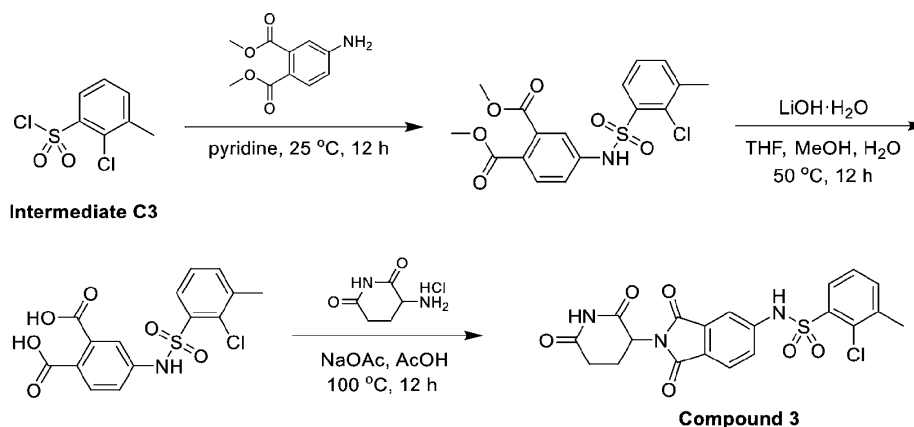
[271] Step 3: 2-(difluoromethoxy)-N-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxoisoindolin-5-yl]-4-fluoro-3-methyl-benzenesulfonamide (Compound 2)

[272] To a solution of **Intermediate C2** (100 mg, 0.364 mmol) and 5-amino-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (99.5 mg, 0.364 mmol) in pyridine (2 mL) was added DMAP (4.45 mg, 0.0364 mmol). The mixture was stirred at 35 °C for 1.5 hours. The reaction mixture was concentrated under reduced pressure and purified by prep-HPLC (Welch Xtimate C18 150*40 mm*10 μm; mobile phase: [water (FA)-ACN]; gradient: 24%-64% B over 25 min). Most of the ACN was removed by concentration under reduced pressure, and then the residual solvent was removed using a freeze dryer to obtain **Compound 2** (16.0 mg, 8.59% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.05-1.99 (m, 1H), 2.20 (s, 3H), 2.63-2.54 (m, 2H), 5.09 (dd, *J* = 5.6, 12.8 Hz, 1H), 7.29-6.92 (m, 1H), 7.41 (t, *J* = 8.8 Hz, 1H), 7.52-7.47 (m, 2H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.92 (dd, *J* = 6.4, 8.8 Hz, 1H), 11.10 (s, 1H), 11.42 (s, 1H); LC/MS (ESI) *m/z* = 512.0 [M+H]⁺.

[273] **Reaction Scheme 3: 2-chloro-N - [2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-benzenesulfonamide**

[280] **[Method 2]**

[281]

[282] Step 1: 4-[(2-chloro-3-methyl-phenyl)sulfonamino]benzene-1,2-dicarboxylic acid

[283] To a solution of **Intermediate C3** (5.00 g, 22.23 mmol) in pyridine (50 mL) was added dimethyl 4-aminobenzene-1,2-dicarboxylic acid (3.1 g, 14.82 mmol). The mixture was stirred at 25 °C for 12 hours. The reaction mixture was concentrated under reduced pressure, diluted with water (300 mL), and extracted with EtOAc (200 mL*3). The organic layer was washed with brine (400 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to obtain a residue. The residue was purified by silica column chromatography (PE : EtOAc = 100 : 1 to 2 : 1) to obtain 4-[(2-chloro-3-methyl-phenyl)sulfonamino]benzene-1,2-dicarboxylic acid (5.3 g, 75% yield, 83% purity) as a yellow solid. LC/MS (ESI) *m/z* = 398.1[M + H]⁺.

[284] Step 2: 4-[(2-chloro-3-methyl-phenyl)sulfonamino]phthalic acid

[285] To a solution of 4-[(2-chloro-3-methyl-phenyl)sulfonamino]benzene-1,2-dicarboxylic acid (5.3 g, 11.06 mmol) in THF (20 mL), MeOH (20 mL) and H₂O (20 mL) was added LiOH·H₂O (1.96 g, 46.59 mmol). The mixture was stirred at 50 °C for 12 hours. The reaction mixture was adjusted to pH 4 to 5 using 2 M HCl. The mixture was diluted with water (120 mL) and extracted with EtOAc (100 mL). The organic layer was washed with brine (200 mL*3), dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to obtain 4-[(2-chloro-3-methyl-phenyl)sulfonamino]phthalic acid (5.8 g, crude product) as a yellow solid. LC/MS (ESI) *m/z* = 369.0 [M + H]⁺.

[286] Step 3: 2-chloro-*N*-

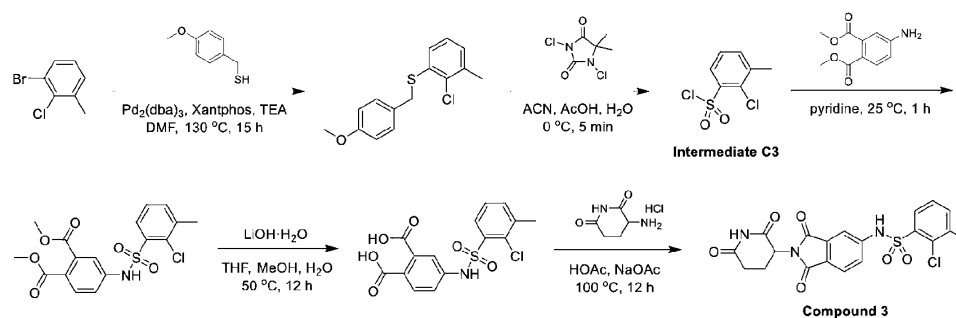
[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-benzenesulfonamide (Compound 3)

[287] To a solution of 4-[(2-chloro-3-methyl-phenyl)sulfonamino]phthalic acid (5 g, 35.31 mmol) and 3-aminopiperidine-2,6-dione (3.31 g, 20.14 mmol, HCl) in AcOH (100 mL) was added NaOAc (1.57 g, 19.19 mmol). The mixture was stirred at 100 °C for 12 hours. The mixture was filtered and concentrated under reduced pressure to obtain a

residue. The residue was purified by prep-HPLC (column: Phenomenex luna C18 250*80 mm*10 um; mobile phase: [water (FA)-ACN]; gradient: 35%-65% B over 20 min) to obtain **Compound 3** (5320.28 mg, 72% yield, 99.18% purity) as a gray solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.95-2.04 (1H, m), 2.36 (3H, s), 2.40-2.48 (1H, m), 2.57 (1H, d, *J*= 18.4 Hz), 2.79-2.94 (1H, m), 5.07 (1H, dd, *J*=12.8, 5.2 Hz), 7.44-7.54 (3H, m), 7.64-7.72 (1H, m), 7.78-7.86 (1H, m), 8.04 (1H, dd, *J* = 8.0, 1.2 Hz), 11.09 (1H, s), 11.61 (1H, s); LC/MS (ESI) *m/z* = 462.2 [*M* + *H*]⁺.

[288] **[Method 3]**

[289]



[290] **Step 1: 2-chloro-*N*-**

1,3-dioxo-isoindolin-5-yl]-3-methyl-benzenesulfonamide

[291] To a solution of 1-bromo-2-chloro-3-methyl-benzene (5 g, 24.33 mmol) and (4-methoxyphenyl)methanethiol (3.94 g, 25.55 mmol) in DMF (100 mL) were added Xantphos (1.41 g, 2.43 mmol), TEA (3.64 g, 35.92 mmol) and Pd₂(dba)₃ (1.56 g, 1.70 mmol) at 25 °C under nitrogen conditions. Thereafter, the reaction mixture was stirred at 130 °C for 15 hours. Water (300 mL) was poured into the reaction mixture and extracted with EtOAc (250 mL x 3). The combined organic layer was washed with brine (300 mL x 3), dried over Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography (SiO₂, petroleum ether/ethylacetate = 100/0 to 100/1) to obtain 2-chloro-1-[(4-methoxyphenyl)methylsulfanyl]-3-methyl-benzene (5 g, 74% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.39 (3H, s), 3.80 (3H, s), 4.11 (2H, s), 6.78-6.91 (2H, m), 7.02-7.14 (3H, m), 7.29 (2H, d, *J* = 8.8 Hz).

[292] **Step 2: 2-chloro-3-methyl-benzenesulfonyl chloride (Intermediate C3)**

[293] To a solution of 2-chloro-1-[(4-methoxyphenyl)methylsulfanyl]-3-methyl-benzene (5 g, 17.93 mmol), acetic acid (5 mL) and water (3 mL) in ACN (120 mL) was added 1,3-dichloro-5,5-dimethyl-imidazolidine-2,4-dione (7.07 g, 35.88 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 5 minutes. Thereafter, the reaction mixture was adjusted to pH 7 using an aqueous sodium hydrogen carbonate solution. The reaction mixture was concentrated, and then water (100 mL) was poured, and extracted with EtOAc (200 mL x 3). The combined organic layer was washed with brine (300 mL),

dried over Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography (SiO_2 , petroleum ether/ethylacetate = 100/0 to 100/1) to obtain 2-chloro-3-methyl-benzenesulfonyl chloride (3.2 g, 79% yield) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 2.53 (3H, s), 7.36-7.42 (1H, m), 7.62 (1H, dd, $J = 7.6$, 0.8 Hz), 8.04 (1H, dd, $J = 8.4$, 1.2 Hz).

[294] Steps 3 to 5: 2-chloro-*N*-

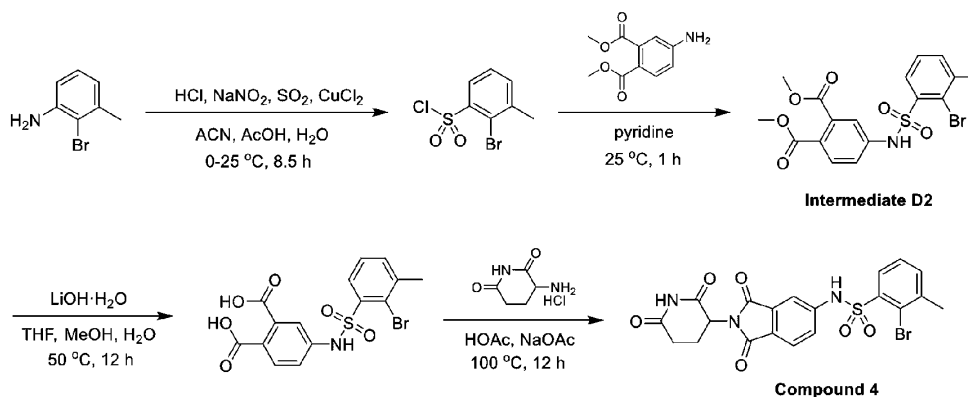
[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-benzenesulfonamide
(Compound 3)

[295] **Compound 3** was obtained by reacting 2-chloro-3-methyl-benzenesulfonyl chloride in the same manner as in Steps 1 to 3 of Method 2.

[296] **Reaction Scheme 4: 2-bromo-*N*-**

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-benzenesulfonamide

[297]



[298] Step 1: 2-bromo-3-methyl-benzenesulfonyl chloride

[299] To a solution of 2-bromo-3-methylaniline (1 g, 5.37 mmol) in acetic acid (3 mL) and ACN (15 mL) was slowly added hydrochloric acid (12 M, 1.71 mL) in water (3 mL). After 10 minutes, a solution of NaNO_2 (371 mg, 5.38 mmol) in water (2 mL) was slowly added at 0 °C. The reaction mixture was stirred at 25 °C for 2 hours to obtain Solution 1. In a separate flask, SO_2 (50 Psi) was bubbled in acetic acid (15 mL) at 10 °C for 20 minutes. Thereafter, a solution of CuCl_2 (723 mg, 5.38 mmol) in water (3 mL) was added to obtain Solution 2. Solution 1 was slowly added to Solution 2 at 0 °C. The reaction mixture was stirred at 25 °C for 6 hours. The reaction mixture was diluted with EtOAc (30 mL), poured into an ice-cold aqueous sodium hydrogen carbonate solution (100 mL), and then extracted with EtOAc (30 mL x 3). The combined organic layer was washed with an aqueous sodium hydrogen carbonate solution (100 mL x 5) and brine (100 mL), dried over Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography (SiO_2 , petroleum ether : ethyl acetate = 100: 0 to 20: 1) to obtain

2-bromo-3-methyl-benzenesulfonyl chloride (0.5 g, 35% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.57 (3H, s), 7.37-7.49 (1H, m), 7.53-7.65 (1H, m), 8.06 (1 H, dd, *J* = 8.0, 0.8 Hz).

[300] Step 2: Dimethyl

4-[(2-bromo-3-methyl-phenyl)sulfonylamino]benzene-1,2-dicarboxylate (**In-
intermediate D2**)

[301] To a solution of 2-bromo-3-methyl-benzenesulfonyl chloride (80 mg, 296.80 μmol) in pyridine (1 mL) was added dimethyl 4-aminobenzene-1,2-dicarboxylate (40 mg, 191.21 μmol). Thereafter, the reaction mixture was stirred at 25 °C for 1 hour. The reaction mixture was concentrated, and then water (10 mL) was poured thereinto, and the reaction mixture was extracted with EtOAc (20 mL x 3). The combined organic layer was washed with brine (30 mL), dried over Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by *prep*-TLC (SiO₂, petroleum ether : ethyl acetate = 2: 1) to obtain **In-
intermediate D2** (80 mg, 92% yield, 97% purity) as a colorless oil. LC/MS (ESI) *m/z* = 442.2 [M + H]⁺.

[302] Step 3: 4-[(2-bromo-3-methyl-phenyl)sulfonylamino]phthalic acid

[303] To a solution of **Intermediate D2** (80 mg, 175.45 μmol) in THF (1 mL), MeOH (1 mL) and water (1 mL) was added LiOH·H₂O (33 mg, 786.46 μmol). Thereafter, the reaction mixture was stirred at 50 °C for 12 hours. Thereafter, the reaction mixture was adjusted to pH 4 to 5 using hydrochloric acid (2 M). Water (20 mL) was poured into the reaction mixture and extracted with EtOAc (30 mL x 3). The combined organic layer was washed with brine (30 mL x 3), dried over Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain 4-[(2-bromo-3-methyl-phenyl)sulfonylamino]phthalic acid (60 mg, crude product) as a white solid. LC/MS (ESI) *m/z* = 396.2 [M - OH]⁺.

[304] Step 4: 2-bromo-*N* -

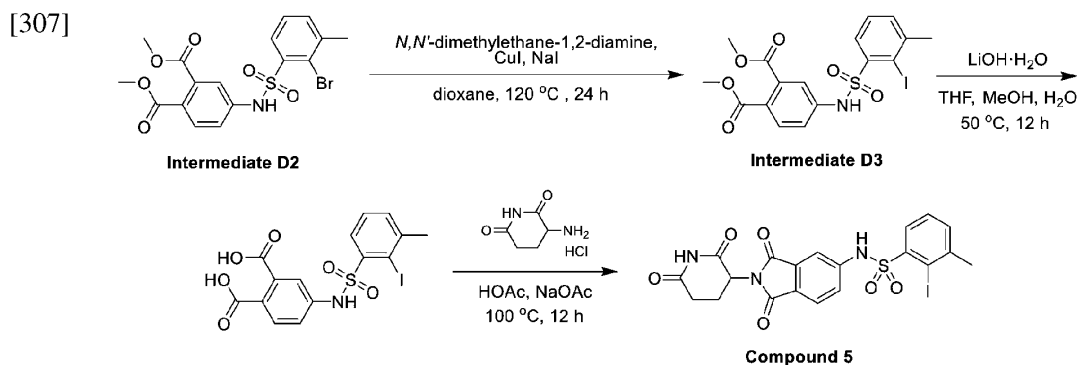
[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-benzenesulfonamide
(Compound 4)

[305] To a solution of 4-[(2-bromo-3-methyl-phenyl)sulfonylamino]phthalic acid (50 mg, 120.71 μmol) and 3-aminopiperidine-2,6-dione (26 mg, 157.97 μmol) in acetic acid (1 mL) was added NaOAc (11 mg, 134.10 μmol). Thereafter, the reaction mixture was stirred at 100 °C for 12 hours. The reaction mixture was concentrated under reduced pressure to obtain a crude product, which was purified by *prep*-HPLC (column: Phenomenex luna C18 150*25 mm* 10 um; mobile phase: [water (FA)-ACN]; B%: 30%-60%, 10 minutes) to obtain **Compound 4** (38.94 mg, 63% yield, 98.71% purity) as a gray solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.94-2.07 (1H, m), 2.40 (3H, s), 2.45 (1H, s), 2.56 (1H, d, *J* = 19.2 Hz), 2.79-2.93 (1H, m), 5.07 (1H, dd, *J* = 12.8, 5.2

Hz), 7.46-7.56 (3H, m), 7.65 (1H, d, $J = 6.8$ Hz), 7.81 (1H, d, $J = 8.4$ Hz), 8.05 (1H, d, $J = 7.2$ Hz), 11.09 (1H, s), 11.61 (1H, s); LC/MS (ESI) $m/z = 506.1$ $[M + H]^+$.

[306] **Reaction Scheme 5: N -**

**[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-2-iodo-3-methyl-benzenesulfo
namide**



[308] Step 1: Dimethyl

4-[(2-iodo-3-methyl-phenyl)sulfonylamino]benzene-1,2-dicarboxylate (**Intermediate D3**)

[309] To a solution of **Intermediate D2** (70 mg, 158.27 μmol), NaI (476 mg, 3.18 mmol) and CuI (35 mg, 183.78 μmol) in 1,4-dioxane (5 mL) was added *N,N'*-dimethylethane-1,2-diamine (31 mg, 351.67 μmol) under nitrogen conditions. Thereafter, the reaction mixture was stirred at 120 °C for 24 hours under nitrogen conditions. The reaction mixture was diluted with EtOAc (20 mL), adjusted to pH = 5 by treatment with hydrochloric acid (1 M), and then extracted with EtOAc (20 mL x 3). The combined organic layer was washed with brine (40 mL), dried over Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography (SiO_2 , petroleum ether : ethyl acetate = 4:1 to 3:1) to obtain **Intermediate D3** (50 mg, 65% yield) as a yellow solid. LC/MS (ESI) $m/z = 490.0$ $[M + H]^+$.

[310] Step 2: 4-[(2-iodo-3-methyl-phenyl)sulfonylamino]phthalic acid

[311] To a solution of **Intermediate D3** (50 mg, 102.19 μmol) in THF (3 mL), MeOH (1 mL) and water (1 mL) was added LiOH·H₂O (13 mg, 309.79 μmol). Thereafter, the reaction mixture was stirred at 50 °C for 12 hours. Thereafter, the reaction mixture was diluted with water (20 mL), and then the reaction mixture was adjusted to pH 5 using hydrochloric acid (1 M) and extracted with EtOAc (15 mL x 3). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain 4-[(2-iodo-3-methyl-phenyl)sulfonylamino]phthalic acid (47 mg, crude product) as a yellow solid. LC/MS (ESI) $m/z = 483.9$ $[M + \text{Na}]^+$.

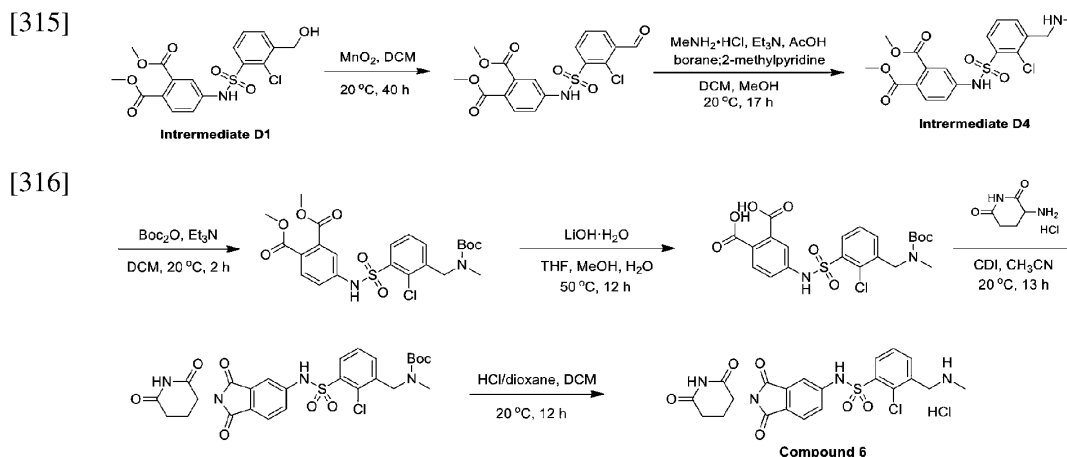
[312] Step 3: N -

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-2-iodo-3-methyl-benzenesulfonamide (Compound 5)

[313] To a solution of 4-[(2-iodo-3-methyl-phenyl)sulfonylamino]phthalic acid (47 mg, 101.90 μmol) and 3-aminopiperidine-2,6-dione (25 mg, 151.89 μmol , hydrochloride) in acetic acid (3 mL) was added NaOAc (11 mg, 134.10 μmol). Thereafter, the reaction mixture was stirred at 100 °C for 12 hours. The reaction mixture was concentrated under reduced pressure to obtain a crude product, which was purified by *prep*-HPLC (formic acid addition, column: Phenomenex luna C18 150*25 mm* 10 μm ; mobile phase: [water (FA)-ACN]; B%: 36%-56%, 10 minutes) to obtain **Compound 5** (35.46 mg, 62% yield, 98.40% purity) as a green solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.92-2.05 (1H, m), 2.46 (3H, s), 2.53-2.62 (2H, m), 2.80-2.91 (1H, m), 5.06 (1H, dd, J = 12.8, 5.6 Hz), 7.44 (2H, s), 7.48-7.54 (1H, m), 7.55-7.61 (1H, m), 7.72-7.83 (1H, m), 7.98 (1H, d, J = 7.2 Hz), 11.09 (1H, s), 11.47-11.72 (1H, m); LC/MS (ESI) m/z = 554.1 $[\text{M} + \text{H}]^+$.

[314] **Reaction Scheme 6: 2-chloro-N -**

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-(methylaminomethyl)benzenesulfonamide



4-[(2-chloro-3-formyl-phenyl)sulfonylamino]benzene-1,2-dicarboxylate

[318] To a solution of **Intermediate D1** (150 mg, 362.47 μmol) in DCM (8 mL) was added MnO_2 (252 mg, 2.90 mmol), and then the reaction mixture was stirred at 20 °C for 40 hours. The reaction mixture was filtered, and then the filtrate was concentrated to obtain dimethyl 4-[(2-chloro-3-formyl-phenyl)sulfonylamino]benzene-1,2-dicarboxylate (150 mg, crude product) as a yellow oil. LC/MS (ESI) m/z = 412.0 $[\text{M} + \text{H}]^+$.

- [319] Step 2: Dimethyl 4-[[2-chloro-3-(methylaminomethyl)phenyl]sulfonylamino]benzene-1,2-dicarboxylate (Intermediate D4)
- [320] To a solution of dimethyl 4-[(2-chloro-3-formyl-phenyl)sulfonylamino]benzene-1,2-dicarboxylate (75 mg, 182.12 μmol) and methanamine (25 mg, 370.27 μmol , hydrochloride) in DCM (3 mL) and MeOH (3 mL) was added Et₃N (36 mg, 355.77 μmol). Thereafter, acetic acid (33 mg, 549.54 μmol) was added. The reaction mixture was stirred at 20 °C for 5 hours, and then borane;2-methylpyridine (39 mg, 364.62 μmol) was added. Thereafter, the reaction mixture was stirred at 20 °C for 12 hours. The reaction mixture was concentrated under reduced pressure to obtain a crude product, which was purified by *prep*-HPLC (formic acid addition, column: Phenomenex luna C18 150*25 mm* 10 μm ; mobile phase: [water (FA)-ACN]; B%: 8%-38% B over 2 min) to obtain **Intermediate D4** (40 mg, 51% yield) as an off-white solid. LC/MS (ESI) m/z = 427.1 (M + H)⁺.
- [321] Step 3: Dimethyl 4-[[3-[[*tert*-butoxycarbonyl(methyl)amino]methyl]-2-chloro-phenyl]sulfonylamino]benzene-1,2-dicarboxylate
- [322] To a solution of **Intermediate D4** (40 mg, 93.71 μmol) in DCM (3 mL) were added Boc₂O (40 mg, 183.28 μmol) and Et₃N (30 mg, 296.47 μmol) and stirred at 20 °C for 2 hours. The reaction mixture was poured into water (10 mL) and extracted with DCM (10 mL x 3). The combined organic layer was washed with brine (20 mL), dried over Na₂SO₄, and concentrated under reduced pressure to obtain a crude product. The crude product was purified by preparative TLC (silica gel plate, petroleum ether : ethyl acetate = 1:1) to obtain dimethyl 4-[[3-[[*tert*-butoxycarbonyl(methyl)amino]methyl]-2-chloro-phenyl]sulfonylamino]benzene-1,2-dicarboxylate (45 mg, 91% yield) as a colorless oil. LC/MS (ESI) m/z = 549.1 [M + Na]⁺.
- [323] Step 4: 4-[[3-[[*tert*-butoxycarbonyl(methyl)amino]methyl]-2-chloro-phenyl]sulfonylamino]phthalic acid
- [324] To a solution of dimethyl 4-[[3-[[*tert*-butoxycarbonyl(methyl)amino]methyl]-2-chloro-phenyl]sulfonylamino]benzene-1,2-dicarboxylate (45 mg, 85.39 μmol) in THF (1 mL), MeOH (1 mL) and water (1 mL) was added LiOH·H₂O (18 mg, 428.94 μmol). The reaction mixture was stirred at 50 °C for 12 hours. Thereafter, the reaction mixture was concentrated under reduced pressure and diluted with water (10 mL), and then the reaction mixture was adjusted to pH 5 using hydrochloric acid (1 M) and extracted with EtOAc (10 mL x 3). The combined organic layer was washed with brine (20 mL), dried over Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain 4-[[3-[[*tert*-butoxycarbonyl(methyl)amino]methyl]-2-chloro-phenyl]sulfonylamino]phthalic acid (43 mg,

crude product) as a colorless oil. LC/MS (ESI) $m/z = 521.1$ $[M + Na]^+$.

[325] Step 5: *tert*-butyl *N* -

[[2-chloro-3-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]sulfamoyl]phenyl]methyl]-*N*-methyl-carbamate

[326] To a solution of 4-[[3-[[*tert*-butoxy-

carbonyl(methyl)amino]methyl]-2-chloro-phenyl]sulfonylamino]phthalic acid (43 mg, 86.18 μmol) in ACN (2 mL) was added CDI (28 mg, 172.68 μmol) and stirred at 20 °C for 30 minutes. Thereafter, 3-aminopiperidine-2,6-dione (18 mg, 109.36 μmol , hydrochloride) was added and stirred at 20 °C for 12.5 hours. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (10 mL x 3). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4 , and concentrated under reduced pressure to obtain *tert*-butyl *N* -

[[2-chloro-3-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]sulfamoyl]phenyl]methyl]-*N*-methyl-carbamate (51 mg, crude product) as a pale yellow oil. LC/MS (ESI) $m/z = 613.2$ $[M + Na]^+$.

[327] Step 6: 2-chloro-*N* -

[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-(methylaminomethyl)benzenesulfonamide (Compound 6)

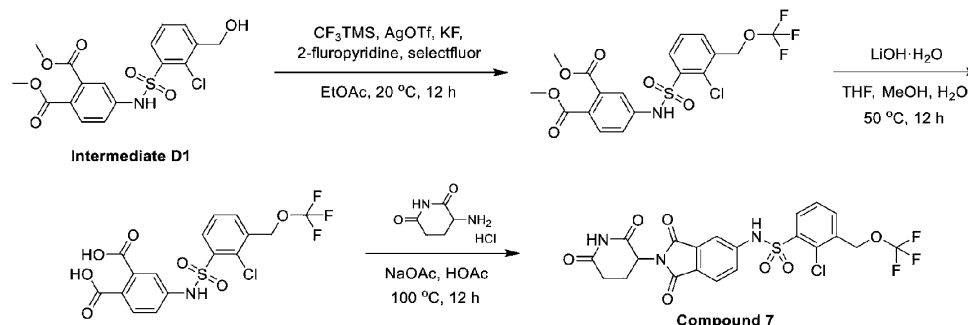
[328] To a solution of *tert*-butyl *N* -

[[2-chloro-3-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]sulfamoyl]phenyl]methyl]-*N*-methyl-carbamate (51 mg, 86.29 μmol) in DCM (1.5 mL) was added hydrochloric acid/dioxane (4 M, 0.5 mL). The reaction mixture was stirred at 20 °C for 12 hours. The reaction mixture was concentrated under reduced pressure and then purified by *prep*-HPLC (hydrochloric acid addition, column: YMC Triart C18 150*25 mm*5 μm ; mobile phase: [water (hydrochloric acid)-ACN]; gradient: 13%-43% B over 10 min) to obtain **Compound 6** (39.07 mg, 85% yield, 99.44% purity, hydrochloride) as an off-white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.92-2.06 (1H, m), 2.40-2.48 (2H, m), 2.58-2.62 (3H, m), 2.76-2.93 (1H, m), 4.22-4.38 (2H, m), 5.08 (1H, dd, $J = 12.8, 5.2$ Hz), 7.47-7.59 (2H, m), 7.70 (1H, t, $J = 8.0$ Hz), 7.83 (1H, d, $J = 8.0$ Hz), 7.97 (1H, d, $J = 7.2$ Hz), 8.25 (1H, d, $J = 6.8$ Hz), 9.16-9.49 (2H, m), 11.12 (1H, s), 11.83 (1H, s); LC/MS (ESI) $m/z = 491.0$ $[M + H]^+$.

[329] **Reaction Scheme 7: 2-chloro-*N* -**

[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-(trifluoromethoxymethyl)benzenesulfonamide

[330]

[331] Step 1: Dimethyl

4-[[2-chloro-3-(trifluoromethoxymethyl)phenyl]sulfonylamino]benzene-1,2-dicarboxylate

[332] To a solution of AgOTf (373 mg, 1.45 mmol), 1-(chloromethyl)-4-fluoro-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (Selectfluor, 257 mg, 725.46 μmol), KF (112 mg, 1.93 mmol), and **Intermediate D1** (200 mg, 483.29 μmol) in EtOAc (2.5 mL) were added 2-fluoropyridine (141 mg, 1.45 mmol) and trimethyl(trifluoromethyl)silane (206 mg, 1.45 mmol) under nitrogen conditions. Thereafter, the reaction mixture was stirred at 20 °C for 12 hours under nitrogen conditions. The reaction mixture was filtered, and then the filtrate was concentrated under reduced pressure and purified by prep TLC (silica gel plate, petroleum ether : ethyl acetate = 1:1) to obtain dimethyl 4-[[2-chloro-3-(trifluoromethoxymethyl)phenyl]sulfonylamino]benzene-1,2-dicarboxylate (15 mg, 6% yield, 97% purity) as a yellow oil. LC/MS (ESI) m/z = 482.0 $[\text{M} + \text{H}]^+$.

[333] Step 2: 4-[[2-chloro-3-(trifluoromethoxymethyl)phenyl]sulfonylamino]phthalic acid

[334] To a solution of dimethyl 4-[[2-chloro-3-(trifluoromethoxymethyl)phenyl]sulfonylamino]benzene-1,2-dicarboxylate (15 mg, 30.20 μmol) in THF (1 mL), MeOH (1 mL) and water (1 mL) was added LiOH·H₂O (7 mg, 166.81 μmol). The reaction mixture was stirred at 50 °C for 12 hours. The reaction mixture was concentrated under reduced pressure to obtain 4-[[2-chloro-3-(trifluoromethoxymethyl)phenyl]sulfonylamino]phthalic acid (14 mg, crude product) as a white solid. LC/MS (ESI) m/z = 436.0 $[\text{M} - \text{OH} + \text{H}]^+$.

[335] Step 3: 2-chloro-N -

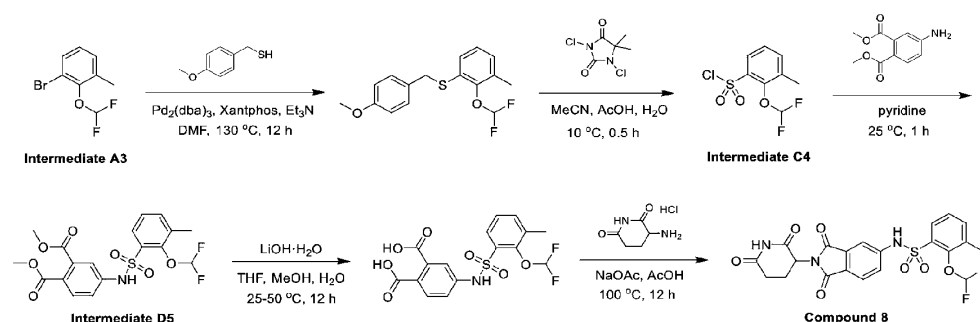
[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-(trifluoromethoxymethyl)benzenesulfonamide (Compound 7)

[336] To a solution of 4-[[2-chloro-3-(trifluoromethoxymethyl)phenyl]sulfonylamino]phthalic acid (14 mg, 30.85 μmol) and 3-aminopiperidine-2,6-dione (8 mg, 48.61 μmol , hydrochloride) in acetic acid (3 mL) was added NaOAc (3 mg, 36.57 μmol). Thereafter, the reaction

mixture was stirred at 100 °C for 12 hours. The reaction mixture was concentrated under reduced pressure to obtain a crude product, which was purified by *prep*-HPLC (formic acid addition, column: Phenomenex luna C18 150*25 mm* 10 um; mobile phase: [water (FA)-ACN]; gradient: 36%-66% B over 10 min) to obtain **Compound 7** (3.09 mg, 18% yield, 98.88% purity) as a green solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.08-2.20 (1H, m), 2.72-2.83 (2H, m), 2.87-2.97 (1H, m), 4.94 (1H, dd, *J* = 12.4, 5.6 Hz), 5.15 (2H, s), 7.46-7.50 (1H, m), 7.51-7.56 (2H, m), 7.60 (1H, s), 7.76 (2H, d, *J* = 8.0 Hz), 7.90-8.04 (1H, m), 8.14-8.26 (1H, m); LC/MS (ESI) *m/z* = 546.0 [M + H]⁺.

[337] **Reaction Scheme 8: 2-(difluoromethoxy)-*N*-**
[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-benzenesulfonamide

[338]



[339] **Step 1: (2-(difluoromethoxy)-3-methylphenyl)(4-methoxybenzyl)sulfane**

[340] To a solution of **Intermediate A3** (4 g, 16.87 mmol), (4-methoxyphenyl)methanethiol (3.12 g, 20.25 mmol), Xantphos (1.46 g, 2.53 mmol), and TEA (3.42 g, 33.75 mmol) in DMF (30 mL) was added Pd₂(dba)₃ (773 mg, 843.74 μmol), and the reaction mixture was stirred at 130 °C for 12 hours. Water (180 mL) was poured into the reaction mixture and extracted with EtOAc (60 mL x 3). The combined organic layer was washed with brine (50 mL x 3), dried over Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography (SiO₂, petroleum ether : ethyl acetate = 1:0 to 10:1) to obtain (2-(difluoromethoxy)-3-methylphenyl)(4-methoxybenzyl)sulfane (4.1 g, 78% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.34 (3H, s), 3.79 (3H, s), 4.08 (2H, s), 6.33-6.74 (1H, m), 6.82 (2H, d, *J* = 8.4 Hz), 7.03-7.12 (2H, m), 7.16-7.23 (3H, m).

[341] **Step 2: 2-(difluoromethoxy)-3-methyl-benzenesulfonyl chloride (**Intermediate C4**)**

[342] To a solution of (2-(difluoromethoxy)-3-methylphenyl)(4-methoxybenzyl)sulfane (4 g, 12.89 mmol) in ACN (30 mL), acetic acid (1.5 mL) and water (1.5 mL) was added 1,3-dichloro-5,5-dimethyl-imidazolidine-2,4-dione (5.08 g, 25.78 mmol), and then the reaction mixture was stirred at 10 °C for 30 minutes. Ice water (30 mL) was added to the reaction mixture, quenched with an aqueous sodium hydrogen carbonate solution

(20 mL), and then extracted with DCM (40 mL x 2). The combined organic layer was dried over Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography (SiO₂, petroleum ether : ethyl acetate = 1:0 to 20:1) to obtain **Intermediate C4** (2 g, 60% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.49 (3H, s), 6.49-6.90 (1H, m), 7.36-7.45 (1H, m), 7.64-7.71 (1H, m), 7.94 (1H, dd, *J* = 8.0, 0.8 Hz).

[343] Step 3: dimethyl 4-((2-(difluoromethoxy)-3-methylphenyl)sulfonamido)phthalate (**Intermediate D5**)

[344] The reaction mixture of **Intermediate C4** (1.96 g, 7.65 mmol) and dimethyl 4-aminobenzene-1,2-dicarboxylate (1.6 g, 7.65 mmol) in pyridine (15 mL) was stirred at 25 °C for 1 hour under nitrogen conditions. The reaction mixture was concentrated and then extracted with EtOAc (20 mL x 3), and the combined organic layer was washed with water (30 mL x 2) and brine (30 mL). Thereafter, it was dried over Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain a crude product, which was purified by silica gel column chromatography (SiO₂, petroleum ether : ethyl acetate = 20:1 to 3:1) to obtain **Intermediate D5** (3 g, 91% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.42 (3H, s), 3.88 (3H, s), 3.92 (3H, s), 6.50-6.91 (1H, m), 7.19 (1H, s), 7.28-7.32 (2H, m), 7.37 (1H, d, *J* = 2.4 Hz), 7.50 (1H, dd, *J* = 7.6, 0.8 Hz), 7.66 (1H, d, *J* = 8.4 Hz), 7.83 (1H, dd, *J* = 7.6, 1.2 Hz); LC/MS (ESI) *m/z* = 430.1 [M + H]⁺.

[345] Step 4: 4-[[2-(difluoromethoxy)-3-methyl-phenyl]sulfonylamino]phthalic acid

[346] To a solution of **Intermediate D5** (3 g, 6.99 mmol) in THF (10 mL), MeOH (10 mL) and water (10 mL) was added LiOH·H₂O (879 mg, 20.96 mmol) at 25 °C. The reaction mixture was stirred at 50 °C for 12 hours. The reaction mixture was diluted with water (100 mL) and then extracted with EtOAc (50 mL x 2). The reaction mixture was adjusted to pH 4-5 by adding hydrochloric acid (2 M) to the water layer and extracted with EtOAc (50 mL x 3). The combined organic layer was washed with brine (30 mL x 2), dried over Na₂SO₄, filtered, and then concentrated under reduced pressure to obtain 4-[[2-(difluoromethoxy)-3-methyl-phenyl]sulfonylamino]phthalic acid (2.7 g, 96% yield) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.30 (3H, s), 6.80-7.21 (1H, m), 7.24 (1H, dd, *J* = 8.4, 2.4 Hz), 7.32 (1H, s), 7.36-7.45 (1H, m), 7.57-7.69 (2H, m), 7.76 (1H, d, *J* = 6.8 Hz), 10.93 (1H, s), 12.38-13.74 (2H, m); LC/MS (ESI) *m/z* = 424.1 [M + Na]⁺.

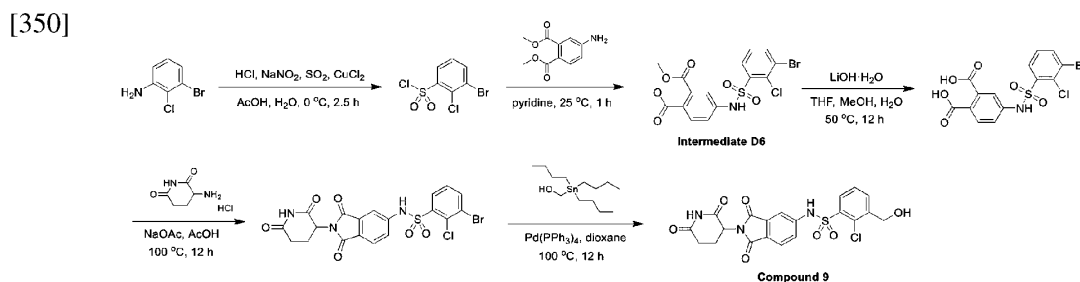
[347] Step 5: 2-(difluoromethoxy)-*N*-

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-benzenesulfonamide (**Compound 8**)

[348] To a solution of 4-[[2-(difluoromethoxy)-3-methyl-phenyl]sulfonylamino]phthalic

acid (1.5 g, 3.74 mmol) and NaOAc (613 mg, 7.47 mmol) in acetic acid (20 mL) was added 3-aminopiperidine-2,6-dione (720 mg, 4.37 mmol, hydrochloride). Thereafter, the reaction mixture was stirred at 100 °C for 12 hours. The reaction mixture was filtered and then concentrated under reduced pressure to obtain a crude product, which was purified by *prep*-HPLC (column: Phenomenex luna C18 150*40 mm* 15 μ m; mobile phase: [water (FA)-ACN]; gradient: 33% - 63% B over 15 min) to obtain **Compound 8** (1.26 g, 67% yield, 98.62% purity) as a pink solid. ^1H NMR (400 MHz, DMSO- d_6) δ ppm 1.96-2.06 (1H, m), 2.30 (3H, s), 2.41-2.48 (1H, m), 2.59 (1H, d, J = 2.8 Hz), 2.79-2.93 (1H, m), 5.08 (1H, dd, J = 12.8, 5.2 Hz), 6.84-7.27 (1H, m), 7.43 (1H, t, J = 8.0 Hz), 7.47-7.55 (2H, m), 7.65 (1H, d, J = 7.2 Hz), 7.78-7.87 (2H, m), 11.10 (1H, s), 11.36 (1H, s); LC/MS (ESI) m/z = 494.2 $[\text{M} + \text{H}]^+$.

[349] **Reaction Scheme 9: 2-chloro-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)-3-(hydroxymethyl)benzenesulfonamide**



[351] **Step 1: 3-bromo-2-chlorobenzenesulfonyl chloride**

[352] To a solution of 3-bromo-2-chloroaniline (2 g, 9.69 mmol) in water (20 mL) were sequentially added hydrochloric acid (12 M, 10.00 mL) and NaNO_2 (804 mg, 11.65 mmol) while stirring. The reaction mixture was stirred at 0 °C for 1 hour to obtain Solution 1. In a separate flask, SO_2 (15 Psi) was bubbled in acetic acid (14 mL) at 0 °C for 20 minutes to obtain Solution 2. Solution 2 was slowly added to Solution 1 at 0 °C and stirred at the same temperature for 10 minutes. CuCl_2 (848 mg, 6.31 mmol) was added, and the reaction mixture was stirred at 0 °C for 1 hour. Water (80 mL) was poured into the reaction mixture and extracted with EtOAc (50 mL x 3). The combined organic layer was washed with brine (50 mL x 3), dried over Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography (SiO_2 , petroleum ether : ethyl acetate = 100:0 to 49:1) to obtain 3-bromo-2-chlorobenzenesulfonyl chloride (0.11 g, 4% yield) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ ppm 7.41 (1H, t, J = 8.0 Hz), 8.03 (1H, dd, J = 8.0, 1.6 Hz), 8.17 (1H, dd, J = 8.0, 1.2 Hz).

[353] **Step 2: dimethyl**

4-[(3-bromo-2-chloro-phenyl)sulfonylamino]benzene-1,2-dicarboxylate (Intermediate

D6)

- [354] To a solution of 3-bromo-2-chlorobenzenesulfonyl chloride (110 mg, 379.36 μmol) in pyridine (2 mL) was added dimethyl 4-aminobenzene-1,2-dicarboxylate (55 mg, 262.91 μmol). The reaction mixture was stirred at 25 °C for 1 hour. The reaction mixture was concentrated, and then water (20 mL) was poured thereinto, and the reaction mixture was extracted with EtOAc (30 mL x 3). The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography (SiO_2 , petroleum ether : ethyl acetate = 10:1 to 4:1) to obtain **Intermediate D6** (60 mg, 46% yield, 93% purity) as a white solid. LC/MS (ESI) $m/z = 431.9$ [$\text{M} - 32 + \text{H}$]⁺.
- [355] Step 3: 4-[(3-bromo-2-chloro-phenyl)sulfonylamino]phthalic acid
- [356] To a solution of **Intermediate D6** (30 mg, 60.30 μmol) in THF (1 mL), MeOH (1 mL) and water (1 mL) was added $\text{LiOH} \cdot \text{H}_2\text{O}$ (11 mg, 266.38 μmol). The reaction mixture was stirred at 50 °C for 12 hours. The reaction mixture was adjusted to pH 4-5 by adding hydrochloric acid (2 M) to the reaction mixture, and then water (20 mL) was added, and the reaction mixture was extracted with EtOAc (10 mL x 3). The combined organic layer was washed with brine (20 mL x 3), dried over Na_2SO_4 , filtered, and then concentrated under reduced pressure to obtain 4-[(3-bromo-2-chloro-phenyl)sulfonylamino]phthalic acid (40 mg, crude product) as a yellow solid. LC/MS (ESI) $m/z = 435.9$ [$\text{M} + \text{H}$]⁺.
- [357] Step 4: 3-bromo-2-chloro-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)benzenesulfonamide
- [358] To a solution of 4-[(3-bromo-2-chlorophenyl)sulfonylamido]phthalic acid (40 mg, 92.03 μmol) and 3-aminopiperidine-2,6-dione (20 mg, 121.51 μmol , hydrochloride) in acetic acid (1 mL) was added NaOAc (10 mg, 121.91 μmol). The reaction mixture was stirred at 100 °C for 12 hours. The reaction mixture was filtered and then concentrated under reduced pressure to obtain a crude product. The crude product was purified by *prep*-HPLC (column: Phenomenex luna C18 150*25 mm* 10 μm ; mobile phase: [water (FA)-ACN]; gradient: 30%-50% B over 10 min) to obtain 3-bromo-2-chloro-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)benzenesulfonamide (8 mg, 17% yield) as a yellow solid. LC/MS (ESI) $m/z = 528.0$ [$\text{M} + \text{H}$]⁺.
- [359] Step 5: 2-chloro-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)-3-(hydroxymethyl)benzenesulfonamide (Compound 9)
- [360] To a solution of tributylstannylmethanol (8 mg, 24.92 μmol) and 3-bromo-2-chloro-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)benzenesulfonamide (8 mg, 15.19 μmol) in 1,4-dioxane (1 mL) was added palladium; triphenylphosphane (562 μg ,

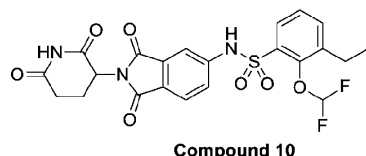
1.52 μ mol) under nitrogen conditions at 25 °C. The reaction mixture was stirred at 100 °C for 12 hours. The reaction mixture was concentrated under reduced pressure to obtain a crude product, which was purified by *prep*-HPLC (column: Phenomenex luna C18 150*25 mm* 10 μ m; mobile phase: [water (TFA)-ACN]; gradient: 28%-48% B over 7 min) to obtain **Compound 9** (5.42 mg, 73% yield, 97.68% purity) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.93-2.07 (1H, m), 2.32-2.44 (1H, m), 2.55-2.69 (1H, m), 2.76-2.93 (1H, m), 4.57 (2H, s), 5.07 (1H, dd, *J* = 12.8, 5.2 Hz), 5.42-5.65 (1H, m), 7.45-7.54 (2H, m), 7.61 (1H, s), 7.82 (2H, t, *J* = 7.2 Hz), 8.10 (1H, d, *J* = 7.6 Hz), 11.10 (1H, s), 11.65 (1H, s); LC/MS (ESI) *m/z* = 478.2 (M + H)⁺.

[361]

[362] **Example 1: 2-(difluoromethoxy)-*N* -**

(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)-3-ethylbenzenesulfonamide (Compound 10)

[363]



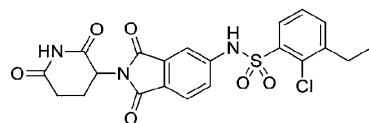
Compound 10

[364] **Compound 10** (53.78 mg, 51% yield, 98.89% purity) was obtained as a yellow solid by reacting **Intermediate A4** in the same manner as in Steps 1 to 5 of Reaction Scheme 8. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.08-1.16 (3H, m), 1.97-2.05 (1H, m), 2.42-2.47 (1H, m), 2.54-2.63 (1H, m), 2.65-2.75 (2H, m), 2.80-2.90 (1H, m), 5.03 (1H, dd, *J* = 12.8, 5.6 Hz), 6.83-7.27 (1H, m), 7.42-7.54 (3H, m), 7.67-7.72 (1H, m), 7.78-7.82 (2H, m), 11.10 (1H, s), 11.32 (1H, s); LC/MS (ESI) *m/z* = 508.2 [M + H]⁺.

[365] **Example 2: 2-chloro-*N* -**

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isindolin-5-yl]-3-ethyl-benzenesulfonamide (Compound 11)

[366]



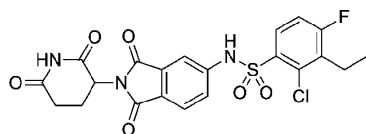
Compound 11

[367] **Compound 11** (7.8 mg, 65% yield, 98.96% purity) was obtained as a yellow solid by reacting **Intermediate A5** in the same manner as in Steps 1 to 5 of Reaction Scheme 8. ¹H NMR (400 MHz, CD₃OD) δ ppm 1.19 (3H, t, *J* = 7.6 Hz), 2.00-2.14 (1H, m), 2.62-2.86 (5H, m), 5.07 (1H, dd, *J* = 12.8, 5.6 Hz), 7.38-7.46 (1H, m), 7.48-7.52 (1H, m), 7.56 (1H, dd, *J* = 7.6, 2.0 Hz), 7.60 (1H, d, *J* = 1.6 Hz), 7.72 (1H, d, *J* = 8.4 Hz), 8.09 (1H, dd, *J* = 8.0, 1.6 Hz); LC/MS (ESI) *m/z* = 476.1 [M + H]⁺.

[368] **Example 3: 2-chloro-*N* -**

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-ethyl-4-fluoro-benzenesulfonamide (Compound 12)

[369]



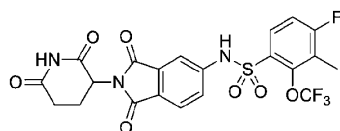
Compound 12

[370] **Compound 12** (69.93 mg, 47% yield, 99.9% purity) was obtained as a white solid by reacting **Intermediate A6** in the same manner as in Steps 1 to 5 of Reaction Scheme 8. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.07 (3H, t, *J* = 7.2 Hz), 1.95-2.05 (1H, m), 2.31-2.46 (1H, m), 2.57-2.70 (1H, m), 2.71-2.80 (2H, m), 2.80-2.91 (1H, m), 5.07 (1H, dd, *J* = 12.8, 5.6 Hz), 7.40-7.55 (3H, m), 7.75-7.87 (1H, m), 8.12 (1H, dd, *J* = 8.8, 5.6 Hz), 11.09 (1H, s), 11.66 (1H, s); LC/MS (ESI) *m/z* = 494.2 [M + H]⁺.

[371] **Example 4: N -**

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-4-fluoro-3-methyl-2-(trifluoromethoxy)benzenesulfonamide (Compound 13)

[372]



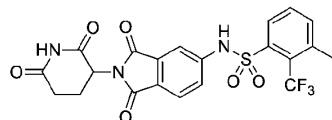
Compound 13

[373] **Compound 13** (13.58 mg, 26% yield, 98.87% purity) was obtained as a green solid by reacting **Intermediate A7** in the same manner as in Steps 1 to 5 of Reaction Scheme 8. ¹H NMR (400 MHz, CDCl₃) δ ppm 2.08-2.19 (1H, m), 2.31 (3H, s), 2.68-2.99 (3H, m), 4.94 (1H, dd, *J* = 12.0, 5.2 Hz), 7.16 (1H, t, *J* = 8.0 Hz), 7.22 (1H, s), 7.44 (1H, d, *J* = 7.2 Hz), 7.56 (1H, s), 7.76 (1H, d, *J* = 8.4 Hz), 7.89-8.02 (2H, m); LC/MS (ESI) *m/z* = 530.2 [M + H]⁺.

[374] **Example 5: N -**

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-2-(trifluoromethyl)benzenesulfonamide (Compound 14)

[375]

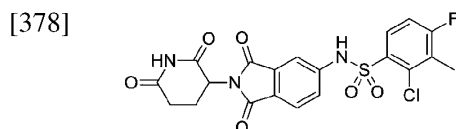


Compound 14

[376] **Compound 14** (9.39 mg, 27% yield, 97.57% purity) was obtained as an off-white solid by reacting commercially available 1-bromo-3-methyl-2-(trifluoromethyl)benzene in the same manner as in Steps 1 to 5 of

Reaction Scheme 8. ^1H NMR (400 MHz, CDCl_3) δ ppm 2.11-2.17 (1H, m), 2.59 (3H, q, $J = 3.2$ Hz), 2.70-2.83 (2H, m), 2.87-2.97 (1H, m), 4.92-4.98 (1H, m), 7.47 (1H, dd, $J = 8.0, 2.0$ Hz), 7.55 (2H, d, $J = 5.2$ Hz), 7.63 (1H, d, $J = 2.0$ Hz), 7.77 (1H, d, $J = 8.0$ Hz), 8.14 (1H, t, $J = 4.8$ Hz); LC/MS (ESI) $m/z = 496.2$ $[\text{M} + \text{H}]^+$.

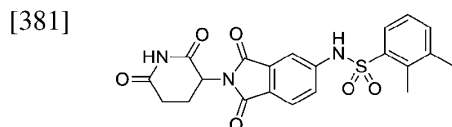
[377] **Example 6: 2-chloro-*N*-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-4-fluoro-3-methyl-benzenesulfonamide (Compound 15)**



Compound 15

[379] **Compound 15** (15.26 mg, 41% yield, 99.84% purity) was obtained as a white solid by reacting commercially available 1-bromo-2-chloro-4-fluoro-3-methyl-benzene in the same manner as in Steps 1 to 5 of Reaction Scheme 8. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.95-2.04 (1H, m), 2.26 (3H, s), 2.58-2.68 (2H, m), 2.80-2.90 (1H, m), 5.07 (1H, m), 7.35-7.54 (3H, m), 7.81 (1H, d, $J = 8.8$ Hz), 8.12 (1H, m), 11.09 (1H, s), 11.47-11.86 (1H, m); LC/MS (ESI) $m/z = 480.2$ $[\text{M} + \text{H}]^+$.

[380] **Example 7: *N*-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-2,3-dimethyl-benzenesulfonamide (Compound 16)**

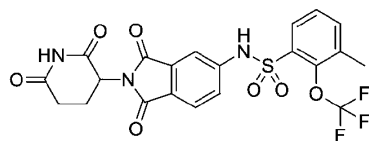


Compound 16

[382] **Compound 16** (74.33 mg, 39% yield, 98.92% purity) was obtained as a white solid by reacting commercially available 1-iodo-2,3-dimethyl-benzene in the same manner as in Steps 1 to 5 of Reaction Scheme 8. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.96-2.04 (1H, m), 2.28 (3H, s), 2.35-2.47 (1H, m), 2.53 (3H, s), 2.57-2.69 (1H, m), 2.80-2.92 (1H, m), 5.07 (1 H, dd, $J = 13.2, 5.6$ Hz), 7.26-7.38 (1H, m), 7.43-7.50 (3H, m), 7.70-7.95 (2H, m), 11.10 (1H, s), 11.46 (1H, s); LC/MS (ESI) $m/z = 442.2$ $[\text{M} + \text{H}]^+$.

[383] **Example 8: *N*-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-methyl-2-(trifluoromethoxy)benzenesulfonamide (Compound 17)**

[384]

**Compound 17**

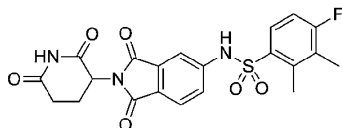
[385] **Compound 17** (1.85 mg, 25% yield, 98.26% purity) was obtained as a white solid by reacting **Intermediate A8** in the same manner as in Steps 1 to 5 of Reaction Scheme 8. ¹H NMR (400 MHz, CD₃OD) δ ppm 2.01-2.26 (1H, m), 2.39 (3H, s), 2.59-2.97 (3H, m), 5.06 - 5.13 (1H, m), 7.40-7.46 (1H, m), 7.48-7.52 (1H, m), 7.58-7.64 (2H, m), 7.71-7.77 (1H, m), 7.91-7.98 (1H, m); LC/MS (ESI) m/z = 512.2 [M + H]⁺. LC/MS t_R = 2.253 min.

[386]

Example 9: N -

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-4-fluoro-2,3-dimethyl-benzenesulfonamide (Compound 18)

[387]

**Compound 18**

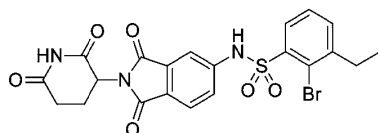
[388] **Compound 18** (79.37 mg, 41% yield, 98.26% purity) was obtained as an off-white solid by reacting commercially available 4-fluoro-2,3-dimethyl-aniline in the same manner as in Steps 1 to 4 of Reaction Scheme 4. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.94-2.04 (1H, m), 2.16 (3H, s), 2.51-2.60 (5H, m), 2.79-2.92 (1H, m), 5.07 (1H, dd, *J* = 12.8, 5.2 Hz), 7.18-7.30 (1H, m), 7.40-7.50 (2H, m), 7.74-7.83 (1H, m), 7.93 (1H, dd, *J* = 8.8, 5.6 Hz), 11.09 (1H, s), 11.50 (1H, br s); LC/MS (ESI) m/z = 460.1 [M + H]⁺.

[389]

Example 10: 2-bromo-N -

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-3-ethyl-benzenesulfonamide (Compound 19)

[390]

**Compound 19**

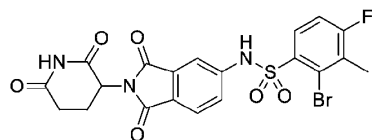
[391] **Compound 19** (73.48 mg, 138.66 μmol, 50% yield, 98.19% purity) was obtained as a yellow solid by reacting **Intermediate B1** in the same manner as in Steps 1 to 4 of Reaction Scheme 4. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.15 (3H, t, *J* = 7.6 Hz),

1.95-2.07 (1H, m), 2.40-2.49 (2H, m), 2.53-2.63 (1H, m), 2.75-2.93 (3H, m), 5.00-5.15 (1H, m), 7.45-7.55 (2H, m), 7.55-7.61 (1H, m), 7.63-7.65 (1H, m), 7.80-7.83 (1H, m), 8.06-8.08 (1H, m), 11.10 (1H, s), 11.61 (1H, m); LC/MS (ESI) m/z = 522.2 $[M + H]^+$.

[392] **Example 11: 2-bromo-*N*-**

[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]-4-fluoro-3-methyl-benzenesulfonamide (Compound 20)

[393]



Compound 20

[394] **Compound 20** (22 mg, 39.94 μ mol, 49% yield, 95.19% purity) was obtained as a yellow solid by reacting **Intermediate B2** in the same manner as in Steps 1 to 4 of Reaction Scheme 4 and separating Compound 20 from the resulting two product mixtures. The structure of the compound was identified by NMR analysis. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 1.97-2.01 (1H, m), 2.28-2.33 (3H, m), 2.40-2.48 (1H, m), 2.57-2.71 (1H, m), 2.81-2.93 (1H, m), 4.91-5.16 (1H, m), 7.39-7.66 (3H, m), 7.76-7.78 (1H, m), 8.11-8.15 (1H, m), 11.09 (1H, s), 11.71 (1H, s); LC/MS (ESI) m/z = 524.1 $[M + H]^+$.

[395]

[396] **[Experimental Examples]**

[397] **1. Cell culture and materials**

[398] The NCI-H1155 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in RPMI 1640 (Roswell Park Memorial Institute Medium 1640; Cytiva) supplemented with 10% FBS, 2 mM L-glutamine, and 100 unit/mL streptomycin-penicillin. The HL-60 cell line was purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea) and maintained in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, and 100 unit/mL streptomycin-penicillin. The NCI-H2023 cell line was purchased from the ATCC and maintained in Dulbecco's Modified Eagle Medium (DMEM / F12 1:1 medium, Cytiva) mixed 1:1 with F12 supplemented with 5% FBS, 0.005 mg/mL insulin, 0.01 mg/mL transferrin, 30 nM sodium selenite, 10 nM hydrocortisone, 10 nM beta-estradiol, and 100 unit/mL streptomycin-penicillin. The HEKa cell line was purchased from the ATCC and maintained in Keratinocyte Growth Kit (ATCC) supplemented with 6 mM L-glutamine, 0.4% bovine pituitary extract, 0.5 ng/mL rh TGF- α , 100 ng/mL hydrocortisone hemisuccinate, 5mg/mL rh insulin, 1 mM epinephrine, and 5 mg/mL apo-transferrin and Dermal Cell Basal Medium (ATCC) supplemented with 100 unit/mL streptomycin-penicillin. The HEK-293 hGSPT1 HiBiT tag cell line was purchased

from Synthego Corporation (Redwood City, CA) and maintained in DMEM medium supplemented with 10% FBS, 2 mM L-glutamine, and 100 unit/mL streptomycin-penicillin.

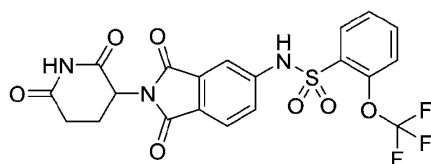
[399] **2. CRBN binding ability analysis**

[400] CRBN binding ability analysis was commissioned by Eurofins and analyzed using the E3scan™ platform. The experimental method used is as follows. The CRBN-DDB1 protein complex was produced using the HEK-293 cell line, and then DNA for qPCR detection was labeled. Streptavidin-coated magnetic beads and a biotinylated small molecule ligand that binds to CRBN were reacted for 30 minutes to produce affinity beads with the small molecule ligand immobilized. The affinity beads with the ligand immobilized were blocked with an excess of biotin and washed with the blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and prevent non-specific binding. The binding reaction was performed by mixing the CRBN-DDB1 ligase, the affinity beads, and the test compound in 1x binding buffer (20% SeaBlock, 0.17x PBS, 0.05% Tween 20, 6 mM DTT). The test compound was prepared in a 100% DMSO solution at a concentration 111 times higher than the final concentration. The solution of the test compound was diluted to 11 concentrations in 3-fold serial dilution. The test compound was dispensed with 100% DMSO using a non-contact dispensing method. Thereafter, the compounds were diluted directly into solutions for experiments to a final concentration of DMSO of 0.9%. All reactions were performed in a polypropylene 384 well plate. The final volume of each reaction solution was set to 0.02 mL. The experimental plate was reacted at room temperature with shaking for about 1 hour, and the affinity beads were washed with the washing buffer (1x PBS, 0.05% Tween 20). Thereafter, the beads were redispersed in the elution buffer (1x PBS, 0.05% Tween 20, 0.5 μM non-biotinylated affinity ligand) and then reacted at room temperature with shaking for about 30 minutes. CRBN-DDB1 concentration in the eluate was measured by qPCR. Kd was calculated by analyzing the solutions of the test compounds at the 11 concentrations and 3 control groups (DMSO).

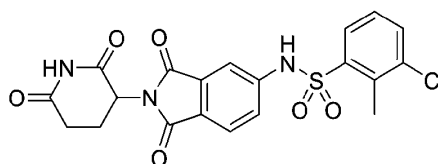
[401] Reference Compound 1 and Reference Compound 2 are compounds having the following structures, described as "Compound 1" and "Compound 5" in International Publication No. WO 2022/066835.

[402]

[Reference Compound 1]



[Reference Compound 2]



[403] The results of analyzing the binding ability to the CRBN-DDB1 complex in this experimental example are shown in Table 1 below.

[404] [Table 1]

Compound No.	Kd (nM)
Reference Compound 1	6
Reference Compound 2	9.9
Compound 2	28
Compound 3	6.2
Compound 4	3.5
Compound 8	7.3
Compound 15	7.9
Compound 16	8.6
Compound 18	7.2

[405] The example compounds showed equal or higher binding ability (lower Kd) to CRBN-DDB1 compared to Reference Compound 1 and Reference Compound 2. However, since the binding ability to CRBN-DDB1 does not necessarily lead to GSPT1 degradation activity, GSPT1 degradation activity was also evaluated below.

[406] **3. Evaluation of GSPT1 degradation ability**

[407] **Production of HEK293 HiBiT-GSPT1 cell line**

[408] The HEK293 cell line expressing HiBiT-GSPT1 was produced by Synthego (California, USA). DNA donor capable of expressing the HiBiT protein fragment consisting of 11 amino acids (VSGWRLFKKIS (SEQ ID NO. 1)(CCACTCCTCTCCGGCCGGGCGCCCCCTGCCTCCATTTCCTGCTCTGTCC ACCACACACACGGCCCCCGGATAATGGTGAGCGGCTGGCGGGCTGTTCAA GAAGATTAGCGGCGGTAGCGATCCGGGCAGTGGCGGCGGCGGCGGGCG GCGGCGGCGGCGGGAGCAGCAGCGGCAGCAGCAGCAGCAGCGACTCGGC (SEQ ID NO. 2), the underlined part is the HiBiT nucleotide sequence) was produced and injected into cells using electroporation together with the CRISPR/Cas9 system that can cause a double-strand break at the N-terminus of the GSPT1 gene in the HEK293 cells. For cell lines in which gene editing was induced, a single clone was obtained through single cell sorting. To verify the correctness of the gene editing, the GSPT1 gene sequence edited from the obtained single clone was amplified using the following two primers [Forward (5'-3'): TTGGCGTTGACGTTGAGTTG (SEQ ID NO. 3), Reverse (5'-3'): ACACGAGGAGGAGGGTTGAG (SEQ ID NO. 4)], and the edited HiBiT-GSPT1 gene sequence was determined by Sanger sequencing method.

[409] **hGSPT1 HiBiT analysis**

[410] The HEK-293 hGSPT1 HiBiT tag cells were seeded in triplicate in a white 96-well analysis plate at a density of 5,000 cells per well. After overnight incubation, a medium mixed with the 3-fold concentration of the test compound was used to treat the cells with the test compound at final concentrations of 1 nM, 3 nM, 10 nM, 30 nM, 100 nM, 1 μ M, 3 μ M, and 10 μ M. After 72 hours of incubation, the level of the GSPT1 HiBiT tag protein was evaluated using the Nano-Glo® HiBiT Lytic Detection System (Promega) according to the manufacturer's instructions. The luminescence signals were measured using a Varioskan LUX multimode microplate reader.

[411] The GSPT1 degradation activity was evaluated in three aspects: EC_{50} , DC_{50} , and D_{max} . D_{max} (%) represents the maximum value for degrading GSPT1 at the highest concentration. In addition, through four parameter logistic regression, the relative EC_{50} values were calculated assuming that the D_{max} value calculated in each experiment was the maximum effect. In this test system, DC_{50} was calculated by defining it as the concentration required to cause the absolute degradation of GSPT1 by 50%. The calculated EC_{50} , DC_{50} , and D_{max} values are shown in Table 2 below.

[412] [Table 2]

Compound No.	EC_{50} (nM)	DC_{50} (nM)	D_{max} (%)
Reference Compound 1	198.3	454.5	78.3
Reference Compound 2	506.0	>10000	42.8
Compound 1	731	>10000	37.3
Compound 2	216.8	557.1	78.4
Compound 3	140	1712.9	59.8
Compound 4	92.3	214	79.9
Compound 5	99.2	131	86.7
Compound 6	2080	>10000	18
Compound 7	337	3090	58
Compound 8	128.4	495.8	73.3
Compound 9	2590	>10000	46.2
Compound 10	289	936	71.4
Compound 11	144.9	2953.8	55.3
Compound 12	253	751	70.5
Compound 13	153	399	76.5
Compound 14	123	118	88.3

Compound 15	116	129	88.6
Compound 16	87.1	>10000	28.1
Compound 17	68.4	318	72.9
Compound 18	288	6140	49.6
Compound 19	123	218	81.9
Compound 20	94.1	104.0	89.5

- [413] For Reference Compound 2, D_{max} was 42.8% and DC₅₀ was greater than 10 μ M. Reference Compound 1 has EC₅₀ = 198.3 nM; DC₅₀ = 454.5 nM; and D_{max} = 78.3%, showing better GSPT1 degradation activity than Reference Compound 2. Meanwhile, the example compounds showed equivalent or improved results in terms of EC₅₀, DC₅₀, and D_{max} compared to Reference Compounds 1 and 2.
- [414] **4. Test method for determining GSPT1 degradation ability through immunoblot analysis**
- [415] In order to determine whether the results of the GSPT1 HiBiT test, a test system artificially created for high-throughput screening of compounds, are reproduced in cancer cell lines and determine the selectivity of the compounds of the present invention for the degradation of various neosubstrates, the NCI-H1155 cell line, a lung cancer cell line, was treated with the example compounds, and then the expression levels of GSPT1 and other known neosubstrates were determined through immunoblot experiments.
- [416] The NCI-H1155 cells were seeded in a 6 well plate (2 x 10⁶ cells per well). After overnight incubation, the cells were treated with Reference Compound 1, Compound 3, Compound 4, and Compound 5 at concentrations of 0 nM, 3 nM, 10 nM, 30 nM, 100 nM, 300 nM, and 1000 nM, respectively. After 6 hours, the cells were harvested. In addition, in order to confirm the change in the amount of GSPT1 degradation over time, the cells were treated with Reference Compound 1, Compound 2, Compound 3, Compound 4, Compound 8, and Compound 11 at a concentration of 100 nM, respectively. After 0, 2, 4, 8, 24, 48, and 72 hours, the cells were harvested. The harvested cells were spun down, washed with PBS, lysed with the RIPA buffer supplemented with Protease Inhibitor Cocktail (Roche) and Phosphatase Inhibitor Cocktail (Roche), and then frozen overnight in a -80 °C cryogenic freezer. The samples thawed on ice were centrifuged, and 1x LDS loading buffer and 1x reducing agent were added, and the samples were heated at 95 °C. The prepared samples were loaded on NuPAGE 4-12% Bis-Tris protein gel and transferred to a nitrocellulose membrane. The membrane was blocked with 5% bovine serum albumin and incubated with primary antibodies overnight, and then the corresponding protein signals were detected using

HRP-linked secondary antibodies and an iBright CL1500 imaging system. The antibodies used were as follows:

- [417] Primary antibodies: rabbit anti-human eRF3/GSPT1 [EPR22908-103] (ab234433, abcam), rabbit anti-human IKAROS (5443, CST), rabbit anti-human HELIOS (D8W4X) (42427, CST), rabbit anti-human AIOLOS (D1C1E) (15103, CST), rabbit anti-human CK1a [EPR1961(2)] (ab108296, abcam), mouse anti-human SALL4 (ab57577, abcam), mouse anti- β -Actin-Peroxidase antibody (A3854, Sigma), and rabbit anti-human N-MYC (D1V2A) (84406, CST)
- [418] Secondary antibodies: goat anti-rabbit IgG, HRP-linked (7074, CST) and horse anti-mouse IgG, HRP-linked (7076, CST).
- [419] The results of measuring the degradation activity of GSPT1 and various neo-substrates are shown in Figures 1 and 2.
- [420] Compound 3, Compound 4, and Compound 5 all showed equivalent or improved GSPT1 degradation activity compared to Reference Compound 1 (see Figure 1). In addition, all the example compounds evaluated had no significant effect on the expression level of other neosubstrates even at the highest concentration. As a result, it was confirmed that the compounds of the present invention exhibit selective degradation activity against GSPT1.
- [421] Meanwhile, when the cancer cell line was treated with Reference Compound 1 at a concentration of 100 nM and the degree of GSPT1 degradation over time was observed, the expression level of GSPT1 was gradually reduced until 24 hours, but after that, from 48 to 72 hours, the expression level of GSPT1 was increased again (see Figure 2). On the other hand, for Compound 2, Compound 3, Compound 4, Compound 8, and Compound 11, a stable decrease in the expression level of GSPT1 was shown throughout the measurement. Therefore, it was confirmed that the compounds of the present invention exhibit much more stable and sustained GSPT1 degradation activity compared to Reference Compound 1.
- [422] **5. Real time cell proliferation analysis**
- [423] In order to determine whether the duration of GSPT1 degradation affects the ability to inhibit cancer cell growth, the cancer cells were treated with Reference Compound 1 and the example compounds, and then the real time cancer cell growth was measured using an Incucyte S3 live cell analysis system (Sartorius, Ann Arbor, MI, USA).
- [424] Real time cell proliferation analysis was performed at Seoul National University. The NCI-H1155 cells were seeded in a transparent 96-well plate at a density of 4,000 cells per well. The cells were cultured overnight in a humidified 5% CO₂ incubator at 37 °C and then treated with Reference Compound 1 and the test compounds at various concentrations. After treatment with the compounds, the cell growth was measured in real time using an Incucyte S3 live cell analysis system (Sartorius, Ann Arbor, MI, USA)

for 72 hours. The measurement results are shown in Figure 3.

[425] When treated with 100 nM of Reference Compound 1, the lung cancer cell line NCI-H1155 cells showed a tendency to inhibit cell growth until 48 hours, but cell growth was rapidly increased after 48 hours (marked as ● in Figure 3). This is consistent with the result (Figure 2) that the expression level of GSPT1 was rebounded from 48 hours when treated with 100 nM of Reference Compound 1. Considering that the EC₅₀ of Reference Compound 1 is 198.3 nM (see Table 2), it is expected from these results that Reference Compound 1 will not show a sustained GSPT1 degradation effect at a dose effective for GSPT1 degradation.

[426] On the other hand, Compound 3, Compound 4, Compound 5, Compound 15, and Compound 19 showed sustained cancer cell growth inhibition at a concentration of 100 nM (marked as ● in Figure 3). These compounds did not show any rebound in cell growth or showed a minimal rebound compared to Reference Compound 1 at 100 nM, even at a lower concentration of 30 nM (marked as ◆ in Figure 3). Overall, it was confirmed that the compounds of the present invention can induce the GSPT1 degradation and cancer cell growth inhibition more consistently and stably than Reference Compound 1.

[427] **6. pH stability**

[428] Approximately 1 mg of Reference Compound 1 (M.W=497.40) powder was added to a 20 mL bottle, 10 mL MeOH was added, and then mixed well. Approximately 2.4 g of acetic acid, 2.48 g of boric acid, and 3.92 g of phosphoric acid were placed in a 500 mL bottle, 500 mL of purified water was added, and then mixed well. The mixed solution was equally divided into 7 bottles and adjusted to pH 2.7, 4.4, 5.2, 6.0, 7.1, 8.1, and 9.1 using 1N HCl or 1N NaOH solution. 10 μL of MeOH solution of Reference Compound 1 was transferred to 7 bottles, 990 μL of buffer solution was added, and then mixed well. Approximately 2 mg of Compound 3 (M.W=461.88) powder was placed in a 2 mL flask and then dissolved by adding 1 mL of DMSO. Approximately 2.4 g of acetic acid, 2.48 g of boric acid, and 3.92 g of phosphoric acid were placed in a 500 mL bottle, 500 mL of purified water was added, and then mixed well. The mixed solution was equally divided into 6 bottles and adjusted to pH 2.0, 4.0, 7.0, 8.0, 9.0, and 11.0 using 1N HCl or 1N NaOH solution. 10 μL of Compound 3 solution (2 mg/ml DMSO solution) was transferred to 6 bottles, 990 μL of buffer solution was added, and then mixed well. HPLC analysis was performed under the conditions described in Tables 3 and 4 below.

[429] [Table 3]

HPLC analysis conditions for Reference Compound 1

Instrument	Agilent 1260
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Instrument NO	CAS-SH-LCMS-BB		
Column	Shiseido C18 (4.6 mm * 150 mm *, 3 µm)		
Mobile phase	Mobile phase A (MPA): 0.1% TFA in water		
	Mobile phase B (MPB): 0.1% TFA in ACN		
Flow rate	1.0 mL/min		
Column temperature	not controlled		
Injection dose	20 µL		
Wavelength	230 nm		
Gradient	Time (min)	MPA%	MPB%
	0	70	30
	5	50	50
	10	50	50
	15	30	70
	16	70	30
	20	70	30

[430] [Table 4]
HPLC analysis conditions for Compound 3

Instrument	Agilent 1260
Instrument NO	CAS-SH-LCMS-BB
Column	Agilent Poroshell 120 EC-C18 2.7 µm 100 *4.6 mm
Mobile phase	Mobile phase A (MPA): 0.1% FA in water
	Mobile phase B (MPB): 0.1% FA in ACN
Flow rate	0.8 mL/min
Column temperature	not controlled
Injection dose	20 µL
Wavelength	248 nm
Cell	60 mm

Gradient	Time (min)	MPA%	MPB%
	0	70	30
	1	70	30
	7	10	90
	10	10	90
	10.1	70	30
	12	70	30

[431] The results of measuring the pH stability of Reference Compound 1 and Compound 3 are shown in Tables 5 and 6, respectively.

[432] [Table 5]

Stability (%) of Reference Compound 1							
Time (hrs)	pH=2.7	pH=4.4	pH=5.2	pH=6.0	pH=7.1	pH=8.1	pH=9.1
0	98.8	98.8	98.8	98.8	98.8	98.8	98.8
1	-	-	-	-	-	-	21.5
2	96.9	96.6	96.7	96.0	92.1	68.6	7.0
4	96.7	96.1	95.5	93.9	87.4	54.4	1.6
8	95.6	95.5	94.7	92.5	84.6	36.6	0.0
24	95.8	87.0	86.5	79.2	54.2	0.0	-

[433] [Table 6]

Stability (%) of Compound 3						
Time (hrs)	pH=2.0	pH=4.0	pH=7.0	pH=8.0	pH=9.0	pH=11.0
0	100.00	100.00	100.00	100.00	100.00	100.00
1	100.80	101.13	100.98	95.20	55.26	0.00
2	103.42	101.88	100.17	89.36	29.29	0.00
3	103.89	102.39	99.27	83.54	15.19	0.00
4	104.73	102.52	98.51	77.82	7.87	0.00
6	105.12	102.93	96.37	68.56	2.48	0.00
8	105.29	102.36	94.17	59.55	0.75	0.00
12	106.00	102.19	89.90	45.42	0.00	0.00
24	107.41	98.94	81.12	25.07	0.00	0.00

- [434] As shown in Table 5, the residual amounts of Reference Compound 1 were only 87%, 86.5%, and 79.2%, respectively, after 24 hours in buffer solutions of pH 4.4, 5.2, and 6.0. Furthermore, Reference Compound 1 began to show instability with a residual amount of 87.4%, at 4 hours in the neutral condition of pH 7.1, and after 24 hours, the residual amount was only 54.2%, indicating that it was very unstable (Table 5). From the above results, it can be seen that Reference Compound 1 is unstable at physiological pH (neutral pH) and will not show sustained drug efficacy. This is consistent with the results showing that the expression level of GSPT1 was restored 48 hours after treatment of the cancer cell line with Reference Compound 1 (Figure 2) and that cell proliferation occurred again (Figure 3).
- [435] On the other hand, Compound 3 was very stable at pH 4.0 for 24 hours and was stable throughout the experiment period even in the neutral condition of pH 7.0 (81.12% of compound remaining after 24 hours). Therefore, it was confirmed that Compound 3 exhibited excellent pH stability compared to Reference Compound 1. From the above results, it can be seen that Compound 3 can act more stably compared to Reference Compound 1 at physiological pH (neutral pH). The above results are consistent with the results shown in Figures 2 and 3. Unlike Reference Compound 1, treatment of cancer cell lines with Compound 3 stably reduced the expression level of GSPT1 even for 72 hours (Figure 2), and the cell proliferation also did not rebound (Figure 3).
- [436] **7. Proteomics analysis using TMT labeling mass spectrometry**
- [437] In order to determine whether the compound of the present invention selectively degrades only GSPT1 at the level of the entire proteome within cells, TMT labeling proteomics analysis was performed.
- [438] The cell lysis solution was prepared by dissolving a tablet containing a protease inhibitor and a phosphatase inhibitor in a mixed solution of 8 M urea, 1% SDS, and 50 mM Tris pH 8.5. The HL60 cell line was treated with 3 samples of DMSO, 4 samples of Compound 3, and 4 samples of Compound 4 for 4 hours, respectively, and then the cells were lysed with the cell lysis solution. The lysed cell solution was treated with an ultrasonic homogenizer three times for 10 seconds on ice and centrifuged at 14,000 g for 15 minutes at 4 °C. Only the supernatant was separated, and proteomics analysis was performed at the Korea Basic Science Institute (KBSI). Each sample was digested into peptide fragments on an S-Trap mini spin column (Protifi, USA) according to the method suggested by the manufacturer. Samples that had undergone pretreatment were each labeled with TMT using the 11 plex TMT labeling kit (Thermo Scientific) according to the method suggested by the manufacturer. Next, mass spectrometry was performed using an LC-MS/MS system consisting of an UltiMate 3000 RSLCnano system (Thermo Fisher Scientific) and a nanoelectrospray source (EASY-Spray

Sources, Thermo Fisher Scientific) equipped with an Orbitrap Eclipse Tribrid mass spectrometer (Thermo Fisher Scientific). The obtained results were analyzed by filtering only confident peptides with a global FDR <1% and first-ranked protein fragments, and only proteins with a minimum spectral count of 4 or more after normalization were used for analysis.

[439] The analysis results of quantifying 6,534 inherent proteins are shown in Figure 4. As shown in Figure 4, Compound 3 and Compound 4 reduced only the abundance of GSPT1 among the entire proteome. These results support that Compound 3 and Compound 4 show a selective profile for GSPT1 at the entire proteome level and have neosubstrate specificity only for GSPT1.

[440] **8. Evaluation of inhibitory ability on protein translation rate**

[441] GSPT1 is an enzyme that mediates the termination step of protein translation, and degradation of GSPT1 slows down protein translation rate. Accordingly, the degradation of GSPT1 is known to induce inhibition of the expression level of cancer-inducing proteins (for example, c-MYC, N-MYC, L-MYC, BCL-2, MCL-1, and the like) whose expression level is maintained at a high level depending on protein translation in cancer cells (Mullard, Nat Rev Drug Discov. 2022, 21: 865-867). Meanwhile, the degradation of GSPT1 is known to activate a cell death mechanism called "integrated stress response" (Surka et al., Blood 2021, 137(5): 661-677). This response is known to be mediated by ATF-4 and ultimately cause cell death in a caspase 3-dependent manner.

[442] In order to determine whether the pharmacodynamic changes were induced by the compound of the present invention in NCI-H1155, a lung cancer cell line where cell death is known to be induced sensitively by GSPT1 degraders, the protein translation rate was measured using puromycin. Puromycin is a substance commonly used as a means to measure protein translation rate because it can be inserted into the protein sequence instead of tyrosyl-tRNA during the protein translation process.

[443] The NCI-H1155 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in RPMI 1640 medium (Cytiva) according to recommendations. The HL-60 cell line was purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea) and cultured in RPMI 1640 (Cytiva) supplemented with 10% FBS, 2 mM L-glutamine, and 100 unit/mL streptomycin-penicillin. The NCI-H2023 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in DMEM / F12 1:1 medium (Cytiva) according to recommendations.

[444] The NCI-H1155 cells, the HL-60 cells, and the NCI-H2023 cells were each seeded in a 6-well plate (2×10^6 cells per well). After overnight incubation, the following experiment was performed.

- [445] First, the NCI-H1155 cells were treated with 1 μ M of Compound 3. 6 hours after the compound treatment, the cells were treated with 1 μ M puromycin. The cells were harvested 0, 10, 20, 30, and 60 minutes after the puromycin treatment, respectively. The NCI-H2023 cells were treated with 1 μ M of Compound 3 and Reference Compound 1, respectively, in a similar manner. 48 hours after the compound treatment, the cells were treated with 1 μ M puromycin. The cells were harvested 0, 30, 60, and 90 minutes after the puromycin treatment, respectively. The DMSO treated group was used as a control group.
- [446] Meanwhile, in order to confirm the effect of protein translation inhibition by Compound 3 on the expression level of the downstream cancer-inducing protein N-MYC, the NCI-H1155 cells were treated with 1 μ M of Compound 3. Protein translation was labeled by treatment with 1 μ M puromycin at 0, 2, 4, 8, and 24 hours after the compound treatment. The cells were harvested 30 minutes after the puromycin treatment. In addition, in order to confirm the expression levels of N-MYC, ATF-4, and cleaved caspase 3, the NCI-H1155 cells and the HL60 cells were treated with 0.3 μ M and 1 μ M of Compound 3, respectively. After 0, 2, 4, 6, 8, and 24 hours, the cells were harvested.
- [447] The cells harvested at each time point were spun down, washed with PBS, lysed with the RIPA buffer supplemented with Protease Inhibitor Cocktail (Roche) and Phosphatase Inhibitor Cocktail (Roche), and then frozen overnight in a -80 °C cryogenic freezer. The samples thawed on ice were centrifuged, and 1x LDS loading buffer and 1x reducing agent were added, and the samples were heated at 95 °C. The prepared samples were loaded on NuPAGE 4-12% Bis-Tris protein gel and transferred to a nitrocellulose membrane. The membrane was blocked with 5% bovine serum albumin and incubated with primary antibodies overnight, and then the corresponding protein signals were detected using HRP-linked secondary antibodies and an iBright CL1500 imaging system. The antibodies used were as follows.
- [448] Primary antibodies: mouse anti-Puromycin (MABE343, sigma), rabbit anti-human eRF3/GSPT1 [EPR22908-103] (ab234433, abcam), rabbit anti-human ATF-4 (D4B8) (11815, CST), rabbit anti-human Cleaved Caspase-3 (Asp175) (9661, CST), mouse anti- β -Actin-Peroxidase antibody (A3854, Sigma)
- [449] Secondary antibodies: horse anti-mouse IgG, HRP-linked (7076, CST), goat anti-rabbit IgG, HRP-linked (7074, CST)
- [450] The analysis results are shown in Figures 5 to 8.
- [451] As shown in Figure 5, when treated with Compound 3, the protein translation rate was significantly reduced along with GSPT1 degradation, compared to the DMSO control group. As shown in Figure 6, the inhibition of protein translation due to GSPT1 degradation was observed about 4 hours after treatment with Compound 3, and the ex-

pression level of the downstream cancer-inducing protein N-MYC was observed to be suppressed 24 hours after treatment with Compound 3, when protein translation was completely inhibited (Figure 6, left). This shows that GSPT1 degradation, the inhibition of protein translation, and the reduction in N-MYC expression level occur sequentially. Even in samples not labeled with puromycin, a decrease in the expression level of N-MYC was observed 24 hours after treatment with Compound 3 (Figure 6, right). In addition, as the expression of GSPT1 is reduced, the expression of ATF-4 protein, which mediates the integrated stress response, is increased, and it was observed that activation (cleavage) of caspase 3, which induces cell death, was sequentially induced 24 hours after treatment with Compound 3 (Figure 6, right). These pharmacodynamic changes were also observed in HL60, an acute myeloid leukemia (AML) cell line (Figure 7).

[452] Meanwhile, in NCI-H2023 cells, where cell death is known not to be induced by GSPT1 degradation, Compound 3 only partially inhibited GSPT1 degradation and accordingly did not inhibit protein translation (Figure 8, left). On the other hand, Reference Compound 1 was observed to exhibit strong GSPT1 degradation activity and inhibit protein translation even in the NCI-H2023 cells (Figure 8, right). The above results support the usefulness of the compound of the present invention as personalized medicine by showing particularly excellent anticancer activity against carcinomas sensitive to GSPT1 degradation.

[453] **9. Cell viability analysis**

[454] The inhibitory efficacy of the compounds of the present invention on cell proliferation was observed using the NCI-H1155 cell line, a lung cancer cell line, and the HL60, MOLM13, MOLM14, MV-4-11, and U937 cells, which are acute myeloid leukemia (AML) cell lines.

[455] The NCI-H1155 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in RPMI 1640 medium (Cytiva) according to recommendations. The exponentially growing NCI-H1155 cells were plated at 6,000 cells per well in a SPL 33596 white 96-well analysis plate and cultured overnight at 37 °C in a humidified 5% CO₂ incubator. Using a medium mixed with the 3-fold concentration of the test compound, the cells were finally treated at concentrations of 1.5 nM, 4.6 nM, 13.7 nM, 41.2 nM, 123.5 nM, 370.4 nM, 1.111 μM, 3.333 μM, and 10 μM. The HL-60 cell line was purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in RPMI 1640 medium (Cytiva) according to recommendations. The exponentially growing HL-60 cells were plated at 4,000 cells per well in a SPL 33596 white 96-well analysis plate and cultured overnight at 37 °C in a humidified 5% CO₂ incubator. Using a medium mixed with the 3-fold concentration of the test compound, the cells were finally treated at concen-

trations of 1.5 nM, 4.6 nM, 13.7 nM, 41.2 nM, 123.5 nM, 370.4 nM, 1.111 μ M, 3.333 μ M, and 10 μ M. The cytotoxicity was measured after 72 hours of incubation using the Promega Cell Titer Glo reagent according to the manufacturer's recommendations. The luminescence signals were measured using a Varioskan LUX multimode microplate reader. Relative IC₅₀ and absolute IC₅₀ were calculated in the same manner as in the experiment in "3. Evaluation of GSPT1 degradation ability" described above. Experiments using MOLM13, MOLM14, MV-4-11, and U937 cell lines were commissioned by Seoul National University and performed using WST-8 reagent under the drug treatment condition for 72 hours. The exponentially growing MOLM13, MOLM14, MV-4-11, and U937 cells were plated at 4,000 cells per well in a SPL 30096 transparent 96-well analysis plate, respectively, and cultured overnight at 37 °C in a humidified 5% CO₂ incubator. Using a medium mixed with the 2-fold concentration of the test compound, the cells were finally treated at concentrations of 1 nM, 3 nM, 10 nM, 30 nM, 100 nM, 300 nM, 1 μ M, 3 μ M, and 10 μ M. The cytotoxicity was measured after 72 hours of incubation using the CELLOMAX™ WST-8 reagent (PRECAREGENE, CM-VA0500) according to the manufacturer's recommendations. The absorbance of light at a wavelength of 450 nm was measured using a SpectraMAX i3x microplate reader (Molecular Devices, SpectraMAX i3x).

[456] The results of evaluating the cell proliferation activity of the compounds in the various cancer cell lines are shown in Table 7 below.

[457] [Table 7]

[458]

Compound No.	Relative IC ₅₀ (nM)						Absolute IC ₅₀ (nM)					
	NSCLC	AML					NSCLC	AML				
	NCI-H1155	HL60	MOLM13	MOLM14	MV4-11	U937	NCI-H1155	HL60	MOLM13	MOLM14	MV4-11	U937
Reference Compound 1	129.0	97.3	944.0	162.0	31.5	486.0	164.1	99.8	1850.0	160.0	35.4	551.0
Reference Compound 2	114.0	347.0	NA	NA	NA	NA	249.0	393.0	NA	NA	NA	NA
Compound 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Compound 2	44.6	55.2	NA	NA	NA	NA	47.8	57.1	NA	NA	NA	NA
Compound 3	43.1	57.1	396	143	18.6	276	56.1	56.3	1760	148	17.8	310
Compound 4	26.7	28.1	168	59.9	12.3	117	31.3	28.1	482	60.7	11	112
Compound 5	24.1	26.6	143	49	11.2	92.6	27	25.4	390	51.6	12.2	94.7
Compound 6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Compound 7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Compound 8	34.5	32.5	NA	NA	NA	NA	43.1	33.6	NA	NA	NA	NA
Compound 9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Compound 10	53.1	67	NA	NA	NA	NA	57.7	67.1	NA	NA	NA	NA
Compound 11	61.4	68.2	NA	NA	NA	NA	96.7	68.6	NA	NA	NA	NA
Compound 12	59.4	51.9	NA	NA	NA	NA	81.2	56.2	NA	NA	NA	NA
Compound 13	34.3	36	NA	NA	NA	NA	34.5	39.5	NA	NA	NA	NA
Compound 14	60.2	34.4	NA	NA	NA	NA	68.3	37.8	NA	NA	NA	NA
Compound 15	38.4	37.9	NA	NA	NA	NA	48.9	36.9	NA	NA	NA	NA
Compound 16	148	NA	NA	NA	NA	NA	329.5	NA	NA	NA	NA	NA
Compound 17	15.7	18	NA	NA	NA	NA	17.7	18.3	NA	NA	NA	NA
Compound 18	100.8	133	NA	NA	NA	NA	120.2	134	NA	NA	NA	NA
Compound 19	30.1	29.5	NA	NA	NA	NA	36.3	28.7	NA	NA	NA	NA
Compound 20	36	26.9	NA	NA	NA	NA	40.2	27.5	NA	NA	NA	NA

[459] As shown in Table 7 above, Compounds 1 to 20 generally showed improved anticancer efficacy compared to Reference Compounds 1 and 2 in the lung cancer cell lines and AML cell lines tested.

[460] Meanwhile, Wuxi Apptec was commissioned to perform cell proliferation inhibition experiments on the following various cell lines of small cell lung cancer (SCLC) and lung adenocarcinoma (LUAD):

[461] Small cell lung cancer (SCLC): NCI-H526, NCI-H69, NCI-H2029, NCI-H889, NCI-H1963, NCI-H524, NCI-H82, NCI-H446, NCI-H211, SHP77

[462] Lung adenocarcinoma (LUAD): NCI-H1975, A549, NCI-H358, NCI-H460

[463] The exponentially growing cells were each plated in a Greiner CELLSTAR #655090 black 96-well analysis plate at the number of cells per well under conditions established by Wuxi Apptec and cultured overnight at 37 °C in a humidified 5% CO₂ incubator. Using a medium mixed with the 10-fold higher concentration of the test compound than the target concentrations, the cells were finally treated at concen-

trations of 1.5 nM, 4.6 nM, 13.7 nM, 41.2 nM, 123.5 nM, 370.4 nM, 1.111 μ M, 3.333 μ M, and 10 μ M. The cytotoxicity was measured after 72 hours of incubation using the Promega Cell Titer Glo reagent according to the manufacturer's recommendations. The luminescence signals were measured using a 2104 EnVision microplate reader (PerkinElmer, 2104 EnVision Multilabel Reader).

- [464] As shown in Figure 9a, Compound 3 and Compound 4 showed minimal cell proliferation inhibition efficacy on the LUAD cell line, while showed overall excellent anticancer effect on the SCLC cell line.
- [465] Since SCLC is mostly known as a carcinoma with a neuroendocrine phenotype (Nat Rev Dis Primers. 2021 Jan 14;7(1):3.), it was confirmed whether the compound of the present invention exhibited anticancer efficacy against lung neuroendocrine cancer (Lung NEC), which has a neuroendocrine phenotype among non-small cell lung cancers (NSCLC), and neuroendocrine prostate cancer (NEPC), which is known to have a neuroendocrine phenotype and have a very poor prognosis among prostate cancers. The experiment was performed by Wuxi Apptec in the same manner as in the small cell lung cancer (SCLC) and lung adenocarcinoma (LUAD)-derived cells.
- [466] Lung neuroendocrine cancer (Lung NEC): NCI-H1770, NCI-H2106
- [467] Neuroendocrine prostate cancer (NEPC): NCI-H660
- [468] The results of calculating the EC₅₀ of Compound 3 and Compound 4 for the LUAD, SCLC, Lung NEC, and NEPC cell lines are shown in Figure 9b. As shown in Figure 9b, Compound 3 and Compound 4 barely inhibited the proliferation of the LUAD cells, but showed excellent cell proliferation inhibition activity against SCLC, Lung NEC, and NEPC. Therefore, it was found that the compound of the present invention, which has excellent GSPT1 degradation activity, exhibits particularly excellent anticancer effect on a carcinoma with a neuroendocrine phenotype. This supports the usefulness of the compound of the present invention as personalized medicine.
- [469] **10. Cytotoxicity test in normal cells and derivation of therapeutic range**
- [470] It was confirmed whether the compound of the present invention exhibited safety against normal cells and the improved therapeutic range accordingly. For this purpose, the cytotoxicity of compounds was compared and evaluated using the HEK293 cells, skin primary cells donated from healthy adults.
- [471] The HEK293 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cells were cultured in Dermal Cell Basal Medium (ATCC) according to recommendations. The HEK293 cells were plated at 10,000 cells per well in a SPL 33596 white 96-well analysis plate and cultured overnight at 37 °C in a humidified 5% CO₂ incubator. Using a medium mixed with the 3-fold concentration of the test compound, the cells were treated at concentrations of 1.5 nM, 4.6 nM, 13.7 nM, 41.2 nM, 123.5 nM, 370.4 nM, 1.111 μ M, 3.333 μ M, and 10 μ M. The cytotoxicity

was measured after 72 hours of incubation using the Promega Cell Titer Glo reagent according to the manufacturer's recommendations. The luminescence signals were measured using a Varioskan LUX multimode microplate reader. Absolute IC₅₀ was calculated in the same manner as in the experiment in "3. Evaluation of GSPT1 degradation ability" described above.

[472] The results of comparing the cytotoxicity of the compounds against normal cells are shown in Table 8 below. The therapeutic index in Table 8 was calculated using the IC₅₀ values of NCI-H1155 and HL60 listed in Table 7.

[473] [Table 8]

Compound No.	Normal cell(HEKa) cytotoxicity absolute IC ₅₀ (nM)	<i>In vitro</i> therapeutic index (absolute IC ₅₀ ratio HEKa/NCI-H1155)	<i>In vitro</i> therapeutic index (absolute IC ₅₀ ratio HEKa/HL60)
Reference Compound 1	873.0	5.3	8.7
Compound 3	>10000	>178.3	>177.5
Compound 4	4090.8	130.8	145.5
Compound 5	1640	60.7	64.5
Compound 8	5210	120.8	155.2
Compound 11	>10000	>103.4	>145.8
Compound 12	5730	70.6	102
Compound 13	259	7.5	6.6
Compound 14	406	5.9	10.7
Compound 15	3160	64.6	85.6
Compound 17	613	34.6	33.5
Compound 19	4690	129.2	163.2
Compound	311	7.7	11.3

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- [474] As shown in Table 8, the compounds of the present invention were confirmed to be safer compounds with a wider therapeutic range due to their overall lower cytotoxicity against normal cells (HEKa cells), compared to Reference Compound 1.
- [475] In addition, the results of comparing the *in vitro* therapeutic index of Reference Compound 1 with those of Compound 3 and Compound 4 are shown in Figure 10.
- [476] As shown in Figure 10, Reference Compound 1 did not show a significant difference between the concentration at which it exhibited anticancer activity against NCI-H1155 or HL60 and the concentration at which it exhibited cytotoxicity against HEKa cells (i.e., it exhibited a low therapeutic index). On the other hand, Compound 3 and Compound 4 showed a much wider therapeutic window (much higher therapeutic index) compared to Reference Compound 1. In particular, Compound 3 showed little cytotoxicity to the HEKa cells, showing a very wide therapeutic window (Figure 10, bottom).
- [477] **11. Evaluation of *in vivo* anticancer efficacy using acute myeloid leukemia (AML) cell line**
- [478] Champions Oncology was commissioned to evaluate the *in vivo* anticancer efficacy using acute myeloid leukemia (AML) cell line and performed the evaluation according to Champions Oncology's internal IACUC guidelines. First, HL60-Luc cell line (5×10^6 cells/0.2 mL PBS), which is HL60 cells, an acute myeloid leukemia cell line, labeled with luciferase, were transplanted by injection into the tail vein of 6 to 8-week-old female NCG mice (Charles River), which were completely immune deficient. Bioluminescence was quantified to measure the progression of acute myeloid leukemia (AML). Bioluminescence was recorded from 4 to 10 days after transplantation. When bioluminescence greater than the background value (bioluminescence of mice not transplanted with HL60 cells) was measured in more than 90% of the total subjects, animals were randomly assigned to each group and administration of Compound 3 was started. Compound 3 was prepared by dissolving it in a mixed solution of 5% DMSO, 10% Solutol HS-15, and 85% sterile saline. Compound 3 was orally administered to each group at a dosage of 5 mg/kg once daily, 15 mg/kg once or twice daily, and 30 mg/kg once daily. Bioluminescence was measured on both the dorsal and ventral sides and was measured based on the total luminescence (Total Flux, photons/sec, number of photons per second). The progression of AML was measured twice a week until the end of medication, and then once a week on both the dorsal and ventral sides. Body weight was measured twice weekly.
- [479] The results of evaluating the drug efficacy of the compound in the HL-60-Luc AML animal model are shown in Figure 11a. As shown in Figure 11a, Compound 3 inhibited

the progression of AML when administered orally at 5 mg/kg daily. Furthermore, at a dose of 15 mg/kg once a day or more, Compound 3 showed tumor remission in addition to the inhibition of the progression of AML.

[480] Meanwhile, follow-up was conducted until the 60th day of the experiment to confirm whether AML progression was still inhibited even after discontinuation of medication. As a result, when Compound 3 was administered at a dose of 15 mg/kg twice a day, AML recurrence did not occur even more than 1 month after discontinuation of medication. Compound 3 was confirmed to be safe as it did not cause body weight loss in mice at the administered dose (Figure 11b). Regardless of medication, body weight loss was observed in the experimental group where AML progressed rapidly. As a result of the above experiment, it was confirmed that Compound 3 is safe and has excellent anticancer efficacy *in vivo*.

[481] **12. Evaluation of *in vivo* anticancer efficacy using lung cancer cell lines**

[482] Seoul National University was commissioned to evaluate the *in vivo* anticancer efficacy using lung cancer cell lines and performed the evaluation according to Seoul National University's internal IACUC guidelines. 5 X 10⁶ H1155 cell lines were transplanted subcutaneously into 5-week-old female Balb/c nude mice. The body weight and tumor volume of the mice were measured every 3 days. Compound 3 was prepared by dissolving it in a mixed solution of 5% DMSO, 10% Solutol HS-15, and 85% sterile saline. Compound 4 was prepared by dissolving it in a mixed solution of 5% NMP, 5% Solutol HS-15, and 90% sterile saline. Compound 3 and Compound 4 were administered starting when the tumor volume reached approximately 100 mm³. Compound 3 was administered at a dose of 5 mg/kg, 15 mg/kg or 30 mg/kg once a day, at a dose of 15 mg/kg twice a day, or at a dose of 30 mg/kg for 5 days with a 9-day break taken. Compound 4 was administered orally at a dose of 3 mg/kg and 10 mg/kg once a day for 6 days. At 6 and 24 hours after the last dose, cancer tissue was removed and pharmacodynamic changes were observed.

[483] The results of evaluating the pharmacodynamics and anticancer efficacy of Compound 3 and Compound 4 in the NCI-H1155 lung cancer animal model are shown in Figures 12 and 13.

[484] When Compound 4 was administered at a dose of 3 mg/kg, the protein expression level of GSPT1 was partially reduced 6 and 24 hours after the end of administration, but no significant change was observed in the expression level of the downstream cancer-inducing protein N-MYC. On the other hand, it was confirmed that when Compound 4 was administered at a dose of 10 mg/kg, the protein expression level of GSPT1 was completely reduced 6 and 24 hours after the end of administration, and the expression level of the downstream cancer-inducing protein N-MYC was also significantly reduced (Figure 12a). In addition, when Compound 4 was administered at a

dose of 10 mg/kg, it was confirmed that the tumor volume was significantly reduced on day 6 (Figure 12b).

[485] Meanwhile, Compound 3 significantly reduced tumor proliferation without significant body weight loss at all doses evaluated (Figure 13). Furthermore, similar to the results in the AML animal model, at a dose of 15 mg/kg once a day or more, Compound 3 inhibited the growth of lung cancer tissue and further showed tumor complete regression. In addition, follow-up was conducted until the 40th day of the experiment to confirm whether recurrence of lung cancer was still inhibited even after discontinuation of medication. As a result, when Compound 3 was administered at a dose of 15 mg/kg twice a day, no recurrence of lung cancer was observed, except for 1 out of 9 animals, even after about 1 month after discontinuation of medication.

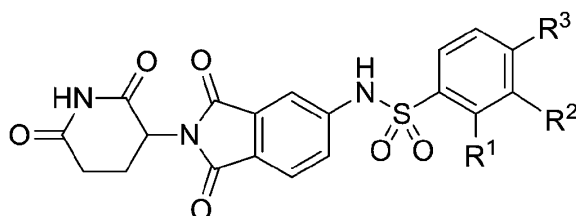
[486] The present invention has been described herein based on exemplary embodiments. However, it should be understood that the present invention is not limited to the described embodiments, and all changes, modifications, alterations, and alternatives that do not depart from the spirit and essential characteristics of the present invention are within the scope of the present invention.

Claims

[Claim 1]

A compound represented by Formula I below, or a stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof:

[Formula I]



in Formula I,

R¹ is halogen, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₁-C₆ haloalkyl, or C₁-C₆ haloalkoxy;

R² is C₁-C₆ alkyl optionally substituted with R^{2a};

R^{2a} is hydroxy, halogen, C₁-C₆ alkoxy, C₁-C₆ haloalkoxy, or -NR'R'';

R' and R'' are each hydrogen or C₁-C₆ alkyl, or R' and R'' may be taken together with the nitrogen atom to which they are attached to form a 3 to 8-membered heterocyclic ring optionally comprising one additional heteroatom selected from N, O, and S; and

R³ is hydrogen or halogen.

[Claim 2]

The compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to claim 1, wherein

R¹ is halogen, C₁-C₆ alkyl, C₁-C₆ haloalkyl, or C₁-C₆ haloalkoxy;

R² is C₁-C₆ alkyl optionally substituted with R^{2a};

R^{2a} is hydroxy or C₁-C₆ haloalkoxy; and

R³ is hydrogen or halogen.

[Claim 3]

The compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to claim 2, wherein

R¹ is halogen or C₁-C₆ alkyl;

R² is C₁-C₆ alkyl; and

R³ is hydrogen or halogen.

[Claim 4]

The compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to claim 3, wherein

R¹ is halogen,

R² is C₁-C₆ alkyl, and

R³ is hydrogen.

[Claim 5]

The compound, or stereoisomer, hydrate, solvate or pharmaceutically

acceptable salt thereof according to claim 2, wherein

R¹ is F, Cl, Br, I, -OCH₂F, -OCHF₂, -OCF₃, -CH₂F, -CHF₂, -CF₃, or -CH₃;

R² is -CH₃, -CH₂CH₃, -CH₂OCF₃, -CH₂OCHF₂, -CH₂OCH₂F, -CH₂OH, or -CH₂CH₂OH; and

R³ is H or F.

[Claim 6]

The compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to claim 1, wherein

R¹ is halogen;

R² is C₁-C₆ alkyl substituted with NR'R'';

R' and R'' are each hydrogen or C₁-C₆ alkyl, or R' and R'' may be taken together with the nitrogen atom to which they are attached to form a 3 to 8-membered heterocyclic ring optionally comprising one additional heteroatom selected from N, O, and S; and

R³ is hydrogen.

[Claim 7]

The compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to claim 6, wherein

R' and R'' are each hydrogen or C₁-C₃ alkyl, or R' and R'' are taken together with the nitrogen atom to which they are attached to form a morpholinyl, thiomorpholinyl, piperazinyl, or piperidinyl ring.

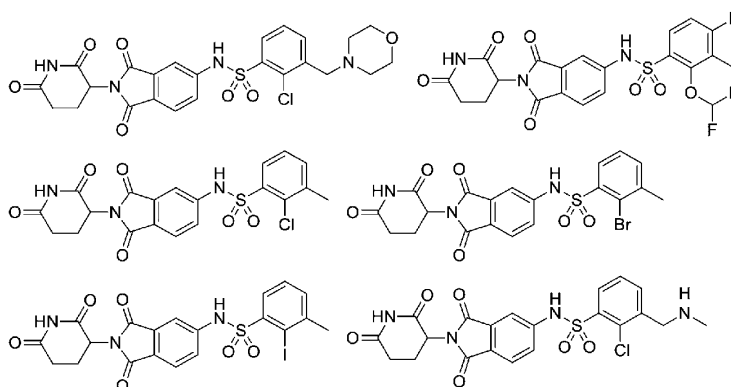
[Claim 8]

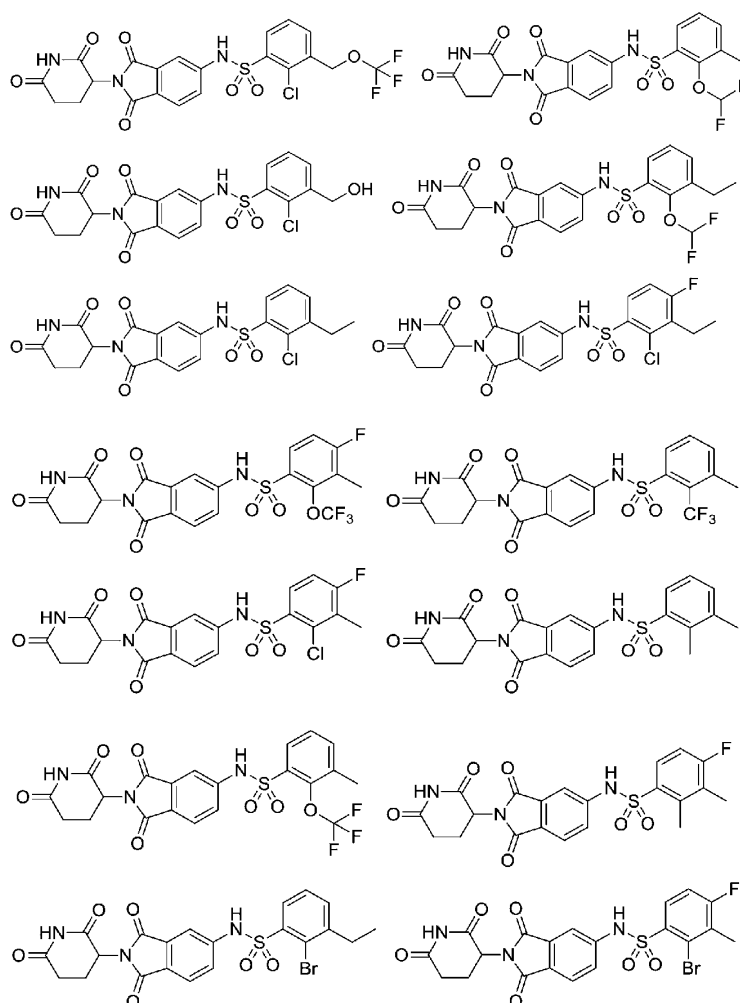
The compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to claim 6, wherein

R² is -CH₂-morpholinyl, -CH₂-CH₂-morpholinyl, -CH₂-NH₂, -CH₂-NH(CH₃), -CH₂-N(CH₃)₂, -CH₂CH₂-NH₂, -CH₂-CH₂NH(CH₃), or -CH₂-CH₂-N(CH₃)₂.

[Claim 9]

The compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to claim 1, wherein the compound is a compound represented by any one of the following formulas:





- [Claim 10] A pharmaceutical composition for treating a disorder of uncontrolled cell proliferation in a mammal, comprising a therapeutically effective amount of the compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to any one of claims 1 to 9.
- [Claim 11] The pharmaceutical composition according to claim 10, wherein the disorder of uncontrolled cell proliferation is cancer.
- [Claim 12] The pharmaceutical composition according to claim 11, wherein the cancer is selected from pediatric acute leukemia, medulloblastoma, brain cancer, lung cancer, blood cancer, bladder cancer, colon cancer, cervical cancer, endometrial cancer, ovarian cancer, squamous cell cancer, kidney cancer, peritoneal cancer, breast cancer, stomach cancer, large intestine cancer, prostate cancer, pancreatic cancer, urogenital tract cancer, lymphatic cancer, laryngeal cancer, skin cancer, malignant

- melanoma, large intestine cancer, endometrial carcinoma, thyroid cancer, rhabdomyosarcoma, liver cancer, and a combination thereof.
- [Claim 13] The pharmaceutical composition according to claim 12, wherein the cancer has a neuroendocrine phenotype.
- [Claim 14] The pharmaceutical composition according to claim 13, wherein the cancer is neuroendocrine prostate cancer, castration-resistant prostate cancer, or lung neuroendocrine tumor.
- [Claim 15] The pharmaceutical composition according to claim 10, wherein the pharmaceutical composition further comprises at least one agent known to treat cancer.
- [Claim 16] The pharmaceutical composition according to claim 15, wherein the at least one agent is selected from:
 uracil mustard, chlormethine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, carmustine, lomustine, streptozocin, dacarbazine, temozolomide, thiotepa, altretamine, methotrexate, 5-fluorouracil, floxuridine, cytarabine, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, pentostatin, bortezomib, vinblastine, vincristine, vinorelbine, vindesine, bleomycin, dactinomycin, daunorubicin, doxorubicin, epirubicin, dexamethasone, clofarabine, cladribine, pemetrexed, idarubicin, paclitaxel, docetaxel, ixabepilone, mithramycin, topotecan, irinotecan, deoxycoformycin, mitomycin-C, L-asparaginase, interferon, etoposide, teniposide 17 α -ethinyl estradiol, diethylstilbestrol, testosterone, prednisone, fluoxymesterone, drostanolone propionate, testolactone, megestrol acetate, tamoxifen, methylprednisolone, methyltestosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, flutamide, toremifene, goserelin, cisplatin, carboplatin, hydroxyurea, amsacrine, procarbazine, mitotane, mitoxantrone, levamisole, navelbine, anastrozole, letrozole, capecitabine, raloxifene, droloxifene, hexamethylmelamine, oxaliplatin, gefitinib, capecitabine, erlotinib, azacitidine, temozolomide, gemcitabine, or vasostatin;
 a DNA methyltransferase inhibitor, a HDAC-inhibitor, a glucocorticoid, a mTOR inhibitor, a cytotoxic agent, a BCL2 inhibitor, a FLT3 inhibitor, an IDH 1/2 inhibitor, a CDK inhibitor, a transcription inhibitor, or a HSP inhibitor; or a combination thereof.
- [Claim 17] The pharmaceutical composition according to claim 16, wherein

the DNA methyltransferase inhibitor is 5-aza-2'-deoxycytidine, 5-azacitidine, zebularine, epigallocatechin-3-gallate, procaine, or a combination thereof;

the HDAC-inhibitor is vorinostat, entinostat, panobinostat, trichostatin A, mocetinostat, belinostat, dacinostat, givinostat, tubastatin A, pracinostat, droxinostat, quisinostat, romidepsin, valproic acid, AR-42 (OSU-HDAC42), tacedinaline, ricolinostat, apicidin, or a combination thereof;

the glucocorticoid is dexamethasone, prednisolone, methylprednisolone, betamethasone, triamcinolone, fludrocortisone, beclomethasone, or a combination thereof;

the mTor inhibitor is BEZ235, everolimus, sirolimus, temsirolimus, rapamycin, AZD8055, or a combination thereof;

the cytotoxic agent is selected from an alkylating agent, an antimetabolite, an antitumor antibiotic, a mitosis inhibitor, or a chemotherapeutic agent selected from anthracycline, cytarabine, a purine analog, sorafenib, gemtuzumab ozogamicin, rituximab, or a combination thereof;

the alkylating agent is selected from carboplatin, cisplatin, cyclophosphamide, chlorambucil, melphalan, carmustine, busulfan, lomustine, dacarbazine, oxaliplatin, ifosfamide, mechlorethamine, temozolomide, thiotepa, bendamustine, and streptozocin;

the antimetabolite is selected from gemcitabine, 5-fluorouracil, capecitabine, hydroxyurea, mercaptopurine, pemetrexed, fludarabine, nelarabine, cladribine, clofarabine, cytarabine, decitabine, pralatrexate, floxuridine, methotrexate, and thioguanine;

the antitumor antibiotic is selected from doxorubicin, mitoxantrone, bleomycin, daunorubicin, dactinomycin, epirubicin, idarubicin, plicamycin, mitomycin, pentostatin, and valrubicin;

the mitosis inhibitor is selected from irinotecan, topotecan, rubitecan, cabazitaxel, docetaxel, paclitaxel, etoposide, vincristine, ixabepilone, vinorelbine, vinblastine, and teniposide;

the BCL2 inhibitor is venetoclax, navitoclax, obatoclax mesylate, sabutoclax, or lisaftoclax;

the FLT3 inhibitor is midostaurin, quizartinib, gilteritinib, sorafenib, crenolanib, or pexidartinib;

the IDH 1/2 inhibitor is ivosidenib, enasidenib, vorasidenib, olutasidenib, AGI-6780, AGI-5198, or GSK321;

the CDK inhibitor is samuraciclib, alvocidib, fadraciclib, seliciclib, zotiraciclib, atuveciclib, enitociclib, voruciclib, SY5609, XL201, Q-901, KRLS-017, GTAEXS-617, TGN-1062, THZ1, THZ2, SY-1365, YKL-5-124, ICEC0942, LY3405105, LDC4297, BS-181, SNS- 32, AT-7519, AZD-4573, KB-0742, AU-07, BTXA-51, GFH-009, JS-101, PRT-2527, QHRD-107, TP-1287, SYHX-1903, CTX-439, KIN-004, SY-12882, THZ-531, CT-7439, AU-003, or AU-004;

the transcription inhibitor is lurbinectedin; and

the HSP inhibitor is pimitespib, luminespib, tanespimycin, alvespimycin, ganetespib, onalespib, geldanamycin, or rocaglamide.

[Claim 18]

A method of regulating a cereblon activity or a GSPT1 activity in at least one cell, comprising contacting the at least one cell *in vitro* with an effective amount of the compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to any one of claims 1 to 9.

[Claim 19]

A method of treating a disorder of uncontrolled cell proliferation, comprising administering a therapeutically effective amount of the compound, or stereoisomer, hydrate, solvate or pharmaceutically acceptable salt thereof according to any one of claims 1 to 9 to a subject.