

CHEMICAL COMPOUNDS AND USES THEREOF

FIELD OF THE INVENTION

Disclosed herein are certain compounds that inhibit Werner Syndrome helicase enzyme (WRN) activity, in particular inhibit ATP dependent helicase domain activity and are therefore useful in treating cancers treatable by inhibition of WRN, including cancers characterized by microsatellite instability (MSI) and/or defective DNA mismatch repair system (dMMR). Also, disclosed are pharmaceutical compositions comprising such compounds and methods of making the same.

SEQUENCE LISTING

This application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML, created on May 28, 2024, is named 70333WO02 Seq List XML 28May2024.xml and is 6,092 bytes in size.

BACKGROUND TO THE INVENTION

Cancer is a leading cause of death throughout the world. A limitation of prevailing therapeutic approaches, e.g., chemotherapy is that their cytotoxic effects are not restricted to cancer cells and adverse side effects can occur within normal tissues. Consequently, novel strategies are needed to better target cancer cells.

Synthetic lethality (SL) arises when a combination of deficiencies in the expression of two or more genes or corresponding loss of function of related gene product proteins (e.g., resulting from one or more chromosomal mutations) leads to cell death, whereas a singular deficiency/loss of function does not. For example, one of the genes (or gene products) can be involved in cell proliferation, whereas the other of the genes (or gene products) can be a non-essential gene. The concept of synthetic lethality originates from studies in drosophila model systems in which a combination of mutations in two or more separate genes leads to cell death (in contrast to viability, or even cell proliferation, which occurs when only one of the genes is mutated or deleted). More recently, studies have explored maladaptive genetic changes in cancer cells that render them vulnerable to synthetic-lethality approaches. These tumor-specific genetic defects can create a vulnerability, which enable the use of targeted agents that are synthetically lethal to such tumor-specific genomic defect and induce the death of tumor cells while sparing normal cells.

Disruptions in DNA repair pathways predispose cells to accumulating DNA damage. Various types of tumors are known to accumulate more mutations in DNA as cancers progress. Therefore, pathways involved in DNA repair can be targeted by cytotoxic treatments based on synthetic lethality, turning dysregulated repair processes against themselves to induce tumor death.

Identifying SL interactions that are relevant in cancers is an area of focus for biological discovery efforts. In a yeast screen looking to uncover SL interactions between tumor suppressor genes and drug targets, *SGS1*, gene encoding a RECQ helicase, was found to be SL with several genes in the screen. In the same study using human cells, Bloom syndrome helicase (BLM), one of five human RECQ helicases, was SL with Check point kinase (CHEK) 1 and 2 (Srivastava R, *et al.*, Mol Cell **2016**;63(3):514-25). During DNA damage repair (DDR), BLM participates in homologous recombination (HR). RECQ helicases are 3' to 5' DNA unwinding DNA-dependent ATPases. Three RECQ helicases, BLM, WRN (WRN) and RECQL4, cause human syndromes that overlap, but are also distinct symptomatically, when their expression is altered or lost (de Renty C, Ellis NA. Ageing Res Rev **2017**;33:36-51). This suggests that they may have overlapping, and distinct functions based on when and where they are expressed in cells, their protein-protein interactions and post-translational modifications.

Another study (DRIVE) using approximately 400 cell lines includes data from which it can be shown that WRN is not broadly essential but that MSI cell lines from large intestine, endometrial and stomach tissues of origin are sensitive to WRN shRNAs (McDonald ER, 3rd, de Weck A, Schlabach MR, Billy E, Mavrakis KJ, Hoffman GR, *et al.*, Cell **2017**;170(3):577-92). The DepMap study, which derives in part from the DRIVE data, also found a pattern of WRN essentiality in MSI cell lines (Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS, *et al.*, Cell **2017**;170(3):564-76). None of the other human RECQ helicases tested in the study showed this MSI SL interaction.

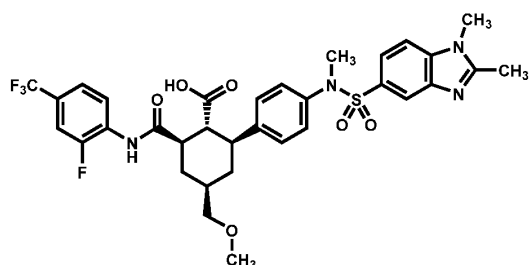
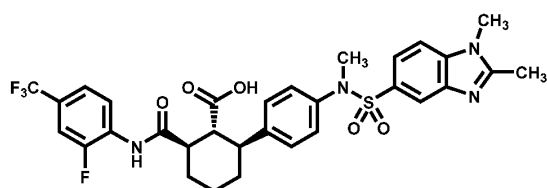
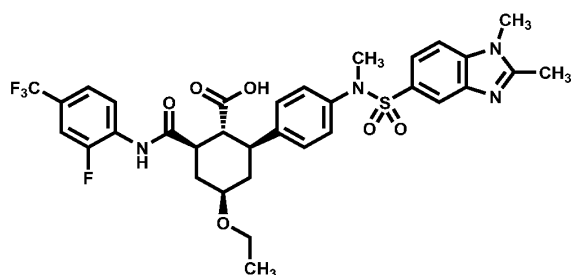
In confirmation of the DRIVE and DepMap studies, it has been reported by several labs that survival of cancer cells with high microsatellite instability are selectively compromised by knockout of WRN (Behan, F.M. *et al.*, Nature **2019**; 568(7753): 511-16, Chan, E.M. *et al.*, Nature **2019**; 568 (551-556), Lieb, S. *et al.*, eLife **2019**;8:e43333, Kategaya, L. *et al.*, iScience **2019**; 13:488-497.) WRN is an enzyme that has both an exonuclease domain and an ATP dependent helicase domain. Kategaya, L. *et al.*, *supra*, identified a synthetic lethality relationship between patients having tumors characterized by high microsatellite instability and, in particular, the ATP dependent helicase domain activity of WRN protein. Identification of

patients having tumors characterized by high microsatellite instability, reflecting a high frequency of microsatellite instability, is known in the art, including for example as disclosed by Dudley, Jonathan C., *et al.*, *Clinical Cancer Research*, 22(4): 813-820, 2016.).

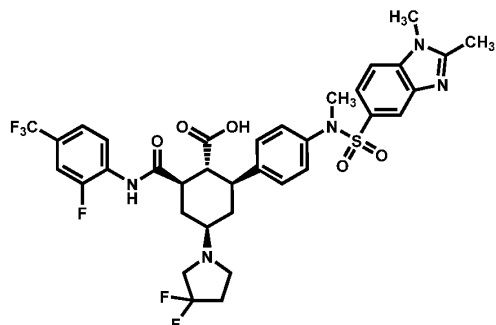
These results indicate that WRN inhibitors may provide a novel therapy for cancer patients having tumors with microsatellite instability (MSI) as a marker of DNA mismatch repair (dMMR), including those patients with MSI-high tumors. Microsatellites are repetitive DNA sequences with varying unit lengths (e.g. ranging from one (mononucleotides) to six bases (di-, tri-, tetra-, penta-, esa-nucleotides)) distributed along coding and/or noncoding regions of the genome. Mutations of such microsatellites, for example, repeat length alterations, can represent microsatellite instability. As known in the art and more fully described herein, MSI can be detected (e.g., directly) by molecular testing (e.g., with respect to certain microsatellites) or (e.g., indirectly) by immunohistochemical evaluation (e.g., with respect to expression of certain MMR proteins). Based on a consensus NCI-Reference Panel (Bethesda, 1998), MSI can be assessed by molecular testing of five microsatellites – including two mononucleotides (BAT25 and BAT26) and three dinucleotides (D2S123, D5S346, D17S250). Tumors can be classified into different subtypes based on such a NCI-Reference Panel approach for molecular testing, including MSI-high (MSI-H) if two or more of the microsatellite markers show instability, MSI-low (MSI-L) if only one microsatellite marker shows instability, and MS-stable (MSS) if none of the five microsatellite markers show instability (i.e., each of the five microsatellite markers are determined as stable). In some instances, for example where molecular testing or immunohistochemical evaluation is not able to distinguish between MSI-L and general chromosomal instability, tumors can be classified as a MSS neoplasms.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a compound selected from the group consisting of:



, and



or a pharmaceutically acceptable salt thereof.

In a second aspect, the present invention provides a pharmaceutical composition comprising a) a compound or pharmaceutically acceptable salt thereof of the invention disclosed herein and b) a pharmaceutically acceptable excipient.

In a third aspect, the present disclosure provides a compound of the invention disclosed herein for use in therapy.

In a fourth aspect, the present disclosure provides a compound of the invention disclosed herein for use in the treatment of cancer.

In a fifth aspect, the present disclosure provides a method of treatment of cancer in a subject in need thereof comprising administering to said subject a therapeutically effective amount of a compound or pharmaceutically acceptable salt thereof of the invention disclosed herein or pharmaceutical composition as disclosed herein.

In a sixth aspect, the present disclosure provides use of a compound of the invention disclosed herein in the manufacture of a medicament for use in the treatment of cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an efficacy study of the compound of Example 138 in mouse model bearing SW48 human colorectal cancer cell line xenografts.

FIG. 2 shows an efficacy study of the compound of Example 83 in mouse model bearing SW48 human colorectal cancer cell line xenografts.

FIG. 3 shows an efficacy study of the compound of Example 250 in mouse model bearing SW48 human colorectal cancer cell line xenografts.

FIG. 4 shows an efficacy study of the compound of Example 306 in mouse model bearing SW48 human colorectal cancer cell line xenografts.

DETAILED DESCRIPTION

Definitions:

As used herein, the terms “a” and “an” are to be construed to encompass both the singular and plural unless otherwise indicated. For example, the phrase “a pharmaceutically acceptable excipient” refers to one or more pharmaceutically acceptable excipients.

The term “pharmaceutically acceptable” refers to those compounds (including salts), materials, compositions, and dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, the term “pharmaceutically acceptable salt” refers to salts that retain the desired biological activity of the subject compound and exhibit minimal undesired toxicological effects.

Such pharmaceutically acceptable salt may be prepared *in situ* during the final isolation and purification of the compound, or by separately reacting the purified compound in its free acid or free base form with a suitable base or acid, respectively. When compounds disclosed herein contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of salts derived from pharmaceutically-acceptable inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic, manganous, potassium, sodium, zinc. Salts derived from pharmaceutically-acceptable organic bases include salts of primary, secondary and tertiary amines, including substituted amines, cyclic amines, naturally-occurring amines, such as arginine, betaine, caffeine, choline, *N,N*-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine. When compounds of the present disclosure contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogen carbonic, phosphoric, monohydrogen phosphoric, dihydrogen phosphoric, sulfuric, monohydrogen sulfuric, hydriodic, or phosphorous acids, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, malonic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic. Also included are salts of amino acids such as arginate, and salts of organic acids like glucuronic or galactunoric acids (see, for example, Berge, S.M., et al, "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, **1977**, 66, 1-19). Certain specific compounds of the present disclosure contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present disclosure.

The present disclosure also includes prodrugs of the compound disclosed herein or a pharmaceutically acceptable salt thereof. As used herein, the term “prodrug” refers to compounds that readily undergo chemical changes under physiological conditions to provide a pharmacologically active parent compound. The term “prodrug moiety” refers to the chemical moiety of a prodrug that is released under physiological conditions to form the active parent compound. An example, without limitation, of a prodrug would be a compound which is administered as an ester (the “prodrug”), but then is metabolically hydrolyzed to the carboxylic acid, the active entity. Additionally, prodrugs can be converted to the compounds of the present disclosure by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present disclosure when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

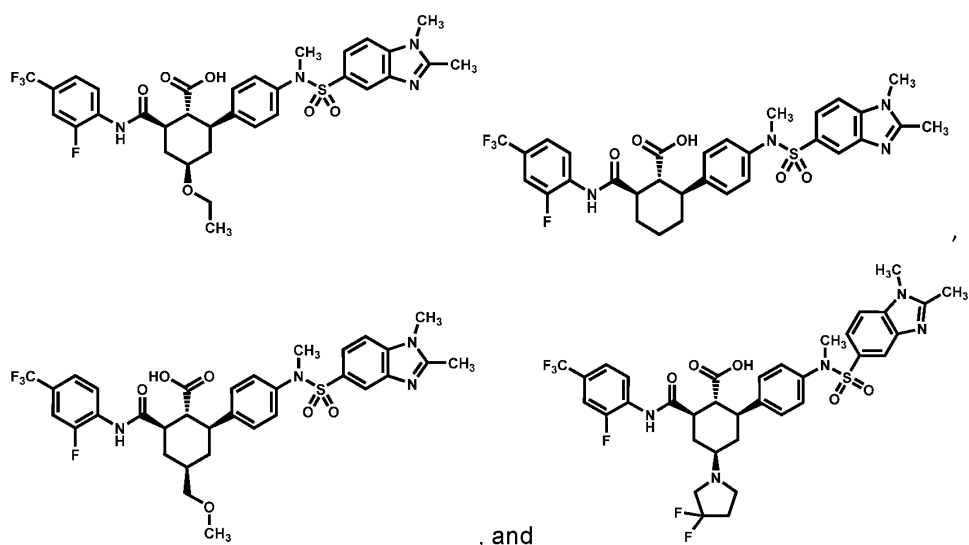
Certain compounds of the present disclosure can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present disclosure. Certain compounds of the present disclosure may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present disclosure and are intended to be within the scope of the present disclosure.

Certain compounds of the present disclosure possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers, regioisomers and individual isomers (e.g., separate enantiomers) are all intended to be encompassed within the scope of the present disclosure. When a stereochemical depiction is shown, it is meant to refer to the compound in which one of the isomers is present and substantially free of the other isomer. “Substantially free of” another isomer indicates at least an 80/20 ratio of the two isomers, more preferably 90/10, or 95/5 or more. In some embodiments, one of the isomers will be present in an amount of at least 99%.

Certain compounds of the present disclosure can exist as tautomers and/or geometric isomers. All possible tautomers and *cis* and *trans* isomers, as individual forms and mixtures thereof are within the scope of this disclosure.

The compounds of the present disclosure may also contain unnatural amounts of isotopes at one or more of the atoms that constitute such compounds. Unnatural amounts of an isotope may be defined as ranging from the amount found in nature to an amount 100% of the atom in question. That differ only in the presence of one or more isotopically enriched atoms.

Exemplary isotopes that can be incorporated into compounds of the present disclosure, such as a compound of the present disclosure include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine, and iodine, such as ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{13}N , ^{15}N , ^{15}O , ^{17}O , ^{18}O , ^{32}P , ^{33}P , ^{35}S , ^{18}F , ^{36}Cl , ^{123}I , and ^{125}I , respectively. Isotopically-labeled compounds (e.g., those labeled with ^3H and ^{14}C) can be useful in compound or substrate tissue distribution assays. Tritiated (i.e., ^3H) and carbon-14 (i.e., ^{14}C) isotopes can be useful for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., ^2H) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements). In some embodiments, in compounds disclosed herein, one or more hydrogen atoms are replaced by ^2H or ^3H , or one or more carbon atoms are replaced by ^{13}C - or ^{14}C -enriched carbon. Positron emitting isotopes such as ^{15}O , ^{13}N , ^{11}C , and ^{15}F are useful for positron emission tomography (PET) studies to examine substrate receptor occupancy. Isotopically labeled compounds can generally be prepared by following procedures analogous to those disclosed in the Schemes or in the Examples herein, by substituting an isotopically labeled reagent for a non-isotopically labeled reagent. Thus, in one embodiment, the present invention includes:



wherein one or more hydrogen atoms attached to carbon atoms are replaced by deuterium.

“Disease” as used herein is intended to be generally synonymous, and is used interchangeably with, the terms “disorder,” “syndrome,” and “condition” (as in medical condition), in that all reflect

an abnormal condition of the human or animal body or of one of its parts that impairs normal functioning, is typically manifested by distinguishing signs and symptoms, and causes the human or animal to have a reduced duration or quality of life.

“Patient” is generally synonymous with the term “subject” and as used herein includes all mammals including humans. Examples of patients include humans, livestock such as cows, goats, sheep, pigs, and rabbits, and companion animals such as dogs, cats, rabbits, and horses. Preferably, the patient is a human.

“In need of treatment” as used herein refers to a judgment made by a physician or other caregiver that a subject requires or will benefit from treatment. This judgment is made based on a variety of factors that are in the realm of the physician’s or caregiver’s expertise.

“Administration” and “administer”, as they apply to, for example, a patient, cell, tissue, organ, or biological fluid, refer to contact of, for example, a compound of the present disclosure or a pharmaceutically acceptable salt thereof, a pharmaceutical composition comprising same, or a diagnostic agent to the subject, cell, tissue, organ, or biological fluid. In the context of a cell, administration includes contact (e.g., in vitro or ex vivo) of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell.

“Therapeutically effective amount” as used herein means the amount of a compound of the present disclosure or a pharmaceutically acceptable salt thereof, which will elicit the desired biological response in a human body. It may vary depending on the compound, the disease and its severity and the age and weight of the subject to be treated. The therapeutically effective amount can be ascertained by measuring relevant physiological effects, and it can be adjusted in connection with the dosing regimen and diagnostic analysis of the subject’s condition. By way of example, measurement of the serum level of a compound of the present disclosure (or, e.g., a metabolite thereof) at a particular time post-administration may be indicative of whether a therapeutically effective amount has been used.

The term “treatment” refers to ameliorating or stabilising the specified condition, reducing or eliminating the symptoms of the condition, slowing or eliminating the progression of the condition, and preventing or delaying reoccurrence of the condition in a previously afflicted patient or subject.

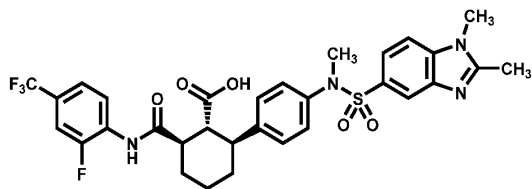
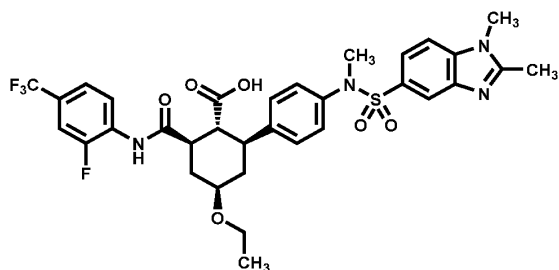
The term “prevention” refers to avoidance of the stated disease in a subject who is not suffering from the stated disease.

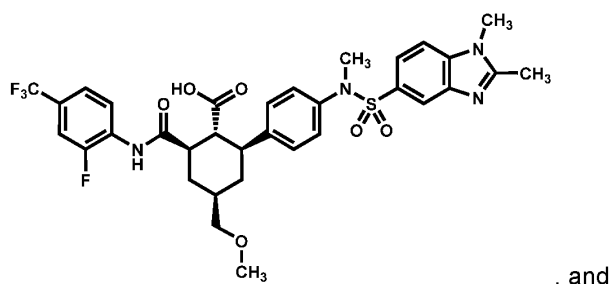
“Inhibiting”, “reducing,” or any variation of these terms in relation of WRN, includes any measurable decrease or complete inhibition to achieve a desired result. For example, there may be a decrease of about, at most about, or at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more, or any range derivable therein, reduction of WRN helicase domain activity compared to its normal activity.

Compounds

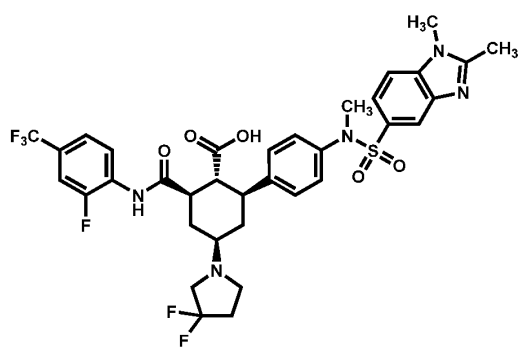
In one aspect, the compound of the present disclosure is selected from the compounds in Table 1, or a pharmaceutically acceptable salt thereof.

In one aspect, the present disclosure provides a compound selected from the groups consisting of:





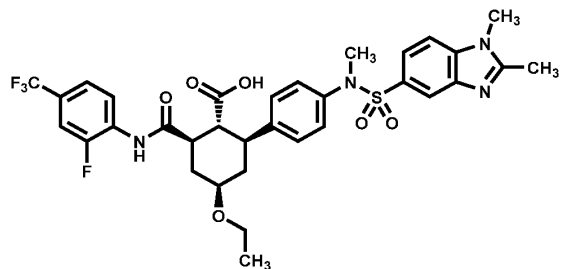
, and



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or a pharmaceutically acceptable salt thereof.

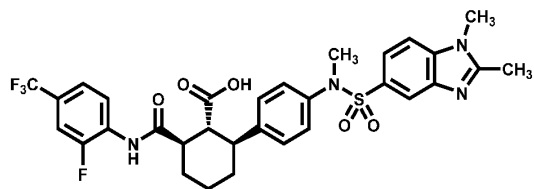
In one embodiment, the present disclosure provides a compound having the structure:



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or a pharmaceutically acceptable salt thereof.

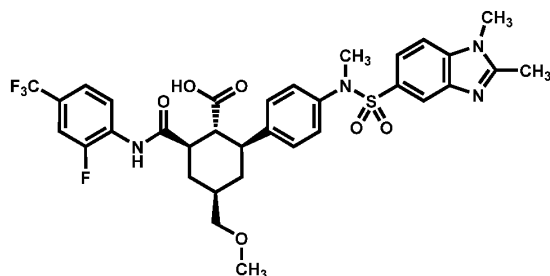
In one embodiment, the present disclosure provides a compound having the structure:



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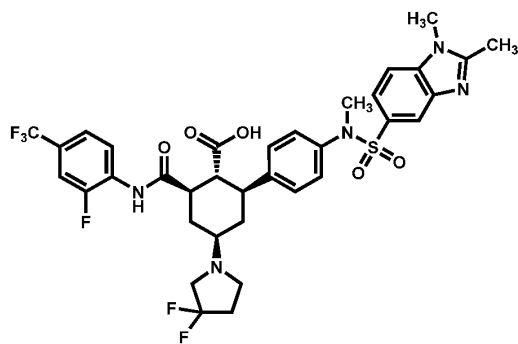
or a pharmaceutically acceptable salt thereof.

In one embodiment, the present disclosure provides a compound having the structure:



or a pharmaceutically acceptable salt thereof.

In one embodiment, the present disclosure provides a compound having the structure:



or a pharmaceutically acceptable salt thereof.

The chemical names in the present application are generated from the corresponding structures using either CHEMDRAW, or CHEMAXON. In some instances, chemical names generated from the structures may give a different structure when using the "Convert Name to Structure" function in CHEMDRAW. For example, CHEMDRAW provides the name "(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[d]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid" based on the structure for the compound of Example 83, where the name provides a different structure when using CHEM DRAW's "Convert Name to Structure" function. An alternative name, "(1*R*,2*R*,6*S*)-2-[[2-fluoro-4-(trifluoromethyl)phenyl]carbamoyl]-6-[4-(*N*-methyl-1,2-dimethyl-1*H*-1,3-benzodiazole)-5-sulfonamido]phenyl)cyclohexane-1-carboxylic acid" provides the correct structure of the

Compound of Example 83 in CHEMDRAW. Therefore, when there is a potential ambiguity, the compounds of the present application should be determined by the structures.

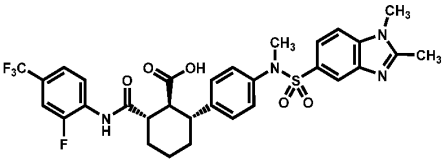
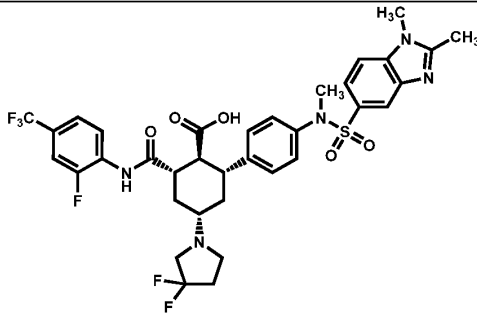
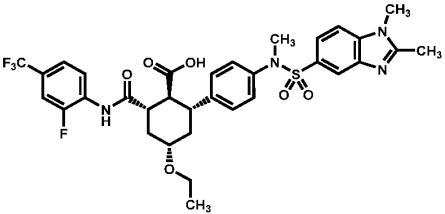
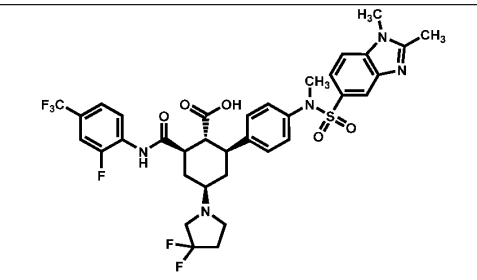
The label “&” in the structures in the present disclosure refers to both chiral centers being present in the mixture. When multiple centers are labelled with “&1” the relative stereochemistry between them is determined. The term “rac” in the chemical names denotes a racemic mixture.

The label “or” in the structures refers to the specific chiral center being a single undefined isomer but absolute stereochemistry was not determined. When multiple centers are labelled with “or1” the relative stereochemistry between them is determined but not the absolute stereochemistry. The term “rel” in the chemical names denotes relative stereochemistry is defined but the absolute chemistry is not determined.

Unless otherwise indicated, Isomer 1 refers to the first eluting isomer and Isomer 2 refers to the second eluting isomer during chiral chromatographic separation.

Table 1.

Ex. No.	Structure	Ex. No.	Structure
81		138	
83		250	

84		305	
137		306	

In another aspect, the present disclosure provides methods of making a compound or pharmaceutically acceptable salt thereof of the invention as disclosed herein and chemical intermediate for making the compound.

In another aspect, the present disclosure provides a pharmaceutical composition comprising a) a compound or pharmaceutically acceptable salt thereof of the invention as disclosed herein and b) a pharmaceutically acceptable excipient. The excipient(s) must be acceptable in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

In another aspect, the present disclosure provides a compound or pharmaceutically acceptable salt thereof of the invention as disclosed herein, for use in therapy.

In another aspect, the present disclosure provides a compound or pharmaceutically acceptable salt thereof of the invention as disclosed herein, for use in the treatment of cancer.

In an embodiment, the cancer is characterised by MSI-H and/or dMMR.

In an embodiment, the cancer is treatable by inhibition of WRN.

In another aspect, the present disclosure provides a method for treating a cancer in a subject in need thereof comprising administering to said subject a therapeutically effective amount of a compound or pharmaceutically acceptable salt thereof of the invention as disclosed herein.

In another aspect, provided is a method for treating a cancer treatable by inhibition of WRN in a patient comprising administering to the patient a therapeutically effective amount of a compound of the present disclosure or a pharmaceutically acceptable salt thereof. In an embodiment, the patient is in recognized need of such treatment. In an embodiment, the compound or a pharmaceutically acceptable salt thereof of the invention as disclosed herein is administered in a pharmaceutical composition.

In another aspect, the present disclosure provides a method of treating a cancer characterized by MSI-H and/or dMMR in a patient comprising administering to the patient, a therapeutically effective amount of a compound of the present invention as disclosed herein, or a pharmaceutically acceptable salt thereof. In an embodiment, the patient is in recognized need of such treatment. In an embodiment, the compound of the present disclosure or a pharmaceutically acceptable salt thereof is administered in a pharmaceutical composition.

In an embodiment, the cancer is characterized by MSI-L or MSI-H according to any method known in the art. For example, a cancer characterized by MSI-L or MSI-H can comprise one or more MSI markers (MSI-L), or preferably two or more MSI markers (MSI-H), in each case selected from the group consisting of BAT25, BAT26, D2S123, D5S346, and D17S250. In an embodiment, the cancer is characterized by dMMR and comprises a mutation that impairs DNA mismatch repair, preferably the cancer comprises a mutation in a MutS homolog and/or a mutation in a MutL homolog. In a subembodiment, the MutS homolog is selected from the group consisting of MSH2, MSH3, and MSH6, and the MutL homolog is selected from the group consisting of MLH1, MLH3, PMS1, and PMS2, preferably a mutation in MLH1, MSH2, and/or PMS2.

In another aspect, the present disclosure provides a pharmaceutical composition comprising a compound of the invention as disclosed herein, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable excipient.

In another aspect, the present disclosure provides a compound of the invention as disclosed herein, or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer treatable by inhibition of WRN.

In another aspect, the present disclosure provides a compound of the invention as disclosed herein, or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer characterized by high MSI and/or dMMR in a patient. In an embodiment, the patient is in recognized need of such treatment. In an embodiment, the compound of the present disclosure or a pharmaceutically acceptable salt thereof is administered in a pharmaceutical composition. In an embodiment, the cancer is characterized by MSI-L or MSI-H according to any method known in the art. For example, a cancer characterized by MSI-L or MSI-H can comprise one or more MSI markers (MSI-L), or preferably two or more MSI markers (MSI-H), in each case selected from the group consisting of BAT25, BAT26, D2S123, D5S346, and D17S250. In an embodiment, the cancer is characterized by dMMR and comprises a mutation that impairs DNA mismatch repair, preferably the cancer comprises a mutation in a MutS homolog and/or a mutation in a MutL homolog. In an embodiment, the MutS homolog is selected from the group consisting of MSH2, MSH3, and MSH6, and the MutL homolog is selected from the group consisting of MLH1, MLH3, PMS1, and PMS2, preferably a mutation in MLH1, MSH2, and/or PMS2.

In another aspect, the present disclosure provides a method of treating a cancer in a patient, comprising:

- (i) determining if the cancer comprises high MSI and/or dMMR; and
- (ii) if the cancer comprises high MSI and/or dMMR, then administering to the patient a therapeutically effective amount of a compound of the invention as disclosed herein or a pharmaceutically acceptable salt thereof.

In an embodiment, provided herein is a compound of the present disclosure, or a pharmaceutically acceptable salt thereof, effective for decreasing WRN helicase activity for use in a method of treating an individual with a proliferative disease, the method comprising determining the presence of a low microsatellite instability (MSI-L) or preferably, a high microsatellite instability (MSI-H), or a marker associated with an MSI-L or an MSI-H, in a population of proliferative cells from the individual, determining a likelihood that the individual will respond to a therapy comprising administering to the individual said compound of the present disclosure, or a pharmaceutically acceptable salt thereof, based on the determination of the presence of MSI-L or MSI-H, respectively, or a marker associated with MSI-L or MSI-H, respectively, in the population of proliferative cells, and administering to the individual said compound of the present disclosure, or a pharmaceutically acceptable salt thereof, if the individual is predicted to respond to the therapy. In some embodiments, the determination of the

presence of MSI-L or MSI-H in the population of proliferative cells comprises determining the presence of one or more MSI markers (MSI-L), preferably two or more MSI markers (MSI-H), in each case selected from the group consisting of BAT25, BAT26, D2S123, D5S346, and D17S250. In some embodiments, the individual is predicted to respond to the therapy if the amount of cells in the population of proliferative cells determined to have at least one of the MSI markers (MSI-L), or preferably at least two of the MSI markers (MSI-H), respectively is above a pre-determined threshold for the proliferative disease. In some embodiments, the individual is predicted not to respond to the therapy if (a) the amount of cells in the population of proliferative cells determined to have at least one of the MSI markers (MSI-L), or preferably at least two of the MSI markers (MSI-H), respectively, below a pre-determined threshold for the proliferative disease; or (b) the population of proliferative cells is determined to have none of the MSI markers (e.g., is considered microsatellite stable (MSS)).

In an embodiment, according to any of the embodiments described above, the determination of the presence of a marker associated with MSI-H in the population of proliferative cells comprises determining the presence of a mutation that impairs DNA mismatch repair. In some embodiments, the mutation comprises a mutation in a MutS homolog and/or a mutation in a MutL homolog. In some embodiments, the MutS homolog is selected from the group consisting of MSH2, MSH3, and MSH6, and the MutL homolog is selected from the group consisting of MLH1, MLH3, PMS1, and PMS2. In some embodiments, the mutation comprises a mutation in MLH1, MSH2, and/or PMS2.

In some embodiments, according to any of the embodiments described above, the determination of the presence of a marker associated with MSI-H in the population of proliferative cells comprises determining the presence of one or more markers of DNA damage. In some embodiments, the one or more markers of DNA damage are selected from the group consisting of high p21 expression and high γ H2AX expression.

In some embodiments, according to any of the embodiments described above, the individual is predicted to respond to the therapy if the amount of cells in the population of proliferative cells determined to have (i) at least one mutation that impairs DNA mismatch repair and/or (ii) at least one marker of DNA damage is above a pre-determined threshold for the proliferative disease. In some embodiments, the at least one mutation that impairs DNA mismatch repair comprises a mutation in MLH1, MSH2, and/or PMS2, and the at least one marker of DNA damage comprises high p21 expression and/or high γ H2AX expression.

In some embodiments, according to any of the embodiments described above, the individual is predicted not to respond to the therapy if (a) the amount of cells in the population of proliferative cells determined to have (i) at least one mutation that impairs DNA mismatch repair and/or (ii) at least one marker of DNA damage is below a pre-determined threshold for the proliferative disease; or (b) the population of proliferative cells is determined to have no mutations that impair DNA mismatch repair and no DNA damage markers.

In another aspect, provided herein is an *in vitro* method for detecting a high microsatellite instability (MSI-H) and the helicase activity of WRN in an individual diagnosed with or thought to have a proliferative disease, the method comprising: (a) contacting a biological sample from the individual with one or more reagents for detecting the presence of an MSI and the helicase activity of WRN; and (b) detecting (i) the presence of an MSI-H; and (ii) the helicase activity of WRN. In some embodiments, the reagent for detecting the presence of an MSI-H in a biological sample comprises a reagent for detecting the presence of two or more MSI markers selected from the group consisting of BAT25, BAT26, D2S123, D5S346, and D17S250.

In another aspect, provided herein is an *in vitro* method for detecting a marker associated with a high microsatellite instability (MSI-H) and the helicase activity of WRN in an individual diagnosed with or thought to have a proliferative disease, the method comprising: (a) contacting a biological sample from the individual with one or more reagents for detecting the presence of a marker associated with an MSI-H and the helicase activity of WRN helicase; and (b) detecting (i) the presence of the marker associated with an MSI-H; and (ii) the helicase activity of WRN helicase. In some embodiments, the reagent for detecting the presence of a marker associated with an MSI-H in a biological sample comprises a reagent for detecting the presence of (i) one or more mutations that impair DNA mismatch repair and/or (ii) one or more markers of DNA damage. In some embodiments, the one or more mutations that impair DNA mismatch repair comprise a mutation in a MutS homolog and/or a mutation in a MutL homolog. In some embodiments, the MutS homolog is selected from the group consisting of MSH2, MSH3, and MSH6, and the MutL homolog is selected from the group consisting of MLH1, MLH3, PMS1, and PMS2. In some embodiments, the one or more mutations comprise a mutation in MLH1, MSH2, and/or PMS2. In some embodiments, the one or more markers of DNA damage are selected from the group consisting of high p21 expression and high γ H2AX expression.

In one approach for this embodiment, the cancer can be characterized by MSI-H according to any method known in the art. For example, a cancer characterized by MSI-H can comprise two or more MSI markers selected from the group consisting of BAT25, BAT26, D2S123, D5S346, and

D17S250. In an embodiment, the cancer is characterized by dMMR and comprises a mutation that impairs DNA mismatch repair, preferably the cancer comprises a mutation in a MutS homolog and/or a mutation in a MutL homolog. In an embodiment, the MutS homolog is selected from the group consisting of MSH2, MSH3, and MSH6, and the MutL homolog is selected from the group consisting of MLH1, MLH3, PMS1, and PMS2, preferably a mutation in MLH1, MSH2, and/or PMS2. In an embodiment, the cancer comprises two or more markers of DNA damage.

In an embodiment, the cancer is endometrial cancer, biliary cancer, gastric cancer, pancreatic cancer, small intestine cancer, breast cancer, prostate cancer, bladder cancer, esophageal cancer, sarcoma, retroperitoneal adenocarcinoma, small lung cancer or renal cell cancer. Preferably, the cancer is uterine corpus endometrial carcinoma, stomach adenocarcinoma, colon adenocarcinoma, rectal adenocarcinoma, prostate adenocarcinoma, adrenal cortical carcinoma, oesophageal carcinoma, liver hepatocellular carcinoma, cervical squamous cell carcinoma, head and neck squamous cell carcinoma, lung squamous cell carcinoma, kidney renal clear cell carcinoma, papillary kidney carcinoma, pancreatic cancer, urothelial bladder cancer, ovarian cancer, breast cancer, glioblastoma multiforme, or low grade glioma (see Isidro Cortes-Ciriano et. al. Nature Communication DOI: 10.1038/ncomms15180).

In another aspect, provided herein is a method of identifying WRN helicase inhibitory activity in a test compound of WRN helicase, said method comprising (i) contacting the test compound with isolated WRN enzyme in an assay buffer to form a WRN reaction pre-mixture; (ii) contacting the WRN reaction pre-mixture with a substrate mixture containing 80 uM ATP, 80 nM dT₅₀ (single-stranded DNA (ssDNA) containing 50 thymine bases), 200 uM NADH, 4 mM PEP, 10 U/mL lactate dehydrogenase and 20 U/mL pyruvate kinase to form a WRN reaction mixture; and (iii) measuring absorbance ($\lambda = 340$ nm) of the WRN reaction mixture, wherein said method further comprises performing steps (i)-(iii) with a positive control sample represented by a compound of the present disclosure.

Pharmaceutical Composition

The compounds of the present disclosure, or a pharmaceutically acceptable salt thereof, provided herein may be in the form of compositions suitable for administration to a subject. In general, such compositions are pharmaceutical compositions comprising a compound of the present disclosure or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable excipients. The pharmaceutical compositions may be used in the

methods disclosed herein; thus, for example, the pharmaceutical compositions can be administered *ex vivo* or *in vivo* to a subject in order to practice the therapeutic methods and uses described herein.

The pharmaceutical compositions can be formulated to be compatible with the intended method or route of administration; exemplary routes of administration are set forth herein. Furthermore, the pharmaceutical compositions may be used in combination with other therapeutically active agents or compounds as described herein in order to treat the diseases, disorders and conditions contemplated by the present disclosure.

The pharmaceutical compositions containing the active ingredient (e.g., a compound of the present disclosure, a pharmaceutically acceptable salt thereof) may be in a form suitable for oral use, for example, as tablets, capsules, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups, solutions, microbeads or elixirs. Pharmaceutical compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions, and such compositions may contain one or more agents such as, for example, sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets and/or capsules contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets and/or capsules. These excipients may be, for example, diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc.

The pharmaceutical compositions typically comprise a therapeutically effective amount of a compound of the present disclosure, or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable excipient. Suitable pharmaceutically acceptable excipients include, but are not limited to, antioxidants (e.g., ascorbic acid and sodium bisulfate), preservatives (e.g., benzyl alcohol, methyl parabens, ethyl or n-propyl, p-hydroxybenzoate), emulsifying agents, suspending agents, dispersing agents, solvents, fillers, bulking agents, detergents, buffers, vehicles, diluents, and/or adjuvants. For example, a suitable vehicle may be physiological saline solution or citrate buffered saline, possibly supplemented with other materials common in pharmaceutical compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Those

skilled in the art will readily recognize a variety of buffers that can be used in the pharmaceutical compositions and dosage forms contemplated herein. Typical buffers include, but are not limited to, pharmaceutically acceptable weak acids, weak bases, or mixtures thereof. As an example, the buffer components can be water soluble materials such as phosphoric acid, tartaric acids, lactic acid, succinic acid, citric acid, acetic acid, ascorbic acid, aspartic acid, glutamic acid, and salts thereof. Acceptable buffering agents include, for example, a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), and N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS).

All the compounds and pharmaceutical compositions provided herein can be used in all the methods provided herein. For example, the compounds and pharmaceutical compositions provided herein can be used in all the methods for treatment and/or prevention of all diseases or disorders provided herein. Thus, the compounds and pharmaceutical compositions provided herein are for use as a medicament.

Routes of Administration

Compounds of the present disclosure, or a pharmaceutically acceptable salt thereof and compositions containing the same may be administered in any appropriate manner. Suitable routes of administration include oral, parenteral (e.g., intramuscular, intravenous, subcutaneous (e.g., injection or implant), intraperitoneal, intracisternal, intraarticular, intracerebral (intraparenchymal) and intracerebroventricular), sublingual, intraocular, and inhalation. Depot injections, which are generally administered subcutaneously or intramuscularly, may also be utilized to administer the compounds of the present disclosure, or a pharmaceutically acceptable salt thereof over a defined period of time. Particular embodiments of the present disclosure contemplate oral administration.

Treatment of Patients Having Tumors Characterized by High Microsatellite Instability

In one aspect, provided herein is a method for decreasing proliferation in a proliferative cell having a microsatellite instability (MSI), comprising decreasing the helicase activity of Werner syndrome ATP-dependent helicase (WRN) in the proliferative cell. In some embodiments, decreasing the helicase activity of Werner syndrome ATP-dependent helicase (WRN) in the proliferative cell is achieved by administering a compound of the present disclosure or a pharmaceutically acceptable salt thereof. In some embodiments, the proliferative cell is

characterized as having MSI low (MSI-L). In some embodiments, the proliferative cell is characterized as having high MSI (MSI-H), used interchangeably with MSH-high. Cells can be characterized as MSI, including MSI-L or MSI-H, or as microsatellite stable (MSS), according to any method known in the art (see, for example, Dudley, Jonathan C., *et al.*, *Clinical Cancer Research*, 22(4): 813-820, 2016.). MSI-H is used to classify tumors as having a high frequency of MSI. A tumor can be classified as MSI, including MSI-low or MSI-high, using polymerase chain reaction (PCR) and/or immunohistochemistry (IHC) assays. As stated in Dudley et al. (*supra*), a tumor is classified as MSI-H by PCR if (i) there is a shift (usually downward) in the size of at least two microsatellite loci from a reference panel of five microsatellite loci in tumor relative to normal, where the reference panel can be the "Bethesda Panel," also referred to herein as the "NCI-Reference Panel (Bethesda, 1998)", which includes two mononucleotide loci (BAT-25 and BAT-26) and three dinucleotide loci (D2S123, D5S346, and D17S250), or alternatively, the reference panel can be Promega Corporation's MSI Analysis System, which includes five mononucleotide loci (BAT-25, BAT-26, NR-21, NR-24, and MONO-27); or (ii) there is a shift in the size of 30% or more microsatellite loci from a reference panel of more than five microsatellite loci in tumor relative to normal. The MSI-H phenotype is associated with germline defects in the mismatch repair genes MLH1, MSH2, MSH6, and PMS2, and is the primary phenotype observed in tumors from patients with HNPCC/Lynch syndrome. A tumor is classified as MSI-H in IHC test if it shows a loss of protein expression for at least 1 of the above 4 mismatch repair genes. Cells can be similarly classified as MSI-H using the tests described herein for tumors.

In some embodiments, a tumor or cell is classified as MSI-H using PCR to amplify the five microsatellite loci of the "Bethesda Panel" (BAT-25, BAT-26, D2S123, D5S346, and D17S250) from both tumor tissue or cells and normal tissue or cells, wherein the tumor or cell is classified as MSI-H if there is a shift in the size of at least two of the microsatellite loci from the tumor tissue or cells relative to the normal tissue or cells. In some embodiments, the shift in size of the microsatellite loci is a downward shift.

In some embodiments, a tumor or cell is classified as MSI-H using PCR to amplify the five microsatellite loci of Promega Corporation's MSI Analysis System (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) from both tumor tissue or cells and normal tissue or cells, wherein the tumor or cell is classified as MSI-H if there is a shift in the size of at least two of the microsatellite loci from the tumor tissue or cells relative to the normal tissue or cells. In some embodiments, the shift in size of the microsatellite loci is a downward shift.

In some embodiments, a tumor is classified as MSI-H using IHC to determine the expression level of the MMR proteins MLH1, MSH2, MSH6, and/or PMS2 in both tumor tissue and normal tissue, wherein the tumor is classified as MSI-H if there is a loss of protein expression for at least one of the MMR proteins in the tumor tissue relative to the normal tissue. In some embodiments, the loss of protein expression is a decrease of at least 20% (such as a decrease of 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more).

In contrast, a tumor is classified as MSI-L by PCR if (i) there is a shift in the size of one microsatellite locus from a reference panel of five microsatellite loci in tumor relative to normal, where the reference panel can be the "Bethesda Panel" or Promega Corporation's MSI Analysis System; or (ii) there is a shift in the size of less than 30% microsatellite loci from a reference panel of more than five microsatellite loci in tumor relative to normal. MSI-L tumors are thought to represent a distinct mutator phenotype with potentially different molecular etiology than MSI-H tumors (Thibodeau, 1998; Wu et al., 1999, Am J Hum Genetics 65:1291-1298). Cells can be similarly classified as MSI-L using the tests described herein for tumors.

Combination Therapy

The present disclosure contemplates the use of compounds of the present disclosure, or a pharmaceutically acceptable salt thereof in combination with one or more active therapeutic agents (e.g., chemotherapeutic agents) or other prophylactic or therapeutic modalities (e.g., radiation). In such combination therapy, the various active agents frequently have different, complementary mechanisms of action. Such combination therapy may be especially advantageous by allowing a dose reduction of one or more of the agents, thereby reducing or eliminating the adverse effects associated with one or more of the agents. Furthermore, such combination therapy may have a synergistic therapeutic or prophylactic effect on the underlying disease, disorder, or condition.

As used herein, "combination" is meant to include therapies that can be administered separately, for example, formulated separately for separate administration (e.g., as may be provided in a kit), and therapies that can be administered together in a single formulation (i.e., a "co-formulation").

In certain embodiments, the compounds of the present disclosure, or a pharmaceutically acceptable salt thereof are administered or applied sequentially, e.g., where one agent is administered prior to one or more other agents. In other embodiments, the compounds of the present disclosure, or a pharmaceutically acceptable salt thereof are administered

simultaneously, e.g., where two or more agents are administered at or about the same time; the two or more agents may be present in two or more separate formulations or combined into a single formulation (i.e., a co-formulation). Regardless of whether the two or more agents are administered sequentially or simultaneously, they are considered to be administered in combination for purposes of the present disclosure.

The present disclosure provides methods for treating cancer with a compound of the present disclosure, or a pharmaceutically acceptable salt thereof and at least one additional therapeutic or diagnostic agent.

In some embodiments, the compound of the present disclosure, or a pharmaceutically acceptable salt thereof is administered in combination with at least one additional therapeutic agent, selected from Temozolomide, Pemetrexed, Pegylated liposomal doxorubicin (Doxil), Eribulin (Halaven), Ixabepilone (Ixempra), Protein-bound paclitaxel (Abraxane), Oxaliplatin, Irinotecan, Venatoclax (bcl2 inhibitor), 5-azacytadine, Anti-CD20 therapeutics, such as Rituxan and obinutuzumab, Hormonal agents (anastrozole, exemestane, letrozole, zoladex, lupon eligard), CDK4/6 inhibitors, Palbociclib, Abemaciclib, CPI (Avelumab, Cemiplimab-rwlc, and Bevacizumab).

In certain embodiments, the present disclosure provides methods for treating cancer comprising administration of a compound of the present disclosure, or a pharmaceutically acceptable salt thereof described herein in combination with a signal transduction inhibitor (STI) to achieve additive or synergistic suppression of tumor growth. As used herein, the term "signal transduction inhibitor" refers to an agent that selectively inhibits one or more steps in a signaling pathway. Examples of signal transduction inhibitors (STIs) useful in methods described herein include, but are not limited to: (i) bcr/abl kinase inhibitors (e.g., GLEEVEC); (ii) epidermal growth factor (EGF) receptor inhibitors, including kinase inhibitors and antibodies; (iii) her-2/neu receptor inhibitors (e.g., HERCEPTIN); (iv) inhibitors of Akt family kinases or the Akt pathway (e.g., rapamycin); (v) cell cycle kinase inhibitors (e.g., flavopiridol); and (vi) phosphatidyl inositol kinase inhibitors. Agents involved in immunomodulation can also be used in combination with one or more compounds of the present disclosure, or a pharmaceutically acceptable salt thereof described herein for the suppression of tumor growth in cancer patients.

In certain embodiments, the present disclosure provides methods for treating cancer comprising administration of a compound of the present disclosure, or a pharmaceutically acceptable salt thereof described herein in combination with a chemotherapeutic agent. Examples of

chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolmelamine; nitrogen mustards such as chiorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitioestanol, mepitioestane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); cyclophosphamide; thiotepa; taxoids, e.g., paclitaxel and doxetaxel; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum and platinum coordination complexes such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT11; topoisomerase inhibitors; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In a particular embodiment, compounds of the present disclosure are

coadministered with a cytostatic compound selected from the group consisting of cisplatin, doxorubicin, taxol, taxotere and mitomycin C. In a particular embodiment, the cytostatic compound is doxorubicin.

Chemotherapeutic agents also include anti-hormonal agents that act to regulate or inhibit hormonal action on tumors such as anti-estrogens, including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, onapristone, and toremifene; and antiandrogens such as flutamide, nilutamide, bicalutamide, enzalutamide, apalutamide, abiraterone acetate, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In certain embodiments, combination therapy comprises administration of a hormone or related hormonal agent.

The present disclosure also contemplates the use of the compounds of the present disclosure, or a pharmaceutically acceptable salt thereof described herein in combination with immune checkpoint inhibitors. The tremendous number of genetic and epigenetic alterations that are characteristic of all cancers provides a diverse set of antigens that the immune system can use to distinguish tumor cells from their normal counterparts. In the case of T cells, the ultimate amplitude (e.g., levels of cytokine production or proliferation) and quality (e.g., the type of immune response generated, such as the pattern of cytokine production) of the response, which is initiated through antigen recognition by the T-cell receptor (TCR), is regulated by a balance between co-stimulatory and inhibitory signals (immune checkpoints). Under normal physiological conditions, immune checkpoints are crucial for the prevention of autoimmunity (i.e., the maintenance of self-tolerance) and also for the protection of tissues from damage when the immune system is responding to pathogenic infection. The expression of immune checkpoint proteins can be dysregulated by tumors as an important immune resistance mechanism. Examples of immune checkpoint inhibitors include but are not limited to CTLA-4, PD-1, PD-L1, BTLA, TIM3, LAG3, OX40, 41BB, VISTA, CD96, TGF β , CD73, CD39, A2AR, A2BR, IDO1, TDO2, Arginase, B7-H3, B7-H4. Cell-based modulators of anti-cancer immunity are also contemplated. Examples of such modulators include but are not limited to chimeric antigen receptor T-cells, tumor infiltrating T-cells, and dendritic-cells.

The present disclosure contemplates the use of compounds of the present disclosure, or a pharmaceutically acceptable salt thereof described herein in combination with inhibitors of the aforementioned immune-checkpoint receptors and ligands, for example ipilimumab, abatacept, nivolumab, pembrolizumab, atezolizumab, dostarlimab, and durvalumab.

Additional treatment modalities that may be used in combination with a compound of the present disclosure, or a pharmaceutically acceptable salt thereof disclosed herein include radiotherapy, a monoclonal antibody against a tumor antigen, a complex of a monoclonal antibody and toxin, a T-cell adjuvant, bone marrow transplant, or antigen presenting cells (e.g., dendritic cell therapy).

The present disclosure contemplates the use of compounds of the present disclosure, or a pharmaceutically acceptable salt thereof described herein for the treatment of glioblastoma either alone or in combination with radiation and/or temozolomide (TMZ), avastin, or lomustine.

Dosing

The compounds of the present disclosure, or a pharmaceutically acceptable salt thereof provided herein may be administered to a subject in an amount that is dependent upon, for example, the goal of administration (e.g., the degree of resolution desired); the age, weight, sex, and health and physical condition of the subject to which the formulation is being administered; the route of administration; and the nature of the disease, disorder, condition or symptom thereof. The dosing regimen may also take into consideration the existence, nature, and extent of any adverse effects associated with the agent(s) being administered.

An effective dose (ED) is the dose or amount of an agent that produces a therapeutic response or desired effect in some fraction of the subjects taking it. The "median effective dose" or ED₅₀ of an agent is the dose or amount of an agent that produces a therapeutic response or desired effect in 50% of the population to which it is administered. Although the ED₅₀ is commonly used as a measure of reasonable expectance of an agent's effect, it is not necessarily the dose that a clinician might deem appropriate taking into consideration all relevant factors. Thus, in some situations the effective amount is more than the calculated ED₅₀, in other situations the effective amount is less than the calculated ED₅₀, and in still other situations the effective amount is the same as the calculated ED₅₀.

EXAMPLES

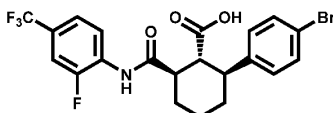
The following examples and references (intermediates) are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present disclosure, and are not intended to limit the scope of what the inventors regard as their disclosure, nor are they intended to represent that the experiments below were performed or that they are all of the experiments that may be performed. It is to be understood that

exemplary descriptions written in the present tense were not necessarily performed, but rather that the descriptions can be performed to generate data of a nature described therein. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.), but some experimental errors and deviations should be accounted for. Some NMR data was processed using computer analysis and may contain artifacts inherent in that process, including reporting of impurity peaks, solvent peaks and miss-integrations.

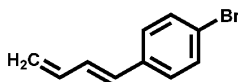
The mass yield of a reaction described herein is the amount of material collected from the reaction but the mmol amount and %yield are adjusted to reflect the purity of the material. For example, if 10 grams of a product (corresponding to 20 mmol if 100% pure) was collected from a reaction but the purity of the product was determined to be 90%, the preparation will be reported as "(10 grams, 18 mmol, 54% yield)". However, when the product is used as a starting material in a subsequent step, for stoichiometry calculation purposes, the product will be considered as 100% pure. For example, the 10 grams product will be described as a starting material of "10 grams, 20 mmol" in a subsequent reaction.

Intermediates

Intermediate 1: (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid



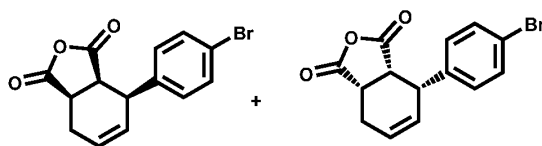
Step 1: (*E*)-1-bromo-4-(buta-1,3-dien-1-yl)benzene



In two separate, equivalent reactions: to a mixture of diethyl allylphosphonate (144 g, 811 mmol) in THF (3000 mL) at 0 °C was added NaH (32.4 g, 811 mmol). After 50 min, 4-bromobenzaldehyde (0.100 kg, 0.540 mol) was added, and the mixtures were allowed to warm to 25 °C for 4 h. The two separate reactions were combined, cooled to 0 °C quenched with aq. NH₄Cl (500 mL), and extracted with EtOAc (500 mL X 3). The combined organic layers were washed with brine (500 mL), dried over Na₂SO₄, filtered and concentrated. The residue was taken up in petroleum ether (500 mL), and 300 g of silica gel (100-200 mesh silica gel) was added. The resulting mixture was concentrated at 40 °C and to give a dry flowing solid, and was loaded to Biotage using 1,500 g silica gel (self-prepared column chromatography, 100-200 mesh silica gel),

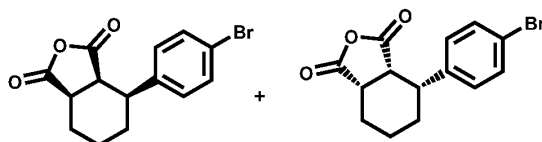
eluting with 0-10% ethyl acetate in petroleum ether to give (*E*)-1-bromo-4-(buta-1,3-dien-1-yl)benzene (90 g, 410 mmol, 38% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 7.43 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 6.76 (dd, *J* = 15.6 Hz, 10.4 Hz, 1H), 6.53 - 6.44 (m, 2H), 5.36 (d, *J* = 17.2 Hz, 1H), 5.21 (d, *J* = 10.0 Hz, 1H).

Step 2: 1:1 mixture of (3a*S*,4*S*,7a*R*)-4-(4-bromophenyl)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione and (3a*R*,4*R*,7a*S*)-4-(4-bromophenyl)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione



In two separate, equivalent reactions: to a mixture of (*E*)-1-bromo-4-(buta-1,3-dien-1-yl)benzene (140 g, 670 mmol) in toluene (1400 mL) at 25 °C was added furan-2,5-dione (65.7 g, 0.670 mol). The mixtures were stirred at 120 °C for 12 h. The two reactions were combined, concentrated, triturated with 2:1 *n*-hexane:ethyl acetate at 20 °C for 30 mins, filtered and washed with *n*-hexane (200 mL X 3) to give a 1:1 mixture of (3a*S*,4*S*,7a*R*)-4-(4-bromophenyl)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione and (3a*R*,4*R*,7a*S*)-4-(4-bromophenyl)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione (189 g, 599 mmol, 44.5% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.52 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 6.22 (m, 1H), 6.17 - 6.15 (m, 1H), 3.83 (d, *J* = 2.0 Hz, 1H), 3.77 - 3.72 (m, 2H), 2.66 - 2.60 (m, 1H), 2.50 - 2.40 (m, 1H).

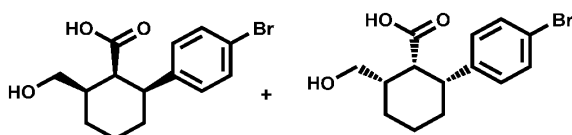
Step 3: 1:1 mixture of (3a*S*,4*S*,7a*R*)-4-(4-bromophenyl)hexahydroisobenzofuran-1,3-dione and (3a*R*,4*R*,7a*S*)-4-(4-bromophenyl)hexahydroisobenzofuran-1,3-dione



In three separate, equivalent reactions: to a 1:1 mixture of (3a*S*,4*S*,7a*R*)-4-(4-bromophenyl)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione and (3a*R*,4*R*,7a*S*)-4-(4-bromophenyl)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione (63.0 g, 205 mmol) in THF (1000 mL) at 25 °C under nitrogen was added platinum (8.00 g, 2.05 mmol). The mixtures were placed under hydrogen atmosphere (2 atm). The reactions were degassed via vacuum evacuation, then backfilling with hydrogen (3X). The mixtures were stirred at 25 °C for 40 min under hydrogen. The three reactions were combined, filtered, and the filter cake was washed with THF (1 L x 3). The combined filtrates were concentrated, triturated with 5:1 *n*-hexane:ethyl acetate (500 mL) at 20 °C for 30 mins, filtered,

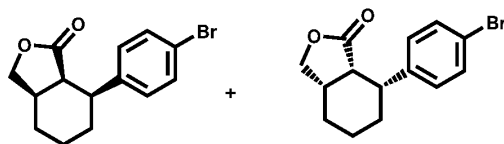
washed with *n*-hexane (100 mL x3) and dried under reduced pressure at 40 °C to provide a 1:1 mixture of (3*aS*,4*S*,7*aR*)-4-(4-bromophenyl)hexahydroisobenzofuran-1,3-dione and (3*aR*,4*R*,7*aS*)-4-(4-bromophenyl)hexahydroisobenzofuran-1,3-dione (164 g, 477 mmol, 78%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.50 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 3.99 (t, *J* = 6.8 Hz, 1H), 3.35 - 3.29 (m, 1H), 3.16 - 3.10 (m, 1H), 2.09 - 2.04 (m, 1H), 1.91 - 1.86 (m, 1H), 1.81 - 1.76 (m, 1H), 1.67 - 1.44 (m, 3H).

Step 4: 1:1 mixture of (3*aS*,4*S*,7*aR*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (3*aR*,4*R*,7*aS*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid



To a 1:1 mixture of (3*aS*,4*S*,7*aR*)-4-(4-bromophenyl)hexahydroisobenzofuran-1,3-dione and (3*aR*,4*R*,7*aS*)-4-(4-bromophenyl)hexahydroisobenzofuran-1,3-dione (164 g, 0.530 mol) in THF (1500 mL) at 0 °C was added NaBH₄ (40.1 g, 1.06 mol), in portions. The reaction was stirred for 1 h, quenched with 2 N HCl (1.00 L) to pH < 3, then was extracted with EtOAc (300 mL X 3). The combined organic layers were washed with brine (500 mL), dried over Na₂SO₄, filtered and concentrated to afford a 1:1 mixture of (3*aS*,4*S*,7*aR*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (3*aR*,4*R*,7*aS*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid (135 g, 346 mmol, 65.2 % yield) as a white solid, which was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.6 (br.s, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 3.34 - 3.30 (m, 1H), 3.23 - 3.18 (m, 1H), 2.88 (t, *J* = 4.4 Hz, 1H), 2.85 - 2.79 (m, 1H), 2.35 - 2.24 (m, 1H), 1.90 - 1.75 (m, 2H), 1.56 - 1.44 (m, 3H), 1.41 - 1.31 (m, 1H).

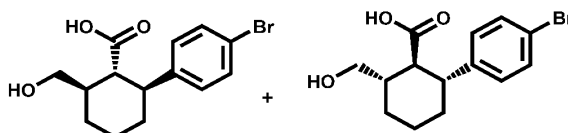
Step 5: 1:1 mixture of (3*aR*,7*S*,7*aS*)-7-(4-bromophenyl)hexahydroisobenzofuran-1(3H)-one and (3*aS*,7*R*,7*aR*)-7-(4-bromophenyl)hexahydroisobenzofuran-1(3H)-one



To a 1:1 mixture of (3*aS*,4*S*,7*aR*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (3*aR*,4*R*,7*aS*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid (135

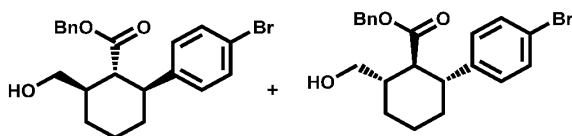
g, 431 mmol) in toluene (1300 mL) at 25 °C was added 4-methylbenzenesulfonic acid (7.42 g, 43.1 mmol). The reaction was stirred at 110 °C for 1 h, cooled to 25 °C, diluted with water (300 mL) and extracted with EtOAc (200 mL X 3). The combined organic layers were washed with NaHCO₃(aq.) (200 mL) and brine (200 mL), dried over Na₂SO₄, filtered and concentrated afford a 1:1 mixture of (3a*R*,7*S*,7a*S*)-7-(4-bromophenyl)hexahydroisobenzofuran-1(3*H*)-one and (3a*S*,7*R*,7a*R*)-7-(4-bromophenyl)hexahydroisobenzofuran-1(3*H*)-one (118 g, 375 mmol, 87 % yield) as a white solid, which was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 4.15 (q, *J* = 4.4 Hz, 1H), 3.84 (d, *J* = 8.4 Hz, 1H), 3.26 (t, *J* = 5.6 Hz, 1H), 3.02 – 2.98 (m, 1H), 2.61 - 2.54 (m, 1H), 1.83 (d, *J* = 9.6 Hz, 3H), 1.49 - 1.34 (m, 2H), 1.12 - 1.06 (m, 1H).

Step 6: 1:1 mixture of (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid



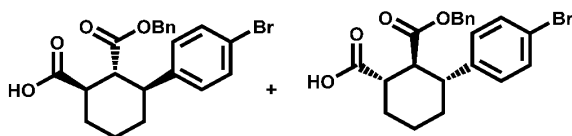
To a 1:1 mixture of (3a*R*,7*S*,7a*S*)-7-(4-bromophenyl)hexahydroisobenzofuran-1(3*H*)-one and (3a*S*,7*R*,7a*R*)-7-(4-bromophenyl)hexahydroisobenzofuran-1(3*H*)-one (118 g, 0.400 mol) in methanol (1.18 L) at 25 °C was added KOH (112 g, 2.00 mol). The reaction was stirred at 75 °C for 3 h, cooled to 0 °C, quenched with 2 N HCl (1.00 L) to pH < 3, and extracted with EtOAc (400 mL X 3). The combined organic layers were washed with brine (500 mL), dried over Na₂SO₄, filtered and concentrated to afford a 1:1 mixture of (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid (105 g, 287 mmol, 71.9 % yield) as a white solid, which was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.8 (s, 1H), 7.46 - 7.41 (m, 2H), 7.18 - 7.15 (m, 2H), 4.50 (s, 1H), 3.40 - 3.36 (m, 1H), 3.20 - 3.16 (m, 1H), 2.71 - 2.64 (m, 1H), 2.25 (t, *J* = 10.8 Hz, 1H), 1.94 - 1.90 (m, 1H), 1.80 - 1.63 (m, 3H), 1.43 - 1.37 (m, 2H), 1.12 - 1.08 (m, 1H).

Step 7: 1:1 mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylate



To a 1:1 mixture of (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylic acid (105 g, 335 mmol) in DMF (1050 mL) at 0 °C were added K₂CO₃ (139 g, 1.01 mol) and (bromomethyl)benzene (86 g, 0.50 mol). The reaction was stirred at 25 °C for 3 h, diluted with water (500 mL), and extracted with EtOAc (200 mL X 3). The combined organic layers were washed with brine (300 mL), dried over Na₂SO₄, filtered, concentrated, triturated with 5:1 *n*-hexane:ethyl acetate (300 mL) at 20 °C for 30 mins, filtered, washed with *n*-hexane (100 mL X 3) and dried under reduced pressure at 40 °C to provide a 1:1 mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylate (98.0 g, 237 mmol, 70.7 % yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.25 - 7.23 (m, 3H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.87 - 6.85 (m, 2H), 4.87 (d, *J* = 12.4 Hz, 1H), 4.69 (d, *J* = 12.8 Hz, 1H), 4.53 (t, *J* = 4.8 Hz, 1H), 3.30 - 3.27 (m, 1H), 3.23 - 3.18 (m, 1H), 2.73 - 2.67 (m, 1H), 2.43 (t, *J* = 10.8 Hz, 1H), 1.88 - 1.85 (m, 1H), 1.81 - 1.70 (m, 3H), 1.52 - 1.36 (m, 2H), 1.18 - 1.09 (m, 1H).

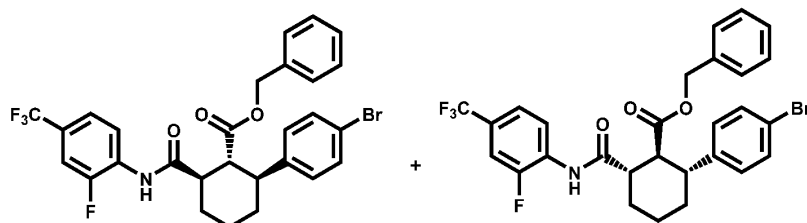
Step 8: 1:1 mixture of (1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,3*R*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid



To a 1:1 mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-(hydroxymethyl)cyclohexane-1-carboxylate (95 g, 0.24 mol) in acetonitrile (950 mL) and water (30 mL) at 0 °C were added sodium periodate (151 g, 707 mmol) and ruthenium(III) chloride (4.89 g, 23.6 mmol). The reaction was stirred at 25 °C for 3 h, diluted with water (500 mL) and extracted with EtOAc (200 mL X 3). The combined organic layers were washed with brine (300 mL), dried over Na₂SO₄, filtered, concentrated, triturated with 3:1 *n*-hexane:ethyl acetate (200 mL) at 20 °C for 30 mins, filtered, washed with *n*-hexane (50 mL x 3) and dried under reduced pressure at 40 °C to provide a 1:1 mixture of (1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid

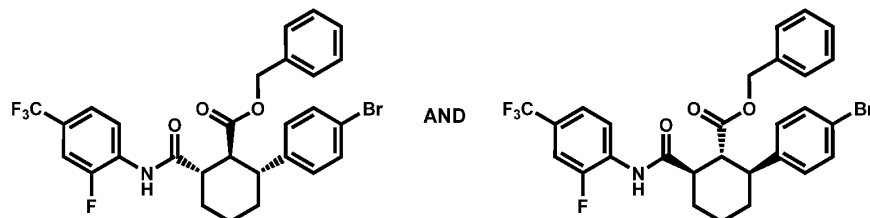
and (1*S*,2*S*,3*R*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid (88 g, 0.21 mol, 88 % yield) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.4 (s, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.25 - 7.23 (m, 3H), 7.17 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 4.4 Hz, 2H), 4.69 (q, *J* = 12.8 Hz, 2H), 2.77 - 2.74 (m, 1H), 2.66 - 2.64 (m, 2H), 2.22 - 2.10 (m, 1H), 1.78 - 1.74 (m, 2H), 1.54 - 1.47 (m, 3H). ES-LCMS *m/z* 439.0 [M+Na]⁺.

Step 9: 1:1 mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate



To a 1:1 mixture of (1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,3*R*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid (85 g, 0.20 mol) in acetonitrile (850 mL) was added 2-fluoro-4-(trifluoromethyl)aniline (73.0 g, 407 mmol) and 1-methyl-1H-imidazole (33.4 g, 407 mmol). After ~2 min, *N*-(chloro(dimethylamino)methylene)-*N*-methylmethanaminium hexafluorophosphate (86 g, 0.31 mol) was added, portion wise. After 12 h at 25 °C, the reaction was diluted with water (200 mL) and extracted with EtOAc (200 mL X 3). The combined organic layers were washed with brine (200 mL), dried over Na₂SO₄, filtered, concentrated, dissolved in EtOAc (500 mL) and combined with silica gel (300 g, 100-200). The resulting mixture was concentrated at 40 °C, loaded onto a silica gel column (300 g, 100-200 mesh silica gel), eluting with 10:1 to 5:1 EtOAc in *n*-heptane to give a 1:1 mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (87 g, 0.15 mol, 73% yield) as a white solid. ¹H NMR (400 MHz, CHLOROFORM-*d*) δ 8.43 (t, *J* = 8.1 Hz, 1H), 7.60 - 7.52 (m, 1H), 7.41 - 7.30 (m, 4H), 7.21 - 7.10 (m, 3H), 7.08 - 7.00 (m, 2H), 6.87 - 6.78 (m, 2H), 4.81 - 4.69 (m, 2H), 3.01 - 2.92 (m, 1H), 2.86 - 2.72 (m, 2H), 2.13 - 2.06 (m, 1H), 2.05 - 1.98 (m, 1H), 1.99 - 1.85 (m, 2H), 1.85 - 1.73 (m, 1H), 1.66 - 1.53 (m, 2H).

Step 10: benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate and benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate



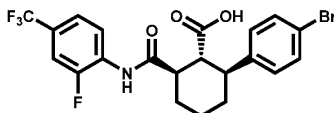
A 1:1 mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (35.0 g, 60.5 mmol) was chirally purified by Chiral-Prep-SFC (Column: DAICEL CHIRALPAK AD [250 mm X 50 mm, 10 μ m]; Mobile Phase A:CO₂, Mobile Phase B:MeOH; Flow rate:100 g/min; Gradient: isocratic 40% B; 220 nm) to afford

First eluting isomer: Isomer 1: benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (15.5 g, 25.1 mmol, 41.5 % yield). Analytical Chiral Chromatography: Column: Chiralpak AD-3, 50×4.6 mm I.D., 3 μ m Mobile phase: A:CO₂ B:MeOH (0.1%IPA, v/v) Gradient: 5-50% B over 1.2 min then 50% B for 1 min) Retention Time = 1.07 min, as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.15 (s, 1H), 8.16 (t, *J* = 8.1 Hz, 1H), 7.72 (dd, *J* = 11.0, 1.5 Hz, 1H), 7.55 (br d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.26 – 7.12 (m, 5H), 6.81 (d, *J* = 6.7 Hz, 2H), 4.77 – 4.62 (m, 2H), 3.13 – 3.03 (m, 1H), 2.96 (t, *J* = 11.2 Hz, 1H), 2.74 (td, *J* = 11.6, 3.4 Hz, 1H), 2.07 (br d, *J* = 8.6 Hz, 1H), 1.95 – 1.84 (m, 1H), 1.82 – 1.73 (m, 1H), 1.72 – 1.47 (m, 3H).

Second eluting isomer: Isomer 2: benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (15.3 g, 26.2 mmol, 43.4 % yield) Analytical Chiral Chromatography: Column: Chiralpak AD-3, 50×4.6 mm I.D., 3 μ m Mobile phase: A:CO₂ B:MeOH (0.1%IPA, v/v) Gradient: 5-50% B over 1.2 min then 50% B for 1 min) Retention Time = 1.36 min, as a white solid. Absolute stereochemistry was determined by co-crystal structure of subsequent final compound with WRN protein and then tracing back to the corresponding enantiopure intermediates.

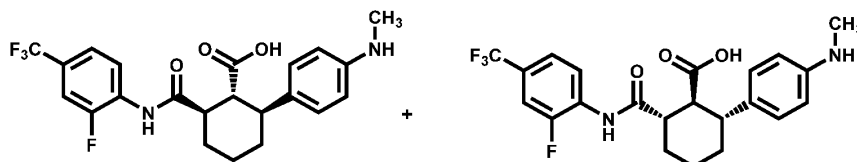
¹H NMR (400 MHz, DMSO-*d*₆) δ 10.15 (s, 1H), 8.16 (t, *J* = 8.1 Hz, 1H), 7.72 (dd, *J* = 10.9, 1.4 Hz, 1H), 7.55 (br d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.27 – 7.09 (m, 5H), 6.81 (d, *J* = 6.7 Hz, 2H), 4.77 – 4.64 (m, 2H), 3.14 – 3.03 (m, 1H), 2.96 (t, *J* = 11.1 Hz, 1H), 2.78 – 2.70 (m, 1H), 2.12 – 2.02 (m, 1H), 1.93 – 1.84 (m, 1H), 1.81 – 1.73 (m, 1H), 1.71 – 1.45 (m, 3H).

Step 11: (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid

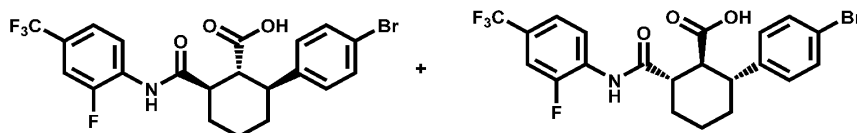


To a mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (0.40 g, 0.69 mmol) in dichloromethane (10 mL) at 0 °C was added BCl₃ (1M in DCM, 1.38 mL, 1.38 mmol). After 3 h the reaction was quenched with cold water (2 mL) and concentrated. The resulting residue was subjected to reverse phase purification (70% MeCN in H₂O, with 10 mM ammonium bicarbonate modifier) to afford (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (0.27 g, 0.51 mmol, 74% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.80 (br s, 1H), 10.13 (s, 1H), 8.21 (t, *J* = 8.0 Hz, 1H), 7.72 (dd, *J* = 10.8, 1.6 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.4 Hz, 2H), 2.97 - 2.95 (m, 1H), 2.78 (t, *J* = 10.8 Hz, 1H), 2.68 - 2.66 (m, 1H), 2.02 - 2.00 (m, 1H), 1.90 - 1.87 (m, 1H), 1.76 - 1.73 (m, 1H), 1.51 - 1.48 (m, 3H). ES-LCMS *m/z* 486.5, 488.5 [M-H]⁻.

Intermediate 6: 1:1 mixture of (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,6*R*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid

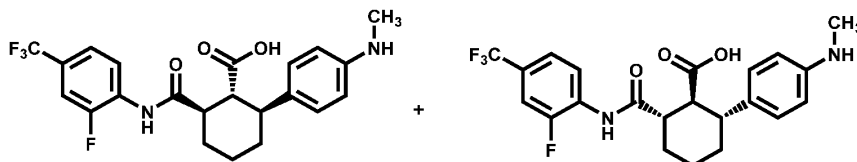


Step 1: 1:1 mixture of (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid



To a 1:1 mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (**Intermediate 1 Step 9**) (896 mg, 1.55 mmol) in dichloromethane (10 mL) at 0 °C was added BCl₃ (1M in DCM, 3.10 mL, 3.10 mmol). After 1 h the reaction was quenched with water and extracted with ethyl acetate. The organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, concentrated, dissolved in DMF (1.5 mL) and purified over silica (40 g C18 column), eluting with 5-100% acetonitrile in water to give 1:1 mixture of (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (340 mg, 0.689 mmol, 44.5% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.76 (s, 1H), 10.09 (s, 1H), 8.20 (t, *J* = 8.0 Hz, 1H), 7.71 (d, *J* = 11.2 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 2.99 (t, *J* = 11.2 Hz, 1H), 2.76 – 2.67 (m, 2H), 2.03 – 2.00 (m, 1H), 1.89 – 1.88 (m, 1H), 1.77 – 1.73 (m, 1H), 1.61 – 1.53 (m, 3H). ES-LCMS *m/z* 488.1 [M+H]⁺.

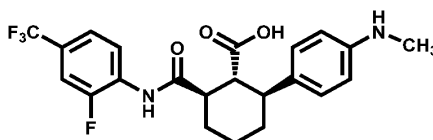
Step 2: 1:1 mixture of (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,6*R*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid



To a degassed solution of a 1:1 mixture of (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,6*S*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (0.339 g, 0.694 mmol) in DMF (3 mL) were added Cs₂CO₃ (679 mg, 2.08 mmol) and *t*-BuBrettPhos Palladacycle Gen. 3 (119 mg, 0.139 mmol). Methanamine (2M in THF, 2.25 mL, 4.50 mmol) was added, and the mixture was heated at 60 °C for 2 h, quenched with water and extracted with ethyl acetate. The organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, concentrated, dissolved in DMF (2 mL) and purified over silica (40 g C18 column), eluting with 5-100% acetonitrile in water to give 1:1 mixture of (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,6*R*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-

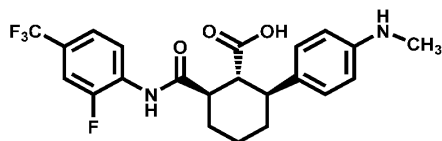
(methylamino)phenyl)cyclohexane-1-carboxylic acid (0.060 g, 0.14 mmol, 51 % yield) as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 11.63 (br, 1H), 10.11 (br, 1H), 8.22 (t, $J = 7.8$ Hz, 1H), 7.71 – 7.68 (m, 1H), 7.54 (d, $J = 8.4$ Hz, 1H), 6.95 (d, $J = 8.4$ Hz, 2H), 6.43 (d, $J = 8.4$ Hz, 2H), 5.39 – 5.37 (m, 1H), 3.00 – 2.93 (m, 1H), 2.70 – 2.67 (m, 2H), 2.64 (d, $J = 4.0$ Hz, 3H), 1.99 – 1.96 (m, 1H), 1.87 – 1.84 (m, 1H), 1.73 – 1.68 (m, 1H), 1.54 – 1.47 (m, 3H). ES-LCMS m/z 439.1 $[\text{M}+\text{H}]^+$.

Intermediate 7: (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid

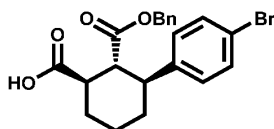


To a degassed solution of (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid **Intermediate 1** (1.50 g, 3.1 mmol) in DMF (30 mL) were added Cs_2CO_3 (3.0 g, 9.2 mmol), methanamine (2M in THF, 30.7 mL, 61.4 mmol) and *t*-BuBrettPhos Palladacycle Gen. 3 (525 mg, 0.614 mmol). The mixture was heated at 60 °C for 2 h, cooled to rt and filtered. The filtrate was concentrated under reduced pressure and the residue was diluted to 100 mL with DMF to give the product as a solution in DMF. Two 9 mL aliquots were removed for subsequent reactions while the remaining 82 mL was concentrated under reduced pressure to yield material that was purified over silica (150 g C18 Gold column), eluting with 30-60% MeCN in water (with 10 nM ammonium bicarbonate and 0.075% ammonium hydroxide) to give (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid (953 mg, 2.17 mmol, 86% yield) as a tan solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.82 - 11.29 (br s, 1H), 10.24 (br s, 1H), 8.21 (t, $J = 8.1$ Hz, 1H), 7.65 (dd, $J = 10.8, 1.5$ Hz, 1H), 7.51 (br d, $J = 8.3$ Hz, 1H), 6.94 (d, $J = 8.8$ Hz, 2H), 6.42 (d, $J = 8.3$ Hz, 2H), 5.61 - 5.14 (m, 1H), 3.06 - 2.82 (m, 1H), 2.69 - 2.41 (m, 2H), 2.63 (s, 3H), 1.93 (br d, $J = 10.3$ Hz, 1H), 1.86 - 1.75 (m, 1H), 1.71 - 1.61 (m, 1H), 1.55 - 1.35 (m, 3H). ES-LCMS m/z 439.2 $[\text{M}+\text{H}]^+$.

Intermediate 7, alternate synthesis: (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclohexane-1-carboxylic acid



Step 1: (1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid



A. Four reactions (850 g x 4) were conducted in parallel:

THF (25.5 L) was added to a mixture of (1*R*)-1-(1-naphthyl)ethanamine (348 g, 2.04 mol) and a 1:1 mixture of (1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,3*R*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid **Intermediate 1 Step 8** (0.850 kg, 2.04 mol) at 25 °C, in one portion. The reaction was heated to 65 °C. After 40 min, the mixture was cooled to 60 °C. After 2 h, the mixture was further cooled to 50 °C, and after 2 h more, the reaction was cooled to 25 °C. After 12 h, the four reactions were combined and filtered, and the filter cake was washed with THF (5.00 L). The filter cake was dried under vacuum at 40 °C to give the undesired salt as a white solid (1.50 kg). The mother liquid (~1.70 kg in mother liquid [25.5 L THF]) was used in the next stage directly.

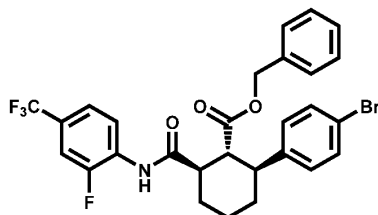
B. Four reactions (425 g x 4) were conducted in parallel.

To the mother liquid from step A (~425 g in mother liquid [6.37 L, THF]) was added (1*S*)-1-(1-naphthyl)ethanamine (174 g, 1.02 mol) at 25 °C in one portion. The mixture was heated to 50 °C for 2 h, then cooled down to 25 °C. After 12 h, the four reactions were combined and filtered, and the filter cake was washed with THF (2.00 L). The filter cake was dried under vacuum at 40 °C to give (1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid, (1*S*)-1-(1-naphthyl)ethanamine salt (1.50 kg, 3.59 mol, 99.6%) as a white solid. This material was divided and used in four reactions (375 g X 4) conducted in parallel.

C. Ethyl acetate (800 mL) was added to the above (1*S*)-1-(1-naphthyl)ethanamine salt (375 g, 899 mmol) at 25 °C in one portion. The pH of the mixture was adjusted to ~1 with 1 M HCl (899 mL, 899 mmol) at 0 ~ 5 °C, and the reaction became clear. The four reactions were combined for work up. The organic layer was separated, and the aqueous phase was extracted with EtOAc (3.00 L X 3). The combined organic layers were washed with H₂O (2.00 L X 3), dried over Na₂SO₄, filtered and concentrated under vacuum at 40 °C to give the crude product as a white solid. This material was triturated with MTBE:EtOAc (3:1, 5 L) at 25 °C for 30 mins, filtered, washed with MTBE (500 mL X 3) and dried under reduced pressure at 40 °C to give (1*R*,2*R*,3*S*)-2-

((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid (1.19 kg, 2.85 mol, 35.0% yield) as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.3 (s, 1H), 7.42 (d, J = 8.4 Hz, 2H), 7.25 - 7.23 (m, 3H), 7.17 (d, J = 8.4 Hz, 2H), 6.83 (d, J = 7.6 Hz, 2H), 4.69 (q, J = 12.8 Hz, 2H), 2.77 - 2.74 (m, 1H), 2.67 - 2.63 (m, 2H), 2.22 - 2.10 (m, 1H), 1.78 - 1.74 (m, 2H), 1.54 - 1.47 (m, 3H). ES-LCMS m/z 439.2 $[\text{M}+\text{Na}]^+$.

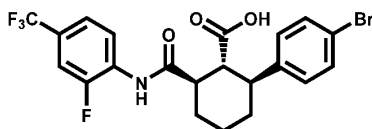
Step 2: benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate



Three reactions (393 g X 3) were conducted in parallel.

To a mixture of (1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)cyclohexane-1-carboxylic acid (393 g, 942 mmol) in acetonitrile (1.70 L) at 0 °C was added 2-fluoro-4-(trifluoromethyl)aniline (337 g, 1.88 mol) and NMI (155 g, 1.88 mol). After ~2 min, TCFH (396 g, 1.41 mmol) was added portionwise. The reaction was stirred at 25 °C for 12 h. The three reactions were combined, concentrated at 40 °C, dissolved in DCM, loaded onto silica gel (100-200 mesh silica gel, self-prepared column chromatography) and eluted with 100:1 to 3:1 EtOAC:petroleum ether to give crude product. This material was triturated with petroleum ether (3.00 L) at 25 °C for 2 h, filtered, washed with petroleum ether (500 mL X 3) and dried under reduced pressure at 40 °C to provide benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (910 g, 1.57 mol, 55.8% yield) as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.1 (s, 1H), 8.15 (t, J = 8.0 Hz, 1H), 7.70 (d, J = 11.2 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.19 - 7.13 (m, 3H), 6.80 (d, J = 6.8 Hz, 2H), 4.68 (q, J = 12.4 Hz, 1H), 3.09 - 3.04 (m, 1H), 2.98 - 2.92 (m, 1H), 2.75 - 2.69 (m, 1H), 2.07 - 2.04 (m, 1H), 1.89 - 1.87 (m, 1H), 1.77 - 1.74 (m, 1H), 1.66 - 1.52 (m, 3H).

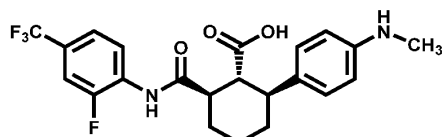
Step 3: (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid



Three reactions (217 g X 3) were conducted in parallel.

To a mixture of benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (217 g, 375 mmol) in DCM (1.20 L) at -30 °C was added BBr₃ (188 g, 0.750 mol). After 1 h the three reactions were combined, cooled to 0 °C, quenched with water (3.00 L) and extracted with EtOAc (2.40 L X 3). The combined organic layers were washed with brine (2.00 L), dried over Na₂SO₄, filtered and concentrated under reduced pressure at 40 °C to give crude residue. The crude product was triturated with 5:1 PE:EA (4.80 L) at 25 °C for 2 h, filtered, washed with PE (500 mL X 3) and dried under reduced pressure at 40 °C to provide (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (0.610 kg, 1.25 mol, 84.9% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.8 (s, 1H), 10.0 (s, 1H), 8.21 (t, *J* = 8.0 Hz, 1H), 7.70 (dd, *J* = 10.8, 1.6 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 3.03 – 2.97 (m, 1H), 2.82 – 2.76 (m, 1H), 2.71 – 2.65 (m, 1H), 2.02 – 2.00 (m, 1H), 1.87 (m, 1H), 1.76 – 1.73 (m, 1H), 1.59 – 1.47 (m, 3H).

Step 4: (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclohexane-1-carboxylic acid

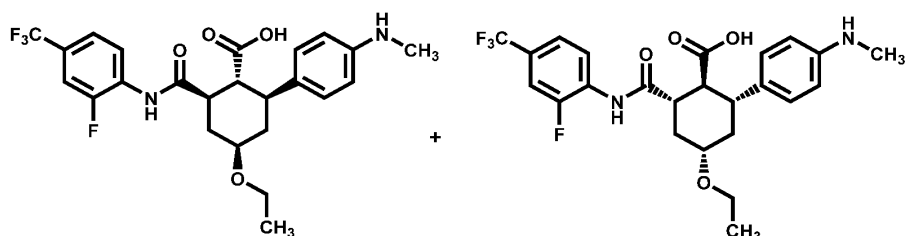


Two reactions (100 g x 2) were conducted in parallel.

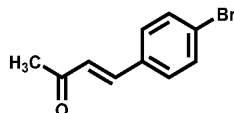
To a mixture of (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (0.100 kg, 204 mmol) in DMF (600 mL) at 20 °C were added Cs₂CO₃ (0.200 kg, 614 mmol), *t*-BuBrettPhos Palladacycle Gen. 3 (35.0 g, 40.9 mmol) and methanamine (426 g, 4.12 mol). The reaction was heated to 60 °C for 2 h. The two reactions were combined, cooled to 20 °C, poured into water (2.40 L) and extracted with MTBE (800 mL X 3). The aqueous phase was adjusted to pH = 6 with 1 M HCl (1.20 L) and extracted with 2-Me-THF (1.50 L X 3). The combined organic layers were washed with brine (1.00 L X 3), dried over Na₂SO₄, filtered and concentrated under reduced pressure at 30 °C to give crude residue. The crude product was triturated with 1:1 EtOAc: MTBE (600 ml) at 20 °C for 30

min, filtered, washed with PE (500 mL X 3) and dried under reduced pressure at 40 °C to give (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclohexane-1-carboxylic acid (0.120 kg, 273 mmol, 66.8% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.6 (s, 1H), 10.0 (s, 1H), 8.21 (t, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 9.6 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 6.42 (d, *J* = 8.4 Hz, 2H), 5.42 (s, 1H), 2.99 - 2.93 (m, 1H), 2.71 - 2.62 (m, 4H), 2.54 - 2.50 (m, 1H), 1.98 - 1.96 (m, 1H), 1.85 - 1.853 (m, 1H), 1.71 - 1.68 (m, 1H), 1.51 - 1.46 (m, 3H).

Intermediate 8: 1:1 mixture of (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,4*S*,6*R*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid

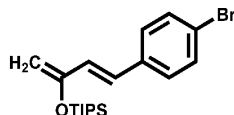


Step 1: (*E*)-4-(4-bromophenyl)but-3-en-2-one



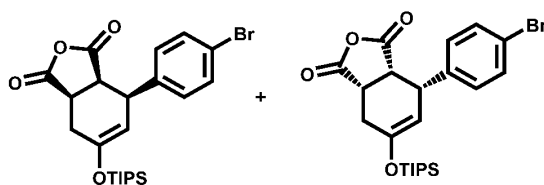
Three reactions were carried out in parallel (1.35 kg x 3): To a mixture of 4-bromobenzaldehyde (1.35 kg, 7.30 mol), acetone (5.4 L) and H₂O (10.8 L) was added NaOH (135 g, 3.37 mol), slowly via a dropping funnel at 15-20 °C. The reaction mixture was stirred at 15-20 °C for 12 h. The three batches were combined, and the reaction mixture was filtered. The filter cake was washed water (1.00 L) and concentrated *in vacuo* at 45 °C to give compound (*E*)-4-(4-bromophenyl)but-3-en-2-one (4.60 kg, 20.0 mol, 89.0% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.68 - 7.57 (m, 5H), 6.83 (d, *J* = 16 Hz, 1H), 2.33 (s, 3H). ES-LCMS *m/z* 224.9 [M+H]⁺.

Step 2: (*E*)-((4-(4-bromophenyl)buta-1,3-dien-2-yl)oxy)triisopropylsilane



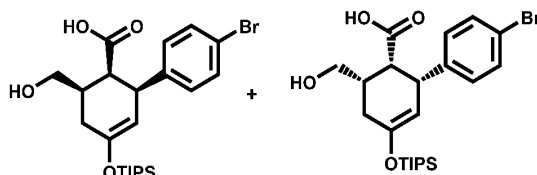
Ten reactions were carried out in parallel (340 g x 10): a mixture of (*E*)-4-(4-bromophenyl)but-3-en-2-one (0.34 kg, 1.51 mol) in 2-methyltetrahydrofuran (2.04 L) was degassed and purged with N₂ 3 times. The reaction was cooled to -20 °C, and TIPSOTf (451 mL, 1.66 mol) and Et₃N (421 mL, 3.02 mol) were added dropwise. The mixture was stirred at 0 °C for 2 h and the reaction was complete. The reactions were combined, poured to ice water (15.0 L) and partitioned between 2-Me-THF and water. The organic layers were dried over MgSO₄ for 12 h, filtered and concentrated below 30 °C *in vacuo* to give (*E*)-((4-(4-bromophenyl)buta-1,3-dien-2-yl)oxy)triisopropylsilane (5.75 kg, 14.8 mol, quantitative yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.51 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 6.83 (d, *J* = 16 Hz, 1H), 6.76 (d, *J* = 17 Hz, 1H), 4.54 (s, 1H), 4.42 (s, 1H), 1.28 - 1.33 (m, 3 H), 1.13 - 1.08 (m, 18H). ES-LCMS *m/z* 381.1 [M+H]⁺.

Step 3: 1:1 mixture of (3a*S*,4*S*,7a*R*)-4-(4-bromophenyl)-6-((triisopropylsilyl)oxy)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione and (3a*R*,4*R*,7a*S*)-4-(4-bromophenyl)-6-((triisopropylsilyl)oxy)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione



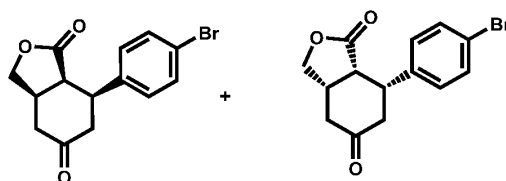
Eighteen reaction were carried in parallel (300 g x 18): To a mixture of furan-2,5-dione (390 g, 3.9 mol) in DCM (2.0 L) was added a solution of (*E*)-((4-(4-bromophenyl)buta-1,3-dien-2-yl)oxy)triisopropylsilane (300 g, 790 mmol) in DCM (1.0 L) slowly over 15 mins at 15 °C. Then the mixture was stirred at 25 °C for 3.5 h. The reactions were combined and concentrated *in vacuo* at 30 °C and triturated with *n*-heptane (5.00 L). The resulting solid product was collected by filtration and washed with *n*-heptane (1.00 L). The filtrate was then concentrated *in vacuo* at 30 °C, and then triturated with *n*-heptane, filtered, and washed with *n*-heptane to recover additional product. The recovery process was repeated 3 more times and the products were combined to provide a 1:1 mixture of (3a*S*,4*S*,7a*R*)-4-(4-bromophenyl)-6-((triisopropylsilyl)oxy)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione and (3a*R*,4*R*,7a*S*)-4-(4-bromophenyl)-6-((triisopropylsilyl)oxy)-3a,4,7,7a-tetrahydroisobenzofuran-1,3-dione (5.60 kg, 8.25 mol, crude) as yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.52 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 5.07 - 5.05 (m, 1 H), 3.92 - 3.80 (m, 3H), 2.66 - 2.53 (m, 2H), 1.08 - 1.03 (m, 21H).

Step 4: 1:1 mixture of (1*S*,2*S*,3*R*)-4'-bromo-3-(hydroxymethyl)-5-((triisopropylsilyl)oxy)-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-carboxylic acid and (1*R*,2*R*,3*S*)-4'-bromo-3-(hydroxymethyl)-5-((triisopropylsilyl)oxy)-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-carboxylic acid



A 1:1 mixture of (3*aS*,4*S*,7*aR*)-4-(4-bromophenyl)-6-((triisopropylsilyl)oxy)-3*a*,4,7,7*a*-tetrahydroisobenzofuran-1,3-dione and (3*aR*,4*R*,7*aS*)-4-(4-bromophenyl)-6-((triisopropylsilyl)oxy)-3*a*,4,7,7*a*-tetrahydroisobenzofuran-1,3-dione (5.50 kg, 11.5 mol) in THF (36.0 L) was degassed and purged with N₂ 3 times. The reaction was cooled to 0 °C, and NaBH₄ (521 g, 13.8 mol) was added to the mixture, in portions, at 0~3 °C. The mixture was stirred at 20 °C for 1 h, and water (962 mL) was added slowly to the reaction at 0-5 °C over 10 min. HCl (1 N) was added to the mixture until pH = 7. The reaction was stirred at 5-10 °C for 20 min, and anhydrous Na₂SO₄ (1.00 kg) was added. The mixture was filtered and concentrated under reduced pressure to give a 1:1 mixture of (1*S*,2*S*,3*R*)-4'-bromo-3-(hydroxymethyl)-5-((triisopropylsilyl)oxy)-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-carboxylic acid and (1*R*,2*R*,3*S*)-4'-bromo-3-(hydroxymethyl)-5-((triisopropylsilyl)oxy)-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-carboxylic acid (3.60 kg, crude) as a white solid, which was used without purification. ES-LCMS *m/z* 483.2 [M+H]⁺.

Step 5: 1:1 mixture of (3*aR*,7*S*,7*aS*)-7-(4-bromophenyl)tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione and (3*aS*,7*R*,7*aR*)-7-(4-bromophenyl)tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione



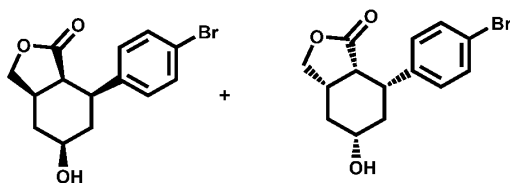
Ten reactions were carried out in parallel (220 g x 10): A 1:1 mixture of (1*S*,2*S*,3*R*)-4'-bromo-3-(hydroxymethyl)-5-((triisopropylsilyl)oxy)-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-carboxylic acid and (1*R*,2*R*,3*S*)-4'-bromo-3-(hydroxymethyl)-5-((triisopropylsilyl)oxy)-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-carboxylic acid (220 g, 4.55 mol) in toluene (1.32 L) was degassed and purged with N₂ 3 times. TsOH (8.70 g, 0.455 mol) was added to the mixture at 20 °C, and the reaction was stirred at 110 °C for 12 h. The mixture was poured into H₂O (1.2 L) at 0 °C and the aqueous layer

was extracted with ethyl acetate (300 mL x 3). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution (150 mL) and brine (150 mL) and dried over sodium sulfate, filtered and concentrated under reduced pressure at 40 °C to provide a crude product.

In a separate reaction, a 1:1 mixture of (1*S*,2*S*,3*R*)-4'-bromo-3-(hydroxymethyl)-5-((triisopropylsilyl)oxy)-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-carboxylic acid and (1*R*,2*R*,3*S*)-4'-bromo-3-(hydroxymethyl)-5-((triisopropylsilyl)oxy)-1,2,3,4-tetrahydro-[1,1'-biphenyl]-2-carboxylic acid (400 g, 827 mmol) in toluene (2.4 L) was degassed and purged with N₂ 3 times. TsOH (15.7 g, 83.0 mmol) was added to the mixture at 20 °C, and the reaction was stirred at 110 °C for 13 h. The mixture was poured into H₂O (500 mL) at 0 °C and the aqueous layer was extracted with dichloromethane (500 mL x 2). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution (500 mL) and brine (500 mL) and dried over sodium sulfate, filtered and concentrated under reduced pressure to provide a crude product.

The two crude products were combined and triturated with isopropyl ether (700 mL) at 20 °C for 30 min. The mixture was filtered, washed with isopropyl ether (100 mL) and dried under reduced pressure to provide a 1:1 mixture of (3*aR*,7*S*,7*aS*)-7-(4-bromophenyl)tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione and (3*aS*,7*R*,7*aR*)-7-(4-bromophenyl)tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione (550 g, 1.78 mol, 33% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.51 - 7.49 (m, 2H), 7.29 - 7.26 (m, 2H), 4.36 - 4.32 (dd, *J* = 5.6 Hz, *J* = 8.8 Hz, 1H), 3.91 - 3.88 (dd, *J* = 2.0 Hz, *J* = 9.2 Hz, 1H), 3.69 - 3.63 (dt, *J* = 4.4 Hz, *J* = 14.0 Hz, 1H), 3.49 - 3.43 (m, 1H), 3.19 - 3.11 (m, 1H), 2.73 - 2.64 (m, 2H), 2.48 - 2.44 (m, 1H), 2.37 - 2.30 (m, 1H). ES-LCMS *m/z* 310.1 [M+H]⁺.

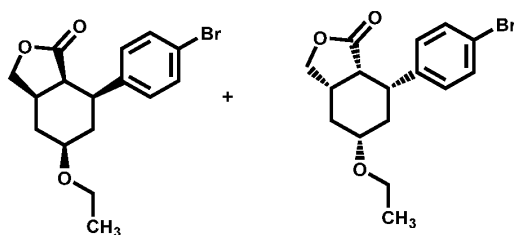
Step 6: 1:1 mixture of (3*aR*,5*R*,7*S*,7*aS*)-7-(4-bromophenyl)-5-hydroxyhexahydroisobenzofuran-1(3*H*)-one and (3*aS*,5*S*,7*R*,7*aR*)-7-(4-bromophenyl)-5-hydroxyhexahydroisobenzofuran-1(3*H*)-one



To a 1:1 mixture of (3*aR*,7*S*,7*aS*)-7-(4-bromophenyl)tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione and (3*aS*,7*R*,7*aR*)-7-(4-bromophenyl)tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione (11.0 g, 35.6 mmol), in THF (100 mL) and methanol (100 mL) at 0 °C was added solid sodium borohydride (3.37 g, 89.0 mmol) portion wise over 5 min. The reaction was stirred at rt for 16 h, quenched with water

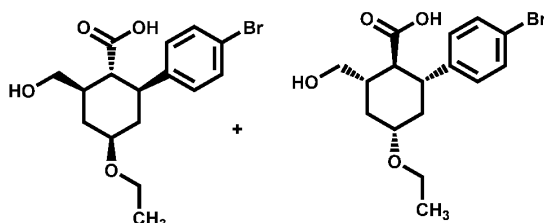
(100 mL) and extracted with EtOAc (500 mL). The EtOAc layer was washed with water (100 mL) and brine (100 mL), dried over sodium sulphate and concentrated to afford a 1:1 mixture of (3a*R*,5*R*,7*S*,7a*S*)-7-(4-bromophenyl)-5-hydroxyhexahydroisobenzofuran-1(3*H*)-one and (3a*S*,5*S*,7*R*,7a*R*)-7-(4-bromophenyl)-5-hydroxyhexahydroisobenzofuran-1(3*H*)-one (7.00 g, 21.6 mmol, 60.7 % yield) as a white solid, which was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.47 (d, *J* = 4 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 4.86 (d, *J* = 4.4 Hz, 1H), 4.20-4.16 (m, 1H), 3.86 (d, *J* = 8.4 Hz, 1H), 3.56-3.55 (m, 1H), 3.21-3.18 (m, 1H), 3.11-3.07 (m, 1H), 2.67-2.64 (m, 1H), 1.99-2.02 (m, 2H), 1.39 (q, *J* = 12.8 Hz, 1H), 1.00 (q, *J* = 12.8 Hz, 1H). ES-LCMS *m/z* 311.1 [M+H]⁺.

Step 7: 1:1 mixture of (3a*R*,5*R*,7*S*,7a*S*)-7-(4-bromophenyl)-5-ethoxyhexahydroisobenzofuran-1(3*H*)-one and (3a*S*,5*S*,7*R*,7a*R*)-7-(4-bromophenyl)-5-ethoxyhexahydroisobenzofuran-1(3*H*)-one



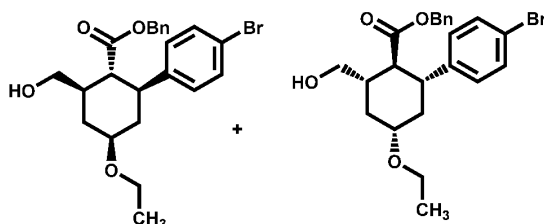
To a 1:1 mixture of (3a*R*,5*R*,7*S*,7a*S*)-7-(4-bromophenyl)-5-hydroxyhexahydroisobenzofuran-1(3*H*)-one and (3a*S*,5*S*,7*R*,7a*R*)-7-(4-bromophenyl)-5-hydroxyhexahydroisobenzofuran-1(3*H*)-one (5.00 g, 16.1 mmol), in *N,N*-dimethylformamide (50 mL) at 0°C was added solid NaH (1.29 g, 32.1 mmol). After 30 min, ethyl iodide (13.0 mL, 161 mmol) was added, and the reaction was stirred at rt for 16 h. The reaction was quenched with 2N HCl (~10 mL) to pH < 3 and extracted with EtOAc (100 mL, 3 times). The combined EtOAc layers were washed with water (20 mL) and brine (20 mL), dried over sodium sulphate and concentrated. The resulting residue was taken up in THF (20 mL) and subjected to reverse phase purification (MeCN in H₂O, 10 mM ammonium bicarbonate modifier, 0-100% gradient) to afford a 1:1 mixture of (3a*R*,5*R*,7*S*,7a*S*)-7-(4-bromophenyl)-5-ethoxyhexahydroisobenzofuran-1(3*H*)-one and (3a*S*,5*S*,7*R*,7a*R*)-7-(4-bromophenyl)-5-ethoxyhexahydroisobenzofuran-1(3*H*)-one (1.70 g, 4.56 mmol, 28.4 % yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.51 - 7.41 (m, 2H), 7.32 - 7.20 (m, 2H), 4.19 (dd, *J* = 8.8, 4.3 Hz, 1H), 3.95 - 3.83 (m, 1H), 3.57 - 3.42 (m, 3H), 3.27 - 3.07 (m, 2H), 2.75 - 2.63 (m, 1H), 2.17 (dd, *J* = 12.3, 4.3 Hz, 2H), 1.44 - 1.21 (m, 2H), 1.14 - 1.06 (m, 3H). ES-LCMS *m/z* 341.0 [M+H]⁺.

Step 8: 1:1 mixture of (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid



To a 1:1 mixture of (3*aR*,5*R*,7*S*,7*aS*)-7-(4-bromophenyl)-5-ethoxyhexahydroisobenzofuran-1(3*H*)-one and (3*aS*,5*S*,7*R*,7*aR*)-7-(4-bromophenyl)-5-ethoxyhexahydroisobenzofuran-1(3*H*)-one (2.70 g, 7.96 mmol) in methanol (30 mL) was added KOH (2.23 g, 39.8 mmol), and the reaction mixture was stirred at 75 °C for 16 h. The reaction was quenched with 2N HCl (~15 mL) to pH < 3, and solid precipitated out. This solid was filtered, washed with water (5 mL, 4 times), washed with petroleum ether (5 mL, 4 times) and dried to afford a 1:1 mixture of (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid (2.40 g, 5.71 mmol, 71.7% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.85 (br s, 1H), 7.46 - 7.42 (m, 2H), 7.22 - 7.14 (m, 2H), 4.58 (br s, 1H), 3.55 - 3.38 (m, 4H), 3.23 - 3.12 (m, 1H), 2.83 - 2.68 (m, 1H), 2.28 - 2.17 (m, 2H), 1.98 (d, *J* = 12.5 Hz, 1H), 1.80 - 1.68 (m, 1H), 1.47 - 1.24 (m, 1H), 1.15 - 1.00 (m, 4H). ES-LCMS *m/z* 357.0 [M+H]⁺.

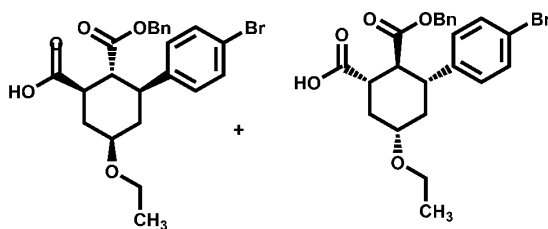
Step 9: 1:1 mixture of benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylate



To a 1:1 mixture of (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid (2.40 g, 6.72 mmol) and potassium carbonate

(2.79 g, 20.2 mmol) in *N,N*-dimethylformamide (25 mL) at 0°C was added benzyl bromide (0.799 mL, 6.72 mmol), dropwise over 1 min. After 3 h, ice water (20 mL) was added, and the reaction was extracted with EtOAc (50 mL X 3). The combined EtOAc layers were washed with water (10 mL) and brine (20 mL), dried over sodium sulphate and evaporated to afford crude material. The crude material was subjected to normal phase purification (ethyl acetate in petroleum ether, 0-100% gradient, 50 min run) to afford a 1:1 mixture of benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylate (2.30 g, 4.63 mmol, 68.9% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.47 - 7.37 (m, 2H), 7.29 - 7.22 (m, 3H), 7.19 - 7.11 (m, 2H), 6.86 (dd, *J* = 7.5, 2.0 Hz, 2H), 4.87 (d, *J* = 12.5 Hz, 1H), 4.70 (d, *J* = 12.5 Hz, 1H), 4.60 (t, *J* = 5.0 Hz, 1H), 3.49 - 3.41 (m, 3H), 3.35 - 3.32 (m, 1H), 3.31 - 3.19 (m, 2H), 2.89 - 2.74 (m, 1H), 2.42 (t, *J* = 11.0 Hz, 1H), 2.21 - 1.95 (m, 2H), 1.90 - 1.77 (m, 1H), 1.42 (q, *J* = 12.0 Hz, 1H), 1.07 (t, *J* = 6.8 Hz, 3H). ES-LCMS *m/z* showed poor ionization.

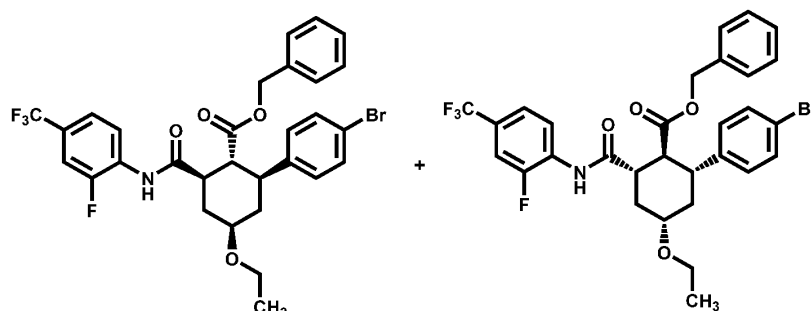
Step 10: 1:1 mixture of (1*R*,2*R*,3*S*,5*R*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-ethoxycyclohexane-1-carboxylic acid and (1*S*,2*S*,3*R*,5*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-ethoxycyclohexane-1-carboxylic acid



To a 1:1 mixture of benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-(hydroxymethyl)cyclohexane-1-carboxylate (2.30 g, 5.14 mmol) in acetonitrile (10 mL) and water (20 mL) at 0 °C was added sodium periodate (3.30 g, 15.4 mmol) and ruthenium(III) chloride (1.07 g, 5.14 mmol). After 1 h, water (100 mL) was added, and the mixture was extracted with EtOAc (500 mL). The EtOAc layer was washed with water (100 mL) and brine (100 mL), dried over sodium sulphate and evaporated concentrated to afford a 1:1 mixture of (1*R*,2*R*,3*S*,5*R*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-ethoxycyclohexane-1-carboxylic acid and (1*S*,2*S*,3*R*,5*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-ethoxycyclohexane-1-carboxylic acid (1.60 g, 2.95 mmol, 57.3% yield) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.54 (br s, 1H), 7.53 - 7.36 (m, 2H), 7.31 - 7.14 (m, 5H), 6.92 - 6.79 (m, 2H), 4.81 - 4.62 (m,

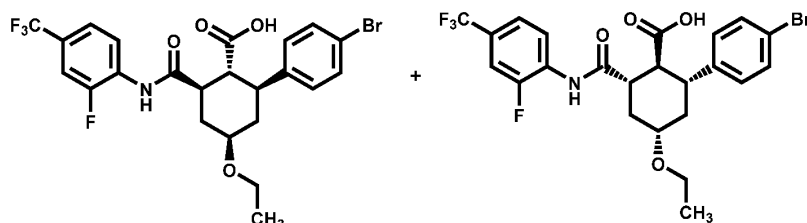
2H), 3.57 - 3.43 (m, 2H), 3.24 - 3.05 (m, 1H), 2.96 - 2.83 (m, 1H), 2.79 - 2.70 (m, 2H), 2.43 - 2.18 (m, 1H), 1.99 (dd, $J = 6.8, 5.8$ Hz, 1H), 1.62 - 1.49 (m, 1H), 1.45 - 1.30 (m, 1H), 1.08 (t, $J = 7.0$ Hz, 3H). ES-LCMS m/z 461.0 $[M+H]^+$.

Step 11: 1:1 mixture of benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate



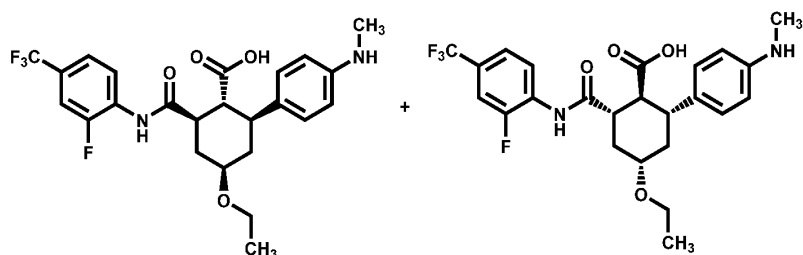
To a 1:1 mixture of (1*R*,2*R*,3*S*,5*R*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-ethoxycyclohexane-1-carboxylic acid and (1*S*,2*S*,3*R*,5*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-ethoxycyclohexane-1-carboxylic acid (1.50 g, 3.25 mmol) in acetonitrile (10 mL) was added 1-methyl-1*H*-imidazole (0.518 mL, 6.50 mmol) and 2-fluoro-4-(trifluoromethyl)aniline (582 mg, 3.25 mmol), sequentially, followed by chloro-*N,N,N',N'*-tetramethylformamidine hexafluorophosphate (1.83 g, 6.50 mmol). After 2 h, the mixture was concentrated and subjected to normal phase purification (ethyl acetate in petroleum ether, 0-80% gradient) to afford a 1:1 mixture of benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (1.20 g, 1.79 mmol, 55.1% yield) as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.17 (s, 1H), 8.17 (t, $J = 8.0$ Hz, 1H), 7.73 (dd, $J = 11.0, 1.5$ Hz, 1H), 7.56 (d, $J = 9.0$ Hz, 1H), 7.46 (d, $J = 7.7$ Hz, 2H), 7.27 - 7.14 (m, 5H), 6.81 (s, 1H), 6.79 (d, $J = 1.5$ Hz, 1H), 4.74 - 4.64 (m, 2H), 3.58 - 3.46 (m, 3H), 3.21 - 3.05 (m, 1H), 2.96 (t, $J = 11.3$ Hz, 1H), 2.82 (td, $J = 12.0, 3.0$ Hz, 1H), 2.39 - 2.34 (m, 1H), 2.12 - 1.97 (m, 1H), 1.69 - 1.53 (m, 1H), 1.52 - 1.40 (m, 1H), 1.10 (t, $J = 7.0$ Hz, 3H). ES-LCMS m/z 622.0 $[M+H]^+$.

Step 12: 1:1 mixture of (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid



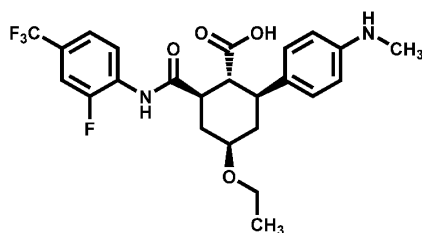
To 1:1 mixture of benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate and benzyl (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylate (0.600 g, 0.964 mmol) in DCM (12 mL) at °C was added a solution of boron trichloride (1.93 mL, 1.93 mmol, 1M in DCM). After 1 h, ice water (2 mL) was added, and the mixture was concentrated. The resulting residue was taken up in THF (5 mL) and subjected to reverse phase purification (MeCN in H₂O, 0.1% ammonium bicarbonate modifier, 0-100% gradient) to afford a 1:1 mixture of (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (350 mg, 0.638 mmol, 66.2% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.27 (br s, 1H), 8.23 (t, *J* = 8.0 Hz, 1H), 7.71 (s, 1H), 7.68 (s, 1H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 3.54 - 3.47 (m, 2H), 3.02 - 2.95 (m, 1H), 2.84 - 2.72 (m, 1H), 2.39 - 2.24 (m, 2H), 2.13 - 1.91 (m, 2H), 1.56 - 1.33 (m, 2H), 1.09 (t, *J* = 7.0 Hz, 3H). ES-LCMS *m/z* 532.0 [M+H]⁺.

Step 13: 1:1 mixture of (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,4*S*,6*R*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid



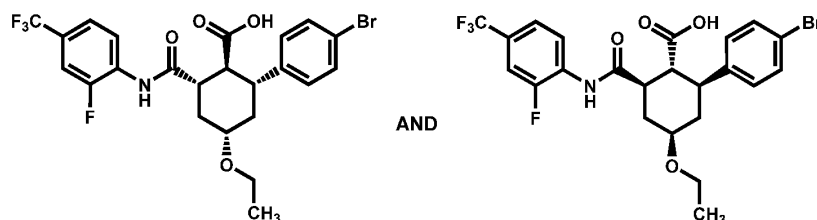
A 1:1 mixture of (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (550 mg, 1.03 mmol) and sodium *tert*-butoxide (298 mg, 3.10 mmol) in 1,4-dioxane (5 mL) was stirred for 5 min. *t*BuXPhos Pd G3 (82.0 mg, 0.103 mmol) was added, and the mixture was degassed for 5 min. Methanamine (2M in THF, 10.3 mL, 20.7 mmol) was added, and the reaction was heated in a Biotage Initiator (microwave) at 100 °C for 1h. The mixture was concentrated, and the resulting residue was subjected to reverse phase purification (MeCN in H₂O, 10 nM ammonium bicarbonate modifier, 0-100% gradient) to afford a 1:1 mixture of (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,4*S*,6*R*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid (305 mg, 0.601 mmol, 58.1% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.70 (br s, 1H), 10.10 (br s, 1H), 8.21 (t, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 9.5 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 6.99 - 6.95 (m, 2H), 6.46 - 6.42 (m, 2H), 5.50 - 5.36 (m, 1H), 3.54 - 3.47 (m, 3H), 3.32 - 3.27 (m, 1H), 3.06 - 2.98 (m, 1H), 2.71 - 2.66 (m, 1H), 2.63 (s, 3H), 2.33 - 2.25 (m, 1H), 2.06 - 1.94 (m, 1H), 1.51 - 1.32 (m, 2H), 1.10 (t, *J* = 7.0 Hz, 3H). ES-LCMS *m/z* 483.2 [M+H]⁺.

Intermediate 9: (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid



Step 1: (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid AND (1*R*,2*S*,4*R*,6*R*)-2-(4-

bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid



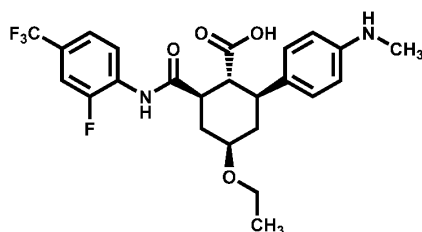
A 1:1 mixture of (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid and (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (**Intermediate 8 Step 12**) (4.50 g, 8.45 mmol) was chirally purified by Chiral-Prep-SFC (Column: YMC Cellulose-SA 250 X 30 mm, 5µm; Mobile Phase: 75:25 CO₂: [MeOH with 0.5% isopropylamine]) to give:

First-eluting isomer (1*S*,2*R*,4*S*,6*S*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (1.20 g, 2.05 mmol, 24.3 % yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.27 (br s, 1H), 8.29 (t, *J* = 8.3 Hz, 1H), 7.73 - 7.64 (m, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 3.50 - 3.47 (m, 2H), 3.09 - 3.02 (m, 1H), 2.98 - 2.90 (m, 1H), 2.81 - 2.71 (m, 1H), 2.52 (br d, *J* = 2.0 Hz, 1H), 2.24 (d, *J* = 12.5 Hz, 1H), 2.04 - 1.94 (m, 1H), 1.40 (qd, *J* = 12.0, 7.0 Hz, 2H), 1.08 (t, *J* = 7.0 Hz, 3H). ES-LCMS *m/z* 532.0 [M+H]⁺.

Second-eluting isomer (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (1.60 g, 2.92 mmol, 34.5 % yield) ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.35 (br s, 1H), 8.28 (t, *J* = 8.0 Hz, 1H), 7.68 (dd, *J* = 11.0, 1.5 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 3.51 - 3.46 (m, 2H), 3.04 (dt, *J* = 12.6, 6.4 Hz, 1H), 2.98 - 2.89 (m, 1H), 2.81 - 2.72 (m, 1H), 2.57 - 2.53 (m, 1H), 2.25 (d, *J* = 11.5 Hz, 1H), 2.02 - 1.95 (m, 1H), 1.47 - 1.34 (m, 2H), 1.09 (t, *J* = 7.0 Hz, 3H). ES-LCMS *m/z* 532.0 [M+H]⁺.

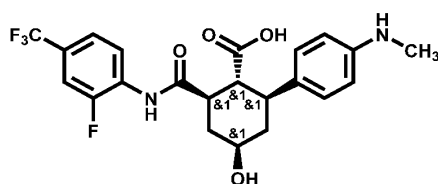
Absolute stereochemistry was determined by co-crystal structure of subsequent final compound with WRN protein and then tracing back to the corresponding enantiopure intermediates.

Step 2: (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid

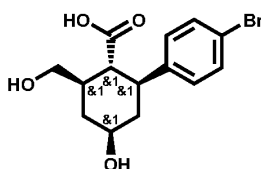


A mixture of (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-ethoxy-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)cyclohexane-1-carboxylic acid (450 mg, 0.845 mmol) and sodium *tert*-butoxide (244 mg, 2.54 mmol) in 1,4-dioxane (4.5 mL) was stirred for 5 min. *t*BuXPhos Pd G3 (67 mg, 0.085 mmol) was added, and the mixture was degassed for 5 min. Methanamine (2M in THF, 8.45 mL, 16.9 mmol) was added, and the reaction was heated in a Biotage Initiator (microwave) at 100 °C for 1h. The mixture was concentrated, and the resulting residue was subjected to reverse phase purification (MeCN in H₂O, 10 nM ammonium bicarbonate modifier, 10-100% gradient) to afford (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid (360 mg, 0.709 mmol, 84.0% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.55 (br s, 1H), 8.29 (t, *J* = 8.0 Hz, 1H), 7.65 (dd, *J* = 11.0, 1.5 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.39 (d, *J* = 8.5 Hz, 2H), 5.32 (br s, 1H), 3.47 (qd, *J* = 7.0, 1.5 Hz, 2H), 3.42 - 3.37 (m, 1H), 2.84 (t, *J* = 10.8 Hz, 1H), 2.69 - 2.58 (m, 4H), 2.44 - 2.35 (m, 1H), 2.23 (d, *J* = 12.5 Hz, 1H), 1.92 (d, *J* = 12.0 Hz, 1H), 1.37 - 1.25 (m, 2H), 1.08 (t, *J* = 7.0 Hz, 3H). ES-LCMS *m/z* 483.2 [M+H]⁺.

Intermediate 11: rac-(1*R*,2*R*,4*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-hydroxy-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid

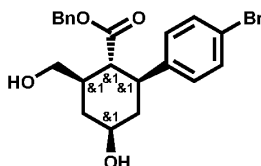


Step 1: rac-(1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-hydroxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid



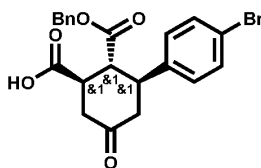
To 1:1 mixture of (3a*R*,5*R*,7*S*,7a*S*)-7-(4-bromophenyl)-5-hydroxyhexahydroisobenzofuran-1(3*H*)-one and (3a*S*,5*S*,7*R*,7a*R*)-7-(4-bromophenyl)-5-hydroxyhexahydroisobenzofuran-1(3*H*)-one **Intermediate 8 Step 6** (3.20 g, 10.3 mmol) was added KOH (2.88 g, 51.4 mmol). The reaction was stirred at 75 °C for 3 h, quenched with 2N HCl (~20 mL) to pH < 3, concentrated then purified by reverse phase chromatography (MeCN in H₂O, 0.1% ammonium bicarbonate modifier, 0-100% gradient) to afford rac-(1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-hydroxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid (2.30 g, 6.92 mmol, 67.0% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.41 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 3.58 - 3.53 (m, 1H), 3.41 - 3.38 (m, 1H), 3.24 - 3.21 (m, 1H), 2.78 - 2.71 (m, 1H), 2.15 - 2.10 (m, 1H), 2.05 (d, *J* = 12.4 Hz, 1H), 1.83 (d, *J* = 12.0 Hz, 1H), 1.73 - 1.68 (m, 1H), 1.29 (q, *J* = 12.4 Hz, 1H), 1.05 (q, *J* = 12.4 Hz, 1H), two protons obscured by solvent peak. ES-LCMS *m/z* 329.0 [M+H]⁺.

Step 2: rac-benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-hydroxy-6-(hydroxymethyl) cyclohexane-1-carboxylate



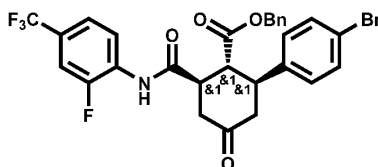
To a mixture of rac-(1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-hydroxy-6-(hydroxymethyl)cyclohexane-1-carboxylic acid (2.30 g, 6.99 mmol) and potassium carbonate (2.90 g, 20.9 mmol) in *N,N*-dimethylformamide (20 mL) at 0 °C was added benzyl bromide (0.83 mL, 7.0 mmol), dropwise over 1 min. The reaction was stirred at rt for 3 h, diluted with ice water (30 mL) and extracted with ethyl acetate (2 X 50 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried over sodium sulphate, concentrated and purified by reverse phase chromatography (MeCN in H₂O, 0.1% ammonium bicarbonate modifier, 0-100% gradient) to afford rac-benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-hydroxy-6-(hydroxymethyl) cyclohexane-1-carboxylate (2.10 g, 4.51 mmol, 65.0% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.96 (s, 1H), 7.45 - 7.41 (m, 2H), 7.27 - 7.22 (m, 3H), 7.16 - 7.12 (m, 2H), 6.89 - 6.85 (m, 2H), 4.88 (d, *J* = 12.8 Hz, 1H), 4.74 - 4.69 (m, 1H), 4.59 - 4.57 (m, 1H), 3.61 (s, 1H), 3.32 - 3.20 (m, 1H), 2.90 (s, 2H), 2.79 - 2.74 (m, 1H), 2.38 (t, *J* = 11.2 Hz, 1H), 2.04 - 1.09 (m, 4H). ES-LCMS *m/z*: poor ionization in MS.

Step 3 : rac-(1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-oxocyclohexane-1-carboxylic acid



To a mixture of rac-benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-4-hydroxy-6-(hydroxymethyl)cyclohexane-1-carboxylate (2.10 g, 5.01 mmol) in acetonitrile (31 mL) and water (15 mL) at -20 °C were added sodium periodate (3.21 g, 15.0 mmol) and ruthenium(III) chloride (1.04 g, 5.01 mmol). After 1h, the reaction was diluted with water (35 mL) and extracted with ethylacetate (4 X 50 mL). The combined organic layers were washed with water (20 mL) and brine (25 mL), dried over sodium sulphate, concentrated then purified by reverse phase chromatography (MeCN in H₂O, 0.1% ammonium bicarbonate modifier, 0-100% gradient) to afford rac-(1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-oxocyclohexane-1-carboxylic acid (1.1 g, 2.4 mmol, 49% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.48 (d, *J* = 1.6 Hz, 2H), 7.28 - 7.23 (m, 5H), 6.88 (dd, *J* = 6.6, 4.0 Hz, 2H), 4.77 (d, *J* = 12.4 Hz, 1H), 4.69 (d, *J* = 12.8 Hz, 1H), 3.25 (t, *J* = 11.2 Hz, 1H), 3.16 - 2.84 (m, 4H), 2.68 (t, *J* = 13.6 Hz, 1H), 2.55 - 2.53 (m, 1H), 2.26 - 2.21 (m, 1H). ES-LCMS *m/z* 429.0 [M-H]⁺.

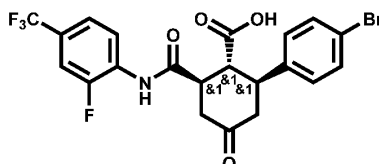
Step 4: rac-benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxocyclohexane-1-carboxylate



To a suspension of rac-(1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-oxocyclohexane-1-carboxylic acid (0.30 g, 0.69 mmol) in acetonitrile (1.5 mL) were added 1-methyl-1*H*-imidazole (0.111 mL, 1.39 mmol) and 2-fluoro-4-(trifluoromethyl)aniline (125 mg, 0.690 mmol) followed by chloro-*N,N,N',N'*-tetramethylformamidine hexafluorophosphate (390 mg, 1.39 mmol). After 2 h, the reaction was concentrated and purified by normal phase chromatography (ethyl acetate in petroleum ether, 0-50% gradient) to afford rac-benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxocyclohexane-1-carboxylate (0.26 g, 0.40 mmol, 57% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.31 (s, 1H), 8.15 (t, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.23 - 7.15 (m, 3H), 6.84 (d, *J* = 6.4 Hz, 2H), 4.73 (q, *J* = 12.8 Hz, 2H), 3.54 - 3.46 (m, 2H), 3.20 - 3.17 (m, 1H), 3.01 (t, *J* = 13.6 Hz,

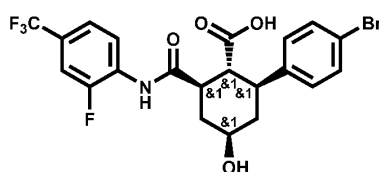
1H), 2.83 (t, $J = 13.2$ Hz, 1H), 2.68 - 2.60 (m, 1H), 2.37 - 2.33 (m, 1H). ES-LCMS m/z 592.0 $[M-H]^+$.

Step 5 : *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxocyclohexane-1-carboxylic acid



To a mixture of *rac*-benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl) phenyl) carbamoyl)-4-oxocyclohexane-1-carboxylate (0.26 g, 0.44 mmol) in dichloromethane (5 mL) at 0 °C was added a solution of boron trichloride (1M in DCM, 0.878 mL, 0.878 mmol), dropwise, over 2 min. After 1 h ,the reaction was quenched with ice water (2 mL), concentrated and purified by reverse phase chromatography (MeCN in H₂O, 0.1% ammonium bicarbonate modifier, 10-100% gradient) to afford *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxocyclohexane-1-carboxylic acid (0.14 g, 0.26 mmol, 60% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.38 (s, 1H), 8.23 (t, $J = 8.0$ Hz, 1H), 7.73 (d, $J = 9.2$ Hz, 1H), 7.57 (d, $J = 8.4$ Hz, 1H), 7.51 (d, $J = 8.4$ Hz, 2H), 7.32 (d, $J = 8.4$ Hz, 2H), 3.42 - 3.41 (m, 1H), 3.29 - 3.23 (m, 1H), 3.13 (t, $J = 4.0$ Hz, 1H), 2.92 (t, $J = 13.6$ Hz, 1H), 2.80 (t, $J = 13.6$ Hz, 1H), 2.55 - 2.52 (m, 1H), 2.46-2.29 (m, 1H). ES-LCMS m/z 499.8 $[M-H]^-$.

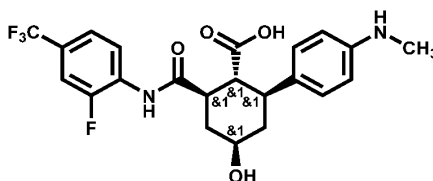
Step 6 : *rac*-(1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl) phenyl)carbamoyl)-4-hydroxycyclohexane-1-carboxylic acid



To a mixture of *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxocyclohexane-1-carboxylic acid (0.14 g, 0.28 mmol) in methanol (2 mL) and tetrahydrofuran (2 mL) at 0°C was added sodium borohydride (26.4 mg, 0.697 mmol) over 1 min. After 3h, the reaction was concentrated then purified by reverse phase chromatography (MeCN in H₂O, 0.1% ammonium bicarbonate modifier, 0-100% gradient) to

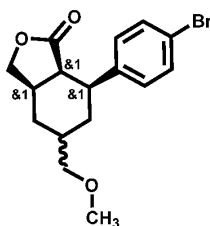
afford *rac*-(1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl) phenyl)carbamoyl)-4-hydroxycyclohexane-1-carboxylic acid (70 mg, 0.14 mmol, 49% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.19 (s, 1H), 8.21 (t, *J* = 8.0 Hz, 1H), 7.71 (dd, *J* = 11.2, 1.6 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.23 (d, *J* = 8.4 Hz, 2H), 4.90 (s, 1H), 3.69 - 3.33 (m, 1H), 3.08 - 3.01 (m, 1H), 2.80 - 2.65 (m, 2H), 2.15 (d, *J* = 12.0 Hz, 1H), 2.09 (d, *J* = 4.8 Hz, 1H), 1.91 - 1.47 (m, 2H). ES-LCMS *m/z* 504.0 [M+H]⁺.

Step 7: *rac*-(1*R*,2*R*,4*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-hydroxy-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid

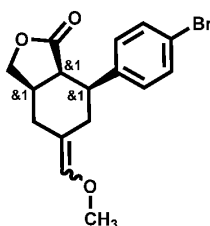


To a mixture of *rac*-(1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl) phenyl)carbamoyl)-4-hydroxycyclohexane-1-carboxylic acid (70.0 mg, 0.140 mmol) and sodium tert-butoxide (40.0 mg, 0.420 mmol) in 1,4-dioxane (2 mL) in a 10 mL microwave vial was added solid *t*BuXPhos Pd G3 (11.03 mg, 0.0140 mmol) and methanamine (2M in THF, 1.39 mL, 2.78 mmol). The reaction was heated at 100 °C for 1 h in an Anton microwave initiator. The reaction was concentrated then purified by reverse phase chromatography (MeCN in H₂O, 0.1% ammonium bicarbonate modifier, 0-100% gradient) to afford *rac*-(1*R*,2*R*,4*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-hydroxy-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid (35.0 mg, 0.0800 mmol, 54% yield) as a white solid. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 10.30 (s, 1H), 8.24 (t, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 10.8 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 6.42 (d, *J* = 8.4 Hz, 2H), 5.40 (s, 1H), 4.77 (d, *J* = 4.0 Hz, 2H), 3.61 (s, 1H), 2.95 (t, *J* = 12.8 Hz, 1H), 2.68 - 2.58 (m, 5H), 2.11 (d, *J* = 10.0 Hz, 1H), 1.84 (d, *J* = 11.2 Hz, 1H), 1.38 (q, *J* = 12.4 Hz, 1H). ES-LCMS *m/z* 455.0 [M+H]⁺.

Intermediate 44: *rac*-(3*aR*,7*S*,7*aS*)-7-(4-bromophenyl)-5-(methoxymethyl)hexahydroisobenzofuran-1(3*H*)-one

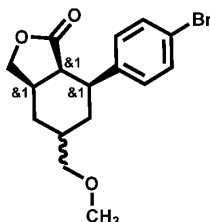


Step 1: *rac*-(3a*R*,7*S*,7a*S*)-7-(4-bromophenyl)-5-(methoxymethylene)hexahydroisobenzofuran-1(3*H*)-one



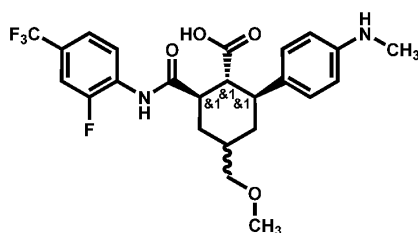
To a mixture of (methoxymethyl)triphenylphosphonium chloride (6.65 g, 19.4 mmol) in tetrahydrofuran (100 mL) at 0 °C was added potassium 2-methylpropan-2-olate (16.17 mL, 16.17 mmol, 1M in THF). After 1 h, *rac*-(3a*R*,7*S*,7a*S*)-7-(4-bromophenyl)tetrahydroisobenzofuran-1,5(3*H*,4*H*)-dione **Intermediate 8 Step 5** (5.0 g, 16.17 mmol) was added portionwise. The reaction was stirred at rt for 14 h, diluted with water (15 mL), concentrated and subjected to reverse phase purification (0-100% MeCN in H₂O, 0.1% ammonium bicarbonate modifier) to afford *rac*-(3a*R*,7*S*,7a*S*)-7-(4-bromophenyl)-5-(methoxymethylene)hexahydroisobenzofuran-1(3*H*)-one (2.0 g, 5.0 mmol, 31% yield) as an off-white solid. (E/Z mixture). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.46 (d, *J* = 8.5 Hz, 2H), 7.28 (dd, *J* = 8.5, 6.0 Hz, 2H), 6.09 - 5.96 (m, 1H), 4.19 (td, *J* = 9.0, 4.5 Hz, 1H), 3.96 - 3.85 (m, 1H), 3.51 (s, 3H), 3.00 (ddt, *J* = 17.4, 13.1, 4.3 Hz, 1H), 2.85 (dd, *J* = 14.0, 6.0 Hz, 1H), 2.77 - 2.61 (m, 1H), 2.39 - 2.21 (m, 1H), 2.19 - 2.06 (m, 1H), 1.74 (dd, *J* = 13.5, 10.5 Hz, 1H), 1.48 (t, *J* = 13.3 Hz, 1H). ES-LCMS *m/z* poor ionization.

Step 2: *rac*-(3a*R*,7*S*,7a*S*)-7-(4-bromophenyl)-5-(methoxymethyl)hexahydroisobenzofuran-1(3*H*)-one

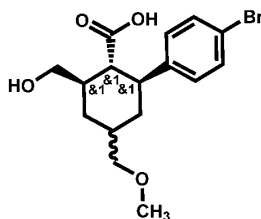


To a mixture of *rac*-(3*aR*,7*S*,7*aS*)-7-(4-bromophenyl)-5-(methoxymethylene)hexahydroisobenzofuran-1(3*H*)-one (2.00 g, 5.93 mmol) in tetrahydrofuran (50 mL) was added platinum on carbon 10 % (0.814 g, 0.415 mmol), under nitrogen. The reaction was placed under a hydrogen atmosphere with a bladder (1 atm). The reaction was degassed via vacuum evacuation, then backfilling with hydrogen, and this process was repeated three times. The mixture was stirred at rt for 16 h, filtered through a Celite pad, concentrated and subjected to reverse phase purification (50-70% MeCN in H₂O, 0.1% ammonium bicarbonate modifier) to afford *rac*-(3*aR*,7*S*,7*aS*)-7-(4-bromophenyl)-5-(methoxymethyl)hexahydroisobenzofuran-1(3*H*)-one (1.5g, 4.0 mmol, 67% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.45 (d, *J* = 8.5 Hz, 2H), 7.28 - 7.23 (m, 2H), 4.20 - 4.15 (m, 1H), 3.89 - 3.81 (m, 1H), 3.41 (t, *J* = 8.0 Hz, 1H), 3.28 - 3.19 (m, 5H), 3.13 - 3.04 (m, 1H), 2.68 - 2.58 (m, 1H), 1.94 - 1.66 (m, 3H), 1.33 - 1.17 (m, 1H), 0.85 (q, *J* = 12.5 Hz, 1H). ES-LCMS *m/z* 339.0 [M+H]⁺.

Intermediate 45: *rac*-(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid



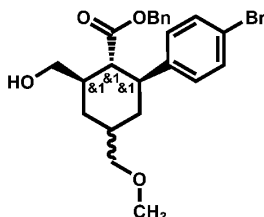
Step 1: *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)-4-(methoxymethyl)cyclohexane-1-carboxylic acid



To a mixture of *rac*-(3*aR*,7*S*,7*aS*)-7-(4-bromophenyl)-5-(methoxymethyl)hexahydroisobenzofuran-1(3*H*)-one **Intermediate 44** (1.50 g, 4.42 mmol) in methanol (15 mL) was added KOH (1.24 g, 22.1 mmol), and the reaction mixture was stirred at 65 °C for 16 h. The reaction was concentrated, quenched with 2N HCl to pH < 3, and solid precipitated out. This solid was filtered, washed with water (5 mL, 4 times), washed with petroleum ether (10 mL, 4 times) and dried to afford *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)-

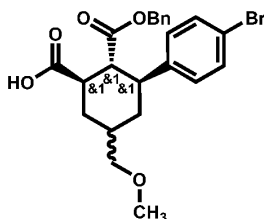
4-(methoxymethyl)cyclohexane-1-carboxylic acid (1.38 g, 3.32 mmol, 75 % yield) as an off-white solid which was used without further purification. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.04 – 11.62 (m, 1H), 7.44 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 4.54 (br dd, J = 4.7, 2.9 Hz, 1H), 3.50 – 3.35 (m, 2H), 3.30 – 3.13 (m, 5H), 2.90 – 2.64 (m, 1H), 2.31 – 2.17 (m, 1H), 2.03 – 1.87 (m, 1H), 1.85 – 1.54 (m, 3H), 1.16 (q, J = 12.5 Hz, 1H), 0.95 – 0.78 (m, 1H). ES-LCMS m/z 357.0 $[\text{M}-\text{H}]^-$.

Step 2: *rac*-benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)-4-(methoxymethyl)cyclohexane-1-carboxylate



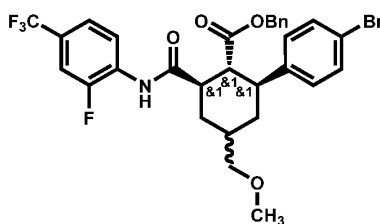
To a mixture of *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)-4-(methoxymethyl)cyclohexane-1-carboxylic acid (1.38 g, 3.86 mmol) and potassium carbonate (1.60 g, 11.6 mmol) in DMF (15 mL) at 0 °C was added benzyl bromide (0.551 mL, 4.64 mmol), dropwise over 1 min. After 14 h at rt, water (50 mL) was added, and the reaction was extracted with EtOAc (50 mL X 4). The combined EtOAc layers were washed with water (20 mL X 4) and brine (20 mL), dried over sodium sulfate and evaporated to afford crude material. The resulting crude material was subjected to normal phase purification (ethyl acetate in petroleum ether, 40-60% gradient, 50 min run) to afford *rac*-benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)-4-(methoxymethyl)cyclohexane-1-carboxylate (1.68 g, 3.67 mmol, 95 % yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.51 – 7.39 (m, 2H), 7.34 – 7.20 (m, 3H), 7.19 – 7.11 (m, 2H), 6.93 – 6.80 (m, 2H), 4.94 – 4.84 (m, 1H), 4.77 – 4.64 (m, 1H), 4.63 – 4.48 (m, 1H), 3.45 (d, J = 7.6 Hz, 1H), 3.32 – 3.15 (m, 6H), 2.90 – 2.70 (m, 1H), 2.47 – 2.35 (m, 1H), 1.95 – 1.61 (m, 4H), 1.28 – 1.20 (m, 1H), 1.02 – 0.84 (m, 1H). ES-LCMS m/z showed poor ionization.

Step 3: *rac*-(1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-(methoxymethyl)cyclohexane-1-carboxylic acid



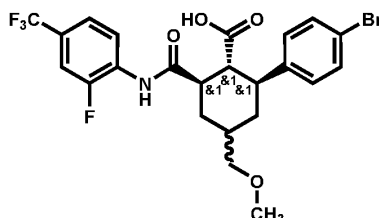
To a mixture of *rac*-benzyl (1*R*,2*S*,4*R*,6*R*)-2-(4-bromophenyl)-6-(hydroxymethyl)-4-(methoxymethyl)cyclohexane-1-carboxylate (1.67 g, 3.73 mmol) in MeCN (25 mL) and water (12 mL) at 0 °C was added sodium periodate (1.60 g, 7.47 mmol) and ruthenium(III) chloride (0.054 g, 0.26 mmol). After 30 min, water (50 mL) was added, and the mixture was extracted with EtOAc (50 mL X 3). The EtOAc layer was washed with water (30 mL X 2) and brine (30 mL), dried over sodium sulfate and concentrated. The residue was triturated (with sonication) in pet ether (25 mL) twice to afford *rac*-(1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-(methoxymethyl)cyclohexane-1-carboxylic acid (1.44 g, 3.08 mmol, 83% yield) as a light brown solid, which was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.45 (br s, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.32 – 7.15 (m, 5H), 6.89 – 6.77 (m, 2H), 4.79 – 4.63 (m, 2H), 3.54 – 3.41 (m, 1H), 3.28 – 3.14 (m, 4H), 2.84 – 2.70 (m, 3H), 2.12 – 2.02 (m, 1H), 1.97 – 1.77 (m, 1H), 1.76 – 1.63 (m, 1H), 1.44 – 1.17 (m, 2H). ES-LCMS *m/z* 458.8 [M-H]⁻.

Step 4: *rac* --benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)cyclohexane-1-carboxylate



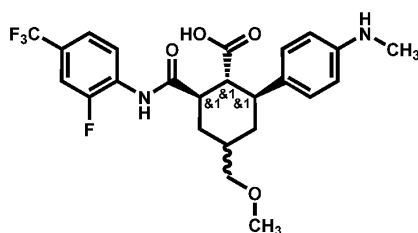
To a mixture of *rac*-(1*R*,2*R*,3*S*)-2-((benzyloxy)carbonyl)-3-(4-bromophenyl)-5-(methoxymethyl)cyclohexane-1-carboxylic acid (0.70 g, 1.5 mmol) in MeCN (14 mL) was added 2-fluoro-4-(trifluoromethyl)aniline (326 mg, 1.82 mmol) (with sonication) and 1-methyl-1*H*-imidazole (747 mg, 9.10 mmol), sequentially, followed after 15 min by chloro-*N,N,N',N'*-tetramethylformamidinium hexafluorophosphate (2.13 g, 7.59 mmol). After 16 h, the mixture was concentrated and subjected to reverse phase purification (MeCN in water with 0.1 % formic acid, 50-80% gradient) to afford a *rac* --benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)cyclohexane-1-carboxylatebenzyl (0.950 g, 1.46 mmol, 96% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.86 (br s, 1H), 10.15 (s, 1H), 8.20 (t, *J* = 8.0 Hz, 1H), 7.73 (br d, *J* = 11.0 Hz, 1H), 7.56 (br d, *J* = 8.6 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.23 (d, *J* = 8.4 Hz, 2H), 3.60 – 3.48 (m, 1H), 3.29 – 3.13 (m, 4H), 3.11 – 2.99 (m, 1H), 2.88 – 2.71 (m, 2H), 2.24 – 2.03 (m, 1H), 1.96 – 1.83 (m, 1H), 1.75 (br d, *J* = 8.6 Hz, 1H), 1.43 – 1.22 (m, 1H). ES-LCMS *m/z* 622.0 [M+H]⁺.

Step 5: *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)cyclohexane-1-carboxylic acid



To a mixture of *rac* --benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)cyclohexane-1-carboxylatebenzyl (0.900 g, 1.45 mmol) in DCM (10 mL) at -10 °C was added boron trichloride (5.78 mL, 5.78 mmol, 1M in DCM). After 45 min, 10% aq sodium bicarbonate (5 mL) was added, and the mixture was concentrated. The resulting residue was taken up in THF (3 mL) and water (5 mL) and subjected to reverse phase purification (MeCN in H₂O, 0.1% formic acid, 80-100% gradient) to afford *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)cyclohexane-1-carboxylic acid (0.750 g, 1.31 mmol, 91% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.86 (br s, 1H), 10.15 (s, 1H), 8.20 (t, *J* = 8.0 Hz, 1H), 7.73 (br d, *J* = 11.0 Hz, 1H), 7.56 (br d, *J* = 8.6 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.23 (d, *J* = 8.4 Hz, 2H), 3.60 – 3.48 (m, 1H), 3.29 – 3.13 (m, 4H), 3.11 – 2.99 (m, 1H), 2.88 – 2.71 (m, 2H), 2.24 – 2.03 (m, 1H), 1.96 – 1.83 (m, 1H), 1.75 (br d, *J* = 8.6 Hz, 1H), 1.44 – 1.21 (m, 1H). ES-LCMS *m/z* 532.0 [M-H].

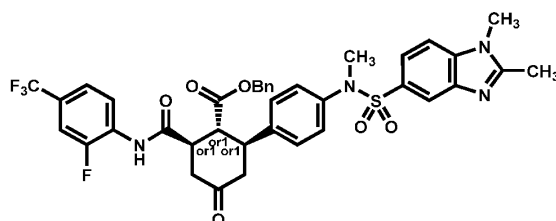
Step 6: *rac*-(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid



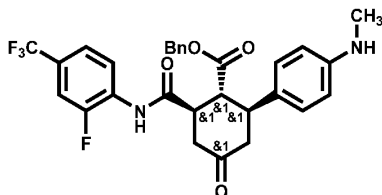
A mixture of *rac*-(1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)cyclohexane-1-carboxylic acid (350 mg, 0.657 mmol) and sodium *tert*-butoxide (190 mg, 1.97 mmol) in dioxane (10 mL) was degassed for 5 min. *t*BuXPhos Pd G3 (52 mg, 0.066 mmol) was added, and the mixture was degassed for 5 min. Methanamine (2M in THF, 6.57 mL, 13.2 mmol) was added, and the reaction was heated at

100 °C for 1 h. The mixture was filtered through Celite, concentrated, and the resulting residue was subjected to reverse phase purification (MeCN in H₂O, 0.1 % formic acid, 30-60% gradient) to afford *rac*-(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid (350 mg, 0.522 mmol, 79% yield) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.10 (s, 1H), 8.19 (t, *J* = 8.3 Hz, 1H), 7.71 (d, *J* = 10.5 Hz, 1H), 7.54 (d, *J* = 9.0 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.42 (d, *J* = 8.5 Hz, 2H), 5.43 (d, *J* = 2.0 Hz, 1H), 3.25 - 3.19 (m, 4H), 3.07 - 2.94 (m, 1H), 2.72 - 2.57 (m, 6H), 2.20 - 1.98 (m, 1H), 1.93 - 1.65 (m, 3H), 1.34 - 1.16 (m, 2H). ES-LCMS *m/z* 483.2 [M+H]⁺.

Intermediate 46A: *rac*-benzyl (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxo-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate



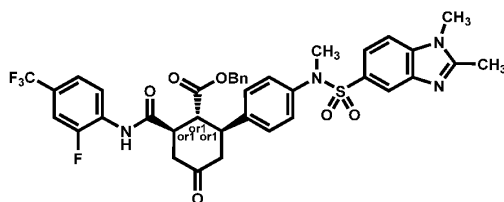
Step 1: *rac*-benzyl (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)-4-oxocyclohexane-1-carboxylate



To a mixture of *rac*-benzyl (1*R*,2*S*,6*R*)-2-(4-bromophenyl)-6-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxocyclohexane-1-carboxylate **Intermediate 11 Step 4** (1.05 g, 1.77 mmol) and sodium 2-methylpropan-2-olate (0.256 g, 2.66 mmol) in dioxane (30 mL) was added *t*BuXPhos Pd G3 (282 mg, 0.354 mmol), and the reaction was degassed for 5 min. Methanamine (2M in THF, 17.7 mL, 35.4 mmol) was added, and the mixture was microwaved at 100 °C. After 1 h, the reaction was concentrated and subjected to normal phase purification (50-100% EtOAc in per ether) to afford *rac*-benzyl (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)-4-oxocyclohexane-1-carboxylate (0.180 g, 0.265 mmol, 15.0% yield) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.26 (s,

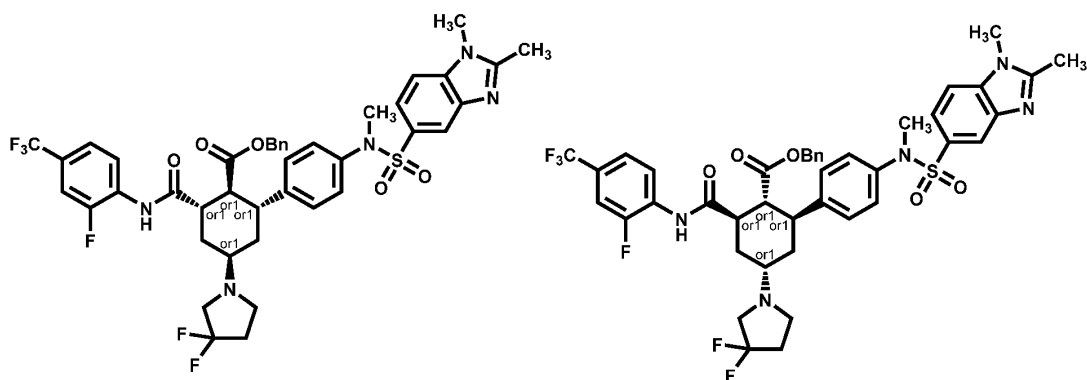
1H), 8.15 (t, $J = 8.0$ Hz, 1H), 7.73 (dd, $J = 11.0, 2.0$ Hz, 1H), 7.56 (d, $J = 8.5$ Hz, 1H), 7.21 - 7.06 (m, 3H), 7.04 (d, $J = 8.5$ Hz, 2H), 6.87 - 6.79 (m, 2H), 6.48 (d, $J = 8.5$ Hz, 2H), 5.61 (q, $J = 5.0$ Hz, 1H), 4.73 (d, $J = 1.5$ Hz, 2H), 3.46 - 3.35 (m, 2H), 3.03 - 2.93 (m, 2H), 2.87 - 2.73 (m, 1H), 2.67 (d, $J = 5.0$ Hz, 3H), 2.58 (d, $J = 1.5$ Hz, 1H), 2.31 - 2.22 (m, 1H). ES-LCMS m/z 541.3 [M-H]⁻.

Step 2: *rac*-benzyl (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxo-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate



To a mixture of *rac*-benzyl (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)-4-oxocyclohexane-1-carboxylate (203 mg, 0.374 mmol) in dichloromethane (10 mL) and pyridine (0.091 mL, 1.1 mmol) at 0 °C was added 1,2-dimethyl-1*H*-benzo[*d*]imidazole-5-sulfonyl chloride (110 mg, 0.450 mmol), portion wise. The reaction was stirred at rt for 45 min, concentrated and subjected to reverse phase purification (0-90% MeCN in H₂O, with 0.1% formic acid modifier) to afford *rac*-benzyl (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxo-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate (145 mg, 0.155 mmol, 41.4 % yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.29 (s, 1H), 8.14 (t, $J = 8.0$ Hz, 1H), 7.74 (dd, $J = 10.8, 1.8$ Hz, 1H), 7.63 - 7.53 (m, 3H), 7.32 - 7.26 (m, 3H), 7.19 - 7.10 (m, 3H), 7.04 (d, $J = 8.5$ Hz, 2H), 6.99 - 6.93 (m, 2H), 4.84 (d, $J = 13.0$ Hz, 1H), 4.62 (d, $J = 13.0$ Hz, 1H), 3.74 (s, 3H), 3.52 - 3.41 (m, 2H), 3.24 - 3.13 (m, 1H), 3.08 (s, 3H), 3.07 - 2.96 (m, 1H), 2.90 - 2.74 (m, 1H), 2.60 (d, $J = 14.0$ Hz, 1H), 2.53 (s, 3H), 2.37 (d, $J = 4.0$ Hz, 1H). ES-LCMS m/z 751.0 [M+H]⁺.

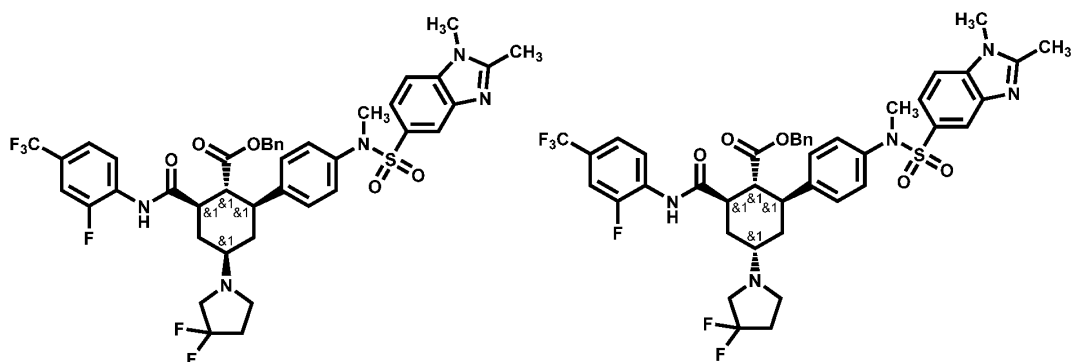
Intermediate 72 and Intermediate 73: *rel*-benzyl (1*R*,2*R*,4*S*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate ISOMER 1 and *rel*-benzyl (1*R*,2*R*,4*S*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate ISOMER 2



ISOMER 1

ISOMER 2

Step 1: *rac*-benzyl (1*R*,2*R*,4*R*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate and *rac*-benzyl (1*R*,2*R*,4*S*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate

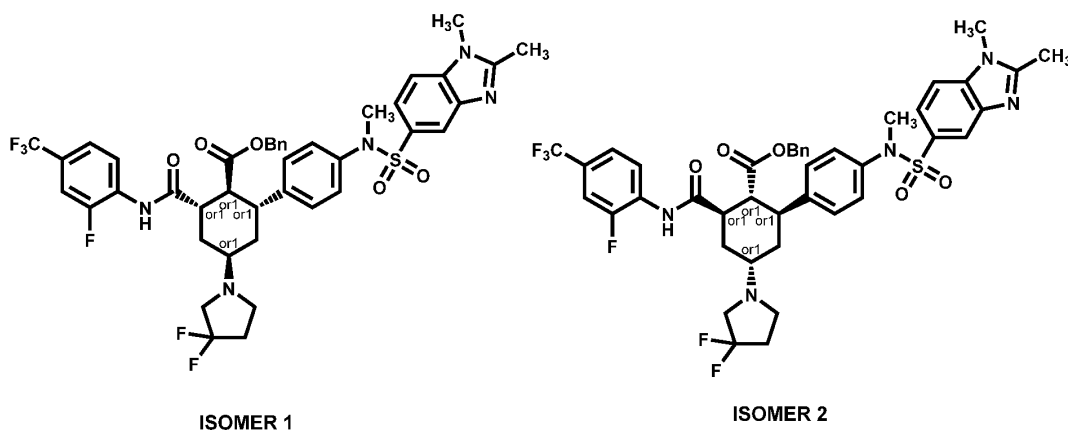


Two reactions were run in parallel: A suspension of *rac*-benzyl (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-oxo-6-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate (**Intermediate 46A**) (200 mg, 0.27 mmol; 100 mg, 0.13 mmol) and 3,3-difluoropyrrolidine (86 mg, 0.80 mmol; 43 mg, 0.40 mmol) in MeOH (0.5 mL; 0.5 mL), THF (0.5 mL; 0.5 mL) and DMA (0.5 mL; 0.5 mL) was heated to 80 °C. After 2 h, sodium cyanoborohydride (25 mg, 0.40 mmol; 13 mg, 0.20 mmol) was added, and the reactions were stirred for 2 h at rt, combined, quenched with water, diluted with MeOH (1 mL) and THF (2 mL), then purified by reverse-phase chromatography (Column: Grace C18 100 g), eluting with 0-100 % MeCN in aq. 0.1% ammonium carbonate to afford:

the first-eluting compound *rac*-benzyl (1*R*,2*R*,4*R*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate (150 mg, 0.16 mmol, 59% yield) as a brown solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.16 (s, 1H), 8.16 (t, *J* = 8.3 Hz, 1H), 7.72 (dd, *J* = 11.0, 2.0 Hz, 1H), 7.64 - 7.44 (m, 3H), 7.36 - 7.14 (m, 3H), 7.19 - 7.05 (m, 3H), 7.03 - 6.88 (m, 4H), 4.80 (d, *J* = 12.5 Hz, 1H), 4.58 (d, *J* = 12.5 Hz, 1H), 3.74 (s, 3H), 3.20 - 3.09 (m, 2H), 3.07 (s, 3H), 3.03 - 2.86 (m, 3H), 2.87 - 2.69 (m, 3H), 2.52 (s, 3H), 2.29 - 2.11 (m, 3H), 1.97 (d, *J* = 12.0 Hz, 1H), 1.61 (q, *J* = 12.2 Hz, 1H), 1.50 - 1.39 (m, 1H). ES-LCMS *m/z* 842.2 [M+H]⁺. Relative stereochemistry was determined by co-crystal structure of subsequent final compound with WRN protein and then tracing back to the corresponding intermediates.

And the second-eluting compound *rac*-benzyl (1*R*,2*R*,4*S*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate (110 mg, 0.13 mmol, 47% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.21 (s, 1H), 8.03 (t, *J* = 7.8 Hz, 1H), 7.70 (dd, *J* = 10.8, 1.3 Hz, 1H), 7.62 - 7.56 (m, 2H), 7.54 (d, *J* = 9.0 Hz, 1H), 7.31 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 7.15 - 7.04 (m, 3H), 7.00 (d, *J* = 9.0 Hz, 2H), 6.91 (d, *J* = 7.0 Hz, 2H), 4.83 (d, *J* = 13.0 Hz, 1H), 4.62 (d, *J* = 13.0 Hz, 1H), 3.74 (s, 3H), 3.40 - 3.38 (m, 2H), 3.07 (s, 3H), 2.94 - 2.83 (m, 3H), 2.76 - 2.67 (m, 3H), 2.55 (s, 3H), 2.42 - 2.28 (m, 2H), 2.13 - 2.01 (m, 1H), 1.92 - 1.71 (m, 3H). ES-LCMS *m/z* 842.2 [M+H]⁺.

Step 2: *rel*-benzyl (1*R*,2*R*,4*S*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-((4-methoxybenzyl)amino)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate ISOMER 1 and *rel*-benzyl (1*R*,2*R*,4*S*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-((4-methoxybenzyl)amino)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate ISOMER 2

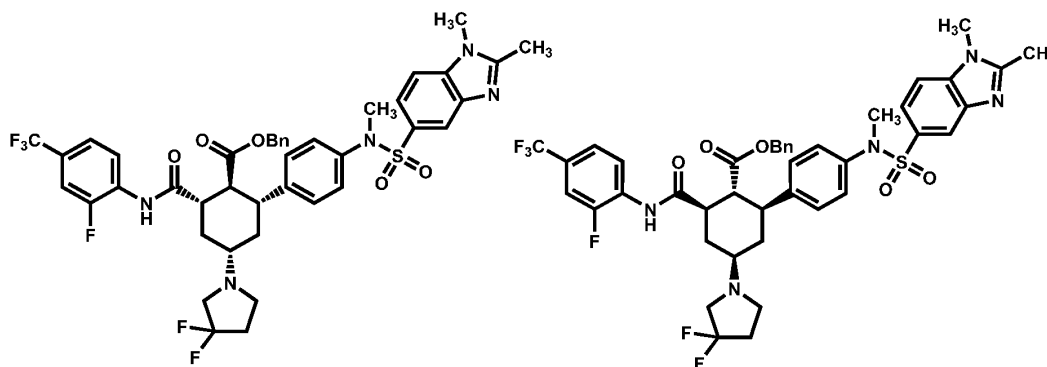


Racemic compound *rac*-benzyl (1*R*,2*R*,4*S*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-((*N*,1,2-trimethyl-1*H*-benzo[d]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate (110 mg) was purified by Chiral-Prep-SFC (Column: YMC Amylose-SA; Mobile Phase A: CO₂, Mobile Phase B: 0.5% isopropylamine in IPA; Gradient: 35% B) to afford:

the first-eluting isomer *rel*-benzyl (1*R*,2*R*,4*S*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-((*N*,1,2-trimethyl-1*H*-benzo[d]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate ISOMER 1 (48 mg, 0.060 mmol, 41% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.21 (s, 1H), 8.03 (t, *J* = 8.0 Hz, 1H), 7.71 (dd, *J* = 11.0, 1.5 Hz, 1H), 7.63 - 7.56 (m, 2H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.31 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 2H), 7.16 - 7.03 (m, 3H), 7.00 (d, *J* = 9.0 Hz, 2H), 6.93 - 6.89 (m, 2H), 4.83 (d, *J* = 13.0 Hz, 1H), 4.62 (d, *J* = 12.5 Hz, 1H), 3.74 (s, 3H), 3.45 - 3.34 (m, 2H), 3.23 - 3.09 (m, 2H), 3.07 (s, 3H), 3.03 - 2.92 (m, 2H), 2.90 - 2.71 (m, 2H), 2.57 (s, 3H), 2.41 - 2.29 (m, 2H), 2.12 - 2.03 (m, 1H), 1.91 - 1.73 (m, 3H). ES-LCMS *m/z* 842.2 [M+H]⁺.

And the second-eluting isomer *rel*-benzyl (1*R*,2*R*,4*S*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-((*N*,1,2-trimethyl-1*H*-benzo[d]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate ISOMER 2 (44 mg, 0.050 mmol, 36 % yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.21 (s, 1H), 8.03 (t, *J* = 8.0 Hz, 1H), 7.71 (dd, *J* = 11.3, 1.8 Hz, 1H), 7.62 - 7.49 (m, 3H), 7.31 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.22 (d, *J* = 8.5 Hz, 2H), 7.16 - 7.04 (m, 3H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.94 - 6.88 (m, 2H), 4.83 (d, *J* = 13.0 Hz, 1H), 4.62 (d, *J* = 12.5 Hz, 1H), 3.74 (s, 3H), 3.46 - 3.37 (m, 2H), 3.22 - 3.10 (m, 2H), 3.07 (s, 3H), 3.05 - 2.95 (m, 2H), 2.93 - 2.84 (m, 1H), 2.75 (d, *J* = 8.0 Hz, 1H), 2.54 (s, 3H), 2.43 - 2.29 (m, 2H), 2.13 - 2.01 (m, 1H), 1.93 - 1.70 (m, 3H). ES-LCMS *m/z* 842.2 [M+H]⁺.

Intermediate 74 and Intermediate 75: benzyl (1*S*,2*S*,4*S*,6*R*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate and benzyl (1*R*,2*R*,4*R*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate



Racemic compound *rac*-benzyl (1*R*,2*R*,4*R*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate from **Intermediate 72 and Intermediate 73**, **Step 1**, the first-eluting compound (150 mg) was purified by Chiral-Prep-SFC (Column: YMC Cellulose-SC; Mobile Phase A: CO₂, Mobile Phase B: 0.5% isopropylamine in IPA; Gradient: 50% B) to afford:

Intermediate 74: the first-eluting isomer benzyl (1*S*,2*S*,4*S*,6*R*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate (75 mg, 0.090 mmol, 48% yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.17 (s, 1H), 8.17 (t, *J* = 8.0 Hz, 1H), 7.73 (dd, *J* = 11.0, 1.5 Hz, 1H), 7.62 - 7.51 (m, 3H), 7.32 - 7.26 (m, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.18 - 7.08 (m, 3H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.97 - 6.90 (m, 2H), 4.81 (d, *J* = 13.0 Hz, 1H), 4.59 (d, *J* = 12.5 Hz, 1H), 3.77 - 3.76 (m, 1H), 3.75 (s, 3H), 3.19 - 3.07 (m, 1H), 3.07 (s, 3H), 3.02 - 2.72 (m, 6H), 2.30 - 2.11 (m, 3H), 2.01 - 1.89 (m, 1H), 1.71 - 1.37 (m, 2H), 3 protons obscured by solvent peaks. ES-LCMS *m/z* 842.2 [M+H]⁺.

and

Intermediate 75: the second-eluting isomer benzyl (1*R*,2*R*,4*R*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate (66 mg, 0.080 mmol, 42 % yield) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.17 (s, 1H), 8.17 (t, *J* = 8.3 Hz, 1H), 7.73 (dd, *J* = 11.0,

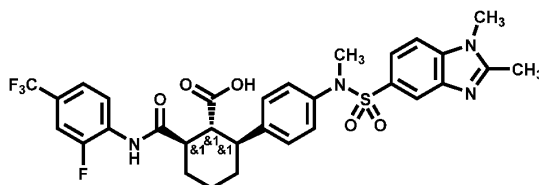
1.5 Hz, 1H), 7.64 - 7.49 (m, 3H), 7.31 (dd, $J = 8.5, 2.0$ Hz, 1H), 7.24 (d, $J = 8.5$ Hz, 2H), 7.18 - 7.07 (m, 3H), 7.00 (d, $J = 8.5$ Hz, 2H), 6.96 - 6.90 (m, 2H), 4.81 (d, $J = 13.0$ Hz, 1H), 4.59 (d, $J = 13.0$ Hz, 1H), 3.75 (s, 3H), 3.84 - 3.66 (m, 1H), 3.20 - 3.11 (m, 1H), 3.07 (s, 3H), 3.03 - 2.75 (m, 6H), 2.53 (s, 3H), 2.31 - 2.09 (m, 3H), 1.97 (d, $J = 12.0$ Hz, 1H), 1.68 - 1.37 (m, 2H). ES-LCMS m/z 842.2 $[M+H]^+$.

Absolute stereochemistry was determined by co-crystal structure of subsequent final compound with WRN protein and then tracing back to the corresponding enantiopure intermediates.

Examples

Example 81

rac-(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid



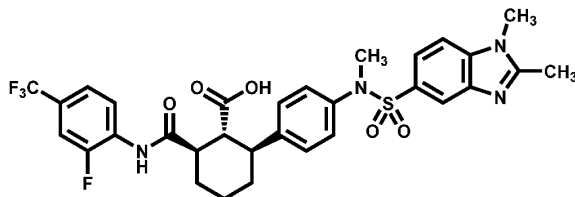
To a 1:1 mixture of (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,6*R*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid **Intermediate 6** (0.0550 g, 0.125 mmol) in DCM (2 mL) at 0 °C were added pyridine (0.030 mL, 0.38 mmol) and 1,2-dimethyl-1*H*-benzo[*d*]imidazole-5-sulfonyl chloride (30.7 mg, 0.125 mmol). After 1 h, the reaction was diluted with water and extracted with EtOAc. The organic layers were washed with brine, dried over anhydrous sodium sulfate, concentrated, dissolved in DMF (2 mL) and purified over silica (40 g C18 column), eluting with 5-100% acetonitrile in water to give rac-(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (0.030 g, 0.045 mmol, 36.0 % yield) as a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ 12.03 (br, 1H), 10.09 (s, 1H), 8.22 (t, $J = 8.0$ Hz, 1H), 7.72 (d, $J = 10.4$ Hz, 1H), 7.63 (d, $J = 8.4$ Hz, 1H), 7.56 (d, $J = 9.2$ Hz, 1H), 7.57 - 7.51 (m, 1H), 7.25 (dd, $J = 6.8, 1.6$ Hz, 1H), 7.18 - 7.17 (m, 2H), 6.97 (d, $J = 8.4$ Hz, 2H), 3.79 (s, 3H), 3.06 (s, 3H), 3.04 - 3.00 (m, 1H), 2.82 - 2.67 (m, 2H), 2.57 (s, 3H), 2.04 - 2.00 (m, 1H), 1.90 - 1.85 (m, 1H), 1.76 - 1.74 (m, 1H), 1.70 - 1.51 (m, 3H). ES-LCMS m/z 647.3 $[M+H]^+$.

Example 83 and Example 84

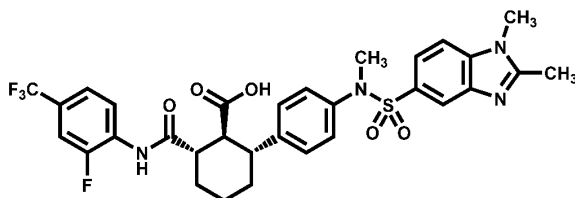
(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid

AND

(1*S*,2*S*,6*R*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid



AND



Rac-(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (**Example 81**) (0.030 g, 0.046 mmol) was chirally purified (Column: CHIRALPAK IE [2 mm X 25 mm, 5 micron]; Mobile Phase: 81:19 hexane [0.2% formic acid]:[1:1 EtOH:DCM]) to give:

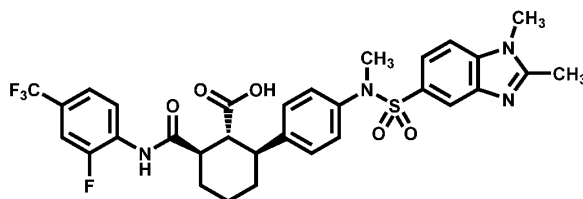
Example 83, the first-eluting isomer: (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (9.0 mg, 0.014 mmol, 30% yield). ¹H NMR (700 MHz, DMSO-*d*₆) δ 12.06 (br s, 1H), 10.10 (s, 1H), 8.22 (t, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 10.9 Hz, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.50 (s, 1H), 7.24 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 3.78 (s, 3H), 3.05 (s, 3H), 3.00 (td, *J* = 11.4, 3.2 Hz, 1H), 2.78 (t, *J* = 11.6 Hz, 1H), 2.67 (td, *J* = 11.8, 3.2 Hz, 1H), 2.56 (s, 3H), 2.05 – 1.97 (m, 1H), 1.91 – 1.84 (m, 1H), 1.79 – 1.72 (m, 1H), 1.65 – 1.57 (m, 1H), 1.53 – 1.51 (m, 1H), 1.53 – 1.50 (m, 1H). ES-LCMS *m/z* 647.2 [M+H]⁺. Absolute stereochemistry was assigned by co-crystallization with WRN protein.

Example 84, the second-eluting isomer: (1*S*,2*S*,6*R*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (8.9 mg, 0.014 mmol, 30% yield). ¹H NMR

(700 MHz, DMF- d_7) δ 12.06 (br s, 1H), 10.11 (s, 1H), 8.22 (t, J = 8.1 Hz, 1H), 7.71 (d, J = 10.9 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.51 – 7.49 (m, 1H), 7.24 (dd, J = 8.5, 1.5 Hz, 1H), 7.19 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 8.4 Hz, 2H), 3.78 (s, 3H), 3.05 (s, 3H), 3.00 (td, J = 11.2, 3.1 Hz, 1H), 2.78 (t, J = 11.2 Hz, 1H), 2.67 (td, J = 11.8, 3.3 Hz, 1H), 2.56 (s, 3H), 2.05 – 1.99 (m, 1H), 1.91 – 1.85 (m, 1H), 1.78 – 1.72 (m, 1H), 1.66 – 1.57 (m, 1H), 1.56 – 1.51 (m, 1H), 1.52 – 1.44 (m, 1H). ES-LCMS m/z 647.2 $[M+H]^+$.

Example 83, second preparation method

(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[d]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid



To a mixture of (1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid (**Intermediate 7**) (0.180 kg, 411 mmol) in MeCN (1.08 L) at 0 °C was added TEA (62.3 g, 616 mmol) and 1,2-dimethyl-1*H*-benzo[d]imidazole-5-sulfonyl chloride (151 g, 616 mmol). The reaction was stirred at 25 °C for 1 h. Water (7.2 L) was added into the mixture slowly at rt. After 1 h, the reaction was filtered, and the resulting solid was washed with water (900 ml) to give a residue (200 g, 309 mmol) as a light blue solid. This residue was triturated with MeCN (1.00 L) at 25 °C for 2 h and filtered. The filter cake was washed with MeCN (100 mL) to give a residue (195 g, 301 mmol) which was dissolved in 5:1 DCM:MeOH (MeOH [650 mL] and DCM [3250 mL]) at 45 °C for 40 min (until the mixture became clear). 21x Palladium Removal Silica Gel was added (30% by weight of crude material) to the mixture, then the mixture was refluxed for 12 hrs. The mixture was filtered while hot, then the filter cake was washed with DCM (1.3 L). The filtrate was concentrated under reduced pressure at 35 °C for 2 h to give a residue (177 g, 274 mmol). This residue was dissolved in 5:1 DCM:MeOH (MeOH [590 mL] and DCM [2950 mL]) at 45 °C for 40 min (until the mixture became clear). 22x Palladium Removal Silica Gel was added (30% by weight of crude material) to the mixture, then the mixture was refluxed for 12 h. The mixture was filtered while hot, then the filter cake was washed with DCM (1.2 L). The filtrate was concentrated under reduced pressure at 35 °C for 2 h to give a residue which was further concentrated under reduced pressure (-0.1 MPa) at 35 °C for 4 h, to remove DCM and MeOH, to give (1*R*,2*R*,6*S*)-2-((2-fluoro-4-

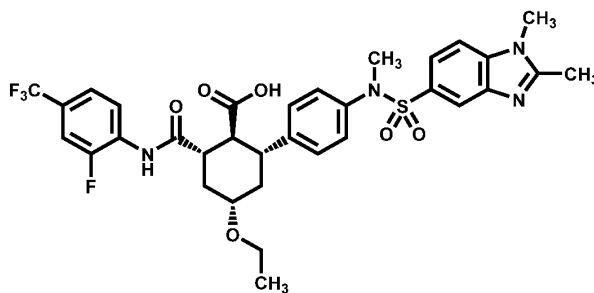
(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[d]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (168 g, 260 mmol, 63.2% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.1 (s, 1H), 8.21 (t, *J* = 8.0 Hz, 1H), 7.70 - 7.73 (m, 2H), 7.60 (d, *J* = 1.2 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.29 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 3.82 (s, 3H), 3.07 (s, 3H), 2.97 - 3.03 (m, 1H), 2.75 - 2.80 (m, 1H), 2.67 - 2.70 (m, 1H), 2.63 (s, 3H), 2.01 - 2.03 (m, 1H), 1.88 - 1.89 (m, 1H), 1.74 - 1.77 (m, 1H), 1.50 - 1.61 (m, 3H). ES-LCMS *m/z* 647.3 [M+H]⁺.

Example 137 and Example 138

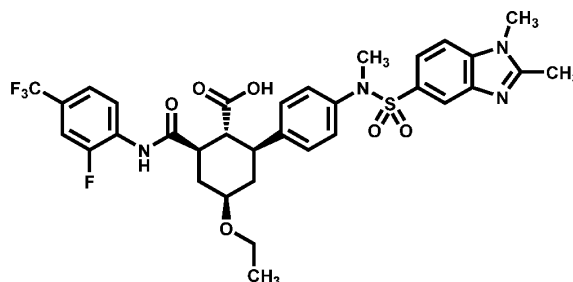
(1*S*,2*S*,4*S*,6*R*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[d]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid

AND

(1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[d]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid



AND



To a 1:1 mixture of (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,4*S*,6*R*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid (**Intermediate 8**) (0.290 g, 0.601 mmol) and pyridine (0.146 mL, 1.80 mmol) in dichloromethane (3 mL) at 0°C was added 1,2-dimethyl-1*H*-benzo[d]imidazole-5-sulfonyl chloride (294 mg, 1.20

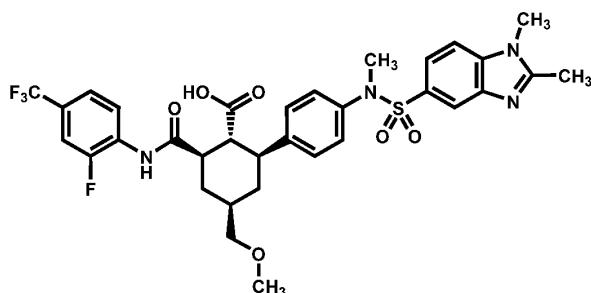
mmol). After 1 h, the reaction was quenched with MeOH (2 mL), concentrated and subjected to reverse phase purification (MeCN in H₂O, 10 mM ammonium bicarbonate modifier, 10-100% gradient) to afford a 1:1 mixture of (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid and (1*S*,2*S*,4*S*,6*R*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (0.300 g, 0.426 mmol, 70.8% yield). This material was chirally purified (Column: YMC Amylose SA, 250 X 20 mm, 5µm; Mobile Phase: 1:1 CO₂:IPA) to give:

Example 137, the first-eluting isomer: (1*S*,2*S*,4*S*,6*R*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (9.0 mg, 0.014 mmol, 30% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.13 (br s, 1H), 10.12 (s, 1H), 8.22 (t, *J* = 8.3 Hz, 1H), 7.77 - 7.68 (m, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.48 (d, *J* = 1.5 Hz, 1H), 7.32 - 7.14 (m, 3H), 6.96 (d, *J* = 9.0 Hz, 2H), 3.78 (s, 3H), 3.60 - 3.42 (m, 3H), 3.05 (s, 3H), 2.79 - 2.64 (m, 2H), 2.56 (s, 3H), 2.33 (dt, *J* = 3.6, 1.9 Hz, 2H), 2.05 (d, *J* = 12.0 Hz, 1H), 1.56 (d, *J* = 11.0 Hz, 1H), 1.51 - 1.34 (m, 1H), 1.10 (t, *J* = 7.0 Hz, 3H). ES-LCMS *m/z* 691.2 [M+H]⁺.

Example 138, the second-eluting isomer: (1*R*,2*R*,4*R*,6*S*)-4-ethoxy-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (0.13 g, 0.19 mmol, 43% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.13 (br s, 1H), 10.12 (s, 1H), 8.22 (t, *J* = 8.3 Hz, 1H), 7.77 - 7.68 (m, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.48 (d, *J* = 1.5 Hz, 1H), 7.32 - 7.14 (m, 3H), 6.96 (d, *J* = 9.0 Hz, 2H), 3.78 (s, 3H), 3.60 - 3.42 (m, 3H), 3.05 (s, 3H), 2.79 - 2.64 (m, 2H), 2.56 (s, 3H), 2.33 (dt, *J* = 3.6, 1.9 Hz, 2H), 2.05 (d, *J* = 12.0 Hz, 1H), 1.56 (d, *J* = 11.0 Hz, 1H), 1.51 - 1.34 (m, 1H), 1.10 (t, *J* = 7.0 Hz, 3H). ES-LCMS *m/z* 691.2 [M+H]⁺. Absolute stereochemistry was assigned by co-crystallization with WRN protein.

Example 250

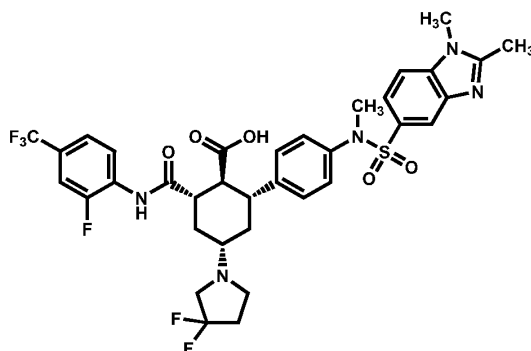
(1*R*,2*R*,4*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid



To a suspension of *rac*-(1*R*,2*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)-6-(4-(methylamino)phenyl)cyclohexane-1-carboxylic acid **Intermediate 45** (325 mg, 0.674 mmol) and 1,2-dimethyl-1*H*-benzo[*d*]imidazole-5-sulfonyl chloride (0.330 g, 1.35 mmol) in dichloromethane (0.5 mL) at 0 °C was added pyridine (0.272 mL, 3.37 mmol). After 10 min the reaction was stirred at rt for 1 h, the mixture was concentrated and subjected to reverse phase purification (25-75% MeCN in H₂O, 10 nM ammonium bicarbonate modifier) to afford *rac*-(1*R*,2*R*,4*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonylamido)phenyl)cyclohexane-1-carboxylic acid (0.150 g, 0.206 mmol, 30.6 % yield) as an off-white solid. The racemic compound was separated by Chiral-Prep-SFC (Column: YMC Amylose-SA 250 X 20 mm, 5µm; 1:1 CO₂: 0.5% isopropylamine in IPA) to afford the fourth-eluting peak (1*R*,2*R*,4*R*,6*S*)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-4-(methoxymethyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonylamido)phenyl)cyclohexane-1-carboxylic acid (16 mg, 0.023 mmol, 11% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.05 (br s, 1H), 10.15 (s, 1H), 8.21 (t, *J* = 8.0 Hz, 1H), 7.72 (dd, *J* = 11.0, 2.0 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.50 (d, *J* = 1.5 Hz, 1H), 7.25 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.19 (d, *J* = 8.5 Hz, 2H), 6.96 (d, *J* = 8.5 Hz, 2H), 3.78 (s, 3H), 3.27 - 3.20 (m, 5H), 3.08 - 3.00 (m, 4H), 2.79 - 2.71 (m, 2H), 2.56 (s, 3H), 2.07 (d, *J* = 12.5 Hz, 1H), 1.95 - 1.83 (m, 1H), 1.80 - 1.69 (m, 1H), 1.36 - 1.23 (m, 2H). ES-LCMS *m/z* 691.2 [M+H]⁺. Relative Stereochemistry was assigned by 2D NMR structure elucidation, and absolute stereochemistry was assigned by known SAR correlation.

Example 305

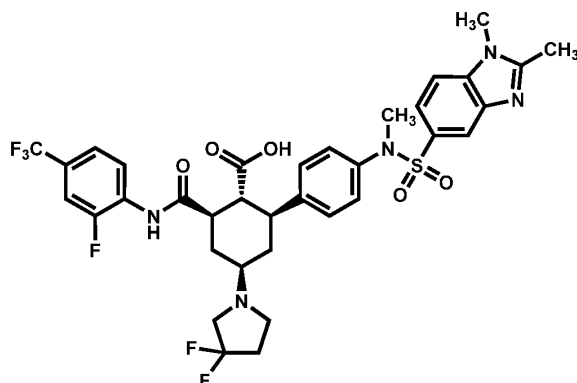
(1*S*,2*S*,4*S*,6*R*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonylamido)phenyl)cyclohexane-1-carboxylic acid



To a mixture of benzyl (1*S*,2*S*,4*S*,6*R*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate **Intermediate 74** (74 mg, 0.088 mmol) in dichloromethane (2 mL) at 0 °C was added boron trichloride (1M in DCM, 0.30 mL, 0.30 mmol). After 2 h at rt, the reaction was quenched with water (2 mL), concentrated and subjected to reverse phase purification to afford (1*S*,2*S*,4*S*,6*R*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (40 mg, 0.053 mmol, 60% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.18 (s, 1H), 10.15 (s, 1H), 8.24 (t, *J* = 8.0 Hz, 1H), 7.72 (dd, *J* = 10.8, 1.8 Hz, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.49 (d, *J* = 1.5 Hz, 1H), 7.27 - 7.18 (m, 3H), 6.96 (d, *J* = 8.5 Hz, 2H), 3.77 (s, 3H), 3.05 (s, 4H), 3.01 - 2.92 (m, 2H), 2.85 - 2.71 (m, 5H), 2.56 (s, 3H), 2.26 - 2.14 (m, 3H), 1.98 - 1.89 (m, 1H), 1.63 - 1.39 (m, 2H). ES-LCMS *m/z* 752.2 [M+H]⁺. Absolute stereochemistry was assigned based on Example 305 being the enantiomer of Example 306.

Example 306

(1*R*,2*R*,4*R*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid



To a mixture of benzyl (1*R*,2*R*,4*R*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylate **Intermediate 75** (64 mg, 0.076 mmol) in dichloromethane (2 mL) at 0 °C was added boron trichloride (1M in DCM, 0.30 mL, 0.30 mmol). After 2 h at rt, the reaction was quenched with water (2 mL), concentrated and subjected to reverse phase purification to afford (1*R*,2*R*,4*R*,6*S*)-4-(3,3-difluoropyrrolidin-1-yl)-2-((2-fluoro-4-(trifluoromethyl)phenyl)carbamoyl)-6-(4-((*N*,1,2-trimethyl-1*H*-benzo[*d*]imidazole)-5-sulfonamido)phenyl)cyclohexane-1-carboxylic acid (22 mg, 0.028 mmol, 37% yield) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.21 (s, 1H), 10.18 (s, 1H), 8.24 (t, *J* = 8.0 Hz, 1H), 7.75 - 7.69 (m, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.57 - 7.47 (m, 2H), 7.28 - 7.18 (m, 3H), 6.96 (d, *J* = 8.5 Hz, 2H), 3.78 (s, 3H), 3.05 (s, 4H), 3.02 - 2.91 (m, 2H), 2.85 - 2.68 (m, 5H), 2.56 (s, 3H), 2.28 - 2.12 (m, 3H), 1.94 (d, *J* = 11.5 Hz, 1H), 1.61 - 1.36 (m, 2H). ES-LCMS *m/z* 752.2 [M+H]⁺. Absolute stereochemistry was assigned by co-crystallization with WRN protein.

List of Selected Abbreviations:

4,4'-dtbbpy)NiCl₂ - [4,4'-Bis(1,1-dimethylethyl)-2,2'-bipyridine] nickel (II) dichloride

Aminosupersilane - *N*-(Adamantan-1-yl)-1,1,1,3,3,3-hexamethyl-2-(trimethylsilyl)trisilan-2-amine

APhOs Pd G3 - palladium G3-(4-(*N,N*-Dimethylamino)phenyl)di-*tert*-butylphosphine, [4-(Di-*tert*-butylphosphino)-*N,N*-dimethylaniline-2-(2'-aminobiphenyl)]palladium(II) methanesulfonate

DBU - 1,8-diazabicyclo(5.4.0)undec-7-ene

DCM - dichloromethane

DMF - *N,N*-dimethylformamide

dppf Pd G3 - methanesulfonato 1,1-ferrocenediyl-bis(diphenylphosphino) (2'-amino-1,1'-biphenyl-2-yl) palladium(II)

EtOAc - ethyl acetate

i-PrOH - isopropanol

IPAm - isopropylamine

[Ir(dtbppy)(ppy)₂](PF₆) - [4,4'-*Bis*(1,1-dimethylethyl)-2,2'-bipyridine-*N*1,*N*1']bis[2-(2-pyridinyl-*N*)phenyl-C]iridium(III) hexafluorophosphate

K₂CO₃ - potassium carbonate

MeCN – acetonitrile

MeOH - methanol

Na₂CO₃ - sodium carbonate

NaH - sodium hydride

NH₄Cl - ammonium chloride

NMI – *N*-methylimidazole

PdCl₂(dppf)-DCM adduct - palladium chloride-1,1-ferrocenediyl-bis(diphenylphosphino)-dichloromethane adduct

PE – petroleum ether

TCFH – *N,N,N',N'*-tetramethylchloroformamidinium hexafluorophosphate

TFA - trifluoroacetic acid

THF - tetrahydrofuran

TIPSOTf - Triisopropylsilyl trifluoromethanesulfonate

TsOH - *p*-Toluenesulfonic acid

Biological Examples

Example A

Functional WRN unwinding activity can be measured using a fluorogenic plate based 384 well assay configured to measure the separation of labeled double stranded DNA substrate. Compounds were dosed out in neat DMSO with a 1:3 serial dilution scheme. 100 nL of compound was stamped into Greiner low volume black assay plates (Greiner Cat#784076) using the Echo Acoustic Dispenser to generate assay ready plates. All solutions were prepared in assay buffer (25 mM TRIS (pH8.0), 5 mM NaCl, 2 mM MgCl₂, 1 mM DTT, 0.05% BSA) for this 10 µL low volume reaction. To prepare the solutions, a 2X WRN Enzyme cocktail was made containing 200 pM of recombinant full-

length WRN protein (1-1432). A 2X Substrate cocktail was made to consist of both 200 μ M ATP (any ultrapure ATP sample) and 12 nM of the fluorescent quenched labeled double stranded DNA oligomer (IDT Custom synthesis; 5'-5IABkFQ (SEQ ID NO. 1)/GCA CTG GCC GTC GTT TTA CGG TCG TGA CT-3' (SEQ ID NO. 2): 5'-TTT TTT ACT TAA CGA CGG CCA GTG C (SEQ ID NO. 3)/36-TAMTSP/-3' (SEQ ID NO. 4)). To start the reaction, 5 μ L of assay buffer was added to a single column to serve as the low control. Following this, 5 μ L of 2X WRN Enzyme was added in all wells except the buffer low control wells. The reaction plate was covered and incubated at ambient temperature for 4 hours to allow for time dependent inhibition if it existed. After 4 hours, the addition of 5 μ L of 2X-ATP/DNA substrate cocktail was added across all wells of the assay plate. This initiated the reaction, as the plate was incubated at ambient temperature for 60 minutes for the unwinding reaction to occur. A 10 mM EDTA solution was prepared and added at 5 μ L across the entire plate after 60 minutes to quench the samples for an endpoint measurement. Fluorescent intensity was measured using excitation and emission wavelengths of 525 nm and 598 nm, respectively. High florescent intensity (DMSO with buffer) represents full inhibition of unwinding activity and low florescent intensity (DMSO with enzyme) represents no inhibition of unwinding activity. The potency of the compounds was determined using a four-parameter inhibition model to generate pIC₅₀, Hill Slope, maximum inhibition (see Table 2).

Example B

SW48 human colorectal cancer cells (MSI-H cancer cells) were obtained from the American Type Culture Collection and maintained in growth medium composed of RPMI-1640 supplemented with 10% fetal bovine serum. Cells were maintained in a humidified 5% CO₂ incubator at 37 °C. Cell dissociation was conducted using TrypLE Select to detach cells from culture flasks. Following dissociation, cells were centrifuged briefly and resuspended in assay medium RPMI-1640 +10% fetal bovine serum. Black 384-well assay plates were prepared from source plates of DMSO and compounds dissolved in DMSO prior to cell dispensation. These source plates were prepared in eleven point, 3-fold serial dilutions from 10 mM stocks stored at 4 °C. Compounds and DMSO controls were dispensed into assay plates at 150 nanoliters per well from source

plates using Labcyte Echo 555 or Echo 655T dispensers with Column 6 and Column 18 containing DMSO only. Cells were then seeded into prepared assay plates in 50 microliters assay medium at 250 cells per well into Columns 1 thru 17 and Columns 19-24. Assay medium without cells was added to column 18 to serve as a surrogate for 100% cell cytotoxicity. Following incubation for 120 hours in a humidified 5% CO₂ incubator at 37 °C, cellular ATP concentration of each well was measured and used as a surrogate of cell viability. This was performed by adding 50 microliters of CellTiter-Glo reagent to each well followed by a 10-minute incubation at room temperature. Luminescence was measured on a Synergy Neo or Synergy Neo2 multimode plate reader. Compound cytotoxic response (%C) was normalized to the robust means of column 6 (Control 1, 0% Cytotoxicity) and column 18 (Control 2, 100% Cytotoxicity) and normalized % cytotoxicity (%C) calculated. $\%C = 100 - 100 * ((\text{Compound} - \text{Control 2}) / (\text{Control 1} - \text{Control 2}))$. These normalized data were then used to determine a 50% cytotoxic response (CC₅₀) using 4-parameter logistic curve model 201 in Activity Base XE:

Four (4) Parameter Logistic Model fit = $A + (B - A) / (1 + (10^C / x)^D)$,

where A = minimum asymptote (% cytotoxicity), B = maximum asymptote (% cytotoxicity), C = Log CC₅₀ (pCC₅₀), D = Hill slope, and x = [inhibitor], (M).

WRN unwinding activity data and cell viability data are shown in Table 2.

Table 2.

Example No.	4 hr UNW pIC ₅₀	SW48 pCC ₅₀
81	8.1	5.9
83	8.6	6.6
84	6.8	5.3
137	6.6	
138	8.5	6.6
250	8.2	6.5
305	6.5	
306	8.3	7.0

Example C

It was observed that the compounds of the present disclosure are bound to cell assay media to various degrees, which would affect the cellular potency analysis in Example B. To measure free drug potency, concentrations used in the cell assays were corrected with the measured free fraction determined in the cell assay media. Compounds were incubated in a rapid equilibrium dialysis (RED) at a nominal concentration of 1 μ M, using a dialysis buffer of phosphate-buffered saline (PBS, 100 mM sodium phosphate + 150 mM sodium chloride, pH 7.4). The test compound was spiked into the medium. Aliquots were added to the donor wells of a RED plate in triplicate, and blank PBS was added to each receiver well, at volumes recommended by the manufacturer. Plates were incubated with shaking at 200 rpm for 4 hours at 37°C. The resultant samples were analysed by LC-MS/MS using a specific and sensitive method. Results were reported as analyte:internal standard peak area ratios (PARs), and the percent unbound was calculated with the following equation:

$$\% \text{Unbound} = 100 \times ((1/D)) / \{ [1 / (\text{Apparent } F_u)] - 1 \} + 1/D,$$

where D = the matrix dilution factor (D = 1 for media); Apparent $F_u = C_{\text{rec}}/C_{\text{don}}$; C_{rec} = PAR in receiver samples; C_{don} = PAR in donor samples.

Percent stability was calculated by:

$$\% \text{Stability} = 100 \times C_{\text{final}}/C_{\text{initial}},$$

where C_{final} = PAR in the T=240 minute sample; C_{initial} = PAR in the T=0 minute sample.

Cell culture media unbound percentage and calculated free drug potency of selected compounds along with their measured potency from Example B are shown in Table 3.

Table 3.

Example No.	SW48 pCC ₅₀	Cell Culture Media Binding (% unbound)	Free Drug SW48 pCC ₅₀
83	6.6	6.5	7.89
138	6.6	6.7	7.77
250	6.5	2.7	8.07
306	7.0	10.3	7.99

Example D

In vivo efficacy study of test compounds in mouse model bearing SW48 human colorectal cancer cell line xenografts

Experiments were performed with female Crl:Nu-Foxn1^{nu} nude mice. Animals were housed under optimized hygienic condition with food and water at libitum and a 12h:12h light:dark cycle. Animals were allowed to acclimatize for at least 4 days before being enrolled in the experimental design.

SW48 human colorectal cancer cells (RRID:CVCL1724) were obtained from ATCC. The cells were cultured in RPMI 1640 with 10% Fetal Bovine Serum at 37°C in atmosphere of 5% CO₂ in air. To establish SW48 xenografts, cells were harvested and re-suspended in serum free RPMI1640 and Matrigel before inoculation and 5 x 10⁶ SW48 tumor cells were injected subcutaneously in 100 µL volume using 27G1/2 needle. Cells were inoculated in the right hind flank of the animals.

Post cell inoculation, tumor growth was monitored daily, and animals were randomized into treatment groups (n=10/group) once the tumor volume reached an appropriate size (80-150 mm³). Throughout the treatment period, both tumor volume and body weight were measured approximately twice a week. The tumor size, in mm³, was computed using the formula: $0.5 \times L \times W^2$, where W = width and L = length of the tumor.

To prepare a test compound for oral administration, between 30-120 mg of the test compound was dissolved in a vehicle solution consisting of 0.5% Methycellulose (cp 400) and 0.5% Tween 80. The compound was freshly prepared one hour prior to daily administration. If turbidity was observed, the solution was vortexed at over 2000 rpm for 10 minutes and mixed with a 1 mL syringe. The resulting solution formulation was utilized for in vivo studies.

Animals received either a vehicle or test compounds at dosage of 30 or 100 mg/kg daily through oral gavage (10ml/kg). Body weight was measured twice a week using the Ohaus electronic scale (STX421) and the data was automatically added to the study log. Moreover, daily clinical observations were performed to closely monitor the animals. Tumor growth inhibition was computed using the following formula:

$$\text{Mean \% } \Delta \text{ Inhibition} = \frac{[(\text{Mean}(C) - \text{Mean}(C_0)) - (\text{Mean}(T) - \text{Mean}(T_0))]}{(\text{Mean}(C) - \text{Mean}(C_0))} \times 100\%$$

where T = current group value; T_0 = Current group initial value; C = Control group Value; C_0 = control group initial value.

Data of tumor volume were exported and analyzed in GraphPad Prism (9.5). The analysis included a two-way ANOVA test, comparing the vehicle control vs. treatments at different time points, followed by a Tukey HSD post hoc test.

The calculation of tumor regression was conducted as per the following formula:

$$\text{Tumor Regression} = (\Delta \text{ tumor volume} / \text{tumor volume at Day 0}) * 100,$$

where Day 0 = treatment start day; Δ tumor volume = mean tumor volume on the evaluation day - mean tumor volume Day 0.

Treatment commenced using the compound of Example 138 at a dosage of 30 mpk, which was administered orally once a day when the average tumor volume reached 117.6 mm³ (n=10/group). The course of treatment with Example 138 lasted for 21 days, after which its overall efficacy was evaluated based on changes in tumor volume, as illustrated in Figure 1. On the 21st day, a mean tumor regression of -74.26% was observed.

The efficacy data associated with the compound of Example 83 is depicted in Figure 2. Treatment was initiated with a daily oral administration of the compound at a dosage of 100 mpk, once the average tumor volume reached 97.2 mm³ (n=10/group). By the 55th day, a significant mean tumor regression of -24.01% was observed in the SW48 tumor. The efficacy data for the compound of Example 250 is depicted in Figure 3. Daily oral administration of the compound at a dosage of 30 mpk was initiated when the average tumor volume reached 103.18 mm³ (n=10/group). By the 21st day, a significant tumor growth inhibition of 72.95% compared to the vehicle control was observed in the SW48 tumors (p<0.0001).

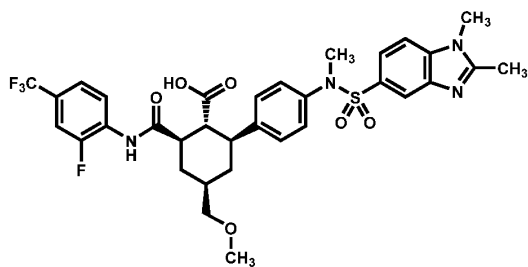
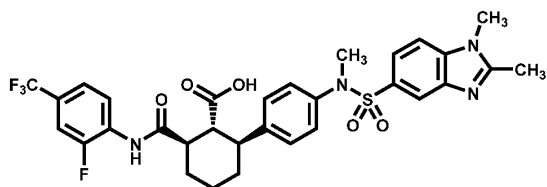
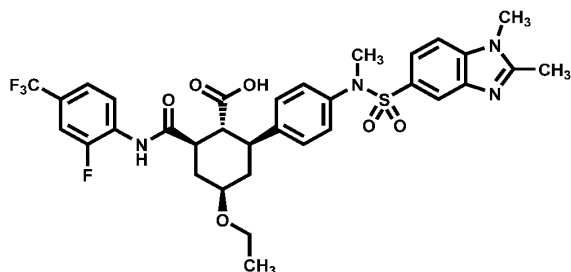
The efficacy data for the compound of Example 306 is depicted in Figure 4. Daily oral administration of the compound at a dosage of 30 mpk was initiated when the average tumor volume was 103.13 mm³ (n=10/group). By the 23rd day, a significant mean tumor regression of -73.2% was observed in the SW48 tumor.

All patent applications, patents, and printed publications cited herein are incorporated herein by reference in the entireties, except for any definitions, subject matter

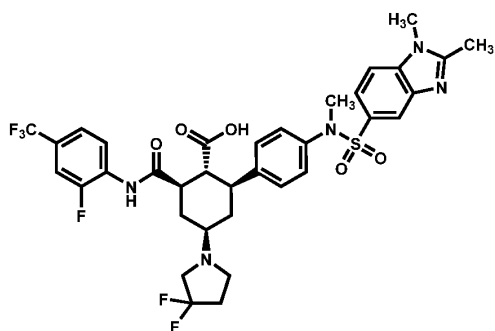
disclaimers or disavowals, and except to the extent that the incorporated material is inconsistent with the express disclosure herein, in which case the language in this disclosure controls.

What is Claimed:

1. A compound which is selected from the group consisting of:

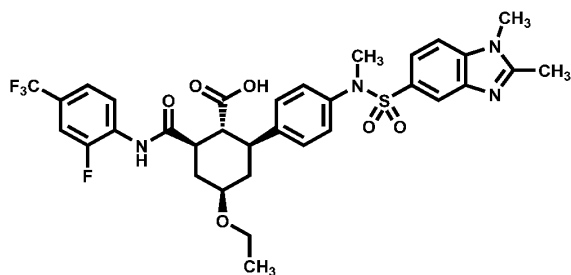


, and



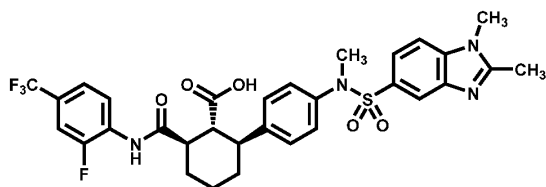
or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1, having the structure:



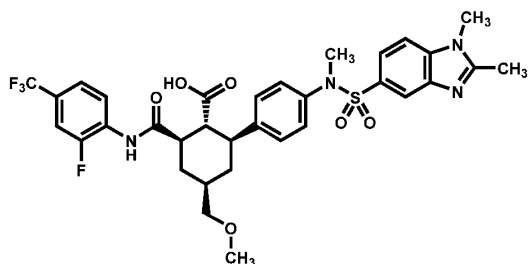
or a pharmaceutically acceptable salt thereof.

3. A compound of claim 1, having the structure:



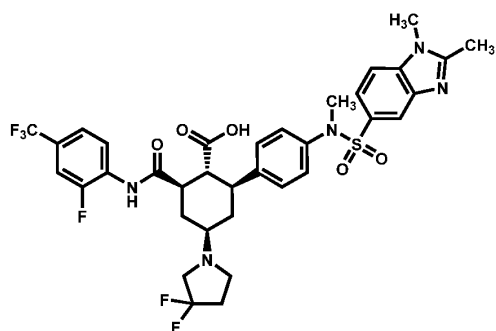
or a pharmaceutically acceptable salt thereof.

4. A compound of claim 1, having the structure:



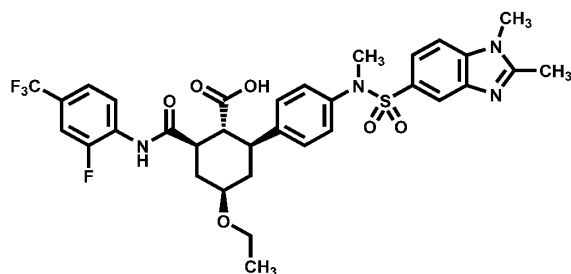
or a pharmaceutically acceptable salt thereof.

5. A compound of claim 1, having the structure:

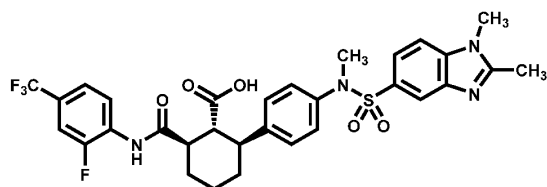


or a pharmaceutically acceptable salt thereof.

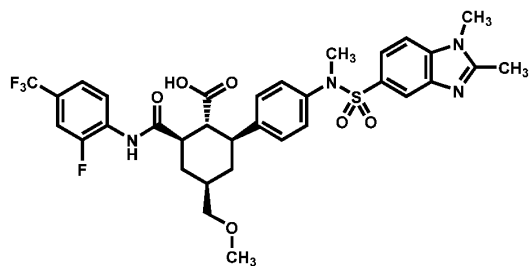
6. A compound of claim 1, having the structure:



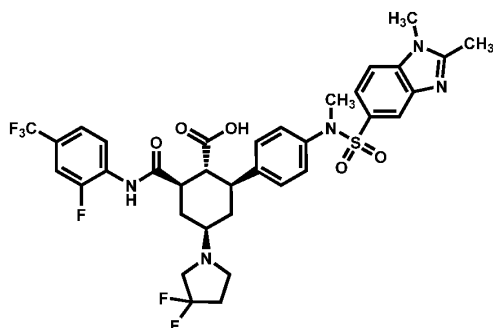
7. A compound of claim 1, having the structure:



8. A compound of claim 1, having the structure:



9. A compound of claim 1, having the structure:



10. A pharmaceutical composition comprising a compound or pharmaceutically acceptable salt thereof of any one of claims 1 to 9 and a pharmaceutically acceptable excipient.
11. A compound or pharmaceutically acceptable salt thereof of any one of claims 1 to 9 or a pharmaceutical composition of claim 10, for use in therapy.
12. A compound or pharmaceutically acceptable salt thereof of any one of claims 1 to 9 or a pharmaceutical composition of claim 10, for use in the treatment of cancer.
13. A method of treatment of cancer in a subject in need thereof comprising administering to said subject a therapeutically effective amount of a compound or pharmaceutically acceptable salt thereof of any one of claims 1 to 9 or a pharmaceutical composition of claim 10.
14. A method of treatment of cancer of claim 13, wherein the cancer is characterised by MSI-H and/or dMMR.
15. A method of treatment of cancer of claim 13, wherein the cancer is treatable by inhibition of WRN.
16. Use of a compound or pharmaceutically acceptable salt thereof of any one of claims 1 to 9 or a pharmaceutical composition of claim 10, in the manufacture of a medicament for use in the treatment of cancer.