

**10,11-DIFLUORO-12-((2-FLUORO-4-IODOPHENYL)AMINO)-5,6-DIHYDROXY-
4,5,6,7-TETRAHYDRO-1H-SPIRO[BENZO[B][1,5,4]OXATHIAZECINE-3,1'-
CYCLOPROPANE] 2,2-DIOXIDE STEREOISOMERS, AND COMPOSITIONS
COMPRISING AND METHODS OF USING THE SAME**

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/722,523, filed November 19, 2024 and of U.S. Provisional Application Serial No 63/618,167, filed January 05, 2024, the disclosures of which are incorporated by reference herein in their entirety.

2. FIELD

[0002] Provided herein are 10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide stereoisomer compounds. Pharmaceutical compositions comprising such compounds and methods of use of such compounds for treating, preventing, and managing various disorders are also provided herein.

3. BACKGROUND

[0003] Mitogen-activated protein kinase (MAPK) is relevant to many cancers. MAPKs specifically phosphorylate serine/threonine residues of proteins, that are activated by a variety of external stimuli (for example, mitogens and growth factors) to manifest its actions inside the cell. The activation of MAPKs regulates many functions of the cells with physiological implications such as cell growth, survival, apoptosis, differentiation, proliferation and gene expression. (G. Pearson, et al., *Endocr. Rev.*, 2001, 153-183.)

[0004] MEK1 and 2 are two human kinases in the middle of the MAPK-cascade involving upstream Rat Sarcoma Virus (RAS)- Rapidly accelerated Fibrosarcoma (RAF) and downstream extracellular regulated kinases (ERK)s.. This signal transduction cascade resulting in phosphorylation of ERKs is extensively studied in cancer pathology. Human cancers frequently harbor mutations in RAS oncogene family members, which drive oncogenesis by increasing cellular proliferation and survival. These are small protein GTPases, regulated by a switch between active GTP-linked and inactive GDP-bound states that is governed by a complex

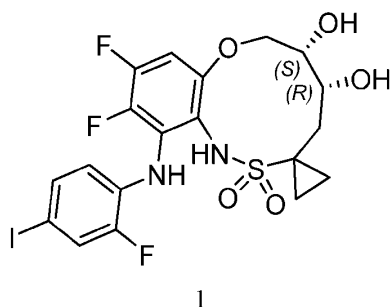
network of guanine exchange factors (GEFs, favoring RAS-GTP) and GTPase activating factors (GAPs, favoring RAS-GDP). (Cox AD, et al., *Nat Rev Drug Discov.* 2014;13:828–51.) RAS activation either due to extrinsic recruitment by transmembrane tyrosine kinase receptors or intrinsic mutations propagates through the downstream RAF-MEK-ERK and PI3K-AKT signaling pathways. Besides RAS-activating mutations that confer independence from physiological regulators, human cancers harbor mutations in other RAS network genes such as NF1 (encoding neurofibromin, a RAS GAP), BRAF, or PTPN11 (encoding the SHP2 tyrosine phosphatase involved in RAS activation). (Decroocq J, et al., *Leukemia* (2022) 36:1237–1252.)

[0005] Several non-ATP-competitive, allosteric MEK1/2 inhibitors have been developed and assessed in clinical studies, primarily for cancers in which ERK1/2 signaling is aberrantly activated and four of them are FDA approved drugs. (Zhao Y, Adjei AA. *Nat Rev Clin Oncol.* 2014;11:385; Caunt CJ, Sale MJ, Smith PD, Cook SJ. *Nat Rev Cancer.* 2015;15:577; Shang J, Lu S, Jiang Y, Zhang J. *Chem Biol Drug Des.* 2016;88:485; Hao C, et al., *Eu. J. Med. Chem.* 2023, 251: 115236; Heinzerling L., *ESMO Open* 2019;4:e000491.doi:10.1136/esmoopen-2019-000491; Stalneck CA et al., *Sci Signal.* 2020, 13(624): 1-14; Echevarria-Vargas IM et al., *Melanoma Manag.* (2017) 4(4), 183–186; Wang A et al., *IUBMB Life*, 2013, 65(9):748–758.). These inhibitors are selective towards MEK1/2, as they bind to non-ATP-competitive allosteric sites. In addition to cancer, MEK1/2 inhibitors have potential to be useful in other diseases.

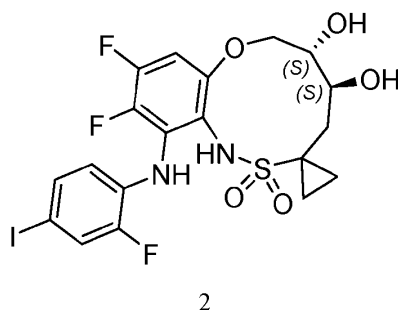
[0006] The compound chemically named 10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide belongs to a novel class of MEK1/2 inhibitors having a macrocyclic scaffold, which has been disclosed in US 9,034,861, which is herein incorporated by reference in its entirety.

4. SUMMARY

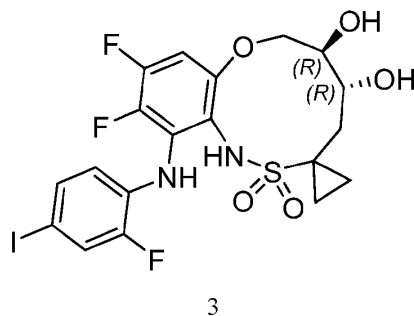
[0007] Provided herein is a pure Compound 1, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof.



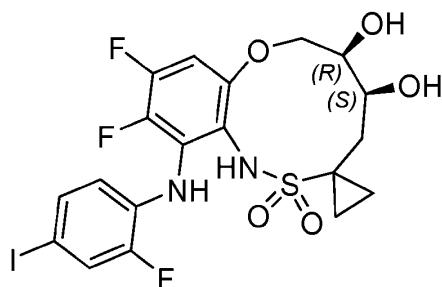
[0008] Also provided herein is a pure Compound 2, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



[0009] Also provided herein is a pure Compound 3, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



[0010] Also provided herein is a pure Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



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[0011] Also provided are pharmaceutical compositions formulated for administration by an appropriate route and means containing effective concentrations of Compound 1, Compound 2, Compound 3, or Compound 4 provided herein, and optionally comprising at least one pharmaceutical carrier.

[0012] In one embodiment, the pharmaceutical compositions deliver amounts effective for the treatment of diseases or disorders in which the RAS pathway is upregulated such as cancer, RASopathies (*e.g.*, Noonan syndrome, Costello syndrome, etc) and laminopathies (*e.g.*, cardiomyopathies, muscular dystrophy, dysplasia, etc.). In one embodiment, the pharmaceutical compositions deliver amounts effective for the treatment and/or prevention of cancer, RASopathies and laminopathies. In one embodiment, the pharmaceutical compositions deliver amounts effective for the amelioration of cancer, RASopathies and laminopathies.

[0013] In one embodiment, provided herein are methods of treating diseases or disorders in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies comprising administering the compounds or pharmaceutical compositions provided herein. Also provided herein are combination therapies using the compounds or pharmaceutical compositions provided herein, in combination with a therapy, *e.g.*, another pharmaceutical agent with activity against diseases or disorders in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies or their symptoms. Examples of therapies within the scope of the methods include, but are not limited to, surgery, chemotherapy, radiation therapy, biological therapy, stem cell transplantation, cell therapy, and combinations thereof.

[0014] These and other aspects of the subject matter described herein will become evident upon reference to the following detailed description.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 provides a PK plasma curve at day 1 of Compound 1 when tested in clinical trial setting.

[0016] FIG. 2 provides a PK plasma curve at day 22 of Compound 1 when tested in clinical trial setting.

6. DETAILED DESCRIPTION

6.1 Definitions

[0017] As used herein, and in the specification and the accompanying claims, the indefinite articles “a” and “an” and the definite article “the” include plural as well as single referents, unless the context clearly indicates otherwise.

[0018] As used herein, the terms “comprising” and “including” can be used interchangeably. The terms “comprising” and “including” are to be interpreted as specifying the presence of the stated features or components as referred to, but does not preclude the presence or addition of one or more features, or components, or groups thereof. Additionally, the terms “comprising” and “including” are intended to include examples encompassed by the term “consisting of”. Consequently, the term “consisting of” can be used in place of the terms “comprising” and “including” to provide for more specific embodiments of the invention.

[0019] The term “consisting of” means that a subject-matter has at least 90%, 95%, 97%, 98% or 99% of the stated features or components of which it consists. In another embodiment the term “consisting of” excludes from the scope of any succeeding recitation any other features or components, excepting those that are not essential to the technical effect to be achieved.

[0020] As used herein, the term “or” is to be interpreted as an inclusive “or” meaning any one or any combination. Therefore, “A, B or C” means any of the following: “A; B; C; A and B; A and C; B and C; A, B and C”. An exception to this definition will occur only when a combination of elements, functions, steps or acts are in some way inherently mutually exclusive.

[0021] As used herein, the term “about” or “approximately” means an acceptable error for a particular value as determined by those skilled in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term “about” or “approximately” means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term “about” or

“approximately” means within 50%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value or range.

[0022] As used herein, and unless otherwise indicated, a chemical compound or composition that is “substantially free” of another chemical compound or composition means that the compound or composition contains, in certain embodiments, less than about 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05%, or 0.01% by weight of the other compound or composition.

[0023] As used herein, and unless otherwise specified, a compound that is “substantially chemically pure” is substantially free from other chemical compounds (*i.e.*, chemical impurities). In certain embodiments, a compound that is substantially chemically pure contains less than about 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05%, or 0.01% of one or more other chemical compounds on a weight basis. The detection of other chemical compounds can be accomplished by any method apparent to a person of ordinary skill in the art, including, but not limited to, methods of chemical analysis, such as, *e.g.*, mass spectrometry analysis, spectroscopic analysis, thermal analysis, elemental combustion analysis and/or chromatographic analysis.

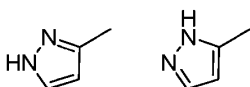
[0024] As used herein and unless otherwise indicated, the terms “pure” when applied to a chiral compound or “stereoisomer” or “stereomerically pure” means one stereoisomer of a compound disclosed herein that is substantially free of other stereoisomers of that compound. For example, a stereomerically pure compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically pure compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, or greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. The compounds disclosed herein have chiral centers and can occur as individual enantiomers or diastereomers, and

mixtures thereof. All such isomeric forms are included within the embodiments disclosed herein, including mixtures thereof.

[0025] The use of stereomerically pure forms of such compounds, as well as the use of mixtures of those forms, are encompassed by the embodiments disclosed herein. For example, mixtures comprising equal or unequal amounts of the enantiomers of a particular compound may be used in methods and compositions disclosed herein. These isomers may be asymmetrically synthesized or resolved using standard techniques such as chiral columns or chiral resolving agents. See, e.g., Jacques, J., *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., *et al.*, *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN, 1972).

[0026] As used herein and unless otherwise indicated, the term “prodrug” means a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (*in vitro* or *in vivo*) to provide an active compound, particularly a compound disclosed herein. Examples of prodrugs include, but are not limited to, derivatives and metabolites of a compound disclosed herein that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. In certain embodiments, prodrugs of compounds with carboxyl functional groups are the lower alkyl esters of the carboxylic acid. The carboxylate esters are conveniently formed by esterifying any of the carboxylic acid moieties present on the molecule. Prodrugs can typically be prepared using well-known methods, such as those described by *Burger's Medicinal Chemistry and Drug Discovery* 6th ed. (Donald J. Abraham *ed.*, 2001, Wiley) and *Design and Application of Prodrugs* (H. Bundgaard *ed.*, 1985, Harwood Academic Publishers Gmhf).

[0027] “Tautomers” refers to isomeric forms of a compound that are in equilibrium with each other. The concentrations of the isomeric forms will depend on the environment the compound is found in and may be different depending upon, for example, whether the compound is a solid or is in an organic or aqueous solution. For example, in aqueous solution, pyrazoles may exhibit the following isomeric forms, which are referred to as tautomers of each other:



[0028] Unless otherwise specified, the terms “solvate” and “solvated,” as used herein, refer to a solid form of a substance which contains solvent. The terms “hydrate” and “hydrated” refer to a solvate wherein the solvent comprises water. The terms “solvate” and “solvated,” as used herein, can also refer to a solvate of a salt, co-crystal, or molecular complex. The terms “hydrate” and “hydrated,” as used herein, can also refer to a hydrate of a salt, co-crystal, or molecular complex.

[0029] The term “isotopologue” of a compound described herein refers to a compound that can contain unnatural proportions of atomic isotopes at one or more of the atoms. For example, an isotopologue of Compounds 1 to 4 may be radiolabeled at one more positions with radioactive isotopes, such as for example tritium (^3H), and/or carbon-14 (^{14}C), or may be isotopically enriched at one or more positions, such as with deuterium (^2H), carbon-13 (^{13}C), oxygen-18 (^{18}O) and/or nitrogen-15 (^{15}N). In certain embodiments, a compound described herein can be radiolabeled at one more positions with radioactive isotopes, such as for example tritium (^3H), and/or carbon-14 (^{14}C), while also being isotopically enriched at one or more positions, such as with deuterium (^2H), carbon-13 (^{13}C), oxygen-18 (^{18}O) and/or nitrogen-15 (^{15}N).

[0030] All isotopic variations of Compounds 1 to 4, whether radioactive or not, are intended to be encompassed within the scope of the embodiments provided herein. In some embodiments, there are provided isotopologues of Compounds 1 to 4, for example, the isotopologues are deuterium, carbon-13, or nitrogen-15 enriched Compounds 1 to 4.

[0031] With regard to the compounds provided herein, when a particular atomic position is designated as having deuterium or “D,” it is understood that the abundance of deuterium at that position is substantially greater than the natural abundance of deuterium, which is about 0.0156%. A position designated as having deuterium typically has a minimum isotopic enrichment factor of, in particular embodiments, at least 100 (1.56% deuterium incorporation), at least 500 (7.8% deuterium incorporation), at least 1000 (15.6% deuterium incorporation), at least 2000 (31.2% deuterium incorporation), at least 3000 (46.8% deuterium incorporation), at least 3500 (54.6% deuterium incorporation), at least 4000 (62.4% deuterium incorporation), at least 4500 (70.2% deuterium incorporation), at least 5000 (78% deuterium incorporation), at least

5500 (85.8% deuterium incorporation), at least 6000 (93.6% deuterium incorporation), at least 6089.7 (95% deuterium incorporation), at least 6217.9 (97% deuterium incorporation), at least 6346.2 (99% deuterium incorporation), or at least 6378.2 (99.5% deuterium incorporation) at each designated deuterium atom.

[0032] The isotopic enrichment and isotopic enrichment factor of the compounds provided herein can be determined using conventional analytical methods known to one of ordinary skill in the art, including mass spectrometry and nuclear magnetic resonance spectroscopy.

[0033] As used herein, and unless otherwise specified, the term “pharmaceutically acceptable salts” refers to salts prepared from pharmaceutically acceptable, relatively non-toxic acids, including inorganic acids and organic acids. In certain embodiments, suitable acids include, but are not limited to, acetic, adipic, 4-aminosalicylic, ascorbic, aspartic, benzenesulfonic, benzoic, camphoric, camphorsulfonic, capric, caproic, caprylic, cinnamic, carbonic, citric, cyclamic, dihydrogenphosphoric, 2,5-dihydroxybenzoic (gentisic), 1,2-ethanedisulfonic, ethanesulfonic, fumaric, galactunoric, gluconic, glucuronic, glutamic, glutaric, glycolic, hippuric, hydrobromic, hydrochloric, hydriodic, isobutyric, isethionic, lactic, maleic, malic, malonic, mandelic, methanesulfonic, monohydrogencarbonic, monohydrogen-phosphoric, monohydrogensulfuric, mucic, 1,5-naphthalenedisulfonic, nicotinic, nitric, oxalic, pamoic, pantothenic, phosphoric, phthalic, propionic, pyroglutamic, salicylic, suberic, succinic, sulfuric, tartaric, toluenesulfonic acid, and the like (*see, e.g., S. M. Berge et al., J. Pharm. Sci.*, 66:1-19 (1977); and *Handbook of Pharmaceutical Salts: Properties, Selection and Use*, P. H. Stahl and C. G. Wermuth, Eds., (2002), Wiley, Weinheim). In certain embodiments, suitable acids are strong acids (*e.g., with pKa less than about 1*), including, but not limited to, hydrochloric, hydrobromic, sulfuric, nitric, methanesulfonic, benzene sulfonic, toluene sulfonic, naphthalene sulfonic, naphthalene disulfonic, pyridine-sulfonic, or other substituted sulfonic acids. Also included are salts of other relatively non-toxic compounds that possess acidic character, including amino acids, such as aspartic acid and the like, and other compounds, such as aspirin, ibuprofen, saccharin, and the like. Acid addition salts can be obtained by contacting the neutral form of a compound with a sufficient amount of the desired acid, either neat or in a suitable solvent.

[0034] As used herein and unless otherwise indicated, the terms “treat,” “treating” and “treatment” refer to alleviating or reducing the severity of a symptom associated with the disease or condition being treated, for example, cancer.

[0035] The term “prevention” includes the inhibition of a symptom of the particular disease or disorder, for example cancer. Generally, the term “preventing” refers to administration of the drug prior to the onset of symptoms, particularly to patients at risk of cancer.

[0036] As used herein and unless otherwise indicated, the term “managing” encompasses preventing the recurrence of the particular disease or disorder, such as cancer, in a patient who had suffered from it, lengthening the time a patient who had suffered from the disease or disorder remains in remission, reducing mortality rates of the patients, and/or maintaining a reduction in severity or avoidance of a symptom associated with the disease or condition being managed.

[0037] As used herein, “subject” or “patient” is an animal, typically a mammal, including a human, such as a human patient.

[0038] As used herein, and unless otherwise specified, the terms “therapeutically effective amount” and “effective amount” of a compound refer to an amount sufficient to provide a therapeutic benefit in the treatment, prevention and/or management of a disease, for example cancer, or to delay or minimize one or more symptoms associated with the disease or disorder to be treated. The terms “therapeutically effective amount” and “effective amount” can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disease or disorder, or enhances the therapeutic efficacy of another therapeutic agent.

[0039] The terms “co-administration” and “in combination with” include the administration of one or more therapeutic agents (for example, a compound provided herein and another cancer agent or supportive care agent) either simultaneously, concurrently or sequentially with no specific time limits. In one embodiment, the agents are present in the cell or in the patient’s body at the same time or exert their biological or therapeutic effect at the same time. In one embodiment, the therapeutic agents are in the same composition or unit dosage form. In another embodiment, the therapeutic agents are in separate compositions or unit dosage forms.

[0040] The term “supportive care agent” refers to any substance that treats, prevents or manages an adverse effect from treatment with Compound 1, or tautomers, or a pharmaceutically acceptable salt thereof.

[0041] In the context of a cancer, inhibition may be assessed by inhibition of disease progression, inhibition of tumor growth, reduction of primary tumor, relief of tumor-related symptoms, inhibition of tumor secreted factors, delayed appearance of primary or secondary tumors, slowed development of primary or secondary tumors, decreased occurrence of primary or secondary tumors, slowed or decreased severity of secondary effects of disease, arrested tumor growth and regression of tumors, increased Time To Progression (TTP), increased Progression Free Survival (PFS), increased Overall Survival (OS), among others. OS as used herein means the time from treatment onset until death from any cause. TTP, as used herein, means the time from treatment onset until tumor progression; TTP does not include deaths. In one embodiment, PFS means the time from treatment onset until tumor progression or death. In one embodiment, PFS means the time from the first dose of compound to the first occurrence of disease progression or death from any cause. In one embodiment, PFS rates will be computed using the Kaplan-Meier estimates. Event-free survival (EFS) means the time from treatment onset until any treatment failure, including disease progression, treatment discontinuation for any reason, or death. In one embodiment, overall response rate (ORR) means the percentage of patients who achieve a response. In one embodiment, ORR means the sum of the percentage of patients who achieve complete and partial responses. In one embodiment, ORR means the percentage of patients whose best response $\geq$ partial response (PR), according to the IMWG Uniform Response Criteria. In one embodiment, duration of response (DoR) is the time from achieving a response until relapse or disease progression. In one embodiment, DoR is the time from achieving a response $\geq$ partial response (PR) until relapse or disease progression. In one embodiment, DoR is the time from the first documentation of a response until to the first documentation of progressive disease or death. In one embodiment, DoR is the time from the first documentation of a response $\geq$ partial response (PR) until to the first documentation of progressive disease or death. In one embodiment, time to response (TTR) means the time from the first dose of compound to the first documentation of a response. In one embodiment, TTR means the time from the first dose of compound to the first documentation of a response $\geq$ partial response (PR). In the extreme, complete inhibition, is referred to herein as prevention or

chemoprevention. In this context, the term “prevention” includes either preventing the onset of clinically evident cancer altogether or preventing the onset of a preclinically evident stage of a cancer. Also intended to be encompassed by this definition is the prevention of transformation into malignant cells or to arrest or reverse the progression of premalignant cells to malignant cells. This includes prophylactic treatment of those at risk of developing a cancer.

[0042] The term “RASopathies” refers to a group of syndromes, also called conditions or disorders, caused by changes in genes that send signals across the Ras/mitogen-activated protein kinase (Ras/MAPK) pathway. While there are different RASopathies (currently, fewer than 10 disorders) and each syndrome has unique features, some common features include heart defects, skin, bone, eye and muscle problems, short stature, learning problems, differences in appearance and an increased risk of developing benign and cancerous tumors. RASopathies include, but are not limited to, Noonan Syndrome, Costello Syndrome, Cardiofaciocutaneous Syndrome, Legius Syndrome, Capillary Arteriovenous Malformation Syndrome, Hereditary gingival fibromatosis, SYNGAP1 syndrome, Leopard Syndrome, neurofibromatosis 1 (NF1), neurofibromatosis 2 (NF2) (*see*, National Cancer Institute: <https://dceg.cancer.gov/research/what-we-study/rasopathies>).

[0043] The term “Laminopathies” refers to a group of rare genetic disorders caused by mutations in genes encoding proteins of the nuclear lamina. They are included in the more generic term nuclear envelopathies that refers to diseases associated with defects of the nuclear envelope. Laminopathies and other nuclear envelopathies have a large variety of clinical symptoms including skeletal and/or cardiac muscular dystrophy, lipodystrophy and diabetes, dysplasia, dermo- or neuropathy, leukodystrophy, and progeria (premature aging). Most of these symptoms develop after birth, typically during childhood or adolescence. Some laminopathies however may lead to an early death, and mutations of lamin B1 (LMNB1 gene) may be lethal before or at birth. Types of known laminopathies and other nuclear envelopathies include, but are not limited to, Atypical Werner syndrome, Buschke–Ollendorff syndrome, Cardiomyopathy, Charcot–Marie–Tooth disease, Emery–Dreifuss muscular dystrophies, Greenberg dysplasia, Hutchinson–Gilford progeria syndrome (HGPS), Leukodystrophy, demyelinating, adult-onset, autosomal dominant (ADLD), Limb-girdle muscular dystrophy type 1B (LGMD1B), Lipodystrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, and

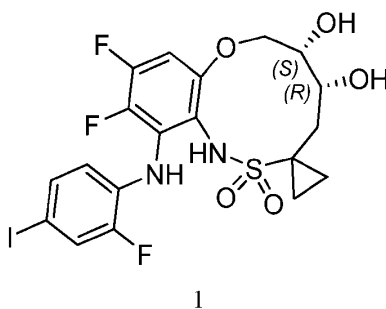
leukomelanodermic papules (LDHCP), Mandibuloacral dysplasia, Pelger–Huet anomaly (PHA), Restrictive dermopathy.

[0044] The term “Neurofibromatosis” or “Neurofibromatoses” refers to a group of genetic disorders, belonging to RASopathies as mentioned above, that cause tumors to form on nerve tissue. These tumors can develop anywhere in the nervous system, including the brain, spinal cord and nerves. There are three types of neurofibromatosis: neurofibromatosis 1 (NF1), neurofibromatosis 2 (NF2) and schwannomatosis. NF1 is usually diagnosed in childhood, while NF2 and schwannomatosis are usually diagnosed in early adulthood. The tumors in these disorders are usually noncancerous (benign), but sometimes can become cancerous (malignant). Symptoms are often mild. However, complications of neurofibromatosis can include hearing loss, learning impairment, heart and blood vessel (cardiovascular) problems, loss of vision, and severe pain. In certain embodiments, a subject who is diagnosed with NF1 has cutaneous and/or plexiform neurofibromas.

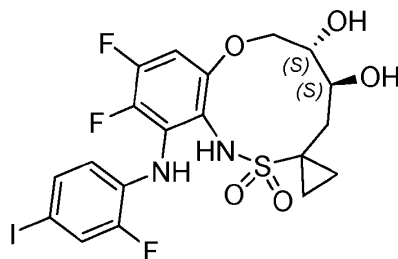
[0045] Unless otherwise specified, to the extent that there is a discrepancy between a depicted chemical structure of a compound provided herein and a chemical name of a compound provided herein, the chemical structure shall control.

COMPOUNDS

[0046] In certain embodiments, provided herein is Compound 1. In certain embodiments, provided herein is pure Compound 1 or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:

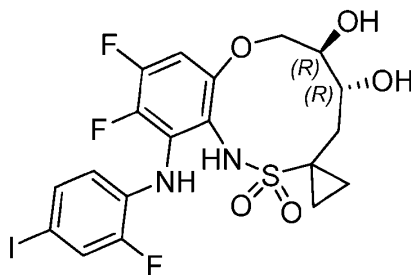


[0047] In certain embodiments, provided herein is Compound 2. In certain embodiments, provided herein is pure Compound 2 or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



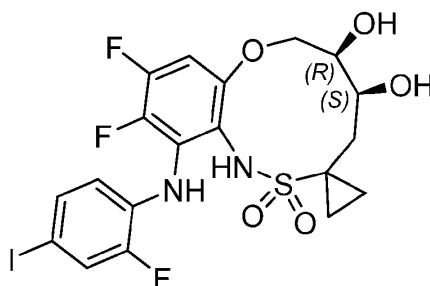
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[0048] In certain embodiments, provided herein is Compound 3. In certain embodiments, provided herein is pure Compound 3 or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



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[0049] In certain embodiments, provided herein is Compound 4. In certain embodiments, provided herein is pure Compound 4 or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



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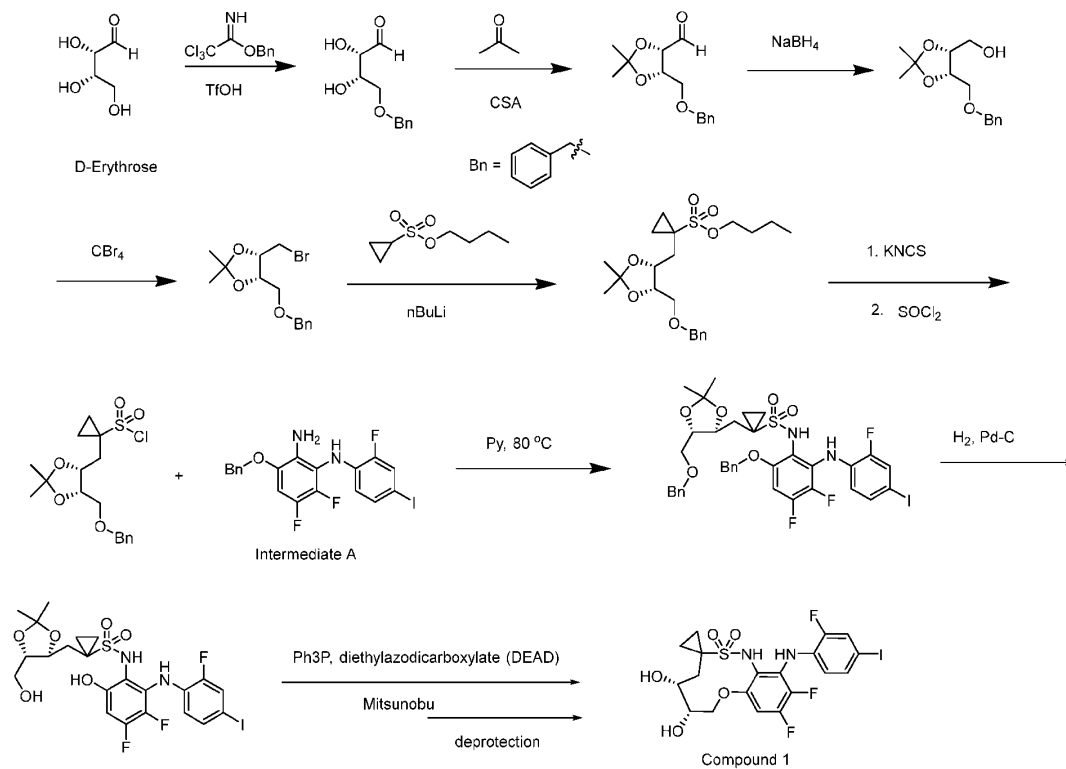
[0050] In certain embodiments, the compound disclosed herein (*e.g.* Compound 1, Compound 2, Compound 3, or Compound 4) is the stereomerically pure compound or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof. In certain embodiments, the compound according to Compound 1 to 4 is the stereomerically pure compound or a pharmaceutically acceptable salt thereof. In certain embodiments, the stereomerically pure compound comprises at least about 80% by weight of the designated stereoisomer and at most about 20% by weight of the other stereoisomer(s), at least about 90% by weight of the designated stereoisomer and at most about 10% by weight of the other stereoisomer(s), at least about 95% by weight of the designated stereoisomer and at most about 5% by weight of the other stereoisomer(s), at least about 96.6% by weight of the designated stereoisomer and at most about 3.4% by weight of the other stereoisomer(s), at least about 97% by weight of the designated stereoisomer and at most about 3% by weight of the other stereoisomer(s), at least about 99% by weight of the designated stereoisomer and at most about 1% by weight of the other stereoisomer(s), or at least about 99.9% by weight of the designated stereoisomer and at most about 0.1% by weight of the other stereoisomer(s). In certain embodiments, the weights are based upon total weight of the compound.

6.2 Methods of Making of the Compounds

[0051] Also provided herein are methods of preparing the compounds disclosed herein.

[0052] In one embodiment, Compound 1, provided herein can be made using conventional organic syntheses and commercially available starting materials. By way of example and not limitation, Compound 1 can be prepared as outlined in Scheme 1 and Scheme 1a, shown below. It should be noted that one skilled in the art would know how to modify the procedures set forth in the illustrative schemes to arrive at the desired product.

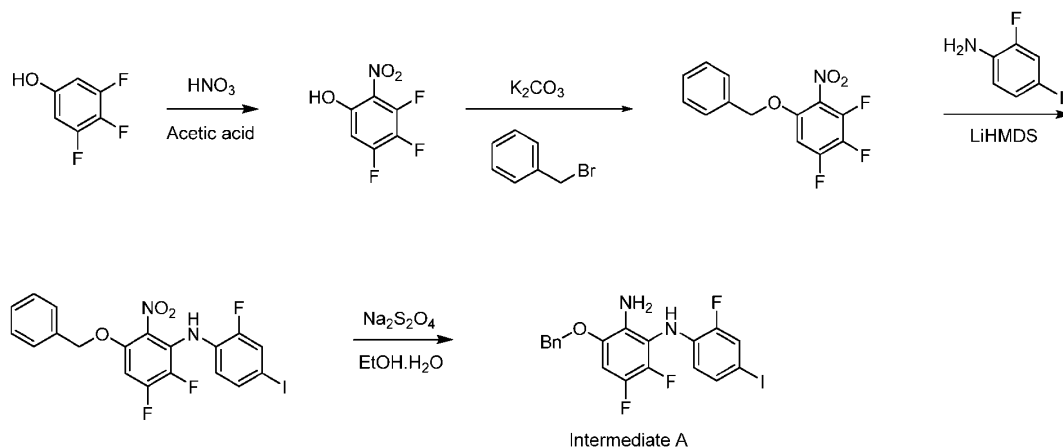
Scheme 1



[0053] It should be understood that the acetonide protecting group in Scheme 1 can be replaced with any other protecting group known in the art suitable for a diol.

Scheme 1a

[0054] Scheme 1a: Synthesis of Intermediate A:

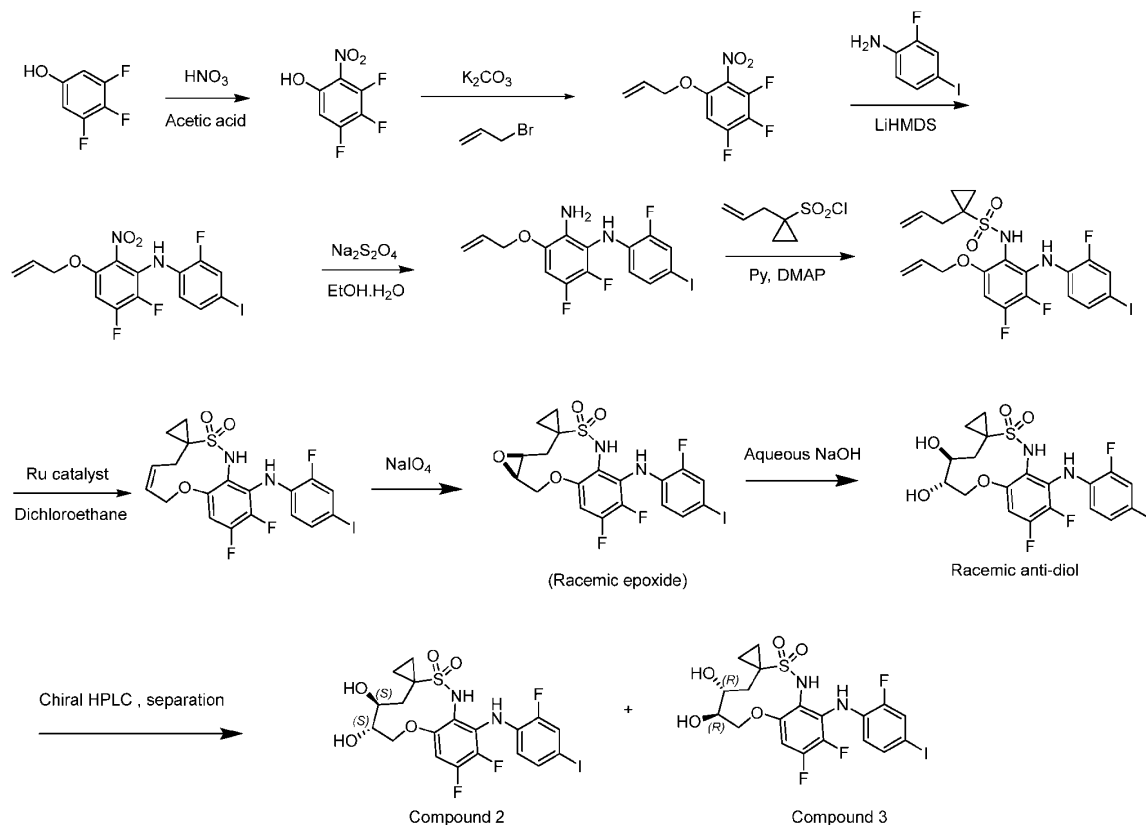


[0055] It should be understood that the benzyl protecting group in Scheme 1a can be replaced with any other protecting group known in the art suitable for a phenol group.

[0056] In one embodiment, Compound 4 can be prepared according to Scheme 1 and Scheme 1a using L-Erythrose instead of D-Erythrose.

[0057] In one embodiment, Compound 2 and Compound 3, provided herein can be made using conventional organic syntheses and commercially available starting materials. By way of example and not limitation, Compound 2 and Compound 3 can be prepared as outlined in Scheme 2, shown below. It should be noted that one skilled in the art would know how to modify the procedures set forth in the illustrative schemes to arrive at the desired products.

Scheme 2



6.3 Methods of Use

[0058] In one embodiment, provided herein is a method of treating a disease or disorder in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies, which comprises administering to a patient a compound disclosed herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In one embodiment, provided herein is a method of treating cancer. In one embodiment, provided herein is a compound disclosed herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) for use in a method of treating a disease or disorder in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies, wherein the method comprises administering said compound to

a patient. In one embodiment, provided herein is a compound disclosed herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) for use in a method of treating cancer.

[0059] In one embodiment, provided herein is a method of preventing a disease or disorder in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies, which comprises administering to a patient a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In one embodiment, provided herein is a method of preventing cancer. In one embodiment, provided herein is a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) for use in a method of preventing a disease or disorder in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies, wherein the method comprises administering said compound to a patient. In one embodiment, provided herein is a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) for use in a method of preventing cancer.

[0060] In one embodiment, provided herein is a method of managing a disease or disorder in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies, which comprises administering to a patient a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In one embodiment, provided herein is a method of managing cancer. In one embodiment, provided herein is a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) for use in a method of managing a disease or disorder in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies, wherein the method comprises administering said compound to a patient. In one embodiment, provided herein is a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) for use in a method of managing cancer.

[0061] In some embodiments, RASopathies include, but are not limited to, neurofibromatosis 1 (NF1), neurofibromatosis 2 (NF2), Noonan Syndrome, Costello Syndrome, Cardiofaciocutaneous Syndrome, Legius Syndrome, Capillary Arteriovenous Malformation Syndrome, Hereditary gingival fibromatosis, or SYNGAP1 syndrome. In certain embodiments, NF1 is associated with cutaneous and/or plexiform neurofibromas.

[0062] In some embodiments laminopathies include, but are not limited to, the Atypical Werner syndrome, Buschke–Ollendorff syndrome, Cardiomyopathy, Charcot–Marie–Tooth disease, Emery–Dreifuss muscular dystrophies, Greenberg dysplasia, Hutchinson–Gilford progeria syndrome (HGPS), Leukodystrophy, demyelinating, adult-onset, autosomal dominant (ADLD), Limb-girdle muscular dystrophy type 1B (LGMD1B), Lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, and leukomelanodermic papules (LDHCP), Mandibuloacral dysplasia, Pelger–Huet anomaly (PHA), or Restrictive dermopathy.

[0063] In one particular embodiment of the methods described herein, the disease or disorder is a neurofibromatosis (NF) related disorder. In some embodiments, the neurofibromatosis is neurofibromatosis 1 (NF1), neurofibromatosis 2 (NF2), or schwannomatosis. In some such embodiments, the disease or disorder is cancer. In one particular embodiment of the methods described herein, the disease or disorder is a Neurofibromatosis Type 1 (NF1)-associated cancer. In one particular embodiment, provided herein is a method of treating NF1 in a subject having NF1. In some embodiments, the methods provided herein comprise treating cutaneous and/or plexiform neurofibromas in NF1 in a subject having NF1. In one embodiment of the methods described herein, the disease or disorder is a Neurofibromatosis Type 2 (NF2)-associated cancer. In one embodiment of the methods described herein, the disease or disorder is schwannomatosis. In one embodiment of the methods described herein, the disease or disorder is a schwannomatosis-associated cancer.

[0064] In other aspects, provided herein is a method for the treatment, prevention or prophylaxis of cancer comprising administering to a subject in need thereof an effective amount of a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In certain embodiments, the cancer may be selected from brain cancer, breast cancer, lung cancer, ovarian cancer, pancreatic cancer, stomach cancer, prostate cancer, renal cancer,

colorectal cancer, melanoma, or leukemia. In further or additional embodiments, the cancer is brain cancer or achenocortical carcinoma. In further or additional embodiments, the cancer is breast cancer. In further or additional embodiments, the cancer is ovarian cancer. In further or additional embodiments, the cancer is pancreatic cancer. In further or additional embodiments, the cancer is stomach cancer. In further or additional emodiments, the cancer is prostate cancer. In further or additional embodiments, the cancer is renal cancer. In further or additional embodiments, the cancer is colorectal cancer. In further or additional embodiments, the cancer is myeloid leukemia. In further or additional embodiments, the cancer is glioblastoma. In further or additional embodiments, the cancer is follicular lymphona. In further or additional embodiments, the cancer is pre-B acute leukemia. In further or additional embodiments, the cancer is chronic lymphocytic B-leukemia. In further or additional embodiments, the cancer is mesothelioma. In further or additional embodiments, the cancer is small cell lung cancer. In a particular embodiment, the cancer is low grade serious ovarian cancer.

[0065] In some embodiments, provided herein is a method of inhibiting proliferation of a cell having a RAS mutation, comprising administering a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In some embodiments, the cancer is associated with a RAS mutation. In certain embodiments, provided herein is a method of inducing apoptosis in a cell having a RAS mutation, comprising administering a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In some embodiments, provided herein is a method of inhibiting proliferation of a cell having a KRAS mutation, comprising administering a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In some embodiments, the cancer is associated with a KRAS mutation. In some embodiments, provided herein is a method of inducing apoptosis in a cell in a cell having a KRAS mutation, comprising administering a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) described herein. In some embodiments, provided herein is a method of inhibiting proliferation of a cell having a NRAS mutation, comprising administering a compound provided

herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In some embodiments, the cancer is associated with a NRAS mutation. In some embodiments, provided herein is a method of inducing apoptosis in a cell having a RAS mutation, comprising administering a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In some embodiments, the KRAS mutation is at codons 12, 13, 59, 61 and/or 146. In some embodiments, the mutant form of the KRAS protein has one or more amino acid substitutions selected from the group consisting of G12C, G12S, G12R, G12F, G12L, G12N, G12A, G12D, G12V, G13C, G13S, G13D, G13V, G13P, S17G, P34S, A59E, A59G, A59T, Q61K, Q61L, Q61R, and Q61H. In some embodiments, the mutant form of the KRAS protein has one or more amino acid substitutions selected from the group consisting of G12C, G12R, G12S, G12A, G12D, G12V, G13C, G13R, G13S, G13A, G13D, G13V, A59E, A59G, A59T, Q61K, Q61L, Q61R, Q61H, K117N, K117R, K117E, A146P, A146T and A146V.

[0066] In certain embodiments, the RAS mutation is a HRAS or MRAS mutation. In one embodiment, the mutant form of the HRAS protein has one or more amino acid substitutions selected from the group consisting of G12C, G12D, G12F, G12N, G12S, G12V, G13C, G13D, G13E, G13R, G13S, G13V, Q61H, Q61K, Q61L, and Q61R.

[0067] In one embodiment, the mutant form of the MRAS protein has one or more amino acid substitutions selected from G23V and T68I.

[0068] In some embodiments, provided herein is a method of treating cancer in a patient having a RAF mutation, comprising administering a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In some embodiments, provided herein is a method of inhibiting proliferation of a cell having a RAF mutation, comprising administering a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof). In some embodiments, the cancer is associated with a RAF mutation. In some embodiments, the BRAF mutation is a K601E mutation. In some embodiments, the BRAF mutation is a V600E mutation.

[0069] In some embodiments, provided herein is a method of treating cancer in a patient having a NF1 mutation, comprising administering a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof).

[0070] In certain embodiments, the cancer is resistant to treatment of one or more MEK protein kinase inhibitors. In other embodiments, the cancer is resistant to treatment of one or more RAF protein kinase inhibitors. In some embodiments, the cancer is resistant to BRAF/MEK inhibition. In some such embodiments, the cancer is an advanced solid tumor. In some embodiments, the cancer is associated with a RAS, RAF, and/or NF1 mutation. In some embodiments, the BRAF mutation is a K601E mutation. In some embodiments, the BRAF mutation is a V600E mutation. In still further embodiments, the resistance is acquired resistance. In other embodiments, the resistance is *de novo* resistance. In further or additional embodiments, the cancer is resistant to an anticancer agent.

[0071] In some embodiments, a compound provided herein can be administered once daily (QD or qd), or divided into multiple daily doses. In addition, the administration can be continuous (*i.e.*, daily for consecutive days or every day), intermittent, *e.g.*, in cycles (*i.e.*, including days, weeks, or months of rest without drug). As used herein, the term “daily” is intended to mean that a therapeutic compound is administered once or more than once each day, for example, for a period of time.

[0072] In some embodiments, administration of a compound or pharmaceutical composition disclosed herein may occur in an amount of between about 0.001 mg/kg of body weight to about 100 mg/kg of body weight per day (administered in single or divided doses), more preferably at least about 0.1 mg/kg of body weight per day. A particular therapeutic dosage can include, *e.g.*, from about 0.01 mg to about 7000 mg of compound, and preferably includes, *e.g.*, from about 0.05 mg to about 2500 mg. The quantity of active compound in a unit dose of preparation may be varied or adjusted from about 0.1 mg to 1000 mg, preferably from about 1 mg to 300 mg, more preferably 10 mg to 200 mg, according to the particular application. In some instances, dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, *e.g.* by dividing such larger doses into several small doses for administration

throughout the day. The amount administered will vary depending on the particular IC_{50} value of the compound used. In combinational applications in which the compound is not the sole therapy, it may be possible to administer lesser amounts of compound and still have therapeutic or prophylactic effect.

[0073] In one embodiment the compound or pharmaceutical composition disclosed herein is administered at a dosage amount of about 1 mg/day, 2 mg/day, 4 mg/day, 5 mg/day, 8 mg/day, 10 mg/day, 15 mg/day, 20 mg/day, 22 mg/day, 30 mg/day, 35 mg/day, 37 mg/day, 45 mg/day, 50 mg/day, 70 mg/day, 100 mg/day, 125 mg/day, 140 mg/day, 175 mg/day, 200 mg/day, 250 mg/day, 280 mg/day, 350 mg/day, 400 mg/day, 450 mg/day, 500 mg/day, 550 mg/day, 600 mg/day, 650 mg/day, 700 mg/day, 750 mg/day, 1000 mg/day or 1400 mg/day. In one embodiment the compound or pharmaceutical composition disclosed herein is administered at a dosage amount of about 2 mg/day, 4 mg/day, 8 mg/day, 15 mg/day, 22 mg/day, 30 mg/day, 37 mg/day, or 45 mg/day.

[0074] For clarity reasons, it is noted that, unless otherwise specified, the compound doses referred to herein refer to the amount of Compound 1, Compound 2, Compound 3, or Compound 4 in its free base form. In case that for example a pharmaceutically acceptable salt of Compound 1, Compound 2, Compound 3, or Compound 4 is used, the amounts given above will need to be adapted accordingly.

6.4 Pharmaceutical Compositions

[0075] Also provided are pharmaceutical compositions formulated for administration by an appropriate route and means containing effective concentrations of Compound 1, Compound 2, Compound 3, or Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof, and optionally comprising at least one pharmaceutical carrier.

[0076] The compounds can be formulated into suitable pharmaceutical preparations such as solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations or elixirs, for oral administration or in sterile solutions or suspensions for ophthalmic or parenteral administration, as well as transdermal patch preparation and dry powder inhalers. Typically the compounds described above are formulated into pharmaceutical compositions using techniques and procedures well known in the art (see, *e.g.*, Ansel

Introduction to Pharmaceutical Dosage Forms, Seventh Edition 1999). In one particular embodiment, a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) can be formulated into capsules. In some such embodiments, a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) is formulated into 2 mg, 4 mg, or 15 mg capsule. In one particular embodiment, a compound provided herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) is formulated into 15 mg capsule.

[0077] In the compositions, effective concentrations of one or more compounds or pharmaceutically acceptable salts is (are) mixed with a suitable pharmaceutical carrier or vehicle. In certain embodiments, the concentrations of the compounds in the compositions are effective for delivery of an amount, upon administration, that treats, prevents, or ameliorates one or more of the symptoms and/or progression of disorders in a subject, such as cancer.

[0078] Typically, the compositions are formulated for single dosage administration. To formulate a composition, the weight fraction of compound is dissolved, suspended, dispersed or otherwise mixed in a selected vehicle at an effective concentration such that the treated condition is relieved or ameliorated. Pharmaceutical carriers or vehicles suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration.

[0079] The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in *in vitro* and *in vivo* systems described herein and then extrapolated therefrom for dosages for humans.

[0080] The concentration of active compound in the pharmaceutical composition will depend on absorption, tissue distribution, inactivation, metabolism and excretion rates of the active compound, the physicochemical characteristics of the compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art. For example, the

amount that is delivered is sufficient to ameliorate one or more of the symptoms of cancer or disease disclosed herein.

[0081] The pharmaceutical composition may be in unit dosage forms suitable for single administration of precise dosages. The pharmaceutical composition will include a conventional pharmaceutical carrier or excipient and a compound according to the invention as an active ingredient. In addition, it may include other medicinal or pharmaceutical agents, carriers, adjuvants, etc.

[0082] Exemplary parenteral administration forms include solutions or suspensions of active compounds in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired.

[0083] Suitable pharmaceutical carriers include inert diluents or fillers, water and various organic solvents. The pharmaceutical compositions may, if desired, contain additional ingredients such as flavorings, binders, excipients and the like. Thus for oral administration, tablets containing various excipients, such as citric acid may be employed together with various disintegrants such as starch, alginic acid and certain complex silicates and with binding agents such as sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often useful for tableting purposes. Solid compositions of a similar type may also be employed in soft and hard filled gelatin capsules.

[0084] Preferred materials, therefore, include lactose or milk sugar and high molecular weight polyethylene glycols. When aqueous suspensions or elixirs are desired for oral administration the active compound therein may be combined with various sweetening or flavoring agents, coloring matters or dyes and, if desired, emulsifying agents or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin, or combinations thereof. Methods of preparing various pharmaceutical compositions with a specific amount of active compound are known, or will be apparent, to those skilled in this art. For examples, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Ester, Pa., 18th Edition (1990).

[0085] In certain embodiments, the effective amount of the compound provided herein (e.g., Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) in the pharmaceutical

composition may be at a level that will exercise the desired effect; for example, about 0.001 mg/kg of a subject's body weight to about 100 mg/kg of a subject's body weight in unit dosage for both oral and parenteral administration.

[0086] The dose of compound to be administered to a subject is rather widely variable and can be subject to the judgment of a health-care practitioner. In any given case, the amount of compound administered will depend on such factors as the solubility of the active component, the formulation used and the route of administration. In one embodiment, application of a topical concentration provides intracellular exposures or concentrations of about 0.01 – 10 mM.

[0087] The administered dose can also be expressed in units other than mg/kg/day. For example, doses for parenteral administration can be expressed as mg/m²/day. One of ordinary skill in the art would readily know how to convert doses from mg/kg/day to mg/m²/day given either the height or weight of a subject or both. For example, a dose of 1 mg/kg/day for a 65 kg human is approximately equal to 38 mg/m²/day.

[0088] The compound disclosed herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) can be administered orally for reasons of convenience. In one embodiment, when administered orally, the compound is administered with a meal and water. In one embodiment, when administered orally, the compound is administered without food. In another embodiment, the compound is dispersed in water or juice (*e.g.*, apple juice or orange juice) and administered orally as a suspension.

[0089] The compound disclosed herein (*e.g.*, Compound 1, Compound 2, Compound 3, Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof) can also be administered intradermally, intramuscularly, intraperitoneally, percutaneously, intravenously, subcutaneously, intranasally, epidurally, sublingually, intracerebrally, intravaginally, transdermally, rectally, mucosally, by inhalation, or topically to the ears, nose, eyes, or skin. The mode of administration is left to the discretion of the health-care practitioner, and can depend in-part upon the site of the medical condition.

[0090] The compounds described herein or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof may be administered as a sole therapy. The compounds described herein or a pharmaceutically acceptable salt, solvate, hydrate,

tautomer, ester, isotopologue, or prodrug thereof may also be administered in combination with another therapy or therapies.

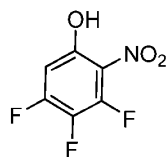
[0091] Other therapies include, but are not limited to administration of other therapeutic agents, radiation therapy or both. Examples of therapies within the scope of the methods include, but are not limited to, surgery, chemotherapy, radiation therapy, biological therapy, stem cell transplantation, cell therapy, and combinations thereof.

[0092] In the instances where the compounds described herein are administered with other therapeutic agents, the compounds described herein need not be administered in the same pharmaceutical composition as other therapeutic agents, and may because of different physical and chemical characteristics, be administered by a different route. For example, the compounds or compositions may be administered orally to generate and maintain good blood levels thereof, while the other therapeutic agent may be administered intravenously. The determination of the mode of administration and the advisability of administration, where possible, in the same pharmaceutical composition, is well within the knowledge of the skilled clinician. The initial administration can be made according to established protocols known in the art, and then, based upon the observed effects, the dosage, modes of administration and times of administration can be modified by the skilled clinician. The particular choice of compound (and where appropriate, other therapeutic agent and/or radiation) will depend upon the diagnosis of the attending physicians and their judgment of the condition of the patient and the appropriate treatment protocol. Other therapeutic agents may include chemotherapeutic agents, such as anti-tumor substances. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate dosing of the individual components of treatment.

7. EXAMPLES

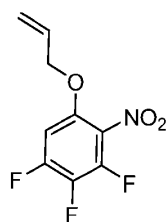
[0093] **Example 1: Reference Synthesis of 10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide**

Step 1: Preparation of 3,4,5-Trifluoro-1-nitrophenol



[0094] 3,4,5-Trifluorophenol (14.81 g, 0.1 mol) was dissolved in glacial acetic acid (50 mL) and cooled to 4 ° C. while concentrated nitric acid (5 mL, 70%) was added dropwise over 15 min, during which time the color of the mixture becomes yellow. Upon complete addition of HNO₃, the reaction mixture was allowed to warm to room temperature and stirred for an additional 30 min. TLC analysis of an aliquot extracted into ethyl acetate indicates that a new non-polar spot was formed and the complete consumption of starting material. The mixture was then diluted with ethyl acetate (200 mL), transferred to separatory funnel, and washed copiously with water (3x 100 mL). The organic layer was finally washed with brine, dried over anhydrous MgSO₄, and evaporated under reduced pressure to afford crude product as a yellowish oil. (17.3 g, 90%). This crude material was used directly in the subsequent reaction. ¹H NMR (400 MHz, CDCl₃): 6.84 (m, 1H, ArH), 10.28 (brs, 1H, OH).

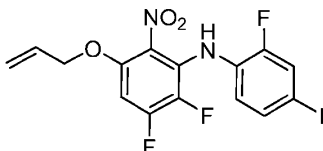
[0095] Step 2: Preparation of 3,4,5-Trifluoro-2-nitro-phenyl Ally Ether



[0096] To a solution of crude 3,4,5-trifluoro-2-nitrophenol (4.8 g, 25 mmol) in acetone (25 mL) was added K₂CO₃ (50 mmol) and allyl bromide (3.6 g, 30 mmol), and the mixture was heated to reflux for 2 h. TLC analysis of the reaction mixture (25% EtOAc:Hexanes) at this time reveals all starting material was consumed and the presence of a non-polar spot. The heating was discontinued and the reaction mixture was allowed to cool. Most of the acetone was evaporated under vacuo, and the remaining residue was diluted with ether (50 mL) and washed successively with water. The organic ether layer was dried over MgSO₄ and concentrated in vacuo by rotary evaporation. The crude yellow-orange oil was further purified by flash column chromatography over silica gel using hexanes to 15% hexanes:ethyl acetate gradient. The homogenous fractions from TLC were collected, combined and evaporated under reduced pressure to yield the allyl

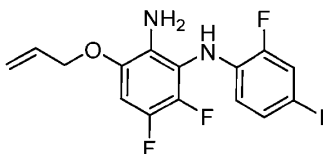
ether product (5.5 g, 95%) ^1H NMR (400 MHz, CDCl_3): 4.65 (dt, $J=1.6, 5.2$ Hz, 2H, OCH_2), 5.39 (d, $J=12.0$ Hz, 1H, $=\text{CH}_2$), 5.45 (d, $J=18.0$ Hz, 1H, $=\text{CH}_2$), 5.98 (m, 1H, $=\text{CH}$), 6.72 (m, 1H, ArH).

[0097] Step 3: Preparation of (3-Allyloxy-5,6-difluoro-2-nitro-phenyl)-(2-fluoro-4-iodo-phenyl)-amine



[0098] To a solution of 2-fluoro-4-iodo-phenylamine (1.1 g, 4.6 mmol) in THF (50 mL) was dropwise added LHMDs solution (6.0 mL, 6.0 mmol, 1 M in THF) at -78°C . After stirring for 1 h at -78°C , a solution of 1-allyloxy-3,4,5-trifluoro-2-nitrobenzene (1.2 g, 5.1 mmol) in THF (10 mL) was dropwise added into the reaction mixture. The reaction mixture was stirred at -78°C for additional 1 h and brought to room temperature and stirred for 16 h. The progress of reaction was monitored by ^1H NMR. After completion, the solvent was removed under reduced pressure. The residue obtained was dissolved in ethyl acetate, washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was triturated with hexane to yield (3-allyloxy-5,6-difluoro-2-nitro-phenyl)-(2-fluoro-4-iodo-phenyl)-amine as a yellow solid (900 mg). ^1H -NMR (400 MHz, CDCl_3): 4.62 (2H, d, $J=4.8$), 5.33-5.36 (1H, d, $J=10$), 5.48 (1H, d, $J=17.2$), 5.98-6.02 (1H, m), 6.22 (1H, dd, $J=2.4, 9.6$), 6.36 (1H, dd, $J=2, 10.4$), 7.04-7.08 (1H, m), 7.45-7.52 (2H, m), 7.79 (1H, s).

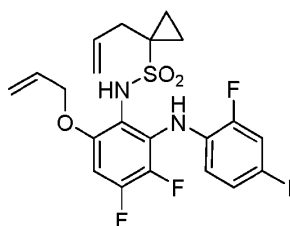
[0099] Step 4: Preparation of 6-Allyloxy-3,4-difluoro-N2-(2-fluoro-4-iodo-phenyl)-benzene-1,2-diamine



[00100] A suspension of (3-allyloxy-5,6-difluoro-2-nitro-phenyl)-(2-fluoro-4-iodo-phenyl)-amine (7, 0.9 g, 2 mmol) in ethanol (12 mL) was stirred at 70°C to obtain a clear solution. To this hot solution, was added a freshly prepared solution of $\text{Na}_2\text{S}_2\text{O}_4$ (1.04 g, 6 mmol) in water (2.5 mL). The reaction mixture was stirred at 90°C for 1 h. The progress of reaction

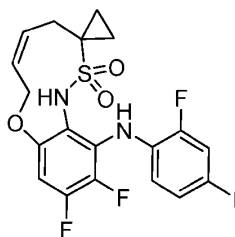
was monitored by TLC. After completion, the solvent was removed under reduced pressure. The residue was diluted with ethyl acetate, washed with water, and the organic phase was dried over anhydrous Na_2SO_4 and concentrated to yield 6-allyloxy-3,4-difluoro-N2-(2-fluoro-4-iodophenyl)-benzene-1,2-diamine as a brown solid (730 mg). $^1\text{H-NMR}$ (400 MHz, CDCl_3): 3.86 (2H, bs), 4.54 (2H, d, $J=5.2$), 5.34 (1H, d, $J=10.8$), 5.42 (1H, d, $J=17.2$), 5.66 (1H, bs), 6.02-6.09 (1H, m), 6.20 (1H, d, $J=8.4$), 6.62-6.66 (1H, m), 7.33 (1H, dd, $J=2, 8.4$), 7.63 (1H, d, $J=2$).

[00101] Step 5: Preparation of 1-Allyl-N-(3,4-difluoro-2-(2-fluoro-4-iodophenylamino)-6-allyloxyphenyl)cyclopropane-1-sulfonamide



[00102] 3-(Allyloxy)-5,6-difluoro-N-(2-fluoro-4-iodophenyl)benzene-1,2-diamine (420.2 mg, 1.0 mmol) is dissolved in anhydrous pyridine (1.0 mL), and to this solution is added 1-allyl-cyclopropyl-1-sulfonyl chloride (250.0 mg, 1.38 mmol, freshly prepared) at room temperature. The mixture is heated in an oil bath under nitrogen for 48 h. The TLC analysis of mixture indicated that a new polar spot is formed when compared with starting material. The reaction mixture is diluted with ethyl acetate and washed with 0.01 M HCl, water, and brine. The organic layer is dried over MgSO_4 and concentrated under reduced pressure. Flash chromatography of crude material over silica gel using 30 to 40% hexanes: ethyl acetate affords pure compound (375 mg, 66%) MS analysis: $[\text{M}+\text{H}]^+$ 565; $^1\text{H NMR}$ (400 MHz, CDCl_3): 0.81 (t, $J=6.0$ Hz, 2H, Cyclopropyl- CH_2), 1.26 (t, $J=6.0$ Hz, 2H, Cyclopropyl- CH_2), 2.73 (d, $J=8.0$ Hz, 2H, $-\text{CH}_2-$), 4.62 (dt, $J=1.2, 5.2$ Hz, 2H, OCH_2), 5.08 (dd, $J=1.2, 16.0$ Hz, 1H, $=\text{CH}_2$), 5.13 (dt, $J=1.6, 8.0$ Hz, 1H, $=\text{CH}_2$), 5.44 (dd, $J=1.6, 8.0$ Hz, 1H, $=\text{CH}_2$), 5.51 (dt, $J=1.6, 8.0$ Hz, 1H, $=\text{CH}_2$), 5.69 (m, 1H, $=\text{CH}$), 6.07 (m, 1H, $=\text{CH}$), 6.12 (1H, s, NH), 6.44 (m, 1H, ArH), 6.56 (dd, $J=4.0, 12.0$ Hz, 1H, ArH), 7.28 (d, $J=8.0$ Hz, 1H, ArH), 7.40 (dd, $J=1.0, 8.0$ Hz, 2H, ArH).

[00103] Step 6: Preparation of (Z)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-4,7-dihydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide



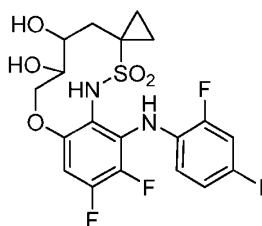
Method A.

[00104] A diluted solution of Zhang catalyst [prepared as described in *Tetrahedron Letters* 46 (2005) 7225-7228] (1.5 mg/mL, 50 μ L) in CH_2Cl_2 was added to a CH_2Cl_2 solution (1.0 mL) of 1-Allyl-N-(3,4-difluoro-2-(2-fluoro-4-iodophenylamino)-6-allyloxyphenyl)cyclopropane-1-sulfonamide from Step 5 (6.3 mg, 0.011 mmol) at room temperature and the mixture was stirred at room temperature for an additional 24 h. The mixture was then concentrated and purified by preparative TLC (silica gel) developing with hexanes:ethyl acetate, and the band corresponding to a new compound was collected and eluted with acetone. The desired compound was isolated as a solid (4.8 mg, 80%). MS analysis: $[\text{m}+\text{H}]^+$ 537; ^1H NMR (400 MHz, CDCl_3): 0.74 (brs s, CH_2), 1.14 (brs, CH_2), 3.14 (m, 2H, CH_2), 4.92 (s, 2H, OCH_2), 5.46 (dd, $J=12.0$ Hz, 1H, $=\text{CH}$), 5.72 (dd, $J=8.0, 12.0$ Hz, 1H, $=\text{CH}$), 6.27 (s, 1H, NH), 6.51 (m, 2H, ArH), 7.18 (s, 1H, NH), 7.29 (d, $J=8.0$, 1H, ArH), 7.41 (d, $J=12.0$ Hz, 1H, ArH).

Method B.

[00105] To a degassed solution of bis-olefin (from Step 5, 930 mg, 1.64 mmol) in dichloroethane (60 mL), Hoveyda-Grubbs 2nd generation catalyst (120 mg, 0.19 mmol, 10 mol%) was added. The reaction mixture was stirred at 70°C . for 3 h. The progress of reaction was monitored by TLC. After completion, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography to yield the desired compound (225 mg).

[00106] Step 7: Preparation of 10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide



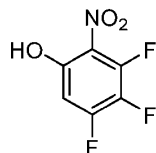
[00107] To a THF solution (0.5 mL) of (E)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-4,7-dihydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide (4.8 mg, 0.009 mmol) was added NMO (5.0 mg) followed by OsO₄ as a solution (5.0 μ L, 4% wt in water), via syringe at room temperature. The mixture was stirred over night (14 h). Starting material is completely consumed by TLC analysis to form a highly polar product (50% hexanes:ethyl cetate). The mixture was diluted with ethyl acetate (5.0 mL), washed with Na₂S₂O₃ (1% solution, 2.0 mL), water, and finally with brine. The organic layer was separated, dried over MgSO₄ and evaporated. The crude compound was purified by preparative TLC, and the most polar band moved by ethyl acetate was collected. Extraction of the collected silica band by acetone yielded racemic diol product (3.8 mg, 74%). MS analysis: [M+H]⁺ 571; ¹H NMR (400 MHz, CDCl₃): 0.68 (brs s, 2H, CH₂), 0.75 (m, 1H), 1.17 (brs, 1H, CH₂), 2.07 (s, 2H, CH₂), 2.12 (s, 2H, CH₂), 3.10-2.50 (m, 3H), 3.65 (m, 1H), 3.85 (d, 1H), 4.04 (brs, 1H), 4.42 (brt, 1H), 6.40 (m, 1H), 6.88 (s, 1H, ArH), 7.28 (d, 1H, ArH), 7.30 (d, J=8.0, 1H, ArH).

[00108] Stereoisomers are obtained using SFC separation under the following conditions: Hexane:Ethanol (90: 10 v/v); Column: Chiralcel OD-H (250x4.6 mm) 5 μ M; Flow Rate: 1.5 ml/min, Temperature: Ambient; Concentration: 1.0 mg/ml, UV Detection: 220 nm.

[00109] Example 2: Enantioselective Synthesis of Compound 1

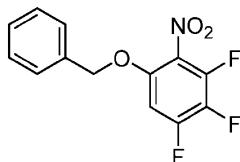
A. Preparation of Intermediate A.

[00110] Step 1: Preparation of 3,4,5-trifluoro-2-nitrophenol



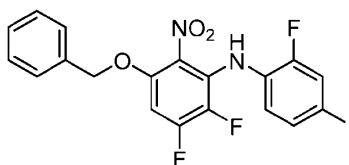
[00111] 3,4,5-trifluorophenol is treated with nitric acid in acetic acid to afford the title compound 3,4,5-trifluoro-2-nitrophenol.

[00112] Step 2: Preparation of 1-(benzyloxy)-3,4,5-trifluoro-2-nitrobenzene



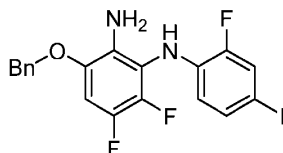
[00113] 3,4,5-Trifluoro-2-nitrophenol is treated with potassium carbonate and benzylbromide to afford the title compound 1-(benzyloxy)-3,4,5-trifluoro-2-nitrobenzene.

[00114] Step 3: Preparation of 3-(benzyloxy)-5,6-difluoro-N-(2-fluoro-4-iodophenyl)-2-nitroaniline



[00115] 1-(Benzyloxy)-3,4,5-trifluoro-2-nitrobenzene from Step 2 is treated with 2-fluoro-4-iodoaniline and Lithium bis(trimethylsilyl)amide to afford the title compound 3-(benzyloxy)-5,6-difluoro-N-(2-fluoro-4-iodophenyl)-2-nitroaniline.

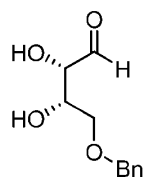
[00116] Step 4: Preparation of 3-(benzyloxy)-5,6-difluoro-N1-(2-fluoro-4-iodophenyl)benzene-1,2-diamine (Intermediate A)



[00117] 3-(Benzyloxy)-5,6-difluoro-N-(2-fluoro-4-iodophenyl)-2-nitroaniline from Step 3 is treated with Sodium Dithionite in Etanol: water to afford the title compound 3-(benzyloxy)-5,6-difluoro-N1-(2-fluoro-4-iodophenyl)benzene-1,2-diamine.

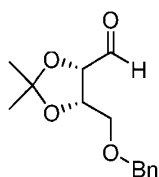
B. Preparation of Compound 1

[00118] Step 1: Preparation of (2S,3S)-4-(benzyloxy)-2,3-dihydroxybutanal



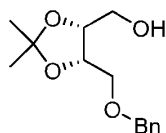
[00119] D-Erythrose is treated with benzyl 2,2,2-trichloroacetimidate in triflic acid to afford the title compound (2S,3S)-4-(benzyloxy)-2,3-dihydroxybutanal.

[00120] Step 2: Preparation of (4S,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde



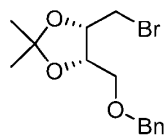
[00121] (2S,3S)-4-(Benzyloxy)-2,3-dihydroxybutanal from Step 1 is treated with acetone and Camphorsulfonic acid to afford the title compound (4S,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde.

[00122] Step 3: Preparation of ((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol



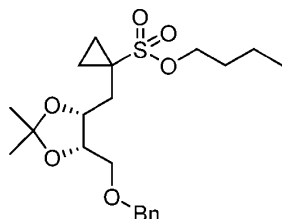
[00123] (4S,5S)-5-((Benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde from Step 2 is treated with sodium borohydride in THF and heated to afford the title compound ((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol.

[00124] Step 4: Preparation of (4S,5S)-4-((benzyloxy)methyl)-5-(bromomethyl)-2,2-dimethyl-1,3-dioxolane



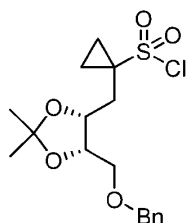
[00125] To ((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol from Step 3 is added tetrabromomethane in a suitable solvent to afford the title compound (4S,5S)-4-((benzyloxy)methyl)-5-(bromomethyl)-2,2-dimethyl-1,3-dioxolane.

[00126] Step 5: Preparation of butyl 1-(((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonate



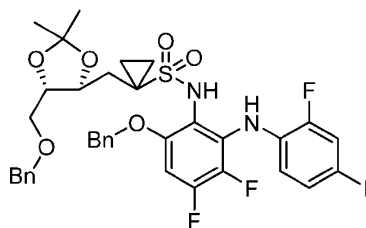
[00127] (4S,5S)-4-((Benzyloxy)methyl)-5-(bromomethyl)-2,2-dimethyl-1,3-dioxolane from Step 4 is treated with butyl cyclopropanesulfonate and nButyl Lithium at low temperature to afford the title compound butyl 1-(((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonate.

[00128] Step 6: Preparation of 1-(((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonyl chloride



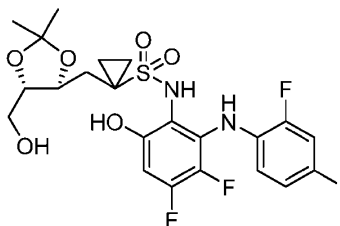
[00129] Butyl 1-(((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonate from Step 5 is treated with potassium thiocyanate (KNCS) followed by thionyl chloride to afford the title compound 1-(((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonyl chloride.

[00130] Step 7: Preparation of N-(6-(benzyloxy)-3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)phenyl)-1-(((4S,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonamide



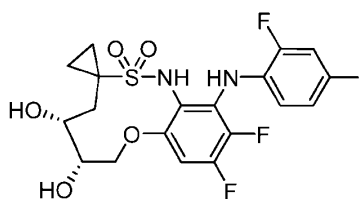
[00131] A solution of Intermediate A and 1-(((4R,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonyl chloride in pyridine is heated to 80°C to afford the title compound of N-(6-(benzyloxy)-3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)phenyl)-1-(((4S,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonamide.

[00132] Step 8: Preparation of N-(3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-6-hydroxyphenyl)-1-(((4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonamide



[00133] N-(6-(Benzyloxy)-3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)phenyl)-1-(((4S,5S)-5-((benzyloxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonamide from Step 7 is treated with palladium on carbon and hydrogen gas in a suitable solvent to afford the title compound N-(3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-6-hydroxyphenyl)-1-(((4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonamide.

[00134] Step 9: Preparation of (5R,6S)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide (Compound 1)

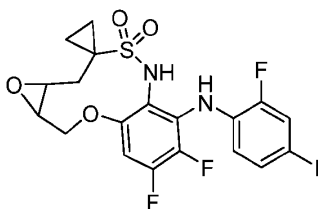


[00135] N-(3,4-Difluoro-2-((2-fluoro-4-iodophenyl)amino)-6-hydroxyphenyl)-1-(((4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)cyclopropane-1-sulfonamide from Step 8 is treated with triphenylphosphine (Ph₃P) and diethylazodicarboxylate (DEAD) in THF followed by a deprotection step to afford the title compound (5R,6S)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide (Compound 1).

[00136] Example 3: Syntheses of Compound 2 and Compound 3

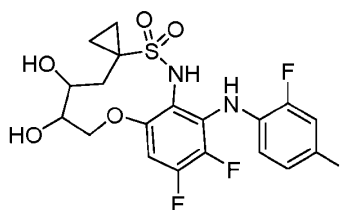
[00137] For the synthesis of Compounds 2 and 3 intermediate (E)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-4,7-dihydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide is prepared according to Example 1, Steps 1-6.

[00138] Step 1: Preparation of 7,8-difluoro-6-((2-fluoro-4-iodophenyl)amino)-1a,2,11,11a-tetrahydro-5H-spiro[benzo[b]oxireno[2,3-h][1,5,4]oxathiazecine-3,1'-cyclopropane] 4,4-dioxide



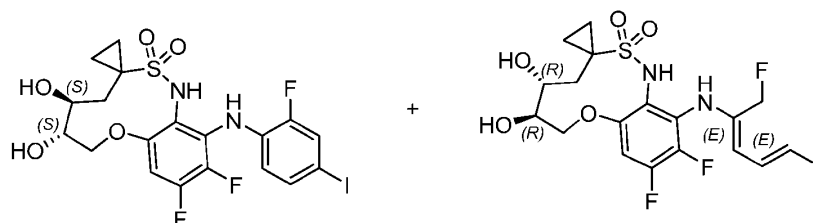
[00139] (E)-10,11-Difluoro-12-((2-fluoro-4-iodophenyl)amino)-4,7-dihydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide from Example 1, Step 6, is treated with sodium periodate to afford the title compound 7,8-difluoro-6-((2-fluoro-4-iodophenyl)amino)-1a,2,11,11a-tetrahydro-5H-spiro[benzo[b]oxireno[2,3-h][1,5,4]oxathiazecine-3,1'-cyclopropane] 4,4-dioxide as a racemic mixture.

[00140] Step 2: Preparation of 10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide



[00141] (1aS,11aR)-7,8-difluoro-6-((2-fluoro-4-iodophenyl)amino)-1a,2,11,11a-tetrahydro-5H-spiro[benzo[b]oxireno[2,3-h][1,5,4]oxathiazecine-3,1'-cyclopropane] 4,4-dioxide from Step 1 is treated with aqueous sodium hydroxide to afford the title compound (5S,6S)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide as a racemic mixture of anti-diol.

[00142] Step 3: Preparation of (5S,6S)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide (Compound 2) and (5R,6R)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide (Compound 3)



[00143] Chiral HPLC separation of racemic 10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide as described in Example 1, Steps 7a and 7b affords the desired compounds (5S,6S)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide (Compound 2) and (5R,6R)-10,11-difluoro-12-((2-fluoro-4-iodophenyl)amino)-5,6-dihydroxy-4,5,6,7-tetrahydro-1H-spiro[benzo[b][1,5,4]oxathiazecine-3,1'-cyclopropane] 2,2-dioxide (Compound 3).

[00144] **Synthesis of Compound 4**

[00145] Compound 4 is prepared according to Example 2, Steps 1-9 by using L-Erythrose instead of D-Erythrose in Step 1.

[00146] **Example 4: In Vitro Cell Proliferation Assay**

[00147] HT29 cells, obtained from ATCC, were grown in DMEM medium supplemented with L-glutamine (Invitrogen) and 10% Fetal Bovine Serum (Hyclone) at 37 °C in a humidified, 5% CO₂ incubator.

[00148] Proliferation assay was done by plating 2,000 cells/well in 100 µL of DMEM/10%FBS in a 96-well plate and incubated overnight at 37 °C in a humidified, 5% CO₂ incubator. Media was replaced with fresh 100 µL of fresh DMEM/10%FBS media containing various concentrations of the compounds. Compounds were added at 3-fold dilutions, concentrations ranging from 3.3 µM to 4.5 nM. After 72 hours of incubation with the compounds at 37 °C in a humidified, 5% CO₂ incubator, cell viability was measured in a luminometer after the addition of 100 µL/well CellTiterGlo reagent (Promega). IC₅₀s were calculated using SoftMax software.

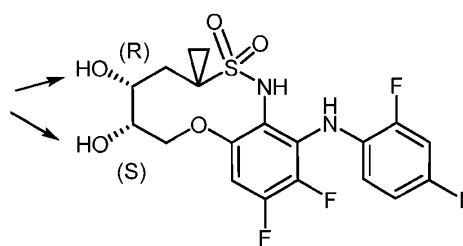
[00149] IC₅₀s obtained for Compound 1 and its enantiomer Compound 4, showed that Compound 1 is 25-fold more potent than its enantiomer Compound 4. Specifically, Compound 1 exhibited IC₅₀ values of 40 nM in the biochemical assay, 16 nM in the HT-29 proliferation assay, 15 nM in the Colo-205 proliferation assay, and 27 nM in the A-375 proliferation assay; whereas Compound 4 exhibited IC₅₀ values of 191 nM in the biochemical assay, 400 nM in the HT-29 proliferation, 358 nM in the Colo-205 proliferation assay, and 451 nM in the A-375 proliferation assay. Reference compound RDEA119 (also known as Refametinib, which is disclosed in US Patent No. 7,759,518), is an acyclic diol compound, which showed an IC₅₀ of 21 nM in biochemical assay and its enantiomer was roughly equipotent having an IC₅₀ of 39 nM under the same conditions. Summary of the data can be found in Table 1.

Table 1: Summary of data comparison between enantiomeric forms

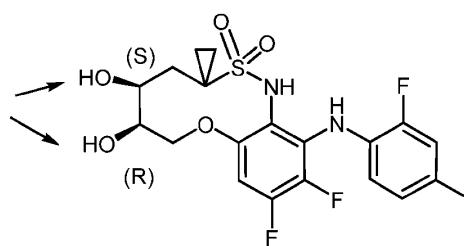
	Biochemical IC ₅₀	pERK, EC ₅₀	HT-29 proliferation, IC ₅₀	Colo-205 proliferation, IC ₅₀	A-375 proliferation, IC ₅₀
Compound 1	40 nM	2 nM	16 nM	15 nM	27 nM

	Biochemical IC ₅₀	pERK, EC ₅₀	HT-29 proliferation, IC ₅₀	Colo-205 proliferation, IC ₅₀	A-375 proliferation, IC ₅₀
Compound 4*	191 nM	46 nM	400 nM	358 nM	451 nM
RDEA-119 (S)	21 nM	4 nM			
Enantiomer (R) of RDEA-119	39 nM	3 nM			

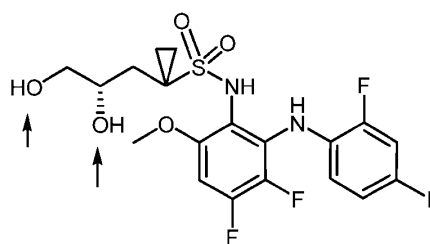
*Compound 4 is the enantiomer of Compound 1



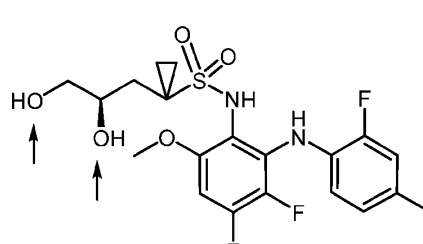
Compound 1



Compound 4



RDEA-119 (S)



Enantiomer (R)

[00150] Therefore, the macrocyclic compounds disclosed herein represent an improvement over the known compounds in the art not only because of their macrocyclic structure as opposed to the acyclic (linear) structure of the known compounds, but also because of the differential behavior of the enantiomers of the macrocyclic diols, which was not observed in the acyclic (linear) diols. Specifically, as noted above, Compound 1 is 25-fold more potent than its enantiomer Compound 4. This result was unexpected in view of the non-differential behavior of the acyclic diols known in the art.

[00151] **Example 5: Phase 1 Clinical Trial of Compound 1**

[00152] **A Phase 1 Open-label Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Compound 1 in Patients With MAPK Pathway-driven Advanced Solid Tumors With a Documented RAS, NF1, or RAF Mutation or Patients Who Have Failed BRAF/MEK Inhibition**

[00153] The Phase 1 clinical trial is a multi-center, open-label, dose escalation 3+3 study design to evaluate the safety, tolerability, pharmacokinetic (PK), pharmacodynamic (PD), and preliminary efficacy of Compound 1 in patients with MAPK pathway driven advanced solid tumors with a documented RAS, NF1 or RAF mutation or patients who have failed BRAF/MEK inhibition.

[00154] Compound 1 was tested in people with advanced solid tumors with rat sarcoma virus (RAS), neurofibromatosis type I (NF1), or rapidly accelerated fibrosarcoma (RAF) mutations.

[00155] Patients with histologically or cytologically diagnosed mitogen-activated protein kinase (MAPK) pathway driven advanced solid tumors with all of the following characteristics qualified for enrollment:

- i. tumor cannot be surgically resected
- ii. patient has failed or is ineligible for standard of care therapy
- iii. patient has no available treatment options with known clinical benefit
- iv. documented evidence of rat sarcoma virus (RAS), neurofibromatosis type I (NF1), and/or rapidly accelerated fibrosarcoma (RAF) mutations. Patients with RAF mutations must have previously failed v-Raf murine sarcoma viral oncogene homolog B (BRAF) / MEK inhibition.

[00156] Patients in the study were administered Compound 1 by mouth as a single dose, followed by one week observation, then once a day during the study, in 28-day cycles.

Participants will continue on daily Compound 1 for up to 2 years, or until:

- i. they decide to withdraw from the study, or
- ii. they experience unacceptable side effects, or
- iii. their disease progresses, or another illness interferes with taking the study drug, or
- iv. the sponsors stops the study.

[00157] In this study, Compound 1 was presented in 1 mg, 4mg, and 10 mg strength capsules, intended for oral administration once daily. Sequential dose escalation is: 2 mg, 4 mg, 8 mg, 15 mg, 22 mg, 30 mg, 37 mg, and 45 mg.

[00158] In some embodiments, the capsule formulation comprises a solid form of Compound 1.

1. **Pharmacokinetic Profile of Compound 1**

1.1 **Interim Phase I Results**

[00159] PK data from the 2 mg and 4 mg cohorts in first-in-human Phase I clinical trial of Compound 1 demonstrated a PK and safety profile that differentiates Compound 1 as a next-generation MEK inhibitor. Compound 1 achieved significant exposures with a favorable safety profile without showing adverse side effects such as rash or GI toxicity, which are typical for MEK inhibitors even at low doses. The long half-life at approximately 70 hours, and the ability to achieve a flat PK curve at steady-state, aim to provide a constant target inhibition while avoiding peak plasma toxicities, which is a unique PK profile among MEK inhibitors used for the treatment of Neurofibromatosis type 1 (NF1).

[00160] Table 5. Pharmacokinetic (PK) data for cohort 1 (2 mg) and cohort 2 (4 mg) at day 1 and day 22 (steady state)

Dose Cohort	C _{max} (ng/mL)	C _{min} (ng/mL)	AUC ₀₋₂₄ (ng*h/mL)**
2 mg Day 1	4.91 (101.3)	N/A	86.3 (89.2)
2 mg Day 22	16.2 (89.7)	10.2 (119.4)	354 (95.2)
4 mg Day 1	6.36 (135.6)	N/A	100 (97.1)
4 mg Day 22	61.3 (10.3)	51.5 (20.8)	1,390 (12.8)

**Data are Geometric Mean (Geometric coefficient of variation (CV)%), C_{max}: highest concentration of drug after a dose is given; C_{min}: lowest concentration of a drug after a dose is given; AUC: area under the concentration-time curve and measures the total drug exposure (the extent) across time.

1.1.1 **PK Results:**

- Plasma exposure increased with an increase in dose and linear PK was observed;

- Long half-life of approximately 70 hours will allow for once daily dosing or longer intervals;
- Prolonged systemic exposure with minimal fluctuation in Compound 1 plasma concentration at steady state (C_{max}/C_{min} ratio of 1.2) indicates a potential to achieve constant target inhibition. *See, FIGs. 1 and 2.*

[00161] At steady-state, drug levels peaked at about 5 hours with a geometric mean maximum concentration (C_{max}) of 16.2 and 61.3 ng/mL for the 2 mg and 4 mg dose groups, respectively. The mean elimination half-life was 67.9 hours supporting once-daily or less frequent oral dosing.

[00162] Compound 1 has a significantly longer half-life compared to early generation MEK inhibitors, particularly those used for the treatment of NF1, which have half-lives of less than 8 hours.

1.1.2 Safety and Tolerability

[00163] In the first 2 dosing cohorts (n=6), Compound 1 was shown to be well-tolerated with a favorable safety profile with no drug-related dose interruptions, reductions or discontinuations. There were no drug-related serious adverse events (SAE) in any dose arm and no protocol-defined stopping criteria were met. Importantly, at the 2 and 4 mg dose levels no rash or skin toxicity, gastrointestinal (GI) toxicity, or ocular toxicity have been observed to date.

[00164] Conclusions:

[00165] Unlike first-generation MEK inhibitors for the treatment of NF1 that require twice-daily dosing (BID) and exhibit short half-lives (<8 hours), Compound 1 has the potential to achieve prolonged target inhibition due to its long half-life of approximately 70 hours with once-daily dosing (QD). The PK profile showed consistent plasma levels at steady-state, as reflected by a low C_{max} to C_{min} ratio, potentially reducing the risks for C_{max} -related toxicity. These findings support the benefit of Compound 1 for both the treatment of NF1, particularly, cutaneous and plexiform neurofibromas in NF1, cancer and other MAPK-driven opportunities.

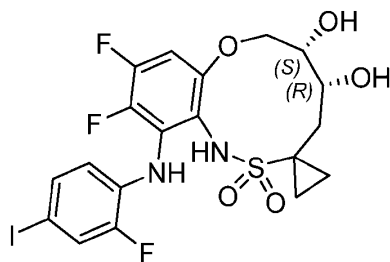
[00166] The embodiments provided herein are not to be limited in scope by the specific embodiments provided in the examples which are intended as illustrations of a few aspects of the provided embodiments and any embodiments that are functionally equivalent are encompassed

by the present disclosure. Indeed, various modifications of the embodiments provided herein are in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the scope of the appended claims.

[00167] A number of references have been cited, the disclosures of which are incorporated herein by reference in their entirety.

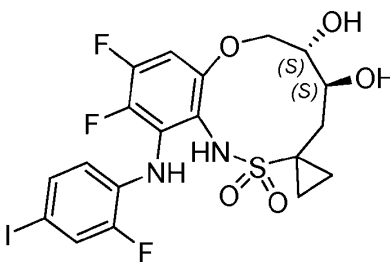
What is claimed is:

1. A pure Compound 1, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



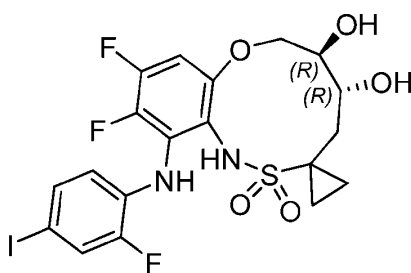
1.

2. A pure Compound 2, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



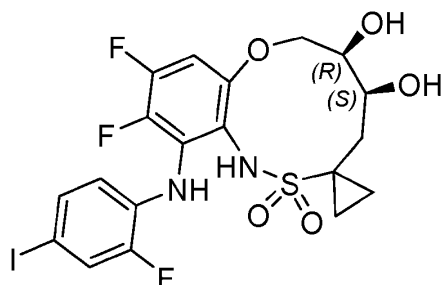
2.

3. A pure Compound 3, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



3.

4. A pure Compound 4, or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof:



4.

5. A pharmaceutical composition comprising a compound of claim 1 or a pharmaceutically acceptable salt, tautomer, ester, isotopologue, or prodrug thereof, and a pharmaceutically acceptable excipient or carrier.
6. A pharmaceutical composition comprising a compound of any one of claims 2 to 4 or a pharmaceutically acceptable salt, solvate, hydrate, tautomer, ester, isotopologue, or prodrug thereof, and a pharmaceutically acceptable excipient or carrier.
7. A method of treating a disease or disorder in which the RAS pathway is upregulated such as cancer, RASopathies and laminopathies comprising administering a therapeutically effective amount of a compound of any one of claims 1 to 4 to a patient having such disease or disorder.
8. The method of claim 7, wherein the disease or disorder is a Neurofibromatosis (NF) related disorder.