

FORMULATIONS AND USES OF ROCK2 INHIBITORS FOR ALS

FIELD

[0001] The present disclosure provides pharmaceutical formulation for oral administration of Rho-associated coiled coil containing protein kinase 2 (ROCK2) inhibitors. The disclosure also provides methods for the treatment of amyotrophic lateral sclerosis (ALS) by administering a therapeutically effective amount of a ROCK2 inhibitor to a patient.

BACKGROUND

[0002] A Rho-associated coiled-coil containing protein kinase (ROCK) is a serine/threonine kinase from the AGC (PKA, PKG, and PKC) kinase family and comprises two isoforms, ROCK1 and ROCK2. The two isoforms are expressed and regulated differently in specific tissues. For example, ROCK1 is ubiquitously expressed at a relatively high level, while ROCK2 is preferentially expressed in certain tissues including heart, brain and skeletal muscle. ROCK is a target of the small GTPase Rho and is involved in diverse cellular activities achieved by phosphorylating downstream effector proteins (MLC, LIMK, ERM, MARCKS, CRMP-2, etc.). Studies have shown that various diseases (e.g., pulmonary fibrosis, cardiac-cerebral vascular disease, neurological disease, cancer, etc.) are related to the pathways mediated by ROCK. As such, ROCK2 has been considered as an important target in the development of potential drug therapies.

[0003] Many potential drugs suffer from low bioavailability when taken orally. The bioavailability of pharmaceutically active substances when taken orally, depends on the extent to which the pharmaceutically active substance is absorbed from the gastro-intestinal environment across the gastro-intestinal mucosa. Lipophilic pharmaceutical substances may be poorly absorbed from the gastro-intestinal tract, inter alia because of their poor solubility and/or dispersibility in water. There is a demand for oral drug formulations for lipophilic and/or poorly soluble drugs.

[0004] Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the motor neurons, the cells that control voluntary muscles of the body. ALS is characterized by the progressive degeneration and eventual death of motor neurons in the brain, brainstem and spinal cord. The neurons involved in ALS facilitate communication between the nervous system and voluntary muscles of the body (motor neurons). As a result, the ability to initiate and control voluntary movement is lost. The neuromuscular junction (NMJ) dismantling

occurs at the early stages of the disease, in most case even prior to motor neurons (MNs) degeneration and onset of the clinical symptoms. ALS affects the muscles needed to move the arms and legs, to speak and swallow, to support the neck and trunk, and to breathe. The symptoms of ALS progress over time and, ultimately, the disease leads to death, generally from respiratory failure as patients lose the ability to control muscles in the chest and diaphragm. The average survival from onset of ALS to death is two to four years, with approximately 50% of patients dying within 30 months of their symptoms beginning.

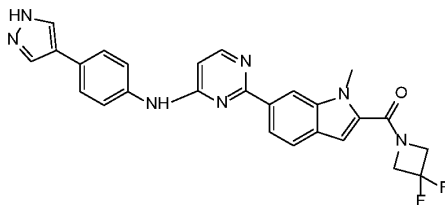
[0005] There is no known cure for ALS. Management of the disease focuses on minimizing symptoms and providing supportive care, with the goal of improving quality of life and slowing the disease progression. Treatments that slow ALS progression include Riluzole, which may extend life by two to three months, and sodium phenylbutyrate/ursodiolcoltaurine, which may extend life by about seven months. The recently approved therapy Tofersen targets only SOD1 mutated patients, which is approximately 2.5% of the total disease population (roughly 330 patients in the US). There is great and ongoing need for improved therapies for ALS and other neurodegenerative diseases.

SUMMARY

[0006] The disclosure provides a pharmaceutical formulation for oral administration of ROCK2 inhibitors.

[0007] In one aspect, the pharmaceutical formulation includes:

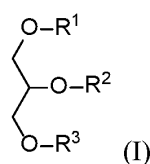
(a) an active pharmaceutical ingredient (API) comprising a compound having a structure:



or a pharmaceutically acceptable salt thereof, and

(b) one or more components selected from:

- (i) a fatty acid component comprising saturated or unsaturated C₈-C₂₄ fatty acids and/or a pharmaceutically acceptable salt thereof;
- (ii) a first glyceride component comprising one or more compounds of having a structure of Formula (I)



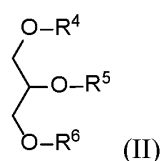
wherein:

each R^1 , R^2 , and R^3 is independently hydrogen or $-\text{C}(\text{O})-\text{R}^{\text{A}}$;

each R^{A} is independently C_7 - C_{21} alkenyl comprising one or two double bonds, provided that at least one of R^1 , R^2 , and R^3 is not hydrogen; and

(iii) a second glyceride component comprising:

one or more compounds having a structure of Formula (II)



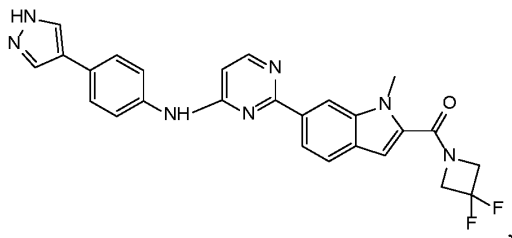
wherein:

each R^4 , R^5 , and R^6 is independently hydrogen, or $-\text{C}(\text{O})-\text{R}^{\text{B}}$;

each R^{B} is independently C_7 - C_{21} alkyl,

provided that at least one of R^4 , R^5 , and R^6 is not hydrogen.

[0008] The API may include a pharmaceutically acceptable salt of



and particularly an HCl salt thereof.

[0009] In some embodiments, the pharmaceutical formulation may suitably include a saturated or unsaturated C_{12} - C_{18} fatty acid (e.g., oleic acid) and/or its pharmaceutically acceptable salt (e.g., sodium oleate).

[0010] In some embodiments, the pharmaceutical formulation may suitably include a first glyceride component including one or more of mono-, di-, and/or tri- fatty acid esters of glycerols, which include at least one of unsaturated fatty acids (e.g., linear or branched C_{13} - C_{21} alkyl chain).

[0011] In some embodiments, the pharmaceutical formulation may suitably include a second glyceride component including one or more of mono-, di-, and/or tri- fatty acid esters of glycerols, which include saturated fatty acids (e.g., linear or branched C₁₃-C₂₁ alkyl chain).

[0012] In one aspect, a method is provided for treating a neurodegenerative disease characterized by the degeneration of motor neurons, the method comprising administering to the subject in need thereof a therapeutically effective amount of a ROCK2 inhibitor. In an embodiment, a method is provided for treating amyotrophic lateral sclerosis (ALS) by administering a therapeutically effective amount of a ROCK2 inhibitor to a patient in need thereof. In an embodiment, the therapeutically effective amount of the ROCK2 inhibitor is administered orally to the patient in a pharmaceutical formulation as described herein. In embodiments, the ROCK2 inhibitor may be selected from Compound A, Compound D, belumosudil (KD025), Zelasudil, Y-27632, Fasudil, GSK429286A, RKI-1447, Azaindole 1 (TC-S 7001), Hydroxyfasudil, GSK269962A, Ripasudil, AT13148, RX007, and derivatives, isomers, hydrates, or pharmaceutically acceptable salts thereof.

[0013] Other aspects of the disclosure are provided *infra*.

BRIEF DESCRIPTION OF THE FIGURES

[0014] FIG. 1 shows mean oral PK profiles for Compound A-HCl formulation in male and female Beagle dogs at 10 mg/kg (F4 100% solid dispersion vs nanosuspension).

[0015] FIG. 2 shows mean oral PK profiles for Compound A-HCl formulations in SD rats at 50 mg/kg (F4, F5 and F6)

[0016] FIG. 3 shows mean oral PK profiles for Compound A-HCl formulations in beagles at 10 mg/kg (F4, F5 and F6).

[0017] FIG. 4 shows mean oral PK profiles for Compound A-HCl formulations in beagles at 10 mg/kg (F4 and F4a)

[0018] FIGS. 5A and 5B show mean oral PK parameters (C_{max} and AUC) for Compound A-HCl formulations in male and female Beagle dogs at 10 mg/kg (F4, F4 50:50 molecular and solid dispersion and F4 100% solid dispersion).

[0019] FIGS. 6A-6P shows the tissue distribution following oral administration of the formulation of Table 7.

[0020] FIG. 7A-7B shows that Compound A extends the survival (FIG. 7A) and delays motor deficit (FIG. 7B) of SOD1^{G93A} mice.

[0021] FIG. 8 shows the lumbar motor neuron number after 13 weeks, 17 weeks and 21 weeks for SOD1^{G93A} mice treated with the ROCK2 inhibitor Compound A.

[0022] FIG. 9 shows the neuromuscular junction (NMJ) innervation (tibialis anterior) after 13 weeks, 17 weeks and 21 weeks for SOD1G93A mice treated with the ROCK2 inhibitor Compound A.

[0023] FIG. 10 neurofilament-light chain (NfL) levels in serum after 13 weeks, 17 weeks and 21 weeks for SOD1G93A mice treated with the ROCK2 inhibitor Compound A.

DETAILED DESCRIPTION

[0024] The present disclosure provides a method for the treatment of neurodegenerative disease in a subject by administering to the subject a therapeutically effective amount of a ROCK2 inhibitor. The neurodegenerative disease may be characterized by the degeneration of motor neurons, and includes amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy, pseudobulbar palsy, and monomelic amyotrophy (MMA). In embodiments, the present disclosure provides a method for the treatment of amyotrophic lateral sclerosis (ALS) comprising administering to a patient in need thereof a therapeutically effective amount of a ROCK2 inhibitor. In embodiments, the ROCK2 inhibitor is (6-(4-((4-(1H-pyrazol-4-yl)phenyl)amino)pyrimidin-2-yl)-1-methyl-1H-indol-2-yl)(3,3-difluoroazetidin-1-yl)methanone (Compound A), or a pharmaceutically acceptable salt thereof. In other embodiments, the ROCK2 inhibitor is belumosudil (2-(3-(4-((1H-indazol-5-yl)amino)quinazolin-2-yl)phenoxy)-N-isopropylacetamide), or a pharmaceutically acceptable salt, thereof. In other embodiments the ROCK2 inhibitor is Zelasudil. The ROCK2 inhibitor, and particularly Compound A, may be administered orally to the patient in a formulation provided herein.

[0025] The present disclosure also provides pharmaceutical compositions and dosage forms for oral administration of ROCK2 inhibitors, and particularly of (6-(4-((4-(1H-pyrazol-4-yl)phenyl)amino)pyrimidin-2-yl)-1-methyl-1H-indol-2-yl)(3,3-difluoroazetidin-1-yl)methanone (Compound A) or a pharmaceutically acceptable salt thereof. The oral pharmaceutical formulations disclosed herein may provide enhanced bioavailability of the ROCK2 inhibitor and/or enhanced exposure of the CNS to the ROCK 2 inhibitor.

Definition

[0026] The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen (N), oxygen (O), and sulfur (S).

[0027] The term "halogen" or "halo" designates -F, -Cl, -Br or -I. Preferred halogens are -F, -Cl and -Br.

[0028] The term "hydroxyl" means -OH.

[0029] The term “oxo” as used herein refers to an oxygen atom that has a double bond to another atom (i.e., the substituent =O), particularly to carbon.

[0030] The term “alkyl,” by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e., unbranched) or branched carbon chain (or carbon), or combination thereof, which is fully saturated. The alkyl may include a designated number of carbons (e.g., C₁-C₁₀ means one to ten carbons). Alkyl is an uncyclized chain. Examples of alkyl radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, methyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like.

[0031] A term “alkoxy” is an alkyl attached to the remainder of the molecule via an oxygen linker (-O-).

[0032] The term “alkenyl” refers to a linear or branched hydrocarbon chain having one or more carbon-carbon double bonds. The alkenyl may include a designated number of carbons (e.g., C₂-C₁₀ means two to ten carbons). Alkenyl is an uncyclized chain. The alkenyl includes vinyl, 1-propenyl, 2-propenyl, 2-butenyl, 3-butenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 2-methyl-2-propenyl, 4-methyl-3-pentenyl, and the like. When the compound of the present disclosure contains an alkenyl group, the compound may exist as the E-form, the Z-form, or any mixture thereof.

[0033] The term “alkynyl” refers to a linear or branched hydrocarbyl having a triple bond and 2-6 carbon atoms (“C₂-C₆ alkynyl”). The alkynyl includes ethynyl, propynyl, and the like.

[0034] The term “cycloalkyl” refers to saturated, carbocyclic groups having from 3 to 7 carbons in the ring. Cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

[0035] The term “aryl” as used herein includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as “aryl heterocycles”, “heteroaromatics” or “heteroaryl”. The term “aryl” also includes 7- to 14-membered polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are “fused rings”) wherein at least one of the rings is aromatic (including heteroaryl), e.g., the other cyclic rings can be fused cycloalkyls, cycloalkenyls, aryls, heteroaryl and/or heterocyclic groups. Single-ring heteroaryl groups may have from 1 to 3 ring heteroatoms

and fused polycyclic heteroaryl groups may have from 1 to 5 ring heteroatoms, wherein the ring heteroatoms are selected from N, O and S.

[0036] The terms "heterocyclyl," "heterocyclic" or "heterocycloalkyl" group refer to 3- to 10-membered ring structures, more preferably 5- or 6-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclic groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like.

[0037] The term "alkylaryl," "aralkyl", as used herein, refers to a C₁-C₆ alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

[0038] As used herein, the definition of each expression, e.g. alkyl, m, n, R, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

[0039] It will be understood that "substituted", "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[0040] The term "pharmaceutically-acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds disclosed herein and inorganic and organic basic addition salts of the compounds disclosed herein. It is understood that the reference to a ROCK2 inhibitor includes the neutral compound and any pharmaceutically acceptable salt for of the ROCK2 inhibitor. The pharmaceutically acceptable salt forms which may be selected on the basis of a chosen route of administration and according to standard pharmaceutical practice.

[0041] As set out above, certain embodiments of the ROCK2 inhibitors may contain a basic functional group, such as amino, and are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. These salts can be prepared in situ in the administration vehicle or the dosage form manufacturing process, or by separately

reacting a purified compound in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", J. Pharm. Sci. 66:1-19).

[0042] The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

[0043] In other cases, the compounds provided in this disclosure may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. These salts can likewise be prepared in situ in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine.

Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al., *supra*).

[0044] Certain compounds provided in this disclosure may exist in particular geometric or stereoisomeric forms. The disclosure contemplates all such compounds, including cis- and trans-isomers, R- and S-enantiomers, diastereomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the disclosure. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are included in this disclosure.

[0045] Unless otherwise specified, all amount percents are in percent by weight (wt %). In reference to the amounts of components in the pharmaceutical formulation (e.g., API, fatty

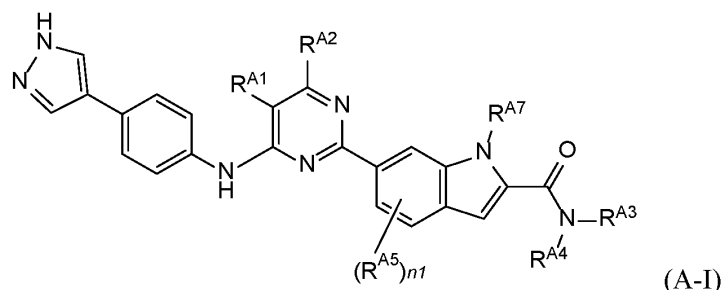
acid component(s), first glyceride component, second glyceride component, etc.), the weight percentage of the component does not include the weight of the capsule shell or coating. In other words, the total weight of the pharmaceutical formulation used for calculation of the wt % for each component includes the fill components (e.g., API, fatty acid component(s), first glyceride component, second glyceride component, etc.), but not the capsule shell or coating.

ROCK2 inhibitors

[0046] The disclosure provides methods and pharmaceutical formulations including one or more ROCK2 inhibitors.

[0047] ROCK2 inhibitors for use in the methods for treating a neurodegenerative disorder, and particularly ALS, and compositions are disclosed in, for example, WO2019/000682, WO 2019/000683, WO2019/001572, WO2020/094111, WO2020/177587, WO2020/259528, WO2022/042711, WO2022/042712, WO2006/105081, WO2019/145729, WO2007/007737, WO90/05723, WO2011/130740, WO2005/037197, WO2008/110846, WO2005/082890, WO2005/037197; US Pat. Nos. 10,323,023, 10,329,282, 11,390,609, 8,357,693, 9,815,820, 10,183,931, 10,696,660, 11,311,541, 11,497,751, 8,193,193, 4,997,834, 9,221,808, 7,547,779, 8,497,294, 7,592,357, 7,547,779; PCT/IB2023/054494 (filed 04/29/2023, entitled Inhibitors of ROCK2) and international application WO 2024/110851 (entitled Inhibitors of ROCK2), the disclosures of each are incorporated herein by reference.

[0048] In one aspect, the ROCK2 inhibitor has the structure of Formula (A-I):



or a pharmaceutically acceptable salt thereof, wherein:

R^{A1} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{A11}, -O-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-OR^{A11}, -NR^{A11}R^{A12}, -O-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)_x-C(=O)R^{A11}, -O-(C₁-C₆ alkyl)_{xl}-C(=O)R^{A11}, -(C₁-C₆ alkyl)_{xl}-C(=O)OR^{A11}, -C(=O)-R^{A11}, -C(=O)OR^{A11}, -(C₁-C₆ alkyl)_{xl}-C(=O)NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)_{xl}-C(=O)R^{A11}, and -NR^{A11}-(C₁-C₆ alkyl)_{xl}-C(=O)OR^{A11},

R^{A2} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{A11}, -O-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-OR^{A11}, -NR^{A11}R^{A12}, -O-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, -O-(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)OR^{A11}, -C(=O)-R^{A11}, -C(=O)OR^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, and -NR^{A11}-(C₁-C₆ alkyl)_{x/}-C(=O)OR^{A11};

alternatively, R^{A1} and R^{A2} are taken together to form a 5- or 6-membered saturated or unsaturated fused ring which may contain from 0 to 2 ring heteroatoms selected from the group consisting of N, O, and S, and which is unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of C₁-C₆ alkyl, halo, -CN, -OH, oxo, -O-(C₁-C₆ alkyl), -O-(C₁-C₆ alkyl)-OH, -O-(C₁-C₆ alkyl)-O-(C₁-C₆ alkyl), -NR^{A11}R^{A12}, -O-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, C₁-C₃ perfluoro alkyl, -NR^{A11}-(C₁-C₆ alkyl)NR^{A11}R^{A12}, and -NR^{A11}-(C₁-C₆ alkyl)-OR^{A11};

R^{A3} and R^{A4} are each independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, 3- to 10-membered heterocyclyl, C₆-C₁₀ aryl, 5- to 14-membered heteroaryl, C₆₋₁₂ aralkyl, -(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)OR^{A11}, and -(C₁-C₆ alkyl)_{x/}-C(=O)NR^{A11}R^{A12};

alternatively R^{A3} and R^{A4} are taken together with the nitrogen to which they are attached to provide (i) a 4- to 6-membered heterocyclic ring having from 0 to 2 additional ring to heteroatoms selected from N, O and S, or (ii) a 5- to 10-membered hetero bicyclic ring system having from 0 to 3 additional ring heteroatoms selected from N, O and S; wherein the heterocyclic ring or the bicyclic ring system are unsubstituted or are substituted with from 1 to 4 substituents selected from the group consisting of halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{A11}, oxo, -O-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-OR^{A11}, -NR^{A11}R^{A12}, -O-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, -O-(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)OR^{A11}, -C(=O)-R^{A11}, -C(=O)OR^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, and -NR^{A11}-(C₁-C₆ alkyl)_{x/}-C(=O)OR^{A11};

the dotted lines represent optional double bonds;

each R^{A5} is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, oxo, -OR^{A11}, -O-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-OR^{A11}, -NR^{A11}R^{A12}, -O-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, -O-(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)OR^{A11}, -C(=O)-R^{A11}, -C(=O)OR^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, and -NR^{A11}-(C₁-C₆ alkyl)_{x/}-C(=O)OR^{A11};

$n/$ is 0 to 3;

R^{A7} is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, -(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)_{x/}-C(=O)R^{A11}, -(C₁-C₆ alkyl)_{x/}-C(=O)OR^{A11}, and -(C₁-C₆ alkyl)_{x/}-C(=O)NR^{A11}R^{A12};

each $x/$ is independently selected from 0 and 1; and

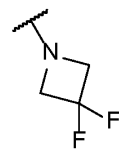
each R^{A11} and R^{A12} are independently selected from the group consisting of H and C₁-C₆ alkyl;

or alternatively, R^{A11} and R^{A12} are taken together when both are attached to the same nitrogen to form a 4- to 7- membered heterocyclic ring having from 0 to 2 additional ring heteroatoms selected from the group consisting of N, O and S, and which heterocyclic ring is unsubstituted or is substituted with 1 to 3 substituents selected from the group consisting of halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, -CN, -NH₂, C₁-C₃ perfluoro alkyl, -OH, -O-(C₁-C₆ alkyl), and -(C₁-C₆ alkyl)-OH.

[0049] In some embodiments, R^{A3} and R^{A4} are taken together with the nitrogen to which they are attached to provide a 4- to 6-membered heterocyclic ring, which is optionally substituted. In some embodiments, -NR^{A3}R^{A4} is



, which may be substituted or unsubstituted. In some embodiments, -NR^{A3}R^{A4} is

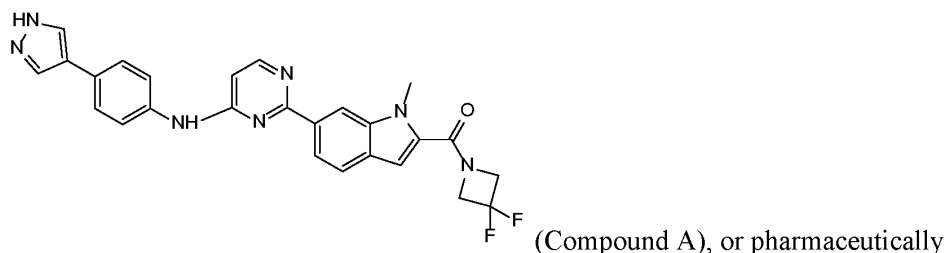


[0050] In some embodiments, R^{A1} and R^{A2} are hydrogen.

[0051] In some embodiments, R^{A7} is unsubstituted C₁-C₃ alkyl. In some embodiments, R^{A7} is unsubstituted methyl.

[0052] In some embodiments, R^{A5} is hydrogen, or n1 is 0.

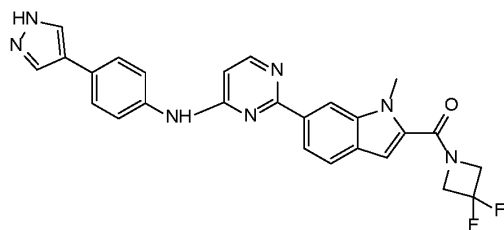
[0053] In some embodiments, the ROCK2 inhibitor has the structure:



acceptable salt thereof. This compound has the chemical name (6-(4-((4-(1H-pyrazol-4-yl)phenyl)amino)pyrimidin-2-yl)-1-methyl-1H-indol-2-yl)(3,3-difluoroazetidin-1-yl)methanone.

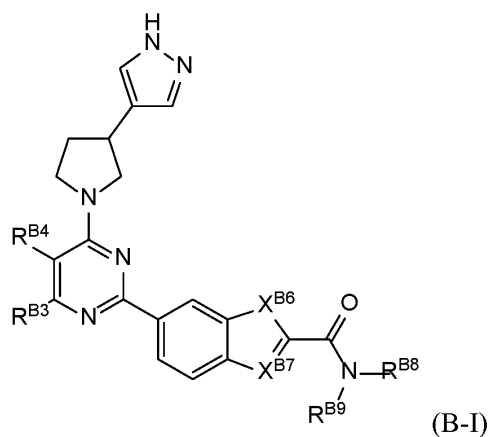
[0054] In some embodiments, the pharmaceutically acceptable salt is HCl.

[0055] In certain embodiments, the API comprises an HCl salt of



[0056] The compounds of Formula (A-I), as well as other disclosed ROCK2 inhibitors, may be synthesized and assayed for ROCK2 inhibition activity *in vitro* as described in, for example, WO2019/000682, WO 2019/000683, WO2019/001572, WO2020/094111, WO2020/177587, WO2020/259528, WO2022/042711, WO2022/042712 and US Pat. No. 10,323,023, 10,329,282 and 11,390,609, the entire contents of which are each incorporated herein by reference.

[0057] In some embodiments, the ROCK2 inhibitor has the structure of Formula (B-I):



or a pharmaceutically acceptable salt thereof, wherein:

R^{B3} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{B11}, -O-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)-OR^{B11}, -NR^{B11}R^{B12}, -O-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -O-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11}, -C(=O)-R^{B11}, -C(=O)OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, and -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11},

R^{B4} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{B11}, -O-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)-OR^{B11}, -NR^{B11}R^{B12}, -O-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -O-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11}, -C(=O)-R^{B11}, -C(=O)OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, and -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11},

alternatively, R^{B3} and R^{B4} are taken together to form a 5- or 6-membered saturated or unsaturated fused ring which may contain from 0 to 2 ring heteroatoms selected from the group consisting of N, O, and S, and which is substituted or unsubstituted with 1 to 3 substituents selected from the group consisting of C₁-C₆ alkyl, halo, -CN, -OH, oxo, -O-(C₁-C₆ alkyl), -O-(C₁-C₆ alkyl)-OH, -O-(C₁-C₆ alkyl)-O-(C₁-C₆ alkyl), -NR^{B11}R^{B12}, -O-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, C₁-C₃ perfluoro alkyl, -NR^{B11}-(C₁-C₆ alkyl)NR^{B11}R^{B12}, and -NR^{B11}-(C₁-C₆ alkyl)-OR^{B11},

X^{B6} is selected from the group consisting of NR^{B10}, O and S;

X^{B7} is selected from the group consisting of CH and N;

R^{B8} and R^{B9} are each independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, 3- to 10-membered heterocyclyl, C₆-C₁₀ aryl, 5- to 14-membered heteroaryl, C₆-₁₂ aralkyl, -(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11}, and -(C₁-C₆ alkyl)_{x2}-C(=O)NR^{B11}R^{B12}, wherein each alkyl, alkenyl, cycloalkyl, heterocyclyl, aryl, heteroaryl and aralkyl may optionally be substituted with one to three substituents selected from C₁-C₃ alkyl, C₁-C₃ perfluoroalkyl, oxo, halo, -CN, -OH, and NH₂;

alternatively R^{B8} and R^{B9} are taken together with the nitrogen to which they are attached provide (i) a 4- to 6-membered heterocyclic ring having from 0 to 2 additional ring heteroatoms selected from N, O and S, or (ii) a 5- to 10-membered hetero bicyclic

ring system having from 0 to 3 additional ring heteroatoms selected from N, O and S; wherein the heterocyclic ring or the bicyclic ring system are unsubstituted or are substituted with from 1 to 4 substituents selected from the group consisting of halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, -CN, C₁-C₆ fluoroalkyl, C₁-C₃ perfluoro alkyl, -OR^{B11}, oxo, -O-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)-OR^{B11}, -NR^{B11}R^{B12}, -O-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -O-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11}, -C(=O)-R^{B11}, -C(=O)OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, and -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11};

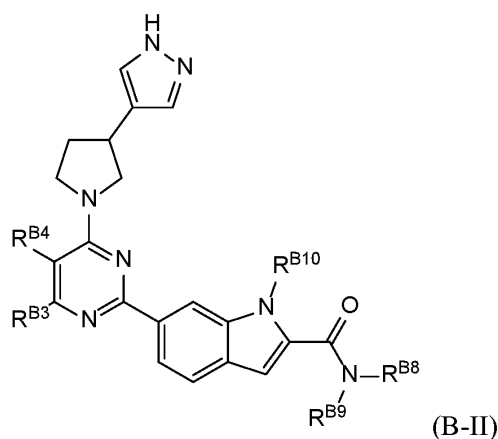
R^{B10} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, C₅-C₁₀ aryl, C₅-C₁₀ heteroaryl, 3- to 10-membered heterocyclyl, -C₁-C₆ alkyl-(C₃-C₇ cycloalkyl), -C₁-C₆ alkyl-(C₅-C₁₀ aryl), -C₁-C₆ alkyl-(C₅-C₁₀ heteroaryl), -C₁-C₆ alkyl-(3- to 10-membered heterocyclyl), -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)NR^{B11}R^{B12}, -(C₁-C₆ alkyl)-OR^{B11}, and -C(=O)-N(R^{B11})(R^{B12}), and wherein each alkyl, alkenyl, cycloalkyl, aryl, heteroaryl and heterocyclyl may optionally be substituted with one to three substituents selected from C₁-C₃ alkyl, C₁-C₃ perfluoroalkyl, oxo, halo, -CN, -OH, and NH₂;

each x₂ is independently selected from 0 and 1; and

each R^{B11} and R^{B12} are independently selected from the group consisting of H and C₁-C₆ alkyl;

or alternatively, R^{B11} and R^{B12} are taken together when both are attached to the same nitrogen to form a 4- to 7- membered heterocyclic ring having from 0 to 2 additional ring heteroatoms selected from the group consisting of N, O and S, and which heterocyclic ring is unsubstituted or is substituted with 1 to 3 substituents selected from the group consisting of halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, -CN, -NH₂, C₁-C₃ perfluoroalkyl, -OH, -O-(C₁-C₆ alkyl), and -(C₁-C₆ alkyl)-OH.

[0058] In some embodiments, the ROCK2 inhibitor has the structure of Formula (B-II):



or a pharmaceutically acceptable salt thereof, wherein:

R^{B3} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{B11}, -O-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)-OR^{B11}, -NR^{B11}R^{B12}, -O-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -O-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11}, -C(=O)-R^{B11}, -C(=O)OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, and -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11};

R^{B4} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{B11}, -O-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)-OR^{B11}, -NR^{B11}R^{B12}, -O-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -O-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11}, -C(=O)-R^{B11}, -C(=O)OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, and -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11};

alternatively, R^{B3} and R^{B4} are taken together to form a 5- or 6-membered saturated or unsaturated fused ring which may contain from 0 to 2 ring heteroatoms selected from the group consisting of N, O, and S, and which is substituted or unsubstituted with 1 to 3 substituents selected from the group consisting of C₁-C₆ alkyl, halo, -CN, -OH, oxo, -O-(C₁-C₆ alkyl), -O-(C₁-C₆ alkyl)-OH, -O-(C₁-C₆ alkyl)-O-(C₁-C₆ alkyl), -NR^{B11}R^{B12}, -O-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, C₁-C₃ perfluoro alkyl, -NR^{B11}-(C₁-C₆ alkyl)NR^{B11}R^{B12}, and -NR^{B11}-(C₁-C₆ alkyl)-OR^{B11};

R^{B8} and R^{B9} are each independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, 3- to 10-membered heterocyclyl, C₆-C₁₀

aryl, 5- to 14-membered heteroaryl, C₆₋₁₂ aralkyl, -(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11}, and -(C₁-C₆ alkyl)_{x2}-C(=O)NR^{B11}R^{B12}, wherein each alkyl, alkenyl, cycloalkyl, heterocyclyl, aryl, heteroaryl and aralkyl may optionally be substituted with one to three substituents selected from C₁-C₃ alkyl, C₁-C₃ perfluoroalkyl, oxo, halo, -CN, -OH, and NH₂;

alternatively R^{B8} and R^{B9} are taken together with the nitrogen to which they are attached provide (i) a 4- to 6-membered heterocyclic ring having from 0 to 2 additional ring heteroatoms selected from N, O and S, or (ii) a 5- to 10-membered hetero bicyclic ring system having from 0 to 3 additional ring heteroatoms selected from N, O and S; wherein the heterocyclic ring or the bicyclic ring system are unsubstituted or are substituted with from 1 to 4 substituents selected from the group consisting of halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, -CN, C₁-C₆ fluoroalkyl, C₁-C₃ perfluoro alkyl, -OR^{B11}, oxo, -O-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)-OR^{B11}, -NR^{B11}R^{B12}, -O-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)-OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -O-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11}, -C(=O)-R^{B11}, -C(=O)OR^{B11}, -(C₁-C₆ alkyl)_{x2}-C(=O)NR^{B11}R^{B12}, -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)R^{B11}, and -NR^{B11}-(C₁-C₆ alkyl)_{x2}-C(=O)OR^{B11};

R^{B10} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, C₅-C₁₀ aryl, C₅-C₁₀ heteroaryl, 3- to 10-membered heterocyclyl, -C₁-C₆ alkyl-(C₃-C₇ cycloalkyl), -C₁-C₆ alkyl-(C₅-C₁₀ aryl), -C₁-C₆ alkyl-(C₅-C₁₀ heteroaryl), -C₁-C₆ alkyl-(3- to 10-membered heterocyclyl), -(C₁-C₆ alkyl)-NR^{B11}R^{B12}, -(C₁-C₆ alkyl)NR^{B11}R^{B12}, -(C₁-C₆ alkyl)-OR^{B11}, and -C(=O)-N(R^{B11})(R^{B12}), and wherein each alkyl, alkenyl, cycloalkyl, aryl, heteroaryl and heterocyclyl may optionally be substituted with one to three substituents selected from C₁-C₃ alkyl, C₁-C₃ perfluoroalkyl, oxo, halo, -CN, -OH, and NH₂;

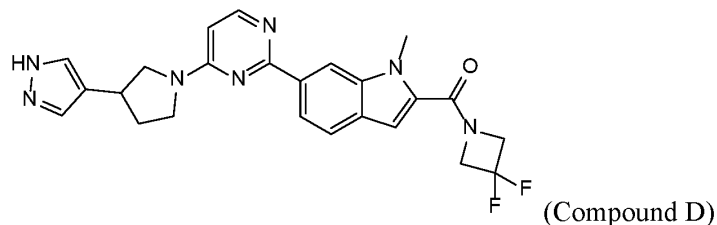
each x2 is independently selected from 0 and 1; and

each R^{B11} and R^{B12} are independently selected from the group consisting of H and C₁-C₆ alkyl;

or alternatively, R^{B11} and R^{B12} are taken together when both are attached to the same nitrogen to form a 4- to 7- membered heterocyclic ring having from 0 to 2 additional ring heteroatoms selected from the group consisting of N, O and S, and which heterocyclic ring is unsubstituted or is substituted with 1 to 3 substituents selected from the group

consisting of halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, -CN, -NH₂, C₁-C₃ perfluoroalkyl, -OH, -O-(C₁-C₆ alkyl), and -(C₁-C₆ alkyl)-OH.

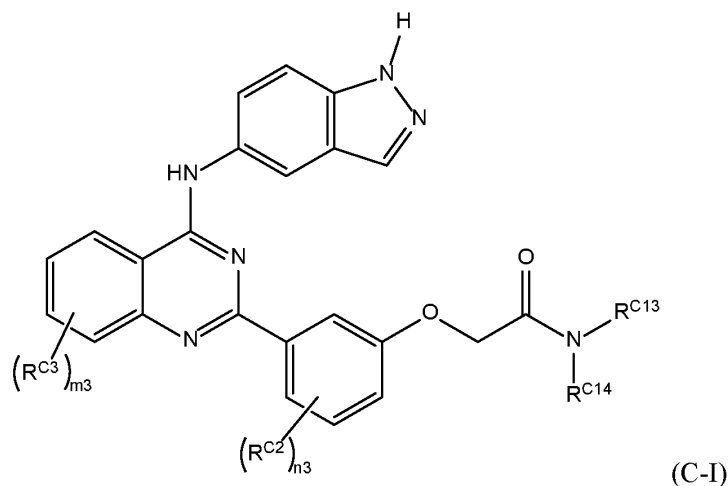
[0059] In some embodiments, the ROCK2 inhibitor has the structure of:



or a pharmaceutically acceptable salt thereof.

[0060] Synthesis and biological assays of Formula (B-I) and its subordinates are described in international application WO 2024/110851 (entitled Inhibitors of ROCK2), the entire disclosure of which is incorporated herein by reference.

[0061] In some embodiments, the ROCK2 inhibitor has the structure of Formula (C-I):



or a pharmaceutically acceptable salt thereof, wherein:

R^{C13} and R^{C14} are independently selected from the group consisting of H, C₁-C₈ alkyl, C₂-

C₈ alkenyl, C₂-C₈ alkynyl, —(C₁-C₆ alkyl)-O—(C₁-C₆ alkyl), —(C₁-C₆ alkyl)-

NR^{C16}R^{C17}, —(C₁-C₆ alkyl)-C(=O)NR^{C16}R^{C17}, aryl, aralkyl, heteroaryl, C₃-

C₇ cycloalkyl, a three to twelve membered heterocyclic ring containing up to 3

heteroatoms, each of which may be optionally substituted by from 1 to 3 substituents

independently selected from halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₃-C₇ cycloalkyl, C₁-

C₆ alkoxy, hydroxy, amino, cyano and C₁-C₃ perfluoro alkyl;

or R^{C13} and R^{C14} may be taken together to form a three to twelve membered heterocyclic ring

having up to 3 heteroatoms which is optionally substituted by from 1 to 3 substituents

independently selected from halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₁-C₆ alkoxy, C₃-C₇ cycloalkyl, oxo, hydroxy, amino, cyano and C₁-C₃ perfluoro alkyl;

R^{C16} and R^{C17} are independently selected from the group consisting of H, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, —(C₁-C₆ alkyl)-O—(C₁-C₆ alkyl), aryl, aralkyl, heteroaryl, C₃-C₇ cycloalkyl, a three to twelve membered heterocyclic ring containing up to 3 heteroatoms, each of which may be optionally substituted by from 1 to 3 substituents independently selected from halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₁-C₆ alkoxy, hydroxy, amino, cyano and C₁-C₃ perfluoro alkyl;

or R^{C16} and R^{C17} may be taken together to form a three to twelve membered heterocyclic ring having up to 3 heteroatoms which is optionally substituted by from 1 to 3 substituents independently selected from halo, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₁-C₆ alkoxy, oxo, hydroxy, amino, cyano and C₁-C₃ perfluoro alkyl;

each R^{C2} is independently selected from the group consisting of lower alkyl, CN, halo, hydroxy, lower alkoxy, amino, and perfluoro lower alkyl;

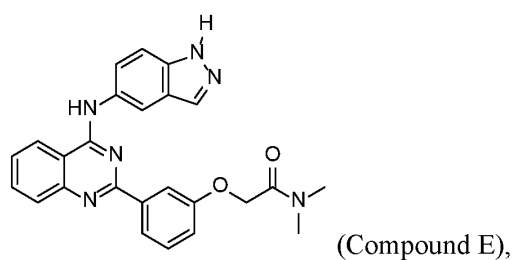
each R^{C3} is independently selected from the group consisting of lower alkyl, CN, halo, hydroxy, lower alkoxy, amino, and perfluoro lower alkyl;

n is selected from 0 to 4; and

m is selected from 0 to 3.

[0062] In some embodiments, in Formula (C-III), each R^{C13} and R^{C14} is independently H or unsubstituted C₁-C₆ alkyl. In some embodiments, each R^{C13} and R^{C14} is independently unsubstituted C₁-C₆ alkyl. In some embodiments, each R^{C13} and R^{C14} is independently methyl or ethyl. In some embodiments, R^{C13} and R^{C14} are methyl.

[0063] In some embodiments, the ROCK2 inhibitor has the structure of

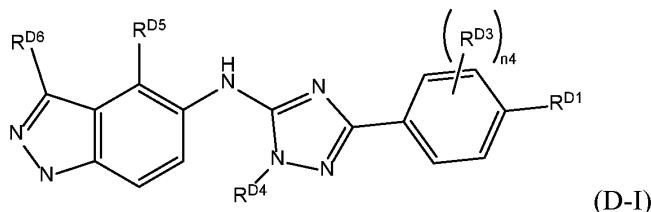


or a pharmaceutically acceptable salt thereof. Compound E is also known by the chemical name 2-(3-(4-((1H-indazol-5-yl)amino)quinazolin-2-yl)phenoxy)-N-isopropylacetamide, and is known as KD025 or belumosudil.

[0064] The compounds of Formula (C-I) may be synthesized and assayed for ROCK2 inhibition activity as described in, for example, U.S. Pat. Nos. 8,357,693, 9,815,820,

10,183,931, 10,696,660, and 11,311,541, the entire contents of which are incorporated herein by reference.

[0065] In one aspect, the ROCK2 inhibitor has the structure of Formula (D-I):



or pharmaceutically acceptable salts thereof, wherein:

R^{D1} is $L-R^{D2}$;

L is a bond or $-L^{D1}-L^{D2}-$,

wherein L^{D1} is selected from the group consisting of a bond, $-(CR^A R^B)_{1-3}-$, $-O(CR^A R^B)_{1-3}-$, $-(CR^A R^B)_{0-3}O-$, and $-NR^C(CR^A R^B)_{1-3}-$; and

L^{D2} is selected from the group consisting of a bond, $-(CR^A R^B)_{1-3}-$, $-O-$, $-NR^D-$, $-C(O)NR^D-$, $-NR^D C(O)-$, $-C(O)O-$, $-OC(O)-$, $-C(O)-$, $-S(O)_2 NR^D-$, $-NR^D S(O)_2-$, $-S(O)_2-$, $-S(O)(NR^D)-$, $-NR^D C(O)NR^E-$, $-OC(O)NR^D-$, and $-C(O)NR^D S(O)_2-$; and

R^{D2} is selected from the group consisting of H, CN, C_1 - C_6 alkyl, C_1 - C_6 haloalkyl, C_1 - C_6 alkyl substituted with $-OR^F$, C_1 - C_6 alkyl substituted with $-NR^F R^G$, C_{1-4} haloalkyl substituted with $-OR^F$, C_{3-8} cycloalkyl substituted with OH, C_{1-4} alkyl substituted with 3 to 8 membered heterocycloalkyl, C_{1-4} alkyl substituted with 6 membered heteroaryl, $-(CR^H R^I)_{1-3} OR^F$, $-(CR^H R^I)_{1-3} NR^F R^G$, $-(CR^N R^O)_{1-3} C(O)OR^F$, $-(CR^N R^O)_{1-3} C(O)NR^F R^G$, C_3 - 10 carbocyclic ring system, and 3 to 10 membered heterocyclic ring system, wherein the carbocyclic ring or heterocyclic ring system is substituted or unsubstituted with: $=O$, $-NR^F R^G$, $-C(O)R^F$, halo, $-CN$, C_{1-4} alkyl, C_{1-4} haloalkyl or C_{1-4} alkyl substituted with $-OR^F$;

R^{D3} is independently selected at each occurrence from the group consisting of halo, C_{1-4} alkyl, C_1 - C_6 haloalkyl, $-CN$, $-OR^J$, $=O$, C_{1-4} alkyl substituted with $-OR^J$, $-NR^J R^K$, C_{1-4} alkyl substituted with $-NR^J R^K$, C_{3-8} cycloalkyl, C_{1-4} alkyl substituted with C_{3-8} cycloalkyl, 3 to 8 membered heterocycloalkyl and C_{1-4} alkyl substituted with 3 to 8 membered heterocycloalkyl; or two R^{D3} are optionally joined to form a phenyl or heteroaryl group may be substituted with halo or C_{1-4} alkyl;

R^{D4} is selected from the group consisting of H, C_{1-4} alkyl, C_{1-4} alkyl substituted with $-OR^L$, C_{1-4} alkyl substituted with $-NR^L R^L$, C_{3-8} cycloalkyl, substituted or unsubstituted phenyl, 3 to 8 membered heterocycloalkyl, C_{1-4} alkyl substituted with C_{3-8} cycloalkyl, C_{1-4} alkyl substituted with 3 to 8 membered heterocycloalkyl and substituted or unsubstituted 5 or 6

membered heteroaryl, wherein the phenyl or heteroaryl group may be substituted with halo or C₁₋₄ alkyl;

R^{D5} is selected from the group consisting of H, halo, -OR^J, C₁₋₄ alkyl, C₁₋₆ haloalkyl, C₁₋₄ alkenyl, —CN, and C₃₋₈ cycloalkyl;

R^{D6} is selected from the group consisting of H, halo, C₁₋₄ alkyl, C₁₋₆ haloalkyl, -CN, and C₃₋₈ cycloalkyl;

n₄ is 0, 1, or 2;

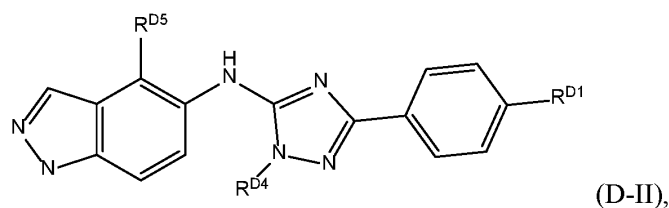
R^A and R^B are selected from the group consisting of H, C₁₋₄ alkyl, or C₁₋₄ haloalkyl or R^A and R^B together with the atom to which they are attached form a 3 to 6 membered cycloalkyl or a 3 to 6 membered heterocycloalkyl;

R^C, R^D, R^E, R^F and R^G are each independently selected from the group consisting of H, C₁₋₄ alkyl and C₁₋₄ haloalkyl;

R^H and R^I are each H except one pair of R^H and R^I on the same carbon atom, together with that carbon atom, form a 3 to 6 membered cycloalkyl or a 3 to 6 membered heterocycloalkyl; and

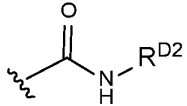
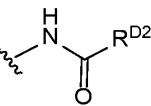
R^J, R^K, R^L, R^M, R^N and R^O are each independently at each occurrence selected from the group consisting of H or C₁₋₄ alkyl.

[0066] In some embodiments, the ROCK2 inhibitor has the structure of Formula (D-II):



or pharmaceutically acceptable salts thereof, wherein R^{D1}, R^{D4} and R^{D5} are provided above for D-I.

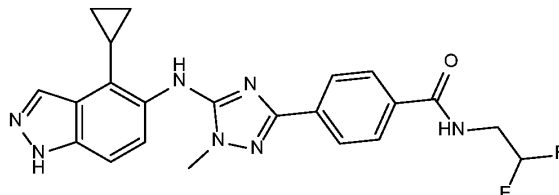
[0067] In some embodiments, in Formula (D-II), R^{D1} is -L^{D1}-L^{D2}-R^{D2}. In some embodiments, L^{D1} is a bond and L^{D2} is -NR^D-, -C(O)NR^D-, -NR^DC(O)-, -C(O)O-. In some

embodiments, R^{D1} is  or . In some embodiments, R^{D2} is C₁₋₆ alkyl (e.g., methyl, ethyl, propyl, isopropyl, butyl, or t-butyl), or C₁₋₆ haloalkyl (e.g., mono, di, or per-fluoro C₁₋₆ alkyl).

[0068] In some embodiments, in Formula (D-II), R^{D4} is hydrogen, or C₁₋₄ alkyl (e.g., methyl, ethyl, propyl, isopropyl, butyl, or t-butyl). In some embodiments, R^{D4} is methyl.

[0069] In some embodiments, in Formula (D-II), R^{D5} is hydrogen, halo, C₁₋₄ alkyl (e.g., methyl, ethyl, propyl, isopropyl, butyl, or t-butyl), C₁₋₆ haloalkyl (e.g., mono, di, or per-fluoro C₁₋₆ alkyl), or C₃₋₈ cycloalkyl (e.g., cyclopropyl, cyclobutyl, or cyclopentyl, cyclohexyl). In some embodiments, R^{D5} is cyclopropyl.

[0070] In some embodiments, the ROCK2 inhibitor has the structure:



or a pharmaceutically acceptable salt thereof, also called zelasudil.

[0071] In embodiments, the ROCK2 inhibitor may be selected from belumosudil (KD025), Zelasudil, Y-27632, Fasudil, GSK429286A, RKI-1447, Azaindole 1 (TC-S 7001), Hydroxyfasudil, GSK269962A, Ripasudil, AT13148, RX007, and derivatives, isomers, hydrates, or pharmaceutically acceptable salts thereof.

[0072] While the exemplary compounds of ROCK2 inhibitors which have been known to the present time are disclosed above, additional compounds shown to have ROCK2 inhibition activities can be used.

[0073] Methods of determining kinase inhibition are disclosed herein. For example, kinase activity of an enzyme and the inhibitory capacity of a test compound can be determined by measuring enzyme specific phosphorylation of a substrate. Commercial assays and kits can be employed. For example, kinase inhibition can be determined using an IMAP® assay (Molecular Devices). This assay method involves the use of a fluorescently tagged peptide substrate. Phosphorylation of the tagged peptide by a kinase of interest promotes binding of the peptide to a trivalent metal-based nanoparticle via the specific, high affinity interaction between the phosphor group and the trivalent metal. Proximity to the nanoparticle results in increased fluorescence polarization. Inhibition of the kinase by a kinase inhibitor prevents phosphorylation of the substrate and thereby limits binding of the fluorescently tagged substrate to the nanoparticle. Such an assay can be compatible with a microwell assay format, allowing simultaneous determination of IC₅₀ of multiple compounds.

Pharmaceutical Formulations

[0074] Provided herein, *inter alia*, are pharmaceutical formulations for oral administration. In one aspect, the pharmaceutical formulation includes (i) an active pharmaceutical ingredient (API) (e.g., ROCK2 inhibitors, particularly Compound A), and (ii)

pharmaceutically acceptable excipients (e.g., lipid formulation including fatty acid components and/or glyceride components).

[0075] The pharmaceutical formulation may suitably include additional excipients such as surfactants, solvents, solubilizing agents, preservatives, anti-oxidants, bulking agents, dissolution enhancers, wetting agents, emulsifiers, suspending agents, antibacterial agents, pH buffering agents, sweeteners, flavoring agents, and combinations thereof.

[0076] The disclosure provides a pharmaceutical formulation that may enhance absorption, controlled release and performance of API, i.e. compounds of Formula (A-I), and particularly Compound A, which may increase oral bioavailability of API. In certain embodiments, the pharmaceutical formulation may improve lymphatic uptake channels and avoid drug uptake via the hepatic portal vein thereby improve first-pass metabolism.

[0077] For example, the pharmaceutical formulations provided herein may improve absorption from the gastro-intestinal tract following oral administration of the compound of Formula IA, and particularly Compound A, and exemplary components of the lipid formulations include, but not limited to, triglycerides (e.g., tri-fatty acid (e.g., saturated or unsaturated) esters of glycerol), monoglycerides (e.g., mono-fatty acid (e.g., saturated or unsaturated) esters of glycerol), saturated or unsaturated fatty acids, phospholipids, bile salts and functionalized lipids (e.g., phospholipids). These lipid components may be fully or partially digested upon, e.g., oral in-take, and ultimately release the fatty acids.

[0078] Absorption of fatty acids taken may depend on characteristic of hydrocarbon chains, e.g., chain length, structure, geometry and the like. For example, short chain (e.g., C₂ to C₆) fatty acids and medium chain (e.g., C₈ to C₁₂) fatty acids may be preferably absorbed through the hepatic portal vein when digested, while long chain (e.g., C₁₄ to C₂₂) fatty acids are rather absorbed via the lymphatic system. So, during digestion of lipidic formulations of an API, mixtures of micelles formed of the fatty acids and derivatives are produced allowing the digested components combined with the API, to be taken up by lipid transporter proteins, resulting in the formation of API-containing lipid droplets in mucosal epithelial cells which are packaged up into nascent lipoproteins called chylomicrons. These API-loaded chylomicrons are then secreted into the lacteals of the enteric lymphatic system where they feedback into the central venous return, allowing the distribution of API around the body.

[0079] The disclosure provides an oral formulation composition for the API including Compound A or its pharmaceutically acceptable salt (e.g., Compound A-HCl), together with the lipidic excipients or components, e.g., a mixture of medium or long chain mono-, di-, or tri- glyceride, long chain fatty acid (C₈ to C₂₂ fatty acids, e.g., oleic acid), medium or long

chain triglyceride (e.g., Gelucire 43/01) and long chain surfactant (C₁₄ to C₂₂ fatty acids salt, e.g., sodium oleate). In some embodiments, the API may be formed in a solid dispersion, molecular dispersion, in a solution, or as a combination thereof.

[0080] The term “molecular dispersion” as used herein refers to a solution (liquid phase) including a solute (e.g., API such as Compound A, Compound A-HCl, etc.) dispersed in a solvent. The dispersed phase (solute, e.g., API) may be present homogeneously dispersed in an aqueous solution or water-based solvent phase or in an oil-based solvent phase.

[0081] The term “solid dispersion” as used herein refers to a system containing hydrophobic ingredient (e.g., API) dispersed in a matrix, which may be prepared by the melt (fusion) method, solvent evaporation method, melt extrusion, lyophilization, electrospinning, or the like. The solid dispersion may comprise particles, e.g., having a particle distribution D₉₀ less than about 100 micron, less than about 90 micron, less than about 80 micron, less than about 70 micron, less than about 60 micron, less than about 50 micron, less than about 40 micron, less than about 30 micron, less than about 20 micron, or less than about 10 micron.

[0082] In certain aspect, the pharmaceutical formulation may comprise the API and one or more of:

- (i) one or more fatty acid components including fatty acids and/or pharmaceutically acceptable salts thereof; and/or
- (ii) a glyceride component including a mixture of mono-, di-, and/or tri- fatty acid esters of glycerol, which include at least one of unsaturated fatty acids (e.g., linear or branched C₇-C₂₁, C₉-C₂₁, C₁₁-C₂₁, C₁₃-C₁₇ or C₁₅-C₁₇ alkenyl chain); and/or
- (iii) a glyceride component including a mixture of mono-, di-, and/or tri- fatty acid esters of glycerol, which are saturated fatty acids (e.g., linear or branched C₇-C₂₁, C₇-C₁₉, C₇-C₁₇, C₉-C₂₁, C₉-C₁₉, C₉-C₁₇, C₁₁-C₂₁, C₁₁-C₁₉ or C₁₁-C₁₇ alkyl chain).

[0083] In certain aspect, the pharmaceutical formulation may comprise the API and each of:

- (i) one or more fatty acid components including fatty acids and/or pharmaceutically acceptable salts thereof;
- (ii) a first glyceride component including a mixture of mono-, di-, and/or tri- fatty acid esters of glycerol, which include at least one of unsaturated fatty acids (e.g., linear or branched C₇-C₂₁, C₉-C₂₁, C₁₁-C₂₁, C₁₃-C₁₇ or C₁₅-C₁₇ alkenyl chain); and
- (iii) a second glyceride component including a mixture of mono-, di-, and/or tri- fatty acid esters of glycerol, which are saturated fatty acids (e.g., linear or branched C₇-

C₂₁, C₇-C₁₉, C₇-C₁₇, C₉-C₂₁, C₉-C₁₉, C₉-C₁₇, C₁₁-C₂₁, C₁₁-C₁₉ or C₁₁-C₁₇ alkyl chain).

[0084] The pharmaceutical formulation may comprise one or more fatty acid components including fatty acids and/or pharmaceutically acceptable salts thereof. The pharmaceutical formulation may also comprise a glyceride component including a mixture of mono-, di-, and/or tri- fatty acid esters of glycerol, which include at least one of unsaturated fatty acids (e.g., linear or branched C₇-C₂₁ alkenyl chain). The pharmaceutical formulation may also comprise a glyceride component including a mixture of mono-, di- and/or tri- fatty acid esters of glycerol, which are saturated fatty acids (e.g., linear or branched C₇-C₂₁ alkyl chain).

[0085] In certain aspect, the pharmaceutical formulation may comprise (i) one or more fatty acid components including fatty acids and/or pharmaceutically acceptable salts thereof; (ii) a first glyceride component including a mixture of mono-, di-, and/or tri- fatty acid esters of glycerol, which include at least one of unsaturated fatty acids (e.g., linear or branched C₇-C₂₁ alkenyl chain); and a second glyceride component including a mixture of mono-, di-, and/or tri- fatty acid esters of glycerol, which are saturated fatty acids (e.g., linear or branched C₇-C₂₁ alkyl chain).

[0086] The fatty acid may suitably include saturated or unsaturated hydrocarbon chains, e.g., saturated or unsaturated C₈-C₂₄ fatty acids.

[0087] The “fatty acid” as used herein refers to a compound or moiety having a carboxylic group (-COOH, -COO⁻) attached to a hydrocarbon chain (e.g., saturated or unsaturated, and linear or branched, preferably linear, C₇-C₂₃, C₁₁-C₂₁, C₁₃-C₁₉, or C₁₅-C₁₇ alkyl or alkenyl chain). The fatty acid may be present in a pharmaceutically acceptable salt form (e.g., sodium salt). As would be understood by a person skilled in the art, the number of carbon atoms specified for a particular fatty acid is one more than the number of carbon atoms in its alkyl or alkenyl chain to account for the carbonyl carbon of the fatty acid (i.e., a C₁₈ fatty acid has a C₁₇ alkyl or alkenyl chain). Accordingly, the fatty acid component may comprise one or more of a C₈-C₂₄ fatty acid, or a C₁₂-C₂₂ fatty acid, or a C₁₄-C₂₀ fatty acid, or a C₁₆-C₁₈ fatty acid, which fatty acid may be saturated or unsaturated, preferably unsaturated, and linear or branched, preferably linear.

[0088] In some embodiments, the fatty acid may be present in a pharmaceutically acceptable salt form (e.g., sodium salt). In some embodiments, the fatty acid components may include a fatty acid. In some embodiments, the fatty acid components may be a pharmaceutically acceptable salt form of a fatty acid (e.g., sodium salt). In some

embodiments, the fatty acid components may comprise both a fatty acid and a pharmaceutically acceptable salt form of a fatty acid (e.g., sodium salt).

[0089] Exemplary saturated C₈-C₂₄ fatty acids includes caprylic acid (CH₃(CH₂)₆COOH), capric acid (CH₃(CH₂)₈COOH), lauric acid (CH₃(CH₂)₁₀COOH), myristic acid (CH₃(CH₂)₁₂COOH), palmitic acid (CH₃(CH₂)₁₄COOH), stearic acid (CH₃(CH₂)₁₆COOH), arachidic acid (CH₃(CH₂)₁₈COOH), behenic acid (CH₃(CH₂)₂₀COOH), or lignoceric acid (CH₃(CH₂)₂₂COOH). Exemplary unsaturated C₈-C₂₄ fatty acids includes myristoleic acid (CH₃(CH₂)₃CH=CH(CH₂)₇COOH), palmitoleic acid (CH₃(CH₂)₅CH=CH(CH₂)₇COOH), sapienic acid (CH₃(CH₂)₈CH=CH(CH₂)₄COOH), oleic acid (CH₃(CH₂)₇CH=CH(CH₂)₇COOH), elaidic acid (CH₃(CH₂)₇CH=CH(CH₂)₇COOH), vaccenic acid (CH₃(CH₂)₅CH=CH(CH₂)₉COOH), linoleic acid (CH₃(CH₂)₄CH=CHCH₂CH=CH(CH₂)₇COOH), linoelaidic acid (CH₃(CH₂)₄CH=CHCH₂CH=CH(CH₂)₇COOH), α-linolenic acid (CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₇COOH), arachidonic acid (CH₃(CH₂)₄CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₃COOH), eicosapentaenoic acid (CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₃COOH), erucic acid (CH₃(CH₂)₇CH=CH(CH₂)₁₁COOH), or docosahexaenoic acid (CH₃CH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CHCH₂CH=CH(CH₂)₂COOH).

[0090] In certain embodiments, the fatty acid(s) may be unsaturated C₈-C₂₄, C₁₂-C₂₂, C₁₄-C₂₀, or C₁₆-C₁₈ fatty acid(s). In certain embodiments, the fatty acid may be one or more unsaturated C₁₂-C₂₄, C₁₂-C₂₂, C₁₂-C₂₀, or C₁₂-C₁₈ fatty acids. In certain embodiments, the fatty acid may be one or more unsaturated C₁₂-C₂₀ fatty acids. In certain embodiments, the fatty acid may be unsaturated C₁₄-C₂₄, C₁₄-C₂₂, C₁₄-C₂₀, or, C₁₄-C₁₈ fatty acids. Preferred fatty acid includes unsaturated C₁₈ fatty acids, and particularly oleic acid (CH₃(CH₂)₇CH=CH(CH₂)₇COOH).

[0091] In certain embodiments, the fatty acid salt comprises one or more unsaturated C₈-C₂₄, C₁₂-C₂₂, C₁₄-C₂₀, or C₁₆-C₁₈ fatty acid sodium salts. In certain embodiments, the fatty acid salt may be unsaturated C₁₂-C₂₄, C₁₂-C₂₂, C₁₂-C₂₀, or C₁₂-C₁₈ fatty acid sodium salt. In certain embodiments, the fatty acid salt may be unsaturated C₁₄-C₂₄, C₁₄-C₂₂, C₁₄-C₂₀, or, C₁₄-C₁₈ fatty acid sodium salt. Preferred fatty acid salt includes unsaturated C₁₈ fatty acid sodium salt, particularly oleic acid sodium salt (CH₃(CH₂)₇CH=CH(CH₂)₇COONa).

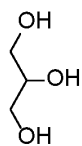
[0092] In embodiments, the fatty acid component comprises a combination of a fatty acid and a fatty acid salt, each for example as provided in the preceding paragraphs.

[0093] In certain aspect, the pharmaceutical formulation comprises the fatty acid component in an amount of about 15 to 50 wt%, about 20 to 45 wt%, about 30 to 45 wt%, about 35 to 45 wt%, or about 35 to 40 wt% based on the weight of the pharmaceutical formulation (i.e., not including the weight of a capsule shell or coating). As used herein, the pharmaceutical formulation refers to the composition comprising the API, the fatty acid and triglyceride components, and any other excipients, but does not include the capsule shell or coating.

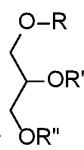
[0094] In embodiments, the fatty acid component comprises a combination of a fatty acid and a fatty acid salt, each for example as provided in the preceding paragraphs. The pharmaceutical formulation may comprise the fatty acid in an amount of about 12 to 48 wt%, about 15 to 45 wt%, of about 25 to 40 wt%, about 30 to 40 wt%, or about 35 to 40 wt% based on the weight of the pharmaceutical formulation. The pharmaceutical formulation may comprise a sodium salt of the fatty acid in an amount of about 1 to 5 wt%, or about 2 to 4 wt%, or about 2 to 3 wt%, based on the weight of the pharmaceutical formulation. The pharmaceutical formulation may comprise oleic acid and sodium oleate.

[0095] The pharmaceutical formulation comprises glyceride components (e.g., a first glyceride component and a second glyceride component), including a mixture of mono-, di-, and/or tri- fatty acid esters of glycerol.

[0096] The term “glycerol” refers to a compound containing triol (three -OH groups) and

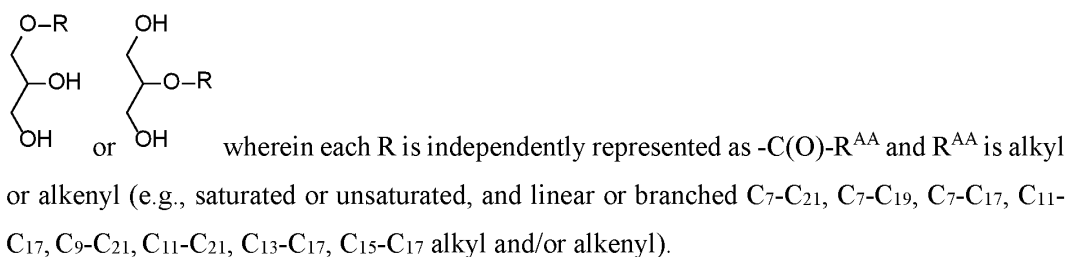


having a structure of . The term “glyceride” as used herein refers to a compound or moiety formed by one to three ester bonds between glycerol and fatty acids (e.g., one, two or

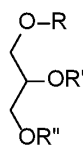


three fatty acids), and having a structure of OR'' wherein each R, R', and R'' is hydrogen or -C(O)-R^{AA} and R^{AA} is alkyl or alkenyl (e.g., saturated or unsaturated, and linear or branched C7-C21, C7-C19, C7-C17, C11-C17, C9-C21, C11-C21, C13-C17, C15-C17 alkyl and/or alkenyl) and at least one of R, R', and R'' is not hydrogen.

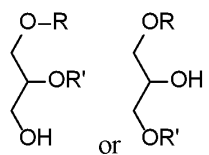
[0097] The term “monoglyceride” or “mono-fatty acid ester of glycerol” as used herein refers to a compound or molecule of glycerol linked to a single fatty acid. The monoglyceride may have a structure of



[0098] The term “diglyceride” or “di-fatty acid esters of glycerol” as used herein refers to a compound or molecule of glycerol linked to two fatty acids, which may be the same or

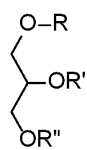


different. The diglyceride may have a structure of $\text{O}-R$ wherein each R, R', and R'' is independently represented as hydrogen or $-C(O)-R^{AA}$, and R^{AA} is alkyl or alkenyl (e.g., saturated or unsaturated, and linear or branched C₇-C₂₁, C₇-C₁₉, C₇-C₁₇, C₁₁-C₁₇, C₉-C₂₁, C₁₁-C₂₁, C₁₃-C₁₇, C₁₅-C₁₇ alkyl and/or alkenyl), and one of R, R', and R'' is hydrogen. The diglyceride may have a structure:



wherein each R and R' is independently represented as $-C(O)-R^{AA}$ and R^{AA} is alkyl or alkenyl (e.g., saturated or unsaturated, and linear or branched C₇-C₂₁ alkyl and/or alkenyl).

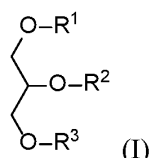
[0099] The term “triglyceride” or “tri-fatty acid esters of glycerol” as used herein refers to a compound or molecule of glycerol linked to three fatty acids, which may be the same or different. The triglyceride may have a structure of



wherein each R, R', and R'' is independently represented as $-C(O)-R^{AA}$ and R^{AA} is alkyl or alkenyl (e.g., saturated or unsaturated, and linear or branched C₇-C₂₁ alkyl and/or alkenyl).

[0100] The pharmaceutical formulation may comprise up to about 75 % of glycerides, or up to about 70 % of glycerides, or up to about 65 % of glycerides, or up to about 60 % of glycerides, or up to about 55 % of glycerides, or up to about 50 % of glycerides. The formulation may comprise at least about 40 % of glycerides, or at least about 45 % of glycerides, or at least about 50 % of glycerides. The glycerides may comprise the first glyceride component and the second glyceride component.

[0101] The first glyceride component may suitably include one or more of mono-, di-, and/or tri- glycerides that include at least one C₇-C₂₁ alkenyl (including one or two double bonds). The first glyceride component comprises one or more glyceride compounds having a structure of Formula (I)



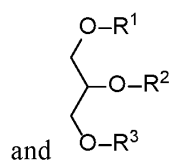
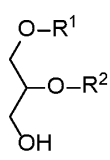
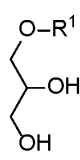
wherein:

each R¹, R², and R³ is independently hydrogen or -C(O)-R^A;

each R^A is independently C₇-C₂₁ alkenyl comprising one or two double bonds, provided that at least one of R¹, R², and R³ is not hydrogen.

[0102] In some embodiments, in Formula (I), each R^A is independently C₇-C₂₁, C₉-C₂₁, C₁₁-C₂₁, C₁₃-C₁₇ or C₁₅-C₁₇ alkenyl (e.g., linear or branched, preferably linear). In some embodiments, in Formula (I), each R^A is independently C₇-C₂₁, C₇-C₁₉, C₁₁-C₁₉, or C₁₁-C₁₇ alkenyl, which includes one or two double bonds. In some embodiments, at least one R^A comprises one or more of C₇ alkenyl, C₉ alkenyl, C₁₁ alkenyl, C₁₃ alkenyl, C₁₅ alkenyl, C₁₇ alkenyl, and combinations thereof. In some embodiments, at least one R^A is C₁₇ alkenyl, which includes one or two double bonds.

[0103] In certain aspect, the first glyceride component includes one or more compounds having one of the following structures:



R¹, R², and R³ are as described above.

[0104] In the first glyceride component, a weight ratio of the compound of mono-, di-, and/or tri- glycerides that include at least one of C₇-C₂₁ alkenyl may be about 32 to 52 : 40 to 55: 5 to 20. In some embodiments, the first glyceride component may include the compound of Formula (I-a), the compound of Formula (I-b), and the compound of Formula (I-c) at a weight ratio of about 32 to 52 : 40 to 55: 5 to 20.

[0105] In the first glyceride component, a weight ratio of mono-, di-, and/or tri- glycerides that include at least one of C₇-C₂₁ alkenyl may be about 32 to 52 : 40 to 55: 5 to 20. In some embodiments, the first glyceride component may alternatively include the compound of

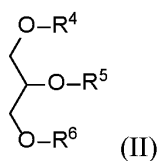
Formula (I-a), the compound of Formula (I-b), and the compound of Formula (I-c) at a weight ratio of about 32 to 52 : 40 to 55: 5 to 20.

[0106] Alternatively, the first glyceride component may dominantly (i.e., greater than 50 wt%) include monoglycerides including at least one of C₇-C₂₁ alkenyl. In some embodiments, the first glyceride component may dominantly include diglycerides including at least one of C₇-C₂₁ alkenyl. In some embodiments, the first glyceride component may dominantly include triglycerides including at least one of C₇-C₂₁ alkenyl.

[0107] In embodiments, the first glyceride component comprises mono-, di-, and tri-glycerides of oleic acid and linoleic acid. The relative amounts of mono-, di-, and tri-glycerides may be about 32-52 % mono-glyceride, about 40-55 % di-glycerides, and about 5-20 % triglycerides. The first glyceride component may be Maisine CC.

[0108] In certain aspect, the pharmaceutical formulation comprises the first glyceride component in an amount of about 30 to 50 wt%, or about 30 to 45 wt%, or about 32 to 40 wt%, or about 35 to 40 wt%, based on the weight of the pharmaceutical formulation.

[0109] The second glyceride component may suitably include one or more of mono-, di-, and/or tri- glycerides of C₈ to C₂₂ saturated fatty acids. The second glyceride component includes one or more glyceride compounds having a structure of Formula (II):



wherein:

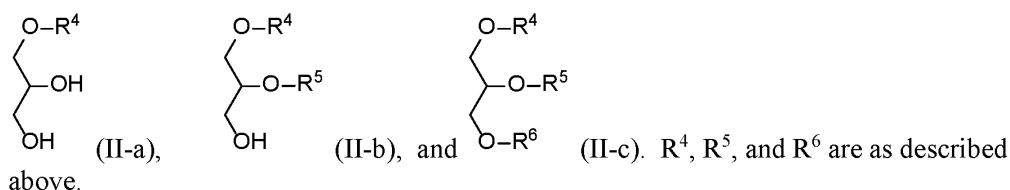
each R⁴, R⁵, and R⁶ is independently hydrogen, or -C(O)-R^B;

each R^B is independently hydrogen or C₇-C₂₁ alkyl,

provided that at least one of R⁴, R⁵, and R⁶ is not hydrogen.

[0110] In some embodiments, in Formula (II), each R^B is independently C₇-C₂₁, C₇-C₁₉, C₇-C₁₇, C₉-C₂₁, C₉-C₁₉, C₉-C₁₇, C₁₁-C₂₁, C₁₁-C₁₉ or C₁₁-C₁₇ alkyl (e.g., linear or branched). In some embodiments, in Formula (II), each R^B is independently C₇-C₂₁, C₇-C₁₉, C₇-C₁₇, C₇-C₁₅, C₇-C₁₃, C₇-C₁₁, C₉-C₂₁, C₁₁-C₁₇, C₁₁-C₂₁, or C₁₃-C₂₁ alkyl. In some embodiments, one of R^B is C₂₁ alkyl. In some embodiments, one of R^B is C₇ alkyl. In some embodiments, one of R^B is C₉ alkyl. In some embodiments, one of R^B is C₁₁ alkyl. In some embodiments, one of R^B is C₁₃ alkyl. In some embodiments, one of R^B is C₁₅ alkyl. In some embodiments, one of R^B is C₁₇ alkyl. In some embodiments, one of R^B is C₁₉ alkyl.

[0111] In certain aspect, the second glyceride component includes one or more compounds having one of the following structures:



[0112] In some embodiments, the second glyceride component may dominantly (i.e., greater than 50 wt%) include monoglycerides including at least one C_7 - C_{21} alkyl. In some embodiments, the second glyceride component may dominantly include diglycerides including at least one C_7 - C_{21} alkyl. In some embodiments, the second glyceride component may dominantly include triglycerides including at least one C_7 - C_{21} alkyl.

[0113] In embodiments, the second glyceride component comprises triglycerides of C_8 to C_{18} saturated fatty acids. The second glyceride component may be Gelucire 43/01.

In certain aspect, the pharmaceutical formulation comprises the second glyceride component in an amount of about 10 to 35 wt%, or about 10 to 30 wt%, or about 10 to 20 wt%, or about 15 to 18 wt%, based on the weight of the pharmaceutical formulation.

[0114] In certain aspect, the pharmaceutical formulation comprises the fatty acid component in an amount of about 32 to 40 wt%, about 33 to 39 wt%, about 34 to 38 wt%, or about 34 to 37 wt%, based on the total weight of the pharmaceutical formulation.

[0115] In some embodiments, the pharmaceutical formulation includes a fatty acid having saturated or unsaturated C_{12} - C_{18} fatty acid in an amount of about 30 to 35 wt%, about 31 to 34 wt%, of about 32 to 34 wt%, based on the total weight of the pharmaceutical formulation.

[0116] In some embodiments, the pharmaceutical formulation includes a sodium salt of a fatty acid having saturated or unsaturated C_{12} - C_{18} fatty acids in an amount of about 2 to 5 wt%, or about 3 to 4 wt%, based on the total weight of the pharmaceutical formulation.

[0117] The certain embodiments, the pharmaceutical formulation includes a fatty acid having saturated or unsaturated C_{12} - C_{18} fatty acid in an amount of about 30 to 35 wt%, about 31 to 34 wt%, of about 32 to 34 wt%; and a sodium salt of a fatty acid having saturated or unsaturated C_{12} - C_{18} fatty acids in an amount of about 2 to 5 wt%, or about 3 to 4 wt%, based on the total weight of the pharmaceutical formulation.

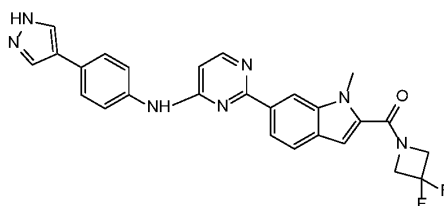
[0118] In certain aspect, the pharmaceutical formulation includes the first glyceride component in an amount of about 30 to 35 wt%, about 31 to 34 wt%, of about 32 to 34 wt%, based on the total weight of the pharmaceutical formulation.

[0119] In certain aspect, the pharmaceutical formulation includes the second glyceride component in an amount of about 25 to 30 wt%, about 26 to 30 wt%, about 27 to 30 wt%, about 28 to 30 wt%, or about 29 to 30 wt% based on the total weight of the pharmaceutical formulation.

[0120] In certain aspect, the pharmaceutical formulation comprises the API and:
 a fatty acid having saturated or unsaturated C₁₂-C₁₈ fatty acid in an amount 30 to 35 wt% (e.g., about 31 to 34 wt%, or about 32 to 34 wt%);
 a sodium salt of a fatty acid having saturated or unsaturated C₁₂-C₁₈ fatty acids in an amount of about 2 to 5 wt% (e.g., about 3 to 4 wt%);
 the first glyceride component in an amount of about 30 to 35 wt% (e.g., about 31 to 34 wt%, or about 32 to 34 wt%); and
 the second glyceride component in an amount of about 25 to 30 wt% (e.g., about 26 to 30 wt%, about 27 to 30 wt%, about 28 to 30 wt%, or about 29 to 30 wt%);
 based on the total weight of the pharmaceutical formulation.

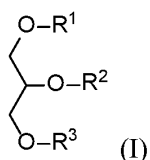
[0121] In certain aspect, the pharmaceutical formulation comprises:

- (i) about 1-15 wt %, or about 1-10 wt %, or about 2-8 wt %, or about 5-7 wt % of the pharmaceutical formulation of a compound having a structure:



or a pharmaceutically acceptable salt thereof, and

- (ii) about 15 to 50 wt%, or about 20 to 45 wt%, or about 30 to 45 wt%, or about 35 to 45 wt%, or about 35 to 40 wt% of the pharmaceutical formulation of a fatty acid component comprising saturated or unsaturated C₈-C₂₄ fatty acids and/or a pharmaceutically acceptable salt thereof;
 (iii) about 30 to 50 wt%, or about 30 to 45 wt%, or about 32 to 40 wt%, or about 35 to 40 wt% of the pharmaceutical formulation of a first glyceride component comprising one or more compounds having a structure of Formula (I)



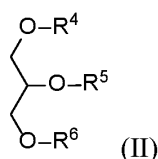
wherein:

each R^1 , R^2 , and R^3 is independently hydrogen or $-C(O)-R^A$;

each R^A is independently C_7-C_{21} alkenyl comprising one or two double bonds, provided that at least one of R^1 , R^2 , and R^3 is not hydrogen; and

(iv) about 10 to 35 wt%, or about 10 to 30 wt%, or about 10 to 20 wt%, or about 15 to 18 wt% of the pharmaceutical formulation of a second glyceride component comprising:

one or more compounds having a structure of Formula (II)



wherein:

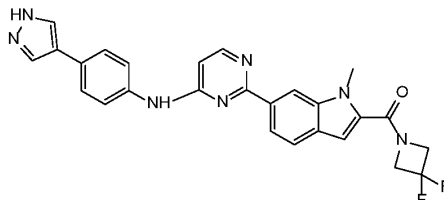
each R^4 , R^5 , and R^6 is independently hydrogen, or $-C(O)-R^B$;

each R^B is independently C_7-C_{21} alkyl,

provided that at least one of R^4 , R^5 , and R^6 is not hydrogen.

[0122] In certain aspect, the pharmaceutical formulation comprises:

(i) about 1-15 wt %, or about 1-10 wt %, or about 2-8 wt %, or about 5-7 wt % of the pharmaceutical formulation of a compound having a structure:



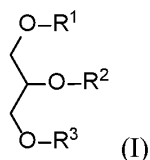
or a pharmaceutically acceptable salt thereof, and

(ii) a fatty acid component comprising:

a saturated or unsaturated C_8-C_{24} fatty acid in an amount of about 12 to 48 wt%, about 15 to 45 wt%, of about 25 to 40 wt%, about 30 to 40 wt%, or about 35 to 40 wt% of the pharmaceutical formulation; and

a sodium salt of a saturated or unsaturated C_8-C_{24} fatty acid in an amount of about 1 to 5 wt%, or about 2 to 4 wt%, or about 2 to 3 wt%, of the pharmaceutical formulation;

- (iii) about 30 to 50 wt%, or about 30 to 45 wt%, or about 32 to 40 wt%, or about 35 to 40 wt% of the pharmaceutical formulation of a first glyceride component comprising one or more compounds having a structure of Formula (I)



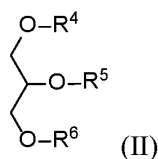
wherein:

each R^1 , R^2 , and R^3 is independently hydrogen or $-\text{C}(\text{O})-\text{R}^{\text{A}}$;

each R^{A} is independently $\text{C}_7\text{-C}_{21}$ alkenyl comprising one or two double bonds, provided that at least one of R^1 , R^2 , and R^3 is not hydrogen; and

- (iv) about 10 to 35 wt%, or about 10 to 30 wt%, or about 10 to 20 wt%, or about 15 to 18 wt% of the pharmaceutical formulation of a second glyceride component comprising:

one or more compounds having a structure of Formula (II)



wherein:

each R^4 , R^5 , and R^6 is independently hydrogen, or $-\text{C}(\text{O})-\text{R}^{\text{B}}$;

each R^{B} is independently $\text{C}_7\text{-C}_{21}$ alkyl,

provided that at least one of R^4 , R^5 , and R^6 is not hydrogen.

Dosage Forms

[0123] The disclosure also provides a semi-solid or liquid-based formulation when formulated as, including, but not limited to, an emulsion, suspension, solution, elixirs, or syrup in which the ROCK2 inhibitor (e.g., compound A) is dissolved and/or suspended.

[0124] The dosage form comprising a ROCK2 inhibitor, such as a compound of the Formula I, and particularly Compound A or its salts, can take the form of solutions, suspensions, emulsion, capsules, soft elastic or hard gelatin capsules, suspensions, and the like preferably in unit dosage forms suitable for simple administration of precise dosages. The composition may take the forms of liquid- or semi-solid filled capsules for oral administration.

[0125] Capsule dosage forms may include soft capsules and hard capsules. Capsules may be used as an oral dosage form for the administration of a ROCK2 inhibitor, such as a compound of the Formula I, and particularly Compound A or its salts. The capsules may be filled with the active ingredient in the form of a liquid, or a powder suspended in liquid. Hard capsules can be made of unplasticized or low-plasticized gelatin and water to form a stiff capsule that can be filled with either powder or liquid. Soft capsules can be made of highly plasticized soft elastic gelatin and can contain a liquid or semisolid ingredient. These capsules are often referred to as “softgel” or “gelcap” capsules.

[0126] As used herein, the term “capsule” refers to any suitable capsular container or case adapted for oral ingestion, e.g., those adapted for use in conjunction with liquid fill compositions. The term “capsule” may include capsules having a shell composed of soft and/or hard materials, such as gelatin, starches, celluloses, cellulose derivatives (e.g., hydroxypropyl methyl cellulose), hydrocolloids, gums, carrageenans, or any other natural or synthetic material which can be used to encapsulate the liquid composition and be ingested by an animal. Optionally, the shell material can be gelatin and/or hydroxypropyl methyl cellulose. In an embodiment, the shell material is gelatin. The term “capsule” also includes a variety of capsule shapes and sizes. The instant disclosure does not limit the dosage form to a specific type or shape. Any commercially available capsule shells or shell materials can be used.

[0127] In an embodiment, the dosage form of the instant disclosure is a soft capsule. In an embodiment, the dosage form of the instant disclosure is a coated liquid-filled soft capsule. The coated capsule can include a liquid fill encapsulated with a soft capsule shell. The exterior surface of the soft capsule shell can be coated with one or more layers of coating.

[0128] Suitable materials for encapsulating the liquid fill may include heat sealable polymers and gelatin. Examples of heat sealable polymers may include, but are not limited to, modified starches, cellulosic polymers and carrageenans. In an embodiment, the material is gelatin. The gelatin can be natural gelatin, chemically modified gelatin, enzymatically modified gelatin, or combinations thereof.

[0129] The material that forms the capsule shell can further includes water. Water can be present in the original material mass before the capsules are made, in an amount sufficient to allow the processing of the material on the encapsulation machine. After the capsules are formed the majority of the moisture can be removed during the drying process.

[0130] The water can have a plasticizing effect on the material. In addition, a non-volatile plasticizer or blend of plasticizers can be added to the material which forms the capsule shell.

The non-volatile plasticizer can be any plasticizer compatible with the material of the capsule shell. For example, the non-volatile plasticizer can be glycerin, maltitol, sorbitan, sorbitol or similar low molecular weight polyhydric alcohols, and mixtures thereof. In embodiments, the ratio of plasticizer to material may determine how hardness or softness the shell.

[0131] The ratio of plasticizer to material in the shell may be sufficient to provide capsules that are not too hard, such that the capsules are brittle and crack if stressed during shipping and handling, and are not too soft, such that the capsules become deformed during shipping and handling. The non-volatile plasticizer can be present in the capsule shell from about 8% to 65% by total weight of the capsule shell, from about 10% to 35% by total weight of the capsule shell.

[0132] The material which forms the capsule shell can further contain extenders. The extender can be any extender which is compatible with the material. Examples of extenders may include natural or modified natural biopolymers and synthetic polymers. Natural biopolymers may include, for instance, cellulose, starch, starch derivatives, bacterial polysaccharides such as xanthan gum and gellan gum and vegetable gums such as guar gum, locust bean gum, gum tragacanth and gum Arabic and animal derived polymers such as chondroitin sulfate, hyaluronic acid, heparin, collagen and chitosan. An example of a modified natural biopolymer may be modified cellulose. Examples of synthetic polymers may include carbon chain polymers of the vinyl and acrylic types as well as heterochains of the polyoxide and polyamine types.

[0133] A coating can be applied on the exterior surface of the soft capsule shell. The coating can contain one or more layers. Any coating suitable for a soft capsule can be applied to the capsule. The coating can provide, for example, waterproofing and sealing, smoothing, polishing, enteric protection and/or delayed release properties to the liquid-filled capsule. The delayed release can be affected by, for example, temperature or pH. In an embodiment, the coating is an enteric coating.

[0134] The coating can be made by any standard coating ingredient known to those skilled in the art. Coating ingredients may include, but are not limited to, fats, fatty acids, waxes, shellac, ammoniated shellac, cellulose acetate phthalates, cellulosics, vinyls, glycols, acrylics and carbohydrate polymers, polymers and co-polymers containing methacrylic acid and methacrylic acid alkyl esters, hydroxypropylmethyl cellulose (HPMC) and combinations thereof.

[0135] The coated capsule can further comprise a finishing coating. In an embodiment, the finishing layer is applied to the coated-capsule. Examples of substance suitable for use in

a finishing coating may include, but are not limited to cellulose, vinyls, glycols, acrylics and carbohydrate polymers and/or combinations thereof.

[0136] The liquid fill or semi-solid fill can be encapsulated with a soft capsule shell by any method known in the art. For example, a soft capsule can be made using a standard rotary die soft gelatin capsule machine as described in *The Theory and Practice of Industrial Pharmacy*, ed. Lachman, et al., 2nd Ed., Pt. II, 404-420, Lea & Febiger, 1976. Additional methods include using a plate process (see *The Theory and Practice of Industrial Pharmacy*, ed. Lachman, et al., 2nd Ed., Pt. II, 405, Lea & Febiger, 1976), as well as Globex type seamless capsule machines, which makes large microcapsules (see U.S. Pat. No. 5,254,294), non-standard rotary die machines, which uses extrusion technology to make gel ribbons (see U.S. Pat. Nos. 6,183,845 and 6,340,473), and other methods for making capsules which use high frequency, ultrasonic, or induction welding to seal the capsules (see U.S. Pat. No. 6,352,719). The above-listed U.S. patents and book are hereby incorporated by reference.

[0137] As used herein, the phrase “liquid hard-shell” refers to a hard capsule encapsulating a liquid or semi-solid formulation. Hard capsules can be single unit dosage forms and may comprise a cap and a body, which can be manufactured separately, and which can be supplied empty for filling with the liquid or semi-solid composition. In some embodiments, hard capsules are made from a polymer such as gelatin. An additional component can be water, which acts as a plasticizer. Another hard capsule may be manufactured from hydroxypropylmethyl cellulose (HPMC). Liquid-fill hard capsule can be filled on a filling machine, such as, for example, a high-speed filling machine.

[0138] In one example, disclosed herein is a method of prepare the filled hard capsule. Empty capsules are supplied to the filling machine in a prelocked condition, wherein the capsule body has a cap which is loosely attached thereto. A series of rings or protrusions are provided in the mating surfaces of the cap or body. These rings are configured to enable the cap to be loosely attached to the body so that the cap and body are held together during storage but would enable the cap to be removed prior to filling of the capsule. Once the capsule has been filled, the cap can be replaced and be forced beyond the prelocked position into a fully locked position. Alternatively, other types of capsule filling machines can be used to accept separate supplies of capsule bodies and caps.

[0139] The capsules may be closed at high speed after filling with the formulated composition. During closure of the capsule, the cap is fitted over the body and the body is pushed up until it locks on the cap. The cap can be close fitting and can be approximately half the length of the body, so the cap can travel for a considerable distance down the capsule

body before locking. This may have the effect of a piston in trapping and pressurizing the capsule. The excess gas can escape through the gap between the cap and the body, and vents may be provided in this region so as to facilitate the escape of excess pressure. Alternatively, the capsule may utilize a particularly tight locking mechanism rather than vents

[0140] In an embodiment, the capsule is banded by applying a band of polymer solution around the junction between the cap and body. The polymer solution can be a solution of the same polymer as the capsule cap and/or body in a solvent therefor. Banding can provide a smooth capsule surface for coating, which may prevent movement between the cap and body of the capsule.

[0141] When preparing the filled capsule that is filled with the composition comprising compound A, it is preferred that the composition is in a liquid form at least during the encapsulation process. In an embodiment, the final capsule contains the composition in the liquid form. In an embodiment, the final capsule contains the composition in semi-solid form at room temperature.

[0142] The dosage and frequency (single or multiple doses) of ROCK2 compounds (e.g., Compound A) administered can vary depending upon a variety of factors, including route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated; presence of other diseases or other health-related problems; kind of concurrent treatment; and complications from any disease or treatment regimen. Other therapeutic regimens or agents can be used in conjunction with the methods and compounds disclosed herein.

[0143] As is well known in the art, therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring compounds effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

Method of Administration

[0144] The administration of the pharmaceutical formulation including ROCK2 inhibitors (e.g., compound A) can be carried out via oral, nasal, intraocular, intravenous, intramuscular, subcutaneous, transdermal, subdermal, sublingual or rectal route of administration, and particularly by oral administration.

[0145] In certain aspect, the route of administration is oral and the pharmaceutical formulation is provided in the form of capsules, such as soft elastic or hard gelatin capsules.

[0146] In an embodiment, the route of administration is in combination with nasal administration. The formulation can be a solution, an aerosol, a liquid suspension, or a liquid dispersion, in the form of a nasal spray, a nasal douche, an inhaler, a nasal drop, and/or a diffuser.

[0147] In embodiments, the route of administration is in combination with dermal administration including but not limited to topical, subcutaneous, subdermal, transdermal, intradermal or dermal patch.

[0148] In embodiments, the route of administration is parenteral, such as by IV injection.

Treatment of neurodegenerative disease

[0149] The method for treating neurodegenerative disease in a subject in need thereof comprises administering to the subject a therapeutically effective amount of a ROCK2 inhibitor. The neurodegenerative disease may be characterized by the degeneration of motor neurons, and includes amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy, pseudobulbar palsy, and monomelic amyotrophy (MMA). The patient may be one that has been recognized or diagnosed to be suffering from the neurodegenerative disease, including ALS, or has been recognized or diagnosed to have a high risk of developing a neurodegenerative disease, and/or that is recognized or diagnosed to be experiencing the symptoms of the onset, or progression towards, the neurodegenerative disease.

[0150] The treatment disclosed herein may slow, reduce and/or prevent the loss of motor neurons in patients diagnosed with a neurodegenerative disease, and particularly ALS.

[0151] The neuromuscular junction (NMJ) dismantling occurs at the early stages of the ALS, in most case even prior to motor neuron degeneration and onset of the clinical symptoms. The treatments disclosed herein may slow, reduce and/or prevent the dismantling of the neuromuscular junction.

[0152] ALS is characterized by the progressive degeneration and eventual death of motor neurons in the brain, brainstem and spinal cord. The neurons involved in ALS facilitate communication between the nervous system and voluntary muscles of the body (motor neurons). As a result, the ability to initiate and control voluntary movement is lost. ALS affects the muscles needed to move the arms and legs, to speak and swallow, to support the neck and trunk, and to breathe. The symptoms of ALS progress over time and, ultimately,

the disease leads to death, generally from respiratory failure as patients lose the ability to control muscles in the chest and diaphragm.

[0153] In embodiments, this disclosure provides a method for the slowing or preventing neuronal loss in a subject in need thereof by administering to the subject a therapeutically effective amount of a ROCK2 inhibitor. The subject may be diagnosed with, or suspected of having, a neurodegenerative disease that is characterized by the loss of neurons, and particularly by the loss of motor neurons.

[0154] The present disclosure provides a method for the treatment of amyotrophic lateral sclerosis (ALS) comprising administering to a patient in need thereof a therapeutically effective amount of a ROCK2 inhibitor, and particularly Compound A. In preferred aspects, the ROCK2 inhibitor, and particularly Compound A, is administered orally to the patient in the formulation provided herein.

[0155] ALS, also known as Lou Gehrig's disease, is a neurodegenerative disease that results in the progressive loss of motor neurons that control voluntary muscles. Motor neuron loss continues until the ability to eat, speak, move, and finally the ability to breathe is lost. Death is usually caused by respiratory failure, with the average survival from onset to death of two to four years. The disease can affect people of any age, but usually starts around the age of 60. Currently, there is no known cure for ALS. Current treatments are directed to slowing the disease and improving symptoms. Current treatments that slow ALS progression extend the life of the patient by about two to seven months.

Method of Treatment

[0156] The present disclosure provides a method for the prevention or treatment of a disease mediated by ROCK2, wherein the method comprises administering to a subject in need thereof an effective amount of a ROCK2 inhibitor as disclosed herein or a pharmaceutically acceptable salt thereof, particularly in a formulation as provided herein.

[0157] In some embodiments, the present disclosure provides methods for the treatment of at least one disease or disorder selected from the group comprising fibrotic diseases, inflammatory diseases, and autoimmune diseases, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound or a composition as defined herein.

[0158] In other embodiments, the disclosure provides methods for the treatment of a cardiovascular disorder, a central nervous system disorder, a neoplastic disease, or a metabolic syndrome, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound or a composition as defined herein.

[0159] In some embodiments, the disease mediated by ROCK2 is an autoimmune disorder including rheumatoid arthritis, systemic lupus erythematosus (SLE; lupus), psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, atopic dermatitis, eczema, or graft-versus-host disease (GVHD; acute and chronic), idiopathic pulmonary fibrosis and scleroderma.

[0160] Other autoimmune disorders that may be treated according to the methods provided in this disclosure include acute disseminated encephalomyelitis (ADEM), acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, alopecia areata, amyloidosis, ankylosing spondylitis, anti-GBM/anti-TBM nephritis, antiphospholipid syndrome (APS), autoimmune angioedema, autoimmune aplastic anemia, autoimmune dysautonomia, autoimmune hepatitis, autoimmune hyperlipidemia, autoimmune immunodeficiency, autoimmune inner ear disease (AIED), autoimmune myocarditis, autoimmune oophoritis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune thyroid disease, autoimmune urticaria, axonal & neuronal neuropathies, Balo disease, Behcet's disease, bullous pemphigoid, cardiomyopathy, Castleman disease, Celiac disease, Chagas disease, chronic inflammatory demyelinating polyneuropathy (CIDP), chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss syndrome, Cogan's syndrome, coxsackie myocarditis, CREST disease, demyelinating neuropathies, dermatitis herpetiformis, dermatomyositis, Devic's disease (neuromyelitis optica), discoid lupus, Dressler's syndrome, eosinophilic esophagitis, eosinophilic fasciitis, erythema nodosum, Evans syndrome, fibrosing alveolitis, giant cell arteritis (temporal arteritis), giant cell myocarditis, glomerulonephritis, Goodpasture's syndrome, granulomatosis with polyangiitis (GPA), Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, hemolytic anemia, Henoch-Schönlein purpura, Hypogammaglobulinemia, idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related sclerosing disease, immunoregulatory lipoproteins, inclusion body myositis, interstitial cystitis, juvenile arthritis, juvenile myositis, Kawasaki syndrome, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, lichen planus, lichen sclerosus, ligneous conjunctivitis, Linear IgA disease (LAD), Meniere's disease, microscopic polyangiitis, mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, myasthenia gravis, myositis, neuromyelitis optica, neutropenia, ocular cicatricial pemphigoid, optic neuritis, palindromic rheumatism, pediatric autoimmune neuropsychiatric disorders associated with streptococcus (PANDAS), paraneoplastic cerebellar degeneration, paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, pars planitis (peripheral uveitis), pemphigus, peripheral neuropathy, perivenous

encephalomyelitis, pernicious anemia, POEMS syndrome, polyarteritis nodosa, autoimmune polyglandular syndromes (type I, II, III), polymyalgia rheumatica, polymyositis, post myocardial infarction syndrome, post pericardiotomy syndrome, progesterone dermatitis, primary biliary cirrhosis, primary sclerosing cholangitis, psoriatic arthritis, pyoderma gangrenosum, pure red cell aplasia, Raynaud's phenomenon, reactive arthritis, reflex sympathetic dystrophy, Reiter's syndrome, relapsing polychondritis, retroperitoneal fibrosis, sarcoidosis, Schmidt syndrome, scleritis, Sjogren's syndrome, Sperm & testicular autoimmunity, stiff person syndrome, subacute bacterial endocarditis (SBE), Susac's syndrome, sympathetic ophthalmia, Takayasu's arteritis, temporal arteritis/Giant cell arteritis, thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, Transverse myelitis, undifferentiated connective tissue disease (UCTD), type-1 autoimmune diabetes, uveitis, vasculitis, vesiculobullous dermatosis, vitiligo, and Wegener's granulomatosis (granulomatosis with polyangiitis; GPA).

[0161] Inflammatory disorders that can be treated by methods provided in this disclosure include, but are not limited to, cardiovascular inflammation, pulmonary inflammation, renal inflammation, arteriosclerosis and sepsis.

[0162] Fibrotic disorders that can be treated by methods provided in this disclosure include idiopathic pulmonary fibrosis, renal fibrosis, kidney fibrosis, ocular fibrosis, cardiac fibrosis, NASH, scleroderma, systemic sclerosis, and cirrhosis.

[0163] In another embodiment, the disclosure provides a method for the treatment of muscular dystrophy (Duchenne muscular dystrophy). In another embodiment, the disclosure provides a method for the treatment of myotonic dystrophy.

[0164] In other embodiments, the ROCK2 inhibitors provided herein may be used to inhibit tumor cell growth and metastasis, and angiogenesis, and are useful for treating neoplastic diseases. Neoplastic diseases include any malignant growth or tumor caused by abnormal or uncontrolled cell division. Neoplastic diseases include lymphoma, carcinoma, leukemia, sarcoma and blastoma. Non-limiting examples include squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, bladder cancer, liver cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic

carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer.

[0165] In other embodiments, the ROCK2 inhibitors provided herein may be used in the treatment of a cardiovascular disorder including hypertension, cardiomyopathy, cardiac remodeling, atherosclerosis, restenosis, cardiac hypertrophy, cerebral ischemia, cerebral vasospasm, and erectile dysfunction.

[0166] In other embodiments, the ROCK2 inhibitors provided herein may be used in the treatment of a pulmonary disorder including idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma.

[0167] In other embodiments, the ROCK2 inhibitors provided herein may be used in the treatment of a central nervous system disorder, including neuronal degeneration or spinal cord injury, traumatic brain injury, cerebral cavernous malformation, Huntington's disease, Parkinson's disease, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), multiple sclerosis, or Duchenne muscular dystrophy.

[0168] In other embodiments, the disclosure provides methods for the treatment of renal diseases including polycystic kidney disease, renal fibrosis, diabetic renal disease, chronic kidney disease and focal segmental glomerulosclerosis.

[0169] In other embodiments, the ROCK2 inhibitors provided herein may be used in the treatment of a metabolic disease including insulin resistance, hyperinsulinemia, type 2 diabetes, obesity, metabolic syndrome and glucose intolerance. The ROCK2 inhibitors may be used to effect weight loss and/or limit weight gain. In an embodiment, a ROCK2 inhibitor is used to reduce or prevent insulin resistance or restore insulin sensitivity.

[0170] In other embodiments, the ROCK2 inhibitors provided herein may be used in the treatment of an ocular disorder including ocular hypertension, age related macular degeneration (AMD; wet and dry), choroidal neovascularization (CNV), choroidal tumor, diabetic macular edema (DME), iris neovascularization, uveitis, glaucoma, primary open-angle glaucoma, acute angle-closure glaucoma, pigmentary glaucoma, congenital glaucoma, normal tension glaucoma, secondary glaucoma, neo vascular glaucoma, geographic atrophy, and retinitis of prematurity (ROP).

[0171] In another embodiment, the disclosure provides methods for the treatment of sickle cell disease.

[0172] In other embodiments, the ROCK2 inhibitors provided herein may be used to treat (i.e., cure or reduce the severity of, etc.) viral infections, particularly coronavirus infections such as SARS-CoV-1, SARS-CoV-2, and MERS-CoV, and to treat or prevent the sequelae

resulting from the viral infection, including the coronavirus infection such as SARS-CoV-1, SARS-CoV-2, and MERS-CoV. In some embodiments, the viral infection is a SARS-CoV-1 infection. In some embodiments, the viral infection is a SARS-CoV-2 infection. In some embodiments, the viral infection is a MERS-CoV infection. In embodiments, the sequelae include one or more of the group consisting of fatigue, dyspnea (difficulty breathing), cough, arthralgia (joint pain), myalgia, headache, chest pain, fever, palpitations, myocardial inflammation, ventricular dysfunction, stroke, pulmonary function abnormalities, fibrosis (such as pulmonary fibrosis), renal dysfunction rash, alopecia, olfactory and/or gustatory dysfunction, sleep dysregulation, cognitive impairment altered, memory impairment, depression, anxiety, changes in mood and combinations thereof. In embodiments, the sequelae include inflammation and/or fibrosis.

EXAMPLES

Example 1: HCl formulation of Compound A (solid dispersion composition)

[0173] The lipid formulation was developed to enhance the absorption, exposure and performance of compounds of Formula I, and particularly Compound A and its salts, which may have challenging oral bioavailability. The formulation targets lymphatic uptake channels, a largely unsaturable uptake channel, permitting an alternative route for drug absorption. It also minimizes drug uptake via the hepatic portal vein, minimizing first-pass metabolism. Both attributes of the formulation may combine to increase drug exposure.

A. Formulation Excipients

[0174] Maisine® CC: This excipient's chemical name is glycerol mono-linoleate, which may enhance lymphatic absorption of lipophilic, poorly absorbed drugs, such as Compound A, increasing oral absorption whilst minimizing various toxicities (e.g. liver).

[0175] Oleic Acid: The fatty acid component of the formulation may also promote lymphatic drug uptake. The efficiency of fatty acid lymphatic uptake may be dependent on multiple factors, including chain length and saturation state, affecting their ability to form mixed micelles. During the digestion process, oleic acid (C18:1) interacts with lipid transporters, maximizing the efficiency of lipid uptake. Oleic acid signals through the CD36 receptor to enhance the formation of chylomicrons, whilst oleic acid-rich diets produce large chylomicrons, so maximizing drug loading capacity.

[0176] Gelucire® 43/01: Falling within the category of 'hard fat', Gelucire® 43/01 as an excipient may be used to ensure that the lipid formulations solidify at room temperature,

ensuring physical stability. Gelucire® 43/01 is a long chain triglyceride, which during digestion releases long chain fatty acids (primarily C₁₂ to C₁₈), which have the right chain length to contribute to lymphatic absorption.

[0177] Sodium Oleate: The use of lipid-based surfactants in the formulation for lymphatic uptake can have variable results and have the potential to negatively affect the absorption. Therefore, is important to select a surfactant in the composition that offers the desired functionality without impacting performance. The performance of sodium oleate is evaluated based on the results of an in-vivo pharmacokinetic study.

B. Lipid solid dispersions

[0178] The use of lipid excipients to enhances the absorption of the API solid dispersions provided herein. We have demonstrated that the lipid composition containing fatty acids and lipid surfactants increased absorption versus an aqueous suspension in Beagle dogs (FIG. 1).

C. *In-vivo* Data Supporting Formulation Development

[0179] Compound A-HCl was formulated into the composition as a fine dispersion. Compound A-HCl API naturally exists as small, low density, non-static, drug particles (typical D90 sub 20 micron). Their dispersion in lipids was initially achieved by adding solid Compound A-HCl to the cooled F4 formulation (ca. 40 °C) and sonicating with a high-powered sonic probe. The composition was evaluated in Beagle dogs in terms of in-vivo pharmacokinetics compared with a Compound A-HCl aqueous nano-suspension formulation.

[0180] Table 1: mean oral PK parameters for Compound A-HCl formulations in male and female beagles at 10 mg/kg

Test formulations	T1/2 Hours	Tmax hours	Cmax ng/ml	AUC last hr*ng/ml
Nanosuspension	0.9	1.8	911.2	3126.5
Lipid solid dispersion	1.5	4	3458.7	13107.6

D. Conclusions

[0181] The sodium oleate-based composition (F4) of Compound A-HCl as 50:50 solid dispersion had superior exposure to the aqueous nanosuspension and that of F4 alone (delivered dose fully solubilized). Therefore, this solid dispersion formulation was selected as the clinical formulation to move into clinical trial supply manufacturing.

[0182] After selection of the F4 50:50 lipid solid dispersion composition (see figure 1), it was found that the unit dose strength needed to be increased as the capsule fill volume needed to be reduced to offer efficiency in large-scale capsule manufacture given that a 00 capsule was selected as appropriate for the Compound A-HCl clinical development pathway with a target dose of 50 mg per capsule. To achieve this dose strength, the amount of Compound A-HCl to be solubilized in the composition needed to be increased by approximately 20%. Initial feasibility investigations increasing the solubilized load of Compound A-HCl were successful, however, on further scale-up a precipitation event occurred, potentially due to pro-longed heating. The F4 100% solid dispersion formulation manufacture does not have a long heating step. Consequently, to achieve the desired 50mg unit dose and avoid potential precipitation problems in the process, the F4 100% solid dispersion formulation was selected as the lead formulation. Feasibility testing for this formulation variant was successfully conducted by the clinical trial supply CMO (Asymchem), supporting the F4 100% solid dispersion's selection as the lead formulation.

Example 2: HCl formulation of Compound A

A. Formulation Excipients

[0183] Maisine® CC: This excipients chemical comprises glycerol mono-linoleate which may be used to enhance lymphatic absorption of highly lipophilic poorly absorbed drugs, increasing oral absorption whilst minimizing various toxicities (e.g. liver).

[0184] Oleic Acid: Fatty acids have been studied to understand their impact on promoting lymphatic drug uptake. The efficiency of fatty acid lymphatic uptake may depend on multiple factors, including chain length and saturation state, affecting their ability to form mixed micelles. During the digestion process, oleic acid (C18:1) interacts fully with lipid transporters maximizing the efficiency of lipid uptake. Oleic acid signals through the CD36 receptor to enhance the formation of chylomicrons, whilst oleic acid-rich diets produce large chylomicrons, so maximizing drug loading capacity.

[0185] Gelucire® 43/01: Falling within the category of 'hard fat', Gelucire 43/01 is an excipient principally used to ensure that liquid lipid formulations solidify at room temperature, ensuring physical stability of highly saturated formulations. Gelucire 43/01 is a long chain triglyceride, which during digestion releases long chain fatty acids (C14 to C18), which have the right chain length to contribute to lymphatic absorption.

[0186] Sodium Oleate: The use of lipid-based surfactants in the formulation of drugs for lymphatic uptake can have variable results and is known to have the potential to negatively affect the absorption. Therefore, is important to select a surfactant in the composition that offers the desired functionality without impacting performance. Such selections can only be made based on the results of in-vivo pharmacokinetic studies as in section 3.0.

B. Lipid Molecular Dispersions versus Suspensions:

[0187] Compound A-HCl was evaluated for its solubility in a range of excipients and formulations to assess how the API might dissolve and what unit dose strengths may be accessible, with an initial focus on achieving stable molecular dispersions as instructed by the literature.

[0188] Formulations testing determined that oleic acid and Maisine® CC offered minimal solubility as solvents for Compound A-HCl, even on heating. To achieve pre-clinical (10 mg/ml and higher) and clinical (30 mg/ml) dose strengths, Compound A-HCl benefitted from lipid-based surfactants allowing the compound to be fully solubilized within the composition. The most effective Compound A-HCl solubilizers for oleic acid and Maisine® CC were sodium oleate and sodium stearate (see tables 1 and 2). Cholic acid required the presence of sodium oleate or stearate to be considered for a functional role.

[0189] Subsequently preclinical formulations (10 mg/ml) were manufactured by heating various oleic acid and Maisine® CC compositions to approximately 130 °C with various lipid-compatible surfactants. Compound A-HCl was added with heating and stirring until solubilized. Lastly, Gelucire® 43/01 was added and the composition was allowed to cool and solidify. Initial formulations were evaluated in in-vivo pharmacokinetic models in rats and compared with the alternative Compound A-HCl nano-suspension formulation.

C. Rat Compositions:

[0190] **Table 2.** Composition of preclinical formulations administered to rats in oral pharmacokinetic studies (see also, FIGS. 2 and 3)

Component	F4 HCl salt	F5 HCl salt	F6 HCl salt
Compound A Solid dispersion	0.00%	0.00%	0.00%
Compound A Molecular dispersion	1.19%	1.19%	1.15%
Oleic acid	33.00%	33.00%	32.02%
Cholic acid	0.00%	0.00%	2.97%

Gelucire® 43/01	29.81%	29.81%	28.92%
Sodium Stearate	0.00%	3.00%	0.00%
Sodium Oleate	3.00%	0.00%	2.91%
Maisine® cc	33.00%	33.00%	32.02%
Total	100.00%	100.00%	100.00%
Solubility	Fully soluble	Fully soluble	Fully soluble

[0191] **Table 3:** mean oral PK parameters for Compound A-HCl formulations in SD rats at 50 mg/kg (F4, F5 and F6).

Test article	T1/2 hours	Tmax hours	Cmax ng/ml	AUC last hr*ng/ml
Nanosuspension	3.56	4.33	1759.18	16929.11
F4	6.23	5.67	2108.35	29445.75
F5	6.47	6.00	2241.74	30990.92
F6	8.55	6.33	1912.34	25502.54

D. Beagle Dog Compositions and Results

Table 4.

Component	F4 HCl salt	F4a HCl salt	F5 HCl salt	F6 HCl salt
Compound A Solid dispersion	0.00%	0.00%	0.00%	0.00%
Compound A Molecular dispersion	1.20%	1.20%	1.17%	1.17%
Oleic acid	32.86%	16.42%	31.90%	31.90%
Cholic acid	0.00%	0.00%	2.92%	2.92%
Gelucire® 43/01	30.10%	30.08%	29.22%	29.22%
Sodium Stearate	0.00%	0.00%	2.89%	0.00%
Sodium Oleate	2.98%	3.04%	0.00%	2.89%
Maisine® cc	32.86%	49.26%	31.90%	31.90%
Total	100.00%	100.00%	100.00%	100.00%
Solubility	Fully soluble	Fully soluble	Fully soluble	Fully soluble

[0192] Table 5: mean oral PK parameters for Compound A-HCl formulations in beagles at 10 mg/kg (F4, F5 and F6)

Test formulation	T1/2 hours	Tmax hours	Cmax ng/ml	AUC last hr*ng/ml
Nanosuspension	0.82	1.33	663.80	2032.14
F4	0.57	2.67	2020.71	6268.92
F5	1.92	2.33	952.35	2821.77
F6	0.71	2.67	904.51	2988.85

[0193] Table 6: mean oral PK parameters for Compound A-HCl formulations in beagles at 10 mg/kg (F4 and F4a)

Test formulation	T1/2 hours	Tmax hours	Cmax ng/ml	AUC last hr*ng/ml
Nanosuspension	1.11	0.92	592.12	1262.94
F4 repeat	0.70	2.50	1342.42	5091.57
F4a	0.70	1.55	467.21	1958.52

E. Molecular/Solid Dispersion Combination Formulations

[0194] The sodium oleate-containing formulation (F4) gave equivalent exposure to a sodium stearate based formulation (F5) in rats (see table 2). However, in a higher mammalian species (Beagle Dog) the highest exposure was clearly achieved by the F4 formulation, suggesting that sodium oleate was not impeding the lymphatic uptake properties of the lipid composition (see FIGS. 3 to 4). Halving the amount of oleic acid (F4a) had no benefit in improving absorption (see FIG. 4).

[0195] As a result, F4 was selected as a lead prototype formulation composition. However, F4 offered limited unit dose strengths in a size 00 capsule (ca. 15-20 mg) when the entire dosage was solubilized. Therefore, in investigate the viability of higher unit dose strengths in clinical compositions for a size 00 capsule, it was decided to assess how the integration of an additional of solid dispersion quantity of Compound A-HCl into the F4 formulation would affect pharmacokinetic parameters for Compound A-HCl.

[0196] The F4 composition, which was selected based on its improved PK performance in the rat and dog, was then studied in-vivo such that various percentages of the delivered dose of Compound A-HCl were formulated as a fine dispersion. Compound A-HCl naturally forms small, low density, non-static drug particles (typical D₉₀ less than 20 micron). Their dispersion in lipids was achieved by adding solid Compound A-HCl to the cooled F4 formulation (ca. 40 deg C) and sonicating with a high-powered sonic probe. Both 50:50 and 100% solid dispersion

F4-based lipid formulations were produced, and their pharmacokinetic performance was compared to F4.

F. Conclusion

[0197] The sodium oleate-based molecular dispersion formulation of Compound A-HCl (F4) containing an additional equal quantity of solid dispersion of Compound A-HCl (50% of the delivered dose in solution – 50% of the delivered dose in suspension) in the formulation had a superior exposure profile to the F4 formulation, purely containing Compound A-HCl as a molecular dispersion. The results were confirmed in multiple experiments across multiple species and multiple doses.

Example 3

[0198] To further improve the capacity of the formulation for the API (Compound A-HCl salt), a further composition was prepared. The dosage form comprises of a hard gelatin capsule where 50% of the API is in solid dispersion. The strength is 50 mg/capsule. The capsules size is 00#. The formulation components and amounts are provided in Table 7, below:

Table 7: Composition of Compound A-HCl Capsules

Ingredient	Amount per capsule
Comp. A- HCl	55.6mg ^a (6.95 %)
Gelucire 43/01	129.9mg (16.24%)
Maisine CC	298.9mg (37.36%)
Oleic Acid	298.9mg (37.36%)
Sodium oleate	16.7mg (2.09%)
Total	800mg
00# Empty Hard Gelatin Capsule	1

^a The strength of Compound A capsules, 50mg is calculated based on the freebase.

[0199] The tissue distribution when this formulation is given orally to the test animals is shown in Figure 6.

Example 4

[0200] Compound A was tested in SOD1^{G93A} mice, a model system for the development of ALS. Compound A was found to extend the survival (FIG. 7A) and delays motor deficit (FIG. 7B) of SOD1^{G93A} mice.

Example 5

[0201] Animal: Wild-type C57Bl/6J and transgenic SOD1^{G93A} (Cat. # 000664 for C57Bl/6J and 004435 for SOD1^{G93A}) mice were obtained from Jackson Laboratory.

[0202] Comp. A or vehicle administration: Comp. A/0.3% Tween-80 or vehicle mixed with peanut butter (1g) was fed to the animal each day accordingly. For the survival experiment, the feeding started at P60 till mice reached terminal stage. For the longitudinal experiment, the feeding started at P50 till mice were sacrificed at P90, P120 and P150.

[0203] Immunohistochemistry of spinal cord: Mice were anaesthetized and perfused transcardially by cold 4% paraformaldehyde in 0.1M PBS at P90, P120, P150 or at the terminal stage. Spinal cords were removed and postfixed in 4% PFA in PBS overnight at 4°C. The L4-L5 segment of the spinal cords were collected, 70-µm-thick vibratome sections were harvested. The sections were then incubated with choline acetyltransferase (ChAT) antibody (1:250, Sigma, AB144P) to label motor neurons and was visualized using Alexa-594 conjugated IgG to count the number of remaining motor neurons in the ventral horn of the spinal cords. To determine whether astrocytes and microglia were active in the spinal cords, antibodies recognize the astrocyte marker (glial fibrillary acidic protein (GFAP)), and microglia marker (ionized calcium-binding adapter molecule 1(Iba-1)) were used. Alexa-488 or -594 conjugated secondary antibody IgGs were used to visualize them and imaging process. The fluorescent intensity in the region-of-interest was measured using Image J to determine the level of activation of astrocytes and microglial cells. The results of this study are shown in Figure 8.

[0204] Neuromuscular junction (NMJ) quantification: Tibialis anterior (TA) muscles were removed from the mice and frozen in OCT with dry ice, and stored at -80 freezer till sectioning. TA muscles were cryosectioned (70 µm) and slide-mounted. NMJs were blocked in 5% donkey serum/0.1% TBS-T for 1 hour and immunolabeled for the presynaptic marker Synaptophysin in 5% donkey serum/0.1% TBS-T at 4°C overnight. The next day, sections were washed 3 times in 0.1% TBS-T, incubated with the postsynaptic marker alpha-bungarotoxin (BTX) in 5% donkey serum/0.1% TBS-T for 3 hr at room temperature, washed three times in 0.1% TBS-T and mounted Fluoromount G. Images were obtained at an Sp8 Leica confocal microscope at 10x (for counting) and 40x (representative images) magnification. NMJ innervation was quantified by identifying BTX-positive NMJs (red) and determined the extent of colocalization with synaptophysin (green). Full innervation was considered when more than 70% overlap of BTX and synaptophysin was observed. At least

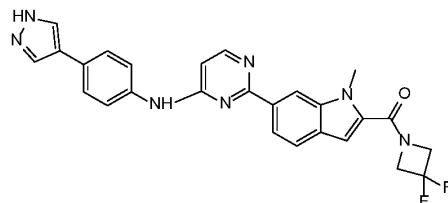
100 NMJs on 20 μ m TA sections were imaged and counted for each group. The results of this study are shown in Figure 9.

[0205] NfL ELISA: Plasma concentrations of neurofilament light chain (NfL), a marker to determine the progression of ALS were determined using enzyme-linked immunosorbent assay (ELISA). To perform ELISA, reagents were equilibrated to room temperature for at least 30 minutes (Cell Signaling Tech 99175). The reagents were prepared and added to the ELISA plate wells according to the instructions, with 100 μ L of standard or test sample per well. After mixing and sealing, the plates were incubated at 37 °C for 2 hours. Post-incubation, the liquid was discarded, and the wells were washed three times with a 2-minute soak of 200 μ L per well. Subsequently, 100 μ L of biotin-labeled antibody working solution was added to each well and incubated for another hour at 37°C. Following this, the wells were washed 5 times, and 100 μ L of horseradish peroxidase-labeled avidin working solution was added to each well. After 1 hour incubation and washing, 90 μ L of substrate solution was added to each well, incubation in the dark for 30 minutes at 37°C. The reaction was stopped by adding stopping solution to each well. The optical density (OD) of each well was measured at a wavelength of 450 nm within 5 minutes after adding the stop solution. The results of this study are shown in Figure 10.

WHAT IS CLAIMED:

1. A pharmaceutical formulation for oral administration comprising:

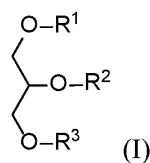
(a) an active pharmaceutical ingredient (API) comprising a compound having a structure:



or a pharmaceutically acceptable salt thereof, and

(b) one or more selected from:

- (i) a fatty acid component comprising saturated or unsaturated C₈-C₂₄ fatty acids and/or a pharmaceutically acceptable salt thereof;
- (ii) a first glyceride component comprising one or more glyceride compounds having a structure of Formula (I)



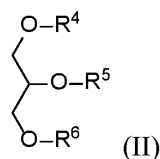
wherein:

each R¹, R², and R³ is independently hydrogen or -C(O)-R^A;

each R^A is independently C₇-C₂₁ alkenyl comprising one or two double bonds, provided that at least one of R¹, R², and R³ is not hydrogen; and

(iii) a second glyceride component comprising:

one or more glyceride compounds having a structure of Formula (II)



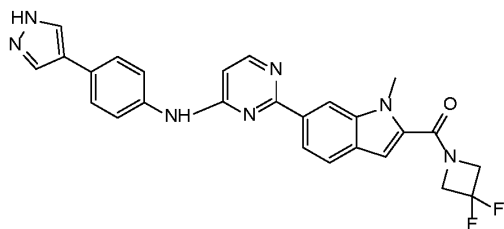
wherein:

each R⁴, R⁵, and R⁶ is independently hydrogen, or -C(O)-R^B;

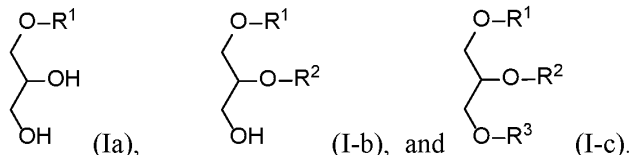
each R^B is independently C₇-C₂₁ alkyl,

provided that at least one of R⁴, R⁵, and R⁶ is not hydrogen.

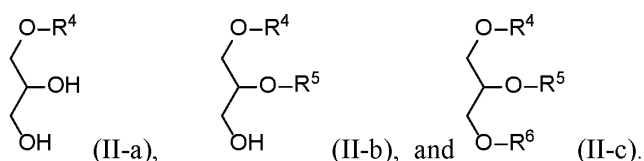
2. The pharmaceutical formulation of claim 1, wherein the API comprises a HCl salt of



3. The pharmaceutical formulation of claim 1, wherein the fatty acid component comprises a saturated or unsaturated C₁₂-C₁₈ fatty acid and/or a pharmaceutically acceptable salt thereof.
4. The pharmaceutical formulation of claim 1, wherein the fatty acid component comprises an oleic acid, sodium oleate, or a combination thereof.
5. The pharmaceutical formulation of claim 1, wherein the first glyceride component comprises the one or more compounds having a structure of Formula (I) and each R^A is independently C₁₃-C₂₁ alkenyl comprising one or two double bonds.
6. The pharmaceutical formulation of claim 1, wherein the first glyceride component comprises the one or more compounds having one of the following structures:

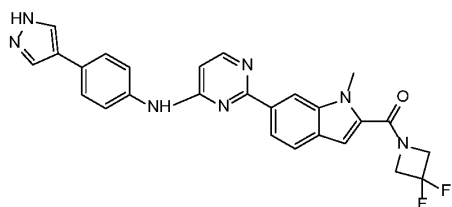


7. The pharmaceutical formulation of claim 6, wherein a ratio of the compound of Formula (I-a), the compound of Formula (I-b), and the compound of Formula (I-c) is about 32 to 52 : 40 to 55: 5 to 20.
8. The pharmaceutical formulation of claim 1, wherein the second glyceride component comprises the one or more compounds having a structure of Formula (II) and each R^B is independently C₇-C₁₇ alkyl.
9. The pharmaceutical formulation of claim 1, wherein the second glyceride component comprises the one or more compounds having one of the following structures:



10. The pharmaceutical formulation of claim 1, wherein the API is formulated in a solid dispersion, in a solution, or as combination thereof.

11. The pharmaceutical formulation of claim 1, wherein the pharmaceutical formulation comprises the compound having a structure of



or a pharmaceutically acceptable salt thereof in

an amount of about 1 to 15 wt% of the pharmaceutical formulation, or about 1-10 wt % of the pharmaceutical formulation, or from about 2-8 wt % of the pharmaceutical formulation, or from about 5-7 wt % of the pharmaceutical formulation.

12. The pharmaceutical formulation of claim 1, wherein the pharmaceutical formulation comprises the fatty acid component in an amount of about 15 to 50 wt% of the pharmaceutical formulation, or about 20 to 45 wt%, about 30 to 45 wt%, about 35 to 45 wt%, or about 35 to 40 wt%, based on the weight of the pharmaceutical formulation.

13. The pharmaceutical formulation of claim 1, wherein the pharmaceutical formulation comprises:

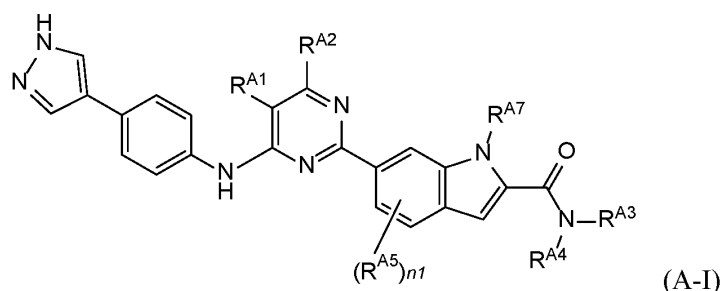
a fatty acid having saturated or unsaturated C₁₂-C₁₈ fatty acid in an amount of 12 to 48 wt%, about 15 to 45 wt%, about 25 to 40 wt%, about 30 to 40 wt%, or about 35 to 40 wt%; and

a sodium salt of a fatty acid having saturated or unsaturated C₁₂-C₁₈ fatty acids in an amount of about 1 to 5 wt%, or about 2 to 4 wt%, or about 2 to 3 wt%,

based on the weight of the pharmaceutical formulation.

14. The pharmaceutical formulation of claim 1, wherein the pharmaceutical formulation comprises the first glyceride component in an amount of about 30 to 50 wt% of the pharmaceutical formulation, or about 30 to 45 wt%, or about 32 to 40 wt%, or about 35 to 40 wt% of the pharmaceutical formulation.

15. The pharmaceutical formulation of claim 1, wherein the pharmaceutical formulation comprises the second glyceride component in an amount of about 10 to 35 wt, or about 10 to 30 wt%, or about 10 to 20 wt%, or about 15 to 18 wt% based on the weight of the pharmaceutical formulation.
16. The pharmaceutical formulation of claim 1, wherein the pharmaceutical formulation comprises:
 - Compound A, or a pharmaceutically acceptable salt in an amount of about 1-15 wt %;
 - a fatty acid having saturated or unsaturated C₁₂-C₁₈ fatty acid in an amount 12 to 48 wt%;
 - a sodium salt of a fatty acid having saturated or unsaturated C₁₂-C₁₈ fatty acids in an amount of about 1 to 5 wt%;
 - the first glyceride component in an amount of about 30 to 50 wt%; and
 - the second glyceride component in an amount of about 10 to 35 wt%, based on the weight of the pharmaceutical formulation.
17. A method for the treatment of a neurodegenerative disease characterized by the degeneration of motor neurons, the method comprising administering to a patient in need thereof a pharmaceutical formulation comprising a therapeutically effective amount of a ROCK2 inhibitor.
18. The method of claim 17, wherein the neurodegenerative disease is amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy, pseudobulbar palsy, or monomelic amyotrophy (MMA).
19. The method of claim 17, wherein the neurodegenerative disease is amyotrophic lateral sclerosis (ALS).
20. The method according to any one of claims 17 to 19, wherein the ROCK2 inhibitor has the structure of Formula (A-I):



or a pharmaceutically acceptable salt thereof, wherein:

R^{A1} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{A11}, -O-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-OR^{A11}, -NR^{A11}R^{A12}, -O-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)_x-C(=O)R^{A11}, -O-(C₁-C₆ alkyl)_x-C(=O)R^{A11}, -(C₁-C₆ alkyl)_x-C(=O)OR^{A11}, -C(=O)-R^{A11}, -C(=O)OR^{A11}, -(C₁-C₆ alkyl)_x-C(=O)NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)_x-C(=O)R^{A11}, and -NR^{A11}-(C₁-C₆ alkyl)_x-C(=O)OR^{A11};

R^{A2} is selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, halo, -CN, C₁-C₃ perfluoro alkyl, -OR^{A11}, -O-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-OR^{A11}, -NR^{A11}R^{A12}, -O-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)_x-C(=O)R^{A11}, -O-(C₁-C₆ alkyl)_x-C(=O)R^{A11}, -(C₁-C₆ alkyl)_x-C(=O)OR^{A11}, -C(=O)-R^{A11}, -C(=O)OR^{A11}, -(C₁-C₆ alkyl)_x-C(=O)NR^{A11}R^{A12}, -NR^{A11}-(C₁-C₆ alkyl)_x-C(=O)R^{A11}, and -NR^{A11}-(C₁-C₆ alkyl)_x-C(=O)OR^{A11};

alternatively, R^{A1} and R^{A2} are taken together to form a 5- or 6-membered saturated or unsaturated fused ring which may contain from 0 to 2 ring heteroatoms selected from the group consisting of N, O, and S, and which is unsubstituted or substituted with 1 to 3 substituents selected from the group consisting of C₁-C₆ alkyl, halo, -CN, -OH, oxo, -O-(C₁-C₆ alkyl), -O-(C₁-C₆ alkyl)-OH, -O-(C₁-C₆ alkyl)-O-(C₁-C₆ alkyl), -NR^{A11}R^{A12}, -O-(C₁-C₆ alkyl)-NR^{A11}R^{A12}, C₁-C₃ perfluoro alkyl, -NR^{A11}-(C₁-C₆ alkyl)NR^{A11}R^{A12}, and -NR^{A11}-(C₁-C₆ alkyl)-OR^{A11};

R^{A3} and R^{A4} are each independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₇ cycloalkyl, 3- to 10-membered heterocyclyl, C₆-C₁₀ aryl, 5- to 14-membered heteroaryl, C₆-C₁₂ aralkyl, -(C₁-C₆ alkyl)-OR^{A11}, -(C₁-C₆ alkyl)-NR^{A11}R^{A12}, -(C₁-C₆ alkyl)_x-C(=O)R^{A11}, -(C₁-C₆ alkyl)_x-C(=O)OR^{A11}, and -(C₁-C₆ alkyl)_x-C(=O)NR^{A11}R^{A12};

alternatively R^{A3} and R^{A4} are taken together with the nitrogen to which they are attached to provide (i) a 4- to 6-membered heterocyclic ring having from 0 to 2 additional ring to heteroatoms selected from N, O and S, or (ii) a 5- to 10-membered hetero bicyclic ring system having from 0 to 3 additional ring heteroatoms selected from N, O and S; wherein the heterocyclic ring or the bicyclic ring system are unsubstituted or are substituted with from 1 to 4 substituents selected from the group consisting of halo, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_7 cycloalkyl, halo, -CN, C_1 - C_3 perfluoro alkyl, $-OR^{A11}$, oxo, $-O-(C_1-C_6 \text{ alkyl})-OR^{A11}$, $-(C_1-C_6 \text{ alkyl})-OR^{A11}$, $-NR^{A11}R^{A12}$, $-O-(C_1-C_6 \text{ alkyl})-NR^{A11}R^{A12}$, $-(C_1-C_6 \text{ alkyl})-NR^{A11}R^{A12}$, $-NR^{A11}-(C_1-C_6 \text{ alkyl})-NR^{A11}R^{A12}$, $-NR^{A11}-(C_1-C_6 \text{ alkyl})-OR^{A11}$, $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)R^{A11}$, $-O-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)R^{A11}$, $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)OR^{A11}$, $-C(=O)-R^{A11}$, $-C(=O)OR^{A11}$, $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)NR^{A11}R^{A12}$, $-NR^{A11}-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)R^{A11}$, and $-NR^{A11}-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)OR^{A11}$;

the dotted lines represent optional double bonds;

each R^{A5} is independently selected from the group consisting of H, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_7 cycloalkyl, halo, -CN, C_1 - C_3 perfluoro alkyl, oxo, $-OR^{A11}$, $-O-(C_1-C_6 \text{ alkyl})-OR^{A11}$, $-(C_1-C_6 \text{ alkyl})-OR^{A11}$, $-NR^{A11}R^{A12}$, $-O-(C_1-C_6 \text{ alkyl})-NR^{A11}R^{A12}$, $-(C_1-C_6 \text{ alkyl})-NR^{A11}R^{A12}$, $-NR^{A11}-(C_1-C_6 \text{ alkyl})-NR^{A11}R^{A12}$, $-NR^{A11}-(C_1-C_6 \text{ alkyl})-OR^{A11}$, $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)R^{A11}$, $-O-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)R^{A11}$, $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)OR^{A11}$, $-C(=O)-R^{A11}$, $-C(=O)OR^{A11}$, $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)NR^{A11}R^{A12}$, $-NR^{A11}-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)R^{A11}$, and $-NR^{A11}-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)OR^{A11}$;

nI is 0 to 3;

R^{A7} is independently selected from the group consisting of H, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_7 cycloalkyl, $-(C_1-C_6 \text{ alkyl})-OR^{A11}$, $-(C_1-C_6 \text{ alkyl})-NR^{A11}R^{A12}$, $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)R^{A11}$, $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)OR^{A11}$, and $-(C_1-C_6 \text{ alkyl})_{xI}-C(=O)NR^{A11}R^{A12}$;

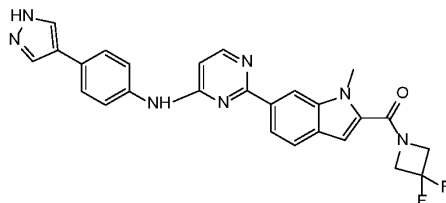
each xI is independently selected from 0 and 1; and

each R^{A11} and R^{A12} are independently selected from the group consisting of H and C_1 - C_6 alkyl;

or alternatively, R^{A11} and R^{A12} are taken together when both are attached to the same nitrogen to form a 4- to 7- membered heterocyclic ring having from 0 to 2 additional ring heteroatoms selected from the group consisting of N, O and S, and which heterocyclic ring is unsubstituted or is substituted with 1 to 3 substituents selected from the group consisting of halo, C_1 - C_6 alkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, C_3 - C_7

cycloalkyl, -CN, -NH₂, C₁-C₃ perfluoro alkyl, -OH, -O-(C₁-C₆ alkyl), and -(C₁-C₆ alkyl)-OH.

21. The method of claim 20, wherein the ROCK2 inhibitor has the formula:



or a pharmaceutically acceptable salt thereof.

22. The method according to any one of claims 17 to 19, wherein the ROCK2 inhibitor is selected from Compound D, belumosudil (KD025), Zelasudil, Y-27632, Fasudil, GSK429286A, RKI-1447, Azaindole 1 (TC-S 7001), Hydroxyfasudil, GSK269962A, Ripasudil, AT13148, RX007, and derivatives, isomers, hydrates, or pharmaceutically acceptable salts thereof.

23. A method for the treatment of a neurodegenerative disease characterized by the degeneration of motor neurons, the method comprising orally administering to a patient in need thereof a pharmaceutical formulation according to any one of claims 1 to 16.

24. The method of claim 23, wherein the neurodegenerative disease is amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), progressive bulbar palsy, pseudobulbar palsy, or monomelic amyotrophy (MMA).

25. The method of claim 24, wherein the neurodegenerative disease is amyotrophic lateral sclerosis (ALS).