

PHARMACEUTICAL COMPOSITIONS AND METHODS OF USE THEREOF**CROSS-REFERENCE TO RELATED PATENT APPLICATION**

[0001] The present application claims priority to, and the benefit of U.S. Provisional Patent Application No. 63/585,862, filed September 27, 2023, which is hereby incorporated by reference in its entirety for all purposes.

BACKGROUND OF THE DISCLOSURE

[0002] Phosphatidylinositol 3-kinases (PI3Ks) comprise a family of lipid kinases that catalyze the transfer of phosphate to the 3-position of the inositol ring of phosphatidylinositol and its derivatives to produce phosphoinositol-3-phosphate (PI(3)P), phosphoinositol-3,4-diphosphate (PI(3,4)P₂) and phosphoinositol-3,4,5-triphosphate (PI(3,4,5)P₃) that, in turn, act as second messengers in signaling cascades by docking proteins containing pleckstrin-homology, FYVE, Phox and other phospholipid-binding domains into a variety of signaling complexes often at the plasma membrane ((Vanhaesebroeck et al., *Annu. Rev. Biochem.* 70:535 (2001); Katso et al., *Annu. Rev. Cell Dev. Biol.* 17:615 (2001)). PI3Ks have been divided into three classes according to their structural characteristics and substrate specificity. Class IA PI3Ks are heterodimers composed of a p110 catalytic subunit and a p85 regulatory subunit. In mammals, there are three genes, PIK3CA, PIK3CB and PIK3CD, encoding p110 catalytic isoforms: p110 α , p110 β and p110 δ , respectively. There are also three genes, PIK3R1, PIK3R2 and PIK3R3, encoding p85 α (and its splicing variants p55 α and p50 α), p85 β and p55 γ regulatory subunits, respectively, collectively called p85. The modular domains of the p85/55/50 subunits include Src Homology (SH2) domains that bind phosphotyrosine residues in a specific sequence context on activated receptor and cytoplasmic tyrosine kinases, resulting in activation and localization of Class IA PI3Ks. Class IB PI3K is a heterodimer composed of a catalytic subunit p110 γ and a regulatory subunit p101. p110 γ is mainly expressed in leukocytes and can be activated directly by GPCRs. Class II PI3Ks are monomers with only a single catalytic subunit. Class III PI3Ks consists of a single catalytic subunit Vps34 (homolog of the yeast vacuolar protein-sorting defective 34). The phospholipid products of class I PI3K link upstream receptors with downstream cellular activities including proliferation, survival, chemotaxis, cellular trafficking, motility, metabolism, inflammatory and allergic responses, transcription and translation (Cantley et al., *Cell* 64:281 (1991); Escobedo and Williams, *Nature* 335:85

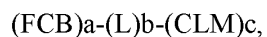
(1988); Fantl et al., Cell 69:413 (1992)). There is this interest and a current need for binding to and regulating PI3K for treatment of various diseases.

[0003] The present disclosure addresses these and other unmet needs.

SUMMARY OF THE INVENTION

[0004] The present invention includes pharmaceutical composition for immediate release comprising a therapeutically effective amount of a compound that forms a covalent bond with a kinase or pseudokinase or a pharmaceutically acceptable salt thereof, and at least one pharmaceutical excipient or methods of use thereof. In one specific embodiment, the kinase is PI3-kinase (PI3K).

[0005] In another embodiment, the compound that forms a covalent bond with a kinase or pseudokinase is a compound having the formula:



or a pharmaceutically acceptable salt thereof, wherein:

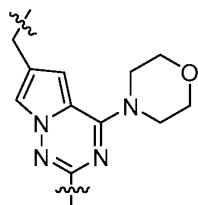
CLM is a covalent linking modality;

L is a linker;

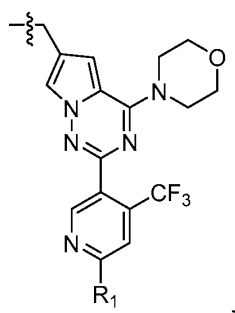
a and c are each independently integers between 1 and 5;

b is an integer between 0 and 5; and

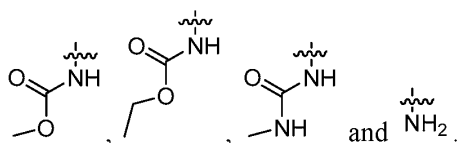
FCB comprises:



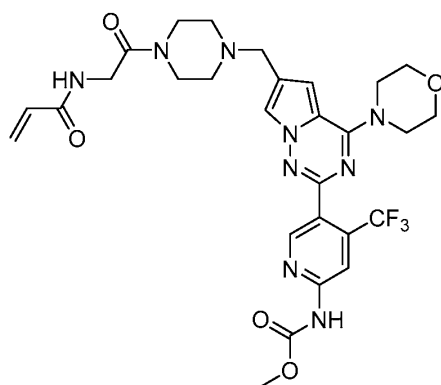
[0006] In a specific embodiment, the FCB has the structure:



wherein R1 is selected from the group consisting of:



[0007] In another specific embodiment, the compound is Compound I:



Compound I.

[0008] Compound I is disclosed in PCT application Publication WO 2021/055747, and PCT application PCT/CN2023/083760, which are hereby incorporated by reference in their entirety. In some embodiments, Compound I is a phosphoinositide 3-kinase (PI3K) inhibitor. In some embodiments, Compound I is an irreversible inhibitor of PI3K. In embodiments, Compound I is an irreversible inhibitor of PI3K α . In other embodiments, Compound I can form a covalent bond with an amino acid of a PI3K. In other specific embodiments, Compound I can form a covalent bond with a cysteine in a PI3K, e.g., PI3K α (e.g., via a Michael reaction).

[0009] The present invention also comprises the administration of the pharmaceutical compositions described herein to a subject. In some embodiments, the method may include treating cancer, a neurodegenerative disease, an autoimmune disorder or aging in a subject in need thereof. In other specific embodiments, the methods may include treating or inhibiting

cancer in a subject. In another specific embodiment, the methods may include treating or ameliorating a cell proliferation disorder in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound that forms a covalent bond with a kinase or pseudokinase or a pharmaceutically acceptable salt thereof before, during, or after the subject has been administered a compound that forms a covalent bond with a kinase or pseudokinase. In a specific embodiment, the kinase is PI3-kinase (PI3K), and preferably PI3K α .

[0010] In another embodiment of treating or ameliorating a cell proliferation disorder in a subject, the cell proliferation disorder is cancer. In some embodiments, the cancer is selected from one or more of the group consisting of leukemia, tumors of the brain, lung (small cell and non-small cell), ovary, prostate, breast, colon, and cancer in a tissue, including intestinal tissue, pancreatic tissue, liver, kidney, prostate, ovary, lung, bone marrow, or breast tissue. In a specific embodiment, the cancer is selected from the group consisting of: heme cancer, colorectal cancer, ovarian cancer, breast cancer, cervical cancer, lung cancer, liver cancer, colon cancer, pancreatic cancer, cancer of the lymph nodes, colon cancer, small intestine, prostate cancer, brain cancer, cholangiocarcinoma, gallbladder carcinoma, cancer of the head and neck, bone cancer, Ewing's sarcoma, skin cancer, kidney cancer, and cancer of the heart. In another embodiment, the cancer is selected from the group consisting of breast cancer, ovarian cancer, pancreatic cancer, colorectal cancer, colon cancer, small intestine and lung cancer. In some embodiments, the cancer is associated with a mutation in the PIK3CA gene. In another embodiment of the present invention, the subject is human. In some embodiments, the subject has cancer with a mutation in the PIK3CA gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1 provides a flowchart outlining a process for manufacturing a pharmaceutical tablet of the present disclosure comprising Compound I.

[0012] Figure 2 shows the Dissolution Profile of Compound I Tablets, 5 mg, Batch 1 in 900 mL in 0.01 M HCl at 37°C, Apparatus II (Paddle), 75 rpm.

[0013] Figure 3 shows the Dissolution Profile of Compound I Tablets, 50 mg, Batch 1 in 900 mL in 0.01 M HCl at 37°C, Apparatus II (Paddle), 100 rpm.

[0014] **Figure 4** shows the Dissolution Profile Compound I Tablets, 1 mg, Batch 2 in 900 mL in 0.01 M HCl at 37°C, Apparatus 1I (Paddle), 75 rpm.

[0015] **Figure 5** shows the Dissolution Profile of Compound I Tablets, 5 mg, Batch 2 in 900 mL in 0.01 M HCl at 37°C, Apparatus 1I (Paddle), 100 rpm.

[0016] **Figure 6** shows the Dissolution Profile of Compound I Tablets, 1mg, Batch 3 in 900 mL in 0.01 M HCl at 37°C, Apparatus 1I (Paddle), 75 rpm.

[0017] **Figure 7** shows the Dissolution Profile of Compound I, 5 mg, Batch 3 in 900 mL in 0.01 M HCl at 37°C, Apparatus 1I (Paddle), 100 rpm.

[0018] **Figure 8** shows the Dissolution Profile of Compound I Tablets, 1mg, Batch 4 in 900 mL in 0.01 M HCl at 37°C, Apparatus 1I (Paddle), 75 rpm.

[0019] **Figure 9** shows the Dissolution Profile of Compound I Tablets, 5 mg, Batch 4 in 900 mL in 0.01 M HCl at 37°C, Apparatus 1I (Paddle), 100 rpm.

DETAILED DESCRIPTIONS OF THE DISCLOSURE

Definitions

[0020] For convenience, certain terms employed in the specification, examples and claims are collected here. Unless defined otherwise, all technical and scientific terms used in this disclosure have the same meanings as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0021] Throughout the present specification, the terms “about” and/or “approximately” may be used in conjunction with numerical values and/or ranges. The term “about” is understood to mean those values near to a recited value. Furthermore, the phrases “less than about [a value]” or “greater than about [a value]” should be understood in view of the definition of the term “about” provided herein. The terms “about” and “approximately” may be used interchangeably.

[0022] Throughout the present specification, numerical ranges are provided for certain quantities. It is to be understood that these ranges comprise all subranges therein. Thus, the range “from 50 to 80” includes all possible ranges therein (e.g., 51-79, 52-78, 53-77, 54-76, 55-75, 60-70, etc.). Furthermore, all values within a given range may be an endpoint for the range encompassed thereby (e.g., the range 50-80 includes the ranges with endpoints such as 55-80, 50-75, etc.).

[0023] The term “composition” and “formulation” can be used interchangeably through out the present specification, which can mean a mixture of materials.

[0024] The term “a” or “an” refers to one or more of that entity; for example, “a PI3-kinase (PI3K) modulator” refers to one or more PI3-kinase (PI3K) modulators or at least one PI3-kinase (PI3K) modulator. In embodiments, the PI3-kinase (PI3K) modulator is a PI3Ka modulator. As such, the terms “a” (or “an”), “one or more” and “at least one” are used interchangeably herein. In addition, reference to “an inhibitor” by the indefinite article “a” or “an” does not exclude the possibility that more than one of the inhibitors is present, unless the context clearly requires that there is one and only one of the inhibitors.

[0025] As used herein, the verb “comprise” as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. The present invention may suitably “comprise”, “consist of”, or “consist essentially of”, the steps, elements, and/or reagents described in the claims.

[0026] It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely", "only" and the like in connection with the recitation of claim elements, or the use of a "negative" limitation.

[0027] The terms “excipient”, “carrier”, and “vehicle” are used interchangeably throughout this application and denote a substance with which a compound of the present invention is administered. The terms “excipient”, “carrier”, and “vehicle” means a material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material involved in carrying or transporting a pharmaceutical agent from one organ, or portion of the body, to another organ or portion of the body.

[0028] The phrase “pharmaceutically acceptable” as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0029] The term “salts” as used herein embraces pharmaceutically acceptable salts commonly used to form addition salts of free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable. The term “salts” also includes solvates of addition salts, such

as hydrates, as well as polymorphs of addition salts. Suitable pharmaceutically acceptable acid addition salts can be prepared from an inorganic acid or from an organic acid.

[0030] The term “pharmaceutically acceptable salts” includes those obtained by reacting the active compound functioning as a base, with an inorganic or organic acid to form a salt, for example, salts of 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, 2-hydroxyethanesulfonic acid, 2-oxoglutaric acid, 4-acetamidobenzoic acid, 4-aminosalicylic acid, acetic acid, adipic acid, ascorbic acid (L), aspartic acid (L), benzenesulfonic acid, benzoic acid, camphoric acid (+), camphor-10-sulfonic acid (+), capric acid (decanoic acid), caproic acid (hexanoic acid), caprylic acid (octanoic acid), carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid (D), gluconic acid (D), glucuronic acid (D), glutamic acid, glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isobutyric acid, lactic acid (DL), lactobionic acid, lauric acid, maleic acid, malic acid, (- L) malonic acid, mandelic acid (DL), methanesulfonic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid, palmitic acid, pamoic acid, phosphoric acid, propionic acid, pyroglutamic acid (- L), salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, tartaric acid (+ L), thiocyanic acid, toluenesulfonic acid (*p*), and undecylenic acid. Those skilled in the art will further recognize that acid addition salts may be prepared by reaction of the compounds with the appropriate inorganic or organic acid via any of a number of known methods.

[0031] As used herein, the phrase “a disorder characterized by cell proliferation” or “a condition characterized by cell proliferation” include, but are not limited to, cancer, benign and malignant tumors. Examples of cancer and tumors include, but are not limited to, cancers or tumor growth of the colorectum, breast, lung, liver, pancreas, lymph node, colon, prostate, brain, head and neck, skin, kidney, blood and heart (e.g., leukemia, lymphoma, carcinoma).

[0032] The term “treating” means one or more of relieving, alleviating, delaying, reducing, improving, or managing at least one symptom of a condition in a subject. The term “treating” may also mean one or more of arresting, delaying the onset (i.e., the period prior to clinical manifestation of the condition) or reducing the risk of developing or worsening a condition.

[0033] As used herein, the terms “inhibiting” or “reducing” cell proliferation is meant to slow down, to decrease, or, for example, to stop the amount of cell proliferation, as measured using

methods known to those of ordinary skill in the art, by, for example, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, when compared to proliferating cells that are not subjected to the methods, compositions, and combinations of the present application.

[0034] An "effective amount" means the amount of a formulation according to the invention that, when administered to a patient for treating a state, disorder or condition is sufficient to effect such treatment. The "effective amount" will vary depending on the active ingredient, the state, disorder, or condition to be treated and its severity, and the age, weight, physical condition and responsiveness of the mammal to be treated.

[0035] "Therapeutically effective amount" means the amount of a compound or a therapeutically active agent that, when administered to a patient for treating a disease or other undesirable medical condition, is sufficient to have a beneficial effect with respect to that disease or condition. The therapeutically effective amount will vary depending on the type of the selected compound or a therapeutically active agent, the disease or condition and its severity, and the age, weight, etc. of the patient to be treated. Determining the therapeutically effective amount of a given compound or a therapeutically active agent is within the ordinary skill of the art and requires no more than routine experimentation.

[0036] The term "therapeutically effective" applied to dose or amount refers to that quantity of a compound or pharmaceutical formulation that is sufficient to result in a desired clinical benefit after administration to a patient in need thereof.

[0037] As used herein, a "subject" can be a human, non-human primate, mammal, rat, mouse, cow, horse, pig, sheep, goat, dog, cat and the like. In embodiments, the subject is human. In embodiments, the subject can be suspected of having or at risk for having a cancer. The terms "subject" and "patient" are used interchangeably throughout the present application.

[0038] "Mammal" includes humans and both domestic animals such as laboratory animals (e.g., mice, rats, monkeys, dogs, etc.) and household pets (e.g., cats, dogs, swine, cattle, sheep, goats, horses, rabbits), and non-domestic animals such as wildlife and the like.

[0039] All weight percentages (i.e., "% by weight" and "wt. %" and w/w and "% wt") referenced herein, unless otherwise indicated, are measured relative to the total weight of the pharmaceutical composition.

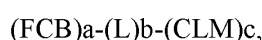
[0040] As used herein, "substantially" or "substantial" refers to the complete or nearly complete extent or degree of an action, characteristic, property, state, structure, item, or result. For example, an object that is "substantially" enclosed would mean that the object is either

completely enclosed or nearly completely enclosed. The exact allowable degree of deviation from absolute completeness may in some cases depend on the specific context. However, generally speaking, the nearness of completion will be so as to have the same overall result as if absolute and total completion were obtained. The use of "substantially" is equally applicable when used in a negative connotation to refer to the complete or near complete lack of action, characteristic, property, state, structure, item, or result. For example, a composition that is "substantially free of" other active agents would either completely lack other active agents, or so nearly completely lack other active agents that the effect would be the same as if it completely lacked other active agents. In other words, a composition that is "substantially free of" an ingredient or element or another active agent may still contain such an item as long as there is no measurable effect thereof.

[0041] As used herein, "D90" refers to the 90% value of particle diameter as determined by sieve analysis, i.e., by the percent of drug particles retained on the mesh screen during the sieve process with a screen of a particular mesh size. For example, if $D_{90} = 100\ \mu\text{m}$, 90% of the drug particles remain on a $100\ \mu\text{m}$ mesh screen during the sieve process. Similarly, "D80" refers to the 80% value of particle diameter, "D70" refers to the 70% value of particle diameter, "D60" refers to the 60% value of particle diameter, "D50" refers to the 50% value of particle diameter, "D40" refers to the 40% value of particle diameter, "D30" refers to the 30% value of particle diameter, "D20" refers to the 20% value of particle diameter, and "D10" refers to the 10% value of particle diameter.

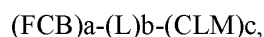
Compounds that form a covalent bond with PI3-kinase (PI3K)

[0042] The compounds, such as those compounds having the formula:



or a pharmaceutically acceptable salt thereof, and/or their subgenera, or Compound I, or salts, solvates, esters and/or prodrugs thereof disclosed herein, can be used in the pharmaceutical composition disclosed herein.

[0043] In another embodiment, the compound that forms a covalent bond with a kinase or pseudokinase is a compound having the formula:



or a pharmaceutically acceptable salt thereof, wherein:

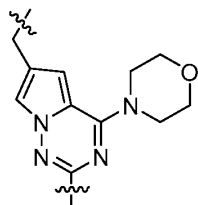
CLM is a covalent linking modality;

L is a linker;

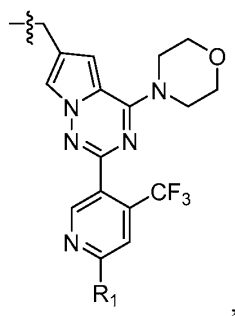
a and c are each independently integers between 1 and 5;

b is an integer between 0 and 5; and

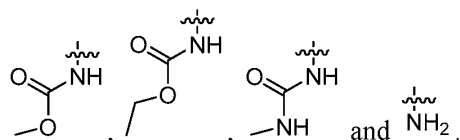
FCB comprises:



[0044] In a specific embodiment, the FCB has the structure:



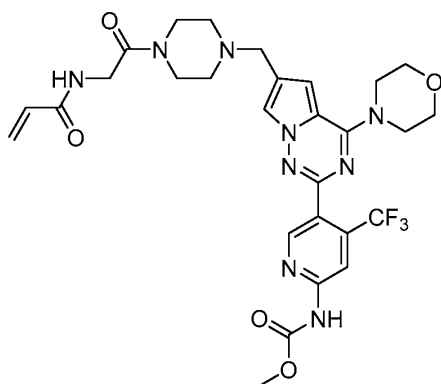
wherein R1 is selected from the group consisting of:



[0045] In some embodiments, the compound that forms a covalent bond with a kinase or pseudokinase (e.g., PI3-kinase (PI3K)) is Compound I. In some embodiments, the compound of formula (FCB)_a-(L)_b-(CLM)_c is compound I.

[0046] In some embodiments, Compound I is a crystalline form of Compound I or a pharmaceutically acceptable salt or solvate of Compound I. In some embodiments, the pharmaceutically acceptable salt selected from the group consisting of a phosphate salt, a hydrochloride salt, a sulfate salt, a mesylate salt, a benzenesulfonate salt, a tosylate salt, a

fumarate salt, a maleate salt, a L-tartrate salt, a citrate salt, and a succinate salt, or a solvate thereof of Compound I:



[0047]

Compound I.

[0048] In a specific embodiment, Compound I is a salt, including a phosphate salt. In some embodiments, the compounds that forms a covalent bond with PI3-kinase (PI3K) is referred to as PI3K compounds.

[0049] The term "CLM" as used herein, refers to any covalent binding modality that is capable of forming a covalent bond with the biological target. The CLM may be linked to an FCB by a bond or by a linker. The CLM may comprise one or more chemical moieties which can form a covalent bond with the biological target. The chemical moieties may be an electrophilic or nucleophilic group.

[0050] The term "biological target", as used herein, refers to any target to which an FCB binds non-covalently to product a therapeutic effect. A CLM binds to the biological target covalently. In some embodiments, the biological target is a protein. Non-limiting examples of biological targets include kinase such as, but not limited to phosphoinositide 3-kinases (PI3Ks) and pseudokinase.

[0051] The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups.

[0052] In some embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C₁-C₃₀ for straight chains, C₃-C₃₀ for branched chains), 20 or fewer, 12 or fewer, or 7 or fewer. Likewise, in some embodiments cycloalkyls have from 3-10 carbon atoms in their ring structure, e.g. have 5, 6 or 7 carbons in the ring structure. The term "alkyl"

(or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having one or more substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents include, but are not limited to, halogen, hydroxyl, carbonyl (such as a carboxyl, alkoxycarbonyl, formyl, or an acyl), thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), alkoxyl, phosphoryl, phosphate, phosphonate, a phosphinate, amino, amido, amidine, imine, cyano, nitro, azido, sulfhydryl, alkylthio, sulfate, sulfonate, sulfamoyl, sulfonamido, sulfonyl, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety.

[0053] Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, or from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Throughout the application, preferred alkyl groups are lower alkyls. In some embodiments, a substituent designated herein as alkyl is a lower alkyl.

[0054] It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include halogen, hydroxy, nitro, thiols, amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF₃, -CN and the like. Cycloalkyls can be substituted in the same manner.

[0055] The term "heteroalkyl", as used herein, refers to straight or branched chain, or cyclic carbon-containing radicals, or combinations thereof, containing at least one heteroatom. Suitable heteroatoms include, but are not limited to, O, N, Si, P, Se, B, and S, wherein the phosphorous and sulfur atoms are optionally oxidized, and the nitrogen heteroatom is optionally quaternized. Heteroalkyls can be substituted as defined above for alkyl groups.

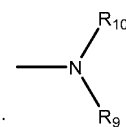
[0056] The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In some embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, and -S-alkynyl. Representative alkylthio groups include methylthio, and ethylthio. The term "alkylthio" also encompasses cycloalkyl groups, alkene and cycloalkene groups, and alkyne groups. "Arylthio" refers to aryl or heteroaryl groups. Alkylthio groups can be substituted as defined above for alkyl groups.

[0057] The terms "alkenyl" and "alkynyl", refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

[0058] The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, and tert-butoxy. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, and -O-alkynyl. Aroxy can be represented by -O-aryl or O-heteroaryl, wherein aryl and heteroaryl are as defined below. The alkoxy and aroxy groups can be substituted as described above for alkyl.

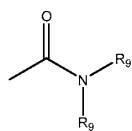
[0059] The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and

substituted amines, e.g., a moiety that can be represented by the general formula:



, wherein R_9 , R_{10} , and R'_{10} each independently represent a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R_8$ or R_9 and R_{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R_8 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In some embodiments, only one of R_9 or R_{10} can be a carbonyl, e.g., R_9 , R_{10} and the nitrogen together do not form an imide. In still other embodiments, the term "amine" does not encompass amides, e.g., wherein one of R_9 and R_{10} represents a carbonyl. In additional embodiments, R_9 and R_{10} (and optionally R'_{10}) each independently represent a hydrogen, an alkyl or cycloalkyl, an alkenyl or cycloalkenyl, or alkynyl. Thus, the term "alkylamine" as used herein means an amine group, as defined above, having a substituted (as described above for alkyl) or unsubstituted alkyl attached thereto, i.e., at least one of R_9 and R_{10} is an alkyl group.

[0060] The term "amido" is art-recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:



, wherein R_9 and R_{10} are as defined above.

[0061] “Aryl”, as used herein, refers to C₅-C₁₀-membered aromatic, heterocyclic, fused aromatic, fused heterocyclic, biaromatic, or biheterocyclic ring systems. Broadly defined, “aryl”, as used herein, includes 5-, 6-, 7-, 8-, 9-, and 10-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, 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” or “heteroaromatics”. The aromatic ring can be substituted at one or more ring positions with one or more substituents including, but not limited to, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino (or quaternized amino), nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, -CF₃, -CN; and combinations thereof.

[0062] The term “aryl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (i.e., “fused rings”) wherein at least one of the rings is aromatic, e.g., the other cyclic ring or rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocycles. Examples of heterocyclic rings include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazoliny, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazoliny, carbazolyl, 4aH carbazolyl, carboliny, chromanyl, chromenyl, cinnoliny, decahydroquinoliny, 2*H*,6*H*-1,5,2-dithiaziny, dihydrofuro[2,3 *b*]tetrahydrofuran, furanyl, furazanyl, imidazolidiny, imidazoliny, imidazolyl, 1*H*-indazolyl, indolenyl, indoliny, indoliziny, indolyl, 3*H*-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindoliny, isoindolyl, isoquinoliny, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholiny, naphthyridiny, octahydroisoquinoliny, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidiny, oxazolyl, oxindolyl, pyrimidiny, phenanthridiny, phenanthroliny, phenaziny, phenothiaziny, phenoxathiny, phenoxaziny, phthalaziny, piperaziny, piperidiny, piperidonyl, 4-piperidonyl, piperonyl, pteridiny, puriny, pyranly, pyraziny, pyrazolidiny, pyrazoliny, pyrazolyl, pyridaziny, pyridoazole, pyridoimidazole, pyridothiazole, pyridiny, pyridyl, pyrimidiny, pyrrolidiny, pyrroliny, 2*H*-pyrrolyl, pyrrolyl, quinazoliny, quinoliny, 4*H*-quinoliziny, quinoxaliny, quinuclidiny, tetrahydrofuranyl, tetrahydroisoquinoliny, tetrahydroquinoliny, tetrazolyl, 6*H*-1,2,5-thiadiaziny, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl,

thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl and xanthenyl. One or more of the rings can be substituted as defined above for "aryl".

[0063] The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

[0064] The term "carbocycle", as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

[0065] "Heterocycle" or "heterocyclic", as used herein, refers to a cyclic radical attached via a ring carbon or nitrogen of a monocyclic or bicyclic ring containing 3-10 ring atoms, and preferably from 5-6 ring atoms, consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and N(Y) wherein Y is absent or is H, O, (C₁-C₁₀) alkyl, phenyl or benzyl, and optionally containing 1-3 double bonds and optionally substituted with one or more substituents. Examples of heterocyclic ring include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazoliny, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazoliny, carbazolyl, 4*aH*-carbazolyl, carboliny, chromanyl, chromenyl, cinnoliny, decahydroquinoliny, 2*H*,6*H*-1,5,2-dithiaziny, dihydrofuro[2,3-*b*]tetrahydrofuran, furanyl, furazanyl, imidazolidiny, imidazoliny, imidazolyl, 1*H*-indazolyl, indolenyl, indoliny, indoliziny, indolyl, 3*H*-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindoliny, isoindolyl, isoquinoliny, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholiny, naphthyridiny, octahydroisoquinoliny, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidiny, oxazolyl, oxepanyl, oxetanyl, oxindolyl, pyrimidiny, phenanthridiny, phenanthroliny, phenaziny, phenothiaziny, phenoxathiny, phenoxaziny, phthalaziny, piperaziny, piperidiny, piperidonyl, 4-piperidonyl, piperonyl, pteridiny, puriny, pyranyl, pyraziny, pyrazolidiny, pyrazoliny, pyrazolyl, pyridaziny, pyridooxazole, pyridoimidazole, pyridothiazole, pyridiny, pyridyl, pyrimidiny, pyrrolidiny, pyrroliny, 2*H*-pyrrolyl, pyrrolyl, quinazoliny, quinoliny, 4*H*-quinoliziny, quinoxaliny, quinuclidiny, tetrahydrofuranyl, tetrahydroisoquinoliny, tetrahydropyranyl, tetrahydroquinoliny, tetrazolyl, 6*H*-1,2,5-thiadiaziny, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl and xanthenyl. Heterocyclic groups can optionally be substituted with one or more substituents at one or more positions as defined above for alkyl and aryl, for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido,

phosphate, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF₃, and -CN.

[0066] The term "carbonyl" is art-recognized and includes such moieties as can be represented

by the general formula: $\text{---}\overset{\text{O}}{\parallel}\text{---X---R}_{11}$ or $\text{---X---}\overset{\text{O}}{\parallel}\text{---R}'_{11}$, wherein X is a bond or represents an oxygen or a sulfur, and R₁₁ represents a hydrogen, an alkyl, a cycloalkyl, an alkenyl, a cycloalkenyl, or an alkynyl, R'₁₁ represents a hydrogen, an alkyl, a cycloalkyl, an alkenyl, a cycloalkenyl, or an alkynyl. Where X is an oxygen and R₁₁ or R'₁₁ is not hydrogen, the formula represents an "ester". Where X is an oxygen and R₁₁ is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R₁₁ is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen and R'₁₁ is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiocarbonyl" group. Where X is a sulfur and R₁₁ or R'₁₁ is not hydrogen, the formula represents a "thioester." Where X is a sulfur and R₁₁ is hydrogen, the formula represents a "thiocarboxylic acid." Where X is a sulfur and R'₁₁ is hydrogen, the formula represents a "thioformate." On the other hand, where X is a bond, and R₁₁ is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R₁₁ is hydrogen, the above formula represents an "aldehyde" group.

[0067] The term "monoester" as used herein refers to an analog of a dicarboxylic acid wherein one of the carboxylic acids is functionalized as an ester and the other carboxylic acid is a free carboxylic acid or salt of a carboxylic acid. Examples of monoesters include, but are not limited to, to monoesters of succinic acid, glutaric acid, adipic acid, suberic acid, sebacic acid, azelaic acid, oxalic and maleic acid.

[0068] The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Examples of heteroatoms are boron, nitrogen, oxygen, phosphorus, sulfur and selenium. Other heteroatoms include silicon and arsenic.

[0069] As used herein, the term "nitro" means -NO₂; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means -SO₂-.

[0070] The term "substituted" as used herein, refers to all permissible substituents of the compounds described herein. In the broadest sense, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic

substituents of organic compounds. Illustrative substituents include, but are not limited to, halogens, hydroxyl groups, or any other organic groupings containing any number of carbon atoms, preferably 1-14 carbon atoms, and optionally include one or more heteroatoms such as oxygen, sulfur, or nitrogen grouping in linear, branched, or cyclic structural formats. Representative substituents include alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, phenyl, substituted phenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, halo, hydroxyl, alkoxy, substituted alkoxy, phenoxy, substituted phenoxy, aroxy, substituted aroxy, alkylthio, substituted alkylthio, phenylthio, substituted phenylthio, arylthio, substituted arylthio, cyano, isocyano, substituted isocyano, carbonyl, substituted carbonyl, carboxyl, substituted carboxyl, amino, substituted amino, amido, substituted amido, sulfonyl, substituted sulfonyl, sulfonic acid, phosphoryl, substituted phosphoryl, phosphonyl, substituted phosphonyl, polyaryl, substituted polyaryl, C₃-C₂₀ cyclic, substituted C₃-C₂₀ cyclic, heterocyclic, substituted heterocyclic, amino acid, peptide, and polypeptide groups.

[0071] Heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. It is understood that “substitution” or “substituted” 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, i.e., a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, or elimination.

[0072] In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein. The permissible substituents can be one or more and the same or different for appropriate organic compounds. The heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms.

[0073] In various embodiments, the substituent is selected from alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carbamate, carboxy, cyano, cycloalkyl, ester, ether, formyl, halogen, haloalkyl, heteroaryl, heterocyclyl, hydroxyl, ketone, nitro, phosphate, sulfide, sulfinyl, sulfonyl, sulfonic acid, sulfonamide, and thioketone, each of which optionally is substituted with one or more suitable substituents. In some embodiments, the substituent is

selected from alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carbamate, carboxy, cycloalkyl, ester, ether, formyl, haloalkyl, heteroaryl, heterocyclyl, ketone, phosphate, sulfide, sulfinyl, sulfonyl, sulfonic acid, sulfonamide, and thioketone, wherein each of the alkoxy, aryloxy, alkyl, alkenyl, alkynyl, amide, amino, aryl, arylalkyl, carbamate, carboxy, cycloalkyl, ester, ether, formyl, haloalkyl, heteroaryl, heterocyclyl, ketone, phosphate, sulfide, sulfinyl, sulfonyl, sulfonic acid, sulfonamide, and thioketone can be further substituted with one or more suitable substituents.

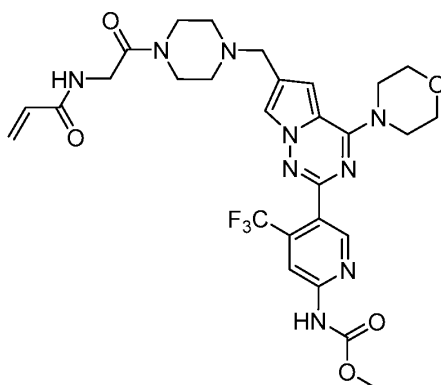
[0074] Examples of substituents include, but are not limited to, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxy, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, thioketone, ester, heterocyclyl, -CN, aryl, aryloxy, perhaloalkoxy, aralkoxy, heteroaryl, heteroaryloxy, heteroarylalkyl, heteroaralkoxy, azido, alkylthio, oxo, acylalkyl, carboxy esters, carboxamido, acyloxy, aminoalkyl, alkylaminoaryl, alkylaryl, alkylaminoalkyl, alkoxyaryl, arylamino, aralkylamino, alkylsulfonyl, carboxamidoalkylaryl, carboxamidoaryl, hydroxyalkyl, haloalkyl, alkylaminoalkylcarboxy, aminocarboxamidoalkyl, cyano, alkoxyalkyl, perhaloalkyl, arylalkyloxyalkyl, and the like. In some embodiments, the substituent is selected from cyano, halogen, hydroxyl, and nitro.

[0075] The terms "polypeptide," "peptide" and "protein" generally refer to a polymer of amino acid residues. As used herein, the term also applies to amino acid polymers in which one or more amino acids are chemical analogs or modified derivatives of corresponding naturally occurring amino acids. The term "protein", as generally used herein, refers to a polymer of amino acids linked to each other by peptide bonds to form a polypeptide for which the chain length is sufficient to produce tertiary and/or quaternary structure. The term "protein" excludes small peptides by definition, the small peptides lacking the requisite higher-order structure necessary to be considered a protein.

[0076] The term "substantially free of" as used herein, means free from therapeutically effective amounts of compounds when administered in suggested doses, but may include trace amounts of compounds in non-therapeutically effective amounts. With respect to compositions of the present invention which are "substantially free of" reducing sugars such as lactose, and/or sugar alcohols such as mannitol, de minimis amounts of such reducing sugars like lactose, and/or mannitol can be present, for example, less than about 10%, less than about 9%, less than about 8%, less than 7%, less than 6%, less than about 5%, less than about 4%, less

than about 3%, less than about 2%, less than about 1%, less than about 0.5%, inclusive of all ranges and subranges therebetween.

[0077] The present disclosure relates to pharmaceutical composition comprising a phosphoinositide 3-kinase (PI3K) inhibitor, such as Compound I, or a pharmaceutically acceptable salt thereof, solvate thereof, or salt solvate thereof.



(Compound I).

[0078] The present invention also includes Compound I or a pharmaceutically acceptable salt thereof as the compound in any combination therapy or method of use or treatment with an additional therapeutically active agent.

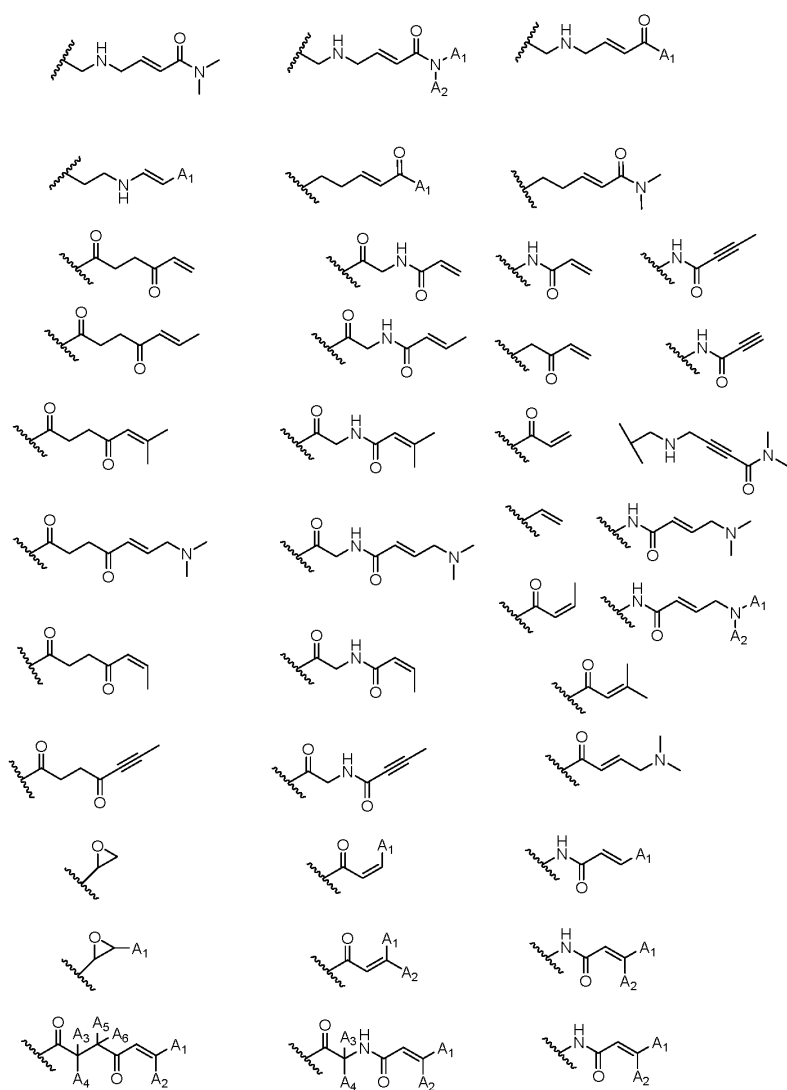
[0079] *CLM*

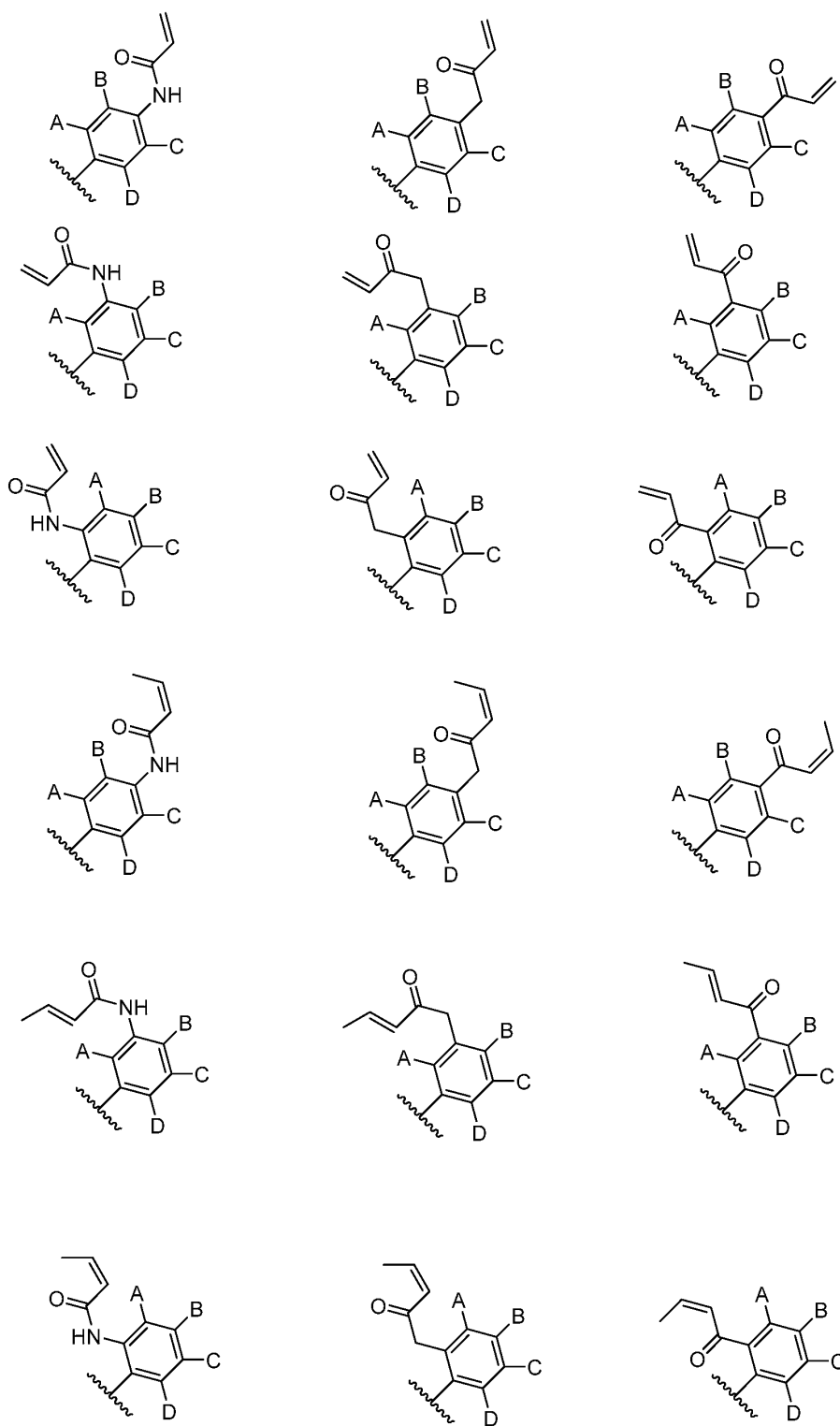
[0080] The compounds of the present disclosure may contain one or more CLM(s). The CLM can be any covalent binding modality that is capable of forming a covalent bond with a biological target. The CLM may comprise one or more chemical moieties, one or more of which are capable of forming a covalent bond with a biological target. In certain embodiments, the CLM may comprise an internal linker or spacer. The internal linker or spacer may combine two parts of the CLM or can be joined to the CLM.

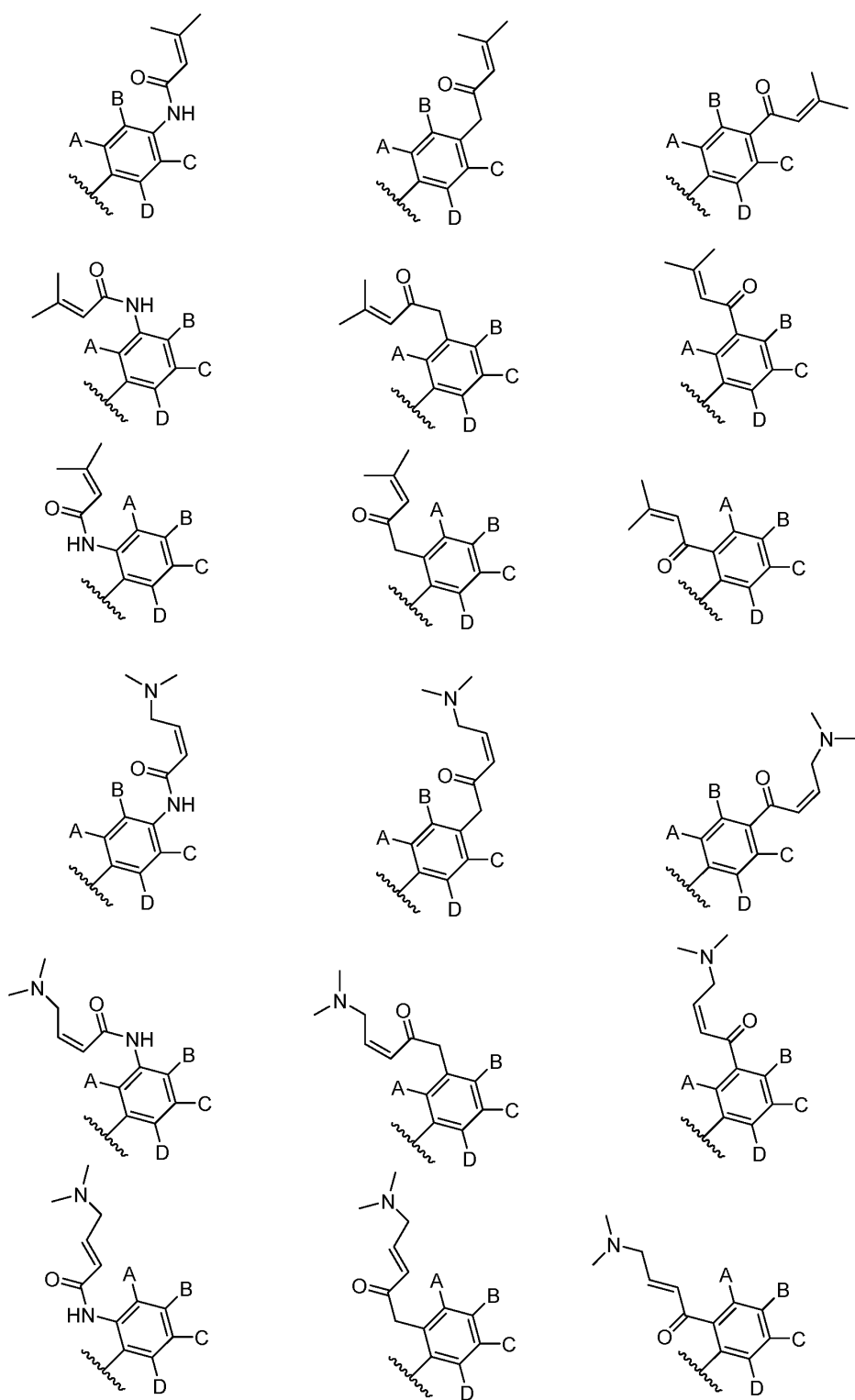
[0081] In some embodiments, the CLM comprises at least one substituted or unsubstituted alkyne. In some embodiments, the CLM comprises at least one substituted or unsubstituted acrylamide. In some embodiments, the CLM comprises at least one substituted or unsubstituted vinyl sulfonamide. In some embodiments, the CLM comprises at least one substituted or unsubstituted vinyl sulfone. In some embodiments, the CLM comprises at least one substituted or unsubstituted fumaramide. In some embodiments, the CLM comprises at least one substituted or unsubstituted acrylate. In some embodiments, the CLM comprises at least one

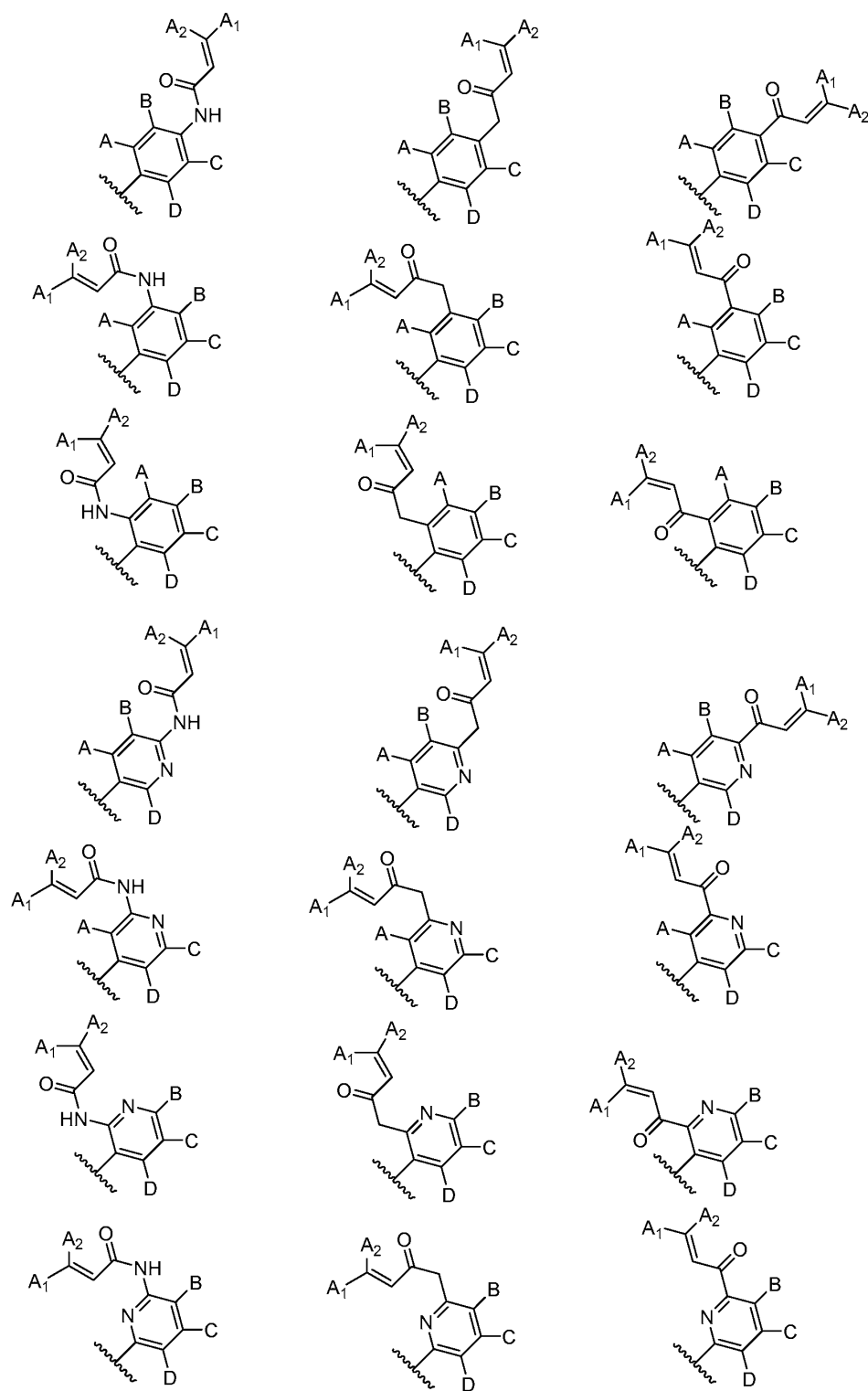
substituted or unsubstituted isothiocyanate. In some embodiments, the CLM comprises at least one substituted or unsubstituted sulfonyl fluoride. In some embodiments, the CLM comprises at least one substituted or unsubstituted fluorosulfate. In some embodiments, the CLM comprises at least one substituted or unsubstituted formyl phenyl boronic acid. In some embodiments, the CLM comprises at least one substituted or unsubstituted boronic acid. In some embodiments, the CLM comprises at least one activated ester. In some embodiments, the CLM comprises at least one substituted or unsubstituted thioester. In some embodiments, the CLM comprises at least one sulfonyl group. In some embodiments, the CLM comprises at least one nitro group. In some embodiments, the CLM comprises at least one substituted or unsubstituted epoxide. In some embodiments, the CLM comprises at least one substituted or unsubstituted formyl phenyl boronic acid. In some embodiments, the CLM comprises at least one substituted or unsubstituted aryl halide. In some embodiments, the CLM comprises at least one substituted or unsubstituted aldehyde. In some embodiments, the CLM comprises at least one substituted or unsubstituted triazine. In some embodiments, the CLM comprises at least one substituted or unsubstituted cyano-acrylamide. In some embodiments, the CLM comprises at least one substituted or unsubstituted chloroacetamide.

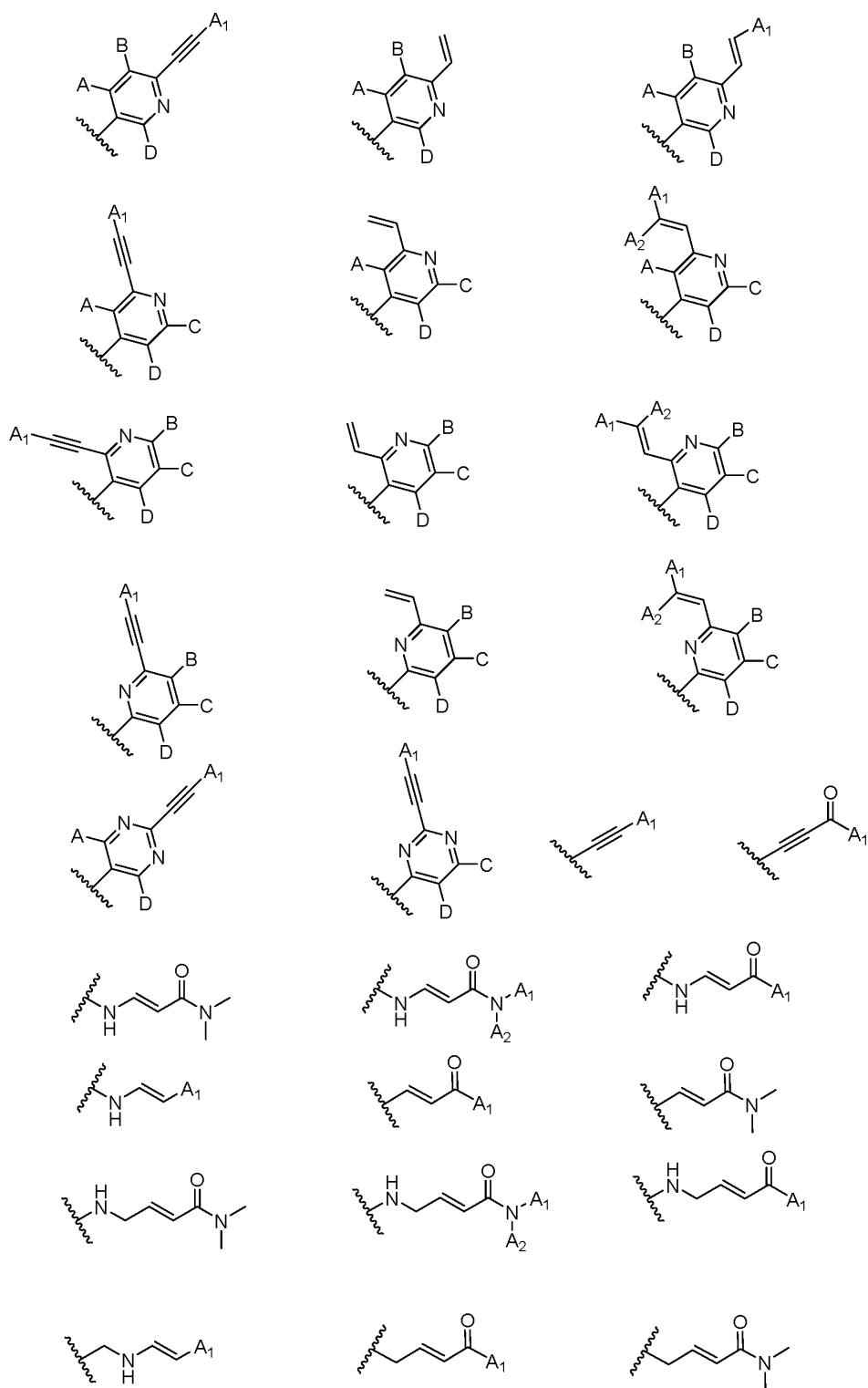
[0082] Exemplary CLMs include, but not limited to





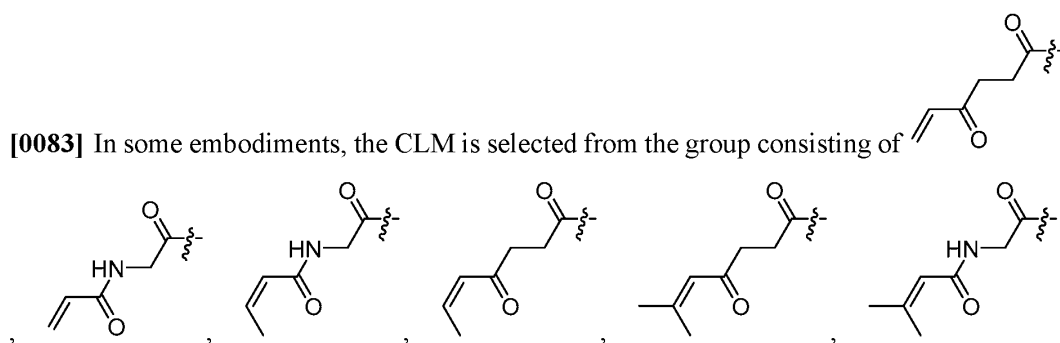


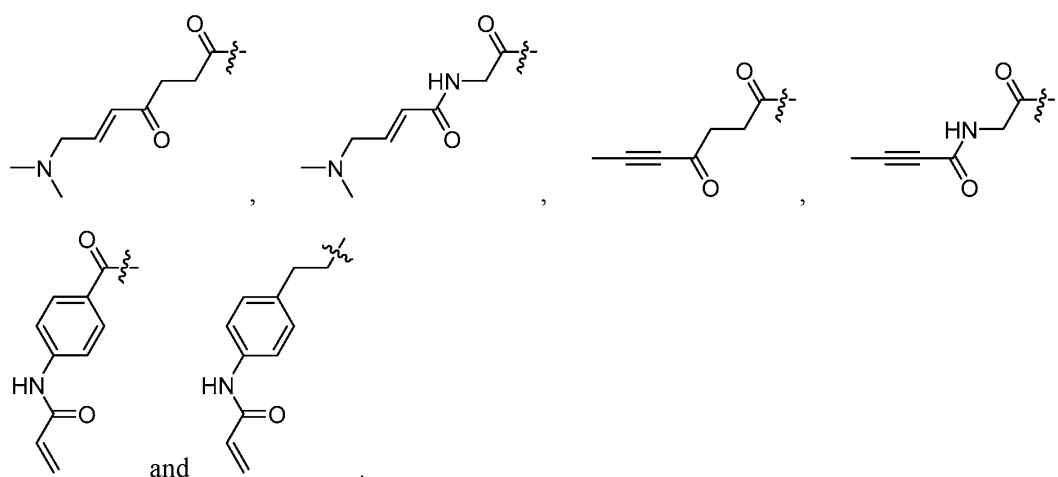




A, B, C and D at each occurrence is independently selected from the group consisting of H, halogen, CF₃, -OH, -NH₂, -SH, -SCH₃, -CN, -NO₂, -CH₂(NH₂), -C(O)OH, -S(O)₂NH₂, -

C(O)NH₂, -C(O)CH₃, NHC(O)-C₁₋₆ alkyl, N(C₁₋₃ alkyl)C(O)-C₁₋₆ alkyl, OC(O)NH₂, OC(O)NH(CH₃), OC(O)N(CH₃)₂, imidazolyl, pyrazolyl, methylimidazolyl, methylpyrazolyl, optionally substituted C₁₋₆ alkyl, optionally substituted C₂₋₆ alkenyl, optionally substituted C₂₋₆ alkynyl, optionally substituted C₃₋₆ cycloalkyl, optionally substituted 5-10 membered heterocycle, optionally substituted aryl, and optionally substituted 5-10 membered heteroaryl, wherein the optional substituents for A, B, C, and D are 1-3 substituents which are independently selected from the group consisting of halogen, OH, NH₂, CH₃, CF₃, -CN, -NO₂, -C(O)OH, -S(O)₂NH₂, -C(O)NH₂, -CH₂NH₂, -C(O)CH₃, SH, -S-CH₃, optionally substituted C₁₋₃ alkyl, and optionally substituted C₃₋₆ cycloalkyl. A₁, A₂, A₃, A₄, A₅, and A₆ at each occurrence are independently selected from the group consisting of H, halogen, CF₃, -OH, -NH₂, -SH, -SCH₃, -CN, -NO₂, -CH₂(NH₂), -C(O)OH, -S(O)₂NH₂, -C(O)NH₂, -C(O)CH₃, NHC(O)-C₁₋₆ alkyl, N(C₁₋₃ alkyl)C(O)-C₁₋₆ alkyl, OC(O)NH₂, OC(O)NH(CH₃), OC(O)N(CH₃)₂, imidazolyl, pyrazolyl, methylimidazolyl, methylpyrazolyl, optionally substituted C₁₋₆ alkyl, optionally substituted C₂₋₆ alkenyl, optionally substituted C₂₋₆ alkynyl, optionally substituted C₃₋₆ cycloalkyl, optionally substituted 5-10 membered heterocycle, optionally substituted aryl, and optionally substituted 5-10 membered heteroaryl, wherein the optional substituents for A₁, A₂, A₃, A₄, A₅, and A₆ are 1-3 substituents which are independently selected from the group consisting of halogen, OH, NH₂, CH₃, CF₃, -CN, -NO₂, -C(O)OH, -S(O)₂NH₂, -C(O)NH₂, -CH₂NH₂, -C(O)CH₃, SH, -S-CH₃, optionally substituted C₁₋₃ alkyl, and optionally substituted C₃₋₆ cycloalkyl, and wherein the C₁₋₃ alkyl and C₃₋₆ cycloalkyl optional substituents are 1-2 substituents, which are independently selected from the group consisting of halogen, OH, NH₂, CH₃, CF₃, -CN, -NO₂, -C(O)OH, -S(O)₂NH₂, -C(O)NH₂, -CH₂NH₂, -C(O)CH₃, SH, and -S-CH₃, or a fragment, derivative or analog thereof.





[0084] *Linkers*

[0085] The compounds in the pharmaceutical compositions of the present disclosure may contain one or more optional linkers connecting the FCB(s) and CLM(s). The linker, L, can be attached to anywhere on FCB and CLM, as long as the efficacy of FCB and the binding of CLM are not significantly affected. In some embodiments, CLM comprises an optional internal linker.

[0086] In some embodiments, the linker (including the internal linker of CLM) is a small molecule. In some embodiments, the linker (including the internal linker of CLM) is selected, but not limited to substituted and unsubstituted C1-C30 alkyl, substituted and unsubstituted C2-C30 alkenyl, substituted and unsubstituted C2-C30 alkynyl, substituted and unsubstituted C3-C30 cycloalkyl, substituted and unsubstituted C1-C30 heterocycloalkyl, substituted and unsubstituted C3-C30 cycloalkenyl, substituted and unsubstituted C1-C30 heterocycloalkenyl, substituted and unsubstituted aryl, and substituted and unsubstituted heteroaryl.

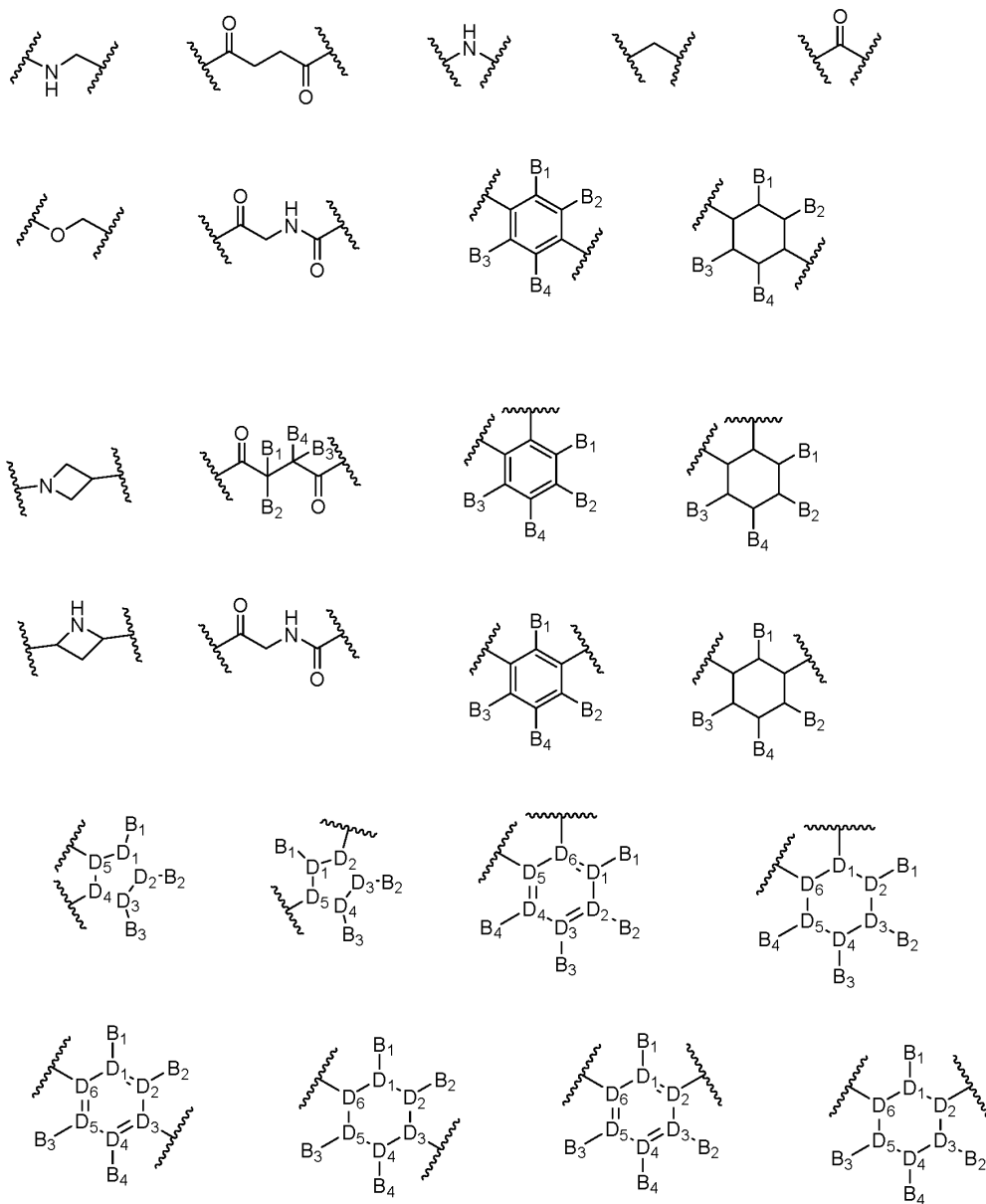
[0087] In some embodiments, the linker (including the internal linker of CLM) can be a C1-C10 straight chain alkyl, C1-C10 straight chain O-alkyl, C1-C10 straight chain substituted alkyl, C1-C10 straight chain substituted O-alkyl, C4-C13 branched chain alkyl, C4-C13 branched chain O-alkyl, C2-C12 straight chain alkenyl, C2-C12 straight chain O-alkenyl, C3-C12 straight chain substituted alkenyl, C3-C12 straight chain substituted O-alkenyl, polyethylene glycol, polylactic acid, polyglycolic acid, poly(lactide-co-glycolide), polycaprolactone, polycyanoacrylate, ketone, aryl, heterocyclic, succinic ester, amino acid, aromatic group, ether, crown ether, urea, thiourea, amide, purine, pyrimidine, bipyridine, indole derivative acting as a cross linker, chelator, aldehyde, ketone, bisamine, bis alcohol, heterocyclic ring structure,

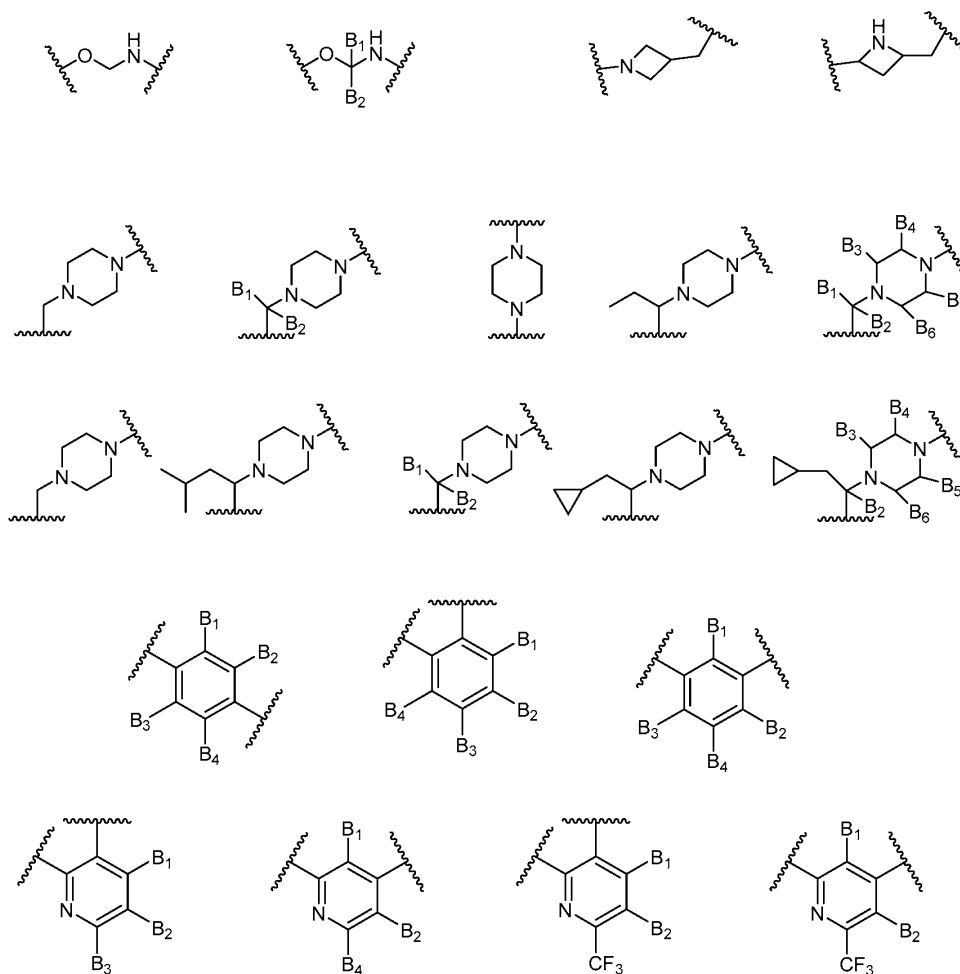
azirine, disulfide, thioether, hydrazone and combinations thereof. For example, the linker can be a C₃ straight chain alkyl or a ketone. The alkyl chain of the linker can be substituted with one or more substituents or heteroatoms. In some embodiments the linker contains one or more atoms or groups selected from -O-, -C(=O)-, -NR-, -O-C(=O)-NR-, -S-, -S-S-. The linker may be selected from dicarboxylate derivatives of succinic acid, glutaric acid or diglycolic acid.

[0088] In some embodiments the alkyl chain of the linker may optionally be interrupted by one or more atoms or groups selected from -O-, -C(=O)-, -NR-, -O-C(=O)-NR-, -S-, -S-S-. The linker may be selected from dicarboxylate derivatives of succinic acid, glutaric acid or diglycolic acid.

[0089] In some embodiments, the linker may be non-cleavable. In some embodiments, the linker may be cleavable. In some embodiments, the linker may be cleaved by an enzyme.

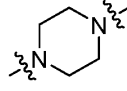
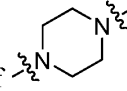
[0090] Non-limiting examples of linkers include

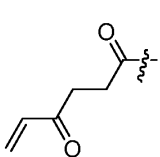
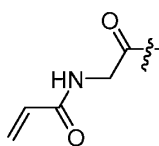
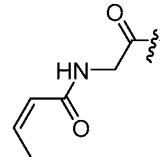
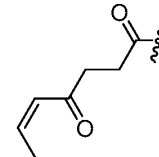
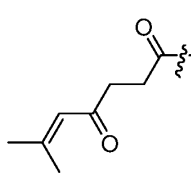
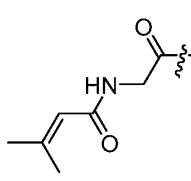
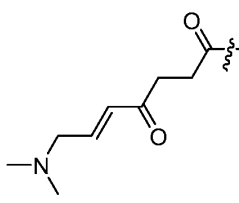
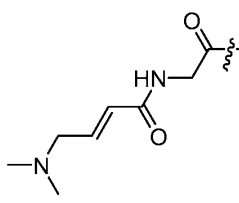
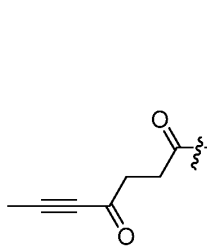
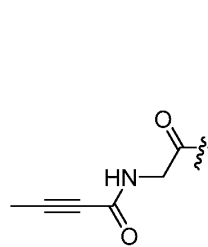
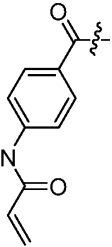
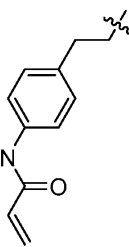




wherein D1, D2, D3, D4, D5 and D6 at each occurrence are independently selected from the group consisting of N, C, O, or S, provided that if D1-6 is a N, the corresponding position is trivalent; if D1-6 is a O or S, the corresponding position is divalent; wherein B1, B2, B3, B4, B5, and B6 at each occurrence is absent or independently selected from the group consisting of H, halogen, CF₃, -OH, -CH₃, -NH₂, -SH, -SCH₃, -CN, -NO₂, -CH₂(NH₂), -C(O)OH, -S(O)₂NH₂, -C(O)NH₂, -C(O)CH₃, NHC(O)-C1-6 alkyl, N(C1-3 alkyl)C(O)-C1-6 alkyl, OC(O)NH₂, OC(O)NH(CH₃), OC(O)N(CH₃)₂, imidazolyl, pyrazolyl, methylimidazolyl, methylpyrazolyl, optionally substituted C1-6 alkyl, optionally substituted C2-6 alkenyl, optionally substituted C2-6 alkynyl, optionally substituted C3-6 cycloalkyl, optionally substituted 5-10 membered heterocycle, optionally substituted aryl, and optionally substituted 5-10 membered heteroaryl, wherein the optional substituents for B1, B2, B3, and B4 are 1-3 substituents independently selected from the group consisting of halogen, OH, NH₂, CH₃, CF₃, -CN, -NO₂, -C(O)OH, -S(O)₂NH₂, -C(O)NH₂, -CH₂NH₂, -C(O)CH₃, SH, -S-CH₃,

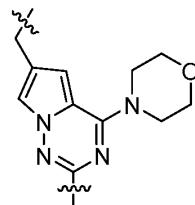
optionally substituted C1-3 alkyl, and optionally substituted C3-6 cycloalkyl, and wherein the C1-3 alkyl and C3-6 cycloalkyl optional substituents are 1-2 substituents independently selected from the group consisting of halogen, OH, NH₂, CH₃, CF₃, -CN, -NO₂, -C(O)OH, -S(O)₂NH₂, -C(O)NH₂, -CH₂NH₂, -C(O)CH₃, SH, and -S-CH₃, or any fragments or analogs thereof.

[0091] In some embodiments, the linker is selected from the group consisting of  and  wherein either end can be connected to the CLM. In some embodiments, the linker selected from the group consisting of  and  is connected to the CLM selected

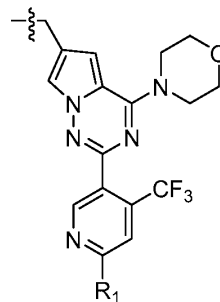
from the group consisting of , , , , , , , , , , , and .

[0092] In some embodiments, the kinase is PI3-kinase (PI3K). The therapeutic conjugate may have a structure of (FCB)_a-(L)_b-(CLM)_c, wherein a and c are, independently, integers between 1 and 5, b is an integer between 0 and 5, and wherein the FCB moiety comprises a PI3K inhibitor, or a fragment, analog or derivative thereof.

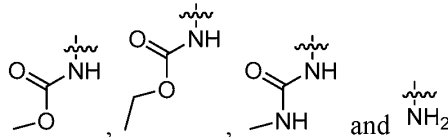
[0093] The FCB moiety, L (linker) moiety, and CLM moieties are discussed in the sections



above. In one non-limiting example, the FCB comprises



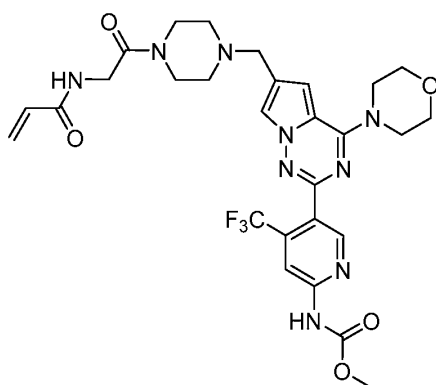
[0094] In some embodiments, the FCB is a compound having the structure,



, wherein R1 is selected from the group consisting of

Pharmaceutical compositions

[0095] In some embodiments, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (FCB)a-(L)b-(CLM)c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof, as disclosed herein, as the active ingredient, combined with a pharmaceutically acceptable excipient or carrier:



[0096]

(Compound I)

[0097] In a specific embodiment, the pharmaceutical composition allows for immediate release of compound I.

[0098] In some embodiments, Compound I is a crystalline form of Compound I or a pharmaceutically acceptable salt or solvate of Compound I. In some embodiments, the pharmaceutically acceptable salt of Compound I is selected from the group consisting of a phosphate salt, a hydrochloride salt, a sulfate salt, a mesylate salt, a benzenesulfonate salt, a tosylate salt, a fumarate salt, a maleate salt, a L-tartrate salt, a citrate salt, and a succinate salt, or a solvate thereof. In some embodiments, Compound I is anhydrous or non-solvated. In another embodiment, the crystalline form is a phosphate salt of Compound I or a solvate thereof. In a specific embodiment, the crystalline form is a hydrate. In another embodiment, the present invention may be an amorphous means of Compound I.

[0099] In a specific embodiment, the form of Compound I in the pharmaceutical compositions describe herein is from PCT application PCT/CN2023/083760, which is incorporated by reference in its entirety herein.

[0100] Pharmaceutical excipients can be added to the pharmaceutical composition or formulation for a variety of purposes, including for immediate release of the compound after oral administration to a patient.

[0101] In some embodiments, the pharmaceutical composition herein can be in a form a tablet. In some embodiments, the tablet herein has a weight range of about 20 mg to about 500 mg, or about 50 mg to about 400 mg, or about 70 mg to about 250 mg, or about 80 mg to about 150 mg. In some embodiments, the tablet herein has a weight of about 100 mg. In some embodiments, the tablet has a diameter of less than about 15 mm, 14 mm, 13 mm, 12 mm, 11 mm, 10 mm, 9 mm, 8 mm, 7 mm, 6 mm, 5 mm, or 4 mm. In some embodiments, the tablet is

a coated tablet. In some embodiment, the tablet has a hardness greater than about 0.5 Kp, about 0.7 Kp, about 1.0 Kp, about 1.2 Kp, about 1.5 Kp, or about 2.0 Kp when compressed using a 6 mm tablet tooling size. In some embodiments, the tablet has a friability less than 3%, less than 2% or less than 1%.

[0102] In some embodiments, the compound that forms a covalent bond with a kinase or pseudokinase, a compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate thereof is in an amount ranging from about 0.1% wt to about 50% wt, or 1% wt to about 40% wt, or about 3% wt to about 20% wt, or about 4% wt to about 10% wt relative to the overall weight of the composition or tablet. In some embodiments, the compound that forms a covalent bond with a kinase or pseudokinase, a compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate thereof is in an amount of about 5% wt.

[0103] In some embodiments, the compound that forms a covalent bond with a kinase or pseudokinase, a compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate thereof is in an amount ranging from about 0.1 mg to about 30 mg per tablet, or about 1 mg to about 20 mg per tablet, or about 3 mg to about 7 mg per tablet, or about 5 mg to about 6 mg per tablet. In some embodiments, the compound that forms a covalent bond with a kinase or pseudokinase, a compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate thereof is in an amount of 5 mg per tablet.

[0104] In some embodiments, suitable pharmaceutical excipients can include filler, binder, disintegrant, glidant, lubricant, coating or any combination thereof.

[0105] In some embodiments, fillers include, for example, materials such as silicon dioxide, titanium dioxide, alumina, talc, kaolin, powdered cellulose, microcrystalline cellulose, urea, sodium chloride, as well as saccharides, or combinations thereof. Any suitable saccharide may be used in the composition of the present disclosure. As used herein, the “saccharides” used in the invention include sugar alcohols, monosaccharides, disaccharides, and oligosaccharides. Exemplary sugar alcohols include, but are not limited to, xylitol, mannitol, sorbitol, erythritol, lactitol, pentitol, and hexitol. Exemplary monosaccharides include, but are not limited to, glucose, fructose, aldose and ketose. Exemplary disaccharides include, but are not limited to, sucrose, isomalt, lactose, trehalose, and maltose. Exemplary oligosaccharides include, but are

not limited to, fructo-oligosaccharides, inulin, galacto-oligosaccharides, and mannan-oligosaccharides. In some embodiments, the saccharide is sorbitol, mannitol, or xylitol. In some embodiments, the saccharide is sorbitol. In some embodiments, the saccharide is sucrose.

[0106] In some embodiments, suitable pharmaceutically acceptable fillers can be selected from microcrystalline cellulose, silicified microcrystalline cellulose, Mannitol EZ, pregelatinized starch, dibasic calcium phosphate dihydrate, lactose monohydrate or any combination thereof.

[0107] In some embodiments, the filler used in the disclosure is in an amount ranging from about 10% wt to about 95% wt, 10% wt to about 80% wt, or about 20% wt to about 70% wt, or about 30% wt to about 60% wt, or about 40% wt to about 60% wt, or about 40% wt to about 50% wt, or about 42% wt to 46% wt of the total weight of the composition or tablet. In some embodiments, the filler used herein is in an amount ranging from about 30 % wt to about 60% wt. In some embodiments, the filler used herein is in an amount of about 30% wt. In some embodiments, the filler used herein is in an amount of about 40% to about 50% wt of the wt of the composition or tablet. In some embodiments, the filler used herein is in an amount of about 45% wt to about 60% wt of the total wt of the composition or tablet. In some embodiments, the filler used herein is in an amount of about 70% wt to about 90% wt of the total wt of the composition or tablet.

[0108] In some embodiments, the filler used herein is in an amount ranging from about 10 mg to about 80 mg per tablet, or about 20 mg to about 70 mg per tablet, or about 30 mg to about 60 mg per tablet, or about 40 mg to about 50 mg per tablet or about 42 mg to about 46 mg per tablet. In some embodiments, the filler used herein is in an amount of 30 mg per tablet. In some embodiments, the filler used herein is in an amount of 42.5 mg per tablet.

[0109] In some embodiments, the filler used herein is in an amount ranging from about 100 mg to about 800 mg per tablet, or about 200 mg to about 700 mg per tablet, or about 300 mg to about 600 mg per tablet, or about 400 mg to about 500 mg per tablet or about 420 mg to about 460 mg per tablet. In some embodiments, the filler used herein is in an amount of 300 mg per tablet. In some embodiments, the filler used herein is in an amount of about 200 mg to about 300 mg per tablet.

[0110] In a specific embodiment, with the ratios and amounts of filler described herein, the filler includes microcrystalline cellulose.

[0111] Binders can be used to impart cohesive qualities to a tablet, and thus ensure that the tablet or tablet layer remains intact after compression. Suitable binder materials include, but

are not limited to, starch (including corn starch and pregelatinized starch), gelatin, sugars (including sucrose, glucose, dextrose and lactose), polyethylene glycol, polyvinyl alcohol, waxes, and natural and synthetic gums, e.g., acacia sodium alginate, polyvinylpyrrolidone, cellulosic polymers (including hydroxypropyl cellulose, hydroxypropyl methylcellulose, methyl cellulose, microcrystalline cellulose, ethyl cellulose, hydroxyethyl cellulose, and the like), and Veegum, and combinations thereof. Examples of polyvinylpyrrolidone include povidone, copovidone and crospovidone.

[0112] Solid pharmaceutical compositions that are compacted into a dosage form, such as a tablet, may include excipients whose functions include helping to bind the active ingredient and other excipients together after compression. Binders for solid pharmaceutical compositions and/or combinations include acacia, alginic acid, carbomer (e.g., carbopol), carboxymethylcellulose sodium, dextrin, ethyl cellulose, gelatin, guar gum, gum tragacanth, hydrogenated vegetable oil, hydroxyethyl cellulose, hydroxypropyl cellulose (e.g., KLUCEL), hydroxypropyl methyl cellulose (e.g., METHOCEL), liquid glucose, magnesium aluminum silicate, maltodextrin, methylcellulose, polymethacrylates, povidone (e.g., KOLLIDON, PLASDONE), pregelatinized starch, sodium alginate, and starch.

[0113] In some embodiments, suitable pharmaceutically acceptable binders can be selected from povidone K29/32, hydroxypropyl cellulose, hydroxypropyl methyl cellulose or any combination thereof.

[0114] In some embodiments, the binder used in this disclosure is in an amount ranging from about 0.1% wt to about 10% wt, or about 1% wt to about 10% wt, or about 2% wt to about 8% wt, or about 2% wt to about 6% wt, or about 2% wt to 5% wt, or about 3% wt to about 4% wt of the total wt of the composition or tablet.

[0115] In some embodiments, the binder used herein is in an amount ranging from about 0.1 mg to about 10 mg per tablet, or about 1 mg to about 10 mg per tablet, or about 2 mg to about 8 mg per tablet, or about 2 mg to about 6 mg per tablet, or about 2 mg to 5 mg per tablet, or about 3 mg to 4 mg per tablet.

[0116] In a specific embodiment, with the ratios and amounts of binder described herein, the binder includes hydroxypropyl cellulose.

[0117] Disintegrants can be used to facilitate disintegration of the tablet, thereby increasing the erosion rate relative to the dissolution rate, and are generally starches, clays, celluloses, algin, gums, or crosslinked polymers (e.g., crosslinked polyvinyl pyrrolidone). Other non-limiting

examples of suitable disintegrants include, for example, lightly crosslinked polyvinyl pyrrolidone, corn starch, potato starch, maize starch and modified starches, croscarmellose sodium, crospovidone, sodium starch glycolate, and combinations and mixtures thereof.

[0118] In some embodiments, pharmaceutically acceptable disintegrants can be selected from Croscarmellose Sodium, Crospovidone XL or any combination thereof.

[0119] In some embodiments, the disintegrant used herein is in an amount ranging from about 0.1% wt to about 10% wt, or about 1% wt to about 10% wt, or about 2% wt to about 8% wt, or about 2% wt to about 6% wt, or about 2% wt to 5% wt, or about 3% wt to 4% wt of the total wt of the composition or tablet. In some embodiments, the integrant used herein is in an amount ranging from about 0.1 mg to about 10 mg per tablet, or about 1 mg to about 10 mg per tablet, or about 2 mg to about 8 mg per tablet, or about 2 mg to about 6 mg per tablet, or about 2 mg to 5 mg per tablet, or about 3 mg to 4 mg per tablet, or about 1 mg to 2 mg per tablet, or about 8 mg to 10 mg per tablet.

[0120] In a specific embodiment, with the ratios and amounts of disintegrant described herein, the disintegrant includes Crospovidone XL.

[0121] Glidants can be added to improve the flowability of a non-compacted solid composition and to improve the accuracy of dosing. Excipients that may function as glidants include, for example, colloidal silicon dioxide, magnesium trisilicate, powdered cellulose, starch, talc, and tribasic calcium phosphate.

[0122] In some embodiments, suitable pharmaceutically acceptable glidants can be selected from Colloidal Silicon Dioxide, Talc or any combination thereof.

[0123] In some embodiments, the glidant is in an amount ranging from about 0.01% wt to about 10% wt, or about 0.1% wt to about 5% wt, or about 0.1% wt to about 3% wt, or about 0.2% wt to about 2% wt, or about 0.3% wt to 1% wt, or about 0.3% wt to 0.6% wt, or about 1% wt to 5% wt, or about 2% wt to 3% wt, or about 0.4% wt to about 0.5% wt of the total wt of the composition or tablet. In some embodiments, the glidant used herein is in an amount ranging from about 0.01 mg to about 10 mg per tablet, or about 0.1 mg to about 5 mg per tablet, or about 0.1 mg to about 3 mg per tablet, or about 0.2 mg to about 2 mg per tablet, or about 0.3 mg to 1 mg per tablet, or about 0.3 mg to 0.6 mg per tablet, or about 0.4 mg to about 0.5 mg per tablet, or about 6 mg to about 10 mg per tablet, or about 7 mg to about 9 mg per tablet.

[0124] In a specific embodiment, with the ratios and amounts of glidant described herein, the glidant includes colloidal silicon dioxide.

[0125] Lubricants can be used to facilitate tablet manufacture, promoting powder flow and preventing particle capping (e.g., particle breakage) when pressure is relieved. Useful lubricants are magnesium stearate, calcium stearate, stearic acid, glyceryl behenate, talc, colloidal silicon dioxide such as Aerosil™ 200, mineral oil (in PEG), hydrogenated vegetable oil (e.g., comprised of hydrogenated and refined triglycerides of stearic and palmitic acids), combinations thereof.

[0126] When a dosage form such as a tablet is made by the compaction of a powdered composition and/or combination, the composition is subjected to pressure from a punch and dye. Some excipients and active ingredients have a tendency to adhere to the surfaces of the punch and dye, which can cause the product to have pitting and other surface irregularities. A lubricant can be added to the composition to reduce adhesion and ease the release of the product from the dye. Lubricants include magnesium stearate, calcium stearate, glyceryl monostearate, glyceryl palmitostearate, hydrogenated castor oil, hydrogenated vegetable oil, mineral oil, polyethylene glycol, sodium benzoate, sodium lauryl sulfate, sodium stearyl fumarate, stearic acid, talc, and zinc stearate.

[0127] In some embodiments, suitable pharmaceutically acceptable lubricants can be selected from magnesium stearate (such as from a vegetable source), Sodium Steryl Fumarate, Glyceryl Behenate or any combination thereof.

[0128] In some embodiments, the lubricant used herein is in an amount ranging from about 0.01% wt to about 10% wt, or about 0.1% wt to about 5% wt, or about 0.1% wt to about 3% wt, or about 0.2% wt to about 2% wt, or about 0.3% wt to 1% wt, or about 0.3% wt to 0.6% wt, or about 0.5% wt to 1.5% wt, or about 0.7% wt to 1.2% wt, or about 0.4% wt to 0.5% wt of the total wt of the composition or tablet.

[0129] In some embodiments, the lubricant used herein is in an amount ranging from about 0.01 mg to about 10 mg per tablet, or about 0.1 mg to about 5 mg per tablet, or about 0.1 mg to about 3 mg per tablet, or about 0.2 mg to about 2 mg per tablet, or about 0.3 mg to 1 mg per tablet, or about 1 mg to about 3 mg per tablet, or about 2 mg to about 3 mg per tablet, or about 0.3 mg to 0.6 mg per tablet, or about 0.4 mg to 0.5 mg per tablet.

[0130] In a specific embodiment, with the ratios and amounts of lubricant described herein, the lubricant includes magnesium stearate.

[0131] In some embodiments, suitable pharmaceutically acceptable coatings can be selected from water-soluble polymers. In some embodiments, suitable pharmaceutically acceptable coatings can be selected from Opadry II White (PVA), Opadry II White (HPMC) or any combination thereof.

[0132] In some embodiments, the coating used herein is in an amount ranging from about 0.1% wt to about 10% wt, or about 1% wt to about 10% wt, or about 2% wt to about 8% wt, or about 2% wt to about 6% wt, or about 2% wt to 5% wt, or about 3% wt to 4% wt of the wt of the composition or tablet.

[0133] In some embodiments, the coating used herein is applied to the tablet for a weight gain of about 1% to about 20% by weight based on the total weight of the tablet. In some embodiments, the coating used herein is applied to the tablet for a weight gain of about 10% or about 7%, or about 5%, or about 3% or about 1% by weight based on the total weight of the tablet.

[0134] In some embodiments, the coating used herein is in an amount ranging from about 0.1 mg to about 10 mg per tablet, or about 1 mg to about 10 mg per tablet, or about 2 mg to about 8 mg per tablet, or about 2 mg to about 6 mg per tablet, or about 2 mg to 5 mg per tablet, or about 3 mg to 4 mg per tablet.

[0135] In some embodiments, the pharmaceutical excipient used herein is selected from Microcrystalline Cellulose (Avicel PH 101®), Lactose Monohydrate (Fast Flo®), Hydroxy Propyl Cellulose (Klucel EXF®), Croscarmellose Sodium (Ac-di-sol®), Colloidal Silicon Dioxide (Cab-O-Sil®), Magnesium Stearate Vegetable or any combination thereof.

[0136] In some embodiments, the pharmaceutical excipient used herein is selected from Microcrystalline Cellulose, Crospovidone XL, Colloidal Silicon Dioxide (Cab-O-Sil®), Magnesium Stearate Vegetable, hydroxypropyl cellulose, or any combination thereof.

[0137] In a specific embodiment, the pharmaceutical composition comprises Microcrystalline Cellulose, hydroxypropyl cellulose, Crospovidone XL, Colloidal Silicon Dioxide (Cab-O-Sil®), and Magnesium Stearate Vegetable. In another specific embodiment, the pharmaceutical composition or formulation is a tablet. In another specific embodiment, the tablet is an oral tablet. In another specific embodiment, the oral tablet is an immediate release oral tablet. In a specific embodiment, the pharmaceutical composition and formulation include a tablet coating. In a specific embodiment, the tablet is coated with Opadry II white.

[0138] In another embodiment, the pharmaceutical composition and formulation may be prepared as described in the examples herein. In a specific embodiment, pharmaceutical composition and formulations may be as described in Table 2, 3, 5, 9, 12 or 15 described herein.

[0139] In a specific embodiment, the pharmaceutical composition and formulation is a tablet with a specific dissolution and disintegration profile. In a specific embodiment, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the drug, such as Compound I is released within about 10 minutes. In another specific embodiment, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of Compound I is released within about 9 minutes, 8 minutes, 7 minutes, 6 minutes, 5 minutes, 4 minutes, 3 minutes, 2 minutes or 1 minute.

[0140] In another specific embodiment, the dissolution of the drug in the tablet is determined using 900 mL of 0.01M HCl at 37.0°C, Apparatus II (paddle), 75 rpm or 100 rpm.

[0141] In another specific embodiment, the pharmaceutical composition comprising a therapeutically effective amount of a compound that forms a covalent bond with a kinase or pseudokinase, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutical excipient, wherein the pharmaceutical composition allows for immediate release of the compound, and the pharmaceutical composition is substantially free of an impurity related to the compound. In a specific embodiment, the compound is compound I, or a pharmaceutically acceptable salt or solvate thereof, and the impurity is related to compound I, i.e., is an analog or the result of a reaction of compound I with an excipient in the composition.

[0142] In another embodiment, the pharmaceutical composition is substantially free of any reducing sugar, i.e., a sugar that has a free aldehyde group or free ketone group. In another embodiment, the pharmaceutical composition is substantially free of lactose, galactose, glucose, maltose, fructose, ribose and/or xylose. In another embodiment, the pharmaceutical composition is substantially free of lactose. In a specific embodiment, the lactose is lactose anhydrate. In a specific embodiment, compositions of the present invention are substantially free of isomers of lactose, such as sucrose, trehalose, maltose, isomaltose, maltulose, isomaltulose, turanose, and cellobiose. In another embodiment, compositions of the present invention are substantially free of isomers of mannitol, such as sorbitol, and compounds closely related to mannitol, such as xylitol, erythritol, lactitol, and maltitol. In another specific embodiment, the compound is Compound I or a pharmaceutically acceptable salt or solvate

thereof. In a specific embodiment, Compound I is in the form of a salt. In another specific embodiment, the salt is a phosphate salt.

[0143] The pharmaceutical formulation of the present disclosure may be prepared by methods known to those skilled in the art, such as mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. As mentioned above, the pharmaceutical compositions of the present disclosure may include one or more pharmaceutically acceptable carriers such as excipients and adjuvants that facilitate processing of active molecules into preparations for pharmaceutical use.

[0144] In some embodiments, the pharmaceutical compositions of the present disclosure are prepared in an oral formulation. For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers known in the art. Such carriers enable the pharmaceutically active ingredient to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject. Pharmaceutical compositions for oral use may be obtained as solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable adjuvants, if desired, to obtain tablets or dragee cores. Such oral pharmaceutical compositions may also be prepared by milling or melt extrusion. Suitable excipients may be any of those disclosed herein and, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose formulation such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP) formulation. Also, disintegrating agents may be employed, such as cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Wetting agents, such as sodium dodecyl sulfate and the like, may be added.

[0145] In some embodiments of the present disclosure, the pharmaceutical composition is prepared for administration by injection (e.g., intravenous, subcutaneous, intramuscular, etc.). In some such embodiments, a pharmaceutical composition comprises a carrier and is formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. In some embodiments, other ingredients are included (e.g., ingredients that aid in solubility or serve as preservatives). In some embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Certain

pharmaceutical compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents and fatty oils, such as vegetable oils (*e.g.*, sesame oil and the like), synthetic fatty acid esters, such as ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, such suspensions may also contain suitable stabilizers or agents that increase the solubility of the pharmaceutical agents to allow for the preparation of highly concentrated solutions.

[0146] The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables. Formulations for intravenous administration can comprise solutions in sterile isotonic aqueous buffer. Where necessary, the formulations can also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachet indicating the quantity of active agent. Where the compound is to be administered by infusion, it can be dispensed in a formulation with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the compound is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

[0147] Suitable formulations further include aqueous and non-aqueous sterile injection solutions that can contain antioxidants, buffers, bacteriostats, bactericidal antibiotics and solutes that render the formulation isotonic with the bodily fluids of the intended recipient; and aqueous and non-aqueous sterile suspensions, which can include suspending agents and thickening agents. Liposomal suspensions containing liposomes that target tissues may be present in suitable formulations.

[0148] Parenteral administration of the formulations of the present invention includes intravenous, subcutaneous and intramuscular administrations of the pharmaceutical compositions described herein. Preparations for parenteral administration includes sterile solutions ready for injection, sterile dry soluble products ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions can be either aqueous or non-aqueous. Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, non-aqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, thickening agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Liposome suspensions are also suitable as pharmaceutically acceptable carriers.

[0149] In some embodiments, of the present disclosure, the pharmaceutical composition is formulated as a depot preparation. Such depot preparations are typically longer acting than non-depot preparations. In some embodiments, such preparations are administered parenterally (e.g., by injection or implantation). For example, subcutaneous or intramuscular depot injections of a sterile solution containing compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, is an effective mode of administration. In some embodiments, depot preparations are prepared using suitable polymeric or hydrophobic materials (for example an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Thus, for example, the pharmaceutical composition may be formulated with suitable polymeric or hydrophobic materials (e.g., an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, e.g., a sparingly soluble salt. In some embodiments, the depot preparation provides a 1-day, 7-day or 30-day depot release profile. In some embodiments such preparations are administered on a once-a-day, once-a-week or once-a-month injection schedule.

[0150] The concentration of the pharmaceutically active compound can be adjusted so that an injection provides an effective amount to produce the desired pharmacological effect. The exact dose depends on the age, weight and condition of the patient or animal, as is known in the art. In some embodiments, the unit-dose parenteral preparations are packaged in an ampoule or a syringe with a needle. All preparations for parenteral administration must be sterile, as is known and practiced in the art. Illustratively, intravenous or intra-arterial infusion of a sterile

aqueous solution containing a compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, is an effective mode of administration.

[0151] In some embodiments, suitable pharmaceutically acceptable carriers can also include, but are not limited to diluents and sterile aqueous or organic solutions. Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, aqueous and non-aqueous solutions. Pharmaceutically acceptable carriers can be aqueous or non-aqueous solutions, suspensions and emulsions. Examples of non-aqueous solvents suitable for use in the present application include, but are not limited to, propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers suitable for use in the present application include, but are not limited to, water, ethanol, alcoholic/aqueous solutions, glycerol, emulsions or suspensions, including saline and buffered media.

[0152] Diluents may be added to the formulations described herein. Diluents can increase the bulk of a solid pharmaceutical composition and/or combination, and may make a pharmaceutical dosage form containing the composition and/or combination easier for the patient and care giver to handle. In various embodiments, diluents for solid compositions include, for example, microcrystalline cellulose (e.g., AVICEL), microfine cellulose, lactose, starch, pregelatinized starch, calcium carbonate, calcium sulfate, sugar, dextrates, dextrin, dextrose, dibasic calcium phosphate dihydrate, tribasic calcium phosphate, kaolin, magnesium carbonate, magnesium oxide, maltodextrin, mannitol, polymethacrylates (e.g., EUDRAGIT[®]), potassium chloride, powdered cellulose, sodium chloride, sorbitol, and talc, and/or mixtures of any of the foregoing. Specific examples of: microcrystalline cellulose include those sold under the Trademark Avicel (FMC Corp., Philadelphia, Pa.), for example, Avicel[™] pH101, Avicel[™] pH102 and Avicel[™] pH112; lactose includes lactose monohydrate, lactose anhydrous and Pharmatose DCL21; dibasic calcium phosphate includes Emcompress.

[0153] Liquid carriers suitable for use in the present application include, but are not limited to, water (partially containing additives, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil).

[0154] Liquid carriers suitable for use in the present application can be used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compounds. In some embodiments, the active ingredient is dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. In some embodiments, the liquid carrier contains other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators.

[0155] Solid carriers suitable for use in the present application include, but are not limited to, inactive substances such as lactose, starch, glucose, methylcellulose, magnesium stearate, dicalcium phosphate, mannitol and the like. A solid carrier can further include one or more substances acting as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier can be a finely divided solid that is in admixture with the finely divided active compound. In tablets, the active compound is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets may contain up to 99% of the active compound. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidone, low melting waxes and ion exchange resins. A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free flowing form such as a powder or granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide delayed or controlled release of the active ingredient therein using, for example, hydroxypropyl methylcellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

[0156] Carriers suitable for use in the present application can be mixed as needed with disintegrants, diluents, granulating agents, lubricants, binders and the like using conventional

techniques known in the art. The carriers can also be sterilized using methods that do not deleteriously react with the compounds, as is generally known in the art.

[0157] The pharmaceutical formulation of the present disclosure may be prepared by methods known to those skilled in the art, such as mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. As mentioned above, the pharmaceutical compositions of the present disclosure may include one or more pharmaceutically acceptable carriers such as excipients and adjuvants that facilitate processing of active molecules into preparations for pharmaceutical use.

[0158] The dissolution rate of a compacted solid pharmaceutical composition in the patient's stomach may be increased by the addition of a disintegrant to the composition and/or combination. Disintegrants include alginic acid, carboxymethylcellulose calcium, carboxymethylcellulose sodium (e.g., AC-DI-SOL and PRIMELLOSE), colloidal silicon dioxide, croscarmellose sodium, crospovidone (e.g., KOLLIDON and POLYPLASDONE), guar gum, magnesium aluminum silicate, methyl cellulose, microcrystalline cellulose, polacrilin potassium, powdered cellulose, pregelatinized starch, sodium alginate, sodium starch glycolate (e.g., EXPLOTAB), potato starch, and starch.

[0159] Flavoring agents and flavor enhancers make the dosage form more palatable to the patient. Common flavoring agents and flavor enhancers for pharmaceutical products that may be included in the composition of the present invention include maltol, vanillin, ethyl vanillin, menthol, citric acid, fumaric acid, ethyl maltol, and tartaric acid.

[0160] Solid and liquid compositions and/or combinations may also be dyed using any pharmaceutically acceptable colorant to improve their appearance and/or facilitate patient identification of the product and unit dosage level.

[0161] In liquid pharmaceutical compositions and/or combinations may be prepared using the excipients where the components are dissolved or suspended in a liquid carrier such as water, vegetable oil, alcohol, polyethylene glycol, propylene glycol, or glycerin.

[0162] Liquid pharmaceutical compositions and/or combinations may contain emulsifying agents to disperse uniformly throughout the composition an active ingredient or other excipient that is not soluble in the liquid carrier. Emulsifying agents that may be useful in liquid compositions and/or combinations of the present invention include, for example, gelatin, egg yolk, casein, cholesterol, acacia, tragacanth, chondrus, pectin, methyl cellulose, carbomer, cetostearyl alcohol, and cetyl alcohol.

[0163] Liquid pharmaceutical compositions and/or combinations may also contain a viscosity enhancing agent to improve the mouth-feel of the product and/or coat the lining of the gastrointestinal tract. Such agents include acacia, alginic acid bentonite, carbomer, carboxymethylcellulose calcium or sodium, cetostearyl alcohol, methyl cellulose, ethylcellulose, gelatin guar gum, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, maltodextrin, polyvinyl alcohol, povidone, propylene carbonate, propylene glycol alginate, sodium alginate, sodium starch glycolate, starch tragacanth, and xanthan gum.

[0164] Sweetening agents such as aspartame, lactose, sorbitol, saccharin, sodium saccharin, sucrose, aspartame, fructose, mannitol, and invert sugar may be added to improve the taste.

[0165] Preservatives and chelating agents such as alcohol, sodium benzoate, butylated hydroxyl toluene, butylated hydroxyanisole, and ethylenediamine tetraacetic acid may be added at levels safe for ingestion to improve storage stability.

[0166] A liquid composition may also contain a buffer such as gluconic acid, lactic acid, citric acid or acetic acid, sodium gluconate, sodium lactate, sodium citrate, or sodium acetate. Selection of excipients and the amounts used may be readily determined by the formulation scientist based upon experience and consideration of standard procedures and reference works in the field.

[0167] The solid compositions of the present invention may include powders, granulates, aggregates and compacted compositions and/or combinations. The dosages include dosages suitable for oral, buccal, rectal, parenteral (including subcutaneous, intramuscular, and intravenous), inhalant and ophthalmic administration. Although the most suitable administration in any given case will depend on the nature and severity of the condition being treated, the most preferred route of the present invention is oral. The dosages may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the pharmaceutical arts.

[0168] Dosage forms include solid dosage forms like tablets, powders, capsules, suppositories, sachets, troches and lozenges, as well as liquid syrups, suspensions, aerosols and elixirs.

[0169] The dosage form of the present invention may be a capsule containing the composition and/or combination, preferably a powdered or granulated solid composition of the invention, within either a hard or soft shell. The shell may be made from gelatin and optionally contain a plasticizer such as glycerin and sorbitol, and an opacifying agent or colorant.

[0170] A composition for tableting or capsule filling may be prepared by wet granulation. In wet granulation, some or all of the active ingredients and excipients in powder form are blended and then further mixed in the presence of a liquid, typically water that causes the powders to clump into granules. The granulate is dried and then screened and/or milled to the desired particle size. The granulate may be tableted, or other excipients may be added prior to tableting, such as a glidant and/or a lubricant.

[0171] A tableting composition may be prepared conventionally by dry granulation. For example, the blended composition of the actives and excipients may be compacted into a slug or a sheet and then comminuted into compacted granules. The compacted granules may subsequently be compressed into a tablet.

[0172] A tableting composition may be prepared conventionally by melt granulation. For example, the blended composition of the actives and excipients may be granulated using a meltable binder into granules. The granules may subsequently be compressed into a tablet.

[0173] In some embodiments, the pharmaceutical composition, such as a compressed tablet composition comprises drug particles or drug-containing granules.

[0174] As an alternative to dry granulation, a blended composition may be compressed directly into a compacted dosage form using direct compression techniques. Direct compression produces a more uniform tablet without granules. Excipients that are particularly well suited for direct compression tableting include microcrystalline cellulose, spray dried lactose, dicalcium phosphate dihydrate and colloidal silica. The proper use of these and other excipients in direct compression tableting is known to those in the art with experience and skill in particular formulation challenges of direct compression tableting.

[0175] In some embodiments, the drug-containing granules or particles comprise a binder, a bulking agent, a disintegrant, and/or a lubricant. In some embodiments, the drug-containing granules are processed by roller compaction, e.g., by the manufacturing parameters disclosed herein. In another specific embodiment, the drug-containing granules or particles may then be additionally compressed after compaction. In a specific embodiment, the drug-containing granules or particles may be compressed into a tablet. In a specific embodiment, the tablet comprises Compound I, or a pharmaceutically acceptable salt, ester, or solvate thereof.

[0176] In some embodiments, the present disclosure provides compressed pharmaceutical tablets prepared by:

(a) blending a mixture of Compound I or a pharmaceutically acceptable salt thereof with input material, such as a first portion of binder, bulking agent, disintegrant, and/or lubricant;

(b) compacting or blending the blended mixture of step (a) using a roller compactor, thereby forming a compacted material;

(c) blending the roller compacted material, i.e., drug-containing granules or particles of step (b) with a second portion of blending material such as disintegrant, lubricant, and/or binder to provide a final blend;

(e) compressing the final blend of step (c) into a compressed tablet; and

(f) optionally coating the compressed tablet with a coating described herein.

[0177] In another specific embodiment, the compressed pharmaceutical tablets are prepared as in Fig. 1.

[0178] In another embodiment, roller compaction processing may vary to affect the particle size or particle size distribution of the drug-containing granules or particles before compression. In a specific embodiment, the particle size distribution of the drug-containing granules or particles may vary in view of the desired composition, i.e., for immediate release. In a specific embodiment, the particle size distribution is determined by sieve analysis.

[0179] In one embodiment, about 2% to about 10% of the drug particles are retained on the sieve at Sieve 20 (841 μm). In another embodiment, about 3% to about 8% of the drug particles are retained on the sieve at Sieve 20 (841 μm). In another embodiment, about 3% to about 6% of the drug particles are retained on the sieve at Sieve 20 (841 μm).

[0180] In one embodiment, an additional about 10% to about 35% of the drug particles are retained on the sieve at Sieve 30 (595 μm). In one embodiment, an additional about 20% to about 30% of the drug particles are retained on the sieve at Sieve 30 (595 μm). In one embodiment, an additional about 25% to about 30% of the drug particles are retained on the sieve at Sieve 30 (595 μm).

[0181] In one embodiment, an additional about 10% to about 20% of the drug particles are retained on the sieve at Sieve 40 (420 μm). In one embodiment, an additional about 12% to about 18% of the drug particles are retained on the sieve at Sieve 40 (420 μm). In one embodiment, an additional about 13% to about 16% of the drug particles are retained on the sieve at Sieve 40 (420 μm).

[0182] In one embodiment, an additional about 0.2% to about 10% of the drug particles are retained on the sieve at Sieve 60 (250 μm). In one embodiment, an additional about 1.0% to about 7% of the drug particles are retained on the sieve at Sieve 60 (250 μm). In one embodiment, an additional about 3.0% to about 6.0% of the drug particles are retained on the sieve at Sieve 60 (250 μm).

[0183] In one embodiment, an additional about 12% to about 25% of the drug particles are retained on the sieve at Sieve 100 (140 μm). In one embodiment, an additional about 15% to about 23% of the drug particles are retained on the sieve at Sieve 100 (140 μm). In one embodiment, an additional about 15% to about 20% of the drug particles are retained on the sieve at Sieve 100 (140 μm).

[0184] In one embodiment, an additional about 10% to about 30% of the drug particles are retained on the sieve at Sieve 230 (63 μm). In one embodiment, an additional about 12% to about 25% of the drug particles are retained on the sieve at Sieve 230 (63 μm). In one embodiment, an additional about 15% to about 20% of the drug particles are retained on the sieve at Sieve 230 (63 μm).

[0185] In another embodiment, D85 value of the drug particles is about 50 μm to about 100 μm . In another embodiment, the D85 value of the drug particles is about 60 μm to about 80 μm . In another embodiment, the D85 value of the drug particles is about 63 μm .

[0186] In another embodiment, the D85 value of the drug particles is greater than about 50 μm , about 60 μm , about 70 μm , about 80 μm , about 90 μm , or about 100 μm .

[0187] In another embodiment, the D65 value of the drug particles is about 100 μm to about 200 μm . In another embodiment, the D65 value of the drug particles is about 120 μm to about 160 μm . In another embodiment, the D65 value of the drug particles is about 140 μm . In another embodiment, the D65 value of the drug particles is greater than about 120 μm , about 130 μm , about 140 μm , about 150 μm , about 160 μm , or about 180 μm .

[0188] In another embodiment, the D45 value of the drug particles is about 300 μm to about 500 μm . In another embodiment, the D45 value of the drug particles is about 350 μm to about 450 μm . In another embodiment, the D45 value of the drug particles is about 420 μm . In another embodiment, the D55 value of the drug particles is greater than about 340 μm , about 360 μm , about 380 μm , about 420 μm , about 440 μm , or about 480 μm . In another embodiment, the drug particles have the characters substantially similar as demonstrated in Table 1. In another

embodiment, the drug particles as described above are before roller compaction. In another embodiment, the drug particles as described above are after roller compaction.

[0189] A capsule filling of the present invention may comprise any of the aforementioned blends and granulates that were described with reference to tableting; however, they are not subjected to a final tableting step.

[0190] The active ingredient and excipients may be formulated into compositions and/or combinations and dosage forms according to methods known in the art.

[0191] In some embodiments, the pharmaceutical compositions of the present disclosure are comprised of the compound of formula (FCB)a-(L)b-(CLM)c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug are stable compositions. In some embodiments of the pharmaceutical topical formulations disclosed herein, the composition, after 40°C/75%RH for 4 weeks, has a total impurities less than 20 % by HPLC. In some embodiments, the composition of the present disclosure, after 40°C/75%RH for 4 weeks, has a total impurities less than 19% by HPLC. In some embodiments, the composition of the present disclosure, after 40°C/75%RH for 4 weeks, has a total impurities less than 18% by HPLC. In some embodiments, the composition of the present disclosure, after 40°C/75%RH for 4 weeks, has a total impurities less than 17% by HPLC. In some embodiments, the composition of the present disclosure, after 40°C/75%RH for 4 weeks, has a total impurities less than 16% by HPLC. In some embodiments, the composition of the present disclosure, after 40°C/75%RH for 4 weeks, has a total impurities less than 15% by HPLC. In some embodiments, the composition of the present disclosure, after 40°C/75%RH for 4 weeks, has a total impurities less than 14% by HPLC. In some embodiments, the composition of the present disclosure, after 40°C/75%RH for 4 weeks, has a total impurities less than 13% by HPLC. In some embodiments, the composition of the present disclosure, after 40°C/75%RH for 4 weeks, has a total impurities less than 12% by HPLC.

Therapeutic Use

[0192] The compounds and the pharmaceutical compositions of the present disclosure find use in any number of methods. For example, in some embodiments the pharmaceutical compositions are useful in methods for modulating a phosphoinositide 3-kinase (PI3K). In embodiments, modulating phosphoinositide 3-kinase (PI3K) activity is in a mammalian cell. In embodiments, modulating phosphoinositide 3-kinase (PI3K) can be in a subject in need thereof (e.g., a mammalian subject) and for treatment of a condition or disease described herein,

including diseases or conditions wherein irreversible inhibition of PI3K provides therapeutic benefit to a subject having the disease or condition.

[0193] In some embodiments, the disclosure provides a method for regulating the activity of a kinase or pseudokinase, comprising administering to a subject in need thereof any pharmaceutical composition disclosed herein. In some embodiments, the method inhibits the activity of a kinase or pseudokinase.

[0194] In some embodiments, the disclosure provides a method for regulating the activity of PI3K comprising administering to a subject in need thereof any pharmaceutical composition disclosed herein.

[0195] In some embodiments, the disclosure provides a method for treating cancer, a neurodegenerative disease, an autoimmune disorder or aging in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition disclosed herein. In some embodiments, the disclosure provides a method for treating cancer, comprising administering to the subject in need thereof the pharmaceutical composition disclosed herein. In some embodiments, the cancer is selected from one or more of the group consisting of leukemia, tumors of the brain, lung (small cell and non-small cell), ovary, prostate, breast, colon, and cancer in a tissue, including intestinal tissue, pancreatic tissue, liver, kidney, prostate, ovary, lung, bone marrow, or breast tissue. In some embodiments, the subject has cancer with a mutation in the PIK3CA gene.

[0196] In embodiments of the present disclosure, a method of reducing, inhibiting, or ameliorating cell proliferation in a patient in need thereof is provided. In embodiments, the reducing, inhibiting, or ameliorating in the method disclosed herein, is in vivo. In another embodiment, the reducing, inhibiting, or ameliorating is in vitro. In embodiments, the cells in the method disclosed herein, are a cancer cells. In embodiments of the present disclosure, a method for reducing or preventing tumor growth, comprising contacting tumor cells with a compound or pharmaceutical composition as disclosed herein. In one embodiment, reducing or preventing tumor growth includes reduction in tumor volume. In one embodiment, reducing or preventing tumor growth includes complete elimination of tumors. In one embodiment, reducing or preventing tumor growth includes stopping or halting the existing tumor to grow. In one embodiment, reducing or preventing tumor growth includes reduction in the rate of tumor growth.

[0197] The present invention also provides treatment of disorders related to proliferation of cells. In one embodiment, the method comprises contacting cancer and/or tumor cells with the pharmaceutical composition comprising compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof and, as disclosed herein.

[0198] In some embodiments, the compound is that forms a covalent bond with a kinase or pseudokinase is Compound I. In some embodiments, the compound of formula (FCB)_a-(L)_b-(CLM)_c is compound I. In some embodiments, Compound I is a crystalline form of Compound I or a pharmaceutically acceptable salt or solvate of Compound I. In some embodiments, the pharmaceutically acceptable salt of Compound I is selected from the group consisting of a phosphate salt, a hydrochloride salt, a sulfate salt, a mesylate salt, a benzenesulfonate salt, a tosylate salt, a fumarate salt, a maleate salt, a L-tartrate salt, a citrate salt, and a succinate salt, or a solvate thereof.

[0199] In another embodiment of treating or ameliorating a cell proliferation disorder in a subject, the cell proliferation disorder is cancer. In a specific embodiment, the cancer is selected from the group consisting of: heme cancer, colorectal cancer, ovarian cancer, breast cancer, cervical cancer, lung cancer, liver cancer, colon cancer, pancreatic cancer, cancer of the lymph nodes, colon cancer, small intestine, prostate cancer, brain cancer, cholangiocarcinoma, gallbladder carcinoma, cancer of the head and neck, bone cancer, Ewing's sarcoma, skin cancer, kidney cancer, and cancer of the heart. In another embodiment, the cancer is selected from the group consisting of wherein the cancer is selected from the group consisting of breast cancer, ovarian cancer, pancreatic cancer, colorectal cancer, colon cancer, small intestine and lung cancer. In another embodiment of the present invention, the subject is human.

[0200] In one embodiment, the present invention provides a method of reducing or inhibiting cell proliferation, and/or a method of treating cancer comprising co-administering to a patient in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof. The present invention also provides methods of treating, preventing, ameliorating and/or alleviating the progression of disorders or conditions characterized by cell proliferation in a subject. More particularly, the methods of the present invention involve administration of an effective amount of the quinolone compounds described herein, in a subject to treat a disorder or a condition characterized by cell proliferation.

[0201] As used herein, administering can be effected or performed using any of the various methods known to those skilled in the art. The pharmaceutical composition comprising the compound of formula (FCB)a-(L)b-(CLM)c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof and can be administered, for example, subcutaneously, intravenously, parenterally, intraperitoneally, intradermally, intramuscularly, topically, enteral (e.g., orally), rectally, nasally, buccally, sublingually, vaginally, by inhalation spray, by drug pump or via an implanted reservoir in dosage formulations containing conventional non-toxic, physiologically acceptable carriers or vehicles.

[0202] Further, the pharmaceutical composition comprising the compound of formula (FCB)a-(L)b-(CLM)c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof can be administered to a localized area in need of treatment. This can be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, transdermal patches, by injection, by catheter, by suppository, or by implant (the implant can optionally be of a porous, non-porous, or gelatinous material), including membranes, such as sialastic membranes or fibers.

[0203] The form in which the compound of formula (FCB)a-(L)b-(CLM)c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof, is administered (e.g., syrup, elixir, capsule, tablet, foams, emulsion, gel, etc.) will depend in part on the route by which it is administered. For example, for mucosal (e.g., oral mucosa, rectal, intestinal mucosa, bronchial mucosa) administration, nose drops, aerosols, inhalants, nebulizers, eye drops or suppositories can be used. The compound of formula (FCB)a-(L)b-(CLM)c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof disclosed herein can be administered together with other biologically active agents, such as anticancer agents, analgesics, anti-inflammatory agents, anesthetics and other agents which can control one or more symptoms or causes of a disorder or a condition characterized by cell proliferation.

[0204] In one embodiment, the compound of formula (FCB)a-(L)b-(CLM)c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof, can be administered together with a second therapeutically active agent or more.

[0205] Additionally, administration can comprise administering to the subject a plurality of dosages over a suitable period of time. Such administration regimens can be determined according to routine methods, upon a review of the instant disclosure.

[0206] In settings of a gradually progressive disorder or condition characterized by cell proliferation, the compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof, are generally administered on an ongoing basis. In certain settings administration of a compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof, disclosed herein can commence prior to the development of disease symptoms as part of a strategy to delay or prevent the disease. In other settings the compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof, disclosed herein is administered after the onset of disease symptoms as part of a strategy to slow or reverse the disease process and/or part of a strategy to improve cellular function and reduce symptoms.

[0207] It will be appreciated by one of skill in the art that dosage range will depend on the particular compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof and its potency. The dosage range is understood to be large enough to produce the desired effect in which the neurodegenerative or other disorder and the symptoms associated therewith are ameliorated and/or survival of the cells is achieved, but not be so large as to cause unmanageable adverse side effects. It will be understood, however, that the specific dose level for any particular patient will depend on a variety of factors including the age, body weight, general health, sex and diet of the individual being treated; the time and route of administration; the rate of excretion; other drugs which have previously been administered; and the severity of the particular disease undergoing therapy, as is well understood by those skilled in the art. The dosage can also be adjusted by the individual physician in the event of any complication. No unacceptable toxicological effects are expected when Compound I disclosed herein are used in accordance with the present application.

[0208] An effective amount the compound of formula (FCB)_a-(L)_b-(CLM)_c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof, disclosed herein comprise amounts sufficient to produce a measurable biological response. Actual dosage levels of active ingredients of the present application can be varied so

as to administer an amount of the compound of formula (FCB)a-(L)b-(CLM)c, and/or their subgenera, or Compound I, or a pharmaceutically acceptable salt, ester, solvate and/or prodrug thereof that is effective to achieve the desired therapeutic response for a particular subject and/or application. Preferably, a minimal dose is administered, and the dose is escalated in the absence of dose-limiting toxicity to a minimally effective amount. Determination and adjustment of a therapeutically effective dose, as well as evaluation of when and how to make such adjustments, are known to those of ordinary skill in the art.

[0209] Throughout this disclosure, various patents, patent applications and publications are referenced. The disclosures of these patents, patent applications and publications in their entireties are incorporated into this disclosure by reference for all purposes in order to more fully describe the state of the art as known to those skilled therein as of the date of this disclosure. This disclosure will govern in the instance that there is any inconsistency between the patents, patent applications and publications cited and this disclosure. The following examples further illustrate the present invention but should not be construed as in any way limiting its scope.

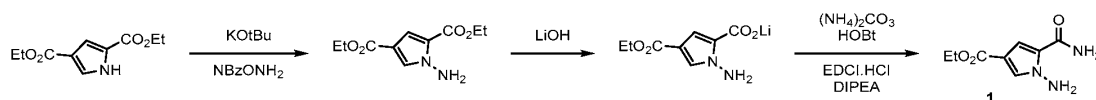
EXAMPLES

[0210] The disclosure now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention and are not intended to limit the invention.

[0211] The examples below are intended to be exemplary and efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, etc.), but some experimental errors and deviations should be accounted for within the knowledge of a person skilled in the art. Unless indicated otherwise, temperature is in degrees Centigrade. Reagents were purchased from commercial suppliers such as Sigma-Aldrich, Alfa Aesar, or TCI, and were used without further purification unless otherwise indicated.

Example 1: Synthesis and Characterization of methyl (5-(6-((4-(acryloylglycyl)piperazin-1-yl)methyl)-4-morpholinopyrrolo[2,1-f][1,2,4]triazin-2-yl)-4-(trifluoromethyl)pyridin-2-yl)carbamate (Compound I)

[0212] Synthesis of Ethyl 1-amino-5-carbamoyl-1H-pyrrole-3-carboxylate (1).

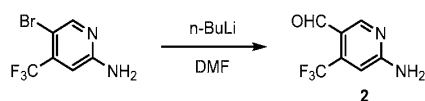


[0213] Diethyl 1-amino-1H-pyrrole-2,4-dicarboxylate. A 20 L reaction vessel was loaded with diethyl 1H-pyrrole-2,4-dicarboxylate (1002 g, 1 equiv, 4.507 mol) and NMP (7.8 L). Then potassium *tert*-butoxide (557.3 g, 1.1 equiv, 4.967 mol) was added, the mixture became pink, and the temperature reached 37 °C. The mixture was stirred until all potassium *tert*-butoxide had dissolved and it was allowed to cool down to 21 °C. Subsequently, O-(4-nitrobenzoyl) hydroxylamine (839.6 g, 1.02 equiv, 4.610 mol) was added portion-wise (exothermic, reached 36 °C). Reaction mixture turns into a dark purple suspension. The reaction mixture was stirred at 45 °C overnight (T slowly drops, mixture turns orange). A sample was taken after 18h and diluted with ACN/water for HPLC analysis. Then, a solution of sodium dithionite (478.6 g, 0.6 equiv, 2.749 mol) in water (2.5 L) was slowly added keeping the temperature below 30 °. The reaction mixture was transferred into a 50 L separation funnel. Toluene (15 L) and water (5 L) were added, and the phases were separated. The aqueous phase was extracted with toluene (3 x 1 L). The combined org phases were washed with water (5 x 1 L), sat. sodium bicarbonate (5 x 1 L) and brine (1 L), dried over sodium sulfate, filtered, and concentrated to give diethyl 1-amino-1H-pyrrole-2,4-dicarboxylate (1077 g, 3.90 mol, 86%) as an orange oil (81% QNMR purity), which solidified upon standing. ¹H NMR (299 MHz, DMSO-d₆) δ 7.52 (d, *J* = 2.1 Hz, 1H), 7.03 (d, *J* = 2.1 Hz, 1H), 6.51 (d, *J* = 1.8 Hz, 2H), 4.34 – 4.06 (m, 4H), 1.38 – 1.14 (m, 6H). LCMS (ESI): found 227.0 [M+H]⁺ (calculated 227.1 [M+H]⁺).

[0214] Lithium 1-amino-4-(ethoxycarbonyl)-1H-pyrrole-2-carboxylate. A 10 L reaction vessel was charged with diethyl 1-amino-1H-pyrrole-2,4-dicarboxylate (1077 g, 1 equiv, 3.90 mol), ethanol (3.8 L) and water (1.9 L). Lithium hydroxide monohydrate (163.9 g, 56 wt%, 0.99 equiv, 3.831 mol) was added and the mixture was stirred at 60 °C. The conversion was monitored by LCMS analysis. The reaction was stopped after 6 hours and cooled down to RT. The mixture was diluted with 2 L toluene and layers were separated. The aqueous phase was washed with toluene (3 x 750 ml) and concentrated at the rotary evaporator at 60 °C. The resulting solids was suspended in TBME (2 L), filtered and washed with TBME (1 L). Lithium 1-amino-4-(ethoxycarbonyl)-1H-pyrrole-2-carboxylate (853.1 g, 3.50 mol, 90%) was obtained as a pale-yellow solid (83% QNMR purity). ¹H NMR (400 MHz, DMSO-D₆) δ 7.37 (s, 2H), 7.09 (d, *J* = 2.2 Hz, 1H), 6.63 (d, *J* = 2.2 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 3H). LCMS (ESI): found 199.0 [M-Li+2H]⁺ (calculated 199.1 [M-Li+2H]⁺).

[0215] Ethyl 1-amino-5-carbamoyl-1H-pyrrole-3-carboxylate (1). To a suspension of lithium 1-amino-4-(ethoxycarbonyl)-1H-pyrrole-2-carboxylate (835.8 g, 1 equiv, 3.399 mmol) in a mixture of DMF (3.4 L) / MeTHF (8.5 mL) was added ammonium carbonate (2939 g, 9.00 equiv, 30.59 mol) followed by HOBt (1041 g, 2.0 equiv, 6.797 mol), EDCI HCl (1303 g, 2.0 equiv, 6.797 mol) and DIPEA (2.96 L, 5 equiv, 16.99 mol). Adding the reagents causes an endotherm. The flask was stirred at room temperature (suspension) for 3 days. The conversion was monitored by LCMS analysis. The solids were filtered off and washed with MeTHF (2 L) and the filtrate was concentrated at 60 °C. The crude material (2 kg) was redissolved in MeTHF (10 L) and washed with a saturated solution of sodium bicarbonate (2 x 2L). Three phases were formed. The top phase (product fraction) was separated, dried over sodium sulfate, filtered and concentrated to dryness. The resulting crude material (908 g) was recrystallized from EtOH (1.8 L). The solids were filtered, washed with EtOH (200 mL) and dried to give the first crop of ethyl 1-amino-5-carbamoyl-1H-pyrrole-3-carboxylate (67 g) as a white solid. The mother liquor was concentrated (794 g) and purified by column chromatography (2 kg silica, gradient: DCM to 5% MeOH). The purified material (505 g) was recrystallized from EtOH (750 mL) and washed with EtOH (100 mL) to give a second crop of ethyl 1-amino-5-carbamoyl-1H-pyrrole-3-carboxylate (68 g). The concentrated mother liquor (435 g) was purified by column chromatography once more (6 kg silica, gradient: DCM to 5% MeOH). Recrystallizing the product fractions resulted into an additional crop 61 g of ethyl 1-amino-5-carbamoyl-1H-pyrrole-3-carboxylate. All crops merged gave ethyl 1-amino-5-carbamoyl-1H-pyrrole-3-carboxylate (**1**) (195.7 g, 992.4 mmol, 29%) as a white solid. ¹H NMR (299 MHz, DMSO-d₆) δ 7.96 (s, 1H), 7.36 (d, *J* = 2.0 Hz, 1H), 7.31 (s, 1H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.86 (d, *J* = 1.8 Hz, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H). LCMS (ESI): found 198.0 [M+H]⁺ (calculated 198.1 [M+H]⁺).

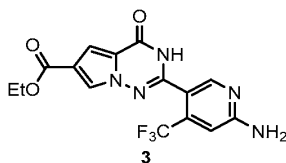
[0216] Synthesis of 6-Amino-4-(trifluoromethyl) nicotinaldehyde



[0217] 6-Amino-4-(trifluoromethyl) nicotinaldehyde (2). A 5L three-necked flask, equipped with a thermometer and under nitrogen, was charged with 5-bromo-4-(trifluoromethyl)-2-pyridylamine (200.0 g, 1 equiv, 829.8 mmol) and dry THF (2000 mL). The solution was cooled down to -70 °C. A 2.5 M solution of n-butyllithium in hexane (995.8 mL, 3.00 equiv, 2.49 mol) was added dropwise over 90 min, keeping the temperature below -60 °C. The mixture was

stirred at -70 °C for 15 min. Then, DMF (160.6 mL, 2.50 equiv, 2.074 mol) was added dropwise over 45 min, keeping the temperature below -60 °C. The mixture was stirred at -70 °C for 30 min. The mixture was warmed-up to -40 °C and carefully quenched with water (74.8 mL, 5 equiv, 4.149 mol). The resulting solution was left to warm up to room temperature and stirred overnight. The mixture was further diluted with water/ethyl acetate (1 L/1 L) and transferred to a separating funnel. The organic layer was washed with water (5 x 300 mL). The combined aqueous phases were extracted with ethyl acetate (3 x 300 mL). The combined organic phases were washed with brine (300 mL), dried over sodium sulfate, filtered and concentrated until a small volume of solvent is left and a solid is formed. The mixture was cooled down to rt and diluted with heptane (300 mL). The mixture was filtered, the solid washed with heptane and dried to give 6-amino-4-(trifluoromethyl) nicotinaldehyde (**2**) (51.5 g, 271 mmol, 33%) as an orange solid. ¹H NMR (299 MHz, DMSO-d₆) δ 9.80 (q, *J* = 1.7 Hz, 1H), 8.63 (d, *J* = 2.1 Hz, 1H), 7.73 (s, 2H), 6.84 (d, *J* = 2.0 Hz, 1H). LCMS (ESI): found 191.0 [M+H]⁺ (calculated 191.0 [M+H]⁺).

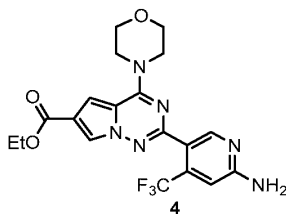
[0218] Step 1: Synthesis of Ethyl 2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-oxo-3,4-dihydropyrrolo[2,1-f] [1,2,4] triazine-6-carboxylate (3**).**



[0219] A 3L 3-neck flask was equipped with a condenser, temperature probe and a mechanical stirrer and charged with a solution of ethyl 1-amino-5-carbamoyl-1H-pyrrole-3-carboxylate (**1**) (195.7 g, 1.00 equiv, 962.7 mmol) and 6-amino-4-(trifluoromethyl) nicotinaldehyde (**2**) (192.7 g, 1.00 equiv, 962.7 mmol) in DMSO (1.9 L). Then cupric chloride dihydrate (213.4 g, 1.30 equiv, 633.4 mmol) was added and the mixture was stirred at 100 °C for 18 hours. The mixture was cooled down to room temperature and poured onto ice water (10 L) causing the precipitation of the product. The suspension was stirred for 30 min before filtering (Buchner filter). The pale brown filter cake was washed with water (3 x 1 L) and TBME (3 x 1 L). The solids were stripped with toluene (3 x 1 L) on a rotary evaporator to remove residues of water. Ethyl 2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-oxo-3,4-dihydropyrrolo[2,1-f] [1,2,4] triazine-6-carboxylate (**3**) (340.6 g, 760.0 mmol, 79%) was obtained as a solid. ¹H NMR (299 MHz, DMSO-d₆) δ 12.20 (s, 1H), 8.08 (d, *J* = 1.8 Hz, 1H), 7.23 (d, *J* = 1.8 Hz, 1H), 7.05 (s,

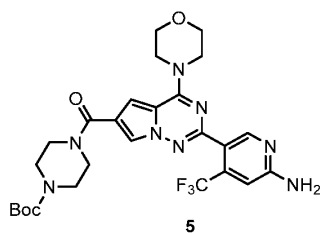
1H), 6.87 (s, 1H), 4.26 (q, $J = 6.9$ Hz, 2H), 1.29 (t, $J = 7.1$, 3H). LCMS (ESI): found 368.0 $[M+H]^+$ (calculated 368.1 $[M+H]^+$); 366.0 $[M-H]^-$ (calculated 366.1 $[M-H]^-$).

[0220] Step 2: Synthesis of Ethyl 2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo[2,1-f] [1,2,4] triazine-6-carboxylate (4).



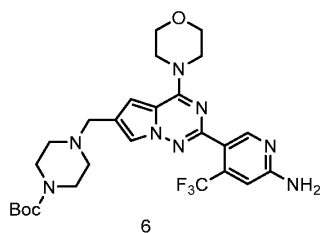
[0221] A 5L three-necked flask, under nitrogen and equipped with a thermometer, was charged with ethyl 2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-oxo-3,4-dihydropyrrolo[2,1-f] [1,2,4] triazine-6-carboxylate (**3**) (335.6 g, 1 equiv, 749.2 mmol), DMF (2.5 L) and morpholine (400 mL, 6.2 equiv, 2.580 mol). Then PyBOP (606.9 g, 1.55 equiv, 1.166 mol) was added in several portions keeping the temperature around 15 °C (exothermic). The mixture was stirred at room temperature for 48 hours. The reaction mixture was transferred into a 20 L vessel and water (12.5 L) was slowly added. The suspension was filtered over a Buchner filter, washed with water (3 x 5 L) and TBME (2 x 5 L). The remaining clay like substance was re-dissolved in ethyl acetate at reflux. The hot suspension was filtered over a glass filter with Celite to remove the copper and the filtrate was concentrated. The remaining solid was suspended in ethyl acetate (1L) at 60 °C. The suspension was centrifuged at 3000 rpm. The resulting solution was decanted and concentrated. The residue was treated with warm ethyl acetate three more times. All organic phases were concentrated, and the obtained solids were filtered, washed with TBME, and dried under vacuum at 60 °C. The solids were stripped with toluene (2 x 1 L) to obtain Ethyl 2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo[2,1-f] [1,2,4] triazine-6-carboxylate (**4**) (206.5 g, 473.2 mmol, 63%) as a pale-yellow solid with a QNMR purity of 91%. ^1H NMR (300 MHz, cdcl_3) δ 8.56 (s, 1H), 8.09 (d, $J = 1.6$ Hz, 1H), 7.19 (d, $J = 1.6$ Hz, 1H), 6.81 (s, 1H), 4.88 (s, 2H), 4.37 (q, $J = 7.1$ Hz, 2H), 4.08 (t, $J = 4.8$ Hz, 4H), 3.84 (t, $J = 4.8$ Hz, 4H), 1.39 (t, $J = 7.1$ Hz, 3H). LCMS (ESI): found 437.2 $[M+H]^+$ (calculated 437.2 $[M+H]^+$).

[0222] Step 3: Synthesis of *tert*-Butyl 4-(2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo [2,1-f] [1,2,4] triazine-6-carbonyl) piperazine-1-carboxylate (5)



[0223] A 3L three-necked flask under nitrogen was charged with ethyl 2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo[2,1-f] [1,2,4] triazine-6-carboxylate (**4**) (206.5 g, 91 wt%, 1 equiv, 473.2 mmol) and THF (2 L). Then *tert*-butyl piperazine-1-carboxylate (440.7 g, 5.0 equiv, 2.366 mol) and TBD (65.87 g, 1.0 equiv, 473.2 mmol) were added and the solution was stirred at 65 °C for 2 days. The conversion was monitored by LCMS and proton NMR analysis. The reaction mixture was concentrated at 50 °C to remove the majority of THF. The material was dissolved in ethyl acetate (1 L) and washed with a 1M solution of potassium bisulfate (2 x 500 mL), water (3 x 500 ml, diluted with brine to enhance separation) and brine (250 mL). The organic phase was dried over sodium sulfate, filtered and concentrated at 50 °C (foaming brown oil). The brown foam was stripped with toluene (1.5 L) to remove traces of ethyl acetate and *tert*-butyl 4-(2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo[2,1-f] [1,2,4] triazine-6-carboxyl) piperazine-1-carboxylate (**5**) (382.0 g, 464 mmol, 98%) was obtained as a brown foam with a QNMR purity of 70%. ¹H NMR (300 MHz, cdcl₃) δ 8.52 (s, 1H), 7.77 (d, *J* = 1.6 Hz, 1H), 6.98 (d, *J* = 1.7 Hz, 1H), 6.80 (s, 1H), 4.06 (t, *J* = 4.9 Hz, 4H), 3.82 (t, *J* = 4.9 Hz, 4H), 3.77 – 3.66 (m, overlaps with a THF signal, but subtraction of another THF signal gives 4H), 3.48 (t, *J* = 5.0 Hz, 4H), 1.47 (m, 27H). LCMS (ESI): found 577.2 [M+H]⁺ (calculated 577.3 [M+H]⁺).

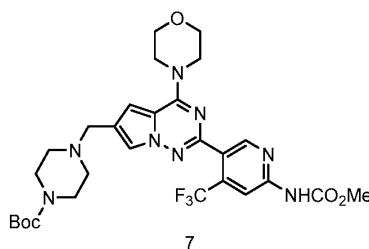
[0224] Step 4: *tert*-Butyl 4-((2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo [2,1-f] [1,2,4] triazin-6-yl) methyl) piperazine-1-carboxylate (6**).**



[0225] A 5 L three-necked flask was charged with *tert*-butyl 4-(2-(6-amino-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo[2,1-f] [1,2,4] triazine-6-carboxyl) piperazine-1-carboxylate (**5**) (271.0 g, 1 equiv, 470.0 mmol) and dry THF (2 L) under nitrogen. The resulting

solution was cooled to 0 °C and a solution of TMS-Cl (107.0n mL, 1.80 equiv, 846.0 mmol) in dry THF (100 mL) was added (mixture forms a brown suspension). The mixture was cooled further to -20 °C and a 2.4 M LiAlH₄ in THF (294.0 mL, 1.50 equiv, 705.0 mmol) was added over 80 min. The reaction mixture was stirred at - 20 °C for 60 minutes. A 2M solution of Rochelle salt (1 L) was slowly added (very exothermic in the beginning, gas evolution) between -20 °C and -10 °C (the mixture become very thick, a solid is formed after 100 ml addition. The solid slowly dissolves at -8 °C and becomes easier to stir again). The mixture was allowed to warm-up to RT overnight. The organic layer was collected, the aq. layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated. The residue was purified by column chromatography (6 kg silica; eluent: DCM/3.5 M NH₃ in MeOH) to give *tert*-butyl 4-((2-(6-amino-4-(trifluoromethyl)pyridin-3-yl)-4-morpholinopyrrolo[2,1-f] [1,2,4] triazin-6-yl) methyl) piperazine-1-carboxylate (**6**) (172.2 g, 306.1 mmol, 65%) as an off-white fluffy solid. ¹H NMR (299 MHz, cdcl₃) δ 8.53 (s, 1H), 7.58 (d, *J* = 1.5 Hz, 1H), 6.80 (s, 1H), 6.67 (d, *J* = 1.6 Hz, 1H), 4.82 (s, 2H), 4.14 – 3.95 (m, 4H), 3.82 (t, *J* = 4.8 Hz, 4H), 3.58 (s, 2H), 3.44 (t, *J* = 5.3 Hz, 4H), 2.43 (t, *J* = 5.1 Hz, 4H), 1.45 (d, *J* = 1.4 Hz, 9H). LCMS (ESI): found 563.3 [M+H]⁺ (calculated 563.3 [M+H]⁺); 561.2 [M-H]⁻ (calculated 561.3 [M-H]⁻).

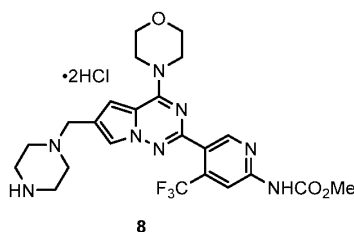
[0226] Step 5: *tert*-Butyl 4-((2-(6-((methoxycarbonyl)amino)-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo[2,1-f] [1,2,4] triazin-6-yl) methyl) piperazine-1-carboxylate (7**).**



[0227] A 3 L three-necked flask was charged with *tert*-butyl 4-((2-(6-amino-4-(trifluoromethyl)pyridin-3-yl)-4-morpholinopyrrolo[2,1-f][1,2,4]triazin-6-yl)methyl)piperazine-1-carboxylate (**6**) (172.2 g, 1 Eq, 306.1 mmol), DCM (1.4 L) and pyridine (74 mL, 3.0 Eq, 918.2 mmol) and the solution was cooled down to 0 °C. Methyl carbonochloridate (26.1 mL, 1.10 Eq, 336.7 mmol) was added to the reaction mixture over 30 min. The mixture was allowed to slowly warm-up to room temperature and stirred overnight. HPLC analysis showed about 90% conversion. Additional methyl carbonochloridate

(2.4 mL, 0.10 Eq, 30.6 mmol) was added dropwise over 5 min and the mixture stirred at room temperature. After 4h, HPLC analysis showed full conversion reach full conversion. The reaction mixture was poured into cold water (500 ml) and transferred to a separating funnel. The organic layer was collected, washed with water (3 × 500 ml) and brine, dried over sodium sulfate, filtered and concentrated. The residue was stripped with toluene to give *tert*-butyl 4-((2-(6-((methoxycarbonyl)amino)-4-(trifluoromethyl) pyridin-3-yl)-4-morpholinopyrrolo[2,1-f][1,2,4] triazin-6-yl) methyl) piperazine-1-carboxylate (7) (174.1 g, 280.5 mmol, 92% yield) as a solid. ¹H NMR (299 MHz, cdcl₃) δ 8.87 (s, 1H), 8.74 (s, 1H), 8.43 (s, 1H), 7.59 (d, *J* = 1.5 Hz, 1H), 6.69 (d, *J* = 1.6 Hz, 1H), 4.05 (t, *J* = 4.8 Hz, 4H), 3.84 (d, *J* = 7.1 Hz, 7H), 3.59 (s, 2H), 3.45 (t, *J* = 5.1 Hz, 4H), 2.44 (t, *J* = 4.9 Hz, 4H), 1.45 (s, 9H). LCMS (ESI): found 621.3 [M+H]⁺ (calculated 621.8 [M+H]⁺); 619.2 [M-H]⁻ (calculated 619.3 [M-H]⁻).

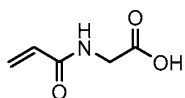
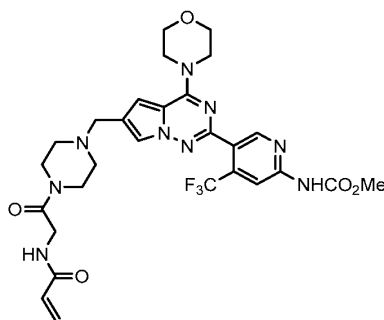
[0228] Step 6: Methyl (5-(4-morpholino-6-(piperazin-1-ylmethyl) pyrrolo[2,1-f][1,2,4] triazin-2-yl)-4-(trifluoromethyl) pyridin-2-yl) carbamate hydrochloride (8).



[0229] A flask was charged, under nitrogen, with *tert*-butyl 4-((2-(6-((methoxycarbonyl)amino)-4-(trifluoromethyl)pyridin-3-yl)-4-morpholinopyrrolo[2,1-f][1,2,4]triazin-6-yl)methyl)piperazine-1-carboxylate (7) (172.1 g, 1 Eq, 277.3 mmol) and CPME (1.0 L) (white suspension) and the mixture was cooled down to 6 °C. A solution of HCl in CPME (1.5 L, 3.0 molar, 16.2 Eq, 4.50 mol) was added over 30 min (only slightly exothermic) and the mixture stirred at room temperature (yellow precipitate immediately formed) overnight. HPLC analysis showed full conversion. The mixture was filtered, the solid washed with 250 ml CPME and 500 ml TBME. The solid was transferred to a flask, but because it was very sticky (probably hygroscopic) MeOH was used to transfer the solid. The mixture was concentrated at the rotary evaporator and the solid dried to give 198 g of crude product. The material was stripped with toluene (2 x 1 L) before recrystallizing. The crude material (191 g) was recrystallized from MeOH (300 mL) The solid was collected by filtration, washed with cold MeOH (50 mL) and dried to give methyl (5-(4-morpholino-6-(piperazin-1-ylmethyl) pyrrolo[2,1-f][1,2,4] triazin-2-yl)-4-(trifluoromethyl) pyridin-2-yl) carbamate

dihydrochloride (**8**) (88.8 g, 150 mmol, 54% yield) as a solid. ^1H NMR (400 MHz, DMSO- D_6) δ 12.13 (s, 1H), 10.92 (s, 1H), 9.59 (s, 2H), 8.73 (s, 1H), 8.31 (s, 1H), 8.07 (s, 1H), 7.38 (s, 1H), 4.41 (s, 2H), 4.02 (t, $J = 4.9$ Hz, 4H), 3.80 – 3.69 (m, 8H), 3.69 – 3.33 (m, 13H), 3.16 (s, 3H). LCMS (ESI): found 521.2 $[\text{M}+\text{H}]^+$ (calculated 521.2 $[\text{M}+\text{H}]^+$).

[0230] **Step 7: Methyl (5-(6-((4-(acryloylglycyl) piperazin-1-yl) methyl)-4-morpholinopyrrolo[2,1-f][1,2,4] triazin-2-yl)-4-(trifluoromethyl) pyridin-2-yl) carbamate (Compound 1).**



Acryloyl glycine. A 2L RBF was charged with glycine (200 g, 1.0 Eq, 2.66 mol), deionized water (88 mL) and sodium hydroxide (710 mL, 30% Wt, 2.0 Eq, 5.32 mol). The mixture was cooled to -5°C before acryloyl chloride (225 mL, 96% Wt, 1.0 Eq, 2.66 mol) was added while maintaining the temperature around 0°C . The mixture was stirred at 0°C for 1 h. After 1h the mixture was acidified with HCl (310 mL, 12 molar, 1.4 Eq, 3.72 mol) until pH 2. The mixture was saturated with sodium sulphate (± 100 g) and diluted with warm MeTHF (300 mL). Phases were separated, and the aqueous phase was extracted with MeTHF (3 x 250 mL). The combined organic layers were washed with brine (150 mL), dried over sodium sulphate and concentrated. At a volume of roughly 500 mL, a solvent swap to ethyl acetate (added 500 mL) was performed. After removing 500 mL ethyl acetate, the solvent switch was repeated twice more. The resulting slurry was agitated at room temperature for 1 h before the solids were collected and rinsed with ethyl acetate (2 x 60 mL), dried under vacuum, and dried further on a rotary evaporator to give **Acryloyl glycine** (76.00 g, 588.6 mmol, 22 % yield) as white solid. ^1H NMR (299 MHz, DMSO- d_6) δ 12.58 (s, 1H), 8.42 (t, $J = 6.0$ Hz, 1H), 6.30 (ddd, $J = 17.1, 10.1, 0.8$ Hz, 1H), 6.10 (ddd, $J = 17.2, 2.3, 0.8$ Hz, 1H), 5.62 (ddd, $J = 10.1, 2.3, 0.8$ Hz, 1H), 3.84 (dd, $J = 6.0, 0.8$ Hz, 2H). LCMS (ESI): found 130.1 $[\text{M}+\text{H}]^+$ (calculated 130.1 $[\text{M}+\text{H}]^+$).

[0231] To a suspension of methyl (5-(4-morpholino-6-(piperazin-1-ylmethyl) pyrrolo[2,1-f] [1,2,4] triazin-2-yl)-4-(trifluoromethyl) pyridin-2-yl) carbamate dihydrochloride (**8**) (73.2 g, 1 Eq, 123 mmol) in THF (750 mL) was added DIPEA (100 mL, 4.7 Eq, 416 mmol) and the mixture slowly became a solution and then turbid again. Acryloyl glycine (23.9 g, 1.5 Eq, 185 mmol) was added followed by HATU (93.8 g, 2 Eq, 247 mmol) and the mixture (yellow) was stirred at room temperature. After 20 min the mixture became a black suspension. After 1 h the reaction was finished and diluted with 600 mL of ethyl acetate and quenched with 600 mL of a sat. solution of sodium bicarbonate. The organic phase was separated and washed with a sat. solution of sodium bicarbonate (600 mL) and brine (2 x 400 mL). The organic phase was dried over sodium sulphate, filtered, and concentrated to give the 147.2 g of crude material as a red sticky oil. The crude material was dissolved in NMP (600 mL) at room temperature and water (1.5 L) was added dropwise. The mixture was stirred for 1 h at room temperature and then filtered off. The residue was extensively washed with water and TBME to remove most of the NMP. The obtained solids were dissolved in DMSO (470 mL) and precipitated by adding water (900 mL). Once again, the residue was extensively washed with water and TBME to remove most of the NMP and DMSO. After drying the material in a circulation oven at 40 °C overnight, methyl (5-(6-((4-(acryloylglycyl) piperazin-1-yl) methyl)-4-morpholinopyrrolo[2,1-f] [1,2,4] triazin-2-yl)-4-(trifluoromethyl) pyridin-2-yl) carbamate (**Compound I**) (57.7 g, 91.4 mmol, 74% yield) was obtained as a solid. ¹H NMR (400 MHz, DMSO-D₆) δ 10.89 (s, 1H), 8.72 (s, 1H), 8.30 (s, 1H), 8.20 (t, *J* = 5.5 Hz, 1H), 7.75 (d, *J* = 1.5 Hz, 1H), 6.97 (d, *J* = 1.6 Hz, 1H), 6.37 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.09 (dd, *J* = 17.1, 2.2 Hz, 1H), 5.59 (dd, *J* = 10.2, 2.2 Hz, 1H), 4.02 (d, *J* = 5.5 Hz, 2H), 3.98 (t, *J* = 4.9 Hz, 4H), 3.74 (d, *J* = 4.4 Hz, 7H), 3.57 (s, 2H), 3.52 – 3.37 (m, 4H), 2.39 (dt, *J* = 17.8, 4.9 Hz, 4H). LCMS (ESI): found 632.4 [M+H]⁺ (calculated 632.3 [M+H]⁺); 630.2 [M-H]⁻ (calculated 630.2 [M-H]⁻).

Example 2: Transfection protocol and readout for NanoBRET screening

[0232] Human embryonic kidney 293-H (HEK 293, Gibco 293-H, #11631017) cell lines were maintained in Dulbecco's Modified Eagle Medium, high glucose, pyruvate (DMEM, Gibco, #11995065) supplemented with 10% fetal bovine serum (FBS, Gibco, #10082147) and 1× penicillin-streptomycin (100× solution, Gibco, #15140148) at 37 °C and 5% CO₂ in a water-saturated incubator. Cell were trypsinized using 0.05% or 0.25% Trypsin-EDTA solution (Trypsin-EDTA, phenol red, Gibco, #25200056 (0.25%) or #25300054). Opti-MEM media supplemented with 10% fetal bovine serum (Opti-MEM I reduced serum media, no phenol red,

Gibco, # 11058021) was used for culturing cells overnight for NanoBRET readout experiments.

[0233] HEK293 cells were cultivated appropriately prior to assay. The medium from cell flask was removed via aspiration, washed 1× with PBS followed by aspiration, trypsinized, and cells were allowed to dissociate from the flask. Trypsin was neutralized using growth medium and cells were pelleted via centrifugation at $200 \times g$ for 5 minutes. The medium was aspirated and the cells were resuspended into a single cell suspension using Opti-MEM I supplemented with 10% FBS. The cell density was adjusted to $2 \times 10^5/\text{mL}$ in Opti-MEM I supplemented with 10% FBS in a sterile, conical tube. The cells were transfected and aliquoted directly in a 96-well plate for the NanoBRET assay the next day, and therefore, the cells were cultured overnight in Opti-MEM. The cells were also transfected in bulk and dispensed into a 96-well plate to allow cells to adhere to the plate overnight, thereby enabling washout studies.

[0234] The lipid:DNA complexes were prepared as follows:

A 10 $\mu\text{g/mL}$ solution of DNA was prepared in Opti-MEM without serum. This solution contains the following ratios of carrier DNA and DNA encoding NanoLuc fused to the biological target. Serial dilution steps may be warranted to accurately dilute the NanoLuc fusion DNA. Added, in order, the following reagents were added to a sterile polystyrene test tube: 1 mL of Opti-MEM without phenol red; 9.0 $\mu\text{g/mL}$ of carrier DNA; 1.0 $\mu\text{g/mL}$ of NanoLuc fusion DNA (for some targets, the amount is less). The reagents were mixed thoroughly. 30 μL of FuGENE HD is added into each mL of DNA mixture to form lipid:DNA complex. Care is taken such that FuGENE HD does not touch the plastic side of the tube and pipetted directly into the liquid in the tube. It is mixed by pipetting up and down 5-10 times and incubated at room temperature for 20 minutes to allow complexes to form. 1 part (e.g. 1mL) of lipid:DNA complex was mixed with 20 parts (e.g. 20mL) of HEK293 cells in suspension at $2 \times 10^5/\text{mL}$ and mixed gently by pipetting up and down 5 times in a sterile, conical tube. Larger or smaller bulk transfections are scaled accordingly, using this ratio. 100 μL cells + lipid:DNA complex was dispensed into a sterile, tissue-culture treated 96-well plate (20,000 cells/well), and incubated at least 16 hours to allow expression. The cells were then incubated in a 37 °C + 5% CO₂ incubator for >16 hrs. The serially diluted Compound I was prepared at 100× final concentration in 100% DMSO. The serially diluted Compound I stock was prepared in PCR plates. 1 μL per well of 100× serially diluted inhibitor/ compound was added to the cells in 96-well plates that have been transiently transfected overnight and mixed by tapping the plate by hand. The plate was incubated at 37 °C + 5% CO₂ incubator overnight.

A 1X solution of substrate mix (500X stock) and appropriate concentration of tracer was prepared in Opti-Mem. The cells were washed by setting a plate washer to the 96 well plate 5X in PBS pH 7.4 by adding 200 μ L PBS each time. The cells were incubated at 37 °C for 2 hours. 100 μ L of the 1X Substrate-Tracer solution was added and the 96 well plate is gently tapped to mix. The plate on plate reader is read every hour for the next 6 hours. The binding assay results show that Compound I provides greater than 80% inhibition of the PI3K target.

Example 3: Cell Proliferation Assay

[0235] The objective of this study was investigate the effect of Compound I on the cell proliferation of 13 cell lines after 3 days treatment, and determine the IC₅₀ of Compound I in each cell line. The cell lines tested included as follows: UM-UC-3 (bladder), KYSE-410 (HN/Esophagus), SW1463 (rectum), Calu-1 (Lung), NCI-H358 (Lung), SW837 (Rectum), SW756 (Cervix), NCI-H2122 (Lung), NCI-H1373 (Lung), NCI-H1792 (Lung), NCI-H23 (Lung), MIA PaCa-2 (Pancreas), and HC44.

[0236] Cells were recovered and maintained in appropriate culture media. The cells were harvested respectively during the logarithmic growth period. The cells were then resuspended and counted using a Vi cell counter (The cell viability be measured by trypan blue exclusion assay.). The cells were then diluted and 90 μ L cell suspensions were used in 96-well plates according to plate map with final cell density. Two duplicate plates were set up. One is for day 0 reading (T0) and the other was cultured in incubator for reading at the end point. The incubated plates were incubated overnight in humidified incubator at 37° C with 5% CO₂.

[0237] At day 0, 10 μ L culture medium to each well for T0 reading. 10 μ L of culture medium was added to each well for T0 reading. Then, 50 μ L CellTiter-Glo® Reagent was added to each well. The contents were then mixed for 2 minutes on an orbital shaker to facilitate cell lysis. The plates were then allowed to incubate at room temperature for 10 minutes to stabilize luminescent signal. Luminescence was then recorded using an EnVision Multi Label Reader. The test compound Compound I, and a control cisplatin, where then diluted at various concentrations from a 10 mM stock solution. Compound I was diluted and 10 μ L of each 10X compound I from working solutions for a concentration ranging from 10 μ M to about 0.005 μ M. Cisplatin was diluted to about 3.33 mM to about 150 nM. The screening plates were then placed back into the incubator for the appropriate treatment time (3 days).

[0238] For the endpoint CTG reading, 50 μ L of CellTiter-Glo® Reagent were added to each well. The contents were then mixed for 2 minutes on an orbital shaker to facilitate cell lysis. The plate was allowed to incubate at room temperature for 10 minutes to stabilize luminescent signal. Luminescence was then recorded using an EnVision Multi Label Reader. IC₅₀'s were determined for each cell line. The assay indicates Compound I has inhibitive effect across multiple cancer cell lines, including a substantial inhibitory effect against NCI-H358 (see Table A).

[0239] **Table A: IC₅₀ Proliferation Assay**

Cell Line Name	Absolute IC ₅₀ (μ M) Compound I	Absolute IC ₅₀ (μ M) Cisplatin
UM-UC-3 (bladder)	> 10	1.8642
KYSE-410 (HN/Esophagus)	2.0231	15.8034
SW1463 (rectum)	3.8582	12.2245
Calu-1 (Lung)	> 10	10.1785
NCI-H358 (Lung)	1.69	12.4619
SW837 (Rectum)	> 10	42.4853
SW756 (Cervix)	7.2855	10.2600
NCI-H2122 (Lung)	3.7651	17.787
NCI-H1373 (Lung)	> 10	8.7241
NCI-H1792 (Lung)	> 10	4.8783
NCI-H23 (Lung)	> 10	3.5848
MIA PaCa-2 (Pancreas)	> 10	7.0279
HCC44 (Lung)	7.92	7.9621

Example 4: Immediate release tablets comprising Compound I

[0240] Compound I was formulated into oral tablets for immediate release after administration to a patient. Weighing, compaction, blending and compression were completed as in **Figure 1** with Compound I as the added drug. Compaction trials of drug particles using different roller compaction process parameters (see Table 1) was also performed and bulk density, tapped density, particle size distribution by sieve analysis, Hausner's ration and compressibility index were determined for each process defined by the respective roller compaction parameters.

[0241] **Table 1: Drug particle analysis after roller compaction trials**

Stage:	Before compaction	Compaction Blend #1	Compaction Blend #2	Compaction Blend #3	Compaction Blend #4	Compaction Blend #5	Final Blend
Process Parameters	N/A	Pressure:40 Bar	Pressure:60 Bar	Pressure:80 Bar	Pressure:60 Bar	Pressure:80 Bar	
		Core-Reducer:40 rpm	Core-Reducer:40 rpm	Core-Reducer:40rpm	Core-Reducer:20rpm	Core-Reducer:10rpm	
Device#		% Retained					
25	0.35	5.50	4.80	5.60	4.50	3.20	3.30
30	2.30	28.20	21.60	27.60	12.40	18.50	26.80
40	0.80	14.00	13.20	18.40	8.80	14.80	13.60
60	0.40	1.20	1.20	0.40	1.60	5.20	4.60
100	20.40	19.20	13.60	24.40	21.60	18.60	17.30
200	16.80	16.80	27.20	15.80	28.60	17.80	14.40
Pan	23.60	13.80	16.80	12.40	25.20	21.80	18.80
Bulk Density (g/ml)	0.46	0.57	0.82	0.88	0.53	0.63	0.57
Tapped Density (g/ml)	0.57	0.73	0.78	0.82	0.68	0.81	0.64
Compressibility Index	21.08	21.92	20.31	19.51	22.08	22.22	20.28
Hausner Ratio	1.27	1.28	1.25	1.24	1.28	1.29	1.25

Example 5: Stability study of formulations comprising Compound I.

[0242] Stability studies of various formulations using various manufacturing methods (e.g., direct blend and granulation) were also conducted on immediate release compositions formulated according to the details in Tables 2 and 3.

[0243] The stability of formulas 1 and 2 (see table 2 and 3) was evaluated by storage over a 4-week period at 40C/75% RH and 50C open container conditions. The samples under open container condition, which represent the worst situation, were tested.

[0244] Aside from some assay variabilities related to sample prep and sampling, no significant increase in degradation products were observed, indicating the formulations are stable.

[0245] Table 2: Formulation 1 (Direct Blend) for Compatibility Evaluation

Ingredients	%w/w range	mg/Tablet range
Compound I	3-10	3-10
Microcrystalline Cellulose (Avicel PH 101)	30-50	30-50
Lactose Monohydrate (Fast Flo)	30-50	30-50
Hydroxy Propyl Cellulose (Klucel EXF)	1.0-5.0	1.0-5.0
Croscarmellose Sodium (Ac-di-sol)	1.0-5.0	1.0-5.0
Colloidal Silicon Dioxide (Cab-O-Sil)	0.1-1.0	0.1-1.0
Magnesium Stearate Vegetable	0.1-1.0	0.1-1.0
Total Weight of Tablet	100.0	100.0

[0246] Table 3: Formulation 2 (Wet Granulation) for Compatibility Evaluation of Compound I

Ingredients	%w/w range	mg/Tablet range
Compound I	3-10	3-10
Microcrystalline Cellulose (Avicel PH 101)	40-60	40-60
Lactose Monohydrate (Fast Flo)	20-40	20-40
Hydroxy Propyl Cellulose (Klucel EXF)	1.0-5.0	1.0-5.0
Croscarmellose Sodium (Ac-di-sol)	1.0-5.0	1.0-5.0
Colloidal Silicon Dioxide (Cab-O-Sil)	0.1-1.0	0.1-1.0
Magnesium Stearate Vegetable	0.1-1.0	0.1-1.0
Purified Water	N/A	Q.S.
Total Weight of Tablet	100.0	100.0

[0247] Excipient compatibility studies were also carried out by mixing Compound I with 18 individual commonly used excipients, respectively. Samples were weighed and mixed in amber vials and stored under two stability conditions (40C/75%RH and 50C) with/without caps. They were evaluated at time points of T0, 2 weeks and 4 weeks. The results confirm compound I is compatible with the excipients in the table below.

[0248] Table 4: Excipients

	Excipient	Function
1	Microcrystalline Cellulose	Filler
2	Silicified Microcrystalline Cellulose	Filler
3	Mannitol EZ	Filler
4	Pregelatinized Starch	Filler
5	Dibasic calcium Phosphate Dihydrate	Filler
6	Lactose Monohydrate	Filler
7	Povidone K29/32	Binder
8	Hydroxypropyl Cellulose	Binder
9	Hydroxypropyl Methyl Cellulose	Binder
10	Croscarmellose Sodium	Disintegrant
11	Crospovidone XL	Disintegrant
12	Colloidal Silicon Dioxide	Glidant
13	Talc	Glidant
14	Magnesium Stearate Vegetable	Lubricant
15	Sodium Steryl Fumarate	Lubricant

16	Glyceryl Behenate	Lubricant
17	Opadry II White (PVA)	Coating
18	Opadry II White (HPMC)	Coating

Example 6: Compound I, 5 mg and 50 mg oral tablets – Batch 1

[0249] 5 mg and 50 mg oral tablets were prepared by a dry granulation process as “Batch 1”.

Table 5 below provides the formulation for each.

[0250] Table 5: Compound I Tablets, 5 mg and 50 mg – Batch 1

Ingredients	Function	mg/unit 5 mg	mg/unit 50 mg
Part I			
Compound I API	Active	4-6	40-60
Microcrystalline cellulose, NF (Avicel PH102)	Filler	15-25	150-250
Lactose Monohydrate, NF (Fast Flo)	Filler	15-20	150-200
Crospovidone XL, NF	Disintegrant	1-2	10-20
Colloidal Silicon Dioxide, NF (Cab-O-Sil)	Glidant	0.1-0.3	1-3
Magnesium Stearate, NF [Vegetable Source]	Lubricant	0.1-0.3	1-3
		94.00	470.00
Part II			
Hydroxypropyl cellulose (Klucel EXF)	Binder	1-2	10-15
Crospovidone XL, NF	Disintegrant	0.1-0.3	1-3
Colloidal Silicon Dioxide, NF (Cab-O-Sil)	Glidant	0.5-2.0	5-20
Magnesium Stearate, NF [Vegetable Source]	Lubricant	0.2-0.3	2-3
Total Core tablet weight		50.00	500.000
Opadry II White (85F18422)	Coating material	1.50	
Purified water	N/A	27.00	
Total Coated tablet weight		103.00	

[0251] The tablets in table 5 were manufactured as follows. Colloidal Silicon Dioxide (Cab-O-Sil) and Crospovidone XL, NF were combined and screened through a #20 mesh sieve. In a Gemco Double Cone Blender (0.5 cu ft): Compound I, Lactose Monohydrate, NF (Fast Flo), screened Colloidal Silicon Dioxide (Cab-O-Sil), Crospovidone XL, and Microcrystalline Cellulose, NF (Avicel PH 102) were added. The ingredients were blended for ten (10) minutes without discharging. Part I Magnesium Stearate was screened through a #30 mesh sieve. The screened Magnesium Stearate was added to the blend and blended for an additional three (3)

minutes. The initial blend underwent compaction using various parameters to evaluate particle size distribution and compression feasibility. The compacted material was milled through a 3.15 mm coarse screen and a 1.0 mm fine screen using a granulator located beneath the rollers.

[0252] The weight of extra-granular excipients was adjusted based on the yield of milled granules. Hydroxypropyl Cellulose (Klucel EXF), Colloidal Silicon Dioxide, NF (Cab-O-Sil), and Crospovidone were screened through a #20 mesh sieve. Magnesium Stearate was screened through a #30 mesh sieve. In a Gemco Double Cone Blender (0.5 cu ft): Approximately half of the milled granules, Colloidal Silicon Dioxide (Cab-O-Sil), Crospovidone, and screened Hydroxypropyl Cellulose (Klucel EXF) were added sequentially along with the remaining milled granules. The mixture was blended for five (5) minutes at 23.5 rpm. The screened Magnesium Stearate was then added to the blend, which was further blended for an additional three (3) minutes at 23.5 rpm. Table 6 shows the particle size distribution of the milled granules and final blend analysis.

[0253] **Table 6: Milled Granules and Final Blend Analysis – Batch 1**

Sample		Milled Granules	Final blend
Bulk Density (gram/cc)		0.596	0.610
Tapped Density (250 taps) (gram/cc)		0.864	0.782
% Individual Amount Retained			
Particle Size Distribution (Sieve Shaker)	20 mesh / 841 microns	6.21	5.87
	40 mesh / 400 microns	33.19	29.77
	60 mesh / 250 microns	6.93	7.72
	80 mesh / 177 microns	8.54	7.32
	100 mesh / 149 microns	5.57	5.03
	200 mesh / 74 microns	18.12	19.11
	Pan	21.56	24.99

[0254] The final blend was then divided into two portions for compression: one for 5 mg strength and the other for 50 mg strength. The blends were compressed using the Globe Pharma tablet press and subsequently tested for weight (n=10), hardness (n=10), thickness (n=10), and friability. The data for 5 and 50 mg are summarized in Tables 7 and 8, respectively. The batches exhibited uniform compression, with consistent weights, hardness, and thickness observed across the batch.

[0255] **Table 7: Compound I, 5 mg Tablet Compression Data – Batch 1**

4.7625 mm Tooling				
In-process sample	Individual Weight, mg (n=10)	Hardness, kp (n=10)	Thickness, inches (n=10)	Friability (%)
	Mean (range)	Mean (range)	Mean (range)	
In-process	51.0 (48.6– 54.0)	5.4 (4.2 – 6.5)	0.112 (0.111 – 0.114)	0.2

[0256] Table 8: Compound I, 50 mg Tablet Compression Data – Batch 1

0.3000 x 0.6000 mm Tooling				
In-process sample	Individual Weight, mg (n=10)	Hardness, kp (n=10)	Thickness, inches (n=10)	Friability (%)
	Mean (range)	Mean (range)	Mean (range)	
In-process	500.2 (495.0 – 504.4)	13.0 (11.1 – 14.0)	0.210 (0.209 – 0.211)	0.4

[0257] The tablets were then loaded into the O'Hara coating pan and coated with Opadry II white (85F18422) film coating solution to a theoretical coating weight gain of 3%. The film coating solution was prepared by placing the required amount of the purified water) was added to the purified water and mixing was continued for a minimum of forty-five (45) minutes. Dissolution testing of the Compound I Tablets 5 mg coated tablets was performed using 500 mL of 0.01M HCl at 37.0°C, Apparatus II (paddle), 75 rpm. The dissolution data is presented in **Figure 2**.

[0258] The dissolution of Batch 1 of more than 75% drug was released within 20 minutes with complete drug release in 70 minutes. The coated tablets were tested for disintegration and completely disintegrated within 30 minutes.

[0259] Dissolution of 50 mg coated tablets from Batch 1 was performed using 900 mL of 0.01M HCl at 37.0°C, Apparatus II (paddle), 100 rpm. The dissolution data is presented in **Figure 3**. The dissolution profile of tablets (50 mg) demonstrated complete release within 10 minutes, confirming adherence to assay and dissolution specifications. However, chromatographic analysis identified the presence of an unidentified impurity in both the 5 mg and 50 mg formulations.

Example 7: Compound I, 1 mg and 5 mg oral tablets – Batch 2

[0260] Batch 2 manufactured to prepare new tablets of 1 mg and 5 mg strengths. In addition, low moisture-grade materials were used to evaluate their impact on an unknown impurity. The

tablets were made using the same approach as that of the previous batch, using a common blend to obtain dose-proportional strengths of 1 mg and 5 mg. Table 9 describes the formulation.

[0261] Table 9: Compound I Oral Tablets 1 mg and 5 mg – Batch 2

Ingredients	Function	mg/unit 1 mg	mg/unit 5 mg
Part I			
Compound I (as Salt)	Active	0.5-1.5	4-6
Microcrystalline cellulose, NF (Avicel PH112)- Part IA	Filler	7-9	35-45
Microcrystalline cellulose, NF (Avicel PH112) - Part IB	Filler	16-20	80-100
Lactose Anhydrous, NF	Filler	16-19	80-90
Crospovidone XL, NF	Disintegrant	1-2	5-10
Colloidal Silicon Dioxide, NF (Cab-O-Sil)	Glidant	0.15-0.3	1-1.5
Magnesium Stearate, NF [Vegetable Source]	Lubricant	0.15-0.3	0.8-2.0
		47.00	235.00
Part II			
Hydroxypropyl cellulose (Klucel EXF)	Binder	1-2	6-8
Crospovidone XL, NF	Disintegrant	0.2-0.3	1-2
Colloidal Silicon Dioxide, NF (Cab-O-Sil)	Glidant	0.5-1.5	4-6
Magnesium Stearate, NF [Vegetable Source]	Lubricant	0.2-0.3	1-1.5
Total Core tablet weight		50.00	250.0
Opadry II White (85F18422)	Coating material	1.50	
Purified water	N/A	27.00	
Total Coated tablet weight		103.00	

[0262] Batch 2 was manufactured to prepare new tablets of 1 mg and 5 mg strengths. In addition, low moisture-grade materials were used to evaluate their impact on an unknown impurity. The tablets were made using the same approach as that of the previous batch, using a common blend to obtain dose-proportional strengths.

[0263] The Manufacturing Process for Batch 2 is as follows: Compound I, Microcrystalline Cellulose, NF (Avicel PH112) (Part IA), and Cab-O-Sil (Part I) were combined and screened through a #30 mesh stainless steel (SS) screen into a properly labeled, double polyethylene-lined container. In a 0.5 cu. ft. Gemco double cone blender, half of the Microcrystalline Cellulose (Avicel PH112) (Part IB) was added. The previously screened materials were then

added. The remaining portion of Microcrystalline Cellulose (Avicel PH112) (Part IB) was then added. The mixture was blended for 10 minutes without engaging the agitator bar.

[0264] The blend was then discharged into a properly labeled, double polyethylene-lined container and screened through a #30 mesh SS screen into another properly labeled, double polyethylene-lined container. In the same 0.5 cu. ft. Gemco double cone blender:

- 1.1. Half of the anhydrous lactose (Part I) was added.
- 1.2. Crospovidone (Part I) was added.
- 1.3. The previously screened blend was added.
- 1.4. The remaining portion of anhydrous lactose (Part I) was added.
- 1.5. The mixture was blended for 10 minutes without using the agitator bar.

[0265] Magnesium Stearate was then weighed and screened through a #30 mesh SS screen into a properly labeled, double polyethylene-lined container.

[0266] In the 0.5 cu. ft. Gemco double cone blender: approximately half of the milled granules were added; Colloidal Silicon Dioxide, NF (Cab-O-Sil) and Crospovidone were added; Screened Hydroxypropyl Cellulose (Klucel EXF) was added; and the remaining milled granules were added.

[0267] The blend was mixed for 5 minutes at 23.5 rpm.

[0268] The screened magnesium stearate was then added to the contents of the blender. The mixture was blended for an additional 3 minutes at 23.5 rpm. A composite sample of 25 grams was collected for both the initial blend (pre-compaction) and the milled granules (post-compaction). The sieve analysis and density results for both pre-compaction and post-compaction granules were documented in Table 10.

[0269] The initial blend was compacted using the Alexanderwerk WP 120 roller compactor at a target pressure of 60 bar. The screw feeder speed was automatically adjusted to maintain a roller gap of 2.0 mm. The compacted material was subsequently milled through a 3.15 mm coarse screen and a 1.0 mm fine screen using the granulator positioned below the rollers. The milled granules were then subjected to a final blending.

[0270] The weight of extra-granular excipients was adjusted based on the yield of the milled granules before weighing. Hydroxypropyl Cellulose (Klucel EXF), Colloidal Silicon Dioxide, NF (Cab-O-Sil), and Crospovidone were screened through a #20 mesh screen. Magnesium Stearate was screened through a #30 mesh screen.

[0271] In the 0.5 cu. ft. Gemco double cone blender: Half of the milled granules were added; Colloidal Silicon Dioxide, NF (Cab-O-Sil), Crospovidone, and screened Hydroxypropyl Cellulose (Klucel EXF) were added; The remaining milled granules were added; and the mixture was blended for 5 minutes at 23.5 rpm. The screened Magnesium Stearate was then added and blended for an additional 3 minutes at 23.5 rpm.

[0272] Table 10: Milled Granules and Final Blend Analysis of Batch 2

Sample		Milled Granules	Final blend
Bulk Density (gram/cc)		0.556	0.610
Tapped Density (250 taps) (gram/cc)		0.730	0.758
% Individual Amount Retained			
Particle Size Distribution (Sieve Shaker)	20 mesh / 841 microns	6.21	5.87
	40 mesh / 400 microns	33.19	29.77
	60 mesh / 250 microns	6.93	7.72
	80 mesh / 177 microns	8.54	7.32
	100 mesh / 149 microns	5.57	5.03
	200 mesh / 74 microns	18.12	19.11
	Pan	21.56	24.99

[0273] The final blend was then divided into two portions for compression: one for 1 mg strength and the other for 5mg strength. The blends were compressed using the Globe Pharma tablet press and subsequently tested for weight (n=10), hardness (n=10), thickness (n=10), and friability. The data is summarized in Table 11. The batch exhibited uniform compression, with consistent weights, hardness, and thickness observed across the batch.

[0274] Table 11: Compound I, 1 mg Tablet Compression Data – Batch 2

4.7625 mm Tooling				
In-process sample	Individual Weight, mg (n=10)	Hardness, kp (n=10)	Thickness, inches (n=10)	Friability (%)
	Mean (range)	Mean (range)	Mean (range)	
Beginning	50.8 (49.6– 52.1)	4.8 (4.4 – 5.7)	0.110 (0.111 – 0.112)	0.1
Middle	50.8 (50.2– 51.4)	5.0 (4.5 – 5.6)	0.110 (0.109 – 0.111)	
End	50.7 (49.7– 52.1)	5.0 (4.6 – 5.3)	0.111 (0.109 – 0.112)	

[0275] The tablets were loaded into the O'Hara coating pan and coated with Opadry II white (85F18422) film coating solution to a theoretical coating weight gain of 3%. The film coating

solution was prepared by placing the required amount of the purified water) was added to the purified water and mixing was continued for a minimum of forty-five (45) minutes. The coating solution was mixed throughout the coating process.

[0276] The dissolution of Compound I Tablets 1 mg coated mini-tablets was performed using 900 mL of 0.01M HCl at 37.0°C, Apparatus II (paddle), 75 rpm. The dissolution data is presented in **Figure 4**. The dissolution was completely released within 10 minutes. The coated tablets were tested for disintegration and completely disintegrated within 30 minutes.

[0277] The dissolution of Compound I Tablets 5 mg coated mini-tablets was performed using 900 mL of 0.01M HCl at 37.0°C, Apparatus II (paddle), 100 rpm. The dissolution data is presented in **Figure 5**. The dissolution profile of Compound I tablets 5mg demonstrated complete release within 10 minutes, confirming adherence to assay and dissolution specifications. However, chromatographic analysis identified the presence of an unidentified impurity in both the 1 mg and 5 mg formulations.

Example 8: Compound I, 1 mg and 5 mg oral tablets by Dry Granulation – Batch 3

[0278] Batch 3 was reformulated by eliminating anhydrous lactose to assess its effect on the unidentified impurity, employing the dry granulation method. Table 12 below shows the formulation.

[0279] **Table 12: Compound I Tablets – Common Blend – Batch 3**

Ingredients	Function	mg/unit 1 mg	mg/unit 5 mg
Part I			
Compound I (as Salt)	Active	0.9-1.2	5-6
Microcrystalline cellulose, NF (Avicel PH112)- Part IA	Filler	7-9	30-40
Microcrystalline cellulose, NF (Avicel PH112) - Part IB	Filler	30-40	150-200
Crospovidone XL, NF	Disintegrant	1-2	7-9
Colloidal Silicon Dioxide, NF (Cab-O-Sil)	Glidant	0.2-0.3	1.25
Magnesium Stearate, NF [Vegetable Source]	Lubricant	0.2-0.3	1.25
		47.00	235.00
Part II			
Hydroxypropyl cellulose (Klucel EXF)	Binder	1-3	6-9
Crospovidone XL, NF	Disintegrant	0.2-0.3	1-2
Colloidal Silicon Dioxide, NF (Cab-O-Sil)	Glidant	0.5-1.5	4-6

Magnesium Stearate, NF [Vegetable Source]	Lubricant	0.2-0.3	1-2
Total Core tablet weight		50.00	250.0
Opadry II White (85F18422)	Coating material	1.50	
Purified water	N/A	27.00	
Total Coated tablet weight		103.00	

[0280] Batch 3 was manufactured as follows by combining and screening Compound I, Microcrystalline Cellulose, NF (Avicel PH112) (Part IA), and Cab-O-Sil (Part I) through a #30 mesh stainless steel (SS) screen into a properly labeled, double polyethylene-lined container.

[0281] In a 0.5 cu. ft. Gemco double cone blender: half of the Microcrystalline Cellulose (Avicel PH112) (Part IB) was added; then added the previously screened materials; and then added the remaining portion of Microcrystalline Cellulose (Avicel PH112) (Part IB); and blended for 10 minutes without using the agitator bar.

[0282] The blend was then discharged into a properly labeled, double polyethylene-lined container. The initial blend was screened through a #30 mesh SS screen into another properly labeled, double polyethylene-lined container.

[0283] In the 0.5 cu. ft. Gemco double cone blend: added half of the screened blend; added Crospovidone (Part I); added the remaining portion of the screened blend; and then blended for 10 minutes without using the agitator bar.

[0284] The blend was then discharged into a properly labeled, double polyethylene-lined container. The initial blend was then screened through a #30 mesh SS screen into another properly labeled, double polyethylene-lined container, then screened the weighed Magnesium Stearate through a #30 mesh SS screen into a properly labeled, double polyethylene-lined container. In the 0.5 cu. ft. Gemco double cone blender: added half of the screened blend; add the screened Magnesium Stearate; added the remaining portion of the screened blend; and then blended for 3 minutes without using the agitator bar.

[0285] The blend was then discharged into a properly labeled, double polyethylene-lined container.

[0286] The initial blend was then compacted using the Alexanderwerk WP 120 roller compactor:

[0287] The blend was compressed into flakes/ribbons at a target pressure of 60 bar. The screw feeder speed was adjusted automatically to maintain a roller gap of 2.0 mm. The compacted

material was then milled through a 3.15 mm coarse screen and a 1.0 mm fine screen using the granulator located below the rollers.

[0288] In the 0.5 cu. ft. Gemco double cone blender: added approximately half of the milled granules; added Colloidal Silicon Dioxide, NF (Cab-O-Sil) and Crospovidone; added screened Hydroxypropyl Cellulose (Klucel EXF); added the remaining milled granules; and blended for 5 minutes at 23.5 rpm. The screened Magnesium Stearate was then added to the contents of the blender and blended for an additional 3 minutes at 23.5 rpm. The sieve analysis and density results of the pre-compaction and post-compaction granules in Table 13.

[0289] Table 13: Milled Granules and Final Blend Analysis – Batch 3

Sample		Milled Granules	Final blend
Bulk Density (gram/cc)		0.568	0.556
Tapped Density (250 taps) (gram/cc)		0.736	0.695
% Individual Amount Retained			
Particle Size Distribution (Sieve Shaker)	20 mesh / 841 microns	28.00	24.00
	40 mesh / 400 microns	22.80	22.80
	60 mesh / 250 microns	16.00	9.20
	80 mesh / 177 microns	10.00	7.60
	100 mesh / 149 microns	4.40	5.60
	200 mesh / 74 microns	4.00	16
	Pan	12.40	12.4

[0290] The final blend was divided into two portions for compression: one for 1 mg strength and the other for 5 mg strength. The blends were compressed using the Globe Pharma tablet press and subsequently tested for weight (n=10), hardness (n=10), thickness (n=10), and friability. The data is summarized in Table 14. The batch exhibited uniform compression, with consistent weights, hardness, and thickness observed across the batch.

[0291] Table 14: Compound I, 1 mg Tablet Compression Data – Batch 3

4.7625 mm Tooling				
In-process sample	Individual Weight, mg (n=10)	Hardness, kp (n=10)	Thickness, inches (n=10)	Friability (%)
	Mean (range)	5.0	Mean (range)	
Beginning	50.2 (48.9 – 51.7)	4.8 (4.7 – 5.4)	0.110 (0.104 – 0.112)	0.0
Middle	50.8 (50.1 – 51.4)	5.3 (4.7 – 6.0)	0.112 (0.109 – 0.115)	

End	49.9 (48.9 – 50.4)	5.0 (4.5 – 5.4)	0.112 (0.109 – 0.115)	
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[0292] The tablets were loaded into the O'Hara coating pan and coated with Opadry II white (85F18422) film coating solution to a theoretical coating weight gain of 3%. The film coating solution was prepared by placing the required amount of the purified water) was added to the purified water and mixing was continued for a minimum of forty-five (45) minutes. The coating solution was mixed throughout the coating process.

[0293] The dissolution of Batch 3 was completely released within 10 minutes. The coated tablets were tested for disintegration and completely disintegrated within 30 minutes.

[0294] Dissolution of Compound 1 Tablets 1mg coated tablets was performed using 900 mL of 0.01M HCl at 37.0°C, Apparatus II (paddle), 75 rpm. The dissolution data of 1 mg is presented in **Figure 6**. The dissolution data of 5 mg tablets is presented in **Figure 7**.

[0295] The dissolution profile of Compound I batch 3 tablets 5 mg demonstrated complete release within 10 minutes, confirming adherence to assay and dissolution specifications. By removing lactose anhydrous from the formulation and analyzing the resulting impact on the unknown impurity, we observed that the impurity was no longer present. Consequently, it was concluded that the unknown impurity was attributable to the presence of lactose.

Example 9: Compound I Oral Tablet 1 mg and 5 mg by Wet Granulation -- Batch 4

[0296] This batch was reformulated by eliminating anhydrous lactose to assess its effect on the unidentified impurity, employing the wet granulation process. Table 15 shows the formulation.

[0297] **Table 15: Compound I, 1 mg and 5 mg Common Blend Tablets**

Ingredients	Function	mg/unit 1 mg	mg/unit 5 mg
Compound I (as Salt)	Active	1.0-1.3	5-6
Microcrystalline cellulose, NF (Avicel PH112)- Part IA	Filler	7-9	30-50
Microcrystalline cellulose, NF (Avicel PH112) - Part IB	Filler	30-40	150-200
Crospovidone XL, NF	Disintegrant	1-2	7-8
Part I			
Hydroxypropyl cellulose (Klucel EXF)	Binder	1-2	7-8
		48.00	240.00
Colloidal Silicon Dioxide, NF (Cab-O-Sil)	Glidant	0.2-0.7	2-3

Crospovidone XL, NF	Disintegrant	0.5-1.5	4-6
Magnesium Stearate, NF [Vegetable Source]	Lubricant	0.2-0.7	2-3
Total Core tablet weight		50.00	250.0
Part II			
Opadry II White (85F18422)	Coating material	1.50	
Purified water	N/A	27.00	
Total Coated tablet weight		103.00	

[0298] Batch 4 was manufactured as follows: Raw materials were weighed into appropriate containers, including double polyethylene-lined containers and HDPE containers. Compound I and Microcrystalline Cellulose, NF (Avicel PH112) (Part IA) were combined and screened through a #30 mesh stainless steel screen into a properly labeled double polyethylene-lined container. The following materials were then transferred into the 0.5 cu. ft. blender:

- 1.1. 50% of the Microcrystalline Cellulose (Avicel PH112) - Part IB
- 1.2. Screened material from Step 2
- 1.3. Hydroxypropyl Cellulose (Klucel EXF)
- 1.4. The remaining portion of Microcrystalline Cellulose (Avicel PH112) - Part IB

[0299] The components were then blended for 5 minutes and then discharged into a stainless-steel bowl. Granulation was then initiated by manually adding water in a controlled manner. Post-granulation, the granules were dried in an oven set at 60°C, and the Loss on Drying (LOD) was measured. The dried granules were then sieved through a #18 mesh and Particle Size Distribution (PSD) testing was performed. A 25-gram composite sample of the initial screened granules was collected.

[0300] The weight of extra-granular excipients was adjusted based on the yield of the milled granules prior to weighing. Colloidal Silicon Dioxide, NF (Cab-O-Sil) and Crospovidone were screened through a #20 mesh screen. Magnesium Stearate was screened through a #30 mesh screen. In a 0.5 cu. ft. Gemco Double Cone Blender, approximately 50% of the screened granules were added. Colloidal Silicon Dioxide, NF (Cab-O-Sil) and Crospovidone were then incorporated. The remaining screened granules were added, and the mixture was blended for 5 minutes at 23.5 rpm. Screened Magnesium Stearate was then added to the blender contents and blending continued for an additional 3 minutes at 23.5 rpm. A 25-gram composite sample of the final blend was then collected. The sieve analysis and density results for the final blend granules are in Table 16.

[0301] **Table 16: Milled granules and Final Blend Analysis – Batch 4**

Sample		Milled Granules	Final blend
Bulk Density (gram/cc)		0.424	0.403
Tapped Density (250 taps) (gram/cc)		0.556	0.510
% Individual Amount Retained			
Particle Size Distribution (Sieve Shaker)	20 mesh / 841 microns	7.55	4.00
	40 mesh / 400 microns	10.57	12.00
	60 mesh / 250 microns	8.30	2.80
	80 mesh / 177 microns	8.30	4.80
	100 mesh / 149 microns	6.79	7.20
	200 mesh / 74 microns	24.91	31.20
	Pan	34.34	36.40

[0302] The final blend was divided into two portions for compression: one for 1 mg strength and the other for 5mg strength. The blends were compressed using the Globe Pharma tablet press and subsequently tested for weight (n=10), hardness (n=10), thickness (n=10), and friability. The data is summarized in Table 17. The batch exhibited uniform compression, with consistent weights, hardness, and thickness observed across the batch.

[0303] Table 17: Compound I, 1 mg Compression Data – Batch 4

4.7625 mm Tooling				
In-process sample	Individual Weight, mg (n=10)	Hardness, kp (n=10)	Thickness, inches (n=10)	Friability (%)
	Mean (range)	Mean (range)	Mean (range)	
Beginning	50.6 (49.6 – 51.8)	5.2 (4.6 – 5.8)	0.119 (0.119 – 0.118)	0.3
Middle	50.4 (49.7 – 51.2)	5.2 (4.6 – 5.6)	0.119 (0.118 – 0.120)	
End	50.4 (49.5 – 51.8)	5.1 (4.6 – 5.8)	0.118 (0.110 - 0.121)	

[0304] The tablets were loaded into the O'Hara coating pan and coated with Opadry II white (85F18422) film coating solution to a theoretical coating weight gain of 3%. The film coating solution was prepared by placing the required amount of the purified water) was added to the purified water and mixing was continued for a minimum of forty-five (45) minutes. The coating solution was mixed throughout the coating process.

[0305] Dissolution of Compound I Tablets 1mg coated tablets was performed using 500 mL of 0.01M HCL at 37.0°C, Apparatus II (paddle), 75 rpm. The dissolution data is presented in

Figure 8. The dissolution of Batch 4 1mg was completely released within 10 minutes. The coated tablets were tested for disintegration and completely disintegrated within 30 minutes.

[0306] Dissolution of Compound I Tablets 5mg coated tablets was performed using 500 mL of 0.01M HCL at 37.0°C, Apparatus II (paddle), 75 rpm. The dissolution data is presented in presented in **Figure 9**.

[0307] The dissolution profile of Compound I tablets 5mg demonstrated complete release within 10 minutes, confirming adherence to assay and dissolution specifications.

Example 10: Clinical study evaluating the safety, tolerability and efficacy of Compound I in Adults with Select Solid Tumors

[0308] This study is conducted in two parts: a dose finding portion to determine the maximum tolerated dose and recommended phase 2 dose (RP2D) of Compound I administered orally with the oral tablets as described herein on once a day (QD) and twice daily (BID) schedules, and a dose expansion portion to evaluate safety and tolerability in tumor-specific cohorts administered Compound I at the recommended phase 2 dose and schedule.

[0309] Intervention Model Description: Part 1(multiple ascending doses, QD or BID): locally advanced, recurrent or metastatic select solid tumors with PIK3CA mutation per local assessment; Part 2 (RP2D determined in Part 1) Patients with locally advanced, recurrent or metastatic select solid tumors with PIK3CA mutation per local assessment will be enrolled protocol defined groups

[0310] Compound I for use in this study can be formulated for oral use and immediate release according to the tablet formulations 1 and 2 described in Examples 5-8 above.

[0311] Adult subjects with histologically confirmed diagnosis of colorectal cancer, gastric cancer, non-small cell lung cancer, human epidermal growth factor receptor 2 (HER2) negative breast cancer, squamous cell carcinoma of the head and neck, urothelial cancer, or select gynecologic cancers (ovarian cancer, cervical cancer, or endometrial cancer) with known PIK3CA mutations or amplifications (as determined at a College of American Pathologists/clinical laboratory improvement amendments [CAP/CLIA]-certified or equivalently accredited diagnostic laboratory using a validated test), who meet all of the eligibility criteria will be enrolled in the Phase 1 portion of the study.

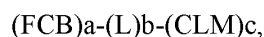
[0312] In the dose finding portion of the study, Compound I will be evaluated as a single-agent at multiple dose levels administered orally until disease progression, unacceptable toxicity, or until meeting any other reason for discontinuation as specified.

[0313] A final dose demonstrates that the immediate release tableted composition comprising Compound I is safe and effective at treating various cancers, including colorectal cancer and breast cancer.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising a therapeutically effective amount of a compound that forms a covalent bond with a kinase or pseudokinase, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutical excipient, wherein the pharmaceutical composition allows for immediate release of the compound.
2. The pharmaceutical composition of claim 1, wherein the kinase is PI3-kinase (PI3K).
3. The pharmaceutical composition of claim 1, wherein the compound that forms a covalent bond with a kinase or pseudokinase is a compound having the formula:



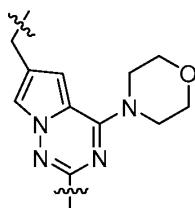
or a pharmaceutically acceptable salt thereof, wherein:

CLM is a covalent linking modality;

L is a linker;

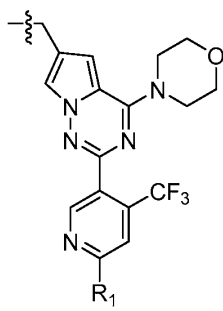
a and c are each independently integers between 1 and 5;

b is an integer between 0 and 5; and

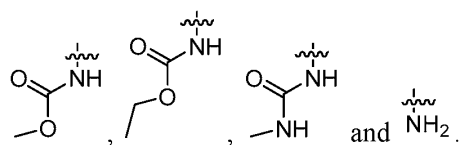


FCB comprises:

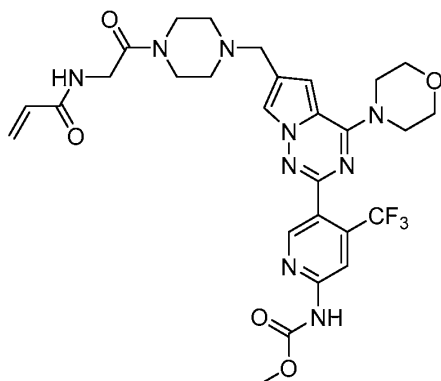
4. The pharmaceutical composition of claim 3, wherein FCB has the structure:



wherein R₁ is selected from the group consisting of:



5. The pharmaceutical composition of any one of claims 1-4, wherein the compound is Compound I:



Compound I or a pharmaceutically acceptable

salt or solvate thereof.

6. The pharmaceutical composition of claim 5, wherein Compound I is a crystalline form of Compound I or a pharmaceutically acceptable salt or solvate of Compound I.

7. The pharmaceutical composition of claim 5, wherein Compound I is an amorphous form of Compound I or its pharmaceutically acceptable salt.

8. The pharmaceutical composition of claim 7, wherein the pharmaceutically acceptable salt of Compound I is selected from the group consisting of a phosphate salt, a hydrochloride salt, a sulfate salt, a mesylate salt, a benzenesulfonate salt, a tosylate salt, a fumarate salt, a maleate salt, a L-tartrate salt, a citrate salt, and a succinate salt, or a solvate thereof.

9. The pharmaceutical composition of any one of claims 1-8, wherein the pharmaceutical excipient is selected from filler, binder, disintegrant, glidant, lubricant, coating or any combination thereof.

10. The pharmaceutical composition of claim 9, wherein the filler is selected from microcrystalline cellulose, silicified microcrystalline cellulose, Mannitol EZ, pregelatinized starch, dibasic calcium phosphate dihydrate, lactose monohydrate or any combination thereof.

11. The pharmaceutical composition of claim 9, wherein the binder is selected from povidone K29/32, hydroxypropyl cellulose, hydroxypropyl methyl cellulose or any combination thereof.
12. The pharmaceutical composition of claim 9, wherein the disintegrant is selected from Croscarmellose Sodium, Crospovidone XL, Sodium starch glycolate, or any combination thereof.
13. The pharmaceutical composition of claim 9, wherein the glidant is selected from Colloidal Silicon Dioxide, Talc or any combination thereof.
14. The pharmaceutical composition of claim 9, wherein the lubricant is selected from Magnesium Stearate Vegetable, Sodium Steryl Fumarate, Glyceryl Behenate, steric acid or any combination thereof.
15. The pharmaceutical composition of claim 9, wherein the coating is water soluble polymers selected from poly vinyl alcohol (PVA)- or hydroxypropyl methylcellulose (HPMC)-based film coating.
16. The pharmaceutical composition of claim 9, wherein the coating is selected from Opadry II White (PVA), Opadry II White (HPMC) or any combination thereof.
17. The pharmaceutical composition of any one of claims 1-16, wherein the compound that forms a covalent bond with a kinase or pseudokinase, or the pharmaceutically acceptable salt thereof is in an amount of greater than 50% wt.
18. The pharmaceutical composition of any one of claims 1-17, wherein compound that forms a covalent bond with a kinase or pseudokinase, or the pharmaceutically acceptable salt thereof is in an amount ranging from about 0.1% wt to about 50% wt, or 1% wt to about 30% wt, or about 1% wt to about 20% wt, or about 1% wt to about 10% wt, or 1% wt to about 4% wt, or about 4% wt to about 3% wt, or about 5% wt to about 6% wt relative to the total core weight of the composition, i.e., the weight before the addition of a coating.
19. The pharmaceutical composition of any one of claims 1-18, wherein the compound that forms a covalent bond with a kinase or pseudokinase, or the pharmaceutically acceptable salt thereof is in an amount of about 2% wt of the total core weight of the composition.
20. The pharmaceutical composition of any one of claims 1-19, wherein the filler is in an amount ranging from about 10% wt to about 95% wt, or about 20% wt to about 90% wt,

or about 30% wt to about 85% wt, or about 40% wt to about 80% wt, or about 40% wt to about 50% wt, or about 42% wt to about 46% wt of the total core weight of the composition.

21. The pharmaceutical composition of any one of claims 1-20, wherein the filler is in an amount ranging from about 70 % wt to about 90% wt of the total core weight of the composition.

22. The pharmaceutical composition of any one of claims 1-20, wherein the filler is in an amount of about 85% wt of the total core weight of the composition.

23. The pharmaceutical composition of any one of claims 1-20, wherein filler is in an amount of about 86% wt of the total core weight of the composition.

24. The pharmaceutical composition of any one of claims 1-20, wherein the filler is in an amount of about 87% wt of the total core weight of the composition.

25. The pharmaceutical composition of any one of claims 1-20, wherein the filler is in an amount of about 88% wt of the total core weight of the composition.

26. The pharmaceutical composition of any one of claims 1-25, wherein the binder is in an amount ranging from about 0.1% wt to about 10% wt, or about 1% wt to about 10% wt, or about 2% wt to about 8% wt, or about 2% wt to about 6% wt, or about 2% wt to 5% wt, or about 2% wt to 4%, about 3% wt to 4% wt of the total core weight of the composition.

27. The pharmaceutical composition of any one of claims 1-26, wherein the binder is in an amount of about 3% wt of the total core weight of the composition.

28. The pharmaceutical composition of any one of claims 1-27, wherein the disintegrant is in an amount ranging from about 0.1% wt to about 10% wt, or about 1% wt to about 10% wt, or about 2% wt to about 8% wt, or about 2% wt to about 6% wt, or about 2% wt to 5% wt, or about 3% wt to 4% wt of the total core weight of the composition.

29. The pharmaceutical composition of any one of claims 1-28, wherein the disintegrant is in an amount of about 3% wt or about 3.5% wt of the total core weight of the composition.

30. The pharmaceutical composition of any one of claims 1-29, wherein the glidant is in an amount ranging from about 0.01% wt to about 10% wt, or about 0.1% wt to about 5% wt, or about 0.1% wt to about 3% wt, or about 0.2% wt to about 2% wt, or about 0.3% wt to 1% wt, or about 0.3% wt to 0.6% wt, or about 0.4% wt to 0.5% wt of the total core weight of the composition.

31. The pharmaceutical composition of any one of claims 1-30, wherein the glidant is in an amount of about 0.5% wt or about 2.5% wt of the total core weight of the composition.

32. The pharmaceutical composition of any one of claims 1-31, wherein the lubricant is in an amount ranging from about 0.01% wt to about 10% wt, or about 0.1% wt to about 5% wt, or about 0.1% wt to about 3% wt, or about 0.2% wt to about 2% wt, or about 0.3% wt to 1% wt, or about 0.3% wt to 0.6% wt, or about 0.4% wt to 0.5% wt of the total core weight of the composition. .

33. The pharmaceutical composition of any one of claims 1-32, wherein the lubricant is in an amount of about 0.5% wt or about 1% of the total core weight of the composition.

34. The pharmaceutical composition of any one of claims 1-33, wherein the coating is in an amount ranging from about 0.1% wt to about 10% wt, or about 1% wt to about 10% wt, or about 2% wt to about 8% wt, or about 2% wt to about 6% wt, or about 2% wt to 5% wt, or about 3% wt to 4% wt of the composition.

35. The pharmaceutical composition of any one of claims 9-31, wherein the coating is in an amount of about 3% wt of the total weight of the composition.

36. The pharmaceutical composition of claim 9-35, wherein the pharmaceutical composition is a in a solid dosage form selected from tablets, powders, capsules, suppositories, sachets, troches and lozenges.

37. The pharmaceutical composition of claim 1-36, wherein the pharmaceutical composition is in a form of tablet.

38. The pharmaceutical composition of claim 37, wherein drug particles before compression into the tablet have a D85 value of about 50 μm to about 100 μm , about 60 μm to about 80 μm or about 63 μm .

39. The pharmaceutical composition of claim 37, wherein drug particles before compression into have a D85 value greater than about 50 μm , about 60 μm , about 70 μm , about 80 μm , about 90 μm , or about 100 μm .

40. The pharmaceutical composition of claim 37, wherein drug particles before compression into the tablet have a D65 value of about 100 μm to about 200 μm , about 120 μm to about 160 μm , or about 140 μm .

41. The pharmaceutical composition of claim 37, wherein drug particles before compression into the tablet have a D65 value greater than about 120 μm , about 130 μm , about 140 μm , about 150 μm , about 160 μm , or about 180 μm .

42. The pharmaceutical composition of claim 37, wherein drug particles before compression into the tablet have a D45 value of about 300 μm to about 500 μm , about 350 μm to about 450 μm , or about 420 μm .

43. The pharmaceutical composition of claim 37, wherein drug particles before compression into the tablet have a D50 value greater than about 340 μm , about 360 μm , about 380 μm , about 420 μm , about 440 μm , or about 480 μm .

44. A method for regulating the activity of a kinase or pseudokinase, comprising administering to a subject in need thereof any pharmaceutical composition any one of claims 1-43.

45. The method of claim 44, wherein the activity of the kinase or pseudokinase is inhibited.

46. The method of claim 44 or 45, wherein the kinase is PI3K.

47. A method for treating cancer, a neurodegenerative disease, an autoimmune disorder or aging in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition of any one of claims 1-43.

48. The method of claim 47, where the subject has cancer.

49. The method of claim 47 or 48, wherein the cancer is selected from one or more of the group consisting of leukemia, tumors of the brain, lung (small cell and non-small cell), ovary, prostate, breast, colon, and cancer in a tissue, including intestinal tissue, pancreatic tissue, liver, kidney, prostate, ovary, lung, bone marrow, or breast tissue.

50. The method of any one of any one of claims 47-49, wherein the subject has cancer with a mutation in the PIK3CA gene.

51. A pharmaceutical composition of any one of claims 1-43, wherein the pharmaceutical composition allows for immediate release of the compound, and the pharmaceutical composition is substantially free of lactose.

52. The pharmaceutical composition of claim 51, wherein the compound is Compound I.

53. The pharmaceutical composition of claim 51 or claim 52, wherein the lactose is lactose anhydrate.
54. A pharmaceutical composition of any one of claims 1-43 or 51-53, wherein the pharmaceutical composition allows for immediate release of the compound, and the pharmaceutical composition is substantially free of any reducing sugar.
55. The pharmaceutical composition of claim 54, wherein the reducing sugar is lactose, galactose, glucose, maltose, fructose, ribose and/or xylose.
56. The pharmaceutical composition of claim 54 or 55, the compound is Compound I.
57. The pharmaceutical composition of any one of claims 51-56, wherein the pharmaceutical excipient is selected from Microcrystalline Cellulose, Crospovidone XL, Colloidal Silicon Dioxide (Cab-O-Sil®), hydroxypropyl cellulose, Magnesium Stearate Vegetable or any combination thereof.
58. The pharmaceutical composition of any one of claims 51-57, wherein the tablet is an oral tablet.
59. The pharmaceutical composition of any claim 58, wherein the oral tablet is an immediate release oral tablet.
60. The pharmaceutical composition of any one of claims 1-43 or 51-59, wherein the composition is as described in Table 2, 3, 5, 9, 12 or 15 described herein.
61. The pharmaceutical composition of claim 60, wherein at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the compound is released within about 10 minutes by a dissolution study described herein.
62. The pharmaceutical composition of claim 61, wherein at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of Compound I is released within about 9 minutes, 8 minutes, 7 minutes, 6 minutes, 5 minutes, 4 minutes, 3 minutes, 2 minutes or 1 minute.
63. The pharmaceutical composition of claim 61 or 62, wherein the dissolution of the Compound in the tablet is determined using 900 mL of 0.01M HCl at 37.0°C, Apparatus II (paddle), 75 rpm or 100 rpm.
64. The pharmaceutical composition of any one of claims 1-43 or 51-63, wherein Compound I is in the form of a salt.

65. A method for regulating the activity of a kinase or pseudokinase, comprising administering to a subject in need thereof any pharmaceutical composition any one of claims 51-64.

66. The method of claim 65, wherein the activity of the kinase or pseudokinase is inhibited.

67. The method of claim 65 or 66, wherein the kinase is PI3K.

68. A method for treating cancer, a neurodegenerative disease, an autoimmune disorder or aging in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition of any one of claims 51-64.

69. The method of claim 68, where the subject has cancer.

70. The method of claim 69, wherein the cancer is selected from one or more of the group consisting of leukemia, tumors of the brain, lung (small cell and non-small cell), ovary, prostate, breast, colon, and cancer in a tissue, including intestinal tissue, pancreatic tissue, liver, kidney, prostate, ovary, lung, bone marrow, or breast tissue.

71. The method of any one of any one of claims 68-70, wherein the subject has cancer with a mutation in the PIK3CA gene.