

Small Molecule Inhibitors and Methods Thereof

Technical Field

- 5 The present invention relates, in general terms, to small molecule inhibitors and their methods of use thereof.

Background

- 10 Branched-chain ketoacid dehydrogenase kinase (BDK) is an enzyme crucial in regulating branched-chain amino acids (BCAAs) catabolism including leucine, isoleucine, and valine, holds significance in protein synthesis and energy metabolism. Disruptions in BCAA metabolism link to various health conditions, such as metabolic disorders (e.g., obesity, insulin resistance, and type 2 diabetes), neurological disorders, muscle-related
15 issues, and liver disease. In addition to its known role in BCAA catabolism, BDK also enhances MEK/ERK signalling, a pivotal pathway driving cell growth and proliferation in cancer.

- BDK emerges as a promising target not only for BCAA-related diseases but also for liver
20 cancer, colorectal cancer and kidney cancer. Virtual screening provides a cost-effective and efficient method to identify candidate compounds for subsequent experimental validation, offering a streamlined approach to explore potential interventions targeting BDK in the context of both metabolic and cancer-related disorders.

- 25 Branched-chain amino acid (BCAA) catabolism is downregulated in liver cancer. The first step of the catabolic pathway is catalysed by branched-chain α -ketoacid dehydrogenase (BCKDH) complex, whose activity is downregulated by BDK through phosphorylation. BDK inhibition promotes BCAA catabolism and reduces the accumulation of BCAAs. Inhibition of the kinase with the selective allosteric inhibitor BT2 or knockdown of BDK
30 in human liver cancer cell lines and mouse liver tumor models slows down liver cancer growth. As dysregulation of BCAA catabolism has been observed in other cancers as well as metabolic diseases such as heart failure and diabetes, interest in developing better BDK inhibitors has accelerated in recent years. This is highlighted by a small molecule BDK inhibitor, PF-07328948, currently undergoing a Phase 1 trial for heart
35 failure with preserved ejection fraction.

BDK regulates the activity of branched-chain α -ketoacid dehydrogenase complex

(BCKD) through phosphorylation and inactivation. This inactivation results in increased branched-chain amino acids (BCAA), which promotes mTOR signaling and fuels cancer cell growth. Therefore, inhibition of BDK is a vital tool to assist with BCAA homeostasis and retard cancer cell growth.

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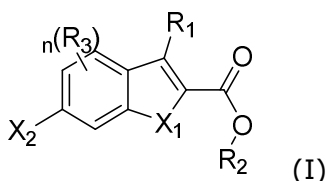
BDK, an atypical kinase, contains a regulatory domain with an allosteric inhibitory site, binds endogenous ketoleucine, previously identified BDK inhibitors including (S)-CPP, phenylbutyrate, BT2, aryl-tetrazoles, PF-07238025, PF-07208254 and PF-07247685. Clofibric acid has an undetermined binding location but may bind to this site as well based on structural similarity. Thiamine pyrophosphate has also been described as a BDK inhibitor; however, its direct interaction with BDK has not been established and is known to be a cofactor. To date, their functions in cancers or metabolic disorders have not been fully investigated either.

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15 It would be desirable to overcome or ameliorate at least one of the above-described problems.

Summary

20 The present disclosure provides a method of treating a disease or condition associated with branched-chain ketoacid dehydrogenase kinase (BDK) in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



25 wherein

R₁ is selected from optionally substituted alkyl, optionally substituted alkoxy, optionally substituted cycloalkyl, aryl optionally substituted with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy; and

R₂ is selected from H, optionally substituted alkyl or optionally substituted aryl;

30 R₃ is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino;

X₁ is selected from O or S;

X₂ is halo; and

n is an integer selected from 0 to 3.

The present disclosure also provides a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof for use in treating a disease or condition associated with BDK.

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The present disclosure also provides a use of a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof in the manufacture of a medicament for treating a disease or condition associated with BDK.

- 10 In some embodiments, the disease or condition associated with BDK is a cancer or a proliferative disease.

In some embodiments, the disease or condition associated with BDK is selected from liver cancer, colorectal cancer, kidney cancer, stomach cancer, and head and neck
15 cancer.

In some embodiments, the compound of Formula (I) is characterised by a cytotoxicity of less than about 20%.

- 20 In some embodiments, the compound of Formula (I) is characterised by an off-target selectivity score of less than 0.005.

In some embodiments, R_1 is selected from optionally substituted C_1 - C_5 alkyl, optionally substituted C_1 - C_5 alkoxy, and phenyl substituted 3 or 4 times with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy.
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In some embodiments, R_1 is phenyl meta substituted with alkyl, alkenyl, oxo, alkoxy or acyloxy, ortho substituted with halo or oxy, and para substituted with halo or oxy.

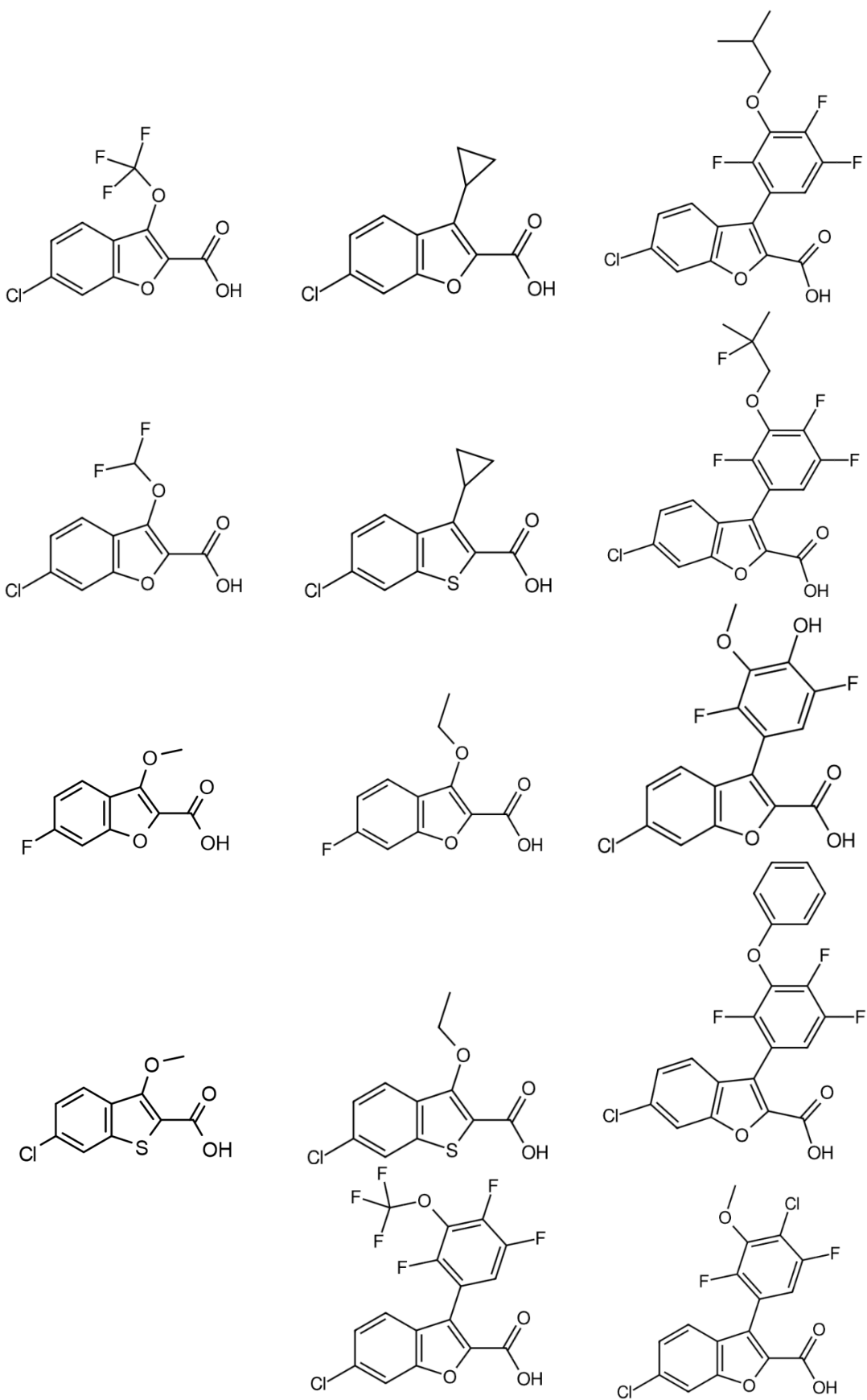
- 30 In some embodiments, R_2 is H.

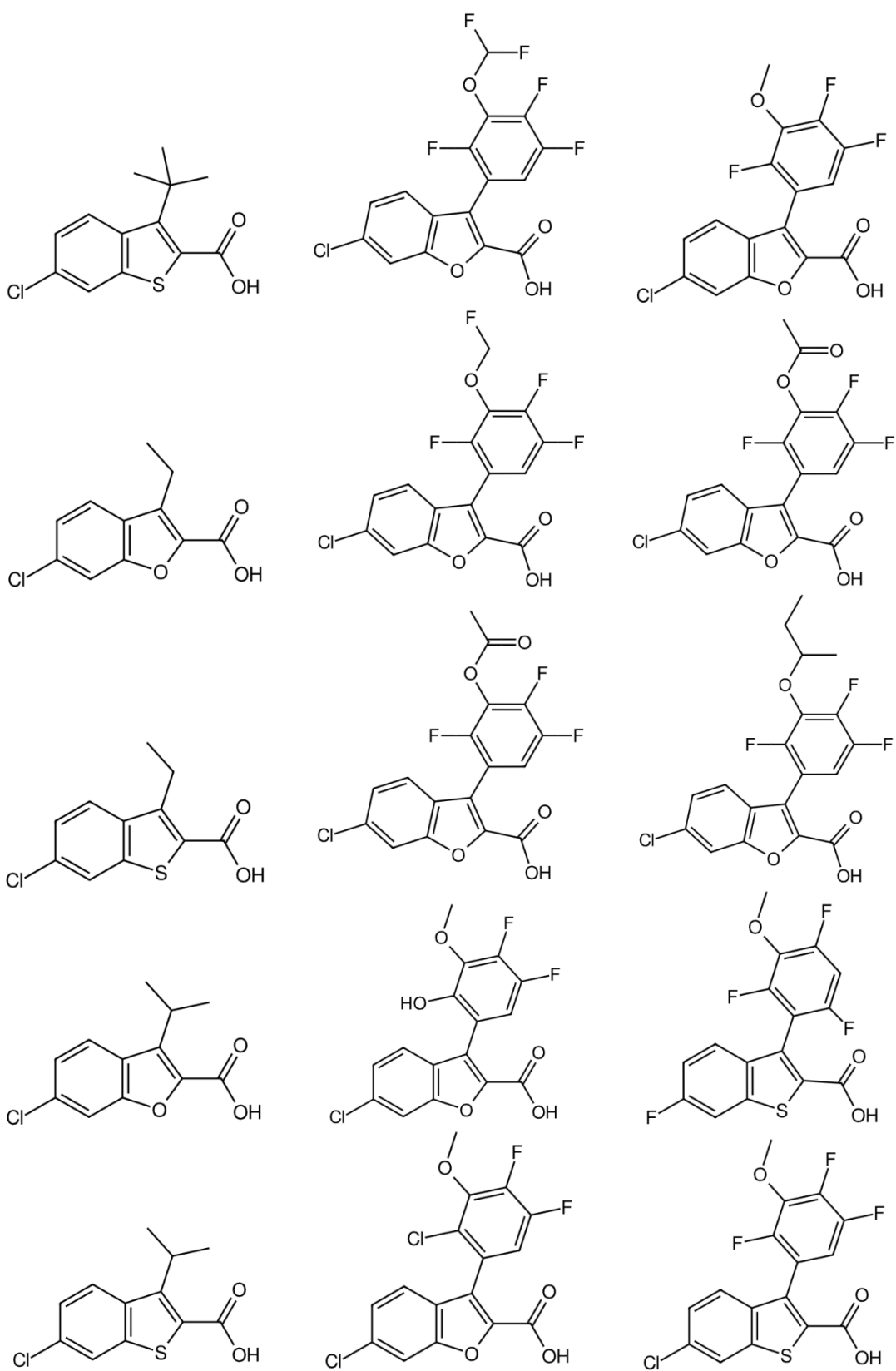
In some embodiments, R_3 is halo.

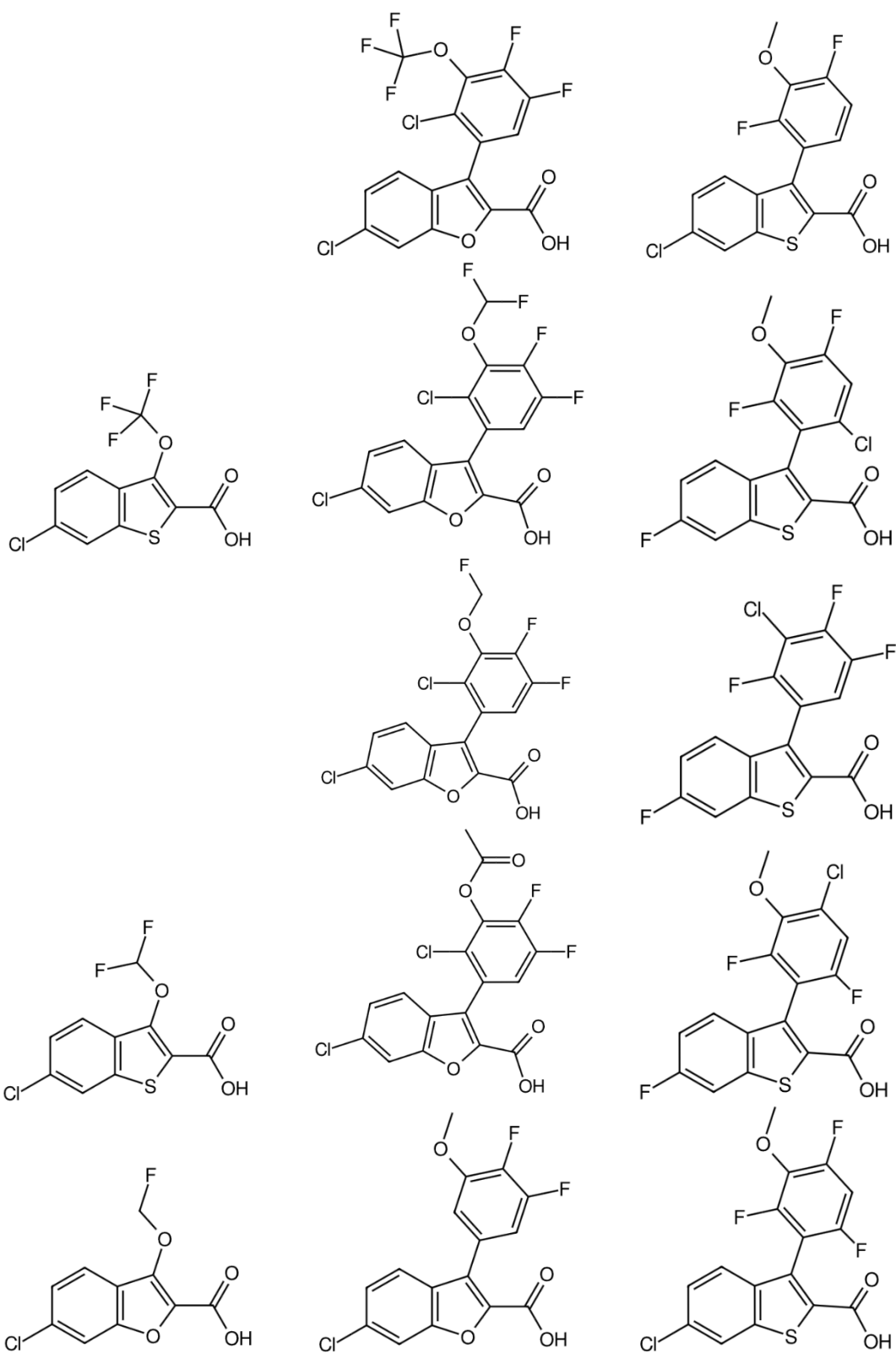
In some embodiments, n is an integer selected from 0 to 1.

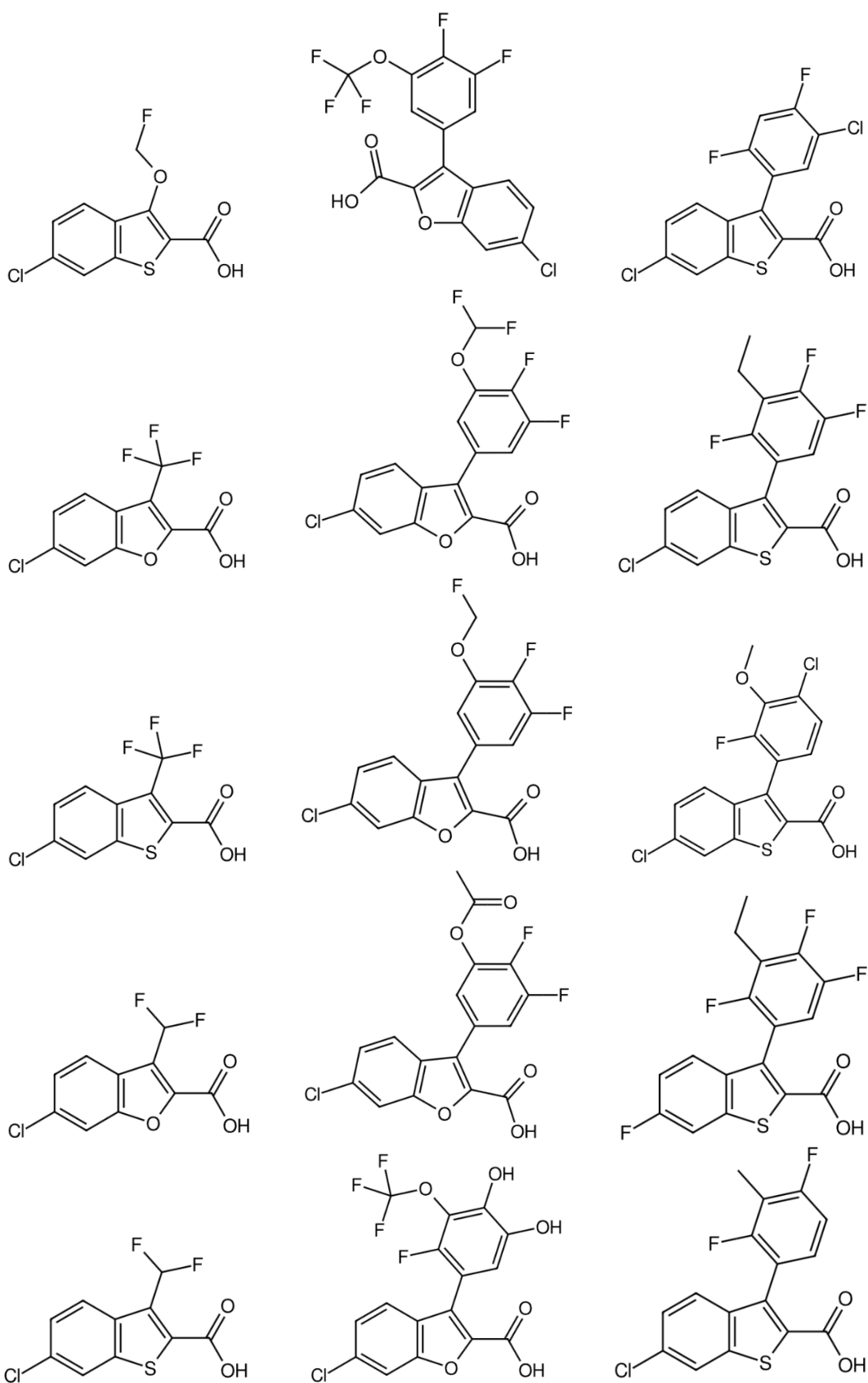
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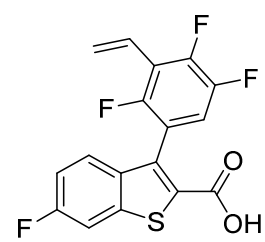
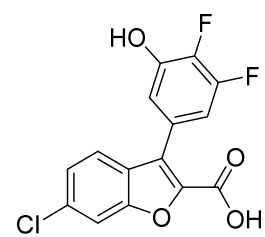
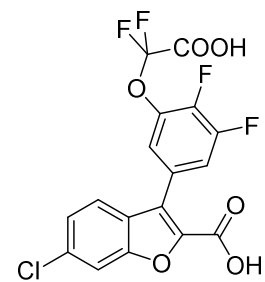
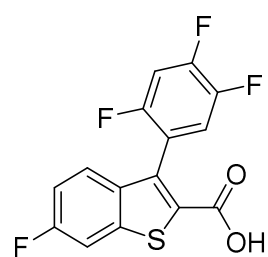
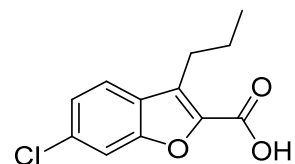
In some embodiments, the compound of Formula (I) is selected from

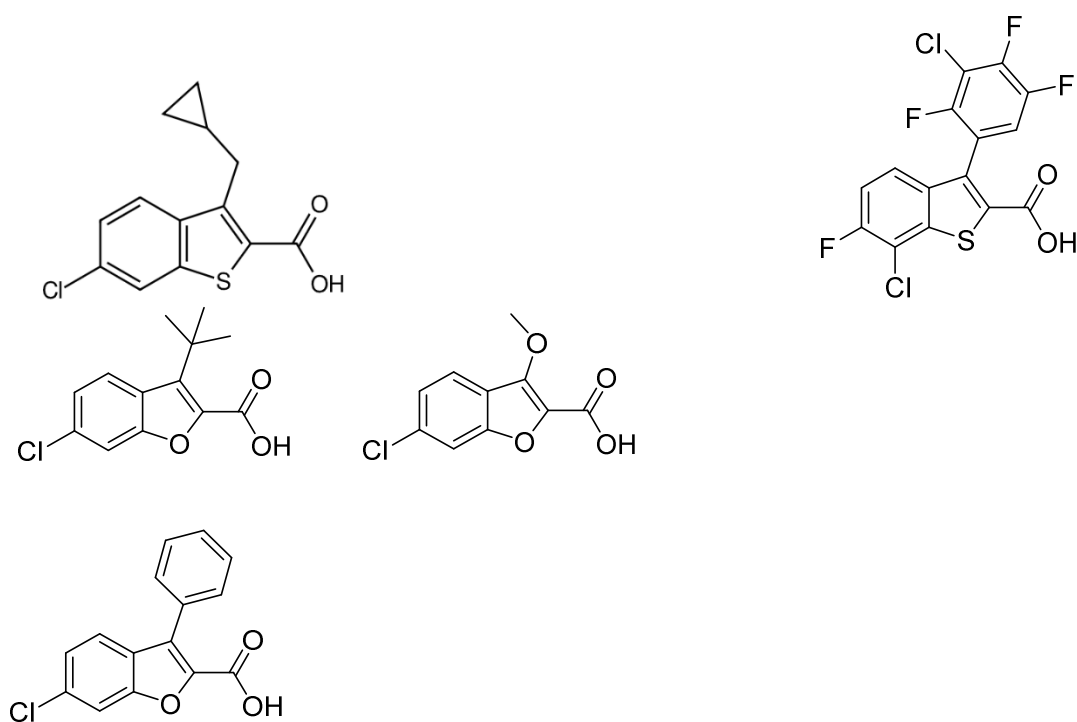




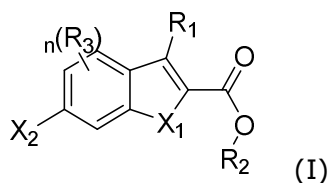








The present disclosure also provides a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof:



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wherein

R_1 is selected from optionally substituted C_2 - C_6 alkyl, optionally substituted C_2 - C_6 alkoxy, optionally substituted C_3 - C_6 cycloalkyl, or aryl optionally substituted 1 to 4 times with alkyl, alkenyl, halo, oxo, alkoxy and acyloxy; and

10 R_2 is selected from H, optionally substituted alkyl or optionally substituted aryl;

R_3 is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino;

X_1 is selected from O or S;

X_2 is selected from Cl or F; and

15 n is an integer selected from 0 to 3.

In some embodiments, R_1 is selected from optionally substituted C_2 - C_6 alkyl, optionally substituted C_2 - C_6 alkoxy, optionally substituted C_3 - C_6 cycloalkyl, or phenyl substituted 3 or 4 times with alkyl, alkenyl, halo, oxo, alkoxy and acyloxy.

In some embodiments, R₁ is phenyl is meta substituted with alkyl, alkenyl, oxo, alkoxy or acyloxy, ortho substituted with halo or oxy, and para substituted with halo or oxy.

In some embodiments, R₂ is H.

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In some embodiments, R₃ is halo.

In some embodiments, n is an integer selected from 0 to 1.

- 10 The present disclosure also provides a pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Brief description of the drawings

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Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

- 20 **Figure 1.** Cell proliferation assays of PF-07208254, MAPLEBDK2, MAPLEBDK4 and MAPLEBDK9 performed in Hep3B cell line with BCKA-enriched medium.

Figure 2. Cell proliferation assays of PF-07208254, MAPLEBDK2, MAPLEBDK4 and MAPLEBDK9 performed in Hep3B BDK knockdown cell line with BCKA-enriched medium.

Figure 3. Cell proliferation assays of PF-07208254, MAPLEBDK2, MAPLEBDK4 and MAPLEBDK9 performed in SNU449 cell line.

- 25 **Figure 4.** Cell proliferation assays of PF-07208254, MAPLEBDK2, MAPLEBDK4 and MAPLEBDK9 performed in HCT-116 cell line.

Figure 5. Toxicology profile of the hits in non-cancerous cell lines.

Figure 6. The docking poses (from AutoDock Vina) of some compounds of the present disclosure.

- 30 **Figure 7.** Cell proliferation assays of PF-07328948, A3 (MAPLEBDK9), A4 (MAPLEBDK4) and other compounds performed in Hep3B cell line with classical DMEM medium.

Figure 8. Cell proliferation assays of PF-07328948, A3 (MAPLEBDK9), A4 (MAPLEBDK4) and other compounds performed in Hep3B BDK knockdown cell line with classical DMEM medium.

- 35 **Figure 9.** Quantification of two sets of Western blot analysis of phosphor-BCKDHA (Ser 293) against total BCKDHA or GAPDH (internal control).

Figure 10. Representative Western blot analysis of phosphor-BCKDHA (Ser 293), total

BCKDHA, BDK and GAPDH of HEK-293T cells exposed to 10uM of listed compounds for 3 days.

Detailed description

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"Alkyl" refers to monovalent alkyl groups which may be straight chained or branched and preferably have from 1 to 10 carbon atoms or more preferably 1 to 6 carbon atoms. Examples of such alkyl groups include methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, and the like.

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"Alkenyl" refers to a monovalent alkenyl group which may be straight chained or branched and preferably have from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms and have at least 1 and preferably from 1-2, carbon to carbon, double bonds. Examples include ethenyl ($-\text{CH}=\text{CH}_2$), *n*-propenyl ($-\text{CH}_2\text{CH}=\text{CH}_2$), *iso*-propenyl ($-\text{C}(\text{CH}_3)=\text{CH}_2$), but-2-enyl ($-\text{CH}_2\text{CH}=\text{CHCH}_3$), and the like.

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"Alkoxy" refers to the group alkyl-O- where the alkyl group is as described above. Examples include, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

20

"Halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

"Aryl" refers to an unsaturated aromatic carbocyclic group having a single ring (eg. phenyl) or multiple condensed rings (eg. naphthyl or anthryl), preferably having from 6 to 14 carbon atoms. Examples of aryl groups include phenyl, naphthyl and the like.

25

"Acyl" refers to groups $\text{H}-\text{C}(\text{O})-$, $\text{alkyl}-\text{C}(\text{O})-$, $\text{cycloalkyl}-\text{C}(\text{O})-$, $\text{aryl}-\text{C}(\text{O})-$, $\text{heteroaryl}-\text{C}(\text{O})-$ and $\text{heterocyclyl}-\text{C}(\text{O})-$, where alkyl, cycloalkyl, aryl, heteroaryl and heterocyclyl are as described herein.

30

"Acyloxy" refers to the groups $-\text{OC}(\text{O})-\text{alkyl}$, $-\text{OC}(\text{O})-\text{aryl}$, $-\text{C}(\text{O})\text{O}-\text{heteroaryl}$, and $-\text{C}(\text{O})\text{O}-\text{heterocyclyl}$ where alkyl, aryl, heteroaryl and heterocyclyl are as described herein.

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"Aminoacyloxy" refers to the groups $-\text{OC}(\text{O})\text{N}(\text{R})_2$, $-\text{OC}(\text{O})\text{NR}''-\text{alkyl}$, $-\text{OC}(\text{O})\text{NR}''-\text{aryl}$, $-\text{OC}(\text{O})\text{NR}''-\text{heteroaryl}$, and $-\text{OC}(\text{O})\text{NR}''-\text{heterocyclyl}$ where R'' is independently

hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl and where each of alkyl, cycloalkyl, aryl, heteroaryl and heterocyclyl is as described herein.

"Heteroaryl" refers to a monovalent aromatic heterocyclic group which fulfils the
5 Hückel criteria for aromaticity (ie. contains $4n + 2$ n electrons) and preferably has from 2 to 10 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen, selenium, and sulfur within the ring (and includes oxides of sulfur, selenium and nitrogen). Such heteroaryl groups can have a single ring (eg. pyridyl, pyrrolyl or N-oxides thereof or furyl) or multiple condensed rings (eg. indoliziny, benzoimidazolyl,
10 coumarinyl, quinolinyl, isoquinolinyl or benzothienyl).

Examples of heteroaryl groups include, but are not limited to, oxazole, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, isoquinoline, quinoline, phthalazine, naphthylpyridine,
15 quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, isothiazole, phenoxazine, phenothiazine, thiazole, thiadiazoles, oxadiazole, oxatriazole, tetrazole, thiophene, benzo[b]thiophene, triazole, imidazopyridine and the like.

20 "Cycloalkyl" refers to cyclic alkyl groups having a single cyclic ring or multiple condensed rings, preferably incorporating 3 to 11 carbon atoms. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, indanyl, 1,2,3,4-tetrahydronaphthalenyl and the
25 like.

"Heterocyclyl" refers to a monovalent saturated or unsaturated group having a single ring or multiple condensed rings, preferably from 1 to 8 carbon atoms and from 1 to 4 hetero atoms selected from nitrogen, sulfur, oxygen, selenium or phosphorous
30 within the ring. The most preferred heteroatom is nitrogen. It will be understood that where, for instance, R_2 or R' is an optionally substituted heterocyclyl which has one or more ring heteroatoms, the heterocyclyl group can be connected to the core molecule of the compounds of the present invention, through a C-C or C-heteroatom bond, in particular a C-N bond.

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Examples of heterocyclyl and heteroaryl groups include, but are not limited to, oxazole, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine,

isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, isothiazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, 5 phthalimide, 1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiadiazoles, oxadiazole, oxatriazole, tetrazole, thiazolidine, thiophene, benzo[b]thiophene, morpholino, piperidiny, pyrrolidine, tetrahydrofuranyl, triazole, and the like.

- 10 When a range of values is specified, for example C₁₋₄ alkyl, it encompasses each value within the range, as well as, all possible intervening ranges. For example, C₁₋₄ alkyl, this includes C₁, C₂, C₃, C₄, C₁₋₄, C₂₋₄, C₃₋₄, C₁₋₃, C₂₋₃, and C₁₋₂ alkyl.

In this specification "optionally substituted" is taken to mean that a group may or may not be further substituted or fused (so as to form a condensed polycyclic group) with 15 one or more groups selected from hydroxyl, acyl, alkyl, alkoxy, alkenyl, alkenyloxy, alkynyl, alkynyloxy, amino, aminoacyl, thio, arylalkyl, arylalkoxy, aryl, aryloxy, carboxyl, acylamino, cyano, halogen, nitro, phosphono, sulfo, phosphorylamino, phosphinyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heterocyclyl, 20 heterocyclylalkyl, heterocycliloxy, oxyacyl, oxime, oxime ether, hydrazone, oxyacylamino, oxysulfonylamino, aminoacyloxy, trihalomethyl, trialkylsilyl, pentafluoroethyl, trifluoromethoxy, difluoromethoxy, trifluoromethanethio, trifluoroethenyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclyl 25 amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, aryl, heteroaryl and heterocyclyl, and the like, and may also include a bond to a solid support material, (for example, substituted onto a polymer resin). For instance, an "optionally substituted amino" group may include amino acid and peptide residues.

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The present disclosure pertains to compounds and their various forms, including ionic forms, tautomers, isomers, polymorphs, pseudopolymorphs, amorphous forms, solvates, co-crystals, chelates, esters, prodrugs, and protected forms. The disclosure also encompasses methods of utilizing these compounds for various purposes. It should 35 be noted that terms like "crystalline form," "polymorph," can be used interchangeably to include all crystalline and amorphous forms, such as polymorphs, pseudopolymorphs, solvates (including hydrates), co-crystals, unsolvated polymorphs (including

anhydrates), conformational polymorphs, amorphous forms, and mixtures thereof, unless a specific crystalline or amorphous form is specified. In certain embodiments, the compounds and their subgroups include polymorphs, solvates, co-crystals, isomers, tautomers, and/or oxides. In other embodiments, they may include polymorphs, solvates, and/or co-crystals.

"Isomer" includes especially optical isomers (for example essentially pure enantiomers, essentially pure diastereomers, and mixtures thereof) as well as conformation isomers (i.e. isomers that differ only in their angles of at least one chemical bond), position isomers (particularly tautomers), and geometric isomers (e.g. cis-trans isomers).

Compounds described herein can comprise one or more asymmetric centers, and thus can exist in various isomeric forms, e.g., enantiomers and/or diastereomers. For example, the compounds described herein can be in the form of an individual enantiomer, diastereomer or geometric isomer, or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. Isomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred isomers can be prepared by asymmetric syntheses. The invention additionally encompasses compounds described herein as individual isomers substantially free of other isomers, and alternatively, as mixtures of various isomers. "Optically-enriched," as used herein, means that the compound is made up of a significantly greater proportion of one enantiomer. In certain embodiments the compound of the present invention is made up of at least about 90% by weight of a preferred enantiomer. In other embodiments the compound is made up of at least about 95%, 98%, or 99% by weight of a preferred enantiomer. Preferred enantiomers may be isolated from racemic mixtures by any method known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts or prepared by asymmetric syntheses. See, for example, Jacques *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen *et al.*, *Tetrahedron* 33:2725 (1977); Eliel, *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972).

Without wanting to be bound by theory, BCKDH activity is suppressed in multiple tumors but not in regenerative or normal tissues and is also suppressed in heart failure (HFpEF)

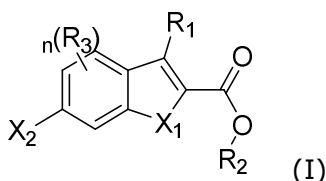
patients. Lower BCKDH activity is also observed in multiple cancer types. BCKDH activity is mainly governed by BDK, a kinase that specifically phosphorylates BCKDH. Phosphorylation of BCKDH results in its decreased activity. By inhibiting BDK, BCKDH activity can be upregulated, leading to therapeutic effects in multiple cancers without
5 affecting regenerating or normal tissues.

Using a Molecular dynamics-Assisted Pocket-to-Ligands Enabler (MAPLE) platform, the inventors designed 86 compounds that have been profiled with molecular docking-based virtual screening. It assesses the binding affinities and interaction profiles of the
10 compounds with the BDK. Cell proliferation assays in both Hep3B wildtype and BDK knockdown cell lines were performed. Accordingly, the inventors have identified several small molecule inhibitors of BDK. These inhibitors exhibit higher specificity for BDK than PF-07208254. Survival assays performed for the compounds show that these compounds do not cause significant toxic effects to non-cancerous cell lines.

15 About 70% of cancers display suppression of the BCAA catabolic pathway. A reduced expression of BCAA catabolic enzymes correlates with tumor progression and aggressiveness. BDK inhibition promotes BCAA catabolism and reduces the accumulation of BCAAs. Inhibition of BDK may have similar therapeutic benefits for
20 kidney, head and neck, and colorectal cancer as these cancers show significantly decreased BCAA catabolism.

Accordingly, the present disclosure provides a method of treating a disease or condition associated with BDK.

25 The present disclosure provides a method of treating a disease or condition associated with branched-chain ketoacid dehydrogenase kinase (BDK) in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof:



30

wherein

R₁ is selected from optionally substituted alkyl, optionally substituted alkoxy, optionally substituted cycloalkyl, aryl optionally substituted with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy; and

R₂ is selected from H, optionally substituted alkyl or optionally substituted aryl;

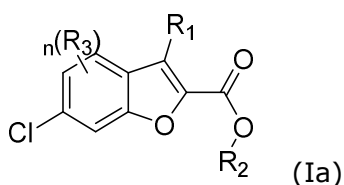
R₃ is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino;

X₁ is selected from O or S;

5 X₂ is halo; and

n is an integer selected from 0 to 3.

The present disclosure provides a method of treating a disease or condition associated with branched-chain ketoacid dehydrogenase kinase (BDK) in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof:



wherein

R₁ is selected from optionally substituted alkyl, optionally substituted alkoxy, aryl optionally substituted with alkyl, halogen; and

R₂ is selected from H, optionally substituted alkyl or optionally substituted aryl;

R₃ is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino; and

n is an integer selected from 0 to 3.

The present disclosure also provides a compound of Formula (I), (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof for use in treating a disease or condition associated with BDK.

The present disclosure also provides a use of a compound of Formula (I), (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof in the manufacture of a medicament for treating a disease or condition associated with BDK.

It was found that a chloro or fluoro substitution on the benzofuran modulates the electron density of the compound sufficiently such that it interacts preferentially with BDK. The binding was found to be better than other halo moieties. Further, the molecular weight of the compound may be larger and causing transport mechanisms into mitochondria to be disrupted. The hydrophilicity of the compound is also improved. In total, this improves the specificity of the compounds to BDK.

In some embodiments, the disease or condition is associated with a dysregulation of BDK. In this regard, BDK may be upregulated or downregulated. The compounds of the present disclosure may inhibit BDK, thus allowing BCKDH activity to be upregulated.

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In some embodiments, the disease or condition is associated with BDK is a cancer or a proliferative disease. A proliferative disease is characterised by excessive proliferation of cells. The turnover of cellular matrix contribute significantly to the pathogenesis of several diseases, including cancer, atherosclerosis, rheumatoid arthritis, psoriasis, idiopathic pulmonary fibrosis, scleroderma and cirrhosis of the liver. The disease or condition may be cancer such as liver cancer, colorectal cancer, kidney cancer, stomach cancer, and head and neck cancer.

15 In particular, BCAA catabolism is believed to be downregulated in liver cancers, and patients with lower levels of key catabolic enzymes have lower survival rates. Accordingly, it is believed that the compounds of the present disclosure may specifically target this class of patients.

20 In some embodiments, the compound of Formula (I) is characterised by a cytotoxicity of less than about 20%. In other embodiments, the cytotoxicity is less than about 15%, less than about 10%, less than about 5%, or less than about 1%.

25 In some embodiments, the compound of Formula (I) is characterised by an off-target selectivity score of less than about 0.01. Off-target effects of a compound refer to unintended interactions and biological activities of the compound with molecules or targets other than its intended target. These interactions may lead to unwanted side effects or toxicity, some of which may be harmful. Minimising off-target effects may improve the safety and specificity of the compound. Off-target selectivity refers to how selectively a compound interacts with targets other than its intended primary target. A low off-target selectivity score indicates that the compound has minimal interactions with off-target compounds, and thus may minimise any potential side effects.

35 In other embodiments, the selectivity score is less than 0.009, less than 0.008, less than 0.007, less than 0.006, less than 0.005, less than 0.004, less than 0.003, less than 0.002, less than 0.001, or 0. In some embodiments, the selectivity score is less than 0.005. In some embodiments, the selectivity score is 0.

In some embodiments, R₁ is selected from optionally substituted alkyl, optionally substituted alkoxy, optionally substituted cycloalkyl, aryl optionally substituted with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy. In some embodiments, R₁ is selected from optionally substituted C₁-C₅ alkyl, optionally substituted C₁-C₅ alkoxy, optionally substituted C₃-C₆ cycloalkyl, aryl substituted 1 to 4 times with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy. In some embodiments, R₁ is aryl substituted 2 to 4 times with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy. In some embodiments, R₁ is aryl substituted 3 or 4 times with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy. In some embodiments, the aryl is phenyl. In some embodiments, the aryl is substituted 2 times with fluoro and another 1 or 2 times with halo, alkyl, alkenyl, oxo, alkoxy or acyloxy. In some embodiments, the aryl is substituted 2 times with fluoro and another 1 or 2 times with chloro, alkyl, alkenyl, oxo, alkoxy or acyloxy. In some embodiments, the aryl is substituted 3 times with fluoro and another 1 time with chloro, alkyl, alkenyl, oxo, alkoxy or acyloxy. In some embodiments, the aryl is substituted 3 times with fluoro. In some embodiments, the aryl is meta substituted with alkyl, alkenyl, oxo, alkoxy or acyloxy. In some embodiments, the aryl is para substituted with alkyl, alkenyl, oxo, alkoxy or acyloxy. In some embodiments, the aryl is either ortho or para substituted with halo. In some embodiments, the aryl is either ortho or para substituted with fluoro or chloro.

20

In some embodiments, the phenyl is meta substituted with alkyl, alkenyl, oxo, alkoxy or acyloxy, ortho substituted with halo or oxy, and para substituted with halo or oxy.

In some embodiments, R₁ is selected from optionally substituted alkyl, optionally substituted alkoxy, and aryl optionally substituted with alkyl, alkenyl and halogen. In some embodiments, R₁ is selected from optionally substituted alkyl, optionally substituted alkoxy, and aryl. In some embodiments, R₁ is selected from optionally substituted C₁-C₅ alkyl, optionally substituted C₁-C₅ alkoxy, and phenyl. In some embodiments, R₁ is selected from optionally substituted C₂-C₅ alkyl, optionally substituted C₂-C₅ alkoxy, and phenyl.

In some embodiments, the optional substituent is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkoxy, optionally substituted amino, optionally substituted acylamino, optionally substituted aminoacyl, optionally substituted heterocyclyl and optionally substituted heteroaryl. In some embodiments, the optional substituent is selected from halo, nitro, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkoxy, and

optionally substituted amino. In some embodiments, the optional substituent is fluoro. The optional substitution may occur 1, 2, 3, 4 or 5 times. In some embodiments, the optional substitution occurs 1 or 2 times. In some embodiments, the optional substitution occurs 1 time.

5

In some embodiments, R_1 is selected from optionally substituted C_1 - C_5 alkyl, optionally substituted C_1 - C_5 alkoxy, and phenyl optionally substituted 1 or 2 times, wherein the optional substituent is selected from halo, nitro, C_1 - C_5 alkyl, C_2 - C_6 alkenyl, C_1 - C_5 alkoxy, and amino optionally substituted with one or two C_1 - C_5 alkyl.

10

In some embodiments, R_1 is phenyl optionally substituted one or two times with halo, nitro, C_1 - C_5 alkyl, C_2 - C_6 alkenyl, C_1 - C_5 alkoxy, and amino optionally substituted with one or two C_1 - C_5 alkyl. In some embodiments, R_1 is phenyl optionally substituted one time with halo, nitro, C_1 - C_5 alkyl, C_2 - C_6 alkenyl, C_1 - C_5 alkoxy, and amino optionally substituted with one or two C_1 - C_5 alkyl. The substitution may be on a para, ortho or meta position relative to the bond to the benzofuran structure.

15

In some embodiments, R_2 is selected from H or optionally substituted alkyl. In some embodiments, R_2 is selected from H or C_1 - C_5 optionally substituted alkyl. In some embodiments, R_2 is H. In some embodiments, R_2 is optionally substituted phenyl.

20

In some embodiments, the optional substituent is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino, optionally substituted acylamino, optionally substituted aminoacyl, optionally substituted heterocyclyl and optionally substituted heteroaryl. In some embodiments, the optional substituent is selected from halo, nitro, optionally substituted alkyl, optionally substituted alkoxy, and optionally substituted amino.

25

In some embodiments, R_3 is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy. In some embodiments, R_3 is selected from halo, oxo, nitro, optionally substituted alkyl. In some embodiments, R_3 is halo. In some embodiments, R_3 is Cl.

30

In some embodiments, X_1 is O. In some embodiments, X_1 is S.

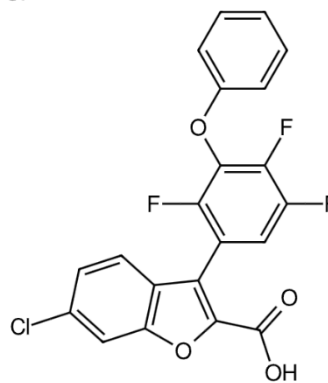
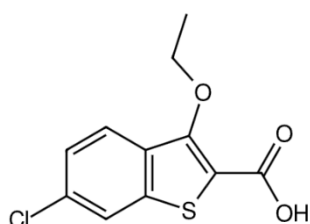
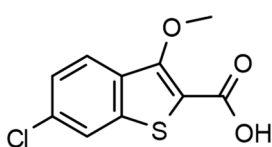
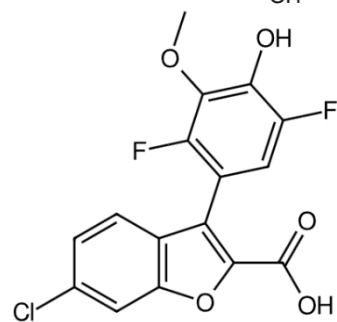
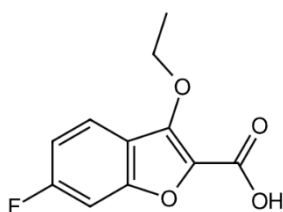
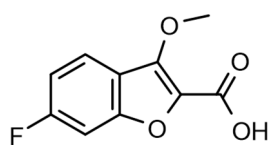
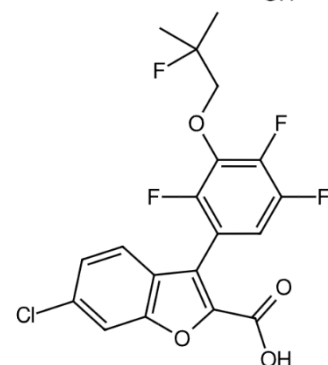
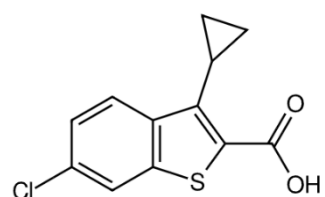
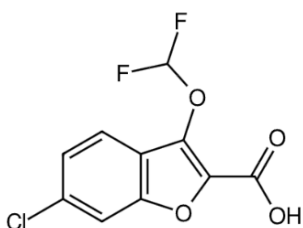
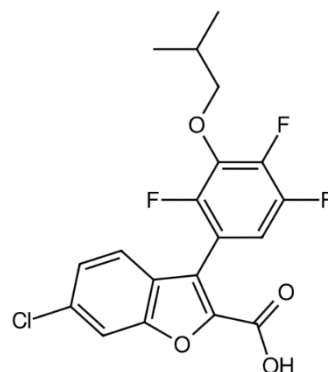
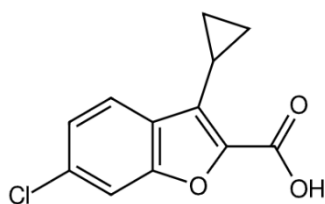
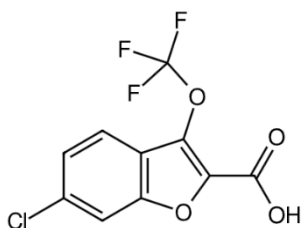
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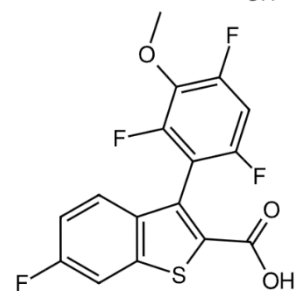
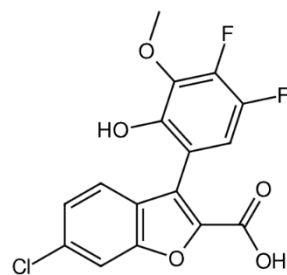
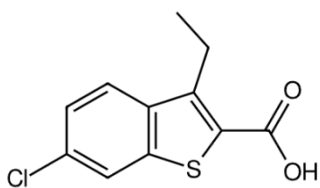
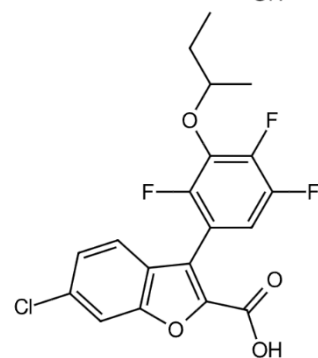
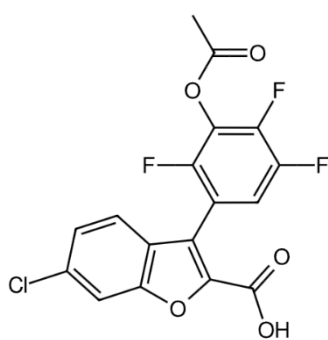
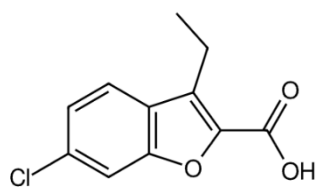
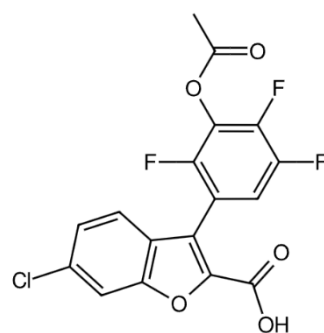
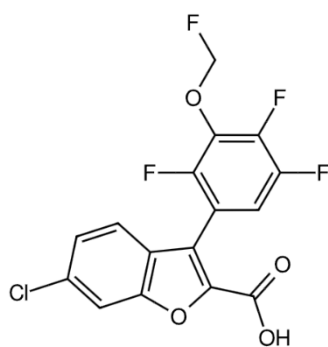
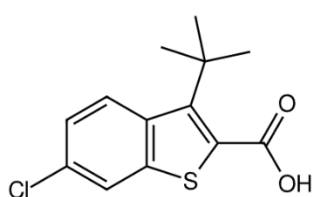
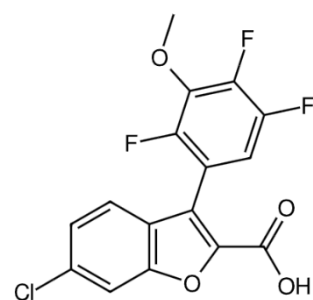
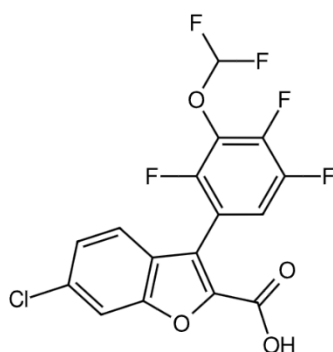
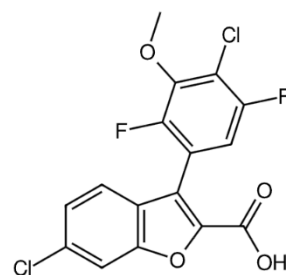
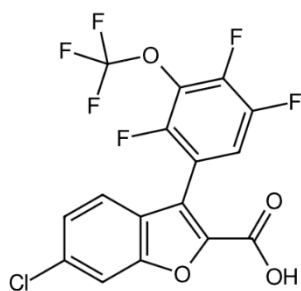
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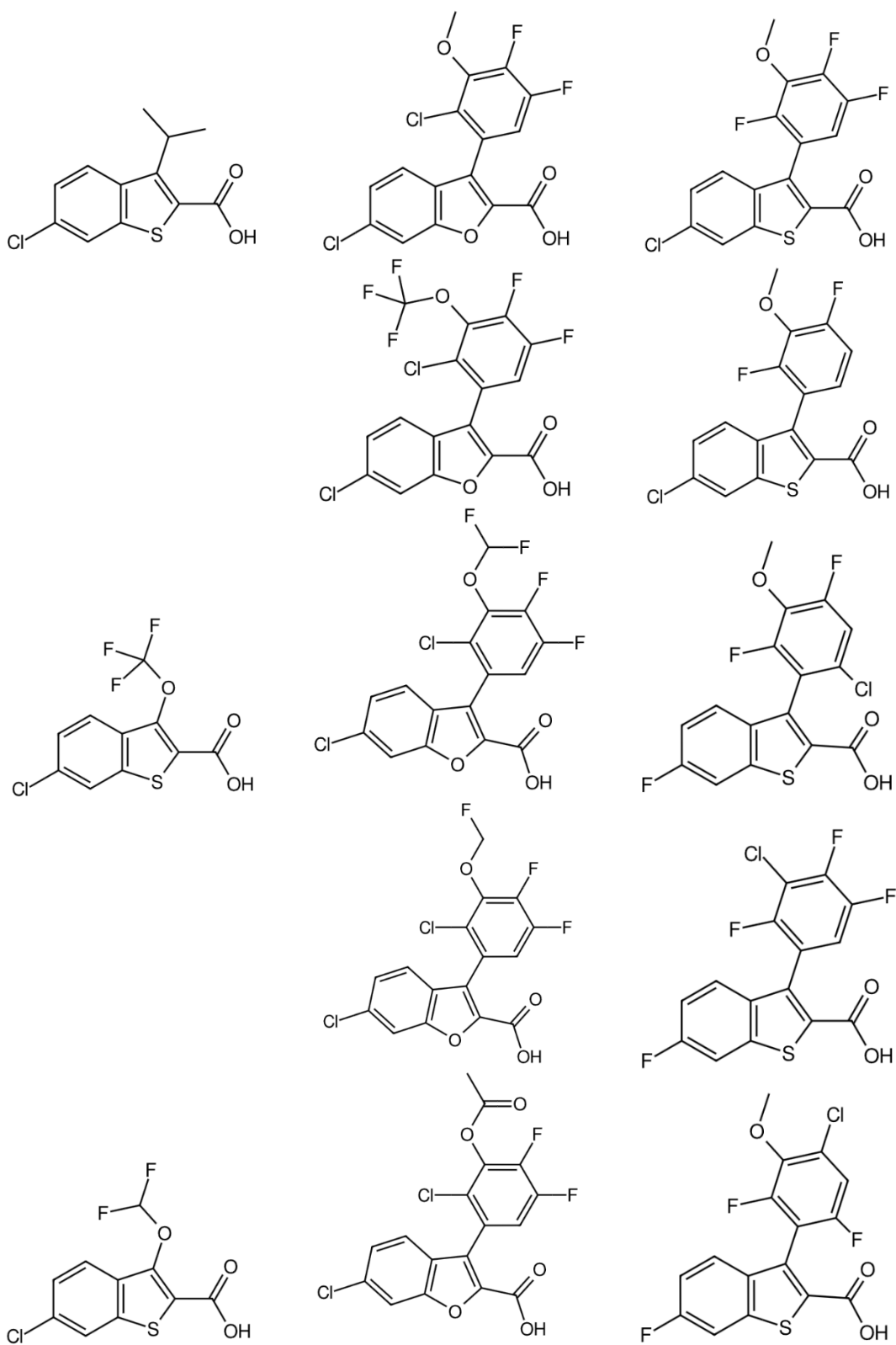
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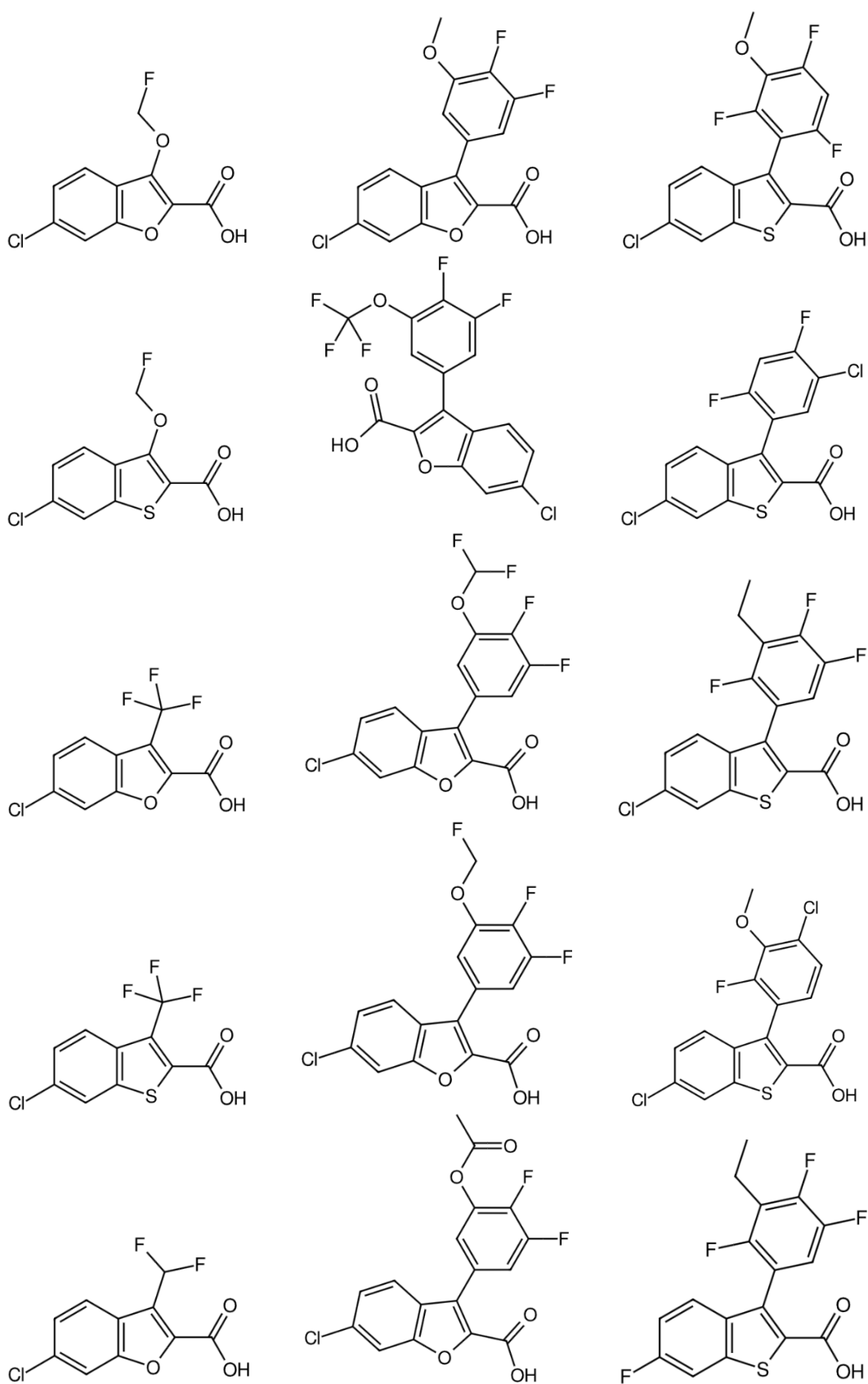
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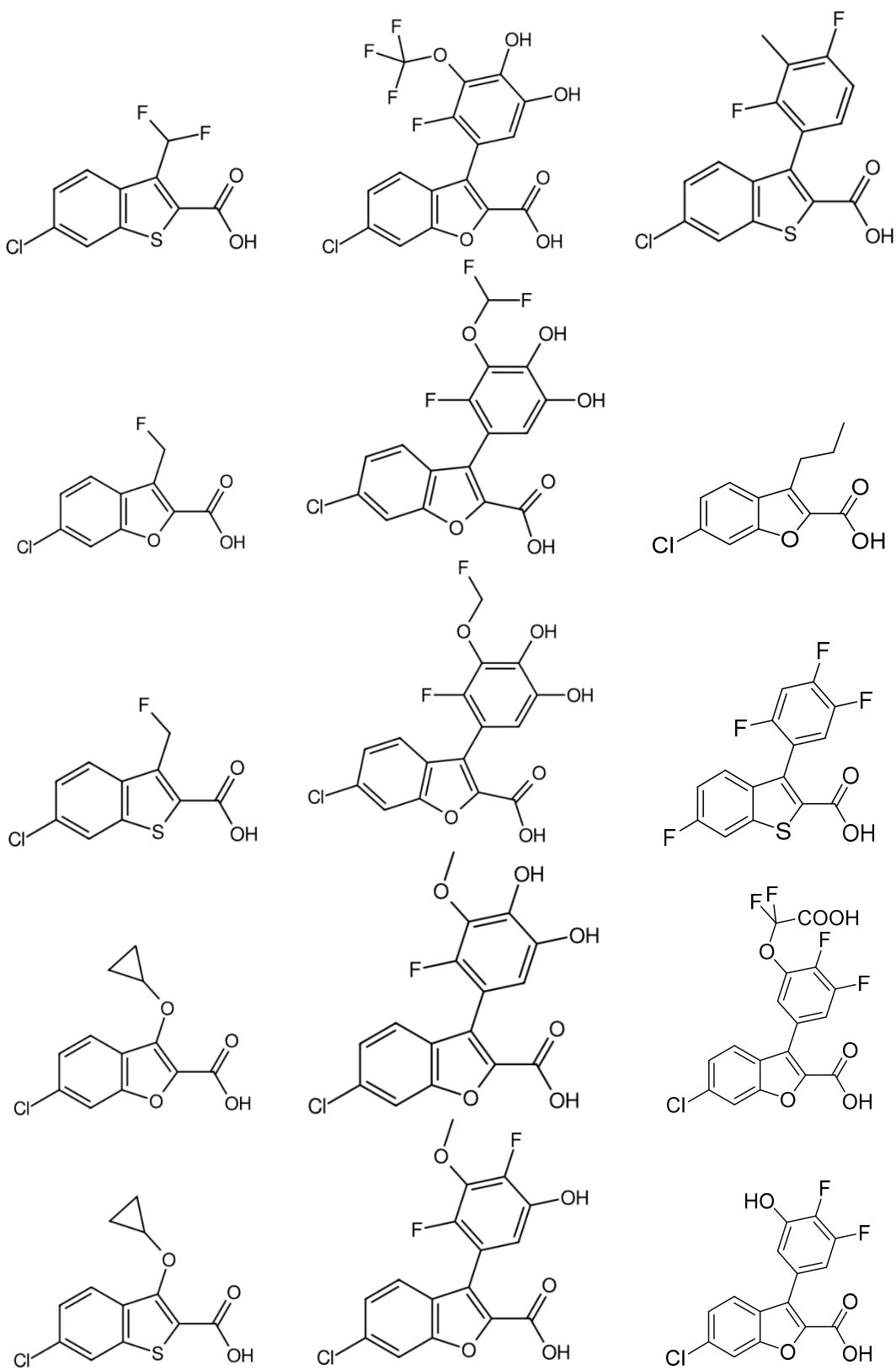
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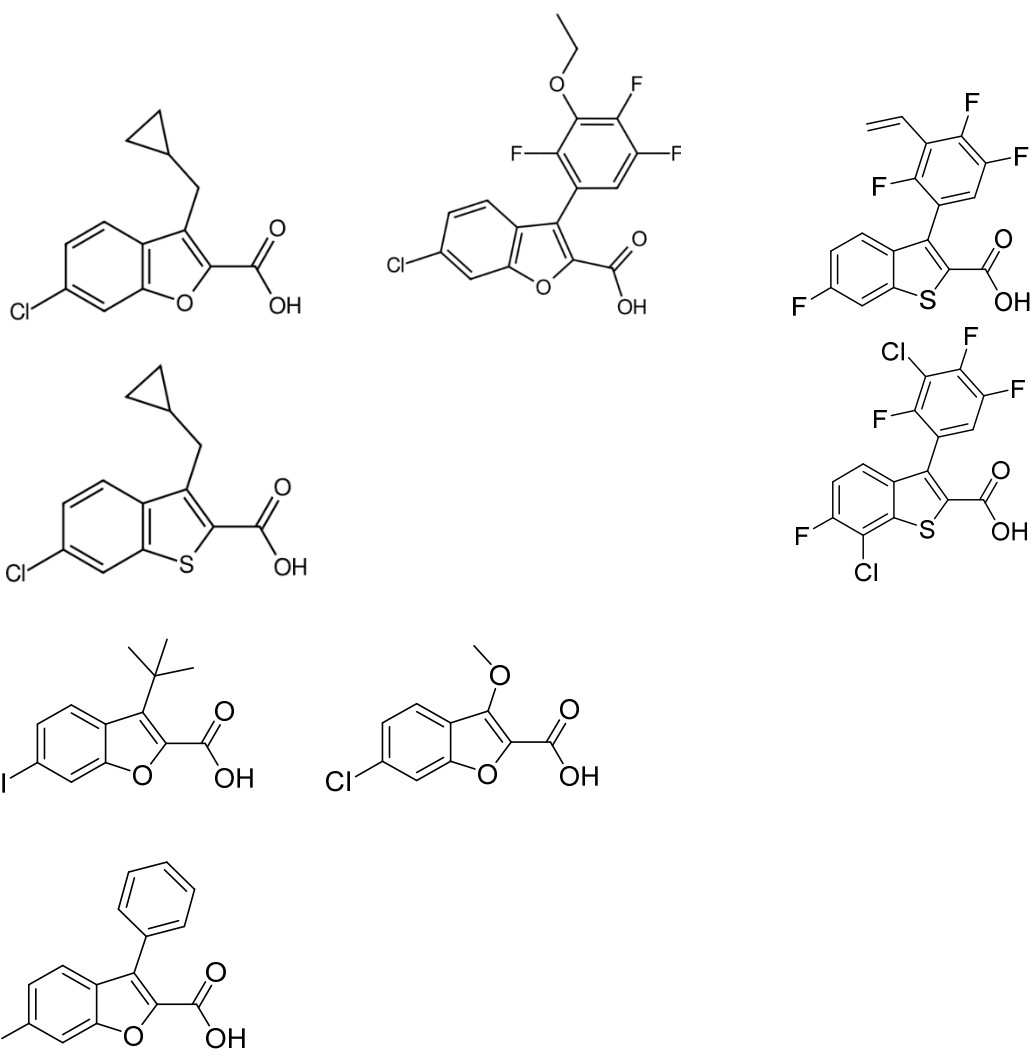




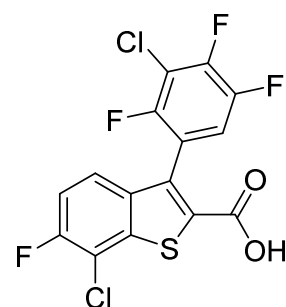
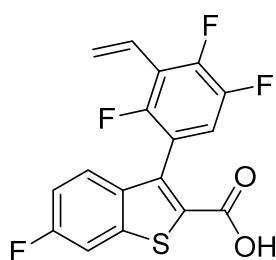
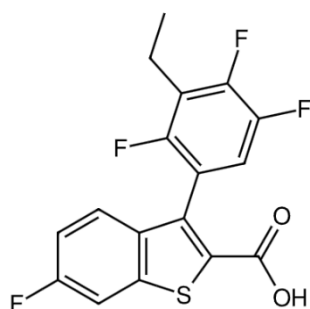
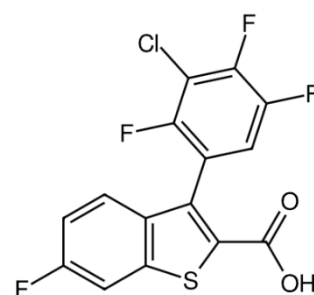
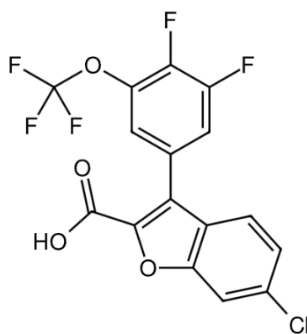
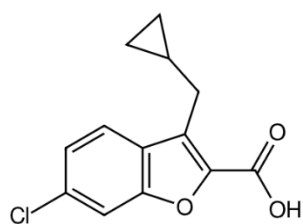
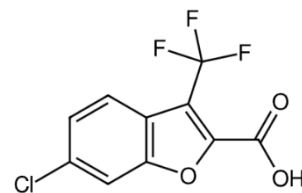
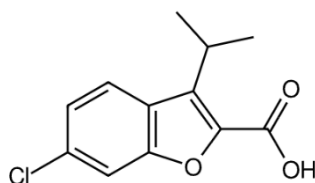
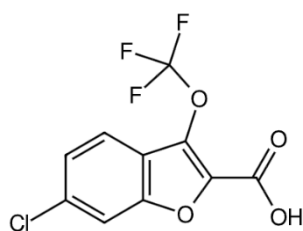




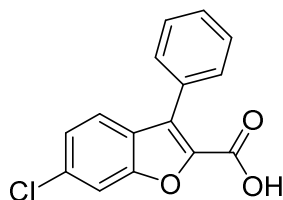
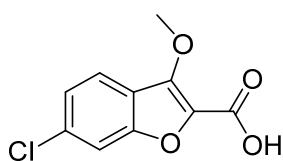
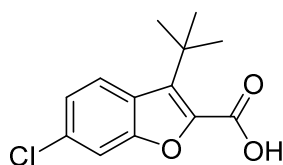




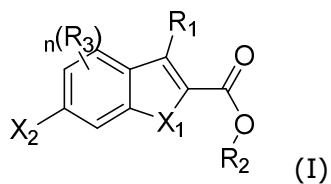
In some embodiments, the compound of Formula (I) is selected from



5 In some embodiments, the compound of Formula (Ia) is selected from



The present disclosure also provides a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof:



wherein

R₁ is selected from optionally substituted C₂-C₆ alkyl, optionally substituted C₂-C₆ alkoxy, optionally substituted C₃-C₆ cycloalkyl, or aryl optionally substituted 1 to 4 times with alkyl, alkenyl, halo, oxo, alkoxy and acyloxy; and

5 R₂ is selected from H, optionally substituted alkyl or optionally substituted aryl;

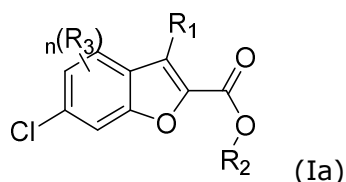
R₃ is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino;

X₁ is selected from O or S;

X₂ is selected from Cl or F; and

10 n is an integer selected from 0 to 3.

The present disclosure also provides a compound of Formula (Ia) or a pharmaceutically acceptable salt, solvate or prodrug thereof:



15 wherein

R₁ is selected from optionally substituted C₂-C₆ alkyl, optionally substituted C₂-C₆ alkoxy, or aryl optionally substituted 1 or 2 times with alkyl, halogen; and

R₂ is selected from H, optionally substituted alkyl or optionally substituted aryl;

R₃ is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted

20 alkoxy, optionally substituted amino; and

n is an integer selected from 0 to 3.

In some embodiments, R₁ is selected from optionally substituted C₂-C₆ alkyl, optionally substituted C₂-C₆ alkoxy, optionally substituted C₃-C₆ cycloalkyl, or phenyl substituted
25 3 or 4 times with alkyl, halo, oxo, alkoxy and acyloxy. In some embodiments, the phenyl is substituted at least one time with halo, and at least one time with alkyl, oxo, alkoxy or acyloxy. In some embodiments, the phenyl is substituted at least two times with halo, and at least one time with alkyl, oxo, alkoxy or acyloxy. In some embodiments, the phenyl is substituted three times with halo, and one time with alkyl, oxo, alkoxy or
30 acyloxy. In some embodiments, the phenyl is substituted at least one times with fluoro, and at least one time with alkyl, oxo, alkoxy or acyloxy. In some embodiments, the phenyl is substituted at least two times with fluoro, and at least one time with alkyl, oxo, alkoxy or acyloxy. In some embodiments, the phenyl is ortho substituted with halo, and meta substituted with alkyl, oxo, alkoxy or acyloxy. In some embodiments, the

phenyl is ortho and para substituted with halo, and meta substituted with alkyl, oxo, alkoxy or acyloxy. In some embodiments, the phenyl is ortho substituted with halo, and meta substituted with alkyl, oxo, alkoxy or acyloxy.

5 In some embodiments, the phenyl is meta substituted with chloro, alkyl or alkoxy. In some embodiments, the phenyl is meta substituted with alkyl or alkoxy. In some embodiments, phenyl is meta substituted with chloro, C₂-C₆ alkyl or C₁-C₆ alkoxy substituted 1-3 times with fluoro.

10 In some embodiments, R₂ is selected from H or optionally substituted alkyl. In some embodiments, R₂ is selected from H or C₁-C₅ optionally substituted alkyl. In some embodiments, R₂ is H. In some embodiments, R₂ is optionally substituted phenyl.

In some embodiments, the optional substituent is selected from halo, oxo, nitro,
15 optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino, optionally substituted acylamino, optionally substituted aminoacyl, optionally substituted heterocyclyl and optionally substituted heteroaryl. In some embodiments, the optional substituent is selected from halo, nitro, optionally substituted alkyl, optionally substituted alkoxy, and optionally substituted amino.

20 In some embodiments, R₃ is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy. In some embodiments, R₃ is selected from halo, oxo, nitro, optionally substituted alkyl. In some embodiments, R₃ is halo. In some embodiments, R₃ is Cl.

25 In some embodiments, n is an integer selected from 0 to 2. In some embodiments, n is an integer selected from 0 to 1. In some embodiments, n is 0. In some embodiments, n is 1.

30 The present disclosure also provides a pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof. The pharmaceutical composition may further comprise a pharmaceutically acceptable excipient.

35 The compound of the invention can be administered to a subject as a pharmaceutically acceptable salt thereof. Suitable pharmaceutically acceptable salts include, but are not limited to salts of pharmaceutically acceptable inorganic acids such as hydrochloric,

5 sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, maleic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

10 Base salts include, but are not limited to, those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium. In particular, the present invention includes within its scope cationic salts eg sodium or potassium salts, or alkyl esters (eg methyl, ethyl) of the phosphate group.

15 Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

20 It will be appreciated that any compound that is a prodrug of the compound of formula (I) is also within the scope and spirit of the invention. Thus, the compound of the invention can be administered to a subject in the form of a pharmaceutically acceptable pro-drug. The term "pro-drug" is used in its broadest sense and encompasses those derivatives that are converted *in vivo* to the compound of the invention. Such derivatives would readily occur to those skilled in the art. Other texts which generally describe prodrugs (and the preparation thereof) include: *Design of Prodrugs*, 1985, H. Bundgaard (Elsevier); *The Practice of Medicinal Chemistry*, 1996, Camille G. Wermuth *et al.*, Chapter 31 (Academic Press); and *A Textbook of Drug Design and Development*, 1991, Bundgaard *et al.*, Chapter 5, (Harwood Academic Publishers).

30 The compound of the invention may be in crystalline form either as the free compound or as a solvate (e.g. hydrate) and it is intended that both forms are within the scope of the present invention. Methods of solvation are generally known within the art.

35 The compound of the invention, or a pharmaceutically acceptable salt, solvate or prodrug thereof is administered to the patient in a therapeutically effective amount. As used herein, a therapeutically effective amount is intended to include at least partially attaining the desired effect, or delaying the onset of, or inhibiting the progression of, or halting or reversing altogether the onset or progression of macular degeneration.

As used herein, the term "effective amount" relates to an amount of compound which, when administered according to a desired dosing regimen, provides the desired therapeutic activity. Dosing may occur at intervals of minutes, hours, days, weeks, 5 months or years or continuously over any one of these periods. Suitable dosages may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage may be in the range of 1 mg to 1000 mg per kg of body weight per dosage. In another embodiment, the dosage may be in the range of 1 mg 10 to 800 mg per kg of body weight per dosage. In yet another embodiment, the dosage may be in the range of 1 mg to 500 mg per kg of body weight per dosage, such as up to 250 mg per body weight per dosage.

Suitable dosage amounts and dosing regimens can be determined by the attending 15 physician and may depend on the severity of the condition as well as the general age, health and weight of the patient to be treated.

The compound of the invention may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is 20 preferable to present it as a composition, preferably as a pharmaceutical composition. The formulation of such compositions is well known to those skilled in the art. The composition may contain any suitable carriers, diluents or excipients. These include all conventional solvents, dispersion media, fillers, solid carriers, coatings, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption 25 agents and the like. It will be understood that the compositions of the invention may also include other supplementary physiologically active agents.

The carrier must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the patient. The 30 compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely 35 divided solid carriers or both, and then if necessary shaping the product.

Injectables for such use can be prepared in conventional forms, either as a liquid solution or suspension or in a solid form suitable for preparation as a solution or suspension in a liquid prior to injection, or as an emulsion. Carriers can include, for example, water, saline (e.g., normal saline (NS), phosphate-buffered saline (PBS),
5 balanced saline solution (BSS)), sodium lactate Ringer's solution, dextrose, glycerol, ethanol, and the like; and if desired, minor amounts of auxiliary substances, such as wetting or emulsifying agents, buffers, and the like can be added. Proper fluidity can be maintained, for example, by using a coating such as lecithin, by maintaining the required particle size in the case of dispersion and by using surfactants. By way of
10 example, the compound, composition or combination can be dissolved in a pharmaceutically effective carrier and be injected into the vitreous of the eye with a fine gauge hollow bore needle (e.g., 30 gauge, 1/2 or 3/8 inch needle) using a temporal approach (e.g., about 3 to about 4 mm posterior to the limbus for human eye to avoid damaging the lens).

15

The compound or composition of the invention may also be suitable for intravenous administration. For example, a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof may be administered intravenously at a dose of up to 100 mg/m².

20

The compound or composition of the invention may also be suitable for oral administration and may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as
25 an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. In another embodiment, the compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug is orally administerable.

30 A tablet may be made by compression or moulding, 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. inert diluent, preservative disintegrant (e.g. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium
35 carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding 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 slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose 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.

5

The compound or composition of the invention may be suitable for topical administration in the mouth including lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatine and glycerin, or sucrose and acacia gum; and
10 mouthwashes comprising the active ingredient in a suitable liquid carrier.

The compound or composition of the invention may be suitable for topical administration to the skin may comprise the compounds dissolved or suspended in any suitable carrier or base and may be in the form of lotions, gel, creams, pastes, ointments and the like.

15 Suitable carriers include mineral oil, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Transdermal patches may also be used to administer the compounds of the invention.

20 The compound or composition of the invention may be suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes which render the compound, composition or combination isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending
25 agents and thickening agents. The compound, composition or combination may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from
30 sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage composition or combinations are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

35

It should be understood that in addition to the active ingredients particularly mentioned above, the composition or combination of this invention may include other agents

conventional in the art having regard to the type of composition or combination in question, for example, those suitable for oral administration may include such further agents as binders, sweeteners, thickeners, flavouring agents disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include cornstarch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

15

Examples

aLMMD Method

Model building. As there were no experimentally resolved structures of human branched-chain alpha-ketoacid dehydrogenase kinase (BDK) at the time the work was done, the crystal structure of *Rattus norvegicus* BDK (PDB ID: 4E01), obtained from the Protein Data Bank (PDB) was used as the template for modelling of human BDK. The sequences of rat (Q00972) and human (O14874) BDK have the same length and share 95.63% identity, differing by only four amino acid residues in the experimentally resolved region of the rat BDK structure. S78A, G91A, S111C and G358A mutations were made on rat BDK using PyMOL to convert it to the human form. All crystallographic ligands were removed. Missing loop residues (337-364) were modelled using MODELLER. The N- and C-termini of BDK were capped with acetyl and N-methyl groups, respectively. PDB2PQR5 was used to determine residue protonation states and add hydrogen atoms.

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System Setup. Using the LEaP module of AMBER 22, each system was solvated with OPC7 water molecules in a periodic truncated octahedron box such that the distance between the protein and box edge was at least 10 Å. Chloride counterions were then added to neutralise the system. A 50-ns conventional molecular dynamics (cMD) simulation of the apo BDK was performed to determine the appropriate number of benzene molecules to be added from the average box volume. Packmol was used to generate 20 different random distributions of benzene probes around the protein. Each

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system was then solvated with OPC water molecules followed by charge neutralisation with chloride ions to give a final benzene concentration of approximately 0.2 M.

MD simulations. For each setup of BDK packed with benzene probes, a 50-ns cMD production simulation run was performed in order to determine boost potential parameters for accelerated molecular dynamics (aMD) simulations. Energy minimisation and MD simulations were performed by the PMEMD module of AMBER 22. The ff19SB force field was used for the BDK protein, while the generalised AMBER force field (GAFF2) was used for benzenes. Atomic charges for benzene were taken from a previous work. Bonds involving hydrogen atoms were constrained using the SHAKE algorithm to enable a time step of 2 fs. A nonbonded interaction cutoff distance of 9 Å was used. Long-range electrostatic interactions were treated using the particle mesh Ewald method under periodic boundary conditions. Energy minimisation was performed for 500 steps with the steepest descent algorithm, followed by another 500 steps with the conjugate gradient algorithm. The system was then heated gradually to 300 K over 50 ps in the NVT ensemble before equilibration in the NPT ensemble at a constant pressure of 1 atm for another 50 ps. Weak harmonic positional restraints with a force constant of 2.0 kcal mol⁻¹ Å⁻² were imposed on heavy atoms during energy minimisation and equilibration steps. Further equilibration without positional restraints was performed for 2 ns, followed by the 50-ns production run at 300 K and 1 atm. The temperature of the system was maintained using a Langevin thermostat with a collision frequency of 2 ps⁻¹, while the pressure was maintained using the Berendsen barostat with a pressure relaxation time of 2 ps.

aMD simulations. The aLMMMD method involves a protein system being packed in a solvated simulation box containing 0.2 M benzenes and subjected to the aMD enhanced sampling method. The aMD enhanced sampling method enables a system to overcome energy barriers more easily. In aMD, a boost potential $\Delta V(r)$ is added to a system's potential energy when the system's potential energy is lower than the threshold energy value E .

$$\begin{aligned} V(r)^* &= V(r) + \Delta V(r), & V(r) < E, \\ V(r)^* &= V(r), & V(r) \geq E \end{aligned}$$

(1)

In this equation above, $V(r)^*$ is the modified potential energy, $V(r)$ is the unmodified original potential energy, and $\Delta V(r)$ is the boost potential. In the dual-boost implementation of aMD, a boost potential is applied to the system's potential energy and dihedral energy as follows:

$$\Delta V(r) = \frac{(E_p - V(r))^2}{(\alpha P + E_p - V(r))} + \frac{(Ed - Vd(r))^2}{(\alpha D + Ed - Vd(r))} \quad (2)$$

In the equation above, E_p is the threshold potential energy, Ed is the threshold dihedral energy, $V(r)$ is the system's potential energy, and $Vd(r)$ is the system's dihedral energy.

- 5 αP is the acceleration factor for the system's potential energy and αD is the acceleration factor for the system's dihedral energy. The boost parameters were calculated as follow:

$$\alpha P = 0.2 \times N_{atoms} \quad (3)$$

$$E_p = V_{PE_avg} + 0.2N_{atoms} \quad (4)$$

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$$\alpha D = \frac{4N_{res}}{5} \quad (5)$$

$$Ed = V_{dihed_avg} + 4N_{res} \quad (6)$$

- 15 In the above equations, N_{atoms} is the total number of atoms, N_{res} is the number of protein residues, V_{PE_avg} is the average potential energy and V_{dihed_avg} is the average dihedral energy. V_{PE_avg} and V_{dihed_avg} for aLMMD were obtained from 50-ns of the corresponding cMD simulations of BDK in benzenes.

- 20 Dual-boost aMD simulations, in which both dihedral energy and total potential energy are boosted, were initiated from the final equilibrated structures of the corresponding cMD simulation runs. Simulations were performed at 300 K and 1 atm. In total, twenty independent 200-ns aLMMD simulations were performed for BDK.

- 25 Trajectory analysis. The 20 individual aLMMD runs for BDK were combined into a single trajectory for analysis. Using the CPPTRAJ module in AMBER 22, the trajectory snapshots saved at 10 ps intervals from the last 70 ns of each simulation were aligned with respect to their Ca atoms before binning the benzene carbon atoms into $1 \text{ \AA} \times 1 \text{ \AA} \times 1 \text{ \AA}$ grid cells to generate benzene occupancy maps. Based on the inverse Boltzmann
30 relationship, the binding free energy associated with a probe atom at voxel i is given by the following equation:

$$\Delta G_i = -RT \ln \frac{N_i}{N_0} \quad (7)$$

In the above equation, R is the gas constant, T is the absolute temperature, and N_i/N_0 is the ratio of the observed occupancy to the expected occupancy. The expected occupancy of a voxel by a probe atom can be obtained using the equation:

$$N_0 = \frac{n_{atoms} \times n_{frames} \times C_p \times N_A}{10^{27}} \quad (8)$$

In the above equation, n_{atoms} is the number of defined probe atoms per molecule ($n_{atoms} = 6$ for benzene), n_{frames} is the total number of trajectory frames being analysed, C_p is the molar concentration of the probe (0.2 M), N_A is the Avogadro constant ($6.02214076 \times 10^{23} \text{ mol}^{-1}$), and 10^{27} is the number of grid cells in 1 L of the simulation box. Benzene occupancy maps were visualised at -1.5 kcal/mol as it has been found to be the best compromise between filtering out spurious binding sites and keeping the true positives. Based on this cutoff, benzenes are approximately at least 12.4 times more likely to be found in the regions indicated by the benzene occupancy maps than the bulk solvent. To compare the overlap of the benzene occupancy maps with known ligands and identify potential new binding sites, ligand-bound structures of BDK were aligned using PyMOL to the respective average protein structure sampled during the simulations.

Pocket clustering. Snapshots from the last 70 ns of the aLMMD runs from human BDK were clustered using the k-means clustering algorithm in the CPPTRAJ1 module of AMBER22. Clustering was based on the heavy-atom root-mean-square deviation (RMSD) of residues that are within $8 \text{ \AA}$ of the ligand in the BT2-bound structure of rat BDK (PDB ID: 4E01). The number of clusters was set to 15 and the centroid of each cluster was selected as the representative structure for visualisation in PyMOL.

25 Compound screening method

Cell survival / proliferation studies

1. Prepare plate for cells seeding.
 - Add 100ul of pbs into each of 96 well plate.
 - Starting seeding density should be around 1,000-2,000 cells per well.
 - Using a multichannel pipette, ensure even cell seeding.
 - Ensure cells are seeded for at least 12 hours before media change into individual conditions.
2. For liver cancer studies, the following modified medium (from original DMEM/RPMI) is used. Branched chain amino acids are substituted with branched chain keto acids. For other cell lines, the original medium is used.

Amino acids	DMEM (Conc. Mm)	RPMI (Conc. Mm)
L-Alanine	NA	NA

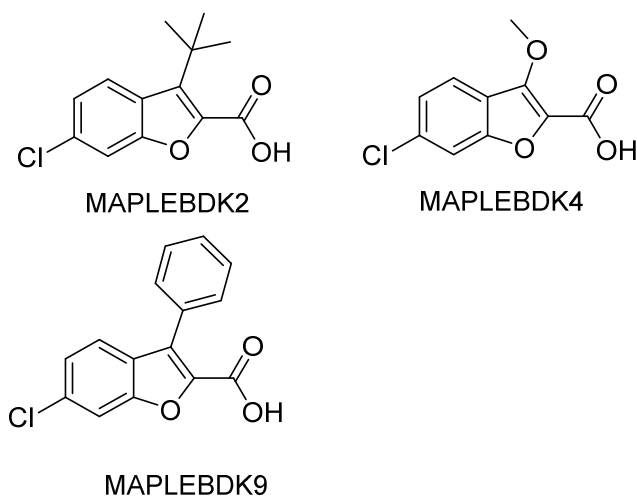
L-Arginine	398	1149
L-Asparagine	NA	379
L-Aspartic acid	NA	150
L-Cystine	201.3	207.7
L-Cysteine	NA	NA
L-Glutamate	NA	136
L-Glutamine	4000	2055
Glycine	400	133
D-Glucose	25000	11111
L-Histidine	200	97
α -ketoisovaleric acid (Ile)	802	382
α -ketoisocaproic acid (Leu)	802	382
L-Lysine	798	219
L-Methionine	201	101
L-Phenylalanine	400	91
L-Proline	NA	174
L-Serine	400	286
L-Threonine	798	168
L-Tryptophan	78	25
L-Tyrosine	399	111
3-methyl-2-oxovaleric acid (Val)	803	171
4-hydroxyproline	NA	152.7

3. Cells are incubated for 3 days with the drugs or DMSO at specified concentrations.
4. Sulforhodamine assay is then conducted to examine the cell survival / proliferation.

5

Identification of inhibitors of human BDK

The MAPLE platform identified 15 potential compounds that have been profiled with molecular docking-based virtual screening, which assesses the binding affinities and interaction profiles of these compounds with the BDK protein. To validate the effectiveness of these compounds, in-vitro experiments were conducted. We have performed cell proliferation assays in both Hep3B wildtype and BDK knockdown cell lines to first assess if these compounds are specific and potent. Three hits **MAPLEBDK2**, **MAPLEBDK4** and **MAPLEBDK9**, showed micromolar potency (Figure 1). In Figure 2, we characterize the growth of Hep3B BDK knockdown cells when exposed to these small molecules at different concentrations. Cell proliferation assays showed that the three hits identified are more specific than PF-07208254.



To further test the effectiveness of the three hits in liver cancer cell lines, we have extended the cell proliferation assays to SNU449 cell line. Consistent with the earlier results, MAPLEBDK2, MAPLEBDK4 and MAPLEBDK9 demonstrate greater potency compared to PF-07208254 (Figure 3).

Further characterization of the three hits in colorectal cancer cell proliferation assays

We recognized that branched chain amino acid catabolism is downregulated in liver cancer but other tumors as well. We further characterize the three hits in colorectal cancer cell line HCT-116. Both MAPLEBDK4 and MAPLEBDK9 outperformed PF-07208254 in restricting the growth of HCT-116 cells (Figure 4). This is consistent with earlier results performed in HCC cell lines.

Toxicology profile of the three hits using non-cancerous cell lines

In addition, we performed survival assays for the compounds in THLE-2 cell line, which is an epithelial liver cell line commonly used for comparison with liver cancer cell lines. We also performed additional studies in ARPE19, an eye cell line and HEK293T, a embryonic kidney cell line. Figure 5 shows that MAPLEBDK4 and MAPLEBDK9 do not cause significant toxic effects on these cell lines while both PF-07208254 and MAPLEBDK2 have minor side effects on these cell lines.

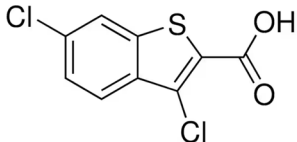
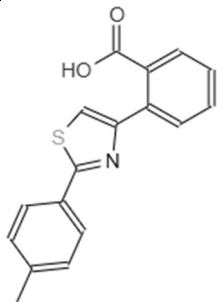
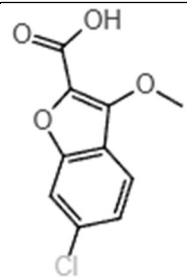
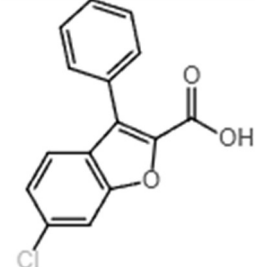
Identification of further inhibitors of human BDK

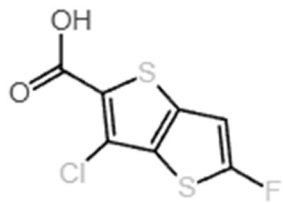
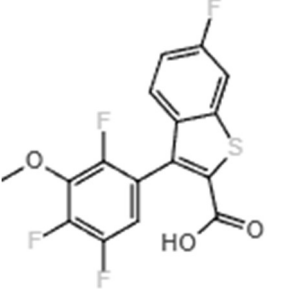
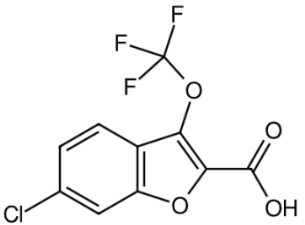
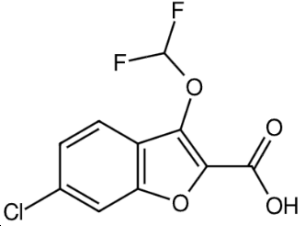
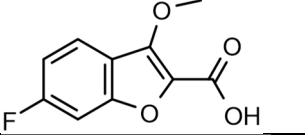
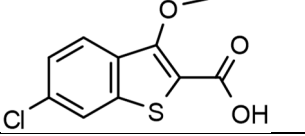
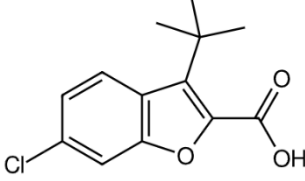
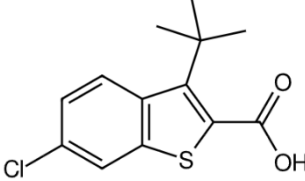
The MAPLE platform has helped us to identify potential compounds that have been profiled with molecular docking-based virtual screening and validated experimentally. To explore further compounds for hit-to-lead optimization, we have performed further computational simulations, namely with AutoDock Vina and DynamicBind simulations. Docking poses of some compounds are shown in Figure 6.

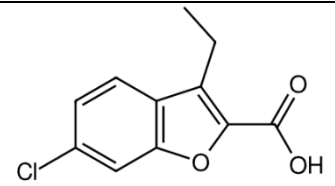
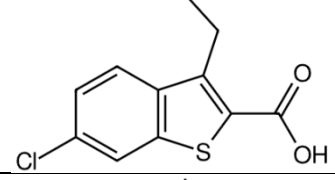
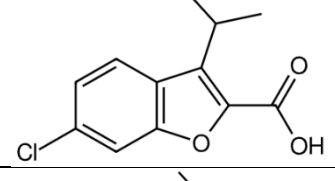
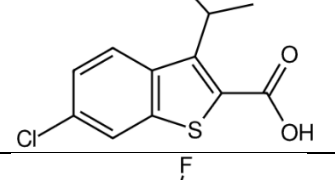
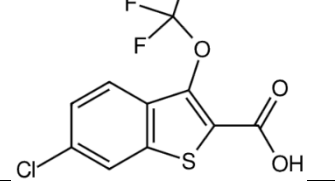
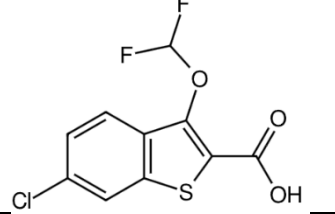
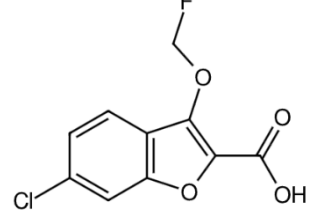
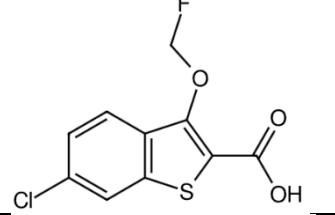
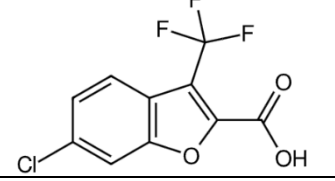
We have included the known BDK inhibitors, BT2, XGT, PF07208254 and PF07328948 as positive controls in our dockings. They have been experimentally validated to inhibit BDK activity via phosphorylation levels of BCKDHA readout. The least negative Autodock Vina score and lowest DynamicBind binding affinity achieved by this set of compounds are -7.3 kcal/mol and 4.926093, respectively, and are used as cutoff values for evaluating our designed compounds. All the compounds reported (excluding the positive controls) in Table 1 dock within the allosteric binding site and score better than one or both of these cutoff values.

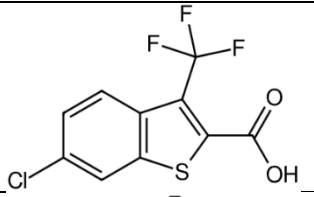
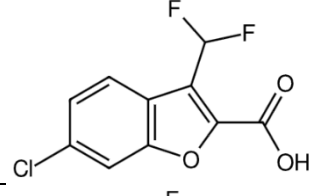
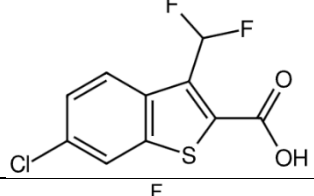
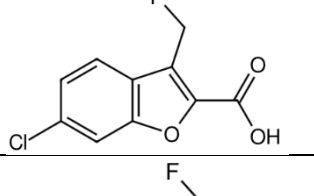
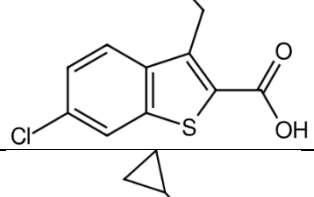
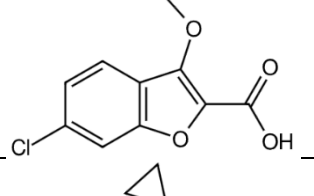
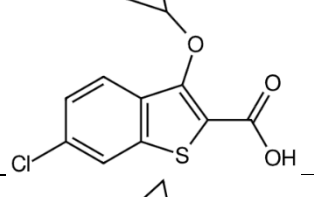
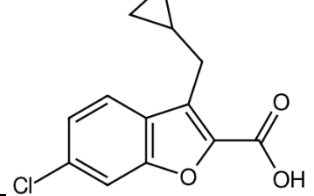
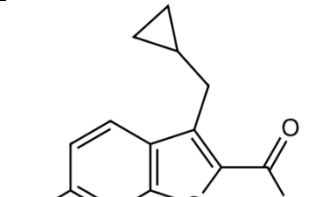
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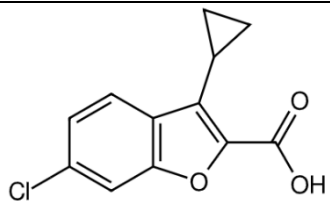
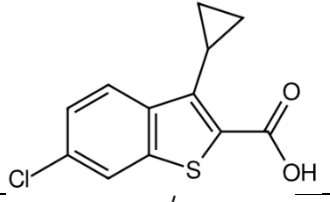
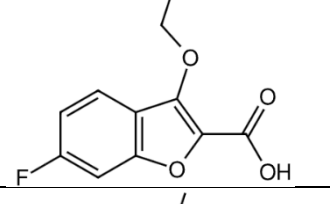
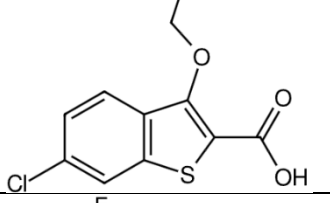
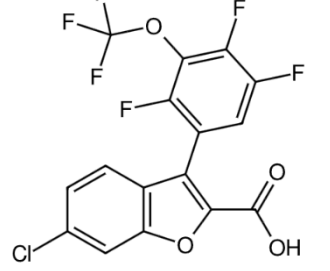
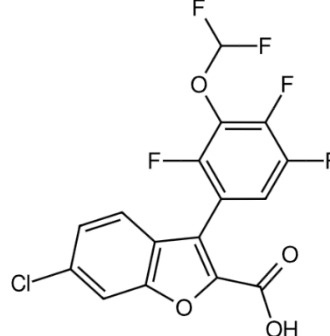
Table 1 indicates the results from the computational simulations. Compounds BT2, XGT, 4, 9, PF07208254 and PF07328948 are known BDK inhibitors.

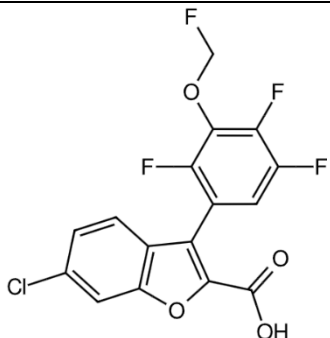
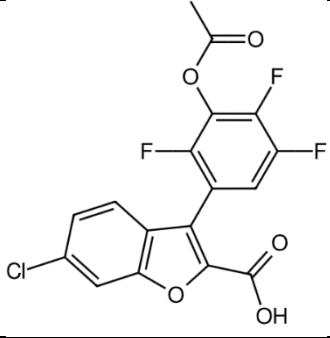
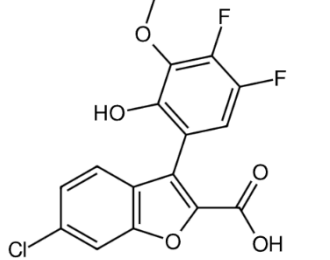
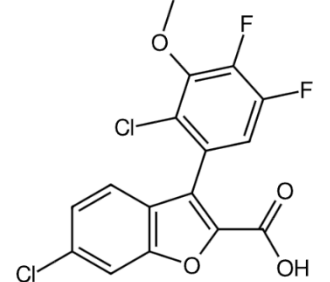
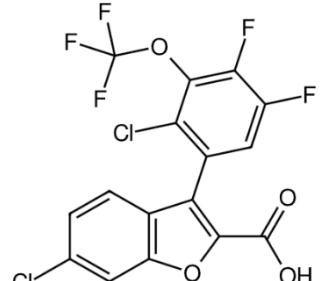
Name	Compound	Autodock Vina dock score (kcal/mol)	DynamicBind cLDDT	DynamicBind binding affinity
BT2		-8.6	0.6968742	4.926093
XGT (8F5S)		-7.3	0.7248195	5.8370786
4		-8.7	0.74873215	4.234713
9		-9.7	0.696963	4.592643

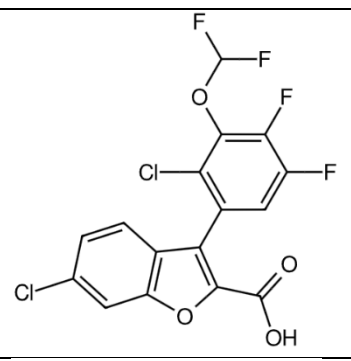
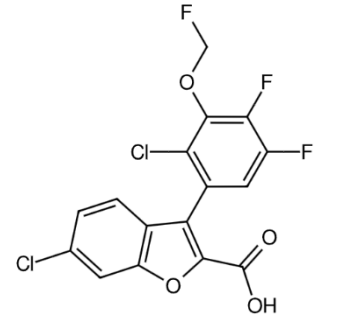
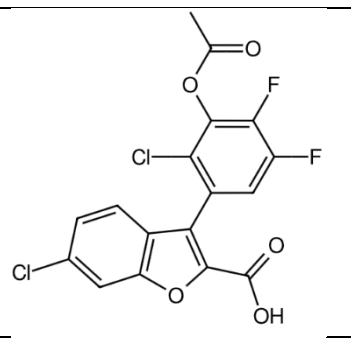
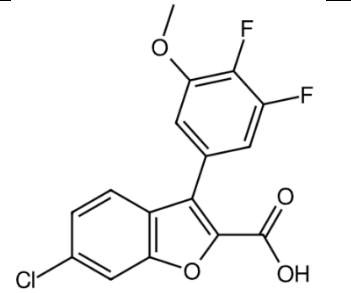
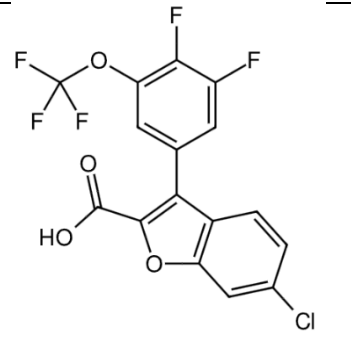
PF07208 254		-7.9	0.70441616	5.420255
PF07328 948		-10	0.6657594	5.607362
4-1		-9.7	0.72343874	4.505844
4-2		-9.3	0.7093725	4.786763
4-3		-8.5	0.7628216	4.428578
4-4		-7.9	0.7073501	4.539552
4-5		-9.5	0.759	4.713
4-6		-8.7	0.755	4.748

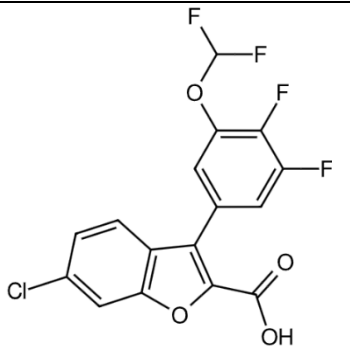
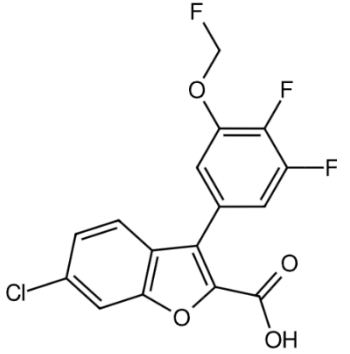
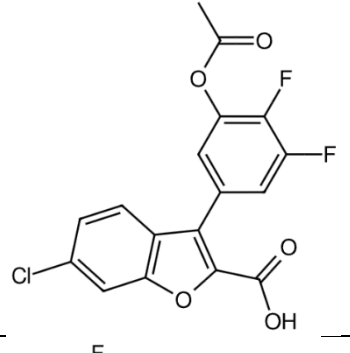
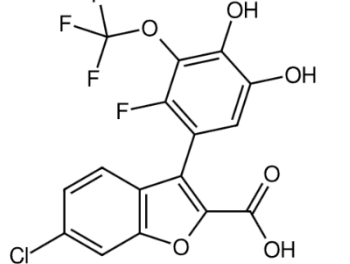
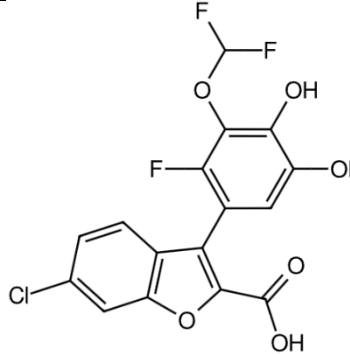
4-7		-9.1	0.726	4.708
4-8		-9.1	0.719	4.761
4-11		-9.9	0.745	4.733
4-12		-8.9	0.726	4.897
4-14		-7.9	0.718	4.61
4-16		-9.1	0.713	4.947
4-17		-8.9	0.729	4.525
4-18		-8.7	0.736	4.556
4-21		-10.2	0.707	4.514

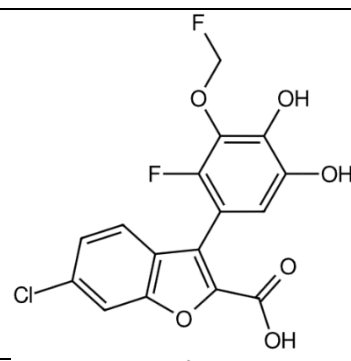
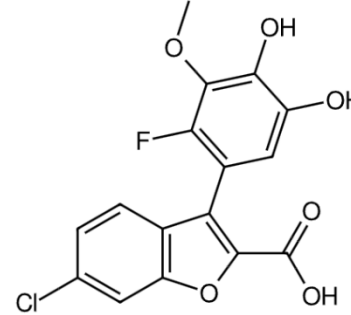
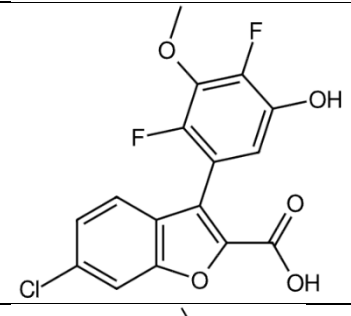
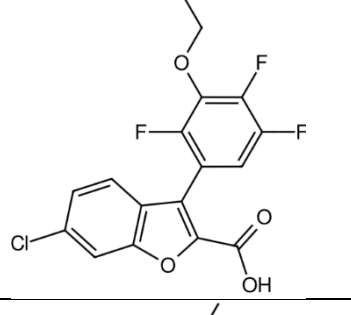
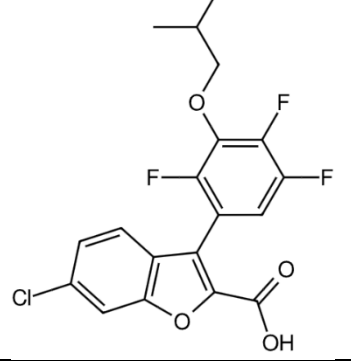
4-22		-9.1	0.705	4.577
4-23		-9.7	0.721	4.501
4-24		-9.2	0.71	4.858
4-25		-8.9	0.73	4.62
4-26		-8.6	0.713	4.801
4-27		-9.4	0.748	4.435
4-28		-8.5	0.746	4.601
4-29		-9.8	0.696	5
4-30		-9	0.718	5.163

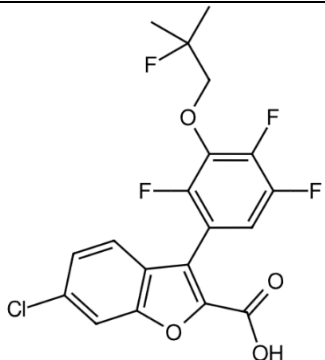
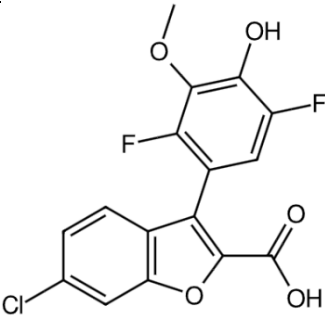
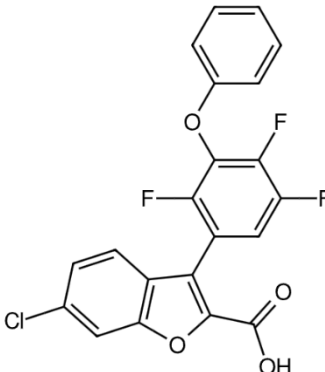
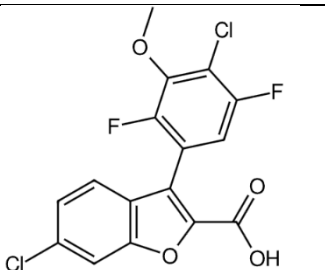
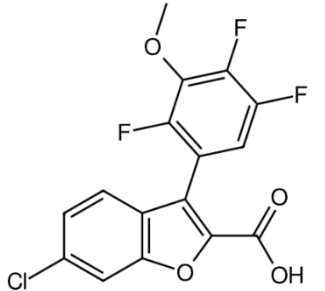
4-31		-9.6	0.706	4.81
4-32		-8.6	0.74	4.86
4-33		-8.9	0.753	4.874
4-34		-8.2	0.738	4.603
9-1		-7.3	0.6127051	5.6721544
9-2		-7.3	0.63904136	5.8004894

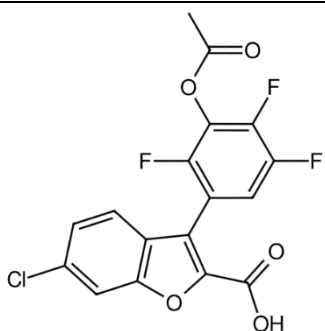
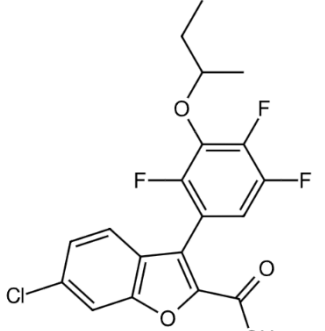
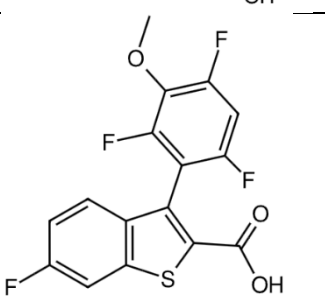
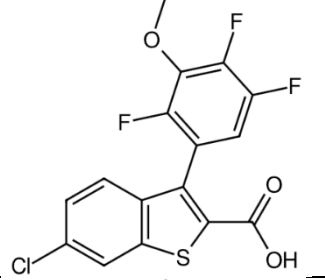
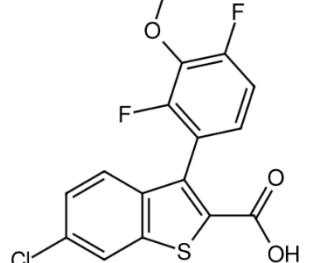
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9-4		-7.4	0.6674723	5.370905
9-5		-8	0.6888136	5.1051903
9-6		-6.9	0.6500763	5.536307
9-7		-6.3	0.63404423	5.5037

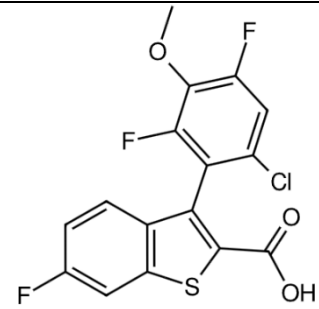
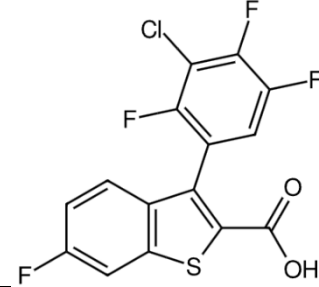
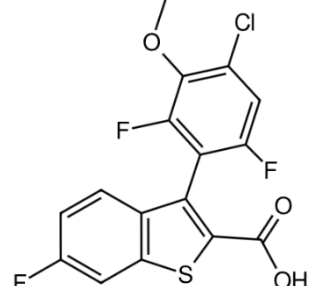
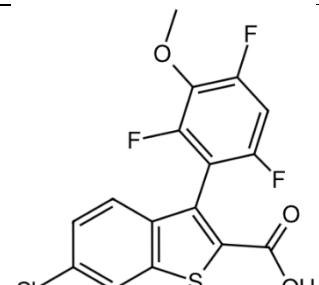
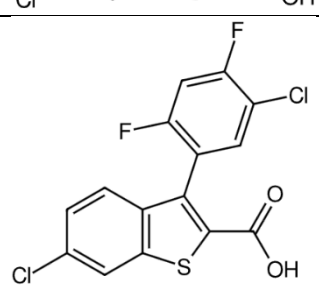
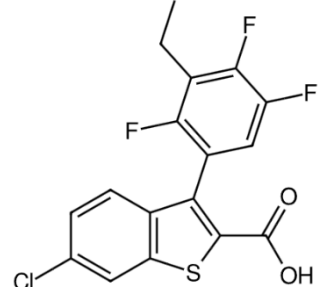
9-8		-8.5	0.6267811	5.776917
9-9		-7.9	0.6600324	5.932401
9-10		-7.2	0.6383678	5.323172
9-11		-7.8	0.6565423	5.31839
9-12		-11.8	0.6261072	5.1936846

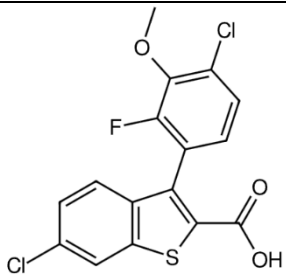
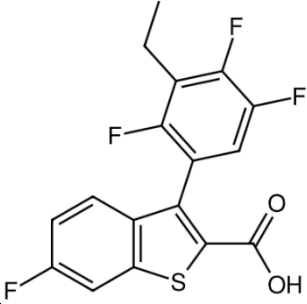
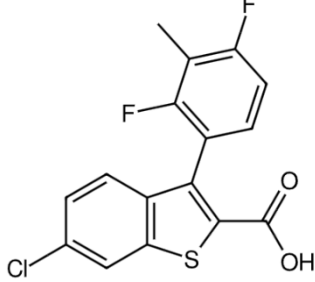
9-13		-8.5	0.6208029	5.7134542
9-14		-7.9	0.66143143	5.511996
9-15		-8.3	0.6511447	4.853844
9-16		-7.1	0.6570821	5.3653493
9-17		-7.4	0.71661323	4.9307704

9-18		-8.4	0.6905448	4.660399
9-19		-8.2	0.67914486	4.2205153
9-20		-6.8	0.65598416	5.4098787
9-21		-7.3	0.6816895	5.5177217
9-22		-7.4	0.6349701	5.672345

9-23		-7.3	0.61619186	5.5929666
9-24		-7	0.73361564	4.678437
9-25		-7.5	0.63431346	5.3217497
9-28		-7.9	0.6568085	5.455569
9-29		-7.6	0.6373642	5.562684

9-30		-7.8	0.642858	5.0401134
9-32		-7.2	0.6593672	5.60506
9-33		-9.3	0.65978074	5.3355327
9-34		-9	0.6625488	5.8591437
9-35		-9.1	0.6463522	5.737741

9-36		-8.1	0.65146554	5.4948072
9-37		-10.2	0.65326977	5.9243965
9-38		-8.1	0.662	5.66
9-39		-8.7	0.657	5.749
9-40		-9	0.669	5.413
9-41		-9.6	0.655	5.809

9-42		-8.3	0.663	5.332
9-43		-10.3	0.68	5.808
9-44		-9.7	0.675	5.635

Experimental validation of compounds after docking experiments

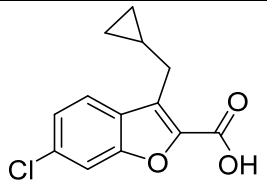
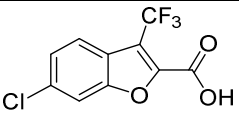
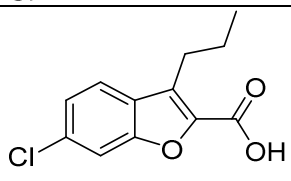
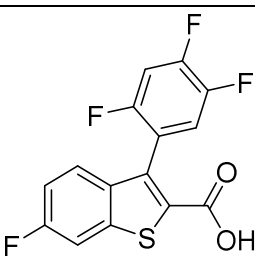
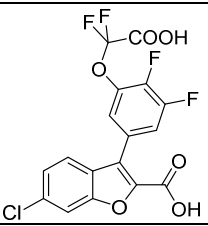
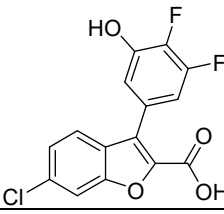
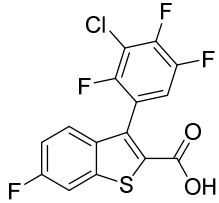
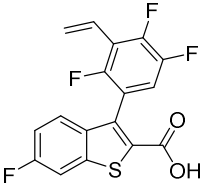
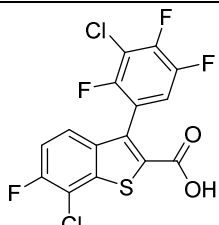
After performing computational analysis on the potential compounds, we selected a few of the compounds and validate them in experiments by performing cell proliferation assays and Western blot analysis.

Firstly, we performed cell proliferation assays in both Hep3B wildtype and BDK knockdown cell lines to assess if these compounds are specific and potent. This time we performed this assay in DMEM classical medium, instead of the BCKA-enriched medium, to ensure ease of reproducibility. We added PF-07328948, which is the clinical phase 1 compound tested by Pfizer. MAPLEBDK4 (A4) showed comparable potency (**Figure 7**). MAPLEBDK9 (A3), M2 and M7 showed significant potency as well. In **Figure 8**, we characterize the growth of Hep3B BDK knockdown cells when exposed to these molecules at different concentrations. Cell proliferation assays showed that the hits identified are more specific than PF-07328948.

Secondly, we performed Western blot analysis to look at the phosphorylation level of BCKDHA, which is directly phosphorylated by BDK. **Figures 9 and 10** showed the

quantification and representative blots, where M2 and M7 showed promising reduction of phosphorylation levels compared to PF-07328948 at 10uM for 3 days.

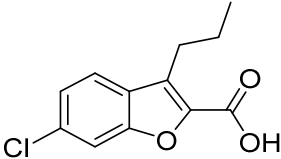
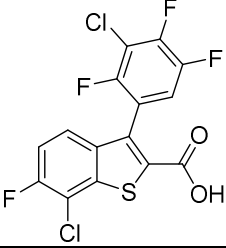
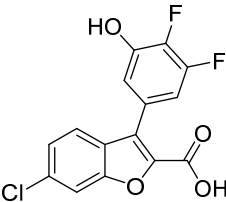
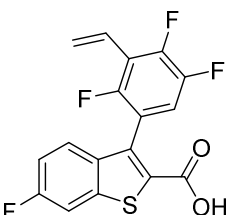
Table 2: Compounds tested for second round of experiments

Cpd Name	Chemical structure	Cpd Name	Chemical structure
A1 (4-29)		M3 (4-21)	
A2		M4	
A3	Compound 9	M5	
A4	Compound 4	M6	
M1 (9-37)		M7	
M2			

5

Table 3: Docking energies for A2, M2, M6, and M7

Compound	Autodock Vina dock score (kcal/mol)	DynamicBind cLDDT	DynamicBind binding affinity

A2 	-8.5	0.722	5.101
M2 	-8.4	0.679	6.261
M6 	-8.5	0.616	6.603
M7 	-10.0	0.690	6.117

We also performed KinomeScan profiling to examine the off-target effects of MAPLEBDK4, MAPLEBDK9 and PF-07328948. Table 4 showed that for 403 targets tested on 10uM of these small molecule inhibitors, MAPLEBDK4 and MAPLEBDK9 showed no target engagement with any of the targets; while PF-07328948 showed slight engagement with two of the targets, DAPK3 and VPS14. Table 5 detailed the interactions percentage, where less than 35% indicated target engagement.

Table 4: Off-target effects of MAPLEBDK4, MAPLEBDK9 and PF-07328948

	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
MAPLEBDK9	S(35)	0	403	10000	0
MAPLEBDK9	S(10)	0	403	10000	0
MAPLEBDK9	S(1)	0	403	10000	0
MAPLEBDK4	S(35)	0	403	10000	0
MAPLEBDK4	S(10)	0	403	10000	0
MAPLEBDK4	S(1)	0	403	10000	0

PF073	S(35)	2	403	10000	0.005
PF073	S(10)	0	403	10000	0
PF073	S(1)	0	403	10000	0

Table 5: Interaction percentage of MAPLEBDK4, MAPLEBDK9 and PF-07328948

DiscoverX Gene Symbol	MAPLEBDK9	MAPLEBDK4	PF-07328948
AAK1	100	100	100
ABL1(E255K)-phosphorylated	76	70	90
ABL1(F317I)-nonphosphorylated	100	100	95
ABL1(F317I)-phosphorylated	90	80	86
ABL1(F317L)-nonphosphorylated	100	100	100
ABL1(F317L)-phosphorylated	100	94	95
ABL1(H396P)-nonphosphorylated	100	100	100
ABL1(H396P)-phosphorylated	100	100	100
ABL1(M351T)-phosphorylated	100	100	100
ABL1(Q252H)-nonphosphorylated	98	92	100
ABL1(Q252H)-phosphorylated	97	100	100
ABL1(T315I)-nonphosphorylated	99	100	94
ABL1(T315I)-phosphorylated	100	94	97
ABL1(Y253F)-phosphorylated	99	100	100
ABL1-nonphosphorylated	100	100	100
ABL1-phosphorylated	100	100	100
ABL2	100	100	100
ACVR1	100	100	100
ACVR1B	99	100	100
ACVR2A	100	100	97
ACVR2B	99	100	100
ACVRL1	72	64	83
ADCK3	100	100	100
ADCK4	100	100	100
AKT1	100	100	100
AKT2	87	87	92
AKT3	100	100	100
ALK	100	100	100
ALK(C1156Y)	100	97	98
ALK(L1196M)	100	100	100
AMPK-alpha1	89	93	100
AMPK-alpha2	88	100	90
ANKK1	85	95	87
ARK5	88	95	100
ASK1	99	100	100
ASK2	68	76	80
AURKA	90	97	100
AURKB	95	86	98
AURKC	100	100	100

AXL	100	100	100
BIKE	100	100	100
BLK	100	100	100
BMPR1A	85	87	81
BMPR1B	91	88	96
BMPR2	88	96	93
BMX	100	100	100
BRAF	95	100	99
BRAF(V600E)	98	100	100
BRK	100	100	100
BRSK1	100	96	96
BRSK2	100	100	100
BTK	78	84	93
BUB1	100	99	100
CAMK1	98	99	96
CAMK1B	97	98	99
CAMK1D	98	99	93
CAMK1G	100	100	100
CAMK2A	100	100	94
CAMK2B	96	100	74
CAMK2D	100	99	100
CAMK2G	100	99	100
CAMK4	79	82	93
CAMKK1	100	100	99
CAMKK2	100	99	100
CASK	93	97	88
CDC2L1	99	100	100
CDC2L2	100	100	100
CDC2L5	100	96	100
CDK11	90	95	50
CDK2	97	100	91
CDK3	100	100	100
CDK4	100	100	100
CDK4-cyclinD1	58	95	100
CDK4-cyclinD3	95	100	100
CDK5	90	96	94
CDK7	93	100	63
CDK8	99	100	62
CDK9	100	100	97
CDKL1	100	99	100
CDKL2	66	87	77
CDKL3	92	98	91
CDKL5	100	98	100
CHEK1	89	87	85
CHEK2	100	100	100

CIT	85	94	76
CLK1	98	98	99
CLK2	100	100	92
CLK3	98	100	100
CLK4	98	100	91
CSF1R	100	100	100
CSF1R-autoinhibited	98	90	98
CSK	97	99	98
CSNK1A1	95	100	94
CSNK1A1L	100	99	100
CSNK1D	100	100	100
CSNK1E	97	95	100
CSNK1G1	100	100	99
CSNK1G2	100	100	100
CSNK1G3	100	99	100
CSNK2A1	98	40	67
CSNK2A2	72	57	68
CTK	100	99	100
DAPK1	97	98	38
DAPK2	98	100	35
DAPK3	88	100	23
DCAMKL1	64	83	72
DCAMKL2	100	89	100
DCAMKL3	76	80	85
DDR1	54	83	89
DDR2	100	100	100
DLK	100	100	100
DMPK	99	100	99
DMPK2	100	100	96
DRAK1	94	100	84
DRAK2	99	100	75
DYRK1A	95	85	88
DYRK1B	74	87	76
DYRK2	100	100	96
EGFR	85	89	100
EGFR(E746-A750del)	97	92	89
EGFR(G719C)	88	98	88
EGFR(G719S)	100	100	100
EGFR(L747-E749del, A750P)	100	100	100
EGFR(L747-S752del, P753S)	100	97	100
EGFR(L747-T751del,Sins)	100	98	95
EGFR(L858R)	89	98	100
EGFR(L858R,T790M)	87	92	76
EGFR(L861Q)	100	97	96
EGFR(S752-I759del)	100	100	99

EGFR(T790M)	100	100	100
EIF2AK1	100	100	100
EPHA1	94	98	93
EPHA2	100	100	100
EPHA3	100	100	98
EPHA4	99	98	100
EPHA5	86	87	88
EPHA6	94	99	100
EPHA7	100	100	100
EPHA8	99	100	100
EPHB1	95	100	100
EPHB2	100	100	100
EPHB3	99	100	99
EPHB4	94	99	98
EPHB6	100	100	100
ERBB2	83	100	100
ERBB3	96	93	89
ERBB4	100	100	100
ERK1	100	100	100
ERK2	94	95	96
ERK3	100	100	100
ERK4	100	100	100
ERK5	100	100	100
ERK8	100	100	91
ERN1	94	98	92
FAK	100	100	100
FER	100	100	100
FES	100	99	100
FGFR1	96	100	98
FGFR2	100	100	100
FGFR3	100	100	100
FGFR3(G697C)	100	100	100
FGFR4	96	100	100
FGR	100	100	100
FLT1	99	100	100
FLT3	100	100	100
FLT3(D835H)	100	100	100
FLT3(D835V)	100	100	100
FLT3(D835Y)	94	97	95
FLT3(ITD)	100	100	100
FLT3(ITD,D835V)	100	100	100
FLT3(ITD,F691L)	42	46	35
FLT3(K663Q)	73	93	85
FLT3(N841I)	91	100	97
FLT3(R834Q)	100	94	98

FLT3-autoinhibited	100	100	100
FLT4	100	100	100
FRK	100	100	100
FYN	100	100	100
GAK	96	100	98
GCN2(Kin.Dom.2,S808G)	76	86	97
GRK1	90	95	100
GRK2	77	82	85
GRK3	81	84	86
GRK4	100	99	100
GRK7	84	87	94
GSK3A	87	100	100
GSK3B	96	94	100
HASPIN	88	80	80
HCK	92	87	99
HIPK1	86	85	50
HIPK2	100	100	100
HIPK3	92	79	78
HIPK4	84	99	99
HPK1	89	94	71
HUNK	83	100	98
ICK	100	98	95
IGF1R	100	100	100
IKK-alpha	91	98	100
IKK-beta	93	100	100
IKK-epsilon	96	100	100
INSR	100	100	100
INSRR	90	94	96
IRAK1	100	100	100
IRAK3	88	85	96
IRAK4	92	97	97
ITK	100	100	100
JAK1(JH1domain-catalytic)	91	94	100
JAK1(JH2domain-pseudokinase)	52	92	92
JAK2(JH1domain-catalytic)	94	93	90
JAK3(JH1domain-catalytic)	100	100	100
JNK1	100	100	100
JNK2	96	100	93
JNK3	90	100	94
KIT	95	93	97
KIT(A829P)	100	89	100
KIT(D816H)	100	95	100
KIT(D816V)	100	100	100
KIT(L576P)	88	95	94
KIT(V559D)	100	100	100

KIT(V559D,T670I)	94	99	98
KIT(V559D,V654A)	77	100	100
KIT-autoinhibited	97	95	91
LATS1	93	100	90
LATS2	94	91	95
LCK	93	100	100
LIMK1	100	100	100
LIMK2	100	100	100
LKB1	86	92	100
LOK	97	98	100
LRRK2	100	100	100
LRRK2(G2019S)	100	96	100
LTK	87	88	92
LYN	91	100	100
LZK	100	100	100
MAK	100	100	100
MAP3K1	99	97	100
MAP3K15	100	100	92
MAP3K2	100	100	100
MAP3K3	77	94	89
MAP3K4	100	100	100
MAP4K2	82	87	72
MAP4K3	100	100	100
MAP4K4	97	100	97
MAP4K5	100	100	100
MAPKAPK2	100	95	94
MAPKAPK5	99	99	100
MARK1	100	100	100
MARK2	100	100	99
MARK3	71	73	50
MARK4	100	100	100
MAST1	98	99	100
MEK1	86	86	87
MEK2	86	83	91
MEK3	100	97	99
MEK4	94	89	100
MEK5	89	100	100
MEK6	100	98	100
MELK	100	99	99
MERTK	100	100	100
MET	93	100	96
MET(M1250T)	99	100	100
MET(Y1235D)	100	100	100
MINK	88	77	85
MKK7	100	94	100

MKNK1	100	100	100
MKNK2	73	95	81
MLCK	100	100	100
MLK1	100	100	96
MLK2	84	100	96
MLK3	85	100	91
MRCKA	100	100	100
MRCKB	100	100	100
MST1	100	97	100
MST1R	93	80	86
MST2	100	100	100
MST3	90	92	80
MST4	78	88	82
MTOR	82	74	78
MUSK	87	93	95
MYLK	87	94	100
MYLK2	90	97	96
MYLK4	100	100	100
MYO3A	96	100	90
MYO3B	93	93	89
NDR1	82	83	86
NDR2	100	100	100
NEK1	100	100	100
NEK10	87	95	62
NEK11	100	97	100
NEK2	100	100	100
NEK3	100	100	93
NEK4	100	95	100
NEK5	100	100	100
NEK6	100	100	97
NEK7	59	91	79
NEK9	96	90	100
NIK	79	74	57
NIM1	95	100	97
NLK	100	100	82
OSR1	69	66	78
p38-alpha	99	100	94
p38-beta	100	100	100
p38-delta	73	73	70
p38-gamma	99	100	100
PAK1	100	100	100
PAK2	100	100	93
PAK3	77	73	83
PAK4	98	100	100
PAK6	100	98	100

PAK7	100	100	95
PCTK1	100	97	100
PCTK2	100	100	100
PCTK3	100	100	100
PDGFRA	98	98	92
PDGFRB	91	97	98
PDPK1	100	100	100
PFCDPK1(P.falciparum)	98	100	100
PFPK5(P.falciparum)	98	100	98
PFTAIRE2	100	100	100
PFTK1	100	100	96
PHKG1	88	99	100
PHKG2	100	100	72
PIK3C2B	74	74	53
PIK3C2G	93	93	70
PIK3CA	77	80	69
PIK3CA(C420R)	100	100	95
PIK3CA(E542K)	97	89	91
PIK3CA(E545A)	100	100	100
PIK3CA(E545K)	91	87	89
PIK3CA(H1047L)	80	88	77
PIK3CA(H1047Y)	60	81	56
PIK3CA(I800L)	93	94	94
PIK3CA(M1043I)	100	98	90
PIK3CA(Q546K)	100	90	93
PIK3CB	97	100	66
PIK3CD	96	96	85
PIK3CG	100	99	61
PIK4CB	100	100	96
PIKFYVE	97	100	100
PIM1	82	94	36
PIM2	70	83	42
PIM3	86	93	46
PIP5K1A	100	100	100
PIP5K1C	91	100	80
PIP5K2B	78	80	70
PIP5K2C	96	97	80
PKAC-alpha	100	100	100
PKAC-beta	100	100	100
PKMYT1	100	100	97
PKN1	87	92	94
PKN2	100	100	100
PKNB(M.tuberculosis)	98	99	89
PLK1	79	79	75
PLK2	88	82	85

PLK3	92	78	90
PLK4	91	90	98
PRKCD	100	100	100
PRKCE	78	100	100
PRKCH	87	97	85
PRKCI	100	100	100
PRKCQ	100	100	96
PRKD1	81	83	94
PRKD2	91	100	100
PRKD3	80	100	95
PRKG1	100	100	100
PRKG2	99	92	100
PRKR	87	80	83
PRKX	76	75	81
PRP4	84	95	100
PYK2	100	100	100
QSK	79	94	88
RAF1	95	96	87
RET	100	100	100
RET(M918T)	100	100	100
RET(V804L)	100	100	100
RET(V804M)	89	83	81
RIOK1	100	100	100
RIOK2	100	90	94
RIOK3	91	81	79
RIPK1	100	100	99
RIPK2	84	100	98
RIPK4	100	100	100
RIPK5	92	88	93
ROCK1	100	98	100
ROCK2	100	100	100
ROS1	98	100	100
RPS6KA4(Kin.Dom.1-N-terminal)	100	100	100
RPS6KA4(Kin.Dom.2-C-terminal)	97	96	47
RPS6KA5(Kin.Dom.1-N-terminal)	100	100	96
RPS6KA5(Kin.Dom.2-C-terminal)	92	100	91
RSK1(Kin.Dom.1-N-terminal)	93	95	93
RSK1(Kin.Dom.2-C-terminal)	97	100	66
RSK2(Kin.Dom.1-N-terminal)	95	98	100
RSK2(Kin.Dom.2-C-terminal)	100	100	99
RSK3(Kin.Dom.1-N-terminal)	100	95	100
RSK3(Kin.Dom.2-C-terminal)	99	95	58
RSK4(Kin.Dom.1-N-terminal)	100	100	100
RSK4(Kin.Dom.2-C-terminal)	94	97	85
S6K1	99	74	87

SBK1	100	78	100
SGK	94	94	94
SgK110	100	100	100
SGK2	97	94	91
SGK3	85	75	68
SIK	94	97	93
SIK2	59	66	68
SLK	100	100	100
SNARK	80	92	91
SNRK	69	79	73
SRC	100	100	100
SRMS	81	92	79
SRPK1	74	88	90
SRPK2	89	94	93
SRPK3	81	78	81
STK16	100	100	100
STK33	100	100	98
STK35	100	100	100
STK36	100	100	100
STK39	80	89	92
SYK	87	95	92
TAK1	100	100	100
TAOK1	87	86	85
TAOK2	82	87	83
TAOK3	94	98	86
TBK1	94	96	97
TEC	97	100	100
TESK1	100	100	100
TGFBR1	100	100	100
TGFBR2	92	88	100
TIE1	98	96	100
TIE2	92	96	88
TLK1	100	100	99
TLK2	100	100	100
TNIK	100	100	99
TNK1	100	100	100
TNK2	100	100	100
TNNI3K	82	100	84
TRKA	100	100	100
TRKB	100	100	100
TRKC	100	97	100
TRPM6	100	100	100
TSSK1B	67	77	88
TSSK3	100	94	82
TTK	91	95	90

TXK	100	100	100
TYK2(JH1domain-catalytic)	100	100	100
TYK2(JH2domain-pseudokinase)	90	85	60
TYRO3	100	97	100
ULK1	96	89	100
ULK2	95	99	100
ULK3	81	76	90
VEGFR2	100	100	99
VPS34	61	72	14
VRK2	89	87	80
WEE1	98	99	100
WEE2	100	100	100
WNK1	100	100	100
WNK2	100	100	100
WNK3	59	67	76
WNK4	86	95	100
YANK1	100	96	100
YANK2	93	100	91
YANK3	81	85	83
YES	100	100	100
YSK1	72	88	72
YSK4	100	97	99
ZAK	100	100	100
ZAP70	90	89	91

Methods for running the computational simulations

Receptor and ligand preparation

The structure of human BDK (PDB ID 8F5J) was used as the receptor structure for docking. Crystallographic bound ligands and buffer ions were removed before it was prepared using AutoDockTools in MGLTools 1.5.7, in which polar hydrogens and Gasteiger charges were added. The 3D structures of the compounds were generated using the LigPrep tool of Schrodinger suite version 2022-3. Outputs for the receptor and ligands were saved in PDBQT format for Autodock Vina docking.

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Docking procedure

Docking of compounds was performed using AutoDock Vina 1.1.2. Exhaustiveness was set to 64 and maximum number of output poses was set to 10. Three rounds of docking were performed on each compound. The docking grid box center coordinates and dimensions were set based on the allosteric binding site in the BDK complex structure (PDB ID 8F5J), with additional allowance given for consideration of pocket expansion and to allow larger compound analogues to fit into the pocket. To enable optimal fitting of ligands to the receptor structure, residues Y99, H132 and R171 were made flexible

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during docking. These residues were selected based on visual inspection of all human BDK crystal structures. All docked poses were visualized in PyMOL and the most negative score from the three replicate runs was reported for each compound.

5 DynamicBind analysis

We utilized DynamicBind6 provided by the Neurosnap platform (<https://neurosnap.ai/service/DynamicBind>) to analyze our compounds. Human BCKDK (PDB ID 8F5S) was used as the receptor structure. The online server generated docking poses, which were visualized using PyMOL5 for each ligand. A list of contact-LDDT (cLDDT) scores and predicted binding affinities were generated. Higher cLDDT values indicate a closer match to the true binding pose and higher binding affinity values indicate a stronger predicted affinity.

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended to embrace all such alterations, modifications, and variations that fall within the spirit and scope of the appended claims.

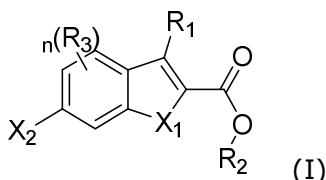
Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase "consisting essentially of", and variations such as "consists essentially of" will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Claims

1. A method of treating a disease or condition associated with branched-chain ketoacid dehydrogenase kinase (BDK) in a subject in need thereof, comprising
5 administering a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof:



wherein

- R_1 is selected from optionally substituted alkyl, optionally substituted alkoxy, optionally
10 substituted cycloalkyl, aryl optionally substituted with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy; and

R_2 is selected from H, optionally substituted alkyl or optionally substituted aryl;

R_3 is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino;

- 15 X_1 is selected from O or S;

X_2 is halo; and

n is an integer selected from 0 to 3.

2. A compound of Formula (I) or a pharmaceutically acceptable salt, solvate or
20 prodrug thereof for use in treating a disease or condition associated with BDK.

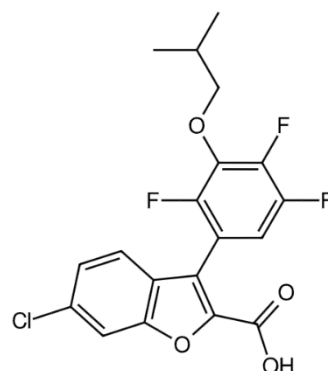
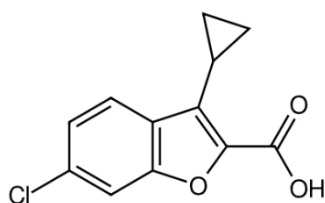
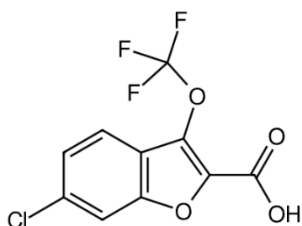
3. Use of a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof in the manufacture of a medicament for treating a disease or condition associated with BDK.

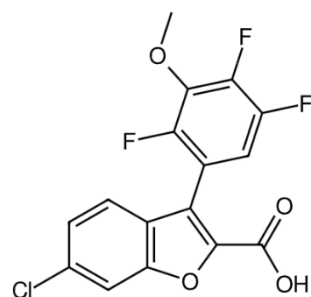
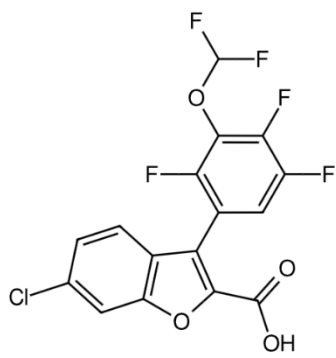
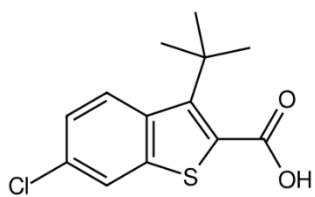
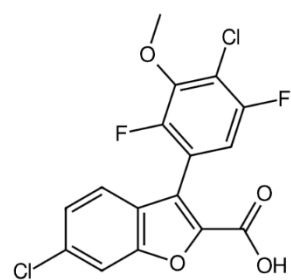
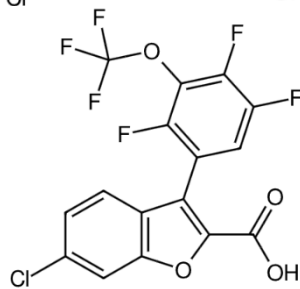
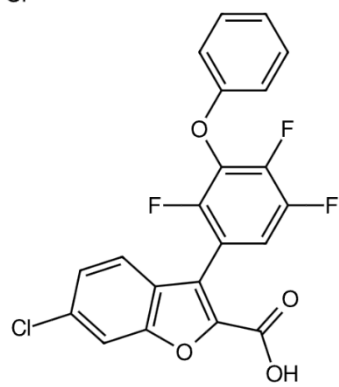
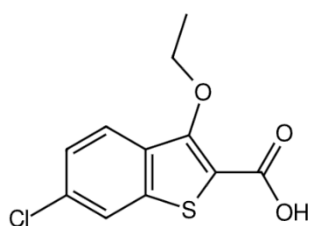
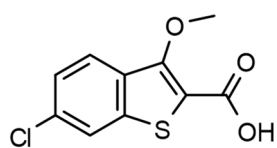
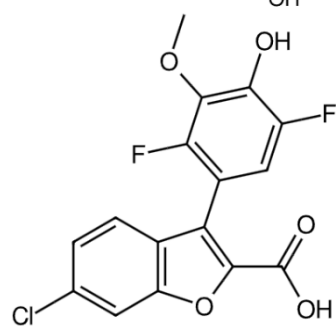
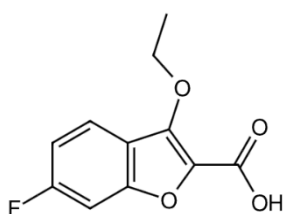
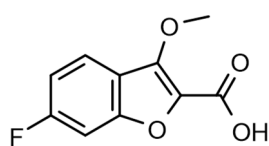
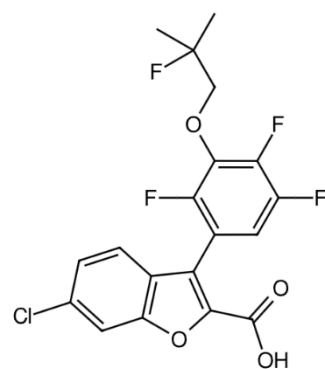
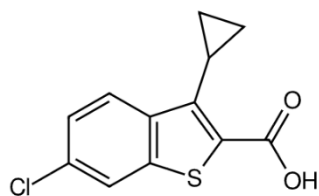
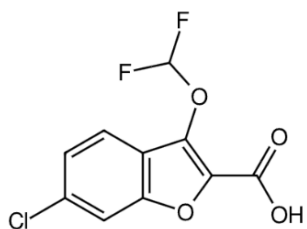
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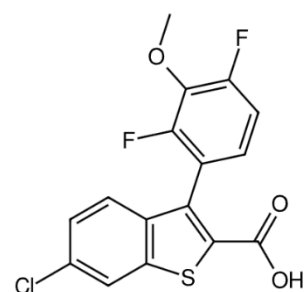
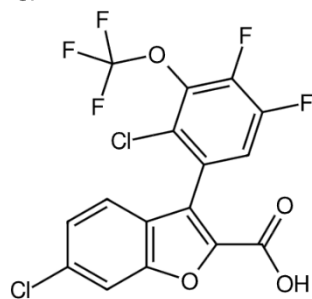
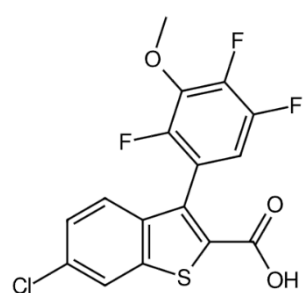
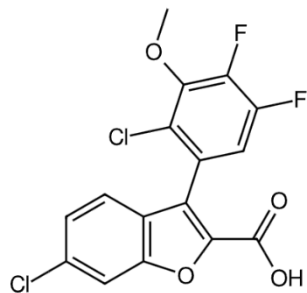
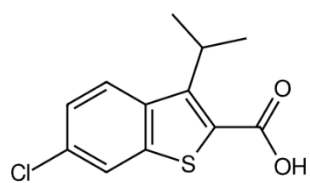
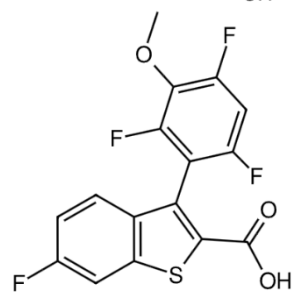
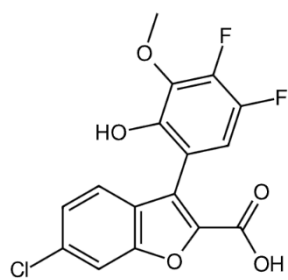
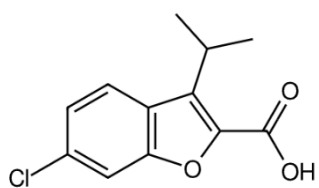
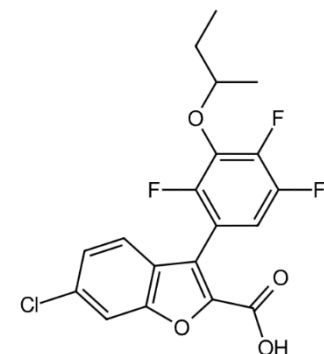
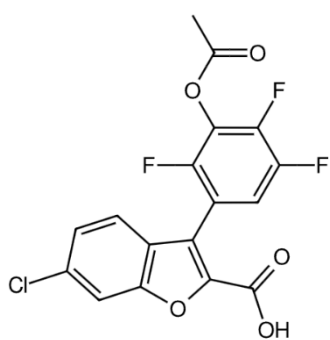
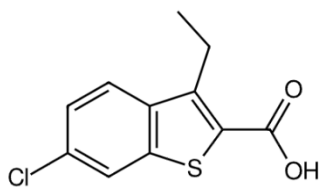
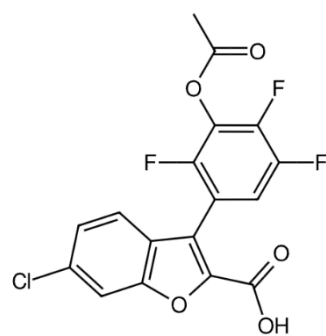
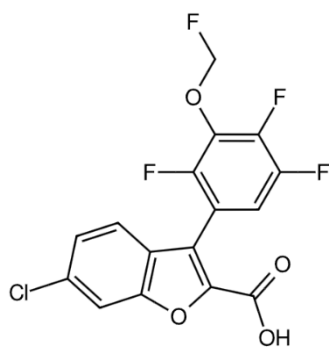
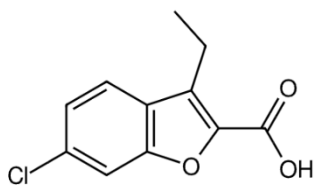
4. The method, compound for use or use according to any one of claims 1 to 3, wherein the disease or condition associated with BDK is a cancer or a proliferative disease.

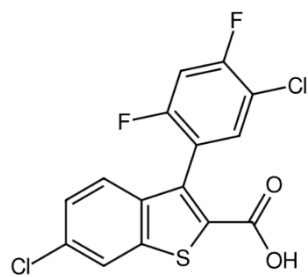
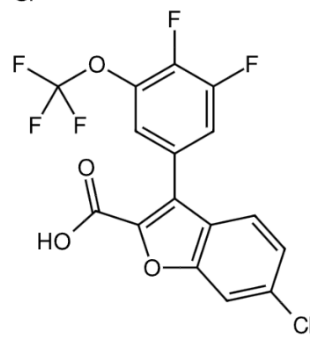
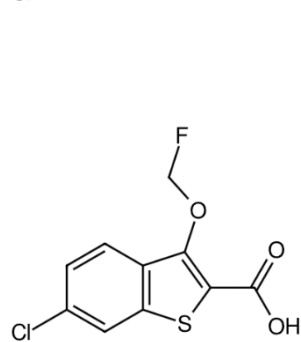
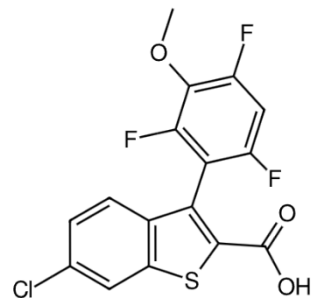
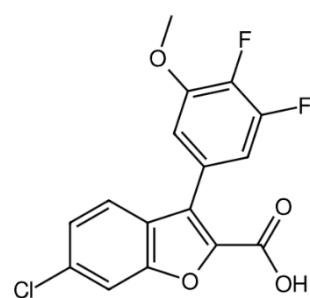
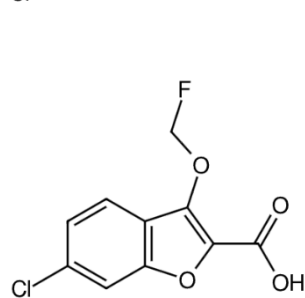
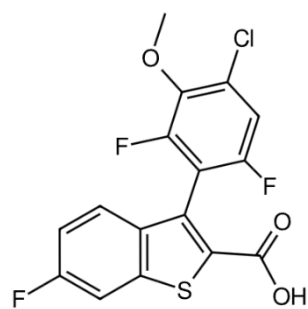
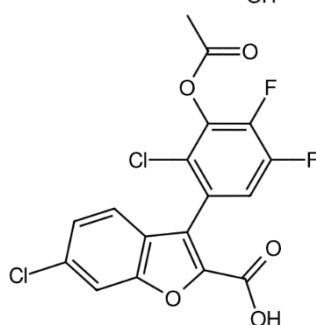
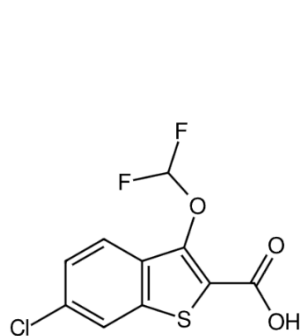
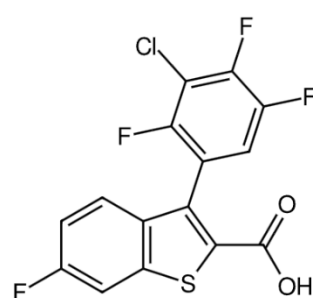
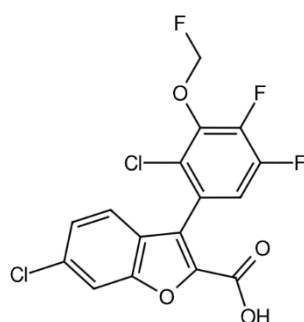
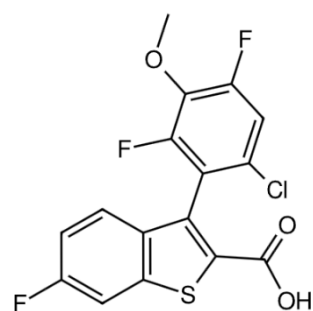
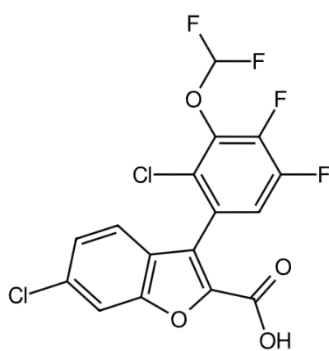
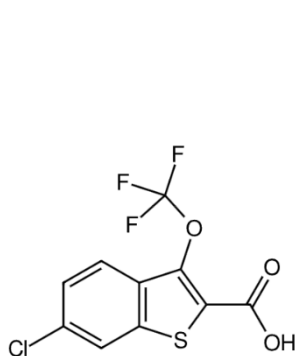
- 30 5. The method, compound for use or use according to any one of claims 1 to 4, wherein the disease or condition associated with BDK is selected from liver cancer, colorectal cancer, kidney cancer, stomach cancer, and head and neck cancer.

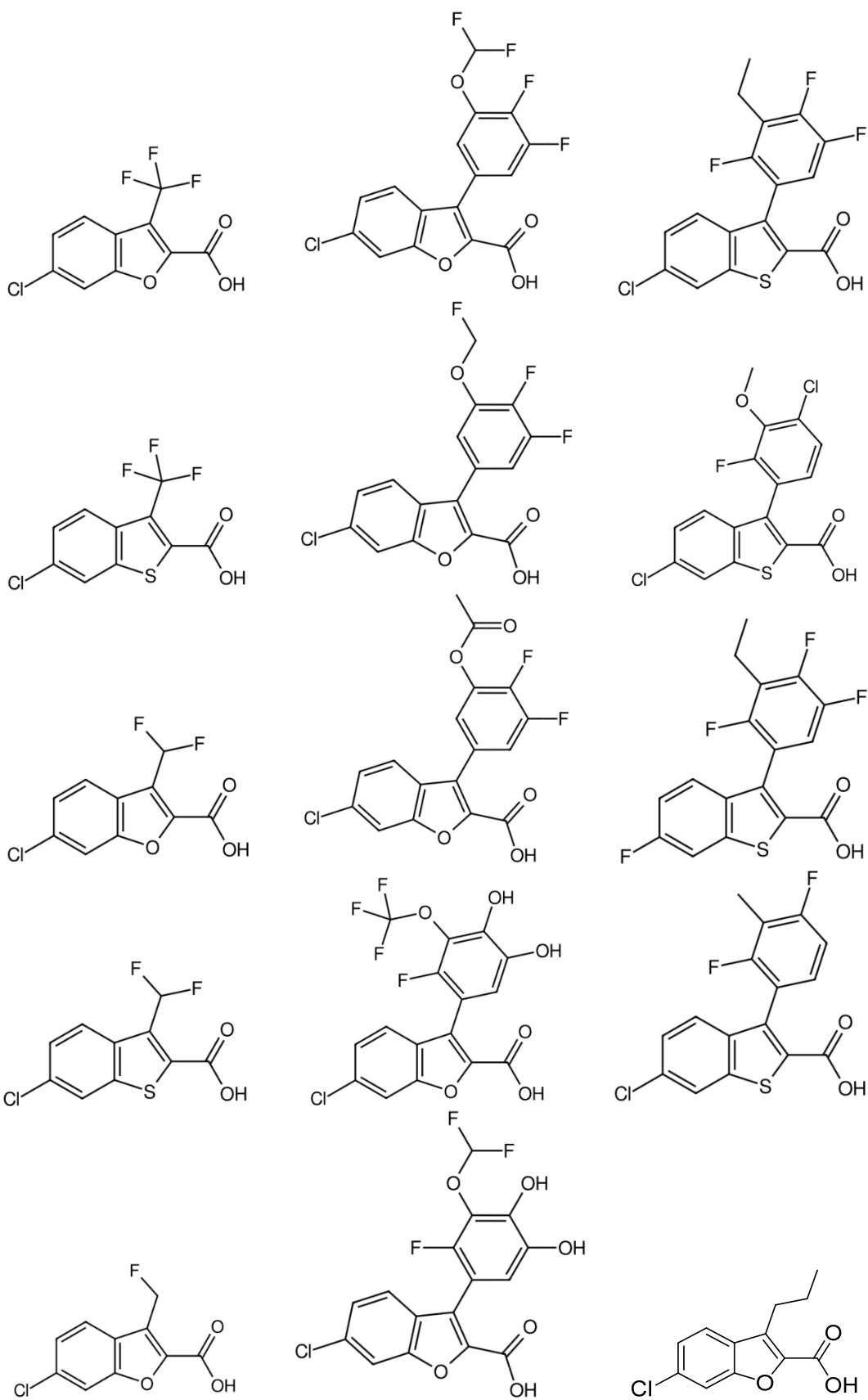
6. The method, compound for use, or use according to any one of claims 1 to 5, wherein the compound of Formula (I) is characterised by a cytotoxicity of less than about 20%.
- 5 7. The method, compound for use, or use according to any one of claims 1 to 6, wherein the compound of Formula (I) is characterised by an off-target selectivity score of less than 0.005.
8. The method, compound for use or use according to any one of claims 1 to 7, wherein R₁ is selected from optionally substituted C₁-C₅ alkyl, optionally substituted C₁-C₅ alkoxy, and phenyl substituted 3 or 4 times with alkyl, alkenyl, halogen, oxo, alkoxy or acyloxy.
- 10 9. The method, compound for use or use according to any one of claims 1 to 8, wherein R₁ is phenyl meta substituted with alkyl, alkenyl, oxo, alkoxy or acyloxy, ortho substituted with halo or oxy, and para substituted with halo or oxy.
- 15 10. The method, compound for use or use according to any one of claims 1 to 9, wherein R₂ is H.
- 20 11. The method, compound for use or use according to any one of claims 1 to 10, wherein R₃ is halo.
12. The method, compound for use or use according to any one of claims 1 to 11, wherein n is an integer selected from 0 to 1.
- 25 13. The method, compound for use or use according to any one of claims 1 to 12, wherein the compound of Formula (I) is selected from

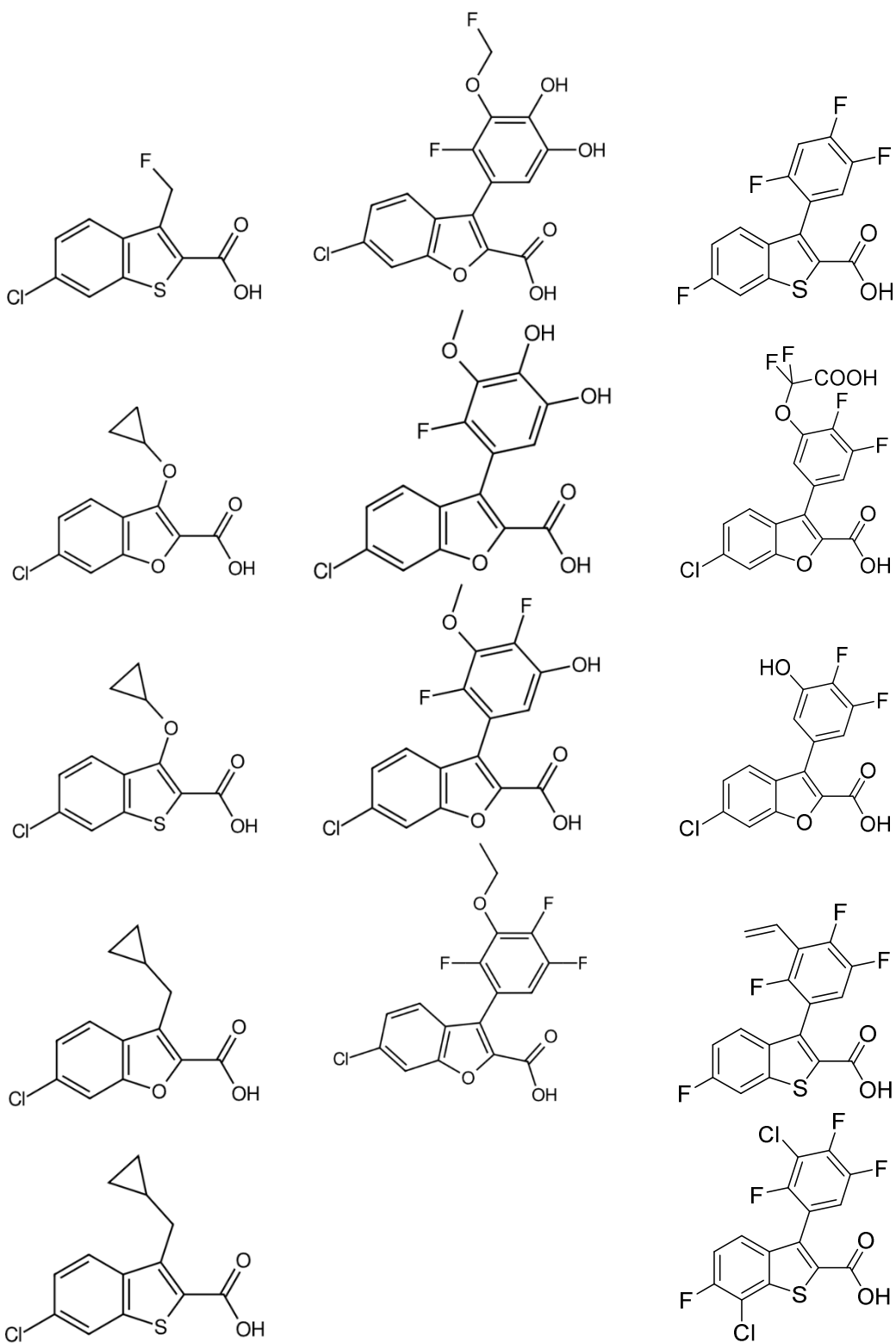


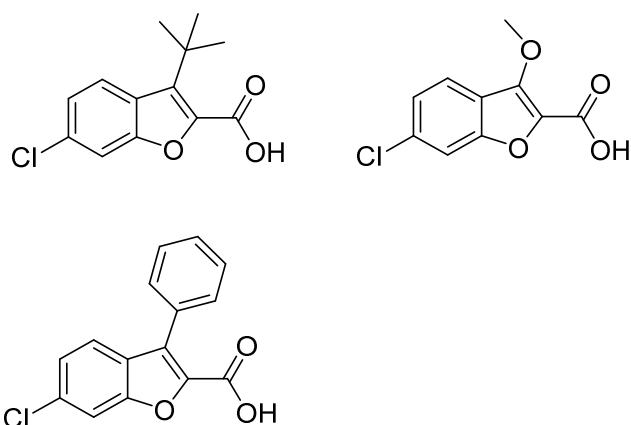




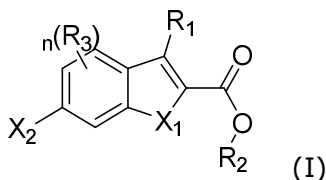








14. A compound of Formula (I) or a pharmaceutically acceptable salt, solvate or prodrug thereof:



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wherein

R₁ is selected from optionally substituted C₂-C₆ alkyl, optionally substituted C₂-C₆ alkoxy, optionally substituted C₃-C₆ cycloalkyl, or aryl optionally substituted 1 to 4 times with alkyl, alkenyl, halo, oxo, alkoxy and acyloxy;

10 R₂ is selected from H, optionally substituted alkyl or optionally substituted aryl;

R₃ is selected from halo, oxo, nitro, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted amino;

X₁ is selected from O or S;

X₂ is selected from Cl or F; and

15 n is an integer selected from 0 to 3.

15. The compound according to claim 14, wherein R₁ is selected from optionally substituted C₂-C₆ alkyl, optionally substituted C₂-C₆ alkoxy, optionally substituted C₃-C₆ cycloalkyl, or phenyl substituted 3 or 4 times with alkyl, alkenyl, halo, oxo, alkoxy and
20 acyloxy.

16. The compound according to claim 14 or 15, wherein phenyl is meta substituted with alkyl, alkenyl, oxo, alkoxy or acyloxy, ortho substituted with halo or oxy, and para substituted with halo or oxy.

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17. The compound according to any one of claims 14 to 16, wherein R₂ is H.

18. The compound according to any one of claims 14 to 17, wherein R_3 is halo.
19. The compound according to any one of claims 14 to 18, wherein n is an integer
5 selected from 0 to 1.
20. A pharmaceutical composition comprising a compound of Formula (I) according to any one of claims 14 to 19, or a pharmaceutically acceptable salt, solvate or prodrug thereof.