
PIPERIDINE INHIBITOR OF SLC6A19 FUNCTION

RELATED APPLICATION

This application claims the benefit of priority to U.S. Provisional Patent Application No. 63/472,979, filed June 14, 2023.

BACKGROUND

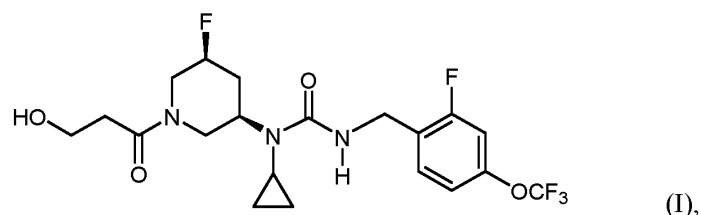
Phenylketonuria (PKU) is an inborn error of metabolism caused by mutations in phenylalanine hydroxylase (PAH), the enzyme responsible for metabolizing phenylalanine. PKU is an autosomal recessive metabolic disorder in which phenylalanine is not properly metabolized and results in abnormally high levels of plasma phenylalanine. People who have PKU have abnormally high blood levels of phenylalanine, which if untreated can lead to irreversible neurological damage resulting in a spectrum of complications such as intellectual disabilities, seizures, neurodevelopmental and behavioral disorders. PKU is difficult to treat because blood levels of phenylalanine are directly related to diet. Patients must adhere to a life-long and strict diet that impacts all aspects of patients' lives. Current standard of care are enzyme co-factor and enzyme substitution therapy, but these therapies are not effective in all patients, and carry potential risk for adverse events.

The enzyme responsible for metabolizing phenylalanine, and thus maintaining phenylalanine homeostasis is phenylalanine hydroxylase (PAH). Loss-of-function (LOF) mutations at PAH gene at chromosome 12q23.2 are known to cause most forms of PKU. These LOF mutations resulting in PKU can be diagnosed as classical PKU (the most severe form), and "mild PKU" or "hyperphe" a less severe form. In addition to PAH, mutations in other enzymes that affect phenylalanine metabolism, such as dihydropteridine reductase (DHPR), the enzyme responsible for synthesis of co-factors required for PAH activity, may also result in elevated levels of phenylalanine. In addition to diet, blood amino acid levels, including levels of phenylalanine, are regulated by SLC6A19. SCL6A19 is located in the proximal tubule of the kidney and is responsible for reabsorption of amino acids back into the blood.

SUMMARY

Disclosed are a compound and pharmaceutically acceptable salts thereof, a pharmaceutical composition, and related methods for treating or preventing a disease or disorder associated with abnormal levels of amino acids via modulation of the SLC6A19 transporter.

One aspect of the invention relates to a compound of Formula (I):



or a pharmaceutically acceptable salt thereof.

Another aspect of the invention relates to methods of treating or preventing a disease or disorder associated with a genetic defect in phenylalanine hydroxylase in a subject in need thereof comprising administering to the subject an effective amount of the compound of Formula (I).

Another aspect of the invention relates to methods of treating or preventing phenylketonuria, hyperphenylalaninemia, tyrosinemia, nonketotic hyperglycinemia, isovaleric acidemia, methylmalonic acidemia, propionic acidemia, maple syrup urine disease, DNAJC12 deficiency, urea cycle disorders, or hyperammonemia in a subject in need thereof comprising administering to the subject an effective amount of the compound of Formula (I).

Another aspect of the invention relates to methods of modulating SLC6A19 transport in a subject in need thereof comprising administering to the subject an effective amount of the compound of Formula (I).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features, objects, and advantages of the invention will be apparent from the detailed description, and from the claims.

DETAILED DESCRIPTION

Definitions

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are collected here. These definitions should be read in light of the remainder of the disclosure and understood as by a person of skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art.

In order for the present invention to be more readily understood, certain terms and phrases are defined below and throughout the specification.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or,

when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e., “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

Certain compounds contained in compositions of the present invention may exist in particular geometric or stereoisomeric forms. In addition, polymers of the present invention may also be optically active. The present invention contemplates all such compounds, including cis-

and trans-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention.

Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

“Geometric isomer” means isomers that differ in the orientation of substituent atoms in relationship to a carbon-carbon double bond, to a cycloalkyl ring, or to a bridged bicyclic system. Atoms (other than H) on each side of a carbon-carbon double bond may be in an E (substituents are on opposite sides of the carbon-carbon double bond) or Z (substituents are oriented on the same side) configuration. “R,” “S,” “S*,” “R*,” “E,” “Z,” “cis,” and “trans,” indicate configurations relative to the core molecule. Certain of the disclosed compounds may exist in “atropisomeric” forms or as “atropisomers.” Atropisomers are stereoisomers resulting from hindered rotation about single bonds where the steric strain barrier to rotation is high enough to allow for the isolation of the conformers. The compounds of the invention may be prepared as individual isomers by either isomer-specific synthesis or resolved from a mixture of isomers. Conventional resolution techniques include forming the salt of a free base of each isomer of an isomeric pair using an optically active acid (followed by fractional crystallization and regeneration of the free base), forming the salt of the acid form of each isomer of an isomeric pair using an optically active amine (followed by fractional crystallization and regeneration of the free acid), forming an ester or amide of each of the isomers of an isomeric pair using an optically pure acid, amine or alcohol (followed by chromatographic separation and removal of the chiral auxiliary), or resolving an isomeric mixture of either a starting material or a final product using various well known chromatographic methods.

If, for instance, a particular enantiomer of compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Percent purity by mole fraction is the ratio of the moles of the enantiomer (or diastereomer) or over the moles of the enantiomer (or diastereomer) plus the moles of its optical isomer. When the stereochemistry of a disclosed compound is named or depicted by structure, the named or depicted stereoisomer is at least about 60%, about 70%, about 80%, about 90%, about 99% or about 99.9% by mole fraction pure relative to the other stereoisomers. When a single enantiomer is named or depicted by structure, the depicted or named enantiomer is at least about 60%, about 70%, about 80%, about 90%, about 99% or about 99.9% by mole fraction pure. When a single diastereomer is named or depicted by structure, the depicted or named diastereomer is at least about 60%, about 70%, about 80%, about 90%, about 99% or about 99.9% by mole fraction pure.

When a disclosed compound is named or depicted by structure without indicating the stereochemistry, and the compound has at least one chiral center, it is to be understood that the name or structure encompasses either enantiomer of the compound free from the corresponding optical isomer, a racemic mixture of the compound or mixtures enriched in one enantiomer relative to its corresponding optical isomer. When a disclosed compound is named or depicted by structure without indicating the stereochemistry and has two or more chiral centers, it is to be understood that the name or structure encompasses a diastereomer free of other diastereomers, a number of diastereomers free from other diastereomeric pairs, mixtures of diastereomers, mixtures of diastereomeric pairs, mixtures of diastereomers in which one diastereomer is enriched relative to the other diastereomer(s) or mixtures of diastereomers in which one or more diastereomer is enriched relative to the other diastereomers. The invention embraces all of these forms.

Structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds produced by the replacement of a hydrogen with deuterium or tritium, or of a carbon with a ^{13}C - or ^{14}C -enriched carbon are within the scope of this invention.

The term “prodrug” as used herein encompasses compounds that, under physiological conditions, are converted into therapeutically active agents. A common method for making a prodrug is to include selected moieties that are hydrolyzed under physiological conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the host animal.

The phrase “pharmaceutically acceptable excipient” or “pharmaceutically acceptable carrier” as used herein means a pharmaceutically acceptable material, composition or vehicle,

such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ or portion of the body, to another organ or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, not injurious to the patient, and substantially non-pyrogenic. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose, and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol, and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer’s solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations. In certain embodiments, pharmaceutical compositions of the present invention are non-pyrogenic, i.e., do not induce significant temperature elevations when administered to a patient.

The term “pharmaceutically acceptable salts” refers to the relatively non-toxic, inorganic and organic acid addition salts of the compound(s). These salts can be prepared in situ during the final isolation and purification of the compound(s), or by separately reacting a purified compound(s) in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts, and the like. (See, for example, Berge et al. (1977) “Pharmaceutical Salts”, *J. Pharm. Sci.* 66:1-19.)

In other cases, the compounds useful in the methods of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term “pharmaceutically acceptable salts” in these instances refers to the relatively non-toxic inorganic and organic base addition salts of a compound(s). These salts can likewise be prepared in situ during the final isolation and

purification of the compound(s), or by separately reacting the purified compound(s) in its free acid form with a suitable base, such as the hydroxide, carbonate, or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary, or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts, and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, and the like (see, for example, Berge et al., *supra*).

The term “pharmaceutically acceptable cocrystals” refers to solid coformers that do not form formal ionic interactions with the small molecule.

A “therapeutically effective amount” (or “effective amount”) of a compound with respect to use in treatment, refers to an amount of the compound in a preparation which, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

The term “prophylactic or therapeutic” treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, (i.e., it protects the host against developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

The term “patient” or “subject” refers to a mammal in need of a particular treatment. In certain embodiments, a patient is a primate, canine, feline, or equine. In certain embodiments, a patient is a human.

An “effective amount” is an amount sufficient to effect beneficial or desired results. For example, a therapeutic amount is one that achieves the desired therapeutic effect. This amount can be the same or different from a prophylactically effective amount, which is an amount necessary to prevent onset of disease or disease symptoms. An effective amount can be administered in one or more administrations, applications or dosages. A therapeutically effective

amount of a composition depends on the composition selected. The compositions can be administered from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the compositions described herein can include a single treatment or a series of treatments.

The terms “decrease,” “reduce,” “reduced,” “reduction,” “decrease,” and “inhibit” are all used herein generally to mean a decrease by a statistically significant amount relative to a reference. However, for avoidance of doubt, “reduce,” “reduction” or “decrease” or “inhibit” typically means a decrease by at least 10% as compared to a reference level and can include, for example, a decrease by at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, up to and including, for example, the complete absence of the given entity or parameter as compared to the reference level, or any decrease between 10-99% as compared to the absence of a given treatment.

The terms “increased,” “increase” or “enhance” or “activate” are all used herein to generally mean an increase by a statically significant amount; for the avoidance of any doubt, the terms “increased,” “increase” or “enhance” or “activate” means an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to a reference level.

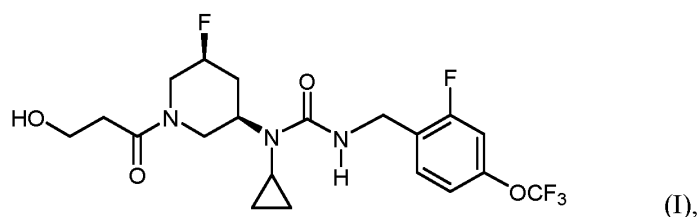
As used herein, the term “modulate” includes up-regulation and down-regulation, e.g., enhancing or inhibiting a response.

A “radiopharmaceutical agent,” as defined herein, refers to a pharmaceutical agent which contains at least one radiation-emitting radioisotope. Radiopharmaceutical agents are routinely used in nuclear medicine for the diagnosis and/or therapy of various diseases. The radiolabelled pharmaceutical agent, for example, a radiolabelled antibody, contains a radioisotope (RI) which serves as the radiation source. As contemplated herein, the term “radioisotope” includes metallic and non-metallic radioisotopes. The radioisotope is chosen based on the medical application of the radiolabeled pharmaceutical agents. When the radioisotope is a metallic radioisotope, a chelator is typically employed to bind the metallic radioisotope to the rest of the molecule. When the radioisotope is a non-metallic radioisotope, the non-metallic radioisotope is typically linked directly, or via a linker, to the rest of the molecule.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover.

Compounds of the Invention

One aspect of the invention relates to a compound of Formula (I):



or a pharmaceutically acceptable salt thereof.

In certain embodiments, the compound has the relative stereochemistry shown.

In certain embodiments, the compound has the absolute stereochemistry shown.

Pharmaceutical Compositions, Routes of Administration, and Dosing

In certain embodiments, the invention is directed to a pharmaceutical composition, comprising a compound of the invention and a pharmaceutically acceptable carrier.

In certain embodiments, a pharmaceutical composition of the invention further comprises at least one additional pharmaceutically active agent other than a compound of the invention. The at least one additional pharmaceutically active agent can be an agent useful in the treatment of ischemia-reperfusion injury.

Pharmaceutical compositions of the invention can be prepared by combining one or more compounds of the invention with a pharmaceutically acceptable carrier and, optionally, one or more additional pharmaceutically active agents.

As stated above, an “effective amount” refers to any amount that is sufficient to achieve a desired biological effect. Combined with the teachings provided herein, by choosing among the various active compounds and weighing factors such as potency, relative bioavailability, patient body weight, severity of adverse side-effects and mode of administration, an effective prophylactic or therapeutic treatment regimen can be planned which does not cause substantial unwanted toxicity and yet is effective to treat the particular subject. The effective amount for any particular application can vary depending on such factors as the disease or condition being treated, the particular compound of the invention being administered, the size of the subject, or the severity of the disease or condition. One of ordinary skill in the art can empirically determine the effective amount of a particular compound of the invention and/or other therapeutic agent without necessitating undue experimentation. A maximum dose may be used, that is, the highest safe dose according to some medical judgment. Multiple doses per day may be contemplated to achieve appropriate systemic levels of compounds. Appropriate systemic levels can be determined by, for example, measurement of the patient’s peak or sustained plasma level of the drug. “Dose” and “dosage” are used interchangeably herein.

In certain embodiments, intravenous administration of a compound may typically be from 0.1 mg/kg/day to 20 mg/kg/day. In one embodiment, intravenous administration of a compound may typically be from 0.1 mg/kg/day to 2 mg/kg/day. In one embodiment, intravenous administration of a compound may typically be from 0.5 mg/kg/day to 5 mg/kg/day. In one embodiment, intravenous administration of a compound may typically be from 1 mg/kg/day to 20 mg/kg/day. In one embodiment, intravenous administration of a compound may typically be from 1 mg/kg/day to 10 mg/kg/day.

Generally, daily oral doses of a compound will be, for human subjects, from about 0.01 milligrams/kg per day to 1000 milligrams/kg per day. It is expected that oral doses in the range of 0.5 to 50 milligrams/kg, in one or more administrations per day, will yield therapeutic results. Dosage may be adjusted appropriately to achieve desired drug levels, local or systemic, depending upon the mode of administration. For example, it is expected that intravenous administration would be from one order to several orders of magnitude lower dose per day. In

the event that the response in a subject is insufficient at such doses, even higher doses (or effective higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of the compound.

For any compound described herein the therapeutically effective amount can be initially determined from animal models. A therapeutically effective dose can also be determined from human data for compounds which have been tested in humans and for compounds which are known to exhibit similar pharmacological activities, such as other related active agents. Higher doses may be required for parenteral administration. The applied dose can be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal efficacy based on the methods described above and other methods as are well-known in the art is well within the capabilities of the ordinarily skilled artisan.

The formulations of the invention can be administered in pharmaceutically acceptable solutions, which may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

For use in therapy, an effective amount of the compound can be administered to a subject by any mode that delivers the compound to the desired surface. Administering a pharmaceutical composition may be accomplished by any means known to the skilled artisan. Routes of administration include but are not limited to intravenous, intramuscular, intraperitoneal, intravesical (urinary bladder), oral, subcutaneous, direct injection (for example, into a tumor or abscess), mucosal (e.g., topical to eye), inhalation, and topical.

For intravenous and other parenteral routes of administration, a compound of the invention can be formulated as a lyophilized preparation, as a lyophilized preparation of liposome-intercalated or -encapsulated active compound, as a lipid complex in aqueous suspension, or as a salt complex. Lyophilized formulations are generally reconstituted in suitable aqueous solution, e.g., in sterile water or saline, shortly prior to administration.

For oral administration, the compounds can be formulated readily by combining the active compound(s) with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to

be treated. Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers, e.g., EDTA for neutralizing internal acid conditions or may be administered without any carriers.

Also specifically contemplated are oral dosage forms of the above component or components. The component or components may be chemically modified so that oral delivery of the derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the component molecule itself, where said moiety permits (a) inhibition of acid hydrolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the component or components and increase in circulation time in the body. Examples of such moieties include: polyethylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. Abuchowski and Davis, "Soluble Polymer-Enzyme Adducts", In: *Enzymes as Drugs*, Hoenberg and Roberts, eds., Wiley-Interscience, New York, N.Y., pp. 367-383 (1981); Newmark et al., *J Appl Biochem* 4:185-9 (1982). Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. For pharmaceutical usage, as indicated above, polyethylene glycol moieties are suitable.

For the component (or derivative) the location of release may be the stomach, the small intestine (the duodenum, the jejunum, or the ileum), or the large intestine. One skilled in the art has available formulations which will not dissolve in the stomach, yet will release the material in the duodenum or elsewhere in the intestine. Preferably, the release will avoid the deleterious effects of the stomach environment, either by protection of the compound of the invention (or derivative) or by release of the biologically active material beyond the stomach environment, such as in the intestine.

To ensure full gastric resistance a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and shellac. These coatings may be used as mixed films.

A coating or mixture of coatings can also be used on tablets, which are not intended for protection against the stomach. This can include sugar coatings, or coatings which make the tablet easier to swallow. Capsules may consist of a hard shell (such as gelatin) for delivery of dry therapeutic (e.g., powder); for liquid forms, a soft gelatin shell may be used. The shell material of cachets could be thick starch or other edible paper. For pills, lozenges, molded tablets or tablet triturates, moist massing techniques can be used.

The therapeutic can be included in the formulation as fine multi-particulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

Colorants and flavoring agents may all be included. For example, the compound of the invention (or derivative) may be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

One may dilute or increase the volume of the therapeutic with an inert material. These diluents could include carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrates include but are not limited to starch, including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as

disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

An anti-frictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

To aid dissolution of the therapeutic into the aqueous environment a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents which can be used and can include benzalkonium chloride and benzethonium chloride. Potential non-ionic detergents that could be included in the formulation as surfactants include laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the compound of the invention or derivative either alone or as a mixture in different ratios.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition,

stabilizers may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For topical administration, the compound may be formulated as solutions, gels, ointments, creams, suspensions, etc. as are well-known in the art. Systemic formulations include those designed for administration by injection, e.g., subcutaneous, intravenous, intramuscular, intrathecal or intraperitoneal injection, as well as those designed for transdermal, transmucosal oral or pulmonary administration.

For administration by inhalation, compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Also contemplated herein is pulmonary delivery of the compounds disclosed herein (or salts thereof). The compound is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. Other reports of inhaled molecules include Adjei et al., *Pharm Res* 7:565-569 (1990); Adjei et al., *Int J Pharmaceutics* 63:135-144 (1990) (leuprolide acetate); Braquet et al., *J Cardiovasc Pharmacol* 13(suppl. 5):143-146 (1989) (endothelin-1); Hubbard et al., *Ann Int Med* 3:206-212 (1989) (α 1-antitrypsin); Smith et al., 1989, *J Clin Invest* 84:1145-1146 (a-1-proteinase); Oswein et al., 1990, "Aerosolization of Proteins", Proceedings of Symposium on Respiratory Drug Delivery II, Keystone, Colorado, March, (recombinant human growth hormone); Debs et al., 1988, *J Immunol* 140:3482-3488 (interferon-gamma and tumor necrosis factor alpha) and Platz et al., U.S. Pat. No. 5,284,656 (granulocyte colony stimulating factor; incorporated by reference). A method and composition for pulmonary delivery of drugs for systemic effect is described in U.S. Pat. No. 5,451,569 (incorporated by reference), issued Sep. 19, 1995 to Wong et al.

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Mo.; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colo.; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, North Carolina; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Mass.

All such devices require the use of formulations suitable for the dispensing of the compounds of the invention. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to the usual diluents, adjuvants and/or carriers useful in therapy. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated. Chemically modified compound of the invention may also be prepared in different formulations depending on the type of chemical modification or the type of device employed.

Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise a compound of the invention (or derivative) dissolved in water at a concentration of about 0.1 to 25 mg of biologically active compound of the invention per mL of solution. The formulation may also include a buffer and a simple sugar (e.g., for inhibitor stabilization and regulation of osmotic pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the compound of the invention caused by atomization of the solution in forming the aerosol.

Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the compound of the invention (or derivative) suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing compound of the invention (or derivative) and may also include a bulking agent, such as lactose, sorbitol, sucrose, or mannitol in amounts which facilitate dispersal of the powder from the device, e.g., 50 to 90% by weight of the formulation. The compound of the invention (or derivative) should advantageously be prepared in particulate form with an average particle size of less than 10 micrometers (μm), most preferably 0.5 to 5 μm , for most effective delivery to the deep lung.

Nasal delivery of a pharmaceutical composition of the present invention is also contemplated. Nasal delivery allows the passage of a pharmaceutical composition of the present invention to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

For nasal administration, a useful device is a small, hard bottle to which a metered dose sprayer is attached. In one embodiment, the metered dose is delivered by drawing the pharmaceutical composition of the present invention solution into a chamber of defined volume, which chamber has an aperture dimensioned to aerosolize and aerosol formulation by forming a spray when a liquid in the chamber is compressed. The chamber is compressed to administer the pharmaceutical composition of the present invention. In a specific embodiment, the chamber is a piston arrangement. Such devices are commercially available.

Alternatively, a plastic squeeze bottle with an aperture or opening dimensioned to aerosolize an aerosol formulation by forming a spray when squeezed is used. The opening is usually found in the top of the bottle, and the top is generally tapered to partially fit in the nasal passages for efficient administration of the aerosol formulation. Preferably, the nasal inhaler will provide a metered amount of the aerosol formulation, for administration of a measured dose of the drug.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethylcellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described above, a compound may also be formulated as a depot preparation. Such long-acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are

suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer R, *Science* 249:1527-33 (1990).

The compound of the invention and optionally other therapeutics may be administered *per se* (neat) or in the form of a pharmaceutically acceptable salt or cocrystal. When used in medicine the salts or cocrystals should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts or cocrystals may conveniently be used to prepare pharmaceutically acceptable salts or cocrystals thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonc, tartaric, citric, methane sulphonc, formic, malonic, succinic, naphthalene-2-sulphonc, and benzene sulphonc. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

Pharmaceutical compositions of the invention contain an effective amount of a compound as described herein and optionally therapeutic agents included in a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration to a human or other vertebrate animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being commingled with the compounds of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficiency.

The therapeutic agent(s), including specifically but not limited to a compound of the invention, may be provided in particles. Particles as used herein means nanoparticles or microparticles (or in some instances larger particles) which can consist in whole or in part of the compound of the invention or the other therapeutic agent(s) as described herein. The particles may contain the therapeutic agent(s) in a core surrounded by a coating, including, but not limited to, an enteric coating. The therapeutic agent(s) also may be dispersed throughout the particles.

The therapeutic agent(s) also may be adsorbed into the particles. The particles may be of any order release kinetics, including zero-order release, first-order release, second-order release, delayed release, sustained release, immediate release, and any combination thereof, etc. The particle may include, in addition to the therapeutic agent(s), any of those materials routinely used in the art of pharmacy and medicine, including, but not limited to, erodible, non-erodible, biodegradable, or nonbiodegradable material or combinations thereof. The particles may be microcapsules which contain the compound of the invention in a solution or in a semi-solid state. The particles may be of virtually any shape.

Both non-biodegradable and biodegradable polymeric materials can be used in the manufacture of particles for delivering the therapeutic agent(s). Such polymers may be natural or synthetic polymers. The polymer is selected based on the period of time over which release is desired. Bioadhesive polymers of particular interest include bioerodible hydrogels described in Sawhney H S et al. (1993) *Macromolecules* 26:581-7, the teachings of which are incorporated herein. These include polyhyaluronic acids, casein, gelatin, gluten, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

The therapeutic agent(s) may be contained in controlled release systems. The term “controlled release” is intended to refer to any drug-containing formulation in which the manner and profile of drug release from the formulation are controlled. This refers to immediate as well as non-immediate release formulations, with non-immediate release formulations including but not limited to sustained release and delayed release formulations. The term “sustained release” (also referred to as “extended release”) is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that preferably, although not necessarily, results in substantially constant blood levels of a drug over an extended time period. The term “delayed release” is used in its conventional sense to refer to a drug formulation in which there is a time delay between administration of the formulation and the release of the drug therefrom. “Delayed release” may or may not involve gradual release of drug over an extended period of time, and thus may or may not be “sustained release.”

Use of a long-term sustained release implant may be particularly suitable for treatment of chronic conditions. “Long-term” release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 7 days, and preferably 30-60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

It will be understood by one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the compositions and methods described herein are readily apparent from the description of the invention contained herein in view of information known to the ordinarily skilled artisan, and may be made without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1: SLC6A19 Isoleucine transport assay

Cell line generation and maintenance

The Flp-In™ T-REx™ 293 cell line was purchased from Thermo Fisher Scientific. The line was used to generate a stable cell line inducibly expressing human SLC6A19 with a C-terminal V5 tag and stably expressing human TMEM27 (also known as Collectrin) with a C-terminal myc-DDK tag. The stable cell line was generated by transfecting SLC6A19- and TMEM27-encoding plasmids using standard protocols, followed by antibiotic selection. Stable cells were maintained in DMEM/F12 supplemented with Glutamax, 10% fetal bovine serum, 100 U/mL penicillin, 100 ug/mL streptomycin, 200 ug/mL hygromycin, 10 ug/mL blasticidin and 300 ug/mL neomycin (Thermo Fisher).

Assay: Isoleucine transport assay in 96-well format

Stable cell lines were seeded at a density of 35,000 cells per well in a poly-D-lysine coated 96-well cell culture-treated plate on day 0. On day 1 the expression of SLC6A19 was

induced by dispensing tetracycline at a final concentration of 1 $\mu\text{g/mL}$ using a Tecan D300e digital dispenser. On day 2 the transport assay was run. Media was removed from the plate using the GentleSpin setting of a Centrifugal Blue Washer (Blue Cat Bio) and cells were washed with 175 μL live cell imaging solution (Thermo Fisher) using the Blue Washer. Following washing, cells were treated with 70 μL of either DMSO, positive control or compound, diluted in Krebs buffer (140 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgCl_2 , 11 mM HEPES, 10 mM Glucose, pH 7.4) at room temperature. After 20-60 minutes 30 μL of a 3.3 mM solution of $^{13}\text{C}_6$, ^{15}N -L-isoleucine (Cambridge Isotope Laboratories) was added. After 20 min incubation with the isoleucine substrate at room temperature cells were washed with 175 μL live cell imaging solution using the Blue Washer. Cells were then lysed in 150 μL of 15 μM D-Leucine- d_{10} (CDN Isotopes) in ultrapure water. Plates were put on a shaker at 700 rpm for a minimum of 40 minutes to facilitate lysis. Following lysis, a standard dilution curve of $^{13}\text{C}_6$, ^{15}N -L-isoleucine was added to wells containing lysates of untreated cells. Plates were returned to the shaker for a minimum of 2 minutes to ensure proper mixing of the standard curve. Plates were then centrifuged for 5 min at 4,000 rpm to pellet cellular debris and precipitate. Supernatants were diluted 1:10 in acetonitrile + 0.1% formic acid in polypropylene plates.

Assay: Isoleucine transport assay in 384-well format

On day 0, stable cell lines were seeded at a density of 20,000 cells per well in a poly-D-lysine coated 384-well cell culture-treated plate in media containing 1 $\mu\text{g/mL}$ tetracycline using a Vialflo 384-well pipette. Transport assays were run the following day (day 1). Media was removed from the plate using the GentleSpin setting of a Centrifugal Blue Washer (Blue Cat Bio) and cells were washed with 80 μL live cell imaging solution (Thermo Fisher) using the Blue Washer. Following washing, cells were treated with 20 μL of either DMSO, positive control or compound, diluted in Krebs buffer (140 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgCl_2 , 11 mM HEPES, 10 mM Glucose, pH 7.4) using a TECAN liquid handler. After 20-60 minutes incubation at room temperature 8.6 μL of a 3.3 mM solution of $^{13}\text{C}_6$, ^{15}N -L-isoleucine (Cambridge Isotope Laboratories) was added. After 20 min incubation with the isoleucine substrate at room temperature cells were washed with 80 μL live cell imaging solution using the Blue Washer. Cells were then lysed in 80 μL of 15 μM D-Leucine- d_{10} (CDN Isotopes) in ultrapure water. Plates were put on a shaker at 700 rpm for a minimum of 2 hours to facilitate

lysis. Following lysis, a standard dilution curve of $^{13}\text{C}_6,^{15}\text{N}$ -L-isoleucine was added to wells containing lysates of untreated cells. Plates were returned to the shaker for a minimum of 5 minutes to ensure proper mixing of the standard curve. Plates were then centrifuged for 10 min at 4,000 rpm to pellet cellular debris and precipitate. Supernatants were diluted 1:10 in acetonitrile + 0.1% formic acid in polypropylene plates.

$^{13}\text{C}_6,^{15}\text{N}$ -L-isoleucine analysis was performed using a RapidFire365-QTOF 6545 (Agilent). Quantitative sample analysis utilizes automated solid-phase extraction (HILIC H6 cartridge) prior to mass spec injection. Samples were loaded using 95% acetonitrile, 0.1% formic acid and eluted from the cartridge with 5% acetonitrile, 0.1% formic acid directly for ESI-MS (electrospray ionization) analysis. Quantification of the analytes were performed using Agilent Masshunter Quant software from the high-resolution full scan data.

Example 2: Human LM clearance

The human liver microsome (LM) clearance was assessed by substrate depletion method. Compound (I) (1 μM) was incubated with human liver microsome (0.5 mg/mL protein) at 37 °C in the presence of 1 mM NADPH and 100 mM phosphate buffer. At various time points spanning from 0 to 60 minutes, an aliquot of the reaction mixture was removed and mixed with 5 volumes of cold acetonitrile (ACN) containing an internal standard (IS) followed by centrifugation. The resulting supernatant was diluted with 1 volume of ultra-pure H_2O and subjected to LC/MS analysis of parent compound. The elimination constant rate, denoted as 'ke', was determined by applying linear regression to the natural log of the percentage the compound (I) remaining versus incubation time curve. The clearance ($\mu\text{L}/\text{min}/\text{mg}$ protein) of compound (I) was calculated using the following equation: $\text{CL}_{\text{int}} = k_e \times \text{incubation volume } (\mu\text{L})/\text{amount of protein (mg)}$

Example 3: CYP3A inhibition

CYP3A inhibition was investigated in human liver microsomes using midazolam as the substrate. Midazolam (1 μM) was incubated with human liver microsomes (0.2 mg/mL protein) at 37 °C for 5 min in the presence of 30 μM compound (I), 1 mM NADPH, and 100 mM phosphate buffer. The reaction was terminated by the addition of 1.5 volume of cold ACN containing internal standards followed by centrifugation. The resulting supernatant was diluted

with 1 volume of ultra-pure H₂O and subjected to LC/MS analysis of 1-hydroxyl midazolam, a major metabolite of CYP3A. The percentage inhibition caused by compound (I) was determined by comparing the decrease in the formation of 1-hydroxyl midazolam to vehicle control.

Example 4: Bidirectional permeability assay in MDCK-MDR1 and Caco2 cells

A bidirectional experiment was performed using caco2 as well as MDCKII cells stably transfected with human MDR1 (MDCKII-MDR1) that encodes Pgp. The cells were cultured in 96-well transwell plates at 37 °C with 5% CO₂. The test compound was prepared in Hank's Balanced Salt Solution with 10 mM HEPES (pH 7.4) and 4% BSA at a concentration of 1 μM for MDCKII-MDR1 assay and 10 μM for Caco2 test. To measure the transport rate from the apical (A) to basolateral (B) side, 75 μL of the compound solution was added to the apical side (donor), and 235 μL of blank solution without the test compound was added to the basolateral compartment (acceptor). To assess the transport rate from the basolateral (B) to apical (A) side, 235 μL of the compound solution was added to the basolateral side (donor), and 75 μL of blank solution without the compound was added to the apical side (acceptor). After a 2-hour incubation at 37 °C, 50 μL samples were taken from both the A and B compartments and mixed to cold ACN containing IS followed by centrifugation. The supernatant was analyzed using LC/MS to detect the parent compound. To further investigate whether the compound is a substrate of Pgp, the bidirectional assay with MDCKII-MDR1 was also conducted in the presence of a Pgp inhibitor, verapamil, at a concentration of 100 μM.

The apparent permeability coefficient (P_{app}), expressed in centimeters per second, can be determined by utilizing the following formula: $P_{app} = (V_A \times [drug]_{acceptor}) / (Area \times Time \times [drug]_{initial,donor})$.

In this equation, V_A represents the volume (in milliliters) in the acceptor well, Area corresponds to the surface area of the membrane (0.143 cm² for Transwell-96 Well Permeable Supports), and time denotes the total transport duration in seconds.

Efflux ratio was determined using the following equation: $Efflux\ Ratio = P_{app}(B \rightarrow A) / P_{app}(A \rightarrow B)$, where $P_{app}(B \rightarrow A)$ indicates the apparent permeability coefficient from basolateral to apical direction. $P_{app}(A \rightarrow B)$ indicates the apparent permeability coefficient from apical to basolateral direction.

Example 5: Measurement of hERG Inhibition

hERG inhibition was assayed using a minor modification of the protocol described in Haraguchi, Y. et al. Electrophysiological analysis of mammalian cells expressing hERG using automated 384-well-patch-clamp. *BMC Pharmacol Toxicol.* 2015; 16: 39.

Cell culture and cell preparation for patch-clamp analysis: In this study, HEK 293-hERG cells (Merck Millipore, Billerica, MA, USA), which are HEK 293 cells stably expressing hERG, and CHO-hERG cells (Merck Millipore), which are CHO cells stably expressing hERG, were used. The HEK 293 cells were cultured in an equal volume mixture of Dulbecco's modified Eagle's medium (DMEM) and Nutrient Mixture F-12 (Invitrogen Life Technologies, CA, USA) supplemented with 10 % fetal bovine serum (FBS) (Invitrogen Life Technologies) and 1 % penicillin/streptomycin (Invitrogen Life Technologies), and the CHO cells were cultured in Ham's F12 medium (Invitrogen Life Technologies) supplemented with 10 % FBS and 1 % penicillin/streptomycin in a humidified 5 % CO₂ atmosphere at 37 °C. For patch-clamp experiments, cultured cells on a polystyrene culture dish (Sumitomo Bakelite, Tokyo, Japan) were detached by Accutase (Innovative Cell Technologies, Inc., CA, USA) at room temperature for several minutes. A viable single cell suspension was obtained by resuspension in patch clamp solution with mild pipetting to avoid cell damage.

Patch-clamp analysis

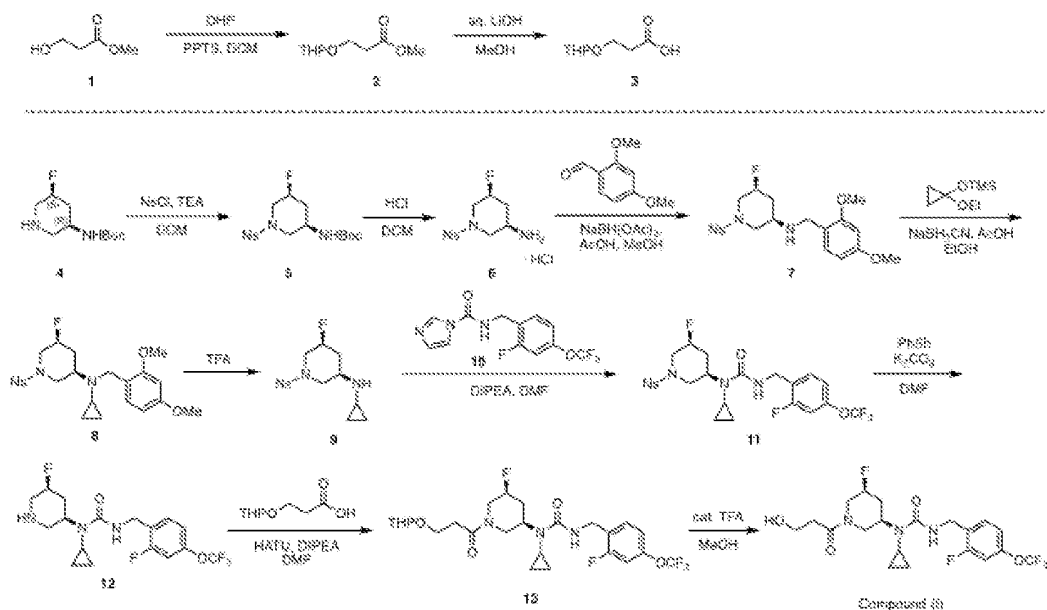
Patch-clamp measurements were performed by an automated multi-well planar patch-clamp system the SyncroPatch 384 Patch Engine (PE) (Nanion Technologies, Munich, Germany) (Fig. 1a). The SyncroPatch 384 PE was a patch-clamp module that was integrated into a liquid handling robot owning a 384 pipetting head, Biomek FX (Beckman Coulter, Brea, CA, USA). The system was controlled by a dedicated software, PatchControl 384 (Nanion Technologies). In this device, 384 cells were measured simultaneously. Patch-clamp recordings were performed at room temperature using an intracellular solution [50 mM KCl, 10 mM NaCl, 60 mM KF, 20 mM EGTA, 10 mM HEPES (pH: 7.2) with 25 µM Escin for perforated patch (HEK 293-hERG cells) or without Escin for whole cell recording (CHO-hERG cells)], and a standard bath solution [140 mM NaCl, 4 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 5 mM D-glucose, 10 mM HEPES (pH: 7.4)]. Suspended single cells and drugs were freshly prepared and applied into teflon reservoirs (Nanion Technologies). In the automated patch-clamp system, the following

procedures were performed automatically. The suspended single cells were aspirated from the reservoir, pipetted into a planar 384-well patch-clamp chip, and entrapped in the holes of the wells by an automatically applied vacuum. Seal generation, the establishment of the perforated or standard whole cell mode and also the electrophysiological recordings were controlled by PatchControl 384. The application of drugs into each well and the washout were also performed automatically by the liquid handling robot. Concentration response curves and IC₅₀ values were calculated automatically by another dedicated software, DataControl 384 (Nanion Technologies). K_v currents were elicited using a voltage step from a holding potential (-80 mV) to +20 mV for 1 s followed by a 1 s step to -50 mV.

Example 6: In Vivo Pharmacokinetics

PK studies were conducted in SD rats (n=3 per group). Inhibitor was administered intravenously (IV) via a 15-minute infusion at 1 mg/kg, and orally (PO) at 10 mg/kg. Blood samples were collected at 0.17, 0.25, 0.42, 0.75, 1, 2, 4, 8, and 24 hours after IV administration, and 0.25, 0.5, 1, 2, 4, 8, and 24 hours after PO administration. The blood samples were centrifuged immediately after collection and separated plasma fraction was subsequently processed for LC/MS analysis. The log-transformed plasma concentrations were plotted against the corresponding sample collection time. The PK parameters were obtained using a non-compartmental method. The IV plasma clearance was calculated by dividing the dose by AUC_{inf}. The terminal half-life of elimination was determined by linear regression of the log-transformed data. The C_{max} was obtained by graphical analysis of the plasma concentration-time data, and the oral bioavailability was determined from dose-adjusted IV and PO AUC_{inf} ratio.

Example 7: Synthesis of the Compound of Formula (I)



Step 1: Synthesis of 2

To a mixture of **1** (1 g, 9.61 mmol) and PPTS (121 mg, 0.48 mmol) in DCM (30 mL) was added DHP (1.13 g, 13.45 mmol) and dropwise at 0 °C. The resulting mixture was stirred at room temperature for 2 hrs. Then the mixture was concentrated under reduced pressure to dryness. The residue was diluted with EtOAc (80 mL) and washed with water (80 mL) and brine (80 mL), the organic layer was separated, dried over anhydrous Na₂SO₄ and concentrated to afford crude **2** (1.78 g, 98.5 % yield) as colorless oil which was used at the next step directly without further purification.

Step 2: Synthesis of 3

To a mixture of **2** (1.78 g, 9.47 mmol) in MeOH (20 mL) and H₂O (8 mL) was added LiOH (453 mg, 18.91 mmol) in portions. The resulting mixture was stirred at room temperature for 3 hrs. Then the mixture was diluted with water (30 mL) and extracted with MTBE (60 mL), the aqueous layer was separated and adjusted to pH=5 with aq. HCl (1 N). Then the mixture was washed with EtOAc (60 mL) twice. The combined organic layers were washed with water (60 mL) and brine (60 mL), dried over anhydrous Na₂SO₄ and concentrated to give crude **3** (1.59 g,

96.5 % yield) as colorless oil which was used at the next step directly without further purification.

Step 3: Synthesis of 5

To a mixture of **4** (4.0 g, 18.35 mmol) and TEA (3.7 g, 36.7 mmol) in anhydrous DCM (50 mL) was added NsCl (4.48 g, 20.19 mmol) dropwise at 0 °C and the resulting mixture was stirred at room temperature for 2 hrs. Then the mixture was diluted with saturated NaHCO₃ solution (100 mL) and extracted with DCM (100 mL) twice. The combined organic layers were washed with brine (100 mL), dried over anhydrous Na₂SO₄, filtered and concentrated to dryness. The residue was purified via flash column chromatography (eluted with PE/EtOAc= 100:0 to 1:1) to give **5** (6.5 g, 87.8% yield) as yellow solid. LC/MS (ESI) m/z: 404 (M+H)⁺.

Step 4: Synthesis of 6

To a solution of **5** (6.5 g, 16.13 mmol) in anhydrous DCM (80 mL) was added HCl (4 M in dioxane, 8 mL, 32.2 mmol) dropwise at 0 °C and the resulting mixture was stirred at room temperature for 2 hr. Then the mixture was concentrated to dryness to give crude **6** (3.9 g, 79.9% yield) as white solid which was used at the next step directly without further purification. LC/MS (ESI) m/z: 304 (M+H)⁺.

Step 5: Synthesis of 7

To a mixture of **6** (3.9 g, 12.85 mmol) and AcOH (77 mg, 1.29 mmol) in MeOH (60 mL) was added 2,4-dimethoxybenzaldehyde (2.35 g, 14.14 mmol) and NaBH(OAc)₃ (5.45 g, 25.7 mmol) slowly at 0 °C. The resulting mixture was stirred at room temperature for 4 h under N₂ atmosphere. Then the mixture was diluted with EtOAc (100 mL) and washed with saturated NaHCO₃ solution (100 mL) and brine (100 mL). The organic layer was separated, dried over anhydrous Na₂SO₄, filtered and concentrated to dryness. The residue was purified via flash column chromatography (eluted with DCM/MeOH= 100:0 to 12:1) to give **7** (5.1 g, 87.9% yield) as yellow solid. LC/MS (ESI) m/z: 454 (M+H)⁺.

Step 6: Synthesis 8

To a mixture of **7** (5.1 g, 11.26 mmol) and AcOH (2.02 g, 33.8 mmol), in EtOH (120 mL) was added (1-ethoxycyclopropoxy)trimethylsilane (3.7 g, 22.52 mmol) and NaCNBH₃ (1.4 g, 22.52 mmol) slowly. The resulting mixture was stirred at 80 °C for 4 hrs under N₂ atmosphere. Then the mixture was concentrated to dryness. The residue was diluted with EtOAc (100 mL) and washed with saturated NaHCO₃ solution (100 mL x 2) and brine (100 mL). The organic layer was separated, dried over anhydrous Na₂SO₄, filtered and concentrated to dryness. The residue was purified via flash column chromatography (eluted with PE/EtOAc= 100:0 to 3:1) to give **8** (4.9 g, 88.3% yield) as yellow solid. LC/MS (ESI) m/z: 494 (M+H)⁺.

Step 7: Synthesis of **9**

8 (4.9 g, 9.94 mmol) was added into TFA (50 mL) and the resulting mixture was stirred at 80 °C for 3 hrs. Then the mixture was concentrated to dryness. The residue was diluted with EtOAc (80 mL) and washed with saturated NaHCO₃ solution (100 mL) and brine (100 mL). The organic layer was separated, dried over anhydrous Na₂SO₄, filtered and concentrated to give crude **9** (2.7 g, 79.4% yield) as yellow solid which was used at the next step directly without further purification. LC/MS (ESI) m/z: 344 (M+H)⁺.

Step 8: Synthesis of **10**

To a solution of CDI (1.54 g, 9.53 mmol) and diisopropylethylamine (3.0 mL, 17.3 mmol) in DMF (17 mL) was added (2-fluoro-4-(trifluoromethoxy)phenyl)methanamine (1.81 g, 8.66 mmol) portion wise. The mixture was stirred at room temp for 2 hr at which LCMS indicated the complete consumption of the starting material. The crude solution of **10** (0.5 M) was directly used for the next step. LC/MS (ESI) m/z: 304 (M+H)⁺.

Step 9: Synthesis of **11**

To a mixture of **9** (2.7 g, 7.87 mmol) and DIPEA (2.0 g, 15.74 mmol) in DMF (50 mL) was added **10** (0.5 M, 8.66 mmol). The resulting mixture was stirred at 70 °C for 6 hrs. Then the mixture was diluted with saturated NH₄Cl solution (100 mL) and extracted with EtOAc (100 mL) twice. The combined organic layers were washed with brine (100 mL), dried over anhydrous Na₂SO₄, filtered and concentrated to dryness. The residue was purified via flash

column chromatography (eluted with PE/EtOAc= 100:0 to 0:100) to give **11** (3.1 g, 68.9% yield) as yellow solid. LC/MS (ESI) m/z: 579 (M+H)⁺.

Step 10: Synthesis of **12**

To a mixture of **11** (3.1 g, 5.36 mmol) and K₂CO₃ (2.22 g, 16.08 mmol) in DMF (50 mL) was added PhSH (1.2 g, 10.72 mmol). The resulting mixture was stirred at 60 °C for 2 hrs. After cooling, the mixture was diluted with water (150 mL) and extracted with EtOAc (100 mL) twice. The combined organic layers were washed with brine (100 mL), dried over anhydrous Na₂SO₄, filtered and concentrated to dryness. The residue was purified via flash column chromatography (eluted with DCM/MeOH= 100:0 to 10:1) to give **12** (1.6 g, 76.2% yield) as colorless oil. LC/MS (ESI) m/z: 394 (M+H)⁺.

Step 11: Synthesis of **13**

To a mixture of **3** (266 mg, 1.53 mmol) and **12** (600 mg, 1.53 mmol) in DMF (6 mL) was added DIPEA (394 mg, 3.05 mmol) and HATU (696 mg, 1.83 mmol). The resulting mixture was stirred at room temperature for 2 hrs. The reaction mixture was concentrated in vacuo and used directly for the next step. LC/MS (ESI) m/z: 572 (M+Na)⁺.

Step 12: Synthesis of (**I**)

To a crude mixture of **13** in MeOH (10 mL) was added TFA (40 mg, 0.30 mmol). The resulting mixture was stirred at room temperature for 2 hrs. Then the solution was concentrated to dryness in vacuo and the residue was purified via prep-HPLC to give (**I**) (390 mg, 54 % yield, over 2 steps) as white solid. LC/MS (ESI) m/z: 466 (M+H)⁺. ¹H NMR (400 MHz, MeOD) δ 7.49 – 7.40 (m, 1H), 7.10 (t, J = 8.5 Hz, 2H), 4.83 – 4.75 (m, 1H), 4.69 – 4.57 (m, 1H), 4.55 – 4.48 (m, 1H), 4.46 – 4.21 (m, 3H), 3.92 – 3.75 (m, 3H), 3.52 (d, J = 32.0 Hz, 1H), 3.18 – 2.94 (m, 1H), 2.73 – 2.63 (m, 1H), 2.62 – 2.52 (m, 3H), 2.45 – 2.25 (m, 2H), 1.04 – 0.91 (m, 2H), 0.88 – 0.70 (m, 2H).

Example 8: Pharmacokinetic (PK) and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Data of the Compound of Formula (**I**)

The compound of Formula (I) was tested in various *in vitro* and *in vivo* PK assays. The results are summarized in **Tables 1** and **2**.

Table 1. ADMET data

Liver Microsomes (CLint)	MDCK (A to B / Efflux Ratio)	CYP3A4 (% Inhibition)	Caco-2 (A to B / Efflux Ratio)	hERG
13	5.4/1.7	<20 @ 30 μ M	5.5/1.7	45@ 30 μ M

Table 2. *In vivo* PK data

IV Clobs (mL/min/kg)	TV t _{1/2} (hours)	PO C _{max} ng/mL	%F
10	1.1	4347	89

Example 9: Pharmacokinetic (PK) and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Data of Comparator Compounds

The comparator compounds 1 and 2 were tested in various *in vitro* and *in vivo* ADMET assays. The results are summarized in **Tables 3** and **4**.

Table 3. *In vitro* PK data

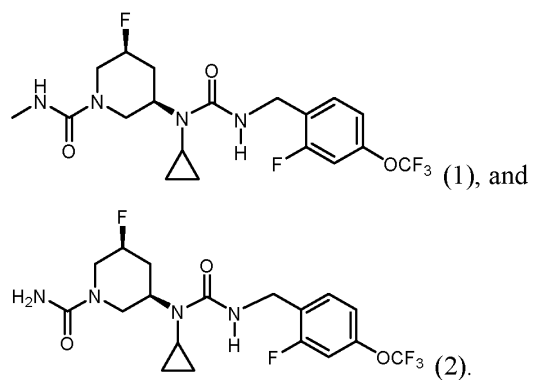
Comparator	Liver Microsomes (CLint)	MDCK (A to B / Efflux Ratio)	CYP3A4 (% Inhibition)	Caco-2 (A to B / Efflux Ratio)	hERG(% Inhibition)
1	21/25	7 / 1.2	<20 @ 10 μ M	5.3/1.4	55@30 μ M
2	11	4/1.8	<20 @ 10 μ M	3.98/1.13	20@10 μ M

Table 4. *In vivo* PK data

Comparator Compound	IV Clobs (mL/min/kg)	IV t _{1/2} (hours)	PO C _{max} ng/mL	%F
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1	20	0.76	2877	68
2	39	0.4	1463	63

The structures of comparator compounds **1** and **2** are:



Example 10: SLC6A19 Isoleucine Transport Assay Data

The compound of Formula (I) and comparator compounds were tested in the isoleucine transport assay. The results are summarized in **Table 5** (below).

Compound	Geomean EC ₅₀ (nM)
(I)	54
Comparator 1	19
Comparator 2	35

INCORPORATION BY REFERENCE

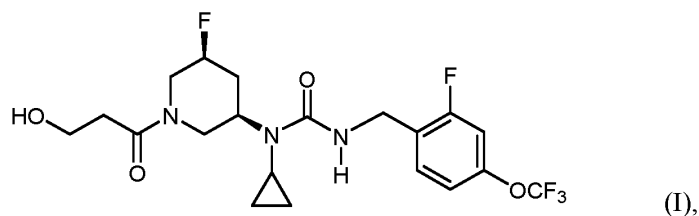
All of the U.S. patents and U.S. and PCT patent application publications cited herein are hereby incorporated by reference.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A compound of Formula (I):



or a pharmaceutically acceptable salt thereof; optionally wherein the compound has the absolute stereochemistry shown.

2. A pharmaceutical composition, comprising the compound of claim 1; and a pharmaceutical acceptable excipient.
3. A method of treating or preventing a disease or disorder associated with a genetic defect in phenylalanine hydroxylase, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.
4. A method of treating or preventing phenylketonuria, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.
5. A method of treating or preventing hyperphenylalaninemia, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.
6. The method of any one of claims 3-5, wherein the compound reduces systemic phenylalanine levels in the subject.
7. A method of treating or preventing tyrosinemia (Type I, II, or III), comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

8. The method of claim 7, wherein the compound reduces systemic tyrosine levels in the subject.
9. A method of treating or preventing nonketotic hyperglycinemia, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.
10. The method of claim 9, wherein the compound reduces systemic glycine levels in the subject.
11. A method of treating or preventing isovaleric acidemia, methylmalonic acidemia, propionic acidemia, maple syrup urine disease, DNAJC12 deficiency, urea cycle disorders, or hyperammonemia, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.
12. A method of treating or preventing diabetes, chronic kidney disease, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, metabolic syndrome, obesity related disorders, or neurodevelopmental and autism-spectrum disorders, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.
13. The method of any one of claims 1-12, wherein the compound inhibits SLC6A19 in the subject.