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(54) Title: COMPOUNDS HAVING ACTIVITY IN INCREASING ION TRANSPORT BY MUTANT-CFTR AND USES THEREOF

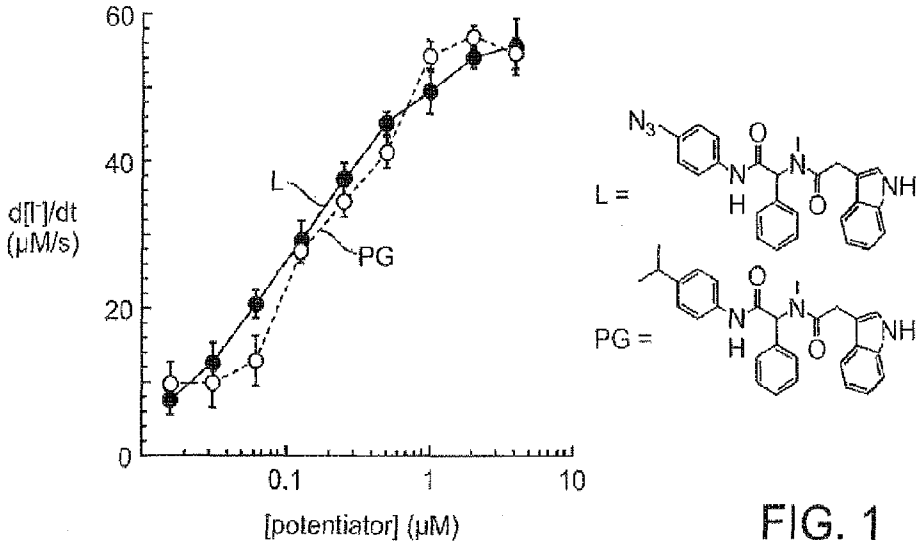

FIG. 1

(57) Abstract: The invention provides compositions, pharmaceutical preparations and methods for increasing activity of a mutant cystic fibrosis transmembrane conductance regulator protein (mutant-CFTR). The compositions pharmaceutical preparations and methods are useful for the study and treatment of disorders associated with mutant-CFTR, such as cystic fibrosis. The compositions and pharmaceutical preparations of the invention may comprise one or more phenylglycine-containing compounds of the invention, or an analog or derivative thereof.

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COMPOUNDS HAVING ACTIVITY IN INCREASING ION TRANSPORT BY MUTANT-CFTR AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119(e) of U.S. Provisional Patent Application No. 60/980,387, filed October 16, 2007, which is herein incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under grant nos. HL73856, EB004 15, HL59198, EY 13574, and DK35 124 awarded by the National Institutes of Health. The government has certain rights in this invention.

Work on this invention was also supported by grants from the Cystic Fibrosis Foundation and/or from Cystic Fibrosis Foundation Therapeutics.

FIELD OF THE INVENTION

The present invention relates to potentiator compounds and methods for increasing ion transport by mutant cystic fibrosis transmembrane conductance regulator protein.

BACKGROUND OF THE INVENTION

The cystic fibrosis transmembrane conductance regulator protein (CFTR) is a cAMP activated chloride ion (Cl^-) channel responsible for Cl^- transport. CFTR is expressed in epithelial cells in mammalian airways, intestine, pancreas and testis. It is there where CFTR provides a pathway for the movement of Cl^- ions across the apical membrane and a key point at which to regulate the rate of transepithelial salt and water transport. Hormones, such as a P-adrenergic agonist, or toxins, such as cholera toxin, lead to an increase in cAMP, activation of cAMP-dependent protein kinase, and phosphorylation of the CFTR Cl^- channel, which causes the channel to open. An increase in the concentration of Ca^{2+} in a cell can also activate different apical membrane channels. Phosphorylation by protein kinase C can either open or shut Cl^- channels in the apical membrane.

Dysfunction of CFTR is associated with a wide spectrum of disease, including cystic fibrosis (CF) and with some forms of male infertility, polycystic kidney disease and secretory diarrhea. CF is a

hereditary disease that mainly affects the lungs and digestive system, causing progressive disability and early death. With an average life expectancy of around 31 years, CF is one of the most common life-shortening, childhood-onset inherited diseases. This disease is caused by mutation of the gene encoding CFTR, and is autosomal recessive. The most common CFTR mutation, deletion of phenylalanine-508 (ΔF508-CFTR), is present in at least one allele in about 90 % of CF patients (Egan et al., (2004) *Science* 304:600-602). ΔF508-CFTR causes Cl^- impermeability because it is not processed correctly, causing it to be retained at the endoplasmic reticulum (rather than the plasma membrane). ΔF508-CFTR also has reduced intrinsic Cl^- conductance relative to wild type CFTR.

Strategies have been investigated to correct the defects in ΔF508-CFTR cellular processing and intrinsic function in cells. Cell growth at low temperature (< 30°C) (Denning et al., (1992) *Nature* 358,761-764) or with high concentrations of chemical chaperones such as glycerol (Sato et al., (1996) *J. Biol. Chem.* 271,635-638; Brown, et al., (1996) *Cell Stress & Chaperones* 1, 1 17-125) corrects partially defective AF508-CFTR cellular processing by a mechanism that may involve improved protein folding and stability (Sharma et al., (2001) *J. Biol. Chem.* 276, 8942-8950). A sustained increase in intracellular calcium concentration by thapsigargin also corrects defective ΔF508-CFTR processing (Egan et al., (2002) *Nature Med.* 8,485-492), possibly by interfering with interactions with molecular chaperones. Compounds like phenylbutryate facilitate ΔF508-CFTR cellular processing by altering chaperone function andlor transcriptional enhancement (Rubenstein et al., (2000) *Am. J. Physio*l. 278, C259-C267; Kang et al., (2002) *Proc. Natl. Acad. Sci.* U.S.A. 99, 838-843). Although these approaches provide insight into mechanisms of ΔF508-CFTR retention at the endoplasmic reticulum, they probably do not offer clinically-useful therapies.

ΔF508-CFTR has significantly impaired channel activity even when present at the cell plasma membrane (Dalemans et al., (1991) *Nature* 354, 526-528). Cell-attached patchclamp measurements showed reduced ΔF508-CFTR open channel probability and prolonged closed times even with maximal cAMP stimulation (Haws et al., (1996) Am. J. Physiol. 270, C1544-C1555; Hwang et al., (1997) Am. J. Physiol. 273, C988-C998). Patch-clamp measurements in excised membranes indicated 7-fold reduced AF508-CFTR activation after phosphorylation compared to wildtype CFTR. Relatively high concentrations of the flavone genistein (>50 pM, Hwang, et al., (1997) *Am. J. Physiol.* 273, C988-C998; Wang et al., (2000) *J. Physiol.* 524,637-638) or the xanthine isobutylmethylxanthine (>1 mM, Drurnrn et al., (1991) *Science* 254, 1797-1799) in combination with cAMP agonists increase ΔF508-CFTR channel activity. Again, these studies have not offered any clinically useful therapies.

Recent identification of small molecule phenylglycine derivatives as potentiators of mutant CFTR has been reported (WO 2005/120497). The phenylglycine derivatives were effective in the high

nanomolar (nM) range. However, the most potent derivative reported in WO 2005/120497 has features that may impact optimal drug activity.

There is accordingly still a need for compounds that can activate mutant CFTR, e.g., ΔF508-CTFR G551D-CFTR, or G1349D-CFTR, and methods of using such compounds for the study and treatment of CF and the treatment and control of other secretory disorders. The present invention addresses these needs, as well as others.

Literature

Phenylglycine derivatives as potentiators of mutant CFTR are reported in International Patent Application Publication (PCT) No. WO 2005/120497. Other PCT publications related to CFTR are represented by WO 01/55106 and WO 2006/10740.

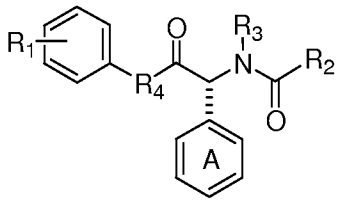
Compounds, formulations and methods of diagnosing and treating mutant-CFTR associated disorders, and related literature of interest are reported in the following US Patent Nos.: 3,953,428; 5,240,846; 5,366,977; 5,407,796; 5,434,086; 5,543,399; 5,582,825; 5,602,110; 5,621,007; 5,635,160; 5,639,661; 5,670,488; 5,674,898; 5,750,571; 5,776,677; 5,834,214; 5,840,702; 5,855,918; 5,863,770; 5,877,179; 5,908,611; 5,939,255; 5,939,536; 5,948,814; 5,958,893; 5,958,907; 5,972,995; 5,976,499; 5,981,178; 5,981,714; 5,989,521; 6,001,588; 6,015,828; 6,030,961; 6,033,688; 6,043,389; 6,063,913; 6,083,954; 6,093,567; 6,110,955; 6,130,248; 6,159,968; 6,201,107; 6,245,735; 6,251,930; 6,281,240; 6,323,187; 6,323,191; 6,329,422; 6,465,494; 6,573,073; 6,599,907; 6,630,482; 6,635,627;6,723,703; 6,730,777; 6,770,739; 6,780,839; 6,902,907; 6,936,618; 6,984,487; 7,118,911; 7,160,729; 7,235,573; 7,238,474; 7,256,210; 7,258,854; 7,259,184; 7,259,250; 7,259,266; 7,261,102; 7,262,200; 7,264,926; 7,264,948; 7,265,088; 7,265,110; 7,265,114; 7,265,148; 7,265,153; 7,267,120; 7,267,652; 7,267,994; 7,268,134; 7,268,155; and 7,268,159.

Reports on the study and correction of defects in CFTR are found in the following references: Denning et al., (1992) *Nature* 358,761-764; Sato et al., (1996) *J. Biol. Chem.* 271,635-638; Brown, et al., (1996) *Cell Stress & Chaperones* 1, 1 17-125; Sharma et al., (2001) *J. Biol. Chem.* 276, 8942-8950; Egan et al., (2002) *Nature Med.* 8,485-492; Rubenstein et al., (2000) *Am. J. Physiol.* 278, C259-C267; Kang et al., (2002) *Proc. Natl. Acad. Sci.* U.S.A. 99, 838-843; Dalemans et al., (1991) *Nature* 354, 526-528; Haws et al., (1996) *Am. J. Physiol.* 270, C1544-C1555; Hwang, et al., (1997) *Am. J. Physiol.* 273, C988-C998; Wang et al., (2000) *J. Physiol.* 524,637-638; and Drurnrn et al., (1991) *Science* 254, 1797-1799.

SUMMARY OF THE INVENTION

The invention provides compositions, pharmaceutical preparations and methods for increasing activity (e.g., ion transport) of a mutant-cystic fibrosis transmembrane conductance regulator protein ("mutant-CFTR") that are useful for the study and treatment of cystic fibrosis ("CF"). The compositions and pharmaceutical preparations may comprise one or more phenylglycine containing compounds of the invention, or an analog or derivative thereof.

The compositions of the invention comprise a compound of formula (I):



(I),

or the salts, solvates, hydrates, and prodrug forms thereof, and stereoisomers thereof, where A is a racemic phenyl comprising L- and D-enantiomers as indicated by the hashed bond; R_1 is hydrogen, a substituted or unsubstituted alkyl, a substituted or unsubstituted heterocycle, or a functional group; R_2 is a substituted or unsubstituted alkoxy, a substituted or unsubstituted phenyl, or a substituted or unsubstituted heterocycle, and is bonded to the R_2 carbonyl either directly or by a spacer comprising 1 to 5 carbons, with the proviso that when R_2 is a heterocycle comprising the spacer and R_4 is NH, then the heterocycle comprising the spacer is substituted with a water soluble group that increases solubility of the compound in water; R_3 is hydrogen or methyl; and R_4 is O or NH.

The pharmaceutical preparations of the invention include an effective amount of a CFTR potentiator compound of the invention. The pharmaceutical compositions can include at least one of a pharmaceutically acceptable carrier, a pharmaceutically acceptable diluent, a pharmaceutically acceptable excipient, and a pharmaceutically acceptable adjuvant.

The methods of the invention include treating a subject having a condition associated with mutant-CFTR, which involves administering to the subject a therapeutically effective amount of a pharmaceutical composition of the invention. The invention also includes a method of increasing ion permeability of a cell producing a mutant-CFTR protein, which involves contacting the cell with an effective amount of the pharmaceutical composition of the invention so as to increase CFTR-mediated ion permeability of the cell.

The invention also provides kits containing one or more compositions of the invention, as well as methods of preparing the compositions.

Advantages of the compounds and compositions of the invention include improved drug like properties such as optical purity and solubility, as well as expanded diversity for generating additional

potentiator compounds. Thus the invention addresses many unmet needs in the development and use of mutant-CFTR potentiator compounds. These and other objects and advantages of the invention will be apparent from the detailed description below.

BREIF DESCRIPTION OF FIGURES

Figure 1: Dose-response curves for R,S racemic PG01 relative to azdio-PG01, where L is racemic azido-PG01and PG is racemic PG01.

Figure 2: Dose-response curves for the S (D-) and R (L-) phenylglycine enantiomers of PG01 relative to the racemic mixture of R,S-PG01, where PG is the PG01 racemic mixture, S is the D-enantiomer of PG01, and R is the L-enantiomer of PG01.

Figure 3: Matrix illustrating a design strategy and the diversity elements employed for D-phenylglycine analog construction, where the panel labeled R_1 shows aniline precursors used in synthesis for generating R_1 diversity, and where the panel labeled R_2 shows the carbonyl precursor used in synthesis for generating R_2 diversity.

Figure 4: Structures of exemplary D-phenylglycine potentiators.

Figure 5: Dose-response curves comparing potentiator activity of exemplary D-phenylglycine potentiators relative to D-enantiomer PG01 (D-PG01 / PG STD).

Figure 6: D-phenylglycine potentiators with improved water solubility.

DETAILED DESCRIPTION OF SPECIFIC EMBODIMENTS

The present invention is based on the discovery of new phenylglycine containing compounds that increase ion transport by mutant cystic fibrosis transmembrane conductance regulator protein ("mutant-CFTR") with high nanomolar potency, and that exhibit a broad range of one or more other properties that find use in the study and treatment of cystic fibrosis ("CF").

The compounds of the invention share a phenylglycine core that includes several structural and functional diversity points that differ from previously reported phenylglycine-based potentiator compounds, such as the PG01 mutant-CFTR potentiator reported in WO 2005/120497. These differences include, for example, resolution and exploitation of non-racemic D-enantiomers of PG01, identification of new diversity elements that replace or tune the aniline and indole substituents of PG01, and modification of the phenylglycine backbone, aniline and / or indole so as to improve water solubility relative to PG01. Accordingly, the subject compounds differ both chemically and structurally to previously known mutant-CFTR potentiator compounds.

By exploiting these different chemical and structural aspects in the design, synthesis and screening of new compound libraries, key structural and chemical features necessary for optimization of compounds containing a phenylglycine core have been identified. The compounds of the invention include one or more of such features so as to impart a pharmacological or biological property that benefits the compound's manufacture, handling, potency, selectivity, and / or pharmacokinetic parameters. The invention also includes compounds with features useful in the study of mutant-CFTR.

As such, the invention provides novel compounds, compositions and pharmaceutical preparations that increase ion transport mediated by mutant-CFTR (e.g., ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR). The invention also features methods of use of such compositions in the treatment of a subject for CF, as well as increasing activity of mutant CFTR in a cell, e.g., by increasing ion transport by mutant CFTR, as well as kits and compound libraries useful for the study and treatment of CF.

Before the present invention and specific exemplary embodiments of the invention are described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Unless defined otherwise, 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 any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, exemplary methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention

is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a "compound" includes a plurality of such compounds and equivalents thereof known to those skilled in the art, and so forth.

Definitions

When describing the compounds, pharmaceutical compositions containing such compounds and methods of using such compounds and compositions, the following terms have the following meanings unless otherwise indicated. It should also be understood that any of the moieties defined forth below may be substituted with a variety of substituents, and that the respective definitions are intended to include such substituted moieties within their scope. By way of non-limiting example, such substituents may include e.g. halo (such as fluoro, chloro, bromo), -CN, $-CF_3$, -OH, $-OCF_3$, C_{2-6} alkenyl, C_{3-6} alkynyl, C_{1-6} alkoxy, aryl and di-C_{1-6} alkylamino.

"Acyl" refers to a radical -C(O)R, where R is hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl or heteroarylalkyl as defined herein. Representative examples include, but are not limited to, formyl, acetyl, cylcohexylcarbonyl, cyclohexylmethylcarbonyl, benzoyl, benzylcarbonyl and the like.

"Acylamino" refers to a radical -NR'C(O)R, where R' is hydrogen, alkyl, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl, heteroarylalkyl and R is hydrogen, alkyl, alkoxy, cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroalkyl, heteroaryl or heteroarylalkyl, as defined herein. Representative examples include, but are not limited to, formylamino, acetylamino, cyclohexylcarbonylamino, cyclohexylmethyl-carbonylamino, benzoylamino, benzylcarbonylamino and the like.

"Acyloxy" refers to the group -OC(O)H, -OC(O)-alkyl, -OC(O)-aryl or -OC(O)-cycloalkyl.

"Aliphatic" refers to hydrocarbyl organic compounds or groups characterized by a straight, branched or cyclic arrangement of the constituent carbon atoms and an absence of aromatic unsaturation. Aliphatics include, without limitation, alkyl, alkylene, alkenyl, alkenylene, alkynyl and alkynylene. Aliphatic groups typically have from 1 or 2 to 6 or 12 carbon atoms. The simplest aliphatic compound is methane and its chemically bonded form methyl (e.g., CH_4, CH_3-, $-CH_2$-, -CH(R)-, $-C(R_i)(R_{ii})$-

). Aliphatics include saturated and unsaturated compounds. Lower aliphatics typically refer to shorter aliphatic compounds having from 1 to 6 carbon atoms.

"Alkanoyl" or "acyl" as used herein refers to the group -C(O)H or -C(O)-alkyl.

"Alkenyl" refers to monovalent olefinically unsaturated hydrocarbyl groups having up to about 11 carbon atoms, particularly, from 2 to 8 carbon atoms, and more particularly, from 2 to 6 carbon atoms, which can be straight-chained or branched and having at least 1 and particularly from 1 to 2 sites of olefinic unsaturation. Particular alkenyl groups include ethenyl (-CH=CH_2), n-propenyl (-CH_2CH=CH_2), isopropenyl (-C(CH_3)=CH_2), vinyl and substituted vinyl, and the like.

"Alkenylene" refers to divalent olefinically unsaturated hydrocarbyl groups particularly having up to about 11 carbon atoms and more particularly 2 to 6 carbon atoms which can be straight-chained or branched and having at least 1 and particularly from 1 to 2 sites of olefinic unsaturation. This term is exemplified by groups such as ethenylene (-CH=CH-), the propenylene isomers (e.g., -CH=CHCH_2- and -C(CH_3)=CH- and –CH=C(CH_3)-) and the like.

"Alkoxy" refers to the group -O-alkyl. Particular alkoxy groups include, by way of example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, *tert*-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

"Alkoxyamino" refers to a radical -N(H)O-alkyl or -N(H)O-cycloalkyl as defined herein.

"Alkoxycarbonyl" refers to a radical -C(O)-alkoxy where alkoxy is as defined herein.

"Alkoxycarbonylamino" refers to the group -NRC(O)OR' where R is hydrogen, alkyl, aryl or cycloalkyl, and R' is alkyl or cycloalkyl.

"Alkyl" refers to monovalent saturated aliphatic hydrocarbyl groups particularly having up to about 11 carbon atoms, more particularly as a lower alkyl, from 1 to 8 carbon atoms and still more particularly, from 1 to 6 carbon atoms. The hydrocarbon chain may be either straight-chained or branched. This term is exemplified by groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, *tert*-butyl, n-hexyl, n-octyl, *tert*-octyl and the like. The term "lower alkyl" refers to alkyl groups having 1 to 6 carbon atoms. The term "alkyl" also includes "cycloalkyls" as defined below.

"Alkylamino" refers to a radical alkyl-NRR', wherein each of R and R' are independently selected from hydrogen and alkyl.

"Alkylarylamino" refers to a radical -NRR' where R represents an alkyl or cycloalkyl group and R' is an aryl as defined herein.

"Alkylene" refers to divalent saturated aliphatic hydrocarbyl groups particularly having up to about 11 carbon atoms and more particularly 1 to 6 carbon atoms which can be straight-chained or branched. This term is exemplified by groups such as methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), the propylene isomers (e.g., $-CH_2CH_2CH_2-$ and $-CH(CH_3)CH_2-$) and the like.

"Alkylthio" refers to a radical -S-alkyl or -S-cycloalkyl group as defined herein that may be optionally substituted as defined herein. Representative examples include, but are not limited to, methylthio, ethylthio, propylthio, butylthio, and the like.

"Alkynyl" refers to acetylenically unsaturated hydrocarbyl groups particularly having up to about 11 carbon atoms and more particularly 2 to 6 carbon atoms which can be straight-chained or branched and having at least 1 and particularly from 1 to 2 sites of alkynyl unsaturation. Particular non-limiting examples of alkynyl groups include acetylenic, ethynyl ($-C\equiv CH$), propargyl ($-CH_2C\equiv CH$), and the like.

"Amide" refers to the radical $-NHC(O)-$ or $-C(O)NH_2$.

"Amino" refers to the radical $-NH_2$.

"Aminocarbonyl" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, aryl or cycloalkyl, or where the R groups are joined to form an alkylene group.

"Aminocarbonylamino" refers to the group -NRC(O)NRR where each R is independently hydrogen, alkyl, aryl or cycloalkyl, or where two R groups are joined to form an alkylene group.

"Aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, aryl or cycloalky, or where the R groups are joined to form an alkylene group.

"Aminohydroxyphosphoryl" refers to the radical $-PO(OH)NH_2$.

"Aralkyl" or "arylalkyl" refers to an alkyl group, as defined above, substituted with one or more aryl groups, as defined above.

"Aromatic" refers to a mono- or polycyclic aromatic hydrocarbon group, and may include one or more heteroatoms in the aromatic ring or ring system termed a heteroaromatic. Also referred to as "aromatic ring" or "aromatic ring system." Simple aromatics comprise from 3-14 carbons, examples of which include arsindole, benzene, benzothiophene, benzo[c]thiophene, benzimidazole, benzoxazole, benzisoxazole, benzothiazole, carbazole, β-carboline, chromane, chromene, cinnoline, furan, imidazole, indazole, indole, indoline, indolizine, isobenzofuran, isochromene, isoindole, isoindoline, isoquinoline, isothiazole, isoxazole, oxadiazole, oxazole, perimidine, phenanthridine, phenanthroline, phenazine, phthalazine, pteridine, purine, pyran, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolizine, purine quinazoline, quinoline, quinolizine, quinoxaline, tetrazole, thiadiazole, thiazole, thiophene, triazole, [1,3,5]triazine and xanthene, as well as fused ring systems such as acridine, anthracene, cinnoline, naphthalene, naphthyridine, quinoline, isoquinoline, quinoxaline and quinazoline.

"Aryl" refers to any functional group or substituent derived from a simple aromatic ring by removal of a hydrogen atom from a carbon atom of a parent aromatic ring system. Typical aryl groups comprises from 6 to 14 carbon atoms. Examples include the radicals of arsindole, benzene, benzothiophene, benzo[c]thiophene, benzimidazole, benzoxazole, benzisoxazole, benzothiazole, carbazole, β-carboline, chromane, chromene, cinnoline, furan, imidazole, indazole, indole, indoline, indolizine, isobenzofuran, isochromene, isoindole, isoindoline, isoquinoline, isothiazole, isoxazole, oxadiazole, oxazole, perimidine, phenanthridine, phenanthroline, phenazine, phthalazine, pteridine, purine, pyran, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolizine, purine quinazoline, quinoline, quinolizine, quinoxaline, tetrazole, thiadiazole, thiazole, thiophene, triazole, [1,3,5]triazine and xanthene, as well as fused ring systems such as acridine, anthracene, cinnoline, naphthalene, naphthyridine, quinoline, isoquinoline, quinoxaline and quinazoline. Examples of radicals denoted by the term "aryl" that are of particular interest include: phenyl, furyl, pyrrolyl, pyrrolidinyl, imidazolyl, isoxazolyl, triazolyl, thiadiazolyl, oxadiazolyl, tetrazolyl, thiatriazolyl, oxatriazolyl, pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, oxazinyl, triazinyl, thiadiazinyl tetrazolo, 1,5-[b]pyridazinyl and purinyl, as well as benzo-fused derivatives, for example, benzoxazolyl, benzthiazolyl, benzimidazolyl and indolyl.

"Arylalkyloxy" refers to an -O-arylalkyl radical where arylalkyl is as defined herein.

"Arylamino" refers to the group aryl-NRR', wherein each of R and R' are independently selected from hydrogen, aryl and heteroaryl.

"Aryloxy" refers to -O-aryl groups wherein "aryl" is as defined herein.

"Arylsulfonyl" refers to a radical $-S(O)_2R$ where R is an aryl or heteroaryl group as defined herein.

"Azide" refers to N_3 or its radical $-N_3$ (also referred to as "azido").

"Carbamoyl" refers to the radical $-C(O)N(R)_2$ where each R group is independently hydrogen, alkyl, cycloalkyl or aryl, as defined herein, which may be optionally substituted as defined herein.

"Carbonyl" refers to the radical -C(O)-.

"Carboxy" refers to the radical -C(O)OH (also referred to as "carboxyl").

"Cyano" refers to the radical -CN.

"Cycloalkenyl" refers to cyclic hydrocarbyl groups having from 3 to 10 carbon atoms and having a single cyclic ring or multiple condensed rings, including fused and bridged ring systems and having at least one and particularly from 1 to 2 sites of olefinic unsaturation. Such cycloalkenyl groups include, by way of example, single ring structures such as cyclohexenyl, cyclopentenyl, cyclopropenyl, and the like.

"Cycloalkyl" refers to cyclic hydrocarbyl groups having from 3 to about 10 carbon atoms and having a single cyclic ring or multiple condensed rings, including fused and bridged ring systems, which optionally can be substituted with from 1 to 3 alkyl groups. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, 1-methylcyclopropyl, 2-methylcyclopentyl, 2-methylcyclooctyl, and the like, and multiple ring structures such as adamantanyl, and the like.

"Cycloheteroalkyl" refers to a stable heterocyclic non-aromatic ring and fused rings containing one or more heteroatoms independently selected from N, O and S. A fused heterocyclic ring system may include carbocyclic rings and need only include one heterocyclic ring. Examples of heterocyclic rings include, but are not limited to, piperazinyl, homopiperazinyl, piperidinyl and morpholinyl, which can be optionally substituted with one or more groups selected from the group consisting of acyl, acylamino, acyloxy, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-. Substituting groups include carbonyl or thiocarbonyl which provide, for example, lactam and urea derivatives. In the examples, M is CR^7, NR^3, O, or S; Q is O, NR^3 or S.

"Dialkylamino" means a radical -NRR' where R and R' independently represent an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, heteroaryl, or substituted heteroaryl group as defined herein.

"Halo" or "halogen" refers to fluoro, chloro, bromo and iodo. Halo groups can be either fluoro or chloro.

"Hetero" when used to describe a compound or a group present on a compound means that one or more carbon atoms in the compound or group have been replaced by a nitrogen, oxygen, or sulfur heteroatom. Hetero may be applied to any of the hydrocarbyl groups described above such as alkyl, e.g. heteroalkyl, cycloalkyl, e.g. cycloheteroalkyl, aryl, e.g. heteroaryl, cycloalkenyl, cycloheteroalkenyl, and the like having from 1 to 5, and especially from 1 to 3 heteroatoms.

"Heterocycle" refers to a closed ring hydrocarbon in which one or more of the atoms in the ring are an element other than carbon (e.g., nitrogen, oxygen, sulfur, etc.). Includes aromatic (aryls and heteroaryls) and non-aromatic (cycloheteroalkyl) rings and systems.

"Heteroaryl" refers to a monovalent heteroaromatic group derived by the removal of a hydrogen atom from an atom of a parent heteroaromatic ring system. Typical heteroaryl groups include, but are not limited to, groups derived from arsindole, benzene, benzothiophene, benzo[c]thiophene, benzimidazole, benzoxazole, benzisoxazole, benzothiazole, carbazole, β-carboline, chromane, chromene, cinnoline, furan, imidazole, indazole, indole, indoline, indolizine, isobenzofuran, isochromene, isoindole, isoindoline, isoquinoline, isothiazole, isoxazole, oxadiazole, oxazole, perimidine, phenanthridine, phenanthroline, phenazine, phthalazine, pteridine, purine, pyran, pyrazine, pyrazole, pyrazine, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolizine, purine quinazoline, quinoline, quinolizine, quinoxaline, tetrazole, thiadiazole, thiazole, thiophene, triazole, [1,3,5]triazine and xanthene, as well as fused ring systems such as acridine, anthracene, cinnoline, naphthalene, naphthyridine, quinoline, isoquinoline, quinoxaline and quinazoline, and the like. In some embodiments, the heteroaryl groups are those derived from thiazole, thiophen, pyrrole, benzothiophene, benzofuran, indole, pyridine, quinoline, imidazole, oxazole and pyrazine.

"Hydroxyl" refers to the radical -OH.

"Nitro" refers to the radical $-NO_2$.

"Phenyl" (often abbreviated as -Ph) is the aryl form of benzene with the functional group, and has the formula $-C_6H_5$, where the six carbon atoms are arranged in an aromatic ring structure.

"Substituted" refers to a group in which one or more hydrogen atoms are each independently replaced with the same or different substituent(s). Typical substituents include, but are not limited to, -X, -R^{14}, -O-, =O, -OR^{14}, -SR^{14}, -S^-, =S, -$NR^{14}R^{15}$, =NR^{14}, -CX_3, -CF_3, -CN, -OCN, -SCN, -NO, -NO_2, =N_2, -N_3, -$S(O)_2O^-$, -$S(O)_2OH$, -$S(O)_2R^{14}$, -$OS(O_2)O^-$, -$OS(O)_2R^{14}$, -$P(O)(O-)_2$, -$P(O)(OR^{14})(O^-)$, -$OP(O)(OR^{14})(OR^{15})$, -$C(O)R^{14}$, -$C(S)R^{14}$, -$C(O)OR^{14}$, -$C(O)NR^{14}R^{15}$, -$C(O)O^-$, -$C(S)OR^{14}$, -$NR^{16}C(O)NR^{14}R^{15}$, -$NR^{16}C(S)NR^{14}R^{15}$, -$NR^{17}C(NR^{16})NR^{14}R^{15}$ and -$C(NR^{16})NR^{14}R^{15}$, where each X is independently a halogen, and where"R^{14}", "R^{15}", "R^{16}", and "R^{17}" are independently hydrogen, alkyl, substituted alkyl, aryl, arylalkyl, cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, heteroalkyl, substituted heteroalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, -$NR^{18}R^{19}$, -$C(O)R^{18}$ or -$S(O)_2R^{18}$ or optionally R^{18} and R^{19} together with the atom to which they are both attached form a cycloheteroalkyl or substituted cycloheteroalkyl ring, and where "R^{18}", "R^{19}", and "R^{22}" are each independently selected from the group consisting of hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloheteroalkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroaryl, and substituted or unsubstituted heteroarylalkyl.

"Substituted aliphatic" includes those groups recited in the definition of "substituted" herein, and particularly refers to aliphatic group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of selected from the group consisting of acyl, acylamino, acyloxy, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-.

"Substituted alkenyl" includes those groups recited in the definition of "substituted" herein, and particularly refers to an alkenyl group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-.

"Substituted alkoxy" includes those groups recited in the definition of "substituted" herein, and particularly refers to an alkoxy group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino,

substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, heteroaryl, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-.

"Substituted alkyl" includes those groups recited in the definition of "substituted" herein, and particularly refers to an alkyl group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, heteroaryl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$-, and aryl-$S(O)_2$-.

"Substituted alkylene" includes those groups recited in the definition of "substituted" herein, and particularly refers to an alkylene group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-.

"Substituted alkynyl" includes those groups recited in the definition of "substituted" herein, and particularly refers to an alkynyl group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-.

"Substituted amino" includes those groups recited in the definition of "substituted" herein, and particularly refers to the group -$N(R)_2$ where each R is independently selected from the group consisting of hydrogen, aliphatic, substituted aliphatic, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, cycloalkyl, substituted cycloalkyl, and where both R groups are joined to form an alkylene group.

"Substituted aryl" includes those groups recited in the definition of "substituted" herein, and particularly refers to an aryl group that may optionally be substituted with 1 or more substituents, for instance from 1 to 5 substituents, particularly 1 to 3 substituents, selected from the group consisting of

acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkenyl, substituted alkenyl, alkoxy, substituted alkoxy, alkoxycarbonyl, alkyl, substituted alkyl, alkynyl, substituted alkynyl, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-. May include heteroaryls and substituted heteroaryls in which one or more carbon atoms of the aromatic ring system is replaced by a group selected from N, O and S. Examples of substituents of particular interest are from one to three halo, trihalomethyl, amino, protected amino, amino salts, mono-substituted amino, disubstituted amino, carboxy, protected carboxy, carboxylate salts, hydroxy, protected hydroxy, salts of a hydroxy group, lower alkoxy, lower allcylthio, alkyl, substituted alkyl, cycloallyl, substituted cycloalkyl, (cycloallcyl)alkyl, substituted (cycloalkyl)allyl, phenyl, substituted phenyl, phenylalkyl, and (substituted pheny1)allyl. Substituents for the heteroaryl group are as heretofore defined, or in the case of trihalomethyl, can be trifluoromethyl, trichloromethyl, tribromomethyl, or triiodomethyl. As used in conjunction with the above substituents for heteroaryl.

"Substituted cycloalkenyl" includes those groups recited in the definition of "substituted" herein, and particularly refers to a cycloalkenyl group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-.

"Substituted cycloalkyl" includes those groups recited in the definition of "substituted" herein, and particularly refers to a cycloalkyl group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-.

"Substituted phenyl" includes those groups recited in the definition of "substituted" herein, and particularly refers to a phenyl group that may optionally be substituted with 1 or more substituents, for instance from 1 to 5 substituents, particularly 1 to 3 substituents. Substituents of the phenyl group include those selected from the group consisting of acyl, acylamino, acyloxy, alkenyl, substituted alkenyl, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkyl, substituted alkyl, alkynyl,

substituted alkynyl, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$-. Substituents of the phenyl group include those that form a fused phenyl ring system in which a heterocycle ring is fused to the phenyl ring, and the heterocycle contains one or more heteroatoms independently selected from N, O and S. Substituents of the phenyl group of particular interest are selected from the group consisting of halogen, hydroxy, protected hydroxy, amino, protected amino, amide, protected amide, thiol, protected thiol, cyano, nitro, azido, trifluoromethyl, C_1 to C_7 alkyl, C_1 to C_7 alkoxy, C_1 to C_7 acyl, C_1 to C_7 acyloxy, carboxy, oxycarboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, (monosubstituted)amino, protected (monosubstituted)amino, (disubstituted)amino, carboxamide, protected carboxarnide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 allyl)carboxamide, N,N-di(C_1 to C_6 allyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino, N-(pl~enylsulfonyl)amino or phenyl, substituted or unsubstituted, such that, for example, a biphenyl or naphthyl group results. Examples of substituted phenyls include a mono- or di(halo)phenyl group such as 2,3 or 4-chlorophenyl, 2,6-dichlorophenyl, 2,5-dichlorophenyl, 3,4-dichlorophenyl, 2,3 or 4-bromophenyl, 3,4-dibromophenyl, 3-chloro-4-fluorophenyl, 2,3 or 4-fluorophenyl and the like; a mono or di(hydroxy)phenyl group such as 2,3, or 4-hydroxyphenyl, 2,4-dihydroxyphenyl, the protected-hydroxy derivatives thereof and the like; a nitrophenyl group such as 2,3, or 4-nitrophenyl; a cyanophenyl group, for example, 2,3 or 4-cyanophenyl; a mono- or di(alky1)phenyl group such as 2, 3, or 4-methylphenyl, 2,4-dimethylphenyl, 2, 3 or 4-(iso-propyl)phenyl, 2,3, or 4-ethylphenyl, 2,3 or 4-(n-propyl)phenyl and the like; a mono or di(alkoxy)phenyl group, for example, 2,6-dimethoxyphenyl, 2,3 or 4-(isopropoxy)phenyl, 2,3 or 4-(t-butoxy)phenyl, 3-ethoxy-4-methoxyphenyl and the like; 2,3 or 4-trifluoromethylphenyl; a mono- or dicarboxyphenyl or (protected carboxy)phenyl group such as 2,3 or 4-carboxyphenyl or 2,4-dibrotected carboxy)phenyl; a mono- or di(hydroxymethy1)phenyl or (protected hydroxymethy1)phenyl such as 2,3 or 4-(protected hydroxymethy1)phenyl or 3,4-di(hydroxymethy1)phenyl; a mono- or di(aminomethy1)phenyl or (protected aminomethy1)phenyl such as 2,3 or 4-(aminomethyl)phenyl or 2,4-(protected aminomethy1)phenyl; or a mono- or dim-(methylsulfonylamino))phenyl such as 2,3 or 4-(N-(methylsulfonylamino))phenyl. Also, the term "substituted phenyl" represents disubstituted phenyl groups wherein the substituents are different, for example, 3-methyl-4-hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 2-methoxy-4-bromophenyl, 4-ethyl-2-hydroxyphenyl, 3-hydroxy-4-nitrophenyl, 2-hydroxy-4-chlorophenyl and the like

"Substituted thioalkoxy" includes those groups recited in the definition of "substituted" herein, and particularly refers to a thioalkoxy group having 1 or more substituents, for instance from 1 to 5 substituents, and particularly from 1 to 3 substituents, selected from the group consisting of acyl, acylamino, acyloxy, aliphatic, substituted aliphatic, alkoxy, substituted alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy,

aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioketo, thiol, alkyl-S(O)-, aryl-S(O)-, alkyl-$S(O)_2$- and aryl-$S(O)_2$.

"Sulfanyl" refers to the radical -SH. "Substituted sulfanyl" refers to a radical such as -SR wherein R is any substituent described herein.

"Sulfone" refers to the group -SO_2R. In particular embodiments, R is selected from H, lower alkyl, alkyl, aryl and heteroaryl.

"Sulfonyl" refers to the divalent radical -$S(O_2)$-. "Substituted sulfonyl" refers to a radical such as R-(O_2)S- wherein R is any substituent described herein. "Aminosulfonyl" refers to the radical $H_2N(O_2)$S-, and "substituted aminosulfonyl" refers to a radical such as $R_2N(O_2)$S- wherein each R is independently any substituent described herein.

"Thioalkoxy" refers to the group -S-alkyl.

"Thioaryloxy" refers to the group -S-aryl.

"Thioketo" refers to the group =S.

"Thiol" refers to the group -SH.

One having ordinary skill in the art will recognize that the maximum number of heteroatoms in a stable, chemically feasible heterocyclic ring, whether it is aromatic or non aromatic, is determined by the size of the ring, the degree of unsaturation and the valence of the heteroatoms. In general, a heterocyclic ring may have one to four heteroatoms so long as the heteroaromatic ring is chemically feasible and stable.

A "mutant cystic fibrosis transmembrane conductance regulator protein" or "mutant-CFTR" is the protein that results from a mutation, e.g., deletion mutation, insertion mutation, or point (substitution) mutation of the CFTR gene product relative to wildtype. A "mutant cystic fibrosis transmembrane conductance regulator protein", or "mutant-CFTR" refers to a dysfunctional CFTR as compared to a functional (e.g., wildtype) CFTR, where the dysfunction can encompass one or more of the following: (i) aberrant CFTR production (e.g., at the level of transcription or translation); (ii) aberrant folding and/or trafficking; (iii) abnormal regulation of conductance; (iv) decreases in chloride conductance; (v) reduction in synthesis; and the like. A "mutant-CFTR gene" is a gene, or coding sequence, which encodes a

mutant-CFTR. For the purposes of this application, the terms "genome" and "gene" are used interchangeably, e.g. "genome that encodes mutant-CFTR" and "gene that encodes mutant-CFTR".

A "gating defective mutant cystic fibrosis transmembrane conductance regulator protein" or "gating defective mutant-CFTR" is a mutant-CFTR that is present on the cell surface and is defective in gating of ions through the channel (e.g., regulation of ion transport). Thus, as used herein a "gating defective mutant-CFTR" encompasses dysfunctions associated with (i) abnormal regulation of conductance; and or (ii) decreases in chloride conductance.

A "mutant-CFTR protein-mediated condition" means any condition, disorder or disease, or symptom of such condition, disorder, or disease that results from or is correlated to the presence of a mutant-CFTR, e.g., ΔF508-CFTR, e.g., chloride ion impermeability caused by reduced activity of ΔF508-CFTR in ion transport relative to a wild-type CFTR. A "mutant-CFTR protein-mediated condition" encompasses conditions in an affected subject which are associated with the presence of a ΔF508-CFTR mutation on at least one allele, thus including subjects that carry a ΔF508-CFTR mutation on both alleles as well as compound heterozygous subjects having two different mutant forms of CFTR, e.g., a subject with one copy of ΔF508-CFTR and a copy of different mutant form of CFTR. Such conditions, disorders, diseases, or symptoms thereof are treatable by specific activation of mutant-CFTR activity, e.g., activation of mutant-CFTR ion transport. ΔF508-CFTR is correlated to the presence of cystic fibrosis (CF), and a description of this disease, including its symptoms, is found in Accession No. 602421 (entitled cystic fibrosis transmembrane conductance regulator; CFTR), and Accession No. 2 19700 (entitled Cystic fibrosis; CF) of the Online Mendelian Inheritance of Man database, as found at the world wide website of the National Institute of Health at ncbi.nlm.nih.gov. Symptoms of mutant-CFTR protein-mediated conditions include meconium ileus, liver disease including biliary tract obstruction and stenosis, pancreatic insufficiency, pulmonary disease including chronic *Pseudomonas aeruginosa* infections and other infections of the lung, infertility associated with abnormal vas deferens development or abnormal cervical mucus, and carcinoma including adenocarcinoma. Many subjects that have a mutant-CFTR protein-mediated condition are homozygous for a gene encoding a ΔF508-CFTR protein.

A "ΔF508-cystic fibrosis transmembrane conductance regulator protein" or "ΔF508-CFTR" is the protein that results from the deletion of a phenylalanine residue at amino acid position 508 of the CFTR gene product. A "ΔF508-CFTR gene" is a gene, or coding sequence, which encodes ΔF508-CFTR. A ΔF508-CFTR gene usually results from deletion of three nucleotides corresponding to the phenylalanine residue at amino acid position 508 of the encoded CFTR gene product. For the purposes of this application, the terms "genome" and "gene" are used interchangeably, e.g. "genome that encodes ΔF508-CFTR and "gene that encodes ΔF508-CFTR". For an example of a gene that encodes ΔF508-CFTR, see, e.g. WO 91102796.

A "mutant-CFTR activator" as used herein is a compound that increases the level of ion transport by a mutant-CFTR relative to ion transport in the absence of the compound, and particularly with respect to transport of chloride ions. CFTR activators of the invention of particular interest are those that are specific mutant-CFTR activators, e.g., compounds that activate mutant-CFTR activity rather than affecting CFTR cellular misprocessing. Mutant-CFTR activators are usually high-affinity mutant-CFTR activators, e.g., have an affinity for mutant-CFTR of at least about one micromolar, about one to five micromolar, about 200 nanomolar to one micromolar, about 50 nanomolar to 200 nanomolar, or below 50 nanomolar.

A "gating defective mutant-CFTR activator" as used herein is a compound that increases the level of ion transport by a gating defective mutant-CFTR relative to ion transport in the absence of the compound, and particularly with respect to transport of chloride ions. CFTR activators of the invention of particular interest are those that are specific gating defective mutant-CFTR activators, e.g., compounds that activate gating defective mutant-CFTR activity rather than affecting, for example, CFTR cellular misprocessing. Gating defective mutant-CFTR activators are usually high-affinity activators of gating defective mutant-CFTRs, e.g., have an affinity for a gating defective mutant-CFTR (e.g., ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR) of at least about one micromolar, about one to five micromolar, about 200 nanomolar to one micromolar, about 50 nanomolar to 200 nanomolar, or below 50 nanomolar.

A "ΔF508-CFTR activator" as used herein is a compound that increases the level of ion transport by ΔF508-CFTR relative to ion transport in the absence of the compound, and particularly with respect to transport of chloride ions. CFTR activators of the invention of particular interest are those that are specific ΔF508-CFTR activators, e.g., compounds that activate ΔF508-CFTR activity rather than affecting CFTR cellular misprocessing. ΔF508-CFTR activators are usually high-affinity ΔF508-CFTR activators, e.g., have an affinity for ΔF508-CFTR of at least about one micromolar, about one to five micromolar, about 200 nanomolar to one micromolar, about 50 nanomolar to 200 nanomolar, or below 50 nanomolar.

As used herein and in the cystic fibrosis field a "potentiator" refers to a compound that increases a basal level of ion transport by a mutant-CFTR (e.g,. ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR), where the mutant CFTR (in the absence of the compound) exhibits aberrantly low levels of ion transport relative to wildtype CFTR. As such, a "mutant-CFTR potentiator" refers to a potentiator compound that provides for increased level of ion transport by a mutant-CFTR relative to ion transport capability of the mutant-CFTR in the absence of the compounds.

As used herein and in the cystic fibrosis field a "mutant-CFTR corrector" is a compound that increases the level of ion transport by a mutant-CFTR relative to ion transport in the absence of the

compound by correcting the underlying defect of the CFTR polypeptide, e.g., a defect that results from post-translational mis-processing (e.g., misfolding). CFTR correctors of the invention of particular interest are those that facilitate correction of specific mutant-CFTRs. Mutant-CFTR correctors are usually exhibit high affinity for one or more mutant-CFTRs,, e.g., have an affinity for mutant-CFTR of at least about one micromolar, about one to five micromolar, about 200 nanomolar to one micromolar, about 50 nanomolar to 200 nanomolar, or below 50 nanomolar.

The term "analog" or "analogue" refers to without limitation any compound which has structural similarity to the compounds of the invention and would be expected, by one skilled in the art, to exhibit the same or similar utility as the claimed and/or referenced compounds.

The term "derivative" refers to without limitation any compound which has a structure derived from the structure of the compounds of the present invention and whose structure is sufficiently similar to those disclosed herein and based upon that similarity, would be expected, by one skilled in the art, to exhibit the same or similar activities and utilities as the claimed and/or referenced compounds.

The term "effective amount" of a compound as provided herein is intended to mean a sufficient amount of the compound to provide the desired utility. The term "therapeutically effective amount" or "efficacious amount" means the amount of a compound that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the compound, the disease and its severity and the age, weight, etc., of the subject to be treated. Thus, as will be pointed out below, the exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition or disease that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact "effective amount." However, an appropriate effective amount may be determined by one of ordinary skill in the art using only routine experimentation.

"Functional group" refers to atoms or small groups of atoms (two to four) that exhibit a characteristic reactivity when treated with certain reagents, and are attached to the carbon backbone of organic molecules. The same functional group will undergo the same or similar chemical reaction(s) regardless of the size of the molecule it is a part of. Examples of functional groups include halogen, hydroxy, carboxy, ester, thioester, amino, oxime, hydrazone, thiol, azide, nitro, nitroso, aldehyde and ketone. The functional groups can be protected or unprotected, activated or unactivated.

The term "in combination with" as used herein refers to uses where, for example, the first compound is administered during the entire course of administration of the second compound; where the first compound is administered for a period of time that is overlapping with the administration of the

second compound, e.g. where administration of the first compound begins before the administration of the second compound and the administration of the first compound ends before the administration of the second compound ends; where the administration of the second compound begins before the administration of the first compound and the administration of the second compound ends before the administration of the first compound ends; where the administration of the first compound begins before administration of the second compound begins and the administration of the second compound ends before the administration of the first compound ends; where the administration of the second compound begins before administration of the first compound begins and the administration of the first compound ends before the administration of the second compound ends. As such, "in combination" can also refer to regimen involving administration of two or more compounds. "In combination with" as used herein also refers to administration of two or more compounds which may be administered in the same or different formulations, by the same of different routes, and in the same or different dosage form type.

The term “isolated” means that a compound which has been substantially separated from, or enriched relative to, other compounds with which it occurs in nature. “Isolated” also refers to the state of a compound separated from all or some of the components that accompany it during manufacture (e.g., chemical synthesis, recombinant expression, culture medium, and the like). Isolated compounds may be present as stereoisomers, and in particular, diastereomers as well as their racemic and resolved, enantiomerically pure forms and salts thereof. Typically, an isolated compound is substantially pure when it is at least 50% to 60%, by weight, free from organic molecules with which it is naturally associated or with which it is associated during manufacture. Generally, the preparation is at least 75%, more usually at least 90%, and generally at least 99%, by weight, of the compound of interest. A substantially pure compound can be obtained, for example, by extraction from a natural source (e.g., bacteria), by chemically synthesizing a compound, or by a combination of purification and chemical modification. A substantially pure compound can also be obtained by, for example, enriching a sample having a particular isomer of a compound of interest. Purity can be measured by any appropriate method, e.g., chromatography, mass spectroscopy, HPLC analysis, etc.

The term "optional" or "optionally" means that the subsequently described event, circumstance, feature or element may, but need not, occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, "heterocyclo group optionally mono- or di- substituted with an alkyl group" means that the allyl may, but need not, be present, and the description includes situations where the heterocyclo group is mono- or disubstituted with an allyl group and situations where the heterocyclo group is not substituted with the alkyl group.

The term "organic group" and "organic radical" means any carbon containing group, including hydrocarbon groups that are classified as an aliphatic group, cyclic group, aromatic group, functionalized derivatives thereof and/or various combination thereof.

The terms “monosubstituted” refers to group with one substituent, “disubstituted” refers to group with two substituents, “trisubstituted” refers a group with three substituents, and so forth. For example, a (monosubstituted)amino refers to an amino group with one substituent, whereas a (disubstituted)amino refers to an amino group with two substituents, and whereas a (trisubstituted)amino refers to an amino group with three substitutents. When two or more substituents are present, they can be the same or different.

The term "pharmaceutically acceptable" refers to a material that is not biologically or otherwise undesirable, i.e., the material is of a medically acceptable quality and composition that may be administered to an individual along with the selected active pharmaceutical ingredient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

The term “pharmaceutically acceptable excipient” as used herein refers to any suitable substance which provides a pharmaceutically acceptable vehicle for administration of a compound(s) of interest to a subject. “Pharmaceutically acceptable excipient” can encompass substances referred to as pharmaceutically acceptable diluents, pharmaceutically acceptable additives and pharmaceutically acceptable carriers. For example, a "pharmaceutically acceptable excipient," "pharmaceutically acceptable diluent," "pharmaceutically acceptable carrier," and "pharmaceutically acceptable adjuvant" includes excipient, diluent, carrier, and adjuvant that are useful in preparing a pharmaceutical composition that are generally safe, non-toxic and neither biologically nor otherwise undesirable, and include an excipient, diluent, carrier, and adjuvant that are acceptable for veterinary use as well as human pharmaceutical use, and may include both one and more than one such excipient, diluent, carrier, and adjuvant.

The term "physiological conditions" is meant to encompass those conditions compatible with living cells, e.g., predominantly aqueous conditions of a temperature, pH, salinity, etc. that are compatible with living cells.

The term "pharmaceutical composition" is meant to encompass a composition suitable for administration to a subject, such as a mammal, especially a human. In general a "pharmaceutical composition” is sterile, and preferably free of contaminants that are capable of eliciting an undesirable response within the subject (e.g., the compound(s) in the pharmaceutical composition is pharmaceutical

grade). Pharmaceutical compositions can be designed for administration to subjects or patients in need thereof via a number of different routes of administration including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, intracheal and the like. In some embodiments the composition is suitable for administration by a transdermal route, using a penetration enhancer other than DMSO. In other embodiments, the pharmaceutical compositions are suitable for administration by a route other than transdermal administration.

The term "pharmaceutically acceptable derivatives" of a compound of the invention include salts, esters, enol ethers, enol esters, acetals, ketals, orthoesters, hemiacetals, hemiketals, acids, bases, solvates, hydrates or prodrugs thereof. Such derivatives may be readily prepared by those of skill in this art using known methods for such derivatization. The compounds produced may be administered to animals or humans without substantial toxic effects and either are pharmaceutically active or are prodrugs.

The term "pharmaceutically acceptable salt" of a compound means a salt that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. Such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoy1)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, glucoheptonic acid, 4,4'-methylenebis-(3-hydroxy-2-ene-1-carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolarnine, tromethamine, N-methylglucamine, and the like.

The term "pharmaceutically acceptable ester" of a compound of the invention means an ester that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound, and includes, but is not limited to, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl and heterocyclyl esters of acidic groups, including, but not limited to, carboxylic acids, phosphoric acids, pbosphinic acids, sulfonic acids, sulfinic acids and boronic acids.

The term "pharmaceutically acceptable enol ether" of a compound of the invention means an enol ether that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound, and includes, but is not limited to, derivatives of formula C=C(OR) where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl or heterocyclyl.

The term "pharmaceutically acceptable enol ester" of a compound of the invention means an enol ester that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound, and includes, but is not limited to, derivatives of formula C=C(OC(O)R) where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl or heterocyclyl.

The term "pharmaceutically acceptable solvate or hydrate" of a compound of the invention means a solvate or hydrate complex that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound, and includes, but is not limited to, complexes of a compound of the invention with one or more solvent or water molecules, or 1 to about 100, or 1 to about 10, or one to about 2,3 or 4, solvent or water molecules.

The terms “polypeptide” and “protein”, used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; fusion proteins with detectable fusion partners, e.g., fusion proteins including as a fusion partner a fluorescent protein, β-galactosidase, luciferase, etc.; and the like. Polypeptides may be of any size, and the term “peptide” refers to polypeptides that are 8-50 residues (e.g., 8-20 residues) in length.

The term "protecting group" means a chemical group introduced into a molecule by chemical modification of a functional group in order to protect or shield the functional group from its normal chemical reactivity. Protecting groups, their addition and removal are well known (W. Green, P. G. M. Wuts, Protective Groups in Organic Synthesis, Wiley-Interscience, New York, 2005). Removal of the protecting group generates the original functional group, which may be referred to as an "unprotected group".

The term "prodrugs" means any compound that releases an active parent drug according to formula (I) in vivo when such prodrug is administered to a mammalian subject. Prodrugs of a compound of formula (I) are prepared by modifying hctional groups present in the compound of formula (I) in such a way that the modifications may be cleaved in vivo to release the parent compound. Prodrugs include

compounds of formula (I) wherein a hydroxy, amino, or sulfhydryl group in compound (I) is bonded to any group that may be cleaved in vivo to regenerate the free hydroxyl, amino, or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to esters (e.g., acetate, formate, and benzoate derivatives), carbamates (e.g., N,N-dimethylaminocarbonyl) of hydroxy functional groups in compounds of formula (I), and the like.

The term "racemic" means a mixture containing approximately equal proportions of enantiomers.

The terms "subject," "host," "patient," and "individual" are used interchangeably herein to refer to any mammalian subject for whom diagnosis or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on. Non-human animal models, particularly mammals, e.g. primate, murine, lagomorpha, etc. may be used for experimental investigations.

As used herein, the terms "determining," "measuring," and "assessing," and "assaying" are used interchangeably and include both quantitative and qualitative determinations.

The term "stereoisomer" means a compound with the same chemical formula and bond structure as a reference compound, but the geometrical positioning of atoms and functional groups in space differs. This class of isomers includes "enantiomers" in which different isomers are non-superimposable mirror-images of each other, and diastereomers when they are not. Enantiomers can be designated by "(+)-" versus "(–)-" when based on optical properties, or "(R)-" versus "(S)-" and or "D-" versus "L-" when based on geometric properties. For example, "D-enantiomer" and "L-enantiomer" refer to the enantiomers of a chiral system, based on the actual geometry of each enantiomer. In the context of amino acids, the enantiomer with geometry based on a naturally occurring amino acid is the L-enantiomer, whereas and the enantiomer based on a non-naturally occurring amino acid is the D-enantiomer. The term "diastereomer" refers to rotational or conformational steroisomers ("rotational isomers" or "rotomers"; and "conformational isomers" or "conformers") when the isomers can interconvert by chemical bond rotations, or cis-trans isomerism ("cis-trans isomers") when this is not possible. Stereoisomers also include "tautomers" which are structural isomers of the same chemical substance that spontaneously interconvert with each other, even when pure. Thus unless indicated otherwise, the description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers and mixtures, racemic or otherwise, thereof. The methods for the determination of stereochemistry and the separation of stereoisomers are well-known in the art (see, e.g., the discussion in Chapter 4 of "Advanced Organic Chemistry", 4th edition J. March, John Wiley and Sons, New York, 1992).

The term "treating" or "treatment" of a condition or disease includes: (1) preventing at least one symptom of the conditions, i.e., causing a clinical symptom to not significantly develop in a mammal that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease, (2) inhibiting the disease, i.e., arresting or reducing the development of the disease or its symptoms, or (3) relieving the disease, i.e., causing regression of the disease or its clinical symptoms.

The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

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

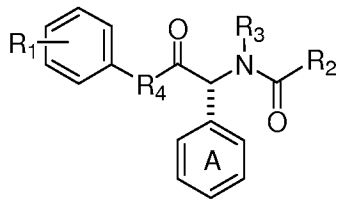
In describing the invention, the structure of the compounds of the invention will be described first. Then, pharmaceutical formulations containing the compounds will be discussed, followed by a description of their methods of use, and kits.

Compounds

The invention provides compounds and compositions containing them that increase ion transport in a mutant-CFTR, such as ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR, and methods of their use in treatment of mutant-CFTR-mediated diseases and conditions, e.g., cystic fibrosis. Such compounds also find use in the study of CFTR ion transport, particularly that of ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, and D1152H-CFTR.

In one embodiment, the invention provides high-affinity small-molecule compounds that increase chloride ion (Cl-) conductance in gating defective mutant-CFTRs, such as ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, and D1152H-CFTR. The compounds comprise a phenylglycine core and multiple diversity points with variable substituents.

The subject compounds of the invention are generally described by formula (I) below.

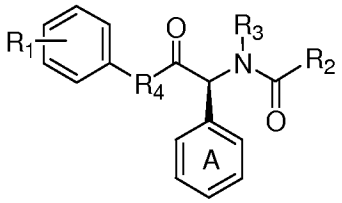


(I)

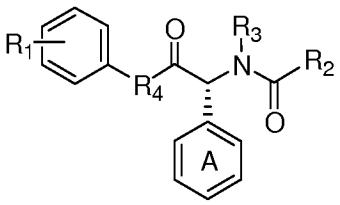
In formula (I), A is a racemic phenyl comprising L- and D-enantiomers as indicated by the hashed bond; R_1 is hydrogen, a substituted or unsubstituted alkyl, a substituted or unsubstituted heterocycle, or a functional group; R_2 is an alkoxy, a substituted or unsubstituted phenyl, or a substituted or unsubstituted heterocycle, and is bonded to the R_2 carbonyl either directly or by a spacer comprising 1 to 5 carbons, with the proviso that when R_2 is a heterocycle comprising the spacer and R_4 is NH, then the heterocycle comprising the spacer is substituted with a water soluble group that increases solubility of the subject compound in water; R_3 is hydrogen or methyl; and R_4 is O or NH. The compounds of formula (I) also encompass the salts, solvates, hydrates, and prodrug forms thereof, as well as stereoisomers thereof. In other embodiments, the phenyl ring A may further include a substituent, such as a detectable label like azide.

In one embodiment, the compound of formula (I) is in a composition that contains the isolated compound. In another embodiment, the composition containing the compound is comprised as a pharmaceutical composition. The compositions generally include an effective amount of a CFTR potentiator compound for potentiating a gating defective mutant-CFTR, such as ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR. In particular, the pharmaceutical compositions generally include a compound that is therapeutically effective in increasing the CFTR-mediated ion permeability of a cell producing a mutant-CFTR, and the composition comprises a therapeutically effective amount of the compound for increasing the CFTR-mediated ion permeability of the cell. When provided as a pharmaceutical composition, the composition may further comprise at least one of a pharmaceutically acceptable carrier, a pharmaceutically acceptable diluent, a pharmaceutically acceptable excipient and a pharmaceutically acceptable adjuvant. The pharmaceutical preparations of the invention are described in further detail herein.

In certain embodiments, the phenyl A ring is non-racemic and consists essentially of either L-enantiomer or D-enantiomer. In a specific embodiment, phenyl A consists essentially of D-enantiomer. Thus, compounds that consist essentially of the L-enantiomer or D-enantiomer version of a phenylglycine compound of formula (I) are provided, and depicted below in formula (II) and formula (III).



D-enantiomer, formula (II)



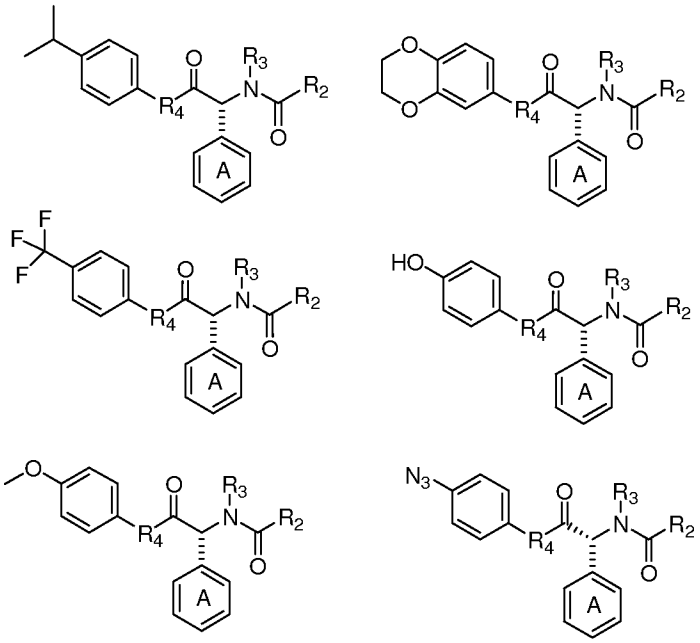
L-enantiomer, formula (III)

Of particular interest are the D-enantiomer compounds of formula (II), which can exhibit more resistance to degradation by enzymes in biological systems, and reduce unwanted immunogenicity in certain embodiments. The specific enantiomer chosen may also exhibit increased potency relative to a racemic mixture or the antipode enantiomer. Thus the compounds of the invention may possess one or more asymmetric centers, and be provided as individual (R)-or (S)- stereoisomers (i.e., (L)- or (D)-stereoisomers, respectively), or as mixtures thereof.

R_1 Substituents

As noted in formula (I), R_1 can be hydrogen, a substituted or unsubstituted alkyl, a substituted or unsubstituted heterocycle, or a functional group. In an exemplary embodiment, R_1 is a substituted or unsubstituted alkyl selected from methyl, ethyl, propyl, isopropyl, methoxy, ethoxy and trifluorormethyl. In another exemplary embodiment, R_1 is a substituted or unsubstituted heterocycle comprising dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4. In yet another embodiment, R_1 is a functional group selected from hydroxy, hydroxymethyl, carboxy, carboxymethyl, acetyl, acetamide, amino, nitro and azide.

Formula (I) compounds of particular interest may comprise an R_1 substituent selected from isopropyl, dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4, trifluoromethyl, hydroxyl, methoxy or azide, as depicted below in the following formulas of Panel A, where R_2, R_3, R_4 and A are as defined for formula (I):



Panel A

A featured aspect of the invention includes compounds of Panel A above in which R_3 is methyl, R_4 is NH, and R_2 is selected from *tert*-butoxy, benzyl, (phenol-2-yl)-ethyl, (phenol-4-yl)-methyl, (phenol-4-yl)-ethyl, (benzoimidazol-2-yl)-aniline-3-yl, pyrrole-1-yl, pyrazine-2-yl, 5-methyl-pyrazine-2-yl, 3-methoxy-2-methyl-2H-indazole-6-yl, pyrrolidin-2-one-5-yl, (5-azidoindole-3-yl)-methyl, (5-carboxymethylindole-3-yl)-methyl, (5-carboxymethoxyindole-3-yl)-methyl, and (5-hydroxyindole-3-yl)-methyl.

Another featured aspect of the invention includes compounds of Panel A above in which R_3 is methyl, R_4 is O, and R_2 is selected from *tert*-butoxy, benzyl, (phenol-2-yl)-ethyl, (phenol-4-yl)-methyl, (phenol-4-yl)-ethyl, (benzoimidazol-2-yl)-aniline-3-yl, pyrrole-1-yl, pyrazine-2-yl, 5-methyl-pyrazine-2-yl, 3-methoxy-2-methyl-2H-indazole-6-yl, pyrrolidin-2-one-5-yl, (indole-3-yl)-methyl, (5-azidoindole-3-yl)-methyl, (5-carboxymethylindole-3-yl)-methyl, (5-carboxymethoxyindole-3-yl)-methyl, and (5-hydroxyindole-3-yl)-methyl.

R_2 Substituents

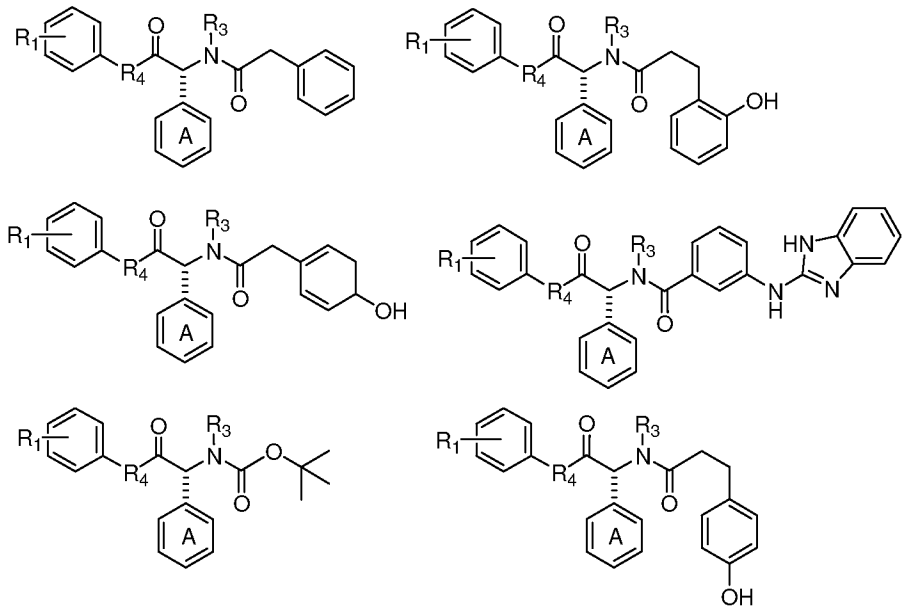
As described above, the R_2 group of formula (I) is a substituted or unsubstituted alkoxy, a substituted or unsubstituted phenyl, or a substituted or unsubstituted heterocycle, and is bonded to the R_2 carbonyl either directly or by a spacer comprising 1 to 5 carbons, provided that when R_2 is a heterocycle bonded to the R_2 carbonyl by a spacer and R_4 is NH, then the heterocycle comprising the spacer is substituted with a water soluble group that increases solubility of the subject compound in water.

The spacer can be straight or branched carbon chains, and may optionally include one or more heteroatoms in the main chain, or contain one or more substituents in place of a hydrogen on a carbon of the spacer. Of particular interest are simple alkyl-based carbon chains that comprise 1 to 5 carbons, 1-4 carbons, and particularly 1-3 carbons or less. The featured spacers comprise a radical selected from -CH_2-, -CH_2-CH_2-, and -CH_2-CH_2-CH_2-.

In one embodiment, R_2 is a substituted or unsubstituted alkoxy group. In a specific embodiment, the alkoxy group is selected from methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, *tert*-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, and 1,2-dimethylbutoxy. A particular alkoxy R_2 group of interest is *tert*-butoxy, which comprises a *t*-butyl carbamate ("Boc") group in the context of the R_2 carbonyl. In other embodiments, R_2 is a substituted alkoxy group, such as an alkoxyamino, alkoxycarbonyl, or alkoxycarbonylamino. Examples of substituted alkoxy include the radicals -N(H)O-alkyl, -N(H)O-cycloalkyl, -C(O)-alkoxy, and -NRC(O)OR' where R is hydrogen, alkyl, aryl or cycloalkyl, and R' is alkyl or cycloalkyl.

In another embodiment, R_2 comprises a phenyl group, which can be an unsubstituted phenyl such as benzyl, or a substituted phenyl, such as phenol (e.g., phenol-2-yl or phenol-4-yl) or aniline (e.g., aniline-3-yl or (benzoimidazol-2-yl)-aniline-3-yl)). In this embodiment, the unsubstituted or substituted phenyl is bonded to the R_2 carbonyl of formula (I) either directly or by a spacer comprising 1 to 5 carbons. For example, in a specific embodiment, R_2 is an unsubstituted phenyl that comprises a one carbon spacer, such as benzyl. In another specific embodiment, the substituted phenyl is a phenol comprising a one carbon spacer, such as (phenol-4-yl)-methyl, or a two carbon spacer such as (phenol-2-yl)-ethyl or (phenol-4-yl)-ethyl. Alternatively, R_2 can be directly attached to the R_2 carbonyl of formula (I), for instance, as with a substituted phenyl comprising the aniline derivative (benzoimidazol-2-yl)-aniline-3-yl.

Thus formula (I) compounds of particular interest may comprise an R_2 substituent exemplified by benzyl, (phenol-2-yl)-ethyl, (phenol-4-yl)-methyl, (phenol-4-yl)-ethyl, *t*-butoxy, or (benzoimidazol-2-yl)-aniline-3-yl, as depicted below in the following formulas of Panel B, where R_1, R_3, R_4 and A are as defined for formula (I):



Panel B

A featured aspect of the invention includes compounds of Panel B above in which R_3 is methyl, R_4 is NH or O, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

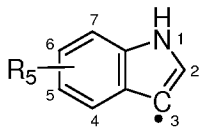
In an additional embodiment, R_2 is a substituted or unsubstituted heterocycle. Thus when R_2 is a heterocycle bonded to the R_2 carbonyl of formula (I) by a spacer and R_4 is NH, the heterocycle is substituted with one or more water soluble groups. Examples of water soluble groups suitable for this purpose include, but are not limited to, hydroxyl, amine, nitro, azido, methoxy, ethoxy, carboxymethyl, carboxyethyl, caboxymethoxy and carboxyethoxy. Specific water soluble groups of interest include a radical selected from -OH, -O-, -C(O)O-and -C(O)OH. As can be appreciated, such water soluble groups can be excluded or incorporated in other substituents of the compounds of the invention, but are only required on the R_2 group when both R_4 is NH and R_2 is a heterocycle bonded to the R_2 carbonyl of formula (I) by a spacer.

Selection of a specific water soluble group can be carried out based on structure activity analysis and by calculating the partition (log P) or distribution (log D) coefficients of the modified versus un-modified parent compound, particularly log P partition coefficients, which are useful in estimating distribution of drugs within the body. Techniques for predicting or measuring partition and distribution coefficients are common (See, e.g., Sangster, J. (1997). *Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry*, Vol. 2 of Wiley Series in Solution Chemistry. Chichester: John Wiley & Sons Ltd; Leo et al., (1995). *Exploring QSAR, Hydrophobic, Electronic, and Steric Constants.*

Washington, DC: American Chemical Society; and Scherrer RA, Howard SM (1977). "Use of distribution coefficients in quantitative structure-activity relationships". *J Med Chem* 20 (1): 53-8).

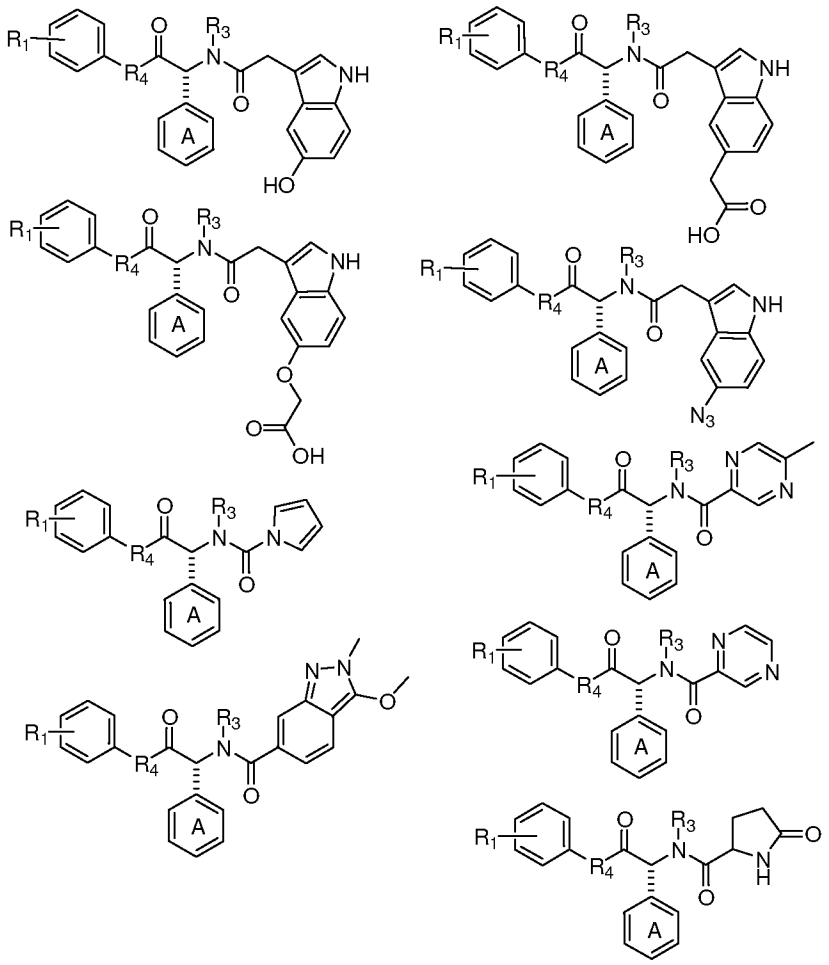
In a specific embodiment, R_2 is a substituted or unsubstituted heterocycle selected from indole, pyrrole, pyrazine, indazole and pyrrolidone, that is connected directly or by a spacer to the R_2 carbonyl of formula (I). Examples of R_2 heterocycle groups without a spacer include, but are not limited to, pyrrole-1-yl, pyrazine-2-yl, indazole-6-yl, pyrrolidone-5-yl, 5-methyl-pyrazine-2-yl, 3-methoxy-2-methyl-2H-indazole-6-yl, and pyrrolidin-2-one-5-yl. Examples of R_2 heterocycle groups that include a spacer and are substituted with a water soluble group include, but are not limited to, (5-azidoindole-3-yl)-methyl, (5-carboxymethylindole-3-yl)-methyl, (5-carboxymethoxyindole-3-yl)-methyl, and (5-hydroxyindole-3-yl)-methyl.

In another specific embodiment, compounds are provided in which R_2 is an indole substituted with a water soluble group that increases solubility of the compound in water. A particular example is an indole-3-yl of the formula:



where R_5 is a water soluble group that increases the solubility of the compound in water, and where C• is a carbon radical at indole position 3 bonded to the R_2-carbonyl of formula (I) by a 1-5 carbon spacer. Particular examples of R_5 are selected from hydroxyl, amine, nitro, azido, methoxy, ethoxy, carboxymethyl, carboxyethyl, caboxymethoxy and carboxyethoxy. In other embodiments, R_5 comprises a radical selected from -OH, -O-, -C(O)O-and -C(O)OH. Of particular interest are substituted indole compounds where R_5 is bonded at indole position 5, with specific examples including (5-azidoindole-3-yl)-methyl, (5-carboxymethylindole-3-yl)-methyl, (5-carboxymethoxyindole-3-yl)-methyl, and (5-hydroxyindole-3-yl)-methyl.

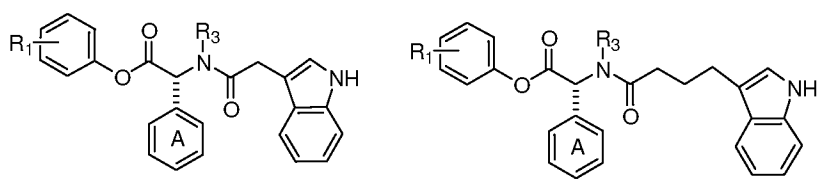
Formula (I) compounds that include R_2 heterocycles with and without spacers are further illustrated in Panel C below and exemplified by R_2 substituents comprising pyrrole-1-yl, pyrazine-2-yl, 5-methyl-pyrazine-2-yl, 3-methoxy-2-methyl-2H-indazole-6-yl, pyrrolidin-2-one-5-yl, (5-carboxymethylindole-3-yl)-methyl, (5-carboxymethoxyindole-3-yl)-methyl, (5-hydroxyindole-3-yl)-methyl, and (5-azidoindole-3-yl)-methyl, and where R_1, R_3, R_4 and A are as defined for formula (I):



Panel C

In a related embodiment, the compound is from Panel C, and R_3 is methyl, R_4 is NH, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4. In another related embodiment, the compound is from Panel C, and R_3 is methyl, R_4 is O, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

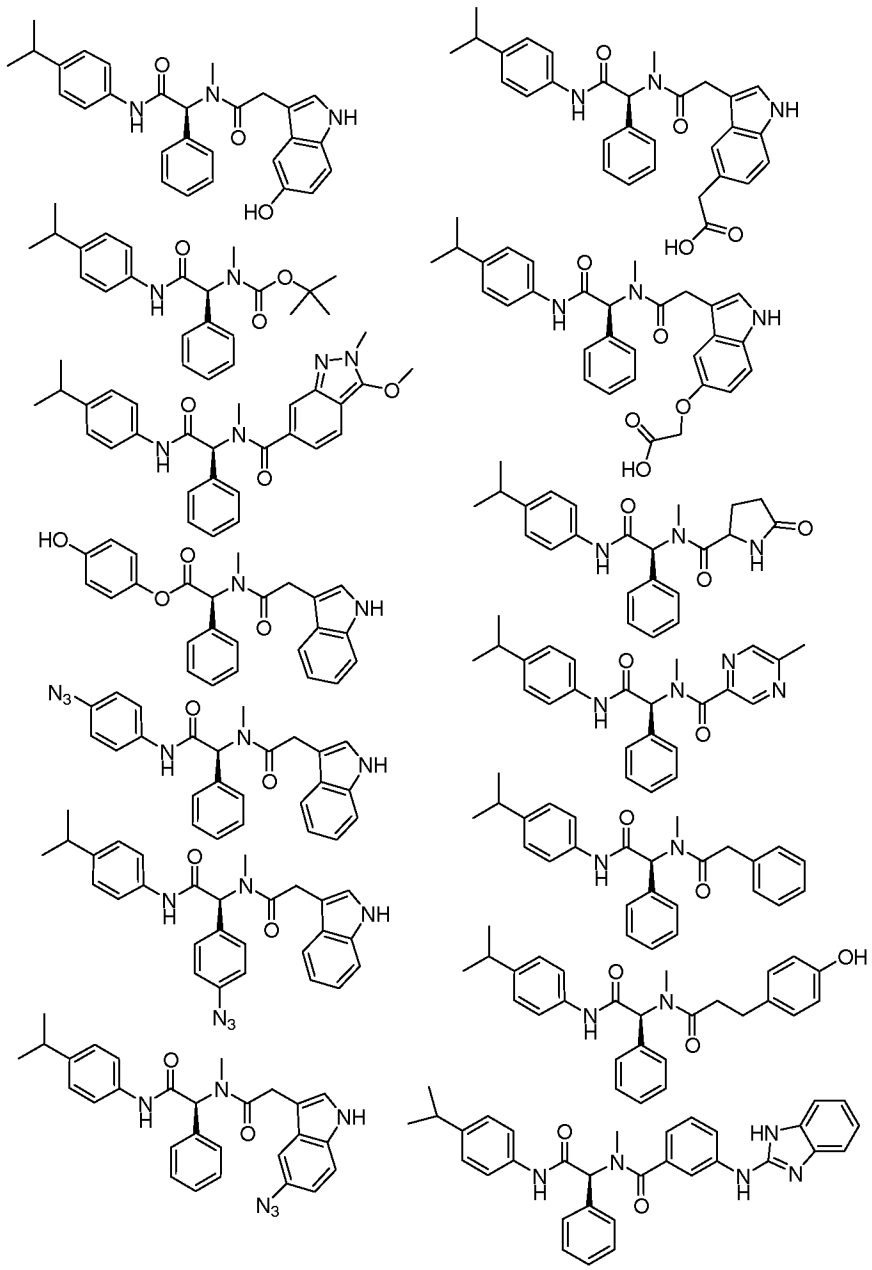
In another embodiment, when R_2 is a heterocycle bonded to the R_2 carbonyl of formula (I) by a spacer and R_4 is O, then the heterocycle may be substituted or unsubstituted, such as depicted in Panel D below, where R_1, R_3 and A are as defined for formula (I):



Panel D

Specific examples include, but are not limited to, a compound from Panel D in which R_3 is methyl, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

Thus is in some embodiments of the invention, the phenylglycine containing compounds may comprise a formula of the following, or the salts, solvates, hydrates, and prodrug forms thereof, and stereoisomers thereof:



Panel E

Analog and Derivative Compounds

Also provided by the invention are analogs and derivatives of the subject compounds described above. The terms "analog" and "derivative" refers to a molecule which is structurally similar or has the same function or activity as the subject phenylglycine containing compounds of the invention. Such analogs and derivatives of the subject compounds can be screened for efficiency in binding to and modulating the activity of a mutant CFTR, such as ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR.

In some embodiments, *in silico* modeling can be used to screen libraries of analog or derivative compounds. For example, protein-ligand docking can be used to predict the position and orientation of a ligand (a small molecule) when it is bound to a protein such as a mutant-CFTR. Docking techniques can be for a variety of purposes, most notably in the virtual screening of large databases of available chemicals in order to select likely drug candidates. An exemplary *in silico* modeling program suitable for use with the subject method is the PREDICTTM 3D Modeling Technology (Predix Pharmaceuticals, Woburn MA), described in greater detail in Becker et al., *PNAS* 101(3 1): 11304-1 1309 (2004).

Pharmaceutical Preparations

Also provided by the invention are pharmaceutical preparations of the subject compounds described above. The subject compounds can be incorporated into a variety of formulations for therapeutic administration by a variety of routes. More particularly, the compounds of the present invention can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers, diluents, excipients andlor adjuvants, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols. In most embodiments, the formulations are free of detectable DMSO (dimethyl sulfoxide), which is not a pharmaceutically acceptable carrier, diluent, excipient, or adjuvant in the context of routes of administration other than transdermal routes. In some embodiments, and particularly where the formulation is for transdermal administration, the compounds can be formulated either without detectable DMSO or with a carrier in addition to DMSO. The formulations may be designed for administration to subjects or patients in need thereof via a number of different routes, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, intratracheal, etc., administration.

Pharmaceutically acceptable excipients usable with the invention, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary

substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985; Remington: The Science and Practice of Pharmacy, A.R. Gennaro, (2000) Lippincott, Williams & Wilkins. The composition or formulation to be administered will, in any event, contain a quantity of the agent adequate to achieve the desired state in the subject being treated.

Dosage forms

In pharmaceutical dosage forms, the subject compounds of the invention may be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

The agent can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes, such as intrapulmonary or intranasal delivery.

Conventional and pharmaceutically acceptable routes of administration include intranasal, intrapulmonary intramuscular, intratracheal, intratumoral, subcutaneous, intradermal, topical application, intravenous, rectal, nasal, oral and other parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the agent andlor the desired effect. The composition can be administered in a single dose or in multiple doses.

In one embodiment of particular interest, the compounds of the invention are administered in aerosol formulation via intrapulmonary inhalation. The compounds of the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

Mechanical devices designed for intrapulmonary delivery of therapeutic products, include but are not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those of skill in the art. 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 I1 nebulizer, manufactured by Marquest Medical Products, Englewood, Colo.; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, North Carolina; the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Mass.; the "standing cloud" device of Inhale Therapeutic Systems, Inc., San Carlos, Calif.; the AIR inhaler manufactured by Alkennes, Cambridge, Mass.; and the AERx pulmonary drug delivery system manufactured by Aradigm Corporation, Hayward, Calif. Of particular interest are the PARI LC PLUS®, the PARI LC STAR®, and the PARI BABY™ nebulizers by PARI Respiratory Equipment, Inc., Monterey, Calif.

Formulations for use with a metered dose inhaler device may generally comprise a finely divided powder. This powder may be produced by lyophilizing and then milling a liquid conjugate formulation and may also contain a stabilizer such as human serum albumin (HSA). Typically, more than 0.5% (wlw) HSA is added. Additionally, one or more sugars or sugar alcohols may be added to the preparation if necessary. Examples include lactose maltose, mannitol, sorbitol, sorbitose, trehalose, xylitol, and xylose. The amount added to the formulation can range from about 0.01 to 200% (wlw), preferably from approximately 1 to 50%, of the conjugate present. Such formulations may then lyophilized and milled to the desired particle size.

The properly sized particles may then 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 may include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant. This mixture may then loaded into the delivery device. An example of a commercially available metered dose inhaler suitable.for use in the present invention is the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, N.C.

Formulations for powder inhalers may comprise a finely divided dry powder containing conjugate 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 particles of the powder may have aerodynamic properties in the lung corresponding to particles with a density of about 1 glcm.sup.2 having a median diameter less than 10 micrometers, preferably between 0.5 and 5 micrometers, most preferably of between 1.5 and 3.5 micrometers. An example of a powder inhaler suitable for use in accordance with the teachings herein is the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Mass. The powders for these devices may be generated andlor delivered by methods disclosed in U.S. Pat. No. 5,997,848, U.S. 5,993,783, U.S. 5,985,248, U.S. 5,976574, U.S. 5,922,354, U.S. 5,785,049 and U.S. 5,654,007.

For oral preparations, the subject compounds can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, and intravenous routes, i.e., any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of the agent. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

Methods of administration of the agent through the skin or mucosa include, but are not necessarily limited to, topical application of a suitable pharmaceutical preparation, transdermal transmission, injection and epidermal administration. For transdermal transmission, absorption promoters or iontophoresis are suitable methods. Iontophoretic transmission may be accomplished using commercially available "patches" which deliver their product continuously via electric pulses through unbroken skin for periods of several days or more.

The subject compounds of the invention can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

The agent can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (e.g., using a suppository) delivery.

Furthermore, the subject compounds can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The compounds of the present invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

Dosages

Depending on the subject and condition being treated and on the administration route, the subject compounds may be administered in dosages of, for example, 0.1 µg to 10 mg/kg body weight per day. The range is broad, since in general the efficacy of a therapeutic effect for different mammals varies widely with doses typically being 20, 30 or even 40 times smaller (per unit body weight) in man than in the rat. Similarly the mode of administration can have a large effect on dosage. Thus, for example, oral dosages may be about ten times the injection dose. Higher doses may be used for localized routes of delivery.

A typical dosage may be a solution suitable for intravenous administration; a tablet taken from two to six times daily, or one time-release capsule or tablet taken once a day and containing a proportionally higher content of active ingredient, etc. The time-release effect may be obtained by capsule materials that dissolve at different pH values, by capsules that release slowly by osmotic pressure, or by any other known means of controlled release.

Those of skill in the art will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

Although the dosage used will vary depending on the clinical goals to be achieved, a suitable dosage range is one which provides up to about 1 µg to about 1,000 µg or about 10,000 µg of subject composition of the to reduce a symptom in a subject animal.

Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more compounds of the invention. Similarly, unit dosage forms for injection or intravenous administration may comprise the compound (s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

Combination therapy

For use in the subject methods, the subject compounds may be formulated with or otherwise administered in combination with other pharmaceutically active agents, including other CFTR-activating agents. The subject compounds may be used to provide an increase in the effectiveness of another chemical, such as a pharmaceutical (e.g., other CFTR-activating agents, or agents that affect cellular misprocessing of mutant-CFTR), or a decrease in the amount of another chemical, such as a

pharmaceutical (e.g., other CFTR-activating agents), that is necessary to produce the desired biological effect.

Examples of other CFTR activating agents include, but are not limited to, enhancers of intracellular cAMP levels, such as for example, but not limited to, forskolin, rolipram, 8-bromo-cAMP, theophylline, papaverine, cAMP and salts, analogs, or derivatives thereof. Other examples include beta agonists, tobramycin (TOBI®, Chiron Inc., Emeryville, Calif.) and curcumin (Egan et al., (2004) *Science* 304:600-603). The compounds of the invention may also be used in combination with specific mutant CFTR activators, such as one or more other correctors and/or potentiators. Examples of mutant-CFTR corrector agents include, but are not limited to, bithiazole containing compounds described WO 2006/101740, which is incorporated herein in its entirety. Corrector compounds of particular interest are described in co-pending US provisional patent application serial no. 60/980,389, filed October 16, 2007, entitled "Compounds Having Activity In Correcting Mutant-CFTR Cellular Processing And Uses Thereof", which is incorporated herein in its entirety.

The compounds described above may also be combined with other therapies for CF, including oral corticosteroids, ibuprofen, ribovarin or antibiotics such as dicloxacillin, cephalosporin, cephalexin,erythromycin, amoxicillin-clavulanate, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol ciprofloxacin, tobramycin, gentamicin, cephalosporins, monobactams and the like.

The compounds described herein for use in combination therapy with the compounds of the present invention may be administered by the same route of administration (e.g. intrapulmonary, oral, enteral, etc.) that the 'compounds are administered. In the alternative, the compounds for use in combination therapy with the compounds of the present invention may be administered by a different route of administration that the compounds are administered.

Methods

Methods for increasing chloride ion permeability of a mutant-CFTR cell

The invention provides methods for increasing ion permeability of a cell that produces mutant-CFTR protein, with cells having a gating defective mutant-CFTR being of interest, with cells having a ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR being of particular interest. In general, the method involves contacting the cell with a compound in an amount effective to activate the mutant-CFTR protein and increase ion permeability of the cell. In one embodiment of particular interest, a compound of the invention is used in the method in combination with a second mutant-CFTR activator or potentiator.

In many embodiments, the cell mutant-CFTR protein is present on the plasma membrane of the cell. Methods of detecting mutant-CFTR protein presence on the plasma membrane are well known in the art and can include but are not limited to, for example, labeling a molecule that binds to CFTR protein with a fluorescent, chemical or biological tag. Examples of molecules that bind to CFTR protein include, without limitation, antibodies (monoclonal and polyclonal), FAB fragments, humanized antibodies and chimeric antibodies. For an example of an antibody that binds to CFTR protein, see, e.g. U.S. Patent No. 6,201,107.

In many embodiments, the cell has increased permeability to chloride ions, and the contacting of the cell with a compound of the invention, particularly when provided in combination with a mutant-CFTR activator or potentiator, increases the rate of chloride ion transport across the plasma membrane of the cell. Contacting the cell with a compound of the invention usually increases the activity of mutant-CFTR protein to increase ion transport.

In most embodiments, the ion transport activity of mutant-CFTR, or the permeability of a cell to ions, is increased by up to about l0%, by up to about 20%, by up to about 50%, by up to about l00%, by up to about 150%, by up to about 200%, by up to about 300%, by up to about 400%, by up to about 500%, by up to about 800%, or up to about 1000% or more. In certain embodiments, where there is no detectable ion transport activity of mutant-CFTR or permeability of a cell to ions, contacting of the cell with a compound of the invention causes detectable activity of mutant-CFTR or permeability of a cell to ions.

Activation of mutant-CFTR andor ion permeability may be measured using any convenient methods that may use molecular markers, e.g., a halide sensitive GFP or another molecular marker (e.g., Galietta et al., (2001) FEBS *Lett.* 499,220-224), patch clamp assays, and short circuit assays.

Suitable cells include those cells that have an endogenous or introduced mutant-CFTR gene. Suitable cells include mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells etc.) harboring constructs that have an expression cassette for expression of mutant-CFTR. The cell used in the subject methods may be a cell present in vivo, ex vivo, or *in vitro.* As used herein, the term "expression cassette" is meant to denote a genetic sequence, e.g. DNA or RNA, that codes for mutant-CFTR protein, e.g., ΔF508-CFTR. Methods of introducing an expression cassette into a cell are well known in the art. See for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, NY, Vols. 1-3 (1989).

Methods of treating cystic fibrosis

The invention also provides methods of treating a subject having a condition associated with mutant-CFTR, e.g., cystic fibrosis. In general, the method involves administering to the subject a

compound of the invention in an amount effective to activate a mutant-CFTR protein to increase ion transport and thereby treat the condition. In an embodiment of particular interest, a compound of the invention is administered in combination with a second mutant-CFTR activator or potentiator, e.g., a compound that enhances intracellular cAMP, e.g., forskolin or a corrector compound, such as the bithiazole containing corrector compounds described WO 2006/101740 and in co-pending US provisional patent application serial no. [to be accorded], filed October 16, 2007, entitled "Compounds Having Activity In Correcting Mutant-CFTR Cellular Processing And Uses Thereof", [**Attorney Docket No. UCSF-358PRV**], each of which are incorporated herein in their entirety.

The compounds disclosed herein are useful in the treatment of a mutant-CFTR mediated condition, e.g., any condition, disorder or disease, or symptom of such condition, disorder, or disease, that results from the presence and/or activity of mutant-CFTR as compared to wild-type CFTR, e.g., activity of mutant-CFTR in ion transport. Such conditions, disorders, diseases, or symptoms thereof are amenable to treatment by activation of mutant-CFTR activity, e.g., activation of mutant-CFTR chloride transport. Cystic fibrosis, a hereditary condition associated with a mutant-CFTR, e.g., ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR, is an example of a condition that is treatable using the compounds of the invention. Use of the compounds of the invention in combination with a second mutant CFTR activator or potentiator is of particular interest.

Cystic fibrosis is predominantly a disorder of infants, children and young adults, in which there is widespread dysfunction of the exocrine glands, characterized by signs of chronic pulmonary disease (due to excess mucus production in the respiratory tract), pancreatic deficiency, abnormally high levels of electrolytes in the sweat and occasionally by biliary cirrhosis. Also associated with the disorder is an ineffective immunologic defense against bacteria in the lungs.

Pathologically, the pancreas shows obstruction of the pancreatic ducts by amorphous eosinophilic concretions, with consequent deficiency of pancreatic enzymes, resulting in steatorrhoea and azotorrhoea and intestinal malabsorption. The degree of involvement of organs and glandular systems may vary greatly, with consequent variations in the clinical picture.

Nearly all exocrine glands are affected in cystic fibroses in varying distribution and degree of severity. Involved glands are of three types: those that become obstructed by viscid or solid eosinophilic material in the lumen (pancreas, intestinal glands, intrahepatic bile ducts, gallbladder, submaxillary glands); those that are histologically abnormal and produce an excess of secretions (tracheobronchial and Brunner's glands); and those that are histologically normal but secrete excessive sodium and chloride (sweat, parotid, and small salivary glands). Duodenal secretions are viscid and contain an abnormal mucopolysaccharide. Infertility occurs in 98% of adult men secondary to maldevelopment of the vas

deferens or to other forms of obstructive azoospermia. In women, fertility is decreased secondary to viscid cervical secretions, but many women with CF have carried pregnancies to term. However, the incidence of maternal complications increases.

Fifty percent of cystic fibrosis patients with pulmonary manifestations usually chronic cough and wheezing associated with recurrent or chronic pulmonary infections. Cough is the most troublesome complaint, often accompanied by sputum, gagging, vomiting, and disturbed sleep. Intercostal retractions, use of accessory muscles of respiration, a barrelchest deformity, digital clubbing, and cyanosis occur with disease progression. Upper respiratory tract involvement includes nasal polyposis and chronic or recurrent sinusitis. Adolescents may have retarded growth, delayed onset of puberty, and a declining tolerance for exercise. Pulmonary complications in adolescents and adults include pneurnothorax, hemoptysis, and right heart failure secondary to pulmonary hypertension.

Pancreatic insufficiency is clinically apparent in 85 to 90% of CF patients, usually presents early in life, and'may be progressive. Manifestations include the frequent passage of bulky, foul-smelling, oily stools; abdominal protuberance; and poor growth pattern with decreased subcutaneous tissue and muscle mass despite a normal or voracious appetite. Rectal prolapse occurs in 20% of untreated infants and toddlers. Clinical manifestations may be related to deficiency of fat-soluble vitamins.

Excessive sweating in hot weather or with fever may lead to episodes of hypotonic dehydration and circulatory failure. In arid climates, infants may present with chronic metabolic alkalosis. Salt crystal formation and a salty taste on the skin are highly suggestive of CF.

Insulin-dependent diabetes develops in 10% of adult patients having CF, and multilobular biliary cirrhosis with varices and portal hypertension develops in 4 to 5% of adolescents and adults. Chronic and/or recurrent abdominal pain may be related to intussusception, peptic ulcer disease, periappendiceal abscess, pancreatitis, gastroesophageal reflux, esophagitis, gallbladder disease, or episodes of partial intestinal obstruction secondary to abnormally viscid fecal contents. Inflammatory complications may include vasculitis and arthritis.

Any of above symptoms of CF may be treated using the compounds of the invention, with use of such compounds in combination with a second mutant-CFTR activator or potentiator being of particular interest.

The above methods may be used to treat CF and its symptoms in humans or in animals. Several animal models for CF are known in the art. For example, Engelhardt et al. *(J. Clin. Invest.* 90: 2598-2607, 1992) developed an animal model of the human airway, using bronchial xenografts engrafted on rat

tracheas and implanted into nude mice. More recently transgenic models of cystic fibrosis have been produced (e.g., Clarke et al., *Science* 257: 1125-1 128, 1992; Dorin et al., *Nature* 359: 21 1-215, 1992). With the recent advances of nuclear transfer and stem cell transformation technologies, the alteration of a wild type CFTR gene in an animal to make it into a mutant-CFTR gene is possible for a wide variety of animals.

Many of these animals show human CF symptoms. In particular, many of these animals showed measurable defects in ion permeability of airway and intestinal epithelia, similar to those demonstrable in human CF tissues, and a susceptibility to bacterial infection. Furthermore, most of the deficient mice had intestinal pathology similar to that of meconium ileus. Also, there appeared to be no prenatal loss from litters produced from crosses between heterozygotes.

Animals suitable for treatment using the subject methods include any animal with a mutant-CFTR related condition, particularly a mammal, e.g., non-human primates (e.g., monkey, chimpanzee, gorilla, and the like), rodents (e.g., rats, mice, gerbils, hamsters, ferrets, and the like), lagomorphs, swine (e.g., pig, miniature pig), equine, canine, feline, and the like. Large animals are of particular interest. Transgenic mammals may also be used, e.g. mammals that have a chimeric gene sequence. Methods of making transgenic animals are well known in the art, see, for example, U.S. Patent No. 5,614,396. For an example of a transgenic mouse with a CFTR defect, see e.g. WO 94104669.

Such animals may be tested in order to assay the activity and efficacy of the subject compounds. Improvement in lung function can be assessed by, for example, monitoring prior to and during therapy the subject's forced vital capacity (FVC), carbon monoxide diffusing capacity (DLco), andlor room air pO_2 >55 mmHg at rest. Significant improvements in one or more of these parameters are indicative of efficacy. It is well within the skill of the ordinary healthcare worker (e.g., clinician) provide adjust dosage regimen and dose amounts to provide for optimal benefit to the patient according to a variety of factors (e.g., patient dependent factors such as the severity of the disease and the like), the compound administered, and the like).

Subjects suitable for treatment

Subjects suitable for treatment with a method of the present invention include individuals having mutant-CFTR protein-mediated condition disorder or disease, or symptom of such condition, disorder, or disease that results from or is correlated to the presence of a mutant-CFTR, usually two alleles of the mutant CFTR. Moreover, subjects suitable for treatment with a method of the present invention include individuals with Cystic Fibrosis (CF). Of particular interest in many embodiments is the treatment of humans with CF.

Symptoms of mutant-CFTR protein-mediated conditions include meconium ileus, liver disease including biliary tract obstruction and stenosis, pancreatic insufficiency, pulmonary disease including chronic *Pseudomonas aeruginosa* infections and other infections of the lung, infertility associated with abnormal vas deferens development or abnormal cervical mucus, and carcinoma including adenocarcinoma.

The compounds of the present invention affect the ion transport capability of the mutant-CFTR by increasing the reduced level of ion transport mediated by a mutant-CFTR, such as the ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR. As such, the compounds of the present invention have particular clinical utility in treating a subset of CF patients that have mutations in the CFTR gene that results a mutant-CFTR that is expressed in the plasma membrane and has reduced chloride conductance capability or has abnormal regulation of conductance (i.e., the mutant-CFTR is gating defective). As such, the compounds of the present invention have clinical utility in treating CF patients having a gating-defective mutant-CFTR, such as ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR. In addition, the compounds of the present invention also have clinical utility in treating CF patients when used in conjunction with compounds that correct cellular misprocessing of a mutant-CFTR, such as ΔF508-CFTR.

CFTR mutations associated with CF are well known in the art. These mutations can be classified in five general categories with respect to the CFTR protein. These classes of CFTR dysfunction include limitations in CFTR production (e.g., transcription and/or translation) (Class I), aberrant folding and/or trafficking (Class II), abnormal regulation of conduction (Class III), decreases in chloride conduction (Class IV), and reductions in synthesis (Class V). Due to the lack of functional CFTR, Class I, II, and III mutations are typically associated with a more severe phenotype in CF (i.e. pancreatic insufficiency) than the Class IV or V mutations, which may have very low levels of functional CFTR expression. A listing of the different mutations that have been identified in the CFTR gene is as found at the world-wide website of the Cystic Fibrosis Mutation Database at genet.sickkids.on.ca/cgi-bin/WebObjects/MUTATION, specifically incorporated by reference herein in its entirety.

A subject suitable for treatment with a method of the present invention may be homozygous for a specific mutant-CFTR, i.e. homozygous subjects with two copies of a specific mutant-CFTR, e.g., AF508-CFTR. In addition, subjects suitable for treatment with a method of the present invention may also be compound heterozygous for two different CFTR mutants, i.e., wherein the genome of the subjects includes two different mutant forms of CFTR, e.g., a subject with one copy of AF508-CFTR and a copy of different mutant form of CFTR.

In some embodiments of the invention, the mutant-CFTR polypeptide is ΔF508-CFTR. In other embodiments of the invention, the mutant-CFTR polypeptide is G551D-CFTR. In yet other embodiments of the invention, the mutant-CFTR polypeptide is G1349D-CFTR. In still other embodiments of the invention, the mutant-CFTR polypeptide is D152H-CFTR. The invention, however, should not be construed to be limited solely to the treatment of CF patients having this mutant form of CFTR. Rather, the invention should be construed to include the treatment of CF patients having other mutant forms of CFTR with similar characteristics, that result in expression of the mutant-CFTR in the plasma membrane and has reduced chloride conductance capability or has abnormal regulation of conductance.

Kits & Systems

Also provided are kits and systems that find use in practicing the subject methods, as described above. The kits typically contain unit doses of the subject compounds, usually in oral or injectable doses. For example, kits and systems for practicing the subject methods may include one or more pharmaceutical formulations that include phenylglycine potentiator compound of the invention, and optionally one or more additional components. As such, in certain embodiments the kits may include a single pharmaceutical composition present as one or more unit dosages. In yet other embodiments, the kits may include two or more separate pharmaceutical compositions, as well as be part of a system. The term "system" as employed herein refers to a collection of components or agents present in single or disparate compositions that are brought together for the purpose of practicing the subject methods.

Thus the kits can include one or more of, depending upon the intended use of the kit, the compositions described herein, such as: a phenylglycine potentiator compound of the invention, and the like. Other optional components of the kit include: buffers, delivery vehicles, delivery systems etc., for administering a phenylglycine potentiator compound, and/or for performing a diagnostic assay. The various components of the kit may be present in separate containers or certain compatible components may be pre-combined into a single container, as desired. The kits also may include one or more additional pharmaceuticals or agents for treating a mutant-CFTR.

In addition to the above components, the subject kits may further include instructions for practicing the subject methods, such as an informational package insert describing the use and attendant benefits of the drugs in treating pathological condition of interest. These instructions may be present in the kits in a variety of forms, one or more of which may be present in or on the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in or on the packaging of the kit, in a package insert, etc. Yet another means would be a computer readable medium, e.g., diskette, CD, etc., on which the information has been recorded. Yet another means that may be present is a website

address which may be used via the internet to access the information at a removed site. Any convenient means may be present in the kits.

In a specific embodiment, a kit is provided for use in treating a subject suffering from cystic fibrosis. This kit includes a pharmaceutical composition comprising phenylglycine compound of the invention, and instructions for the effective use of the pharmaceutical composition in a method of treating a subject suffering from cystic fibrosis. Such instructions may include not only the appropriate handling properties, dosing regiment and method of administration, and the like, but further include instructions to optionally screen and type the subject for mutant-CFTR (e.g., ΔF508-CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR). This aspect of the invention assists the practitioner of the kit in tracking or gauging the potential responsiveness of the subject to treatment with a composition of the invention. Thus in another embodiment, the kit may further include a system for characterizing mutant-CFTRs, such as described in WO 2005/120497. In another embodiment, the kit includes one or more phenylglycine compositions that are detectably labeled.

EXAMPLES

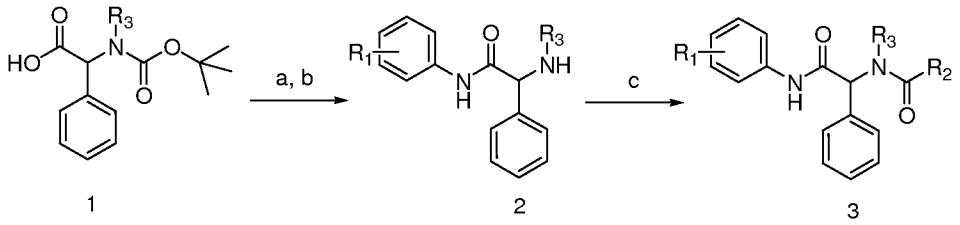
The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric. Thus it is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: General materials and methods

The following materials and methods illustrate the synthesis, analysis and testing of CFTR potentiator compounds.

Compounds Phenylglycine compounds were synthesized, purified, and confirmed by NMR and liquid chromatography / mass spectrometer following standard protocols. **Scheme 1** illustrates the general synthesis method.

Scheme 1: Synthesis of phenyl glycine derivatives.



1 2 3

Scheme 1 conditions: (a). p-isopropylaniline, EDCI, cat. (catalytic amount) DMAP, CH_2Cl_2, 22°C, 2 h, yield 92%; (b). TFA, 22°C, 15 min, 98%; and (c). indole-3-acetic acid, EDCI, cat. DMAP, CH_2Cl_2, 22°C, 2 h, 92%. R_3 is hydrogen or methyl depending on the phenylglycine synthesis core chosen for elaboration. R_1 represents the diversity point of the aniline derivative. R_2 represents the diversity point of the carbonyl derivative.

The following illustrates synthesis of standard compound PG01 (racemic). To a solution of N-*tert*-butoxycarbonyl-N-methylphenylgycine (compound 1) (1.26 g, 4.75 mmol) at room temperature is added *p*-isopropylaniline (705 mg, 5.22 mmol), 4-(N,N-dimethylamino) pyridine (DMAP) (1 16 mg, 0.92 mmol) in CH_2Cl_2 (25 mL), and 1-ethyl-3-[3-(dimethylamino)-propyllcarbodiimide (EDCI, 1 .OO g, 5.22 mmol). The reaction mixture is stirred for 2 hours and then quenched by pouring over saturated NH_4Cl. After extraction with CH_2Cl_2 the organic layer is washed successively with water and brine, dried (Na_2SO_4), and concentrated in vacuo. Column chromatography of the crude residue gave [(4-isopropylphenylcarbamoyl)- phenylmethyl-methylcarbamic acid *tert*-butyl ester (compound 1a) as a white solid (typical yield 1.67 g, 92%). Compound 1a (300 mg, 0.785 mmol) is dissolved in a minimal quantity of trifluoroacetic acid (TFA), maintained at room temperature for 15 min, poured over aqueous $NaHCO_3$, and extracted with CH_2Cl_2. Washing, drying and evaporation of the organic layer gives compound 2 as a yellow oil (218 mg, 98%). To a mixture of compound 2 (177 mg, 0.620 mmol), indole-3-acetic acid (1 14 mg, 0.651 mmol) and DMAP (15 mg, 0.124 mmol) in CH_2Cl_2 (5 mL), EDCI (131 mg, 0.682 mmol) is added at room temperature to yield crude compound 3. The reaction mixture is worked up as for compound 2a and re-crystallized from CH_2Cl_2:MeOH (9:1) to give the standard PG01 compound as a white solid (typical yield 1.67g, 92%).

Biological Assays The compounds are tested for biological activity by various methods. Dose-response assays measuring I^- influx are performed on ΔF508-CFTR transfected Fischer rat thyroid (FRT)

epithelial cells. Other cell lines are the "class III" gating defective CFTR mutants G551D-CFTR and G1349D-CFTR, which are potentiator specific variants (Gregory et al., MCB 11 :3886-3893 (1991). These mutations affect the glycine residues in NBDI and NBD2 that are highly conserved in ATP-binding cassette proteins (Hyde et al., 1990; Logan et al., 1994). The gating defective mutant G551D-CFTR represents the most common gating defect causing cystic fibrosis. Other assays include patch-clamp analysis to assess electrophysiological mechanism of ΔF508-CFTR activation, and transepithelial current measurements to confirm activation of ΔF508-CFTR Cl^- currents. Primary cultures are used to assess activity of the compounds in a native lung airway system, such as nasal epithelial cells from a ΔF508 homozygous subject, and nasal polyp epithelial cells from a CF patient with the G551D-CFTR mutation. Direct interaction between the test compounds with ΔF508-CFTR is performed by measuring cellular cAMP concentrations to confirm that synergy of the test compounds with forskolin is not due to cAMP elevation.

Cell lines Clonal populations of Fischer rat thyroid (FRT) epithelial cells stably co-expressing human ΔF508-CFTR and the high-sensitivity halide-sensing green fluorescent analog YFP-H148QI152L (Galietta et al., A.S. (2001) FEBS Lett. 499,220-224) are generated by liposome transfection and limiting dilution with ZeocidG418 selection. Clones are evaluated for high fluorescence and ΔF508-CFTR plasma membrane targeting after growth at 27°C for 24 hours. For screening, cells are cultured on plastic in Coon's modified F12 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 pglml streptomycin, and plated on black 96-well microplates (Corning-Costar 3904) at 30,000 cells/well. For short-circuit measurements cells are cultured on Snapwell permeable supports (Coming-Costar) at 500,000 cells/insert. Human nasal epithelium cells from CF patients are cultured on Snapwell inserts and allowed to differentiate in a hormone-supplemented medium (Galietta et al., Am. J. Physiol., 275: 19723- 19728 (1 998)). Other measurements are done using stably transfected FRT cells expressing YFP-H148Q and wildtype- or G551ID-CFTR (Galietta et al., (2001) J. Biol. Chem. 276, 19723-19728). Patch clamp experiments are done on AF508-CFTR-expressing FRT cells plated in 35-mm Petri dishes.

Iodine Influx Rate Compounds are tested for CFTR potentiator activity using a Beckman integrated system containing a 3-meter robotic arm, CO_2 incubator containing microplate carousel, plate-washer, liquid handling workstation, bar code reader, delidding station, plate sealer, and two FluoStar fluorescence plate readers (Galaxy, BMG Lab Technologies), each equipped with dual syringe pumps and HQ500/20X (500 ± 10 nm) excitation and HQ535130M (535 ± 15 nm) emission filters (Chroma). Software written in VBA (Visual Basic for Applications) is used to compute baseline-subtracted fluorescence slopes (giving halide influx rates).

For assay of ΔF508-CFTR potentiator activity, the incubator (27°C, 90% humidity, 5% CO_2/ 95% air) is loaded with forty-to-sixty 96-well plates containing FRT cells. After an 18-24 hour incubation plates are washed 3 times with PBS (300 µl / wash) leaving 50 µl PBS. 10 µl of PBS containing 120 µM forskolin is added, and after 5 min test compounds (0.6 µl of 0.25 nM DMSO solution) are added to each well to give 2.5 µM final compound concentrations. After 15 min, 96-well plates are transferred to a plate reader for fluorescence assay. Each well is assayed individually for iodine ion (I^-) influx by recording fluorescence continuously (200 ms per point) for 2 seconds (baseline) and then for 12 seconds after rapid (4 s) addition of 160 µL of isosmolar PBS in which 137 mM Cl^- is replaced by I^-. I^- influx rates are computed from initial fluorescence versus time-curve slopes (determined by 3rd order polynomial regression) after normalization for total fluorescence (background subtracted initial fluorescence). All compound plates contain negative control (e.g., DMSO vehicle alone) and positive controls (e.g., genistein, 5µM and 50µM). Assay analysis typically indicate a Z'-factor of >0.7 (Zhang et al., J. Biomol. Screen 4:67-73 (1 999)).

For example, FRT epithelial cells stably coexpressing human ΔF508-CFTR and the high-sensitivity halide-sensing green fluorescent protein YFP-H148Q/I152L (Galietta et al., FEBS Lett. (2001) 499:220-224) were carried out as described previously (Pedemonte et al., J. Clinical Investigation (2005) 115:2564-2571). Cells were grown at 37°C (90% humidity; 5% CO2) for 24 hours and then incubated for 20 hours with 50 µl of medium containing test compounds. At the time of the assay, cells were washed with PBS and then incubated with PBS containing forskolin (20 µM) and genistein (50 µM) for 20 min. Measurement was carried out using FLUOstar fluorescence plate readers (Optima; BMG LABTECH Gmbh), each equipped with 500 ± 10 nm excitation and 535 ± 15 nm emission filters (Chroma Technology Corp.). Each well was assayed individually for I^- influx by recording fluorescence continuously (200 ms per point) for 2 seconds (baseline) and then for 12 seconds after rapid (<1 second) addition of 165 µl PBS in which 137 mM Cl^- was replaced by I^-. The I^- influx rate was computed by fitting the final 11.5 seconds of the data to an exponential for extrapolation of initial slope and normalizing for background-subtracted initial fluorescence. All experiments contained negative controls (DMSO vehicle) and positive controls (PG01).

Whole-cell patch-clamp The cell-attached configuration of the patch-clamp technique is performed on FRT cells expressing ΔF508-CFTR as follows. Cells are seeded at a density of 10^4 cells/well and grown at 37°C for 24-48 hours and then incubated for 24-48 hours at 27°C to allow trafficking of the ΔF508 protein to the plasma membrane. Borosilicate glass pipettes are fire polished to obtain tip resistances of 2-4 MΩ. Currents are sampled at 500 Hz using a patch-clamp amplifier (EPC-7, List, Darrnstadt) and low-pass filtered using a 4-pole Bessel filter set at a cutoff frequency of 250 Hz and digitized at 500Hz using an ITC-16 data translation interface (Instrutech). The extracellular (bath) solution is prepared to contain (in mM): 150 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 mannitol, and 10 TES (pH

7.4). The pipette solution is prepared to contain (in mM): 120 CsCl, 1 $MgCl_2$, 10 TEA-Cl, 0.5 EGTA, 1 Mg-ATP, and 10 Hepes (pH 7.3). Membrane conductances are monitored by alternating the membrane potential between +80 and -100 mV. Current-voltage relationships are generated by applying voltage pulses between -100 and +100 mV in 20 mV steps. Analysis of open channel probability (P_o), mean channel open time (T_o), and mean channel closed time (T_c) are done using recordings of at least three minute intervals (Taddei et al., FEBS Lett. 55852-56 (2004)).

Transepithelial current measurements Chamber experiments for short-circuit transepithelial measurements are performed 7-9 days after plating ΔF508-CFTR expressing FRT cells on Snapwell inserts. The basolateral solution is prepared to contain (in mM): 130 NaCl,2.7 KCl, 1.5 KH_2PO_4, 1 $CaCl_2$, 0.5 $MgCl_2$, 10 glucose, 10 Na-Hepes (pH 7.3). In the apical bathing solution 65 mM NaCl is replaced by Na gluconate, and $CaCl_2$ increased to 2 mM. Solutions are bubbled with air and maintained at 37°C. The basolateral membrane is permeabilized with 250 µg/ml amphotericin B. The hemichambers are connected to a DVC-1000 voltage clamp (World Precision Instruments) via Ag/AgCl electrodes and 1 M KCl agar bridges for recording short-circuit current.

Assay of cAMP cAMP activity is measured using the BIOTRAK enzymatic immunoassay (Arnersham) of FRT cell lysates after incubation with the compounds for 10 minutes in the presence of 0.5 pM forskolin.

Pharmacokinetics To increase compound solubility when necessary, test articles are dissolved in a liposomal formulation containing 5 mg potentiator in 2 1.3 mg hydrogenated soy phosphatidylcholine, 5.2 mg cholesterol, 8.4 mg distearoylphosphatidylglycerol, and 90 mg sucrose in 5 ml PBS. A bolus of potentiator containing solution (5 mg/kg) is administered intravenously in rats over 1 min (male Sprague-Dawley rats, 360-420 grams) by a jugular vein catheter. Arterial blood samples (~1 ml) are obtained at predetermined times for LCMS analysis.

Liquid Chromatography / Mass Spectrometry (LCMS) For analysis of blood samples, collected plasma is chilled on ice, and ice-cold acetonitrile (2: 1 v:v) added to precipitate proteins. Samples are centrifuged at 4°C at 20,000g for 10 min. Supernatants (supplemented with sulforhodamine 101 as internal standard) are analyzed for test compounds by extraction with C-18 reversed-phase cartridges (1 ml, Alltech Associates, Inc. Deerfield, IL) by standard procedures. The eluate is evaporated, and the residue reconstituted in 100 µl of mobile phase for HPLC analysis. Reversed-phase HPLC separations are carried out using a Supelco C18 column (2.1 x 100 mm, 3 µm particle size) connected to a solvent delivery system (Waters model 2690, Milford, MA). The solvent system consists of a linear gradient from 20% CH_3CN/10 mM KH_2PO_4, pH 3 to 95% CH_3CN/10mM KH_2PO_4, pH 3 over 10 min, followed by 6 min at 95% CH_3CN/20 mM NH_4OAc (0.2 ml/min flow rate). Test compounds are detected at 256 nm, after

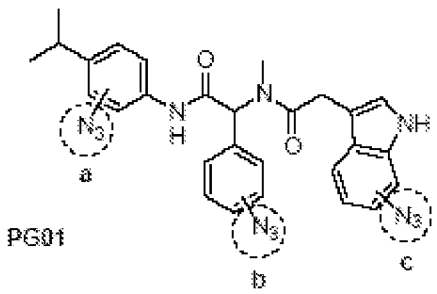
establishing a linear standard calibration curve in the range of 20-5000 nM. The typical detection limit is 10 nM and recovery >90%. Mass spectra are acquired on a mass spectrometer (Alliance HT 2790 + ZQ) using negative ion detection, scanning from 200 to 800 Da (Sonawane et al., J. Pharm. Sci. 94:134-143 (2004)).

Stability in hepatic microsomes To predict hepatic clearance of test compounds, in vitro incubations are done with rat hepatic microsomes in the absence (control) and presence of NADPH as follows. Test compounds (1 0 µM each) are incubated separately with a phosphate buffered (100 mM) solution of rat liver microsomes (2 mg proteinlml, Sigma) containing NADPH (0 or 1 mM) for 60 min at 37°C. After 60 min the mixture is chilled on ice, and 0.5 ml of ice-cold acetonitrile added to precipitate the proteins for LCMS analysis as described above.

Example 2: Synthesis of detectably-labeled Potentiator PG01 in support of biological studies.

Initial screening of a 150,000 small molecule compound library against transfected epithelial cells containing mutated CFTR protein via an iodide uptake assay identified potentiators of defective ΔF508-CFTR gating with nanomolar potency and were active in human ΔF508 and G551D cells. The most potent compound of the class was a phenylglycine derivative, 2-[(2–1H-indol-3-ylacetyl)methylamino]-N-(4-isopropyl-phenyl)-2-phenylacetamide (PG01). The PG01 compound was found to reversibly activate ΔF508-CFTR in the presence of forskolin with Ka ~ 70 nM and also activate the CFTR gating mutants G551D and G1349D with Ka values of ~1100 and 40 nM, respectively. Biochemical studies suggested a mechanism of action involving improved ΔF508-CFTR folding at the ER and stability at the cell surface.

To probe the interaction of the highly active phenylglycine potentiators on ΔF508-CFTR, a panel of photoaffinity labeled derivatives of potentiator PG01 was designed. Incorporation of an azido functional group on one of the aryl groups was chosen for this purpose. Proper placement of the azido functional group such that the activity of the derivative was not compromised was an important issue in designing target derivatives.

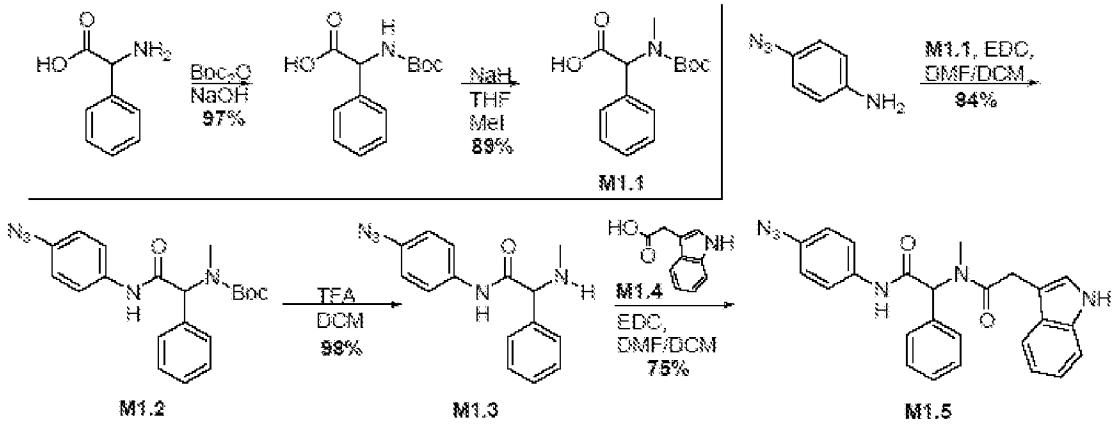


Retrosynthetic analysis of the three options illustrated above as PG01 a, b and c indicated that incorporation of an azido group at either the aniline ring or the indole ring gave the most direct synthetic

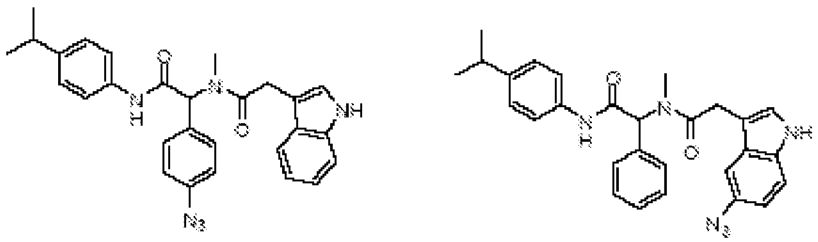
approach. Commercial availability of 4-azidoaniline favored an initial synthesis of N-(4-azido-phenyl)-2-[(2-1H-indol-3-yl-acetyl)-methyl-amino]-2-phenyl-acetamide to give the azido-PG01 derivative M1.5.

The synthesis of azido-PG01 derivative M1.5 began with EDC coupling of 4-azidoaniline with Boc-Me-aminophenylglycine M1.1. *N*-Boc deprotection of M1.2 gave amine M1.3 which was coupled to 3-indole acetic acid M1.4 with EDC (Scheme 2) to give the targeted photoaffinity labeled derivative M1.5. Care was taken to avoid heat and light exposure to prevent decomposition of the azide.

Scheme 2: Azido-PG01 synthesis



Testing of the azido-phenylglycine derivative against PG01 indicated no loss in activity by incorporation of an azido functionality in the aniline ring (Figure 1). Use of the azido-PG01 derivatives in photoaffinity studies aid in generating structure-activity relationship (SAR) data for the design and selection of CFTR potentiator derivatives. Structures for two other photoaffinity labeled derivatives of PG01 are depicted below.

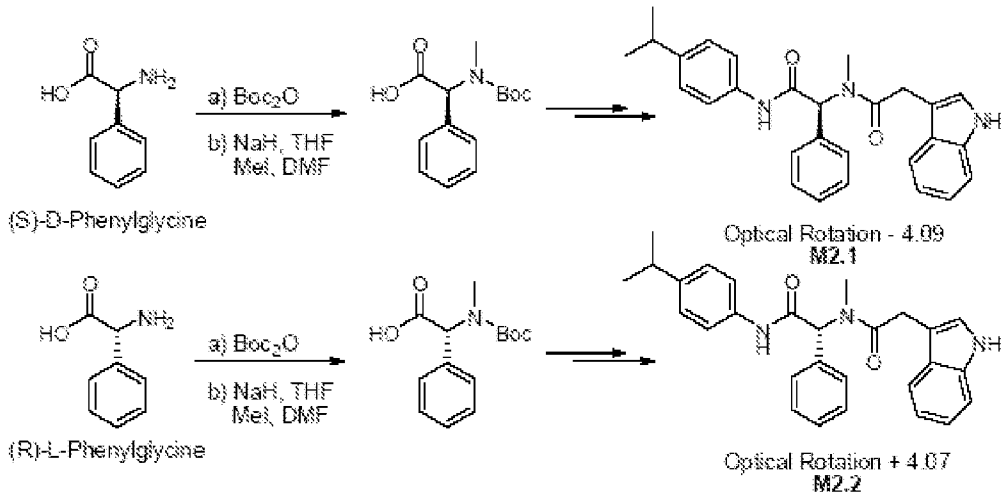


Example 3: R- and S-Antipodes of Potentiator PG01.

Original PG01 is a racemic mixture. To determine which enantiomer was contributing to the high nM potency, both enantiomers of PG01 were synthesized and their activity compared to the racemic mixture. The enantiomers were synthesized from their corresponding D/L-amino acids via the method

used to synthesize M1.1. The optical rotations of M2.1 and M2.2 were -4.09 and +4.07, respectively. This indicated that the products were chiral.

Scheme 3: Synthesis route to R- and S-PG01



Both R/S enantiomers M2.1 and M2.2 were screened against the racemic PG01 compound and found to have similar activities, with the D-enantiomer appearing marginally better than the racemic mixture (Figure 2). In addition, the S-enantiomer, which comes from the unnatural D-amino acid configuration ((S)-D-phenylglycine; D-enantiomer), may be more resistant to enzymatic degradation than the R-enantiomer ((R)-L-phenylglycine; L-enantiomer). Accordingly, the D-enantiomer phenylglycine core was chosen for further study.

Example 4: Design and synthesis of analogs around the D-enantiomer of Potentiator PG01.

Structure activity relationship (SAR) data was reviewed for phenylglycines from initial library screenings. It was found that all of the highly active compounds identified in the initial screening had H-bond donor characteristics on the indole ring and contained a methyl group on the nitrogen of phenylglycine. Comparing all ~200 phenylglycines screened to PG01, it appeared that there was not much variation in place of the indole ring, but some potential to vary the aniline ring.

To expand the analog platform around the D-enantiomer of PG01 ("D-PG01"), acids were selected which had an acidic hydrogen for proton donation. Additionally, 4-isopropylaniline and 2,3-dihydrobenzodioxinaniline were included due to their prevalence in the original ten most active potentiators. Figure 3 illustrates the basic design strategy and the diversity elements employed for analog construction, where the panel labeled R_1 shows aniline precursors used for synthesis of phenylglycine derivatives for generating R_1 diversity, and where the panel labeled R_2 shows the carbonyl precursor

used for synthesis of phenylglycine derivatives for generating R_2 diversity. Eight D-phenylglycine derivatives were initially constructed following the design in Figure 3, and are depicted in Figure 4 relative to new potentiator compound D-PG01 (labeled PG-STD in Figure 3).

Although not as active as D-PG01, all eight compounds were found to exhibit potentiator activity, indicating some variability in R_2. The three best examples are with M3.3, M3.7, and M3.8. All three have an aromatic system. Two are heterocycles. Of the two heterocycles, one is a 2H-indazole derivative – M3.8 which is very similar in structure to the indole ring in D-PG01 but lacks hydrogen bond donation – and the other is a pyrazine derivative – M3.3 which is also lacking hydrogen bond donation. This may indicate that the indole heterocycle might be binding in an aromatic or hydrophobic pocket. Photoaffinity studies are designed to generate SAR data to expand on these findings.

Example 5: Synthesis of derivatives of the D-enantiomer of Potentiator PG01 with improved water solubility

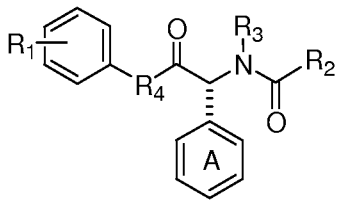
While D-PG01 is a highly active potentiator, its water solubility is, however, poor. Accordingly, analogs were designed to improve aqueous solubility while maintaining the potency of D-PG01. Log P calculations of D-PG01 indicate a value of 5.21 in which a decreasing value is increasingly water soluble. Synthesis of compounds M5.1 and M5.2 which, as indicated by preliminary log P calculations, are designed to have better water solubility and high nM potency. Another approach involves changing the amide linkage in D-PG01 to an ester linkage in which a phenol group is installed (e.g., analog M5.3). See Figure 6. The compounds are synthesized and the activity characterized relative to D-PG01 as described above.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

That which is claimed is:

1. A composition comprising a compound of formula (I):



(I),

or the salts, solvates, hydrates, and prodrug forms thereof, and stereoisomers thereof, wherein:

A is a racemic phenyl comprising L- and D-enantiomers as indicated by the hashed bond;

R_1 is hydrogen, a substituted or unsubstituted alkyl, a substituted or unsubstituted heterocycle, or a functional group;

R_2 is a substituted or unsubstituted alkoxy, a substituted or unsubstituted phenyl, or a substituted or unsubstituted heterocycle, and is bonded to the R_2 carbonyl either directly or by a spacer comprising 1 to 5 carbons, with the proviso that when R_2 is a heterocycle bonded to the R_2 carbonyl by a spacer and R_4 is NH, then said heterocycle bonded to the R_2 carbonyl by a spacer is substituted with a water soluble group that increases solubility of said compound in water;

R_3 is hydrogen or methyl; and

R_4 is O or NH.

2. The composition of claim 1, wherein the composition further comprises at least one of a pharmaceutically acceptable carrier, a pharmaceutically acceptable diluent, a pharmaceutically acceptable excipient and a pharmaceutically acceptable adjuvant.

3. The composition of claim 1, wherein phenyl A is non-racemic and consists essentially of either L-enantiomer or D-enantiomer.

4. The composition of claim 3, wherein said phenyl A consist essentially of D-enantiomer.

5. The composition of claim 1, wherein said spacer comprises 1 to 3 carbons.

6. The composition of claim 5, wherein said spacer is a radical selected from $-CH_2-$, $-CH_2-CH_2-$, and $-CH_2-CH_2-CH_2-$.

7. The composition of claim 1, wherein R_3 is methyl.

8. The composition of claim 1, wherein R_4 is O.

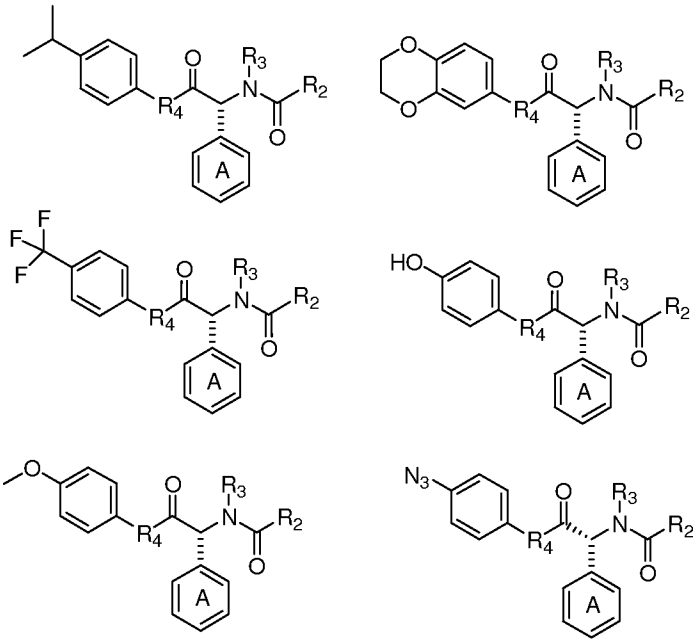
9. The composition of claim 1, wherein R_4 is NH.

10. The composition of claim 1, wherein R_1 is a substituted or unsubstituted alkyl selected from methyl, ethyl, propyl, isopropyl, methoxy, ethoxy and trifluormethyl.

11. The composition of claim 1, wherein R_1 is a substituted or unsubstituted heterocycle comprising dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

12. The composition of claim 1, wherein R_1 is a functional group selected from hydroxy, hydroxymethyl, carboxy, carboxymethyl, acetyl, acetamide, amino, nitro and azide.

13. The composition of claim 1, wherein the compound is selected from:



wherein R_2, R_3, R_4 and A are as defined for formula (I).

14. The composition of claim 13, wherein R_3 is methyl, R_4 is NH, and R_2 is selected from *tert*-butoxy, benzyl, (phenol-2-yl)-ethyl, (phenol-4-yl)-methyl, (phenol-4-yl)-ethyl, (benzoimidazol-2-yl)-aniline-3-yl, pyrrole-1-yl, pyrazine-2-yl, 5-methyl-pyrazine-2-yl, 3-methoxy-2-methyl-2H-indazole-6-yl, pyrrolidin-2-one-5-yl, (5-carboxymethylindole-3-yl)-methyl, (5-carboxymethoxyindole-3-yl)-methyl, and (5-hydroxyindole-3-yl)-methyl.

15. The composition of claim 13, wherein R_3 is methyl, R_4 is O, and R_2 is selected from *tert*-butoxy, benzyl, (phenol-2-yl)-ethyl, (phenol-4-yl)-methyl, (phenol-4-yl)-ethyl, (benzoimidazol-2-yl)-aniline-3-yl, pyrrole-1-yl, pyrazine-2-yl, 5-methyl-pyrazine-2-yl, 3-methoxy-2-methyl-2H-indazole-6-yl, pyrrolidin-2-one-5-yl, (indole-3-yl)-methyl, (5-carboxymethylindole-3-yl)-methyl, (5-carboxymethoxyindole-3-yl)-methyl, and (5-hydroxyindole-3-yl)-methyl.

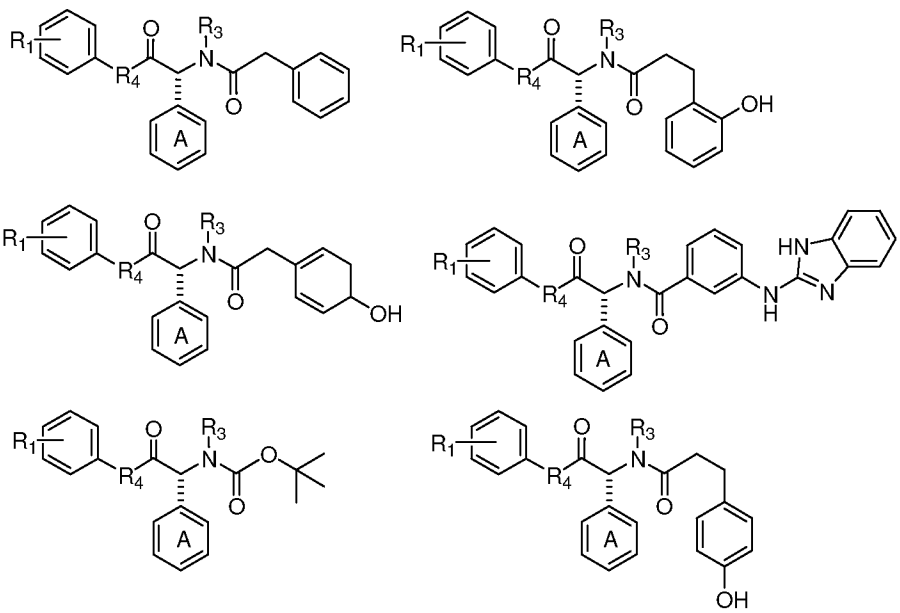
16. The composition of claim 1, wherein R_2 is an unsubstituted phenyl, or a substituted phenyl selected from phenol and aniline, and wherein said phenyl is bonded to the R_2 carbonyl of formula (I) either directly or by a spacer comprising 1 to 5 carbons.

17. The composition of claim 16, wherein said phenol is selected from phenol-2-yl and phenol-4-yl, and said aniline is selected from aniline-3-yl and (benzoimidazol-2-yl)-aniline-3-yl.

18. The composition of claim 16, wherein said phenol is selected from (phenol-2-yl)-ethyl, (phenol-4-yl)-methyl and (phenol-4-yl)-ethyl.

19 The composition of claim 1, wherein R_2 is an alkoxy selected from methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, *tert*-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, and 1,2-dimethylbutoxy.

20. The composition of claim 1, wherein said compound is selected from:



wherein R_1, R_3, R_4 and A are as defined for formula (I).

21. The composition of claim 20, wherein R_3 is methyl, R_4 is NH, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

22. The composition of claim 20, wherein R_3 is methyl, R_4 is O, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

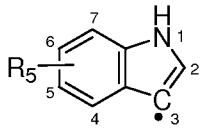
23. The composition of claim 1, wherein R_2 is a substituted or unsubstituted heterocycle selected from indole, pyrrole, pyrazine, indazole and pyrrolidone, and wherein said heterocycle is bonded to the R_2 carbonyl of formula (I) either directly or by a spacer comprising 1 to 5 carbons, and with the proviso that when said heterocycle is bonded to the R_2 carbonyl by a spacer and R_4 is NH, then said heterocycle bonded to the R_2 carbonyl by a spacer is substituted with said water soluble group.

24. The composition of claim 23, wherein said pyrrole is pyrrole-1-yl, said pyrazine is pyrazine-2-yl, said indazole is indazole-6-yl, and said pyrrolidone is pyrrolidone-5-yl.

25. The composition of claim 23, wherein said substituted or unsubstituted heterocycle is selected from pyrrole-1-yl, pyrazine-2-yl, 5-methyl-pyrazine-2-yl, 3-methoxy-2-methyl-2H-indazole-6-yl, and pyrrolidin-2-one-5-yl.

26. The composition of claim 23, wherein R_2 is an indole substituted with a water soluble group that increases solubility of said compound in water.

27. The composition of claim 26, wherein said indole is an indole-3-yl of the formula:



wherein R_5 is said water soluble group, and wherein C• is a carbon radical at indole position 3 bonded to the R_2-carbonyl of formula (I) by said spacer.

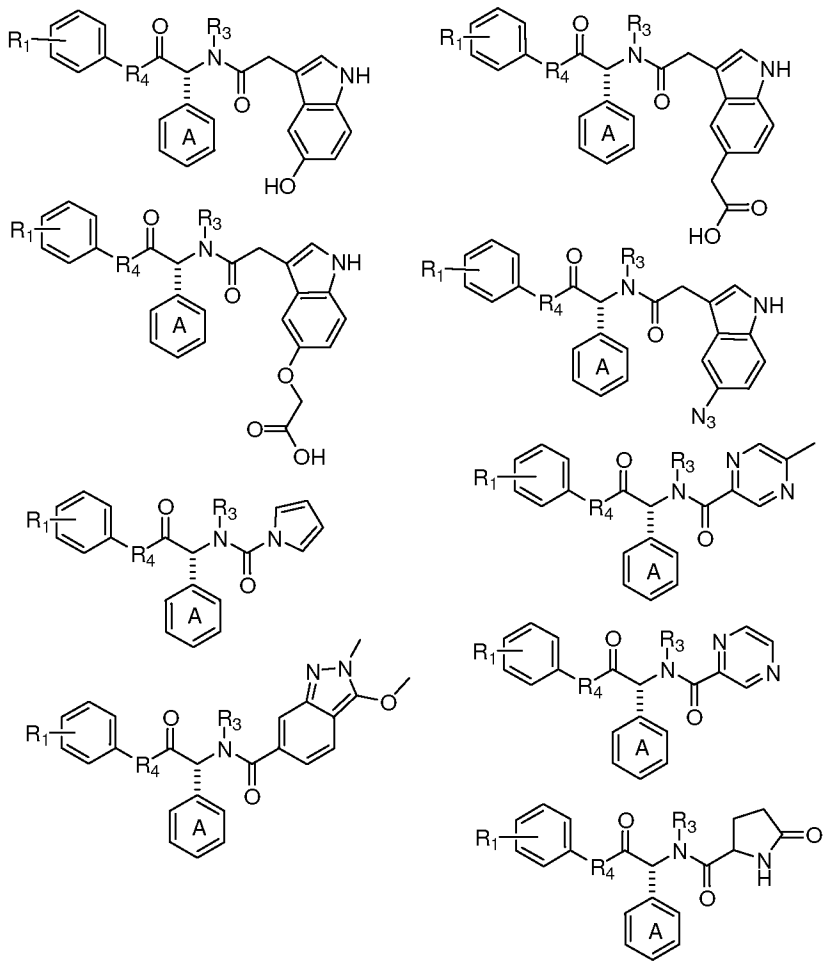
28. The composition of claim 27, wherein R_5 is selected from hydroxyl, amine, nitro, azido, methoxy, ethoxy, carboxymethyl, carboxyethyl, caboxymethoxy and carboxyethoxy.

29. The composition of claim 27, wherein R_5 comprises a radical selected from -OH, -O-, -C(O)O-and -C(O)OH.

30. The composition of claim 27, wherein R_5 is bonded at indole position 5.

31. The composition of claim 30, wherein said indole is selected from (5-carboxymethylindole-3-yl)-methyl, (5-carboxymethoxyindole-3-yl)-methyl, and (5-hydroxyindole-3-yl)-methyl.

32. The composition of claim 1, wherein said compound is selected from:

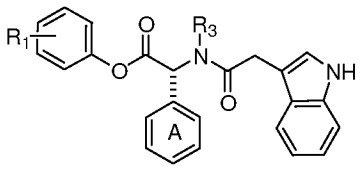


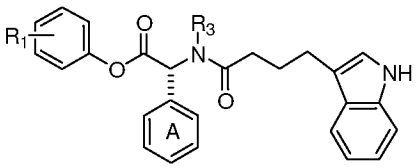
wherein R_1, R_3 R_4 and A are as defined for formula (I).

33. The composition of claim 32, wherein R_3 is methyl, R_4 is NH, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

34. The composition of claim 32, wherein R_3 is methyl, R_4 is O, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

35. The composition of claim 1, wherein said compound is selected from:



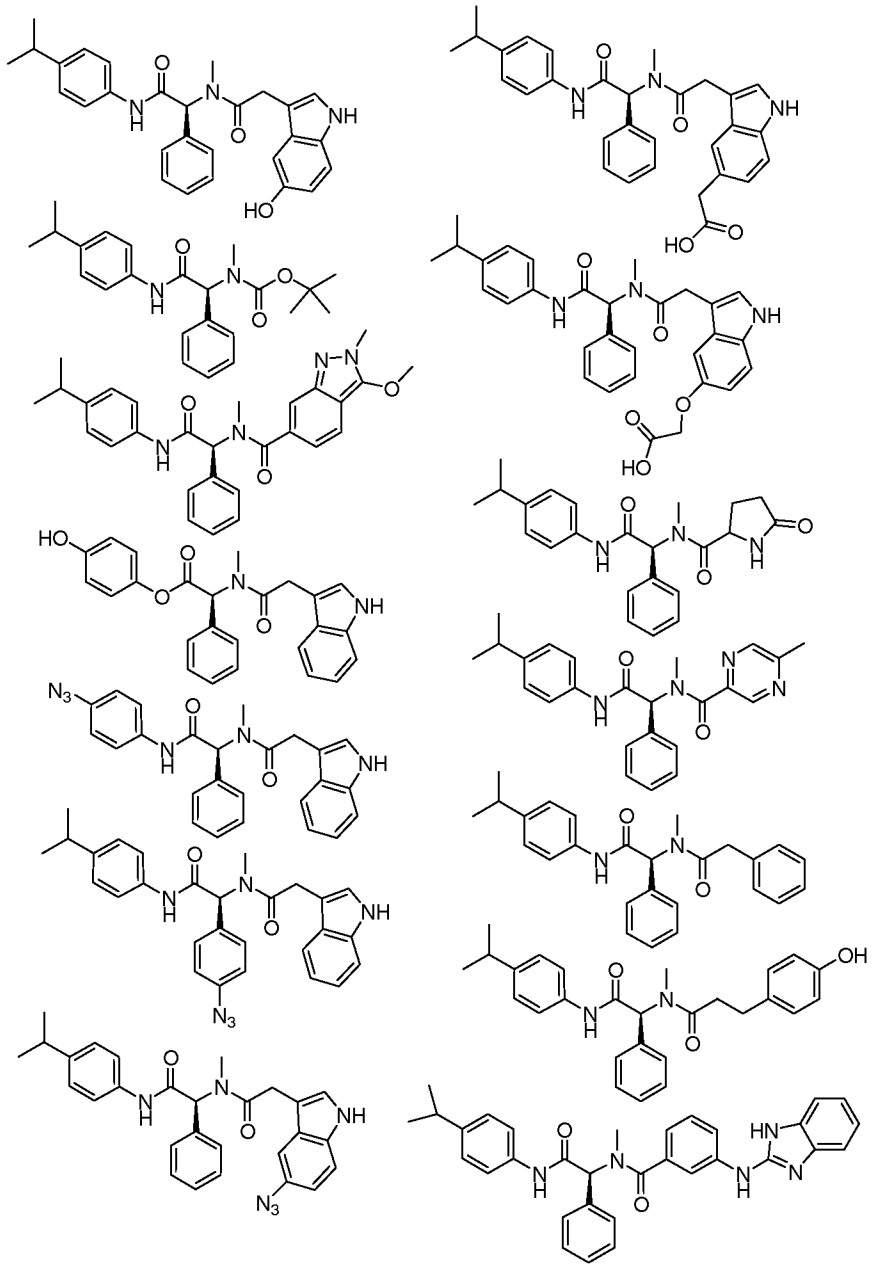


wherein R_1, R_3 and A are as defined for formula (I).

36. The composition of claim 35, wherein R_3 is methyl, and R_1 is selected from hydroxyl, isopropyl, methoxy, trifluormethyl, azide, and dioxane fused to the R_1-phenyl so as to comprise benzodioxane bonded to R_4.

37. The composition of claim 1, wherein said phenyl ring A further comprises a detectable label comprising azide.

38. A composition comprising an effective amount of a CFTR potentiator compound selected from:



or the salts, solvates, hydrates, and prodrug forms thereof, and stereoisomers thereof.

39. The composition of claim 1, wherein the compound is therapeutically effective in increasing the CFTR-mediated ion permeability of a cell producing a mutant-CFTR, and said composition is a pharmaceutical composition that comprises a therapeutically effective amount of said compound.

40. The composition of claim 39, wherein the pharmaceutical composition further comprises at least one of a pharmaceutically acceptable carrier, a pharmaceutically acceptable diluent, a pharmaceutically acceptable excipient and a pharmaceutically acceptable adjuvant.

41. The composition of claim 39, wherein the mutant-CFTR is ΔF508-CFTR.

42. A method of treating a subject having a condition associated with mutant-CFTR, said method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition of Claim 1.

43. The method of claim 42, wherein said condition is cystic fibrosis.

44. The method of claim 42, wherein the subject, after treatment, has a decrease in mucous or bacterial titer in their lungs, a decrease in coughing or wheezing, a decrease in pancreatic insufficiency, or a decrease in electrolyte levels in their sweat.

45. The method of claim 42, wherein said subject is a non-human animal.

46. The method of claim 45, wherein the animal is a mammal.

47. The method of claim 42, wherein the mutant-CFTR is a ΔF508-CFTR.

48. A method of increasing ion permeability of a cell producing a mutant-CFTR protein, said method comprising:

contacting said cell with an effective amount of the pharmaceutical composition of one of Claim 1, said contacting being effective to increase CFTR-mediated ion permeability of said cell.

49. The method of claim 48, wherein said cell contains a recombinant expression cassette that encodes said mutant-CFTR protein.

50 The method of claim 48, wherein said cell contains a genome that encodes said mutant-CFTR protein.

51. The method of claim 48, wherein the mutant-CFTR is a ΔF508-CFTR.

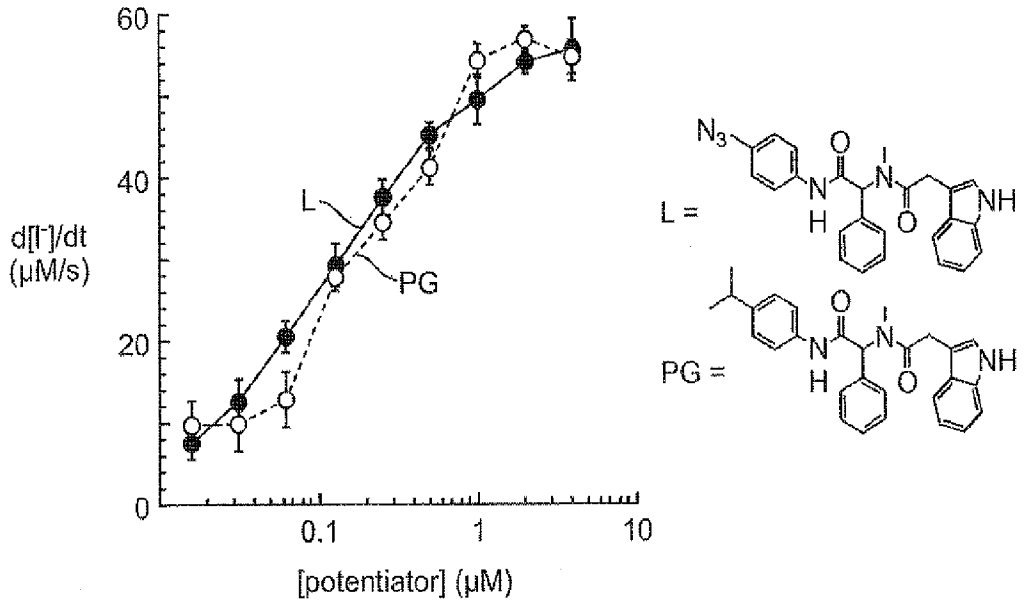

60
40
20
0
d[I⁻]/dt
(μM/s)
L
PG
0.1
1
10
[potentiator] (μM)
L =
PG =
N3
NH

FIG. 1

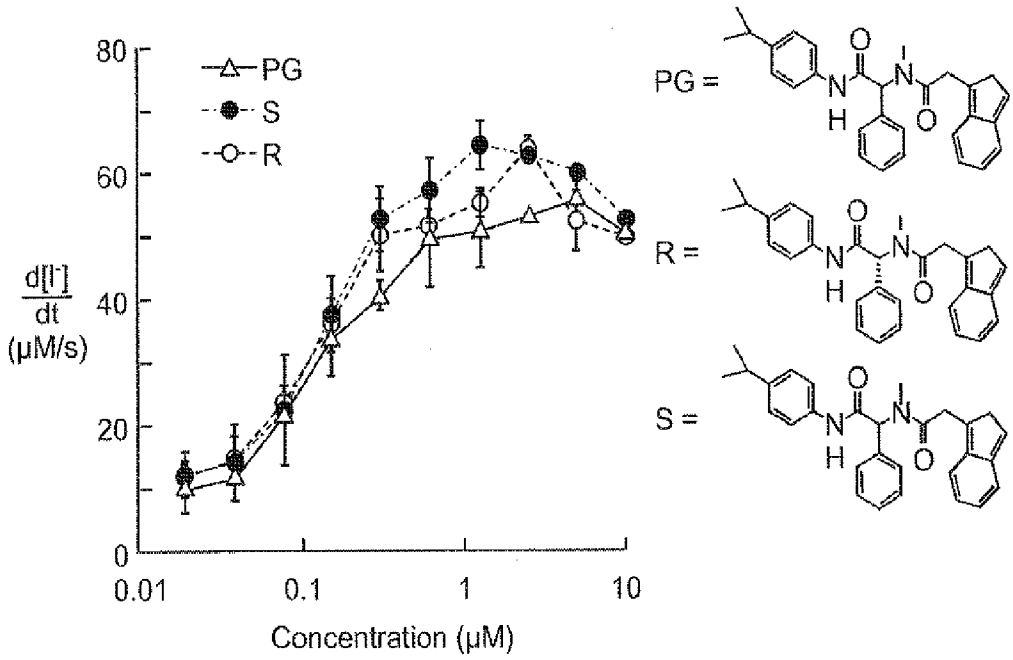

80
60
40
20
0
d[I⁻]
dt
(μM/s)
PG
S
R
0.01
0.1
1
10
Concentration (μM)
PG =
R =
S =

FIG. 2

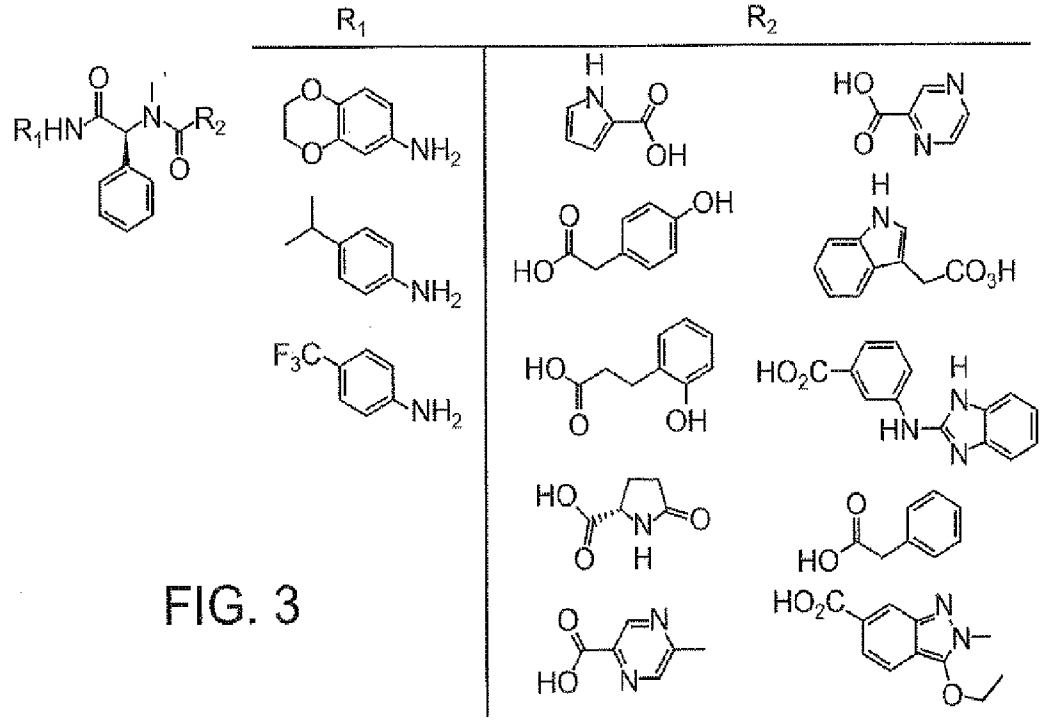
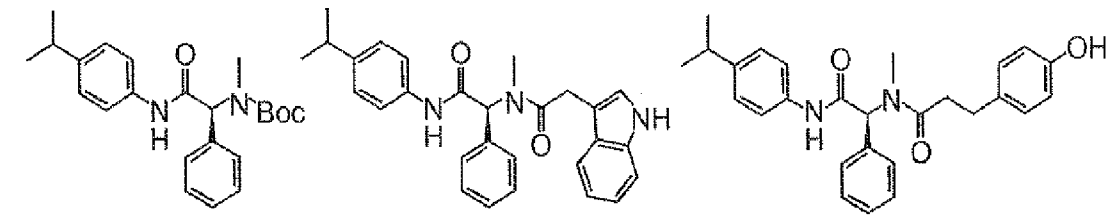
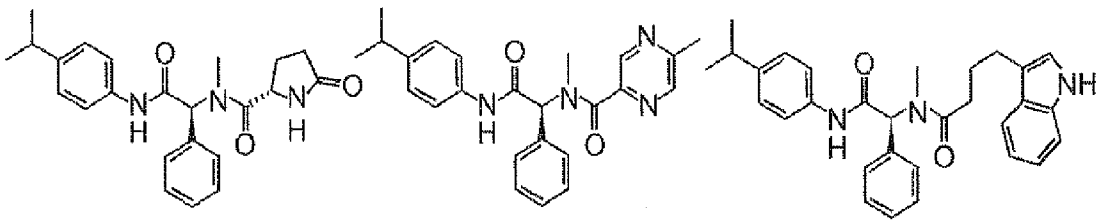


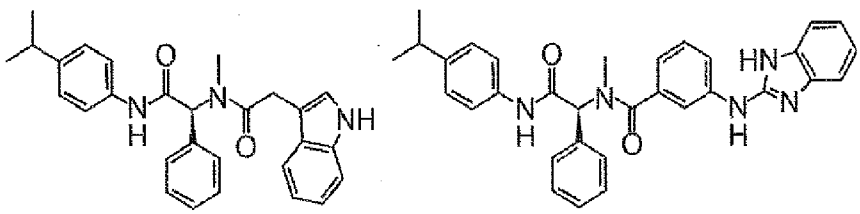
FIG. 3



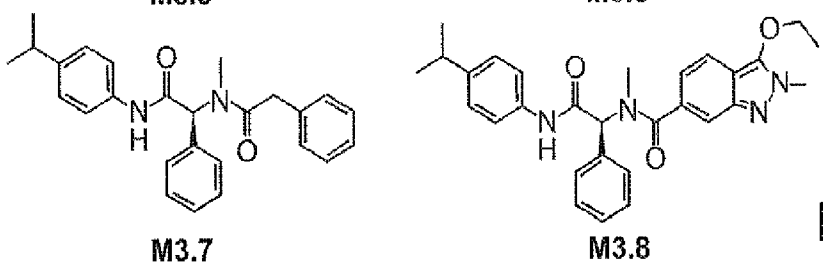
PG-BocN PG-STD M3.1



M3.2 M3.3 M3.4



M3.5 M3.6



M3.7 M3.8

FIG. 4

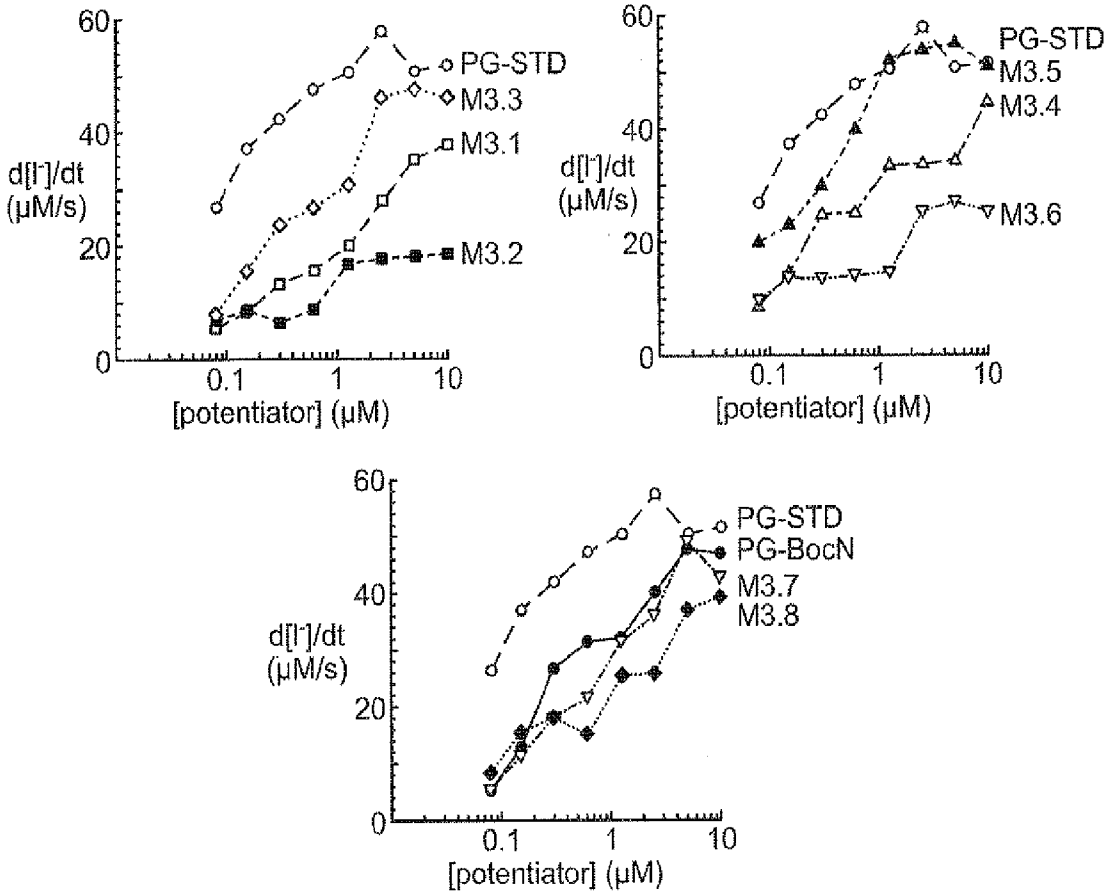

60
40
20
0
d[I⁻]/dt
(μM/s)
PG-STD
M3.3
M3.1
M3.2
0.1
1
10
[potentiator] (μM)
PG-STD
M3.5
M3.4
M3.6
PG-STD
PG-BocN
M3.7
M3.8

FIG. 5

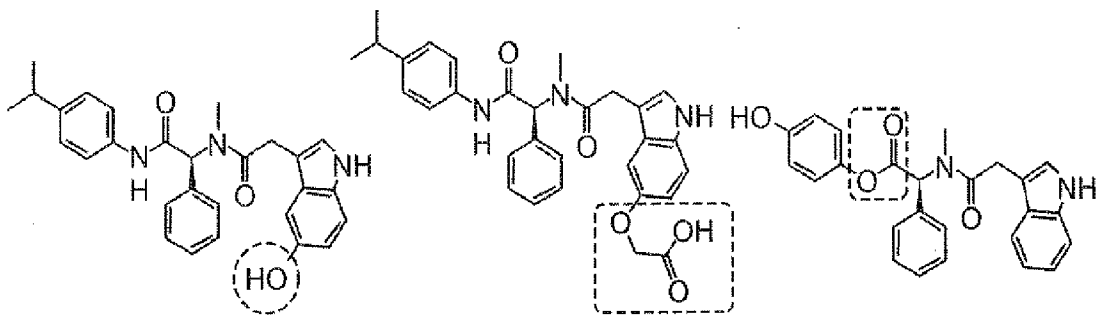

M5.1
Log P: 4.14
M5.2
Log P: 3.67
M5.3
Log P: 3.59

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/075505

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D209/18 C07D231/56 C07D241/24 C07D207/27 C07D207/327
A61K31/4015 A61K31/404 A61K31/416 A61K31/495 C07D235/30
A61K31/167 A61K31/4168 C07C233/65

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/120497 A (UNIV CALIFORNIA [US]; VERKMAN ALAN [US]; GUY R KIPLIN [US]; PEDEMONTE) 22 December 2005 (2005-12-22) cited in the application claims 1-8,24-40; examples p1,p6; table 1	1-51
A	WO 02/074730 A (MERCK PATENT GMBH [DE]; SCHADT OLIVER [DE]; JONCZYK ALFRED [DE]; STAEH) 26 September 2002 (2002-09-26) claims 1-9	1-51
A	WO 01/55106 A (MELACURE THERAPEUTICS AB [SE]; LUNDSTEDT TORBJOERN [SE]; SKOTTNER ANNA) 2 August 2001 (2001-08-02) cited in the application claims 1-45	1-51

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2009	05/02/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kyriakakou, Georgia

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/075505

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NICOLETTA PEDEMONTE ET AL: "Phenylglycine and sulfonamide correctors of defective DeltatF508and G551D cystic fibrosis transmembrane conductance regulator chloride-channel gating" MOLECULAR PHARMACOLOGY, BALTIMORE, MD, US, vol. 67, no. 5, 1 May 2005 (2005-05-01), pages 1797-1807, XP002492468 ISSN: 0026-895X page 1797 - page 1801; examples PG-01,PG-06; table 1 -----	1-51

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/075505

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		MX PA02007289 A	22-09-2003
		US 2003195212 A1	16-10-2003