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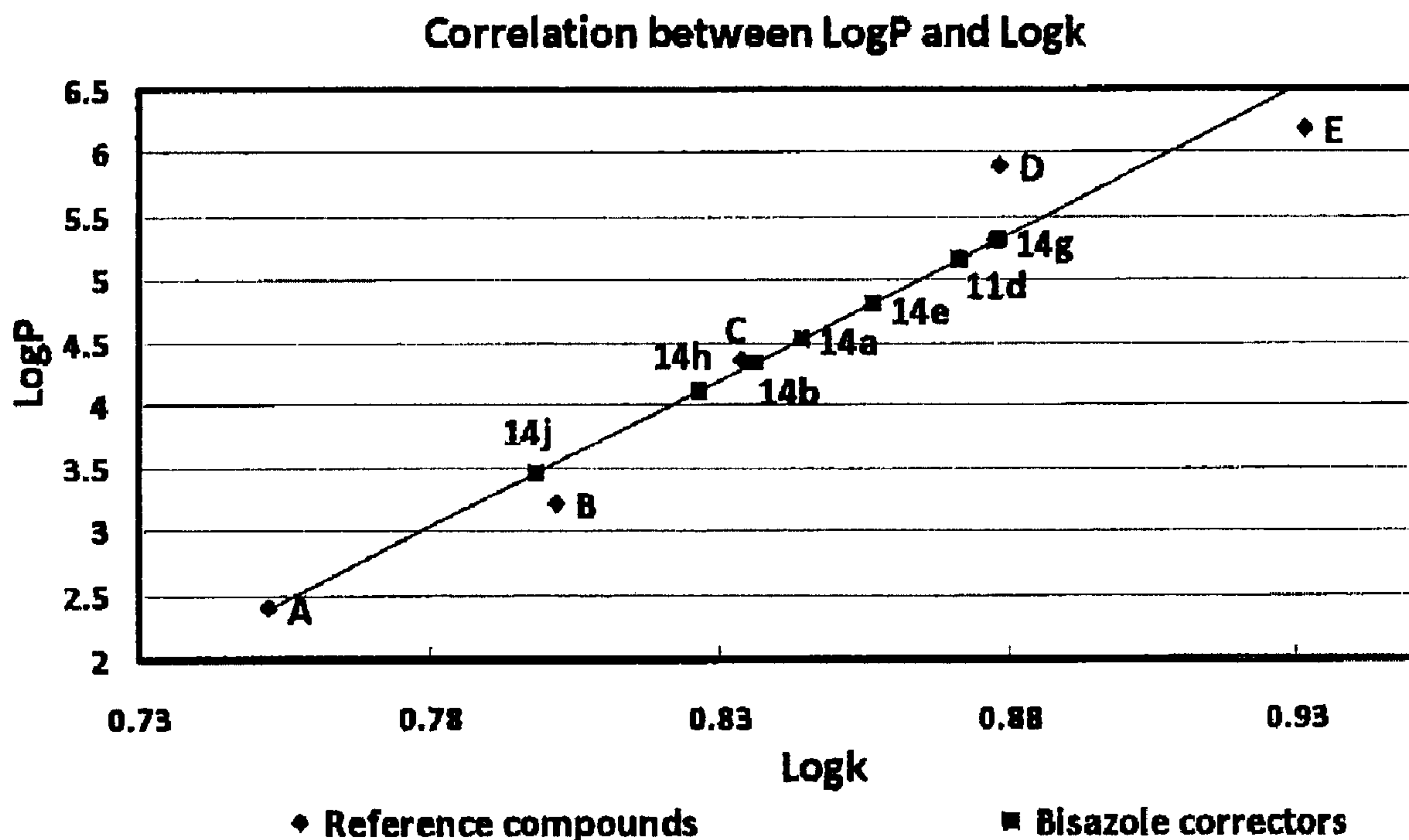
(71) Demandeur/Applicant:
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,
US

(72) Inventeurs/Inventors:
VERKMAN, ALAN S., US;
KURTH, MARK J., US

(74) Agent: SMART & BIGGAR

(54) Titre : PYRAZOLYLTHIAZOLES EN TANT QUE CORRECTEURS DU REGULATEUR DE LA CONDUCTIVITE
TRANSMEMBRANAIRE DE LA PROTEINE $\Delta F508$ DE LA FIBROSE KYSTIQUE

(54) Title: PYRAZOLYLTHIAZOLE COMPOUNDS AS $\Delta F508$ -CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE
REGULATOR CORRECTORS



(57) Abrégé/Abstract:

The present disclosure 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 pyrazolylthiazole-containing compounds of the embodiments, or an analog or derivative thereof.

**PYRAZOLYLTHIAZOLE COMPOUNDS AS Δ F508-CYSTIC FIBROSIS TRANSMEMBRANE
CONDUCTANCE REGULATOR CORRECTORS**

ABSTRACT

5 The present disclosure 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
10 pyrazolylthiazole-containing compounds of the embodiments, or an analog or derivative thereof.

5 This invention was made with government support under grant nos. DK072517 and GM076151 awarded by the National Institutes of Health and grant nos. CHE-0614756, CHE-0449845, and CHE-9808183 awarded by the National Science Foundation. The government has certain rights in the invention.

The present disclosure relates to corrector compounds and methods for correcting cellular processing of mutant cystic fibrosis transmembrane conductance regulator protein.

15 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 β -adrenergic
20 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 ($\Delta F508$ -CFTR), is present in at least one allele in about 90 % of CF patients (Egan et al., (2004) *Science* 304:600-602). $\Delta F508$ -CFTR causes Cl^- impermeability because it is not processed correctly, causing it to be retained at the endoplasmic reticulum (rather than the plasma membrane). $\Delta F508$ -CFTR also has reduced intrinsic Cl^- conductance relative to wild type CFTR.

Strategies have been investigated to correct the defects in Δ F508-CFTR cellular processing and intrinsic function in cells. Cell growth at low temperature ($< 30^{\circ}\text{C}$) (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 $\Delta F508$ -CFTR processing (Egan et al., (2002) *Nature Med.* 8,485-492), possibly by interfering with interactions with molecular chaperones. Compounds like phenylbutyrate facilitate $\Delta F508$ -CFTR cellular processing by altering chaperone function and/or transcriptional enhancement (Rubenstein et al., (2000) *Am. J. Physiol.* 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 $\Delta F508$ -CFTR retention at the endoplasmic reticulum, they probably do not offer clinically-useful therapies.

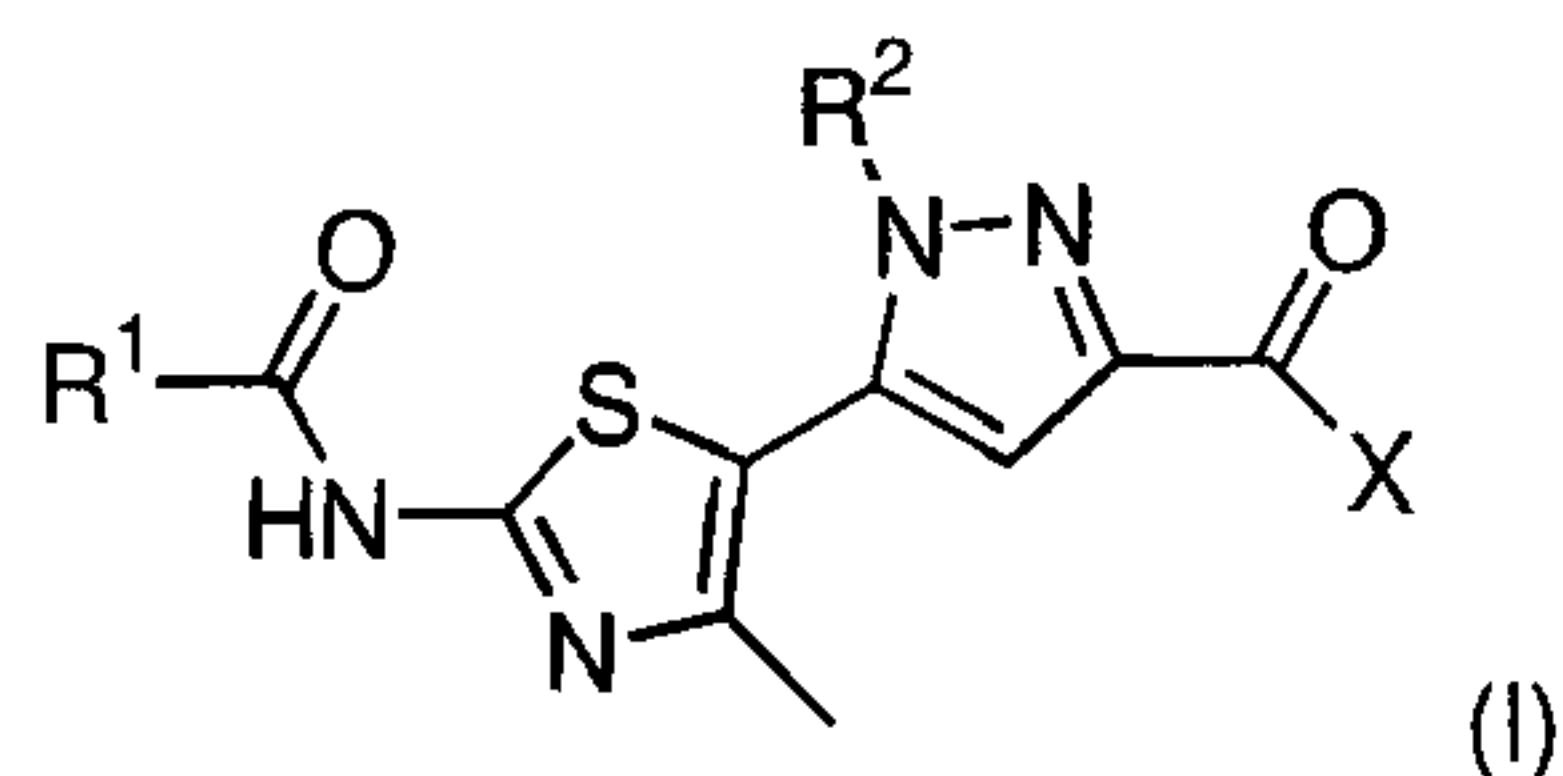
$\Delta F508$ -CFTR has significantly impaired channel activity even when present at the cell plasma membrane (Dalemans et al., (1991) *Nature* 354, 526-528). Cell-attached patchclamp measurements showed reduced $\Delta F508$ -CFTR open channel probability and prolonged closed times even with maximal 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 $\Delta F508$ -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 $\Delta F508$ -CFTR channel activity. Again, these studies have not offered any clinically useful therapies.

There is accordingly still a need for compounds that can correct cellular processing or folding of mutant CFTR, e.g., $\Delta F508$ -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 disclosure addresses these needs, as well as others.

SUMMARY

The present disclosure 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 compounds of the present disclosure, or an analog or derivative thereof.

The present disclosure provides a compound of formula (I):



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

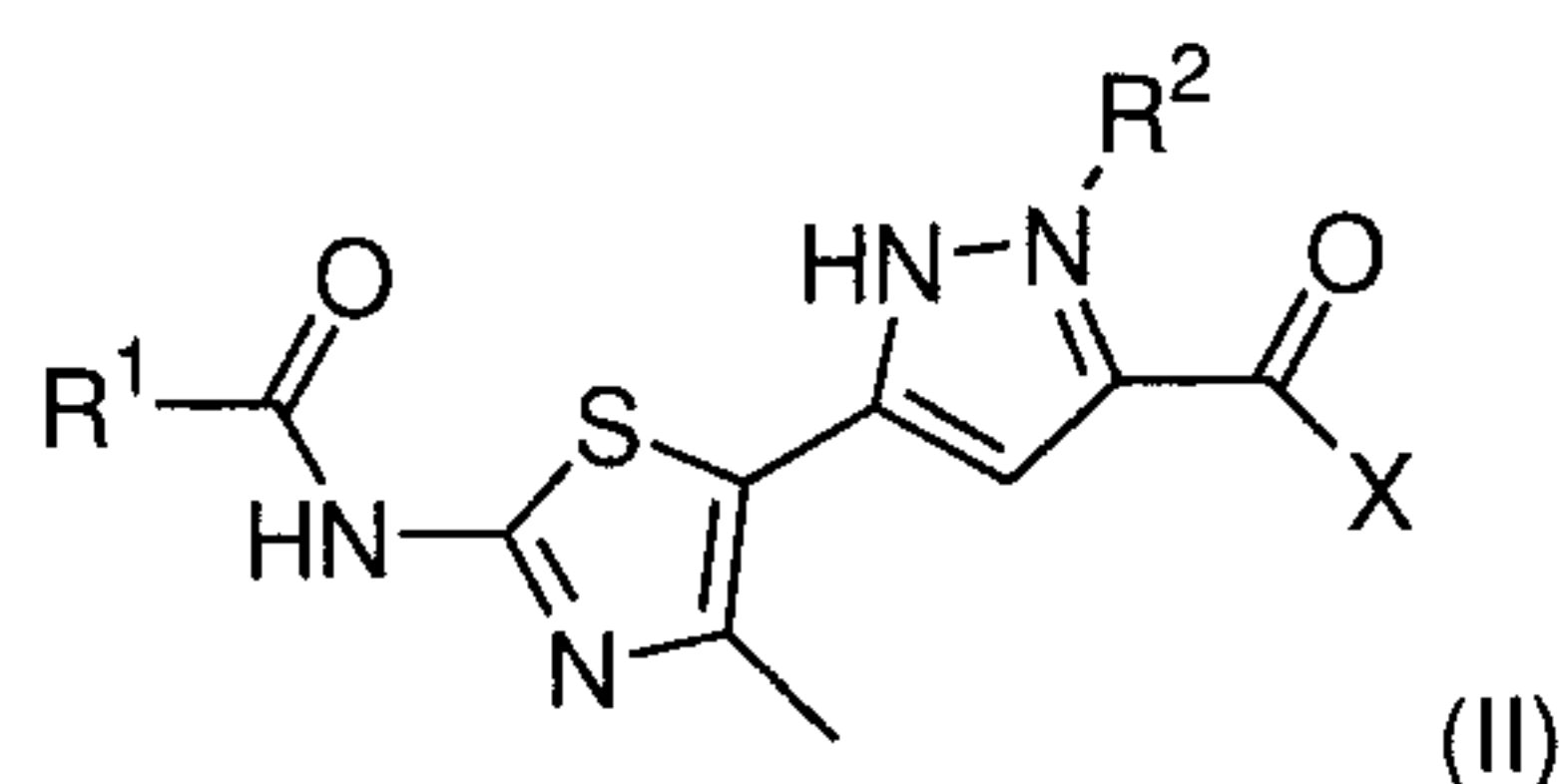
wherein:

R¹ is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl;

R² is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl; and

X is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

The present disclosure provides a compound of formula (II):



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

R¹ is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl;

R² is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl; and

X is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

The present disclosure provides pharmaceutical compositions comprising an effective amount of a disclosed compound. 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 present disclosure provides methods of treating a subject having a condition associated with mutant-CFTR, which involves administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a disclosed compound. The present disclosure provides a method of increasing ion permeability of a cell producing a mutant-CFTR protein, which involves contacting the cell with an effective amount of a composition comprising a disclosed compound so as to increase CFTR-mediated ion permeability of the cell.

The present disclosure provides kits containing one or more compositions comprising a disclosed compound, as well as methods of preparing the compositions.

Advantages of the compounds and compositions include improved drug like properties such as increased potency and solubility, as well as expanded diversity for generating additional corrector compounds. The compounds also are useful in the study of mutant-CFTR related disorders. Thus the present disclosure addresses many unmet needs in the development and use of mutant-CFTR corrector compounds. These and other objects and advantages of the present disclosure will be apparent from the detailed description below.

BRIEF DESCRIPTION OF FIGURES

Figure 1. Dose-response relation for increased I^- influx in ΔF508 -CFTR cells treated with pyrazolylthiazoles: **14g** (in solid) and **14h** (in dashed).

Figure 2. Standard calibration curve correlating experimentally determined capacity factor k with $\log P$. Data are shown for reference compounds ($\blacklozenge$: A = 4-chlorophenol, B = 2,4-dichlorophenol, C = 3,4,5-trichlorophenol, D = pentachlorophenol, E = p,p'-DDT), Compounds 11d/14a/14b/14e/14g/14h/14j.

DETAILED DESCRIPTION

The present disclosure is based on the discovery of pyrazolylthiazole and related compounds that correct cellular processing or folding of 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 disorders related to mutant-CFTR, such as cystic fibrosis ("CF").

The compounds share a pyrazolylthiazolyl core which includes a pyrazole ring that can accommodate attachment of functionalities on the core ring.

By exploiting the chemical and structural aspects in the design, and synthesis and screening of compound libraries of the present disclosure, features for optimization of compounds containing a pyrazolylthiazolyl core structural motif have been identified. The compounds of the present disclosure 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 present disclosure also includes compounds with features useful in the study of mutant-CFTR.

As such, the present disclosure provides novel compounds, compositions and pharmaceutical preparations that correct cellular processing or folding of mutant-CFTR (e.g., ΔF508 -CFTR). The present disclosure 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 correcting cellular processing or folding of 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 embodiments. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the embodiments, 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 embodiments.

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.

Terms

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, -CF₃, -OH, -OCF₃, C₂₋₆ alkenyl, C₃₋₆ alkynyl, C₁₋₆ alkoxy, aryl and di-C₁₋₆ 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, cyclohexylcarbonyl, 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., CH₄, CH₃-, -CH₂-, -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=CH₂), n-propenyl (-CH₂CH=CH₂), isopropenyl (-C(CH₃)=CH₂), 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=CHCH₂- and -C(CH₃)=CH- and -CH=C(CH₃)-) 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.

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

"Alkoxy carbonylamino" 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.

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

5 "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 (-CH₂-), ethylene (-CH₂CH₂-), the propylene isomers (e.g., -CH₂CH₂CH₂- and -CH(CH₃)CH₂-) and the like.

10 "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.
15 Particular non-limiting examples of alkynyl groups include acetylenic, ethynyl (-C≡CH), propargyl (-CH₂C≡CH), and the like.

"Amide" refers to the radical -NHC(O)- or -C(O)NH₂.

"Amino" refers to the radical -NH₂.

20 "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 cycloalkyl, or where the R groups are joined to form an alkylene group.

25 "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
30 carbons, examples of which include arsinole, 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,
35 quinoline, quinolizine, quinoxaline, tetrazole, thiadiazole, thiazole, thiophene, triazole, [2,4,6]triazine and xanthene, as well as fused ring systems such as acridine, anthracene, cinnoline, naphthalene, naphthyridine, quinoline, isoquinoline, quinoxaline and quinazoline.

40 "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, [2,4,6]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 N₃ or its radical -N₃ (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 CR⁷, NR³, O, or S; Q is O, NR³ 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," "heterocyclic," "heterocycloalkyl," and "heterocyclyl" refer to a saturated or unsaturated group having a single ring or multiple condensed rings, including fused bridged and spiro ring systems, and having from 3 to 15 ring atoms, including 1 to 4 hetero atoms. These hetero atoms are selected from the group consisting of nitrogen, sulfur, or oxygen, wherein, in fused ring systems, one or more of the rings can be cycloalkyl, aryl, or heteroaryl, provided that the point of attachment is through the non-aromatic ring. In certain embodiments, the nitrogen and/or sulfur atom(s) of the heterocyclic group are optionally oxidized to provide for the N-oxide, -S(O)-, or -SO₂- moieties.

"Heteroaryl" by itself or as part of another substituent, refers to a monovalent heteroaromatic radical derived by the removal of one hydrogen atom from a single atom of a heteroaromatic ring system. Typical heteroaryl groups include, but are not limited to, groups derived from acridine, arsindole, carbazole, β -carboline, chromane, chromene, cinnoline, furan, imidazole, indazole, indole, indoline, indolizine, isobenzofuran, isochromene, isoindole, isoindoline, isoquinoline, isothiazole, isoxazole, naphthyridine, oxadiazole, oxazole, perimidine, phenanthridine, phenanthroline, phenazine, phthalazine, pteridine, purine, pyran, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, pyrrole, pyrrolizine, quinazoline, quinoline, quinolizine, quinoxaline, tetrazole, thiadiazole, thiazole, thiophene, triazole, xanthene, benzodioxole and the like. In certain embodiments, the heteroaryl group is from 5-20 membered heteroaryl. In certain embodiments, the heteroaryl group is from 5-10 membered heteroaryl. In certain embodiments,

heteroaryl groups are those derived from thiophene, pyrrole, benzothiophene, benzofuran, indole, pyridine, quinoline, imidazole, oxazole and pyrazine.

"Hydroxyl" refers to the radical -OH.

"Nitro" refers to the radical -NO₂.

5 "Phenyl" (often abbreviated as -Ph) is the aryl form of benzene with the functional group, and has the formula -C₆H₅, 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
 10 are not limited to, -X, -R¹⁴, -O-, =O, -OR¹⁴, -SR¹⁴, -S⁻, =S, -NR¹⁴R¹⁵, =NR¹⁴, -CX₃, -CF₃, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -S(O)₂O⁻, -S(O)₂OH, -S(O)₂R¹⁴, -OS(O₂)O⁻, -OS(O)₂R¹⁴, -P(O)(O-)₂, -P(O)(OR¹⁴)(O⁻), -OP(O)(OR¹⁴)(OR¹⁵), -C(O)R¹⁴, -C(S)R¹⁴, -C(O)OR¹⁴, -C(O)NR¹⁴R¹⁵, -C(O)O⁻, -C(S)OR¹⁴, -NR¹⁶C(O)NR¹⁴R¹⁵, -NR¹⁶C(S)NR¹⁴R¹⁵, -NR¹⁷C(NR¹⁶)NR¹⁴R¹⁵ and -C(NR¹⁶)NR¹⁴R¹⁵, where each X is independently a halogen, and where "R¹⁴", "R¹⁵", "R¹⁶", and
 15 "R¹⁷" are independently hydrogen, alkyl, substituted alkyl, aryl, arylalkyl, cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, heteroalkyl, substituted heteroalkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, -NR¹⁸R¹⁹, -C(O)R¹⁸ or -S(O)₂R¹⁸ or optionally R¹⁸ and R¹⁹ together with the atom to which they are both attached form a cycloheteroalkyl or substituted cycloheteroalkyl ring, and where "R¹⁸", "R¹⁹", and "R²²" are each
 20 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.

25 "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, alkoxy carbonyl, alkoxy carbonylamino, amino, substituted amino, aminocarbonyl,
 30 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
 35 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, alkoxy carbonyl, alkoxy carbonylamino, amino, substituted amino, aminocarbonyl, aminocarbonylamino, aminocarbonyloxy, aryl, aryloxy, azido, carboxyl, cyano, cycloalkyl, substituted cycloalkyl, halogen, hydroxyl, keto, nitro, thioalkoxy, substituted thioalkoxy,
 40 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, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, 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, substituted 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 alkylthio, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, (cycloalkyl)alkyl, substituted (cycloalkyl)allyl, phenyl, substituted phenyl, phenylalkyl, and (substituted phenyl)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, 5 protected amide, thiol, protected thiol, cyano, nitro, azido, trifluoromethyl, C₁ to C₇ alkyl, C₁ to C₇ alkoxy, C₁ to C₇ acyl, C₁ to C₇ acyloxy, carboxy, oxycarboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, (monosubstituted)amino, protected (monosubstituted)amino, (disubstituted)amino, carboxamide, 10 protected carboxamide, N-(C₁ to C₆ alkyl)carboxamide, protected N-(C₁ to C₆ alkyl)carboxamide, N,N-di(C₁ to C₆ alkyl)carboxamide, trifluoromethyl, N-((C₁ to C₆ alkyl)sulfonyl)amino, N-(phenylsulfonyl)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, 15 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(alkyl)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 20 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-dibromoprotected carboxyphenyl; a mono- or di(hydroxymethyl)phenyl or (protected hydroxymethyl)phenyl such as 2,3 or 4-(protected hydroxymethyl)phenyl or 3,4-di(hydroxymethyl)phenyl; a mono- or 25 di(aminomethyl)phenyl or (protected aminomethyl)phenyl such as 2,3 or 4-(aminomethyl)phenyl or 2,4-(protected aminomethyl)phenyl; or a mono- or di(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- 30 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 35 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 -SO₂R. In particular embodiments, R is selected from H, lower alkyl, alkyl, aryl and heteroaryl.

5 "Sulfonyl" refers to the divalent radical -S(O₂)-. "Substituted sulfonyl" refers to a radical such as R-(O₂)S- wherein R is any substituent described herein. "Aminosulfonyl" refers to the radical H₂N(O₂)S-, and "substituted aminosulfonyl" refers to a radical such as R₂N(O₂)S- wherein each R is independently any substituent described herein.

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

10 "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".

30 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 heterozygous subjects having two

different mutant forms of CFTR, e.g., a subject with one copy of $\Delta F508$ -CFTR and a copy of different form of CFTR, e.g., a non-mutant CFTR or a different mutant 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. $\Delta F508$ -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. In certain embodiments, subjects that have a mutant-CFTR protein-mediated condition are homozygous for a gene encoding a $\Delta F508$ -CFTR protein. In certain embodiments, subjects that have a mutant-CFTR protein-mediated condition are heterozygous for a gene encoding a $\Delta F508$ -CFTR protein.

A " $\Delta F508$ -cystic fibrosis transmembrane conductance regulator protein" or " $\Delta F508$ -CFTR" is the protein that results from the deletion of a phenylalanine residue at amino acid position 508 of the CFTR gene product. A " $\Delta F508$ -CFTR gene" is a gene, or coding sequence, which encodes $\Delta F508$ -CFTR. A $\Delta F508$ -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 $\Delta F508$ -CFTR" and "gene that encodes $\Delta F508$ -CFTR". For an example of a gene that encodes $\Delta F508$ -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 embodiments 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 embodiments 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., Δ F508-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 " Δ F508-CFTR activator" as used herein is a compound that increases the level of ion transport by Δ F508-CFTR relative to ion transport in the absence of the compound, and particularly with respect to transport of chloride ions. CFTR activators of the embodiments are those that are specific Δ F508-CFTR activators, e.g., compounds that activate Δ F508-CFTR activity rather than affecting CFTR cellular misprocessing. Δ F508-CFTR activators are usually high-affinity Δ F508-CFTR activators, e.g., have an affinity for Δ F508-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., Δ F508-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 embodiments 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.

As used herein, a "mutant-CFTR corrector-potentiator" is a compound that exhibits both mutant-CFTR corrector and potentiator activity, and 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 parent compound and would be expected, by one skilled in the art, to exhibit the same or similar utility as the parent compound.

The term "derivative" refers to without limitation any compound which has a structure derived from the structure of the parent compound 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 parent compound.

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.

5 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

10 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

15 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;

20 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

25 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

30 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 or different routes, and in the same or different dosage form type.

35 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

40 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 alkyl may, but need not, be present, and the description includes situations where the heterocyclo group is mono- or disubstituted with an alkyl 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 substituents. 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.

The term "pharmaceutically acceptable derivatives" of a compound 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-hydroxybenzoyl)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, triethanolamine, tromethamine, N-methylglucamine, and the like.

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 in vivo when such prodrug is administered to a mammalian subject. Prodrugs of a compound are prepared by modifying functional groups present in the compound in such a way that the modifications may be cleaved in vivo to release the parent compound. Prodrugs include compounds wherein a hydroxy, amino, or sulfhydryl group in the compound 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 the compounds, 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 stereoisomers ("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 embodiments 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 embodiments depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

It is understood that in all substituted groups defined above, polymers arrived at by defining substituents with further substituents to themselves (e.g., substituted aryl having a substituted aryl group as a substituent which is itself substituted with a substituted aryl group, which is further substituted by a substituted aryl group, etc.) are not intended for inclusion herein. In such cases, the maximum number of such substitutions is three. For example, serial substitutions of substituted aryl groups are limited to substituted aryl-(substituted aryl)-substituted aryl.

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 embodiments, the structure of the compounds 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 present disclosure provides compounds and compositions containing them that correct cellular processing or folding of mutant-CFTR, such as $\Delta F508$ -CFTR, and methods of their use in treatment of mutant-CFTR-mediated diseases and conditions, e.g., cystic fibrosis.

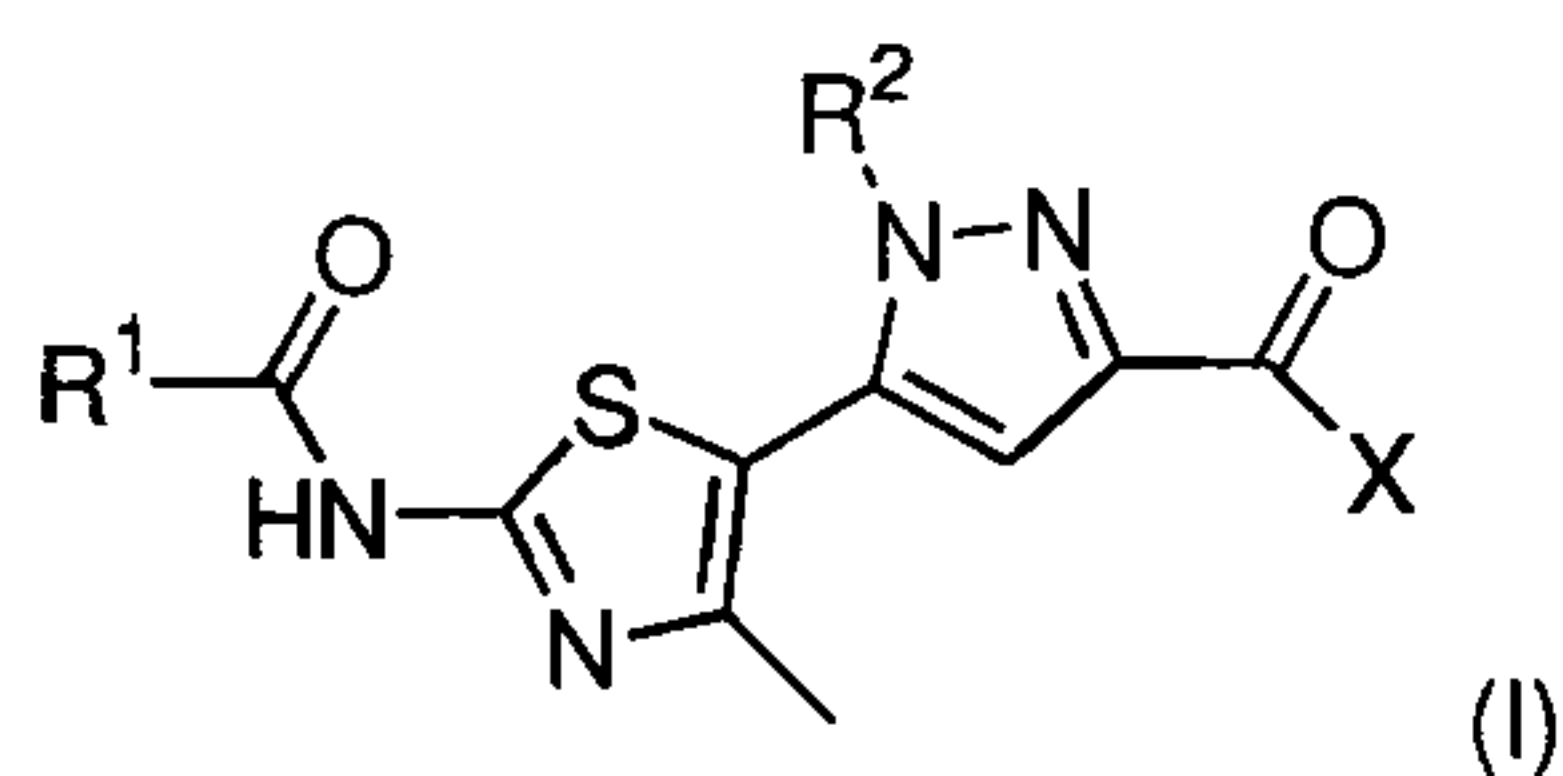
Such compounds also find use in the study of CFTR ion transport, particularly that of $\Delta F508$ -CFTR.

In one embodiment, the present disclosure provides high-affinity small-molecule compounds that increase chloride ion (Cl^-) conductance in cellular processing and folding defective mutant-CFTRs, such as $\Delta F508$ -CFTR. The compounds comprise a pyrazolylthiazolyl
5 core and multiple diversity points of substituents.

The compositions of the present disclosure include compounds of Formulae I-VII, shown below. Pharmaceutical compositions and methods of the present disclosure also contemplate compounds of Formulae I-VII.

10 **Formula I**

The present disclosure provides a compound of formula (I):



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

15 R^1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl;

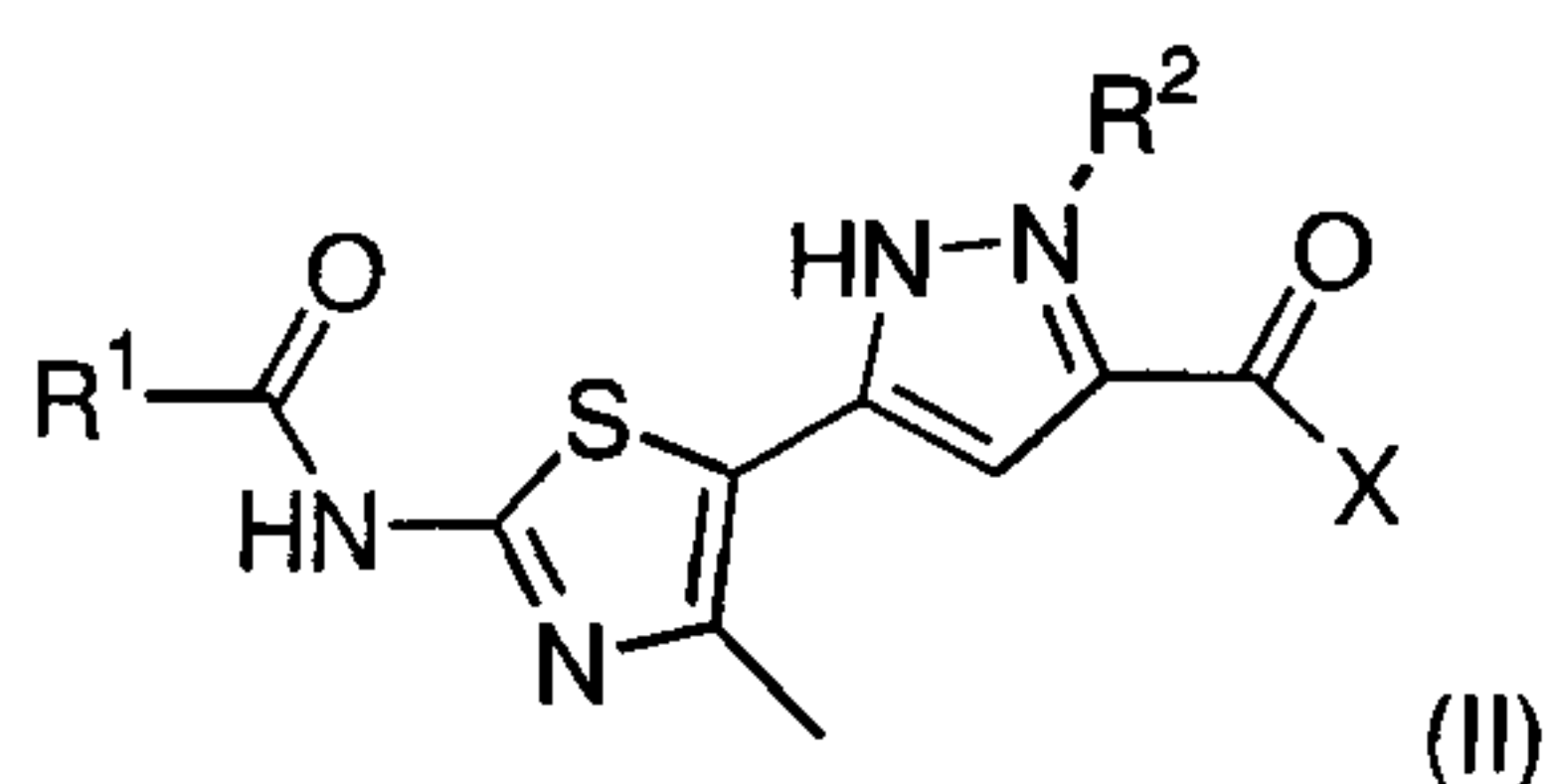
R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted
20 heteroaryl, heterocyclyl, and substituted heterocyclyl; and

X is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

25

Formula II

The present disclosure provides a compound of formula (II):



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

30 R^1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl;

R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl; and

X is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

In Formulae (I) and (II), R^1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl.

In certain embodiments, R^1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R^1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl. In certain embodiments, R^1 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

In certain embodiments, R^1 is alkyl. In certain embodiments, R^1 is substituted alkyl. In certain embodiments, R^1 is alkenyl. In certain embodiments, R^1 is substituted alkenyl. In certain embodiments, R^1 is alkynyl. In certain embodiments, R^1 is substituted alkynyl. In certain embodiments, R^1 is aryl. In certain embodiments, R^1 is substituted aryl. In certain embodiments, R^1 is cycloalkyl. In certain embodiments, R^1 is substituted cycloalkyl. In certain embodiments, R^1 is heteroaryl. In certain embodiments, R^1 is substituted heteroaryl. In certain embodiments, R^1 is heterocyclyl. In certain embodiments, R^1 is substituted heterocyclyl.

In Formulae (I) and (II), R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl.

In certain embodiments, R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, and substituted aryl.

In certain embodiments, R^2 is alkyl. In certain embodiments, R^2 is substituted alkyl. In certain embodiments, R^2 is alkenyl. In certain embodiments, R^2 is substituted alkenyl. In certain embodiments, R^2 is alkynyl. In certain embodiments, R^2 is substituted alkynyl. In certain embodiments, R^2 is aryl. In certain embodiments, R^2 is substituted aryl. In certain embodiments, R^2 is cycloalkyl. In certain embodiments, R^2 is substituted cycloalkyl. In certain embodiments, R^2 is heteroaryl. In certain embodiments, R^2 is substituted heteroaryl. In certain embodiments, R^2 is heterocyclyl. In certain embodiments, R^2 is substituted heterocyclyl.

In Formulae (I) and (II), X is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl,

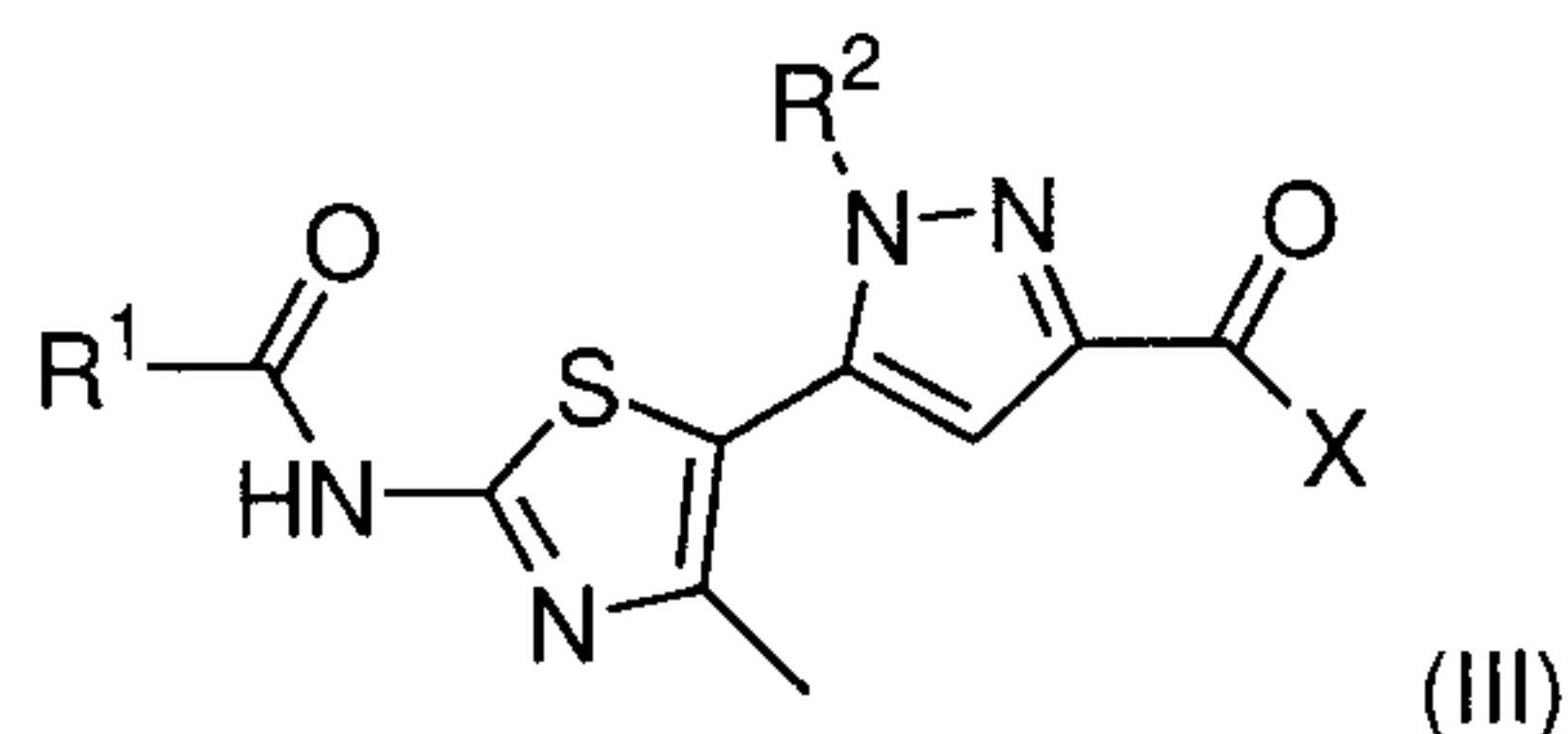
heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

In certain embodiments, X is selected from alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl. In certain
5
embodiments, X is selected from aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl. In certain embodiments, X is selected from heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

10 In certain embodiments, X is alkyl. In certain embodiments, X is substituted alkyl. In certain embodiments, X is alkenyl. In certain embodiments, X is substituted alkenyl. In certain embodiments, X is alkynyl. In certain embodiments, X is substituted alkynyl. In certain embodiments, X is aryl. In certain embodiments, X is substituted aryl. In certain embodiments, X is cycloalkyl. In certain embodiments, X is substituted cycloalkyl. In certain embodiments, X is
15 heteroaryl. In certain embodiments, X is substituted heteroaryl. In certain embodiments, X is heterocyclyl. In certain embodiments, X is substituted heterocyclyl. In certain embodiments, X is alkoxy. In certain embodiments, X is substituted alkoxy. In certain embodiments, X is amino. In certain embodiments, X is substituted amino. In certain embodiments, X is hydroxyl.

Formula III

The present disclosure provides a compound of formula (III):



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

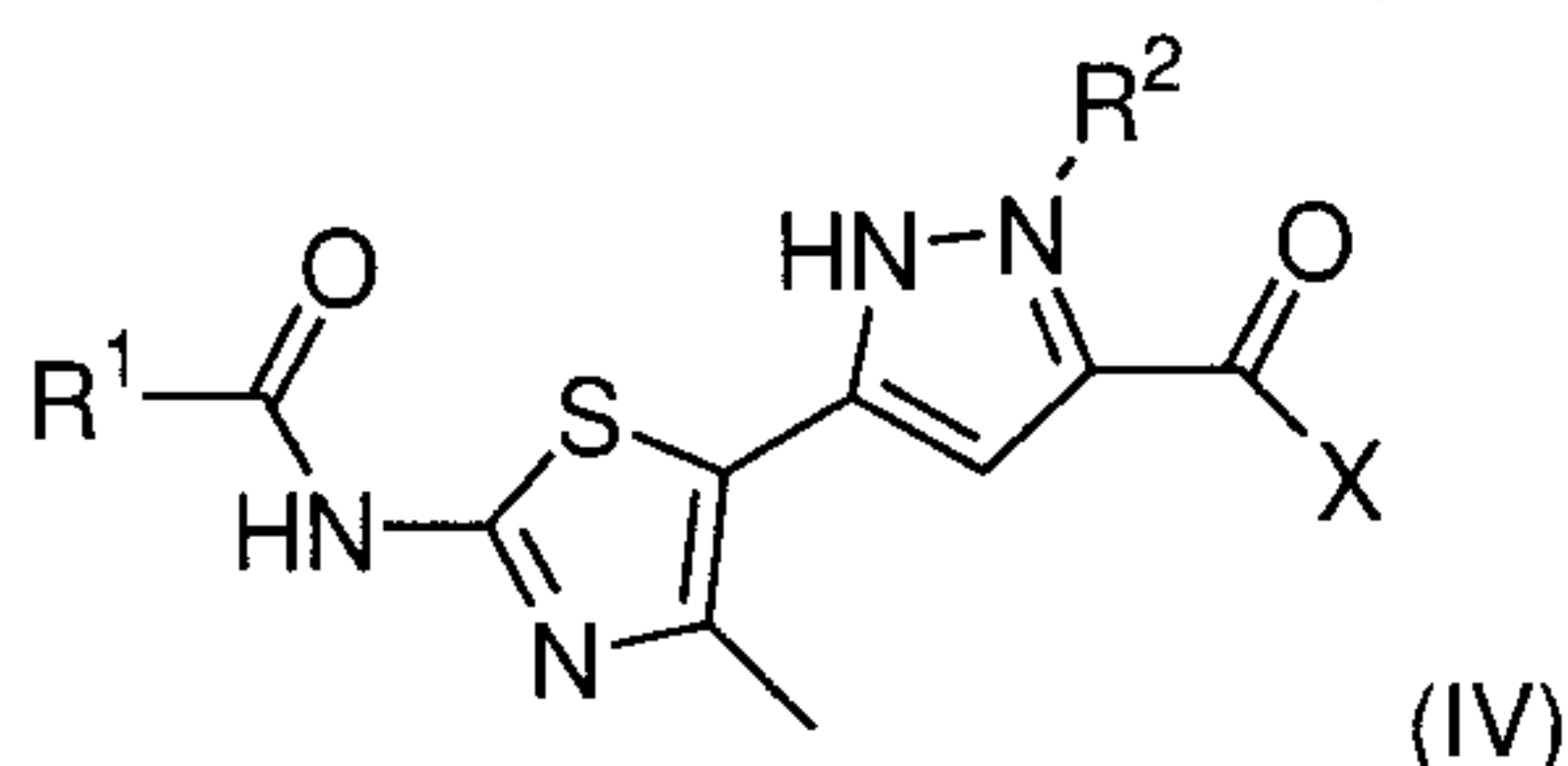
R¹ is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

R² is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, and substituted aryl; and

X is selected from heterocyclyl, substituted heterocyclyl, amino, substituted amino, and hydroxyl.

Formula IV

The present disclosure provides a compound of formula (IV):



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

R^1 is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, and
5 substituted aryl; and

X is selected from heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy,
amino, substituted amino, and hydroxyl.

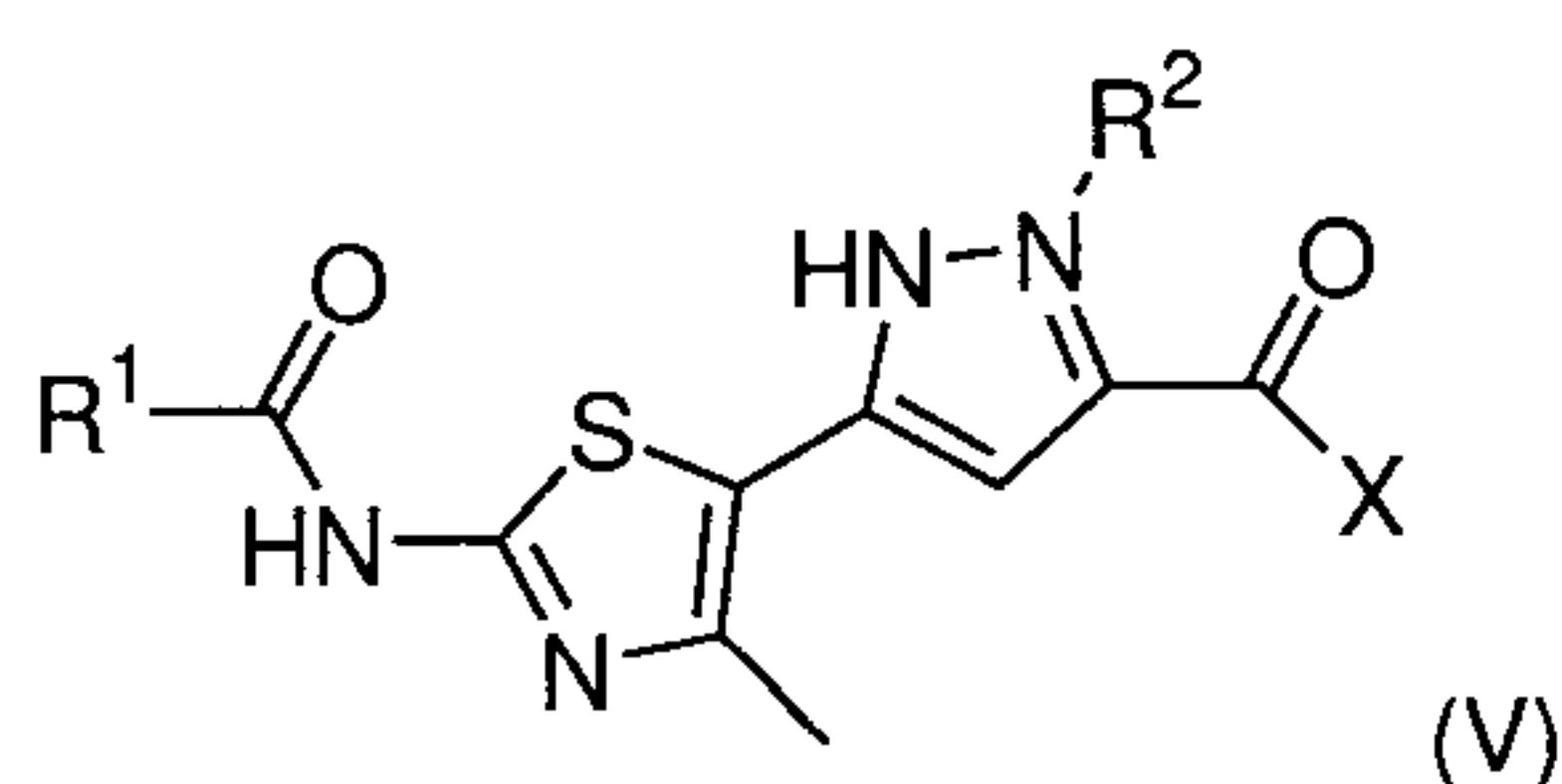
In formulae (III) and (IV), R^1 is selected from alkyl, substituted alkyl, aryl, and substituted
10 aryl. In certain embodiments, R^1 is alkyl. In certain embodiments, R^1 is substituted alkyl. In
certain embodiments, R^1 is aryl. In certain embodiments, R^1 is substituted aryl.

In formulae (III) and (IV), R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted
alkenyl, aryl, and substituted aryl. In certain embodiments, R^2 is alkyl. In certain embodiments,
 R^2 is substituted alkyl. In certain embodiments, R^2 is alkenyl. In certain embodiments, R^2 is
15 substituted alkenyl. In certain embodiments, R^2 is aryl. In certain embodiments, R^2 is substituted
aryl.

In formulae (III) and (IV), X is selected from heterocyclyl, and substituted heterocyclyl,
alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl. In certain embodiments, X is
heterocyclyl. In certain embodiments, X is substituted heterocyclyl. In certain embodiments, X is
20 alkoxy. In certain embodiments, X is substituted alkoxy. In certain embodiments, X is amino. In
certain embodiments, X is substituted amino. In certain embodiments, X is hydroxyl.

Formula V

The present disclosure provides a compound of formula (V):



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

R^1 is selected from alkyl and substituted alkyl;

R^2 is selected from alkyl, substituted alkyl, alkenyl, and substituted alkenyl; and

30 X is selected from amino and substituted amino.

In formula (V), R^1 is selected from alkyl and substituted alkyl. In certain embodiments, R^1
is alkyl. In certain embodiments, R^1 is substituted alkyl. In certain embodiments, R^1 is a tert-butyl
group.

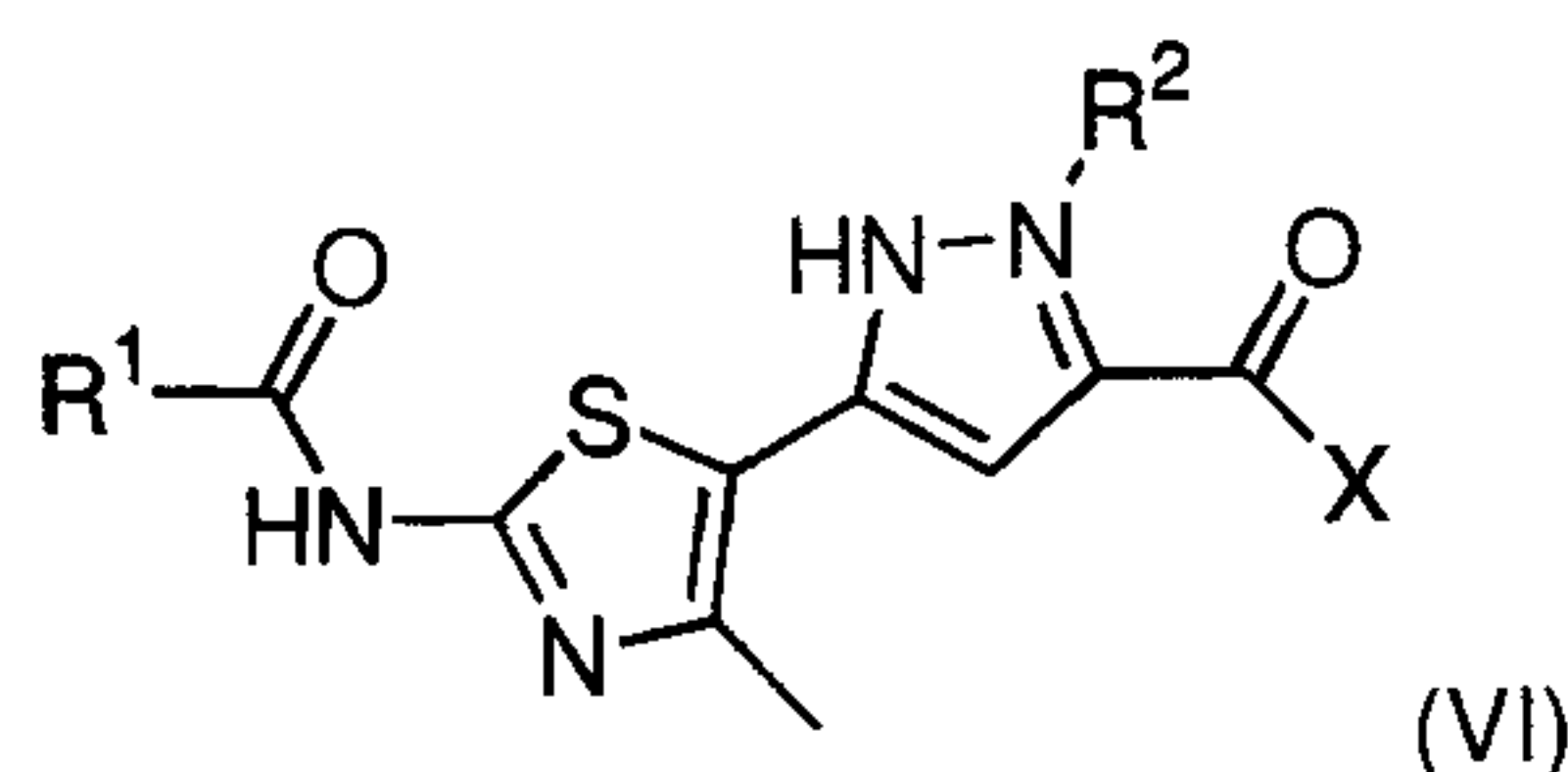
35 In formula (V), R^2 is selected from alkyl, substituted alkyl, alkenyl, and substituted
alkenyl. In certain embodiments, R^2 is alkyl. In certain embodiments, R^2 is substituted alkyl. In

certain embodiments, R^2 is alkenyl. In certain embodiments, R^2 is substituted alkenyl. In certain embodiments, R^2 is allyl. In certain embodiments, R^2 is benzyl. In certain embodiments, R^2 is $-\text{CH}_2\text{C}_6\text{H}_3(2\text{-OMe}/4\text{-Cl})$.

In formula (V), X is selected from amino and substituted amino. In certain embodiments, X is amino. In certain embodiments, X is substituted amino. In certain embodiments, X is $\text{NHC}_6\text{H}_4(4\text{-OMe})$. In certain embodiments, X is $\text{NHCH}_2\text{C}_6\text{H}_5$. In certain embodiments, X is $\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$.

Formula VI

The present disclosure provides a compound of formula (VI):



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

R^1 is selected from alkyl and substituted alkyl;
 R^2 is selected from alkyl, substituted alkyl, alkenyl, and substituted alkenyl; and
 X is selected from alkoxy, substituted alkoxy, amino, and substituted amino.

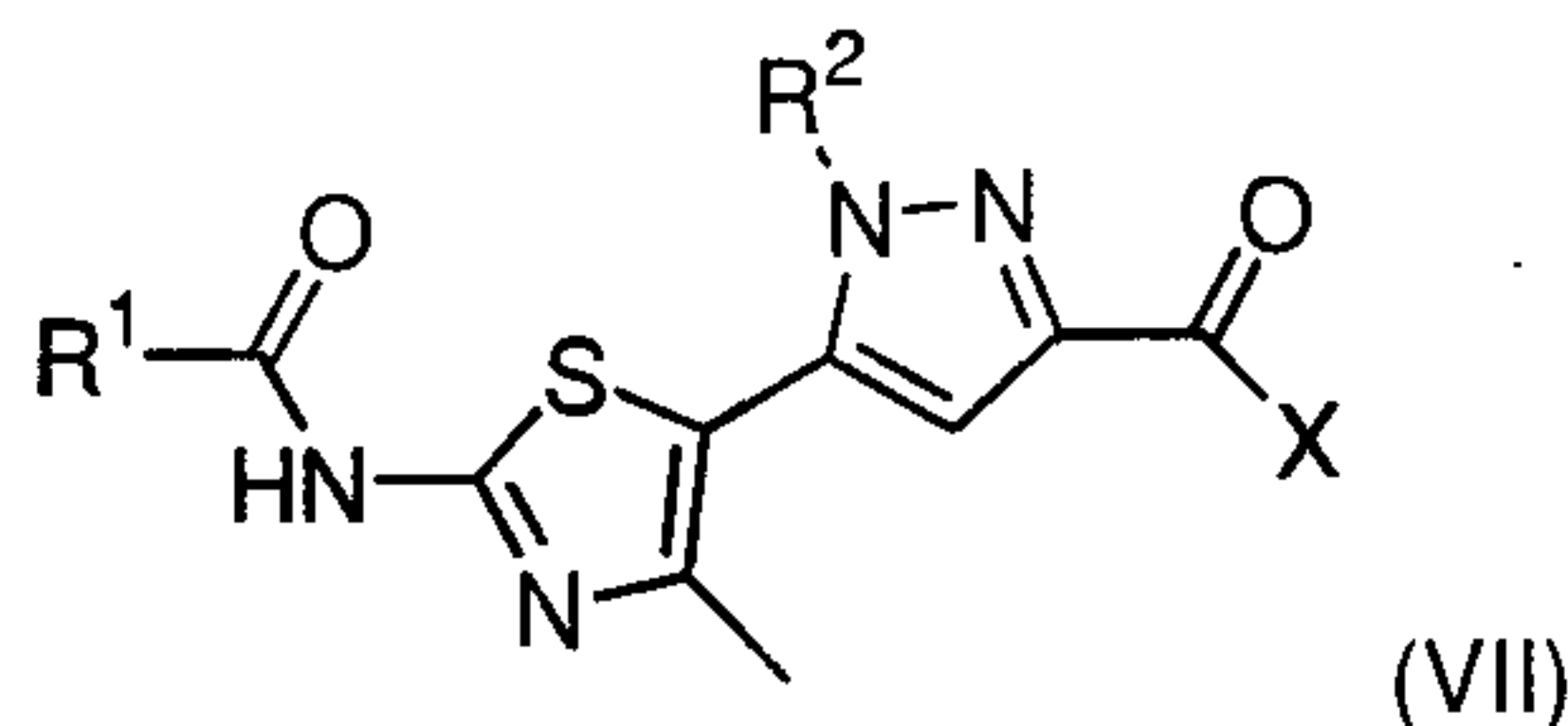
In formula (VI), R^1 is selected from alkyl and substituted alkyl. In certain embodiments, R^1 is alkyl. In certain embodiments, R^1 is substituted alkyl. In certain embodiments, R^1 is a tert-butyl group.

In formula (VI), R^2 is selected from alkyl, substituted alkyl, alkenyl, and substituted alkenyl. In certain embodiments, R^2 is alkyl. In certain embodiments, R^2 is substituted alkyl. In certain embodiments, R^2 is alkenyl. In certain embodiments, R^2 is substituted alkenyl. In certain embodiments, R^2 is benzyl.

In formula (VI), X is selected from alkoxy, amino and substituted amino. In certain embodiments, X is alkoxy. In certain embodiments, X is amino. In certain embodiments, X is substituted amino. In certain embodiments, X is ethoxy.

Formula VII

The present disclosure provides a compound of formula (VII):



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

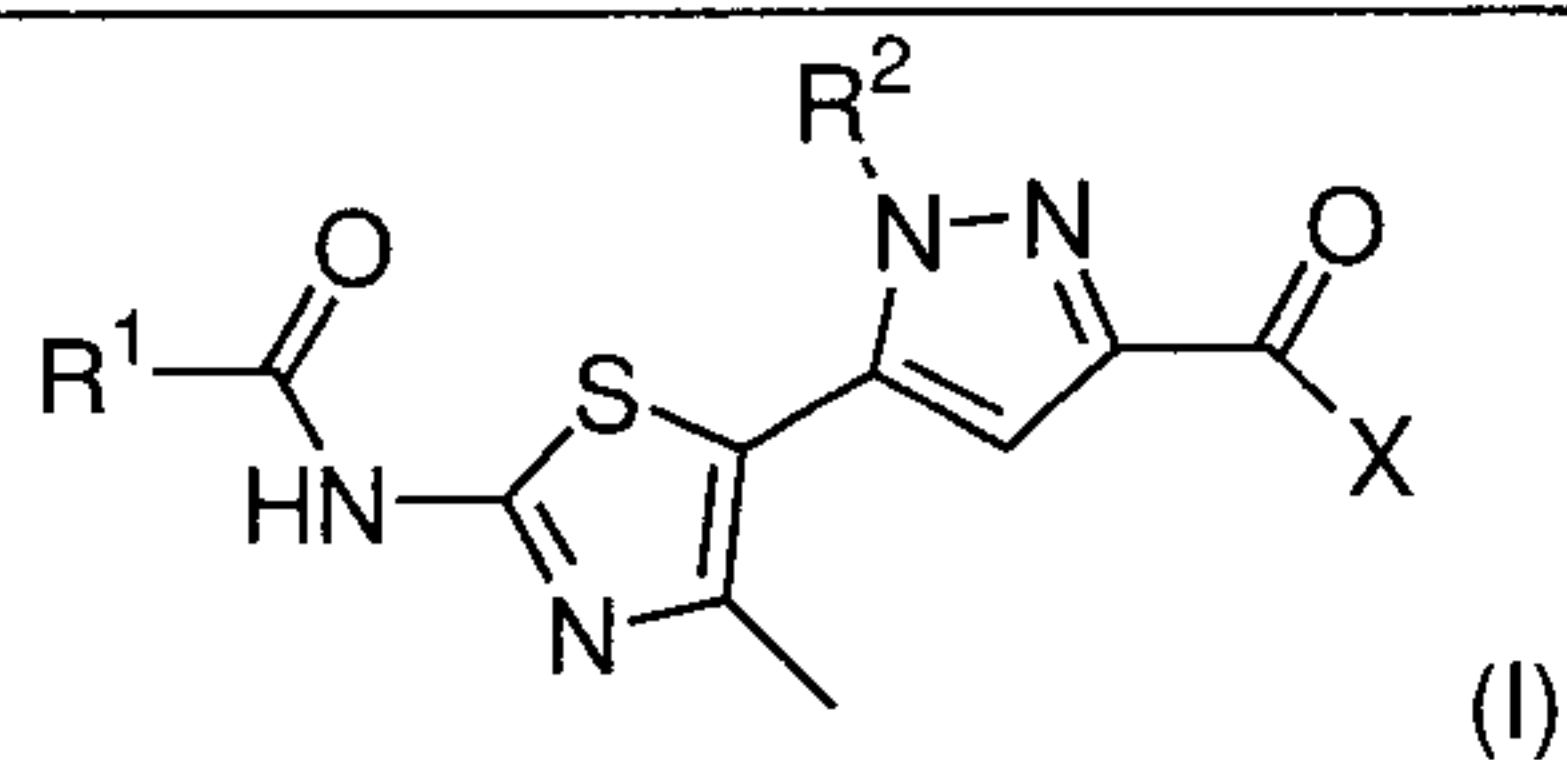
R¹ is selected from alkyl and substituted alkyl;
R² is selected from aryl and substituted aryl; and
X is selected from amino and substituted amino.

5 In formula (VII), R¹ is selected from alkyl and substituted alkyl. In certain embodiments, R¹ is alkyl. In certain embodiments, R¹ is substituted alkyl. In certain embodiments, R¹ is a tert-butyl group.

 In formula (VII), R² is selected from aryl and substituted aryl. In certain embodiments, R² is aryl. In certain embodiments, R² is substituted aryl. In certain embodiments, R² is C₆H₄(4-Br).

10 In formula (VII), X is selected from amino and substituted amino. In certain embodiments, X is amino. In certain embodiments, X is substituted amino. In certain embodiments, X is NHC₆H₄(4-OMe).

Particular compounds of interest are illustrated in the following tables.



Compound	R ¹	R ²	X
11a	C(CH ₃) ₃	CH ₂ CH=CH ₂	NHC ₆ H ₄ (4-OMe)
11b	C(CH ₃) ₃	CH ₂ CH=CH ₂	NHCH ₂ C ₆ H ₅
11c	C(CH ₃) ₃	CH ₂ CH=CH ₂	N(CH ₂ CH ₂) ₂ O
11d	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	NHC ₆ H ₄ (4-OMe)
11e	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	NHCH ₂ C ₆ H ₅
11f	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	N(CH ₂ CH ₂) ₂ O
11g	C(CH ₃) ₃	CH ₂ C ₆ H ₅	NHC ₆ H ₄ (4-OMe)
11h	C(CH ₃) ₃	CH ₂ C ₆ H ₅	NHCH ₂ C ₆ H ₅
11i	C(CH ₃) ₃	CH ₂ C ₆ H ₅	N(CH ₂ CH ₂) ₂ O
11j	C(CH ₃) ₃	CH ₂ CH ₂ OH	NHC ₆ H ₄ (4-OMe)
11k	C(CH ₃) ₃	CH ₂ CH ₂ OH	NHCH ₂ C ₆ H ₅
11l	C(CH ₃) ₃	CH ₂ CH ₂ OH	N(CH ₂ CH ₂) ₂ O
11m	C ₆ H ₅	CH ₂ CH=CH ₂	NH(CH ₂)OH
11n	C ₆ H ₅	C ₆ H ₄ (4-Br)	NH(CH ₂)OH
11o	C ₆ H ₅	CH ₂ C ₆ H ₅	NH(CH ₂)OH
11p	C ₆ H ₅	CH ₂ CH ₂ OH	NH(CH ₂)OH
11q	C ₆ H ₅	CH ₂ CH=CH ₂	NHC ₆ H ₄ (4-OMe)
11r	C ₆ H ₅	C ₆ H ₄ (4-Br)	NHC ₆ H ₄ (4-OMe)
11s	C ₆ H ₅	CH ₂ C ₆ H ₅	NHC ₆ H ₄ (4-OMe)
11t	C ₆ H ₅	CH ₂ CH ₂ OH	NHC ₆ H ₄ (4-OMe)
11u	C ₆ H ₅	CH ₂ CH=CH ₂	N(CH ₂ CH ₂) ₂ O
11v	C ₆ H ₅	C ₆ H ₄ (4-Br)	N(CH ₂ CH ₂) ₂ O
11w	C ₆ H ₅	CH ₂ C ₆ H ₅	N(CH ₂ CH ₂) ₂ O
11x	C ₆ H ₅	CH ₂ CH ₂ OH	N(CH ₂ CH ₂) ₂ O
12a	C(CH ₃) ₃	CH ₂ CH=CH ₂	OH
12b	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	OH
12c	C(CH ₃) ₃	CH ₂ C ₆ H ₅	OH
12d	C ₆ H ₅	CH ₂ CH=CH ₂	OH
12e	C ₆ H ₅	C ₆ H ₄ (4-Br)	OH
12f	C ₆ H ₅	CH ₂ C ₆ H ₅	OH
12g	C ₆ H ₅	CH ₂ CH ₂ OH	OH

(II)			
Compound	R ¹	R ²	X
10b	C(CH ₃) ₃	CH ₂ C ₆ H ₅	OEt
14a	C(CH ₃) ₃	CH ₂ CH=CH ₂	NHC ₆ H ₄ (4-OMe)
14b	C(CH ₃) ₃	CH ₂ CH=CH ₂	NHCH ₂ C ₆ H ₅
14c	C(CH ₃) ₃	CH ₂ CH=CH ₂	N(CH ₂ CH ₂) ₂ O
14d	C(CH ₃) ₃	CH ₂ C ₆ H ₅	NHC ₆ H ₄ (4-OMe)
14e	C(CH ₃) ₃	CH ₂ C ₆ H ₅	NHCH ₂ C ₆ H ₅
14f	C(CH ₃) ₃	CH ₂ C ₆ H ₅	N(CH ₂ CH ₂) ₂ O
14g	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	NHC ₆ H ₄ (4-OMe)
14h	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	N(CH ₂ CH ₂) ₂ O
14i	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	NHCH ₂ C ₆ H ₅
14j	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	NH(CH ₂)OH
15a	C(CH ₃) ₃	CH ₂ CH=CH ₂	OH
15b	C(CH ₃) ₃	CH ₂ C ₆ H ₅	OH

Particular compound of interest, and salts or solvates or stereoisomers thereof, include: Compounds 10b, 11d, 14a, 14b, 14e, 14g, 14h, and 14j.

- Particular compound of interest, and salts or solvates or stereoisomers thereof, include:
- 5 Compounds 14a, 14e, 14g, and 14h.

Analog and Derivative Compounds

Also provided by the present disclosure 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 pyrazolylthiazolyl-containing compounds. 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 $\Delta F508$ -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 present disclosure are pharmaceutical preparations of the subject compounds described above. Pharmaceutically acceptable derivatives include those that retain

the essential characteristic of the parent compound, namely, the ability to activate a mutant-CFTR, such as $\Delta F508$ -CFTR. The pharmaceutically acceptable derivatives may further include one or more of additional features that impart a pharmacological or biological property that benefits the compound's manufacture, handling, potency, selectivity, and / or pharmacokinetic parameters.

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 embodiments can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers, diluents, excipients and/or 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. 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 embodiments, 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 embodiments 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 embodiments 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 and/or the desired effect. The composition can be administered in a single dose or in multiple doses.

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

10 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 the embodiments 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, 15 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 Alkermes, 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, 20 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% (w/w) HSA is added. Additionally, one or more sugars or sugar alcohols may be 25 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% (w/w), 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 30 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 35 delivery device. An example of a commercially available metered dose inhaler suitable for use in the embodiments 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 40 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 g/cm² 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 and/or delivered by methods disclosed in U.S. Pat. No. 5,997,848, U.S. 5,993,783, U.S. 5,985,248, U.S. 5,976,574, 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 embodiments 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 embodiments 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,
 5 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.
 10 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
 15 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.

20 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,
 25 tablet or suppository, contains a predetermined amount of the composition containing one or more compounds of the embodiments. 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

30 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 potentiate gating defective mutant-CFTR), or a decrease in the amount of another
 35 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
 40 include beta agonists, tobramycin (TOBI®, Chiron Inc., Emeryville, Calif.) and curcumin (Egan et

al., (2004) *Science* 304:600-603). The compounds of the embodiments may also be used in combination with specific mutant CFTR activators, such as correctors and/or potentiators. Examples of mutant-CFTR potentiating agents include, but are not limited to, phenylglycine containing compounds and sulfonamide containing compounds described in WO 2005/120497 and co-pending US provisional patent application serial no. 60/980,387, filed October 16, 2007, entitled "Compounds Having Activity In Increasing Ion Transport By Mutant-CFTR And Uses Thereof", each of which are incorporated herein by reference in its entirety.

The compounds described above may also be combined with other therapies for CF, including oral corticosteroids, ibuprofen, ribovarín 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 embodiments 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 embodiments 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 present disclosure provides methods for increasing ion permeability of a cell that produces mutant-CFTR protein, with cells having a folding or processing defective mutant-CFTR being of interest, with cells having a $\Delta F508$ -CFTR being of particular interest. In general, the method involves contacting the cell with a compound in an amount effective to correct the folding or processing defect of a mutant-CFTR protein and increase ion permeability of the cell. In some embodiments, the cell contains a recombinant expression cassette that encodes said mutant-CFTR protein. In other embodiments, the cell contains a genome that encodes said mutant-CFTR protein. In yet other embodiments, the mutant-CFTR is a $\Delta F508$ -CFTR. In another embodiment of interest, a compound of the embodiments is used in the method in combination with a second mutant-CFTR activator or potentiator.

The present disclosure provides methods for 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 embodiments. The present disclosure also provides 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 embodiments so as to increase CFTR-mediated ion permeability of the cell. In some embodiments, the condition is cystic fibrosis. In some embodiments, 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. In some embodiments, the subject is a non-human animal. In some embodiments, the subject is human.

In embodiments of particular interest, the animal is a mammal. In some embodiments, the mutant-CFTR is a $\Delta F508$ -CFTR.

In many embodiments, the 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 embodiments, particularly when provided in combination with another 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 embodiments 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 10%, by up to about 20%, by up to about 50%, by up to about 100%, 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 embodiments causes detectable activity of mutant-CFTR or permeability of a cell to ions.

Activation of mutant-CFTR and/or 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., Galletta 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., $\Delta F508$ -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 present disclosure 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 embodiments 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 embodiments 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 potentiator compound, such as the phenylglycine and sulfonamide containing potentiator compound described in WO 2005/120497 and co-pending US provisional patent application serial no. 60/980,387, filed October 16, 2007, entitled "Compounds Having Activity In Increasing Ion Transport By Mutant-CFTR And Uses Thereof", each of which are incorporated herein by
 5 reference in its 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
 10 symptoms thereof are amenable to treatment by correction of cellular processing or folding of a mutant-CFTR, e.g., activation of mutant-CFTR chloride transport. Cystic fibrosis, a hereditary condition associated with a mutant-CFTR, e.g., $\Delta F508$ -CFTR, is an example of a condition that is treatable using the compounds of the embodiments. Use of the compounds of the embodiments in combination with a second mutant CFTR activator or potentiator is of particular interest,
 15 including a corrector compound of the embodiments.

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
 20 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: 1125-1128, 1992; Dorin et al., *Nature* 359: 211-215, 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.

25 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.

30 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
 35 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
 40 and during therapy the subject's forced vital capacity (FVC), carbon monoxide diffusing capacity

(DLco), and/or 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 embodiments 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 embodiments include individuals with 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 embodiments 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 $\Delta F508$ -CFTR. As such, the corrector compounds of the embodiments 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 due to folding or cellular processing (i.e., the mutant-CFTR is folding or cellular processing defective). As such, the corrector compounds of the embodiments have clinical utility in treating CF patients having a folding or cellular processing mutant-CFTR, such as $\Delta F508$ -CFTR. In addition, the corrector compounds of the embodiments also have clinical utility in treating CF patients when used in conjunction with compounds that activate or potentiate a gating mutant-CFTR, such as $\Delta F508$ -CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-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 embodiments 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 embodiments 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 certain embodiments, the mutant-CFTR polypeptide is Δ F508-CFTR. In certain embodiments, the mutant-CFTR polypeptide is G551D-CFTR. In certain embodiments, the mutant-CFTR polypeptide is G1349D-CFTR. In certain embodiments, the mutant-CFTR polypeptide is D152H-CFTR. The present disclosure, however, should not be construed to be limited solely to the treatment of CF patients having this mutant form of CFTR. Rather, the present disclosure 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 corrector compound of the embodiments, 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. This includes systems libraries of the compounds of the embodiments as well as individual compounds of the embodiments.

Thus the kits can include one or more of, depending upon the intended use of the kit, the compositions described herein, such as: a corrector compound of the embodiments. Other optional components of the kit include: buffers, delivery vehicles, delivery systems etc., for administering a corrector 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.

5 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 corrector compound of the embodiments 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,
10 but further include instructions to optionally screen and type the subject for mutant-CFTR (e.g., $\Delta F508$ -CFTR, G551D-CFTR, G1349D-CFTR, or D1152H-CFTR). This aspect assists the practitioner of the kit in tracking or gauging the potential responsiveness of the subject to treatment with a composition of the embodiments. In another embodiment, the kit includes one or more corrector compositions that are detectably labeled.

15

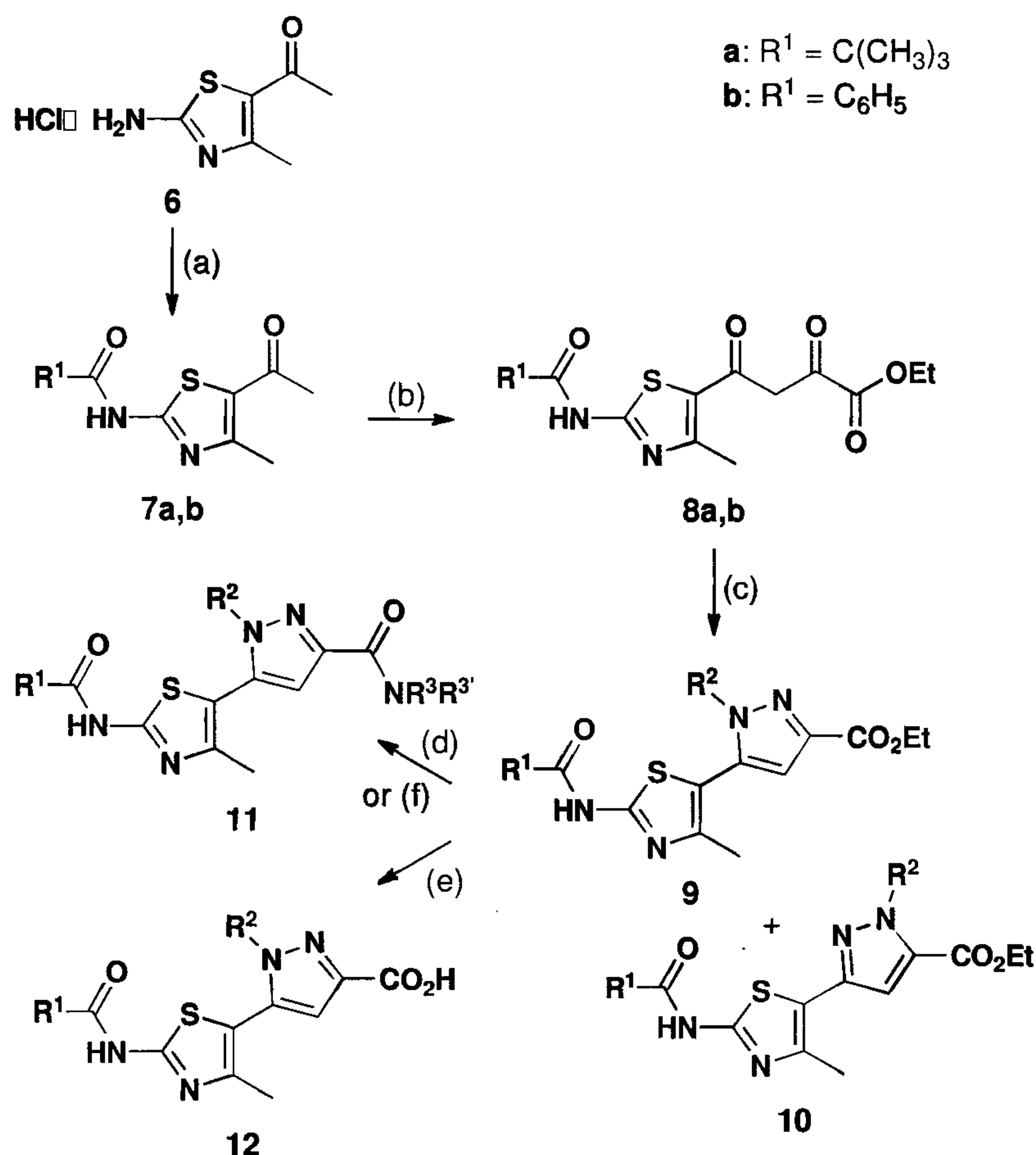
EXAMPLES

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 embodiments, and are not intended to limit the scope of what the inventors regard as their invention nor are they
20 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
25 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.

30 All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

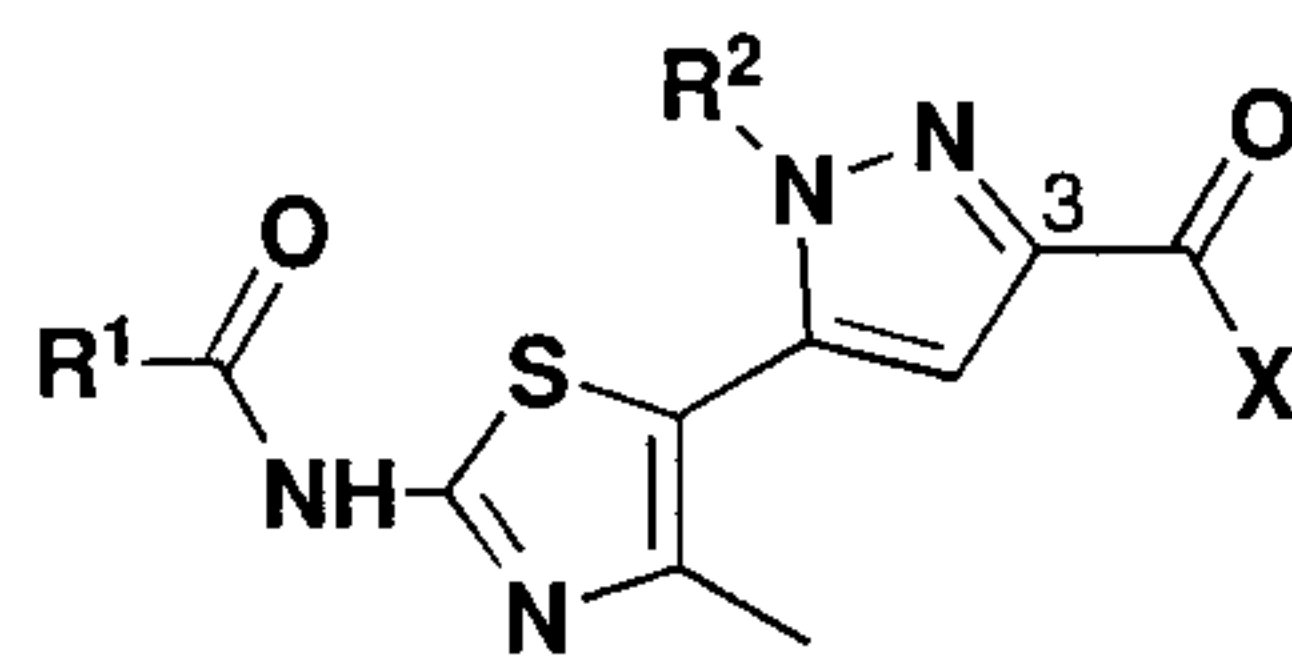
Chemistry

35 General and integrated synthetic methods to access a series of functionally and regioisomerically diversified pyrazolylthiazoles is disclosed herein.

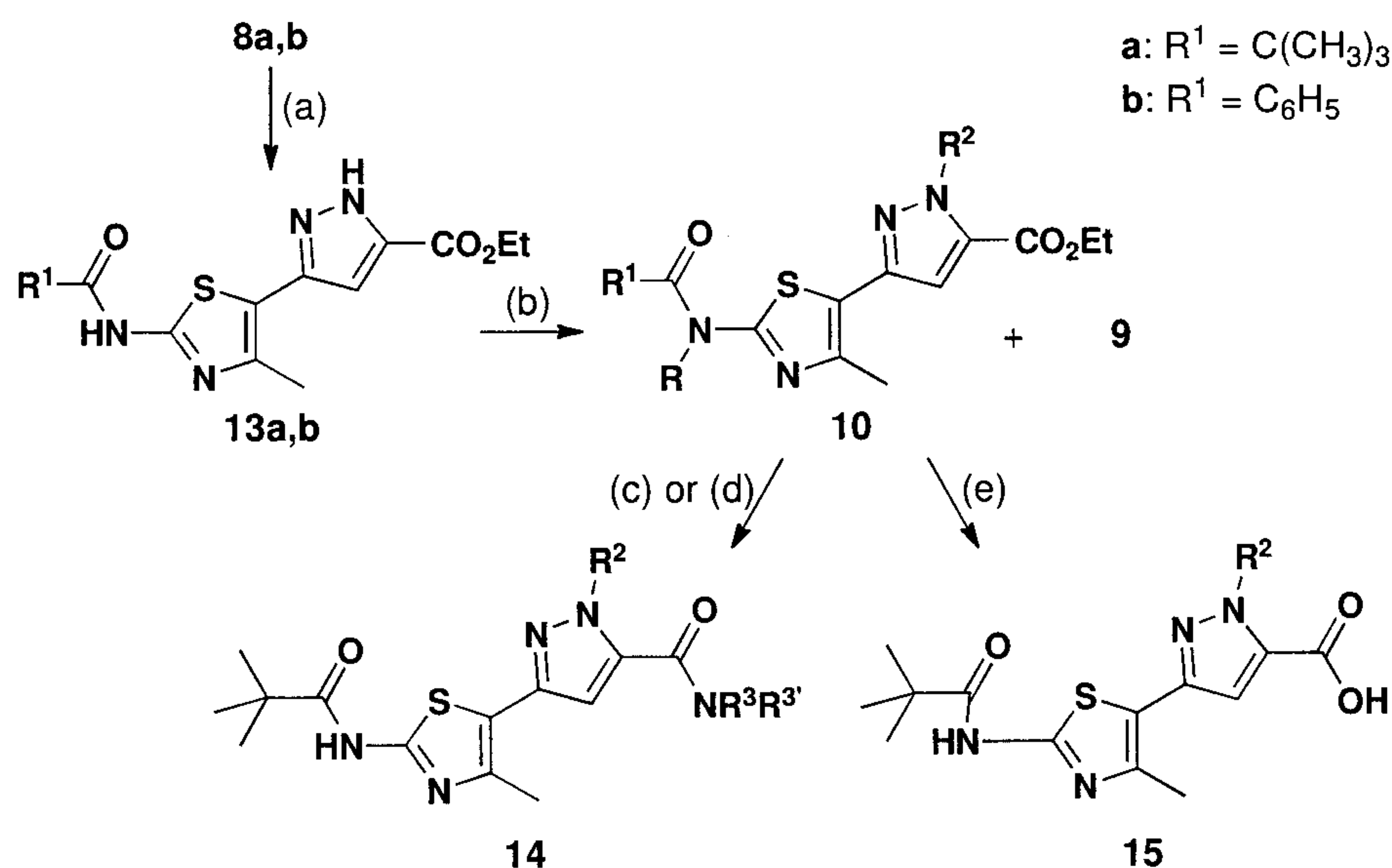
Scheme 1. Synthesis of N γ -substituted pyrazolylthiazoles.

Reagents and conditions: (a) $\text{R}^1\text{CO}_2\text{H}$, CDI, DMF, 85 °C (b) Diethyl oxalate, LHMDs, THF, -78 °C (c) R^2NHNH_2 , EtOH (d) $\text{R}^3\text{R}^3'\text{NH}$, AlMe_3 , DCM, 0 °C (e) NaOH, THF/ H_2O (f) Ethanol amine, MW, 160 °C, 30 Min.

As shown in Scheme 1, synthetic effort began with conversion of the amino group of **6** into amide **7** through a CDI-mediated coupling reaction, which occurred in greater than 79% yield. Amide **7** was then subjected to Claisen reaction with diethyl oxalate in the presence of 2.1 equivalents of LHMDs, giving 1,3-diketone **8** in excellent yield (>90%). Next, the condensation reaction between **8** and substituted hydrazines proceeded with excellent selectivity to deliver the N γ regioisomers **9** in good yield (>87%; **9**:**10**>10:1). A minor by-product was found to be **7**, indicating that the 2,4-dioxobutanoate moiety is susceptible to a reverse Claisen reaction in the presence of hydrazine nucleophiles. The excellent regioselectivity of this condensation reaction reflects the fact that the two carbonyl groups in **8** have very different electrophilicity. From pyrazolylthiazole **9**, the final diversification in this series was achieved by converting the ester moiety into the corresponding amide ($\rightarrow$ **11**) via aminolysis in good yield (>73%) or acid ($\rightarrow$ **12**) by saponification. Thirty-nine N γ -substituted pyrazolylthiazoles were prepared (see Table 1) by varying three diversity points (two R^1 inputs, four R^2 inputs, and four $\text{NR}^3\text{R}^3'$ inputs).

Table 1. N γ -Substituted pyrazolylthiazole analogs.
 $R^1 = \text{C}(\text{CH}_3)_3, \text{C}_6\text{H}_5$
 $R^2 = \text{CH}_2\text{CH}=\text{CH}_2, \text{C}_6\text{H}_4(4\text{-Br}), \text{CH}_2\text{C}_6\text{H}_5, (\text{CH}_2)_2\text{OH}$
 $X = \text{OEt}, \text{NHC}_6\text{H}_4(4\text{-OMe}), \text{NHCH}_2\text{C}_6\text{H}_5, \text{NH}(\text{CH}_2)_2\text{OH}, \text{N}(\text{CH}_2\text{CH}_2)_2\text{O}, \text{OH}$

Compd	R ¹	R ²	X	Compd	R ¹	R ²	X
9a	C(CH ₃) ₃	CH ₂ CH=CH ₂	OEt	11m	C ₆ H ₅	CH ₂ CH=CH ₂	NH(CH ₂)OH
9b	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	OEt	11n	C ₆ H ₅	C ₆ H ₄ (4-Br)	NH(CH ₂)OH
9c	C(CH ₃) ₃	CH ₂ C ₆ H ₅	OEt	11o	C ₆ H ₅	CH ₂ C ₆ H ₅	NH(CH ₂)OH
9d	C(CH ₃) ₃	CH ₂ CH ₂ OH	OEt	11p	C ₆ H ₅	CH ₂ CH ₂ OH	NH(CH ₂)OH
9e	C ₆ H ₅	CH ₂ CH=CH ₂	OEt	11q	C ₆ H ₅	CH ₂ CH=CH ₂	NHC ₆ H ₄ (4-OMe)
9f	C ₆ H ₅	C ₆ H ₄ (4-Br)	OEt	11r	C ₆ H ₅	C ₆ H ₄ (4-Br)	NHC ₆ H ₄ (4-OMe)
9g	C ₆ H ₅	CH ₂ C ₆ H ₅	OEt	11s	C ₆ H ₅	CH ₂ C ₆ H ₅	NHC ₆ H ₄ (4-OMe)
9h	C ₆ H ₅	CH ₂ CH ₂ OH	OEt	11t	C ₆ H ₅	CH ₂ CH ₂ OH	NHC ₆ H ₄ (4-OMe)
11a	C(CH ₃) ₃	CH ₂ CH=CH ₂	NHC ₆ H ₄ (4-OMe)	11u	C ₆ H ₅	CH ₂ CH=CH ₂	N(CH ₂ CH ₂) ₂ O
11b	C(CH ₃) ₃	CH ₂ CH=CH ₂	NHCH ₂ C ₆ H ₅	11v	C ₆ H ₅	C ₆ H ₄ (4-Br)	N(CH ₂ CH ₂) ₂ O
11c	C(CH ₃) ₃	CH ₂ CH=CH ₂	N(CH ₂ CH ₂) ₂ O	11w	C ₆ H ₅	CH ₂ C ₆ H ₅	N(CH ₂ CH ₂) ₂ O
11d	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	NHC ₆ H ₄ (4-OMe)	11x	C ₆ H ₅	CH ₂ CH ₂ OH	N(CH ₂ CH ₂) ₂ O
11e	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	NHCH ₂ C ₆ H ₅	12a	C(CH ₃) ₃	CH ₂ CH=CH ₂	OH
11f	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	N(CH ₂ CH ₂) ₂ O	12b	C(CH ₃) ₃	C ₆ H ₄ (4-Br)	OH
11g	C(CH ₃) ₃	CH ₂ C ₆ H ₅	NHC ₆ H ₄ (4-OMe)	12c	C(CH ₃) ₃	CH ₂ C ₆ H ₅	OH
11h	C(CH ₃) ₃	CH ₂ C ₆ H ₅	NHCH ₂ C ₆ H ₅	12d	C ₆ H ₅	CH ₂ CH=CH ₂	OH
11i	C(CH ₃) ₃	CH ₂ C ₆ H ₅	N(CH ₂ CH ₂) ₂ O	12e	C ₆ H ₅	C ₆ H ₄ (4-Br)	OH
11j	C(CH ₃) ₃	CH ₂ CH ₂ OH	NHC ₆ H ₄ (4-OMe)	12f	C ₆ H ₅	CH ₂ C ₆ H ₅	OH
11k	C(CH ₃) ₃	CH ₂ CH ₂ OH	NHCH ₂ C ₆ H ₅	12g	C ₆ H ₅	CH ₂ CH ₂ OH	OH
11l	C(CH ₃) ₃	CH ₂ CH ₂ OH	N(CH ₂ CH ₂) ₂ O				

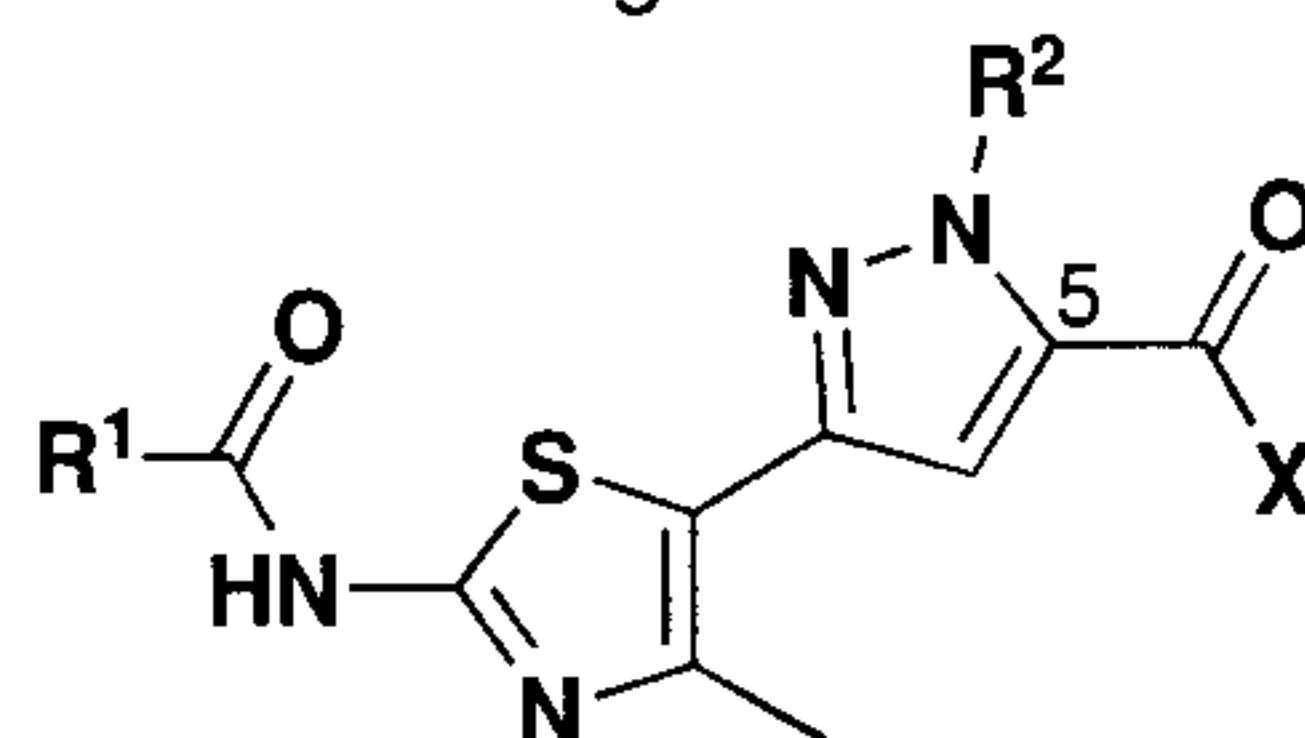
Scheme 2. Synthesis of N β -substituted pyrazolythiazole.

Reagents: (a) NH₂NH₂•H₂O, EtOH (b) R²Br, K₂CO₃, Acetone, 60 °C (c) R³R^{3'}NH, AlMe₃, DCM, MW, 100 °C (d) NH₂(CH₂)₂OH, EtOH, MW 180 °C (e) NaOH, THF/H₂O.

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Scheme 2 shows a synthetic route to access the N β -substituted pyrazole regioisomer. First, **8** was reacted with hydrazine monohydrate to deliver pyrazole **13** in 63% yield. The N β position of **13** was then selectively alkylated, giving mainly **10** (R = H) in 59-66% yield when R¹ = pivaloyl (**10**:**9** = 8:1). The major by-product of this reaction is N-alkylation of the amide moiety leading to formation of bis-alkylated product (**10**: R = R²). This complication was serious with the benzamide analog of **13** (R¹ = C₆H₅) that the major product was, in fact, the bisalkylation product (**10**: R = R²); the desired mono-alkylated product (**10**: R = H) was obtained in 20% yield. Therefore, final diversification on the N β -substituted isomers was mainly focused on pivaloyl amide analogs of **10** and included hydrolysis or aminolysis, such as microwave irradiation in the AlMe₃-mediated aminolysis, to deliver **14** and **15**, respectively. In total, fifteen N β -substituted pyrazolythiazoles were prepared for this focused library (see Table 2).

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Table 2. N β -Substituted pyrazolylthiazole analogs.
 $R^1 = C(CH_3)_3$
 $R^2 = CH_2CH=CH_2, C_6H_4(4-Br), CH_2C_6H_3(2-OMe/4-Cl)$
 $X = OEt, NHC_6H_4(4-OMe), NHCH_2C_6H_5, N(CH_2CH_2)_2O, NH(CH_2)_2OH, OH$

Compd	R ¹	R ²	X	Compd	R ¹	R ²	X
10a	C(CH ₃) ₃	CH ₂ CH=CH ₂	OEt	14f	C(CH ₃) ₃	CH ₂ C ₆ H ₅	N(CH ₂ CH ₂) ₂ O
10b	C(CH ₃) ₃	CH ₂ C ₆ H ₅	OEt	14g	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	NHC ₆ H ₄ (4-OMe)
10c	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	OEt	14h	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	N(CH ₂ CH ₂) ₂ O
14a	C(CH ₃) ₃	CH ₂ CH=CH ₂	NHC ₆ H ₄ (4-OMe)	14i	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	NHCH ₂ C ₆ H ₅
14b	C(CH ₃) ₃	CH ₂ CH=CH ₂	NHCH ₂ C ₆ H ₅	14j	C(CH ₃) ₃	CH ₂ C ₆ H ₃ (2-OMe/4-Cl)	NH(CH ₂) ₂ OH
14c	C(CH ₃) ₃	CH ₂ CH=CH ₂	N(CH ₂ CH ₂) ₂ O	15a	C(CH ₃) ₃	CH ₂ CH=CH ₂	OH
14d	C(CH ₃) ₃	CH ₂ C ₆ H ₅	NHC ₆ H ₄ (4-OMe)	15b	C(CH ₃) ₃	CH ₂ C ₆ H ₅	OH
14e	C(CH ₃) ₃	CH ₂ C ₆ H ₅	NHCH ₂ C ₆ H ₅				

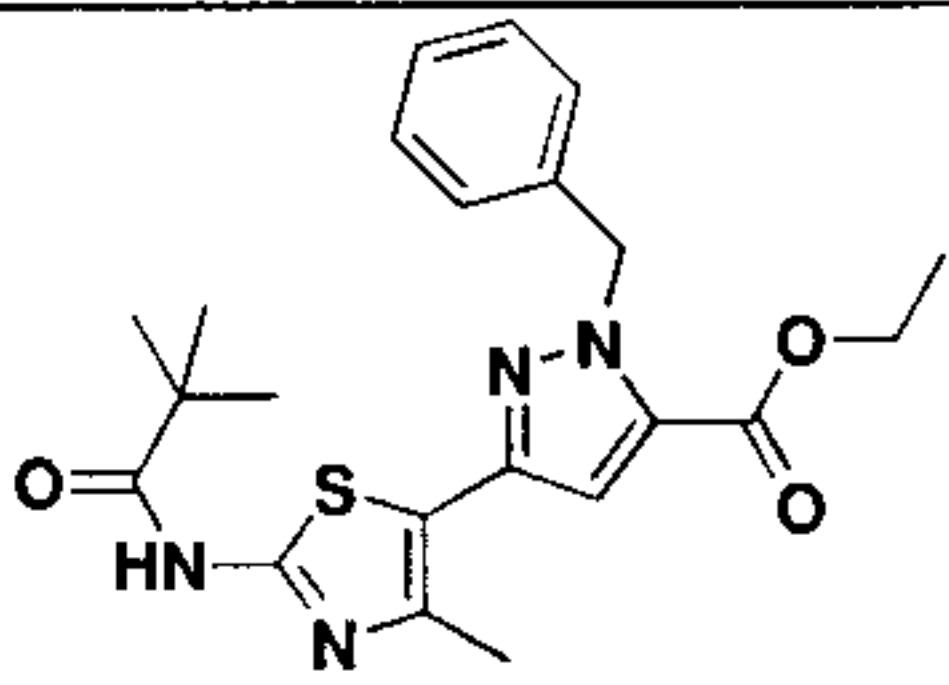
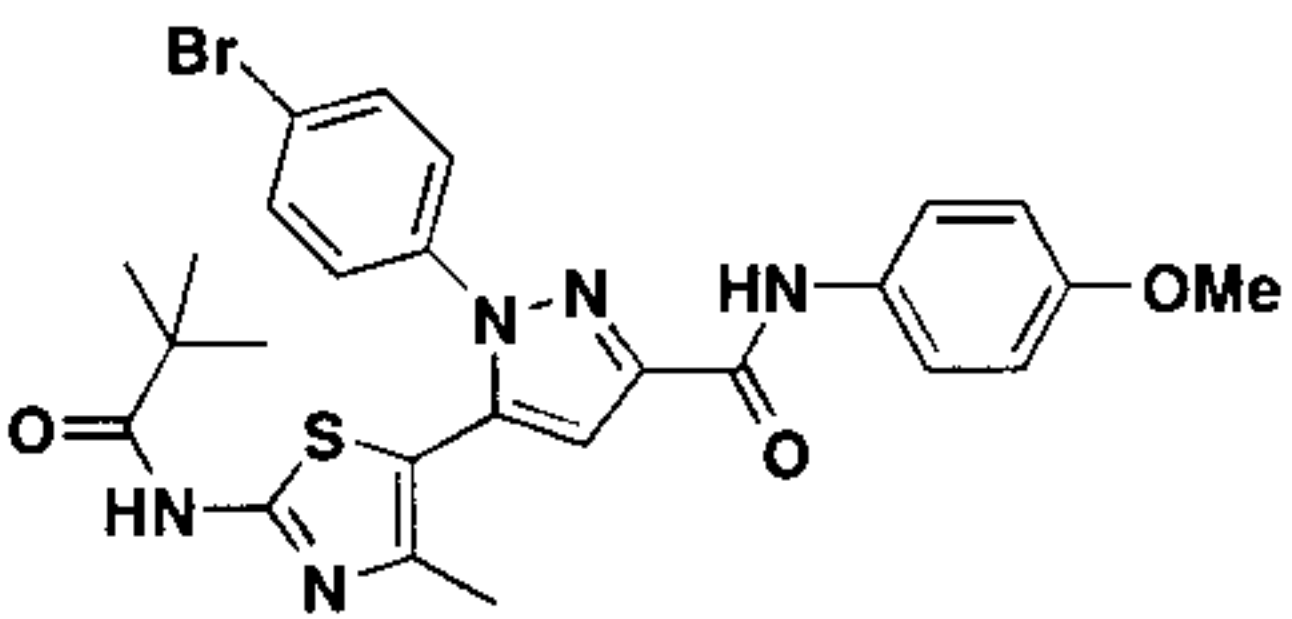
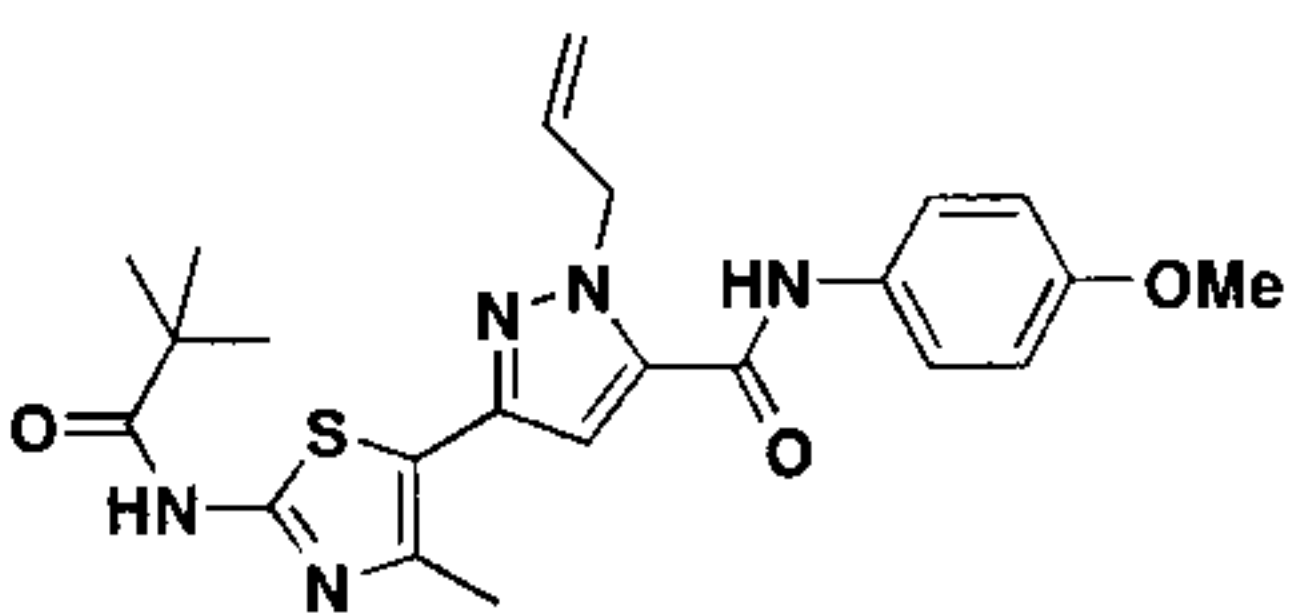
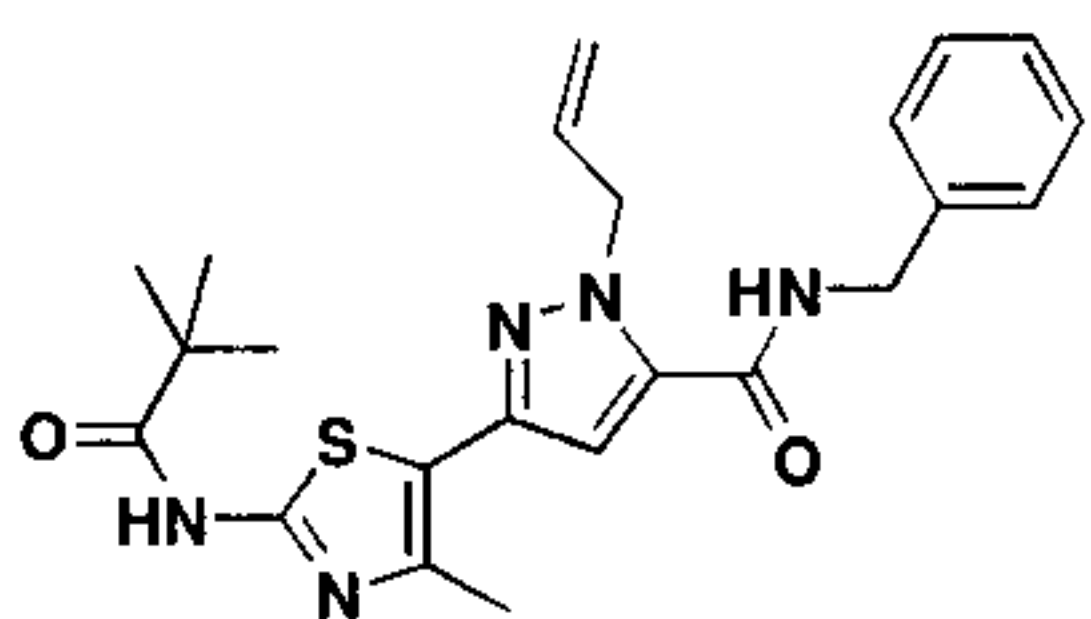
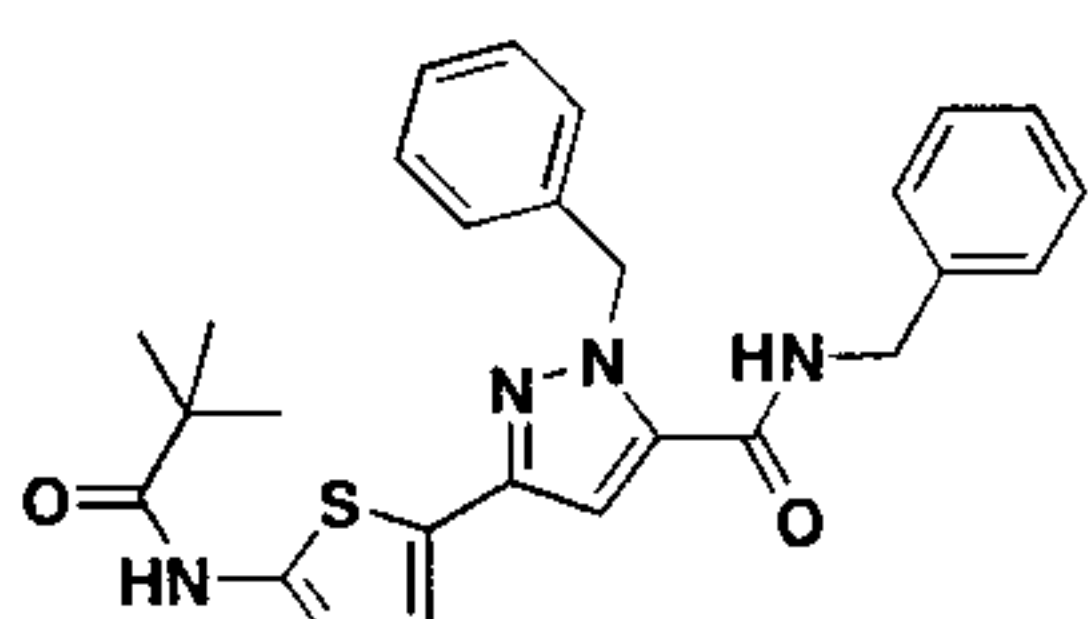
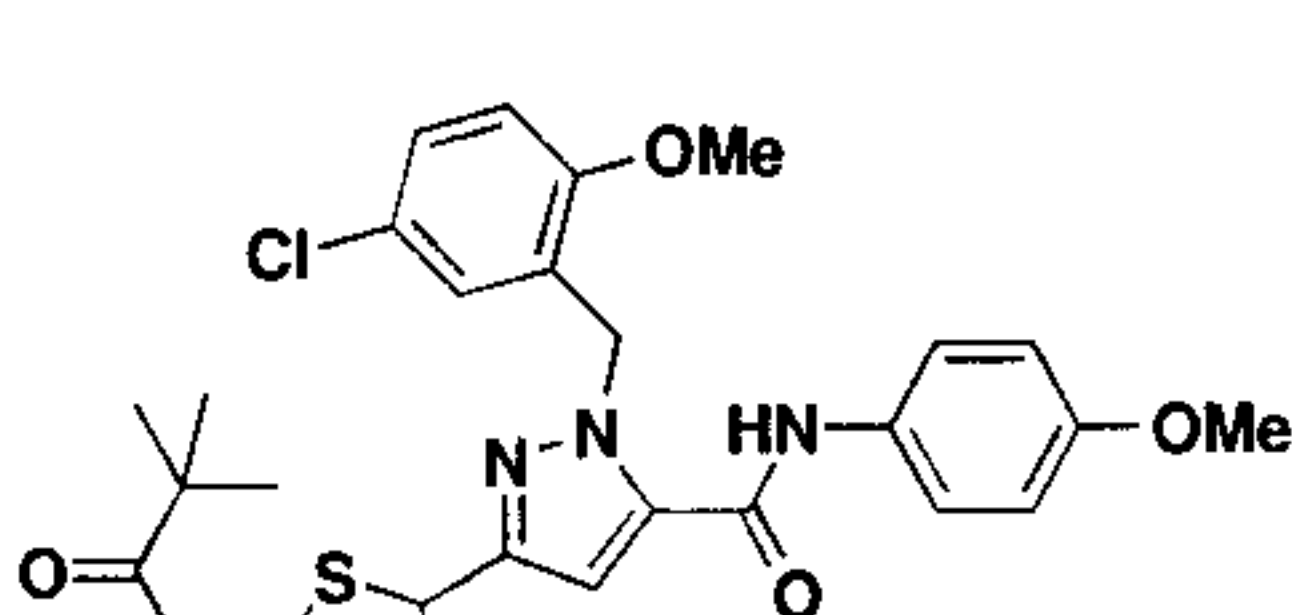
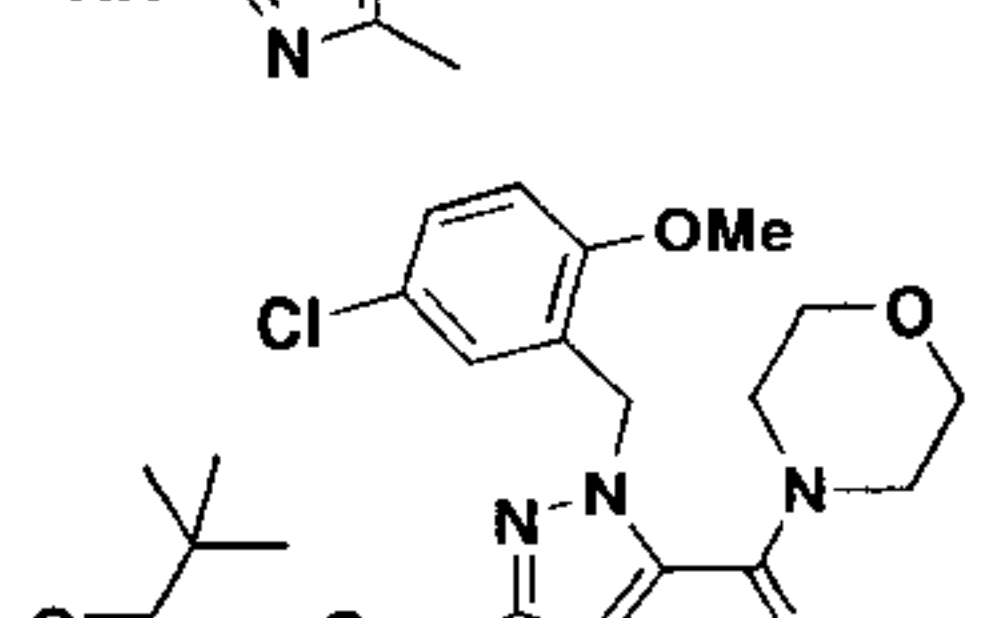
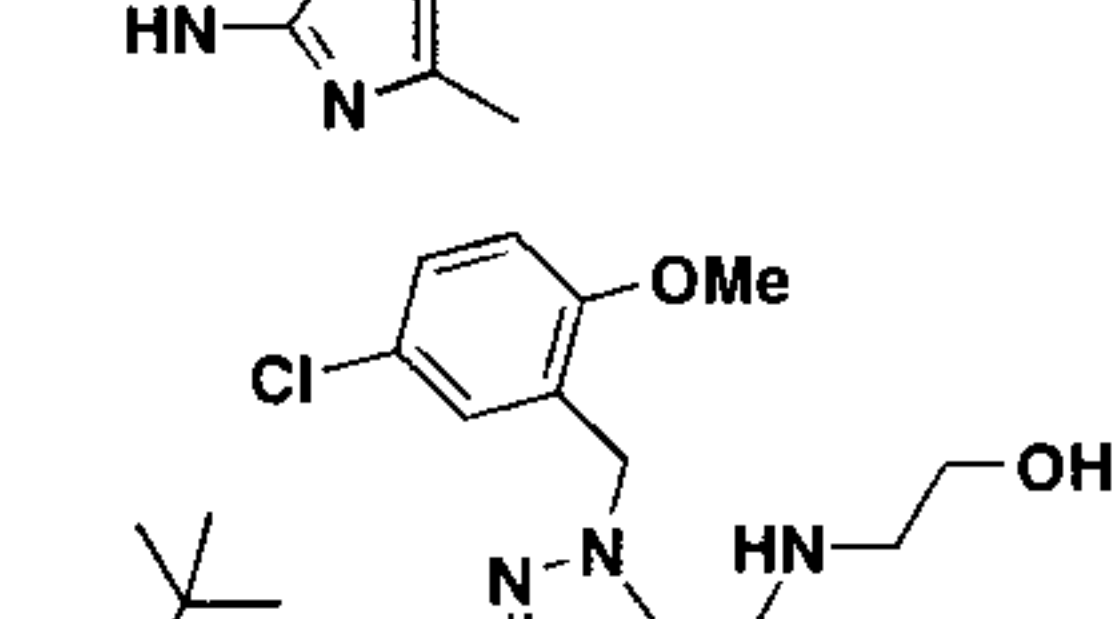
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Structure-Activity Relationships

The N γ - and N β -regioisomeric pyrazolylthiazoles were assayed for $\Delta F508$ -CFTR corrector activity. An established cell-based corrector assay was used in which I⁻ influx was measured in FRT cells coexpressing human $\Delta F508$ -CFTR and the I⁻-sensitive fluorescent sensor YFP-H148Q/I152L. Following 24 h incubation with test compounds, I⁻ influx was determined from the kinetics of YFP-H148Q/I152L quenching in response to I⁻ addition in cells treated with a cAMP agonist and the potentiator genistein. Out of the fifty-four compounds tested, eight had significant corrector activity as judged by concentration-dependent increases in I⁻ influx as exemplified in Figure 1 for **14g** and **14h**.

Structures for these compounds as well as their EC₅₀ and V_{max} values from ion influx data are summarized in Table 3. Their EC₅₀ values range from 0.93 to 8.5 μ M while increasing I⁻ influx up to 7.3 μ M/s (note: increased I⁻ influx is a quantitative measure of effective $\Delta F508$ -CFTR corrector activity). As illustrated in Table 3, pyrazolylthiazole **14h** is a corrector with good activity among the eight hits. Of the eight active compounds, **10b**, an ester, and **11d**, N γ -substituted pyrazole, are less effective in the assay used as pyrazolylthiazole correctors. Considering that ester-containing pyrazolylthiazoles **9** and **10** are inactive in the assay used except for **10b**, the carboxamide group at C5 of the pyrazole ring seems to be a determinant of activity. Another observation from Table 3, that shows the eight hits, is that the majority of active pyrazolylthiazoles are N β -substituted pyrazole isomers; there is an active N γ -substituted corrector (**11d**); and there is an active ester corrector (**10b**).

Table 3. Pyrazolylthiazole $\Delta F508$ -CFTR corrector activity.

	corrector structure	corrector number	EC_{50} (μM) ^a	V_{max} ($\mu M/s$) ^b
5		10b	8.5	2.6
		11d	3.4	2.5
		14a	0.93	0.5
		14b	3.0	1.4
		14e	0.75	1.0
10		14g	1.0	6.1
		14h	1.0	7.26
		14j	3.0	2.4

^a Concentration where the increased I^- influx is 50% of V_{max} .^b V_{max} is maximum increase in I^- influx due to compound effect.

LogP measurements

LogP represents a compound's partition coefficient log value determined from octanol versus water, where a smaller logP correlates with better water solubility. LogP is a well-established parameter for the ADME profiling as it has implications in solubility, absorption, distribution, metabolism, and excretion – which are important for orally administered drugs – and, according Lipinski's rule of 5, should generally be <5 for good bioavailability.

LogP can be related to the experimentally determined capacity factor k by measuring the retention time of the compound using reverse-phase HPLC. A standard calibration curve is constructed using compounds with known logP values and experimentally determined logk values (see diamond data points in Figure 2); this standard calibration curve is then used to correlate logk with logP for each pyrazolylthiazole corrector. These data for **11b** and **14a/b/e/g/h/j** are depicted in Figure 2. Most of the active pyrazolylthiazole correctors (squares in Figure 2) have logP values of less than 5. The most active pyrazolylthiazole, **14h**, has a logP value of 4.1 and **14j** has a logP of 3.5.

Experimental Section

Ye et al., J. Med. Chem. 2010, 53, 3773-3781 is hereby incorporated by reference in its entirety.

All purchased starting materials and reagents were used without further purification.

Product purification was performed either on an automated flash chromatography system (Combiflash by Teledyne: 35 min of elution with linear gradient from 100% hexane to 100% EtOAc solvent) with silica gel columns or on an HPLC system [Waters:15 mL/min flow rate, linear gradient elution with 0.1% TFA-containing H₂O/MeCN from 5-95% MeCN in 20 min. Xterra Prep MS C₁₈ OBD column (19 mm x 100 mm) and dual wavelength absorbance detector]. NMR spectra (¹H at 600 MHz; ¹³C at 151 MHz) were recorded in CDCl₃ solvent on a Varian 600. Chemical shifts are expressed in parts per million relative to internal TMS or solvent. Coupling constants are expressed in units of hertz (Hz). Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and bs (broad singlet). LC/MS (Waters Micromass ZQ) specifications are as follows: electrospray (+) ionization, mass ranging from 100 to 900 Da, 20-V cone voltage. LC: Xterra MS C₁₈ column (2.1 mm x 50mm x 3.5 μm), 0.2 mL/min water/acetonitrile (containing 0.1% TFA), 30 min linear gradient 0-100% acetonitrile. The LC/MS UV detector is a diode array with 200-400nm wave length range. Purity is based on the peak area percentage of the UV diode array signals. Compound purities by RP-HPLC were ≥95%.

Δ508-CFTR corrector activity assay.

FRT epithelial cells stably coexpressing human ΔF508-CFTR and the high-sensitivity halide-sensing fluorescent protein YFP-H148Q/I152L were used as described previously. Cells were grown at 37° C (95% air / 5% CO₂) for 24 h and then incubated for 16 - 20 h with 50 μL of medium containing the test compound. 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.

Measurements were carried out using aFLUOstar fluorescence plate reader (Optima; BMG

LABTECH GmbH) 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 s (baseline) and then for 12 s after rapid (<1 second) addition of 165 μL PBS in which 137 mM Cl^- was replaced by I^- . Initial I^- influx rate was computed exponential regression. All experiments contained negative control (DMSO vehicle) and positive control [*N*-(2-(5-chloro-2-methoxyphenylamino)-4'-methyl-4,5'-bithiazol-2'-yl)benzamide].

1-(2-Amino-4-methylthiazol-5-yl)ethanone HCl (6).

Thiazole **6** was prepared as described in Hantzsch A., Thiazoles from thiamides. Justus Liebigs Ann. Chem. 1889, 250, 257-73, which is hereby incorporated by reference in its entirety.

General procedure A: Preparation of 8 via CDI-mediated Amide Formation.

Carboxylic acid (1.75 equiv) was dissolved in DMF (3.3 mL/mmol of carboxylic acid) and carbonyldiimidazole (CDI; 1.75 equiv) was added slowly to manage CO_2 evolution. After all the CDI had been added, the solution was stirred for an additional 15 min at which point thiazole HCl salt **6** (1 equiv). The reaction mixture was warmed to 85°C and stirred for 20 h. After the reaction was complete, the reaction mixture was cooled to room temperature and poured into water (17 mL/mmol of carboxylic acid) to precipitate the product, which was then collected by filtration, washed with water (3x200mL), and dried under vacuum at 100°C for 18 h to deliver **7**.

N-(5-acetyl-4-methylthiazol-2-yl)pivalamide (7a).

Pivalic acid (5.0 g, 49 mmol) was reacted with the thiazole•HCl **6** (5.4 g, 28 mmol) by general procedure A and **7a** was obtained as an off-white solid (5.3 g, 79%). ^1H NMR (600 MHz, CDCl_3) δ 9.06 (s, 1H), 2.64 (s, 3H), 2.51 (s, 3H), 1.34 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 190.66, 176.46, 158.72, 155.21, 125.30, 39.29, 30.44, 27.16 18.08; LC/MS: cal. $[\text{M}+\text{H}^+]= 241.10$, found 241.12.

(Z)-Ethyl 2-hydroxy-4-(4-methyl-2-pivalamidothiazol-5-yl)-4-oxobut-2-enoate (8a).

To a solution of LHMDS (1M; 50.3 mL, 50.3 mmol) in THF (50 mL) cooled to -78°C was slowly added **7a** (5.5 g, 22.9 mmol) in dry THF (100 mL) via a syringe and the mixture was stirred for 30 min. Diethyl oxalate (3.7 mL, 27.4 mmol) was added quickly in one portion and the resulting mixture was stirred at -78°C for 2 h before being allowed to warm to room temperature for another 2 h. When the reaction was completed, water (50 mL) and 1N aq. HCl (50 mL) were sequentially added to the reaction mixture and the product was extracted with EtOAc (3 x 100 mL). The combined organic extract was washed with brine twice and dried over MgSO_4 . Filtration and solvent removal under vacuum delivered the crude product which was purified on a silica gel column with automated flash chromatography (solvent system: gradient hexane/ethyl acetate) to give **8** as a yellow solid (7.22 g, 92%). ^1H NMR (600 MHz, CDCl_3) δ 9.08 (s, 1H), 6.70 (s, 1H), 4.39 (q, $J = 6$, 2H), 2.71 (s, 3H), 1.40 (t, $J = 6$, 3H), 1.35 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 186.24, 176.56, 165.29, 162.01, 160.00, 157.46, 123.06, 101.63, 62.57, 39.33, 27.08, 18.60, 14.12; LC/MS: ESI-MS, cal. $[\text{M}+\text{H}^+]= 341.12$, found 341.04.

General Procedure B: Preparation of Ny-Substituted Pyrazoles, 9 via Cyclocondensation with 8.

A mixture of substituted hydrazine (or hydrazine hydrochloride; 1.05 equivalent) and **8** in absolute ethanol (3.3 mL/mmol of **8**) was stirred at room temperature for 18 h. After the starting material was consumed as indicated by TLC, the solvent was removed by rotoevaporation and the concentrate was extracted with ethyl acetate (3.3 mL/mmol of **8**) and washed with water. The ethyl acetate layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to deliver the crude Ny-substituted pyrazole product, which was then purified by silica gel column chromatography (Combiflash).

Ethyl 1-(4-bromophenyl)-5-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-3-carboxylate (9b).

Para-bromophenylhydrazine (0.14 g, 0.64 mmol) was reacted with **8a** (0.217 g, 0.63 mmol) by general procedure B. After purification, an off-white product was obtained (0.27 g, 87%). ¹H NMR (600 MHz, CDCl₃) δ 8.80 (s, 1H), 7.50 (d, J=6, 2H), 7.27 (d, J= 6, 2H), 7.02 (s, 1H), 4.46 (q, J = 6, 2H), 2.01 (s, 3H), 1.42 (t, J = 6, 3H), 1.33 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 176.30, 162.19, 158.25, 147.48, 144.95, 138.29, 135.34, 132.52, 126.59, 122.64, 113.14, 112.69, 61.58, 39.34, 27.37, 15.90, 14.61; LC/MS: cal. [M+H⁺] and [M+2+H⁺] =491.08 and 493.08, found 490.97 and 492.86.

General Procedure C: Preparation of 11a-I via Aminolysis of Ny-substituted Pyrazole Ester 9.

Pyrazolythiazole **9** (1 equiv) was dissolved in dry DCM (15 mL/mmol of **9**) and cooled to 0 °C for 10 min. AlMe₃ (2.0 equiv) in hexane (1.0 M) was added and the resulting mixture was stirred at room temperature for 12 h. When the reaction was complete, water was added followed by 0.1N aq. HCl to neutralized the mixture which was then extracted with EtOAc. The EtOAc layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give the crude amide **10**, which was then purified by silica gel column chromatography with Combiflash.

1-(4-Bromophenyl)-N-(4-methoxyphenyl)-5-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-3-carboxamide (11d).

Pyrazolythiazole **9b** (100 mg, 0.20 mmol) was reacted with anisidine (28 mg, 0.22 mmol) by general procedure C and an off-white solid product was obtained (85 mg, 73% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.84 (s, 1H), 8.64 (s, 1H), 7.61 (d, J = 9.0, 2H), 7.54 (d, J = 8.8, 2H), 7.28 (d, J = 8.8, 2H), 7.11 (s, 1H), 6.91 (d, J = 9.0, 2H), 3.82 (s, 3H), 2.05 (d, J = 5.6, 3H), 1.32 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 176.29, 159.15, 158.35, 156.63, 147.90, 147.53, 138.28, 136.09, 132.67, 131.03, 126.38, 122.64, 121.74, 114.46, 112.81, 111.50, 77.43, 77.22, 77.01, 55.71, 39.34, 27.36, 15.93; LC/MS: cal. [M+H⁺] = 568.10, found 568.10.

Synthesis of ethyl 3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxylate (13).

2,4-Dioxo-4-(thiazol-5-yl)butanoate **8** (2.0 g, 5.9 mmol) and hydrazine hydrate (0.40 mL, 6.4 mmol) were dissolved in absolute ethanol (20 mL) and the mixture was stirred at room temperature for 20 h. When the reaction was over, the reaction mixture was concentrated to half volume by rotoevaporation and the resulting solid was collected by filtration. This solid residue

was washed with cold ethanol (x3), dried under vacuum, and used in the next step without further purification. A small portion of product remained in the ethanol filtrate, which was concentrated under reduced pressure and the resulting solid collected. The two portions of product were combined and weighed (1.66 g, 83%). ¹H NMR (600 MHz, DMSO+CDCl₃) δ 13.79 and 11.99 (s and s, 1H), 11.77 (s, 1H), 6.97 and 6.85 (s and s, 1H), 4.34 and 4.29 (q and q, *J* = 7.1, 2H), 2.46 and 2.37 (s and s, 3H), 1.31 (triplet overlapping, *J* = 7.1, 3H), 1.23 (s, 9H); ¹³C NMR (151 MHz, DMSO+CDCl₃) δ 176.83(minor) and 176.53, 161.81(minor) and 158.79, 156.90(minor) and 156.00, 144.96 (minor) and 144.87, 143.11(minor) and 143.07, 135.30(minor) and 134.57, 116.84, 106.91 and 106.22(minor), 60.93, and 60.15(minor), 40.05(minor) and 38.74, 26.59, 16.38 and 15.92(minor), 14.24 (minor) and 14.15; LC/MS: cal. [M+H⁺] = 337.13, found 337.05.

General procedure D: Preparation of 10 via Alkylation of the Pyrazolylthiazole 13.

Pyrazolylthiazole **13** (1 equiv), K₂CO₃ (0.75~0.85 equiv) and an alkylating agent were dissolved in acetone (3.3 mL/mmol of **13**). The mixture was then refluxed (60 °C) for 24 h. When the reaction was complete, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. Water was added to the resulting mixture, which was then extracted with EtOAc (3x). The organic extracts were combined, dried over MgSO₄, filtered, and concentrated by rotoevaporation. The resulting crude product was then purified by silica gel chromatography (Combiflash; Hexane/EtOAc gradient elution).

Ethyl 1-allyl-3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxylate (10a, R=H, R²=Allyl).

Allyl bromide (0.172 mL, 1.98 mmol) was reacted with pyrazolylthiazole **13** (0.5 g, 1.49 mmol) by general procedure D. Product **10a** was obtained after purification (0.33 g, 59% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.71 (s, 1H), 6.95 (s, 1H), 6.04 (m, 1H), 5.19 (m, 3H), 5.14 (dd, *J* = 1.1, 17.0, 1H), 4.37 (q, *J* = 7.1 2H), 2.52 (s, 3H), 1.39 (t, *J* = 7.1, 3H), 1.33 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 175.75, 159.36, 155.65, 143.29, 143.19, 133.10, 133.09, 118.20, 117.74, 109.61, 61.21, 54.15, 39.11, 27.21, 16.35, 14.23; LC/MS: cal. [M+H⁺]=377.17, found 377.13.

Ethyl 1-benzyl-3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxylate (10a, R=H, R²=Bn).

Benzyl chloride (0.12 mL, 0.96 mmol) was reacted with pyrazolylthiazole **13** (0.27 g, 0.80 mmol) by general procedure D. Product **10b** was obtained after purification (0.23 g, 66%). ¹H NMR (600 MHz, CDCl₃) δ 8.74 (s, 1H), 7.31 (m, 5H), 6.96 (s, 1H), 5.76 (s, 2H), 4.33 (q, *J* = 7.1, 2H), 2.51 (t, *J* = 7.1 3H), 1.33 (s, 9H); ¹³C NMR (151 MHz, CDCl₃) δ 175.98, 159.62, 155.77, 143.82, 143.61, 137.08, 133.27, 128.89, 128.70, 128.46, 128.06, 128.05, 127.96, 118.51, 109.99, 61.40, 60.59, 55.22, 39.31, 27.43, 21.26, 16.73, 14.41, 0.21; LC/MS: cal. [M+H⁺] = 427.18, found 427.08.

Ethyl 1-(5-chloro-2-methoxybenzyl)-3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxylate (10a, R=H, R²=CH₂(2-MeO-5-Clphenyl)).

2-(Bromomethyl)-4-chloro-1-methoxybenzene (0.37 g, 1.57 mmol) was reacted with **13** (0.5 g, 1.49 mmol) by general procedure D. Product **10c** was obtained after purification (0.37 g, 51%). ¹H NMR (600 MHz, CDCl₃) δ 8.70 (s, 1H), 7.17 (dd, *J* = 2.6, 8.7, 1H), 7.02 (s, 1H), 6.79 (d,

$J = 8.7$, 1H), 6.59 (d, $J = 2.6$, 1H), 5.76 (s, 2H), 4.32 (q, $J = 7.1$, 2H), 3.86 (s, 3H), 2.53 (s, 3H), 1.34 (t, $J = 7.1$, 3H), 1.32 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 175.90, 159.47, 155.74, 155.25, 144.02, 143.96, 134.06, 128.38, 128.00, 127.27, 125.79, 118.39, 111.59, 110.01, 61.48, 56.01, 50.28, 39.31, 27.45, 16.75, 14.36; LC/MS: purity and Calculated $[\text{M}+\text{H}^+]$ and $[\text{M}+2+\text{H}^+] = 491.15$ and 493.14, found 491.10 and 493.08.

General Procedure E: Preparation of 14a-i via Aminolysis of 10 with non-alcoholic amines.

To a solution of ethyl ester **10** (1 equiv) in dry DCM (10 mL/mmol of **10**), which was chilled at 0 °C in a sealed microwave reaction vessel, was added 2.0 M trimethyl aluminum (1.2 equiv) in hexanes. The resulting mixture was stirred at 0 °C for 10 min and then the amine reactant (1.2 equiv) in dry DCM (1 mL/mmol of **10**) was injected into the mixture. The reaction tube was then mounted to the microwave reactor and irradiated with microwave at 100 °C for 40 min. After cooling, water and 0.1N aq. HCl were added to the reaction mixture sequentially to neutralize the solution. DCM extraction (3x), drying over MgSO_4 , filtration, and rotoevaporation gave a residue which purified by silica gel chromatography (CombiFlash).

1-Allyl-N-(4-methoxyphenyl)-3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxamide (14a).

Ethyl ester **10a** (100 mg, 0.27 mmol) was reacted with anisidine (40 mg, 33 mmol) by general procedure E and gave **14a** (45 mg, 67%). ^1H NMR (600 MHz, CDCl_3) δ 8.80 (s, 1H), 7.83 (s, 1H), 7.50 (d, $J = 8.7$, 2H), 6.91 (d, $J = 9.0$, 2H), 6.73 (s, 1H), 6.08 (ddd, $J = 5.8$, 10.9, 16.1, 1H), 5.23-5.10 (m, 4H), 3.81 (s, 3H), 2.51 (s, 3H), 1.32 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 176.02, 157.72, 157.19, 155.66, 143.92, 143.35, 136.48, 133.56, 130.21, 124.50, 122.59, 118.31, 118.09, 114.53, 105.41, 77.46, 77.25, 77.04, 55.74, 54.19, 39.33, 27.44, 16.71; LC/MS: cal. $[\text{M}+\text{H}^+] = 454.19$, found 454.16.

1-allyl-N-benzyl-3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxamide (14b).

Ethyl ester **10a** (100 mg, 0.27 mmol) was reacted with benzylamine (36 μL , 33 mmol) by general procedure E and gave **14b** (85 mg, 91% yield). ^1H NMR (600 MHz, CDCl_3) δ 8.93 (s, 1H), 7.30 (m, 4H), 6.87 (t, $J = 5.6$, 1H), 6.64 (s, 1H), 6.02 (ddt, $J = 5.7$, 11.3, 17.0, 1H), 5.12 (m, 4H), 4.59 (d, $J = 5.8$, 2H), 2.46 (s, 3H), 1.29 (t, $J = 2.9$, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 176.09, 159.69, 155.66, 143.74, 143.20, 137.89, 136.22, 133.66, 128.98, 127.98, 127.89, 118.34, 117.82, 105.52, 54.04, 43.75, 39.28, 27.38, 16.63; LC/MS: cal. $[\text{M}+\text{H}^+] = 438.20$, found 438.13.

Synthesis of N,1-dibenzyl-3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxamide (14e).

Ethyl ester **10b** (100 mg, 0.23 mmol), benzylamine (0.51 mL, 4.7 mmol), and sodium cyanide (5.3 mg, 0.1 mmol) were mixed in MeOH (8 mL) and refluxed for 18 h. Upon cooling, the methanol was removed by rotoevaporation and the residue was taken up in EtOAc (50 mL), washed with water (50 mL; 3x) and 0.1N aq. HCl (50 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The concentrate was purified by silica gel chromatography (CombiFlash), giving pure product of **14e** (31.9 mg, 34.41%). ^1H NMR (600 MHz, CDCl_3) δ 8.71 (s, 1H), 7.37-7.23 (m, 10H), 6.58 (s, 1H), 6.24 (t, $J = 5.6$, 1H), 5.79 (s, 2H), 4.58 (d, $J = 5.8$, 2H), 2.50 (s, 3H), 1.32 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 175.94, 159.67, 155.63, 143.79, 143.36,

137.70, 137.31, 136.12, 129.07, 128.71, 128.33, 128.00, 127.99, 127.94, 118.46, 105.32, 54.98, 43.80, 39.31, 27.44, 16.74; LC/MS: cal. $[M+H]^+$ = 488.21, found 488.20.

1-(5-chloro-2-methoxybenzyl)-N-(4-methoxyphenyl)-3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxamide (14g).

5 Ethyl ester **10c** (30 mg, 0.061 mmol) was reacted with anisidine (12 mg, 0.098 mmol) by general procedure E to give **14g** (26 mg, 75%). ^1H NMR (600 MHz, CDCl_3) δ 8.83 (s, 1H), 7.76 (s, 1H), 7.48 (d, J = 8.5, 2H), 7.16 (dd, J = 2.6, 8.7, 1H), 6.89 (d, J = 9.0, 2H), 6.79 (d, J = 2.5, 1H), 6.75 (d, J = 8.7, 2H), 5.78 (s, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 2.51 (s, 3H), 1.32 (s, 9H). ^{13}C NMR (151 MHz, CDCl_3) δ 176.00, 157.68, 157.18, 155.73, 155.47, 144.06, 143.84, 130.26, 10
128.52, 128.15, 127.91, 125.76, 122.42, 118.27, 114.53, 111.67, 105.38, 55.97, 55.71, 49.90, 39.32, 27.43, 16.71; LC/MS: cal. $[M+H]^+$ = 568.18, found 568.17.

N-(5-(1-(5-chloro-2-methoxybenzyl)-5-(morpholine-4-carbonyl)-1H-pyrazol-3-yl)-4-methylthiazol-2-yl)pivalamide (14h).

Ethyl ester **10c** (30 mg, 0.061 mmol) was reacted with morpholine (7 μL , 0.091 mmol) by general procedure E to give **14h** (28 mg, 87%). ^1H NMR (600 MHz, CDCl_3) δ 8.74 (s, 1H), 7.19 (dd, J = 2.6, 8.7, 1H), 6.86 (d, J = 2.6, 1H), 6.77 (d, J = 8.7, 1H), 6.38 (d, J = 9.6, 1H), 5.50 (s, 2H), 3.79 (d, J = 12.0, 3H), 3.66 (d, J = 24.8, 4H), 3.45 (s, 4H), 2.50 (s, 3H), 1.31 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 175.95, 160.92, 155.76, 155.55, 143.95, 143.31, 136.09, 128.96, 128.83, 127.45, 125.67, 118.40, 111.95, 105.67, 66.82, 56.16, 49.29, 39.31, 27.43, 16.72; 15
20 LC/MS: cal. $[M+H]^+$ = 532.18, found 532.14.

General procedure F for Preparation of amide 11m-p and 14j: Aminolysis of 9 or 10 with alcoholic amine.

Pyrazolylthiazole **9** or **13** was dissolved in dry ethanol (4 mL) in a 10 mL microwave reaction tube. Ethanolamine (20 equivalent) was added to the solution, the tube was sealed, placed in a microwave reactor, and the reaction mixture was heated at 180 $^\circ\text{C}$ for 30 minutes. After the tube had cooled, ethanol was removed under reduced pressure, aq. NH_4Cl was added, and the mixture was extracted with chloroform (x3). The chloroform extracts were combined and the solvent removed under reduced pressure to give a crude product, which was purified with HPLC. 25

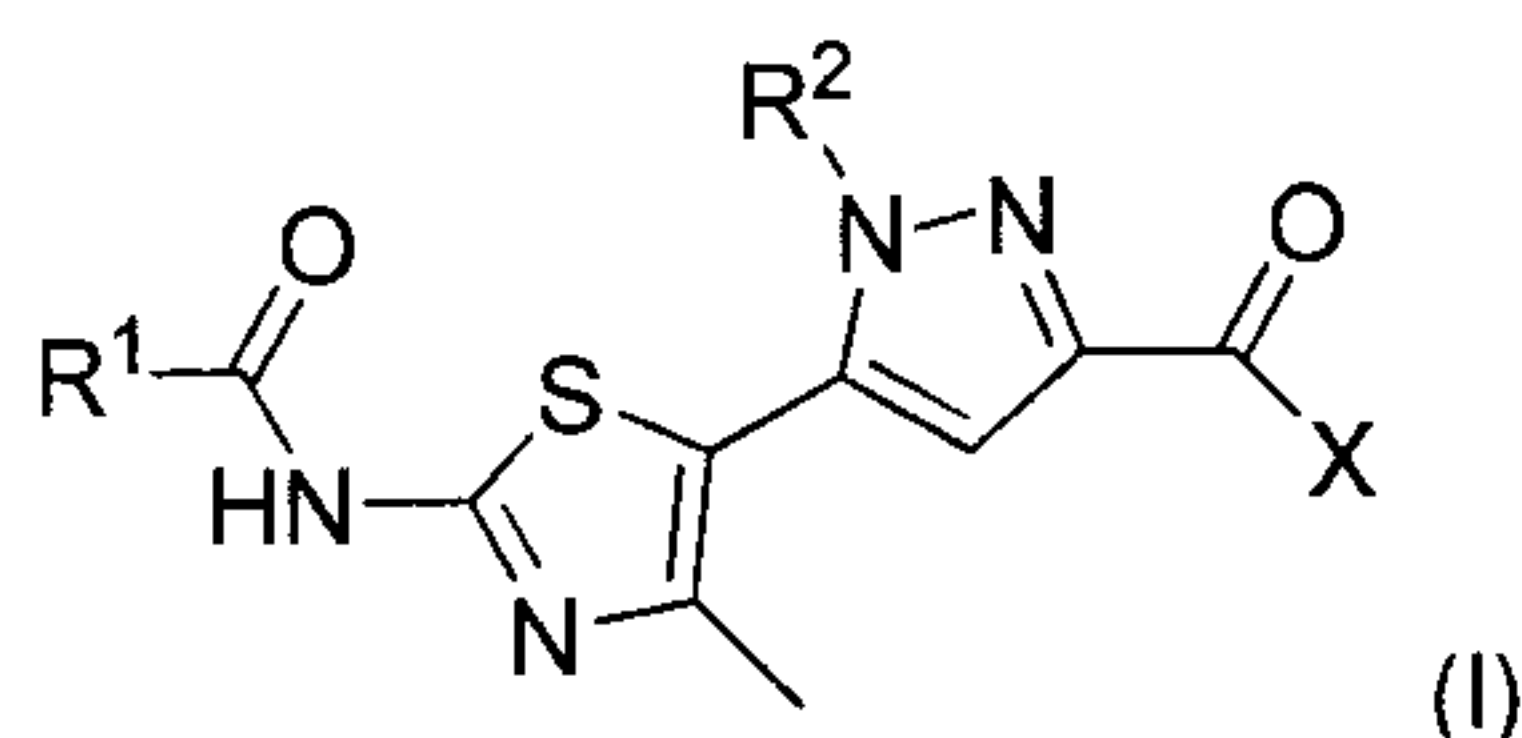
1-(5-chloro-2-methoxybenzyl)-N-(2-hydroxyethyl)-3-(4-methyl-2-pivalamidothiazol-5-yl)-1H-pyrazole-5-carboxamide (14j).

Following general procedure F, **14j** was obtained in 33% yield. ^1H NMR (600 MHz, CDCl_3) δ 7.19 (dd, J = 2.6, 8.7, 1H), 6.80 (d, J = 8.8, 1H), 6.73 (d, J = 2.6, 1H), 6.72 (s, 1H), 6.52 (s, 1H), 5.76 (s, 2H), 3.85 (s, 3H), 3.82 (t, J = 5.4, 2H), 3.59 (dd, J = 5.5, 10.3, 2H), 2.60 (s, 3H), 1.37 (s, 9H); ^{13}C NMR (151 MHz, CDCl_3) δ 178.26, 160.36, 159.63, 155.31, 140.97, 137.49, 134.35, 128.57, 127.81, 127.17, 125.52, 117.96, 111.65, 104.79, 77.35, 77.22, 77.01, 76.80, 61.75, 55.85, 49.93, 42.12, 39.94, 26.56, 13.43; LC/MS: cal. $[M+H]^+$ = 506.16, found 506.15. 35

CLAIMS

That which is claimed is:

1. A compound of formula (I):



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

R^1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl;

- 10 R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl; and

- X is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl,
15 heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

2. The compound of Claim 1, wherein R^1 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl.

- 20 3. The compound of Claim 1, wherein R^1 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

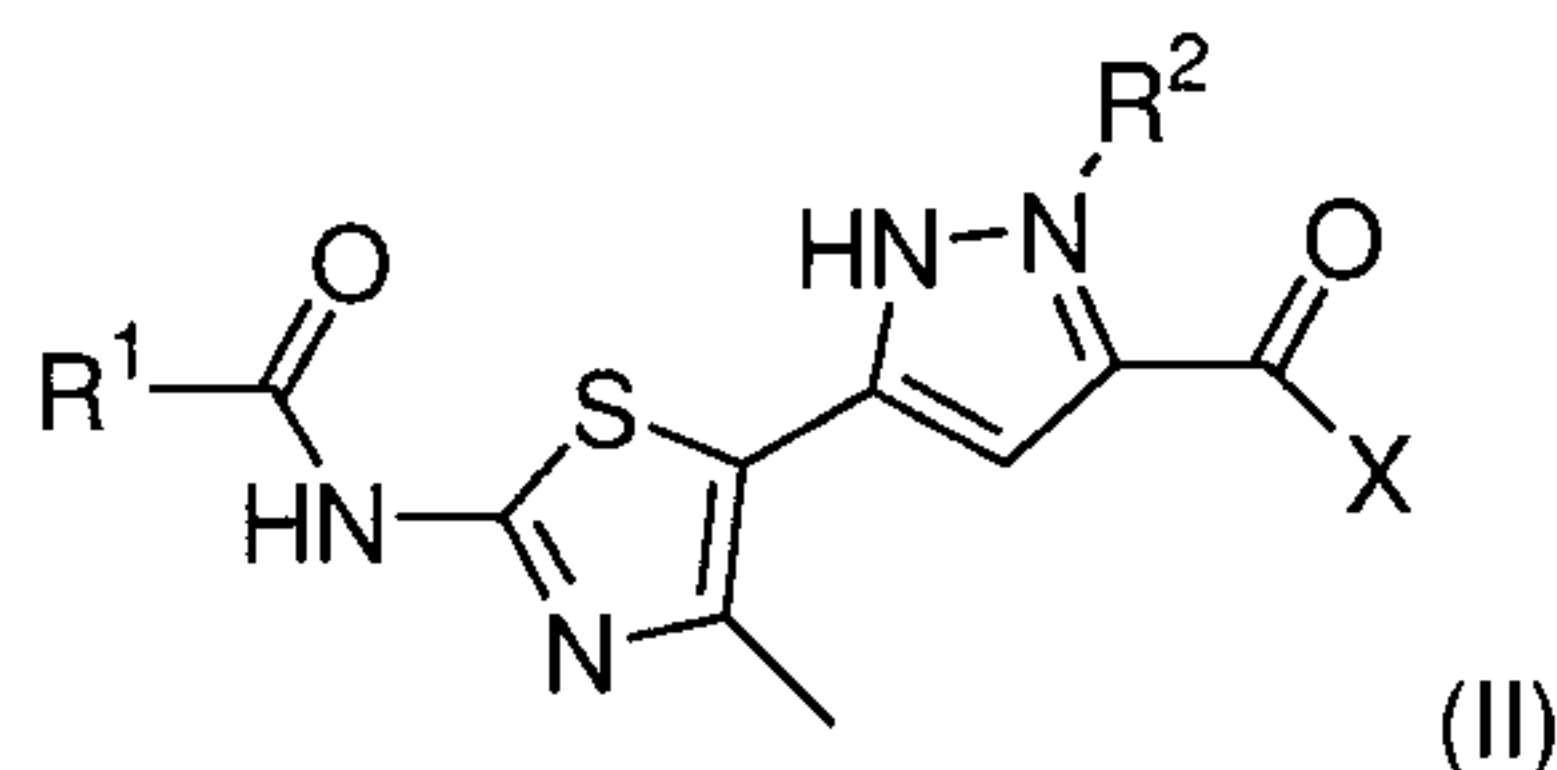
4. The compound of Claim 1, wherein R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl.

5. The compound of Claim 1, wherein R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, and substituted aryl.

- 30 6. The compound of Claim 1, wherein X is selected from aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, amino, substituted amino, and hydroxyl.

- 35 7. The compound of Claim 1, wherein X is selected from heterocyclyl, substituted heterocyclyl, amino, substituted amino, and hydroxyl.

8. A compound of formula (II):



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

5 wherein:

R¹ is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl;

10 R² is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl; and

15 X is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

9. The compound of Claim 8, wherein R¹ is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl.

20 10. The compound of Claim 8, wherein R¹ is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

25 11. The compound of Claim 8, wherein R² is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, and substituted heterocyclyl.

12. The compound of Claim 8, wherein R² is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, and substituted aryl.

30 13. The compound of Claim 8, wherein X is selected from aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, substituted heterocyclyl, amino, substituted amino, and hydroxyl.

35 14. The compound of Claim 1, wherein X is selected from heterocyclyl, substituted heterocyclyl, alkoxy, substituted alkoxy, amino, substituted amino, and hydroxyl.

15. A compound selected from Compounds 10b, 11d, 14a, 14b, 14e, 14g, 14h, and 14j.
16. A compound selected from Compounds 14a, 14e, 14g, and 14h.
17. A pharmaceutical composition comprising an effective amount of a compound of any of Claims 1-16.
18. 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 comprising a compound of any of Claims 1-16.
19. The method of Claim 18, wherein said condition is cystic fibrosis.
20. The method of Claim 18, 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.
21. The method of Claim 18, wherein said subject is human.
22. The method of Claim 18, wherein the mutant-CFTR is $\Delta F508$ -CFTR.
23. A method of increasing ion permeability of a cell producing a mutant-CFTR protein, said method comprising:
 - 25 contacting said cell with an effective amount of a pharmaceutical composition comprising a compound of any of Claims 1-16, said contacting being effective to increase CFTR-mediated ion permeability of said cell.
24. The method of Claim 23, wherein said cell contains a recombinant expression cassette that encodes mutant-CFTR protein.
25. The method of Claim 23, wherein said cell contains a genome that encodes said mutant-CFTR protein.
26. The method of Claim 23, wherein the mutant-CFTR is $\Delta F508$ -CFTR.

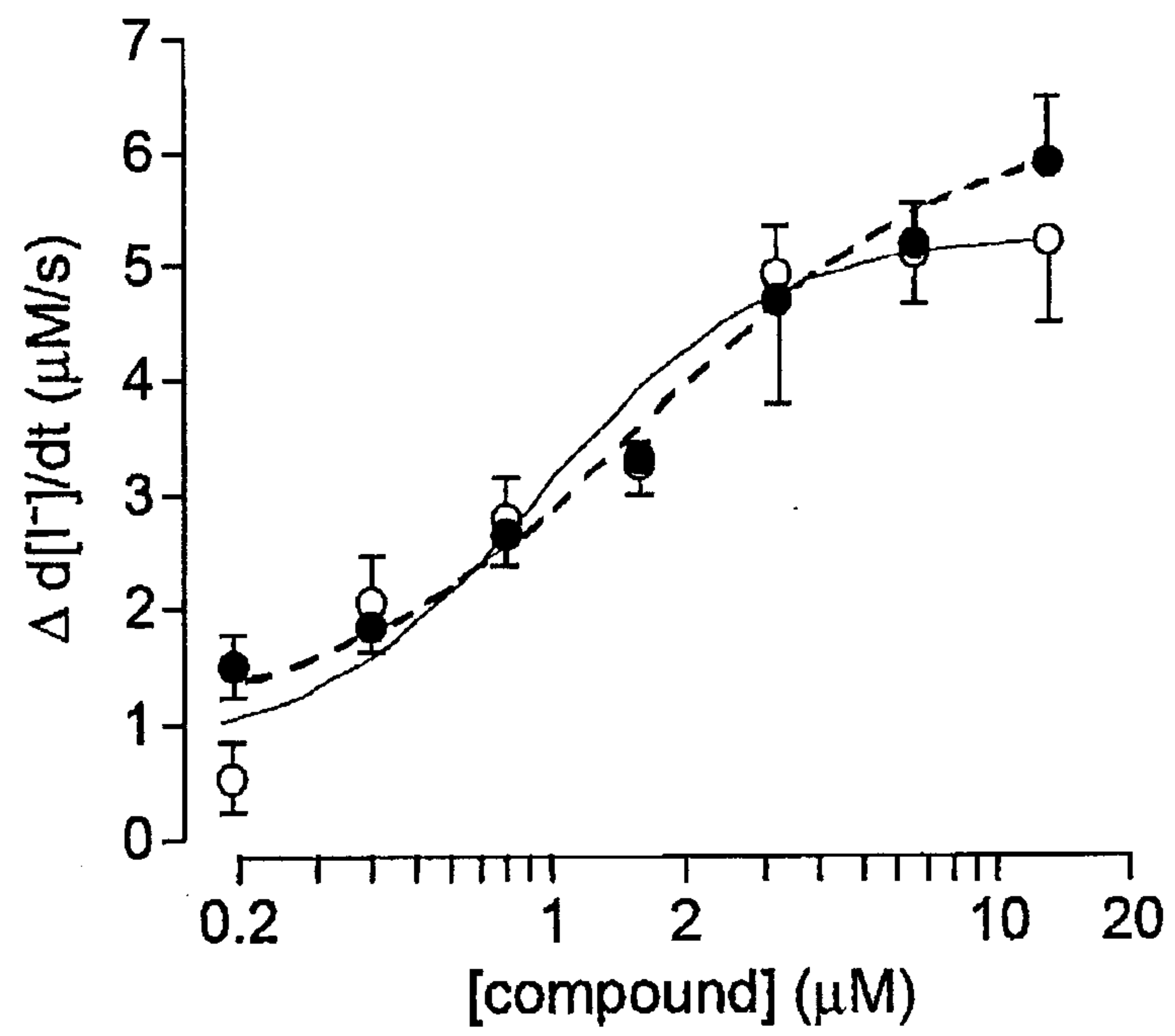


Figure 1

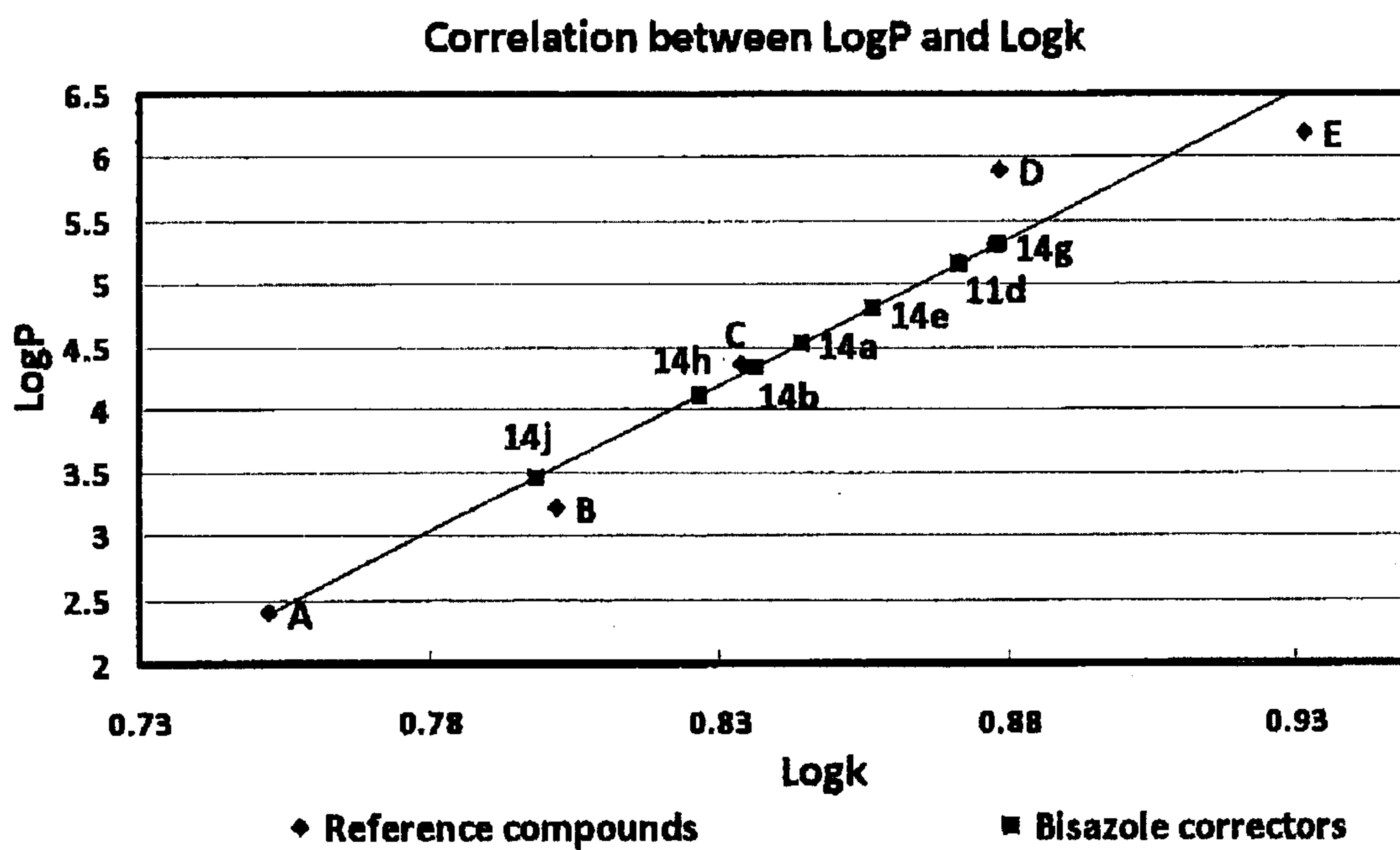


Figure 2

Correlation between LogP and Logk

