(10) International Publication Number
WO 2014/058974 A1

(19) World Intellectual Property Organization

(21) International Application Number:
PCT/US20 13/064052

(22) International Filing Date:
9 October 2013 (09.10.2013)

(24) Filing Language:
English

(25) Filing Language:
English

(26) Publication Language:

(27) Priority Data:
61/711,982 10 October 2012 (10.10.2012) US

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(51) International Patent Classification:
A61K 31/195 (2006.01) A61K 9/20 (2006.01)
A61K 31/19 (2006.01) A61K 9/16 (2006.01)
A61K 31/70 (2006.01) A61P 29/00 (2006.01)

(54) Title: METHODS OF MANAGING INFLAMMATION USING GLYCOLYSIS PATHWAY INHIBITORS

(57) Abstract: This disclosure relates to methods of managing inflammation using glycolysis pathway inhibitors. In certain embodiments, the disclosure relates to treating or preventing diseases or conditions associated with epithelial airway inflammation comprising administering an effective amount of a glycolysis pathway inhibitor to a subject in need thereof.

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Declarations under Rule 4.17:
— as to applicant’s entitlement to apply for and be granted a patent (Rule 4.17(H))
— of inventorship (Rule 4.17(iv))
Published:
— with international search report (Art. 21(3))
METHODS OF MANAGING INFLAMMATION USING GLYCOLYSIS PATHWAY INHIBITORS

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

This invention was made with government support under contract number 7R01HL109362-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

This Application claims priority to U.S. Provisional Application Number 61/711,982 filed October 10, 2012, hereby incorporated by reference in its entirety.

BACKGROUND

Cystic fibrosis (CF) is a genetic lung disease associated with mutations in the cystic fibrosis (CF) transmembrane regulator channel (CFTR). A dysfunctional CFTR protein on the epithelial cell surface of lung tissue decreases the transport of chloride through the channel. Excessive airway inflammation, mucus build-up, and decreased mucociliary clearance are typically reported in patients with CF. Inflammation diminishes lung function and controlling inflammation slows lung deterioration. Airway epithelial cells play a role in the inflammatory signaling and have been reported to exhibit a number of dysfunctional signaling cascades that modulate inflammation. Ziady et al., report that interaction with CREB binding protein modulates the activities of Nrf2 and NF-κB in cystic fibrosis airway epithelial cells. See Am J Physiol Lung Cell Mol Physiol, 2012, 302: L1221-L1231.

There is no cure for cystic fibrosis and limited treatment options. A patient with cystic fibrosis typically takes antibiotics to prophylatically suppress infections; however, lung transplantation often becomes necessary over time. Ivacaftor is an FDA approved medication for CF patients with a G551D mutation in CFTR. See Jih & Hwang, Proc Natl Acad Sci U S A, 2013, 110(11):4404-9. Only small percentage of patients have CF due to a G551D mutation. Thus, there is a need to identify additional therapies.

Lumacaftor is a medication being tested for CF patients that have a mutation resulting in a missing phenylalanine in position 508 of CFTR. Van Goor et al., Proc Natl Acad Sci U S A, 2011, 108(46): 18843-8.
Glycolysis inhibition has been reported for anticancer treatment. See Pelicano et al., Oncogene, 2006, 25, 4633-4646.


Tannahill et al., report that succinate is an inflammatory signal that induces IL-1b through HIF-la. See Nature, 2013, 496:238-243.

References cited herein are not an admission of prior art.

SUMMARY

This disclosure relates to methods of managing inflammation using glycolysis pathway inhibitors. In certain embodiments, the disclosure relates to inhibition of glycolysis as an ant-inflammatory therapy. In certain embodiments, the disclosure relates to treating or preventing inflammation comprising administering an effective amount of a glycolysis pathway inhibitor to a subject in need thereof. In certain embodiments, the disclosure relates to treating or preventing diseases or conditions associated with epithelial airway inflammation comprising administering an effective amount of a glycolysis pathway inhibitor to a subject in need thereof.

In certain embodiments, the disclosure relates to methods of treating or preventing cystic fibrosis comprising administering a pharmaceutical composition comprising a glycolic inhibitor to a subject in need thereof.

In certain embodiments, the subject is diagnosed with, exhibiting symptoms of, or at risk of fibrosis. In certain embodiments, the glycolic inhibitor is 3-bromopyruvate, 2-deoxyglucose, 6-aminonicotinamide (6-aminonic), oxythiamine, lonidamine, genistein, 5-thiogluucose, mannoheptulose, a-chlorohydrin, ornidazole, oxalate or salts thereof. In certain embodiments, the glycolysis pathway inhibitor is arsenic, oxamate, sodium fluoride (NaF), Nal, glufosfamide, imatinib, or alternative salts thereof.

In certain embodiments, the pharmaceutical composition is administered in combination with a second fibrosis agent. In certain embodiments, the fibrosis agent is tobramycin (TOBI), Pulmozyme, Cayston Creon or combinations thereof.

In certain embodiments, the disclosure relates to the use of a glycolysis in the production of a medicament for the treatment or prevention of fibrosis.
In certain embodiments, the pharmaceutical composition is a powder, pill, tablet, capsule, or aqueous saline buffer.

In certain embodiments, the disclosure relates to a pharmaceutical composition comprising a glycolic inhibitor and a second fibrosis agent.

In certain embodiments, the disclosure relates to methods of treating or preventing inflammation by administering glycolysis inhibitors disclosed herein to a subject diagnosed with, at risk of, or exhibiting symptoms of diseases or conditions associated with epithelial airway inflammation such as cystic fibrosis, asthma, chronic obstructive pulmonary disease (COPD), emphysema, bronchitis, atherosclerosis, acute respiratory distress syndrome (ARDS), or chronic coughing.

In certain embodiments, the disclosure relates to methods of treating or preventing a disease or conditions associated with liver inflammation comprising administering a glycolysis pathway inhibitor to a subject in need thereof. In certain embodiments, the subject is diagnosed with hepatitis and/or cirrhosis.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 illustrates the glycolysis pathway and differences in metabolomic (primary human tracheal epithelial (HTE) cells only) and proteomic analysis of glycolysis in both primary HTE (n=6 CF and nonCF) and primary neutrophils (n=6 CF and nonCF).

Proteomic analyses indicated significant increases in the expression of glycolysis enzymes in both neutrophils and epithelia. Increased expression of Hexokinase was only in CF neutrophils. Decreased expression of pyruvate dehydrogenase was only in CF epithelia. Metabolomic analysis indicated increases connoted by arrows in circles.

Figure 2 shows data on normalized levels of steady state intracellular glucose in the 9HTEo cell pair. Levels of glucose were measured in pCEP (nonCF model) and pCEPR (CF model) cells. Cells were trypsinized and washed 5 times in cold saline then lysed and assayed by glucose oxidase assay. *significantly different (p<0.05) from nonCF control.

Figure 3 shows data on IL6 levels in media following inflammatory stimulation of 9HTEo cells in culture. Cells were incubated with or without inhibitors of glycolysis for 30 minutes and then treated with (stim) or without 10 ng/ml TNFa and IL-1β for 24 hours. At 24 hours media was collected and assayed by ELISA for IL6 levels. * significantly different (p<0.05) from stimulated RPMI control.
Figure 4 shows data on IL8 levels in media following inflammatory stimulation of 9HTEo cells in culture. Cells were incubated with or without inhibitors of glycolysis for 30 minutes and then treated with (stim) or without 10 ng/ml TNFa and IL-1β for 24 hours. At 24 hours media was collected and assayed by ELISA for IL8 levels. * significantly different (p<0.05) from stimulated RPMI control.

Figure 5 shows data on IL6 levels in media following inflammatory stimulation of HuH7 cells in culture. Cells were incubated with or without inhibitors of glycolysis for 30 minutes and then treated with (stim) or without 10 ng/ml TNFa and IL-1β for 24 hours. At 24 hours media was collected and assayed by ELISA for IL6 levels. * significantly different (p<0.05) from stimulated RPMI control.

Figure 6 shows data on IL8 levels in media following inflammatory stimulation of HuH7 cells in culture. Cells were incubated with or without inhibitors of glycolysis for 30 minutes and then treated with (stim) or without 10 ng/ml TNFa and IL-1β for 24 hours. At 24 hours media was collected and assayed by ELISA for IL8 levels. * significantly different (p<0.05) from stimulated RPMI control.

**DETAILED DISUSSION**

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and 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 disclosure will be limited only by the appended claims.

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

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of immunology, medicine, organic chemistry, biochemistry, molecular biology, pharmacology, and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings unless a contrary intention is apparent.

Prior to describing the various embodiments, the following definitions are provided and should be used unless otherwise indicated.

Reference to any compound, medication, or similar therapeutic includes the parent compound and any alternative salt forms.

As used herein, "subject" refers to any animal, typically a human patient, livestock, or domestic pet.

As used herein, the terms "prevent" and "preventing" include the prevention of the recurrence, spread or onset. It is not intended that the present disclosure be limited to complete prevention. In some embodiments, the onset is delayed, or the severity of the disease is reduced.

As used herein, the terms "treat" and "treating" are not limited to the case where the subject (e.g., patient) is cured and the disease is eradicated. Rather, embodiments, of the present disclosure also contemplate treatment that merely reduces symptoms, and/or delays disease progression.
Metabolic shift towards glycolysis in CF cells contributes to inflammation

Inflammation is a hallmark of CF. Early in life, patients exhibit exaggerated inflammation in the lungs in response to infection. Over time, these responses become persistent and contribute to the lung destruction, pulmonary function deterioration, respiratory failure and death. It is unclear whether dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) in itself results in aberrant cell signaling that gives rise to abnormal inflammatory responses, or whether some stimulus is required. However, most investigators observe increases in pro-inflammatory pathways and decreases in pathways that are anti-inflammatory, even in the absence of bacteria. Controlling inflammation provides significant benefit to patients.

Shifts CF cells to an increase rate of glycolytic metabolism have been discovered in primary CF epithelia and neutrophils from CF patients’ blood. Through genome wide transcription profiling of human blood neutrophils, compared to controls, CF neutrophils differentially express a host of transcripts involved in glucose metabolism. Independent proteomic and metabolomic studies in both primary airway epithelia and blood neutrophils demonstrate that loss of CFTR function alone promotes a switch to a glycolytic phenotype. These data suggest that a metabolic switch towards glycolysis is a primary defect of CF airway epithelia and neutrophils.

Glucose metabolism plays a key role in neutrophil priming and activation, and epithelial cells inflammatory signaling, and redox signaling, which are relevant to CF pathology. Glycolytic metabolism has been linked to dysregulated inflammatory responses in a number of diseases including cancer, and Alzheimer’s disease. Experimental data disclosed herein shows an increase in the gene and protein expression of the enzymes of indicating that CFTR dysfunction increases glycolytic metabolism, which in turn accentuates pro-inflammatory signaling in neutrophils and epithelial cells.

Inhibition of glycolysis reduces IL6 and IL8 following stimulation with TNFa and IL-1β in CF cells

To examine the impact of glucose metabolism on inflammatory signaling, the production of IL6 and IL8 by epithelial cell lines was measured in culture. Inhibition of glycolysis significantly reduces the levels of both IL6 (Fig. 3) and IL8 (Fig. 4) following
stimulation with TNFa and IL-1β. These results support the notion that glycolysis is a
target for anti-inflammatory therapy. Modifiers of glycolysis are viable therapeutic
option in CF. Interestingly, inhibition of glycolysis with the inhibitors we used did not
completely block the production of IL6 or IL8. Completely blocking the production of
IL6 or IL8 may be undesirable in CF.

In certain embodiments, the disclosure relates to treating or preventing diseases or
conditions associated with epithelial airway inflammation comprising administering an
effective amount of a glycolysis pathway inhibitor to a subject in need thereof.

In certain embodiments, diseases or conditions associated with epithelial airway
inflammation include cystic fibrosis, asthma, bronchitis, chronic obstructive pulmonary
disease (COPD), atherosclerosis, or acute respiratory distress syndrome (ARDS).

In certain embodiments, the subject is diagnosed with, exhibiting symptoms of, or
at risk of fibrosis. In certain embodiments, the glycolic inhibitor is 3-bromopyruvate, 2-
deoxyglucose, 6-aminonicotinamide (6-aminonic), oxythiamine, lonidamine, genistein, 5-
epigallocatechin gallate, thiglucose, mannoheptulose, a-chlorohydrin, ornidazole, oxalate or salts thereof. In
certain embodiments, the glycolysis pathway inhibitor is arsenic, oxamate, sodium
fluoride (NaF), Nal, glufosfamide, imatinib, or alternative salts thereof.

**Glycolysis inhibition in hepatocytes**

A significant decrease in cytokine production is observed with glycolysis
inhibition in hepatocytes in culture. The effect of inhibiting glycolysis on inflammatory
cytokine production by hepatocytes was tested. Inhibition of inflammation in HUH7 cells
was observed (Figures 5 and 6). These data support indicate that the inhibition of
glycolysis is efficient in significantly reducing inflammation in non-airway cells. Because
inhibition of glycolysis in liver tissues is anti-inflammatory, glycolysis inhibition may be
used to treat or prevent liver conditions or diseases such as hepatitis and cirrhosis.

In certain embodiments, the disclosure relates to methods of treating or preventing
a disease or condition associated with liver inflammation comprising administering a
glycolysis pathway inhibitor to a subject in need thereof. In certain embodiments, the
subject is diagnosed with hepatitis and/or cirrhosis.

As used herein, "hepatitis" refers to a medical conditions characterized by liver
inflammation. The cause may be infectious or non-infections, e.g., alcohol consumptions,
drug induced, the result of metabolic disorders, autoimmune disease, Wilson's disease,
Alpha 1-antitrypsin deficiency fatty liver disease, a viral Hepatitis A, B, C, D, or E infection, systemic lupus erythematosus. In certain embodiments, the disclosure contemplates methods used to treat or prevent these medical conditions and the subject may be at risk of or diagnosed with one of the causes of hepatitis. Cirrhosis, fibrous scarring of the liver, is often the result of chronic inflammation, typically caused by alcoholism, hepatitis B and hepatitis C, and fatty liver disease.

**Combinations**

As used herein, the term "combination with" when used to describe administration with an additional treatment means that the agent may be administered prior to, together with, or after the additional treatment, or a combination thereof.

In certain embodiments, the pharmaceutical composition is administered in combination with a second fibrosis agent. In certain embodiments, the fibrosis agent is tobramycin (TOBI), Pulmozyme, Cayston Creon or combinations thereof.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with an antibiotic. In certain embodiments, the antibiotic is selected from tobramycin, azithromycin, amikacin, aztreonam, vancomycin, levofloxacin, fosfomycin, ciprofloxacin, alternative salts, or combinations thereof.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with alpha 1 microglobulin.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with dornase alfa.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with ivacaftor.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with lumacaftor.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with ivacaftor and lumacaftor.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with denufosol.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with burlulipase.
In certain embodiments, the glycolysis pathway inhibitor is administered in combination with fat-soluble vitamins selected from (A, D, E, K) or combinations thereof. In certain embodiments, the glycolysis pathway inhibitor is administered in combination with antioxidants, e.g., one selected from beta-carotene, tocopherols (different forms of vitamin E), coenzyme Q10 (CoQ10), carotenoids (lutein, lycopene and zeaxanthin), zinc, selenium or combinations thereof.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with [N6022] 3-(5-(4-(1H-imidazol-1-yl)phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoic acid.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with ataluren.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with simvastatin.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with N-acetylcysteine.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with a pancreatic enzyme or pancrecarb.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with bronchodilator, e.g., one selected from tiotropium, ipratropium bromide, theophylline, salbutamol and albuterol, levosalbutamol and levalbuterol, pirbuterol, ephedrine, terbutaline, salmeterol, clenbuterol, formoterol, bambuterol, indacaterol, alternative salts, or combinations thereof.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with an anti-inflammatory agent, e.g., one selected from methotrexate, hydroxychloroquine, sildenafil, pioglitazone alternative salts, or combinations thereof.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with a proton pump inhibitor, omeprazole, nexeum.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with insulin and/or repaglinide.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with mannitol.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with sulforaphane.
In certain embodiments, the glycolysis pathway inhibitor is administered in combination with alendronate.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with gluthathione, glutamine.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with an anti-viral agent. In certain embodiments, the anti-viral agent is selected from lamivudine, adefovir, tenofovir, telbivudine, boceprevir, telaprevir, ribavirin, and entecavir.

In certain embodiments, the glycolysis pathway inhibitor is administered in combination with immune system modulators, e.g., interferon alpha-2a and PEGylated interferon alpha-2a.

In certain embodiments, the anti-viral agent boceprevir or telaprevir is with ribavirin and peginterferon alfa.

Pharmaceutical Compositions

In certain embodiments, the disclosure relates to the use of a glycolysis in the production of a medicament for the treatment or prevention of inflammation or cystic fibrosis. In certain embodiments, the pharmaceutical composition is a powder, pill, tablet, capsule, or aqueous saline buffer.

In certain embodiments, the disclosure relates to a pharmaceutical composition comprising a glycolic inhibitor and pharmaceutically acceptable excipient and/or a second agent.

Pharmaceutical compositions disclosed herein may be in the form of pharmaceutically acceptable salts, as generally described below. Some preferred, but non-limiting examples of suitable pharmaceutically acceptable organic and/or inorganic acids are hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, acetic acid and citric acid, as well as other pharmaceutically acceptable acids known per se (for which reference is made to the references referred to below).

When the compounds of the disclosure contain an acidic group as well as a basic group, the compounds of the disclosure may also form internal salts, and such compounds are within the scope of the disclosure. When a compound contains a hydrogen-donating heteroatom (e.g. NH), salts are contemplated to covers isomers formed by transfer of said hydrogen atom to a basic group or atom within the molecule.
Pharmaceutically acceptable salts of the compounds include the acid addition and base salts thereof. Suitable acid addition salts are formed from acids which form non-toxic salts. Examples include the acetate, adipate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, cyclamate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulphate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, pyroglutamate, saccharate, stearate, succinate, tannate, tartrate, tosylate, trifluoroacetate and xinofoate salts. Suitable base salts are formed from bases which form non-toxic salts. Examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts. Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts. For a review on suitable salts, see Handbook of Pharmaceutical Salts: Properties, Selection, and Use by Stahl and Wermuth (Wiley-VCH, 2002), incorporated herein by reference.

The compounds described herein may be administered in the form of prodrugs. A prodrug can include a covalently bonded carrier which releases the active parent drug when administered to a mammalian subject. Prodrugs can be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or in vivo, to the parent compounds. Prodrugs include, for example, compounds wherein a hydroxyl group is bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl group. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol functional groups in the compounds. Methods of structuring a compound as prodrugs can be found in the book of Testa and Mayer, Hydrolysis in Drug and Prodrug Metabolism, Wiley (2006). Typical prodrugs form the active metabolite by transformation of the prodrug by hydrolytic enzymes, the hydrolysis of amide, lactams, peptides, carboxylic acid esters, epoxides or the cleavage of esters of inorganic acids.

Pharmaceutical compositions for use in the present disclosure typically comprise an effective amount of a compound and a suitable pharmaceutical acceptable carrier. The preparations may be prepared in a manner known per se, which usually involves mixing the at least one compound according to the disclosure with the one or more
pharmaceutically acceptable carriers, and, if desired, in combination with other
pharmaceutical active compounds, when necessary under aseptic conditions. Reference is
again made to U.S. Pat. No. 6,372,778, U.S. Pat. No. 6,369,086, U.S. Pat. No. 6,369,087
and U.S. Pat. No. 6,372,733 and the further references mentioned above, as well as to the
standard handbooks, such as the latest edition of Remington's Pharmaceutical Sciences.

Generally, for pharmaceutical use, the compounds may be formulated as a
pharmaceutical preparation comprising at least one compound and at least one
pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally
one or more further pharmaceutically active compounds.

The pharmaceutical preparations of the disclosure are preferably in a unit dosage
form, and may be suitably packaged, for example in a box, blister, vial, bottle, sachet,
ampoule or in any other suitable single-dose or multi-dose holder or container (which may
be properly labeled); optionally with one or more leaflets containing product information
and/or instructions for use. Generally, such unit dosages will contain between 1 and 1000
mg, and usually between 5 and 500 mg, of at least one compound of the disclosure,
e.g. about 10, 25, 50, 100, 200, 300 or 400 mg per unit dosage.

The compounds can be administered by a variety of routes including the oral,
ocular, rectal, transdermal, subcutaneous, intravenous, intramuscular or intranasal routes,
depending mainly on the specific preparation used. The compound will generally be
administered in an "effective amount", by which is meant any amount of a compound that,
upon suitable administration, is sufficient to achieve the desired therapeutic or
prophylactic effect in the subject to which it is administered. Usually, depending on the
condition to be prevented or treated and the route of administration, such an effective
amount will usually be between 0.01 to 1000 mg per kilogram body weight of the patient
per day, more often between 0.1 and 500 mg, such as between 1 and 250 mg, for example
about 5, 10, 20, 50, 100, 150, 200 or 250 mg, per kilogram body weight of the patient per
day, which may be administered as a single daily dose, divided over one or more daily
doses. The amount(s) to be administered, the route of administration and the further
treatment regimen may be determined by the treating clinician, depending on factors such
as the age, gender and general condition of the patient and the nature and severity of the
disease/symptoms to be treated. Reference is again made to U.S. Pat. No. 6,372,778, U.S.
Pat. No. 6,369,086, U.S. Pat. No. 6,369,087 and U.S. Pat. No. 6,372,733 and the further
references mentioned above, as well as to the standard handbooks, such as the latest edition of Remington's Pharmaceutical Sciences.

Depending upon the manner of introduction, the compounds described herein may be formulated in a variety of ways. Formulations containing one or more inhibitors can be prepared in various pharmaceutical forms, such as granules, tablets, capsules, suppositories, powders, controlled release formulations, suspensions, emulsions, creams, gels, ointments, salves, lotions, or aerosols and the like. Preferably, these formulations are employed in solid dosage forms suitable for simple, and preferably oral, administration of precise dosages. Solid dosage forms for oral administration include, but are not limited to, tablets, soft or hard gelatin or non-gelatin capsules, and caplets. However, liquid dosage forms, such as solutions, syrups, suspension, shakes, etc. can also be utilized. In another embodiment, the formulation is administered topically. Suitable topical formulations include, but are not limited to, lotions, ointments, creams, and gels. In a preferred embodiment, the topical formulation is a gel. In another embodiment, the formulation is administered intranasally.

Formulations containing one or more of the compounds described herein may be prepared using a pharmaceutically acceptable carrier composed of materials that are considered safe and effective and may be administered to an individual without causing undesirable biological side effects or unwanted interactions. The carrier is all components present in the pharmaceutical formulation other than the active ingredient or ingredients. As generally used herein "carrier" includes, but is not limited to, diluents, binders, lubricants, disintegrators, fillers, pH modifying agents, preservatives, antioxidants, solubility enhancers, and coating compositions.

Carrier also includes all components of the coating composition which may include plasticizers, pigments, colorants, stabilizing agents, and glidants. Delayed release, extended release, and/or pulsatile release dosage formulations may be prepared as described in standard references such as "Pharmaceutical dosage form tablets", eds. Liberman et. al. (New York, Marcel Dekker, Inc., 1989), "Remington - The science and practice of pharmacy", 20th ed., Lippincott Williams & Wilkins, Baltimore, MD, 2000, and "Pharmaceutical dosage forms and drug delivery systems", 6th Edition, Ansel et al, (Media, PA: Williams and Wilkins, 1995). These references provide information on carriers, materials, equipment and process for preparing tablets and capsules and delayed release dosage forms of tablets, capsules, and granules.
Examples of suitable coating materials include, but are not limited to, cellulose polymers such as cellulose acetate phthalate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate and hydroxypropyl methylcellulose acetate succinate; polyvinyl acetate phthalate, acrylic acid polymers and copolymers, and methacrylic resins that are commercially available under the trade name EUDRAGIT® (Roth Pharma, Westerstadt, Germany), zein, shellac, and polysaccharides.

Additionally, the coating material may contain conventional carriers such as plasticizers, pigments, colorants, glidants, stabilization agents, pore formers and surfactants.

Optional pharmaceutically acceptable excipients present in the drug-containing tablets, beads, granules or particles include, but are not limited to, diluents, binders, lubricants, disintegrants, colorants, stabilizers, and surfactants. Diluents, also referred to as "fillers," are typically necessary to increase the bulk of a solid dosage form so that a practical size is provided for compression of tablets or formation of beads and granules.

Suitable diluents include, but are not limited to, dicalcium phosphate dihydrate, calcium sulfate, lactose, sucrose, mannitol, sorbitol, cellulose, microcrystalline cellulose, kaolin, sodium chloride, dry starch, hydrolyzed starches, pregelatinized starch, silicone dioxide, titanium oxide, magnesium aluminum silicate and powdered sugar.

Binders are used to impart cohesive qualities to a solid dosage formulation, and thus ensure that a tablet or bead or granule remains intact after the formation of the dosage forms. Suitable binder materials include, but are not limited to, starch, pregelatinized starch, gelatin, sugars (including sucrose, glucose, dextrose, lactose and sorbitol), polyethylene glycol, waxes, natural and synthetic gums such as acacia, tragacanth, sodium alginate, cellulose, including hydroxypropylmethylcellulose, hydroxypropylcellulose, ethylcellulose, and veegum, and synthetic polymers such as acrylic acid and methacrylic acid copolymers, methacrylic acid copolymers, methyl methacrylate copolymers, aminoalkyl methacrylate copolymers, polyacrylic acid/polymethacrylic acid and polyvinylpyrrolidone.

Lubricants are used to facilitate tablet manufacture. Examples of suitable lubricants include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, glycerol behenate, polyethylene glycol, talc, and mineral oil.

Disintegrants are used to facilitate dosage form disintegration or "breakup" after administration, and generally include, but are not limited to, starch, sodium starch
glycolate, sodium carboxymethyl starch, sodium carboxymethylcellulose, hydroxypropyl cellulose, pregelatinized starch, clays, cellulose, algine, gums or cross linked polymers, such as cross-linked PVP (Polyplasdone XL from GAF Chemical Corp).

Stabilizers are used to inhibit or retard drug decomposition reactions which include, by way of example, oxidative reactions.

Surfactants maybe anionic, cationic, amphoteric or nonionic surface active agents. Suitable anionic surfactants include, but are not limited to, those containing carboxylate, sulfonate and sulfate ions. Examples of anionic surfactants include sodium, potassium, ammonium of long chain alkyl sulfonates and alkyl aryl sulfonates such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium bis-(2-ethylthioxy)-sulfosuccinate; and alkyl sulfates such as sodium lauryl sulfate. Cationic surfactants include, but are not limited to, quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, cetrimonium bromide, stearyl dimethylbenzyl ammonium chloride, polyoxyethylene and coconut amine. Examples of nonionic surfactants include ethylene glycol monostearate, propylene glycol myristate, glycercyl monostearate, glycercy1 stearate, polyglyceryl-4-oleate, sorbitan acylate, sucrose acylate, PEG-150 laurate, PEG-400 monolaurate, polyoxyethylene monolaurate, polysorbates, polyoxyethylene octylphenylether, PEG-1000 cetyl ether, polyoxyethylene tridecyl ether, polypropylene glycol butyl ether, Poloxamer® 401, stearoyl monoisopropanolamide, and polyoxyethylene hydrogenated tallow amide. Examples of amphoteric surfactants include sodium N-dodecyl-beta-alanine, sodium N-lauryl-beta-aminodipropionate, myristoamphoacetate, lauryl betaine and lauryl sulfobetaine.

If desired, the tablets, beads, granules, or particles may also contain minor amount of nontoxic auxiliary substances such as wetting or emulsifying agents, dyes, pH buffering agents, or preservatives.

The concentration of the inhibitor(s) to carrier and/or other substances may vary from about 0.5 to about 100 wt.% (weight percent). For oral use, the pharmaceutical formulation will generally contain from about 5 to about 100% by weight of the active material. For other uses, the pharmaceutical formulation will generally have from about 0.5 to about 50 wt. % of the active material.

The compositions described herein can be formulation for modified or controlled release. Examples of controlled release dosage forms include extended release dosage
forms, delayed release dosage forms, pulsatile release dosage forms, and combinations thereof.

The extended release formulations are generally prepared as diffusion or osmotic systems, for example, as described in "Remington - The science and practice of pharmacy" (20th ed., Lippincott Williams & Wilkins, Baltimore, MD, 2000). A diffusion system typically consists of two types of devices, a reservoir and a matrix, and is well known and described in the art. The matrix devices are generally prepared by compressing the drug with a slowly dissolving polymer carrier into a tablet form. The three major types of materials used in the preparation of matrix devices are insoluble plastics, hydrophilic polymers, and fatty compounds. Plastic matrices include, but are not limited to, methyl acrylate-methyl methacrylate, polyvinyl chloride, and polyethylene. Hydrophilic polymers include, but are not limited to, cellulotic polymers such as methyl and ethyl cellulose, hydroxyalkylcelluloses such as hydroxypropyl-cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and Carbopol® 934, polyethylene oxides and mixtures thereof. Fatty compounds include, but are not limited to, various waxes such as carnauba wax and glyceryl tristearate and wax-type substances including hydrogenated castor oil or hydrogenated vegetable oil, or mixtures thereof.

In certain preferred embodiments, the plastic material is a pharmaceutically acceptable acrylic polymer, including but not limited to, acrylic acid and methacrylic acid copolymers, methyl methacrylate, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, aminoalkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), methacrylic acid alkylamine copolymer poly(methyl methacrylate), poly(methacrylic acid)(anhydride), polymethacrylate, polyacrylamide, poly(methacrylic acid anhydride), and glycidyl methacrylate copolymers.

**EXAMPLES**

**Metabolic Dysfunction in CF Cells:**

A genome-wide expression profile was performed on the main effector cell causing CF lung damage, the neutrophil, in order to identify potential mechanistic pathways causing acceleration in lung decline in CF. Peripheral blood neutrophils were studied from CF patients and from healthy controls aged 16 to 40 years. Peripheral blood was collected and neutrophils separated by Ficoll method and selected by CD16+ magnetic microbeads. RNA was immediately extracted from the neutrophils and analysis was done
using the Illumina HT12 microarray platform. We studied 11 healthy controls and 26 CF patients.

The results showed that of the 48,000 probes in the Illumina platform, about 10,000 were expressed above background in neutrophils. When comparing CF to controls, there were several hundred genes that were differentially expressed at p < 0.01 and many of these were enriched for glucose and carbohydrate metabolism. Specifically CF neutrophils compared to controls had significantly increased expression of transcripts for the glycolysis pathway which is characteristic of a metabolic shift to a glycolytic phenotype. In addition, CF neutrophils differentially expressed to a greater degree than controls, two glucose transporters (GLUT1 and GLUT3).

This data indicate a previously undescribed abnormality in the metabolism of CF peripheral blood neutrophils. These metabolic manifestations appear to be related to CFTR dysfunction as evidenced by independent studies conducted in different cell types in two different labs. Using proteomics and metabolomics, a number of biochemical profiles of primary human tracheal airway epithelial (HTE) cells were found to be significantly increased upon pharmacological inhibition of CFTR function using the inhibitor CFTRi_{a172}, and the most pronounced CF effect was increased glucose metabolism (Fig 1). The proteomic and metabolomic data indicate that CFTR inhibition results in the increased expression, as well as function, of proteins in the glycolytic pathway. Proteomic studies in human neutrophils also revealed the increased expression of glycolytic enzymes in CF versus non-CF. These data mirror data observed in CF neutrophils using gene array analyses (Table 1). The similarities in the regulation of glycolysis in CFTR inhibited primary airway epithelia and CF neutrophils (2 very different cell types) suggest that the metabolic shift toward a glycolytic phenotype is due to CFTR dysfunction and is a basic defect in CF cells. The data on a metabolic shift toward glycolysis in CF cells has now been generated in 2 CF cell types, using transcriptomic, proteomic, and metabolomic analyses that have been confirmed by biochemical measures. The implication of this phenomenon is that CFTR dysfunction is linked to a metabolic abnormality (glycolytic shift), which in non-CF related studies has been extensively shown to affect redox status and inflammatory signaling.

The elevation of expression of glycolytic enzymes in CF cells is coupled to a significant increase in the levels of glycolysis intermediates, indicating that the rate of glycolysis may be significantly increased. Biochemical measurement of glucose in
tracheal epithelial airway cell lines revealed a decrease of -40% in the level of steady state glucose in CF cells (Fig. 2). These data further suggest that glucose metabolism is markedly elevated in CF epithelia.

Materials and methods for studies on inhibition of cytokines in CF cells and hepatocytes

Cell culture

Cells were maintained at 37°C, 5%CO2. HUH 7.5 cells (ATCC) were grown in RPMI 1640 medium containing 10% fetal calf serum, 2 g/L glucose and without antibiotics. Transformed human tracheal cell lines pCEP (9/HTEo(-) AS) and pCEPR (9/HTEo(-) pCEP-R) were grown on collagen collated cultureware in DMEM medium containing 10% fetal calf serum, 4.5 g/L glucose and 50 µg/mL hygromycin. Primary human bronchial epithelial cell cultures derived from CF and healthy lungs were purchased from ChanTest Corporation, Cleveland, OH and were maintained at an air-liquid interface with basolateral medium containing 1:1 DMEM:Ham's F12, 1% v/v Ultroser G (Pall Corporation, Port Washington, NY), 100 U/mL penicillin, 100µg/mL streptomycin, 50 µg/mL gentamicin, 250ng/mL amphotericin B and 200 µg/mL fluconazole.

Cellular glucose determinations

Cell lines were passaged into regular culture medium or culture medium containing 1 g/L glucose and grown to 75% confluence. Cells were treated with trypsin and the trypsin was neutralized by addition of culture medium. The cells were collected, centrifuged and washed 3 times with ice cold PBS. Cells were resuspended in 60 mM NaF and lysed by repeated freeze thaw cycles followed by sonication at 4 watts for 10 sec on ice. Lysates were assayed for glucose and protein.

Glycolysis inhibitors and cytokine/chemokine release

Cells at 75% confluence were preincubated for 1 hr in culture medium containing either 5 µM 6-aminonicotinamide, 100 µM arsenic, 2g/L 6-deoxyglucose+2 g/L glucose, 9 mM sodium oxomate or 5 mM NaF before addition of 10 ng/nL TNFa + 10ng/mL ILip. Culture medium was collected after 24hr, quenched on ice and protease inhibitor cocktail added to yield 10µg/ml leupeptin, 5 µg/mL aprotinin and 4 µg/mL pepstatin. Collected medium samples were centrifuged at 18kxg for 10min at 4°C and the debris free supernatants were immediately frozen at -20°C. After collection of medium, attached cells were lysed in Reporter Lysis Buffer (Promega) and the lysates were sonicated at 4 watts
for 10 sec on ice. The lysates were centrifuged and the debris free supernatants were stored as above. Medium samples were assayed for IL6 and IL8 and cell lysates were assayed for protein.

5 Glucose, cytokine, chemokine and protein assays
Cell lysate glucose concentrations were measured using an Amplex Red glucose assay according to the manufacturer’s protocol (Invitrogen). Fluorescence was measured and concentrations calculated using a Molecular Dynamics M2 spectrophotometer and SoftMax Pro software. Diluted aliquots of medium supernatants were assayed in duplicate for IL6 and IL8 using ELISA kits according to manufacturer’s protocols (eBioscience, BMS213/2MST and BMS204/3MST). Assay plates were read with a BioTek Synergy II reader and concentrations were calculated using 4 parameter curve fitting. The data were normalized to protein content. Protein was measured directly using a BCA protein assay (Thermo Scientific) or after acetone precipitation using a RC DC Protein assay (Bio-Rad Laboratories).

References
Glycolysis inhibitors and concentrations:
Deoxyglucose induces Akt phosphorylation via a mechanism independent of LKB 1/AMP-activated protein kinase signaling activation or glycolysis inhibition Mol Cancer Ther 7:809-817.

pCEP/R cells:
CLAIMS

1. A method of treating or preventing cystic fibrosis comprising administering a pharmaceutical composition comprising a glycolic inhibitor a subject in need thereof.

2. The method of Claim 1, wherein the subject is diagnosed with, exhibiting symptoms of, or at risk of fibrosis.

3. The method of Claims 1-2, wherein the glycolic inhibitor is 3-bromopyruvate, 2-deoxyglucose, 6-aminonicotinamide, oxythiamine, lonidamine, genistein, 5-thioglucone, mannoheptulose, a-chlorohydrin, ornidazole, oxalate or salts thereof.

4. The method of Claims 1-3, wherein the pharmaceutical compositions is administered in combination with a second fibrosis agent.

5. The method of Claim 4, wherein the fibrosis agent is TOBI, Pulmozyme, Cayston Creon or combinations thereof.

6. Use of a compound as provided in Claim 3 in the production of a medicament for the treatment or prevention of fibrosis.

7. The method of Claim 1, wherein the pharmaceutical compositions is a powder, pill, tablet, capsule, or aqueous saline buffer.

8. A pharmaceutical composition comprising a glycolic inhibitor and a second fibrosis agent.
Fig. 1
FIG. 2
FIG. 3
FIG. 4
FIG. 5
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
A61K 31/195(2006.01)i, A61K 31/19(2006.01)i, A61K 31/70(2006.01)i, A61K 9/20(2006.01)i, A61K 9/16(2006.01)i, A61P 29/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/195; A61K 31/19; A61K 31/70; A61K 9/16; A61P 29/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
eKOMPASS(KXPO internal) & Keywords: fibrosis, glycolic inhibitor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search
17 December 2013 (17.12.2013)

Date of mailing of the international search report
17 December 2013 (17.12.2013)

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
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Form PCT/ISA/210 (second sheet) (July 2009)
Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **X** Claims Nos.: 1-5, 7  
   because they relate to subject matter not required to be searched by this Authority, namely:  
   Claims 1-5 and 7 pertain to a method for treatment of the human by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. **X** Claims Nos.: 5, 6  
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
   Claims 5 and 6 are unclear, since they refer to one of claims which are not drafted in accordance with PCT Rule 6.4(a) (PCT Article 6).

3. **X** Claims Nos.: 3, 4  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. **-** As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. **-** As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. **-** As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 

4. **-** No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 

**Remark on Protest**  
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Form PCT/ISA/210 (continuation of first sheet (2))  (July 2009)
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