(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 December 2020 (24.12.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/257093 A1

(51) International Patent Classification:
A61P 11/04 (2006.01) *C07C 317/48* (2006.01)
C07C 317/26 (2006.01) *C07C 317/50* (2006.01)
C07C 317/28 (2006.01)

(21) International Application Number:
PCT/US2020/037696

(22) International Filing Date:
15 June 2020 (15.06.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/862,434 17 June 2019 (17.06.2019) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) Title: METHOD FOR TREATING ASTHMA

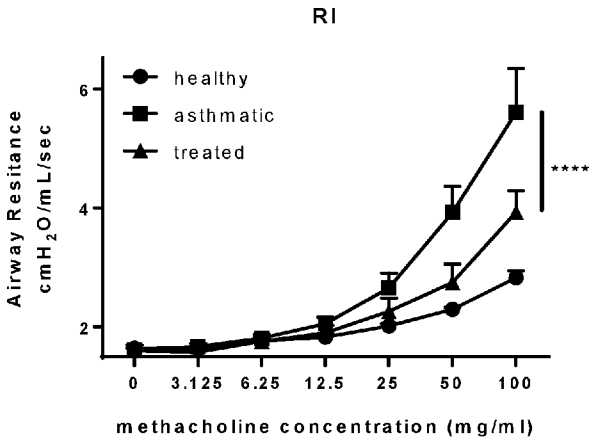


FIG. 4

(57) Abstract: The present invention is directed to a method for treating Asthma. The method comprises administering to a subject in need thereof 3-methanesulfonylpropionitrile (dapansutrile), or a pharmaceutically acceptable solvate thereof, in an effective amount. The preferred route of administration is oral administration or local administration.

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METHOD FOR TREATING ASTHMA

FIELD OF THE INVENTION

The present invention relates to using 3-methanesulfonylpropionitrile (dapansutrile), or its pharmaceutically acceptable solvates, for treating asthma.

BACKGROUND

Asthma is a common chronic disorder of the airways characterized by variable and recurring symptoms, reversible airway obstruction, bronchial hyperresponsiveness, and an underlying inflammation. Acute symptoms of asthma include cough, wheezing, shortness of breath and nocturnal awakening. Asthma is regarded as a chronic disease based on a condition of chronic airway inflammation together with airway hyperresponsiveness, with at least partially reversible airway obstruction.

Central to the pathophysiology of asthma is the presence of underlying airway inflammation mediated by the recruitment and activation of multiple cell types including mast cells, eosinophils, T lymphocytes, macrophages, dendritic cells and neutrophils. Type 2 T-helper (Th2) cells appear to play a central role in the activation of the immune cascade that results in inflammation. Th2-derived cytokines include IL-5, which is needed for eosinophil differentiation and survival, and IL-4 which is important for Th2 cell differentiation and with IL-13 is important for IgE formation and leads to overproduction of IgE and eosinophilia. IgE-driven activation of mucosal mast cells releases bronchoconstrictor mediators such as histamine and cysteinyl-leukotrienes as well as inflammatory cytokines. Eosinophils contain inflammatory enzymes, generate leukotrienes, and express a wide variety of pro-inflammatory cytokines. Airway epithelial cells also play a role in the inflammatory process via release of cytokines such as eotaxin that direct and modify the inflammatory response. Acute and chronic inflammation can affect not only the airway caliber and airflow but also can increase the existing bronchial hyperresponsiveness to a variety of stimuli, which enhances susceptibility to bronchospasm.

As a consequence of airway inflammation and the generation of growth factors, the airway smooth muscle cell can undergo proliferation, activation, contraction, and hypertrophy; which are events that can influence airway airflow limitation. In asthma, the dominant physiological event leading to clinical symptoms is airway narrowing and a subsequent interference with airflow. In acute exacerbations of asthma, bronchial smooth

muscle contraction (bronchoconstriction) occurs quickly to narrow the airways in response to exposure to a variety of stimuli including allergens or irritants. Allergen-induced acute bronchoconstriction results from an IgE-dependent release of mediators from mast cells that includes histamine, tryptase, leukotrienes, and prostaglandins that directly contract airway smooth muscle. The mechanisms influencing airway hyperresponsiveness are multiple and they include inflammation, dysfunctional neuroregulation, and airway remodeling. Airway remodeling involves structural changes such as thickening of the sub-basement membrane, subepithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, blood vessel proliferation and dilation with consequent permanent changes in the airway that increase airflow obstruction.

Airway epithelium and endothelial cell function are also critically involved in asthma. Upon disease progression, epithelial subbasement membranes thicken with mucus hypersecretion and the formation of mucus plugs. Changes in endothelial cell integrity lead to edema, another key pathophysiology defining asthmatic change of the airway. These factors serve to further limit airflow.

Asthma is characterized by dominant T helper type 2 (Th2) immune responses, including enhanced IL-4, IL-5 and IL-13 responses, allergen-specific immunoglobulin production, eosinophilia, airway inflammation, bronchoconstriction, and airway hyperresponsiveness. Current standard therapies for asthma are a combination of corticosteroids and β_2-agonists (anti-inflammatory and bronchodilator drugs). These drugs provide acceptable control of the disease for many asthmatics. However, it is estimated that 5 to 10% of the asthma patients have symptomatic disease despite treatment with this combination of corticosteroids and β_2-agonists.

There is a need to develop a new method for effectively treating asthma. The method should be effective with minimal side effects.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows total numbers (mean ± SEM) of leukocyte subpopulations (macrophage, lymphocyte, neutrophil, and eosinophil, from left to right) in bronchoalveolar lavage fluids on Day 29, 24 hours after the final ovalbumin (OVA) aerosol challenge and intraperitoneal dapansutrile dose. ****$p < 0.0001$, eosinophil, between asthmatic and treated mice (n=8 per group). Mice were treated with 60 mg/kg dapansutrile intraperitoneally.

FIG. 2 shows volume of inflammatory cells infiltrate per basal membrane in lung tissue of mice treated with dapansutrile. **$p < 0.01$ between asthmatic and treated mice (n=8 per group). Mice were treated with 60 mg/kg dapansutrile intraperitoneally.

FIG. 3 shows area of epithelial basal membrane (mean ± SEM) covered by goblet cells in healthy, asthmatic, and treated mice (n=8 per group). **$p < 0.01$ between asthmatic and treated mice. Mice were treated with 60 mg/kg dapansutrile intraperitoneally.

FIG. 4 shows mean ± SEM airway resistance toward methylcholine for in healthy, asthmatic, and dapansutrile-treated mice. ****$p < 0.0001$ between asthmatic and treated mice. MCh (acetyl-β-methylcholine chloride) provocation testing started with PBS, followed by MCh aerosols with increasing concentrations from 0 to 100 mg/mL (n=8 per group). Mice were treated with 60 mg/kg dapansutrile intraperitoneally.

FIG. 5 shows total numbers of leukocyte subpopulations (macrophage, lymphocyte, neutrophil, and eosinophil, from left to right, mean ± SEM) in bronchoalveolar lavage fluids on Day 29, 24 hours after the final ovalbumin (OVA) aerosol challenge and oral dapansutrile dose. ****$p < 0.0001$, eosinophil, between asthmatic and treated mice (n=8 per group).

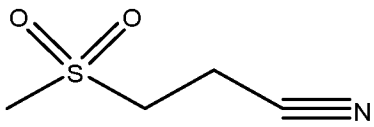
FIG. 6 shows volume of inflammatory cells infiltrate per basal membrane in lung tissue of mice orally treated with dapansutrile. *$p < 0.05$ between asthmatic and treated mice (n=8 per group).

FIG. 7 shows mean ± SEM airway resistance toward methylcholine for in healthy, asthmatic, and dapansutrile-treated mice. **$p < 0.01$ between asthmatic and treated mice. MCh (acetyl-β-methylcholine chloride) provocation testing started with PBS, followed by MCh aerosols with increasing concentrations from 0 to 100 mg/mL (n=8 per group). Mice were orally treated with dapansutrile in feed at 7.5 g/kg food.

DETAILED DESCRIPTION OF THE INVENTION

The inventor has discovered that 3-methanesulfonylpropionitrile, which reduces the levels of IL-1β and IL-6 in several whole animal models of local and systemic inflammation, is effective in treating asthma, reducing airway inflammation, reducing airway resistance, improving lung function, ameliorating asthma symptoms, and improving patient's quality of life.

The present invention is directed to a method of treating asthma. The method comprises the step of administering to a subject in need thereof an effective amount of a compound of 3-methanesulfonylpropionitrile (dapansutrile), or a pharmaceutically acceptable solvate thereof, to treat asthma.



3-methanesulfonylpropionitrile (Dapansutrile)

"Solvates," as used herein, are addition complexes in which the compound is combined with an acceptable solvent in some fixed proportion. Acceptable solvents include, but are not limited to, water, acetic acid, ethanol, and other appropriate organic solvents.

In one embodiment, the present method is effective in prophylactic treatment, which is a process of protecting against the development of asthma by a treatment of dapansutrile before the onset of asthma to affect pathogenesis. By prophylactic treatment, dapansutrile is administered to a patient in need thereof, before the onset of asthma.

In another embodiment, the present method is effective in therapeutic treatment after the onset of asthma, when the patient starts to show clinical signs and/or symptoms.

The main functional changes of the lungs associated asthma include malfunctioning of the immune system, cellular infiltration composed primarily of eosinophils and neutrophils, acute and chronic inflammation, fluid accumulation (edema), excessive secretion of mucus, and changes in the airway walls that could lead to bronchial epithelial injury, fibrosis, and increased sensitivity to agents that cause bronchial constriction. These features need to be considered in order to develop treatments of the underlying disease process. Small animal models can be designed to mimic the airway inflammation, increased responsiveness to bronchial constrictors, changes in the airway wall, and changes in the migration of the eosinophils and neutrophils to the lungs. A mouse model of asthma via ovalbumin sensitization (Lunding, 2015b), for example, can be used to evaluate bronchodilator efficacy of dapansutrile.

The present method for treating asthma is based on the properties of dapansutrile to reduce at least one of the following processes contributing to pathophysiology that accompanies this disorder: inflammation, excessive cell proliferation, airway and/or lung tissue edema, airway hyperreactivity, and bronchoconstriction.

Indicia of efficacy for treating asthma by the present method include demonstrable improvement in measurable signs, symptoms and other variables clinically relevant to asthma. Such improvements include increased blood oxygen saturation, decreased hypoxia and hypercapnia, decreased need for supplemental oxygen, decreased frequency of coughing and/or wheezing, improved forced expiratory volume in one second (FEV_1), improved forced

vital capacity (FVC) or other physiologically relevant parameter of respiratory function, decreased need for mechanical ventilation, decreased amount of inflammatory cells infiltrating the lung, decreased levels of proinflammatory cytokines and chemokines, improved alveolar fluid clearance rate, decreased pulmonary edema as determined by any radiographic or other detection method such as amount of epithelial lining fluid, wet to dry lung weight, alveolar fluid clearance and/or radiographic visualization methods, increase in general quality of life, patient-reported or physician-observed signs such as ease of breathing, or decrease in severity of coughing and/or wheezing.

The present method treats asthma by (i) improving symptoms (daytime and nocturnal symptoms, limitation of activities, use of rescue medications), (ii) improving lung function such as peak expiratory flow (PEF) and/or forced expiratory volume in one second (FEV1), and/or (iii) reducing exacerbations (rate and severity). The present method improves Asthma Quality of Life Questionnaire (AQLQ) scores, which include the scores of symptoms, activity limitation, emotional function, and environmental exposure.

The present invention has demonstrated that dapansutrile reduced airway resistance, reduced inflammatory cells (eosinophils and neutrophils) and mucus hyperproduction in broncho-alveolar lavage fluid, and reduced airway inflammation, in ovalbumin-induced allergic airway inflammation in mice.

Pharmaceutical Compositions

The present invention provides pharmaceutical compositions comprising one or more pharmaceutically acceptable carriers and an active compound of 3-methanesulfonylpropionitrile, or a pharmaceutically acceptable solvate thereof. The active compound or its pharmaceutically acceptable solvate in the pharmaceutical compositions in general is about 1-90% for a tablet formulation, about 1-100% for a capsule formulation, about 0.01-20%, or 0.05-20%, or 0.1-20%, or 0.2-15%, or 0.5-10%, or 1-5% (w/w), for a topical formulation; about 0.1-5% for an injectable formulation, 0.1-5% for a patch formulation,. The active compound used in the pharmaceutical composition in general is at least 90%, preferably 95%, or 98%, or 99% (w/w) pure.

In one embodiment, the pharmaceutical composition is in a dosage form such as tablets, capsules, granules, fine granules, powders, syrups, suppositories, injectable solutions, patches, or the like.

In one embodiment, the pharmaceutical composition is in the form of an aerosol suspension of respirable particles comprising the active compound, which the subject inhales.

The respirable particles can be liquid or solid, with a particle size sufficiently small to pass through the mouth and larynx upon inhalation. In general, particles having a size of about 1 to 10 microns, preferably 1-5 microns, are considered respirable. The respirable particles including dapansutrile can be prepared into dry powder using well-known art of super critical fluid technology. In such a case, the compound is admixed with appropriate excipients and milled into a homogenous mass using suitable solvents or adjuvants. Following this, this mass is subjected to mixing using super critical fluid technology and suitable particle size distribution achieved. The particles in the formulation need to be within a desired particle size range such that the particles can be directly inhaled into the lungs using a suitable inhalation technique or introduced into the lungs via a mechanical ventilator.

Pharmaceutically acceptable carriers, which are inactive ingredients, can be selected by those skilled in the art using conventional criteria. Pharmaceutically acceptable carriers include, but are not limited to, non-aqueous based solutions, suspensions, emulsions, microemulsions, micellar solutions, gels, and ointments. The pharmaceutically acceptable carriers may also contain ingredients that include, but are not limited to, saline and aqueous electrolyte solutions; ionic and nonionic osmotic agents such as sodium chloride, potassium chloride, glycerol, and dextrose; pH adjusters and buffers such as salts of hydroxide, phosphate, citrate, acetate, borate; and trolamine; antioxidants such as salts, acids and/or bases of bisulfite, sulfite, metabisulfite, thiosulfite, ascorbic acid, acetyl cysteine, cysteine, glutathione, butylated hydroxyanisole, butylated hydroxytoluene, tocopherols, and ascorbyl palmitate; surfactants such as lecithin, phospholipids, including but not limited to phosphatidylcholine, phosphatidylethanolamine and phosphatidyl inositiol; poloxamers and poloxamines, polysorbates such as polysorbate 80, polysorbate 60, and polysorbate 20, polyethers such as polyethylene glycols and polypropylene glycols; polyvinyls such as polyvinyl alcohol and povidone; cellulose derivatives such as methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose and hydroxypropyl methylcellulose and their salts; petroleum derivatives such as mineral oil and white petrolatum; fats such as lanolin, peanut oil, palm oil, soybean oil; mono-, di-, and triglycerides; polymers of acrylic acid such as carboxypolymethylene gel, and hydrophobically modified cross-linked acrylate copolymer; polysaccharides such as dextrans and glycosaminoglycans such as sodium hyaluronate. Such pharmaceutically acceptable carriers may be preserved against bacterial contamination using well-known preservatives, these include, but are not limited to, benzalkonium chloride, ethylenediaminetetraacetic acid and its salts, benzethonium chloride, chlorhexidine, chlorobutanol, methylparaben,

thimerosal, and phenylethyl alcohol, or may be formulated as a non-preserved formulation for either single or multiple use.

For example, a tablet formulation or a capsule formulation of the active compound may contain other excipients that have no bioactivity and no reaction with the active compound. Excipients of a tablet may include fillers, binders, lubricants and glidants, disintegrators, wetting agents, and release rate modifiers. Binders promote the adhesion of particles of the formulation and are important for a tablet formulation. Examples of binders include, but not limited to, carboxymethylcellulose, cellulose, ethylcellulose, hydroxypropylmethylcellulose, methylcellulose, karaya gum, starch, starch, and tragacanth gum, poly(acrylic acid), and polyvinylpyrrolidone.

For example, a patch formulation of the active compound may comprise some inactive ingredients such as 1,3-butylene glycol, dihydroxyaluminum aminoacetate, disodium edetate, D-sorbitol, gelatin, kaolin, methylparaben, polysorbate 80, povidone (polyvinylpyrrolidone), propylene glycol, propylparaben, sodium carboxymethylcellulose, sodium polyacrylate, tartaric acid, titanium dioxide, and purified water. A patch formulation may also contain skin permeability enhancer such as lactate esters (e.g., lauryl lactate) or diethylene glycol monoethyl ether.

Topical formulations including the active compound can be in a form of gel, cream, lotion, liquid, emulsion, ointment, spray, solution, and suspension. The inactive ingredients in the topical formulations for example include, but not limited to, lauryl lactate (emollient/permeation enhancer), diethylene glycol monoethyl ether (emollient/permeation enhancer), DMSO (solubility enhancer), silicone elastomer (rheology/texture modifier), caprylic/capric triglyceride, (emollient), octisalate, (emollient/UV filter), silicone fluid (emollient/diluent), squalene (emollient), sunflower oil (emollient), and silicone dioxide (thickening agent).

Method of Administration

The present invention is directed to a method of treating Asthma. The method comprises the steps of first identifying a subject suffering from asthma or has a propensity to develop asthma, and administering to the subject the active compound dapansutrile, in an amount effective to treat asthma. “An effective amount,” as used herein, is the amount effective to treat asthma by ameliorating the pathological condition, reducing airway inflammation, reducing airway hyperresponsiveness, improving lung function, and/or reducing the symptoms of asthma.

Any method for delivering the compound to the tissues of the lung, including local administration and systemic administration, is suitable for the present invention.

Systemic administration includes oral, parenteral (such as intravenous, intramuscular, subcutaneous or rectal), and other systemic routes of administration. In systemic administration, the active compound first reaches plasma and then distributes into target tissues.

In one embodiment, the active compound is delivered by local administration to the lung. Local administration includes inhalation and targeted drug delivery. Methods of inhalation include liquid instillation, instillation as a pressurized fluid preparation via metered dose inhaler or equivalent, inhalation of an aerosolized solution via nebulizer, inhalation of dry powder, and directing soluble or dried material into the air stream during mechanical ventilation.

In one embodiment, the pharmaceutical composition is administrated to a subject by inhalation of an aerosol suspension of respirable particles comprising the active compound. The respirable particles can be liquid or solid (e.g., dry powder), with a particle size sufficiently small to pass through the mouth and larynx upon inhalation; in general, particles ranging from about 1 to 10 microns, but more preferably 1-5 microns, in size are considered respirable. The surface concentrations of active compounds delivered via inhalation can vary according to compounds; but are generally $1x10^{-10}$-$1x10^{-4}$ moles/liter, and preferably $1x10^{-8}$-$1x10^{-5}$ moles/liter.

In one embodiment, the pharmaceutical composition is administrated orally to the subject. The dosage for oral administration is generally at least 0.1 mg/kg/day and less than 100 mg/kg/day or 200mg/kg/day. For example, the dosage for oral administration is 1-100, or 5-50, or 10-50 mg/kg/day, for a human subject. For example, the dosage for oral administration is 100-10,000 mg/day, and preferably 500-2000, 500-4000, 500-4000, 1000-5000, 2000-5000, 2000-6000, or 2000-8000 mg/day for a human subject. The drug can be orally taken once, twice, three times, or four times a day.

In one embodiment, the pharmaceutical composition is administrated intravenously to the subject. The dosage for intravenous bolus injection or intravenous infusion is generally 0.03 to 20 and preferably 0.03 to 10 mg/kg/day.

In one embodiment, the pharmaceutical composition is administrated subcutaneously to the subject. The dosage for subcutaneous administration is generally 0.3-20, and preferably 0.3-3 mg/kg/day.

Those of skill in the art will recognize that a wide variety of delivery mechanisms are also suitable for the present invention.

The present invention is useful in treating a mammal subject, such as humans, horses, and dogs. The present invention is particularly useful in treating humans.

The following examples further illustrate the present invention. These examples are intended merely to be illustrative of the present invention and are not to be construed as being limiting.

EXAMPLES

Example 1. Dapansutrile treatment (intraperitoneal) reduced allergic airway inflammation and mucus production in mice

A well-established mouse model of experimental allergic asthma (Sel 2008, Wegmann 2005, Wegmann 2007, Lunding 2015a) was used to evaluate dapansutrile as a therapeutic option in allergic bronchial asthma and to determine if dapansutrile would have an impact on airway inflammation and the development of airway hyperresponsiveness (AHR). Inflammation in this model is characterized by the infiltration of eosinophils as well as of TH2 cells and involves the subsequent development of AHR and mucus hyperproduction so that this model resembles the major pathophysiologic hallmarks of human bronchial asthma.

Methods and Materials

C57BL/6j mice were sensitized to OVA (ovalbumin) by three intraperitoneal (i.p.) injections of 10 µg OVA adsorbed to 150 mg aluminum hydroxide on days 1, 14, and 21. This sensitization results in an adoptive immune response against OVA with OVA-specific TH2 cells and the production of OVA-specific antibodies of the subclasses IgE and IgG4.

To induce acute allergic airway inflammation, mice were exposed three times to an OVA aerosol (1% w/v in PBS) on days 26, 27, and 28.

Healthy control animals (healthy group) were sham sensitized to PBS and subsequently challenged with PBS aerosol. Non-drug treated animals (asthmatic group) and drug-treated animals (treated group) were sensitized by OVA aerosol and subsequently challenged with OVA aerosol. The treatment group were treated with dapansutrile at 60 mg/kg at days 25, 26, 27, and 28 by intraperitoneal (i.p). injection, whereas the healthy group and the asthmatic group were administered with saline by intraperitoneal injection on days 25, 26, 27, and 28.

Eight animals per group were used. All animals were sacrificed on day 29. The following readouts were measured according to Lunding, 2015b.

- General infiltration of inflammatory cells into the broncheoalveolar lumen
- Determination of the specific infiltration of eosinophils, neutrophils and lymphocytes by histologic differentiation of the bronchoalveolar lavage (BAL) cells.
- Goblet cell hyperplasia by CAST system (Computer Assisted Stereological Toolbox), including making microscopic pictures of the airways.
- Airway resistance in response to methacholine to determine the airway hyperresponsiveness.

Specifically, inflammatory cell subpopulations (eosinophils, macrophages, neutrophils, lymphocytes) infiltrating the bronchoalveolar lumen were quantified using cyto-spinned and quick-diff-stained cells from bronchoalveolar lavage fluids (BALF). Further, inflammatory cell infiltration was recorded from hematoxylin and eosin (HE)-stained lung/airway cross-sections. AHR was assessed by recording airway resistance during a methacholine (MCh) provocation test in mice mechanically ventilated by a Buxco FinePoint RC unit. Mucus hyperproduction was quantified in PAS (periodic acid-Schiff)-stained airway cross-sections undergoing systematic, uniform random sampling and subsequent stereologic analysis of mucus amount in the airways and mucus producing goblet cells in the airway mucosa.

Results

Allergic airway inflammation and mucus hyperproduction were assessed on Day 29, 24 hours after the final OVA aerosol challenge and dapansutrile dose. FIG. 1 shows total numbers of leukocyte subpopulations (macrophage, lymphocyte, neutrophil, and eosinophil, from left to right) on Day 29.

Dapansutrile (60 mg/kg), administered as 4 i.p. injections one day prior and concurrently with three daily OVA aerosol challenges, led to a significant reduction in eosinophils in BALF (FIG. 1). Comparing to asthmatic mice, treatment with dapansutrile resulted in an approximately 60% reduction in eosinophils (from 21.96 x 10^4 cells/ml to 7.30 x 10^4 cells/ml; ****$p < 0.0001$), a 70% reduction in neutrophils (from 2.41 x 10^4 cells/ml to 0.70 x 10^4 cells/ml; $p < 0.01$), and a 32% reduction in lymphocytes (from 0.80 x 10^4 cells/ml to 0.55 x 10^4 cells/ml) in BALF. Macrophage numbers showed no significant reduction (FIG. 1).

FIG. 2 shows that the number of inflammatory cells in lung tissue was significantly lower in asthmatic mice vs. dapansutrile-treated mice (**$p < 0.01$). The label on the y-axis reads "volume of inflammatory cell infiltrate per basal membrane area ($\mu m^3/\mu m^2$)". The inflammatory cells were counted within a specific distance around the airways using a microscope with the computer assisted stereological toolbox (CAST) system. These counts were set in ratio to the basal membrane to normalize within each microscopic slide to avoid slide-dependent differences.

FIG. 3 shows area of epithelial basal membrane covered by goblet cells in healthy, asthmatic, and treated mice. Comparing to asthmatic mice, Dapansutrile-treated animals displayed a significant reduction of goblet cells covering the airway mucosa (22.74% reduced to 17.67%, $p < 0.01$) as quantified by stereology of PAS-stained airway cross-sections.

The results of FIGs. 1-3 show that dapansutrile treatment in OVA-induced allergic airway inflammation model resulted in significant reduction of allergic airway inflammation and mucus production.

Airway hyperresponsiveness was determined by measuring airway resistance on Day 29 in response to 100 mg/mL methacholine. The results are shown in FIG. 4.

The airway resistance in response to methacholine was 5.61 cm $H_2O \cdot sec \cdot ml^{-1}$ in asthmatic mice and 3.93 cm $H_2O \cdot sec \cdot ml^{-1}$ in dapansutrile-treated mice. Dapansutrile treatment reduced airway resistance by about 60% when comparing with asthmatic mice. ****$p < 0.0001$ between asthmatic and treated mice. Baseline airway resistance of healthy animals was 2.83 cm $H_2O \cdot sec \cdot ml^{-1}$.

Example 2. Cytokines in BALF

Cytometric bead arrays were used as a method to assess the concentration of cytokines of IFN-γ, TNFα, IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, and IL17A) in the BALF of Example 1. The beads in this array were coated with antibodies specific against a variety of cytokines, some relevant and known to be affected by NLRP3 signaling. The concentrations of all measured cytokines showed some reduction between asthmatic mice and dapansutrile-treated mice. Both IL-1β and IL-6 concentration showed a statistically significant reduction between asthmatic mice and dapansutrile-treated mice ($p<0.001$ and $p<0.05$, respectively).

Example 3. Dapansutrile treatment (oral) reduced allergic airway inflammation and airway resistance in mice

The experimental protocols of the mouse model were the same as those described in Example 1, except dapansutrile was administered orally by food.

The already-sensitized mice were fed ad libidum with food pellets containing 7.5 g/kg dapansutrile starting on day 22 for one week; the first aerosol challenge was on day 26. Mice typically consume about 4 g of food per day, resulting in an approximate daily dose of 0 mg/kg/day for control groups and 1,000 mg/kg/day for the treatment groups. This food pellet concentration (7.5g/kg of dapansutrile in food) in mouse chow resulted in a blood level nearly the same as that of humans treated orally with dapansutrile at doses of 1000 mg/day (40 μg/mL blood level; Marchetti 2018b)

Sham-sensitized, OVA-challenged mice were used as healthy controls (healthy). OVA-sensitized, OVA-challenged asthmatic controls (asthmatic) were fed with control food pellets without dapansutrile.

Comparing to asthmatic mice, treatment with dapansutrile (food) resulted in an approximate 75% reduction in eosinophils (from 15.93 x 10^4 cells/ml to 3.77 x 10^4 cells/ml; $p < 0.0001$), an approximate 75% reduction in neutrophils (from 1.74 x 10^4 cells/ml to 0.43 x 10^4 cells/ml; $p < 0.05$), and an approximate 75% reduction in lymphocytes (from 1.00 x 10^4 cells/ml to 0.26 x 10^4 cells/ml, $p< 0.05$) in BALF. Macrophage numbers showed no significant reduction (FIG. 5).

FIG. 6 shows that the number of inflammatory cells in lung tissue was significantly lower in asthmatic mice vs. dapansutrile-treated mice ($p< 0.05$). The label on the y-axis reads "volume of inflammatory cell infiltrate per basal membrane area ($\mu m^3/\mu m^2$)". The inflammatory cells were counted within a specific distance around the airways using a microscope with the computer assisted stereological toolbox (CAST) system. These counts were set in ratio to the basal membrane to normalize within each microscopic slide to avoid slide-dependent differences.

Comparing to asthmatic mice, Dapansutrile-treated mice also displayed a prominent reduction of goblet cells covering the airway mucosa (-29%; 15.75% reduced to 11.16%), as quantified by stereology of PAS-stained airway cross-sections. (data not shown).

The airway resistance of dapansutrile-treated animals in response to MCh was significantly lowered and revealed reductions of approximately 60% at 100 mg/mL MCh, as compared to the sham-treated asthmatic controls (4.57 cm H_2O.s/mL reduced to 3.38 cm

H_2O.s/mL) (FIG. 6). **p < 0.01 between asthmatic and treated mice. Baseline airway resistance of healthy animals was 2.58 cm H_2O.s/mL at 100 mg/mL MCh.

The results show that dapansutrile-treatment by food intake resulted in significant reduction of allergic airway inflammation.

References:

Lunding L, Webering S, Vock C, *et al.* IL-37 requires IL-18Ra and SIGIRR/IL-1R8 to diminish allergic airway inflammation in mice. *Allergy* 2015a Apr;70(4):366-73.

Lunding LP, Webering S, Vock C, *et al.* Poly(inosinic-cytidylic) acid-triggered exacerbation of experimental asthma depends on IL-17A produced by NK cells. J Immunol 2015b;194:5615-5625 .

Marchetti C, Swartzwelter B, Koenders MI, *et al.* The NLRP3 Inflammasome Inhibitor OLT1177™ Suppresses Joint Inflammation in Murine Models of Acute Arthritis. *Arthritis Research and Therapy* 2018b;20:169.

Sel S, Wegmann M, Dicke T, *et al.* Effective prevention and therapy of experimental allergic asthma using a GATA-3-specific DNAzyme. *J Allergy Clin Immunol* 2008;121:910-916.e5.

Wegmann M, Fehrenbach H, Held T, et al. Involvement of distal airways in a chronic model of experimental asthma. *Clin Exp Allergy* 2005 Oct;35(10):1263-71.

Wegmann M, Göggel R, Sel S, et al. Effects of a low-molecular-weight CCR-3 antagonist on chronic experimental asthma. *Am J Respir Cell Molec Bio* 2007;36(1):61-7.

The invention, and the manner and process of making and using it, are now described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, to make and use the same. It is to be understood that the foregoing describes preferred embodiments of the present invention and that modifications may be made therein without departing from the scope of the present invention as set forth in the claims. To particularly point out and distinctly claim the subject matter regarded as invention, the following claims conclude the specification.

WHAT IS CLAIMED IS:

1. A method for treating asthma, comprising the step of:

administering dapansutrile or a pharmaceutically acceptable solvate thereof, to a subject in need thereof, in an amount effective to treat asthma.

2. The method according to Claim 1, which reduces airway inflammation, reduces airway hyperresponsiveness, improves lung function, and/or reducing the symptoms of asthma.

3. The method according to Claim 1, wherein said compound is administered by oral administration.

4. The method according to Claim 1, wherein said compound is administered by local administration.

5. The method according to Claim 4, wherein said compound is administered by inhalation.

6. The method according to Claim 1, wherein the treating is a therapeutic treatment, and dapansutrile is administered to the subject when the subject shows clinical signs and/or symptoms of asthma.

7. The method according to Claim 1, wherein the treating is a prophylactic treatment, and dapansutrile is administered to the subject before the onset of asthma.

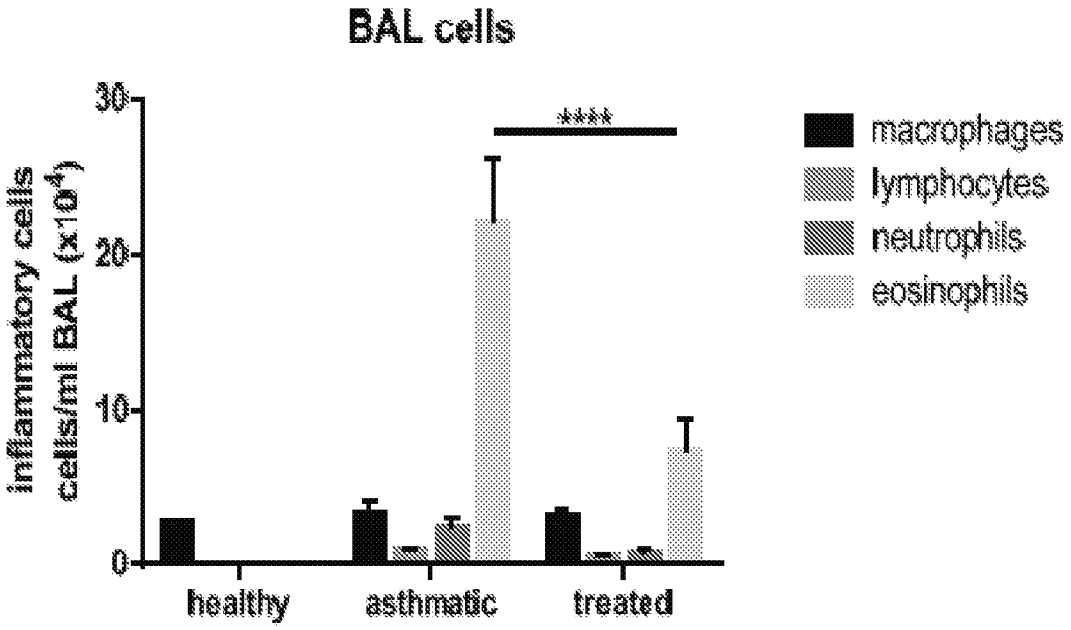

BAL cells
inflammatory cells
cells/ml BAL (x10^4)
30
20
10
0
****
macrophages
lymphocytes
neutrophils
eosinophils
healthy
asthmatic
treated

FIG. 1

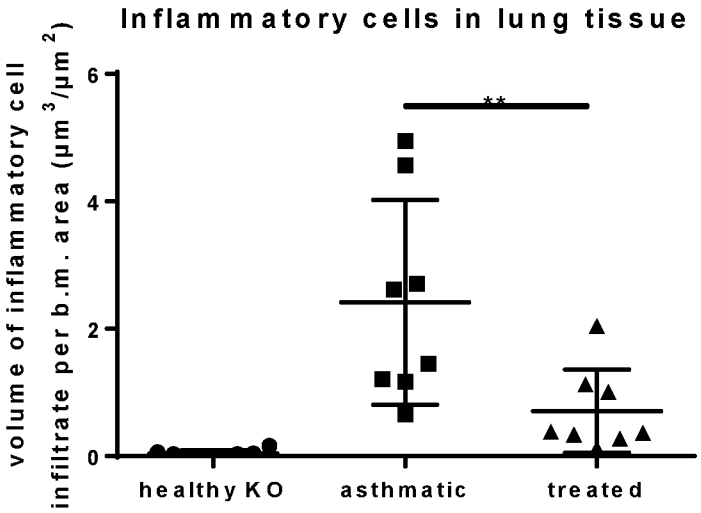

Inflammatory cells in lung tissue
volume of inflammatory cell
infiltrate per b.m. area (µm³/µm²)
6
4
2
0
**
healthy KO
asthmatic
treated

FIG. 2

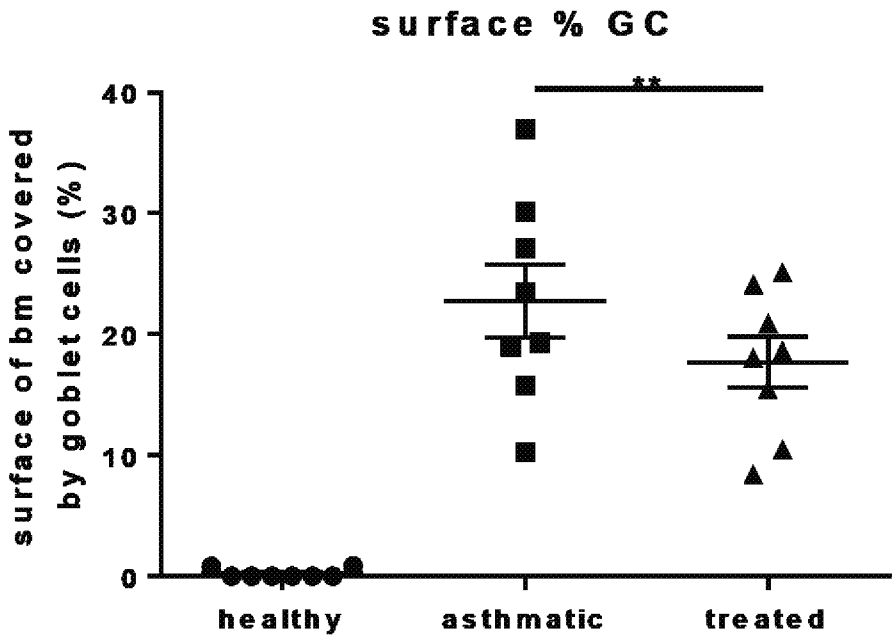

surface % GC
surface of bm covered by goblet cells (%)
40
30
20
10
0
**
healthy
asthmatic
treated

FIG. 3

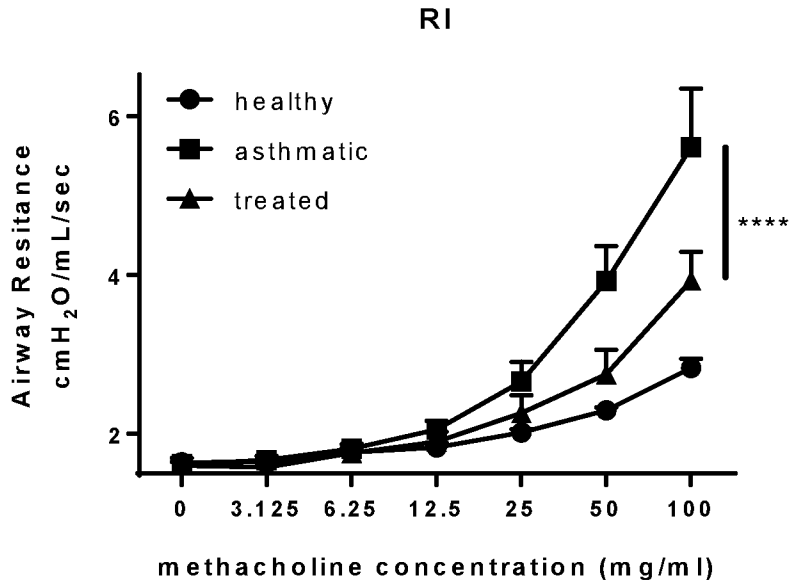

RI
Airway Resitance
cmH2O/mL/sec
6
4
2
healthy
asthmatic
treated
****
0
3.125
6.25
12.5
25
50
100
methacholine concentration (mg/ml)

FIG. 4

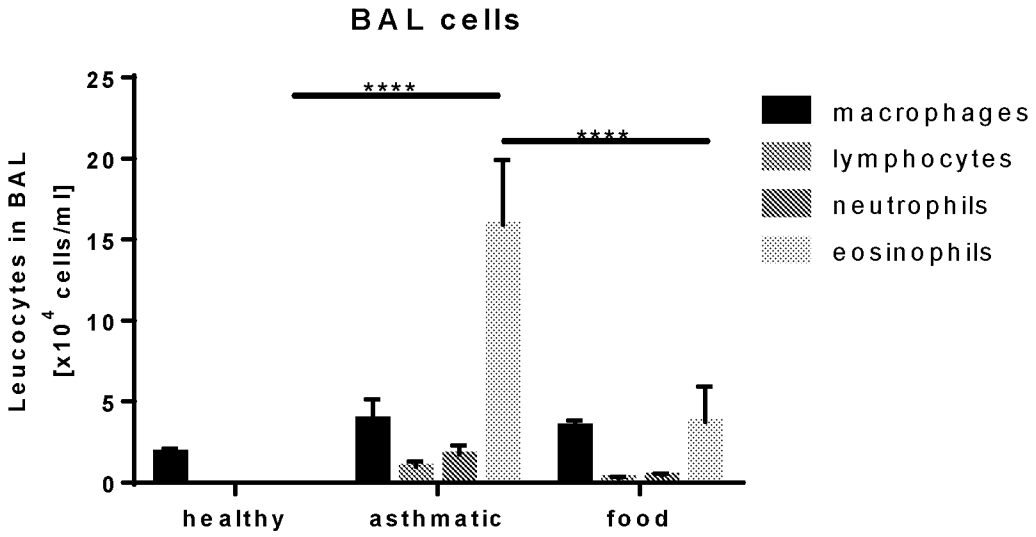

BAL cells
Leucocytes in BAL
[x10^4 cells/ml]
25
20
15
10
5
0
****
****
macrophages
lymphocytes
neutrophils
eosinophils
healthy
asthmatic
food

FIG. 5

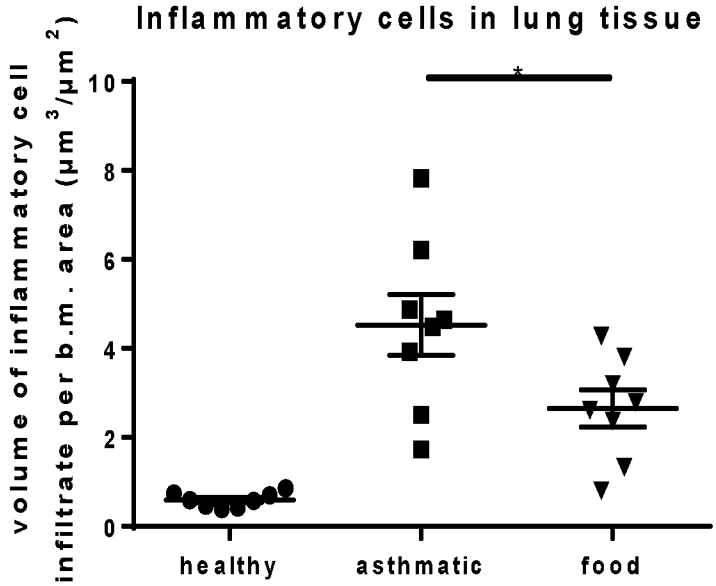

Inflammatory cells in lung tissue
volume of inflammatory cell infiltrate per b.m. area (µm³/µm²)
10
8
6
4
2
0
*
healthy
asthmatic
food

FIG. 6

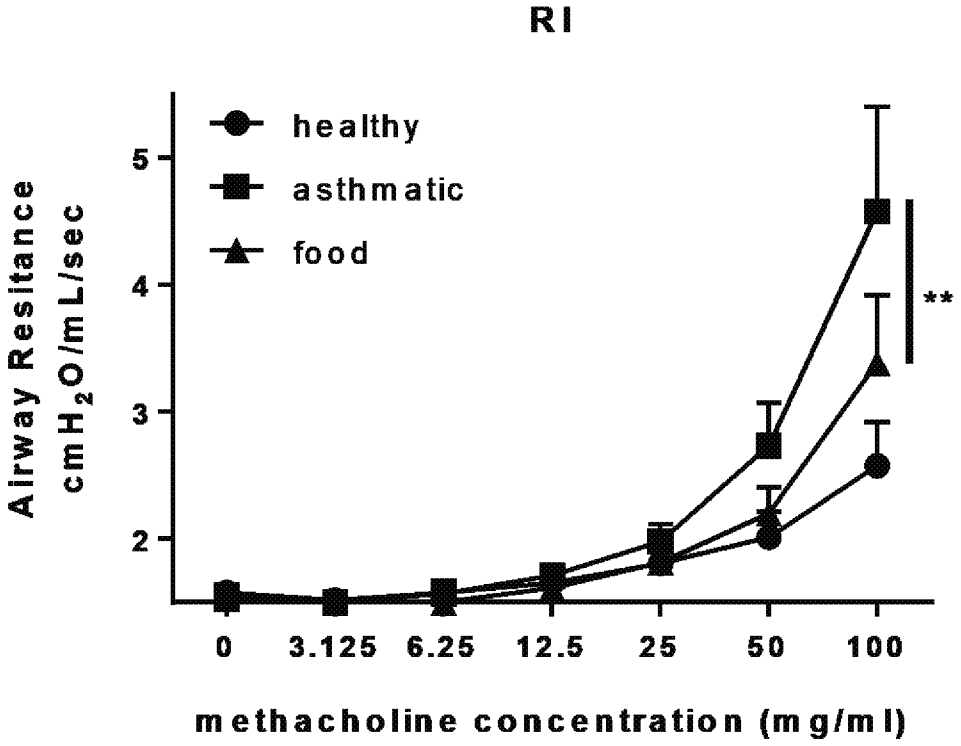

RI
healthy
asthmatic
food
Airway Resitance
cmH2O/mL/sec
5
4
3
2
**
0
3.125
6.25
12.5
25
50
100
methacholine concentration (mg/ml)

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/037696

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 11/06; C07C 317/26; C07C 317/28; C07C 317/48; C07C 317/50 (2020.01)
CPC - A61P 11/06; C07C 317/26; C07C 317/28; C07C 317/48; C07C 317/50 (2020.08)

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)
see Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2019/0119203 A1 (IFM TRE INC) 25 April 2019 (25.04.2019) entire document	1-7
Y	MARCHETTI et al. OLT1177, a β-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation, PNAS, 29 January 2018, Pgs. E1530-E1539	1-7
Y	WO 2017/129897 A1 (GALDERMA RESEARCH & DEVELOPMENT) 03 August 2017 (03.08.2017) see machine translation	7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 2020	28 SEP 2020
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