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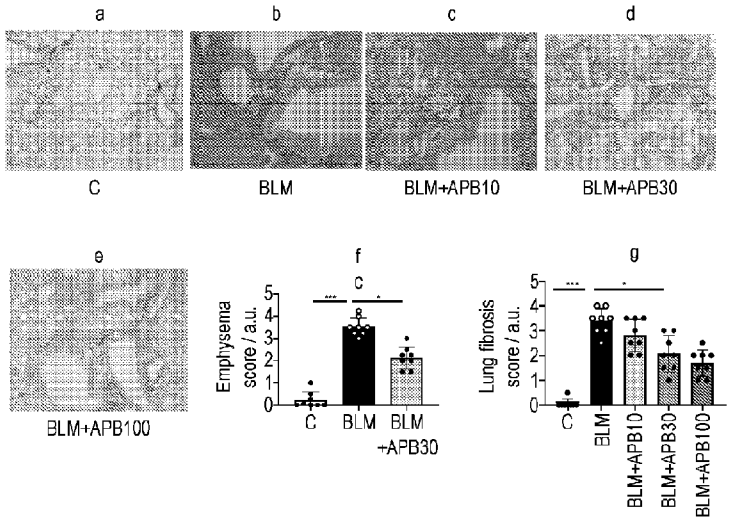

FIG 3

(57) Abstract: The present invention relates to 4 -phenylbutyric acid deriva f ives, namely the compounds *N*- (4-phenylbutanoyl-D-alanine) and/ or *N*- (4-phenylbutanoyl-L-alanine), for use in the treatment of fibrosis. The invention further relates to said 4- phenyl-butyric acid derivatives for use in the treatment of in terstitial lung disease and for use in the prevention of lung cancer.

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4-Phenylbutyric acid derivatives for use in the treatment of fibrosis

The present invention relates to 4-phenylbutyric acid derivatives for use in the treatment of fibrosis. It further relates to the 4-phenylbutyric acid derivatives for use in the treatment of interstitial lung disease and for use in the prevention of lung cancer.

Fibrosis is a pathological feature of many chronic diseases. Fibrosis is defined by the accumulation of excess extracellular matrix components. Fibrosis is similar to the process of scarring. Both involve stimulated fibroblasts laying down connective tissue, including collagen and glycosaminoglycans. Fibrosis is initiated when immune cells such as macrophages release soluble factors that stimulate fibroblasts.

A well-known pro-fibrotic mediator is TGF-β, which is released by macrophages as well as any damaged tissue between surfaces called interstitium. Other soluble mediators of fibrosis include connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), and interleukin 10 (IL-10). These initiate signal transduction pathways such as the AKT/mTOR (AKT: protein kinase B; mTOR: mammalian target of rapamycin) and SMAD (suppressor of mothers against decapentaplegic) pathways that ultimately lead to the proliferation and activation of fibroblasts, which deposit extracellular matrix into the surrounding connective tissue. This process of tissue repair is a complex one, with tight regulation of extracellular matrix synthesis and degradation ensuring maintenance of normal tissue architecture. However, the entire process, although necessary, can lead to a progressive irreversible fibrotic response if tissue injury is severe or re-

petitive, or if the wound healing response itself becomes deregulated.

If highly progressive, the fibrotic process eventually leads to organ malfunction and death. Fibrosis can affect nearly every tissue in the body. Affected tissues in the body include lung tissue, liver tissue, kidney tissue, brain tissue, heart tissue, arterial tissue, tissue from joints, skin tissue, bone marrow tissue and gastrointestinal tissue.

There is currently no cure for fibrosis. Treatment is aimed at slowing the course of the disease. The choice of treatment for fibrosis depends on the cause of the fibrosis and the tissue affected.

Fibrosis occurs in the context of a wide variety of diseases and there are many different subtypes of fibrosis:

Scleroderma is an autoimmune rheumatic disease characterised by fibrosis in the skin and internal organs and by injuries to small arteries. It is a rare connective tissue disorder with unknown and complex pathogenesis. It can be divided into two forms, localised scleroderma, or systemic sclerosis, which can further be classified as either limited systemic sclerosis (formerly known as the CREST syndrome comprising of calcinosis, Raynaud phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasia) or diffuse systemic sclerosis based on clinical and serological criteria. Common treatments of scleroderma include medication to improve circulation, medicines that reduce the activity of the immune system and slow the progression of the condition, steroids to relieve joint and muscle problems, and moisturising affected areas of skin.

Skin fibrosis, or dermal fibrosis or cutaneous fibrosis, is a symptom common to a number of diseases including scleroderma. Causes include chemical exposure, trauma and irradiation. It is characterised by an increase of fibrous connective tissues in the dermis or subcutis. It is characterized by proliferation of fibroblasts and collagen fibres in the dermis or around hair follicles, typically oriented parallel to the epidermis. In more severe cases, the fibrosis can extend deeper into the dermis and subcutis. Corticosteroids, immunotherapy drugs, immunoglobulins, and anti-fibrotic drugs are commonly used in the treatment for cutaneous fibrosis.

Interstitial lung disease (ILD) describes a large group of disorders, most of which cause progressive scarring of lung tissue. The scarring associated with interstitial lung disease eventually affects your ability to breathe and get enough oxygen into the bloodstream. High blood pressure in the lungs (pulmonary hypertension), respiratory failure and right-sided heart failure are common consequences of ILD. All interstitial lung diseases affect the lung interstitium, which is a lace-like network of tissue that goes through both lungs. It supports the lung's tiny air sacs, called alveoli. Types of ILD include interstitial pneumonia, idiopathic pulmonary fibrosis, nonspecific interstitial pneumonitis, hypersensitivity pneumonitis, cryptogenic organising pneumonia (COP), acute interstitial pneumonitis, desquamative interstitial pneumonitis, sarcoidosis and asbestosis.

The cause of most ILD is unknown. Bacteria, viruses, and fungi can cause interstitial pneumonia. Inhalation of lung sensitising substances and particles can also be a cause of ILD. These substances and particles include asbestos, bird excretions, coal dust, metal dust, grain dust, silica dust, pesticides, ozone and

talc. Some antibiotics, anti-inflammatory drugs, chemotherapy, radiotherapy and heart medications can also be the cause of ILD.

The treatment a patient receives depends on the type of ILD the patient has and its cause. Antibiotics and antifungal drugs treat most interstitial pneumonias caused by bacteria and fungi, respectively. Corticosteroids cause the immune system's activity to slow, which can cause slowing down the course of the disease. In advanced ILD, a lung transplant may be required.

Pulmonary fibrosis, or lung fibrosis, is one of many ILDs. In many cases, the exact cause of pulmonary fibrosis cannot be determined. In this case, the condition is called idiopathic pulmonary fibrosis. In other cases, the cause of pulmonary fibrosis is known. The causes for pulmonary fibrosis are the same as for ILD in general mentioned above. It can also be caused by a number of conditions including dermatomyositis, polymyositis, mixed connective tissue disease, systemic lupus erythematosus, rheumatoid arthritis, sarcoidosis, scleroderma and pneumonia. The influences described can lead to cell death with destruction of the alveolar wall leading to emphysema, fibroblast activation and proliferation. This results in TGF-β driven fibre and matrix deposition, defective alveolar cell repair and severe fibrosis.

As pulmonary fibrosis progresses, it may lead to complications such as blood clots in the lungs, a collapsed lung or lung infections. Also, the risk of developing lung cancer is increased in pulmonary fibrosis patients.

There is a range of comorbidities of pulmonary fibrosis. Comorbidity is the presence of one or more additional conditions often co-occurring with a primary condition. The additional condition(s) can be the cause of the primary condition, or the addi-

tional condition(s) can be caused by the primary condition, or the conditions exist simultaneously regardless of their causal relationship. In the last case, the conditions might have a common cause. Typical comorbidities of pulmonary fibrosis, and idiopathic pulmonary fibrosis in particular, are pulmonary hypertension and acute exacerbation.

In summary, the treatment options for all types of fibrosis and diseases associated with fibrosis as described are extremely limited and there is a great need for new treatments that can at least alleviate the symptoms and signs arising from fibrosis and/or that can prevent fibrosis at least partially from developing.

The sodium salt of 4-phenylbutyric acid, sodium 4-phenylbutyrate is used in the treatment of urea cycle disorders. In addition, it has also been described in patents and in the scientific literature for a number of medical uses. These uses encompass a variety of illnesses, such as benign prostatic hyperplasia, cancer, HIV, kidney failure and thalassemia. For example, WO 9510271 A2 (US GOV HEALTH & HUMAN SERV, 20 April 1995) discloses compositions and methods using 4-phenylbutric acid derivatives for therapy and prevention of a number of pathologies. Furthermore, EP 2599767 A1 (LUNAMED AG, 05 June 2013) describes a number of 4-phenylbutric acid derivatives for use in cancer therapy and other pharmaceutical applications. EP 2389932 A1 (LUNAMED AG, 30 November 2011) refers to pharmaceutical compositions comprising the histone hyperacetylating agent phenylbutyric acid for use in the treatment of a genetic disorder, like treatment of depression, vaginitis and varicosis or the prevention of Sudden Infant Death Syndrome with a genetic disorder background. T.A. Gudasheva et al. (Design and synthesis of cholecystokinin-4 dipeptide analogues with anxiolytic and anxio-

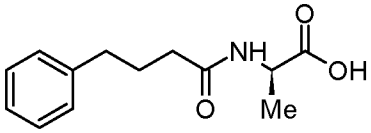
genic activities. Russ. J. Bioorganic Chem. 2007, Vol. 33, No. 4, pages 383–389) disclose the design and synthesis of cholecystokinin-4 dipeptide analogues with anxiolytic and anxiogenic activities. However, the scope of medical uses for 4-phenylbutyric acid and derivatives thereof is still not fully explored.

The 4-phenylbutyric acid derivatives *N*-(4-phenylbutanoyl-D-alanine) (Formula 1.1) and *N*-(4-phenylbutanoyl-L-alanine) (Formula 1.2) have been found to be effective in the treatment of bowel diseases in EP 3283066 B1 (PHENOTEC AG, 11 May 2022). Diseases treatable with the 4-phenylbutyric acid derivatives of Formula 1.1 and/or Formula 1.2 comprise Crohn's disease, ulcerative colitis, inflammatory diseases of the bile ducts, vulvovaginitis, varicose veins, major depressive disorder and tinnitus.

It is an object of the present invention to provide an improved treatment of various types of fibrosis, in particular pulmonary fibrosis, and diseases associated with fibrosis. In particular, it is an object to at least alleviate the symptoms and signs arising from fibrosis and/or to prevent fibrosis at least partially from developing.

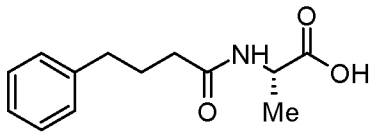
The problems are solved with 4-phenylbutyric acid derivatives according to the independent claims.

A first aspect of the invention relates to a 4-phenylbutyric acid derivative, namely:
N-(4-phenylbutanoyl-D-alanine),



(Formula 1.1); and/or

N-(4-phenylbutanoyl-L-alanine),



(Formula 1.2)

or pharmaceutically acceptable salts, solvates and/or hydrates thereof for use in the treatment of fibrosis.

In the context of the present invention, the term "or" is to be understood as an exclusionary disjunction. "A or B" states that exactly one of the two statements is true (if the disjunction is true).

The above-mentioned 4-phenylbutric acid derivatives may be employed as a single stereoisomer or as a mixture of stereoisomers. Such stereoisomers can be enantiomers. The compounds may be used as a racemate. However, preferably they are used in enantiomerically pure form. Furthermore, the 4-phenylbutric acid derivatives may be employed in form of the free acid, the free base, as a salt, as a solvate, or as a hydrate.

In the context of the present invention, the term "pharmaceutically acceptable salt" is used to refer to an ionisable active pharmaceutical ingredient (API) that has been combined with a counterion to form a neutral complex. A pharmaceutically acceptable salt is a salt of an API that is clinically equivalent to the API, meaning that the addition of the counterion of the resulting salt does not lead to clinically severe adverse events; or substantially the same maximum venous blood serum concentration, c_{max}, is achieved; or it takes substantially the same time until the maximum venous blood serum concentration is reached, t_{max}; or the area under the curve, AUC, based on venous

blood serum concentration as a function of time is substantially the same; or the API and its salt have substantially the same partition coefficient, log P; or the API and its salt have substantially the same dissociation constant, pK_a; or the API and its salt have substantially the same melting point; or any combination of these conditions. Preferably, pharmaceutically acceptable salts comprise salts of the compounds in Formula 1.1 and Formula 1.2 with a counterion selected from the group consisting of aluminium, arginine, benzathine, calcium, chloroprocaine, choline, diethanolamine, ethanolamine, ethylenediamine, lysine, magnesium, histidine, lithium, meglumine, potassium, procaine, sodium, triethylamine, zinc, acetate, aspartate, benzenesulfonate, benzoate, besylate, bicarbonate, bitartrate, bromide, camsylate, carbonate, chloride, citrate, decanoate, edetate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolate, hexanoate, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, octanoate, oleate, pamoate, pantothenate, phosphate, polygalacturonate, propionate, salicylate, stearate, acetate, succinate, sulfate, tartrate, teoclate and tosylate.

A solvate of a compound is the crystal structure of said compound where a solvent is incorporated into the lattice. In the context of the present invention, a pharmaceutically acceptable solvate is a solvate of an API that is clinically equivalent to the API, meaning that the solvent incorporated into the lattice does not lead to clinically severe adverse events; or substantially the same maximum venous blood serum concentration, c_{max}, is achieved; or it takes substantially the same time until the maximum venous blood serum concentration is reached, t_{max}; or the area under the curve, AUC, based on venous blood serum concentration as a function of time is substantially the same; or the

API and its solvate have substantially the same melting point; or any combination of these conditions. Preferably, pharmaceutically acceptable solvates comprise solvates of the compounds in Formula 1.1 and Formula 1.2 with an incorporated solvent selected from the group consisting of H_2O, ethanol, DMSO, propanediol, acetone, 1-propanol, isopropanol or any combination thereof.

In the context of the present invention, a pharmaceutically acceptable hydrate is a pharmaceutically acceptable solvate, wherein H_2O is the solvent incorporated into the crystal lattice.

In the context of the present invention, a treatment of a disease refers to an effect as a result of the administration of a compound to a living organism, wherein the effect is the prevention or delay of onset of symptoms; or amelioration of symptoms; or prevention or delay of onset of signs; or amelioration of signs; or a combination thereof.

As it is understood in the art, a symptom is something felt or experienced, such as pain or dizziness. As it is understood in the art, a sign is an objective observable indication of a disease, injury, or abnormal physiological state that may be detected during a physical examination, examining the patient history, or diagnostic procedure. Disease biomarkers are a type of sign. Signs and symptoms are not mutually exclusive.

In the case of pulmonary fibrosis, for example, these symptoms and signs comprise shortness of breath (dyspnoea), a dry cough, fatigue, unintended weight loss, aching muscles (myalgia), aching joints (arthralgia), widening and rounding of the tips of the fingers and/or toes (clubbing), the presence of fibrosis biomarkers, histopathological confirmation of the presence of fibrotic tissue, spirometrical confirmation of reduced forced ex-

piratory volume in 1 second (FEV1), and spirometrical confirmation of reduced forced vital capacity (FVC).

Organs where fibrotic tissue can form include the lungs, heart, liver, skin, cardiovascular vessels, brain, gastrointestinal tract, bone marrow and kidneys.

It has been found that the 4-phenylbutyric acid derivatives according to the invention attenuate fibrosis. Even though these compounds have been found to be effective in the treatment of the bowel diseases described in in EP 3283066 B1 (PHENOTEC AG, 11 May 2022), it was surprising that these compounds would also be effective in the treatment of fibrosis.

Without wishing to be bound by any theory, it is believed that the 4-phenylbutyric acid derivatives described in the present invention exert their effect at a point in the biochemical cascade of formation of fibrosis, which plays a role in all types of fibrosis or at least in most types of fibrosis.

4-phenylbutric acid derivatives according to the invention are physiologically well tolerated.

In a preferred embodiment of the first aspect of the invention, the fibrosis is accompanied by scleroderma.

As described above, scleroderma is an autoimmune rheumatic disease. Among other characteristics, it can comprise development of fibrosis of the skin and other organs such as the lung.

Another preferred embodiment relates to the 4-phenylbutyric acid derivative according to the first aspect of the present invention for use in the treatment of skin fibrosis.

A second aspect of the present invention relates to a 4-phenylbutyric acid derivative, namely:
N-(4-phenylbutanoyl-D-alanine) (Formula 1.1); and/or
N-(4-phenylbutanoyl-L-alanine) (Formula 1.2);
or pharmaceutically acceptable salts, solvates and/or hydrates thereof for use in the treatment of interstitial lung disease.

As described above, interstitial lung disease (ILD) comprises several disorders causing progressive scarring of lung tissue. It has been found that the compound(s) according to the invention can be used for the treatment of said progressive scarring of lung tissue.

A preferred embodiment relates to the 4-phenylbutyric acid derivative according to the first and/or second aspect of the present invention for use in the treatment of pulmonary fibrosis.

It was found that signs of pulmonary fibrosis, as both a type of fibrosis and a type of ILD, can be attenuated using the 4-phenylbutyric acid derivatives according to the invention. In particular, the treatment reduces the number of dead epithelial cells in the bronchoalveolar lavage (BAL) accompanied by a reduction in total protein and DNA. Moreover, the treatment leads to a significant reduction of macrophages and neutrophils and concomitant myeloperoxidase in the BAL. Therefore, the treatment reduces the acute respiratory barrier injury that can be induced by certain chemotherapeutic agents, for example. Acute respiratory barrier injury results often in the development of fibrotic tissue during the repair process. If the signs of the acute respiratory barrier injury are reduced, the resulting formation of fibrosis is limited, too.

In addition, the treatment reduces the typical biomarkers of lung fibrosis. These biomarkers include TGF-β in the BAL, IL-1β in the BAL fluid (BALF), IL-6 in the BALF, TIMP-1 in the BALF, MMP-9 in the BALF, and collagen in the BAL. Also, histological data indicate the reduction of lung emphysema and a reduction in the formation of fibrotic tissue.

Another preferred embodiment relates to the 4-phenylbutyric acid derivative according to the first and/or second aspect of the invention for use in the treatment of idiopathic pulmonary fibrosis (IPF).

If the cause of the development of fibrotic tissue in the lung is unknown, the condition is called idiopathic pulmonary fibrosis.

A further preferred embodiment relates to the 4-phenylbutyric acid derivative according to the first and/or second aspect of the invention for use in the treatment of pulmonary fibrosis, wherein the pulmonary fibrosis is induced by chemotherapeutic agents and/or by radiotherapy, in particular by chemotherapeutic agents selected from the group consisting of bleomycin, mitomycin C, busulfan, cyclophosphamide, chlorambucil, melphalan, methotrexate, 6-mercaptopurine, azathioprine, cytosine arabinoside, gemcitabine, fludarabine, bis(chloroethyl) nitrosourea, chloroethyl cyclohexyl nitrosourea, methyl chloroethyl cyclohexyl nitrosourea, etoposide, paclitaxel, docetaxel, all-trans retinoic acid, gefitinib, imatinib mesylate, irinotecan, interferons, interleukin-2, tumour necrosis factor-α, or any combination thereof; more in particular, wherein the pulmonary fibrosis is induced by bleomycin.

Radiation cancer therapy can result in tissue damage known as radiation fibrosis. Similarly, a wide variety of chemotherapeutic agents have been associated with pulmonary toxicities. One of the effects of those agents can be the development of severe fibrosis in the lungs resulting in respiratory insufficiency and possibly death (LIMPER, A. H. Chemotherapy-induced lung disease. Clin. Chest. Med. 2004, Vol. 25, pages 53-64). It was found that the 4-phenylbutyric acid derivatives according to the invention can attenuate the adverse effects of such chemotherapeutic agents.

A yet further preferred embodiment relates to the 4-phenylbutyric acid derivative according to the first and/or second aspect of the present invention, wherein the pulmonary fibrosis or idiopathic pulmonary fibrosis is accompanied by pulmonary hypertension as a complication.

Pulmonary hypertension happens when the pressure in the blood vessels leading from the heart to the lungs is too high.

Another preferred embodiment relates to the 4-phenylbutyric acid derivative according to the first and/or second aspect of the present invention for use in the treatment of the syndrome of combined pulmonary fibrosis and emphysema (CPFE).

Emphysema, the presence of air-filled spaces caused by the breakdown of the walls of the alveoli, is relatively common in patients with fibrotic ILD. The co-occurrence of these conditions is known as the syndrome of CPFE.

A further preferred embodiment relates to the 4-phenylbutyric acid derivative according to the first and/or second aspect of the present invention, wherein the pulmonary fibrosis or idio-

pathic pulmonary fibrosis is accompanied by acute exacerbation as a complication.

Acute exacerbation is a sudden worsening of symptoms of pulmonary diseases. Those symptoms include shortness of breath and increased amount of phlegm.

A preferred embodiment relates to the 4-phenylbutyric acid derivative according to the first and/or second aspect of the present invention, wherein fibrosis formation occurs on an inflammation-independent immune pathway.

Even though inflammatory processes play a role in certain immune pathways of fibrosis formation, inflammation and fibrosis can represent two distinct processes requiring the activation of two different immune pathways, at least in certain types of fibrosis (Lo Re, S. et al. Uncoupling between Inflammatory and Fibrotic Responses to Silica, PLOS 2014, Vol. 9, No. 7, pages 1-10). Without wishing to be bound by any theory, it is believed that the mode of action the 4-phenylbutyric derivatives according to the invention takes place in immune pathways that lead to fibrosis formation, which occur independently of inflammation.

A third aspect of the present invention relates to a 4-phenylbutyric acid derivative, namely:
N-(4-phenylbutanoyl-D-alanine) (Formula 1.1); and/or
N-(4-phenylbutanoyl-L-alanine) (Formula 1.2);
or pharmaceutically acceptable salts, solvates and/or hydrates thereof for use in the prevention of lung cancer.

Lung cancer is an important comorbidity encountered in ILD patients, especially in pulmonary fibrosis patients. Although the causal relationship between lung cancer and ILD is not yet com-

pletely understood, the scientific literature suggests that ILD may promote lung cancer development. Specifically in IPF, some molecular and genetic features of its pathogenesis and progression are linked to mechanisms that favour development of malignancy (KEWALRAMANI, N. et al. Lung cancer in patients with fibrosing ILDs, ERJ Open Res. 2022, Vol 8, 00115-2022).

Since scientific data suggest an increased risk of lung cancer in patients with ILD, in particular IPF, the 4-phenylbutyric acid derivatives according to the invention are suitable for reducing the risk of lung cancer.

A preferred embodiment relates to the 4-phenylbutyric acid derivative according to any one aspect of the present invention, wherein the treatment is a preventive and/or curative treatment, wherein the 4-phenylbutyric acid derivative is administered at a dose of between 1 mg/kg/day and 500 mg/kg/day, preferably between 10 mg/kg/day and 200 mg/kg/day, more preferably between 20 mg/kg/day and 200 mg/kg/day, and most preferably 30 mg/kg/day.

In the context of the present invention, a preventive treatment is a treatment, wherein an active pharmaceutical ingredient (API) is administered prior to onset of symptoms and/or signs of a particular disease to reduce the risk of developing that disease or prevent that disease entirely. For example, if a subject is exposed to conditions that may increase the likelihood of developing a disease, the API may be administered as a preventive measure. If, for example, a patient is treated with a chemotherapeutic agent that can induce the development of fibrotic tissue, the 4-phenylbutyric acid derivatives according to the invention can be administered to prevent development of fibrosis.

In the context of the present invention, a curative treatment is a treatment, wherein an API is administered after onset of symptoms and/or signs of a particular disease to prevent or slow down the development of the disease.

In the context of the present invention, a dose of, for example, 30 mg/kg/day indicates that, within one day, an amount of 30 mg of the API is administered per kg bodyweight of the subject.

Another preferred embodiment relates to the 4-phenylbutyric acid derivative according to any one aspect of the present invention, wherein the 4-phenylbutyric acid derivative is administered

- orally, in particular as a tablet to swallow, or as a sirup;
- intramuscularly;
- intraperitoneally;
- intravenously;
- transdermally, in particular as a transdermal patch;
- rectally, in particular as a suppository; or
- any combination thereof.

The medicament of the present invention may for example be administered partially in combination with conventional injectable liquid carriers, such as water or suitable alcohols. Conventional pharmaceutical recipients for injection, such as stabilizing agents, solubilizing agents, and buffers, may be included in such injectable compositions. These compositions may contain conventional ingredients such as binding agents, fillers, lubricants, and acceptable wetting agents. The compositions may take any convenient form, such as tablets, pellets, granule, capsules, lozenges, aqueous or oily solutions, suspensions, emulsions or dry powdered forms suitable for reconstitution with water or other suitable liquid media before use. The multiparticulate forms, such as pellets or granules, may be filled

into a capsule, compressed into tablets or suspended in a suitable liquid. Furthermore, suitable controlled release formulations and methods for their preparations are known from the prior art.

The dosage form depends in particular on the disease to be treated. For example, if pulmonary fibrosis is to be treated, oral or intravenous administration is suitable. However, if the active substance according to the invention is to be used in the case of already advanced pulmonary fibrosis, access to the active substance via the blood capillaries may possibly be hindered. Instead of intravenous administration or administration via the gastrointestinal tract, a transdermal patch applied on the chest of a subject is suitable in such a case, for example, whereby the active substance can reach the lung tissue through the skin.

Further advantages and aspects of the present invention become apparent from the description of the following examples.

General

The aim of the following implementation examples was to test the pharmacological efficacy of APB in the mouse model of bleomycin (BLM)-induced lung fibrosis by daily gavage for up to 14 days.

Pulmonary fibrosis is modelled experimentally by a single intratracheal administration of bleomycin (BLM; 1.5 mg/kg) in 8 weeks to 10 weeks old C57BI mice characterised by an acute injury of the alveolar epithelium followed by emphysema, repair and lung fibrosis.

APB powder was dissolved in physiological saline and administered in a volume of 200 µl per gavage.

The mice were observed daily for clinical signs and adverse effects and body weights were recorded before each gavage to adjust the APB dose.

The mice were sacrificed at day 15 for full analyses:

Bronchoalveolar lavage (BAL) was performed by rinsing the lungs four times with 0.5 ml physiological saline solution at room temperature. After centrifugation at 400 g for 10 min at 4 °C, the supernatant (cell-free BAL fluid) was stored at -20 °C for cytokine analysis. The total cells were counted and differential cell counts were assessed on Giemsa stained cytospins.

Cytokines measurement in BALF: Levels of CXCL1, IL-1β, IL-6, TIMP-1, MMP-9 and MPO in BALF were determined by enzyme-linked immunosorbent assay (ELISA), using commercial kits from R&D (Abingdon, UK). Total protein, DNA and collagen levels were assessed by Sircol assay as described before (BESNARD, A.-G. et al. CXCL6 antibody neutralization prevents lung inflammation and fibrosis in mice in the bleomycin model. J. Leukoc. Biol. 2013, Vol. 94, p. 1317-1323).

Microscopic analyses of lungs: The right lungs were fixed in 4 % buffered paraformaldehyde, processed, embedded in paraffin, cut at 3 µm and stained with haematoxylin and eosin. The microscopic slides were analysed for morphological alterations, assessed by a semi-quantitative score 0-5 (with increasing severity) as described (FANNY, M. et al. The IL-33 Receptor ST2 Regulates Pulmonary Inflammation and Fibrosis to Bleomycin. Front. Immunol.

2018, Vol. 9, p. 1-12). The left lung was frozen at -80° for potential future analyses.

Short description of the figures

Fig. 1a-1f: Treatment with APB (*N*-(4-phenylbutanoyl-D-alanine)) attenuates the BLM-induced cell recruitment in bronchoalveolar lavage (BAL). Mice were challenged by a single intratracheal administration of bleomycin in saline (BLM; 1.5 mg/kg) and were treated with or without APB at 10 mg/kg, 30 mg/kg, or 100 mg/kg daily for 14 days, control mice received intratracheally the vehicle only. Eight mice/group, mean and SD.

Fig. 2a-2f: Treatment with APB attenuates the BLM-induced increased cytokine levels in the bronchoalveolar fluid (BALF). After an intratracheal instillation of bleomycin (BLM) received daily APB at 10 mg/kg, 30 mg/kg, or 100 mg/kg for 14 days and were analysed at day 15. Control mice received intratracheally the vehicle only. Eight mice/group, mean and SD.

Fig. 3a-3g: Treatment with APB attenuates lung fibrosis by microscopy. After a single intratracheal instillation BLM daily APB at 10 mg/kg, 30 mg/kg, or 100 mg/kg for 14 days mice were analysed at day 15. Control mice received intratracheally the vehicle only. Eight mice/group, mean and SD.

Fig. 4a-4f: Curative treatment with APB attenuates the emphysema and fibrosis of the lungs. After an intratracheal instillation of bleomycin (BLM) the mice received from day 7 APB daily at 30 mg/kg and were analysed at day 14. Control mice received intratracheally the vehicle only. Six mice/group, mean and SD.

Example 1: Reduction of BLM-induced cell recruitment

C57Bl mice with BLM-induced pulmonary fibrosis were treated with APB (*N*-(4-phenylbutanoyl-D-alanine)) at 10 mg/kg/day, 30 mg/kg/day, or 100 mg/kg/day for 14 days. The mice were followed daily for clinical adverse effects. Body weight was taken before compound administration. At day 15, the mice were sacrificed for inflammatory cells recruitment and microscopic analysis.

Fig. 1 shows levels of biomarkers for acute respiratory barrier injury of the control group (C), the group that received BLM only (BLM) and the groups that received both BLM and APB at varying doses (10 mg/kg APB per day: BLM+APB10; 30 mg/kg APB per day: BLM+APB30; 100 mg/kg APB per day: BLM+APB100), after 14 days. The total cells in the BAL (Fig. 1a; $x10^4$), the number of neutrophils in the BAL (Fig. 1b; $x10^4$), the number of macrophages in the BAL (Fig. 1c; $x10^5$), the number of lymphocytes in the BAL (Fig. 1d; $x10^4$), the DNA in the BAL (Fig. 1e; ng/ml) and the total protein in the BAL (Fig. 1f; µg/ml) were measured.

The test compound APB was given daily by gavage at 0 mg/kg/day, 10 mg/kg/day, 30 mg/kg/day and 100 mg/kg/day for 14 days. APB at 100 mg/kg/day was well tolerated. No clinical adverse effects related to the administration of the test compound was observed.

DNA and total protein levels were increased by BLM as a sign of disruption of the respiratory barrier. As described above, this acute respiratory barrier injury often leads to fibrosis. The data shows that daily APB gavage dose-dependently reduces BLM-induced total cells, macrophages, lymphocytes and neutrophils recruitment. Therefore, APB reduces the acute respiratory barrier injury caused by BLM.

Example 2: Reduction of the typical biomarkers of pulmonary fibrosis

Mice with BLM-induced fibrosis were treated with APB at doses of 10 mg/kg/day, 30 mg/kg/day and 100 mg/kg/day for 14 days. Biomarkers indicative for pulmonary fibrosis were measured after 14 days.

Fig. 2 shows levels of biomarkers for fibrosis of the control group (C), the group that received BLM only (BLM) and the groups that received both BLM and APB at varying doses (10 mg/kg APB per day: BLM+APB10; 30 mg/kg APB per day: BLM+APB30; 100 mg/kg APB per day: BLM+APB100), after 14 days. CXCL1 in the BALF (Fig. 2a; pg/ml), IL-1β in the BALF (Fig. 2b; pg/ml), IL-6 in the BAL (Fig. 2c; pg/ml), TIMP-1 in the BALF (Fig. 2d; pg/ml), MMP-9 in the BALF (Fig. 2e; ng/ml) and collagen in the BAL (Fig. 2f; µg/ml) were measured.

BLM leads within 14 days to increased cytokine production such as TGF-β, IL-1β, IL-6, TIMP-1, MMP-9, and collagen levels in the BALF, which are involved in the fibrotic disease process in mice. APB had a dose dependent effect on CXCL1, IL-1β, IL-6 levels. Further, the remodelling factors TIMP-1 and MMP-9 as well as collagen were reduced by APB in the BALF.

Example 3: Reduction of lung emphysema and pulmonary fibrosis

The lung tissue of the mice in example 2 was analysed histologically after 14 days.

Fig. 3a-e show histological images of the lung tissue of the control group (C), the group that received BLM only (BLM) and the groups that received both BLM and APB at varying doses

(10 mg/kg APB per day: BLM+APB10; 30 mg/kg APB per day: BLM+APB30; 100 mg/kg APB per day: BLM+APB100), after 14 days. Fig. 3f shows an emphysema score determined from the histological images, and Fig. 3g shows a lung fibrosis score determined from the histological images.

Microscopic analysis of the lung tissue at 14 days after single BLM administration reveals a destruction and repair of the alveolar cells resulting in emphysema and distinct focal fibrotic areas with proliferation of fibroblasts and interstitial matrix deposition. Treatment with APB significantly reduced the lung fibrosis score and the emphysema score. This reduction is dose-dependent, as the effect increases with higher doses.

Example 4: Curative treatment of APB on BLM-induced pulmonary fibrosis

While the daily treatment with APB in mice with BLM-induced pulmonary fibrosis was already started prior to any symptoms and/or signs of pulmonary fibrosis (preventive treatment) in examples 1-3, treatment with APB (30 mg/kg/day) started at day 7 after intratracheal instillation of BLM in example 4, to investigate the effect of a delayed APB administration (curative treatment). Without wishing to be bound by theory, it is assumed that fibrotic tissue and fibrosis biomarkers have already developed after 7 days.

Fig. 4 shows levels of biomarkers for fibrosis, the lung fibrosis score and the emphysema score of the control group (C), the group that received BLM only (BLM) and the group that received both BLM and APB (BLM+APB30; APB treatment starting at day 7), after 14 days. TIMP-1 in the BALF (Fig. 4a; pg/ml), MMP-9 in the BALF (Fig. 4b; ng/ml), collagen in the BAL (Fig. 4c; µg/ml),

lung fibrosis score (Fig. 4d; a.u.), emphysema score (Fig. 4e; a.u.), and collagen in the lung (Fig. 4f; µg/ml) were measured.

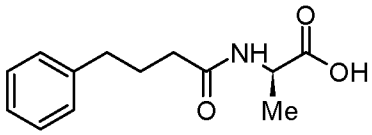
Reduced amounts of fibrotic mediators such as TIMP-1, MMP-9 and soluble collagen in BAL were found. Furthermore, microscopy analysis of the lung showed attenuated fibrosis and emphysema and reduced collagen in the lung tissue. Thus, APB is an effective treatment by either preventive or curative administration in the murine fibrosis model.

The data of examples 1-4 demonstrate that the daily oral administration of APB at has a dose-dependent preventive and curative effect attenuating lung fibrosis in the BLM mouse model of lung fibrosis.

Claims

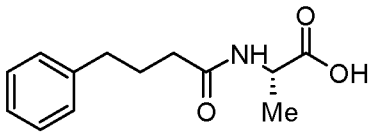
1. 4-Phenylbutyric acid derivative, namely:

- *N*-(4-phenylbutanoyl-D-alanine),



(Formula 1.1); and/or

- *N*-(4-phenylbutanoyl-L-alanine),



(Formula 1.2)

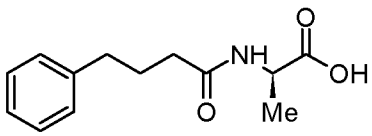
or pharmaceutically acceptable salts, solvates and/or hydrates thereof for use in the treatment of fibrosis.

2. 4-Phenylbutyric acid derivative according to claim 1, wherein the fibrosis is accompanied by scleroderma.

3. 4-Phenylbutyric acid derivative according to any one of the preceding claims for use in the treatment of skin fibrosis.

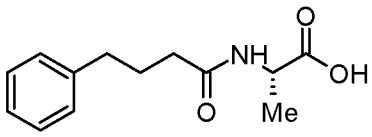
4. 4-Phenylbutyric acid derivative, namely:

- *N*-(4-phenylbutanoyl-D-alanine),



(Formula 1.1); and/or

– *N*-(4-phenylbutanoyl-L-alanine),



(Formula 1.2)

or pharmaceutically acceptable salts, solvates and/or hydrates thereof for use in the treatment of interstitial lung disease.

5. 4-Phenylbutyric acid derivative according to any of claims 1, 2 or 4 for use in the treatment of pulmonary fibrosis.

6. 4-Phenylbutyric acid derivative according to claim 5 for use in the treatment of idiopathic pulmonary fibrosis.

7. 4-Phenylbutyric acid derivative according to claim 5, wherein the pulmonary fibrosis is induced by chemotherapeutic agents and/or by radiotherapy, in particular by chemotherapeutic agents selected from the group consisting of bleomycin, mitomycin C, busulfan, cyclophosphamide, chlorambucil, melphalan, methotrexate, 6-mercaptopurine, azathioprine, cytosine arabinoside, gemcitabine, fludarabine, bis(chloroethyl) nitrosourea, chloroethyl cyclohexyl nitrosourea, methyl chloroethyl cyclohexyl nitrosourea, etoposide, paclitaxel, docetaxel, all-trans retinoic acid, gefitinib, imatinib mesylate, irinotecan, interferons, interleukin-2, tumour necrosis factor-α, or any combination thereof; more in particular, wherein the pulmonary fibrosis is induced by bleomycin.

8. 4-Phenylbutyric acid derivative according to any of claims 5 or 6, wherein the pulmonary fibrosis or idiopathic pulmonary fibrosis is accompanied by pulmonary hypertension as a

complication.

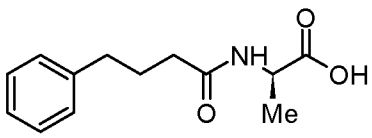
9. 4-Phenylbutyric acid derivative according to any of claims 5 or 6 for use in the treatment of the syndrome of combined pulmonary fibrosis and emphysema.

10. 4-Phenylbutyric acid derivative according to any of claims 5 or 6, wherein the pulmonary fibrosis or idiopathic pulmonary fibrosis is accompanied by acute exacerbation as a complication.

11. 4-Phenylbutyric acid derivative according to any one of the preceding claims, wherein fibrosis formation occurs on an inflammation-independent immune pathway.

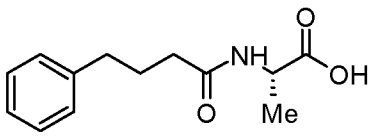
12. 4-Phenylbutyric acid derivative, namely:

- *N*-(4-phenylbutanoyl-D-alanine),



(Formula 1.1); and/or

- *N*-(4-phenylbutanoyl-L-alanine),



(Formula 1.2)

or pharmaceutically acceptable salts, solvates and/or hydrates thereof for use in the prevention of lung cancer.

13. 4-Phenylbutyric acid derivative according to any one of the preceding claims, wherein the treatment is a preventive and/or curative treatment, wherein the 4-phenylbutyric acid

derivative is administered at a dose of between 1 mg/kg/day and 500 mg/kg/day, preferably between 10 mg/kg/day and 200 mg/kg/day, more preferably between 20 mg/kg/day and 200 mg/kg/day, and most preferably 30 mg/kg/day.

14. 4-Phenylbutyric acid derivative according to any one of the preceding claims, wherein the 4-phenylbutyric acid derivative is administered

- orally, in particular as a tablet to swallow, or as a sirup;
- intramuscularly;
- intraperitoneally;
- intravenously;
- transdermally, in particular as a transdermal patch;
- rectally, in particular as a suppository; or
- any combination thereof.

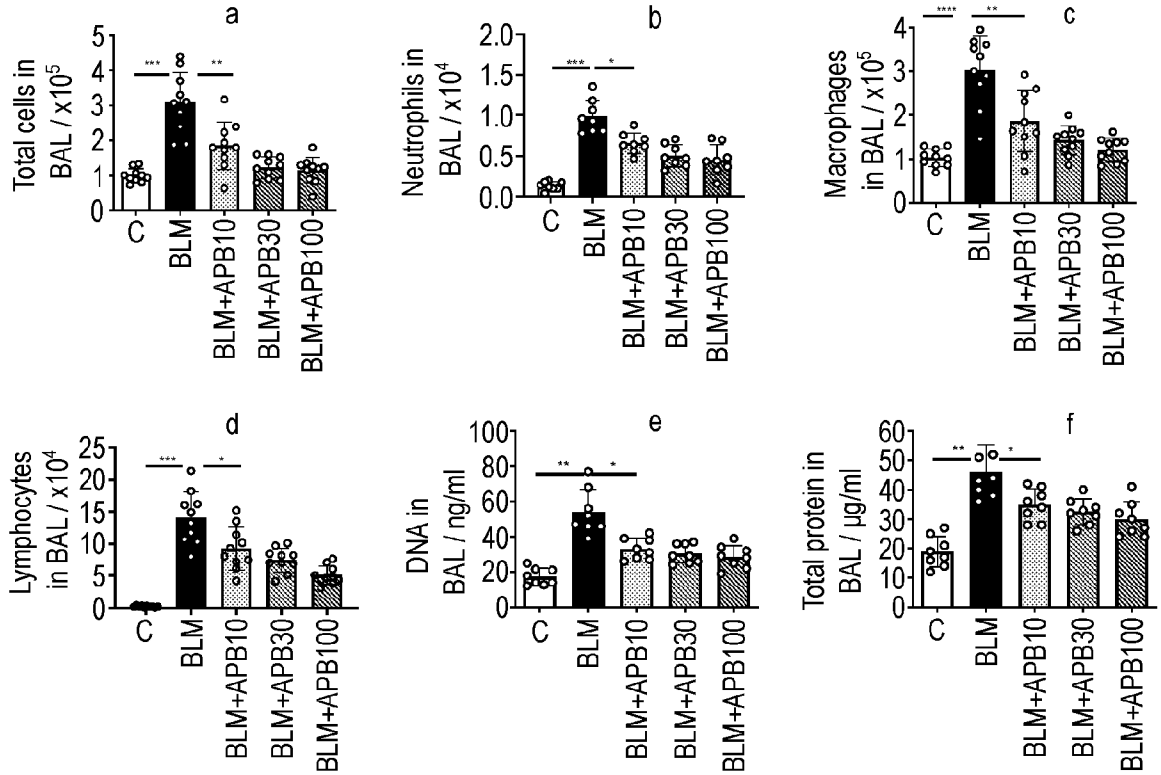

a
Total cells in BAL / x10^5
b
Neutrophils in BAL / x10^4
c
Macrophages in BAL / x10^5
d
Lymphocytes in BAL / x10^4
e
DNA in BAL / ng/ml
f
Total protein in BAL / µg/ml
C
BLM
BLM+APB10
BLM+APB30
BLM+APB100

FIG 1

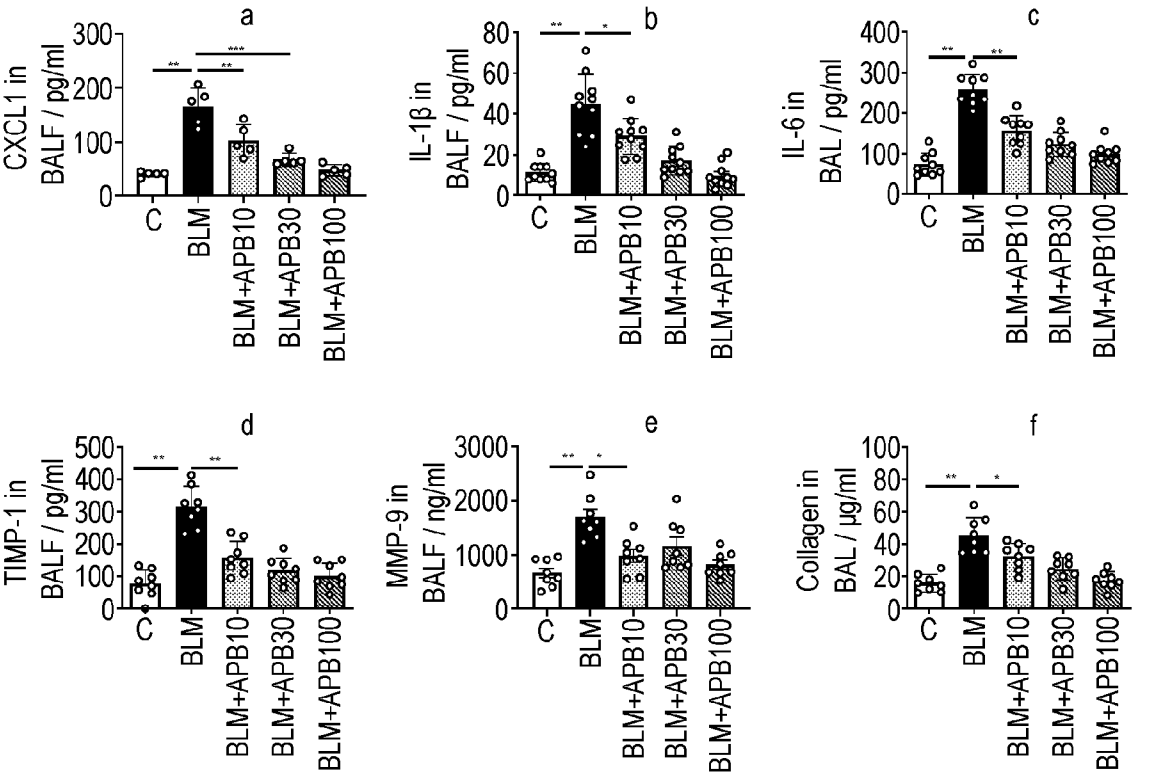

a
CXCL1 in BALF / pg/ml
b
IL-1β in BALF / pg/ml
c
IL-6 in BAL / pg/ml
d
TIMP-1 in BALF / pg/ml
e
MMP-9 in BALF / ng/ml
f
Collagen in BAL / µg/ml
C
BLM
BLM+APB10
BLM+APB30
BLM+APB100

FIG 2

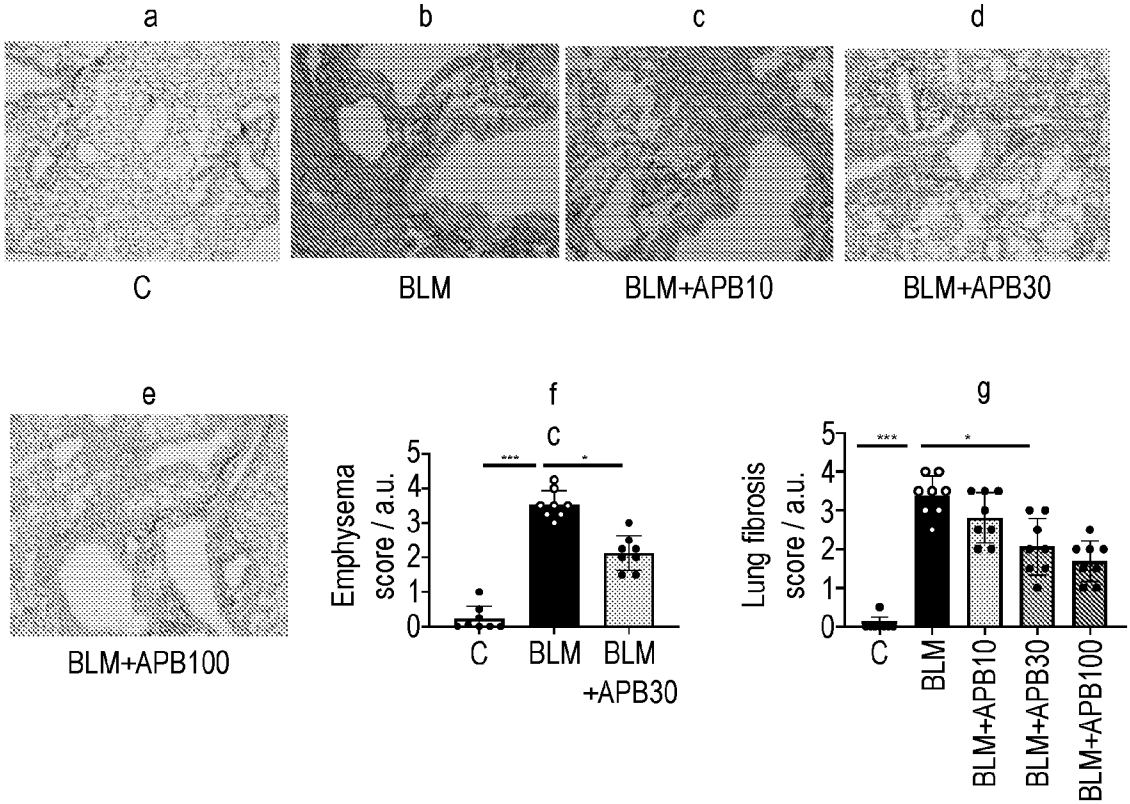

a
b
c
d
C
BLM
BLM+APB10
BLM+APB30
e
BLM+APB100
f
Emphysema score / a.u.
C
BLM
BLM +APB30
g
Lung fibrosis score / a.u.
C
BLM
BLM+APB10
BLM+APB30
BLM+APB100

FIG 3

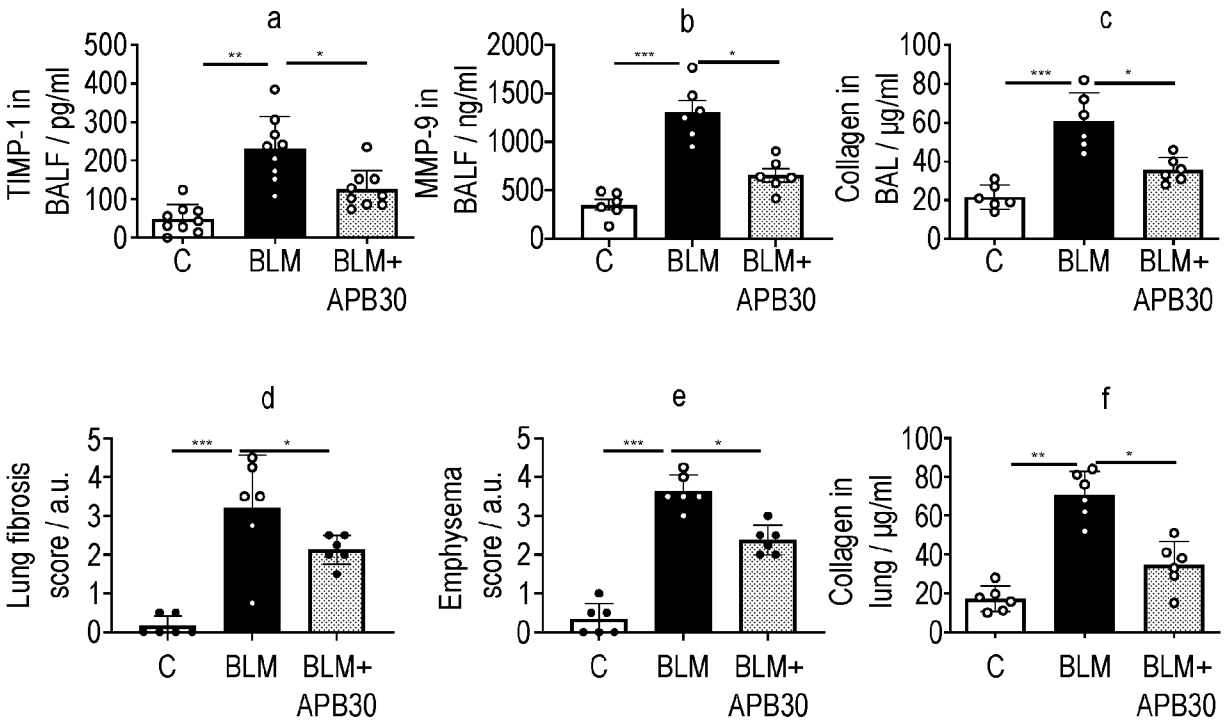

a
TIMP-1 in BALF / pg/ml
C
BLM
BLM+ APB30
b
MMP-9 in BALF / ng/ml
c
Collagen in BAL / µg/ml
d
Lung fibrosis score / a.u.
e
Emphysema score / a.u.
f
Collagen in lung / µg/ml

FIG 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/000354

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/192 A61K31/198 A61P11/00 A61P17/00 A61P35/00
A61P43/00
ADD.
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 A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 599 767 A1 (LUNAMED AG [CH]) 5 June 2013 (2013-06-05) cited in the application	1-8, 10-14
Y	paragraph [0033] examples 3, 9 claims 1, 12, 15	1-14

X	WO 2019/197015 A1 (TRUOG PETER [CH]) 17 October 2019 (2019-10-17) example 1 claims 1-3, 6	12-14

Y	WO 2016/165770 A1 (TRUOG PETER [CH]) 20 October 2016 (2016-10-20) claims 1, 7, 17, 20	1-14

☐ 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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2023	15/01/2024

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

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2023/000354

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2599767 A1	05-06-2013	NONE	
WO 2019197015 A1	17-10-2019	TW 201943687 A	16-11-2019
		WO 2019197015 A1	17-10-2019
WO 2016165770 A1	20-10-2016	DK 3283066 T3	08-08-2022
		EP 3283066 A1	21-02-2018
		JP 6609322 B2	20-11-2019
		JP 2018513167 A	24-05-2018
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		WO 2016165770 A1	20-10-2016