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(54) Title: 5'-ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN KINASE (AMPK) ACTIVATORS FOR TREATING PULMONARY HYPERTENSION

(57) Abstract: The invention relates to 5'-adenosine monophosphate-activated protein kinase (AMPK) activators such as biguanide derivatives and 5-aminoimidazole-4-carboxamide riboside (AICAR) or derivatives thereof for use in preventing and/or treating pulmonary hypertension.



WO 2010/103040 A1

5'-ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN KINASE (AMPK) ACTIVATORS FOR TREATING PULMONARY HYPERTENSION

FIELD OF THE INVENTION

5 The invention relates to 5'-adenosine monophosphate-activated protein kinase (AMPK) activators for use in preventing and/or treating pulmonary hypertension.

BACKGROUND OF THE INVENTION

10 Pulmonary hypertension is a serious disease characterized by a progressive increase of pulmonary vascular resistance and pulmonary arterial pressure (PAP), leading to right ventricle hypertrophy and death (HUMBERT, M. (2008). Update in pulmonary arterial hypertension 2007. *Am J Respir Crit Care Med*, 177, 574-579 ; RUBIN, L.J. (2006). Pulmonary arterial hypertension. *Proc Am Thorac Soc*, 3, 111-115.).

15 In 2003, the 3rd World Symposium on Pulmonary Arterial Hypertension was convened in Venice to attempt classification of pulmonary hypertension based on new understandings of disease mechanisms. Classification system can be summarized as follows (WHO stands for World Health Organization):

- 20 • WHO Group I - Pulmonary arterial hypertension (PAH)
 - Idiopathic (IPAH)
 - Familial (FPAH)
 - Associated with other diseases (APAH): collagen vascular disease (e.g. scleroderma), congenital shunts between the systemic and pulmonary circulation, portal hypertension, HIV infection, drugs, toxins, or other diseases or disorders
 - 25 ○ Associated with venous or capillary disease
- WHO Group II - Pulmonary hypertension associated with left heart disease
 - Atrial or ventricular disease
 - Valvular disease (e.g. mitral stenosis)
- 30 • WHO Group III - Pulmonary hypertension associated with lung diseases and/or hypoxemia
 - Chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD)

- Sleep-disordered breathing, alveolar hypoventilation
- Chronic exposure to high altitude
- Developmental lung abnormalities
- WHO Group IV - Pulmonary hypertension due to chronic thrombotic and/or embolic disease
- Pulmonary embolism in the proximal or distal pulmonary arteries
- Embolization of other matter, such as tumor cells or parasites
- WHO Group V - Miscellaneous

Common symptoms of pulmonary hypertension are shortness of breath, fatigue, non-productive cough, angina pectoris, fainting or syncope, peripheral edema (swelling of the limbs which commonly manifests around the ankles and feet), and rarely hemoptysis (coughing up blood). Therefore, patients with pulmonary hypertension can also be classified according to their ability to function (provided by the New York Heart Association):

- Class I: Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnea or fatigue, chest pain or near syncope.
- Class II: Patients with pulmonary hypertension resulting in slight limitation of physical activity. These patients are comfortable at rest, but ordinary physical activity causes undue dyspnea or fatigue, chest pain or near syncope.
- Class III: Patients with pulmonary hypertension resulting in marked limitation of physical activity. These patients are comfortable at rest, but less than ordinary physical activity causes undue dyspnea or fatigue, chest pain or near syncope.
- Class IV: Patients with pulmonary hypertension resulting in inability to perform any physical activity without symptoms. These patients manifest signs of right heart failure. Dyspnea and/or fatigue may be present at rest, and discomfort is increased by any physical activity.

The WHO group 1 of pulmonary hypertension, defined as PAH disease is currently incurable and its prognosis is bad: PAH mortality is estimated at about 50 % for 5 years, with a poor quality of life for the half of patients treated by monotherapy. We estimate that about 100,000 persons are suffering of PAH all over the world. The frequency of PAH is greater for women of child bearing age and for

children, but the reasons of this predisposition have not been elucidated. In Europe, primary or idiopathic PAH is causing about 200 deaths per year, and its incidence is about 3 cases for one million people per year. Other pulmonary hypertensions, i.e. pulmonary hypertension which causes are known, are however very more prevalent, and their frequency increases. Recently, American CDC (Centres for Disease Control and Prevention) underlined that pulmonary hypertension has to be considered not as a rare disease any more, but as an emergent chronic disease.

Although major advances in the understanding of disease development and treatment have been achieved over the last 15 years, the pathogenesis of pulmonary hypertension remains not clearly understood. All forms of pulmonary hypertension, including idiopathic (IPAH), familial (FPAH) and connective tissue disease-associated forms of pulmonary hypertension (APAH), display similar pathologic changes and are considered to share common pathogenic mechanisms, which involve endothelial dysfunction, endothelial and smooth muscle cell proliferation and increased vasoconstriction (HUMBERT, 2008, *ibid.*).

Current therapies based on the use of drugs that improve endothelial function, through the endothelin-1, the NO, or the prostacyclin pathways have shown benefits for this group of patients. Current therapies may be:

- continuous intravenously injection of Prostacycline or Prostaglandine I₂: for example epoprosterone (Flolan®);
- Prostacycline analog: for example iloprost (Ventavis®), treprostinil (Remodulin®);
- endothelin receptor antagonist: for example bosentan (Tracleer®), sitaxentan;
- phosphodiesterase-5 inhibitors: for example sildenafil (Revatio®).

However, these treatments failed to improve the long-term survival and their use is hampered by either undesired side effects or inconvenient drug administration routes (HUMBERT, M., SITBON, O. & SIMONNEAU, G. (2004). Treatment of pulmonary arterial hypertension. *N Engl J Med*, 351, 1425-1436).

In view of the foregoing, there remains a need in the art to provide new treatments against pulmonary hypertension. Surprisingly, it was found that compounds that activate 5'-adenosine monophosphate-activated protein kinase (AMPK) such as biguanide derivatives and 5-aminoimidazole-4-carboxamide

riboside (AICAR) present the characteristic of protecting against and treating pulmonary hypertension.

Biguanide derivative drugs, for instance metformin, have been widely used for more than 40 years for the treatment of hyperglycemia in patients with type II diabetes mellitus. Their action consists in reducing hyperglycemia by improving peripheral sensitivity to insulin, reducing gastrointestinal glucose absorption, and decreasing hepatic glucose production (BORST, S.E. & SNELLEN, H.G. (2001). Metformin, but not exercise training, increases insulin responsiveness in skeletal muscle of Sprague-Dawley rats. *Life Sci*, 69, 1497-1507 ; CASPARY, W.F. & CREUTZFELDT, W. (1971). Analysis of the inhibitory effect of biguanides on glucose absorption: inhibition of active sugar transport. *Diabetologia*, 7, 379-385 ; HUNDAL, R.S., KRSSAK, M., DUFOUR, S., LAURENT, D., LEBON, V., CHANDRAMOULI, V., INZUCCHI, S.E., SCHUMANN, W.C., PETERSEN, K.F., LANDAU, B.R. & SHULMAN, G.I. (2000). Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes*, 49, 2063-2069). According to current knowledge, there is no relationship between diabetes and pulmonary hypertension.

Besides, metformin has recently been shown to lower other cardiovascular risk factors (MCALISTER, F.A., EURICH, D.T., MAJUMDAR, S.R. & JOHNSON, J.A. (2008). The risk of heart failure in patients with type 2 diabetes treated with oral agent monotherapy. *Eur J Heart Fail*, 10, 703-708). The cardioprotective effect of metformin has very recently been shown (GUNDEWAR, S., CALVERT, J.W., JHA, S., TOEDT-PINGEL, I., JI, S.Y., NUNEZ, D., RAMACHANDRAN, A., ANAYA-CISNEROS, M., TIAN, R. & LEFER, D.J. (2009). Activation of AMP-activated protein kinase by metformin improves left ventricular function and survival in heart failure. *Circulation Research*, 104, 403-411). In humans, metformin, at therapeutic concentration, has anti-hypertensive properties in hypertensive obese women and in patients with type II diabetes (GIUGLIANO, D., DE ROSA, N., DI MARO, G., MARFELLA, R., ACAMPORA, R., BUONINCONTI, R. & D'ONOFRIO, F. (1993). Metformin improves glucose, lipid metabolism, and reduces blood pressure in hypertensive, obese women. *Diabetes Care*, 16, 1387-1390). The word "hypertension" without a qualifier normally refers to systemic arterial hypertension. Hypertension and pulmonary hypertension are not the same diseases, and their

causes are different. It was therefore not obvious that metformin presents a protective effect against pulmonary hypertension.

SUMMARY OF THE INVENTION

5 Applicant demonstrates that compounds that activate AMPK (which may be referred to herein as “AMPK activators” present (have, possess, or display) a protective and curative effect against pulmonary hypertension.

 The present invention pertains to AMPK activators that prevent and/or treat pulmonary hypertension.

10 The invention relates to a method for preventing and/or treating pulmonary hypertension comprising the administration to a patient of a composition comprising one or more AMPK activators in a suitable pharmaceutical medium.

 It is also relates to the use of a composition comprising one or more AMPK activators in a suitable pharmaceutical medium for the manufacture of a medicament
15 for preventing and/or treating pulmonary hypertension.

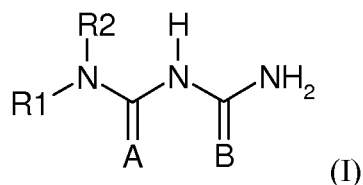
DETAILED DESCRIPTION OF THE INVENTION

 The present invention provides methods for preventing and/or treating pulmonary hypertension by administering one or more AMPK activators to a patient
20 in need of such prevention or treatment. Examples of AMPK activators include but are not limited to various biguanide derivatives as described herein, and the compound AICAR, or suitable derivatives thereof. AICAR is “5-aminoimidazole-4-carboxamide riboside”, and is also known as “5-aminoimidazole-4-carboxamide-1- β -4-ribofuranoside”. AICAR is also referred to as “acadesine”. According to the
25 invention, one or more of these AMPK activators, including combinations of biguanide derivatives and AICAR (or AICAR derivatives), are used to make pharmaceutical formulations that are acceptable for administration.

 Biguanide derivatives are a group of drugs that are useful in the treatment of hyperglycemia, and which comprise a substituted biguanide structure.

30 In a particular embodiment, the biguanide derivatives have the following formula (I):

6



wherein

R1 and R2 are independently selected from hydrogen or a linear, branched or
5 cyclic, saturated or unsaturated (C1-C4) alkyl group, or an aralkyl group;

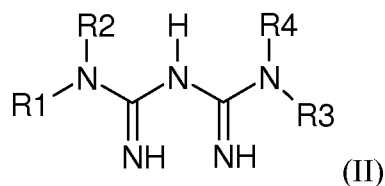
A and B are independently selected from NH or N-CHR3-N, wherein R3 is
selected from hydrogen or a linear, branched or cyclic, saturated or unsaturated (C1-
C4) alkyl group;

and also the tautomeric, enantiomeric, diastereoisomeric and epimeric forms,
10 the solvates and the pharmaceutically acceptable salts thereof.

Preferably, the linear, branched or cyclic, saturated or unsaturated (C1-C4)
alkyl group is methyl, ethyl, n-propyl, i-propyl, n-butyl or i-butyl. Preferably, the
aralkyl group is benzyl or phenethyl.

According to this particular embodiment, preferred biguanide derivatives
15 suitable for use in the present invention include, but are not limited to, those
substituted on one or both of their primary amine groups (R1 and/or R2) by at least
one alkyl or aralkyl group such as methyl, ethyl, n-propyl, n-butyl or phenethyl, and
preferably include metformin, phenformin and buformin. More preferably, the
biguanide derivative according to the invention is metformin.

20 In another particular embodiment, the biguanide derivatives have the following
formula (II) as described in the international patent application WO 02/074740, the
complete contents of which is hereby incorporated by reference:



25

wherein

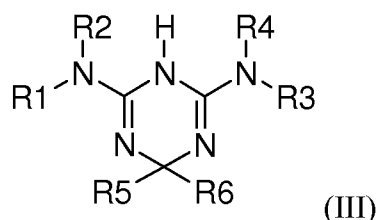
R1 and R2 are independently selected from a branched or unbranched (C1-C6)
alkyl chain, or

R1 and R2 together form a 3-to 8-membered ring including the nitrogen atom to which they are attached,

R3 and R4 together form a ring selected from the group aziridine, pyrrolyl, imidazolyl, pyrazolyl, indolyl, indolinyl, pyrrolidinyl, piperazinyl and piperidyl including the nitrogen atom to which they are attached,

and also the tautomeric, enantiomeric, diastereoisomeric and epimeric forms, the solvates and the pharmaceutically acceptable salts thereof.

In still another particular embodiment, the biguanide derivatives have the following formula (III) as described in the international patent application WO 01/55122, the complete contents of which is hereby incorporated by reference:



wherein

R1, R2, R3 and R4 are independently selected from the groups:

H,

(C1-C20)alkyl optionally substituted with halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C3-C8)cycloalkyl,

(C2-C20)alkylene substituted or otherwise with halogen, (C1-C5)alkyl, (C1-C5)alkoxy,

(C2-C20)alkyne substituted or otherwise with halogen, (C1-C5)alkyl, (C1-C5)alkoxy,

(C3-C8)cycloalkyl substituted or otherwise with (C1-C5)alkyl, (C1-C5)alkoxy,

(C3-C8)heterocycloalkyl carrying (including) one or more heteroatoms chosen

from N, O, S and substituted or otherwise with (C1-C5)alkyl, (C1-C5)alkoxy,

(C6-C14)aryl(C1-C20)alkyl substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl-(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

(C6-C14)aryl substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

5 (C1-C13)heteroaryl carrying one or more heteroatoms chosen from N, O, S and substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl-

10 it being possible for R1 and R2, on the one hand, and R3 and R4, on the other hand, to form with the nitrogen atom to which they are connected (bonded) an n-membered ring (n ranging from 3 to 8) comprising or otherwise one or more heteroatoms chosen from N, O, S and being capable of being substituted with one or more of the following groups: amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-
15 C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

R5 and R6 are independently selected from the groups:

H,

20 (C1-C20)alkyl substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

25 (C2-C20)alkylene substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

30 (C2-C20)alkyne substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

(C3-C8)cycloalkyl substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-

C14)aryloxy, (C6-C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

(C3-C8)heterocycloalkyl carrying one or more heteroatoms chosen from N, O, S and substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl-(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

(C6-C14)aryl substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

(C1-C13)heteroaryl carrying one or more heteroatoms chosen from N, O, S and substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl-(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

(C6-C14)aryl(C1-C5)alkyl substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl-(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

it being possible for R5 and R6 to form with the carbon atom to which they are attached an m-membered ring (m ranging from 3 to 8) comprising or otherwise one or more heteroatoms chosen from N, O, S and being capable of being substituted with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

or being capable of forming with the carbon atom to which they are attached a C10-C30 polycyclic residue substituted or otherwise with amino, hydroxyl, thio, halogen, (C1-C5)alkyl, (C1-C5)alkoxy, (C1-C5)alkylthio, (C1-C5)alkylamino, (C6-C14)aryloxy, (C6-C14)aryl(C1-C5)alkoxy, cyano, trifluoromethyl, carboxyl, carboxymethyl or carboxyethyl,

it being possible for the nitrogen atom of a hetero-cycloalkyl or heteroaryl group to be substituted with a (C1-C5)alkyl, (C3-C8)cycloalkyl, (C6-C14)aryl, (C6-C14)aryl(C1-C5)alkyl or (C1-C6)acyl group,

and also the tautomeric, enantiomeric, diastereoisomeric and epimeric forms and the pharmaceutically acceptable salts thereof.

With respect to AICAR, this compound, or derivatives (analogs, precursors, etc.) thereof that retain the ability to activate AMPK, are intended to be encompassed by the invention, as are various isomeric and stereoisomeric forms and pharmaceutically acceptable salts thereof. Examples of such derivatives are described, for example, in WO2005/98465, WO2005/038068, WO2004/043957, US2009/0105293 and US2007/0265223, the complete contents of each of which are hereby incorporated by reference.

The term "tautomeric forms" is meant to include isomers that are interconverted by a tautomerization reaction. The term "enantiomeric forms" is meant to include stereoisomers that are mirror images of each other, i.e. that are non-superposable, that is, not identical. The term "diastereoisomeric forms" is meant to include stereoisomers that are not enantiomers. When two diastereoisomers differ from each other at only one stereocenter, they are called epimeric forms or epimers. The term "solvates" is meant to include the biguanide derivative dissolved in a solvent.

The term "pharmaceutically acceptable salts" is meant to include salts of the active compound which are prepared with relatively non toxic acids. Acid addition salts can be obtained by contacting the neutral form of the compound with a sufficient amount of the corresponding acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include, but are not limited to, those derived from inorganic acids like hydrochloric, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulphuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively non-toxic organic acids like acetic, propionic, isobutyric, oxalic, maleic, malonic, benzoic, p-chlorophenoxyacetic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, embonic (or pamoic) and the like. Also included are salts of amino-acids such as arginate and the like, and salts of organic acids like glucuronic or galacturonic acids and the like. In a preferred embodiment, the metformin salt is chosen in the group consisting of metformin hydrochloride, metformin p-chlorophenoxyacetate and metformin embonate.

As used herein, the term “patient” denotes an animal, such as birds (e.g. poultry), and mammals (e.g. a rodent, a feline, a canine, or a primate). Preferably, a patient according to the invention is a human.

5 In the context of the invention, the term “treating” or “treatment”, as used herein, refers to a method that is aimed at reversing, alleviating, inhibiting, slowing down or stopping the progression, aggravation or deterioration of the symptoms of the pathology, at bringing about ameliorations of the symptoms of the pathology, and/or at curing the pathology. The terms “preventing” or “prevention”, as used
10 herein, refer to a method that is aimed at delaying or preventing the onset of the pathology.

The composition of the present invention is suitable for use in a variety of drug delivery systems, and is intended for enteral, parenteral, topical, oral or local administration.

15 In certain aspect, the composition can be formulated in a solid form. In a preferred embodiment, the AMPK activator is administered orally. Solid form preparations include but are not limited to powders, tablets, pills, capsules, cachets, lozenges, and dispersible granules. According to this aspect, the term “suitable pharmaceutical medium” is meant to include solid carriers, but also substances that may act as diluents, flavouring agents, binders, preservatives, tablets disintegrating
20 agents, or encapsulating material, etc. The powder and tablets preferably contain from about 5 % to about 95 % of an AMPK activator. Suitable carriers include but are not limited to magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatine, tragacanth, methylcellulose, a low melting wax, cocoa butter, and their mixtures.

25 In another aspect, the composition is administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. The term “suitable pharmaceutical medium” in this aspect is meant to include an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used including, for example, water, buffered water, 0.4 % saline, 0.3 % glycine, hyaluronic acid and
30 their mixtures. These compositions may be sterilized by conventional, well-known sterilization techniques or may be sterile filtered. The composition may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, including pH adjusting and buffering agents, tonicity

adjusting agents, wetting agents and the like, such as, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

According to others aspects, the composition may also be administered in other forms, for example, as suppositories, aerosols, transdermal patches, etc.

The composition comprising an AMPK activator in a suitable pharmaceutical medium may be in the form of a film-coated tablet. By "film-coated tablet" we mean a tablet or pill covered or coated with one or more layers of substances (such as various sugars, cellulose ethers, cellulose acetates, various polymers and copolymers, etc., as known to those of skill in the art), the layers typically dissolving upon contact with an aqueous environment such as within the body, thereby releasing the active agents in the tablet. Preferably, an AMPK activator may be administered in a composition in the form of a film-coated tablet. The composition comprising an AMPK activator in a suitable pharmaceutical medium in the form of a film-coated tablet may comprise, for example, metformin chlorhydrate, polyvinylpyrrolidone, magnesium stearate, hydroxypropyl methylcellulose, polyethylene glycol and optionally a colouring agent.

For example, the composition used according to the invention can be GLUCOPHAGE® of Merck-Lipha. For example, the medium may include polyvinylpyrrolidone (Povidone K30®), magnesium stearate, hydroxypropyl methylcellulose (Hypromellose®), polyethylene glycol (Macrogol 400®, macrogol 8000®) and colouring agent (Opadry®).

In therapeutic use for the treatment of diabetes, metformin is typically administered at the initial dosage of about 0.001 mg/kg to about 500 mg/kg daily. A daily dose range of about 0.1 mg/kg to about 150 mg/kg is preferred. The dosage however may be varied depending upon the requirements of the patient and the severity of the condition being treated. For instance, a human being may be treated with an initial dose of 500 mg, and this dose may be increased to 3 g/day.

According to the invention, the composition comprising an AMPK activator (e.g. a biguanide derivative and/or AICAR or AICAR derivative) in a suitable pharmaceutical medium is administered with an initial daily dosage of about 0.001 mg/kg to about 500 mg/kg. The invention pertains to all pulmonary hypertension types from any origins. In one embodiment, the invention consists in

(provides) an AMPK activator for treating and/or preventing pulmonary hypertension, wherein pulmonary hypertension is pulmonary arterial hypertension (PAH). PAH corresponds to pulmonary hypertension belonging to the group I of the WHO clinical classification of pulmonary hypertension. This includes idiopathic PAH (IPAH), familial PAH (FPAH) and PAH associated with drugs, toxins, other diseases or infections (APAH). In another embodiment, the invention consists in an AMPK activator for treating and/or preventing pulmonary hypertension, wherein pulmonary hypertension is pulmonary hypertension associated with left heart disease. "Pulmonary hypertension associated with left heart disease" represents group II within WHO clinical classification system. In another embodiment, the invention consists in an AMPK activator for treating and/or preventing pulmonary hypertension, wherein pulmonary hypertension is pulmonary hypertension associated with lung diseases and/or hypoxia. "Pulmonary hypertension associated with lung diseases and/or hypoxia" represents group III within WHO clinical classification system. In another embodiment, the invention consists in an AMPK activator for treating and/or preventing pulmonary hypertension, wherein pulmonary hypertension is pulmonary hypertension due to chronic thrombotic and/or embolic disease. "Pulmonary hypertension due to chronic thrombotic and/or embolic disease" represents group IV within WHO clinical classification system.

According to the classification provided by the New York Heart Association, patients with pulmonary hypertension can be classified according to their ability to function (class I to class IV). The invention pertains to the treatment and/or the prevention of pulmonary hypertension to patients belonging to any of these classes.

According to one embodiment of the invention, the medicament is used in association with another treatment against pulmonary hypertension, preferably involving the administration of another active agent suitable for preventing and/or treating pulmonary hypertension. According to another embodiment, the composition of the invention further comprises an additional active agent suitable for preventing and/or treating pulmonary hypertension. In yet another embodiment, two or more AMPK activators may be used (e.g. together or in sequence) to treat pulmonary hypertension.

In one embodiment, the additional active agent is an endothelin receptor antagonist. According to this embodiment, the endothelin receptor antagonist is selected from the group consisting of bosentan or sitaxentan.

5 In another embodiment, the additional active agent is a phosphodiesterase-5 inhibitor. According to this embodiment, the phosphodiesterase-5 inhibitor is sildenafil.

10 In still another embodiment, the additional active agent is a prostacycline or a prostacycline analog. According to this embodiment, the prostacycline is epoprostenol and the prostacycline analog is selected from the group consisting of iloprost or treprostinil.

It must be further noted that three (or more) active agents may also be combined such as at least one AMPK activator, an endothelin receptor antagonist and a phosphodiesterase-5 inhibitor (for example metformin, sitaxentan and sildenafil).

15 In these embodiments, said additional active agents may be contained in the same composition or administrated separately.

20 Therefore, the invention relates preferably to a combination for simultaneous or separate administration comprising metformin and bosentan in a suitable pharmaceutical medium. The invention relates to a product comprising metformin and bosentan as a combined preparation for simultaneous, separate or sequential use in the treatment of pulmonary hypertension.

25 The invention further relates to a combination for simultaneous or separate administration comprising metformin, sitaxentan and sildenafil in a suitable pharmaceutical medium. The invention relates to a product comprising metformin, sitaxentan and sildenafil as a combined preparation for simultaneous, separate or sequential use in the treatment of pulmonary hypertension.

Moreover, if they are contained in different compositions, these compositions may be administered to the patient at the same time, or successively, or alternatively.

30 All of the biguanide derivatives of the formula I, II and III can be prepared by the procedures described in international patent applications WO 01/55122 and WO 02/074740, the complete contents of both of which are hereby incorporated by reference.

Another aspect of the invention relates to a method for preventing and/or treating pulmonary hypertension comprising administering to a subject in need thereof an AMPK activator such as a derivative biguanide as described above.

5 In a particular embodiment, the method for preventing and/or treating pulmonary hypertension comprises administering to a subject in need thereof an AMPK activator in combination with an additional active agent suitable for preventing and/or treating pulmonary hypertension, as described above.

10 The invention will further be illustrated in view of the following figures and examples, which should not be construed as limiting the invention in any way.

FIGURES

Figure 1: Metformin prevents chronic hypoxic-induced pulmonary hypertension. (a) Mean PAP, (b) right ventricular wall thickness, (c) pulmonary artery flow acceleration and (d) (RV/LV+S) ratio determined in control rats (normoxia), rats chronically treated for 21 days with metformin ($100 \text{ mg kg}^{-1} \text{ day}^{-1}$), rats exposed to hypoxia for 21 days, and metformin-treated rats exposed to hypoxia, (e) Mean PAP and (f) [RV/(LV+S)] ratio determined in rats exposed to hypoxia for 21 days non-treated (0) or treated with metformin doses ranging from 0.1 to $100 \text{ mg kg}^{-1} \text{ day}^{-1}$.
20 Dotted lines indicated the control values in normoxic rats. ($^{\#}P < 0.001$ vs control, $^*P < 0.001$ vs hypoxia, $n = 5-10$).

Figure 2: Metformin limits progression of PAH in hypoxic rats. (a) Mean PAP, (b) right ventricular wall thickness, (c) pulmonary artery flow acceleration and (d) [RV/(LV+S)] ratio determined in control rats (normoxia), rats chronically treated for 7 days with metformin ($100 \text{ mg kg}^{-1} \text{ day}^{-1}$), rats exposed to hypoxia for 21 days (hypoxia) non-treated and treated with metformin for the last 7 days of hypoxia. ($^{\#}P < 0.001$ vs control, $^{\S}P < 0.05$ vs untreated, $n = 5-9$).

30 **Figure 3:** Metformin prevents MCT-induced pulmonary hypertension. (a) Mean PAP and, (b) (RV/LV+S) ratio determined in control rats, rats chronically treated for 30 days with metformin ($100 \text{ mg kg}^{-1} \text{ day}^{-1}$), MCT-injected rats at day 30 post-MCT injection, and MCT-injected rats at day 30 post-MCT injection treated for 30 days by

metformin ($^{\#}P<0.001$ vs control, $^{*}P<0.001$ vs MCT, $n = 5-10$). (c) Survival rates of metformin-treated MCT-injected rats (grey) versus untreated MCT-injected rats.

Figure 4: Metformin prevents pulmonary hypertension-associated pulmonary arterial wall remodelling. Representative sections of lung tissue (a) and quantification of the relative thickness of small pulmonary artery (20-60 μm) wall (b) and percentages of distal muscularization of normally nonmuscular arteries (c) in samples from control, hypoxic (21 days) and MCT-injected rats (day 30), untreated and treated by metformin ($^{\#}P<0.001$ vs control, $^{*}P<0.001$ vs hypoxia, $^{\S}P<0.001$ vs MCT).

Figure 5: Metformin improves endothelial function and reduces pulmonary artery contractility. (a) ACC phosphorylation and expression, eNOS phosphorylation and expression, Rho kinase activity (assessed by the extent of phosphorylation of MYPT) and RhoA expression have been analyzed by western blot in pulmonary artery from control rats and rats exposed to hypoxia for 21 days, untreated and treated with metformin (3 different samples representative of each condition). β -actin amounts were also assessed in each sample. (b) Cumulative concentration-response curves for CCh-induced relaxation of PhE (1 μM)-contracted pulmonary artery rings from hypoxic (21 days, ■) and metformin-treated hypoxic rats (●). Tension is expressed as percentage of the amplitude of the PhE-induced contraction. (c) Cumulative concentration-response curves for the contraction induced by PhE in pulmonary artery rings from hypoxic (21 days, ■) and metformin-treated hypoxic rats (●). (d) Cumulative concentration-response curves for the contraction induced by PhE in pulmonary artery rings from normoxic rats under control condition (■) and in the presence of metformin (4 mM, ●).

Figure 6: Metformin decreased pulmonary arterial cell proliferation associated with hypoxic PH. (a) Representative PCNA staining and quantification of the percentage of PCNA-positive pulmonary artery sections in lungs from normoxic rats (normoxia), hypoxic (21 days) and metformin-treated hypoxic rats. ($^{\#}P<0.001$ vs control, $^{*}P<0.001$ vs hypoxia). (b) ERK, p38 and JNK phosphorylation and expression analyzed by western blot in pulmonary arteries from control rats, rats exposed to hypoxia for 21 days untreated and treated with metformin (3 different

samples representative of each condition). β -actin amounts were also assessed in each sample.

Figure 7: Metformin inhibits PDGF-induced pulmonary artery smooth muscle cell proliferation. (a) Analysis by western blot of the expression of PCNA in rat pulmonary artery smooth muscle cells under basal condition and stimulated by PDGF for 24h (20 ng ml⁻¹), without or with metformin 4 mM. (b) Typical micrographs of BrdU labelling in rat pulmonary artery smooth muscle cells under basal condition and stimulated by PDGF (20 ng ml⁻¹) for 24h, without or with metformin 4 mM. Quantification of proliferation was expressed as the percentage of BrdU positive pulmonary arterial smooth muscle cells. ([#] $P < 0.001$ vs control, * $P < 0.001$ vs PDGF).

EXAMPLES

All experiments were conducted in accordance with international guidelines for the care and use of laboratory animals.

Values are expressed as mean $\pm$ SEM (standard error of the mean). In experiments with comparison of two conditions, a non paired Student's t-test was used. Differences among multiple groups were tested with ANOVA (one-way ANOVA, Fisher's test, Holm-Sidak method). $P < 0.05$ was considered significant.

Example 1: hypoxic pulmonary hypertension

Male Wistar rats (250 g) were used. The normoxic rats were housed in room air at normal atmospheric pressure. Chronic hypoxic pulmonary hypertension was obtained by housing animals in a hypobaric chamber at 480 mmHg (Vacucell 111L, Medcenter, Munich, Germany) for 21 days. Metformin (Calbiochem) was administered by daily intraperitoneal (IP) injections at the dosage of 100 mg.kg⁻¹.day⁻¹ starting either at the 1st or at the 14th day of the 21 days-hypoxia. Four groups of rats were used: normoxic rats (control group), normoxic rats receiving metformin, hypoxic rats receiving saline, and hypoxic rats treated by metformin.

Analyses:

• Cardiac ultrasonography: Cardiac ultrasonography (SONOS 5500 echocardiograph, Philips, 12-MHz sector scare transducer) was performed by the same operator on anesthetized rats, placed in the left lateral decubitus position after thorax epilation. The wall thickness of the right ventricle was measured. The peak tricuspid regurgitation velocity was measured by continuous-wave. Pulmonary artery flow was recorded by pulsed-wave Doppler echocardiography and the acceleration time of pulmonary artery blood flow was measured from the onset of ejection to the time of peak velocity.

• Haemodynamic measures: Haemodynamic measures were made as previously described (GUILLUY, C., SAUZEAU, V., ROLLI-DERKINDEREN, M., GUERIN, P., SAGAN, C., PACAUD, P. & LOIRAND, G. (2005). Inhibition of RhoA/Rho kinase pathway is involved in the beneficial effect of sildenafil on pulmonary hypertension. *Br J Pharmacol*, 146, 1010-8.). Rats were anaesthetized by IP injection of ketamine and xylazine. Haemodynamic parameters were measured using a venous catheter inserted in the right jugular vein. The catheter was introduced in the right atrium, the right ventricle and then in the pulmonary artery. Systolic right ventricle pressure, systolic, diastolic and mean PAP were then measured (Hewlett-Packard, M1106B).

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• Measure of right ventricular hypertrophy: After sacrifice, heart was removed and washed from blood. Right ventricular (RV) hypertrophy was measured using the ratio of RV weight to left ventricle (LV) plus interventricular septum weight (RV/LV+S).

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• Histologic analysis: The left lung was removed. Left bronchus was slowly injected with 4 % paraformaldehyde in PBS to distend air-cells and the entire piece was put in the same fixation solution to be processed for paraffin-embedded sections (10 μ m). Three transversal sections per lung were stained with haematoxylin-eosin-saffron and Weigert's staining. A total of 15-30 arterial vessels (20 to 60 μ m in diameter) were examined in each section. The wall thickness was quantified by measuring the area using Metaview software (Universal Imaging Co., West Chester, PA, U.S.A.). The ratio area of the lumen (LA) to the area of the entire vessel (arterial

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wall plus lumen=VA) x 100 was calculated. The medial area (MA, in %) was calculated using the formula $MA = 100 - AL/AW \times 100$. Muscularity of distal pulmonary arteries was assessed as previously described (CHASSAGNE, C., EDDAHIBI, S., ADAMY, C., RIDEAU, D., MAROTTE, F., DUBOIS-RANDE, J.L., ADNOT, S., SAMUEL, J.L. & TEIGER, E. (2000). Modulation of angiotensin II receptor expression during development and regression of hypoxic pulmonary hypertension. *Am J Respir Cell Mol Biol*, 22, 323-32.) and expressed as a percentage of the total number of arteries examined (30-60 in each condition). Proliferation was assessed using immunohistochemistry on lung sections stained with anti-proliferating cell nuclear antigen (PCNA) antibody (Dako, Glostrup, Denmark). All these analyses were performed by one observer blinded to the group from which the sections were taken.

Results are presented on Figure 1. Rats maintained in hypobaric chamber for 21 days displayed and increased hematocrit ($66.0 \pm 1.4\%$ vs 45.4 ± 1.9 in controls, $n=10$, $P<0.001$) attesting the hypoxic condition. The rats exposed to chronic hypoxia developed pulmonary hypertension characterized by an increase in mean PAP (30.2 ± 2.63 mmHg vs 10.2 ± 1.2 mm Hg in normoxic rats, $n=16$, $P<0.001$), thickening of the right ventricle wall (0.292 ± 0.034 mm vs 0.107 ± 0.017 in normoxic rats, $n=10$, $P<0.001$), and decrease of the pulmonary flow acceleration time (0.016 ± 0.002 s vs 0.037 ± 0.003 s, $n=10$, $P<0.001$) (Figure 1a-c). Right ventricular remodelling in hypoxic rats was also attested by the strong increase in the ratio of RV weight to LV plus septum (RV/LV+S) (0.44 ± 0.03 vs 0.20 ± 0.01 , $n=16$, $P<0.001$) (Figure 1d). Metformin treatment ($100 \text{ mg.kg}^{-1}.\text{day}^{-1}$) applied daily for the entire duration of hypoxia exposure almost completely prevented PH. Mean PAP, right ventricle wall thickness, and the RV/LV+S ratio remained all to near normal levels (Figure 1a, b and d), and the pulmonary flow acceleration time was partially normalized (Figure 1c). The protective action of metformin in hypoxic rats depended of the dose used as shown by the gradual increase in the effect of metformin concentration ranging from 0.1 to $100 \text{ mg kg}^{-1} \text{ d}^{-1}$ on mean PAP and RV/(LV+S) (Figure 1e and f).

To further define the effect of metformin in the progression of PAH in hypoxic rats, we also assessed the effect of metformin treatment administrated during the last 7 days of a 21days-hypoxia. Mean PAP, right ventricle wall thickness, the

RV/(LV+S) ratio and the pulmonary flow acceleration time were all significantly reduced by metformin administration (Figure 2).

These results indicate that metformin effectively prevents pulmonary hypertension induced by hypoxic conditions.

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The effects of a curative metformin treatment, started after the establishment of pulmonary artery were also analyzed using (based on) the same parameters. Animals were exposed to hypoxia for 4 weeks and metformin treatment was started after 2 weeks of hypoxia. The results show that the symptoms of established pulmonary hypertension were ameliorated by the administration of metformin.

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Example 2: monocrotaline induced pulmonary hypertension

For monocrotaline (MCT)-induced pulmonary hypertension, rats received a subcutaneous injection of saline (controls) or MCT (Sigma, Saint Quentin Fallavier, France; 60 mg/kg/rat) to pulmonary hypertension. Experimental groups were further divided in untreated rats (receiving saline), and treated rats that received daily intraperitoneal IP injections of metformin ($100 \text{ mg.kg}^{-1}.\text{day}^{-1}$), starting at the day of MCT injection (day 0). Rats were examined at day 30. Survival curves were established from day 0 to day 55.

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The same analyses as in Example 1 have been run.

Results are presented in Figure 3. The rats challenged with MCT developed severe pulmonary hypertension characterized by an elevated mean PAP (Figure 3a) and an increase in the RV/LV+S ratio (Figure 3b). MCT-induced pulmonary hypertension is associated with a low survival rate (30 % at day 30, Figure 3c). Metformin treatment improved all the parameters of MCT-induced pulmonary hypertension with a significant decrease of mean PAP at day 30 (Figure 2a), decrease of the RV/LV+S ratio (Figure 3b), and a strong improvement of the survival rate to 61 % at day 30 (Figure 3c).

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Example 3: Effect of metformin on pulmonary arterial cell proliferation and excessive vasoconstriction

Figure 4 shows a comparison of the effect of metformin on both hypoxic pulmonary hypertension and MCT-induced PH, according to the histological analysis of pulmonary arteries.

5 Histologic examination of pulmonary vasculature showed that medial wall thickness of small pulmonary arteries (20-60 μ m in diameters) and distal muscularization were markedly increased in hypoxic rat at day 21 (Figure 4a and b). Treatment with metformin resulted in a normalization of pulmonary arterial wall thickness (Figure 4a and b).

10 Similarly, lung specimens from MCT-treated rats (30 days) displayed severe thickening and muscularization of the arterial wall and metformin treatment also significantly reduced pulmonary arterial remodeling in MCT-treated rats (Figure 4a and b). The progressive arterial wall thickening occurring in pulmonary hypertension resulted from both pulmonary arterial cell proliferation and excessive vasoconstriction.

15 We thus assessed the effect of metformin on these two different processes.

Western blot analyses have been made on pulmonary arteries from sacrificed rats of examples 1 and 2:

20 Pulmonary arteries (extralobar) were harvested and put in NETF buffer (100 mM NaCl, 2 mM EGTA, 50 mM Tris-Cl, pH 7.4 and 50 mM NaF) containing 1 % NP-40, 2 mM orthovanadate, protease and phosphatase inhibitor cocktails (Sigma). Lysates were obtained using Polytron material and then sonicated. Protein concentration of each sample was determined and equal amounts of protein were loaded in each lane of polyacrylamide/SDS gels. After electrophoresis and transfer to
25 nitrocellulose membrane, samples were analysed by western blot with antibodies against acetyl CoA carboxylase (ACC) and phosphor-ACC (Cell Signaling, Cell Signaling Technology, Inc., Danvers, MA, U.S.A.), eNOS and phospho-eNOS (Cell Signaling, Cell Signaling Technology, Inc., Danvers, MA, U.S.A.), RhoA (Santa Cruz Biotechnology, Santa cruz, CA, U.S.A.), phospho-ERK (Santa Cruz), phospho-
30 JNK (Cell Signaling), phospho-p38 (Cell Signaling) and PCNA (proliferating cell nuclear antigen). Rho kinase activity was quantified by western blot analysis for phosphorylated MYPT using a rabbit polyclonal anti-phospho-MYPT (Thr696) (Upstate, Euromedex, Munolsheim, France). Equal loading was confirmed by

reprobing the membrane with anti-beta-actin antibody (Sigma, Saint Quentin Fallavier, France). Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibody and subsequent ECL kit detection (Amersham Pharmacia Biotech, Orsay, France). Quantification was made by densitometric analysis with QuantityOne (Biorad, Hercules, CA, U.S.A.).

Results are presented in Figures 5a and 6.

To analyze the potential effect of metformin on contractile properties of the pulmonary artery, expression and activity of markers of endothelial function and arterial contraction in lysates of pulmonary artery from control and hypoxic rats, treated or not by metformin were analysed. As metformin has been shown to stimulate AMPK activity (ZHOU, G., MYERS, R., LI, Y., CHEN, Y., SHEN, X., FENYK-MELODY, J., WU, M., VENTRE, J., DOEBBER, T., FUJII, N., MUSI, N., HIRSHMAN, M.F., GOODYEAR, L.J. & MOLLER, D.E. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*, 108, 1167-74.), the efficiency of metformin treatment was first checked by monitoring the level of phosphorylation of acetyl CoA carboxylase (ACC), a representative downstream target of AMPK. The increased in ACC phosphorylation observed in pulmonary arteries from hypoxic rats treated with metformin attested the efficiency of the treatment (Figure 5a). Phosphorylation of eNOS was used as a marker of endothelial function and phosphorylation of MYPT as a marker of RhoA/Rho kinase activation and arterial smooth muscle cell contraction (Figure 5a). Metformin treatment strongly increased the level of eNOS phosphorylation in hypoxic rat (7.6 ± 1.2 -fold over hypoxic rats, $n=8$, $P<0.001$) (Figure 5a). This effect was associated with a marked decreased in hypoxia-induced MYPT phosphorylation (4.8 ± 0.9 -fold over control in hypoxic rats vs 0.8 ± 0.8 -fold over control in metformin treated rats, $n=8$, $P<0.001$), suggesting that metformin decreased of RhoA/Rho kinase activity, without change of RhoA expression (Figure 5a).

These data therefore indicate that metformin treatment limits pulmonary artery vasoconstriction in hypoxic mammals such as rats.

Further, potential effect of metformin on cell proliferation is analysed.

In lung sections from hypoxic rats, PCNA labelling showed cell proliferation in pulmonary arteries as compared with control (Figure 6a), and attested by an strong increase in the number of PCNA positive pulmonary artery sections (Figure 6a). In parallel to normalization of vessel morphology, a number of PCNA positive vessels recovered basal level in hypoxic rats treated with metformin (Figure 6a). To get further insights into the antiproliferative properties of metformin, we next examined by western blot the activation of the main members of the three major subgroups of the mitogen-activated protein kinases (MAPK) family, namely extracellular regulated kinase (ERK), p38 MAPK (p38), and C-Jun NH₂-terminal kinase (JNK), in pulmonary artery lysates. As previously described (Welsh *et al.*, 2001), hypoxia induced activation of ERK (3.3 ± 0.8 -fold over control, n=8), a strong increase in p38 activity (6.3 ± 1.1 -fold over control, n=8) and no significant change in JNK activity (Figure 6b). However, hypoxia-induced activation of ERK and p38 was strongly reduced in metformin-treated rats (1.1 ± 0.5 and 2.3 ± 0.7 -fold over control, respectively, n=8, $p < 0.001$) (Figure 6b).

These data therefore indicate that metformin has antiproliferative properties.

Example 4: *ex vivo* contraction assay

Pulmonary arteries from rats of example 1 and 2 were collected in physiological saline solution (in mM; 130 NaCl, 5.6 KCl, 1 MgCl₂, 2 CaCl₂, 11 glucose, 10 Tris, pH 7.4 with HCl) and cut into rings. Rings of pulmonary arteries were suspended under isometric conditions, connected to a force transducer (Pioden controls Ltd, Canterbury, UK) and set up at a tension of 300-500 mg in Krebs-Henseleit solution at 37°C bubbled with 95 % O₂-5 % CO₂. After equilibration, the response to KCl 60 mM was determined. Endothelial-dependent relaxation was tested by adding increasing concentrations of carbachol (CCh) to rings pre-contracted by phenylephrine (PhE) (1 µM). Concentration-response curves to PhE were obtained by measuring the amplitude of the contractile responses to increasing PhE concentrations. For the analysis of the *ex-vivo* effect of metformin, pulmonary arterial rings from normoxic rats were treated by metformin (4 mM, 2.5 h) and contraction measurements were performed in the continuous presence of 4 mM metformin. Amplitude of the PhE-induced contraction was expressed in mg per mg of tissue (mg/mg).

Results are presented in Figures 5b, c and d. We functionally assessed the contractile properties of pulmonary artery rings from untreated and metformin-treated hypoxic rats (Figure 5b). Endothelial NO releasing capacity of the endothelium was assessed by measuring CCh-induced relaxation of pulmonary artery rings contracted by PhE (1 μ M). As shown in Figure 5b, CCh-induced relaxation was increased in metformin-treated hypoxic rat, indicating that metformin limited hypoxia-induced endothelial dysfunction. This observation is in agreement with the increase phosphorylation of eNOs observed in pulmonary arteries from metformin-treated hypoxic rats (Figure 5a). Cumulative contraction-response curve to phenylephrine further showed that contractile responses were significantly reduced in pulmonary artery rings from metformin treated hypoxic rats (Figure 5c), in agreement with the reduced RhoA/Rho kinase activation observed in pulmonary arteries from metformin-treated hypoxic rats (Figure 5a). This inhibitory effect of metformin on pulmonary artery contraction was also observed in control pulmonary arteries, treated *ex vivo* by metformin (Figure 5d). The maximal amplitude of PhE-induced contraction was reduced by 50 % in pulmonary arterial rings treated by metformin (4 mM, 2.5 h).

Taken together, these results provide evidence that metformin-induced inhibition of pulmonary vasoconstriction and improvement of endothelial function participates in its beneficial effect on pulmonary hypertension.

To analyze whether metformin directly affects pulmonary artery smooth muscle cell proliferation, we next analyzed *in vitro* rat pulmonary artery smooth muscle cell proliferation induced by PDGF.

Example 5: *in vitro* proliferation assay

Smooth muscle cells from rat pulmonary artery explants were cultured in DMEM with 10 % foetal calf serum, 100 U.ml⁻¹ penicillin and 100 μ g.ml⁻¹ streptomycin. Secondary cultures were obtained by serial passages after the cells were harvested with 0.5 g.l⁻¹ trypsin and 0.2 g.l⁻¹ EDTA and reseeded in fresh medium. After dissociation, cells at passage 2 were seeded on a 6 well-plate, washed and maintained in serum-free medium for 24 h, and then stimulated by platelet

derived growth factor (PDGF) (Peprotech France, Levallois Perret) during 24 h, with or without 4 mM metformin. Proliferation was assessed using both Western blot on cell lysates with antibody against PCNA and BrdU (bromodeoxyuridine) proliferation assay.

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PDGF (20 ng ml⁻¹) induced pulmonary artery smooth muscle cell proliferation, was attested by Western blot by a 3-fold increase in PCNA expression (Figure 6a) and an increased number of BrDU positive cells (Figure 7a). Both PCNA expression and the number of BrdU positive cells were strongly reduced by metformin (4 mM), indicating that metformin directly inhibited pulmonary artery smooth muscle cell proliferation (Figures 7a and b).

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This antiproliferative action of metformin could thus partly account for its beneficial effect on pulmonary artery remodeling and hypertension.

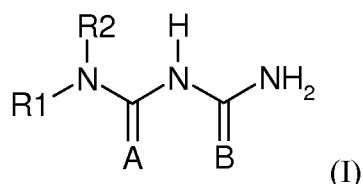
CLAIMS

1. A 5'-adenosine monophosphate-activated protein kinase (AMPK)
5 activator for use in preventing and/or treating pulmonary hypertension.

2. An AMPK activator according to claim 1, wherein the AMPK activator
is chosen from biguanide derivatives, 5-aminoimidazole-4-carboxamide riboside
(AICAR) and derivatives thereof.

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3. An AMPK activator according to claim 2, wherein the biguanide
derivative is selected from the compounds of formula (I):



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wherein

R1 and R2 are independently selected from hydrogen or a linear, branched or
cyclic, saturated or unsaturated (C1-C4) alkyl group, or an aralkyl group;

20 A and B are independently selected from NH or N-CHR3-N, wherein R3 is
selected from hydrogen or a linear, branched or cyclic, saturated or unsaturated (C1-
C4) alkyl group;

and the tautomeric, enantiomeric, diastereoisomeric and epimeric forms, the
solvates and the pharmaceutically acceptable salts thereof.

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4. An AMPK activator according to claim 2 or 3, wherein the biguanide
derivative is selected from the group consisting of metformin, phenformin and
buformin.

30 5. An AMPK activator according to any of claims 2 to 4, wherein the
biguanide derivative is metformin or one of its pharmaceutically acceptable salts.

6. An AMPK activator according to claim 5, wherein the metformin salt is selected from the group consisting of metformin hydrochloride, metformin p-chlorophenoxyacetate and metformin embonate.

5 7. An AMPK activator according to any of claim 1 to 6, wherein the AMPK activator is administered orally.

8. An AMPK activator according to any of claims 1 to 7, wherein the AMPK activator is administered in a composition in the form of a film-coated tablet.

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9. An AMPK activator according to any of claims 1 to 8, wherein pulmonary hypertension is pulmonary arterial hypertension (PAH).

10. An AMPK activator according to any of claims 1 to 8, wherein
15 pulmonary hypertension is pulmonary hypertension associated with left heart disease.

11. An AMPK activator according to any of claims 1 to 8 wherein
20 pulmonary hypertension is pulmonary hypertension associated with lung diseases and/or hypoxia.

12. An AMPK activator according to any of claims 1 to 8 wherein
25 pulmonary hypertension is pulmonary hypertension due to chronic thrombotic and/or embolic disease.

25

13. Product comprising metformin and bosentan as a combined preparation for simultaneous, separate or sequential use in the treatment of pulmonary hypertension.

14. Product comprising metformin, sitaxentan and sildenafil as a combined
30 preparation for simultaneous, separate or sequential use in the treatment of pulmonary hypertension.

15. A method for preventing and/or treating pulmonary hypertension comprising administering to a subject in need thereof an AMPK activator.

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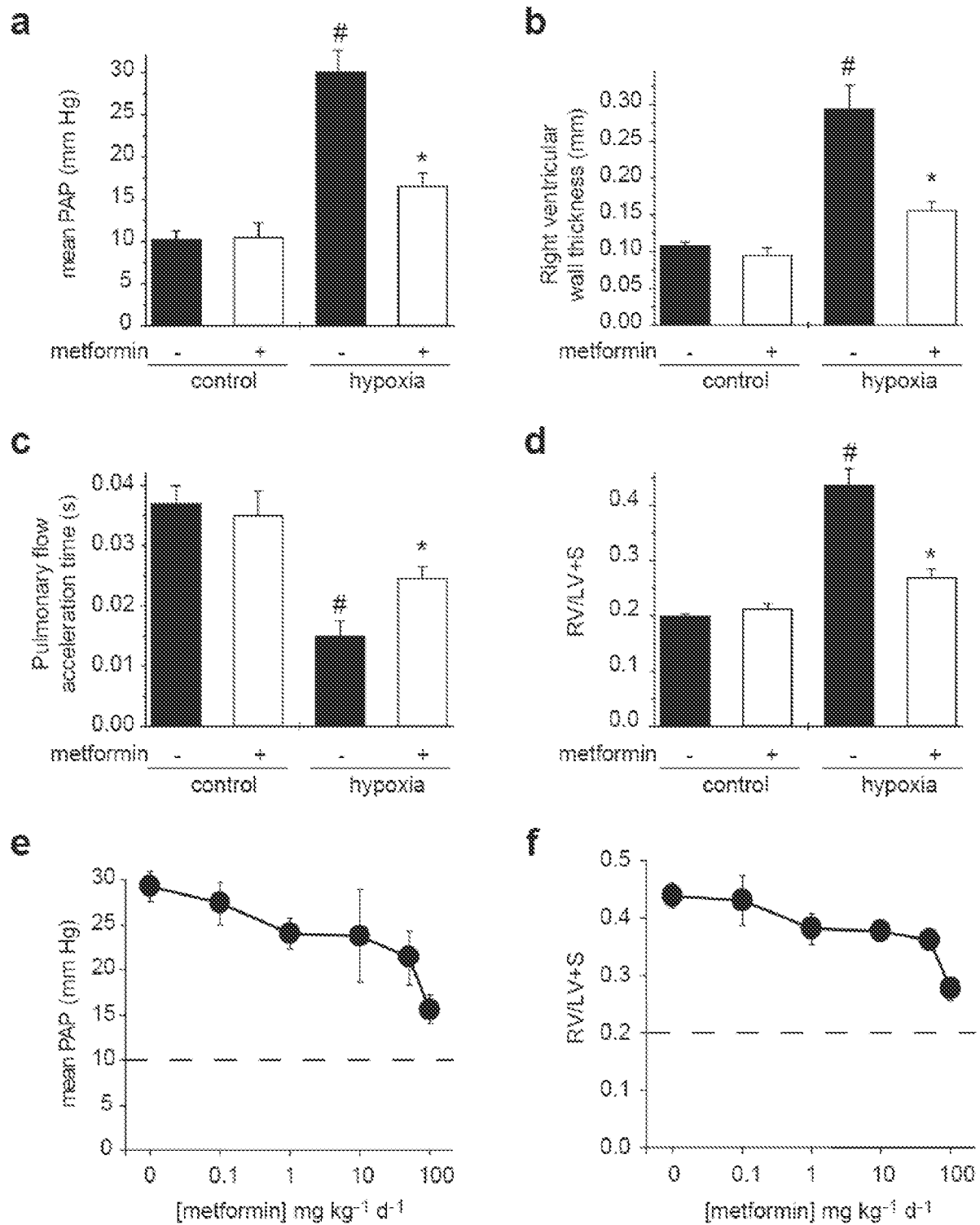


Figure 1

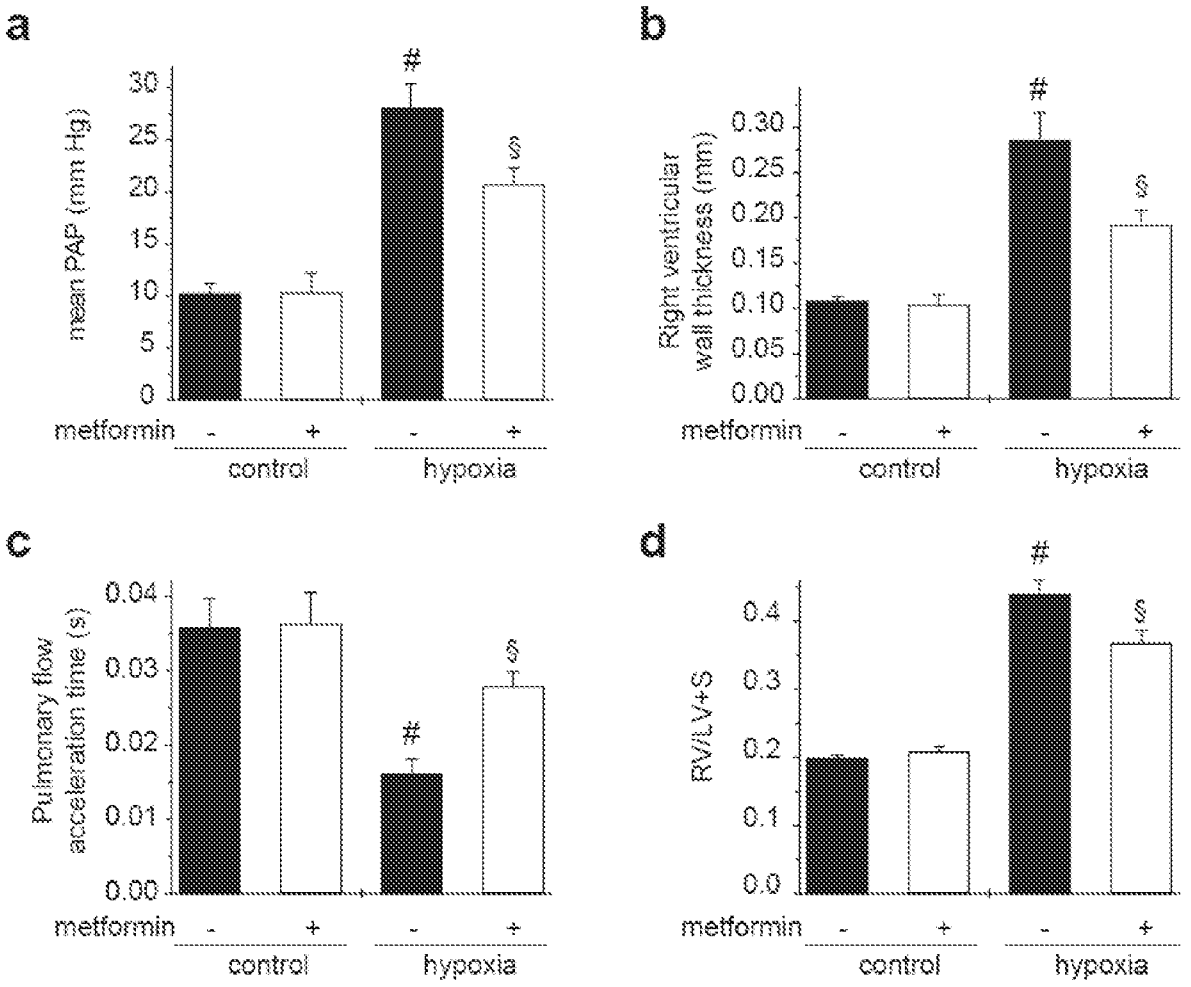


Figure 2

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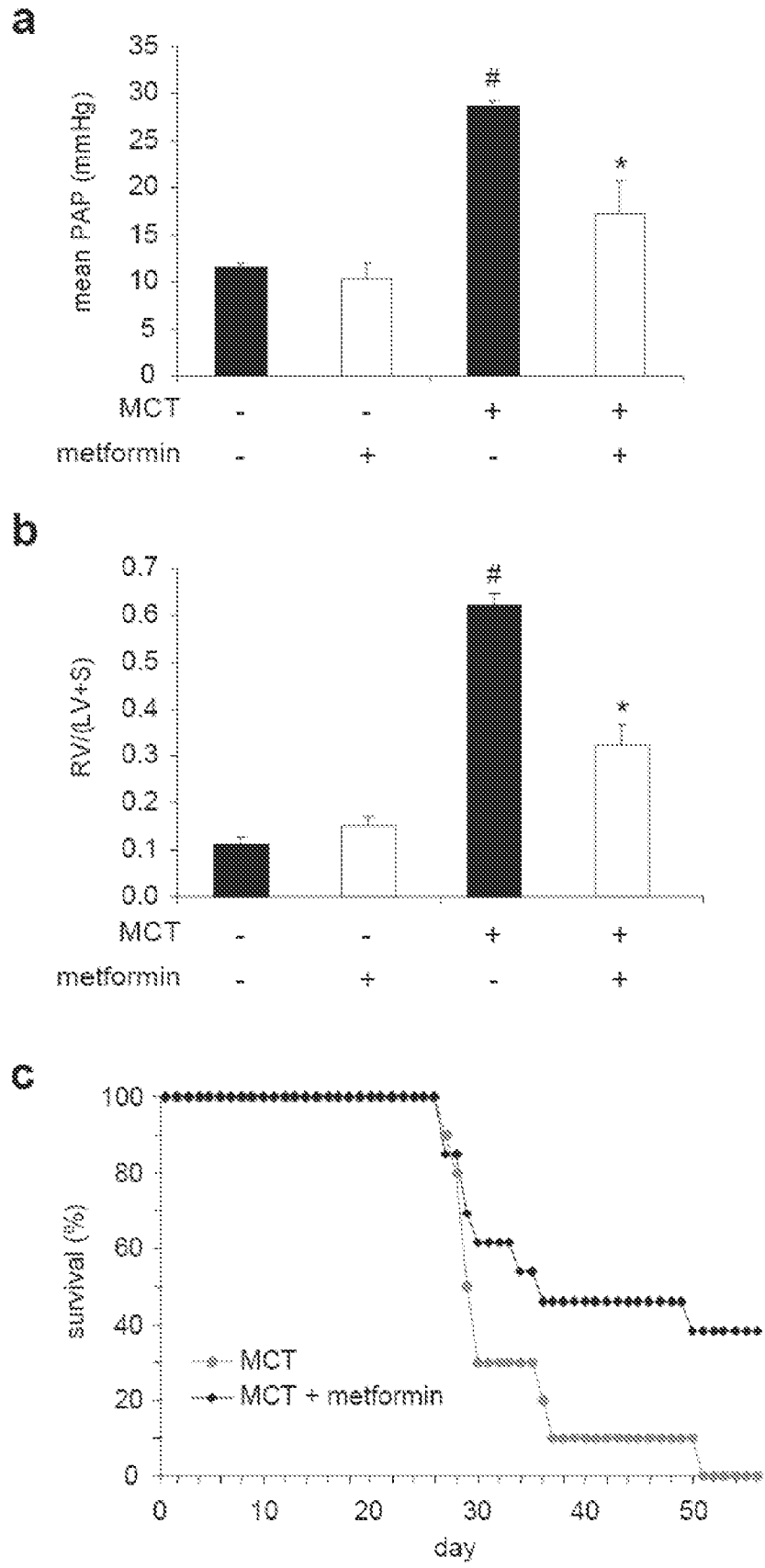


Figure 3

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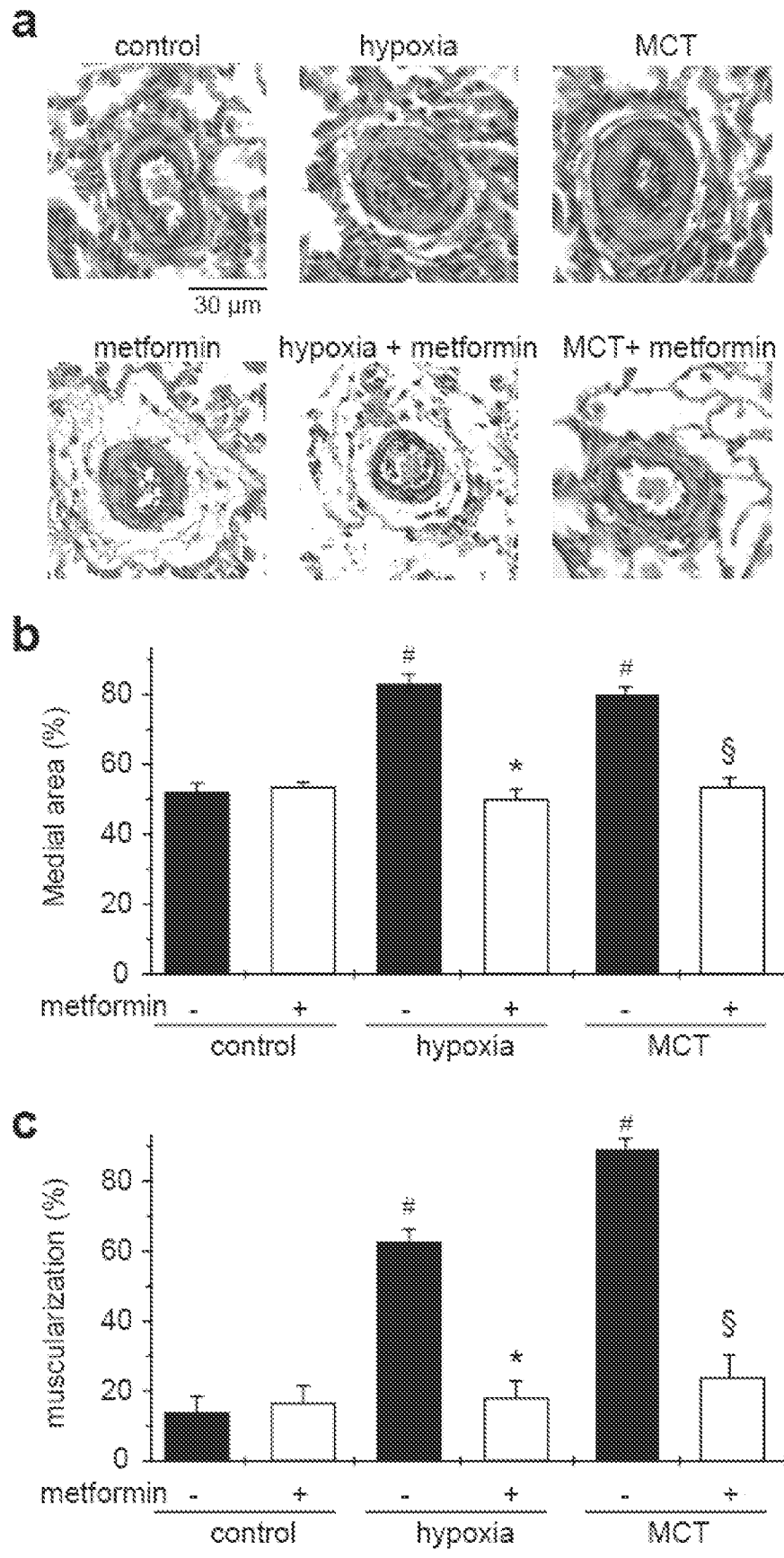


Figure 4

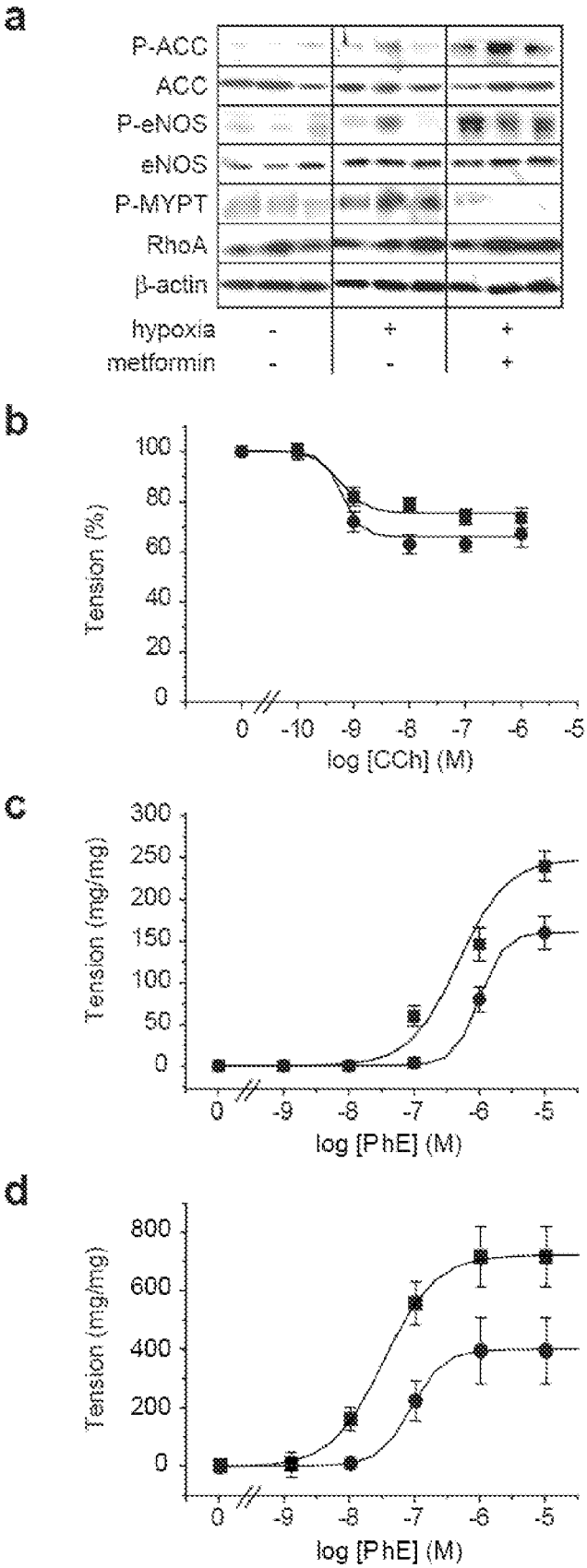


Figure 5

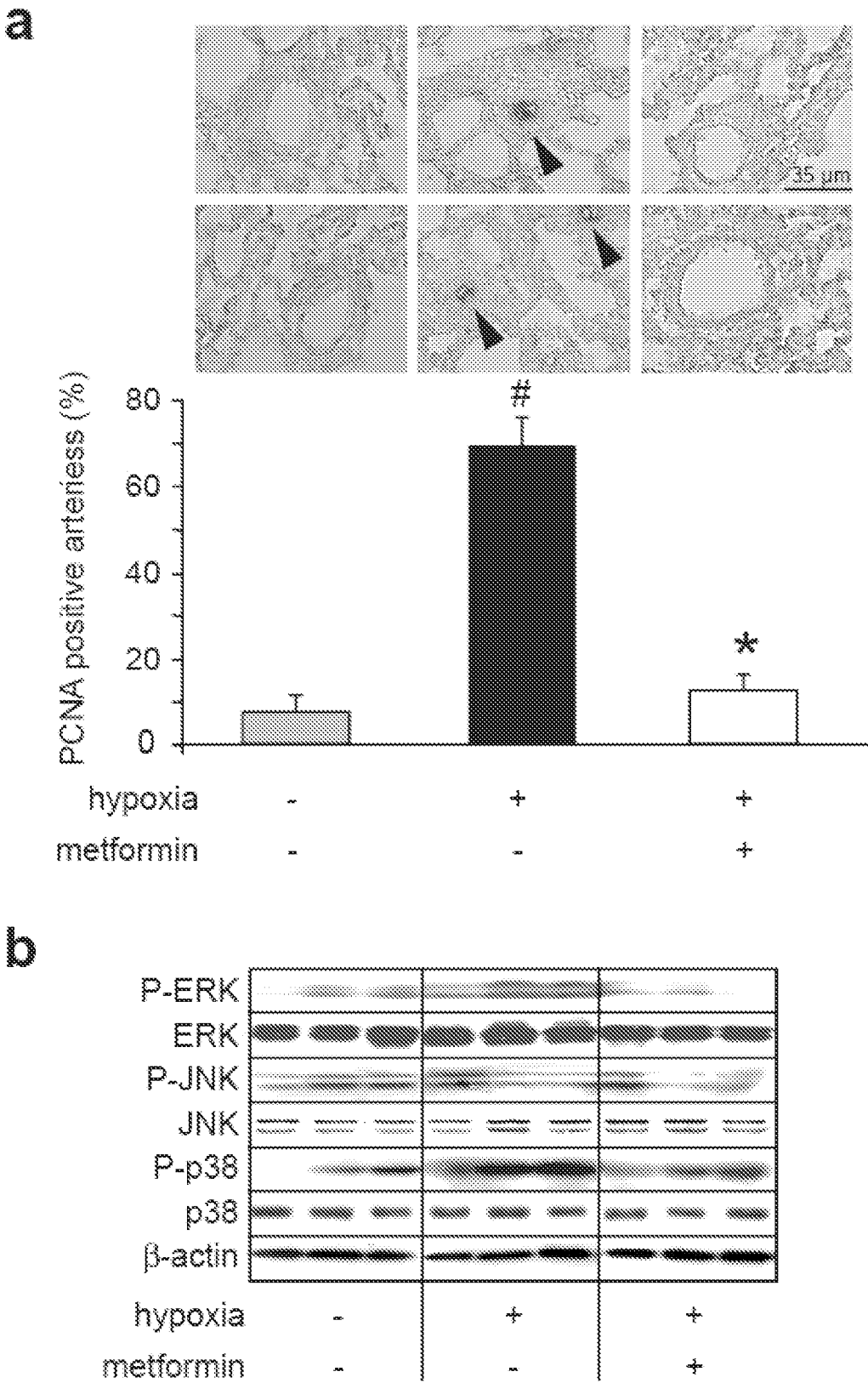


Figure 6

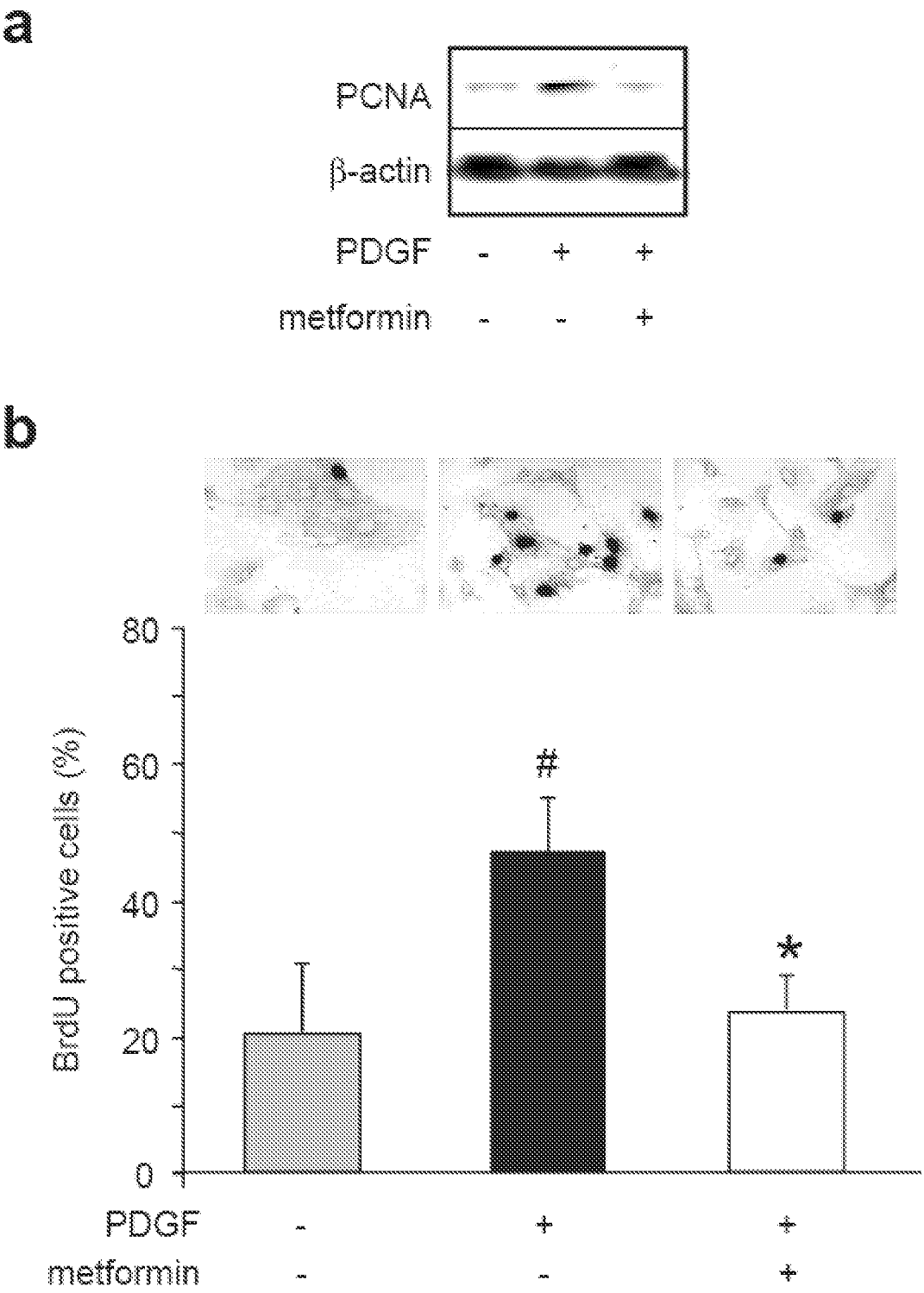


Figure 7

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/053034

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/155 A61K31/7056 A61P9/12
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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, PASCAL, SCISEARCH, 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	WO 2008/066770 A (UNIV FLORIDA [US]; OSTROV DAVID A [US]; RAIZADA MOHAN K [US]; HERNANDE) 5 June 2008 (2008-06-05) page 13, line 22 - line 24 page 28, line 11 - line 21 page 37, line 16 - line 22 * page 55, table 1, compound "NSC152085" *	1,2, 7-12,15
X	SASAKI ET AL: "Metformin Attenuates the Progression of Heart Failure Induced by Rapid Pacing in Dogs" JOURNAL OF CARDIAL FAILURE, CHURCHILL LIVINGSTONE, NAPERVILLE, IL, US, vol. 13, no. 6, 11 July 2007 (2007-07-11), page S54, XP022140088 ISSN: 1071-9164	1-12,15
Y	the whole document ----- -/--	13,14



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)

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"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

26 April 2010

Date of mailing of the international search report

04/05/2010

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	FAHLÉN M: "Phenformin as a cause of primary pulmonary hypertension." BRITISH MEDICAL JOURNAL 11 DEC 1976, vol. 2, no. 6049, 11 December 1976 (1976-12-11), page 1450, XP009118621 ISSN: 0007-1447 the whole document -----	1-4, 7-12,15
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X	WO 00/28076 A1 (ST VINCENTS INST MED RES [AU]; STAPLETON DAVID IAN [AU]; CHEN ZHIPING) 18 May 2000 (2000-05-18) page 6, line 16 - line 22 -----	1,7-12, 15
Y	EP 1 902 732 A1 (TAKEDA PHARMACEUTICAL [JP]) 26 March 2008 (2008-03-26) page 5, paragraph 24 -----	2-6
Y	WO 2007/050783 A2 (COTHERIX INC [US]; FONG BENSON [US]) 3 May 2007 (2007-05-03) claims -----	2-6
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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