Abstract:
The invention relates to the treatment of pulmonary hypertension, and in particular to the use of SERIOO and related compounds for the treatment of pulmonary hypertension. In particular, the invention relates to the use of SERIOO (Ac-RYYR - WK-KKKKKK-NH2) and related compounds for the treatment of pulmonary hypertension.
Materials and methods for treatment of pulmonary hypertension

Field of the invention
The invention relates to the treatment of pulmonary hypertension, and in particular to the use of SER100 and related compounds for the treatment of pulmonary hypertension.

Background to the invention
Pulmonary hypertension (PH) is a rare lung disorder. Patients are normally severely affected, with a life expectancy of only a few years after the first symptoms have occurred. In the 1980s, the median survival time was 2-3 years, although the survival time has been doubled in recent years due to novel treatment schedules and medication (McLaughlin et al., 2005; Bensa et al., 2010).

PH is a chronic, progressive disease characterized by elevated blood pressure in the pulmonary arteries. This elevated pressure ultimately results in right ventricular failure and death (McLaughlin et al., 2006).

In this respect, PH is distinct from other forms of hypertension because it affects the right side of the heart, while other forms of hypertension affect the left side of the heart.

PH is often asymptomatic in the beginning and is typically diagnosed late in its course. The first symptom of PH is usually shortness of breath with everyday activities, such as climbing stairs. Fatigue, dizziness and fainting spells can also be symptoms, as can irregular heartbeat (palpitations or strong, throbbing sensation), racing pulse, progressive shortness of breath during exercise or activity, and difficulty breathing at rest. Swelling of the ankles, abdomen or legs, bluish lips and skin and chest pain may occur as strain on the heart increases (cor pulmonale). In more advanced stages of the disease, even minimal activity will produce some of the symptoms. Eventually, it may become difficult to carry out any activities as the disease worsens.

Symptoms range in severity, although a given patient may not have all of the symptoms.

Despite improvements in the diagnosis and management of PH over the past 2 decades with the introduction of targeted medical therapies leading to improved survival, the disease continues to have a poor long-term prognosis, whilst mortality and hospitalisation rates continue to increase (Mehari et al., 2014). Consequently there is a need for further clinical options for treatment of PH.
Summary of the invention

The invention provides a compound for use in a method of treating pulmonary hypertension, wherein the compound has the formula:

$$R^1-Z-X-Z'-R^2$$

wherein:

- $R^1$ is H (hydrogen), $C_{1-4}$ alkyl, acetyl (Ac), formyl, benzoyl or trifluoroacetyl (Tfa);
- $R^2$ is OH or NR$_3$R$_4$ where each of $R_3$ and $R_4$ independently represents H (hydrogen), C$_{1-6}$ alkoxy, aryl or C$_{1-6}$ alkyl;
- $X$ is a hexapeptide having the amino acid sequence (RK)YY(RK)(WI)(RK), where parentheses indicate alternative residues which may be present at positions 1, 4, 5 and 6, and wherein each amino acid residue in said hexapeptide may be in the L or D form;
- $Z$ and $T$ are independently absent or a charged peptide chain of from 4 to 20 amino acid residues, wherein each amino acid residue in $Z$ or $Z'$ may be in the L or D form, providing that not both of $Z$ and $Z'$ are absent;
- or is a retro form thereof;
- or is a pharmaceutically acceptable salt, solvate or hydrate of either.

Thus the peptide may consist of:

(i) entirely L-amino acids (all-L);
(ii) a mixture of L- and D-amino acids; or
(iii) entirely D-amino acids (all-D);

or may be a retro form of any of those alternatives (including retro-all-L and retro-all-D).

The hexapeptide $X$ may be selected from the group consisting of KYYRWR, KYYRWK, RYYRWR, RYYRWK, RYYRK (all-D), RYYYK, RYYYR, RYYYK, RYYYK, RYYYK, and RYYYWK, or a retro or retro-all D form thereof, e.g. KWRYR or KWRYYK. In some preferred embodiments the hexapeptide $X$ is RYYRWK or KYYRWK, e.g. RYYRWK.

It may be desirable that all amino acid residues in the hexapeptide $X$ are in the L-form.

The number of amino acid residues in each of $Z$ and $Z'$ is preferably in the range of 4-10, e.g. 4, 5, 6 or 7.
Z, where present, is typically negatively charged. It is preferred that the amino acid residues of Z are selected from the group consisting of Q, T, S, P, N, E, and D. For example, the N-terminal amino acid of Z may be selected from the group consisting of Q T, N and S and the remaining amino acid residues are selected from the group consisting of P, D and E.

Specific examples of sequences for Z include N(E)$_7$, N(E)$_5$, N(E)$_3$, S(E)$_7$, S(E)$_5$, S(E)$_3$, N(P(E)$_4$, N(P(E)$_5$, N(D)$_7$, N(D)$_5$, N(D)$_3$, Q(E)$_7$, Q(E)$_5$, Q(E)$_3$, Q(N(D)$_7$, Q(D)$_6$, Q(D)$_5$, and Q(D)$_3$. Preferably Z is N(E)$_5$.

Z', where present, is typically positively charged. It is preferred that the amino acid residues of Z' are selected from the group consisting of A, G, K, and R, preferably K. Specific examples of sequences for Z' include A(K)$_4$G, K$_5$G, A(K)$_5$, H$_6$, K$_7$, K$_8$, K$_9$, K$_6$, K$_7$, K$_6$, K$_5$, and K$_4$. K$_4$, K$_5$, K$_6$, K$_7$ and K$_8$; may be particularly preferred, especially K$_6$.

It may be desirable that all amino acid residues of Z and Z' are in the L-form.

It may be desirable that the compound is all-L or all-D. For example, if it contains D-amino acids, then it is all-D.

Additionally or alternatively, it may be desirable that the peptide is not a retro peptide.

In certain embodiments, Z is absent and Z' is present.

Examples of conjugates Z-X-Z' are

RYYRWK-KKKKKK
KKKKKK-RYYRWK
NEEEEE-RYYRWK-KKKKKK
RYYRWK-KKKKKK (all D)
KYYRWK-KKKKKK
RYYRK-KKKKKK
RYYRWK-AKKKKK
RYYRWK-KKKKK
RYYRWK-KKKKKK
and retro and retro-all-D forms thereof such as KKKKKK-KWRYYR and KKKKKK-KWRYYR (all-D).
It may be preferred that $R^1$ is H, Ac or Tfa, especially Ac. Additionally or alternatively, $R^2$ may be $\text{NH}_2$.

Specific exemplary compounds include:

- Ac-RYYRWK-KKKKKK-NH$_2$ (SER100)
- Ac-KKKKKK-RYYRWK-NH$_2$
- H-NEEEEE-RYYRWK-KKKKKK-NH$_2$
- Ac-RYYRWK-KKKKKK-NH$_2$ (all D)
- AC-KYYRWK-KKKKKK-NH$_2$
- AC-RYYRIK-KKKKKK-NH$_2$
- AC-RYYRWK-KKKKKK-NH$_2$
- AC-RYYRK-KKKKKK-NH$_2$
- AC-RYYRWK-KKKKKK-NH$_2$
- Ac-RYYRWK-KKKKKK-NHz
- Tfa-RYYRWK-KKKKKK-NH$_2$
- AC-KKKKKK-KWRYYR-NH$_2$
- AC-KKKKKK-KWRYYR-NH$_2$ (all D)

and pharmaceutically acceptable salts, hydrates and solvates thereof.

Any appropriate pharmaceutically acceptable salt may be employed, especially acid addition salts. Suitable examples of counterions include acetate, trifluoroacetate, chloride, sulphite, maleate and oleate, although acetate and chloride may be particularly preferred.

Examples of acid addition salts of SER100 are:

- Ac-RYYRWK-KKKKKK-NH$_2$ x $9\text{CH}_3\text{COOH}$
- Ac-RYYRWK-KKKKKK-NH$_2$ x $9\text{HCl}$.

The invention also provides the use of a compound as described above in the preparation of a medicament for the treatment of PH.

The invention further provides a method of treatment of PH in a subject in need thereof, comprising administering a compound as described above to said subject.

In all aspects of the invention, the compounds are useful in the treatment of pulmonary hypertension (PH). They may be of use in the treatment of any sub-group of PH, including Group 1 PH (also referred to as pulmonary arterial hypertension; PAH), Group V PH, Group 2 PH, Group 3 PH, Group 4 PH and Group 5 PH.
The invention will now be described in more detail, by way of example and not limitation, by reference to the accompanying drawings and examples.

**Brief description of the figures**

**Figure 1. Schematic representation of the experimental groups and interventions**

**Figure 2. Effect of SER100 on right ventricular systolic pressure (RVSP)**
Right ventricular systolic pressure (RVSP) in normoxic mice and animals exposed to hypoxia (10% O2; 2 or 5 weeks) plus semaxanib (20mg/kg, subcutaneously at day 0, 7 & 14) in the absence and presence of SER100 (1mg/kg/day; osmotic minipump). Interventions were started at day 14. Data are expressed as mean±se. ***P<0.001 v Normoxia; ###P<0.001 v Hypoxia/semaxanib (5 weeks); $P<0.05 v$ Hypoxia/ semaxanib (2 weeks). n=7-8.

**Figure 3. Effect of SER100 on right ventricular hypertrophy (RVH)**
Right ventricle:body weight ratio (RV/BW) and right ventricle to left ventricle plus septum ratio (RV/[LV+S]) in normoxic mice and animals exposed to hypoxia (10% O2; 2 or 5 weeks) plus semaxanib (20mg/kg, subcutaneously at day 0, 7 & 14) in the absence and presence of SER100 (1mg/kg/day; osmotic minipump). Interventions were started at day 14. Data are expressed as meanisem. *** P<0.001 v Normoxia; ###P<0.01 v Hypoxia/semaxanib (5 weeks). n=7-8.

**Figure 4. Effect of SER100 on mean arterial blood pressure (MABP)**
Mean arterial blood pressure (MABP) in normoxic mice and animals exposed to hypoxia (10% O2; 2 or 5 weeks) plus semaxanib (20mg/kg, subcutaneously at day 0, 7 & 14) in the absence and presence of SER100 (1mg/kg/day; osmotic minipump). Interventions were started at day 14. Data are expressed as mean±sem. n=7-8.

**Figure 5. Effect of SER100 on pulmonary vascular remodeling**
Pulmonary vascular re-modelling in normoxic mice and animals exposed to hypoxia (10% O2; 5 weeks) plus semaxanib (20mg/kg, subcutaneously at day 0, 7 & 14) in the absence and presence of SER100 (1mg/kg/day; osmotic minipump). Interventions were started at day 14. n=6.

**Figure 6. Effect of SER100 on Aquaporin (AQP)-1 expression in lung homogenates**
Aquaporin (AQP)-1 expression in whole lung homogenates from normoxic mice and animals exposed to hypoxia (10% O₂; 5 weeks) plus semaxanib (20mg/kg, subcutaneously at day 0, 7 & 14) in the absence and presence of SER100 (1mg/kg/day; osmotic minipump). Interventions were started at day 14. Data are expressed as mean±sem. n=6. *P<0.05 v Normoxia.

Figure 7. Effect of SER100 on pulmonary microvascular endothelial cell proliferation
Proliferation of pulmonary microvascular endothelial cells from mice exposed to hypoxia (10% O₂; 5 weeks) plus semaxanib (20mg/kg, subcutaneously at day 0, 7 & 14) in the absence (control) and presence of SER100 (10nM-10µM). Cells were plated in 96 well plates at a density of 10k cells/well and proliferation measured by BrdU incorporation at 24hr. Data are expressed as mean±sem. n=4.

Figure 8. Effect of SER100 on pulmonary microvascular endothelial cell migration (subcutaneous delivery)
Migration of pulmonary microvascular endothelial cells from mice exposed to hypoxia (10% O₂; 5 weeks) plus semaxanib (20mg/kg, subcutaneously at day 0, 7 & 14) in the absence (control) and presence of SER100 (10nM-10µM). Cells were seeded at a density of 25k cells/well and a scratch made with a 1ml pipette tip. Data are expressed as mean±sem. Right panel shows data for SER100 (1µM) alone for clarity. n=4. *P<0.05 versus Control.

Figure 9. Effect of SER100 on pulmonary microvascular endothelial cell migration (1mg/kg/day osmotic minipump)
Migration of pulmonary microvascular endothelial cells from mice exposed to hypoxia (10% O₂; 5 weeks) plus semaxanib (20mg/kg, subcutaneously at day 0, 7 & 14) in the absence (control) and presence of SER100 (1mg/kg/day; osmotic minipump). Cells were seeded at a density of 10k cells/well and a scratch made with a 1ml pipette tip. Data are expressed as mean±sem. n=4. ** P<0.05 versus Hypoxia

Detailed description of the invention
Throughout the description and claims the one letter code for natural amino acids is used as well as the three letter code for natural amino acids and generally accepted three letter codes for other a-amino acids, such as ornithine (Orn), 2,4-diaminobutanoic acid (Dab) and 2,3-diaminopropanoic acid (Dapa). Where the L or D form has not been specified it is to be understood that the amino acid in question has the L form. Where nothing is specified it is to be understood that the C-terminal amino acid of a compound of the invention exists as the free carboxylic acid, which may also be specified as "-OH".
D-amino acids are unnatural amino acids which are stable in a protease-rich environment. Thus, a useful way of stabilising peptides against proteolytic degradation is to substitute L-amino acids with corresponding D-amino acids.

The term "all-D form" of a peptide refers to a peptide wherein all amino acid units are in the D-form. Similarly, "all-L form" refers to a peptide where all amino acids units are in the L-form.

The term "retro form" of a peptide refers to a peptide having the reversed sequence to that shown, i.e. the relevant amino sequence (e.g. Z-X-Z', Z-X or X-Z) runs from C- to N-terminus instead of the conventional N- to C-terminus. For the avoidance of doubt, R^1 and R^2 groups remain at the actual N and C-termini of the molecule. Alternatively, of course, the sequence of the peptide as written may be reversed, so that it is shown in the conventional N- to C- direction.

The term "retro-all-D form" of a peptide refers to a peptide which has the reversed sequence to that shown (as described above) and is composed entirely of D-amino acids. A "retro-all-D" peptide is sometimes known as a "retro-inverso" peptide. Retro-all-D peptides may be superior mimics of conventional L-peptides than all-D or retro forms of those peptides.

The term "C_{1-6} alkyl" includes methyl, ethyl, propyl, iso-propyl, butyl, pentyl and hexyl and all possible isomers thereof. C_{1-4} alkyl, especially methyl and ethyl, may be preferred.

The term "C_{1-6} alkoxy" refers to an ester group of the formula R-O- wherein R represents C_{1-6} alkyl. R may preferably be C_{1-4} alkyl, especially methyl and ethyl.

The term "aryloxy" refers to an ester group of the formula R-O- wherein R represents phenyl or naphthyl optionally substituted with a C_{1-6} alkyl group, e.g. a C_{1-4} alkyl group, as described above.

Suitable methods of synthesis for the therapeutic compounds used in the invention are described, for example, in W098/11125 and WO01/98324.

**Pulmonary hypertension**

PH is defined by a mean pulmonary arterial pressure ≥ 25 mmHg at rest, as assessed by right heart catheterization (RHC). Normal pressure at rest is 10-20 mmHg. A definition of ≥
30 mmHg during exercise is sometimes applied, but is controversial (Guidelines for the
diagnosis and treatment of pulmonary hypertension (2009)).

Current clinical classification, as laid down at the 4\textsuperscript{th} World Symposium on Pulmonary
Arterial Hypertension (2008), identifies a number of different sub-groups of pulmonary hypertension.

Group 1 is designated "pulmonary arterial hypertension" (PAH) and is characterised by pre-
capillary PH in the absence of other causes of pre-capillary PH such as lung disease,
chronic thromboembolic PH, or other rare diseases.

PAH may be idiopathic PAH, heritable PAH, drug and toxin-induced PAH, or persistent
pulmonary hypertension of the new-born. PAH may also be associated with connective
tissue disease, HIV infection, portal hypertension, congenital heart disease, schistosomiasis
or chronic haemolytic anaemia.

A number of genetic abnormalities have been identified as being associated with or
conferring a predisposition to heritable PAH, with the gene encoding bone morphogenetic
protein receptor 2 being the most commonly affected.

Group 5 is defined as PH due to pulmonary veno-occlusive disease (PVOD) and/or
pulmonary capillary haemangiogenesis.

Group 2 is defined as PH due to left-sided heart disease or failure, including systolic
dysfunction, diastolic dysfunction and valvular disease. It may be associated with
cardiomyopathy, mitral stenosis, mitral regurgitation, aortic stenosis and aortic regurgitation.

Group 3 is defined as PH due to lung disease and/or hypoxia. For example it may be
associated with chronic obstructive pulmonary disease (COPD), interstitial lung disease,
other pulmonary diseases, sleep disordered breathing, alveolar hypoventilation disorders,
chronic exposure to high altitude and developmental abnormalities.

Group 4 encompasses PH due to pulmonary embolus or pulmonary thrombosis.

Group 5 encompasses PH having unclear or multifactorial mechanisms which do not fit into
any of the other categories.
For reviews of classification, etiology and diagnosis, see Simonneau et al., 2009 and the Guidelines for the diagnosis and treatment of pulmonary hypertension (2009).

Any of the sub-groups of PH (groups 1, 1', 2, 3, 4 and 5), as well as any of the individual sub-types of PH within these groups, may be separately suitable for treatment according to the invention.

Pulmonary arterial smooth muscle cell (PASMC) proliferation, hypertrophia, and migration in response to hypoxia is thought to be a key component of the vascular remodeling that occurs in chronic hypoxic pulmonary hypertension. However, a number of other factors may be implicated in PASMC proliferation (Eddahibi et al., 1999; Stiebellehner et al., 2003), including aquaporins (AQPs).

13 types of aquaporins have been identified in mammals to date, of which six are present in the kidney. Various types are also present in the lung and CNS amongst other tissues (Nielsen and Acre, 1995, Zhang et al., 2010). The most well-characterised at present are AQP1, 2, 3 and 4.

AQP1, AQP4 and AQP7 have been shown to be present in pulmonary artery smooth muscle, with AQP1 being the predominant species. Accumulating evidence indicates that proliferation, hypertrophia, and migration of certain cell types in the pulmonary artery system is regulated by AQP1 (Monzani et al., 2009). Leggett et al. (2012) have suggested a possible role of AQP1 in mediating pulmonary vascular remodeling during the pathogenesis of pulmonary hypertension. However, the actual role (if any) of AQP1 in PH remains unclear.

During acute hypoxia, the amount of AQP1 in the pulmonary artery smooth muscles is increased. However, no increase in mRNA was observed and no changes were seen in the level of other AQPs present in the pulmonary artery bed (Leggett et al., 2012).

In contrast, in chronic hypoxia, where migration of pulmonary artery smooth muscle cells is elevated, both AQP1 mRNA and AQP1 protein levels were upregulated (Leggett et al., 2012). These increases were also seen after hypertonic stress (Leitch et al., 2001). Modulation of AQP1 localization was found within the cell (Conner et al., 2012).

The upregulation of AQP1 in chronic hypoxia seems to be cell type-specific (Yamamoto et al., 2001, Leggett et al., 2012). Since AQP1 mRNA levels were not altered during acute
hypoxic exposure, Leggett et al. found it unlikely that changes in AQP1 transcription are involved; these changes may instead be a result of altered AQP1 protein synthesis and/or stability.

**SER100 and related compounds**

The compounds described herein are partial agonists of the nociceptin receptor (also known as the orphanin FQ receptor or opioid receptor-like 1, and often designated NOP or ORL1). They were described in WO01/98324.

Hexapeptides having the sequence Ac-(RK)YY(RK)(WI)(RK)-NH₂ were found by Dooley et al. (J. Pharmacol. Exp. Ther. 283(2): 735-741, 1997) to have partial agonist activity at the nociceptin receptor but were insufficiently stable to be viable clinical candidates. The compounds described in WO01/98324 have significantly increased stability, and may also display increased solubility, compared to those hexapeptides. In addition, the compounds used in the present invention appear to have a reduced capacity to cross the blood-brain barrier as compared to both the unconjugated hexapeptides and nociceptin itself, thus reducing or eliminating their activity in the central nervous system and limiting their effects more specifically to the periphery.

The compound Ac-RYYRWK-KKKKKK-NH₂ is referred to in this specification as SER100. It has previously been known as ZP120. It has been shown to have considerable aquaretic effects, i.e. promoting excretion of water while conserving electrolytes such as sodium and potassium. It was initially developed for treatment of acute/sub-acute and chronic heart failure. However, in clinical trials, it decreased the systolic blood pressure (SBP) in healthy individuals, and produced a more pronounced decrease in SBP in patients with heart failure. It has subsequently been developed for use in treatment-resistant isolated systolic hypertension (TR-ISH), which is a major risk factor for stroke, acute myocardial infarction, heart and renal failure.

SER100 has been shown to decrease AQP2 (aquaporin 2) protein level and alter AQP2 protein localisation in different parts of the nephron in a vasopressin-independent manner (Hadrup et al., 2007). These effects on AQP2 are believed to be independent of SER100 binding to the ORL1 receptor, and thus appear to be mediated by a separate mechanism to the nociceptin pathway. It was suggested that SER100 might down-regulate AQP2 protein level by increasing degradation or urinary excretion, rather than by a direct effect on expression.
The present inventors came to investigate the effects of SER100 on PH motivated by a belief that SER100 and related compounds may be capable of modulating the expression or distribution of AQP1, including AQP1, in the pulmonary vasculature (e.g. in the pulmonary artery smooth muscle cells (PASMC), or pulmonary endothelium cells), and may therefore represent a candidate for use in the treatment of PH. In particular, it was thought that by down-regulating AQP1 protein level, or modulating AQP1 distribution, SER100 and related compounds may inhibit the stimulatory effect of AQP1 on PASMC proliferation and/or migration. It was thought that the compounds may act to down-regulate the levels of active AQP1 protein, or modulate cellular or sub-cellular localisation of AQP1, in pulmonary artery, e.g. in pulmonary artery smooth muscle. This expected effect on AQP1 may have been exerted via the compounds' agonist effect at the nociceptin receptor (ORL1) or may be independent thereof. However, whatever the effect (if any) on AQP1, it may be desirable that the compounds have agonist activity at the nociceptin receptor.

SER100 was indeed found to have alleviatory effects on indicators of PH, including a lowered pulmonary artery pressure (RVSP), suppressed pulmonary vascular remodelling (i.e. reduced muscularisation), and suppressed right ventricle/left ventricle ratio in models of PH. However, it was surprisingly found that SER100 did not downregulate expression of AQP1. In fact, hypoxia was found to elevate AQP1 expression in untreated animals, and this increased expression was further enhanced in animals treated with SER100. This suggests that AQP1 expression may actually represent an endogenous defence mechanism against the effects of hypoxia, which is further reinforced by SER100.

**Pharmaceutical compositions**

For use in the present invention, therapeutically effective compounds are typically formulated as pharmaceutical compositions for storage or administration. Such a composition typically comprises a therapeutically effective amount of a compound of the invention, in the appropriate form, in a pharmaceutically acceptable carrier.

The therapeutically effective amount will depend on the specific compound, the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration can be tailored to achieve optimal efficacy, and may depend on such factors as weight, diet, concurrent medication and other factors, well known to those skilled in the medical arts. The dosage sizes and dosing regimen most appropriate for human use may be guided by the results obtained by the present invention, and may be
confirmed in properly designed clinical trials. The compounds of the present invention may be particularly useful for treatment of humans.

The term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers. Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). For example, sterile saline and phosphate-buffered saline at slightly acidic or physiological pH may be used. pH buffering agents may be phosphate, citrate, acetate, Tris(hydroxymethyl)aminomethane (TRIS), N-Tris(hydroxymethyl)methyl-3-aminopropanesulphonic acid (TAPS), ammonium bicarbonate, diethanolamine, histidine, which is a preferred buffer, arginine, lysine, or acetate or mixtures thereof. The term further encompasses any agents listed in the US Pharmacopeia for use in animals, including humans.

The term "pharmaceutically acceptable salt" refers to a salt of any one of the compounds of the invention. Salts include pharmaceutically acceptable salts such as acid addition salts and basic salts. Examples of acid addition salts include hydrochloride salts, citrate salts and acetate salts. Examples of basic salts include salts where the cation is selected from alkali metals, such as sodium and potassium, alkaline earth metals, such as calcium, and ammonium ions +N(R^3)_3R^4, where R^3 and R^4 independently designates optionally substituted C_1_6-alkyl, optionally substituted C_2_7-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl. Other examples of pharmaceutically acceptable salts are described in "Remington's Pharmaceutical Sciences", 17th edition. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, U.S.A., 1985 and more recent editions, and in the Encyclopaedia of Pharmaceutical Technology.

"Treatment" is an approach for obtaining beneficial or desired clinical results. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures in certain embodiments. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. By treatment is meant inhibiting or reducing
an increase in pathology or symptoms when compared to the absence of treatment, and is not necessarily meant to imply complete cessation of the relevant condition.

The pharmaceutical compositions can be in unit dosage form. In such form, the composition is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparations, for example, in solution form, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms. It may be provided in single dose injectable form, for example in the form of a pen. In certain embodiments, packaged forms include a label or insert with instructions for use. Compositions may be formulated for any suitable route and means of administration. Pharamaceutically acceptable carriers or diluents include those used in formulations suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, and transdermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

Compositions of the invention may further be compounded in, or attached to, for example through covalent, hydrophobic and electrostatic interactions, a drug carrier, drug delivery system and advanced drug delivery system in order to further enhance stability of the compound, increase bioavailability, increase solubility, decrease adverse effects, achieve chronotherapy well known to those skilled in the art, and increase patient compliance or any combination thereof. Examples of carriers, drug delivery systems and advanced drug delivery systems include, but are not limited to, polymers, for example cellulose and derivatives, polysaccharides, for example dextran and derivatives, starch and derivatives, poly(vinyl alcohol), acrylate and methacrylate polymers, polyactic and polyglycolic acid and block co-polymers thereof, polyethylene glycols, carrier proteins, for example albumin, gels, for example, thermogelling systems, for example block co-polymeric systems well known to those skilled in the art, micelles, liposomes, microspheres, nanoparticulates, liquid crystals and dispersions thereof, L2 phase and dispersions thereof, well known to those skilled in the art of phase behaviour in lipid-water systems, polymeric micelles, multiple emulsions, self-emulsifying, self-microemulsifying, cyclodextrins and derivatives thereof, and dendrimers.

An effective dosage and treatment protocol may be determined by conventional means, starting with a low dose in laboratory animals and then increasing the dosage while
monitoring the effects, and systematically varying the dosage regimen as well. Numerous factors may be taken into consideration by a clinician when determining an optimal dosage for a given subject. Such considerations are known to the skilled person.

Suitable routes and dosages for SER100 and related compounds may include intravenous (i.v.) infusion, e.g. at a level of 0.90-255 Mg/kg, e.g. for between 1 and 60 hours, e.g. between 6 and 48 hours. Alternatively, SER100 and related compounds may be given subcutaneously (s.c), e.g. in a single bolus of 5-30 mg (for a 70 kg adult), e.g. once or twice daily. Oral formulations may also be suitable, e.g. in an amount of 1-50 mg (for a 70 kg adult), e.g. once or twice or three times daily. It may be desirable to begin with a course of administration by intravenous infusion, followed by a course of subcutaneous or oral administration as described.

**Example 1: Experimental model of pulmonary hypertension**

Sprague Dawley rats (180-200 g) are randomly divided into three groups:

Treatment group 1 (placebo group) is treated with distilled water.

Treatment group 2 (MCT/hypoxia group) is treated with monocrotaline (MCT) (Hanmi Pharmacy, Seoul, Korea) and hypoxia by being housed in a cage with low O₂ tension and elevated CO₂ concentration. MCT is given as a single dose at 60 mg/kg, subcutaneously (n=7).

Treatment group 3 (MCT+S group) is treated with MCT and hypoxia as the MCT/hypoxia group. Additionally, SER100 at 20 mg/kg is administered subcutaneously twice daily for 28 days (n=7).

On day 26 or 27, right ventricular pressure is measured via a PE 50 catheter (Becton Dickinson, Franklin Lakes, NJ, USA) inserted into the right ventricle via the internal jugular vein and a fluid-filled PE-50 tube connected to a pressure transducer (Grass polygraph, Grass instrument CO, Quincy, MA, USA).

After 28 days, the animals are anaesthetized by intraperitoneal injection of ketamine (100 mg/kg). For animals in all three groups, the right ventricle (RV) free wall is dissected from the left ventricle (LV) and septum (S) and weighed. The RV remodeling is assessed by the RV to (LV plus S) weight ratio.
Vascular remodeling is studied by fixation of the left lung with a transcardiac infusion of 4% paraformaldehyde. The perfused lung is removed and paraffin-embedded. Serial coronal sections 5 µm thick are obtained from the lung tissue, followed by deparaffinization of the samples and staining with hematoxylin-eosin (H&E).

The medial wall thickness (MWT) is measured at pulmonary arterioles 50-100 µm in external diameter and at the peribronchiolar muscular arteries. The MWT ratio, which is an index of medial wall hypertrophy, is determined as the average data of 10 to 15 fields per slice and the external diameter-internal diameter/external diameter is calculated for all slices.

Lung and kidney tissues are removed and snap-frozen at -70°C for Western Blot analysis of AQP1 and AQP2. The tissue samples are homogenized in ten volumes of homogenizing buffer (0.32 M sucrose, 25 mM imidazole and 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.2) containing 8.5 mM leupeptin and 1 mM phenylmethylsulfonyl fluoride), for 10 s using a polytron. Aliquots are stored at -70°C. Samples of the homogenate are run on 7.5% polyacrylamide mini gels (Bio-rad Mini Protean).

For each gel, an identical gel is run in parallel and subjected to Coomassie staining to determine loading. After electrophoresis, the protein is transferred to nitrocellulose paper for 2 hours at 400 mA and 120 V in a BioRad transblot system. After transfer, the protein bands will be identified by Ponceau S and destained with distilled water. The nitrocellulose sheets are washed in Tween phosphate buffered saline (PBST) and then incubated with rabbit anti AQP1 (Alomone, Jerusalem, Israel) and rabbit anti-AQP2 (Alomone, Jerusalem, Israel) overnight at 4°C.

The labeling is visualized with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) using an enhanced chemiluminescence (ECL) system (Amersham Pharmacia Biotech, Little Chalfont, UK).

It is expected that the RV/LV ratio will increase in the MCT/Hypoxia group compared to the placebo group as an indicator of the increased pressure in the pulmonary artery. Correspondingly, the right ventricular pressure is expected to be elevated compared to placebo.
In the MCT+S group, the RV/LV is expected to be lower than MCT/Hypoxia group. The right ventricular pressure is expected to be lower than the MCT/Hypoxia group compared to placebo as an indicator of lower pressure in the pulmonary artery.

It was originally expected that the amount of AQP1 in the various lung artery samples would be significantly lower in the MCT+S group compared to the MCT/Hypoxia group. It was not expected that SER100 would have a significant influence on the amount on AQP2 in the lung.

Example 2: Effect of SER100 in a pre-clinical model of hypoxia-induced pulmonary hypertension

Male C57BL/6 mice (Charles River, UK; 8-10 weeks) were randomly assigned to one of 5 groups, n=8 per group, as described below and depicted in Figure 1:

1. Normoxia
2. Normoxia + SER100 (1mg/kg/day; osmotic minipump)
3. Hypoxia + semaxanib (2 weeks)
4. Hypoxia + semaxanib (5 weeks)
5. Hypoxia + semaxanib (5 weeks) + SER100 (1mg/kg/day; osmotic minipump)

Normoxic animals (Groups 1-2) were maintained at 21% O2 throughout. Hypoxic animals were exposed to 10% O2 in a normobaric chamber for 14 days (Group 3) or 35 days (Groups 4-5), and administered the vascular endothelial growth factor (VEGF) receptor antagonist semaxanib (Z-3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-2-indolinone; Sugen 5416) (suspension in 0.5% [w/v] carboxymethylcellulose sodium, 0.9% [w/v] sodium chloride, 0.4% [v/v] polysorbate 80, 0.9% [v/v] benzyl alcohol in deionized water; 20mg/kg, subcutaneously, 1x/week for 3 weeks). SER100 (1mg/kg/day) was delivered by osmotic minipump (model 1004; Alzet, USA) implanted subcutaneously at day 14 once the PH phenotype had established ('reversal protocol').

Hemodynamic measurements
At 14 days (Group 3) or 35 days (Groups 1-2 & 4-5), the right ventricular systolic pressure (RVSP) and mean arterial blood pressure (MABP) were recorded. Animals were removed from the chamber individually and anaesthetized immediately to minimize the
time spent outside the hypoxic environment prior to haemodynamic measurement. Mice were anaesthetized with 1.5% isofluorane, dissolved in oxygen and delivered at 0.4L/min, and placed supine on a thermostatically controlled heating blanket (37°C). To measure MABP, the left common carotid artery was isolated and a fluid filled catheter introduced into the vessel. To measure RVSP, the right jugular vein was isolated and a Millar micromanometer tipped catheter (Millar MicroTip 1.4F catheter, Millar Instruments, USA) introduced into the superior vena cava and then advanced into the right ventricle. Both MABP and RVSP were recorded onto a pre-calibrated PowerLab system (ADInstruments, Australia) running Labchart 6.0 software.

Following measurement of pulmonary and systemic haemodynamics, animals were sacrificed by anaesthetic overdose and exsanguination, the heart removed, and heart chamber weights measured to evaluate right ventricular hypertrophy (RVH; right ventricle to left ventricle plus septum ratio; RV/[LV+S] and right ventricle to body weight ratio; RV/BW). In 6 animals, the left lung lobe was isolated by ligation, removed and snap frozen in liquid N2. The remaining four lung lobes were cannulated via the trachea and fixed by inflation with 4% paraformaldehyde in PBS at physiological pressure before paraffin embedding and sectioning.

The right lung was fixed by inflation with 10% formalin in PBS before paraffin embedding and sectioning. The remaining lung tissue and kidney were dissected and snap frozen in liquid N2. Transverse formalin-fixed lung sections were stained with an anti-smooth muscle actin (clone 1A4; Dako, UK) antibody. Pulmonary vascular muscularisation (i.e. remodelling) was determined by counting vessels of less than 100pm in diameter in each lung section, and defining according to degree of muscularization: fully muscularized (two distinct and continuous elastic lamina), partially muscularized (second elastic lamina not continuous), and non-muscularized (single elastic lamina). Approximately 75 vessels were counted per section from 6 animals in each group and the proportion of vessels in each category was expressed as a percentage of total vessels counted.

**Effect of SER100 on whole lung aquaporin (AQP)-1 expression in hypoxia-induced pulmonary hypertension**

Mice exposed to normoxia or 5 weeks hypoxia in the absence and presence of SER100 (1mg/kg/day) were killed by cervical dislocation, the lungs extracted and placed into cold DMEM/F12 media, and homogenates generated using a Precellys Tissue Homogenizer (Precellys, UK). Aquaporin (AQP)-1 protein expression was determined by immunoblot using primary anti-AQP-1 antibody (Abeam, UK; 1:500) and secondary horse-radish peroxidase conjugated goat anti-rabbit IgG antibody (Dako, UK 1:2000). Bands were
quantitated by densitometry using ImageJ and normalized to the loading control (anti-actin, 1:5000, Millipore, UK; secondary antibody horse-radish peroxidase conjugated anti-mouse IgG, 1:1000; Dako, UK).

Effect of SER100 in vivo on migration of pulmonary microvascular endothelial cells from mice with hypoxia-induced pulmonary hypertension

Primary murine pulmonary endothelial cells were isolated as described previously (Khambata et al. 2011. Br. J. Pharmacol., 164:584-97). Briefly, mice exposed to normoxia or 5 weeks hypoxia in the absence and presence of SER100 (1mg/kg/day) were killed by cervical dislocation and lungs were extracted and placed into cold DMEM/F12 media. Lungs were minced using scissors and incubated in collagenase (type 1a, 0.1%, Sigma-Aldrich, UK) for 1 hour at 37°C, filtered (70µm; Millipore, UK) and re-suspended in endothelial cell growth medium (ECGM) containing DMEM/F1 2 (Gibco, UK), 20% bovine serum (Gibco, UK), 50 U/ml penicillin and 0.5 mg/ml streptomycin (Sigma-Aldrich, UK), 50Mg/ml endothelial cell growth supplement (Sigma-Aldrich UK) and 3 Mg/ml endothelial cell growth serum/heparin (Promocell, UK) and were plated in a 75cm² gelatin-coated flask (Corning® Biocoat, UK). Cells were grown to ~80% confluence, before undergoing positive selection for endothelial cells using magnetic bead separation (Dynabeads, sheep anti-rat IgG, 4x106 beads/ml, Life Technologies, UK) coated with 50 ng/ml CD31 (anti-mouse, Affymetrix, UK), re-plated and grown to ~80% confluence before a second positive selection using 5 Mg/ml CD102 (BD Pharmingen, UK). For all experiments, endothelial cells were removed from flasks using trypsin and re-suspended in appropriate medium.

To assess proliferation, cells were seeded onto 96-well plates at a density of 1 x 105 cells/well, grown for 24h, and starved in low serum for 24h. Cells were then incubated in medium containing 20% FBS in the absence and presence of SER100 (10nM to 10MM) and counted at 24 h using BrdU incorporation (Roche Diagnostics, UK).

Primary pulmonary microvascular endothelial cells were isolated as above. Endothelial cells were plated in gelatin-coated wells of a 96 well plate (Corning® Biocoat, UK) at a density of 2.5 x 105 cells/well and grown to confluence. 12h prior to and for the duration of the experiment, cells were incubated in a media containing 1% serum. A scratch was performed using a 10µl sterile pipette and images were taken at regular intervals over a 24h period to monitor scratch closure. Cells were treated with vehicle (DMEM/F1 2 alone) or SER100 (10nM to 10µM, dissolved in DMEM/F1 2).

Results are expressed as mean±SEM. Statistical analyses were performed by one-way ANOVA, with Bonferroni post-hoc tests, with the exception of the endothelial cell migration which was analysed by 2-way ANOVA with repeat measures. All analyses were
conducted using GraphPad Prism version 5. P<0.05 denotes significance. The n value denotes the number of animals in each group.

**Results**

Hypoxia induced a significant increase in RVSP at 2 weeks (normoxia: 21.7±0.9mmHg versus hypoxia: 48.1±1.5mmHg) and 5 weeks (hypoxia: 51.7±1.3mmHg) that was significantly reversed in the presence of SER100 (42.8±1.1mmHg; Figure 2). SER100 had no significant effect on RVSP under normoxic conditions (18.3±1.2mmHg), although did tend to reduce RVSP modestly.

Hypoxia induced a significant increase in RVH whether determined as right ventricle to body weight ratio (RV/BW; normoxia: 0.0008±0.00002, 2 weeks hypoxia: 0.0012±0.00004, 4 weeks hypoxia: 0.004±0.00006) or right ventricle to left ventricle plus septum ration (RV/[LV+S]; normoxia: 0.26±0.004, 2 weeks hypoxia: 0.38±0.013, 5 weeks hypoxia: 0.45±0.018; Figure 3). SER100 produced a significant reduction in RVH following 5 weeks hypoxia (RV/BW: 0.0012±0.00005, RV/[LV+S]: 0.38±0.02; Figure 3), but did not influence RV size in normoxic mice.

No significant differences in mean arterial blood pressure were observed across all groups, although SER100 produced a modest reduction in MABP (Figure 4).

Normoxic animals had a low percentage of partially and fully muscularised pulmonary small arteries (Figure 5). Exposure to hypoxia caused pulmonary vascular re-modelling characteristic of pulmonary hypertension with a significantly greater percentage of vessels becoming fully muscularised, with a smaller number of partially muscularised arteries. This re-modelling was modestly reversed by SER100, with a smaller number of fully muscularised arteries and a greater number of non-muscularised vessels compared to hypoxia alone; however, this trend did not reach statistical significance.

Aquaporin (AQP)-1 protein expression tended to be up-regulated in lungs from hypoxic mice when compared to normoxic controls (Figure 6). Interestingly, AQP-1 expression was further, and significantly increased in hypoxic animals receiving SER100 (1mg/kg/day; osmotic minipump).

SER100 (10nmM-10pM) produced a concentration-dependent augmentation of pulmonary microvascular endothelial cell growth from mice exposed to 5 weeks hypoxia (10% O2), although this effect failed to reach statistical significance (Figure 7). If anything, it appeared...
as if this effect might be bell-shaped, in that at the highest concentration SER100 tended to
decrease cell proliferation compared to lower concentrations of the compound.

SER100 (10 nM-10 µM) produced a concentration-dependent increase in pulmonary microvascular endothelial cell migration from mice exposed to 5 weeks hypoxia such that at 1 µM SER100 there was a statistically-significant augmentation (10% O2; Figure 8). Interestingly, in animals treated with SER100 (1 mg/kg/day; osmotic minipump) in vivo, the basal migration of pulmonary microvascular endothelial cells was significantly greater than mice exposed to hypoxia in the absence of SER100.

SER100 appears to be an effective intervention for the reversal of hypoxia-induced PH. The compound, at a dose of 1 mg/kg/day, causes a significant reduction in the development of elevated pulmonary artery pressure (i.e. RVSP) and also abrogates the accompanying RVH. The effect of SER100 against pulmonary vascular re-modelling (i.e. muscularisation of pulmonary small arteries) was less impressive, although in this context there was a trend towards reversal of the muscularisation of the small pulmonary arteries that characterize this disorder. SER100 produced a pulmonary-selective effect since MABP remained unchanged; indeed, it may be possible to increase the dose of SER100 to accentuate the beneficial effects on the pulmonary vasculature and right heart without causing significant systemic hypotension, thereby increasing the efficacy of the compound. SER100 causes enhanced proliferation of pulmonary microvascular endothelial cells isolated from hypoxic mice in vitro. Furthermore, the same cells from hypoxia animals treated with SER100 in vivo exhibit a markedly increased intrinsic rate of growth. With regard to involvement of AQP-1 as potentially underlying the effects of SER100 these data provide some support for such an interaction. That is, AQP-1 expression was modestly increased in lung homogenates from animals exposed to hypoxia when compared to normoxic controls. However, a significant increase in AQP-1 expression was observed in the same cells from mice exposed to hypoxia but treated with SER100 in vivo. These findings warrant further study to demonstrate a role for AQP-1 in the pathogenesis of pulmonary hypertension, and the ability of SER100 to modulate the activity of this protein to exert a therapeutic action.

In summary, SER100 reverses several indices of disease severity in well-established experimental models of pulmonary hypertension.

While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the
invention set forth are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention. All documents cited herein are expressly incorporated by reference.

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Leopold Stiebellehner, Maria G. Frid, John T. Reeves, 1Robert B. Low, Meena Gnanasekharan, Kurt R. Stenmark

Elena Monzani, Riccardo Bazzotti, Carla Perego, Caterina A. M. La Porta

Søren Nielsen, Peter Acre

Guowei Zhang, Xing Zeng*, Ling Han, Jian-an Wei, Haiding Huang


Virginia Leitch, Peter Agre, Landon S. King

Rapid Aquaporin Translocation Regulates Cellular Water Flow

Naoki Yamamoto, Kazuhiro Yonedaa, Kiyofumi Asaia, Kazuya Sobueb, Toyohiro Tadad, Yoshiihito Fujita, Hirotada Katsuya, Masataka Fujita, Noritaka Aihara, Mitsuhiito Mase, Kazuo Yamada, Yutaka Miura, Taiji Kato
Alterations in the expression of the AQP family in cultured rat

Claims

1. A compound for use in a method of treating pulmonary hypertension, wherein the compound has the formula:
   \[ R^1-Z-X-Z'-R^2 \]
   wherein:
   - \( R^1 \) is H (hydrogen), \( C_{1-4} \) alkyl, acetyl (Ac), formyl, benzoyl or trifluoroacetyl (Tfa);
   - \( R^2 \) is OH or \( NR_3R_4 \) where each of \( R_3 \) and \( R_4 \) independently represents H (hydrogen), \( C_{(1-6)} \) alkoxy, aryloxy or \( C_{(1-6)} \) alkyl;
   - \( X \) is a hexapeptide having the amino acid sequence (RK)YY(RK)(WI)(RK), where parentheses indicate alternative residues which may be present at positions 1, 4, 5 and 6, and wherein each amino acid residue in said hexapeptide may be in the L or D form;
   - \( Z \) and \( Z' \) are independently absent or a charged peptide chain of from 4 to 20 amino acid residues, wherein each amino acid residue in \( Z \) or \( Z' \) may be in the L or D form, providing that not both of \( Z \) and \( Z' \) are absent;
   - or is a retro form thereof;
   - or is a pharmaceutically acceptable salt, solvate or hydrate of either.

2. A compound for use according to claim 1 wherein \( X \) is selected from the group consisting of KYYRWR, KYYRK, RYYRWR, RYYRWK, RYYRWK (all-D), RYYRIK, RYYRIR, RYYKIK, RYYKIR, RYYKWR, RYYKWK, KWRYYR and KWRYYK.

3. A compound for use according to claim 2 wherein \( X \) is RYYRWK or KYYRWK.

4. A compound for use according to any one of the preceding claims wherein the number of amino acid residues in \( Z \) and \( Z' \) is in the range of 4-10.

5. A compound for use according to any one of the preceding claims wherein \( Z \) is negatively charged.

6. A compound for use according to any one of the preceding claims wherein the amino acid residues of \( Z \) are selected from the group consisting of Q, T, S, P, N, E, and D.

7. A compound for use according to claim 6 wherein the N-terminal amino acid of \( Z \) is selected from the group consisting of Q, T, N and S and the remaining amino acid residues are selected from the group consisting of P, D and E.

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8. A compound for use according to claim 7 wherein Z is selected from the group consisting of N(E), N(E)$_5$, N(E)$_3$, S(E)$_7$, S(E)$_6$, S(E)$_5$, S(E)$_3$, NP(E)$_4$, NP(E)$_5$, N(D)$_7$, N(D)$_6$, N(D)$_5$, N(D)$_3$, Q(E)$_7$, Q(E)$_5$, Q(E)$_3$, QN(D)$_7$, Q(D)$_6$, Q(D)$_5$, and Q(D)$_3$.

9. A compound for use according to any one of the preceding claims wherein Z' is positively charged.

10. A compound for use according to any one of the preceding claims wherein the amino acid residues of Z' are selected from the group consisting of A, G, K, and R.

11. A compound for use according to claim 10 wherein Z' is selected from the group consisting of A(K$_4$)G, K$_5$G, A(K$_5$)$_2$, H, K$_{10}$, K$_8$, K$_7$, K$_6$, K$_5$, and K$_4$.

12. A compound for use according to any one of claims 1 to 4 or 9 to 11 as dependent on any one of claims 1 to 4 wherein Z is absent and Z' is present.

13. A compound for use according to any one of the preceding claims wherein Z-X-Z' is selected from the group consisting of:

- RYYRWK-KKKKKK;
- KKKKKK-RYYRWK;
- NEEEEE-RYYRWK-KKKKKK;
- RYYRWK-KKKKKK (all D);
- KYYRWK-KKKKKK;
- RYYYIK-KKKKKK;
- RYYRWK-AKKKKK;
- RYYRWK-KKKKKK;
- RYYRWK-KKKKKK;
- RYYRWK-KKKKKK;
- KKKKKK-KWRYYR; and
- KKKKKK-KWRYYR (all D).

14. A compound for use according to any one of the preceding claims wherein R' is H, Ac or Tfa, e.g. Ac.

15. A compound for use according to any one of the preceding claims wherein R$^2$ is NH$_2$. 
16. A compound for use according to any one of the preceding claims wherein the compound is selected from the group consisting of:

- Ac-RYYRWK-KKKKKK-NH$_2$;
- Ac-KKKKKK-RYYRWK-NH$_2$;
- H-NEEEEE-RYYRWK-KKKKKK-NH$_2$;
- AC-RYYRWK-KKKKKK-NH$_2$ (all D);
- Ac-KYYRWK-KKKKKK-NH$_2$;
- Ac-RYYRIK-KKKKKK-NH$_2$;
- Ac-RYYRWK-AKKKKK-NH$_2$;
- Ac-RYYRWK-KKKKK-NH$_2$;
- Ac-RYYRWK-KKKKKC-NH$_2$;
- Tfa-RYYRWK-KKKKKK-NH$_2$;
- Ac-KKKKKK-KWRYYR-NH$_2$; and
- AC-KKKKKK-KWRYYR-NH$_2$ (all D)

and pharmaceutically acceptable salts, hydrates and solvates thereof.

17. A compound for use according to claim 16 which is
- Ac-RYYRWK-KKKKKK-NH$_2$ x 9CH$_3$COOH; or
- AC-RYYRWK-KKKKKK-NH$_2$ x 9HCl.

18. Use of a compound as described in any of claims 1 to 17 in the preparation of a medicament for the treatment of PH.

19. A method of treatment of PH in a subject in need thereof, comprising administering a compound as described in any one of claims 1 to 17 to said subject.

20. A compound for use, use, or method according to any one of the preceding claims wherein the PH is Group 1 PH.

21. A compound for use, use, or method according to claim 20 wherein the PH is selected from the group consisting of idiopathic pulmonary arterial hypertension (PAH), heritable PAH, drug or toxin-induced PAH, persistent pulmonary hypertension of the newborn, PAH associated with connective tissue disease, PAH associated with HIV infection, PAH associated with portal hypertension, PAH associated with congenital heart disease, PAH associated with schistosomiasis and PAH associated with chronic haemolytic anaemia.
22. A compound for use, use, or method according to any one of claims 1 to 19 wherein the PH is Group V PH.

23. A compound for use, use, or method according to claim 22 wherein the PH is due to pulmonary veno-occlusive disease (PVOD) or pulmonary capillary haemangiogenesis.

24. A compound for use, use, or method according to any one of claims 1 to 19 wherein the PH is Group 2 PH.

25. A compound for use, use, or method according to claim 24 wherein the PH is associated with cardiomyopathy, mitral stenosis, mitral regurgitation, aortic stenosis or aortic regurgitation.

26. A compound for use, use, or method according to any one of claims 1 to 19 wherein the PH is Group 3 PH.

27. A compound for use, use, or method according to claim 26 wherein the PH is associated with chronic obstructive pulmonary disease (COPD), interstitial lung disease, other pulmonary diseases, sleep disordered breathing, alveolar hypoventilation disorders, chronic exposure to high altitude or developmental abnormalities.

28. A compound for use, use, or method according to any one of claims 1 to 19 wherein the PH is Group 4 PH.

29. A compound for use, use, or method according to claim 28 wherein the PH is PH due to pulmonary embolus or PH due to pulmonary thrombosis.

30. A compound for use, use, or method according to any one of claims 1 to 19 wherein the PH is Group 5 PH.
1. Normoxia 21% O₂

2. Normoxia 21% O₂
   - SER100 (1mg/kg/day)

3. Hypoxia (2 weeks) 10% O₂
   - Semaxanib (20mg/kg; s.c.)

4. Hypoxia (5 weeks) 10% O₂
   - Semaxanib (20mg/kg; s.c.)

5. Hypoxia (5 weeks) + SER100 10% O₂
   - Semaxanib (20mg/kg; s.c.)
   - SER100 (1mg/kg/day)

WEEK 0 1 2 3 4 5

ENDPOINT MEASUREMENTS

FIGURE 1
FIGURE 3
FIGURE 3, CONT.
FIGURE 4
AQP-1 expression (normalised to β-actin)

- Normoxia
- Hypoxia/Semaxanib (5 weeks)
- Hypoxia/Semaxanib (5 weeks) + SER 100

FIGURE 6
FIGURE 7
FIGURE 9
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. X forming part of the international application as filed:
      - □ in the form of an Annex C/ST.25 text file.
      - □ on paper or in the form of an image file.
   b. □ furnished together with the international application under PCT Rule 13fer1 (a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. □ furnished subsequent to the international filing date for the purposes of international search only:
      - □ in the form of an Annex C/ST.25 text file (Rule 13fer1 (a)).
      - □ on paper or in the form of an image file (Rule 13fer1 (b) and Administrative Instructions, Section 7:13).

2. □ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K7/06 A61K38/08

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)
C07K 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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No.

A COLETTE T DOOLEY ET AL: "Bi ndi ng and In Vi tro Acti vities of Pepti des w ith High Affi nity for the Noci cepti n/Orphan n FQ Receptor, 0RL1", THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 283, no. 2, 14 July 1997 (1997-07-14), pages 735-741, XP055277955,
the whole document

A WO 01/98324 A1 (ZEALAND PHARMACEUTICALS AS [DK]; LARSEN BJARNE DUE [DK]; PETERSEN JORG) 27 December 2001 (2001-12-27)
claims

***/-/-**

* 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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Date of the actual completion of the international search
7 June 2016

Date of mailing of the international search report
16/06/2016

Name and mailing address of the ISA/

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Authorized officer

Vogt, Titus
## INTERNATIONAL SEARCH REPORT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>A</td>
<td>wo 02/28412 AI (ZEALAND PHARMACEUTICALS AS [DK]; PETERSEN JORGEN SOBERG [DK]; KAPUSTA) 11 April 2002 (2002-04-11) cl aims</td>
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<td>STUART RICH: &quot;The effects of vasodilators in pulmonary hypertension: pulmonary vascular or peripheral vascular?&quot;, CIRCULATION. HEART FAILURE (PRINT), vol. 2, no. 2, 1 March 2009 (2009-03-01), pages 145-150, XP055278263, United States ISSN: 1941-3289, DOI: 10.1161/CIRCHEARTFAILURE.108.805374 abstract; table 1</td>
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