Abstract:
The present invention discloses non peptide prokineticin 1 receptor agonists and their uses for the treatment of PKR1 mediated disorders, in particular for the treatment of vascular diseases, neurodegenerative diseases, diseases involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotropic hypogonadism and disturbances of circadian rhythm, and to prevent or limit the toxicity, in particular the cardiotoxicity and neurotoxicity, of drugs. The present invention also discloses a prokineticin receptor-1 agonist for use for promoting the differentiation of cardiac epicardin+ progenitor cells into cardiac myocytes in a subject affected with a cardiac disease and/or the differentiation of renal epicardin+ progenitor cells into vasculogenenic and/or glomerular cells in a subject affected with a renal disease. The present invention further discloses a prokineticin receptor-1 agonist for use for treating or preventing insulin resistance, in particular associated with type II diabetes.
Prokineticin 1 receptor agonists and their uses

Field of the Invention

The present invention relates to non peptide prokineticin receptor-1 (PKRI) agonists and pharmaceutical compositions comprising thereof. The present invention also relates to the fields of cardiology, nephrology and endocrinology.

Background of the Invention

Prokineticin-1, also called endocrine gland-derived vascular endothelial growth factor (EG-VEGF) and prokineticin-2, also called Bv8, are two secreted proteins from the AVIT secreted protein family that are widely express among mammalian tissues. These molecules exert their biological functions through activation of two closely related G-protein-coupled receptors, the prokineticin receptors 1 and 2 (PKRI and PKR2) (Lin et al., 2002; Masuda et al., 2002; Soga et al., 2002). Both PKRI and PKR2 are highly expressed in human and rat endocrine tissues, including thyroid, pituitary, and adrenal glands, as well as in the ovary and testis. PKR2 and to a lesser extent PKRI are expressed in the brain. PKRI, originally called GPR73, is mainly expressed in spleen, prostrate, pancreas, monocytes leukocytes, and in human heart (Urayama et al., 2007 and Parker et al., 2000).

Prokineticins exhibit different affinities for their receptors, prokineticin-2 being the most potent agonist for both receptors (Chen et al. 2005). PKRI and PKR2 share 85% amino acid identity and diverge mainly in their N-terminal sequences. PKRI is encoded by a gene located on human chromosome region 2q14, whereas the PKR2 gene is located on 2p13 (Parker et al., 2000).

Prokineticins are involved in regulating various biological processes that include gastrointestinal motility (Li et al., 2001), pain sensitization (Negri et al., 2005), angiogenesis (LeCouter et al., 2001; LeCouter et al., 2003), circadian rhythms (Cheng et al., 2002), olfactory bulb activation (Ng et al., 2005), hematopoiesis (LeCouter et al., 2004), monocyte differentiation (Dorsch et al., 2005), and macrophage activation (Martucci et al., 2006). In cardiomyocytes, prokineticin-2 via PKR1 protects cardiomyocytes against hypoxia induced apoptosis (Urayama et al., 2007). Moreover, transient PKRI gene transfer reduces mortality and preserves left ventricular function by
promoting angiogenesis and cardiomyocyte survival after the coronary ligation as a mouse model of myocardial infarction (Urayama et al., 2007).

Recent studies have revealed that prokineticins and their receptors are associated with the pathologies of various human diseases and genetic syndromes including, for example, heart failure (Urayama et al. 2007), abdominal aortic aneurysm (Choke et al. 2009) and Kallmann syndrome (Hardelin and Dode 2008), and familial hyperthyroidism (Warner et al., 2006).

A designed PK2β peptide possessing only 47 amino acids of the N-terminus of PK2 has been described as a potent agonist for PKRl (WO2005/097826). However, the use of peptide drugs is limited due to the instability (proteolytic cleavage of the peptide backbone) and the low bioavailability (poor membrane transport capability) of these molecules. Furthermore, peptide drugs have usually to be administered by the parenteral route because of insufficient absorption from the gastrointestinal tract.

Consequently, there is a strong need of developing non peptide agonists for PKRl that could be administered by the oral route to treat PKRl mediated disorders.

**Summary of the Invention**

The first object of the present invention is to provide non peptide prokineticin receptor-1 (PKRl) agonists.

In a first aspect, the present invention provides a pharmaceutical composition comprising a PKRl agonist of formula (I)

![Formula Image](attachment)

wherein

A is R1 or

![Alternative Structure Image](attachment)
m is selected from 0 and 1;
n is selected from 0, 1 and 2;

R^1 is selected from the group consisting of a hydrogen atom; a halogen atom; a (C_5-C_6)-alkyl, a (C_2-C_5)-alkenyl and a (C_2-C_6)-alkynyl group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; a (C_6-C_12)-aryl; a 5 to 7-membered-ring heterocycle; a (C_6-C_6)-alkoxy, a (C_2-C_6)-acyl, a (C_2-C_6)-ester, an amino group, a (C_6-C_6)-amine, a (C_6-C_6)-amide, a (C_6-C_6)-imine, a (C_6-C_6)-nitrile, a (C_6-C_6)-thioalkyl, a (C_6-C_6)-sulfone and a (C_6-C_6)-sulfoxide group wherein the alkyl part of the group is optionally interrupted by one of several heteroatoms chosen among N, O and S, and is optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol;

R^2 is selected from the group consisting of a (C_6-C_12)-aryl group and a 5 to 7-15 membered-ring heterocycle, preferably from phenyl and furan, optionally substituted by at least one substituent selected from the group consisting of a (C_6-C_6)-alkyl, a (C_2-C_6)-alkenyl, a (C_2-C_6)-alkynyl, a non substituted (C_6-C_12)-aryl, a 5 to 7-membered-ring heterocycle, a (C_6-C_6)-alkoxy, a (C_2-C_6)-acyl, a (C_6-C_6)-amine, an amino group, a (Q-C_6^-amide, a (C_6-C_6)-thioalkyl, a (C_6-C_6)-sulfone and a (C_6-C_6)-sulfoxide group; and

R^3 is selected from the group consisting of a (C_6-C_6)-alkyloxyalkyl, a (C_2-C_6)-alkenyl, a (C_2-C_6)-alkynyl and a (C_6-C_6)-amine group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and

25 a (C_6-C_12)-aryl and a 5 to 7-membered-ring heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (C_6-C_6)-alkyloxyalkyl, a (C_6-C_6)-alkoxy group and a halogen atom;

or any pharmaceutically acceptable salt thereof.

The PKR1 agonist may be a compound of formula (I) wherein

\[ A \text{ is } R^1 \]
and $R^1$ is selected from the group consisting of a hydrogen atom; a halogen atom; a (Ci-C$_3$)-alkyl, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; a (Ci-C$_3$)-alkoxy, a (C$_2$-C$_3$)-acyl, a (C$_2$-C$_3$)-ester, a (Ci-C$_3$)-amine, an amino group, a (Ci-C$_3$)-amide and a (C$_1$-C$_3$)-thioalkyl, group wherein the alkyl part of the group is optionally interrupted by one of several heteroatoms chosen among N, O and S, and is optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol. Preferably, $R^1$ is selected from the group consisting of a hydrogen atom, a halogen atom, an amino group, a (Ci-C$_3$)-alkyl group and a (Ci-C$_3$)-alkoxy group.

The PKR1 agonist may be a compound of formula (I) wherein $R^2$ may be selected from the group consisting of a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, preferably a 5-membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C$_3$)-alkyl, a (C$_1$-C$_3$)-alkoxy, a (C$_2$-C$_3$)-acyl, a (Ci-C$_3$)-alcohol, a carboxylic group, a (C$_2$-C$_3$)-ester, a (C$_1$-C$_3$)-amine, an amino group, a (Ci-C$_3$)-amide, a hydroxyl, an aldehyde, a (C$_1$-C$_3$)-halogenoalkyl, a thiol, a (Ci-C$_3$)-thioalkyl and a halogen atom. Preferably, $R^2$ is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C$_3$)-alkyl and a (Ci-C$_3$)-alkoxy group.

The PKR1 agonist may be a compound of formula (I) wherein $R^3$ is selected from the group consisting of a (Ci-C$_6$)-alkyl and a (Ci-C$_6$)-amine group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C$_3$)-alkyl, a (Ci-C$_3$)-alkoxy group and a halogen atom. Preferably, $R^3$ is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C$_3$)-alkyl group, preferably methyl, a (C$_1$-C$_3$)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine.

In a particular embodiment, the PKR1 agonist is a compound of formula (I) wherein
R\textsuperscript{1} is selected from the group consisting of a hydrogen atom and an amino group; R\textsuperscript{2} is selected from the group consisting of a 5-membered-ring heterocycle and a phenyl group, preferably from a furan, a thiophene and a phenyl group, optionally substituted by a (C\textsubscript{i}-C\textsubscript{3})-alkoxy group, preferably a methoxy group; and R\textsuperscript{3} is selected from the group consisting of a (C\textsubscript{i}-C\textsubscript{6})-alkyl, preferably a tert-butyl group, and a phenyl group, optionally substituted by a substituent selected from the group consisting of a (C\textsubscript{i}-C\textsubscript{3})-alkoxy group and a halogen atom, preferably from a methoxy group and chlorine. More preferably, R\textsuperscript{1} is selected from the group consisting of a hydrogen atom and an amino group; R\textsuperscript{2} is selected from the group consisting of an unsubstituted furan group or a phenyl group, optionally substituted by one or two methoxy groups; and R\textsuperscript{3} is selected from the group consisting of a tert-butyl group and a phenyl group, optionally substituted by a methoxy group or chlorine.

The PKR\textsubscript{1} agonist may also be a compound of formula (I) wherein

R\textsuperscript{2} is selected from the group consisting of a 5-membered-ring heterocycle and a phenyl group, preferably from a furan, a thiophene and a phenyl group, optionally substituted by a (C\textsubscript{i}-C\textsubscript{3})-alkoxy group, preferably a methoxy group; and R\textsuperscript{3} is selected from the group consisting of a (C\textsubscript{i}-C\textsubscript{6})-alkyl, preferably a tert-butyl group, and a phenyl group, optionally substituted by a substituent selected from the group consisting of a (C\textsubscript{i}-C\textsubscript{3})-alkoxy group and a halogen atom, preferably from a methoxy group and chlorine. Preferably, R\textsuperscript{2} is selected from the group consisting of a phenyl group, optionally substituted by a (C\textsubscript{i}-C\textsubscript{3})-alkoxy group, preferably a methoxy group, and a furan group; and R\textsuperscript{3} is a phenyl group, optionally substituted by a substituent selected from the group consisting of a (C\textsubscript{i}-C\textsubscript{3})-alkoxy group and a halogen atom, preferably from a methoxy group
and chlorine. More preferably, \( R^2 \) is a non substituted furan group and \( R^3 \) is a non substituted phenyl group.

In particular, the present invention concerns a PKRI agonist selected from the group consisting of IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS 10, IS 11, IS 12, IS 14, IS 15, IS 16, IS 17, IS 18, IS 19, IS 20, IS 21, IS 22, IS 23, IS 26, IS 27, IS 28, IS 30, IS 34 and IS 35.

The present invention also concerns a PKRI agonist as described above as a medicament.

The present invention further concerns a PKRI agonist as described above as a cardioprotective agent.

The present invention further concerns a PKRI agonist as described above as a neuroprotective agent.

In another aspect, the present invention concerns a pharmaceutical composition comprising a PKRI agonist as described above and a pharmaceutically acceptable carrier and/or excipient. The pharmaceutical composition according to the invention can further comprise one or more other active compounds associated with pharmaceutically acceptable excipients and/or carriers.

In another aspect, the present invention concerns a pharmaceutical composition or a PKRI agonist as described above for use for the prevention or treatment of a disease or a disorder selected from the group consisting of a vascular disease, a neurodegenerative disease, a disease involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotropic hypogonadism and disturbances of circadian rhythm.

In a further aspect, the present invention concerns a pharmaceutical composition or a PKRI agonist as described above for use to prevent or to limit the neurotoxicity and/or cardiotoxicity of a compound, in particular of an antiviral or antineoplastic agent.

In last aspect, the present invention concerns a product containing a PKRI agonist of the invention and an antineoplastic agent, as a combined preparation for simultaneous, separate or sequential use in the treatment of cancer.

Another object of the present invention is to provide new therapeutic approaches for treating cardiac diseases and/or renal diseases. In particular, the present invention offers the possibility to treat patients with cardiac and/or renal necrosis, in particular due to ischemic diseases, and to provide a regeneration of necrotic areas.
In a first aspect, the present invention concerns a prokineticin receptor-1 agonist for use for promoting the differentiation of cardial epicardin+ progenitor cells (cEPPC) into cardiomyocytes in a subject affected with a cardiac disease and/or the differentiation of renal epicardin+ progenitor cells (rEPPC) into vasculogenic and/or glomerular cells in a subject affected with a renal disease. In an embodiment, the cardiac disease is selected from the group consisting of heart failure, myocardial infarction, ischemic heart disease, diabetes-mediated cardiovascular complications and the cardiorenal syndrome. In another embodiment, the renal disease is selected from the group consisting of chronic renal disease, renal artery stenosis, renal failure, acute kidney injury, acute-on-chronic renal failure, ischemic nephropathy, Churg-Strauss syndrome, Wegener's granulomatosis, diabetes-mediated renal complications and the cardiorenal syndrome.

In a second aspect, the present invention concerns a prokineticin receptor-1 agonist for use for preventing fat tissue development in heart and/or kidney in a subject, in particular in a subject affected with obesity or being susceptible to develop obesity.

In another aspect, the present invention concerns an in vitro or ex vivo method of producing cardiomyocytes, wherein said method comprises the step of contacting cardial epicardin+ progenitor cells with a prokineticin receptor-1 agonist. The present invention also concerns an in vitro or ex vivo method of producing glomerular cells, wherein said method comprises the step of contacting renal epicardin+ progenitor cells with a prokineticin receptor-1 agonist.

A further object of the present invention is to provide new therapeutic approaches for treating or preventing insulin resistance.

Thus, in an aspect, the present invention concerns a prokineticin receptor-1 agonist for use for treating or preventing insulin resistance in a subject. The insulin resistance may be associated with type II diabetes, obesity, hypertension, cardiac and/or renal disorders, and/or other metabolic disorder. In an embodiment, the insulin resistance is associated with type II diabetes. In another embodiment, the insulin resistance is associated with obesity. In a further embodiment, the insulin resistance is associated with hypertension. In another embodiment, the insulin resistance is associated with cardiac and/or renal disorders.

In another aspect, the present invention concerns a prokineticin receptor-1 agonist for use for treating or preventing type II diabetes in a subject.

In an embodiment, the PKR1 agonist is prokineticin -2 or an active fragment thereof.
In a preferred embodiment, the PKR1 agonist is a compound of formula (I) according to the invention.

**Legends of the Figures**

**Figure 1**: Prokineticin-2 via PKR1 inhibits the differentiation of cEPPC into 5 adipocytes. **Fig. 1A.** Oil red staining after pro-adipogenic treatment (MIX), and pre-treatment with prokineticin-2 with pro-adipogenic treatment (PK-2+MIX). **Fig. 1B.** (*) Shows difference between MIX and PK-2+MIX treated wild type cEPPC (p<0.05). In PKR1-KO mice prokineticin-2 was not able to inhibit adipogenesis.

**Figure 2**: Characterisation of adipogenic gene expression in cEPPC differentiating into 10 adipocytes. Quantitative RT-PCR analysis of cEPPC treated by prokineticin-2 (PK-2), pro-adipogenic cocktail (MIX) or PK-2+MIX during 1 week or 2 weeks. (*) Shows difference between PK-2 and MIX treatment (p<0.05), (#) shows difference between MIX and PK-2+MIX.

**Figure 3**: Prokineticin-2 treatment of the cEPPC induced mature, and spontaneously beating cardiomyocytes as beating frequency is increased by beta adrenergic stimuli (isoproterenol) (Fig. 3A and 3B) and as they are positively stained by troponin, a cardiomyocyte marker (Fig. 3C).

**Figure 4**: **Fig. 4A.** Gross morphology of kidneys. **Fig. 4B.** Mallory tetrachrome staining of cryosectioned kidneys. **Fig. 4C.** Kidney weight to body weight ratio (n=8, 20 p<0.05). **Fig. 4D.** Paraffin-sectioned neonatal kidneys stained with Mallory tetrachrome. **Fig. 4E.** Representative illustration of TUNEL analyses on kidneys. The histogram and photographs show larger numbers of TUNEL-positive apoptotic cells in mutant glomeruli (n=4, p<0.05). **Fig. 4F.** Representative illustration of PECAM-1-positive capillaries in glomeruli. The histogram shows that there were fewer PECAM-1-positive endothelial cells in mutant glomeruli (n=3, p<0.01).

**Figure 5**: **Fig. 5A.** Mallory tetrachrome staining on cryosectioned kidney samples. (Upper) Electron microscopy analyses on kidney samples. Thickening of the glomerular basement membrane (gbm) was evident in mutant kidneys (lower). **Fig. 5B.** Histological analyses showed enlarged tubules (tb) and severe fibrosis between the tubules in mutant 30 kidneys. Electron microscopy revealed disorganized tubular (tb) and dysmorphic peritubular vessels (ptv) and collagen (c) deposition in mutant kidneys. **Fig. 5C.** Mutants had fewer mitochondria in their tubules, with fewer granulocyte and crista structures. **Fig. 5D.**
5D. Representative mitochondrial function analyses based on SDH staining in cryosectioned kidneys.

**Figure 6:** Urine and serum analyses (24-week-old mice, n=6 for each group)

**Figure 7:** Expression of PKR1 in mouse kidney. Impaired glomerular endothelial cells (ec) with fenestration and podocyte structures (pod) in 36 weeks old mutant mice kidneys (-/-) demonstrated by electron microscopic analysis.

**Figure 8:** **Fig. 8A.** Representative illustration of capillary density by an endothelial marker, PECAM-1 (n=3) staining in cryosectioned neonatal (PI) kidneys. Original magnification=X40. Quantification of capillary density calculated as numbers of PECAM-1 positive cells/dapi+ cells corrected with 100 per high power field. Histograms show the quantitative changes between wild type (+/+ and null mutants (-/-), n=4, p<0.05. **Fig. 8B.** HIF-1α transcript levels in RNA derived from neonatal (left) and 3 weeks old kidneys (right, n=4, p<0.05). **Fig. 8C.** Western blot analyses with HIF-lor and GAPDH antibodies on kidney protein confirm an increase in HIF-lor levels in 3 weeks old mutant kidneys (n=3, duplicated, p<0.05). **Fig. 8D.** Histogram represents RT-PCR analysis showing that expression of pro-angiogenic factors, PDGF, FGF and VEGF were differently altered in mutant kidneys at the age of 3 weeks (n=4). **Fig. 8E.** Western Blot analysis and histogram show diminished levels of phosphorylated-Akt form in neonatal mutant kidneys (n=4).

**Fig. 8F.** Co-immunostaining of kidney sections with active caspase-3 antibody, endothelium specific PECAM-1, progenitor cell specific epicardin antibodies in glomerulus. **Fig. 8G.** VEGF immunostaining of glomerulus (glm) and tubules (tbl).

**Figure 9:** **Fig. 9A.** Epicardin+ progenitor cells in glomeruli. Original magnification=X40. Quantification of epicardin positive cells were calculated as numbers of epicardin positive cells/dapi+ cells corrected with 100 per high power field (n=5, 25 p<0.05). **Fig. 9B.** Representative illustration of epicardin, Ki67, PECAM-1 and a-SMA positive cell number in the renal EPDCs derived from kidney explants treated with vehicle or prokineticin-2 (5 or 10 nM) for 24 h. Original magnification=X63. Histograms show the quantitative changes between wild type (+/+ and null mutants (-/-). Quantification of the specific cell number/dapi positive cell number corrected with 100 per high power field (n=4, p<0.05).

**Figure 10:** Formulas of PKR1 agonists IS1 to IS12 and IS14 to IS19.

**Figure 11:** Synthesis of non peptide PKR1 agonists.

**Figure 12:** Induction of in vitro angiogenesis by prokineticin-2 and IS1 compound. **Fig.
12A: tube-like formation of the endothelial cells on Matrigel. **Fig. 12B:** quantification of the tube-like formation of the endothelial cells on Matrigel.

**Figure 13:** Induction of *in vitro* angiogenesis by prokineticin-2 and IS compounds. H5V cells were infected with adenovirus carrying PKR1 cDNA in order to dominantly express PKR1. **Fig. 13A:** 1 µM prokineticin-2 or IS compound. **Fig. 13B:** 10 µM prokineticin-2 or IS compound.

**Figure 14:** intracellular calcium release detected by fura-4 labeled CHO utilizing confocal microscope analyses.

**Figure 15:** PKR1 internalization in CHO cells GFP-PKR1.

**Figure 16:** **Fig. 16A:** PKR1 and PECAM-1 co-staining of the aortas (upper) and cultures endothelial cells, showing that loss of PKR1 was observed only in ec-PKR<sup>r</sup> aortas and endothelial cells, (upper panel). **Fig. 16B:** Representative in vitro angiogenesis assay on matrigel with endothelial cells derived from control (L2L2<sup>+/v</sup>) and ec-PKR<sup>r</sup> hearts. **Fig. 16C:** Proliferation rate of the endothelial cells derived from L2L2<sup>+/v</sup> or ec-PKR<sup>r</sup> hearts in response to FGF or prokineticin-2.

**Figure 17:** **Fig. 17A:** Electron microscopic analyses on capillaries of heart, kidney, pancreas and adipose tissues. **Fig. 17B:** Representative illustration of PECAM-1 staining on heart, kidney, pancreas and adipose tissue, indicating reduced capillary formation in all these organs.

**Figure 18:** **Fig. 18A:** Metabolic cage analyses revealed that mutant mice displayed increased food, and water intakes, and urine volume. **Fig. 18B:** GTT and ITT analyses show impaired glucose clearance and insulin tolerance. **Fig. 18C:** Increased serum insulin and decreased plasma glucose levels in mutant were evident. **Fig. 18D:** Insulin and betacatenin immunostainings revealed increased insulin density and increased beta cell surface in mutant pancreas, respectively. Tunel staining on pancreas sections revealed increased apoptosis in mutant pancreas. Electron microscopic analyses show, abnormal mitochondria, and apoptotic endothelial cells in mutant pancreas. **Fig. 18E:** The histograms show quantification of insulin+ cells number and cell surfaces, and the apoptotic cells.

**Figure 19:** **Fig. 19A:** Semi-thin sections of adipose tissue revealed an unequal size of mutant adipocytes. **Fig. 19B:** RT-PCR analyses demonstrated an increase in expression of HIFla but not VEGF in mutant adipocytes. **Fig. 19C:** Collagen and alpha-SMA co-staining of cryosectioned adipocytes exhibit increased collagen deposition in the mutant.
adipocytes. qPCR verified shows the levels of fibrosis gene expression in mutant adipocytes over wild adipocytes. **Fig. 19D:** Tunel staining of the adipocyte section shows abundant apoptotic cells in mutant adipocytes. **Fig. 19E:** Serum FFA levels were increased in mutant mice. **Fig. 19F:** qPCR shows expression levels of insulin receptor (IR) insulin receptor signal (IRS), FFA transporters. **Fig. 19G:** Impaired Akt phosphorylation 20 min after insulin administration (i.p.) in mutant adipocytes.

**Figure 20:** **Fig. 20A:** PKR1 gene reintroduction rescues metabolic alterations such as GTT and ITT in mutant mice. **Fig. 20B:** PKR1 gene transfer corrects the abnormal gene expression in mutant adipocytes. **Fig. 20C:** PECAM and a-SMA co-staining of adipose tissues. PKR1 gene transfer modifies angiogenic profile in adipose tissue.

**Figure 21:** Prokineticin-2 inhibits adipogenesis in 3T3-L1 cells. **Fig. 21A:** Representative in vitro adipogenesis assay in 3T3-L1 cells treated with control (ctr), adipogenic cocktail (AC) or pretreatment with 5 nM of prokineticin-2 (+PK-2) 10h before adipogenic cocktail treatment. **Fig. 21B:** Histogram shows pretreatment of adipocytes with prokineticin-2 reduced number of adipocytes detected by Oil red staining. qPCR analyses show increased levels of adipogenic genes, resistin and adiponectin by adipogenic cocktail that was inhibited by prokineticin-2. **Fig. 21C:** qPCR analyses reveal that PPARα and C/EBPa were significantly, PPARγ slightly reduced by prokineticin-2 pretreatment. **Fig. 21D:** In the PKR1 knock-down 3T3-L1 cells (siRNA-PKR1), prokineticin-2 (5 nM) 20 pretreatment did not able to inhibit adipogenesis induced by adipogenic cocktail, (* p<0.05, n=4).

**Figure 22.** Prokineticin-2 inhibits proliferation of 3T3-L1 cells. **Fig. 22A:** 10% FCS increased cell numbers within 24 h as compare to cell numbers in 0.5% serum treated cells (control), reaching to maximum within 4 days. Treatment of the cells with prokineticin-2 (5nM) significantly reduced cell numbers as compare to that of 10% FCS alone. Ki67 positive proliferating cells in S phase were increased 1.5 times with % FCS treatment of the 3T3-L1 cells (** p<0.05, n=4). Pretreatment of the cells with prokineticin-2 completely abolished Ki67 positive cell number (*p<0.05, n=4). **Fig. 22B:** Representative illustration of Ki67 positive cells and dapi positive total cells in each group. **Fig. 22C:** qPCR analyses for cycling gene expression 10 hours (left) and 48 hours after 10% FCS and 10% FCS+Prokineticin-2 treatment. Transcript of Cyclin D and E levels were reduced 10h after treatment of the prokineticin-2, whereas cdk2 and c-Myc were reduced 48h after prokineticin-2 treatment (*p<0.05, n=4). **Fig. 22D:** Representative illustration of Tunel...
positive cells and dapi positive total cells in each group. No detectible apoptotic cells were observed in either group.

**Figure 23.** PKR1 null mutant mice (PKR1/-) exhibit obesity at the later age. **Fig. 23A:** Food intake, body, WAT and pancreas (pancr) weight of the PKR1-null mutant (PKR1/-) 5 and wild type (WT) mice (*p<0.05, n=6). **Fig. 23B:** Representative illustration of histology of adipose tissue derived from PKR1/- and WT mice. **Fig. 23C:** Representative illustration of Ki67 positive cells and dapi positive total cells in adipose tissue of PKR1-null mutant and wild type mice. **Fig. 23D:** Quantification of adipose numbers/High Power Field (HPF, 10X objective) (*p<0.05, n=20 HPF) and % of Ki67 positive proliferating 10 adipocytes/dapi positive total cell number/HPF in the PKR1/- and WT mice (*p<0.05, n=20 HPF, 40X objective). Histograms show the number of proliferating adipocytes was increased in PKR1/- mice

**Figure 24.** PKR1 null mutant mice (PKR1/-) exhibit impaired adipocyte structure and function. **Fig. 24A:** Representative illustration of PECAM-1 positive capillary endothelial 15 cells and dapi positive total cell numbers. Quantification of % of PECAM-1 positive endothelial cells/dapi positive total cell number/HPF in the PKR1/- and WT mice. Histogram shows no alteration on adipocytes capillary network between PKR1-null mutant (PKR1/-) and wild type (WT) mice (p>0.05, n=20 HPF, 40X objective). **Fig. 24B:** Electron microscopic analyses on adipose tissues. The necrotic nucleus (nc), lipid (lp) 20 accumulation in the cytoplasm, abnormal mitochondrial (mtc) structures and small blood vessels (bv) were observed in the adipose tissue of the PKR1/- mice. **Fig. 24C:** Western blot analyses on lysates derived from the adipose tissue of both group of mice 0 or 20 min after i.p. 0.75 U/I insulin injection. Western blot analyses revealed that insulin-stimulated phosphorylation of Akt (at serine and threonin) were significantly lower in PKR1/- 25 adipocytes as compare to WT adipocytes (* p<0.05, n=3).

**Figure 25:** Formulas of PKR1 agonists IS20 to IS23, IS26 to IS28, IS30, IS34 and IS35.

**Figure 26:** Synthesis of the PKR1 agonist IS28.

**Figure 27:** In vitro pro-angiogenic activity of prokineticin-2 and the PKR1 agonists 30 ISI, IS20, IS27, IS28 and IS30 at 1, 10 and 100 nM (quantification of the tube-like formation of the endothelial cells on Matrigel).

**Figure 28:** In vitro pro-angiogenic activity of prokineticin-2 and the PKR1 agonists ISI, IS21, IS22, IS23 and IS26 at 100 nM (quantification of the tube-like formation of the
endothelial cells on Matrigel).

**Figure 29:** In vitro pro-angiogenic activity of prokineticin-2 and the PKRI agonists ISI, IS34 and IS35 at 0.1, 1, 10 and 100 nM (quantification of the tube-like formation of the endothelial cells on Matrigel).

## 5 Detailed description of the invention

### Non peptide PKRI agonists

The inventors disclose herein non peptide prokineticin receptor-1 (PKRI) agonists. They demonstrated that these agonists are able to activate the PKRI signalling pathway by specific binding on PKRI.

"Prokineticin receptor 1" or "PKRI", as used herein, refers to a G-protein-coupled receptor that could be activated by the binding of prokineticin-1 or prokineticin-2. It may be also named GPR73 or PROKR1. The reference amino acid sequence for human PKRI can be retrieved from the Genbank Accession No. NP_620414. The reference entry for human PKRI in the transcriptome database UniGene is Hs.683430.

As used herein, the term "prokineticin receptor-1 agonist" or "PKRI agonist" refers to any compound that is able to bind and activate the prokineticin receptor-1, as described above. The PKRI agonist activity of a compound may be assessed by any method known by the skilled person. For example, the PKRI agonist activity may be assessed by any of the methods disclosed in the experimental section: the measure of the capacity to induce in vitro angiogenesis, the measure of the capacity to activate the PKRI signalling pathway (e.g. by measurement of the intracellular calcium), or the capacity to induce to internalization of the receptor (example lc).

The present invention discloses a PKRI agonist of formula (I)

![Chemical structure](image)
A is

\[
\begin{array}{c}
\text{R}^1 \\
\begin{array}{c}
\text{N} \\
\end{array}
\end{array}
\]

or

\[
\begin{array}{c}
\text{H}_2\text{N}
\end{array}
\]

5 m is selected from 0 and 1;

n is selected from 0, 1 and 2;

R^1 is selected from the group consisting of a hydrogen atom; a halogen atom; a (C_1-C_6)-alkyl, a (C_2-C_6)-alkenyl and a (C_2-C_6)-alkynyl group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; a (C_6-C_12)-aryl; a 5 to 7-membered-ring heterocycle; a (Ci-C_6)-alkoxy, a (C_2-C_6)-acyl, a (C_2-C_6)-ester, an amino group, a (Ci-C_6)-amine, a (Ci-C_6)-amide, a (Ci-C_6)-imine, a (Ci-C_6)-nitrile, a (Ci-C_6)-thioalkyl, a (Ci-C_6)-sulfone and a (CrC^-imine, a (Ci-C_6)-sulfoxide group wherein the alkyl part of the group is optionally interrupted by one of several heteroatoms chosen among N, O and S, and is optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol;

R^2 is selected from the group consisting of a (C_6-C_12)-aryl group and a 5 to 7-membered-ring heterocycle, preferably from phenyl and furan, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C_6)-alkyl, a (C_2-C_6)-alkenyl, a (C_2-C_6)-alkynyl, a (C_6-C_12)-aryl, a 5 to 7-membered-ring heterocycle, a (Ci-C_6)-alkoxy, a (C_2-C_6)-acyl, a (Ci-C_6)-alcohol, a carboxylic group, a (C_2-C_6)-ester, a (CrC^-imine, a (Ci-C_6)-amine, an amino group, a (Ci-C_6)-amide, a (CrC^-imine, a (Ci-C_6)-nitrile, a hydroxyl, an aldehyde, a (C_1-C_6)-halogenoalkyl, a thiol, a (C_1-C_6)-thioalkyl, a (Q-C^-sulfone, a (C_1-C_6)-sulfoxide group and a halogen atom; and

R^3 is selected from the group consisting of a (Ci-C_6)-alkyl, a (C_2-C_6)-alkenyl, a (C_2-C_6)-alkynyl and a (Ci-C_6)-amine group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a (C_6-C_12)-aryl and a 5 to 7-membered-ring heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C_6)-alkyl, a (Ci-C_6)-alkoxy group and a halogen atom;

or any pharmaceutically acceptable salt thereof.

As used herein, the term "alkyl" refers to a univalent radical containing only carbon and hydrogen atoms arranged in a chain. The alkyl group may be linear or branched. More
specifically, the term "alkyl" means a group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heneicosyl, docosyl and the other isomeric forms thereof. (Ci-C₆)-alkyl includes methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl and the other isomeric forms thereof. (Ci-C₃)-alkyl includes methyl, ethyl, propyl, or isopropyl. The term "Me" as used herein, refers to a methyl group. The term "Et" as used herein, refers to an ethyl group.

As used herein, the term "alkoxy" corresponds to an alkyl group bonded to the molecule by an -O-(ether) bond. (CrC₆)-alkoxy includes methoxy, ethoxy, propyloxy, butyloxy, pentyloxy, hexyloxy and the other isomeric forms thereof. (Ci-C₃)-alkoxy includes methoxy, ethoxy, ethyloxy, and propyloxy.

The term "alkenyl" refers to an alkyl group having at least one unsaturated ethylene bond and the term "alkynyl" refers to an alkyl group having at least one unsaturated acetylene bond. (C₂-C₆)-alkenyl includes ethenyl, propenyl (1-propenyl or 2-propenyl), 1-2 or 2-methylpropenyl, butenyl (1-butenyl, 2-butenyl, or 3-butenyl), methylbutenyl, 2-ethylpropenyl, pentenyl (1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl), hexenyl (1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl), and the other isomeric forms thereof. (C₂-C₃)-alkenyl includes ethenyl, 1-propenyl and 2-propenyl. (C₂-C₆)-alkynyl includes ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl, 1-pentynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl and the other isomeric forms thereof. (C₂-C₃)-alkynyl includes ethynyl, 1-propynyl and 2-propynyl.

The "aryl" groups are monocyclic, bicyclic, or polycyclic aromatic hydrocarbons. Examples include phenyl, biphenyl, a-naphthyl, β-naphthyl, anthracenyl, phenanthrenyl, fluorenyl, indenyl, pentalenyl, azulenyl, biphenylenyl and the like. This term is also intended to include the partially hydrogenated derivatives of the aromatic hydrocarbons groups enumerated above. The aryl groups of the present invention can be substituted or unsubstituted. Preferably, when unspecified, the groups are unsubstituted. (C₆-Ci₂)-aryl includes phenyl, biphenyl, indenyl, pentalenyl and azulenyl.

The term "heterocycle", as used herein, refers to 5-10 membered heterocyclic ring systems comprising one or more heteroatoms, preferably 1 to 5 endocyclic heteroatoms. They may be mono-, bi- or tri-cyclic systems. They may be aromatic or not. Examples of aromatic heterocycles include pyridine, pyridazine, pyrimidine, pyrazine, furan, thiophene,
pyrrole, oxazole, thiazole, isothiazole, imidazole, pyrazole, oxadiazole, triazole, thiadiazole and triazine groups. Examples of non-aromatic heterocycles include piperazine and piperidine. Examples of 5 to 7-membered-ring heterocycles include pyrrolidine, tetrahydrofuran, tetrahydrothiophene, pyrrole, furan, thiophene, piperidine, 5 tetrahydropyran, thiane, pyridine, pyrylium, thiopyran, azepane, oxepane, thiepane, azepine, oxepine and thiepene.

The term "heteroatom" refers to any atom that is not carbon or hydrogen. In particular embodiments, this term refers to N, S, or O.

"Acyl" groups correspond to alkyl groups bonded to the molecule by a -CO- bond. (C₂-C₆)-acyl includes acetyl, propylacetyl, butylacetyl, hexylacetyl and the other isomeric forms thereof. (C₃-C₄)-acyl includes acetyl, propylacetyl and isopropylacetyl.

"Alcohol" groups correspond to alkyl groups containing at least one hydroxyl group. Alcohol can be primary, secondary or tertiary. (Ci-C₆)-alcohol includes methanol, ethanol, propanol, butanol, pentanol, hexanol and the other isomeric forms thereof. (Ci-C₃)-alcohol includes methanol, ethanol, propanol and isopropanol.

"Ester" groups correspond to alkyl groups bonded to the molecule by a -COO- (ester) bond. (C₂-C₆)-ester includes methylester, ethylester, propylester, butylester, pentylester and the other isomeric forms thereof. (C₂-C₃)-ester includes methylester and ethylester.

"Amine" groups correspond to alkyl groups bonded to the molecule by a -N- (amine) bond. (Ci-C₆)-amine includes methylamine, ethylamine, propylamine, butylamine, pentyamine, hexylamine and the other isomeric forms thereof. (Ci-C₃)-amine includes methylamine, ethylamine, and propylamine.

"amide" groups correspond to alkyl groups containing at least one carbonyl (CO) group bound to a nitrogen atom. (Ci-C₆)-amide includes methylamide, ethylamide, propylamide, butylamide, pentylamide, hexylamide and the other isomeric forms thereof. (Ci-C₃)-amide includes methylamide, ethylamide and propylamide.

"Imine" groups correspond to alkyl groups having a (-C≡N-) bond. (Ci-C₆)-imine includes methylimine, ethylimine, propylimine, butylimine, pentylimine, hexylimine and the other isomeric forms thereof. (Ci-C₃)-imine includes methylimine, ethylimine, and propylimine.

"Nitrile" groups correspond to alkyl groups having a (-C≡N-) bond.

The halogen can be CI, Br, I, or F, preferably CI, Br or F, more preferably CI.
"Halogenoalkyl" groups correspond to alkyl groups having at least one halogen. The groups can be monohalogenated or polyhalogenated, containing the same or different halogen atoms. For example, the group can be an trifluoroalkyl (CF₃-R). (Ci-C₆)-halogenoalkyl includes halogenomethyl, halogenoethyl, halogenopropyl, halogenobutyl, halogenopentyl, halogenohexyl and the other isomeric forms thereof. (Ci-C₃)-halogenoalkyl includes halogenomethyl, halogenoethyl, and halogenopropyl.

"Thioalkyl" groups correspond to alkyl groups bonded to the molecule by a -S-(thioether) bond. (Ci-C₆)-thioalkyl includes thiomethyl, thioethyl, thiopropl, thiobutyl, thiopentyl, thiohexyl and the other isomeric forms thereof. (Ci-C₃)-thioalkyl includes thiomethyl, thioethyl, and thiopropl.

"Sulfone" groups correspond to alkyl groups bonded to the molecule by a -SOO-(sulfone) bond. (Ci-C₆)-sulfone includes methylsulfone, ethylsulfone, propylsulfone, butylsulfone, pentylsulfone, hexylsulfone and the other isomeric forms thereof. (Ci-C₃)-sulfone includes methylsulfone, ethylsulfone and propylsulfone.

"Sulfoxide" groups correspond to alkyl groups bonded to the molecule by a -SO-(sulfoxide) group. (Ci-C₆) sulfoxide includes methylsulfoxide, ethylsulfoxide, propylsulfoxide, butylsulfoxide, pentylsulfoxide, hexylsulfoxide and the other isomeric forms thereof. (Ci-C₃) sulfoxide includes methylsulfoxide, ethylsulfoxide, propylsulfoxide and isopropylsulfoxide.

The term "pharmaceutically acceptable salt" refers to salts which are non-toxic for a patient and suitable for maintaining the stability of a therapeautic agent and allowing the delivery of said agent to target cells or tissue. Pharmaceutically acceptable salts are well known in the art (Berge et al., 1977). These salts can be prepared in situ during the final isolation and purification of the agonists of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate,
lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like.

In a first aspect, A is

![Diagram](image)

In this aspect, the PKR1 agonist has thus the formula (Ia)

In particular, the agonist of formula (I) is defined as above, with the proviso that when m is 0, n is 1, R¹ is a hydrogen atom and R² is a furan group, then R³ is not a phenyl group or a phenyl group substituted in para by a methoxy group; and when m is 0, n is 0, R¹ is a hydrogen atom and R² is a furan group, then R³ is not a phenyl group.

In an embodiment, R² is selected from the group consisting of a (C₆-C₁₂)-aryl group and a 5 to 7-membered-ring heterocycle, preferably from phenyl and furan, optionally substituted by at least one substituent selected from the group consisting of a (C₁-C₆)-alkyl, a (C₂-C₆)-alkenyl, a (C₂-C₆)-alkynyl, a non substituted (C₆-C₁₂)-aryl, a 5 to 7-membered-ring heterocycle, a (C₁-C₆)-alkoxy, a (C₂-C₆)-acyl, a (C₁-C₆)-alcohol, a carboxylic group, a (C₂-C₆)-ester, a (C₁-C₆)-amine, an amino group, a (C₁-C₆)-amide, a (C₁-C₆)-imine, a (C₁-C₆)-nitrile, a hydroxyl, an aldehyde, a (C₁-C₆)-halogenoalkyl, a thiol, a (C₁-C₆)-thioalkyl, a (C₁-C₆)-sulfone and a (C₁-C₆)-sulfoxide group; and

R³ is selected from the group consisting of a (C₁-C₆)-alkyl, a (C₂-C₆)-alkenyl, a (C₂-C₆)-alkynyl and a (C₁-C₆)-amine group, optionally substituted by at least one substituent
selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a \((C_6-C_{12})\)-aryl and a 5 to 7-membered-ring heterocycle, optionally substituted by at least one substituent selected from the group consisting of a \((C_6-C_5)\)-alkyl, a \((C_3-C_1)\)-alkoxy group and a halogen atom.

5 In another embodiment, \(R^1\) is selected from the group consisting of a hydrogen atom; a halogen atom; a \((C_3-C_1)\)-alkyl, a \((C_2-C_3)\)-alkenyl and a \((C_2-C_3)\)-alkynyl group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; a \((C_6-C_{12})\)-aryl, preferably a phenyl group; a 5 to 7-membered-ring heterocycle; a \((C_3-C_1)\)-alkoxy, a \((C_2-C_3)\)-acyl, a \((C_2-C_3)\)-ester, an amino group, a \((C_2-C_3)\)-amine, a \((C_1-C_3)\)-nitrile, a \((C_1-C_3)\)-thioalkyl, a \((C_1-C_3)\)-sulfone and a \((C_1-C_3)\)-sulfoxide group wherein the alkyl part of the group is optionally interrupted by one of several heteroatoms chosen among N, O and S, and is optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol;

10 \(R^2\) is selected from the group consisting of a phenyl group and a 5 to 7-membered-ring \(N\)-, \(O\)- or \(S\)-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl, a \((C_2-C_3)\)-alkenyl, a \((C_2-C_3)\)-alkynyl, a phenyl group, a 5-membered-ring heterocycle, a \((C_3-C_1)\)-alkoxy, a \((C_2-C_3)\)-acyl, a \((C_3-C_1)\)-alcohol, a carboxylic group, a \((C_2-C_3)\)-ester, a \((C_3-C_1)\)-amine, an amino group, a \((C_3-C_3)\)-nitrile, a \((C_3-C_3)\)-imine, a \((C_3-C_3)\)-nitrile, a hydroxyl, an aldehyde, a \((C_3-C_3)\)-halogenoalkyl, a thiol, a \((C_3-C_3)\)-thioalkyl, a \((C_3-C_3)\)-sulfone, a \((C_3-C_3)\)-sulfoxide group and a halogen atom; and

20 \(R^3\) is selected from the group consisting of a \((C_6-C_5)\)-alkyl, a \((C_2-C_3)\)-alkenyl, a \((C_3-C_3)\)-amine and a \((C_2-C_3)\)-alkynyl group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and

25 a phenyl group and a 5 to 7-membered-ring heterocycle, optionally substituted by at least one substituent selected from the group consisting of a \((C_3-C_3)\)-alkyl, a \((C_3-C_3)\)-alkoxy group and a halogen atom.

In this embodiment, preferably, \(R^2\) is selected from the group consisting of a phenyl group and a 5 to 7-membered-ring \(N\)-, \(O\)- or \(S\)-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a \((C_3-C_3)\)-alkyl, a \((C_2-C_3)\)-alkenyl, a \((C_2-C_3)\)-alkynyl, a non substituted phenyl group, a 5-membered-ring heterocycle, a \((C_3-C_3)\)-alkoxy, a \((C_2-C_3)\)-acyl, a \((C_3-C_3)\)-alcohol, a carboxylic group, a \((C_2-C_3)\)-ester, a \((C_3-C_3)\)-amine, an amino group, a \((C_3-C_3)\)-amide, a \((C_3-C_3)\)-imine, a \((C_3-C_3)\)-nitrile, a
hydroxyl, an aldehyde, a (Ci-C₃)-halogenoalkyl, a thiol, a (Ci-C₃)-thioalkyl, a (C₁-C₃)-sulfone and a (Ci-C₃)-sulfoxide group.

In an embodiment, R¹ is selected from the group consisting of a hydrogen atom; a halogen atom; a (Ci-C₃)-alkyl, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; a (C₁-C₃)-alkoxy, a (C₂-C₃)-acyl, a (C₂-C₃)-ester, an amino group, a (Ci-C₃)-amine, a (Ci-C₃)-amide and a (Ci-C₃)-thioalkyl, group wherein the alkyl part of the group is optionally interrupted by one of several heteroatoms chosen among N, O and S, and is optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol.

In a particular embodiment, R¹ is selected from the group consisting of a hydrogen atom, a halogen atom, an amino group, a (Ci-C₃)-alkyl group and a (Ci-C₃)-alkoxy group, said (Ci-C₃)-alkyl group and/or (Ci-C₃)-alkoxy group being optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol.

In a more particular embodiment, R¹ is selected from the group consisting of a hydrogen atom, a halogen atom, an amino group, a (Ci-C₃)-alkyl group and a (C₁-C₃)-alkoxy group. Preferably, R¹ is selected from the group consisting of a hydrogen atom, a halogen atom, an amino group, a methyl group and a methoxy group. More preferably, R¹ is selected from the group consisting of a hydrogen atom, a halogen atom, a methyl group and a methoxy group. Even more preferably, R¹ is a hydrogen atom or an amino group, more preferably a hydrogen atom.

In an embodiment, R² is selected from the group consisting of a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, preferably a 5-membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C₃)-alkyl, a (Ci-C₃)-alkoxy, a (C₂-C₃)-acyl, a (Ci-C₃)-alcohol, a carboxylic group, a (C₂-C₃)-ester, a (Ci-C₃)-amine, an amino group, a (Ci-C₃)-amide, a hydroxyl, an aldehyde, a (Ci-C₃)-halogenoalkyl, a thiol, a (Ci-C₃)-thioalkyl and a halogen atom. Preferably, R² is selected from the group consisting of a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, preferably a 5-membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C₃)-alkyl, a (Ci-C₃)-alkoxy, a (C₂-C₃)-acyl, a (Ci-C₃)-alcohol, a
carboxylic group, a \((C_2-C_3)\)-ester, a \((C_1-C_3)\)-amine, an amino group, a \((C_1-C_3)\)-amide, a hydroxyl, an aldehyde, a \((C_1-C_3)\)-halogenoalkyl, a thiol and a \((C_1-C_3)\)-thioalkyl.

In a particular embodiment, \(R^2\) is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl, a \((C_1-C_3)\)-alkoxy, a \((C_2-C_3)\)-acyl, a \((C_1-C_3)\)-alcohol, a carboxylic group, a \((C_2-C_3)\)-ester, a \((C_1-C_3)\)-amine, an amino group, a \((C_1-C_3)\)-amide, a hydroxyl, an aldehyde, a \((C_1-C_3)\)-halogenoalkyl, a thiol, a \((C_1-C_3)\)-thioalkyl and a halogen atom. Preferably, \(R^2\) is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl, a \((C_1-C_3)\)-alkoxy, a \((C_2-C_3)\)-acyl, a \((C_1-C_3)\)-alcohol, a carboxylic group, a \((C_2-C_3)\)-ester, a \((C_1-C_3)\)-amine, an amino group, a \((C_1-C_3)\)-amide, a hydroxyl, an aldehyde, a \((C_1-C_3)\)-halogenoalkyl, a thiol and a \((C_1-C_3)\)-thioalkyl.

In a more particular embodiment, \(R^2\) is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl and a \((C_1-C_3)\)-alkoxy group, preferably methyl and methoxy. Preferably, \(R^2\) is selected from the group consisting of a phenyl group, optionally substituted by one or two methoxy, a furan and a thiophene group. More preferably, \(R^2\) is selected from the group consisting of a non substituted furan group and a phenyl group, optionally substituted by one or two methoxy.

In an embodiment, \(R^3\) is selected from the group consisting of a \((C_1-C_6)\)-alkyl and a \((C_1-C_3)\)-amine group optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl, a \((C_1-C_3)\)-alkoxy group and a halogen atom.

In a particular embodiment, \(R^3\) is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl group, preferably methyl, a \((C_1-C_3)\)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine.

In a more particular embodiment, \(R^3\) is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; and a
phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (C1-C3)-alkyl group, preferably methyl, a (C1-C3)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine. Preferably, R3 is selected from the group consisting of a tert-butyl group and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a methyl group, a methoxy group and chlorine. More preferably, R3 is selected from the group consisting of a tert-butyl, a phenyl, a methoxyphenyl and a chlorophenyl group.

In an embodiment, R1 is selected from the group consisting of a hydrogen atom; a halogen atom; a (C1-C3)-alkyl, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; a (C1-C3)-alkoxy, a (C2-C3)-acyl, a (C2-C3)-ester, an amino group, a (C1-C3)-amine, a (C1-C3)-amide and a (C1-C3)-thioalkyl, group wherein the alkyl part of the group is optionally interrupted by one of several heteroatoms chosen among N, O and S, and is optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol;

R2 is selected from the group consisting of a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, preferably a 5-membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (C1-C3)-alkyl, a (C1-C3)-alkoxy, a (C1-C3)-acyl, a (C1-C3)-alcohol, a carboxylic group, a (C2-C3)-ester, a (C1-C3)-amine, an amino group, a (C1-C3)-amide, a hydroxyl, an aldehyde, a (C1-C3)-halogenoalkyl, a thiol, a (C1-C3)-thioalkyl and a halogen atom; and

R3 is selected from the group consisting of a (C1-C6)-alkyl and a (C1-C6)-amine group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (C1-C3)-alkyl, a (C1-C3)-alkoxy group and a halogen atom.

In a particular embodiment, R1 is selected from the group consisting of a hydrogen atom, a halogen atom, an amino group, a (C1-C3)-alkyl group and a (C1-C3)-alkoxy group, said (C1-C3)-alkyl group and/or (C1-C3)-alkoxy group being optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol;
$R^2$ is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (C$_3$-alkyl), a (C$_3$-alkoxy), a (C$_2$-C$_3$)-acyl, a (C$_3$)-alcohol, a carboxylic group, a (C$_2$-C$_3$)-ester, a (C$_3$)-amine, an amino group, a (C$_3$)-amide, a hydroxyl, an aldehyde, a (C$_3$)-halogenoalkyl, a thiol, a (C$_3$)-thioalkyl and a halogen atom; and

$R^3$ is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (C$_3$)-alkyl group, preferably methyl, a (C$_3$)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine.

In another embodiment, $R^1$ is selected from the group consisting of a hydrogen atom, a halogen atom, an amino group, a (C$_3$)-alkyl group and a (C$_3$)-alkoxy group, said (C$_3$)-alkyl group and/or (C$_3$)-alkoxy group being optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol;

$R^2$ is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (C$_3$)-alkyl, a (C$_3$)-alkoxy, a (C$_2$-C$_3$)-acyl, a (C$_3$)-alcohol, a carboxylic group, a (C$_2$-C$_3$)-ester, a (C$_3$)-amine, an amino group, a (C$_3$)-amide, a hydroxyl, an aldehyde, a (C$_3$)-halogenoalkyl, a thiol and a (C$_3$)-thioalkyl; and

$R^3$ is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (C$_3$)-alkyl group, preferably methyl, a (C$_3$)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine.

In a more particular embodiment, $R^1$ is selected from the group consisting of a hydrogen atom, a halogen atom, an amino group, a (C$_3$)-alkyl group and a (C$_1$-C$_3$)-alkoxy group, preferably from the group consisting of a hydrogen atom, a halogen atom, an amino group, a methyl group and a methoxy group, more preferably from the group consisting of a hydrogen atom and an amino group, and even more preferably is a hydrogen atom;
R\(^2\) is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a \((\text{C}_1-\text{C}_3)\)-alkyl and a \((\text{C}_1-\text{C}_3)\)-alkoxy, preferably from the group consisting of a phenyl group, optionally substituted by one or two methoxy groups, a furan and a thiophene group, more preferably is a non substituted furan group or a phenyl group optionally substituted by one or two methoxy groups; and

R\(^3\) is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a \((\text{C}_1-\text{C}_3)\)-alkyl group, a \((\text{C}_1-\text{C}_3)\)-alkoxy group and a halogen atom, preferably from the group consisting of a tert-butyl, a phenyl, a methoxyphenyl and a chlorophenyl group.

In an embodiment, the PKR1 agonist has the formula (II)

\[
\begin{array}{c}
\text{R}^1 \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{m} \quad \text{CH}_2 \quad \text{n} \\
\text{R}^2 \\
\text{R}^3
\end{array}
\]

\[\text{(II)}\]

m, n, R\(^1\), R\(^2\) and R\(^3\) being as defined in formula (Ia) and in the above disclosed embodiments.

In another embodiment, the PKR1 agonist has the formula (III)

\[
\begin{array}{c}
\text{R}^1 \\
\text{N} \quad \text{m} \quad \text{CH}_2 \quad \text{n} \\
\text{R}^2 \\
\text{R}^3
\end{array}
\]

\[\text{(III)}\]

m, n, R\(^1\), R\(^2\) and R\(^3\) being as defined in formula (Ia) and in the above disclosed embodiments.
In another embodiment, the PKR1 agonist has the formula (IV)

![Formula Image]

m, n, R₁, R₂ and R₃ being as defined in formula (Ia) and in the above disclosed embodiments.

In an embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV) and R¹ is a hydrogen atom or an amino group; R² is selected from the group consisting of a 5-membered-ring heterocycle and a phenyl group, preferably from a furan, a thiophene and a phenyl group, optionally substituted by a (Ci-C₃)-alkoxy group, preferably a methoxy group; and R³ is selected from the group consisting of a (Ci-C₆)-alkyl, preferably a tert-butyl group, and a phenyl group, optionally substituted by a substituent selected from the group consisting of a (Ci-C₃)-alkoxy group and a halogen atom, preferably from a methoxy group and chlorine. Preferably, R¹ is a hydrogen atom.

In another embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV), and R¹ is a hydrogen atom or an amino group; R² is selected from the group consisting of a 5-15 membered-ring heterocycle and a phenyl group, preferably from a furan, a thiophene and a phenyl group, optionally substituted by a (Ci-C₃)-alkoxy group, preferably a methoxy group; and R³ is a phenyl group. Preferably, R¹ is a hydrogen atom. Preferably, in this embodiment, the PKR1 agonist has the formula (II).

In a particular embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV), and R¹ is selected from the group consisting of a hydrogen atom and an amino group; R² is a non substituted furan group or a phenyl group optionally substituted by one or two methoxy; and R³ is a tert-butyl group or a phenyl group optionally substituted by a methoxy group or chlorine. Preferably, R¹ is a hydrogen atom.

In a particular embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV), and m is 0; n is selected from 1 and 2; R¹ is a hydrogen atom; R² is a 5-membered-ring O-heterocycle, preferably a furan group; and R³ is a phenyl group. Preferably, in this embodiment, the PKR1 agonist has the formula (II).
In another particular embodiment, the PKR1 agonist has the formula (la), (II), (III) or (IV), and m is 1; n is selected from 0 and 2; \( R^1 \) is a hydrogen atom; \( R^2 \) is selected from the group consisting of a 5-membered-ring O- or S-heterocycle and a phenyl group, preferably from a furan, a thiophene and a phenyl group, optionally substituted by a (Ci-C3)-alkoxy 5 group, preferably a methoxy group; and \( R^3 \) is a phenyl group. Preferably, in this embodiment, the PKR1 agonist has the formula (II).

In another particular embodiment, the PKR1 agonist has the formula (la), (II), (III) or (IV), and m is 1; n is 2; \( R^1 \) is a hydrogen atom; \( R^2 \) is selected from the group consisting of a 5-membered-ring O- or S-heterocycle and a phenyl group, preferably from a furan, a thiophene and a phenyl group, optionally substituted by a (Ci-C3)-alkoxy group, preferably a methoxy group; and \( R^3 \) is a phenyl group. Preferably, in this embodiment, the PKR1 agonist has the formula (II).

In another embodiment, the PKR1 agonist has the formula (la), (II), (III) or (IV), and m is 0; n is selected from 0, 1 and 2; \( R^1 \) is a hydrogen atom; \( R^2 \) is a 5-membered-ring O-heterocycle, preferably a furan group; and \( R^3 \) is selected from the group consisting of a phenyl and a (Ci-C6)-alkyl group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C3)-alkoxy group and a halogen atom, preferably from methoxy and chlorine. Preferably, in this embodiment, the PKR1 agonist has the formula (III).

In a particular embodiment, the PKR1 agonist has the formula (la), (II), (III) or (IV), m is 0; n is selected from 1 and 2; \( R^1 \) is a hydrogen atom; \( R^2 \) is a 5-membered-ring O-heterocycle, preferably a furan group; and \( R^3 \) is selected from the group consisting of a phenyl and a (Q-C\(^n\)-alkyl group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C3)-alkoxy group and a halogen atom, preferably from methoxy and chlorine. Preferably, in this embodiment, the PKR1 agonist has the formula (III).

In another particular embodiment, the PKR1 agonist has the formula (la), (II), (III) or (IV), and m is 0; n is selected from 1 and 2; \( R^1 \) is a hydrogen atom; \( R^2 \) is a 5-membered-ring O-heterocycle, preferably a furan group; and \( R^3 \) is a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C3)-alkoxy group and a halogen atom, preferably from methoxy and chlorine. Preferably, in this embodiment, the PKR1 agonist has the formula (III).
In another particular embodiment, the PKRl agonist has the formula (Ia), (II), (III) or (IV), and m is 0; n is selected from 1 and 2; R¹ is a hydrogen atom; R² is a 5-membered-ring O-heterocycle, preferably a furan group; and R³ is a phenyl group. Preferably, in this embodiment, the PKRl agonist has the formula (III).

5 In another particular embodiment, the PKRl agonist has the formula (Ia), (II), (III) or (IV), and m is 0; n is selected from 1 and 2; R¹ is a hydrogen atom; R² is a 5-membered-ring O-heterocycle, preferably a furan group; and R³ is a phenyl group substituted by a (C₁-C₃)-alkoxy group, preferably a methoxy group. Preferably, in this embodiment, the PKRl agonist has the formula (III).

10 In another particular embodiment, the PKRl agonist has the formula (Ia), (II), (III) or (IV), and m is 0; n is selected from 1 and 2; R¹ is a hydrogen atom; R² is a 5-membered-ring O-heterocycle, preferably a furan group; and R³ is a phenyl group substituted by a halogen atom, preferably chlorine. Preferably, in this embodiment, the PKRl agonist has the formula (III).

15 In another particular embodiment, the PKRl agonist has the formula (Ia), (II), (III) or (IV), m is 0; n is selected from 1 and 2; R¹ is a hydrogen atom; R² is a 5-membered-ring O-heterocycle, preferably a furan group; and R³ is a (C₃⁻alkyl) group, preferably a tert-butyl group. Preferably, in this embodiment, the PKRl agonist has the formula (III).

In another embodiment, the PKRl agonist has the formula (Ia), (II), (III) or (IV), and m is 0; n is selected from 0 and 1; R¹ is a hydrogen atom; R² is a 5-membered-ring O-heterocycle, preferably a furan group; and R³ is a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (C₁⁻C₃)-alkoxy group and a halogen atom, preferably from methoxy and chlorine. Preferably, in this embodiment, the PKRl agonist has the formula (IV).

25 In a particular embodiment, the PKRl agonist has the formula (Ia), (II), (III) or (IV), m is 0; n is 1; R¹ is a hydrogen atom; R² is a 5-membered-ring O-heterocycle, preferably a furan group; and R³ is a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (C₁⁻C₃)-alkoxy group and a halogen atom, preferably from methoxy and chlorine. Preferably, in this embodiment, the PKRl agonist has the formula (IV).

30 In another particular embodiment, the PKRl agonist has the formula (Ia), (II), (III) or (IV), and m is 0; n is 1; R¹ is a hydrogen atom; R² is a 5-membered-ring O-heterocycle,
preferably a furan group; and \( R^3 \) is a phenyl group. Preferably, in this embodiment, the PKR1 agonist has the formula (IV).

In another particular embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV), and \( m = 0; n = 0; R^1 \) is a hydrogen atom; \( R^2 \) is a 5-membered-ring O-heterocycle, preferably a furan group; and \( R^3 \) is a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (C1-C3)-alkoxy group and a halogen atom, preferably from methoxy and chlorine. Preferably, in this embodiment, the PKR1 agonist has the formula (IV).

In another particular embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV), and \( m = 0; n = 0; R^1 \) is a hydrogen atom; \( R^2 \) is a 5 to 7-membered-ring O-heterocycle, preferably a furan group; and \( R^3 \) is a phenyl group. Preferably, in this embodiment, the PKR1 agonist has the formula (IV).

In an embodiment, the PKR1 agonist has the formula (II) or (IV), \( m \) is selected from 0 and 1; \( n \) is selected from 0, 1 and 2; \( R^1 \) is a hydrogen atom; \( R^2 \) is selected from the group consisting of a furan group and a phenyl group, optionally substituted by a methoxy group; and \( R^3 \) is a phenyl group.

In a particular embodiment, the PKR1 agonist has the formula (II) or (IV), \( m \) is selected from 0 and 1; \( n \) is selected from 0, 1 and 2; \( R^1 \) is a hydrogen atom; \( R^2 \) is selected from the group consisting of a furan group and a phenyl group substituted by a methoxy group; and \( R^3 \) is a phenyl group.

In a particular embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV), and \( m = 0; n = 1; R^1 \) is selected from the group consisting of a hydrogen atom and an amino group; \( R^2 \) is selected from the group consisting of a furan group and a phenyl group, preferably a phenyl group, optionally substituted by a methoxy group; and \( R^3 \) is a phenyl group. Preferably, in this embodiment, the PKR1 agonist has the formula (III).

In another particular embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV), and \( m = 0; n = 1; R^1 \) is an amino group; \( R^2 \) is a phenyl group, preferably substituted by a methoxy group; and \( R^3 \) is a phenyl group. Preferably, in this embodiment, the PKR1 agonist has the formula (III).

In an embodiment, the PKR1 agonist of formula (Ia), (II), (III) or (IV) has one or several of the following features: a) \( m = 0; b) n = 1; c) R^1 \) is a hydrogen atom; d) \( R^2 \) is a furan group; and e) \( R^3 \) is a phenyl group. In another embodiment, the PKR1 agonist of formula (Ia), (II), (III) or (IV) has one or several of the following features: a) \( m = 1; b) n =
2; c) R₁ is a hydrogen atom; d) R₂ is a phenyl group; and e) R₃ is a phenyl group. In another embodiment, the PKRI agonist of formula (Ia), (II), (III) or (IV) has one or several of the following features: a) m is 1; b) n is 2; c) R₁ is a hydrogen atom; d) R₂ is a phenyl group substituted by a (Ci-C₃)-alkoxy group, preferably methoxy; and e) R₃ is a phenyl group. In another embodiment, the PKRI agonist of formula (Ia), (II), (III) or (IV) has one or several of the following features: a) m is 0; b) n is 1; c) R₁ is a hydrogen atom; d) R₂ is a furan group; and e) R₃ is a phenyl group. In another embodiment, the PKRI agonist of formula (Ia), (II), (III) or (IV) has one or several of the following features: a) m is 0; b) n is 1; c) R₁ is a hydrogen atom; d) R₂ is a furan group; and e) R₃ is a phenyl group substituted by a (Ci-C₃)-alkoxy group, preferably methoxy. In another embodiment, the PKRI agonist of formula (Ia), (II), (III) or (IV) has one or several of the following features: a) m is 0; b) n is 1; c) R₁ is a hydrogen atom; d) R₂ is a furan group; and e) R₃ is a (Ci-C₆)-alkyl group, preferably a tert-butyl group. In an embodiment, the PKRI agonist of formula (IV) has one or several of the following features: a) m is 0; b) n is 1; c) R₁ is a hydrogen atom; d) R₂ is a furan group; and e) R₃ is a phenyl group.

The PKRI agonist of formula (Ia), (II), (III) or (IV) may meet one of the above-listed features. It may also meet two of these features, for instance a) and b); a) and c); a) and d); a) and e); b) and c); b) and d); b) and e); c) and d); c) and e); or d) and e). The PKRI agonist may meet three of these features, for instance a) and c); a) and d); a) and e); b) and c); b) and d); b) and e); c) and d); c) and e); or d) and e). The PKRI agonist may meet four of these features, for instance a) and c); a) and d); a) and e); b) and c); b) and d); b) and e); c) and d); c) and e); or a) and c); d) and e). The PKRI agonist may also meet all of these features, i.e. a), b), c), d) and e).

In a second aspect, A is

\[
\begin{align*}
N & \quad NH \\
& \quad H_2N
\end{align*}
\]
In this aspect, the PKR1 agonist has thus the formula (lb)

\[
\text{\begin{align*}
\text{R}^2 & \text{ is selected from the group consisting of a } (C_6-C_2)-\text{aryl group and a } 5 \text{ to } 7\text{-membered-ring heterocycle, preferably from phenyl and furan, optionally substituted by at least one substituent selected from the group consisting of a } (C_6-\text{alkyl, a } (C_2-C_6)\text{-alkenyl, a } (C_2-C_6)\text{-alkynyl, a } (C_6-\text{aryl group, a } (C_2-C_6)\text{-alkoxy, a } (C_2-C_6)\text{-acyl, a } (C_6-\text{alkoxy, a } (C_6)\text{-ester, a } (C_6)\text{-amine, an amino group, a } (C_6)\text{-amide, a } (C_6)\text{-imine, a } (C_6)\text{-nitrile, a hydroxyly, an aldehyde, a } (C_6)\text{-halogenoalkyl, a thiol, a } (C_6)\text{-thioalkyl, a } (C_6)\text{-sulfone, a } (C_1-20)C_6\text{-sulfoxide group and a halogen atom;}

\text{R}^3 & \text{ is selected from the group consisting of a } (CrC^\wedge)\text{-alkyl, a } (C_2-C_6)\text{-alkenyl, a } (C_2-C_6)\text{-alkynyl and a } (C_6)\text{-amine group, optionally substituted by at least one substituent selected from a hydroxyly, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a } (C_6-C_{12})\text{-aryl and a } 5 \text{ to } 7\text{-membered-ring heterocycle, optionally substituted by at least one substituent selected from the group consisting of a } (CrC^\wedge)\text{-alkyl, a } (C_6)\text{-alkoxy group and a halogen atom.}

\text{In an embodiment, } R^2 \text{ is selected from the group consisting of a phenyl group and a } 5 \text{ to } 7\text{-membered-ring } N-, O- \text{ or } S\text{-heterocycle, preferably a } 5 \text{-membered-ring } N-, O- \text{ or } S\text{-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a } (C_1-C_3)\text{-alkyl, a } (C_1-C_3)\text{-alkoxy, a } (C_2-C_3)\text{-acyl, a } (C_6)\text{-alkoxy, a carboxylic group, a } (C_2-C_3)\text{-ester, a } (C_1-C_3)\text{-amine, an amino group, a } (C_1-C_3)\text{-amide, a hydroxyly, an aldehyde, a } (C_1-C_3)\text{-halogenoalkyl, a thiol, a } (C_1-C_3)\text{-thioalkyl and a halogen atom.}
\end{align*}}
\]
In a particular embodiment, \( R^2 \) is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl, a \((C_1-C_3)\)-alkoxy, a \((C_2-C_3)\)-acyl, a \((C_1-C_3)\)-alcohol, a carboxylic group, a \((C_2-C_3)\)-ester, a \((C_1-C_3)\)-amine, an amino group, a \( \text{phenyl} \) group, a \( \text{hydroxyl} \) group, a \( \text{pyrrole} \), a \( \text{furan} \) and a \( \text{thiophene} \) group.

In a more particular embodiment, \( R^2 \) is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl and a \((C_1-C_3)\)-alkoxy \( N\)-, \( O\)- or \( S\)-heterocycle, preferably a 5-membered-ring \( N\)-, \( O\)- or \( S\)-phenyl, a hydroxyl, an aldehyde, a \( (C_1-C_3)\)-halogenoalkyl, a thiol, a \( (C_1-C_3)\)-thioalkyl and a halogen atom.

In an embodiment, \( R^3 \) is selected from the group consisting of a \( (C_1-C_6)\)-alkyl and a \( (C_1-C_5)\)-amine group optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a phenyl group and a 5 to 7-membered-ring \( N\)-, \( O\)- or \( S\)-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl, a \((C_1-C_3)\)-alkoxy group and a halogen atom.

In a particular embodiment, \( R^3 \) is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl group, preferably methyl, a \((C_1-C_3)\)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine.

In a more particular embodiment, \( R^3 \) is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a \((C_1-C_3)\)-alkyl group, preferably methyl, a \((C_1-C_3)\)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine. Preferably, \( R^3 \) is selected from the group consisting of a tert-butyl group and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a methyl group, a methoxy group and chlorine. More preferably, \( R^3 \) is selected from the group consisting of a tert-butyl, a phenyl, a methoxyphenyl and a chlorophenyl group.

In an embodiment, \( R^2 \) is selected from the group consisting of a phenyl group and a 5 to 7-membered-ring \( N\)-, \( O\)- or \( S\)-heterocycle, preferably a 5-membered-ring \( N\)-, \( O\)- or \( S\)-
heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (C_i-C_3)-alkyl, a (C_i-C_3)-alkoxy, a (C_2-C_3)-acyl, a (C_i-C_3)-alcohol, a carboxylic group, a (C_2-C_3)-ester, a (C_i-C_3)-amine, an amino group, a (C_i-C_3)-amide, a hydroxyl, an aldehyde, a (C_i-C_3)-halogenoalkyl, a thiol, a (C_i-C_3)-thioalkyl and a halogen atom; and

R^3 is selected from the group consisting of a (C_i-C_6)-alkyl and a (C_i-C_6)-amine group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (C_i-C_3)-alkyl, a (C_i-C_3)-alkoxy group and a halogen atom.

In another embodiment, R^2 is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (C_i-C_3)-alkyl, a (C_i-C_3)-alkoxy, a (C_2-C_3)-acyl, a (C_i-C_3)-alcohol, a carboxylic group, a (C_2-C_3)-ester, a (C_i-C_3)-amine, an amino group, a (C_i-C_3)-amide, a hydroxyl, an aldehyde, a (C_i-C_3)-halogenoalkyl, a thiol, a (C_1-C_3)-thioalkyl and a halogen atom; and

R^3 is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (C_i-C_3)-alkyl group, preferably methyl, a (C_i-C_3)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine.

In a further embodiment, R^2 is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (C_i-C_3)-alkyl and a (C_i-C_3)-alkoxy, preferably from the group consisting of a phenyl group, optionally substituted by a methoxy, a furan and a thiophene group; and

R^3 is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (C_i-C_3)-alkyl group, a (C_1-C_3)-alkoxy group and a halogen atom, preferably from the group consisting of a tert-butyl, a phenyl, a methoxyphenyl and a chlorophenyl group.
In an embodiment, the PKRI agonist of formula (lb) has one or several of the following features: a) \( m = 0 \); b) \( n = 1 \); c) \( R^2 \) is a furan group; and d) \( R^3 \) is a phenyl group.

In another embodiment, the PKRI agonist of formula (lb) has one or several of the following features: a) \( m = 1 \); b) \( n = 2 \); c) \( R^2 \) is a phenyl group; and d) \( R^3 \) is a phenyl group.

In another embodiment, the PKRI agonist of formula (lb) has one or several of the following features: a) \( m = 1 \); b) \( n = 2 \); c) \( R^2 \) is a phenyl group substituted by a \((C_1-C_3)-alkoxy\) group, preferably methoxy; and d) \( R^3 \) is a phenyl group. In another embodiment, the PKRI agonist of formula (lb) has one or several of the following features: a) \( m = 1 \); b) \( n = 0 \); c) \( R^2 \) is a furan group; and d) \( R^3 \) is a phenyl group. In another embodiment, the PKRI agonist of formula (lb) has one or several of the following features: a) \( m = 1 \); b) \( n = 1 \); c) \( R^2 \) is a furan group; and d) \( R^3 \) is a phenyl group. In another embodiment, the PKRI agonist of formula (lb) has one or several of the following features: a) \( m = 1 \); b) \( n = 2 \); c) \( R^2 \) is a phenyl group substituted by a halogen atom, preferably chlorine. In another embodiment, the PKRI agonist of formula (lb) has one or several of the following features: a) \( m = 0 \); b) \( n = 1 \); c) \( R^2 \) is a furan group; and d) \( R^3 \) is a phenyl group substituted by a \((C_1-C_3)-alkoxy\) group, preferably methoxy. In another embodiment, the PKRI agonist of formula (lb) has one or several of the following features: a) \( m = 0 \); b) \( n = 1 \); c) \( R^2 \) is a furan group; and d) \( R^3 \) is a phenyl group.

The PKRI agonist of formula (lb) may meet one of the above-listed features. It may also meet two of these features, for instance a) and b); a) and c); a) and d); b) and c); b) and d); or c) and d). The PKRI agonist may meet three of these features, for instance a), b) and c); a), b) and d); a), c) and d); or b), c) and d). The PKRI agonist may also meet all of these features, i.e. a), b), c) and d).
The PKR1 agonist may be selected from the group consisting of

<table>
<thead>
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<th>Name of agonist</th>
<th>Formula</th>
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<tbody>
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</tr>
<tr>
<td>IS2</td>
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<tr>
<td>IS3</td>
<td><img src="image" alt="Formula IS3" /></td>
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<td>IS4</td>
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Preferably, the PKR1 agonist is selected from the group consisting of IS1 to IS3, IS5 to IS7, IS9 to IS12, IS14, IS15, IS17 to IS23, IS26 to IS28, IS30, IS34 and IS35. More preferably, the PKR1 agonist is selected from the group consisting of IS1, IS3, IS5 to IS7, IS9, IS11, IS12, IS14, IS17 to IS23, IS26 to IS28, IS34 and IS35. The PKR1 agonist may also be selected from the group consisting of IS1, IS3, IS5, IS6, IS9, IS12, IS14, IS20, IS21, IS26 to IS28, IS34 and IS35. In a particular embodiment, the PKR1 agonist is selected from the group consisting of IS1, IS20, IS27, IS28, IS34 and IS35. In another particular embodiment, the PKR1 agonist is selected from the group consisting of IS27, IS28 and IS35. In a further embodiment, the PKR1 agonist is selected from the group consisting of IS1, IS20 and IS34.

In a particular embodiment, the PKR1 agonist has the formula (Ia), (II), (III) or (IV), m, n, R1, R2 and R3 are defined as in formula (Ia) and in the above disclosed embodiments, with the proviso that when m is 0, n is 0, R1 is a hydrogen atom, R2 is a furan group, R3 is a 1 phenyl group then the agonist has the formula (II) or (IV).

The present invention also concerns a PKR1 agonist of formula (Ia), (II), (III) or (IV), wherein m, n, R1, R2 and R3 have the same meaning as in the above disclosed embodiments, with the proviso that when m is 0, n is 1, R1 is a hydrogen atom and R2 is a furan group, then R3 is not a phenyl group or a phenyl group substituted in para by a methoxy group; and when m is 0, n is 0, R1 is a hydrogen atom and R2 is a furan group, then R3 is not a phenyl group.

The present invention further concerns a PKR1 agonist of formula (Ia), (II), (III) or (IV), wherein n, R1, R2 and R3 have the same meaning as in the above disclosed embodiments, and m is 1. In an embodiment, m is 1 and n is 2. In a particular embodiment, m is 1; n is selected from 0 and 2, R1 is a hydrogen atom; R2 is selected from the group consisting of 5-membered-ring O- or S-heterocycle and a phenyl group, optionally substituted by a (C1-C3)-alkoxy group, preferably a methoxy group; and R3 is a phenyl group. Preferably, R2 is selected from the group consisting of a furan, a thiophene, a phenyl and a methoxyphenyl group. More preferably, R2 is selected from the group consisting of a furan and a methoxyphenyl group.

The present invention further concerns a PKR1 agonist of formula (Ia), (II), (III) or (IV), wherein m, R1, R2 and R3 have the same meaning as in the above disclosed embodiments, and n is 2, with the proviso that when m is 0, R1 is a hydrogen atom, R2 is a
furan group and $R^3$ is a phenyl group then the agonist has the formula (III) or (IV). In an embodiment, $R^1$ is a hydrogen atom; $R^2$ is selected from the group consisting of a 5-membered-ring O- or S-heterocycle and a phenyl group, optionally substituted by a (C$_1$-C$_3$)-alkoxy group, preferably a methoxy group; and $R^3$ is a phenyl group. Preferably, $R^2$ is selected from the group consisting of a furan, a thiophene, a phenyl and a methoxyphenyl group. More preferably, $R^2$ is selected from the group consisting of a furan and a methoxyphenyl group.

The present invention further concerns a PKR1 agonist of formula (Ia), (II), (III) or (IV), wherein $m$, $R^1$, $R^2$ and $R^3$ have the same meaning as in the above disclosed embodiments, and $n$ is 2, with the proviso that when $m$ is 0, $R^1$ is a hydrogen atom, $R^2$ is a furan group and $R^3$ is a phenyl group then the agonist has the formula (II) or (IV), preferably (IV).

The present invention further concerns a PKR1 agonist of formula (Ia), (Ib), (II), (III) or (IV), wherein $m$, $n$, $R^1$ and $R^2$ have the same meaning as in the above disclosed embodiments, and $R^3$ is a (Q-C$_n^\alpha$-alkyl group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol. In an embodiment, $R^3$ is a (CrC$_n^\alpha$-alkyl group. In a particular embodiment, $R^3$ is a tert-butyl group. In a more particular embodiment, $R^1$ is a hydrogen atom; $R^2$ is selected from the group consisting of a 5-membered-ring O- or S-heterocycle, preferably a furan group, and a phenyl group, optionally substituted by a (Ci-C$_3$)-alkoxy group, preferably a methoxy group; and $R^3$ is a (CrC$_n^\alpha$-alkyl group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol. Preferably, $R^3$ is a (Q-C$_n^\alpha$-alkyl group. More preferably, $R^3$ is a tert-butyl group.

The present invention further concerns a PKR1 agonist of formula (Ia), (Ib), (II), (III) or (IV), wherein $m$, $n$, $R^1$ and $R^2$ have the same meaning as in the above disclosed embodiments, and $R^3$ is selected from the group consisting of a phenyl group and a 5-membered-ring heterocycle, substituted by at least one substituent selected from the group consisting of a (Ci-C$_3$)-alkyl, a (Ci-C$_3$)-alkoxy group and a halogen atom, with the proviso that when $m$ is 0, $n$ is 1, $R^1$ is a hydrogen atom, $R^2$ is a furan group and $R^3$ is a phenyl group substituted by a methoxy, then the methoxy group is in meta- or ortho-position. In an embodiment, $R^3$ is selected from the group consisting of a phenyl group and a phenyl group substituted by at least one substituent selected from the group consisting of a (C$_1$-C$_3$)-alkyl, a (Ci-C$_3$)-alkoxy group and a halogen atom, preferably from the group...
consisting of methyl, methoxy and chlorine.

The present invention further concerns a PKR1 agonist of formula (la), (lb), (II), (III) or (IV), wherein m, n, R1 and R3 have the same meaning as in the above disclosed embodiments, and R2 is selected from the group consisting of a 5 to 7-membered ring 5 heterocycle and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C6)-alkyl, a (Ci-C5)-alkoxy group or a halogen atom. In an embodiment, R2 is selected from the group consisting of a 5-membered ring S-heterocycle, preferably a thiophene group, a phenyl and a methoxyphenyl group. In a particular embodiment, m is 1; n is 2; R1 is a hydrogen atom; R2 is selected from the group consisting of a 5-membered ring S-heterocycle, preferably a thiophene group, a phenyl and a methoxyphenyl group; and R3 is a phenyl group.

The present invention further concerns a PKR1 agonist as described above as a medicament. The PKR1 agonist can be a PKR1 agonist according to any of the above embodiments and can optionally be selected from the group consisting of IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS10, IS11, IS12, IS14, IS15, IS16, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS30, IS34 and IS35, preferably from the group consisting of IS1, IS3, IS6, IS7, IS9, IS11, IS12, IS14, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS34 and IS35, more preferably from the group consisting of IS1, IS9, IS19, IS20, IS27, IS28, IS34 and IS35. The PKR1 agonist may also be selected from the group consisting of IS3, IS5, IS6 and IS12.

The present invention also concerns a PKR1 agonist as described above as a cardioprotective agent. As used herein, the term "cardioprotective agent" refers to an agent that protects cardiomyocytes from damages, in particular from cell death. The PKR1 agonist can be a PKR1 agonist according to any of the above embodiments and can optionally be selected from the group consisting of IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS10, IS11, IS12, IS14, IS15, IS16, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS30, IS34 and IS35, preferably from the group consisting of IS1, IS3, IS6, IS7, IS9, IS11, IS12, IS14, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS34 and IS35, more preferably from the group consisting of IS1, IS9, IS19, IS20, IS27, IS28, IS34 and IS35. The PKR1 agonist may also be selected from the group consisting of IS3, IS5, IS6 and IS12.

The present invention also concerns a PKR1 agonist as described above as a neuroprotective agent. As used herein, the term "neuroprotective agent" refers to an agent
that protects neuronal cells from damages, in particular from cell death. The PKR1 agonist can be a PKR1 agonist according to any of the above embodiments and can optionally be selected from the group consisting of IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS10, IS11, IS12, IS14, IS15, IS16, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS30, IS34 and IS35, preferably from the group consisting of IS1, IS3, IS6, IS7, IS9, IS11, IS12, IS14, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS34 and IS35, preferably from the group consisting of IS1, IS9, IS19, IS20, IS27, IS28, IS34 and IS35. The PKR1 agonist may also be selected from the group consisting of IS3, IS5, IS6 and IS12.

The PKR1 agonists of the invention can be synthesized by any process known by the man skilled in the art. Such processes are described, for example, in the articles of Erlenmeyer and Plochl (Erlenmeyer, 1893; Plochl, 1884). As illustrative example, the synthesis of the agonists IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS10, IS11, IS12, IS14, IS15, IS16, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS30, IS34 and IS35, is detailed in the present application in the experimental section.

Pharmaceutical composition comprising a non peptide PKR1 agonist

The present invention also concerns a pharmaceutical composition comprising a non peptide PKR1 agonist as described above and a pharmaceutically acceptable carrier and/or excipient.

The pharmaceutical composition of the invention may comprise one or several PKR1 agonists according to any of the above embodiments. For example, the composition may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 PKR1 agonists as described above.

In a particular embodiment, the pharmaceutical composition comprises one or several PKR1 agonist selected from the group consisting of IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS9, IS11, IS12, IS14, IS15, IS16, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS30, IS34 and IS35. Preferably, the pharmaceutical composition comprises one or several PKR1 agonist selected from the group consisting of IS1, IS3, IS6, IS7, IS9, IS11, IS12, IS14, IS17, IS18, IS19, IS20, IS21, IS22, IS23, IS26, IS27, IS28, IS34 and IS35. More preferably, the pharmaceutical composition comprises one or several PKR1 agonists selected from the group consisting of IS1, IS9, IS19, IS20, IS27, IS28, IS34 and IS35. In a preferred embodiment, the pharmaceutical composition comprises one or several PKR1 agonists selected from the group consisting of IS1, IS20, IS27, IS28, IS34 and IS35.
In another embodiment, the pharmaceutical composition comprises one or several PKR1 agonist selected from the group consisting of IS6, IS7, IS8, IS11, IS12, IS14, IS15, IS17, IS18 and IS19.

In a preferred embodiment, the pharmaceutical composition of the invention comprises a) IS5, b) IS6, c) IS3, d) IS5 and IS6, e) IS6 and IS3, f) IS5 and IS3 or g) IS 6, IS3 and IS5.

The pharmaceutical composition of the invention is formulated in accordance with standard pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York) known by a person skilled in the art. Possible pharmaceutical compositions include those suitable for oral, rectal, topical (including transdermal, buccal and sublingual), or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. For these formulations, conventional excipient can be used according to techniques well known by those skilled in the art. The compositions for parenteral administration are generally physiologically compatible sterile solutions or suspensions which can optionally be prepared immediately before use from solid or lyophilized form. Adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle and a surfactant or wetting agent can be included in the composition to facilitate uniform distribution of the active ingredient. For oral administration, the composition can be formulated into conventional oral dosage forms such as tablets, capsules, powders, granules and liquid preparations such as syrups, elixirs, and concentrated drops. Non toxic solid carriers or diluents may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. For compressed tablets, binders, which are agents which impart cohesive qualities to powdered materials are also necessary. For example, starch, gelatine, sugars such as lactose or dextrose, and natural or synthetic gums can be used as binders. Disintegrants are also necessary in the tablets to facilitate break-up of the tablet. Disintegrants include starches, clays, celluloses, algins, gums and crosslinked polymers. Moreover, lubricants and glidants are also included in the tablets to prevent adhesion to the tablet material to surfaces in the manufacturing process and to improve the flow characteristics of the powder material during manufacture. Colloidal silicon dioxide is most commonly used as a glidant and
compounds such as talc or stearic acids are most commonly used as lubricants. For transdermal administration, the composition can be formulated into ointment, cream or gel form and appropriate penetrants or detergents could be used to facilitate permeation, such as dimethyl sulfoxide, dimethyl acetamide and dimethylformamide. For transmucosal administration, nasal sprays, rectal or vaginal suppositories can be used. The active compound can be incorporated into any of the known suppository bases by methods known in the art. Examples of such bases include cocoa butter, polyethylene glycols (carbowaxes), polyethylene sorbitan monostearate, and mixtures of these with other compatible materials to modify the melting point or dissolution rate. In a preferred embodiment, the pharmaceutical composition of the invention is suitable for parenteral or oral administration.

Pharmaceutical composition according to the invention may be formulated to release the active drug substantially immediately upon administration or at any predetermined time or time period after administration.

In a particular embodiment, the pharmaceutical composition according to the invention comprises 10 mg to 2 g of PKR1 agonist of the invention. Preferably, pharmaceutical composition according to the invention comprises 100 mg to 800 mg of PKR1 agonist of the invention.

The pharmaceutical composition according to the invention can further comprise one or more other active compounds associated with pharmaceutically acceptable excipients and/or carriers. For example, the agonists of the invention can be associated with other PKR agonists such as prokineticin-2 or the prokineticin 2β peptide as described in the international patent application WO 2005/097826. They also can be associated with other drugs such as drugs used for the treatment of vascular diseases, neurodegenerative diseases, diseases involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotropic hypogonadism, disturbances of circadian rhythm or cancers.

The agonists of the invention can be associated with drugs used for the treatment of vascular diseases. Examples of such drugs include, but are not limited to, ACE inhibitors (e.g., enalapril, captopril, lisinopril and ramipril), beta-blockers (e.g. alprenolol, bucindolol, carteolol, carvedilol, labetalol, nadolol, penbutolol, pindolol, propranolol, acebutolol, atenolol, betaxolol, bisoprolol, celiprolol and esmolol), aldosterone antagonists
(e.g. spironolactone, eplerenone, canrenone, prorenone and mexrenone) or vasodilators, or a combination thereof.

The agonists of the invention can also be associated with drugs used for the treatment of neurodegenerative diseases. Examples of such drugs include, but are not limited to, riluzole, acetylcholinesterase inhibitors (e.g. donepezil, galantamine and rivastigmine), memantine, Levodopa (or L-DOPA), dopamine agonists (e.g. bromocriptine, pergolide, pramipexole, ropinirole, piribedil, cabergoline, apomorphine, and lisuride), MAO-B inhibitors (e.g. selegiline and rasagiline), tetrabenazine, amantadine or valproic acid, or a combination thereof.

The agonists of the invention can also be associated with drugs used for the treatment of diseases involving impaired gastrointestinal motility, obesity. Examples of such drugs include, but are not limited to, mesalazine, immunosuppressants (e.g. prednisone, TNF inhibition, azathioprine, methotrexate and 6-mercaptopurine), proton pump inhibitors (e.g. omeprazole, esomeprazole, pantoprazole, lansoprazole and rabeprazole), mosapride or laxatives, or a combination thereof.

The agonists of the invention can be associated with one or several hormones used to treat Kallman syndrome and normosmic hypogonadotropic hypogonadism such as human chorionic gonadotropin (hCG), testosterone, estrogen or progestins, or a combination thereof.

The agonists of the invention can also be associated with orlistat used to treat obesity.

The agonist of the invention can be associated with one or several drugs used to treat cardiac diseases and/or renal diseases, with one or several drugs used to treat or to prevent insulin resistance, or with one or several drugs used to treat or to prevent type II diabetes, obesity or hypertension.

In a particular embodiment, the PKR1 agonist is associated with one or several drugs used to treat or prevent type II diabetes. Examples of such drugs include, but are not limited to, insulin; sulfonylureas such as Carbutamide, Acetohexamide, Chlorpropamide, Tolbutamide, Tolazamide, Glipizide, Gliclazide, Glibenclamide, Gliquidone, Glycleypramide or Glimepiride; nonsulfonylurea secretagogues such as repaglinide or nateglinide; alpha glucosidase inhibitors such as miglitol or acarbose; and thiazolidinediones.
The other active compound associated with the PKRI agonist of the invention may also be a drug having cardiotoxic and/or neurotoxic side effects.

In a particular embodiment, the pharmaceutical composition according to the invention comprises at least one PKRI agonist of the invention and an antiviral agent, in particular an antiviral agent having cardiotoxic and/or neurotoxic side effects. The antiviral agent may be selected from the group consisting of D4T (2'-3'-didehydro-2'-3' dideoxythymidine or Stavudine), ddl (2'3'-dideoxyinosine or Didanosine) and ddC (2'-3'-dideoxycytidine or Zalcitabine).

In another particular embodiment, the pharmaceutical composition according to the invention comprises at least one PKRI agonist of the invention and an antineoplastic agent, in particular an antineoplastic agent having cardiotoxic and/or neurotoxic side effects. As used herein, the term "antineoplastic agent" refers to an agent with anti-cancer activity that inhibits or halts the growth of cancerous cells or immature pre-cancerous cells, kills cancerous cells or immature pre-cancerous cells, increases the susceptibility of cancerous or pre-cancerous cells to other antineoplastic agents, and/or inhibits metastasis of cancerous cells. These agents may include chemical agents as well as biological agents. The antineoplastic agent may be selected from the group consisting of antineoplastic agents belonging to platinum, taxane, anthracycline and vinca alkaloid families. In particular, the antineoplastic agent may be selected from the group consisting of cisplatin, carboplatin, oxaplatin, picoplatin, tetraplatin, satraplatin, paclitaxel, docetaxel, doxorubicin, daunorubicin, idarubicin, detorubicin, carminomycin, epirubicin, morpholinodoxorubicin, morpholinodaunorubicin, methoxymorpholinyldoxorubicin, vinblastine, vincristine, vindesine, vinorelbine, cyclophosphamide, fluorouracil, rituximab, arsenic trioxide, trastuzumab, thalidomide, etoposide, pentastatin, cytarabine, interferons, busulfan and derivatives and combinations thereof.

The PKRI agonists of the invention can also be associated with other neuroprotective and/or cardioprotective agents.

The present invention also concerns a product containing a PKRI agonist of the invention and an antineoplastic agent, as a combined preparation for simultaneous, separate or sequential use in the treatment of cancer.

Uses of PKRI agonists and pharmaceutical compositions comprising thereof

Previous studies have demonstrated that:
- Prokineticin-2 prevents the apoptotic death of cardiomyocytes induced by oxidative stress and promotes cardiomyocyte survival and angiogenesis (Urayama et al., 2007);
- Prokineticins have a role in appetite regulation. In particular, prokineticin 2 has anorectic effect and can thus potently inhibit food intake (Gardiner et al., 2010);
- Prokineticins potently and specifically stimulate the contraction of gastrointestinal smooth muscle through the activation of the prokineticin receptor (Li et al., 2001);
- Prokineticin-2 supports neuronal survival and protects neurons against excitotoxic death, and is involved in postnatal and adult olfactory bulb neurogenesis (Melchiorri et al., 2001; Ng et al., 2005);
- The administration of prokineticin-2 in adult rat brain results in increased neurogenesis (patent US 7,323,334);
- Prokineticins and their receptors are associated with Kallmann syndrome and normosmic hypogonadotrophic hypogonadism (Hardelin and Dode, 2008; Abreu et al., 2010);
- Prokineticins and their receptors are associated with disturbances of circadian rhythm (Cheng et al., 2002).

Furthermore, the inventors have herein demonstrated that prokineticin-2 via PKRI plays a role as an anti-adipogenic determinant regulating adipocyte proliferation and differentiation.

Consequently, the present invention concerns a pharmaceutical composition or a PKRI agonist as described above for use for the prevention or treatment of a disease or a disorder selected from the group consisting of a vascular disease, a neurodegenerative disease, a disease involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotrophic hypogonadism, hyperthyroidism, in particular Familial isolated hyperparathyroidism (FIHP), disturbances of circadian rhythm, sleeping disorders and a pregnancy and placental function-related disorder. Preferably, the disease or disorder is selected from the group consisting of a vascular disease, a neurodegenerative disease, a disease involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotrophic hypogonadism, and disturbances of circadian rhythm.

As used herein, the term "prevention or treatment of a disease" or "prevention or treatment of a disorder" or "to prevent or to treat a disease or "to prevent or to treat a disorder" refers to any act intended to ameliorate the health status of patients such as
therapy, prevention, prophylaxis and retardation of the disease or the disorder. In certain embodiments, such term refers to the amelioration or eradication of the disease or the disorder, or symptoms associated with said disease or disorder. In other embodiments, this term refers to minimizing the spread or worsening of the disease or disorder resulting from the administration of one or more therapeutic agents to a subject with said disease or disorder.

As used herein, the term "patient" or "subject" refers to any mammal, preferably a human being.

The cardiovascular disease may be selected from the group consisting of myocardial infarction, acute coronary syndrome, ischemic stroke, abdominal aorta aneurysm, cerebral aneurysm, ischemic heart disease, coronary heart disease, peripheral arterial disease, restenosis, angina pectoris, diabetic angiopathy and diabetes-mediated cardiovascular complications.

The neurodegenerative disease may be selected from the group consisting of Parkinson's disease and other parkinsonian disorders, Alzheimer's disease and other dementing neurodegenerative disorders, amyotrophic lateral sclerosis, multiple sclerosis, Creutzfeldt-Jakob disease, Huntington's disease and neuronal degeneration associated to multiple sclerosis.

The disease involving impaired gastrointestinal motility may be selected from the group consisting of irritable bowel syndrome, diabetic gastroparesis, postoperational ileus, chronic constipation, and gastroesophageal reflux disease, Hirschsprung's disease (congenital colonic aganglionosis) and chronic dyspepsia.

The pregnancy and placental function-related disorder may be selected from the group consisting of placental dysfunction, preeclampsia, fetal inflammatory response syndrome and antiphospholipid syndrome.

The present invention also concerns a pharmaceutical composition or a PKR1 agonist as described above for use for the prevention or treatment of a disease associated with insufficient prokineticin receptor activity.

The present invention also concerns the use of a PKR1 agonist of the invention for preparing a medicament for preventing or treating a disease or a disorder selected from the group consisting of a vascular disease, a neurodegenerative disease, a disease involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotropic hypogonadism, hyperthyroidism, in particular Familial isolated
hyperparathyroidism (FIHP), disturbances of circadian rhythm, sleeping disorders and a pregnancy and placental function-related disorder.

In another aspect, the present invention further concerns a method for preventing or treating a disease or a disorder in a subject, wherein said disease or disorder is selected from the group consisting of a vascular disease, a neurodegenerative disease, a disease involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotrophic hypogonadism, hyperthyroidism, in particular Familial isolated hyperparathyroidism (FIHP), disturbances of circadian rhythm, sleeping disorders and a pregnancy and placental function-related disorder, and comprising administering a therapeutically active amount of a PKRI agonist according to the invention to said subject. By a "therapeutically effective amount" is intended an amount of PKRI agonist of the invention administered to a patient that is sufficient to provide a therapeutic effect. It is to be understood that the total amount of the PKRI agonist of the invention may vary depending on the volume of blood plasma of the patient. Suitable means and measures to determine the therapeutically effective amount are available to the person skilled in the art. In a particular embodiment, the method of the invention for preventing or treating a disease or a disorder in a subject, wherein said disease or disorder is selected from the group consisting of a vascular disease, a neurodegenerative disease, a disease involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotrophic hypogonadism, hyperthyroidism, in particular Familial isolated hyperparathyroidism (FIHP), disturbances of circadian rhythm, sleeping disorders and a pregnancy and placental function-related disorder, comprises administering 0.2 to 30 mg / kg of body weight / day of a PKRI agonist of the invention to said subject. All embodiments as disclosed above for a pharmaceutical composition or a PKRI agonist of the invention for use for the prevention or treatment of a disease or a disorder selected from the group consisting of a vascular disease, a neurodegenerative disease, a disease involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotrophic hypogonadism, hyperthyroidism, in particular Familial isolated hyperparathyroidism (FIHP), disturbances of circadian rhythm, sleeping disorders and a pregnancy and placental function-related disorder, are also encompassed in this aspect.

Due to their cardioprotective and neuroprotective activities, the PKRI agonists of the invention and the pharmaceutical compositions comprising thereof, may be used to prevent or to limit the neurotoxicity and/or cardiotoxicity of a compound, in particular of an
antiviral or antineoplastic agent. The antiviral agent may be selected from the group consisting of D4T (2'-3’-didehydro-2'-3’-dideoxythymidine or Stavudine), ddl (2’3’-dideoxyinosine or Didanosine) and ddC (2’-3’-dideoxycytidine or Zalcitabine). The antineoplastic agent may be selected from the group consisting of antineoplastic agents belonging to platinum, taxane, anthracycline and vinca alkaloid families. In particular, the antineoplastic agent may be selected from the group consisting of cisplatin, carboplatin, oxaplatin, picoplatin, tetraplatin, satraplatin, paclitaxel, docetaxel, doxorubicin, daunorubicin, idarubicin, detorubicin, carminomycin, epirubicin, morpholinodoxorubicin, morpholinodaunorubicin, methoxymorpholinyl doxorubicin, vinblastine, vincristine, vindesine, vinorelbine, cyclophosphamide, fluorouracil, rituximab, arsenic trioxide, trastuzumab, thalidomide, etoposide, pentastatin, cytarabine, interferons, busulfan and derivatives and combinations thereof.

The present invention also concerns the use of a PKR1 agonist of the invention for preparing a medicament for preventing or limiting the cardiotoxicity and/or neurotoxicity of a compound, in particular the cardiotoxicity and/or neurotoxicity of an antiviral or antineoplastic agent.

In another aspect, the present invention further concerns a method for preventing or limiting the cardiotoxicity and/or neurotoxicity of a compound in a subject, in particular the cardiotoxicity and/or neurotoxicity of an antiviral or antineoplastic agent, comprising administering a therapeutically active amount of a PKR1 agonist according to the invention. In this aspect, by a "therapeutically effective amount" is intended an amount of PKR1 agonist of the invention administered to a patient that is sufficient to prevent the cardiomyocyte and/or neuronal cell death. It is to be understood that the total amount of the PKR1 agonist of the invention may vary depending on the volume of blood plasma of the patient. Suitable means and measures to determine the therapeutically effective amount are available to the person skilled in the art. In a particular embodiment, the method of the invention for preventing or limiting the cardiotoxicity and/or neurotoxicity of a compound in a subject comprises administering 0.2 to 30 mg / kg of body weight / day of a PKR1 agonist of the invention to said subject.

30 Effects of PKR1 agonists on epicardin positive progenitor cells

Cardiovascular diseases are a major health risk throughout the industrialized world. Among these diseases, myocardial infarction, commonly known as a heart attack, is one of
the most well-known types and is the most common cause of ischemia in heart. Myocardial infarction is caused by a sudden and sustained lack of blood flow to an area of the heart, commonly caused by narrowing of a coronary artery. Without adequate blood supply, the tissue becomes ischemic, leading to the death of myocytes and vascular structures. This area of necrotic tissue is referred to as the infarct site, and will eventually become scar tissue. Survival is dependent on the size of this infarct site, with the probability of recovery decreasing with increasing infarct size.

Current treatments for myocardial infarction focus on reperfusion therapy, which attempts to start the flow of blood to the affected area to prevent the further loss of tissue. These treatments may succeed in reestablishing the blood supply, however tissue damages that occurred before the reperfusion treatment began has been thought to be irreversible and organ transplantation may be needed to replace nonfunctional tissue.

Significant effort has been invested in stem-cell-based therapies and multipotent progenitor cells have been recently identified in the heart. Among these cells, epicardially derived cells (EPDC) are characterized as the stem cell-derived multipotential vascular progenitors (Wessels et al., 2004). EPDCs are positive for the epicardial-specific transcription factor, epicardin. They can either form endothelial cells, in response to a combination of myocardial vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) signaling, or differentiate into smooth muscle cells, on exposure to platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), and bone morphogenetic protein-2 (BMP-2) (Kruithof et al., 2006). Thymosin β4 and prokineticin-2 were identified as signaling factors for adult EPDC mobilization and differentiation into endothelial and smooth muscle cells promoting neovasculogenesis (Smart et al., 2007; Urayama et al., 2008).

However, there is still a strong need for improvement of stem-cell-based therapies for heart diseases, and in particular for a method for promoting cardiomyocyte regeneration.

The inventors have herein demonstrated that prokineticin receptor 1 agonists, such as prokineticin-2, promote the differentiation of cardiac epicardin positive progenitor cells (cEPPC) into cardiomyocytes and, in the same time, block the differentiation of these cells into adipocytes. The inventors have also provided evidence that epicardin positive progenitor cells are present in kidney (rEPPC) and that prokineticin receptor 1 agonists, in particular prokineticin-2, induce the differentiation of these renal epicardin positive
progenitor cells (rEPPC) into endothelial and vascular smooth muscle cells, thereby promoting renal angiogenesis.

In an aspect, the present invention concerns a prokineticin receptor-1 agonist for use for promoting the differentiation of cardiac epicardin+ progenitor cells (cEPPC) into cardiomyocytes, in particular in a subject affected with a cardiac disease, and/or the differentiation of renal epicardin+ progenitor cells (rEPPC) into vasculogenic cells and/or glomerular cells, in particular in a subject affected with a renal disease.

As used herein, the term "epicardin positive progenitor cells" or "epicardin+ progenitor cells" refers to progenitor cells expressing the transcription factor epicardin, also named capsulin, PODI or TCF-21. Progenitor cells are undifferentiated cells with a limited capacity of self-renewal via a limited number of cell divisions. As used herein, the term "progenitor cells" refers to multipotent cells that can differentiate into only few cell types. In particular, cardiac epicardin+ progenitor cells have the ability to differentiate into cardiomyocytes, adipocytes and vasculogenic cells. Renal epicardin+ progenitor cells (rEPPC) have the ability to differentiate into glomerular cells, adipocytes and vasculogenic cells. The expression of epicardin may be assessed by any method known by the skilled person such as immunochemistry using an anti-epicardin antibody or quantitative RT-PCR. Cardiac and renal epicardin+ cells can be isolated from cardiac or renal tissue by any method known by the skilled person. For example, they can be isolated by using affinity binding with an anti-epicardin antibody, as illustrated in the experimental section. It has to be understood that progenitor cells are not embryonic stem cells, and in particular human embryonic stem cells.

As used herein, the term "patient" or "subject" refers to any mammal, preferably a human being.

The cardiac disease may be any cardiac disease inducing cardiomyocyte lesion, injury, death, necrosis or apoptosis. In a particular embodiment, the cardiac disease is selected from the group consisting of heart failure, myocardial infarction, ischemic heart disease, the cardiorenal syndrome and cardiovascular complications of diabetes. In a more particular embodiment, the cardiac disease is myocardial infarction or ischemic heart disease, preferably myocardial infarction.

The renal disease may be a renal disease inducing lesion, injury, death, necrosis or apoptosis of glomerular cells and/or an ischemic renal disease. In a particular embodiment, the renal disease is selected from the group consisting of chronic renal disease, renal artery
stenosis, renal failure, acute kidney injury, acute-on-chronic renal failure, ischemic nephropathy, Churg-Strauss syndrome, Wegener's granulomatosis, the cardiorenal syndrome and renal complications of diabetes. In a more particular embodiment, the renal disease is acute kidney injury or ischemic nephropathy.

In a preferred embodiment, the subject is affected with a cardiorenal syndrome. This syndrome is a condition characterized by kidney failure and heart failure.

In this aspect, the PKR1 agonist may be a peptide or a non peptide PKR1 agonist. Preferably, the PKR1 agonist is a non peptide PKR1 agonist, more preferably, a non peptide PKR1 agonist of formula (I) as disclosed above. All the embodiments of the non peptide PKR1 agonist as described above are also contemplated in this use.

In an embodiment, the PKR1 agonist is selected from the group consisting of prokineticin-2 (PK2) and prokineticin-1 (PK1), and an active fragment thereof, i.e. a fragment that is able to bind and activate PKR1. PK2 and PK1 may be used in their precursor or mature form. The NCBI accession number for the sequence of the human PK1 precursor is NP_115790.1. The NCBI accession number for the sequence of the human PK2 precursor is NP_001119600.1. In a particular embodiment, the PKR1 agonist is an active fragment of PK2. This fragment may be a peptide comprising 47 amino acids of the N-terminus of PK2 as described in the SEQ ID NO.: 1 and 2 of the international patent application WO 2005/097826.

In another embodiment, the PKR1 agonist is an antibody or a fragment thereof. As used herein, the term "antibody" is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE, and humanized or chimeric antibody. In certain embodiments, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and they are most easily manufactured. The term "a fragment thereof" is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')2, single domain antibodies (DABs), Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means for preparing and characterizing antibodies are also well known in the art (See, e.g., Harlow and Lane, 1988). A "humanized" antibody is an antibody in which the constant and variable framework region of one or more human immunoglobulins is fused with the binding region, e.g. the CDR, of an animal immunoglobulin. "Humanized" antibodies contemplated in the present invention are chimeric antibodies from mouse, rat, or other
species, bearing human constant and/or variable region domains, bispecific antibodies, recombinant and engineered antibodies and fragments thereof. Such humanized antibodies are designed to maintain the binding specificity of the non-human antibody from which the binding regions are derived, but to avoid an immune reaction against the non-human antibody. A "chimeric" antibody is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. The antibody may be, for example, an anti-mouse Bv8 monoclonal antibody (Genentech).

The peptidic agonist may comprise modifications inducing an improved resistance to proteolysis such as a -CONH- amide peptide bond replaced by a (CH₂-NH) reduced bond, a (NH-CO) retro-inverso bond, a (CH₂-0) methylene-oxo bond, a (CH₂-S) thiomethylene bond, a (CH₂-CH₂) carba bond, a (CO-CH₂) ceto-methylene bond, a (CHOH-CH₂) hydroxyethylene bond, a (N-N) bond, a E-alcene bond or a -CH=CH- bond. The peptidic agonist can also present either carboxylic (i.e. -COO⁻) or carboxyl-amidated (i.e. -CONH₂) C-terminal extremity.

In embodiments wherein the PKR1 agonist is a peptidic compound, the agonist may be administered to the subject in the form of a nucleic acid sequence encoding said agonist. The sequence encoding the peptidic agonist may be obtained by cloning, by PCR amplification or by chemical synthesis according to the conventional techniques in common use and using the literature data. The nucleic acid may be inserted in a recombinant vector allowing its expression in eukaryote cells. The structure and composition of such vectors is well-known by the skilled person. The expression vector may be a plasmid or a viral vector. In particular, it may be derived from a lentivirus, a poxvirus, an adenovirus, a retrovirus, a herpesvirus or an adenovirus-associated virus. In this vector, the encoding sequence is placed under the control of elements necessary for its expression in a eucaryote host cell or organim, and in particular in human. These elements include elements regulating transcription as well as signals for initiation and termination of translation. For instance, the promoter may be selected from the group consisting of SV40 (Simian Virus 40) promoter, the HMG (Hydroxy-Methyl-Glutaryl-coenzyme A) promoter,
the TK (Thymidine Kinase) promoter, the CMV (cytomegalovirus) promoter, the RSV (Rous Sarcoma Virus) promoter and the MLP promoter (Major Late Promoter).

The PKRl agonist may be used alone, in combination with one or several other PKRl agonists and in combination with other active substances, such as drugs used to treat cardiac diseases and/or renal diseases.

The present invention also concerns the use of a PKRl agonist for preparing a medicament for promoting the differentiation of cardial epicardin+ progenitor cells into cardiomyocytes in a subject affected with a cardiac disease and/or the differentiation of renal epicardin+ progenitor cells into vasculogenic and/or glomerular cells in a subject affected with a renal disease. The PKRl agonist may be any PKRl agonist as described above.

The present invention also concerns a method for promoting the differentiation of cardial epicardin+ progenitor cells into cardiomyocytes in a subject affected with a cardiac disease and/or the differentiation of renal epicardin+ progenitor cells into vasculogenic and/or glomerular cells in a subject affected with a renal disease, comprising administering a therapeutically active amount of a PKRl agonist to said subject. By a "therapeutically effective amount" is intended an amount of PKRl agonist administered to a patient that is sufficient to provide a therapeutic effect, i.e. that is sufficient to promote the differentiation of epicardin+ progenitor cells into cardiomyocytes and/or renal cells. In a particular embodiment, the therapeutically effective amount is 0.2 to 30 mg / kg of body weight / day. It is to be understood that the total amount of the PKRl agonist may vary depending on the volume of blood plasma of the patient. Suitable means and measures to determine the therapeutically effective amount are available to the person skilled in the art. The PKRl agonist may be any PKRl agonist as described above.

The present invention also concerns a PKRl agonist for use for stimulating the regeneration of cardiomyocytes in a subject having an ischemic heart disease. The regeneration of cardiomyocytes is obtained from the differentiation of cardial epicardin+ progenitor cells. In an ischemic heart disease, and in particular in cardiac infarction, the lack of oxygen caused by obstruction of the tissue's blood supply induces necrosis of cardiomyocytes leading to irreversible injuries. The stimulation of the regeneration of cardiomyocytes allows limiting or preventing these injuries and thus limiting infarction sequelae. The PKRl agonist may be any PKRl agonist as described above.
The present invention further concerns a PKRl agonist for promoting renal angiogenesis, in particular intra- and extra-glomerular angiogenesis. The renal angiogenesis is obtained from the differentiation of renal epicardin+ progenitor cells into renal vasculogenic cells. Promoting renal angiogenesis is particularly important in 5 ischemic renal diseases such as renal artery stenosis which induces intrarenal microvascular and tissue remodeling that may lead to irreversible injury and progressive deterioration of renal function. The PKRl agonist may be any PKRl agonist as described above.

The present invention further concerns a PKRl agonist for use for the prevention or 10 the treatment of a renal disease. The renal disease is as defined above. The PKRl agonist may be any PKRl agonist as described above.

The inventors have herein demonstrated that PKRl agonists promote the differentiation of cardiac epicardin positive progenitor cells (cEPPC) into cardiomyocytes and, in the same time, block the differentiation of these cells into adipocytes. Accordingly, 15 the present invention concerns a PKRl agonist for use for preventing fat tissue development in heart and/or kidney in a subject, in particular a subject affected with obesity or being susceptible to develop obesity. Obesity is a disease due to excess body fat leading to reduced life expectancy and increased health problems. As used herein, the term "subject affected with obesity" or "obese subject" refers to a subject having a Body Mass 20 Index (BMI) that is greater than 30 kg/m², preferably 35 kg/m², more preferably 40 kg/m². The PKRl agonist may be any PKRl agonist as described above.

The present invention also concerns a PKRl agonist for use for preventing epicardial fat tissue development in heart in a subject affected with or being susceptible to a cardiac disease. In a particular embodiment, the subject affected with or being susceptible to a 25 cardiac disease is an obese subject. The present invention also concerns a PKRl agonist for use for preventing fat tissue development in kidney in a subject affected with or being susceptible to a renal disease. In a particular embodiment, the subject affected with or being susceptible to a renal disease is an obese subject.

The present invention further concerns an in vitro or ex vivo method of producing 30 cardiomyocytes, wherein said method comprises the step of contacting cardial epicardin+ progenitor cells with a PKRl agonist. The present invention also concerns an in vitro or ex vivo method of producing glomerular cells, wherein said method comprises the step of contacting renal epicardin+ progenitor cells with a PKRl agonist.
The culture medium which may be used during the step of contacting epicardin+ progenitor cells with a PKR1 agonist is designed to support the growth and the differentiation of these cells. This medium generally is changed every day and comprises a carbon source, a nitrogen source, antibiotics to prevent fungi and bacteria growth, a buffer to maintain pH and specific growth factors. This medium may be easily designed by the skilled person in the art. In an embodiment, the culture medium comprises compounds for stimulating cell differentiation selected from the group consisting of dexamethasone, ascorbic acid (vitamin C) and glycerophosphate, and combinations thereof. In a preferred embodiment, the culture medium comprises dexamethasone, ascorbic acid and glycerophosphate. The PKR1 agonist may be any PKR1 agonist as described above.

A pharmaceutical composition comprising cardiomyocytes and/or glomerular cells obtained with the method of the invention may be administered to a subject in need thereof, i.e. in a subject affected with a cardiac and/or renal disease. Cells may be administered with a pharmacologically acceptable carrier that is compatible with the cells and may be, for instance, cell culture medium (such as Eagle's minimal essential media), phosphate buffered saline, Krebs-Ringer buffer, and Hank's balanced salt solution with glucose (HBSS). The pharmaceutical composition comprising cells is formulated in accordance with standard pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York) known by a person skilled in the art. In a preferred embodiment, pharmaceutical composition is suitable for parenteral administration. In a particular embodiment, the pharmaceutical composition comprises cells obtained by the method of the invention encapsulated in a biocompatible matrix known in the art. A variety of encapsulation technologies have been developed (e.g. Qi et al., 2008 and WO 91/10425).

The present invention also concerns a method of treating cardiac disease in a subject in need thereof, said method comprising steps consisting of:

- obtaining cardiac epicardin+ progenitor cells;
- contacting said epicardin+ progenitor cells with a PKR1 agonist for promoting the differentiation of said cells into cardiomyocytes;
- transplanting a therapeutically effective amount of cardiomyocytes obtained by differentiation of said epicardin+ progenitor cells into said subject.
The present invention further concerns a method of treating renal disease in a subject in need thereof, said method comprising steps consisting of
- obtaining renal epicardin+ progenitor cells;
- contacting said epicardin+ progenitor cells with a PKR1 agonist for promoting the differentiation of said cells into glomerular cells;
- transplanting a therapeutically effective amount of glomerular cells obtained by differentiation of said epicardin+ progenitor cells into said subject.

The PKR agonist may be any PKR agonist as described above.

In a preferred embodiment, cardiomyocytes or glomerular cells are administered by injection. Preferably, these cells are injected directed into the heart or into the kidney.

According to the origin of epicardin+ progenitor cells, the transplantation may be autologous, isogeneic, allogeneic or xenogeneic. As used below, the "donor" is the donor of epicardin+ progenitor cells and the "recipient" is the subject who receives the transplantation. The transplantation may be isogeneic, i.e. the donor and recipient are genetically identical; allogeneic, i.e. the donor and recipient are of the same species; xenogeneic, i.e. the donor and recipient are of different species; or autologous, i.e. the donor and recipient are the same subject. Allogeneic and xenogeneic transplantations may require the administration of antirejection drugs. For isogeneic, allogeneic and xenogeneic transplantations, the donor may be alive or deceased. In a preferred embodiment, the transplantation is autologous.

The present invention concerns a PKR agonist for use for preventing or limiting the nephrotoxicity of a compound, in particular of a drug. The present invention also concerns the use of a PKR agonist for preparing a medicament for preventing or limiting the nephrotoxicity of a compound, in particular of a drug. The present invention further concerns a method for preventing or limiting the nephrotoxicity of a compound in a subject, in particular the nephrotoxicity of a drug, comprising administering a therapeutically active amount of a PKR agonist to said subject. In this aspect, by a "therapeutically effective amount" is intended an amount of PKR agonist administered to a subject that is sufficient to limit or prevent an alteration of the renal function. For example, the nephrotoxic compound may be selected from the group consisting of aminoglycoside antibiotics such as amikacin, arbekacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, rhodostreptomycin, streptomycin, tobramycin, and apramycin; diuretics; angiotensin-converting enzyme inhibitors such as Captopril;
cyclosporin A; FK-506; Amphotericin B; Cisplatin; Nonsteroidal anti-inflammatory drugs such as aspirin, ibuprofen; antibacterial sulfonamides; and antineoplastic agents, in particular anthracyclines. The PKR1 agonist may be any PKR1 agonist as described above.

**Effects of PKR1 agonists on insulin resistance**

Insulin resistance is a physiological condition where tissues such as muscle, liver and fat in the body respond poorly to insulin. As a result, the ability of insulin to decrease blood glucose levels is reduced. This leads to compensatory hyperinsulinemia. However, when this compensatory increase is insufficient or does not occur, blood glucose concentrations increase and type II diabetes occurs. Insulin resistance can also cause or contribute to other metabolic disorders such as hypertension, obesity and atherosclerosis.

Commonly, the primary treatment for insulin resistance is exercise and weight loss to reduce risk of complications, in particular of diabetes. Medications that reduce insulin resistance include, for example, metformin and the thiazolidinediones. However, the use of these drugs is not recommended for patients with isolated insulin resistance. Then, there is still a strong need for improvement of treatment of insulin resistance, associated or not with other metabolic disorders, such as type II diabetes.

The inventors have herein demonstrated that endothelial prokineticin receptor 1 is a positive regulator of insulin sensitivity.

The present invention thus also concerns a prokineticin receptor-1 agonist for use for treating or preventing insulin resistance in a subject.

In this aspect, the PKR1 agonist may be any peptide or non peptide PKR1 agonist as described above. Preferably, the PKR1 agonist is a non peptide PKR1 agonist, more preferably, a non peptide PKR1 agonist of formula (I) as disclosed above. All the embodiments of the non peptide PKR1 agonist as described above are also contemplated in this aspect.

As used herein, the term "insulin resistance" refers to a physiological condition where fat and/or muscle cells fail to respond adequately to circulating insulin. This resistance leads to an increase of blood glucose level. The insulin resistance can be detected in a subject by any method known by the skilled person such as glucose tolerance test (GTT) or insulin tolerance test (ITT).

The insulin resistance may be associated with one or several other disorders such as type II diabetes, obesity, hypertension, cardiac disorder or renal disorder.
In an embodiment, the insulin resistance is associated with type II diabetes. In another embodiment, the insulin resistance is associated with obesity. In a further embodiment, the insulin resistance is associated with hypertension. In another embodiment, the insulin resistance is associated with a cardiac and/or renal disorder.

5 The present invention thus also concerns a prokineticin receptor-1 agonist for use for treating or preventing type II diabetes in a subject. As used herein, the term "patient" or "subject" refers to any mammal, preferably a human being.

In an embodiment, the PKR1 agonist is used in combination with one or several drugs used to treat or to prevent insulin resistance.

10 In another embodiment, the PKR1 agonist is used in combination with one or several drugs used to treat or to prevent type II diabetes, obesity or hypertension.

In a particular embodiment, the PKR1 agonist is used in combination with one or several drugs used to treat or prevent type II diabetes. Examples of such drugs include, but are not limited to, insulin; sulfonylureas such as Carbutamide, Acetohexamide, Chlorpropamide, Tolbutamide, Tolazamide, Glipizide, Gliclazide, Glibenclamide, Gliquidone, Glyclopyramide or Glimepiride; nonsulfonylurea secretagogues such as repaglinide or nateglinide; alpha glucosidase inhibitors such as miglitol or acarbose; and thiazolidinediones.

The present invention also concerns the use of a PKR1 agonist for preparing a medicament for treating or preventing insulin resistance in a subject. The present invention also concerns a method for treating or preventing insulin resistance in a subject, said method comprising the step consisting of administering a therapeutically effective amount of a PKR1 agonist to the subject in need thereof. In this aspect, by a "therapeutically effective amount" is intended an amount of PKR1 agonist administered to a subject that is sufficient to increase insulin sensitivity. The PKR1 agonist may be any PKR1 agonist as described above.

The present invention also concerns the use of a PKR1 agonist for preparing a medicament for treating or preventing type II diabetes. The present invention also concerns a method for treating or preventing type II diabetes in a subject, said method comprising the step consisting of administering a therapeutically effective amount of a PKR1 agonist to the subject in need thereof. In this aspect, by a "therapeutically effective amount" is intended an amount of PKR1 agonist administered to a subject that is sufficient to decrease
blood glucose level by increasing insulin sensitivity. The PKR1 agonist may be any PKR1 agonist as described above.

The following examples are given for purposes of illustration and not by way of limitation.

5 Examples

**Example 1: Non peptide PKR1 agonists**

*Synthesis and NMR characterization of PKR1 agonists*

IS compounds (Figures 10 and 25) were synthesized according to a general method originally developed by Erlenmeyer and Plochl (Erlenmeyer, 1893; Plochl, 1884). Condensation of acylated glycine 1 with an aldehyde 2 in the presence of acetic anhydride afforded the azlactone 3, which was condensed to an amine to afford 4 (Figure 11).

**IS1 to IS3, IS6 to IS12, IS14 to IS21, IS26 and IS34**

An amine (4 mmol) and an azlactone 3 (4 mmol) in suspension in CHiCl/ EtOH (8 mL, 1/1) was sonicated for 10 min. After stirring overnight at room temperature, the solvent was removed under vacuum, and the residue was purified by flash chromatography (EtOAc to EtOAc/EtOH, 80/20) to give the expected product a white solid (purity > 97%).

**IS1**

\[
\begin{align*}
\text{H NMR (400 MHz, DMSO-d}_6\text{): } & 9.77 (s, 1H), 8.68 (m, 1H), 8.58 (s, 1H), 8.47 (d, J = 4.6 Hz, 1H), 8.13 (m, 2H), 7.77 (d, J = 7.8 Hz, 1H), 7.62 (m, 2H), 7.55 (m, 2H), 7.33 (m, 2H), 6.69 (d, J = 3.4 Hz, 1H), 6.52 (m, 1H), 4.49 (d, J = 6.0 Hz, 2H); \\
\text{RMN } & 1^3\text{C (100 MHz, DMSO): 40.3, 111.7, 113.5, 117.7, 122.8, 126.8, 127.7, 127.8, 131.2, 133.6, 134.7, 143.6, 147.5, 148.5, 149.6, 164.4, 165.7 ppm. }
\end{align*}
\]

**IS2**

\[
\begin{align*}
\text{H NMR (400 MHz, CDCl}_3\text{): } & 8.57 (s, 1H), 8.42 (d, J = 4.9 Hz, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.61 (m, 2H), 7.53 (d, J = 7.8 Hz, 1H), 7.43 (m, 3H), 7.32 (d, J = 7.8 Hz, 1H), 7.11 (m, 1H), 6.94 (s, 1H), 6.49 (d, J = 3.5, 1H), 6.41 (dd, J = 1.9, 3.6 Hz, 1H), 4.33 (d, J = 5.3 Hz, 2H); \\
\text{1^3C NMR (100 MHz, }
\end{align*}
\]
IS3

$^1$H NMR (300 MHz, CDC$_3$:): 8.59 (s, 1H), 8.49 (m, 2H), 7.93 (m, 2H), 7.56 (m, 3H), 7.47 (m, 3H), 7.25 (s, 1H), 7.12 (m, IH), 6.94 (s, IH), 6.52 (d, $J = 3.4$ Hz, IH), 6.44 (m, IH), 4.53 (d, $J = 6.2$ Hz, 2H); $^1$C NMR (100 MHz, DMSO-d$_6$): 165.9, 164.7, 149.5, 148.9, 148.1, 143.5, 133.3, 131.2, 127.6, 127.5, 126.8, 121.7, 117.3, 113.6, 111.6, 41.7. LC-MS (ESI): calculated: 347.1; found: 348.2 (M+H$^+$).

IS6

$^1$H NMR (400 MHz, CDC$_3$:): 8.75 (s, 1H), 7.87 (m, 3H), 7.58 (m, IH), 7.48 (t, $J = 7.4$ Hz, IH), 7.38 (m, 3H), 7.24 (m, IH), 6.69 (m, 4H), 6.38 (m, 4H), 5.29 (m, IH), 3.46 (m, 4H); $^1$C NMR (100 MHz, CDC$_3$:): 166.4, 165.8, 150.4, 147.6, 143.9, 137.3, 133.3, 132.4, 128.8, 127.8, 127.6, 114.2, 113.3, 112.8, 112.2, 108.6, 41.1, 41.0. LC-MS (ESI): calculated: 376.2; found: 377.2 (M+H$^+$).

IS8

$^1$H NMR (400 MHz, CDC$_3$:): 8.46 (s, 2H), 8.41 (m, IH), 7.91 (m, 2H), 7.51 (m, 5H), 7.15 (m, IH), 6.83 (s, IH), 6.48 (m, 3H), 3.60 (q, $J = 6.7$ Hz, 2H), 2.91 (q, $J = 7$ Hz, 2H); $^1$C NMR (100 MHz, CDC$_3$:): 166.5, 165.2, 150.4, 150.3, 148.0, 144.1, 136.7, 134.8, 133.3, 132.6, 129.0, 127.8, 127.7, 123.7, 114.6, 113.6, 112.3, 41.1, 33.0. LC-MS (ESI): calculated: 361.1; found: 362.2 (M+H$^+$).

IS9

RMN $^1$H (400 MHz, CDC$_3$:): 8.041 (s, IH), 8.18 (d, $J = 4.3$ Hz, 1H), 7.90 (d, $J = 7.4$ Hz, 2H), 7.51 (m, 5 H), 7.40 (d, $J = 1.8$ Hz, 1H), 7.14 (d, $J = 7.8$ Hz, 1H), 6.99 (ddd, $J = 0.9, 5.0, 7.5$ Hz, 1H), 6.92 (s, 1H), 6.48 (d, $J = 3.4$ Hz, 1 H), 6.41 (ddd, $J = 1.8, 3.5$ Hz, IH), 3.73 (dd, $J = 5.9, 12.4$ Hz, 2H), 2.99 (t, $J = 6.3$Hz, 2H); $^1$C NMR (100 MHz, CDC$_3$:): 166.3, 164.7, 160.0, 150.6, 149.1, 144.0, 136.8, 133.7, 132.4, 128.9, 127.8, 127.6, 123.8, 121.6, 114.4, 114.0, 112.3, 39.4, 36.8.
LC-MS (ESI): calculated: 361.1; found: 362.2 (M+H+).

**IS10**

\(^1\)H NMR (400 MHz, CDCl\(_3\)): 8.41 (s, 1H), 7.93 (d, 7 = 7.7 Hz, 2H), 7.82 (d, 7 = 4.6 Hz, 1H), 7.73 (s, 1H), 7.47 (m, 2H), 7.32 (m, 4H), 7.15 (d, 7 = 3.6 Hz, 1H), 6.99 (m, 1H), 6.44 (m, 1H), 6.32 (d, 7 = 8.4 Hz, 1H), 5.16 (s, 1H), 3.38 (s, 4H);

\(^13\)C NMR (100 MHz, CDCl\(_3\)): 167.2, 165.9, 159.0, 147.6, 137.6, 136.8, 133.2, 132.4, 132.2, 129.5, 128.8, 128.0, 127.4, 126.2, 125.1, 113.0, 108.3, 41.4, 41.3. LC-MS (ESI): calculated: 392.1; found: 393.2 (M+H+)⁺.

**IS11**

\(^1\)H NMR (400 MHz, CDCl\(_3\)): 8.10 (s, 1H), 7.89 (m, 1H), 7.81 (d, 7 = 7.5 Hz, 2H), 7.57 (s, 1H), 7.50 (m, 1H), 7.33 (m, 8H), 6.93 (s, 1H), 6.47 (m, 1H), 6.40 (d, 7 = 8.4 Hz, 1H), 5.06 (t, 7 = 5.4 Hz, 1H), 3.53 (m, 4H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): 167.0, 166.7, 158.9, 147.6, 137.4, 134.1, 133.0, 132.2, 129.9, 129.4, 128.9, 128.8, 128.7, 127.9, 127.5, 112.9, 108.4, 41.2. LC-MS (ESI): calculated: 386.2; found: 387.2 (M+H+)⁺.

**IS12**

\(^1\)H NMR (400 MHz, CDCl\(_3\)): 8.20 (s, 1H), 7.88 (m, 1H), 7.83 (d, 7 = 7.4 Hz, 2H), 7.56 (s, 1H), 7.49 (m, 1H), 7.34 (m, 5H), 6.93 (s, 1H), 6.79 (d, 7 = 8.8 Hz, 2H), 6.46 (m, 1H), 6.38 (d, 7 = 8.4 Hz, 1H), 5.11 (m, 1H), 3.48 (m, 4H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): 167.3, 166.8, 160.0, 158.9, 147.6, 137.4, 133.0, 132.1, 131.2, 128.6, 128.1, 127.9, 127.7, 126.5, 114.1, 112.8, 108.4, 55.4, 41.2, 41.0. LC-MS (ESI): calculated: 416.2; found: 417.2 (M+H+)⁺.

**IS14**

\(^1\)H NMR (400 MHz, CDCl\(_3\)): 8.51 (d, 7 = 1.6 Hz, 1H), 8.46 (dd, 7 = 1.1, 4.6 Hz, 1H), 8.05 (s, 1H), 7.70 (d, 7 = 7.8 Hz, 1H), 7.45 (s, 1H), 7.23 (dd, 7 = 4.8, 7.8 Hz, 1H), 6.90 (t, 7 = 5.3 Hz, 1H), 6.73 (s, 1H), 6.46 (m, 2H), 4.52 (d, 7 = 5.9 Hz, 2H), 1.29 (s, 9H). \(^13\)C NMR (100 MHz, CDCl\(_3\)): 178.2, 165.5, 150.5, 149.2, 148.9, 143.8, 135.8, 134.2, 127.9, 123.8, 114.5, 112.8, 112.4, 41.6, 39.6, 27.6. LC-MS (ESI): calculated: 327.2; found: 328.2 (M+H+)⁺.
**IS 15**

H NMR (400 MHz, CDCl$_3$): 8.65 (s, 1H), 8.41 (s, 1H), 8.35 (dd, $J = 7.5, 4.8$ Hz, 1H), 7.68 (d, $J = 7.7$ Hz, 2H), 7.41 (s, 1H), 7.36 (d, $J = 3.8$ Hz, 2H), 7.31 (m, 1H), 7.17 (dd, $J = 4.8$, 7.8 Hz, 1H), 7.12 (t, $J = 5.9$ Hz, 1H), 7.06 (s, 1H), 6.56 (d, $J = 3.4$ Hz, 1H), 6.42 (dd, $J = 1.9$, 3.4 Hz, 1H), 4.49 (d, $J = 5.8$ Hz, 2H); $^1$C NMR (100 MHz, CDCl$_3$): 166.0, 164.8, 150.0, 149.1, 148.7, 144.5, 135.9, 134.5, 134.0, 132.0, 131.0, 130.4, 130.1, 127.5, 125.5, 123.8, 120.2, 116.6, 115.6, 112.4, 41.6. LC-MS (ESI): calculated: 381.1; found: 382.0 (M+H$^+$).

**IS 16**

$^1$H NMR (400 MHz, DMSO-d$_6$): 9.72 (s, 1H), 8.71 (t, $J = 5.8$ Hz, 1H), 8.53 (s, 1H), 8.43 (d, $J = 4.1$ Hz, 1H), 8.05 (d, $J = 8.5$ Hz, 2H), 7.72 (m, 2H), 7.33 (dd, $J = 4.8$, 7.5 Hz, 1H), 7.19 (s, 1H), 7.06 (d, $J = 8.7$ Hz, 2H), 6.68 (d, $J = 3.3$ Hz, 1H), 6.56 (s, 1H), 4.40 (d, $J = 5.8$ Hz, 2H), 3.84 (s, 3H). $^1$C NMR (100 MHz, DMSO-d$_6$): 165.4, 164.7, 162.0, 149.8, 148.6, 147.8, 144.4, 135.2, 134.9, 129.9, 127.5, 126.1, 123.2, 117.6, 113.7, 113.5, 112.2, 55.4, 40.4. LC-MS (ESI): calculated: 377.1; found: 378.2 (M+H$^+$).

**IS 17**

$^1$H NMR (400 MHz, DMSO-d$_6$): 9.94 (s, 1H), 8.75 (t, $J = 6.0$ Hz, 1H), 8.52 (d, $J = 1.8$ Hz, 1H), 8.43 (dd, $J = 1.4$, 4.6 Hz, 1H), 8.07 (d, $J = 8.5$ Hz, 2H), 7.75 (d, $J = 1.6$ Hz, 1H), 7.70 (d, $J = 7.8$ Hz, 1H), 7.62 (d, $J = 8.5$ Hz, 2H), 7.34 (dd, $J = 4.8$, 7.8 Hz, 1H), 7.23 (s, 1H), 6.72 (d, $J = 3.4$ Hz, 1H), 6.57 (dd, $J = 1.6$, 3.3 Hz, 1H), 4.40 (d, $J = 6.0$ Hz, 2H). $^1$C NMR (100 MHz, DMSO-d$_6$): 165.0, 164.4, 149.7, 148.6, 147.9, 144.8, 136.5, 135.1, 134.9, 132.7, 129.9, 128.4, 126.8, 123.3, 118.3, 114.4, 112.3, 40.3.

**IS 18**

$^1$H NMR (400 MHz, CDCl$_3$): 9.96 (s, 1H), 8.48 (d, $J = 2.1$ Hz, 1H), 8.39 (dd, $J = 1.6$, 4.8 Hz, 1H), 8.14 (dd, $J = 1.9$, 7.9 Hz, 1H), 7.72 (t, $J = 1.9$, 7.8 Hz, 1H), 7.45 (m, 1H), 7.37 (d, $J = 1.8$ Hz, 1H), 7.18 (m, 2H), 7.02 (t, $J = 7.4$ Hz, 1H), 6.96 (d, $J = 8.3$ Hz, 1H), 6.80 (s, 1H), 6.45 (d, $J = 3.4$ Hz,
IS19

**1H NMR** (400 MHz, CDC13): 8.84 (s, 1H), 8.43 (d, J = 2.3 Hz, 1H), 8.34 (dd, J = 1.6, 4.8 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.48 (t, J = 6.0 Hz, 1H), 7.43 (m, 2H), 7.36 (d, J = 1.8 Hz, 1H), 7.27 (t, J = 8.2 Hz, 1H), 7.12 (dd, J = 4.8, 7.8 Hz, 1H), 6.80 (s, 1H), 6.43 (d, J = 3.5 Hz, 1H), 6.36 (dd, J = 1.9, 3.5 Hz, 1H), 4.41 (d, J = 6.0 Hz, 2H), 3.76 (s, 3H); **13C NMR** (100 MHz, CDC13): 166.5, 165.6, 160.0, 150.2, 149.0, 148.6, 144.1, 135.8, 134.6, 134.3, 129.8, 127.1, 123.7, 119.7, 118.7, 114.8, 114.7, 112.9, 112.3, 55.6, 41.4. **LC-MS (ESI):** calculated: 377.1; found: 378.2 (M+H)+.

IS20

**1H NMR** (400 MHz, DMSO-d6): 3.75 (s, 3H), 4.41 (d, J = 6.1 Hz, 2H), 6.94 (d, J = 8.8 Hz, 2H), 7.33 (s, 3H), 7.52-7.60 (m, 5H), 8.08 (d, J = 8.0, 2H), 8.5 (d, J = 6.3 Hz, 2H), 8.71 (t, J = 6.1 Hz, 1H), 9.98 (s, 1H); **RMN** 13C (100 MHz, DMSO): 41.7, 55.2, 114.0, 122.0, 126.5, 127.6, 128.3, 129.8, 131.1, 131.6, 133.7, 148.9, 149.3, 159.7, 166.4, 166.2. **LC-MS (ESI):** calculated: 377.1; found: 378.2 (M+H)+.

IS21

**NMR** **1H** (400 MHz, DMSO-d6) δ 3.74 (s, 3H), 4.41 (d, J = 5.9 Hz, 2H), 6.93 (d, J = 9.1 Hz, 2H), 7.36 (m, 2H), 7.60 (m, 5H), 7.73 (d, J = 7.7 Hz, 1H), 8.05 (d, J = 7.4 Hz, 2H), 8.44 (d, J = 6.1 Hz, 1H), 8.54 (s, 1H), 8.68 (s, 1H), 9.93 (s, 1H); **NMR** 13C (100 MHz, DMSO-d6) δ 30.8, 55.4, 114.2, 123.4, 126.7, 128.4, 129.9, 131.3, 131.8, 133.9, 135.0, 135.5, 148.0, 148.8, 159.8, 165.5, 166.1. **LC-MS (ESI):** calculated: 377.1; found: 378.2 (M+H)+.

IS26

**NMR** **1H** (400 MHz, CDC13) δ 3.53 (s, 1H), 3.81 (s, 1H), 3.94 (s, 3H); **13C NMR** (100 MHz, CDC13): 165.7, 164.4, 157.8, 150.4, 149.2, 148.7, 143.7, 135.8, 134.3, 134.0, 132.8, 127.6, 123.6, 121.6, 120.5, 113.7, 113.5, 112.2, 111.5, 56.1, 41.4. **LC-MS (ESI):** calculated: 377.1; found: 378.2 (M+H)+.
4.47 (d, J = 5.9 Hz, 2H), 6.74 (d, J = 8.9 Hz, 1H), 6.92 (d, J = 5.5 Hz, 2H), 7.12 (s, 1H), 7.20-7.24 (m, 1H), 7.41 (t, J = 7.5 Hz, 2H), 7.52 (t, J = 7.3 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 7.5 Hz, 2H), 8.35 (s, 1H), 8.40 (d, J = 4.6 Hz, 1H), 8.48 (s, 1H); NMR 1 H (100 MHz, CDCl₃) δ 41.8, 56.0, 56.3, 112.2, 111.3, 111.4, 112.2, 124.0, 127.6, 128.1, 129.2, 130.2, 132.8, 136.2, 148.9, 149.2, 149.3, 150.3, 166.9, 167.2. LC-MS (ESI): calculated: 417.5; found: 418.2 (M+H⁺).

IS4, IS5 and IS7

**IS4:** A solution of 3-aminopyridine (94 mg, 1 mmol) and azlactone 3a (0.24 g, 1 mmol) in CH₃CN (2 mL) was heated under reflux for 3h and allowed to cool to room temperature. The precipitate, was filtered, washed with MeOH and Et₂O and recrystallized 5 in EtOH to afford 30 mg of IS4 as a white solid.

**IS4**

1 H NMR (300 MHz, CDCl₃): 9.28 (s, 1H), 8.72 (m, 2H), 8.23 (dd, J = 1.6, 4.8 Hz, 1H), 8.10 (d, J = 8.3 Hz, 1H), 7.93 (d, J = 7.2 Hz, 2H), 7.57 (m, 1H), 7.47 (t, J = 7.2, 1H), 7.37 (s, 1H), 7.16 (dd, J = 4.7, 8.3 Hz, 1H), 6.80 (s, 1H), 6.37 (m, 2H); 13 C NMR (100 MHz, DMSO-d₆): 165.7, 164.0, 149.6, 144.8, 144.4, 141.9, 135.8, 133.6, 131.8, 128.4, 127.9, 127.4, 127.2, 123.4, 117.4, 114.5, 112.4. LC-MS (ESI): calculated:
IS5: A solution of 3-aminopyridine (47 mg, 0.5 mmol) and azlactone 3a (120 mg, 0.5 mmol) in DMF (1 mL) was heated at 130°C for 1h and concentrated in vacuo. The residue was dissolved in AcOEt (50 ml), washed with water, a solution of Na₂C₅O₃ and brine. Two recrystallisations in AcOEt afforded 55 mg of IS5 as a white solid.

**IS5**

![Structural diagram of IS5]

**H NMR (400 MHz, DMSO-d₆)**: 10.44 (s, IH), 10.03 (s, IH), 8.44 (dd, J = 1.6, 4.9 Hz, 2H), 8.07 (d, J = 7.2 Hz, 2H), 7.83 (d, J = 1.6 Hz, IH), 7.72 (dd, J = 1.6, 4.9 Hz, 2H), 7.58 (m, 3H), 7.13 (s, IH), 6.83 (d, J = 3.5 Hz, IH), 6.63 (dd, J = 1.9, 3.5 Hz, IH); **1³C NMR (100 MHz, CDCl₃)**: 165.7, 164.5, 150.2, 149.5, 145.9, 144.9, 133.4, 131.8, 128.4, 127.9, 127.4, 117.4, 114.7, 113.9, 112.4. LC-MS (ESI): calculated: 333.1; found: 334.0 (M+H)+.

IS7: A solution of 2-hydrazinylpyridine (654 mg, 6 mmol) and azlactone 3a (960 mg, 4 mmol) in CH₂Cl₂/EtOH (16 mL, 1/1) was heated under reflux for 1h and concentrated in vacuo. The residue was purified by flash chromatography (Et₂O followed by EtOAc) and recrystallized in Et₂O to afford 0.57 g of IS7 as a white solid.

**IS7**

![Structural diagram of IS7]

**H NMR (400 MHz, MeOD-d₄)**: 9.89 (s, IH), 9.44 (s, IH), 8.01 (m, 3H), 7.45 (m, 6H), 7.16 (s, IH), 6.82 (d, J = 8.4Hz, IH), 6.64 (m, IH), 6.59 (d, J = 3.4 Hz, IH), 6.41 (dd, J = 1.8, 3.5 Hz, IH); **1³C NMR (100 MHz, DMSO-d₆)**: 165.7, 164.0, 159.2, 149.2, 146.7, 143.3, 136.9, 133.0, 131.0, 127.6, 127.2, 125.0, 116.9, 114.6, 113.6, 111.4, 106.4. LC-MS (ESI): calculated: 348.1; found: 349.0 (M+H)+.

IS22

A suspension of azlactone 4d (1 g, 3.59 mmol) and 4-aminopyridine (0.35 g, 3.77 mmol) in DMF (8 ml) was stirred at 130°C overnight. After concentration under vacuum, water was added and the compound was extracted with EtOAc (2 x50 mL), dried over MgSO₄, concentrated *in vacuo* and recrystallized in EtOH to afford 540 mg (56 %) of adduct IS22 as white crystals.
IS22

NMR $^1$H (300 MHz, DMSO-d$_6$) $\delta$ 3.77 (s, 3 H), 6.99 (d, $J$ = 8.8 Hz, 2 H), 7.26 (s, 1 H), 7.55 (t, $J$ = 7.3 Hz, 2 H), 7.61 (d, $J$ = 7.3 Hz, 1 H), 7.66 (d, $J$ = 8.8 Hz, 2 H), 7.77 (d, $J$ = 6.3 Hz, 2 H), 8.07 (d, $J$ = 7.4 Hz, 2 H), 8.44 (d, $J$ = 6.3 Hz, 2 H), 10.17 (s, 1 H), 10.62 (s, 1 H); NMR $^{13}$C (100 MHz, DMSO-d$_6$) $\delta$ 55.2, 113.8, 144.0, 126.4, 126.4, 127.9, 128.2, 128.3, 129.7, 131.4, 131.8, 133.4, 146.0, 150.1, 165.4, 166.0. LC-MS (ESI): calculated: 373.3; found: 374.2 (M+H$^+$).

IS23

A suspension of azlactone 4d (1.12 g, 4 mmol) and 3-aminomethyl-pyridine (0.43 g, 4 mmol) in CH$_2$Cl$_2$/EtOH (8 ml, 1/1) was heated at 70°C for 1 h and cooled down at rt. The precipitate was filtered and purified by flash chromatography (CH$_2$Cl$_2$/Acetone 9/land 5 CH$_2$Cl$_2$/EtOH 9/1) to afford the expected product as a white solid.

IS27

A suspension of aminoguanidinium carbonate (1.71 g, 12.54 mmol) and azlactone 3a (9.6 g, 4 mmol) in EtOH (8 ml) was stirred under reflux overnight. After cooling down to room temperature, the precipitate was filtered off. The solvent was the removed under vacuum, and the residue was purified by flash chromatography (EtOAc to EtOAc/EtOH, 80/20) and recrystallized in water to give the expected product IS27 (106 mg, 7 %) as a white solid (purity > 97%).

IS27

$^1$H NMR (400 MHz, DMSO-d$_6$): 4.80 (s, 1 H), 4.95 (s, 2 H),
5.28 (d, J = 7.4 Hz, 1 H), 6.24 (d, J = 3.1 Hz, 1 H), 6.39 (s, 1 H), 7.47 (t, J = 7.8 Hz, 2 H), 7.50-7.53 (m, 2 H), 7.61 (s, 1 H), 7.67 (d, J = 7.3 Hz, 2 H); **RMN** 13C (100 MHz, DMSO): 109.6, 110.4, 126.9, 128.5, 131.6, 133.7, 143.7, 148.7, 161.0, 166.1, 171.8. LC-MS (ESI): calculated: 353.3; found: 315.0 (M-38).

**IS28**

The synthesis of IS28 is illustrated in Figure 26.

**Synthesis of intermediate 5**: A suspension of ethylenediame (2.51 g, 41.8 mmol) and azlactone 3a (1 g, 4.2 mmol) in suspension in CH2Cl2/EtOH (16 mL, 1/1) was stirred at r.t. for 4 h. After concentration under vacuum, water was added and the adduct was extracted with CH2Cl2, dried over MgSO4 and concentrated under vacuum to afford 5 (367 mg, 29 %). 1H NMR (400 MHz, DMSO-d6): 2.61 (t, J = 6.3 Hz 2H), 3.17 (q, J = 6.0 Hz), 6.55-6.56 (m, 1H), 6.68 (d, J = 3.5 Hz, 1H), 7.14 (s, 1H), 7.55 (t, J = 7.7 Hz, 2H), 7.62 (t, J = 7.3 Hz, 1H), 7.73 (s, 1H), 8.06 (d, J = 7.0 Hz, 3H); 13C NMR (100 MHz, DMSO-d6): δ 41.2, 42.8, 112.2, 113.6, 117.1, 127.6, 127.9, 128.3, 131.6, 133.9, 144.3, 149.9, 164.3.

**Synthesis of intermediate 6**: a mixture of 5 (0.15 g, 0.50 mmol), 1,3-bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (0.50 mmol), HgCl2 (0.13 g, 0.50 mmol) and Et3N (68 μL, 1.5 mmol) in CH2Cl2/EtOH (8 ml, 1/1) was heated at 70°C for 1 h and concentrated. The residue was purified by flash chromatography (AcOEt/Pentane, 1/1) to afford 6 (0.45 g, 17%) as a white solid (%). 1H NMR (400 MHz, CDC13): 1.37 (s, 9H), 1.43 (s, 9H), 1.99 (s, 1H), 3.49-3.58 (m, 4H), 6.39-6.44 (m, 2H), 6.68 (s, 1H), 7.24 (s, 1H), 7.39-7.50 (m, 4H), 7.91 (d, J = 7.3 Hz, 2H), 8.6 (d, J = 25 Hz, 2H); RMN 13C (100 MHz, CDC13): 21.5, 28.5, 30.1, 40.5, 40.9, 60.8, 79.8, 112.5, 112.6, 114.2, 128.1, 128.3, 129.1, 132.6, 133.8, 144.0, 149.4, 150.8, 153.4, 157.4, 163.7, 166.1.

**Synthesis of IS28**: A solution of 6 (45 mg, 0.5 mmol) and TFA (200 μl) in CH2Cl2 (800 μl) was stirred at r.t. for 4 hours, concentrated, washed with Et2O and dried under vacuum to afford IS28 (16 mg, 94 %) as a white powder.

**IS 28**

1H NMR (400 MHz, DMSO-d6): 3.21-3.31 (m, 4H), 6.58 (s, 1H), 6.71 (s, 1H), 7.16 (s, 4H), 7.55 (t, J = 8.0 Hz, 3H), 7.62 (t, J = 6.8 Hz, 1H), 7.56 (s, 1H), 8.05 (d, J = 7.4Hz, 2H),
8.25 (s, 1H), 9.85 (s, 1H); RMN \(^{13}\)C (100 MHz, DMSO-d6): 38.5, 40.5, 112.3, 114.1, 117.6, 127.0, 127.9, 128.3, 131.7, 133.7, 144.7, 149.6, 157.0, 165.3, 165.7. LC-MS (ESI): calculated: 341.2; found: 342.2 (M+H\(^+\)).

IS30

A suspension of azlactone 4d (0.83 g, 3 mmol) and 2-amino-5-aminomethyl-pyridine (0.43 g, 4 mmol) was heated under reflux in CH\(_2\)Cl\(_2\) (10 ml) for 1 h, concentrated in vacuo and purified by flash chromatography (EtOAc to EtOAc/EtOH, 80/20) to afford the expected product IS30 (655mg, 54 %) as a white solid.

IS 30

\[\text{NMR H (400 MHz, DMSO-d}_6\text{)} \delta \text{ 3.72 (s, 3 H), 4.21 (d, J = 5.9 Hz, 2 H), 5.45 (s, 2 H), 6.41 (d, J = 8.4 Hz, 1H), 6.82 (d, J = 8.8 Hz, 2 H), 7.25 (s, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.50 (m, 5H), 7.84 (s, 1H), 8.01 (d, J = 7.7Hz, 1H), 8.03 (s, 1H), 8.28 (s, 1H), 9.72 (s, 1H); NMR \(^{13}\)C (100 MHz, DMSO-d6) \delta 40.3, 54.7, 107.5, 113.5, 122.9, 126.4, 127.4, 127.7, 129.4, 130.7, 131.1, 133.5, 136.7, 146.4, 158.2, 159.3, 165.0, 165.1. LC-MS (ESI): calculated: 402.2; found: 403.2 (M+H\(^+\)).]

IS35

A solution of azlactone 3a (0.37 g, 1.5 mmol) and guanidine carbonate (1.44 mmol) in EtOH (40 mL) was stirred under reflux for 18 h. After concentration under vacuum, the adduct underwent a first purification by flash chromatography CH\(_2\)Cl\(_2\)/MeOH (8/2) and a second one by HPLC (Symetry shield RP18, 7 \(\mu\)m, 19x300 mm, with a flow rate of 10 mL/min using a 50 min gradient from water (0.1% TFA) to CH\(_3\)CN (0.1% TFA) yielded IS35 (52 mg, 13 %) as a white solid. \textbf{H} NMR (400 MHz, CDC\(_3\)): 6.42-6.51 (m, 2 H furyan), 6.70-6.14 (br m,2 NH guanidine), 6.15 (s, C=CH), 7.45-7.56 (m, 4 ArH), 7.90-7.94 (m, 2 ArH), 8.70-8.79 (br m, NH) ; RMN \(^{13}\)C (100 MHz, DMSO-d6): 112.2, 127.8, 116.4, 127.6, 128.4, 131.0, 131.4, 134.4, 143.7, 162.4, 164.5, 173.8. LC-MS (ESI): calculated: 298.1; found: 299.1 (M+H\(^+\)).
**IS 35**

\[
\begin{align*}
\text{HN} & \quad \text{NH}_{2} \\
\text{O} & \quad \text{O} \\
\text{HN} & \quad \text{NH}_{2}
\end{align*}
\]

\(^1\text{H}\) NMR (400 MHz, CDC\(_3\)): 6.42-6.51 (m, 2 H furan), 6.70-6.14 (br m, 2 NH guanidine), 6.15 (s, C=CH), 7.45-7.56 (m, 4 ArH), 7.90-7.94 (m, 2 ArH), 8.70-8.79 (br m, NH); \(^{13}\)C (100 MHz, DMSO-d\(_6\)): 112.2, 127.8, 116.4, 127.6, 128.4, 131.0, 131.4, 134.4, 143.7, 162.4, 164.5, 173.8. LC-MS (ESI): calculated: 298.1; found: 299.1 (M+H\(^+\))^+. 

**Cell culture**

Coronary endothelial cells (H5V cells) were maintained in DMEM supplemented with 10% heat inactivated Fetal Calf Serum (FCS), glutamine (ImM) under 5% CO\(_2\) at 37°C.

**Example la: Induction of in vitro angiogenesis:**

In order to evaluate the potential angiogenic activity of the PKRI agonists, the test of Growth Factor Reduced Matrigel (GFR Matrigel) 24 well plate was performed. Matrigel leads to the differentiation of many cell types and induces the formation and organization of endothelial cells into capillary tubules. Twenty-four-well culture plates were coated with Matrigel and endothelial cells (H5V) were trypsin-harvested and seeded onto the coated plates at 10\(^5\) cells per well in the serum free assay medium with PKRI agonists, ISI and prokineticin-2, and incubated at 37°C for 24h. Tube formation as two dimensional branched structures were observed using an inverted phase contrast microscope (Zeiss), and the number of branching points were quantified in per well from each sample. Each experiment was repeated at least three times. Images were captured at a magnification of X10 with a digital microscope camera system (Urayama K et al., 2007).

**Results**

The results are presented in Figures 12, 13, 27, 28 and 29. They demonstrate that the IS compounds (cf. Figures 10 and 25) are able to induce in vitro angiogenesis as the well known PKRI agonist, prokineticin-2.
**Example lb: Activation of PKR1 signaling pathway - Measurement of intracellular calcium**

CHO-K1 (Chinese Hamster Ovary) cells were infected with a adenovirus carrying PKR1 cDNA. Cells were then stimulated, 48h after infection, with or without ISI agonists, at 37°C.

Calcium imaging was conducted essentially as previously described (Horinouchi et al., 2007) In brief, Intracellular free calcium $[\text{Ca}^{2+}]_i$ was measured with the fluorescent $\text{Ca}^{2+}$ indicator dye Fluo 4-AM, the membrane-permeant acetoxy methyl ester form of Fluo 4 (Invitrogen). Changes in $[\text{Ca}^{2+}]_i$ were determined from variations in the fluorescence intensity of Fluo 4-AM. Briefly, trypsinized CHO-K1-PKR1 cells were seeded onto glass bottom culture dishes (MatTek, MA, USA) for 24 h. Immediately before $\text{Ca}^{2+}$ indicator loading, cells were gently washed with HBSS (invitrogen) and then incubated in Fluo 4-AM working solution (Fluo 4-AM 2.5 $\mu$M and 2.5 mM probenecid dissolved in standard buffer) for 30 min at 37 °C in cell incubator. Afterwards, cells were washed with HBSS including probenecid to remove extracellular Fluo 4-AM. Dishes were then placed on the stage of the laser confocal microscope (Leica). The fluorescence in the cells was excited at a wavelength of 488 nm and a series of images (total exposure time: 300 s) were acquired at 0.5s intervals at emission wavelength 518 nm. Data are presented as the fluorescence intensity of 3 randomly chosen cells within a randomly selected 40x field. To evaluate the effect of ISI compound on $[\text{Ca}^{2+}]_i$ in CHO-K-PKR1, the maximum change in fluorescence intensity upon addition of IS 1 was measured and normalized to the baseline fluorescence obtained before IS 1 addition.

**Results**

The results are presented in Figure 14. They demonstrate that the ISI compound is able to activate the PKR1 signalling pathway in cells expressing PKR1.

**Example lc: Specificity of the agonists to PKR1 - Assessment of PKR1 internalization**

The capacity of agonist compounds to induce internalization of PKR1 was assessed. Therefore, CHO-K1 cells transfected with a plasmid encoding GFP-PKR1 (Green Fluorescent Protein-PKR1), in the presence of Lipofectamine2000 (Invitrogen), were used. Cells were stimulated, 48h after transfection, with or without agonist compound, at 37°C. GFP is a fluorescent protein fusion (26.9 kDa) which has a major excitation peak at a wavelength of 396 nm and a minor one at 472 nm. Its emission peak is at 504 nm which is
in the lower green portion of the visible spectrum. Fluorescence allowed tracking the localization of PKR1 after stimulation by the agonist (membrane or subcellular). Cells were seeded in glass-bottom coated with polyL-lysine and receptor studies were performed in the presence of various PKR1-ligands. Samples were observed under a Leica confocal microscope (SP2 AOBS MP) with objective 63x at $37^\circ$C. Images were automatically recorded during 20 min, with increasing time intervals to avoid bleaching effects because of repetitive scanning. Reconstituted videos contained 42 images and lasted 2 second.

**Results**

The results are presented in Figure 15. They demonstrate that the IS1 compound specifically interacts with PKR1 and induces its internalization in cells expressing PKR1.

**Conclusion**

These experiments demonstrate that the agonists of the invention, and in particular IS compounds (cf. Figures 10 and 25) are able to specifically activate the PKR1 signalling pathway inducing the internalization of the receptor and angiogenesis.

**Example 2: Effects of PKR1 agonists on cardiac epicardin positive progenitor cells**

**Materials and Methods**

**Extraction and culture of cEPPC**

Hearts were removed from P1 neonate C57BL/B6 mice or PKR1 null mutant mice, cut into pieces (approximately 1 mm$^3$), rinsed in PBS to remove excess blood and plated onto 0.1 % gelatin petri dish in DMEM containing GlutaMax, 4.5 g/L glucose, penicillin, streptomycin and 15 % fetal calf serum. Cultures were maintained with minimum disturbance to allow explants to adhere. All cells were maintained at $37^\circ$C in a humidified air 5% CO$_2$ atmosphere. After 4 days explants were removed and cells were gently washed and fresh medium with DMEM containing GlutaMax, 4.5 g/L glucose, penicillin, streptomycin and 10 % fetal calf serum serum was added. The cells were cultured during 10 days and trypsinized for experimentation.

**Adipogenic treatment**

For adipogenesis, cells were plated at the density of 2x10$^4$ onto 0.1% gelatin coated culture chamber in DMEM containing GlutaMax, 4.5 g/L glucose, penicillin, streptomycin
and 10 % fetal calf serum. After 4 days the fresh medium was added and adipocyte
differentiation was induced on confluent cells by treatment with adipogenic cocktail
comprising 0.25μM Dexamethasone, 0.5mM 3-isobutyl-l-methylxanthine, 10 μg/ml
insulin during 48 hours at 37°C in a humidified air 5% C0₂ atmosphere. After 48 hours the
fresh medium was added and the adipogenesis was maintained by adding 10μg/ml insulin
during 48 hours. After this time the medium was changed and the adipogenesis was
continued during 48 hours. The induction of adipogenesis was repeated by a new treatment
with 0.25 μM Dexamethasone, 0.5mM 3-isobutyl-l-methylxanthine, 10μg/ml insulin
during 48 hours following by 10μg/ml insulin treatment during 48 hours and finally the
cells were maintained in fresh medium for 48 hours. To test the role of Prokineticin-2 in
adipogenic differentiation of EPPC, the cells were treated with prokineticin-2, 12 hours
before the adipogenesis beginning (including the treatment by 0.25 μM Dexamethasone,
0.5 mM 3-isobutyl-l-methylxanthine, 10 μg/ml insulin) for the two steps.

**Oil red O staining**

Cells were fixed in 3.7 % formaldehyde at 4°C during 1 hour. The accumulation of
neutral lipids can be detected by staining the cells in solution of 0.5% Oil red O in 60%
isopropanol at room temperature during 30 minutes. To quantify the cells differentiation
DAPI (a marker of nucleus) was added at 1/100 in water. The pictures were taken with
inverted microscope at x40 objectif.

**RNA Isolation**

After adipogenic treatment the total RNA from cells was isolated using TRI
Reagent (Molecular Research Center) and treated with DNase using the RNase-Free DNase
Set. The quantitative PCR reactions were carried out in the presence of a fluorescent dye
(Sybrgreen, BioRad). Relative abundance of mRNA was calculated after normalisation to
36B4. The expression of PPAR alpha and PPAR gamma was analyzed.

<table>
<thead>
<tr>
<th>Mouse 36B4 forward</th>
<th>5’AGA TTC GGG ATA TGC TGT TGG 3’ (SEQ ID NO. :1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse 36B4 reverse</td>
<td>5’AAA GCC TGG AAG AAG GAG GTC 3’ (SEQ ID NO. :2)</td>
</tr>
<tr>
<td>Mouse PPAR alpha forward</td>
<td>5’ACG ATG CTG TCC TCC TTG ATG 3’ (SEQ ID NO. :3)</td>
</tr>
<tr>
<td>Mouse PPAR alpha reverse</td>
<td>5’GTG TGA TAA AGC CAT TGC CGT 3’ (SEQ ID NO. :4)</td>
</tr>
<tr>
<td>Mouse PPAR gamma forward</td>
<td>5’CCA TTC TGG CCC ACC AAC 3’ (SEQ ID NO. :5)</td>
</tr>
<tr>
<td>Mouse PPAR gamma reverse</td>
<td>5’AAT GCG AGT GGT CTT CCA TCA 3’ (SEQ ID NO. :6)</td>
</tr>
</tbody>
</table>
Cardiomyocyte treatment

For cardiomyogenesis, cells were plated at 2x10^4 onto 1% gelatin coated culture chamber in DMEM containing GlutaMax, 4.5 g/L glucose, penicillin, streptomycin and 10% fetal calf serum. The cells were maintained in culture during six days and medium were change every 2 days. After 6 days, cardiomyocyte differentiation can be induced using culture medium supplemented with 1µM dexamethasone, 50µg/ml ascorbic acid, 10mM glycerophosphate without or with 10 nM of prokineticin-2. The medium was change every 2 days during 36 days. After this time, the contractility of cardiomyocyte was taken with time laps microscopy at 5% CO₂ 37°C, and 800 frames take per cardiomyocyte group (10ms/frame). To test the contractility of cardiomyocyte (beating cells), a beta adrenergic receptor agonist was applied into the medium at the concentration of 25 nM for Isoproterenol and then time laps analyses were performed.

Immunostaining

Cells were fixed in 3.7% formaldehyde at 4°C 1 hours. After fixation, the cells were blocked with in PBS containing 10% donkey serum and 0.5% Triton-X100 for 1 hour and then incubated with antibody against Troponin C (Santa Cruz), containing 1% donkey serum and 0.05% Triton-X100 at 4°C overnight. The following secondary antibodies were used Alexa 488-conjugated anti goat antibody. Finally, cells were covered with mounting medium from Vector Laboratories containing 4, 6-diamidino-2- phenylindol dihydrochloride (DAPI). The immunofluorescence was detected by Leica TCSNT fluorescent microscope.

Results

Cardiac epicardin positive progenitor cell (cEPPC) differentiation into adipocytes

Neonatal EPPC were subjected to the standard differentiation program including dexamethasone, 3-isobutyl-1-methylxanthine, and insulin. For the first time, it was shown that the stimulation of cEPPC by proadipogenic treatment leads to adipocyte formation (Fig. 1A, upper line, left panel; Fig. 1B).

It was also determined whether prokineticin-2 alters cEPPC differentiation into adipocyte. To explore this phenomenon, cEPPC were pre-treated with prokineticin-2
several hours before pro-adipogenic treatment. It was thus demonstrated that prokineticin-2 pre-treatment inhibits adipocyte formation in cEPPC (Fig. 1A, upper line, right panel; Fig. IB).

**Involvement of PKR1 in inhibition of adipogenesis**

The role of PKR1 in inhibition of adipogenesis by prokineticin-2 was tested by using the same differentiation protocol for cEPPC derived from PKR1 null mutant mice (cEPPC-PKR1-/-). It was thus shown that there was no difference in adipocyte formation in cEPPC-PKR1-/- without or with prokineticin-2 pretreatment during adipogenic stimuli. These data demonstrated that prokineticin-2 acts via PKR1 to inhibit the adipocyte formation in cEPPC (Fig. 1A, lower line; Fig. IB).

**Regulation of adipogenic gene expression**

Since the program of adipocyte differentiation involves up-regulation of a subset of gene such as pPAR gamma and pPAR alpha, that are specific for maturing or mature adipocyte, it was tested whether the expression of mRNAs of these genes correlated with the inhibition of differentiation. The inventors showed that a pro-adipogenic treatment in cEPPC was correlated with up-regulation of adipogenic genes such as PPAR gamma in early stage of adipogenesis following by PPAR alpha in late stage of adipogenesis. Interestingly, they also showed that prokineticin-2 pretreatment leading to adipocyte regression involved in suppression of PPAR gamma gene expression in early stage of adipogenesis. They revealed in the same condition, that prokineticin-2 pretreatment inhibits up-regulation of PPAR alpha in the late stage of cEPPC adipogenesis (Figure 2).

**Cardiomyocyte differentiation**

The ability of cEPPC to differentiate into cardiomyocyte lineage was tested. The inventors showed that a treatment comprising Dexamethasone, acid ascorbic and glycerophosphat alone can not lead to differentiation of cEPPC into functional cardiomyocytes. Surprisingly, they demonstrated that a treatment of prokineticin-2 leads to differentiation of cEPPC into functional cardiomyocytes (Figure 3).

**Conclusion**

The inventors have herein demonstrated that PKR1 agonists stimulate the differentiation of cardiac EPPC in cardiomyocytes and block their differentiation in
adipocytes.

**Example 3: Effect of genetic inactivation of PKR1 and activation of PKR1 signaling pathway in kidney**

**Materials and Methods**

5 Generation of knockout mice

To conditionally disrupt the GPR73 gene encoding PKR1, embryonic stem (ES) cells were electroporated with the targeting vector pGPR73 L3 that encompasses GPR73 exon 2 and contains a loxP site in the intron located upstream of exon 2, whereas a loxP-flanked (floxed) neomycin (neo) selection cassette is present in the intron downstream of exon 2.

To excise floxed DNA segments of the GPR73 L3 allele, GPR73 L3/+ mice were bred with CMV-Cre transgenic mice that express Cre recombinase in germ cells. PCR analysis of tail DNA revealed a Cre-dependent GPR73 L- allele excision, yielding GPR73 L+/L- heterozygous animals. After heterozygous interbreeding, GPR73 L-/+ mice were recovered at Mendelian frequency, survived to adulthood, and were fertile. These mice have a reduced life span by 6% as compared to wild type.

TUNEL Analysis

Briefly, frozen sections were fixed in 4% freshly prepared paraformaldehyde in PBS for 20 minutes and incubated in permeabilization solution (0.1% Triton, 0.1% sodium citrate), followed by incubation with TUNEL reaction mixture at 37°C for 60 minutes in a dark, humidified chamber. Sections were then washed, counterstained with DAPI (0.5 µg/mL in PBS), and mounted with VECTASHIELD medium (Vector Laboratories, Inc, Burlingame, Calif).

For staining the tissue section for succinate dehydrogenase activity, the kidneys were frozen, and 5-µm sections were incubated in 0.1 M phosphate buffer (pH 7.4) containing 0.1 M sodium succinate and 0.1% nitro blue tetrazolium at 37 °C for 5 min. The staining intensity (blue color) of each section was quantified by using Image software. 6 to 10 images per kidney (3 to 4 animals per genotype group) were acquired with a fluorescence microscope (Leica). Results are expressed as the percentage of apoptotic cells. The TUNEL labeling index was calculated as the mean number of DAPI-stained TUNEL-30 positive nuclei/glomerulus, for 50 glomeruli per kidney (n=3) for the various groups of
mice.

Glomerular pathology analyses were based on the assessment of 50 glomeruli/kidney cross-section. Tubule damage (consisting of at least one of the following: dilatation, atrophy, necrosis) was assessed by scoring 100 renal proximal tubules/kidney cross-section on randomly selected high-power microscopy fields (×40).

Frozen tissue sections for the immunofluorescence staining of structural proteins were fixed, blocked and stained with primary antibodies against PECAM-1, podocin, NaPi, Wt1, Dystrophin, N-cadherine and PKR2, (Santa Cruz); LC3, and epicardin (abeam), α-SMA (sigma), active capase-3 (Millipore), and PKR1 (IGBMC, Illkirch). Antibody binding was detected by incubation with Fluorescein, Alexa 555- or Alexa 488-conjugated secondary antibodies. Finally, the nuclei were stained with DAPI. Fluorescence was analyzed on a Leica Microsystems TCS SP5 laser scanning confocal microscope. The number of PECAM-1-positive, DAPI-stained cells per glomerulus, was determined by counting 50 glomerular fields per kidney section for each group of mice.

For staining the tissue section for oil red O, cryosectiones (5 μm) were fixed in 10% formalin for 30 min, and then washed in distilled water and stained with 0.5% oil red O in 36% Triethylphosphat (Sigma-Aldrich) for 30 min to identify neutral lipids, cholesterol, and fatty acids (red colour). After rinsing with water, nuclei were counterstained (blue) with Mayer's hematoxylin for 5 min and washed in distilled water for 3 times. Finally, slides were covered in aqueousmount under a coverslip for viewing with a light microscope at equal light intensity. Images from the stained slides of mice were initially acquired using a 24-bit file format. ImageJ 1.37v software (National Institutes of Health) was used to convert bright-field (24-bit) images of Oil red O stainings to 8-bit images. Threshold values were chosen that maximize selection of the Oil red O positive tissue while minimizing background interference. Thus, the total number of lipid drops from each image (percent) was quantified.

**RNA Extraction, Quantification, and Reverse-Transcription Polymerase Chain Reaction Analysis**

A minimum of 3 mouse kidney samples for each of the genotype groups were analyzed in each experiment. Extracted RNA samples were used as the template for cDNA prepared via a reverse-transcription polymerase chain reaction that was performed in duplicate for all RNA samples. Semi-quantitative or real-time reverse-transcription polymerase chain
reaction (MiQ, Biorad) was then used to determine the mRNA expression of indicated genes. The GAPDH housekeeping gene was used as a control. Results were analyzed with Bio-Rad qPCR software version 2.0.

**In situ hybridization**

DNA templates for producing mouse PKR1 riboprobes were generated from PCR expression vector encoding full length PKR1 after the linearization with BamHI or EcoRV and transcription with T7 or Sp6 RNA polymerases to generate the antisense and sense probes, respectively. Digoxigenin-labeled (DIG-RNA labeling Mix, Roche) riboprobes were transcribed in vitro in antisense or sense orientations using T7, Sp6, or T3 RNA polymerases as indicated above. Some of the experiments were performed using a Tecan GenePaint Robot and a Tyramide signal amplification method (for details, see www.eurexpress.org and www.genepaint.org). In each hybridization experiment, control sense probes were included, allowing the determination of possible false-positive signal.

**Western blot analysis**

Tissues were lysed in lysis buffer. Total protein was separated by SDS-PAGE in 7 or 12% acrylamide gels and blotted onto PVDF membranes. The membrane was blocked and incubated with primary antibodies, troponin C, HIF1α (Santa Cruz); phosho-Akt and total Akt (Cell Signaling). It was then incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary immunoglobulins. The signal was then detected by enhanced chemiluminescence (Pierce, Rockford, IL), according to the manufacturer's protocol. Akt activity was evaluated and quantified by Western blot analysis and densitometry, using antibodies specific for Akt kinase at the residues (Ser-473) that are phosphorylated upon activation.

**Biochemical analyses on blood and urine samples**

After an overnight fast, blood was collected at 8 a.m. by retro-orbital puncture after a short anesthesia with isoflurane (Forene®, Abbott France). 300 μl of blood was collected into a heparinized tube for biochemical analysis. Urine was obtained from individual mice housed in metabolic cages for 24 hours. Urine and plasma creatinine concentrations were determined by a modification of Jaffe's reaction method. The parameters were determined using an Olympus analyzer with kits and controls supplied by Olympus or other suppliers (see EMPReSS Website).
Kidney explants, Isolation of Epicardin + progenitor cells

Kidneys were removed from PI neonate C57BL/B6 mice, cut into pieces (approximately 1 mm³), rinsed in PBS to remove excess blood and plated onto 0.1 % gelatin-coated culture chamber in DMEM containing GlutaMax™, 4.5 g /L glucose, 5 penicillin, streptomycin and 15 % fetal calf serum (FCS) in the presence of prokineticin-2 (5 nM-10 nM) or its vehicle (PBS only). Cultures were maintained with minimum disturbance to allow explants to adhere. After 2 or 4 days of culture, explants were removed and cells were maintained in DMEM containing Glutamax, 4.5g/L glucose, penicillin streptomycin without serum during 12 hours at 37°C in a humidified air 5% CO₂ atmosphere. Next, cells were cultured in the presence or absence of prokineticin-2 (5 or 10nM) during 24 hours. Cells were fixed in 3.7% formaldehyde at 4°C and cell types were assessed by immunofluorescence.

Primary renal-EPDC cells migrated away to form a monolayer surrounding the remaining kidney explants were utilized and further cultured (16 days) in DMEM containing GlutaMax™, 4.5 g /L glucose, penicillin, streptomycin and 10% FCS, and trypsinized and resuspend in the isolation Buffer (0.1%BSA, 2mM EDTA in PBS) and incubated with biotynilated anti-TCF21 for 20 minutes at room temperature then incubated with 85μl of FlowComp Dynabeads (Dynabeads R FlowCompTM Flexi, invitrogen ) for 30 minutes at room temperature and then placed in the magnet for 2 minutes. The cells attached to Dynabeads were collected with ImL FlowComp Release Buffer and then cells were resuspend in medium containing 10% Fetal calf serum with DMEM 4.5g/L glucose, glutamax, pencilline, streptomycin. After 12 hours cells were washed with PBS and fresh medium without serum were added during 12 hours. Next, cells were cultured in the presence or absence of prokineticin-2 (5 or10nM) during 48 hours. Cells were fixed in 3.7% formaldehyde at 4°C and cell types were assessed by immunofluorescence staining.

Statistical analysis

Data are expressed as means ± SEM. Multigroup comparisons were carried out by one-way ANOVA with post hoc correction. Student's t test was used for pairwise comparisons between groups. For all analyses, values of P<0.05 were considered significant.
Results

PKR1 deficiency-induced renal abnormalities accompanied apoptosis and reduced capillary lumen in glomeruli

Macroscopic inspection showed the kidneys of PKR1 null mutant mice to be massively enlarged, with an irregular surface presenting multiple cortical swellings (Figure 4A). In some cases, one kidney was atrophied, or displayed massive urine retention (Figure 4A and B). Overall, kidney weight with urine in the cortical swellings to body weight ratio significantly increased in PKR1-null mice as compared to wild type mice at the age of 24 weeks (Figure 4C). The inventors also examined neonatal kidneys, to determine the clinical course of the disease. Kidney hypoplasia was also evident in neonatal mutants (Figure 4D). TUNEL assays in kidney sections from mutant and wild-type mice revealed that the glomeruli of mutant kidneys contained significantly more TUNEL-positive cells than those of wild-type kidneys, at the age of 24 weeks (Figure 4E). Accordingly, mutant glomeruli contained fewer PECAM-1-positive visible capillary endothelial cells (Figure 4F).

Renal ultrastructural analysis and abnormal mitochondria

Systematic histological examinations of PKR1 null mutant mice showed dilatation of the Bowman's spaces in glomeruli (Figure 5A, upper panels). Electron micrographs showed considerable thickening of the lamina densa of the glomerular base membrane (GBM), with the presence of numerous protusions on the podocytic side (Figure 5A, lower panels). Mallory tetrachrome staining revealed dilated renal tubules and severe interstitial fibrosis between the tubules (Figure 5B, upper panels). In the mutant kidney, peritubular capillaries were occupied by structures with multiple lumens and prominent interstitial cells, often in loose associations, corresponding to dysmorphogenic capillaries (Figure 5B, middle panels). The number and density of mitochondria in proximal tubule cells was significantly lower in mutant than in wild-type animals. These abnormalities in mitochondrial density were accompanied by changes in mitochondrial structure, such as mitochondrial swelling, and decreases in the numbers of cristae and granulocytes (Figure 5C, lower panels). Consistent with the observed defects in mitochondrial morphology, mutant kidneys displayed only 75% of the SDH activity as compared to wild-type kidneys (n=6, Figure 5D).
PKR1 deficiency-induced renal dysfunction

Renal functions of 24 weeks old mice were assessed by blood and urine chemistry. The 24-h urine flow of PKR1 null mutant mice was significantly lower than that of wild-type mice. However, no differences in water and food intake and blood glucose levels were observed (Figure 6). No significant changes in urinary potassium, sodium and chloride excretion rates were observed between mutant and wild-type mice, consistent with the values obtained for serum (Figure 6). Mutant mice had a significantly lower glomerular filtration rate, as estimated by creatinine clearance. Lower creatinine concentrations (mmol/24-h/g) in the urine of mutant mice were associated with higher concentrations in the serum of these mice. However, the urine phosphate/creatinine, but not calcium/creatinine ratio was significantly higher in mutant mice than in wild-type controls. Mutant mice displayed higher levels of absolute renal phosphate (Pi) excretion, associated with hypophosphatemia (Figure 6), indicating that mutant mice displayed poor Pi retention in the kidney and poor stabilization of serum Pi concentration. Mutant mice exhibit protein urea at the age of 36 weeks old (total proteins in urine were 6.625+0.7 and 10.72+2.4g/l, for wild type and mutant, respectively, n=5, p<0.05), consistent with fenestrated endothelial cell structures at this age (Figure 7).

Characterization of cardiac and renal function impairment

The potential defect in vascularization was determined by determining numbers of PECAM-1+capillary density in kidneys. Neonatal PKR1-null mutant mice exhibit reduced numbers of capillary number ad density as detected by PECAM-1 staining in kidneys as compared to their wild-type littermates (Figure 8A). A reduction of angiogenesis was observed in mutant glomerulus by 39% (n=4, p<0.05). Hypoxia inducible factor (HIF-1 a) transcript levels did not significantly increase in the mutant kidneys at the postnatal day 1. However, at the age of three weeks, inductions of HIF-1 a transcript as well as protein were observed in kidneys of mutants (Figure 8B, C). Parallel to HIF-loc induction at this age, pro-angiogenic factors, PDGF-B and FGF-2 transcripts were increased in mutant kidneys. Furthermore, enhancement of VEGF transcripts were significantly in the kidneys of the mutants (Figure 8D). This data indicates the initiation of a compensatory signaling at these time points.

Next, the inventors investigated whether PKR1 survival pathway is impaired in the neonatal kidney. Indeed, activation of Akt kinase appeared reduced in mutant kidneys as
compared to wild type, indicated by 60% or greater reductions in phospho-Akt (Figure 8F), confirming the increased cell death in this organ at the early stage. Co-staining of the apoptotic marker, active caspase-3 and cell specific markers illustrated that glomerular progenitor cells (epicardin+) and glomerular endothelial cells (pecam+) were also positive for active caspase-3 (Fig. 8F) in only mutant kidneys at the 24 weeks-old-age. These data clearly indicate that the angiogenic defects in mutant are ameliorated over time but cannot compensate for the survival deficit in these cells. Supporting this findings, the inventors found that VEGF level is increased in extraglomerular area, but not in glomerular zone of the mutant kidneys (Figure 8G).

Interestingly, epicardin+ cells in the mutant glomerulus were significantly lower than those in the wild-type tissues (Figure 9A). To assess the possible direct effect of PKR1 signaling on renal-epicardin+ cells as shown for cardiac-epicardin+ cells (Urayama et al., 2008), the inventors used explant cultures from wild-type and mutant kidneys from postnatal day 1 (PI). In untreated kidney explants, 50+6 % of the cells proliferates, as shown by Ki67 staining (Figure 9B). The treatment of kidney explants with prokineticin-2 (5-10 nM) stimulated the extensive outgrowth of cells (Figure 9B, histogram). 90% of the emerging epithelial cells were positive for epicardin. Prokineticin-2 induced differentiation of renal-epicardin+ cells into PECAM-1+ endothelial and αSMA+ vascular smooth muscle cells that were blocked in the mutant renal-epicardin+ cells (Figure 9B, histogram).

Similar results were obtained in epicardin+ isolated cell cultures derived from kidney explants.

Conclusion

The inventors have herein provided the first evidence that angiogenic PKR1 signaling regulates heart kidney functions. They further showed that PKR-1 null mice develop also renal functional abnormalities.

The inventors have also herein provided the first evidence that epicardin+ progenitor cells can be found in kidney. The data presented here are also the first in vivo report that PKR1 signaling prevents apoptosis in glomerular endothelial and progenitor cells. The inventors have further demonstrated that PKR1 signaling controls epicardin+ progenitor cell differentiation that is involved in glomerular angiogenesis.
Example 4 : Effect of genetic inactivation of PKR1 on insulin resistance

Materials and methods

*Generation endothelial specific inactivation of PKR1 in mice*

Ec-PKR1<sup>−/−</sup> mice were generated using the Cre-loxP system. Mice carrying a *PKR1* gene in which exon 2 is flanked by lox sites were bred with a transgenic mice expressing *Cre* recombinase under control of the *Tie2* promoter-enhancer. B6.Cg-Tg(Tek-cre)12Flv/J mice (stock no. 004128) were purchased from the The Jackson Laboratories (Bar Harbor, ME). Previous studies have shown that *Tie2* expression is limited to vascular endothelial cells and the endocardial cushion. Note that PKR1 does not express in the endocardial cushion. As a result of the breeding, all the mice in these experiments have a C57B1/6 genetic background. Mice with *PKR1* gene flanked by *lox* sites (*PKR1<sup>lox/lox</sup>*) were generated as previously described (Boulberdaa et al. 2011). Homozygous *pKR1<sup>lox/lox</sup>* (L2/L2) mice were bred with *tie2* promoter-driven Cre-recombinase transgenic mice to generate *rie2-Cre:PKR1<sup>lox/WT</sup>* mice. The obtained mice were bred with *PKR1<sup>lox/lox</sup>* mice to generate *tie2-Cre:PKR1<sup>lox/lox</sup>* (ec-PKR1<sup>−/−</sup>) mice. The genotype of each mouse was confirmed by PCR. The littermates from same breeding pairs were used as controls (L2/L2). Animals were housed on a 12-h light/dark cycle and were fed a standard rodent chow. All protocols for animal use and euthanasia were in accordance with European guidelines. All experiments were carried out in male mice.

20 *Endothelial cell isolation and culture.*

The isolation of endothelial cells by an immunobead protocol has been reported elsewhere (Lim and Luscinskas, 2006). Briefly, mouse aortae were digested in collagenase, filtered through 100-μm cell strainers, centrifuged, and washed twice with medium. Cell suspensions were incubated with a monoclonal antibody (rat anti-mouse) against platelet endothelial cell adhesion molecule 1 (PECAM-1) for 30 min at 4°C. The cells were washed twice with buffer to remove unbound antibody and were resuspended in a binding buffer containing washed magnetic beads coated with sheep anti-rat immunoglobulin G. Attached cells were washed four to five times in cell culture medium and then were digested with trypsin-EDTA to detach the beads. Bead-free cells were centrifuged and resuspended for culture. Endothelial cells were cultured on gelatine (BD Biosciences, Franklin Lakes, NJ) coated plates and incubated in DMEM-F12 medium (GIBCO/BRL)
supplemented with 15% FBS, 100 units/ml of penicillin, 100 µg/ml of streptomycin, 90 units/ml of heparin, 10 µg/ml of mouse epidermal growth factor (EGF), and 150 µg/ml of endomitogen (Biological Technologies, Stoughton, MA). Endothelial cells were confirmed by positive staining with platelet-endothelial cell adhesion molecule (PECAM) antibody (Santa Cruz Biotechnology). Cell proliferation and in vitro angiogenesis assays were performed as previously described (Guilini et al., 2010).

**Histological and electron microscopy analyses**

Organs were removed from 3, 12 or 24-week-old mice, dissected and frozen for the cutting of frozen sections (5 µm), which were stained with Mallory tetrachrome. For electron microscopy, hearts were fixed by immersion in glutaraldehyde, postfixed by incubation in osmium tetroxide and embedded in epoxy resin, by routine methods (Urayama et al., 2008). Glomerular pathology analyses were based on the assessment of 50 glomeruli/kidney cross-section. Tubule damage (consisting of at least one of the following: dilatation, atrophy, necrosis) was assessed by scoring 100 renal proximal tubules/kidney cross-section on randomly selected high-power microscopy fields (x40).

**TUNEL assay and immunohistochemistry**

TUNEL (terminal dUTP nick end-labeling) assays were performed with an in situ cell-death detection kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. The inventors acquired 6 to 10 images per heart or kidney (3 to 4 animals per genotype group) with a fluorescence microscope (Leica). Results are expressed as the percentage of apoptotic cells. The TUNEL labeling index was calculated as the mean number of DAPI-stained TUNEL-positive nuclei/glomerulus, for 50 glomeruli per kidney (n=3) or of DAPI-stained TUNEL-positive nuclei in 10 high-power microscopic fields (x 40) per heart section (n=3), for the various groups of mice.

Frozen tissue sections for the immunofluorescence staining of structural proteins were fixed, blocked and stained with primary antibodies against a-SMA (sigma), and PKR1 (IGBMC, nikirch), PECAM, Insulin and beta-catenin (Santa Cruz), ZO-1 (Invitrogen), Collagen type VI (Abeam), CD68 (Serothec). Antibody binding was detected by incubation with Fluorescein, Alexa 555- or Alexa 488-conjugated secondary antibodies. Finally, the nuclei were stained with DAPI. Fluorescence was analyzed on a Leica Microsystems TCS SP5 laser scanning confocal microscope. The number of PECAM-1-positive, DAPI-stained cells per heart, pancreas and kidney was determined.
Aortia sections were stained with oil red O and sodium succinate buffer for succinate dehydrogenase activity. The intensity of staining of each section was quantified with Image J software.

**RNA extraction, quantification, and reverse-transcription polymerase chain reaction analysis**

Total RNA was isolated from neonatal and adult mouse organs with TRI®Reagent (Molecular Research Center) and treated with DNase, using the RNase-Free DNase Set. The GAPDH housekeeping gene was used as a control. Results were analyzed with Bio-Rad qPCR software version 2.0. Primer sequences are shown in the Table below.

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Biochemical analyses on urine samples

Urine samples were collected and analyzed as outlined on the ICS website (http://www.ics-mci.fr)

Western Blot—Assay of Protein Production.

Tissue samples were homogenized in lysis buffer. Approximately 20 μg of protein for each were separated by 12% SDS/PAGE, transferred to a nitrocellulose filter, and incubated with antibodies directed against phosphor- Akt and total Akt (Cell signaling) (1:300 dilution). Samples were treated with peroxidase-conjugated secondary antibody (1:1000 dilution), and immunoreactivity was detected by using the ECL chemiluminescent.
detection kit according to the manufacturer's specifications (Amersham Pharmacia). The intensity of the resulting bands was quantified by densitometry.

**Intraperitoneal insulin tolerance test and intraperitoneal glucose tolerance test.**

At age 12, 24, 36 weeks, the mice underwent an intraperitoneal insulin tolerance test (ITT) after a 4-h fast or intraperitoneal glucose tolerance test (GTT) without fasting. Insulin (Umaline®, Rapide) 0.75 units/kg body wt, or glucose, 1 g/kg body wt in normal saline (0.9% NaCl), was administered intraperitoneally. Blood samples were taken from the tail vein at 0, 15, 30, 60, 90 and 120 min after loading, and plasma glucose levels were measured using Countour TS (Bayer) glucometer. For measurement of the plasma insulin levels, 4h after fasting, 2mg/g glucose was administered intraperitoneally. Blood samples were also taken at 0, 20 min for measurement of plasma glucose and insulin levels. Plasma glucose levels were determined using the glucose oxidation method (Yuen and McNeill, 2000). Insulin levels were determined using enzyme-linked immunosorbent assay kits with mouse insulin as standard outlined on the ICS website (http://www.ics-mci.fr).

15 **Statistical analysis**

Data are expressed as means ± SEM. Multigroup comparisons were carried out by one-way ANOVA with post hoc correction. Student's t test was used for pairwise comparisons between groups. For all analyses, values of P<0.05 were considered significant.

20 **Results**

**Endothelial dysfunction and impaired angiogenesis in Ec-PKR† Organs**

To prove loss of PKR1 expression on protein level, co-expression of endothelial marker protein PECAM-1 and PKR1 was performed by immunofluorescence staining in aorta and primary endothelial cells (ECs) isolated from hearts. Unlike the wild aorta and ECs, the PKR1 protein was undetectable in ec-PKR† aorta and ECs ((Figure 16A). Next, the capability of primary ECs to form tube-like structures was investigated upon cultivation on Matrigel (Figure 16B). Control ECs formed capillary-like structures that connected to anastomosing networks within 16 h in culture. In sharp contrast ec-PKR† ECs largely failed to generate tubes, but instead formed cellular aggregates, even if cultured for longer time periods. To investigate whether defective tube formation is due to
impaired proliferation; thus, the proliferation rate of control and ec-PKR1−/− ECs was determined in response to prokineticin and FGF by counting cell number, which revealed considerable difference (Figure 16C). Collectively, analysis of proliferation, reveal first signs for a mechanism that could explain the phenotype of PKR1-deficient cells.

Electron microscopy of the vessels in heart, kidney, pancreas and adipose tissues revealed that endothelial cells from mutant vessels had necrotic nuclei (heart), and a dramatic increase of fenestrations and contained an elevated amount of vesiculovacuolar organelles (kidney), apoptosis (pancreas) and hypervariable diameter of vessels (adipocyte) (Figure 17A). To assess the impact of these endothelial structural defects in angiogenesis, adult mutant and control heart, kidney, pancreas and adipose tissues were immunohistochemically stained for PECAM-1 on cryosections. Significantly low capillary formation was observed in all these organs of mutant mice as compare to these of control mice (Figure 17B) at the age of 12 weeks. These analyses underline the crucial role of PKR1 as a regulator of angiogenic programs in adult tissues.

**Endothelial cell (EC)-specific ablation of PKR1 triggers insulin resistance.**

Metabolic cage analyses revealed significant increases in water and food intake in ec-PKR+/− mice as compared to control mice at the 24 week old age (Figure 18A). Moreover ec-PKR+/− mice had high basal serum insulin levels and low fasting plasma glucose levels (Fig. 18B). The animals also showed higher levels of serum insulin during glucose tolerance test (GTT), which reflects the higher glucose levels in these animals under GTT conditions and is consistent with their prediabetic state.

To determine whether insulin resistance induces compensatory β cell mass expansion, pancreatic β cell number and insulin intensity in ec-PKR+/− and L2L2+/− mice were measured. However, insulin positive beta cell number was not significantly difference between these two groups of mice (Figure 18D and 18E histogram). In contrast, insulin positive cells were enlarged as detected by beta-catenin staining in the mutant pancreas (Figure 18D and 18E, histogram). Next, the inventors considered whether the failure to expand β cell mass in this model might be due to decreased cell survival. Indeed, ec-PKR+/− pancreas displayed approximately 3-fold increase in apoptosis (Figure 18D and 18E histogram). Electron microscopic analyses revealed abnormal mitochondrial structures, including swollen mitochondria and disorganized cristae, in mutant beta cells, confirming hypoxic pancreas (Figure 18F).
Mutant mice exhibit a concomitants decrease in visceral fat mass (1.281± 0.19) as compare to control fat mass (1.93±0.19, n=6, p<0.05). Consistent that mutant mice display slightly decreased body weights as compare to control group (L2L2+/+) at 36-week old age. Ec-PKR" mice showed unequal size in white adipocytes with very pronounced "streaks" interspersed among the adipocytes (Figure 19A). Indeed, HIFa levels increased in ec-PKR"/adipocyte by 80% at the expense of proangiogenic gene such as VEGF induction (Figure 19B). Ec-PKR" mice displayed an increased deposition of fibrillar collagen in the adipose tissue that is indeed partly a result of an increased level of expression of Lysyl oxidase (LOX), collagen Iα, 1α2 and 3a (Figure 19C). The abundance of adipocyte death was detected by TUNEL assay in the mutant adipose tissue (Figure 19D). The resistance to insulin action in adipocytes was also evident by higher serum free fatty acid (FFA) levels in mutant mice than those of control mice (L2L2+/+), suggestive of increased lipolysis in ec-PKR" mice. A decrease in FFA transporters, FATP-1 and FATP-4 expression in mutant adipocytes indicates that FFA uptake by adipocytes was decreased (Figure 19F).

Next, the inventors investigated whether increased FFA impairs insulin signaling pathway in the mutant visceral adipocytes. The expression of the mRNAs encoding IRS-1 and IPβ was decreased 1.5-fold (Figure 19F). This insulin resistance was paralleled by significantly reduced tyrosine and serine phosphorylation of Akt in the adipose tissues of the ec-PKRI-/- mice as compared to control mice in the insulin stimulated state (Figure 19G). During fasting Ec-PKRI-/- skeletal muscle exhibit normal insulin receptor expression and insulin-mediated Akt phosphorylation. However, glucose transport (Glut 1 and 4) levels were significantly decreased in the Ec-PKRI-/- skeletal muscle (data not shown). Thus, ec-PKRI-/- mice exhibit a peripheral insulin resistance.

**Reversal of metabolic disorders in ec-PKRI" mice by adenovirus-mediated expression of PKRI**

To test if PKR1 treatment would reverse metabolic disorders in ecPKRI-/- mice, recombinant adenovirus carrying PKR1 cDNA was injected into ec-PKR-/- mice as well as wild-type mice. Intravenous injection of recombinant adenovirus expressing PKR1 led to an approximate threefold increase of PKR1 expression levels in adipocytes, without altering the food intake and body weight in the adenovirus-control infected mice. In each case, adenovirus-control infected mice were identical with the non-treated mice (data not shown). Injection of PKR1 adenovirus in control mice did not significantly alter glucose
tolerance and insulin sensitivity, as shown in GTTs and ITTs as compared to control mice. Rescue of PKR1 expression by adenovirus injection in ec-PKR1-/- mice amended glucose intolerance and insulin resistance in these mice (Figure 20A). The food and water intake and urine volume were also reduced significantly in ec-PKR1 +/- mice as compared to non-treated ec-PKR1 +/- mice. Accordingly, the gene expressions that are involved in fatty acid uptakes, IR and IRS were significantly improved in ec-PKR1 +/- mice treated with adenovirus PKR1. These results indicate that endothelial PKR1 is a positive regulator of insulin sensitivity.

Example 5: PKR1 agonists as anti-adipogenic factors

Obesity is a risk factor for various disorders, e.g., type 2 diabetes mellitus and cardiovascular diseases and contributes to a rising incidence of mortality and morbidity. Obesity is characterized by expansion of adipose tissue mass that results from increased fat cell number (hyperplasia) and/or increased fat cell size (hypertrophy). An increased number of adipocytes are caused by proliferation and/or differentiation of preadipocytes. An increased size of adipocytes is due to lipid accumulation.

Identification of the factors involved in adipocyte hyperplasia and adipogenesis is important for elucidating the pathophysiology of obesity. Prokineticin-2 is an angiogenic and anorexigenic hormone that activate two G protein-coupled receptors: PKR1 and PKR2. Prokineticin-2 regulates food intake and energy metabolism via central mechanisms. However, the peripheral effect of prokineticin-2 on adipocytes have not been studied until now. Since adipocytes express mainly prokineticin receptor-1 (PKR1), the inventors investigated the role of PKR1 receptor on adipocyte functions.

Materials and methods

Cell culture and differentiation

Murine preadipocytes, 3T3-L1 cells were obtained from American Type Culture Collection (Rockville, MD) and were differentiated into adipocytes, as previously described (Xie et al., 2006). Cells were grown at 37°C in 5% CO₂ in DMEM containing 100 U Gentamycine with 10% FBS and were seeded in six-well plates. Differentiation into mature adipocytes was induced by exposing the cells to the medium supplemented with 0.5 mM IBMX, 1 µM dexamethasone, and 10 µg/ml insulin for 2 days. Cells were then
incubated with medium containing 10 μg/ml insulin. After 2 days, medium was replaced with the medium containing 10 μg/ml insulin.

**Adipocyte differentiation by oil red O staining**

Intracellular triglyceride accumulation was evaluated using Oil Red O staining (Rochford et al., 2004). 3T3-L1 cells cultured on six-well plates were washed with PBS, fixed for at least 30 min with 10% formaldehyde, and stained for 30 min with Oil Red O, which was prepared by diluting 0.5% Oil Red O (Sigma-Aldrich) in triethyphosphate with ¾ 0 (3:2 vol/vol). After cells were washed three times with PBS, stained triglycerides were visually compared. For quantitative analysis of Oil Red O staining, oil red positive cells were detected and calculated as % of total dapi positive cells in the at least 20 random fields from each samples, using an inverted phase contrast microscope (Leica). Each experiment was repeated at least four times. Images were captured at a magnification of 40x with a digital microscope camera system.

**RNA interference**

The RNA interference technique was used for down-regulating PKR1 gene expression as previously described. Small interfering RNAs (siRNAs) were obtained from ambion. SiRNA mixture was prepared by diluting siPORT Amine transfection agent into Opti-MEM and incubated at room temperature for 10 minutes. Then PKR1 siRNA or nonspecific siRNA was diluted into Opti-MEM and was combined at the same ratio with siPORT amine transfection agent mixture prepared before. The 3T3-L1 cells were trypsinized then resuspended into in DMEM containing 100 U gentamycin with 10% FCS, 4.5g/L glucose and transfected with PKR1 siRNA or nonspecific siRNA (100nM) prepared before. The cells were cultured during 48 hours until confluence in DMEM containing 100 U gentamycin with 10% FCS supplemented with 4.5g/l glucose and were seeded in six-well plates. After 48 hours medium was replaced with induction medium to induce differentiation into mature adipocyte or to isolate RNA for detection of PKR1 levels by PCR.

**Cell counting**

3T3-L1 grown in 0.5 or 10 % FBS were treated with prokineticin-2 and then counted every 24 h to evaluate mitotic clonal expansion (Guilini et al., 2010). Total cell number per well was measured every 24h from d 0-4. 3T3-L1 cells were detached from six-well plates.
using 0.25% trypsin (Invitrogen), and were resuspended in condition medium, followed by counting under a light microscope with a hemocytometer. Each experiment was repeated at least four times in duplication.

**Analysis of Gene expression by Quantitative PCR**

Total RNA was prepared from 3T3-L1 cells using a Tri-Reagent (MRC, Cincinnati, OH) according to the manufacturer's instructions. Total RNA (5µg) was reverse-transcribed with Super Script II Reverse Transcription Reagents (Invitrogen). The resultant cDNA was subjected to real-time quantitative PCR, in which a specific primer and probe for mouse adiponectin, resistin, PPARα, PPARγ, C/EBPα, cyclin D, E, cdk2, c-Myc were used (Table below). As an internal control, beta-actin (for 3T3-L1 cells) was used. PCR assays were conducted with the MylQ amplification system (Biorad). Each experiment was repeated at least four times in duplication.

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**Genetically manipulated animal models**

Male PKR1<sup>−/−</sup> mice in C57BL/6 gene background were originally made by homologous recombination for analysis of PKR1<sup>−/−</sup> function in cardiovascular system (Boulberdaa et al., 2011). All of the mice were housed in the animal facility at the Ecole Superieure de Biotechnologie de Strasbourg (ESBS, Illkirch) with a 12:12-h light-dark cycle and constant temperature (22-24°C). The mice had free access to water and diet. The mice at the age of 6 weeks received the High fat diet (Research Diet, New Brunswick, NY) contains 60% kcal in fat or the chow diet (5.1% kcal in fat) as the normal fat diet for 34 weeks. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the French Animal Care Committee, with European regulation-approved protocols. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Perfecture du Bas-Rhin (Permit Number: 67-274). All efforts were made to minimize suffering.

**Evaluation of DNA synthesis and apoptosis**

DNA synthesis was evaluated with Ki67 Labeling (Santa Cruz), as previously described protocol (Urayama et al., 2007; Urayama et al., 2008) apoptosis was detected by the TdT-mediated dUTP nick end-labeling (TUNEL) assay utilizing Apoptag fluorescein in situ apoptosis detection kit (Milipore) according to the manufacturer’s protocol (Urayama et al., 2007; Urayama et al., 2008). 3T3-L1 cells were seeded in 8 chambers culture slides. Preadipocytes grown in 0.5% or 10% FCS were treated with prokineticin-2 for 10 hours. Slides were wash twice in PBS, and fixed in 3,7% formaldehyde for 30min at room temperature. Antigen reassessment for Ki67 was achieved by heating the slides in a water bath to 95°C for 45min in 10mM citric acid, pH 6.0. Non specific binding was blocked by 10% donkey serum for 2 hours at room temperature. Immunostaining was performed by incubating the slides with goat antibodies (dilution 1/100) directed against the Ki67 protein for overnight at 4°C. Then, Alexa 488-conjugated donkey anti-goat secondary antibodies (dilution 1/300 ; Invitrogen) were finally added to the sample for 30 min at room
temperature and slides were mounted in Vectashield mounting medium (Abcys, Paris, France) containing Dapi (dilution 1/500). For TUNEL analysis of fragmented DNA, after being fixed and permeabilized, slides were incubated with TdT terminal transferase and fluorescein-dUTP. Cryosectioned mice adipose tissue samples were obtained and then, 5 Ki67 or TUNEL assay were performed following the manufacturer's protocol. Cells were scored for Ki67 or TUNEL-positive nuclei corresponding to condensed DAPI stained nucleus. The percentage of Ki67 or TUNEL-positive cells was evaluated by viewing each field at x40 magnification. Generally, 10 different microscopic fields containing 10-15 cells each were recorded for each sample. Each experiment was repeated at least three times. Frozen tissue sections were fixed, blocked and stained with primary antibodies against PECAM-1. Antibody binding was detected by incubation with Fluorescein, Alexa 555-conjugated secondary antibodies. Finally, the nuclei were stained with DAPI. Fluorescence was analyzed on a Leica Microsystems TCS SP5 laser scanning confocal microscope. The inventors determined the number of PECAM-1-positive, DAPI-stained 15 cells, by counting 20 fields at x40 magnification per adipocyte section for each group of mice.

**Western blot analysis**

The adipose tissues were taken 20 min after i.p. injection of insulin (50 mg/kg). Proteins from adipose tissue were extracted by lysing the tissues in Tris-buffered saline (50 mM Tris-HCl and 100 mM NaCl, pH 7.5) containing 1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 1 mM EDTA, and 1 mM Sodium vanadate and protease inhibitor cocktails (Roche). Protein concentrations were determined with a BCA Protein Assay Kit (Pierce, Rockford, IL). Lysates were diluted with Laemmli's buffer and were subjected to SDS-PAGE. Electrophoresed proteins were transferred onto a PVDF membrane and then blocked with PBS containing 5% BSA for 1 h, incubated with rabbit anti phosphor-Akt polyclonal antibody (1:1000 in dilution) and total Akt polyclonal antibody (1:500 in dilution) (Cell Signaling Technology, Danvers, MA, USA) overnight at 4 C, followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG (Santa Cruz) (1:5000 in dilution) as secondary antibody for 1 h at room temperature. After the reaction, proteins were visualized with an ECL plus kit (GE Healthcare).
Statistic analysis

Data are expressed as mean ± SEM. Multigroup comparisons were performed using one-way ANOVA with post hoc correction. Comparisons between two groups were made using unpaired Student's t test. For all analyses, P < 0.05 was considered significant utilizing Prism.

Results

Prokineticin-2 inhibits adipogenic differentiation of 3T3-L1 preadipocytes

To investigate the effects of prokineticin-2 on preadipocyte differentiation, 3T3-L1 cells were grown to confluence and then differentiated with adipogenic cocktail (insulin, dexamethasone, and isobutylmethylxanthine) following pretreatment of the cells with prokineticin-2 or its vehicle. Pretreatment of the 3T3-L1 with prokineticin-2 (5 nM) induced a significant decrease in adipogenic cocktail-mediated lipid accumulation, measured by oil red O staining (Figure 21A). Accordingly, prokineticin-2 induced a marked decrease in the expression of molecular markers of adipocyte differentiation, such as adiponectin and resistin (Figure 21B) and also significantly reduced mRNA levels of the key transcription factor of adipogenesis, PPARα, PPARγ and C/EBPα (Figure 21C).

Finally, to study a potential role of PKR1, the inventors studied the effects of prokineticin-2 in 3T3-L1 adipogenesis after acute knock-down of PKR1, utilizing siRNA for PKR1 (3T3-PKR1-/-). PKR1 mRNA was significantly decreased to ~70% by transfection with PKR1 siRNA in comparison with nonspecific siRNA transfection from day 48. In these conditions, prokineticin-2-mediated inhibition of adipogenesis was completely alleviated, as shown by microscopic analysis of Oil red O accumulation in Figure 21D. Interestingly spontaneous adipocyte formation was observed in 3T3-PKR1-/- cells. Taken together, these data show that prokineticin-2 inhibits adipose conversion by activating PKR1.

Prokineticin-2 inhibits proliferation of 3T3-L1 cells

They also investigated involvement of different mechanisms into the prokineticin-2/PKR1 signaling-mediated inhibition of adipogenesis. In control 3T3-L1 cells, the number of cells began to increase from about 24 h after induction with 10% FCS. However, when compared with 10% FCS treated cells, the number of prokineticin-2 treated cells was lower 24-72 h after induction (Fig. 22A). Next, they assessed the cell
cycle via Ki67 staining. Entry into S phase increased within 10h in cells grown in 10 % FBS (Fig. 22B) as compare to cells grown in 0.5% FCS. However, the increase in Ki67 positive cell number by 10% FCS was inhibited by prokineticin-2 treatment. Accordingly, prokineticin-2 induced a marked decrease in the expression of molecular markers of cell cycle, such as cyclin E, cyclin D within 10 hours, Cdk2, and c-Myc within 48 hours (Figure 22C). There were no differences between prokineticin-2 treated and control cells in culture medium supplemented with 0.5% FCS, indicating that prokineticin-2 has no cytotoxic effect. A direct apoptotic effect of prokineticin-2 was excluded by TUNEL staining, which did not reveal any detectable cell mortality 48 hours after treatment of the 10 cells with prokineticin-2 (Figure 22D). Prokineticin-2 was also downregulated cycling gene expression 48 hours after adipogenic cocktail (insulin, dexamethasone, and isobutylmethylxanthine).

**PKRl null mutant (PKRl/-) mice develop obesity**

PKRl/- mice exhibit severe obesity after 40 weeks age on a normal chow diet (5.1% kcal in fat). Compare to the wild type, mutant mice exhibit a ~40% increase in body weight at 40 week-old ages (Figure 23A). The visceral fat tissues were increased by ~2.6 times in weight in the PKRl/- mice relative to the wild-type littermates on a normal chow diet (Figure 23A). However, the mutant mice consumed identical amounts of food on the chow diet to that of the wild type mice. Semi-thin sections of adipose tissues revealed that the number of the adipocyte was increased by approximately 1.5 times in the mutant adipose tissues (Figure 23B). In concert to that, the abundance of adipocyte proliferation was detected by Ki67 immunoassay in the mutant adipose tissue (Figure 23C). These results show that inactivation of PKRl leads to an increase in the body fat content.

Although the proliferation rate is increased in PKRl/- adipose tissue, PECAM-1 staining revealed that the number of capillary network in PKRl/- adipose tissue remained unchanged as compare to wild adipose tissue (Figure 24A). PKRl/- adipocytes exhibited ultrastructural features of necrosis (i.e., ruptured basal membranes, organelle degeneration), but no detectable features of apoptosis (Figure 24B upper). Small cytoplasmic lipid droplets and swollen mitochondria with loss of cristae were evident in the PKRl/- adipocytes (Figure 24B lower). Consistent with these findings, the Hypoxia inducible factor, HIF transcript was significantly increased in the PKRl/- adipocytes, indicating a development of hypoxia. Next the inventors investigated whether insulin
response in PKRl-/- adipose tissue is impaired. Indeed, insulin-stimulated phosphorylation of Akt kinase at threonin 308 and serine 478 was severely attenuated in PKRl-/- adipose tissue (Figure 24C).

**Conclusion**

These results show for the first time that prokineticin-2 via PKRl plays a role as an anti-adipogenic determinant regulating adipocyte proliferation and differentiation, using 3T3-L1 cells and PKRl +/- mice.

Utilizing 3T3-L1 preadipocytes and PKRl-null mutant mice, the inventors identified that prokineticin-2 via PKRl signaling inhibits adipogenesis that is due to inhibition of adipocyte hyperplasia. In 3T3-L1 cells prokineticin-2 inhibits adipogenesis induced by adipogenic stimuli. Knock down of PKRl expression by small-interfering RNA in these cells abrogated decreases in adipogenesis by prokineticin-2, indicating PKRl involvement. Prokineticin-2 also inhibits proliferation of 3T3-L1 cells that was confirmed by adipogenic and cell cycling gene expressions in these cells. PKRl -deficient mice start to become obese under a normal fat diet at the age of 40 weeks due to an increase in number of proliferating adipocytes.

**References**

Chen et al. (2005) Mol Pharmacol 67:2070-2076
LeCouter et al. (2001) Nature 412, 877-884;
Lim and Luscinskas, Methods in Molecular Biology, 2006. Volume 341, 141-154
Martucci et al. (2006) Br. J. Pharmacol. 147, 225-234
Ng et al. (2005) Science 308. 1923-1927
Plochl J. (1884) Chem. Ber. 17: 1623
Urayama et al., (2007) FASEB J. 21, 2980-2993
Claims

1. A pharmaceutical composition comprising a PKR1 agonist of formula (I)

\[
\text{A} \quad \begin{array}{c}
\text{H} \\
\text{N} \\
\text{m} \\
\text{CH}_2 \\
\text{n} \\
\text{R}_1 \\
\text{N} \\
\text{R}_2 \\
\text{R}_3 \\
\text{NH} \\
\text{H}_2\text{N}
\end{array}
\]

wherein

- A is selected from 0 and 1;
- m is selected from 0 and 1;
- n is selected from 0, 1 and 2;
- \( R^1 \) is selected from the group consisting of a hydrogen atom; a halogen atom; a \((C_1-20)\)-alkyl, a \((C_2-C_6)\)-alkenyl and a \((C_2-C_6)\)-alkynyl group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; a \((C_6-C_{12})\)-aryl; a 5 to 7-membered-ring heterocycle; a \((Ci-C_6)\)-alkoxy, a \((C_2-C_6)\)-acyl, a \((C_2-C_6)\)-ester, an amino group, a \((Ci-C_6)\)-amine, a \((Ci-C_6)\)-amide, a \((Ci-C_6)\)-imine, a \((Ci-C_6)\)-nitrile, a \((Ci-C_6)\)-thioalkyl, a \((Ci-C_6)\)-sulfone and a \((Ci-C_6)\)-sulfoxide group wherein the alkyl part of the group is optionally interrupted by one of several heteroatoms chosen among N, O and S, and is optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol;
- \( R^2 \) is selected from the group consisting of a \((C_6-C_{12})\)-aryl group and a 5 to 7-membered-ring heterocycle, preferably from phenyl and furan, optionally substituted by at least one substituent selected from the group consisting of a \((Ci-C_6)\)-alkyl, a \((C_2-C_6)\)-alkenyl, a \((C_2-C_6)\)-alkynyl, a non substituted \((C_6-C_{12})\)-aryl, a 5 to 7-membered-ring heterocycle, a \((Ci-C_6)\)-alkoxy, a \((C_2-C_6)\)-acyl, a \((Ci-C_6)\)-alcohol, a carboxylic group, a \((C_2-C_6)\)-
C₆)-ester, a (Ci-C₆)-amine, an amino group, a (Ci-C₆)-amide, a (Ci-C₆)-imine, a (CrC₆)-nitrile, a hydroxyl, an aldehyde, a (Ci-C₆)-halogenoalkyl, a thiol, a (Ci-C₆)-thioalkyl, a (Ci-C₆)-sulfone and a (Ci-C₆)-sulfoxide group;

R³ is selected from the group consisting of a (Ci-C₆)-alkyl, a (C₂-C₆)-alkenyl and a 5 (C₂-C₆)-alkynyl and a (Ci-C₆)-amine group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a (C₆-C₂)-aryl and a 5 to 7-membered-ring heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C₆)-alkyl, a (C₁-C₃)-alkoxy group and a halogen atom; or any pharmaceutically acceptable salt thereof.

2. The pharmaceutical composition according to claim 1, wherein

A is \( \mathbf{R}^1 \)

and

\( \mathbf{R}^1 \) is selected from the group consisting of a hydrogen atom; a halogen atom; a (Ci-C₃)-alkyl, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; a (Ci-C₃)-alkoxy, a (C₂-C₃)-acyl, a (C₂-C₃)-ester, a (Ci-C₃)-amine, an amino group, a (Ci-C₃)-amide and a (Ci-C₃)-thioalkyl, group wherein the alkyl part of the group is optionally interrupted by one of several heteroatoms chosen among N, O and S, and is optionally substituted by at least one substituent selected from an hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol.

3. The pharmaceutical composition according to claim 2, wherein \( \mathbf{R}^1 \) is selected from the group consisting of a hydrogen atom, a halogen atom, an amino group, a (Ci-C₃)-alkyl group and a (Ci-C₃)-alkoxy group.

4. The pharmaceutical composition according to any one of claims 1 to 3, wherein \( \mathbf{R}^2 \) is selected from the group consisting of a phenyl group and a 5 to 7-membered-ring N-, O- or S-heterocycle, preferably a 5-membered-ring N-, O- or S-heterocycle, optionally...
substituted by at least one substituent selected from the group consisting of a (Ci-C₃)-alkyl, a (Ci-C₃)-alkoxy, a (Ci-C₃)-acyl, a (Ci-C₃)-alcohol, a carboxylic group, a (C₂-C₃)-ester, a (Ci-C₃)-amine, an amino group, a (Ci-C₃)-amide, a hydroxyl, an aldehyde, a (C₁-C₃)-halogenoalkyl, a thiol, a (Ci-C₃)-thioalkyl and a halogen atom.

5. The pharmaceutical composition according to any one of claims 1 to 4, wherein R² is selected from the group consisting of a phenyl, a pyrrole, a furan and a thiophene group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C₃)-alkyl and a (Ci-C₃)-alkoxy group.

6. The pharmaceutical composition according to any one of claims 1 to 4, wherein R³ is selected from the group consisting of a (Ci-C₆)-alkyl and a (Ci-C₆)-amine group, optionally substituted by at least one substituent selected from a hydroxyl, a carboxylic group, an aldehyde, a halogen atom and a thiol; and a phenyl group and a 5 to 7-15 membered-ring N-, O- or S-heterocycle, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C₃)-alkyl, a (Ci-C₃)-alkoxy group and a halogen atom.

7. The pharmaceutical composition according to any one of claims 1 to 6, wherein R³ is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C₃)-alkyl group, preferably methyl, a (Ci-C₃)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine.

8. The pharmaceutical composition according to claim 1, wherein

A is \[ \text{R}^1 \]

\[ \text{R}^1 \text{ is selected from the group consisting of a hydrogen atom and an amino group; } \text{R}^2 \text{ is selected from the group consisting of a 5-membered-ring heterocycle and a phenyl group, preferably from a furan, a thiophene and a phenyl group, optionally substituted by a (Ci-C₃)-alkoxy group, preferably a methoxy group; and } \text{R}^3 \text{ is selected from the group } \]

9. The pharmaceutical composition according to any one of claims 1 to 8, wherein

A is selected from the group consisting of a methyl, an ethyl, a propyl, an isopropyl, a butyl, an isobutyl, a tert-butyl group; and a phenyl group, optionally substituted by at least one substituent selected from the group consisting of a (Ci-C₃)-alkyl group, preferably methyl, a (Ci-C₃)-alkoxy group, preferably methoxy, and a halogen atom, preferably chlorine.
consisting of a (C<sub>i</sub>-C<sub>6</sub>)-alkyl, preferably a tert-butyl group, and a phenyl group, optionally substituted by a substituent selected from the group consisting of a (C<sub>i</sub>-C<sub>3</sub>)-alkoxy group and a halogen atom, preferably from a methoxy group and chlorine.

9. The pharmaceutical composition of claim 1, wherein

A is

\[
\begin{array}{c}
\text{NH} \\
\text{H}_2\text{N}
\end{array}
\]

10. R<sup>2</sup> is selected from the group consisting of a 5-membered-ring heterocycle and a phenyl group, preferably from a furan, a thiophene and a phenyl group, optionally substituted by a (C<sub>i</sub>-C<sub>3</sub>)-alkoxy group, preferably a methoxy group; and R<sup>3</sup> is selected from the group consisting of a (C<sub>i</sub>-C<sub>6</sub>)-alkyl, preferably a tert-butyl group, and a phenyl group, optionally substituted by a substituent selected from the group consisting of a (C<sub>1</sub>-C<sub>3</sub>)-alkoxy group and a halogen atom, preferably from a methoxy group and chlorine.

10. The pharmaceutical composition of claim 9, wherein R<sup>2</sup> is selected from the group consisting of a phenyl group, optionally substituted by a (C<sub>i</sub>-C<sub>3</sub>)-alkoxy group, preferably a methoxy group, and a furan group; and R<sup>3</sup> is a phenyl group, optionally substituted by a substituent selected from the group consisting of a (C<sub>i</sub>-C<sub>3</sub>)-alkoxy group and a halogen atom, preferably from a methoxy group and chlorine.

11. The pharmaceutical composition of claim 1, wherein the PKR1 agonist is selected from the group consisting of

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<td>IS22</td>
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<td>------</td>
<td>------</td>
</tr>
<tr>
<td><img src="image1" alt="IS21" /></td>
<td><img src="image2" alt="IS22" /></td>
</tr>
</tbody>
</table>

The images are chemical structures.
12. The pharmaceutical composition according to any one of claims 1 to 11, further comprising one or more other active compounds associated with pharmaceutically acceptable excipients and/or carriers.

13. A pharmaceutical composition according to any one of claims 1 to 12 for use for the prevention or treatment of a disease selected from the group consisting of a vascular disease, a neurodegenerative disease, a disease involving impaired gastrointestinal motility, obesity, Kallmann syndrome, normosmic hypogonadotropic hypogonadism, hyperthyroidism, in particular Familial isolated hyperparathyroidism (FIHP), disturbances of circadian rhythm, sleeping disorders and a pregnancy and placental function-related disorder.

14. The pharmaceutical composition according to claim 13, wherein the disease is a vascular disease selected from the group consisting of myocardial infarction, acute
coronary syndrome, ischemic stroke, abdominal aorta aneurysm, cerebral aneurysm, ischemic heart disease, coronary heart disease, peripheral arterial disease, restenosis, angina pectoris and diabetic angiopathy.

15. The pharmaceutical composition according to claim 14, wherein the disease is a neurodegenerative disease selected from the group consisting of Parkinson's disease and other parkinsonian disorders, Alzheimer's disease and other dementing neurodegenerative disorders, amyotrophic lateral sclerosis, multiple sclerosis, Creutzfeldt-Jakob disease, Huntington's disease and neuronal degeneration associated to multiple sclerosis.

16. The pharmaceutical composition according to claim 13, wherein the disease is a disease involving impaired gastrointestinal motility selected from the group consisting of irritable bowel syndrome, diabetic gastroparesis, postoperative ileus, chronic constipation, and gastroesophageal reflux disease, Hirschsprung's disease (congenital colonic aganglionosis) and chronic dyspepsia.

17. The pharmaceutical composition according to any one of claims 1 to 12 for use for promoting the differentiation of cardial epicardin+ progenitor cells into cardiomyocytes in a subject affected with a cardiac disease and/or the differentiation of renal epicardin+ progenitor cells into vasculogenic and/or glomerular cells in a subject affected with a renal disease.

18. The pharmaceutical composition according to claim 17, wherein the cardiac disease is selected from the group consisting of heart failure, myocardial infarction, ischemic heart disease and the cardiorenal syndrome.

19. The pharmaceutical composition according to claim 17, wherein the renal disease is selected from the group consisting of chronic renal disease, renal artery stenosis, renal failure, acute kidney injury, acute-on-chronic renal failure, ischemic nephropathy, Churg-Strauss syndrome, Wegener's granulomatosis and the cardiorenal syndrome.
20. The pharmaceutical composition according to any one of claims 1 to 12 for use for preventing fat tissue development in heart and/or kidney in a subject affected with obesity

21. An *in vitro* or *ex vivo* method of producing cardiomyocytes, wherein said method comprises the step of contacting cardial epicardin+ progenitor cells with a PKRl agonist according to any one of claims 1 to 12.

22. An *in vitro* or *ex vivo* method of producing glomerular cells, wherein said method comprises the step of contacting renal epicardin+ progenitor cells with a PKRl agonist according to any one of claims 1 to 12.

23. The pharmaceutical composition according to any one of claims 1 to 12 for use for treating or preventing insulin resistance in a subject.

24. The pharmaceutical composition according to claim 23, wherein the insulin resistance is associated with type II diabetes.

25. The pharmaceutical composition according to claim 23, wherein the insulin resistance is associated with obesity.

26. The pharmaceutical composition according to claim 23, wherein the insulin resistance is associated with hypertension.

27. The pharmaceutical composition according to claim 23, wherein the insulin resistance is associated with cardiac and/or renal disorders.

28. The pharmaceutical composition according to any one of claims 1 to 12 for use for treating or preventing type II diabetes in a subject.
Cardiomyocyte beating frequency

Before isoproterenol

After isoproterenol

Figure 3A

Troponin/dapi staining

Vehicle

PK-2 treated

Figure 3C
Figure 4
Figure 5
### Urine analysis

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<th>Wild type PKR1(+/+)</th>
<th>Null mutant PKR1(−/−)</th>
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<tr>
<td>Urea (mmol/l)</td>
<td>1291.5±156.19</td>
<td>1265.12±137.91</td>
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<tr>
<td>Creatinine (µmol/l)</td>
<td>4729.87±776.07</td>
<td>4611.13±367.58</td>
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<td>Creatinine (mg/24h)</td>
<td>1.18±0.15</td>
<td>0.76±0.07*</td>
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<tr>
<td>Creatinine (µM/24h/g BW)</td>
<td>336.89±37.5</td>
<td>225.11±21.9*</td>
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<td>Urea/Creat (mol/mol)</td>
<td>279.74±13.12</td>
<td>264.44±13.24</td>
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<td>Phos (mmol/l)</td>
<td>31.91±2.29</td>
<td>51.49±5.68*</td>
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<tr>
<td>Phos/Creat (mol/mol)</td>
<td>7.67±1.08</td>
<td>11.14±0.88*</td>
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<tr>
<td>Phos excretion (nmol/(mg Creat/ g BW))</td>
<td>2.07±0.28</td>
<td>2.99±0.23*</td>
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<td>Ca (mmol/l)</td>
<td>1.59±0.26</td>
<td>2.05±0.38</td>
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<td>Ca/Creat (mol/mol)</td>
<td>0.37±0.07</td>
<td>0.43±0.07</td>
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<td>Na (mmol/l)</td>
<td>180.38±19</td>
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<td>Na/Creat (mol/mol)</td>
<td>40.11±3.2</td>
<td>40.69±2.16</td>
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<td>Na excretion (mg Creat/g BW)</td>
<td>10.57±0.69</td>
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<td>K (mmol/l)</td>
<td>335±44.92</td>
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<td>K/Creat (mol/mol)</td>
<td>71.3±3.36</td>
<td>69.23±4.15</td>
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<td>K excretion (mg Creat/g BW)</td>
<td>19.39±0.74</td>
<td>18.52±0.89</td>
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<td>Na/K</td>
<td>0.56±0.03</td>
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<td>Cl (mmol/l)</td>
<td>233.88±25.08</td>
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<td>Cl/Creat (mol/mol)</td>
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<td>Cl excretion (mg Creat/g BW)</td>
<td>13.88±0.65</td>
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<td>Glucose (mmol/l)</td>
<td>2.04±0.29</td>
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<td>Total protein units (g/l)</td>
<td>6.25±1.16</td>
<td>3.93±0.71*</td>
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### Blood analysis

<table>
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<th></th>
<th>Wild type PKR1(+/+)</th>
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<td>Urea (mmol/l)</td>
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<td>Creatinine (µmol/l)</td>
<td>12.28±0.47</td>
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<td>Phos (mmol/l)</td>
<td>2.24±0.11</td>
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<td>Phos/Creat (mol/mol)</td>
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<td>Na (mmol/l)</td>
<td>151.75±1.05</td>
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<td>Na/Creat (mol/mol)</td>
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<td>10119.99±853.53*</td>
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<td>K (mmol/l)</td>
<td>7.68±0.29</td>
<td>7.33±0.19</td>
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<tr>
<td>K/Creat (mol/mol)</td>
<td>630.94±28.12</td>
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<td>Na/K</td>
<td>19.93±0.73</td>
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<td>Cl (mmol/l)</td>
<td>112.75±0.82</td>
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<td>Cl/Creat (mol/mol)</td>
<td>9.28±0.35</td>
<td>7.60±0.63*</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>11.33±2.0</td>
<td>9.73±0.84</td>
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<tr>
<td>Total protein units (g/l)</td>
<td>51.67±11.34</td>
<td>58.2±2.31</td>
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Creat:Creatinine, Phos: Phosphate, BW: body weight * P<0.05

Figure 6
Figure 9
Figure 10
Figure 11

Vehicle

IS1

In vitro angiogenesis

Figure 12

<table>
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<tr>
<th>Compound</th>
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<tr>
<td>3b</td>
<td>Ar = 2-thiophenyl, R = Ph</td>
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<tr>
<td>3c</td>
<td>Ar = R = Ph</td>
</tr>
<tr>
<td>3d</td>
<td>Ar = (4-MeO)-Ph, R = Ph</td>
</tr>
<tr>
<td>3e</td>
<td>Ar = 2-furyl, R = t-Bu</td>
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<td>3f</td>
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<td>3g</td>
<td>Ar = 2-furyl, R = (4-MeO)-Ph</td>
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<td>3h</td>
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</tr>
<tr>
<td>3i</td>
<td>Ar = 2-furyl, R = (2-MeO)-Ph</td>
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<td>3j</td>
<td>Ar = 2-furyl, R = (3-MeO)-Ph</td>
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<tr>
<td>3e</td>
<td>Ar = [3,4-(MeO)₂]-Ph, R = Ph</td>
</tr>
</tbody>
</table>
Calcium release

Figure 14

PKR1 internalization

Figure 15
Figure 16
Figure 17
Figure 18
Figure 19
Figure 20
Figure 21
Figure 22
Figure 23
Figure 24
Figure 25
Figure 28

Fold increase over negative control

Figure 29

Fold increase over negative control
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
   a. (means)
      □ on paper
      ☑ in electronic form
   b. (time)
      ☑ in the international application as filed
      □ together with the international application in electronic form
      □ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in 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**

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

- A61K
- A61P

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

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

- EPO-Internal
- BIOSIS
- CHEM ABS Data
- EMBASE
- WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Further documents are listed in the continuation of Box C.

See patent family annex.

**Date of the actual completion of the international search**

1 March 2012

**Date of mailing of the international search report**

08/03/2012

Name and mailing address of the ISA:

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV RIJSWIJK

Tel. (+31-70) 340-2040,

Fax: (+31-70) 340-3016

Authorized officer

Hoff, Philippe
<table>
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### INTERNATIONAL SEARCH REPORT

**Form PCT/ISA/210 (continuation of second sheet) (April 2008)**

**PCT/EP2011/073474**

### DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>SHODA, MIYUKI ET AL: &quot;Virtual screening leads to the discovery of an effective antagonist of lymphocyte function-associated antigen-1&quot;, CHEMMEDCHEM, vol. 2, no. 4, 2007, pages 515-521, XP002636100, ISSN: 1860-7179 tables 2-4</td>
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