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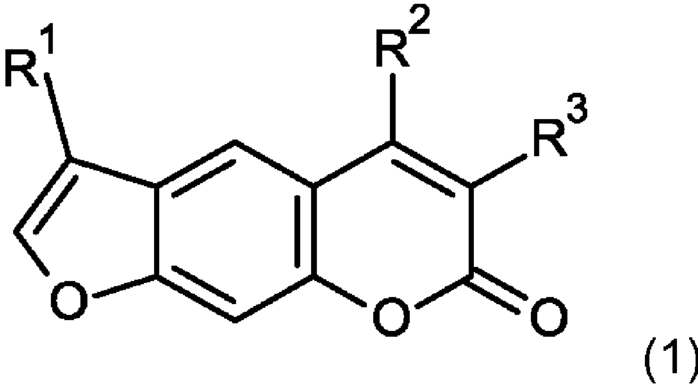
(54) **Title**: PSORALEN DERIVATIVES FOR PREVENTING OR TREATING HEART FAILURE OR CARDIAC HYPERTROPHY



(57) **Abstract**: The present invention relates to compound characterized by a general formula 1, wherein R^1 is a aryl or a heteroaryl, and - R^2 and R^3 independently of each other are hydrogen or a C_1-C_5 alkyl, but at least one of R^2 and R^3 is not hydrogen, or together are a C_3 or C_4 alkyl forming a 5- or 6 membered ring, for use in a method for treating of heart failure, hypertension, cardiac hypertrophy or cancer.

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Psoralen derivatives for preventing or treating heart failure or cardiac hypertrophy

Description

The present invention relates to the use of psoralen derivatives in a method for preventing or treating heart failure, hypertension or cardiac hypertrophy, particularly caused by α-adrenergic-receptor-induced hypertrophy of cardiomyocytes.

Protein kinase A (PKA) is a cAMP-dependent Ser/Thr kinase that plays a central role in cAMP-dependent signal transduction pathways. PKA consists of a dimer of regulatory (R) subunits, each of which binds one catalytic (C) subunit. The cAMP/PKA pathway controls a plethora of physiological processes.

AKAPs are a family of around 50 proteins. The defining characteristic of an AKAP is a PKA binding domain that interacts with the R subunit dimer of PKA. Some AKAPs interact with PKA RI type subunits, some with PKA RII type subunits, and some are dual specific interacting with both R subtypes.

The A kinase anchoring protein AKAP-Lbc tethers protein kinase A (PKA) and possesses a guanine nucleotide exchange factor (GEF) activity, which stimulates the small GTP-binding protein RhoA. In particular, AKAP-Lbc catalyzes the exchange of GDP to GTP in RhoA promoting its activation. RhoA controls a plethora of cellular processes including gene expression, cellular growth and cytoskeletal dynamics. Pathological changes of its function, in particular with regard to cytoskeletal structures, are often responses to chronic stress signals, a common characteristic of many diseases including chronic heart failure.

It has been shown that AKAP-Lbc is critical for activating RhoA and transducing hypertrophic signals downstream of 1-adrenergic receptors (ARs). Moreover, suppression of AKAP-Lbc expression by infecting rat neonatal ventricular cardiomyocytes with lentiviruses encoding AKAP-Lbc specific short hairpin RNAs strongly reduces both α1-AR-mediated RhoA activation and hypertrophic responses (Appert-Collin et al. PNAS, 104(24), 10140-10145, 2007).

Garazd et al. (Chemistry of Natural Compounds 2002, 38, 230-242) show annulated furocoumarins having cardiotropic activity, but fail to distinctly point towards a specific medical utility.

CN101307056B (to SHANGHAI INST MATERIA MEDICA) shows 4-alkyl-4'-phenyl-substituted psoralen derivatives for the treatment of diabetes mellitus.

Based on this background, it is the objective of the instant invention to provide novel inhibitors of the guanine nucleotide exchange factor activity of AKAP-Lbc for use in a method for preventing or treating heart failure, hypertension or cardiac hypertrophy.

It was surprisingly found that psoralen derivatives can specifically inhibit the guanine nucleotide exchange factor activity of AKAP-Lbc. Additionally, it has been found that by inhibition of the nucleotide exchange factor activity and the resulting inhibition of the RhoA activation, the psoralen derivatives of the present invention inhibit the α1-adrenergic induced increase in beating frequency of cardiomyocytes. The compounds provided by the present invention represent not only valuable tools, but also enable novel approaches for the treatment of diseases that involve AKAP-Lbc-mediated activation of RhoA such as chronic heart failure, hypertension or cardiac hypertrophy.

In general, the psoralen derivatives of the invention are characterized by having a mononuclear C_6 aryl or pentacyclic or hexacyclic heteroaryl in the 4' position of the psoralen scaffold (R^1), and optionally an alkyl substitution in position 3 or 4 (R^3 and/or R^2).

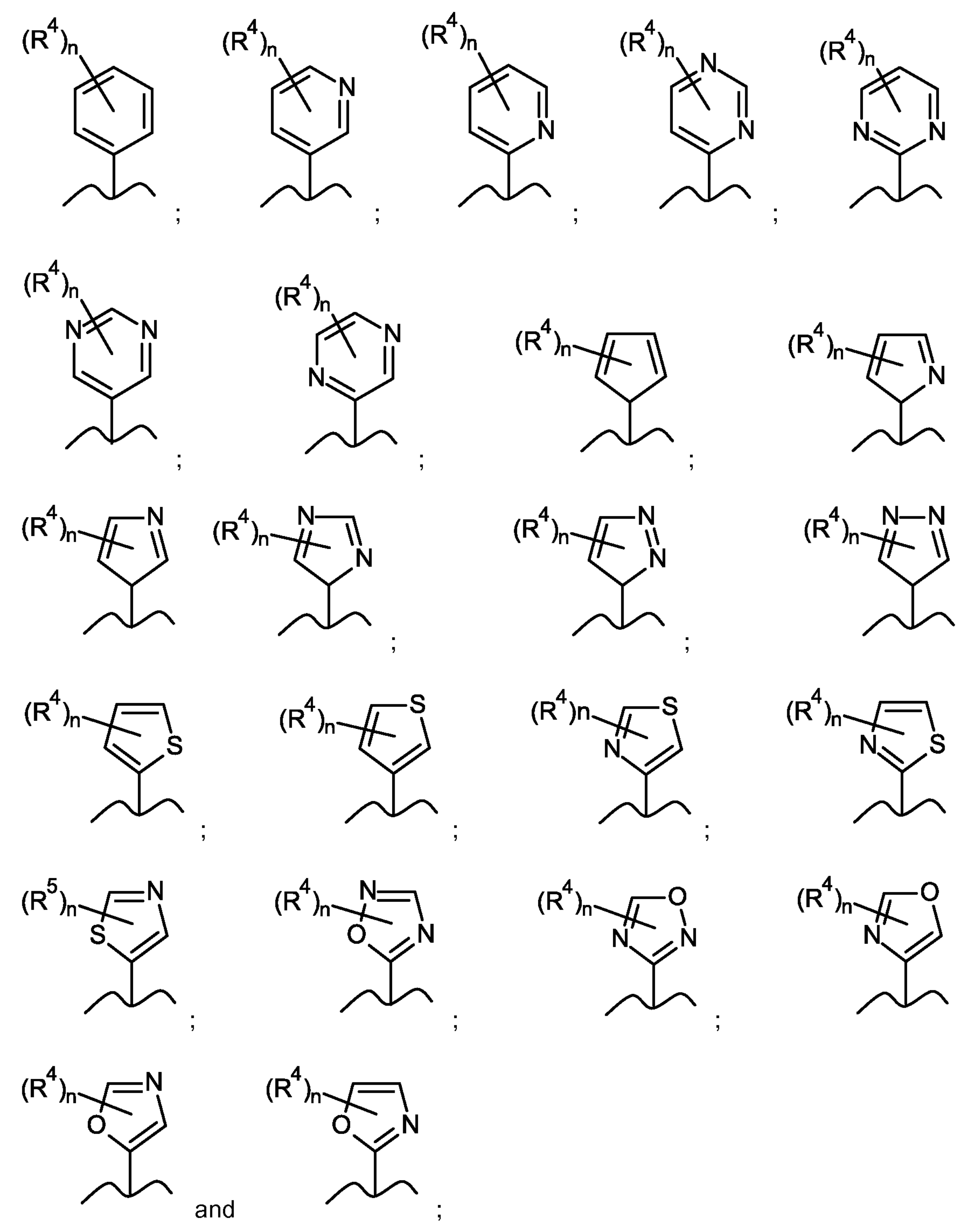
The term aryl in the context of the present invention signifies a cyclic aromatic hydrocarbon. A heteroaryl in the context of the present invention refers to an aryl that comprises one or several nitrogen, oxygen and/or sulphur atoms. The aryl substitution in position 4' may be substituted by one or more functional groups as defined below. Such functional group may enhance the solubility in an aqueous medium of the compound of the invention.

According to one aspect of the invention, a compound characterized by a general formula 1

R1 R2 R3 O O O (1),

wherein

- R^1 is aryl or heteroaryl selected from the group comprised of:



wherein

n is 0, 1, 2, 3 or 4, and

- each R^4 independently from any other is $COOR^5$ (carboxylic acid or ester), $CONR^5_2$ (amide), $C(NH)NR^5_2$ (substituted or unsubstituted amidine), CN_4H_2, NR^5, COR^5, OR^5, CF_3, OCF_3, CN, NO_2, F, Cl or Br, or
- two R^4 together are a dioxyalkyl forming a five- or six-membered ring,

  and

- R^2 and R^3

- independently of each other are hydrogen or a C_1-C_5 alkyl, but at least one of R^2 and R^3 is not hydrogen, or
- together are a C_3 or C_4 alkyl, forming a 5- or 6 membered ring,

  wherein each R^2 and R^3 independently of the other bears 0, 1 or 2 substituents R^6, with any non-substituted position being taken by hydrogen or fluorine, each R^6 being selected, independently from any other, from $COOR^5$, $CONR^5_2$, $C(NH)NR^5_2$, CN_4H_2, NR^5_2, COR^5, OR^5, CF_3, OCF_3, CN, NO_2, Cl and Br,
- with each R^5 independently from any other being hydrogen or a C_1-C_4 alkyl,

is provided for the use in a method for preventing or treating cardiac hypertrophy, hypertension or heart failure.

A C_1-C_4 alkyl in the context of the present invention signifies a saturated linear or branched hydrocarbon having 1, 2, 3 or 4 carbon atoms, wherein one carbon-carbon bond may be unsaturated and one CH_2 moiety may be exchanged for oxygen (ether bridge). Non-limiting examples for a C_1-C_4 alkyl are methyl, ethyl, propyl, prop-2-enyl, n-butyl, 2-methylpropyl, *tert*-butyl, but-3-enyl, prop-2-inyl and but-3-inyl.

A C_1-C_5 alkyl in the context of the present invention signifies a saturated linear or branched hydrocarbon having 1, 2, 3, 4 or 5 carbon atoms, wherein one carbon-carbon bond may be unsaturated and one CH_2 moiety may be exchanged for oxygen (ether bridge). Non-limiting examples for a C_1-C_5 alkyl include the examples given for C_1-C_4 alkyl above, and additionally 3-methylbut-2-enyl, 2-methylbut-3-enyl, 3-methylbut-3-enyl, n-pentyl, 2-methylbutyl, 3-methylbutyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 1,2-dimethylpropyl and pent-4-inyl.

In some embodiments, each R^5 independently from any other R^5 is hydrogen, CH_3, C_2H_5, C_3H_7 or C_4H_9.

In some embodiments, R^1 is substituted by one R^4 group (n is 1). In some embodiments, R^1 is substituted by two R^4 groups (n is 2).

In some embodiments, R^1 is phenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl or 5-pyrimidyl. In some embodiments, R^1 is a six-membered ring substituted by one or two R^4 substituents in para and/or ortho position to the attachment position of R^4.

In some embodiments, R^1 is selected from phenyl, 2-pyridyl, 3-pyridyl, 2-pyrimidyl or 5-pyrimidyl, and R^1 is substituted by one R^4 group in para-position, with R^4 selected from methoxy (OCH_3) or ethoxy (OC_2H_5), with all other substituents of R^1 being H.

In some embodiments, R^1 is an unsubstituted 3-pyridyl group.

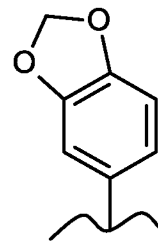
In some embodiments, R^4 is a methoxy group (OCH_3). In some embodiments, R^1 is methoxyphenyl.

In some embodiments, R^1 is

- 2,3-dihydrobenzodioxine:

, or

- 1,3-benzodioxole: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In some embodiments, one of R^2 and R^3 is C_2H_5, C_3H_7, C_4H_9 or C_5H_{11} and the other one is hydrogen or CH_3.

In some embodiments, one of R^2 and R^3 is $O(CH_xF_{2-x})_mCH_yF_{3-y}$ or $(CH_2)_mZ$, wherein Z is selected from CH_3, $(CH_2)_xOR^7$, $COOR^7$, $CONR^7$, with m being 0, 1, 2 or 3, x being 0, 1 or 2, y being 0, 1, 2 or 3, and R^7 being hydrogen, CH_3 or C_2H_5.

In some embodiments, R^2 is methyl, ethyl, propyl, prop-2-enyl, n-butyl, 3-methylbut-2-enyl or 3-methylbut-3enyl.

In some embodiments, R^3 is hydrogen or methyl.

In some embodiments, R^2 is methyl, ethyl, propyl, prop-2-enyl, n-butyl, 3-methylbut-2-enyl or 3-methylbut-3-enyl, and R^3 is hydrogen or methyl.

In some embodiments, R^1 is either

- phenyl, 2-pyridyl, 3-pyridyl, 2-pyrimidyl or 5-pyrimidyl, and R^1 is a (mono-) para-positioned methoxy (OCH_3) or ethoxy (OC_2H_5) group, with all other substituents being H, or
- R^1 is an unsubstituted 3-pyridyl group, and

R^2 is methyl, ethyl, propyl, prop-2-enyl, n-butyl, 3-methylbut-2-enyl or 3-methylbut-3enyl, and R^3 is hydrogen or methyl.

In some embodiments, R^2 or R^3 is substituted by one functional group selected from $C(NH)NR^5_2$, CN_4H_2, NR^5_2, $COOR^5$, $CONR^5_2$, COR^5, CF_3, OCF_3, OR^5, CN, NO_2, F, Cl, and Br,

wherein each R^5 independently from any other is hydrogen or a C_1-C_4 alkyl. In some embodiments, the functional group is in ω-position (terminal position on the alkyl chain).

In some embodiments, R^2 or R^3 is substituted by a COOR", CONR"$_2$, CN_4H_2,, OR", OCF_3 or CF_3, wherein each R" independently from another is hydrogen, CH_3, C_2H_5, C_3H_7, or C_4H_9.

5 In some embodiments, R^2 is ω-hydroxy-n-propyl, n-propanol, ω-hydroxy-n-butyl or 2-hydroxy-2-methylpropyl. In some embodiments, R^2 is ω-carboxy-n-propyl, ω- carboxy-n-butyl or 2-carboxy -2-methylpropyl.

In some embodiments, R^2 is a C_3 or C_4 alkyl and R^3 is hydrogen.

In some embodiments, R^2 is a C_3 or C_4 alkyl and R^3 is methyl. In some embodiments, R^2 is
10 methyl and R^3 is methyl.

According to some embodiments of the invention, a compound characterized by the general formula 1

(1),

wherein

15 - R^1 is phenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl or 5-pyrimidyl, and R^1 is substituted by n substituents, n being 0, 1 or 2, and each substituent independently from any other is OR^7, COR^7, $COOR^7$, $CONR^7{}_2$, CN, OCF_3, CF_3, F, Cl, Br, CN_4H_2, $C(NH)NR^7{}_2$ or NO_2 ,
- R^2 is hydrogen, $O(CH_xF_{2-x})_mCH_yF_{3-y}$ or $(CH_2)_mZ$, wherein Z is selected from CH_3,
20 $(CH_2)_xOR^7$, $COOR^7$, $CONR^7$, with
  - m being 0, 1, 2 or 3,
  - x being 0, 1 or 2,
  - y being 0, 1, 2 or 3, and
  - R^7 being hydrogen, CH_3 or C_2H_5,
25 - R^3 is hydrogen, methyl, CH_2OH, CH_2COOH, ethyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, propyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, butyl, $(CH_2)_3OH$, and $(CH_2)_3COOH$ or
- R^2 and R^3 together are a C_3 or C_4 alkyl forming a 5- or 6 membered ring,

is provided for use in a method for treating or preventing heart failure, hypertension or cardiac hypertrophy.

30 In some embodiments, R^1 is

- phenyl, 2-pyridyl, 3-pyridyl, 2-pyrimidyl or 5-pyrimidyl, and R^1 is a (mono-) para-positioned methoxy (OCH_3) or ethoxy (OC_2H_5) group, with all other substituents being H, or
- an unsubstituted 3-pyridyl group.

In some embodiments, R^2 is hydrogen, $O(CH_xF_{2-x})_mCH_yF_{3-y}$ or $(CH_2)_m CH_3$, with

- m being 0, 1, 2 or 3,
- x being 0, 1 or 2, and
- y being 0, 1, 2 or 3.

In some embodiments, R^3 is selected from hydrogen, methyl, CH_2OH, ethyl, $(CH_2)_2OH$, propyl, $(CH_2)_3OH$, and $(CH_2)_2COOH$.

In some embodiments:

- R^1 is methoxyphenyl,
- R^2 is methyl, ethyl, propyl or butyl and
- R^3 is selected from hydrogen, methyl, CH_2OH, CH_2COOH, ethyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, propyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, butyl, $(CH_2)_3OH$, and $(CH_2)_3COOH$.

In one embodiment, the compound is selected from the group comprised of 3-(4-methoxyphenyl)-5-propyl-furo[3,2-g]chromen-7-one, 3-(4-methoxyphenyl)-5,6-dimethyl-furo[3,2-g]chromen-7-one, 5-butyl-3-(4-methoxyphenyl)furo[3,2-g]chromen-7-one, 5,6-dimethyl-3-(3-pyridyl)furo[3,2-g]chromen-7-one and 3-[3-(4-methoxyphenyl)-5-methyl-7-oxo-furo[3,2-g]chromen-6-yl]propanoic acid.

The compounds shown in table 1 are embodiments of any aspect of the invention.

Table 1:

Compound	Formula	Name
31413 (scaff10) (IC50=25.9 µmol/L)		3-(4-methoxyphenyl)-5,6-dimethyl-furo[3,2-g]chromen-7-one

31864 (IC50=39.9 µmol/L)		3-(4-methoxyphenyl)-5-propyl-furo[3,2-g]chromen-7-one
31892 (IC50=78.9 µmol/L)		5-butyl-3-(4-methoxyphenyl)furo[3,2-g]chromen-7-one
Scaf-10-3		5,6-dimethyl-3-(3-pyridyl)furo[3,2-g]chromen-7-one
Scaf-10-8		3-[3-(4-methoxyphenyl)-5-methyl-7-oxo-furo[3,2-g]chromen-6-yl]propanoic acid

The compounds listed in table 1 were analysed in a guanine nucleotide exchange (GEF) assay format (Cytoskeleton Inc., Denver, CO, USA) according to the instructions given by the manufacturer and/or in a Rhotekin pull down assay (see below, Examples).

According to one aspect of the invention, a pharmaceutical composition for preventing or treating heart failure, hypertension or cardiac hypertrophy is provided, comprising a compound according to the above aspect or embodiments of the invention. Pharmaceutical compositions for enteral administration, such as nasal, buccal, rectal or, especially, oral

administration, and for parenteral administration, such as subcutaneous, intravenous, intrahepatic or intramuscular administration, may be used. The pharmaceutical compositions comprise from approximately 1% to approximately 95% active ingredient, preferably from approximately 20% to approximately 90% active ingredient.

5 According to one aspect of the invention, a dosage form for preventing or treating heart failure, hypertension or cardiac hypertrophy is provided, comprising a compound according to the above aspect or embodiments of the invention. Dosage forms may be for enteral administration, such as nasal, buccal, rectal, transdermal or oral administration, or as an inhalation formulation or suppository. Alternatively, dosage forms may be for parenteral 10 administration, such as intravenous, intrahepatic, or especially subcutaneous, or intramuscular injection forms. Optionally, a pharmaceutically acceptable carrier and/or excipient may be present.

According to one aspect of the invention, a method for manufacture of a medicament for preventing or treating heart failure, hypertension or cardiac hypertrophy is provided, 15 comprising the use of a compound according to the above aspect or embodiments of the invention. Medicaments according to the invention are manufactured by methods known in the art, especially by conventional mixing, coating, granulating, dissolving or lyophilizing.

According to one aspect of the invention, a method for preventing or treating heart failure, hypertension or cardiac hypertrophy is provided, comprising the administration of a 20 compound according to the above aspect or embodiments of the invention to a patient in need thereof.

The treatment may be for prophylactic or therapeutic purposes. For administration, a compound according to the above aspect of the invention is preferably provided in the form of a pharmaceutical preparation comprising the compound in chemically pure form and 25 optionally a pharmaceutically acceptable carrier and optionally adjuvants. The compound is used in an amount effective against heart failure, hypertension or cardiac hypertrophy. The dosage of the compound depends upon the species, the patient age, weight, and individual condition, the individual pharmacokinetic data, mode of administration, and whether the administration is for prophylactic or therapeutic purposes. The daily dose administered 30 ranges from approximately 1 µg/kg to approximately 1000 mg/kg, preferably from approximately 1 µg to approximately 100 µg, of the active agent according to the invention.

According to one aspect of the invention, a compound according to the above aspect or embodiments of the invention is provided for use in a method for preventing or treating cancer.

According to one aspect of the invention, the use of a compound as medicament is provided, wherein the compound is selected form the group comprised of 3-(4-methoxyphenyl)-5-propyl-furo[3,2-g]chromen-7-one, 3-(4-methoxyphenyl)-5,6-dimethyl-furo[3,2-g]chromen-7-one and 5-butyl-3-(4-methoxyphenyl)furo[3,2-g]chromen-7-one, 5,6-dimethyl-3-(3-pyridyl)furo[3,2-g]chromen-7-one and 3-[3-(4-methoxyphenyl)-5-methyl-7-oxo-furo[3,2-g]chromen-6-yl]propanoic acid.

The compounds disclosed herein show a distinct and novel utility for the indications claimed, particularly cardiac hypertrophy and cancer. Garazd et al. (ibid.) indicates that the 2,3-annelated derivative (in the substituent usage of the present specification, R^2 and R^3 together are a C_3 alkyl forming a 5 membered ring) slows cardiac frequency when applied at high concentrations. According to the observations of the present inventors, compound 32413 of the present invention (at 2.5, 10, 25 and 50 µmol/L) prevents the phenylephrine/alpa-adrenoceptor-induced increase in cardiac myocyte beating frequency. The compound does not, however, affect the adrenalin, i.e. beta-adrenoceptor-induced increase in cardiac myocyte beating frequency. This argues for a specific effect on the alpha-adrenoceptor pathway on part of the compounds of the present invention. In addition, the data do not indicate a link between beating frequency and cardiac hypertrophy.

Wherever reference is made herein to an embodiment of the invention, and such embodiment only refers to one feature of the invention, it is intended that such embodiment may be combined with any other embodiment referring to a different feature. For example, every embodiment that defines R^1 may be combined with every embodiment that defines R^2 or R^3, to characterize a group of compounds of the invention or a single compound of the invention with different properties.

The invention is further characterized, without limitations, by the following examples, from with further features, advantages or embodiments can be derived. The examples do not limit but illustrate the invention.

Description of the figures

Fig. 1 shows the concentration dependent inhibition of the nucleotide exchange by the compounds or the invention 31413, 31864 and 31892.

Fig. 2 shows microscopic images and determined fluorescence intensities that illustrate the effect of compound 31413 on the formation of stress fibres.

Fig. 3 shows the determination of the amount of active RhoA-GTP with SDS-PAGE.

Fig. 4 shows the determination of the inhibitory effect of compound 31413 on different GEFs by measurement of luciferase activities.

Fig. 5 shows the measurements of beat rate of neonatal cardiomyocytes in presence or absence of compound 31413.

5 Fig. 6 shows the determination of the α-actin 1 mRNA amount in presence and absence of PE (phenylephrin) and compound 31413.

Fig. 7 shows the determination of the α-actin 1 mRNA amount in presence and absence of PE, ISO (isoproterenol) and compound 31413.

Fig. 8 and 9 show proliferation experiments in cell culture (see Example 6)

10 **Examples**

Example 1: Inhibition of RhoA-GEF exchange

Following the protocol of the RhoGEF exchange assay Biochem Kit, a nucleotide exchange experiment for AKAP-Lbc and RhoA was established. The fluorescence labelled nucleotide analogues 2'(3')-O-(N-methylanthraniloyl)-guanosine diphosphate und 2'(3')-O-(N-
15 methylanthraniloyl)-guanosine triphosphate, hereinafter referred to mGDP and mGTP, were used for tracing the nucleotide exchange.

a) Nucleotide exchange of mGTP against GTPγS

25µl depletion buffer (100 mmol/L sodium phosphate, pH 7.4, 5 mmol/L EDTA, 200 mmol/L ammonium sulfate) with an excess of mGDP (5 mmol/L) were added to 200 µg recombinant
20 his6-RhoA (25 µl) and incubated one hour at room temperature. Then, magnesium chloride ($MgCl_2$) was added to a final concentration of 10 mmol/L.

By centrifugation with Vivaspin® 500 concentrator vials (MWCO 5 kDa, 10 min, 10000 rpm) and buffer (100 mmol/L sodium phosphate, pH 7.4, 100 mmol/L NaCl, protease inhibitor [cOmplete, Mini, EDTA-free protease inhibitor cocktaile]) RhoA-mGDP was washed twice
25 and reduced to 100µl. 1 µmol/L RhoA-mGDP and 0,5 µmol/L AKAP-Lbc DHPH in nucleotide exchange buffer (40 mmol/L Tris pH 7.5; 100 mmol/L NaCl; 20 mmol/L $MgCl_2$, 100 µg/ml BSA) were used for the nucleotide exchange. The fluorescence measurements were performed in a micro plate reader (TECAN Safire) over a period of 15 min, whereby the fluorophor of mGDP was excited at 360 nm and the resulting fluorescence signal was
30 determined at 440 nm.

b) Nucleotide exchange of GDP against mGTP

At a concentration relation of 2 µmol/L protein and 1 µmol/L mGTP, an ideal fluorescence signal-to-noise-ratio was found in the nucleotide exchange buffer (40 mM Tris pH 7.5, 100 mmol/L NaCl, 20 mmol/L $MgCl_2$, 100 µg/ml BSA). The fluorescence signal for mGTP was measured analogous to mGDP (λ_{exc}=360 nm; λ_{em}=440 nm) in a micro plate reader (TECAN Safire) over a period of 15 min. The experiment was performed in a 384-well plate. The compounds 31413, 31864 and 31892 (table 1) showed a fifty percent inhibition of nucleotide exchange in concentration between 25.9 µmol/L and 78.9 µmol/L (Fig. 1).

Example 2: inhibition of stress fibre formation

For the initial analysis, 3T3 cells were used to evaluate the cellular effects of the compounds of the present invention. In these cells changes in the cytoskeleton caused by GTPase activation are well observable, because the cells have a G protein coupled receptor for LPA. A stimulation of 3T3 cells with LPA leads to an activation of RhoA and thereby to the formation of fibrillar actin filaments (F-actin, stress fibres). The participation of AKAP-Lbc as an exchange factor for RhoA within the signal transduction via a G protein of the $G_{\alpha 12/13}$ family was shown.

3T3 mouse fibroblasts were seeded on 30 mm cover slips (in 6 well plates) in a cell density of $2x10^5$ cells/well. After one day, the cells were cultivated without serum for 16 h to minimize the serum-induced formation of stress fibres. On the following day, the cells were treated with 30 µmol/L of compounds 31413, 31864 and 31892, respectively, for 30 min at 37 °C. After the treatment the formation of stress fibres were stimulated with 300 nM of lysophosphatide acid (LPA) for 10 min at 37 °C.

Following that, the cells were washed with phosphate buffer (Dulbecco's Phosphate-Buffered Saline ++) and fixed in 2.5 % paraformaldehyde in sodium cacodylate buffer (100 mmol/L sodium cacodylate, 100 mmol/L sucrose, pH 7.4) for 15 min. Then, the cells were permeabilized with 0.5 % Triton X-100. After incubation in a blocking solution (0.3 ml 45 % *fish skin g*elatine/100 ml PBS) for 30 min at 37 °C, the cells were treated with TRITC-Phalloidin. Phalloidin binds irreversibly to fibrillar actin (F-actin) and provides, coupled to the fluorescent dye tetramethyrhodamine isothiocyanate (TRITC), the visualization of the actin skeleton. Another dye, 4′,6-diamidin-2-phenylindole (DAPI), binds to AT-rich region of the DNA and visualizes the nuclei. The stained specimens were bedded into a Immu-Mount 45 and stored in a dark, cooled place until the microscopic analysis. For the microscopic investigations, images were taken with two different fluorescence channels. The excitation was performed with argon-ion laser (364 nm) for DAPI and with a helium-neon-laser (543 nm) for TRITC-phalloidin.

Without LPA stimulation no stress fibres could be identified after addition of DMSO instead of the compounds of the invention (Fig. 2A, a). After treatment with LPA a clear formation of stress fibres could be detected (Fig. 2A, b). The *Clostridium limosum* C3-ADP-ribosyltransferase (C3lim) was used as negative control, which inhibits RhoA by ADP-
5 ribosylation at Asp 41. With C3lim the polymerization of actin and the formation of stress fibres were inhibited in the presence of LPA (Fig. 2A, c). Compound 31413 also showed a suppression of the stress fibre formation (Fig. 2A, d). The graph in Fig. 2B shows results of the immuno fluorescence measurement after quantification of the mean fluorescence intensity and supports the visual impression. All confocal images are representative
10 examples of at least three independent experiments. The quantification of the stress fibre formation were performed with the software package ImageJ by the determination of the overall fluorescence of the TRITC-phalloidin. The statistical analysis was performed according to the method of analysis of variance (ANOVA). The significant difference to the untreated sample was ***, $p < 0.001$ and *, $p < 0.05$, respectively. The significance between
15 the groups DMSO-LPA (Fig. 2B, b), C3slim+LPA (Fig. 2B, c) and 31413+LPA (Fig. 2B, d) was $p<0,001$, respectively.

Example 3: Inhibition *in vitro* assays:

A luciferase reporter assay and a rhotekin precipitation assay were performed to investigate the RhoA activation in cells.

20 a) Rhotekin-based precipitation

HEK293 cells were seeded in 6-well plates. One day later the cells were transfected with the following plasmid DNAs:

Vector	
pEGFP-N1	empty
pEGFP-N1	human AKAP-Lbc (incl. N-term. Flag-tag)
pCMV2B	p63RhoGEF
pcDNA	GαqRC (constitutively active)
pcDNA	Gα12QL (constitutively active)

After additional 24 hours the cells were treated with compound 31413 (50 µmol/L) or DMSO for 30 min at 37 °C. Subsequently, the cells were lysed in precipitation buffer (50 mM Tris
25 pH 7.4, 150 mmol/L NaCl, 4 mmol/L $MgCl_2$; 10 % v/v glycerol, 1 % octylphenoxypolyethoxyethanol [IPEGAL]) and centrifugated 20000 g for two minutes to

remove cell debris. 40 µl of the lysate were taken for the determination of the total RhoA content. The residual lysate was incubated with 40 µl GSH-sepharose coupled GST-rhotekin for one hour at 4 °C. Then, the GSH-sepharose was washed twice with precipitation buffer, enriched with SDS-PAGE loading buffer and heated 5 min at 90 °C. The analysis of the
5 precipitated RhoA-GTP was performed by SDS-PAGE and subsequent immunoblotting with anti-RhoA antibodies (sc-418). The ratio between total RhoA and Rho-GTP was densitometrically determined.

The activation state of RhoA was determined with a Rho binding region of the rhotekin protein, a Rho effector, immobilised on a sepharose bead in the presence and absence of
10 compound 31413. Rhotekin only binds to the activated form of RhoA, RhoA-GTP. To investigate the effects of compound 31413 on G protein coupled receptor signals, HEK293 cells were transfected with a constitutively active form of the Gα12 subunit, with AKAP-Lbc (for the Gα12 mediated RhoA activation), with a constitutively active Gαq subunit (GαqRC) and with p63RhoGEF, respectively. p63RhoGEF contains the DNA sequence of a RhoA
15 selective exchange factor and connects the Gαq coupled receptor signal with the activation of RhoA. The cells were treated with compound 31413 twenty four hours after transfection.

GTP-bound RhoA was detected by rhotekin precipitation (Fig.3). The precipitation of RhoA from transfected HEK293 cells was performed with a rhotekin sepharose, and analysed with SDS-PAGE and immunoblotting with a RhoA specific antibody. In contrast to the control cells
20 treated with DMSO, the cells treated with compound 31413 shows a significantly decreased amount of activated GTP-bound RhoA (Fig.3A). The precipitated RhoA-GTP was separated with SDS-PAGE, blotted and detected with a specific anti-RhoA antibody.

For Gα12 coupled and AKAP-Lbc mediated activation of RhoA, less RhoA-GTP was precipitated in the presence of compound 31413 (Fig. 3, black bar) then in presence of
25 DMSO (Fig. 3, white bar). For GαqRC and p63RhoGEF, no difference could be observed in the precipitated amount of RhoA-GTP of samples treated or untreated with compound 31413 (50 µmol/L). This result indicates that compound 31413 influences the Gα12 coupled activation of RhoA and not the Gαq coupled activation.

b) Luciferase-reporter assay

30 The experiments were performed following the protocol of the dual luciferase reporter assay (Promega). HEK293 cells were transfected with plasmid DNAs of two luciferases and plasmids of different exchange factors. A serum response element was located ahead of the coding luciferase sequences. Activated RhoA activates the serum response element, which enables the expression of the firefly luciferase. After 4 hours the cells were treated with
35 compound 31413. After additional twenty four hours the cells were lysed in passive lysis buffer (Promega). The luciferase activity in the cell lysate was determined by measurement

of the luminescence signal according to above mentioned protocol with 96 well plate (OptiPlate 96) and *LarII*- and. *Stop-&Glow-*substrate solutions in a micro plate reader

To rule out that other RhoGEF are influenced by the inventive compounds in their activity, HEK293 cells were transfected with different RhoGEF constructs. Additionally, as
5 normalization control, two luciferase plasmids were added, which code for a serum response element (SRE) coupled firefly luciferase and for a *Renilla* luciferase, respectively. The GEF mediated activation of RhoA leads to a stimulation of the transcription factor serum response factor (SRF), which switches on the expression of the SRE-coupled luciferase. The cells were treated with compound 31413 four hours after transfection and lysed after twenty hours.
10 The luciferase activities were determined in the lysate. First, the effect of compound 31413 on the AKAP-Lbc mediated RhoA/SRF activation was investigated (Fig. 4A). The luciferase activity was inhibited concentration dependently by compound 31413 with a determined IC_{50} value of approx. 25 µmol/L, which is comparable to the results from the nucleotide exchange experiment. Further, the luciferase activity of the indicated RhoGEFs was analysed in
15 absence or presence of 50 µmol/L compound 31413 (Fig. 4 B). None of the investigated GEF shows a significant reduction of the luciferase activity after treatment with compound 31413, when compared to the DMSO treated control. The data indicate the specific inhibition of the AKAP-Lbc mediated RhoA activation by the inventive compounds.

Example 4: Effect of inventive compounds on cardiomyocytes

20 For cultivation of primary neonatal rat cardiomyocytes, hearts of two to three days old rats were obtained and quickly stored on ice in ADS buffer (116.4 mM NaCl, 5.4 mM KC, 5.6 mM dextrose,10.9 mM NaH_2PO4, 405.7 µmol/L MgSO4, 20 mM Hepes, pH 7.3). Under sterile conditions, the hearts were cleaned from blood and vessels, cut into small pieces and transferred into falcon tubes. The ADS buffer was exchanged against an enzyme solution
25 (see table below). Within four cycles each with five ml enzyme solution and an enzyme mix comprising collagenase and pancreatin, isolated cells were released from the tissue (12 min at 37 °C in a water bath with approx. 220 rpm).

Animal count	**Collagenase**	**Pancreatin**	**in ADS-Puffer**	**FKS**	**Plat. Med.**
Up to 20	12 mg	14 mg	18 ml (4 x 4 ml)	2,5 ml	5 ml

The supernatant with the isolated cells was collected, transferred in fetal calf serum (FCS,
30 2.5 ml on ice) and subsequently centrifugated in FCS (100 g for 3 min at 4 °C). The supernatant of the centrifugation was discarded and the pellet was resuspended in 5 ml cold plating medium. After an additional centrifugation, the pellet was resuspended in 15 ml medium, transferred into a dish and incubated for two hours at 37 °C to allow the settling of the fibroblasts. The cardiomyocytes in the supernatant were counted with a Scepter

Handheld Automated Cell Counter and transferred in dishes coated with gelatine. On the following day the medium was changed.

The neonatal cardiomyocytes were seeded in 6-well plates with $1,5x10^6$ cell per well. The stimulation with 100 µmol/L phenylephrine (PE) was performed for six or twenty four hours at 37 °C. Untreated cells served as control. The effect of compound 31413 on the α-adrenergic stimulation of the cardiomyocytes was determined after incubation with the compound for three hours. To do this, the contraction of the cells was counted within a minute and different conditions were compared.

The frequency of contraction could be determined in a similar manner with a calcium sensor (Fluo-4). In a laser-scanning microscope, Fluo-4 was excited with an argon laser (488 nm) and the emission signals were detected and collected at a wavelength of 505 nm. The fluorescence intensity represented the intracellular concentration of calcium ions. Usually, the line-scan mode was used for image recording, and the line-scan images were recorded with a speed of 1.54 or 1.92 ms per line along the longitudinal axis of the cell. After the sequential image recording, a two-dimensional image with 512x100 lines or 512x2000 lines was generated and saved for later analysis.

Isolated neonatal cardiomyocytes were used to investigate the relation between AKAP-Lbc induced RhoA activation and the contractility of the heart. After a chronic stimulation of the α1-adreno receptors (AR) with the specific agonist PE for six hours, the beats of the cardiomyocytes were counted over a period of 15 s and a beat rate per minute (bpm) was calculated. An increase of the beat rate of the cardiomyocytes from 100 bpm to 160 beats per minute could be observed. The solvent control (DMSO+PE) showed a similar result. The treatment of the cells with 2.5 µmol/L compound 31413 led to a normalization of the beat rate. With significantly elevated concentrations of compound 31413 (25 µmol/L and 50 µmol/L), an arrest could be achieved (Fig. 5A). The beat rate of neonatal cardiomyocytes was counted with help of a light microscope.

Additional calcium measurements were performed. To do this, the cardiomyocytes were pre-incubated with 2.5 µmol/L compound 31413. The fluorescence dye Fluo-4 was added after 20 min. Fluo-4 is a calcium sensor, which is used for investigating the calcium increase in cells. In cardiomyocytes the dye serves for examining the contractility. During contraction and relaxation, the calcium concentrations differ in the cytosol of cardiomyocytes. The fluorescence intensity of Fluo-4 increases with an increasing calcium concentration, and a decreasing calcium concentration will cause reduced fluorescence intensity. A frequency could be calculated from the fluo-4 fluorescence oscillation by a time depended analysis, wherein the fluorescence was measured over a period of two hours. The ground state at the moment before application (black), the period 10 to 30 sec after application (dark grey) and

70 to 110 sec after application (light grey) were evaluated (Fig. 5). Application refers to the adding of PE or adrenaline. The stimulation with PE and adrenaline leads to an increase of the frequency from 100 bpm to 150 bpm for PE and 200 bpm for adrenaline, respectively. The pre-incubation with 2.5 µmol/L compound 31413 inhibited only the PE induced increase of beat rate and not the adrenaline induced one. This indicates an influence of compound 31413 on the α-AR-induced signals, because adrenaline mainly affects the β-adrenoreceptors. The results of the manual determination of the beat rate (Fig. 5A) are consistent with those of the calcium determination (Fig. 5B) and show a negative chronotropic effect of compound 31413 after α-adrenergic stimulation. The statistical analysis was performed according to the method of analysis of variance (ANOVA). The significance between the groups PE and PE+31413 was $p<0,001$.

Example 5: Inhibition of cardiac hypertrophy marker expression

Primary neonatal cardiomyocytes were prepared as described above. The cardiomyocytes were stimulated for 24 or 48 hours with 100 µmol/L phenylephrin (PE) or 10 µmol/L isoproterenol (ISO) in absence or presence of 10 µmol/L or 50 µmol/L of compound 31413.

After 24 hours or 48 hour of stimulation, the medium was removed, and every well was washed with 2 ml phosphate buffered saline and fixed with 200 µl TRIzol. The cells were removed from each well and transferred into a 1.5 ml Eppendorf tube, respectively.

For cell breakdown, 40 µl chloroform was added to each Eppendorf tube. The broken cells were centrifuged for 15 min at 1400 g and 4 °C. The supernatant containing RNA/DNA was taken, mixed with 100 µl ispropanol and centrifugated for 20 min at 1500 g and 4 °C. The supernatant was discarded and the pellet was resuspended in 500 µL ethanol. The RNA from the pellet was purified by chromatography. The concentration of the RNA was determined by NANODROP.

The quantification of the hypertrophy marker α-sceletal muscle actin (α-actin 1) mRNA was performed by quantitative real-time PCR. First, the RNA was transcribed to a cDNA with a SuperScript™III First-Strand Synthesis SuperMix (Invitrogen) and random hexamer primers. The cDNA was amplified in a PCR reaction in an Applied Biosystem StepOne™ Real-Time PCR System. Glycerinealdehydephosphate dehydrogenase and NTC (no template control) was used as controls. All samples were triplicates. The following primers have been used:

Primer	Applied Biosystem catalogue number
ANF (Nppa)	4351372, Rn00664637_g1
α-sceletal muscle actin (ACTA1)	4331182, Rn01426628_g1

rat GAPD (GAPDH) endogenous Control 4352338E
(VIC®/MGB Probe, Primer Limited)

After 24 hours of stimulation wit PE (100 µmol/L), a threefold increase in the relative amount of the hypertrophy marker α-sceletal muscle actin (α-actin 1) mRNA could be observed (Fig. 6). Without PE stimulation, the addition of compound 31413 caused no significant change in the relative amount of α-actin 1 mRNA. After PE stimulation and addition of compound 31413, a concentration dependent decrease of the α-actin 1 mRNA amount. This indicates the anti hypertrophic efficacy of compound 31413. The statistical analysis was performed according to the method of analysis of variance (ANOVA). The significance between the groups PE and PE+31413 was $p<0,001$.

A significant decrease of the α-actin 1 mRNA amount could also be observed in the presence of 10 µmol/L and 50 µmol/L compound after stimulation with ISO for 48 hours (Fig. 7). This indicates that beta-adrenergic induced hypertrophy can also be inhibited by the compounds of the invention. These data underline that control of beat frequency and hypertrophy are different (see above).

Example 6: Inhibition of cancer cell proliferation in cell culture.

MCF7 cells (mamma carcinoma-derived), HEK293 cells (human embryo kidney-derived), HeLa (cervical carcinoma-derived) and A549 cells (lung carcinoma-derived) were seeded into 24 well plate culture dishes and kept under standard mammalian cell culture conditions. On day 2 of the experiment, cells were either left untreated, incubated with DMSO, or with Scaff-10 (10, 30 or 50 µmol/L) (applied in DMSO solution) for the indicated periods of time. At the indicated time points the cells were trypsinized to disassociate them from the surface of the culture dishes (0.25 % trypsin in EDTA, 3 min), and cell numbers were determined in a *Neubauer* counting chamber. Cell numbers are expressed as cell number per ml (ZZ/ml).

Example 7: Synthesis of 5,6-dimethyl-3-(3-pyridyl)furo[3,2-g]chromen-7-one

HO, O, O + Br, O, H−Br, N — 2,5 eq K_2CO_3 / Aceton, 50-55°C

O, O, O, O, N — 1N NaOH / 5h, 100°C — O, O, O, N

7-Hydroxy-3,4-dimethyl 2H-chromen-2-one was dissolved in acetone and heated to 50-55°C. K_2CO_3 was added with strong stirring. 3-(Bromoacetyl)-pyridine hydrobromide was added and stirring was continued at 50-55°C. 1N NaOH was added to the resulting product. The solution was heated to 100°C for 5 hours and precipitated with 1N H_2SO4.

5 **Example 8: Rhotekin assay (Rho pull-down assay and western blotting)**

The continuous stimulation of α-adrenoceptors on the surface of cardiac myocytes can lead to cardiac myocyte hypertrophy. α-Adrenoceptor stimulation e.g. with phenylephrine causes activation of the guanine nucleotide exchange (GEF) activity of AKAP-Lbc, which in turn activates specifically RhoA (Appert-Collin et al, 2007, ***Proc Natl Acad Sci U S A* 104: 10140-**
10 **10145**). This AKAP-Lbc-dependent pathway plays a critical role in the development of α-adrenoceptor-dependent cardiac myocyte hypertrophy.

The activity of RhoA can be measured in Rhotekin pull down assays. The pull-down of active GTP-bound RhoA from cardiac myocytes was essentially carried out as described previously (Ren & Schwartz, 2000, Methods Enzymol 325: 264-272; Tamma et al, 2003, J Cell Sci 116:
15 3285-3294). In brief, neonatal cardiac myocytes were grown in 60 mm dishes and were left untreated, incubated with phenylephrine to stimulate α-adrenoceptors and in consequence the guanine nucleotide exchange (GEF) activity of AKAP-Lbc, which in turn activates specifically RhoA. The active form of RhoA is the GTP-bound RhoA. GTP-RhoA was precipitated from lysates using the GST-Rhotekin fusion protein (20-30 mg) coupled to
20 glutathion Sepharose 4B. GTP-RhoA was eluted by boiling the precipitate in Laemmli buffer (10 minutes) containing DTT (40 mM). Total RhoA in lysates and precipitated GTP-RhoA were detected by Western blot analysis using commercially available anti-RhoA monoclonal antibodies (Santa Cruz Biotechnology, Heidelberg, Germany) and peroxidase-conjugated anti-mouse secondary antibodies. Signals were visualized using the Odessey Western blot
25 detection system. To quantify the amount of active RhoA, signal densities were determined and related to the signal densities obtained for total RhoA. Ratios obtained for the various experimental conditions were normalized to those ratios obtained for control cells. Statistical analysis was carried out using the Newman-Keuls multiple comparison test.

Analysis of the results obtained in presence of the compounds of table 1 showed (at
30 concentration of 30µmol/L) a reduced activity of between 30 and 50% of the control group (phenylephrine concentration 100µmol/L)

Claims

1. A compound characterized by a general formula 1

(1),

wherein

- R^1 is an aryl or a heteroaryl selected from the group comprised of:

; ; ; ;

; ; ; ;

; ; ;

; ; ;

; ; ; ;

; and ;

wherein

n is 0, 1, 2, 3 or 4, and

- each R^4 independently from any other is $COOR^5$, $CONR^5_2$, $C(NH)NR^5_2$, CN_4H_2, NR^5_2, COR^5, OR^5, CF_3, OCF_3, CN, NO_2, F, Cl or Br, or
- two R^4 together are a dioxylalkyl forming a five- or six-membered ring, and

- R^2 and R^3
  - independently of each other are hydrogen or a C_1-C_5 alkyl, or
  - together are a C_3 or C_4 alkyl forming a 5- or 6 membered ring,

    wherein R^2 and/or R^3 independently of one another bears 0, 1 or 2 substituents R^6, with any non-substituted position being hydrogen or fluorine, each R^6 being selected, independently from any other R^6, from $COOR^5$, $CONR^5_2$, $C(NH)NR^5_2$, CN_4H_2, NR^5_2, COR^5, OR^5, CF_3, OCF_3, CN, NO_2, Cl and Br,
  - with each R^5 independently from any other R^5 being hydrogen or a C_1-C_4 alkyl,

  and
  - at least one of R^2 and R^3 is not hydrogen,

for use in a method for preventing or treating heart failure, hypertension or cardiac hypertrophy.

2. A compound characterized by the general formula 1,

(1),

wherein

- R^1 is phenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl or 5-pyrimidyl, and R^1 is substituted by n substituents, n being 0, 1 or 2, and each substituent

independently from any other is OR^7, COR^7, $COOR^7$, $CONR^7_2$, CN, OCF_3, CF_3, F, Cl, Br, CN_4H_2, $C(NH)NR^7_2$ or NO_2 ,

- R^2 is hydrogen, $O(CH_xF_{2-x})_mCH_yF_{3-y}$ or $(CH_2)_mZ$, wherein Z is selected from CH_3, $(CH_2)_xOR^7$, $COOR^7$, and $CONR^7$, with
  - m being 0, 1, 2 or 3,
  - x being 0, 1 or 2,
  - y being 0, 1, 2 or 3, and
  - R^7 being hydrogen, CH_3 or C_2H_5,
- R^3 is selected from hydrogen, methyl, CH_2OH, CH_2COOH, ethyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, propyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, butyl, $(CH_2)_3OH$, and $(CH_2)_3COOH$, or
- R^2 and R^3 together are a C_3 or C_4 alkyl forming a 5- or 6 membered ring,

for use in a method for preventing or treating heart failure, hypertension or cardiac hypertrophy.

3. A compound according to claim 1 or 2, wherein
   - R^1 is
     - phenyl, 2-pyridyl, 3-pyridyl, 2-pyrimidyl or 5-pyrimidyl, and R^1 is a (mono-) para-positioned methoxy (OCH_3) or ethoxy (OC_2H_5) group, with all other substituents being H, or
     - an unsubstituted 3-pyridyl group.
4. A compound according to any of the preceding claims, wherein
   - R^2 is hydrogen, $O(CH_xF_{2-x})_mCH_yF_{3-y}$ or $(CH_2)_m$ CH_3, with
   - m being 0, 1, 2 or 3,

   x being 0, 1 or 2, and

   y being 0, 1, 2 or 3,
   - R^3 is selected from hydrogen, methyl, CH_2OH, CH_2COOH, ethyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, propyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, butyl, $(CH_2)_3OH$, and $(CH_2)_3COOH$,

   for use in a method for preventing or treating heart failure, hypertension or cardiac hypertrophy.
5. A compound according to any of the preceding claims, wherein
   - R^1 is methoxyphenyl,
   - R^2 is methyl, ethyl, propyl or butyl and
   - R^3 is selected from hydrogen, methyl, CH_2OH, CH_2COOH, ethyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, propyl, $(CH_2)_2OH$, $(CH_2)_2COOH$, butyl, $(CH_2)_3OH$, and $(CH_2)_3COOH$.

6. A compound according to any of the preceding claims, selected form the group comprised of:
   - 3-(4-methoxyphenyl)-5-propyl-furo[3,2-g]chromen-7-one;
   - 3-(4-methoxyphenyl)-5,6-dimethyl-furo[3,2-g]chromen-7-one;
   - 5-butyl-3-(4-methoxyphenyl)furo[3,2-g]chromen-7-one
   - 5,6-dimethyl-3-(3-pyridyl)furo[3,2-g]chromen-7-one
   - 3-[3-(4-methoxyphenyl)-5-methyl-7-oxo-furo[3,2-g]chromen-6-yl]propanoic acid.
7. A pharmaceutical composition for use in a method for preventing or treating heart failure, hypertension of cardiac hypertrophy, comprising a compound according to any one of claims 1 to 6.
8. A dosage form for use in a method for preventing or treating heart failure, hypertension or cardiac hypertrophy, comprising a compound according to any one of claims 1 to 6.
9. A method for making a medicament for preventing or treating heart failure, hypertension or cardiac hypertrophy, comprising the use of a compound according to any one of claims 1 to 6.
10. A method for treating heart failure or cardiac hypertrophy, comprising the administration of a compound according to any one of claims 1 to 6 to a patient in need thereof.
11. A compound according to any one of claims 1 to 6 for use in a method for treating cancer.
12. Use of a compound as a medicament, said compound selected form the group comprised of:
   - 3-(4-methoxyphenyl)-5-propyl-furo[3,2-g]chromen-7-one;
   - 3-(4-methoxyphenyl)-5,6-dimethyl-furo[3,2-g]chromen-7-one;
   - 5-butyl-3-(4-methoxyphenyl)furo[3,2-g]chromen-7-one
   - 5,6-dimethyl-3-(3-pyridyl)furo[3,2-g]chromen-7-one
   - 3-[3-(4-methoxyphenyl)-5-methyl-7-oxo-furo[3,2-g]chromen-6-yl]propanoic acid.

Fig 1

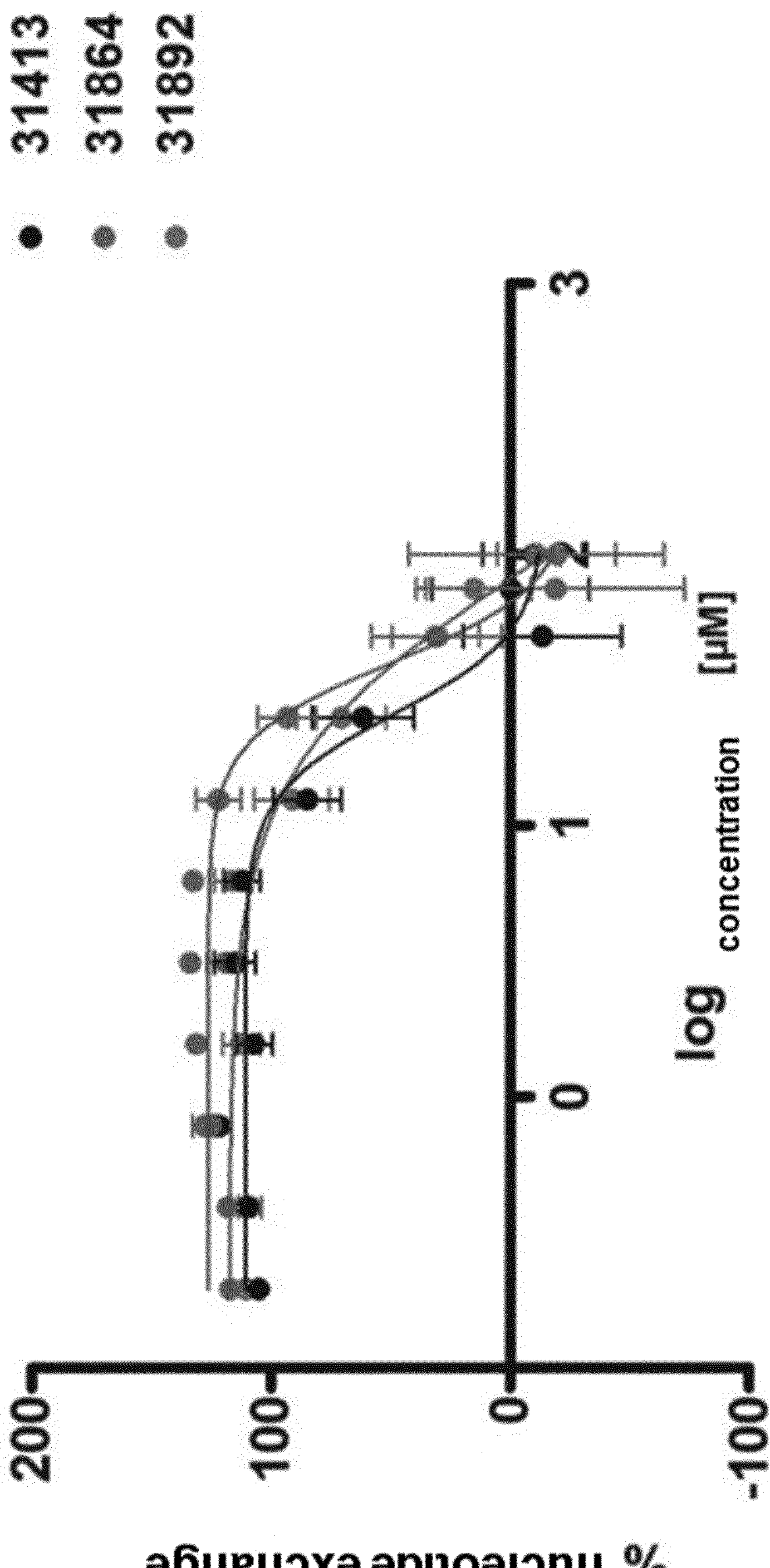
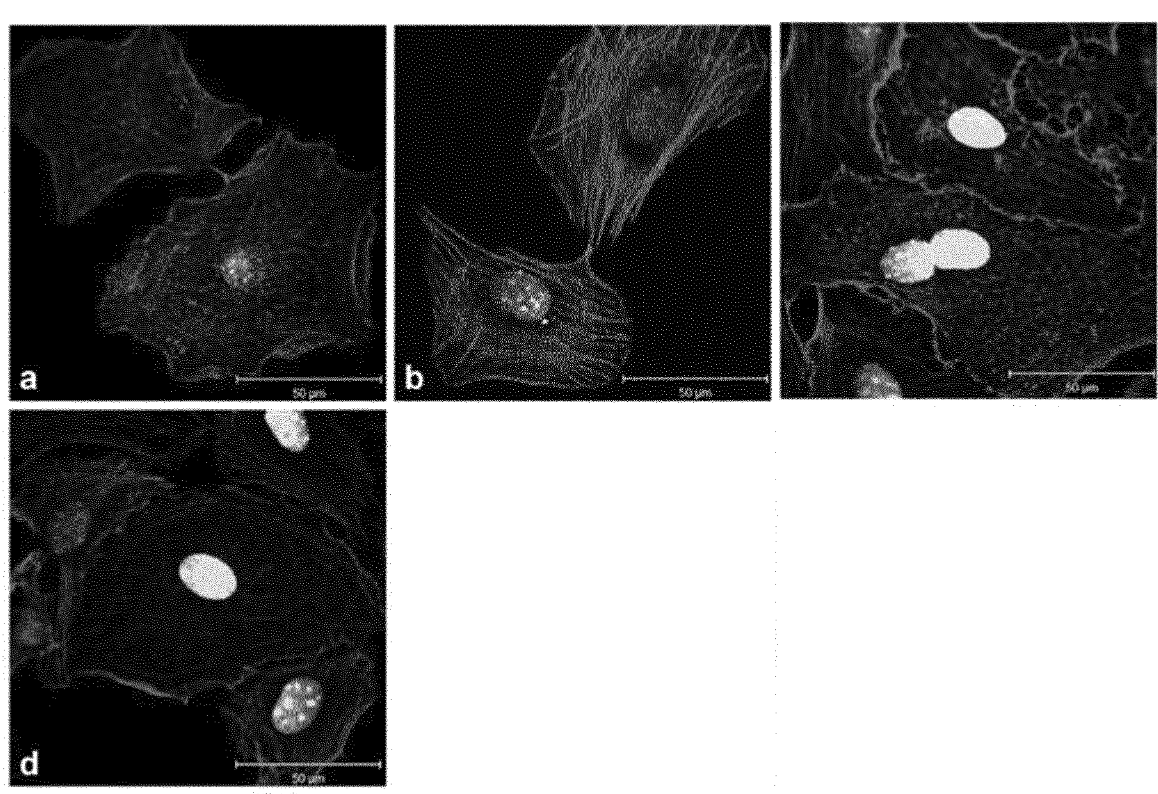


Fig. 2

A)



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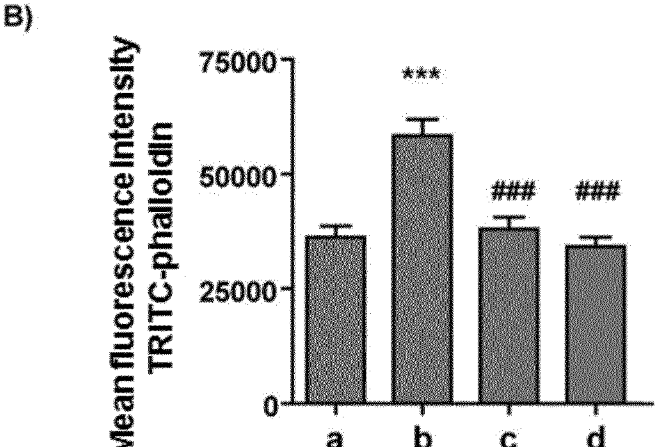
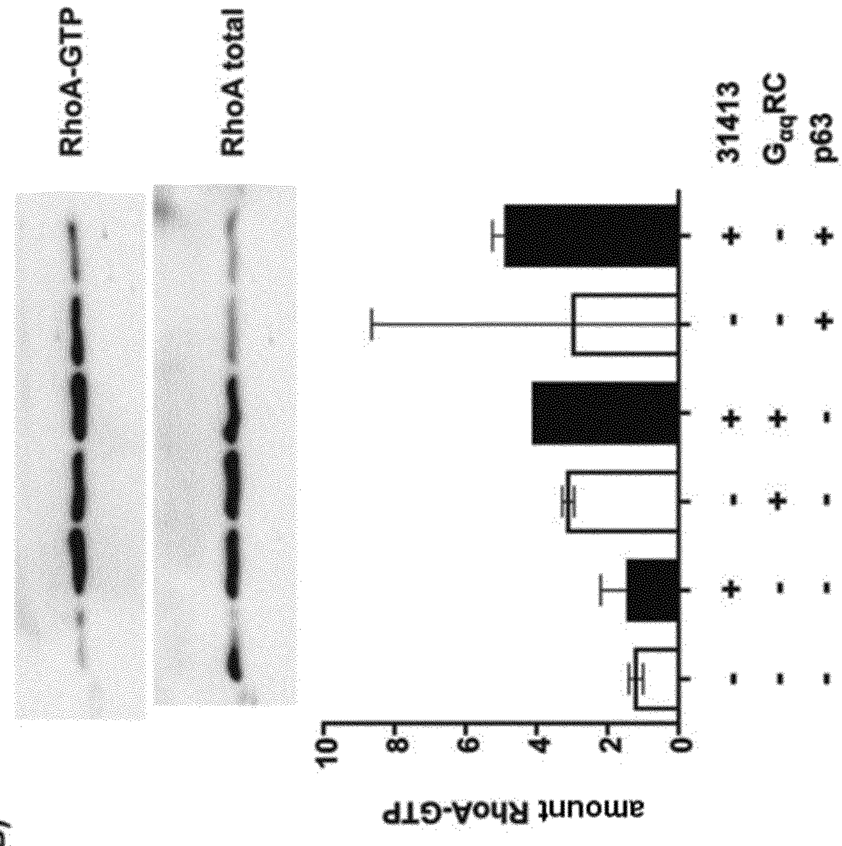


Fig. 3



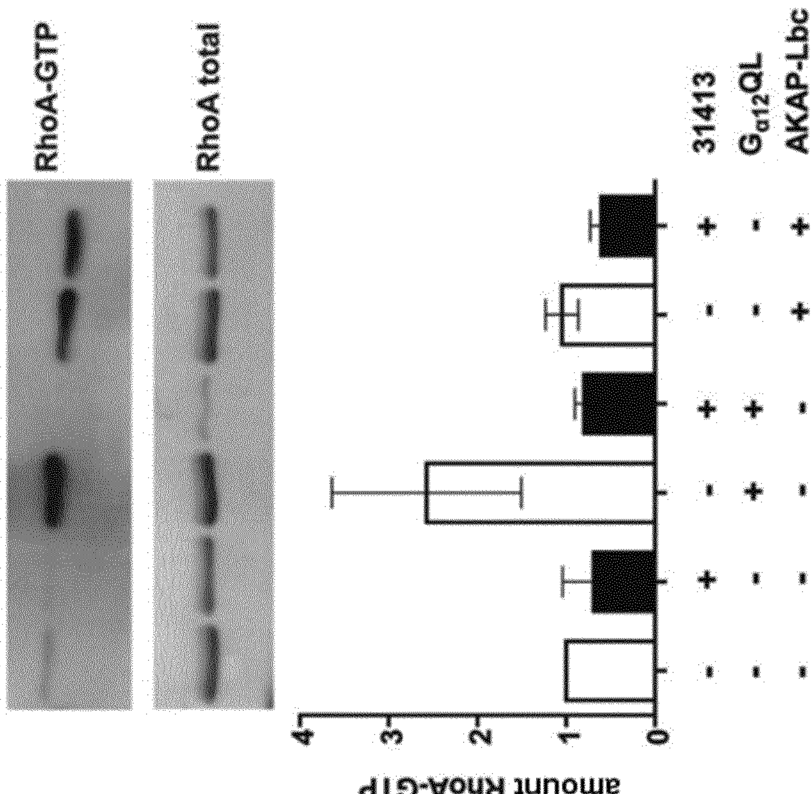


Fig. 4

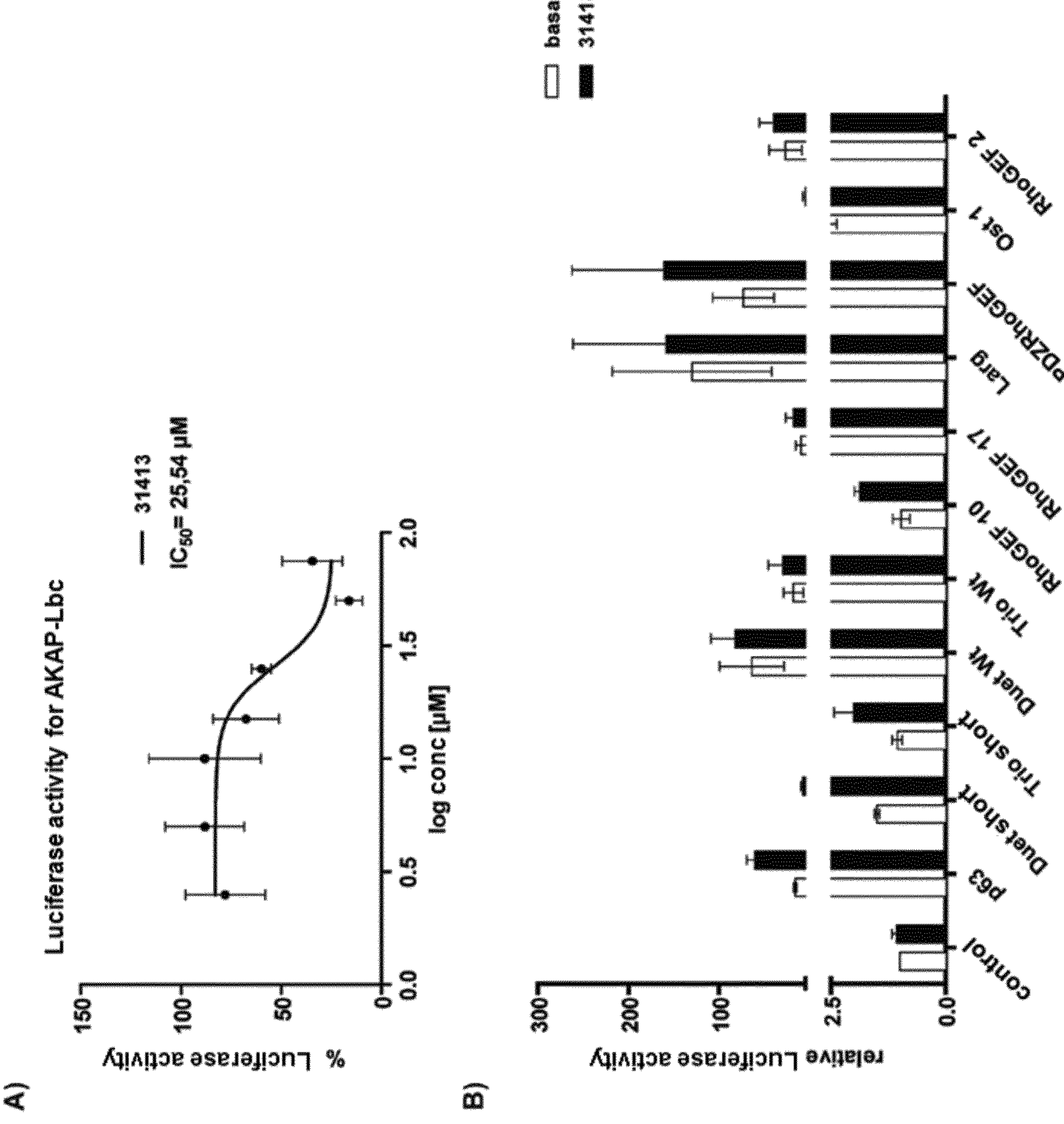


Fig 5

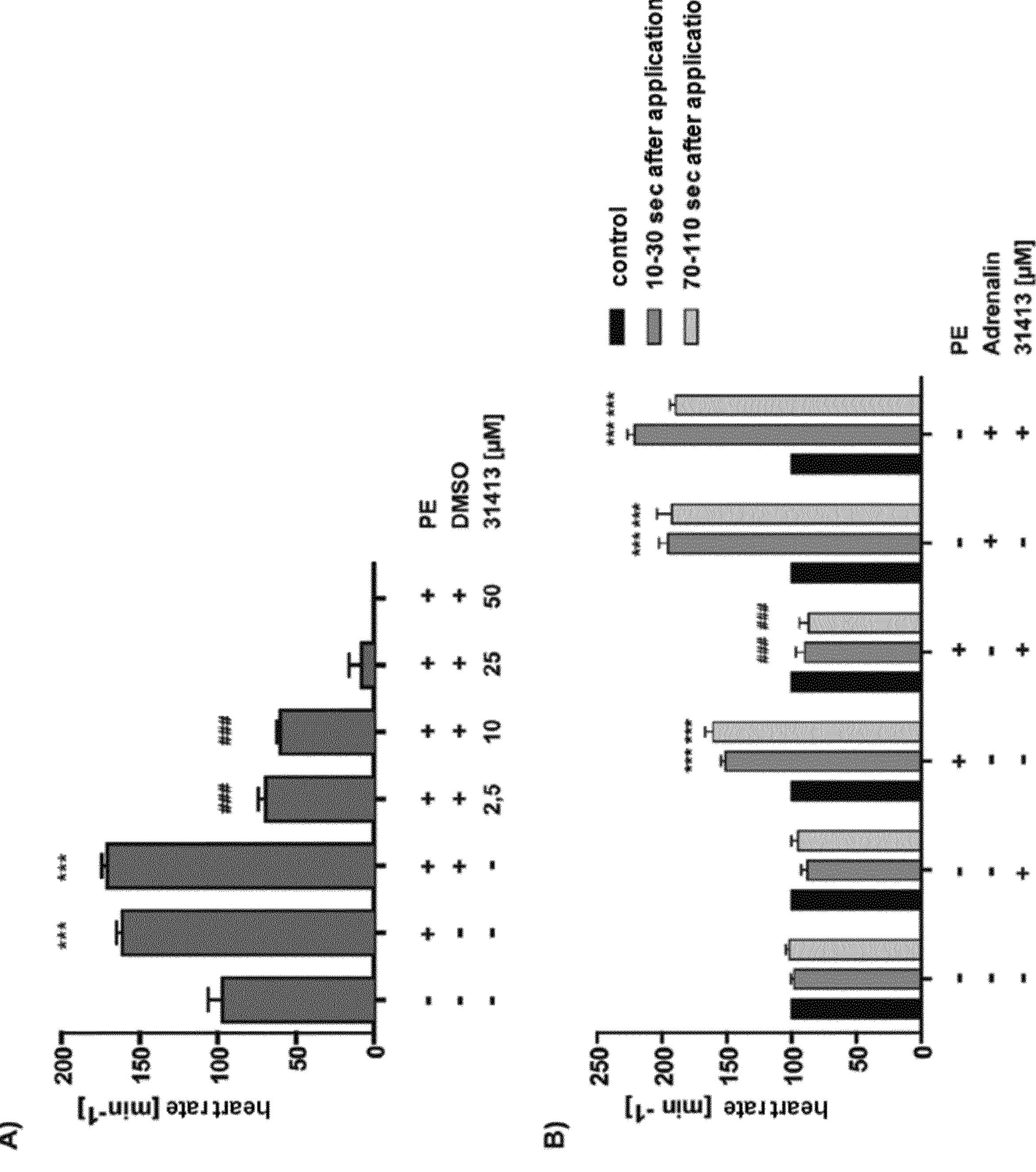


Fig. 6

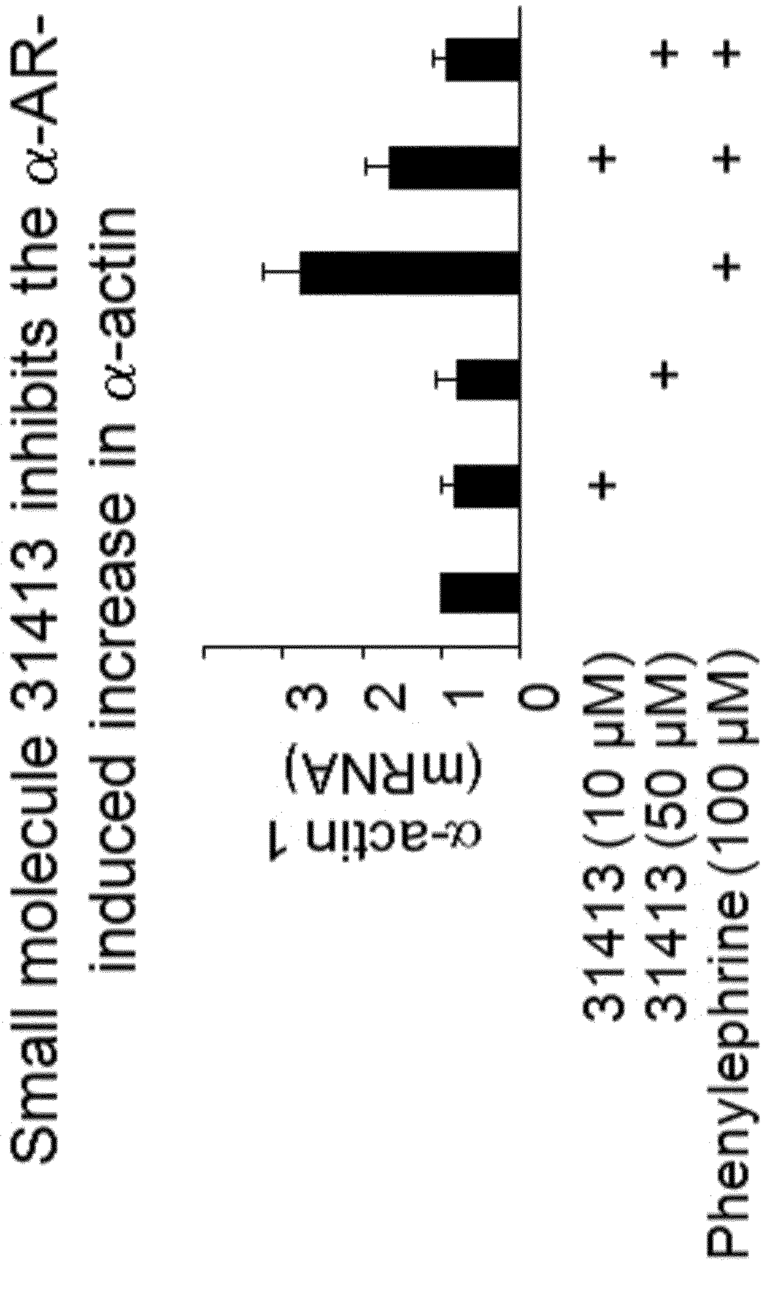


Fig. 7

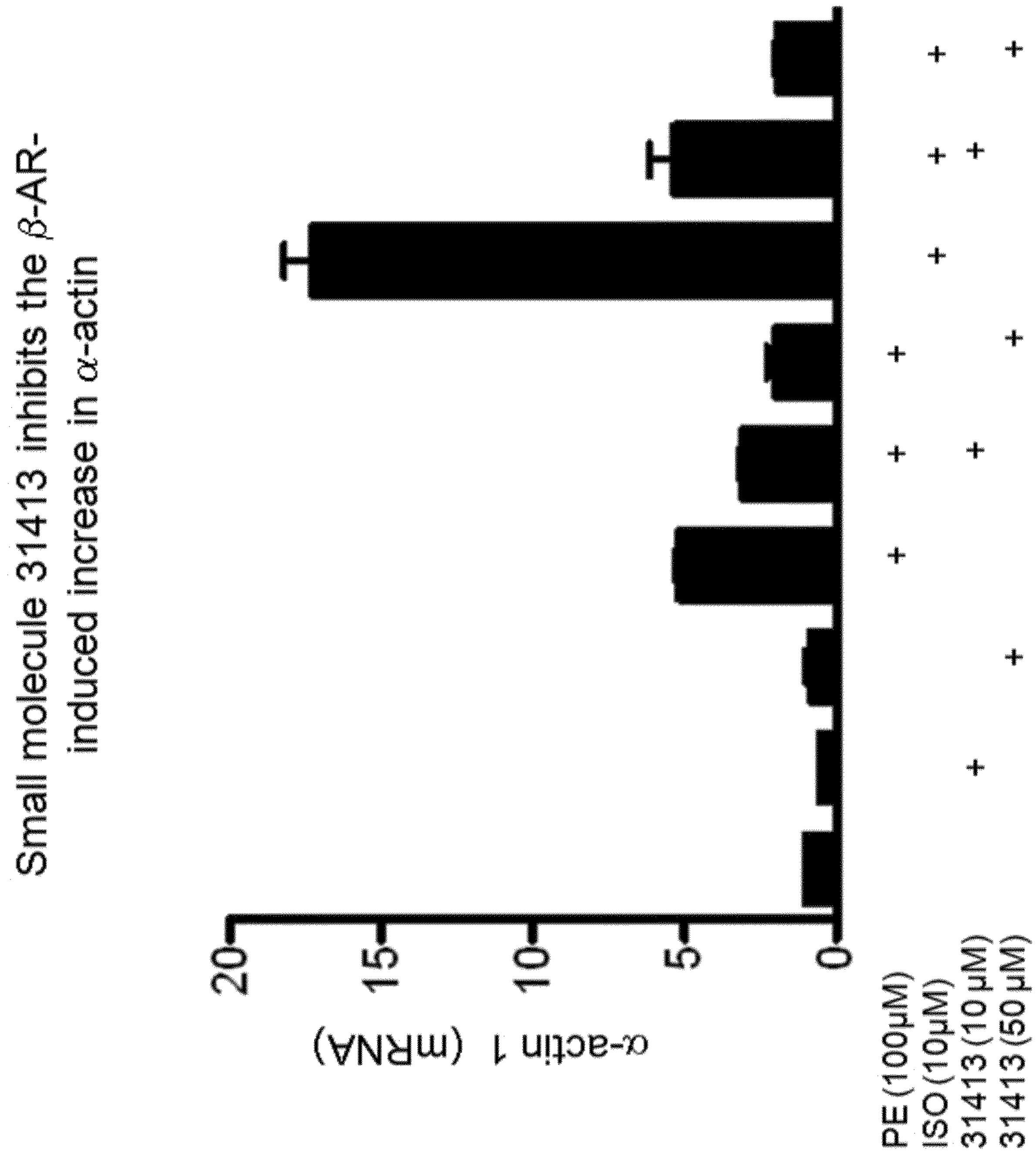
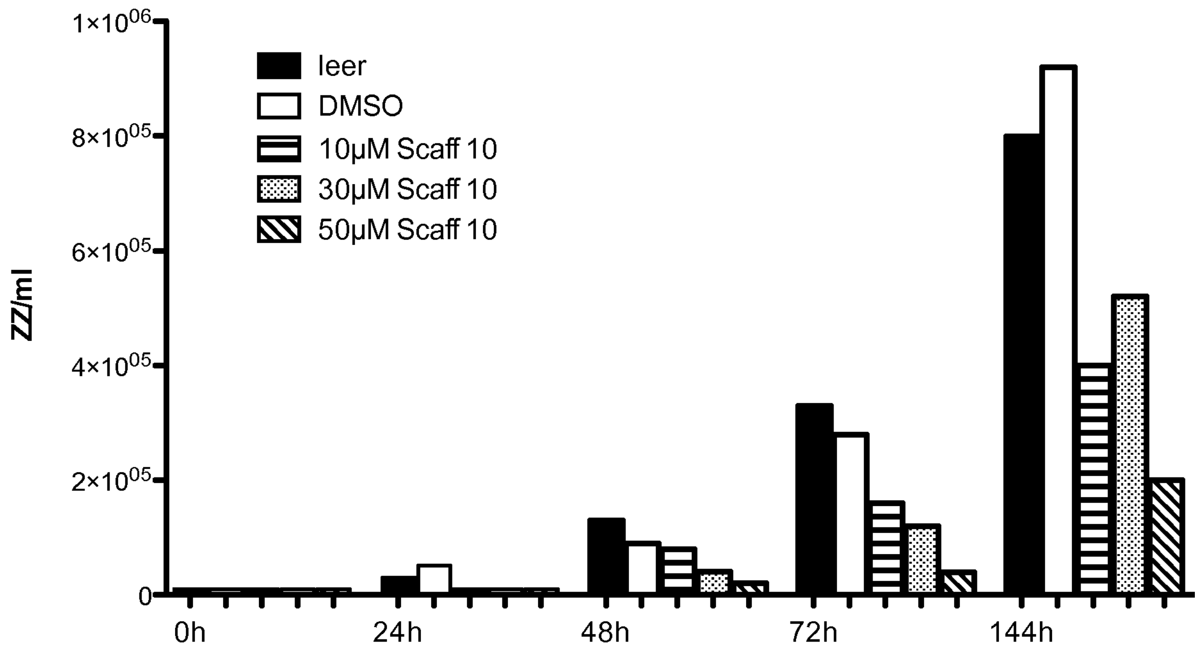


Fig. 8 A



B

HEK293
Incubation Compound Scaff 10
27.05.2013

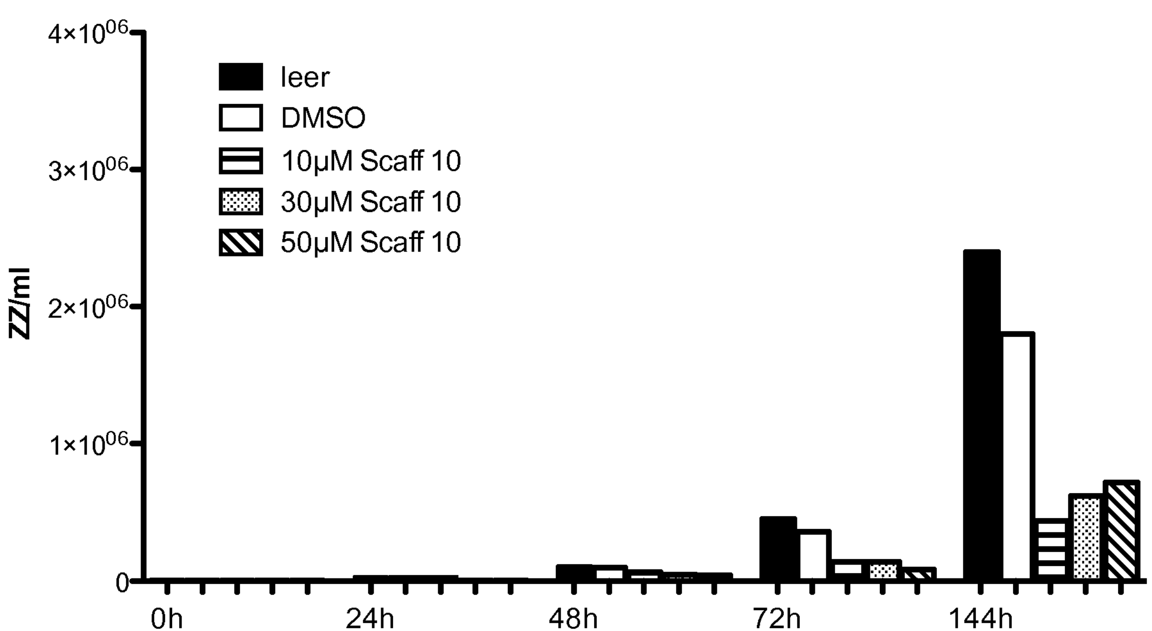
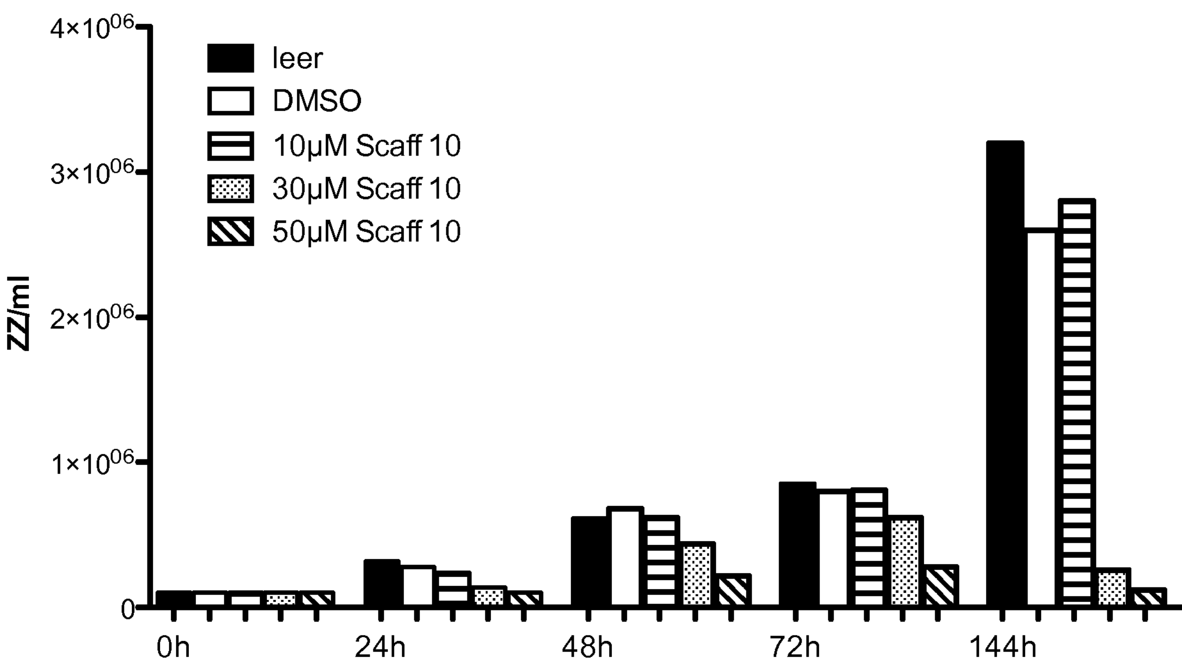


Fig. 9 A

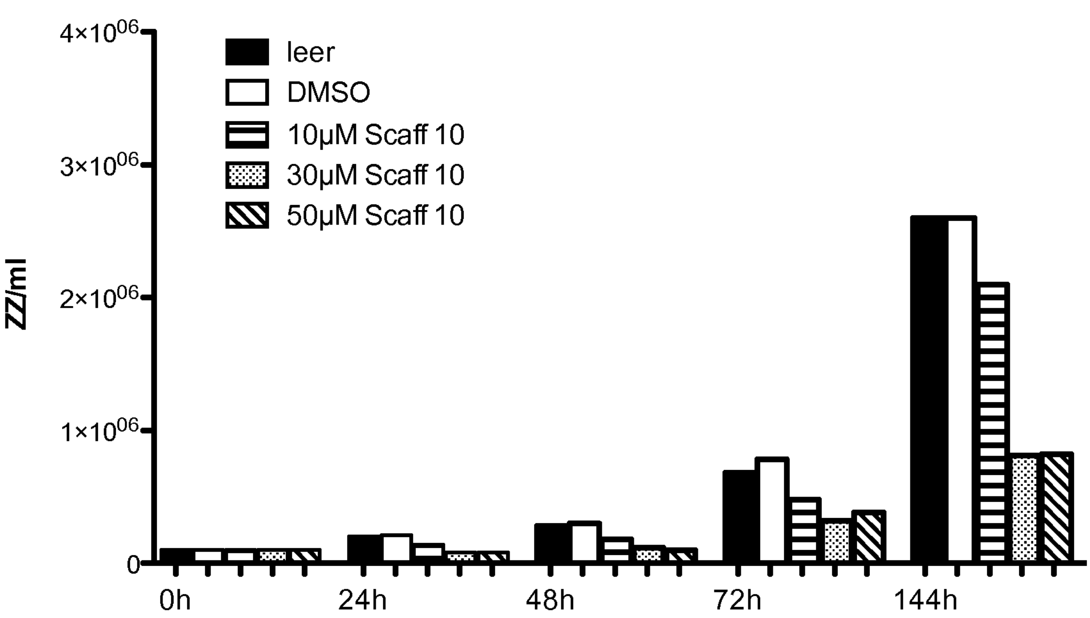


B

A549

Incubation Compound Scaff 10

27.05.2013



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/063938

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/35 A61P9/00 A61P9/04 A61P9/12
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

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

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. M. GARAZD ET AL: CHEMISTRY OF NATURAL COMPOUNDS, vol. 38, no. 3, 1 January 2002 (2002-01-01), pages 230-242, XP055037978, ISSN: 0009-3130, DOI: 10.1023/A:1020423826071 See the compounds of page 231, and in particular compound N.45, and its activity as cardiotropic agent (see page 233) -----	1-10,12
Y	WO 03/084530 A1 (BIOSYNERGEN INC [KR]; SONG DONG-KEUN [KR]; SON JONG-KEUN [KR]) 16 October 2003 (2003-10-16) See claims and page 12, lines 5-6: psoralene derivatives and their use to treat hypertension and chronic heart failure -----	1-10,12
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2013	04/12/2013

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

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/160597 A1 (UNIV XI AN JIAOTONG [CN]; HE LANGCHONG [CN]; ZHANG JIE [CN]; LI NA [CN) 29 December 2011 (2011-12-29) See abstract and claims: psoralene derivatives and their use as antihypertensive agents	1-10,12
A	ALINE APPERT-COLLIN ET AL: "The A-kinase anchoring protein (AKAP)-Lbc-signaling complex mediates alpha1 adrenergic receptor-induced cardiomyocyte hypertrophy", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 104, no. 24, 12 June 2007 (2007-06-12), pages 10140-10145, XP002680702, ISSN: 0027-8424, DOI: 10.1073/PNAS.0701099104 [retrieved on 2007-05-30] See abstract: relevance of AKAP-protein kinase A as a target for the treatment of chronic heart failure	1-10,12
A	FRANK CHRISTIAN ET AL: "Small Molecule AKAP-Protein Kinase A (PKA) Interaction Disruptors That Activate PKA Interfere with Compartmentalized cAMP Signaling in Cardiac Myocytes", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 286, no. 11, 18 March 2011 (2011-03-18), pages 9079-9096, XP002664288, ISSN: 0021-9258, DOI: 10.1074/JBC.M110.160614 [retrieved on 2010-12-22] See abstract: involvment of AKAP-Lbc in the process leading to cardiac hypertrophy	1-10,12

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CA [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 2008, HU, LIHONG ET AL: "Preparation of furanocoumarin derivatives for treatment of diabetes mellitus", XP002683315, retrieved from STN Database accession no. 2008:1414229 See the derivatives of formula (I) where R6 is phenyl, an din particular compound RN: 215865-92-2, as agonist of GLUT4 and GLUT4 expression and their use to treat diabetes & CN 101 307 056 A (SHANGHAI INSTITUTE OF MATERIA MEDICA, CHINESE ACADEMY OF SCIENCES, PEO) 19 November 2008 (2008-11-19) -----	9
A	DATABASE CA [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 2008, MIN, DAE JIN ET AL: "Skin preparations containing PPAR-activating substances from plant", XP002683316, retrieved from STN Database accession no. 2008:347592 See the claimed compounds, and particular compound having RN: 777857-59-7, and their use as PPAR agonists, for treating, e.g. inflammatory conditions & KR 806 162 B1 (AMOREPACIFIC CORP., S. KOREA) 22 February 2008 (2008-02-22) -----	1-10,12
X	PICCAGLI LAURA ET AL: "Virtual screening against nuclear factor [kappa]B (NF-[kappa]B) of a focus library: Identification of bioactive furocoumarin derivatives inhibiting NF-[kappa]B dependent biological functions involved in cystic fibrosis.", BIOORGANIC & MEDICINAL CHEMISTRY 1 DEC 2010 LNKD- PUBMED:20980154, vol. 18, no. 23, 1 December 2010 (2010-12-01), pages 8341-8349, XP002683317, ISSN: 1464-3391 See abstract, figure 1, compound 10f, column 8344, right hand column, 8345 right hand column, table 3: compound 10f as inhibitor of NK-kb and relevance for treatment cystic fibrosis -----	9
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International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 2001, XP002683318, Database accession no. 374762-77-3 See compound having RN: 37462-77-3 -----	1-10,12

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/063938

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10, 12

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-10, 12

   Compounds of formula (I) for use in the treatment of cardiovascular diseases as defined in claims 1, 2, 4, 7, 9.

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2. claim: 11

   Compounds of formula (I) for use in the treatment of cancer, as defined in claim 11.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/063938

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03084530 A1	16-10-2003	AU 2003219580 A1	20-10-2003
		KR 20030079484 A	10-10-2003
		WO 03084530 A1	16-10-2003
WO 2011160597 A1	29-12-2011	CN 101857598 A	13-10-2010
		WO 2011160597 A1	29-12-2011