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(54) Title: NEW RACl INHIBITORS AS POTENTIAL PHARMACOLOGICAL AGENTS FOR HEART FAILURE TREATMENT

(57) Abstract: The present invention finds application in the field of medicine and, in particular, to new compounds particularly active in inhibiting Racl member of the Rho family. Pharmaceutical preparations containing them useful for the treatment and/or prevention of heart failure are disclosed as well.

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DESCRIPTION OF THE INVENTION

NEW RAC1 INHIBITORS AS POTENTIAL PHARMACOLOGICAL AGENTS FOR HEART FAILURE TREATMENT

The present invention finds application in the field of medicine and, in particular, to new compounds useful for the treatment and/or prevention of heart failure.

BACKGROUND

Heart failure constitutes one of the leading worldwide causes of morbidity and mortality. Only, in Italy, it has been estimated that 1 million , which approximates 1.7 % of the population and about 22 million of people in the world, accounting for about 0.036% of the population, is affected by heart failure. Clinical monitoring has observed that 50% of people suffering from said pathology die within 5 years from the diagnosis. Thus, a large number of patients will benefit from the development of new pharmacological treatments. At present, pharmacological intervention includes the use of β-adrenergic receptor antagonists, inhibitors of angiotensin II, aldosterone and diuretics, which are currently employed in standard treatments for heart failure. Introduction of these pharmacological interventions have substantially increased the patient's survival and decreased morbidity. However, these agents are far from ideal due to their side effects.

Accordingly, the identification of new molecular targets and the development of pharmacological agents for the treatment of heart failure represent an important scientific goal. Recent *in vitro* and *in vivo* evidences and preliminary studies in clinic have indicated a pivotal role of Rad in the development of heart failure agents and, thus, it has become a leading target for the treatment of heart failure (Sah, V.P., et al., 1999. Cardiac-specific overexpression of

RhoA results in sinus and atrioventricular nodal dysfunction and contractile failure. J *Clin Invest.* 103(12): p. 1627-34. Maack C , et al., 2003.Oxygen free radical release in human failing myocardium is associated with increased activity of racl-GTPase and represents a target for statin treatment. *Circulation.* 108(13): p. 1567-74. Satoh , M., et al., 2OO6.Requirement of Racl in the development of cardiac hypertrophy. *Proc Natl Acad Sd USA.* 103(19): p. 7432-7). In particular, Rad is a member of a RhoGTPase subfamily (Rac1-Rac4) that transduces extracellular signals from G-coupled protein receptors (GPCR), integrins and growth factor receptors to effector molecules that modulate multiple signalling pathways.

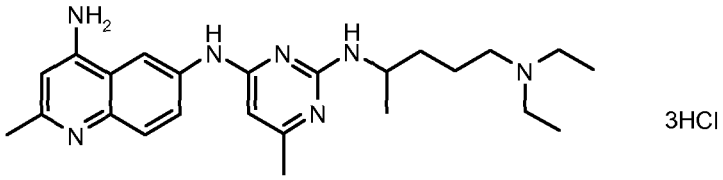
The Rho family comprises 22 genes encoding at least 25 proteins in humans; among them Rho, Rac, and Cdc42 proteins have been studied in the most detail. All Rho family members bind GTP (Guanosine-5'-triphosphate), which is an energy transfer molecule within the cells, and most exhibit GTPase activity and cycle between an inactive GDP-bound form and an active GTP-bound form. Said cycling activity is finely regulated by 3 groups of proteins: the guanine nucleotide exchange factors (GEFs) as activators, and the GTPase activating proteins (GAPs) and GDP dissociation inhibitors (GDIs) as negative regulators.

When bound to GTP, Rho GTPases interact with their downstream effectors, which include protein kinases, regulators of actin polymerization and other proteins with adaptive functions. The selective interaction of the different Rho GTPases with a variety of effectors determines the final outcome. For instance, the interaction of the Rho isoforms RhoA, RhoB, and/or RhoC with ROCK family kinases affects the actin organization, whereas their interaction with Dial

stimulates actin polymerization. The p21-activated kinase (PAK) family of proteins, on the other hand, act downstream of both Rac and Cdc42.

A classical approach to pharmacologically inhibit the Rho's activity is based on antagonizing the GTP binding to the GTP-binding domain of Rho proteins. However, high homology of the GTP binding domain among the Rho proteins hampers a specific pharmacological inhibition of a particular Rho protein. This specificity of action is of particular interest since some of the biological actions of these proteins have opposite cellular effects thus causing undesired side effects.

Recently, a first-generation of specific inhibitor of Rac GTPase has been developed. In particular, the chemical compound NSC23766 (see here below)



NSC23766

has been identified by a structure-based virtual screening of compounds fitting into a surface groove of Rac1 known to be critical for GEF specification. *In vitro,* it effectively inhibits Rac1 binding and activation by the Rac-specific GEF Trio or Tiami in a dose-dependent manner without interfering with the closely related Cdc42 or RhoA binding or activation by their respective GEFs. NSC23766, interferes with the binding between Rac1 and GEF, thus directly affecting the exchange of GDP with GTP and the activation of its major effector PAK1. However, relevant chemical modification of the structure of inhibitors is necessary in order to achieve better fitting into the groove on the Rac1 surface, thus increasing the docking affinity, the inhibitory potency on Rac1 activity, and

obtaining more effective and specific drugs which would show lower side effects. In addition, compounds showing a selectivity of action against Rac1 than versus other Rho family proteins are highly desirable. In fact, the activation of RhoA leads to bradycardic effect, thus its inhibition might have a detrimental action in patients affected by heart failure (Sah, V.P., et al., 1999). In contrast the Rac1 activation has been associated with hydrogen peroxide production and development of heart failure in clinical and preclinical studies (Maack, C , et al., 2003. Oxygen free radical release in human failing myocardium is associated with increased activity of rac1-GTPase and represents a target for statin treatment. *Circulation.* 108(13): p. 1567-74. Satoh, M., et al., 2006. Requirement of Rac1 in the development of cardiac hypertrophy. *Proc Natl Acad Sci U S A.* 103(19): p. 7432-7. Sussman, M.A., et al., 2000. Altered focal adhesion regulation correlates with cardiomyopathy in mice expressing constitutively active rad. *J Clin Invest.* 105(7): p. 875-86). Thus, the selective inhibition of Rad may have a positive effect on patients affected by heart failure.

OBJECT OF THE INVENTION

Accordingly, it is a first object of the invention a compound as per claim 1 and dependent claims thereto.

As a second object, the compounds of the invention are used as a medicament, according to claim 5.

In a preferred embodiment, said compounds may particularly be used for the treatment or the prevention of human heart failure, cancers, hypertension, inflammation, and atherosclerosis, as per claim 6.

As a further object, the present invention concerns a pharmaceutical composition comprising the compounds of the invention, as per claims 7-9.

SUMMARY OF THE INVENTION

The present invention relates to new Rac1 inhibitors showing a selective inhibitory action for Rac1 with respect to RhoA. The process for the chemical synthesis of said compounds, as well as the use of these compounds as potential drugs for the treatment of heart failure are disclosed as well.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the binding mode of complexes of Rac1 with CWB024 and the referenced compound NSC23766

FIG. 2 shows the results of the CWB024/02054 inhibition of the intracellular content of Rad -GTP.

FIG. 3-7 show the relative remaining LC/MS peak areas of $[M+H]^+$ ^{13}C -isotope peaks for CWB018, CWB020, CWB021 , CWB022, CWB024 in 0 and 60 min incubations, with and without cofactors. Co-factors = NADPH, UDPGA, PAPS and GSH.

FIG. 8 shows the metabolic reactions and metabolites detected for the invention compounds with Rad inhibitory activity.

FIG. 9 which shows the relative remaining enzyme activities (%) with 10 µM CWB021/01039 (white) and 10 µM CWB024/02054 (black) in comparison to solvent control samples. The probe metabolites as in experimental. For CYP2C19, a=omeprazole demethylation and b=omeprazole 5-hydroxylation; for CYP3A4, a=omeprazole 3-hydroxylation, b= omeprazole sulfonation, c=testosterone 6β-hydroxylation and d= midazolam 1'-hydroxylation.

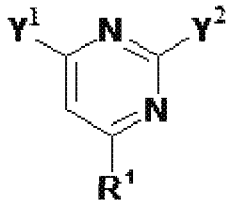
FIG. 10 shows the concentration dependent effect of CWB024/02054 on Rad GTP intracellular levels determined by G-LISA assay.

FIG. 11 shows the concentration dependent effect of CWB024/02054 on RhoA GTP intracellular levels determined by G-LISA assay.

FIG. 12 shows the video-microscopy analysis of cells incubated with CWB024/02054 for 16h in the presence or absence of PDGF-BB (20 ng/ml).

DETAILED DESCRIPTION OF THE INVENTION

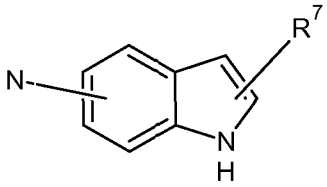
According to a first embodiment, the present invention concerns a compound having the following general formula (I):



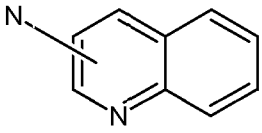
(I)

wherein

a) when Y^2 is -NR $^2R^3$ and R^2 is H and R^3 is -CH-(CH_3)(CH2)3-N(CH_2CH3)2 then Y^1 is -NR $^4R^5$ wherein R^4 is H and R^5 is i) indole optionally substituted to the pyrrole ring with a R^7 group, being the R^7 group a methyl (-CH_3) or a hydroxyl (-OH) group and said indole being attached to any available position on the benzene ring to the N atom of the Y^1 residue such as



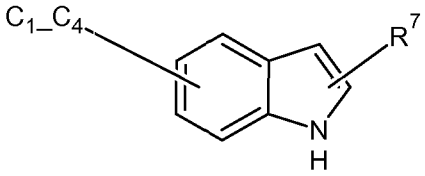
or R^5 is ii) an unsubstituted quinoline attached to any available position on the pyridinic ring to the rest of the Y^1 moiety such as



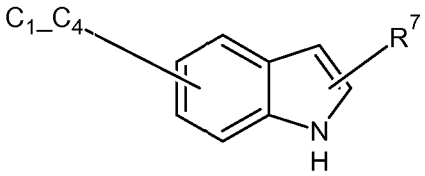
;or wherein

b) when Y^2 is -NR $^2R^3$ and R^2 is H and R^3 is a Ci-C_4 alkyl chain substituted with indole optionally substituted at any available position on the pyrrole ring with a

R^7 group as above defined and said indole being attached to any available position on the benzene ring to the N atom of the Y^2 moiety such as

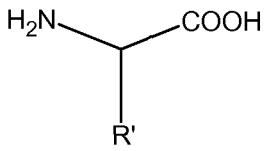


then Y^1 is -NR $^4R^5$ wherein R^4 is H and R^5 is a Ci-C_4 alkyl chain substituted with indole optionally substituted at any available position on the pyrrole ring with a R^7 group as above defined and said indole being attached to any available position on the benzene ring to the rest of the Y^1 moiety such as



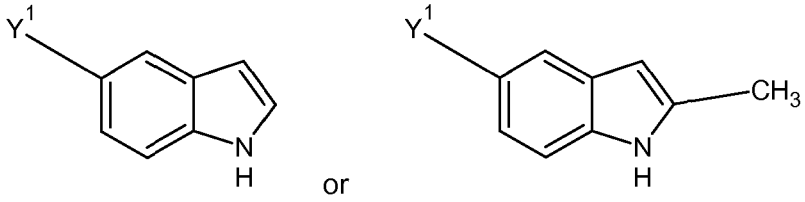
;or wherein

c) when Y^2 is -NR $^2R^3$ and R^2 is H and R^3 is an unsubstituted or methyl (-CH_3) or hydroxyl (-OH) substituted N containing C_9-Ci_0 heterocycle attached to any available position on the benzene ring to the N atom of the Y^2 moiety then Y^1 is -CO(OR ^{8})R^9 wherein R^8 and R^9 are each independently a CrC_4 alkyl chain or R^9 is the R' chain of a natural amino acid of formula

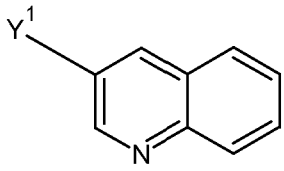


; and wherein in formula (I) R^1 is H or methyl (-CH_3); or any salts thereof.

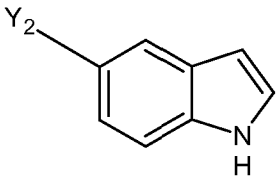
Preferably, the compounds of the invention are those of formula (I) above wherein in a) Y^1 is -NR $^4R^5$ wherein R^4 is H and R^5 is i) an unsubstituted or a indole substituted at the position 2 with a methyl (-CH_3) group, being the indole attached to the N atom of the Y^1 residue at the position 5 such as



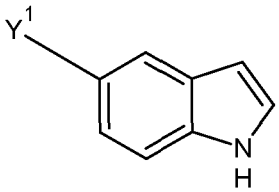
or R^5 is ii) an unsubstituted quinoline attached to the N atom of the Y^1 residue at the position 3 such as



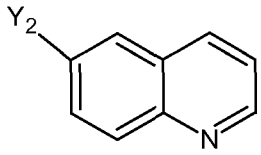
or the compounds of the invention are those of formula (I) above wherein in b) Y^2 is -NR $^2R^3$ and R^2 is H and R^3 is a Ci-C_4 alkyl chain, preferably a methylene (-CH_2-) substituted with an unsubstituted indole and said indole being attached to the Y^2 residue at the position 5 such as



and Y^1 is-NR $^4R^5$ wherein R^4 is H and R^5 is a CrC $_4$ alkyl chain, preferably a methylene (-CH_2-) substituted with an unsubstituted indole and said indole being attached to the Y^1 residue at the position 5 such as



or the compounds of the invention are those of formula (I) above wherein in c) Y^2 is -NR $^2R^3$ wherein R^2 is H and R^3 is an unsubstituted quinoline attached to the position 6 to the N atom of the Y^2 residue such as



and Y^1 is -CO(OR ^{8})R^9 wherein R^8 is methy (-CH_3) and R^9 is -CH_2-$CH(CH_3)_2$ and R^1 is methyl (-CH_3).

Even more preferably, the compounds of the invention are:

4-Methyl-2-[6-methyl-2-(quinolin-6-ylamino)-pyrimidin-4-ylamino]-pentanoic acid methyl ester;

N2,N4-Bis-(1 H-indol-5-ylmethyl)-pyrimidine-2,4-diamine;

N2-(4-Diethylamino-1-methyl-butyl)-N4-(2-methyl-1 H-indol-5-yl)-pyrimidine-2,4-diamine;

N2-(4-Diethylamino-1-methyl-butyl)-N4-quinolin-3-yl-pyrimidine-2,4-diamine;

N2-(4-Diethylamino-1-methyl-butyl)-N4-(1 H-indol-5-yl)-pyrimidine-2,4-diamine;

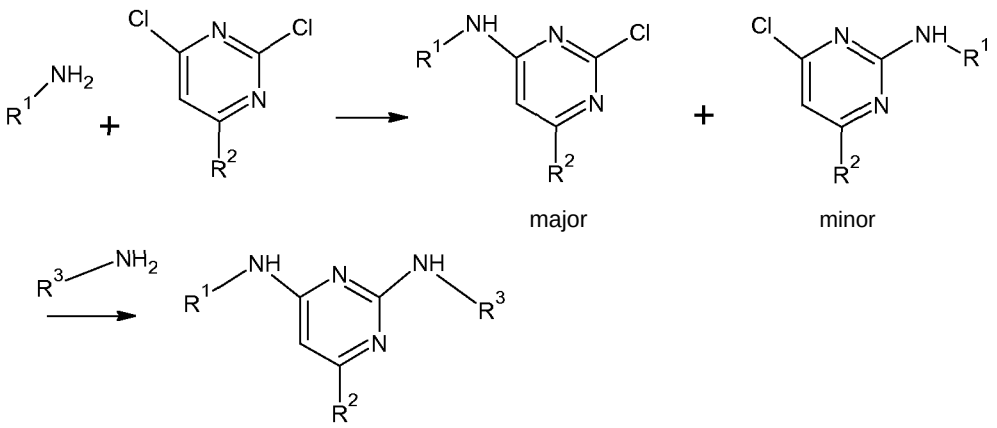
or any pharmaceutically acceptable salts thereof.

The C_9-C_{10} "heterocyclic group" or "heterocycles" above mentioned refers to unsatured cyclic group of 9 or 10 carbon atoms comprising a nitrogen atom; preferred heterocycles of the present invention are as indole and quinoline.

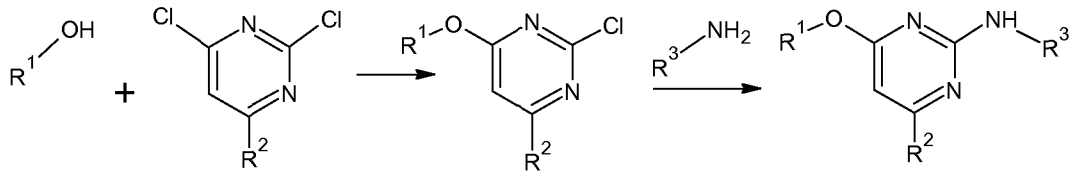
"Any natural aminoacid" refer to the naturally occurring amino acid, selected among glycine, alanine, cysteine, lysine, valine, proline, leucine, arginine, threonine, tryptophan, phenylalanine, tyrosine, glutamic acid, asparagine, aspartic acid, glutamine, being glycine the most preferred one (R^9 being H).

As for the preparation of the compounds of the invention, they can be synthesized according to Scheme 1 and Scheme 2 below.

Scheme 1



Scheme 2



Reactions in Scheme 1 are reactions between amines RI-NH_2, R3-NH_2 and 2,4-dichloropyrimidine which are amination reactions onto a haloaromatic ring. The major product after the first step is the amination product on 4-position of 2,4-dichloropyrimidine.

The first step in Scheme 2 is aroxylation of haloaromatic ring, the reaction between aromatic alcohol and 2,4-dichloropyrimidine. The second step is the same amination reaction onto a haloaromatic ring as in Scheme 1.

The synthesis of the specific compounds is given in the following Examples 1 to 10.

The present invention also concerns a pharmaceutical preparation comprising one or more of the compounds of the invention.

In particular, said compounds are in the form of a salt and, preferably, in the form of a pharmaceutically acceptable salt.

"Pharmaceutically acceptable salt" is intended to include any salts suitable to be administered to human or animal and having suitable technological properties, such as, for instance, sodium, potassium, ammonium, zinc salt or any salts with amino acids, such as with lysine (see, for a general reference, Remington's Pharmaceutical Sciences Handbook, Mack Pub. Co., N.Y., USA 17th edition, 1985).

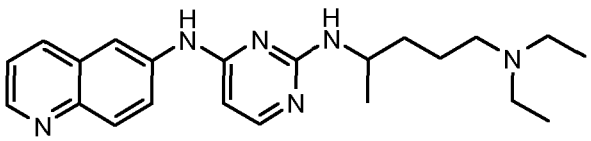
As per a preferred embodiment, the compounds of the present invention are in the form of hydrochloride.

The pharmaceutical preparations of the invention comprise in addition to one or more of the compounds of the invention, and according to the type of formulation to be used, other pharmaceutical additives and/or excipients conventional in the art, such as, for instance, diluents, solvents, bulking agents, fillers, reological modifiers, stabilizers, binders, lubricants, disintegrants, preservatives, pH adjusting agents, buffers, antioxidants, chelating agents, plasticizers, polymers, emulsifiers, edulcorants, flavoring agents, etc., alone or in combination.

Comparative Example 1

N2-(4-Diethylamino-1-methyl-butyl)-N4-quinolin-6-yl-pyrimidine-2,4-diamine

(CWB004/01022)



The mixture of 6-aminoquinoline (200 mg, 1.39 mmol), 2,4-dichloropyrimidine (249 mg, 1.67 mmol) and diisopropylethylamine (274 mg, 2.09 mmol) in

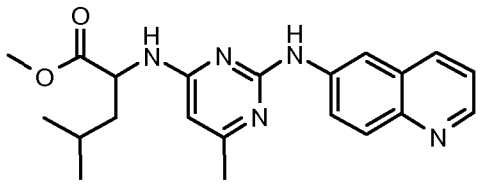
isopropanol (4 ml.) was stirred in a pressure bottle at 120 ^{0}C for 19 h. The reaction mixture was evaporated under vacuum and the residue was purified by silica gel column using EtOAc/heptane 2/8, 1/1 and pure EtOAc as eluent to give 200 mg (56 %) of 6-(2-chloro-pyrimidin-4-ylamino)quinoline.

The mixture of 6-(2-chloro-pyrimidin-4-ylamino)quinoline (200 mg, 0779 mmol) and 2-amino-5-diethylaminopentane (148 mg, 0.935 mmol) in ethylene glycol (4 ml.) was stirred in a pressure bottle at 110 ^{0}C for 2 days. Saturated $NaHCO_3$ solution was added to the reaction mixture and it was extracted with EtOAc, the organic layer was dried over Na_2SO_4, filtrated and evaporated under vacuum. The residue was purified by silica gel column using EtOAc, 10 % MeOH in EtOAc and EtOAc/MeOH/Et_3N 7:3:0.5 as eluent to give 200 mg (68 %) of the titled compound.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.83 (dd, 1H), 8.10-7.99 (m, 4H), 7.66 (dd, 1H), 7.38 (dd, 1H), 6.81 (s, 1H), 6.08 (d, 1H), 4.96 (d, 1H), 4.11 (m, 1H), 2.59-2.44 (m, 6H), 1.61-1.57 (m, 4H), 1.28 (d, 3H), 1.07-1.00 (m, 6H).

Example 2

4-Methyl-2-r6-methyl-2-(quinolin-6-ylamino)-pyrimidin-4-ylamino1-pentanoic acid methyl ester (CWB01 8/01 034)

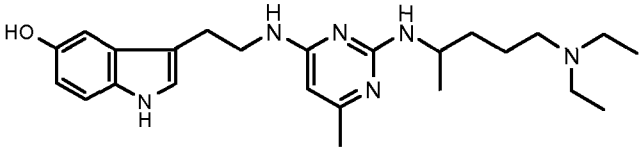


The title compound was prepared from L-leucine methyl ester as described in Example 1. 6-aminoquinoline was used instead of 2-amino-5-diethylaminopentane.

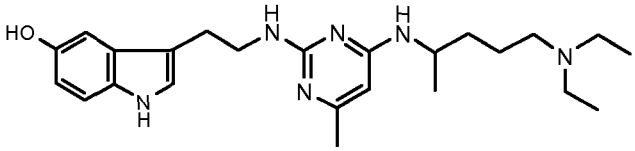
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.76 (dd, 1H), 8.39 (d, 1H), 8.14 (d, 1H), 8.00 (d, 1H), 7.65 (dd, 1H), 7.35 (dd, 1H), 7.14 (s, 1H), 5.87 (s, 1H), 4.96 (d, 1H), 4.79 (br. s, 1H), 3.68 (s, 3H), 2.28 (s, 3H), 1.82-1 .67 (m, 3H), 0.99 (dd, 6H).

Comparative Example 3

3-{2-[2-(4-Diethylamino-1-methyl-butylamino)-6-methyl-pyrimidin-4-ylamino1-ethyll-1 H-indol-5-ol (CWB025/02056) and 3-{2-r4-(4-diethylamino-1-methyl-butylamino)-6-methyl-pyrimidin-2-ylamino]-ethyl)-1 H-indol-5-ol (CWB027/02058)



CWB025/02056



CWB027/02058

The mixture of serotonin hydrochloride (1.0 g, 4.72 mmol), 2,4-dichloro-6-methylpyrimidine (1.0 g, 6.13 mmol) and diisopropylethylamine (1.0 g, 10.0 mmol) in isopropanol (8 mL) was stirred in a pressure bottle at 120 ^{0}C overnight. The reaction mixture was evaporated under vacuum and the residue was purified by silica gel column using CH_2Cl_2/MeOH as eluent to give 500 mg (50 %) of 3-(2-chloro-6-methyl-pyrimidin-4-ylamino)-1 H-indol-5-ol and 200 mg (14 %) of 3-(4-chloro-6-methyl-pyrimidin-2-ylamino)-1 H-indol-5-ol.

The first title compound was prepared from 3-(2-chloro-6-methyl-pyrimidin-4-ylamino)-1 H-indol-5-ol as described in Example 1.

^{1}H-NMR (300 MHz, $CDCl_3$ + CD_3OD): δ 7.16(d, 1H), 6.94-6.96 (m, 2H), 6.76 (dd, 1H), 5.47 (s, 1H), 4.01 (m, 1H), 3.50 (m 2H), 2.92 (t, 2H), 2.47-2.61 (m,

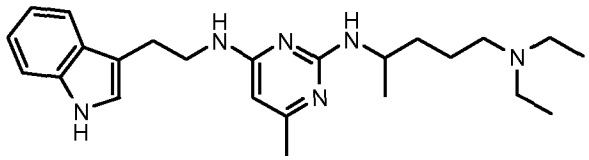
6H), 2.10 (s, 3H), 1.44-1.67 (m, 4H), 1.13 (d, 3H), 0.98-1.09 (m, 6H) (CWB025).

The second title compound was prepared from 3-(4-chloro-6-methyl-pyrimidin-2-ylamino)-1H-indol-5-ol as described in Example 3.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.21 (br, 1H), 7.11(d, 1H), 6.98(d, 1H), 6.87 (s, 1H), 6.75 (dd, 1H), 5.50 (s, 1H), 5.08 (br, 1H), 4.67 (br, 1H), 4.01 (br, 1H), 3.67 (m, 1H), 3.57 (m, 1H), 2.57 (t, 2H), 2.47-2.61 (m, 6H), 2.15 (s, 3H), 1.49-1.57 (m, 4H), 1.13 (d, 3H), 1.02 (t, 6H) (CWB027).

Comparative Example 4

N2-(4-Diethylamino-1-methyl-butyl)-N4-r2-(1 H-indol-3-yl)-ethyl)-6-methylpyrimidine-2,4-diam ine (CWB002/02038)

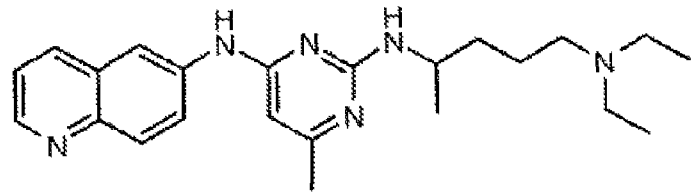


The title compound was prepared from tryptamine hydrochloride as described in Example 1.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.55(br, 1H), 7.60 (d, 1H), 7.37 (d, 1H), 7.17 ddd, 1H), 7.10 (ddd, 1H), 7.03 (d, 1H), 5.51 (s, 1H) 4.63 (br, 2H), 4.06 (m, 1H), 3.62 (m, 2H), 3.05 (t, 2H), 2.42-2.57 (m, 6H), 2.15 (s, 3H), 1.43-1.57 (m, 4H), 1.17 (d, 3H), 1.01 (t, 6H).

Comparative Example 5

N2-(4-Diethylamino-1-methyl-butyl)-6-methyl-N4-quinolin-6-yl-pyrimidine-2,4-diamine (CWB003/01020)

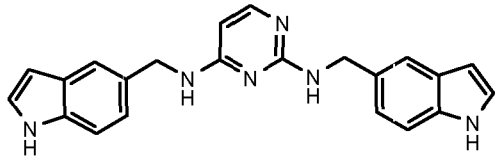


The title compound was prepared from 6-aminoquinoline as described in Example 1.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.83 (dd,1 H), 8.09-8.05 (m, 3H), 7.66 (dd, 1H), 7.39 (dd, 1H), 6.74 (s, 1H), 5.97 (s, 1H), 4.90 (d, 1H), 4.12 (m, 1H), 2.67-2.47 (m, 6H), 1.60-1 .58 (m, 4H), 1.28 (d, 3H), 1.04-0.99 (m, 6H).

Example 6

N2,N4-Bis-(1 H-indol-5-ylmethyl)-pyrimidine-2,4-diamine (CWB020/010 4 1)

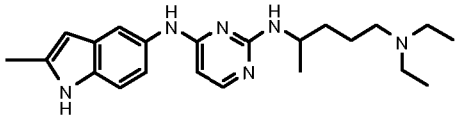


The title compound was prepared from 5-(aminomethyl)-indole and 2,4-dichloropyrimidine as described in Example 3.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (m, 2H), 7.86 (d, 1H), 7.61 (d, 2H), 7.33 (m, 2H), 7.23-7.15 (m, 4H), 6.51 (m, 2H), 5.75 (d, 1H), 5.18 (m, 1H), 4.96 (m, 1H), 4.69 (d, 2H), 4.59 (d, 2H).

Example 7

N2-(4-Diethylamino-1-methyl-butyl)-N4-(2-methyl-1 H-indol-5-yl)-pyrimidine-2,4-diamine (CWB02 1/0 1039)



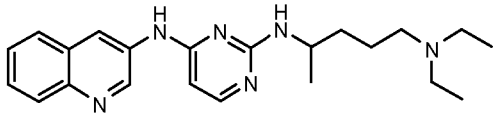
The title compound was prepared from 5-amino-2-methylindole, 2,4-dichloropyrimidine and 2-amino-5-diethylaminopentan as described in Example 3.

^{1}H NMR (300 MHz, CD_3OD): δ 7.68 (d, 1H), 7.58 (s, 1H), 7.22 (d, 1H), 7.06 (dd, 1H), 6.09 (s, 1H), 5.92 (d, 1H), 4.1 1-4.08 (m, 1H), 2.67-2.54 (m, 6H), 2.42 (s, 3H), 1.61-1 .53 (m, 4H), 1.22 (d, 3H), 1.03 (t, 6H).

Example 8

N2-(4-Diethylamino-1-methyl-butyl)-N4-quinolin-3-yl-pyrimidine-2,4-diamine

(CWB022/02052)



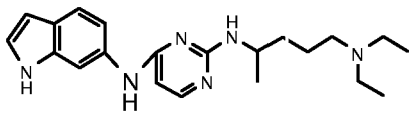
The title compound was prepared fro m 3-aminoquinoline, 2,4-dichloropyrimidine and 2-amino-5-diethylaminopentan as described in Example 3.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.92 (d, 1H), 8.74 (s, 1H), 8.17 (br, 1H), 8.00 (m, 2H), 7.74 (dd, 1H), 7.27-7.56 (m, 2H), 5.89 (d, 1H), 5.58 (br, 1H), 4.00 (m, 1H), 2.42-2.57 (m 6H), 1.60 (m, 4H), 1.28 (d, 3H), 1.00 (t, 6H).

Comparative Example 9

N2-(4-Diethylamino-1-methyl-butyl)-N4-(1 H-indol-6-yl)-pyrimidine-2,4-diamine

(CWB023/02053)



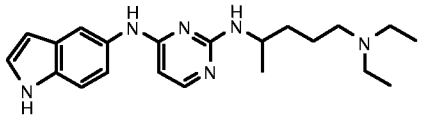
The title compound was prepared from 6-aminoindole, 2,4-dichloropyrimidine and 2-amino-5-diethylaminopentan as described in Example 3.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86(d, 1H), 7.82 (br, 1H), 7.55 (d, 1H), 7.19 (m, 1H), 6.81 (d, 1H), 6.63 (br, 1H), 6.50 (s, 1H), 5.89 (d, 1H), 4.81 (d, 1H), 3.97 (m, 1H), 2.53-2.71 (m 6H), 1.67-1 .96 (m, 4H), 1.18 (d, 3H), 1.04 (t, 6H).

Example 10

N2-(4-Diethylamino-1-methyl-butyl)-N4-(1 H-indol-5-yl)-pyrimidine-2,4-diamine

(CWB024/02054)



The title compound was prepared from 5-aminoindole, 2,4-dichloropyrimidine and 2-amino-5-diethylaminopentan as described in Example 3.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.84 (br, 1H), 7.83(d, 1H), 7.56 (d, 1H), 7.36 (d, 1H), 7.24 (m, 1H), 7.08 (dd, 1H), 6.96 (br, 1H), 6.51 (s, 1H), 5.88 (d, 1H), 4.68 (d, 1H), 4.03 (m, 1H), 3.1 1 (br, 1H), 2.56 (q, 4H), 2.45 (m 2H), 1.42-1 .52 (m, 4H), 1.15 (d, 3H), 1.03 (t, 6H).

Docking experiments

In order to analyse the Rad inhibitory effect of the compounds of the invention, a docking analysis has been performed utilizing the crystal structure of Rad .

A docking analysis includes the analysis of the orientation of one molecule with respect to a second molecule to which it is bound forming a complex.

Table 1 below summarizes the protein-protein interface GlideScores for all the compounds of the invention.

The GlideScore, in particular, provides a measure of the binding affinity (ΔGb) between Rad and a ligand/compound of the invention. It measures the

electrostatic interactions between molecules, including polar-polar contacts, hydrogen bonds and ionic bridges, as well as van der Waals interactions, torsional and desolvation energies, in addition to steric clashes. The efficiency measure of ΔGb/P.S.A. was also developed and computed, wherein P.S.A. is the polar surface area in Angstromm ^{2} of a particular ligand. The deeper the negative GlideScore/P.S.A values are, the stronger is the expected binding affinity of the different molecules to Rac1. The results obtained for the referenced compound NSC23766 are also included.

Table 1: Docking results of compounds with Rac1 inhibitory activity (software Glide).

COMPOUND	ΔGb	ΔGb/P.S.A.
NSC23766	-9.16	-0.1753
CWB020/01041	-10.25	-0.1788
CWB021/01039	-9.14	-0.1986
CWB024/02054	-7.77	-0.1689

As from the results shown in Table 1, compared to the NSC23766 which has a ΔGb/P.S.A ratio equal to -0.1753, compounds CWB020/01041, CWB021/01039, and CWB024/02054have similar ΔGb/P.S.A value and are expected to elicit a significant inhibitory action on Rac1 activity.

The structure of the docked ligands occupied the same binding site as NSC23766 on the Rad surface.

Analysis ofRad inhibitory activity

To determine the effect of the compounds of the invention on Rad activity we have utilized human cultured cells. The cells have been incubated for 4 hours with a fixed concentration of each invention compound and then Rad activity

has been stimulated by adding 10 ng/ml of PDGF-BB to the culture medium. The intracellular amount of Rac1-GTP has been determined using the GLISA™ assay (Cytoskeleton, Inc). The GLISA™ assay uses a 96-well plate coated with RBD domain of Rac1-family effector protein PAK1 . The active GTP-bound form of Rac1 , but not the inactive GDP-bound form from a total cell lysates of human cells binds to the plate. Accordingly, bound active Rac-1 protein was then detected by incubation with a specific primary antibody followed by a secondary antibody conjugated to horseradish peroxidase (HRP). The signal is then developed with O-phenylenediaminereagent (OPD reagents). A significant inhibition on Rac1 activity should decrease the intracellular levels of Rac1-GTP and therefore reduce the percentage of Rac1-GTP detected compared to control untreated cells.

Table 2 below shows the Rac1 inhibitory activity of the tested compounds measured in human smooth muscle cells by G-LISA assay on a double two experiments base.

Compound	Concentration (µM)	Rac1 inhibitory activity (% vs control)
Control PDGF- BB10 ng/ml		100
NSC23766	50	89.9
CWB018/01034	50	34.2
CWB020/01041	50	17.1
CWB021/01039	50	81.1
CWB022/02052	50	77.6
CWB024/02054	50	58.3

The shown compounds have a significant higher efficiency than the reference compound NSC23766.

Thus, compound CWB018, 020, 021 , 022, and 024 showed a significant end efficient inhibitory action on Rac1 activity and are selected for further investigation.

Under our experimental conditions we observed only an 11% inhibitory effect of NSC23766 on Rad activity compared to 18.9÷82.3% inhibition of the compounds of the invention. Accordingly, compounds CWB018/01034, CWB020/01041 , CWB021/01039, CWB022/02052, and CWB024/02054, which show the highest inhibitory activity, have been selected for further analysis. Thus, a classic pull down assay was also performed in human cells incubated with 50 and 100 μM concentration of CWB024/02054.

As shown in Figure 2, CWB024/02054 very efficiently reduced the intracellular content of Rad -GTP by over 90%.

Anti-apoptotic effect of selected compounds on ischemic cardiac myocytes in culture

To establish a cardiomyocytes based apoptosis assay, which mimics the post-ischemic damage observed after myocardial infarction, we have utilized a cardiac muscle cell line, HL-1 cells, derived from the AT-1 mouse atrial cardiomyocyte tumor lineage. The HL-1 cells were incubated in glucose deficient medium in the presence of hydrogen peroxide and either in presence or absence of compounds of the invention. Apoptosis rate of ischemic HL-1 cells was normalised to the apoptosis rate of the 8h control cells under non-ischemic conditions, thus incubated with normal concentration of glucose in the absence of hydrogen peroxide. Both non-ischemic and ischemic HL-1 cultures were harvested after 8 hours. Analyzed concentration of NSC23766 and the selected compounds with Rad inhibitory activity, CWB021/01039, CWB022/02052, and CWB024/02054 was 100 μM. The apoptotic rates were

then evaluated by TUNEL assay, a method for detecting DNA strand breaks in apoptotic cells by flow cytometer. The solvent of the compounds, i.e. DMSO, has no influence on the apoptosis rate. As shown in Table 3 below, apoptosis is increased in ischemic cultures compared with the corresponding control culture. NSC23766 and the compounds of the invention reduced the apoptotic rate in ischemic cultures compared to the 8 h ischemia.

Table 3. Effect of newly synthesized compounds with Rac1 inhibitory activity on apoptosis rate in HL-1 cells normalized to control (TUNEL-assay). The data are representative of two experiments.

	Relative rate of apoptosis normalised to control (%)
8h Control DSMO Control	100 94.6
8 h Ischemia	701.6
Ischemia+NSC26766	546.5
Ischemia+CWB021/01039	511.6
Ischemia+CWB022/02052	607.8
Ischemia+CWB024/02054	319.4

From the above data, the compound CWB024/02054 showed to have the best inhibitory effect with approximately 50% reduction of relative apoptosis.

Toxicity profile of selected compounds with Rac1 inhibitory activity

To measure the cytotoxic potential of the compounds of the invention the so called resazurine assay was applied which investigates the metabolic activity as biological endpoint. Compounds CWB01 8/01 034, CWB020/01 041 , CWB021/01039, CWB022/02052, and CWB024/02054 were prepared as 100 mM stock solutions in acetonitrile and incubated with the cultured cells at 8 different concentrations starting with 1 mM and followed by 1:2 dilution steps (1 mM, 500 µM, 250 µM, 125 µM, 62.5 µM, 31.25 µM, 15.625 µM, and 7.8125 µM). For a pre-screening of the compounds they were first tested in human

hepatocellular carcinoma cells (HepG2 cells)for 24 h and to gain further information about the cytotoxic potential more specific target tissues like the mouse cardiomyocytes HL-1 and primary rat cardiomyocytes were used additionally. In Table 4 below the calculated EC_5Ovalues (dose with half maximal effect) determined from the dose-response curves, maximal viability concentration (MVC) pointing out the first concentration with clear reduction of metabolic activity and classification are indicated. A classification scheme was previously set up by screening known reference compounds and calculation of their EC_{50} values. Accordingly, the compounds can be ranked into high toxic ($EC_{50} \leq 10$ µM), medium toxic (10 µM < $EC_{50} \leq 100$ µM), low toxic (100 µM < $EC_{50} \leq 500$ µM) and not toxic ($EC_{50} > 500$ µM). Compound NSC23766 which was tested in HepG2 can be classified as low toxic and towards HL-1 cells as not toxic to low toxic since it was not possible to calculate an EC50 due to only minor effects up to the maximal test concentration whereas whereas compound CWB018/01034 has a low toxic potential in HepG2 cells and all other compounds can be classified as medium toxic having EC_{50} values ranging from 21 µM to 64 µM. In HL-1 and primary rat cardiomyocytes all the compounds of the invention can be classified as medium toxic. Compound CWB01 8/0 1034 had a higher toxic potential in H1-1 and primary rat cardiomyocytes compared to HepG2, all other compounds reached the same toxic classification in the three tested cell types.

Table 4 shows the summary of the cytotoxicity profile and classification for compounds of the invention with Rac1 inhibitory activity. The EC_{50}, MVC determined for the endpoint metabolic activity in HepG2, HL-1 and primary rat cardiomyocytes after 24 h of compound exposure and the consequent

classification is displayed (the data are representative of two repeated experiments).

		HepG2			HL-1			Primary Rat Cardiomyocytes	
		EC50 (μM)	MVC (μM)		EC50 (μM)	MVC (μM)		EC50 (μM)	MVC (μM)
NCS23766	+	~224	31.2	No/+	>100	50	-	-	-
CWB018/01034	+	162	62.5	++	68	15.6	++	85	31.2
CWB020/1041	++	64	7.8	++	39	15.6	++	17	3.9
CWB021/01039	++	21	7.8	++	24	31.2	++	13	31.3
CWB022/02052	++	32	7.8	++	60	31.2	++	15	7.8
CWB024/02054	++	33	7.8	++	56	7.8	++	17	1.9

Cytotoxicity-Classification:

EC50 ≤ 10 μM, high (+++)

10 μM < EC50 ≤ 100 μM, medium (++)

100 μM < EC50 ≤ 500, low (+)

EC50 > 500 μM, (no)

The toxicity classification has been determined by using drugs currently utilized in clinic with known toxicity profile.

Analysis of permeation properties of selected compounds with Rac1 inhibitory activity

The permeation characteristics of the Rac1 inhibitors were determined first in the artificial system PAMPA (Parallel Artificial Membrane Permeability Assay) which uses a membrane covered with a lipid to simulate the lipid bilayer of various cell types, including intestinal epithelium and second *in vitro* across Caco2, a human colon carcinoma cell line seeded as monolayers onto polycarbonate membranes. The PAMPA is able to predict the ability of a compound to permeate by a passive transcellular transport through an artificial

lipid layer assembled on a membrane and also allows a classification into low (flux <20%), medium (20%< flux <70%), and high (flux >70%) absorbing substances. The Caco2 assay provides a well-established *in vitro* model to predict the human intestinal absorption. In their differentiated phase, Caco2 cells exhibit the morphological and physiological properties of the human small intestine, e.g. barrier function, active transport systems and efflux systems. According to the compounds apparent permeation, i.e Papp values which are expressed as $*10^{-6}$ cm/s, in Caco2 cells, they can be ranked into 3 categories:

- compounds with a Papp < 1 is predicted to be poorly absorbed (0 - 20% in vivo human absorption);
- compounds with Papp between 1 and 10 are moderately absorbed (20 - 70%); and
- compounds with a Papp >10 are predicted to be well absorbed (70 - 100%).

Both assays are important in the early drug discovery, as they may help predicting oral absorption, a drug's most prominent way of entry into the blood stream and a key success factor in the drug development process.

The flux rate of the compounds of the invention and the reference NSC23766 was measured from a donor compartment to an acceptor compartment across the lipid layer at a start concentration of 250 µM over a diffusion period of 16 h. According to the PAMPA-classification scheme, the tested compounds can be ranked as follows:

- low permeable compounds: NSC23766, CWB021/01039 and CWB024/02054;
- medium permeable is CWB022/02052; and
- high permeable compounds are CWB01 8/01 034 and CWB020/01041 .

The calculations of the apparent permeation across Caco2 monolayers over a period of 90 min with a start concentration of 50 µM resulted in the classification of the tested compounds as follows:

- medium penetration was detected for NSC23766 and CWB022/02052
- high permeable are CWB018/01034, CWB020/01041 , CWB021/01039 and CWB024/02054.

For those compounds with a low diffusion rate across the artificial membrane in the PAMPA but a medium or high permeability across Caco2 layers, i.e. NSC23766, CWB021/01039 and CWB024/02054, an active transport mechanism is assumed. Thus, compounds CWB01 8/01 034, CWB020/01041 , CWB021/01 039 and CWB024/02054 show better permeation characteristics compared to the reference NSC23766. Results are depicted in table 5.

Table 5 shows PAMPA flux values, Papp-values of the invention compounds with Rac1 inhibitory activity, and their classification.

	PAMPA flux [%] ± SD	PAMPA Classification	Papp [x10^{-6}cm/s] ± SD	Caco2 Classification
NSC23766	11±4	low	2.4±4.2	medium
CWB018/01034	85±11	high	20.5±1.9	high
CWB020/01041	54±4	high	65.9±3.1	high
CWB021/01039	5±1	low	17.1±12.5	high
CWB022/02052	34±4	medium	8.9±2.7	medium
CWB024/02054	6±2	low	32.1±12.4	high

Determination of metabolic stability of selected compounds with Rac1 inhibitory activity

The determination of the metabolic stability of compounds of the invention with Rac1 inhibitory activity were carried out by incubating the tested compounds with liver homogenates in the presence of appropriate cofactors.

The basic incubation mixture with total volume of 500 µl consisted of the following components: 100 µl liver homogenate, compound in DMSO (dimethyl sulfoxide) and 1 mM NADPH (reduced nicotinamide adenine dinucleotide phosphate), 1 mM UDPGA (uridine 5'-diphosphoglucuronic acid), 1 mM PAPS (3'-phosphoadenosine-5'-phosphosulfate) and 1 mM GSH (glutathione). The substrate concentration used was 10 µM. Two parallel incubations, one with cofactors and one without, were made. Final DMSO concentration in the incubation was 1 µM. Each reaction mixture was preincubated for 2 minutes at +37°C in a shaking incubator block (Eppendorf Thermomixer 5436, Hamburg, Germany). Reaction was started by addition of a cofactor mixture and stopped by adding 250 µl of ice-cold acetonitrile.

The remaining LC/MS peak areas (relative to 0 min incubation samples, %) detected for the substrate were as follows:

CWB01 8/01 034 disappear completely in the 60 minute incubation time, the disappearance being completely non-cofactor dependent.

For CWB022/02052 5% of LC/MS peak area was remaining after 60 minute incubation, the disappearance being mainly cofactor dependent and thus dominated by CYP-related metabolism.

For CWB020/01041 and CWB021/01 039, about 33 - 41% LC/MS peak area was remaining after 60 min incubation, the detected disappearance being mainly cofactor dependent, even thought some disappearance was detected also without cofactors for some of the compounds. CWB024/02054 was the most stable of the substances in the incubations, having about 91% remaining peak area after 60 minute incubation and disappearance is clearly non-cofactor dependent.

The remaining peak areas after 60 minutes incubation was also determined after incubation with increasing concentrations of compounds CWB021/01039 and CWB024/02054. The remaining peak areas for CWB021/01 039 with 10 µM, 1 µM and 0.1 µM initial substrate concentrations were 95%, 81% and 69%, respectively, while the corresponding values for CWB024/02054 were 86%, 87% and 76%, respectively. The disappearances for both study compounds were also clearly cofactor dependent and thus metabolism related . Calculations of pharmacokinetic (PK) parameters on the basis of substrate depletion for both study compounds (1 µM, 60 min time point) in human liver microsomes are shown in Table 6.

Table 6. Kinetic *in vitro - in vivo* extrapolations of study compound clearances and half-lifes in human, wherein: intrinsic clearance (whole liver) = CL_{int}; hepatic clearance = CL_H;
total (systemic) clearance = CL_{to}tai; half-life = $t_{1/2}$.

Substance	CL_{int}(L/min)	CL_h(L/h)	CL_{tot}(L/h)	$t_{1/2}$(h)
CWB0021	0.31	15.14	15.32	7.3
CWB0024	0.2	10.63	10.81	10.34

All the detected metabolites were tentatively identified according to the accurate mass data and in-source fragment ion data. Accordingly, the main metabolic reactions for all the compounds were hydroxylations and dealkylations and their combinations.

The relative percentage amount of each metabolite from total combined metabolite LC/MS peak areas for the CWB1 8/01 034, CWB020/01 041 , CWB021/01039, CWB022/02052, and CWB024/02054 are shown in the Table 7 below.

Table 7. LC/ESI/TOF-MS data obtained for the substrate and its metabolites. The % means the relative share of the total combined metabolite peak area for the particular compound.

Compound	RT [min]	Identification	Meas.(m/z) $[M+H]^+$	Calc. (m/z)	%
CWB018	3.95	Parent	380.2049	380.2087	
M1	3.79	demethylation	366.1908	366.1930	98.5
M2	3.74	Demethylation+hydroxylation	382.1874	382.1879	0.6
M3	4.03	Demethylation+decarboxylation	322.2084	322.2032	0.5
M4	3.20	N-dealkylation (loss of methyl 4-methylpentanoate)	252.1230	252.1249	0.5
CWB020	4.03	Parent	369.1803	369.1828	
M1	3.85	Hydroxylation	385.1804	385.1777	75.0
M2	3.79	2x hydroxylation	401.1762	401.1726	13.6
M3	3.92	Hydroxylation+sulphate conjugation	465.1374	465.1345	11.4
CWB021	3.68	Parent	381.2791	381.2767	
M1	3.63	Deethylation	353.2480	353.2454	100
CWB022	3.61	Parent	379.2646	379.2610	
M1	3.61	Deethylation	351.2313	351.2297	6.3
M2	3.47	Hydroxylation	395.2572	395.2559	8.0
M3	3.60	Hydroxylation	395.2560	395.2559	61.8
M4	3.63	2x hydroxylation	411.2525	411.2508	7.0
M5	3.58	Deethylation + hydroxylation	367.2274	367.2246	16.9
CWB024	3.63	Parent	367.2627	367.2610	
M1	3.58	Deethylation	339.2326	339.2297	60.0
M2	3.37	Hydroxylation	383.2607	383.2559	36.0
M3	3.21	2x hydroxylation	399.2553	399.2508	4.0

Inhibition towards CYP enzymes

The effects of 10 µM of the invention compounds on major drug metabolising Cytochrome P450 (CYP) activities were measured. Neither of the invention compounds did notably inhibit any of the model activities studied, and consequently, the IC_{50} values of both CWB021/01039 and CWB024/02054 against major drug metabolising CYPs were above 10 µM. The activity affected

most with both study compounds was associated with CYP2D6, but was still inhibited only by 35% and 30% by 10 μM CWB021/01039 and CWB024/02054, respectively. Thus, the likelihood that CWB021/01 039 and CWB024/02054 could cause major drug-drug interactions *in vivo* is relatively low.

Selectivity of Rad inhibitory activity

For compound CWB024/02054, other G-LISA assays was performed in order to calculate the actual IC50 value of this compound on Rad activity and to determine the selective action towards other members of the same class of family proteins, such as RhoA. As from Fig. 10, CWB024/02054 showed a concentration dependent inhibitory action of the expression levels of Rad GTP with a calculated IC50 value of approximately 138 μM.

Subsequently, the effect of CWB024/02054 on RhoA activity was determined by G-LISA assay by incubating cultured cells with increasing concentrations of the compound. As shown in Fig. 11 the compound did not have any significant effect on RhoA GTP levels in cells after PDGF-BB stimulation. In contrast, we observed a significant compensatory induction of RhoA activity by up to two fold at 25 μM and above.

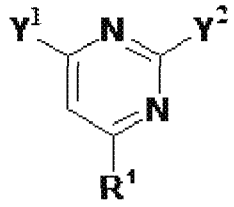
Since Rad is involved in membrane ruffle formation and lamellipodia extension, both subcellular structures involved in cell migration, the effect of CWB024/02054 on cell movement in the absence and presence of PDGF-BB has been monitored by video-microscopy analysis. As shown in Fig 12, the incubation of CWB024/02054 for 16h in the presence of PDGF-BB significantly reduced cell movement at 50 and 100 μM concentration. It is important to point out that at the same concentration the compound significantly affected Rad GTP levels in the same cell type (see Fig 10).

Finally, the effect of CWB024/02054 on cytoskeleton organization after stimulation with PDGF 20ng/ml has been investigated. In the presence of 50 μM compound CWB024/02054 PDGF stimulation only marginally induced lamellipodia formation at the cell edges, as compared to control untreated cells. These results further indicate that CWB024/02054 is effective in inhibiting Rac1-mediated cellular events.

From the above disclosure it is clear that the compound according to the invention are useful in the treatment of the heart failure and are advantageously selective for Rac1 with respect to RhoA, thus being particularly useful in the treatment of patients affected by hear failure.

CLAIMS:

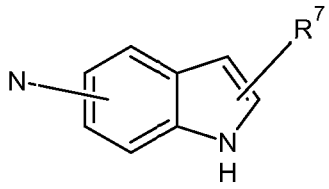
1. A compound having the following general formula (I):



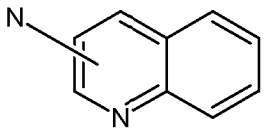
(I)

wherein

a) when Y^2 is -NR $^2R^3$ and R^2 is H and R^3 is -CH-(CH_3)(CH2)3-N(CH_2CH3)2 then Y^1 is -NR $^4R^5$ wherein R^4 is H and R^5 is i) indole optionally substituted to the pyrrole ring with a R^7 group, being the R^7 group a methyl (-CH_3) or a hydroxyl (-OH) group and said indole being attached to any available position on the benzene ring to the N atom of the Y^1 residue such as

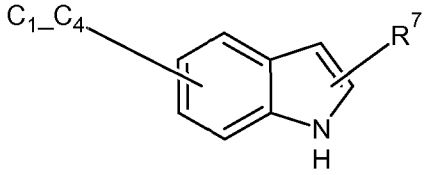


or R^5 is ii) an unsubstituted quinoline attached to any available position on the pyridinic ring to the N atom of the Y^1 moiety such as

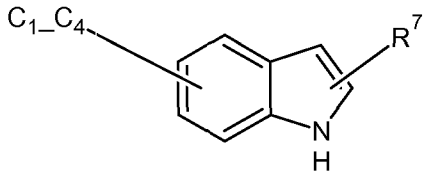


;or wherein

b) when Y^2 is -NR $^2R^3$ and R^2 is H and R^3 is a C_r C_4 alkyl chain substituted with indole optionally substituted at any available position on the pyrrole ring with a R^7 group as above defined and said indole being attached to any available position on the benzene ring to the rest of the Y^2 moiety such as

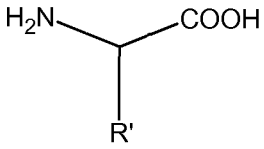


then Y^1 is -NR ^{4}R^5 wherein R^4 is H and R^5 is a CrC $_4$ alkyl chain substituted with indole optionally substituted at any available position on the pyrrole ring with a R^7 group as above defined and said indole being attached to any available position on the benzene ring to the rest of the Y^1 moiety such as



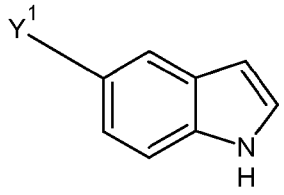
;or wherein

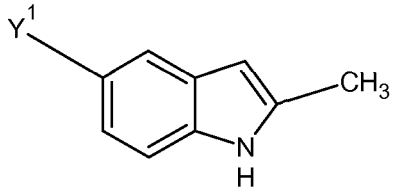
c) when Y^2 is -NR ^{2}R^3 and R^2 is H and R^3 is an unsubstituted or methyl (-CH_3) or hydroxyl (-OH) substituted N containing C9-C$_1$0 heterocycle attached to any available position on the benzene ring to the N atom of the Y^2 moiety then Y^1 is -CO(OR ^{8})R^9 wherein R^8 and R^9 are each independently a C$_r$ C_4 alkyl chain or R^9 is the R' chain of a natural amino acid of formula



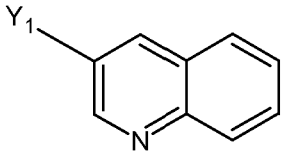
; and wherein in formula (I) R^1 is H or methyl (-CH_3); or any salts thereof.

2. A compound according to claim 1, wherein in a) Y^1 is -NR ^{4}R^5 wherein R^4 is H and R^5 is i) an unsubstituted or a indole substituted at the position 2 with a methyl (-CH_3) group, being the indole attached to the N atom of the Y^1 residue at the position 5 such as

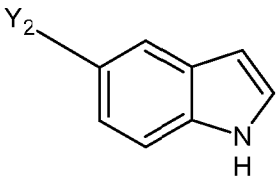


or


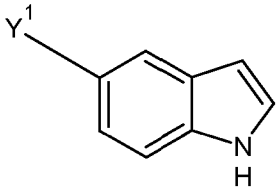
or R^5 is ii) an unsubstituted quinoline attached to the N atom of the Y^1 residue at the position 3 such as



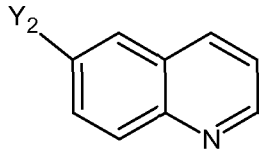
or the compounds of the invention are those of formula (I) above wherein in b) Y^2 is -NR $^2R^3$ and R^2 is H and R^3 is a Ci-C_4 alkyl chain, preferably a methylene (-CH_2-) substituted with an unsubstituted indole and said indole being attached to the Y^2 residue at the position 5 such as



and Y^1 is-NR $^4R^5$ wherein R^4 is H and R^5 is a Ci-C_4 alkyl chain, preferably a methylene (-CH_2-), substituted with an unsubstituted indole said indole being attached to the Y 1 residue at the position 5 such as



or the compounds of the invention are those of formula (I) above wherein in c) Y^2 is -NR $^2R^3$ wherein R^2 is H and R^3 is an unsubstituted quinoline attached to the position 6 to the N atom of the Y^2 residue such as



and Y^1 is -CO(OR ^{8})R^9 wherein R^8 is methyl (-CH_3) and R^9 is -CH_2-$CH(CH_3)_2$ and wherein R^1 is methyl (-CH_3).

3. A compound according to any one of the preceding claims, which is 4-Methyl-2-[6-methyl-2-(quinolin-6-ylamino)-pyrimidin-4-ylamino]-pentanoic acid methyl ester or N2,N4-Bis-(1 H-indol-5-ylmethyl)-pyrimidine-2,4-diamine or N2-(4-Diethylamino-1-methyl-butyl)-N4-(2-methyl-1 H-indol-5-yl)-pyrimidine-2,4-diamine or N2-(4-Diethylamino-1-methyl-butyl)-N4-quinolin-3-yl-pyrimidine-2,4-diamine or N2-(4-Diethylamino-1 -methyl-butyl)-N4-(1 H-indol-5-yl)-pyrimidine-2,4-diamine; or a pharmaceutically acceptable salts thereof.

4. A compound according to any one of the preceding claims, which is N2-(4-Diethylamino-1-methyl-butyl)-N4-(1 H-indol-5-yl)-pyrimidine-2,4-diamine or a pharmaceutically acceptable salt thereof.

5. A compound according to any one of the preceding claims as a medicament.

6. A compound according to claim 5 as a medicament for the treatment or the prevention of the human heart failure, cancers, hypertension, inflammation, and atherosclerosis.

7. A pharmaceutical composition comprising one or more compounds of any one of claims 1 to 4 and pharmaceutical additives and/or excipients.

8. The pharmaceutical composition according to claim 7, wherein said additives and/or excipients are selected in the group comprising diluent, solvents, bulking agents, fillers, reological modifier, stabilizers, binders, lubricants, disintegrant, preservatives, pH adjusting agents, buffers,

antioxidant, chelating agents, plasticizer, polymers, emulsifiers, edulcorants, flavoring agents, alone or in combination.

9. The pharmaceutical composition according to claim 7 or 8 comprising one or more of 4-Methyl-2-[6-methyl-2-(quinolin-6-ylamino)-pyrimidin-4-ylamino]-pentanoic acid methyl ester or N2,N4-Bis-(1 H-indol-5-ylmethyl)-pyrimidine-2,4-diamine or N2-(4-Diethylamino-1 -methyl-butyl)-N4-(2-methyl-1 H-indol-5-yl)-pyrimidine-2,4-diamine or N2-(4-Diethylamino-1 -methyl-butyl)-N4-quinolin-3-yl-pyrimidine-2,4-diamine or N2-(4-Diethylamino-1-methyl-butyl)-N4-(1 H-indol-5-yl)-pyrimidine-2,4-diamine; or a pharmaceutically acceptable salts thereof.

FIG.1

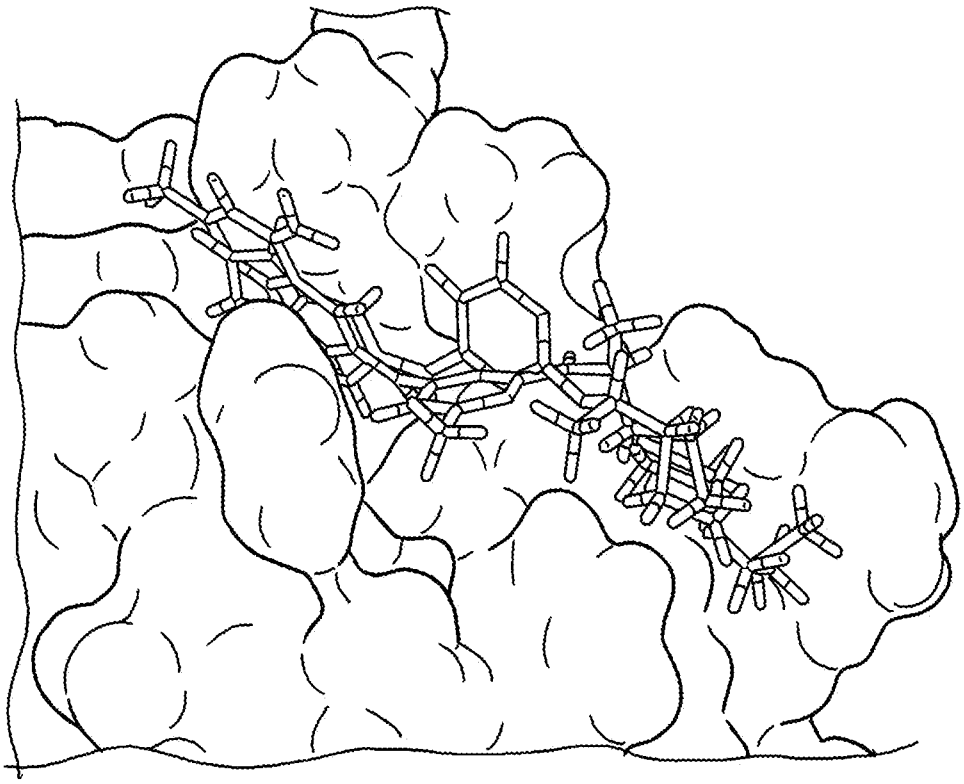


FIG. 2

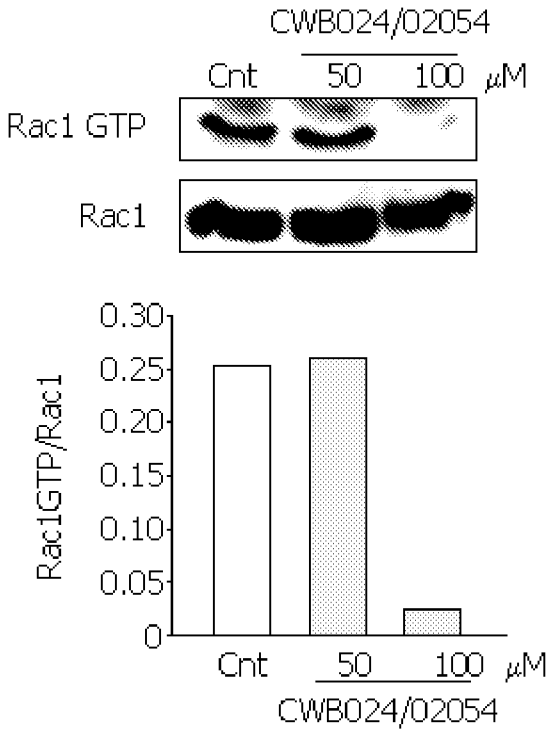

CWB024/02054
Cnt
50
100
μM
Rac1 GTP
Rac1
Rac1GTP/Rac1
0.30
0.25
0.20
0.15
0.10
0.05
0
Cnt
50
100
μM
CWB024/02054

FIG. 3

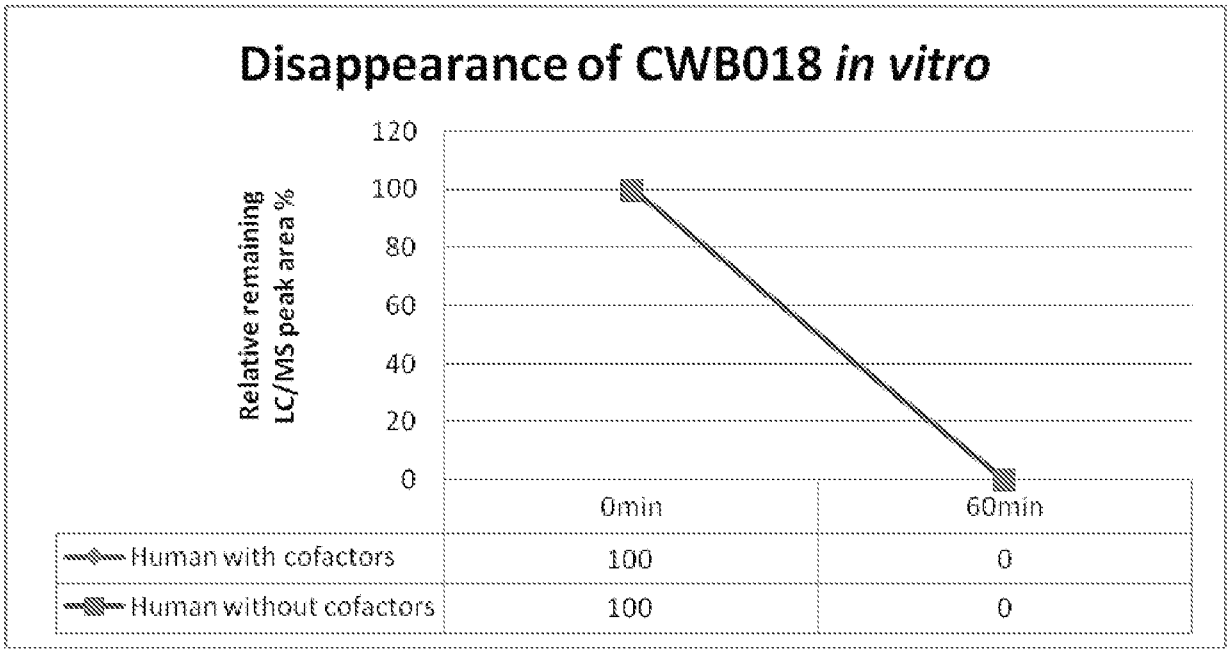

Disappearance of CWB018 in vitro
Relative remaining LC/MS peak area %
120
100
80
60
40
20
0
0min
60min
Human with cofactors
100
0
Human without cofactors
100
0

FIG. 4

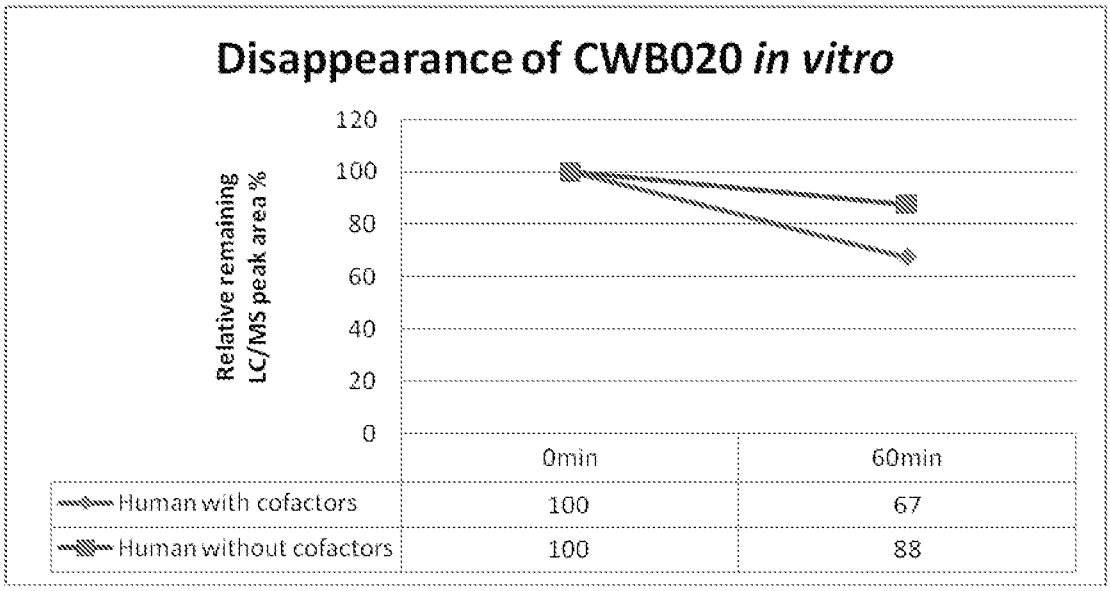

Disappearance of CWB020 *in vitro*
Relative remaining LC/MS peak area %
120
100
80
60
40
20
0
0min
60min
Human with cofactors
100
67
Human without cofactors
100
88

FIG. 5

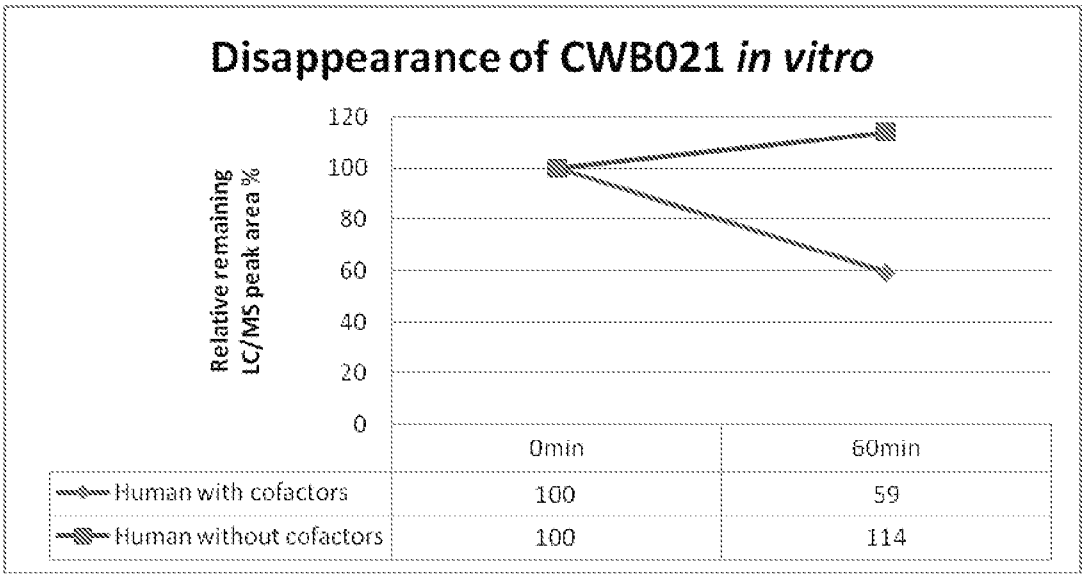

Disappearance of CWB021 in vitro
Relative remaining LC/MS peak area %
120
100
80
60
40
20
0
0min
60min
Human with cofactors
100
59
Human without cofactors
100
114

FIG. 6

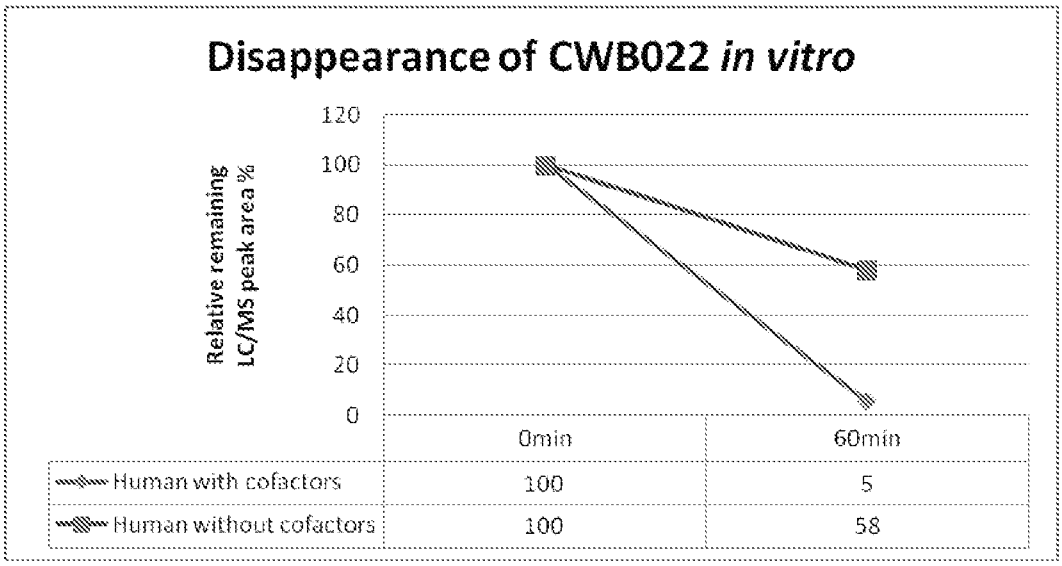

Disappearance of CWB022 in vitro
Relative remaining LC/MS peak area %
120
100
80
60
40
20
0
0min
60min
Human with cofactors
100
5
Human without cofactors
100
58

FIG. 7

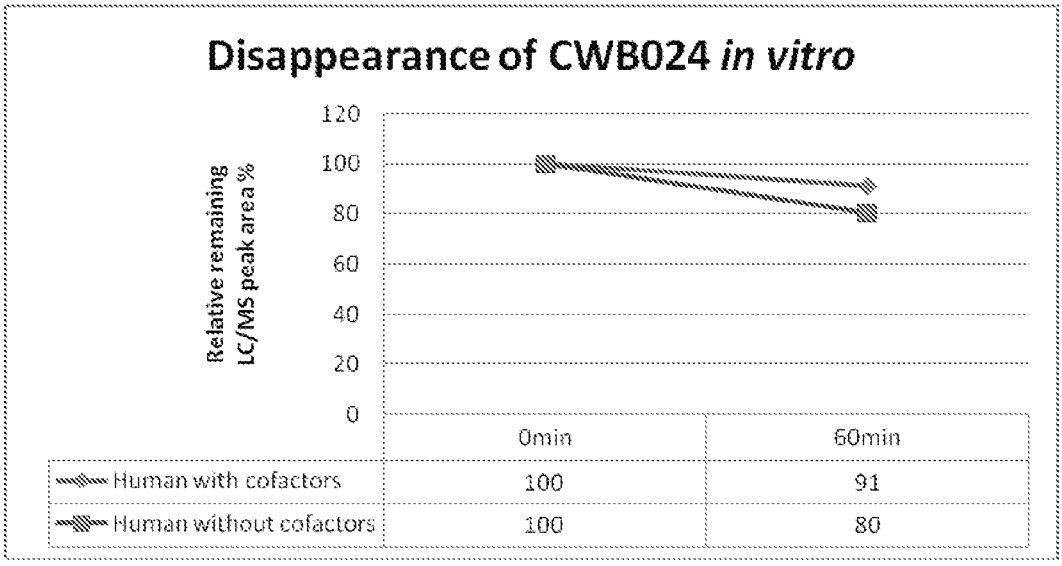

Disappearance of CWB024 in vitro
Relative remaining LC/MS peak area %
120
100
80
60
40
20
0
0min
60min
Human with cofactors
100
91
Human without cofactors
100
80

FIG. 8

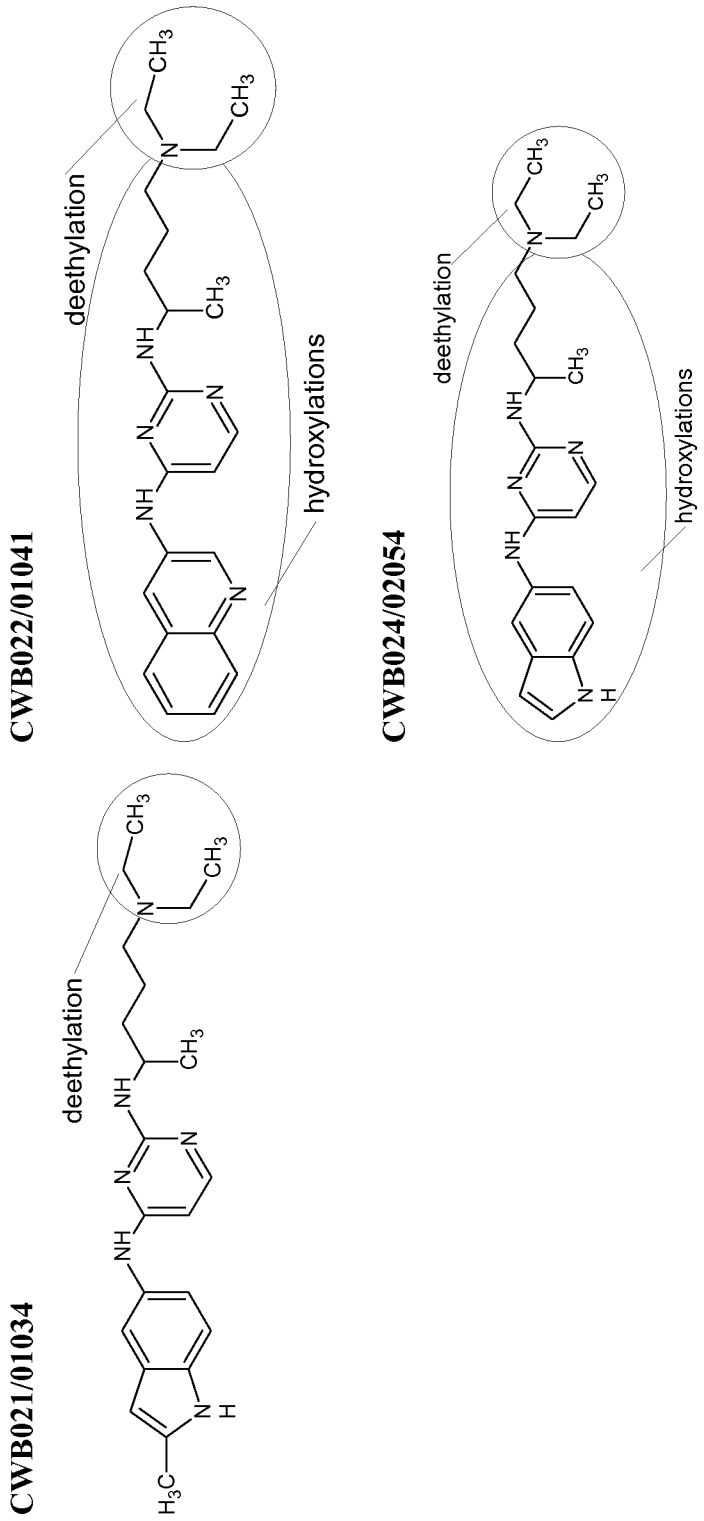

CWB022/01041
deethylation
hydroxylations
CWB024/02054
deethylation
hydroxylations
CWB021/01034
deethylation

FIG. 9

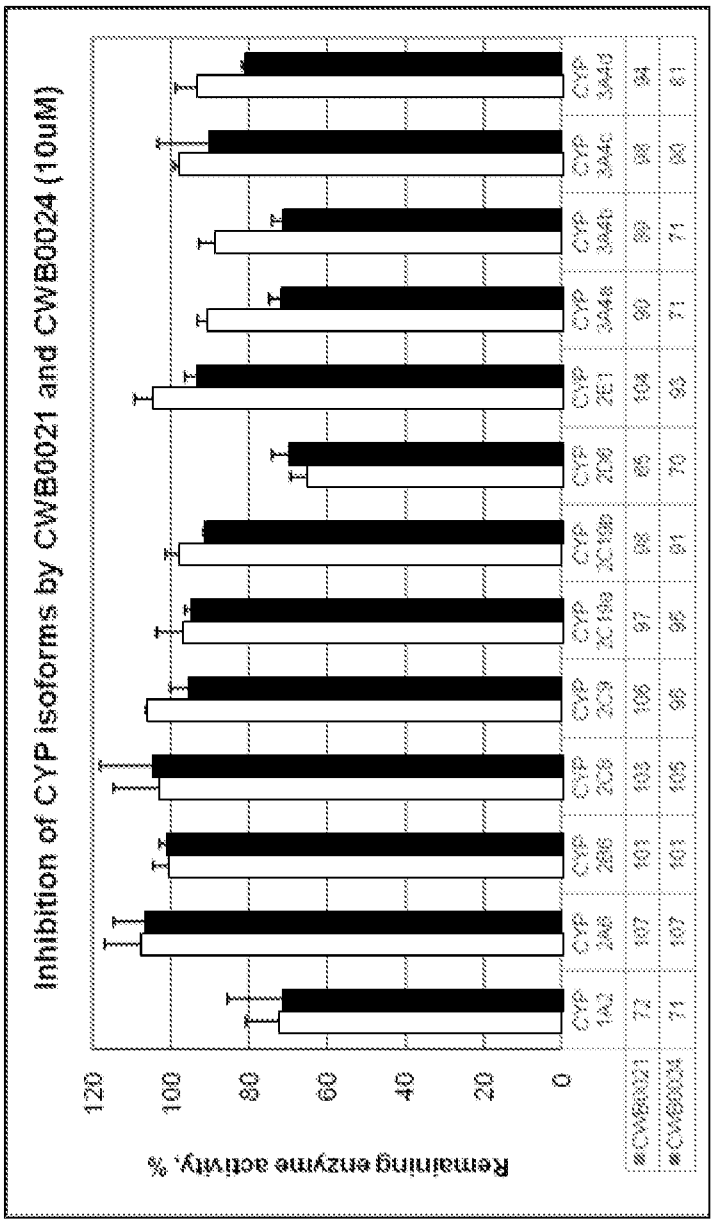

Inhibition of CYP isoforms by CWB0021 and CWB0024 (10uM)
Remaining enzyme activity, %
120
100
80
60
40
20
0
CWB0021
CWB0024
72
71
107
107
101
101
103
105
108
96
97
95
91
88
86
70
104
99
80
71
89
71
88
80
94
81
CYP 1A2
CYP 2A6
CYP 2B6
CYP 2C9
CYP 2C19
CYP 2C18
CYP 2D6
CYP 2E1
CYP 3A4
CYP 3A4b
CYP 3A5
CYP 3A7

FIG. 10

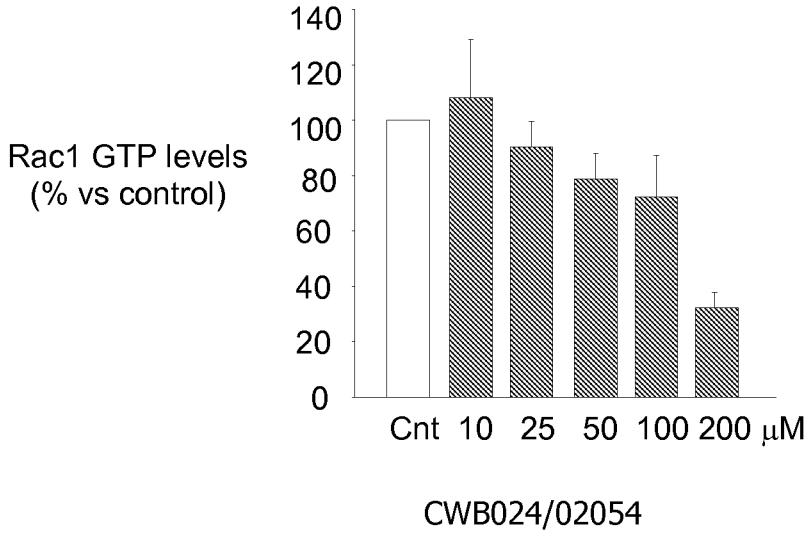

Rac1 GTP levels
(% vs control)
140
120
100
80
60
40
20
0
Cnt 10 25 50 100 200 μM
CWB024/02054

FIG.11

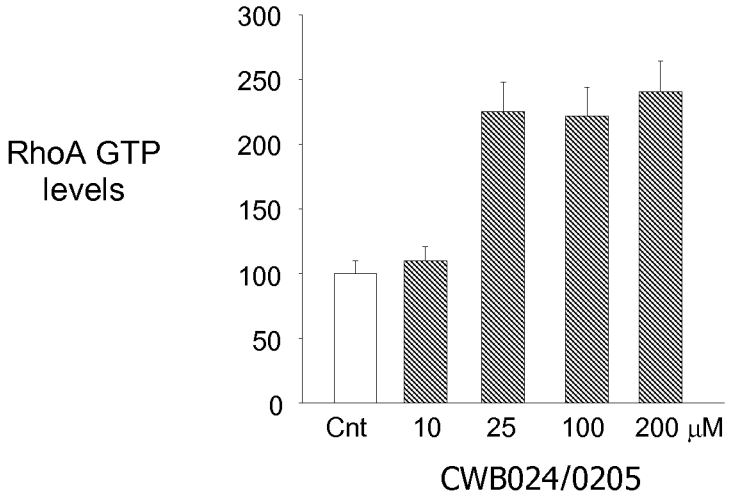
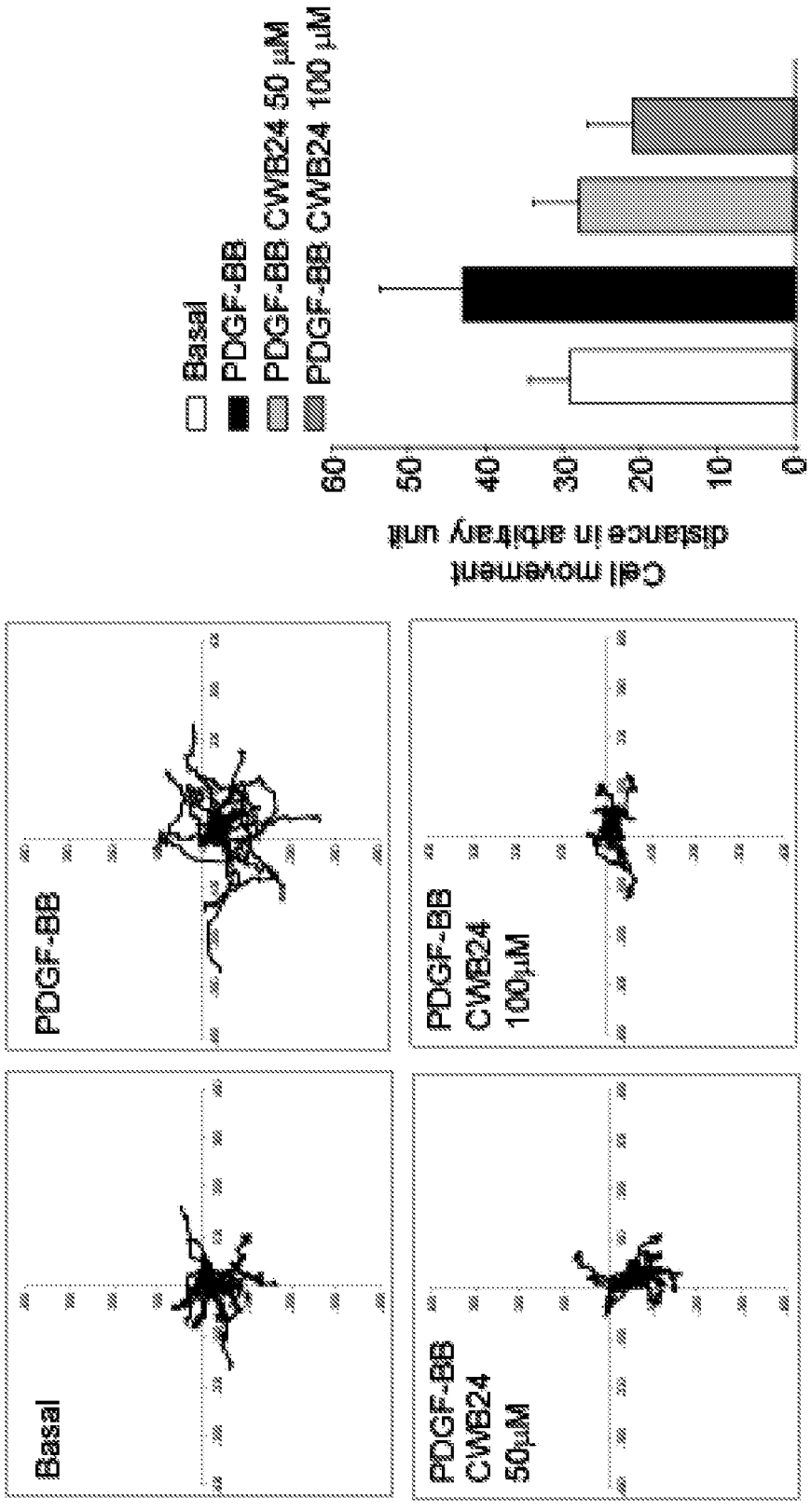

RhoA GTP levels
300
250
200
150
100
50
0
Cnt
10
25
100
200 μM
CWB024/0205

FIG.12


Basal
PDGF-BB
PDGF-BB CWB24 50 µM
PDGF-BB CWB24 100 µM
Cell movement
distance in arbitrary unit
0
10
20
30
40
50
60
Basal
PDGF-BB
PDGF-BB
CWB24
50µM
PDGF-BB
CWB24
100µM

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/054865

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D239/47 C07D239/48 C07D401/12 C07D403/12 A61K31/506 A61P9/04
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)
C07D 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 , BEILSTEIN Data, 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
A	WO 2005/051392 A (CHILDRENS HOSP MEDICAL CENTER [US]; ST JUDE CHILDRENS RES HOSPITAL [US) 9 June 2005 (2005-06-09) paragraph [0001]; claims 1,4,6	1-9

☐ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categones of cited documents

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

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

"L" document which may throw doubts on prtonty 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 prionty 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 documenl 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 docu¬ments, 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
28 May 2010	07/06/2010

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	Mates Valdiviel so, J

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/054865

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005051392 A	09-06-2005	CA 2546727 Al	09-06-2005
		EP 1691812 Al	23-08-2006
		JP 2007512363 T	17-05-2007