HU000025884T2

(19) **HU**

(11) Lajstromszám: **E 025 884**

(13) **T2**

MAGYARORSZÁG
Szellemi Tulajdon Nemzeti Hivatala

EURÓPAI SZABADALOM
SZÖVEGÉNEK FORDÍTÁSA

(21) Magyar ügyszám: **E 09 782777**
(22) A bejelentés napja: **2009. 09. 08.**

(96) Az európai bejelentés bejelentési száma:
EP 20090782777
(97) Az európai bejelentés közzétételi adatai:
EP 2331537 A1 **2010. 03. 18.**
(97) Az európai szabadalom megadásának meghirdetési adatai:
EP 2331537 B1 **2015. 10. 28.**

(51) Int. Cl.: ***C07D 417/14*** *(2006.01)*
A61K 3144/39 *(2006.01)*
A61P 35/00 *(2006.01)*

(86) A nemzetközi (PCT) bejelentési szám:
PCT/EP 09/061644
(87) A nemzetközi közzétételi szám:
WO 10029082

(30) Elsőbbségi adatok:
08164104 **2008. 09. 10.** **EP**
96674 P **2008. 09. 12.** **US**

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(54) **Proliferatív betegségek kezelésében alkalmas 1-((5-heteroariltiazol-2-il)-amino-karbonil)pirrolidin-2-karboxamid származékok, mint foszfatidilinozitol 3-kináz (P13K) inhibitorok**

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 331 537 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
28.10.2015 Bulletin 2015/44

(21) Application number: **09782777.8**

(22) Date of filing: **08.09.2009**

(51) Int Cl.:
C07D 417/14 *(2006.01)* ***A61K 31/4439*** *(2006.01)*
A61P 35/00 *(2006.01)*

(86) International application number:
PCT/EP2009/061644

(87) International publication number:
WO 2010/029082 (18.03.2010 Gazette 2010/11)

(54) 1-((5-HETEROARYLTHIAZOL-2-YL)AMINOCARBONYL)PYRROLIDINE-2-CARBOXAMIDE DERIVATIVES AS PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) INHIBITORS USEFUL IN THE TREATMENT OF PROLIFERATIVE DISEASES

1-((5-HETEROARYLTHIAZOL-2-YL)AMINOCARBONYL)PYRROLIDIN-2-CARBOXAMID-DERIVATE ALS PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) INHIBITOREN NÜTZLICH BEI DER BEHANDLUNG VON PROLIFERATIVER KRANKHEITEN

DÉRIVÉS DE 1-((5-HÉTÉROARYLTHIAZOL-2-YL)AMINOCARBONYL)PYRROLIDINE-2-CARBOXAMIDE COMME INHIBITEURS DE PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) UTILES DANS LE TRAITEMENT DE MALADIES PROLIFÉRATIVES

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR
Designated Extension States:
AL BA RS

(30) Priority: **10.09.2008 EP 08164104**
12.09.2008 US 96674 P

(43) Date of publication of application:
15.06.2011 Bulletin 2011/24

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(56) References cited:
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EP 2 331 537 B1

Description

[0001] The present invention relates to a specific 2-carboxamide cycloamino urea derivative, as a new, alpha-selective phosphatidylinositol (PI) 3-kinase inhibitor compound. This invention also relates to compositions, either alone or in combination with at least one additional therapeutic agent, and optionally in combination with a pharmaceutically acceptable carrier. This invention still further relates to the compound for use, either alone or in combination with at least one additional therapeutic agent, in the treatment of a number of diseases, in particular, those mediated by one or more of abnormal activity of growth factors, receptor tyrosine kinases, protein serine/heroine kinases, G protein coupled receptors and phospholipid kinases and phosphatases.

[0002] Phosphatidylinositol 3-kinases (PI3Ks) comprise a family of lipid kinases that catalyze the transfer of phosphate to the D-3' position of inositol lipids to produce phosphoinositol-3-phosphate (PIP), phosphoinositol-3,4-diphosphate (PIP_2) and phosphoinositol-3,4,5-triphosphate (PIP_3) that, in turn, act as second messengers in signaling cascades by docking proteins containing pleckstrin-homology, FYVE, Phox and other phospholipid-binding domains into a variety of signaling complexes often at the plasma membrane ((Vanhaesebroeck et al., Annu. Rev. Biochem 70:535 (2001); Katso et al., Annu. Rev. Cell Dev. Biol. 17:615 (2001)). Of the two Class 1 PI3Ks, Class 1A PI3Ks are heterodimers composed of a catalytic p110 subunit (α, β, δ isoforms) constitutively associated with a regulatory subunit that can be p85α, p55α, p50α, p85β or p55γ. The Class 1B sub-class has one family member, a heterodimer composed of a catalytic p110γ subunit associated with one of two regulatory subunits, p101 or p84 (Fruman et al., Annu Rev. Biochem. 67:481 (1998); Suire et al., Curr. Biol. 15:566 (2005)). The modular domains of the p85/55/50 subunits include Src Homology (SH2) domains that bind phosphotyrosine residues in a specific sequence context on activated receptor and cytoplasmic tyrosine kinases, resulting in activation and localization of Class 1A PI3Ks. Class 1B PI3K is activated directly by G protein-coupled receptors that bind a diverse repertoire of peptide and non-peptide ligands (Stephens et al., Cell 89:105 (1997)); Katso et al., Annu. Rev. Cell Dev. Biol. 17:615-675 (2001)). Consequently, the resultant phospholipid products of class I PI3K link upstream receptors with downstream cellular activities including proliferation, survival, chemotaxis, cellular trafficking, motility, metabolism, inflammatory and allergic responses, transcription and translation (Cantley et al., Cell 64:281 (1991); Escobedo and Williams, Nature 335:85 (1988); Fantl et al., Cell 69:413 (1992)).

[0003] In many cases, PIP2 and PIP3 recruit Akt, the product of the human homologue of the viral oncogene *v-Akt,* to the plasma membrane where it acts as a nodal point for many intracellular signaling pathways important for growth and survival (Fantl et al., Cell 69:413-423(1992); Bader et al., Nature Rev. Cancer 5:921 (2005); Vivanco and Sawyer, Nature Rev. Cancer 2:489 (2002)). Aberrant regulation of PI3K, which often increases survival through Akt activation, is one of the most prevalent events in human cancer and has been shown to occur at multiple levels. The tumor suppressor gene *PTEN,* which dephosphorylates phosphoinositides at the 3' position of the inositol ring and in so doing antagonizes PI3K activity, is functionally deleted in a variety of tumors. In other tumors, the genes for the p110α isoform, *PIK3CA,* and for *Akt* are amplified and increased protein expression of their gene products has been demonstrated in several human cancers. Furthermore, mutations and translocation of p85α that serve to up-regulate the p85-p110 complex have been described in human cancers. Finally, somatic missense mutations in *PIK3CA* that activate downstream signaling pathways have been described at significant frequencies in a wide diversity of human cancers (Kang at el., Proc. Natl. Acad. Sci. USA 102:802 (2005); Samuels et al., Science 304:554 (2004); Samuels et al., Cancer Cell 7:561-573 (2005)). These observations show that deregulation of phosphoinositol-3 kinase and the upstream and downstream components of this signaling pathway is one of the most common deregulations associated with human cancers and proliferative diseases (Parsons et al., Nature 436:792 (2005); Hennessey at el., Nature Rev. Drug Disc. 4:988-1004 (2005)).

[0004] In view of the above, inhibitors of PI3K alpha would be of particular value in the treatment of proliferative disease and other disorders.

[0005] WO2004/096797 discloses certain thiazole derivatives as inhibitors of PI3K gamma and their pharmaceutical use, particularly for the treatment of inflammatory and allergic conditions.

[0006] WO 2005/021519 also discloses certain thiazole derivatives as inhibitors of PI3K gamma and their pharmaceutical use, particularly for the treatment of inflammatory and allergic conditions.

[0007] WO 2006/051270 also discloses certain thiazole derivatives as inhibitors of PI3K alpha and their pharmaceutical use, particularly due to anti-tumor activity.

[0008] WO 2007/129044 also discloses certain thiazole derivatives as inhibitors of PI3K alpha and their pharmaceutical use, particularly due to anti-tumor activity.

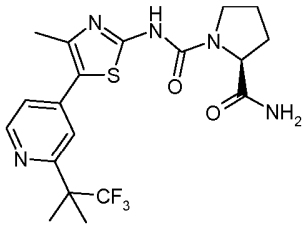
[0009] In view of the prior art, there is a need to provide further compounds suitable for treatment of proliferative diseases, particularly to provide compounds having improved selectivity and / or higher / improved activity.

[0010] It has now been found that the 2-carboxamide cycloamino urea derivative of the invention given below has advantageous pharmacological properties and inhibits, for example, PI3K (phosphatidylinositol 3-kinase). In particular, this compound shows selectivity for PI3K alpha with respect to beta and/or, delta and/or gamma subtypes. Hence, the compound of the invention is suitable, for example, to be used in the treatment of diseases depending on PI3 kinases (in particular PI3K alpha, such as those showing overexpression or amplification of PI3K alpha, somatic mutation of

PIK3CA or germline mutations or somatic mutation of PTEN or mutations and translocation of p85α that serve to up-regulate the p85-p110 complex), especially proliferative diseases such as tumor diseases and leukaemias.

[0011] Further, this compound preferably shows improved metabolic stability and hence reduced clearance, leading to improved pharmacokinetic profiles.

[0012] In a first aspect, the present invention provides the compound (S)-Pyrrolidine-1,2 dicarboxylic acid 2-amide 1-({4-methyl-5-[2-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-pyridin-4-yl]-thiazol-2-yl}-amide), of structure:



in free form or in pharmaceutically acceptable salt form.

[0013] The invention may be more fully appreciated by reference to the following description, including the following glossary of terms and the concluding examples. As used herein, the terms "including", "containing" and "comprising" are used herein in their open, non-limiting sense.

[0014] Any formula given herein is intended to represent hydrates, solvates, and polymorphs of such compounds, and mixtures thereof.

[0015] Any formula given herein is also intended to represent unlabeled forms as well as isotopically labeled forms of the compounds. Isotopically labeled compounds have structures depicted by the formulas given herein except that one or more atoms are replaced by an atom having a selected atomic mass or mass number. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, and chlorine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{18}F ^{31}P, ^{32}P, ^{35}S, ^{36}Cl, ^{125}I respectively. The invention includes various isotopically labeled compounds as defined herein, for example those into which radioactive isotopes, such as ^{3}H, ^{13}C, and ^{14}C, are present. Such isotopically labelled compounds are useful in metabolic studies (preferably with ^{14}C), reaction kinetic studies (with, for example ^{2}H or ^{3}H), detection or imaging techniques, such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) including drug or substrate tissue distribution assays, or in radioactive treatment of patients. In particular, an ^{18}F or labeled compound may be particularly preferred for PET or SPECT studies. Isotopically labeled compounds of this invention and prodrugs thereof can generally be prepared by carrying out the procedures disclosed in the schemes or in the examples and preparations described below by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

[0016] Further, substitution with heavier isotopes, particularly deuterium (i.e., ^{2}H or D) may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements or an improvement in therapeutic index. It is understood that deuterium in this context is regarded as a substituent of a compound of thethe invention. The concentration of such a heavier isotope, specifically deuterium, may be defined by the isotopic enrichment factor. The term "isotopic enrichment factor" as used herein means the ratio between the isotopic abundance and the natural abundance of a specified isotope. If a substituent in a compound of this invention is denoted deuterium, such compound has an isotopic enrichment factor for each designated deuterium atom of at least 3500 (52.5% deuterium incorporation at each designated deuterium atom), at least 4000 (60% deuterium incorporation), at least 4500 (67.5% deuterium incorporation), at least 5000 (75% deuterium incorporation), at least 5500 (82.5% deuterium incorporation), at least 6000 (90% deuterium incorporation), at least 6333.3 (95% deuterium incorporation), at least 6466.7 (97% deuterium incorporation), at least 6600 (99% deuterium incorporation), or at least 6633.3 (99.5% deuterium incorporation). In the compounds of this invention any atom not specifically designated as a particular isotope is meant to represent any stable isotope of that atom. Unless otherwise stated, when a position is designated specifically as "H" or "hydrogen", the position is understood to have hydrogen at its natural abundance isotopic composition. Accordingly, in the compounds of this invention any atom specifically designated as a deuterium (D) is meant to represent deuterium, for example in the ranges given above.

[0017] Where the plural form (e.g. compounds, salts) is used, this includes the singular (e.g. a single compound, a single salt). "A compound" does not exclude that (e.g. in a pharmaceutical formulation) more than one compound of the invention (or a salt thereof) is present.

[0018] The following general definitions shall apply in this specification, unless otherwise specified:

Halogen (or halo) denotes fluorine, bromine, chlorine or iodine, in particular fluorine, chlorine. Halogen-substituted groups and moieties, such as alkyl substituted by halogen (haloalkyl) can be mono-, poly- or per-halogenated.

"Treatment" includes prophylactic (preventive) and therapeutic treatment as well as the delay of progression of a disease or disorder.

"PI3 kinase mediated diseases" (especially PI3K alpha mediated diseases or diseases mediated by overexpression or amplification of PI3K alpha, somatic mutation of PIK3CA or germline mutations or somatic mutation of PTEN or mutations and translocation of p85α that serve to up-regulate the p85-p110 complex), are especially such disorders that respond in a beneficial way (e.g. amelioration of one or more symptoms, delay of the onset of a disease, up to temporary or complete cure from a disease) to the inhibition of a PI3 kinase, especially inhibition of PI3Kalpha or a mutant form thereof (where among the diseases to be treated, proliferative diseases such as tumor diseases and leukaemias may be especially mentioned).

"Salts" (which, what is meant by "or salts thereof" or "or a salt thereof"), can be present alone or in mixture with free compound of the invention and are preferably pharmaceutically acceptable salts. Such salts are formed, for example, as acid addition salts, preferably with organic or inorganic acids, from compounds of the invention with a basic nitrogen atom, especially the pharmaceutically acceptable salts. Suitable inorganic acids are, for example, halogen acids, such as hydrochloric acid, sulfuric acid, or phosphoric acid. Suitable organic acids are, e.g., carboxylic acids or sulfonic acids, such as fumaric acid or methansulfonic acid. For isolation or purification purposes it is also possible to use pharmaceutically unacceptable salts, for example picrates or perchlorates. For therapeutic use, only pharmaceutically acceptable salts or free compounds are employed (where applicable in the form of pharmaceutical preparations), and these are therefore preferred. In view of the close relationship between the novel compounds in free form and those in the form of their salts, including those salts that can be used as intermediates, for example in the purification or identification of the novel compounds, any reference to the free compounds hereinbefore and hereinafter is to be understood as referring also to the corresponding salts, as appropriate and expedient. The salts of compounds of the invention are preferably pharmaceutically acceptable salts; suitable counter-ions forming pharmaceutically acceptable salts are known in the field.

"Combination" refers to either a fixed combination in one dosage unit form, or a kit of parts for the combined administration where a compound of the the invention and a combination partner (e.g. another drug as explained below, also referred to as "therapeutic agent" or "co-agent") may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, e.g. synergistic effect. The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected combination partner to a single subject in need thereof (e.g. a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time. The term "pharmaceutical combination" as used herein means a product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term "fixed combination" means that the active ingredients, e.g. a compound of the invention and a combination partner, are both administered to a patient simultaneously in the form of a single entity or dosage. The term "non-fixed combination" means that the active ingredients, e.g. a compound of the invention and a combination partner, are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of three or more active ingredients.

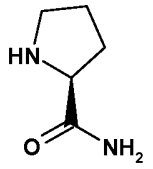
[0019] In preferred embodiments, which are preferred independently, collectively or in any combination or sub-combination, the invention relates to a compound of the invention, in free base form or in acid addition salt form, wherein the substituents are as defined herein.

[0020] Pharmaceutically acceptable prodrugs of a compound of the invention may be provided.

[0021] Pharmaceutically acceptable metabolites of a compound of the invention may be provided.

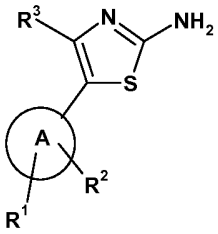
[0022] The present invention also relates to processes for the production of a compound of the invention. In principle all known processes which convert two different amines into a corresponding urea derivative are suitable and may be applied by using the respective starting material.

[0023] Thus, the invention in particular relates to a process for manufacturing a compound of the invention, which comprises reacting a compound of formula (II)



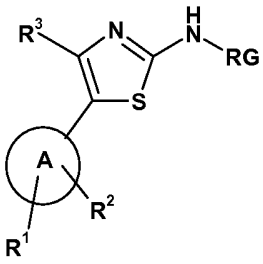
(II)

either with a compound of formula (IIIA)



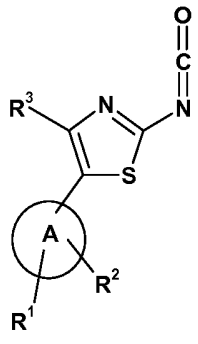
(IIIA)

wherein the substituents and A are defined as corresponding with the compound of the invention herein and R^3 may additionally represent Chloro, in the presence of an activating agent ("method A"), or with a compound of formula (IIIB)



(IIIB)

wherein R^1 and R^2 are defined as corresponding with the compound of the invention herein, R^3 is defined as corresponding with the compound of the invention herein and may additionally represent Chloro and RG represents a reactive group, such as imidazolylcarbonyl, which can react directly or via the formation of the isocyanate intermediate of formula (IIIE) ("method B"),



(IIIE)

wherein the substituents are as defined in (IIIB),

[0024] in each case optionally in the presence of a diluent and optionally in the presence of a reaction aid and

[0025] recovering the resulting compound of the invention in free form or in form of a salt and, optionally converting a compound of the invention obtainable according to method A or method B into a different compound, and/or converting an obtainable salt of a compound of the invention into a different salt thereof, and/or converting an obtainable free compound of the invention into a salt thereof, and/or separating an obtainable isomer of a compound of the invention

from one or more different obtainable isomers of the compound of the invention.

Reaction conditions

[0026] The process may be performed according to methods known in the art, or as disclosed below in the Examples. For example a compound of formula II may be reacted with a compound of formula III in a solvent, e.g. dimethylformamide, in the presence of a base e.g. an organic amine, e.g. triethylamine.

[0027] Where temperatures are given hereinbefore or hereinafter, "about" has to be added, as minor deviations from the numeric values given, e.g. variations of ±10 %, are tolerable.

[0028] All reactions may take place in the presence of one or more diluents and/or solvents. The starting materials may be used in equimolar amounts; alternatively, a compound may be used in excess, e.g. to function as a solvent or to shift equilibrium or to generally accelerate reaction rates.

[0029] Reaction aids, such as acids, bases or catalysts may be added in suitable amounts, as known in the field, required by a reaction and in line with generally known procedures.

Protecting groups

[0030] If one or more other functional groups, for example carboxy, hydroxy, amino, sulfhydryl or the like are or need to be protected in a starting material as described herein or any other precursor, because they should not take part in the reaction or disturb the reaction, these are such groups as are usually used in the synthesis of peptide compounds, and also of cephalosporins and penicillins, as well as nucleic acid derivatives and sugars. Protecting groups are such groups that are no longer present in the final compounds once they are removed, while groups that remain as substituents are not protecting groups in the sense used here which are groups that are added at a starting material or intermediate stage and removed to obtain a final compound.

[0031] The protecting groups may already be present in precursors and should protect the functional groups concerned against unwanted secondary reactions, such as acylations, etherifications, esterifications, oxidations, solvolysis, and similar reactions. It is a characteristic of protecting groups that they lend themselves readily, i.e. without undesired secondary reactions, to removal, typically by acetolysis, protonolysis, solvolysis, reduction, photolysis or also by enzyme activity, for example under conditions analogous to physiological conditions, and that they are not present in the end-products. The specialist knows, or can easily establish, which protecting groups are suitable with the reactions mentioned above and below.

[0032] The protection of such functional groups by such protecting groups, the protecting groups themselves, and their removal reactions are described for example in standard reference works, such as J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973, in T. W. Greene, "Protective Groups in Organic Synthesis", Third edition, Wiley, New York 1999, in "The Peptides"; Volume 3 (editors: E. Gross and J. Meienhofer), Academic Press, London and New York 1981, in "Methoden der organischen Chemie" (Methods of organic chemistry), Houben Weyl, 4th edition, Volume 15/I, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jescheit, "Aminosäuren, Peptide, Proteine" (Amino acids, peptides, proteins), Verlag Chemie, Weinheim, Deerfield Beach, and Basel 1982, and in Jochen Lehmann, "Chemie der Kohlenhydrate: Monosaccharide und Derivate" (Chemistry of carbohydrates: monosaccharides and derivatives), Georg Thieme Verlag, Stuttgart 1974.

Optional Reactions and Conversions

[0033] Salts of a compound of the inventino with a salt-forming group may be prepared in a manner known *per se*. Acid addition salts of compounds of the invention may thus be obtained by treatment with an acid or with a suitable anion exchange reagent. A salt with two acid molecules (for example a dihalogenide of a compound of the invention) may also be converted into a salt with one acid molecule per compound (for example a monohalogenide); this may be done by heating to a melt, or for example by heating as a solid under a high vacuum at elevated temperature, for example from 130 to 170°C, one molecule of the acid being expelled per molecule of a compound of the invention. Salts can usually be converted to free compounds, e.g. by treating with suitable basic compounds, for example with alkali metal carbonates, alkali metal hydrogencarbonates, or alkali metal hydroxides, typically potassium carbonate or sodium hydroxide.

[0034] Stereoisomeric mixtures, e.g. mixtures of diastereomers, can be separated into their corresponding isomers in a manner known *per se* by means of suitable separation methods. Diastereomeric mixtures for example may be separated into their individual diastereomers by means of fractionated crystallization, chromatography, solvent distribution, and similar procedures. This separation may take place either at the level of a starting compound or in a compound of the invention itself. Enantiomers may be separated through the formation of diastereomeric salts, for example by salt formation with an enantiomer-pure chiral acid, or by means of chromatography, for example by HPLC, using chroma-

tographic substrates with chiral ligands.

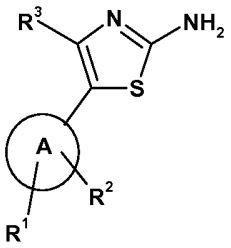
[0035] It should be emphasized that reactions analogous to the conversions mentioned in this chapter may also take place at the level of appropriate intermediates (and are thus useful in the preparation of corresponding starting materials).

Starting materials:

[0036] The starting materials of the formulae II and III, as well as other starting materials mentioned herein, e.g. below, can be prepared according to or in analogy to methods that are known in the art, are known in the art and/or are commercially available. Insofar as the production of the starting materials is not particularly described, the compounds are either known or may be prepared analogously to methods known in the art, e.g. in WO 05/021519 or WO04/096797, or as disclosed hereinafter. Novel starting materials, as well as processes for the preparation thereof, are likewise an embodiment of the present invention. In the preferred embodiments, such starting materials are used and the reaction chosen are selected so as to enable the preferred compounds to be obtained.

[0037] In the starting materials (including intermediates), which may also be used and/or obtained as salts where appropriate and expedient, the substituents are preferably as defined for a compound of the invention.

[0038] The disclosure herein also relates to compounds of formula (IIIA) or a salt thereof

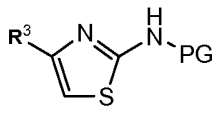


(IIIA)

wherein the substituents are as defined for a compound of the invention.

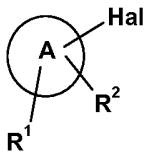
[0039] The present disclosure further relates to processes for the production of a compound of formula (IIIA). In principle, all known processes which couple two aryl / heteroaryl components (such as Heck-type reactions) into a corresponding urea derivative are suitable and may be applied by using the respective starting material. The invention thus also relates to a process for preparing a compound of formula (IIIA), which comprises

(Step 1) reacting a compound of formula (IV)



(IV)

wherein R^3 is defined as corresponding with a compound of the invention herein and may additionally represent halo, PG represents a protection group, such as an acyl group, with a compound of formula (V)



(V)

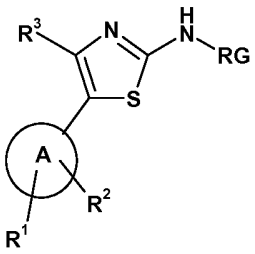
wherein R^1, R^2, A are defined as corresponding with a compound of the invention herein and Hal represents halo, such as bromo, under Heck conditions; optionally in the presence of a diluent and optionally in the presence of a reaction aid;

(Step 2) followed by removal of the protective group, e.g. under acidic conditions; optionally in the presence of a diluent and optionally in the presence of a reaction aid; and

recovering the resulting compound of formula (IIIA) in free form or in form of a salt and,

optionally converting a compound of the formula (IIIA) obtained into a different compound of the formula (IIIA), and/or converting an obtained salt of a compound of the formula (IIIA) into a different salt thereof, and/or converting an obtainable free compound of the formula (IIIA) into a salt thereof, and/or separating an obtainable isomer of a compound of the formula (IIIA) from one or more different obtained isomers of the formula (IIIA).

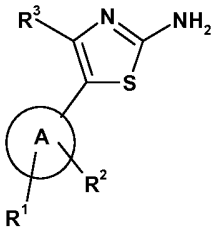
[0040] The present disclosure further relates to compounds of formula (IIIB) or a salt thereof



(IIIB)

wherein R^1, R^2, A are defined as corresponding with a compound of the invention , RG represents a reactive group, particularly imidazolylcarbonyl which can react directly or via the formation of the isocyanate intermediate of formula (IIIE), and R^3 is defined as corresponding with a compound of the invention herein and may additionally represent halo.

[0041] The present disclosure further relates to processes for the production of a compound of formula (IIIB). In principle, all known processes which convert an amine or salt thereof into a corresponding activated derivative are suitable and may be applied by using the respective starting material. The invention thus also relates to a process for preparing a compound of formula (IIIB), which comprises reacting a compound of formula (IIIA)



(IIIA)

wherein the substituents are as defined herein, with an activating reagent, such as 1,1'-carbonyldiimidazole, optionally in the presence of a diluent and optionally in the presence of a reaction aid; and
recovering the resulting compound of formula (IIIB) in free form or in form of a salt, and
optionally converting a compound of the formula (IIIB) obtained into a different compound of the formula (IIIB), and/or converting an obtained salt of a compound of the formula (IIIB) into a different salt thereof, and/or converting an obtainable free compound of the formula (IIIB) into a salt thereof, and/or separating an obtainable isomer of a compound of the formula (IIIB) from one or more different obtained isomers of the formula (IIIB).

[0042] The invention relates in one embodiment to compositions for human or veterinary use where inhibition of PI3K is indicated.

[0043] The invention relates to the treatment of cellular proliferative diseases such as tumor and/or cancerous cell growth mediated by PI3K. Diseases may include those showing overexpression or amplification of PI3K alpha, somatic mutation of PIK3CA or germline mutations or somatic mutation of PTEN or mutations and translocation of p85α that serve to up-regulate the p85-p110 complex. In particular, the compounds are useful in the treatment of human or animal (e.g., murine) cancers, including, for example, sarcoma; lung; bronchus; prostate; breast (including sporadic breast cancers and sufferers of Cowden disease); pancreas; gastrointestinal cancer; colon; rectum; colon carcinoma; colorectal adenoma; thyroid; liver; intrahepatic bile duct; hepatocellular; adrenal gland; stomach; gastric; glioma; glioblastoma; endometrial; melanoma; kidney; renal pelvis; urinary bladder; uterine corpus; uterine cervix; vagina; ovary; multiple myeloma; esophagus; a leukaemia; acute myelogenous leukemia; chronic myelogenous leukemia; lymphocytic leukemia; myeloid leukemia; brain; a carcinoma of the brain; oral cavity and pharynx; larynx; small intestine; non-Hodgkin lymphoma; melanoma; villous colon adenoma; a neoplasia; a neoplasia of epithelial character; lymphomas; a mammary carcinoma; basal cell carcinoma; squamous cell carcinoma; actinic keratosis; tumor diseases, including solid tumors; a tumor of the neck or head; polycythemia vera; essential thrombocythemia; myelofibrosis with myeloid metaplasia; and Walden stroem disease..

[0044] The condition or disorder (e.g. PI3K-mediated) may also be selected from the group consisting of: polycythemia vera, essential thrombocythemia, myelofibrosis with myeloid metaplasia, asthma, COPD, ARDS, Loffler's syndrome, eosinophilic pneumonia, parasitic (in particular metazoan) infestation (including tropical eosinophilia), bronchopulmonary aspergillosis, polyarteritis nodosa (including Churg-Strauss syndrome), eosinophilic granuloma, eosinophil-related disorders affecting the airways occasioned by drug-reaction, psoriasis, contact dermatitis, atopic dermatitis, alopecia areata,

erythema multiforme, dermatitis herpetiformis, scleroderma, vitiligo, hypersensitivity angiitis, urticaria, bullous pemphigoid, lupus erythematosus, pemphisus, epidermolysis bullosa acquisita, autoimmune haematogical disorders (e.g. haemolytic anaemia, aplastic anaemia, pure red cell anaemia and idiopathic thrombocytopenia), systemic lupus erythematosus, polychondritis, scleroderma, Wegener granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory bowel disease (e.g. ulcerative colitis and Crohn's disease), endocrine opthalmopathy, Grave's disease, sarcoidosis, alveolitis, chronic hypersensitivity pneumonitis, multiple sclerosis, primary biliary cirrhosis, uveitis (anterior and posterior), interstitial lung fibrosis, psoriatic arthritis, glomerulonephritis, cardiovascular diseases, atherosclerosis, hypertension, deep venous thrombosis, stroke, myocardial infarction, unstable angina, thromboembolism, pulmonary embolism, thrombolytic diseases, acute arterial ischemia, peripheral thrombotic occlusions, and coronary artery disease, reperfusion injuries, retinopathy, such as diabetic retinopathy or hyperbaric oxygen-induced retinopathy, and conditions characterized by elevated intraocular pressure or secretion of ocular aqueous humor, such as glaucoma.

[0045] For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.03 to about 100.0 mg/kg per body weight, e.g. about 0.03 to about 10.0 mg/kg per body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 3 g, e.g. about 5 mg to about 1.5 g, conveniently administered, for example, in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from ca. 0.1 to about 500 mg, e.g. about 1.0 to about 500 mg active ingredient.

[0046] The compound may be administered by any conventional route, in particular enterally, e.g. orally, e.g. in the form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, topically, e.g. in the form of lotions, gels, ointments or creams, by inhalation, intranasally, or in a suppository form.

[0047] The compound may be administered in free form or in pharmaceutically acceptable salt form e.g. as indicated above. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds.

[0048] Consequently, the invention also provides:

- a compound of the invention , in free form or in a pharmaceutically acceptable salt form as a pharmaceutical, e.g. in any of the methods as indicated herein.
- a compound of the invention in free form or in pharmaceutically acceptable salt form for use as pharmaceutical, e.g. in any of the methods as indicated herein, in particular for the use in one or more phosphatidylinositol 3-kinase mediated diseases.
- the use of a compound of the invention in free form or in pharmaceutically acceptable salt form in any of the methods as indicated herein, for the manufacture of a medicament for the treatment of one or more phosphatidylinositol 3-kinase mediated diseases.

[0049] PI3K serves as a second messenger node that integrates parallel signaling pathways, evidence is emerging that the combination of a PI3K inhibitor with inhibitors of other pathways will be useful in treating cancer and proliferative diseases in humans.

[0050] Approximately 20-30% of human breast cancers overexpress Her-2/neu-ErbB2, the target for the drug trastuzumab. Although trastuzumab has demonstrated durable responses in some patients expressing Her2/neu-ErbB2, only a subset of these patients respond. Recent work has indicated that this limited response rate can be substantially improved by the combination of trastuzumab with inhibitors of PI3K or the PI3K/AKT pathway (Chan et al., Breast Can. Res. Treat. 91:187 (2005), Woods Ignatoski et al., Brit. J. Cancer 82:666 (2000), Nagata et al., Cancer Cell 6:117 (2004)).

[0051] A variety of human malignancies express activitating mutations or increased levels of Her1/EGFR and a number of antibody and small molecule inhibitors have been developed against this receptor tyrosine kinase including tarceva, gefitinib and erbitux. However, while EGFR inhibitors demonstrate anti-tumor activity in certain human tumors (e.g., NSCLC), they fail to increase overall patient survival in all patients with EGFR-expressing tumors. This may be rationalized by the fact that many downstream targets of Her1/EGFR are mutated or deregulated at high frequencies in a variety of malignancies, including the PI3K/Akt pathway. For example, gefitinib inhibits the growth of an adenocarcinoma cell line in in vitro assays. Nonetheless, sub-clones of these cell lines can be selected that are resistant to gefitinib that demonstrate increased activation of the PI3/Akt pathway. Down-regulation or inhibition of this pathway renders the resistant subclones sensitive to gefitinib (Kokubo et al., Brit. J. Cancer 92:1711 (2005)). Furthermore, in an in vitro model of breast cancer with a cell line that harbors a PTEN mutation and over-expresses EGFR inhibition of both the PI3K/Akt pathway and EGFR produced a synergistic effect (She et al., Cancer Cell 8:287-297(2005)). These results indicate that the combination of gefitinib and PI3K/Akt pathway inhibitors would be an attractive therapeutic strategy in cancer.

[0052] The combination of AEE778 (an inhibitor of Her-2/neu/ErbB2, VEGFR and EGFR) and RAD001 (an inhibitor of mTOR, a downstream target of Akt) produced greater combined efficacy that either agent alone in a glioblastoma xenograft model (Goudar et al., Mol. Cancer. Ther. 4:101-112 (2005)).

[0053] Anti-estrogens, such as tamoxifen, inhibit breast cancer growth through induction of cell cycle arrest that requires the action of the cell cycle inhibitor p27Kip. Recently, it has been shown that activation of the Ras-Raf-MAP Kinase pathway alters the phosphorylation status of p27Kip such that its inhibitory activity in arresting the cell cycle is attenuated, thereby contributing to anti-estrogen resistance (Donovan, et al, J. Biol. Chem. 276:40888, (2001)). As reported by Donovan et al., inhibition of MAPK signaling through treatment with MEK inhibitor reversed the aberrant phosphorylation status of p27 in hormone refractory breast cancer cell lines and in so doing restored hormone sensitivity. Similarly, phosphorylation of p27Kip by Akt also abrogates its role to arrest the cell cycle (Viglietto et al., Nat Med. 8:1145 (2002)).

[0054] Accordingly, the compound may be used in the treatment of hormone dependent cancers, such as breast and prostate cancers. By this use, it is aimed to reverse hormone resistance commonly seen in these cancers with conventional anticancer agents.

[0055] In hematological cancers, such as chronic myelogenous leukemia (CML), chromosomal translocation is responsible for the constitutively activated BCR-Abl tyrosine kinase. The afflicted patients are responsive to imatinib, a small molecule tyrosine kinase inhibitor, as a result of inhibition of Abl kinase activity. However, many patients with advanced stage disease respond to imatinib initially, but then relapse later due to resistance-conferring mutations in the Abl kinase domain. In vitro studies have demonstrated that BCR-Ab1 employs the Ras-Raf kinase pathway to elicit its effects. In addition, inhibiting more than one kinase in the same pathway provides additional protection against resistance-conferring mutations.

[0056] Accordingly, the compound may be used in combination with at least one additional agent selected from the group of kinase inhibitors, such as Gleevec®, in the treatment of hematological cancers, such as chronic myelogenous leukemia (CML). By this use, it is aimed to reverse or prevent resistance to said at least one additional agent.

[0057] Because activation of the PI3K/Akt pathway drives cell survival, inhibition of the pathway in combination with therapies that drive apoptosis in cancer cells, including radiotherapy and chemotherapy, will result in improved responses (Ghobrial et al., CA Cancer J. Clin 55:178-194 (2005)). As an example, combination of PI3 kinase inhibitor with carboplatin demonstrated synergistic effects in both in vitro proliferation and apoptosis assays as well as in in vivo tumor efficacy in a xenograft model of ovarian cancer (Westfall and Skinner, Mol. Cancer Ther. 4:1764-1771 (2005)).

[0058] In addition to cancer and proliferative diseases, there is accumulating evidence that inhibitors of Class 1A and 1 B PI3 kinases would be therapeutically useful in others disease areas. The inhibition of p110β, the PI3K isoform product of the PIK3CB gene, has been shown to be involved in shear-induced platelet activation (Jackson et al., Nature Medicine 11:507-514 (2005)). Thus, a PI3K inhibitor that inhibits p110β would be useful as a single agent or in combination in anti-thrombotic therapy. The isoform p110δ, the product of the PIK3CD gene, is important in B cell function and differentiation (Clayton et al., J. Exp. Med. 196:753-763 (2002)), T-cell dependent and independent antigen responses (Jou et al., Mol. Cell. Biol. 22:8580-8590 (2002)) and mast cell differentiation (Ali et al., Nature 431:1007-1011 (2004)). Thus, it is expected that p110δ-inhibitors would be useful in the treatment of B-cell driven autoimmune diseases and asthma. Finally, the inhibition of p110γ, the isoform product of the PI3KCG gene, results in reduced T, but not B cell, response (Reif et al., J. Immunol. 173:2236-2240 (2004)) and its inhibition demonstrates efficacy in animal models of autoimmune diseases (Camps et al., Nature Medicine 11:936-943 (2005), Barber et al., Nature Medicine 11:933-935 (2005)).

[0059] The invention further provides pharmaceutical compositions comprising the compound, together with a pharmaceutically acceptable excipient suitable for administration to a human or animal subject, either alone or together with other anticancer agents.

[0060] The compound of the invention may be used in methods of treating human or animal subjects suffering from a cellular proliferative disease, such as cancer. The methods of treating a human or animal subject in need of such treatment, comprise administering to the subject a therapeutically effective amount of a compound of the invention either alone or in combination with one or more other anticancer agents. In particular, compositions will either be formulated together as a combination therapeutic or administered separately. Suitable anticancer agents for use with a compound of the invention include, but are not limited to, one or more compounds selected from the the group consisting of kinase inhibitors, anti-estrogens, anti androgens, other inhibitors, cancer chemotherapeutic drugs, alkylating agents, chelating agents , biological response modifiers, cancer vaccines, agents for antisense therapy as set forth below:

A. Kinase Inhibitors:_Kinase inhibitors for use as anticancer agents in conjunction with the compound of the the invention include inhibitors of Epidermal Growth Factor Receptor (EGFR) kinases such as small molecule quinazolines, for example gefitinib (US 5457105, US 5616582, and US 5770599), ZD-6474 (WO 01/32651), erlotinib (Tarceva®, US 5,747,498 and WO 96/30347), and lapatinib (US 6,727,256 and WO 02/02552); Vascular Endothelial Growth Factor Receptor (VEGFR) kinase inhibitors, including SU-11248 (WO 01/60814), SU 5416 (US 5,883,113 and WO 99/61422), SU 6668 (US 5,883,113 and WO 99/61422), CHIR-258 (US 6,605,617 and US 6,774,237), vatalanib or PTK-787 (US 6,258,812), VEGF-Trap (WO 02/57423), B43-Genistein (WO-09606116), fenretinide (retinoic acid p-hydroxyphenylamine) (US 4,323,581), IM-862 (WO 02/62826), bevacizumab or Avastin® (WO 94/10202), KRN-951, 3-[5-(methylsulfonylpiperadine methyl)-indolyl]-quinolone, AG-13736 and AG-13925, pyrrolo[2,1-f][1,2,4]triazines, ZK-304709, Veglin®, VMDA-3601, EG-004, CEP-701 (US 5,621,100), Cand5 (WO

04/09769); Erb2 tyrosine kinase inhibitors such as pertuzumab (WO 01/00245), trastuzumab, and rituximab; Akt protein kinase inhibitors, such as RX-0201; Protein Kinase C (PKC) inhibitors, such as LY-317615 (WO 95/17182), and perifosine (US 2003171303); Raf/Map/MEK/Ras kinase inhibitors including sorafenib (BAY 43-9006), ARQ-350RP, LErafAON, BMS-354825 AMG-548, and others disclosed in WO 03/82272; Fibroblast Growth Factor Receptor (FGFR) kinase inhibitors; Cell Dependent Kinase (CDK) inhibitors, including CYC-202 or roscovitine (WO 97/20842 and WO 99/02162); Platelet-Derived Growth Factor Receptor (PDGFR) kinase inhibitors such as CHIR-258, 3G3 mAb, AG-13736, SU-11248 and SU6668; and Bcr-Abl kinase inhibitors and fusion proteins such as STI-571 or Gleevec® (imatinib).

B. Anti-Estrogens:_Estrogen-targeting agents for use in anticancer therapy in conjunction with the compoound of the invention include Selective Estrogen Receptor Modulators (SERMs) including tamoxifen, toremifene, raloxifene; aromatase inhibitors including Arimidex® or anastrozole; Estrogen Receptor Downregulators (ERDs) including Faslodex® or fulvestrant.

C. Anti-Androgens:_Androgen-targeting agents for use in anticancer therapy in conjunction with the compound of the invention include flutamide, bicalutamide, finasteride, aminoglutethamide, ketoconazole, and corticosteroids.

D. Other Inhibitors:_Other inhibitors for use as anticancer agents in conjunction with the compound of the invention include protein farnesyl transferase inhibitors including tipifarnib or R-115777 (US 2003134846 and WO 97/21701), BMS-214662, AZD-3409, and FTI-277; topoisomerase inhibitors including merbarone and diflomotecan (BN-80915); mitotic kinesin spindle protein (KSP) inhibitors including SB-743921 and MKI-833; proteasome modulators such as bortezomib or Velcade® (US 5,780,454), XL-784; and cyclooxygenase 2 (COX-2) inhibitors including non-steroidal antiinflammatory drugs I (NSAIDs).

E. Cancer Chemotherapeutic Drugs: Particular cancer chemotherapeutic agents for use as anticancer agents in conjunction with the compound of the invention include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

F. Alkylating Agents:_Alkylating agents for use in conjunction with the compound of the invention include VNP-40101M or cloretizine, oxaliplatin (US 4,169,846, WO 03/24978 and WO 03/04505), glufosfamide, mafosfamide, etopophos (US 5,041,424), prednimustine; treosulfan; busulfan; irofluven (acylfulvene); penclomedine; pyrazoloacridine (PD-115934); O6-benzylguanine; decitabine (5-aza-2-deoxycytidine); brostallicin; mitomycin C (MitoExtra); TLK-286 (Telcyta®); temozolomide; trabectedin (US 5,478,932); AP-5280 (Platinate formulation of Cisplatin); porfiromycin; and clearazide (meclorethamine).

G. Chelating Agents:_Chelating agents for use in conjunction with the compound of the invention include tetrathiomolybdate (WO 01/60814); RP-697; Chimeric T84.66 (cT84.66); gadofosveset (Vasovist®); deferoxamine; and bleomycin optionally in combination with electorporation (EPT).

H. Biological Response Modifiers:_Biological response modifiers, such as immune modulators, for use in conjunction with the compound of the invention include staurosprine and macrocyclic analogs thereof, including UCN-01, CEP-701 and midostaurin (see WO 02/30941, WO 97/07081, WO 89/07105, US 5,621,100, WO 93/07153, WO 01/04125, WO 02/30941, WO 93/08809, WO 94/06799, WO 00/27422, WO 96/13506 and WO 88/07045); squalamine (WO 01/79255); DA-9601 (WO 98/04541 and US 6,025,387); alemtuzumab; interferons (e.g. IFN-a, IFN-b etc.); interleukins, specifically IL-2 or aldesleukin as well as IL-1, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, and active biological variants thereof having amino acid sequences greater than 70% of the native human sequence; altretamine (Hexalen®); SU 101 or leflunomide (WO 04/06834 and US 6,331,555); imidazoquinolines such as resiquimod and imiquimod (US 4,689,338, 5,389,640, 5,268,376, 4,929,624, 5,266,575, 5,352,784, 5,494,916, 5,482,936, 5,346,905, 5,395,937, 5,238,944, and 5,525,612); and SMIPs, including benzazoles, anthraquinones, thiosemicarbazones, and tryptanthrins (WO 04/87153, WO 04/64759, and WO 04/60308).

I. Cancer Vaccines:_Anticancer vaccines for use in conjunction with the compound of the invention include Avicine® (Tetrahedron Lett. 26:2269-70 (1974)); oregovomab (OvaRex®); Theratope® (STn-KLH); Melanoma Vaccines; GI-4000 series (GI-4014, GI-4015, and GI-4016), which are directed to five mutations in the Ras protein; GlioVax-1;

MelaVax; Advexin® or INGN-201 (WO 95/12660); Sig/E7/LAMP-1, encoding HPV-16 E7; MAGE-3 Vaccine or M3TK (WO 94/05304); HER-2VAX; ACTIVE, which stimulates T-cells specific for tumors; GM-CSF cancer vaccine; and Listeria monocytogenes-based vaccines.

J. Antisense Therapy: Anticancer agents for use in conjunction with the compound of the invention also include antisense compositions, such as AEG-35156 (GEM-640); AP-12009 and AP-11014 (TGF-beta2-specific antisense oligonucleotides); AVI-4126; AVI-4557; AVI-4472; oblimersen (Genasense®); JFS2; aprinocarsen (WO 97/29780); GTI-2040 (R2 ribonucleotide reductase mRNA antisense oligo) (WO 98/05769); GTI-2501 (WO 98/05769); liposome-encapsulated c-Raf antisense oligodeoxynucleotides (LErafAON) (WO 98/43095); and Sirna-027 (RNAi-based therapeutic targeting VEGFR-1 mRNA).

[0061] The compound can also be combined in a pharmaceutical composition with bronchiodilatory or antihistamine drugs substances. Such bronchiodilatory drugs include anticholinergic or antimuscarinic agents, in particular ipratropium bromide, oxitropium bromide, and tiotropium bromide, and β-2- adrenoreceptor agonists such as salbutamol, terbutaline, salmeterol, carmoterol, milveterol and, especially, formoterol or indacaterol. Co-therapeutic antihistamine drug substances include cetirizine hydrochloride, clemastine fumarate, promethazine, loratadine, desloratadine diphenhydramine and fexofenadine hydrochloride.

[0062] A combination may also be provided comprising the compound and one or more compounds that are useful for the treatment of a thrombolytic disease, heart disease, stroke, etc. Such compounds include aspirin, a streptokinase, a tissue plasminogen activator, a urokinase, a anticoagulant, antiplatelet drugs (e.g, PLAVIX; clopidogrel bisulfate), a statin (e.g., LIPITOR or Atorvastatin calcium), ZOCOR (Simvastatin), CRESTOR (Rosuvastatin), etc.), a Beta blocker (e.g., Atenolol), NORVASC (amlodipine besylate), and an ACE inhibitor (e.g., lisinopril).

[0063] A combination may also be provided comprising the compound and one or more compounds that are useful for the treatment of antihypertension. Such compounds include ACE inhibitors, lipid lowering agents such as statins, LIPITOR (Atorvastatin calcium), calcium channel blockers such as NORVASC (amlodipine besylate).

[0064] A combination may also be provided comprising the compound and one or more compounds selected from the group consisting of fibrates, beta-blockers, NEPI inhibitors, Angiotensin-2 receptor antagonists and platelet aggregation inhibitors.

[0065] A combination may also be provided comprising the compound and a compound suitable for the treatment of inflammatory diseases, including rheumatoid arthritis. Such compound may be selected from the group consisting of TNF-α inhibitors such as anti-TNF-α monoclonal antibodies (such as REMICADE, CDP-870) and D2E7 (HUMIRA) and TNF receptor immunoglobulin fusion molecules (such as ENBREL), IL-1 inhibitors, receptor antagonists or soluble IL-1Rα (e.g. KINERET or ICE inhibitors), nonsterodial anti-inflammatory agents (NSAIDS), piroxicam, diclofenac, naproxen, flurbiprofen, fenoprofen, ketoprofen ibuprofen, fenamates, mefenamic acid, indomethacin, sulindac, apazone, pyrazolones, phenylbutazone, aspirin, COX-2 inhibitors (such as CELEBREX (celecoxib), PREXIGE (lumiracoxib)), metalloprotease inhibitors (preferably MMP-13 selective inhibitors), p2x7 inhibitors, α2α inhibitors, NEUROTIN, pregabalin, low dose methotrexate, leflunomide, hydroxyxchloroquine, d-penicillamine, auranofin or parenteral or oral gold.

[0066] A combination may also be provided comprising the compound and a compound suitable for the treatment of osteoarthritis. Such compound may be selected from the group consisting of standard non-steroidal anti-inflammatory agents (hereinafter NSAID's) such as piroxicam, diclofenac, propionic acids such as naproxen, flurbiprofen, fenoprofen, ketoprofen and ibuprofen, fenamates such as mefenamic acid, indomethacin, sulindac, apazone, pyrazolones such as phenylbutazone, salicylates such as aspirin, COX-2 inhibitors such as celecoxib, valdecoxib, lumiracoxib and etoricoxib, analgesics and intraarticular therapies such as corticosteroids and hyaluronic acids such as hyalgan and synvisc.

[0067] A combination may also be provided comprising the compound and an antiviral agent and/or an antisepsis compound. Such antiviral agent may be selected from the group consisting of Viracept, AZT, acyclovir and famciclovir. Such antisepsis compound may be selected from the group consisting of Valant.

[0068] A combination may also be provided comprising the compound and one or more agents selected from the group consisting of CNS agents such as antidepressants (sertraline), anti-Parkinsonian drugs (such as deprenyl, L-dopa, Requip, Mirapex; MAOB inhibitors (such as selegine and rasagiline); comP inhibitors (such as Tasmar); A-2 inhibitors; dopamine reuptake inhibitors; NMDA antagonists; Nicotine agonists; Dopamine agonists; and inhibitors of neuronal nitric oxide synthase).

[0069] A combination may also be provided comprising a compound of the invention and one or more anti-Alzheimer's drugs. Such anti-Alzheimer Drug may be selected from the group consisting of donepezil, tacrine, α2δinhibitors, NEUROTIN, pregabalin, COX-2 inhibitors, propentofylline or metryfonate.

[0070] A combination may also be provided comprising a compound of the invention and anosteoporosis agents and/or an immunosuppressant agent. Such osteoporosis agents may be selected from the group consisting of EVISTA (raloxifene hydrochloride), droloxifene, lasofoxifene or fosomax. Such immunosuppressant agents may be selected from the group consisting of FK-506 and rapamycin.

[0071] Kits that include the compound the invention and a combination partner as disclosed herein may also be

provided. Representative kits include a PI3K inhibitor compound (e.g., the compound of the invention) and a package insert or other labeling including directions for treating a cellular proliferative disease by administering a PI3K inhibitory amount of the compound.

[0072] In general, the compound of the invention will be administered in a therapeutically effective amount by any of the accepted modes of administration for agents that serve similar utilities. The actual amount of the compound of the invention , i.e., the active ingredient, will depend upon numerous factors such as the severity of the disease to be treated, the age and relative health of the subject, the potency of the compound used, the route and form of administration, and other factors. The drug can be administered more than once a day, preferably once or twice a day. All of these factors are within the skill of the attending clinician. Therapeutically effective amounts of compounds of formulas I may range from about 0.05 to about 50 mg per kilogram body weight of the recipient per day; preferably about 0.1-25 mg/kg/day, more preferably from about 0.5 to 10 mg/kg/day. Thus, for administration to a 70 kg person, the dosage range would most preferably be about 35-70 mg per day.

[0073] In general, the compoundof the invention will be administered as pharmaceutical compositions by any one of the following routes: oral, systemic (e.g., transdermal, intranasal or by suppository), or parenteral (e.g., intramuscular, intravenous or subcutaneous) administration. The preferred manner of administration is oral using a convenient daily dosage regimen that can be adjusted according to the degree of affliction. Compositions can take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, suspensions, elixirs, aerosols, or any other appropriate compositions. Another preferred manner for administering the compoundof the invention is inhalation. This is an effective method for delivering a therapeutic agent directly to the respiratory tract.

[0074] The choice of formulation depends on various factors such as the mode of drug administration and bioavailability of the drug substance. For delivery via inhalation the compound can be formulated as liquid solution, suspensions, aerosol propellants or dry powder and loaded into a suitable dispenser for administration. There are several types of pharmaceutical inhalation devices-nebulizer inhalers, metered dose inhalers (MDI) and dry powder inhalers (DPI). Nebulizer devices produce a stream of high velocity air that causes the therapeutic agents (which are formulated in a liquid form) to spray as a mist that is carried into the patient's respiratory tract. MDI's typically are formulation packaged with a compressed gas. Upon actuation, the device discharges a measured amount of therapeutic agent by compressed gas, thus affording a reliable method of administering a set amount of agent. DPI dispenses therapeutic agents in the form of a free flowing powder that can be dispersed in the patient's inspiratory air-stream during breathing by the device. In order to achieve a free flowing powder, the therapeutic agent is formulated with an excipient such as lactose. A measured amount of the therapeutic agent is stored in a capsule form and is dispensed with each actuation.

[0075] The invention also relates to formulations wherein the particle size of a compound of formula (I) between 10 - 1000 nm, preferably 10 - 400 nm. Such pharmaceutical formulations have been developed especially for drugs that show poor bioavailability based upon the principle that bioavailability can be increased by increasing the surface area i.e., decreasing particle size. For example, U.S. 4,107,288 describes a pharmaceutical formulation having particles in the size range from 10 to 1,000 nm in which the active material is supported on a crosslinked matrix of macromolecules. U.S. 5,145,684 describes the production of a pharmaceutical formulation in which the drug substance is pulverized to nanoparticles (average particle size of 400 nm) in the presence of a surface modifier and then dispersed in a liquid medium to give a pharmaceutical formulation that exhibits remarkably high bioavailability. Both documents are included by reference.

[0076] In a further aspect, the invention provides pharmaceutical compositions comprising a (therapeutically effective amount) of a compound of the invention, and at least one pharmaceutically acceptable excipient. Acceptable excipients are non-toxic, aid administration, and do not adversely affect the therapeutic benefit of the compound of the invention . Such excipient may be any solid, liquid, semi-solid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

[0077] Solid pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like.

[0078] Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. Preferred liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols.

[0079] Compressed gases may be used to disperse a compound of the invention in aerosol form. Inert gases suitable for this purpose are nitrogen, carbon dioxide, etc. Other suitable pharmaceutical excipients and their formulations are described in Remington's Pharmaceutical Sciences, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990). The amount of the compound in a formulation can vary within the full range employed by those skilled in the art. Typically, the formulation will contain, on a weight percent (wt%) basis, from about 0.01-99.99 wt% of a compound of the invention based on the total formulation, with the balance being one or more suitable pharmaceutical excipients. Preferably, the compound is present at a level of about 1-80 wt%.

[0080] The invention further relates to pharmaceutical compositions comprising (i.e. containing or consisting of) at

least one compound of the invention and at least one pharmaceutically acceptable excipient.

[0081] Pharmaceutical compositions comprising a compound of the invention in free form or in pharmaceutically acceptable salt form in association with at least one pharmaceutical acceptable excipient (such as a carrier and/or diluent) may be manufactured in conventional manner by mixing the components.

[0082] Combined pharmaceutical compositions comprising a compound of the invention in free form or in pharmaceutically acceptable salt form and further comprising a combination partner (either in one dosage unit form or as a kit of parts) in association with at least one pharmaceutical acceptable carrier and/or diluent may be manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier and/or diluent with said active ingredients.

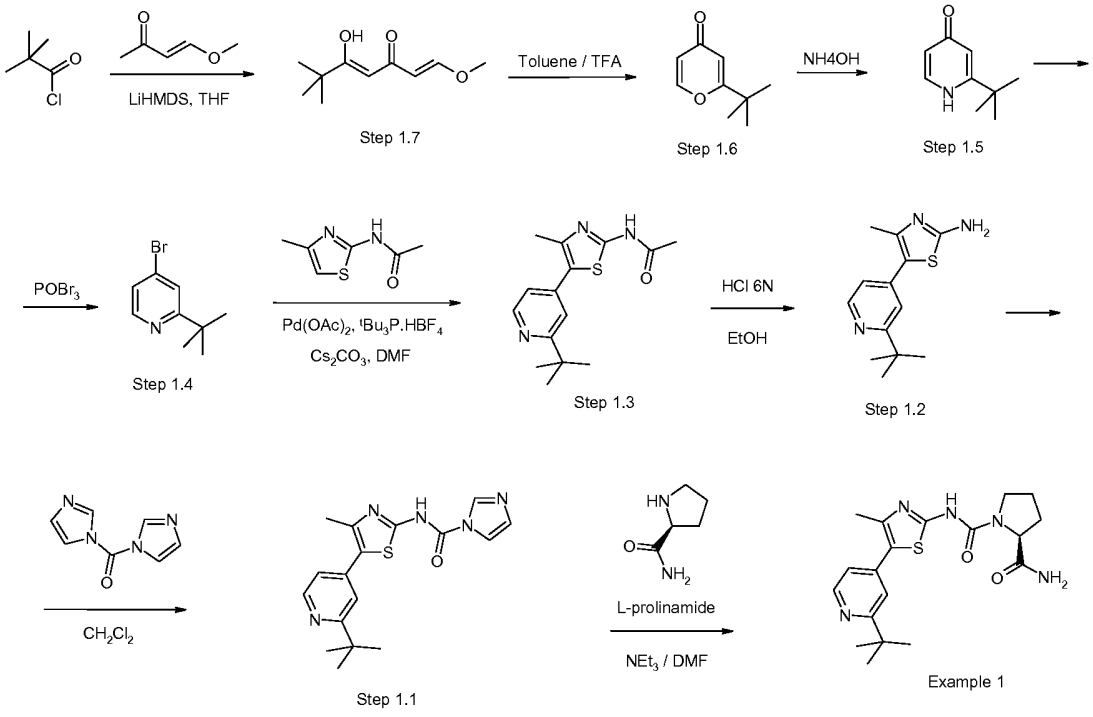
[0083] Consequently, the invention provides in further aspects

- a combined pharmaceutical composition, e.g. for use in any of the methods described herein, comprising a compound of the invention in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent and/or carrier.
- a combined pharmaceutical composition comprising a compound of the invention in free form or in pharmaceutically acceptable salt form as active ingredient; one or more pharmaceutically acceptable carrier material(s) and / or diluents and optionally one or more further drug substances. Such combined pharmaceutical composition may be in the form of one dosage unit form or as a kit of parts.
- a combined pharmaceutical composition comprising a therapeutically effective amount of a compound of the invention in free form or in pharmaceutically acceptable salt form and a second drug substance, for simultaneous or sequential administration.
- a compound of the invention for use in a method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective non-toxic amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof, and at least a second drug substance, e.g. as indicated above.
- a pharmaceutical combination, e.g. a kit, comprising a) a first agent which is a compound of the invention as disclosed herein, in free form or in pharmaceutically acceptable salt form, and b) at least one co-agent, e.g. as indicated above; whereby such kit may comprise instructions for its administration.

[0084] The following examples illustrate the invention. Example 1 is provided for reference purposes. Methods for preparing such compounds are described hereinafter.

Example 1: (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-{[5-(2-*tert*-butyl-pyridin-4-yl)-4-methyl-thiazol-2-yl]-amide}

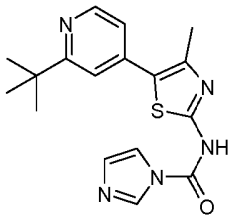
[0085]



[0086] Et_3N (1.54 mL, 11.1 mmol, 3 eq) is added to a solution of imidazole-1-carboxylic acid [5-(2-*tert*-butyl-pyridin-4-yl)-4-methyl-thiazol-2-yl]-amide (Step 1.1) (1.26 g, 3.7 mmol) and L-prolinamide (0.548 g, 4.8 mmol, 1.3 eq) in DMF (25 mL), under an argon atmosphere. The reaction mixture is stirred for 14 h at rt, quenched by addition of a saturated solution of $NaHCO_3$, and extracted with EtOAc. The organic phase is washed with a saturated solution of $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated. The residue is purified by silica gel column chromatography (DCM/MeOH, 1:0 → 94:6), followed by trituration in Et_2O to afford 1.22 g of the title compound as an off-white solid: ESI-MS: 388.1 $[M+H]^+$; t_R 2.35 min (System 1); TLC: R_f = 0.36 (DCM/MeOH, 9:1). ^1H NMR (400 MHz, DMSO-d6) δ(ppm): 1.32 (s, 9 H) 1.75-1.95 (m, 3 H) 1.97 - 2.13 (m, 1 H) 2.39 (s, 3 H) 3.38-3.50 (m, 1 H) 3.52-3.65 (m., 1 H) 4.10-4.40 (m, 1 H) 6.94 (br. s., 1 H) 7.22 (d, 1 H) 7.30 - 7.48 (m, 2 H) 8.49 (d, 1 H) 10.87 (br. s., 1 H)

Step 1.1: Imidazole-1-carboxylic acid [5-(2-*tert*-butyl-pyridin-4-yl)-4-methyl-thiazol-2-yl]-amide

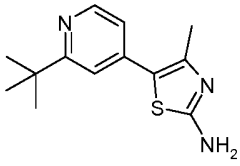
[0087]



[0088] A mixture of 5-(2-*tert*-butyl-pyridin-4-yl)-4-methyl-thiazol-2-ylamine (Step 1.2) (1 g, 4.05 mmol) and 1,1'-carbonyldiimidazole (0.984 g, 6.07 mmol, 1.5 eq) in DCM (50 mL) is stirred for 4 h at reflux and allowed to cool. The resulting precipitate is collected by filtration to provide 1.26 g of the title compound as white solid: ESI-MS: 340.2 $[M-H]^-$; t_R= 2.85 min (System 1).

Step 1.2: 5-(2-*tert*-Butyl-pyridin-4-yl)-4-methyl-thiazol-2-ylamine

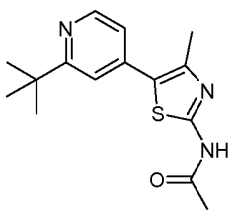
[0089]



[0090] A mixture of N-[5-(2-*tert*-butyl-pyridin-4-yl)-4-methyl-thiazol-2-yl]-acetamide (Step 1.3) (2 g, 7 mmol), a 6N aqueous solution of HCl (10 mL) and EtOH (50 mL) is stirred for 2 h at 85°C, allowed to cool, quenched by addition of a saturated solution of $NaHCO_3$ and extracted with DCM/MeOH (9:1, v/v). The organic phase is washed with a saturated solution of $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated. The residue is purified by silica gel column chromatography (DCM/MeOH, 1:0 → 96:4) to afford 1.21 g of the title compound as a yellow solid: ESI-MS: 248.1 $[M+H]^+$; TLC: R_f = 0.36 (DCM/MeOH, 9:1).

Step 1.3: N-[5-(2-*tert*-Butyl-pyridin-4-yl)-4-methyl-thiazol-2-yl]-acetamide

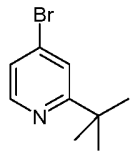
[0091]



[0092] A mixture of 2-acetamido-4-methylthiazole (1.2 g, 7.7 mmol, 1.1 eq), cesium carbonate (4.55 g, 14 mmol, 2 eq), tri-*tert*-butylphosphinium tetrafluoroborate (0.406 g, 1.4 mmol, 0.2 eq), palladium (II) acetate (0.15 g, 0.7 mmol, 0.1 eq) and 4-bromo-2-*tert*-butyl-pyridine (Step 1.4) (1.5 g, 7 mmol) in DMF (50 mL) is stirred for 1.5 h at 90°C under an argon atmosphere, allowed to cool, quenched by addition of a saturated solution of $NaHCO_3$ and filtered through a pad of celite. The filtrate is extracted with EtOAc. The organic phase is washed with a saturated solution of $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated. The residue is purified by silica gel column chromatography (DCM/MeOH, 1:0 → 97:3) to afford 2.02 g of the title compound as a yellow solid: ESI-MS: 290.1 $[M+H]^+$; TLC: R_f = 0.35 (DCM/MeOH, 9:1).

Step 1.4: 4-Bromo-2-*tert*-butyl-pyridine

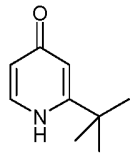
[0093]



[0094] A mixture of 2-*tert*-butyl-1H-pyridin-4-one (Step 1.5) (4.25 g, 28 mmol) and $POBr_3$ (8.88 g, 31 mmol, 1.1 eq) is heated to 120°C, stirred for 15 min, allowed to cool, quenched by addition of a saturated solution of $NaHCO_3$ and extracted with DCM/MeOH (9:1, v/v). The organic phase is washed with a saturated solution of $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated. The residue is purified by silica gel column chromatography (Hex/EtOAc, 95:5) to afford 5.18 g of the title compound as a yellow oil: ESI-MS: 214.0 / 216.0 $[M+H]^+$; t_R 2.49 min (System 1); TLC: R_f = 0.35 (Hex/EtOAc, 1:1).

Step 1.5: 2-*tert*-Butyl-1H-pyridin-4-one

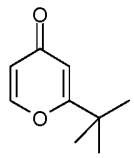
[0095]



[0096] A mixture of 2-*tert*-butyl-pyran-4-one (Step 1.6) (5.74 g, 37.7 mmol) and a 30% aqueous solution of ammonium hydroxide (100 mL) is stirred for 1 h at reflux, allowed to cool and concentrated. The residue is triturated with MeOH (200 mL) and filtered. The filtrate is concentrated and the residue purified by silica gel column chromatography (DCM/MeOH/NH_3^{aq}, 94:5:1 → 92:7:1) to afford 4.46 g of the title compound as a yellow solid: ESI-MS: 152.0 $[M+H]^+$; t_R 1.45 min (System 1); TLC: R_f = 0.11 (DCM/MeOH, 9:1).

Step 1.6: 2-*tert*-Butyl-pyran-4-one

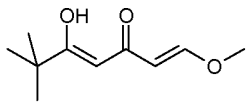
[0097]



[0098] A mixture of 5-hydroxy-1-methoxy-6,6-dimethyl-hepta-1,4-dien-3-one (Step 1.7) (6.8 g, 36.9 mmol) and TFA (5.65 mL, 74 mmol, 2 eq) in benzene (250 mL) is stirred for 14 h at rt and concentrated. Purification of the residue by silica gel column chromatography (Hex/EtOAc, 1:0 → 75:25) provides 5.74 g of the title compound as a yellow oil: ESI-MS: 153.1 $[M+H]^+$; t_R 3.21 min (System 1); TLC: R_f = 0.22 (Hex/EtOAc, 1:1).

Step 1.7: 5-Hydroxy-1-methoxy-6,6-dimethyl-hepta-1,4-dien-3-one

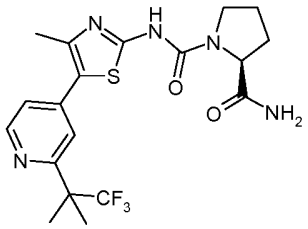
[0099]



[0100] LiHMDS (1M in THF, 100 mL, 2 eq) is added dropwise to a cold (-78°C) solution of 4-methoxy-3-buten-2-one (10 mL, 100 mmol, 2 eq) in THF (400 mL). After a 30 min stirring at - 78°C, a solution of pivaloyl chloride (6.12 mL, 50 mmol) in THF (100 mL) is added. The resulting mixture is allowed to warm to rt over 2 h and quenched by addition of a saturated solution of NH_4Cl. THF is removed under vacuum. The concentrated mixture is extracted with Et_2O. The organic phase is washed with brine, dried (Na_2SO_4), filtered and concentrated. The residue is purified by silica gel column chromatography (Hex/EtOAc, 1:0 → 85:15) to afford 6.83 g of the title compound as a yellow oil: ESI-MS: 185.1 $[M+H]^+$; TLC: R_f = 0.87 (Hex/EtOAc, 1:1).

Example 15: (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-({4-methyl-5-[2-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-pyridin-4-yl]-thiazol-2-yl}-amide)

[0101]



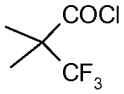
[0102] The title compound is prepared in analogy to the procedure described in Example 1 but with the following modifications. In Step 1.1, the reaction mixture is stirred for 14 h at reflux. In Step 1.2, the reaction mixture is stirred for 1 h at 85°C and extracted with EtOAc after being quenched. In Step 1.3, the reaction mixture is stirred for 2.5 h at 120°C. In Step 1.4, the reaction mixture is stirred for 1 h at 83°C and extracted with EtOAc after being quenched. In Step 1.5, the reaction mixture is stirred for 1 h at 65°C and trituration in MeOH is not performed. In Step 1.6, the crude product is not purified. In Step 1.7, 3,3,3-trifluoro-2,2-dimethyl-propionyl chloride (Step 12.1) is used.

[0103] Title compound: ESI-MS: 442.0 $[M+H]^+$; t_R 3.02 min (System 1); TLC: R_f = 0.35 (DCM/MeOH, 9:1). ^1H NMR (400 MHz, DMSO-d6) δ(ppm): 1.60 (s, 6 H) 1.70-1.95 (m, 3 H) 1.99 - 2.16 (m, 1 H) 2.40 (s, 3 H) 3.38 - 3.51 (m, 1 H) 3.51 - 3.69 (m, 1 H) 4.10-4.40 (m, 1 H) 6.95 (br. s., 1 H) 7.39 (d, 2 H) 7.53 (s, 1 H) 8.58 (d, 1 H) 10.93 (br. s., 1 H)

[0104] In an alternative procedure the title compound is prepared in analogy to the procedure described in Example 1 but with the following modifications: N,N-Dimethylacetamide is used instead of DMF and the mixture is stirred at 65 °C for 2 h. In Step 1.1, phenyl chloroformate (added slowly) is used instead of 1,1'-carbonyldiimidazole and the reaction is carried out in THF in the presence of N,N-diethyl-isopropylamine at room temperature (1.5 h). In Step 1.2, the reaction mixture is heated under stirring for 5 h under (reflux) and extracted with EtOAc after being quenched. In Step 1.3, the reaction mixture is stirred for 2 h at 100°C. In Step 1.4, the reaction is run in toluene using 1.1 equivalents of $POBr_3$ and 1.1 equivalents of tripropylamine and the mixture is stirred for 2 h at 80°C and extracted with EtOAc after being quenched. In Step 1.5, the reaction mixture is stirred for 1 h at 65°C and trituration in MeOH is not performed. In Step 1.6, toluene is used instead of benzene and the crude product is not purified. In Step 1.7, 3,3,3-trifluoro-2,2-dimethyl-propionyl chloride (Step 12.1) is used.

Step 12.1: 3,3,3-Trifluoro-2,2-dimethyl-propionyl chloride

[0105]



[0106] The title compound is prepared in analogy to the procedure described in Step 5.1 but using 3,3,3-trifluoro-2,2-dimethyl-propionic acid.

Step 5.1:1-Methyl-cyclopropanecarbonyl chloride

[0107]

[0108] A mixture of 1-methyl-cyclopropanecarboxylic acid (10 g, 100 mmol) and oxalyl chloride (10.49 ml, 120 mmol, 1.2 eq) in $CHCl_3$ (80 ml) is stirred for 4 h at 70 °C. The reaction mixture is concentrated to afford 11.8 g of the title compound as a yellow oil which is used without further purification.

Analytical HPLC conditions:

[0109] Linear gradient 20-100% solvent A in 5 min + 1.5 min 100% solvent A; detection at 215 nm, flow rate 1 mL/min at 30°C. Column: Nucleosil 100-3 C18 (70 x 4.0 mm). Solvent A = CH_3CN + 0.1 % TFA; Solvent B = H_2O + 0.1% TFA.

MS conditions:

[0110] Instrument: Micromass Platform II, eluent: 15% methanol in water containing 0.2% of a 25% ammonium hydroxide solution

[0111] ^{1}H-NMR spectra were measured on a Varian Mercury 400 Spectrometer in the indicated solvents. Abbreviations: br: broad; s: singlet; d: doublet; t: triplet; q: quartet; ppm: part per million

HPLC/MS conditions:

[0112] Instrument: Hewlett Packard Agilent 1100 series, column: XBridge™ C18 2.5 microm 3.0 X 30 mm, temperature: 50 °C, eluent: 2 channel system: Channel A 5% acetonitrile in water, Channel B acetonitrile containing 1.0% formic acid

Time (minutes)	% channel B	Flow (ml/minute)
0	5	1.4
3.7	95	1.4
4.4	95	2.4
4.45	95	2.4

[0113] detection: Agilent 1100 DAD 210-350 nm and Waters Micromass ZQ 2000 ESI+ and ESI-.

Preparative HPLC:

[0114] Instrument: Gilson preparative HPLC system, column: Sunfire™ Prep C18 OBD™ 5 microm 30 X 100 mm, temperature: 25 °C, eluent: gradient from 5 - 100% acetonitrile in 0.05% aqueous trifluoroacetic acid over 20 minutes, flow rate: 30 ml/minute, detection: UV 254 nm.

Abbreviations and Acronyms:

[0115]

BBr_3	boron tribromide

$^tBuP.HBF_4$	tri-*tert*-butylphosphinium tetrafluoroborate
DCM	dichloromethane
DIEA	diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	dimethyl formamide
DMP	1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone
DMSO	dimethylsulfoxide
Hex	hexane
L	liter(s)
LiHMDS	lithium bis(trimethylsilyl)amide
m.p.	melting point
MPLC	medium pressure liquid chromatography
NBS	N-bromosuccinimide
NMP	1-methyl-2-pyrrolidone
$PdCl_2(dppf)$	[1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II)
$Pd(PPh_3)_4$	tetrakis(triphenylphosphine)palladium(0)
R_f	ratio of fronts (TLC)
rt	room temperature
TFA	Trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
t_R	time of retention
v	volume
wt.	weight

Example A: efficiency as PI3 kinase inhibitors

[0116] **PI3K KinaseGlo assay**: 50 nL of compound dilutions were dispensed onto black 384-well low volume Non Binding Styrene (NBS) plates (Costar Cat. No. NBS#3676). L-a-phosphatidylinositol (PI), provided as 10 mg/ml solution in methanol, was transferred into a glass tube and dried under nitrogen beam. It was then resuspended in 3% OctylGlucoside (OG) by vortexing and stored at 4°C. The KinaseGlo Luminescent Kinase Assay (Promega, Madison/WI, USA) is a homogeneous HTS method of measuring kinase activity by quantifying the amount of ATP remaining in solution following a kinase reaction.

[0117] 5 μL of a mix of PI/OG with the PI3K subtype were added (Table 1). Kinase reactions were started by addition of 5 μl of ATP-mix containing in a final volume 10 μl 10 mM TRIS-HCl pH 7.5, 3mM $MgCl_2$, 50 mM NaCl, 0.05% CHAPS, 1 mM DTT and 1 μM ATP, and occurred at room temperature. Reactions were stopped with 10 μl of KinaseGlo and plates were read 10 mins later in a Synergy2 reader using an integration time of 0.1 seconds per well. 2.5 μM of a pan-class 1 PI3 kinase inhibitor (standard) was added to the assay plates to generate the 100% inhibition of the kinase reaction, and the 0% inhibition was given by the solvent vehicle (90% DMSO in water). The standard was used as a reference compound and included in all assay plates in the form of 16 dilution points in duplicate.

Table 1 PI3Ks by KinaseGlo: assay conditions and reagent protocol

Vol (10 μL)	Enzyme (nM)	ATP (μM)	PI/OG (μM/ μg/ml)	NaCl (mM)	Mg^{2+} (mM)	CHAPS (%)	DTT (mM)	time (mins)
PI3Ka	**10**	**1**	**11/10**	**50**	**3**	**0.05**	**1**	**30**
PI3Kβ	**25**	**1**	**11/10**	**50**	**3**	**0.05**	**1**	**30**
PI3Kγ	**150**	**1**	**22/20**	**50**	**3**	**0.05**	**1**	**90**
PI3Kd	**10**	**1**	**11/10**	**50**	**3**	**0.05**	**1**	**30**

Cloning of PI3Ks

[0118] The PI3Kα, PI3Kβ and PI3Kδ constructs are fusion of p85α iSH2 domain and the respective p110 isoforms. The p85α fragment and p110 isoform genes were generated by PCR from first strand cDNA generated by RT-PCR from commercial RNA from placenta, testis and brain as described below. The PI3Kγ construct was obtained from Roger

Williams lab, MRC Laboratory of Molecular Biology, Cambridge, UK (November, 2003) and is described (Pacold, Michael E.; Suire, Sabine; Perisic, Olga; Lara-Gonzalez, Samuel; Davis, Colin T.; Walker, Edward H.; Hawkins, Phillip T.; Stephens, Len; Eccleston, John F.; Williams, Roger L. Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. Cell (2000), 103(6), 931-943).

PI3Kα constructs and proteins

[0119]

PI3Kα wt	BV1075	p85iSH2(461-568)-GGGGGGGGGGGG-p110α (21-1068)-His

[0120] BV1075: The construct for Baculovirus BV-1075 was generated by a three-part ligation comprised of a p85 fragment and a p110α fragment cloned into vector pBlueBac4.5. The p85 fragment was derived from plasmid p1661-2 digested with Nhe/Spe. The p110α fragment derived from is clone was verified by sequencing and used in a LR410 as a SpeI/HindIII fragment. For the generation of the baculovirus expression vector LR410 the gateway LR reaction to transfer the insert into the Gateway adapted pBlueBac4.5 (Invitrogen) vector was used. The cloning vector pBlueBac4.5 (Invitrogen) was digested with Nhe/HindIII. This resulted in the construct PED 153.8. The p85 component (iSH2) was generated by PCR using ORF 318 (described above) as a template and one forward primer KAC1028 (5'-GCTAGCAT-GCGAGAATATGATAGAT-TATATGAAG-AATATACC) (SEQ ID NO: 1) and two reverse primers, KAC1029 (5'-GCCTC-CACCAC-CTCCGCCTG-GTTTAATGCTGTTCATACGTTTGTC) (SEQ ID NO: 2) and KAC1039 (5'- TACTAGTC-CGCCTCCAC-CACCTCCGCCTCCACCACCTCCGCC) (SEQ ID NO: 3). The two reverse primers overlap and incorporate the 12x Gly linker and the N-terminal sequence of the p110α gene to the SpeI site. The 12x Gly linker replaces the single Gly linker in the BV1052 construct. The PCR fragment was cloned into pCR2.1 TOPO (Invitrogen). Of the resulting clones, p1661-2 was determined to be correct by sequencing. This plasmid was digested with Nhe and SpeI and the resulting fragment was gel-isolated and purified for sub-cloning.

[0121] The p110α cloning fragment was generated by enzymatic digest of clone LR410 (see above) with Spe I and HindIII. The SpeI site is in the coding region of the p110α gene. The resulting fragment was gel-isolated and purified for sub-cloning. The cloning vector, pBlueBac4.5 (Invitrogen) was prepared by enzymatic digestion with Nhe and HindIII. The cut vector was purified with Qiagen column and then dephosphorylated with Calf Intestine alkaline phosphatase (CIP) (BioLabs). After completion of the CIP reaction the cut vector was again column purified to generate the final vector. A three-part ligation was performed using Roche Rapid ligase and the vendor specifications. The final plasmid was verified by sequencing.

Kinase domain.

Protein sequence of BV 1075:

[0122]

```
   1 MREYDRLYEE YTRTSQEIQM KRTAIEAFNE TIKIFEEQCQ TQERYSKEYI EKFKREGNEK
  61 EIQRIMHNYD KLKSRISEII DSRRRLEEDL KKQAAEYREI DKRMNSIKPG GGGGGGGGGG
 121 GLVECLLPNG MIVTLECLRE ATLITIKHEL FKEARKYPLH QLLQDESSYI FVSVTQEAER
 181 EEFFDETRRL CDLRLFQPFL KVIEPVGNRE EKILNREIGF AIGMPVCEFD MVKDPEVQDF
 241 RRNILNVCKE AVDLRDLNSP HSRAMYVYPP NVESSPELPK HIYNKLDKGQ IIVVIWVIVS
 301 PNNDKQKYTL KINHDCVPEQ VIAEAIRKKT RSMLLSSEQL KLCVLEYQGK YILKVCGCDE
 361 YFLEKYPLSQ YKYIRSCIML GRMPNLMLMA KESLYSQLPM DCFTMPSYSR RISTATPYMN
 421 GETSTKSLWV INSALRIKIL CATYVNVNIR DIDKIYVRTG IYHGGEPLCD NVNTQRVPCS
 481 NPRWNEWLNY DIYIPDLPRA ARLCLSICSV KGRKGAKEEH CPLAWGNINL FDYTDTLVSG
 541 KMALNLWPVP HGLEDLLNPI GVTGSNPNKE TPCLELEFDW FSSVVKFPDM SVIEEHANWS
 601 VSREAGFSYS HAGLSNRLAR DNELRENDKE QLKAISTRDP LSEITEQEKD FLWSHRHYCV
 661 TIPEILPKLL LSVKWNSRDE VAQMYCLVKD WPPIKPEQAM ELLDCNYPDP MVRGFAVRCL
 721 EKYLTDDKLS QYLIQLVQVL KYEQYLDNLL VRFLLKKALT NQRIGHFFFW HLKSEMHNKT
 781 VSQRFGLLLE SYCRACGMYL KHLNRQVEAM EKLINLTDIL KQEKKDETQK VQMKFLVEQM
 841 RRPDFMDALQ GFLSPLNPAH QLGNLRLEEC RIMSSAKRPL WLNWENPDIM SELLFQNNEI
 901 IFKNGDDLRQ DMLTLQIIRI MENIWQNQGL DLRMLPYGCL SIGDCVGLIE VVRNSHTIMQ
 961 IQCKGGLKGA LQFNSHTLHQ WLKDKNKGEI YDAAIDLFTR SCAGYCVATF ILGIGDRHNS
1021 NIMVKDDGQL FHIDFGHFLD HKKKKFGYKR ERVPFVLTQD FLIVISKGAQ ECTKTREFER
1081 FQEMCYKAYL AIRQHANLFI NLFSMMLGSG MPELQSFDDI AYIRKTLALD KTEQEALEYF
1141 MKQMNDAHHG GWTTKMDWIF HTIKQHALNE LGGAHHHHHH
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(SEQ ID NO: 4)

PI3Kβ constructs and proteins

[0123]

PI3Kβ	BV949	p85iSH2(461-N58K-568)-GGGGGG-p110β(2-1070)-His

[0124] BV949: PCR products for the inter SH2 domain (iSH2) of the p85 PI3Kα, PI3Kβ and PI3Kδ subunit and for the full-length p110β subunit were generated and fused by overlapping PCR. The iSH2 PCR product was obtained from first strand cDNA generated by RT-PCR from commercial human RNA from placenta, testis and brain (Clontech), initially using primers gwG130-p01 (5'-CGAGAATATGATAGATTATATGAAGAAT-3') (SEQ ID NO: 5) and gwG130-p02 (5'-TGGTTT-AATGCTGTTCATACGTTTGTCAAT-3') (SEQ ID NO: 6). Subsequently, in a secondary PCR reaction Gateway recombination AttB1 sites and linker sequences were added at the 5'end and 3'end of the p85 iSH2 fragment respectively, using primers gwG130-p03 (5'-GGGACAAGTT-TGTACAAAAAAGCAGGCTACGAAGGAGATATACATATGCGA-GAATATGATAGATTATATG AAGAAT-3') (SEQ ID NO: 7) and gwG130-p05 (5'-ACTGAAGCATCCTCCTC-CTCCTC-CT-CCTGGTTTAATGCTGTTCATACGTTTGTC-3') (SEQ ID NO: 8). The p110β fragment was obtained by PCR using as template a p110β clone (from unknown source that was sequence verified) using primers gwG130-p04 (5'-ATTAAAC-CAGGAGGAGGAGGAGGAGGATGCTT-CAGTTTCATAATGCCTCCTGCT -3') (SEQ ID NO: 9) which contains linker sequences and the 5'end of p110β and gwG130-p06 (5'-AGCTCCGTGATGGTGATGGTGATGTGCTCCAGATC-TG-TAGTCTTTCCGAA-CTGTGTG-3') (SEQ ID NO: 10) which contains sequences of the 3'end of p110-β fused to a Histidine tag. The p85-iSH2/ p110β fusion protein was assembled by an overlapping PCR a reaction of the linkers at the 3'end of the iSH2 fragment and the 5'end of the p110β fragment, using the above mentioned gwG130-p03 primer and a primer containing an overlapping Histidine tag and the AttB2 recombination sequences (5'-GGGACCACTTTGTACAAGAAAGCTGGGTTTAAGCTCCGTGATGGTGATGGTGATGTGCT CC-3') (SEQ ID NO: 11). This final product was recombined in a Gateway (Invitrogen) OR reaction into the donor vector pDONR201 (Invitrogen) to generate the ORF253 entry clone. This clone was verified by sequencing and used in a Gateway LR reaction (Invitrogen) to transfer the insert into the Gateway adapted pBlueBac4.5 (Invitrogen) vector for generation of the baculovirus expression vector LR280. This LR280 has an amino acid mutation in the p85 sequence.

Kinase domain.

Protein sequence of BV949:

[0125]

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   1 MREYDRLYEE YTRTSQEIQM KRTAIEAFNE TIKIFEEQCQ TQERYSKEYI EKFKREGKEK
  61 EIQRIMHNYD KLKSRISEII DSRRRLEEDL KKQAAEYREI DKRMNSIKPG GGGGGCFSFI
 121 MPPAMADILD IWAVDSQIAS DGSIPVDFLL PTGIYIQLEV PREATISYIK QMLWKQVHNY
 181 PMFNLLMDID SYMFACVNQT AVYEELEDET RRLCDVRPFL PVLKLVTRSC DPGEKLDSKI
 241 GVLIGKGLHE FDSLKDPEVN EFRRKMRKFS EEKILSLVGL SWMDWLKQTY PPEHEPSIPE
 301 NLEDKLYGGK LIVAVHFENC QDVFSFQVSP NMNPIKVNEL AIQKRLTIHG KEDEVSPYDY
 361 VLQVSGRVEY VFGDHPLIQF QYIRNCVMNR ALPHFILVEC CKIKKMYEQE MIAIEAAINR
 421 NSSNLPLPLP PKKTRIISHV WENNNPFQIV LVKGNKLNTE ETVKVHVRAG LFHGTELLCK
 481 TIVSSEVSGK NDHIWNEPLE FDINICDLPR MARLCFAVYA VLDKVKTKKS TKTINPSKYQ
 541 TIRKAGKVHY PVAWVNTMVF DFKGQLRTGD IILHSWSSFP DELEEMLNPM GTVQTNPYTE
 601 NATALHVKFP ENKKQPYYYP PFDKIIEKAA EIASSDSANV SSRGGKKFLP VLKEILDRDP

 661 LSQLCENEMD LIWTLRQDCR EIFPQSLPKL LLSIKWNKLE DVAQLQALLQ IWPKLPPREA
 721 LELLDFNYPD QYVREYAVGC LRQMSDEELS QYLLQLVQVL KYEPFLDCAL SRFLLERALG
 781 NRRIGQFLFW HLRSEVHIPA VSVQFGVILE AYCRGSVGHM KVLSKQVEAL NKLKTLNSLI
 841 KLNAVKLNRA KGKEAMHTCL KQSAYREALS DLQSPLNPCV ILSELYVEKC KYMDSKMKPL
 901 WLVYNNKVFG EDSVGVIFKN GDDLRQDMLT LQMLRLMDLL WKEAGLDLRM LPYGCLATGD
 961 RSGLIEVVST SETIADIQLN SSNVAAAAAF NKDALLNWLK EYNSGDDLDR AIEEFTLSCA
1021 GYCVASYVLG IGDRHSDNIM VKKTGQLFHI DFGHILGNFK SKFGIKRERV PFILTYDFIH
1081 VIQQGKTGNT EKFGRFRQCC EDAYLILRRH GNLFITLFAL MLTAGLPELT SVKDIQYLKD
1141 SLALGKSEEE ALKQFKQKFD EALRESWTTK VNWMAHTVRK DYRSGAHHHH HHGA
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(SEQ ID NO: 12)

Kinase domain.

PI3Kγ construct and protein

[0126]

PI3Kγ	BV950	p110γ(Δ143-[Met144-1102])-His

[0127] Construct obtained from Roger Williams lab, MRC Laboratory of Molecular Biology, Cambridge, UK (November, 2003). Description of the construct in (Pacold, Michael E.; Suire, Sabine; Perisic, Olga; Lara-Gonzalez, Samuel; Davis, Colin T.; Walker, Edward H.; Hawkins, Phillip T.; Stephens, Len; Eccleston, John F.; Williams, Roger L. Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. Cell (2000), 103(6), 931-943). Constructs lacking the N-terminal 144 aa.

Protein sequence of BV950:

[0128]

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  1 MSEESQAFQR QLTALIGYDV TDVSNVHDDE LEFTRRGLVT PRMAEVASRD PKLYAMHPWV
 61 TSKPLPEYLW KKIANNCIFI VIHRSTTSQT IKVSPDDTPG AILQSFFTKM AKKKSLMDIP
121 ESQSEQDFVL RVCGRDEYLV GETPIKNFQW VRHCLKNGEE IHVVLDTPPD PALDEVRKEE
181 WPLVDDCTGV TGYHEQLTIH GKDHESVFTV SLWDCDRKFR VKIRGIDIPV LPRNTDLTVF
241 VEANIQHGQQ VLCQRRTSPK PFTEEVLWNV WLEFSIKIKD LPKGALLNLQ IYCGKAPALS
301 SKASAESPSS ESKGKVRLLY YVNLLLIDHR FLLRRGEYVL HMWQISGKGE DQGSFNADKL
361 TSATNPDKEN SMSISILLDN YCHPIALPKH QPTPDPEGDR VRAEMPNQLR KQLEAIIATD
421 PLNPLTAEDK ELLWHFRYES LKHPKAYPKL FSSVKWGQQE IVAKTYQLLA RREVWDQSAL
481 DVGLTMQLLD CNFSDENVRA IAVQKLESLE DDDVLHYLLQ LVQAVKFEPY HDSALARFLL
541 KRGLRNKRIG HFLFWFLRSE IAQSRHYQQR FAVILEAYLR GCGTAMLHDF TQQVQVIEML
601 QKVTLDIKSL SAEKYDVSSQ VISQLKQKLE NLQNSQLPES FRVPYDPGLK AGALAIEKCK
661 VMASKKKPLW LEFKCADPTA LSNETIGIIF KHGDDLRQDM LILQILRIME SIWETESLDL
721 CLLPYGCIST GDKIGMIEIV KDATTIAKIQ QSTVGNTGAF KDEVLNHWLK EKSPTEEKFQ
781 AAVERFVYSC AGYCVATFVL GIGDRHNDNI MITETGNLFH IDFGHILGNY KSFLGINKER
841 VPFVLTPDFL FVMGTSGKKT SPHFQKFQDI CVKAYLALRH HTNLLIILFS MMLMTGMPQL
901 TSKEDIEYIR DALTVGKNEE DAKKYFLDQI EVCRDKGWTV QFNWFLHLVL GIKQGEKHSA
961 HHHHHH (SEQ ID NO: 13)
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PI3Kδ construct and protein

[0129]

PI3Kδ	BV1060	p85iSH2(461-568)-GGGGGG-p110δ(2-1044)-His

[0130] BV1060: PCR products for the inter SH2 domain (iSH2) of the p85 subunit and for the full-length p110δ subunit were generated and fused by overlapping PCR. The iSH2 PCR product was generated by using as a template the ORF318 (see above) and the primers gwG130-p03 (5'- GGGACAAG-TTTGTACAAAAAAGCAGGCTACGAAGGAGA-TATACATATGC-GAGAATATGATAGATTATATGAAGAAT-3') (SEQ ID NO: 7) and gwG154-p04 (5'-TCCTCCTCCT-CCTCCTCCTGGTTTAATGCTGTTCATACGTTTGTC-3') (SEQ ID NO: 14). The p110δ fragment was obtained from first strand cDNA generated by RT-PCR from commercial human RNA from placenta, testis and brain (Clontech), using initially primers gwG154-p01 (5'- ATGCCCCCTGGGGTGGACTGCCCCAT-3') (SEQ ID NO: 15) and gwG154-p02 (5'-CTACTGCCTGT-TGTCTTTGGACACGT-3') (SEQ ID NO: 16). In a subsequent PCR reaction linker sequences and a Histidine tag was added at the 5'end and 3'end of the p110δ fragment respectively, using primers gw154-p03 (5'-ATTAAACCAGGAGGAGGAGGAGGAGGACCCCCTGGGGTGGAC-TGCCCCATGGA-3') (SEQ ID NO: 17) and gwG154-p06 (5'-AGCTCCGTGATGGTGATGGTGAT-GTGCT-CCCTGCCTGTTGTCTTTGGACACGTTGT-3') (SEQ ID NO: 18). The p85-iSH2/ p110δ fusion protein was assembled in a third PCR reaction by the overlapping linkers at the 3'end of the iSH2 fragment and the 5'end of the p110δ fragment, using the above mentioned gwG130-p03 primer and a primer containing an overlapping Histidine tag and the Gateway (Invitrogen) AttB2 recombination sequences (5'-GGG-ACCACTTTGTACAAGAAAGCTGGGTTTAA-GCTCCGTGATGGTGATGGTGAGTGCTCC-3') (SEQ ID NO: 19). This final product was recombined in a Gateway OR reaction into the donor vector pDONR201 (Invitrogen) to generate the ORF319 entry clone. This clone was verified by sequencing and used in a Gateway LR reaction (Invitrogen) to transfer the insert into the Gateway adapted pBlueBac4.5 (Invitrogen) vector for generation of the baculovirus expression vector LR415.

Protein sequence of BV1060:

[0131]

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   1 MREYDRLYEE YTRTSQEIQM KRTAIEAFNE TIKIFEEQCQ TQERYSKEYI EKFKREGNEK
  61 EIQRIMHNYD KLKSRISEII DSRRRLEEDL KKQAAEYREI DKRMNSIKPG GGGGGPPGVD
 121 CPMEFWTKEE NQSVVVDFLL PTGVYLNFPV SRNANLSTIK QLLWHRAQYE PLFHMLSGPE
 181 AYVFTCINQT AEQQELEDEQ RRLCDVQPFL PVLRLVAREG DRVKKLINSQ ISLLIGKGLH
 241 EFDSLCDPEV NDFRAKMCQF CEEAAARRQQ LGWEAWLQYS FPLQLEPSAQ TWGPGTLRLP
 301 NRALLVNVKF EGSEESFTFQ VSTKDVPLAL MACALRKKAT VFRQPLVEQP EDYTLQVNGR
 361 HEYLYGSYPL CQFQYICSCL HSGLTPHLTM VHSSSILAMR DEQSNPAPQV QKPRAKPPPI

 421 PAKKPSSVSL WSLEQPFRIE LIQGSKVNAD ERMKLVVQAG LFHGNEMLCK TVSSSEVSVC
 481 SEPVWKQRLE FDINICDLPR MARLCFALYA VIEKAKKARS TKKKSKKADC PIAWANLMLF
 541 DYKDQLKTGE RCLYMWPSVP DEKGELLNPT GTVRSNPNTD SAAALLICLP EVAPHPVYYP
 601 ALEKILELGR HSECVHVTEE EQLQLREILE RRGSGELYEH EKDLVWKLRH EVQEHFPEAL
 661 ARLLLVTKWN KHEDVAQMLY LLCSWPELPV LSALELLDFS FPDCHVGSFA IKSLRKLTDD
 721 ELFQYLLQLV QVLKYESYLD CELTKFLLDR ALANRKIGHF LFWHLRSEMH VPSVALRFGL
 781 ILEAYCRGST HHMKVLMKQG EALSKLKALN DFVKLSSQKT PKPQTKELMH LCMRQEAYLE
 841 ALSHLQSPLD PSTLLAEVCV EQCTFMDSKM KPLWIMYSNE EAGSGGSVGI IFKNGDDLRQ
 901 DMLTLQMIQL MDVLWKQEGL DLRMTPYGCL PTGDRTGLIE VVLRSDTIAN IQLNKSNMAA
 961 TAAFNKDALL NWLKSKNPGE ALDRAIEEFT LSCAGYCVAT YVLGIGDRHS DNIMIRESGQ
1021 LFHIDFGHFL GNFKTKFGIN RERVPFILTY DFVHVIQQGK TNNSEKFERF RGYCERAYTI
1081 LRRHGLLFLH LFALMRAAGL PELSCSKDIQ YLKDSLALGK TEEEALKHFR VKFNEALRES
1141 WKTKVNWLAH NVSKDNRQEL GGAHHHHHH (SEQ ID NO: 20)
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Purification of PI3Kα, PI3Kβ and PI3Kγ constructs

[0132] PI3Kα, PI3Kβ and PI3Kγ were purified in two chromatographic steps: immobilized metal affinity chromatography (IMAC) on a Ni sepharose resin (GE Healthcare) and gel filtration utilizing a Superdex 200 26/60 column (GE Healthcare). All buffers were chilled to 4°C and lysis was performed chilled on ice. Column fractionation was performed at room temperature. All buffers used to purify PI3Kβ contained 0.05% Triton X100 in addition to what is described below.

[0133] Typically frozen cells from 10 L of Tn5 cell culture were resuspended in "Lysis Buffer" 20 mM Tris-Cl, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole, 1 mM NaF, 0.1 ug/mL okadaic acid (OAA), 5 mM BME, 1 x Complete protease inhibitor cocktail - EDTA-free (20 tablets/1 L buffer, Roche Applied Sciences), benzonase (25U/mL buffer, EMD Biosciences) at a ratio of 1:6 v/v pellet to Lysis Buffer ratio, and mechanically lysed by douncing 20 strokes using a tight-fitting pestle. The lysate was centrifuged at 45,000 g for 30 minutes, and the supernatant was loaded onto a pre-equilibrated IMAC column (3 mL resin/100 mL lysate). The column was washed with 3-5 column volumes of Lysis Buffer, followed by a second wash of 3-5 column volumes with 20 mM Tris-Cl, pH 7.5, 500 mM NaCl, 5% glycerol, 45 mM imidazole, 1 mM NaF, 0.1μg/mL OAA, 5 mM BME, 1x Complete protease inhibitor cocktail - EDTA-free. Protein was eluted with 20 mM Tris-Cl, pH 7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole, 1 mM NaF, 0.1μg/mL OAA, 5 mM BME, 1x Complete protease inhibitor cocktail - EDTA-free. Pertinent fractions were analyzed by SDS-PAGE and pooled accordingly. The protein was further purified by gel filtration on a Superdex 200 26/60 column equilibrated in 20 mM Tris-Cl, pH 7.5, 0.5 M NaCl, 5% glycerol, 1 mM NaF, 5 mM DTT, 1x Complete protease inhibitor cocktail - EDTA-free. Pertinent fractions were analyzed by SDS-PAGE and pooled accordingly. An equal volume of Dialysis Buffer (20 mM Tris-Cl, pH 7.5, 500 mM NaCl, 50% glycerol, 5 mM NaF, 5 mM DTT) was added to the pool and than dialyzed against Dialysis Buffer two changes (one change overnight). Protein was stored at - 20°C.

Purification of PI3Kδ

[0134] PI3Kδ was purified in three chromatographic steps: immobilized metal affinity chromatography on a Ni Sepharose resin (GE Healthcare), gel filtration utilizing a Superdex 200 26/60 column (GE Healthcare), and finally a ion exchange step on a Q-HP column (GE Healthcare). All buffers were chilled to 4°C and lysis was performed chilled on ice. Column fractionation was performed at room temperature.

[0135] Typically frozen cells from 10 L of Tn5 cell culture were resuspended in "Lysis Buffer" 20 mM Tris-Cl, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole, 1 mM NaF, 0.1μg/mL okadaic acid (OAA), 5 mM BME, 1 x Complete

protease inhibitor cocktail - EDTA-free (20 tablets/1 L buffer, Roche Applied Sciences), benzonase (25U/mL lysis buffer, EMD Biosciences) at a ratio of 1:10 v/v pellet to Lysis Buffer ratio, and mechanically lysed by douncing 20 strokes using a tight-fitting pestle. The lysate was centrifuged at 45,000 g for 30 minutes, and the supernatant was loaded onto a pre-equilibrated IMAC column (5 mL resin/100 mL lysate). The column was washed with 3-5 column volumes of Lysis Buffer, followed by a second wash of 3-5 column volumes with 20 mM Tris-Cl, pH 7.5, 500 mM NaCl, 5% glycerol, 40 mM imidazole, 1 mM NaF, 0.1 ug/mL OAA, 5 mM BME, 1 x Complete protease inhibitor cocktail - EDTA-free. Protein was eluted with 20 mM Tris-Cl, pH 7.5, 500 mM NaCl, 5% glycerol, 250 mM imidazole, 1 mM NaF, 0.1μg/mL OAA, 5 mM BME, 1 x Complete protease inhibitor cocktail - EDTA-free. Pertinent fractions were analyzed by SDS-PAGE and pooled accordingly. The protein was further purified by gel filtration on a Superdex 200 equilibrated in 20 mM Tris-Cl, pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM NaF, 0.1 ug/mL OAA, 5 mM DTT, 1 x Complete protease inhibitor cocktail - EDTA-free. Pertinent fractions were analyzed by SDS-PAGE and pooled accordingly. These fractions were diluted 1:10 v/v pool volume to buffer ratio with "Buffer A" 20 mM Tris-Cl, pH 8.2, 5% glycerol, 1 mM NaF, 0.1 μg/mL OAA, 5 mM DTT and loaded onto a prepared Q-HP column. After sample loading is completed we wash with Buffer A and 5% "Buffer B" 20 mM Tris-Cl, pH 8.2, 1 M NaCl, 5% glycerol, 1 mM NaF, 0.1 ug/mL OAA, 5 mM DTT for 3-5 column volumes. We elute the protein using a 5%-30% gradient of Buffer B. Typically the protein elutes at -200 mM NaCl. Pertinent fractions were analyzed by SDS-PAGE and pooled accordingly. An equal volume of Dialysis Buffer (20 mM Tris-Cl, pH 7.5, 500 mM NaCl, 50% glycerol, 1 mM NaF, 0.1μg/mL OAA, 5 mM DTT) was added to the pool and then dialyzed against Dialysis Buffer two changes (one change overnight). Protein was stored at -20°C.

[0136] The following results were obtained using the above described assays.

ex.	PI3Ka / IC50 [umol l-1]	PII3Kb / IC50 [umol l-1]	PI3Kd / IC50 [umol l-1]	PI3Kg / IC50 [umol l-1]
15	0.008	1.212	0.077	1.097

[0137] The compound of the present invention shows selectivity for PI3K alpha with respect to beta and/or delta and/or gamma subtypes e.g. as measured in a biochemical assay.

Example B: Determination of *in vitro* metabolic clearance

Abreviations

[0138]

ACN	Acetonitrile
ADME	Absorption, Distribution, Metabolism and Excretion
ALP	Automated labware positioner
%B	% of HPLC solvent B
BT	Biotransformation (or metabolic stability or microsomal stability)
[C]t=0	Initial (time zero) *In vitro* concentration of TA
CLh	Hepatic clearance (mL/min/kg)
CLint	Intrinsic clearance rate (μL reaction volume/min/mg microsomal protein)
CLint,s	Intrinsic clearance rate scaled for liver mass (mL reaction volume/min/g liver)
Cyno	Cynomolgous
CYP(s)	Cytochrome P450(s)
DiH2O	Deionized water
ERh	Hepatic extraction ratio
ESI	Electrospray ionization
fub	Free fraction of drug in blood or plasma
fum	Free fraction of drug in microsomes
IS	Internal standard
*k*mic	Elimination rate in microsomes
KPi	0.05M Potassium phosphate buffer, pH 7.4
LC-MS/MS	Liquid chromatography coupled tandem mass spectrometry
LOD	Limit of detection
M	Microsomal protein content in the incubation (mg/mL)
NADPH	β-Nicotinamide adenine dinucleotide phosphate, reduced form
NCE	New chemical entity

NSB	Non-specific binding
Qh	Portal blood flow (mL/min/kg)
RPM	Rotations per minute
SD	Standard deviation
S-D	Sprague-Dawley
SF1	Scaling factor: mg microsomal protein per gram liver
SF2	Scaling factor: gram of liver per kg animal body weight
t1/2	*In vitro* clearance half-life (min)
TA	Test article
UDPGA	Uridine 5'diphosphoglucuronic acid
UGT	Uridine 5'diphosphate glucuronosyltransferases
V	Reaction incubation volume (μL)

[0139] Final test article and protein concentrations, as well as incubation duration, are shown below (Table 1). Low test article concentrations were selected to comply with the assumption that reaction kinetics are evaluated at a concentration less than (or approximately equal to) *K*m. DMSO is known to have an inhibitory effect on CYP activity. Therefore, the concentration of DMSO in the incubation media has been restricted to 0.01% (*v*/*v*) so that interference to the metabolic process is minimized.

Table 1 Final reaction components and concentrations in metabolic clearance incubations

Reaction component	Final reaction concentration
Potassium phosphate (KPi) buffer, pH 7.4	50 mM
MgCl2	2.0 mM
NADPH	1.0 mM
UDPGA[a]	1.0 mM
Alamethacin[a]	25 μg/mg liver microsomes
Liver microsomes	0.5 mg/mL
Test article	1.0 μM
CAN	0.06% (*v*/*v*)
DMSO (test article solvent)	0.01% (*v*/*v*)

[a]Optional components required only to reconstitute UGT (Uridine 5'diphosphate glucuronosyltransferases) activity.

[0140] A typical experiment is performed in 96-well format with shaking incubation at 37 °C. The *in vitro* metabolic clearance rate is derived from data collected at four time points (eg. 0, 5 , 15 and 30 minutes) in a reaction including cofactor(s) (NADPH and/or UDPGA). A 30 minutes negative control incubation (minus cofactor) is also performed to assess CYP-unrelated stability issues (eg, chemical instability, CYP-independent metabolism).

[0141] In general, TA's in 10 mM DMSO are diluted 1:1000 into 0.6% ACN (*v*/*v*) in DiH2O to 10 μM. Immediately prior to the start of the experiment, 1.25 mg/mL of microsomal protein is suspended in 50 mM KPi. For evaluation of UGT-mediated metabolism, the suspension may be first pretreated by 5 min incubation on ice with alamethicin (25 μg/mg of microsomal protein). TA (35 μL) is added to 140 μL of the microsomal suspensions for 175 enzyme-substrate mixture. This enzyme•substrate mixture is preincubated for 15 min at 37 °C. The 30 min negative control incubation is processed by combining 25 μL of enzyme•substrate mixture with an equal volume of 50 mM KPi containing 4 mM MgCl2. Following a 30 minute incubation at 37 °C, the mix is quenched by adding 50 μL of ACN containing the MS internal standard (2 μM alprenolol). The T=0 min time point is processed by combining 25 of enzyme•substrate mixture directly with 50 μL of ACN containing the MS internal standard (2 μM alprenolol). 25 μL of the cofactor solution is added (2mM NADPH in 50 mM KPi plus 4 mM MgCl2; optionally including 2 mM UDPGA for CYP+UGT assays) to simulate the complete quenched reaction mixture.

[0142] The bulk reactions for the remaining time points are initiated by addition of 125 μL of cofactor solution (2 mM NADPH in 50 mM KPi plus 4 mM MgCl2) to the remaining 125 μL of enzyme•substrate mixture. For UGT metabolism, 2 mM UDPGA, is included in the cofactor solution, as well. At specific reaction time points (eg. 5 , 15, 30 minutes), reaction aliquots (50 μL) are removed and reactions are terminated by addition of acetonitrile (50 μL containing mass spectrometry internal standard (2 μM alprenolol). All the samples are centrifuged at ~3400x*g* at 4 oC for 10 min and the supernatants are analyzed by LC-MS/MS for quantitation of remaining TA. The percentage of TA remaining, relative to 0 minutes, is used to estimate *in vitro* elimination-rate constant (*k*mic) which can be used to calculate *in vitro* metabolic clearance rates.

[0143] Analysis of samples is performed on a high performance liquid chromatography-tandem massspectrometry (LC/MS) system consisting of a Waters Quattro Premiere mass spectrometer, an ESI ion source, a CTC-HTS Pal autosampler, and an Agilent LC Pump. Samples are separated on an Atlantic C18 column, 2.1x30 mm, 3.5 micron using the fast mobile phase gradient outlined in Table 2. Mobile phase A consists of purified water containing 10 mM ammonium formate. Mobile phase B consists of acetonitrile containing 0.01% formic acid. The flow rate is 1 mL/min. The injection volume is 10 μL. The first 30 seconds of elute is diverted to waste for sample clean-up. Compounds are detected using the software MassLynx/QuanLynx which collects intensity data for all fragments related to the molecular weight of the test compound. After collection of the raw data, the software may combine the profiles of up to 3 quality fragments if needed. Generally, the fragment peak of strongest intensity is integrated.

Table 2 Mobile phase gradient for HPLC

Time (min)	%B
0.0	5
0.2	5
0.85	95
1.02	95
1.05	5

[0144] Each microsomal elimination rate, *k*mic, is based on a 4-point elimination curve tested in singlet. LC-MS/MS raw data for a reaction plate is returned as integrated analyte peak areas for the TA and IS. These values may be converted to analyte:IS peak area ratios to standardize data comparisons.

[0145] The reaction time point (eg. 0, 5, 20 or 30 min) is plotted versus the natural logarithm of

[0146] percent TA remaining relative to 0 minutes (based on relative peak area ratio). The slope of this clearance plot, *k*mic, is used to calculate the *in vitro* half-life, *t*1/2, as shown in Eq. (1). In order to focus on linear reaction kinetics, whenever possible, data points representing <10% TA remaining are generally excluded from the definition of the clearance plot slope. The reaction *t*1/2 is the core experimental value used for calculating CLint (Eq. 2)

$$\text{Eq. (1): } t½ = 0.693/-k_{mic}$$

$$\text{Eq (2): } Clint = 0.693/-k_{mic} \cdot V/M$$

[0147] The following results were obtained using the above described procedure:

example	CYP MetCL-Ra / CL(int) [ul min-1 mg-1]	CYP MetCL-Hu / CL(int) [ul min-1 mg-1]
comparative example,		
WO2004/096797, no 133	56	37
example according to this invention		
15	29	33

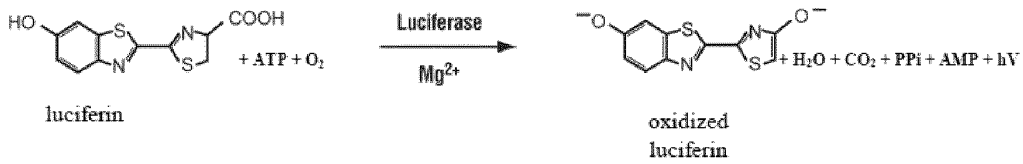
Example C: Inhibition of PI3K alpha mutants E545K and H1047R determined in a luciferase luminescence assay

[0148] Luminescence is a well established readout to determine ATP concentrations and can thus be used to follow the activity of many kinases regardless of their substrate. The KinaseGlo Luminescent Kinase Assay (Promega, Madison/WI, USA) is a homogeneous HTS method of measuring kinase activity by quantifying the amount of ATP remaining in solution following a kinase reaction.

[0149] Phosphoinositide 3-kinase was incubated at room temperature in 50-μl medium containing 1 μM ATP, 5 mM MgCl2, 50 mM NaCl, 5 μg/ml soybeen phosphatidylinositol (Avanti Polar lipids, Cat. Nr 840044C), 0.015% octoglucoside (Sigma, Cat. Nr 09882), 0.01 % CHAPS, 1 mM DTT, 2.5 % DMSO, and 10 mM Tris-HCl pH 7.5. The kinase reaction was initiated by the addition of ATP (15 min preincubation of enzyme with inhibitor) and stopped after 1 h with 50 μl KinaseGlo® (Promega, cat. Nr V6714) and luminescence was measured by a Victor II reader (0.1 s integration). Curves were fitted by non-linear regression using the logistic equation (model 205 of XLfit®, ID Business Solutions, Guildford, UK).

Assay Principle of Luminescence Assay (KinaseGlo):

[0150]



[0151] Using above assay system and using PI3K proteins obtained from constructs shown in the following table

Type	Code	Construct
Wild type	BV1075	p85iSH2(461-568)-GGGGGGGGGGG-p110α (21-1068)-His
E545K	BV1147	p85iSH2(461-568)-GGISGGGGGIMV-p110α(21-E542K-1068)-His
H1047R	BV1097	p85iSH2(461-568)-GGISGGGGGIMV-p110α(21-H1047R-1068)-His

the inhibitory activity against wild type and mutant PI3Kalpha was assessed. The results are shown in the following table.

Inhibition of wild type and mutant PI3Kalpha:

[0152]

Example	PI3Kalpha wild type	PI3Kalpha E545K	PI3Kalpha H1047R
		IC50s in nM	
15	4.6	4.0	4.8

[0153] BV1147: The activating mutation E545K found in many cancers was introduced into ORF318 by site directed mutagenesis with the QuickChange XL mutagenesis kit (Stratagene). Uusing the method recommended by the manufacturer and the mutagenic primers gwG152-p15 (5'-CTCTCTGAAATCACTAAGCAGGAGAAAGATTTT-3') (SEQ ID NO: 21) and gwG152-p16 (5'-AAAATCTTTCT-CCTGCTTAGTGATTTCAGAGAG-3') (SEQ ID NO: 22) ORF544 was generated. This clone was verified by sequencing and used in a Gateway LR reaction (Invitrogen) to transfer the insert into the Gateway adapted pBlueBac4.5 (Invitrogen) vector for generation of the baculovirus expression vector LR561.

Kinase domain.

Protein sequence of BV 1147:

[0154]

1 MREYDRLYEE YTRTSQEIQM KRTAIEAFNE TIKIFEEQCQ TQERYSKEYI EKFKREGNEK
61 EIQRIMHNYD KLKSRISEII DSRRRLEEDL KKQAAEYREI DKRMNSIKPG GISGGGGGIM
121 VLVECLLPNG MIVTLECLRE ATLITIKHEL FKEARKYPLH QLLQDESSYI FVSVTQEAER
181 EEFFDETRRL CDLRLFQPFL KVIEPVGNRE EKILNREIGF AIGMPVCEFD MVKDPEVQDF
241 RRNILNVCKE AVDLRDLNSP HSRAMYVYPP NVESSPELPK HIYNKLDKGQ IIVVIWVIVS
301 PNNDKQKYTL KINHDCVPEQ VIAEAIRKKT RSMLLSSEQL KLCVLEYQGK YILKVCGCDE
361 YFLEKYPLSQ YKYIRSCIML GRMPNLMLMA KESLYSQLPM DCFTMPSYSR RISTATPYMN
421 GETSTKSLWV INSALRIKIL CATYVNVNIR DIDKIYVRTG IYHGGEPLCD NVNTQRVPCS
481 NPRWNEWLNY DIYIPDLPRA ARLCLSICSV KGRKGAKEEH CPLAWGNINL FDYTDTLVSG
541 KMALNLWPVP HGLEDLLNPI GVTGSNPNKE TPCLELEFDW FSSVVKFPDM SVIEEHANWS
601 VSREAGFSYS HAGLSNRLAR DNELRENDKE QLKAISTRDP LSEITKQEKD FLWSHRHYCV
661 TIPEILPKLL LSVKWNSRDE VAQMYCLVKD WPPIKPEQAM ELLDCNYPDP MVRGFAVRCL

721 EKYLTDDKLS QYLIQLVQVL KYEQYLDNLL VRFLLKKALT NQRIGHFFFW HLKSEMHNKT
781 VSQRFGLLLE SYCRACGMYL KHLNRQVEAM EKLINLTDIL KQEKKDETQK VQMKFLVEQM
841 RRPDFMDALQ GFLSPLNPAH QLGNLRLEEC RIMSSAKRPL WLNWENPDIM SELLFQNNEI
901 IFKNGDDLRQ DMLTLQIIRI MENIWQNQGL DLRMLPYGCL SIGDCVGLIE VVRNSHTIMQ
961 IQCKGGLKGA LQFNSHTLHQ WLKDKNKGEI YDAAIDLFTR SCAGYCVATF ILGIGDRHNS
1021 NIMVKDDGQL FHIDFGHFLD HKKKKFGYKR ERVPFVLTQD FLIVISKGAQ ECTKTREFER
1081 FQEMCYKAYL AIRQHANLFI NLFSMMLGSG MPELQSFDDI AYIRKTLALD KTEQEALEYF
1141 MKQMNDAHHG GWTTKMDWIF HTIKQHALNE LGGAHHHHHH (SEQ ID NO: 23).

[0155] BV1097: The activating mutation H1047R found in many cancers was introduced into ORF318 by site directed mutagenesis with the QuickChange XL mutagenesis kit (Stratagene).

[0156] Using the method recommended by the manufacturer and the mutagenic primers gwG152-p07 (5'-CAAAT-GAATGATGCACGTCATGGTGGCTGGACA-3') (SEQ ID NO: 24)and gwG152-p11 (5'-TGTCCAGCCA-CCATGACGT-GCATCATTCATTTG-3') (SEQ ID NO: 25) ORF396 was generated. This clone was verified by sequencing and used in a Gateway LR reaction (Invitrogen) to transfer the insert into the Gateway adapted pBlueBac4.5 vector (Invitrogen) for generation of the baculovirus expression vector LR480.

Kinase domain.

Protein sequence of BV 1097:

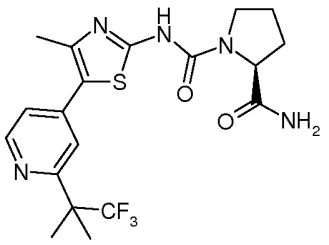
[0157]

1 MREYDRLYEE YTRTSQEIQM KRTAIEAFNE TIKIFEEQCQ TQERYSKEYI EKFKREGNEK
61 EIQRIMHNYD KLKSRISEII DSRRRLEEDL KKQAAEYREI DKRMNSIKPG GISGGGGGIM
121 VLVECLLPNG MIVTLECLRE ATLITIKHEL FKEARKYPLH QLLQDESSYI FVSVTQEAER
181 EEFFDETRRL CDLRLFQPFL KVIEPVGNRE EKILNREIGF AIGMPVCEFD MVKDPEVQDF
241 RRNILNVCKE AVDLRDLNSP HSRAMYVYPP NVESSPELPK HIYNKLDKGQ IIVVIWVIVS
301 PNNDKQKYTL KINHDCVPEQ VIAEAIRKKT RSMLLSSEQL KLCVLEYQGK YILKVCGCDE
361 YFLEKYPLSQ YKYIRSCIML GRMPNLMLMA KESLYSQLPM DCFTMPSYSR RISTATPYMN
421 GETSTKSLWV INSALRIKIL CATYVNVNIR DIDKIYVRTG IYHGGEPLCD NVNTQRVPCS
481 NPRWNEWLNY DIYIPDLPRA ARLCLSICSV KGRKGAKEEH CPLAWGNINL FDYTDTLVSG
541 KMALNLWPVP HGLEDLLNPI GVTGSNPNKE TPCLELEFDW FSSVVKFPDM SVIEEHANWS
601 VSREAGFSYS HAGLSNRLAR DNELRENDKE QLKAISTRDP LSEITEQEKD FLWSHRHYCV
661 TIPEILPKLL LSVKWNSRDE VAQMYCLVKD WPPIKPEQAM ELLDCNYPDP MVRGFAVRCL
721 EKYLTDDKLS QYLIQLVQVL KYEQYLDNLL VRFLLKKALT NQRIGHFFFW HLKSEMHNKT
781 VSQRFGLLLE SYCRACGMYL KHLNRQVEAM EKLINLTDIL KQEKKDETQK VQMKFLVEQM
841 RRPDFMDALQ GFLSPLNPAH QLGNLRLEEC RIMSSAKRPL WLNWENPDIM SELLFQNNEI
901 IFKNGDDLRQ DMLTLQIIRI MENIWQNQGL DLRMLPYGCL SIGDCVGLIE VVRNSHTIMQ
961 IQCKGGLKGA LQFNSHTLHQ WLKDKNKGEI YDAAIDLFTR SCAGYCVATF ILGIGDRHNS

1021 NIMVKDDGQL FHIDFGHFLD HKKKKFGYKR ERVPFVLTQD FLIVISKGAQ ECTKTREFER
1081 FQEMCYKAYL AIRQHANLFI NLFSMMLGSG MPELQSFDDI AYIRKTLALD KTEQEALEYF
1141 MKQMNDARHG GWTTKMDWIF HTIKQHALNE LGGAHHHHHH (SEQ ID NO: 26).

Claims

1. The compound (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-({4-methyl-5-[2-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-pyridin-4-yl]-thiazol-2-yl}-amide), of structure:



in free form or in pharmaceutically acceptable salt form.

2. The compound according to claim 1 , in free form or in pharmaceutically acceptable salt form, for use as a pharmaceutical.

3. Use of the compound according to claim 1 , in free form or in pharmaceutically acceptable salt form, for the manufacture of a medicament for the treatment of cancer.

4. Use according to claim 3, wherein the cancer is selected from sarcoma; lung; bronchus; prostate; breast; pancreas; gastrointestinal cancer; colon; rectum; colon carcinoma; colorectal adenoma; thyroid; liver; intrahepatic bile duct; hepatocellular; adrenal gland; stomach; gastric; glioma; glioblastoma; endometrial; melanoma; kidney; renal pelvis;

urinary bladder; uterine corpus; uterine cervix; vagina; ovary; multiple myeloma; esophagus; a leukaemia; acute myelogenous leukemia; chronic myelogenous leukemia; lymphocytic leukemia; myeloid leukemia; brain; a carcinoma of the brain; oral cavity and pharynx; larynx; small intestine; non-Hodgkin lymphoma; melanoma; villous colon adenoma; a neoplasia; a neoplasia of epithelial character; lymphomas; a mammary carcinoma; basal cell carcinoma; squamous cell carcinoma; actinic keratosis; tumor diseases, including solid tumors; a tumor of the neck or head; polycythemia vera; essential thrombocythemia; myelofibrosis with myeloid metaplasia; and Walden stroem disease.

5. A pharmaceutical composition comprising a therapeutically effective amount of the compound according to claim 1, in free form or in pharmaceutically acceptable salt form and one or more pharmaceutically acceptable excipients.

6. A combined pharmaceutical composition, adapted for simultaneous or sequential administration, comprising a therapeutically effective amount of the compound according to claim 1 in free form or in pharmaceutically acceptable salt form and a therapeutically effective amount of one or more combination partners; and one or more pharmaceutically acceptable excipients.

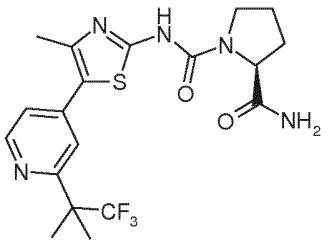
7. A pharmaceutical composition according to claim 5 or a combined pharmaceutical composition according to claim 6 for use in the treatment of cancer.

8. A compound according to claim 1 or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer.

9. A compound according to claim 8 or a pharmaceutically acceptable salt thereof, for use in the treatment of a cancer selected from sarcoma; lung; bronchus; prostate; breast; pancreas; gastrointestinal cancer; colon; rectum; colon carcinoma; colorectal adenoma; thyroid; liver; intrahepatic bile duct; hepatocellular; adrenal gland; stomach; gastric; glioma; glioblastoma; endometrial; melanoma; kidney; renal pelvis; urinary bladder; uterine corpus; uterine cervix; vagina; ovary; multiple myeloma; esophagus; a leukaemia; acute myelogenous leukemia; chronic myelogenous leukemia; lymphocytic leukemia; myeloid leukemia; brain; a carcinoma of the brain; oral cavity and pharynx; larynx; small intestine; non-Hodgkin lymphoma; melanoma; villous colon adenoma; a neoplasia; a neoplasia of epithelial character; lymphomas; a mammary carcinoma; basal cell carcinoma; squamous cell carcinoma; actinic keratosis; tumor diseases, including solid tumors; a tumor of the neck or head; polycythemia vera; essential thrombocythemia; myelofibrosis with myeloid metaplasia; and Walden stroem disease.

Patentansprüche

1. Die Verbindung (S)-Pyrrolidin-1,2-dicarbonsäure-2-amid-1-({4-methyl-5-[2-(2,2,2-trifluor-1,1-dimethylethyl)pyridin-4-yl]thiazol-2-yl}amid) der Struktur:



in freier Form oder in Form eines pharmazeutisch zulässigen Salzes.

2. Verbindung nach Anspruch 1 in freier Form oder in Form eines pharmazeutisch zulässigen Salzes zur Verwendung als ein Arzneimittel.

3. Verwendung der Verbindung nach Anspruch 1 in freier Form oder in Form eines pharmazeutisch zulässigen Salzes zur Herstellung eines Medikaments zur Behandlung von Krebs.

4. Verwendung nach Anspruch 3, wobei der Krebs ausgewählt ist aus Sarkom, Lungen-, Bronchial-, Prostata-, Brust-, Bauchspeicheldrüsen-, Magen-Darm-, Darm-, Mastdarmkrebs, Kolonkarzinom, kolorektalem Adenom, Schilddrüsen-, Leber-, intrahepatischem Gallengangkrebs, hepatozellulärem Krebs, Nebennieren-, Magenkrebs, gastrischem

Krebs, Gliom, Glioblastom, Endometriumkrebs, Melanom, Nieren-, Nierenbecken-, Harnblasen-, Gebärmutterkörper-, Gebärmutterhals-, Vaginal-, Eierstockkrebs, multiplen Myelomen, Speiseröhrenkrebs, einer Leukämie, akuter myeloischer Leukämie, chronischer myeloischer Leukämie, lymphatischer Leukämie, myeloischer Leukämie, Gehirnkrebs, einem Gehirnkarzinom, Mundhöhlen- und Rachen-, Kehlkopf-, Dünndarmkrebs, Non-Hodgkin-Lymphom, Melanom, villösem Kolonadenom, einer Neoplasie, einer Neoplasie mit epithelialem Charakter, Lymphomen, einem Mammakarzinom, Basalzellkarzinom, Plattenepithelkarzinom, aktinischer Keratose, Tumorerkrankungen, einschließlich solider Tumore, einem Hals-oder Kopftumor, Polycythaemia vera, essentieller Thromobzythämie, Myelofibrose mit myeloischer Metaplasie und Morbus Waldenström.

5. Pharmazeutische Zusammensetzung, umfassend eine therapeutisch wirksame Menge der Verbindung nach Anspruch 1 in freier Form oder in Form eines pharmazeutisch zulässigen Salzes und einen oder mehrere pharmazeutisch zulässige(n) Hilfsstoff(e).

6. Kombinierte pharmazeutische Zusammensetzung, angepasst an eine gleichzeitige oder aufeinanderfolgende Verabreichung, umfassend eine therapeutisch wirksame Menge der Verbindung nach Anspruch 1 in freier Form oder in Form eines pharmazeutisch zulässigen Salzes und einer therapeutisch wirksamen Menge von einem oder mehreren Kombinationspartner(n); und einen oder mehrere pharmazeutisch zulässige(n) Hilfsstoff(e).

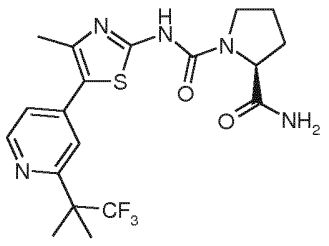
7. Pharmazeutische Zusammensetzung nach Anspruch 5 oder eine kombinierte pharmazeutische Zusammensetzung nach Anspruch 6 zur Verwendung bei der Behandlung von Krebs.

8. Verbindung nach Anspruch 1 oder ein pharmazeutisch zulässiges Salz davon zur Verwendung bei der Behandlung von Krebs.

9. Verbindung nach Anspruch 8 oder ein pharmazeutisch zulässiges Salz davon zur Verwendung bei der Behandlung von Krebs, der ausgewählt ist aus Sarkom, Lungen-, Bronchial-, Prostata-, Brust-, Bauchspeicheldrüsen-, Magen-Darm-, Darm-, Mastdarmkrebs, Kolonkarzinom, kolorektalem Adenom, Schilddrüsen-, Leber-, intrahepatischem Gallengangkrebs, hepatozellulärem Krebs, Nebennieren-, Magenkrebs, gastrischem Krebs, Gliom, Glioblastom, Endometriumkrebs, Melanom, Nieren-, Nierenbecken-, Harnblasen-, Gebärmutterkörper-, Gebärmutterhals-, Vaginal-, Eierstockkrebs, multiplen Myelomen, Speiseröhrenkrebs, einer Leukämie, akuter myeloischer Leukämie, chronischer myeloischer Leukämie, lymphatischer Leukämie, myeloischer Leukämie, Gehirnkrebs, einem Gehirnkarzinom, Mundhöhlen- und Rachen-, Kehlkopf-, Dünndarmkrebs, Non-Hodgkin-Lymphom, Melanom, villösem Kolonadenom, einer Neoplasie, einer Neoplasie mit epithelialem Charakter, Lymphomen, einem Mammakarzinom, Basalzellkarzinom, Plattenepithelkarzinom, aktinischer Keratose, Tumorerkrankungen, einschließlich solider Tumore, einem Hals-oder Kopftumor, Polycythaemia vera, essentieller Thromobzythämie, einer Myelofibrose mit myeloischer Metaplasie und Morbus Waldenström.

Revendications

1. Composé 1-({4-méthyl-5-[2-(2,2,2-trifluoro-1,1-diméthyl-éthyl)-pyridin-4-yl]thiazole-2-yl}-amide) du 2-amide de l'acide (S)-pyrrolidine-1,2-dicarboxylique, de structure :



sous forme libre ou sous forme de sel pharmaceutiquement acceptable.

2. Composé selon la revendication 1, sous forme libre ou sous forme de sel pharmaceutiquement acceptable, destiné à être utilisé comme produit pharmaceutique.

3. Utilisation du composé selon la revendication 1, sous forme libre ou sous forme de sel pharmaceutiquement acceptable, dans la fabrication d'un médicament pour le traitement du cancer.

4. Utilisation selon la revendication 3, dans laquelle le cancer est choisi parmi un sarcome ; le cancer du poumon ; le cancer des bronches ; le cancer de la prostate ; le cancer du sein ; le cancer du pancréas ; le cancer gastro-intestinal ; le cancer du côlon ; le cancer du rectum ; le carcinome du côlon ; l'adénome colorectal ; le cancer de la thyroïde ; le cancer du foie ; le cancer du canal biliaire intrahépatique ; le cancer hépatocellulaire ; le cancer de la glande surrénale ; le cancer de l'estomac ; le cancer gastrique ; le cancer du gliome ; le cancer du glioblastome ; le cancer de l'endomètre ; le mélanome ; le cancer du rein ; le cancer bassinet du rein ; le cancer de la vessie urinaire ; le cancer du corps utérin ; le cancer du col de l'utérus ; le cancer du vagin ; le cancer de l'ovaire ; le myélome multiple ; le cancer de l'oesophage ; une leucémie ; la leucémie myéloïde aiguë ; la leucémie myéloïde chronique ; la leucémie lymphocytaire ; la leucémie myéloïde ; le cancer du cerveau ; un carcinome du cerveau ; le cancer de la cavité orale et du pharynx ; le cancer du larynx ; le cancer de l'intestin grêle ; le lymphome non hodgkinien ; le mélanome ; l'adénome des villosités du côlon ; une néoplasie ; une néoplasie à caractère épithélial ; les lymphomes ; un carcinome mammaire ; le carcinome basocellulaire ; le carcinome à cellules squameuses ; la kératose actinique ; les maladies tumorales, y compris les tumeurs solides ; une tumeur du cou ou de la tête ; la maladie de Vaquez ; la thrombocytémie essentielle ; myélofibrose avec métaplasie myéloïde ; et la maladie de Waldenström.

5. Composition pharmaceutique comprenant une quantité thérapeutiquement efficace du composé selon la revendication 1, sous forme libre ou sous forme de sel pharmaceutiquement acceptable et un ou plusieurs excipients pharmaceutiquement acceptables.

6. Composition pharmaceutique combinée, adaptée à une administration simultanée ou séquentielle, comprenant une quantité thérapeutiquement efficace du composé selon la revendication 1 sous forme libre ou sous forme de sel pharmaceutiquement acceptable et une quantité thérapeutiquement efficace d'un ou plusieurs partenaires de combinaison ; et un ou plusieurs excipients pharmaceutiquement acceptables.

7. Composition pharmaceutique selon la revendication 5 ou composition pharmaceutique combinée selon la revendication 6 destinée à être utilisée dans le traitement du cancer.

8. Composé selon la revendication 1 ou sel pharmaceutiquement acceptable de celui-ci, destiné à être utilisé dans le traitement du cancer.

9. Composé selon la revendication 8 ou un sel pharmaceutiquement acceptable de celui-ci, pour une utilisation dans le traitement d'un cancer choisi parmi un sarcome ; le cancer du poumon ; le cancer des bronches ; le cancer de la prostate ; le cancer du sein ; le cancer du pancréas ; le cancer gastro-intestinal ; le cancer du côlon ; le cancer du rectum ; le carcinome du côlon ; l'adénome colorectal ; le cancer de la thyroïde ; le cancer du foie ; le cancer du canal biliaire intrahépatique ; le cancer hépatocellulaire ; le cancer de la glande surrénale ; le cancer de l'estomac ; le cancer gastrique ; le cancer du gliome ; le cancer du glioblastome ; le cancer de l'endomètre ; le mélanome ; le cancer du rein ; le cancer bassinet du rein ; le cancer de la vessie urinaire ; le cancer du corps utérin ; le cancer du col de l'utérus ; le cancer du vagin ; le cancer de l'ovaire ; le myélome multiple ; le cancer de l'oesophage; une leucémie ; la leucémie myéloïde aiguë ; la leucémie myéloïde chronique ; la leucémie lymphocytaire ; la leucémie myéloïde ; le cancer du cerveau ; un carcinome du cerveau ; le cancer de la cavité orale et du pharynx ; le cancer du larynx ; le cancer de l'intestin grêle ; le lymphome non hodgkinien ; le mélanome ; l'adénome des villosités du côlon ; une néoplasie ; une néoplasie à caractère épithélial ; les lymphomes ; un carcinome mammaire ; le carcinome basocellulaire ; le carcinome à cellules squameuses ; la kératose actinique ; les maladies tumorales, y compris les tumeurs solides ; une tumeur du cou ou de la tête ; la maladie de Vaquez ; la thrombocytémie essentielle ; myélofibrose avec métaplasie myéloïde ; et la maladie de Waldenström.

REFERENCES CITED IN THE DESCRIPTION

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- WO 04096797 A **[0036]**
- US 5457105 A **[0060]**
- US 5616582 A **[0060]**
- US 5770599 A **[0060]**
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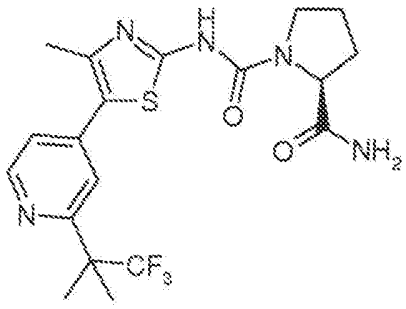
Proliferatív betegségek kezelésében alkalmas 1-((5-heteroariltiazol-2-il)-amino-karbonil)pirrolidin-2-karboxamid származékok, mint foszfatidilinozitol 3-kináz (PI3K) inhibitorok

Szabadalmi igénypontok

1. A



képletű (S)-pirrolidin-1,2-dikarbonsav-2-amid-1-({4-metil-5-[2-(2,2,2-trifluor-1,1-dimetil-etil)-piridin-4-il]-tiazol-2-il}-amid) szabad vagy gyógyászatilag elfogadható sója formájában.

2. Az 1. igénypont szerinti vegyület szabad vagy gyógyászatilag elfogadható sója formájában gyógyszerként történő alkalmazásra.

3. Az 1. igénypont szerinti vegyület szabad vagy gyógyászatilag elfogadható sója formájában történő alkalmazása rák kezelésére szolgáló gyógyszer előállítására.

4. A 3. igénypont szerinti alkalmazás, ahol a rák szarkóma; tüdő; bronchus; prosztata; mell; hasnyálmirigy; gastrointestinalis rák; vastagbél; végbél; colon karcinoma; colorectalis adenoma; pajzsmirigy; máj; intrahepaticus epevezeték; hepatocellularis; mellékvese mirigy; gyomor; gastricus; glioma; glioblastoma; endometrialis; melanoma; vese; renalis pelvis; húgyhólyag; uterin corpus; uterin cervix; vagina; petefészek; myeloma multiplex; nyelőcső; leukémia; akut myelogen leukémia; krónikus myelogen leukémia; lymphocyta leukémia; myeloid leukémia; agy; agy karcinoma; szájüreg és garat; gége; vékonybél; non-Hodgkin lymphoma; melanoma; bolyhos colon adenoma; egy neoplasia; egy epithelialis jellegű neoplasia; lymphomák; egy emlő karcinoma; basalis sejt karcinoma; pikkelysejt karcinoma; actinikus keratosis; tumoros betegségek, beleértve a szilárd tumorokat; a nyak és fej tumora; polycythemia vera;

thrombocythemia is; myeloid metaplasiaval járó myelofibrosis; és Walden Stroem betegség közül választott.

5. Gyógyászati készítmény, mely terápiásan hatékony mennyiségű 1. igénypont szerinti vegyületet szabad vagy gyógyászatilag elfogadható sója formájában, és egy vagy több gyógyszerészetileg elfogadható adalékanyagot tartalmaz.

6. Egyidejű vagy egymást követő adagolásra kialakított kombinált gyógyászati készítmény, mely terápiásan hatékony mennyiségű 1. igénypont szerinti vegyületet szabad vagy gyógyászatilag elfogadható sója formájában, és egy vagy több terápiásan hatásos mennyiségű kombinációs partnert, és egy vagy több gyógyszerészetileg elfogadható adalékanyagot tartalmaz.

7. Az 5. igénypont szerinti gyógyászati készítmény vagy a 6. igénypont szerinti kombinált gyógyászati készítmény rák kezelésében történő alkalmazásra.

8. Az 1. igénypont szerinti vegyület vagy gyógyászatilag elfogadható sója rák kezelésében történő alkalmazásra.

9. Az 1. igénypont szerinti vegyület vagy gyógyászatilag elfogadható sója szarkóma; tüdő; bronchus; prosztata; mell; hasnyálmirigy; gastrointestinalis rák; vastagbél; végbél; colon karcinoma; colorectalis adenoma; pajzsmirigy; máj; intrahepaticus epevezeték; hepatocellularis; mellékvese mirigy; gyomor; gastricus; glioma; glioblastoma; endometrialis; melanoma; vese; renalis pelvis; húgyhólyag; uterin corpus; uterin cervix; vagina; petefészek; myeloma multiplex; nyelőcső; leukémia; akut myelogen leukémia; krónikus myelogen leukémia; lymphocyta leukémia; myeloid leukémia; agy; agy karcinoma; szájüreg és garat; gége; vékonybél; non-Hodgkin lymphoma; melanoma; bolyhos colon adenoma; egy neoplasia; egy epithelialis jellegű neoplasia; lymphomák; egy emlő karcinoma; basalis sejt karcinoma; pikkelysejt karcinoma; actinikus keratosis; tumoros betegségek, beleértve a szilárd tumorokat; a nyak és fej tumora; polycythemia vera; thrombocythemia is; myeloid metaplasiaval járó myelofibrosis; és Walden Stroem betegség közül választott rák kezelésében történő alkalmazásra.

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