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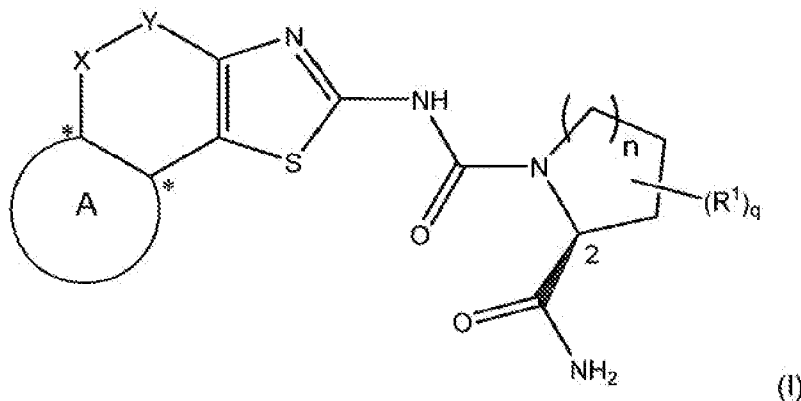
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(54) Title: 2-CARBOXAMIDE CYCLOAMINO UREAS USEFUL AS PI3K INHIBITORS



(57) Abstract: The present invention relates to compounds of formula (I) and salts thereof, wherein the substituents are as defined in the description, to compositions and use of the compounds in the treatment of diseases ameliorated by inhibition of phosphatidylinositol 3-kinase.

2-CARBOXAMIDE CYCLOAMINO UREAS USEFUL AS PI3K INHIBITORS

The present invention relates to substituted 2-carboxamide cycloamino ureas, as new phosphatidylinositol (PI) 3-kinase inhibitor compounds, their pharmaceutically acceptable salts, prodrugs thereof and processes for their production. This invention also relates to compositions of these compounds, 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 methods of use of these compounds, either alone or in combination with at least one additional therapeutic agent, in the prophylaxis or 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/threonine kinases, G protein coupled receptors and phospholipid kinases and phosphatases.

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 (PIP₂) and phosphoinositol-3,4,5-triphosphate (PIP₃) 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 p85 α , p55 α , p50 α , p85 β or p55 γ . The Class 1B sub-class has one family member, a heterodimer composed of a catalytic p110 γ 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)).

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

5 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

10 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 p110 α 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 p85 α that serve to up-regulate the p85-p110

15 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 et al., *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

20 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 et al., *Nature Rev. Drug Disc.* 4:988-1004 (2005)).

In view of the above, inhibitors of PI3Ks would be of particular value in the treatment of

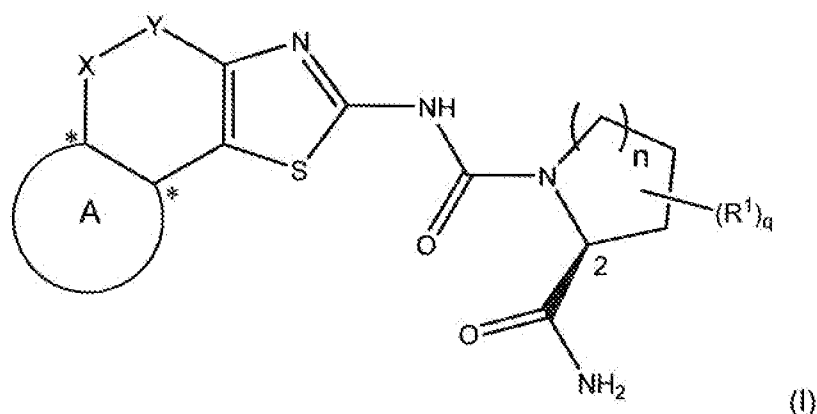
25 proliferative disease and other disorders. Selectivity towards the PI3K α isoform is desirable, and further desirable properties include improved pharmacokinetic properties and/or chemical stability.

WO2004/096797 discloses certain thiazole derivatives as inhibitors of PI3 kinase and their
30 use as pharmaceutical.

WO 2005/021519 also discloses certain thiazole derivatives as inhibitors of PI3 kinase and their use as pharmaceutical.

It has now been found that the 2-carboxamide cycloamino ureas of the formula I given below have advantageous pharmacological properties and inhibit, for example, the PI3 kinases (phosphatidylinositol 3-kinase). In particular, preferably, these compounds show selectivity for PI3K alpha versus beta and/or delta and/or gamma subtypes in the biochemical and/or in the cellular assay. A further property which is preferably desirable for compounds of formula I includes improved stability, for example, improved chemical stability e.g. in solid form and/or in buffer solution. Hence, the compounds of formula I are suitable, for example, to be used in the treatment of diseases depending on the PI3 kinase (in particular PI3K alpha, such as those showing somatic mutation of PIK3CA or germline mutations or somatic mutation of PTEN), especially proliferative diseases such as tumor diseases and leukaemias.

In a first aspect, the present invention provides compounds of formula I,



wherein,

A an unsubstituted or substituted aryl ring or unsubstituted or substituted heterocyclic ring fused to the rest of the molecule at the positions indicated by the symbol *;

X-Y is $(CH_2)_r$ or $O(CH_2)_t$ or $(CH_2)_tO$ wherein,

r is 1, 2 or 3;

t is 1 or 2;

n is 0, 1 or 2;

q is 0, 1, 2, 3 or 4;

R^1 represents, independently at each occurrence,

halo;

hydroxy;

unsubstituted or substituted aryl;

unsubstituted or substituted amino;

unsubstituted C₁-C₇-alkyl;

C₁-C₇-alkyl, which is substituted one or more times by

hydroxy, C₁-C₇-alkoxy, unsubstituted or substituted amino, aryl or

5 heterocyclyl, and wherein aryl may be mono or poly-substituted by halo; or

two R¹ substituents together form an alkandiyl to form a cyclic moiety, optionally substituted by hydroxy or halo;

10 or a salt, solvate, hydrate or prodrug thereof.

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
15 sense.

Any formula given herein is intended to represent compounds having structures depicted by the structural formula as well as certain variations or forms. In particular, compounds of any formula given herein may have asymmetric centers and therefore exist in different
20 stereoisomeric forms such as different enantiomeric forms. If at least one asymmetrical carbon atom is present in a compound of the formula I, such a compound may exist in optically active form or in the form of a mixture of optical isomers, e. g. in the form of a racemic mixture. Thus an asymmetric carbon atom may be present in the (*R*)-, (*S*)- or (*R,S*)-configuration, preferably in the (*R*)- or (*S*)-configuration. All optical isomers and their
25 mixtures, including the racemic mixtures, are part of the present invention. Thus, any given formula given herein is intended to represent a racemate, one or more enantiomeric forms, one or more diastereomeric forms, one or more atropisomeric forms, and mixtures thereof. Furthermore, certain structures may exist as geometric isomers (e.g. *cis* and *trans* isomers), as tautomers, or as atropisomers. For example, substituents at a double bond or a ring may
30 be present in *cis*- (=Z-) or *trans* (=E-) form. The compounds of the invention may thus be present as mixtures of isomers or preferably as pure isomers, preferably as enantiomer-pure diastereomers or pure enantiomers.

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

Any formula given herein is also intended to represent unlabeled forms as well as

5 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 ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{15}N , ^{31}P , ^{32}P , ^{18}F ,
10 ^{35}S , ^{36}Cl , ^{125}I respectively. Various isotopically labeled compounds of the present invention, for example those into which radioactive isotopes such as ^3H , ^{13}C , and ^{14}C are incorporated. Such isotopically labelled compounds are useful in metabolic studies (preferably with ^{14}C), reaction kinetic studies (with, for example ^2H or ^3H), detection or imaging techniques, such as positron emission tomography (PET) or single-photon emission computed tomography
15 (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. Further, substitution with heavier isotopes such as deuterium (i.e., ^2H) may afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements. Isotopically labeled compounds
20 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.

25 Further, substitution with heavier isotopes, particularly deuterium (i.e., ^2H 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 in the compound of the formula (I). The concentration of such a heavier isotope, specifically
30 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),
5 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
10 designated as a deuterium (D) is meant to represent deuterium, for example in the ranges
given above.

When referring to any formula given herein, the selection of a particular moiety from a list of
possible species for a specified variable is not intended to define the moiety for the variable
15 appearing elsewhere. In other words, where a variable appears more than once, the choice
of the species from a specified list is independent of the choice of the species for the same
variable elsewhere in the formula (where one or more up to all more general expressions in
embodiments characterized as preferred above or below can be replaced with a more
specific definition, thus leading to a more preferred embodiment of the invention,
20 respectively).

Where the plural form (e.g. compounds, salts, pharmaceutical preparations, diseases and
the like) is used, this includes the singular (e.g. a single compound, a single salt, a single
pharmaceutical preparation, a single disease, and the like). "A compound" does not exclude
25 that (e.g. in a pharmaceutical formulation) more than one compound of the formula (I) (or a
salt thereof) is present.

Salts are preferably the pharmaceutically acceptable salts of compounds of formula (I) if they
are carrying salt-forming groups. Acids/bases required to form the salts are generally known
30 in the field.

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 (halogenalkyl) can be mono-, poly- or per-halogenated.

- 5 **Hetero atoms** are atoms other than Carbon and Hydrogen, preferably nitrogen (N), oxygen (O) or sulfur (S), in particular nitrogen.

- 10 "Alkyl" refers to a straight-chain or branched-chain alkyl group, and includes C₁₋₇alkyl and more preferably C₁₋₄alkyl. Such alkyl groups include, for example, methyl, ethyl, n- or iso-propyl, n-, iso-, sec- or tert-butyl, n-pentyl, n-hexyl, n-heptyl, with particular preference given to methyl, ethyl, n-propyl, iso-propyl, n-butyl and iso-butyl. Alkyl may be unsubstituted or substituted. Exemplary substituents include, but are not limited to hydroxy, alkoxy, halogen (especially fluoro), amino and di-substituted amino, mono- or di-alkyl substituted amino, acetamino, morpholinyl, aryl. An example of a substituted alkyl is trifluoromethyl.
- 15 Cycloalkyl may also be a substituent to alkyl. An example of such a case is the moiety (alkyl)-cycloalkyl, such as (alkyl)-cyclopropyl or (alkyl)-cyclobutyl, e.g. methyl-cyclopropyl or methyl-cyclobutyl. A more specific example of an (alkyl)-cycloalkyl moiety includes geminal-type of substitution pattern, e.g. 1-alkyl cycloalkyl, such as 1-methyl cyclopropyl. Another example of cycloalkyl as a substituent to alkyl is alkandiyl-cycloalkyl, such as alkandiyl-
- 20 cyclopropyl, e.g. -CH₂-cyclopropyl. C₁-C₇-alkyl is alkyl with from and including 1 up to and including 7 carbon atoms, preferably from and including 1 up to and including 4 carbon atoms (C₁-C₄-alkyl), and is linear or branched; preferably, lower alkyl is butyl, such as n-butyl, sec-butyl, isobutyl, tert-butyl, propyl, such as n-propyl or isopropyl, ethyl or preferably methyl.

25

Each alkyl part of other groups like "alkoxy", "alkoxyalkyl", "alkoxycarbonyl", "alkoxy-carbonylalkyl", "alkylsulfonyl", "alkylsulfoxyl", "alkylamino", "halogenalkyl" shall have the same meaning as described in the above-mentioned definition of "alkyl".

- 30 "**C₃₋₇-Cycloalkyl**" refers to a saturated or partially saturated, monocyclic, fused polycyclic, or Spiro polycyclic, carbocycle having from 3 to 7 ring atoms per carbocycle. Illustrative examples of cycloalkyl groups include the following moieties: cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. C₃-C₇-cycloalkyl may be unsubstituted or substituted; exemplary

substituents are provided in the definition for alkyl. C₃-C₇-cycloalkyl may also be a substituent on other groups, e.g. on an alkyl group.

5 "Aryl" refers to an unsaturated carbocyclic aromatic ring system, preferably, having a ring system of not more than 16 carbon atoms, especially not more than 10 carbon atoms, e.g. having 6 to 16, preferably 6 to 10 ring carbon atoms, is preferably mono- or bi-cyclic, and is unsubstituted or substituted. For example, aryl is unsubstituted or substituted phenyl.

10 "Heterocyclyl" refers to a heterocyclic radical that is unsaturated (in particular maximally unsaturated, eg. carrying the highest possible number of conjugated double bonds in the ring(s)) e.g. heteroaryl), saturated or partially saturated in the bonding ring and is preferably a monocyclic or in a broader aspect of the invention bicyclic ring; has 3-16 ring atoms, more preferably 4-10 ring atoms, wherein at least in the ring bonding to the radical of the molecule of formula (I) one or more, preferably 1-4 ring atoms, especially one or two ring atoms are a
15 heteroatom selected from the group consisting of nitrogen, oxygen and sulfur; the bonding ring preferably having 4-12 ring atoms, especially 4-7 ring atoms, for example 6-10 ring atoms, especially for heteroaryl, such as 5, 6, 9 or 10 ring atoms. The heterocyclyl may be unsubstituted or substituted by one or more, especially 1 to 3, substituents independently selected from the group consisting of alkyl or the substituents defined above for substituted
20 alkyl and / or from one or more of the following substituents: oxo (=O), thiocarbonyl (=S), imino(=NH), imino-lower alkyl, and, for nitrogen containing heteroaryls, including N-oxides thereof.

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

30 "PI3 kinase mediated diseases" (especially PI3K alpha mediated diseases) 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 (where the diseases to be treated may include those showing somatic mutation of PIK3CA or germline mutations or somatic mutation of PTEN). Diseases to be treated include especially proliferative diseases such as tumor diseases, including solid tumors, leukaemias, glioblastoma, breast cancer and prostate cancer may be 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 formula I and are preferably pharmaceutically acceptable salts. Salt-forming groups in a compound of formula (I) are groups or radicals having basic or acidic properties. Compounds having at least one basic group or at least one basic radical, e.g., amino; a secondary amino group not forming a peptide bond or a pyridyl radical, may form acid addition salts, e.g., with inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid; or with suitable organic carboxylic or sulfonic acids, e.g., aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid or oxalic acid; or amino acids, such as arginine or lysine; aromatic carboxylic acids, such as benzoic acid; 2-phenoxy-benzoic acid; 2-acetoxy-benzoic acid; salicylic acid; 4-aminosalicylic acid; aromatic-aliphatic carboxylic acids, such as mandelic acid or cinnamic acid; heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid; aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxyethanesulfonic acid; or aromatic sulfonic acids, e.g., benzene-, *p*-toluene- or naphthalene-2-sulfonic acid. When several basic groups are present mono- or poly-acid addition salts may be formed.

Compounds of formula (I) having acidic groups, a carboxy group or a phenolic hydroxy group, may form metal or ammonium salts, such as alkali metal or alkaline earth metal salts, e.g., sodium, potassium, magnesium or calcium salts; or ammonium salts with ammonia or suitable organic amines, such as tertiary monoamines, e.g., triethylamine or tri(2-hydroxyethyl)-amine, or heterocyclic bases, e.g., *N*-ethyl-piperidine or *N,N'*-dimethylpiperazine. Mixtures of salts are possible.

Compounds of formula (I) having both acidic and basic groups can form internal salts.

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.

Compounds of the present invention may also form **solates and hydrates**, and as such any reference to a compound of formula (I) is therefore to be understood as referring also to the corresponding solvate and/or hydrate of the compound of formula (I), as appropriate and expedient.

The present invention also relates to **pro-drugs** of a compound of formula (I) that convert *in vivo* to the compound of formula (I) as such. Any reference to a compound of formula (I) is therefore to be understood as referring also to the corresponding pro-drugs of the compound of formula (I), as appropriate and expedient.

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 formula I and a combination partner (e.g. an other 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 formula I 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 formula I 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.

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

As shown in formula I, the alpha-amide substituent is at the 2-position on the pyrrolidine ring and the stereochemistry at this position is as drawn.

- 5 The ring A is preferably an unsubstituted or substituted 5- or 6-membered heterocyclic (preferably a heteroaryl) ring containing 1 or 2 heteroatoms selected from N, S or O, wherein at least one heteroatom is N.

More preferably, ring A is selected from an unsubstituted or substituted pyridine ring,
10 unsubstituted or substituted pyrimidine ring, unsubstituted or substituted thiazole ring, unsubstituted or substituted pyrazole ring or unsubstituted or substituted oxazole ring. More preferred is an unsubstituted or substituted pyridine ring, unsubstituted or substituted pyrimidine ring or unsubstituted or substituted thiazole ring.

- 15 Preferably, ring A is fused to the rest of the molecule of formula I through carbon atoms of ring A.

Ring A is preferably substituted by one, two or three R^2 groups, preferably two R^2 groups, most preferably one R^2 group, independently selected at each occurrence from,

- 20 unsubstituted or substituted C_1 - C_7 -alkyl;
unsubstituted or substituted amino;
unsubstituted or substituted C_3 - C_7 -cycloalkyl.

Preferably, R^2 is selected from

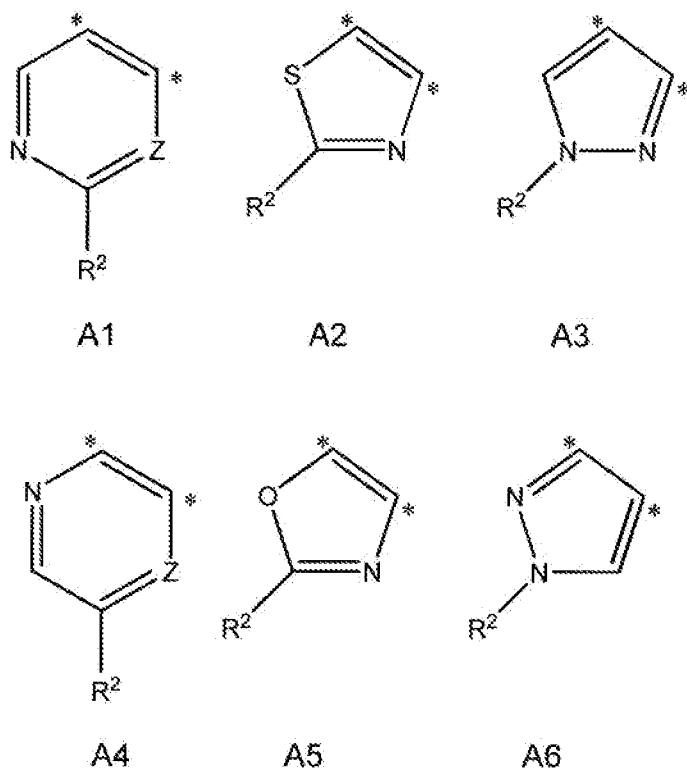
- 25 unsubstituted C_1 - C_7 -alkyl;
di(C_1 - C_7 -alkyl)amino;
 C_1 - C_7 -alkyl substituted one or more times by C_3 - C_7 -cycloalkyl or halo
(preferably fluoro);
unsubstituted C_3 - C_7 -cycloalkyl;
30 C_3 - C_7 -cycloalkyl which is substituted one or more times by halo (preferably fluoro), (halo- C_1 - C_7 -alkyl) or C_1 - C_7 -alkyl;

More preferably, R^2 is selected from methyl, t-butyl, diethylamino, cyclopropylmethyl, 2-fluoro-1,1-dimethylethyl or 2,2,2-trifluoro-1,1-dimethyl-ethyl.

In another embodiment, R^2 is selected from methyl, t-butyl, diethylamino, cyclopropylmethyl or 2-fluoro-1,1-dimethylethyl.

The ring A is more preferably selected from A1 or A2 or A3 or A4 or A5 or A6:

5



wherein,

Z is N or CH and R^2 is defined as above.

10

Preferably, ring A is selected from A1 or A2.

X-Y preferably represents $(CH_2)_r$ or $O(CH_2)_t$ wherein,

r is 1, 2 or 3;

15

t is 1 or 2;

X-Y more preferably represents $(CH_2)_r$ or $O(CH_2)_t$ wherein r is 2 and t is 1. For the avoidance of doubt, that is, X-Y is preferably $-CH_2-CH_2-$ or $-O-CH_2-$, such that in the latter case, the X in X-Y is the O in $-O-CH_2-$.

20 R^1 preferably represents, independently at each occurrence,

halo;

hydroxy;

unsubstituted or substituted phenyl;

di(C₁-C₇-alkyl)amino;

5 unsubstituted C₁-C₇-alkyl;

C₁-C₇-alkyl, which is substituted one or more times by

hydroxy, C₁-C₇-alkoxy, di(C₁-C₇-alkyl)amino, di-(perdeuteroC₁-C₇-alkyl)amino, phenyl, morpholinyl, acetamino, or *N*-(C₁-C₇-alkyl)-*N*-(phenylC₁-C₇-alkyl)amino, and wherein independently each phenyl may be mono or poly-substituted by halo.

10

R¹ more preferably represents, independently at each occurrence,

fluoro;

hydroxy;

unsubstituted phenyl;

15 dimethylamino;

methyl;

methyl, which is substituted one or more times (preferably substituted once) by

hydroxy, methoxy, dimethylamino, di-(perdeuteromethyl)amino, phenyl, morpholinyl, acetamino, or *N*-(methyl)-*N*-(phenylmethyl)amino, and wherein independently each phenyl

20 may be mono or poly-substituted by fluoro.

R¹ most preferably represents, independently at each occurrence,

fluoro;

hydroxy;

25 unsubstituted phenyl;

dimethylamino;

methyl;

hydroxy methyl;

methoxy methyl;

30 dimethylamino methyl;

di-(perdeuteromethyl)amino methyl;

benzyl;

morpholin-4-yl methyl;

N-acetamino methyl;

N-(methyl)-*N*-(3-fluoro-phenylmethyl)amino methyl.

An embodiment of the present invention includes compounds of the formula I wherein *n* is 0 or 1. Preferably, *n* is 1.

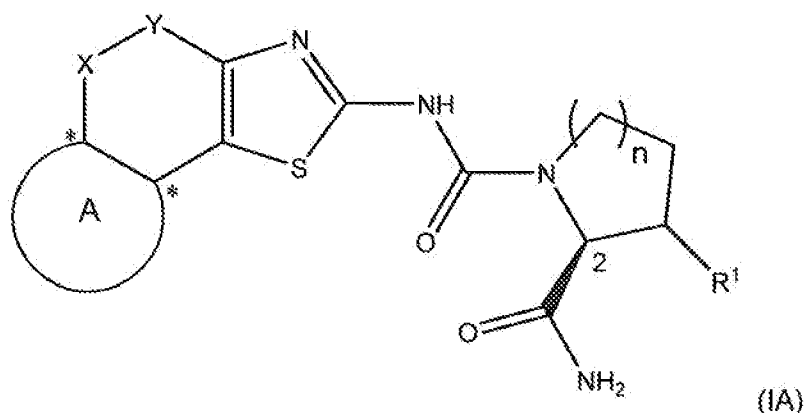
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Another embodiment of the present invention includes compounds of the formula I wherein *q* is 0, that is, wherein the nitrogen containing heterocyclic ring is substituted only by the amide at position 2. In this embodiment, it is preferred that *n* is 0 or 1, more preferably 1.

10 Another embodiment of the present invention includes compounds of the formula I wherein *q* is 1, that is, wherein the nitrogen containing heterocyclic ring is substituted only by the amide at position 2 and a single *R*¹ group. In this embodiment it is preferred that *n* is 0 or 1, more preferably 1. In this embodiment, the *R*¹ group may be substituted at position 2- (i.e. on the same carbon as that which is substituted by the amide group) or position 3- or position 4- or

15 position 5- of the nitrogen containing heterocyclic ring.

Preferably, in this embodiment, the *R*¹ group is substituted at position 3 of the nitrogen containing heterocyclic ring, i.e. compounds of formula IA:



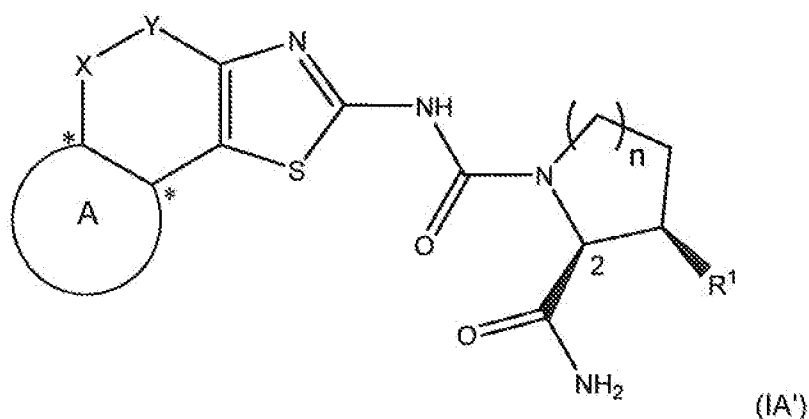
20 wherein the substituents are defined as for a compound of formula (I).

Preferably in compounds of formula IA, *n* is 1, thus providing compounds wherein the nitrogen containing heterocyclic ring is a pyrrolidine ring, substituted at the 2-position by an amide having the drawn stereochemistry, and in the 3-position by an *R*¹ group.

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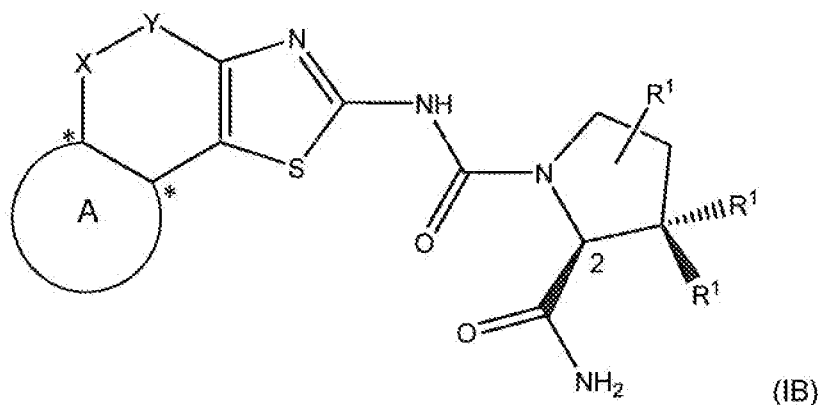
Preferably, the *R*¹ group has a stereochemistry which is *cis*- to the amide at position 2, i.e. compounds according to formula (IA'):

- 15 -



wherein the substituents are defined as for a compound of formula (I).

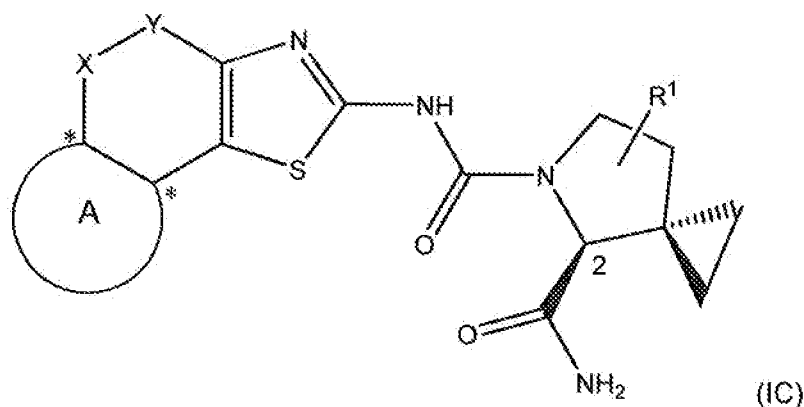
- 5 Preferably in compounds of formula IA', n is 1, thus providing compounds wherein the nitrogen containing heterocyclic ring is a pyrrolidine ring, substituted at the 2-position by an amide having the drawn stereochemistry, and in the 3-position by an R¹ group having the drawn stereochemistry, thus the amide and R¹ groups are cis- relative to each other.
- 10 A further embodiment of the present invention includes compounds of the formula I wherein q is 2 or 3, thus at least two R¹ substituents are present, each R¹ independently selected from the groups defined as for formula I herein. In this embodiment, it is preferred that at least each of two R¹ is bonded at position 3 of the pyrrolidine ring, and an optional third R¹ group, if present, is bonded elsewhere on the nitrogen containing heterocyclic ring. It is
- 15 further preferred that n is 1 and the third R¹ group, if present, is bonded at the 4- or 5- position of the resultant pyrrolidine ring, i.e. to provide compounds of formula IB:



wherein the substituents are defined as for a compound of formula (I).

In compounds according to formula IB, it is preferred that the third R^1 is bonded at position 4- of the pyrrolidine ring.

A further embodiment of the present invention includes compounds of the formula I wherein n is 1, and wherein two R^1 groups are bonded at position 3 of the pyrrolidine ring, and, together form an alkandiyl, preferably a C_3 - C_8 -cycloalkyl, in particular a cyclopropyl, i.e. to provide compounds of formula IC:



wherein the substituents are defined as for a compound of formula (I) and the third R^1 group is optional, and if present, is preferably bonded at position 4- of the pyrrolidine ring.

In any of the formulae (IA), (IA'), (IB) or (IC), the preferred definitions, if not otherwise stated, for ring A, X-Y, R^1 and n can also apply.

The invention further relates to pharmaceutically acceptable prodrugs of a compound of formula (I), (IA), (IA'), (IB) and/or (IC).

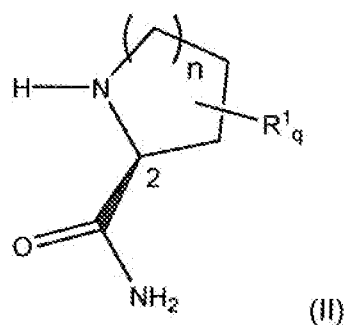
The invention further relates to pharmaceutically acceptable metabolites of a compound of formula (I), (IA), (IA'), (IB) and/or (IC).

The invention relates especially to the compounds of the formula (I), (IA), (IA'), (IB) and/or (IC) given in the Examples, as well as the methods of manufacture described herein.

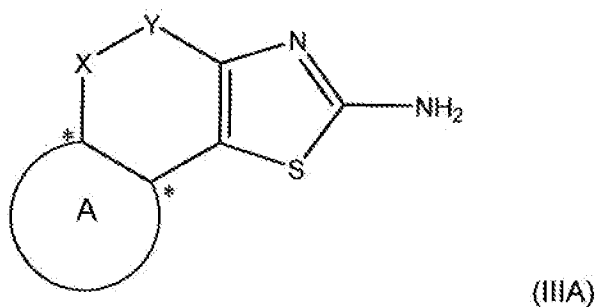
The present invention also relates to processes for the production of a compound of formula (I), (IA), (IA'), (IB) and/or (IC). In principle all known processes which convert two different

amines into a corresponding urea derivative are suitable and may be applied by using the respective starting material.

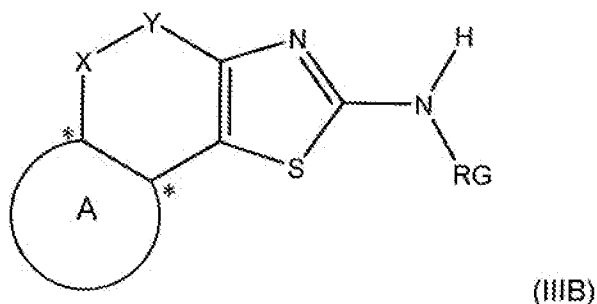
Thus, the invention in particular relates to a process which comprises reacting a compound
 5 of formula II



wherein the substituents are as defined above, either with a compound of formula IIIA



wherein the substituents are as defined above, in the presence of an activating agent
 10 ("method A") or with a compound of formula IIIB



wherein R^1 is as defined above; RG represents a reactive group (such as imidazolylcarbonyl)
 ("method B"),

15 in each case optionally in the presence of a diluent and optionally in the presence of a reaction aid and

recovering the resulting compound of formula I in free form or in form of a salt and,

optionally converting a compound of the formula I obtainable according to method A or method B into a different compound of the formula I, and/or converting an obtainable salt of a compound of the formula I into a different salt thereof, and/or converting an obtainable free compound of the formula I into a salt thereof, and/or separating an obtainable isomer of a compound of the formula I from one or more different obtainable isomers of the formula I.

Reaction conditions

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 IIIA or IIIB in a solvent, e.g. dimethylformamide, in the presence of a base e.g. an organic amine, e.g. triethylamine.

Where temperatures are given hereinbefore or hereinafter, "about" has to be added, as minor deviations from the numeric values given, e.g. variations of $\pm 10\%$, are typically tolerable.

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.

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

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. Also in the case of conversions of a compound of the formula (I), (IA), (IA'), (IB) and/or (IC) into a different compound of the formula (I), (IA), (IA'), (IB) and/or (IC), protecting groups may be introduced and removed, if useful or required.

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.

- 10 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

- 25 A compound of the formula (I), (IA), (IA'), (IB) and/or (IC) may be converted into a different compound of the formula (I), (IA), (IA'), (IB) and/or (IC).

- 30 In a compound of the formula (I), (IA), (IA'), (IB) and/or (IC) wherein a substituent carries an amino or amino-C₁-C₇-alkyl substituent, the amino can be converted into acylamino, e.g. C₁-C₇-alkanoylamino, by reaction with a corresponding C₁-C₇-alkanoylhalogenide, e.g. a corresponding chloride, in the presence of a tertiary nitrogen base, such as triethylamine or pyridine, in the absence or presence of an appropriate solvent, such a methylene chloride, for example at temperatures in the range from -20 to 50 °C, e.g. at about room temperature.

Salts of a compound of formula (I), (IA), (IA'), (IB) and/or (IC) with a salt-forming group may be prepared in a manner known *per se*. Acid addition salts of compounds of formula (I), (IA), (IA'), (IB) and/or (IC) 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 formula (I), (IA), (IA'), (IB) and/or (IC)) 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 formula (I), (IA), (IA'), (IB) and/or (IC). 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.

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 formula (I), (IA), (IA'), (IB) and/or (IC) 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 chromatographic substrates with chiral ligands.

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

Starting materials:

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.

- 5 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 formula (I), (IA), (IA'), (IB) and/or (IC).

Pharmaceutical compositions, uses and methods of treatment

- 10 The present invention also relates to use of the compounds of formula (I), (IA), (IA'), (IB) and/or (IC) as disclosed herein as pharmaceuticals. The present invention includes in one embodiment compositions comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC), e.g. for human or veterinary use, e.g. where inhibition of PI3K is indicated.
- 15 In one embodiment, the invention relates to the treatment of cellular proliferative diseases such as tumor (benign or malignant) and/or cancerous cell growth, e.g. mediated by PI3K. Diseases may include those showing somatic mutation of PIK3CA or germline mutations or somatic mutation of PTEN. In particular, the compounds may be useful in the treatment of human or animal (e.g., murine) cancers, including, for
- 20 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;
- 25 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
- 30 carcinoma; actinic keratosis; tumor diseases, including solid tumors; a tumor of the neck or head; polycythemia vera; essential thrombocythemia; and myelofibrosis with myeloid metaplasia.

In other embodiments, the condition or disorder (e.g. PI3K-mediated) is selected from the group consisting of: an epidermal hyperproliferation, prostate hyperplasia, a neoplasia, a neoplasia of epithelial character, Cowden syndrome, Lhermitte-Duclos disease or Bannayan-Zonana syndrome, asthma, COPD, ARDS, Loeffler'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 angitis, urticaria, bullous pemphigoid, lupus erythematosus, pemphigus, epidermolysis bullosa acquisita, autoimmune haematological 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 ophthalmopathy, 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.

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 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 1 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 500 mg active ingredient.

The compounds of formula (I), (IA), (IA'), (IB) and/or (IC) 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.

The compounds of formula (I), (IA), (IA'), (IB) and/or (IC) 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.

Consequently, the invention also provides:

- a method for preventing or treating conditions, disorders or diseases mediated by the activation of the PI3, e.g. the PI3 kinase alpha enzyme e.g. such as indicated above, in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula (I), (IA), (IA'), (IB) and/or (IC) or a pharmaceutically acceptable salt thereof
- use of a compound of formula (I), (IA), (IA'), (IB) and/or (IC), 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 formula (I), (IA), (IA'), (IB) and/or (IC) 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 formula (I), (IA), (IA'), (IB) and/or (IC) in free form or in pharmaceutically acceptable salt form in any of the methods as indicated herein, in particular for the treatment of one or more phosphatidylinositol 3-kinase mediated diseases.
- the use of a compound of formula (I), (IA), (IA'), (IB) and/or (IC) in free form or in pharmaceutically acceptable salt form in any of the methods as indicated herein, in particular for the manufacture of a medicament for the treatment of one or more phosphatidylinositol 3-kinase mediated diseases.

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.

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)).

A variety of human malignancies express activating 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 sub-clones 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.

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 than either agent alone in a glioblastoma xenograft model (Goudar et al., Mol. Cancer. Ther. 4:101-112 (2005)).

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)).

Accordingly, the present invention provides, in a further aspect, compounds of formulae (I), (IA), (IA'), (IB) and/or (IC) for use 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.

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.

Accordingly, in another aspect, the present invention provides the compounds of formulae (I), (IA), (IA'), (IB) and/or (IC) for use 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.

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)).

In addition to cancer and proliferative diseases, there is accumulating evidence that

5 inhibitors of Class 1A and 1B PI3 kinases would be therapeutically useful in others disease

areas. The inhibition of p110 β , 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 p110 β would be useful as a single

agent or in combination in anti-thrombotic therapy. The isoform p110 δ , the product of the

10 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 p110 δ -inhibitors would be useful in the treatment of

B-cell driven autoimmune diseases and asthma. Finally, the inhibition of p110 γ , the isoform

15 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)).

20 The invention further provides pharmaceutical compositions comprising at least one

compound of formula (I), (IA), (IA'), (IB) and/or (IC), together with a pharmaceutically

acceptable excepiant suitable for administration to a human or animal subject, either alone

or together with another therapeutic agent, for example another anticancer agent.

25 The invention further provides methods of treating human or animal subjects suffering from a

cellular proliferative disease, such as cancer. The invention thus provides methods of

treating a human or animal subject in need of such treatment, comprising administering to

the subject a therapeutically effective amount of a compound of formula (I), (IA), (IA'), (IB)

and/or (IC) either alone or in combination with one or more other therapeutic agents, e.g.

30 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 formula I include, but are not limited to, one or more compounds selected

from 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 a

- 5 compound of the formula (I), (IA), (IA'), (IB) and/or (IC) 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,
10 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)-
15 indolyl]-quinolone, AG-13736 and AG-13925, pyrrolo[2,1-f][1,2,4]triazines, ZK-304709, Veglin[®], VMMA-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
20 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-
25 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 a compound of formula (I), (IA), (IA'), (IB) and/or (IC) include Selective Estrogen Receptor Modulators (SERMs) including tamoxifen, toremifene, raloxifene; aromatase inhibitors including Arimidex[®] or anastrozole; Estrogen Receptor Downregulators (ERDs)
30 including Faslodex[®] or fulvestrant.

C. Anti-Androgens: Androgen-targeting agents for use in anticancer therapy in conjunction with a compound of formula (I), (IA), (IA'), (IB) and/or (IC) include flutamide, bicalutamide, finasteride, aminoglutethamide, ketoconazole, and corticosteroids.

D. Other Inhibitors: Other inhibitors for use as anticancer agents in conjunction with a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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 a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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, Cosmegen), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®, US 2004073044), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitabine, Gemcitabine (difluorodeoxycytidine), 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 a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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, iroflufen (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 (Platinite formulation of Cisplatin); porfiromycin; and clearazide (meclorethamine).

G. Chelating Agents: Chelating agents for use in conjunction with a compound of formula (I), (IA), (IA'), (IB) and/or (IC) include tetrathiomolybdate (WO 01/60814); RP-697; Chimeric T84.66 (cT84.66); gadofosveset (Vasovist®); deferoxamine; and bleomycin optionally in combination with electroporation (EPT).

H. Biological Response Modifiers: Biological response modifiers, such as immune modulators, for use in conjunction with a compound of formula (I), (IA), (IA'), (IB) and/or (IC) include staurosporine 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 a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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 a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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).

- 5 A compound of formula (I), (IA), (IA'), (IB) and/or (IC) may also be combined in a pharmaceutical composition with bronchodilatory or antihistamine drugs substances. Such bronchodilatory drugs include anticholinergic or antimuscarinic agents, in particular glycopyrrolate, ipratropium bromide, oxitropium bromide, and tiotropium bromide, OrM3, aclidinium, CHF5407, GSK233705 and β -2- adrenoreceptor agonists such as salbutamol,
- 10 terbutaline, salmeterol, carmoterol, milveterol and, especially, indacaterol and formoterol. Co-therapeutic antihistamine drug substances include cetirizine hydrochloride, clemastine fumarate, promethazine, loratadine, desloratadine diphenhydramine and fexofenadine hydrochloride.
- 15 The invention provides in a further aspect a combination comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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),
- 20 ZOCOR (Simvastatin), CRESTOR (Rosuvastatin), etc.), a Beta blocker (e.g., Atenolol), NORVASC (amlodipine besylate), and an ACE inhibitor (e.g., lisinopril).

The invention provides in a further aspect a combination comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) and one or more compounds that are useful for the treatment

25 of antihypertension. Such compounds include ACE inhibitors, lipid lowering agents such as statins, LIPITOR (Atorvastatin calcium), calcium channel blockers such as NORVASC (amlodipine besylate).

The invention provides in a further aspect a combination comprising a compound of formula

30 (I), (IA), (IA'), (IB) and/or (IC) and one or more compounds selected from the group consisting of fibrates, beta-blockers, NEPI inhibitors, Angiotensin-2 receptor antagonists and platelet aggregation inhibitors.

The invention provides in a further aspect a combination comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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

5 REMICADE, CDP-870) and D2E7 (HUMIRA) and TNF receptor immunoglobulin fusion molecules (such as ENBREL), IL-1 inhibitors, receptor antagonists or soluble IL-1R α (e.g. KINERET or ICE inhibitors), nonsteroidal anti-inflammatory agents (NSAIDS), piroxicam, diclofenac, naproxen, flurbiprofen, fenoprofen, ketoprofen, ibuprofen, fenamates, mefenamic acid, indomethacin, sulindac, apazone, pyrazolones, phenylbutazone, aspirin, COX-2
10 inhibitors (such as CELEBREX (celecoxib), PREXIGE (lumiracoxib)), metalloprotease inhibitors (preferably MMP-13 selective inhibitors), p2x7 inhibitors, α 2 α inhibitors, NEUROTIN, pregabalin, low dose methotrexate, leflunomide, hydroxychloroquine, d-penicillamine, auranofin or parenteral or oral gold.

15 The invention provides in a further aspect a combination comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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
20 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.

25 The invention provides in a further aspect a combination comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) and an antiviral agent and/or an antiseptis compound. Such antiviral agent may be selected from the group consisting of Viracept, AZT, acyclovir and famciclovir. Such antiseptis compound may be selected from the group consisting of Valant.

30 The invention provides in a further aspect a combination comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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).

The invention provides in a further aspect a combination comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) and one or more anti-Alzheimer's drugs. Such anti-Alzheimer Drug may be selected from the group consisting of donepezil, tacrine, $\alpha 2\delta$ inhibitors, NEUROTIN, pregabalin, COX-2 inhibitors, propentofylline or metryfonate.

The invention provides in a further aspect a combination comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) and an osteoporosis 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.

In another aspect of the preferred embodiments, kits that include one or more compound of formula (I), (IA), (IA'), (IB) and/or (IC) and a combination partner as disclosed herein are provided. Representative kits include a PI3K inhibitor compound (e.g., a compound of formula (I), (IA), (IA'), (IB) and/or (IC)) and a package insert or other labeling including directions for treating a cellular proliferative disease by administering a PI3K inhibitory amount of the compound(s).

In general, the compounds of formula (I), (IA), (IA'), (IB) and/or (IC) 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 formula (I), (IA), (IA'), (IB) and/or (IC), 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.

In general, compounds of formula (I), (IA), (IA'), (IB) and/or (IC) 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 compounds of the formula I is inhalation. This is an effective method for delivering a therapeutic agent directly to the respiratory tract.

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.

The inventions 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.

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In a further aspect, the invention provides pharmaceutical compositions comprising a (therapeutically effective amount) of a compound of formula (I), (IA), (IA'), (IB) and/or (IC), 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 formula (I), (IA), (IA'), (IB) and/or (IC). 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.

10

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.

15

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.

20

Compressed gases may be used to disperse a compound of the formula I 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

25

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 formula I 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%.

30

The invention further relates to pharmaceutical compositions comprising (i.e. containing or consisting of) at least one compound of formula (I), (IA), (IA'), (IB) and/or (IC) and at least one pharmaceutically acceptable excipient.

Pharmaceutical compositions comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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.

5

Combined pharmaceutical compositions comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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
10 manufactured in conventional manner by mixing with a pharmaceutically acceptable carrier and/or diluent with said active ingredients.

Consequently, the invention provides in further aspects

- 15 * a combined pharmaceutical composition, e.g. for use in any of the methods described herein, comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) in free form or pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent and/or carrier.
- 20 * a combined pharmaceutical composition comprising a compound of formula (I), (IA), (IA'), (IB) and/or (IC) 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.
- 25 * a combined pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I), (IA), (IA'), (IB) and/or (IC) in free form or in pharmaceutically acceptable salt form and a second drug substance, for simultaneous or sequential administration.
- 30 * 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), (IA), (IA'), (IB) and/or (IC) 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 formula (I), (IA), (IA'), (IB) and/or (IC) 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.

The following examples of compounds formula (I), (IA), (IA'), (IB) and/or (IC) illustrate the invention without limiting the scope thereof. Methods for preparing such compounds are described.

Temperatures are measured in degrees Celsius. Unless otherwise indicated, the reactions take place at rt and the MS are obtained with ESI. The following HPLC/MS methods are used in the preparation and analysis of the Intermediates and Examples:

Methods A1 to A3 (LCMS: analytical HPLC/MS):

System: Agilent 1100 Series with Waters Micromass ZQ 2000 ESI+ and/or ESI-

Column: XBridge C18, 3 x 30 mm, 2.5 micron

Temperature: 50 °C

Eluent A: H₂O, containing 5% CH₃CN and 0.8% HCOOH

Eluent B: CH₃CN, containing 0.6% HCOOH

Flow Rate: 1.2-2.4 mL/min

Method A1: method Polar4a_p_100-900 and method Polar4a_pn_100-900:

Gradient: 0 – 2.9 min: 1% to 95% of B

Method A2: method Fast4_p_100-900 and method Fast4a_pn_100-900:

Gradient: 0 – 2.4 min: 10% to 95% of B

Method A3: method Slow4a_pn_100-900:

Gradient: 0 – 4.4 min: 5% to 95% of B

Method B (preparative HPLC) Instrument: Waters preparative HPLC system, column:

Sunfire™ Prep C18 OBD™ 5 micron 30 X 100 mm, temperature: 25 °C, eluent: gradient from 5 - 100% CH₃CN in 0.05% aqueous TFA over 20 minutes, flow rate: 30 ml/minute, detection: UV 254 nm.

Method C (preparative HPLC) Instrument: Waters preparative HPLC system, column: Sunfire™ Prep C18 OBD™ 5 microm 30 X 100 mm, temperature: 25 °C, eluent: gradient from 5 - 50% CH₃CN in 0.05% aqueous TFA over 20 minutes, flow rate: 30 ml/minute, detection: UV 254 nm.

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Method D (Analytical HPLC): Linear gradient 2-100% CH₃CN (0.1%TFA) and H₂O (0.1% TFA) in 5 min + 1.5 min 100% CH₃CN (0.1%TFA); detection at 215 nm, flow rate 1 mL/min at 30 °C. Column: Nucleosil 100-3 C18 (70 x 4mm)

10 Method E (preparative HPLC/MS) Instrument: Gilson preparative HPLC system, column: Sunfire™ Prep C18 OBD™ 5 micron 30 X 100 mm, temperature: 25 °C, eluent: gradient from 5 - 100% CH₃CN in 0.05% aqueous TFA over 20 minutes, flow rate: 30 ml/minute, detection: UV 254 nm.

15 Method F (Analytical HPLC) Instrument: Shimadzu SIL-10A, Method: Linear gradient 2-100% CH₃CN (0.1%TFA) and H₂O (0.1% TFA) in 4 min + 2 min 100% CH₃CN (0.1%TFA); back to -100% CH₃CN (0.1%TFA) in 3 min.; detection at 215 nm, flow rate 2 mL/min at RT. Column: Nucleosil OD-5-100 C18 (150 x 4.6 mm)

20 Method G (Analytical HPLC) Instrument:

System: Agilent 1100 Series

Column: HP Hypersil BDS C18, 4 x 125 mm, 5 micron

Temperature: 25°C

Eluent A: H₂O, containing 0.1% v/v TFA

25 Eluent B: CH₃CN, containing 0.1% v/v TFA

Gradient: 10% → 100% B in 5 min, 2.5 min with 100% B, then → 10% B in 1 min

Flow Rate: 1.5 mL/min

Detection: UV 215 nm

30 Method H (Analytical HPLC) Instrument:

System: Agilent 1100 Series

Column: Macherey-Nagel Nucleosil 100-3 C18HD, 4 x 125 mm, 3 micron

Temperature: 30°C

Eluent A: H₂O, containing 0.1% v/v TFA

Eluent B: CH₃CN, containing 0.1% v/v TFA

Gradient: 2% → 100% B in 7 min, 2 min with 100% B, then → 2% B in 1 min

Flow Rate: 1.0 mL/min

Detection: UV 215 nm

5

Method I (LCMS: analytical HPLC/MS):

System: Waters Acquity UPLC with Waters Micromass ZQ 2000 ESI+/-

Column: Acquity HSS T3 C18, 2.1 x 50 mm, 1.8 micron

Temperature: 50 °C

10 Eluent A: H₂O, containing 0.05% v/v HCOOH and 3.75 mM ammonium acetate

Eluent B: CH₃CN, containing 0.04% HCOOH

Gradient: 2% → 98% B in 4.3 min, 0.7 min with 98% B, then → 2% B in 0.1 min and 0.9 min with 2% B

Flow Rate: 1.0 mL/min

15

Method J (LCMS: analytical HPLC/MS):

System: Agilent 1100 Series; MS: G1946D

Column: Symmetry C8, 2.1 x 50mm, 3.5 micron

Eluent A: H₂O, containing 0.1% v/v HCOOH

20 Eluent B: CH₃CN, containing 0.1% v/v HCOOH

Gradient: 0 – 3.3 min: 5% to 95% of B

Flow Rate: 1.0 mL/min

Method K (LCMS: analytical HPLC/MS):

25 System: Waters Acquity UPLC

Column: Acquity HSS T3 C18, 2.1 x 50 mm, 1.8 micron

Eluent A: H₂O, containing 0.05% v/v HCOOH and 0.05% ammonium acetate

Eluent B: acetonitrile, containing 0.04% HCOOH

Gradient: 2% → 98% B in 1.7 min, 0.45 min with 98% B, then → 2% B in 0.04 min

30 Flow Rate: 1.2 mL/min

Method L (LCMS: analytical HPLC/MS):

System: Waters Acquity UPLC; MS: Waters AQ Detector

Column: Acquity HSS, 1.8 µm 2.1 x 50mm, 3/µm

Eluent A: H₂O, containing 0.1% v/v HCOOH

Eluent B: CH₃CN, containing 0.1% v/v HCOOH

Gradient: 0 – 1.5 min: 10% to 95% of B, then 1 min: 95% B

Flow Rate: 1.2 mL/min

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ESI-MS:

Instrument: Micromass Platform II

Eluent: 15% v/v MeOH in H₂O containing 0.2% v/v of a 25% ammonium hydroxide solution

Flow Rate: 0.05 mL/min

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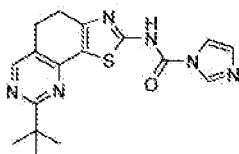
In the following examples, the abbreviations given below are used:

	atm.	atmosphere
	CDI	1,1'-carbonyldiimidazole
	CH ₃ CN	acetonitrile
15	DAST	diethylaminosulfur trifluoride
	DCE	1,2-dichloroethane
	DCM	dichloromethane
	DMF	N,N-dimethylformamide
	DMSO	dimethyl sulfoxide
20	Et ₂ O	diethyl ether
	EtOAc	ethyl acetate
	EtOH	ethanol
	eq	equivalent(s)
	h	hour(s)
25	H ₂ O	water
	HPLC	High Performance Liquid Chromatography
	HV	high vacuum
	LCMS	liquid chromatography coupled with mass spectrometry
	LiHMDS	lithium bis(trimethylsilyl)amide
30	MeOH	methanol
	mL	milliliter(s)
	min	minute(s)
	MS-ESI	electrospray ionisation mass spectrometry
	MW	microwave

- 40 -

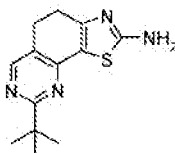
	NaHCO ₃	sodium bicarbonate
	Na ₂ SO ₄	sodium sulfate
	NH ₄ Cl	ammonium chloride
	RM	reaction mixture
5	R _f	ratio of fronts in TLC
	rt	room temperature
	TEA	triethylamine
	TFA	trifluoroacetic acid
	THF	tetrahydrofuran
10	TLC	thin layer chromatography
	t _R	retention time
	UV	Ultraviolet

15 Intermediate A: Imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide



20 8-tert-Butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-ylamine (**Stage A.1**, 0.319 g, 1.225 mmol) and CDI (278 mg, 1.715 mmol) were added to DCM (5 ml) and DMF (0.25 ml) under an argon atm. After 18 h the residue was cooled to 4 °C and the precipitate was collected by filtration. The solid was dried at 50 °C in high vacuo to give the title compound as a pale yellow solid. LCMS: t_R 0.95 min and M+H 319.0 (method A2) for (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-carbamic acid methyl ester; the product of the reaction of the title compound with MeOH during the preparation of the sample as MeOH solution.

25 **Stage A.1:** 8-tert-Butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-ylamine

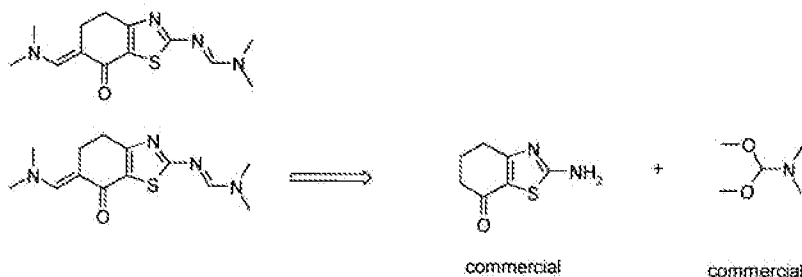


Intermediate A.1 was obtained by two different routes. Both routes start from 2-amino-5,6-dihydro-4H-benzothiazol-7-one and described below:

Route 1:

To a mixture of N'-{6-[1-dimethylamino-meth-(E)-ylidene]-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl}-N,N-dimethyl-formamidinium (**Stage A.2**, 2.2 g, 7.90 mmol) in 2-methoxyethanol (20 mL) was added at rt sodium hydroxide (1.185 g, 29.6 mmol) and 2,2-dimethyl-propionamidinium hydrochloride (1.620 g, 11.85 mmol). The RM was stirred at 125 °C for 3 h. After cooling to rt, the RM was diluted with MeOH, adsorbed onto silica gel and purified by flash chromatography (CombiFlash® Companion system®, with RediSep® silica gel column, eluent: DCM / MeOH / Ammonia 95:5:0.5). LC: t_R 3.64 min (method D). MS: $M+H = 261$. ^1H -NMR in DMSO- d_6 : 8.24 (s, 1H); 7.70 (s, 2H); 2.89 - 2.84 (m, 2H); 2.76 - 2.71 (m, 2H); 1.28 (s, 9H).

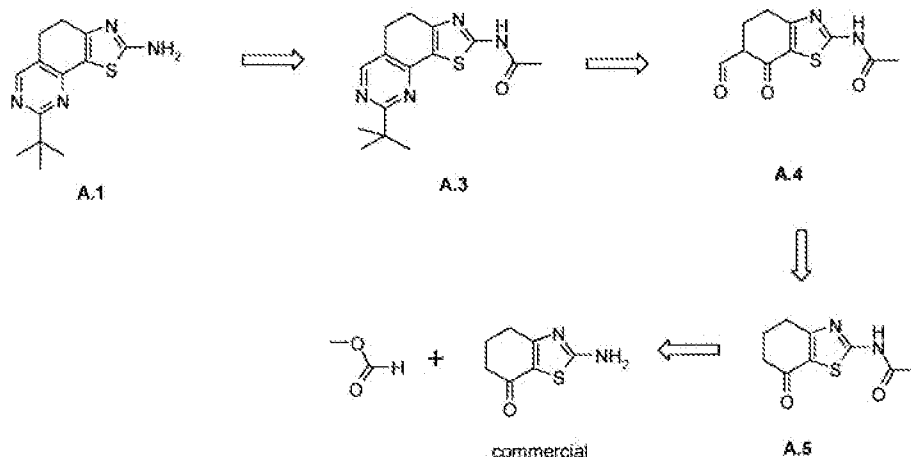
Stage A.2: N'-{6-[1-Dimethylamino-meth-(E)-ylidene]-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl}-N,N-dimethyl-formamidinium



A suspension of 2-amino-5,6-dihydro-4H-benzothiazol-7-one (3.5 g, 20.81 mmol) in dimethoxymethyldimethylamine (12 mL, 90 mmol) was heated at 100 °C with stirring for 65 h. The RM was then evaporated to dryness in vacuo and the residue was suspended in EtOAc. After 1 h at 4 °C, the solid was filtered off, washed with EtOAc and then dried under high vacuum at 60 °C to give the pure title product as brown crystals. LC: t_R 3.25 min (method D). MS: $M+H = 279$. ^1H -NMR in DMSO- d_6 : 8.40 (s, 1H); 7.23 (s, 1H); 3.15 (s, 3H); 3.05 (s, 6H); 2.97 (s, 3H); 2.91 (t, 2H); 2.67 (t, 2H).

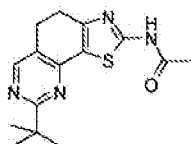
Route 2:

- 42 -



Potassium carbonate (0.434 g, 3.14 mmol) was added to a mixture of N-(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-acetamide (**Stage A.3**, 0.38 g, 1.257 mmol) in MeOH. The RM was stirred at 50 °C for 52 h, then cooled to rt and evaporated in vacuo to give a red mass. Water (20 mL) was added and the mixture was stirred at rt for a further 3 h. The red suspension was then cooled down to 4 °C and filtered to give after drying under high vacuum the title compound as a beige solid. LCMS: t_R 0.99 min and $M+H = 261$ (method A3). ^1H -NMR in DMSO- d_6 , 400 MHz: 8.24 (s, 1H); 7.70 (s, 2H); 2.89 - 2.84 (m, 2H); 2.76 - 2.71 (m, 2H); 1.28 (s, 9H).

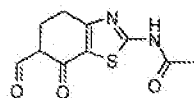
Stage A.3: N-(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-acetamide



Pyridine (13 mL) was added to N-(6-formyl-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide (**Stage A.4**, 3.05 g, 12.8 mmol) and tert-butylamine hydrochloride (1.788 g, 12.8 mmol) and the mixture was heated in a sealed vessel at 160 °C for 6.5 h. After cooling the reaction mixture was filtered to give a solid. The filtration mother liquor was evaporated to give further solid material. The combined solids were triturated repeatedly with hot CH_3CN and the CH_3CN mother liquors evaporated to give a solid which was shown to be predominantly the title product. The crude product was dissolved in ca. 10 ml of a 10 % DMSO in MeOH to give a slightly hazy orange solution which was filtered and added dropwise to water (100 ml) at rt with stirring. The precipitate solid was collected by filtration to give the title compound as an orange solid. LCMS: t_R 1.37 min and $M+H = 303.0$ (method

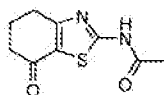
A3). $^1\text{H-NMR}$ in DMSO-d_6 , 400 MHz: 12.40 (s, 1H); 8.44 (s, 1H); 2.88 – 3.00 (m, 4H); 2.17 (s, 3H); 1.32 (s, 9H).

Stage A.4: N-(6-Formyl-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide



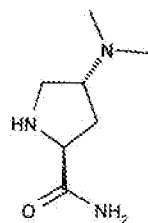
LiHMDS solution (1 M, 27.7 mL) was added over 10 min to a suspension of N-(7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide (**Stage A.5**, 2.0 g, 9.23 mmol) in dry THF (20 mL) cooled at -78°C under an argon atm. The RM was then stirred at -78°C for 2.5 h and methyl formate (2.308 mL, 36.9 mmol) added dropwise over 30 min. The RM was then warmed slowly to rt and was then stirred 18 h at rt. The RM was drawn out into aqueous 1 M HCl (70 mL) and extracted 3X with DCM, dried over Na_2SO_4 and evaporated to give a the title compound as a solid. HPLC: t_R 3.65 min (method D). MS: $M-H = 237$. $^1\text{H-NMR}$ in DMSO-d_6 (400 MHz): 12.50 (s, br, 1H); 7.55 (s, 1H); 2.90 – 2.60 (m, 4H); 2.15 (s, 3H).

Stage A.5: N-(7-Oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide



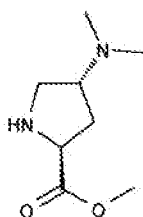
To acetic anhydride (80 mL) was added at rt 2-amino-5,6-dihydro-4H-benzothiazol-7-one (10 g, 59.4 mmol) and the resulting suspension was heated to reflux. After 1.75 h stirring at reflux, the RM was allowed to cool with stirring and stirred for 18 h at rt before further cooling with an ice / NaCl bath, and a solid was collected by filtration. The solid was then triturated twice with refluxing acetone (10 mL then 15 mL) before filtering and drying under vacuum at 40°C to give the title product as a beige solid. HPLC: t_R 3.47 min (method D). MS: $M-H = 211.1$. $^1\text{H-NMR}$ in DMSO-d_6 (600 MHz): 12.55 (s, br, 1H); 2.84 (t, 2H); 2.48 (t, 2H); 2.17 (s, 3H); 2.065 (qt, 2H).

Intermediate B: (2S,4R)-4-Dimethylamino-pyrrolidine-2-carboxylic acid amide



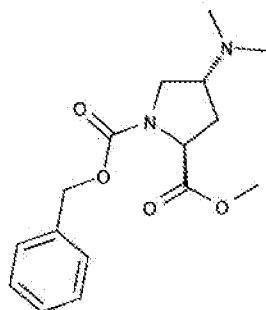
A solution of (2S,4R)-4-dimethylamino-pyrrolidine-2-carboxylic acid methyl ester (**Stage B.1**, 225 mg) and 7 M ammonia in MeOH (7 mL) was stood for 18 h at rt in a sealed vessel. Evaporation and trituration with Et₂O gave the title compound as a white solid.

5 **Stage B.1** (2S,4R)-4-Dimethylamino-pyrrolidine-2-carboxylic acid methyl ester



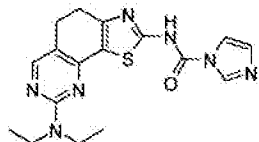
A mixture of (2S,4R)-4-dimethylamino-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester (**Stage B.2**, 420 mg), 10% palladium on carbon (80 mg) and MeOH (10 mL) was stirred for 16 h under an atm. of hydrogen. Filtration and evaporation gave the title compound which was used without purification in the following steps.

Stage B.2 (2S,4R)-4-Dimethylamino-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester



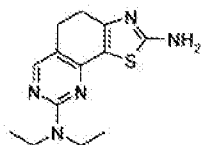
- 15 Sodium cyanoborohydride (200 mg) was added to a mixture of (2S,4R)-4-amino-pyrrolidine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester (400 mg), formalin (0.68 ml), acetic acid (0.72 ml), triethylamine (0.2 ml) and MeOH (2 ml) and the mixture was stirred for 2 h at rt. The RM was then partitioned between DCM and aqueous NaHCO₃ solution, the DCM layers evaporated and purified by normal phase chromatography, eluent; gradient from EtOAc to 20 % EtOH in EtOAc, to give the predominant UV-active component. The chromatographed material was taken up with 1 M HCl, washed 2X with Et₂O, the aqueous layer basified with NaHCO₃, 3X extracted with Et₂O, dried over Na₂SO₄ and evaporated to give the title compound as a pale yellow oil.
- 20

Intermediate C: Imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide



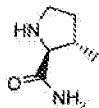
To a mixture of 2-amino-8-N,N-diethylamino-4,5-dihydrothiazolo[4,5-h]quinazoline (**Stage C.1**, 1 g, 3.63 mmol) in DCM (35 mL) was added CDI (1.178 g, 7.26 mmol). The RM was stirred at 40 °C for 90 h. After cooling to rt, the solid was collected by filtration to give the title compound. HPLC: t_R 4.11 min (method D). MS: $M+H = 334$ for (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-carbamic acid methyl ester as the product of the reaction of the title compound with MeOH.

Stage C.1: 2-Amino-8-diethylamino-4,5-dihydrothiazolo[4,5-h]quinazoline



To N'-(6-[1-dimethylamino-meth-(E)-ylidene]-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-N,N-dimethyl-formamidinium (**Stage A.2**, 1 g, 3.59 mmol) in 2-methoxyethanol (10 mL) was added NaOH (0.539 g, 13.47 mmol) and N,N-diethylguanidine (0.454 g, 3.94 mmol) under argon at rt. The RM was stirred for 3.5 h at 125 °C and then cooled to rt. After evaporation in vacuo the residue was dissolved in 0.1 M HCl (50 mL) and washed with EtOAc. The aqueous layer was then basified with 6 N NaOH and extracted 3 X with EtOAc. the organic layers were dried over Na_2SO_4 , evaporated and dried under high vacuum at 60 °C to give the title compound as orange crystals. LC: t_R 3.60 min (method D). MS: $M+H = 276$. ^1H -NMR in DMSO- d_6 : 7.91 (s, 1H); 7.59 (s, 2H); 3.50 (q, 4H); 2.69 (dd, 4H); 1.07 (t, 6H).

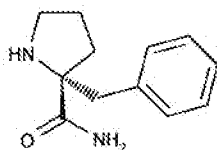
Intermediate D: (2S,3S)-3-Methyl-pyrrolidine-2-carboxylic acid amide



A 4 M solution of HCl in 1,4-dioxan (1.5 mL) was added to a suspension of (2S,3S)-3-methylpyrrolidine-2-carboxylic acid (0.5 g) in EtOH (5 mL) at rt and the mixture heated at reflux for 20 h. The RM was evaporated and a 7 M solution of ammonia in MeOH (5.6 mL) added. The RM was stood at rt for 6 days then evaporated, the residue triturated with MeOH

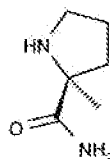
(0.5 mL) and filtered and washed with cold MeOH (2 mL) to give the title compound as a white solid. $^1\text{H-NMR}$ (d_6 -DMSO, 400 MHz): 8.06 (s, 1H), 7.67 (s, 1H), 3.60 (d, 1H), 3.25-3.14 (m, 2H), 2.24-2.15 (m, 1H), 2.09-1.98 (m, 1H), 1.57-1.45 (m, 1H), 1.13 (d, 3H).

5 Intermediate E: (R)-2-Benzyl-pyrrolidine-2-carboxylic acid amide



A mixture of (3R,7aR)-7a-benzyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (1.40 g, prepared as described by Wang and Germanas *Synlett* **1999**, 33-36.) and 7 M ammonia in MeOH (15 ml) was heated at 50 °C for 3 days in a sealed vessel. The cooled RM was
10 then evaporated and triturated with chloroform to give the title compound as a white solid.

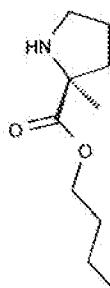
Intermediate F (S)-2-Methyl-pyrrolidine-2-carboxylic acid amide



A solution of (S)-2-methyl-pyrrolidine-2-carboxylic acid butyl ester (**Stage F.1**, 2.3 g) in a 7 M
15 solution of ammonia in MeOH (22.2 ml) was heated in a bomb at 70 °C for 10 days.

Evaporation of the RM and trituration with hexanes (20 mL) gave the title compound as an off-white solid. $^1\text{H-NMR}$ (d_6 -DMSO, 400 MHz): 7.40 (s, 1H), 6.89 (s, 1H), 2.95-2.84 (m, 1H), 2.72-2.60 (m, 1H), 2.06-1.95 (m, 1H), 1.66-1.44 (m, 2H), 1.42-1.30 (m, 1H), 1.22 (s, 3H).

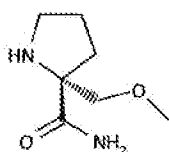
20 **Stage F.1**: (S)-2-Methyl-pyrrolidine-2-carboxylic acid butyl ester



Concentrated HCl (2 ml) was added to a suspension of (S)-2-methyl-pyrrolidine-2-carboxylic acid (2 g) in butan-1-ol (50 ml) which was heated at 60 °C for 18 h then at reflux for 4 days. The RM was evaporated, partitioned between saturated aqueous NaHCO_3 and DCM,

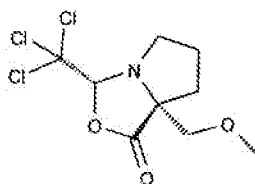
extracted 3X with DCM, dried over Na₂SO₄ and evaporated. The isolated oil was then kugelrohr distilled at 10 mbar to give the title compound as a clear colorless oil from the fraction distilling at an oven temperature of 100-120 °C.

5 Intermediate G: (R)-2-Methoxymethyl-pyrrolidine-2-carboxylic acid amide



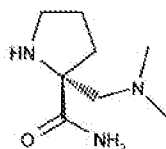
A mixture of (3R,7aR)-7a-methoxymethyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (**Stage G.1**, 0.6 g) and 7 M ammonia in MeOH (6 mL) was stood at rt for 2 days in a sealed vessel. The RM was then evaporated to give the title compound as a pale yellow oil which was used without further purification.

Stage G.1: (3R,7aR)-7a-Methoxymethyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one



15 A 1M of solution of lithium diisopropylamide in a 3:5 mixture of hexanes / THF (8.25 ml) was added dropwise to (3R,7aS)-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (1.51 g, prepared as described by Wang and Germanas *Synlett* 1999, 33-36.) in THF (5 ml) at -78 °C. After stirring 30 minutes at -78 °C methoxymethylchloride (1.14 ml) was added. The RM was then allowed to warm to -30 °C over 3 h and water was added. The aqueous layer
20 was extracted with DCM, the combined organic layers evaporated and the residue was then purified by normal phase chromatography, eluting with DCM, to give the title compound as a pale yellow oil.

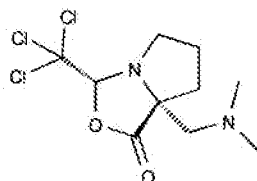
Intermediate H: (R)-2-Dimethylaminomethyl-pyrrolidine-2-carboxylic acid amide



A mixture of (3R,7aR)-7a-dimethylaminomethyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (**Stage H.1**, 0.26 g) and 7 M ammonia in MeOH (4 mL) was heated at 50 °C for 3 days in a sealed vessel. The cooled RM was then evaporated to give the title compound as a brown oil which was used without further purification. MS: M+H = 172.1.

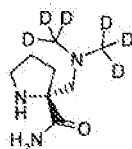
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Stage H.1: (3R,7aR)-7a-Dimethylaminomethyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one



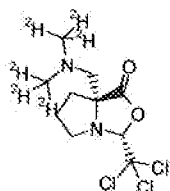
A 1 M solution of lithium diisopropylamide in a 3:5 mixture of hexanes / THF (8.25 ml) was added dropwise to (3R,7aS)-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (1.51 g, prepared as described by Wang and Germanas *Synlett* 1999, 33-36.) in THF (5 ml) at -78 °C. After stirring 30 minutes at -78 °C Eschenmoser's salt (2.78 g) was added. The RM was then allowed to warm to -40 °C with vigorous stirring over 1 h and maintained for 2 h at -40 °C. Water was then added and the aqueous layer extracted with DCM, the combined organic layers dried over Na₂SO₄ and evaporated. The residue was then purified by normal phase chromatography eluting with a gradient from DCM to 20 % EtOAc in DCM to give the title compound as a pale yellow oil (M+H = 301/303/305 3:3:1).

Intermediate I: d₆-(R)-2-Dimethylaminomethyl-pyrrolidine-2-carboxylic acid amide



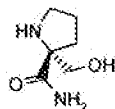
d₆-(3R,7aR)-7a-Dimethylaminomethyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (**Stage I.1**, 440 mg, 1.430 mmol) was dissolved in ammonia in MeOH (10.2 mL, 71.4 mmol) in a sealed vessel and heated at 75 °C for 5 days. The reaction mixture was evaporated and triturated 2X with CHCl₃ to give the title product as a pale brown solid.

Stage I.1: d₆-(3R,7aR)-7a-Dimethylaminomethyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one



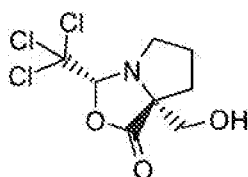
(3R,7aR)-1-Oxo-3-trichloromethyl-dihydro-pyrrolo[1,2-c]oxazole-7a-carbaldehyde (obtained as described in *J. Org. Chem.* **2006**, *71*(1), 97-102, 1 g, 3.67 mmol), dimethylamine- d_7 in THF (1.0 mL, 14.09 mmol), acetic acid (0.525 mL, 9.17 mmol) and DCE (4 mL) were combined under argon and sodium triacetoxyborohydride (1.089 g, 5.14 mmol) was added portionwise. After stirring at rt for 3 h the RM was taken up in DCM, and partitioned with 1 M NaOH extracting once more with DCM. The organic layers were combined then washed twice with water before being dried over Na_2SO_4 . The solution was evaporated to half of its volume and 1 M HCl in H_2O was added, the layers separated and the aqueous layer washed twice with DCM. The pH of the aqueous layers were adjusted to ~8 with saturated Na_2CO_3 solution and extracted three times with DCM. The organic layers were dried over Na_2SO_4 and evaporated, to give the desired title product as a pale yellow oil.

Intermediate J: (R)-2-Hydroxymethyl-pyrrolidine-2-carboxylic acid amide



(3R,7aR)-7a-Hydroxymethyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (**Stage J.1**, 308 mg, 1.122 mmol) was added to 7 M ammonia in MeOH (8.014 mL, 56.1 mmol) under argon and heated at 75 °C in a sealed vessel for 72 h and then evaporated to give the title compound as a viscous pale brown oil which was used without further purification.

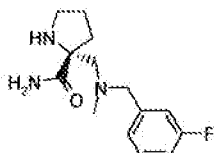
Stage J.1: (3R,7aR)-7a-Hydroxymethyl-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one



Sodium triacetoxyborohydride (544 mg, 2.57 mmol) was added to (3R,7aR)-1-oxo-3-trichloromethyl-dihydro-pyrrolo[1,2-c]oxazole-7a-carbaldehyde (obtained as described in *J. Org. Chem.* **2006**, *71*(1), 97-102, 500 mg, 1.835 mmol) in DCE (4 mL). The RM was stirred

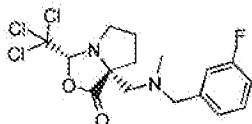
18 h at rt then taken up in DCM, the organic layer washed with water, dried over Na₂SO₄ and evaporated. The crude product was purified using a 20 g RediSep® silica gel column (eluent DCM to 10% MeOH in DCM) to give the title compound as a clear pale yellow oil.

- 5 **Intermediate K:** (R)-2-([(3-Fluoro-benzyl)-methyl-amino]-methyl)-pyrrolidine-2-carboxylic acid amide



- (3R,7aR)-7a-([(3-Fluoro-benzyl)-methyl-amino]-methyl)-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (**Stage K.1**, 385 mg, 0.973 mmol) was added to 7 M ammonia in MeOH (6.950 mL, 48.7 mmol) under argon and heated in a sealed vessel at 50 °C for 6 days and then at 75 °C for 5 days. The RM was then evaporated and the residue purified by flash chromatography using a 12 g RediSep® silica gel column (eluent 5 % MeOH in DCM) to give the title compound as a yellow oil.

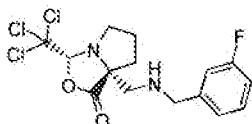
- 15 **Stage K.1:** (3R,7aR)-7a-([(3-Fluoro-benzyl)-methyl-amino]-methyl)-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one



- Sodium triacetoxymethylborohydride (889 mg, 4.19 mmol) was added to a mixture of (3R,7aR)-7a-([(3-fluoro-benzylamino)-methyl]-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one (**Stage K.2**, 400 mg, 1.048 mmol), formaldehyde (37 % in H₂O, 0.102 mL, 1.36 mmol) and acetic acid (0.150 mL, 2.62 mmol) in DCE (4 mL) under an argon atm. After 2 h stirring at rt the RM was partitioned between DCM and water. The combined organic layers were dried over Na₂SO₄ and evaporated to give the title compound as a pale brown oil which was used without further purification.

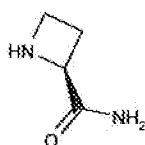
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- Stage K.2:** (3R,7aR)-7a-([(3-Fluoro-benzylamino)-methyl]-3-trichloromethyl-tetrahydro-pyrrolo[1,2-c]oxazol-1-one



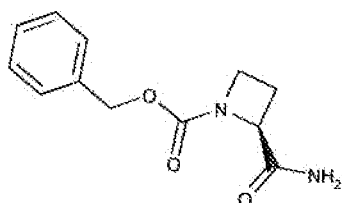
Sodium triacetoxyborohydride (544 mg, 2.57 mmol) was added to (3R,7aR)-1-oxo-3-trichloromethyl-dihydro-pyrrolo[1,2-c]oxazole-7a-carbaldehyde (obtained as described in *J. Org. Chem.* **2006**, *71*(1), 97-102, 500 mg, 1.835 mmol), 3-fluorobenzylamine (0.251 mL, 2.20 mmol) and acetic acid (0.263 mL, 4.59 mmol) in DCE (4 mL) under an argon atm. The RM was stirred at rt for 3 h, then partitioned between water and DCM, the combined organic dried over Na₂SO₄ and evaporated to give the title compound as a pale yellow oil which was used without further purification.

Intermediate L: (S)-Azetidine-2-carboxylic acid amide



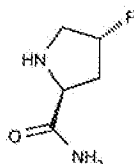
A mixture of (S)-2-carbamoyl-azetidine-1-carboxylic acid benzyl ester (**Stage L.1**, 1.8 g) and 10% palladium on carbon (0.2 g) in MeOH (25 ml) was stirred under a hydrogen atm. at rt for 5 h. Filtration and evaporation gave the title compound which was used without further purification.

Stage L.1: (S)-2-Carbamoyl-azetidine-1-carboxylic acid benzyl ester



A mixture of (S)-azetidine-1,2-dicarboxylic acid 1-benzyl ester 2-methyl ester (2.5 g) and 7 M ammonia in MeOH (10 ml) was stood at rt for 18 h in a sealed vessel. The RM was then evaporated to give the title compound as a white solid which was used without further purification. MS: M+H 235.1 and M-H 233.1.

Intermediate M: (2S,4R)-4-fluoro-pyrrolidine-2-carboxylic acid amide

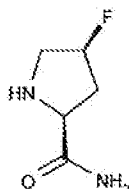


A 1.25 M solution of HCl in EtOH (2.3 ml) was added to a suspension of (2S,4R)-4-fluoro-pyrrolidine-2-carboxylic acid (0.25 g) in EtOH (2 ml) at rt and the mixture heated for 62 h at 55 °C. The RM was evaporated and a 7 M solution of ammonia in MeOH (5.6 ml) added.

The RM was stood at rt for 36 h then evaporated, the residue triturated with MeOH (0.5 ml) and filtered to give the title compound as a white solid. ¹H-NMR (d₆-DMSO, 400 MHz): 7.68 (s, 1H), 7.37 (s, 1H), 5.30 (d, 1H), 3.96 (t, 1H), 3.40-3.12 (m, 2H), 2.48-2.31 (m, 1H), 2.02-1.81 (m, 1H).

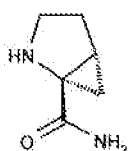
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Intermediate N: (2S,4S)-4-Fluoro-pyrrolidine-2-carboxylic acid amide



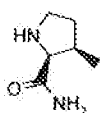
10 A mixture of (2S,4S)-2-carbamoyl-4-fluoro-pyrrolidine-1-carboxylic acid tert-butyl ester (1.0 g), conc. HCl (0.6 ml) and 1-butanol (10 ml) was heated for 48 h at 50 °C. The RM was evaporated and partitioned DCM and aqueous NaHCO₃, the DCM layers were dried over Na₂SO₄ and evaporated. A solution of 7 M ammonia in methanol (10 ml) was added to the residue and the mixture was stood for 60 h at rt in a sealed vessel. Evaporation and trituration with EtOH gave the title compound as a white solid.

15 Intermediate O: (1S,5R)-2-Aza-bicyclo[3.1.0]hexane-1-carboxylic acid amide



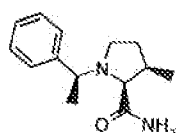
20 A mixture of (1S,5R)-2-aza-bicyclo[3.1.0]hexane-1-carboxylic acid ethyl ester (2.5 g, prepared by the procedure of Hercouet *Tetrahedron Asymmetry* **1996**, 7, 1267-1268.) and 7 M ammonia in MeOH (20 ml) was heated in a sealed vessel at 80 °C for 5 days. The cooled RM was evaporated and triturated with hexanes / DCM to give the title compound as a beige solid. ¹H-NMR (DMSO-d₆, 400 MHz): 7.15 (s, 1H), 7.04 (s, 1H), 3.00 – 2.91 (m, 1H), 2.67 (q, 1H), 1.94 - 1.83 (m, 1H), 1.74 – 1.67 (m, 1H), 1.64 – 1.55 (m, 1H), 1.38 – 1.31 (m, 1H), 0.90 (t, 1H).

25 Intermediate P: (2S,3R)-3-Methyl-pyrrolidine-2-carboxylic acid amide



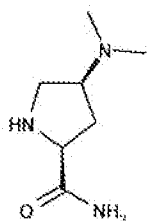
(2S,3R)-3-Methyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide (**Stage P.1**, 1.1 g, 4.76 mmol) and Pd on charcoal 10 % (0.101 g, 0.947 mmol) in MeOH (20 mL) was shaken under a H₂ atm. for 46 h at rt. The RM was then filtered through a Fluoropore Membrane Filter (0.2µm FG) and evaporated. The residue was dissolved in DCM and evaporated to dryness to give the title compound as white crystals MS: M+H = 129.0. ¹H-NMR (d₆-DMSO, 600 MHz): 7.34 (s, br, 1H), 7.10 (s, br, 1H), 3.48 (d, 1H), 3.02 – 2.97 (m, 1H), 2.80 – 2.75 (m, 1H), 2.35– 2.28 (m, 1H), 1.88 – 1.81 (m, 1H), 1.39 – 1.32 (m, 1H), 0.83 (d, 3H).

10 **Stage P.1:** (2S,3R)-3-Methyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide



Trimethylaluminum in toluene (2 M, 3.23 mL) was added dropwise to a mixture of ammonium chloride (0.346 g, 6.47 mmol) in toluene (3.2 mL) at 0 °C under an argon atm., with the formation of methane gas. The RM was then allowed to warm to rt, and stirred at rt for a further 15 min. before (2S,3R)-3-methyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester (prepared as described in *Tetr. Lett.* **1997**, 38 (1), 85-88; 1.6 g, 6.47 mmol) was slowly added. The RM was stirred at rt for 56 h, 1 M HCl was then added with cooling and the RM washed 3X with DCM. The aqueous phase was basified with Na₂CO₃, extracted 3X with DCM and the combined organic layers dried over Na₂SO₄. Evaporation gave the title compound as a yellowish oil. MS: M+H = 233.2. HPLC: t_R 3.17 min (method D). ¹H-NMR (d₆-DMSO, 600 MHz): 7.37 – 7.33 (m, 2H), 7.30 (t, 2H), 7.25 (s, br, 1H), 7.21 (t, 1H), 7.12 (s, br, 1H), 3.55 (q, 1H), 3.40 (d, 1H), 2.71 (t, 1H), 2.28– 2.22 (m, 1H), 2.21 – 2.16 (m, 1H), 1.71 (qt, 1H), 1.38 – 1.31 (m, 1H), 1.21 (d, 3H), 0.90 (d, 3H).

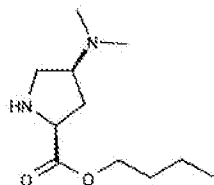
25 Intermediate Q: (2S,4S)-4-Dimethylamino-pyrrolidine-2-carboxylic acid amide



A solution of (2S,4S)-4-dimethylamino-pyrrolidine-2-carboxylic acid butyl ester (**Stage Q.1**, 326 mg) and 7 M ammonia in MeOH (8 ml) was stood for 18 h at rt in a sealed vessel.

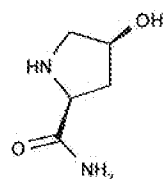
Filtration, evaporation and trituration with Et₂O / MeOH gave the title compound as a beige solid.

Stage Q.1: (2S,4S)-4-Dimethylamino-pyrrolidine-2-carboxylic acid butyl ester



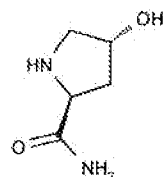
Concentrated HCl (0.3 ml) was added to a mixture of (2S,4S)-4-dimethylamino-pyrrolidine-2-carboxylic acid methyl ester dihydrochloride (400 mg) and 1-butanol (4 ml) and heated for 18 h at 115 °C. After cooling the RM was evaporated then partitioned between DCM and aqueous NaHCO₃ solution and the DCM layers dried and evaporated to give the title compound as a brown oil which was used without further purification.

Intermediate R: (2S,4S)-4-Hydroxy-pyrrolidine-2-carboxylic acid amide



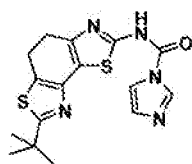
A solution of (2S,4S)-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester hydrochloride (1 g) in a 7M solution of ammonia in MeOH (10 ml) was stirred for 18 h then evaporated and trituated with Et₂O. The residue was dissolved in the minimum volume of hot MeOH and stood at 4 °C for 4 h. The title compound was isolated by filtration as a white solid.

Intermediate S: (2S,4R)-4-hydroxy-pyrrolidine-2-carboxylic acid amide



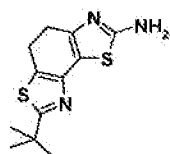
A solution of (2S,4R)-4-hydroxy-pyrrolidine-2-carboxylic acid benzyl ester (1 g) in 880 ammonia (5 ml) was stirred for 18 h then evaporated and trituated with Et₂O to give the title compound as a white solid. ¹H-NMR (d₆-DMSO, 400 MHz): 9.15 (s, br, 1H), 8.04 (s, 1H), 7.63 (s, 1H), 5.56 (s, 1H), 4.40 (s, 1H), 4.27-4.16 (m, 1H), 3.27 (d, 1H), 3.02 (d, 1H), 2.33-2.19 (m, 1H), 1.89-1.76 (m, 1H).

Intermediate T: Imidazole-1-carboxylic acid (7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide



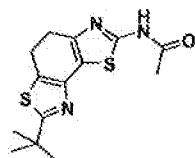
- 5 7-tert-Butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-ylamine (**Stage T.1**, 175 mg, 0.659 mmol) was dissolved in DCM (10 mL), CDI (297 mg, 1.648 mmol) was then added, and the RM was stirred at rt for 2 h. The title compound was isolated by filtration, washed with DCM and dried under HV.

- 10 **Stage T.1:** 7-tert-Butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-ylamine



- N-(7-tert-Butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-acetamide (**Stage T.2**, 225 mg, 0.732 mmol) was dissolved in EtOH (10 mL), HCl 36% (1.48 g, 14.64 mmol) was then added, and the RM heated to reflux. After 18 hours at reflux the RM was cooled to rt and
 15 adjusted to pH 8-9 by the addition of 5% aqueous sodium bicarbonate solution, extracted with EtOAc, and washed two times with H₂O. The organic layer was dried over Na₂SO₄ and evaporated to give the title product (t_R 4.408 min (Method F)).

Stage T.2: N-(7-tert-Butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-acetamide

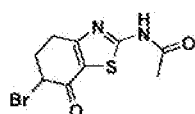


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- N-(6-Bromo-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide (**Stage T.3**, 473 mg, 1.635 mmol) was dissolved in MeOH (10 mL), 2,2-dimethylthio propionamide (230 mg, 1.962 mmol) and ammonium phosphomolybdate (307 mg, 0.164 mmol) were added, and the RM was stirred at 25 °C for 20 h. The RM was then stood for 2 days before stirring at 50 °C for
 25 24 h. The mixture was extracted with EtOAc/H₂O. The organic layers were dried over Na₂SO₄ and evaporated. The crude material was chromatographed with 30 g of silica gel,

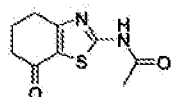
eluent DCM/MeOH=99:1. Fractions containing the product were evaporated and lyophilized from dioxane to give 225 mg of the title compound as a white solid ($M+H = 308$; $M-H = 306$; t_R 5.525 min (Method F)).

5 **Stage T.3: N-(6-Bromo-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide**



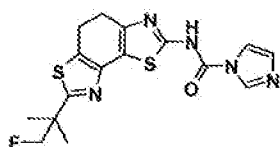
N-(7-Oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide (**Stage T.4**, 2.286 g, 10.87 mmol) was dissolved in AcOH (60 ml), then bromine (1.74 g, 10.87 mmol) dissolved in AcOH (10 mL) were slowly added and the RM was heated to 75 °C for 20 h. The color changed from red to beige. The mixture was evaporated and the residue was dissolved in MeOH (10 mL) and precipitated with H₂O. The mixture was filtered and dried on HV. The crude material was chromatographed with MPLC C18 H₂O 0.1% TFA / CH₃CN 0.1% TFA, gradient 0-50%. Fractions containing the product were neutralized with NaHCO₃, extracted with EtOAc and lyophilized from dioxane to give of the title compound as a white solid ($M+H = 291$; $M-H = 289$; t_R 4.29 (Method F)). ¹H-NMR (d₅-DMSO, 600.13 MHz): 12.75 (s, 1H) 4.95 (t, 1H), 2.95-2.84 (m, 2H), 2.62-2.52 (m, 1H), 2.40-2.30 (m, 1H), 2.20 (s, 3H).

Stage T.4: N-(7-Oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide



To acetic anhydride (80 mL) was added at rt 2-amino-5,6-dihydro-4H-benzothiazol-7-one (10 g, 59.4 mmol) and the yellow suspension was heated to reflux. After 1.75 h stirring at this temperature, the RM was allowed to cool with stirring, and stirred overnight at rt. After further cooling with an ice / NaCl bath, the suspension was filtered. The solid was then refluxed twice with acetone (10 mL then 15 mL) and filtered. The resulting solid was dried under vacuum at 40 °C overnight to afford the title compound as a beige solid (HPLC: t_R 3.47 min (Method A3), $M-H = 211.1$), ¹H-NMR in DMSO-d₆ (600 MHz): 12.55 (s, br, 1H); 2.84 (t, 2H); 2.48 (t, 2H); 2.17 (s, 3H); 2.065 (qt, 2H)).

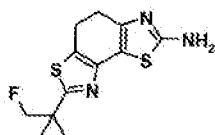
Intermediate U: Imidazole-1-carboxylic acid [7-(2-fluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl]-amide



7-(2-Fluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-amine (**Stage U.1** 61.6 mg, 0.217 mmol) was dissolved in DCM (2 mL), and CDI (297 mg, 1.648 mmol) added to give a clear colorless solution. The RM stood overnight at rt to give a white suspension.

- 5 The mixture was cooled at 4 °C for 1 h and then filtered, washed with DCM and dried under vacuum to give of the title compound as a white solid (analysis of a sample in MeOH; M+H = 342.0 showed methyl carbamate product in MS; t_R 2.14 min (Method A3)).

Stage U.1 7-(2-Fluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-amine



10

3-Fluoro-2,2-dimethyl-thiopropionamide (**Stage U.2**, 394 mg, 2.331 mmol) was dissolved in EtOH, N-(6-bromo-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide (**Stage T.3**, 473 mg, 1.635 mmol) and ammonium phosphomolybdate (80 mg, 0.042 mmol) were then added to give a yellow suspension. The RM was heated to reflux to give a dark blue-green suspension. The reaction mixture was stirred at 65 °C 3 days. The RM was filtered and the filtrate evaporated. The residue was taken up in DMF and purified by prep-HPLC (method E). Fractions containing the product were combined and evaporated to give the title compound as a white solid (M+H = 284.1; t_R 1.30 min (Method A3)).

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20 **Stage U.2** 3-Fluoro-2,2-dimethyl-thiopropionamide

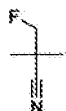


The title compound was prepared by the procedure of Boys, M. L.; Downs, V. L. *Synth. Commun.* **2006**, 36, 295. Sodium hydrogen sulphide hydrate (3.89 g, 69.4 mmol, hygroscopic) was added to a solution of 3-fluoro-2,2-dimethyl-propionitrile (**Stage U.3**, 1.17 g, 11.57 mmol) and diethyl amine hydrochloride (7.61 g, 69.4 mmol) in 1,4-dioxane (7 mL) and H₂O (7 mL) at rt. The RM was heated to 55 °C and then stirred for 3 days at this temperature. The reaction mixture was diluted with water (50 ml) and extracted 5X with EtOAc (50 mL). The organic layers were dried over Na₂SO₄ and evaporated to give an

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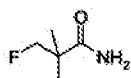
orange oil. DCM (5 mL) was then added to give a white suspension. The suspension was filtered and the filtrate was purified with flash chromatography on silica gel eluting with DCM. The product containing fractions were evaporated to give the title compound as a pale yellow oil (M+H = 136.1, M-H = 134.1; t_R 0.69 min (Method A3)). ^{19}F -NMR (d_6 -DMSO, 400 MHz) 218ppm (t, 1F).

Stage U.3 3-Fluoro-2,2-dimethyl-propionitrile



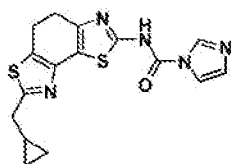
3-Fluoro-2,2-dimethyl-propionamide (**Stage U.4**, 1.82 g, 15.28mmol) and phosphorus pentoxide (2.168 g, 15.28 mmol) were combined to give a free flowing white powder which was heated with an oil bath to a bath temperature of 180 °C over 50 minutes under an argon atmosphere. A 300 mbar vacuum was then slowly applied distilling a mobile clear colourless oil which formed a low melting waxy solid on standing of the title compound. ^{19}F -NMR (d_6 -DMSO, 400 MHz) 219.5 ppm (t, 1F).

Stage U.4 3-Fluoro-2,2-dimethyl-propionamide



3-Fluoro-2,2-dimethyl-propionyl fluoride (DE3326874 and DE3611195, 2.5 g, 20.47 mmol) was added at 0 °C to a mixture of aqueous ammonia (10 mL) and THF (20 mL). The RM was then allowed to warm to rt and stood overnight at rt. The volume was reduced by 50% under vacuum to give a thick white suspension. The suspension was extracted with DCM/H₂O. The organic layers were dried over Na₂SO₄ and evaporated to give the title compound as a white crystalline solid.

Intermediate V: Imidazole-1-carboxylic acid (7-cyclopropylmethyl-4,5-dihydro-benzo [1,2-;3,4-d']bisthiazol-2-yl)-amide

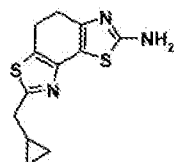


7-Cyclopropylmethyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-ylamine (**Stage V.1**, 72 mg, 0.273 mmol) was dissolved in 5 mL DCM, CDI (73.9 mg, 0.410 mmol) was added, and the

RM stirred at rt for 20 h. Additional CDI (37 mg, 0.205 mmol) was added and the RM stirred at rt for a further 2 hours. The mixture was filtered, washed with DCM and filtrate was evaporated to give the title compound ($M+H = 322.1$; $M-H = 320.2$ MS-ES in MeOH shows the methyl carbamate product).

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Stage V.1 7-Cyclopropylmethyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-ylamine

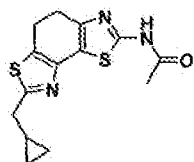


N-(7-Cyclopropylmethyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-acetamide (**Stage V.2**, 180 mg, 0.589 mmol) was dissolved in EtOH (10 mL) and 36% HCl (1.193g, 11.79

10 mmol) added. The RM was heated to 90 °C for 16 h then cooled and extracted with EtOAc/H₂O. The organic layer was dried over Na₂SO₄ and evaporated. The crude material was chromatographed with MPLC C18 H₂O 0.1% TFA / CH₃CN 0.1% TFA, gradient 0-50%. Product containing fractions were neutralized with NaHCO₃, extracted with EtOAc and lyophilized from dioxane to give the title compound ($M+H = 264.2$; $M-H = 262.1$; t_R 4.183

15 min (Method F)).

Stage V.2 N-(7-Cyclopropylmethyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-acetamide

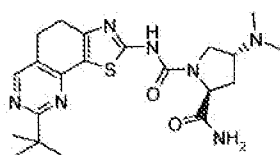


N-(6-Bromo-7-oxo-4,5,6,7-tetrahydro-benzothiazol-2-yl)-acetamide (**Stage T.3**, 347 mg,

20 1.200 mmol) was dissolved in MeOH (10 mL) and 2-cyclopropyl-thioacetamide (*Can. J. Chem* **1995 Vol.73** 1468-1477, 166 mg, 1.440 mmol) and ammonium phosphomolybdate (225 mg, 0.120 mmol) were added. The RM was stirred at rt for 20 h then heated at 50 °C for 3 h, 60 °C for 2 h and then refluxed for 24 h. The RM was partitioned between EtOAc/H₂O. The organic layer was dried over Na₂SO₄ and evaporated. The raw material was

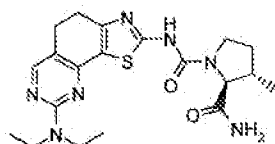
25 chromatographed with MPLC C18 H₂O 0.1% TFA / CH₃CN 0.1% TFA, gradient 0-50%. Product containing fractions were neutralized with NaHCO₃, extracted with EtOAc and lyophilized from dioxane to give the title compound as a white solid ($M+H = 306.2$; $M-H = 304.2$; t_R 5.12 min (Method F)).

Example 1: (2S,4R)-4-Dimethylamino-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



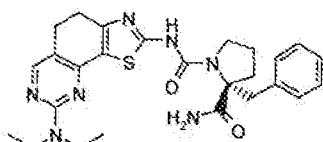
- 5 To a solution of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**, 45.1 mg, 0.127 mmol) in DMF (1 mL) was added (2S,4R)-4-dimethylamino-pyrrolidine-2-carboxylic acid amide (**Intermediate B**, 22 mg, 0.140 mmol) and triethylamine (0.053 ml, 0.382 mmol). The RM was then stirred at rt for 17 h and purified by preparative HPLC (method B). The product containing fractions were combined and eluted through a Bond Elut - SCX, 300 mg cartridge. The cartridge was then washed with a 7 M solution of ammonia in MeOH and evaporated to give the title compound. MS: M+H = 444. HPLC: t_R 3.17 min (method D).

- 15 **Example 2:** (2S,3S)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



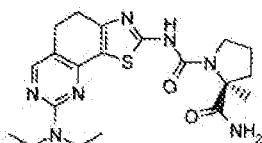
- A mixture of (2S,3S)-3-methyl-pyrrolidine-2-carboxylic acid amide (**Intermediate D**, 6.24 mg, 0.049 mmol), imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 18 mg, 0.049 mmol) and triethylamine (0.020 mL, 0.146 mmol) in DMF (0.4 mL) was stirred at rt for 6 h. The RM was then directly purified by preparative HPLC (method B). The product containing fractions were then filtered through a 300 mg Bond Elut-SCX cartridge. The cartridge was then eluted with a 7 M ammonia solution in MeOH and the eluent evaporated to give the title compound as a yellow solid. MS: M+H = 430.1. HPLC: t_R 3.72 min (method D). ^1H -NMR (DMSO- d_6 , 600 MHz): 11.05 (s, 1H); 8.04 (s, 1H); 7.44 (s, br, 1H); 6.95 (s, br, 1H); 3.78 (s, br, 1H); 3.60 – 3.50 (m, 6H); 2.82 (s, br, 4H); 2.18 (s, br, 1H); 2.03 (s, br, 1H); 1.55 (s, br, 1H); 1.10 (t, 6H); 1.05 (d, 3H).

Example 3: (R)-2-Benzyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



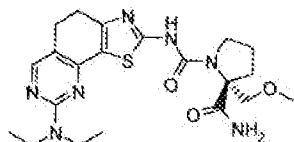
To a mixture of imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 50 mg, 0.135 mmol) in DMF (1 mL) was added (R)-2-benzyl-pyrrolidine-2-carboxylic acid amide (**Intermediate E**, 33.2 mg, 0.162 mmol) and triethylamine (0.057 mL, 0.406 mmol). The RM was stirred for 21 h at 40 °C and then directly purified with preparative HPLC (method B). The product containing fractions were eluted through a 300 mg Bond Elut - SCX, cartridge. The cartridge was then eluted with 7 M ammonia-solution in MeOH and evaporated give the title compound as a yellow solid. MS: M+H = 506.1. HPLC: t_R 4.38 min (method D).

Example 4: (S)-2-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



To a mixture of imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 75 mg, 0.203 mmol) in DMF (2 mL) was added (S)-2-methyl-pyrrolidine-2-carboxylic acid amide (**Intermediate F**, 39 mg, 0.305 mmol) and triethylamine (0.085 mL, 0.609 mmol). The RM was stirred for at 40 °C 17 h and then directly purified with preparative HPLC (method B). The product containing fractions were eluted through a 300 mg Bond Elut - SCX cartridge. The cartridge was then eluted with 7 M ammonia-solution in MeOH and evaporated to give the title compound as a yellow solid MS: M+H = 430.2. HPLC: t_R 3.80 min (method D).

Example 5: (R)-2-Methoxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

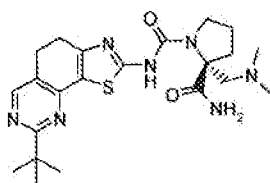


A mixture of imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 75 mg, 0.203 mmol), (R)-2-methoxymethyl-

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pyrrolidine-2-carboxylic acid amide (**Intermediate G**, 35.3 mg, 0.223 mmol) and triethylamine (0.085 mL, 0.609 mmol) in DMF (2 mL) was stirred for 6 h at 40 °C. After cooling to rt, the RM was dissolved in MeOH (1 mL) and directly purified by preparative HPLC (method B). The product containing fractions were eluted through a 300 mg Bond Elut
 5 - SCX cartridge. The cartridge was then eluted with 7 M ammonia solution in MeOH and evaporated to give the title product as a yellow solid. MS: M+H = 460.1. HPLC: t_R 3.94 min (method D).

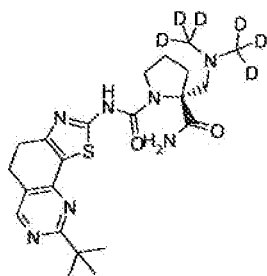
Example 6: (R)-2-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]
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Imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**, 132 mg, 0.372 mmol) was added to a mixture of (R)-2-dimethylaminomethyl-pyrrolidine-2-carboxylic acid amide (**Intermediate H**, 70 mg, 0.409
 15 mmol) and triethylamine (0.155 mL, 1.115 mmol) in DMF (1 mL) at rt. After 18 hours MeOH (0.5 mL) was added, the RM filtered through a PTFE membrane filter and purified by preparative HPLC (method B). The product containing fractions were combined and evaporated to remove CH₃CN and then basified by the addition of solid NaHCO₃ to give a yellow white precipitate. After cooling to 4 °C, the solid was recollected by filtration and then
 20 further purified by preparative HPLC (method C). The product containing fractions were eluted through a 300 mg Bond Elut - SCX cartridge. The cartridge was then eluted with a 7 M ammonia in MeOH solution and evaporated to give the title product as a yellow amorphous glass. LCMS: t_R 0.98 min and M+H = 458.1 (method A3). ¹H-NMR (CD₃OD, 400 MHz): 8.28 (s, 1H); 3.92 – 3.82 (m, 1H); 3.67 – 3.44 (m, 2H); 3.04 – 2.88 (m, 4H); 2.85 –
 25 2.71 (m, 1H); 2.56 (s, br, 6H); 2.29 – 2.19 (m, 1H); 2.08 – 1.87 (m, 3H); 1.37 (s, 9H).

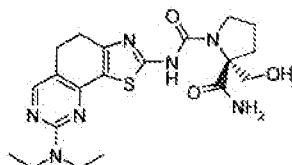
Example 7: d₆-(R)-2-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

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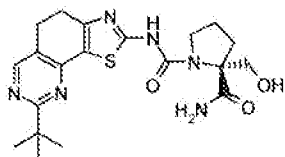
Imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**, 91 mg, 0.256 mmol) and triethylamine (0.036 mL, 0.256 mmol) were added to d₅-(R)-2-dimethylaminomethyl-pyrrolidine-2-carboxylic acid amide (**Intermediate I**, 50 mg, 0.282 mmol) suspended in DMF (2 mL) under an argon atm. The RM was then stirred for 2.75 h at 40 °C and then purified directly by preparative HPLC twice (method B and then method C). Each time, fractions containing the product were combined and eluted through a 300 mg Bond Elut - SCX cartridge. The cartridge was then eluted with a 7 M ammonia in MeOH solution and evaporated to give a yellow solid. The solid was suspended in DCM, filtered and dried in vacuo to give the title compound as a yellow solid. LCMS: t_R 1.21 min and M+H = 464.0 (method A1).

Example 8: (R)-2-Hydroxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



Imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 50 mg, 0.135 mmol) was added to a mixture of (R)-2-hydroxymethyl-pyrrolidine-2-carboxylic acid amide (**Intermediate J**, 25.4 mg, 0.176 mmol) and triethylamine (0.047 mL, 0.338 mmol) in DMF (1 mL) at rt. After 18 h at rt the RM was filtered through a PTFE membrane and purified by preparative HPLC (method B). Product containing fractions were combined and filtered through a Bond Elut - SCX cartridge. The cartridge was then eluted with a 7 M ammonia in MeOH solution and evaporated to give an orange glass which was recrystallized from MeOH / water to afford the title compound as a yellow solid. LCMS: t_R 1.09 min and M+H = 446.0 (method A3).

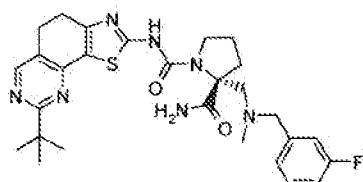
Example 9: (R)-2-Hydroxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



Imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide

5 **(Intermediate A**, 124 mg, 0.350 mmol) was added to a mixture of (R)-2-hydroxymethyl-pyrrolidine-2-carboxylic acid amide (**Intermediate J**, 63 mg, 0.350 mmol) and triethylamine (0.049 mL, 0.350 mmol) in DMF (2 mL). The RM was stirred for 2 h at 40 °C then stood for 18 h at rt and then directly purified by preparative HPLC (method B). The product containing
10 fractions were eluted through a 300 mg Bond Elut - SCX cartridge. The cartridge was then eluted with 7 M solution of ammonia in MeOH and evaporated to give a yellow solid. The isolated solid was then suspended in DCM, filtered and dried to give title compound as a pale yellow / white solid. LCMS: t_R 1.31 min and $M+H = 430.9$ (method A1).

Example 10: (R)-2-[(3-Fluoro-benzyl)-methyl-amino]-methyl-pyrrolidine-1,2-dicarboxylic
15 acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

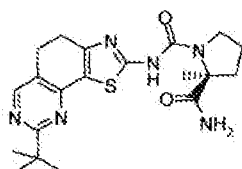


Imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide

(Intermediate A, 40.1 mg, 0.113 mmol) and triethylamine (0.047 mL, 0.339 mmol) were added to (R)-2-[(3-fluoro-benzyl)-methyl-amino]-methyl-pyrrolidine-2-carboxylic acid amide
20 **(Intermediate K**, 30 mg, 0.113 mmol) suspended in DMF (1 mL) at rt under argon. The RM was stirred for 2.5 h at 40 °C and then directly purified by preparative HPLC (method C). The product containing fractions were eluted through a Bond Elut - SCX, 300 mg cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH and evaporated to give a yellow solid. The solid was triturated with DCM, filtered and dried to give the title compound
25 as a pale yellow solid. LCMS: t_R 0.81 min and $M+H = 552.3$ (method A2).

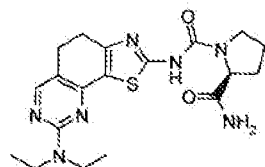
Example 11: (S)-2-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

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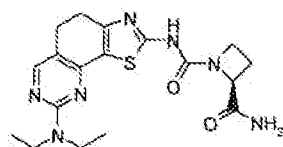
(S)-2-Methyl-pyrrolidine-2-carboxylic acid amide (**Intermediate F**, 22.14 mg, 0.169 mmol) and triethylamine (0.035 mL, 0.254 mmol) were added to imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**, 30 mg, 0.085 mmol) in DMF (1 mL) under argon at rt. After stirring 16 h at rt the RM was directly purified by preparative HPLC (method B). The product containing fractions were eluted through a 300 mg Bond Elut - SCX cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH and evaporated to give the title compound as a yellow crystalline solid. MS: M+H = 415.1. HPLC: t_R 3.73 min (method D).

Example 12: (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



(S)-Pyrrolidine-2-carboxylic acid amide (148 mg, 1.299 mmol) and triethylamine (0.272 mL, 1.949 mmol) were added to imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 240 mg, 0.65 mmol) and (DMF (1 mL) under argon at rt. The RM was stirred for 15.5 h at rt and then purified with preparative HPLC (method C). Product containing fractions were combined and filtered through a Bond Elut - SCX cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH and evaporated to give the title compound as orange crystals. MS: M+H = 416.1. HPLC: t_R 3.56 min (method D).

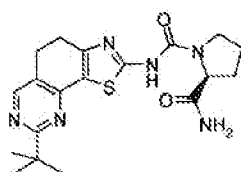
Example 13: (S)-Azetidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



A mixture of (S)-azetidine-2-carboxylic acid amide (**Intermediate L**, 26.4 mg, 0.264 mmol), imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide

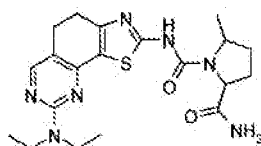
(**Intermediate C**, 75 mg, 0.203 mmol) and triethylamine (0.085 mL, 0.609 mmol) in DMF (2 mL) was stirred at 40 °C for 2 h. The RM was then purified directly by preparative HPLC (method B). The product containing fractions were eluted through a 300 mg Bond Elut - SCX cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH and
 5 evaporated to give the title compound as a white crystalline solid. MS: M+H = 402.1. HPLC: t_R 3.56 min (method D).

Example 14: (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



(S)-Pyrrolidine-2-carboxylic acid amide (16 mg, 0.14 mmol) was added to a mixture of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**, 45 mg, 0.127 mmol) and triethylamine (0.053 mL, 0.381 mmol) in DMF (1 mL) at rt. The RM was stood overnight at rt, then evaporated and the residue crystallized
 15 from MeOH and water. The title compound was collected by filtration. MS: M+H = 401.1 (MS-ESI). ¹H-NMR (CD₃OD, 400 MHz): 8.31 (s, 1H), 4.46 (d, 1H), 3.77 – 3.54 (m, 2H), 3.08 – 2.90 (m, 4H), 2.33 – 2.23 (m, 1H), 2.13 – 2.00 (m, 3H), 1.38 (s, 9H).

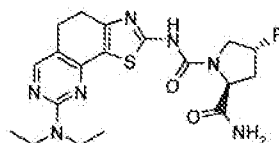
Example 15: 5-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



To a suspension of imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 75 mg, 0.203 mmol) in DMF (2 mL) was added 5-methyl-pyrrolidine-2-carboxylic acid amide (prepared as described in *Arch. Pharm.*, **1936**,
 25 274, 40; 39 mg, 0.305 mmol) and triethylamine (0.085 mL, 0.609 mmol). The RM was stirred at 40 °C for 2.5 h. and then purified directly by preparative HPLC (method B). The product containing fractions were eluted through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH, evaporated and the residue triturated

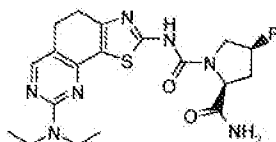
with DCM to give the title compound as a yellow solid. LCMS: t_R 0.77 min and $M+H = 430.0$ (method A2).

Example 16: (2S,4R)-4-Fluoro-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



To imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 75 mg, 0.203 mmol) in DCM (2 mL) was added (2S,4R)-4-fluoro-pyrrolidine-2-carboxylic acid amide (**Intermediate M**, 40.2 mg, 0.305 mmol) and triethylamine (0.085 mL, 0.609 mmol). The RM was stirred at 40 °C for 17.5 h and then directly purified by preparative HPLC (method B). The product containing fractions were eluted through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH and evaporated to give the title compound as a yellow solid. HPLC: t_R 3.66 min (method D). MS: $M+H = 434.1$.

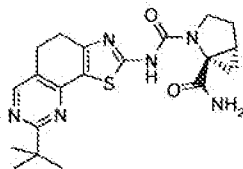
Example 17: (2S,4S)-4-Fluoro-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



To imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 17 mg, 0.046 mmol) in DMF (0.4 mL) was added (2S,4S)-4-fluoro-pyrrolidine-2-carboxylic acid amide (**Intermediate N**, 6.08 mg, 0.046 mmol) and triethylamine (0.019 mL, 0.138 mmol). The RM was stirred at 40 °C for 17 h and then directly purified by preparative HPLC (method B). The product containing fractions were eluted through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH and evaporated to give the title compound as a yellow solid. HPLC: t_R 3.55 min (method D). MS: $M+H = 434.1$.

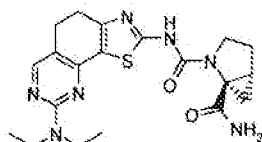
Example 18: (1S,5R)-2-Aza-bicyclo[3.1.0]hexane-1,2-dicarboxylic acid 1-amide 2-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

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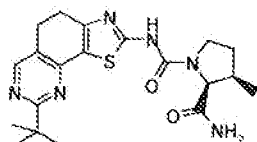
(1S,5R)-2-Aza-bicyclo[3.1.0]hexane-1-carboxylic acid amide (**Intermediate O**, 208 mg, 1.65 mmol) was added to a stirred mixture of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**, 532 mg, 1.5 mmol) and triethylamine (0.627 mL, 4.50 mmol) in DMF (4 mL) at rt. After 56 and 80 hours additional portions of (1S,5R)-2-aza-bicyclo[3.1.0]hexane-1-carboxylic acid amide (**Intermediate O**, 104 mg, 0.825 mmol) were added and the RM stood 18 h at rt. The RM was then filtered through a PTFE membrane and purified by preparative HPLC (method B). The product containing fractions were eluted through a Bond Elut – SCX cartridge. The cartridge was then eluted with 7 M ammonia-solution in MeOH and evaporated. The residue was purified for a second time by preparative HPLC (method B) and isolated in the same manner. The crude product was then recrystallised from a 1:1 mixtre of water / MeOH to give the title compound as a yellow crystalline solid. LCMS: t_R 1.22 min and $M+H = 413.1$ (method A3). ^1H -NMR (d_6 -DMSO, 400 MHz): 11.22 (s, 1H), 8.40 (s, 1H); 7.33 (s, br, 1H); 7.05 (s, br, 1H); 3.97 – 3.84 (m, 1H); 3.64 – 3.52 (m, 1H); 3.01– 2.83 (m, 4H); 2.28 – 2.13 (m, 1H); 1.95 – 1.74 (m, 3H); 1.33 (s, 9H); 1.04 – 0.97 (m, 1H)).

Example 19: (1S,5R)-2-Aza-bicyclo[3.1.0]hexane-1,2-dicarboxylic acid 1-amide 2-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



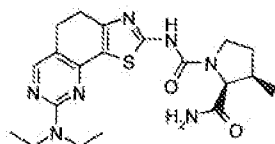
To imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 75 mg, 0.203 mmol) in DCM (2 mL) was added (1S,5R)-2-aza-bicyclo[3.1.0]hexane-1-carboxylic acid amide (**Intermediate O**, 38.4 mg, 0.305 mmol) and triethylamine (0.085 mL, 0.609 mmol). The RM was stirred at 40 °C for 17.5 h and then directly purified by preparative HPLC (method B). The product containing fractions were eluted through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH and evaporated to give the title compound as a yellow solid. HPLC: t_R 3.73 min (method D). MS: $M+H = 428.1$.

Example 20: (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



To imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide
 5 **(Intermediate A**, 60 mg, 0.169 mmol) in DMF (1 mL) was added (2S,3R)-3-methyl-pyrrolidine-2-carboxylic acid amide **(Intermediate P**, 32.5 mg, 0.254 mmol) and triethylamine (0.071 mL, 0.508 mmol). The RM was stirred under argon for 2 h at rt and then directly purified by preparative HPLC (method B). The CH₃CN was evaporated from the product containing fractions and the remaining liquid then eluted through a Bond Elut – SCX
 10 cartridge. The cartridge was then eluted with a 7 M solution of ammonia in MeOH and evaporated. The residue was triturated in methanol and the title compound collected as white crystals following filtration and drying. HPLC: *t_R* 3.66 (method D). MS: M+H = 415.1. ¹H-NMR (CD₃OD, 600 MHz): 7.96 (s, 1H), 4.04 (d, 1H), 3.47 (t, 1H), 3.18 (q, 1H), 2.71 – 2.67 (m, 2H), 2.64 – 2.58 (m, 2H), 2.24 – 2.15 (m, 1H), 1.76 (s, br, 1H), 1.53 (s, br, 1H), 1.03
 15 (s, 9H), 0.78 (d, 3H).

Example 21: (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

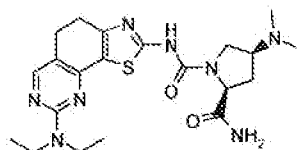


To imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide **(Intermediate C**, 82 mg, 0.222 mmol) in DMF (1.5 mL) was added under argon (2S,3R)-3-methyl-pyrrolidine-2-carboxylic acid amide **(Intermediate P**, 42.7 mg, 0.333 mmol) and triethylamine (0.093 mL, 0.666 mmol). The RM was stirred at rt for 2 h and then directly purified by preparative HPLC (method B). The CH₃CN was removed under vacuum
 25 from the product containing fractions and the remaining solution then eluted through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M ammonia solution in MeOH, evaporated and the residue triturated with methanol to give the title compound as a white crystalline solid. HPLC: *t_R* 3.74 (method D). MS: M+H = 430.1. ¹H-NMR (CD₃OD, 600 MHz):

- 70 -

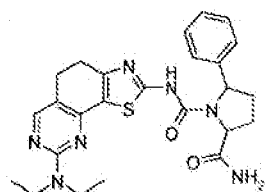
7.62 (s, 1H), 4.03 (d, 1H), 3.46 (t, 1H), 3.26 (q, 4H), 3.17 (q, 1H), 2.54 (s, 4H), 2.24 – 2.15 (m, 1H), 1.80 – 1.72 (m, 1H), 1.58 – 1.47 (m, 1H), 0.83 (t, 6H), 0.77 (d, 3H).

Example 22: (2S,4S)-4-Dimethylamino-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



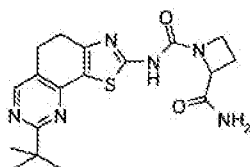
To imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 60 mg, 0.162 mmol) in DMF (1.5 mL) was added (2S,4S)-4-dimethylamino-pyrrolidine-2-carboxylic acid amide (**Intermediate Q**, 28.1 mg, 0.179 mmol) and triethylamine (0.068 mL, 0.487 mmol). The RM was stirred at 40 °C for 17 h and then directly purified by preparative HPLC (method B). The product containing fractions were passed through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M ammonia solution in MeOH. The solution was evaporated to give the title compound. HPLC: t_R 3.23 min (method D). MS: $M+H = 459.1$.

Example 23: 5-Phenyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



To a suspension of imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 65 mg, 0.176 mmol) in DMF (2 mL) were added 5-phenyl-pyrrolidine-2-carboxylic acid amide (prepared as described in patent US 3164597, Example 42, 50.2 mg, 0.264 mmol) and triethylamine (0.074 mL, 0.528 mmol). The RM was stirred at 40 °C for 2 h and then directly purified by preparative HPLC (method B). The product containing fractions were passed through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M ammonia solution in MeOH. The solution was evaporated and the residue was triturated in DCM to give the title compound as a yellow solid. LCMS: t_R 0.99 min and $M+H = 492.0$ (method A2).

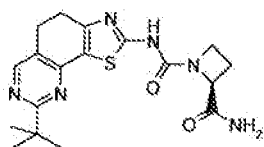
Example 24: Azetidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



To a solution of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-

- 5 h]quinazolin-2-yl)-amide (**Intermediate A**, 100 mg, 0.282 mmol) in DMF (2 mL) were added azetidine-2-carboxylic acid amide (56.5 mg, 0.564 mmol) and triethylamine (0.118 mL, 0.846 mmol). The RM was stirred under argon atm. at rt for 16 h and then directly purified by preparative HPLC (method B). The product containing fractions were passed through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M ammonia solution in MeOH.
- 10 The solution was evaporated and the residue purified by flash chromatography on silica gel, eluting with DCM / MeOH 95:5 containing 0.5 % 880 HN₃ to give the title compound as a yellow solid. HPLC: t_R 3.55 min (method D). MS: M+H = 387.1.

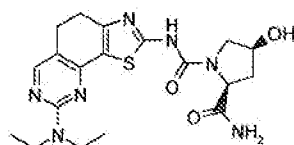
Example 25: (S)-Azetidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



- (S)-Azetidine-2-carboxylic acid amide (**Intermediate L**, 68.1 mg, 0.680 mmol) was added to a stirred mixture of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**, 241 mg, 0.680 mmol) and triethylamine (0.284
- 20 mL, 2.040 mmol) in DMF (3 mL) at rt. The RM was allowed to stand 66 h at rt and then directly purified by preparative HPLC (method B). The product containing fractions were evaporated to remove the CH₃CN and then basified with NaHCO₃. The aqueous phase was then extracted 4X with 10 % MeOH in DCM, the combined organic layers dried over Na₂SO₄ and evaporated to give a solid which was triturated with MeOH / water to give the title
- 25 compound as an off-white crystalline solid. LCMS: t_R 1.08 min and M+H = 386.9 (method A3).

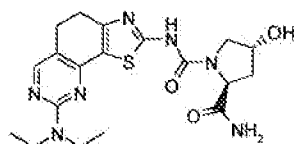
Example 26: (2S,4S)-4-Hydroxy-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

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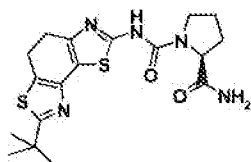
To a suspension of imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 75 mg, 0.203 mmol) in DMF (2 mL) were added (2S,4S)-4-hydroxy-pyrrolidine-2-carboxylic acid amide (**Intermediate R**, 39.6 mg, 0.305 mmol) and triethylamine (0.113 mL, 0.812 mmol). The RM was stirred at 40 °C for 2.5 h and then directly purified by preparative HPLC (method B). The product containing fractions were passed through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M ammonia solution in MeOH. The solution was evaporated to give the title compound as a yellow solid. HPLC: t_R 3.44 min (method D). MS: $M+H = 432.1$.

Example 27: (2S,4R)-4-Hydroxy-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



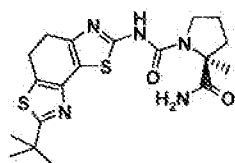
To a suspension of imidazole-1-carboxylic acid (8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate C**, 75 mg, 0.203 mmol) in DMF (2 mL) were added (2S,4R)-4-hydroxy-pyrrolidine-2-carboxylic acid amide (**Intermediate S**, 39.6 mg, 0.305 mmol) and triethylamine (0.113 mL, 0.812 mmol). The RM was stirred at 40 °C for 4.5 h and then directly purified by preparative HPLC (method B). The product containing fractions were passed through a Bond Elut – SCX cartridge. The cartridge was then eluted with a 7 M ammonia solution in MeOH. The solution was evaporated and the residue was recrystallised from DCM to give the title compound as a yellow solid. HPLC: t_R 3.31 min (method D). MS: $M+H = 432.1$.

Example 28: (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]



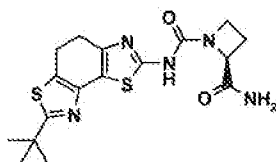
L-Prolinamide (59.4 mg, 0.521 mmol) and TEA (0.121 mL, 0.868 mmol) were added to imidazole-1-carboxylic acid (7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide (**Intermediate T**, 156 mg, 0.434 mmol) in DMF (1 mL). The RM was stirred at rt for 10 min. The mixture was extracted with EtOAc/H₂O. The organic layer was dried over Na₂SO₄ and evaporated. The crude material was chromatographed with MPLC C18 H₂O 0.1% TFA / CH₃CN 0.1% TFA, gradient 0-50%. Product containing fractions were neutralized with NaHCO₃, extracted with EtOAc and lyophilized from dioxane to give the title compound as a white solid (M+H = 406; M-H = 404; t_R 4.75 min (Method F). ¹H-NMR (d₆-DMSO, 600.13 MHz) 10.80 (s, 1H) 7.40 (s, 1H), 6.98 (s, 1H), 4.30 (s, 1H) 3.65-3.55 (m 1H), 3.48-3.38 (m, 1H), 3.10 (t, 2H), 2.94 (t, 2H), 2.14-2.05 (m, 1H), 1.95-1.78 (m, 3H), 1.40 (s, 9H)).

Example 29: (S)-2-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]



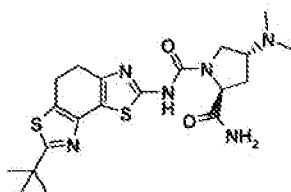
(S)-2-Methyl-pyrrolidine-2-carboxylic acid amide (**Intermediate F**, 7.7 mg, 0.060 mmol) and TEA (0.014 mL, 0.100 mmol) were added to imidazole-1-carboxylic acid (7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide (**Intermediate T**, 18 mg, 0.050 mmol) in DMF (3 mL). The RM was stirred at rt for 10 min, then extracted with EtOAc/H₂O. The organic layer was dried over Na₂SO₄ and evaporated. The crude material was chromatographed with MPLC C18 H₂O 0.1% TFA / CH₃CN 0.1% TFA, gradient 0-50%. Product containing fractions were neutralized with NaHCO₃, extracted with EtOAc and lyophilized from dioxane to give the title compound as a white solid (M+H = 420; M-H = 418; t_R 4.875 min (Method F). ¹H-NMR (d₆-DMSO, 600.13 MHz) 10.60 (s, br, 1H) 7.68 (s, 1H), 6.86 (s, 1H), 3.68-3.60 (m 1H), 3.60-3.50 (m 1H), 3.08 (t, 2H), 2.90 (t, 2H), 2.10-1.98 (m, 1H), 1.90-1.68 (m, 3H), 1.48 (s, 3H), 1.37 (s, 9H)).

Example 30: (S)-Azetidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]



(S)-Azetidine-2-carboxylic acid amide (**Intermediate L**, 37.1 mg, 0.370 mmol) and TEA (0.14 mL, 1.010 mmol) were added to imidazole-1-carboxylic acid (7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide (**Intermediate T**, 121 mg, 0.337 mmol) in DMF (2 mL). The RM was stirred for 5 min and then stood for 2 days at rt. The RM was filtered and
 5 directly purified by Prep.HPLC (method E). Product containing fractions were combined and filtered through a Bond Elute - SCX, 300 mg cartridge. The cartridge was then washed with 7 M ammonia-solution in MeOH (2 ml). The filtrate was evaporated to give the title compound as a white solid (M+H = 391.8; M-H = 389.8; t_R 1.84 min (Method A3). ^1H -NMR (d_6 -DMSO, 400 MHz) 11.12 (s, 1H), 7.52 (s, 1H), 7.30 (s, 1H), 4.74-4.62 (m, 1H), 3.92 (t,
 10 2H), 3.08 (t, 2H), 2.90 (t, 2H), 2.46-2.36 (m, 1H), 2.18-2.02 (m, 1H), 1.39 (s, 9H)).

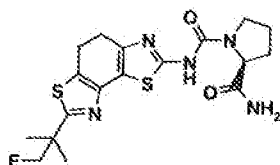
Example 31: (2S,4R)-4-Dimethylamino-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]



(2S,4R)-4-Dimethylamino-pyrrolidine-2-carboxylic acid amide (**Intermediate B**, 9.33 mg, 0.059 mmol) and TEA (0.019 ml, 0.135 mmol) were added to imidazole-1-carboxylic acid (7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide (**Intermediate T**, 19.4 mg, 0.054 mmol) in DMF (1 ml). The RM was stirred for 5 min and then stood overnight at rt. The RM was filtered and directly purified by Prep.HPLC (method E). Fractions containing the
 20 product were combined and filtered through a Bond Elute - SCX, 300 mg cartridge. The cartridge was then washed with 7 M ammonia-solution in MeOH. The filtrate was evaporated to give the title compound as a white solid (M+H = 449.1; M-H = 447.3; t_R 1.47 min (Method A3). ^1H -NMR (d_6 -DMSO, 400 MHz) 7.40 (s, 1H), 7.00 (s, 1H), 4.44-4.25 (m, 1H), 3.86-3.76 (m 1H), 3.35-3.25 (m 1H), 3.08 (t, 2H), 2.92 (t, 2H), 2.96-2.78 (m, 1H), 1.18 (s, br, 6H),
 25 2.08-1.85 (m, 2H), 1.38 (s, 9H)).

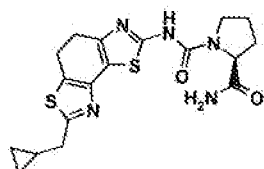
Example 32: (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-(2-fluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]

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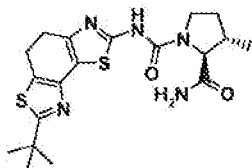
L-Prolinamide (23.98 mg, 0.210 mmol) and TEA (0.080 mL, 0.573 mmol) were added to imidazole-1-carboxylic acid [7-(2-fluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl]-amide (**Intermediate U**, 72.1 mg, 0.191 mmol) in DMF (1 mL). The RM was stirred for 5 min and then stood overnight at rt. The RM was evaporated and then triturated with MeOH (2 mL) and H₂O (1 mL). The mixture was cooled to 4 °C. The suspension was filtered and washed with cold MeOH/H₂O=2:1 and the solid dried under HV at 40 °C to give the title compound as a white solid (M+H = 424.0; M-H = 422.1; t_R 1.7 min (Method A3). ¹H-NMR (d₆-DMSO, 400MHz) 7.19 (s, 1H), 6.96 (s, 1H), 4.58 (s, 1H) 4.44 (s, 1H), 4.28 (s, br, 1H) 3.64-3.50 (m 1H), 3.48-3.38 (m 1H), 3.10 (t, 2H), 2.92 (t, 2H), 2.18-1.98 (m, 1H), 1.94-1.78 (m, 3H), 1.38 (s, 6H) ¹⁹F-NMR (d₆-DMSO, 600.13 MHz) 219ppm (t, 1F)).

Example 33: (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-cyclopropylmethyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]



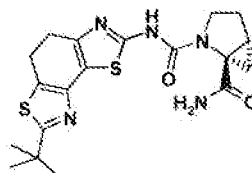
L-Prolinamide (16.7 mg, 0.147 mmol) and TEA (0.027 mL, 0.196 mmol) were added to imidazole-1-carboxylic acid (7-cyclopropylmethyl-4,5-dihydro-benzo [1,2-;3,4-d']bisthiazol-2-yl)-amide (**Intermediate V**, 35 mg, 0.098 mmol) in DMF (1.5 mL). The RM was stirred at rt for 15 min and then extracted with EtOAc/H₂O. The organic layer was dried over Na₂SO₄ and evaporated. The crude material was chromatographed with MPLC C18 H₂O 0.1% TFA / CH₃CN 0.1% TFA, gradient 0-50%. Product containing fractions were neutralized with NaHCO₃, extracted with EtOAc and lyophilized from dioxane to give the title compound as a white solid (M+H = 404; M-H = 402; t_R 4.49 min (Method F). ¹H-NMR (d₆-DMSO, 600.13 MHz) 10.78 (s, 1H) 7.40 (s, 1H), 6.98 (s, 1H), 4.28 (s, br, 1H) 3.65-3.55 (m, 1H), 3.48-3.38 (m, 1H), 3.08 (t, 2H), 2.97 (t, 2H), 2.84 (d, 2H), 2.14-2.04 (m, 1H), 1.93-1.72 (m, 3H), 1.10 (q, 1H), 0.60-0.50 (m, 2H), 0.40-0.38 (m, 2H)).

Example 34: (2S,3S)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]



(2S,3S)-3-Methyl-pyrrolidine-2-carboxylic acid amide (**Intermediate D**, 13.37 mg, 0.104 mmol) and TEA (0.039 mL, 0.278 mmol) were added to imidazole-1-carboxylic acid (7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide (**Intermediate T**, 25 mg, 0.070 mmol) in DMF (2 mL). The RM was stirred at rt for 10 min and then extracted with EtOAc/H₂O. The organic layer was dried over Na₂SO₄ and evaporated. The crude material was lyophilized from dioxane without further purification to give the title compound as a white solid (M+H = 420.1; M-H = 418.1; t_R 4.85 min (Method F). ¹H-NMR (d₆-DMSO, 600.13 MHz) 10.78 (s, br, 1H) 7.44 (s, br, 1H), 6.97 (s, br, 1H), 3.90-3.70 (m, 1H) 3.62-3.42 (m, 2H), 3.08 (t, 2H), 2.94 (t, 2H), 2.22-2.12 (m, 1H), 2.06-1.94 (m, 1H), 1.58-1.48 (m, 1H), 1.39 (s, 9H), 1.04 (d, 3H)).

Example 35: (1S,5R)-2-Aza-bicyclo[3.1.0]hexane-1,2-dicarboxylic acid 1-amide 2-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]

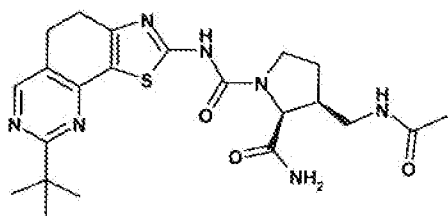


The title compound was synthesized in a similar manner as described for **Example 34** using **intermediate O** instead of **intermediate D**.

(M+H = 418.0; M-H = 416.1; t_R 4.90 min (Method F). ¹H-NMR (d₆-DMSO, 600.13 MHz) 10.78 (s, br, 1H) 7.40 (s, br, 1H), 7.06 (s, br, 1H), 3.92-3.82 (m, 1H) 3.62-3.52 (m, 1H), 3.08 (t, 2H), 2.94 (t, 2H), 2.26-2.14 (m, 1H), 1.92-1.72 (m, 3H), 1.39 (s, 9H), 1.05-0.95 (m, 1H)).

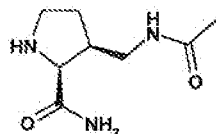
Example 36: (2S,3S)-3-(Acetylamino-methyl)-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

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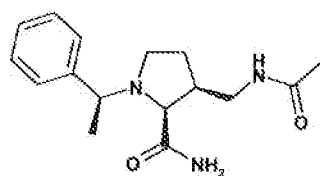
Triethylamine (0.339 mmol) was added to a solution of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**) (0.113 mmol) and (2S,3S)-3-(acetamino-methyl)-pyrrolidine-2-carboxylic acid amide (**Stage 36.1**) (0.135 mmol) at rt. After stirring for 85 min, the reaction mixture was concentrated. The residue was purified by silica gel column chromatography followed by trituration with Et₂O to afford the title compound as a yellow solid. HPLC: t_R = 4.20 min (method H); LCMS: t_R = 1.48 min, [M+H]⁺ 472 (method I); TLC: R_f = 0.14 (9:1 CH₂Cl₂/MeOH); ¹H-NMR (d₆-DMSO, 600 MHz): 11.15 (br s, 1H), 8.41 (s, 1H), 7.81 (br s, 1H), 7.53 (br s, 1H), 7.13 (br s, 1H), 4.28 (m, 1H), 3.74 (m, 1H), 3.40 (m, 1H), 3.25 (m, 1H), 2.98 (m, 2H), 2.93 (m, 3H), 2.42 (m, 1H), 2.02 (m, 1H), 1.82 (s, 3H), 1.74 (m, 1H), 1.33 (s, 9H).

Stage 36.1: (2S,3S)-3-(Acetylamino-methyl)-pyrrolidine-2-carboxylic acid amide



A mixture of (2S,3S)-3-(acetamino-methyl)-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide (**Stage 36.2**) (0.48 mmol) and 10% Pd on charcoal, wet with 50% H₂O (Aldrich 330108) (0.096 mmol) in MeOH (5 mL) was hydrogenated for 6.5 h at rt. The reaction mixture was then filtered through a Fluoropore Membrane Filter (0.2 μm FG) and evaporated. The residue was dissolved in CH₂Cl₂ and evaporated to dryness to afford the title compound as an off-white solid. ESI-MS: [M+H]⁺ 186; TLC: R_f = 0.08 (200:20:1 CH₂Cl₂/MeOH/conc. NH₄OH).

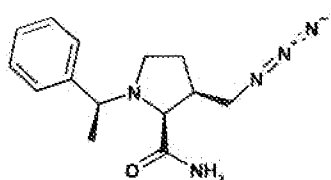
Stage 36.2: (2S,3S)-3-(Acetylamino-methyl)-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide



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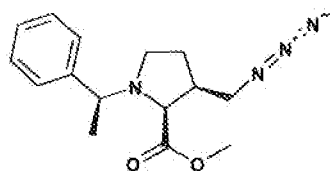
Thioacetic acid (2.312 mmol) was added to (2S,3S)-3-azidomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide (**Stage 36.3**) (0.578 mmol) at rt with the formation of nitrogen gas. After stirring for 16 h, the reaction mixture was diluted with Et₂O, the solids were removed by filtration and the filtrate was concentrated. The residue was purified by silica gel column chromatography to afford the title compound as a light yellow oil (thiol odor). HPLC: t_R = 3.71 min (method H); LC-MS: t_R = 0.64 min, [M+H]⁺ 290 (method I); TLC: R_f ≈ 0.38 (200:20:1 CH₂Cl₂/MeOH/conc. NH₄OH).

Stage 36.3: (2S,3S)-3-Azidomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide



Trimethylaluminum in toluene (2 M, 15.95 mmol) was added dropwise to a mixture of NH₄Cl (15.95 mmol) in toluene (2 mL) at 0 °C with the formation of methane gas. The reaction mixture was allowed to warm to rt, stirred for a further 15 min and then slowly treated with a solution of (2S,3S)-3-azidomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester (**Stage 36.4**) (7.98 mmol) in toluene (8 mL). Additional reagent prepared from NH₄Cl (15.95 mmol) in toluene (2 mL) and trimethylaluminum in toluene (2 M, 15.95 mmol) at 0 °C was added after 18 h. After stirring for 44 h, the mixture was cooled to 0 °C, quenched with 1M HCl and then washed with CH₂Cl₂ (3X). The aqueous phase was basified with a 1:1 saturated solution of NaHCO₃/saturated solution of Rochelle's salt and extracted with THF (10X). The combined organic layers were dried (Na₂SO₄), filtered and concentrated. The residue was purified using a RediSep[®] silica gel column to afford the title compound as a yellow oil. HPLC: t_R = 2.50 min (method G); ESI-MS: [M+H]⁺ 274; TLC: R_f = 0.26 (3:1 Hex/EtOAc).

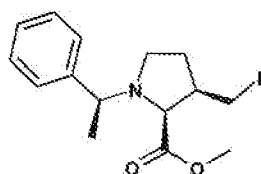
Stage 36.4: (2S,3S)-3-Azidomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester



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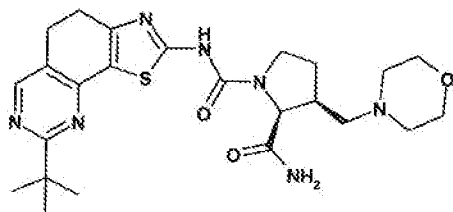
Sodium azide (5.34 mmol) was added to a solution of (2S,3R)-3-iodomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester (**Stage 36.5**) (3.56 mmol) in DMF (30 mL) at rt. After 18 h, the reaction mixture was poured onto water and extracted with MTBE (2X). The combined organic phases were washed with brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified using a RediSep[®] silica gel column to afford the title compound as a brown oil. HPLC: t_R = 3.26 min (method G); ESI-MS: $[\text{M}+\text{H}]^+$ 289.

Stage 36.5: (2S,3R)-3-iodomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester



A solution of [but-3-enyl-((S)-1-phenyl-ethyl)-amino]-acetic acid methyl ester [432555-77-6] (20.22 mmol) in THF (10 mL) was slowly added to a solution of lithium diisopropylamide (24.26 mmol) in 1:2 hexanes/THF (30 mL) at -78°C . The reaction mixture was warmed to 0°C , stirred for 1 h and then re-cooled to -78°C . A solution of zinc bromide (50.5 mmol) in Et_2O (40 mL) was added and the reaction mixture was then warmed to rt. After stirring for 1 h, the mixture was cooled to 0°C and iodine (22.24 mmol) was added in portions. The reaction mixture was stirred at 0°C for 2 h and at rt for another 2 h, diluted with Et_2O and then successively washed with a saturated solution of $\text{Na}_2\text{S}_2\text{O}_3$ and a saturated solution of NH_4Cl . The aqueous layers were each back-extracted with Et_2O . The combined organic phases were dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography to afford the title compound as a red oil. HPLC: t_R = 3.46 min (method G); ESI-MS: $[\text{M}+\text{H}]^+$ 374.

Example 37: (2S,3S)-3-Morpholin-4-ylmethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

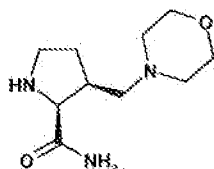


Triethylamine (0.423 mmol) was added to a solution of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**) (0.141 mmol) and

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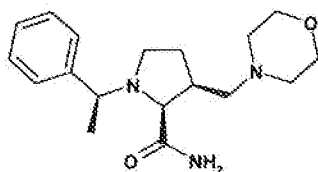
(2S,3S)-3-(acetylamino-methyl)-pyrrolidine-2-carboxylic acid amide (**Stage 37.1**) (0.155 mmol) in DMF (0.5 mL) at rt. After stirring for 3 h, the reaction mixture was concentrated. The residue was purified by silica gel column chromatography to afford the title compound as a white solid. HPLC: t_R = 4.04 min (method H); LCMS: t_R = 1.36 min, $[M+H]^+$ 500 (method I); TLC: R_f = 0.09 (19:1 $CH_2Cl_2/MeOH$); ^1H -NMR (d_6 -DMSO, 600 MHz): 11.15 (br s, 1H), 8.39 (m, 1H), 7.37 (br s, 1H), 7.05 (s, 1H), 4.27 (m, 1H), 3.70 (m, 1H), 3.59 (m, 4H), 3.42 (m, 1H), 2.97 (m, 2H), 2.90 (m, 2H), 2.58 (m, 1H), 2.38 (m, 4H), 2.35 (m, 1H), 2.17 (m, 1H), 2.02 (m, 1H), 1.75 (m, 1H), 1.32 (s, 9H).

10 Stage 37.1: (2S,3S)-3-Morpholin-4-ylmethyl-pyrrolidine-2-carboxylic acid amide



A mixture of (2S,3S)-3-morpholin-4-ylmethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide (**Stage 37.2**) (1.046 mmol) and 10% Pd on charcoal, wet with 50% H₂O (Aldrich 330108) (0.105 mmol) in MeOH (5 mL) was hydrogenated for 6.5 h at rt. The reaction mixture was then filtered through a Fluoropore Membrane Filter (0.2 μ m FG) and evaporated. The residue was dissolved in CH_2Cl_2 and evaporated to dryness to afford the title compound as colorless oil. ESI-MS: $[M+H]^+$ 214; TLC: R_f = 0.14 (200:20:1 $CH_2Cl_2/MeOH/conc. NH_4OH$).

20 Stage 37.2: (2S,3S)-3-Morpholin-4-ylmethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide

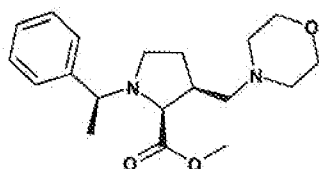


Trimethylaluminum in toluene (2 M, 2.89 mmol) was added dropwise to a mixture of NH₄Cl (2.89 mmol) in toluene (3 mL) at 0 °C with the formation of methane gas. The reaction mixture was allowed to warm to rt, stirred for a further 15 min and then slowly treated with a solution of (2S,3S)-3-morpholin-4-ylmethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester (**Stage 37.3**) (1.444 mmol) in toluene (9 mL). Additional reagent prepared

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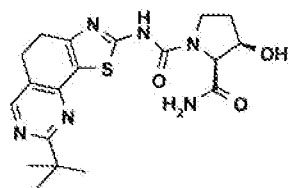
from NH_4Cl (2.89 mmol) in toluene (2 mL) and trimethylaluminum in toluene (2 M, 2.89 mmol) at 0°C was added after 18 h. After stirring for 60 h, the mixture was cooled to 0°C , quenched with 1M HCl and then washed with CH_2Cl_2 (3X). The aqueous phase was basified with a 1:1 saturated solution of NaHCO_3 /saturated solution of Rochelle's salt and extracted with THF (10X). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography to afford the title compound as a yellow oil. HPLC: $t_R = 2.18$ min (method G); ESI-MS: $[\text{M}+\text{H}]^+$ 318.

Stage 37.3: (2S,3S)-3-Morpholin-4-ylmethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester



A mixture of (2S,3R)-3-iodomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester (**Stage 36.5**) (7.07 mmol), K_2CO_3 (21.22 mmol) and morpholine (10.61 mmol) in CH_3CN (24 mL) was stirred at 50°C for 62 h. The reaction mixture was poured onto ice water and extracted with EtOAc (3X). The combined organic layers were successively washed with water and brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified using a RediSep[®] silica gel column to afford the title compound as a yellow oil. HPLC: $t_R = 2.56$ min (method G); ESI-MS: $[\text{M}+\text{H}]^+$ 333.

Example 38: (2S,3R)-3-Hydroxy-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]

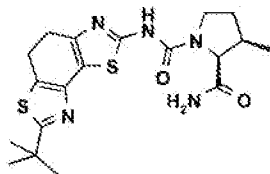


The title compound was synthesized using methodology as described for **Example 20** using (2S,3R)-3-hydroxy-pyrrolidine-2-carboxylic acid amide (H. Fukushima et al. Bioorg. Med. Chem. **2004**, 12, 6053; H. Ji et al. J. Med. Chem. **2006**, 49(21), 6254) instead of intermediate P.

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LCMS: $t_R = 1.45$ min, $M+H = 417.0$, $M-H = 415$ (method J). ^1H -NMR (d_6 -DMSO, 600.13 MHz): 12-11 (s, br, 1H) 8.4 (s, 1H), 7.2-6.9 (m, 2H), 5.2 (s, 1H), 4.4 (s, 1H), 4.25 (s, br, 1H), 3.6 (m, 1H); 3.45 (m, 1H), 2.95 (m, 2H), 2.9 (m, 2H), 2.0 (m, 1H), 1.9 (m, 1H), 1.3 (s, 9H).

- 5 **Example 39:** (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]

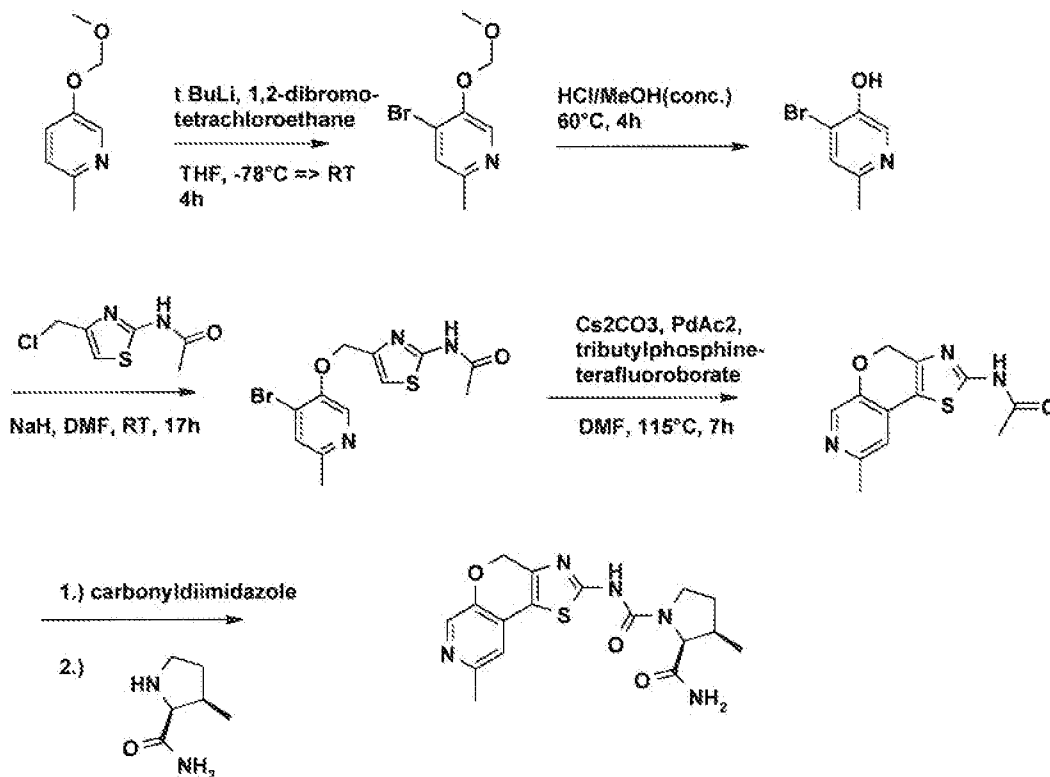


The title compound was synthesized using methodology as described for **Example 34** using (2S,3R)-3-methyl-pyrrolidine-2-carboxylic acid amide (**intermediate P**) instead of

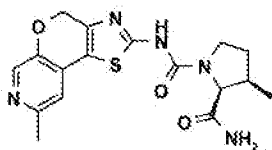
- 10 **intermediate D.**

LCMS: $t_R = 1.32$ min, $M+H = 420.0$, $M-H = 418.1$ (method J). ^1H -NMR (d_6 -DMSO, 400 MHz): 10.65 (s, br, 1H) 7.39 (s, br, 1H), 6.99 (s, br, 1H), 4.2 (m, 1H), 3.67 (t, 1H), 3.3 (m, 1H), 3.08 (t, 2H), 2.93 (t, 2H), 2.38 (m, 1H), 1.95 (m, 1H), 1.7 (m, 1H), 1.39 (s, 9H), 0.97 (d 3H).

- 15 **Synthesis scheme:**



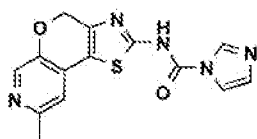
Example 40: (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-methyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide]



- 5 The title compound was prepared starting from imidazole-1-carboxylic acid (8-methyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide (**Stage 40.1**) using synthetic methodology described in the preparation of Example 20.

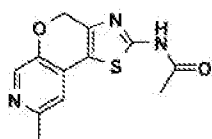
LCMS: $t_R = 0.29$ min, $M+H = 374.0$, $M-H = 372$ (method J). ^1H -NMR ^1H -NMR (CD_3OD , 400 MHz): 7.9 (s, 1H), 7.0 (s, 1H), 5.27 (s, 2H), 4.38 (d, 1H), 3.8 (dd, 1H), 3.5 (m, 1H), 2.5 (m, 1H), 2.4 (s, 1H), 2.08 (m, 1H), 1.95 (m, 1H), 1.12 (d, 3H).

Stage 40.1: Imidazole-1-carboxylic acid (8-methyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide



- 15 Imidazole-1-carboxylic acid (8-methyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide was prepared from N-(8-methyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-acetamide (**Stage 40.2**) using synthetic methodology as described for the preparation of intermediate A.

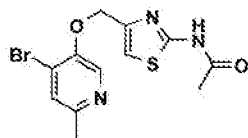
- 20 **Stage 40.2:** N-(8-methyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-acetamide



- A mixture of 463 mg (1.53 mmol) N-[4-(4-bromo-6-methyl-pyridin-3-yloxymethyl)-thiazol-2-yl]-acetamide (**Stage 40.3**), 900 mg (2.71 mmol) cesium carbonate, 30.4 mg (0.135 mmol) palladium acetate and 81 mg (0.271 mmol) tributylphosphine tetrafluoroborate in 3 mL DMF was stirred at 115°C for 7 h under argon. Then the reaction mixture was poured onto water, EtOAc was added and, after filtration over a layer of Hyflo Super Gel medium (Fluka 56678), the filtrate was extracted with EtOAc (2X). The combined organic layers were washed with

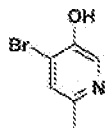
water and brine, dried (MgSO_4) and filtered. The filtrate was concentrated in vacuo to leave a residue that was purified on silica gel ($\approx$ 100% EtOAc in heptane) to afford 93 mg of the title compound as a solid. LCMS: $t_R = 0.27$ min, $M+H = 262$, $M-H = 260$ (method J).

5 Stage 40.3: N-[4-(4-Bromo-6-methyl-pyridin-3-yloxymethyl)-thiazol-2-yl]-acetamide



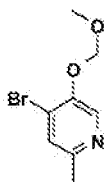
To a solution of 270 mg (1.44 mmol) 4-bromo-6-methyl-pyridin-3-ol (**Stage 40.4**) in DMF (5 mL), 44.8 mg NaH (1.87 mmol) was added and this mixture was stirred for 1 h at rt. 301 mg (1.58 mmol) 2-acetamido-4-(chloromethyl)-1,3-thiazole (Apollo OR15549) was added and stirring was continued at rt for another 17 h. The reaction mixture was poured on water and extracted with EtOAc (2X). The combined organic layers were washed with water and brine, dried (MgSO_4) and filtered. The filtrate was concentrated in vacuo to leave 350 mg of the title compound (white solid) considered sufficiently pure without further purification. LCMS: $t_R = 1.36$ min, $M+H = 342$ (^{79}Br), 344 (^{81}Br) (method J).

15 Stage 40.4: 4-Bromo-6-methyl-pyridin-3-ol



920 mg (3.05 mmol) 4-bromo-5-methoxymethoxy-2-methyl-pyridine (**Stage 40.5**) were dissolved in 5 mL MeOH, HCl conc. (1 mL, 12 mmol) was added and the reaction mixture was stirred for 2 h at rt. While concentrating the mixture in vacuo, the title compound (as hydrochloride) crystallized. Filtration afforded 570 mg white crystals. $^1\text{H-NMR}$ (d_6 -DMSO, 400 MHz): 8.19 (s, 1H), 8.04 (s, 1H), 2.53 (s, 3H).

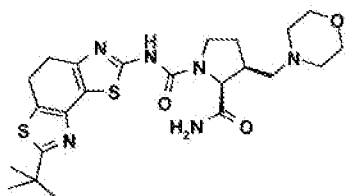
25 Stage 40.5: 4-Bromo-5-methoxymethoxy-2-methyl-pyridine



A solution of 1000 mg (6.53 mmol) 5-methoxymethoxy-2-methyl-pyridine (J.-P. Behr et al. Bioorg. Med. Chem. Lett. **2003**, 13(10), 1713) in 10 mL THF was cooled to -78°C , where

4.03 mL (6.85 mmol) *t*-BuLi (1.7M solution in pentane) was added. The resulting mixture was stirred under argon for 1 h, then 2.126 g (6.53 mmol) 1,2-dibromotetrachloroethane (in 5 mL THF) was added. Stirring was continued for 1 h at -78°C and the reaction mixture was warmed to rt. Saturated NH₄Cl solution was added and the aqueous mixture was extracted with EtOAc (2X). The combined organic layers were washed with H₂O and brine, dried (MgSO₄) and filtered. The filtrate was concentrated in vacuo to leave a residue that was purified on silica gel (EtOAc / hexane = 1:1) to afford 920 mg of the title compound as an oil. LCMS: *t*_R = 0.29 min, M+H = 232 (⁷⁹Br), 234 (⁸¹Br) (method J).

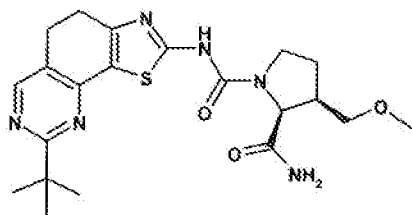
- 10 **Example 41:** (2S,3S)-3-Morpholin-4-ylmethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]



The title compound was synthesized using methodology as described for **Example 34** using (2S,3S)-3-morpholin-4-ylmethyl-pyrrolidine-2-carboxylic acid amide (**Stage 37.1**) instead of **intermediate D**.

LCMS: *t*_R = 0.68 min, M+H = 505.3.0, M-H = 503.2 (method J). ¹H-NMR (d₆-DMSO, 600.13 MHz): 10.65 (s, b, 1H), 7.4 (s, b, 1H), 7.05 (s, b, 1H), 4.3 (s, b, 1H), 3.7 (m, 1H), 3.6 (m, 4H), 3.3 (m, 1H), 3.1 (t, 2H), 2.9 (t, 2H), 2.6 (m, 1H), 2.4 (m, 1H), 2.35 (m, 4H), 2.15 (m, 1H), 2.0 (m, 1H), 1.75 (m, 1H), 1.35 (s, 9H).

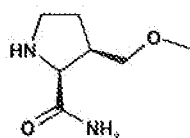
- 20 **Example 42:** (2S,3R)-3-Methoxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



Triethylamine (0.525 mmol) was added to a solution of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**) (0.175 mmol) and (2S,3R)-3-methoxymethyl-pyrrolidine-2-carboxylic acid amide (**Stage 42.1**) (0.192 mmol) in DMF (0.7 mL) at rt. After stirring for 3 h, the reaction mixture was concentrated. The residue

was purified by silica gel column chromatography to afford the title compound as a white solid. HPLC: t_R = 2.84 min (method G); LCMS: t_R = 1.71 min, $[M+H]^+$ 445 (method I); TLC: R_f = 0.41 (19:1 CH_2Cl_2 /MeOH); ^1H -NMR (d_6 -DMSO, 600 MHz): 11.15 (br s, 1H), 8.40 (s, 1H), 7.42 (br s, 1H), 7.05 (br s, 1H), 4.27 (m, 1H), 3.74 (m, 1H), 3.46 (m, 1H), 3.42 (m, 1H), 3.24 (s, 3H), 3.16 (m, 1H), 2.98 (m, 2H), 2.91 (m, 2H), 2.54 (m, 1H), 2.03 (m, 1H), 1.77 (m, 1H), 1.32 (s, 9H).

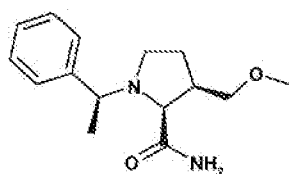
Stage 42.1: (2S,3R)-3-Methoxymethyl-pyrrolidine-2-carboxylic acid amide



The title compound was prepared in analogy to the procedure described in Stage 37.1 but (2S,3R)-3-methoxymethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide (**Stage 42.2**) was used instead of (2S,3S)-3-morpholin-4-ylmethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide.

The title compound was obtained as a white solid. ESI-MS: $[M+H]^+$ 159.

Stage 42.2: (2S,3R)-3-Methoxymethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide

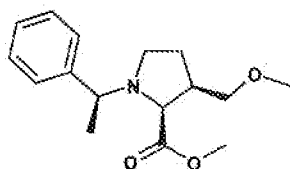


The title compound was prepared in analogy to the procedure described in Stage 37.2 but (2S,3R)-3-methoxymethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester (**Stage 42.3**) was used instead of (2S,3S)-3-morpholin-4-ylmethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester.

The title compound was obtained as a yellow oil. HPLC: t_R = 2.35 min (method G); LC-MS: t_R = 0.47 min, $[M+H]^+$ 263 (method K); TLC: R_f = 0.05 (1:1 Heptanes/EtOAc).

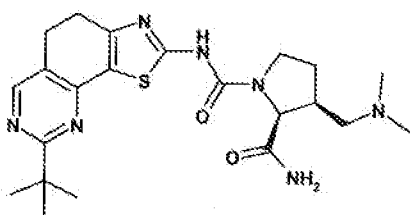
Stage 42.3: (2S,3R)-3-Methoxymethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid methyl ester

- 87 -



A mixture of (3aR,6aS)-1-((S)-1-phenyl-ethyl)-hexahydro-furo[3,4-b]pyrrol-6-one [805246-48-4] (17.05 mmol), KOH (71.60 mmol) and iodomethane (68.20 mmol) in toluene (79 mL) was stirred at 80°C for 1.5 h. The reaction mixture was cooled to rt and partitioned between water and MTBE. The aqueous layer was extracted with MTBE (3X). The combined organic layers were dried (Na₂SO₄), filtered and concentrated. The residue was purified by silica gel column chromatography to afford the title compound as a yellow oil. HPLC: t_R = 2.98 min (method G); LC-MS: t_R = 0.69 min, [M+H]⁺ 278 (method K); TLC: R_f = 0.25 (1:3 Heptanes/EtOAc).

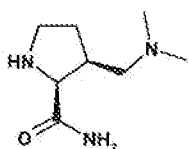
- 10 **Example 43:** (2S,3S)-3-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]



Triethylamine (0.305 mmol) was added to a solution of imidazole-1-carboxylic acid (8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide (**Intermediate A**) (0.102 mmol) and (2S,3S)-3-dimethylaminomethyl-pyrrolidine-2-carboxylic acid amide (**Stage 43.1**) (0.102 mmol) in DMF (0.3 mL) at rt. After stirring for 0.5 h, the reaction mixture was concentrated and dried overnight under vacuum at 50°C. The residue was suspended in EtOAc (1 mL), filtered and dried under vacuum to afford the title compound as a white solid. HPLC: t_R = 4.01 min (method H); LCMS: t_R = 1.33 min, [M+H]⁺ 458 (method I); TLC: R_f = 0.08 (4:1

- 20 CH₂Cl₂/MeOH); ¹H-NMR (d₆-DMSO, 600 MHz): 11.14 (br s, 1H), 8.40 (s, 1H), 7.42 (br s, 1H), 7.06 (br s, 1H), 4.27 (m, 1H), 3.71 (m, 1H), 3.42 (m, 1H), 2.98 (m, 2H), 2.90 (m, 2H), 2.52 (m, 1H), 2.33 (m, 1H), 2.18 (m, 1H), 2.18 (s, 6H), 2.01 (m, 1H), 1.72 (m, 1H), 1.32 (s, 9H).

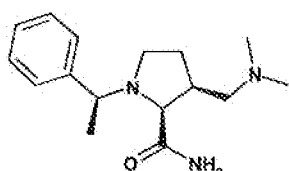
Stage 43.1: (2S,3S)-3-Dimethylaminomethyl-pyrrolidine-2-carboxylic acid amide



The title compound was prepared in analogy to the procedure described in Stage 37.1 but (2S,3S)-3-dimethylaminomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide (**Stage 43.2**) was used instead of (2S,3S)-3-morpholin-4-ylmethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide. Also, the hydrogenation was performed under 4 bar pressure.

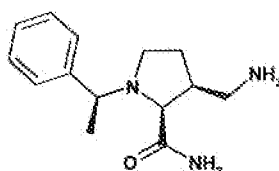
The title compound was obtained as a yellow oil. ESI-MS: $[M+H]^+$ 172.

Stage 43.2: (2S,3S)-3-Dimethylaminomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide



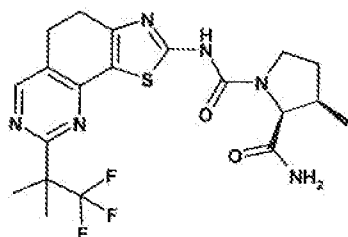
A mixture of (2S,3S)-3-aminomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide (**Stage 43.3**) (0.418 mmol), sodium cyanoborohydride (2.86 mmol) and 37% aqueous formaldehyde (2.14 mmol) in MeOH (3.3 mL) was stirred at 55°C for 16 h. The reaction mixture was cooled to rt and concentrated. The residue was purified using a RediSep® silica gel column to afford the title compound as a white foam. HPLC: t_R 3.59 min (method H); LC-MS: t_R = 0.86 min, $[M+H]^+$ 276 (method I); TLC: R_f = 0.13 (9:1 CH_2Cl_2 /MeOH).

Stage 43.3: (2S,3S)-3-Aminomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide



A mixture of (2S,3S)-3-azidomethyl-1-((S)-1-phenyl-ethyl)-pyrrolidine-2-carboxylic acid amide (**Stage 36.3**) (0.723 mmol) and triphenylphosphine (0.867 mmol) in THF (3 mL) was stirred at rt for 25 h. The reaction mixture was concentrated to afford the crude title compound as a light brown solid. HPLC: t_R 3.53 min (method H); ESI-MS: $[M+H]^+$ 248.

Example 44: (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl]-amide}

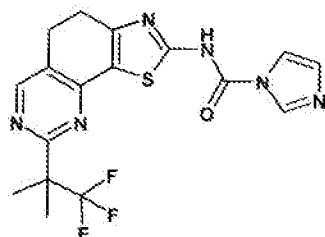


Triethylamine (1.714 mmol) was added to a solution of imidazole-1-carboxylic acid [8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl]-amide (**Stage 44.1**) (0.490 mmol) and (2S,3R)-3-methyl-pyrrolidine-2-carboxylic acid amide (**intermediate P**)

(0.979 mmol) in DMF (1.5 mL) at rt. After stirring for 1.5 h, the reaction mixture was concentrated. The residue was diluted with a saturated solution of NaHCO₃ and extracted with EtOAc (2X). The combined organic layers were successively washed with water and brine, dried (Na₂SO₄), filtered and concentrated. The residue was purified using a RediSep[®] silica gel column to afford the title compound as a beige solid. HPLC: t_R = 5.77 min

(method H); LCMS: t_R = 2.18 min, $[M+H]^+$ 469 (method I); TLC: R_f = 0.16 (19:1 CH₂Cl₂/MeOH); ¹H-NMR (d₆-DMSO, 600 MHz): 11.19 (br s, 1H), 8.50 (s, 1H), 7.43 (br s, 1H), 7.01 (br s, 1H), 4.18 (m, 1H), 3.72 (m, 1H), 3.40 (m, 1H), 3.03 (m, 2H), 2.94 (m, 2H), 2.38 (m, 1H), 1.97 (m, 1H), 1.70 (m, 1H), 1.58 (s, 6H), 0.99 (d, 3H).

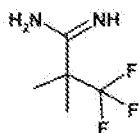
Stage 44.1: Imidazole-1-carboxylic acid [8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl]-amide



The title compound was prepared in analogy to the procedure described for Intermediate A but in Stage A.3 3,3,3-trifluoro-2,2-dimethyl-propionamidine hydrochloride (**Stage 44.2**) was used instead of tert-butylamidine hydrochloride.

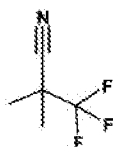
The title compound was obtained as a beige solid. HPLC: t_R = 6.73 min (method H); LCMS: t_R = 1.11 min, $[M+H]^+$ 373 (method J). Note : for characterization purposes, the title compound was dissolved in MeOH --> methyl carbamate derivative.

Stage 44.2: 3,3,3-Trifluoro-2,2-dimethyl-propionamidine hydrochloride



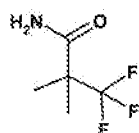
3,3,3-Trifluoro-2,2-dimethyl-propionitrile (**Stage 44.3**) (12.40 mmol) was added to a solution of sodium methoxide (freshly prepared from 48.40 mmol sodium metal and 102 mL MeOH) at rt. After 4 h, acetic acid (48.40 mmol) and then NH_4Cl (14.88 mmol) were slowly added and the reaction mixture was then heated to 70°C for 40 h. After concentrating (40°C , 100 mbar) the reaction mixture, the residue was suspended in acetone, filtered and dried under vacuum to afford the title compound as a white solid. ESI-MS: $[\text{M}+\text{H}]^+$ 155.

Stage 44.3: 3,3,3-Trifluoro-2,2-dimethyl-propionitrile



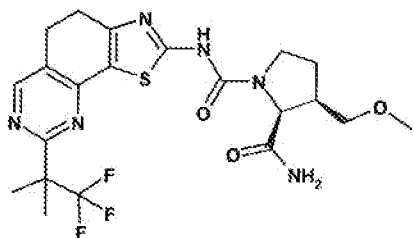
A mixture of 3,3,3-trifluoro-2,2-dimethyl-propionamide (**Stage 44.4**) (121.2 mmol) and phosphorus pentoxide (121.2 mmol) was slowly heated to 200°C and the resulting distillate collected. The title compound was obtained as a colorless liquid.

Stage 44.4: 3,3,3-Trifluoro-2,2-dimethyl-propionamide



Oxalyl chloride (140.8 mmol) was added dropwise to a solution of 3,3,3-trifluoro-2,2-dimethyl-propionic acid [889940-13-0] (128 mmol) in CH_2Cl_2 (128 mL) at 0°C . Added a few drops of DMF until gas evolution was observed and then continued stirring for 30 min. After warming to rt and stirring overnight, the reaction mixture was concentrated (40°C , 00 mbar). The residue was dissolved in THF (128 mL), cooled to 0°C and then slowly treated with a solution of concentrated aqueous ammonia (64 mL). After stirring at 0°C for 30 min and then at rt for 4 h, the reaction mixture was concentrated to half its volume to give a thick white suspension. After filtering and drying, the title compound was obtained as a white solid. ESI-MS: $[\text{M}+\text{H}]^+$ 156.

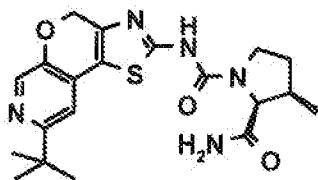
Example 45: (2S,3R)-3-Methoxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl]-amide]



Triethylamine (1.714 mmol) was added to a solution of imidazole-1-carboxylic acid [8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl]-amide (**Stage 44.1**) (0.490 mmol) and (2S,3R)-3-methoxymethyl-pyrrolidine-2-carboxylic acid amide (**Stage 42.1**) (0.0979 mmol) in DMF (1.5 mL) at rt. After stirring for 2 h, the reaction mixture was

concentrated. The residue was diluted with a saturated solution of NaHCO_3 and extracted with EtOAc (2X). The combined organic layers were successively washed with water and brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified using a RediSep[®] silica gel column to afford the title compound as a beige solid. HPLC: $t_R = 5.71$ min (method H); LCMS: $t_R = 2.15$ min, $[\text{M}+\text{H}]^+ 499$ (method I); TLC: $R_f = 0.40$ (19:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$); $^1\text{H-NMR}$ (d_6 -DMSO, 600 MHz): 11.23 (br s, 1H), 8.50 (s, 1H), 7.42 (br s, 1H), 7.06 (br s, 1H), 4.27 (m, 1H), 3.74 (m, 1H), 3.46 (m, 1H), 3.42 (m, 1H), 3.24 (s, 3H), 3.16 (m, 1H), 3.03 (m, 2H), 2.94 (m, 2H), 2.55 (m, 1H), 2.03 (m, 1H), 1.77 (m, 1H), 1.58 (s, 6H).

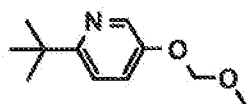
Example 46: (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide]



LCMS: $t_R = 0.50$ min, $\text{M}+\text{H} = 416$, $\text{M}-\text{H} = 414$ (method L). $^1\text{H-NMR}$ (d_6 -DMSO, 400 MHz): 8.05 (s, 1H), 7.37 (bs, 1H), 7.09 (s, 1H), 6.97 (bs, 1H), 5.31 (s, 2H), 4.18 (m, 1H), 3.69 (dd, 1H), 3.39 (m, 1H), 2.35 (m, 1H), 1.95 (m, 1H), 1.71 (m, 1H), 1.27 (s, 9H), 0.975 (d, 3H).

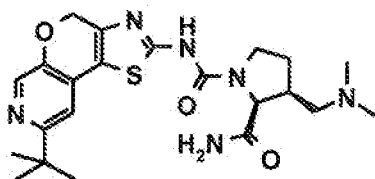
The title compound was prepared from 2-tert-butyl-5-methoxymethoxy-pyridine using synthetic methodology as described for the preparation of **Example 40**. The starting material, 2-tert-butyl-5-methoxymethoxy-pyridine was prepared from 2-bromo-5-methoxymethoxy-pyridine as described below:

2-tert-Butyl-5-methoxymethoxy-pyridine:



6.69g (74.7 mmol) copper(I)cyanide in 15 ml dry THF was cooled to -75°C, where 149 ml (149 mmol) of a 1M tert-butylmagnesium chloride solution was added drop-wise. Stirring was continued for 40 min. After that, 4.07g (18.67 mmol) 2-bromo-5-methoxymethoxy-pyridine (Zhong, W. et al. WO2008147547) dissolved in 30 ml dry THF were added drop-wise. The reaction mixture was stirred at -75°C for 1 h and then allowed to reach room temperature, where stirring was continued for another 6 h. Then 30ml of 25% aqueous ammonia was added to the reaction mixture. The mixture was filtered and extracted twice with CH₂Cl₂. The combined organic layers were washed with water, dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by chromatography on silicagel (EtOAc, heptane) to yield 2.64 g of the pure title compound as an oil. LCMS: t_R = 0.55 min, M+H = 196 (method K).

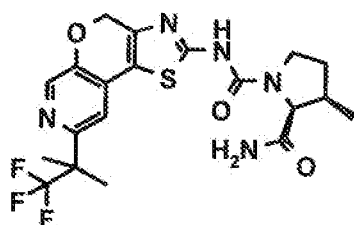
Example 47: (2S,3S)-3-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide]



The title compound was prepared using synthetic methodology described for the preparation of Example 46 and using (2S,3S)-3-dimethylaminomethyl-pyrrolidine-2-carboxylic acid amide (**Stage 43.1**) instead of (2S,3R)-3-methyl-pyrrolidine-2-carboxylic acid amide in the last step of the synthesis.

LC-MS: t_R = 0.24 min, M-H = 457 (method L). ¹H-NMR (d₆-DMSO, 400 MHz): 11.15 (bs, 1H), 8.03 (s, 1H), 7.35 (s, 1H), 7.07 (s, 1H), 7.0 (s, 1H), 5.28 (s, 2H), 4.23 (m, 1H), 3.65 (m, 1H), 3.40 (m, 1H), 2.6-2.45 (m, 2H), 2.30 (m, 1H), 2.17 (s, 6H), 2.15 (m, 1H), 1.70 (m, 1H), 1.27 (s, 9H).

Example 48: (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide]

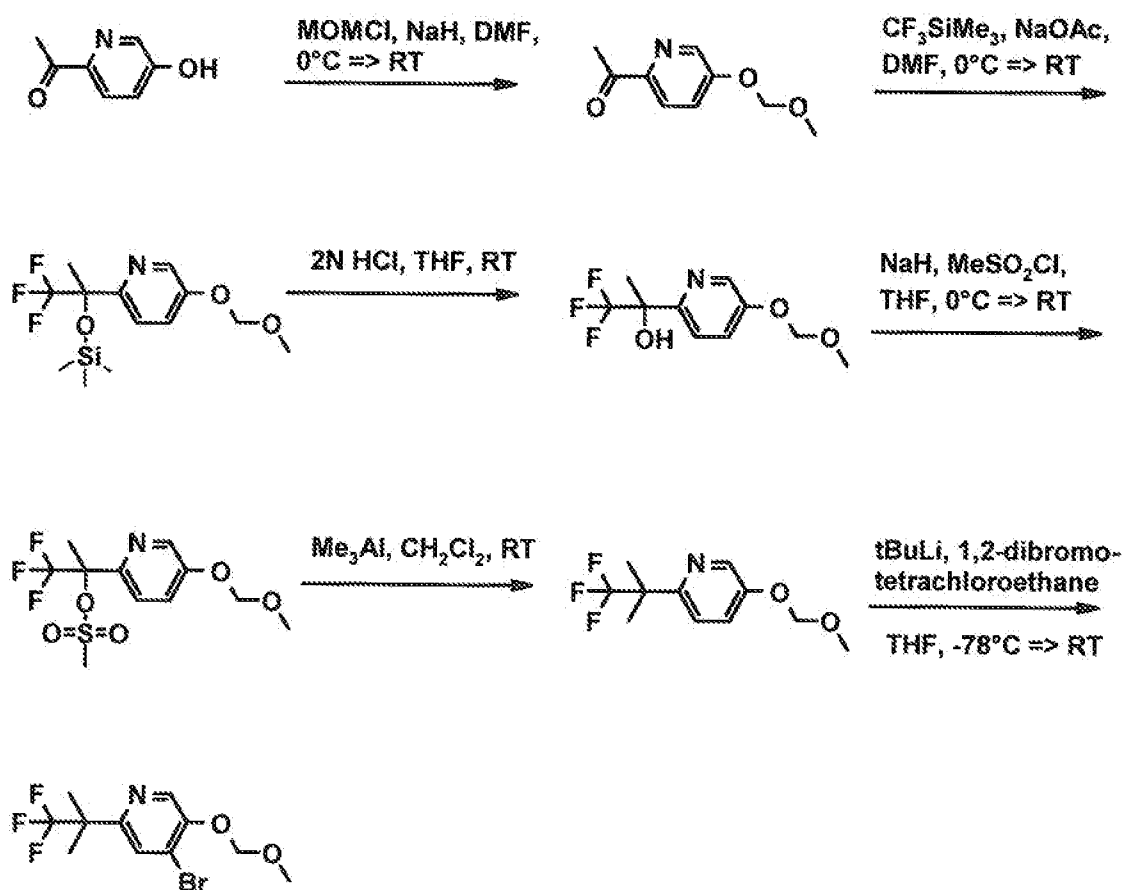


LC-MS: $t_R = 1.59$ min, $M+H = 470$ (method F). ^1H -NMR (d_6 -DMSO, 400 MHz): 11.25 (bs, 1H), 8.15 (s, 1H), 7.42 (s, 1H), 7.34 (s, 1H), 7.00 (s, 1H), 5.40 (s, 2H), 4.19 (s, 1H), 3.71 (m, 1H), ca. 3.4 (m, 1H), 2.5-2.35 (m, 1H), 1.99 (m, 1H), 1.68 (m, 1H), 1.56 (s, 6H), 0.99 (d, 3H).

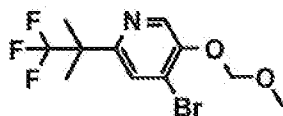
5

The title compound was prepared starting from 4-bromo-5-methoxymethoxy-2-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-pyridine using synthetic methodology as described for the preparation of **Examples 40 and 42**.

- 10 4-Bromo-5-methoxymethoxy-2-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-pyridine was prepared as follows:



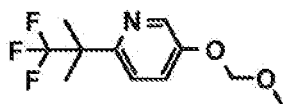
a) 4-Bromo-5-methoxymethoxy-2-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-pyridine:



The title compound was prepared using synthetic methodology as described for the

5 preparation of **Example 40, stage 40.5** as an oil. LCMS (method L): $t_R = 1.26$ min, $M+H = 328$ (^{79}Br), 330 (^{81}Br) .

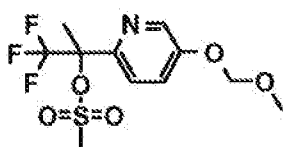
b) 5-Methoxymethoxy-2-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-pyridine



10 1.7 g (4.13 mmol) methanesulfonic acid 2,2,2-trifluoro-1-(5-methoxymethoxy-pyridin-2-yl)-1-methyl-ethyl ester was dissolved in 20 ml dichloromethane. The reaction mixture was cooled to 0°C where 2.065 ml (4.13 mmol) trimethylaluminum was added slowly. The reaction mixture was stirred at room temperature for 20 h. After that water was added slowly and the mixture was extracted 2 times with EtOAc. The combined organic layers were washed with

15 water and brine, dried (MgSO_4), filtered and concentrated in vacuo. The residue was purified by chromatography on silicagel (EtOAc, heptane) to yield to afford 0.55 g of the pure title compound. LCMS (method L): $t_R = 1.09$ min, $M+H = 250$.

c) Methanesulfonic acid 2,2,2-trifluoro-1-(5-methoxymethoxy-pyridin-2-yl)-1-methyl-ethyl ester



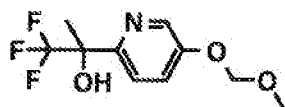
To a stirred mixture of 0.213 g (8.44 mmol) sodium hydride in 6 ml dry THF, 1.06 g (4.22 mmol) 1,1,1-trifluoro-2-(5-methoxymethoxy-pyridin-2-yl)-propan-2-ol (dissolved in 4.9 ml dry THF) was added dropwise at 0°C. After completion of the addition, stirring was continued for

25 3 h at room temperature. Then methanesulfonyl chloride (dissolved in 7.3 ml dry THF) was added at room temperature and stirring was continued for another 2 h. Then water and sat. NaHCO_3 soln. were added. This mixture was extracted 2 times with EtOAc. The combined organic layers were washed with water and brine, dried (MgSO_4), filtered and concentrated

in vacuo to afford 1.7 g of the title compound, which was used directly in the next step.

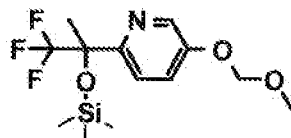
LCMS (method L): $t_R = 0.96$ min, $M+H = 330$.

d) 1,1,1-Trifluoro-2-(5-methoxymethoxy-pyridin-2-yl)-propan-2-ol



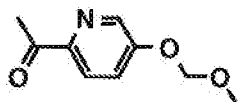
A mixture of 1.48 g (4.58 mmol) 5-methoxymethoxy-2-(2,2,2-trifluoro-1-methyl-1-trimethylsilyloxy-ethyl)-pyridine and 5 ml (10 mmol) 2N HCl in 15 ml THF was stirred at room temperature for 2 h. After that the reaction mixture was poured on water and a pH of 1-2 was adjusted by the addition of 2N HCl. The reaction mixture was extracted 2 times with EtOAc. The combined organic layers were washed with water and brine, dried ($MgSO_4$), filtered and concentrated in vacuo to afford 1.06 g of the title compound as an oil (88% pure), which was used in the next step without purification. LCMS (method L): $t_R = 0.88$ min, $M+H = 252$.

e) 5-Methoxymethoxy-2-(2,2,2-trifluoro-1-methyl-1-trimethylsilyloxy-ethyl)-pyridine



A mixture of 1.12 g (6.18 mmol) 1-(5-methoxymethoxy-pyridin-2-yl)-ethanone, 1.055 g (7.42 mmol) trimethyl(trifluoromethyl)silane and 0.025 g (0.309 mmol) sodium acetate in 9 ml DMF was stirred for 1 h at 0°C and 1 h at room temperature. After that the reaction mixture was poured on water and a pH of 1-2 was adjusted by the addition of 2N HCl. The reaction mixture was extracted 2 times with EtOAc. The combined organic layers were washed with water and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by chromatography on silicagel (EtOAc, heptane) to yield 1.87 g of the pure title compound as an oil. LCMS (method L): $t_R = 1.38$ min, $M+H = 324$.

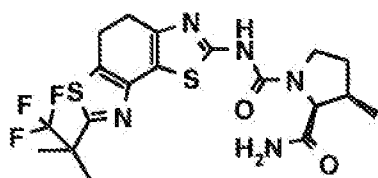
f) 1-(5-Methoxymethoxy-pyridin-2-yl)-ethanone:



NaH was added to a solution of 1 g (7.29 mmol) 1-(5-hydroxy-pyridin-2-yl)-ethanone (Anichem) in 15 ml DMF at 0°C. After stirring this mixture for 1 h, 0.78 ml (8.75 mmol)

MOMCl was added at 0°C. Then the reaction mixture was stirred at room temperature for 2h. The reaction mixture was poured on water and extracted 2 times with EtOAc. The combined organic layers were washed with water and brine, dried (MgSO₄), filtered and concentrated in vacuo. The residue was purified by chromatography on silicagel (EtOAc, heptane) to yield 1.12 g of the pure title compound as an oil. LCMS (method L): t_R = 0.66 min, M+H = 182.

Example 49: (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[7-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl]-amide}

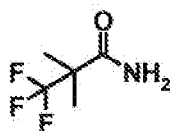


LCMS: t_R = 0.97 min, M+H = 474, M-H = 414 (method L). ¹H-NMR (d₆-DMSO, 400 MHz): 10.7 (bs, 1H), 7.37 (s, 1H), 6.97 (s, 1H), 4.18 (m, 1H), 3.69 (dd, 1H), 3.39 (m, 1H), 3.14 (t, 2H), 2.95 (t, 2H), 2.34 (m, 1H), 1.94 (m, 1H), 1.68 (m, 1H), 1.62 (s, 6H), 0.965 (d, 3H).

The title compound was prepared from 2,2-dimethyl-thiopropionamide using synthetic methodology as described for the preparation of **Example 39**. The starting material, 3,3,3-trifluoro-2,2-dimethyl-thiopropionamide was prepared as described below:

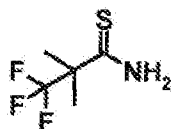
Preparation of 3,3,3-trifluoro-2,2-dimethyl-thiopropionamide:

a) 3,3,3-Trifluoro-2,2-dimethyl-propionamide



A mixture of 2 g (12.81 mmol) 3,3,3-trifluoro-2,2-dimethylpropionic acid (Aldrich), 2.077 g (12.81 mmol) carbonyl-diimidazole and 5.55 ml (64.1 mmol) aqueous ammonia in 50 ml CH₂Cl₂ was stirred at room temperature for 20 h. After that, diethyl ether was added and the resulting mixture was washed with 0.1 N HCL, 0.1 N NaOH, water and brine. The organic layer was dried (MgSO₄), filtered and concentrated in vacuo to afford 1.15 g of the title compound as white crystals, that were used in the next step without further purification. MS: M+H = 156.

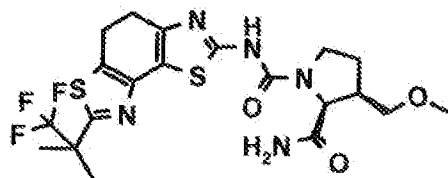
b) 3,3,3-Trifluoro-2,2-dimethyl-thiopropionamide



A mixture of 1.05 g (6.77 mmol) 3,3,3-trifluoro-2,2-dimethyl-propionamide and 1.48 g (3.66 mmol) Lawesson's reagent in 32 ml dry THF was stirred at reflux temperature for 20 h. After
 5 that the reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was purified by chromatography on silicagel (heptane, EtOAc) to afford 0.81 g of the title compound as pure white crystals. MS: M+H = 172.

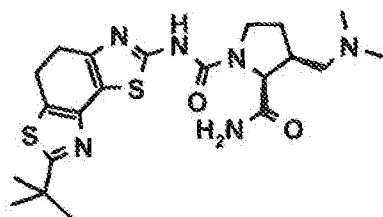
Using (2S,3R)-3-methoxymethyl-pyrrolidine-2-carboxylic acid amide (**Stage 42.1**) in the last
 10 step of the synthesis the following example was prepared in the same way:

Example 50: (2S,3R)-3-Methoxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[7-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl]-amide]



15 LC-MS: $t_R = 0.96$ min, M+H = 504, M-H = 502 (method L). $^1\text{H-NMR}$ (d_6 -DMSO, 400 MHz): 10.76 (bs, 1H), 7.34 (bs, 1H), 7.02 (bs, 1H), 4.29 (m, 1H), 3.45 (m, 1H), 3.42 (m, 2H), 3.22 (s, 3H), 3.14 (m, 3H), 2.97 (m, 2H), 2.48 (m, 1H), 1.97 (m, 1H), 1.75 (m, 1H), 1.62 (s, 6H).

Example 51: (2S,3S)-3-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[7-(tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide]
 20



The title compound was prepared using synthetic methodology described for the preparation of Example 39 and using (2S,3S)-3-dimethylaminomethyl-pyrrolidine-2-carboxylic acid amide (**Stage 43.1**) instead of (2S,3R)-3-methyl-pyrrolidine-2-carboxylic acid amide in the
 25 last step of the synthesis.

LC-MS: $t_R = 0.91$ min, $M+H = 463$, $M-H = 461$ (method L). ^1H -NMR (d_6 -DMSO, 400 MHz): 10.7 (bs, 1H), 7.36 (s, 1H), 7.01 (bs, 1H), 4.28 (m, 1H), 3.67 (dd, 1H), 3.39 (m, 1H), 3.07 (m, 2H), 2.92 (m, 2H), 2.5-2.44 (m, 2H), 2.30 (m, 1H), 2.14 (s, 6H), 1.95 (m, 1H), 1.40 (m, 1H), 1.36 (s, 9H).

5

Efficiency as PI3 kinase inhibitors

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- α -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.

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20

5 μ L of a mix of PI/OG with the PI3K subtype were added (Table 1). Kinase reactions were started by addition of 5 μ L of ATP-mix containing in a final volume 10 μ L 10 mM TRIS-HCl pH 7.5, 3mM $MgCl_2$, 50 mM NaCl, 0.05% CHAPS, 1mM DTT and 1 μ M ATP, and occurred at room temperature. Reactions were stopped with 10 μ L 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 μ M 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 μ L)	Enzyme (nM)	ATP (μ M)	PI/OG (μ M/ μ g/ml)	NaCl (mM)	Mg^{2+} (mM)	CHAPS (%)	DTT (mM)	time (mins)
PI3Ka	10	1	11/10	50	3	0.05	1	30
PI3K β	25	1	11/10	50	3	0.05	1	30
PI3K γ	150	1	22/20	50	3	0.05	1	90
PI3K δ	10	1	11/10	50	3	0.05	1	30

Cloning of PI3Ks

The PI3K α , PI3K β and PI3K δ constructs are fusion of p85 α iSH2 domain and the respective p110 isoforms. The p85 α 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 PI3K γ construct was
 5 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.
 10 Cell (2000), 103(6), 931-943).

PI3K α constructs and proteins

PI3K α wt	BV1075	p85iSH2(461-568)-GGGGGGGGGGG-p110 α (21-1068)-His
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BV1075: The construct for Baculovirus BV-1075 was generated by a three-part ligation comprised of a p85 fragment and a p110 α fragment cloned into vector pBlueBac4.5. The p85 fragment was derived from plasmid p1661-2 digested with
 15 Nhe/Spe. The p110 α 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
 20 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'-GCTAGCATGCGAGAATATGATAGAT-TATATGAAG-AATATACC) (SEQ ID No. 1) and two reverse primers, KAC1029 (5'-GCCTCCACCAC-CTCCGCCTG-GTTTAATGCTGTTTCATACGTTTGTC) (SEQ ID No. 2) and KAC1039 (5'-
 25 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 p110 α 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

- 100 -

correct by sequencing. This plasmid was digested with Nhe and SpeI and the resulting fragment was gel-isolated and purified for sub-cloning.

The p110 α 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 p110 α 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:

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1 MREYDRLYEE YTRTSQEIQM KRTAIEAFNE TIKIFEEQCQ TQERYSKYEI EKFKREGNEK
15 61 EIQRIMHNYD KLKSRISEII DSRRLLEEDL KKQAAEYREI DKRMNSIKPG GGGGGGGGGG
121 GLVECLLPNG MIVTLECLRE ATLITIKHEL FKEARKYPLH QLLQDESSYI FVSVTQEAER
181 EEFFDETRRL CDLRFLQPFLL KVIEPVGNRE EKILNREIGF AIGMPVCEFD MVKDPEVQDF
241 RRNILNVCKE AVDLRDLNSP HSRAMYVYFP NVESPELPEK HIYNKLDKGQ IIVVIWVIVS
301 PNNDKQKYTL KINHDCVPEQ VIAEAIRKKT RSMLLSSEQL KLCVLEYQGK YILKVCGCDE
20 361 YFLEKYPLSQ YKYIRSCIML GRMPNMLMA KESLYSQLPM DCFTMPSYSR RISTATPYMN
421 GETSTKSLWV INSALRIKIL CATYVNVNIR DIDKIYVRTG IYHGGEPLCD NVNTQRVPCS
481 NPRWNEWLNY DIYIPDLRA ARLCLSICSV KGRKGAKKEH CPLAWGNINL FDYTDTLVSG
541 KMALNLWPVP HGLEDLLNPI GVTGSNPNKE TPCLELEFDW FSSVVKFPDM SVIEEHANWS
601 VSREAGFSYS HAGLSNRLAR DNELRENDKE QLKAISTRDP LSEITEQEKD FLWSHRHYCV
25 661 TIPEILPKLL LSVKWNRSDE VAQMYCLVKD WPPIKPEQAM ELLDCNYPDP MVRGFAVRCL
721 EKYLTDCKLS QYLIQLVQVL KYEQYLDNLL VRFLKALKALT NQRIGHFFFW HLKSEMINKT
781 VSQRFGLLLE SYCRACGMYL KHLNRQVEAM EKLINLTDIL KQEKDETQK VQMKFLVEQM
841 RRPDFMDALQ GFLSPLNPAH QLGNLRLLEC RIMSSAKRPL WLNWENPDIM SELLFQNNET
901 IFKNGDDLRLQ DMLTLQIIRI MENIWQNQGL DLRMLPYGCL SIGDCVGLIE VVRNSHTIMQ
30 961 IQCKGGLKGA LQFNSHTLHQ WLKDKNKGEI YDAAIDLFTR SCAGYCVATF ILGIGDRHNS
1021 NIMVKDDGQL FHIDFGHFLD HKKKKFGYKR ERVPFVLTDQ FLIVISKGAQ ECTKTREFER
1081 FQEMCYKAYL AIRQHANLFI NLFSSMLGSG MPELQSFDDI AYIRKTLALD KTEQEALEYF
1141 MKQMNDAHHG GWPTKMDWIF HTIKQHALNE LGGAHHHHHH (SEQ ID No. 4)

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PI3K β constructs and proteins

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.

5 Protein sequence of BV949:

```

1 MREYDRLYEE YTRTSQEIQM KRTAIEAFNE TIKIFEEQCQ TQERYSKYEI EKFKREGKEK
61 EIQRIMHNYD KLKSRRISEII DSRRRLLEEDL KKQAAEYREI DKRMNSIKPG GGGGGCFSFI
121 MPPAMADILD IWAVDSQIAS DGSIPVDFLL PTGIYIQLEV PREATISYIK QMLWKQVHNY
181 PMFNLLMDID SYMFACVNQT AVYEELEDET RRLCDVRPFL PVLKLVTRSC DPGEKLDISKI
10 241 GVLIGKGLHE FDSLKDPEVN EFRRKMRKFS EEKILSLVGL SWMDWLKQTY PPEHEPSIPE
301 NLEDKLYGGK LIVAVHFENC QDVFSFQVSP NMNPIKVNEL AIQKRLTIHG KEDEVSPYDY
361 VLQVSGRVEY VFGDHPLIQF QYIRNCVMNR ALPHFILVEC CKIKKMYEQE MIATEAAINR
421 NSSNLPLPLP PKKTRIISHV WENNNPFQIV LVKGNKLNTE ETVKVHVRAG LFHGTTELLCK
481 TIVSSEVSGK NDHIWNEPLE FDINICDLPR MARLCFAVYA VLDKVKTKKS TKTINPSKYQ
15 541 TIRKAGKVHY PVAWVNTMVF DFKGQLRTGD IILHSWSSFP DELEEMLNPM GTVQTNPYTE
601 NATALHVKFP ENKKQPYYPY PFDKIIEKAA EIASSDSANV SSRGGKKFLP VLKEILDNDP
661 LSQLCRNEMD LIWTLRQDCR EIFPQSLPKL LLSIKWNKLE DVAQLQALLQ IWPKLPPREA
721 LELLDNFNPD QYVREYAVGC LRQMSDEELS QYLLQLVQVL KYEPFLDCAL SRFILLERALG
781 NRRIGQFLFW HLRSEVHIPA VSVQFGVILE AYCRGSGVGHM KVLSKQVEAL NKLKTLNLSI
20 841 KLNVAKLNRA KGKEAMHTCL KQSAYREALS DLQSPLNPCV ILSELYVEKC KYMDSKMKPL
901 WLVDNNKVFV EDSVGVIFKN GDDLRLQDMLT LQMLRLMDLL WKEAGLDLRM LFPYGLATGD
961 RSGLTIEVST SETIADIQLN SSNVAAAAAF NKDALLNWLK EYNSGDDLDL AIEEFTLSCA
1021 GYCVASYVLG IGDRHSDNIM VKKTGQLFHI DFGHILGNFK SKFGIKRERV PFILTYDFIH
1081 VIQQGKTGNT EKFGFRFQCC EDAYLILRRH GNLFITLFLAL MLTAGLPELT SVKDIQYLKD
25 1141 SLALGKSEEE ALKQFKQKFD EALRESWTTK VNWMAHTVRK DYRSGAHHHH HHGA (SEQ

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ID No. 12)

Kinase domain.

PI3Ky construct and protein

PI3Ky	BV950	p110γ(Δ143-[Met144-1102])-His
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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:

```

1 MSEESQAFQR QLTALIGYDV TDVSNVHDDE LEFTRRGLVT PRMAEVASRD PKLYAMHPWV
5 61 TSKPLPEYLW KKIANNCFI VIHRSTTSQT IKVSPDDTPG AILQSFFTKM AKKKSIMDIP
121 ESQSEQDFVL RVCGRDEYLV GETPIKNFQW VRHCLKNGEE INHVLDTPPD PALDEVKKEE
181 WPLVDDCTGV TGYHEQLTIH GKDHESVFTV SLWDCDRKFR VKIRGIDIPV LPRNTDLTVF
241 VEANIQHGGQ VLCQRRTPSPK PFTEEVLMNV WLEFSIKIKD LPKGALLNLQ IYCGKAPALS
301 SKASAESPS ESKGKVRLLY YVNLILLIDHR FLLRRGEYVL HMWQISGKGE DQGSFNADKL
10 361 TSATNPDKEN SMSISILLDN YCHPIALPKH QTPDPPEGDR VRAEMPQLR KQLEAIIATD
421 PLNPLTAEDK EELWHFRYES LKHPKAYPKL FSSVKWGQQE IVAKTYQLLA RREVWDQSAL
481 DVGLTMQLLD CNFSDENVRA IAVQKLESLE DDDVLHYLLQ LVQAVKFEPY HDSALARFLL
541 KRGLRANKRIG HFLFWFLRSE IAQSRHYQQR FAVILEAVLR GCGTAMLDHF TQQVQVLEML
601 QKVTLDIKSL SAEKYDVSSQ VISQLKQKLE NLQNSQLPES FRVPYDPGLK AGALAIKCK
15 661 VMASKKKPLW LEFKCADPTA LSNETIGTIF KHGDDLQDM LILQILRIME SIWETESLDL
721 CLLPYGCIST GDKIGMIEIV KDATTIAKIQ QSTVGNTGAF KDEVLNHWLK EKSPTEEFQ
781 AAVERFVYSC AGYCVATFVL GIGDRHNDNI MITETGNLFH IDEGHILGNY KSFLGINKER
841 VPFVLTDFEL FVMGTSCKKT SPHFQKFQDI CVKAYLALRH HTNLLIILFS MMLMTGMPQL
901 TSKEDIEYIR DALTVGKNEE DAKKYFLDQI EVCRDKGWTV QFNWFLHLVL GIKQGEKHS
20 961 HHHHHH (SEQ ID No. 13)

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PI3K δ construct and protein

PI3K δ	BV1060	p85iSH2(461-568)-GGGGGG-p110 δ (2-1044)-His
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BV1060: PCR products for the inter SH2 domain (iSH2) of the p85 subunit and for the full-length p110 δ 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-TTTGTACAAAAAGCAGGCTACGAAGGAGATATACATATGC-GAGAATATGATAGATTATATGAAGAAT-3') (SEQ ID No. 7) and gwG154-p04 (5'-TCCTCCTCCT-CCTCCTCCTGGTTTAATGCTGTTCATACGTTTGTC-3') (SEQ ID No. 14). The p110 δ 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'-ATGCCCCCTGGGGTGGACTGCCCAT-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

Protein sequence of BV1060:

	1	MREYDRLYEE	YTRTSQEIOM	KRTAIEAFNE	TIKIFEEQCO	TQERYSKEYI	EKFKREGNEK
20	61	ETQRIMHNYD	KLKSRISEII	DSRRLEEDL	KKQAAEYREI	DKRMNSIKPG	GGGGGPPGVD
	121	CFMEFWTKEE	NQSVVVDFLL	PTGVYLNFPV	SRNANLSTIK	QLLWHRAQYE	PLFHMLSGPE
	181	AYVFTCINQT	AEQQELEDEQ	RRLCDVQPFL	PVLRVLVAREG	DRVKKLINSQ	ISLLIGKGLH
	241	EFDSLCDPEV	NDFRAKMCQF	CEEAARRQO	LGWEAWLQYS	FPLQLEPSAQ	TWGPGLRLP
	301	NRALLVNVKF	EGSEESFTFQ	VSTKDVPLAL	MACALRKKAT	VFRQPLVEQP	EDYTLQVNGR
25	361	HEYLYGSYPL	CQFQYICSL	HSGLTPHMTM	VHSSSILAMR	DEQSNPAPQV	QKPRAKPPPI
	421	PAKKPSSVSL	WSLEQPFRIE	LIQGSKVNAD	ERMKLVVQAG	LFHGNEMLCK	TVSSSEVSVC
	481	SEPVWKQRLE	FDINICDLPR	MARLCFALYA	VIEKAKKARS	TKKKSKKADC	PIAWANLMLF
	541	DYKDQMLTGE	RCLYMWPSVP	DEKGELNPT	GTVRSNPNTD	SAAALLICLP	EVAPHPVVP
	601	ALEKILEIGR	HSECVHVTEE	EQLQLREILE	RRGSGELYEH	EKDLVWKL RH	EVQEHFPEAL
	661	ARLLLVTKWN	KHEDVAQMLY	LLCSWPPELV	LSALELLDFS	FPDCHVGSFA	IKSLRKLTDG
30	721	ELFQYLLQLV	QVLKYESYLD	CELTKFLLDR	ALANRKIGHF	LFWHLRSEMH	VPSVALRFGI
	781	ILEAYCRGST	HHMKVLMKQG	EALSKLKALN	DFVKLSSQKT	PKPQTKELMH	LCMRQEAYLE
	841	ALSHLQSPLD	PSTLLAEVCV	EQCTFMDSKM	KPLWIMYSNE	EAGSGGSGVI	IFKNGDDLRO
	901	DMLTLQMIQL	MDVLWKQEGE	DLRMTFYGCL	PTGDRGTGLIE	VVLRSDTIAN	IQLNKSNNMAA
	961	TAAFNKDALL	NWLKSKNPGE	ALDRAIEEFT	LSCAGYCVAT	VVLGIGDRHS	DNIMIRESGQ
35	1021	LFHIDFGHFL	GNFKTKFGIN	RERVPFILTY	DFVHVIOQOK	TNNSEKPERF	RGYCERAYTI
	1081	LRRHGLFLH	LFALMEAAGL	PELSCSKDIO	YLKDSLALGK	TEEEALKHFR	VKFNEALRES

1141 WKTKVNWLAH NVSKDNRQEL GGAHHHHHH (SEQ ID No. 20)

Purification of PI3K α , PI3K β and PI3K γ constructs

PI3K α , PI3K β and PI3K γ were purified in two chromatographic steps: immobilized metal affinity chromatography (IMAC) on a Ni sepharose resin (GE Healthcare) and gel filtration
5 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 PI3K β contained 0.05% Triton X100 in addition to what is described below.

Typically frozen cells from 10 L of Tn5 cell culture were resuspended in "Lysis Buffer" 20 mM
10 Tris-Cl, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole, 1 mM NaF, 0.1 μ g/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
15 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, 0.1 μ g/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
20 mM imidazole, 1 mM NaF, 0.1 μ g/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
25 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 then dialyzed against Dialysis Buffer two changes (one change overnight). Protein was stored at -20°C.

Purification of PI3K δ

30 PI3K δ 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.

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, 0.1µg/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, 0.1µg/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, 0.1µg/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, 0.1µg/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.

The following results were obtained using the above described assays.

Example no.	PI3Kalpha / IC50 [µmol l ⁻¹]	PI3Kbeta / IC50 [µmol l ⁻¹]	PI3Kgamma / IC50 [µmol l ⁻¹]	PI3Kdelta / IC50 [µmol l ⁻¹]
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Example no.	PI3Kalpha / IC50 [umol l-1]	PI3Kbeta / IC50 [umol l-1]	PI3Kgamma / IC50 [umol l-1]	PI3Kdelta / IC50 [umol l-1]
1	0.004	1.238	0.067	0.18
2	0.0055	0.332	0.09	0.03
3	0.045	0.127	1.59	0.74
4	0.0065	0.143	0.176	0.22
5	0.0055	0.047	0.153	0.046
6	0.016	1.63	0.50	0.47
7	0.0235	3.136	0.233	0.051
8	0.0095	0.276	0.180	0.198
9	0.0085	0.087	0.141	0.072
10	0.018	0.449	0.282	0.054
11	0.005	0.047	0.129	0.018
12	0.006	0.516	0.230	0.053
13	0.009	0.277	0.104	0.118
14	0.004	0.268	0.191	0.016
16	0.0055	0.297	0.074	0.060
17	0.008	0.389	0.153	0.236
18	0.0105	0.374	0.155	0.028
19	0.0055	0.588	0.123	0.077
20	0.0035	0.155	0.080	0.011
21	< 0.003	0.519	0.237	0.036
22	0.0235	6.905	0.262	1.895
24	0.007	0.135	0.098	0.024
25	0.0055	0.346	0.122	0.045
26	0.0065	0.328	0.138	0.058
27	0.0045	0.397	0.097	0.072
28	0.0155	2.035	0.336	0.331
29	0.008	0.721	1.358	0.892

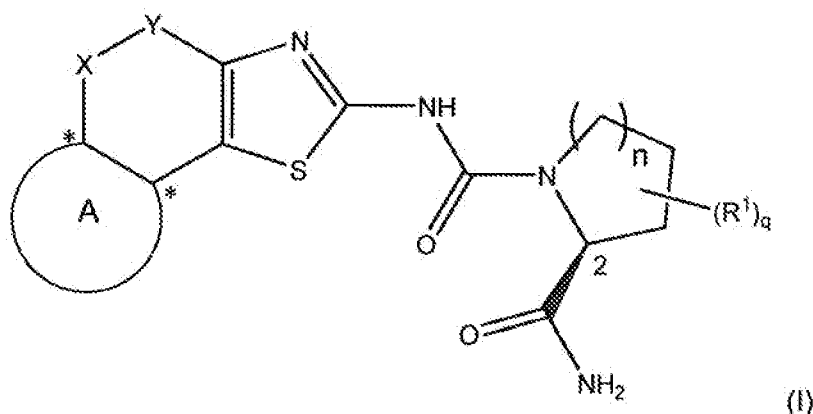
- 108 -

Example no.	PI3Kalpha / IC50 [umol l-1]	PI3Kbeta / IC50 [umol l-1]	PI3Kgamma / IC50 [umol l-1]	PI3Kdelta / IC50 [umol l-1]
30	0.023	2.135	0.119	0.294
31	0.0515	5.825	1.226	1.940
32	0.007	1.092	0.131	0.043
33	0.0695	5.079	0.909	0.860
34	0.0125	1.818	0.166	0.156
35	0.009	2.908	0.195	0.354
36	0.012	0.51	n.d.	0.04
37	0.004	0.182	n.d.	0.019
38	0.009	0.866	n.d.	0.044
39	0.013	1.612	0.334	0.265
41	0.024	3.046	n.d.	0.496
42	0.008	0.409	n.d.	0.032
43	0.005	0.771	n.d.	0.045
44	0.007	0.073	n.d.	0.049
45	0.0065	0.061	n.d.	0.024
46	0.0055	1.148	n.d.	0.157
47	0.003	6.168	n.d.	1.620
48	0.006	0.920	n.d.	0.019
49	0.004	1.248	n.d.	0.101
50	0.009	0.555	n.d.	0.113
51	0.005	3.285	n.d.	0.278

n.d. = not done.

CLAIMS

1. A compound of formula I



wherein,

A is an unsubstituted or substituted aryl ring or unsubstituted or substituted heterocyclic ring fused to the rest of the molecule at the positions indicated by the symbol *;

X-Y is $(CH_2)_r$ or $O(CH_2)_t$ or $(CH_2)_tO$ wherein,

r is 1, 2 or 3;

t is 1 or 2;

n is 0, 1 or 2;

q is 0, 1, 2, 3 or 4;

R^1 represents, independently at each occurrence,

halo;

hydroxy;

unsubstituted or substituted aryl;

unsubstituted or substituted amino;

unsubstituted C_1 - C_7 -alkyl;

C_1 - C_7 -alkyl, which is substituted one or more times by

hydroxy, C_1 - C_7 -alkoxy, unsubstituted or substituted amino, aryl or

heterocyclyl, and wherein aryl may be mono or poly-substituted by halo; or

two R^1 substituents together form an alkandiyl to form a cyclic moiety, optionally substituted by hydroxy or halo,

or a salt thereof.

2. A compound according to Claim 1, wherein

Ring A is substituted by one, two or three R^2 groups, independently selected at each occurrence from,

unsubstituted or substituted C_1 - C_7 -alkyl;

unsubstituted or substituted amino;

unsubstituted or substituted C_3 - C_7 -cycloalkyl,

or a salt thereof.

3. A compound according to claim 1 or claim 2, wherein

R^2 is selected from

unsubstituted C_1 - C_7 -alkyl;

di(C_1 - C_7 -alkyl)amino;

C_1 - C_7 -alkyl substituted one or more times by C_3 - C_7 -cycloalkyl or halo;

unsubstituted C_3 - C_7 -cycloalkyl;

C_1 - C_7 -cycloalkyl which is substituted one or more times by halo, (halo- C_1 - C_7 -alkyl) or C_1 - C_7 -alkyl,

or a salt thereof.

4. A compound according to any preceding claim, wherein

ring A is an unsubstituted or substituted 5- or 6-membered ring containing 1 or 2

heteroatoms selected from N, S or O, wherein at least one heteroatom is N,

or a salt thereof.

5. A compound according to any preceding claim, wherein

ring A is selected from an unsubstituted or substituted pyridine ring, unsubstituted or substituted pyrimidine ring or unsubstituted or substituted thiazole ring,

or a salt thereof.

6. A compound according to any preceding claim, wherein

X-Y represents $(CH_2)_r$ or $O(CH_2)_t$ wherein r is 2 and t is 1.

7. A compound according to any preceding claim, wherein

R^1 represents, independently at each occurrence,

halo;

hydroxy;
 unsubstituted or substituted phenyl;
 di(C₁-C₇-alkyl)amino;
 unsubstituted C₁-C₇-alkyl;

- 5 C₁-C₇-alkyl, which is substituted one or more times by
 hydroxy, C₁-C₇-alkoxy, di(C₁-C₇-alkyl)amino, di-(perdeuteroC₁-C₇-alkyl)amino, phenyl,
 morpholinyl, acetylamino, or *N*-(C₁-C₇-alkyl)-*N*-(phenylC₁-C₇-alkyl)amino, and wherein
 independently each phenyl may be mono or poly-substituted by halo.

- 10 8. A compound according to any preceding claim, wherein
 n is 1 and q is 1.

9. A compound according to claim 1, selected from:

(2*S*,4*R*)-4-Dimethylamino-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-
 15 dihydro-thiazolo[4,5-*h*]quinazolin-2-yl)-amide];

(2*S*,3*S*)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-
 dihydro-thiazolo[4,5-*h*]quinazolin-2-yl)-amide];

(*R*)-2-Benzyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-
 thiazolo[4,5-*h*]quinazolin-2-yl)-amide];

20 (*S*)-2-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-
 thiazolo[4,5-*h*]quinazolin-2-yl)-amide];

(*R*)-2-Methoxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-
 dihydro-thiazolo[4,5-*h*]quinazolin-2-yl)-amide];

25 (*R*)-2-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-
 4,5-dihydro-thiazolo[4,5-*h*]quinazolin-2-yl)-amide];

*d*₆-(*R*)-2-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-
 4,5-dihydro-thiazolo[4,5-*h*]quinazolin-2-yl)-amide];

(*R*)-2-Hydroxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-
 dihydro-thiazolo[4,5-*h*]quinazolin-2-yl)-amide]

30 (*R*)-2-Hydroxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-
 dihydro-thiazolo[4,5-*h*]quinazolin-2-yl)-amide]

(*R*)-2-[(3-Fluoro-benzyl)-methyl-amino]-methyl-pyrrolidine-1,2-dicarboxylic acid 2-
 amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-*h*]quinazolin-2-yl)-amide];

(S)-2-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

5 (S)-Azetidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

10 5-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,4R)-4-Fluoro-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,4S)-4-Fluoro-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

15 (1S,5R)-2-Aza-bicyclo[3.1.0]hexane-1,2-dicarboxylic acid 1-amide 2-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(1S,5R)-2-Aza-bicyclo[3.1.0]hexane-1,2-dicarboxylic acid 1-amide 2-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

20 (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,4S)-4-Dimethylamino-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

25 5-Phenyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

Azetidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide]; (S)-Azetidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

30 (2S,4S)-4-Hydroxy-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,4R)-4-Hydroxy-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-diethylamino-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

(S)-2-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

5 (S)-Azetidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

(2S,4R)-4-Dimethylamino-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

10 (S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[7-(2-fluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl]-amide];

(S)-Pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-cyclopropylmethyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

(2S,3S)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

15 (1S,5R)-2-Aza-bicyclo[3.1.0]hexane-1,2-dicarboxylic acid 1-amide 2-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

(2S,3S)-3-(Acetylamino-methyl)-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

20 (2S,3S)-3-Morpholin-4-ylmethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,3R)-3-Hydroxy-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

25 (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-methyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide];

(2S,3S)-3-Morpholin-4-ylmethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide];

30 (2S,3R)-3-Methoxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,3S)-3-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl)-amide];

(2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl]-amide];

(2S,3R)-3-Methoxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-thiazolo[4,5-h]quinazolin-2-yl]-amide];

(2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide];

5 (2S,3S)-3-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(8-tert-butyl-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl)-amide];

(2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[8-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4H-5-oxa-1-thia-3,7-diaza-cyclopenta[a]naphthalen-2-yl]-amide];

10 (2S,3R)-3-Methyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[7-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl]-amide];

(2S,3R)-3-Methoxymethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[[7-(2,2,2-trifluoro-1,1-dimethyl-ethyl)-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl]-amide];

(2S,3S)-3-Dimethylaminomethyl-pyrrolidine-1,2-dicarboxylic acid 2-amide 1-[(7-tert-butyl-4,5-dihydro-benzo[1,2-d;3,4-d']bisthiazol-2-yl)-amide].

15

10. A pharmaceutical composition comprising a compound of formula (I), according to any of Claims 1 to 9, or a pharmaceutically acceptable salt thereof, and optionally a further therapeutic agent, together with a pharmaceutically acceptable carrier.

20

11. A compound of the formula (I), according to any one of Claims 1 to 9, or a pharmaceutically acceptable salt thereof, for use in the treatment of a lipid and/or protein kinase dependent disease.

25

12. Use of a compound of formula (I), according to any one of Claims 1 to 9, or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition for use in the treatment of a lipid and/or protein kinase dependent disease.

30

13. A method of treatment of a disease that responds to inhibition of a lipid and/or protein kinase, which comprises administering a prophylactically or therapeutically effective amount of a compound of formula (I) according to any one of Claims 1-9, or a pharmaceutically acceptable salt thereof, to a warm-blooded animal in need of such treatment.

14. A compound for use according to Claim 11, or the use of a compound according to claim 12, or a method of treatment according to claim 13, wherein the disease is a lipid kinase dependent disease dependent on a Class I PI3K.
- 5 15. A compound for use according to Claim 11, or the use of a compound according to claim 12, or a method of treatment according to claim 13, wherein the disease is a lipid kinase dependent disease dependent on a Class I PI3K selected from the group consisting of PI3Kalpha, PI3Kbeta, PI3Kdelta, PI3Kgamma.
- 10 16. A compound for use according to Claim 11, or the use of a compound according to claim 12, or a method of treatment according to claim 13, wherein the disease is a proliferative disease; a benign or malignant tumor; a cancer selected from 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; 15 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 20 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; and myelofibrosis with myeloid metaplasia.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/059254

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D513/04 C07D513/14 A61P35/00 A61K31/428 A61K31/429
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D A61K A61P

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

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

EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006/106013 A1 (BREITFELDER STEFFEN [DE] ET AL) 18 May 2006 (2006-05-18) paragraph [0003] - paragraph [0007]; claims 1, 31; examples A-200, C-380, C-381 -----	1-16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

10 August 2010

Date of mailing of the international search report

17/08/2010

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Authorized officer

Johnson, Claire

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/059254

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006106013 A1	18-05-2006	AR 055273 A1	15-08-2007
		AU 2005293607 A1	20-04-2006
		BR PI0516557 A	09-09-2008
		CA 2579279 A1	20-04-2006
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		WO 2006040279 A1	20-04-2006
		JP 2008515852 T	15-05-2008
		KR 20070064660 A	21-06-2007
		US 2006100254 A1	11-05-2006
		US 2009156554 A1	18-06-2009
		US 2010113414 A1	06-05-2010
